

# SOLID TUMOR ANCILLARY TESTING

## **BREAST SERVICE:**

NOTE: For all metastatic breast cases, please give the case to breast service (WW breast service or SM breast attendings) to sign out the biomarkers. The case can be signed out by the original service (according to organ), and then given to breast service to put an addendum for biomarkers.

<b>Primary Breast</b>	<b>Immunohistochemistry</b>	<b>FISH</b>
All primary carcinomas (including metaplastic)	ER, PR, HER2, Ki-67	HER2
Ductal carcinoma in situ (DCIS)*	ER, PR*	

<b>Metastatic Breast</b>	<b>Immunohistochemistry</b>	<b>FISH</b>
All breast carcinomas (including metaplastic)	ER, PR, HER2, Ki-67	HER2

\*Performed only on resection/excision specimen

- Smart text: .breastbiomarkers

## **LUNG SERVICE**

<b>Primary Lung</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
Adenocarcinoma	PD-L1, ALK	None	EGFR (Idylla), Tempus (xT)
Squamous cell carcinoma	PD-L1		Tempus (xT)
Mesothelioma	PD-L1, BAP1		
Large cell, LCNEC, or any other type of rare carcinoma	PD-L1, ALK	None	EGFR (Idylla), Tempus (xT)
Carcinoid tumors	None	None	None
Small cell carcinoma	None	None	None
<b>Metastatic Lung</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
All carcinomas	PD-L1 (Clinician request)	per request	per request

- Smart text: .IMPDL1

## **GENITOURINARY SERVICE**

<b>Metastatic Tumors</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
Urothelial carcinoma	PD-L1		
<b>pT2 Tumors or Above</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
Urothelial carcinoma	PD-L1		

- Smart text: .IMPDL1GU

## **GASTROINTESTINAL SERVICE**

<b>Primary Colorectal</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
Adenocarcinoma (Resection)	MMR (does not need to be repeated if performed on initial biopsy specimen) <sup>2</sup>		MSI <sup>1</sup>
Adenocarcinoma (Biopsy)	MMR (Perform on all cases if adequate tissue present) <sup>2</sup>		
<b>Metastatic Colorectal</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
Adenocarcinoma	HER2*, MMR <sup>1</sup>		KRAS*

<b>Primary Pancreas</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
Ductal adenocarcinoma			
<b>Metastatic Pancreaticobiliary</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
Metastatic pancreatic ductal adenocarcinoma	MMR <sup>1, 3</sup>		
Metastatic cholangiocarcinoma	MMR <sup>1, 3</sup>		
<b>GI Stromal Tumor &amp; Neuroendocrine</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
GI neuroendocrine tumor (all sites, including metastasis)	Ki-67		
Primary GIST (all sites)			PDGFRA/c-kit
<b>Primary Small Bowel</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
Adenocarcinoma (biopsy and resection)	MMR <sup>3</sup>		
<b>Primary Gastric</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
Adenocarcinoma (biopsy and resection)	HER2, PD-L1 <sup>^</sup> , MMR <sup>3</sup>	HER2 (only if IHC is 2+)	
<b>Primary GEJ</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
Adenocarcinoma (biopsy and resection)	HER2, PD-L1 <sup>^</sup> , MMR <sup>3</sup>	HER2 (only if IHC is 2+)	
<b>Primary Esophageal</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
Locally advanced (inoperable), recurrent, or metastatic adenocarcinoma	HER2, PD-L1, MMR <sup>3</sup>	HER2 (only if IHC is 2+)	
Squamous cell carcinoma	PD-L1, MMR <sup>3</sup>		
<b>Primary Ampulla</b>	<b>Immunohistochemistry</b>	<b>FISH</b>	<b>Molecular</b>
Adenocarcinoma (biopsy and resection)	CK20, CDX2, MUC1 and MUC2, MMR <sup>3</sup>		

\* If KRAS result is wild type, order reflex CRC Panel sequencing analysis (BRAF, KRAS, NRAS, PIK3CA, and AKT1). In addition, order reflex HER2 IHC. Order HER2 FISH if IHC score is 2+.

<sup>1</sup> All metastatic GI malignancies (including pancreaticobiliary malignancies) are being tested for MMR deficiency by IHC given the approval of PD1 inhibitors in all GI cancers that are MSI-H

<sup>1</sup> MSI by PCR is indicated if MMR IHC results are equivocal or questionable. If IHC has already been done on a biopsy and a normal expression pattern is observed, MSI PCR will not be performed on the resection specimen from the same patient **UNLESS**:

- 1) Patient age under 50
- 2) Personal hx of Lynch-related tumor(s) – may need to be informed by clinicians
- 3) Family hx of CRC or Lynch syndrome – may also need to be informed by clinicians
- 4) Histologic features suggestive of MSI on resection specimens (mucinous, poorly differentiated, medullary, tumor infiltrating lymphocytes, Crohn-like peritumoral lymphoid response)

<sup>2</sup> BRAF mutational analysis is indicated for cases with loss of MLH1 expression.

<sup>3</sup> MLH1 promoter methylation test is indicated for cases with loss of MLH1 expression. Please email [SurgicalPathologySendouts@mednet.ucla.edu](mailto:SurgicalPathologySendouts@mednet.ucla.edu) to order the test

<sup>^</sup>Use the PD-L1 stain Smart text: .PDLGEJ, can be used for all GI PD-L1 IHC reports (CPS)

c-KIT/PDGFR $\alpha$  is a send out test. Please email [SurgicalPathologySendouts@mednet.ucla.edu](mailto:SurgicalPathologySendouts@mednet.ucla.edu) to order the test (see “Ancillary Send Out Tests” below).

Smart phrase for reporting in Beaker is .HER2CRC

**HER2 IHC TESTING FOR COLORECTAL CARCINOMA (MODIFIED FROM HERACLES CRITERIA)<sup>a</sup>**

Staining	Pattern	Interpretation	FISH	Eligibility to HERACLES trial
No staining (0)	-	Negative	No	No
Faint staining (1+), any cellularity	Any	Negative	No	No
Moderate (2+), <50% cells	Any	Negative	No	No
Moderate (2+), $\geq$ 50% cells	Circumferential, basolateral, or lateral	Equivocal	Yes	Yes if amplified
Intense (3+), $\leq$ 10% cells	Circumferential, basolateral, or lateral	Negative	No	No
Intense (3+), >10% <50% cells	Circumferential, basolateral, or lateral	Equivocal <sup>b</sup>	Yes	Yes if amplified
Intense (3+), $\geq$ 50% cells	Circumferential, basolateral, or lateral	Positive	No <sup>c</sup>	Yes

<sup>a</sup> Valtorta E, et al. Assessment of a HER2 scoring system for colorectal cancer: results from a validation study. Mod Pathol. 2015;28(11):1481-91.

<sup>b</sup> This was interpreted as “positive” in the above publication, but could be confusing because FISH is required for this category. It is better to interpret it as “equivocal”.

<sup>c</sup> In the above publication, FISH is not mandatory for this category, but recommended for research purposes.

**DERMATOLOGY SERVICE**

<u>Tumor</u>	<u>Immunohistochemistry</u>	<u>FISH</u>	<u>Molecular</u>
Melanoma			BRAF (per request)
Metastatic melanoma			BRAF (per request)

## GYN SERVICE

<b>Primary Ovarian</b>	<b>Immunohistochemistry</b>	<b>FISH</b>
Endometrioid and clear cell and other uterine carcinomas	MMR*, ER, PR, p53	
Most high grade carcinomas	p53, WT1, p16, ER, PR	

<b>Metastatic Ovarian</b>	<b>Immunohistochemistry</b>	<b>FISH</b>
Endometrioid and clear cell type carcinomas	MMR, ER, PR (per clinician request and if not performed on primary)	
All high grade carcinomas (includes MMMT)	p53, ER, PR (per clinician request and if not performed on primary)	

<b>Primary Uterine</b>	<b>Immunohistochemistry</b>	<b>FISH</b>
All endometrial carcinomas	MMR, ER, PR (MMR performed on endometrial biopsy or curettage specimens for <b>all</b> endometrial cancer results, when adequate tissue available)	
Uterine high grade serous carcinoma	p53, WT1, p16, ER, PR	Her2/Neu

<b>Metastatic Uterine</b>	<b>Immunohistochemistry</b>	<b>FISH</b>
All endometrial carcinomas	MMR, ER, PR (per clinician request and if not performed on primary)	
All high grade carcinomas (includes MMMT)	HER2, p53 (per clinician request and if not performed on primary)	HER2 (per clinician)

<b>Primary Cervical</b>	<b>Immunohistochemistry</b>	<b>FISH</b>
Squamous cell carcinoma	PD-L1 (at the time of diagnosis) P16, Ki-67, and HPV ISH (if needed)	
Adenocarcinoma	PD-L1 (at the time of diagnosis) P16, Ki-67, and HPV ISH (if needed)	

<b>Metastatic Cervical</b>	<b>Immunohistochemistry</b>	<b>FISH</b>
Squamous cell carcinoma	P16, Ki-67, and HPV ISH (if needed)	
Adenocarcinoma	P16, Ki-67, and HPV ISH (if needed)	

<b>Uterine leiomyoma</b>	<b>Immunohistochemistry</b>	<b>FISH</b>
Leiomyomas with characteristic morphologic features in patients of any age <sup>+</sup> (see two references)	Fumarate Hydratase (FH)	
All leiomyoma in young patients (under 35)	Fumarate Hydratase (FH)	

\* Performed on excision/resection only, unless requested by clinician to be performed on initial biopsy specimen.

- MLH1 promoter hypermethylation studies are ordered for cases with loss of MLH1 expression. Please email [SurgicalPathologySendouts@mednet.ucla.edu](mailto:SurgicalPathologySendouts@mednet.ucla.edu) to order the test (see below).

<sup>+</sup> References:

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4830748/>

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5106328/>

## NEUROPATHOLOGY SERVICE

<u>Tumor</u>	<u>Immunohistochemistry</u>	<u>FISH</u>	<u>Molecular</u>
<b>GBM (Grade IV)</b>	GFAP, Ki67, IDH1 R132H, ATRX (Add Olig2 if concerned for PNET or ependymoma)	PTEN, EGFR	MGMT methylation IDH1/2#
<b>Infiltrating glioma (Grade II, III)</b>	GFAP, Ki67, IDH1 R132H, ATRX, p53	1p/19q, CDKN2A (if astrocytic)	MGMT methylation
<b>Midline glioma</b> (Infiltrating glioma in thalamus, cerebellum, brainstem, spinal cord)	H3K27M and H3K27me3 (in addition to infiltrating glioma workup, see above)		
<b>Pilocytic astrocytoma</b>	BRAF V600E, GFAP, Ki-67, neurofilament, synaptophysin, IDH1 R132H	BRAF duplication	
<b>Ganglioglioma</b>	BRAF V600E, GFAP, Ki-67, neurofilament, synaptophysin		
<b>Pleomorphic xanthoastrocytoma</b>	BRAF V600E, GFAP, Ki-67, neurofilament, synaptophysin		
<b>Pituitary adenoma</b>	IM PIT panel (LH, FSH, TSH, GH, prolactin, ACTH, Ki-67) *		
<b>Chordoma</b>	S100, Ker AE1/3, Brachury		
<b>Meningioma vs Solitary Fibrous Tumor/Hemangiopericytoma</b>	EMA, SSTR2A, STAT6, Ki67 (Classic or usual meningioma does <b>not</b> require these stains)		
<b>Medulloblastoma</b> ^	Beta catenin, synaptophysin, GFAP, Ki67, YAP1, GAB1, INI1	N-MYC, C-MYC	
<b>Ependymoma</b>	GFAP, EMA, Ki67 if classic (neurofilament, EMA, CD99, Olig2 if distinguishing from astrocytic tumors). H3K27me3 in posterior fossa		

# if IDH1 IHC is negative and patient is less than 54 years old, order IDH1/IDH2 PCR unless Foundation Medicine genomic profiling is ordered.

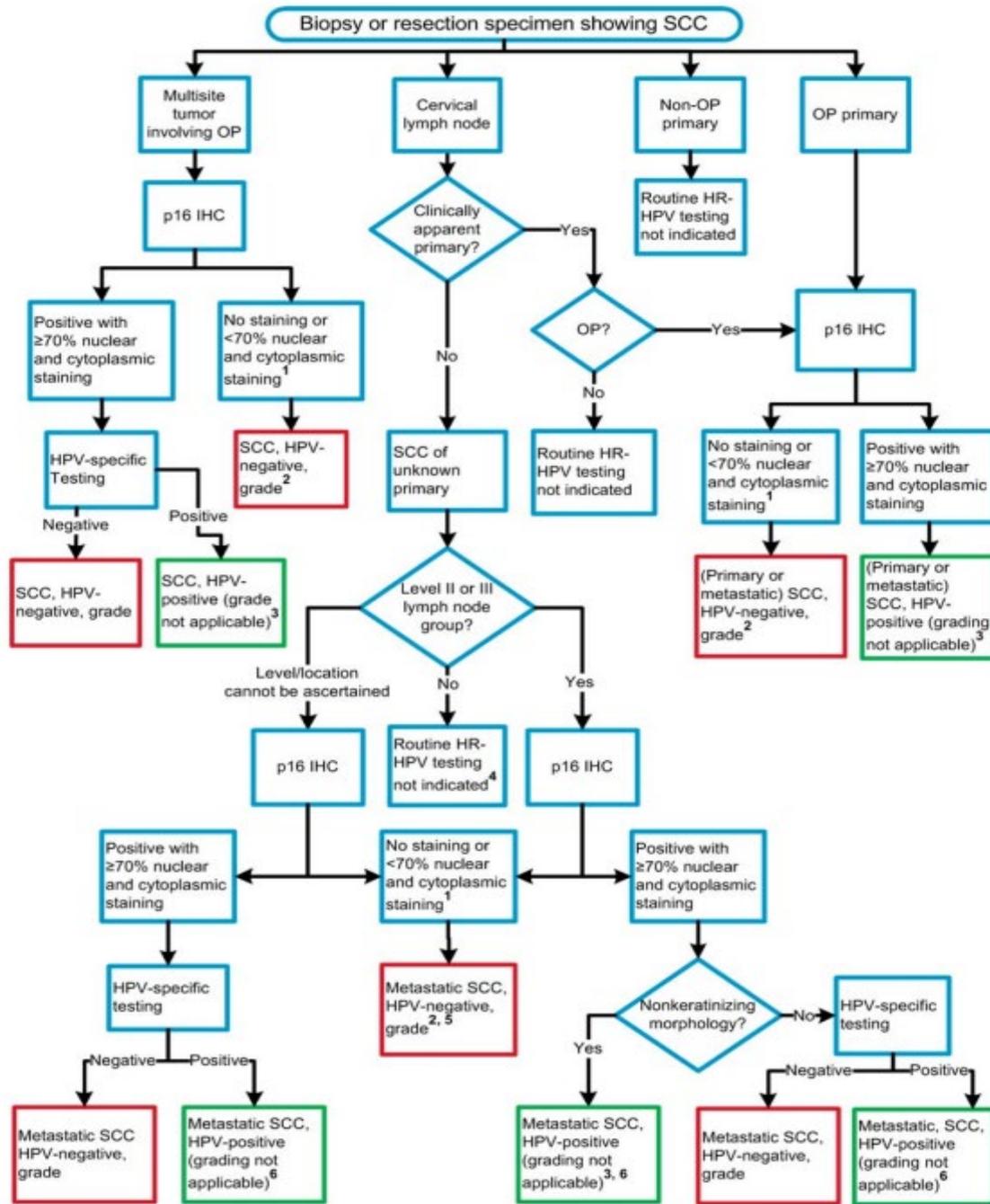
\* PIT1, SF1, synaptophysin IHC if hormone stains from the initial hormone panel are negative

^ If nodular/desmoplastic variant suspected add reticulin special stain.

^ Add INI1 IHC if AT/RT is suspected or less than 10 years old; Foundation Medicine genomic profiling via Neurooncology recommended.

MGMT methylation studies is a send out test. Please email [SurgicalPathologySendouts@mednet.ucla.edu](mailto:SurgicalPathologySendouts@mednet.ucla.edu) to order the test (see below).

# HEAD AND NECK SERVICE: p16/HPV ISH staining flow chart



**Figure 1.** High-risk human papillomavirus (HR-HPV) testing in head and neck squamous cell carcinomas (SCCs). Abbreviations: IHC, immunohistochemistry; OP, oropharyngeal. <sup>1</sup>Consider HR-HPV-specific testing for equivocal p16 results (50%–70% nuclear and cytoplasmic staining). <sup>2</sup>May also be reported as p16 negative with a comment specifying that the tumor is very likely HPV negative. <sup>3</sup>May also be reported as p16 positive with a comment specifying that the tumor is very likely HPV positive. <sup>4</sup>HR-HPV may be indicated in patients where the clinical suspicion for an HPV-positive SCC is high. <sup>5</sup>Consider Epstein-Barr encoding region (EBER) in situ hybridization for Epstein-Barr virus for the rare metastatic nonkeratinizing squamous cell carcinoma that is HR-HPV negative. <sup>6</sup>Include comment, “Likely oropharyngeal primary.”

## **ANCILLARY SEND OUT TESTS:**

<b><u>Test Name</u></b>	<b><u>Department</u></b>	<b><u>Location</u></b>
MGMT (Molecular)	NP	NeoGenomics
BRAF V600E (Molecular)	NP	NeoGenomics
BRAF Rearrangement FISH for PA	NP	NeoGenomics
MLH1 Promoter Methylation (Molecular)	GYN	NeoGenomics
cKIT w/reflex to PDGFRa	GI/BST	UW
cKIT for Melanoma	GI/Derm	UW or NeoGenomics
Gene Trails Panel for Hematologic Malignancies	HemePath	NeoGenomics
Iron Quant	Liver	Mayo
Copper Quant	Liver	Mayo
Amyloid Protein ID	H/L, GI/Liver	Mayo
PLA2R IF	Renal	Mayo
Alport Staining for Collagen IV	Renal	Dr. Laura Flynn, Seattle Children's
AFB, Bacterial, Fungal Broad-range PCR	All	Univ. of Washington
MAML2 FISH	Head/Neck	Mayo
MYD88	HemePath	NeoGenomics

For all send out tests please e-mail [SurgicalPathologySendouts@mednet.ucla.edu](mailto:SurgicalPathologySendouts@mednet.ucla.edu) with the required test, case number and case block. Please check with them if a circled H&E slide is required.