

Mutation Profiling of Clinically Advanced Cancers Using Next-Generation Sequencing for Targeted Therapy: A Lifespan Experience

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ABSTRACT

The application of modern molecular tests such as next-generation sequencing (NGS) to human malignancies has led to better understanding of tumor biology and the design of targeted molecular therapies. In the research setting, important genomic alterations in tumors have been discovered with potential therapeutic implications but data regarding the impact of this technology in a real world oncology practice is limited. As a result, we decided to review the results of NGS in 144 advanced-stage cancer patients referred to the oncology practices of Lifespan-affiliated centers in Rhode Island. Most cancers revealed genomic alterations in genes commonly mutated in cancer. However, several unexpected genomic alterations were discovered in certain cancers with potential therapeutic intervention. Most cancers contained “actionable” genomic alterations despite being of advanced stage. Our experience demonstrates that application of NGS in the clinical setting contributes both to increasing the therapeutic armamentarium as well as our understanding of tumor biology.

KEYWORDS: Next generation sequencing, genomic alterations, targeted therapy, actionable

INTRODUCTION

The biology of cancer is incredibly complex. An important concept in carcinogenesis is the presence of alterations in the tumor genome, which lead to various degrees of prolonged cell survival, decreased cell death, and changes to the tissue microenvironment. Cancer genomes are often riddled with a myriad of mutations, but only a few mutations are believed to drive a cell toward uncontrolled clonal expansion; the so-called “driver” mutations. Mutations in “driver” genes confer a growth advantage to the cell, are causally implicated in cancer development and have thus been positively selected for.^[1] Other “passenger” mutations may develop along the way, but these are generally not thought to confer a biologic advantage to the cancer cell.

Understanding the importance of driver mutations paves the road for targeted molecular therapy. Successful examples already abound in the field of oncology and include *BRAF* mutations in melanoma, *ERBB2/HER2*-Neu amplification

in breast and gastroesophageal cancer, and *ALK* fusions in lung cancer among many others. Recently, with the advent of newer technologies such as Next Generation Sequencing (NGS) and the cooperation of large international consortia such as The Cancer Genome Atlas (TCGA), genomic alterations and therapeutic targets are increasingly being identified.

What separates NGS from prior technologies is the ability to sequence up to millions of fragments of DNA simultaneously, referred to as “massively parallel” sequencing. The technology begins by fragmenting tumor DNA into small single-stranded fragments. The DNA fragments are modified by attaching “adapters,” or short DNA segments of a known sequence, which are then bound to a surface, usually a glass slide or a well depending on the platform. The DNA strands are then amplified and ready to be sequenced. As nucleotides are added and incorporated into the DNA strands, a signal is released and recorded by the instrument. Each commercial platform has a proprietary approach to the chemistry used and method of detection. The final result is a set of sequencing data that requires tremendous bioinformatics support and interpretation. For utilization in clinical oncologic practice, only genes implicated in the carcinogenesis of solid tumors require sequencing and so most commercial NGS platforms use a targeted gene panel.^[2]

Comprehensive evidence on improved treatment outcomes using NGS technology to detect genomic alterations in solid tumors is still lacking. However, oncologists, insurers and medical organizations generally agree that NGS plays a valuable role in detecting possible actionable genomic alterations in patients with advanced cancer as well as contributing to our understanding of cancer biology. To that end, we decided to assess our institutional experience with a targeted NGS gene panel in 144 patients with advanced solid tumors.

MATERIALS AND METHODS

A total of 144 patients with metastatic or treatment refractory tumors treated at Lifespan partner hospitals (Rhode Island Hospital, The Miriam Hospital, and Newport Hospital) between 2012 and 2015 were included. Consent was obtained at the time of visit with the oncologist responsible for their care. Tumor type was confirmed by routine histology, immunohistochemistry and clinical/radiologic correlation. Tissue samples included primary resections, biopsies,

and cytology specimens. Formalin-fixed paraffin embedded (FFPE) tissue sections of tumor were sent to Foundation One (Cambridge, MA) and analyzed using a customized next-generation sequencing assay.^[3] The current assay interrogates at least 315 genes (more than 4,500 exons) as well as introns of 28 genes known to be somatically mutated in human cancers. All of the genes included are either unambiguous drivers of carcinogenesis based on current knowledge and/or validated targets for therapy (FDA-approved and/or in clinical trials). The types of alterations include base substitutions, insertions/deletions, copy number alterations, and rearrangements. *Actionable* genomic alterations (or mutations) are defined as either linked to: (I) an FDA approved therapy in the patient's tumor type; (II) an FDA-approved therapy outside the patient's tumor type, or; (III) non-FDA approved therapies in clinical trials or preclinical testing.^[4]

RESULTS

A total of 144 tumors were submitted for analysis, including tumors from 80 males (56%) and 64 females (44%) with a mean age of 62.9 years. There were a total of 4 (2.8%) sample failures due to inadequate tissue volume or failure during the analytic phase. Therefore, data was available for 140 tumors. A summary distribution of tumor types submitted for NGS is represented in **Figure 1**.

A total of 620 genomic alterations were detected in 171 genes. The most common genomic alterations were in *TP53* (13.5%), *APC* (7.9%), *KRAS* (7.3%), *CDKN2A* (4.7%), and *ARID1A* (2.1%) (**Figure 2**). An average of 4.5 genomic alterations were detected per tumor (range 0–24). No reportable genomic alterations were detected in 2 tumors (1 ovarian serous carcinoma, 1 colon cancer) and these were not included in the subsequent analysis.

Colorectal carcinoma

A total of 203 genomic alterations were identified in 83 genes in 41 colorectal adenocarcinomas with an average number of 5.0 alterations per tumor. The most common genomic alterations included *TP53* (85%), *APC* (75%), *KRAS* (48%), *PTEN* (15%), *SMAD4* (13%), *FBXW7* (13%), *ARID1A* (13%), *PIK3CA* (10%), and *BRAF* (8%). Our cohort had higher rates of *TP53*, *APC*, and *KRAS* mutations compared to the Catalogue of Somatic Mutations in Cancer (COSMIC) database of all colorectal adenocarcinomas (**Table 1**).

Pancreatic ductal adenocarcinoma

A total of 68 genomic alterations were identified within 21 genes in 18 pancreatic ductal adenocarcinomas with an average number of 3.8 alterations per tumor. The most common genetic alterations included *KRAS* (100%), *TP53* (76%), *CDKN2A* (29%), *SMAD4* (18%) and *ATM* (12%). Concurrent loss of *CDKN2A* and *CDKN2B* occurred in 3 tumors (18%). Our cohort had higher rates of *KRAS*, *TP53*, *ATM* and *CDKN2A* alterations compared to the COSMIC database

Figure 1. Most common tumors submitted for analysis

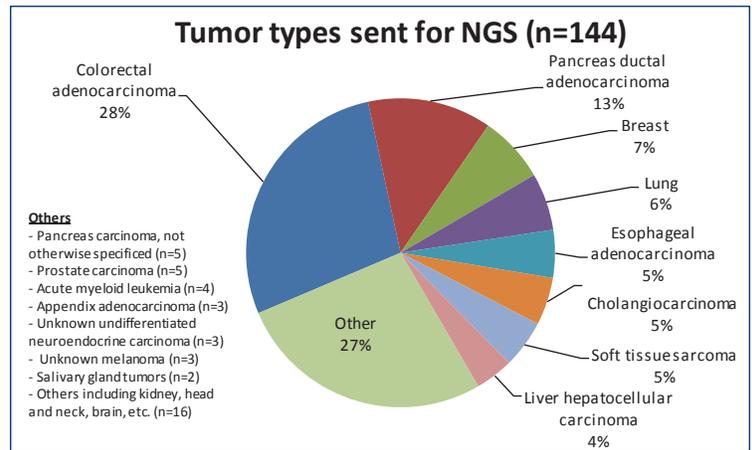


Figure 2. Most common genomic alterations in all cancers

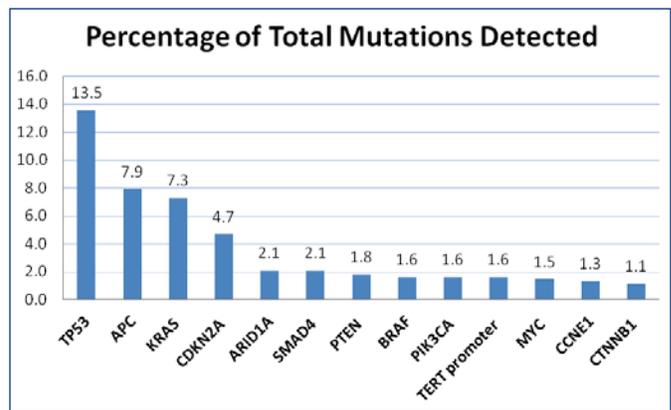


Table 1. Differences in colorectal adenocarcinoma genomic alterations between Lifespan and COSMIC cohorts

Genes altered in colorectal adenocarcinoma (n=41)	Lifespan (% cases with mutation)	COSMIC Database (% cases with mutation)
<i>TP53</i>	85	48
<i>APC</i>	75	42
<i>KRAS</i>	48	35
<i>PTEN</i>	15	25
<i>SMAD4</i>	13	23
<i>FBXW7</i>	13	20
<i>ARID1A</i>	13	17
<i>PIK3CA</i>	10	22
<i>BRAF V600E</i>	8	14

of all pancreatic adenocarcinomas (Table 2). Interestingly, *CDK6* and *MYST3* alterations were detected in 12% of tumors in our cohort and not found in any of the tumors included in COSMIC.

Other carcinomas

Many tumors had alterations in genes known to be recurrently mutated for that tumor type. Carcinomas of the breast frequently contained *PIK3CA*, *PTEN*, *TP53* and *GATA3* alterations. Esophageal adenocarcinomas contained *TP53*, *CDKN2A* (p16), *KRAS*, and *ERBB2/Her2-Neu* alterations. Cholangiocarcinomas contained *TP53*, *IDH1*, *MLL3*, and *ARID1A* alterations. Hepatocellular carcinomas contained frequent alterations in *TERT*, *CTNNB1* (β-catenin) and *CDKN2A* (p16). Prostate adenocarcinomas contained *TP53*, *PTEN*, and *TMPRSS1-ERG* fusion alterations. All unknown primary melanomas contained *BRAF* mutations.

Table 2. Differences in pancreatic adenocarcinoma genomic alterations between Lifespan and COSMIC cohorts

Genes altered in pancreatic ductal adenocarcinoma (n=18)	Lifespan (% cases with mutation)	COSMIC Database (% cases with mutation)
<i>KRAS</i>	100	71
<i>TP53</i>	76	49
<i>CDKN2A</i>	29	22
<i>SMAD4</i>	18	20
<i>ATM</i>	12	3
<i>CDK6</i>	12	0
<i>MYST3</i>	12	0

Table 3. Actionable mutations by tumor type

Cancer	FDA Tx ^a	FDA-GA ^b	NonFDA Tx ^c	NonFDA-GA ^d	Clinical trials ^e
Total (n=138)	19 (14%)		93 (67%)		129 (93%)
Appendix adenocarcinoma (n=3)	0	-	1	<i>KIT, KRAS</i>	2 (67%)
Colorectal adenocarcinoma (n=41)	3	<i>BRAF</i>	27	<i>KRAS, FLT3, NF1, ALK fusion, ERBB3, ERBB4, FGFR1, MAP2K1, BRCA1</i>	40 (98%)
Cholangiocarcinoma, intra- and extrahepatic (n=8)	0	-	6	<i>BRAF, DDR2, PTEN, IDH2</i>	8 (100%)
Acute myelogenous leukemia (n=4)	0	-	3	<i>DNMT3A, IDH2, PTPN11, NF1, NRAS, TET2</i>	3 (75%)
Brain; anaplastic astrocytoma (n=1)	0	-	0		1 (100%)
Breast carcinoma; ductal, lobular and NOS (n=9)	6	<i>PIK3CA, AKT1, NF1, PTEN</i>	7	<i>FGFR2, FGFR4, GNAS, MET, NF1</i>	9 (100%)
Esophagus and gastroesophageal junction adenocarcinoma (n=8)	2	<i>ERBB2/Her2-neu</i>	5	<i>EGFR, TOP2A, KRAS, CCND1, PIK3CA</i>	8 (100%)
Head and neck squamous cell carcinoma (n=1)	0	-	0	-	1 (100%)
Kidney carcinoma; clear cell and urothelial (n=4)	2	<i>VHL, STK11</i>	4	<i>DDR2, ERBB3</i>	4 (100%)
Hepatocellular carcinoma (n=6)	0	-	2	<i>TSC2, PTEN, STK11</i>	5 (83%)
Lung carcinoma; including adeno-, small cell and squamous (n=9)	2	<i>MET, ERBB2</i>	5	<i>PIK3CA, STK11, PTEN, FLT3, KRAS</i>	9 (100%)
Pancreas; including adenocarcinoma and NOS (n=23)	0	-	22	<i>KRAS, ERBB2, PIK3RA, STK11, FBXW7</i>	22 (100%)
Prostate carcinoma (n=3)	0	-	1	<i>PTEN</i>	3 (100%)
Salivary gland tumors (n=2)	0	-	1	<i>BRIP1</i>	2 (100%)
Soft tissue tumors (n=7)	0	-	2	<i>NF1, PTEN</i>	5 (70%)
Stomach adenocarcinoma (n=1)	0	-	1	<i>MET, BRAF</i>	1 (100%)
Thyroid medullary carcinoma (n=1)	1	<i>RET, VHL</i>	1	<i>RET, VHL</i>	1 (100%)
Unknown primary adenocarcinoma (n=2)	0	-	2	<i>BRAF, PIK3CA</i>	2 (100%)
Unknown primary melanoma (n=3)	3	<i>BRAF</i>	3	<i>BRAF</i>	3 (100%)
Unknown primary undifferentiated neuroendocrine carcinoma (n=3)	0	-	0	-	0 (0%)

a – FDA-Tx: Number of cases with FDA-approved therapies for genomic alterations in patient’s tumor type

b – FDA-GA: Genomic alterations in patient’s tumor with FDA-approved targeted therapies.

c – NonFDA-Tx: Number of cases with FDA-approved therapies for a genomic alteration present in the patient’s tumor but approved for a different tumor type

d – NonFDA-GA: Genomic alterations in patient’s tumor with FDA-approved therapies for a different tumor type

e – Clinical trials – Number of cases containing a targetable genomic alteration being investigated in a clinical trial as of this writing

Actionable mutations

Of 138 tumors, 130 (94%) had actionable genomic alterations. These included 19 (14%) with an FDA-approved therapy for the specific tumor type, 93 (67%) with a mutation for which an FDA-approved therapy exists for a different tumor type, and 129 (93%) with mutations being studied in clinical trials. There were 8 patients (6%) that had no actionable genomic alterations. A summary of actionable mutations specific for tumor type is presented in **Table 3**.

DISCUSSION

This analysis provides a unique appraisal of a single health system's experience using NGS for identifying potential therapeutic genomic targets in patients with metastatic and treatment-resistant cancers. Out of 138 patients with advanced or metastatic cancer, 94% had potentially actionable genomic alterations in their tumors. Most of these included clinical trials studying a targeted therapy with regards to the tumor specific mutation and 67% of all cases had FDA-approved therapy for the patient's specific tumor mutation but in a different tumor. Nonetheless, NGS discovered that 14% of the patients in our cohort had genomic alterations with FDA-approved therapies their specific tumor type.

The tumors with FDA-approved therapies, and therefore, of most clinical interest included colon, breast, esophagus, kidney, lung, thyroid, and melanoma. Twenty (48%) patients with colonic adenocarcinomas had mutations in *KRAS* and therefore would not benefit from anti-EGFR therapy.^[5] Three colonic adenocarcinomas had activating mutations in *BRAF*, a gene which promotes cell proliferation via the MAPK signaling pathway. Present in about 8–15% of colon cancers, *BRAF* mutations in advanced stage colon cancer have been associated with decreased overall survival, lack of response to anti-EGFR therapy, and decreased sensitivity to vemurafenib.^[6-8] However, regorafenib has been FDA-approved for the treatment of metastatic colon cancer and has shown increased survival benefit in patients with metastatic, previously treated disease.^[9, 10] The lack of response to *BRAF* inhibition may be due, in part, to *EGFR* activation, and early evidence suggests that dual inhibition therapy may have clinical benefit.^[11] Other targeted FDA-approved therapies were discovered in 16 patients including 6 breast carcinomas (*PIK3CA*, *AKT1*, *NF1*, *PTEN* mutations), 2 gastroesophageal adenocarcinomas (*ERBB2/Her2-neu* mutations), 2 kidney carcinomas (*VHL*, *STK11*), 2 lung adenocarcinomas (*MET*, *ERBB2/Her2-neu*), 1 medullary carcinoma of the thyroid (*RET*, *VHL*) and 3 melanomas (*BRAF*). All of the therapies have been approved because they target the mutant protein (or more commonly the downstream effector protein) and have showed various degrees of success.

Two-thirds of patients had genomic alterations in their tumors with targetable FDA-approved therapies but for a

different histologic tumor type. Unexpected or uncommon mutations accounted for a subset of these patients. One patient had an *ALK* fusion positive colon cancer, which has rarely been described and has not undergone enough clinical testing to merit treatment as is in *ALK* mutated lung cancers.^[12] Two intrahepatic cholangiocarcinomas were positive for the *BRAF* V600E mutation and *ERBB2/Her2-neu* amplification. Bonilla and colleagues reported an excellent response to *BRAF* inhibitors in a patient with a *BRAF* V600E mutated poorly differentiated intrahepatic cholangiocarcinoma with multiple metastases.^[13] A similar dramatic response was seen in a patient with *ERBB2/HER2-neu* amplified metastatic cholangiocarcinoma treated with trastuzumab.^[14] Alterations in *ERBB2/Her2-neu* were discovered in 2/18 (11%) of pancreatic carcinomas. Although uncommon (<1% of all pancreatic carcinomas in COSMIC), there is some evidence that *ERBB2* alterations are associated with higher rates of brain and lung metastases.^[15] In the single patient with gastric adenocarcinoma in our cohort, a *BRAF* V600E mutation was detected; the significance of which is yet unclear.^[16] Interestingly, two patients with unknown primary adenocarcinoma had actionable genomic alterations (*BRAF*, *PIK3CA*). Traditionally thought of as having poor prognoses, patients with carcinomas of unknown primary may benefit from NGS targeted gene panels although systematic evidence is still in its infancy.^[17]

The applications of NGS are not just limited to targeted therapeutic information, but have far reaching implications with regards to cancer biology, genotype-phenotype correlations, and prognostics. Our colorectal carcinoma cohort had significantly more *TP53*, *APC* and *KRAS* mutations compared to the COSMIC database. In addition, 100% of our pancreatic adenocarcinoma cohort had *KRAS* mutations compared to 71% in the COSMIC cohort. While these data are not surprising given our cohort consisted entirely of advanced stage/treatment resistant cancers, it underscores the importance these driver mutations play in cancer progression, especially since they are present even in precancerous lesions.^[18, 19] Not all driver mutations, however, imply a biologic or therapeutic significance. In melanomas, for example, *BRAF* mutations are usually mutually exclusive of other mutations, which may explain the success of *BRAF* and downstream MEK inhibitors, either alone or in combination.^[20] However, in our small cohort of 3 *BRAF* mutated colon cancers, each case had anywhere from 3 to 23 additional mutations in several important cancer promoting genes such as *TP53*, *SMAD4* and *PTEN*. Thus while common driver mutations exist across many cancer types, one cannot assume that the same applies for therapeutic effect. Additional complications arise from molecular heterogeneity within the same tumor. Some studies have shown that mutations may vary by more than 50% depending on the region of tumor sampled as well as information regarding treatment options and prognosis.^[21, 22]

CONCLUSION

For the 144 patients in the Lifespan system with advanced cancers that have progressed on therapy, next generation sequencing using a targeted gene panel detected a clinically actionable genomic alteration in nearly all patients. Most of the opportunities consisted of clinical trials and off-label therapies, but at least 14% of patients had FDA-approved therapies for a mutation in their specific tumor. Next generation sequencing technology can profile a tumor's genomic landscape and generate important clinical and biological information but is not without limitations. The true clinical utility of NGS needs to be explored in rigorously designed clinical trials and with clinical outcomes in institutions implementing the technology, including the Lifespan system.

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