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Treatment inferred from mutations identified using massive parallel sequencing leads to clinical benefit in some heavily pretreated cancer patients

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Abstract

Molecular portraits of numerous tumors have flooded oncologists with vast amounts of data. In parallel, effective inhibitors of central pathways have shown great clinical benefit. Together, this promises potential clinical benefits to otherwise end-stage cancer patients. Here, we report a clinical service offering mutation detection of archived samples using the ion Ampliseq cancer panel coupled with clinical consultation.

A multidisciplinary think tank consisting of oncologists, molecular-biologists, genetic counselors, and pathologists discussed 67 heavily pretreated, advanced cancer patient cases, taking into account mutations identified using ion Ampliseq cancer panel, medical history, and relevant literature.

The team generated a treatment plan, targeting specific mutations, for 41 out of 64 cases. Three patients died before results were available. For 32 patients, the treating oncologists chose not to include the panel recommendation in the treatment plan for various reasons. Nine patients were treated as recommended by the panel, 5 with clinical benefit, and 4 with disease progression.

This study suggests that routine use of massive parallel tumor sequencing is feasible and can judiciously affect treatment decisions when coupled with multidisciplinary team-based decision making. Administration of personalized based therapies at an earlier stage of disease, expansion of genetic alterations examined, and increased availability of targeted therapies may lead to further improvement in the clinical outcome of metastatic cancer patients.

Abbreviations: FFPE = formalin fixed paraffin embedded, Mb = million bases, PGM = Personal Genome Machine.

Keywords: DNA, high-throughput nucleotide sequencing, mutation, neoplasms, precision medicine

1. Introduction

In recent years, molecular profiles of tumors such as breast,[1,2] prostate,[3] colon,[4] lung,[5] ovary,[6] and glioblastoma[7] have been reported. In parallel, inhibitors of molecular pathways are commonly used in oncological practice including inhibitors of ABL1,[8] Adenylyl cyclase,[9] ALK,[10] BRAF,[11,12] CDK4/6,[13] DNMT,[14] EGFR,[15–18] HER2,[19–22] JAK,[23] KIT,[24] MEK,[25] mTOR,[26] RET,[5] ROS,[27] SMO,[28] VEGF,[29,30] and VEGFR.[31,32] Some of these inhibitors have shown clinical activity in diverse organs—HER2 inhibition in HER2-positive breast[22] and gastric tumors;[33] CKIT inhibition in gastrointestinal stroma tumor[24] and melanoma,[34] and mTOR inhibition in renal cell carcinoma,[35] Astrocytoma,[26] pancreatic neuroendocrine tumors,[36] and ER-positive breast cancer.[37] These reports, in conjunction with phase II,[38] phase I[39] and case reports[40] where patients derived clinical benefit from pathway inhibition, provide the clinical rationale for testing mutations in tumor samples and utilizing mutation analysis to choose a pathway inhibitor to treat patients. Several academic institutions[41,42] and commercial companies[43,44] offer a molecular profiling service[41,42] that hundreds of cancer patients in Israel have chosen to utilize, indicating an unmet need.

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In this report, we describe a comprehensive molecular service based in an academic hospital setting. We detail the validation of the molecular technique, patient population and mutations found, as well as the decision-making process, clinical decisions taken by the molecular oncology forum and clinical outcome.

2. Methods

2.1. Patient population

Patients were referred by their treating physician, at their discretion after a detailed discussion with the patient where the possible benefits and expected limitations were carefully reviewed prior to ordering this service. The clinical service included mutation detection, data analysis, and panel treatment recommendation. Patients receiving off-label treatment signed an informed consent (29c) that was approved by the head of the Hadassah Medical Center ethics (Helsinki) committee prior to treatment.

2.2. Molecular profiling

Formalin fixed paraffin embedded (FFPE) tissue was examined by a pathologist to identify the region for sampling and percentage of tumor cells in the analyzed region. DNA was extracted using QIamp DNA FFPE Tissue Kit and the Ion Ampliseq cancer panel was applied. Up to 4 samples were loaded on a 314 chip (10 million bases (Mb)capacity) or up to 8 samples were loaded on a 316 chip (100 Mb capacity) and run on an Ion Torrent Personal Genome Machine (PGM) System. Mutations were identified by the Ion Variant caller as previously described.[43] The V1 panel amplifies 13,311 bp in *ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2/4, FBXW7, FGFR1/2/3, FLT3, GNAS, HNF1A, HRAS, IDH1, JAK2/3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NMP1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB11, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53* and *VHL*. The V2 panel amplifies 22,027 bp in the same genes and also in *EZH2, GNA11, GNAQ*, and *IDH2*. Sanger sequencing was performed as previously described.

2.3. Data interpretation

All variants were (manually) visualized using the integrative genome viewer.[44] Noncoding and synonymous variants were not investigated further. All variants with allelic fraction of 100% ± 3% or 50% ± 3% were perceived as potential germ line changes. If a variant was previously identified, in the study population, as a known germline variant, it was appraised as such. All others were perceived as somatic changes. Non-synonymous somatic variants were examined using the COSMIC database,[45] and variants not identified in the database were not further evaluated. The variants identified in COSMIC were investigated by a literature review initiated by references found in the COSMIC database. A report including a summary of the case, the variant caller report, and review of the literature was sent to the treating physician. Based on the treating physician's remarks, a revised report was sent to the molecular oncology forum members including molecular-biologists, genetic counselors, oncologists, and pathologists. Each case was presented, reviewed, and discussed to reach a consensus recommendation.

2.4. FFPE-based somatic panel validation

To validate the test, we sequenced 20 samples, 19 of the samples tested positive for KRAS, BRAF, or EGFR and 1 sample was positive for several mutations. In 19 out of 20 samples we succeeded in generating amplified DNA amenable for massive parallel sequencing. The average number of bases read was 229 Mb per chip which resulted in average coverage of 3503X. Sanger sequencing was performed on previously unknown mutations for further validation. The previously known mutations in all samples were identified.

Reproducibility was tested using duplicates prepared separately from the same DNA sample. There was full concordance between variants called, a total of 14 pairs. The average difference of variant allelic fraction (i.e., the percentage of the DNA reads that are mutated) in the duplicates was 1.6% with a median of 0.5%. A sample of normal tissue was analyzed and the variations found were either 50% ± 3% or 100% ± 3%, all perceived as germline. Based on these results, a clinical service was established where each tumor sample is tested twice, and certain mutations are regarded as germline.

3. Results

3.1. Patient population

Table 1 reports the patients' characteristics. The median number of previous treatments is 2. The advanced stage of disease in this population is demonstrated by the fact that 3 patients died while the test was processed, in a span of weeks (Table 1). In 64 cases the test was performed on existing FFPE samples. In 3 cases where no tissue was available for testing, test-designated biopsies were performed, 2 from lung metastases, and 1 from the primary gastric tumor. DNA was extracted from the tumor primary site (n = 33), local recurrences or distant metastasis (n = 29). The tumors were either naive to chemotherapy (n = 47), or previously treated (n = 16).

3.2. Molecular profile

Some samples tested harbored known mutations; 3 BRAF V600E, 3 KRAS G12D, 1 KRAS G13C, and 1 IDH1 R132H were reidentified. A KRAS G12D positive case was reclassified as KRAS wild type, a KRAS negative case was reclassified as KRAS A146T positive, and a BRAF V600E negative case was reclassified as BRAF V600E positive.

3.3. Clinical outcome

Of 67 patients assessed, 3 died before results were processed. Of the remaining patients, for 23 patients no novel perceived actionable somatic mutations were detected; in 41 patients, 75 novel actionable somatic mutations were detected with a median of 1 mutation per sample (range 1–3). One sample harboring hundreds of somatic mutations is not described. Actionable mutations are listed in Table 2. Of the 41 patients with actionable mutations that led to treatment recommendations, 9 patients received the treatment recommended by the forum. In 4 patients the disease progressed, however in 5, following the recommended treatment a clinical benefit, stable disease for more than 2 months or partial response was achieved (Table 3).[46–74] In 32 patients, treatment was deferred due to a combination of reasons including availability of pathway inhibitors in clinical trials outside the country, poor clinical condition, and other available treatment options. In cases where germline mutations were suspected, genetic consultation was recommended.

Table 1

Clinical characterization of patients examined.

Median age	**58 (range 16–82)**
Gender	(%)
Male	54
Female	46
Primary site	(n) Pathology
Skin	15 (14 melanoma, squamous cell carcinoma)
CNS	13 (6 glioblastoma multiforme, 2 glioma, 1 granular cell tumor, 2 oligodendrogioma, 2 anaplastic astrocytoma)
Large intestine	9 (6 adenocarcinoma, 2 mucinous carcinoma, 1 carcinoma)
Gastric	7 (4 adenocarcinoma, 3 signet ring cell carcinoma)
Pancreatic	5 (4 adenocarcinoma, 1 adeno-squamous carcinoma)
H&N	5 (3 squamous cell carcinoma, 2 undifferentiated carcinoma)
Gynecological	4 (1 uterine leiomyosarcoma, 1 ovarian serous carcinoma, 1 mixed serous carcinoma and endometrioid carcinoma of endometrium, 1 endometrioid carcinoma
Other	9 (2 unknown adenocarcinoma, 2 lung adenocarcinoma, 1 adenoid cystic carcinoma,1 small bowel adenocarcinoma, 1 transitional cell carcinoma, 1 urethra transitional cell carcinoma, 1 uveal melanoma)
Number of previous treatments	(n)
0	4
1	17
2	15
3	14
4	8
5	5
6	1
7	2

Sixty-seven patients were examined; the median age of the patient population is 58 years with nearly equal distribution between male and female patients. Sixty-four of the patients suffered from metastatic cancer or had brain tumors. The median of the number of previous treatments is 2 (range 0–7).

4. Discussion

This series of 67 metastatic cancer or brain tumor patients whose tumors were tested for actionable mutations demonstrates that in the majority of patients, actionable mutations can be identified. When the recommended treatment was applied, clinical benefit was achieved in a significant portion of the patients. This work has several limitations, including being conducted in a single institution, retrospective study, limited accessibility to pathway inhibitors, a small heterogeneous population, and lack of clear indication.

A proof that such a service prolongs the life of patients in a randomized prospective study was not found.[75] The extendibility of such a proof will be hard to come by, as the paradigm in oncology is shifting from large randomized trials to highly tailored small trials,[76] and following the perception that each patient's cancer is unique and genomic characterization of the tumor can have clinical significance in treating cancer patients[76] in a patient-centered research approach.[77,78] An impetus to establishing and applying this test clinically was the ever-increasing utilization of genomic tests performed by private companies. It was felt that a service that includes a validated test followed by a discussion by a multidisciplinary forum should be established.

There is limited availability to pathway inhibitors recommended by the forum, as phase I/II trials targeting molecular pathways are currently sparsely available in Israel.[79] The recommended treatment options often include treatments that may not be covered by the health insurance, and when purchased privately, may cost thousands of dollars a month.

The small patient population described is very heterogonous as to cancer site, number of treatments, and clinical statuses. It also does not represent the general patient population as these patients were able to pay for the service and were selected at the treating physician's discretion. Patient selection could have led to deference of treatments proposed as some patients were on one hand too ill to receive treatment, or on the other hand had other treatment options. The limitations of this work mirror the realty of implementing tumor biology into day-to-day clinical practice. These include, among other things, complicated issues involving ethics, drug accessibly, and clinical indication.[80]

This study highlights the growing ethical dilemmas a treating oncologist is faced with daily,[78] and questions such as whether it is ethical to offer an unproven test or treatment to patients that are suffering from end stage cancer? Is it ethical to deny a test that may decrease suffering and prolong life? As with others,[81] in our experience, it is essential to conduct a detailed discussion with the patient where the possible benefits and expected limitations are carefully reviewed prior to ordering this service.

For all patients with an actionable mutation, a clinical trial outside of Israel could be found using www.clinicaltrials.gov.[79] This option is considered not relevant by the molecular oncology forum due to the effort and suffering of advanced stage cancer patients traveling to a foreign country and living there, the very high costs and the inherently unknown clinical benefit. As another option, the concept of suitable off-trial possibilities was opted.[81] It is clear that this treatment concept is inferior to including patients in clinical trials. As molecular characterization of tumors has been democratized, increasing access to molecular inhibitors should be the next challenge of the pharmaceutical, research, and clinical community. This approach may help solve poor accrual as once uniform clinical entities are fragmented to an assortment of rare tumors with hundreds of compounds and thousands of combinations waiting to be tested in phase I/II trials.[82]

Other groups have recently published the clinical results of harnessing molecular profiling to metastatic cancer patients. A study of 1283 advanced metastatic cancer patients tested FFPE tumor tissue using targeted sequencing of hotspot regions in *PIK3CA, BRAF, KRAS, NRAS, PTEN, EGFR, KIT, GNAQ* and *MET*. Using these tests, clinical targets were found in 40% of the patients. Sixteen percent received targeted treatment with 4% of the total population achieving a clinical response. A similar group of patients who received nontargeted therapy had an inferior response rate, time to treatment failure, and overall survival.[83] In 2 studies including 109 and 423 metastatic breast cancer patients, fresh tissue biopsies were tested for amplifications and deletions using comparative genome hybridization and hot spot sequencing of *AKT1* and *PIK3CA*. Using these tests, clinical targets were found in 50% and 46% of the patients respectively. Sixteen percent and 13% received targeted treatment; the treatment was outside a clinical trial protocol in 40% of the patients, with a total of 8% and 3% respectively achieving clinical benefit.[84,85] Another study of 11 advanced metastatic cancer patients tested fresh tissue biopsies using whole genome

Table 2

Actionable mutations identified in 67 cancer patients.

Gene	Mutation	Primary site	Treatment suggested
ABL1	G250R	Gastric, buccal	Nilotinib; dasatinib; Ponatinib
	G251D	Buccal	Nilotinib; dasatinib; Ponatinib
APC	E1317Q	Bladder	CDK 4/6 inhibitor
	R1450*	Colon	Gentamycin; Macrolid
ATM	Q355*	Appendix	Everolimus; Platinum compound; PARP inhibitors; Ataluren
	P568S	Melanoma	Everolimus; Platinum compound; PARP inhibitors; Ataluren
BRAF	G469V	Colon	MEK inhibitor
	D594A	Gastric	Sorafenib; MEK inhibitor
	D594G	Melanoma	Sorafenib in combination with a MEK inhibitor
	V600E	Colon	BRAF inhibitor in combination with EGFR inhibitor and MEK inhibitor; Irinotecan in combination with Cetuximab; BRAF inhibitor in combination with an EGFR inhibitor
CDKN2A	R58*	Head and neck	CDK 4/6 inhibitor
	V59G	Melanoma	Palbociclib
	D74N	Lung	CDK 4/6 inhibitor
	P135Q	Melanoma	CDK 4/6 inhibitor
EGFR	C595Y	Brain	EGFR inhibitor
	G598V	Brain	EGFR inhibitor
	D761N	Lymphoma	Erlotinib
	V774M	Brain	EGFR inhibitor
ERBB2	P761del	Unknown	Neratinib
	V842I	Gastric	Lapatinib
ERBB4	P594L	Melanoma	Lapatinib
GNAQ	Q209L	Melanoma	MEK inhibitor
GNAS	R201H	Appendix	Somatostatin
IDH1	R132H	Brain	5-Azacytadine
	R132C	Colon	5-Azacytadine
JAK3	V722I	Melanoma	Revision of pathology; Tofacitinib
KIT	D816V	Melanoma	Nilotinib
KRAS	G12D	Endometrial; pancreas; small bowel; brain	mTOR inhibitor in combination with MEK inhibitor; MEK inhibitor; PIK3CA inhibitor; Bevacizumab
	A146T	Colon	FOLFIRI in combination with Bevacizumab; FOLFOX in combination with Bevacizumab; MEK inhibitor; Temsirolimus
MET	N375S	Head and neck	Crizotinib
	T1010I	Melanoma	MET inhibitor
	Exon 14 splice site	Melanoma	MET inhibitor
NOTCH1	L2457V	Brain	NOTCH1 inhibitor
NRAS	G12A	Melanoma	MEK inhibitor
	T50I	Melanoma	MEK inhibitor
	Q61R	Melanoma	MEK162; MEK inhibitor
PDGFRA	G859E	Melanoma	imatinib
PIK3CA	E81K	Bladder	mTOR inhibitor
	R88Q	Endometrial	mTOR inhibitor in combination with MEK inhibitor
	I391M	Sarcoma	PIK3CA pathway inhibitor
	E542K	Gastric	mTOR inhibitor
	E545K	Urethra	mTOR inhibitor
	Q546R	Brain	PIK3CA inhibitor; AKT inhibitor; mTOR inhibitor
PTEN	H61R	Adrenal	mTOR inhibitor
	R130*	Brain	PIK3CA inhibitor; mTOR inhibitor
	P233*	Adrenal	mTOR inhibitor
	L325F	Endometrial	mTOR inhibitor in combination with MEK inhibitor
SMAD4	V354L	Pituitary	Rosiglitazone
TP53	P72R	Sarcoma	Wee-1 inhibitor
	V97F	Melanoma	Wee-1 inhibitor
	P151S	Skin	Wee-1 inhibitor
	R306X	Colon	Wee-1 inhibitor

In 41 patients, 75 novel actionable somatic mutations were detected with a median of 1 mutation per sample (range 1–3). One sample harboring hundreds of somatic mutations is not described.
* Stop.

sequencing and whole transcriptome sequencing. Using these tests, clinical targets were found in 89% of the patients. One patient was treated according to the targets identified with a short-lived partial response.[86] Initiatives such as the AURORA trial where hundreds of metastatic breast cancer patients will be subjected to molecular characterization and treated per mutation with a pathway inhibitor[87] and the NCI-MATCH trial that aims at recruiting 2400 metastatic cancer patients who will be treated in 24 different arms based on somatic mutations identified in the tumor sample will better quantify the benefit of this approach.

Table 3

Clinical outcome of patients treated as recommended.

#	Age	Gender	Primary site	Stage	Previous treatment
1	31	Male	Buccal	IV	TPF (Paclitaxel, Cisplatin, 5-Flurouracil (5-FU)); 70 GY concurrent with cisplatin; 5-FU, leucovorin, cisplatin and Cetuximab; and paclitaxel.
2	51	Female	Tongue	IV	TPF; radiotherapy, 48 GY, concurrent with mitomycin-c, bleomycin, vincristine and Cetuximab; weekly Adriamycin and cyclophosphamide.
3	58	Female	Colon	IV	XELOX (Capecitabine, oxaliplatin) and Bevacizumab; FOLFIRI (5-FU ,Leucovorin, Irinotecan) and Bevacizumab; Irinotecan and Panitumumab; FOLFOX (5-FU, Leucovorin, oxaliplatin) and Panitumumab; regorafenib and Mitomycin-C.
4	63	male	Melanoma	IV	Ipilimumab; pembrolizumab in combination with adoptive cell therapy.
5	67	Female	Appendix	IV	Hyperthermic intraperitoneal chemotherapy; FOLFOX in combination with Bevacizumab; Irinotecan.
6	63	Female	Uterus	IV	Adriamycin in combination with Ifosfamide; Gemcitabine in combination with paclitaxel; pazopanib; pegylated doxorubicin and Trabectedin.
7	69	Male	Colon	IV	FOLFOX and FOLFIRI in combination with Bevacizumab.
8	59	Male	Melanoma	IV	A clinical trial of Nivolumab or Nivolumab plus Ipilimumab Versus Ipilimumab Alone; and paclitaxel in combination with carboplatin.
9	44	Female	Melanoma	IV	Radiosurgery to the brain; Cisplatin, Decarbazine and interleukin-2.

#	Gene	Mutation	Variant frequency	Treatment suggested	Treatment applied	Maximal Response	Progression free survival	Overall survival
1	ABL1	NM_005157 c.748G>A G250R[*]	7%	Nilotinib; dasatinib; Ponatinib	Nilotinib	Progressive disease	2 wk	2 wk
		NM_005157 c.752 G>A G251D	7%					
2	MET	NM_000245 c.1124 A>G N375S[†]	76%, 76%	Crizotinib	Crizotinib	Progressive disease	5 wk	5 wk
3	BRAF	NM_004333 c.1406 G>T G469V[‡]	41%, 39%	Sorafenib together with a MEK inhibitor or gentamycin	Gentamycin was added to mitomycin-C	Progressive disease	2 mo	11 mo
	APC	NM_000038 c.4348 C>T R 1450[§,*]	67%, 67%					
4	NRAS	NM_002524 c.181 C>A Q61R[‖]	18%, 18%	A MEK inhibitor, Nilotinib, or combination of both.	trametinib was added to treatment with pembrolizumab	Progressive disease	2 mo	5 mo, alive
	KIT	NM_000222 c.2447 A>T D816V[¶]	10%, 13%					
5	GNAS	NM_000516 c.602 G>A R201H[#]	26%	Somatostatin analog, Lanreotide	Lanreotide	Clinical benefit	9 wk	9 wk
	ATM	NM_000051 c.1063 C>T Q355[*]	16%					
	KRAS	NM_004985 c.35 G>A G12D	19%					
6	PIK3CA	NM_006218 c.1173 A>G I391M[**]	56%	Examination of the variations in germline DNA. If the mutation proved to be somatic, treatment with Sirolimus in combination with Decarbazine	Sirolimus	Stable disease	3 mo	6 mo
	TP53	NM_000546 c.215 C>G P72R	79%					
7	BRAF	NM_004333 c.1799 T>A V600E[‡†]	23%, 25%	Combination of triple inhibition of BRAF, EGFR and MEK as one option, irinotecan and Cetuximab as a second option, or treatment with a BRAF inhibitor in combination with an EGFR inhibitor.	Vemurafenib in combination with panitumumab	Partial response	13 mo	20 mo
	TP53	NM_000546 c.574 C>A Q192K	62%, 63%					
8	PDGFRA	NM_006206 c.1706 G>A G589E[‡‡]	41%, 38%	Imatinib	Paclitaxel in combination with carboplatin and imatinib	Two months of treatment, underwent a metastasectomy		21 mo, alive
	CDKN2A	NM_058195c.404 C>A P135Q	87%, 86%					
	MET	NM_000245 g.99655 G>T splice junction	40%, 36%					
	SMAD4	exon 14	41%, 50%					
		NM_005359 c.747 G>C Q249H						
9	NRAS	NM_002524 c.182 A>G Q61R[‖]	44%, 46%	MEK162	MEK162	Partial response	16 mo	30 mo, alive[§§]
	ATM	NM_000051 c.1810 C>T P568S	18%, 20%					

Nine patients were treated as recommended, 4 with progressive disease and 5 with clinical benefit.

[*] Previously described in Chronic Myeloid Leukemia. In a phase I trial 81 previously treated Chronic Myeloid Leukemia Patients were treated with Ponatinib, 100% of chronic phase patients achieved an hematological response, and 36% of blast phase patients including patients with the G250E mutation.[8] Inhibition of this mutant may also be achieved by Nilotinib or Dasatinib.[46]

[†] The variant frequency may reflect a triplication of the mutant allele reminiscent of previously described hereditary papillary renal cell carcinoma.[47] c-MET is a proto-oncogene that codes for the hepatocyte growth factor (HGF) receptor, a tyrosine kinase frequently mutated in multiple tumors including H&N cancers. In experimental models, the N375S mutation leads to decreased binding of HGF receptor to its ligand, and decreased sensitivity to treatment with a cMET inhibitor.[48] In a phase II trial, 14 metastatic H&N patients were treated with the c-MET inhibitor foretinib without knowledge of c-MET mutation status. By RECIST criteria there was no response (decrease in tumor size by 25%) and 50% of patients had a stable disease (SD) thus the trial was halted.[49] Two clinical trials that studied c-MET inhibitors, foretinib and cabozantinib, in patients harboring mutations in this gene showed tumor responses.[50,51]

[‡] The *BRAF* G469 V is an activating mutation with a transformation rate similar to that of V600E[52] and has been reported in colon cancer.[53] Signaling induced by non-V600E BRAF mutations, such as BRAF L597R/Q/S and K601E mutants, is suppressed by a MEK inhibitor, and a metastatic melanoma patient harboring a BRAF L597R/Q/S mutation treated with a MEK inhibitor achieved a partial response.[54] A phase II study, randomizing 69 second or third-line metastatic colon cancer patients to Capecitabine or Selumetinib, a MEK inhibitor, in a 1:1 ratio, shows an equivalent clinical benefit, 29% stable disease and a progression-free survival of 2 mo.[55]

[§] Correction of the APC gene function by induced read-through of premature termination codons using gentamycin or a macrolide was demonstrated in experimental models.[56]

[‖] MAPK pathway activation can occur through mutations in *NRAS*. In a phase II trial, 30 previously treated NRAS-mutated metastatic melanoma patients were treated with the MEK1 and MEK2 inhibitor MEK162 with 10% partial response and 63% disease control rate; two patients with brain metastasis had tumor shrinkage.[57] In a phase IIB trial, previously treated metastatic melanoma patients, of whom 17% are *NRAS* mutated, were randomized to treatment with oral MEK1/2 inhibitor selumetinib or with temozolomide. Patients had a 5.8% partial response and 52% disease control rate with no difference found between treatment arms.[58]

[¶] The KIT exon 17 mutation results in the substitution of aspartic acid at residue 816 in the phosphotransferase domain. This substitution is present in the vast majority of adult mastocytosis. Unlike the other c-kit oncogenic mutations, KIT-D816 V fails to transform cells in some in vitro assays. For these reasons and because of the indolent nature of most mastocytosis pathologies, KIT-D816 V is thought to favor cell survival and proliferation, but the oncogenic potential of KIT-D816 V mutant is questioned.[59] In a phase 2 trial, 61 patients with systemic mastocytosis were treated with Nilotinib. Out of 37 patients with aggressive disease, all of whom harbored a D816 V mutation, 8 had a response.[60] Six melanoma patients with exon 11 KIT mutations were treated with Nilotinib and 2 achieved a partial response.[61] Nilotinib synergizes with MEK inhibitors to kill drug-resistant CML cells and block tumor growth in mice.[62] As a result of this finding, a phase I/II trial of Nilotinib and MEK-162 in CML patients was conducted (NCT02225574).

[#] GNAS encodes the stimulatory G-protein alpha subunit. The R201H is a frequent mutation in low-grade appendiceal mucinous neoplasms causing activation of cAMP-PKA pathway, inducing MUC2 and MUC5AC expression and mucin production.[63] Somatostatin binds the 5 Somatostatin receptors 1–5 that activate the inhibitory G protein, thus inhibiting Adenylyl cyclase.[64]

[**] PIK3CA is an oncogenic kinase upstream the mTOR pathway. The biological activity of the I391 M mutation has not been described and the variant frequency left the origin of the mutation, germline or somatic, in question.

[††] The most frequent *BRAF* mutation in colon cancer is V600E, a poor prognostic factor as it confers resistance to Cetuximab as a single agent, and treatment with Vemurafenib, a specific BRAF V600E inhibitor, is ineffective.[65] In the PRIME study, 53 BRAF V600E mutated patients were randomized to FOLFOX vs. FOLOX in combination with panitumumab. No difference in progression-free survival (PFS) or overall survival (OS) was found; the BRAF mutation conferred a worse OS when compared to wild-type group.[66] The resistance to BRAF inhibitors may be mediated via activation of CRAF via the KRAS/EGFR pathway.[67] In vitro, this activation could be inhibited either through a MEK inhibitor like selumetinib, a combination of Vemurafenib and Gefitinib, an EGFR inhibitor[67] or a BRAF inhibitor and Cetuximab.[68] A case report of an elderly metastatic mucinous colon cancer patient harboring a BRAF V600E mutation describes treatment with capecitabine, raltitrexed, Cetuximab, and Cetuximab in combination with Irinotecan, with progressive disease (PD). He was then treated with a combination of Cetuximab and Vemurafenib with a partial remission (PR) clinically that lasted for 6–7 mo; a subsequent PET-CT indicated that the disease progressed when Cetuximab treatment was stopped. The side effects of the treatment were a rash and a skin cancer.[69] A case report of a colon cancer patient with metastatic adenocarcinoma with mucinous features harboring a BRAF V600E mutation describes treatment with FOLFOX in combination with Bevacizumab with PR, and treatment with FOLFIRI in combination with Cetuximab with PD. The patient was treated with a combination of Cetuximab and Sorafenib with a mixed response and remained in excellent clinical condition for 7 mo. She was then treated with regorafenib as a single agent for 3.5 mo and then in combination with panitumumab. No side effects were described.[70] In a phase II clinical trial, 35 metastatic colon cancer patients who progressed after 1 line of treatment were randomized to treatment with Cetuximab alone or in combination with Sorafenib. No clinical benefit was found; more hand and foot syndrome was reported.[71]

[‡‡] PDGFRA is mutated in 5% of melanoma patients, with 29% of the mutations in exon 12.[72] The G589E mutation on exon 12 has not been described. Growth of cells transfected with exon 12-mutated PDGFRA was inhibited by imatinib and crenolanib.[72] Of 2 GIST patients harboring a PDGFRA exon 12 mutation, 1 had not progressed or died after 31 mo of imatinib treatment[73] and the other had a partial response with imatinib treatment.[74]

[§§] After progression, she was treated with pembrolizumab and achieved complete response.

Molecular profiling in the NCI-MATCH is based on the Oncomine Cancer Panel assay, using AmpliSeq chemistry and the PGM sequencer. Using this assay achieved an overall sensitivity of 96.98% and 99.99% specificity in detecting mutations. High reproducibility in detecting all reportable variants was observed, with a 99.99% mean interoperator pairwise concordance.[88]

Our experience is in line with these studies, putative targets are identified in most patients, and clinical benefit is achieved in modest numbers. This study suggests that routine use of massive parallel tumor sequencing is feasible and can judiciously affect treatment decisions when coupled with multidisciplinary team based decision making. Administration of personalized therapies at earlier stages of therapy, expansion of genetic alterations examined, and availability of targeted therapies may lead to further improvement in the clinical outcome of patients.

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