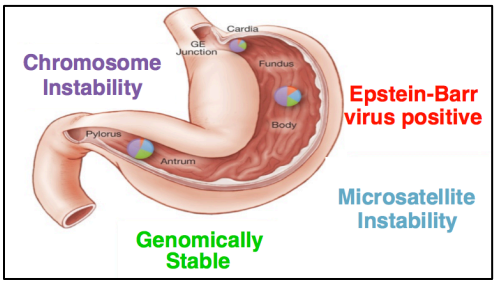
## GastroGenus Gastric Cancer Classifier (v2)

The UNC Molecular Genetics Laboratory offers genomic analysis of paraffin embedded tumor tissue to classify gastric adenocarcinoma in a manner that assists with selection of experimental therapy.

**Rationale for testing:** The Cancer Genome Atlas Network study reveals gastric adenocarcinoma is not one disease but rather is comprised of four major classes: *Epstein-Barr Virus-positive (EBV), Microsatellite Instability (MSI), Genomically Stable, and Chromosome Instability.* Somatic gene variants may influence response to targeted therapy. Clinical trials are available for EBV-positive cancers, MSI cancers, and for cancers having evidence of signalling pathway activation (e.g. *PIK3CA* or *ERBB2* mutation*).*




The *GastroGenus Gastric Cancer Classifier* uses massive parallel sequencing to interrogate selected regions of 26 human genes, along with Q-PCR measurement of Epstein-Barr virus DNA, and a test for *MLH1* CpG methylation status. Results may qualify a patient for experimental targeted therapy, and tumor markers may be helpful in evaluating response to intervention.

**Clinical Indications for GastroGenus Gastric Cancer Classifier (v2):** Patient with recurrent or metastatic gastric adenocarcinoma, including gastroesophageal junction cancer, in whom knowledge of pertinent gene variants would facilitate patient management and inform options for clinical trial enrollment

**Specimen Requirements**:
Primary or metastatic gastric adenocarcinoma tissue is suitable (formalin fixed, paraffin embedded biopsy or resection, or fine needle aspirate cell block; avoid acid decalcification rather make alcohol-fixed touch preps of bony mets). Prepare 18 unstained sections on plain, uncoated glass slides (5 to 10 uM thick). An accompanying H&E stained slide is reviewed by a pathologist who circles areas for dissection (>2mm square having >20% malignant cells). A copy of the surgical pathology report is requested.

**Method:**
Amplicons are generated using reagents in the TruSight Tumor Sequencing Panel (Illumina) that are then sequenced on a Miseq. *MLH1* promoter methylation status is tested by pyrosequencing bisulfite-treated DNA. EBV DNA is measured by Q-PCR, and *EBER in situ* hybridization is done to localize EBV infection if indicated. Results are interpreted by a pathologist in the context of clinicopathologic features.

**Gene regions sequenced:**

AKT1 exon 2
ALK exon 23
APC exon 15
BRAF exon 11
BRAF exon 15
CDH1 exon 8
CDH1 exon 9
CDH1 exon 12
CTNNB1 exon 2
EGFR exons 18-21
ERBB2 exon 20
FBXW7 exons 7-11
FGFR2 exon 6
FOXL2 exon 1
GNAQ exons 4-6
GNAS exon 6
GNAS exon 8
KIT exon 9
KIT exon 11
KIT exon 13
KIT exon 17
KIT exon 18
KRAS exons 1-4
MAP2K exon 2
MET exon 1
MET exon 4
MET exon 13
MET exon 15
MET exon 16
MET exon17
MET exon 18
MET exon 20
MSH6 exon 5
NRAS exons 1-4
PDGFRA exon 12
PDGFRA exon 14
PDGFRA exon 18
PIK3CA exon 1
PIK3CA exon 2
PIK3CA exon 7
PIK3CA exon 9
PIK3CA exon 20
PTEN exons 1-7
PTEN exon 9
SMAD4 exon 8
SMAD4 exon 11
SRC exon 10
STK11 exon 1
STK11 exon 4
STK11 exon 6
STK11 exon 8
TP53 exons 2-11

**Limitations:** The assay is sensitive to most gene variants at >10% mutant allele frequency. Gene amplification, translocation, or insertion or deletion over 25 bases in length, are not detected. Variants predicted to be non-deleterious (such as synonymous coding changes and common population variants) are not reported. Normal tissue is not tested to prove whether a variant is somatic (acquired) or germline (heritable). Genetic counseling is recommended if the patient has evidence of a heritable cancer syndrome (e.g. more than one tumor type, early age of onset, family history); to make a patient appointment call the UNC Cancer Genetics Clinic at (919) 843-8724.

***Valid clinical uses:**

***PIK3CA* mutation -** Detect *PIK3CA* mutation to predict drug response or to evaluate options for clinical trial enrollment (e.g. NCT01613950, NCT01226316, NCT01708161, NCT01928459, NCT01449370). Related PI3K pathway inhibitor trials are available (e.g. for *PIK3CA, AKT1, STK11* or *PTEN* mutation).

***EBV*-positive -** Assign EBV status of the malignancy for prognosis and to explore options for clinical trial enrollment (e.g. viral lytic induction therapy: NCT00982449, NCT02080416)

***MLH1* methylated -** Assign *MLH1* promoter methylation status of the malignancy for response to 5-FU and to explore options for clinical trial enrollment (e.g. PD-1 antibody pembrolizumab in NCT01876511; PARP inhibitor veliparib in NCT01264432)

***TP53 mutated -*** Detect *TP53* mutation to explore options for clinical trial enrollment (e.g. NCT02042989)

***ERBB2 (Her2)* mutation -** Detect *ERBB2* kinase domain (exon 20) mutation to predict drug efficacy and to evaluate options for clinical trial enrollment (e.g. NCT01602406). Optional ERBB2 (Her2) histochemistry is available upon request (overexpression by immunohistochemistry, and/or FISH; requires 4 extra 5uM paraffin sections) to qualify patients for standard of care Her2-targeted therapy.

***KRAS* mutation –** Evaluate options for clinical trials (e.g. NCT02022982)

**Prognosis -** Emerging data suggests a worse prognosis for "*Chromosome Instability*" class, or mutated *KRAS* or *NRAS* in proximal stomach cancer; and a better prognosis for "*Microsatellite Instability*" class or for *PIK3CA* mutation in intestinal histology.

**References:**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2. The Cancer Genome Atlas Network. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; 513: 202-9.
3. Gulley ML. Genomic Assays for Epstein-Barr Virus-Positive Gastric Adenocarcinoma. Experimental and Molecular Medicine, 47:e134, 2015. PMID: 25613731
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6. An JY, Kim H, Cheong JH, Hyung WJ, Kim H, Noh SH. Microsatellite instability in sporadic gastric cancer: its prognostic role and guidance for 5-FU based chemotherapy after R0 resection. Int J Cancer. 2012; 131:505-11. PMID:21898388
7. Yan M, et al. HER2 aberrations in cancer: implications for therapy. Cancer Treat Rev. 2014 40:770-80, PMID: 24656976
8. Warneke VS, Behrens HM, Haag J, Balschun K, Böger C, Becker T, Ebert MP, Lordick F, Röcken C. Prognostic and putative predictive biomarkers of **gastric** cancer for personalized medicine. Diagn Mol Pathol. 2013, 22(3):127-37. PMID:23846438
9. clinicaltrials.gov


**To consult a pathologist** about indications for testing or the significance of a result, call the Molecular Genetics Lab at (984) 974-1825 or Dr. Gulley at (919) 843-4595. Email: margaret_gulley@med.unc.edu

Website, http://www.uncmedicalcenter.org/uncmc/professional-education-services/mclendon-clinical-laboratories/directory/molecular-pathology-and-genetics