Current Status of Biomarkers (including DNA Tumor Markers and Immunohistochemistry in the Laboratory Diagnosis of Tumors)

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Outline

- Update to DNA Testing in cervical dysplasia/cancer
- Breast cancer molecular markers
- Colon cancer molecular markers

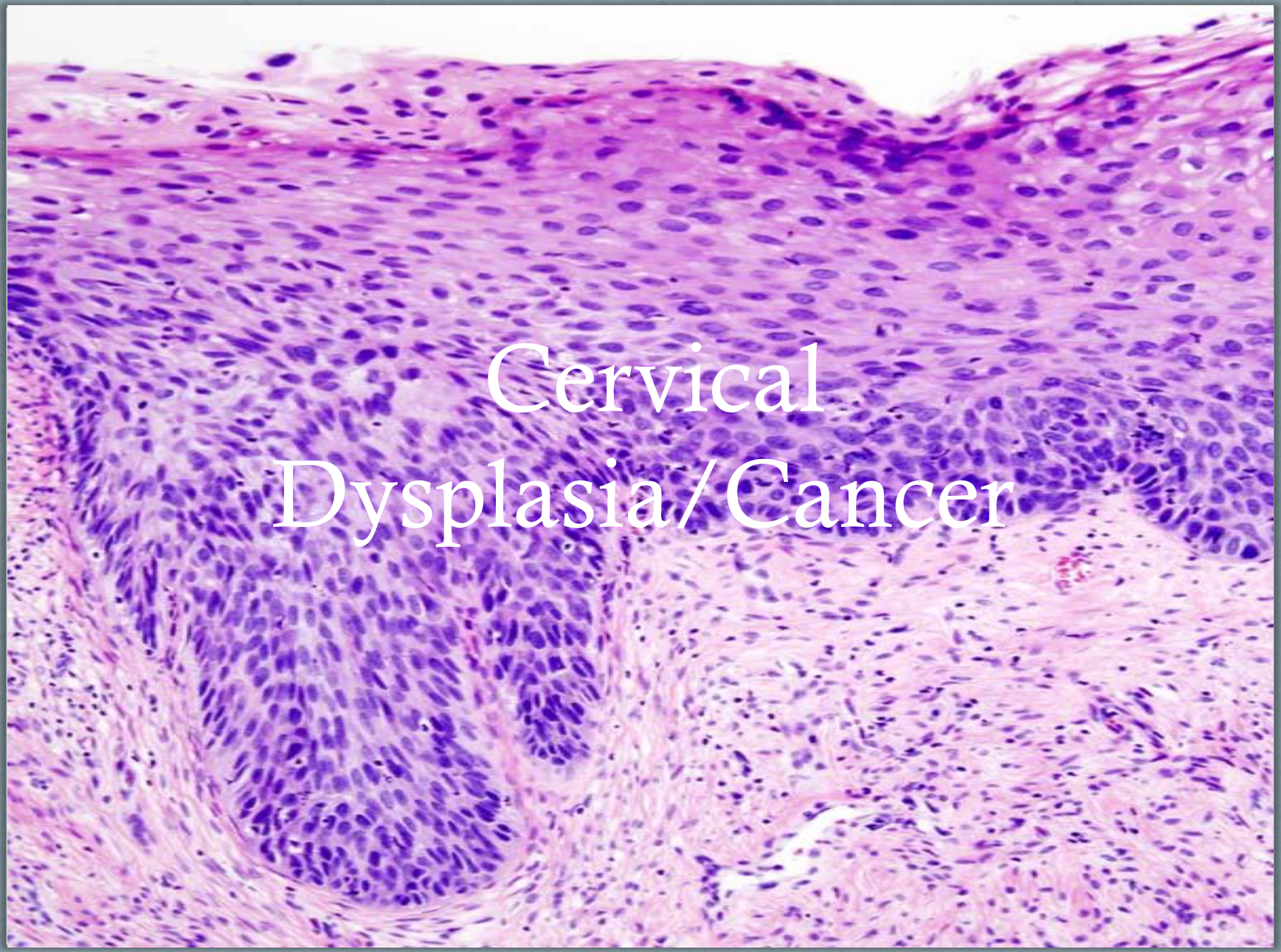


Image from: http://commons.wikimedia.org/wiki/File:Cervical_intraepithelial_neoplasia_(4)_CIN3.jpg

Cervical Dysplasia and Cancer

- High Risk Human Papillomavirus (hrHPV):
  - Presence required for development of cervical cancer.
  - Vast majority of hrHPV is self limited and cleared within 2 years.
  - Small percentage (10%) persists and becomes oncogenic.
  - HPV 16 and 18 types cause 70% of cervical cancer.

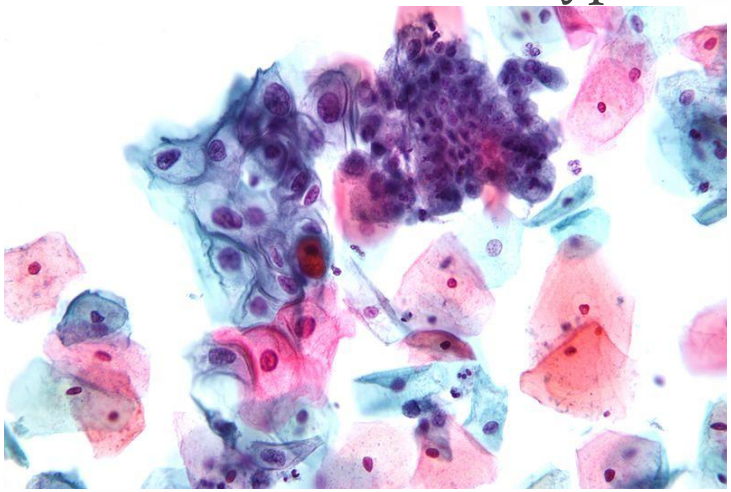


Image from: http://commons.wikimedia.org/wiki/File:Low-grade_sil_and_endocx.jpg

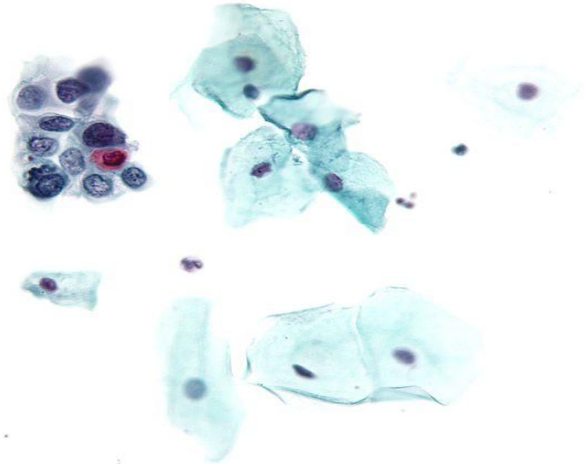
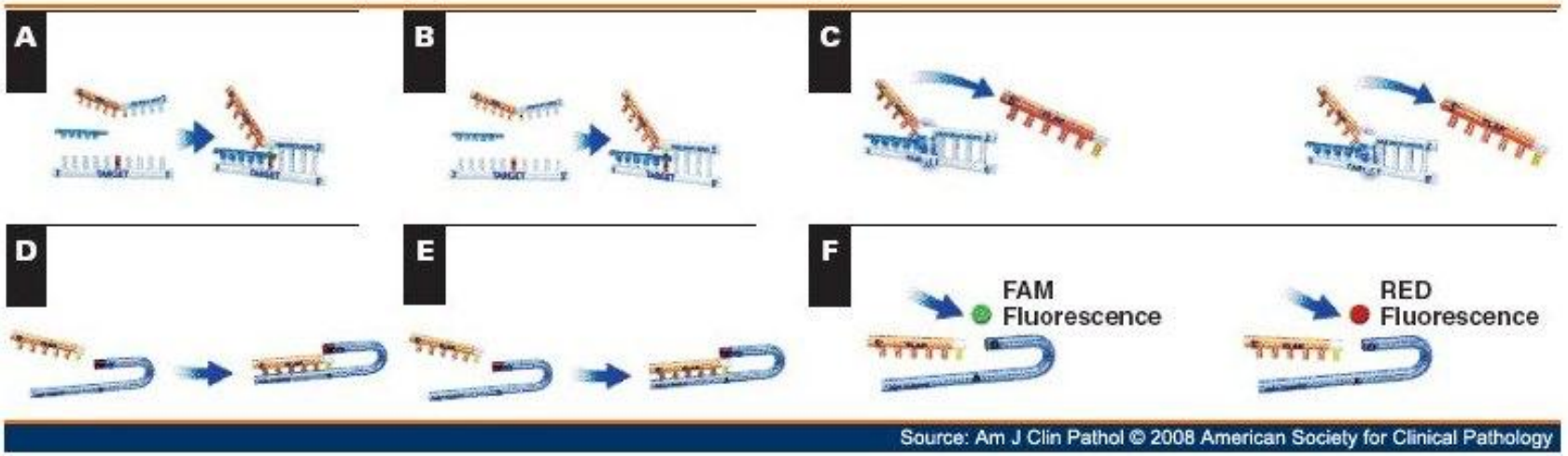


Image from: http://commons.wikimedia.org/wiki/File:High-grade_squamous_intraepithelial_lesion.jpg

Cervical Dysplasia and Cancer

- High Risk DNA Testing:
  - Approved for use by the FDA in 2011
  - Specifically detects HPV 16 and 18 along with 12 other less common hrHPV types.
    - Identifies 14 hrHPV types

Figure 1. Graphic Representation of an Invader-based HPV Reaction.



Source: Am J Clin Pathol © 2008 American Society for Clinical Pathology

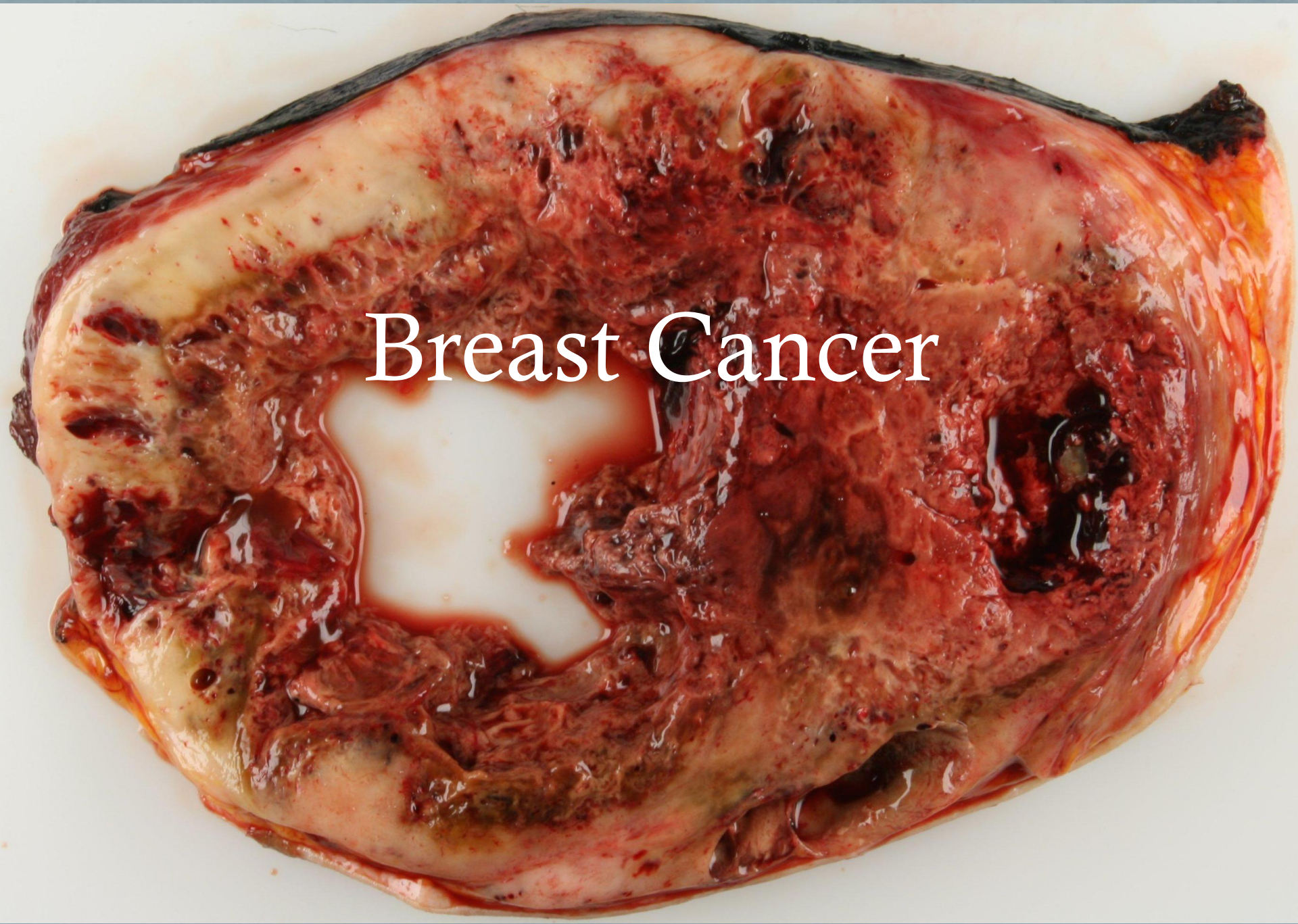
Current Recommendations

Aged <21 y	521-522	No screening		HPV testing should not be used for screening or management of ASC-US in this age group
Aged 21-29 y	522-523	Cytology alone every 3 y	HPV-positive ASC-US[†] or cytology of LSIL or more severe: Refer to ASCCP guidelines[2] Cytology negative or HPV-negative ASC-US[†]: Rescreen with cytology in 3 y	HPV testing should not be used for screening in this age group
Aged 30-65 y	523-529	HPV and cytology "cotesting" every 5 y (preferred)	HPV-positive ASC-US or cytology of LSIL or more severe: Refer to ASCCP guidelines[2] HPV positive, cytology negative: Option 1: 12-mo follow-up with cotesting Option 2: Test for HPV16 or HPV16/18 genotypes • If HPV16 or HPV16/18 positive: refer to colposcopy • If HPV16 or HPV16/18 negative: 12-mo follow-up with cotesting Cotest negative or HPV-negative ASC-US: Rescreen with cotesting in 5 y	Screening by HPV testing alone is not recommended for most clinical settingst
		Cytology alone every 3 y (acceptable)	HPV-positive ASC-US[†] or cytology of LSIL or more severe: Refer to ASCCP guidelines[2] Cytology negative or HPV-negative ASC-US[†]: Rescreen with cytology in 3 y	
Aged >65 y	529-531	No screening following adequate negative prior screening		Women with a history of CIN2 or a more severe diagnosis should continue routine screening for at least 20 y
After hysterectomy	531	No screening		Applies to women without a cervix and without a history of CIN2 or a more severe diagnosis in the past 20 y or cervical cancer ever
HPV vaccinated	531-533	Follow age-specific recommendations (same		

Saslow, D; et all; American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening Guidelines for the Prevention and Early Detection of Cervical Cancer; 2012 American Jnl of Clin Path, 137, 516-542.

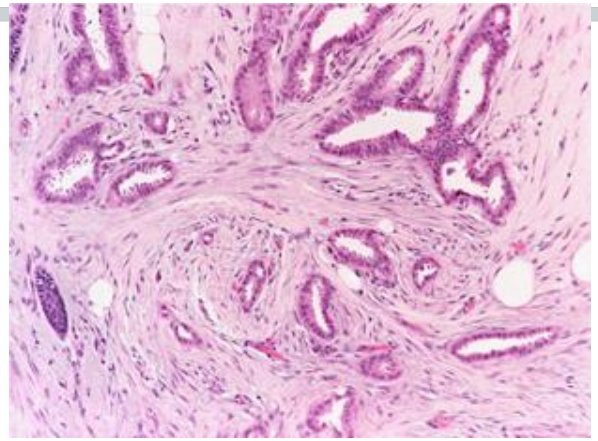
Cervical Dysplasia and Cancer

- hrHPV DNA testing
  - April 24 2014, approved by FDA for a screening tool for cervical cancer.
  - Can be used alone for women 25 years and older.
  - If HPV 16 or 18 is detected, the FDA recommends a colposcopy.
  - If the less common hrHPV types are found, the FDA recommends a PAP smear to determine if a colposcopy is needed.


Breast Cancer

Breast Cancer

- Currently, invasive breast cancer is evaluated in the following manner:
  - How much glandular formation is present in the tumor?
    - Given a score of 1 to 3; 1 means most tumor cells in gland formation; 3 means few to no tumor cells in gland formation.
  - How pleomorphic are the tumor cells?
    - Given a score of 1 to 3; 1 means tumor cells are small and uniform; 3 means tumor cells are very pleomorphic.
  - How mitotically active is the carcinoma?
    - Given a score of 1 to 3; 1 means little to no mitotic activity, 3 means prominent mitotic activity.
    - Histologic grading:
  - Total of the histologically observed tumor features gives the histologic grade of the tumor.
    - Total score of 3-5 = Well differentiated carcinoma.
    - Total score of 6-7 = Moderately differentiated carcinoma.
    - Total score of 8-9 = Poorly differentiated carcinoma.
  - Histologic grade strongly correlates with Disease Free Survival and Overall Survival



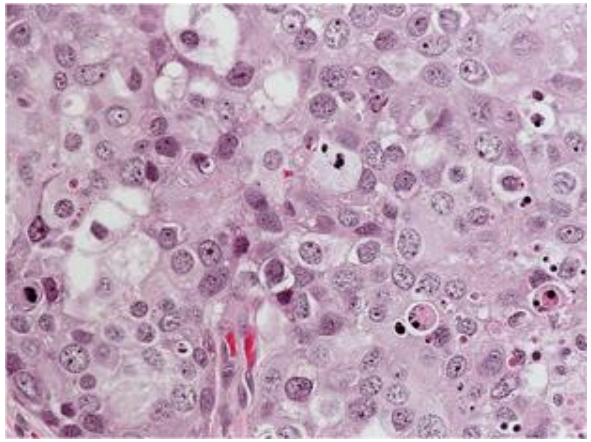


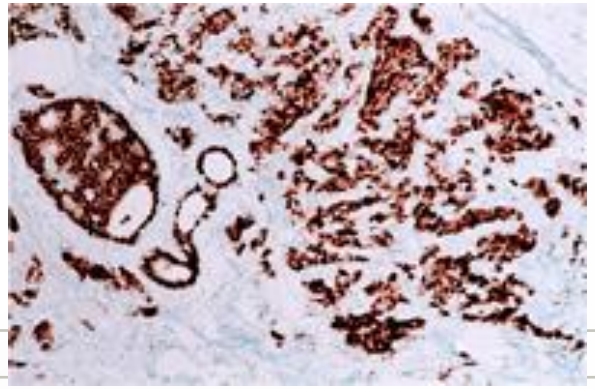
Image from: http://pathology.jhu.edu/breast/grade.php

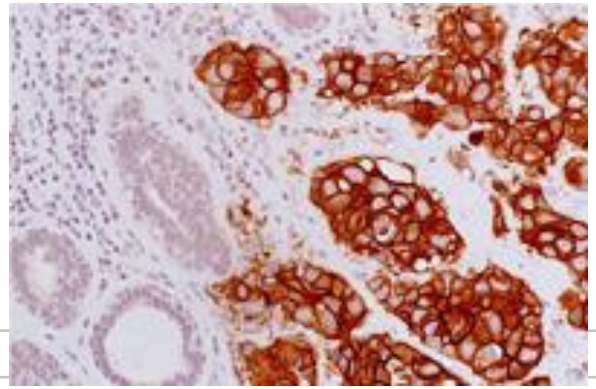
Breast Cancer

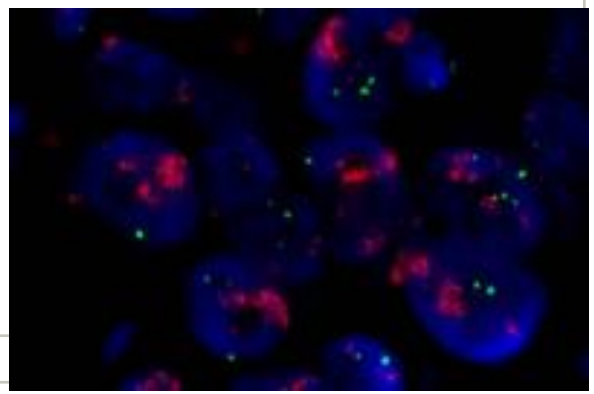
- Other ways of to look at breast cancer prognosis:
  - Molecular classification.
  - Multigene classifiers.
    - i.e. Oncotype Dx

Breast Cancer

- Do the tumor cells express estrogen (ER) progesterone (PR) or herceptin (HER2) receptors?
  - Graded on percentage of tumor cells expressing each receptor and the strength of the immunohistochemical (IHC) staining seen on the cells.



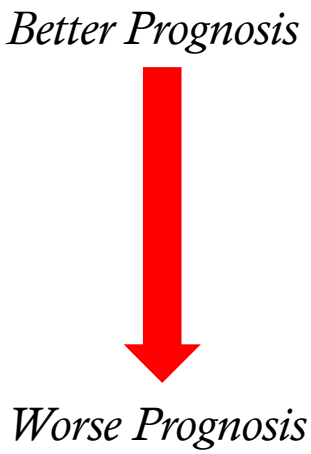




Images from: http://pathology.jhu.edu/breast/biomarker-testing.php

Breast Cancer

- Molecular Classification:
  - ER+/HER2-; low mitotic rate: Luminal A
  - ER+/HER2-; high mitotic rate: Luminal B
  - ER+/HER2+: Luminal HER2
  - ER-/HER2+: HER2 Enriched
  - ER-/PR-/HER2-: Basal-like
  - ER-/PR-/HER2-: Normal-like



Breast Cancer

- Multigene Classifiers
  - Looking for recurrent amplifications and deletions in genes by IHC, FISH, and RNA.
    - If present, represents a more aggressive clinical course (High risk)
  - Not ready for everyday clinical use.
    - Dependent on proliferation.
    - Don't take into account genetic or racial differences.
    - Has not been shown to be significantly better than traditional prognostic markers.
    - Expensive.

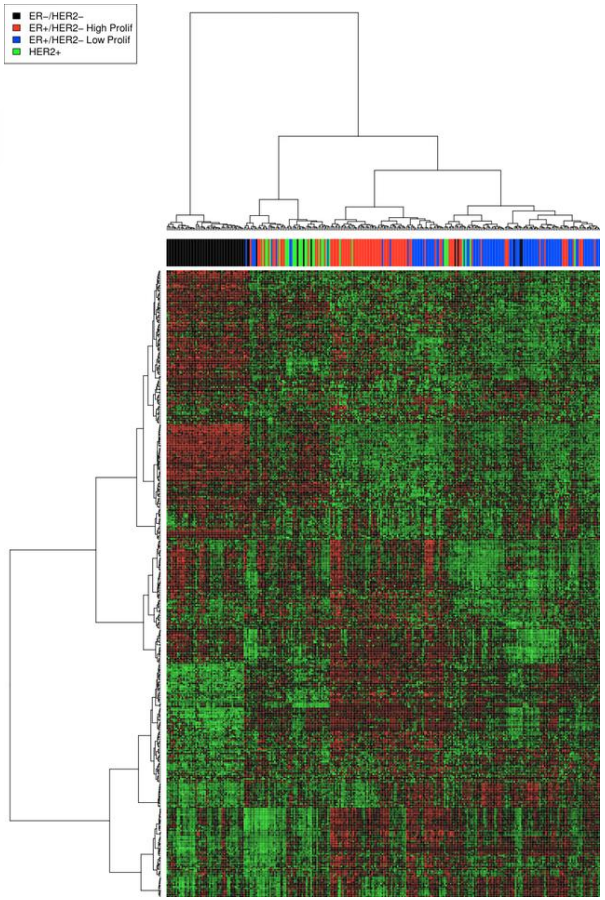


Image from: http://en.wikipedia.org/wiki/Breast_cancer_classification

Breast Cancer

- How is this information being used?
  - ER, PR, and HER2 receptor presence or absence leads to use of different therapy.
    - Endocrine therapy can be used in a patient with any strength of ER staining in greater than 1% of invasive tumor cells.
    - Anti-HER2 therapy can be used in a patient with:
      - HER2 positivity by IHC
        - Defined as circumferential membrane staining that is complete and intense, observed in a homogeneous population and within greater than 10% of the invasive tumor cells (as defined by ASCO-CAP guidelines).
      - HER 2 positivity by in situ hyberization (ISH)
        - Defined as HER2/CEP17 greater than 2.0 (as defined by ASCO-CAP guidelines)

Breast Cancer

- How is this information currently being used?
  - ER, PR, and HER2 receptor presence or absence leads to use of different therapy.
    - Chemotherapy can be used:
      - In HER2+ patients (with anti-HER2 therapy).
      - Triple negative disease (ER-, PR-, HER2-).
      - ER+/HER2-…Sometimes.

When to Use Chemotherapy in ER+/HER2- Patients

	Relative indications for chemoendocrine therapy	*Factors not useful for decision*	*Relative indications for endocrine therapy alone*
Clinicopathological features			
ER and PgR	**Lower ER and PgR level**		**Higher ER and PgR level**
Histological grade	**Grade 3**	**Grade 2**	**Grade 1**
Proliferation	**Higha**	**Intermediatea**	**Lowa**
Nodes	**Node positive (four or more involved nodes)**	**Node positive (one to three involved nodes)**	**Node negative**
PVI	**Presence of extensive PVI**		**Absence of extensive PVI**
pT size	**>5 cm**	**2.1–5 cm**	**≤2 cm**
Patient preference	**Use all available treatments**		**Avoid chemotherapy-related side-effects**
Multigene assays			
	High score	**Intermediate score**	**Low score**

Goldhirsch A, et al; Thresholds for therapies: highlights of St. Gallen International Expert Consensus on the Primary Therapy of early breast Cancer 2009; Annals of Oncology, 20:1319-1329(2009

Colon Cancer

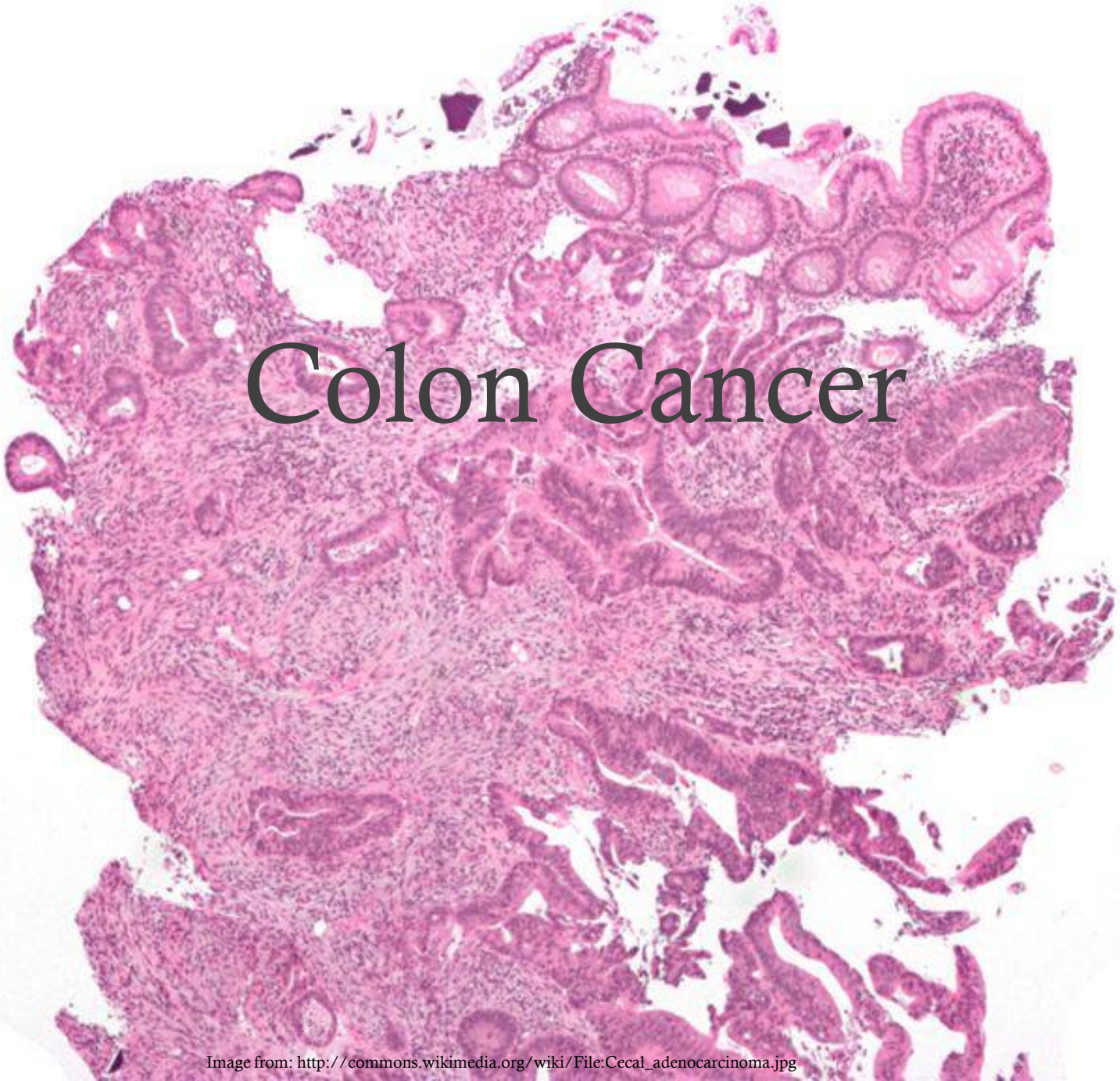


Image from: http://commons.wikimedia.org/wiki/File:Cecal_adenocarcinoma.jpg

Colon Cancer

- DNA mismatch repair (MMR) system
  - Works to repair errors that occur during replication.
    - MMR proteins can be identified using IHC.
  - A deficiency of these proteins can lead to colon cancer.
    - May be a spontaneous deficiency (most common) or a result of Lynch Syndrome.
    - 15% of colorectal cancers are caused by MMR protein deficiency.

Colon Cancer

- MMR deficiency is present when:
  - A high number DNA replication errors are present in the tumor cells.
  - High levels of DNA microsatellite instability (MSI-H) is present.
    - Microsatellites are sequences of DNA that are repeated.
    - MMR deficiency leads to variation in the number of repeats (could increase or decrease numbers).
    - MSI-H is defined by instability in greater than or equal to 30% of microsatellite loci.

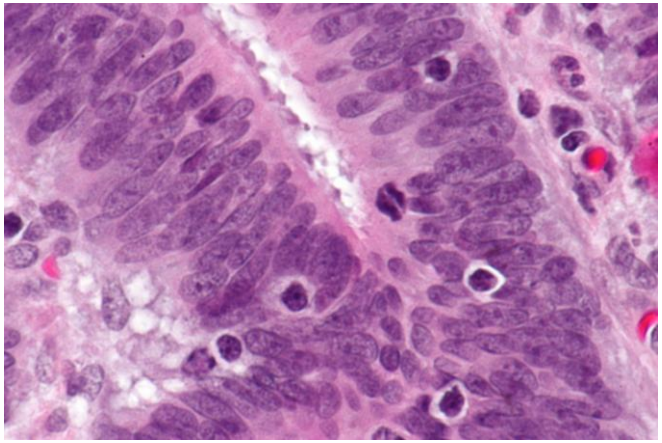


Image from: http://en.wikipedia.org/wiki/Microsatellite_instability

Colon Cancer

- MMR protein expression tested by IHC.
- Presence of MSI tested by Polymerase chain reaction (PCR).

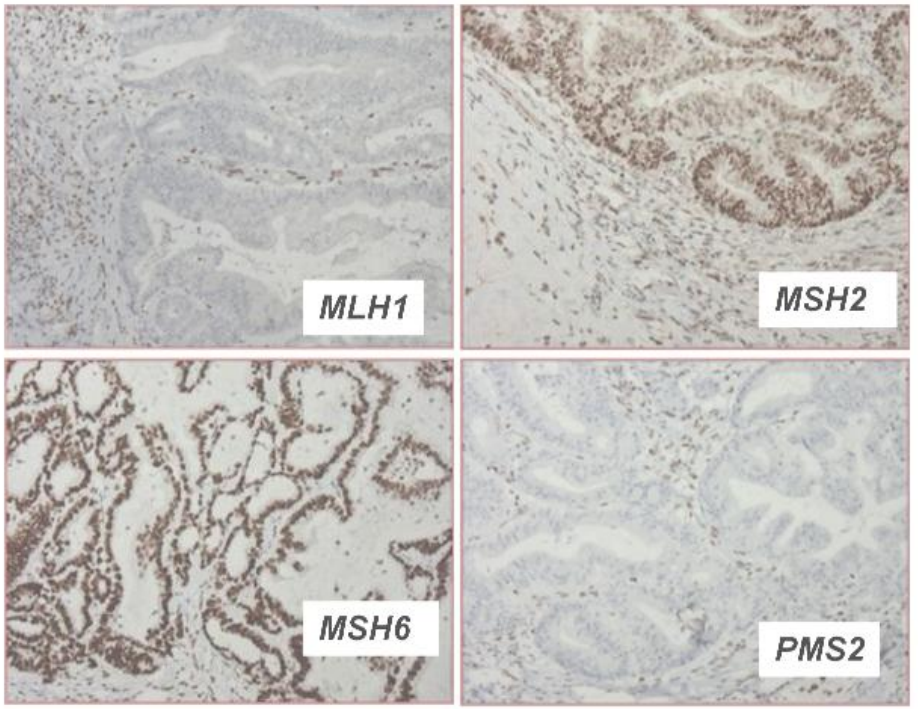


Image from:http://www.lynchscreening.net/implementation/immunohistochemistry-ihc-only-2/

Colon Cancer

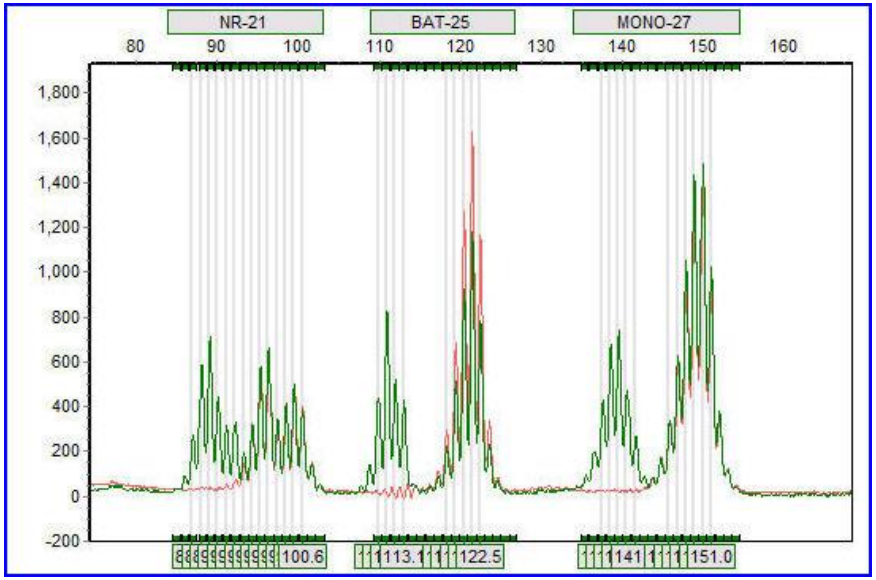


Image from: http://commons.wikimedia.org/wiki/File:Microsatellite_Instability_in_GeneMarker.jpg

- So why do we care about MMR deficiency?
  - Patients with MSI-H have a longer survival rate compared to low MSI and stable microsatellites.
  - Fluorouracil (5-FU) based chemotherapy is less beneficial for patients with MSI-positive tumors.
    - Some studies suggest it may even be harmful.

Colon Cancer

- KRAS/NRAS
  - Works as molecular on/off switch.
    - When turned on, it recruits and activates proteins for propagation of growth factor.
  - KRAS is mutated in approximately 35-45% of colorectal cancers.
    - Mutation causes KRAS to accumulate in the on position which activates the downstream pro-proliferative signaling pathways.
  - NRAS works similarly to KRAS but in different sites of the cell.

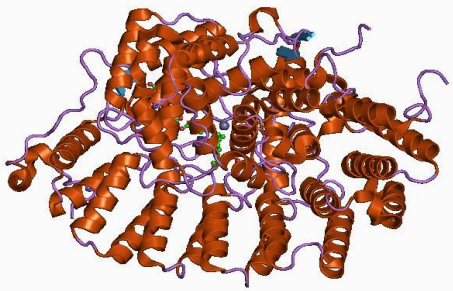


Image from: http://commons.wikimedia.org/wiki/File:PDB_1d8d_EBI.jpg

Colon Cancer

- So why do we care about KRAS/NRAS?
  - If a mutation in KRAS/NRAS is present, EGFR inhibitors will be less effective in colon cancer.
    - Cetuximab and panitumimab are first line therapy for unresectable, inoperable, or metastatic colon cancer.
    - The target pathway is already activated.

Colon Cancer

- BRAF
  - Protein kinase that works downstream of RAS.
  - Mutation occurs at V600E.
    - Leads to increased kinase activity which causes downstream activation of a pathway that leads to cell division.
    - Also important for melanoma.
  - BRAF V600E is present in 5-10% of colon cancers.

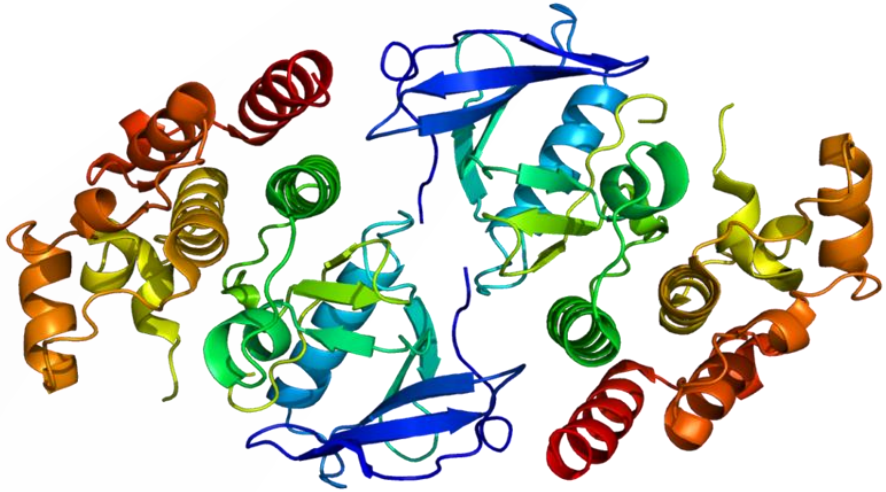


Image from: http://commons.wikimedia.org/wiki/File:Protein_BRAF_PDB_1uwh.png

Colon Cancer

- So why do we care about BRAF V600E?
  - Worse overall prognosis.
  - Gives clues to origin of MSI-H.
    - If present, unlikely to be Lynch Syndrome.
  - Limited response to BRAF inhibiting therapy.
    - Only 5% of metastatic colorectal carcinomas with BRAF V600E have a response compared to 81% in BRAF V600E positive advanced stage melanoma.
  - Cetuximab and panitumimab less effective in patients with BRAF V600E.

Resources

- College of American Pathologists; Molecular Resource Guide; Version 5.0, Issue No. 1, 2014
- FDA News Release; FDA approves first human papillomavirus test for primary cervical cancer screening; http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm394773.htm
- Saslow, D; et all; American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening Guidelines for the Prevention and Early Detection of Cervical Cancer; 2012 American Jnl of Clin Path, 137, 516-542.
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