Submitted by: Mark D. Hiatt, MD, MBA, MS
Company: Guardant Health *(505 Penobscot Drive, Redwood City, CA 94063)*
Contact: mhiatt@guardanthealth.com; 903-343-1188

Date of request: August 8, 2021

Guidelines Panels: *Gastric Cancer* and *Esophageal and Esophagogastric Junction Cancers*[1]

FDA status: Guardant Health's **Guardant360 CDx** is the first plasma-based comprehensive genomic profiling (CGP) test approved by the FDA to profile tumor mutations in all solid malignant neoplasms[2,3,4] and serve as a companion diagnostic (CDx) for osimertinib, sotorasib, and amivantamab-vmjw in non-small cell lung cancer (NSCLC) and is also certified, accredited, and approved by the Clinical Laboratory Improvement Act, College of American Pathologists, and New York State Department of Health, respectively.

On behalf of Guardant Health, I thank the *Gastric Cancer* and *Esophageal and Esophagogastric Junction Cancers* Panels and staff for their rapid and thorough updates to the Guidelines, which incorporate the best and latest science pertaining to treatment selection.

In our common interest in updating these Guidelines, I respectfully request that the Panel consider the following suggestions (also incorporated in the table below):

1. **Next-generation sequencing (NGS) as a first-tier test at diagnosis**. Please consider recommending a well validated NGS assay, including plasma-based assays such as Guardant360 CDx and Guardant360, within the main algorithms (GAST-9 and ESOPH-10 and 19) to be considered at the diagnosis of advanced disease for the evaluation of biomarkers. Removing the requirement of exhausting tissue testing via sequential methods aligns with other guidelines, increases access to complete genotyping, and enables characterization of the primary and metastatic sites with results in only 7 to 10 days (in the case of Guardant360 assays).[3]

2. **Liquid biopsy as a tool to profile more patients**. Please consider expanding this use of a well validated circulating tumor DNA (ctDNA) assay beyond "patients who are unable to undergo a traditional biopsy" to give physicians greater autonomy in their choice of testing modality. In patients whose diagnosis is already established by histopathology, plasma-based testing may obviate the need for a repeat invasive biopsy *even for patients who are able to undergo one*, as Guardant360 has been shown to identify targetable alterations at similar rates with similar matched therapy outcomes as tissue-based profiling.[5,6,7,8,9,10,11,12,13,14,15,16] In this setting, tissue can be saved for testing that requires it (like PD-L1 or diagnostic stains).

Requests and **Rationale**:

Page	**Revision** (suggested in ***blue***, focused on *gastric* cancer, but applicable to *esophageal*)	**Rationale**
GAST- 9 ESOPH-10 and 19	• ~~*If sufficient tissue is available after the above testing has been completed,*~~ *NGS may be considered via tissue or a well validated plasma assay*	Due to spatial and temporal tumor heterogeneity, both tissue testing (via PCR/IHC/NGS) and ctDNA have limitations and incomplete sensitivity in cancer of the stomach and esophagus. As tissue testing may be a barrier due to biopsy of multiple sites and demands on tissue for histologic and PD-L1 analysis, oncologists benefit from the choice to pursue liquid biopsy at diagnosis or progression to obtain genotyping results as fast as 7 days while tissue is used for other important tests.

[1] NCCN Clinical Practice Guidelines in Oncology, *Gastric Cancer* and *Esophageal and Esophagogastric Junction Cancers*, both v. 4.2021 – August 3, 2021.
[2] Correspondence dated August 7, 2020, to Guardant Health from the FDA.
[3] https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpma/pma.cfm?id=P200010, accessed August 25, 2020.
[4] https://www.fda.gov/news-events/press-announcements/fda-approves-first-liquid-biopsy-next-generation-sequencing-companion-diagnostic-test, accessed August 12, 2020.
[5] Willis J, Lefterova MI, Artyomenko A, et al. Validation of Microsatellite Instability Detection Using a Comprehensive Plasma-Based Genotyping Panel. *Clin Cancer Res* 2019;25(23):7035-45.
[6] Catenacci DVT, Kang Y-K, Park H, et al. Margetuximab plus pembrolizumab in patients with previously treated, HER2-positive gastro-oesophageal adenocarcinoma (CP-MGAH22–05): a single-arm, phase 1b–2 trial. *Lancet Oncol* 2020;21:1066-76.
[7] Kim ST, Banks KC, Pectasides E, et al. Impact of genomic alterations on lapatinib treatment outcome and cell-free genomic landscape during HER2 therapy in HER2+ gastric cancer patients. *Ann Oncol* 2018;29:1037-48.
[8] Pectasides E, Stachler MD, Derks S, et al. Genomic heterogeneity as a barrier to precision medicine in gastroesophageal adenocarcinoma. *Cancer Discov* 2018;8:37-48.
[9] Janjigian YY, Maron SB, Chatila WK, 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):821-31.
[10] Kim ST, Banks KC, Lee S-H, et al. Prospective Feasibility Study for Using Cell-Free Circulating Tumor DNA–Guided Therapy in Refractory Metastatic Solid Cancers: An Interim Analysis. *JCO Precis Oncol* 2017;(1):1-15.
[11] Parikh AR, Leshchiner I, Elagina L, et al. Liquid versus tissue biopsy for detecting acquired resistance and tumor heterogeneity in gastrointestinal cancers. *Nat Med* 2019;25:1415–21.
[12] Maron SB, Chase LM, Lomnicki S, et al. Circulating Tumor DNA Sequencing Analysis of Gastroesophageal Adenocarcinoma. *Clin Cancer Res* 2019;25(23):7098-112.
[13] Drilon A, Laetsch TW, Kummar S, et al. Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. *N Engl J Med* 2018;378(8):731-9.
[14] Hong DS, Bauer TM, Lee JJ, et al. Larotrectinib in adult patients with solid tumours: a multi-centre, open-label, phase I dose-escalation study. *Ann Oncol* 2019;30(2):325-31.
[15] Kato S, Okamura R, Baumgartner JM, et al. Analysis of Circulating Tumor DNA and Clinical Correlates in Patients with Esophageal, Gastroesophageal Junction, and Gastric Adenocarcinoma. *Clin Cancer Res* 2018;24(24):6248-56. doi:10.
[16] Kim ST, Cristescu R, Bass AJ, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. *Nat Med* 2018;24(9):1449-58.

		Guardant360 assays are highly sensitive and specific with high concordance to tissue for biomarkers such as HER2, *NTRK* fusions, and MSI. FDA Summary of Safety and Effectiveness Data demonstrate that when compared to an externally validated NGS assay, Guardant360 CDx had a positive percent agreement (PPA) of 88% and negative percent agreement (NPA) of 100% for HER2 amplifications among 356 patients with advanced solid cancers, and a 100% PPA and NPA for *NTRK1* fusions in the same cohort.[3] Guardant360 has reliably identified patients with *NTRK* fusions likely to respond to larotectinib.[13,14] The clinical utility of liquid biopsy in gastroesophageal cancer is known, as a response rate of 67% and disease control rate of 100% were achieved in patients with advanced gastric cancer treated as directed by Guardant360 alone without tissue.[10] In 1,630 patients with advanced gastric or esophageal cancer, Guardant360 identified more actionable, characterized alterations than tissue testing did in the primary and metastatic tumors.[12] Plasma ctDNA has the added benefit for patients with metastatic disease of capturing heterogeneity when the primary tumor and its metastases differ in HER2 expression, a known phenomenon characterized by the use of a ctDNA such as Guardant360. Discordant heterogeneity between the primary and metastasis is shown for gastroesophageal adenocarcinoma (GEA), and Guardant360 reliably detects targetable metastatic mutations (87.5%), obviating the need for tissue sampling or biopsy.[8]
GAST-B *3 of 6* ESOPH-B *3 of 6*	Assessment of Overexpression or Amplification of HER2 in Gastric Cancer For patients with inoperable locally advanced, recurrent, or metastatic adenocarcinoma of the stomach for whom trastuzumab[e] therapy is being considered, assessment for tumor HER2 overexpression using immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) or other in situ hybridization (ISH) method is recommended.[4] NGS, via plasma ("liquid biopsy") or tissue, offers the opportunity to assess numerous mutations simultaneously, along with other molecular events such as amplification, deletions, tumor mutation burden, and microsatellite instability status. NGS can be considered instead of sequential testing for single biomarkers ~~when limited diagnostic tissue is available or when the patient is unable to undergo a traditional biopsy.~~, especially by means of a comprehensive panel that has been validated in gastric cancer.	The 2017 ASCO/CAP/ASCP *HER2* testing guidelines specifically pertain to IHC and FISH methodologies performed on tissue specimen(s). These guidelines issued no recommendation related to molecular genomic testing, as in 2016 evidence was unavailable. Robust evidence supports that a well validated plasma-based NGS panel, such as Guardant360, can reliably detect *HER2* amplifications and mutations identifying patients who may benefit from targeted therapies. Comparable outcomes for HER2 amplifications detected by NGS performed on plasma (as in "liquid biopsy") have been reported. In advanced GEA, HER2 amplification detected by Guardant360 was associated with improved overall response to trastuzumab vs. HER2 negative patients. Loss of HER2 was a common post-treatment phenomenon detectable by Guardant360 and not requiring post-progression biopsy.[6] ctDNA and metastatic lesion concordance for ERBB2 was 100%.[15]
GAST-B *3 of 6* ESOPH-B *3 of 6*	Assessment of Overexpression or Amplification of HER2 in Gastric Cancer ~~It should be noted that NGS has several inherent limitations and thus whenever possible, the use of gold standard assays (IHC/ISH) should be performed first and if sufficient tissue is available, additional NGS testing may be considered.~~	Neither IHC/ISH nor NGS via tissue have 100% sensitivity in detecting advanced cancer of the stomach or esophagus due to many biological limitations. Thus, the statement about the limitations of NGS should be removed or clarified with a discussion of limitations associated all modalities. Failure to include NGS via plasma ctDNA may limit the number of patients completely genotyped and guided thereby to targeted therapies and clinical trials.
	Or consider this replacement: Biomarker testing may be considered at clinical or radiological progression for patients with advanced/metastatic gastric cancer.	Post-progression biopsy is difficult to mandate in clinical practice and ERBB2 amplification by ctDNA could be used during second-line treatment to reassess HER2 status.[6]
GAST-B *4 of 6* ESOPH-B *4 of 6*	Microsatellite Instability (MSI) or Mismatch Repair (MMR) Testing[h] • Universal testing for MSI by polymerase chain reaction (PCR), next generation sequencing (NGS), or MMR by IHC should be performed for all newly	Willis et al. validated Guardant360 against 1,145 tissue-matched pan-cancer patients for MSI status of various modalities: PCR, NGS, and IHC. Results demonstrated high analytical performance, overall accuracy of 98.4%, and diagnostic accuracy of 87% for MSI-High and 99.5% for MSI-stable. Notably, several

	diagnosed gastric cancers.[5] The testing is performed on formalin-fixed, paraffin-embedded (FFPE) tissue and results are interpreted as MSI-high (MSI-H) or mismatch repair-deficient (dMMR) in accordance with CAP DNA Mismatch Repair Biomarker Reporting Guidelines.[6] Testing via a well validated plasma circulating tumor DNA ("liquid biopsy") assay may be considered.	patients with MSI-High tumors were missed by IHC, but detected by Guardant360. Outcomes data for immunotherapy in gastric MSI-High by cfDNA (63% (10/16) patients achieved complete or partial remission w/ sustained clinical benefit.[5]
GAST-B *5 of 6* ESOPH-B *5 of 6*	Next-Generation Sequencing (NGS): ~~It should be noted that NGS has several inherent limitations and thus whenever possible, the use of gold standard assays (IHC/ISH/targeted PCR) should be performed and if sufficient tissue is available, additional NGS testing may be considered.~~ *Or consider this addition:* A negative tissue result should be interpreted with caution, as such does not exclude the presence of tumor mutations or amplifications.	Neither IHC/ISH, nor NGS via tissue have 100% sensitivity in clinical practice. Thus, this statement should be removed or clarified with a discussion of the limitations associated with each modality. Failure to include NGS via plasma ctDNA may limit the number of patients completely genotyped and thereby guided to targeted therapies and clinical trials.
GAST-B *5 of 6* ESOPH-B *5 of 6*	Liquid Biopsy[7,8] • The genomic alterations of solid cancers may be identified by evaluating circulating tumor DNA (ctDNA) in the blood, hence a form of "liquid biopsy." Liquid biopsy is being used more frequently in patients with advanced disease, particularly who are unable to have a clinical biopsy for disease surveillance and management. The detection of mutations/alterations in DNA shed from gastric carcinomas can identify targetable alterations or the evolution of clones with altered treatment response profiles. Therefore, for patients who have metastatic or advanced gastric cancer ~~and are unable to undergo a traditional biopsy~~, testing using a validated NGS-based comprehensive genomic profiling assay performed on plasma in a CLIA-approved laboratory may be considered. A negative result should be interpreted with caution, as this does not exclude the presence of tumor mutations or amplifications.	As high concordance, sensitivity, and specificity are demonstrated in well validated ctDNA assays such as Guardant360, utilization of liquid biopsy in lieu of tissue testing is a valid approach, particularly in a metastatic setting in which ctDNA may result in enhanced detection of heterogeneous drivers. Further, Guardant360 is concordant with tissue-based MSI and TMB in metastatic gastric cancer, obviating need for biopsy to detect patients eligible for pembrolizumab.[16]

Summaries of **Relevant Studies**:

- In a prospective clinical utility study of 86 patients with GEA, 56% had HER2 amplification at baseline by Guardant360. HER2+ patients had better response compared to HER2- patients (OR 7.3). Thirty-five (42%) of 83 patients had no detectable HER2 amplification by ctDNA post-treatment by trastuzumab, suggesting HER2 loss at baseline for the treatment studied, margetuximab and pembrolizumab. Presence of HER2 amplification by ctDNA at time of initiating second-line study treatment was associated with improved clinical outcomes compared with absence of HER2 amplification. The authors suggest that post-progression biopsy is difficult to mandate in clinical practice and ERBB2 amplification by ctDNA could be used during second-line treatment to reassess HER2 status.[6]

- In a prospective study evaluating the efficacy of combining lapatinib with capecitabine and oxaliplatin in untreated HER2 amplified/overexpressing gastric cancers, patients were also assessed with Guardant360 and tumor NGS assays. Given high plasma-to-tissue concordance for key driver alterations, plasma-based ERBB2 amplification was a robust predictor of response to lapatinib plus chemotherapy in line with previous studies on MET and FGFR2 inhibitor in gastric cancer.[7]

- In a retrospective multicenter study evaluating the extent of genomic heterogeneity in patients with newly diagnosed metastatic GEA, prior to receipt of systemic therapy, tissue and ctDNA were evaluated. In 11 patients, tissue whole-exome sequencing in the primary tumor and distant metastasis revealed significant discordance in mutations (42% discordant) and amplifications (63%). Striking heterogeneity within the primary tumor was also noted, when multiple areas were assessed (a rarity in clinical practice). In five discordant cases of primary tumor negative and metastasis positive, Guardant 360 identified all alterations present in the metastatic lesion, all of which were targeted in the PANGEA trial. Correlation of genomic alterations between tumors and ctDNA (Guardant360) was also evaluated. In 87.5% of cases in which the primary tumor was negative and metastasis was positive, Guardant360 also identified the alterations. This study reported high concordance of ctDNA profiling, with 17 out of 20 (85%) targetable gene amplifications (MET, ERBB2, FGFR2, *EGFR*, and

KRAS) in the metastasis detected in ctDNA. Two clinical cases highlight how ctDNA may identify missed opportunities to treat metastatic gastroesophageal cancer, and the challenge in using primary tumor tissue as the gold standard in concordance studies of ctDNA.[8]

- In a prospective multiple-parallel-cohort, open-label clinical trial using Guardant360-guided matched therapy when tissue was insufficient or unobtainable for NGS, 76 patients with gastric cancer were evaluated. ctDNA was detected in 78% (59/76), of which 33% (25 of 76) had actionable mutations. The overall distribution of alterations was similar between tumor-tissue sequencing in the Cancer Genome Atlas. Among patients with gastric cancer treated based on ctDNA results, a response rate of 67% and disease control rate of 100% were observed. This prospective ctDNA-guided molecular testing program is the first of its type with objective response evaluated in solid tumors. This program guided patients in whom biopsy was not readily available or in whom tumor material was not sufficient for comprehensive sequencing to genomically matched therapies available in practice or clinical trials. The authors concluded "among patients with insufficient tumor tissue for sequencing, ctDNA testing can be a feasible option to guide molecularly matched therapy."[10]

- In a retrospective multi-center study of 1,630 patients with GEA on whom Guardant360 was performed, the genomic alteration landscape was described and additional analysis of ctDNA, primary tumor tissue, and metastatic tumor tissue concordance/heterogeneity was performed. CtDNA identified more actionable, characterized alterations than did tissue testing in the primary and metastatic tumors. Discordance was moderate, as expected for GEA due to spatial and temporal heterogeneity (see Figures 4A and B). The authors concluded "Combining tissue-NGS and ctDNA-NGS increased sensitivity for detection of HER2, EGFR, FGFR2, and MET alterations (Figure 4C). This conclusion highlights the complementary benefit of using ctDNA-NGS together with tissue-NGS to overcome the inherent false-negative rates of either test, either due to spatial heterogeneity (inherent to tissue) or technical shedding limitations (as pertaining to ctDNA).[12]

- In an independent, prospective trial, 55 pediatric and adult patients with *NTRK* fusions identified by tissue or Guardant360 in a pan-cancer basket trial were targeted with larotrectinib. The response rate was 75% (95% confidence interval, 61 to 85), according to independent review. Twenty-nine had a complete response rate. In addition to detecting the initial *NTRK* driver fusions, Guardant360 detected on-target resistance mutation that may be theoretically overcome with a next-generation TRK inhibitor in clinical trials.[13]

- In a multicenter, phase 1 dose-escalation study of larotrectinib, 70 patients were enrolled, including eight with *NTRK*-fusion-positive tumors to receive six dose cohorts. Larotrectinib demonstrated activity in all patients with *NTRK* fusions.[14]

- In an independent retrospective study of 55 patients with GEA with Guardant360, 76% of patients had relevant ctDNA detected. Of characterized alterations, 97% were considered by the authors potentially actionable with agents approved by the FDA or with ones in clinical trials. Tissue NGS was performed in 31 patients. Concordance studies in GEA are expected to be less than 100% due to known heterogeneity between the tumor and its metastasis and as response to treatment. The overall concordance rate for the four common alterations was 76.5%. Notably, ctDNA and metastatic lesion concordance for ERBB2 was 100%. In terms of clinical outcomes, overall survival was worse when ERBB2 alterations were present and the percentage of ctDNA was greater than 2.3%.[15]

Thank you for considering these suggestions.

Sincerely,

Mark D. Hiatt, MD, MBA, MS
Vice President, Medical Affairs | Guardant Health