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Predictive Biomarkers and Personalized Medicine

Clinical Cancer Research

Integrated Next-Generation Sequencing and Avatar Mouse Models for Personalized Cancer Treatment

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Abstract

Background: Current technology permits an unbiased massive analysis of somatic genetic alterations from tumor DNA as well as the generation of individualized mouse xenografts (Avatar models). This work aimed to evaluate our experience integrating these two strategies to personalize the treatment of patients with cancer.

Methods: We performed whole-exome sequencing analysis of 25 patients with advanced solid tumors to identify putatively actionable tumor-specific genomic alterations. Avatar models were used as an *in vivo* platform to test proposed treatment strategies.

Results: Successful exome sequencing analyses have been obtained for 23 patients. Tumor-specific mutations and copy-number variations were identified. All samples profiled contained relevant genomic alterations. Tumor was implanted to create an Avatar model from 14 patients and 10 succeeded. Occasionally, actionable alterations such as mutations in *NF1*, *PI3KA*, and *DDR2* failed to provide any benefit when a targeted drug was tested in the Avatar and, accordingly, treatment of the patients with these drugs was not effective. To date, 13 patients have received a personalized treatment and 6 achieved durable partial remissions. Prior testing of candidate treatments in Avatar models correlated with clinical response and helped to select empirical treatments in some patients with no actionable mutations.

Conclusion: The use of full genomic analysis for cancer care is encouraging but presents important challenges that will need to be solved for broad clinical application. Avatar models are a promising investigational platform for therapeutic decision making. While limitations still exist, this strategy should be further tested. *Clin Cancer Res; 20(9); 2476–84. ©2014 AACR.*

Introduction

Cancer is considered a disease caused and driven by the accumulation of genetic aberrations (1). Virtually every cancer has its unique set of molecular changes, and the knowledge of such alterations in the clinical arena could ultimately facilitate an individualized approach to cancer treatment (2, 3). Recent advances in timeliness and cost of next-generation sequencing (NGS) technologies allow for the characterization of the cancer genome in a time frame that is compatible with treatment decisions, offering the opportunity to potentially increase the therapeutic efficacy by targeting the genomic aberrations driving tumor behavior (4–6).

There are, however, still significant challenges to integrate genomic testing into cancer treatment decision-making as the interpretation of the genomic information is still defying. On the one end, for most cancers there are a large number of mutations considered to be relevant (7, 8). While many of those are not drug targets, it is common to find several potential treatment opportunities for each given patient. How to prioritize these potential treatments is an unresolved issue (9). At present, the ability to generate genomic data supersedes the capacity to draw inferences from prior experiences and make informed treatment recommendations that can benefit the profiled individual patient. Novel tools to integrate genomic information with traditional clinical and pathologic data in an iterative manner are still needed (10). Here, we present our experience using a combined approach of exome sequencing and personalized xenografting to define patient therapy. A key component of our approach is the development of patient-derived xenografts, so-called Avatar mouse models, that

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Translational Relevance

Despite the clear potential of tailoring cancer treatment using genomics data and the appearance of exciting technological advances, a plethora of challenges remain to be resolved before wide-spread implementation of personalized therapy. The masses of data generated by high-throughput technologies are challenging to manage, visualize, and convert to the knowledge required to improve patient outcomes. Personalized xenografts developed in mice from patients' tumor tissues could aid in the process of interpreting genomic analyses, identifying actionable leads, and relating these to the drug space. This work describes one of the first experiences to apply exome sequencing and patient-derived xenografts, so-called Avatar mouse models, to personalizing cancer treatment in the clinic in real time. This approach is of clear interest as a means to better define optimal therapy for patients with advanced cancers.

permits bench testing of treatment strategies derived from the genomic analysis (11, 12).

Materials and Methods

This is a retrospective analysis of the patients that have received in our centers a personalized treatment approach tailored by the integration of exome sequencing and Avatar mouse models during the past 4 years. It represents a proof-of-concept case report as it demonstrates the feasibility of combining both technologies in the clinical setting and guide individual patient treatment. The protocol was Institutional Review Board approved and all patients signed informed consent.

Overview of personalized treatment approach

Patients had an exome characterization of tumor and normal tissue and bioinformatic analysis to determine the most biologically relevant somatic mutations. Simultaneously, we attempted to generate an Avatar mouse model from the same patient. Using genomic analysis, we integrated this information to help manually select a group of 5 to 10 treatments, which were then bench tested in the Avatar mouse model to select the most effective treatment candidate for the patient. Figure 1 shows a study schema.

Patient eligibility

All patients were adults with noncurable advanced cancer with an Eastern Cooperative Oncology Group (ECOG) performance status 0–1 and adequate bone marrow, liver, and renal function to receive chemotherapy. Either archival tumor tissue (preferentially frozen), xenograft tissue from the patient's tumor, or tumor lesions suitable for a tumor biopsy were used.

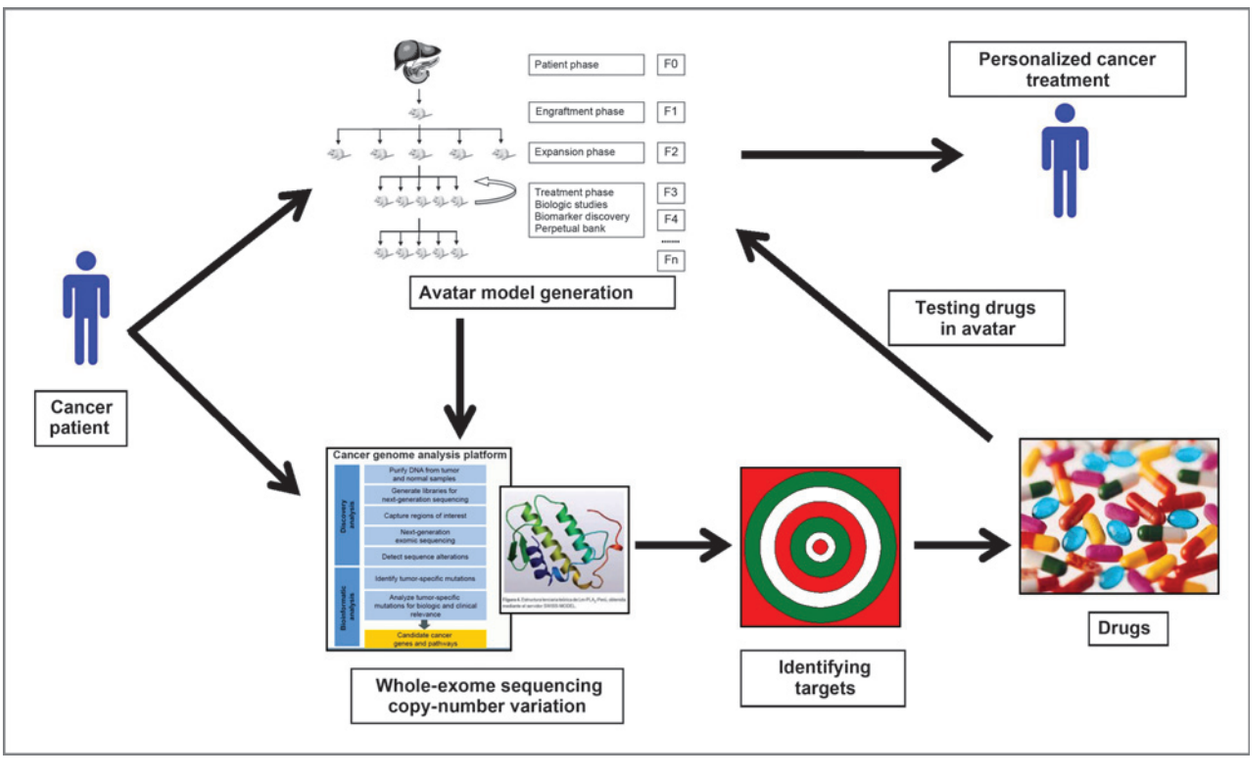

Figure 1. Study design schema.

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Genomic and bioinformatics analysis

After pathologic review, thin sections were obtained for specialized dissection and purification of the tumor DNA to enrich for tumor purity. Tumor formalin-fixed paraffin-embedded blocks were cut in 3 μm thick sections, stained with hematoxylin and eosin, and assessed by a pathologist to confirm tumor type and mark regions predominantly containing neoplasic cells and normal tissue. An adjusted number of consecutive unstained slides of 8 to 10 μm thickness were used for macro-dissection in each case to yield approximately 250 ng of DNA. DNA samples were enriched for coding regions in the genome using custom DNA capture approaches. Matching normal DNA was obtained from blood. Genomic DNA from tumor and normal samples were fragmented and used for Illumina TruSeq library construction (Illumina). Exonic regions were captured in solution using the Agilent SureSelect 51 Mb Kit (version 4) according to the manufacturer's instructions (Agilent). These include the coding exons of ~20,000 genes covering >50 Mb of the genome. Paired-end sequencing, resulting in 100 bases from each end of the fragments, was performed using a HiSeq 2000 Genome Analyzer (Illumina). Exome sequencing was performed at depths of 75× to >200× depending on the tumor purity. The tags were aligned to the human genome reference sequence (hg18) using the Eland algorithm of CASAVA 1.7 software (Illumina). The chastity filter of the BaseCall software of Illumina was used to select sequence reads for subsequent analysis. The ELAND algorithm of CASAVA 1.7 software (Illumina) was then applied to identify point mutations and small insertions and deletions.

The resulting alterations are compared among tumor and normal sequence data as well as to databases of known variants to distinguish common variants, private (rare) germline changes, and potential somatic alterations. Known polymorphisms recorded in dbSNP were removed from the analysis. Potential somatic mutations were filtered and visually inspected. We used three *in silico* methods (Polyphen, SIFT, SNP&GO) to estimate the functional significance of a given confirmed mutation.

Generation of Avatar mouse models

We attempted to establish an Avatar model from each of the patients following the methodology previously published by our group (12, 13). Mice used in this research have been treated humanely according to the regulations laid down by the Bioethics Committee and the relevant EC guidelines (directive 86/609/EEC), with due consideration to the alleviation of distress and discomfort. More detailed information about the Avatar generation protocols can be found in the Supplementary Material. Briefly, a tumor specimen obtained by a tumor biopsy was transplanted and propagated in nude mice. Avatar models were mostly generated by specimens obtained from fresh biopsies of metastasis, as they were generally more accessible than the primary tumors and generally represent a more advanced tumor clone with additional driver/aggressive mutations. Once the tumor specimen was in an exponential growth phase, cohorts of mice with tumor sizes of 0.15 to 0.3 mL were randomized to several treatment groups. The xenograft provided a mechanism to test for the most effective agent if there were several candidate agents identified with exome sequencing and to formulate treatment recommendations for patients in whom the genomic analysis was not contributory.

Patient treatments and follow-up

The patients included in the study started receiving conventional treatment while exome sequencing and Avatar models were being generated and tested. Those that afterwards presented with progressive disease received the personalized treatment accordingly to the results.

A team integrated by biologists, clinicians, and bioinformaticians performed the decision-making process of choosing the most appropriate molecular treatment for the Avatars, and the most "actionable" Avatar-suggested treatment for the patients. This was performed by (i) the prioritization of candidate driver mutations, (ii) the interpretation of the mutations at the pathway/metabolic level, and (iii) selecting the drugs potentially related with those pathways and processes.

Patients were treated with either standard-of-care regimens or referred to clinical trials with new drugs. Several medical and technical issues influenced the use of standard or personalized treatments approach such as the finding or not of a druggable target, the viability of obtaining a specific treatment, and the timing and condition of the patients when the data were available. Treatments administration, toxicity monitoring, and management and efficacy assessment were performed as per current practice guidelines or as specified in the research protocols.

Results

General results

A total of 25 patients were included. Table 1 summarizes the most relevant clinical characteristics of these patients. Successful exome sequencing analyses were obtained for 23 patients. Two patients with pancreatic cancer progressed rapidly and the procedures were aborted. An Avatar mouse model was successfully generated in 10 of 14 patients. In 9 patients, a model could not be generated due to either technical reasons or patient refusal. A total of 13 patients received a cancer treatment based on the genomic and/or Avatar mouse model data to date. Three additional patients are still in response with up front chemotherapy and will be treated at the time of progression with a personalized regimen. The remaining 7 patients have not received a personalize therapy because of failure to find a suitable druggable alteration (2 cases), poor performance status that precluded patient treatment (1 case), or lack of access to investigational agents (4 cases).

Genomic analysis

More than 30 million bases of target DNA were analyzed in the tumor and normal samples in every case, with

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an average of at least 70 distinct reads at each base. Supplementary Table S1 lists, for each one of these patients, tumor-specific mutations and copy-number variations (CNV) as determined by bioinformatics analysis. For each patient, the mutated genes symbol, gene description, functional group and pathway, transcript accession, mutation position, the genomic, transcript and protein level, and the mutation type is listed.

The number of somatic mutations and CNVs ranged from 5 to 952 and from 0 to 965, respectively. The median number of mutations was 45 and median CNV was 6. From this list of candidates, we manually extracted the most relevant alterations and from this, the clinical actionable genetic alterations that could be targeted with current drug armamentarium. As shown in Table 2, these results varied significantly from one patient to another with patients such as patient #1 with a low grade intestinal neuroendocrine tumor having only one targetable mutation in *CREB3L3* and patient #7 with malignant melanoma with mutations in more than 10 well accepted drug targets such as *IGF1R*, *MET*, *PI3K*, and *FGFR*.

Table 1. Patient characteristics

Characteristics	Number of patients (%)
Sex	
Male	15 (60)
Female	10 (40)
Age, y	
Median	55
Range	35–74
Number of prior treatment regimens	
Median	3
Range	1–8
Primary tumor type	
Colorectal	3 (12)
Glioblastoma	2 (8)
Pancreas	7 (28)
NSCLC	5 (20)
Melanoma	3 (12)
Other[a]	5 (20)
Attempted Avatar generation	14 (61)
Engraftment success	10 (71)
Successful exome sequencing obtained	23 (92)
Successful CNV obtained	21 (84)
Received personalized treatment	13 (57)
Not received personalized treatment	12 (43)
On standard first-line treatment	3 (30)
Not suitable druggable alteration	2 (20)
Poor performance status	3 (10)
Difficult access to drug	4 (40)

[a]Other, 1 duodenal, 1 oesophageal, 1 SCLC, 1 intestinal neuroendocine, and 1 renal.

Avatar mouse models

To empirically test the tumor response to theoretical treatments and make individual patient treatment decisions, we generated Avatar mouse models from 14 of these patients. Supplementary Table S2 lists the specific regimens, dosing details, and responses observed in the Avatar.

The Avatar models proved valuable to help interpret the genomic information. This is well illustrated in patient #3. Genomic analysis of this patient showed 62 somatic mutations and 6 CNV. Exome sequencing detected the p.F909C mutation in the catalytic domain of the phosphoinositide 3-kinase protein, leading to a volume change (from bulky F to a smaller C) with the introduction of possible S-S bonds. The severity of this mutation was estimated to be high based on structural information (Fig. 2A), and two other mutations involving *PI3KCA* F909 were reported by the Cosmic database (p.F909L and F909S). Furthermore, the presence of *GNG11* amplification suggested activation of the Ras-Raf-MEK pathway in this tumor with wild-type *RAS* and *RAF* genes (14). To evaluate these therapeutic options, we treated the patient Avatar model. As shown in Fig. 1C, treatment with a PI3K inhibitor alone did not show evidence of tumor control and indeed there was no evidence of activation of the PI3K pathway in this tumor despite the presence of a *PI3K* mutation (Fig. 2B). Interestingly, the use of the Avatar model enabled a more complete analysis, testing drug cocktails, and showed the combination of a PI3K and MEK inhibitors as a possible effective approach (Fig. 2C). Unfortunately, we did not find access to such a combination in the clinic to offer the patient. Gemcitabine was also effective, but the patient had already failed this treatment previously as he had initially been diagnosed as a pancreatic adenocarcinoma in another center. In addition, this tumor also had a somatic mutation of discoidin domain receptor 2 (*DDR2*), which had been just recently reported to be associated with increased sensitivity to dasatinib (15). The patient was started on dasatinib, as due to time restrains we could not wait to obtain the Avatar results. In both the patient and the xenograft, treatment failed. (Fig. 2D).

Likewise, the glioblastoma of patient #2 had 63 mutations and 23 CNVs detected including a mutation in Neurofibromin 1 (*NF1*). Inactivation of *NF1* gene has been related to an increased activity of downstream RAS pathways which was demonstrated in this case (Fig. 2B). As hyperactivation of RAS turns signals through the RAF/MEK/ERK and PI3K/mTOR pathways to regulate cell growth and survival (16, 17), a battery of treatments, including PI3K and MEK inhibitors, were tested (Fig. 2E and F). The most effective treatment in the Avatar model was the combination of everolimus and erlotinib (reported to be effective in low-grade gliomas with NF1 inactivation (16) that slowed tumor growth but did not result in tumor shrinkage. Patient treatment was stopped 3 months later due to lack of clinical benefit without clear radiologic progressive disease.

Avatar models also proved useful for a direct assessment between potential targeted therapies based on genomic information and potentially active chemotherapy regimens selected from a long list of phase II studies. This is well seen

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Table 2. Genomics analysis

N#	Patient Id	Tumor type	N# Mutations	Relevant somatic mutations	N# CNV	Relevant CNV	Putative targets
1	PGDX4	Neuroendocrine tumor	5	*CREB3L3*, *ITPR2*, *MYO5B*	0	0	CREB3L3
2	PGDX30	Glioblastoma	63	*EPHA3*, *NF1*, *PTPN11*, *FAS*, *CDKN2A*	0	0	NF1
3	PGDX7	High grade pancreatic neuroendocrine tumor	62	*ARID1A*, *ARID1B*, *JAKMIP2*, *JARID2*, *PIK3C2A*, *PIK3CA*, *SSTR2*, *DDR2*, *TP53*	6	*GNG11*	PI3KCA, DDR2
4	PGDX11	Pancreatic Adenocarcinoma	38	*KRAS*, *UBA1*, *FAM83H*, *SMAD4*, *SLC15A2*, *PIWIL3*, *SLC3A2*, *SLC22A17*, *TP53*	10	0	Not found
5	PGDX61	Melanoma Uveal	5	*GNA11*, *TAOK3*	0	0	GNA11
6	PGDX68	Colon cancer	71	*APC*, *DICER 1*, *TP53*, *CHEK1*, *SOS1*	63	0	CHEK1
7	PGDX76	Melanoma	952	*BRCA1*, *EZH2*, *FGFR2*, *FN1*, *IGF1R*, *KDR*, *KRAS*, *MET*, *MPL*, *PRKCB*, *PIK3C2G*, *PTK2B*	0	0	FGFR2, IGF1R, PIK3C2G, MET, BRCA1
8	PGDX135	Melanoma	29	*BAI3*, *DNAH5*, *MDN1*, *NRAS*	2	*SKT19*	NRAS
9	PGDX368	Pancreatic Adenocarcinoma	18	*SMAD4*, *KRAS*, *ERBB2IP*	0	0	ERBB2IP
10	PGDX331	Pancreatic Adenocarcinoma	21	*KRAS*, *XPC*, *P53*	0	0	XPC
11	PGDX369	Pancreatic Adenocarcinoma	29	*KRAS*, *P53*, *SMAD4*	3	0	Not found
12	PGDX379T2	Renal Carcinoma	25	*BAP1*	965	*ZAP70*, *FGFR3*, *NOTCH1*, *TERT*, *STK11*, *GNA11*, *ZNF668*, *SOCS1*, *IRS2*	BAP1, FGFR3, NOTCH1, STK11, GNA11
13	PGDX330	Glioblastoma	64	*MLLT10*, *PBRM1*	27	*EGFR*, *CDKN2A*, *ERRFI1*	EGFR
14	PGDX27	NSCLC	45	*EGFR*, *EZH2*, *TPR*, *TP53*, *RHOH*	0	0	EGFR
15	PGDX17	NSCLC	69	*ARID4B*, *PIK3R6*, *PTPRC*, *TP53*, *TOPO2A*	13	0	PTPRC, KIF5B-RET Fusion STK11 Whole Gene Deletion
16	PGDX140	NSCLC	391	*BUB1B*, *CYLD*, *EPHB6*, *EGFR*, *KRAS*, *KEAP1*, *MSH6*, *NTRK1*, *NTRK3*, *MUTYH*, *RUNX1T1*, *TP53*	12	*CDKN2A*	EGFR
17	PGDX24	SCLC	32	*PAPPA2*	Not done	Not done	Not found
18	PGDX60	NSCLC	9	*NOCHT2*, *MML3*	6	0	NOCTH2
19	PGDX3	NSCLC	38	*DLK1*, *JAK3*	Not done	Not done	JAK3
20	PGDX48	Duodenal cancer	127	*CARD11*, *CHL1*, *FANCD2*, *IRS1*, *NRAS*, *SMARCA4*, *TP53*, *PIK3R1*	25	0	NRAS, PIK3R1
21	PGDX39	Colon cancer	172	*APC*, *EGFR*, *FN1*, *GRM1*, *TP53*, *TSC2*	0	0	EGFR, FN1, TSC2
22	PGDX327	Colon cancer	108	*APC*, *AXIN2*, *EGFR*, *KDM6A*, *PTEN*, *PI3KCA*, *TP53*	32	*RECQL4*	EGFR, PI3KCA, PTEN
23	PGDX310	Esophageal cancer	96	*PIK3R1*, *NF1*, *TP53*	65	*CCND1*	NF1, PIK3R1

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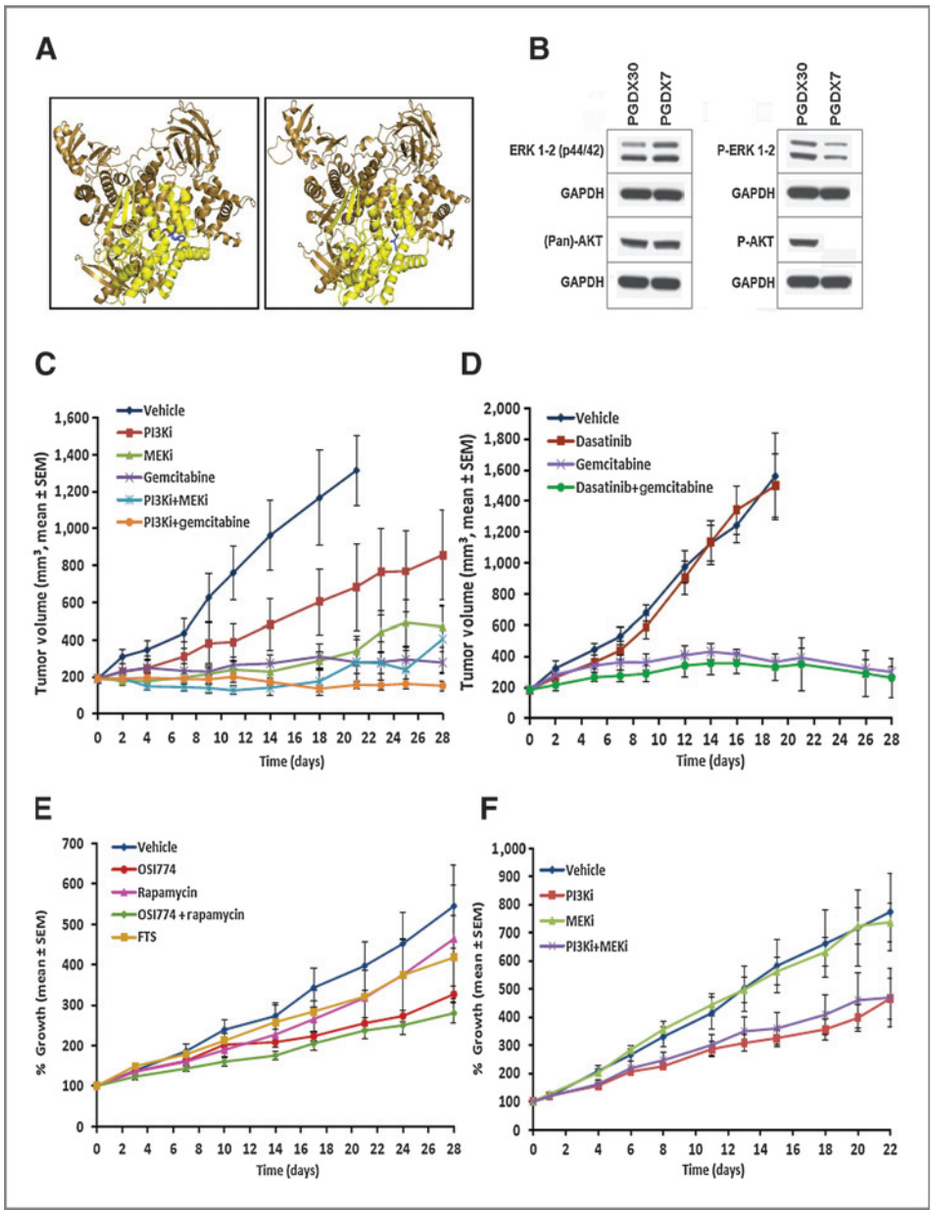

Figure 2. A, structural models of PI3K. Kinase domain in yellow. Mutated aa in blue. Right, original protein with original aa. Left, predictive model of structural changes caused by the mutation. Severity of the mutation estimated to be high by computational analysis. B, total and activated ERK and AKT were evaluated by Western blot (WB) analysis in total protein extracts of the high-grade pancreatic neuroendocrine (PGDX7) and the glioblastoma (PGDX30) xenografted tumors. Left, total ERK and AKT proteins; right, phosphorylated forms of ERK and AKT, in the analyzed samples. GAPDH was used in all cases as loading control (*P* = phospho). C and D, representative tumor growth curve of Avatar PGDX7 treated with the studied agents. PI3i: 20 mg/kg *per os*; everyday, Monday–Friday (M-F), for 28 days. MEKi: 4mg/kg *per os*; everyday, M-F, for 28 days. PI3Ki + MEKi: 20 mg/kg *per os* + 4 mg/kg *per os*; everyday, M-F, for 28 days. PI3Ki+Gemcitabine: 20 mg/kg *per os*; everyday, M-F, for 28 days + 100 mg/kg i.p.; twice a week for 28 days. E and F, representative tumor growth curve of Avatar PGDX30 treated with the studied agents. OSI774 (erlotinib): 50 mg/kg i.p.; daily for 28 days. Rapamycin: 4 mg/kg *per os*; daily, for 10 days. FTS: 100 mg/kg *per os*; daily, for 28 days. OSI774 (erlotinib) + rapamycin: 50 mg/kg i.p.; daily, for 28 days + 4 mg/kg *per os*; daily for 28 days. PI3Ki: 20 mg/kg *per os*; daily M-F, for 28 days. MEKi: 4 mg/kg *per os*; daily M-F, for 28 days. PI3Ki + MEKi: 20 mg/kg *per os*; daily M-F + 4 mg/kg *per os*; daily M-F for 28 days.

in patient #16 with non–small cell lung cancer (NSCLC). Genomic analysis showed a mutation in *EGFR* not described previously (p.A1158V). A battery of treatments was tested in the Avatar, including treatment with erlotinib; however, other agents such as everolimus and nab-paclitaxel were more effective. Based on these data, the patient was treated with nab-paclitaxel and everolimus achieving a partial response.

Furthermore, Avatar models were used to test empirically potential active drugs for individualized patient treatment when there were no druggable alterations identified. This is well illustrated in patient #17 diagnosed of advanced small-cell lung cancer (SCLC). NGS revealed 32 mutations, none were actionable. She received two consecutive personalized treatments based solely on her personal Avatar results obtaining a favorable outcome both times (Table 3).

Clinical outcome

A total of 13 patients have received a treatment based on the genomic and/or Avatar model data (Table 3), including 5 patients who received more than one sequential tailored treatment. Six patients achieved partial remissions and 7 patients are currently on treatment with at least disease stabilization. The Avatar proved to be useful guiding a successful therapy in 5 patients. In 4 patients, the treatment was based exclusively on Avatar mouse models (#15, 16, 17, and 20), receiving multiple sequential guided treatments. On the whole, 13 treatments were Avatar directed, and in 11

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Table 3. Patients, treatments, and outcome

Patient #	Primary tumor	Putative target on NGS	Avatar best treatment	Patient-tailored treatment	Best response (RECIST)	Time on treatment (months)	Present status
1	Neuroendocrine tumor	*CREB3L3* mutation	No engraftment	Sandostatin + metformin	SD Complete metabolic response by PET	18+	On treatment
2	Glioblastoma	*NF1* mutation	Rapamycine + erlotinib	Everolimus + erlotinib + bevacizumab	PD	3	Dead
3	High grade pancreatic neuroendocrine tumor	*PI3KCA*, *DDR2* mutations	MEK inh + Pi3K inh	Dasatinib	PD	3	Dead
5	Uveal melanoma	*GNA11* mutation	No engraftment	1st: Protein kinase C inhibitor	SD	4	Other treatment
				2nd: Carboplatin + paclitaxel + Pi3k inhibitor			
10	PDAC	*XPC* mutation	Not performed	Mitomycin C	PD	3	Dead
12	Renal	*BAP1* mutation	Not performed	Mitomycin C + irinotecan	SD	3+	On treatment
13	Glioblastoma	*EGFR* amplification	Not performed	Erlotinib	SD	3+	On treatment
14	NSCLC	*EGFR* mutation	No engraftment	Erlotinib	PR	24+	On treatment
15	NSCLC	*PTPRC* mutation. *KIF5B-RET* Fusion	-Irinotecan + pemetrexed + bevacizumab	1st: Irinotecan + pemetrexed + bevacizumab	1st: CR	1st: 13	On treatment
		STK11 Whole Gene Deletion	-Nab-paclitaxel + everolimus	2nd: Nab-paclitaxel + everolimus	2nd: PR	2nd: 4	
				3rd: Sunitinib	3rd: SD	3rd: 2+	
16	NSCLC	*EGFR*	-Cisplatin + gemcitabine	1st: Cisplatin + gemcitabine	1st: PR	1st: 6	On treatment
			-Nab-paclitaxel -Everolimus	2nd: Nab-paclitaxel + everolimus	2nd: PR	2nd: 3+	
17	SCLC	No target	-Irinotecan	1st: Irinotecan	1st: PR	1st: 6	Dead
			-Nab-paclitaxel	2nd: Nab-paclitaxel	2nd: SD	2nd: 4	
20	Duodenal	*NRAS* and *PIK3R1* mutation	-Gemcitabine + everolimus	1st: Irinotecan + cetuximab	1st: PR	1st: 3	Dead
			-Eribuline	2nd: Eribuline	2nd: PD	2nd: 1	
			-Irinotecan + cetuximab	3rd: Gemcitabine	3rd: PD	3rd: 1	
22	Colorectal	*EGFR* and *PIK3CA*	Irinotecan + panitumumab	Irinotecan + panitumumab	PR	2+	On treatment

Abbreviations: CR, complete response; PD, progressive disease; PDAC, pancreatic adenocarcinoma; PR, partial response; SD, stable disease.

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the Avatar response mimicked the patient response, predicting 2 progressive disease, 1 complete response, 6 partial response, and 2 stable disease (Table 3). In 3 patients, no potential targetable alteration was found, including two pancreatic adenocarcinoma (PDAC) with a *KRAS* mutation and no animal model and the SCLC described above which received Avatar-guided treatment.

Discussion

This report summarizes our experience of personalizing treatment of advanced cancer patients by integrating data obtained by NGS techniques and Avatar mouse models developed from the patient's own tumor. This work is one of the first experiences to apply these technological advances to patient care and shows the feasibility of the approach. At this time, 57% patients have received a personalized therapy and out of them 77% have experienced a clinical benefit (stable disease or partial response) with the tailored treatment. Several aspects are worth discussing.

All tumor samples profiled contained biologically or clinically potentially meaningful genomic alterations, including several that might predict sensitivity or resistance to targeted agents. Thus, exome-sequencing analysis provides a comprehensive approach for the detection of multiple categories of actionable genetic alterations. However, the use of this information is complex because of the high number of somatic alterations encountered and the lack of biologic testing or data for most identified alterations. Functional validation is the gold standard for assessing the mutation significance and in this sense, personalized xenografts developed in mice from patients' tumor tissues, such as Avatar models, offer a tool to test and validate the hypothesis generated by the genetic analysis (18, 19). This is best illustrated in patient #3 in whom the exome analysis of his tumor showed a plausibly actionable alteration in the *PIK3CA* gene. Bioinformatic tools predicted the relevance of the mutation to be high; however, PI3K inhibitors failed to offer activity in the animal model.

It is becoming clear that predicting treatment response to known oncogenes is complex and requires detailed information of how different genetic backgrounds function and about how the neoplastic stroma will contribute to drug response. The redundancies of the proliferative signaling pathways may underlay the lack of response in some patients whose tumors express oncogenic targets, and are consequently treated with matched targeted drugs but fail to obtain a therapeutic benefit (20). For example, in patient #3 the activation of the MAPK pathway due to the amplification of GNG11 could explain the failure of PI3K inhibitors and highlights the importance in obtaining CNVs in addition to assessing gene mutations. To fine-tune therapies to be efficacious in each individual, not only common driver mutations will have to be analyzed but we will also need to develop a deeper understanding of the individual diversity in the biology of cancer among patients with *a priori* similar tumors. A systems approach will be necessary to determine if analyzing alterations in signaling pathways, as opposed to directly targeting mutated genes, can prove to be more useful. In our cases, for example, the DNA repair pathway alterations found in patients #10 and #12 could be targeted mechanistically using drugs that take advantage of these altered DNA repair mechanisms.

Avatar models may help resolve some of the above mentioned issues as they can help channel the genomic analysis results into appropriate empiric testing. As seen in our results, they are an accurate *in vivo* platform to test proposed treatment strategies, showing an existing remarkable correlation between drug activity in the Avatar and clinical outcome in the patients, in terms of both drug resistance and sensitivity. Moreover, in most cases when genomic analysis provided little insight or targeting pathways failed, more conventional drugs and combinations were appropriately selected only because of the Avatar model.

There are different technologies that allow us to interrogate the genomic profile of a tumor, such as sequencing only a target panel of genes, which could facilitate the interpretation and analysis of the results. However, the advantages of sequencing exome rather than selected targets are several, including the possible identification of a larger number of druggable mutated genes and discovering possible new genes involved in the tumorigenesis that allow a more comprehensive analysis. In addition, the costs of exome sequencing are continuing to diminish and the results can be obtained in a relatively short time.

There are limitations with this combined approach that continue to challenge its broad clinical application. First, important technical issues regarding tumor profiling remain to be solved to obtain readily interpretable results, for example: choosing the most appropriate technique (target sequencing vs. exome sequencing vs. whole-genome sequencing; ref. 21), observed tumor heterogeneity (22), subclonal evolution (23), or selecting primary tumor versus metastatic tumors (24). Second, the generation of a personalized xenograft model has limitations, requiring large amounts of fresh tumor material and intense resources. Engraftment failure is still an issue that can be improved.

The development and propagation of the Avatar model and drug testing takes 4 to 6 months and, in addition, the failure rate in tumorgraft establishment is also a drawback. Finally, there are also practical issues in the everyday clinical setting that have to be considered. Examples are patient #3 in which the optimal tailored treatment (the combined PI3K and MEK inhibitors) could not be given or patient #8 having a melanoma with an *NRAS* mutation but not meeting eligibility criteria to participate in a clinical study. Difficult access to the drug or combination treatment can be a major drawback.

In summary, here we describe our experience using a new approach to determine the optimal treatment for an individual patient with cancer. The sample size remains at the time too small and heterogeneous to conclude if this approach will be better than the standard-of-care approach to select therapy. However, the work represents an important advance showing that the analysis of somatic genetic alterations plus the use of the patient's tumor growing in

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nude mice can be performed in the clinical setting and can guide to specific treatments in a significant fraction of patients. The detection of actionable tumor-specific genomic alterations in the clinical setting is at the time feasible; however, predicting treatment response to known oncogenes is still complex. Bench testing of candidate treatments in patient-derived xenografts correlates with clinical response and may help to select treatment in some of the patients with no actionable mutations, helping in the challenge of linking confirmed mutations to biologic function and ultimately to clinical response and utility. Despite limitations in efficiency, speed, and cost; our current data suggest that further studies applying the Avatar-Exome integrating approach might yield promising results to the arising field of personalized cancer medicine.

Disclosure of Potential Conflicts of Interest

L.A. Diaz is an officer and board member for and has ownership interest (including patents) in Personal Genome Diagnostics; has received speakers bureau honoraria from Illumina; and is a consultant/advisory board member for Amgen. V.E. Velculescu is employed on the board of directors of, is CSO for, and has ownership interest (including patents) in Personal Genome Diagnostics. D. Sidransky is a chairman of, has ownership interest (including patents) in, and is a consultant/advisory board member for Champions Oncology. M. Hidalgo has ownership interest (including patents) in Champions Oncology. No potential conflicts of interest were disclosed by the other authors.

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