

# 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.<sup>1</sup> 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

Sjjoblom 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.<sup>2,3</sup> 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 BRAFV600E were noted.<sup>4</sup>

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-glucuronosyl transferase 1A1 (UGT1A1)	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.<sup>5</sup>

**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.<sup>6</sup> Recent retrospective analysis is suggesting an adverse prognostic impact.<sup>7</sup>

**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.<sup>8</sup>

**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.<sup>9</sup> 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.<sup>10</sup> 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-fluorouracil, two chemotherapeutic drugs commonly used to treat colorectal cancer.<sup>11</sup>

**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.<sup>1</sup> However, the presence of the mutation or deficiency of the enzyme does not always dictate clinical outcome.<sup>12</sup>

Uridine diphosphate-glucuronosyl transferase 1A1 (UGT1A1) is an enzyme that mediates glucouronidation. This enzyme enables conjugation of glucuronic acid to the active form of irinotecan, SN-38. Therefore if UGT1A1 is mutated resulting in a quantitative deficiency of the UGT1A1 enzyme, there will be a decreased rate of irinotecan metabolism resulting in clinically significant neutropenia and diarrhea.<sup>13</sup>

**Gene Expression Assays:** Oncotype Dx Colon, ColoPrint, ColDX are clinically available examples.<sup>14</sup> The aforementioned assays assess 18 genes or more, the data is then used to predict an individual tumor's risk of recurrence.<sup>15</sup> 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%).<sup>16</sup> 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).<sup>17</sup>

**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.<sup>18</sup>

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.<sup>19</sup>

### 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 in PIK3CA, FGF19 and RPS6KA3.<sup>20</sup> 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.<sup>21</sup>

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.<sup>22</sup>

### 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 morbidity and mortality from Lynch Syndrome in relatives. *Genet Med*. 2009; 11(1): 35-41
10. Sinicrpe 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 Pharmacological Sciences*. 2006 Aug. 27(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. *JNCL*. 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, Noshio 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](mailto:kperez@lifespan.org)