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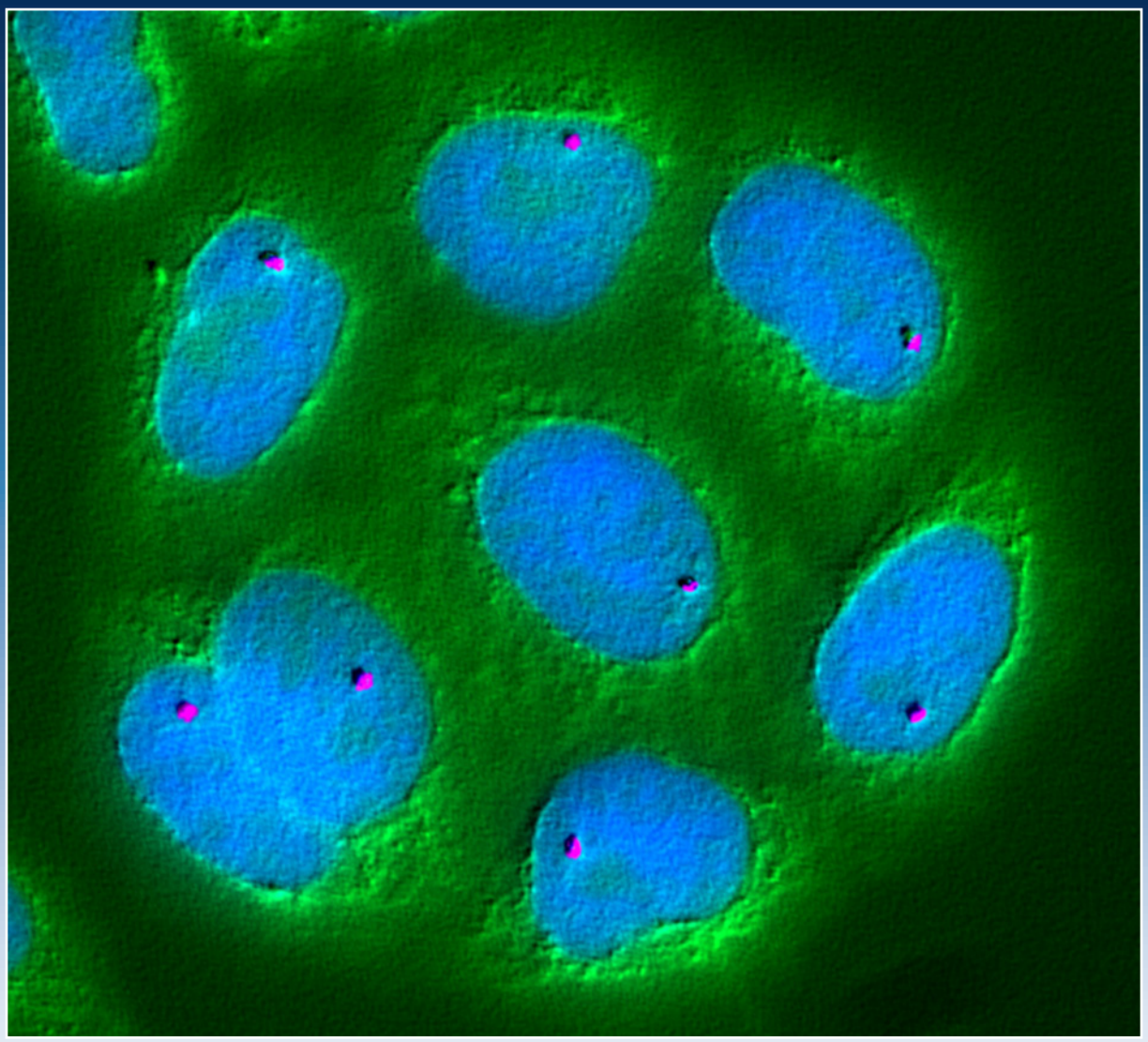

SPECIAL SECTION

UPDATES *in* ONCOLOGY: PART I

GUEST EDITORS KIMBERLY PEREZ, MD; MURRAY B. RESNICK, MD, PhD

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M. Resnick, MD, PhD

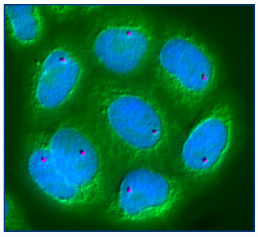

K. Perez, MD


H. Khurshid, MD


H. Safran, MD

Updates in Oncology: Part 1

KIMBERLY PEREZ, MD
MURRAY B. RESNICK, MD, PhD
GUEST EDITORS


TOM MISTELI, NCI CENTER FOR CANCER RESEARCH (2014)

On the cover: Mapping the position of genes in the cell nucleus sheds light on basic principles governing the genome. Here, a single gene called Pem (purple) has been localized using fluorescence in situ hybridization. DNA is stained blue; the cell cytoplasm is stained green. This image was originally submitted as part of the 2015 NCI Cancer Close Up project and selected for exhibit. See also https://visualsonline.cancer.gov/closeup.

Advances in the Molecular Profiling of Tumor Tissue

KIMBERLY PEREZ, MD; MURRAY B. RESNICK, MD, PhD

In the realm of cancer care, complete sequencing of the human genome has supported a move away from the traditional paradigm in which histopathologically defined disease is treated primarily with cytotoxic chemotherapy, toward the use of molecularly targeted drugs. The cancer genome typically contains numerous mutations; however, a select number are considered "driver" mutations. When specific drugs are developed either targeting these driver mutations, or pathways associated with these mutations, they are also termed "actionable" mutations. Early success stories demonstrated in breast cancer with trastuzumab in ERBB2 amplified cancer, imatinib in Philadelphia chromosome positive chronic myelogenous leukemia, erlotinib in EGFR mutated non-small cell lung cancer, cetuximab in KRAS wild-type colorectal cancer and vemurafenib in BRAF-mutant melanoma. The Cancer Genome Atlas is a comprehensive program in cancer genomics that began in 2006 and has been the foundation of the molecular profiling movement. As a result of the associated and consequential genomic discoveries, there are now hundreds of compounds in clinical development targeting more than 100 actionable mutations in cancer-related genes representing multiple cellular pathways.

Cancer treatment has entered a new frontier. The advent of new sequencing technologies such as next generation sequencing (NGS) allows for rapid, relatively inexpensive profiling of individual cancer genomes. This technologic advancement offers the opportunity to "individualize" cancer care.

Despite all of the hype regarding molecular profiling of individual tumors certain caveats need to be considered: 1: The fact that a given mutation is actionable in a tumor from one organ does not necessarily mean it is actionable in another. For example, encouraging results have been obtained targeting the BRAF mutation in melanoma; however, these same compounds are not active against BRAF mutated colonic cancer. 2: It appears that many tumors are able to develop resistance towards drugs targeting single mutations and in all likelihood combination therapy targeting several mutations or multiple steps along a single pathway will be necessary to combat resistance. 3: Several other cancer-associated pathways other than actionable mutations are being successfully targeted. Angiogenesis, the immune microenvironment, tumor sensitization, induction of cell death, tumor vaccines and monoclonal antibody delivery of toxic molecules are but a few of these novel therapeutic pathways.

The success of molecular profiling will require continued collaboration between oncologists, biostatisticians, pathologists, geneticists, policy-makers and members of the biopharmaceutical industry in order to develop new clinical models that enable rapid translation of many new biomarkers and cancer targets into new clinical tests and therapeutic interventions to benefit cancer patients.

Guest Editors

Murray B. Resnick, MD, PhD, is Professor of Pathology at the Alpert Medical School of Brown University and Vice Chair of Pathology and Director of Gastrointestinal Pathology at Lifespan/Rhode Island Hospital. He is also director of the molecular pathology core facility of the COBRE Center for Cancer Research Development.

Kimberly Perez, MD, is an Assistant Professor of Medicine, Division of Hematology/ Oncology, at The Warren Alpert Medical School of Brown University.

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

KENNETH FRIEDMAN, MD; HOWARD SAFRAN, MD; MURRAY B. RESNICK, MD, PhD

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

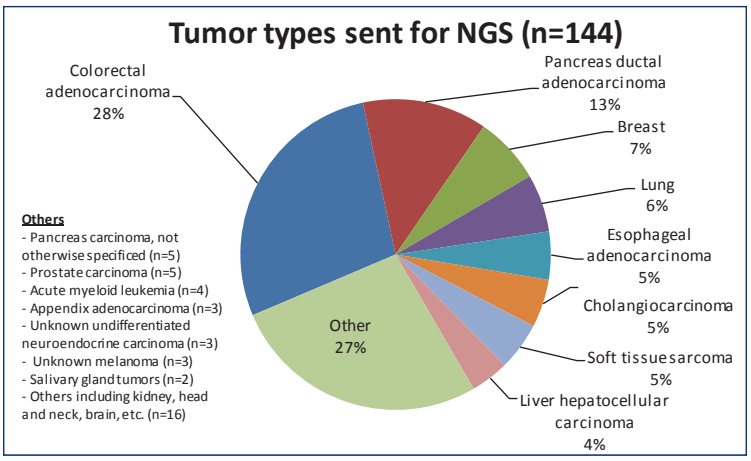


Figure 2. Most common genomic alterations in all cancers

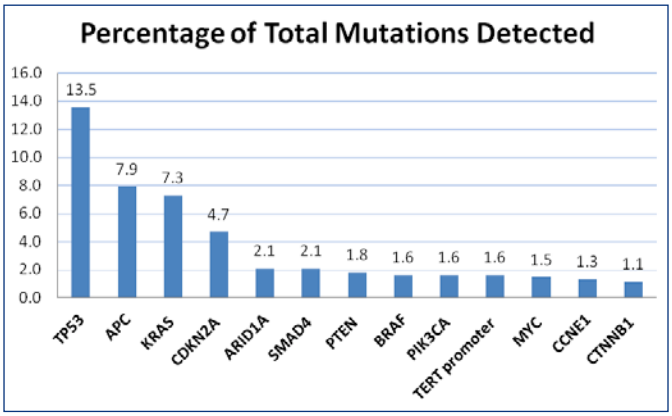


Table 1. Differences in colorectal adenocarcinoma genomic alterations between Lifespan and COSMIC cohorts

Genes altered in colorectal adenocarcinoma (n=41)	Lifespan (% cases with mutation)	COSMIC Database (% cases with mutation)
TP53	85	48
APC	75	42
KRAS	48	35
PTEN	15	25
SMAD4	13	23
FBXW7	13	20
ARID1A	13	17
PIK3CA	10	22
BRAF V600E	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 *TMPRSST-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)
KRAS	100	71
TP53	76	49
CDKN2A	29	22
SMAD4	18	20
ATM	12	3
CDK6	12	0
MYST3	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	*KIT, KRAS*	2 (67%)
Colorectal adenocarcinoma (n=41)	3	*BRAF*	27	*KRAS, FLT3, NF1, ALK fusion, ERBB3, ERBB4, FGFR1, MAP2K1, BRCA1*	40 (98%)
Cholangiocarcinoma, intra- and extrahepatic (n=8)	0	-	6	*BRAF, DDR2, PTEN, IDH2*	8 (100%)
Acute myelogenous leukemia (n=4)	0	-	3	*DNMT3A, IDH2, PTPN11, NF1, NRAS, TET2*	3 (75%)
Brain; anaplastic astrocytoma (n=1)	0	-	0		1 (100%)
Breast carcinoma; ductal, lobular and NOS (n=9)	6	*PIK3CA, AKT1, NF1, PTEN*	7	*FGFR2, FGFR4, GNAS, MET, NF1*	9 (100%)
Esophagus and gastroesophageal junction adenocarcinoma (n=8)	2	*ERBB2/Her2-neu*	5	*EGFR, TOP2A, KRAS, CCND1, PIK3CA*	8 (100%)
Head and neck squamous cell carcinoma (n=1)	0	-	0	-	1 (100%)
Kidney carcinoma; clear cell and urothelial (n=4)	2	*VHL, STK11*	4	*DDR2, ERBB3*	4 (100%)
Hepatocellular carcinoma (n=6)	0	-	2	*TSC2, PTEN, STK11*	5 (83%)
Lung carcinoma; including adeno-, small cell and squamous (n=9)	2	*MET, ERBB2*	5	*PIK3CA, STK11, PTEN, FLT3, KRAS*	9 (100%)
Pancreas; including adenocarcinoma and NOS (n=23)	0	-	22	*KRAS, ERBB2, PIK3RA, STK11, FBXW7*	22 (100%)
Prostate carcinoma (n=3)	0	-	1	*PTEN*	3 (100%)
Salivary gland tumors (n=2)	0	-	1	*BRIP1*	2 (100%)
Soft tissue tumors (n=7)	0	-	2	*NF1, PTEN*	5 (70%)
Stomach adenocarcinoma (n=1)	0	-	1	*MET, BRAF*	1 (100%)
Thyroid medullary carcinoma (n=1)	1	*RET, VHL*	1	*RET, VHL*	1 (100%)
Unknown primary adenocarcinoma (n=2)	0	-	2	*BRAF, PIK3CA*	2 (100%)
Unknown primary melanoma (n=3)	3	*BRAF*	3	*BRAF*	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.

References

1. Greenman C, et al. Patterns of somatic mutation in human cancer genomes. Nature, 2007. 446(7132): p. 153-8.
2. Futreal PA, et al. A census of human cancer genes. Nat Rev Cancer, 2004. 4(3): p. 177-83.
3. Frampton, GM, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol, 2013. 31(11): p. 1023-31.
4. Levy MA, Lovly CM, Pao W. Translating genomic information into clinical medicine: lung cancer as a paradigm. Genome Res, 2012. 22(11): p. 2101-8.
5. Douillard JY, Rong A, Sidhu R. RAS mutations in colorectal cancer. N Engl J Med, 2013. 369(22): p. 2159-60.
6. Roth AD, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. J Clin Oncol, 2010. 28(3): p. 466-74.
7. Mao C, et al. BRAF V600E mutation and resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer: a meta-analysis. Mol Biol Rep, 2011. 38(4): p. 2219-23.
8. Prahallad A, et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature, 2012. 483(7387): p. 100-3.
9. Grothey A, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet, 2013. 381(9863): p. 303-12.
10. Kim, et al. CONCUR trial. ESMO 2014, 2014(Abstract 5000).
11. Connolly K, et al. Anticancer activity of combination targeted therapy using cetuximab plus vemurafenib for refractory BRAF (V600E)-mutant metastatic colorectal carcinoma. Curr Oncol, 2014. 21(1): p. e151-4.
12. Aisner DL, et al. ROS1 and ALK fusions in colorectal cancer, with evidence of intratumoral heterogeneity for molecular drivers. Mol Cancer Res, 2014. 12(1): p. 111-8.
13. Loaiza-Bonilla A, et al. Dramatic response to dabrafenib and trametinib combination in a BRAF V600E-mutated cholangiocarcinoma: implementation of a molecular tumour board and next-generation sequencing for personalized medicine. Ecancermedicalscience, 2014. 8: p. 479.
14. Law LY. Dramatic response to trastuzumab and paclitaxel in a patient with human epidermal growth factor receptor 2-positive metastatic cholangiocarcinoma. J Clin Oncol, 2012. 30(27): p. e271-3.
15. Chou A, et al. Clinical and molecular characterization of HER2 amplified-pancreatic cancer. Genome Med, 2013. 5(8): p. 78.
16. van Grieken NC, et al. KRAS and BRAF mutations are rare and related to DNA mismatch repair deficiency in gastric cancer from the East and the West: results from a large international multicentre study. Br J Cancer, 2013. 108(7): p. 1495-501.
17. Ross JS, et al. Comprehensive genomic profiling of carcinoma of unknown primary site: New routes to targeted therapies. JAMA Oncology, 2015.
18. Kanda M, et al. Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. Gastroenterology, 2012. 142(4): p. 730-733 e9.
19. Fearon ER. Molecular genetics of colorectal cancer. Annu Rev Pathol, 2011. 6: p. 479-507.
20. Robert C, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. N Engl J Med, 2015. 372(1): p. 30-9.
21. Gerlinger M, et al. Intratumor heterogeneity and branched evolution revealed by multiregion sequencing. N Engl J Med, 2012. 366(10): p. 883-92.
22. Yap TA, et al. Intratumor heterogeneity: seeing the wood for the trees. Sci Transl Med, 2012. 4(127): p. 127ps10.

Authors

Kenneth Friedman, MD, is the Gastrointestinal Pathology Fellow in the Department of Pathology at Lifespan/Rhode Island Hospital.

Murray B. Resnick, MD, PhD, is Professor of Pathology at the Alpert Medical School of Brown University and Vice Chair of Pathology and Director of Gastrointestinal Pathology at Lifespan/Rhode Island Hospital.

Howard Safran, MD, is the Director of the Brown University Oncology Group and Professor of Medicine at the Alpert Medical School of Brown University.

Correspondence

Kenneth Friedman, MD
Department of Pathology
Rhode Island Hospital
593 Eddy Street
Providence, RI 02903
401-444-8524
Kentheus@gmail.com

Updates in Tumor Profiling in Gastrointestinal Cancers

KIMBERLY PEREZ, MD; HOWARD SAFRAN MD

ABSTRACT

In the last decade there has been a focus on biomarkers that play a critical role in understanding molecular and cellular mechanisms which drive tumor initiation, maintenance and progression of cancers. Characterization of genomes by next-generation sequencing (NGS) has permitted significant advances in gastrointestinal cancer care. These discoveries have fueled the development of novel therapeutics and have laid the groundwork for the development of new treatment strategies. Work in colorectal cancer (CRC) has been in the forefront of these advances. With the continued development of NGS technology and the positive clinical experience in CRC, genome work has begun in esophagogastric, pancreatic, and hepatocellular carcinomas as well.

KEYWORDS: Tumor profiling, colorectal carcinoma, esophagogastric carcinoma, pancreatic carcinoma, hepatocellular carcinoma

INTRODUCTION

The prognosis for patients with gastrointestinal cancers is currently based on tumor-node-metastasis (TNM) staging; however, outcomes for patients with the same histologic-clinical staging can be heterogeneous. As a result, research efforts have shifted from identification of mutations of individual genes to genome-wide identification of genetic abnormalities in cancer. Identification of these somatic mutations and evaluation of gene expression patterns is key to understanding the molecular mechanism of cancer and the development of novel therapeutics.

The application of next generation sequencing (NGS) technology – the rapid sequencing of large stretches of DNA – has been in development in gastrointestinal malignancies. In the forefront is colorectal cancer, where there has been an improvement in mortality rates because of improvements in treatment as a result of several predictive and prognostic biomarkers.[1] The experience in CRC, as well as lung cancer, breast cancer and melanoma, has fostered the pursuit of genome profiles in other cancers as well.

TUMOR TYPES AND MUTATIONS

Colorectal Cancer

Sjoblom and Wood and colleagues were the first to perform exome-wide mutation analysis by sanger-sequencing to demonstrate the genomic profile of CRC, which included high-frequency mutated genes such as APC, KRA, TP53.[2,3] With the development of NGS, The Cancer Genome Atlas (TCGA) network further expanded the genome profile. They demonstrated 32 somatic recurrently mutated genes, among the somatic mutations identified in 24 genes. The most frequent mutated genes were APC, TP53, KRAS, PIK3CA, FBXW7, SMAD4, TCF/L2, NRAS, ACVR2A, APC, TGFBR2, MSH3, MSH6, SLC9A9, TCF7L2 and BRAF V600E were noted.[4]

At the current time, there are previously identified genes, which are directing clinical treatment options or are used as prognostic indicators (see **Table 1**):

Table 1. Colorectal Cancer Molecular Profile

Tumor Type	Gene	Incidence	Clinical Implications
Colorectal Cancer	EGFR	70%	Therapeutic – anti-EGFR monoclonal antibodies Prognostic -none
	K-ras exon 2 (codon 12,13,61)	40%	Therapeutic – If wild-type, increased susceptibility to anti-EGFR monoclonal antibodies Prognostic – If mutated, associated poorer prognosis
	B-raf V600E	5%	Therapeutic – If mutated, decrease response to EGFR monoclonal antibodies Prognostic – If mutated associated poorer prognosis
	Microsatellite Instability	15%	Therapeutic – If present insensitive to fluorouracil but sensitive to irinotecan Prognostic – If present, good prognosis
	Dihydro-pyrimidine dehydrogenase (DPD)	3–5%	Therapeutic – If present patient is unable to metabolize fluorouracil Prognostic – none
	Uridine diphosphate-glucoronosyl transferase 1A1 (UGTA1A1)	3–5%	Therapeutic – If present patient is unable to metabolize irinotecan Prognostic – none

EGFR gene expression: The epidermal growth factor receptor is a transmembrane receptor which is expressed in 70% of CRCs. Anti-EGFR monoclonal antibodies such as cetuximab competitively inhibit EGFR by preventing its binding to endogenous ligands and prolong survival when given in combination with chemotherapy.[5]

K-ras (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog)*:* Ras is an oncogene. K-ras is found in adenocarcinomas that transduce extracellular signals from the EGFR to the nucleus. Forty percent (40%) of CRC are positive for mutation in K-ras exon 2, which includes codons 12,13,61. A mutation in any of these sites in K-ras are currently the only predictive biomarker, which denotes anti-EGFR monoclonal antibody efficacy in CRC.[6] Recent retrospective analysis is suggesting an adverse prognostic impact.[7]

B-raf (v-raf murine sarcoma viral oncogene homolog B) V600E gene mutation: B-raf is an oncogene that encodes a protein which belongs to the raf/mil family of serine/threonine protein kinases. This protein plays a role in regulating the MAP kinase/ ERKs signaling pathway, which affects cell division, differentiation, and secretion. B-raf mutations are found in 5–95% of CRC. A B-raf mutation has been associated with a negative response to EGFR inhibitors. Also its presence has been associated with overall poor prognosis [8]

Microsatellite Instability (MSI): MSI is caused by defects in DNA mismatch repair genes, which include MLH1, MSH2, MSH3, PMS1, PMS2, and MSH6. Loss of function or expression of these genes leads to a higher than normal frequency of frameshift mutations and base-pair substitutions in regions of short tandem repeated nucleotide sequences found throughout the genome, also known as microsatellites.[9] Approximately 15% of CRC show MSI. The following phenotypic characteristics have been described: location in the proximal bowel, and characteristic histologic findings (poor differentiation, mucinous, and marked lymphocytic infiltration). MSI has also been described in Lynch syndrome (LS) – a hereditary syndrome which is associated with increased risk of colorectal cancer. BRAF mutations are found in up to 50-70% of MLH1 mutated tumors, while LS rarely has BRAF mutations. Therefore if a patient is found to have a MLH1 mutation, an associated mutation in BRAF(V600E) supports a sporadic etiology.[10] There is also data, which supports a role in pharmacogenomics by MSI. Presence of MSI has been associated with better prognosis and chemo sensitivity to irinotecan but Ribic and colleagues demonstrated an associated poorer response to 5-fluoruracil, two chemotherapeutic drugs commonly used to treat colorectal cancer.[11]

Pharmacogenomic data: Two genes have been identified that impact metabolism of fluorouracil and irinotecan. A mutation in Dihydropyrimidine dehydrogenase (DPD) results in deficiency of the DPD enzyme, which is a major catabolizing enzyme of fluorouracil. It has been detected in 3-5% of the general population.[1] However, the presence of the mutation or deficiency of the enzyme does not always dictate clinical outcome.[12]

Uridine diphosphate-glucuronosyl transferase 1A1 (UGTA1A1) is an enzyme that mediates glucouronidation. This enzyme enables conjugation of glucuronic acid to the active form of irinotecan, SN-38. Therefore if UGTA1A1 is mutated resulting in a quantitative deficiency of the UGTA1A1 enzyme, there will be a decreased rate of irinotecan metabolism resulting in clinically significant neutropenia and diarrhea.[13]

Gene Expression Assays: Oncotype Dx Colon, ColoPrint, ColDX are clinically available examples.[14] The aforementioned assays assess 18 genes or more, the data is then used to predict an individual tumor's risk of recurrence.[15] At this time trials have demonstrated the role of these assays in predicting recurrence risk for stage I-III CRC but none have proven to be reliable indicators of response to adjuvant therapy.

Associated genes of unclear clinical relevance:

In recent studies analyzing the correlation of treatment response and molecular profile, the data demonstrated mutational frequencies of: KRAS (45%), NRAS (5%), BRAF(7%), PIK3CA (9%), PTEN (6%), TP53(60%), EGFR (1%), AKT1(<1%) and CTNNB1 (2%).[16] The role of these mutations individually is unclear but gene signatures have demonstrated some prognostic and therapeutic relevance. For example, Yu and colleagues characterized CRC genomes by NGS. A five-gene-signature (CDH10, COL6A3, SMAD4, TMEM132D, VCAN) was devised. In an analysis of 22 patients with CRC, a mutation in one or more of these genes was associated with a significant improvement in overall survival independent of tumor-node-metastasis (TNM) staging. The data demonstrate a median survival time of 80.4months in the mutant group versus 42.4 months in the wild type group (p=0.0051).[17]

Pancreatic and Biliary Cancers

To date, few DNA sequencing-based studies have been carried out to define the predominant mutations in pancreatic cancer. Due to the low survival rates and high proportion of late-stage and metastatic diagnoses associated with pancreatic cancer, it has proven difficult to assess prognostic and therapeutic markers. Therefore, currently there are no Food and Drug Administration (FDA) drug options that exploit known genomic alterations. Current implicated genes include KRAS, TP53, SMAD/DPC4 (SMAD family member 4/ deletion target in pancreatic carcinoma 4 homolog), and CDKN2A (cyclin-dependent kinase inhibitor 2A; p16).

Jones and colleagues were the first to characterize the tumor profile of pancreatic adenocarcinoma. The data demonstrated an average of 63 genetic alterations, resulting in dysregulation of 12 cellular signaling pathways in most tumors. Although this analysis identified frequently mutated genes, ie KRAS, there was no common mutation profile limiting our ability to further decipher the molecular carcinogenesis of pancreatic cancer. [18]

As with pancreatic cancer comprehensive genomic profiling is underway to further characterize intrahepatic

cholangiocarcinoma (IHCCA), extrahepatic cholangiocarcinoma(EHCCA) and gallbladder carcinomas (GBCA). Ross and colleagues performed comprehensive genomic profiling of the above tumors, which included 182 cancer-related genes. The most common genes identified included CDKN2B and ARID1A. Unique to IHCCA were FGFR, IDH1/2, BRAF and MET. EHCCA and GBCA shared common mutations in ERBB2, but differed in the frequency of KRAS mutations.[19]

Hepatocellular Carcinoma

As with the biliary cancers, initial analysis of the genetic landscape of hepatocellular carcinoma (HCC), as well as the related signaling pathways, is underway. Four pathways have been linked to HCC pathogenesis. The first is the Wnt/B-catenin pathway, which is now considered the main oncogenic pathway in HCC. The genes most commonly associated with pathway activation include CTNNB1 and AXIN1. The second is interruption of cellular regulatory mechanisms, which has been linked to recurrent mutations in ARID1A and ARID2 (AT-rich interactive domain 1A and 2). The third is NRF2/KEAP1 pathway, if activated, results in transcription of antioxidant genes, thereby giving proliferative and survival advantages to tumor cells. The fourth is the PI3K/Akt/mTOR and Ras/Raf/MAP kinase pathways, which are activated by mutation sin PIK3CA, FGF19 and RPS6KA3.[20] Work is in progress to target these four pathways but none of the targeted therapy options have been approved yet.

EsophagoGastric Cancers

Esophagogastric carcinomas are heterogeneous, with multiple environmental etiologies and alternative pathways of carcinogenesis. With NGS the genes implicated in dysfunction of this pathway in gastric cancer include: ARID1A, MLL3, MLL, PIK3CA, FAT4 and MSI. As with CRC, microsatellite instability (MSI) has been identified as underlying a distinctive carcinogenic pathway in 15% of all gastric cancers.[21]

One of the most recent breakthroughs in targeted therapy is the use of HER2 antibody, Trastuzumab, in gastric cancer. HER2 overexpression is observed in 7–34% of gastric cancers; however, resistance within this cohort to targeted therapy is present. The culprits of resistance include alterations in HER2 structure and surroundings, dysregulation of HER2 downstream signal effectors and interaction of HER2 with other membrane receptors. Mutations in PIK3CA and PTEN can impact the PI3K-Akt pathway, which is a downstream signaling pathway of HER2. However at this time little is known about the association between HER2 expression and PI3K-Akt pathway alterations.[22]

CONCLUSION

At this time large cancer sequencing initiatives, International Cancer Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA), have demonstrated heterogeneity in gastrointestinal malignancies. However, unlike CRC, the technology has not yet elucidated the significant genetic downstream effectors in the other gastrointestinal malignancies. At this time CRC remains an example of where this technology can take disease prognostication and therapeutic medicine.

References

1. Chung C, Christianson M. Predictive and prognostic biomarkers with therapeutic targets in breast, colorectal and non-small cell lung cancers: a systematic review of current development, evidence and recommendation. J Oncol Pharm Practice. 2014; 20(1): 11-28.
2. Sjoblom T, et al. The consensus coding sequences of human breast and colorectal cancer. Science. 2006; 314: 268-74.
3. Wood LD, et al. The genomic landscapes of human breast and colorectal cancers. Science. 2007; 318: 1108-1113.
4. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature. 2012; 487: 330-337.
5. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. NEJM. 2004; 351: 337-345.
6. Lee D, Kim K, Han S et al. KRAS mutation is associated with worse prognosis in stage III or high-risk stage II colon cancer patients treated with adjuvant FOLFOX. Ann Surg Oncol. 2015 Jan; 22(1): 187-94.
7. Van Cutsem E, Kohne C, Lang I, et al. Cetuximab plus irinotecan, fluorouracil, and Leucovorin as first-line treatment for metastatic colorectal cancer: Updated Analysis of overall survival according to tumor KRAS and BRAF mutation status. JCO. 2011 Apr; EPub ahead of print.
8. Kim R, Xu H, Myllykangas S et al. Genetic-based biomarkers and next-generation sequencing : the future of personalized care in colorectal cancer. Per Med. 2011 May; 8(3): 331-345.
9. Recommendations from the EGAPP Working Group: Genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbiditiy and mortality from Lynch Syndrome in relatives. Genet Med. 2009; 11(1): 35-41
10. Sinicrope F, Sargent D. Molecular pathways: microsatellite instability in colorectal cancer: prognostic, predictive and therapeutic implications. Clinical Cancer Research: An Official Journal of the American Association for Cancer Research. 2012; 18: 1506-12.
11. Fallik D, Borrini F, Boige V, et al. Microsatellite instability is a predictive factor of the tumor response to irinotecan in patients with advanced colorectal cancer. Cancer Research. 2003; 63: 5738-44.
12. Maitland M, Vasisht K, Ratain M. TPMT, UGT1A1 and DPYD: genotyping to ensure safer cancer therapy? Trends in Phamacological Sciences. 2006 Aug. 77(8): 432-437.
13. Kalia M. Biomarkers for personalized oncology: recent advances and future challenges. Metabolism Clinical and Experimental. 2014 Epub ahead of print.
14. Di Narzo A, Tejpar S, Rossi S, et al. Test of four colon cancer risk-scores in formalin fixed paraffin embedded microarray gene expression data. JNCI. 2014 Oct. 106(1).
15. Esplin E, Snyder M. Genomic era diagnosis and management of hereditary and sporadic colon cancer. World Journal of Clinical Oncology. 2014 Dec. 5(5): 1036-1047.
16. Ciombar K, Haraldsdottir S, Goldberg R. How can Next-generation sequencing (Genomics) help us in treating colorectal cancer? Curr Colorectal Cancer Rep. 2014; 10:372-379.
17. Yu J, Wu W, Li X, et al. Novel recurrently mutated genes and a prognostic mutation signature in colorectal cancer. Gut. 2013. EPub ahead of print.
18. Jones S, Zhang X, Parsons D, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science. 2008 Sept; 321(5987): 1801-1806.

19. Ross J, Wang K, Catenacci D, et al. Comprehensive genomic profiling of biliary tract cancers to reveal tumor-specific differences and genomic alterations. J Clin Oncol. 2015; 33(suppl 3; abstr 231)
20. Nault J, Zucman-Rossi J. Genetics of Hepatocellular Carcinoma: The Next Generation. Journal of Hepatology. 2014; 60: 224-226.
21. Yamamoto H, Watanabe Y, Maehata T, et al. An updated review of gastric cancer in the next-generation sequencing era: Insights from bench to bedside and vice versa. World J Gastroenterol. 2014 April; 20(14): 3927-3937.
22. Sukawa Y, Yamamoto H, Nosho K, et al. Alterations in the human epidermal growth factor receptor 2-phosphatidylinositol 3-kinase-v-Akt pathway in gastric cancer. World J Gastroenterol. 2012; 18: 6577-6586.

Authors

Kimberly Perez, MD, is an Assistant Professor of Medicine at The Warren Alpert Medical School of Brown University; Department of Medicine, Division of Hematology/Oncology, Providence, RI.

Howard P. Safran, MD, is the Research Director of the Comprehensive Cancer Center at Rhode Island, The Miriam and Newport hospitals and the medical director for the Brown University Oncology Group. He is a Professor of Medicine at The Warren Alpert Medical School of Brown University.

Correspondence

Kimberly Perez, MD
Department of Medicine
The Rhode Island Hospital
593 Eddy Street
Providence, RI 02903
401-444-4538
Fax 401-444-8918
kperez@lifespan.org

NSCLC: An Update of Driver Mutations, Their Role in Pathogenesis and Clinical Significance

ROBERT C. BLACK, MD; HUMERA KHURSHID, MD

ABSTRACT

Lung cancer is the most common malignancy in the US and causes the most cancer-related deaths. Non-small-cell lung carcinoma (NSCLC) accounts for the majority of cases. NSCLC historically was considered one entity, reflected by platinum-based therapy as the standard of care; however, with the discovery of EGFR mutations and ALK rearrangements, the landscape of treatment has become more personalized reflecting genomic heterogeneity. The molecular basis for tumor genesis was recognized and became a new method of classification. The availability of tumor sequencing and testing for these mutations is also becoming more accessible outside of major academic institutions. Targeted therapies offer alternatives to dangerous cytotoxic chemotherapy with equal or better efficacy. With these changes, driver mutations will play an increasing role in the diagnosis and treatment of NSCLC. In this review we will examine the characteristics of several NSCLC driver mutations and gene rearrangements and emerging data on therapies directed against them.

KEYWORDS: NSCLC, Driver Mutation, EGFR, ALK, ROS1, RET, BRAF, FGFR, MET, Targeted therapy

BACKGROUND

Lung cancer is the most common malignancy in the US and is also responsible for the most cancer related deaths. The American Cancer Society estimates there will be 221,200 new cases and 158,000 deaths from of lung cancer in 2015. Approximately 85% of all lung cancer is NSCLC and the median age of diagnosis for NSCLC is approximately 70 years. Worse yet, more than half (approximately 57%) of all lung cancers are at an advanced stage at diagnosis. While the treatment of early stage lung cancer remains surgical resection and close follow up, advanced lung cancer is still a very mortal disease requiring aggressive and toxic treatments. This presents as a problem in many patients, as they do not have a performance status compatible with the aggressive standard-of-care chemotherapy and radiation regimens. However, many of the existing targeted therapies are better tolerated than standard chemotherapy and this approach may provide more treatment options for these frail patients than currently exist.

Following the human genome project, many genes were implicated in the development of cancer. Continued research into the genetics of lung cancer has led to the discovery of mutations and gene rearrangements influencing oncogenesis also known as, "Driver Mutations." (**Table 1**) This phenomenon is best described in Non-Small Cell Lung Cancer (NSCLC), specifically adenocarcinoma. As a result, many prognostic tools and medications have been developed. In this review we discuss the most prominent driver mutations and gene rearrangements of NSCLC and the current agents both available and under development which target them.[1]

EGFR

EGFR is the most well established driver mutation in NSCLC. Epidermal Growth Factor Receptor is a cell signaling, trans-membrane protein intimately involved in proliferation. Mutations occur in the kinase region and lead to unregulated phosphorylation and activation of cell survival/proliferation pathways. There are multiple therapies available that work against this mutation: Erlotinib and Afatinib are FDA-approved for EGFR mutated tumors.

The incidence is highest in female, non-smokers, with adenocarcinoma histology. Though this gene has multiple known mutations, 90% of those present in adenocarcinoma are of exon 19 and L858R point mutation in exon 21.[2] This mutation is favored in Asian populations with its frequency reaching 62%,[3] as opposed to 10% in US populations.[4]

Table 1. Common Genomic Alterations in NSCLC

	Type of alteration	Frequency	Therapy FDA Approved?	Implications
EGFR	Mutation	10-35%	Yes	Prognostic and Predictive
ALK	Rearrangement	3-7%	Yes	Predictive, Not prognostic
ROS1	Rearrangement	1%	Yes, but for a different mutation	Predictive, Not prognostic
RET	Rearrangement	1%	Yes, but only for other cancers	Not Predictive or Prognostic
BRAF	Mutation	1-3%	Yes, but only for other cancers	Not Predictive or Prognostic
FGFR-1	Amplification	20%	No	Prognostic (Negatively) only
MET	Amplification	2-4%	Yes, but for a different mutation	Prognostic (Negatively) only

NCCN guidelines now recommend routine testing for this mutation for all new cases of metastatic adenocarcinoma, with consideration in squamous cell patients who are never smokers or have mixed histology. Treatment with Erlotinib or Afatinib should be offered as upfront therapy to all patients who harbor this mutation.

It should be noted that insertions to exon 20 have been deemed tyrosine kinase inhibitor (TKI) resistant through clinical trials and EGFR inhibitors will be far less effective in patients with these mutations.[5] For patients with this insertion, conventional chemotherapy is favored.

A mutation of the 790th amino acid from T to M is found in up to 50% of patients who initially responded to treatment with erlotinib but subsequently progressed despite continued treatment.[6] T790M can be the sole driver mutation in EGFR mutated cancers; however, this is rare and only accounts for approximately 5% of all EGFR mutated NSCLC.[7] New TKIs that are mutant specific and can specifically target this mutation are currently under development; rociletinib is furthest along in development but currently there are no approved agents for use against the T790M mutation.[8]

ALK

Anaplastic Lymphoma Kinase, is a CD receptor (CD246) that plays a large role in the development of neurons. The ALK gene products are known to promote cell growth/proliferation and inhibit apoptosis at baseline. Rearrangement and fusion of this gene with EML4 is amplified and leads to a fusion protein product that is produced in an unregulated quantity and subsequently causes excessive cell proliferation. It is found in 5–7% of NSCLC. Again found predominantly in never-smokers and almost exclusively in adenocarcinoma,[9] this is the second, and only other genetic aberration other to have an FDA-approved therapy in advanced NSCLC.[10] Crizotinib, an ALK inhibitor, was approved for treatment of metastatic NSCLC in 2011 and response rates were comparable to EGFR positive tumors being treated with Erlotinib.

NCCN guidelines now recommend routine testing for this gene fusion (2p23) by FISH for all new diagnoses of metastatic lung adenocarcinoma. (**Fig. 2 and 3**) Treatment with crizotinib should be offered as upfront therapy to all patients.

In ASCO 2014, Siraj M Ali et al, presented their experience with *ALK* rearranged lung carcinomas (LC) as detected by a clinical next generation sequencing (NGS)-based assay. Genomic profiling of 1,070 lung carcinomas was performed. Of 1,070 total lung carcinomas profiled, 47 harbored *ALK* rearrangements (4.4%). Of the 28 *ALK* rearranged specimens also tested by *ALK* FISH, [9] (32%) were negative, and 19 were positive. Twenty-two patients were treated with crizotinib and had response data available; 19 responded by investigator assessment. Of the 9 cases which were negative by FISH, 5 patients responded to crizotinib, 2 patients did not, and

Figure 1. Known mutations to EGFR gene

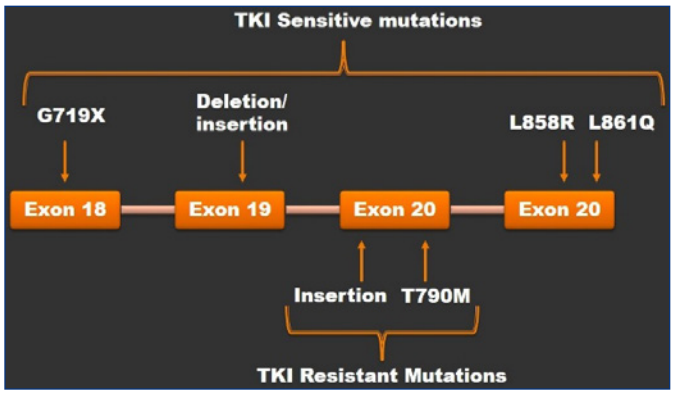


Figure 2. Fluorescence in situ hybridization (FISH) for ALK gene (Arrows indicate wild type)

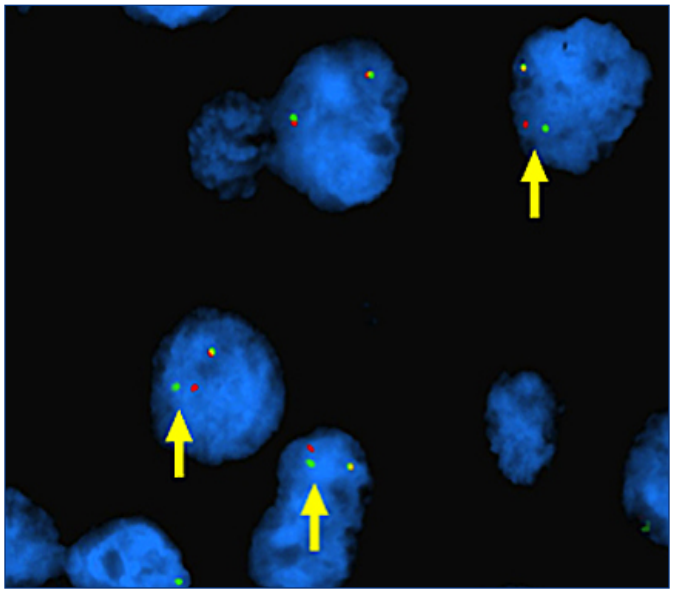
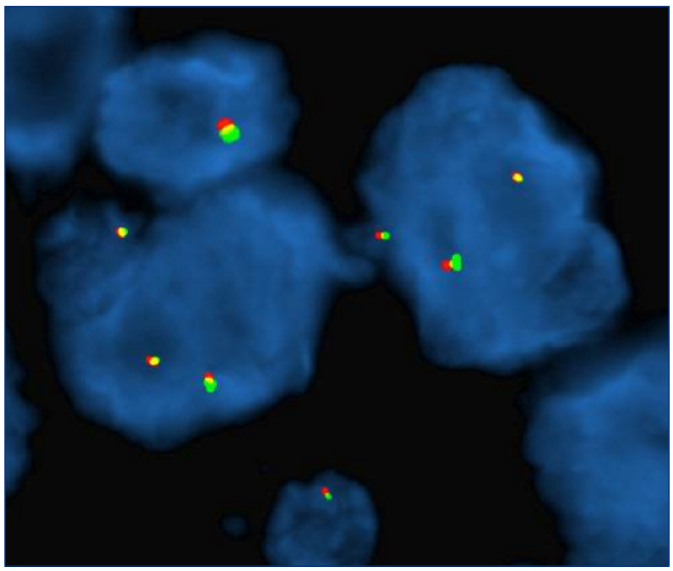


Figure 3. Fluorescence in situ hybridization (FISH) for ALK gene (fusion genes)



the response data for the remaining 2 patients is unavailable. Targeted NGS may be more sensitive for the detection of *ALK* rearrangements than FISH. In light of the responsiveness of *ALK* NGS+/FISH- tumors to crizotinib, the use of FISH as the gold standard for *ALK* detection in LC warrants prospective study.[11]

Several resistance mutations have been described.[12] In April 2014 a second agent, ceritinib, was approved for treatment of ALK positive NSCLC. Ceritinib is a second generation TKI that is approximately 20 times more potent than crizotinib.[13] Preclinical studies suggested that ceritinib had significant activity against cells that were either sensitive or resistant to crizotinib, including resistant tumors with the most common L1196M and G1269A resistance mutations.[14]

ROS1

ROS1 is a tyrosine kinase receptor belonging to the insulin family. Having striking similarity to ALK, this gene promotes proliferation and inhibits apoptosis, develops mutations via a rearrangement that leads to unregulated production of fusion proteins which retain the kinase domain,[15] and preferentially affects young, never-smokers with adenocarcinoma. ROS1 translocations are identified by a FISH break-apart assay, again much like ALK. Though similar to ALK in many respects, the notable difference is the frequency at which ROS1 is found in tumors which is 1–2% of all NSCLC.

Interestingly, this gene rearrangement has increased sensitivity to the well-established ALK inhibitor, crizotinib.[16] Retrospective analysis of crizotinib suggest that it may actually be a stronger inhibitor of ROS1 than it is of ALK. Initial data is so promising that it is highly likely that this will be the third mutation to be formally included in national guidelines for the treatment of advanced adenocarcinoma of the lung following EGFR and ALK.

RET

RET is a proto oncogene that becomes pathologic upon rearrangement with other genes leading to fusion proteins.[17] Normally RET, a tyrosine kinase, responds to growth factors and promotes cell proliferation. RET mutations are present in 1–2 % of lung adenocarcinoma and not found in SCC. This pathway is currently better understood in thyroid cancer and the only FDA-approved agent against this mutation is the multiple kinase inhibitor vandetanib (which targets RET among other kinases).[18] Initial trials of this agent in RET positive NSCLC were disappointing. At this time there are no recommended treatments for RET positive NSCLC patients. Clinical trials with other RET inhibitors are ongoing.

BRAF

BRAF is a member of the Raf kinases which regulate the MAP kinase pathway. Better known for its role in melanoma, BRAF mutations are seen in 1–4% of lung adenocarcinoma.[19] There are several mutations identified,[20] of which the most clinically significant is a point mutation, V600E. This mutation causes phosphorylation in the absence of normal signal and unregulated cell growth. V600E accounts for 90% of BRAF positive melanoma, but only accounts for 50% of BRAF positive lung adenocarcinoma. This is problematic as the only two FDA approved BRAF targeting drugs, vemurafenib and dabrafenib, were developed to target this specific mutation. A phase II trial treating V600E positive lung adenocarcinoma with dabrafenib demonstrated clinical efficacy. Responses were relatively durable and the side-effect profile was consistent with what is observed patients with melanoma. This data suggests that current BRAF inhibitors could be as efficacious in V600E positive lung adenocarcinoma as they are in melanoma.

FGFR1

Fibroblast growth factor receptor-1 is another tyrosine kinase which plays a role in cell proliferation. Contrary to other mutations, FGFR1 amplification is associated with smoking and with worse overall survival. This amplification is found in approximately 15–20% of lung squamous cell carcinomas.[21] Actionable driver mutations are rare in lung SCC compared to adenocarcinoma and there is heightened interest in FGFR1 amplification. Gene copy number is evaluated by fluorescent in situ hybridization, though the number of copies needed for the gene to be significant is not known.[22]

TKIs for this mutation are currently under development and in phase 1 trials. A study of the TKI "BGJ398" in patients with FGFR1-amplified lung SCC showed partial response in 15% of patients. There are no approved FGFR inhibitors at this time.

MET

MET amplification is found in 2-4% of untreated NSCLC but found in up to 20% of EGFR positive cancers that have developed resistance to TKIs.[23] Subsequent studies confirmed that amplification of MET is integral to development of this resistance.[24] MET is located on chromosome 7 and amplification can be detected by fluorescent in situ hybridization. In a recent phase I trial, crizotinib showed activity in patients with MET amplified NSCLC and had a response rate of 33%.[25] This may evolve into a new second line treatment for TKI resistant EGFR positive patients.

FINAL THOUGHTS

There exist other mutations not mentioned in this article. DDR2, PIK3C, AKT and PTEN mutations are all suspected to be driver mutations and are currently under investigation as well. As yet there are no clear therapies or prognostic implications for tumors positive for these mutations and further development is needed. Additionally, these mutations are found more commonly in squamous cell lung cancer and may offer hope of targeted therapy for a tumor type that currently lacks effective agents.

Although individual rates of mutations are small, when added together they account for a large percentage of new NSCLC diagnoses, particularly in non-smokers with adenocarcinoma. Many new agents offer equivalent or better OS and PFS with less toxicity than chemotherapy. NGS analysis is a useful tool for discovery of these mutations and potential treatments on or off clinical trials. We recommend its use in patients who have progressed through standard therapy but retain a good performance status. Personalized medicine is the future of oncology and genomic analysis will play a large role in cancer prognosis and therapy.

References

1. Lovly C, Horn L, Pao W. 2015. Molecular Profiling of Lung Cancer. My Cancer Genome http://www.mycancergenome.org/content/disease/lung-cancer/ (Updated February 6).
2. Ladanyi M, Pao W. Lung adenocarcinoma: guiding EGFR-targeted therapy and beyond. Mod Pathol. 2008;21 Suppl 2:S16-22.
3. Shi Y, Au JS, Thongprasert S, et al. A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). J Thorac Oncol. 2014;9(2):154-62.
4. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004;350(21):2129-39.
5. Yasuda H, Kobayashi S, Costa DB. EGFR exon 20 insertion mutations in non-small-cell lung cancer: preclinical data and clinical implications. Lancet Oncol. 2012;13(1):e23-31.
6. Kobayashi S, Boggon TJ, Dayaram T, et al. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med. 2005;352(8):786-92.
7. Inukai M, Toyooka S, Ito S, et al. Presence of epidermal growth factor receptor gene T790M mutation as a minor clone in non-small cell lung cancer. Cancer Res. 2006;66(16):7854-8.
8. Sequist, et al. First-in-human evaluation of CO-1686, an irreversible, highly selective tyrosine kinase inhibitor of mutations of EGFR (activating and T790M). J Clin Oncol. 32:5s, 2014 (suppl; abstr 8010^).
9. Wong DW, Leung EL, So KK, et al. The EML4-ALK fusion gene is involved in various histologic types of lung cancers from nonsmokers with wild-type EGFR and KRAS. Cancer. 2009;115(8):1723-33.
10. Shaw AT, Kim DW, Nakagawa K, et al. Crizotinib versus chemotherapy in advanced ALK-positive lung cancer. N Engl J Med. 2013;368(25):2385-94.
11. Siraj MA, et al. Identifying ALK rearrangements that are not detected by FISH with targeted next generation sequencing of lung carcinoma. J Clin Oncol 32;5s, 2014 (suppl; abstr 8049).
12. Choi YL, Soda M, Yamashita Y, et al. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. N Engl J Med. 2010;363(18):1734-9.
13. Kim et al. Ceritinib in advanced anaplastic lymphoma kinase (ALK)-rearranged (ALK+) non-small cell lung cancer (NSCLC): Results of the ASCEND-1 trial. J Clin Oncol 32:5s, 2014 (suppl; abstr 8003^).
14. Iacono D, Chiari R, Metro G, et al. Future options for ALK-positive non-small cell lung cancer. Lung Cancer. 2015;
15. Rikova K, Guo A, Zeng Q, et al. Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. Cell. 2007;131(6):1190-203.
16. Ou et al. Efficacy and safety of crizotinib in patients with advanced ROS1-rearranged non-small cell lung cancer (NSCLC). *J Clin Oncol* 31, 2013 (suppl; abstr 8032).
17. Ju YS, Lee WC, Shin JY, et al. A transforming KIF5B and RET gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing. *Genome Res*. 2012;22(3):436-45.
18. Wells SA, Gosnell JE, Gagel RF, et al. Vandetanib for the treatment of patients with locally advanced or metastatic hereditary medullary thyroid cancer. *J Clin Oncol*. 2010;28(5):767-72.
19. Cardarella S, Ogino A, Nishino M, et al. Clinical, pathologic, and biologic features associated with BRAF mutations in non-small cell lung cancer. *Clin Cancer Res*. 2013;19(16):4532-40.
20. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature*. 2002;417(6892):949-54.
21. Dutt A, Ramos AH, Hammerman PS, et al. Inhibitor-sensitive FGFR1 amplification in human non-small cell lung cancer. *PLoS ONE*. 2011;6(6):e20351.
22. Heist RS, Sequist LV, Engelman JA. Genetic changes in squamous cell lung cancer: a review. *J Thorac Oncol*. 2012;7(5):924-33.
23. Bean J, Brennan C, Shih JY, et al. MET amplification occurs with or without T790M mutations in EGFR mutant lung tumors with acquired resistance to gefitinib or erlotinib. Proc Natl Acad Sci USA. 2007;104(52):20932-7.
24. Engelman JA, Zejnullahu K, Mitsudomi T, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science*. 2007;316(5827):1039-43.
25. Camidge DR et al. Efficacy and safety of crizotinib in patients with advanced c-MET-amplified non-small cell lung cancer (NSCLC). *J Clin Oncol* 32:5s, 2014 (suppl; abstr 8001).

Authors

Robert C. Black, MD, is a Fellow in the Div. of Hematology/Oncology at the Alpert Medical School of Brown University.

Humera Khurshid, MD, is a Medical oncologist at the Comprehensive Cancer Center at Rhode Island Hospital and an Assistant Professor at The Warren Alpert Medical School of Brown University.

Correspondence

Robert C. Black, MD
Rhode Island Hospital
593 Eddy Street
Providence, RI 02903
401-444-2073
Fax 401-444-2330
rblack@lifespan.org