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Next Generation Sequencing in Oncological Diagnostics: Hype or Hope?

Rana Hallak[1,2], Manfred Kuepper[2,3], Amer Al Chikh Youssef[4]

[1]Department of Biochemistry, Faculty of Pharmacy, Al Rasheed University, Ghabareb, Syria
[2]Rana Hallak Clinical Laboratory, Damascus, Kafar Sousseh, Syria
[3]Department of Biochemistry, Faculty of Pharmacy, Arab International University, Ghabareb, Syria
[4]Doctors Cancer Care Center Damascus, Damascus, Syria
Email: ayoussef2@hotmail.com

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Abstract

The understanding of how genetic and epigenetic factors influence tumorigenesis, progression and invasion, is vastly growing since new technologies allow the analysis of the functional genome namely the exome, the transcriptome and the epigenome, besides enabling genome-wide assessment of genetic variations. With the advent of new drugs that are indicated tissue agnostic, depending on certain mutations, there is a growing demand for fast and cost-effective genetic diagnosis. The method in focus that already became an indispensable tool in viral diagnosis is next-generation sequencing (NGS). This approach allows sequencing of literally every DNA molecule in the sample and can either be used to assess numerous genetic markers of one patient at a time, or to assess fewer markers of many patients in parallel, which reduces costs. We submitted 23 samples of different tumor entities to four diagnostic companies with different analysis profiles. The results as disclosed and discussed in this report indicate that so far, the main application of NGS is rather in cancer research than in diagnosis, as none of the reports had a real impact on the therapeutic scheme. We are perfectly aware that such a small cohort cannot be generalized, but considering the costs vs. benefits, NGS should be engaged upon a very stringent evaluation only. However, in cases where obtaining a tissue biopsy is impossible or unfavorable, analysis of liquid biopsy by NGS provides a vital alternative.

Keywords

Oncology, Next Generation Sequencing, Tumor Diagnosis, Personalized Medicine

1. Introduction

Cancer is a genetic disorder and as such caused by alterations in protein-coding or regulatory parts of the DNA that drive tumorigenesis, progression or invasion. Thus, these mutations can be of diagnostic or prognostic value or have an impact on the therapeutic regimen. The past decades since the publication of the first draft of the human genome provided growing insight into cellular processes which are involved in the pathobiology of cancer and we are beginning to understand the impact of individual genetic changes on the clinical outcome.

The growing understanding of genetic processes in cancer also has a significant impact on the therapeutic options. With Vitrakvi (Larotrectinib) as the first drug to be approved as tissue-agnostic, and Keytruda (Pembrolizumab) being reassessed for tissue-agnostic use shortly after, the treatment has changed in the direction to abandon "one-size-fits-all" regimens in favor of personalized therapy.

Assessing all these requires an enormous sequencing capacity, yet it has to be done in the most cost-effective way. Next-generation sequencing (NGS) was designed to reveal the sequence of literally every individual DNA molecule within the sample allowing assessment of many genetic parameters in parallel for very low per-base-costs.

Currently, several companies offer NGS services for cancer diagnosis. We employed four different companies for the assessment of various tumor entities and would like to share our experiences and opinions in this report. We may emphasize that with respect to the high costs for the patient we are making a rather rigorous evaluation of costs vs. benefits. Rather than giving recommendations, we intend to initiate a discussion on how to implement this undoubtedly powerful method into our clinical routine in the most beneficial way.

2. Materials and Methods

A total of 23 samples from ten different tumor entities (**Table 1**) including 10 male (average age 60.3 years), and 13 female patients (average age 50.3 years) were assessed for copy number variants (CNVs), and small variants including indels, sequence variations and structural changes, such as translocations, by massive parallel NGS engaging four different service providers located in the US and Europe: Exact Sciences Company (Phoenix, AZ, USA), Omicure (France), Agendia (Amsterdam, The Netherlands), and Neoscreen (Athens, Greece).

Exact Sciences Corp. (Phoenix, AZ, USA) disclosed a panel of 235 genes under its corporate brand Paradigm; the current panel disclosed under their brand Oncotype MAP assesses 258 genes. Both Paradigm and Oncotype MAP calculate the tumor mutational burden (TMB) according to methods described elsewhere [1]-[7], determine microsatellite instability (MSI), detail mutations that are relevant with respect to therapy, and report genomic findings "of unknown significance", *i.e.* somatic or germline non-reference alleles found in less than 1% of the population. Unfortunately, the proportion of the mutant alleles, *i.e.*, the per-

centage of confirmed reads with that respective sequence variant from the total number of reads is not disclosed. The same is true for the other companies except for Neoscreen. Based on the mutation status and TDM, alternative therapy regimens are suggested and recruiting clinical studies are detailed, in which the patient might be enrolled.

Omicure (France), *i.e.* its contractor Eurofins Scientific SE (Luxemburg), discloses a panel of 590 genes of which the entire protein-coding part is examined. For another five genes only the promoter region is analyzed and 22 genes are investigated for gene fusions. Additionally, 24 microRNAs are also being assessed. Like Exact Sciences Corp., Omicure calculates the TMB, summarizes the mutation status of the most relevant genes, discloses identified mutations in other genes that are relevant with respect to therapy, and details DNA alterations of unknown significance. MSI, however, is not assessed. Based on the mutations identified in the sample and the TMB, alternative regimens are suggested and information on recruiting clinical trials is provided.

Table 1. Patients, age at time of sampling, tumor entity, type of sample and site of collection.

Patient	Sex	Age	Tumor Entity	Sample	Collection Site	Laboratory
A.B.	f	50	Mamma Ca.	FFPE	mamma	Agendia
K.B.	f	65	Mamma Ca.	FFPE	mamma	Agendia
G.H.	f	29	Mamma Ca.	FFPE	mamma	Neoscreen
N.Bi.	f	60	Mamma Ca. (invasive lobular ca. grade III)	FFPE	mamma	Oncotype
M.S.	f	67	Mamma High-Grade Ca.	FFPE	axillar lymph node	Oncotype
J.D.	f	38	Mamma Ca. (liver metastasis)	FFPE	liver	Paradigm
N.Ba.	f	36	Mamma Ca.	FFPE	mamma	Omicure
M.A.H.*	m	72	Lung Cancer	blood	peripheral blood	Omicure
Z.Z.	f	54	NSCLC (brain metastasis)	blood	peripheral blood	Omicure
S.L.	m	68	NSCLC	FFPE	lung	Oncotype
A.A.R.	m	78	Adenocarcinoma of the Lung	blood	peripheral blood	Omicure
S.Y.	f	63	Sqamous Cell Ca.	FFPE	nasopharynx	Oncotype
M.Sh.	m	25	Malignant Neoplasm	FFPE	nasopharynx	Oncotype
M.H.	m	42	Naso-pharyngal Undifferentiated Ca	FFPE	nasopharynx	Omicure
A.G.	m	63	Hepatocellular Ca.	FFPE	liver	Oncotype
M.A.	m	71	Intrahepatic Cholangiocarcinoma	FFPE	liver	Oncotype
W.M.	f	48	Spindle Cell Sarcoma	FFPE	left upper arm	Oncotype
M.K.	m	77	Sarcoma	FFPE	left arm	Omicure
S.T.	f	63	Thyroid Cancer, Papillary Ca.	FFPE	thyroid	Omicure
F.M.	f	63	Colorectal Adenocarcinoma	FFPE	colon	Oncotype
L.H.	f	56	Ovarian Ca. (serous high grade)	blood	peripheral blood	Omicure
S.A.	m	36	Gastric Cancer	blood	peripheral blood	Omicure
M.Ka.	m	71	Bladder Transitional Cell Ca.	FFPE	bladder	Omicure

Agendia (Amsterdam, The Netherlands) runs a non disclosed panel of 70 genes specifically for assessment of metastatic risk of breast cancer. Results are provided as a proprietary MammaPrint Risk-of-Recurrence score estimating the risk of recurrence after 5 and 10 years without adjuvant systemic treatment, and a proprietary MammaPrint Index evaluating the risk of distant metastases in patients with invasive breast cancer. Unlike Exact Sciences Company and Omicure, TMB is not calculated; MSI is not assessed nor are any identified mutations disclosed, nor are alternative therapy regimens suggested.

Neoscreen (Athens, Greece) assesses point mutations such as base substitutions and small deletions and duplications in the coding regions of BRCA1 and BRCA2 genes including the intron-exon boundaries by massive parallel NGS. In a second step, identified mutations are assessed in genomic DNA from peripheral blood by Sanger sequencing in order to discriminate hetero, from homozygous mutations and to proof presence in the germline. The report describes mutations in detail and gives information about the quality of the NGS analysis such as the coverage.

3. Results

Nineteen FFPE (formalin fixed paraffin embedded) and 4 liquid biopsy samples from a total of 23 patients were processed and assessed by four different companies as described afore.

The report issued by Agendia for breast cancer patients A.B. and K.B. states a nearly identical MammaPrint Index defining a low risk of recurrence and metastasis without giving any further details. In particular, no mutations are disclosed that may be linked to reduced efficacy of certain drugs, nor are data provided that allow for stratification with respect to subjecting the patient to endocrine therapy alone or in combination with chemotherapy.

Neoscreen subjected patient G.H. (mamma ca.) to ultra-deep NGS on BRCA1 and 2. A specific mutation in the BRCA1 gene was identified with an allele frequency of 16.37% and confirmed by Sanger sequencing from peripheral blood as being heterozygous. This finding came with the very general recommendation to set up a tailored surveillance scheme for this patient and to consider testing of first-degree relatives.

NGS analysis of the other 20 samples revealed 51 mutations of immediate relevance in 30 genes with no clustering within specific entities. The most frequently mutated genes were PALB2 with 5 sequence variants in 4 cases across 3 different tumor entities and PTEN with 3 allelic losses and 1 sequence variant in 4 cases of 3 different entities. BRCA1, CHEK2, and TP53 showed each 3 sequence variants in 3 cases across 3 tumor entities (**Table 2**).

Microsatellites were stable in all cases where assessed (14/23), except for patient M.Ka. for whom a high level of instability was reported. TMB, assessed in 20 cases, was low in 16 and high in 4 cases (patients M.A.: 11/Mb, S.L.: 12/Mb, M.S.: 15/Mb, M.Ka. 22, 7/Mb).

Table 2. Therapeutic relevant allelic variants.

Gene	Variant	Entity	Patient
ACVR1	W245Rfs*7	Mamma Ca.	N.Bi.
ATM	loss	Mamma Ca.	M.S.
	p.D273DX	NSCLC	Z.Z.
APC	Q222*	NSCLC	Z.Z.
	Q1429*, R876*	Colon Ca.	F.M.
ARID1A	W1073*	Liver Ca.	A.G.
BAP1	loss c.784-3_784del p.?	Liver Ca.	A.G.
BRCA1	c.3756_3759delGTCT	Mamma Ca.	G.H.
	p.Q356R	Nasopharyngal	M.H.
	p.R1699W	Ovarian Ca.	L.H.
BRCA2	S1064lfs*12	NSCLC	S.L.
CHEK2	p.Q69X	NSCLC	Z.Z.
	p.D438Y	Mamma Ca.	N.Ba.
	p.Q36X	Sarcoma	M.K.
EGFR	loss	Lung Ca.	A.A.R.
ESR1	Y537N	Mamma Ca.	J.D.
ERBB2	p.P944X	Lung Ca.	A.A.R.
ERBB3	E731Q	Mamma Ca.	M.S.
FGF4	gain	Nasopharyngal	M.Sh.
FGFR1	gain	Mamma Ca.	J.D.
FGFR2	H242D	Mamma Ca.	M.S.
FLT4	gain	Nasopharyngal	M.Sh.
GATA3	P408Afs*99	Mamma Ca.	N.Bi.
GNAS	Q870L	NSCLC	S.L.
KEAP1	V980fs*55	NSCLC	S.L.
KRAS	G12D	Liver Ca.	M.A.
	G13D	Colon Ca.	F.M.
JAK3	V722I	Mamma Ca.	N.Bi.
PALB2	p.L1143H, p.?-1002	Mamma Ca.	S.T.
	p-130-131X	Mamma Ca.	N.Ba.
	p-130-131X	Sarcoma	M.K.
	p-130-131X	Gastric Ca.	S.A.
PBRM1	loss V530del	Liver Ca.	A.G.
PIK3CA	p.E542K	Bladder Ca.	M.Ka.
PTEN	loss	Mamma Ca.	M.S.
	c.209+1G>A	Mamma Ca.	J.D.
	loss	Nasopharyngal	M.Sh.

Continued

	loss	Sarcoma	W.M.
RAD51B	p.G96X	Mamma Ca.	M.H.
	p.K243R	Mamma Ca.	N.Ba.
RAD54L	p.S657C	Bladder Ca.	M.Ka.
SF3B1	K700E	Mamma Ca.	J.D.
SMAD4	loss	NSCLC	S.L.
TP53	c.357_357 + 2del p.?	NSCLC	S.L.
	c.994-1G > Cp.?	Mamma Ca.	M.S.
	R273H	Colon Ca.	F.M.

3.1. Therapy Recommendations

Based on the afore mentioned mutations of therapeutic relevance (Table 2) and immunohistochemistry (IHC), in particular for PD-L1 and HER-2, therapeutic regimes with supposedly improved benefit have been suggested for 19 of 20 patients; however, only in four cases these regimens are FDA or NCCN approved: Pembrolizumab was suggested for treatment of the PD-L1 positive malignant nasopharyngeal neoplasm of patient M.Sh. Based on positive IHC for estrogen receptor (ER) of the invasive lobular mamma carcinoma grade III of patient N.Bi, a total of 17 FDA/NCCN approved regimens are provided. With respect to the high TMB, Pembrolizumab is recommended for patient S.L. (NSCLC), as for patient M.K. (sarcoma) due to high TMB and MSI.

Also in four cases, regimens with potentially reduced benefit based on specific mutations were disclosed.

3.2. Allelic Variants of Unknown Significance

Both Exact Sciences Company and Omicure reported allelic variants ("Other Genomic Findings", respectively "Other DNA Alterations") in 288 out of 654 genes without further explanation.

A possibly interesting finding is that the metastatic NSCLC of patient Z.Z., and the lung cancer sample of patient M.A.H., both assessed by liquid biopsy, as well as the adenocarcinoma of the lung of patient A.A.R., share allelic variants in 6 genes: CYP2D6, IGSF10, GXYLT1, KMT2C, MUC16, and SPTA1. IGSF10 and SPTA1 show identical allelic variants across these three cases, CYP2D6 show identical variants between patients Z.Z. and M.A.H. but partly different variants in patient A.A.R., and sequence variants in GXYLT1, KMT2C, and MUC16 are partly shared. Moreover, patients Z.Z. and M.A.H. share identical mutations in 4 genes, namely BCR, FGFR4, FLT3, and SLC22A1, and patients Z.Z. and A.A.R. share identical variants in 3 more genes, namely CHD2, HNF1A, and LTK (Table 3).

Furthermore, GXYLT1, IGSF10, KMT2C, MUC16, exhibit sequence variants in all 10 cases where these genes had been assessed, and SPTA1 shows variations in 80% of all cases where it has been assessed. IGSF10 however, unanimously

Table 3. Shared allelic variants of unknown significance in lung cancer.

Gene	Z.Z.	M.A.H.	A.A.R.
BCR	p.S1092SGX p.A1204G	p.S1092SGX	
CHD2	p.-1388-1389X		p.-1388-1389X
CYP2D6	p.P34S p.R365H p.E211GX p.L213P	p.P34S p.R365H p.E211GX p.L213P	p.P34S p.E211GX p.L213P
FGFR4	p.G388R	p.G388R	
FLT3	p.T227M	p.T227M	
GXYLT1	p.D153V, p.D228Y, p.E249G, p.E249K, p.H117L, p.R126S, p.R258L, p.R261S, p.Y264*, p.Y264N, p.Y265C	p.D153V, p.D228Y, p.E249G, p.E249K, p.H117L, p.R126S, p.R258L, p.R261S, p.Y264*, p.Y264N, p.Y265C, p.S212*	p.D153V, p.E249G, p.E249K, p.H117L, p.R126S, p.R258L, p.R261S
HNF1A	p.P289X		p.288-289X
IGSF10	p.Y150D	p.Y150D	p.Y150D
KMT2C	p.D348N, p.G838S p.Y987H	p.G838S, p.R909K p.Y987H	p.D348N, p.G838S, p.C391*, p.G315S, p.Y987H
LTK	p.D535N		p.D535N
MUC16	p.D1229H, p.I4034F, p.S11154F, p.T2891I, p.T10155I, p.T7063A	p.D1229H, p.S1953P, p.S11154F, p.T2891I	p.D1229H, p.I4034F, p.S11154F, p.T2891I, p.S5885F, p.L1833F, p.R1015G, p.S2058P, p.P3289T, p.T7063A, p.S9687*
SLC22A1	p.425-7	p.-425-?	
SPTA1	p.L1858V	p.L1858V	p.L1858V

shows only the variant p.Y150D, rather pointing to a common allelic variant in Syria.

Little is known about the function of the BCR gene product apart from its role as fusion partner of ABL in the Philadelphia chromosome. Diekmann *et al*. however showed that the *bcr*-encoded protein (Bcr) is a GAP protein for the Ras-related GTP-binding protein, $p21^{rac}$ [8].

The CYP2D6 mutations in both lung cancers are identical to one another and the same is true for FGFR4, were both lung cancers show the same genetic change.

Cytochrome P450, Subfamily IID, Polypeptide 6 (CYP2D6) belongs to the P450II superfamily and is as such involved in the metabolism of the vast majority of prescribed and over-the-counter drugs [9]. Mutations within the CYP2D6 gene have been found to be associated with the poor metabolizer (PM) phenotype [10] [11]. Allelic variant c.1316-11C > A was identified in mamma ca. patient J.D., while both lung cancer patients M.H. and Z.Z. show an identical array of variations (p.P34S, p.R365H, p.E211GX, p.L213P).

Fibroblast Growth Factor Receptor 4 (FGFR4) is a protein-tyrosine kinase, which as such can be a factor in carcinogenesis [12] [13]. Intrahepatic cholangiocarcinoma patient M.A. exhibits variant S137, while both lung cancers show the identical variant p.G388R.

FLT3 is a member of the type III receptor tyrosine kinase family that includes KIT, FMS, and platelet-derived growth factor receptor. Since its expression in human blood and marrow was found to be restricted to CD34+ hematopoietic stem or progenitor cells, it is also referred to as Stem Cell Tyrosine Kinase 1 (STK1) [14]. Abu-Duhier, *et al*. found FLT3 mutations to be the strongest prognostic factor for overall survival in adult AML patients under the age of 60 years; however, there is no described link to lung cancer [15].

IGSF10 belongs to the immunoglobulin (Ig) superfamily and appears to play a role in early migration of GNRH-expressing neurons [16].

Solute Carrier Family 22, Member 1 (SLC22A1), also known as Organic Cation Transporter 1 (OCT1) is a polyspecific organic cation transporter system expressed in human only in liver [17]. Polyspecific organic cation transporters are generally found in the liver, kidney, and intestine and are critical for elimination of many endogenous amines as well as a wide array of drugs and environmental toxins.

SPTA1 encodes spectrin which is the predominant component of the membrane skeleton of the red blood cell and involved in determining the properties of the membrane including its shape and deformability.

Glucoside Xylosyltransferase 1 (GXYLT1) is a xylosyltransferase that adds the first xylose to O-glucose-modified residues in the epidermal growth factor (EGF) repeats of proteins such as NOTCH1 and 2 but had not been linked to cancer so far [18]. However, in a recent study, Peng *et al*. report that expression levels are significantly elevated in colorectal cancer (CRC) tissues compared to normal tissues and report that mRNA levels gradually increase with tumor progression and differ significantly between tumor stages [19]. Kaplan-Meier plots showed that patients with elevated GXYLT1 levels had a shorter disease-free survival or overall survival than those with low GXYLT1 levels, indicating that elevated GXYLT1 mRNA expression goes together with a poor prognosis in CRC patients. Mutations within the GXYLT1 gene were found in 18 of 45 (40%) samples of CRC, with the nonsense mutation GXYLT1 S212*, that we identified in one of the lung cancer samples (patient M.H.) as one of the most frequently occurring mutations. Overexpression of wild type (WT) GXYLT1 and GXYLT1 S212* in CRC cells *in vitro* activated the Notch pathway. Moreover, GXYLT1 promoted migration and invasion *in vitro* and metastasis *in vivo*, with the GXYLT1 S212* mutant having a much greater effect. Functionally, both GXYLT1 and GXYLT1 S212* interact with ERK2. While WT GXYLT1 induced metastasis involving the Notch and MAPK pathways, the GXYLT1 S212* mutant mainly promoted metastasis by activating the MAPK pathway. The authors propose that GXYLT1 acts as a novel metastasis-associated driver gene and GXYLT1 S212* might serve

as a potential indicator for therapies targeting the MAPK pathway in CRC.

The KMT2A-D proteins are methyltransferases responsible for methylation of histone 3 lysine 4 (H3K4me), which together with H3K79me is a key step in euchromatin formation, allowing transcription and giving access to DNA repair proteins [20] [21]. KMT2 mutations were initially associated with pediatric Mixed Lineage Leukaemias (MLL) [22]. Meanwhile, mutations of all 4 KMT2 genes have been linked to a variety of cancers, with a majority of somatic heterozygous loss-of-function mutations, suggesting that haploinsufficiency for these epigenetic regulators may underlie disease [23]. Heterozygous somatic mutations in the paralogous MLL3/KMT2C and MLL2(4)/KMT2D genes are now among the most frequent mutations in human cancer as revealed by large-scale tumor DNA sequencing studies, which revealed KMT2C/D alterations in over 40% of squamous cell cancers of the lung, and up to 30% of adenocarcinoma of the lung [22]. KMT2 mutations occur frequently in NSCLC and are associated with higher TMB and poor survival [21]. Shi *et al*. however, reported that patients with KMT2C mutations have increased PD-L1 expression levels and showed a longer median progression-free survival (PFS) compared to wild-type patients, which is particularly true for NSCLC patients with TP53/KMT2C co-mutations who were subjected to immune checkpoint inhibitor (ICI) treatment, resulting in a greater durable clinical benefit [24]. The authors conclude that KMT2C or KRAS mutations combined with TP53 mutations may represent a powerful biomarker with respect to ICI therapy. The study of Chang *et al*. suggests that KMT2C/D mutations that disrupt homologous recombination (HR)-mediated DNA repair, sensitize NSCLC to Poly (ADP-Ribose) Polymerase inhibitors (PARPi), whose efficacy is still unclear in NSCLC [21]. According to them, high-frequency KMT2C/D mutations may serve as biomarkers for PARPi therapies in NSCLC and other cancers with infrequent BRCA1/2 mutations. Na *et al*. showed that deficiency of KMT2C in extensive-stage SCLC was found to promote multiple-organ metastases in mice [25]. Moreover they revealed that KMT2C directly regulates the expression of DNMT3A, a de novo DNA methyltransferase and that epigenetic reprogramming by concerted KMT2C- and DNMT3A-mediated histone and DNA hypomethylation seems to underlie SCLC metastasis, suggesting a potential epigenetic therapeutic vulnerability.

4. Discussion

NGS in cancer diagnosis promises to provide the basis for individual, personalized therapy by genome-wide assessment of mutations linked to the respective tumor entity. However, this requires a nearly complete understanding of both the physiological functions of all of our genes, and their particular role in cancer, especially with respect to progression and metastasis. While we are beginning to have insight into tumorigenesis and genetic factors that determine the clinical course, we are far from such in-depth knowledge.

With this in mind it's no wonder that from 19 patients with mutations in re-

levant genes (Table 2), only in four cases FDA/NCCN-approved alternative therapies have been suggested, albeit in all of these cases based on either IHC (PD-L1, ER, Her-2), TMB high, or MSI high and not on mutations assessed by NGS, *i.e.* the same result could have been achieved in a standard diagnostic setting for a fraction of the costs. The suggested therapies for the remaining 16 cases are off-label and therefore of doubtful value.

Yet, the mutation profile of four cases revealed therapeutic regimes with a reduced benefit, providing the chance to avoid futile therapeutic approaches with possible progress under therapy. This alone cannot be overestimated.

So far, the authors must state that the results of the NGS analysis did not provide significant clinical benefit compared to standard diagnostic procedures, with the exception, that only NGS on a liquid biopsy for the brain metastasis of NSCLC (patient Z.Z.) allowed assessment of the mutation status of the tumor. With respect to the very high costs of the method and the limited impact on the individual therapeutic regimens, we'd suggest restricting NGS profiling to liquid biopsies for primary and metastatic tumors for which obtaining a tissue biopsy is difficult or impossible. Also the analysis has to be carried out in a transparent way, disclosing the panel of assessed genes, and reporting identified mutations besides giving information about regimens with potentially increased and such with possibly decreased efficacy.

That the place for NGS so far is rather in cancer research than in diagnosis becomes clear when looking at the numerous reported findings of unknown significance; yet, they are in part quite interesting indeed.

First of all, it is necessary to recognize that the reference sequences from which the frequencies of allelic variants are established are mainly based on data obtained from Caucasians. Therefore, a rare variant in these references could still be frequent in the Arabic setting. Therefore it seems advised to build a database on allelic variants in Arabic countries for a more accurate evaluation of their significance. With this in mind, we could think that different allelic variants across multiple samples, such as for GXYLT1, IGSF10, KMT2C, MUC16, and SPTA1 rather represent "normal" genetic variations in our population. However, that two tumor samples from the same tissue of origin, *i.e.*, NSCLC and adenocarcinoma of the lung, share identical mutations might hint at some significance of these variants, especially, since the other entities that we've assessed exhibited different allelic variants.

From the genes with shared allelic variants between the two lung cancer samples detailed afore, four could be indeed of interest.

While the protein-tyrosine kinase FGFR4 can as such be a factor in carcinogenesis the p.G388R variant identified in both lung cancer patients has been linked to inferior prognosis in several cancer types, including lung cancer. Quintanal-Villalonga *et al*. report that this particular FGFR4 variant activates MAPK and STAT3 and induces N-cadherin protein expression, causing pro-oncogenic effects in NSCLC *in vitro* and *in vivo* [26]. The N-cadherin expression

appears essential for the pro-tumorigenic role and higher N-cadherin expression levels correlated with poorer outcomes. Hence, the FGFR4 p.G388R variant alone or in combination with N-cadherin expression levels appears as a promising prognostic marker in NSCLC and possibly other lung cancers. Since FGFR4 p.G388R activates MAPK, this signal transduction pathway might also be a new therapeutic target in NSCLC and possibly other lung cancer entities.

Another gene that can promote migration and invasion via the MAPK pathway is GXYLT1. It would be of interest to address the question of whether the S212* variant identified in patient M.H., is also related to the promotion of lung cancer and what the impact of the other numerous allelic GXYLT1 variants is that we identified in both lung cancer samples. In such a case, the MAPK pathway would probably be a therapeutic target in GXYLT1 mutated lung cancers.

Mutations in the receptor tyrosine kinase gene FLT3 are an important prognosis factor in AML and are linked to inferior outcomes. Future studies should explore whether the p.T227M variant that is present in both lung cancer samples as the only allelic variant also has prognostic value in these entities.

Finally the significance of the KMT2C methyltransferase gene variants found in both lung cancer samples and the efficacy of PARPi treatment in NSCLC should be elucidated in future, multicentric studies among Arabic patients, as well as the impact of TP53/ KMT2C co-mutations as biomarkers for PFS and benefit of ICI treatment.

In conclusion, although NGS deep-sequencing did not provide a direct benefit with respect to therapy, it revealed mutations in four genes with potential significance in lung cancers. In the future, NGS is expected to become an essential building block in oncology as it became for instance in virology, especially since the future trend for new drugs in oncology goes clearly towards tissue-agnostic, mutation and gene expression-depended approval.

Providing access to targeted oncology drugs and eliminating uncertainties in drug matching requires not only an understanding of gene function but also knowledge about the frequencies of allelic variants in the Arabic population and their impact on progression and invasion, which have to be determined in a multi-centric cross-Arabic setting. It would be highly desirable if the necessary laboratory infrastructure would be established in a national centre with access to all professionals in this field in order to improve therapy and move towards individualized oncology care.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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