UCDAVIS HEALTH SYSTEM

M. Tomic, M.D.
Department of Pathology and Laboratory Medicine
University of California Davis Medical Center

A HEALTHIER WORLD THROUGH BOLD INNOVATION

Screening for Lynch Syndrome in Endometrial Carcinoma: Practical Considerations

Financial Disclosures

- NONE!

Lynch Syndrome: Definition

- Autosomal dominant genetic disorder that confers an increased risk of cancer
  - 2-3% of endometrial carcinomas are Lynch syndrome associated
  - In women, endometrial cancer and colorectal cancer are equally likely to be the sentinel cancer

Lynch Syndrome: Genetics

- Germline mutation that leads to loss of function of a mismatch repair (MMR) protein

Lynch Syndrome: Mismatch Repair

- The MMR system recognizes and repairs base pair mismatches. The consequence of impaired MMR is an **increased mutation rate** with biallelic loss.
  - Recognition
    - MutS-alpha heterodimer: **MSH2** and **MSH6**
    - MutS-beta heterodimer: MSH2 and MSH3
  - Repair
    - MutL-alpha heterodimer: **MLH1** and **PMS2**
    - MutL-beta heterodimer: MLH1 and PMS1
    - MutL-gamma heterodimer: MLH1 and MSH3
  - **EpCAM**
    - Upstream from MSH2, large deletions in 3' end cause silencing of MSH2

Lynch Syndrome: Molecular Phenotype

- Microsatellite instability
  - MSI testing
- Loss of protein expression
  - Immunohistochemistry

Lynch Syndrome: Molecular Phenotype

- Microsatellite instability
  - Lynch syndrome carcinomas show high frequency microsatellite instability (**MSI-H**)
  - Microsatellites are regions of repetitive nucleotide sequences
  - These regions are particularly prone to DNA mismatches
  - Mismatch leads to lengthening or shortening of the microsatellite

Lynch Syndrome: Molecular Phenotype

- Microsatellite instability
  - Testing employs PCR to amplify regions containing microsatellites in tumor *and* normal tissue. Amplification products are separated by capillary electrophoresis
    - MSI-High is defined by instability in ≥30% of examined microsatellites (or 2 loci in a 5 marker panel)
    - MSI-Low is defined by instability of <30% of examined microsatellites
    - MSS (Microsatellite stable) is defined by no instability

Not All MSI-H Tumors are Lynch Associated!!!

- Sporadic MSI-H colorectal and endometrial carcinomas
  - Silencing of MLH1 due to promoter methylation
  - Sporadic MSI-H colorectal carcinomas
    - BRAF V600E present in sporadic but ***not*** Lynch associated carcinomas
  - Sporadic MSI-H endometrial carcinomas
    - Lack the BRAF V600E mutation so must assess for promoter methylation

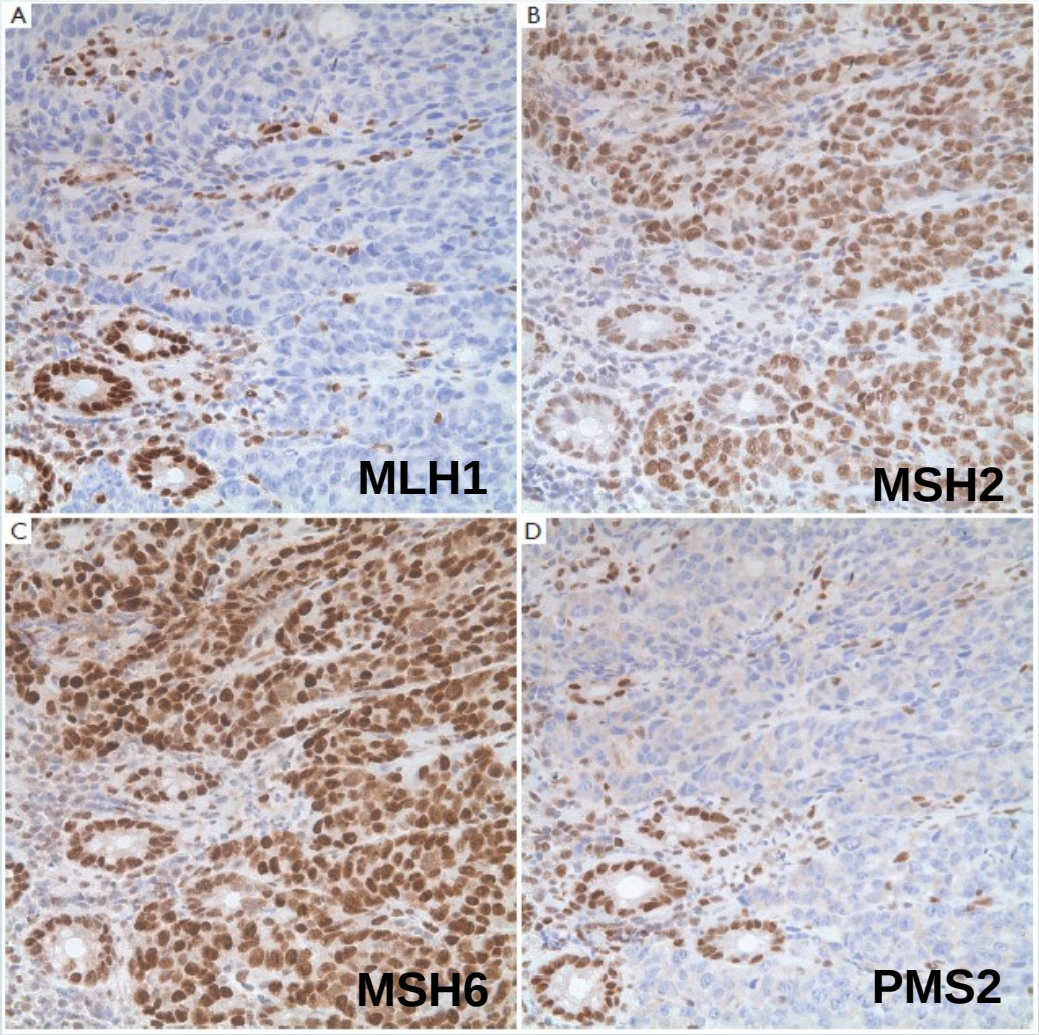
MSI Caveats

- **MSS and MSI-L can be seen in cases with germline mutation of MSH6**
- **Sensitivity is likely affected by the composition of microsatellite markers used**
  - **Mononucleotide repeats are more sensitive**

Lynch Syndrome: Molecular Phenotype

- Loss of protein expression
  - Detected by immunohistochemistry for MLH1, PMS2, MSH2, MSH6 performed on tumor
    - Four patterns
      - No staining for MLH1 and PMS2 due to loss of MLH1
      - No staining for PMS2 due to loss of PMS2
      - No staining for MSH2 and MSH6 due to loss of MSH2 (or EPCAM)
      - No staining for MSH6 due to loss of MSH6

Lynch Syndrome: Molecular Phenotype



Colonic adenocarcinoma:
IHC pattern consistent with loss of MLH1

Fleming M. et al. Journal of Gastrointestinal Oncology 2012;3:153-173.

MMR IHC Caveats

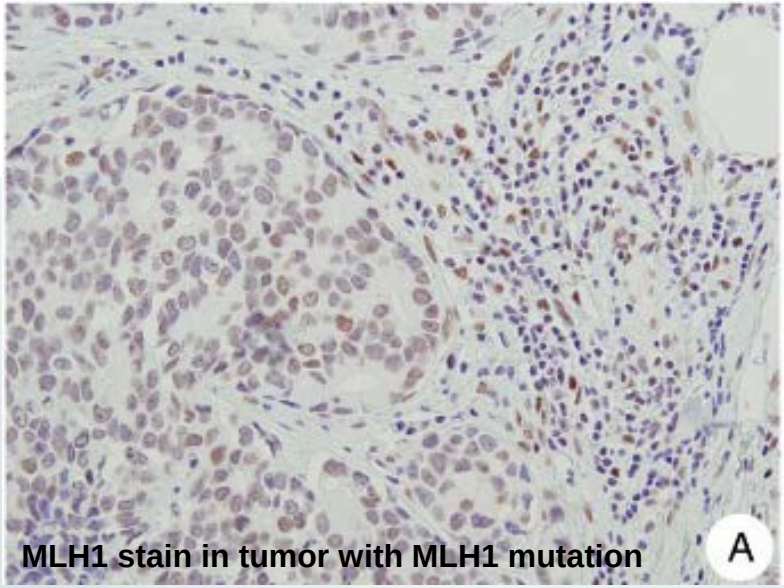
- Lack of clearly defined and agreed upon criteria for interpretation
- United Kingdom National External Quality Assessment Service: 2008 Recommendations
  - ***Normal***: similar staining intensity in tumor and internal control
  - ***Abnormal***: no staining in tumor with positive staining in internal control, ***or*** patchy or weak staining in tumor compared to internal control

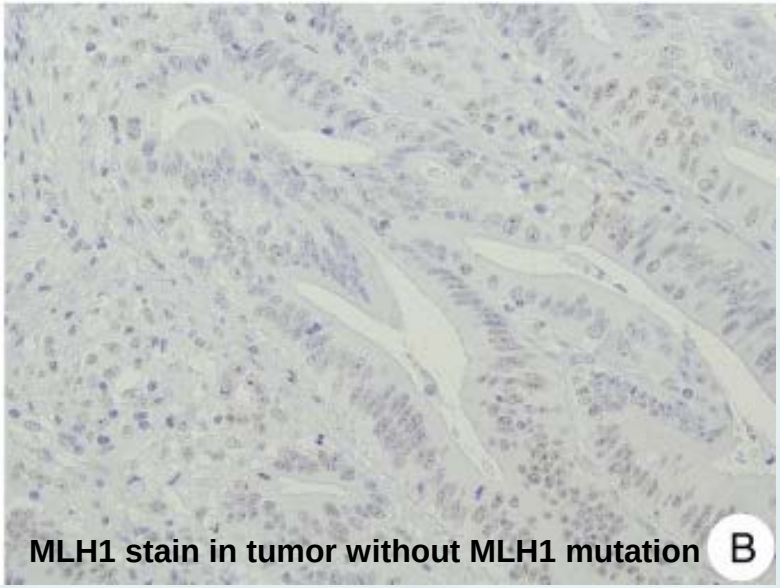
MMR IHC Caveats

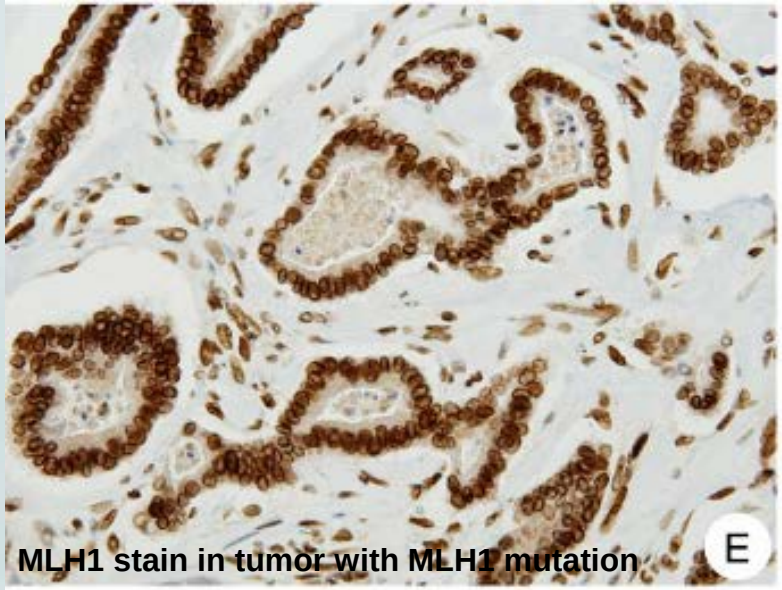
	Overbeek et al. 2008 % of cases with consensus interpretation (5/7 pathologists)	Klarskov et al. 2010 % of cases with consensus interpretation (5/6 pathologists)
MLH1	89%	83%
PMS2	96%	91%
MSH2	93%	94%
MSH6	87%	76%

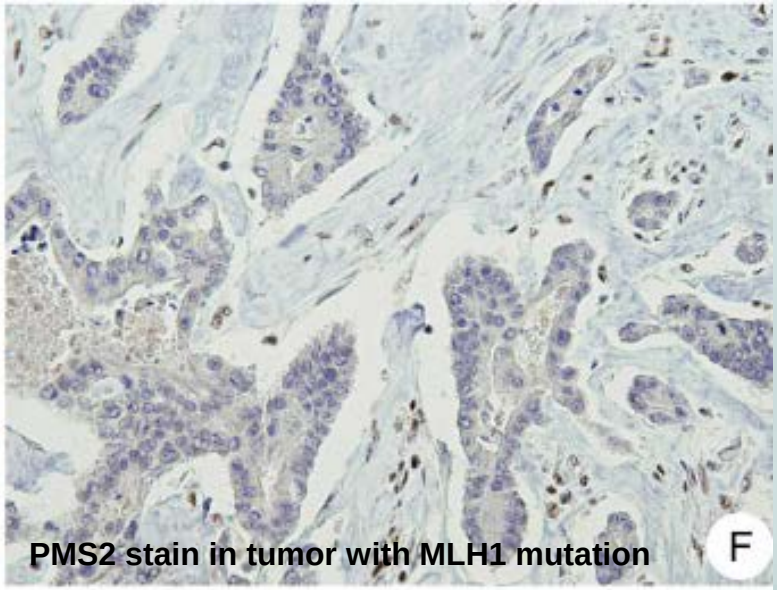
- Klarskov et al. identified weak staining as primary cause of lack of consensus
- Stratified weak staining into
  - Technically weak – weak staining in tumor and internal control
  - Biologically weak – weaker staining in tumor compared to control
    - 19 tumors showed a biologically weak pattern, 8 carried a disease predisposing mutation

Overbeek J. et al. Human Pathology 2008;39:116-125.
Klarskov et al. Human Pathology 2010;41:1387-1396.









Klarskov et al. Human Pathology 2010;41:1387-1396.

Findings from the EGAPP (Evaluation of Genomic Applications in Practice and Prevention) Working Group

- Clinical Validity
  - MSI
    - MLH1 or MLH2
      - Sensitivity 80-91%, Specificity 90%
    - MSH6 or PMS2
      - Sensitivity 55-77%, Specificity 90%
  - IHC
    - Sensitivity 83%, Specificity 89%
    - Regardless of MMR protein
  - BRAF V600E
    - Absent in virtually 100% of Lynch syndrome individuals
    - Present in 68% of individuals without Lynch syndrome

EGAPP Working Group. Genetics in Medicine 2009;11:35-41.

Identification of Lynch-Related Carcinomas

- Targeted vs. universal screening?
- Which screening strategies to implement?

Lynch Syndrome: Clinical Phenotype

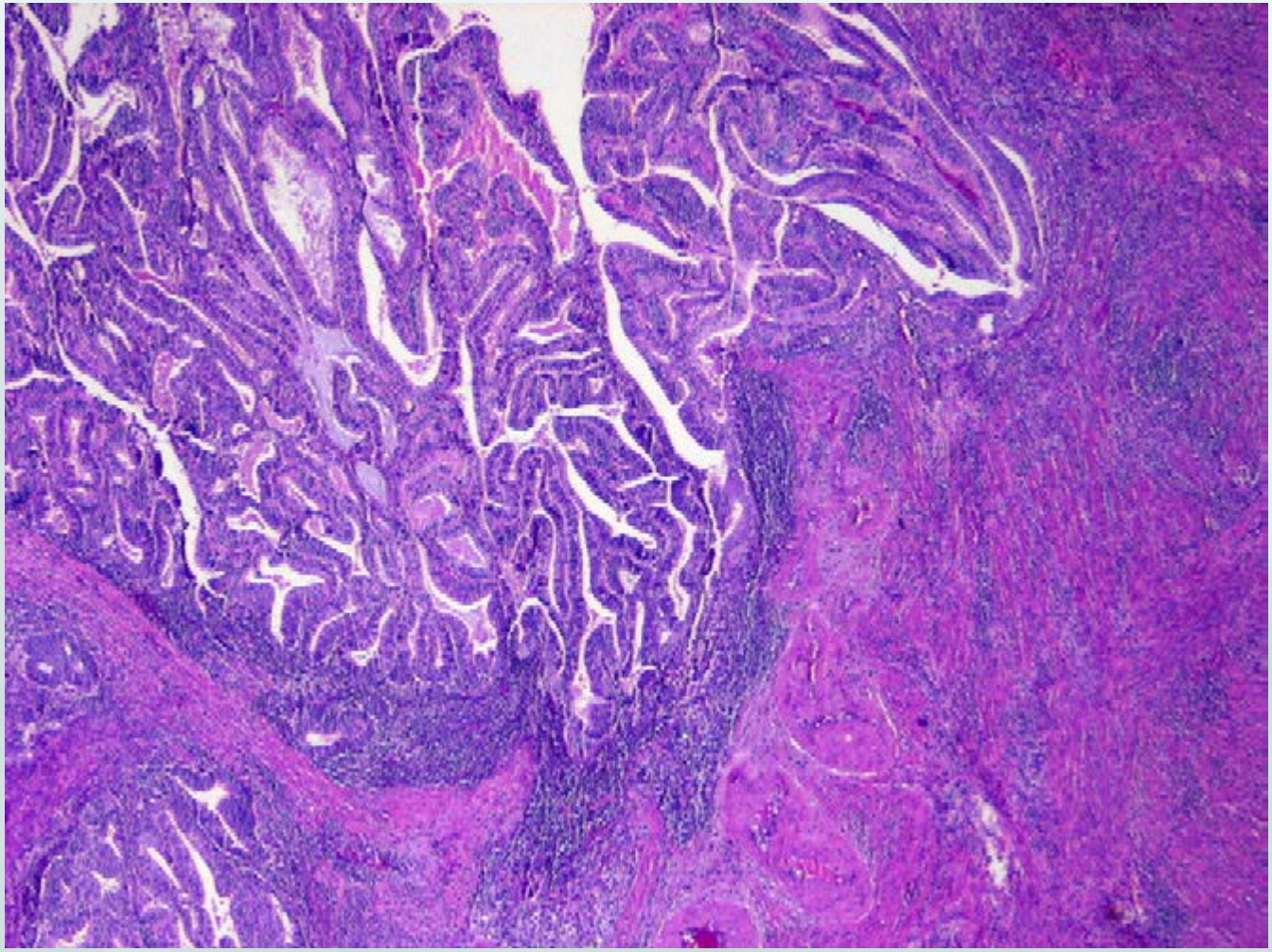
- Positive family history, prior cancer history, younger age at diagnosis
  - Amsterdam Criteria (1999)
  - Bethesda Guidelines (2004)
- Tumor site
  - Endometrial carcinoma - lower uterine segment (LUS)
    - In a series of 1,009 cases, 10 (29%) of the 35 (3.5%) LUS endometrial carcinomas were Lynch Syndrome associated*

*Westin SN et al. Journal of Clinical Oncology 2008;36:5965-5971.

Lynch Syndrome: Histologic Phenotype

- Shia J. et al. Human Pathology 2008;39:116-125.
  - 102 endometrial carcinoma cases; 52 MSI-H & 50 non MSI-H
    - Host inflammatory response
    - Cytologic grade
    - Architecture
    - Invasion pattern
    - Metaplasia
    - Necrosis
    - LVI
    - Background endometrium
    - Tumor infiltrating lymphocytes (OR 3.1) and peritumoral lymphocytes (OR 2.8) only features that were significant predictors of MSI status

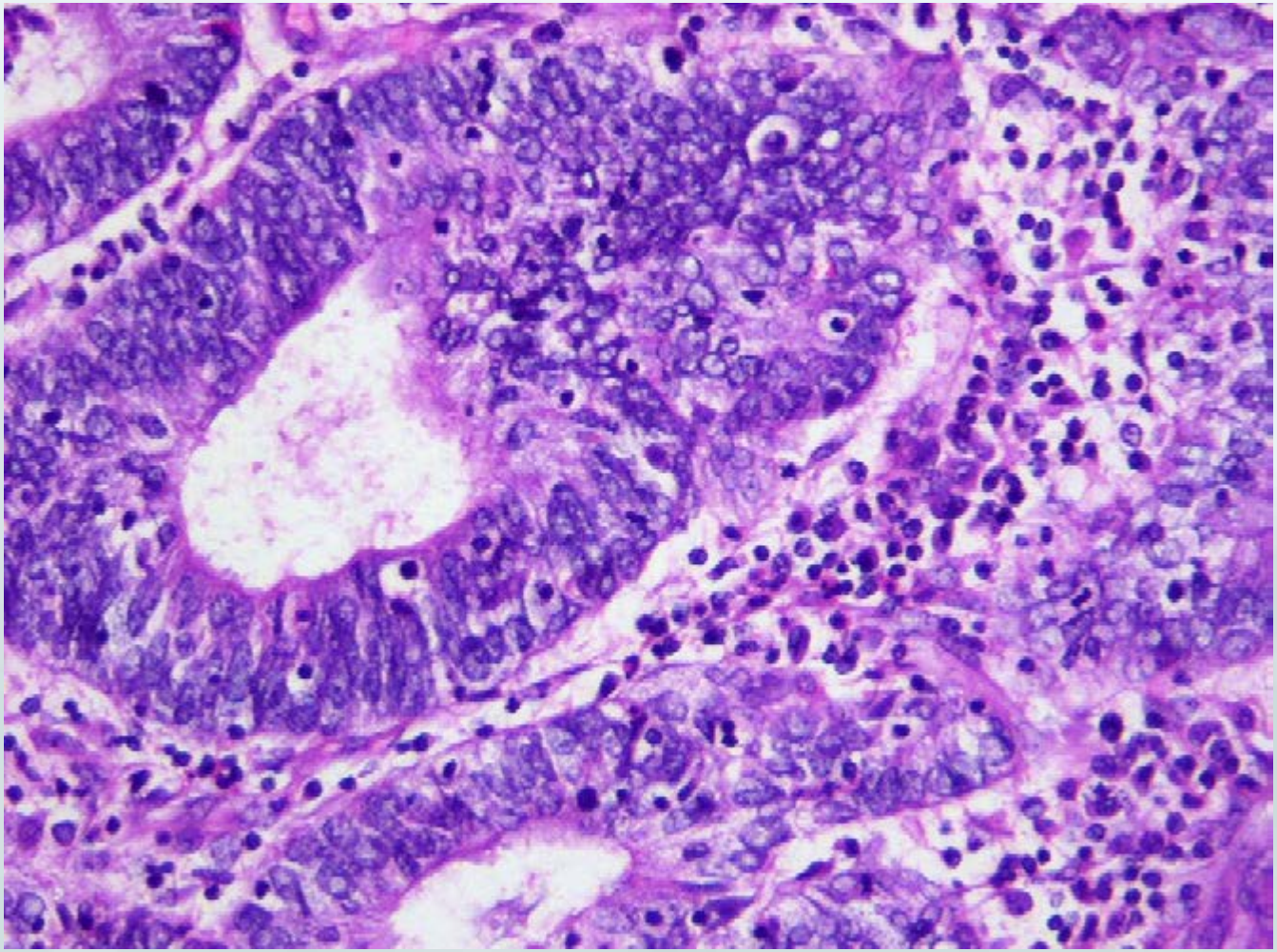
Lynch Syndrome: Histologic Phenotype



Endometrioid
FIGO 1
with peritumoral lymphocytes

Shia J. et al. Human Pathology 2008;39:116-125.

Lynch Syndrome: Histologic Phenotype



Endometrioid FIGO 1 with tumor infiltrating lymphocytes

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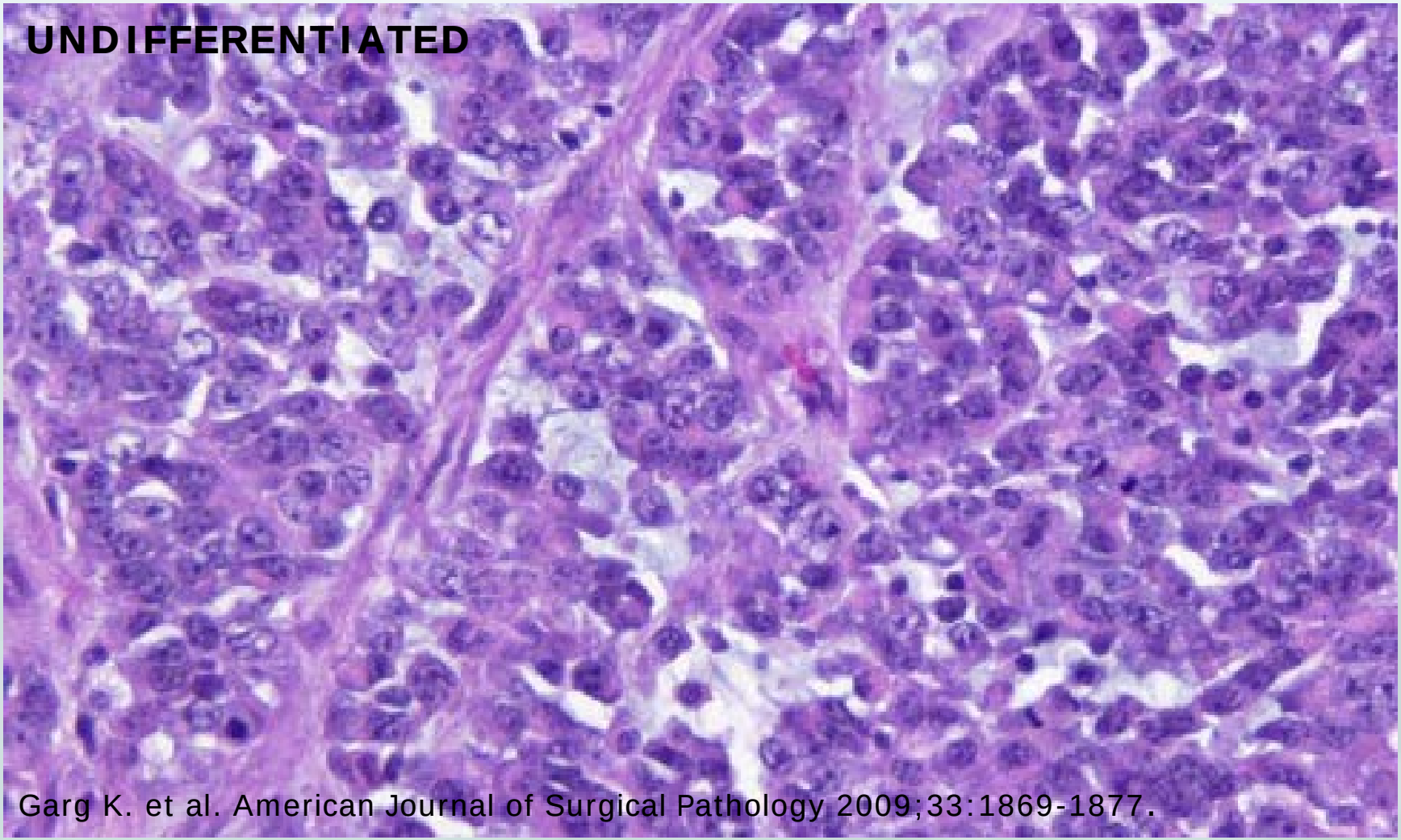
Lynch Syndrome: Histologic Phenotype



Garg K. et al. American Journal of Surgical Pathology 2009;33:1869-1877.

Lynch Syndrome: Histologic Phenotype

Histologic type in Lynch Syndrome associated tumors

	Broadus et al., 2006, N=50		Carcangiu et al., 2010, N=23	
	Endometrioid	Non-endometrioid	Endometrioid	Non-endometrioid
MLH1	3 (6%)	0	5 (22%)	2 (9%)
MSH2	40 (80%)	7 (14%)	7 (30%)	8 (35%)
MLH1 & MSH2	0	0	1 (4%)	0

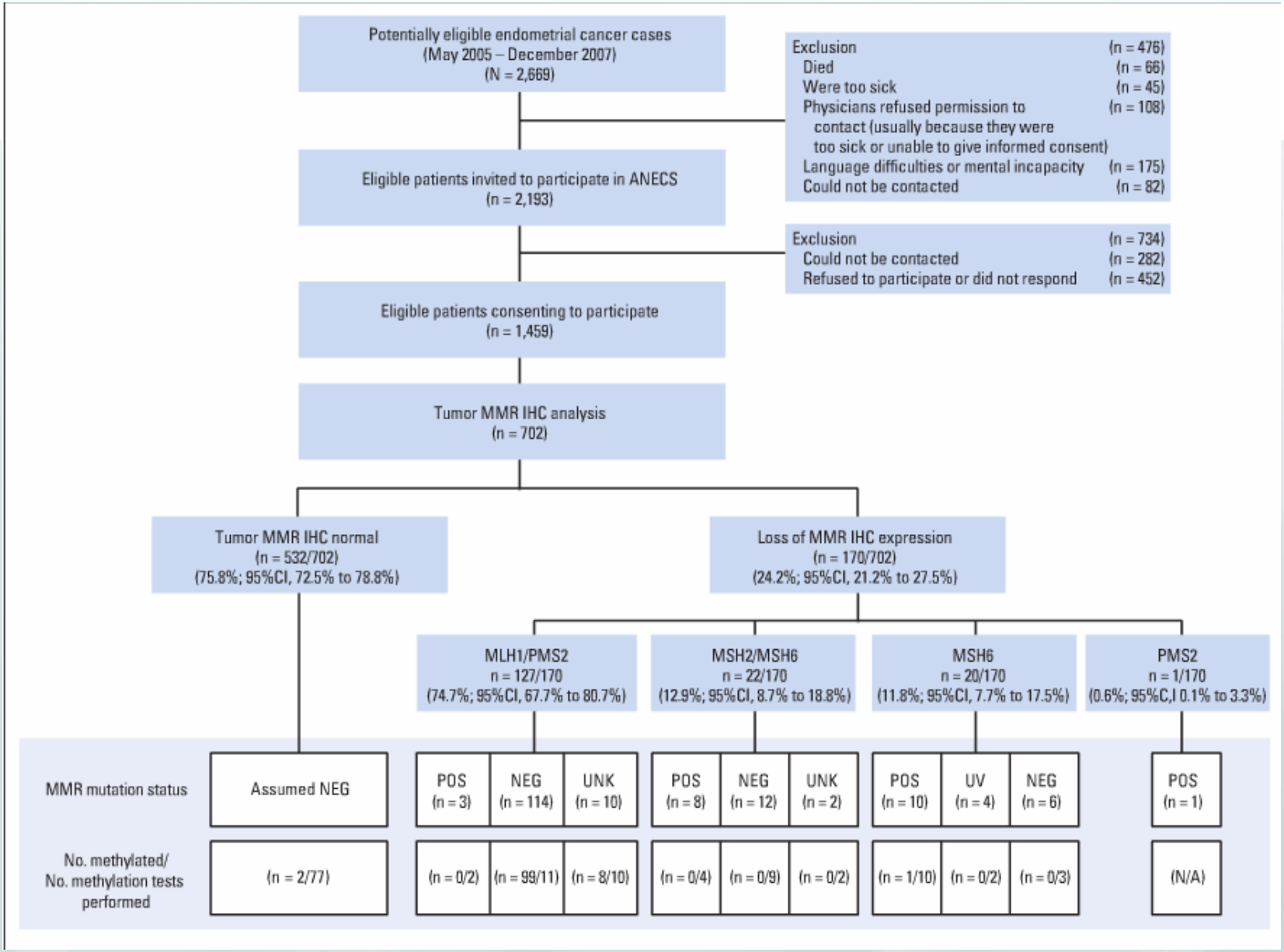
Broaddus R. et al. Cancer 2006;106:87-94.
Carcangiu M. et al. International Journal of Surgical Pathology;18:21-26

Recommendations for Lynch Syndrome Screening in Endometrial Carcinoma

- NCCN
  - Patient < 50 or meets Amsterdam or Bethesda Criteria
- Society of Gynecologist Oncologists
  - "In summary, all women who are diagnosed with endometrial cancer should undergo systematic clinical screening for Lynch syndrome (review of personal and family history) and/or molecular screening. Molecular screening of endometrial cancers for Lynch syndrome is the preferred strategy when resources are available."

Lynch syndrome Screening Strategies in Endometrial Carcinoma

- Buchanan DD et al. Journal of Clinical Oncology 2014;32:90-100
  - Comparison of the frequencies of MLH1, PMS2, MSH2, and MSH6 mutations in LS associated endometrial carcinoma
  - Comparison of the performance characteristics of various screening strategies
  - Validation of MLH1 promoter methylation as a negative predictive marker for Lynch syndrome



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Lynch syndrome Screening Strategies in Endometrial Carcinoma

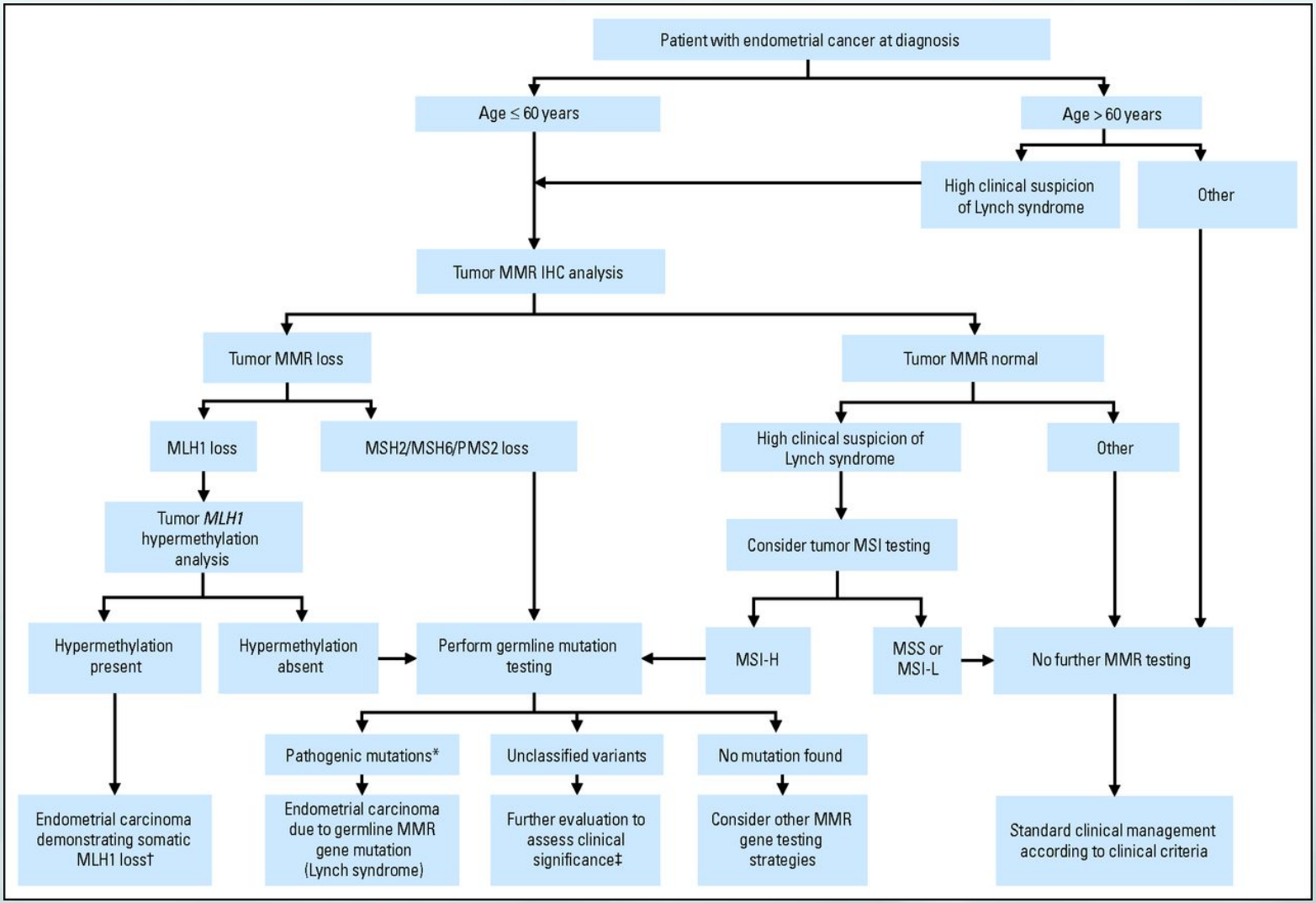
Performance characteristics of selected clinical criteria and tumor tests

Criteria	% Fulfilling Criteria	% of Mutation Carriers Fulfilling Criteria	Positive Predictive Value	
			%	95% CI
Amsterdam II	3.6%	14.3%	12.0	3.2-32.3
Bethesda	36.3%	76.2%	6.3	3.8-10.3
Age < 60	40.8%	85.7%	6.3	3.9-10.0
MMR IHC loss <60	10.2%	85.7%	25.4	16.1-37.3
MMR IHC loss <60 MLH1 unmethylated	5.6%	85.7%	46.2	30.4-62.6
	N=698	N=21		

**Negative predictive values ranged from 97.3-100.0%

Buchanan DD et al. Journal of Clinical Oncology 2014;32:90-100

Proposed Algorithm for Endometrial Carcinoma



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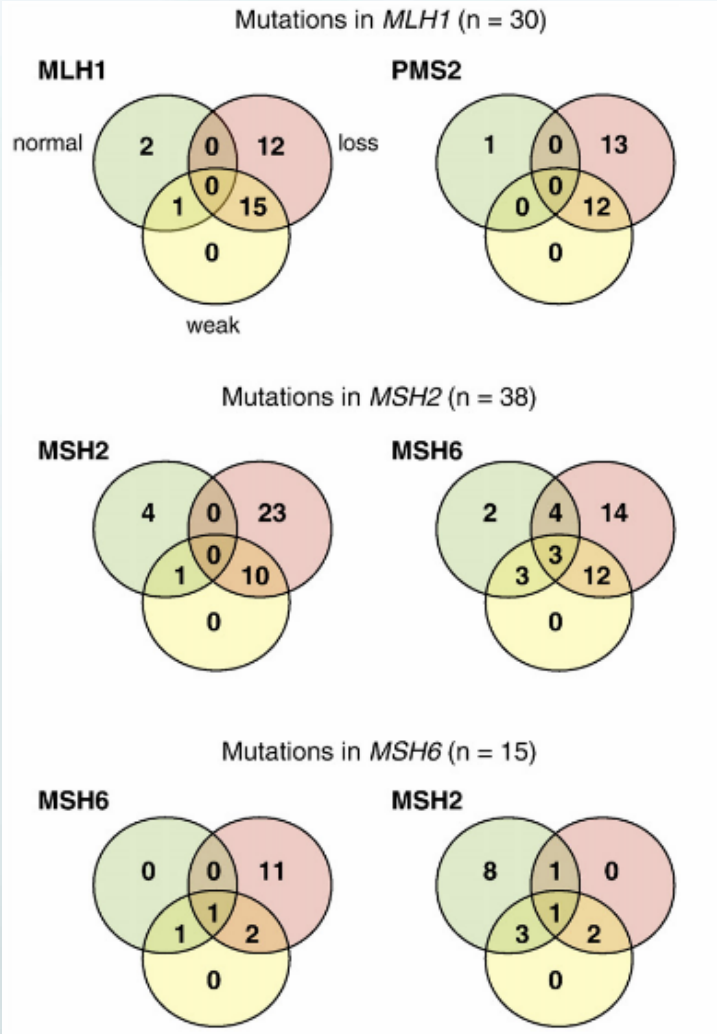
Towards a Rational, Comprehensive Approach to Genetic Screening For Lynch Syndrome and Beyond

- A well-coordinated interdisciplinary approach is essential to success
  - To address questions of informed consent
  - To ensure implementation of reporting and referral protocols that result in appropriate patient follow up
- Prospective research
  - To engage in an on-going assessment of the clinical utility and cost effectiveness of adopted testing

Lynch Syndrome Screening in Endometrial Carcinoma

QUESTIONS???

Interobserver variability in MMR immunohistochemistry interpretation in Lynch associated carcinomas.



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