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Acta Medica Academica

Journal of Department of Medical Sciences
of Academy of Sciences and Arts of Bosnia and Herzegovina

Genomics in Hematology and Oncology Practice

ISSN 1840-1848 (Print) Volume 48 Number 1 April 2019 ISSN 1840-2879 (Online)

Online First www.ama.ba

Acta Medica Academica

Contents
2019 Vol. 48, No. 1

Acta Medica Academica

www.ama.ba

AIMS AND SCOPE

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SUBSCRIPTION

Acta Medica Academica is published triannually. The annual subscription fee is € 50 outside of Bosnia and Herzegovina.

PUBLISHER CONTACT INFORMATION

Academy of Sciences and Arts of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina. Contact person: Husref Tahirović, E-mail: husref.tahirovic@untz.ba

COVER PHOTO PICTURE

Anna Bayerová (1853-1924; the first official female doctor in Bosnia and Herzegovina) by Jan Vilímek (1860-1938), created on 7 June 1889.

AUTHOR INFORMATION

Instructions to authors can be found at http://www.ama.ba/forms/AMA-2019-instrukcija.pdf. Home page of the Journal www.ama.ba offers free access to all articles.

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PRINT

Dobra knjiga, Sarajevo, BA. Printed on acid-free paper.

CIRCULATION

500 copies.

THE JOURNAL IS INDEXED IN

Medline/PubMed; EBSCOhost; Index Copernicus; CAB Abstract/Global Health Databases; IndexScholar.com; DOAJ; CrossRef; InfoBase Index.

Print and electronic issues of AMA are covered in Scopus and Embase through Medline.

Acta Medica Academica (ISSN 1840-1848) is an international peer-reviewed triannual journal which is printed as a continuation of the journal Works of the Academy of Sciences and Arts of Bosnia and Herzegovina, Department of Medical Sciences, founded in 1953. *Acta Medica Academica* Online (ISSN 1840-2879) offers free access to all articles at www.ama.ba.

Editorial
Acta Medica Academica 2019;48(1):1-5
DOI: 10.5644/ama2006-124.237

Genomics in Hematology and Oncology Practice

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Received: 24 April 2019
Accepted: 25 April 2019

Key Words: Genomics ▪ Practice ▪ Education ▪ Future.

"What can and doesn't have to be always, at the end, surrenders to something that has to be."
Ivo Andric. Signs by the roadside (1)

Most of my colleagues when writing commentaries or overviews quote Shakespeare, and deservedly so. I have to confess, with embarrassment though, that my understanding and command of great Shakespeare's poetry is very limited. I was raised and learned to love great Russian classics and our only Nobel prize laureate in literature, Ivo Andric. Definitely, because he wrote about places and people I recognize and understand; partially because he was born and schooled in Bosnia and Herzegovina. His writing once in private conversation with my friends was described as "mighty, slow, wide river" which immediately brought in my mind Mississippi in New Orleans where my American adventure started. Power of that unstoppable force that carries everything with and in front of it always fascinated me. That is how I see progress in oncology with the introduction of genomics and prospect of precision medicine and individualized treatments. Genomics offers the potential for deeper understanding of disease pathophysiology, prognostication, identification of predictors of therapeutic responses, depth of responses, discovery of new targets for the treatment and ultimately improvement in quality of life and prospect for the cure.

In this special issue of Acta Medica Academica (AMA) we attempted to describe ways how introduction of genomics has influenced the practice of Oncology and Malignant Hematology from diagnosis to planning and coordination of care to treatment and outcomes itself. Readers will decide if we succeeded.

The main question we will have to answer in the future is: Do genomic alterations always or in the most of the cases lead to oncogenic pathway activation and how do we target multiple potential drivers and how to address development of resistance pathways in order to achieve optimal antitumor efficacy? The preliminary results from National Cancer institute Molecular Analysis for Therapeutic Choice (NCI-MATCH) trial presented at 2018 American Society of Clinical Oncology (ASCO) Annual meeting described results of 3 cohorts with no agent reaching the prespecified threshold of notable clinical activity (2). That prompted edi-

torial in JAMA Oncology by Eckhardt and Lieu: Is precision medicine an oxymoron? (3). Authors asked very legitimate question if these results indicate that a molecularly driven agnostic approach is a failure. Their response is qualified yes and no. My reading of preliminary NCI-MATCH results is that they are exactly what they are: preliminary. These are early nascent results pointing to the fact that drugs T-DM1, AZD4547 or taselisib in patients harboring ERBB2/HER2 amplification, FGFR alterations or PIK3CA mutations, respectively, did not provide meaningful clinical effects when addressed as isolated mutations. Nothing more or nothing less. It is true that those results also pointed to the possible shortcoming of addressing individual mutations with monotherapy. Recently published study I-PREDICT from University of San Diego presented results of targeting larger fraction of identified molecular alterations, yielding a higher "matching score" (4). This novel approach showed significantly improved disease control rates, as well as longer progression-free survival (PFS) and overall survival (OS) compared to targeting of fewer somatic alterations. Despite innovative approach and excellent results seen in this study, this is just scratching the surface. We have to keep in mind that in this study, as well as in other studies to the present, we addressed only molecular or gene alterations that have available FDA approved or experimental treatments, so called "drugable" alterations. Consequently, with increased availability of therapies targeting other possibly driving alterations, expected outcomes could, and I believe will become more and more clinically relevant.

Therefore, the articles in this issue are describing the translation of increasing knowledge and understanding of genomics to the clinical practice, having medical students, residents, fellows as well practicing hematologists and oncologists in mind. Our goal was not to give comprehensive review of present understanding of clinical genomics in Oncology and Malignant Hematology, but to provide basic premises of changes that genomics use brings to every day practice of malignant disease patient care.

We begin with an article by Trivedi et al. (5) that summarizes changing landscape of clinical practice, from empirical to evidence-based to biology-based personalized medicine. In this article authors are describing technological and intellectual advances that led to explosion of new treatments in the therapy on malignant diseases. They are describing evolution of critical thinking in oncology and development of linkage between genomic and clinical data as well as computational biology expertise required for data analysis. Ultimately, authors are painting a picture of future developments and the need for changes in education of physicians in order to fulfill promise of most appropriate care for each individualized patient.

Article by Audeh et al. (6) opens for readers almost magical world of thinking behind development of 70-gene assay MammaPrint, first FDA cleared genomic assay for breast cancer. The quality of this paper is not so much in clinical data connected with use of MammPrint, although they are very impressive. Authors in this article took "roads less travelled". They opened window into thought process and decision making behind MINDACT trial design and goals definition. Paper illustrates meticulous decision making process and weighing between what can be and what needs to be done. This paper is not relevant only for those interested in genomics in the oncological and hematological practice, but also for everybody who wants to learn about scientific process and design of research studies.

Although our understanding of molecular processes involved in the development of acute myelogenous leukemia (AML) are greatly advanced by genomic medicine,

there are still "great unknowns". Madanat et al. (7) summarized data on commonly mutated genes and genomic pathways in AML. This data is now increasingly being used for disease classification, risk stratification, and clinical care of patients. Review highlights major updates in the World Health Organization (WHO) classification, including cytogenetic re-classification, provisional entities (AML with mutated RUNX1 and AML with BCR/ABL1) and updates to the European Leukemia Net (ELN) AML group risk stratification (RUNX1, ASXL1 and TP53). Future of treatment could be driven by complex interactions between different mutations. Assessment of minimal residual disease (MRD) also could improve risk stratification and selection of post remission therapy.

Strategic decision making required to optimize laboratory work up of lymphomas is focus of article by Shi et al. (8). Authors are discussing lure and attraction of "new shiny" tests for multiple genomic abnormalities and their significance for patient care and practical world use. Danger of over testing is real and with commercial entities pressure and financial influence we, as ultimate providers of the best care, need to have clear guidance which testing is necessary, and which will be affordable from patients' and societal view point. Authors are providing simplified algorithm for the work up of Diffuse large B-cell lymphoma (DLBCL) and High-grade B-cell lymphoma (HGBCL) which is rational and practical and could be used almost universally. As the field evolves, new tests and panels could become more affordable and clinically relevant and will become the standard of care (SOC).

Although mutational landscape of multiple myeloma (MM) seems hard to decipher due to significant heterogeneity, it is also almost perfect model case for evolution of genetic changes. MM is unique among hematological malignancies in its universal evolution from pre-malignant stages to proliferation of malignant cells. Due to that, significant body of literature is available examining impact of genomics on MM risk stratification and treatment. I applaud Castaneda and Baz (9) for their effort to make it easier for readers to navigate through richness of data and for summarizing them in this article. They also explained very nicely predictive value of genomic testing and possible use of this testing for evaluation of specific agents resistance development. In addition, it seems that Next generations sequencing (NGS) will become universally accepted and used tests for evaluation of Minimal Residual Disease (MRD) in MM patients. Negative MRD is now shown to correlate with better OS in MM (PRIMER study)(10).

Devitt and Dreicer (11) focus on role of genomics in Genitourinary (GU) malignancies. They described most common germ line mutations associated with prostate cancer, as well renal cell carcinoma. It is very important to emphasize that prostate cancer is one of the most heritable forms of malignancies. That is reason for recommendation for all patients with metastatic prostate cancer to be referred for genetic counselling and testing. Mutations in the genes HOXB13 and BRCA 1 or 2 have been associated with family clusters of prostate cancers. Authors also explained potential predictive role of mutation testing in the treatment of prostate cancer. Prognostic utility of mutations in PBRM1 and BAP1 in renal cell carcinoma seems to be in concordance with biological and clinical features of this disease. However, it seems that wider use of genomics in GU cancer is still in its nascent stages and more opportunities will become available in the future. That is becoming more obvious in urothelial cancers, as molecular subtyping using gene expression profiling has emerged as a prognostic and predictive tool.

Use of genomic testing completely changed landscape of lung cancer treatments. Body of literature dealing with this

topic is growing exponentially and Ankur Parikh (12) summarized present state of knowledge and open questions waiting for answers. Presently, defining treatment for lung cancer is almost impossible without analysis of targetable genes altered in non-small cell cancer (NSCLC). Treating patients without knowledge of EGFR, ALK, ROS1, BRAF, MET, HER2, RET and NTRK1 mutations is now considered far outside of SOC. Big data analysis and long term results from ongoing genomics based clinical trials will open other, at the present, uncovered avenues for more effective treatments of this deadly disease.

SOC treatments are also rapidly evolving in the arena of Gynecologic (GYN) malignancies. Maurie Markman (13) reviewed present state of art approaches to treatment of ovarian cancer and changing landscape based on BRCA mutations. In addition multiple other potentially "driver" mutations (PIK3CA, ADAMTS, DICER1, BRAF, KRAS, ARIDA1A and others), although presently still not targetable or "drugable" could become soon very effective targets with potentially less toxicity and better clinical outcomes. Use of check point inhibitors is becoming more and more standard in the treatment of GU malignancies, and genomic analysis could potentially define patients who are good candidates for these treatments.

Saadeh et al. (14) took task to review very important topic of application of precision medicine in oncology pharmacy practice. This topic is not frequently covered in oncology literature and, in my opinion, this is very important contribution to education of practicing oncologists. Explosion of new malignancies targeting medications is creating new challenges and new opportunities for clinical pharmacists, particularly those specializing in Pharmacogenomics (PGx). This component of precision medicine is based on polymorphisms and strongly impacts drugs selection and dosing. Although new medications are very effective, they bring potentially new and different toxicities that could be reduced and minimized by using pharmacogenomics-based dosing recommendations. It is very important to emphasize critical role of clinical pharmacists in supporting multidisciplinary approach to the care of patients with cancers.

Very interesting case report describing crucial role of comprehensive genomic profiling in the treatment of patient with high stage uterine mesenchymal tumor is presented by Lee et al. (15). Testing showed to be essential for establishing correct diagnosis as well as uncovering until then unrecognized ALK mutation used subsequently as target for effective treatment. This shows how effective analysis can change life of oncology patients, one patient at time.

Last, but not least is genomics practice article by Trivedi et al. (16). Oncology patient whose tissue is analyzed by next generation sequencing (NGS) can show targetable mutations or be candidates for genomics based clinical trial. However, significant number of patients will not fit into either of these groups and additional options need to be investigated. That is where Molecular Tumor Board (MTB), multidisciplinary panel discussion comes into play opening other possible avenues based on in depth analysis of growth pathways and role of mutated genes in signal transduction. In this article, results of MTB in mid-size cancer center are described. It illustrates the advantages provided to patients by finding treatments when no other options are available, as well as missed opportunities based on physicians' and patients' preferences or biases. It also emphasizes the need for education of patients, physicians and general public about incredible advantages and possible shortcomings of genomics testing and concepts of precision medicine. I honestly hope this issue of AMA could help making that educational task easier.

Acknowledgment: Author would like thank Miss. Kathryn Edwards and Dr. Harsha Trivedi for assistance with manuscript preparation.

Conflict of Interest: The author declares that he has no conflict of interest.

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Narrative Review
Acta Medica Academica 2019;48(1):6-17
DOI: 10.5644/ama2006-124.238

Changing Landscape of Clinical-Genomic Oncology Practice

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Received: 25 September 2018
Accepted: 29 April 2019

Key Words: Genomics ▪ Breast Cancer ▪ Clinical Trials ▪ Precision Medicine ▪ Personalized Medicine.

The current paper discusses the use of genomics in the context of the changing landscape of clinical practice and modern medicine. Medical practice has shifted considerably over the past few decades, from empirical to evidence-based to personalized medicine, and the transition from reliance on observation to measureable parameters. Scientific innovation is required to collect an ever-increasing number and variety of data points and sophisticated analyses capable of distilling vast datasets into meaningful information. The next phase of innovation seeks to personalize disease management, in particular through genomics in oncology. With expanding use of genomics in medicine, and several initiatives collecting genomic data at the population level, education of patients and physicians is critical for data utility. By combining genomic and clinical data, bioinformatics approaches can be applied to developing individualized or targeted therapies. Breast cancer provides an example through which to understand the evolution of genomic data from pure science to clinical utility. From intrinsic subtype classification to development of multigene panels estimating recurrence risk, new studies, such as the FLEX trial, will expand to evaluate the whole transcriptome of tumours. This approach will enable discovery of novel gene signatures and ultimately pave the way toward a personalized approach to breast cancer management. **Conclusion.** Despite the potential for genomics to personalize treatments, a number of challenges remain to fully integrate these types of large datasets in a manner that provides clinicians and patients with meaningful, actionable information. However, if challenges are addressed, precision medicine has the capacity to transform patient care.

Introduction

For centuries, empirical evidence dominated the practice of medicine. Historically, clinical decision making was largely based on clinical experience, gained through trial and error. Despite the long history of research in medicine, approaches to incorporating the knowledge gained from basic and clinical research into the practice of medicine were generally non-standardized and subjective until recently. The utilization of research evidence in diagnosing and treating patients was determined by the individual physician, and, if incorporated, would have been alongside clinical experience and personal beliefs.

The use of evidence-based methods for both teaching and practicing medicine is founded in clinical epidemiology, and chronologically follows just behind evidence-based policies and guidelines, first published by David Eddy in a series of papers in the *Journal of the American Medical*

Association (1-4). It concurrently de-emphasizes intuition and unsystematic clinical experience as rationale for clinical decision making (5). A shift toward evidence-based medicine has gained attention over the past two decades (6), seeking to integrate clinical expertise, the patient's personal preferences and the best available evidence in making health care decisions. Now an umbrella term "evidence-based medicine" captures both population- and individual-level decisions, this practice emphasizes the importance of incorporating evidence from formalized research into clinical decision making.

Evidence-based medicine is not without shortcomings (7). Evidence used in practice has been collected from large cohorts of patients, from which data are summarized into an expected response of the average patient from a given population (8). Although far more informative and accurate than its predecessors of intuition and the "art of medicine", the unfortunate consequence of this approach is that outliers are not represented, and they may be unlikely to respond similarly to the average patient for any given treatment.

Precision or personalized medicine, in contrast, focuses on the individual and seeks to improve health outcomes by integrating a huge variety and number of data points, from genomics to environmental and lifestyle factors, in order to provide an individualized approach to health care. Despite a lofty set of long-term goals, including earlier detection and better monitoring of disease symptoms, prediction of disease in asymptomatic individuals, more accurate prediction of treatment responses, improved disease surveillance, and prevention of disease, when possible, precision medicine has its own set of challenges and limitations. Some of these include small sample sizes ("n of 1" studies), the technological capability required to compile large datasets, such as gene expression data or full exome sequencing, and the informatics needed to distil vast amounts of data into clinically useful measurements.

There are strengths and weaknesses to both of these approaches, and integration of the strengths of both toward 'evidence-based precision medicine' will be complimentary and provide the best possible treatment for patients. Nonetheless, there are challenges to the integration of these two approaches. These include revisions to medical education and training programs, including training additional experts in clinical bioinformatics to interpret the large data sets that will be generated, and education of clinical professionals to stay current with the ever changing body of knowledge and to address the increasingly multidisciplinary practice of medicine. However, the benefits of successful integration of these approaches may be an ultimate shift in emphasis from reactive to proactive medicine, and a focus on prevention, rather than treatment, of disease (7).

Here, we will explore the challenges and progress of integrating genomics into clinical practice and several initiatives in various countries for large-scale genomic data collection at the population level. We will use breast cancer as an example through which to demonstrate the progression of genomic data into clinical utility and highlight how genomics and clinical data are being integrated for the discovery of new genomic signatures with the potential to provide individualized insights into disease management.

Medical Science: From Observation to Measurement

In addition to shifting paradigms to the practice of medicine, the science of medicine has transitioned from dependence on observation, empirical knowledge, and patient-reported symptoms to measurable parameters. This changing landscape has paralleled scientific and technological advances

that continue to propel the field forward and allow for the collection of an ever-increasing number and variety of data points, enabling improved understanding of health and disease, as well as treatment options. For example, in a span of only a few decades, diagnostic tests have graduated from use of the microscope to visualize changes in cellularity that might indicate infection or inflammation to modern techniques, such as molecular testing, rapid detection and quantification of pathogens, automated blood chemistry panels, genetic and genomic testing, and sophisticated medical imaging. Furthermore, novel ways of measuring outcomes are moving from the idea of treating the disease to improving the patient's quality of life. Hospitals and healthcare systems are becoming more interested in patient-reported outcomes as a way of measuring progress and success. All of these datasets require sophisticated and sometimes complex analyses capable of distilling the mountain of information down to an actionable, or at least measurable, outcome.

Advances in Science and Technology

Advances in technology are making it possible to diagnose diseases, including cancer, earlier than ever before. For example, improvements in radiology and medical imaging have led to an uptick in early breast cancer diagnoses (9). Cancer Research UK reports that 31% of female invasive breast cancer cases in England are now detected by screening (10). Women diagnosed by this method are overwhelmingly Stage I-II, and with early treatment have observed steadily improving breast cancer survival since the 1990s (11). Despite these statistics, the American Medical Association (AMA) recommendation of regular mammographic screening for all women starting at age 40 has become controversial in the last decade. In November 2009, the US Preventative Services Task Force (USPSTF) recommended that screening mammograms should start at age 50 instead of 40 for women of average risk (12). The rationale was that although screen detection has improved the diagnosis of breast cancer, the rate of false-positive results was more common in women aged 40-49, resulting in unintended consequences such as psychological harm and unnecessary imaging tests and biopsies in women without cancer. The Breast Cancer Surveillance Consortium recently evaluated 1,682,504 digital screening mammograms from 2007 to 2013 and confirmed that to be true (13). They determined that the while screen detection has increased the rate at which abnormal findings (AIR) are identified, since switching from film to digital images (10.0% in 2008 to 11.6%, Table 3), the proportion of these cases correctly detecting invasive cancer has declined from 90.3% specificity in 2008 to 88.9% in the current study.

Although many more women are now being treated for breast cancer, there has been no concomitant increase in survival benefit as a population (11). The USPSTF suggested that mammograms now detect small tumours, which previously would have gone undetected in the patient's lifetime, and unnecessary early treatment is pervading. In a pre-screening era these tumours, even untreated, may have posed minimal risk to the patient. Now the challenge is to identify which tumours are biologically low risk to appropriately spare patients from over-treatment, versus the tumours that are caught at a very early stage but are biologically high risk and require treatment.

In order to accurately distinguish high risk from low risk tumours, as well as to identify effective therapies, appropriate biological markers are needed. The same mutations may predominate in multiple cancers, and initiatives like The Cancer Genome Atlas (National Institutes of Health) are showing that there is substantial heterogeneity of ma-

lignant drivers, even within the same 'type' of cancer (8), which is still classified according to its site of origin, as it has been historically. Heterogeneity of treatment responses can be found across all cancer types, but has the potential to be resolved with new biological marker discovery. Biological markers of cancer aggressiveness or treatment response have been identified in nearly every cancer and correlated to survival. Expanding availability of targeted therapies provide further opportunity to correlate genomic changes in the tumour with treatment responses, leading to improved understanding of targetable pathways involved in disease progression. The discovery of accurate biological markers has the potential to ultimately change the way cancer is described, diagnosed and treated, from a disease classified by its tissue of origin to an individualized classification based on a particular set of biological characteristics.

Although the development of novel and targeted therapeutics in some cancers has been made possible by the discovery of single gene mutations (genetics), other cancers rely on expression profiles that are composed of many genes (genomics). In breast cancer, advances have been driven by expression profiles more than single gene mutations. Although the heterogeneity and complexity of breast cancer has been recognized for some decades, the field shifted substantially with the publication of microarray-based gene expression profiles demonstrating this heterogeneity at the molecular level and the sub classification of breast cancer into molecular subtypes (14-16). These subtypes, luminal A, luminal B, normal breast-like, HER2, and basal-like (15, 17, 18), have distinct clinical-pathological features, risk factors, responses to therapy, and clinical outcomes, demonstrating the clinical utility of molecular profiling. Following this discovery was the development of multigene panels to assess molecular characteristics of tumours and predict risk of recurrence in patients with early stage disease. Historically, clinical-pathological factors (patient age, tumour size, histopathologic features, lymph node involvement) have been used to estimate probability of breast cancer recurrence; however, there has been substantial interest in developing molecular assays that more accurately predict clinical outcome, thereby selecting patients who will most benefit from more aggressive therapies, while avoiding overtreatment in patients with comparatively low risk of distant recurrence. Presumably, selection of a combination of genes that provide information about a tumour's metastatic potential will most accurately predict distant recurrence in that patient. Several commercially-available molecular assays have been developed with the aim of providing this information (19). These assays use a variety of platforms, including reverse-transcriptase PCR (OncotypeDX, Breast Cancer Index, EndoPredict assays), Nanostring technology (Prosigna assay), and microarray technology (MammaPrint assay), for quantification of gene expression (19).

The first FDA-cleared multigene test, MammaPrint, uses a combined profile of 70 genes to assess metastatic potential of the tumour (20), thereby predicting a patient's risk of distant recurrence. The genes that comprise this signature function in proliferation, cell cycle dysregulation, invasion, angiogenesis, growth, and resistance to apoptosis (21). Genes were selected from the full transcriptomes of archived tumour specimens from patients without any systemic therapy (endocrine or chemotherapy) who either had a poor prognosis (distant metastasis within the first five years) or a good prognosis (no distant metastasis within the first five years). Using unsupervised hierarchical clustering, a signature of 70 genes was developed, representing the most differentially expressed genes between these two groups

of patients (20). The 70-gene signature was further validated in subsequent studies (22-26), and clinical utility was assessed in the prospective, randomized MINDACT trial, which compared outcomes following risk assessment by genomic or clinical parameters (27). The MINDACT trial demonstrated that patients at low genomic risk of recurrence by the 70-gene signature could safely forego chemotherapy (27). The companion molecular subtyping assay to MammaPrint uses an 80-gene signature (BluePrint) to sub-categorize breast tumours as Luminal-, HER2-, or Basal-type (28-30). Compared with standard clinical subtyping, molecular assessment provides information about the functionality of the dominant molecular pathway in a tumour, and has been shown in studies to provide more accurate classification, as indicated by chemotherapy responses (29, 31). These assays demonstrate the clinical utility of genomics in breast cancer.

Biomarker discovery provides new opportunities for treatment personalization with advancing targeted therapeutics. Although using the immune system to treat cancer has a history dating from the late nineteenth century (32), cancer immunotherapy research and available therapies have exploded in recent years (33, 34). Immunotherapies, including checkpoint inhibitors, have shown remarkable success in generating durable responses in some patients, even in those for whom no other treatments were effective. However, these therapies have been underwhelming in others, and identification of biomarkers that accurately predict which patients will benefit from particular therapies will be crucial to ensuring more widespread success of these agents. Like other targeted therapies, immunotherapy is not a 'one size fits all' approach to cancer treatment. Although treatment success rates are higher in some cancers than others, immune checkpoint inhibitors are also not cancer site-specific. A variety of factors likely contribute to its success or failure, and understanding these factors will enable improved patient selection. The first FDA approval of an immune checkpoint inhibitor, pembrolizumab (Keytruda), which targets programmed death protein 1 (PD-1) on the surface of immune cells, thereby improving immune responses, was for patients with advanced melanoma. Since then, however, this inhibitor has been approved for use in at least 12 types of cancer and in patients with a common biomarker (tumours identified as microsatellite instability-high or mismatch repair deficient), irrespective of the cancer's site of origin (https://www.fda.gov/news-events/newsroom/pressannouncements/ucm560167.htm). With improved accuracy of biological markers and patient selection, it is possible that the success of immunotherapies will be expanded into cancers for which these treatment regimens have previously had limited success, including breast cancer (35, 36). It is likely that identifying the most predictive biomarkers or combination of biomarkers will require integration of tumour biology and host immune factors, merging genomics and immunology.

Innovation in Data Capture and Analysis

Integration of datasets consisting of imaging, molecular, genetic/genomic, cellular, organismal, environmental, family history and lifestyle data will be required in order to truly personalize medicine. However, learning and integrating the ever-growing mass of scientific discoveries and synthesizing it into an actionable recommendation will require immense data processing capacity. IBM Research announced in 2007 that the computer IBM Watson was taking on medical science. Watson would apply its DeepQA open-domain question answering technology to provide an evidence-based clinical decision support system. Watson uses natural

language processing and machine learning to analyze unstructured information, overcoming the challenges with the structured data of traditional expert systems. It generates a list of possible questions and uses abductive reasoning to generate hypotheses and produce possible answers from the available information. In the healthcare setting, each potential answer then receives a confidence rating based on the supporting clinical and scientific evidence. If we can improve diagnostic accuracy, it could potentially be the most directly impactful step to improving our healthcare system.

Now that we are beginning to implement ways of integrating large and sometimes disparate pieces of information, the next challenge involves the production of content (data) necessary to generate meaningful conclusions and inform treatment recommendations. High throughput platforms, such as gene expression microarrays and next generation sequencing, produce thousands to millions of pieces of information per patient. Tied to clinical information, this big data provides the base from which questions can be asked and, one day, answered.

Several notable initiatives, including The Cancer Genome Atlas, the Cancer Moonshot, the 100,000 Genomes Project (United Kingdom) (37), the Sweden Cancerome Analysis Network - Breast (SCAN-B)[38], the NIH's All of Us research program, and France Genomic Medicine 2025, seek to enable scientific discovery through the integration of genomics and medicine on a large-scale, population-based level to improve collaboration, transparency and patient outcomes. France's Genomic Medicine 2025 aims to place France as a leader among major countries engaged in genomic medicine within the next 10 years. The first two genomic platforms were selected in 2017; 10 additional platforms are planned over the next five years. One will serve to meet the needs of patients suffering from cancer or rare diseases; the other is meant to begin sequencing genomes from the general population. Equipment and resources are planned to sequence the equivalent of 18,000 genomes per year. The 100,000 Genomes Project, in partnership with the National Health Service England, launched in late 2012, plans to sequence 100,000 genomes from NHS patients, targeting patients with rare diseases and those with cancer. The program aims to benefit patients and enable new scientific discovery, while also driving the UK genomics industry (37). The SCAN-B study was initiated in 2010 as a multicenter observational study to evaluate genomic profiles of breast cancer by whole transcriptome RNA-sequencing; by 2017, they had enrolled >10,000 patients and generated RNAseq data on >7500 specimens (38). The Cancer Genome Atlas (TCGA), a collaboration between the National Cancer Institute and the National Human Genome Research Institute to accelerate the understanding of the molecular basis of cancer through genome analysis technologies, collected data from 11,000 patients on 33 different tumor types during an 11 year period of study (https://cancergenome.nih.gov/abouttcga/overview). President Obama announced the Cancer Moonshot Initiative in January 2016, acknowledging the need to accelerate discovery and encouraging change. The five strategic goals of the initiative are to catalyze new scientific breakthroughs, unleash the power of data, accelerate bringing new patent therapies to patients, strengthen prevention and diagnosis, and to improve patient access and care (https://www.cancersupportcommunity.org/sites/default/files/uploads/policy-and-advocacy/article/cancer_moonshot_report_final.pdf). Notably, Vice President Joe Biden emphasized the need to engage patients as partners in research. The "All of us" research program was announced in 2016 by NIH to advance precision medicine. It has a goal to enroll 1 million or more

volunteers from diverse backgrounds and lay the scientific foundation for a new era of personalized health care. Data will be collected from participants by surveys, their electronic records, and some participants will provide urine and blood samples. Data will be analyzed to learn about the health disparities and different health conditions.

New Technologies Require Education

Patients are already exhibiting a growing desire to participate in their own health/disease management, as well as to connect with others afflicted by the same disease or condition. The internet and modern social media tools provide endless resources to patients and their families, changing the dynamic of the patient-physician relationships. More information can be a double-edged sword, since there is just as much misinformation as reliable, verifiable information. It is now the job of health professionals and care givers to not only disseminate information, but to also contextualize and curate information given to them by patients. All of these new technologies require education, and despite the unprecedented wealth of information provided in the current era of genomics in medicine, many physicians do not have the training/expertise to interpret results from the deluge of genetic/genomic tests performed (39).

Through clinical trials, physicians and patients can work together to advance medical science. The traditional view of clinical trials is that a study is designed to answer a single question, usually in reference to a new drug compared to the current standard of care. Of late, the failure of trial after trial to produce a positive result has magnified some major flaws of traditional trial design, namely that trials take too long to show efficacy. Although counter-intuitive, it actually becomes even harder to show efficacy in disease states in which treatments are currently very effective. Adaptive trial designs are pushing the envelope by moving patients in and out of different treatment arms based on their response (40). Unlike the traditional model, patients who do not benefit from an experimental treatment are quickly offered alternatives. In savvy adaptive trials, clinical and genomic data from responders are used to then identify other patients with similar characteristics for future randomizations in order to optimize the chance of finding the right patient for the right therapeutic. The goal of the trial has now changed from examining *how* will a patient respond to an experimental therapeutic, to identifying *who* will most likely benefit from an experimental therapeutic. By linking genomics and clinical data we can apply bioinformatics approaches to developing individualized or targeted treatments, using novel genomic signature and biomarkers to identify patients who will respond, or just as importantly patients who will not respond.

Linking Genomics and Clinical Data: the FLEX Study

Advances in technology have enabled this paradigm shift in medicine, recognizing the importance of converging clinical factors and genomic pathway dependencies. Likewise, the ability to electronically capture clinical data efficiently has opened the door to novel trial designs that are accelerating the rate of insight discovery and hypothesis testing. For example, testing for the 70-gene MammaPrint (MP) signature has become standard of care in many early stage breast cancers, but largely unrealized is that MP is still tested on a microarray platform, similar to how it was developed. This has allowed for the untested genes that reside on the microarray platform, but are outside of the MP algorithm, to be tested against clinical outcomes at a cost-effective rate, allowing the scale of research to not be limited by traditional trial funding.

This scalable model is the foundation of the FLEX Registry protocol.

FLEX (MammaPrint, BluePrint and Full-genome Data Linked with Clinical Data to Evaluate New Gene Expression Profiles: An Adaptable Registry) is a large-scale, population based prospective registry, sponsored by Agendia, Inc. The study is open to Stage I, II or III breast cancer patients and began enrolling patients in 2017. The FLEX study matches full-genome expression data (Figure 1) with comprehensive clinical data on patients enrolled in the study. Full genome profiling includes results from Agendia's two genomic signature assays, MammaPrint and BluePrint. FLEX is patient-centered, agnostic to breast cancer subtype, management plan and treatment regimen. The study aims to collect 10 years of follow-up data on participating patients. By design, the study will not follow a defined endpoint, but uses a changing heuristic model driven by matching genomic data with comprehensive clinical data.

Unlike traditional trials, often designed without the downstream input of those who will later try to analyze the data (41), in FLEX, all participating investigators have the ability to propose concepts for investigation, which will shape the method and

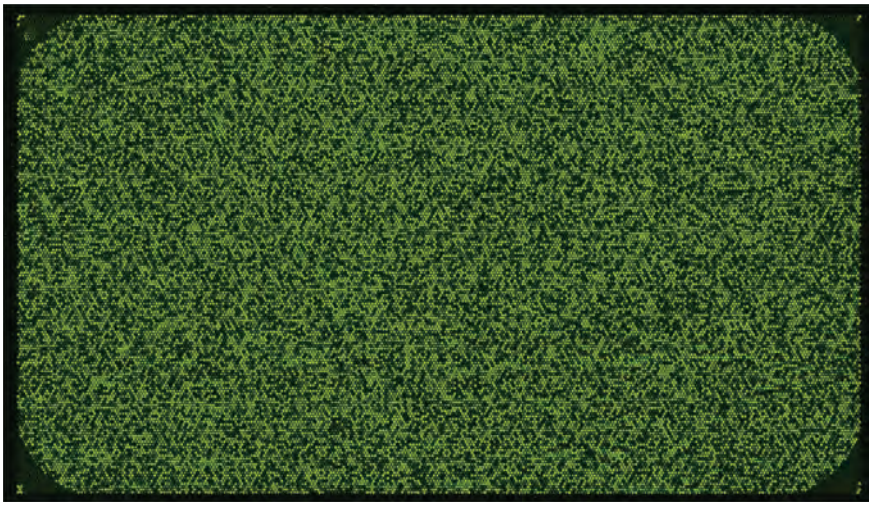

Figure 1. Representative pseudo-color image of microarray for full transcriptome.

Figure 2. Model of the FLEX registry research cycle in which new gene signatures, discovered through retrospective analysis, can be validated in a larger population and used to create personalized breast cancer profiles in the future.

structure of the clinical and genomic data collection of the trial, which will evolve over time (Figure 2). The scale of FLEX is also not limited to a defined enrollment target, which will allow obscure and yet-undefined clinical and genomic subsets to be adequately powered for study. This accelerated heuristic technique that FLEX employs has already produced compelling proof-of-concept arguments. Preliminary data analysis was performed on a subset of enrolled patients (n=43) from a single location. A heatmap of quintile normalized gene intensities for top 25% of most variable genes from full genome transcriptome, across 44 samples (one patient had two submissions) clustered by Pearson correlation is shown in Figure 3. Several clinical factors are included for comparison, demonstrating that the most differentially expressed genes in the breast tumors from these patients do not cluster by tumor grade, histopathologic tumor type, or lymph node involvement (Figure. 3). This method of clustering analysis applied over the non-limited scale and proliferative trial objectives will reveal genomic signatures relevant to breast cancer management, as well as the landscape of clinical-genomic oncology practice.

Summary/Conclusions

Technological developments in genomic medicine are advancing at a breathtaking rate. The potential for precision medicine to match patients to gene-targeted therapy is a very intriguing promise, but simultaneously creates many challenges. One of the main challenges to implementing genomic medicine into clinical practice is conquering the knowledge gap arising from the extensive breadth and depth of data available

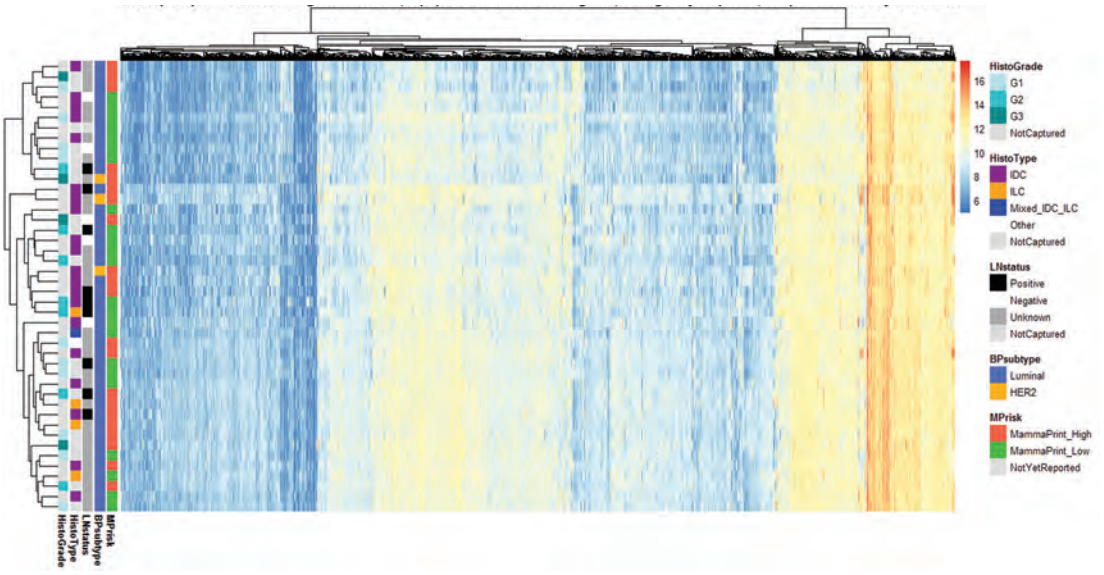

Figure 3. Heatmap of quantile normalized gene intensities for top 25% of most variable genes from full genome transcriptome, across 44 samples clustered by Pearson correlation. Visual representation of the relative amount of expression of all genes across the array for all tumor samples included in the analysis (n=44). Expression of all genes in all tumor samples is relative to all other genes in order to visualize expression patterns among all tumor samples. Gene expression intensity is represented by colors, with blue indicating low expression and red indicating high expression. Each row includes the pattern of expression for all genes for a single tumor sample. Similar gene expression profiles are clustered more closely together, so that the most similar profiles are in closest proximity. Clinical characteristics (grade, tumor type, lymph node status, BluePrint subtype, MammaPrint result) associated with each tumor sample are indicated by the legend on the right and color-coded accordingly on the left side of the heatmap.

through genomic testing. The complexity is then compounded by wide variations in the genomic landscape of tumors, both within and between cancer types. Most of our presently practicing oncologists were educated and trained in the era of empiric and "evidence-based" oncology and few have comprehensive training in techniques of rapidly advancing genomic medicine. Even fewer have the knowledge and skills necessary to embark on "data mining" of large datasets provided by gene expression profiling or full exome sequencing to distil vast amounts of data into clinically useful measurements.

In contrast, practicing oncologists have the clinical experience and expertise in patient care necessary to fill gaps that modern genomic analysis and massive data production create. Therefore, the future of oncology will rely on interdisciplinary collaboration between physicians, biologists, geneticists, bioinformatics specialists, and patients and their families. All participants will have to acquire and retain new knowledge and skills in order to provide the best individualized care to patients. To succeed, genomics must be included in the medical education of future physicians. This training process will undoubtedly be ongoing, facilitated and complicated by fast moving technological advances. The practice of medicine is changing along with advances that revise the science of medicine. In a new era of genomics and informatics, "one size fits all" treatments may soon be replaced with therapies that are truly personalized to an individual's unique combination of genes, environment, and lifestyle. Oncology is at the forefront of these advances; however, with all of the fervor these advances bring, there are challenges to be addressed in order to fully realize the potential that genomics and personalized medicine have in transforming patient care.

Authors' Contributions: Conception and design: TT, HMK, WA and GS; Acquisition, analysis and interpretation of data: HMK and TT; Drafting the article HT, HMK and TT; Revising it critically for important intellectual content: HT, HMK, TT, WA and GS; Approved final version of the manuscript: HT, HMK, TT, WA and GS.

Conflict of Interest: HMK, TT and WA are employees of Agendia, Inc.

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Narrative Review
Acta Medica Academica 2019;48(1):18-34
DOI: 10.5644/ama2006-124.239

Prospective Validation of a Genomic Assay in Breast Cancer: The 70-gene MammaPrint Assay and the MINDACT Trial

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Received: 15 October 2018
Accepted: 29 April 2019

Key words: Breast Cancer ▪ Genomics ▪ MammaPrint ▪ MINDACT.

MammaPrint was the first genomic assay in breast cancer to be validated with a prospective randomized trial, the MINDACT trial. The 70 gene MammaPrint assay was developed to determine the risk of distant metastasis in early stage breast cancer through gene expression analysis and was the first FDA cleared genomic assay for breast cancer. The assay identifies primary breast cancers likely to metastasize within the first five years of diagnosis and has clinical utility for helping to determine the expected benefit from adjuvant chemotherapy. The MINDACT Trial was the first trial of a genomic assay in breast cancer to provide prospective, randomized evidence of clinical utility for this important clinical question, identifying a significant proportion of patients who could safely forgo chemotherapy within a cohort of patients with high risk clinical characteristics. Nearly half of all patients (46%) who would have been advised chemotherapy according to clinical guidelines were identified genomically by MammaPrint as being low risk and found to have equivalent rates of freedom from metastasis at 5 years with or without chemotherapy. Based upon the MINDACT trial, the ASCO Biomarker Guidelines now approve the use of MammaPrint to inform decisions regarding chemotherapy for women with clinically high-risk ER+ breast cancer, and as the only approved assay for use in women with 1-3 involved lymph nodes. Recent studies suggest information obtained from the 70-gene assay may also help inform decisions regarding endocrine therapy, as well as chemotherapy, targeted therapy and immunotherapy. **Conclusion.** The power of gene expression analysis in breast cancer, effectively illustrated with MammaPrint in the MINDACT trial, is now being explored through examination of the full transcriptome in breast cancer.

Introduction – Bringing Genomic Anatomy to the Clinic

The widespread availability and awareness of mammographic screening has led to the fortunate circumstance that most women diagnosed with breast cancer are diagnosed at a curable stage (1). The challenge for clinicians is therefore not only to achieve cure but do so with as little harm as possible for the individual with breast cancer. Given the well-known acute and long-term toxicities of chemotherapy, a fundamental requirement for precision oncology is to identify with certainty those patients who will benefit from chemotherapy and those who will not, in order to appropriately apply such therapy. The advent of rapid genomic tech-

nology has allowed the development of clinically available genomic information, through gene expression microarray testing performed on cancer specimens. The 70-gene MammaPrint assay was the first such test to reveal the genomic "anatomy" of breast cancer, as it related to the most important aspect of early stage breast cancer, the likelihood of metastasis. The ability to detect this critical element of cancer biology carried clear implications for clinical utility when compared to classical morphology-based pathology (2). The ultimate proof of clinical utility for a genomic assay, however, requires a randomized prospective trial, and this has been achieved with the (Microarray in Lymph Node Negative and 1-3 Lymph Node Positive Disease May Avoid Chemotherapy) Trial MINDACT (3). This paper will review the basis for the MINDACT trial, the outcomes of the trial, and the implications it has provided for further application of genomic profiling in early breast cancer.

MINDACT Trial Overview

The MINDACT trial is the first prospective randomized trial reporting outcomes which illustrate the importance of genomic information for making appropriate treatment decisions in early stage breast cancer (3). The ability to routinely and rapidly analyze the genomic anatomy of breast cancers has revealed a level of information never before applied to a large adjuvant therapy trial, and the additional precision and clinical utility provided by gene expression analysis was clearly proven by the MINDACT trial (4). The primary finding from MINDACT, that a large proportion of women may be safely spared chemotherapy, avoiding its associated toxicities and costs without affecting their health outcomes, has broad implications for the quality of life of women with early stage breast cancer, as well as for health care costs (5). The significance of the findings in the trial has continued to be appreciated since the publication in the New England Journal of Medicine in August 2016, with recognition of the practice-changing data by ASCO, NCCN, St Gallen, AJCC, and the health care insurance industry (6-9). The MINDACT trial has, however, also been considered complex in its design and extensive in the extent of data it generated, prompting the need for a broad and in-depth overview of the origins, rationale, and outcomes of this landmark trial.

The Question To Be Answered by the MINDACT Trial

The MINDACT Trial was designed to determine whether gene expression information from newly diagnosed early stage breast cancer (ESBC) could be used to identify breast cancers which were unlikely to benefit from chemotherapy and could safely avoid overtreatment and the associated toxicity (10). The importance of this question was emphasized by the fact that major clinical guidelines such as NCCN and St Gallen advised chemotherapy for a large proportion of ESBC to reduce the risk of metastatic recurrence based on clinical features and pathology (8, 9). This was particularly true for estrogen receptor positive (ER+) cancers, in which chemotherapy was added in addition to endocrine therapy based on clinical features alone, without clear evidence that chemotherapy was needed or beneficial in all cases. The toxicity and cost of chemotherapy mandated more concrete justification for its use, in the new era of genomic or "precision" oncology, where further information beyond simple immunohistochemical factors could be routinely obtained by assessing patterns of gene expression in primary breast cancers.

Genomic Anatomy Viewed Through the 70-Gene MammaPrint Assay

The gene expression microarray utilized in MINDACT to provide this essential genomic information was the 70-gene panel known as MammaPrint, which had been developed and validated to predict the biological potential for metastasis in a primary breast cancer (2, 11-13). The 70 genes which make up the MammaPrint genomic assay were discovered through an exhaustive, unbiased analysis of a cohort of breast cancers, collected and stored by the Netherlands Cancer Institute, from women who had undergone surgery, but had not received any systemic therapy for their cancers. Although the breast cancers from these women all appeared to be clinically and histologically similar, it was observed through long-term clinical follow-up that some women had remained entirely free of metastatic disease for many years, while others had experienced metastatic recurrences within the first five years after diagnosis (see Figure 1). Without the effect of systemic therapy to alter the outcome, this cohort provided a rare opportunity to identify the true biology of the potential for metastasis, a feature which could not be definitively identified by classical pathology alone. The genomic "anatomy", where the true pathology of cancer lies, was assessed with microarray technology, and gene expression from the entire genome was assessed for all cancers (2).

The gene expression pattern in cancers from women who did not have any incidence of metastatic recurrence within 5 years of diagnosis was compared to the gene expression pattern from cancers which had recurred with metastatic disease during that period, seeking differences which could distinguish the two groups. From this innovative and ground-breaking scientific study, 70 genes were identified, whose expression patterns could distinguish the non-metastasizing "Low Risk" breast cancers, (which may not have benefitted from adjuvant chemotherapy had it been given), from those early-metastasizing "High Risk" breast cancers, which clearly required systemic therapy. The 70 genes in the MammaPrint assay were found to be components of seven functional pathways involved in the metastatic process, providing the basis for their ability to predict the potential for metastasis (14, 15). The initial discovery was then validated in two other, larger cohorts (12, 13), and the consistent differences in clinical outcome were profound: an approximately 10% risk

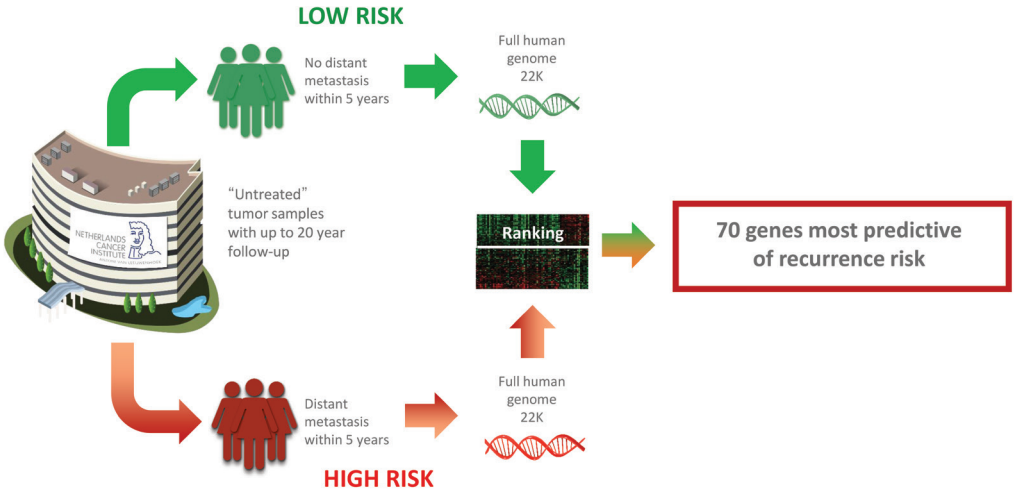

Figure 1. Development of the 70-gene MammaPrint signature.

of metastasis at 10 years without any systemic therapy for cancers with a Low Risk 70 gene MammaPrint profile, and a nearly 30% risk of metastasis at 10 years with the High-Risk profile. With these data, MammaPrint became the first genomic assay in breast cancer to achieve FDA clearance (16). The 70 genes, in their respective patterns of under-expression or over-expression, effectively and accurately separated breast cancers into either of the two groups, without overlap; a breast cancer either had a significant likelihood of metastasis within the first five years, or it did not, as a binary quality.

The clear distinction between genomic High Risk and genomic Low Risk cancers, in the propensity to metastasize, paralleled the binary clinical decision-making process, in which a decision is made to administer chemotherapy or not, based on a clinically-derived assessment of metastatic risk. With the advent of this powerful genomic technology, readily accessible in the clinic, it was then necessary to ask which method of risk-assessment, clinical or genomic, was better able to predict the risk of metastasis, and therefore the need for systemic chemotherapy. This was the origin of the MINDACT trial.

MINDACT Trial Design

MINDACT was designed to determine if gene expression could identify individuals with genomic "Low Risk" breast cancers who were unlikely to benefit from chemotherapy and could safely avoid it. Importantly, MINDACT was not designed (or powered) to illustrate the extent of benefit of chemotherapy for genomically "High Risk" cancers, in part because cancers with a high risk of metastasis may or may not be chemo sensitive, may require additional targeted therapies in some cases, and may unfortunately relapse even with aggressive systemic therapy. The optimal treatment for such cancers continues to be the subject of intense research, although chemotherapy remains the standard of care at this time. In order to determine the answer to the MINDACT question, it was necessary to identify breast cancers which appeared to require chemotherapy based on clinical risk assessment ("clinically High Risk") but would not be predicted to benefit from chemotherapy by genomic, MammaPrint risk assessment (genomically Low Risk). If both methods of risk assessment were in agreement in all patients, it would then be clear that genomic assessment added nothing to standard clinical assessment, and no improvement could be made in the selection of patients requiring chemotherapy. However, if there were a substantial proportion of patients in which clinical and genomic risk assessment disagreed, it would be possible to determine the relative accuracy of both methods by randomizing such patients to have the chemotherapy decision based on either the clinical or genomic risk and compare the outcomes.

Determining the Clinical Risk Assessment

In order to make such a comparison, a standardized and reproducible method for clinical risk assessment was required. MINDACT was to be conducted in 9 European countries, with different languages and cultures. The solution to this was the use of a computer-based, universal algorithm for clinical risk assessment, Adjuvant!Online (17). This program, a compendium of clinical data from numerous large prospective adjuvant therapy trials in breast cancer had been available and familiar to all clinicians for many years and provided an estimate of Overall Survival without chemotherapy at 10 years. (For the MINDACT trial, a modified version which also integrated HER2 was employed).

The question also arose as to what level of "clinical risk" warranted the use of chemotherapy. In addition to the diversity of

language and culture across the MINDACT study sites, there were different regional thresholds for the level of risk which justified the administration of chemotherapy, as well as individual physician opinions regarding when chemotherapy was warranted. The common thread amongst all breast cancer clinicians, however, was acknowledging the importance of integrating the patient's own preferences when making the important chemotherapy decision (18, 19). The risks and toxicity of chemotherapy were of course of great concern to patients; temporary risks such as hair loss, fatigue, nausea; more permanent risks such as neuropathy and cognitive dysfunction, as well as the rare life-threating risks of acute leukemia and cardiac disease. The patient-based threshold for enduring chemotherapy to reduce the risk of breast cancer recurrence versus the risk of toxicity of chemotherapy was therefore assessed through polling women regarding their opinions. Prior studies from the United States, Australia and Europe had documented wide variation in patient thresholds for the necessary magnitude of chemotherapy benefit, ranging from 0.5% to over 5% (18-21). The result obtained from the women polled for the design of the MINDACT trial was that chemotherapy would be worth the toxicity for most women with ESBC if it provided a greater than 2% benefit for breast cancer specific survival (BCSS). This threshold then required calculation of the absolute level of risk which is improved by at least 2% with the use of chemotherapy, based on prior clinical studies. The generally-recognized benefit of chemotherapy, as reported by the Early Breast Cancer Trialist's Collaborative Group (EBCTCG) in the so-called Oxford Overview, is to improve survival by approximately 25%, based on data from three decades of adjuvant trials in breast cancer (22, 23). A 2% absolute benefit of chemotherapy, would be obtained when the overall risk is 8%, as this would constitute a relative benefit of 25%. With 8% risk being the minimum risk for which chemotherapy is justified, any patients with a clinical estimate of Overall Survival, with endocrine therapy but without chemotherapy, of 92% or higher at 10 years by Adjuvant!Online would be categorized as Clinical Low Risk, and not likely to derive meaningful benefit from adjuvant chemotherapy. The clinical characteristics of the Clinical Low Risk group included T1a and T1b tumors of any grade, T1c tumors of Grade 1 or 2, and Grade 1 tumors of 3 cm or less. All others with an expected benefit of chemotherapy of greater than 2% would be classified as Clinical High Risk, with the potential benefit sufficient to advise chemotherapy (10).

MINDACT Trial Methods

How often were clinical Risk (according to Adjuvant! Online and genomic risk, (according to MammaPrint risk assessment) in agreement, and when they disagreed, which method of risk assessment was better able to predict the need for chemotherapy? This central question for the MINDACT trial were answered by conducting both clinical and genomic risk assessment on every patient enrolled in the trial. If there was agreement, or concordance, between the clinical and MammaPrint methods for identifying a Low Risk patient (clinically Low/genomically Low, or cL/gL), then no chemotherapy would be advised, while if both agreed in identifying a High-Risk patient (clinically High/genomically High, or cH/gH), then chemotherapy would be routinely advised, and no randomization would be required. However, for those patients classified as clinically High Risk by Adjuvant!Online but were identified as genomically Low Risk by MammaPrint (cH/gL), i.e. discordant, these patients would be randomly assigned to have the chemotherapy decision based on clinical risk or genomic risk. For those

patients whose treatment decision would be based on genomic Low Risk with MammaPrint, and no chemotherapy given, their rates of recurrence with distant metastasis needed to be at least as low as those patients whose treatment was determined by their clinical High-Risk category and did receive chemotherapy. Therefore, MINDACT was a "non-inferiority" trial, designed to determine whether the outcome of the treatment decision based on genomically-assessed risk would be as good as, or not inferior to, the outcome when the therapy decision was based on clinical risk assessment. In terms of statistical significance, the non-inferiority goal would be a lack of statistically significant difference between the clinical outcomes of the two groups.

The Relevant Clinical Endpoint in MINDACT: Distant Metastasis

Because the purpose of chemotherapy is to reduce metastatic recurrence, the main life-threatening aspect of breast cancer, the optimal endpoint for making the comparison between the groups in MINDACT is the incidence of distant metastasis (24). Many clinical endpoints are collected in clinical trials, but are less relevant to the question of whether chemotherapy is needed to reduce the rate of distant metastatic recurrence: Disease Free Survival or DFS includes events such as second primary cancers and local in-breast recurrence, clinical events which, while undesirable, are not the primary reason chemotherapy is given, and Overall Survival, or OS, includes deaths from any cause, and does not distinguish those who have experienced metastatic recurrence from those who have not. As described in a consensus statement in the Journal of Clinical Oncology regarding relevant endpoints:

"The separation of distant as a specific end point is also very important for ancillary studies involving microarray analysis and for developing genetic panels for use in determining prognosis and/or response to treatment. In these situations, distant disease recurrence is often used as a marker for survival to increase statistical power because there is such a strong correlation between these end points and because there will be more distant events than deaths. Using a combined regional/distant end point would dilute the correlation with survival and weaken the discriminatory power of the analysis" (24).

The optimal endpoint, therefore, in a study to validate the clinical utility of a genomic assay, is one which registers distant metastasis or death ("distant metastasis free survival" or DMFS). If distant metastases and only deaths due to breast cancer are registered, the endpoint is "distant metastasis free interval", or DMFI. The MINDACT trial was therefore designed and powered to determine whether DMFS for the clinically High Risk/MammaPrint Low Risk group would be the same at five years for those who received chemotherapy and those who did not. The assumption was made by the MINDACT investigators that the minimum acceptable outcome for DMFS for the cohort not receiving chemotherapy was required to be at least 92% at 5 years, and if it appeared that the rate of metastatic recurrence during the conduct of the trial appeared to exceed this rate, the trial could be stopped early, for patient safety.

The Role of Chemotherapy in Reducing Metastatic Recurrence of Breast Cancer

The Early Breast Cancer Trialist's Collaborative Group (EBCTCG) conducts periodic meta-analyses of the long-term outcomes of three decades of adjuvant therapy trials in breast cancer, the results of which have formed the basis of the standard of care and clinical treatment guidelines for many years

(22, 23). Such meta-analyses provided proof of the overall benefit chemotherapy in reducing metastatic recurrence and improving overall survival, although only for a small proportion of women with breast cancer, as well as the incremental benefit of the addition of anthracyclines and taxanes to chemotherapy regimens. A major observation obtained from over 15 years of follow-up of tens of thousands of women with ESBC, is that the effect of chemotherapy in reducing metastatic recurrence is seen primarily during the first five years after diagnosis. The five years of follow-up reported in the MINDACT trial were therefore considered sufficient to identify all patients benefitting from chemotherapy. Although metastatic recurrences continue to occur after five years, almost exclusively in estrogen receptor positive breast cancer, such "late" recurrences occur at the same rate in women who received chemotherapy as in those who did not, indicating the need for interventions other than chemotherapy to reduce these recurrences, such as extended endocrine therapy or targeted agents.

MINDACT Trial Enrollment

From 2007 through 2011, 6,693 patients with breast cancer were enrolled, across 9 European countries, in over 110 individual sites. Enrolled patients were required to have a pathology-confirmed diagnosis of breast cancer, a tumor stage of T1, T2 or operable T3, and from 0 to 3 positive lymph nodes. The majority of enrolled patients had ER+ breast cancer (88.4%), and ranged in age from 23 to 71, with the median age being 55. Importantly, one third (33.2%) of MINDACT patients were less than 50 years of age.

Main Results of the MINDACT Trial

Figure 2 shows the distribution of patients classified according to clinical risk by Adjuvant!Online and genomic risk by MammaPrint. Approximately half of all patients were classified as clinically Low Risk, and half as Clinically High Risk, while MammaPrint identified 64% as genomically Low Risk, and 36% as genomically High Risk. The comparison of clinical risk assessment with genomic risk assessment revealed agreement, or concordance, in two thirds (68%) of all patients, with 41% clinically and genomically Low Risk (cl/gL) and 27% clinically and genomically High Risk (cH/gH), supporting the continued importance of clinical factors in estimating the risk of metastatic recurrence. However, clinical risk was primarily concordant with genomic risk in identifying Low Risk patients, who were

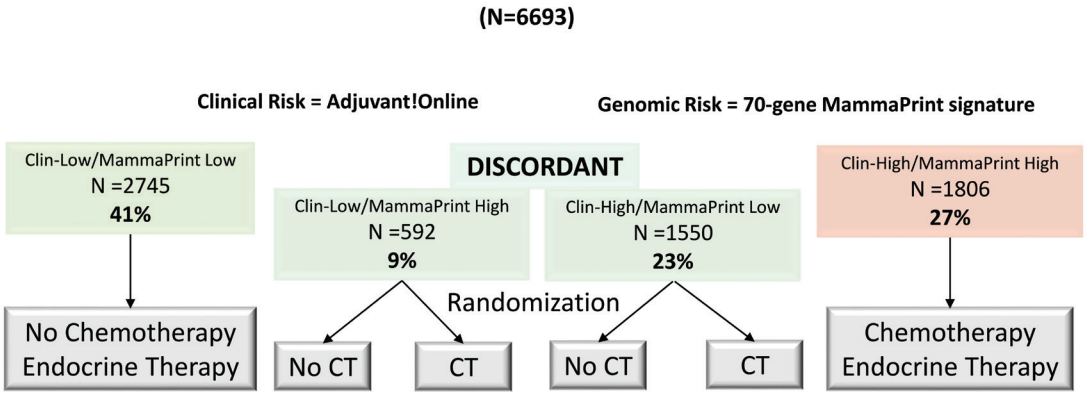

Figure 2. Distribution of Clinical Risk and Genomic Risk in the MINDACT Trial. From Ref. 3 Cardoso (2016).

unlikely to benefit from chemotherapy, with 4 out of 5 being concordant, and in only 1 one out of 5 did genomic analysis provide additional information. In contrast, for the 50% of MINDACT patients identified to be clinically High Risk, in only 1 of 2 was there concordance with the genomic risk, with an equal proportion having a discordant, genomic Low Risk. This finding supports the original rationale of the MINDACT trial, to seek to identify, by gene expression patterns, those patients who may be potentially overtreated when the decision to administer chemotherapy is based on clinical risk assessment alone.

The Primary Test Group in MINDACT: Clinically High Risk but MammaPrint Low Risk Patients

1550 patients were in the discordant cH/gL group, with half randomly assigned to receive chemotherapy based on the clinical High-Risk assessment, or not to receive chemotherapy, based on their genomic Low Risk assessment. Figure 3a shows the outcome for the cH/gL group which did not receive chemotherapy (with 100% compliance "Per Protocol" with this treatment decision). The DMFS at 5 years for this group was 94.7%, with a confidence interval ranging from 92.5% to 96.2%, well above the threshold of 92% set by the MINDACT investigators. The MINDACT trial therefore met its required endpoint and is considered a positive trial.

Most important, however, was the comparison of the DMFS at 5 years with the cH/gL cohort who did receive chemotherapy. Shown in Figure 3b is this comparison, using the "Intent to Treat" cohorts, taking into account the small numbers of patients (approximately 12.7%) in both groups who did not follow their assigned treatment. The reasons for not following the treatment randomly assigned by the protocol were described in the Supplementary Section of the NEJM publication (3), and were primarily due to patient preference; some assigned to chemotherapy declined it, and some assigned to no chemotherapy requested to be treated. The DMFS for the group assigned to receive chemotherapy was 95.9%, while the group assigned to no chemotherapy was 94.4%, a numerical difference of 1.5% which was not statistically significant, with a p-value of 0.267. The MINDACT trial

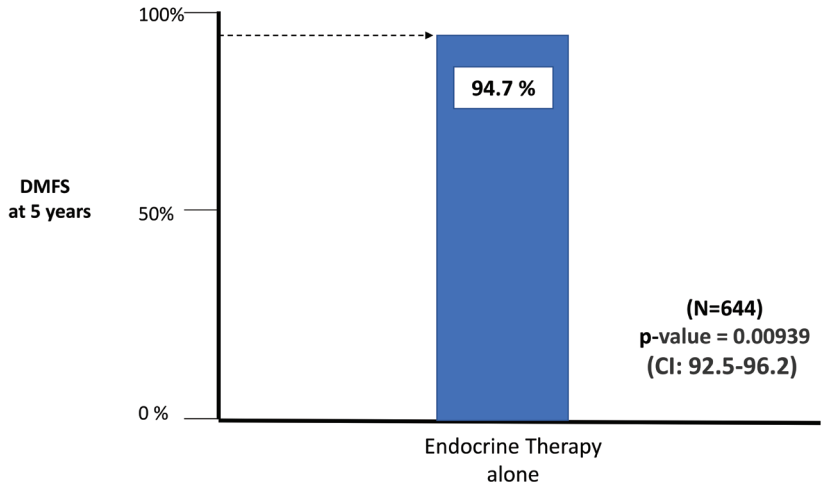

Figure 3a: Clinical High Risk/MammaPrint Low Risk Treated Without Chemotherapy (Per Protocol; (Distant Metastasis Free Survival). From ref. 3 Cardoso (2016).

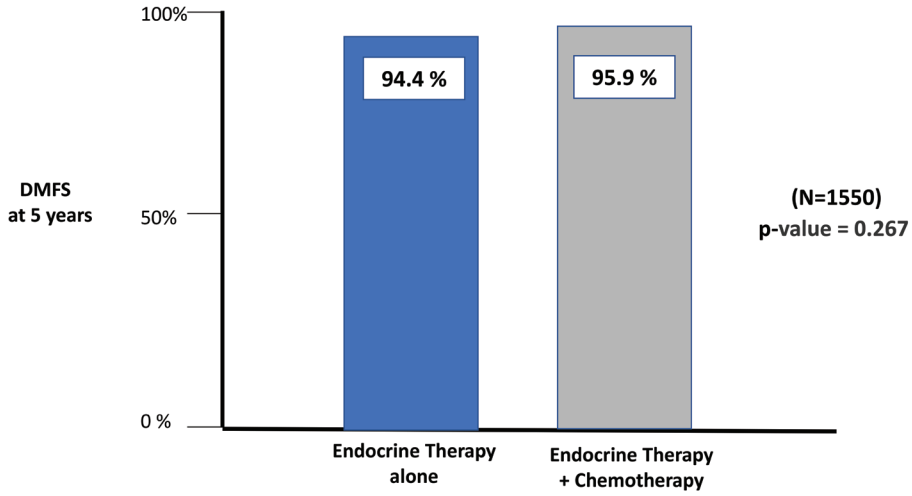

Figure 3b. Clinical High Risk/MammaPrint Low Risk treated with/without chemotherapy (intent to treat; (Distant Metastasis Free Survival). From ref. 3 Cardoso (2016).

had sufficient patients enrolled, and recurrence events observed, to detect a clinically meaningful difference, if it existed, between the chemotherapy and no chemotherapy groups. The lack of a statistically significant difference in the frequency of metastatic recurrence between these groups supported the hypothesis that MammaPrint Low Risk patients could safely avoid chemotherapy, even when clinical High-Risk factors were present.

The "Non-Significant" 1.5%

In the Editorial by Drs Hudis and Dickler which accompanied the MINDACT trial publication in the NEJM, the issue of the 1.5% numerical difference was addressed (4). In their words, "a difference of 1.5%, if real, might mean more to one patient than to another". Acknowledging that the threshold for accepting the toxicity of chemotherapy in return for some degree of protection from recurrence is a matter of individual preference, they agree that MammaPrint can identify patients "in whom any plausible benefit of chemotherapy would be modest". Drs Hudis and Dickler concluded that "On the basis of the MINDACT study, clinicians may consider ordering the 70-gene signature for patients in line for chemotherapy who hope to forgo it on the basis of a possibly low genomic risk."

The Lymph Node Positive Patients in MINDACT

Nearly 1400 patients with 1-3 involved lymph nodes were enrolled in MINDACT, the largest cohort of node positive patients reported in a randomized controlled trial involving genomic profiling of breast cancer. Node positive patients represented 21% of the entire enrolled population. However, within the important primary test group of clinically High Risk/MammaPrint low risk patients, 48%, or 709 were node positive. Figure 3c shows the DMFS rates at 5 years for the 1-3 Lymph Node positive patients, with those receiving chemotherapy at 96.3% and those not receiving chemotherapy at 95.6% DMFS. As with the cH/gL group as a whole, the p-value of 0.724 indicated no statistically significant benefit to the addition of chemotherapy for this large cohort with lymph node positive breast cancer.

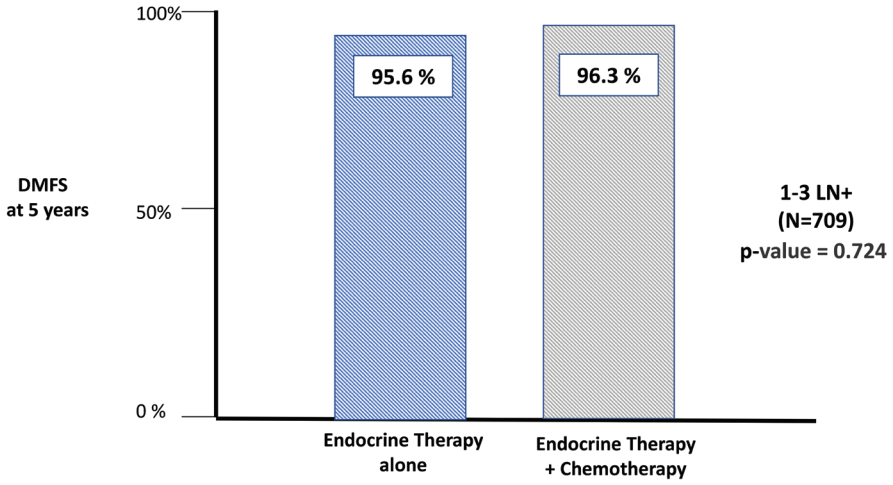

Figure 3c. Clinical High Risk/MammaPrint Low Risk treated with/without chemotherapy (intent to treat) 1-3 LN+. From ref. 3 Cardoso (2016).

The implications of this critical finding are that cancers with the biological capacity to reach regional lymph nodes do not always have the capacity for distant metastases, and my not require chemotherapy, in contrast to recommendations in most clinical guidelines. MINDACT provides the first and only prospective, randomized data in over 700 patients with 1-3 lymph node positive breast cancer in which genomic profiling can identify those patients who can safely avoid chemotherapy.

The Effect of Genomic Risk Assessment in Early Stage Breast Cancer

Additional endpoints beyond the primary goal of identifying clinically high-risk patients who could safely avoid chemotherapy were also analyzed. The effect of determining the need for chemotherapy for all patients based either on clinical risk assessment or genomic risk assessment as measured by DMFS was assessed. For those MINDACT patients in whom clinical risk assessment was used to determine the need for chemotherapy, the DMFS at 5 years was 95%, while those patients treated according to their MammaPrint risk assessment had a 5-year DMFS of 94.7%. There was no statistically significant difference in these clinical outcomes; however, the MammaPrint risk assessment allowed 46% of clinically high-risk women to safely avoid chemotherapy. The benefits of avoiding unnecessary, toxic chemotherapy, for quality of life are likely to be substantial, as well as the health economic benefits of avoiding costly therapy and managing its side effects. Cost-effectiveness data from MINDACT are being analyzed, although previous studies have already documented the cost-effectiveness of the 70 gene MammaPrint assay (5, 25).

Randomization of Chemotherapy Regimen

Patients receiving chemotherapy in either the concordant High-Risk cohort or the discordant cohorts randomized to receive chemotherapy were offered an optional secondary randomization to either standard of care anthracycline-containing regimens or the non-anthracycline study regimen, docetaxel/capecitabine. Of the 2877 pa-

tients receiving chemotherapy, 1806 (63%) were in the clinical High/ Genomic High-Risk group, 775 (27%) were Clin High/Genomic Low, and 292 (10%) were Clin Low/ Genomic High Risk. Not all patients participated in the secondary randomization, with 2227 (77%) receiving standard of care regimens, and 650 (23%) treated with docetaxel/ capecitabine. The choice of regimen did not affect clinical outcomes, which were similar in the standard and non-standard arms (26).

Questions Arising from MINDACT: Is MammaPrint Predictive of Chemotherapy Benefit?

MINDACT was designed and patient enrollment numbers calculated to answer the question of whether MammaPrint could identify women with genomically Low Risk ESBC who could safely avoid chemotherapy. It was not designed to answer whether chemotherapy would benefit women with genomically High Risk breast ESBC. The design of MINDACT was such that all patients with discordance between clinical and genomic risk assessment were randomized to have the decision to administer chemotherapy based on one of the two methods. Although the largest discordant group was the primary test group described above, Clinically High Risk and Genomically Low Risk, (n=1550, 23% of total), there was also a small cohort in which the Clinical Risk was Low and Genomic Risk High (n=592, 9% of total). The clinical characteristics of this group were low risk due primarily to small tumor size (98% T1) and low to intermediate grade (85%). Although the majority of patients in this cohort were estrogen receptor positive, approximately 12% were also HER2+, while 9% were classified clinically as "triple negative".

This discordant group also underwent randomization, per the protocol design, with the group receiving chemotherapy based on genomic High Risk having a 98.1% DMFI at 5 years, versus DMFI of 95.6% for the group not receiving chemotherapy due to Low Clinical Risk, a numerical difference of 2.5% which did not reach statistical significance (p value 0.282) (Figure 4). Due to the small benefit of chemotherapy predicted by the clinical Low Risk classification, a significantly larger number of randomized patients, at least 2000, would have been required to de-

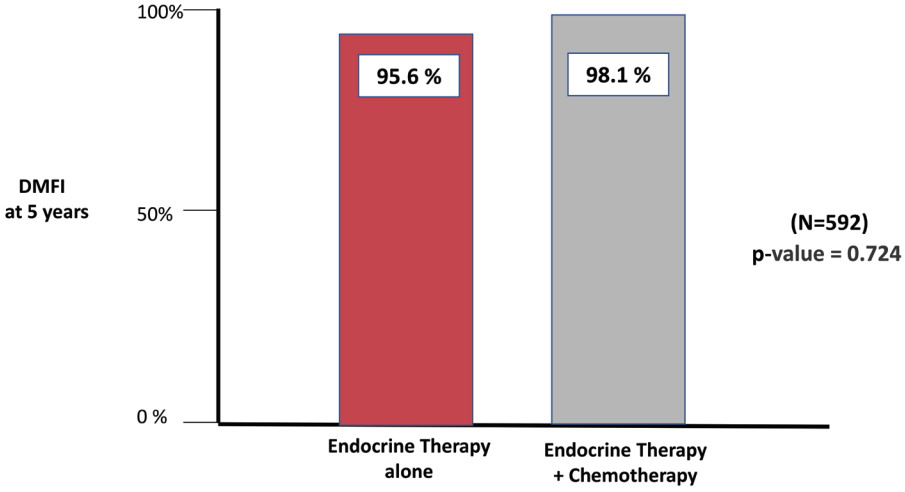

Figure 4. Clinical Low Risk/MammaPrint High Risk treated with/without chemotherapy (intent to treat) Distant Metastasis Free Interval (DMFI). From ref. 3 Cardoso (2016).

tect a statistically significant benefit of chemotherapy in the MammaPrint High Risk cohort (27). As the MINDACT trial was not powered or designed to determine the benefit of chemotherapy in MammaPrint High Risk patients, MINDACT does not provide definitive data to rule in or rule out the benefit of chemotherapy in MammaPrint High Risk patients. However, the lack of statistical significance for the benefit of chemotherapy in clinically low risk patients with a High Risk MammaPrint index does not affect the predictive value of the assay.

When Is a Test "Predictive"?

Diagnostic tests are generally used to predict the likelihood of a medical condition and have both a positive predictive value (PPV) and a negative predictive value (NPV) for predicting the presence of the condition. Diagnostic tests may also be used to predict the likelihood of responding or not responding to a specific therapy. A PPV in this context is defined as the proportion of patients with a "positive" test result who will benefit from a therapy, while the NPV is the proportion of patients with a "negative" test result who will not benefit from the therapy. Most diagnostic tests have significantly different predictive value if the test is "negative" or "positive".

In breast cancer, the use of molecular tests for the presence of estrogen receptor protein (ER) and amplification of the HER2 gene are considered standard of care in predicting response to anti-estrogen therapy and HER2-targeted therapy, respectively. Yet the NPV and PPV of these tests vary significantly. The presence of ER predicted a 60% rate of response in ER+ metastatic breast cancer (28), while when ER was absent, the rate of response was 5-8%. Therefore, the PPV of the ER test was 60%, while the NPV, the ability to identify non-responders, was far greater, at 92-95%. For the prediction of response to endocrine therapy, the NPV of ER is of greater utility and accuracy.

With detection of amplification of HER2 as a predictive test for the likelihood of response to HER2-targeted therapy, the original studies of single agent trastuzumab in metastatic breast cancer observed a 35% response rate in HER2-amplified (FISH+) patients, and a 7% response rate in patients without HER2 amplification (29). Therefore, the PPV of the presence of amplification of HER2 for predicting response to trastuzumab was 35%. The NPV of the absence of HER2 amplification was far greater, at 93% in this trial, and approaching 99% in recent trials (30), predicting lack of benefit of trastuzumab.

The Predictive Value of MammaPrint

In this context, the question of predictive value may also be applied to MammaPrint. MammaPrint is indeed predictive. The MINDACT trial tested the NPV of MammaPrint for the potential benefit from chemotherapy in preventing distant metastasis. In other words, would a Low Risk MammaPrint index predict the absence of benefit from chemotherapy? The answer from the MINDACT trial was "yes", in that the administration of chemotherapy to patients with a MammaPrint Low Risk index did not yield a statistically significant difference in freedom from metastasis, although a numerical difference of 1.5% in DMFS was reported. Therefore, the NPV of MammaPrint for chemotherapy benefit is 98.5%, in that MP correctly identified 98.5% of ER+, clinically High-Risk patients who would not derive benefit from chemotherapy. The NPV of MammaPrint for predicting absence of chemotherapy benefit is equal to the NPV of ER or HER2 in predicting the absence of benefit to tamoxifen or trastuzumab, respectively.

Does a High-Risk MP score predict the presence of benefit from chemotherapy? In

other words, what is the PPV of MammaPrint? This question cannot be answered by the MINDACT trial, as this would have required that all enrolled patients with a High-Risk MP score be randomized to receive either chemotherapy or no chemotherapy, even those with concordant clinical high risk, a design which would have been unethical by 21st century clinical trial standards. The clinically Low Risk cohort (greater than 92% overall survival at 10 years without chemotherapy, according to clinical/pathologic features) within the MammaPrint High Risk group were randomized, but with such a good clinical prognosis, the ability to detect a further benefit was exceedingly small. Of these clinically Low Risk patients, 95.6% were free of distant metastases (DMFI) with endocrine therapy alone after 5 years, while the addition of chemotherapy increased the proportion without metastasis to 98.1%, a difference of 2.5%. Since the remaining risk of metastasis, despite endocrine therapy, was 4.4%, the MP High Risk patients benefitted from chemotherapy by 2.5% of the 4.4%, a relative risk reduction of 56% of the residual risk. A very similar magnitude of relative risk reduction has been observed in other, non-randomized MammaPrint cohorts, in which a pooled series of 541 ER+ patients with both clinical high and low risk treated with chemotherapy and endocrine therapy had a distant disease free survival (DDFS) of 88%, in comparison to 76% DDFS for those receiving only endocrine therapy (31), an absolute reduction of 12%, and a relative risk reduction of 50%, in keeping with the MINDACT data.

ASCO Clinical Practice Guidelines, NCCN Guidelines, and the MINDACT Trial

The results of the MINDACT trial were reviewed by the American Society of Clinical Oncology (ASCO) Clinical Practice Guideline Expert Panel in an update in 2017 devoted entirely to the trial (6)). Based on these data, the updated guidelines stated that MammaPrint may be used in clinically high-risk ER+, HER2-, Lymph Node negative and 1-3 Lymph node positive breast cancer to "inform decisions in withholding adjuvant systemic chemotherapy". For women with 1-3 positive lymph nodes, the guidelines further specified that "such patients should be informed that a benefit of chemotherapy cannot be excluded, particularly in patients with greater than one involved lymph node." However, the guideline recommended that other genomic assays should not be used in lymph node positive patients. MammaPrint was identified as the first and only genomic assay which could be used in this group.

In 2018, over two years after the publication of the MINDACT trial data, the NCCN updated their Breast Cancer Guideline to acknowledge that with the MINDACT trial, MammaPrint was the only risk of recurrence genomic assay with Level 1 evidence in both lymph node negative and lymph node positive breast cancer (32). The only other risk of recurrence genomic assay recognized to also have Level I evidence limited only to lymph node negative breast cancer, was the 21-gene Oncotype Dx assay, based upon the Trial Assigning Individualized Options for Treatment (TAILORx) (33).

Prospective Randomized Trials of Genomic Assays in Breast Cancer: MINDACT and TAILORx

MINDACT was the first reported prospective randomized trial of a risk of recurrence genomic assay in breast cancer. Two years after the publication of the MINDACT trial, the second, and largest such trial of this kind, (with over 10,000 patients enrolled), TAILORx which evaluated the 21-gene OncotypeDx assay, also reported the prospective and randomized arms of the trial (33).

Unlike MINDACT, in which the primary goal was to confirm the safety of withholding chemotherapy in clinically high risk/ MammaPrint low risk patients, TAILORx was instead intended to clarify whether a specific risk of recurrence "score" (RS) could identify patients who could safely avoid chemotherapy. Unlike MINDACT, TAILORx was limited to lymph node negative patients. Clinical risk category, as had been determined by the MINDACT trial, was assessed in TAILORx, but was not used in the randomization or patient selection for enrollment. The randomized cohort was not balanced for clinical risk, in that 74% of the randomized patients were clinical "Low Risk" as defined in the MINDACT trial. The cohorts randomized to receive chemotherapy followed by endocrine or endocrine therapy alone, were limited to those with RS 11-25, with no randomization for RS 0-10, or RS 26 and above. The results, without subset analysis, showed no benefit in DFS with the addition of chemotherapy with RS 11-25. However, subset analysis revealed that these findings did not apply equally to all ER+, lymph node negative women, with a chemotherapy benefit of 5.8% seen for women 50 years old and younger with RS 16-25. The low event rate in this study led some (34) to question whether the predominance of clinical low risk patients precludes drawing any definitive conclusions regarding the utility of an intermediate RS for determining the need for chemotherapy with this assay.

Future Directions for MammaPrint and Gene Expression Profiling in Breast Cancer

The MINDACT trial proved the clinical utility of stratifying breast cancers as having a Low or High Risk MammaPrint Index, through the expression patterns of 70 genes, for identifying patients who could safely forgo chemotherapy. Within the Low and High-Risk categories, further stratification of the range of the MammaPrint index has provided additional information with clinical utility which could not be obtained through clinical features or pathology. Within the Low Risk MammaPrint Range, from >0.00 to +1.00, all patients may safely forgo chemotherapy, and ER+ patients are routinely treated with 5 years of endocrine therapy alone. However, a subset of these MammaPrint Low Risk patients, with indices from >0.355 to +1.00 appear to have an extremely low risk of recurrence, with or without 5 years of endocrine therapy, for over 20 years from diagnosis (35). Post-menopausal women with node negative, ER+ cancers 3cm or less were randomized on the Stockholm Tamoxifen Trial (STO-3) to either 2 or 5 years of Tamoxifen, or no systemic therapy. MammaPrint was performed on stored tumor samples from these patients. The breast cancer specific survival (BCSS) for the untreated patients with MammaPrint indices of >0.355 was 94% at 20 years, compared to 97% for those receiving 2 or more years of Tamoxifen. For those women with Low Risk MammaPrint indices not in this "ultra low" or Late Recurrence Low Risk (LRLR) range, endocrine therapy was highly beneficial and significantly improved survival. Pathology features such as grade and Ki67 were unable to identify these subsets.

Within the MammaPrint High Risk range, indices 0.00 to-1.00, endocrine therapy appears to be inadequate for substantially reducing the risk of recurrence, and additional therapy is needed. Data from the neoadjuvant I-SPY 2 Trial (Investigation of Serial Studies to Predict Your Therapeutic Response With Imaging And molecular Analysis 2) has identified differential response to chemotherapy and targeted therapies for cancers with MammaPrint High Risk indices at the lower portion of the range ("High 1") compared to those at the upper portion of the range ("High2") (36-38). Cancers

with a MammaPrint High 2 index were highly likely to obtain pathologic complete remission(pCR) with the PARP inhibitor veliparib combined with carboplatin (36, 37), as well as to immunotherapy with the pembrolizumab combined with paclitaxel (38). MammaPrint High2 identified ER+ breast cancers likely to respond to pembrolizumab, a therapy with little activity in unselected ER+ breast cancers.

Conclusion

The MINDACT trial provided the first reported prospective randomized data supporting the clinical utility of the MammaPrint 70 gene assay in early stage breast cancer and will provide a rich source of additional data in the years to come as further sub studies and additional follow-up are performed. Gene expression profiling with MammaPrint has the ability to identify the risk of early metastasis, the likelihood of long term disease specific survival without therapy, and the likelihood of response to targeted therapies. With such clinically important information derived from only 70 genes, the potential information which could be obtained from analysis of the full transcriptome may be extraordinary. Full transcriptome expression data can now be obtained from any breast cancer also undergoing MammaPrint testing, offering the opportunity to explore a virtually unlimited array of important questions in breast cancer. This valuable information is now being collected through a registry trial sponsored by Agendia known as FLEX (Full-genome Data Linked with Clinical Data to Evaluate New Gene Expression Profiles) in which full transcriptome data will be correlated with extensive clinical annotation (see Trivedi et al, in this issue). Breast cancer clinicians may eventually rely on access to the anatomy of the full genome for the practice of precision oncology, a process which began with the 70 gene MammaPrint assay.

Authors' Contributions: Conception and design: WA, LB, HK, GS and HT; Acquisition, analysis and interpretation of data: WA, LB and HK; Drafting the article: WA; Revising it critically for important intellectual content: WA, LB and HK; Approved final version of the manuscript: WA, LB, HK, GS and HT.

Conflict of Interest: Dr William Audeh: Chief Medical Officer, Agendia, Inc.; Dr Lisa Blumencranz, Senior Medical Science Liaison, Agendia, Inc.; Dr Heather Kling, Medical Science Liaison, Agendia, Inc. Other authors declare that they have no conflict of interest.

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Narrative Review
Acta Medica Academica 2019;48(1):35-44
DOI: 10.5644/ama2006-124.240

Advances in Acute Myeloid Leukemia Genomics, Where Do We Stand in 2018?

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Received: 15 October 2018
Accepted: 27 February 2019

Key Words: AML ▪ NGS, Biology ▪ Myeloid ▪ Mutation.

The aim of this review is to summarize the data on commonly mutated genes and genomic pathways in acute myeloid leukemia (AML) with a focus on recently approved targeted therapies. AML is a heterogeneous disease with recurrent cytogenetic and genomic abnormalities that define the disease biology and pathogenesis. Classification of the disease categories and their prognostication was updated in the past 2 years to reflect the most recent advances in understanding the complex disease biology of AML. This review highlights major updates in the World Health Organization classification, including cytogenetic re-classifications, provisional entities, and updates to the European Leukemia Net (ELN) AML risk group stratification. An overview of pivotal studies that used novel sequencing techniques to define the mutational landscape of AML is also provided. In these studies, mutations are classified into subgroups based on functional pathways and are used to understand various interactions and mutual exclusivity of some mutations, suggesting important roles in disease evolution and AML pathogenesis. The complex interactions between mutations can dictate outcomes as well as possibly predict disease phenotypes after correcting for clinical variables. **Conclusion.** Genomic testing in AML using next generation sequencing has become widely available and a new standard of care for all patients. Therefore, it is vital to use novel methods to incorporate these data in clinical decision making.

Introduction

Acute myeloid leukemia (AML) is a clonal hematopoietic disorder affecting the myeloid cell lineage and is characterized by the expansion of undifferentiated immature myeloid precursors, which result in rapid progression of peripheral cytopenias and bone marrow failure (1). Cytogenetic and molecular heterogeneity can define AML phenotype and affect disease classification, prognosis, response to therapy, and treatment strategy (2). Recent advances in sequencing techniques have allowed the incorporation of genomic abnormalities in decision-making, diagnosis, and changes in treatment recommendations for AML patients (3, 4). Updates to the World Health Organization (WHO) 2016 criteria for AML classification and consensus guidelines have incorporated genomic data into AML classifications and prognostic systems (2, 5). Genomic data can also be used to evaluate minimal residual disease (MRD), identify specific targets for therapy such as *FLT3* and *IDH1/IDH2* inhibitors, and develop novel targeted therapies.

In this review, we discuss the genomic landscape of AML and the impact of the

commonly mutated genes and pathways on AML biology and prognosis.

AML Classifications

Cytogenetic Characterization

Cytogenetic analysis remains one of the most important diagnostic and risk stratification tools in AML. Specific balanced translocation or inversions have been described in AML and can be used as both diagnostic and prognostic tools. These abnormalities include:

- AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1,*
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11,*
- APL with *PML-RARA,*
- AML with t(9;11)(p21.3;q23.3); *MLLT3-KMT2A,*
- AML with t(6;9)(p23;q34.1); *DEK-NUP214 and*
- AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2).

The detection of some of these abnormalities (translocation (8;21), inversion 16/t(16;16) and AML with *PML-RARA*) were recognized by the WHO classification as sufficient to diagnose AML even in the absence of bone marrow blasts of ≧20%. In the 2016 WHO classification, a new provisional entity was added to recognize AML with BCR-ABL1 (5).

Although the distinction between *de novo* AML with *BCR-ABL1 vs.* blast phase chronic myeloid leukemia (BP-CML) is difficult, data suggest that the deletion of specific genes such as *IKZF1*, *CDKN2A*, T cell receptor genes, and immunoglobulins may support a *de novo* AML diagnosis over BP-CML (6, 7). Additionally, the 2016 WHO refined the definition of AML with myelodysplasia-related changes (AML-MRC); patients diagnosed with AML-MRC must have ≥50% of dysplastic cells in at least 2 cell lines, have a history of myelodysplastic syndrome or have specific cytogenetic abnormalities that define this disease entity. Of note, patients who meet dysplastic marrow criteria but concurrently carry an *NPM1* or bi-allelic *CEPBA* mutation, are not classified as AML-MRC. The AML-MRC cytogenetic abnormalities include 3 broad categories: complex karyotype, i.e. ≥3 abnormalities; unbalanced karyotypic abnormalities, such as monosomy 7 or 13; and balanced abnormalities as t(11;16) or t(3;21) amongst others (5). Additionally, minor updates in the WHO classification included renaming the *MLL* gene *KMT2A*, as well as recognizing that inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) is merely a gene rearrangement, without a gene fusion. AML with inv(3) or t(3:3) was noted to be associated with *GATA2/MECOM* and was shown to have aberrant expression of the stem cell regulator *ETV1*. Both of these 3q gene rearrangements reposition a *GATA2* enhancer and lead to *ETI1* activation/*MECOM* expression and functional *GATA2* haploinsufficiency. These studies showed how the repositioning of a single gene enhancer leads to AML development (8, 9).

The above cytogenetic abnormalities are found in approximately 20-30% of AML patients, and although they are grouped together, they have significant heterogeneity in their outcomes. Significant advance have been made in our understanding of the genomic landscape of AML since the completion of the human genome sequencing project. These advances have led to the recognition of several somatic mutations that play an important role in AML pathogenesis, prognosis, and the development of targeted therapies.

Genomic Landscape of AML

Several large scale genomic studies that included whole genome sequencing (WGS), whole exome sequencing (WES), RNA sequencing and other sequencing technolo-

gies have helped define the genomic landscape of AML (10-12).

In a study of 200 de novo AML samples from the Cancer Genome Atlas Project, analysis of WGS, WES, RNA and microRNA sequencing and DNA methylation identified at least one driver mutation in each AML sample and highlighted the complex interplay between the genomic abnormalities in each sample (12). Somatic mutations were classified into 9 functional groups in decreasing frequency (see Table 1).

In this study, mutational co-occurrence and exclusivity were investigated. Transcription factor fusions genes such as *PML-RARA, MYH11-CBFB*, and *MLL* containing fusion were found to be mutually exclusive of *DNMT3A, NPM1, CEPBA, IDH1, IDH2* and *RUNX1* mutations. These relationships suggest that such mutations may carry similar functions in AML initiation to fusion genes. Additionally, *RUNX1* and *TP53* mutations were noted to be mutually exclusive of *FLT3* and *NPM1* mutations. Mutual exclusivity was found within each biologic/functional gene group such as mutual exclusivity within cohesin complex genes, spliceosome proteins, signaling proteins, and histone-modifying proteins. These findings suggested that a single mutation in each of these pathways is adequate for AML pathogenesis. Clonal evolution plays a significant role in AML relapse and resistance to cytotoxic chemotherapy (12).

In a larger cohort of approximately 1500 AML patients, 5234 driver mutations were identified involving 76 genes, of those, point mutations accounted for the majority of alterations in 73% of the cases. Furthermore, 86% of samples had 2 or more driver mutations. A Bayesian model was used to reclassify AML into subtypes based on mutual exclusivity and co-occurrence of mutations. Eleven subtypes were identified, these include:

- NPM1-mutated AML (27% of cohort),
- AML with mutated chromatin and/or RNA-splicing genes (18%) which include (*RUNX1, MLL, SRSF2, ASXL1, STAG2*),
- AML with *TP53* mutations and/or chromosomal aneuploidy (13%),
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB–MYH11 (5%),
- AML with biallelic *CEBPA* mutations (4%),
- AML with t(15;17)(q22;q12); *PML–RARA* (4%),
- AML with t(8;21)(q22;q22); *RUNX1–RUNX1T1* (4%),
- AML with *MLL* fusion genes; t(x;11)(x;q2) (3%),
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *GATA2, MECOM(EVI1)* (1%),

Table 1. Genomic Functional Groups and Frequency in AML*

Functional Group	Gene	Percentage†
Signaling genes	*FLT3, KIT, KRAS, NARS, PTPN11*	59
DNA-methylation genes	*DNMTA3/B, DNMT1, TET1, IDH1, IDH2*	44
Chromatin modifying genes	*KMT2A* fusions, *ASXL1, EZH2, KDM6A*	30
Nucleophosmin gene	*NPM1*	27
Transcription-factor genes	*RUNX1, CEBPA*	22
Transcription-factor fusions	*PML-RARA, RUNX1-RUNX1T1, MYH11-CEBFB*	18
Tumor-suppressor genes	*TP53, WT1, PHF6*	16
Spliceosome-complex genes	*SF3B1, SRSF2, U2AF1, ZRSR2*	14
Cohesin-complex genes	*STAG2, RAD21, SMC3/5*	13

*Adapted from Cancer Genome Atlas Project (12); †Frequency in Cohort.

– AML with *IDH2*R172 mutations and no other class-defining lesions (1%) and
– AML with t(6;9)(p23;q34); *DEK–NUP214* (1%).

However 11% of patients had AML driver mutations without a detected class-defining lesion and 4% had AML without a detected mutational driver. Only 4% of patient samples met criteria for 2 or more genomic subgroups, most of which fell in the *TP53*–aneuploidy and chromatin–spliceosome subgroups. The study also showed specific clinical characteristics and different outcomes for each of the subgroups. As expected, the *TP53*–aneuploidy subgroup had dismal outcomes. Patients in the chromatin–spliceosome group had lower white blood cell and blast counts, were older and had low responses and higher relapses leading to poor outcomes as well. Although patients in the *IDH2*^{R172} subgroup constituted only 1%, their outcomes were better and similar to *NPM1*-mutated AML. In that cohort, mutations in *DNMT3A*, *ASXL1*, *IDH1/2,* and *TET2* genes were often acquired early and often found in association with other genetic abnormalities, suggesting that they are not likely driver mutations of AML, however they are mutations that confer an increased risk for clonal hematologic disorders (11).

In an analysis that compared the mutational profile of patients with de novo AML to therapy related and secondary AML, spliceosome mutations (*SRSF2, SF3B1, U2AF1, ZRSR2*) and mutations in *ASXL1, EZH2, BCOR, or STAG2* were >95% specific to define secondary AML compared to de novo, but in this study the analysis was not adjusted for cytogenetics and other important clinical variables (13). In another study of 465 patients with secondary and primary AML, clinical variables such as age, cytogenetics, and WBC changed the specificity of some of the mutations to AML phenotype and changed the impact of these mutations on outcomes suggesting that other clinical variables should be taken into account when analyzing genomic data (14). Mutations in AML can be categorized and affect several cellular pathways (Table 1).

FLT3

FMS-like tyrosine kinase 3 (*FLT3*) is a receptor tyrosine kinase and gene mutations are found in up to 30% of AML patients. There are 2 main types of mutations in *FLT3*, internal tandem duplications *(FLT3-ITD*), which are more common and occur in the juxtamembrane domain of the receptor in about 25% of AML, and point mutations in the activation loop of the tyrosine kinase domain *(FLT3-TKD*) in about 5-7% of AML (15). The impact of each mutation on outcomes is different; where *FLT3-ITD* mutations have adverse outcomes in AML with normal karyotype, whereas *FLT3-TKD* mutations have a controversial prognostic value, likely secondary to its lower frequency and smaller number of patients in studies to date. Importantly, incorporating *FLT3-ITD* allelic ratio plays a role in re-classifying disease risk in AML as well as the co-occurrence of *NPM1* mutation per the ELN criteria discussed below (2).

In 2017, the U.S. Food and Drug Administration (FDA) granted regulatory approval for midostaurin, a *FLT3* inhibitor, for newly diagnosed patients with *FLT3*-mutated AML patients during induction and consolidation chemotherapy (Figure 1A). The phase III randomized clinical trial that led to its approval was performed over a decade, accruing a total of 717 patients. The overall survival was significantly longer in the midostaurin group than in the placebo group with a hazard ratio for death of 0.78 95% CI, 0.63 to 0.96; one-sided P=0.009 by stratified score test (16). This can be used as a bridge to transplant, however it is not yet approved in the post-transplant maintenance setting (Table 2).

Table 2. Targeted Therapies in AML

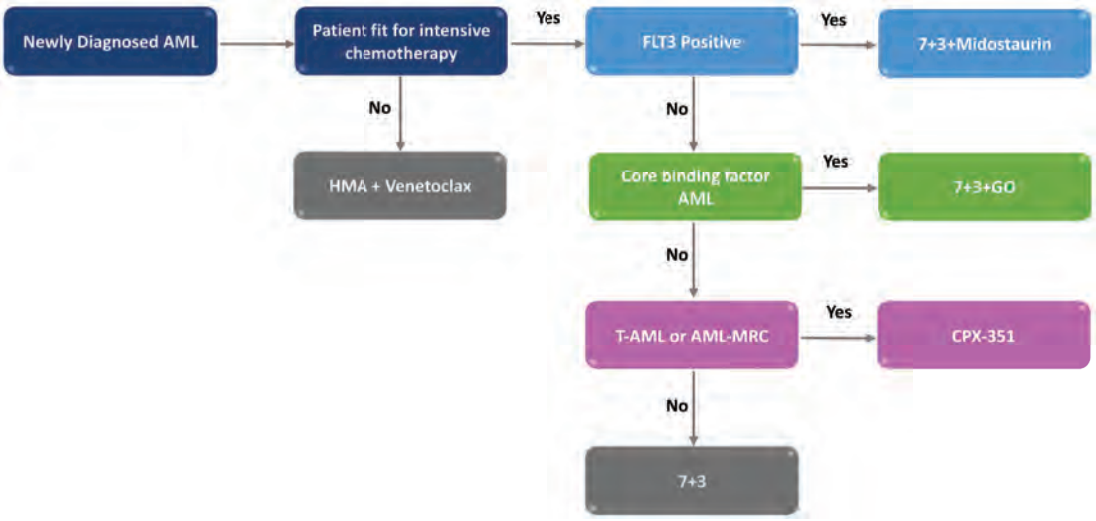
Drug	Mechanism of action	FDA Status/Date of Approval	Newly diagnosed vs R/R AML	Number Of Patients	Trial Phase	Outcomes
Midostaurin	*FLT3* Inhibitor	Approved 4/28/2017	Newly Dx	717 (360 midostaurin arm)	Phase III	mOS 74.7 m *vs* 24.5 m for PL. HR for death, 0.78; P=0.009
Gilteritinib	*FLT3* Inhibitor	Approved 11/28/2018 (Interim analysis)	R/R	138 (Gilteritinib arm)	Phase III	CR/CRh 21%
Quizartinib	*FLT3-ITD* Inhibitor	Breakthrough designation 8/1/2018	R/R	367 (245 Quizartinib arm)	Phase III	mOS 27 wks vs 20.4 wks for standard of care
Enasidenib	*IDH2* Inhibitor	Approved 8/1/2017	R/R	176	Phase I/II	ORR/CR 40%/19% mOS 9.3m
Ivosidenib	*IDH1* Inhibitor	Approved 7/20/2018	R/R	179	Phase I/II	ORR/CR 42%/24%
Gemtuzumab ozogamicin + chemotherapy	CD33 Antibody	9/1/2017 (reapproved)	Newly Dx	280	Phase III	2 year OS 53·2% vs 41·9% (HR ·69 P=0.0368)
Gemtuzumab ozogamicin (GO)	CD33 Antibody	9/1/2017 (reapproved)	Newly Dx	237 (118 on GO arm)	Phase III	mOS 4.9 m vs 3.6 m (HR 0.69, P=0.005)
Gemtuzumab ozogamicin (GO)	CD33 Antibody	9/1/2017 (reapproved)	R/R	57	Phase II	CR 26% mRFS 11.6 m
Venetoclax	BCL2 Inhibitor	Not Approved	R/R	32	Phase II	ORR 19% mOS 4.7 m

FDA=Food and drug administration; R/R=Relapsed/refractory; ORR=Overall response rate; CR=Complete remission; CRi=Complete remission with incomplete count recovery; CRh=Complete remission with partial hematologic recovery; RFS=Relapse free survival; mOS=Median overall survival; PL=Placebo, m=months, HR=Hazard ratio.

NPM1

Nucleophosmin is a nucleolar phosphoprotein encoded by the *NPM1* gene and regulates multiple cellular processes. The *NPM1* gene has 12 exons and the protein product shuttles between the cytoplasm and the nucleus, although primarily residing in the nucleus. Nucleophosmin has a role in ribosome biogenesis, p53-dependent stress response, genomic stability and modulation of other growth-suppression pathways. Mutations in *NPM1* involving exon 12 in the C-terminus of the protein lead to the expression of mutant cytoplasmic NPMc+, which is the *most* common mutation in AML and is always heterozygous (17). Recently, a study evaluating the exact leukemogenic effects of NPMc+ revealed that it also dislocates a transcription factor driver of monocyte differentiation PU.1 (also known as SPI1) into cytoplasm with it preventing collaboration with other master transcription factors CEBPA and RUNX1, thereby repressing terminal granulocytic differentiation (18).

NPM1 mutations are found in about 30% of AML patients and generally carry a favorable prognosis in the absence of *FLT3-ITD*, or when the *FLT3-ITD* allelic ratio is low.



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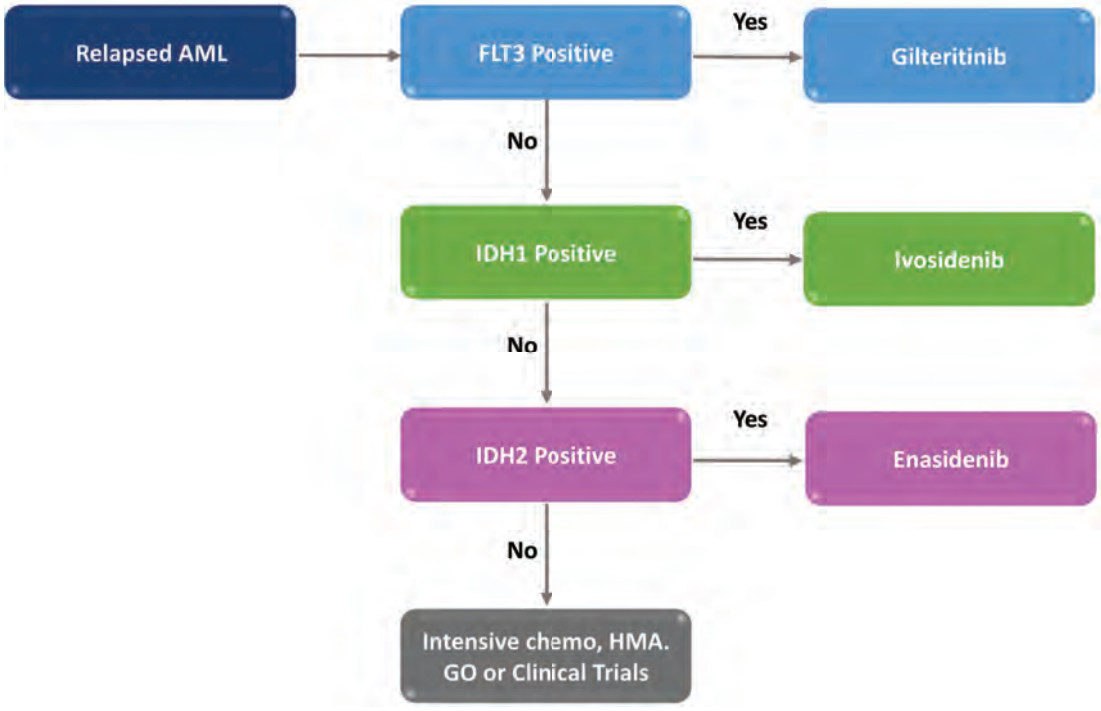

1B

Figure 1. Treatment algorithm for patients with Newly Diagnosed (1A) and Relapsed AML using approved targeted therapies (1B). HMA= Hypomethyating agents; GO=Gemtuzumab ozogamicin; tAML=Therapy related AML; AML-MRC=AML with myelodysplasia changes.

NPM1 mutations can co-occur with *DNMT3A, FLT3-ITD, IDH1/2* and *TET2* but are mutually exclusive of *RUNX1, CEPBA* and *TP53* (12, 17, 19).

CEBPA

CCAAT/enhancer binding protein α (*CEBPA)* mutations occur in 5-10% of AML patients. Bi-allelic mutations constitute two

thirds of mutations, involve mutations in the N- and C- terminus, and carry a favorable prognosis in AML (20). *CEBPA* is a transcription factor that binds both promoter and enhancer regions and plays a role in neutrophil differentiation (21). It is most commonly seen in patients with a normal karyotype and is recognized as a separate entity in the recent WHO classification (5, 22).

Mutations in Methylation Pathway

DNMT3A

DNA (cytosine-5)-methyltransferase 3A is an enzyme that transfers methyl groups to specific CpG structures in the DNA regulating epigenetic changes. DNMT3A mutations occur in about 20 - 25% of patients with AML (23). The majority of somatic DNMT3A mutations occur at a single amino acid, R882. DNMT3A can co-occur with FLT3-ITD, NPM1, and IDH1/2 mutations is also rarely associated with transcription factor fusions such as t(8:21), inv16 or t(15;17). Most studies have associated DNMT3A mutations with worse outcomes, however some data suggest this can be overcome with higher anthracycline doses (24, 25).

TET2

Ten-eleven translocation 2 (*TET2)* is a methylcytosine dioxygenase 2 gene that encodes a protein involved in epigenetic modification by hydroxylation of the 5' end of the methyl cytosine residues (26). Mutations in *TET2* are found in about 10% of AML, although *TET2* mutations can occur in patients without evidence of hematologic malignancies where the incidence increases with age. This clonal hematopoiesis was shown to increase the risk of cardiovascular disease and death (27). The impact of *TET2* mutations on outcomes and response to therapy in AML remains controversial (28).

IDH1/IDH2

Isocitrate dehydrogenase 1 and 2 are enzymes that catalyze the oxidative decarboxylation of isocitrate to 2-oxoglutarate and control DNA methylation and histone modification. Mutations in IDH1/2 lead to increased level of 2-hydroxyglutarate. *IDH1* mutations occur in 5-10% affecting the arginine at either R132 or R170 residues and are exclusive of one another. *IDH2* mutations occur about 10% of AML affecting arginine residues R140 or R172 (29). Both *IDH1* and *IDH2* inhibitors have been developed and approved by the FDA for treatment of relapsed/refractory AML (Figure 1B), where they can be used as bridge for allogeneic hematopoietic cell transplantation in eligible patients (30, 31).

In August 2017, the first in class oral inhibitor of isocitrate dehydrogenase 2 (*IDH2*) enzyme, enasidenib (AG-221) received regulatory approval for relapse/refractory AML with an *IDH2* mutation. Patients with relapsed/refractory AML received oral treatment daily and achieved an overall response rate of 40.3% with a median duration of response of 5.8 months (95% CI 3.9 – 7.4). The median overall survival for all patients was 9.3 months (8.2-10.9 m), however median overall survival reached 19.7 months (11.6 m to not reached) for those who achieved a complete remission (19.3%) (30) (Table 2). More recently, in July 2018, ivosidenib, an *IDH1* inhibitor was approved by the FDA for the treatment of adult patients with *IDH1* mutated relapsed/refractory AML. The overall response rate was 41.6% with 21.6% of patients achieving a CR. The median duration of responses were 6.5 months (95% CI, 4.6 to 9.3) and 9.3 months (95% CI, 5.6 to 18.3) respectively (31)(Table 2).

Chromatin Modifying Genes

ASXL1

Additional sex combs-like (*ASXL*) 1 gene is a chromatin modifying gene that encodes a

binding protein what enhances or represses gene transcription. *ASXL1* mutations occur in about 5% of de-novo AML and up to a quarter of patients with secondary AML. ASXL1 mutations have a negative impact on OS and have been classified as poor risk AML in the 2017 ELN risk stratification (2), although some reports showed that this impact may be lost when controlling for clinical and chromosomal abnormalities (32, 33).

Tumor Suppressor Genes

TP53

Tumor protein p53 (TP53) is a tumor suppressor gene located on the short arm of chromosome 17 and is involved in cell cycle regulation. Mutations are more common in secondary AML and are found in a quarter of patients, but only found in 5% of patients with de novo disease (12). *TP53* mutations are often associated with complex karyotype and carry a poor prognosis (34).

WT1

Wilms tumor (WT) 1 gene is a tumor suppressor gene. Overexpression of *WT1* is common in hematopoietic myeloid malignancies and confers a higher chance of relapse and poor outcomes, even in the post allogeneic transplant setting. Some studies are investigating its use as a minimal residual disease marker to predict early relapses (35, 36).

AML Risk Stratification (ELN 2017)

The updated European Leukemia Net (ELN) recommendations for the diagnosis and management of AML were revised and published in 2017. The 2010 ELN risk stratification included mutations in *CEBPA, NPM1* and *FLT3.* In the update version, 3 additional genomic abnormalities into the AML prognostication risk groups; these include *ASXL1, RUNX1* and *TP53*. Additionally, there has been a distinction in risk stratification based on *FLT3-ITD* allelic ratio, low defined as allelic ratio <0.5 or *FLT3-ITD* and high allelic ratio where the mutation burden is more than 0.5.Thus, the current AML risk stratification includes 3 risk categories based on genetics (i.e. cytogenetic and molecular abnormalities). Favorable risk group includes patients with core binding factor leukemia [t(8;21 and inv(16) or t(16;16)], patients with biallelic mutated *CEPBA* as well as mutated *NPM1* without *FLT3-ITD* or with *FLT3-ITD* allelic ratio <0.5. The intermediate risk group includes patients with mutated *NPM1* and *FLT3-ITD* allelic ratio of >0.5, wild type *NPM1* with *FLT3-ITD* negative of ratio <0.5 and t(9;11) and all other cytogenetic abnormalities that are not classified as favorable or adverse. The adverse risk group includes patients with the following cytogenetic abnormalities [t(6;9), t(v;11q23.3)/*KMT2A* rearranged, t(9;22), inv(3), chromosome 5, 7 or 17p abnormalities] and molecular abnormalities [wild type *NPM1* and *FLT3-ITD* ratio >0.5, mutated *RUNX1*, *ASXL1* and *TP53*] (2). This stratification does not yet account for other co-occurring mutations or other mutational interactions, which may change in future prognostication systems.

Conclusions

Understanding genomics in AML is prudent for risk stratification and making treatment decisions; further characterization of mutational interactions, the impact on prognosis and treatment responses as well as translating genomic testing, such as MRD testing, into clinically meaningful therapeutic interventions is necessary to further advance therapies and improve outcomes. To date, the risk stratification of AML is dependent on incorporating a few mutations as *FLT3, NPM1, CEBPA, RUNX1,* and *TP53* into each

risk category, thus mutational analysis using widely available myeloid gene panels has become standard of care for all newly diagnosed patients. These are important to guide optimal outcomes, treatment planning for upfront hematopoietic cell transplantation. The co-occurrence of gene mutations and disease heterogeneity mandate the use of newer analytic techniques to better personalize management for each of our patients.

Authors' Contributions: Conception and design: YFM, AN and MEK; Acquisition, analysis and interpretation of data: YFM, AN and MEK; Drafting the article: YFM; Revising it critically for important intellectual content: MEK and AN; Approved final version of the manuscript: YFM, MEK and AN.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Narrative Review
Acta Medica Academica 2019;48(1):45-56
DOI: 10.5644/ama2006-124.241

Laboratory Work-Up of Chronic B-Cell Lymphoid Malignancies – A Value-Based Approach

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Received: 26 September 2018
Accepted: 3 January 2019

Key Words: Lymphoma ▪ Pathology ▪ Prognosis.

The aim of study was to summarize recent developments in laboratory work-up of lymphomas and discuss their clinical relevance. Diagnosis of lymphoma requires tissue biopsy with adequate work-up by pathologists. Recent developments in laboratory testing have raised the bar for establishing the diagnosis: more and more testing seems to be required, while the lines between research and clinical practice are being blurred. Academic medical practice is designed to push boundaries and test new hypotheses, which eventually result in improved patient care. Ability to (relatively) cheaply screen for multiple genomic abnormalities using new technologies is luring. Often, however, no change in patient management is pursued based on these results. It is therefore useful to review which testing is truly necessary from the patient's point of view. **Conclusions.** The laboratory work-up of lymphomas in a regular clinical practice requires relatively few tests. Many new tests have prognostic value, but do not necessarily contribute to the patient management.

Introduction

Malignant lymphomas are neoplasms of mature lymphocytes, involving lymph nodes as well other hematopoietic (bone marrow, spleen) and non-hematopoietic tissues (gastrointestinal tract, skin, etc.). In the United States, lymphomas account for approximately 4-5% of all new cases of malignant diseases. About 90% of all lymphomas are of B-cell lineage.

The new WHO classification of lymphoid malignancies continued in the steps of previous versions by keeping the multifactorial approach in disease definition (1). *Clinical features* are represented by aggressiveness in presentation and localization. *Morphologic findi*ngs include cell size and shape, presence and distribution of nucleoli, quality and quantity of cytoplasm, and the pattern of growth. *Immunophenotype* of the cells is critical in assigning lineage and differentiation pattern, linking the malignant cells to their normal counterparts. *Cytogenetic findings* are sometimes used as disease-defining events, in cases of the recurrent translocations (involving *MYC*, *CYCLIN D1*, or *BCL2* loci, for example), in the right clinical and morphologic context. Finally, detection of *molecular abnormalities* includes clonality assays for immunoglobulin or T-cell receptor gene rearrangements, and a plethora of novel point mutations. The newly described mutations are most commonly discussed as prognostic variables, with very few of them approaching the level of disease-defining events (MYD88 L265P for lymphoplasma-

cytic lymphoma; BRAF V600E for hairy cell leukemia).

When ordering laboratory test, such as gene mutation profile for lymphoma, it is important to keep in mind the ultimate purpose, whether it is to confirm/establish a diagnosis, identify a prognostically important marker, or to guide decision on therapy choice and duration. It is also necessary to be clear whether the testing is done in the routine clinical practice, well-controlled clinical trial environment, or as a purely research endeavor. In reality, these different scenarios are often intermingled, which leads to a lot of unnecessary testing, resulting in increased burden for the laboratory operations and increased health care costs.

In this review, we will assess the current knowledge and utility of laboratory testing in the most common B-cell lymphomas.

Chronic Lymphocytic Leukemia/ Small Lymphocytic Lymphoma

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is the most common B-cell lymphoproliferative disorder, accounting for almost 20% of all NHL cases (2). Most cases are diagnostically straightforward: high lymphocyte count in the peripheral blood (need $\geq 5\times10^9$ clonal B-cells/L for the diagnosis of CLL), typical morphology with small lymphocyte size and "checkered" chromatin, and typical immunophenotype showing dim CD20 expression and co-expression of CD5, CD23, and CD200. Because of its high prevalence, and the ease of obtaining the specimen from peripheral blood, CLL/SLL is probably the most studied hematologic disease. There is an "overabundance" of prognostic markers available. For example, serum LDH, beta2-microglobulin, thymidine kinase, vitamin D, circulating CD26, BAFF, and vitamin D are all predictive of the behavior of the disease (3-5). In particular, LDH level, as a correlate to the cell turnover, is predictive of Richter's transformation (6). Similarly, looking at malignant cell morphology and phenotype, there are numerous variables of poor prognosis, including CD38 expression in ≥30% of cells, ZAP70 expression in ≥ 20-30% of cells, CD49d expression in ≥30-45% of cells, and high proliferative rate measured by DNA staining or mitotic activity (7, 8). RNA-based assays can also be employed in prognostication of CLL/SLL. For example RNA levels of ZAP70 and CD38 correlate with expression of these markers and poor prognosis. In addition, gene expression profiling can identify microRNA expression signatures which are associated with more aggressive disease course (9). Detection of chromosomal abnormalities is a mainstay of prognostic factor determination in CLL/SLL, with del(17p), del(11q), trisomy 12 and complex karyotype (≥3 clonal abnormalities) typically associated with poor outcome (10, 11). Single gene sequencing assays are employed to determine the level of somatic hypermutation (IgVH; >2% difference from the germline is associated with better prognosis) (12), to "stereotype" immunoglobulin gene into prognostic subsets (13), and to assess the presence of TP53 inactivating mutations (14-16). In addition, recently a number of additional genes with prognostic significance have been identified. For example, mutations in NOTCH1 are associated with a more aggressive clinical course, including higher likelihood of Richter's transformation and diminished responsiveness to rituximab (17). SF3B1 and BIRC3 mutations are similarly associated with a more aggressive disease (18).

As the number of potential prognostic factors for CLL/SLL is ever-increasing, it is important to try to integrate them into an actionable model for patients requiring treatment (19). A number of models have been proposed throughout the years, starting with Binet and Rai staging schemes from

1970s. The most recent prognostic models include staging information, clinical and laboratory findings, and a limited number of variables. International Prognostic Index (CLL-IPI) was developed by the International CLL Working Group in 2016 (4). It includes patient age, Rai/Binet stage, beta2-microglobulin level, IgVH status, and 17p/TP53 abnormalities. In fact, the prognostic model is weighed, so that 17p/TP53 abnormalities contribute the most to the overall prognostic score, see Tables 1 and 2.

Table 1. CLL-IPI Score Sheet (4)

Parameter	Points
FISH 17p- or TP53 mutation	4
IGVH unmutated	2
Beta-2-microglobulin >3.5mg/dL	2
Rai stage I-IV/Binet B-C	1
Age >60 years	1
Total max	10

CLL-IPI=Chronic lymphocytic leukemia - International Prognostic Index; FISH=Fluorescent in-situ hybridization; TP53; IGVH=Immunoglobulin heavy chain variable region.

Table 2. Treatment-Free Survival in CLL/SLL by CLL-IPI Score.

Points	Risk category	Treatment (%)*
0-1	Minimal	78
2-3	Low	54
4-6	Intermediate	32
7-10	High	0

CLL/SLL= Chronic lymphocytic leukemia/small lymphocytic lymphoma; CLL-IPI=Chronic lymphocytic leukemia – International Prognostic Index; *5 year treatment – free survival, Mayo Clinic, Rochester, unpublished data.

Based on this score, patients are stratified into the prognostic subgroups with clear difference in survival. While the mutation profiling is useful in research and clinical study settings, at this point it appears that for a regular clinical practice parameters covered in CLL-IPI would suffice for the management of patients requiring treatment, to select the most appropriate treatment combination. From pathology work-up perspective, that would limit the evaluation of CLL/SLL cells to IgVH, FISH for 17p deletion, and TP53 mutation analysis.

Patients who have undergone treatment, and are in remission by standard techniques (CT scan-negative, blood lymphocytes <4000/microL, bone marrow lymphocytes <30%) could undergo minimal residual disease (MRD) testing by flow cytometry immunophenotyping or molecular analysis (PCR, next generation sequencing) (19). The detection of the MRD is associated with shorter progression-free and overall survival. Patients in clinical trials are regularly tested for the presence of MRD. Outside clinical trials MRD testing is sporadic and should be restricted to potentially curative treatments.

Follicular Lymphoma

Follicular lymphoma (FL) is a neoplasm of follicle center (germinal center) B-cells, which accounts for about 20% of all lymphomas (20). Pathologic evaluation of FL is relatively straight forward and consists of establishing (I) neoplastic nature of the lymphocytes and (II) their follicular origin. A simple hematoxylin/eosin stain is often enough to establish both of these, when a lymph node is completely effaced by uniform population of follicles without polarity. However, immunostains are very helpful for evaluation of less obvious cases, particularly needle biopsy specimens. BCL2 expression on germinal center cells (defined by CD10 and/or BCL6) is diagnostic of follicular lymphoma. In addition, BCL2 staining is necessary for the detection of in-situ follicular neoplasia. Overexpression of BCL2 is a result of a IGH/BCL2 translocation t(14;18)(q32;q21); however FISH for this abnormality is rarely needed in general clinical practice if adequate evaluation by immunohistochemistry is done.

Prognostically, the most important factors are grade (1/2 vs. 3A vs. 3B; established by counting the number of centroblasts per high power field) and clinical stage, usually defined by FLIPI scores. FLIPI (Follicular Lymphoma International Prognostic Index) and FLIPI2 scores integrate several clinical and laboratory parameters, including age, LDH level, size of the largest lymph node, bone marrow involvement, and hemoglobin level (21, 22). Therefore, routine work-up of follicular lymphomas does not require sophisticated molecular or cytogenetic testing. The two exceptions are cases of pediatric-type follicular lymphoma and large B cell lymphoma with IRF4 rearrangement which may have follicular architecture. Pediatric-type FL is usually found in children and young adults, in the head and neck region (23). These tumors show high grade morphology (FL grade 3A or 3B) and high proliferation rate but lack BCL2 staining and by definition do not have rearrangements of BCL2, BCL6 or IRF4 loci by FISH. On the other hand, they often have deletion of 1p36 region or mutations in TNFRSF14 gene (24). Large B-cell lymphoma with IRF4 rearrangement has a similar epidemiology and localization to pediatric-type follicular lymphoma, and, as its name suggests, is defined by the translocations involving IRF4 locus, most commonly juxtaposed to IGH locus (25). Both pediatric type FL and large B-cell lymphoma with IRF4 rearrangement are relatively indolent diseases, the former rarely requiring systemic therapy. If there is a high level of suspicion for one of these 2 types of follicular lymphomas (young patient, high grade follicular morphology), it is prudent to perform FISH for BCL2, BCL6 and IRF4 rearrangements.

There are numerous studies showing a potential for different gene mutations to be helpful in the prognosis of FL, including m7-FLIPI panel, and p53 mutations (26, 27). However, usefulness of these tests is not yet widely accepted, and more studies are needed before they can be recommended for a routine clinical practice.

Mantle Cell Lymphoma

Mantle cell lymphoma (MCL) is an aggressive neoplasm of small mature B-cells, characterized by CCND1/IGH translocation and the resulting cyclin D1 overexpression (28). The diagnosis of a typical mantle cell lymphoma is usually straight forward: the involved lymph node is effaced by a monotonous infiltrate of small lymphocytes with hyperchromatic irregular nuclei. The typical phenotype of MCL is $CD20^{bright}$ $CD5^{+}CD23^{-}$ $CD200^{-}$. This phenotype can be overlapping with that of CLL/SLL, as well as marginal zone lymphoma or lymphoplasmacytic lymphoma. Therefore it is necessary to prove either CCND1/IGH translocation, or uniform nuclear expression of cyclin D1, but usually there is no need to do both. It is, however, necessary to correlate the morphology and FISH findings with the immunophenotype, as cyclin D1 can frequently be seen overexpressed in plasma cell neoplasms (with CCND1/IGH translocation) and hairy cell leukemia (without CCND1/IGH translocation). Lymph nodes with preserved follicular architecture may contain in-situ mantle cell neoplasia, which is a rare indolent disorder that can be recognized only when staining slides for cyclin D1.

Similar to CLL/SLL and FL, prognosis of MCL is guided by staging. Mantle cell lymphoma international prognostic index (MIPI) is based on patient age, ECOG performance status, LDH level and the WBC count in the peripheral blood (29). From pathology perspective, there are three important parameters to consider. First, it is necessary to assess morphology to determine if there is a pleomorphic or blastic appearance of the cells, both of which are associated with a more aggressive disease. Second,

expression of SOX11 should be evaluated. Classical, nodal-based MCL is positive for SOX11, and in rare cases of cyclin D1-negative MCL, SOX11 is a key diagnostic marker (30, 31). In contrast, SOX11-negative mantle cell lymphoma is typically an indolent disease, involving peripheral blood, bone marrow and spleen, and sparing the lymph nodes (32-34). Finally, it is important to assess proliferative activity, by Ki67 immunostain, since there is a correlation between proliferative activity and poor prognosis. For simplification, a cutoff of 30% has been validated as a useful addition to MIPI score (35), but other cutoffs have also been used. Overexpression of TP53 is also associated with a more aggressive disease (36). There is a potential value of additional cytogenetic and molecular testing in MCL, mostly to determine predictors of poor prognosis, such as 9p and 17p deletions. In addition, TP53 mutations are also predictive of aggressive clinical course, and even the indolent form of MCL may turn into a very aggressive disease if TP53 mutations are acquired. Other potential genes of interest include CCND1 and BIRC3. Mutations in these 2 genes have been associated with ibrutinib resistance (37, 38), though it is still unclear whether finding these mutations requires a different clinical approach.

Lymphoplasmacytic Lymphoma

Lymphoplasmacytic lymphoma (LPL) is a neoplasm of small lymphocytes, plasmacytoid lymphocytes and mature plasma cells, all derived from a single B-cell clone (39). It usually affects bone marrow, and often spleen and lymph nodes as well. Most cases are associated with increased IgM production, leading to the clinical syndrome of Waldenström macroglobulinemia (WM). The distinction between LPL and marginal zone lymphoma (MZL) with plasmacytic differentiation is somewhat arbitrary as no definitive morphologic, immunophenotypic or genetic markers are present. Recently identified mutation in the adaptor protein MYD88 (L265P) is found in >90% of LPL cases, but can also be seen in cases of MZL and DLBCL (particularly of immunoprivileged sites) (40). Therefore, molecular testing for this mutation is helpful, but the positive result has to be interpreted in the context of the morphologic findings. Presence of MYD88 L265P mutation is associated with a good response to ibrutinib therapy; however about 30% of LPL also contains CXCR4 mutations which confer resistance to ibrutinib (41). LPL is usually an indolent disease, treated based on the disease stage and other significant pathologic findings to determine prognosis or therapy are established. In fact, the most important part of the pathologic evaluation is to exclude multiple myeloma (MM) from the differential diagnosis, as the treatment approach is completely different between MM and LPL. Plasma cells in LPL are usually positive for CD19 and CD45 and negative for CD56 and cyclin D1, in contrast to plasma cells in MM. In addition, MYD88 L265P mutation has not been identified in MM.

Multiple Myeloma

Multiple myeloma (MM, plasma cell myeloma) is a neoplasm of mature plasma cells (≥10%) involving the bone marrow, and is usually associated with the presence of monoclonal (M) protein in the serum and urine. The diagnostic criteria for the active MM have been updated recently (42), to include so-called biomarkers of malignancy (≥60% clonal plasma cells in the bone marrow, free light chain ration of ≥100 and >1 focal lesion on MRI); any one of these features, as well as previously recognized "CRAB" findings, excludes the possibility of smoldering multiple myeloma (SMM) and warrants treatment. The quantification of plasma cells is crucial in establishing the

diagnosis of MM. This is best done by morphology (on the aspirate slide), and/or by immunohistochemical stain for CD138 or MUM (on the biopsy slide). Flow cytometry is not a reliable method of quantifying plasma cells in the diagnostic specimen for MM. Plasma cells are usually underestimated by flow cytometry immunophenotyping, as they tend to be associated with the lipid phase of the bone marrow aspirate, which gets lost during the staining and washing steps in sample preparation. On the other hand, flow cytometry is an excellent method for evaluating the phenotype: plasma cells in multiple myeloma are cytoplasmic light chain restricted (or rarely negative), usually dim for CD38, negative for CD19 and CD45, and often positive for CD56 and CD117.

Prognostically, MM is stratified into a high risk and standard risk disease. The criteria for high risk include presence of t(4;14), t(14;16), t(14;20), del(17p), add(1p) and TP53 mutations. In addition, high stage disease, high risk gene expression profiling signature and high plasma cell proliferative rate are also risk factors which fulfill criteria for the high risk MM (43). In practice, very few institutions employ all of the prognostic markers, but FISH studies remain essential. Numerous point mutations have been found in multiple genes in MM samples (44). The usefulness of gene mutation screens is not yet well established, and they are mostly utilized in relapsed/refractory disease for which innovative treatment options are explored. The exception may be detection of biallelic TP53 mutations/17p deletions, which are associated with a particularly aggressive disease (45, 46).

Similarly to CLL/SLL, MM patients who have undergone therapy and are in complete remission may benefit from performing MRD study on the bone marrow aspirate, by flow cytometry or molecular techniques (47). MRD status is a strong predictor of survival, but at this point it is not used to guide treatment adjustment.

Diffuse Large B-Cell Lymphoma and Aggressive B-Cell Neoplasms

Diffuse large B-cell lymphoma (DLBCL) is a neoplasm of medium-to-large B-cells with a diffuse growth pattern. The size of nuclei has to be at least the same or larger than size of macrophage nuclei, or twice the size of the nuclei of small lymphocytes (48). DLBCL accounts for about 30% of all non-Hodgkin lymphomas, and it is considered an intermediate grade disorder, which requires treatment. Historically, the term DLBCL has been an all-encompassing "waste-basket" for any B-neoplasm with large cells. Over time, more specific entities have differentiated into separate diseases. For example, the latest WHO classification recognizes following separate entities: large B-cell lymphoma with IRF4 rearrangement, T-cell/histiocyte-rich large B-cell lymphoma, primary DLBCL of the CNS, primary cutaneous DLBCL, leg type, EBV-positive DLBCL, NOS, DLBCL associated with chronic inflammation, lymphomatoid granulomatosis grade 3, primary mediastinal (thymic) large B-cell lymphoma, intravascular large B-cell lymphoma, ALK-positive large B-cell lymphoma, HHV8-positive DLBCL, plasmablastic lymphoma, and primary effusion lymphoma (1). The remaining cases fulfilling diagnostic criteria for DLBCL are designated as DLBCL, not otherwise specified (NOS).

As with other lymphomas, the prognosis of DLBCL is primarily determined by clinical features and staging studies. Biologically, based on cell of origin/postulated normal counterpart, DLBCL cases can be divided into two groups: germinal center B cell type (GCB) and activated B-cell/post-germinal center type (ABC). The two groups differ in their gene expression patterns, chromosomal abnormalities, recurrent mutations, and, to a certain extent, prognosis and therapy response. WHO classification of lymphoid neoplasms requires subtyping of DLBCL,

NOS at diagnosis, by cell of origin. Originally, the cell of origin grouping was achieved by gene expression profiling (GEP) (49, 50). This was followed by development of multiple immunohistochemical algorithms which were able to match GEP classification in the majority of cases. The most commonly used is Hans algorithm (51), which requires staining of DLBCL cases with CD10, MUM1 and BCL6. CD10 and BCL6 staining indicate GBC type; MUM1 staining is associated with ABC type; the order of importance is CD10>MUM1>BCL6; 30% staining is used as a cutoff. Although immunohistochemical staining for cell of origin lacks in reproducibility and accuracy, it is now widely used to classify DLBCL cases. Recently, the GEP platform for cell of origin classification has become commercially available (52), but the cost and logistics still prevent it from widespread use. Other important prognostic markers used in pathology workup of DLBCL include staining for CD5 (positive staining associated with a more aggressive disease (53)), BCL2, MYC (MYC and BCL2-double expressors have worse prognosis (54)), and, possibly, EBV (EBV positivity removes the case from DLBCL, NOS to EBV-positive DLBCL, with worse prognosis in older patients, and better in younger patients; therapeutic options may also differ based on the immunosuppression context). Numerous single gene mutations have been identified in DLBCL. Many of them are associated with ABC phenotype and constitutive activation of B-cell receptor and NFκB signaling pathway (55). While there is a potential use of the mutation panels in determining prognosis, their therapeutic value awaits results from ongoing clinical trials.

Burkitt lymphoma (BL) is an aggressive neoplasm of medium-size mature B-lymphocytes with blastic-appearing chromatin (56). It mainly affects children and young adults. Endemic BL is EBV-driven and limited to equatorial Africa and Papua New Guinea. Sporadic BL is distributed worldwide, including Europe and USA, and is relatively rare (1-2% of all lymphomas). Immunophenotype of BL is fairly typical: the cells have a mature B-phenotype (CD20-positive, light chain-restricted, TdT-negative), show germinal center B-cell differentiation (CD10 and BCL6-positive), and are also CD43-positive and BCL2-negative. A hallmark of BL is the presence of MYC rearrangements, usually in the form of MYC/IGH translocation t(14;18)(q24;q32). A relatively small fraction of BL cases don't have MYC rearrangement by available techniques. Some of these cases likely have unusual breakpoints, not detectable by common FISH probes. Rare cases of BL have aberrations of 11q region instead (57). BL is a potentially curable disease with aggressive chemotherapy.

High grade B-cell lymphoma (HGBCL) with MYC and BCL2 and/or BCL6 abnormalities is a relatively new term designating an aggressive lymphoma occurring mostly in the elderly people (58). In the past 20 years there has been an increasing recognition of a subgroup of aggressive B-cell lymphomas which do not satisfy strict criteria for DLBCL of BL. It was subsequently discovered that many of these cases have two or even three major genetic events that are driving their behavior. Invariably, these neoplasms have MYC rearrangement, which can be paired with BCL2 and/or BCL6 rearrangement ("double or triple-hit lymphoma"). The behavior of these neoplasms is significantly more aggressive than that of DLBCL, and R-CHOP therapy is associated with short survival (59, 60). More aggressive therapeutic approaches are being tested in clinical trials. Importantly, the morphology of HGBCL with MYC and BCL2 and/or BCL6 rearrangement can be blastoid/Burkitt-like, large cell/DLBCL-like, or fall somewhere in between the two. Therefore, any case of DLBCL may contain these abnormalities, although having a germinal

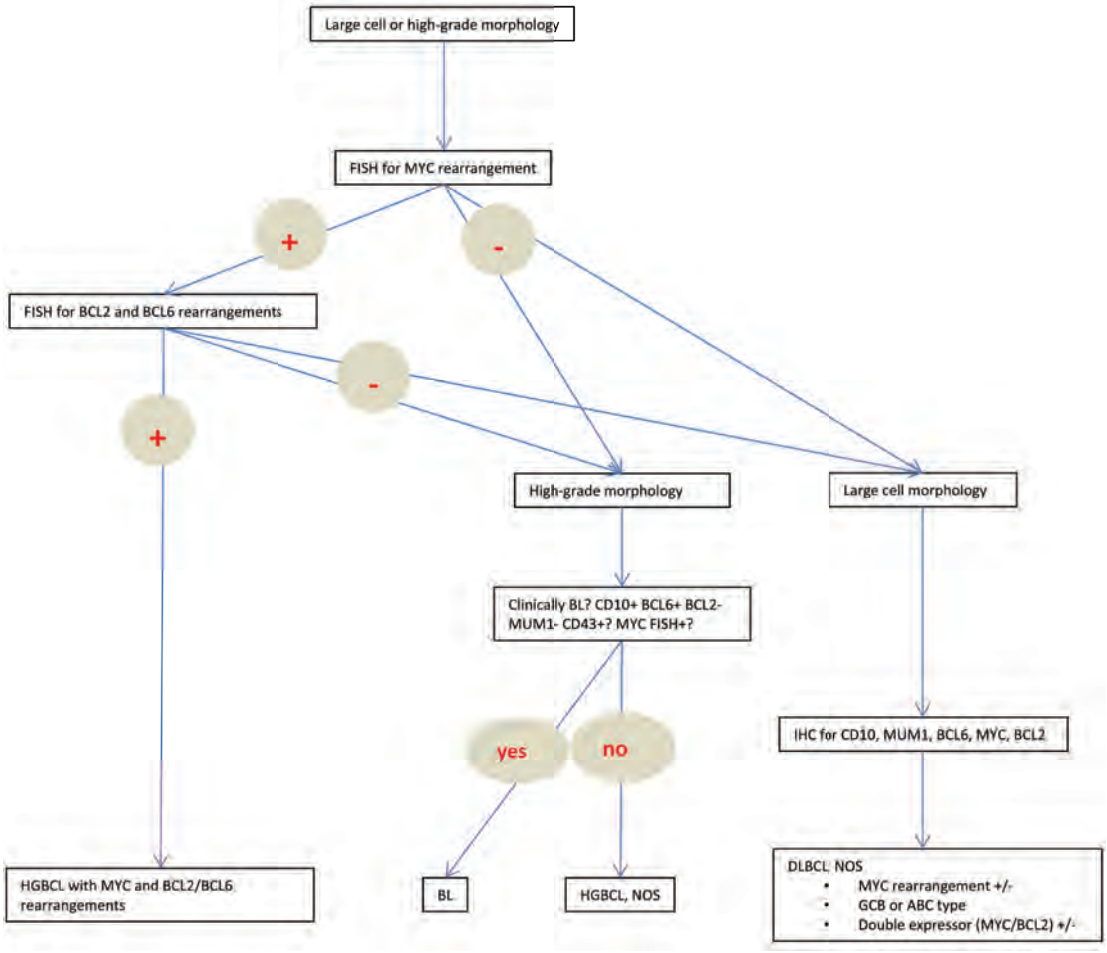

Figure 1. A Simplified Algorithm for the Work-Up of DLBCL and HGBCL.

center phenotype increases their likelihood. As a result of these findings, it is practically impossible to diagnose DLBCL without excluding the possibility of a "double or triple-hit lymphoma" by FISH. An example of a rational approach to the pathology workup of DLBCL and high grade B-cell lymphomas is shown in Figure 1.

Conclusion

Application of new technologies has been exponentially increasing our knowledge of genetics and pathology of lymphomas. Clinical utility for many of these findings is lagging behind for several reasons. First and foremost, the rationale for ordering a test in a routine clinical practice has to be that the result will change the clinical approach (choice of therapy, frequency of follow-up). With still limited therapeutic options, the benefit for the patients from undergoing advanced genetic testing is questionable. In addition, there are limited resources available. Even large academic centers, which have testing infrastructure on-site, struggle to offer advanced genetic testing due to the fact that the reimbursement by insurance companies is, at best, inconsistent. This review summarizes the rational approach to pathology workup of most common lymphoid malignancies, in a routine clinical practice. In Table 3, we listed commonly used ancillary testing in lymphoid neoplasms. As the field

Table 3. Commonly Used Immunophenotypic, Cytogenetic, and Molecular Tests in a Non-Clinical Trial Setting

Disease type	Immunophenotyping	Fish studies	Molecular studies
CLL/SLL	Typical phenotype: Positive for CD20 (dim), CD5, CD23, CD200, Negative for CD10	Del(17p)	IgVH, TP53
FL	-	Rearrangements of BCL2, BCL6, and MUM1*	-
MCL	Cyclin D1, SOX11 Ki67, TP53	t(11;14)†	TP53
LPL	-	-	MYD88 L265P mutation
MM	Typical phenotype: Positive for CD138, CD38, CD56, CD117, Negative for CD19, CD45	t(4;14), t(14;16), t(14;20), del(17p), add(1p)	
DLBCL	CD10‡, MUM1‡, BCL6‡, BCL2, MYC	Rearrangements of MYC, BCL6, and BCL2	Cell-of-origin stratification by GEP
HGBCL	TdT (to exclude lymphoblastic lymphoma)	Rearrangements of MYC, BCL6, BCL2; aberrations of 11p§	-

CLL/SLL=Chronic lymphocytic leukemia/Small lymphocytic lymphoma; FL=Follicular lymphoma; MCL=Mantle cell lymphoma; LPL=lymphoplasmacytic lymphoma; MM=Multiple myeloma; DLBCL=Diffuse large B-cell lymphoma; HGBCL=High grade B-cell lymphoma; IgVH=Immunoglobulin heavy chain hypermutation; GEP=Gene expression profiling. *If pediatric-type FL is in differential diagnosis; †Not necessary if cyclin D1 strongly positive by immunohistochemistry; ‡If cell-of-origin stratification by GEP is not available; §If MYC-negative BL is under consideration.

evolves, new tests and panels will be developed, with better prognostic and therapeutic values. Increasingly, pharmaceutical companies and clinician-initiated clinical trials are trying to target a subset of patients based on the results of genetic testing. Whether the results of these clinical trials will rapidly change general clinical practice remains to be seen.

Authors' Contributions: Conception and design: MS, LD and DJ; Acquisition, analysis and interpretation of data: DJ; Drafting the article: DJ; Revising it critically for important intellectual content: MS and LD; Approved final version of the manuscript: MS, LD and DJ.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Narrative Review
Acta Medica Academica 2019;48(1):57-67
DOI: 10.5644/ama2006-124.242

Multiple Myeloma Genomics – A Concise Review

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Received: 15 October 2018
Accepted: 25 January 2019

Key Words: Chromosome Aberration ▪ Clonal Evolution ▪ High-Throughput Nucleotide Sequencing.

The aim of this review is to summarize the current knowledge of genomic information in multiple myeloma. Multiple myeloma is a genetically complex plasma cell neoplasm that evolves from pre-malignant stages following genomic evolution leading to the proliferation of malignant plasma cells and the production of monoclonal immunoglobulin. The outcomes of patients with myeloma have dramatically improved over the past decade with the introduction of novel agents. Nevertheless, the disease is considered incurable and displays considerable heterogeneity in clinical presentation, course and survival. This heterogeneity can often be traced to cytogenetic abnormalities in the malignant clone. Accordingly, a large body of literature has examined the impact of genomics on myeloma and risk stratification based on cytogenetics has been adopted. In this review, we will focus on the cytogenetics of multiple myeloma and the prognostic significance as well as possible predictive implications. We will briefly review the existing methodologies relevant to myeloma but explore in greater depth the more novel molecular tools as applied to this disease. **Conclusion.** The field of genomics in multiple myeloma is rapidly evolving however more translational research is needed to accurately use genomic data as a tool of precision medicine.

Introduction

Multiple myeloma (MM) is a clonal malignancy of terminally differentiated plasma cell representing the second most common hematological malignancy (10% of all hematological malignancies) after non-Hodgkin lymphoma with a globally marked increase in incident cases over that past 25 years (1, 2). This genetically complex disease develops in a multistep process that evolves from pre-malignant disease states such as monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM) following primary genetic events including chromosomal translocations involving the immunoglobulin heavy-chain genes (IGH) and aneuploidy. Subsequently, secondary genetics events including copy number abnormalities, DNA hypomethylation and acquired mutations lead to tumor progression (3, 4). Genetic events detected at the MGUS stage are considered primary events involved in tumor development and events present at the MM stages that were absent in MGUS are thought to be secondary events leading to tumor progression (4, 5).

Since the early 2000s we have observed an accelerated growth of knowledge pertaining to genomic and molecular characterization of MM evolving from metaphase karyotyping and Fluorescent In Situ Hybridization (FISH) to more high-throughput technologies such as gene expression profil-

ing (GEP) and next generation sequencing The advances in genomic techniques has led to a better appreciation of the underlying genetic abnormalities of multiple myeloma not only at the chromosomal level but at the single gene level showing that multiple myeloma is not a single disease but a collection of diseases with a common clinical phenotype (3). Moreover, myeloma displays significant clonal heterogeneity which is also characterized by different clones having different genomic abnormalities which can impact presentation and drug sensitivity (6). The myeloma plasma cells for the most part reside in the bone marrow but they can also be seen in the peripheral blood and other extramedullary sites. However, it is noteworthy that most of the genomic work has focused on the bone marrow compartment although some investigators have examined the biology of extramedullary disease (7) as well as the genomic characterization of the disease by the means of circulating tumor DNA analysis aiming to accommodate the clonal heterogeneity and multifocal nature of the disease (spatial heterogeneity) however most of this studies are limited by small sample sizes and the lack of consensus about a platform capable of identifying existing and new subclones (8, 9). Accordingly, this complex genomic landscape is not yet fully elucidated.

The focus of this review is to summarize the current knowledge of genomic information in MM. For the sake of brevity, we will not focus on already establish methodologies such as metaphase cytogenetics and FISH but rather explore emerging data with new molecular techniques. In addition, we will not review the genomics of other plasma cell dyscrasias including monoclonal gammopathy of undetermined significance, amyloidosis, osteosclerotic myeloma or plasma cell leukemia.

Genetic Alterations

Using metaphase cytogenetics and FISH, the primary genetic abnormalities in MM include translocations and trisomies commonly involving odd-numbered chromosomes which are each noted in about 40% of patients with some overlap (10, 11). The primary translocations (>90%) in MM usually involve the immunoglobulin heavy chain (IgH) gene locus on chromosome 14 (14q32.33) and one of several partner chromosomes including chromosomes 4, 6, 11, 14, and 20 (Table 1). Less common chromosomes partners include chromosome 12 and 8. Primary trisomies typically involve the odd-numbered chromosomes 3, 5, 7, 9, 11, 15, 19 and/or 21 leading to a hyperdiploid karyotype (4, 10-13). In one series, harboring of trisomy 3 or trisomy 5 has significantly better overall survival whereas trisomy 21 was associated with worse outcome (14). Secondary genomic events include chromosomal translocations (MYC), copy number variations and single-nucleotide variants. Monosomy of chromosome 13 and del 13q are the most common secondary cytogenetic abnormalities in MM being detected in 35-40% and 6-10% of patients respectively (12, 15). Early reports suggested that monosomy 13 or deletion 13q was associated with worse outcome. However, more recent data in patients treated with bortezomib and/or lenalidomide, the impact of this abnormality is no longer prognostic (16) and it appears that this historical link between del 13q is a surrogate of its association with high risk cytogenetics lesions. Other abnormalities commonly observed in MM include del 1p, gain 1q, del 17p, and monosomy 17 (3, 10) (Table 1).

The presence of t(4;14) observed in ~15% of patients with MM results in deregulation of the expression of fibroblast growth factor (FGFR3) and multiple myeloma SET (MMSET) and is associated with adverse

Table 1. Genomic Alterations in Multiple Myeloma*

Genomic Event	Genes Involved	Frequency in MM† (%)	Prognostic Value
Primary Abnormalities			
Trisomies	Odd-numbered chromosomes: 3, 5, 7, 9, 11, 15, 19 or 21	~45	Favorable‡
Translocations	t(11;14): CCND1	15	Neutral
	t(4;14): FGR3/MMSET	15	Adverse
	t(6;14): CCND3	2	Neutral
	t(14;16): MAF	5	Adverse
	t(14;20): MAFB	1	Adverse
Secondary Abnormalities			
Chromosome gains	1q: MCL1, CKS1B, ANP32E or BCL9	40	Adverse
	8q: MYC	15	Neutral
	11q: CCND1	15	Neutral
Chromosome losses	1p: CDKN2C or FAM46C	30	Adverse
	12p: CD27	15	Adverse
	14q: TRAF3	10	Not determined
	16q: CYDL or WWOX	30	Neutral
	17p:TP53	10	Adverse
	13q: RB1, DIS3, mir15a or mir16.1	40	Neutral
Translocations	Affecting MYC: t(8;14), t(8;11)	15	Adverse

*Adapted from Manier S, et al. and Kumar SK (4, 12); †Multiple myeloma; ‡Trisomy 21 may be associated with worse outcome.

prognosis with poor PFS and OS in different clinical settings (4, 17, 18). It's worth to mention that despite the poor prognosis associated with t(4;14) it appears that early treatment of such patients with a proteasome inhibitor may result in survival improvement (19). In terms of prognosis, the most important chromosome arm alteration given its associated aggressive clinical course, poor overall survival and development of extramedullary disease is the monoallelic deletion of 17p13 (locus of tumor suppressor gene *p53*) (20, 21). Additional chromosomal changes modulate the outcome of patients with t(4;14) and del(17p) which accounts for the degree of heterogeneity observed in the survival of these high risk patients. OS is impacted in patients harboring t(4;14) when associated with del(13q14), del(1p32) and chromosomal structural changes (>30). Del(1p32) has been also associated with worsening prognosis in patient with del(17p) (22). Most recently Walker et al (23) in a genome-wide analysis of the largest set (1273 NDMM patients) of molecular and clinical data established to date from NDMM, as part of the Myeloma Genome Project, have identified genetic drivers that adversely impact prognosis. Multivariate analysis identified biallelic inactivation of TP53 and gain or amplification of 1q as being associated with poor PFS (15.4 months) and OS (20.7 months).

Importantly, genetic alterations are further modulated by clinical parameters such as the international staging system (ISS) and serum lactate dehydrogenase (LDH) to impact prognosis. Accordingly, patients with t(4;14) and ISS1 and normal LDH are expected to fare better than patients with the t(4;14) and ISS3 for example. This is the basis of the revised ISS (17). Based on the revised

ISS, cytogenetic abnormalities considered to be associated with high risk disease include deletion 17p, t(4;14) and t(14;16).

While there is overall agreement about the prognostic impact of cytogenetic abnormalities in myeloma used to define high risk, it remains unclear whether these abnormalities represent predictive biomarkers. Of the abnormalities detected by FISH, only t(11;14) has the potential to be used as a predictive marker. As such, myeloma patients harboring the t(11;14) have a single agent response rate of nearly 40% with the bcl2 inhibitor venetoclax, whereas patients without this translocations are unlikely to respond (24-26).

Gene Expression Profiling

High-throughput genomic tools such as gene expression profiling (GEP) have been extensively investigated with the goal of predicting patient's outcomes. An initial attempt of molecular classification of MM using GEP identified 5 recurrent translocations, specific trisomies, and expression of cyclin D genes conforming 8 subgroups (11q13, 6p21, 4p16, MAF, D1, D1+D2, and D2) based on cyclin D gene expression and various 14q32 recurrent translocations (27). On the basis of gene expression profiling studies, several subgroups of multiple myeloma have been identified, which further reflects the genetic heterogeneity of the disease.

In the past years, plasma cell gene-expression signatures designed to specifically identify patients with poor outcome have been developed by several groups including the University of Arkansas for Medical Science (UAMS), Intergroupe Francophone du Myélome (IFM), Skyline 92-HOVON and others (28-30). The UAMS group identified a 70-gene signature by GEP to molecularly define high-risk disease under the treatment platform of Total Therapy 2 (TT2) suggesting that altered transcriptional regulation of genes (nearly half of which map to chromosome 1) may contribute to disease progression. Subsequent multivariate analysis revealed that a 17-gene subset could predict outcomes as well as the 70-gene model (28). Logistic regression analysis of the 70-gene score in relation to event free and overall survival data from UAMS TT2/3 series was performed and published in 2016 showing that the 70-gene prognostic risk score is continuously associated with increased risk of 5-year relapse and death (31). Importantly, these GEP signatures were externally validated in datasets including patients treated on various clinical trials and showing continued prognostic significance (32).

An attempt to combine biological and clinical parameters as a prognostic tool was published by Kuiper R, et al (33). Using clinical data of 4750 patients (from the HO65/GMMG-HD4, UAMS-TT2, UAMS-TT3, MRC-IX, assessment of proteasome inhibition for extending remissions (APEX), and IFM trials) the value of 20 existing risk markers was evaluated. Other than FISH and ISS, gene expression classifiers were used (EMC92, UAMS17, UAMS70, UAMS80, IFM15, MRC-IX6, HM19 and GPI50) showing that combining GEP and ISS data is useful to identify low and high risk MM. Overall while several GEP-based signatures have been developed, there is no consensus on which platform and signature is best and most clinical risk stratification still relies on standard karyotypic analysis and FISH (32). The UAMS has also developed an 80 gene signature (GEP80) by performing GEP analysis in a training set of 142 UAMS-TT3A patients which was subsequently validated in 128 patients in the UAMS-TT3B. The GEP80 signature showed insights into novel mechanisms of resistance to bortezomib with the potential of helping predict response to the agent (34). In addition, while those GEP signature may be prognostic it is unclear if they are predictive and affect the choice of therapy.

Molecular Information from DNA Sequencing

Next-generation sequencing has shown a lack of a universal driver mutation in multiple myeloma, and the presence of coexistent subclones of malignant plasma cells with some degree of overlap (3). Investigators have reported on results of various DNA-based high-throughput technologies better known as next generation sequencing (NGS) including whole-genome sequencing (WGS) and whole-exome sequencing (WES) to distinguish polymorphisms and characterize the biology of MM. In all studies (summarized in Table 2), a heteroge-

Table 2. Landmark Studies in Multiple Myeloma Genomics

Author	Technique(s)	Mutated Gene	Potentially Actionable
Walker et al. (19)	WES*	KRAS	MEK inhibitor
		NRAS	MEK inhibitor
		TP53	PRIMA-1 analog
		DIS3	-
		FAM46C	-
		TRAF3	-
		BRAF	BRAF kinase inhibitor
		RB1	-
		CYLD	-
		IRF4	-
		MAX	-
		HIST1H1E	-
		EGR1	-
		LTB	-
		FGFR3	Masitinib
Lohr et al. (18)	WES*	KRAS	MEK inhibitor
	WGS†	NRAS	MEK inhibitor
		TP53	PRIMA-1 analog
		DIS3	-
		FAM46C	-
		BRAF	BRAF kinase inhibitor
		TRAF3	-
		RB1	-
		CYLD	-
		PRDM1	-
		ACTG1	-
Chapman et al. (17)	WES*	NRAS	MEK inhibitor
	WGS†	KRAS	MEK inhibitor
		FAM46C	-
		DIS3	-
		TP53	PRIMA-1 analog
		CCND1	CDK inhibitor
		PNRC1	-
		ALOX12B	-
		HLA-A	-
		MAGED1	-

*Whole exome sequencing; †Whole genome sequencing.

neous mutational landscape was observed and while clonal heterogeneity is an established feature in MM, the subclonal evolution associated with disease progression has not been well explored.

Chapman et al. (35) reported the first results of next generation sequencing in samples of patients with MM. They studied 38 MM patients (WGS in 22 pts, WES in 15 pts and 1 patient sample analyzed by both approaches). This study identified 10 statistically significant protein-coding mutations in MM including NRAS, KRAS, FAM46C, DIS3, TP53, CCND1, PNRC1, ALOX12B, HLA-A and MAGED1 but at a low frequency. One of the thirty eight patients harbored a BRAF kinase mutation leading to the genotyping of an additional 161 MM patients and found BRAF mutations in 7 patients (4%). The gene set mutation spectrum included genes involved the nuclear factor Kappa B (NF-κB) pathway, histone methylation, protein translation, and homeostasis.

In 2014, a study by Lohr et al. (36) was designed to address some of the limitations of the Chapman et al study (35) that was only powered to detect commonly mutated genes and didn't examine copy number alterations or clonal heterogeneity due to the small sample size and modest sequence coverage. Parallel sequencing of paired tumor/normal samples from 203 MM patients showed that eleven genes were recurrently mutated including KRAS, NRAS, TP53, FAM46C, DIS3, BRAF, TRAF3, PRDM1, CYLD, RB1 and ACTG1. Among the 11 significantly mutated genes were five genes (KRAS, NRAS, FAM46C, DIS3 and TP53) previously identified by Chapman et al. (35). The previously tested gene set hypotheses including the mutations of genes in the NF-kB pathway, histone-modifying enzymes and the coagulation cascade retained statistical significance across all 203 patients ($p<0.05$) when tested as individual hypotheses.

The largest comprehensive molecular analysis was reported by Walker et al. (20) that performed WES in 463 patients enrolled in a phase III trial (National Cancer Research Institute Myeloma XI Trial) identifying 15 significantly mutated genes: IRF4, KRAS, NRAS, MAX, HIST1H1E, RB1, EGR1, TP53, TRAF3, FAM46C, DIS3, BRAF, LTB, CYLD, and FGFR3. In this cohort the RAS/MAPK pathway was the most frequently mutated pathway (KRAS: 21.2%, NRAS: 19.4%, BRAF: 6.7%) making up a total of 43.2%. Moreover, mutational activation of the NF-kB pathway genes were identified in 17% of cases. In this study FGFR3 was found to be mutated solely in the t(4;14) group and the transcriptional regulator EGR1 mutation in the hyperdiploid samples.

Interestingly, the only recurrent mutations that significantly affect survival outcomes are those observed in TP53. The coexistence of del 17p, and *TP53* mutations (25-40% of patients harboring del 17p) appears to cumulatively increase the risk of poor outcomes highlighting the hierarchical interaction between abnormalities of different types (4, 12, 21). Most chromosome 17 deletions are hemizygous and are observed in around 10% of patients with newly diagnosed MM however, the frequency increases up to 80% in advanced disease. The TP53 gene is located within a minimally deleted region on 17p13 and functions as a transcriptional regulator influencing cell-cycle arrest, DNA repair, and apoptosis in response to DNA damage (4, 21, 37).

These sequencing studies have examined the mutational landscape in MM showing that despite genetic heterogeneity the most frequently mutated genes belong to a limited number of pathways (Table 3) as well as the lack of a universal driver mutation and the presence of coexistent subclones. More importantly, in the current era of personalized medicine when physicians aim to tailor the appropriate therapy to each patient on the

Table 3. Frequently Mutated Genes in Multiple Myeloma*

Gene	Frequency (%)	Function
KRAS	20-25	MAPK signaling pathway (cell survival and growth)
NRAS	23-25	MAPK signaling pathway (cell survival and growth)
TP53	8-15	Tumor suppressor involved in response to DNA damage and apoptosis
DIS3	11	Exosome endoribonuclease
FAM46C	~11	Unclear
BRAF	6-15	MAPK signaling pathway (cell survival and growth)
TRAF3	3-6	NF-κB signaling pathway (cell survival and proliferation)
ROBO1	2-5	Transmembrane receptor involved cell growth though crosstalk with MET signaling
CYLD	2-3	NF-κB signaling pathway (cell survival and proliferation)
EGR1	4-6	Transcription factor
SP140	5-7	Antigen-response mechanisms in mature B cells
FAT3	4-7	Cadherin superfamily member (cell adhesion)
CCND1	3	Cell cycle progression

*From Kumar SK (12).

basis of genomic data, the identification of driver mutations in MM is promising especially for those patients harboring actionable mutations/pathways. To date however, the only evidence of precision medicine is related to BRAF mutation found in about 4% of patients and case reports had noted response with the BRAF inhibitor, vemurafenib (38, 39).

Clonal Evolution and Subclonality

Despite being characterized by the secretion of a unique monoclonal protein in the majority of patients, a degree of heterogeneity is observed at the molecular level, which suggests a Darwinian evolution of MM (35, 36, 6, 40). This heterogeneity is observed as soon as the monoclonal gammopathy of undetermined significance stage, meaning that immortalized plasma cells diverge very early in their evolution (40, 41). Keats et al. have examined the genomic changes over time in 28 patients with multiple myeloma (42). They noted that about a third of the patients (especially low risk hyperdiploid patients) had stable genome over time. The rest of the patients had clonal changes characterized by either clonal heterogeneity at diagnosis or linear evolution. Of interest, patients with high risk cytogenetics had more genomic changes and only one high risk patient had genomic stability. The same authors studied the clonal dynamics of 1 patient with t(4;14) at 7 time points and noted the clonal dynamics in the face of treatment selection.

Next generation sequencing has shown a lack of universal driver mutation, presence of coexistent subclones and oligoclonality in MM which leads to various type of evolution of the disease over time (3, 40). Clonal evolution in multiple myeloma before and after therapy can follow several patterns including branching clonal development, subclonal shift, linear clonal pattern and clonal stability (4). In patients with branching clonal evolution (estimated 30% of patients), one or more subclones appear at a later time point, whereas others subclones have disappeared. In patients with a subclonal shift, the subclones at diagnosis are also present at relapse, but the frequency of the subclones has changed throughout the disease course and one clone has become more dominant than another. In patients with a linear clonal

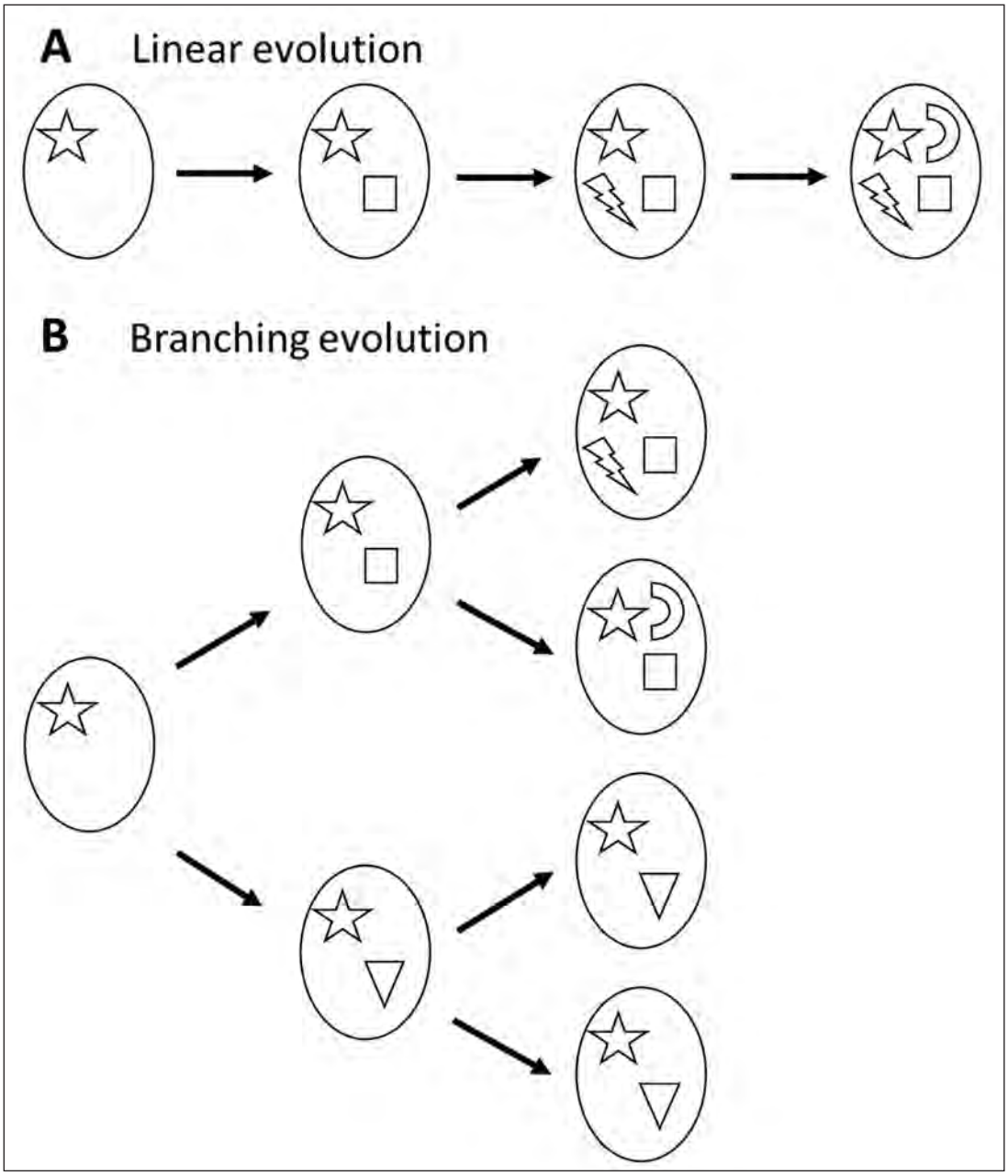

Figure 1. Myeloma is characterized by two types of subclonal evolution (A) a linear one with acquisition of novel mutations over time in the clone and (B) a branching evolution where subclones diverge with subclonal acquisition of novel mutations. From Robiou du Pont S (40).

evolution (~40% of patients), a new subclone has emerged between diagnosis and relapse, which was absent at diagnosis (40). In patients with clonal stability (approximately 30% of patients) the same composition of clonal and subclonal heterogeneity is found before and after treatment suggesting that all subclones are affected by therapy and will repopulate equivalently (4) (Figure 1). As noted by Keats et al. this may be more

commonly noted in patients with low risk disease.

The therapeutic implications of clonal heterogeneity and clonal evolution are key areas in multiple myeloma and may have therapeutic implications as previously noted by Fakhri et al. (41) including subclone drug resistant allowing future dominance, targeted therapy of the main clone to maximize effectiveness, combination therapy to overcome branching clonal evolution and therapy recycling based on reemergence of clone(s). Targeted agents will more than likely have to be combined with standard-of-care agents, and such regimens might need to be adjusted over time based on the evolving clonal architecture, while maintaining the broadly active backbone combinations (12).

Summary

The availability of more affordable and high throughput genomic tools has led to an improved understanding of the genomic landscape of multiple myeloma. While the complexity and heterogeneity of the disease continue to make personalized medicine a challenge for myeloma patients, it is our opinion that this genomic revolution will undoubtedly lead to precision medicine in myeloma in the near future. Importantly, in addition to an improved understanding of tumor genomics, an in-depth assessment of the tumor microenvironment (including the immune microenvironment) and the host are needed to more completely characterize the disease, identify new targets and develop better therapies for myeloma patients.

Authors' Contributions: Conception and design: OC and RB; Acquisition, analysis, and interpretation of data: OC and RB; Drafting the article: OC; Revising it critically for important intellectual content: RB; Approved final version of the manuscript: OC and RB.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Narrative Review
Acta Medica Academica 2019;48(1):68-77
DOI: 10.5644/ama2006-124.243

Evolving Role of Genomics in Genitourinary Neoplasms

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Received: 4 September 2018
Accepted: 8 February 2019

Key Words: Biomarkers ▪ Risk ▪ Prostate Cancer ▪ Renal Cell Carcinoma ▪ Urothelial Carcinoma.

The aim of this article is to review the current role of genomic testing in the risk, prognosis, and treatment of genitourinary malignancies. The authors selected guidelines, publications, and abstracts relevant to the current and emerging role of genomics in genitourinary cancers. The risk of developing genitourinary cancer can be stratified based on genomic data. Prostate cancer has the strongest degree of heritability, with *BRCA1/2* and *HOXB13* mutations playing a role in familial disease. Genomic data is on the verge of informing treatment decisions across genitourinary cancers. mCRPC has diverse genomic alterations that represent potential therapeutic targets, including alterations in the AR pathway, DNA damage and repair pathways, cell cycle pathways, PI3K pathway, and Wnt signaling. Genomic alterations in clear cell renal cell carcinoma can inform prognosis and mutations in mTOR pathways predict response to mTOR inhibitors. Urothelial carcinoma can be classified into different subtypes based on gene expression profiling, which provides prognostic information and predicts response to chemotherapy and immunotherapy. Specific mutations have been identified that predict response to therapy including *ERCC2* mutations and cisplatin, DNA damage and repair mutations and checkpoint inhibitors, and *FGFR3* mutations and FGFR tyrosine kinase inhibitors such as erdafitinib. **Conclusion.** Genitourinary malignancies have not felt the impact of genomic data as greatly as other cancer types. The majority of benefit lies in identifying patients at high risk of genitourinary cancer. Fortunately, breakthroughs are on the horizon that will result in a greater incorporation of genomic information into treatment decisions for patients with genitourinary cancer.

Introduction

Genomic testing has altered the practice of oncology, informing multiple facets of the care of patients with a variety of neoplasms. The myriad of publications from the Human Genome project has led to a paradigm shift and changes in our ability to correlate genetic information with biologic behavior. Rapid developments in technology resulting in more cost effective and faster sequencing of genetic information, led to the swift, albeit still evolving integration of this information into clinical practice. Genetic information may help identify populations at a high risk of genitourinary malignancies and can increasingly inform treatment decisions.

In this review, we aim to provide an overview of the role of genomic testing in the care of patients with genitourinary malignancies, focusing on prostate, kidney, and urothelial cancers, and touch on some of the emerging applications of genomics that may evolve into standards of care.

Prostate Cancer

Genomics Inform Risk

Prostate cancer represents one of the most heritable forms of malignancy. The Nordic Twin Study of Cancer showed an increase in the relative risk of developing prostate cancer of up to 5.69 times and 1.7 times for monozygotic and dizygotic twins respectively (1, 2). Men with a positive family history of prostate cancer in a first degree relative are at higher risk of prostate cancer, which further increases if more than one first degree relative is affected (3). Mutations in the genes homeobox B13 (*HOXB13*) and breast cancer susceptibility types 1 and 2 (*BRCA1/2*) have been associated with familial clusters of prostate cancer. *HOXB13* is a tumor suppressor gene that encodes for a DNA-binding domain. The mutation G48E in *HOXB13* was associated with familial prostate cancer in 2012 (4) and has subsequently been shown to confer an approximately 4.5 fold increased risk in the development of prostate cancer for carriers of the mutation (5). *BRCA1/2* encode for DNA repair machinery and have been implicated in the hereditary breast and ovarian cancer (HBOC) syndrome. Men carrying mutations in *BRCA1* or *BRCA2* have an increased risk of developing prostate cancer by up to 3.8 fold and 8.6 fold respectively (6). In addition, *BRCA2* mutations have been associated with an earlier onset of prostate cancer (7, 8) and both *BRCA1* and *BRCA2* mutations with more aggressive clinicopathologic disease (9, 10). The DNA mismatch repair proteins encoded by the genes *MSH2*, *MLH1*, and *MSH6*, have also been associated with an increased risk of prostate cancer in the Danish HNPCC registry (11). However, these genes account for a small proportion of the heritable risk. Genome wide association studies have shown nearly 170 single nucleotide polymorphisms (SNPs) that are associated with an increased risk of prostate cancer (12). No individual SNP variant carries substantial risk on its own to be of clinical utility.

Men with a known family history of Lynch syndrome or mutation in *HOXB13* or *BRCA1/2* should be informed of the risks associated with these mutations and the utility of germline testing if they do not have prostate cancer. This may provide information relevant to the risk and benefit counseling regarding the utility of prostate specific antigen (PSA) screening. A family history suggestive of the HBOC syndrome should also prompt as discussion regarding the utility of germline testing for the presence of *BRCA1/2*, preferably with a genetic counselor when available (13).

Clinical Decision Making

Genomic testing can help inform prognosis and treatment in patients with an established diagnosis of prostate cancer. The utility of genomic testing has been primarily shown in the metastatic castrate-resistant prostate cancer (mCRPC). mCRPC harbors a multitude of genomic variations. Mutations affecting the androgen receptor pathway, DNA mismatch repair machinery, cell cycle pathway, phosphatidylinositol-3-kinase (PI3K) pathway, and Wnt signaling have been identified (14). Therapeutic agents targeting each of these pathways have been developed or are in development and several are being tested in prostate cancer.

Mutations in the androgen receptor (AR) pathway are present frequently in prostate cancer, occurring in over 70% of mCRPC cases in a series of 150 patients (14), confirming the importance of the AR pathway in the pathogenesis of prostate cancer. This dependence on AR signaling, even in the advanced castrate-resistant state, establishes a biologic rationale for the efficacy of next generation anti-androgens such as abiraterone acetate and enzalutamide. Ge-

nomic alterations in the AR pathway can lead to resistance to these agents. AR-V7 is a splice variant of the androgen receptor that leads to resistance to abiraterone and enzalutamide and develops in nearly a third of men treated with these agents (15). Recently presented data from the prospective, multicenter PROPHECY trial showed that patients with AR-V7 positivity determined by circulating tumor cells were unlikely to respond to abiraterone or enzalutamide and had shortened progression free survival and overall survival (16). AR-V7 does not confer resistance to non-AR targeted therapies and there is evidence that treatment with docetaxel may eliminate AR-V7 clones (17). Whether this can re-sensitize a patient to AR-targeted therapy remains in question.

Mutations in DNA-repair genes have been identified as an important and potentially under-identified contributor to prostate cancer. In mCRPC, mutations in DNA repair may be present in up to 22.7% of patients (14). More importantly, germline mutations in these genes are fairly common and under-identified. In a population of 692 men with metastatic prostate cancer, germline mutations in the DNA-repair genes *BRCA2, ATM, CHEK2, BRCA1, RAD51D,* and *PALB2* were identified in 11.8% of patients (18). Current guidelines recommend testing men for the presence of *BRCA1/2* mutations in men with prostate cancer with a Gleason score of 7 or greater and one of the following: close relative with ovarian or breast cancer diagnosed at age 50 or younger; or at least two relatives with breast, ovarian, or prostate cancer (Gleason 7 or greater) at any age (13). However, recent data suggests that as many as 40% of men with prostate cancer and germline mutations do not meet criteria for testing under the current guidelines (19), which likely contributes to the under-recognition of these germline abnormalities. In addition, guidelines only focus on the testing for *BRCA1/2* and do not include recommendation regarding broad testing for the other potential germline mutations. Several classes of therapeutics have potential activity in patients with defective DNA repair genes, including immunotherapy, PARP inhibitors, and platinum chemotherapy. Defects leading to microsatellite instability represent a prostate cancer population that has a much greater chance of response to immune checkpoint inhibitors but are found in <2.5% of patients (20). Patients with Lynch syndrome associated prostate cancers have a higher likelihood of microsatellite instability (11). Platinum-based chemotherapy has also been associated with improved responses in patients with DNA repair defects in the *BRCA2* gene (21). The role of PARP inhibitors in the treatment of prostate cancer is an area of active research. Early data showed that in patients with known defects in DNA repair machinery who had progressed on standard therapies, olaparib elicited an overall response rate of 33% with response defined as either PSA decline of 50% or greater, radiographic response by RECIST 1.1, or a reduction in volume of circulating tumor cells (22). Multiple phase III trials investigating the role of PARP inhibitors in mCRPC are ongoing, both in selected and unselected patient populations, as well as single agent and combination therapy.

PI3K pathway mutations may be present in almost 50% of mCRPC (14). The mutated genes in this pathway included *PTEN, PIK-3CA, AKT1,* and *PIK3CB*. The use of PI3K inhibitors has been studied in unselected patients with mCRPC. Buparlisip, a pan-class I PI3K inhibitor, did not yield PSA responses in men with mCRPC who had progressed on docetaxel and/or enzalutamide in a single arm phase II trial (23). In a small phase I trial, a novel inhibitor of PI3Kβ showed some evidence of activity in mCRPC patients whose tumors harbored *PI3KCB* abnormalities (24). This is an emerging area of research.

Summary

Genomic alterations have become increasingly important to our understanding of the biology of prostate cancer (Table 1). Genetics clearly play a role in an individual's risk of developing prostate cancer, with connections between mutations in *HOXB13* and *BRCA1/2* to familial clusters of prostate cancer. For men who have a diagnosis of mCRPC, the presence of mutations in DNA repair genes is under-recognized and is clinical useful. Standard therapies such as platinum chemotherapy and immunotherapy for MSI-high tumors can be considered in patients harboring DNA repair abnormalities. Additional mutations are on the cusp of demonstrating clinical utility and many future therapies will likely be dependent on the presence of genomic biomarkers. Ongoing research is needed to further define optimal use of germ-line and somatic testing of patients with advanced prostate cancer.

Kidney Cancer

Genetic Risk

The Nordic Twin Study of Cancer suggested that kidney cancer showed evidence of heritability, though the familial risk estimation was quite low in dizygotic twins (1). Several genes have been implicated in inherited syndromes that are associated with an increased risk of developing kidney cancer (Table 2). These inherited syndromes represent a small proportion of newly diagnosed renal cancers, but have informed our understanding of the biology of kidney cancer. Unfortunately, no established screening paradigm exists for kidney cancer. Patients with a known inherited syndrome are frequently recommended, based on expert opinion, to undergo annual cross sectional imaging for surveillance with either computed tomography or magnetic resonance imaging of the abdomen.

Table 1. Prostate Cancer Summary

Pathway/Gene	Biologic Role	Clinical Implications
HOXB13	Tumor suppressor gene encoding a DNA-binding domain	Germline mutations confers 4.5 times increased risk of developing prostate cancer; Informs shared decision making for prostate cancer screening; genetic counseling when germline mutation detected
BRCA1	Tumor suppressor gene encoding DNA repair machinery	Germline mutations confers 3.8 times increased risk of developing prostate cancer; Informs shared decision making for prostate cancer screening; genetic counseling when germline mutation detected; more aggressive clinicopathologic disease; potential for treatment with PARP inhibitors and immunotherapy
BRCA2	Tumor suppressor gene encoding DNA repair machinery	Germline mutations confers 8.6 times increased risk of developing prostate cancer; Informs shared decision making for prostate cancer screening; genetic counseling when germline mutation detected; earlier onset and more aggressive clinicopathologic disease; potential for treatment with PARP inhibitors and immunotherapy
AR-V7 splice variant	Confers resistance to next generation anti-androgen therapy	Predicts lack of response to abiraterone/enzalutamide
DNA Damage Response and Repair Pathway (*BRCA2, ATM, CHEK2, BRCA1, RAD51D, PALB2, MSH2, MLH1, MSH6)*	Encode for cellular machinery responsible for the recognition and repair of DNA damage	DDR-deficient tumors predict response to immunotherapy with checkpoint inhibitors of the PD1 pathway; may predict response to PARP inhibitors; germline mutations are common and patients should receive genetic counseling when detected
PI3K Pathway *(PTEN, PIK3CA, AKT1, PIK3CB)*	Intracellular signaling pathway that regulates cell cycle	May predict benefit to PI3K inhibitors

Table 2. Syndromes Associated with Increased Risk of Renal Cell Carcinoma

Syndrome	Mutation	Clinical Features
Clear Cell Carcinoma		
Von Hippel Lindau	VHL	CNS hemangioblastomas, pheochromocytoma, pancreatic neuroendocrine tumor
PTEN Hamartoma Syndrome	PTEN	Lipomas, fibromas, acral keratosis, GI polyps; increased risk of cancers of the breast, thyroid, and endometrium; papillary renal carcinoma also seen
Familial Clear Cell with Chromosome 3 Translocation	Translocation chromosome 3	Clear cell kidney cancer
BAP1 Mutant Disease	BAP1	Melonoma, mesothelioma, epithelioid atypical Spitz tumors
Papillary Carcinoma		
Hereditary Leiomyomatosis	FH	Cutaneous and uterine leiomyomata; Papillary renal carcinoma type 2
Hereditary Papillary Renal Cancer	MET	Papillary renal carcinoma type 1
Other Histology		
Tuberous Sclerosis Complex	*TSC1, TSC2*	Facial angiofibromas, subependymal giant cell astrocytoma, subependymal nodules, CNS cortical tubules; renal angiomyolipoma
SDH-associated Renal Cancer	*SDHB, SDHC, SDHD*	Paraganglioma, pheochromocytoma; renal clear cell, chromophobe, or oncocytoma
Lynch Syndrome	*MLH1, MSH2*	Familial history of cancer, primarily colon endometrial, ovarian, small bowel, upper urinary tract urothelial carcinoma, pancreatic; clear cell and papillary renal carcinoma described (25)

Clinical Decision Making

Genomic testing currently has no established role in the prognosis and treatment of kidney cancer, though some genomic factors are emerging in clear cell carcinoma of the kidney that may contribute to clinical decision making in the future. Clear cell renal cell carcinoma (ccRCC) is characterized by loss or inactivation of the *VHL* gene (26), located on the short arm of chromosome 3. The development of haplo insufficiency at 3p appears to be an early event in the genetic evolution of ccRCC (27). A second hit to *VHL* typically occurs later in life via mutation or methylation events that result in a decrease or loss of expression of VHL (28). This results in downstream signaling changes promoting tumorigenesis. Given the early and nearly ubiquitous presence of *VHL* abnormalities in ccRCC, other genomic markers have been explored as potential prognostic and predictive markers.

Mutations in *PBRM1* and *BAP1*, which are also located on 3p, have been shown to have prognostic utility (29). Expression of PBRM1 and BAP1 from resected ccRCC specimens stratified outcomes into four distinct groups. PBRM1+/BAP1+ specimens had the longest relapse free and disease specific survival, while PBRM-/BAP1- tumors had the worst outcomes. These genetic findings correlated with traditional pathologic characteristics that are associated with poor outcomes, such as tumor size, TNM stage, nuclear grade, and tumor necrosis. While this very nicely ties the genetic abnormalities to the biology and clinical features of the disease, it does not provide a superior methodology for estimating risk.

The *MTOR* pathway has been a target of treatment in ccRCC, with both temsirolimus and everolimus approved. Mutations in *TSC1, TSC2,* and *MTOR* have been demonstrated in ccRCC, with *TSC1* muta-

tions associated with the development of higher grade tumors (30). These mutations have also been associated with response to mTOR inhibitors (31), which have been approved for use in poor risk patients. Mutations in *PBRM1* have been associated with response to the immune checkpoint inhibitors, though the strength and mechanism of this interaction is unclear (32).

Summary

While our understanding of the genomic factors underlying the clinical behavior of ccRCC has made tremendous progress in the last decade, genomic testing in kidney cancer has little role in the treatment of patients outside the context of clinical trials. A deeper understanding of the genomic alterations driving biological behavior is emerging. In patients with a strong family history of syndromes associated with an increased risk of kidney cancer or clinical characteristics of these syndromes, consideration of germline testing should be given and discussed with the patient.

Urothelial Carcinoma

Genetic Risk

Bladder cancer has evidence of heritable risk factors, though exact genomic mechanisms of this risk are less well understood. Urothelial carcinoma is associated with hereditary non-polyposis colorectal cancer, also known as the Lynch syndrome. Defects in *MSH2* in particular were associated with an increased risk of developing upper tract urothelial carcinoma in a Danish cohort of families with Lynch syndrome (33). Renal pelvis and ureteral urothelial carcinoma is included in the Amsterdam II criteria as a Lynch syndrome-associated cancer. Patients who present with upper tract disease should undergo detailed family history to explore for evidence of heritable mismatch repair defects and have germline testing performed as appropriate (34).

Clinical Decision Making

Molecular subtyping of urothelial cancer utilizing gene expression profiling techniques has emerged as a potential prognostic and predictive tool. Four subtypes emerged from the Cancer Genome Atlas project via a hierarchical clustering analysis, originally described as clusters I, II, III, and IV (35). Clusters I and II refer to luminal subtypes and clusters III and IV to basal subtypes. Other groups have divided the subtypes differently, but similarities exist between the various categories. Basal subtypes have been shown to be more aggressive and are associated with worse survival in chemotherapy-naïve urothelial carcinoma (36). Interestingly, basal subtypes have also been shown to have the best outcomes after administration of neoadjuvant chemotherapy (37). Molecular subtyping may also provide predictive information regarding response to immunotherapy. Utilizing the Cancer Genome Atlas (TCGA) subtyping classification, cluster III subtypes had the highest response rate compared with other subtypes at 30% following treatment with nivolumab in the second line setting (38). Cluster III subtypes were also associated with the strongest interferon-γ expression signature, which also correlated with a higher likelihood of response (38). The same TCGA subtyping was utilized in the corresponding clinical trial for atezolizumab following platinum based chemotherapy. Cluster II subtypes showed the best response rate to therapy at 34%. However, PD-L1 expression levels and CD8 T cell gene expression markers were higher in the cluster III subtype (39). These data come from smaller phase II trials and will be explored in larger phase III trials.

In addition to broad molecular subtyping, specific genetic mutations have been identified that may impact clinical decision making. Somatic mutations in the nucleotide excision repair gene *ERCC2* are present in 7-12% of

urothelial carcinoma (35, 40). Mutations in *ERCC2* are associated with a distinct molecular subtype (41) that overlaps with luminal subtypes. The presence of *ERCC2* mutations was enriched in patients who responded to cisplatin chemotherapy (40) and later validated as a biomarker of platinum sensitivity (42). Mutations in other DNA damage response and repair (DDR) machinery have also been identified in urothelial carcinoma as a biomarker of response to immunotherapy. In a series of 60 patients, 46.7% had an alteration in DDR genes with 25% having deleterious alterations (43). Deleterious DDR mutations were found in *ATM, POLE, BRCA2, ERCC2, FANCA*, and *MSH6*. The presence of DDR mutations were associated with higher response rates, longer progressive free survival, and longer overall survival with immunotherapy. Mutations in *FGFR3* have been identified as an intriguing target for therapy. Cluster I subtypes are enriched with *FGFR3* mutations (35) and are generally associated with immunologically cold tumors that do not respond to checkpoint inhibitors (38, 39). Small molecule tyrosine kinase inhibitors of FGFR3 are currently in development and showing promising results. The FGFR3 TKI erdafitinib was recently reported in a phase II trial to have an overall response rate of 40% (44). Given that this is a population that has poorer responses to immunotherapy and minimal therapeutic options, erdafitinib represents a potential breakthrough for patients with urothelial cancer.

Summary

Urothelial carcinoma has some association with the Lynch syndrome, especially when presenting with upper urinary tract disease, and a detailed family history should be obtained in these patients. Referral to genetic counselor may be warranted if history suggests the presence of a heritable syndrome. Molecular profiling of urothelial carcinoma has revealed distinct subgroups of disease with differing clinical behavior. There are early signs that molecular subtyping may play a role in prognosis and treatment selection (Table 3), but this is currently not applicable outside the clinical trial setting. *ERCC2* mutations are useful in predicting response to platinum chemotherapy, are not specific enough to warrant omitting administration of cisplatin to patients who are oth-

Table 3. Urothelial Cancer Summary

TCGA Molecular subtyping		
Pathway/Gene	Biologic Role	Clinical Implications
Cluster I	Luminal subtype	Less aggressive, less benefit from neoadjuvant chemotherapy
Cluster II	Luminal subtype	Less aggressive, less benefit from neoadjuvant chemotherapy; higher response rates to atezolizumab
Cluster III	Basal subtype	More aggressive, better response to neoadjuvant chemotherapy; higher response rates to nivolumab
Cluster IV	Basal subtype	More aggressive, better response to neoadjuvant chemotherapy
DNA Damage Response and Repair Pathway *(ATM, POLE, BRCA2, ERCC2, FANCA, MSH6)*	Encode for cellular machinery responsible for the recognition and repair of DNA damage	Higher response rates to checkpoint inhibitors
ERCC2	Nucleotide excision repair	Predicts higher response rates to cisplatin
FGFR3	Fibroblast growth factor receptor	Predicts response to FGFR3 tyrosine kinase receptor inhibitors (erdafitinib)

TCGA=The Cancer Genome Atlas.

erwise eligible. This is also true of defects in DDR genes as they relate to administration of checkpoint inhibitors. *FGFR3* alterations will likely become a key piece of information, as targeted therapy is likely to be approved for patients with these alterations in the near future pending completion of phase III studies.

Conclusions

Clinical breakthroughs resulting from a deeper understanding of the genetic influences in cancer have been a leap forward in the field of oncology. Genitourinary malignancies have not felt the impact as significantly. Breast and thoracic malignancies are prime examples of the potential power of genomic data, as genomic data influences decisions on treatment in a large proportion of patients. Genomic data is currently most relevant in identifying patients carrying high risk germline alterations for genitourinary cancers. The role of genomic testing to influence treatment decisions is currently limited. Fortunately, the field is changing rapidly and breakthroughs driven by genomic information are on the horizon.

Authors' Contributions: Conception and design: MED and RD; Acquisition, analysis and interpretation of data: MED; Drafting the article: MED; Revising it critically for important intellectual content: MED and RD; Approved final version of the manuscript: MED and RD.

Conflict of Interest: Dr. Devitt has received compensation as advisor/speaker for Bayer Pharmaceuticals. Dr. Dreicer has received compensation as adviser for Incyte, Astra Zeneca, Seattle Genetics, EMD Serono, Pfizer.

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Review Article

Narrative Review
Acta Medica Academica 2019;48(1):78-83
DOI: 10.5644/ama2006-124.244

Lung Cancer Genomics

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Received: 26 December 2018
Accepted: 28 April 2019

Key Words: Genomics ▪ Lung Cancer ▪ Targeted Therapy ▪ Immunotherapy ▪ Next-Generation Sequencing.

The landscape of lung cancer treatment is rapidly evolving with the use of genomic testing which helps identify specific mutations or resistance mutations for these heterogenous tumors. Advanced lung cancer has a very poor prognosis but identifying other treatment options based on genomic profiling of the tumor can lead to improved outcomes. Evidence of benefit for genomic testing in lung cancer has now resulted in this test becoming part of national guidelines. There are challenges with genomic testing which need to be understood as well as understanding how to apply test results. These results can help identify treatment options or may serve as predictors to respond to specific therapies. **Conclusion.** In the current era of precision medicine, it is imperative clinicians be familiar with genomic testing and be able to offer it to their cancer patients, specifically those with advanced lung cancer.

Introduction

Cancer is a leading cause of death worldwide with lung cancer representing the highest with an estimated 2.09 million cases in 2018. The most common cause of cancer death is from lung cancer representing 1.76 million deaths in 2018 as well (1). Tobacco use is responsible for approximately 22% of all cancer deaths and causes nearly 90% of lung cancers (2, 3). Lung cancer is categorized as small cell lung cancer and the more common non-small cell lung cancer (NSCLC) which represents 80-85% of lung cancer cases. Non-small cell lung cancer is further sub-divided based on histologic types to adenocarcinoma, squamous cell carcinoma, large-cell neuroendocrine carcinoma, and pulmonary carcinoid tumors (4). In the U.S., the 5-year survival rates for all people with all types of lung cancer is 18% (5). The survival rate is directly related to the stage at diagnosis with people diagnosed at earlier stages having a higher rate of survival; 92% 5-year survival rate for stage IA versus 10% 5-year survival rate for stage IVA (6). The traditional modalities of treatment have included surgery, radiation and chemotherapy. However, the landscape of cancer treatment, specifically in lung cancer, has been rapidly evolving over the past 5 years to now incorporate immuno-oncology treatments as well as targeted therapies based on molecular alterations. Immuno-oncology treatments and targeted therapies for patients with known driver mutations have led to improved responses when compared to chemotherapy alone. These successes have led to utilizing these therapies in the first-line setting for certain patient populations. These newer advances have led to better outcomes as well

as improved quality of life in patients with late-stage lung cancers.

Large-scale comprehensive sequencing efforts such as The Cancer Genome Atlas (CGA) have led to the discovery of various mutations and pathways which may play a role in the pathogenesis of lung cancer and may offer a target for potential treatment (7). These sequencing efforts, or genomic testing, have helped clinicians understand the heterogenous nature of lung cancer as well as expanded the field of precision oncology.

This review article will highlight the role of genomic testing in making treatment decisions for patients with lung cancer.

Genomic Sequencing

Since the early days of the Human Genome Project, there has been a continuous decrease in costs for next-generation sequencing (NGS) with more attention towards clinical implementation of whole genomes. Increased adoption has resulted in increased actionable therapeutic insights (8). As a result, more clinicians have utilized NGS for their patients with advanced disease especially when other treatment options are no longer available. One main question is when the appropriate time is to order NGS for a patient.

Although this answer is less clear for other cancers, the National Comprehensive Cancer Network (NCCN) guidelines recommends broad molecular profiling upfront in advanced or metastatic patients with the goal of identifying rare driver mutations for which effective drugs or clinical trials may be available (9). Several targetable genes are known to be altered in NSCLC including *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, *HER2*, *RET* and *NTRK1*. Upfront NGS can be more cost-effective and faster than multiple single gene or limited gene testing. A study presented at the 2018 American Society of Clinical Oncology Annual Meeting predicted that in the United States, using Centers for Medicare and Medicaid Services reimbursement, NGS resulted in a savings of almost $1.4 million compared with exclusionary testing, $1.5 million compared with sequential testing, and more than $2.1 million compared with panel testing. NGS was also less expensive with commercial payers as well (10).

Solid Tumor vs. Liquid Biopsy

Another consideration when ordering NGS is the method to which to obtain the testing. Genomic analysis of tumor tissue is the standard technique for identifying DNA alterations in malignancies (11). However, obtaining tumor tissue is always not feasible and in some instances, major complication rates with thoracic biopsies have been reported at 5.2% (12). NGS of circulating tumor cell-free DNA (cfDNA) represents a relatively non-invasive method of identifying potential targetable mutations from peripheral blood. In a retrospective study of twenty-eight patients with advanced solid tumors with paired NGS tissue and cfDNA, concordance was 91.9-93.9%, however the concordance rate decreased to 11.8-17.1% when considering only genes with reported genomic alterations in either assay (11). A prospective study evaluating plasma cfDNA in detecting oncogenic drivers for lung cancer demonstrated a tissue NGS concordance of 96.1% that directly led to matched targeted therapy in 21.9% (46/210) with clinical response. The authors also noted a shorter turnaround time for plasma NGS compared to tissue NGS with median time to result of 7 days compared to 20, respectively (13). One limitation of plasma NGS genotyping included the low concentration of cfDNA shed into the peripheral circulation (14). This would then suggest that a negative finding on cfDNA may not exclude the presence of a targetable driver. Despite the differences in concordance, the U.S. Food and Drug Administration (FDA) did approve the first

liquid biopsy test which can detect the presence of a T790M mutation in patients with metastatic epidermal growth factor receptor (EGFR) mutation-positive non-small cell lung cancer, who have progressed on or after an EGFR tyrosine kinase inhibitor (15).

Results from Genomic Testing

NGS has helped identify many genomic alterations in lung cancers. According to researchers from the CGA and others, the most commonly mutated oncogenes in lung adenocarcinoma are *KRAS* (in 33% of tumors), *EGFR* (in 14%), *BRAF* (in 10%), *PIK3CA* (in 7%), and *MET* (in 7%). Data from the CGA have also shown a higher prevalence of *EGFR* mutations than of other mutations in specimens from groups with a low rate of transversion (16). Table 1 demonstrates recurrent molecular alterations in lung adenocarcinoma, squamous-cell carcinoma and small-cell carcinoma.

Genomic analyses can also discover clonal evolution as well as resistance genes. Subclones may be intermixed within one tumor sample or regionally separated within a primary tumor and metastatic sites (17). One possible scenario is when an *ALK* fusion-positive tumor treated with an *ALK* inhibitor continues to progress due to evolution of an *EGFR* mutation-driven subclonal cancer cell population (18). Figure 1 demonstrates three scenarios for evolution of the *ALK* fusion after *ALK* inhibition.

Long-term treatment results of the impact of NGS on treatment decisions and patient outcomes are still underway. Clinical trials such as NCI-MATCH and ASCO's TAPUR studies will provide some outcome data once available. However, smaller studies have been published including a retrospective study of 234 stage IIIb/IV NSCLC patients who had NGS testing in Israel. 62% performed tissue NGS and 38% performed

Table 1. Recurrent Molecular Alterations in Lung Adenocarcinoma, Squamous-Cell Carcinoma, and Small-Cell Carcinoma.*

Type of Alteration	Adenocarcinoma	Squamous-Cell Carcinoma	Small-Cell Carcinoma
Cell-cycle mutations	*TP53* (46%), *CDKN2A*(4%)	*TP53* (91%), *CDKN2A* (17%), *RB1* (7%)	*TP53* (92%), *RB1* (75%)
	RTK/PI3K-MTOR signaling	*RTK/PI3K-MTOR* signaling	*RTK/PI3K-MTOR* signaling: *PTEN* (5%)
	KRAS (33%), *EGFR* (14%), *BRAF* (10%), *STK11* (17%), *MET* (8%), *NF1* (11%), *PIK3CA* (7%), *RIT1* (2%)	*PIK3CA* (16%), *PTEN* (8%), *HRAS* (3%)	
Other mutations	Oxidative stress response: *KEAP1* (17%), *MYC* pathway; *MGA* (8%)	Oxidative stress response: *CUL3* (6%), *KEAP1* (12%), *NFE2L2* (15%)	Epigenetic deregulation: *EP300* (11%), *CREBBP* (10%)
	Aberrant splicing: *U2AF1* (3%), *RBM10* (8%)	Squamous differentiation: *NOTCH1* (8%), *ASCL4* (3%), *NOTCH2* (5%)	Neuroendocrine differentiation: *NOTCH1* (15%), *NOTCH2* (5%), and *NOTCH3* (9%)
Rearrangements	*ALK* (3–8%), *ROS1* (2%), *RET* (1%), *NTRK1* (3%), *NRG1* (2%), *BRAF* (3% in those who never smoked), *ERBB4* (1%)	*FGFRs* (rare)	*RB1* (13%), *TP73* (7%), *CREBBP* (4%), *PTEN* (4%), *RBL1* (3%)
Amplifications	*TTF1* (14%), *TERT* (18%), *EGFR* (7%), *MET* (4%), *KRAS* (6%), *ERBB2* (3%), *MDM2* (8%)	Chr3q: *SOX2* (43%), *TP63* (29%), *PIK3CA* (38%), *HES1* (26%)†	*MYC* family members (16%): *MYC, MYCN, MYCL1, SOX2* (27%), *FGFR1* (8%), *IRS2* (2%)
Deletions	*CDKN2A* (20%)	*CDKN2A* (27%), *PTEN* (3%)	*TP53, RB1, CDKN2A,* Chr3p (e.g., *FHIT, ROBO1*)†
Commonly altered pathways	MAPK and PI3K signaling, oxidative stress response, cell-cycle progression, RNA splicing and processing, nucleosome remodeling	Squamous-cell differentiation, oxidative stress response, MAPK and PI3K signaling	Cell-cycle regulation, PI3K signaling, regulation of nucleosome transcriptional and remodeling, NOTCH signaling and neuroendocrine differentiation

* Percentages represent the prevalence of mutation and were obtained from the cBioPortal for Cancer Genomics (www.cbioportal.org).[10,11]
† Chromosomes 3q and 3p are cytogenetic bands.

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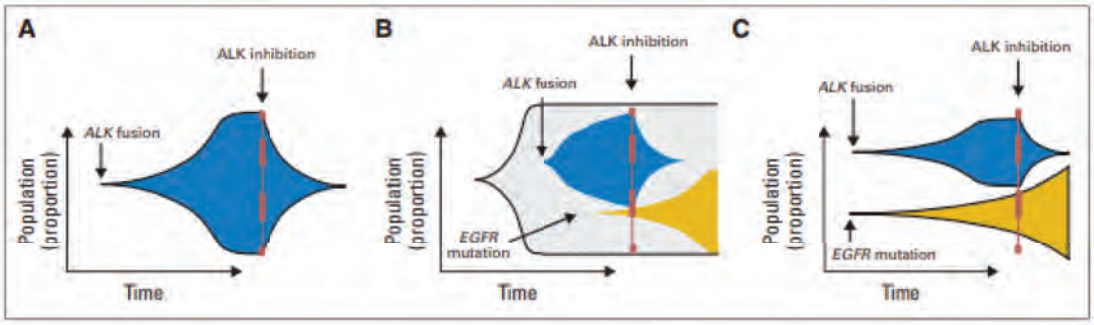

Figure 1. These scenarios for evolution of an anaplastic lymphoma kinase gene (*ALK*) fusion after *ALK* inhibition. (A) *ALK* fusion is a truncal event shared by all cancer cells, and *ALK* inhibition is effective. (B) *ALK* fusion and epidermal growth factor receptor gene (*EGFR*) mutation are later branched events that are only present in a fraction of the cancer cells. *ALK* inhibition clears cancer cells that carry the *ALK* fusion but leaves *ALK* fusion-negative cancer cells, including cells that carry *EGFR* activating mutations, to proliferate. (C) *ALK* fusion and *EGFR* mutation are both trunk events in separate primary tumors and progress in close proximity. *ALK* inhibition attenuates the growth of the primary tumor that carries *ALK* fusion leaves the *EGFR* mutated primary to progress. Reprinted with permission from Journal of Clinical Oncology (18).

liquid NGS. 91 patients had received targeted therapy based on NGS analysis, 75 received therapy based on NCCN guidelines, 9 off-protocol, and 7 received immunotherapy due to high tumor mutational burden (TMB) found on NGS. Median overall survival for this group was 25.7 months (19). Numerous case reports and case studies have also been reported in the literature highlighting positive responses to genomic-based therapy. Our group published a report on a patient with metastatic NSCLC who harbored a *PTEN* and *STK11* mutation from NGS testing who had a response to temsirolimus for almost 20 months (20). Although each case is unique and not all patients will benefit from NGS based therapy, these results highlight the heterogenous nature of metastatic lung cancer and will help identify specific patient populations that will benefit from such treatment.

In addition to providing genomic mutation results, NGS now also provides biomarkers which can help identify those patients who may respond to immunotherapy. Aside from the correlation of PD-L1 expression and response to checkpoint inhibitors, other markers are also present which may serve as predictors to respond to immunotherapy. Lung cancer genomes have a high tumor mutational burden (TMB) compared to other cancer types which is attributed to cigarette smoke exposure (21). Recent data reviewing 151 patients with any type of cancer who underwent NGS, had a TMB assessment and treated with immunotherapy were reviewed for response rate (RR), progression-free survival (PFS) and overall survival (OS). Higher TMB was independently associated with better outcome parameters. The RR for high TMB (>/= 20 mutations/mb) vs. low to intermediate TMB was 58% vs. 20%, median PFS was 12.8 vs. 3.3 months and median OS was not reached in the high TMB group vs. 16.3 for the low to intermediate group (22). A phase III trial specific to non-small-cell lung cancer showed that 1-year PFS rate was 42.6% with nivolumab plus ipilimumab versus 13.2% with chemotherapy in patients with a higher TMB (≥10 mutations per megabase) (23). Other predictors to respond to immunotherapy seen on NGS testing include identification of repair pathway defects such as MMR deficiency and mutation in DNA polymerases POLE

and POLD1 which are surrogate markers for TMB (24).

Obtaining these data points are instrumental in helping to identify which patients will respond to targeted or immunotherapy. One study demonstrated that out of 4064 patients with non-small cell lung cancer, 871 (21.4%) had an alteration in *EGFR*, *ALK* or *ROS1*. Among those with a driver alteration, improved OS was observed in those treated vs not treated with targeted therapies (median, 18.6 months vs 11.4 months, respectively). TMB of 20 or more was also associated with improved OS when treated with checkpoint inhibitors (16.8 months vs. 8.5 months, respectively). This study further illustrates the positive value of genomic testing in improving treatment responses in select patients as well as the importance of genomic databases for data collection and interpretation (25).

Discussion

As we continue to gain a better insight into the heterogenous nature of lung cancers, we must accept that treatment is no longer "one-size fits all." Standard treatments with surgery, radiation and chemotherapy certainly still have their place, however, it is essential to deepen our understanding of each unique cancer patient's disease so we can offer them the best treatment option available. The field of precision medicine is rapidly growing and NGS is a significant part of that growth. As costs for NGS testing has decreased this has allowed greater access for clinicians and patients. The turnaround time can vary usually between 7-21 days depending on the test ordered and whether it is a solid tumor biopsy or liquid. Results can also be difficult to interpret if clinicians do not have much experience. Developing molecular tumor boards can help create a platform where cases are discussed, and treatments are reviewed based on current evidence (26). This may also help enroll patients into more clinical trials as well. In the future, with the increase usage of NGS, more relevant mutations can be discovered which can lead to further drug development. In addition, databases can capture multiple data points and outcome data to help create potential algorithms to identify patients most likely to respond to a specific therapy.

Conclusion

Long-term data from current clinical trials such as NCI-MATCH and TAPUR will be available to help identify successful targetable mutations or biomarkers for various cancers. The past 5 years have seen rapid growth in the field of oncology, specifically in lung cancer treatments. With the success of immunotherapy and targeted therapies, we will without doubt see patients with advanced lung cancers living longer.

Conflict of Interest: The author declares that he has no conflict of interest.

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Narrative Review
Acta Medica Academica 2019;48(1):84-89
DOI: 10.5644/ama2006-124.245

Genomic-Based Therapy of Gynecologic Malignancies

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Received: 9 October 2018
Accepted: 15 March 2019

Key Words: Gynecologic Malignances ▪ Ovarian Cancer ▪ PARP Inhibitors ▪ Microsatellite Instability ▪ Endometrial Cancer.

This paper will review the current status of genomic-based therapy of gynecologic malignancies. The routine "standard-of-care" delivery of targeted therapeutics based on the presence of specific molecular biomarkers in the management of the gynecologic malignancies has been delayed compared to the substantial progress made in several other tumor types. However, relatively recently reported and rather robust phase 3 trial data have confirmed a potentially major role for PARP inhibitors as both active treatment and maintenance therapy of advanced ovarian cancer. Further, data demonstrating the presence of a specific molecular phenotype (micro-satellite (instability high – MSI-H) is a valid biomarker for the potential clinical utility of checkpoint inhibitor immunotherapy has relevance for all gynecologic malignancies, and particularly in the setting of metastatic or recurrent endometrial cancer. **Conclusions.** The introduction of PARP inhibitors into the oncology armamentarium has substantially impacted standard-of-care strategies in the management of ovarian cancer. It is anticipated that the results of ongoing and future trials will further define the role of genomic-based therapy in ovarian cancer and other gynecologic malignancies.

Introduction

Advances in the chemotherapeutic management of ovarian cancer over the past several decades subsequently led to the introduction of several important anti-neoplastic drugs (cisplatin, carboplatin, paclitaxel) into the standard-of-care management of multiple malignancies (1). Unfortunately, the same cannot be stated for the role played by ovarian cancer, or any other gynecologic malignancy in the early development of molecularly-based ("genomic") therapeutic strategies. However, over just the past several years rapidly evolving paradigm-changing concepts of precision-cancer medicine have entered the arena of the gynecologic cancers and these changes are on the verge of transforming the fundamental management of ovarian cancer and other female pelvic malignancies.

The goal of this review will be to highlight recent advances in the delivery of targeted therapeutics in the management of the gynecologic malignancies.

BRCA (BReast CAncer) Mutations and PARP (poly-[ADP-ribose] polymerase) Inhibitors

Mutations within the Breast Cancer Susceptibility Gene (BRCA) 1 and 2 gene family have long been recognized to be responsible for the majority of ovarian cancers discovered to have a hereditary relationship

(2). While data suggest such mutations are uncommon within the general population (0.5% incidence in one large unselected patient study) as many as 10% – 15% of women who develop ovarian cancer will be discovered to possess a germline BRCA abnormality (2, 3). An additional 5-7% of ovarian cancers will be found to have a somatic mutation in BRCA with a wildtype BRCA within the germline.

Of considerable interest to the question of possible therapeutic implications of the presence of a BRCA mutation investigators in several centers noted that the overall survival of patients treated with platinum-based chemotherapy and whose ovarian cancers contained this defect appeared to be somewhat superior to the much larger population of women with a wild-type (normal) BRCA gene (4, 5). (Note: Evidence also exists that the presence of a BRCA mutation may favorably impact the outcome associated with non-platinum-based chemotherapy (6, 7).

Subsequent pre-clinical investigative efforts revealed the major role of BRCA gene products in the DNA repair process and the impaired ability of malignant cells to adequately repair damage (including that produced by exposure to platinum agents) in the presence a mutation in the BRCA genes (8, 9). In experimental models poly (ADP-ribose) polymerase (PARP) was shown to be a second critical component of the DNA repair process. In fact, in a series of elegant studies investigators demonstrated that inhibition of PARP function in the presence of a genetic deficiency of BRCA produced a rather profound degree of tumor cell kill, while cells possessing a wild type BRCA were substantially less susceptible to the effects of PARP inhibition (1000-fold less sensitive) (8, 9).

This observation quickly led academic researches and biotech/pharma companies to initiate robust efforts to develop clinically useful inhibitors of PARP. Several agents in this class have been examined in trials in ovarian cancer and other malignances. Three PARP inhibitors are currently commercially available in the United States with regulatory approval granted for their administration in the management of ovarian cancer. Two PARP inhibitors (olaparib, rucaparib) are currently specifically approved for delivery as "therapy" of recurrent or persistent disease following several lines ($\geq$2 for rucaparib; $\geq$3 for olaparib) of cytotoxic chemotherapy in the presence of a BRCA mutation. Objective response rates have been reported to range between 30-70+% in this setting with the greatest opportunity to achieve clinical benefit where there is also likely persistent sensitivity to platinum agents (10-17). It is important to acknowledge here that in the absence of formal randomized trial comparisons between the various PARP agents it is not possible to make any definitive statement regarding the relative clinical effectiveness of the individual drugs.

Three drugs (niraparib, olaparib, rucaparib) are approved as a "maintenance" approach following attainment of a clinical response (complete or partial) to a platinum-based second-line (or later) cytotoxic chemotherapy regimen (Table 1) (18-22). "*Maintenance therapy*" in this setting implies knowledge that the cancer remains present with the therapeutic goal to extend ("maintain") an achieved response with acceptable treatment-related side effects. The general concept is to continue therapy for an indefinite period, or until subsequent progression is documented, unacceptable toxicity develops, or a patient desires to discontinue treatment.

It is relevant to acknowledge here that the trials of PARP inhibitors in ovarian cancer have revealed the very impressive "sensitivity" and documented clinical benefit associated with tumors possessing either germline or somatic BRCA mutations (18, 23). However, even patients with a wild type

Table 1. PARP Inhibitor Maintenance Therapy in Epithelial Ovarian Cancer

Drug (trial design)	Patient population	Median PFS (in months)*	Hazard Ratio†
Niraparib (randomized phase 3) second-line (or later) (28)	Germline BRCA mutation	21.0 vs. 5.5	0.27
	Wildtype BRCA	9.3 vs. 3.9	0.45
Olaparib (randomized phase 2) second-line (or later) (19, 20)	Overall population	8.4 vs. 4.8	0.35
	Germline BRCA mutation	11.2 vs. 4.3	0.18
	Wildtype BRCA	7.4 vs 5.5	0.54
Olaparib (randomized phase 3) second-line (or later) (21)	Germline BRCA mutation	19.1 vs 5.5	0.30
Olaparib (randomized phase 3) front-line (25)	Germline BRCA mutation	(Not reached) vs. 13.8	0.30
Rucaparib (randomized phase 3) second-line (or later) (22)	Germline BRCA mutation	16.6 vs. 5.4	0.23
	Intention to treat	10.8 vs. 5.4	0.36

PARP=Poly (ADP-ribose) polymerase; PFS=Progression-free survival; *Active Treatment vs. Placebo Control (all differences noted are "statistically significant"); †Treatment vs. Control; BRCA= Breast Cancer Susceptibility Gene.

BRCA (and no evidence of a somatic mutation) can respond to this class of drugs. The older term "BRCAness" had been employed to suggest the presence of additional poorly defined molecular abnormalities that interfered with DNA repair, in a manner like a BRCA mutation, and which might render cancer cells more susceptible to PARP inhibitors and platinum agents (24).

Researchers and molecular diagnostic companies are actively exploring possible algorism that may be employed in this clinical setting to identify such cancers that exhibit "homologous recombination deficiency" (HRD) independent of the presence of a documented BRCA mutation. However, while the presence of HRD with existing diagnostic platforms does appear to identify a population of individuals more likely to respond to a PARP inhibitor there remain a considerable percentage of patients whose cancers fail to exhibit this phenotype but who also achieve evidence of clinical benefit (based on the randomized phase 3 trial results compared to placebo). As a result, the FDA approval of the three available PARP drugs specifically did not require the presence of a BRCA mutation or of a HRD molecular phenotype to prescribe these agents.

Results of the first completed phase 3 randomized trial employing a PARP inhibitor (olaparib) as "maintenance" therapy for patients with a germline BRCA mutation in the front-line setting following the completion of a platinum and taxane regimen have recently been reported (25). Compared to "placebo" maintenance, treatment with olaparib resulted in a 70% reduction in the risk of disease progression or death. At three years follow-up 60% of patients treated with olaparib had not progressed compared to 27% who received placebo.

While the available oral PARP inhibitors differ somewhat in their toxicity profiles and schedule of administration they have all been shown to be reasonably well tolerated in the clinical trials setting, including the performance of formal quality-of-life assessments (26, 27). However, it is relevant to note that the administration of these agents results in a high incidence (approximately 70%) of so-called "*low grade*" nausea which in the context of daily oral therapy anticipated to be taken for several years may be far more serious to an individual patient's overall quality-of-life than the existing toxicity scale terminology might suggest. And the question to be asked here is of the willingness of a patient to continue to take an oral medication for this extended duration if she experiences "low grade" nausea every day.

Other Genomic Abnormalities of Therapeutic Relevance in the Gynecologic Malignancies

Both high grade and low grade epithelial ovarian malignancies are characterized by a reasonably high incidence of molecular abnormalities including genes known or suspected to be "driver mutations" (e.g., PI3KCA, ADAMTS, DICER1, BRAF, KRAS, ARIDA1A; MEK; AKT2; PTEN, FBXW7) (28-34). However, either effective targeted therapeutics do not currently exist for these molecular events or the utility of agents revealed to be effective in other malignancies have not yet been shown to be relevant in ovarian cancer. Unfortunately, a similar conclusion can be drawn for the status of molecular targeting therapeutics in endometrial cancer (35).

A phase 2 study which examined the clinical activity in ovarian cancer of the established lung cancer anti-neoplastic, gefitinib (a tyrosine kinase inhibitor of EGFR) revealed only a single response (4% overall response rate) (36). However, this response occurred in the one patient in the trial whose cancer possessed an activating mutation in EGFR known to characterize the responding lung cancer patient population. The investigators also examined the incidence of such molecular events in ovarian cancer and revealed a rate of 3.5% (2 of 57 patients). Unfortunately, but certainly not surprisingly due to the low incidence of such mutations in ovarian cancer, this potentially highly relevant observation has not been followed-up with further essential clinical studies.

Similarly, an older phase 2 trial of trastuzumab in ovarian cancer discovered an overall incidence of overexpression of the Her2 receptor of only 11.4%, a far lower proportion of patients than observed in breast cancer (37). In fact, a total of 837 patients were required to be screened to find the 45 patients who entered this phase 2 study. While the objective response rate was only 7.3% it must be noted that the cancers of only 14 patients, 31% of study participants had +3 staining by immunohistochemistry for overexpression of Her2, the patient population with the highest probability of achieving a clinical response. The report did not describe the relationship between Her2 tissue staining and response, so it remains unknown today if the small subgroup of ovarian cancer patients (<2%; 14 of 837 screened individuals) who strongly overexpress Her-2 within their cancers might be reasonable candidates to receive one or more of several known highly clinically active anti-Her2 therapeutic agents.

The documented presence of micro-satellite instability (MSI-H) is associated with the presence of multiple molecular abnormalities within a cancer cell and this event and has been shown represent a malignant phenotype with a reasonably high statistical probability (approximately 40% – 50%) of responding to an immune modulatory checkpoint inhibitor (38, 39). Based on data from several small, but impressive studies, the FDA has approved the commercial use of this strategy in the presence of the MSI-H phenotype, completely agnostic to the specific tumor type. (Note: This was the first time the FDA has approved the use of an anti-neoplastic agent completely independent of the site of tumor origin.

Overall, approximately 17%, 2% and 3.5% of endometrial, ovarian, and cervical cancers, respectively, will be found to be MSI-H if the tumors are subjected to specific molecular testing for the presence of this abnormality or the diagnostic platform utilized examines for multiple genomic events (40).

Conclusions

While to date genomic abnormalities within two genes (BRCA 1 and 2) has dominated both research efforts and clinical use of precision medicine within the domain of the fe-

male pelvic malignancies, it can be anticipated that this situation will substantially change over the coming years due to efforts by the pharmaceutical/biotech industry to develop novel products designed to effectively "target" both relatively common and unique molecular events within this group of cancers.

Conflict of Interest: The author declares that he has no conflict of interest.

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Narrative Review
Acta Medica Academica 2019;48(1):90-104
DOI: 10.5644/ama2006-124.246

Precision Medicine in Oncology Pharmacy Practice

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Received: 30 September 2018
Accepted: 12 December 2018

Key Words: Pharmacogenomics ▪ Pharmacogenetics ▪ Oncology Pharmacy.

The objective of this review is to provide an overview of the components, process and resources available to apply precision medicine strategies to drug therapy in cancer medicine, with an emphasis on oncology pharmacy practice. Precision medicine initiatives in oncology take into account individual variability in genes, environment and lifestyle factors. Genomic assays of patient tumors is now the standard of care in oncology and recommendations for targeted drug therapies are often formulated by interprofessional teams. Pharmacogenomics (PGx) is a component of precision medicine based on polymorphisms that impact medication selection and/or dosing. Several oncolytic agents used in the treatment of cancer and supportive care have pharmacogenomic-based dosing recommendations to minimize potential toxicities. Several resources are reviewed here to guide treatment options in oncology as they relate to somatic mutations and PGx. Examples include: OncoKB is a precision oncology knowledge base that offers evidence-based information for somatic mutations. The Clinical Pharmacogenetics Implementation Consortium provides PGx-based guidelines for several oncolytic therapies used to treat cancer and for supportive care. Pharmacists can be integral members of the interprofessional team in many practice settings in precision medicine. Involvement can include membership in molecular tumor boards, PGx dosing services and provide patient education. **Conclusion.** Precision medicine is a rapidly evolving field in oncology that requires an interprofessional approach of drug therapy experts.

Introduction

In 2015, a new Precision Medicine Initiative was launched in the United States to accelerate progress and research toward curing diseases and providing access to personalized information (1). Precision medicine is an emerging approach that takes into account individual variability in genes, environment, and lifestyle factors (1, 2). Precision medicine more accurately predicts which treatment option will work better for a particular disease in a specific group of people. Although used synonymously with personalized medicine, precision medicine is more recently the preferred term as some may misinterpret the word personalized to imply treatments developed uniquely for each individual (versus which approach is best for a specific group of individuals) (2). Oncology has certainly been leading the forefront of precision medicine as many molecular (or somatic) alterations have been identified that drive cancer (1). These somatic mutations are not inherited, are sporadic, and account for the majority of cancers. Many targeted and immunologic therapies have been developed against these mutations and

are successfully being used in the treatment of a variety of cancers.

Pharmacogenomics (PGx) is a component of precision medicine that is defined as the study of how genetic variations may influence an individual's response to drug therapy (3). Pharmacogenetics, on the other hand, often refers to how a single gene may influence a person's response to a drug. Despite differences in definition, pharmacogenomics and pharmacogenetics are commonly used synonymously in general practice. PGx is based on inherited (or germline) polymorphisms in drug metabolizing enzymes or other targets and is currently being used by some oncology practices to improve clinical outcomes, reduce adverse effects, and decrease costs associated with drug therapies (4).

Drug manufacturers have been incorporating pharmacogenomics into the drug development, labeling, and approval processes for several years (5, 6). According to the PharmGKB database at the time of writing, there are 509 annotated drug labels that contain pharmacogenetic information approved by the United States Food and Drug Administration (FDA), European Medicines Agency (EMA), Pharmaceuticals and Medical Devices Agency, Japan (PMDA), and Health Canada (Sante Canada) (HCSC) (5). Of these, there are 95 annotated drug labels specific to oncology. Biomarker information contained within may include germline polymorphisms, somatic gene mutations, and others. There are multiple PGx dosing guidelines available that have been published by the Clinical Pharmacogenetics Implementation Consortium (CPIC), the Royal Dutch Association for the Advancement of Pharmacy – Pharmacogenetics Working Group), the Canadian Pharmacogenomics Network for Drug Safety and others (7). At the time of writing, each group has produced findings related to oncology practice. For example, there are currently 6 CPIC guidelines available for 10 drugs specifically used in oncology practice (8).

Many other factors affecting drug response should also be taken into account when individualizing drug therapy in a patient with cancer (9). Pharmacokinetic factors such as drug-drug or drug-disease interactions, enzyme inhibition or induction, and environmental factors such as smoking, alcohol, and diet are important considerations. Patient specific factors such as age, sex, renal and hepatic function, performance status, medication adherence, and medication access (financial considerations) should also be assessed. In many clinical pharmacy practices across the country, pharmacists are conducting comprehensive medication therapy reviews, also known as medication therapy management (MTM), in which an individual patient's medications are assessed for interactions, optimal dosing, strategies to minimize adverse drug reactions, minimize costs, and collaborate with other providers to improve treatment outcomes (10). Integration of precision medicine, including pharmacogenomics, with MTM offers a prime opportunity for pharmacists to collaborate with oncologists to further optimize drug selection, dosage, and clinical outcomes. The overall goal is to target the right drug to the right tissue for the right patient while minimizing toxicity.

The focus of this paper is to provide an overview of the components, process, and resources available to apply precision medicine strategies to the most common drug therapies and their pharmacogenomic targets in cancer medicine, with an emphasis on oncology pharmacy practice for both oncolytic and supportive therapies. Specific mutations associated with the tumor itself are briefly reviewed, however just as important are genetic markers associated with the individual patient.

The PubMed biomedical database was searched from 2008 through August 2018

using the Medical Subject Heading (MeSH) keywords: precision medicine, oncology pharmacy practice, pharmacogenomics, and pharmacogenetics (limited to humans, English language, and review articles). OncoKB, PharmGKB, and CPIC guidelines were manually searched for additional references.

Precision Medicine and Oncolytic Drug Therapy Selection

Tumor sequencing of patient tumors is now considered the standard of care in oncology (11). Somatic mutations not only serve as a driver in the development of cancer but also as a therapeutic target for treating cancer. Of the 95 annotated drug labels pertaining specifically to oncology that contain pharmacogenetic information, the FDA requires genetic testing for 49 (5). This field of oncology is rapidly evolving with many new FDA approvals in the pipeline. A new comprehensive precision oncology knowledge base is available (OncoKB) that offers evidence-based information about individual somatic mutations to assist in guiding optimal treatment decisions (11, 12). This knowledge base contains information on FDA approved therapies and agents in clinical trials from a variety of resources and leading experts. Potentially actionable mutations are assigned to one of four levels based on available clinical and laboratory data that support the use of the mutation as a predictive biomarker of drug sensitivity to FDA-approved or investigational agents for a specific indication (11, 12). Table 1 provides a summary of level 1 [biomarker presence recognized by FDA as responsive to FDA-approved drugs for specified indication(s)], level 2 [biomarker presence recognized by standard care as disease responsive to FDA-approved drugs for specified indication(s)], and level R1 [biomarker presence indicates resistance to FDA-approved drugs for specified indication(s)] FDA approved drugs and their associated genes (12). Several other useful genomic knowledge bases are also available that provide information regarding the relevance of genes and their variants (7, 13-15). These databases are updated on a continual basis.

Many precision medicine initiatives are being conducted through the use of interprofessional molecular tumor boards (MTBs) or precision medicine clinics.[4] The goal is often to make clinical recommendations for targeted therapies based on next-generation sequencing (NGS) panels. The interprofessional team may use NGS results to develop an individual patient treatment plan in which a patient may be recommended for standard therapy (an FDA approved targeted therapy), nonstandard FDA-approved targeted therapy (off-label use), or a clinical trial. MTBs often vary in their composition but may include medical oncologists, radiation oncologists, clinical oncology pharmacists, clinical laboratory scientists, molecular genetic scientists, clinical nurses, financial strategists, data managers, coordinators, and others. Some centers are utilizing MTBs as an opportunity to provide interprofessional education for medical oncology fellows, pathology residents, geneticists, pharmacy residents, and students from multiple healthcare professions (4). Several leading cancer centers in the U.S. have published their experiences and outcomes related to their MTBs (16-23).

Pharmacists can have a key role as an interdisciplinary team member in precision medicine. In some cancer centers, clinical pharmacists may be coordinators of MTBs, assist with drug procurement, provide information for and assist with financial assistance programs, manage investigational drug services, participate in data collection and research, and provide comprehensive patient and caregiver education (4). The majority of the oral targeted therapies are

Table 1. Gene Interactions with FDA Approved Oncolytic Agents (11)*

Drug	Disease Indication (s)	Gene (s)
Level 1: Biomarker presence recognized by FDA as responsive to FDA-approved drugs for specified indication (s)		
Ado-trastuzumab emtansine	Breast cancer	ERBB2
Afatinib	Non-small cell lung cancer	EGFR
Alectinib	Non-small cell lung cancer	ALK
Binimetinib + encorafenib	Melanoma	BRAF
Brigatinib	Non-small cell lung cancer	ALK
Ceritinib	Non-small cell lung cancer	ALK
Cetuximab	Colorectal cancer	KRAS
Cobimetinib + vemurafenib	Melanoma	BRAF
Crizotinib	Non-small cell lung cancer	ALK, ROS1
Dabrafenib	Melanoma	BRAF
Dabrafenib + trametinib	Anaplastic thyroid cancer Non-small cell lung cancer Melanoma	BRAF
Dacomitinib	Non-small cell lung cancer	EGFR
Dasatinib	Acute lymphoid leukemia Chronic myelogenous leukemia	ABL1
Enasidenib	Acute myeloid leukemia	IDH2
Erlotinib	Non-small cell lung cancer	EGFR
Everolimus	CNS cancer	TSC1, TSC2
Gefitinib	Non-small cell lung cancer	EGFR
Imatinib	Acute lymphoid leukemia Chronic myelogenous leukemia Gastrointestinal stromal tumor Leukemia Myelodysplasia Myeloproliferative neoplasm Dermatofibrosarcoma protuberans	ABL1, KIT, PDGFRA, PDGFRB
Ivosidenib	Acute myeloid leukemia	IDH1
Lapatinib	Breast cancer	ERBB2
Lapatinib + trastuzumab	Breast cancer	ERBB2
Neratinib	Breast cancer	ERBB2
Nilotinib	Chronic myelogenous leukemia	ABL1
Niraparib	Ovarian cancer	BRCA1, BRCA2
Nivolumab	Colorectal cancer	Microsatellite instability-high
Osimertinib	Non-small cell lung cancer	EGFR
Panitumumab	Colorectal cancer	KRAS
Pembrolizumab	All solid tumors	Microsatellite instability-high
Pertuzumab + trastuzumab	Breast cancer	ERBB2
Regorafenib	Gastrointestinal stromal tumor Colorectal cancer	KIT, KRAS
Rucaparib	Ovarian cancer	BRCA1, BRCA2
Sunitinib	Gastrointestinal stromal tumor	KIT
Trametinib	Melanoma	BRAF

Drug	Disease Indication (s)	Gene (s)
Trastuzumab	Breast cancer, Esophagogastric cancer	ERBB2
Vemurafenib	Non-Langerhans cell histiocytosis/ Erdheim-Chester disease, Melanoma	BRAF
Level 2: Biomarker presence recognized by standard care as disease responsive to FDA-approved drugs for specified indication(s)		
Abemaciclib	Dedifferentiated liposarcoma Well-differentiated liposarcoma	CDK4
Cabozantinib	Renal cell carcinoma Non-small cell lung cancer	MET, RET
Ceritinib	Inflammatory myofibroblastic tumor	ALK
Crizotinib	Inflammatory myofibroblastic tumor Non-small cell lung cancer	ALK, MET
Dasatinib	Gastrointestinal stromal tumor	PDGFRA
Everolimus	Renal cell carcinoma	TSC1, TSC2
Imatinib	Melanoma Gastrointestinal stromal tumor	KIT, PDGFRA
Olaparib	Ovarian cancer	BRCA1, BRCA2
Palbociclib	Dedifferntiated liposarcoma Well-differentiated liposarcoma	CDK4
Sorafenib	Gastrointestinal stromal tumor Thymic tumor	KIT
Sunitinib	Thymic tumor	KIT
Vandetanib	Non-small cell lung cancer	RET
Level R1 – Biomarker presence indicates resistance to FDA-approved drugs for specified indication(s)		
Afatinib	Non-small cell lung cancer	EGFR
Cetuximab	Colorectal cancer	KRAS, NRAS
Erlotinib	Non-small cell lung cancer	EGFR
Gefitinib	Non-small cell lung cancer	EGFR
Imatinib	Gastrointestinal stromal tumor	PDGFRA
Panitumumab	Colorectal cancer	KRAS, NRAS

*Last updated November 5, 2018; ERBB2=Erb-b2 receptor kinase 2; EGFR=Epidermal growth factor receptor; ALK=anaplastic lymphoma kinase; BRAF=B-Raf proto-oncogene serine/threonine-protein kinase; KRAS=Kirsten rat sarcoma viral oncogene homolog; ROS1=ROS proto-oncogene 1; ABL1=Abelson tyrosine-protein kinase 1; IDH2=Isocitrate dehydrogenase 2; TSC1=Tuberous sclerosis 1; TSC2=Tuberous sclerosis 2; KIT=KIT proto-oncogene receptor tyrosine kinase; PDGFRA= Platelet-derived growth factor receptor alpha; PDGFRB=Platelet-derived growth factor receptor beta; IDH1=Isocitrate dehydrogenase 1; BRCA1=Breast cancer type 1 susceptibility gene; BRCA2=Breast cancer type 2 susceptibility gene; CDK4=Cyclin dependent kinase 4; MET=MET proto-oncogene; RET=Ret proto-oncogene; TSC1=TSC complex subunit 1; TSC2=TSC complex subunit 2;NRAS=NRAS proto-oncogene

dispensed from a specialty pharmacy, often separate from the site of the precision medicine clinic or healthcare system. Pharmacists in specialty pharmacies dispense medications that are considered to be complex with high associated cost. They often assist with locating programs for patients who require financial assistance, provide MTM services, and assess for medication adherence (24). In many clinical oncology and specialty pharmacy practice settings, pharmacists provide comprehensive patient and/or caregiver education. For those patients receiving oral oncolytic therapy at home, patient and

caregiver education should not only include information specific to administration of the oral oncolytic and expected side effects, but also how to prevent and manage any side effects in the home setting and safe handling of these medications. Monitoring adherence to oral oncolytic therapy is crucial to the efficacy of these medications (25-27). Pharmacists, nurses, and/or other providers in specialty pharmacies and out-patient cancer clinics perform routine patient phone calls to follow up on medication adherence and to check for medication tolerability and side effects. Pharmacists and other healthcare providers are continually in touch with their patients and provide additional resources as needed.

Once therapy decisions are individualized for a patient, the pharmacist should verify that the chemotherapy regimen is appropriate per protocol (checking for appropriate dose, frequency, and duration of therapy), check for any dose adjustments that may be needed based on renal and/or hepatic function, and review all medications for drug-drug and drug-food interactions. Grapefruit and grapefruit juice commonly interact with oral oncolytic therapies (such as erlotinib, imatinib, lapatinib, palbociclib, and several others) (28). Co-administration of strong CYP3A4 (cytochrome P450 family 3 subfamily A member 4) inhibitors (such as itraconazole, grapefruit/grapefruit juice, and others) can significantly increase the concentration of the oral oncolytic placing the patient at risk of increased toxicity. It is therefore recommended to avoid the concurrent administration of strong CYP3A4 inhibitors with many oral oncolytic agents (28). Appropriate drug-specific supportive care modalities, such as antiemetics, antimicrobials, anti-diarrheals, moisturizers, and others, should also be incorporated into a patient's treatment plan and education. All patients should be prescribed an appropriate antiemetic regimen based on guidelines and individual patient factors (29, 30).

Cancer treatments are very complex, expensive, and require appropriate monitoring and follow up, especially in the home setting for oral oncolytic therapy. Pharmacists are an integral part of the healthcare team in multiple practice settings in precision medicine.

Pharmacogenomic Applications in Oncology

PGx is an evolving field, especially in the realm of clinical pharmacy practice. Many institutions in the United States have established multidisciplinary pharmacogenomic services led by pharmacists (31). Several models of PGx programs have been published both for the in-patient and community setting and more are emerging in the area of oncology practice (32, 33). There are many different enzymes involved in the metabolism of drugs, the most common being the cytochrome P450 (CYP) enzymes. There are approximately 57 CYP genes that encode for their respective enzyme proteins and an extensive number of gene variants which can result in a decrease, loss, or gain of enzyme function (31, 34) Phenotypes based on these gene variants are often organized into simpler groups. For example, CYP2D6 (cytochrome P450 family 2 subfamily D member 6) variants are classified into the following phenotypes: poor, intermediate, extensive, and ultrarapid metabolizers at the time of the CYP2D6 and Codeine CPIC guideline release, though further clarification to five phenotypes has since been made by CPIC and further work is being done to standardize the genotype to phenotype translation at the time of writing (31, 35, 36). Therefore, PGx variants may run the spectrum of causing minimal changes in clinical decision making, to rendering a drug unusable from an efficacy standpoint (e.g., a drug cannot be sufficiently bioactivated for it to be efficacious), to rendering a drug unusable from

a safety standpoint (e.g., a drug produces substantially greater effect such that dangerous side effects or toxicity may emerge) (31). Other drug metabolizing enzymes such as dihydropyrimidine dehydrogenase (DPD), thiopurine methyltransferases, and glucose-6-phosphate dehydrogenase (G6PD) have an important role in PGx. Additionally, other pharmacokinetic and pharmacodynamic effects may be seen via PGx-based changes in receptors and transporters. Pharmacogenomic assessment of metabolizing enzymes can potentially optimize dosing in oncology and minimize drug toxicities. The CPIC guidelines have been designed to help translate genetic laboratory results into actionable prescribing decisions for affected drugs, ultimately optimizing drug therapy (8). Included here is an overview of the CPIC guidelines for drugs used in oncology practice. Although irinotecan is not currently included in CPIC, the PGx of irinotecan will be reviewed briefly. Table 2 provides a summary of pharmacogenomic information for agents used in oncology that are assigned CPIC Level A. Level A indicates that genetic information should be used to change prescribing of the affected drug and recommendations are based from moderate to high level of evidence (8).

5-Fluorouracil, Capecitabine, and Tegafur

5-Fluorouracil (5-FU) and capecitabine are fluoropyrimidine analogues used to treat a variety of solid tumors. Numerous genetic variants of DPYD, the gene that encodes for dihydropyrimidine dehydrogenase (DPD), have been identified (37). DPD is the first and rate-limiting enzyme for fluoropyrimidine catabolism of 5-FU, capecitabine, and tegafur to dihydrofluorouracil (same metabolite for all three fluoropyrimidine analogues). Some of the genetic variants of DPYD do not affect DPD activity, whereas others can significantly decrease enzyme

Table 2. Pharmacogenomic Overview of Oncolytic Agents Assigned CPIC Level A (5, 8)*

Oncolytic Agent	Gene	PGx Level per FDA Labeling†
Oncolytic Agents Used in the Treatment of Cancer		
Capecitabine	DPYD	Actionable PGx
Fluorouracil	DPYD	Actionable PGx
Irinotecan	UGT1A1	Actionable PGx
Mercaptopurine	TPMT, NUDT15	Testing recommended
Tamoxifen	CYP2D6	None
Tegafur‡	DPYD	None
Thioguanine	TPMT, NUDT15	Testing recommended
Agents Used for Supportive Care		
Allopurinol	HLA-B	None
Ondansetron	CYP2D6	Informative PGx
Rasburicase	G6PD	Testing required
Tropisetron§	CYP2D6	None

*Level A indicates that genetic information should be used to change prescribing; †Testing required: testing should be conducted before using this drug; Testing recommended: testing is recommended before using this drug; Actionable PGx: label does not discuss genetic or other testing for gene/protein/chromosomal variants, but does contain information about changes in efficacy, dosage or toxicity due to such variants; Informative PGx: label mentions a gene or protein is involved in the metabolism or pharmacodynamics of the drug, but there is no information to suggest that variation in these genes/proteins leads to different response; ‡Tegafur is assigned CPIC level C: no prescribing actions are recommended; §Tropisetron is not FDA approved in the United States; DPYD=Gene encoding for dihydropyrimidine dehydrogenase; UGT1A1=uridine diphosphate glucuronosyltransferase 1As; TPMT=Thiopurine methyltransferase; NUDT15=Nudix hydrolase 15; CYP2D6=Cytochrome P450 family 2 subfamily D member 6; HLA-B=Human leukocyte antigen B; G6PD=Glucose-6-phosphate dehydrogenase

function placing patients at very high risk of toxicity (nausea/vomiting, neutropenia, diarrhea, stomatitis, mucositis, and/or hand-foot syndrome). Four genetic variants of clinical significance have been identified and include c.1905+1G>A (rs3918290, also known as DPYD*2A, DPYD: IVS14+1G>A); c.1679T>G (rs55886062, DPYD*13, p.I560S); c.2846A>T (rs67376798, p.D949V); and c.1129-5923C>G (rs75017182, HapB3) (37). Approximately 7% of Europeans carry at least one decreased function DPYD variant, the most common variant being HapB3 (4.7%), followed by DPYD*2A (1.6%), and p.D949V (0.7%). In African ancestry, the c.557A>G (rs115232898, p.Y186C) decreased function variant is relatively common with an estimated carrier frequency of 3-5%.

The benefits of genotype-based dosing have been demonstrated in clinical studies, indicating a decreased incidence of 5-FU related toxicities and toxicity-related deaths (37). Dosing recommendations are based on genotype and associated gene activity scores (AS). Carriers with 2 normal function alleles are assigned an AS of 2 and phenotypically are categorized as a normal DPYD metabolizer. Here, normal DPD activity and normal risks for fluoropyrimidine toxicities would be expected and therefore no dosage or therapy adjustments are required. Carriers of one no function or decreased function allele are considered intermediate metabolizers with associated AS of 1 and 1.5 respectively. Significantly reduced DPD activity and severe toxicities can be expected, therefore it is recommended to empirically reduce the initial dose of 5-FU or capecitabine. The recommended dose reductions for intermediate metabolizers are 50% for an AS of 1, and 25% for an AS of 1.5. DPYD poor metabolizers, characterized as carriers with two no function variants, are considered to have complete DPD deficiency and are at very high risk of severe or fatal drug toxicity. 5-FU and capecitabine therapy should be avoided, especially for those with an AS of 0. For those with an AS of 0.5, 5-FU or capecitabine can be considered at a significantly reduced dose if other therapeutic options are not viable. Drug labels for 5-FU and capecitabine currently include warnings and precautions about DPD deficiency (38, 39). The clinical utility for testing for other gene variants that have a role in 5-FU metabolism, such as TYMS and MTHFR, has not been established at this time (8).

Tegafur is a prodrug of 5-FU and is metabolized by the same enzyme pathway as described above for 5-FU and capecitabine. The impact of DPYD variants is limited and dosing adjustments have not been established. Currently there are no PGx recommendations to guide clinical practice for tegafur (37).

Mercaptopurine and Thioguanine

The thiopurines, mercaptopurine (MP) and thioguanine (TG), are commonly used in the treatment of acute lymphoblastic leukemia (ALL) and some autoimmune disorders. Thiopurine methyltransferase (TPMT) is the enzyme responsible for methylation of MP and TG into the respective inactive metabolites, methyl-mercaptopurine and methyl-thioguanine (40). However, in the deficiency or absence of TPMT, the metabolic pathway of MP and TG is shifted to favor the formation of active thioguanine nucleotide (TGN) metabolites which can accumulate and result in an increased potential for severe adverse effects, especially life-threatening myelosuppression. Although TPMT variants are rare overall, ethnic differences in the frequency of low-activity variant alleles have been reported in black, white, and Asian populations (approximately 6, 5, and 3% respectively) (41). Azathioprine is also metabolized through the same pathway as MP and TG, however this drug will not be reviewed here due to its limited utility in oncology.

TPMT status should be tested prior to initiation of therapy with appropriate starting doses of MP or TG adjusted based on genotype (40). Clinical studies have indicated that dose adjustments based on genotype have reduced severe toxicities while maintaining therapeutic effects (40). For patients who are homozygous wild-type or normal TPMT alleles it is expected that there will be lower levels of TGN metabolites and therefore full doses of MP or TG may be initiated. Patients with intermediate activity (heterozygous for TPMT alleles) are not able to tolerate full doses therefore it is suggested to reduce the dose to 30-70% of full starting dose for MP and a reduction of 30-50% of full dose for TG. Patients who are homozygous variant, mutant, low or deficient activity have a 100% risk of developing life-threatening myelosuppression, therefore a 10-fold reduction in dose is recommended along with a decrease in frequency of administration from daily to three times a week. Further monitoring, titration, and dosage adjustments should be considered based on patient response and tolerability. More dosing information is available in the CPIC guidelines (40).

Recent studies have identified variants in the nudix hydrolase 15 (NUDT15) gene that have been strongly associated with thiopurine-related myelosuppression in patients with inflammatory bowel diseases and children with ALL (42). NUDT15 is one of the pathways that converts active thiopurine metabolites (TdGTP and TGTP) to inactive metabolites (TdGMP and TGMP) (41). Patients with defective NUDT15 alleles are at risk of accumulation of these thiopurine active metabolites and therefore thiopurine toxicity (42). Low function alleles are more common in those of Asian ancestry and Hispanic ethnicity (43). Dosing recommendations based on NUDT15 genotypes are currently in process (43).

Tamoxifen

Tamoxifen is a selective estrogen receptor modulator that has a variety of indications in the prevention and treatment of breast cancer (44). Tamoxifen undergoes extensive hepatic metabolism by 2 major pathways, both of which are mediated by CYP2D6 and CYP3A4 enzymes (45). The two major metabolites, endoxifen and 4-hydroxytamoxifen (4HT), have significantly more antiestrogenic activity than the parent compound tamoxifen. Lower endoxifen concentrations and higher risk of breast cancer recurrence have been observed in patients who have low CYP2D6 enzyme activity as a result of CYP2D6 polymorphisms (45, 46). It has been estimated that 17-21% of the Caucasian population may be CYP2D6 poor metabolizers (31). Co-administration of strong CYP2D6 inhibitors (such as fluoxetine or paroxetine) may also significantly reduce endoxifen concentrations. Although other clinical studies have shown conflicting results regarding outcomes and CYP2D6 polymorphisms, the CPIC guidelines indicate that there is uniformly strong evidence that CYP2D6 poor metabolizers (AS = 0) have lower endoxifen concentrations compared to normal metabolizers in the adjuvant setting (45). These patients may be at a higher risk of breast cancer recurrence and worse event-free survival. Due to these increased risks, alternative hormonal therapy for CYP2D6 poor metabolizers is therefore recommended (45). It should be noted that higher doses of tamoxifen (40 mg daily) may not increase endoxifen concentrations equivalent to normal metabolizers and coadministration of weak to strong CYP2D6 inhibitors should be avoided. A moderate recommendation to use alternate hormonal therapy is suggested for those who are intermediate CYP2D6 metabolizers (AS 0.5) and normal metabolizers with the presence

of the *10 allele (AS 1). For those who have no *10 allele present (either intermediate or normal metabolizer, AS 1) the recommendation to consider alternate hormonal therapy is optional at the time of publication (45). No dosage adjustments are needed for CYP2D6 normal metabolizers (AS 1.5 – 2) or ultrarapid metabolizers (AS > 2), however co-administration of moderate to strong CYP2D6 inhibitors should be avoided.

Variation in CYP2C9, CYP3A4, and CYP3A5 genes have been associated with altered 4HT and endoxifen concentrations however clinical outcomes have not been fully elucidated (45). CYP2C19 genotyping has been studied more extensively but conflicting clinical results have not led to any therapeutic recommendations at this time.

Irinotecan

Irinotecan is a key chemotherapeutic agent in the treatment of colon cancer and a variety of other solid tumors. SN-38, the active metabolite of irinotecan, is glucuronidated by uridine diphosphate glucuronosyltransferase (UGT) family, primarily UGT1A1 (47). Genetic variants in the UGT1A1 gene, especially the UGT1A1*28 allele, have been found to be associated with severe toxicities, notably neutropenia and diarrhea. The UGT1A1*28 allele is common in Caucasians (29-45%), Africans (42-51%), and Asians (16%) and has been implicated in Gilbert's syndrome and Crigler-Najjar syndrome. Studies have indicated that patients who are heterozygous and homozygous for UGT1A1*28 had lower maximum tolerated doses of irinotecan compared to those with wild-type alleles (47). Dose reductions for this patient population have not been fully elucidated. The FDA labeling for irinotecan indicates that a reduction in the starting dose of irinotecan by at least one level should be considered for patients who are known to be homozygous for UGT1A1*28 allele (48). CPIC guidelines for irinotecan use in clinical practice have not yet been established.

Rasburicase

Rasburicase is FDA approved for prophylaxis and treatment of hyperuricemia during chemotherapy in adults and children with lymphoma, leukemia, and solid tumors (49, 50). However, rasburicase carries a black box warning indicating that patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency should not receive this drug (49). It is estimated that 5% of the world's population has G6PD deficiency and in certain populations (throughout Asia and Africa) the prevalence may be as high as 30% (50). G6PD is an enzyme important in the pathway associated with nicotinamide adenine dinucleotide phosphate (NADPH) production, a substance that protects erythrocytes from oxidative stress. Erythrocytes that are deficient in G6PD produce lower amounts of NADPH and are therefore at higher risk of oxidative stress and drug-induced hemolytic anemia. Rasburicase is a urate oxidase enzyme that oxidizes uric acid to allantoin and hydrogen peroxide. The administration of rasburicase in patients with known G6PD deficiency has resulted in severe and fatal cases of hemolytic anemia and methemoglobinemia (50). Because of this, rasburicase is contraindicated by the FDA and other agencies in patients with known G6PD deficiency (49, 50). It is recommended that testing for G6PD deficiency should be conducted prior to rasburicase therapy in patients who are at higher risk for G6PD deficiency, such as those with African or Mediterranean ancestry (50). There may be other ancestries, however, that are also at a higher risk of being G6PD deficient. Quantitative enzyme assay should be the preferred screening method due to the variability in G6PD variants that are included in genotype-only tests and high intrasubject variability in females.

Allopurinol

In oncology, allopurinol is often used in the management of hyperuricemia in patients receiving chemotherapy for the treatment of lymphoma, leukemia, and solid tumors (51). The most common indication for allopurinol is for the management of patients with signs and symptoms of primary or secondary gout. In patients with gout, severe cutaneous adverse reactions (SCARs) such as hypersensitivity reactions, Stevens-Johnson syndrome, and toxic epidermal necrolysis have been strongly associated with the human leukocyte antigen (HLA)-B (HLA-B*58:01) variant allele (52). The HLA molecules and genes have an important role in the immune system and HLA-B in particular has been noted to be one of the most polymorphic genes associated with adverse drug reactions. The estimated risk of developing SCAR with allopurinol administration is between 0.1-0.4%, however the mortality risk can be very high (reported up to 25%). The populations at highest risk of HLA-B*58:01 variant and allopurinol-induced SCAR include Taiwan Han-Chinese, Japanese, Korean, Thai, and Europeans (France) (52, 53). Genotyping results are currently reported as HLA-B*58:01 positive (at least one copy of HLA-B*58:01 is present) or negative (no copies of HLA-B*58:01 are detected). For those who are HLA-B*58:01 positive, allopurinol is contraindicated, while patients who are negative may receive standard doses of allopurinol (52, 53). At the time of this writing the FDA has not included HLA-B*58:01 testing in the allopurinol prescribing information (51-53). Drug labeling in Taiwan includes recommendations for HLA-B*58:01 allele testing and Japanese labeling contains precautions (53). It should also be noted that the CPIC guidelines primarily focus on allopurinol therapy for gout (52, 53).

Ondansetron and Tropisetron

The 5-hydroxytryptamine type-3 (5-HT_3) receptor antagonists are used extensively in oncology for the prevention and treatment of chemotherapy and radiotherapy induced nausea and vomiting. Ondansetron is metabolized by the CYP3A4, CYP1A2, and CYP2D6 enzymes to 4 inactive metabolites, whereas tropisetron is metabolized primarily by CYP2D6 to inactive metabolites (54). Other 5-HT_3 receptor antagonists (dolasetron, granisetron, palonosetron, and ramosetron) are metabolized through a variety of other CYP enzymes. The most recent CPIC guideline indicates that patients who are CYP2D6 ultrarapid metabolizers, receiving either ondansetron or tropisetron, may have a higher risk of nausea and vomiting due to increased metabolism of the parent compound (54). If the CYP2D6 genotype is known, it is recommended to consider using an agent that is not predominantly metabolized by CYP2D6, such as granisetron (54). Normal metabolizers may receive standard doses of either ondansetron or tropisetron. There is insufficient evidence for recommendations for CYP2D6 intermediate or poor metabolizers, however these patients could potentially have elevated blood levels of ondansetron placing them at higher risk of QT prolongation (54). Further clinical studies are needed in order to determine this association.

Challenges in Precision Medicine

Based on experiences at other institutions, there have been a multitude of challenges encountered in precision medicine (16, 17, 20, 21, 23, 55). Molecular tumor boards, precision medicine clinics and pharmacogenomic services all require a multidisciplinary approach with appropriate financial, staff, and educational resources. Integration of a good information technology (IT) plat-

form is a critical element to provide genomic information that can be readily shared and interpreted across disciplines. Examples of some limitations and challenges that have frequently been encountered include the following: significant lag time to obtain genomic test results (especially if analysis is conducted off site) and therefore delay in implementation of therapy, timing of genomic testing is often conducted in patients with late stage cancer who have exhausted all other standard therapies, interpretation of test results can be overwhelming and complex, and limited access to targeted therapies and/or clinical trials (16, 17, 20, 21, 23, 55). Genomic test reimbursement and inconsistent payer policies are also significant challenges. Oncolytic drug therapies are very expensive and may be cost prohibitive for many patients and payers. Pharmacogenomic services have not yet been embraced by many institutions and/or oncology pharmacists. Challenges also exist here with respect to costs of tests and lack of reimbursement, turn around time, and interpretation of results. Educational efforts need to continue to improve as many practitioners, even oncologists, have not been adequately trained in molecular biology. There is a compelling need to modernize the genetics content in college curriculums and continuing education efforts to keep practitioners abreast of this ever-evolving field.

Conclusions

Pharmacists are in a prime position to support multi-disciplinary teams in precision medicine by applying PGx to cutting-edge patient centered cancer care. Numerous somatic mutations have been identified that are known to drive cancer. As a result, many therapeutic targets have been developed and many more are in the pipeline. Additionally, PGx is an important and evolving component of precision medicine that can be applied to other medications as well. Dosing strategies based on polymorphisms are important in order to prevent undue toxicities and decrease side effects patients may experience, while still maintaining the medication's clinical efficacy. Numerous opportunities exist in oncology pharmacy practice for precision medicine – molecular tumor boards, MTM, PGx dosing services, patient/caregiver education, continuous professional development, and education for healthcare providers and students. We believe that pharmacists can be a valuable member of the interprofessional team in precision oncology.

Authors' Contributions: Conception and design: CS and DB; Acquisition, analysis, and interpretation of data: CS, DB and DR; Drafting the article: CS, DB and DR; Revising it critically for important intellectual content: CS, DB and DR; Approved final version of the manuscript: CS, DB and DR.

Conflict of Interest: Authors 1: Funded research with Genemarkers, LLC; Author 2: Funded research with Genemarkers, LLC; author of educational materials for RxGenomix and American Pharmacists Association: Author 3: None.

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Original Article
Acta Medica Academica 2019;48(1):105-115
DOI: 10.5644/ama2006-124.247

Implementation and Outcomes of a Molecular Tumor Board at Herbert-Herman Cancer Center, Sparrow Hospital

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Received: 27 December 2018
Accepted: 19 April 2019

Key Words: Molecular Tumor Board ▪ Precision Medicine ▪ Targeted Therapy ▪ Genomic Profiling.

Objective. This paper describes our experience and outcomes from 54 cases presented to the (Molecular tumor board) MTB. **Methods.** 54 Cases presented between July 2017 and April 2018 were included in this analysis. These patients had different types of cancers that had either failed standard therapy or were expected to fail and physicians were looking for future options for anticipated progression. Patients who had obvious mutations and were candidates for Targeted Agent and Profiling Utilization Registry or Molecular Analysis for Treatment Choice clinical trials were not included. Oncologists presented the cases virtually and Foundation Medicine scientific and clinical team discussed the molecular pathways to find targeted options or trials. Tumor board attendees included oncologists, nurses, pharmacists, mid-level providers, residents and staff of the Cancer Center. **Results.** Amongst the 54 cases presented 81% had one or more potentially actionable alteration. 12 (22%) patients received genomically matched therapy as per MTB recommendations. Additional 13 (24%) patients have options available when they progress. Out of 12 patients who got treatment six are alive at the time of this analysis**.** Genomically matched therapy or Clinical Trials option were offered to the 46% of patients based on the MTB discussion. **Conclusion.** More widespread use of molecular diagnostics, better physician education and multidisciplinary collaboration between the staff involved in diagnosis and treatment, as well as third party payers are necessary for consensus on treatment and care of oncology patients.

Introduction

Precision medicine provides new options for cancer treatments and has become an integral part of oncology clinical practice. For some cancers, such as non-small cell lung cancer (NSCLC), precision medicine and genomic profiling is routinely used to integrate targeted treatments (1). Clinical trials with enrollment based on precision medicine have shown us the utility of targeted therapies to block specific molecular pathways activated in cancer (2-5). This is partly possible due to the availability of tumor genomic sequencing technology. These technologies have become more affordable and prevalent, which has led to increasing incorporation of next generation sequencing (NGS)-based comprehensive genomic profiling (CGP) in routine clinical practice. However, the use of CGP for treatment decision guidance is complex for oncologists as it often requires

complex interpretation of molecular biology and genomic results. With the increase in number of approved and investigational drugs, as well as the number of clinical trials incorporating the expanding knowledge of precision medicine, there is an increasing gap that needs to be filled.

Bridging this gap is largely possible in the setting of molecular tumor boards (MTBs). Multidisciplinary tumor boards in oncology are widely acceptable practice. MTBs include participants with a diverse spectrum of expertise and can provide guidance to oncologists seeking to implement such genomic-based personalized targeted therapy in practice (6-10). MTB review also serves as an educational tool, allowing for evidence-based interpretation of the genomic alterations found in each report. When supported by expert genomicists, bioinformatics specialists, pathologists and molecular oncologists, such discussions can provide rapid and accurate data analyses, comprehensive clinical assessment, as well as consideration of up-to-date availability of relevant clinical trials. Indeed, such MTBs are being established and successfully implemented for treatment decision support and for the guidance of optimal utilization of CGP in the clinic (6, 7, 9, 10).

This article describes the experience of a multidisciplinary MTB, which reviewed molecular profiling reports (Foundation Medicine, Cambridge, MA) of 54 advanced cancer patients with solid tumors who had exhausted or were likely to exhaust standard of care (SOC) options including available clinical trials at our own institution. All patients discussed at the Sparrow Hospital Herbert-Herman Cancer Center (HHCC) MTB between July 2017 and April 2018 are included in this analysis. The tumor board weighed evidence for actionability of genomic alterations identified by the molecular profiling and discussed possible treatment options.

Methods

The MTB at our cancer center was launched in July 2017 and met twice a month for 60 minutes each month in 2017 and then switched to once a month in 2018. The MTB comprised of medical and radiation oncologists, nursing, pharmacy and clinical trials staff from Sparrow Hospital, and was done virtually with the Foundation Medicine (FM) team including a genomicist and molecular oncologist. At each session four to five cases were presented and discussed in detail. These cases were referred by the treating oncologists. All information was de-identified in compliance with the Health insurance portability and accountability act (HIPAA). Patients and families were informed about the MTB decision making process when their case was referred for the discussion. The recommendations from the MTB were sent to each physician individually by email and maintained on the shared drive for future reference. This was discussed with the patients/families by the treating oncologist. If there was any change in treatment based on the MTB recommendation the new therapy was started only after the patients were educated by the nurses or pharmacist and patients were consented for the treatment.

The patient's treating physician or the senior oncologist, a clinical trials director, or a designated representative (e.g. physician assistant or Clinical trials specialist) presented the patient's case giving concise medical history including the date of diagnosis, type of tumor, therapies received and the relevant markers. This was followed by discussion from the FM genomics scientist and molecular oncologist of the molecular profiling results and implications for each case. Information discussed included the alterations detected in a given sample, their level of characterization and potential actionability. Targeted or immunotherapies

therapies matched to each alteration detected and approved in the patient's tumor type or in another tumor type, as well as openly enrolling genomically-matched clinical trials were also discussed. This was solely an advisory discussion. The ultimate decision to choose the therapy was left to the treating physicians.

Patients whose cases were selected for the MTB discussion had a range of different solid tumor types (n=53) or lymphoma (n=1). At the time of the MTB they had either failed standard therapy or were expected to fail and their physicians were looking for future options for anticipated progression. Patients who were obvious candidates for any of the open clinical trials at our site including the Targeted Agent and Profiling Utilization Registry (TAPUR) and the National Cancer Institute's Molecular Analysis for Treatment Choice (NCI-MATCH) Study were not selected for MTB discussion. Similarly, patients with clear matches to Food and Drug Administration (FDA) approved therapies in their tumor type were not selected for MTB discussion. Only the cases where the specific genomic mutation was not a direct match to an approved treatment or available clinical trial were selected for presentation to the MTB. By a direct match we meant if the patient's genomic mutation directly matched with the approved therapy. For example, if it was EGFR positive then treat with EGFR targeted treatment or if it was MSI high we will treat with FDA approved Immunotherapy. If after screening patients were eligible based on the genomic target to the list of available drugs on TAPUR or Match they would be enrolled on one of the clinical trials.

Hybrid capture-based comprehensive genomic profiling (Foundation Medicine, Cambridge, MA) was performed on 56 samples from 55 unique patients for 315 genes on submitted FFPE tissue samples (n=50), for 405 genes on whole blood (n=3), or for 62 genes on circulating tumor DNA isolated from submitted blood samples (n=3) as previously described (11-13). Most of the patients were sent for genomics when they progressed. However, if it was not possible to get fresh tissue, archival tissue was used from the initial diagnosis. Genomic alterations including base substitutions, insertions/deletions, copy number changes, and rearrangements were assessed, as well as determination of tumor mutational burden (TMB) and microsatellite instability (MSI) status (14, 15).

Results

Patient Characteristics

Patients presented from July 2017 to April 2018 were included in this analysis. CGP results for a total of 55 patients were presented for MTB discussions. One patient discussed in the MTB had lymphadenopathy only and did not have cancer so was excluded from analysis. All other patients (n = 54) were heavily pretreated advanced cancer patients who had exhausted or were likely to exhaust SOC options including available clinical trials at our own institution. Only those patients whose oncologist could not easily identify an appropriate genomically-matched treatment option from the CGP report and thus required the knowledge of the genomics and bioinformatics team were selected for MTB discussion (Figure 1).

Among the tumor types presented the majority of cases were gynecological malignancies (28%, 15/54) followed by breast carcinoma (17%, 9/54), colorectal carcinoma (9%, 5/54), non-small cell lung carcinoma (9%, 5/54), or other tumor types (37%, 20/54).

Demographics of the patients discussed are represented in Table 1. Median age was 64 years (range 37-82) and 69% (37/54) were females. Patients discussed at this MTB had an average of 2.4 prior lines of therapy be-

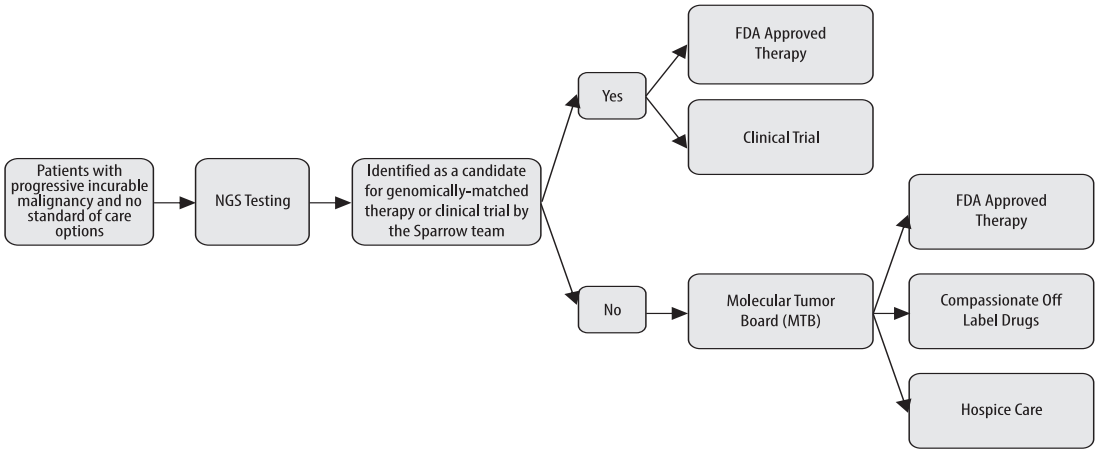

Figure 1. Flow chart depicting selection of patient cases for the Sparrow Health MTB and resulting treatment options.

Table 1. Patient Demographics and Disease History

Demographics	MTB Patients (%)
No. patients	54
Median Age (years)	64
≥65 years	26 (47)
<65 years	28 (53)
Gender (Male:Female)	17 (31):37 (69)
Disease histology	
Gynecological	15 (28)
Breast	9 (17)
NSCLC	5 (9)
CRC	5 (9)
Other	20 (38)
Number of prior lines of therapy	
Mean	2.39
Median	2
1 line	14 (26)
2 lines	18 (33)
3 lines	14 (26)
4 lines	5 (9)
5 lines	1 (2)
6 lines	2 (4)

MTB=Molecular tumor board; NSCLC=Non-small cell lung cancer; CRC=Colorectal cancer.

fore CGP was performed; 74% (40/54) of patients had received ≥1 line of therapy and 15% (8/54) of patients received ≥ 3 lines of prior therapies. At the time of analysis 32 patients (59%) were still alive.

Genomic Alterations and Potential Treatment Options Identified

Of the 54 total patients, 100% had potentially actionable alteration(s) identified by CGP. An actionable alteration is defined by being linked as either a positive or negative biomarker for an approved therapy or enrollment criteria for an open clinical trial. (Personal communication) Thirteen patients (24%) had alterations with matched therapy in their tumor type, 25 patients (46%) had alterations with matched therapy in another tumor type, and 16 patients (30%) were identified with alterations with a genomically matched clinical trial options (Figure 2). In 76% (41/54) of cases, more than one potentially actionable alteration was identified.

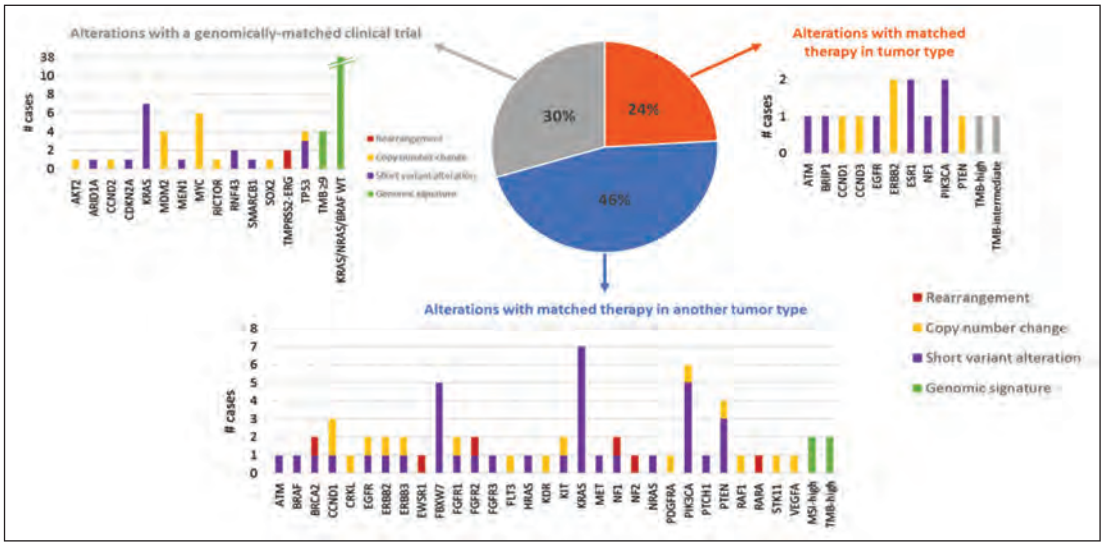

Figure 2. Distribution of potentially actionable alterations identified using CGP. Actionability was assessed at the time of reporting for a given case. Note: a therapy may be approved in a patient's tumor type, but the patient still may not qualify for the therapy based on the specific FDA label. TMB ≥9 mutations/Mb is used as a designator for clinical trial eligibility specifically due to the enrollment criteria for the TAPUR trial pembrolizumab arm, which was open at the time of this study. TMB-intermediate and TMD-high designations for therapy associations were made by Foundation Medicine.

The distribution of potentially actionable alterations identified is shown in Figure 2.

Treatment Assignments and Patient Outcomes

We further analyzed how MTB discussions influenced the implementation of treatments in our patients. Twelve (22%) patients received a genomically-matched therapy based on CGP results and MTB discussion (Table 2). Of note, 2 patients received what was assessed to be a genomically-matched treatment based on MTB discussion and the treating physician's discretion, but the therapy received was not listed on the CGP report (Table 2, patients 8 and 11). Out of these 12 patients, 9 had stable disease (SD) as their best response to matched therapy, and 3 had progressive disease (PD) as assessed by recist 1.1 criteria. At the time of follow-up 9 had progressed and 3 maintained SD. The median follow-up period was 17 months. Patients who eventually progressed stayed on treatments between 3 and 15 months. Average time to progression was 7.6 months. Six out of 8 patients who had progressed were alive at the time of analysis (median time to follow up=17 months). An additional 13/54 (24%) patients are anticipated to receive matched treatment options when they progress on current SOC therapy.

Five out of 54 (9%) patients had at least one potential genomically-matched therapy option identified, but we could not get approval from insurance (n=4) or the patient did not qualify for available trial(s) primarily due to poor performance status (n=1). Three patients received treatment on label as recommended by MTB. The treating physician did not recognize the direct match and referred to the MTB and the tumor board discussed and recognized the match to the therapies. If those patients were not presented at MTB they would not have gotten these therapies. Six patients (11%) had genomically-matched options available, but the treating physician chose a different option. This was due to other available agents judged to be more effective than targeted

Table 2. Clinical and Genomic Characteristics of Patients' Treatment Based on MTB Discussion

Patient	Diagnosis	Matched alteration	Matched targeted therapy	PFS (months)	Best Response
1	Anus SCC	KRAS/NRAS/ BRAF WT	Cetuximab on trial	15	PD
2	Uterus Endometrial Adeno	ERBB2 amplification	Trastuzumab on clinical trial	6	SD
3	Ovary granulosa cell tumor	CDKN2A p16INK4a A60fs*89	Palbociclib on trial	3	PD
4	Ovary serous carcinoma	ATM D2721M	Olaparib (FDA-approved on-label)	SD	SD
5	Breast carcinoma (NOS)	BRCA2 V1988I	Olaparib (FDA-approved off-label)	8	SD
6	Lung adenocarcinoma	EGFR exon 19 del + T790M	Osimertinib (FDA-approved on-label)	SD	SD
7	Lung SCC	KRAS/NRAS/BRAF WT	Cetuximab on trial	4	PD
8	Adrenal gland cortical	FGFR2-CIT fusion	Sunitinib on trial*	5	SD
9	Breast ILC	ESR1 Y537N	Fulvestrant on label	10	SD
10	Ovary serous carcinoma	MSI-H and TMB 19 mutations/Mb	Pembrolizumab on trial	12	SD
11	Colon Adenocarcinoma	TMB 8 mutations/Mb	Pembrolizumab off label†	SD	SD
12	Breast carcinoma (NOS)	CCND1 amplification	Palbociclib on label	5	SD

MTB=Molecular tumor board; PFS=Progression free survival; SCC=Squamous cell carcinoma; WT=Wild Type; PD=Progressive disease; SD=Stable disease; NOS=Not otherwise specified; ILC=Invasive lobular carcinoma; MSI-H=Microsatellite instability high; TMB=Tumor mutational burden; Mb=Megabase. *This patient was approved for the sunitinib arm of the TAPUR trial based on the FGFR2-CIT fusion alteration detected; however, sunitinib was not one of the matched therapies listed on the CGP report. †This patient was approved for insurance coverage of off label pembrolizumab based on MTB discussion and TMB of 8 mutations/Mb; however, pembrolizumab was not listed as one of the matched therapies on the CGP report.

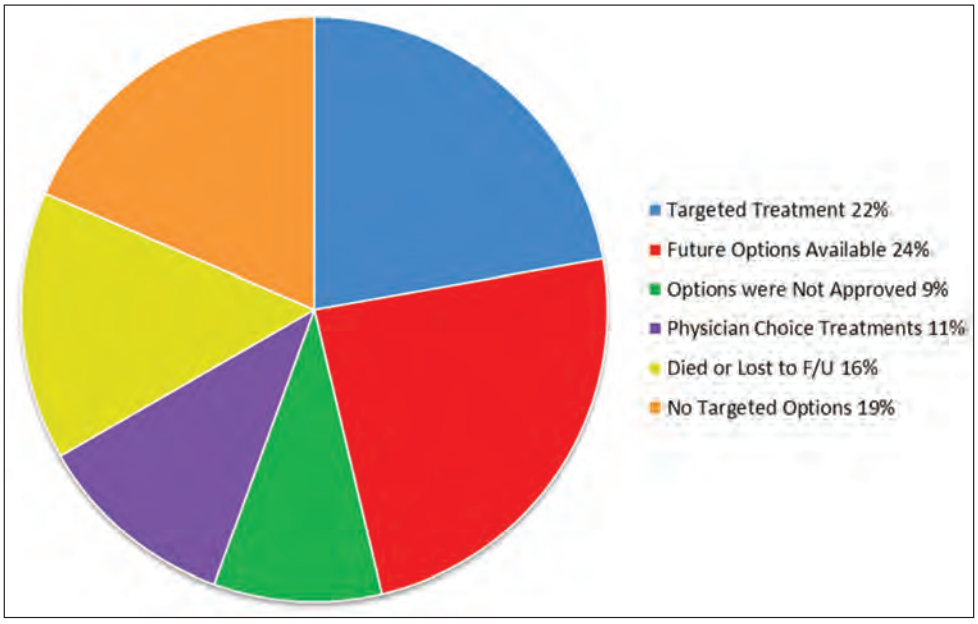

Figure 3. Patient Treatment Assignments Based on Molecular Tumor Board Discussions.

therapy or inability of the patient to travel to far away sites for treatments. Eight patients (16%) either died prior to planned treatment or refused further treatments. One patient was lost to follow up and ten patients (19%) did not have targetable options, even though they had mutations (Figure 3). In total, a genomically matched therapy or clinical trial option was able to be offered to the patient in 81% (44/54) of cases based on the MTB discussion.

Discussion

There is an increasing body of evidence based on prospective and retrospective studies, case reports, and clinical practice showing that matching targeted agents with genomic alterations improves patient outcomes (16). Clinical reports suggest that 30%-80% of advanced solid tumors harbor potentially actionable genomic variants (17). Meta-analysis of 570 Phase II studies of new anticancer agents, done on 32,149 patients showed that personalized approach correlated with statistically significant higher median responses rates, prolonged median progression free survival and improved overall survival (18). Additional Meta analyses by Schwaederle M et al. also demonstrated benefit for patients treated with personalized matched therapy (19, 20). Prospective molecular profiling studies by Stockley et al. demonstrated that treatment with genotype matching in early phase was associated with an increased objective tumor response (13). Wheeler et al. reported that use of CGP to assign therapies in patients with multiple genomic aberrations was associated with longer time to treatment failure and stable disease in patients with refractory malignancies (21). In the multicenter open label phase 2 trial (SHIVA) authors showed that molecularly targeted agents did not improve significantly medium progression free survival (PFS) when compared to physicians' choice of treatment. However, there was a signal for very slight improvement in the PFS, 2.3 versus 2 month in experimental group vs. the control group. This French trial limited molecular alterations to ones identified within 3 molecular pathways (hormone receptor, PI3K/AKT/mTOR, RAF/MEK) which is the limitation of this study (22).

Another prospective trial, emulating clinical benefits of high throughput genomic analysis in clinical practice, MOSCATO – 01 showed that high throughput genomics could improve outcomes in a subset of patients with hard-to-treat cancers. Although only 7% of successfully screened patients benefited from this approach, we think that with the further refinement of this approach higher or larger number of patients will benefit (23).

However, implementation of genomic-based precision medicine in oncology represents major challenge due to depth of knowledge and expertise required to make decisions which will benefit patients. Obstacles to implementation of precision medicine in clinical practice are particularly high in community practices. They include time-consuming analyses of results of molecular testing, determining clinical trial eligibility, molecular test selection, determining the optimal time for molecular testing, financial concerns, genetic counseling and particularly patient attitudes. MTBs overcome some of those obstacles by providing necessary expertise in a multidisciplinary setting. The MTB at HHCC was established in July 2017 as cooperative multidisciplinary board in association with FM and in a short period showed to be of great benefit for our patients. We found targetable non-KRAS alterations in 81% of cases. This is similar to results reported by other molecular tumor boards (39-86%) (10, 24, 25). These percentages depend upon definition of actionable alterations, and are sensitive to selection bias, since it is expected that physicians will most often

submit cases for discussion at MTBs they believe have potentially actionable alterations detected by CGP. With advancement in standardization of variant calling and reporting we can expect that differences and biases will be reduced and results from different studies will become more comparable. In the case of our MTB, patients were selected when they did not have a clear choice for a genomically-matched clinical trial open at HHCC and did not have a direct match to an approved targeted therapy. However, we also discussed and recommended future treatments for patients who were still stable or responding to present treatment. This may be specific for our MTB and could skew results toward higher numbers.

Patients treated based on recommendations from our MTB (n=12) benefited from treatment and those who ultimately progressed (n=9) stayed on treatments between 3 and 15 months (mean 7.6 months). Mean progression free survival on the prior therapy for these patients (n=9) was 4 months. Six out of 8 patients who eventually progressed were still alive at the time of analysis. An additional 3 patients are still being treated with matched therapy and have Stable disease (SD). All these patients had very advanced disease and the only other option was symptom control and Hospice. Data from 126,620 patients extracted from the electronic medical records of 10 hospices in the CHOICE network (Coalition of Hospices Organized to Investigate Comparative Effectiveness) showed that 93.6% of those patients died within 6 months (26).

One of the characteristics of our MTB was that 13/54 patients were still responding or were stable on previous treatment at the time of the MTB. NGS testing in these patients was done mostly due to patients', families' and physician's anxiety and need to have other available options. Similar observations were made by Schwaederlea et al. and could be considered as a limitation related to the current use of molecular diagnostics. The authors believe that early, and maybe premature testing is related to the time to obtain results (in their case median of 27 days). Consequently, physicians are ordering tests before patients have failed previous treatment (8). In the case of our patients all 13 have potential molecular targets identified by CGP when they progress. We expect that with better and more efficient work flow between local pathology and molecular diagnostic companies' time to obtain results will be significantly reduced and delays will be eliminated. That will taper patients' and physicians' anxiety and bring more appropriate timing of testing.

One of the main concerns from analysis of our MTB results was that 6/54 patients had available molecular targets, but were still treated with chemotherapy by their treating physicians. In addition, 8/54 patients refused recommended molecular treatment or died before it could be applied. Patient's refusal at least partially can be explained by physician's hesitance to use molecular targeted therapy. This is not unexpected since most of the presently practicing oncologists are trained in the era of the "evidence-based medicine" and use of cytotoxic chemotherapy. Although far more informative and accurate than its predecessors of intuition and the "art of medicine", the unfortunate consequence of the approach of "evidence-based medicine" is that outliers are not represented, and they may be unlikely to respond similarly to the average patient for any given treatment. Precision or personalized medicine, in contrast, focuses on the individuals and seeks to improve health outcomes by integrating a huge variety and number of data points, from genomics to environmental and lifestyle factors, in order to provide an individualized approach to health care (27). Although molecular diagnostics use and practice at HHCC is considered advanced, it is still necessary to improve education and

participation of all treating physicians. MTBs by their structure represent ideal vessels for education, collegiate interaction, multidisciplinary discussion and finally creation of consensus on treatment and care of patients. However, they require full participation of and interaction between all involved participants. Otherwise, opportunities will be missed. There are definite obstacles that need to be overcome, in particular limited available time, especially in busy practices where physicians' income is based on number of patients seen. In order to resolve this important issue, it is necessary to have better understanding of precision medicine by policy makers, third-party payers, hospital administrations, patients and the general public. Development of clinical decision algorithms based on molecular testing and available targeted therapies will make resolution easier. Expected results from precision medicine trials including the National Cancer Institute NCI-MATCH and IMPACT (1-3), and ASCO-TAPUR (17) could help to clarify the role of precision medicine and consequently MTBs in the every day s care of oncology patients.

Need for education and collaboration between providers and third-party payers is emphasized by the number of patients who had molecular targeted options identified, but were refused treatment coverage by payers (n=4), as well as patients who refused treatment (8/54). The main reason for these decisions are, in our opinion, costs of the medications, out-of-pocket costs for patients and/or overall costs for third party payers. Bryce et al. (28) had similar experience with their patients at a Mayo MTB where 6% of the patients with targetable mutations were not able to receive targeted therapy due to insurance denying payments. Hopefully, the increasing trend to incorporate molecular testing and targeted therapy into National Comprehensive Cancer Network (NCCN) and American Society of Clinical Oncology (ASCO) guidelines will facilitate approval in these cases. It is also our recent experience that some, but not all, third party payers are more inclined to approve targeted therapy based on valid molecular testing.

In 10/54 cases patients did not have targetable options as assessed by the MTB. In these cases therapy or trials were identified linked to a KRAS mutation only (low level of evidence for efficacy) and none of these patients received genomically matched therapy. In 1 additional case the only "genomic match" for actionability was not a directly targetable alteration, but rather option for the KRAS/NRAS/BRAF wild type (WT) cetuximab TAPUR arm. These data argue that CGP identifies potentially actionable alterations in a large majority of patients, but more published evidence for genomically-matched targeted therapy, better access to drugs and trials, more investment into education, better collaboration between all parties vested into patients' care and possibly more appropriate timing of NGS testing (so patients do not die before getting treatment) is needed.

Conclusion

The MTB is multidisciplinary platform for discussion, treatment recommendations and knowledge acquisition related to genomic testing and precision oncology. Although precision medicine is progressing in breathtaking pace, practice of MTB's is lagging behind. In most of the cases it is limited to large Academic centers. This paper presents model of collaboration between community cancer center and sophisticated technology company that ultimately improves oncology patients' care. This model can be used, with local modifications, in other community centers and bring advantages of precision medicine to more than 80% of all oncology patients, who are treated in their local communities.

Acknowledgement: We acknowledge Foundation Medicine team for their help throughout the establishment, operation and scientific discussions of Molecular Tumor board case. Their help and feedback in the preparation of this manuscript is appreciated. We acknowledge the help of Kathryn Edwards, Clinical Research Assistant, Clinical Trials Office, Sparrow Herbert-Herman Cancer Center for the preparation of the manuscript.

Authors' Contributions: Conception and design: GS and HT; Acquisition, analysis and interpretation of data: GS, HT, DA, UC, JM, HA-A, MH and JH; Drafting the article: GS and HT; Revising it critically for important intellectual content: GS and HT; Approved final version of the manuscript: GS, HT, DA, UC, JM, HA-A, MH and JH.

Conflict of Interest: Dr. Srkalovic is on the speakers bureau of Foundation Medicine. None of the other authors have declared any conflict of interest associated with this work.

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Case Report
Acta Medica Academica 2019;48(1):116-120
DOI: 10.5644/ama2006-124.248

TNS1-ALK Fusion in a Recurrent, Metastatic Uterine Mesenchymal Tumor Originally Diagnosed as Leiomyosarcoma

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Received: 16 September 2018
Accepted: 30 April 2019

Key Words: Alk ▪ Leiomyosarcoma ▪ IMT ▪ Kinase Fusion ▪ CGP.

Objective. We report a female patient diagnosed with a leiomyosarcoma and who harbored a druggable target as identified by comprehensive genomic profiling in the course of clinical care. **Case Report.** The patient progressed five years after curative intent surgery and adjuvant treatment. After failure of multiple lines of chemotherapy,she was enrolled in a trial of an ALK inhibitor based on comprehensive genomic profiling (CGP) identifying an TNS1-ALK fusion. **Conclusion.** In this case, identification of the ALK kinase fusion permitted enrollment in a matched mechanism driven clinical trial after exhausting standard of care treatment options. CGP raises the possibility of uterine inflammatory myofibroblastic tumor as an alternative diagnosisto leiomyosarcoma, highlighting the complementary role of CGP beyond immunohistochemical analyses.

Introduction

Leiomyosarcoma (LMS) is a malignant tumor arising from smooth muscle, typically of uterine origin (1). Management of disseminated or recurrent disease is challenging, with cytotoxic chemotherapy being the backbone of treatment despite poor outcomes with a median survival of 27 months from first disease recurrence (2). Diagnosis of LMS, especially of the myxoid type, may also be challenging since a subset of cases may be re-classified as either inflammatory myofibroblastic tumor (IMT) or high-grade endometrial stromal sarcoma (ESS) based on both morphologic, immunohistochemical and molecular features (3). Distinction of these entities may affect therapeutic decision-making as IMT and ESS may be less aggressive and more amenable to targeted therapies than LMS. Specifically, IMTs frequently harbor fusions of the ALK kinase that may be susceptible to ALK inhibitors, well established and approved agents in non-small cell lung cancer (4-6). A complementary role for molecular testing in advanced tumors is established, and comprehensive genomic profiling (CGP) has demonstrated ability to both re-classify tumors and distinguish recurrence from a second primary (7).

Here we present a case of a malignant and high stage uterine mesenchymal tumor, originally diagnosed as LMS, which after progression on LMS standard therapy was

found to harbor an ALK gene fusion by genomic profiling. This molecular finding enabled the patient to be enrolled in a clinical trial with the ALK-inhibitor crizotinib and suggested that the tumor was potentially a previously under-recognized IMT.

Methods

Comprehensive genomic profiling was performed on the Foundation One Heme panel (Foundation Medicine, Cambridge, MA, USA) in a CLIA certified laboratory. This method consists of the analysis of the coding DNA sequences of 405 cancer-related genes, selected introns of 31 genes involved in chromosomal rearrangements, and the RNA sequences of 265 genes commonly involved in fusions. The genes were sequenced to a median coverage of 859X and the sequences were analyzed for base substitutions, indels, copy number alterations, and rearrangements.

Case Presentation

A 39-year old African-American woman initially presented with a diagnosis of leiomyoma and ovarian cyst though concern for invasive disease remained. Therefore, a TAH-LSO was performed, and microscopic examination of the specimen identified a leiomyosarcoma. A completion BSO, omentectomy and tumor debulking was then performed, and LMS involving the omentum, right fallopian, and multiple colonic nodules was confirmed based on immunohistochemical and morphologic examination. She received six cycles of adjuvant gemcitabine and paclitaxel followed by radiation therapy. She was under surveillance with bi-annual imaging when a pelvic/bowel recurrence was identified 4 years from her original diagnosis. After surveillance imaging suspicious for recurrent disease, she underwent a PET-CT notable for a left pelvic mass superior to the bladder measuring 3.9cm with an SUV 10 and a cul de sac mass measuring 4.2 cm with an SUV of 5.3. She underwent a salvage partial colectomy and pathologic examination identified distal sigmoid colon and proximal rectal masses that were morphologically consistent with metastatic leiomyosarcoma. She was treated with palliative doxorubicin and olaratumab but imaging after the second cycle showed progression with an enlarging pelvis mass measures 5.8 × 5.6 cm with SUV 7.3, previously 2.9 × 3.1 cm with SUV 6.7. Two months after the third cycle of treatment, a pelvic mass was resected, which was again diagnosed as metastatic LMS and adherent to the small bowel serosa, without bowel wall involvement. A nodule on the sigmoid colon was also interpreted as LMS. Two months after surgery, adjuvant pazopanib at 400 mgs daily was initiated, and then switched to 400/600 mg alternating daily due to poor tolerance and fatigue.

After 12 months on pazopanib she presented to an emergency room with pain and was found to have progressive disease. She was transitioned to trabectedin but tolerated it poorly, and imaging after two cycles confirmed further disease progression. In an effort to explore all possible treatment options a specimen from the salvage colectomy was sent for comprehensive genomic profiling as previously described (8). CGP identified a predicted oncogenic TNS1-ALK fusion which was also observed in a complementary circulating tumor DNA assay (Figure 1). Based on her CGP results she was screened for a precision medicine clinical trial (NCT02693535). Owing to impaired renal function from extrinsic compression by the large pelvic mass (Figure 1A) she was deemed ineligible. In the absence of ability to get on trial she was transitioned to Brigatinib off-label. Within 2 weeks she had significant improvement in pain and was able to come off all opioids. First imaging re-

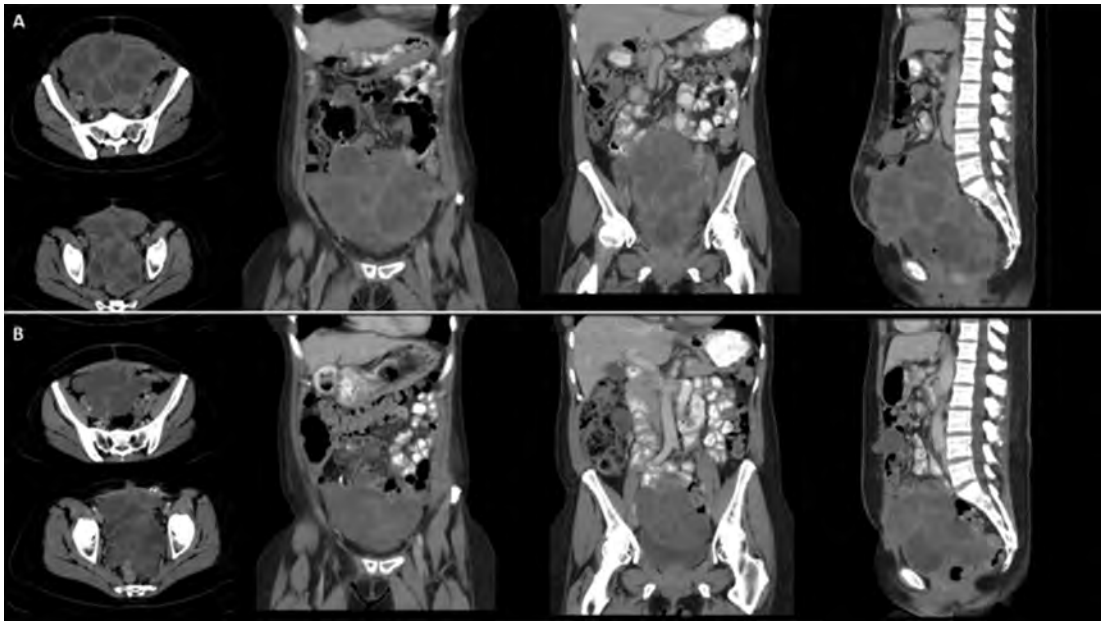

Figure 1. Rapid treatment response in a symptomatic LMS patient whose tumor was found to have an ALK-TNS1 fusion on comprehensive genomic profiling. Images are shown: (A) prior to brigatinib and (B) after only 35 days on brigatinib 180 mg by mouth daily.

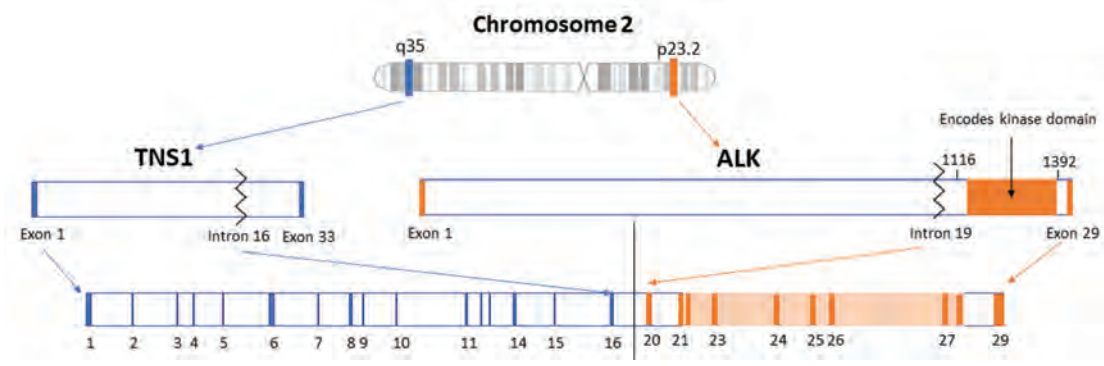

Figure 2. ALK fusion in Leiomyosarcoma. A fusion between TNS1 and ALK with breakpoints identified in intron 16 and 19 respectively was identified in the salvage colectomy specimen.

vealed radiographic partial response (Figure 1B) which has continued for 9 months at the time of submission. There were no adverse events on Brigatinib 180 mg once daily.

Discussion

Morphologic and immunohistochemical (IHC) analyses remain important tools in classifying solid tumors, but can be complicated by inter-observer variability, overlapping IHC and morphology in rare tumors. Molecular testing is a complementary tool, capable of both identifying pathognomonic molecular alterations that aid in diagnosis while simultaneously exploring rare variants that exist across tumor types. The case presented here supports the clinical utility of CGP in advanced, recurrent, and refractory cancers.

Recent work has demonstrated that inflammatory myofibroblastic tumor (IMT) is strongly associated with kinase fusions, particularly ALK fusions (9, 10). Notably, IMTs often arise in the uterus, and as such can be

misdiagnosed as leiomyosarcoma, particularly the myxoid variety (11). A case reported previously had such myxoid features and was diagnosed as a smooth muscle tumor of uncertain malignant potential, but harbored an ALK fusion and was ultimately revised to a diagnosis of uterine IMT (12). In a large series of myxoid LMS, 4 of 30 cases (13%) were potentially under-recognized IMT based on either ALK positivity by immunohistochemistry or ALK gene rearrangements (3). The diagnosis of each is based on morphologic and immunohistochemical characteristics, but the identification of an ALK fusion/rearrangement may greatly aid in the diagnosis of difficult cases. Indeed, after re-review of morphology of available H&E image and molecular profile, this particular tumor may be best re-classified as an IMT. At present, there are multiple reports of uterine IMT patients who harbor an ALK fusion and who have responded to an ALK inhibitor (10, 13, 14). Molecular profiling of recurrent, metastatic uterine mesenchymal tumors may potentially aid in the diagnosis of difficult cases and enable patient enrollment in appropriate clinical trials. Owing to the rare nature of LMS and other uterine sarcomas compounded by the relative rarity of actionable alterations, randomized prospective trials are unlikely to be completed. Multi-arm basket trials matching treatments to genomic alterations, such as the ASCO TAPUR study, may be the optimal mechanism for prospective efficacy assessment as was done for this patient.

Conclusion

Identification of the TNS1-ALK fusion through CGP allowed for this tumor, originally diagnosed as LMS, to be recharacterized as an IMT. The patient also benefitted from targeted therapy, which highlights the dual role that genomic profiling can play in aiding with classifying diagnostically challenging cases and enabling patients to benefit from matched targeted therapy typically within a mechanism driven clinical trial.

What Is Already Known on this Topic

Diagnosis of leiomyosarcoma based off morphologic and immunohistochemical characteristics can be challenging and a number of cases may be reclassified as IMTs as IMTs frequently harbor kinase fusions, particularly those of ALK, which can also allow for treatment with matched targeted therapy.

What this Study Adds

We report a patient with a tumor originally diagnosed as LMS that failed multiple lines of chemoradiation. CGP revealed that the tumor harbored a TNS1-ALK fusion that led to the revision of the patient's diagnosis to uterine IMT and subsequent enrollment in an ALK inhibitor trial.

Authors' Contributions: Conception and design: SMA and SJK; Acquisition, analysis, and interpretation of data: JL, AS, SMA, DIL and SJK; Drafting the article: SMA and JL; Revising it critically for important intellectual content: SMA, JL and SJK; Approved final version of the manuscript: JL, AS, SMA, DIL and SJK.

Conflict of Interest: JL, DIL, and SMA are employees of and have equity interest in Foundation Medicine, a wholly owned subsidiary of Roche Pharmaceuticals. SMA serves on the SAB of Incysus Inc., and consulting with Revolution Medicines and Takeda (non-oncology). AS declares honoraria/consultant fees from Eli Lily, Novartis, Daiichi Sankyo, and serves on the Board of Directors for Certis Oncology Solutions. SJK declares honoraria from Foundation Medicine, Inc., and consultant/advisory fees from Eli Lilly, Astellas, Boston Biomedical, and has ownership/equity in TP Therapeutics.

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Historical Article

Narrative Review
Acta Medica Academica 2019;48(1):121-126
DOI: 10.5644/ama2006-124.249

Dr. Anna Bayerová: The First Official Female Doctor in Bosnia and Herzegovina

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Received: 1 April 2019
Accepted: 25 April 2019

Key Words: Medical History of Austro-Hungarian Occupied BH ▪ Treatment of Muslim Women by Female Physicians ▪ Anna Bayerová ▪ Bayerová's Medical Practice in Tuzla ▪ Bayerová's Political Activities in Sarajevo.

This biographical note details Anna Bayerová's (1853–1924) activities as the first female Austro-Hungarian health officer in 1878 to1918 occupied Bosnia and Herzegovina (BH). Anna Bayerová is known as a heroine of Czech feminism and the 'first Czech female physician', though she only practised in the Czech lands from 1913 to 1916. In 1891, Bayerová was enrolled as the first Austro-Hungarian female health officer and assigned to treat Muslim women in the district of Tuzla, Bosnia. She pursued this mission for the first three months of 1892, had herself transferred to Sarajevo in the summer, and soon thereafter quitted the service. Her biographers point to a series of political and personal motivations to abandon her mission in Bosnia, which, from the viewpoint of Czech feminists, included fulfilling her professional duties in an exemplary way. She spent most of her professional life as a physician in Switzerland and did not request Austrian recognition of her medical degree until 1913. Bayerová died in Prague in 1924. **Conclusion.** Bayerová, partly for political reasons and partly due to her panic-fuelled fear of catching tuberculosis, quitted her role as the first Austro-Hungarian female health officer in BH soon after her arrival in 1892.

Introduction

Anna Bayerová (1853–1924), a heroine of Czech feminism, is known as the 'first Czech female physician'. Several detailed biographies have been dedicated to her memory (1-4), and she appears in contemporaneous Czech (5) and German (6, 7) national physicians' listings and international female physicians' encyclopaedias (8). In Switzerland, the German Empire and Austria, her name was once Germanised as 'Anna Bayer'.

Bayerová finished her medical studies in Berne in 1881 and spent most of her active live in Switzerland where she became involved into the anti-alcohol campaigning linked to contemporaneous European movements of temperance and social reform (9). She owed her prominence to her close ties with the Czech women's movement and her lead role in Czech feminists' and Austrian social democrats' campaigns to admit women to academic, particularly medical, educational institutions. These parties felt that female physicians would improve poor women's and children's access to healthcare (10). Bayerová helped to articulate this mission in *Ženské Listy,* the journal of the Czech women's movement, from the early 1880s (11).

When in 1891 Bénjamin de Kállay, Austria-Hungary's Joint Minister of Finance and de-facto governor of the occupied Ottoman province Bosnia and Herzegovina (BH), created a position for an Austro-Hungarian female health officer, Bayerová was considered predestined to pioneer the broader implementation of a feminist concept of public health and women's studies in Austria (12). Austro-Hungarian universities did not admit female students until 1897, but Kállay was convinced that the prioritized modernization of BH required extraordinary measures, not least of which included employing female physicians to educate the rural (female) population about public health and hygiene (13).

First, Kállay agreed with contemporary international (14) and Czech feminist arguments that the improvement of working class and poor rural women's and children's health depended on a public health system with state-employed female physicians (14). Second, a systematic Austro-Hungarian health census of the Bosnian population had revealed the spread of infectious diseases such as peculiarly (not sexually transmitted) endemic syphilis (*frenjak*), the eradication of which was considered another Austro-Hungarian priority regarding the 'occupied territory' (15). Third, the fact that 35 percent of Bosnia's population was Muslim (16) justified the argument that women's healthcare be performed by female physicians to accommodate 'religious modesty' (17). Thus, Kállay avoided any protest from Austro-Hungarian medical bodies, while the Czech women's movement succeeded in placing Anna Bayerová as the first female Austro-Hungarian health officer in BH. However, Bayerová is not sufficiently recognized in the medical historiography of BH (18-20), so this paper will be the first article written only about Dr. Anna Bayerova published in BH.

This paper aims to present a short biography of Bayerová and her work as the first female doctor in BH, based on the vast Czech literature on her life and activities (21, 22, 13).

Bayerová's Short Biography

Bayerová was born into a lower-middle-class family in Melník, a small town 30 kilometres north of Prague, on November 4, 1853.[1] She attended the first Czech collegiate school for girls, which had been founded by the Czech Women Professionals' Association in Prague (Ženský Výrobní Spolek Český v Praze). The doyenne of the Czech womens's movement and editor in chief of *Ženské* Listy, Eliška Krásnohorská herself seems to have encouraged Bayerová to realize her 'great dream' of becoming a physician and raised funds for her to attend a Swiss university (23).

In 1874, Bayerová took the general qualification for university entrance exam (*Matura*) in Switzerland and enrolled at the University of Zurich. In 1877, she transferred to the University of Berne and earned her medical degree in 1881, one year after her compatriot Bohuslava Kecková (1854–1911) was awarded a degree in Zurich. However, because of Bayerová's involvement in the Czech women's movement, she was the one who would be celebrated in *Ženské Listy* and the *Journal of Czech Physicians* as the 'first Czech female physician' in 1881.

In 1882, Bayerová volunteered as an assistant doctor at the Royal Maternity Clinic in Dresden, directed by the renowned German gynaecologist Franz von Winckel (1837–1911), who supported 'women's need of female physicians' ('Aerztinnen für Frauen' in German) (24). Subsequently, Bayerová took over a medical office in Teufen, a village near St. Gallen in Switzerland, which proved to be poorly attended. In 1887, she

[1]Bayerová's earlier biographers gave 1854 as the year of her birth, while more recent international biographies state it as 1852. Following Czech sources, the correct year is 1853.

requested and received nationalization in Switzerland in order to establish her own practice in Berne (23).

In 1891, Krásnohorská urged Bayerová to apply for the advertised position of a female physician in Tuzla, a small town in the north-east region of BH. She was accepted by de-facto governor Kállay, who summoned her for a confidential conversation in Vienna in December. According to Bayerová, Kállay instructed her personally to educate Bosnian Muslim women about hygiene (21). She was immediately transferred to Sarajevo, where she was sworn into the office of 'provincial female physician' (*Landesärztin*) (21) in the rank of a captain of the Austro-Hungarian army (Picture 1).

Picture 1. Anna Bayerová (1891) in the uniform of the Austro-Hungarian army while serving as a 'provincial female physician' of Bosnia (*Landesärztin in Bosnien*). Source: Navrátil M. Almanach Českych Lékařů, Praha 1913, s.p. (5).

At that time, living conditions in BH were very poor. The level of education, especially among the female Muslim population, was insufficient; there were many epidemics of infectious diseases, and the mortality rate was high. On January 1, 1892, Bayerová assumed her duties in Tuzla (21). According to her own official report, between January and March she treated 118 female patients, of whom 47 were Muslim, and visited 213 Muslim women of the district in their homes (23). While she wrote in private letters to her friends that she was delighted with her work with 'those uneducated and friendly women' (12), she was continuously involved in conflicts with her superiors from the very beginning of her service. She complained, with good reason, about the disorganization surrounding her ad hoc created office and her low remuneration. The Austro-Hungarian army had supplied her with neither accommodation nor an office. Bayerová was forced to organise both facilities, for which her salary proved to be insufficient. The question of whether she was expected to charge patients a fee remained unresolved, and when she decided to treat poor women cost free, she personally had to pay for their medication (21). According to Nečas, she sought continuously to practise her own concept of a female physician's duty, to treat and help 'all women', while her superiors urged her to restrict her caregiving to Muslim women. She was also required to visit the district's Muslim villages on horseback, though she had never learned to ride (21).

Bayerová considered her work thwarted by the Austro-Hungarian army and sought the intervention of Kállay, who was ready to support her claims and ideas. At Bayerová's instigation, the Minister created the role of 'female health officer' (*Amtsärztin*) at a fixed, adjusted remuneration. He also decreed that an Austro-Hungarian female health officer was entitled to treat patients regardless of their religion, nationality or gender (21).

However, Bayerová had alienated herself from her Austro-Hungarian surroundings to the point that, after three months, she requested a transfer to 'higher located' Sarajevo on account of her poor health. During her short residence in Tuzla she had caught an infection with diphtheria and come down with influenza twice (12). Not least due the fact that her mother and her brother had died young of "emaciation" she suffered from an excessive fear to contract tuberculosis which instigated her to avoid low situated "unhealthy" settings throughout her life (23). The transfer was authorized in August 1892. She expected to function as a female health officer in Sarajevo, but soon complained in letters to her friends that her superiors used her for paperwork (21).

In Sarajevo, Bayerová moved in with Adelina Paulina Irby (1831–1912) and Priscilla Johnston (25), who had established a girls' secondary school in Sarajevo in 1871 (which the Austro-Hungarian authorities reluctantly tolerated because of the ladies' reputation as enthusiastic supporters of Serb nationalism).[2] Against this background, the Austro-Hungarian authorities treated her as an unwanted person who had to quit her service, as she expressed in a letter to Krásnohorská. Her father's death in the late autumn of 1892 provided her with a strong argument to leave the country, despite Kállay's appeals that she continued her work and 'help the villages' (21).

[2]For the earlier cooperation of Czech feminists with Irby's project during the 'Herzegovina uprising' in Bosnia (1875-1877), see Anderson D. Two Women Travellers in the Balkans in the 1860s: Georgina Muir Mackenzie, Adeline Paulina Irby. *Proceedings of the BRLSI. Bath Roval Literay and Scientific Institution)* 8 (2004); http://www.brlsi.org/proceed04/lunch200311.htm (accessed on October 3, 2008); see also McVicker MF. Georgina Mary Muir Mackenzie (Lady Sebright, ?-1874, and Adeline Paulina Irby, 1831-1911, p. 105-108. In: McVicker MF. Women Adventurers, 1750-1900. A Biographical Dictionary, with Excerpts from Selected Travel Writings. Jefferson, London: McFarland and Company Inc., Publishers; 2008; p. 107.

Several Czech studies have explored why Bayerová did not pursue her mission in BH (13, 21, 23). Anna Honzaková (13), the third female Czech physician, invokes Bayerová's fear that her health was seriously at risk after she had contracted several infectious diseases in Tuzla. The Czech historian Ctibor Nečas (21) points to the inevitable national and political antagonism between a radical Czech feminist and the Austro-Hungarian authorities in BH, while her most recent biographer Marie Bahenská (21) depicts Bayerová as an irresolute personality who, despite her political radicalism, might have preferred a quiet middle-class life in more comfortable and lofty located 'healthy' settings to the fulfilment of her 'historical mission'.

In 1893, Bayerová returned to Prague to dedicate herself to a campaign for Austrian 'women's need of female physicians,' which was eventually supported by the Austrian 'German' Social Democracy. Bayerová's Bosnia experience inspired the slogan that 'not just religious, but also natural modesty' should be considered sufficient justification for accrediting female physicians in Austria (26, 10).

Later in 1893, Bayerová returned to Switzerland, where she worked as a medical educator in girls' schools in Berne. In 1900, financial problems compelled her to work again as a physician in a sanatorium near Geneva (22). Since women's admission to Austrian medical schools in 1897, Krásnohorská had repeatedly urged Bayerová to have her degree recognised in Austria and to practise in Prague. But in 1900, Bayerová finally refused, and her mentor parted ways with her (23, 27)

In 1910, Bayerová returned to Prague and took up employment as a school physician. However, the low remuneration compelled her to request Austrian recognition of her degree in 1913. She worked at a mental hospital in Bohnice, near Prague, until 1916.

Bayerová died in 1924 at the home of her friend Libuše Bráfová, with whom she lived upon her return to the Czech lands. Her burial was attended by thousands of compatriots who wished to honour the popular 'Czech heroine'.

Bayerová's Publishing Activities

Bayerová restricted her research activities to her thesis on the blood counts of infants and new-borns, which was quoted repeatedly in the *Journal of Czech Physicians* (28). From the early 1880s, she predominantly wrote popular scientific articles in *Ženské Listy*, most of which concerned women's health issues, hygiene and hygiene education. She was also the author of a popular Swiss booklet against alcoholism and its negative consequences in form of male domestic violence against women and children which had been edited by the Swiss temperance movement since 1897 (9). In 1907, she edited and published Anna Fischer-Dückelmann's popular medical book *Die Frau als Hausärztin* (German, 1901, *Women as Family Doctors*) under the title Žena lékařkou in Czech translation (29). As the title indicates, the book elucidated the human anatomy, pregnancy, the necessity of healthy nutrition, attire and sports, childhood illnesses, modern education, domestic remedies, medicinal herbs and female sexuality for common women. The book's liberal, feminist-maternalist character is evident in its provision of a chapter on contraception, which recommended the diaphragm (*pessar*) as a new contraceptive method under female control (30).

Concluding Remarks

As a symbol of the Czech women's movement, Anna Bayerová was expected to pioneer women's education and medicine by scrupulously performing the duties of an Austro-Hungarian female health officer in BH. While the 1891 introduction of the office was based officially on the presence of a female Muslim population, Bayerová obviously was not ready to accept such restrictions. Though she succeeded in redefining her role, she left the country after one year, due in large part to the fact that her gender and her liberal attitudes made her an outsider.

Authors' Contributions: Conception and design: HT and BF; Acquisition, analysis and interpretation of data: BF and HT; Drafting the article: BF and HT; Revising it critically for important intellectual content: HT and BF; Approved final version of the manuscript: BF and HT.

Conflict of Interest: The authors declare that they have no conflict of interest.

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DOI: 10.5644/ama2006-124.250

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by Nerma Tanović

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Acknowledge. Anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. List the source(s) of funding for the study and for the manuscript preparation in the acknowledgements section.

Authors' contributions (eg. Authors' contributions: Conception and design: XX and YY; Acquisition, analysis and interpretation of data: YY and ZZ; Drafting the article: XX, YY and ZZ; Revising it critically for important intellectual content: XX, ZZ; Approved final version of the manuscript: XX, YY and ZZ.

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Sample references

Articles in journals

Standard journal article (*List the first six authors followed by et al.*):

Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002;347(4):284-7.

More than six authors:

Rose ME, Huerbin MB, Melick J, Marion DW, Palmer AM, Schiding JK, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res. 2002;935(1-2):40-6.

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ISSN 1840-1848