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Next Generation Sequencing in Cancer Diagnosis and Treatment Is Coming of Age

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Abstract

Next-generation sequencing has long since emerged as the method of choice for whole genome sequencing as well as for assessing genetic changes in a vast set of genes. Hence, it was bound to propagate the approach of personalized medicine that came forth, esp. in oncology. Instead of subjecting patients with a certain tumor entity to a certain tumor-specific therapy, patients even diagnosed with different tumor entities may receive identical, yet patient-specific treatments based on certain mutations identified in the tumor. In a nutshell, gene mutations that affect key metabolic pathways and are believed to be causative, *i.e.*, driver mutations, direct the therapy towards the respective lost or gained function and allow intervention at the root cause, provided that the respective drug is available. This holds the promise to increase therapeutic success while limiting adverse side effects, esp. those of generalized chemotherapy. With respect to the high costs of NGS, it is crucial to obtain data about how many patients actually benefit in what proportion of cases, valid therapeutic recommendations are based on NGS and not on more conventional and thus cheaper diagnostic procedures. Two years after scrutinizing a cohort of 20 patients with rather mixed results, we'd like to come forth with a larger cohort of 43 patients and in our opinion the perspective has vastly improved.

Keywords

NGS, Personalized Cancer Therapy, Mutations, FDA-Actionable Therapy, Off-Label Therapy

1. Introduction

The concept of cancer as a genetic disorder, where so called "driver" mutations

propagate malignant transformation while an array of so called "passenger" mutations influences prognosis and therapeutic success is well established and is currently leading to a paradigm change towards personalized treatment [1]. This, of course, requires assessing genetic variations across a vast gene panel for which massive parallel next-generation sequencing (NGS) has emerged as the method of choice [2] [3].

We previously compared the panels as assessed by different companies available to physicians in the Near East with respect to the number of genes covered and genetic variations assessed. Moreover, we determined the proportion of cases from our own centers in which NGS did provide information about drug schemes that are expected to show an increased or decreased efficiency; however, with respect to the low proportion of cases that did benefit from NGS, we advised to select patients cautiously [4].

Now, two years later, we decided to have another look into this matter, since the understanding of the therapeutic impact of mutations has broadened since.

2. Materials and Methods

Data were collected prospectively. Patients refractory to standard chemotherapy who had already received third-line therapy or higher were included in the study.

Thirty-one FFPE and twelve liquid biopsy samples encompassing 17 different tumor entities from 43 patients (Table 1), including 25 male (average age 53.9 years), and 18 female patients (average age 52.8 years) were assessed by NGS for a variety of genetic variations, such as sequence variations, copy number variants (CNVs), indels, and structural changes, such as translocations. In addition, Omicure (France) and Cryogene (Lebanon) assess tumor mutational burden (TMB) and microsatellite instability (MSI).

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
I. A.	f	68	uterus ca.	FFPE	uterus	Cryogene
A. Gh.	m	76	prostate ca.	liquid	peripheral Blood	Cryogene
R. J.	f	50	mamma ca., high grade, metastatic	FFPE	neck metastasis	Cryogene
L. H.	f	58	ovarian ca., high grade, metastatic	liquid	peripheral Blood	Omicure
N. Ba.	f	36	mamma ca., metastatic	FFPE	mamma	Omicure
A. A. R.	m	78	adenocarcinoma of the lung	liquid	peripheral blood	Omicure
M. H.	m	42	naso-pharyngal undifferentiated ca	FFPE	nasopharynx	Omicure
M. K.	m	77	sarcoma	FFPE	left arm	Omicure

Continued

H. A.	m	76	prostate ca.	liquid	peripheral blood	Cryogene
S. Sh.	f	61	naso-pharyngal ca., high grade, metastatic	FFPE	axillar lymph node	Cryogene
O. T.	m	53	adenocarcinoma lung, metastatic	liquid	peripheral blood	Omicure
T. Kh.	m	52	chondrosarcoma, metastatic	liquid	peripheral blood	Omicure
B. A.	f	63	mamma ductal adenocarcinoma, metastatic	FFPE	breast	Omicure
M. K.	m	71	bladder ca.	FFPE	bladder	Omicure
S. A.	m	36	gastric cancer	liquid	peripheral blood	Omicure
S. T.	f	63	Thyroid cancer, papillary, metastatic	FFPE	thyroid	Omicure
Z. Z.	f	54	NSCLC, metastastic	liquid	peripheral blood	Omicure
M. A. H.*	m	72	lung cancer	liquid	peripheral blood	Omicure
H. H.	m	70	bile-duct ca., metastatic	FFPE	abdominal mass	Omicure
J. J.	m	59	squamous cell ca.	liquid	peripheral blood	Cryogene
Kh. A.	m	37	lung ca., metastatic	FFPE	lung	Omicure
Y. O.	m	53	pancreas ca.	FFPE	pancreas	Omicure
A. S.	f	57	hepatocellular ca.	FFPE	liver	Cryogene
O. A.	m	46	adenocarcinoma	liquid	peripheral blood	Cryogene
Z. D.	m	61	adenocarcinoma	FFPE	liver	Cryogene
Z. M.	f	69	adenocarcinoma colon	FFPE	colon	Cryogene
M. U.	m	51	adenocarcinoma prostate	FFPE	prostate	Cryogene
I. S.	m	45	colorectal ca., metastatic	FFPE	Mesenteric node	Omicure
A. A.	f	49	adenosquamous ca., metastatic	FFPE	bronchi	Omicure
R. N.	f	51	colorectal ca., metastatic	FFPE	rectum	Omicure
N. M.	f	59	adenocarcinoma colon, metastatic	FFPE	liver	Cryogene
M. AR.	f	45	adenocarcinoma stomach	FFPE	stomach	Cryogene
A. K.	m	60	adenocarcinoma colon, metastatic	FFPE	sacrococygeal	Cryogene
I. M.	m	52	adenocarcinoma pancreas, metastatic	FFPE	liver	Cryogene
D. R.	f	44	adenocarcinoma mamma, metastatic	liquid	peripheral blood	Cryogene

Continued

M. A.	f	55	adenocarcinoma pancreas, metastatic	FFPE	liver	Cryogene
F. M.	f	26	adenocarcinoma colon	FFPE	colon	Cryogene
A. A.	m	32	Ewing sarcoma, metastatic	FFPE	lung	Cryogene
Q. A.	m	6	sarcoma	FFPE	lung	Cryogene
Sh. A.	f	41	adenocarcinoma, metastatic	FFPE	lymph node	Cryogene
Kh. E.	m	45	fibromyxoid sarcoma	FFPE	soft tissue left hip	Cryogene
A. A. A.	m	59	NSCLC	FFPE	lung	Cryogene
N. S. S.	m	39	NSCLC	FFPE	lung	University St. Josef

Nineteen samples were submitted to Omicure, 23 to Cryogene and one was processed at St. Joseph University, Beirut, Lebanon. The panel of 590 genes assessed by Omicure has previously been detailed [4], Cryogene discloses a panel of 648 genes, and University St. Joseph Beirut a panel of 335 genes.

3. Results

NGS identified mutations of immediate relevance, *i.e.*, gene mutations that possibly have therapeutic impact, in 54 genes; mutations in 14 of these genes were the base for the recommendation of FDA/NCCN-approved therapeutic regimens for a total of 12 patients representing 27.9% of cases. For another 9 cases (20.9%) FDA/NCCN-approved therapies were suggested on the base of high TMB, MSI or immunohistochemistry (IHC); in five of these NGS data additionally point to off-label therapies, *i.e.*, therapeutic regimens for other indications bearing the same genetic mutations. Thus, for a total of 21 patients (48.8%) patient specific, FDA/NCCN-approved therapies are available.

For 9 patients (20.9%) NGS data suggested only off-label therapies, and for 2 patients (4.7%) suggested off-label therapies were solely based on high TMB [5] [6], MSI or IHC, respectively. For 11 patients (25.6%) no therapeutic options could be given.

This means in turn, that for a total of 32 out of 43 patients (74.4%) therapies with potentially increased effectiveness are available; in 26 of these cases (60.5%) the recommendations are based on NGS data.

In our previously published cohort of 20 patients [4] therapeutic regimes with supposedly improved benefit have been available for 19 patients, yet, in 15 cases (75%) the recommended therapies were off-label. Only in four cases (20%) the regimens were FDA or NCCN approved, however, these recommendations were solely based on TMB high, or MSI high and not on mutations assessed by NGS.

Compared to these findings we can say that the table has remarkably turned in

favor of NGS.

The mutations are detailed in Table 2 and Table 3. The all-over most frequently mutated gene is TP53 with genetic variants found in 15 cases (34.9%) across 9 tumor entities, followed by PALB2 with mutations in 10 cases (23.3%) identified in 8 entities. PIK3CA mutations are found in 6 patients (14%) from 6 entities, while ATM, BRCA1 and BRCA2 mutations manifest also in 6 cases, yet across 5 tumor entities. Both NF1 and KRAS scored mutations in five patients (11.6%) found in four, resp. three entities. Four patients (9.3%) show mutations in RAD51B and BRAF identified in three, resp. two entities. However, there was no clustering of specific gene variants.

Table 2. Gene mutations identified by NGS.

No.	Gene	Cases	% Cases	Entities	% Entities
1	TP53	15	34.9%	9	50.0%
2	PALB2	10	23.3%	8	44.4%
3	PIK3CA	6	14.0%	6	33.3%
4	ATM	6	14.0%	5	27.8%
5	BRCA1	6	14.0%	5	27.8%
6	BRCA2	6	14.0%	5	27.8%
7	NF1	5	11.6%	4	22.2%
8	KRAS	5	11.6%	3	16.7%
9	RAD51B	4	9.3%	3	16.7%
10	BRAF	4	9.3%	2	11.1%
11	CHEK2	3	7.0%	3	16.7%
12	BRIP1	3	7.0%	2	11.1%
13	CDK12	3	7.0%	2	11.1%
14	APC	3	7.0%	1	5.6%
15	ARID1A	2	4.7%	2	11.1%
16	CDKN2A	2	4.7%	2	11.1%
17	ERBB2	2	4.7%	2	11.1%
18	EGFR	2	4.7%	1	5.6%
19	NTRK2	2	4.7%	1	5.6%
20	AKT1	1	2.3%	1	5.6%
21	BCORL1	1	2.3%	1	5.6%
22	CCND1	1	2.3%	1	5.6%
23	CDKN1B	1	2.3%	1	5.6%
24	CUL3	1	2.3%	1	5.6%

Continued

25	DNMT3A	1	2.3%	1	5.6%
26	ERBB3	1	2.3%	1	5.6%
27	ETV6	1	2.3%	1	5.6%
28	EWSR1-FLI1	1	2.3%	1	5.6%
29	FANCD2	1	2.3%	1	5.6%
30	FANCL	1	2.3%	1	5.6%
31	FGF3	1	2.3%	1	5.6%
32	GATA3	1	2.3%	1	5.6%
33	IDH1	1	2.3%	1	5.6%
34	KMT2C	1	2.3%	1	5.6%
35	MAP3K1	1	2.3%	1	5.6%
36	MET	1	2.3%	1	5.6%
37	NCOR1	1	2.3%	1	5.6%
38	NRAS	1	2.3%	1	5.6%
39	NTRK1	1	2.3%	1	5.6%
40	PBRM1	1	2.3%	1	5.6%
41	PIK3R1	1	2.3%	1	5.6%
42	PTEN	1	2.3%	1	5.6%
43	PTPRD	1	2.3%	1	5.6%
44	RAD51D	1	2.3%	1	5.6%
45	RAD54L	1	2.3%	1	5.6%
46	ROS1	1	2.3%	1	5.6%
47	SMARCA4	1	2.3%	1	5.6%
48	SMARCE1	1	2.3%	1	5.6%
49	SOCS1	1	2.3%	1	5.6%
50	SPOP	1	2.3%	1	5.6%
51	TCF7L2	1	2.3%	1	5.6%
52	TERT	1	2.3%	1	5.6%
53	TET2	1	2.3%	1	5.6%
54	TYRO3	1	2.3%	1	5.6%

Table 3. Mutations with therapeutic impact.

Gene	Cases
ATM	4
PIK3CA	4

Continued

BRCA1	2
BRCA2	2
BRIP1	2
EGFR	2
KRAS	2
AKT1	1
CDK12	1
CHEK2	1
ERBB2	1
NRAS	1
PALB2	1
RAD51B	1

Among the 26 cases that are the base for the suggested FDA/NCCN-approved therapies, the most frequently mutated gene is ATM, which appears in 4 cases across three entities. PIK3CA mutations are found in 4 cases across four tumor entities. Mutant alleles of BRCA1, BRCA2, BRIP1, EGFR and KRAS are identified in each 2 cases; in this patient cohort EGFR mutations are found in 2 cases of lung cancer and KRAS in 2 colorectal cancers.

Microsatellites were stable in all but one cases where assessed (25/26). TMB, assessed in 42 cases, was low in 33, high in 8 cases and unknown in one case.

Within the nine patients for which only off-label therapies ("for other indications") were suggested, the most frequently mutated gene is PALB2, found in 6 cases across 5 entities, followed by BRCA1 identified in 3 patients across 3 entities. Mutations in ATM and CHEK2 are found in 2 cases and BRCA2, IDH1, PIK3CA, RAD51B and RAD54L score only once.

"Therapies for other indications" means that a specific mutation identified in a gene approves prescription of a specific therapeutic regimen, yet in a different entity, while for the tumor entity of the respective patient prescription of that particular regimen is not (yet) approved by FDA/NCCN. Still, that treatment is a valid option as the mechanism of the drug is related to the function of the target gene, affected by that very same mutation. Off-label recommendations in our patient cohort encompass the PI3K inhibitor [7] alpelisib and the PARP inhibitors [8]-[10] olaparib and niraparib.

4. Discussion

The FDA approval of the immunotherapy drug pembrolizumab (Keytruda™) in 2017 for tumors with a specific genetic change, regardless of the cancer type, started the trend of "agnostic" drug prescription which is being applied to an ever-

growing number of therapeutic agents ever since. In a nutshell, this approach is based on the assumption that a drug that effectively treats a certain tumor entity which exhibits certain mutations within specific genes should also be effective against another entity showing the same pattern of gene mutations. Although this therapeutic approach basically has an experimental character, it is promising and should be considered whenever canonical regimens come to their limits.

In this context, massive parallel next-generation sequencing is indispensible for investigating a vast number of target genes and the subsequent detection of characteristic mutations or patterns of mutations.

Two years ago, we probed the benefit of NGS in oncology with respect to its impact on providing possible alternative therapeutic approaches [4], yet, the results were somewhat sobering. While for 19 of 20 patients in the cohort, regimes with supposedly improved benefit were available, only 20% of these were FDA or NCCN approved but not based on NGS data, instead on TMB high, or MSI high. The suggested therapies for the remaining 15 cases were reasoned on NGS results but not approved by FDA or NCCN, hence off-label. Our conclusion back then was that cases to be subjected to NGS analysis must be selected very carefully and stringent with respect to the rather unfavorable costs/benefit ration.

Meanwhile, it appears that the table has turned in favour of NGS, since in our latest study presented here, patient specific FDA/NCCN-approved therapies are recommended for a total of 21 patients (48.8%). For 12 of which, the recommendations are based on NGS data, while for the remaining nine patients, the alternative regimens are based on either high TMB, MSI or immunohistochemistry (IHC), respectively. However, for five of these nine cases, NGS data additionally reveal off-label therapies. For another nine patients (20.9%) NGS data unlock off-label therapies, so that a total of 26 patients (60.5%) benefits from NGS analysis.

Moreover, in five cases where a specific, FDA/NCCN approved therapy could be recommended and in two cases where only off-label therapies could be suggested, the data were obtained from liquid biopsy and would thus not be available by other means of analysis but NGS. The impact of this approach is nicely reviewed by Ho *et al.* [11].

Still, the costs are very high and in our recent cohort 11 patients representing 25.6% did not benefit from the analysis, yet, in turn, almost 75% of patients did, either from the NGS data or from the assessment of TMB and MSI as part of the total analysis. The ongoing improvement of the techniques will significantly reduce the process costs, making the approach available to more patients, but still, patients must be thoroughly selected; Mosele *et al.* [12] forward guidelines for the use of NGS in the context of precision medicine.

The number of recommended alternative therapeutic approaches can also be expected to increase with our growing understanding of the tumor metabolism and function of genes in the context of control vs malignant transformation.

However, the authors do believe, that a future breakthrough is likely to occur when NGS data are combined with transcriptome analysis, since matching data

on patterns of somatic mutations with data on patterns of gene activity would add a new level of information, acknowledging that tumor behavior and, in this respect, the projected clinical outcome is best predicted by assessing changes in the genetic programming of the tumor. In this, we fully support the views of Cilento *et al.* [13].

5. Conclusion

NGS provided FDA-actionable or off-label therapeutic options in 74.4% of refractory cancer cases (60.5% NGS-derived). Thus, in comparison with the older cohorts, this study supports the growing role and clinical utility of NGS in guiding personalized cancer therapy when balanced with careful patient selection.

Author Contributions

Rana Hallak: Writing—original draft, Formal analysis, Methodology, review & editing.

Yasmin Alchikh Youssef: Data curation, Visualization, Investigation, Writing—review & editing.

Mohamad Amer Al Chikh Youssef: Conceptualization, Resources, Supervision, Writing—review & editing.

Conflicts of Interest

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

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