

Molecular Profiling of Exceptional Responders to Cancer Therapy

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Exceptional responders • Immune system • Precision medicine • Genomic instability • DNA damage repair

ABSTRACT

Background. The vast majority of metastatic cancers cannot be cured. Palliative treatment may relieve disease symptoms by stopping or slowing cancer growth and may prolong patients' lives, but almost all patients will inevitably develop disease progression after initial response. However, for reasons that are not fully understood, a very few patients will have extraordinary durable responses to standard anticancer treatments.

Materials and Methods. We analyzed exceptional responders treated at Fox Chase Cancer Center between September 2009 and November 2017. An exceptional response was defined as a complete response lasting more than 1 year or a partial response or stable disease for more than 2 years. Tumor samples were analyzed using an Ambry Genetics test kit with a 142-gene panel. Messenger RNA expression was evaluated using NanoString's nCounter PanCancer Pathways Panel and Immune Profiling Panel and compared with matched controls for gender, age, and cancer type.

Results. Twenty-six exceptional responders with metastatic bladder, kidney, breast, lung, ovarian, uterine, and colon cancers were enrolled. Mutations were identified in 45 genes. The most common mutation was an *EPHA5* non-synonymous mutation detected in 87.5% of patients. Mutations in DNA damage repair pathway genes were also frequent, suggesting increased genome instability. We also found varying expression of 73 genes in the Pathways panel and 85 genes in the Immune Profiling panel, many of them responsible for improvement in tumor recognition and anti-tumor immune response.

Conclusion. The genomic instability detected in our exceptional responders, plus treatment with DNA damage compounds combined with favorable anticancer immunity, may have contributed to exceptional responses to standard anticancer therapies in the patients studied. *The Oncologist* 2021;26:186–195

Implications for Practice: With recent advances in the treatment of cancer, there is increased emphasis on the importance of identifying molecular markers to predict treatment outcomes, thereby allowing precision oncology. In this study, it was hypothesized that there is a “specific biologic signature” in the biology of the cancer in long-term survivors that allows sensitivity to systemic therapy and durability of response. Results showed that DNA damage repair pathway alterations, combined with favorable anticancer immunity, may have contributed to exceptional responses. It is very likely that an in-depth examination of outlier responses will become a standard component of drug development in the future.

INTRODUCTION

Some patients with metastatic cancer will have markedly better responses to treatment than other patients receiving the same treatment. The National Cancer Institute (NCI) defines exceptional responders as patients with cancer who

have had a complete response (CR) or partial response (PR) for at least 6 months to a treatment in clinical trials in which less than 10% of patients responded overall [1]. Exceptional responders are not often studied because they

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are quite rare and, within a given trial, form too small a subset for statistical analysis. There is now, however, a growing interest in studying exceptional responders. The NCI's Exceptional Responders pilot clinical trial (NCT02243592) enrolled 100 exceptional responders to analyze tumor tissue and clinical data and to determine whether certain molecular features can predict responses to anticancer drugs. Study accrual goals were met by November 2017, and analysis is ongoing. (The study reported here is separate from NCT02243592).

In the last decade, a number of major advances in molecular biology technology have increased our ability to characterize cancer genes and their structure and expression. There has also been an increasing effort to translate individual genomic information into personalized medicine, with the goal of optimizing survival [2]. A fascinating story of the discovery of somatic gain-of-function mutations in hyper-eosinophilic syndrome came from a group of community practice oncologists who reported a dramatic response to the off-label use of imatinib [3]. This initial observation generated the hypothesis that hypereosinophilic syndrome is driven by clonal activation of an imatinib-responsive tyrosine kinase, which has been confirmed later by several trials [4]. Similarly, investigation of a small group of patients with non-small-cell lung cancer who had extraordinary responses to gefitinib led to the discovery of a culprit epithelial growth factor receptor (EGFR) mutation [5].

In this single-center study, we aimed to identify specific molecular signatures among exceptional responders and to unravel molecular patterns that could explain how exceptional responders beat the odds. The hope is that molecular alterations detected in our patients could be explored in others and eventually become a predictor of treatment responses.

SUBJECTS, MATERIALS, AND METHODS

Study Population

We defined an exceptional responder as a patient with a metastatic solid tumor who had a CR lasting more than 1 year following systemic anticancer therapy or PR or stable disease (SD) lasting more than 2 years at any time during the disease course. Patients with hematologic malignancies (including lymphomas), curable solid tumors (e.g., germ cell tumors), and curable nonmetastatic disease were ineligible for this study. Potential participants were identified and referred by their treating medical oncologists. All patients were aged at least 18 years, had an Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 1 , and had available formalin-fixed paraffin-embedded (FFPE) archival tumor tissue. This study was approved by the institutional review board at Fox Chase Cancer Center in September 2014 (IRB 14-083).

Clinical Data

Each patient gave informed consent, including permission to access their archived tumor samples and collect relevant clinical data from electronic medical records. Information obtained included the patient's age, sex, tumor type and location, date of diagnosis, stage, ECOG performance status, treatments received, duration of best response (CR, PR, SD), and date of recurrence or death if applicable. Response assessment was obtained

using the RECIST version 1.1 [6]. Progression-free survival (PFS) was defined as the time from the first dose of the treatment associated with best response until documented radiological progression or death from any cause. Duration of response was defined as the time from the documented response of the treatment associated with best response until documented progression. The treatment associated with the best response that allowed for inclusion of the patient in the study was determined based on a review of clinical data. Data cutoff date for analysis was November 30, 2017.

Molecular Profiling

Next-generation sequencing was performed on 16 patients who had a sufficient amount of archived tissue. DNA and RNA were extracted from sections of FFPE tumor specimens from biopsies or surgical resections. If multiple archived tumor specimens were available, the most recent was reviewed. The minimum acceptable tumor cellularity was 10%. Genomic profiling was initially performed to identify molecular aberrations potentially predictive of treatment response and targetable for therapeutics. The samples were analyzed using an Ambry Genetics test kit with a 142-gene panel for solid tumors. Exons and a small number of select introns from the 142 genes were enriched using a hybridization-based capture approach and sequenced by next-generation sequencing on a HiSeq system (Illumina; San Diego, CA). The target average coverage per sample was greater than 250 \times to allow high-confidence variant calling [7]. Genomic profiles were compared between different tumor types and treatments associated with the best response. ANNOVAR [8] was used for variant annotation. The functional consequences of nonsynonymous single nucleotide variants and splice variants were predicted using PolyPhen-2 (Polymorphism Phenotyping v2), SIFT, LRT, MutationTaster, MutationAssessor, and CADD [9]. Variants leading to non-synonymous changes in exonic regions of encoded proteins or splice variants were selected if they received scores indicating a protein-damaging function with at least three of six in silico predictors. We also applied a population filter of less than 1% to focus on only rare mutations, and known sequence artifacts were also removed. For indel variants, we only kept those causing frameshifting insertions or deletions. Next, we queried the International Cancer Genome Consortium (ICGC) database with the most commonly observed variants in our analysis (variants present in more than one patient) to look for their distribution in The Cancer Genome Atlas (TCGA) bladder, kidney, breast, and lung cancers, because those are the most common tumors among our data set.

Messenger RNA Expression

Tumor samples from 23 exceptional responders and 23 matched controls were tested for levels of messenger RNA (mRNA) expression using NanoString's nCounter PanCancer panels (Pathways and Immune Profiling). Microdissection of tissue was not performed, and all tissue on unstained slides was used for RNA extraction. Each sample from exceptional responders was paired with a control from a patient matched for age, sex, and diagnosis, and levels of mRNA expression were compared between the two samples. Protected health information related to the control patients was acquired from the Fox Chase Biosample Repository. Twenty 10- μ m

slides were prepared for analysis from each FFPE tissue block. RNA extraction was performed using a High Pure FFPET RNA Isolation Kit (Roche; Penzburg, Germany). Each 50-ng RNA sample was hybridized with the CodeSets of nCounter PanCancer Pathways and Immune Profiling panels. The hybridized samples and data acquisition were processed with the NanoString Prep Station and Digital Analyzer, respectively. For both panels, NanoString expression counts were normalized using NanoString nSolver [10] using default settings (background subtraction based on geometric mean of negative controls, positive control normalization based on geometric mean of positive control probes, and standard CodeSet normalization). Genes with a maximum log₂-scale normalized expression of <6 were excluded as uniformly low expressors. For comparison of gene expression in patient tumor samples and matched controls, the pairwise *t* test was performed and a gene was defined as significant if the false discovery rate <.05 (Benjamini-Hochberg method [11]). The significant genes were plotted according to the order of gene expression fold-change between tumor and control samples.

RESULTS

Patients

Twenty-six exceptional responders (13 male and 13 female) were identified and enrolled in this study. Seven patients had urothelial carcinoma of the bladder, seven had kidney cancer, five had non-small-cell lung cancer, four had breast cancer, and one patient had ovarian, uterine, and colon cancer. CR lasting more than 1 year was observed in 15 patients; 11 patients had either PR or SD. The mean age was 71 years (range, 33–93). Median progression-free survival was 67.5 months (range, 24–142). Baseline characteristics are summarized in Table 1. Table 2 summarizes patients' gender, age, cancer type, treatment that led to the extraordinary response, duration of treatment, and duration of response. Two of our patients received concurrent therapies that could potentially modulate response. Patient 11 (Table 2) was diagnosed with clear cell renal cell carcinoma (RCC) metastatic to the adrenal gland and experienced a PR with sorafenib for 88 months. After 69 months of initial sorafenib treatment, the patient underwent adrenalectomy. Sorafenib was held for 1 month, and after surgery he continued on therapy for another 18 months, when he experienced progression and changed therapy. Patient 13 had clear cell RCC metastatic to soft tissue in his right thigh. After 47 months of initial therapy with sunitinib, he received radiotherapy in the right thigh mass. After radiotherapy, he continued sunitinib for another 5 months, when he had progression and changed therapy. The rest of our patients did not receive any therapy that could potentially modulate response, such as metastasectomy, radiation, or any other systemic treatment, except therapies listed in Table 2.

Molecular Profiling and Targeted Gene Sequencing

Targeted gene sequencing using an Ambry Genetics test kit with a 142-gene panel for solid tumors was performed on 16 patients. After initial analysis, 191 variants were obtained. The variants predicted to be damaging were

Table 1. Demographic data on exceptional responders

Characteristic	Exceptional responders (n = 26)
Gender, n (%)	
Male	13 (50)
Female	13 (50)
Age, years, median (range)	71 (33–93)
Tumor types, n (%)	
Urothelial carcinoma of the bladder	7 (26.9)
Kidney	7 (26.9)
Breast	4 (15.5)
Lung	5 (19.2)
Ovarian	1 (3.8)
Uterine	1 (3.8)
Colon	1 (3.8)
Response type, n	
Complete response (>1 yr)	15
Partial response/stable disease (>2 yr)	11
Duration of response, months, median (range)	
Complete response	88 (25–132)
Partial response/stable disease	45.5 (24–138)

identified by three methods. Mutations were identified in 45 genes (Fig. 1), with a total of 76 rare damaging variants (supplemental online Table 1). The most common mutation was an *EPHA5* nonsynonymous mutation, identified in 14 patients (87.5%). *NF1* splicing mutation was observed in 11 patients (68.7%). This mutation had a population frequency of 1.5%, which is slightly above the cutoff value of 1% and seemed to be damaging by MutationTaster and FATHMM methods but not by ClinVar. Because of the fact that it was present in 68.7% of our patients, we felt that this mutation should be included here. Other mutations also occurred in a high percentage of our patient population. A *FOXL2* nonsynonymous mutation was identified in nine patients (56.2%), *ABL1* mutations (frameshift and nonsynonymous) were identified in seven patients (43.8%), *TP53* mutations (frameshift, nonsynonymous, and stop codon) were identified in six patients (37.5%), *ATM* frameshift and nonsynonymous mutations were identified in four patients (25%), and *ABL2* frameshift mutation, *BRCA2* (frameshift, nonsynonymous, and stop codon), *VHL* (nonsynonymous), *NOTCH4* (nonsynonymous), and *NOTCH 1* (nonsynonymous) mutations were each identified in three patients (18.7%).

A total of seven damaging variants were observed in more than one patient in our analysis (supplemental online Table 1). These variants were observed in genes *EPHA5*, *FOXL2*, *ABL1*, *ABL2*, *APC*, *ATM*, and *VHL*. We queried the ICGC database with these seven variants, and no specific variant level data could be found in TCGA bladder, kidney, breast, and lung data sets, showing that these variants are indeed rare. Looking at gene levels, we also observed that the mutations were rare in the four tumors analyzed from

Table 2. Exceptional responders' treatment and response

Patient number	Gender	Age	Cancer type	Rx	Rx duration, mo	Best response	Duration of response, mo	Status	Sequencing	NanoString
1	M	79	Bladder	Cetuximab + paclitaxel	3	CR	88	Alive	Yes	Yes
2	M	76	Bladder	MVAC	4	CR	98	Alive	No	Yes
3	M	71	Bladder	Gem	2	CR	72	Alive	Yes	Yes
4	M	75	Bladder	Gem + cis	3	CR	79	Alive	No	Yes
5	F	78	Bladder	ddMVAC	3	CR	39	Died	Yes	No
6	M	84	Bladder	Gem + carbo	3	CR	25	Died	Yes	Yes
7	M	93	Bladder	Gem + cis	4	CR	90	Alive	Yes	Yes
8	M	86	Papillary RCC	Tivozanib	41	PR	41	Alive	No	Yes
9	F	50	Clear cell RCC	Axitinib	24	PR	24	Died	No	Yes
10	M	58	Anaplastic RCC	Sunitinib	39	PR	39	Alive	Yes	Yes
11	M	68	Clear cell RCC	Sorafenib	88	PR	88	Died	No	Yes
12	M	57	Clear cell RCC	Sunitinib	20	PR	50	Alive	No	Yes
13	M	59	Clear cell RCC	Sunitinib	52	PR	52	Died	Yes	Yes
14	M	53	Clear cell RCC	Sunitinib	138	PR	138	Died	Yes	No
15	F	77	TNBC	Paclitaxel + bev	6	CR	99	Alive	No	Yes
16	F	33	ER/PR-, HER2+ BC	Paclitaxel + trastuzumab	Paclitaxel: 6, trastuzumab: 93	CR	93	Alive	Yes	Yes
17	F	48	TNBC	Paclitaxel	6	CR	142	Alive	Yes	Yes
18	F	48	ER/PR-, HER2+ BC	Paclitaxel + trastuzumab	Paclitaxel: 6, trastuzumab: 132	CR	132	Alive	Yes	Yes
19	F	59	Lung adenocarcinoma (KRAS mut.)	Carbo + pemetrexed	Carbo: 8, pemetrexed: 50	PR	50	Alive	Yes	Yes
20	F	71	Lung adenocarcinoma (EGFR mut.)	Osimertinib	24	PR	24	Alive	No	Yes
21	M	83	Lung adenocarcinoma (EGFR mut.)	Erlotinib	2	PR	25	Alive	Yes	Yes
22	F	65	Lung adenocarcinoma	Cis + gem	3	CR	117	Alive	No	Yes
23	F	79	Lung adenocarcinoma	Carbo + paclitaxel	4	CR	62	Died	No	Yes
24	F	71	Uterine carcinosarcoma	Ifosfamide	4	SD	32	Died	Yes	Yes
25	F	84	High-grade serous ovarian	Carbo + gem	5	CR	78	Alive	Yes	Yes
26	F	64	Colon cancer KRAS wt	FOLFIRI + cetuximab	11	CR	63	Alive	Yes	No

Abbreviations: BC, breast cancer; bev, bevacizumab; carbo, carboplatin; cis, cisplatin; CR, complete response; dd, dose-dense; ER, estrogen; F, female; FOLFIRI, folinic acid, fluorouracil, and irinotecan; gem, gemcitabine; M, male; mut, mutation; MVAC, methotrexate, vincristine, doxorubicin, and dislatin; mut, mutation; PR-, progesterone negative; PR, partial response; RCC, renal cell carcinoma; Rx, prescription; SD, stable disease; TNBC, triple negative breast cancer; wt, wild type

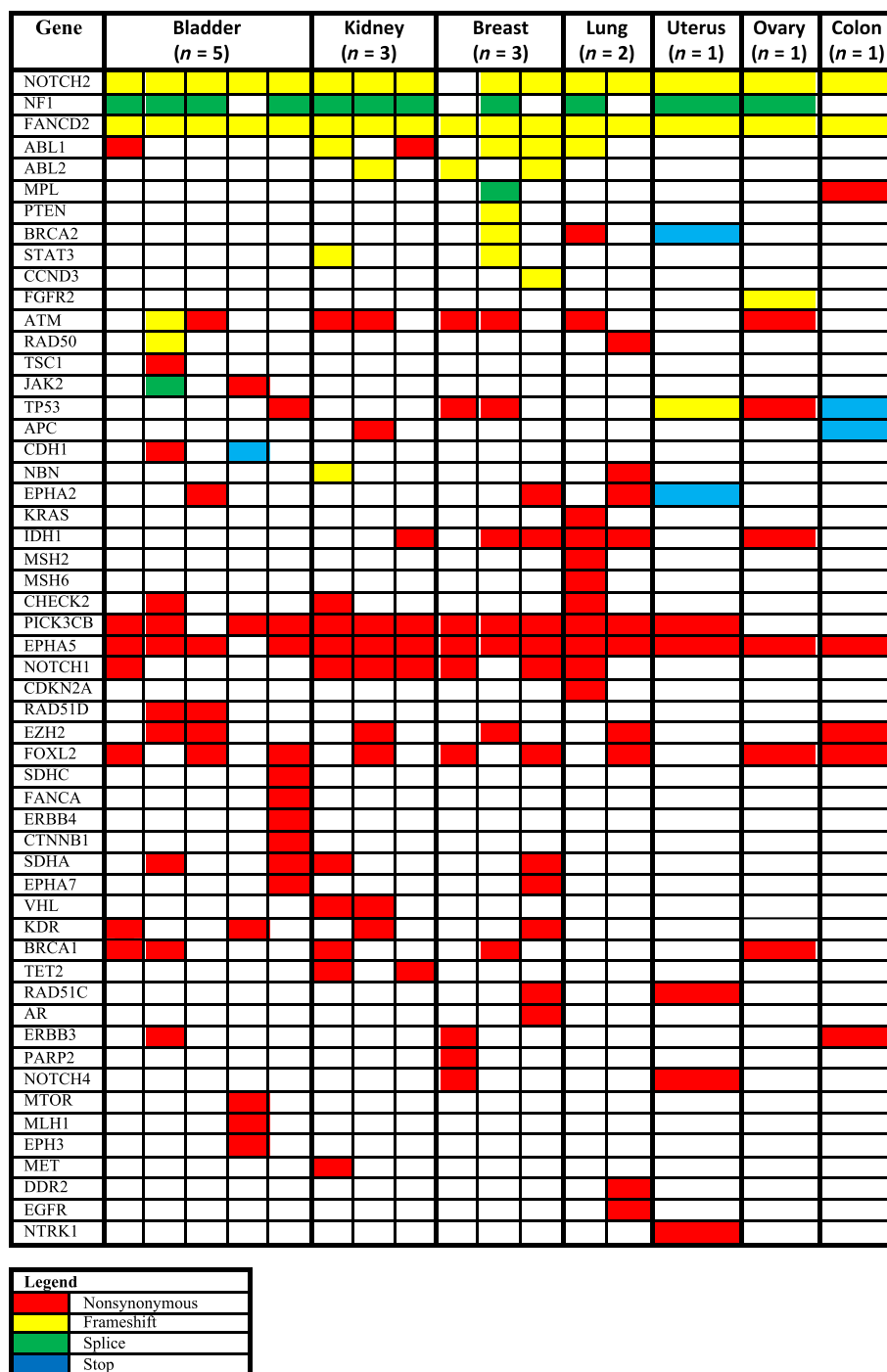


Figure 1. Genomic alterations. Genomic data are from sequencing using Ambry Genetics test kit with a 142-gene panel for solid tumors. The target average coverage per sample was greater than 250x to allow high-confidence variant calling.

the ICGC database (bladder, kidney, breast, and lung), with the exception of a *VHL* mutation in kidney cancer (supplemental online Table 2).

NanoString nCounter System Panels

Twenty-three exceptional responders and 23 control patients matched by age, gender, and disease underwent gene expression analysis using NanoString's PanCancer Immune Profiling Panel and PanCancer Pathways Panel. These panels analyzed 770 genes involved in immune response and cancer-associated

canonical pathways, respectively. Among the genes analyzed, the level of expression significantly differed between case and control in 73 genes in the Pathways Panel and 85 genes in the Immune Profiling Panel.

The Pathways Panel analyzes 770 genes from 13 cancer-associated canonical pathways involved in the hallmarks of cancer; some genes are involved in more than one pathway. Genes with the most significantly different expression are shown in Figure 2. The three most overexpressed genes were tenascin-C (*TNC*) and thrombospondin-4 (*THBS4*; PI3K

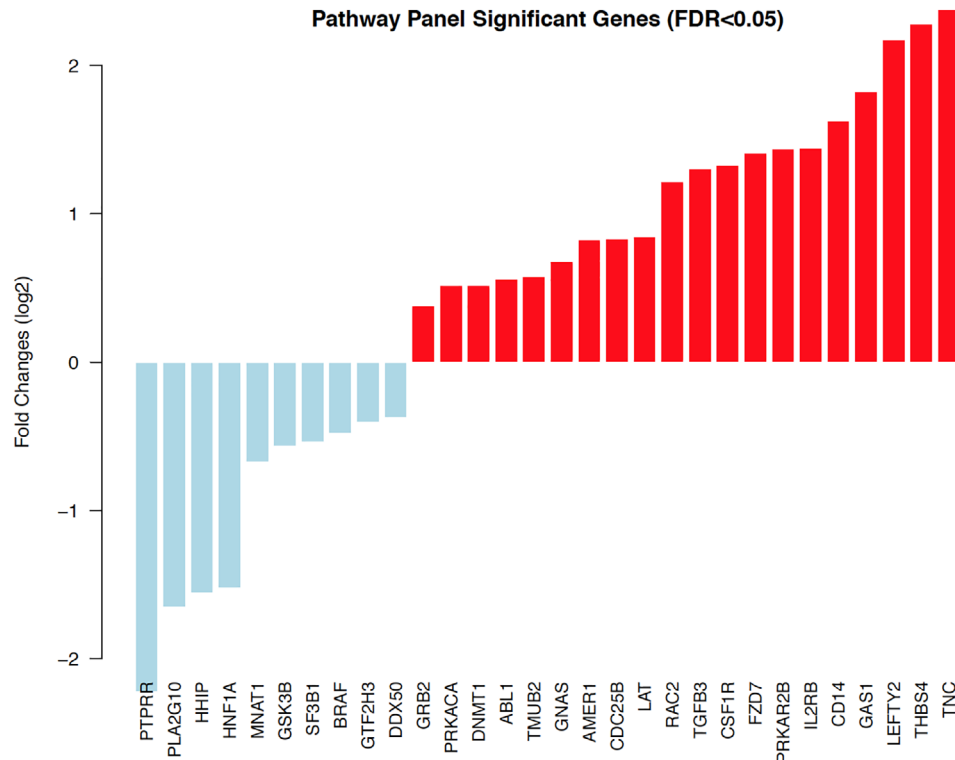


Figure 2. Significant genes from Pathway Panel. Gene expression of 23 paired patient tumor samples and matched control samples were obtained using NanoString nCounter PanCancer Pathway Panel. The pairwise *t* test was performed for the tumor samples and matched controls, and the cutoff of FDR <0.05 was used to define significantly differentially expressed genes. Red bar, overexpressed; blue bar, underexpressed.

Abbreviation: FDR, false discovery rate.

pathway) and *LEFTY2* (TGF- β pathway). We found six overexpressed genes in the MAPK pathway (*CD14*, *TGFB3*, *RAC2*, *CDC25B*, *PRKACA*, and *GRB2*) and six in the RAS pathway (*CSF1R*, *RAC2*, *LAT*, *ABL1*, *PRKACA*, and *GRB2*). Five overexpressed genes are cancer drivers (*CSF1R*, *AMER1*, *GNAS*, *ABL1*, and *DNMT1*). Five of the overexpressed genes we found are in the PI3K pathway (*TNC*, *THBS4*, *IL2RB*, *CSF1R*, and *GRB2*) and the cell cycle and apoptosis pathway (*PRKAR2B*, *TGFB3*, *CDC25B*, *ABL1*, and *PRKACA*). These pathways are involved in deregulating cellular energetics and cell-death resistance, respectively. Three genes were overexpressed in the WNT pathway (*FZD7*, *RAC2*, and *PRKACA*) and the transcription misregulation pathway (*CD14*, *IL2RB*, and *CSF1R*), which are involved in replicative immortality and genome instability, respectively. Finally, two genes were overexpressed in the Hedgehog pathway (*GAS1* and *PRKACA*), two in the TGF- β pathway (*LEFTY2* and *TGFB3*), and two in the JAK-STAT pathway (*PAKAR2B* and *GRB2*). These pathways are involved in cell-death resistance, growth suppressor evasion, and sustained proliferative signaling, respectively. The most underexpressed gene in the Pathways Panel was *PTPRR* (greater than twofold), followed by *PLA2G10*, *HHIP*, and *HNF1A* (Fig. 2). Three of the underexpressed genes are cancer drivers (*BRAF*, *SF3B1*, and *HNF1A*). Two genes were underexpressed in the Hedgehog pathway (*GSK3B* and *HHIP*), two in the DNA damage repair pathway (*GTF2H3* and *MNAT1*), and two in the MAPK pathway (*BRAF* and *PTPRR*).

Immune Profiling Panel analyzes genes related to the immune system. The most overexpressed of these was

TNFRSF8 (CD30 receptor expressed by activated T and B cells), followed by *CYBB*, *CCL2*, *SIGLEC1*, *CD14*, *FAS*, *FCER1G*, *CSF1R*, *LY96*, *ITGAM*, *LAIR2*, *IL2RB*, *TNFSF13B*, *IL21R*, *SELPLG*, *CD74*, *TNFRSF4*, *ITGB2*, and *C1QB*. The most underexpressed genes were *NOS2*, *ARG2*, *REPS1*, and *ATG5* (Fig. 3).

DISCUSSION

Identifying molecular alterations in exceptional responders' tumors was a complex process that involved sequencing targeted genes and assessing gene expression on tumors in exceptional responders and matched controls. The most interesting discovery was identifying the same non-synonymous mutation of the *EPHA5* gene in 87.5% of exceptional responders. Mutations in other genes in the *EPHA* family were also identified: *EPHA2*, *EPHA3*, and *EPHA7*. Another commonly mutated gene was *FOXL2*, a forkhead family transcriptional factor observed in nine patients (56%). Although its role in cancer is not fully understood, recent evidence has suggested that *FOXL2* and its target genes participate in the DNA damage repair (DDR) pathway. Our study also identified additional mutations in DDR pathway such as *BRCA2*, *ATM*, *RAD50*, and *FANCA*. NanoString analysis also demonstrated change in expression of DDR pathway genes: expression of *GTF2H3* (involved in the nucleotide excision repair process) and *FANCL* was reduced in bladder cancer exceptional responders

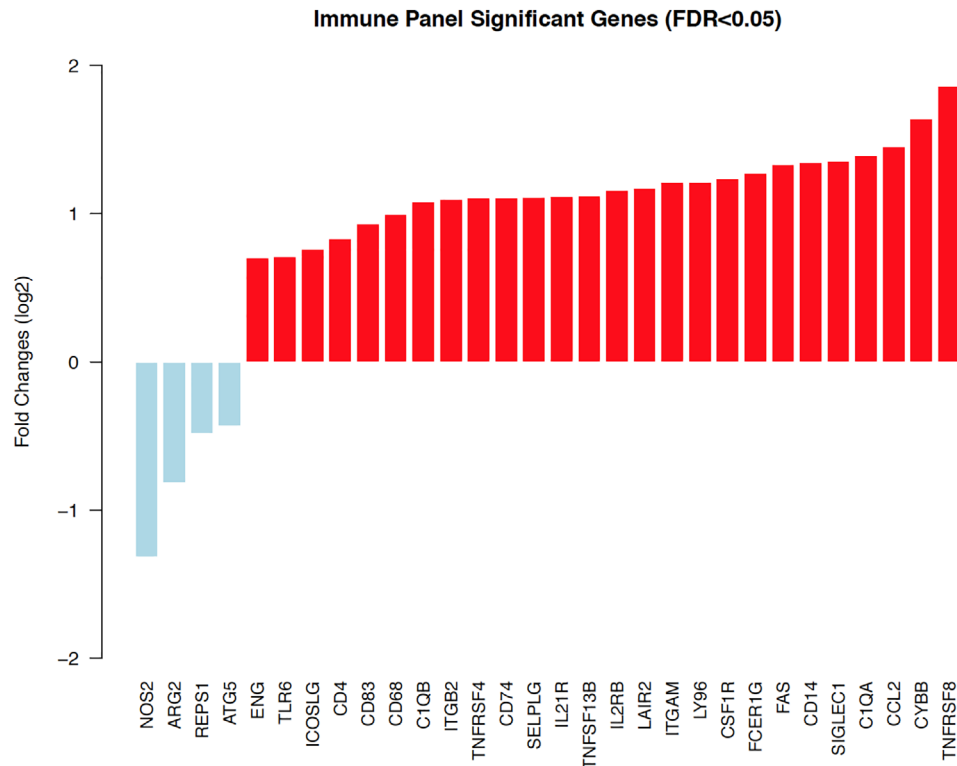


Figure 3. Significant genes from Immune Panel. Gene expression of 23 paired patient tumor samples and matched control samples were obtained using NanoString nCounter PanCancer Immune Panel. The pairwise *t* test was performed for the tumor samples and matched controls, and the cutoff of FDR <0.05 was used to define significantly differentially expressed genes. Red bar, overexpressed; blue bar, underexpressed.

Abbreviation: FDR, false discovery rate.

(supplemental online Figs. 1, 2) in comparison to matched control patients. Other underexpressed DDR genes were *SF3B1* [12], *GSK3B* [13], *MINAT1* [14] (Fig. 2), and *ATG5* (Fig. 3). *ATG5* is a key gene in the autophagy process, playing an important role in the repair and clearance of DNA damage [15].

Eph receptors are the largest family of receptor tyrosine kinases and are known to play an important role in tumor immunity by regulating chemotaxis and migration and enhancement of CD8+ T-cell activity against tumor cells [16–19]. A recently study showed that *EPHA5* mutations were associated with increased tumor mutation burden, tumor-infiltrating lymphocytes, and durable responses in patients with lung adenocarcinoma treated with immunotherapy [20]. This study also reported that *EPHA5*-mutated tumors were associated with high mutation frequencies of DDR genes and had a tumor microenvironment that was enriched with tumor-infiltrating CD8+ T cells, CD4+ activated memory T cells, and macrophage M1 cells [20]. Additionally, *EPHA5*-mutated tumors had an immune signature characterized by increased expression levels of chemokines and cytolytic activity-associated gene signatures [20]. Another study indicated that *EPHA5* plays a direct role in DDR and interacts with ATM at sites of DNA repair and increases sensitivity to anticancer therapies [21].

Our results revealed increased expression of genes related to antitumor immunity. We identified overexpression of several genes involved in the complex interaction between

cancer and the host immune system (Fig. 3). *IL21R* improves T-cell responses and activates natural killer (NK) cells. Interleukin (IL)-21 also improves antigen presentation, and its overexpression is associated with improved outcomes in pre-clinical models of breast cancer [22]. Similarly, *SIGLEC1* (CD169) plays a crucial role in inducing a cytotoxic T-cell response [23]. Exceptional responders also overexpressed *CD4*, a membrane glycoprotein of T lymphocytes that interacts with the major histocompatibility complex II antigens [24], and *IL2RB*, which enriches intratumoral T and NK cells [25] (Fig. 3). *TNFRSF4* (OX-40) promotes the survival and expansion of CD4 and CD8 T cells, enhancing CD8+ T-cell cytotoxic activity and decreasing T-cell exhaustion by inhibiting IL-10 and regulating T-regulatory cells [26]. OX-40 agonists are currently being tested in multiple clinical trials [27–29]. Interestingly, the most overexpressed gene in exceptional responders, analyzed with the Immune panel, was *TNFRSF8* (CD30), a member of the TNF receptor superfamily. *TNFRSF8* is a positive regulator of apoptosis and is expressed only by activated T and B cells. Other pathways implicated in tumor immunity were also differently expressed in the cohort of exceptional responders. *NOTCH1* and *NOTCH2* affect the activation of CD8+ T cells [30] and promote activation of the M1 phenotype that has proinflammatory antitumor activity [31]. *NOTCH2* was overexpressed in exceptional responders with bladder cancer (supplemental online Figs. 1, 2). Interestingly, among exceptional responders, *ARG2* and *NOS2*

(genes involved in immune escape mechanisms) were significantly underexpressed (Fig. 3).

Huang et al. demonstrated that *EPHA5*-mutated lung adenocarcinomas had durable responses to immunotherapy likely related to alterations in the DDR pathway, increased tumor mutational burden, and a favorable antitumor immune signature [20]. We found similar results in our exceptional responders. Some molecular alterations found in our population might help to explain their exceptional responses to chemotherapy. It is well known that tumors with DDR alterations can achieve good responses when treated with DNA damage compounds such as alkylating agents, platinum-based agents, gemcitabine, paclitaxel, irinotecan, and 5-fluorouracil, which almost all of our patients received [32]. Additionally, the most overexpressed gene in our cohort, analyzed through NanoString's pathway panel, was *TNC*, followed by *THBS4*, *LEFTY2*, and *GAS1* (Fig. 2). *TNC* is an extracellular matrix molecule that has pleiotropic effects on tumor and stromal cells. It may also affect drug responsiveness and DNA repair through its interaction with fibronectin [33]. *THBS4*, a tumor-suppressor gene, is a member of the extracellular calcium-binding protein family. Overexpression of *THBS4* in vitro significantly suppresses cancer-cell growth [34]. *LEFTY2* is a regulator of tumor proliferation whose overexpression is related to sensitivity to cisplatin in preclinical studies [35, 36]. In addition, several studies have shown that *GAS1* regulates cell-growth arrest, apoptosis, and sensitivity to cisplatin [37, 38]. Our study demonstrated an overexpression of the *LEFTY2* gene, especially in bladder cancer exceptional responders treated with platinum agents (supplemental online Figs. 1, 2).

Our kidney cancer exceptional responders were treated with antiangiogenic TKIs alone. Antiangiogenic treatment has demonstrated clinical benefit and has been approved for front line treatment of clear cell renal cell carcinoma [39, 40]. Recently, combination trials of TKIs and immunotherapy have shown excellent durable responses [41, 42]. The mechanisms that led our patients to experience these durable responses are not clear but could be related to antitumor immune response. The impact of TKI treatment in the setting of DDR impairment is not known. Our working hypothesis is that TKIs in addition to underlying favorable tumor microenvironment are capable of maintaining durable PRs, similar to the combination of TKIs and immune checkpoint inhibitors. All these patients had *EPHA5* mutation, and the RNA analyzes showed overexpression of genes related to chemotaxis and activation of immune response, such as *IL6*, *COLEC12IL1B*, *CXCL2*, and *CXCL3*; (supplemental online Figs. 3, 4).

Our results demonstrate that outliers have a higher mutational burden in the DDR pathway than is found in an average population of patients with cancer, and mRNA expression analyses confirmed those findings. DDR pathway impairment leads to an accumulation of cytosolic double-strand DNA in the presence of DNA damage agents. This could lead to a proinflammatory state and a shift in cancer immunoeediting toward elimination of tumor cells. The genomic instability detected in our outliers, plus treatment with DNA damage compounds such as alkylating agents, platinum-based agents, gemcitabine, paclitaxel, irinotecan,

and 5-fluorouracil (in almost all patients), and a favorable immune system could result in durable responses. We also found that outliers overexpressed multiple genes responsible for tumor recognition and tumor-cell killing by cytotoxic CD8+ T and NK cells.

Because of several limitations of this study, our results should be interpreted cautiously. This single-center, retrospective pilot study is subject to recall bias because we did not search the entire electronic medical record to identify all exceptional responders. In addition, we had to exclude some patients from the analysis because of a lack of viable archived tumor tissue for next-generation sequencing. Furthermore, not all patients in this study underwent mRNA expression analysis. Finally, next-generation sequencing was done using a novel Ambry Genetics platform not certified by the Clinical Laboratory Improvement Amendments at the time of analyses, allowing for the possibility that sequencing data sets may have some artifactual mutations. Another important consideration is that some of our patients who met the eligibility criteria for exceptional response had been treated with targeted therapies that are known to induce durable responses, such as trastuzumab for HER2-positive breast cancer and osimertinib and erlotinib for lung adenocarcinomas with activating *EGFR* mutation. Therefore, the exceptional responses could be explained by targeted inhibition of these driver mutations. Nevertheless, these tumors shared similar molecular profiles with the rest of our population, and these patients did better than would be expected for a median duration of response with the same therapies. In addition, two of our patients received metastasis-directed local therapy during the course of systemic therapy that could have influenced the duration of response. However, the metastasis-directed therapies were given after a long time of response to systemic therapy (69 and 47 months), which would not exclude these patients based on our exceptional responder criteria.

CONCLUSION

Cancer development involves alterations in distinct pathways that confer the common cancer characteristics of uncontrolled growth, neoangiogenesis, immune evasion, and the potential to metastasize [43]. Our results may help to elucidate the complex interaction of antineoplastic drugs and molecular characteristics leading to exceptional responses. *EPHA5* mutations may contributed to exceptional responses along with other molecular alterations in the DDR pathway and a favorable antitumor immune signature. This is interesting because of the fact that the U.S. Food and Drug Administration approved combination of cytotoxic drugs and immune checkpoint inhibitors, based on their ability to produce more profound and durable responses [44]. This study also highlights the potential for sustained therapeutic response in cancers with a mutation-disabled DDR pathway with DNA-damaging therapy when there is an active anticancer immune response. It also demonstrates the value of interrogating RNA expression beyond tumor mutation profiling to elucidate better therapeutic strategies for patients with any cancer. Further exploration

of molecular mechanisms that drive exceptional responses is warranted.

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DISCLOSURES

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For Further Reading:

Masayuki Takeda, Takayuki Takahama, Kazuko Sakai et al. Clinical Application of the FoundationOne CDx Assay to Therapeutic Decision-Making for Patients with Advanced Solid Tumors. *The Oncologist* First published: 16 December 2020.

Implications for Practice:

This prospective cohort study was initiated to investigate the feasibility and utility of clinical application of FoundationOne CDx. A total of 181 samples were processed for genomic testing between September 2018 and June 2019, with data being successfully obtained for 175 of these samples, yielding a success rate of 96.7%, and 24 individuals (14%) received matched targeted therapy.