Circulogene

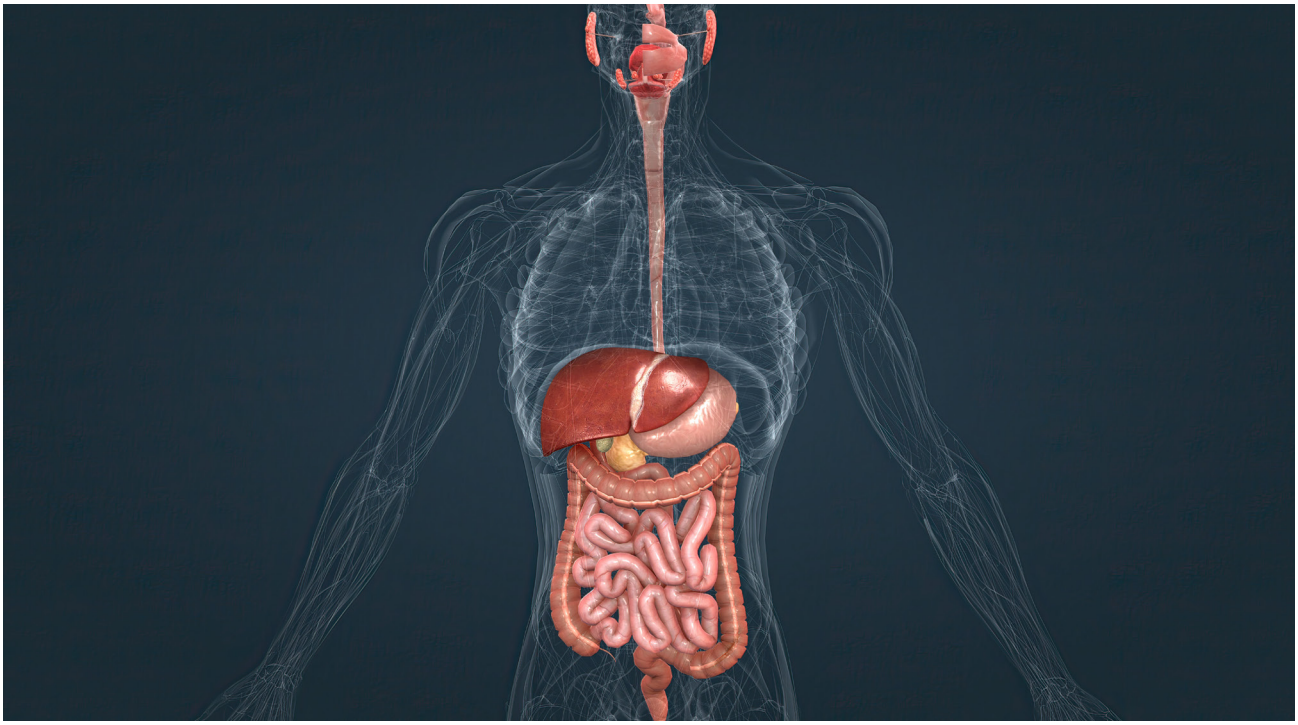
Programmatic Upstream Liquid Biopsy Molecular Testing in GI Cancers

Gastrointestinal (GI) cancers represent 26% of the global cancer incidence and 35% of all cancer deaths.[1] GI cancers represent multiple different anatomical cancers.

Even more so, within each anatomical GI cancer, there will be a unique molecular tumor biology warranting different treatment approaches. These advances in tumor biology are so important for individual treatment, that the American Society of Clinical Oncology (ASCO) named Molecular Profiling Drives Progress in GI Cancers as the ASCO cancer advance for the year of 2021.[2] The role of liquid biopsy plasma NGS testing in colorectal and non-colorectal GI cancers has continued to evolve and expand.[3-5]

When tumor biology is treated, survival outcomes in advanced GI cancers improve. **Survival in advanced microsatellite instability-high (MSI-H) cancers treated with immune therapy can exceed 70% at 4-years.[6]** Targeting NTRK and other oncogenic fusions can achieve durable disease control.[7] Even in pancreatic cancer, matching individual treatment to the identified tumor biology can extend median overall survival (OS) by over a year compared to unmatched group therapies.[8] Just as important as knowing the right therapy, it is equally important to avoid the wrong therapy. MSI-H GI cancers can have a detrimental survival benefit if given chemotherapy.[9] GI cancers with oncogenic fusions do very poorly with standard group chemotherapy compared to potential outcomes with the targeted therapy.[10]

Not testing for, or not knowing, driver mutations, fusion targets, or MSI status in GI cancers, will miss the tremendous survival benefit of targeted and immune therapy for those patients. Molecular testing is necessary in all advanced GI cancers and is now becoming equally important in the earlier curative stages of GI cancers.



NGS has facilitated the ease of molecular testing in GI cancers

Next-generation technology (NGS) makes molecular testing efficient and more cost effective than single-gene testing approaches. Liquid biopsy with plasma NGS molecular testing has further extended the ease of full molecular testing with a simple blood test.[11] Fragments of circulating tumor DNA (ctDNA) and RNA (ctRNA) can identify specific pathogenic driver mutations, gene rearrangement fusions, and gene amplifications.

Although tissue and plasma NGS testing remains complementary, comparative simultaneous tissue and plasma NGS testing has unexpectedly identified that tissue molecular testing will miss up to one-third of the driver mutations present, whereas plasma testing will identify more of these targetable alterations guiding treatment and improving survival outcomes.[12-14]

Tissue is still the 'gold standard' in making a diagnosis of cancer. However, given this data, the true 'gold standard' of molecular tumor biology testing has evolved to plasma. More complete molecular findings and a much quicker turnaround time of the molecular tumor biology results make a liquid biopsy with plasma NGS an ideal molecular testing approach for GI cancers.

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Problems with the current model of molecular testing

1 Molecular testing not getting done

The biggest problem with the current molecular testing approach is that the molecular testing is not getting done. Chart review data continues to show National Comprehensive Cancer Network guideline recommended molecular testing is not being performed by medical oncologists in most patients. In a chart review of colorectal cancer, only half of patients had MSI testing and full guideline recommended molecular testing was only performed in 28% of patients before initiating anti-EGFR combination therapy.[15]

The National Cancer Database identified only 28% of all adults and only 43% of younger adults with CRC underwent germline mismatch repair deficiency testing.[16]

2 Time from DX to RX matters

Across all cancers and all stages, the time from diagnosis to treatment matters. It is not the turnaround time of a molecular test that matters. It is the time from diagnosis of the cancer to starting treatment that matters. **Studies identify a 4 to 6-week window from the time from diagnosis to starting treatment as the critical period before survival outcomes begin to fall.** Not because of any treatment difference, but simply the delay in starting treatment. A meta-analysis of thirty-four studies across seven major cancer types noted a significant association between increased cancer mortality and delaying cancer treatment beyond 4 weeks from diagnosis.[17] Data from the National Cancer Database show a delay beyond 6 weeks in initiating the curative treatment in six cancers, including early-stage pancreatic cancer, resulted in a significantly reduced OS, with an estimated 1.2 to 3.2% increased risk of cancer mortality per week delay.[18]

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3 Tissue only or first approaches are limiting

A tissue only or first approach of molecular testing is also a limiting factor in the implementation of therapeutic advances in treating GI cancers. Tissue NGS testing takes too long with turnaround times of 3 weeks, and longer when ordered after pathology review, leading to treatment start times greater than 30 days from diagnosis. Tissue testing is fraught with spatial heterogeneity of pathogenic targetable mutations, gene rearrangement fusions, and gene amplifications. Sufficient tissue acquisition for full molecular testing in and of itself can be a limiting barrier to molecular testing in GI cancers. Additionally, any time a cancer recurs or progresses, repeat molecular testing is needed as molecular tumor biology has changed due to clonal evolution. Repeat tissue testing is not always practical in the clinic.

A tissue only or first approach of molecular testing is also a **limiting factor in the implementation** of therapeutic advances in treating GI cancers.

Liquid biopsies with plasma NGS molecular testing can overcome these tissue heterogeneity and acquisition barriers. With a simple blood test, a liquid biopsy for plasma NGS testing can efficiently and quickly identify a cancer's clonal evolution and resistant pathways guiding a more effective change in treatment for that individual.

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The clinical utility of liquid biopsy in GI cancers

1 Microsatellite instability is enriched in GI cancers

MSI-H is a recognized pan-cancer predictive immune biomarker warranting testing in all cancers. MSI-H cancers are enriched in GI cancers. MSI-H findings can be germline (Lynch Syndrome) or somatic (tumor) origin. Frequency can range from **15% in colorectal cancers**, **25% in small bowel adenocarcinomas**, **5-15% in GEJ**, and **up to 5% in pancreaticobiliary cancers**, and GI neuroendocrine tumors.[19] MSI-H is also found more frequently in early-stage cancers than advanced stages.[20]

MSI-H cancers have a unique tumor biology resulting in detrimental outcomes with chemotherapy, yet remarkable benefit from immune therapy alone. This immune therapy benefit is irrespective of the primary tissue site and is achieved in early and advanced GI cancers. In metastatic colorectal cancers, first-line immune therapy with the immune checkpoint inhibitor (ICI) pembrolizumab demonstrated a superior median progression free survival PFS compared to standard group chemotherapy.[21] Notably when the chemotherapy treated patients received salvage immune therapy, their outcomes were still significantly inferior to first-line immune therapy. Results from the phase 3 CheckMate-8HW trial also support a first-line dual anti-PD-1/anti-CTLA-4 chemotherapy free nivolumab/ipilimumab immune therapy approach.[22] **4-year OS in MSI-H metastatic colorectal and gastroesophageal junction (GEJ) cancers also exceeds 70%, far better than chemotherapy.**[9]

Frequency can range from

15% in colorectal cancers

25% in small bowel adenocarcinomas

5-15% in GEJ

Up to 5% in pancreaticobiliary cancers

The impact of treating the ICI sensitive MSI-H tumor biology in early-stage cancers is now recognized. MSI-H rectal cancers receiving immune therapy alone achieved a 100% complete response obviating the need for concurrent chemoradiation therapy or a surgical resection.[23] In the resectable MSI-H colon cancer NICHE-2 trial, **neoadjuvant ICI therapy achieved a 95% major pathologic response (<10% viability) and 67% pathologic complete response.**[24] In a study of neoadjuvant dual ICI therapy in gastric or GEJ adenocarcinomas, all patients had an R0 resection with 59% having surgical pathologic complete responses.[25]

It is oncologically clear that knowing the MSI status before any treatment step, whether advanced or early resectable stages, is essential for patients with GI cancers to get their best potential outcomes.

2 NTRK and other alterations are primary tissue-site agnostic

Oncogenic fusions with parts of two genes undergoing rearrangement are increasingly becoming identified and can be targeted with oral tyrosine kinase inhibitors (TKI). NTRK1-2-3 fusions have an FDA treatment indication of targeted NTRK TKI treatment irrespective of the primary anatomical cancer site. NTRK fusions can be present in colon, cholangiocarcinoma, pancreatic, esophageal, and gastric carcinomas as well as GIST tumors.[26] Oncogenic RET fusions have been identified in esophageal, gastric, and colorectal adenocarcinomas.[27] ALK and ROS1 fusions have also rarely been identified in GI cancers. Although rare by group incidence, for everyone with a pathogenic and targetable fusion, it is a 100% finding for that individual. In MSI-H BRAF wild-type CRC, fusions are enriched up to a reported 5-7% incidence.[28]

The treatment impact of testing for and potentially identifying fusions is clinically profound. Chemotherapy is notably poorly effective in oncogenic fusion cancers. **In fusion driven metastatic CRC,median OS with standard chemotherapy was just 15.6 months. That compares to 33.7 months in non-fusion metastatic CRC (10).** Oncogenic fusion driven GI cancers are best treated with the specific fusion targeted TKI agents. BRAF V600E mutations also have a tumor agnostic testing indication with a combinational targeted therapy approach.[29]

3 Different anatomical GI cancers have a different molecular tumor biology

Different ctDNA/RNA alterations are typically different in different cancer histologies. Unique to GI cancers, beyond the primary tissue-site agnostic MSI and fusion testing, there is a different molecular tumor biology in the different anatomical GI cancers.

The different treatment approaches in right-side versus left-side CRC is not anatomical sidedness driven, but molecular tumor biology driven. Sidedness is a group surrogate for the individual's specific underlying tumor biology. **Right-sided CRC is more frequent MSI-H than left-sided CRC, 22% versus 4%.**[30] There is an important clinical caveat supporting the importance of liquid biopsy plasma NGS testing in CRC. Even when the primary tumor is RAS mutant, if the plasma ctDNA is not demonstrating circulating RAS/RAF ctDNA mutations, there can still be a benefit of anti-EGFR monoclonal antibody therapies.[31] PIK3CA mutations although not directly targetable indicate an OS benefit of simple ASA after standard of care treatment and a more durable benefit with liver metastases radioembolization.[32,33]

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GEJ cancers demonstrating HER2 gene amplification are best treated with anti-HER2 monoclonal antibody and now in combination with immune checkpoint inhibitors (ICI).[34] Plasma ctDNA HER2 copy number can identify effective anti-HER2 therapy.[35] Clinical trials have shown that plasma HER2 amplification overcomes tissue spatial heterogeneity is a better predictor of anti-HER2 /ICI treatment benefit than relying solely on tissue HER2 testing results.[34]

Pancreaticobiliary and small bowel adenocarcinomas are typically fraught with limited tissue for full molecular testing. Biliary tract cancers are especially unique with uniquely specific molecular tumor biology identifiable in ctDNA.[36] Extrahepatic and intrahepatic cholangiocarcinoma carry unique molecular tumor biologies. FGFR1-3 fusions and IDH1/2 mutations are frequent enough to the point of being pathognomonic for an intrahepatic cholangiocarcinoma.[37] Plasma NGS testing can be helpful in diagnostically differentiating an intrahepatic cholangiocarcinoma from a hepatocellular carcinoma and therapeutically guiding treatment for either entity.[38] In the Know Your Tumor pancreatic cancer registry, actionable molecular findings guiding matched **treatment were found in 26% of patients** and a matched treatment approach extended OS by 1 year and improved 3-year OS by an absolute 30% compared to patients not receiving treatment based upon the molecular tumor biology.[8]

Plasma NGS testing of small bowel adenocarcinomas has identified multiple potential therapeutic targets beyond MSI-H.[39] Even in GIST tumors, the specific KIT mutation exon and PDGFRA mutation guides specific TKI treatment sensitivity and resistance. KIT exons 11 and 9 are primary imatinib sensitive. Exon 11 is most durable with exon 9 benefitting from double dose imatinib. However, PDGFRA exon 18 D842V are imatinib resistant yet sensitive to Avapritinib.[40] KIT and PDGFRA negative GIST may have NF1 mutations indicating a genetic syndrome.[41]

Molecular findings in GI neuroendocrine tumors (NET) and carcinomas (NEC) can also be distinct. Plasma ctDNA/RNA alterations were identified in 87% of 320 neuroendocrine neoplasms, including 165 pancreatic NET and 52 GI NEC, sampled patients.[42] These findings can be helpful to discern the underlying tumor biology. KRAS would be far more typical of pancreatic adenocarcinoma than pancreatic NET. TP53, SMAD4, and RB1 mutations are not seen in well-differentiated but are frequent in poorly differentiated cases. Even in GI neuroendocrine malignancies, plasma NGS has a role in diagnosis and treatment.

PD-L1 expression is a predictive immune biomarker in GEJ and biliary tract cancers. Positive plasma cell-free RNA PD-L1 findings can overcome the limitations of necrotic tissue sampling and subsequent tissue PD-L1 protein negative or unknown results.

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4 ctDNA can guide treatment decisions in GI cancers

Plasma NGS can identify targetable oncogene drivers and MSI-H tumor biology guiding first-line treatment. But also, a liquid biopsy identification of any genomic shedding of ctDNA into plasma is a powerful prognostic indicator of the underlying tumor biology aggressiveness for all GI cancers.[43] The more ctDNA mutations being shed into plasma, the more aggressive the tumor biology of that cancer. That can guide potential aggressiveness of what and timing of and multi-disciplinary treatment options.

Plasma ctDNA can also be particularly useful and helpful in monitoring treatment in GI cancers.[4,5] Clearance of baseline ctDNA or a large decrease of the ctDNA variant allele fraction percentage is very predictive of survival and benefit of treatment.[44] Liquid biopsy can enhance therapeutic decision making even when tissue molecular testing has been done.[45] In the proof of principle CHRONOS trial, ctDNA was effectively used to guide rechallenge with panitumumab in metastatic colorectal cancer.[46] Another advantage of plasma NGS ctDNA/RNA testing is not only to monitor treatment response, detect a cancer recurrence or progression, but to identify resistant pathways and identify potential new treatment targets.[47,48]

Plasma NGS can easily provide vital treatment information and guidance, that tissue molecular testing cannot, across the spectrum of treating GI cancers.

Molecular testing impactful in the treatment of earlier stage GI cancers

Molecular tumor biology also matters in earlier stage GI cancers. The proof of principle trials of neoadjuvant immune therapy in MSI-H colorectal and GEJ cancers are just the beginning of the clinical utility and impact of molecular testing in early-stage GI cancers.

Pre- and post-operative plasma NGS testing can guide and monitor neoadjuvant/adjuvant treatment.[49,50] Baseline shedding of ctDNA or post-operative persistence of ctDNA is associated with poorer outcomes in resected GI cancers.[51] Clearance of the baseline shedding ctDNA with neoadjuvant treatment can overcome this poor tumor biology.[5]

1 Tissue-informed liquid biopsy testing is limited by tumor biology

There is a role, albeit limited, for tissue-informed liquid biopsy assays in resected CRC. **A tissue informed liquid biopsy assay is individually developed by identifying mutations in the resected surgical primary tumor specimen.** It is just assessing those primary tumor specific mutations. It is not assessing the clonal evolution that occurs when a cancer recurs or progresses. This has been useful in clinically discerning patients with stage II CRC who need, or who can forego, adjuvant treatment. However, these tissue-informed liquid biopsy assays are limited to that one point in time post-surgical decision making. These assays are not designed to be used in a repeat testing approach. The active clonal evolution with new genomic alterations with cancer recurrence/progression renders the primary tumor mutations irrelevant in this metastatic setting. Nor do they provide precision oncology of new mutations or resistant pathways guiding treatment. In a non-industry supported study comparing a tissue informed liquid biopsy assay to standard CEA and CT monitoring in resected CRC, CEA/CT monitoring outperformed the tissue informed liquid biopsy assay in identifying more recurrences nor did the tissue informed assay identify recurrences any earlier than standard CEA/CT monitoring.[52]

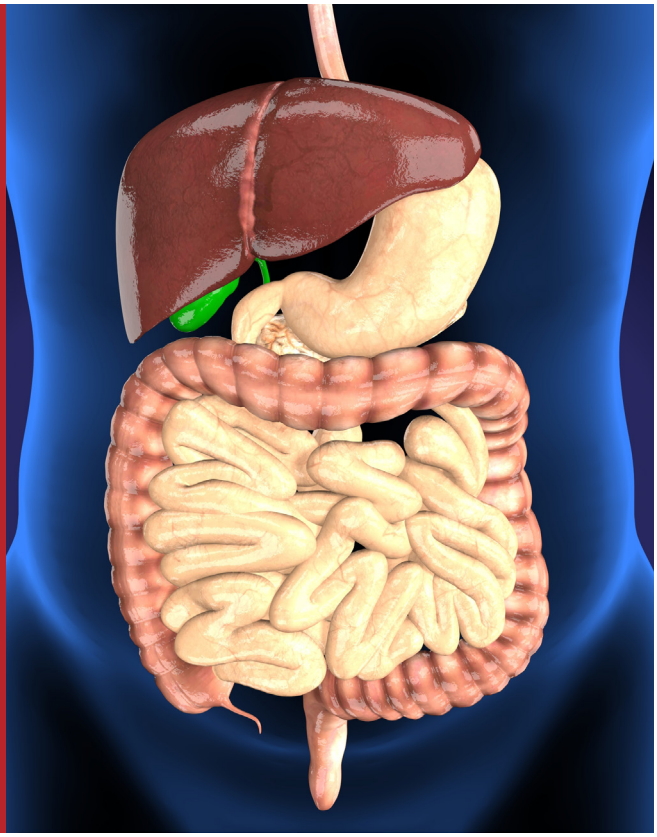
A tissue informed liquid biopsy assay is individually developed by identifying mutations in the resected surgical primary tumor specimen.

Germline testing is underutilized in GI cancers

Germline testing to identify, or exclude, hereditary cancer syndrome remains an underutilized part of GI cancer care. Lynch syndrome with germline defective DNA mismatch repair is the classic hereditary GI cancer syndrome occurring in **3-5%** of CRC patients. **Associated Lynch Syndrome cancers are wide-ranging including endometrial, small intestine, stomach, pancreas, biliary tract, ovary, brain, and upper urinary tract with lifetime risks ranging from 20-70% in CRC and endometrial cancers and lesser 10-15% risks of gastric, ovary and the other extra-colonic cancers.**[53] Finding a Lynch Syndrome impacts management of the individual with a direct therapeutic impact of immune therapy treatment given the resulting MSI-H tumor biology and surveillance follow-up of metachronous CRC and other primaries. There is also a vitally important impact of focused cancer screenings of affected family members.

Lifetime risks ranging from 20-70% in CRC and endometrial cancers

Lesser 10-15% risks of gastric, ovary and the other extra-colonic cancers



Programmatic molecular testing makes a difference across all stages of GI cancers

Implementing a programmatic approach to molecular testing and treatment of GI cancers is a vital foundation for any 'center of excellence' GI cancer program. The survival outcome benefits that precision oncology, immune oncology, and aggressive multi-disciplinary treatment of all anatomical GI cancers provide will be lost if molecular testing is not fully done. With a consistent programmatic approach of molecular testing, all members of the GI cancer program team will know what needs to be done, when it needs to be done, and will ensure it gets done. This will provide the needed molecular tumor biology when treatment discussions and recommendations are being made. A programmatic approach to molecular testing will allow one to see the molecular tumor biology never seen or known before. Just as we think and provide better cancer care and treatment together as a multi-disciplinary team, having a consistent programmatic approach to molecular testing will ensure the needed molecular testing gets done. Without molecular tumor biology findings, precision oncology and personalized cancer treatment does not exist.

Drawing the liquid biopsy for plasma NGS testing at the time of the tissue biopsy ensures the molecular tumor biology is known and available at the time of treatment discussions and decision making. Even though the final stage is frequently unknown at the time of diagnosis of GI cancers, all stages need and can benefit from molecular testing. Any ctDNA in an anatomical early-stage GI cancer indicates a more aggressive tumor biology warranting more aggressive treatment approaches. Just as not fully staging for metastatic disease with PET imaging and locoregional staging with endoscopic ultrasound when indicated, can lead to a wrong treatment and poorer outcome, not knowing the molecular tumor biology of a cancer may miss the right and best multi-disciplinary treatment approach and potentially lead to a wrong treatment and patient survival outcomes suffer.

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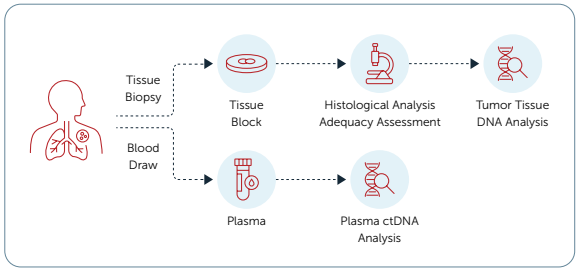
Programmatic plasma NGS testing in GI cancers...

Plasma AND tissue NGS COMPLEMENTARY testing

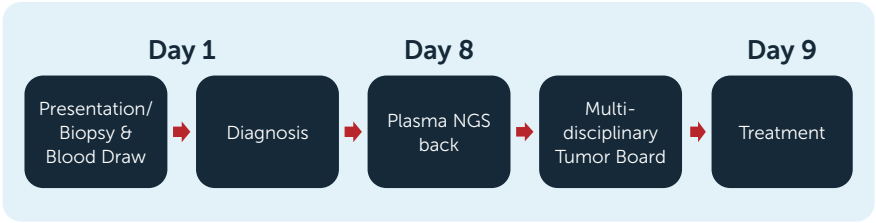


Block sent after cancer DX for tissue NGS testing

3-5 days with pathologist - 3 days to be sent out - tissue NGS 3-week TAT > 30 days

Blood-based Tumor Profiling: Presentation to Treatment

- **TISSUE for DX**
- **Plasma NGS for TUMOR BIOLOGY RX**



References:

1. Arnold M, Abnet C, Neale R, et al. Global Burden of 5 Major Types of Gastrointestinal Cancer. Gastroenterology 2020;159:335-349.
2. Smith S, Wachter K, Burris H, et al. Clinical Cancer Advances 2021: ASCO's Report on Progress Against Cancer. J Clin Oncol 2021; https://doi.org/10.1200/JCO.20.03420.
3. Mukherji R, Yin C, Hameed R, et al. The current state of molecular profiling in gastrointestinal malignancies. Biology Direct 2022;17:15 https://doi.org/10.1186/s13062-022-00322-0
4. Patelli G, Mauri G, Tosi F, et al. Circulating Tumor DNA to Drive Treatment in Metastatic Colorectal Cancer. Clin Cancer Res 2023;29(22):4530-4539.
5. Lee M, Kaseb A, Pant S. The Emerging Role of Circulating Tumor DNA in Non-Colorectal Cancers. Clin Cancer Res 2023;29(17):3267-3274.
6. Andre T, Lonardi S, Wong K, et al. Nivolumab plus low-dose ipilimumab in previously treated patients with microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: 4-year follow-up from CheckMate 142. Ann Oncol 2022;33(10):1052-1060.
7. Hong D, DuBois S, Kummar S, et al. Larotrectinib in patients with TRK fusion-positive solid tumours: a pooled analysis of three phase 1/2 clinical trials. Lancet Oncol 2020;21:531-540.
8. Pishvaian M, Blais E, Brody J, et al. Overall survival in patients with pancreatic cancer receiving matched therapies following molecular profiling: a retrospective analysis of the Know Your Tumor registry trial. Lancet Oncol 2020;21(4):508-518.
9. Chao J, Fuchs C, Shitara K, et al. Assessment of Pembrolizumab Therapy for the Treatment of Microsatellite Instability-High Gastric or gastroesophageal Junction Cancer Among patients in the KEYNOTE-059, KEYNOTE-061, and KEYNOTE-062 Clinical Trials. JAMA Oncol 2023;7(6):895-902.
10. Pietrantonio F, Di Nicolantonio F, Schrock A, et al. ALK, ROS1, and NTRK Rearrangements in Metastatic Colorectal Cancer. J Natl Cancer Inst 2017;109(12):djx089.
11. Corcoran R, Chabner B. Application of Cell-free DNA Analysis to Cancer Treatment. N Engl J Med 2018;379(18):1754-1765.
12. Leighl N, Page R, Raymond V, et al. Clinical Utility of Comprehensive Cell-free DNA Analysis to Identify Genomic Biomarkers in Patients with Newly Diagnosed Metastatic Non-small Cell Lung Cancer. Clin Cancer Res 2019;25(15):4691-4700.
13. Palmero R, Taus A, Viteri S, et al. Biomarker Discovery and Outcomes for Comprehensive Cell-Free Circulating tumor Versus Standard-of-Care Tissue Testing in Advanced Non-Small-Cell Lung Cancer. JCO Precis Oncol 2021;5:93-102.
14. Vidula N, Niemierko A, Malvarosa G, et al. Tumor Tissue- versus Plasma-based Genotyping for Selection of Matched Therapy and Impact on Clinical Outcomes in Patients with Metastatic Breast Cancer. Clin Cancer Res 2021;27:1-10.
15. Gutierrez M, Price K, Lanman R, et al. Genomic Profiling for KRAS, NRAS, BRAF, Microsatellite Instability, and Mismatch Repair Deficiency Among Patients With Metastatic Colon Cancer. JCO Precis Oncol 2019;htpps://doi.org/10.1200/PO.19.00274.

16. Papke D, Lindeman N, Schrag D, et al. Underutilization of Guideline-Recommended Mismatch Repair/Microsatellite Instability Biomarker Testing in Advanced Colorectal Cancer. Cancer Epidemiol Biomarkers Prev 2022;31:1746-1751.

17. Hanna T, King W, Thibodeau S, et al. Mortality due to cancer treatment delay: systemic review and meta-analysis. British Med J 2020;371:m4087.

18. Khorana A, Tullio K, Elson P, et al. Time to initial cancer treatment in the United States and association with survival over time: An observational study. PLoS ONE 2019;14(3):e0213209.

19. Bonneville R, Krook M, Kautto E, et al. Landscape of microsatellite Instability Across 39 Cancer types. JCO Precis Oncol 2017;htpps://doi.org/10.1200/PO.17.00073.

20. Le D, Durham J, Smith K, et al. Mismatch-repair deficiency predicts response of solid tumors to PD-1 blockade. Science 2017;357(6349):409-413.

21. Andre T. Shiu K, Kim T, et al. Pembrolizumab in Microsatellite-Instability-high advanced Colorectal Cancer. N Engl J Med 2020;383:2207-2218.

22. Andre T, Elez E, Van Cutsem E, et al. Nivolumab (NIVO) plus ipilimumab (IPI) vs chemotherapy (chemo) as first0line (1L) treatment for microsatellite instability-high/mismatch repair-deficient (MSI-H/dMMR) metastatic colorectal cancer (CRC): First results of the CheckMate 8HW study. Presented at the 2024 Gastrointestinal Cancers Symposium; San Francisco, CA, January 18-20, 2024. LBA 768.

23. Cercek A, Lumish M, Sinopoli J, et al. PD-1 Blockade in Mismatch Repair-Deficient, Locally Advanced Rectal Cancer. N Engl J Med 2022;386:2363-2376.

24. Emiloju O, Sinicrope F. Neoadjuvant Immune Checkpoint Inhibitor Therapy for Localized Deficient mismatch Repair Colorectal Cancer. JAMA Oncol 2023;9(12):1708-1715.

25. Andre T, Tougeron D, Piessen G, et al. Neoadjuvant Nivolumab Plus Ipilimumab and Adjuvant Nivolumab in Localized Deficient Mismatch Repair/Microsatellite Instability-High Gastric or Esophagogastric Junction Adenocarcinoma; The GERCOR NEONIPIGA Phase II Study. J Clin Oncol 2022;41:255-265.

26. Westphalen C, Krebs M, Le Tourneau C, et al. Genomic context of NTRK1/2/3 fusion-positive tumours from a large real-world population. npj Precis Oncol 2021;5:69.

27. Subbiah V, Cote G. Advances in Targeting RET-Dependent Cancers. Cancer Discov 2020;10:498-505.

28. Cocco E, Benhamida J, Middha S, et al. Colorectal Carcinomas Containing Hypermethylated MLH1 Promoter and Wild-Type BRAF/KRAS Are Enriched for Targetable Kinase Fusions. Cancer Res 2019;79(6):1047-1053.

29. Tabernero J, Ros, J, Elez E. The Evolving Treatment Landscape in BRAF V600E-Mutated Metastatic Colorectal Cancer. 2022 ASCO Educational Book. https://doi.org/10.1200/EDBK_349561.

30. Salem M, Weinberg B, Xiu J, et al. Comparative molecular analysis of left-sided colon, right sided colon, and rectal cancers. Oncotarget 2017;8(49):86536-86368.

31. Bouchahda M, Saffroy R, Karaboue A, et al. Undetectable RAS-Mutant Clones in Plasma: Possible Implication for Anti-EGFR Therapy and Prognosis in Patients With RAS-Mutant Metastatic Colorectal Cancer. JCO Precis Oncol 2020;4:1070-1079.

32. Liao X, Lochhead P, Nishihara R, et al. Aspirin Use, Tumor PIK3CA Mutation, and Colorectal-Cancer Survival. N Engl J Med 2012;367:1596-1606.

33. Ziv E, Bergen M, Yarmohammadi H, et al. PI3K pathway mutations are associated with longer time to local progression after radioembolization of colorectal liver metastases. Oncotarget 2017;8(14):23529-23538.

34. Jannigian Y, Maron S, Chatila W, et al. First-line pembrolizumab and trastuzumab in HER2-positive oesophageal, gastric, or gastro-oesophageal junction cancer: an open-label, single-arm, phase 2 trial. Lancet Oncol 2020;21(6):821-831.

35. Nakamura Y, Okamoto W, Kato T, et al. Circulating tumor DNA-guided treatment with pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer: a phase 2 trial. Nat Med 2021;htpps://doi.org/10.1038/s41591-021-01553-w.

36. Berchuck J, Facchinetti F, DiToro D, et al. The clinical landscape of cell-free DNA alterations in 1671 patients with advanced biliary tract cancer. Ann Oncol 2022;33(12):1269-1283.

37. Goldstein D, Lemech C, Valle J. New molecular and immunotherapeutic approaches in biliary cancer. ESMO Open 2017;2:e000152.

38. Cowzer D, White J, Chou J, et al. Targeted Molecular Profiling of Circulating Cell-Free DNA in Patients With Advanced Hepatocellular Carcinoma. JCO Precis Oncol 2023;7:e2300272.

39. Yu D, Wang J, Zheng B, et al. Comprehensive genomic profiling of small bowel adenocarcinoma by tissue and plasma biopsy. Genomics 2024;116:110766.

40. Banerjee S, Kumar A, Lopez N, et al. Cost-effectiveness Analysis of Genetic Testing and Tailored First-Line Therapy for Patients With Metastatic Gastrointestinal Stromal Tumors. JAMA Network Open 2020;3(9):e2013565.

41. Gasparotto D, Rossi S, Polano M, et al. Quadruple-negative GIST Is a Sentinel for Unrecognized Neurofibromatosis type 1 Syndrome. Clin Cancer Res 2016;23(1):273-282.

42. Zakka K, Nagy R, Drusbosky L, et al. Blood-based next generation sequencing analysis of neuroendocrine neoplasms. Oncotarget 2020; 11(19):1749-1757.

43. Vu P, Khagi Y, Riviere P, et al. Total Number of Alterations in Liquid Biopsies Is an Independent Predictor of Survival in Patients With Advanced Cancers. JCO Precis Oncol 2020;4:192-201.

44. Urbini M, Marisi G, Azzali I, et al. Dynamic Monitoring of Circulating Tumor DNA in Patients With metastatic Colorectal Cancer. JCO Precis Oncol 2023;7:e2200694.

45. Manca P, Corallo S, Busico A, et al. The Added Value of Baseline Circulating Tumor DNA Profiling in Patients with molecularly Hyperselected, leftsided metastatic Colorectal Cancer. Clin Cancer Res 2021;27(9):2505-2514.

46. Sartore-Bianchi A, Pietrantonio F, Lonardi S, et al. Circulating tumor DNA to guide rechallenge with panitumumab in metastatic colorectal cancer: the phase 2 CHRONOS trial. Nat Med 2022;28: 1612-1618.

47. Parikh A, Leshchiner I, Elagina L, et al. Liquid versus tissue biopsy for detecting acquired resistance and tumor heterogeneity in gastrointestinal cancers. Nat Med 2019;25:1415-1421.

48. Hsiehchen D, Bucheit L, Yang D, et al. Genetic features and therapeutic relevance of emergent circulating tumor DNA alterations in refractory non-colorectal gastrointestinal cancers. Nat Comm 2022;13:7477.

49. Kotani D, Oki E, Nakamura Y, et al. Molecular residual disease and efficacy of adjuvant chemotherapy in patients with colorectal cancer. Nat med 2023;29:127-134.

50. Morris V, George T. using Circulating Tumor DNA for Colon Cancer Adjuvant Therapy: To Be or not to Be? Clin Cancer Res 2022;28(3):438-440.

51. Hsu P, Singh K, Dhiman A, et al. Utility of Perioperative measurement of Cell-free DNA and Circulating Tumor DNA in Informing the Prognosis of GI Cancers: A Systematic Review. JCO Precis Oncol 2022; https://doi.org/10.1200/PO.21.00337

52. Fakih M, Sandhu J, Wang C, et al. Evaluation of Comparative Surveillance Strategies of Circulating Tumor DNA, Imaging, and Carcinoembryonic Antigen Levels in Patients with Resected Colorectal Cancer. JAMA Network Open 2022;5(3):e221093.

53. Lynch H, Snyder C, Shaw T, et al. Milestones of Lynch Syndrome: 1895-2015. Nat Rev Cancer 2015;15:181-194.