

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 give to breast service to put an addendum for biomarkers.

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

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

*Performed only on resection/excision specimen

- Smart text: .breastbiomarkers

GASTROINTESTINAL SERVICE

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

Primary Pancreas	Immunohistochemistry	FISH	Molecular
Ductal adenocarcinoma			

Metastatic Pancreaticobiliary	Immunohistochemistry	FISH	Molecular
Metastatic pancreatic ductal adenocarcinoma	MMR ¹		
Metastatic cholangiocarcinoma	MMR ¹		

GI Stromal Tumor & Neuroendocrine	Immunohistochemistry	FISH	Molecular
GI neuroendocrine tumor (all sites, including metastasis)	Ki-67		
Primary GIST (all sites)			PDGFRA/c-kit

Primary Gastric	Immunohistochemistry	FISH	Molecular
Adenocarcinoma	HER2, PD-L1 [^]	HER2 (only if IHC is 2+)	
For all <u>untreated</u> adenocarcinoma (biopsy and resection)	Add MMR ¹		

Primary GEJ	Immunohistochemistry	FISH	Molecular
Adenocarcinoma	HER2, PD-L1 [^]	HER2 (only if IHC is 2+)	
For locally advanced (inoperable), recurrent, or metastatic adenocarcinoma	Add MMR		

Primary Esophageal	Immunohistochemistry	FISH	Molecular
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Locally advanced (inoperable), recurrent, or metastatic adenocarcinoma	HER2, PD-L1, MMR	HER2 (only if IHC is 2+)	
Squamous cell carcinoma (by request)	PD-L1, MMR		

Primary Ampulla	Immunohistochemistry	FISH	Molecular
Adenocarcinoma (resection)	CK7, CK20, CDX2, MUC1 and MUC2		

* 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+.

[†] 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

¹ 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)

² BRAF mutational analysis is indicated for cases with loss of MLH1 expression.

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

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

LUNG SERVICE

Primary Lung	Immunohistochemistry	FISH	Molecular
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
Metastatic Lung	Immunohistochemistry	FISH	Molecular
All carcinomas	PD-L1 (Clinician request)	per request	per request

- Smart text: .IMPD1

GENITOURINARY SERVICE

Metastatic Tumors	Immunohistochemistry	FISH	Molecular
Urothelial carcinoma	PD-L1		
pT2 Tumors or Above	Immunohistochemistry	FISH	Molecular
Urothelial carcinoma	PD-L1		

- Smart text: .IMPD1GU

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

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

<u>Metastatic Ovarian</u>	<u>Immunohistochemistry</u>	<u>FISH</u>
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)	

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

<u>Metastatic Uterine</u>	<u>Immunohistochemistry</u>	<u>FISH</u>
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)

<u>Primary Cervical</u>	<u>Immunohistochemistry</u>	<u>FISH</u>
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)	

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

<u>Uterine leiomyoma</u>	<u>Immunohistochemistry</u>	<u>FISH</u>
Leiomyomas with characteristic morphologic features in patients of any age ⁺ (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 to order the test (see below).

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

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

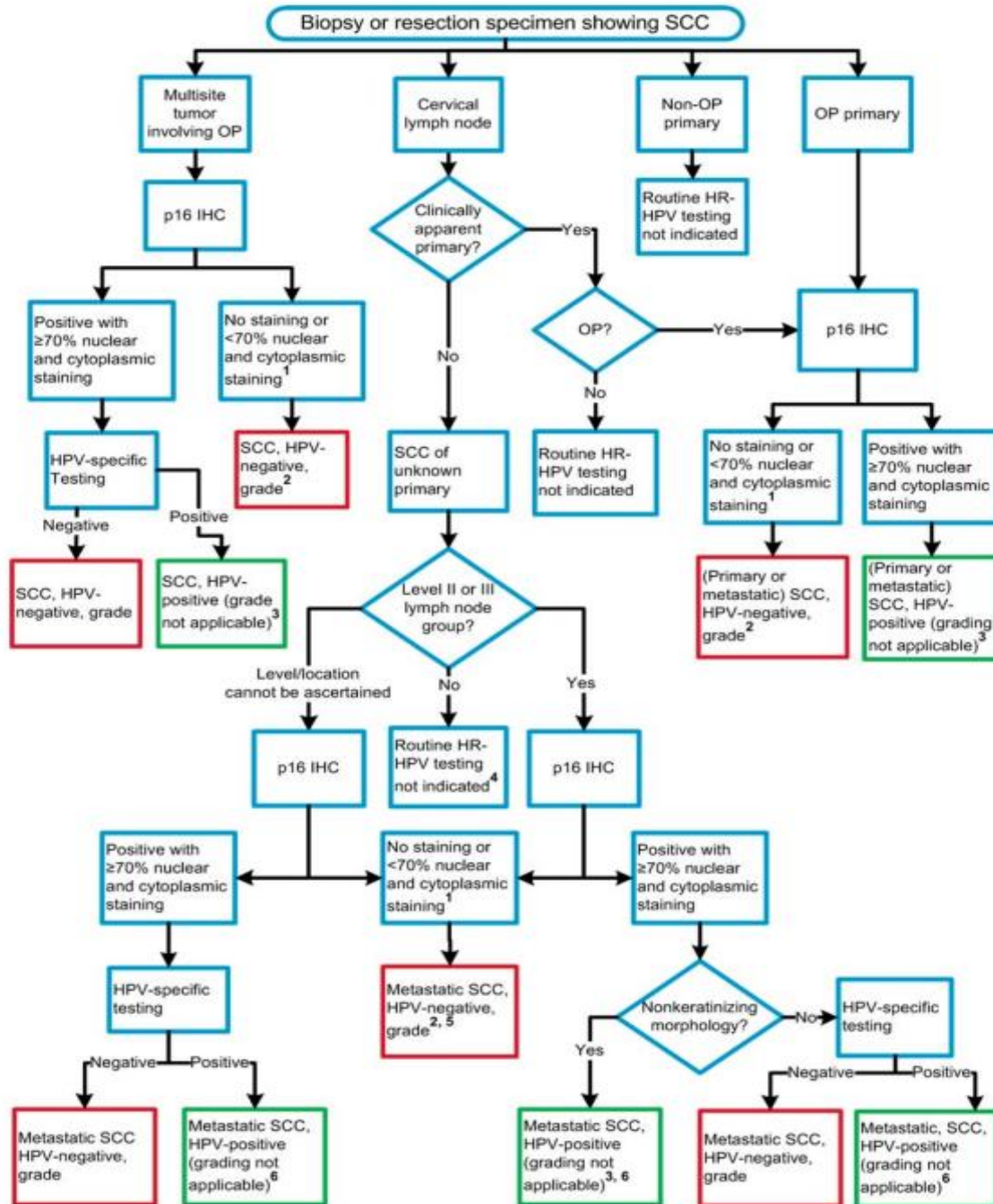


Figure 1. High-risk human papillomavirus (HR-HPV) testing in head and neck squamous cell carcinomas (SCCs). Abbreviations: IHC, immunohistochemistry; OP, oropharyngeal. ¹Consider HR-HPV-specific testing for equivocal p16 results (50%–70% nuclear and cytoplasmic staining). ²May also be reported as p16 negative with a comment specifying that the tumor is very likely HPV negative. ³May also be reported as p16 positive with a comment specifying that the tumor is very likely HPV positive. ⁴HR-HPV may be indicated in patients where the clinical suspicion for an HPV-positive SCC is high. ⁵Consider 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. ⁶Include comment, "Likely oropharyngeal primary."

ANCILLARY SEND OUT TESTS:

<u>Test Name</u>	<u>Department</u>	<u>Location</u>
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 with the required test, case number and case block. Please check with them if a circled H&E slide is required.