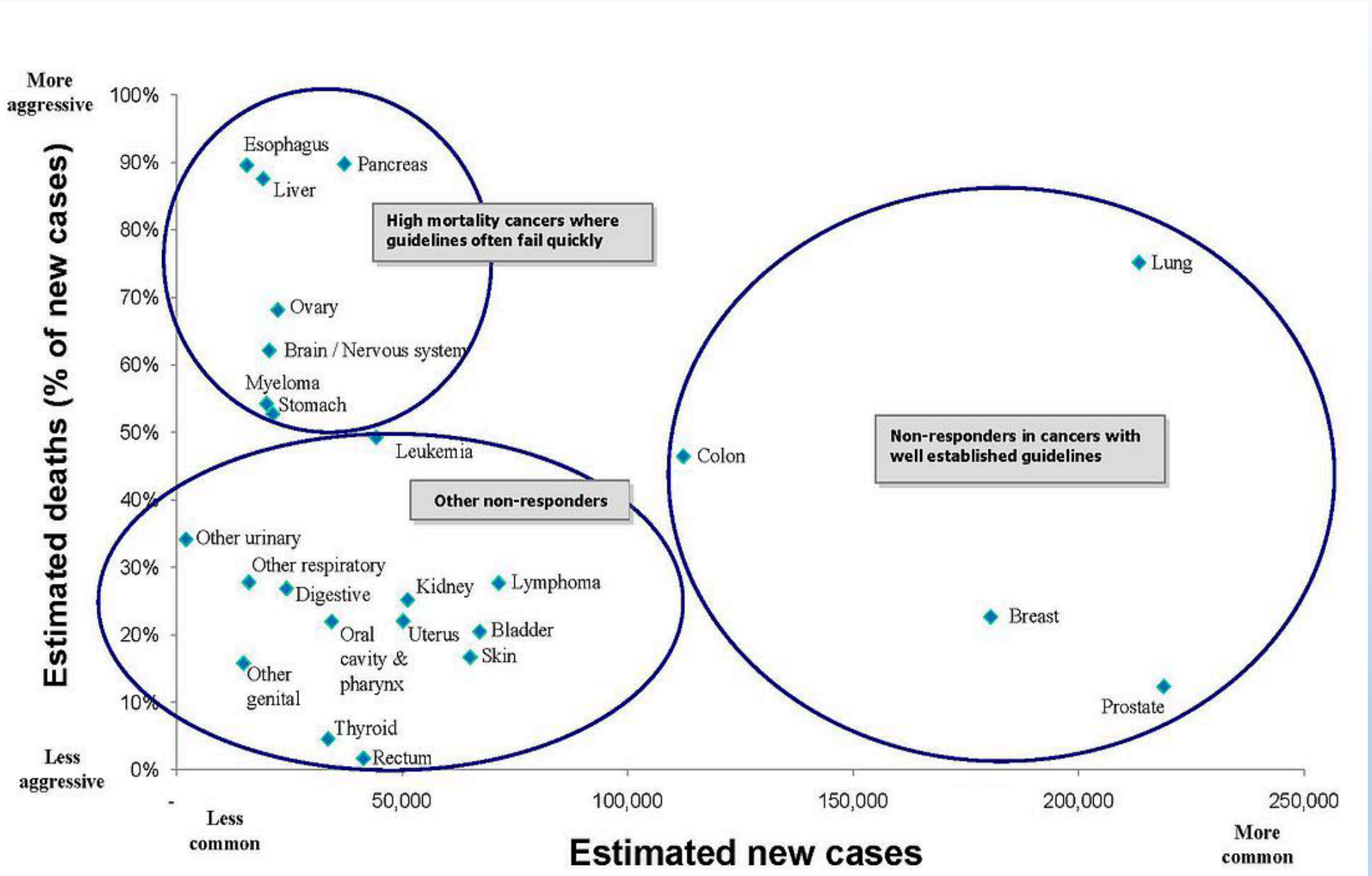
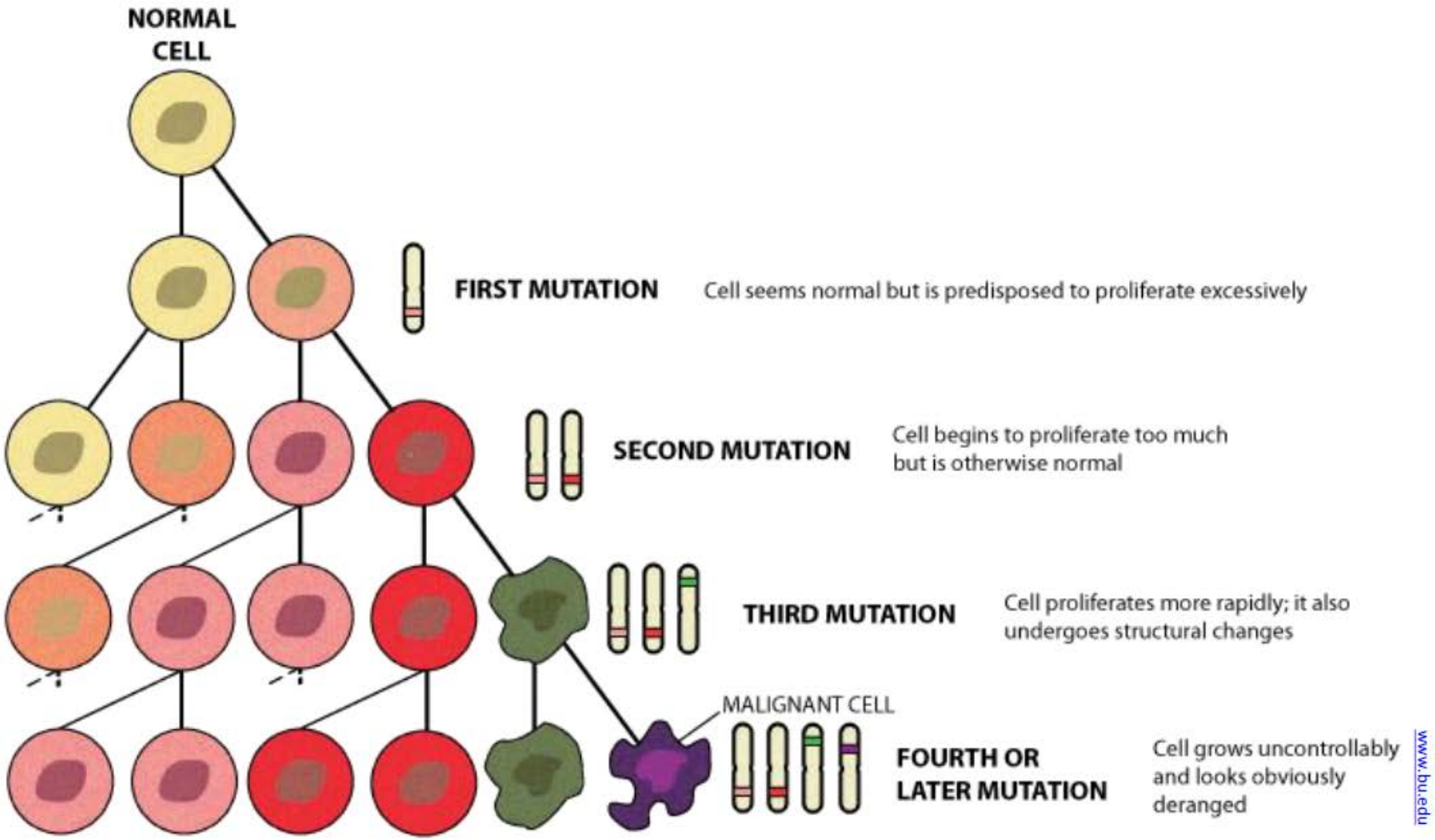
TUMOR GENOMICS

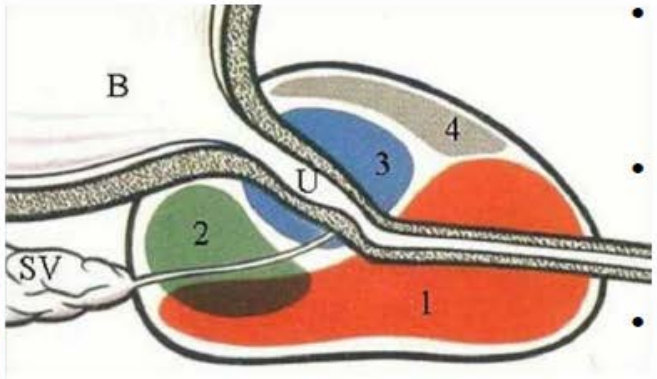

More aggressive
Estimated deaths (% of new cases)
Less aggressive
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Esophagus
Pancreas
Liver
High mortality cancers where guidelines often fail quickly
Ovary
Brain / Nervous system
Myeloma
Stomach
Leukemia
Other non-responders
Other urinary
Other respiratory
Digestive
Kidney
Lymphoma
Oral cavity & pharynx
Uterus
Bladder
Skin
Other genital
Thyroid
Rectum
Lung
Non-responders in cancers with well established guidelines
Colon
Breast
Prostate
-
50,000
100,000
150,000
200,000
250,000
Less common
Estimated new cases
More common

How Cancer is Formed

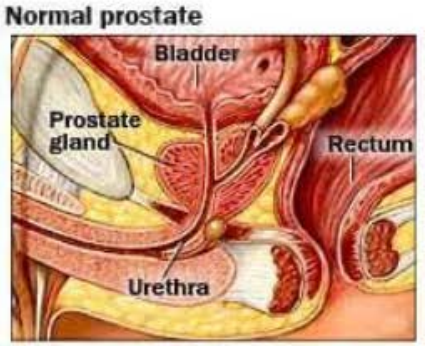


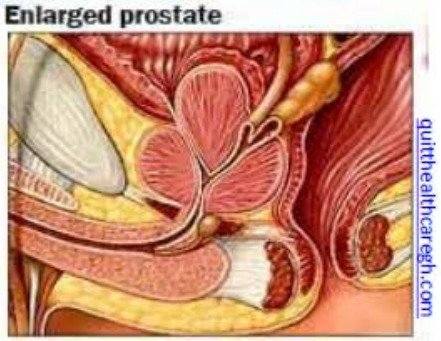
Prostate Cancer

- **Prostate comprised of 4 zones**
  - Peripheral – 1
    - 70% of prostatic volume
    - Site of 80% of prostate cancers
  - Transition– 2
    - 5% of prostatic volume
    - Site of 10% of prostatic cancers
  - Central -3
    - 25% of prostatic volume
    - Site of 5% of prostatic cancers
  - Anterior fibromuscular - 4

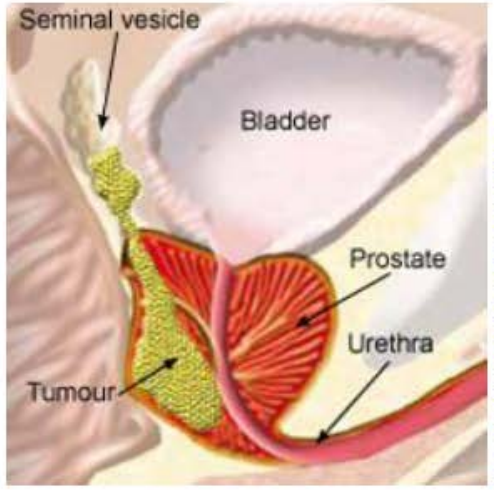


www.endotext.com

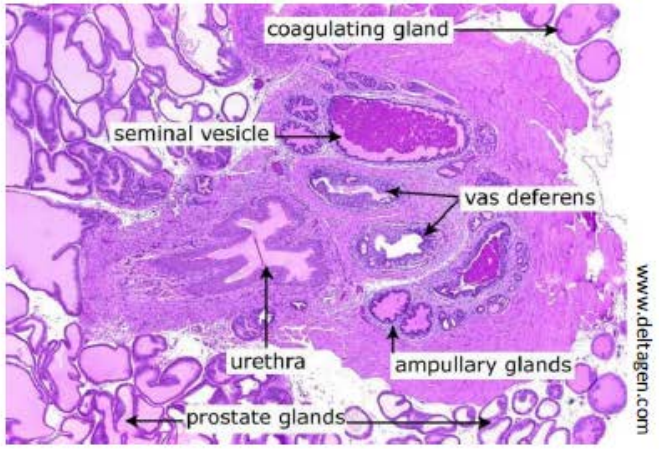




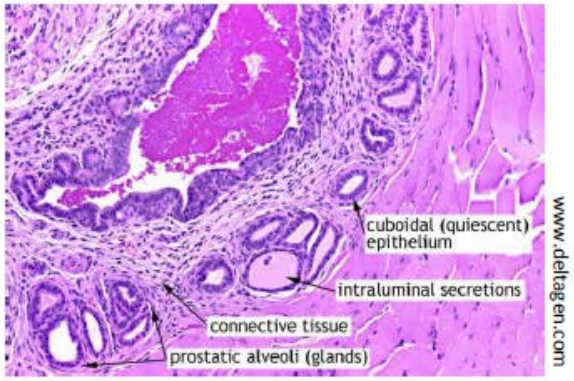
quitthealthcaregh.com



www.prostatebrachytherapycentre.com

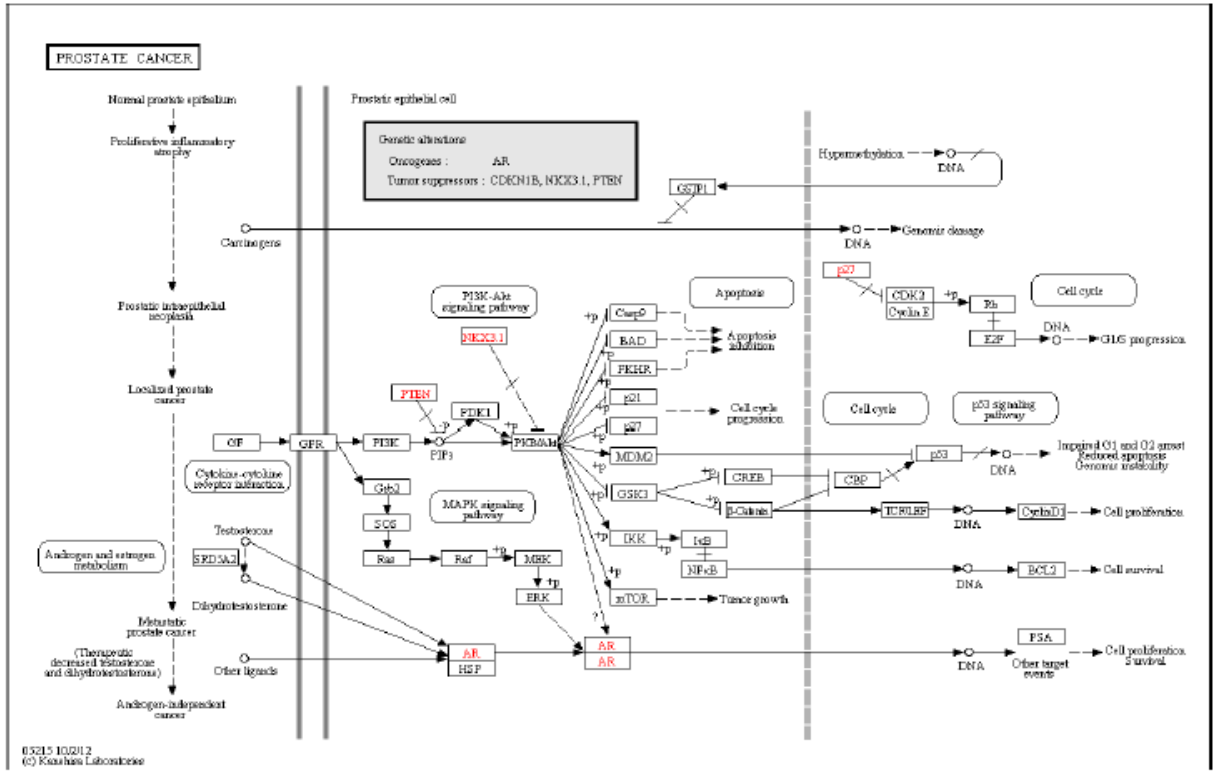


www.deltagen.com



www.deltagen.com

Prostate Cancer Pathway



Drug Therapy

Leuprolide
Goserelin
Triptorelin
Flutamide

- Key molecular alterations in prostate-cancer cells as critical determinants of the phenotype of prostate-cancer cells
  - Carcinogen defenses (GSTP1)
    - Glutathione S-transferases (GSTP1) are detoxifying enzymes
    - Cells of prostatic intraepithelial neoplasia are devoid of GSTP1
  - Growth-factor-signaling pathways (NKX3.1, PTEN, and p27) - regulate growth and survival of normal prostate cells
    - Inadequate levels of PTEN and NKX3.1 lead to a reduction in p27 levels and to increased proliferation and decreased apoptosis
  - Androgens (AR)
    - Transcription factor that is normally activated by its androgen ligand
      - If altered activity of AR co-activators → emergence of androgen-independent prostate cancer

PROSTATE CANCER PANEL

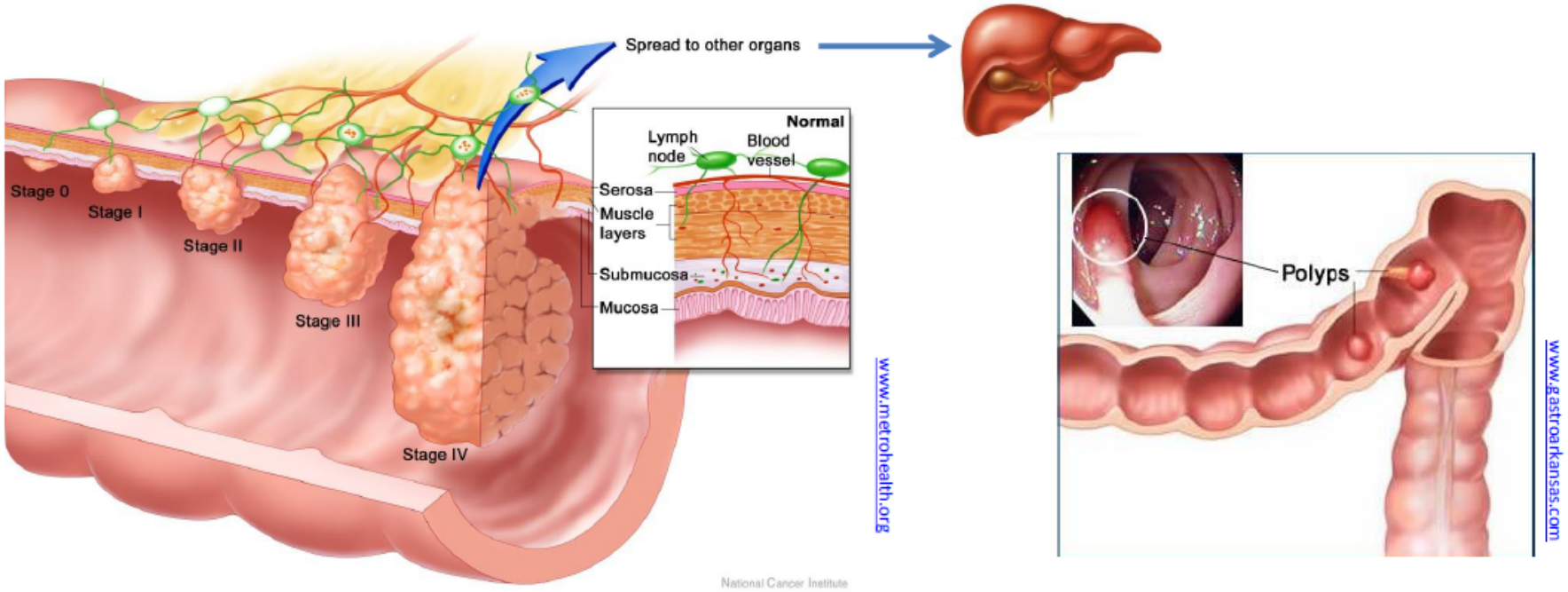
- This test is performed on select exons for the genes listed unless another method is noted: AKT1, ATF4, BIRC5, CD24, CSK2, CYP19A1, EGFR, ESR1, FZD1, FZD2, FZD7, FZD9, H2AFZ, HDAC1, HSPB1, ID1, IFITM1, JUN, MDM2, MMP-11, MYC, PKM2, PTEN, P53, RAC1, RAP1A, RHOC, RRM2, SFN, SNAI2, TGFB1, YBX1, YWHAZ, TMPRSS2-ERG FISH, and NKX3.1 FISH, PTEN FISH.

BLADDER TUMOR PROFILE

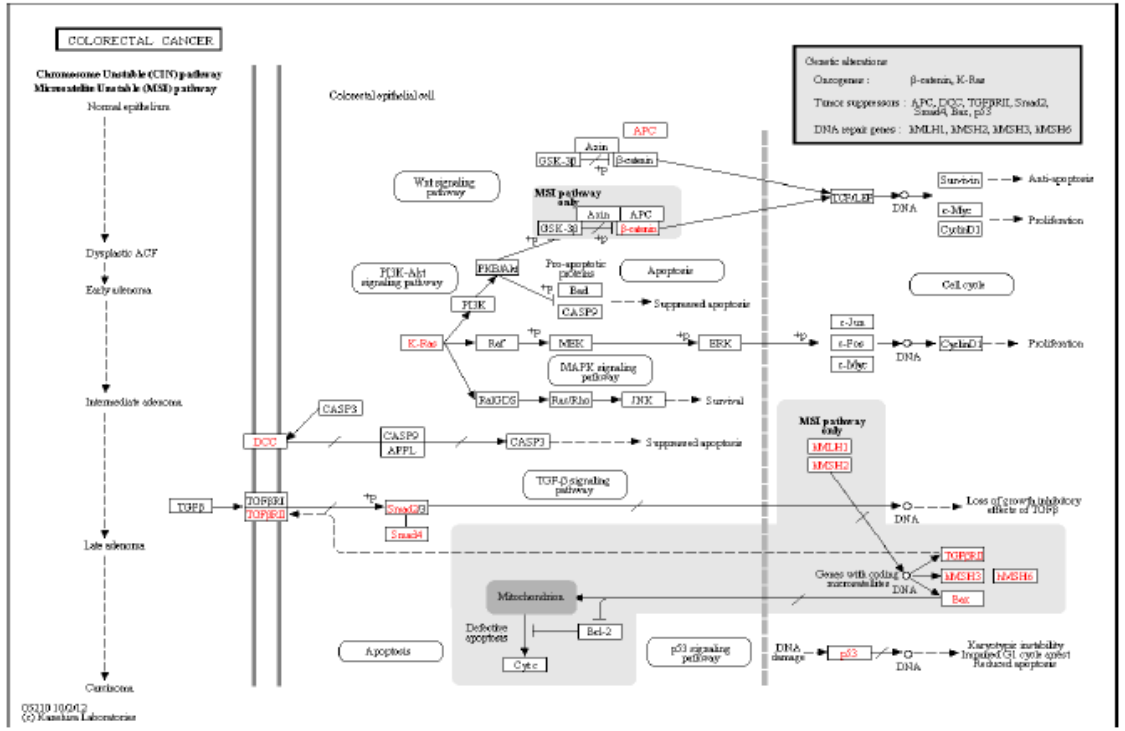
- This test is performed on select exons for the genes listed unless another method is noted: BIRC5, CDC25B, COL4A1, FABP4, KPNA2, MBNL2, MSN, COL18A1, COLA3BP, NEK1, SKAP2, UBE2C.

Colorectal Cancer

- Colorectal cancer (CRC) is the second largest cause of cancer-related deaths in Western countries.
- Colorectal cancer is a disease originating from the epithelial cells lining the colon or rectum
- Mutations in the Wnt signaling pathway artificially increase signaling activity
- Most commonly mutated gene in all colorectal cancer is the APC gene
- APC protein is a "brake" on the accumulation of β-catenin protein
    - Transcription factor for stem cell renewal and differentiation



Colorectal Cancer Pathway



Drug Therapy
Oxaliplatin
5-Fluorouracil (5-FU)
Leucovorin (FOLFOX4)
Bevacizumab [
Irrinotecan
Cetuximab
Capecitabine

- Chromosomal instability (CIN),
  - Activation of oncogenes such as K-ras and inactivation of TSG such as p53, DCC/Smad4, and APC.
- Microsatellite instability (MSI)
  - Inactivation of the DNA mismatch repair genes MLH1 and/or MSH2 by hypermethylation of their promoter
  - Secondary mutation of genes with coding microsatellites, such as transforming growth factor receptor II (TGF-RII) and BAX
- Hereditary syndromes have germline mutations in specific genes
    - APC on chromosome 5q in FAP (Familial Adenomatous Polyposis)
    - Mutated DNA mismatch repair genes in HNPCC (Hereditary Non Polyposis Colon Cancer [Lynch's Syndrome])

Colorectal Cancer

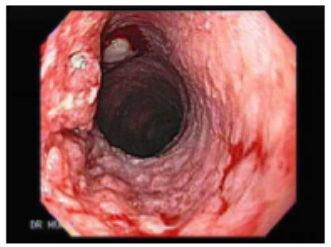
- Colorectal cancer is the second leading cause of cancer related mortality in the United States, with an estimated 143,460 new cases and 51,690 deaths anticipated in 2012 (ACS 2012). With the advent of more chemotherapy options and with the availability of biologic therapies in the recent past, mortality rates are declining, and patients are living longer. Over the past 20 years, survival in metastatic colorectal cancer has more than doubled. Nonetheless, colorectal cancer remains the second leading cause of cancer related death in the United States. New therapeutic strategies are clearly needed.
- The main histologic subtype of colorectal cancer is adenocarcinoma. Colorectal adenocarcinomas arise through the acquisition of a series of mutations that occur over the space of many years, and results in the evolution of normal epithelium to adenoma to carcinoma to metastasis (Fearon and Vogelstein 1990). In the past two decades, there has been increasing recognition that some somatic mutations may be prognostic or predictive markers for specific therapies available in colorectal cancer. These mutations involve genes such as KR AS, BRAF, PIK3CA, AKT1, SMAD4, PTEN, NRAS, and TGFBR2 (Baba et al. 2011; De Roock et al. 2010; Dienstmann et al. 2011; Fernandez-Peralta et al. 2005; Haigis et al. 2008; Negri et al. 2010; Papageorgis et al. 2011; Sartore-Bianchi et al. 2009).
- Furthermore, there has been increasing recognition that some of these mutant gene products may be targets for drug development. (De Roock et al. 2010; Huang et al. 2008; Thenappan et al. 2009).

COLORECTAL TUMOR PROFILE

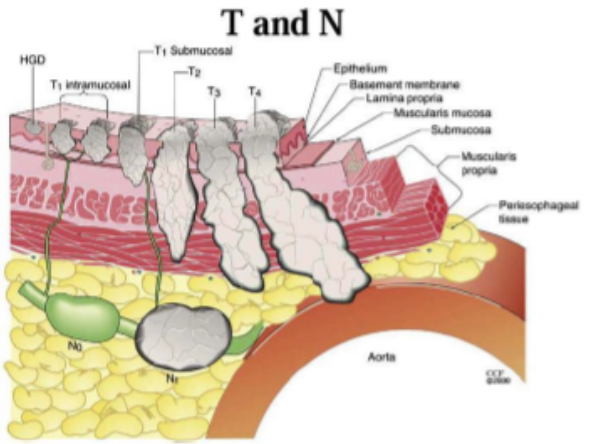
- This test is performed on select exons for the genes listed unless another method is noted: AKT1, APC, BRAF, CDH1, EGFR, HRAS, KIT, KRAS, MET, Microsatellite Instability (MSI), NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, SMAD4, SMO, TP53, MET FISH, PTEN FISH.

Gastric Esophageal Cancer

- 9th leading cancer in the world and is associated with a 5-year survival rate under 25%
- One of the deadliest malignancies
- The most common histologic types
  - Squamous cell carcinoma (SCC)
  - Adenocarcinoma (AC)
  - Together constitute more than 90% of esophageal malignancies.
- Smoking and heavy alcohol intake are important risk factors for the development of SCC

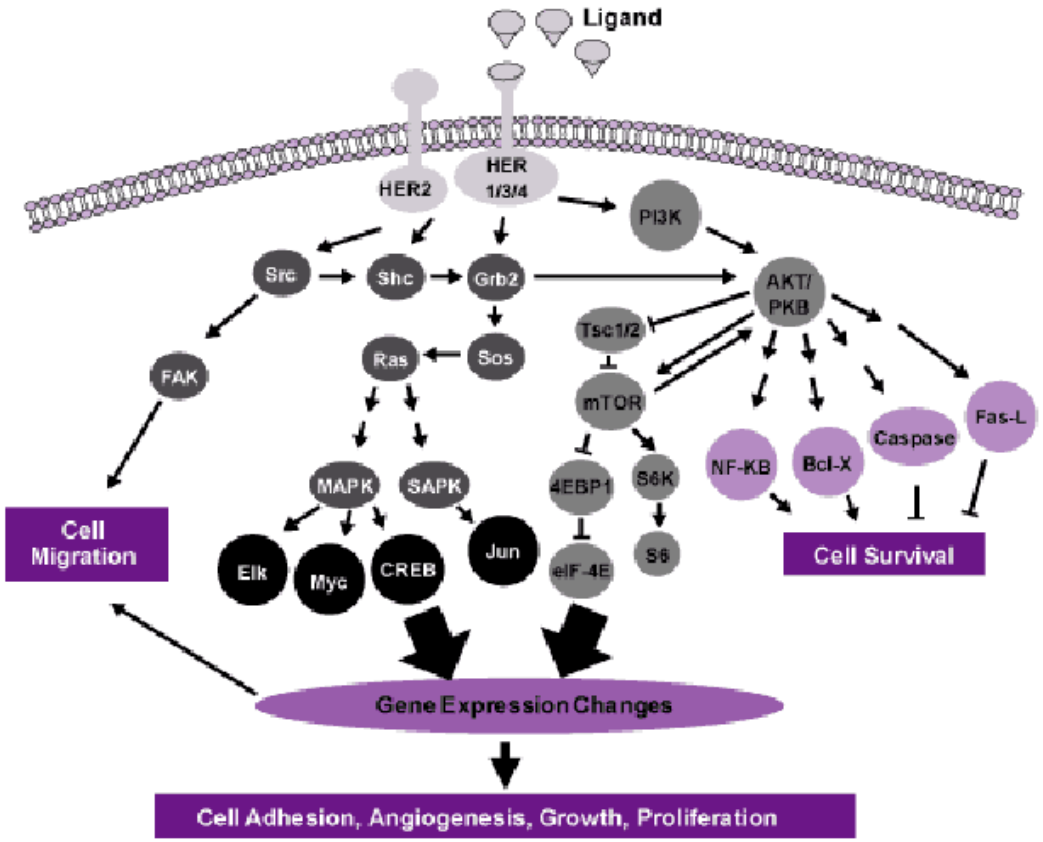


toxancer.com



www.clevelandclinicmeded.com

Gastric Esophageal Cancer Pathway



Drug Therapy
Fluorouracil
Cisplatin
Mitomycin

- Tumorigenesis at the cellular level - multiple genetic alterations
  - Allelic losses at chromosomes 4q, 5q, 9p, 9q, and 18q and abnormalities of *p53, Rb, cyclin D1,* and *c-myc*

GASTRIC ESOPHAGEAL CANCER

- Gastric cancer is the fourth most commonly diagnosed cancer and the second most common cause of cancer death worldwide, with an estimated 989,600 new cases and 738,000 deaths in 2008 (Kamangar, Dores, and Anderson 2006; ACS 2011). Gastric cancer incidence varies throughout the world, with Japan and Korea having the highest incidences (Crew and Neugut 2006). In the U.S., 21,320 new cases and 10,540 deaths are estimated for 2012 (ACS 2012). There are two main sites of gastric cancer: cardia (proximal, gastroesophageal junction) and noncardia (fundus, body, distal, and lesser or greater curvature). The incidence of noncardia tumors is decreasing, possibly due to lower incidence of H. Pylori infection caused by improved diet, food storage, and overall sanitation (Parsonnet et al. 1991). H. Pylori infection is a major etiologic factor in the development of intestinal type gastric cancer (Parsonnet et al. 1991). Nonetheless, the incidence of proximal tumors has been increasing since the 1970s, suggesting etiologic heterogeneity among gastric malignancies (Wu et al. 2009).
- Most patients with this tumor present with inoperable, locally advanced, or metastatic disease (SEER Stat Fact Sheet: Stomach, accessed 2012). Diagnosis is often delayed because many patients with early stage disease present with vague, nonspecific symptoms or no symptoms at all. Late stage disease at presentation, relative chemo-resistance, and frequent comorbidities causing poor functional status have contributed to poor overall survival (Okines and Cunningham 2010; Kim et al. 2012; Bang et al. 2010). Even patients with operable disease will only have about a one in three chance of surviving 5 years (McDonald et al. 2001; Cunningham et al. 2006). Metastatic disease is treated with systemic chemotherapy and supportive measures.

GASTRO ESOPHOGEAL TUMOR PROFILE

- This test is performed on select exons of the genes listed unless another method is noted: BRAF, CTNNB1, ERBB2, ERBB4, HRAS, KIT, KRAS, MET, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, SMO, TP53, HER2, MET FISH, and PTEN FISH.

Gastrointestinal Stromal Tumor

- Gastrointestinal stromal tumor (GIST) is the most common mesenchymal neoplasm of the gastrointestinal tract, if not the most common sarcoma overall (Reichardt et al. 2009). GIST is believed to arise from the interstitial cells of Cajal or their precursors. These pacemaker cells of the bowel have features of smooth muscle cells, fibroblasts, and neurons to various degrees (Huizinga et al. 1995).
- GIST characteristically stains positive for the KIT receptor tyrosine kinase by immunohistochemistry. At the genomic level, mutations in KIT or the receptor tyrosine kinase PDGFRA are the hallmark of this diagnosis (Hirota et al. 1998). KIT and PDGFRA are mutated in ~85% and ~5%, respectively, of GIST. Mutations are also rarely found in the serinethreonine kinase, BRAF (<1%).
- The incidence of GIST is on the order of 10–15/million (3000–4500 cases/year in the US) (Nilsson et al. 2005), although autopsy series may identify as many as 10% of people examined with microscopic GIST.

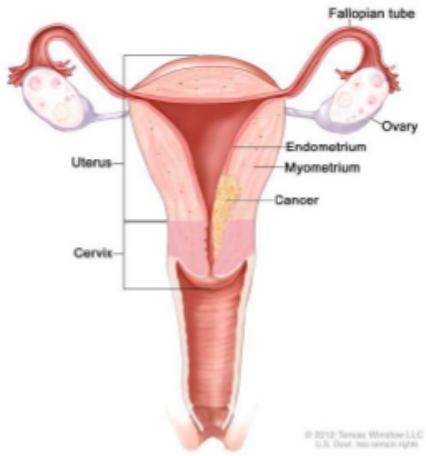
Gastrointestinal Stromal Tumor Profile

- This test is performed on select exons of the genes listed unless another method is noted: BRAF, EGFR, HRAS, KIT, KRAS, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, SMO, TP53, HER2, MET FISH, and PTEN FISH.

Cervical and Uterine Cancer

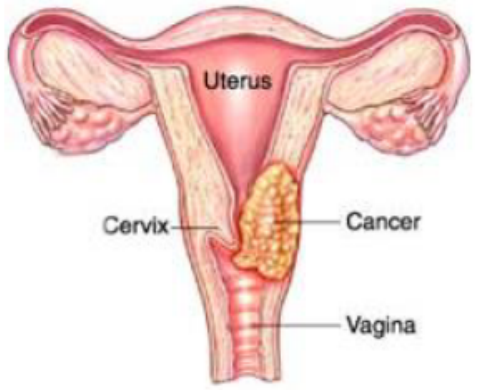
- Both types of cancer begin in different part of uterus
  - Uterine cancer begins in the cells of the endometrium
    - Also called endometrial cancer.
  - Cervical cancer originates in the thin, flat cells on the surface of the cervix, the lower neck-like portion of the uterus
    - Transformation zone (Squamo-columnar junction)
      - Squamous and columnar cells meet)
    - Abnormal growth or dysplasia develop

Uterine (endometrial) Cancer



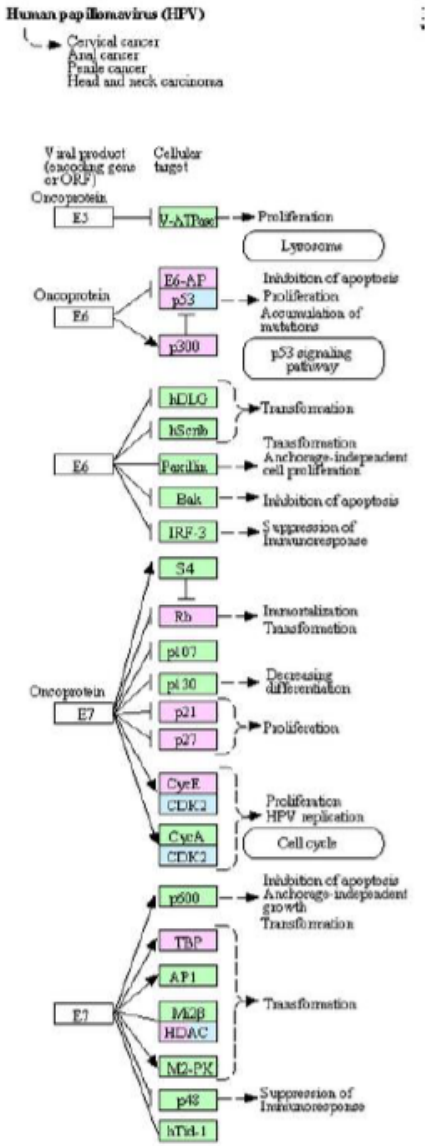
www.cancer.gov

Cervical Cancer



www.womenfitness.net

Cervical Cancer Pathway

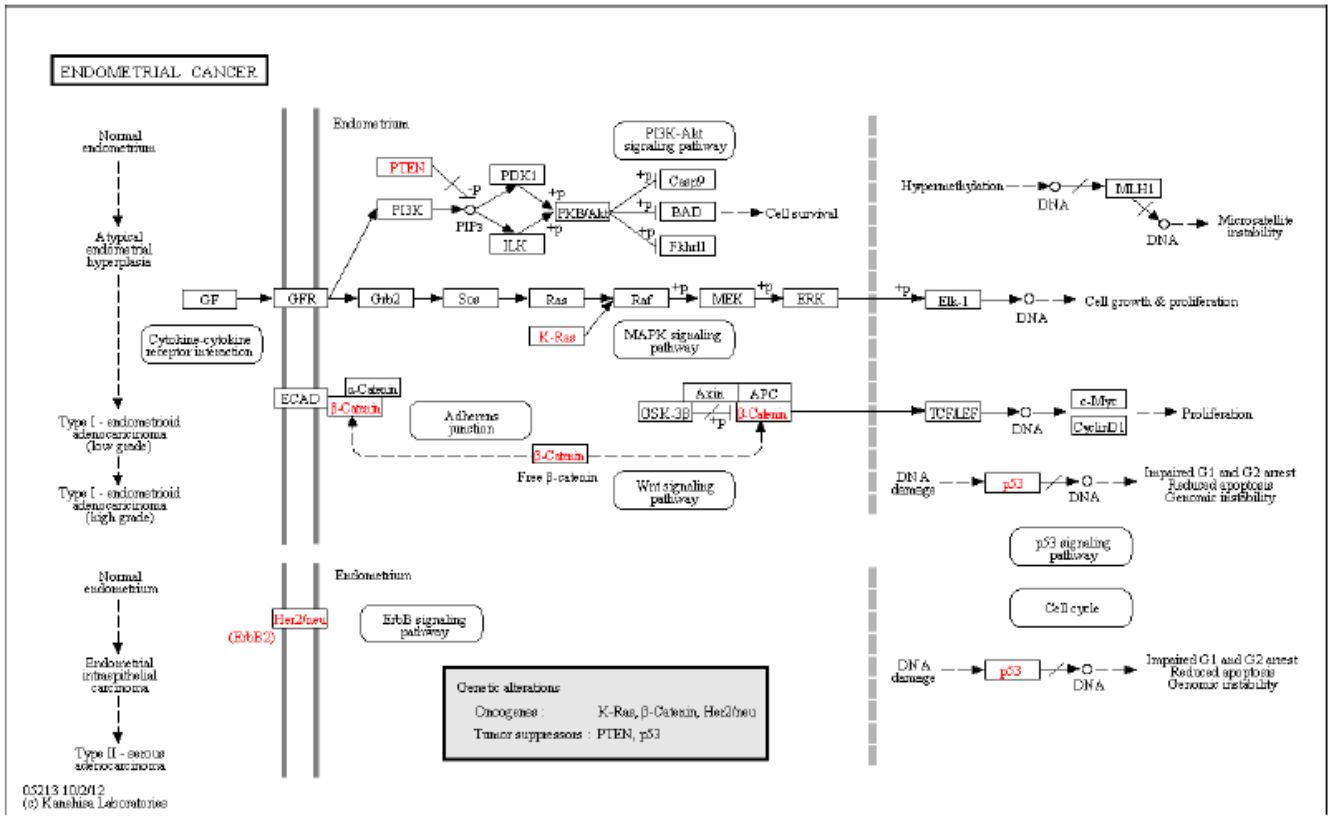


Drug Therapy

Methotrexate, Doxorubicin, Cisplatin, Vinblastine, Mitomycin, Bleomycin, Vincristine

- Specific subset of human papillomavirus (HPV) types
  - Types 16, 18, 33 and 42
- E6 and E7 genes of these high risk HPVs are oncogenes that deregulate key cell cycle controls
- E6 and E7 oncoproteins bind respectively to the p53 and Retinoblastoma (Rb) tumor suppressor proteins
  - Involved in the regulation of growth control
- Other cellular genes playing a role in carcinogenesis and the aggressiveness of cervical tumors
  - Mutation in the ras family of genes
  - Amplification in EGFR and ERBB2

Uterine Cancer Pathway



Drug Therapy
Progestin
Tamoxifen

- **Type-I carcinoma**
  - Hyperestrogenism by association with endometrial hyperplasia
  - Frequent expression of estrogen and progesterone receptors
  - Younger age
  - Defects in DNA-mismatch repair and mutations in PTEN, K-ras, and beta-catenin
- **Type-II carcinoma**
  - Unrelated to estrogen - associated with atrophic endometrium
  - Frequent lack of estrogen and progesterone receptors
  - Older age
  - Aneuploidy, p53 mutations, and her2/neu amplification

CERVICAL TUMOR PROFILE

- This test is performed on select exons for the genes listed unless another method is noted: BRAF, CTNNB1, EGFR, ERBB2, ERBB4, HRAS, KRAS, MET, NOTCH1, NRAS, PDGFRA, PIK3CA, PTEN, SMO, TP53, MET FISH, PTEN FISH.

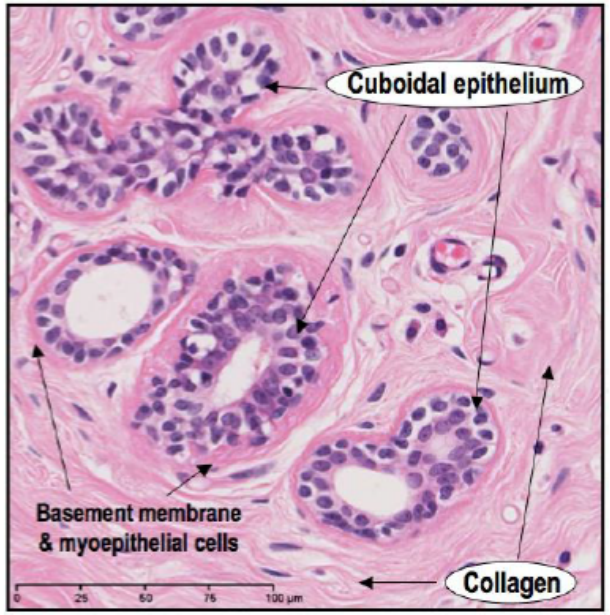
ENDOMETRIAL TUMOR PROFILE

- This test is performed on select exons for the genes listed unless another method is noted: BRAF, EGFR, HRAS, KIT, KRAS, MET, Microsatellite Instability (MSI), NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, SMO, TP53, MET FISH, PTEN FISH.

Breast Cancer

- Breast cancer affects one in eight women
- Breast cancer originating from ducts - ductal carcinomas
- Breast cancer originating from lobules - lobular carcinomas

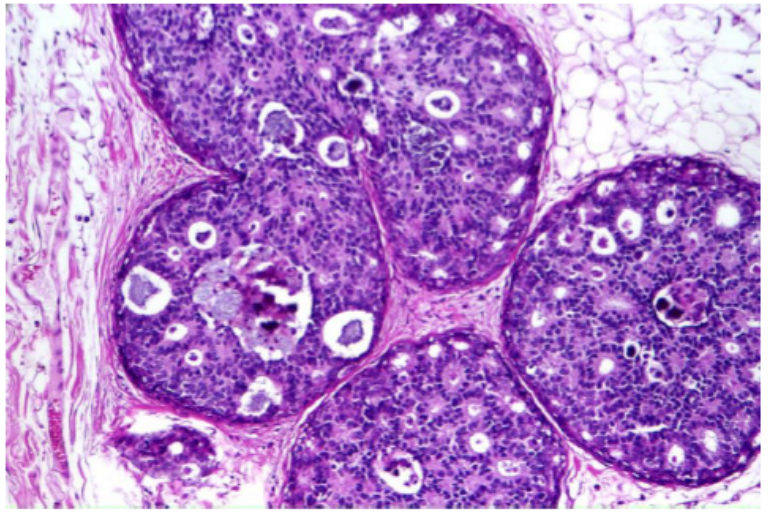
Normal Ducts



micro2tele.com

The ducts are lined with a different type of epithelium called stratified cuboidal (square-ish) epithelium. Normal ducts are lined by two cell layers, and the outer cells are different from those in the inner layer.

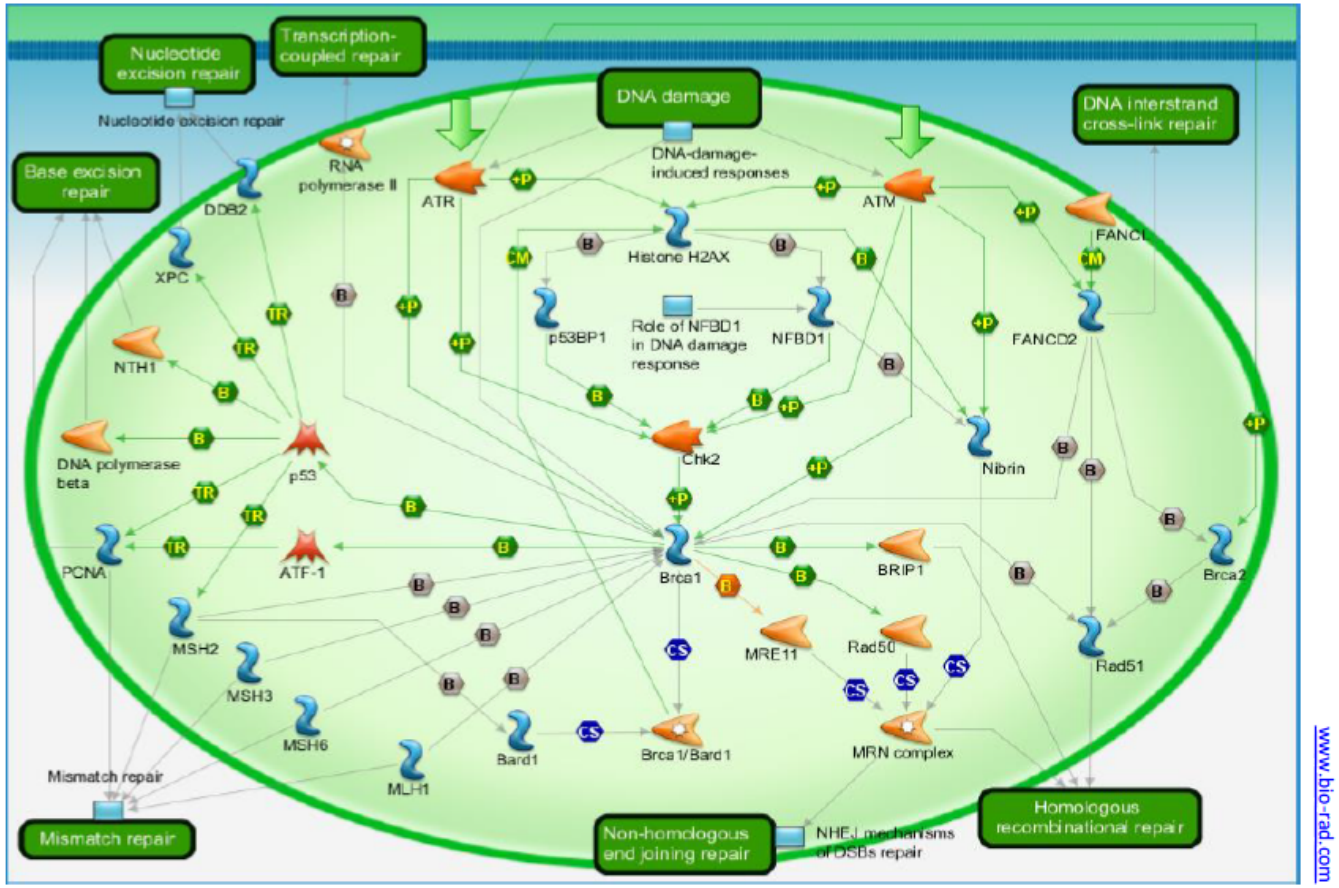
Ductal Carcinoma



breastpath.com

Clustered large ducts that are filled with many cells. This cellular proliferation in the duct or tubule, causes it to expand and enlarge. These malignant cells are all abnormal, and there are no layers.

Breast Cancer Pathway



Drug Therapy

Doxorubicin
Epirubicin
5-FU
Cyclophosphamide
Docetaxel
Paclitaxel
Methotrexate
Trastuzumab
Tamoxifen
Anastrozole
Letrozole
Exemestane

- The hereditary breast cancer syndrome includes genetic alterations in various susceptibility genes such as p53, PTEN, BRCA1, and BRCA2.
- Brca1 plays a central role in DNA repair by facilitating cellular response to DNA repair
- Both Brca1 and Brca2 are implicated in HRR via RAD51.
- Sporadic breast cancers result from a serial stepwise accumulation of acquired and uncorrected mutations in somatic genes MYC, CCND1 (Cyclin D1) and ERBB2 (HER2/neu).

Breast Cancer

- Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in women worldwide, accounting for 23% (1.38 million) of the total new cancer cases and 14% (458,400) of the total cancer deaths in 2008 (Jemal et al. 2011; Jemal, Siegel, and Ward 2010). In the U.S., 229,060 new cases and 39,920 deaths are estimated for 2012 (ACS 2012). Classically, treatment decisions have been based upon histology of the tumor and on the status of three main biomarkers: estrogen receptor (ER), progesterone receptor (PR), and HER2/neu. Despite significant improvements in the treatment of breast cancer, novel therapies and treatment strategies are needed.

Molecular alterations involving the PI3K/AKT pathway (Figure 1) occur in over 30% of invasive breast tumors. Alterations in breast cancer resulting in hyperactivity of the PI3K pathway include gain-of-function mutations in PIK3CA (the gene encoding the PI3K catalytic subunit p110α), mutations in AKT1, amplification of AKT2, and loss of the phosphatase PTEN (Engelman, Luo, and Cantley 2006). Mutations in PIK3CA cluster in two major 'hot spots' located in the helical (E542K and E545K in exon 9) and catalytic (H1047R in exon 20) domains (Bachman et al. 2004; Saal et al. 2005). Expression of these mutant p110α isoforms confers growth factor-independent proliferation and protection from anoikis (a form of cell death) and chemotherapy.

Both genetic and biochemical data suggest that activation of the PI3K/AKT survival pathway contributes to breast cancer development and tumorigenesis. PIK3CA mutations in primary breast tumors have been associated with lymph node metastases, presence of ER and PR, and HER2 overexpression (Saal et al. 2005; Stemke-Hale et al. 2008).

The impact of these mutations and of PTEN loss on the virulence of breast cancer and patient outcome is not completely clear yet. However, PI3K hyperactivity has been associated with resistance to anti-HER2 and anti-estrogen therapies. For example, presence of activating PIK3CA mutations and loss of PTEN in HER2-overexpressing cancers correlates with a lower response to trastuzumab and lapatinib (Berns et al. 2007; Nagata et al. 2004; Serra et al. 2008). Prospective studies to confirm these findings are in progress. These data also suggest that inhibitors of the PI3K pathway currently in clinical development (Brachmann et al. 2009; Engelman 2009) can be used to reverse acquired and de novo drug resistance.

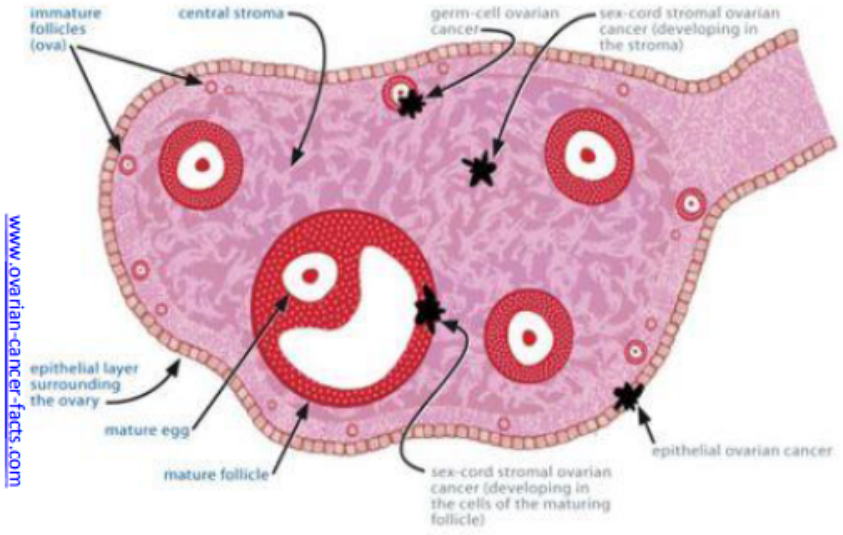
Currently, there are many kinds of PI3K/AKT pathway inhibitors in clinical development. Preclinical studies suggest the main activity of these drugs will be limited to tumors with PIK3CA mutations (Brachmann et al. 2009; O'Brien et al. 2010; She et al. 2008). Agents include PI3K inhibitors, AKT inhibitors, mTOR inhibitors, and dual PI3K/mTOR inhibitors. Although these small molecules block different elements within the same cellular signaling pathway, their differential selectivity may have distinct therapeutic impact in patients with breast cancer.

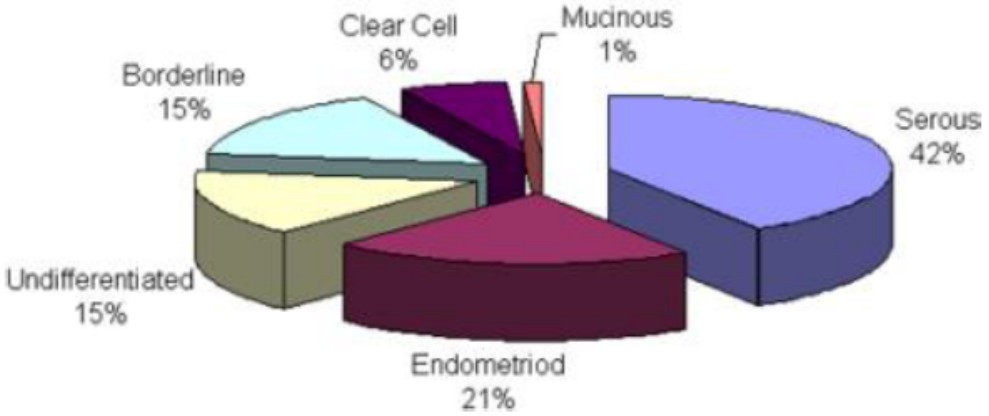
BREAST CANCER PROFILE

- This test is performed on select exons for the genes listed unless another method is noted: AKT1, APC, BRAF, CDH1, CTNNB1, EGFR, ERBB2, ERBB4, FGFR1, HRAS, KIT, KRAS, MET, NOTCH1, NRAS, NPM1, PIK3CA, PTEN, SMO, TP53, HER2, MET FISH, PTEN FISH.

Ovarian Cancer

- > 90% ovarian cancers are classified as "epithelial"
- Other types may arise from the egg cells (germ cell tumor) or supporting (stroma) cells



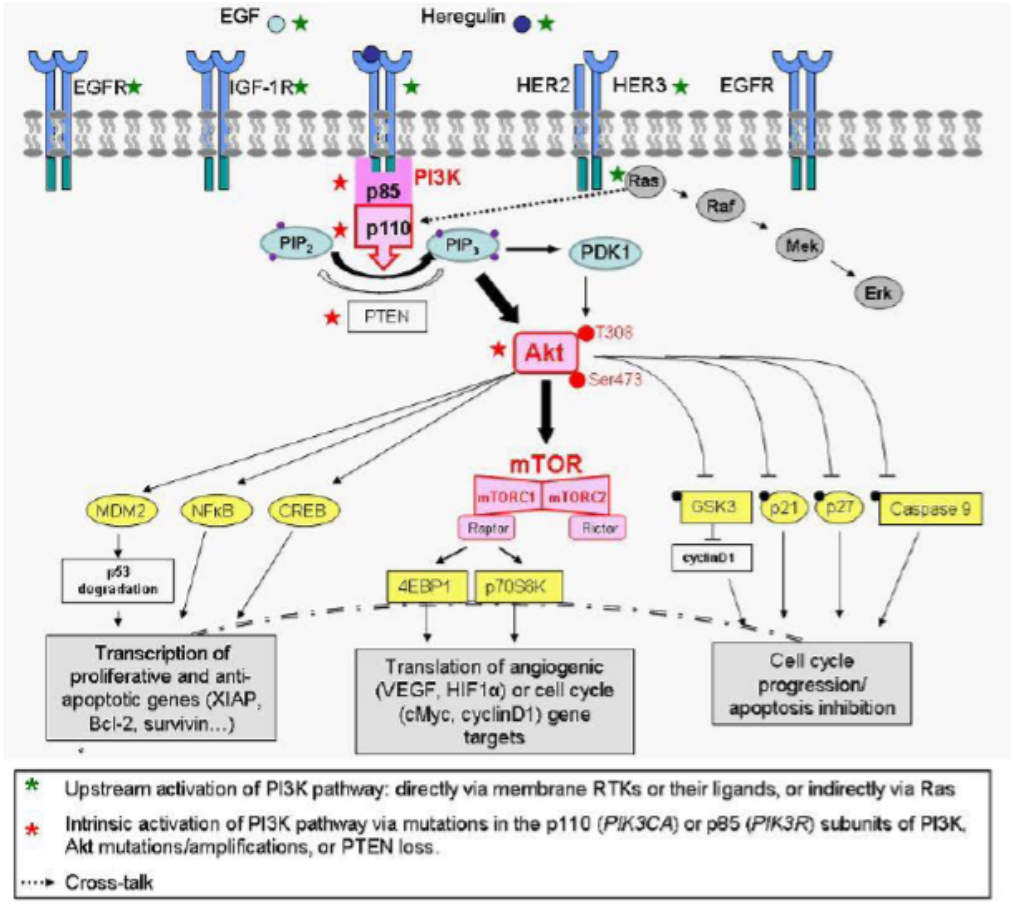


Germ cell carcinoma tumor - makes up about five percent of ovarian cancer cases, and begins in the cells that form eggs

Stromal carcinoma tumors - ovarian stromal carcinoma accounts for about five percent of ovarian cancer cases

Serous – the most common type of epithelial ovarian cancer
Endometrioid – the second most common type of epithelial ovarian cancer
Undifferentiated – the cells of this type of tumor do not share characteristics with any specific type of ovarian tissue cells
Borderline tumors – cells have characteristics of both benign (non-cancerous) and malignant (cancerous) tissue.
Clear cell – this type of ovarian cancer gets its name because the center of the cells appear clear when viewed through a microscope
Mucinous – most commonly found in early stages, these tumors often present as large pelvic or abdominal masses

Ovarian Cancer Pathway



The PI3K/Akt/mTOR Pathway in Ovarian Cancer: Biological Rationale and Therapeutic Opportunities
By Alexandra Leary, Edouard Auclin, Patricia Pautier and Catherine Lhomme DOI: 10.5772/54170

Drug Therapy
Paclitaxel
Cisplatin
Carboplatin

- 10% of ovarian cancers due to inherited mutations in cancer susceptibility genes (BRCA1 or BRCA2)
- The vast majority of ovarian cancers are sporadic
  - Accumulation of genetic damage over a lifetime
  - Several specific genes involved in ovarian carcinogenesis have been identified,
    - p53 tumor suppressor gene
    - ERBB2 and PIK3CA oncogenes.

Ovarian Cancer

- Epithelial ovarian cancer (EOC) is the most common cause of gynecological cancer death in the United States, with an estimated 22,280 new cases and 15,500 deaths estimated for 2012 (ACS 2012). The vast majority of women are diagnosed with advanced stage EOC. Current practice consists of aggressive surgical removal of tumors, followed by platinum–taxane based chemotherapy (Muggia 2009). Despite initial aggressive treatment, most tumors recur, and the overall 5-year survival rate is 44% (Siegel, Naishadham, and Jemal 2012).

Emerging knowledge about underlying molecular alterations in ovarian cancer could allow for more personalized diagnostic, predictive, prognostic, and therapeutic strategies. Approximately 10–20% of high grade ovarian cancers are associated with germline mutations in BRCA1/2 (Pal et al. 2005). Somatic alterations in BRCA1/2 and other genes associated with DNA repair are seen in approximately 50% of high grade ovarian cancers (TCGA 2011) and tumors with a ‘BRCAness’ molecular profile are relatively sensitive to treatment with DNA damaging agents cisplatin and PARP inhibitors (Konstantinopoulos et al. 2010).

More recently, EOC tumors have been broadly classified into two distinct groups with unique histological, clinical and molecular profiles. Type I tumors have low grade serous, clear cell, endometrioid, and mucinous histological features. Typically, these tumors are slow growing and confined to the ovary, and are less sensitive to standard chemotherapy. BRAF and KRAS somatic mutations are relatively common in these tumors, which may have important therapeutic implications.

Type II tumors are high grade serous cancers of the ovary, peritoneum, and fallopian tube. Other high grade endometrioid and poorly differentiated ovarian cancers as well as carcinosarcomas are included in the type II group. These tumors are clinically aggressive and are often widely metastatic at the time of presentation. High grade serous EOC tumors display high levels of genomic instability with few common mutations, other than TP53, which is altered in over 90% of the cases (Kurman and Shih 2011; Landen, Birrer, and Sood 2008; TCGA 2011). PIK3CA and RAS signaling pathways are altered in 45% of the cases, but somatic mutations are rare and gene amplifications are far more common (TCGA 2011).

Currently, the most common ‘actionable’ alterations with potential for small molecule targeted therapy in EOC tumors are in the PIK3CA/PTEN and KRAS/BRAF signaling pathways.

OVARIAN TUMOR PROFILE

- This test is performed on select exons for the genes listed unless another method is noted: BRAF, CTNNB1, EGFR, ERBB2, ERBB4, HRAS, KRAS, MET, NRAS, PIK3CA, PTEN, SMO, TP53, MET FISH, PTEN FISH.