Colorectal Cancer

Getting colorectal cancer patients on the right treatment, faster

CIRCULOGENE
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According to the American Cancer Society, approximately 147,950 individuals will be diagnosed with CRC and 53,200 will die from the disease in 2020, including 17,930 cases and 3,640 deaths in individuals aged younger than 50 years. Colorectal cancer is the third most commonly diagnosed cancer in men and women and is the second leading cause of cancer death when men and women are combined. Getting these patients on the right treatment faster can make all the difference. The adoption of precision medicine can have a substantial effect on survival in patients with colorectal cancer!

The Oncologist 2020; 25:404-413

"The sidedness of colon cancer has a therapeutic impact. Right sided colon cancers are enriched with microsatellite instability high (MSI-H) and aberrant EGFR pathways, with BRAF and PIK3CA portending a better benefit for bevacizumab with anti-EGFR monoclonal antibodies preferential in left sided colon cancer. However, it is not the group sidedness, it is individual tumor biology that matters and guides the best treatment benefit for that individual.

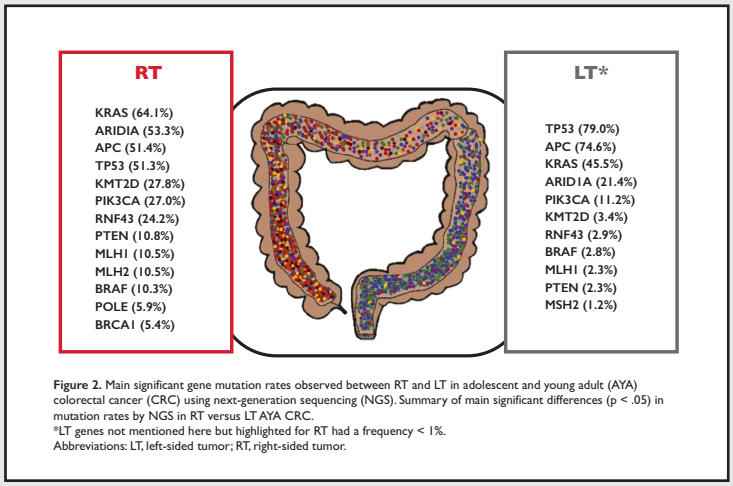


Figure 2. Main significant gene mutation rates observed between RT and LT in adolescent and young adult (AYA) colorectal cancer (CRC) using next-generation sequencing (NGS). Summary of main significant differences ($p < .05$) in mutation rates by NGS in RT versus LT AYA CRC.
*LT genes not mentioned here but highlighted for RT had a frequency < 1%.
Abbreviations: LT, left-sided tumor; RT, right-sided tumor.

"Notably, clinicopathologic and molecular features of CRC are different between AYA (adolescent and young adults) and older patients. AYA patients more often present with advanced-stage disease (stage III or IV), and their tumors are likely to appear more historically aggressive by way of mucinous or signet ring features and/or poor differentiation. Nodal involvement in early stage rectal cancer is also more frequent in patients under 50 years of age compared with older individuals."

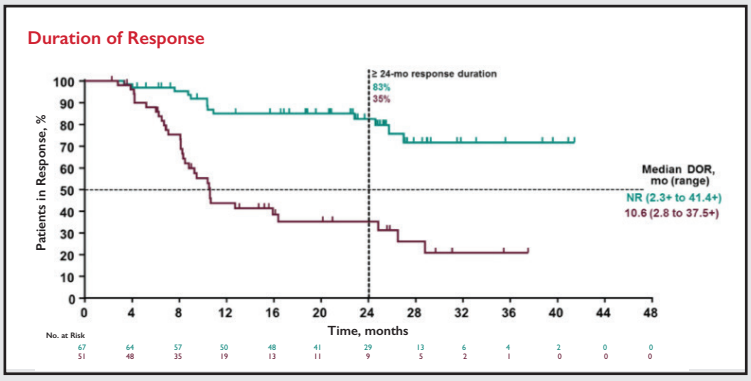
JCO Precis. Oncol.

"Liquid biopsy has emerged as a minimally invasive tool to genotype tumors, to assess patient prognosis and detect MRD, to monitor treatment efficacy, and to track the dynamism of clonal evolution over time and therapies. Whereas tissue biopsies catch single snapshots of the tumor in a specific spatiotemporal fragment, liquid biopsy may more comprehensively depict the intrinsic and dynamic intratumoral heterogeneity."-2019;3:1–14

"Despite guideline recommendations and significant therapeutic implications, overall biomarker testing rates in mCC remain suboptimal. Adherence to guideline-recommended biomarker testing would potentially reduce exposure to expensive and ineffective therapies, resulting in improved patient outcomes."-2019 Dec 6;3:PO.19.00274

ASCO 2020 – "First-line FDA approval immune therapy MSI-H colon cancer"

"In MSI-H patients, immunotherapy responses are very durable compared to chemotherapy."



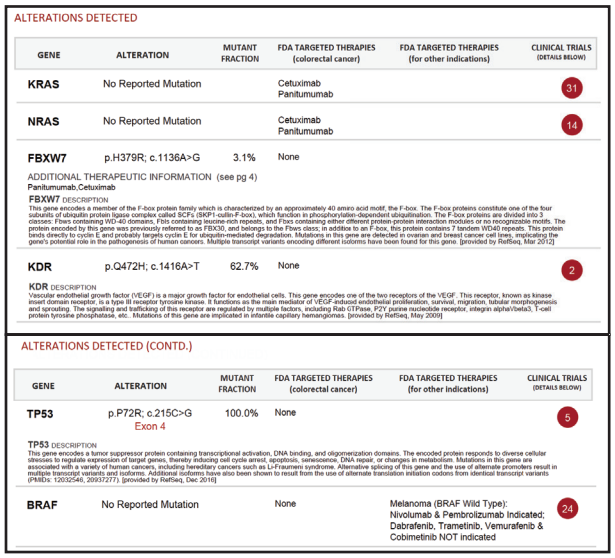
CIRCULOGENE'S comprehensive colorectal panel is a noninvasive technique that can be combined with traditional tissue biopsy to track cell-free DNA and detect disease biomarkers in blood faster and more accurately.

Sidedness case – 54-Year-Old Male

DESCENDING COLON PRIMARY WITH LUNG AND LIVER METASTASES

We learn from groups, yet we take care of individuals. Treatment stratification factors only define a group. Tumor biology reflects the individual. The sidedness of treating colon cancer straddles this paradox with right and left sided colon cancers reflecting a different group tumor biology. Right sided colon cancers are enriched with microsatellite instability high and aberrant EGFR pathways, with BRAF and PIK3CA portending a better benefit for bevacizumab with anti-EGFR monoclonal antibodies preferential in left sided colon cancer. However, it is not the group sidedness—but individual tumor biology—that matters and guides the best treatment benefit for that individual.

This case presents a group sidedness treatment approach countered by the individual tumor biology to guide the biologic precision oncology treatment decision.



ALTERATIONS DETECTED

GENE	ALTERATION	MUTANT FRACTION	FDA TARGETED THERAPIES (colorectal cancer)	FDA TARGETED THERAPIES (for other indications)	CLINICAL TRIALS (DETAILS BELOW)
KRAS	No Reported Mutation		Cetuximab Panitumumab		31
NRAS	No Reported Mutation		Cetuximab Panitumumab		14
FBXW7	p.H379R; c.1136A>G	3.1%	None		

ADDITIONAL THERAPEUTIC INFORMATION (see pg 4)
Panitumumab,Cetuximab

FBXW7 DESCRIPTION
This gene encodes a member of the F-box protein family which is characterized by an approximately 40 amino acid motif, the F-box. The F-box proteins constitute one of the four subunits of ubiquitin protein ligase complex called SCFs (SKP1-cullin-F-box), which function in phosphorylation-dependent ubiquitination. The F-box proteins are divided into 3 classes: Fbws containing WD-40 domains, Fbls containing leucine-rich repeats, and Fbxs containing either different protein-protein interaction modules or no recognizable motifs. The protein encoded by this gene was previously referred to as FBX30, and belongs to the Fbws class; in additive to an F-box, this protein contains 7 tandem WD40 repeats. This protein binds directly to cyclin E and probably targets cyclin E for ubiquitin-mediated degradation. Mutations in this gene are detected in ovarian and breast cancer cell lines, implicating the gene's potential role in the pathogenesis of human cancers. Multiple transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Mar 2012]

KDR	p.Q472H; c.1416A>T	62.7%	None		2

KDR DESCRIPTION
Vascular endothelial growth factor (VEGF) is a major growth factor for endothelial cells. This gene encodes one of the two receptors of the VEGF. This receptor, known as kinase insert domain receptor, is a type III receptor tyrosine kinase. It functions as the main mediator of VEGF-induced endothelial proliferation, survival, migration, tubular morphogenesis and sprouting. The signalling and trafficking of this receptor are regulated by multiple factors, including Rab GTPase, P2Y purine nucleotide receptor, integrin alpha/beta3, T-cell protein tyrosine phosphatase, etc.. Mutations of this gene are implicated in infantile capillary hemangiomas. [provided by RefSeq, May 2009]

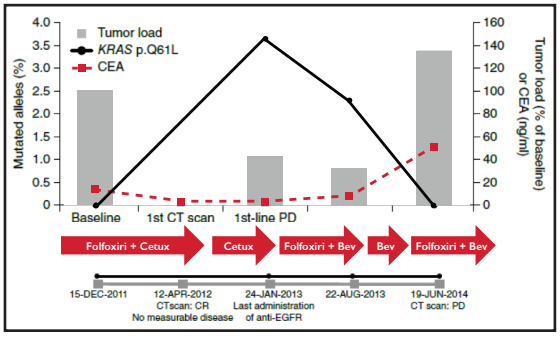
ALTERATIONS DETECTED (CONTD.)

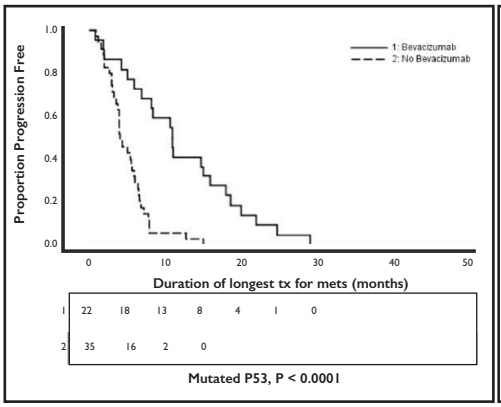
GENE	ALTERATION	MUTANT FRACTION	FDA TARGETED THERAPIES (colorectal cancer)	FDA TARGETED THERAPIES (for other indications)	CLINICAL TRIALS (DETAILS BELOW)
TP53	p.P72R; c.215C>G Exon 4	100.0%	None		5

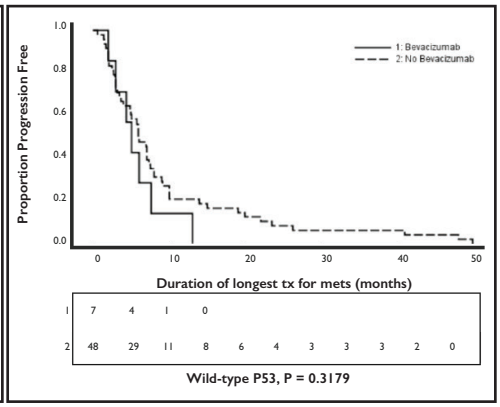
TP53 DESCRIPTION
This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded protein responds to diverse cellular stresses to regulate expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism. Mutations in this gene are associated with a variety of human cancers, including hereditary cancers such as Li-Fraumeni syndrome. Alternative splicing of this gene and the use of alternate promoters result in multiple transcript variants and isoforms. Additional isoforms have also been shown to result from the use of alternate translation initiation codons from identical transcript variants (PMIDs: 12032546, 20937277). [provided by RefSeq, Dec 2016]

BRAF	No Reported Mutation		None	Melanoma (BRAF Wild Type): Nivolumab & Pembrolizumab Indicated; Dabrafenib, Trametinib, Vemurafenib & Cobimetinib NOT indicated	24

The left side primary and KRAS/BRAF wild-type biology would support an anti-EGFR approach of either cetuximab or panitumumab with chemotherapy. However, the KDR gene and p53 mutations indicate an actionable precision tumor biology. The KDR gene encodes VEGFR-2 function and mutations are associated with elevated VEGFA levels. TP53 mutations are also associated with elevated VEGFA levels. KDR mutations have been associated with responses to the multi-targeted VEGF TKI regorafenib in colon cancer. TP53 mutations have also been associated with a significant benefit with the addition of bevacizumab to chemotherapy regimens across a spectrum of solid tumors compared to p53 wild-type patients. This makes a very compelling case for treating the tumor biology with anti-VEGF bevacizumab as opposed to the group-indicated anti-EGFR biologic.







It is also very important to be aware that if the bedside oncologic decision is for anti-EGFR with chemotherapy due to anti-angiogenesis contraindications, KRAS mutations can evolve with clonal suppression, inducing anti-EGFR resistance. In that setting, an anti-angiogenesis therapeutic approach with bevacizumab, ramucirumab or regorafenib would be far differently and more favorably balanced to keep going forward.

Case Study Prepared by Doctor Paul Walker
Chief Medical Officer, Former Director of Thoracic Oncology at East Carolina University

Sources:
- Oncotarget, 2017, Vol. 8, (No. 49)
- Oncotarget, May, Vol. 4, (No 5)
- Cancer Biology & Therapy, 21:1, 95-100, Mutated TP53 is a marker of increased VEGF expression: analysis of 7,525 pan-cancer tissues
- Nature Medicine, Volume 21, Number 27, Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients

Colorectal Cancer – PIK3CA Mutations Case Study

Real Patients, Real Results

72-YEAR-OLD FEMALE PATIENT

TRANSVERSE COLON PRIMARY AND SOLITARY LIVER AND LUNG METASTASIS

PIK3CA mutations may not yet be directly targetable in colon cancer; however, these mutations still carry impactful actionable therapeutic decisions.

PIK3CA mutations play a role in guiding individual decisions regarding the effectiveness of anti-EGFR monoclonal antibodies. With the availability of broad next-generation sequencing (NGS) testing, PIK3CA mutations are now identified as one of several pathways beyond the basic RAS/RAF resistance pathways that predicts cetuximab or panitumumab resistance.

PIK3CA mutations also have a predictive impact of a liver radioembolization benefit in treating colorectal liver metastasis. Patients with chemotherapy refractory colon liver metastases with PIK3CA mutations achieved a much higher response rate and markedly longer local control than colon cancers without PIK3CA mutations.

The most dramatic survival benefit of PIK3CA mutations is with simple aspirin use. Published back in 2012, the regular use of aspirin after definitive cancer treatment for PIK3CA mutated colon cancers had a dramatic reduction in cancer-specific and overall mortality, whereas the use of aspirin had no impact in the PIK3CA wild type cancers. So simple but so impactful!

The evolution of liquid biopsy NGS technology is now readily available and can easily overcome the tissue heterogeneity limitations and tissue acquisition barriers to accurately identify the tumor biology that can then guide a more precise and better treatment for patients.

SUMMARY OF RESULTS

Gene(s) Tested:	50
Alteration(s) Detected:	3
FDA-Approved Targeted Therapies:	2
Additional Therapies:	0
Open Clinical Trials: see pg 6	48

The following 50 genes were tested:
ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL

These mutations, relevant in Colorectal Cancer, were tested for and determined to be **absent**:
BRAF V600 mutation (Not Found)
KRAS codons 12, 13, 59, 61, 117 or 146 (Not Found) NRAS codons 12, 13, 59, 61, 117 or 146 (Not Found)

IMMUNOTHERAPY TEST RESULTS — FDA GUIDANCE

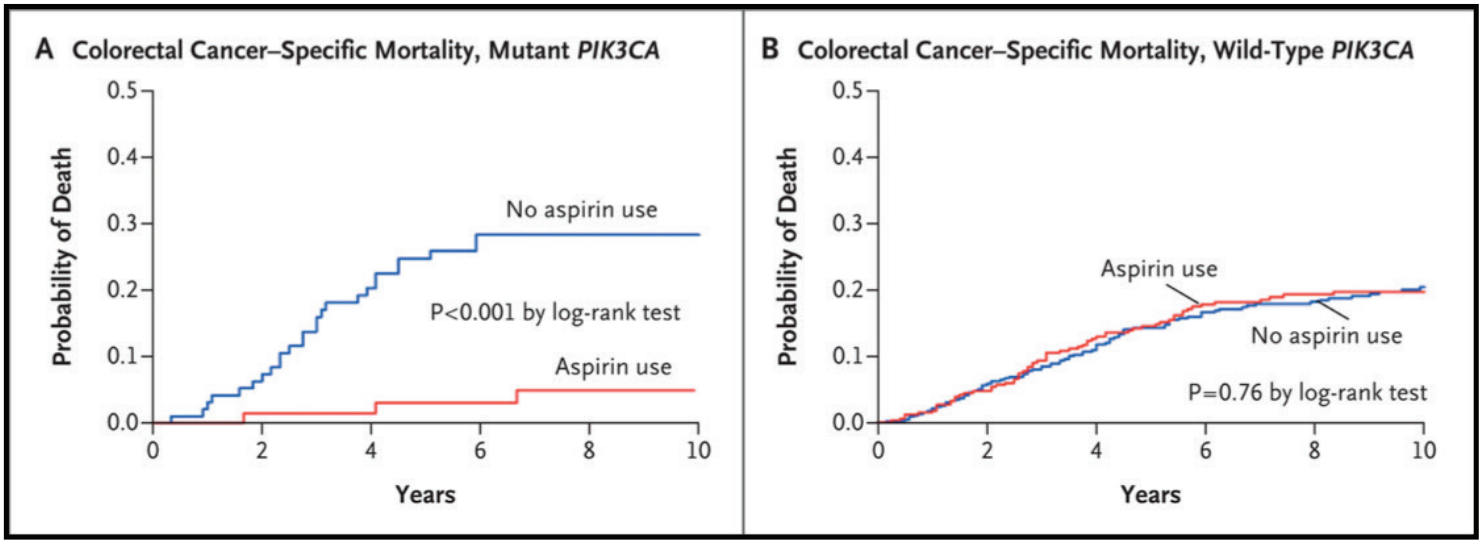
MSI-H	Not Detected

ALTERATIONS DETECTED

GENE	ALTERATION	MUTANT FRACTION	FDA TARGETED THERAPIES (colorectal cancer)	FDA TARGETED THERAPIES (for other indications)	CLINICAL TRIALS (DETAILS BELOW)
KRAS	No Reported Mutation		Cetuximab Panitumumab		31
NRAS	No Reported Mutation		Cetuximab Panitumumab		14
KIT	p.M541L; c.1621A>C	62.8%	None		1
PIK3CA	p.I391M; c.1173A>G	47.3%	None		7

KIT DESCRIPTION
This gene encodes the human homolog of the proto-oncogene c-kit. C-kit was first identified as the cellular homolog of the feline sarcoma viral oncogene v-kit. This protein is a type 3 transmembrane receptor for MGF (mast cell growth factor, also known as stem cell factor). Mutations in this gene are associated with gastrointestinal stromal tumors, mast cell disease, acute myelogenous lukemia, and piebaldism. Multiple transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Jul 2008]

PIK3CA DESCRIPTION
Phosphatidylinositol 3-kinase is composed of an 85 kDa regulatory subunit and a 110 kDa catalytic subunit. The protein encoded by this gene represents the catalytic subunit, which uses ATP to phosphorylate PtdIns, PtdIns4P and PtdIns(4,5)P2. This gene has been found to be oncogenic and has been implicated in cervical cancers. A pseudogene of this gene has been defined on chromosome 22. [provided by RefSeq, Apr 2016]



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Sources:
- American Association for Cancer Research, Cancer Res 2009;69
- Lupini et al. BMC Cancer (2015) 15:808
- N Engl J Med 2012;367:1596-606
  DOI: 10.1056/NEJMoa1207756
- Oncotarget, 2017, 8(14):23529-23538
- Clin Cancer Res; 23(16) August 15, 2017

Real Patients, Real Results

53-YEAR-OLD MALE PATIENT

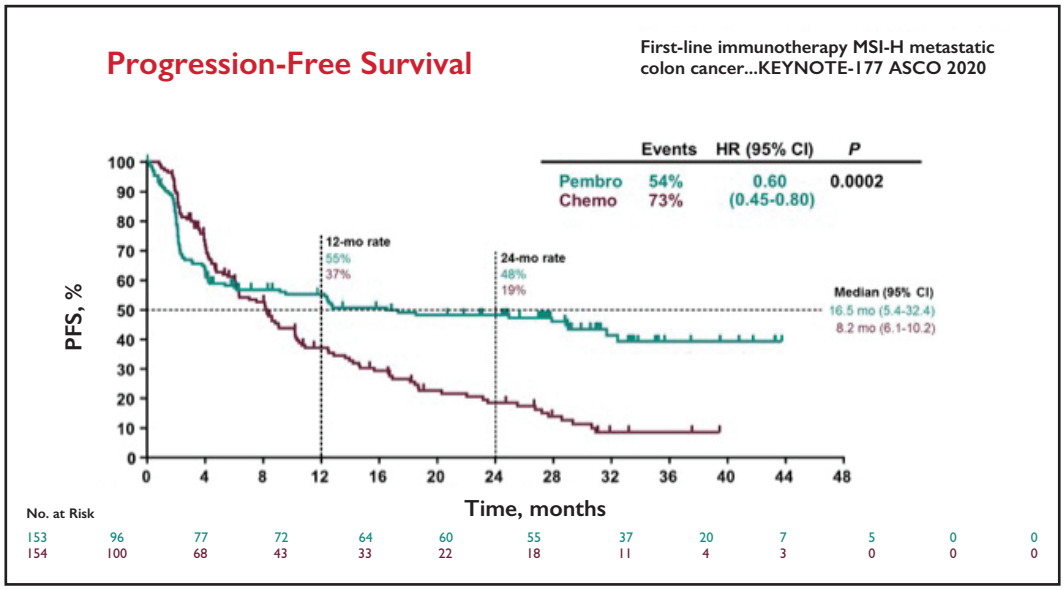
MSI-H METASTATIC COLON CANCER

Microsatellite instability high (MSI-H) cancers carry a unique tumor biology with chemotherapy resistance yet anti-angiogenesis and immune checkpoint inhibition (ICI) therapeutic sensitivity. MSI-H cancers clearly need to be treated differently.

Adjuvant 5-fluorouracil-based chemotherapy has long been known to be ineffective in patients with MSI-H colon cancers who would otherwise benefit from adjuvant chemotherapy. MSI-H colon cancers demonstrated a better benefit with the addition of anti-angiogenesis therapy with bevacizumab in both the metastatic treatment setting but also in the adjuvant setting. In the KRAS wild-type metastatic trial CALGB/SWOG 80405, the addition of bevacizumab to the mFOLFOX6 chemotherapy backbone more than doubled the median overall survival compared to cetuximab in MSI-H patients, with no biologic agent differential impact in the MSS patients. Likewise, in the adjuvant NSABP-08 trial, the addition of bevacizumab to adjuvant mFOLFOX6 was of no benefit in the overall group. However, in patients with mismatch repair deficiency colon cancers and the unique MSI tumor biology, there was significant survival benefit of adding bevacizumab to the adjuvant chemotherapy.

Most notable now is the sensitivity of MSI-H cancers to immune therapy with ICIs. The second-line FDA tissue-agnostic approval of anti-PD-1 therapy in 2017—solely based upon the MSI-H tumor biology irrespective of the anatomical tissue of origin—has now evolved into first-line FDA approval of immune therapy in metastatic MSI-H colon cancers in 2020. At ASCO 2020, single-agent pembrolizumab in MSI-H colorectal cancer achieved a much higher response rate and progression free survival with marked durability compared to standard chemotherapy. This same marked benefit of ICI in MSI-H colorectal cancer patients has now entered into earlier stage use, with one study achieving a 100% major pathologic response!

It is vitally important that MSI is assessed on all colorectal cancers. MSI-H is a tumor biology with dramatic benefit when treated with immune therapy. MSI status can now easily and quickly be assessed by a liquid biopsy. Clearance of the plasma MSI is also a dynamic marker to monitor immune therapy response, whereas persistence is a harbinger of progressing disease. This has tremendous treatment impact and benefit for your patients and also sparks the need for hereditary Lynch Syndrome testing with potential lifesaving impact for their families.



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Sources:
- Nature Medicine
- J Clin Oncol 37:1217-1227
- J Natl Cancer Inst;2013;105:989–992
- J Gastrointest Oncol 2020;11(4):826-828
- N Engl J Med 2015;372:2509-20