Colorectal Cancer Molecular Diagnostics

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Molecular CRC Testing

- MSI, MMR IHC
- KRAS
- BRAF
- PIK3CA
- PTEN
- APC, SMAD4, BMPRIA,STK11
- Septin 9

MSI Testing

- MSI-H Sporadic (15%)
- MSI-H Lynch (2-3%)

Lynch Syndrome Cancers

- Colorectal CA 80%
- Endometrial CA 50%
- Pancreatic, gastric, small bowel, sebaceous skin, ovarian, genitourinary, GBM cancers
- Screening: Age 25 or 10 years < than youngest

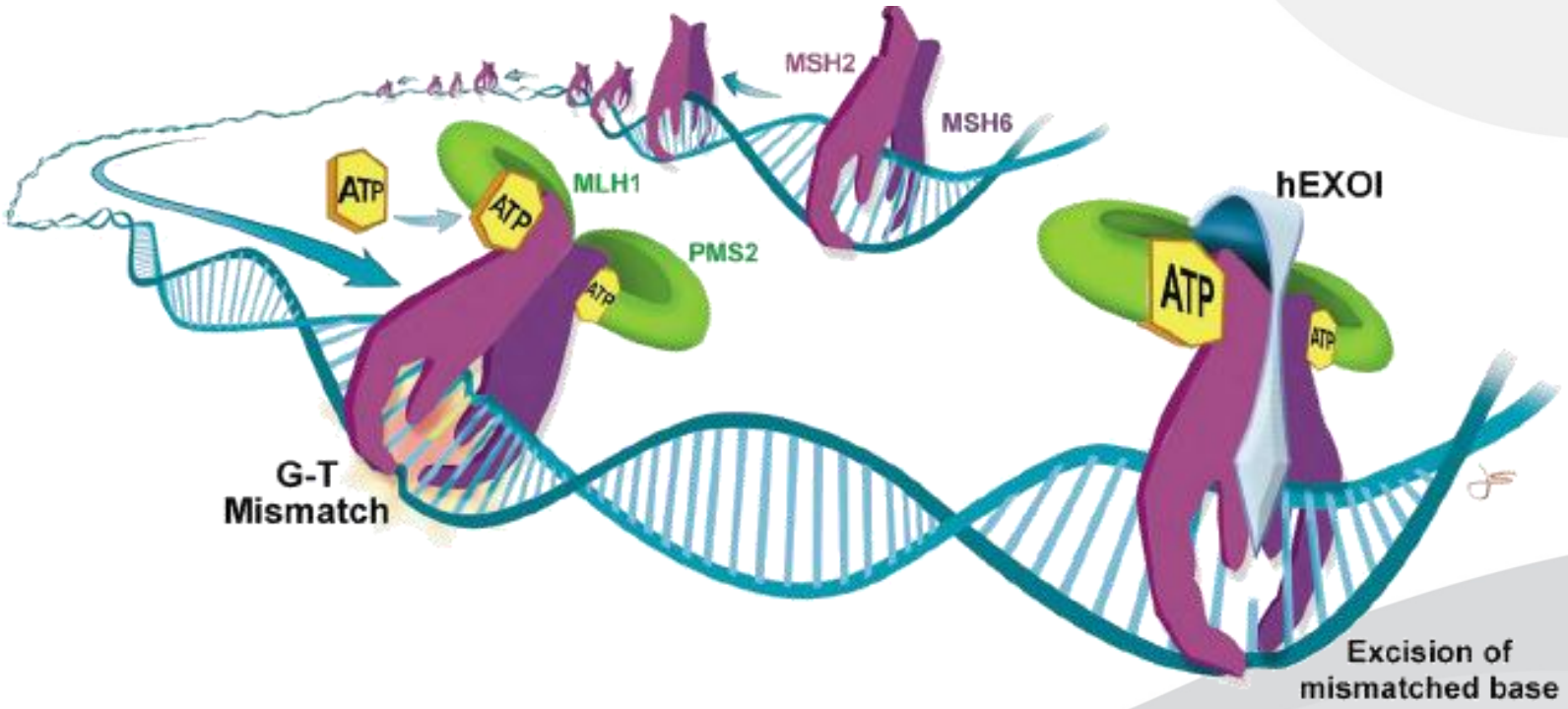
  Annual colonoscopy & endometrial bx, periodic EGD, EUS of pancreas, pelvic exam, brain scans, urine cytology

HUGE & LIFELONG IMPACT ON LYNCH PATIENTS: DX IS CRITICAL

Microsatellite instability (MSI)

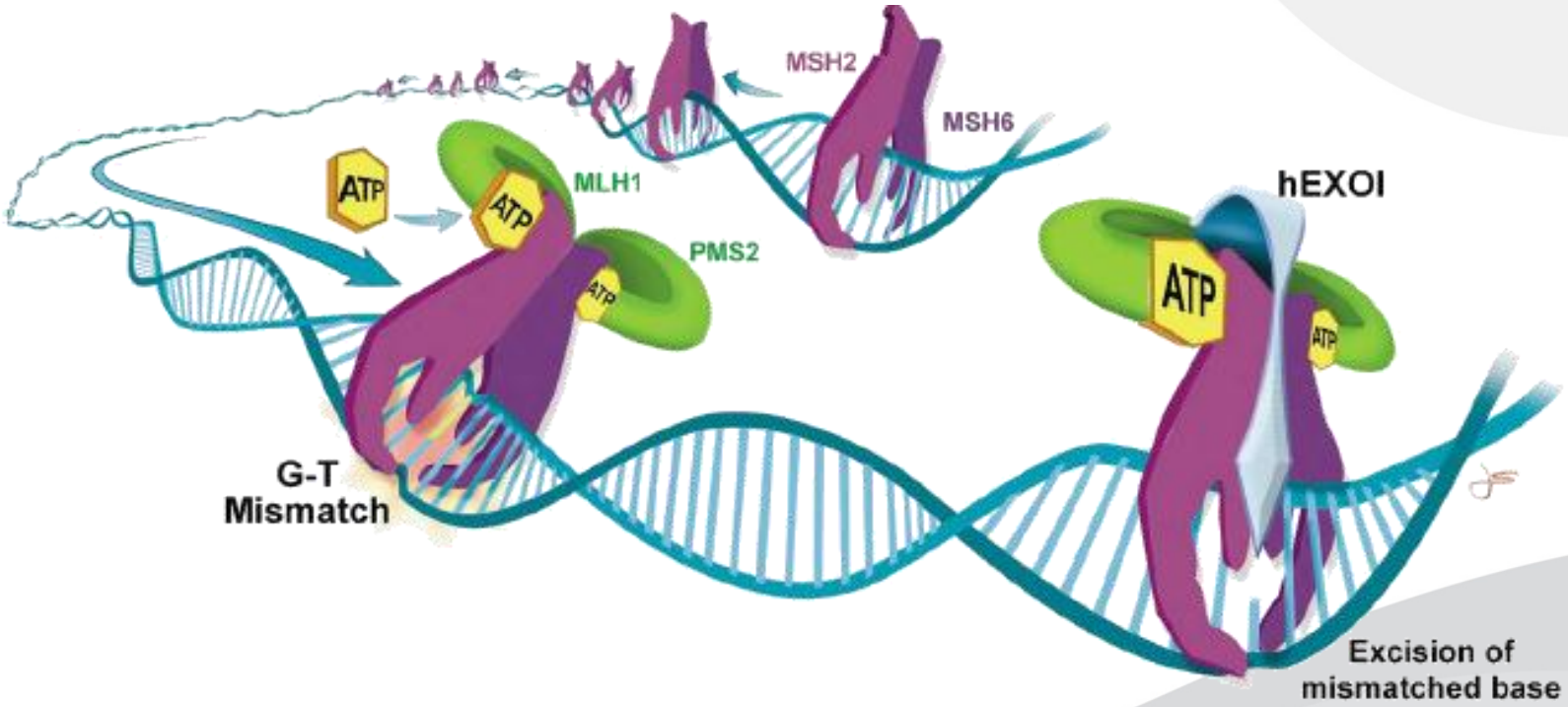
Microsatellites: Short, repetitive DNA sequences prone to error during replication

Normally repaired by MMR gene proofreading complex



Microsatellite instability (MSI)

- Mutations in MMR genes lead to accumulation of altered length microsatellites (MSI)
- MLH-1, MSH-2, MSH-6, PMS-2 alterations cause MSI-H cancer

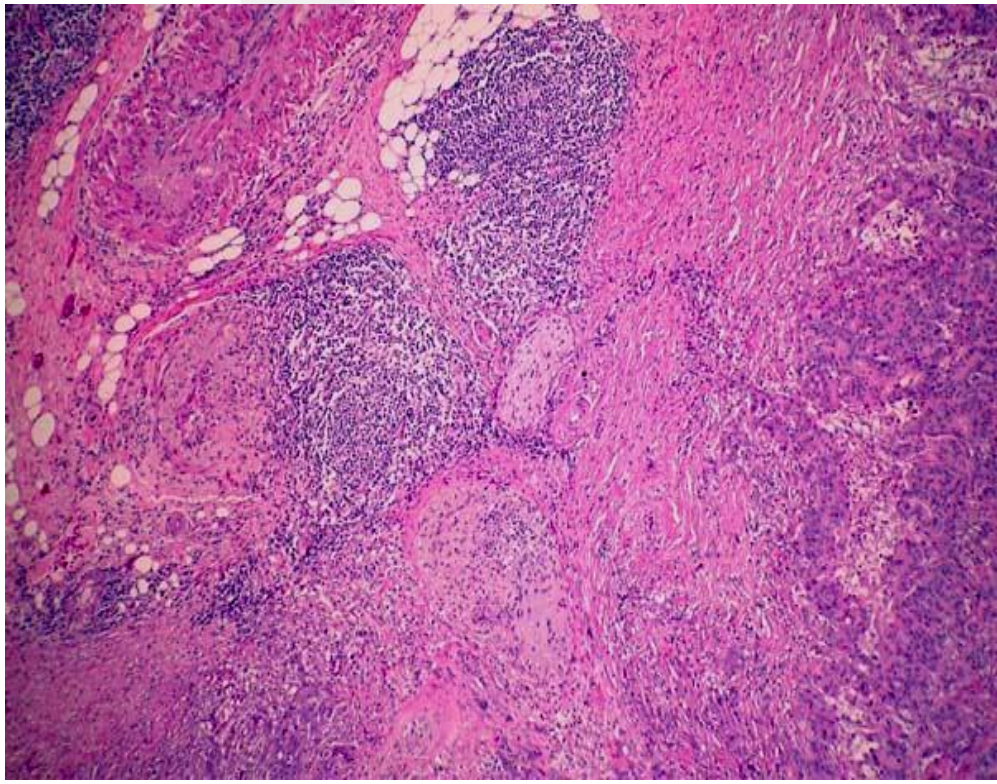


MSI-High Colon Cancer

- Sporadic: ~100% MLH1 (methylated)
- Lynch: 60% MLH1, 35% MSH2, 5% PMS2, MSH6, other
- Familial & sporadic path: identical

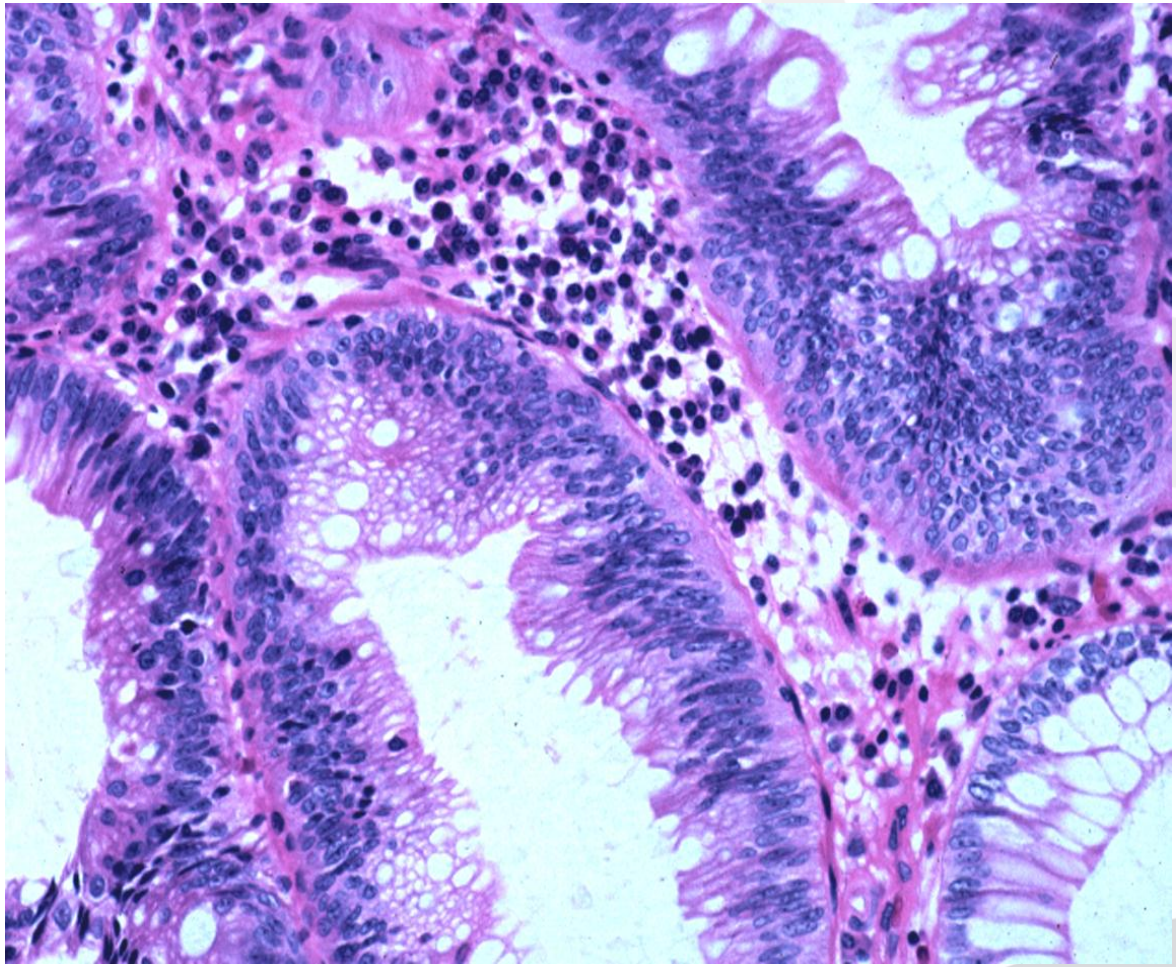
MSI-H CRC: Clinicopathologic Features

Right-sided location
Age < 50 years (Lynch)
Poor differentiation
Absence of dirty necrosis
>2 tumor infiltr lymphs/hpf
Mucinous change
Crohn's-like lymphoid reaction



Greenson JK, et al. *Am J Surg Pathol* 27:563-570, 2003.

Duodenal or Gastric Adenoma



Consider FAP and Lynch Syndrome

Reasons to Diagnose MSI-H CRC

- Hereditary and syndromic components of Lynch
- Prognosis
- Therapy

Lynch Testing

Tumor screening assays (90% sens)

- Detect affected patients with tumor
- MSI by PCR (paraffin works well)
- MMR Immunohistochemistry:
- MLH-1, MSH-2, MSH-6, PMS2

Blood germline mutation analysis

- Detect affected family members without tumor

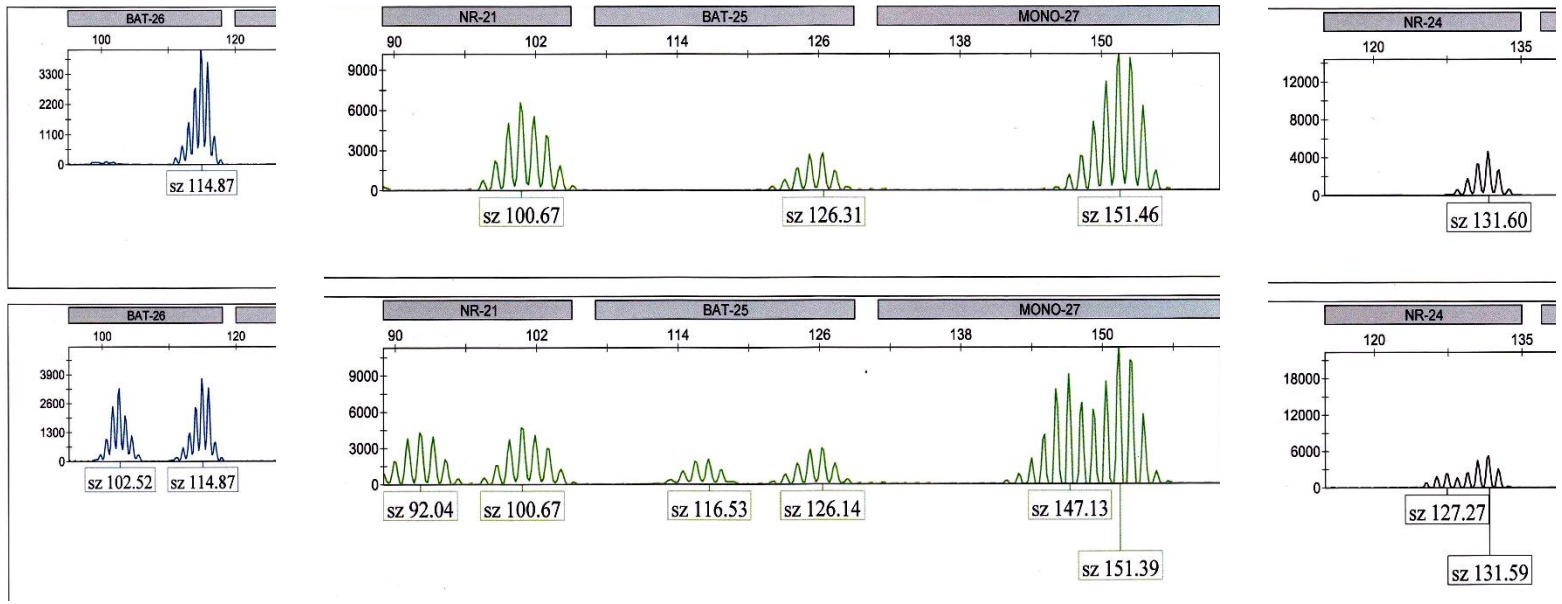
MSI/IHC: Other Tumors

- Non-colon tumor tissue
  - Endometrial neoplasia
  - Other syndromic tumors
- Fresh or fixed (formalin)

MSI Requirements

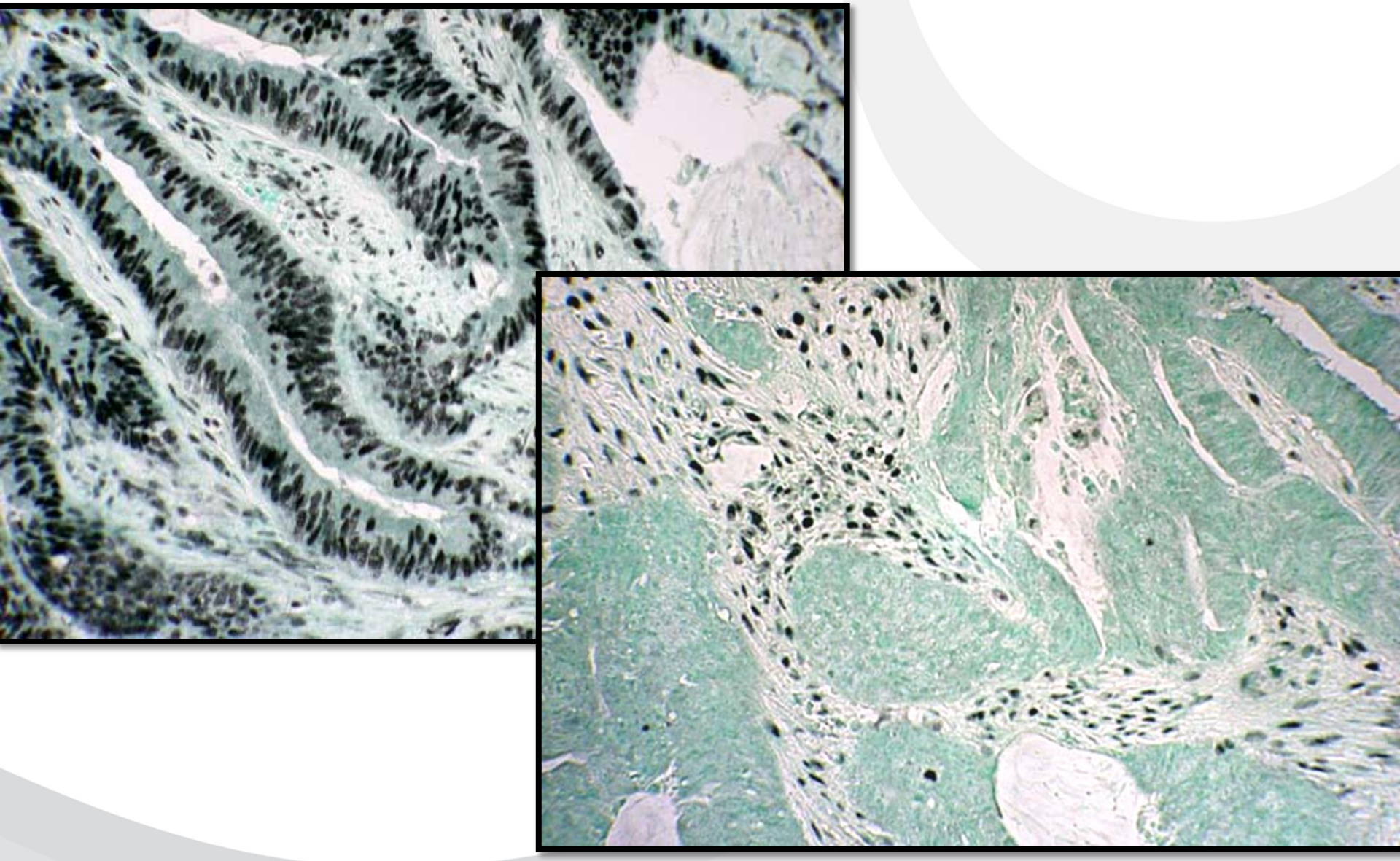
- *Also* need constitutional (normal) DNA
  - Non-tumor paraffin tissue
  - Blood
  - Buccal swab

MSI Electropherogram Results



Microsatellite-High (MSI-H)	**Instability at ≥ 2 loci**
Microsatellite-Low (Indeterminate)	**Instability at 1 locus**
Microsatellite stable (MSS)	**None of 5 loci unstable**

IHC in Lynch Syndrome



Sporadic vs. Lynch CRC

- Family history
- MSH2, MSH6, PMS2 IHC loss
- Adenoma involvement
- MLH1 promoter methylation
- BRAF point mutation (V600E)
- Germline MMR gene mutation

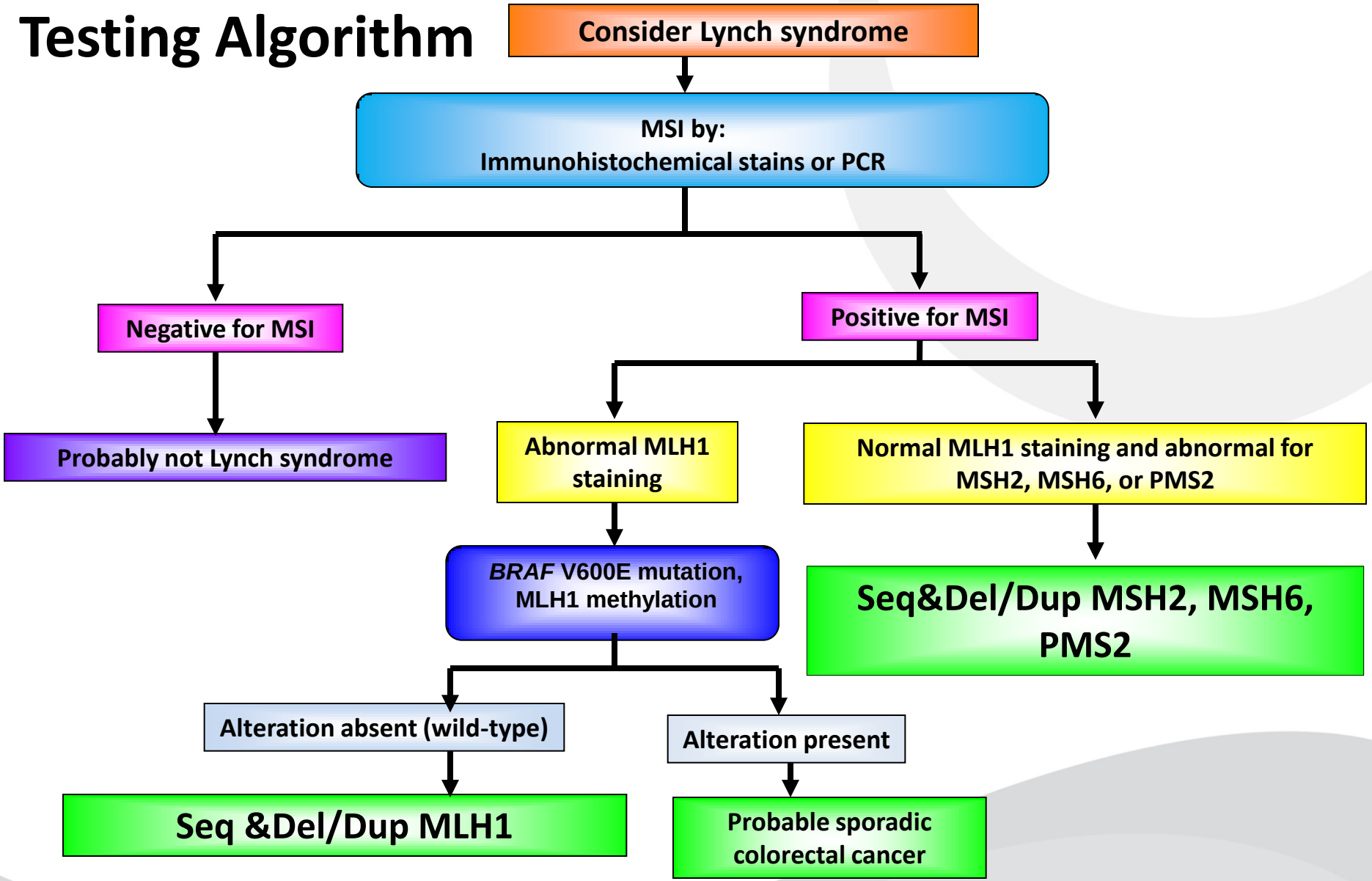
MSI: Prognosis & Adjuvant Rx

MSI-H in randomized *stage-matched* sporadic tumors predicts:

- Longer survival
- Chemotherapy

Zaanan A, et al. Clin Cancer Res 17:7470;2011
Ribic CM, et al. NEJM 349:247;2003

Lynch Syndrome Testing Algorithm



KRAS Testing

The Metastasis Problem

- 50-60% CRC patients present with or develop metastases
- 5-yr survival

  Stage I + II (N0) → 91%

  Stage III (N1,2) → 70%

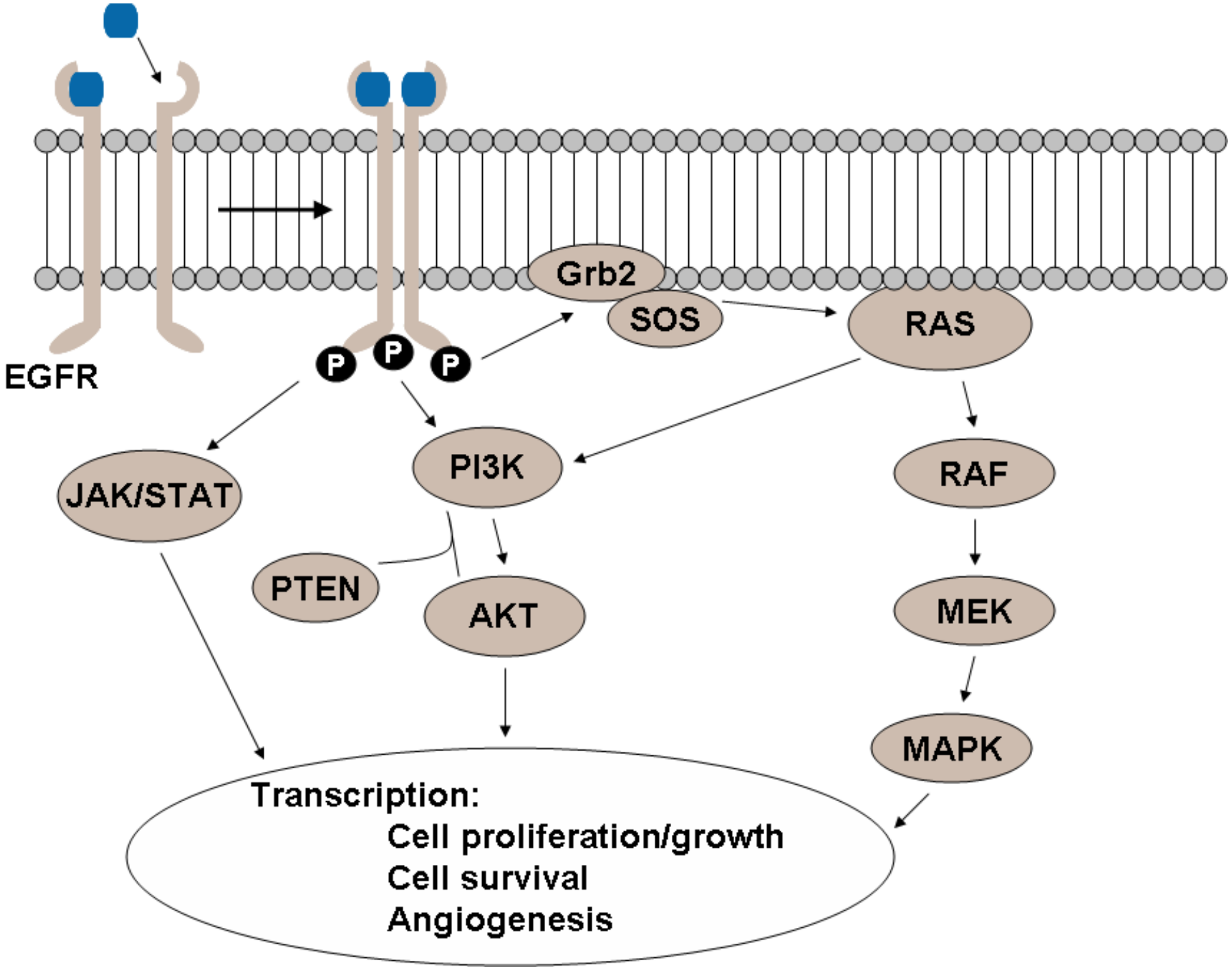
  Stage IV (M1) → 11%

Search for alternate Rx's

5 FU/Leucovorin mainstay for decades

After 2000 → new therapies

- Oxaliplatin (FOLFOX)
- Irinotecan (FOLFIRI)
- Anti-VEGF (bevacizumab)
- Anti-EGFR (cetuximab, panitumumab)


EGFR
P
P
P
Grb2
SOS
RAS
JAK/STAT
PI3K
RAF
PTEN
AKT
MEK
MAPK
Transcription:
Cell proliferation/growth
Cell survival
Angiogenesis

KRAS mutation

- <1% response rate to anti-EGFR Rx with codon 12 or 13 or 61 mutations (~40% of CRC)
- ~40% response rate with KRAS WT (~60% of CRC)
- But…. ~ 60% KRAS WT will not respond
- Other markers play a role

Cost (savings)

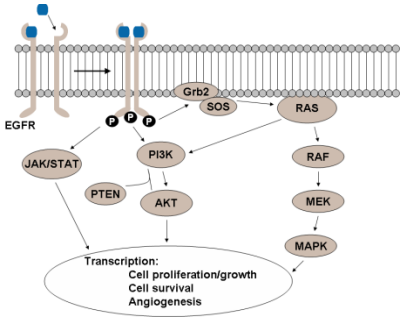
- ~30,000 new metastatic CRC annually
- KRAS testing = $13 million ($452/pt)
- Cetuximab Rx= $2.1 *billion* ($71,120/pt)
- Mutated KRAS (~40%) excluded from cetuximab
- Cost savings: ~$750 million annually
- High toxicity; ~2 month added survival

No need for normal tissue for KRAS testing

KRAS Methods

- All methods applicable to formalin-fixed paraffin-embedded tissue
- Tumor microdissection
- Sequencing, Sequenom, allele specific PCR, and melt curve analysis

Future



- Impact of *specific* KRAS 12/13 mutations?
- Other predictors of anti-EGFR response
  - Other KRAS mutations: codon 61, others?
  - BRAF
  - EGFR copy number (FISH, CISH, PCR), specific mutations
  - PTEN
  - PIK3CA

KRAS Summary

- KRAS mutations in 30-40% CRC's
- Highly predictive of lack of response to anti-EGFR Rx
- Laboratory plays key role determining proper and most cost-effective Rx in stage III-IV CRC
- BRAF, PIK3CA, PTEN downstream markers may be useful in KRAS wild type tumors
- Additional biomarkers expected

BRAF Testing

BRAF

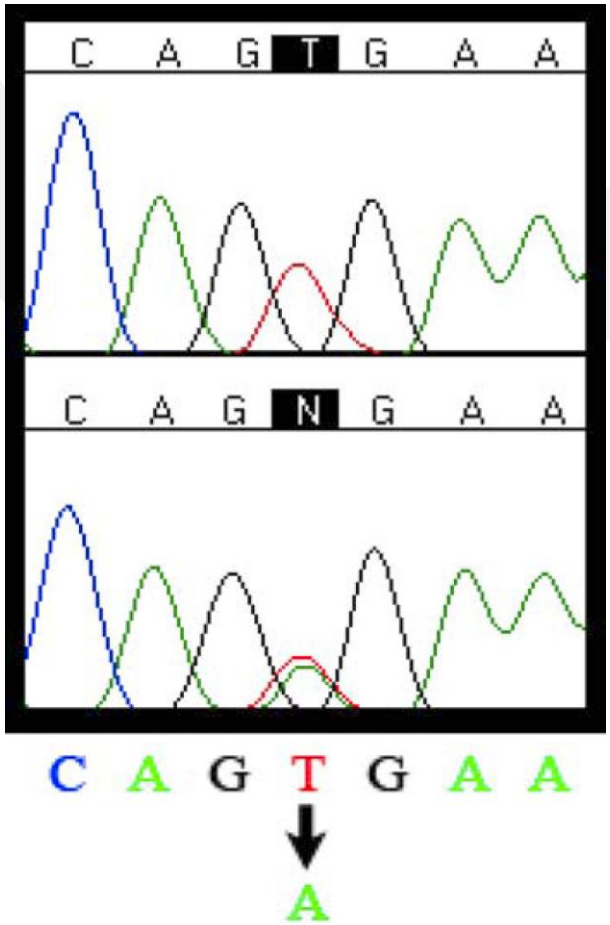
- ~ 10% colorectal cancers have BRAF mutations
- Predicts anti-EGFR non-response in KRAS WT

Study	Pts	BRAF Mutation %	BRAF Wild Type Response	BRAF Mutated Response
Cappuzzo '08	79	5%	17%	0%
De Roock '08	113	6%	27%	17%
Di Nicolantonio '08	79	14%	32%	0%

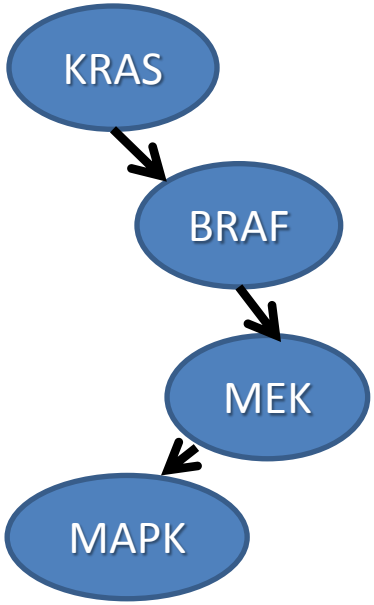
BRAF mutation

T to A transversion

Valine to glutamate at codon 600 (BRAF V600E)



BRAF: Downstream from KRAS



BRAF and KRAS mutations:

Mutually exclusive

KRAS-WT/BRAF-MUT

Anti-EGFR therapy non-responders

Chemoradiation Rx: Does it affect GI cancer molecular testing?

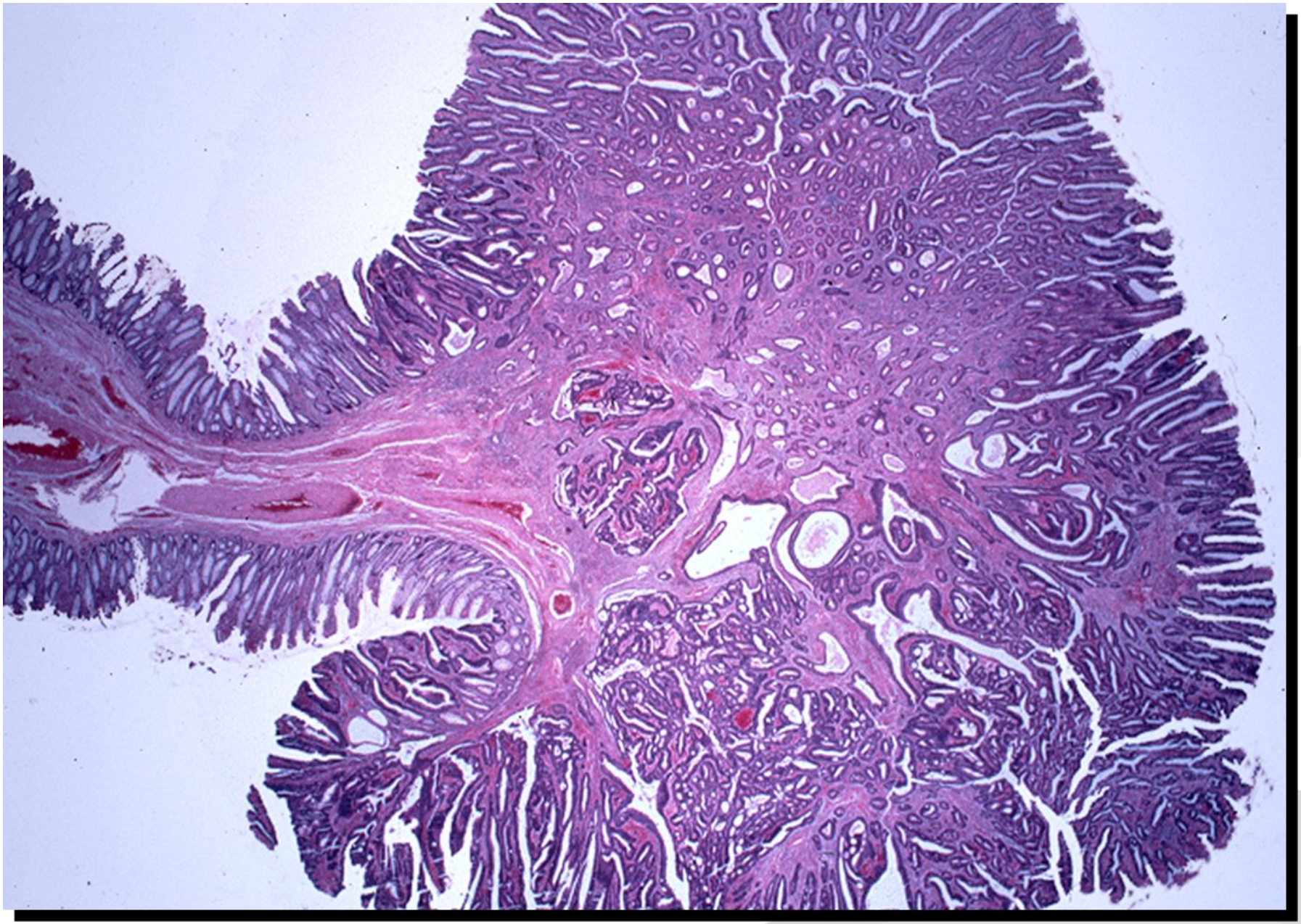
- Neoadjuvant Rx common in rectal & esophageal adenocarcinomas
- Ondrejka SL, et al. *Am J Surg Pathol* 35:1327,2011
  - Pre and Post neoadjuvant Rx
  - No change in 18 patients for MSI PCR
  - No change in 18 patients for KRAS mutations by Sanger sequencing

Gastrointestinal Polyposes Predisposing to CRC

Case 1

- 18 yo boy currently asymptomatic but strong family history of colon cancer
- Colonoscopy reveals hundreds of colonic polyps
- Colectomy is performed


cm
1
2
3
4



Familial Adenomatous Polyposis

- Sequencing reveals APC knockout mutation
- Risk of colorectal cancer 100%, average age onset 39 years
- Extra-colonic intestinal manifestations: duodenal & jejunal adenomas/ carcinomas, gastric polyps (?cancer)

Case 2

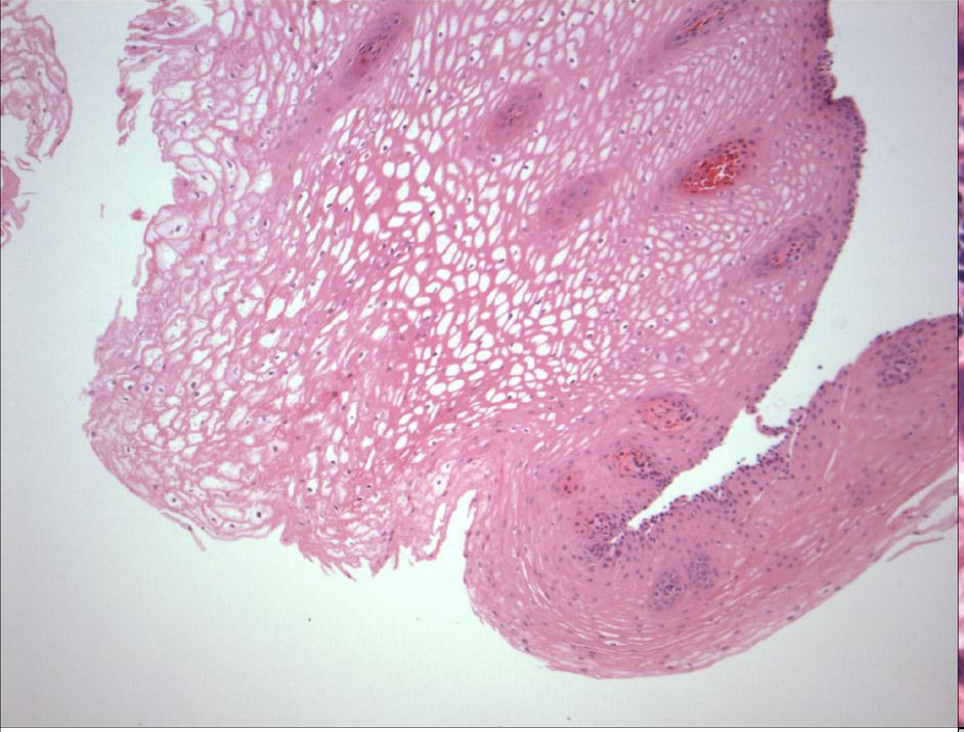
- 45 yo man with 85 adenomas
- No APC mutation in germline
- Family history of colon cancer, recessive inheritance
- MUTYH gene is sequenced; compound Y179C & G396D germline mutation detected

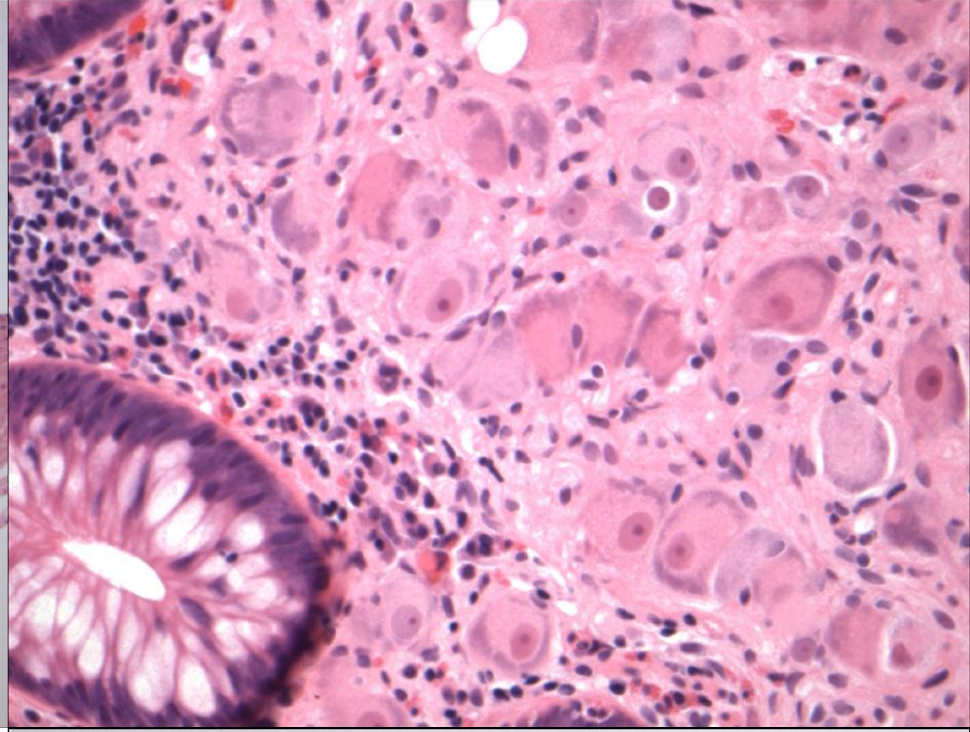
MYH-assc polyposis (MAP)

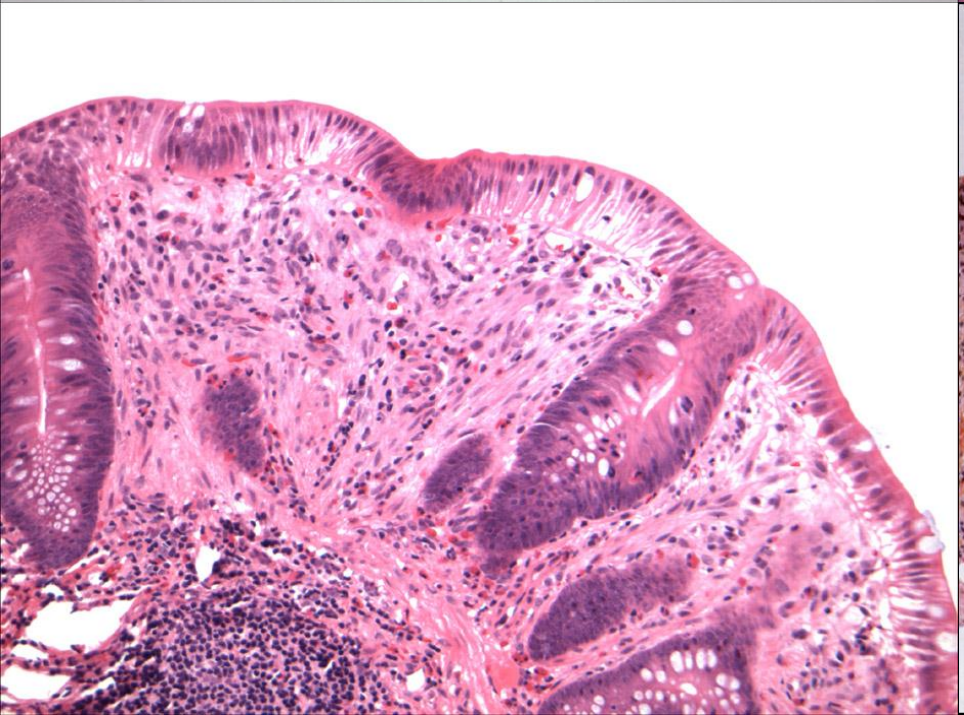
- Colon polyps usually like attenuated FAP
- Extra-colonic: duodenal ad/ca, cancer of ovary, bladder, skin, sebaceous glands
- Most homozy or compound heterozy of Y179C and G396D mutations

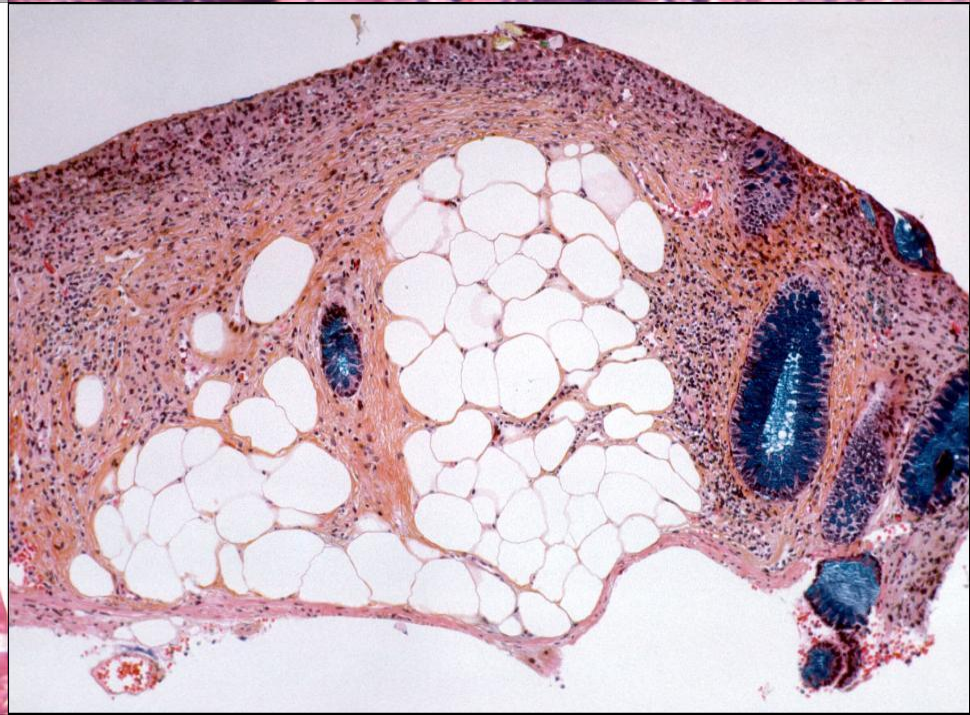
Case 3

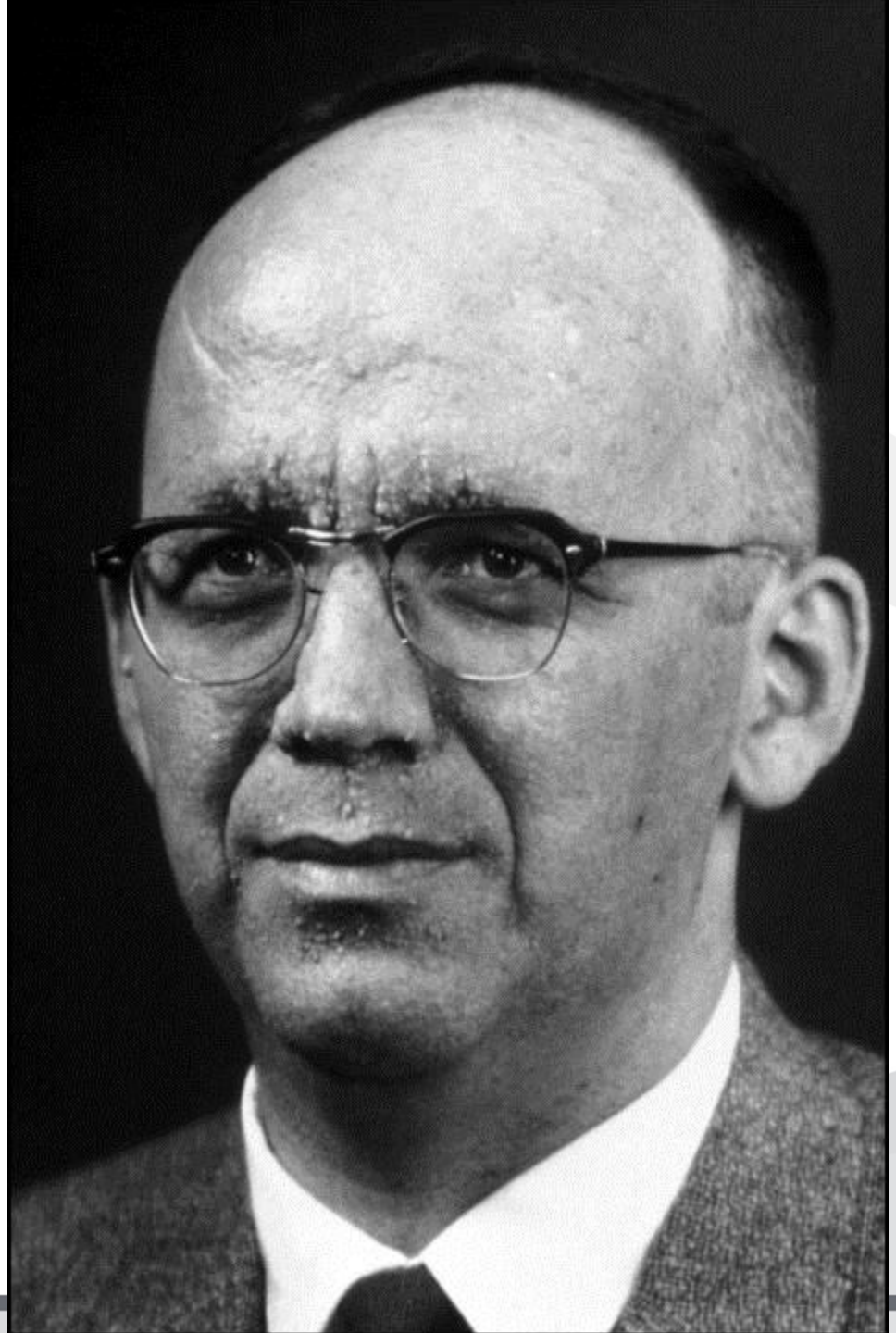
- 35 yo woman with breast & thyroid cancer
- Sister with ovarian cancer in her 20's, thyroid nodule at 32, & childhood colon polyps
- FH of endometrial cancer
- Endoscopy reveals colonic, gastric & esophageal polyps









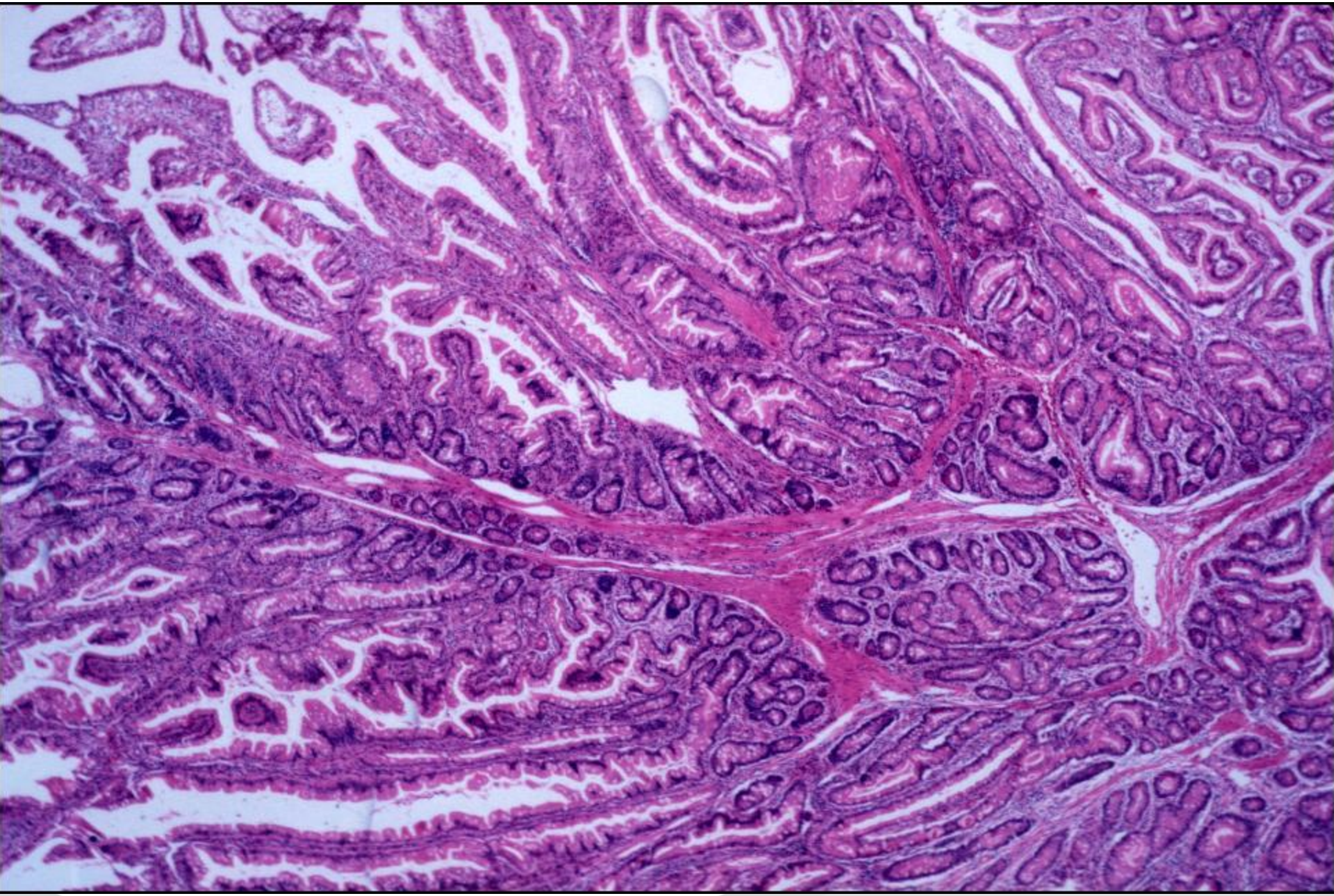


Cowden's syndrome

- Personal medical and FH history implicating Cowden's syndrome
- PTEN mutation testing to reveal pathogenic germline change (R335X) of Cowden's
- Emerging literature on colon cancer risk, plus previously known breast, thyroid, endometrial CA risks

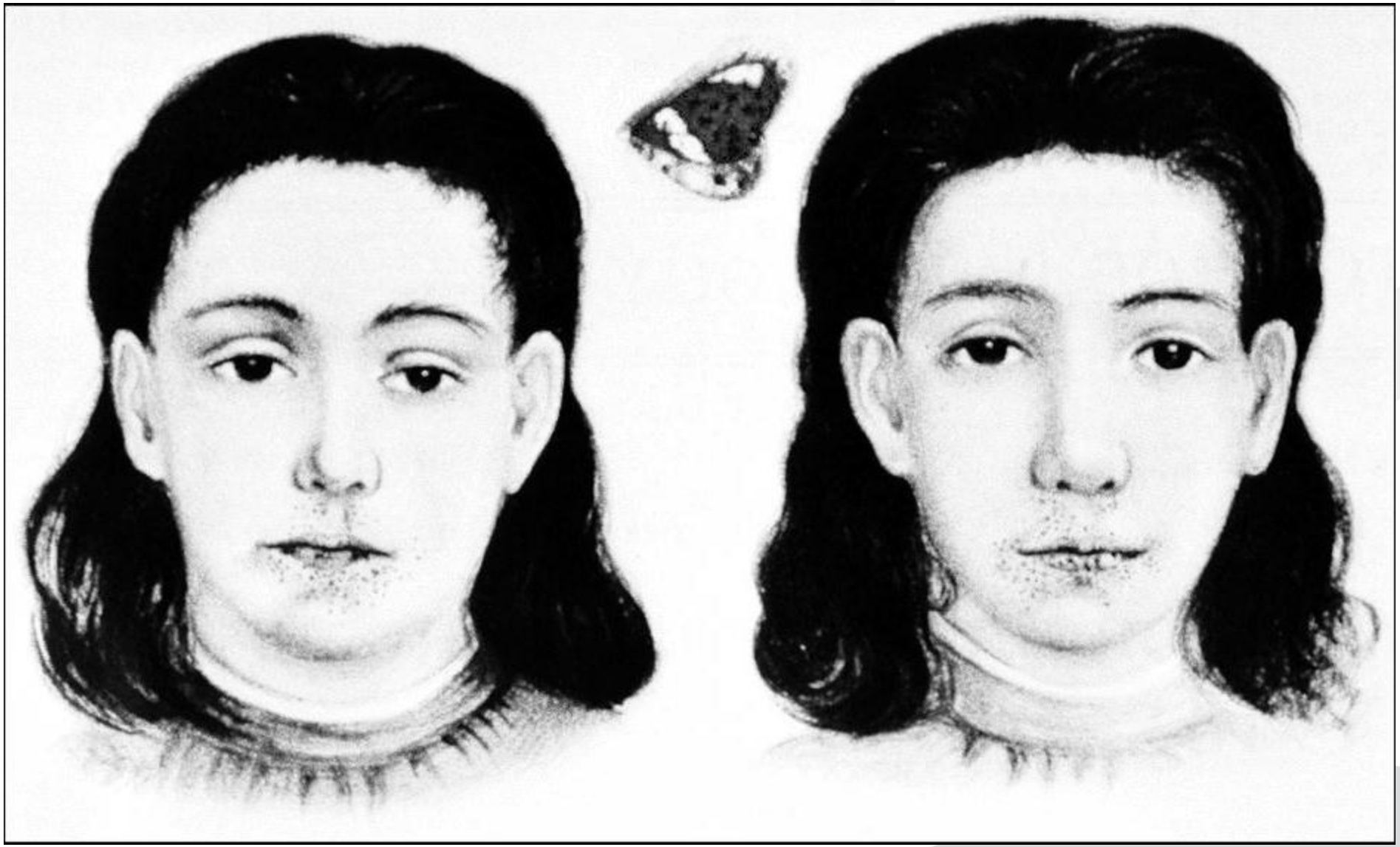
Case 4

- 15 yo girl presents with rectal bleeding & small bowel obstruction
- Jejunal intussusception & polyposis
- Bowel resected



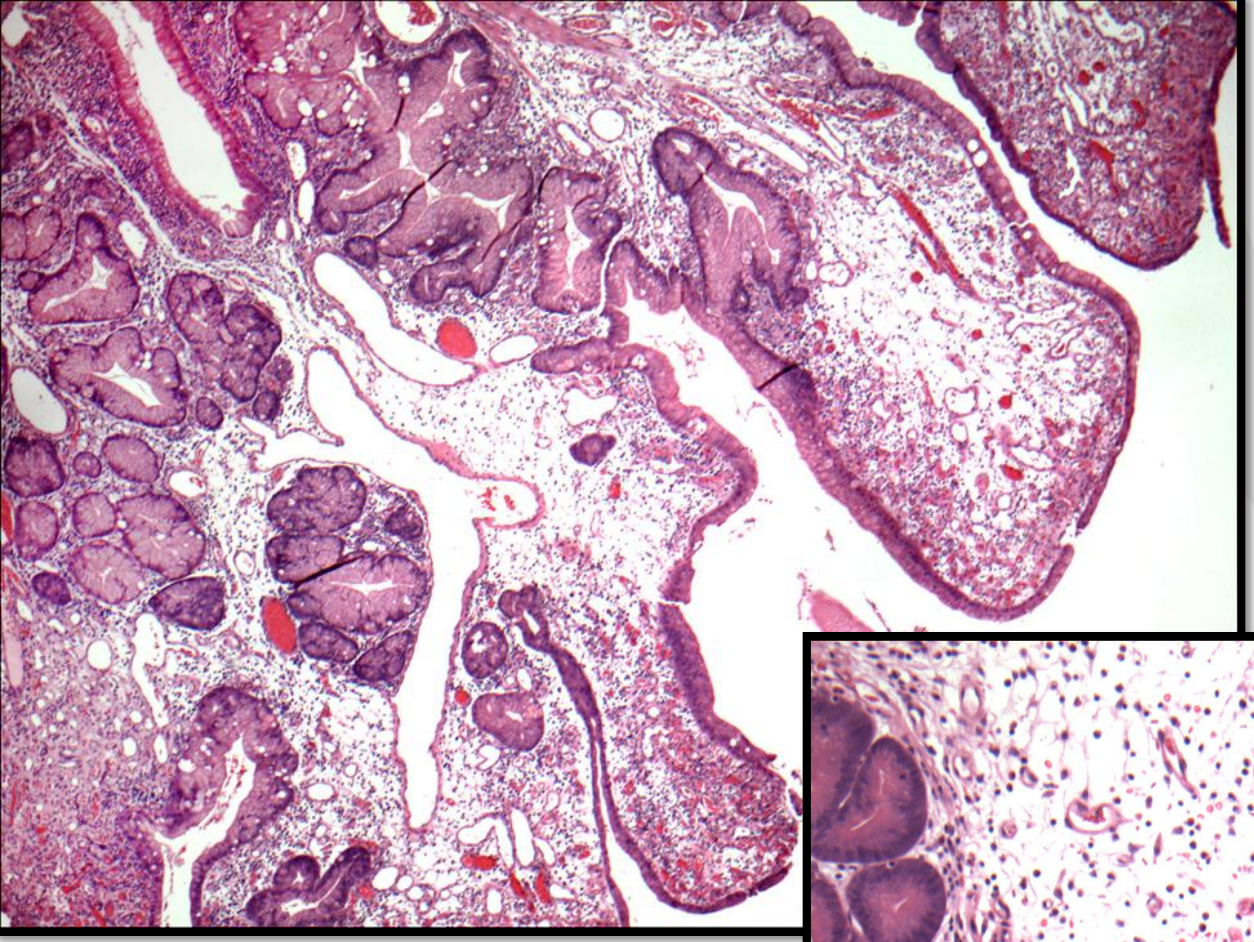
Peutz-Jeghers

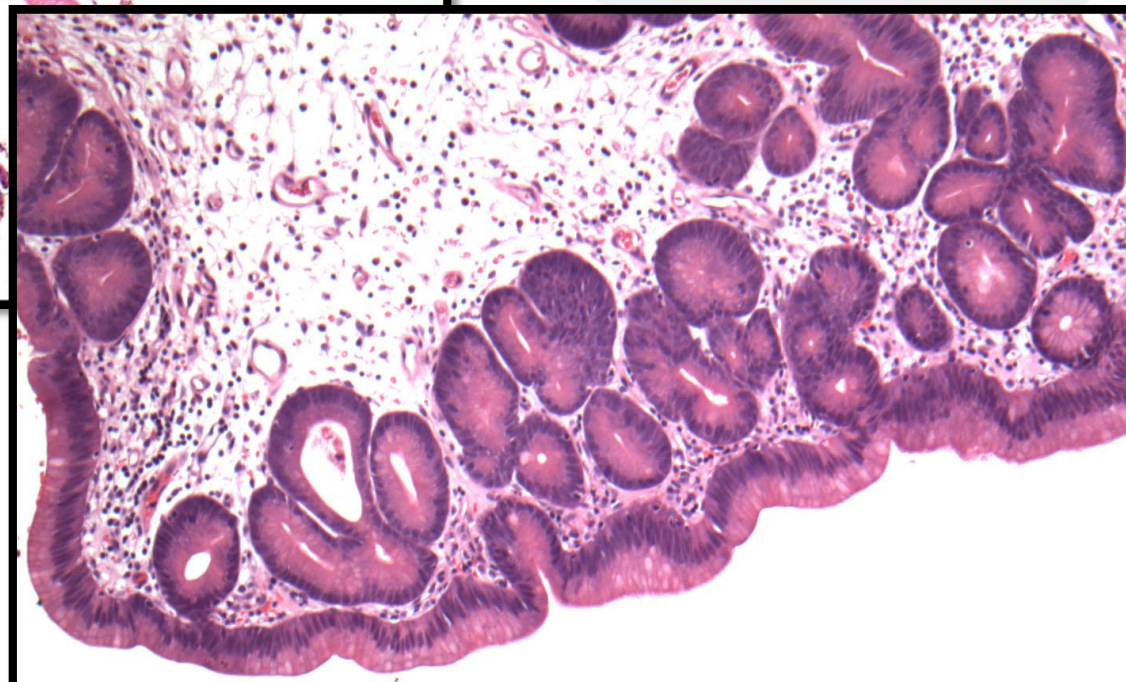
- Hamartomatous polyps, mostly small bowel (jejunum), also stomach and colon
- Mucocutaneous hyperpigmentation
- Autosomal dominant mutation in STK11 (LKB1) gene (50-90 %)
- Multi-organ cancer syndrome: breast, colon, pancreas, stomach, lung, gyn, testes: *93% lifetime cancer risk*

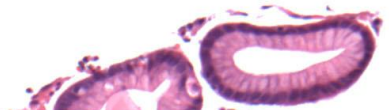


Case 5

- 57 yo man with upper GI bleeding
- Upper GI endoscopy revealed 5x6 cm gastric mass & multiple smaller polyps throughout stomach (biopsied as hyperplastic polyps)
- Mass & few smaller polyps resected







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Juvenile Polyposis

- 28% SMAD4, 24% BMPR1A
- SMAD4 also causes hereditary hemorrhagic telangiectasia (HHT)
- SMAD4 mutations may also have severe gastric polyposis & ↑ gastric cancer risk
- JP at high risk for colon (20-70%) & gastric cancer (mostly SMAD4 for gastric)

Summary: CRC Molecular Dx: Current Impact on Practice: 15 Genes

- Metastatic CRC for Anti-EGFR Rx (50-60%): KRAS
- KRAS WT for Anti-EGFR Rx (40%): BRAF, PIK3CA, PTEN
- Sporadic MSI-H CRC (15%): MMR IHC, MSI, MLH1 Methylation, BRAF
- Lynch MSI-H (2-3%): MMR IHC, MSI, MLH1 Methylation, BRAF
- FAP (<1%): APC
- Juvenile Polyposis, Peutz-Jeghers, Cowden's (<1%): SMAD4, BMPRIA, STK11, PTEN

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