Molecular applications in Gynecologic Pathology

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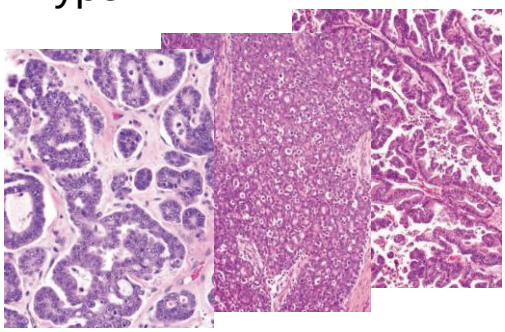
THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

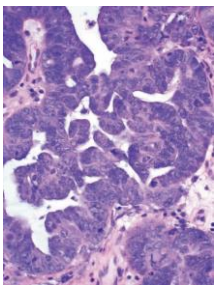
Ovarian cancer

– Morphologic classification

Type I



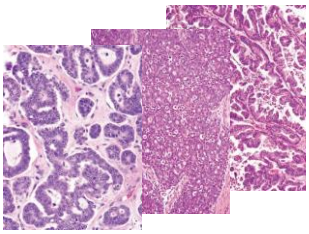
Type II



Ovarian cancer

- **Type I tumors**

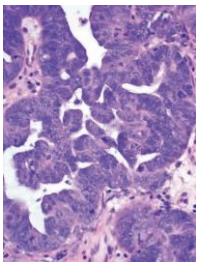
  Progress from borderline tumors
  Usually low grade
  Ras pathway mutations common
  BRCA wild type
  Usually *TP53* wild type
  Chromosomally stable
  Frequently platinum resistant
  MEK inhibitors



Ovarian cancer

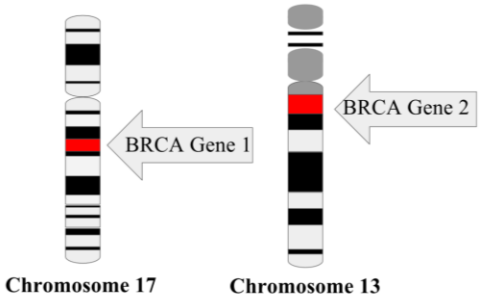
- **Type II tumors** – High grade serous carcinoma

  Distal fallopian tube origin
  High grade
  Ras wild type
  BRCA dysfunction
  TP53 mutant
  Copy number changes
  Often/usually platinum sensitive



Gynecologic malignancies in genetic syndromes

- Hereditary breast and ovarian cancer syndrome
  - germline mutation in *BRCA1* and *BRCA2*
  - significantly increased risk of cancer
    - breast
    - ovary
    - prostate
    - pancreas



Ovarian cancer

- Homologous recombination repair pathway alterations
- *BRCA1* and *BRCA2*
  - germline mutations
  - somatic mutations
  - epigenetic silencing

Ovarian cancer

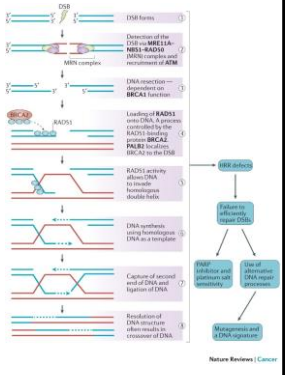
- Homologous repair pathway alterations
- *BRCA1* and *BRCA2*
  - germline mutations
  - somatic mutations
  - epigenetic silencing (promoter methylation)

BRCA-ness

Ovarian cancer

- Homologous repair pathway alterations

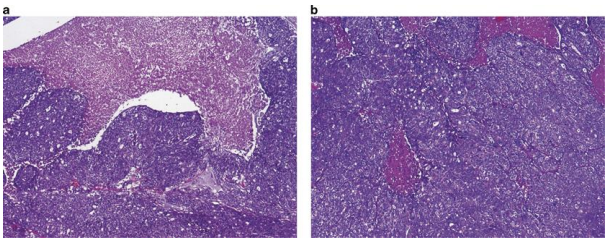
  50% of High-grade serous carcinoma
  - inability to repair ds DNA breaks
  - high levels of genomic instability
  - relatively better outcome compared to tumors with intact BRCA pathway
  - susceptibility to DNA-damaging agents
    - platinum based therapy
    - poly (ADP-ribose)/ PARP inhibitors



Ovarian cancer

Pathologic features of high-grade serous carcinoma with *BRCA* mutations

- SET-phenotype (Solid, pseudoEndometrioid, and Transitional cell carcinoma-like morphology
- Higher mitotic index
- More TILs (tumor infiltrating lymphocytes; *BRCA1*)
- Necrosis (geographic or comedo)

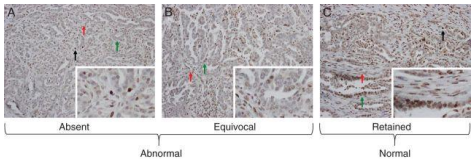


Modern Pathology (2012) 25, 625–636

Ovarian cancer

Testing for BRCA

- Mutational analysis (germline and somatic)
- Immunohistochemistry (BRCA1)



- Functional assays – HR deficiency

Ann Oncol. 2014 Dec; 25(12): 2372–2378

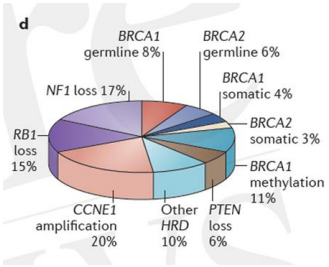
Ovarian cancer

THE CANCER GENOME ATLAS
National Cancer Institute
National Human Genome Research Institute

- Molecular classification
  - Mutational profiles (germline and somatic)
  - Gene expression patterns
  - Immunoprofiling

Ovarian cancer
Molecular classification

- High grade serous carcinoma
  - ➢ TP53 mutations - nearly ubiquitous
  - ➢ point mutations in other genes - relatively uncommon
  - ➢ prevalent structural variations
  - ➢ high levels of genomic instability

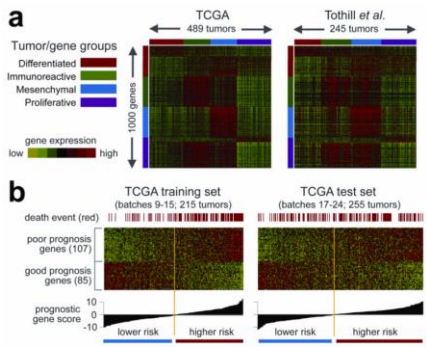


Class C malignancy

Nat Rev Cancer. 2015 Nov; 15(11): 668–679

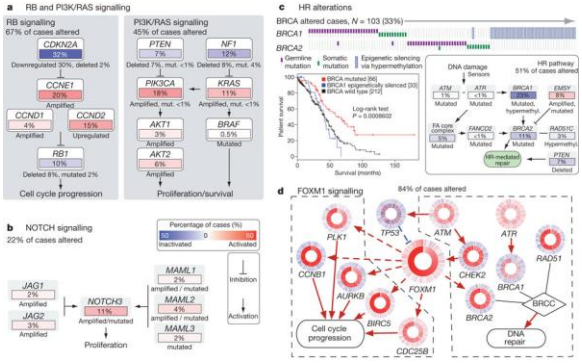
Ovarian cancer
Molecular classification

- Gene expression profiles - 4 types
  - C1 – Mesenchymal
  - C2 – Immunoreactive
  - C4 – Differentiated
  - C5 – Proliferative



Nature. 2011; 474:609–615

Altered pathways in HGS-OvCa



D Bell *et al. Nature* **474**, 609-615 (2011) doi:10.1038/nature10166

nature

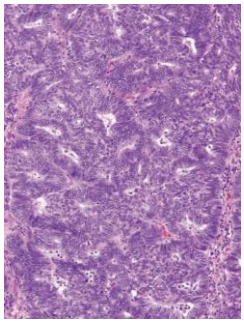
Ovarian cancer

- Non-high-grade serous carcinoma
  - Clear cell carcinoma: *PIK3CA, ARID1A,* occasionally *TP53*
  - Endometrioid carcinoma: *CTNNB1, ARID1A*, and *PIK3CA*
  - Mucinous carcinoma: *KRAS, ERBB2*/her2
  - Low-grade serous carcinoma: *RAS/RAF, ERBB2*/her2

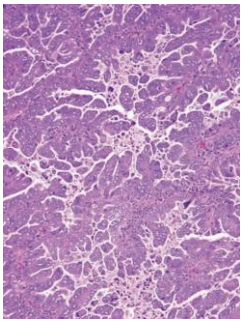
Endometrial cancer

– Morphologic classification

Type I – endometrioid

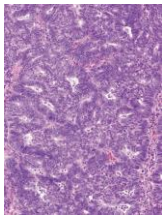


Type II –serous



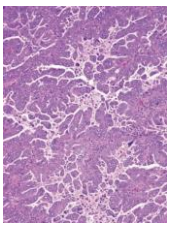
Endometrial cancer

- **Type I**
  - wide age span
  - associations: obesity, unopposed estrogenic stimulation
  - express ER and PR
  - precursor: endometrial hyperplasia
  - PTEN mutation prevalent

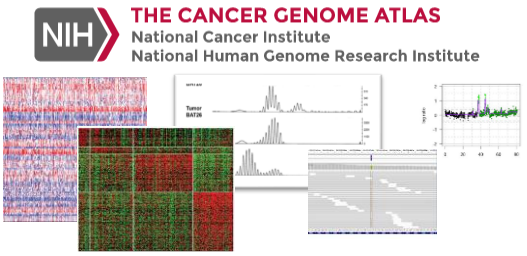


Endometrial cancer

- **Type II**
  - postmenopausal (over 60 years of age)
  - loss of ER and PR expression
  - background: atrophic endometrium
  - precursor: endometrial intraepithelial carcinoma
  - TP53 mutation nearly ubiquitous

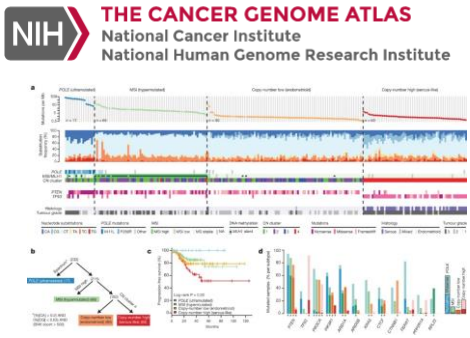


The Cancer Genome Atlas - TCGA



- ❑Mutational repertoire
- ❑Copy number alterations
- ❑Methylation profiles
- ❑Expression profiles
- ❑Microsatellite instability

Endometrial cancer
Molecular classification



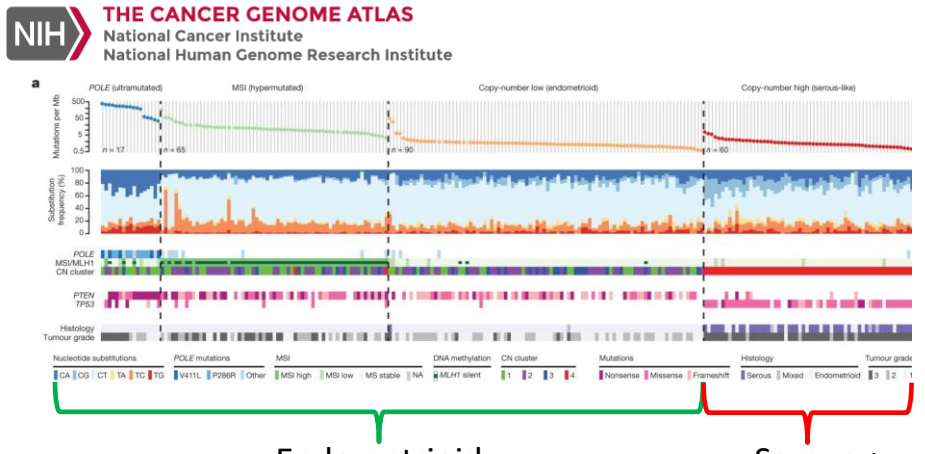
nature

G Getz *et al. Nature* 497, 67-73 (2013)

Samples – 373 tumors:
- ❑ 307- endometrioid
- ❑ 66 - serous

Four molecular subtypes:
- ❑ POLE - ultramutated
- ❑ MSI - hypermutated
- ❑ Copy-number low
- ❑ Copy-number high

Endometrial cancer
Molecular classification



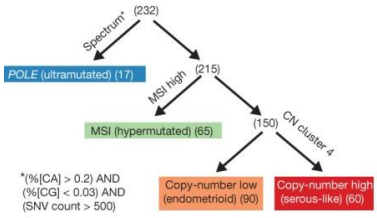
nature

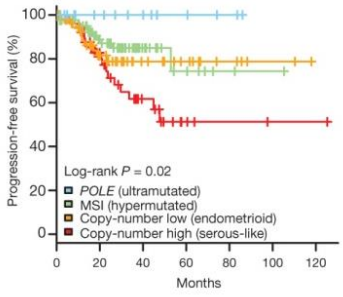
G Getz *et al. Nature* 497, 67-73 (2013)

Endometrial cancer
Molecular classification

THE CANCER GENOME ATLAS
National Cancer Institute
National Human Genome Research Institute

Integrative analysis – 4 types
- POLE (ultramutated)
- MSI group (hypermutated)
- Copy number low (endometrioid)
- Copy number high (serous-like)





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Endometrial cancer
Molecular classification

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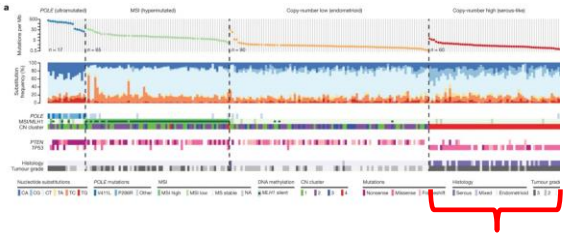

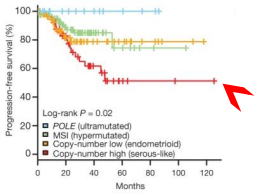

Copy-number high – serous-like
- extensive copy number alterations
- lowest mutation rate
- serous + some, grade 3 endometrioid
- *TP53* mutations – 90%

Endometrial cancer
Molecular classification

THE CANCER GENOME ATLAS
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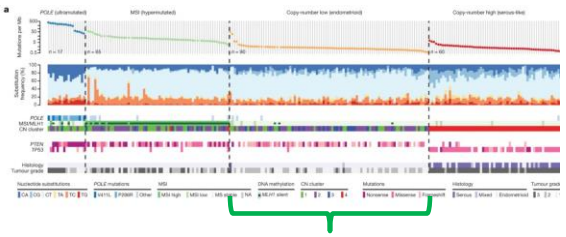
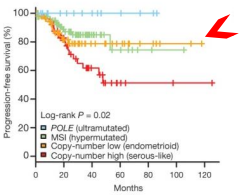
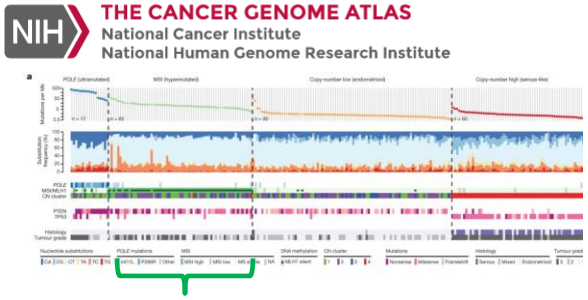
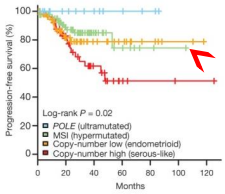




Copy-number low - endometrioid
- Microsatellite stable
- Low mutation rate
- Low level copy number alterations
- *CTNNB1 (*β - catenin*)* mutations ~ 50%

Endometrial cancer
Molecular classification

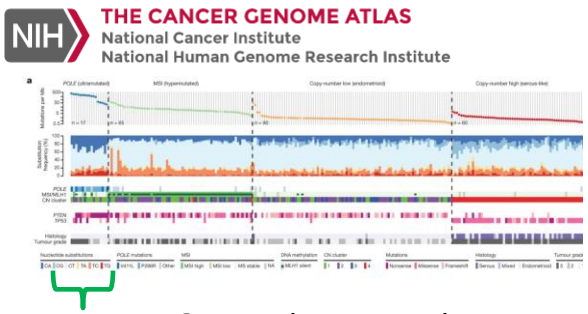


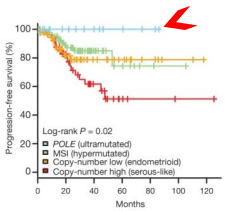


MSI – hypermutated

- Microsatellite instable, MLH1 methylation
- high mutation rate – median ~ 600
- few copy number alterations
- endometrioid histology
- *PTEN, KRAS* mutations

Endometrial cancer
Molecular classification



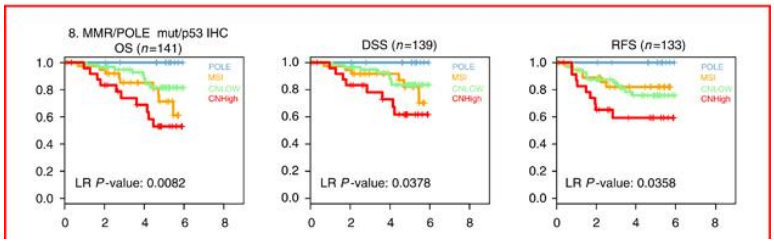


POLE – ultramutated

- hot spot mutations in *POLE*
- very high mutation rate – median ~ 9000
- high grade/ ambiguous histology
- *TP53* mutations – up to 35%

Endometrial cancer
Molecular classification

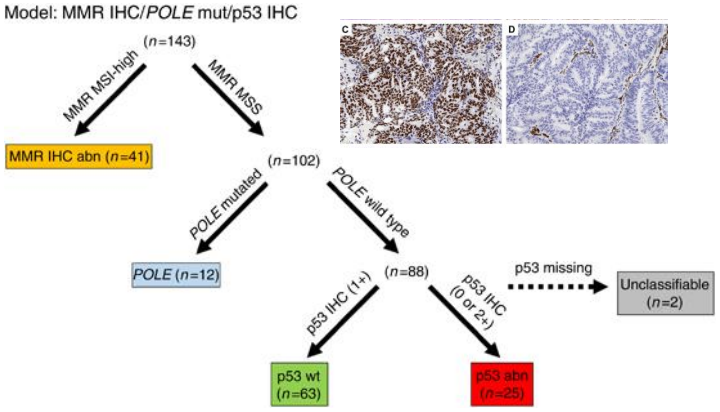
- TCGA classification - reproduced
  - POLE mutational analysis
  - MMR immunohistochemistry
  - p53 immunohistochemistry



British Journal of Cancer (2015) 113, 299–310

Endometrial cancer
Molecular classification

- Clinically applicable algorithm -



British Journal of Cancer (2015) 113, 299–310

Lynch syndrome – Hereditary nonpolyposis colorectal carcinoma (HNPCC)

- Autosomal dominant (highly penetrant)
- Defects in mismatch repair system (MMR)
- Implicated genes: MLH1, MSH2, MSH6, and PMS2 (also deletions of EpCAM)
- Endometrial cancer – 2-5% Lynch syndrome
- Risk of endometrial cancer through age of 70 years
  16-61%
- Risk of ovarian cancer through age of 70 years
  5-10%

Lynch syndrome (HNPCC)

- Risk of endometrial cancer by the age of 70 years
  - MLH1 mutation carriers – 20-54%
  - MSH2 mutation carriers – 21-49%
  - MSH6 mutation carriers – 16-61%
    (later onset)
- At least 50% of documented cases – presenting cancer in gyn tract
- A decade earlier than colon cancer (if presenting with endometrial cancer)

Lynch syndrome (HNPCC)

- Clinical and pathologic features
  - younger age at presentation
    (mean age 47-49 years)
  - location – lower uterine segment (10-30%)
  - most commonly endometrioid type
    non-endometrioid histology is also seen,
    undifferentiated/dedifferentiated type
  - tumor heterogeneity
  - tumor infiltrating lymphocytes/ peritumoral lymph(s)

Lynch syndrome (HNPCC)

- Clinical features
  - younger age at presentation

Insufficient predictive value in identifying Lynch syndrome

  - most commonly endometrioid type
    non-endometrioid histology is also seen,
    undifferentiated/dedifferentiated
  - tumor heterogeneity
  - tumor infiltrating lymphocytes/ peritumoral lymph(s)

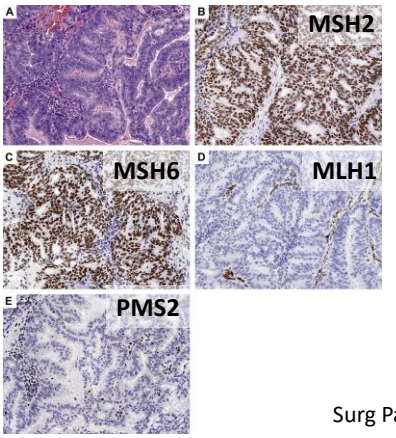
Endometrial cancer
Microsatellite instability (MSI)

- Loss of MMR function ⇨ MSI-H
- Causes of MSI-H
  - germline mutations in MMR genes (Lynch)
  - epigenetic alterations
    - MLH1 promoter methylation
- 20-25% of all endometrial cancers are MSI-H
  - of these 75% due to MLH1 promoter methylation

Endometrial cancer
Microsatellite instability (MSI)

- Lynch syndrome screening

  Immunohistochemistry - MMR gene products



Surg Pathol Clin. 2016 Jun;9(2):201-14

Endometrial cancer
Microsatellite instability (MSI)

- Lynch syndrome screening

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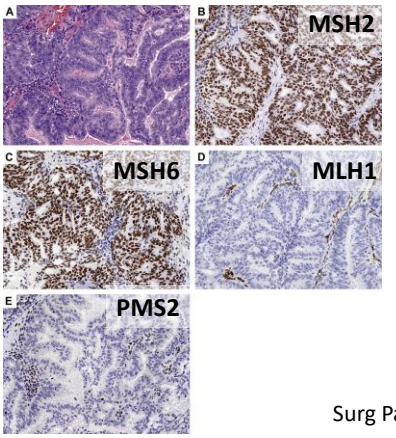


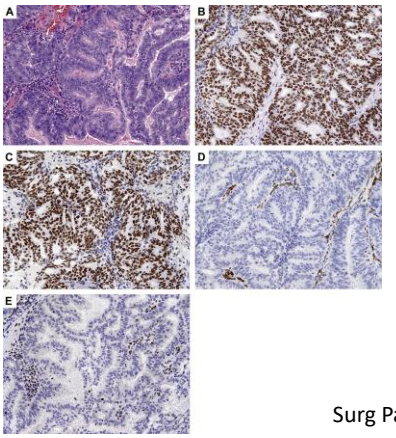
MLH1 promoter methylation

Surg Pathol Clin. 2016 Jun;9(2):201-14

Endometrial cancer
Microsatellite instability (MSI)

- Lynch syndrome screening

  Immunohistochemistry - MMR gene products



MLH1 promoter methylation

? BRAF mutational analysis

Surg Pathol Clin. 2016 Jun;9(2):201-14

Endometrial cancer
Microsatellite instability (MSI)

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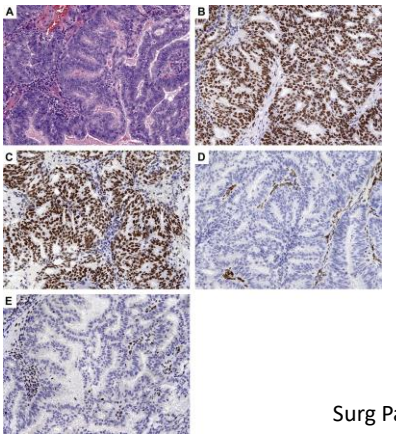
  - Immunohistochemistry - MMR gene products



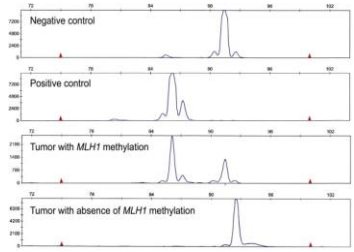
MLH 1 promoter methylation

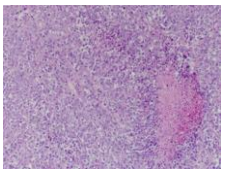
BRAF mutational analysis

Surg Pathol Clin. 2016 Jun;9(2):201-14

Endometrial cancer
Microsatellite instability (MSI)

- MLH1 promoter methylation
  - 75% of MSI-H cancers
  - Indicator of sporadic nature of cancer
  - Older age of onset
  - Higher grade endometrioid tumors
  - Undifferentiated histology





Endometrial cancer
Microsatellite instability (MSI)

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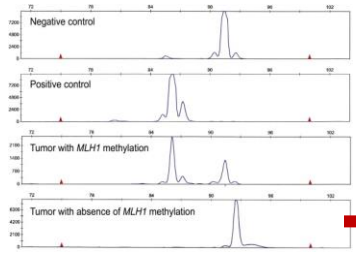
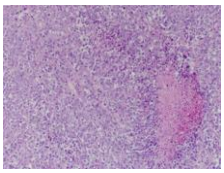
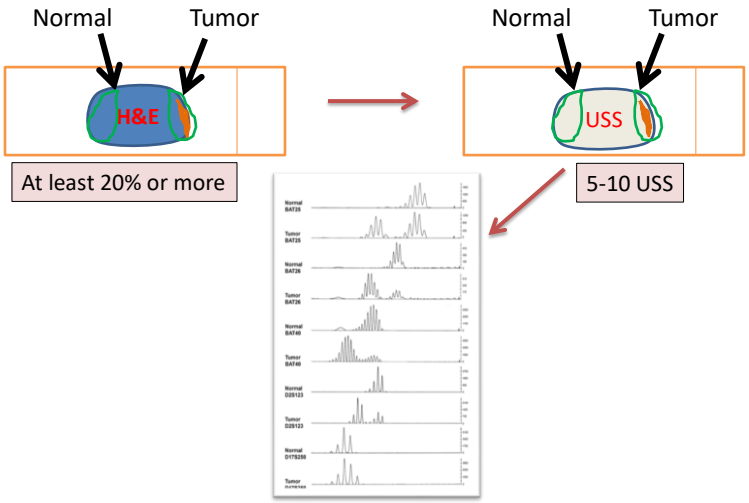




Further testing

Endometrial cancer
Microsatellite instability (MSI)



Endometrial cancer
Microsatellite instability (MSI)

- Testing of all endometrial carcinomas
  vs.
  Testing endometrial cancer before age 60
- IHC
  vs.
  PCR-based method
  vs.
  Both

Endometrial cancer
Microsatellite instability (MSI)

- Testing of all endometrial carcinomas
  vs.
  Testing endometrial cancer before age 60
- IHC
  vs.
  PCR-based method
  vs.
  Both

Endometrial cancer
Microsatellite instability/ Lynch syndrome

For patients with Lynch syndrome

- Early screening for colorectal cancer
- Endometrial cancer screening and prophylaxis
  - f/u with regular endometrial sampling
  - hormonal suppression (OCP or progestins)
  - hysterectomy

For patients with MSI endometrial cancer
Lynch and sporadic

- Treatment decisions = personalized medicine

Endometrial cancer
Microsatellite instability (MSI)

- Significant responses to anti-PD-1 inhibitors in patients who failed conventional therapy
- Somatic hypermutation and neoepitope formation correlates with response to immunotherapy
- MSI - as a biomarker for PD-1 blockade

FDA U.S. FOOD & DRUG ADMINISTRATION

FDA News Release

FDA approves first cancer treatment for any solid tumor with a specific genetic feature

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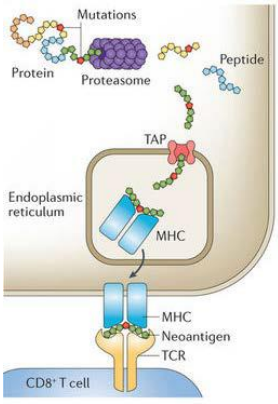
For Immediate Release May 23, 2017

<u>Pembrolizumab/ Keytruda ©</u>

For any adult or pediatric solid tumor with dMMR/MSI-H that have progressed on prior therapy

Why MSI-H tumors respond to immunotherapy?

Mutations → **Neoantigens** → **Recognition by immune system**



Nat Reviews Genetics (2016) vol 17

Mutational signatures

Mutational signature – mutation types arising from a specific cause/mutagenic process
- DNA replication errors
- Genotoxins/mutagens
- DNA repair errors

Mutational signatures associated with high TMB
- MMR - small indels at mononucleotide repeats - MSI
- *POLE* - C > A (in TpCpT; TCT > TAT)
- UV exposure
- Smoking

Mutational signatures

Mutational signature – mutation types arising from a specific cause/mutagenic process
- DNA replication errors
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Mutational signatures associated with high TMB
- MMR
- *POLE*

} common in endometrial and colon cancer

- UV exposure
- Smoking

Tumor mutational burden

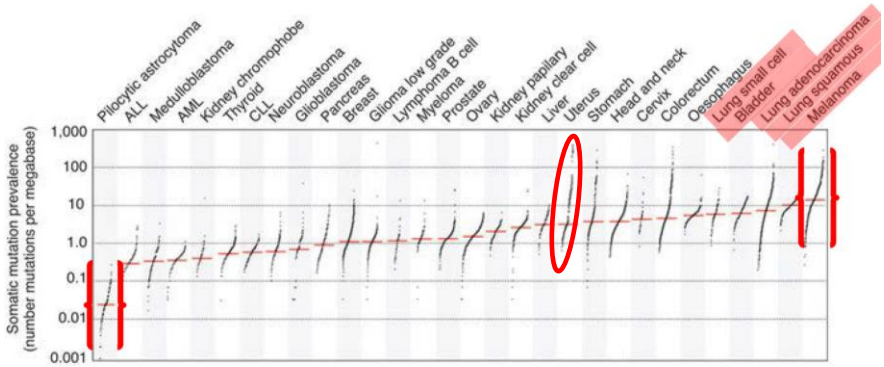
TMB – number of non-synonymous mutations per tumor exome (genome coding regions)
Measured as – number of mutations/Mb

What is TMB – high?
>200 exome
> 10 or 20/Mb

Do we need to sequence exome?

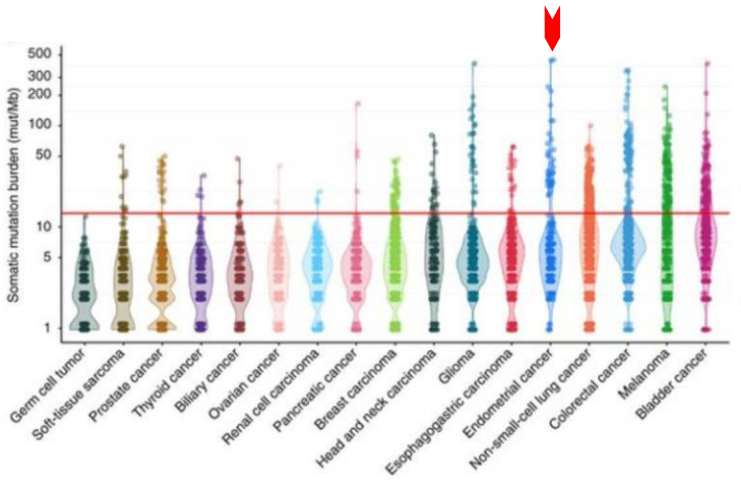
Does targeted panel provides enough information?

Tumor mutation burden



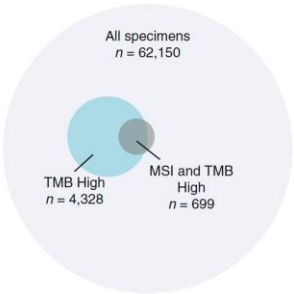
Nature biotechnology 34, 1019-1024 (2016)

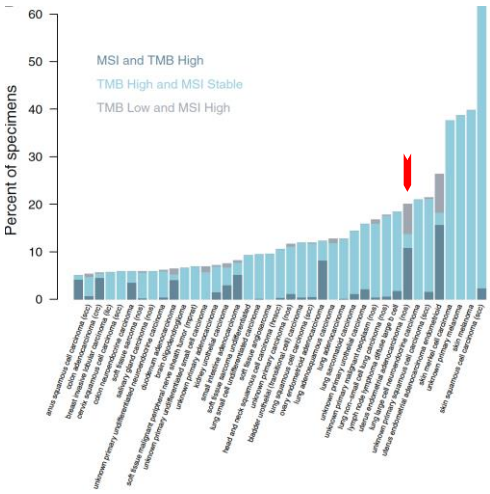
Tumor mutation burden



Nature Medicine (2017); 23(6): 703

TMB and MSI correlation





Genome Medicine (2017), 9:34

Other potential targets

- BRCA mutation carriers/HRD
- PTEN mutations
- PiK3CA mutations
- ARID1A mutations
- FGFR2 mutations
- etc….

Endometrial cancer Clinical trials

Table 5
On-going clinical trials in advanced and/or recurrent endometrial cancer.

Drug(s)	Phase	Patient eligibility	Trial number
C + T ± Metformin	II, III	Stage III, IVA, IVB or recurrent EMCA	NCT02065687
Bev + C + T vs. C + Bev + Ixabepilone vs. T + C + Temsirolimus	II	Stage III, IVA, IVB or recurrent EMCA	NCT00977574
A + P + T vs. C + T	III	Stage III, stage IV or recurrent EMCA, estrogen receptor (ER)/progesterone receptor (PR) status	NCT00602992
Fulvestrant	II	Recurrent, persistent, or metastatic endometrial cancer that is not curable with surgery or radiotherapy	NCT00000903
C + T + Dasatinib	I	Stage III, stage IV, or recurrent EMCA	NCT01440998
P + T + C + RT	III	Stage III or IVA, stage I or II clear cell or serous carcinoma with positive peritoneal cytology	NCT00942357
Nivolumab ± Cabozantinib	II	Advanced, recurrent or metastatic EMCA	NCT03307741
P + RT + AZD1775	I	Stage I-III vaginal cancer, stage I-IIIB cervical cancer, stage I-IIIC uterine cancer	NCT03401784
Edibulin mesylate + G	I	Recurrent or Stage III-IV endometrial or ovarian cancer	NCT00410553
Dasatinib + P	II	Stage IVb or recurrent EMCA	NCT01401750
Everolimus + Letrozole vs. Tamoxifen + Medroxyprogesterone	II	Stage III or IV or recurrent EMCA	NCT02220881
Letrozole + RADOO1 (Everolimus)	II	Advanced or recurrent EMCA	NCT01068349
Metformin + Letrozole + Everolimus	II	Advanced or recurrent EMCA	NCT01797523
T + C + Selinexor	I	Ovarian, fallopian tube or primary peritoneal cancer, endometrial cancer. EMCA must be stage IVB or recurrent disease	NCT02269293
Ribociclib + Everolimus + Letrozole	II	Advanced or recurrent endometrial carcinoma that is refractory to curative treatment	NCT03008408
Pembrolizumab + A	II	Advanced EMCA, prior platinum-based chemotherapy for first-line treatment has failed	NCT03276013
Enzalutamide + C + T	II	Stage III or IV or recurrent endometrioid EMCA	NCT02684227
Olaparib + metformin + cyclophosphamide	I, II	Recurrent EMCA after platinum-based therapy	NCT02755844
Nintedanib + C + T	II	Stage IIIC1, IVA, IVB and recurrent EMCA	NCT02730416
Letrozole ± Palbociclib	II	Advanced or recurrent EMCA	NCT02730429
Pembrolizumab + T + C	II	Stage III or IV or recurrent EMCA	NCT02549209
MEDI0612 + T + C	I,II	Advanced, metastatic or recurrent EMCA and SCLC	NCT03366480
IMGN853 + Bev vs. IMGN853 + C vs. IMGN853 + PLD vs. IMGN853 + Pembrolizumab	I	Advanced ovarian cancer, primary peritoneal cancer, fallopian tube cancer, or EMCA	NCT02606305
T ± MLN0128, MLN0128 ± MLN1117	II	Advanced, recurrent, or persistent EMCA that has relapsed or is refractory	NCT02725268
C + cyclophosphamide + atezolizumab	I	Advanced breast or advanced gynecologic cancer	NCT02914470
Temsirolimus + Cediranib	I	Advanced gynecologic malignancies	NCT01865162
Vigil + Durvalumab	II	Locally advanced or metastatic women's cancer	NCT02725489
GSK2	I	Advanced or recurrent gynecologic malignancies	NCT02433626
Temsirolimus + nivolumab vs. Irinotecan + nivolumab vs. Irinotecan + capecitabine + nivolumab	I,II	Metastatic tumors (including EMCA)	NCT02423954
Pembrolizumab	II	Persistent or recurrent EMCA	NCT02880783
Niraparib	II	Recurrent EMCA	NCT03016338
LY3023414	II	Persistent or recurrent EMCA	NCT02549989
Brachytherapy + RT ± P	II	Recurrent EMCA	NCT00492778
Durvalumab ± tremelimumab	II	Recurrent or persistent EMCA	NCT03015129
Lenvatinib + T	I	Recurrent EMCA and ovarian cancer	NCT02780700
Tazemetostat	II	Recurrent ovarian, primary peritoneal and EMCA	NCT03348631
Transtinib ± GSK2141795	I	Recurrent or persistent EMCA	NCT01930973
Sunitinib malate	II	Recurrent or metastatic EMCA	NCT00459420
Durvalumab + RT ± tremelimumab	I	Metastatic or unresectable gynecologic cancers	NCT03277482
Letrozole + ribociclib	II	Recurrent ovarian, fallopian tube, primary peritoneal or EMCA	NCT02657928
G + nivrerolimab (BIGN853)	I	Flt3-positive recurrent ovarian, primary peritoneal, fallopian tube, endometrial, triple negative breast cancer	NCT02996825
Surgery + HIPEC P + C or T or PLD or G (P or IV at the discretion of the oncologist)	–	Stage IIcIV primary or recurrent ovarian, fallopian tube, peritoneal carcinoma or EMCA	NCT01970722
Dasatinib	II	Recurrent or persistent ovarian, fallopian tube, endometrial or peritoneal cancer.	NCT02059265
Sodium cridanimod	II	Recurrent or persistent EMCA	NCT02064725
Epacadostat + pembrolizumab	II	Recurrent/metastatic EMCA	NCT03333967
Avelumab	II	MSI, MSI-H and POLE-mutatyr recurrent or persistent EMCA	NCT02912572
ONC201	II	Metastatic or recurrent EMCA	NCT03099499
Olaparib + AZD2014 (2 dosing regimens) vs. Olaparib + AZD5363	I,II	Recurrent endometrial, triple negative breast and ovarian, primary peritoneal or fallopian tube cancer	NCT02208375
AL3818	I,II	Recurrent or metastatic endometrial, ovarian or cervical cancer	NCT02558348
Pembrolizumab + T + C	II	Stage III or IV or recurrent	NCT02549209
C + T vs. trastuzumab	II	Advanced/recurrent uterine serous papillary carcinoma	NCT01307902
AL3818 + C + T	I,II	Recurrent, persistent or metastatic endometrial, ovarian, fallopian, primary peritoneal or cervical carcinoma	NCT02584478

C = carboplatin, T = paclitaxel, A = Adriamycin/doxorubicin, Bev = bevacizumab, P = cisplatin RT = radiation therapy, PLD = pegylated liposomal doxorubicin hydrochloride, HIPEC = hyperthermic intraperitoneal chemotherapy, G = gemcitabine.

Adopted from Arend et al Gynecol Oncol (2018)

Thank you!