OncoNext Liquid™

Scan and Monitor

LIQUID BIOPSY FOR THE DETECTION AND MONITORING OF CANCER: ANALYSIS OF HOTSPOTMUTATIONS VIA PLASMA DERIVED CIRCUL ATING TUMOR DNA

Cancer and somatic mutations

The majority of cancers arise after a series of somatic gene mutations that accumulate during an individual's lifetime[1,2]. Somatic mutations occur spontaneously and can originate in any type of cell. These alterations may result from errors during DNA replication or from contact with detrimental environmental factors due to accidental or occupational exposures, or life style choices. Unlike inheritable pathogenic variants found in the germline, acquired somatic mutations are not passed on from one generation to the next.

Thousands of somatic mutations that can drive cancer progression, metastasis, treatment response and resistance have been catalogued by researchers and clinical laboratories. Identifying and understanding the somatic alterations in an individual's tumor can be crucial in cancer diagnosis and in planning personalized cancer treatment, monitoring response to therapy, and identifying cancer recurrence. Moreover, as a tumor progresses, it continues to acquire additional alterations that can affect the response to therapeutic agents such as chemotherapy or targeted therapies.

Established methods including fine needle aspiration, tissue biopsy, microscopic examination, in situ DNA or RNA hybridization, and immunohistochemical stains are routinely used to identify somatic changes in various cancer types. However, a tissue biopsy is an invasive procedure that can be difficult to obtain, and frequently involves co-morbidities for the patient. Moreover, it is costly to perform sequential tissue biopsies to assess residual tumor burden and changes in genetic composition during and after cancer treatment[3,4]. Also, assessment by a tumor biopsy is only a viable option when the primary sites of the tumor or metastases are known and surgically accessible.

Novel methods of somatic mutation analysis, known collectively as a **liquid biopsy**, measure cell-free DNA (cfDNA), more specifically **circulating tumor DNA** (**ctDNA**), or **circulating tumor cells** (**CTC**) from a blood sample. **Plasma** obtained from the blood sample, instead of tumor tissue obtained via a biopsy or fine needle aspiration, now allows non-invasive, highly sensitive and specific detection of somatic mutations in various cancers.[36-39]

Circulating Tumor DNA (ctDNA)

All cells, including tumor cells and non-malignant cells, shed DNA, called **cell-free DNA** (**cfDNA**), into the circulatory system (Diaz and Bardelli 2014). Cancer and other conditions, such as renal failure and myocardial infarction, often result in higher levels of cfDNA than in healthy patients (Diaz and Bardelli 2014). **Circulating tumor DNA** (**ctDNA**) is cfDNA that is shed from tumor cells into the circulatory system[4]. The mechanisms whereby tumor cells release DNA into the blood are not well understood; DNA may enter the bloodstream via secretion from viable tumor cells as either free DNA or in cell-derived vesicles known as exosomes, via secretion from phagocytes post-engulfment of tumor cells, or as a result of tumor cell death through necrosis and apoptosis (Aarthy et al. 2015; Chaudhuri et al. 2015; Diaz and Bardelli 2014; Ignatiadis, Lee, and Jeffrey 2015; Polivka, Pesta, and Janku 2015). Once in the bloodstream, the DNA persists only for a short time ($t_{1/2}$ of ~2 hours) (Diaz and Bardelli 2014; Diehl et al. 2008). Most cfDNA and ctDNA are between 180-200 base pairs (bp) in length (Diaz and Bardelli 2014; Diehl et al. 2005; Diehl et al. 2008; Fan et al. 2008; Jahr et al. 2001; Mouliere et al. 2011). ctDNA can be distinguished from other cfDNA by the presence of somatic mutations, but in the case of solid malignancies ctDNA makes up only a **small fraction** (often only **<1%**)(Diehl et al. 2005; Diehl et al. 2008; Holdhoff et al. 2009); in hematological malignancies (e.g., leukemia), on the other hand, the blood

contains much higher percentages of cfDNA derived from cancer cells. The contributing fraction of ctDNA to the total cfDNA increases with increasing tumor burden (Diaz and Bardelli 2014; Diehl et al. 2008; Newman et al. 2014) and, as such, the amount recovered may vary greatly among patients.

What is a liquid biopsy?

The term "***liquid biopsy***" describes non-invasive, highly sensitive and cost effective methods of isolating and detecting these cfDNA fragments, including circulating tumor DNA (ctDNA), from the plasma from patients diagnosed with cancer or from individuals who may have cancer. Liquid biopsies are thought to capture the entire tumor genome[5,6]. When liquid biopsy techniques are combined with deep sequencing technologies, a new set of tools is created that identify somatic genomic alterations in tumors. These data can subsequently be used for personalized treatment and monitoring[7-9].

Thus, by analyzing cell-free DNA isolated from a patient's blood, we can identify clinically relevant genomic alterations in ctDNA and match these alterations to targeted therapies and clinical trials.

Liquid biopsies offer a potential alternative to surgical tumor biopsy and histological assessment, eliminating many of the difficulties and concerns associated with traditional tests (**Table 1**) as well as a means of augmenting imaging studies and other diagnostic methods.

Given these advantages, as more studies are reported demonstrating the correlation between mutations in tumor tissue and ctDNA, ctDNA may have an increasing utility in the clinical setting for the investigation of solid tumors as a diagnostic and prognostic tool (Aarthy et al. 2015; Chaudhuri et al. 2015; Diaz and Bardelli 2014; Ignatiadis, Lee, and Jeffrey 2015; Jovelet et al. 2016; Polivka, Pesta, and Janku 2015). Because tumors are temporally and spatially heterogeneous (Gerlinger et al. 2012; Hiley et al. 2014; Ichihara and Lovly 2015; Nik-Zainal et al. 2012; Piotrowska et al. 2015; Wang et al. 2014), a tissue biopsy may only give a "snapshot" of one portion of one tumor at one time. Considering that blood collection is easier than a tissue biopsy, and the fact that ctDNA likely derives from all tumor sites, liquid biopsy has the potential to more accurately monitor a patient's disease burden and progression in real time by allowing detection of DNA characterizing intra-tumor and inter-tumor heterogeneity (Aarthy et al. 2015; Chaudhuri et al. 2015; Diaz and Bardelli 2014; Ichihara and Lovly 2015; Ignatiadis, Lee, and Jeffrey 2015; Piotrowska et al. 2015; Polivka, Pesta, and Janku 2015). Further, ctDNA has been detected in a number of other bodily fluids, such as urine, stool, cerebrospinal fluid, and saliva; so ctDNA testing may involve tumor monitoring utilizing these sample sources as well (Patel and Tsui 2015).

Table 1: Advantages of liquid biopsy over tumor biopsy

Tumor Biopsy	Liquid Biopsy
Invasive and expensive	Non invasive and less expensive
Specific to localized tumor site	Less dependent on original tumor site since tumor from both primary and metastatic sites release DNA into the bloodstream
Assessment of tumor heterogeneity limited to section of biopsy analyzed	Can capture tumor heterogeneity
A limited amount of tissue may be obtained for immunohistochemical and genomic analysis	A few copies of mutant ctDNA are sufficient for analysis
Difficult to biopsy some organs	Easy to collect sample from blood
Not viable if primary tumor has been resected or if the tumor cannot be easily visualized via imaging studies	Allows for serial evaluation in absence of detectable primary tumor or metastases
Serial biopsies are difficult to tolerate	Patient can tolerate serial blood draws for evaluation; may lead to greater compliance
	New tool that can be applied for evaluation of response to therapy and for detection of residual disease
	May allow for evaluation of development of resistance
	May aid in early detection of cancer

The Clinical Utility of ctDNA in Monitoring Cancer and Directing Therapy

Research has shown that somatic mutations in a defined set of genes are often the underlying drivers of the development of cancer across different tumor types (**Table 2**)[1]. These genes include, but are not limited to, *BRAF*, the *RAS* gene family, *EGFR*, *PIK3CA*, *FOXL2*, and *TP53*. Somatic mutations in *BRAF* gene are commonly associated with malignant melanoma, non- Hodgkin lymphoma, colorectal cancer, papillary thyroid carcinoma, non-small-cell lung carcinoma, and adenocarcinoma of the lung, while somatic *EGFR* mutations are observed in lung cancers[11]. Additionally, *PIK3CA* mutations are more frequent in breast and colorectal cancer[12]. *FOXL2* mutations are commonly seen in granulosa cell tumors, and *TP53* mutations are detected in almost all cancer types[9]. Additionally, recurrent alterations have been found in specific tumor types and across different cancers. These mutations occur at "***hotspots***" or places in the DNA sequence that are both vulnerable to mutagenesis and of significant impact on the protein function in a way that alters cellular growth and life cycle.

Knowing that a patient's tumor has developed one or more of these hotspot mutations can help the physician develop a personalized treatment plan while monitoring disease response and potential drug resistance. For example, in metastatic melanoma patients found to harbor a specific somatic *BRAF* mutation (V600E) in their tumor, treatment with BRAF inhibitors dabrafenib, trametinib and vemurafenib either alone or in combination is often recommended[13]. In addition, the EGFR inhibitors cetuximab and panitumumab are most useful in the lung cancer patients whose tumors are *KRAS* wild type (which means they do not have a mutation in the *KRAS* gene) and whose tumors express EGFR protein. Additionally, several large clinical trials have also shown that the EGFR tyrosine kinase inhibitors (TKIs) afatinib and erlotinib are useful only for treating patients whose tumors are found to carry EGFR kinase domain mutations[14].

Recent studies have shown the feasibility of using liquid biopsies to monitor tumor dynamics. Several studies have shown that the somatic mutations identified through a liquid biopsy of a patient's blood correlate with those found in the tumor specimen obtained either through biopsy or surgical resection[15,16]. Utilizing ctDNA to identify somatic mutations in tumors has been shown to correlate with clinical and radiologic outcomes for a patient as well as to predict the overall survival of the patient in some cases[12,13,15-17]. Additionally, several studies have shown that the reappearance or rising levels of ctDNA can be seen months before clinical signs or symptoms or imaging changes become apparent. Thus, serial evaluation of ctDNA has been shown to be helpful in tracking disease progression, as well as in identifying the appearance of additional somatic mutations, which may be associated with drug sensitivity and resistance, in several different cancer types[18]. A recent study by Perrone et al. (2014) showed promising results for the application of ctDNA mutation analysis as a screening tool for individuals at high risk of developing colorectal cancer.[40]

ctDNA has now been used as a biomarker in an increasing number of cancer types, including lymphoma, melanoma, GIST, thyroid cancer, breast cancer, colon, and lung cancer monitoring for common mutations, (e.g., those found in BRAF, EGFR , KRAS, PIK3CA, TP53, KIT, PDGFRA), and has informed prognosis and treatment decisions (Gonzalez-Cao et al. 2015; Kang et al. 2015; Lubitz et al. 2016; Piotrowska et al. 2015; Roschewski et al. 2015; Schiavon et al. 2015; Sefrioui et al. 2015; Spindler et al. 2015; Xu et al. 2015; Yoo et al. 2014). As a result of these promising initial studies, ctDNA analysis is now being incorporated into several ongoing clinical trials (Polivka et al. 2015). ctDNA analysis is likely to be useful for tumor molecular profiling as it has several potential advantages over traditional tumor biopsy. It is possible that ctDNA analysis will be used to monitor tumors over time in response targeted therapeutics, to monitor the development of resistance, and for the detection of minimal residual disease (Arnedos et al. 2015; Bordi et al. 2015; Diaz and Bardelli 2014; Garcia-Murillas et al. 2015;Saliou et al. 2015; Siravegna et al. 2015; Yoo et al. 2014). Further, the amount and type of ctDNA recovered may be indicative of tumor stage and burden (Diaz and Bardelli 2014; Ocana et al. 2015; Saliou et al. 2015), and thus potentially used for tumor staging. Further, in addition to gene specific mutation detection, ctDNA has been used to detect other tumor-specific genetic alterations, including microsatellite instability, loss of heterozygosity, and epigenetic changes (Aarthy et al. 2015). Importantly, ctDNA may be one day be used in cancer screening and for early detection of disease (Amant et al. 2015; Bianchi et al. 2015).

Table 2 – Frequency of somatic mutations by genes and cancer types

Cancer type	Gene name	Frequency of somatic mutations
Breast cancer	*PIK3CA*	26%
	TP53	23%
Colorectal cancer	*BRAF*	11%
	KRAS	36%
	NRAS	5%
	PIK3CA	14%
	TP53	45%
Endometrial cancer	*KRAS*	14%
	PIK3CA	21%
	TP53	17%
Granulosa cell tumor	*FOXL2*	97%
Head & Neck cancer	*EGFR*	2%
	PIK3CA	7%
	TP53	38%
Kidney cancer	*TP53*	5%
Lung cancer	*BRAF*	1-4% 1% in Non Small Cell Lung Cancer (NSCLC)
	EGFR	29%
	KRAS	17%
	PIK3CA	4%
	TP53	34%
Melanoma	*BRAF*	45%
	NRAS	18%
	TP53	12%
Ovarian cancer	*BRAF*	7%
	FOXL2	18%
	KRAS	12%
	PIK3CA	9%
	TP53	46%
Pancreatic cancer	*BRAF*	2%
	KRAS	57%
	PIK3CA	2%
	TP53	36%
Prostate cancer	*BRAF*	1%
	EGFR	3%
	KRAS	4%
	PIK3CA	2%
	TP53	14%
Testicular cancer	*BRAF*	2%
	FOXL2	2%
	KRAS	4%
	NRAS	2%
	TP53	5%
Thyroid cancer	*BRAF*	41%
	GNAS	3%
	KRAS	2%
	NRAS	7%
	PIK3CA	3%
	TP53	6%

References: COSMIC database (http://cancer.sanger.ac.uk/cosmic) accessed 07/28/2015 [19-25].

OncoNext Liquid™ test

A liquid biopsy, such as GENOMA's **OncoNext Liquid™** test, involves a blood collection (one tubes x10 ml blood). Once collected, the blood sample is centrifuged to separate the plasma containing the cell-free DNA from other components. The cfDNA is then extracted, amplified, and then analyzed for the specific somatic mutations of interest by next-generation sequencing (NGS).
Being able to analyze the liquid biopsy for multiple mutations simultaneously allows the clinician to better understand the tumor profile and adapt treatment appropriately. Blood samples can be drawn from a patient before, during, and/or after cancer treatment, or at regular intervals. Liquid biopsy has the potential for continual monitoring, which is a major advantage in cancer care.

Potential indications for the OncoNext Liquid™ test

OncoNext Liquid™ ***Monitor*** **test** is meant for patients who have been **diagnosed with cancer**, in order to:

- **Provide tumor profiling for precision medicine**: The **OncoNext Liquid™** ***Monitor*** **test** can provide physicians with valuable information about a patient's tumor profile (somatic mutations present in the tumor), which can be utilized in the development of a personalized treatment plan.
- **Monitor treatment efficacy in patients**. When a patient starts a new treatment, **OncoNext Liquid™** ***Monitor*** **test** provides a novel way of reviewing the treatment's effectiveness (e.g. monitoring the presence of mutations prior to and during treatment).
- **Monitor residual disease e/o recurrence in patients with known mutations in the primary tumor**: in instances where patients have undergone a resection of their tumor and/or have gone into disease remission, serial analysis of ctDNA burden utilizing the **OncoNext Liquid™** ***Monitor*** **test**, can help check the development of disease reoccurrence or progression.
- **Monitor disease progression and tumor evolution**: While patients are undergoing cancer treatment, oncologists can use the **OncoNext Liquid™** ***Monitor*** **test** to check the development of the patient's tumor progression and/or tumor evolution (changes in the type of mutations within a tumor). It is important to evaluate tumor evolution throughout treatment as it can lend information about potential drug sensitivity and resistance.
- **Help the physician explore other options of treatment when the patient is resistant to current therapies**.
- **Provide an alternative method for biopsy** when tissue is difficult to obtain or not available, or when the primary site of metastatic disease is unknown, or when the quantity of tissue obtained in a biopsy sample is limited and traditional molecular genotyping is requested.
- Provide prognostic information for some patients.
- **Clinical Trial Matching:** it is an additional feature of the **OncoNext Liquid™** ***Monitor*** **test**, which gives patients the option to receive personalized information about clinical trials that may be best suited for him or her, based on their tumor's profile.

OncoNext Liquid™ ***Scan*** is meant for **preventative surveillance of high-risk populations**, in order to screen for mutations that could indicate early disease. Examples of high risk populations include, but are not limited to:

- **Significant family history of one of the cancers that are known to harbor the mutations in the genes within the screen** (i.e. a mother and grandmother diagnosed with colon cancer).
- **Known genetic predisposition for one of the screened cancer types** (i.e. the individual carries a BRCA1 pathogenic variant).
- **Personal history of smoking, or prolonged exposure to second-hand smoke**.
- **Exposure to known carcinogens**, like radon.
- **Prolonged radiation or UV light exposure**.
- **History of hormone use** (fertility drugs, progestogen-containing hormone replacement therapy).
- **Certain reproductive and dietary factors**.
- **History of certain infectious diseases or chronic health conditions**.

(Cancer types included in the screen: breast, colorectal, lung, melanoma, and ovarian cancers).

OncoNext Liquid™ test technology

The **OncoNext Liquid™** test is designed for the detection of hotspot somatic mutations in a set of **23 driver genes**

involved primarily in **breast**, **ovarian**, **lung**, and **colorectal cancers**, and **melanoma**. The assay requires a blood sample (plasma) for cell free DNA isolation, which is used for PCR amplification of both the wild type and mutant DNA. The mutant DNA is sequenced on next-generation sequencing platforms.

Reported Genes and Mutations

GENOMA's **OncoNext Liquid™** test detects frequently occurring **hotspot mutations** in **23** cancer driver genes: **AKT1**, **ALK**, **AR**, **BRAF**, **CTNNB1**, **EGFR**, **ERBB2**, **ESR1**, **FOXL2**, **GNA11**, **GNAQ**, **KIT**, **KRAS**, **MEK1 (MAP2K1)**, **MET**, **NRAS**, **PDGFRA**, **PIK3CA**, **PTEN**, **RET**, **ROS1**, **SMAD4** and **TP53**, implicated in melanoma, and lung, colorectal and gastric, prostate, breast and ovarian cancers (**Table 3**).

The gene content was carefully selected to include content cited by industry organizations such as the National Comprehensive Cancer Network (NCCN)[59] and the European Society for Medical Oncology (ESMO)[60]. These genes and gene regions include single nucleotide variants (SNV) and insertions and deletions (indels) that have demonstrated involvement in tumors.

Research studies and clinical trials have shown that these mutations may reflect tumor burden, treatment response and resistance, and disease prognosis. The majority of somatic mutations are activating mutations, meaning they increase the activity of the protein coded by the gene, which leads to continuous release of growth signals, increased cell proliferation and may contribute to tumor formation.

Table 3: Genes and gene regions investigated

Gene	Associated Cancers
AKT1	Breast, Lung, Colon*
ALK	Lung, Neuroblastoma, Rhabdomyosarcoma
AR	Prostate
BRAF	Melanoma*, Colon* Lung, Ovary, Gastric, Glioma, Thyroid, Pancreas, Prostate
CTNNB1	Melanoma
EGFR	Lung *; Head & Neck, Prostate
ERBB2	Breast, Lung
ESR1	Breast
FOXL2	Ovary
GNA11	Melanoma
GNAQ	Melanoma
KIT	Gastric, Melanoma*, Thymic Carcinoma
KRAS	Colon *, Gastric, Lung *, Ovary, Thyroid, Endometrial, Pancreas, Prostate
MEK1 (MAP2K1)	Melanoma, Lung, Ovary, Colon.
MET	Lung*, Colon, Gastric
NRAS	Colon *, Lung, Melanoma, Thyroid
PDGFRA	Gastric, Melanoma,
PIK3CA	Lung, Breast, Prostate, Colon, Ovary, Head & Neck, Pancreas, Thyroid
PTEN	Breast, Lung,
RET	Lung*, Thyroid
ROS1	Lung
SMAD4	Colon
TP53	Lung, Melanoma, Ovary, Colon, Breast; Endometrial, Head & Neck, Renal, Pancreas, Prostate, Thyroid

***** *Indicates in NCCN guideline for cancer type;* **§** *frequently occurring hotspot mutations.*

Possible outcomes

GENOMA's **OncoNext Liquid™** test reports on the absence or presence of each of the hotspot mutations above 2 mutant DNA copies per patient plasma sample. Input DNA is the total amount of cfDNA from the provided patient plasma sample used in the assay. The total number of detected mutant copies of ctDNA are reported. Input and mutant DNA content are variable. Mutant DNA percentage is also reported relative to input, with reference to limit of detection (LOD). Personalized interpretation of the result based on the individual's clinical history is provided. Optional clinical trial matching based on the results may be requested for patients with advanced disease tested via **OncoNext Liquid™ Monitor** analysis.

Clinical utility

In a large study of subjects with various cancers, Bettegowda et al (2014) showed that ctDNA was detected in more than 75% of patients with advanced (metastatic) disease (pancreatic, ovarian, colorectal, bladder, gastroesophageal, breast, melanoma, hepatocellular, and head and neck cancers) and less than 50% of primary (Stage 1) cancers (brain, renal, prostate, or thyroid cancers)[26]. In a separate group of 206 metastatic colorectal cancer patients, the authors showed high sensitivity and specificity of ctDNA detection for clinically relevant *KRAS* gene mutations (87.2% and 99.2%, respectively).

Kidess et al (2015) detected somatic mutation in 68% of colorectal cancer patients (n=38) in a 46 mutations panel which included *BRAF, KRAS, EGFR* and *PIK3CA* [8]. 54% early (Stage I-III) and 93% advanced (Stage IV) cancer patients showed presence of mutations. Fifty percent patients had *KRAS*, 16% *PIK3CA* and 8% had *BRAF* mutations. No *EGFR* mutations were detected in the study. These data were in concordance with mutations identified in the tumors. In patients undergoing liver metastatectomy, ctDNA levels predicted tumor recurrence earlier than carcinoembryonic antigen (CEA) value or imaging. Four of the healthy individuals (n=47) also showed signals at or near the limit of detection of ctDNA.

In one of the largest multicentric clinical trial (CORRECT), effect of a multikinase inhibitor, regorafenib was evaluated by assessing ctDNA levels in metastatic colorectal cancer patients[18]. Mutation analysis was performed in a total of 760 patients which included 505 regorafenib treated and 255 placebo. Comparison of patient matched archived tumor specimen and fresh plasma showed concordant mutations ranging from 76% - 97% for the three genes. *KRAS* mutations were identified in 69%, *PIK3CA* mutations in 84% and *BRAF* mutations in 3% of the patients. In the regorafenib treated group, patients with *KRAS* mutations showed a significantly reduced progression free and overall survival as compared to the placebo. Interestingly, in the placebo group, patients with higher ctDNA had poor overall and progression free survival. The study supports the use of ctDNA to establish tumor genotypes at the time of treatment.

Oshiro et al (2015) reported that *PIK3CA* mutant ctDNA positive patients had significantly lower recurrence free survival (RFS) than patients with negative ctDNA in a study conducted on stage I to III breast cancer patients (n=313)[27]. The patients with higher counts of *PIK3CA* mutant ctDNA (>29 alleles) showed significant lower recurrence free and overall survival. Additionally, Beaver et al (2014) detected *PIK3CA* mutations in 12 out of 29 breast cancer patients (stage I-III patients). One patient with persistent ctDNA following initial treatment developed clinically apparent metastasis 23 months later[28].

Dawson et al (2013) detected ctDNA in nearly 97% of the metastatic breast cancer patients, and showed a higher sensitivity and specificity of ctDNA measurements in detecting tumor as compared to the CTCs and CA15-3 levels[29]. *PIK3CA* and *TP53* point mutations correlated with disease and the patients with higher level of ctDNA showed poor prognosis.

Janku et al (2015) tested 21 mutations in *BRAF, EGFR, KRAS* and *PIK3CA* in 157 patients with advanced cancers (including colorectal, melanoma, NSCLC, appendiceal cancer, ovarian and uterine cancers) who progressed on systemic therapy[17]. The authors found significant similarity in the mutations detected in archival tissue and mutations identified in ctDNA. They also found that forty-one patients with more than 1% of KRAS mutant cfDNA had a shorter median survival compared to the 20 patients with ≤ 1% of KRAS mutant DNA (4.8 vs. 7.3 months, p = 0.008). Similarly, 67 patients with >1% of mutant cfDNA (*BRAF, EGFR, KRAS*, or *PIK3CA*) had a shorter median survival compared to 33 patients with ≤ 1% of mutant cfDNA (5.5 vs. 9.8 months, p = 0.001).

Obtaining enough tissue to perform histology on a biopsy may be difficult in advanced pancreatobiliary

carcinoma patients. Zill et al (2015) used ctDNA measurements to analyze mutations in a set of 54 genes. *KRAS* and *TP53* were the most commonly mutated genes, with *APC, SMAD4, GNAS, FBXW7*, and *BRAF* also being recurrently mutated in the patient ctDNA[16]. Across these five genes (*KRAS, TP53, APC, FBXW7,* and *SMAD4)* the average sensitivity was 92.3%, specificity was 100%, and average diagnostic accuracy was 97.7%. The authors also identified actionable mutations during the follow-up of the patients which otherwise was undetected due to the failure of initial tissue biopsy.

The growing evidence in the field of liquid biopsy suggests that these types of biomarker analyses can be applied to patients with multiple different types of cancers[8, 9, 16, 17, 26, 27, 29-35]. More and more, ongoing clinical trials are now incorporating liquid biopsies to evaluate therapy response (www.clinicaltrials.gov).

Limitations and warnings

GENOMA's OncoNext Liquid™ (Monitor or Scan) *test* is a plasma-based hotspot mutation panel to aid clinicians in the identification of plasma mutation tumor burden, monitoring of cancer patients, treatment planning and preventative surveillance of high risk cancer populations for early detection of disease.
Cancer is heterogeneous disease that can occur as a result of somatic mutations in various driver genes. Pathway's OncoNext Liquid™ (Monitor or Scan) identifies somatic cancer derived hotspot mutations in 23 cancer driver genes. This test is not meant to diagnose cancer, and is only meant to screen for a possible malignancy as an adjunct to other medical examinations and interventions. It will not detect all cancers, and has not been designed to find very small tumors. No test can replace a physician's examination, imaging studies, and tissue biopsies as the gold-standard for cancer diagnosis. It is possible that mutations in these or other genes not tested in GENOMA's OncoNext Liquid™ (Monitor or Scan) test may be involved in the patient's disease. Therefore, a negative test result, where no mutations are detected, does not eliminate involvement of other genes and/or mutations. Furthermore, a positive test result needs to be interpreted in the context of individual's clinical history including stage of disease, imaging results, therapeutic details, and other laboratory data.
Results could be misinterpreted if clinical information provided is inaccurate or incomplete. Improper blood sampling and handling could result in error. Genetic counseling or medical consultation is recommended for the individual tested.

TECHNICAL INFORMATION

Assay Method

OncoNext Liquid™ (Monitor or Scan) test identifies hotspot somatic mutations (**table 4**) in a set of 23 genes commonly involved in, but not limited to, breast, lung, colorectal, and ovarian cancers and melanoma. Cell free DNA containing circulating tumor DNA is isolated from plasma, quantitated and PCR amplified. The mutant DNA pool is then PCR amplified for sequencing on Illumina's next-generation sequencing platforms.

Sequencing

Libraries from plasma cfDNA, were sequenced on a NGS sequencer.

Range

OncoNext Liquid™ (Monitor or Scan) test reports on the absence or presence of each of the hotspot somatic mutations with > 2 mutant DNA copies per patient plasma sample. Input DNA is the total amount of cfDNA from the provided patient plasma sample used in the assay. The total number of detected mutant copies of ctDNA are reported. Input and mutant DNA content are variable. Mutant DNA % is also reported relative to input, with reference to limit of detection (LOD). Databases queried include Catalogue of Somatic Mutations in Cancer (COSMIC), The Cancer Genome Atlas (TCGA), cBioPortal, National Center of Biotechnology Information (NCBI), locus specific databases and other

public databases. Personalized interpretation of the result based on the individual's clinical history is provided. Optional clinical trial matching based on the results for patients with advanced disease may be requested.

Expected Values

0, 1 or more variants

Technical limitations

Gene amplifications, translocations, and insertions or deletions over 25 bases in length are not detectable by this assay. Variants predicted to be non-deleterious (such as synonymous coding changes and common population variants) are not reported.
In validation studies, the analytical sensitivity and specificity of the targeted cancer gene assay were > 99% and > 99.9%, respectively. These may be lower for structural alterations and vary depending on the quality of the specimen. Next generation sequencing approaches may provide incorrect sequence or mutational data due to insufficient coverage in specific regions of the genome, inability to distinguish highly related human sequences, and sequencing errors.
The analysis of sequence specific alterations can also be hampered by three aspects related to the tumor DNA. First, the quality of tumor DNA obtained; second, the quantity of DNA obtained can be very low, limiting the amount of DNA molecules that can be successfully analyzed by next generation sequencing. Third, the purity of tumor DNA can be a factor, as a significant portion of the DNA analyzed in the tumor sample may be derived from contaminating normal tissues. These three aspects can reduce the chance of detecting somatic sequence and copy number alterations and rearrangements.
Genetic alterations are defined as clinically significant based on published literature and other evidence. Literature references are not comprehensive and there may be other studies that relate to the test results. This test, meant to identify somatic mutations, is not intended to detect the presence or absence of germline mutations.

Target Coverage

Coverage is the number of times a region is sequenced (the number of reads) within a single run. In general, the deeper the coverage of a targeted region, the more sensitive and reliable the assay is. For variant calling, 25.000x coverage is required for reliable detection of mutations occurring at frequencies as low as 0.1%. To pass quality control (QC) metrics for the **OncoNext Liquid™** test, samples should yield > 25.000x coverage on > 93.5% of bases targeted by the assay. Libraries generated from analytical cfDNA and clinical cfDNA performed with > 99.8% of amplicons yielding coverage ≥25.000x (**Table 4**).

Mutant Allele Fraction (MAF)

The mutant allele fraction is the frequency of the mutant allele identified in the sample and is reported for base substitutions, insertions and deletions.

Performance specifications

Mutant Allele Frequency (MAF) / Tumor Fraction	Sensitivity	Positive Predictive Value (PPV)
≥0.1%	99% (97.2%-100%)*	99.9% (99.4%-100%)*

*95% Confidence Interval

Disclaimer

Results presented in this report are intended for use solely by a qualified health care professional. Any diagnosis, counseling, or treatment determination made as a result of data presented in the report

should be made by a qualified health care professional in conjunction with other individual patient health information, including clinical presentation and other test reports. Information contained within the report is current as of the report date; a qualified health professional should reassess these data as relevant literature becomes available.

Table 4: Hotspot mutations investigated with OncoNext Liquid™ 23 genes

Gene	Mutation	Exon	Nucleotide Variation
AKT1	E17K	3	c.49 G>A
ALK	D1091N	20	c.3271G>A
ALK	I1171N	22	c.3512T>A
ALK	T1151M	22	c.3452C>T
ALK	F1174C	23	c.3521T>G
ALK	F1174I	23	c.3520T>A
ALK	F1174L	23	c.3522C>A
ALK	F1174V	23	c.3520T>G
ALK	D1225N	24	c.3673G>A
ALK	F1245C	24	c.3734T>G
ALK	F1245L	24	c.3735C>G
ALK	F1245V	24	c.3733T>G
ALK	R1275Q	25	c.3824G>A
ALK	Y1278S	25	c.3833A>C
ALK	1151Tins		
ALK	C1156Y		
ALK	G1202R		
ALK	G1269A		
ALK	L1152R		
ALK	L1196M		
ALK	L1198F		
ALK	S1206Y		
AR	L702H	4	c.2105T>A
AR	W742C	5	c.2226G>T
AR	H875Y	8	c.2623C>T
AR	F877L	8	c.2631C>A
AR	T878A	8	c.2632A>G
BRAF	G466V	11	c.1397G>T
BRAF	G469A	11	c.1406G>C
BRAF	G469E	11	c.1406G>A
BRAF	G469L	11	c.1405_1406delGGinsTT
BRAF	G469V	11	c.1406G>T
BRAF	Y472C	11	c.1415A>G
BRAF	D594E	15	c.1782T>A
BRAF	D594E	15	c.1782T>G
BRAF	D594G	15	c.1781A>G
BRAF	D594H	15	c.1780G>C
BRAF	D594N	15	c.1779_1780delTGinsGA
BRAF	D594N	15	c.1780G>A
BRAF	D594V	15	c.1781A>T
BRAF	G596R	15	c.1786G>C
BRAF	K601E	15	c.1801A>G
BRAF	L597Q	15	c.1790T>A
BRAF	L597R	15	c.1790T>G
BRAF	L597S	15	c.1789_1790delCTinsTC
BRAF	L597V	15	c.1789C>G
BRAF	V600D	15	c.1799_1800delTGinsAT

BRAF	V600E	15	c.1799T>A
BRAF	V600E	15	c.1799_1800delTGinsAA
BRAF	V600G	15	c.1799T>G
BRAF	V600K	15	c.1798_1799delGTinsAA
BRAF	V600M	15	c.1798G>A
BRAF	V600R	15	c.1798_1799delGTinsAG
CTNNB1	S37F	3	c.110C>T
CTNNB1	S37Y	3	c.110C>A
CTNNB1	S45P	3	c.133T>C
CTNNB1	S45F	3	c.134C>T
CTNNB1	S45Y	3	c.134C>A
EGFR	G719A	18	c.2156G>C
EGFR	G719C	18	c.2155G>T
EGFR	G719S	18	c.2155G>A
EGFR	Exon 19 Deletions	19	
EGFR	Exon 19 Insertions	19	
EGFR	A763_Y764insFQEA	20	c.2290_2291ins
EGFR	Exon 20 Insertions	20	
EGFR	S768I	20	c.2303G>T
EGFR	T790M	20	c.2369C>T
EGFR	L858R	21	c.2573T>G
EGFR	L861Q	21	c.2582T>A
EGFR	**E746_A750>IP**		c.2235_2248delGGAATTAAGAGAAGinsAATTC
EGFR	**E746_A750del**		c.2235_2249delGGAATTAAGAGAAGC
EGFR	**E746_A750del**		c.2236_2250delGAATTAAGAGAAGCA
EGFR	**E746_P753>VS**		c.2237_2257del21insTCT
EGFR	**E746_S752>A**		c.2237_2254del18
EGFR	**E746_S752>D**		c.2238_2255del18
EGFR	**E746_S752>I**		c.2235_2255delinsAAT
EGFR	**E746_S752>V**		c.2237_2255delinsT
EGFR	**E746_T751>A**		c.2237_2251del15
EGFR	**E746_T751>I**		c.2235_2252delinsAAT
EGFR	**E746_T751>IP**		c.2235_2251delinsAATTC
EGFR	**E746_T751>V**		c.2237_2252delinsT
EGFR	**E746_T751>VA**		c.2237_2253delinsTTGCT
EGFR	**E746_T751del**		c.2236_2253del18
EGFR	**K745_E749del**		c.2233_2247del15)
EGFR	**L747_A750>P**		c.2238_2248delATTAAGAGAAGinsGC
EGFR	**L747_A750>P**		c.2239_2248delTTAAGAGAAGinsC
EGFR	**L747_E749del**		c.2239_2247delTTAAGAGAA
EGFR	**L747_P753>Q**		c.2239_2258delinsCA
EGFR	**L747_S752>Q**		c.2239_2256delinsCAA
EGFR	**L747_S752del**		c.2239_2256del18
EGFR	**L747_T751>Q**		c.2238_2252delinsGCA
EGFR	**L747_T751>S**		c.2240_2251del
EGFR	**L747_T751del**		c.2238_2252del
ERBB2(HER2)	G309A	8	c.926G>C
ERBB2(HER2)	D769H	19	c.2305G>C
ERBB2(HER2)	D769Y	19	c.2305G>T
ERBB2(HER2)	G776S	19	c.2326 G>A
ERBB2(HER2)	L755_T759del	19	c.2264_2278del
ERBB2(HER2)	L755S	19	c.2264T>C

ERBB2(HER2)	Exon 20 Insertions	20	
ERBB2(HER2)	G778_P780dup	20	c.2339_2340ins
ERBB2(HER2)	V777L	20	c.2329G>T
ERBB2(HER2)	V842I	21	c.2524G>A
ERBB2(HER2)	R896C	22	c.2686C>T
ERBB2(HER2)	c.2263_2264delTTinsCC		c.2263_2264delTTinsCC
ERBB2(HER2)	c.2322_2334dupATACGTGATGGC		c.2322_2334dupATACGTGATGGC
ERBB2(HER2)	c.2328_2336dupTGTGGGCTC		c.2328_2336dupTGTGGGCTC
ESR1	S463P		
ESR1	V534E		
ESR1	P535H		
ESR1	L536Q		
ESR1	L536R		
ESR1	Y537C		
ESR1	Y537S		
ESR1	Y537N		
ESR1	D538G		
FOXL2	C134W	1	c.402 C>G
GNA11	R183C	4	c.546_547delCCinsTT
GNA11	R183C	4	c.547C>T
GNA11	Q209L	5	c.626A>T
GNA11	Q209P	5	c.626A>C
GNAQ	R183Q	4	c.548G>A
GNAQ	Q209L	5	c.626A>T
GNAQ	Q209P	5	c.626A>C
GNAQ	Q209R	5	c.626A>G
KIT	A502–Y503insFA	9	c.1507_1508insTTGCCT
KIT	E490K	9	c.1468G>A
KIT	Exon 9 Mutation	9	
KIT	F504L	9	c.1510T>C
KIT	556 ins L	11	
KIT	575 ins PE	11	
KIT	Del 554–558	11	
KIT	Del 554–559	11	
KIT	Del 566–572	11	
KIT	Del 566–574	11	
KIT	Del 579	11	
KIT	Del V559	11	
KIT	E583_E589dupPYDHKWE	11	
KIT	Exon 11 Mutation	11	
KIT	G565V	11	
KIT	K550N	11	
KIT	K558N	11	
KIT	L576P	11	c.1727T>C
KIT	N566D	11	
KIT	P577_D579del	11	c.1730_1738del
KIT	V559A	11	c.1676T>C
KIT	V559D	11	c.1676T>A
KIT	V559G	11	
KIT	V560A	11	
KIT	V560D	11	c.1727T>C (V560D)
KIT	V560del	11	c.1679_1681del
KIT	V560G	11	
KIT	V569G	11	
KIT	W557R	11	c.1669T>A

KIT	W557R	11	c.1669T>C
KIT	Y553N	11	c.1657T>A
KIT	Exon 13 Mutation	13	
KIT	K642E	13	c.1924A>G
KIT	N655	13	
KIT	N655S	13	
KIT	R634W	13	
KIT	V654A	13	
KIT	Exon 14 Mutation	14	
KIT	H697Y	14	c.2089C>T
KIT	D816H	17	c.2446G>C
KIT	D816V	17	
KIT	D820E	17	c.2460T>A
KIT	D820V	17	
KIT	D820Y	17	
KIT	Exon 17 Mutation	17	
KIT	N822I	17	
KIT	N822K	17	
KIT	N822Y	17	
KIT	Y823D	17	
KIT	A829P	18	
KIT	I841V	18	
KIT	S864F	18	
KIT	Other KIT mutations		
KRAS	G12A	2	c.35G>C
KRAS	G12C	2	c.34G>T
KRAS	G12D	2	c.35G>A
KRAS	G12R	2	c.34G>C
KRAS	G12S	2	c.34G>A
KRAS	G12V	2	c.35G>T
KRAS	G13A	2	c.38G>C
KRAS	G13C	2	c.37G>T
KRAS	G13D	2	c.38G>A
KRAS	G13R	2	c.37G>C
KRAS	G13S	2	c.37G>A
KRAS	G13V	2	c.38G>T
KRAS	Q22K	2	c.64C>A
KRAS	Q61H	3	c.183A>C
KRAS	Q61H	3	c.183A>T
KRAS	Q61H	3	c.183A>C
KRAS	Q61K	3	c.181C>A
KRAS	Q61L	3	c.182A>T
KRAS	Q61P	3	c.182A>C
KRAS	Q61R	3	c.182A>G
KRAS	A146P	4	c.436G>C
KRAS	A146T	4	c.436G>A
KRAS	A146V	4	c.437C>T
KRAS	K117N	4	c.351A>C
KRAS	K117N	4	c.351A>T
MEK1 (MAP2K1)	D67N	2	c.199G>A
MEK1 (MAP2K1)	F53L	2	c.157T>C
MEK1 (MAP2K1)	K57N	2	c.171G>T

MEK1 (MAP2K1)	Q56P	2	c.167A>C
MEK1 (MAP2K1)	C121S	3	c.362G>C
MEK1 (MAP2K1)	E203K	3	c.607G>A
MEK1 (MAP2K1)	I111S	3	c.332T>G
MEK1 (MAP2K1)	N382H	3	c.1144A>C
MEK1 (MAP2K1)	P124L	3	c.371C>T
MEK1 (MAP2K1)	P124S	3	c.370C>T
MEK1 (MAP2K1)	P264S	3	c.790C>T
MET	c.2888-6_29del	14	c.2888-6_29del
MET	c. 3028G>C	14	c. 3028G>C
MET	c.2887-18_2887-7del12	14	c.2887-18_2887-7del12
MET	c.2888delA	14	c.2888delA
MET	c.3001_3021delGTAGACTACCGAGC TACTTTT	14	c.3001_3021delGTAGACTACCGAGCT ACTTTT
MET	c.3024_3028+7delAGAAGGTATATT	14	c.3024_3028+7delAGAAGGTATATT
MET	c.3028+1G>T	14	c.3028+1G>T
MET	c.3028G>A	14	c.3028G>A
MET	c.3028G>T	14	c.3028G>T
MET	L1213V	18	c.3637 C>G
MET	V1206L	18	c.3616 G>T
NRAS	G12A	2	c.35G>C
NRAS	G12C	2	c.34G>T
NRAS	G12D	2	c.35G>A
NRAS	G12R	2	c.34G>C
NRAS	G12S	2	c.34G>A
NRAS	G12V	2	c.35G>T
NRAS	G13A	2	c.38G>C
NRAS	G13C	2	c.37G>T
NRAS	G13D	2	c.38G>A
NRAS	G13R	2	c.37G>C
NRAS	G13V	2	c.38G>T
NRAS	Q61E	3	c.181C>G
NRAS	Q61H	3	c.183A>C
NRAS	Q61H	3	c.183A>T
NRAS	Q61H	3	c.183A>T
NRAS	Q61K	3	c.181C>A
NRAS	Q61L	3	c.182A>T
NRAS	Q61L	3	c.182_183delAAinsTG
NRAS	Q61P	3	c.182A>C
NRAS	Q61R	3	c.182A>G
NRAS	Q61R	3	c.182_183delAAinsGG
PDGFRA	c.1679_1693delGGGTCATTGAATCAA		
PDGFRA	c.1681_1682insAGAGGG		
PDGFRA	c.1696_1713del18		
PDGFRA	c.2526_2537delCATCATGCATGA		
PDGFRA	c.2533_2544delCATGATTCGAAC		
PDGFRA	D842V	18	c.2525 A>T

PDGFRA	D846Y (c.2536 G>T)	18	
PDGFRA	Exon 12 Mutation	12	
PDGFRA	Exon 14 Mutation	14	
PDGFRA	Exon 18 Mutation	18	
PDGFRA	V561D (c.1682 T>A)		
PDGFRA	Y555C (c.1664 A>G)		
PIK3CA	D549N	9	c.1645G>A
PIK3CA	E542K	9	c.1624G>A
PIK3CA	E545G	9	c.1634A>G
PIK3CA	E545K	9	c.1633G>A
PIK3CA	E545Q	9	c.1633G>C
PIK3CA	E545V	9	c.1634A>T
PIK3CA	Q546E	9	c.1636C>G
PIK3CA	Q546K	9	c.1636C>A
PIK3CA	Q546L	9	c.1637A>T
PIK3CA	Q546P	9	c.1637A>C
PIK3CA	Q546R	9	c.1637A>G
PIK3CA	H1047R	20	c.3140A>G
PIK3CA	H1047L	20	c.3140A>T
PIK3CA	H1047Y	20	c.3139C>T
PIK3CA	M1043I	20	c.3129G>A
PTEN	R130*	5	c.388C>T
PTEN	R130fs*4	5	c.389delG
PTEN	R130G	5	c.388C>G
PTEN	R130Q	5	c.389G>A
PTEN	R159S	6	c.477G>T
PTEN	K267fs*9	7	c.800delA
PTEN	P248fs*5	7	c.741dupA
PTEN	R233*	7	c.697C>T
PTEN	N323fs*2	8	c.968supA
PTEN	N323fs*21	8	c.968delA
RET	C634 Mutations	11	
RET	M918T	16	
ROS1	G2032R		
ROS1	D2033N		
ROS1	L2155S		
SMAD4	E330A		c.989A>C
SMAD4	D351H		c.1051G>C
SMAD4	D351N		c.1051G>A
SMAD4	D355E		c.1065C>A
SMAD4	R361C		c.1081C>T
SMAD4	R361S		c.1081C>A
SMAD4	R361H		c.1082G>A
SMAD4	D537Y		c.1609G>T
TP53	Whole coding region	Exons 2-11	

AKT1

Biological function: The AKT1 gene provides instructions for making a protein called AKT1 kinase. This protein is found in various cell types throughout the body, where it plays a critical role in many signaling pathways. For example, AKT1 kinase helps regulate cell growth and division (proliferation), the process by which cells mature to carry out specific functions (differentiation), and cell survival. AKT1 kinase also helps control apoptosis, which is the self-destruction of cells when they become damaged or are no longer needed.
Signaling involving AKT1 kinase appears to be essential for the normal development and function of the nervous system. Studies have suggested a role for AKT1 kinase in cell-to-cell communication among nerve cells (neurons), neuronal survival, and the formation of memories.
The AKT1 gene belongs to a class of genes known as oncogenes. When mutated, oncogenes have the potential to cause normal cells to become cancerous.

The **E17K** is a missense mutation, resulting in an amino acid change at position 17 in AKT1, from a glutamic acid (E) to a lysine (K). This mutation occurs within the pleckstrin homology domain (PHD) of AKT1 and results in activation of the phosphatidylinositol 3-kinase (PI3K) pathway. In vitro studies suggest that the AKT1 E17K mutation is less sensitive than wild type AKT1 to inhibition by the experimental AKT inhibitor VIII, a non-ATP competitive agent which requires a functional pleckstrin homology domain (Carpten et al. 2007). Other AKT inhibitors, both allosteric and catalytic, are in clinical development. Preclinical studies have also shown that AKT1 E17K promotes cell growth, colony formation, and migration and invasion in cell lines, as well as tumor formation in mice (Beaver et al., 2013; 23888070).

Incidence in disease: AKT1 mutations have been reported in 1.4% (20/1413) of colorectal carcinoma samples analyzed in COSMIC (Aug 2015) and in less than 1% (2/223) of sequenced tumors in the Colorectal Adenocarcinoma TCGA dataset (cBioPortal for Cancer Genomics, Aug 2015). A study reported AKT1 mutations in 1.3% (4/311) colorectal carcinoma samples analyzed (Stachler et al., 2015; 25683705).

Role in disease: Although Akt activation has been shown to be insufficient to induce tumor formation on its own, it has been shown to accelerate tumor formation and increase metastasis in animal models of cancer (Hutchinson et al., 2001; 11238953, Dillon et al., 2009; 19491266, Hutchinson et al., 2004; 15126356). Overexpression of phosphorylated Akt (p-Akt) is thought to occur early in colorectal carcinoma tumorigenesis and to play a role in tumor progression (Roy et al., 2002; 11756242, Itoh et al., 2002; 12115344, Johnson et al., 2010; 20421047, Henderson-Jackson et al., 2010; 21151390). Preclinical studies suggest that Akt signaling may regulate epithelial-mesenchymal transition in colorectal cancer (Suman et al., 2013; 24000138).

Effect on drug sensitivity: Numerous targeted therapies are under investigation for the PI3K/Akt/mTOR pathway. AKT1 E17K mutants are not expected to be sensitive to inhibition of PI3K, because the aberration in the PH domain allows them to be recruited to the cell membrane independent of PI3K activity (Carpten et al., 2007; 17611497). However, inhibitors of Akt itself, or downstream mTOR inhibitors, may be effective. Drugs that block that catalytic activity of Akt are currently being examined in clinical trials. Additional small molecules that block the recruitment of Akt to the membrane are under preclinical investigation (Jo et al., 2011; 21464312, Kim et al., 2010; 20068047). The mTOR inhibitors everolimus and temsirolimus, which have been approved by the FDA in some tumor types, as well as other mTOR inhibitors, are being tested in clinical trials, both alone and in combination with other therapies. Targeting mTORC1/2 is another treatment strategy that is currently under investigation, and several inhibitors targeting the mTORC1/Raptor and mTORC2/Rictor complexes are being tested in early phase clinical trials (Grunt and Mariani, 2013; 23215720).

AKT1 in breast cancers

Somatic mutations in AKT1 have been found in a fraction of breast cancers.

Gene Mutation	Invasive Breast Cancer	Hormone Receptor Positive (ER+ and/or PR+) Invasive Breast Cancer	HER2 positive Invasive Breast Cancer	Triple-negative Invasive Breast Cancer
AKT1	4% (O'Brien et al. 2010)	3.2% (Stemke-Hale et al. 2008)	<1% (Stemke-Hale et al. 2008)[a]	<1% (Stemke-Hale et al. 2008)[a]

AKT1 in Lung Cancer

Somatic mutations in *AKT1* have been found in ~ 1% of all NSCLC (Bleeker et al. 2008; Do et al. 2008; Malanga et al. 2008), in both adenocarcinoma and squamous cell carcinoma histology. Preclinical data have shown that the presence of this mutation results in cellular transformation in vitro and in vivo (Carpten et al. 2007). Specific clinical characteristics of lung cancer patients harboring *AKT1* mutations have yet to be described.

In the vast majority of cases, *AKT1* mutations are non-overlapping with other oncogenic mutations found in NSCLC (e.g., *EGFR* mutations, *ALK* rearrangements, etc.).

In preclinical experiments using a cell line harboring BRAF V600E and AKT1 E17K mutations, the AKT1 E17K mutation was associated with sensitivity to GSK2141795B (a tool compound related to the AKT1 inhibitor uprosertib, GSK214179; Lassen et al. 2014).

AKT1 in Colorectal Cancer

Somatic mutations in AKT1 have been found in <1–6% of all colorectal cancer (Carpten et al. 2007; COSMIC; Fumagalli et al. 2008; Kim et al. 2008). In colorectal cancer, the only AKT1 mutation observed up to this time is the E17K mutation, which has also been observed in other types of cancer. This mutation in the Pleckstrin homology domain alters the ligand binding site and leads to constitutive kinase activity. Preclinical data have shown that the presence of this activating mutation results in cellular transformation in vitro and in vivo (Carpten et al. 2007). Specific clinical characteristics of colorectal cancer patients harboring AKT1 mutations have yet to be described. AKT1 mutations and PTEN mutations appear to be mutually exclusive. Likewise, AKT1 mutations and PI3K mutations appear to be mutually exclusive.

BRAF

Biological function: The BRAF gene (OMIM – 164757), also known as B-raf proto-oncogene, encodes a protein in the raf/mil family of serine/threonine protein kinases. BRAF plays a role in regulating the RAS/RAF/MAP/MEK signaling pathway that affects cell division, differentiation and secretion.[41] Somatic mutations in BRAF result in an activated protein, which causes a continuous release of growth signals and may contribute to cancers by allowing abnormal cell growth and division (Brose et al., 2002; 12460918, Bettegowda et al., 2014; 24553385, Rothschild 2015; 26018876, Davies et al., 2002; 12068308).

Clinical relevance: Somatic mutations in BRAF gene are detected in melanoma (45%), thyroid (41%), colorectal (11%), ovarian (7%), pancreatic (2%), testicular (2%), and prostate cancers (1%), and in 1% of non-small cell lung carcinoma (http://cancer.sanger.ac.uk/cosmic).[42] Activation of Braf may predict sensitivity to inhibitors of Braf or components of the MAPK pathway, including MEK (Solit et al., 2006; 16273091, Flaherty et al., 2010; 20818844, Flaherty et al., 2012; 23020132, Menzies and Long, 2014; 24583796). BRAF V600E and V600K are the most common mutations detected in cancers. The high frequency of these activating mutations led to the development of tyrosine kinase inhibitors for the treatment of cancer. The V600E-specific inhibitors, vemurafenib and dabrafenib, and MEK inhibitor trametinib are approved for use in melanoma (FDA drug label).[43] In contrast to the V600E mutation, low-activity BRAF mutations like G469A have been shown to signal through CRAF rather than BRAF to activate

the MAPK pathway.[44] In addition, these types of mutations have been reported to confer resistance to some MEK inhibitors, and to confer sensitivity to sorafenib. The multi-tyrosine kinase inhibitors regorafenib and sorafenib that target BRAF and Raf-1 are approved to treat colorectal cancer and hepatocellular carcinoma, and sorafenib is also approved for the treatment of advanced thyroid cancer. These drugs are in clinical trials for multiple tumor types. Metastatic melanoma, non-small cell lung cancer, and renal cell cancer patients with wild type BRAF or with BRAF V600 mutations have shown complete or partial response to the monoclonal antibodies targeting PD1 receptors (programmed cell death 1).[45]
In some studies, BRAF V600E mutation has been correlated with lack of response to approved anti-Egfr therapies such as cetuximab and panitumumab in CRC; patients with wild-type BRAF experienced a higher response rate when cetuximab was added to chemotherapy, compared to treatment with chemotherapy alone (Di Nicolantonio et al., 2008; 19001320, Cui et al., 2014; 24390240).

BRAF in Lung Cancer

Somatic mutations in *BRAF* have been found in 1–4% of all NSCLC (Brose et al. 2002; Cardarella et al. 2013; Davies et al. 2002; Naoki et al. 2002; Paik et al. 2011; Pratilas et al. 2008), most of which are adenocarcinomas. *BRAF* mutations are more likely to be found in former/current smokers (Paik et al. 2011; Pratilas et al. 2008)
In contrast to melanoma where the majority of *BRAF* mutations occur at valine 600 (V600) within exon 15 of the kinase domain, *BRAF* mutations in lung cancer also occur at other positions within the kinase domain. In one study of 697 patients with lung adenocarcinoma, *BRAF* mutations were present in 18 patients (3%). Of these 18 patients, the *BRAF* mutations identified were V600E (50%), G469A (39%), and D594G (11%; Paik et al. 2011).
In the vast majority of cases, *BRAF* mutations are non-overlapping with other oncogenic mutations found in NSCLC (e.g., *EGFR* mutations, *ALK* rearrangements, etc.).

BRAF in Melanoma

Somatic mutations in BRAF have been found in 37-50% of all malignant melanomas (COSMIC; Davies et al. 2002; Hodis et al. 2012; Krauthammer et al. 2012; Maldonado et al. 2003). BRAF mutations are found in all melanoma subtypes but are the most common in melanomas derived from skin without chronic sun-induced damage (Curtin et al. 2005; Maldonado et al. 2003). In this category of melanoma, BRAF mutations are found in ~59% of samples (Curtin et al. 2005).
The most prevalent BRAF mutations detected in melanoma are missense mutations that introduce an amino acid substitution at valine 600. Approximately 80–90% of V600 BRAF mutations are V600E (valine to glutamic acid; COSMIC; Lovly et al. 2012; Rubinstein et al. 2010) while 5-12% are V600K (valine to lysine; COSMIC; Lovly et al. 2012; Rubinstein et al. 2010), and 5% or less are V600R (valine to arginine) or V600D (valine to aspartic acid; COSMIC;Lovly et al. 2012; Rubinstein et al. 2010). The result of these mutations is enhanced BRAF kinase activity and increased phosphorylation of downstream targets, particularly MEK (Wan et al. 2004). In the vast majority of cases, BRAF mutations are non-overlapping with other oncogenic mutations found in melanoma (e.g., NRAS mutations, KIT mutations, etc.).
While BRAF inhibitor therapy is associated with clinical benefit in the majority of patients with BRAF V600E-mutated melanoma, resistance to treatment and tumor progression occurs in nearly all patients, usually in the first year (Chapman et al. 2011; Sosman et al. 2012). A variety of mechanisms have been implicated in primary and acquired resistance to BRAF inhibitors, primarily through reactivation of the MAP kinase pathway and other cell signaling pathways. Secondary BRAF mutations have not been described. Mechanisms of resistance are described below (Table 1); the frequencies of each of these mechanisms of resistance are not yet known. Possible second-line and greater treatment options supported by preclinical rationale are listed, although clinical data are mostly lacking. First-line combination therapy with BRAF and MEK inhibitor therapy may delay or prevent some of the mechanisms below (Flaherty et al. 2012). Additionally, BRAF inhibitors have been investigated in combination with MEK inhibitors in subsets of patients with BRAF V600E-mutated melnaoma previously resistant to BRAF inhibitors (Johnson et al. 2014; Ribas et al. 2014).

BRAF in Colorectal Cancer

Approximately 8–15% of colorectal cancer (CRC) tumors harbor BRAF mutations (De Roock et al. 2009; Rizzo et al. 2010; Tejpar et al. 2010). The presence of BRAF mutation is significantly associated with right-sided colon cancers and is associated with decreased overall survival (Roth et al., 2010). Several studies have reported that patients with metastatic CRC (mCRC) that harbor BRAF mutations do not respond to anti-EGFR antibody agents cetuximab or panitumumab in the chemotherapy-refractory setting (Bardelli and Siena 2010; Folprecht et al. 2010; Gravalos et al. 2010; Lievre, Blons, and Laurent-Puig 2010). Based on these findings, BRAF mutations were suggested to be a negative predictor of response to anti-EGFR therapy (De Roock et al. 2009; Mao et al. 2011; Rizzo et al. 2010; Sharma and Gulley 2010; Tejpar et al. 2010).

The most frequently reported BRAF mutation is an activating missense mutation in which the amino acid glutamic acid is substituted for valine at amino acid position 600 (V600E; Mao et al. 2011; Rizzo et al. 2010). This mutation is also associated with unresponsiveness to anti-EGFR therapy in wild type KRAS patients with mCRC, as indicated by the results of a meta-analysis by Mao et al. (2011).

While BRAF V600-mutated melanomas are sensitive to vemurafenib (Sosman et al. 2012), BRAF V600-mutated CRCs may not be as sensitive (Kopetz et al. 2010; Prahallad et al. 2012). Activation of EGFR in colorectal cancer could explain why colorectal cancers generally have a lower response to BRAF inhibitors (Corcoran et al. 2012; Prahallad et al. 2012).

BRAF in Ovarian Cancer

Somatic mutations in BRAF have been found in a fraction of ovarian cancers and are associated with Type I tumors (see Table). The most common variant is V600E in 95% of cases (COSMIC).

Table. Frequency of Somatic Gene Mutations in Epithelial Ovarian Cancer (EOC)

	EOC Overall	Type I				Type II
Gene Mutation		**Low Grade Serous**	**Clear Cell**	**Endometrioid**	**Mucinous**	**High Grade Serous**
BRAF	11% (Kurman and Shih 2011)	24–33% (Singer et al. 2003; Nakayama et al. 2006)	1% (Kuo et al. 2009)	24% (Singer et al. 2003)	50–75% (Gemignani et al. 2003)	<1% (TCGA 2011)

BRAF in Gastrointestinal Stromal Tumor (GIST)

Somatic mutations in BRAF have been found in <1% of GIST (Agaimy et al. 2009), and are similar to those seen in melanoma. In particular, the most common BRAF mutations are missense mutations which introduce an amino acid substitution at valine 600, in particular V600E (valine to glutamic acid). The result of these mutations is enhanced BRAF kinase activity and increased phosphorylation of downstream targets, particularly MEK (Hubbard 2004).

As in melanoma, BRAF mutations in GIST are non-overlapping with other oncogenic mutations found in GIST (e.g., KIT or PDGFRA mutations). BRAF mutations appear to be associated with a high risk of malignancy and resistance to presently available KIT/PDGFRA tyrosine kinase inhibitors (Agaram et al. 2008).

EGFR

Biological function: The EGFR (Epidermal Growth Factor Receptor) gene (OMIM-131550) encodes a cell surface glycoprotein, which is a member of the protein kinase superfamily involved in binding of epidermal growth factor.[41] Binding of EGFR protein to a ligand induces receptor dimerization and tyrosine auto phosphorylation, which in turn leads to cell proliferation, cell cycle progression, differentiation, increased cell invasiveness and apoptosis (Bettegowda et al., 2014; 24553385, Rothschild 2015; 26018876, Greenman et al., 2007; 17344846).

Incidence in cancer and prognosis: EGFR somatic mutations are detected in lung (29%), prostate (3%) and head and neck (2%) cancers (http://cancer.sanger.ac.uk/cosmic).[42] Most mutations occur in exons 18-21 which encode part of the kinase domain. Exon 19 deletions are most frequently found (about

40%), followed by L858R (36.2%), T790M (3.8%) and exon 20 (1.3%) mutations. EGFR alterations have been reported in 13-35% of lung adenocarcinomas. EGFR amplification has been documented in up to 62% of non-small cell lung cancer (NSCLC), and has been correlated with EGFR protein expression as measured by immunohistochemistry, although this correlation is not consistent for low level gene amplification. EGFR protein expression or overexpression has been reported in up to 70% of NSCLC tumors. EGFR mutations have been reported to predict improved survival in patients with resected Stage 1-3 lung adenocarcinoma15 or resected Stage 1 NSCLC.
Patients with EGFR mutations in non-small cell lung cancer are most effectively treated using EGFR tyrosine kinase inhibitors (TKIs). In several large clinical trials, it has been demonstrated that afatinib and erlotinib are useful in patients with exon 19 deletions and exon 21 (L858R) mutations. Treatment with the EGFR-targeting antibody panitumumab is indicated in lung cancer patients who have EGFR mutations and are negative for KRAS and NRAS codon 12/13 mutations.[46] Cetuximab alone or in combination with other drugs is approved for the treatment of head and neck cancer and EGFR-expressing wild type KRAS colorectal cancer patients. Erlotinib is indicated as the first, second and third line drug fortreatment in colorectal cancer patients with EGFR-activating mutations. Erlotinib is also approved for advanced pancreatic cancers.[47]
Acquisition of a secondary mutation in EGFR T790M-positive lung cancers is the most common mechanism of resistance in patients treated with first generation TKIs.[48] Studies have shown that tumors with EGFR exon 19 in-frame deletions are responsive to erlotinib, while the EGFR L858R mutation is a resistance marker and the patients with this mutation have poor disease-free survival.[49]

EGFR in Non-Small Cell Lung Cancer (NSCLC)
Approximately 10% of patients with NSCLC in the US and 35% in East Asia have tumor associated *EGFR* mutations (Lynch et al. 2004; Paez et al. 2004; Pao et al. 2004). These mutations occur within EGFR exons 18–21, which encodes a portion of the EGFR kinase domain (Figure 1). *EGFR* mutations are usually heterozygous, with the mutant allele also showing gene amplification (Soh et al. 2009). Approximately 90% of these mutations are exon 19 deletions or exon 21 L858R point mutations (Ladanyi and Pao 2008). These mutations increase the kinase activity of EGFR, leading to hyperactivation of downstream pro-survival signaling pathways (Sordella et al. 2004).
Regardless of ethnicity, *EGFR* mutations are more often found in tumors from female never smokers (defined as less than 100 cigarettes in a patient's lifetime) with adenocarcinoma histology (Lynch et al. 2004; Paez et al. 2004; Pao et al. 2004). However, *EGFR* mutations can also be found in other subsets of NSCLC, including in former and current smokers as well as in other histologies.
In the vast majority of cases, *EGFR* mutations are non-overlapping with other oncogenic mutations found in NSCLC (e.g., *KRAS* mutations, *ALK* rearrangements, etc.).

Effect on drug sensitivity: The presence of a sensitizing EGFR mutation in a tumor is the strongest biological predictor of sensitivity to an Egfr tyrosine kinase inhibitor (TKI), including osimertinib, afatinib, erlotinib, gefitinib, cetuximab, panitumumab, and lapatinib (Mok et al., 2009; 19692680, Rosell et al., 2009; 19692684, Tsao et al., 2005; 16014883, Rosell et al., 2012; 22285168).
The Egfr TKIs erlotinib, gefitinib, and afatinib have been approved by the FDA for the treatment of EGFR mutant non-small cell lung cancer (NSCLC) (Shepherd et al., 2005; 16014882, Rosell et al., 2012; 22285168, Sequist et al., 2013; 23816960). Other Egfr inhibitors are also approved in other indications, including the dual Egfr/Her2 inhibitor lapatinib and the anti-Egfr monoclonal antibodies cetuximab and panitumumab (Geyer et al., 2006; 17192538, Cunningham et al., 2004; 15269313, Vermorken et al., 2008; 18784101, Van Cutsem et al., 2007; 17470858).
Resistance to EGFR inhibition may arise by reactivation of the MAPK pathway, and preclinical evidence suggests that co-targeting EGFR and MAPK signaling may retard the development of acquired resistance to third-generation EGFR inhibitors. Necitumumab is an anti-EGFR antibody that is FDA approved to treat metastatic squamous NSCLC in combination with gemcitabine and cisplatin. Addition of necitumumab increased overall and progression-free survival in patients with squamous NSCLC relative to chemotherapy alone; however, it exhibited a poor tolerability profile in non-squamous NSCLC, and EGFR expression has not been demonstrated to be predictive of

clinical benefit in NSCLC. Preclinical studies have reported that EGFR-mutant cells are sensitive to HSP90 inhibitors. Clinical studies of HSP90 inhibitors, alone and in combination with EGFR inhibitors, have reported response rates ranging from 0% to 18% in patients with NSCLC harboring EGFR mutations (Garon et al., 2012; ASCO Abstract 7543), although combination treatment was deemed too toxic. The reovirus Reolysin, which targets cells that harbor activated RAS signaling due to alterations in RAS genes or upstream activators such as EGFR, is also in clinical trials in some tumor types. A trial of Reolysin in combination with paclitaxel and carboplatin in patients with NSCLC harboring activating KRAS or EGFR alterations reported significantly improved response and survival rates compared to assumed historical data for paclitaxel and carboplatin alone.

ERBB2 (Her2)

Gene and Alteration: ERBB2 (also known as HER2) encodes a receptor tyrosine kinase which is in the same family as EGFR. Amplification or overexpression of ERBB2 can lead to excessive proliferation and tumor formation[61].

Frequency and Prognosis: In the TCGA datasets, ERBB2 amplification or mutation was observed in 6% of lung adenocarcinoma cases[62]. HER2 overexpression has been documented in 11-32% of non-small cell lung cancers (NSCLC), and is higher in lung adenocarcinomas (38%) than in squamous cell (16%) and large cell (17.9%) tumors[63,64]. A tendency toward shorter survival has been observed in patients with NSCLC harboring ERBB2 amplification and strong HER2 protein expression[65].

Potential Treatment Strategies: Based on extensive clinical evidence, ERBB2 amplification or activating mutation may predict sensitivity to therapies targeting HER2, including antibodies such as trastuzumab[66-71], pertuzumab in combination with trastuzumab6[8,72,73], and ado-trastuzumab emtansine (T-DM1)[74], as well as dual EGFR/HER2 kinase inhibitors such as lapatinib[75-78], afatinib[71,79-82], neratinib[83,84], and dacomitinib[85]. In patients with breast cancer, concurrent PIK3CA or PTEN alterations that activate the PI3K pathway have been associated with resistance to therapies that target HER2, including trastuzumab and lapatinib[86-90]. However, other studies have reported conflicting results, with one study suggesting that neither PIK3CA nor PTEN alteration is associated with trastuzumab resistance[91], and another study reporting a correlation between PIK3CA mutation and increased clinical response to the combination of letrozole and lapatinib[92]. Clinical trials of agents aimed at preventing or overcoming resistance to anti-HER2 therapies are under way, including agents targeting the PI3K-AKT pathway or HSP90.[93,94]

HER2 (ERBB2) in Breast Cancer

Human epidermal receptor growth factor 2 (HER2, ERBB2) overexpression occurs in 18–20% of breast cancer (Owens et al. 2004; Slamon et al. 1987; Yaziji et al. 2004). HER2 overexpression arises from multiple mechanisms; gene amplification is the most common. Overexpression in general is measured by IHC and gene amplification is measured by FISH methods. Activating mutations in HER2 are estimated to occur at a frequency of 1.6–2.0% in breast cancer (Bose et al. 2013; COSMIC).

HER2 overexpression in breast cancer carries prognostic and predictive significance. In the adjuvant setting, HER2 status is prognostic for outcomes and predictive for outcomes with HER2-targeting therapies such as trastuzumab- based therapy and with anthracycline-based therapies (NCCN 2012). In the metastatic setting, HER2 status predicts outcomes with trastuzumab and HER2-targeting agents (NCCN 2012).

Recently, in patients without HER2 gene amplification, activating HER2 mutations have also been identified (Bose et al. 2013). Preclinical studies have indicated that some HER2 mutations may result in sensitivity or resistance to trastuzumab, neratinib, or lapatinib, depending on the specific mutation (Bose et al. 2013).

Gene or Protein	Invasive Breast Cancer	Hormone Receptor Positive (ER+ and/or PR+) Invasive Breast	HER2 positive Invasive Breast	Triple-negative Invasive Breast

		Cancer	Cancer	Cancer
HER2 amplification	18% (Slamon et al. 1987)	18% (Slamon et al. 1987)	100%	N/A
HER2 overexpression	18–20% (Owens et al. 2004; Yaziji et al. 2004)	8% (Blows et al. 2011)	100%	N/A
HER2 mutations	1.6–2.0% (25 cases reported in Bose et al. 2013; COSMIC)	8 cases reported (Bose et al. 2013)	One case reported (Bose et al. 2013)	No cases reported (Bose et al. 2013)

NOTE: ER = estrogen receptor; PR = progesterone receptor; N/A = not applicable

HER2 (ERBB2) in Lung Cancer

HER2 mutations are detected in approximately 2–4% of NSCLC (Buttitta et al. 2006; Shigematsu et al. 2005; Stephens et al. 2004). The most common mutation is an in-frame insertion within exon 20. *HER2* mutations appear to be found more commonly in never smokers (defined as less than 100 cigarettes in a patient's lifetime) with adenocarcinoma histology (Buttitta et al. 2006; Shigematsu et al. 2005; Stephens et al. 2004). However, *HER2* mutations can also be found in other subsets of NSCLC, including in former and current smokers as well as in other histologies (Buttitta et al. 2006; Shigematsu et al. 2005; Stephens et al. 2004). The exon 20 insertion results in increased HER2 kinase activity and enhanced signaling through downstream pathways, resulting in increased survival, invasiveness, and tumorigenicity (Wang et al. 2006).

HER2 amplification does not appear to coincide with HER2 mutation (Buttitta et al. 2006; Stephens et al. 2004). Unlike in breast cancer, there is currently no role for *HER2* amplification as a prognostic or predictive marker in NSCLC.

In the vast majority of cases, *HER2* mutations are non-overlapping with other oncogenic mutations found in NSCLC (e.g., *EGFR* mutations, *ALK* rearrangements, etc.).

FOXL2

Biological function: The FOXL2 (Forkhead Transcription Factor) gene (OMIM – 605597) encodes a transcription factor critical for granulosa cell development. FOXL2 is a member of the forkhead--winged helix family of transcription factors containing a highly conserved DNA-binding forkhead domain.[41] It is one of the earliest markers of ovarian differentiation, and its expression persists into adulthood. FOXL2 is required for the normal development of granulosa cells and shows strong expression in granulosa cells and moderate expression in stromal cells; no expression has been detected in oocytes (Amberger et al., 2015; 25428349, Shah et al., 2009; 19516027).

FOXL2 has been reported to positively or negatively regulate a number of target genes, including genes involved in steroidogenesis, inflammation, apoptosis, and detoxification (Escudero et al., 2010; 20167115, Batista et al., 2007; 17360647, Pisarska et al., 2004; 15059956).

Incidence in cancer: FOXL2 somatic mutations are detected in 18% ovarian and 2% of testicular cancers (http://cancer.sanger.ac.uk/cosmic).[42] A subtype of ovarian and testicular cancer, granulosa cell tumors (GCT), account for 5% of ovarian malignances.[50] In one study, FOXL2 mutations were detected in 97% adult-type GCTs (97%), in 3 of 14 thecomas (21%), and in 1 of 10 juvenile-type GCTs (10%).[50] The most common mutation in the FOXL2 gene is a missense mutation 402C>G (C134W), which leads to protein activation.

Targeted therapies: Currently, no therapies directly targeting FOXL2 alterations are approved or are under investigation in clinical trials.

GNA11

Guanine nucleotide binding proteins (G proteins) are a family of heterotrimeric proteins which couple

seven transmembrane domain receptors to intracellular cascades, including neurotransmitter, growth factor, and hormone signaling pathways (for a recent review, see Rosenbaum, Rasmussen, and Kobilka 2009). Heterotrimeric G proteins are composed of three subunits, Ga, Gß, and Gγ (Figure 1); each of the subunits has many different family members. The GNA11 gene encodes the alpha-11 subunit (Ga11). Receptor activation catalyzes the exchange of GDP (guanosine diphosphate) to GTP (guanosine triphosphate) on the Ga subunit, resulting in the dissociation of the Ga subunit from Gßγ. Both Ga and Gßγ can then activate downstream cellular signaling pathways. The signal is terminated when GTP is hydrolyzed to GDP by the intrinsic GTPase activity of the Ga subunit. Oncogenic mutations result in a loss of this intrinsic GTPase activity, resulting in a constitutively active Ga subunit (Kalinec et al. 1992; Landis et al. 1989).

GNA11 in Melanoma

Somatic mutations in *GNA11* have been found in up to 34% of primary uveal melanomas and up to 63% of uveal melanoma metastases (Van Raamsdonk et al. 2010). In all malignant melanoma, *GNA11* mutations are found in about 1.2% of samples (COSMIC). *GNA11* mutations have not been detected in extraocular melanoma (Van Raamsdonk et al. 2010).
The majority of melanoma-associated mutations in *GNA11* have been detected at codon 209 within exon 5 of the gene, a region within the catalytic (GTPase) domain of *GNA11*. Mutation at this site inactivates the GTPase domain, resulting in a constitutively active GNA11 protein which is 'locked' in the GTP bound form (Kalinec et al. 1992; Landis et al. 1989). Expression of GNA11 Q209L in mice results in melanocyte transformation and increased signaling through the MAPK pathway (Van Raamsdonk et al. 2010).
In the vast majority of cases, GNA11 mutations are non-overlapping with other oncogenic mutations found in melanoma (e.g., BRAF mutations, KIT mutations, etc.). Currently, there are no direct anti-GNA11 therapies available.

GNAQ

Guanine nucleotide binding proteins (G proteins) are a family of heterotrimeric proteins which couple seven transmembrane domain receptors to intracellular cascades, including neurotransmitter, growth factor, and hormone signaling pathways (for review, see Neves, Ram, and Iyengar 2002 and Rosenbaum, Rasmussen, and Kobilka 2009). Heterotrimeric G proteins are composed of three subunits, Ga, Gß, and Gγ (Figure 1); each of the subunits has many different family members. The GNAQ gene encodes the Gq alpha subunit (Gag). Receptor activation catalyzes the exchange of GDP (guanosine diphosphate) to GTP (guanosine triphosphate) on the Ga subunit, resulting in the dissociation of the Ga subunit from Gßγ. Both Ga and Gßγ can then activate downstream cellular signaling pathways. The signal is terminated when GTP is hydrolyzed to GDP by the intrinsic GTPase activity of the Ga subunit. Oncogenic mutations result in a loss of this intrinsic GTPase activity, resulting in a constitutively active Ga subunit (Kalinec et al. 1992; Landis et al. 1989).

GNAQ in Melanoma

Somatic mutations in *GNAQ* have been found in ~50% of primary uveal melanomas and up to 28% of uveal melanoma metastases (Onken et al. 2008; Van Raamsdonk et al. 2009; van Raamsdonk et al. 2010). In all malignant melanoma, *GNAQ* mutations are found in about 1.3% of samples (COSMIC). *GNAQ* mutations are rare in extraocular melanoma (Van Raamsdonk et al. 2009).
The majority of melanoma-associated mutations in *GNAQ* have been detected at codon 209 within exon 5 of the gene, a region within the catalytic (GTPase) domain of *GNAQ*. Mutation at this site inactivates the GTPase domain, resulting in a constitutively active GNAQ protein, which is 'locked' in the GTP bound form (Kalinec et al. 1992; Landis et al. 1989). Expression of GNAQ Q209L in mice results in melanocyte transformation and increased signaling through the MAPK pathway (Van Raamsdonk et al. 2009).
In the vast majority of cases, GNAQ mutations are non-overlapping with other oncogenic mutations found in melanoma (e.g., BRAF mutations, KIT mutations, etc.). Currently, there are no direct anti-GNAQ therapies available.

KIT

KIT (also called CD117) is a receptor tyrosine kinase (RTK) expressed on a wide variety of cell types. The ligand for KIT is stem cell factor (SCF). The binding of SCF to the extracellular domain of KIT induces receptor dimerization and activation of downstream signaling pathways, including the PI3K-AKT-mTOR pathway, the RAS-RAF-MEK-ERK pathway, and the signal transducer and activator of transcription 3 (acute-phase response factor), or STAT3, pathway, all of which are involved in mediating pro-growth and pro-survival signals within the cell (Figure 1).
Mutant KIT has been implicated in the pathogenesis of several cancers including melanoma, acute leukemia, and gastrointestinal stromal tumor (GIST; Heinrich et al. 2003; Hirota et al. 1998).
The discovery of KIT mutations revolutionized the treatment of GISTs. The use of imatinib mesylate (Gleevec), an oral KIT inhibitor leads to rapid, substantial, and durable tumor responses (Demetri et al. 2002). Not all KIT mutations are associated with equal sensitivity to imatinib (Heinrich et al. 2008); some are more sensitive to second-generation KIT inhibitors.

KIT in Melanoma

Somatic mutations in KIT have been found in 2–8% (Beadling et al. 2008; COSMIC; Curtin et al. 2006; Handolias et al. 2010; Willmore-Payne et al. 2005) of all malignant melanoma. KIT mutations may be found in all melanoma subtypes but are the most common in acral melanomas (10–20%) and mucosal melanomas (15–20%; Beadling et al. 2008; Curtin et al. 2006; Satzger et al. 2008; Torres-Cabala et al. 2009). Among mucosal melanomas, KIT mutations are more common in anorectal and vulvo-vaginal primaries (15–25%) than in sinonasal/oropharyngeal tumors (~7%).
Somatic point mutations in melanoma tumor specimens have been detected predominantly in the juxtamembrane domain but also in the kinase domain of KIT. They can induce ligand-independent receptor dimerization, constitutive kinase activity, and transformation (Growney et al. 2005; Hirota et al. 1998; Hirota et al. 2001; Kitayama et al. 1995). The spectrum of mutations overlaps with those found in gastrointestinal stromal tumor (GIST).
An increasing number of case reports, retrospective studies, and phase II clinical trials have demonstrated clinical responses of KIT mutated melanoma to imatinib (Carvajal et al. 2011; Guo et al. 2011; Hodi et al. 2013), sunitinib (Minor et al. 2012; Zhu et al. 2009), sorafenib (Quintas-Cardama et al. 2008), and nilotinib (Lebbe et al. 2014). In one case study, a patient with melanoma harboring a KIT L576P mutation demonstrated a response to everolimus after acquiring resistance to imatinib (Si et al. 2012).
In the majority of cases, KIT mutations are non-overlapping with other oncogenic mutations found in melanoma (e.g., NRAS mutations, BRAF mutations, etc.; Beadling et al. 2008). In addition, in rare cases the KIT genotype of a primary lesion may differ from its metastases (Terheyden et al. 2010).

KIT in Gastrointestinal Stromal Tumor (GIST)

KIT is mutated in ~85% of GIST (Heinrich et al. 2003). The vast majority of *KIT* mutations are found in exon 11 (juxtamembrane domain; ~70%), exon 9 (extracellular dimerization motif; 10–15%), exon 13 (tyrosine kinase 1 (TK1) domain; 1–3%), and exon 17 (tyrosine kinase 2 (TK2) domain and activation loop; 1–3%; Heinrich et al. 2003). Secondary *KIT* mutations in exons 13, 14, 17, and 18 are commonly identified in post-imatinib biopsy specimens, after patients have developed acquired resistance.

KRAS

Biological function: KRAS (Kirsten Rat Sarcoma Viral Oncogenic homolog) gene (OMIM – 190070) is part of the RAS/RAF/ MEK/MAP signal transduction pathway downstream from epidermal growth factor receptor (EGFR).[41] The KRAS protein relays signals for cell growth or cell differentiation. The abnormal KRAS protein is always active and can direct cells to grow and divide in an uncontrolled way (Bettegowda et al., 2014; 24553385, Rothschild, 2015; 26018876; Greenman et al., 2007; 17344846, Kidess et al., 2015; 25575824, Vogelstein et al., 2013; 23539594, Tabernero et al., 2015; 26184520).
Role in disease: The KRAS gene is one of the most commonly mutated genes in human malignancies, with high incidences in pancreatic, colorectal, and lung cancers (Farber et al., 2011; 22016105, Feldmann et al., 2007; 17520196, Han et al., 2011; 22011285).

Activating KRAS mutations can result in constitutive activation of the Ras/Raf/MEK/ERK and PI3K/Akt pathways (Nakano et al., 1984; 6320174, Pylayeva-Gupta et al., 2011; 21993244). Studies using xenograft models or cell lines of colorectal cancer have reported that activating KRAS mutations, often assessed in the context of other genetic alterations, can play a role in the development and progression of colorectal cancer (Davies et al., 2014; 24293351, Cagnol and Rivard, 2013; 22430215, Trobridge et al., 2009; 19208363).

Incidence in cancer: KRAS gene mutations are common in pancreatic (57%), colorectal (36%), lung (17%), endometrial (14%), ovarian (12%), gastric (6%), prostate (4%) testicular (4%) and thyroid (2%) cancers (http://cancer.sanger.ac.uk/ cosmic).[42] In colorectal cancer, KRAS and NRAS mutations occur in about 50% of the patients and predict a poor response to the EGFR targeting therapies.[51] Codon 12/13 mutations are seen in nearly 95% of KRAS mutated cancers. Per NCCN guidelines, testing for KRAS codon 12/13 mutations is recommended before using EGFR inhibitors like Erbitux (cetuximab), Vectibix (panitumumab) for the treatment of non-small cell lung and colorectal cancers. Circulating tumor DNA analysis shows that acquired resistance to the anti-EGFR pathway is associated with emergence of RAS pathway mutations and these mutations can be detected in plasma before disease progression clinically manifests.[52]
Activating mutations in KRAS result in activation of downstream pathways, including the RAF/MEK/ERK pathway. The MEK inhibitor trametinib has been approved for use in BRAF V600-mutant melanoma. Trametinib and other MEK inhibitors, alone or in combination therapy, are in clinical trials, as are multiple other approaches to targeting KRAS signaling.
KRAS activating mutations, including G12C, G12V and G12D, are observed in approximately 10-38% of non-small cell lung cancer (NSCLC) samples, predominantly in lung adenocarcinoma cases (COSMIC, May 2014) (Aviel-Ronen et al., 2006; 16870043, Rekhtman et al., 2013; 23619604, Stella et al., 2013; 23644698, Stjernström et al., 2014; 24500884, Cai et al., 2013; 23495083, Kim et al., 2013; 23307237, Ragusa et al., 2013; 23357969, Russell et al., 2013; 23486266, Villaruz et al., 2013; 23526491, Yip et al., 2013; 23392229). KRAS mutations have been found to be mutually exclusive with ALK rearrangements and EGFR mutations in NSCLC (Gainor et al., 2013; 23729361, Shigematsu et al., 2005; 15741570, Shigematsu et al., 2005; 15753357). However, case studies of NSCLC patients harboring ALK mutations or EML4-ALK fusions have reported the emergence of KRAS mutations upon acquired resistance to crizotinib, demonstrating a role for KRAS in crizotinib resistance in NSCLC (Doebele et al., 2012; 22235099, Rossing et al., 2013; 24279718).

KRAS in Non-Small Cell Lung Cancer (NSCLC)

Approximately 15–25% of patients with lung adenocarcinoma have tumor associated *KRAS* mutations. *KRAS* mutations are uncommon in lung squamous cell carcinoma (Brose et al. 2002). In the majority of cases, these mutations are missense mutations which introduce an amino acid substitution at position 12, 13, or 61. The result of these mutations is constitutive activation of *KRAS* signaling pathways.
In the vast majority of cases, *KRAS* mutations are found in tumors wild type for *EGFR* or *ALK*; in other words, they are non-overlapping with other oncogenic mutations found in NSCLC. Therefore, *KRAS* mutation defines a distinct molecular subset of the disease. *KRAS* mutations are found in tumors from both former/current smokers and never smokers. They are rarer in never smokers and are less common in East Asian vs. US/European patients (Riely et al. 2008; Sun et al. 2010).
The role of *KRAS* as either a prognostic or predictive factor in NSCLC is unknown at this time. Very few prospective randomized trials have been completed using *KRAS* as a biomarker to stratify therapeutic options in the metastatic setting. Unlike in colon cancer, *KRAS* mutations have not yet been shown in NSCLC to be negative predictors of benefit to anti-EGFR antibodies. However, *KRAS* mutations are negative predictors of radiographic response to the EGFR tyrosine kinase inhibitors, erlotinib and gefitinib [for review, see (Riely and Ladanyi 2008; Riely, Marks, and Pao 2009)]. Currently, there are no direct anti-KRAS therapies available.

KRAS in Colorectal Cancer

Approximately 36–40% of patients with colorectal cancer have tumor-associated KRAS mutations (Amado et al. 2008; COSMIC; Faulkner et al. 2010; Neumann et al. 2009). The concordance between

primary tumor and metastases is high (Cejas et al. 2009; Mariani et al. 2010; Santini et al. 2008), with only 3–7% of the tumors discordant. The majority of the mutations occur at codons 12, 13, and 61 of the KRAS gene. The result of these mutations is constitutive activation of KRAS signaling pathways.
Multiple studies have now shown that patients with tumors harboring mutations in KRAS are unlikely to benefit from anti-EGFR antibody therapy, either as monotherapy (Amado et al. 2008) or in combination with chemotherapy (Bokemeyer et al. 2009; Bokemeyer et al. 2011; Douillard et al. 2010; Lievre et al. 2006; Peeters et al. 2010). Further, in trials of oxaliplatin based chemotherapy, the patients with KRAS mutated tumors appeared to do worse when treated with EGFR antibody therapy combined with an oxaliplatin based chemotherapy compared to the patients treated with an oxaliplatin based treatment alone.

KRAS in Ovarian Cancer

KRAS mutations are found in approximately 40% of patients with Type I EOC tumors. In the majority of cases, these mutations are missense mutations which introduce an amino acid substitution at position 12, 13, or 61. The result of these mutations is constitutive activation of KRAS signaling pathways. The most common mutation is KRAS G12D c.35G>A (COSMIC).
The role of KRAS as either a prognostic or predictive factor in EOC is unknown at this time. In one study KRAS mutations were associated with poor prognosis in metastatic Type I tumors (Jones et al. 2012). Another study showed that low grade tumors with KRAS mutations had a better outcome (Wong et al. 2010). Currently, there are no direct anti-KRAS therapies available. However, patients with KRAS mutated ovarian tumors may benefit from treatment with MEK inhibitors (Nakayama et al. 2008).

Table. Frequency of Somatic Gene Mutations in Epithelial Ovarian Cancer (EOC)

	EOC Overall	Type I				Type II
Gene Mutation		**Low Grade Serous**	**Clear Cell**	**Endometrioid**	**Mucinous**	**High Grade Serous**
KRAS	14% (COSMIC)	33% (Singer et al. 2003; Nakayama et al. 2006)	<1–7% (Kuo et al. 2009; Singer et al. 2003)	<1% (Singer et al. 2003)	50–75% (Gemignani et al. 2003)	<1% (TCGA 2011)

Effect on drug sensitivity: Many of the current attempts to target K-Ras are directed against its downstream signaling pathways, Raf/MEK/ERK and PI3K/Akt/mTOR (Yeh et al., 2009; 19372556, Britten, 2013; 23443307). The MEK inhibitors trametinib and cobimetinib (in combination with vemurafenib) have been FDA-approved for BRAF V600E- and V600K-mutant melanoma and are under investigation in clinical trials (Flaherty et al., 2012; 22663011, Larkin et al., 2014; 25265494). A novel clinical approach for KRAS-positive tumors, based on synthetic lethal interactions that occur in the presence of a KRAS mutation and either diminished Cdk4 activity or diminished Bcl-2/ Bcl-xL activity, is a treatment combination of MEK inhibition and either Cdk4/6 inhibition or Bcl-2/Bcl-xL inhibition (Mao et al., 2014; 24496383, Puyol et al., 2010; 20609353, Tan et al., 2013; 23475955, Corcoran et al., 2013; 23245996).

Effect on drug resistance: In some cancer types, such as colorectal cancer and non-small cell lung cancer, activating KRAS mutations and KRAS amplification have been associated with resistance to Egfr-targeted therapies (Eberhard et al., 2005; 16043828, Linardou et al., 2008; 18804418, Ramos et al., 2008; 19064407, Campos-Parra et al., 2013; 23538866, De Roock et al., 2011; 21163703, Douillard et al., 2013; 24024839, Valtorta et al., 2013; 23404247, Li et al., 2014; 25155261).
For colorectal carcinoma patients with metastatic disease and tumors harboring a KRAS or NRAS mutation, the NCCN guidelines (v.1.2016) recommend against the use of cetuximab and panitumumab.

MET

The *MET* gene (MNNG-HOS transforming gene; Cooper et al. 1984) located on chromosome 7, encodes a receptor tyrosine kinase (RTK) belonging to the MET/RON family of RTKs. Binding of its ligand, hepatocyte growth factor (HGF; also called scatter factor (SF)), induces a conformational change in the MET receptor that facilitates receptor phosphorylation and activation. Activated MET then phosphorylates its substrates, resulting in activation of multiple downstream pathways within the cell, including the PI3K-AKT-mTOR pathway, which is involved in cell survival, and the RAS-RAF-MEK-ERK pathway, which is involved in cell proliferation. In the context of malignancy, aberrant signaling through the MET receptor promotes pleiotrophic effects including growth, survival, invasion, migration, angiogenesis and metastasis (Birchmeier et al. 2003; Peruzzi and Bottaro 2006).
The MET receptor and/or its ligand HGF have been reported to be aberrantly activated in many human cancers. Germline mutations in the tyrosine kinase domain of *MET* occur in 100% of hereditary papillary renal cell carcinoma, and somatic mutations in *MET* are found in 10–15% of sporadic papillary renal cell carcinoma (Schmidt et al. 1997). Mutations in *MET* have been reported at low frequencies in head and neck squamous cell carcinoma (Di Renzo et al. 2000), childhood hepatocellular carcinoma (Park et al. 1999), NSCLC (Kong-Beltran et al. 2006; Ma et al. 2003) and small cell lung cancer (Ma et al. 2003). Amplification of *MET* has been reported in gastric cancer (Nakajima et al. 1999), esophageal cancer (Miller et al. 2006), colorectal cancer (Umeki, Shiota, and Kawasaki 1999), gliomas (Beroukhim et al. 2007), clear cell ovarian cancer (Yamamoto et al. 2011) and NSCLC (Bean et al. 2007; Cappuzzo et al. 2009; Chen et al. 2009; Engelman et al. 2007; Kubo et al. 2009; Okuda et al. 2008; Onozato et al. 2009).

MET in Non-Small Cell Lung Cancer (NSCLC)

In non-small cell lung cancer (NSCLC), multiple mechanisms of MET activation have been reported, including gene amplification (Bean et al. 2007; Cappuzzo et al. 2009; Chen et al. 2009; Engelman et al. 2007; Kubo et al. 2009; Okuda et al. 2008; Onozato et al. 2009) and mutation (Kong-Beltran et al. 2006; Ma et al. 2003).
Nonsynonymous *MET* mutations occurring in the juxtamembrane and semaphorin domains have been described in NSCLC and SCLC (Kong-Beltran et al. 2006; Ma et al. 2003; Ma et al. 2005a). However, some of these were recently identified in corresponding germline DNA (Krishnaswamy et al. 2009). The activity of MET inhibitors in NSCLC or SCLC tumors with non-kinase domain *MET* mutations is not yet known. By contrast, responses to foretanib (XL880 or GSK136308), an oral inhibitor of MET and other tyrosine kinases including VEGFR2, have been described in patients with papillary renal cell cancer (Eder et al. 2007). 100% of hereditary papillary renal cell carcinomas harbor germline activating mutations in the tyrosine kinase domain of MET (Schmidt et al. 1997).
MET protein expression may also be abnormal in tumors. Overexpression of MET protein in tumor tissue relative to adjacent normal tissues occurs in 25–75% of NSCLC and is associated with poor prognosis (Benedettini et al. 2010; Ichimura et al. 1996; Liu and Tsao 1993; Ma et al. 2005b; Nakamura et al. 2007; Olivero et al. 1996; Siegfried et al. 1998; Xu et al. 2010). In a recent phase II study in which patients with NSCLC were randomized to MetMab (an anti-MET antibody) plus erlotinib vs erlotinib alone, increased expression of MET protein was associated with improved progression free survival and overall survival in patients who received MetMAb and erlotinib (Spigel et al. 2011). Increased MET expression was defined as more than 50% of the tumor having moderate or high MET expression assessed by immunohistochemistry using a specific anti-MET antibody (Ventana CONFIRM anti-CMET clone SP44).

NRAS

Biological function: NRAS (Neuroblastoma Ras Viral Oncogenic homolog) gene (OMIM 164790), like KRAS, is part of the mitogen activation protein kinase (MAPK) pathway (Greenman et al., 2007; 17344846, Kidess et al., 2015; 25575824).[41] The encoded protein, which has intrinsic GTPase activity, is activated by a guanine nucleotide-exchange factor and inactivated by a GTPase activating protein.

Incidence in cancer: NRAS mutations are detected in metastatic melanoma (18%), thyroid (7%), and testicular (2%) cancers (http://cancer.sanger.ac.uk/cosmic).[42] NRAS is also mutated in approximately

3-5% of colorectal cancer.[53] Approximately 56% of the mutations reported in the NRAS gene in colorectal cancer are located in codon 61, with Q61R and Q61K substitutions the most frequently reported. Recent data has shown that NRAS mutation status may be predictive for anti-EGFR therapy response in metastatic melanoma and colorectal cancer.[53,54]

N-Ras activation may predict sensitivity to inhibitors of the Raf/MEK/ERK, PI3K/Akt, and other downstream pathways (Wang et al., 2013; 23274911, Migliardi et al., 2012; 22392911, Pylayeva-Gupta et al., 2011; 21993244). In some cancer types, such as melanoma and colorectal cancer, NRAS mutation may predict resistance to therapies (Trunzer et al., 2013; 23569304, Douillard et al., 2013; 24024839, Linardou et al., 2008; 18804418). Activating NRAS mutations have been associated with resistance to Egfr inhibitors and/or antibodies in colorectal cancer (Linardou et al., 2008; 18804418, Ramos et al., 2008; 19064407, De Roock et al., 2011; 21163703, Douillard et al., 2013; 24024839). Current NCCN guidelines (2014) recommend against the use of cetuximab or panitumumab in colorectal cancer patients with any known NRAS mutation (NCCN.org).

NRAS in Non-Small Cell Lung Cancer (NSCLC)

Somatic mutations in *NRAS* have been found in ~1% of all NSCLC (Brose et al. 2002; Ding et al. 2008; Ohashi et al. 2013). *NRAS* mutations are more commonly found in lung cancers with adenocarcinoma histology and in those with a history of smoking (Ohashi et al. 2013). In the majority of cases, these mutations are missense mutations that introduce an amino acid substitution at position 61. Mutations at position 12 have also been described (Ohashi et al. 2013). The result of these mutations is constitutive activation of NRAS signaling pathways. Currently, there are no direct anti-NRAS therapies available, but preclinical models suggest that MEK inhibitors may be effective (Ohashi et al. 2013).
In the vast majority of cases, *NRAS* mutations are non-overlapping with other oncogenic mutations found in NSCLC (e.g., *EGFR* mutations, *ALK* rearrangements, etc.).

NRAS in Colorectal Cancer

NRAS mutations occur in ~1–6% of colorectal cancers (COSMIC; De Roock et al. 2010; Irahara et al. 2009; Janku et al. 2007; Vaughn et al. 2011).
Wild type NRAS, together with wild type BRAF and KRAS, is associated response to EGFR antibody therapy (De Mattos-Arruda, Dienstmann, and Tabernero 2011; De Roock et al. 2010).
Several studies have shown that patients with NRAS-mutated tumors are less likely to respond to cetuximab or panitumumab, but this may not have an effect on PFS or overall survival (De Mattos-Arruda, Dienstmann, and Tabernero 2011; De Roock et al. 2010; Peeters et al. 2010).

NRAS in Melanoma

Somatic mutations in NRAS have been found in ~13–25% of all malignant melanomas (Ball et al. 1994; Curtin et al. 2005; van 't Veer et al. 1989). In the majority of cases, these mutations are missense mutations which introduce an amino acid substitution at positions 12, 13, or 61. The result of these mutations is constitutive activation of NRAS signaling pathways. NRAS mutations are found in all melanoma subtypes, but may be slightly more common in melanomas derived from chronic sun-damaged (CSD) skin (Ball et al. 1994; van 't Veer et al. 1989). Currently, there are no direct anti-NRAS therapies available.
In the vast majority of cases, NRAS mutations are non-overlapping with other oncogenic mutations found in melanoma (e.g., BRAF mutations, KIT mutations, etc.).

PDGFRA

The platelet derived growth factor receptor alpha (PDGFRA) belongs to a family of receptor tyrosine kinases (RTKs) that include PDGFRA and PDGFRB. The binding of ligands, such as platelet derived growth factor (PDGF), induces a conformational change that facilitates receptor homo- or heterodimer formation, thereby resulting in activation of PDGFRA tyrosine kinase activity. Activated PDGFRA then phosphorylates its substrates, resulting in activation of multiple downstream pathways within the cell, including the PI3K-AKT-mTOR pathway, which is involved in cell survival, and the RAS-RAF-MEK-ERK pathway, which is involved in cell proliferation (Figure 1).

Mutant PDGFRA has been implicated in the pathogenesis of a number of cancers. For example, mutations are found in gastrointestinal stromal tumors (GIST; Corless et al. 2005; Heinrich et al. 2003), and fusions are found in hypereosinophilic syndrome (Cools et al. 2003). In dermatofibrosarcoma protuberans, PDGFRA is activated by a PDGFB fusion protein; as a result, imatinib, as a PDGFRA inhibitor, has shown activity and is approved for clinical use (Labropoulos and Razis 2007; Simon et al. 1997).

PDGFRA in Gastrointestinal Stromal Tumor (GIST)

PDGFRA is mutated in ~5% of GIST, most frequently in gastric GIST. Specifically, *PDGFRA* mutations are found mostly in exons 18 (tyrosine kinase 2 (TK2) domain; ~5%), 12 (juxtamembrane domain; 1%) and 14 (tyrosine kinase 1 (TK1) domain; <1%). Mutations except for D842V in exon 18 are sensitive to imatinib (Corless et al. 2005). In gastrointestinal stromal tumor, detection of mutant PDGFRA predicts responsiveness to tyrosine kinase inhibitor therapy.

PIK3CA

Biological function: PIK3CA (Phosphatidylinositol 3-Kinase, Catalytic Alpha) gene (OMIM – 171834) is a member of the PI3K-PTEN-AKT pathway, downstream from both the EGFR and the RAS/RAF/MAPK pathways, which regulate cell proliferation, growth, apoptosis, autophagy, invasion and proliferation (Samuels et al., 2005; 15950905, Engelman, 2009; 19629070).
All mutations in PIK3CA are activating and result in an activated PI3K (phosphatidylinositol 3-kinase) protein that may result in abnormal cell proliferation.

Incidence in cancer: PIK3CA somatic mutations are detected in breast (26%), endometrial (21%), colorectal (14%), gastric (10%), ovarian (9%), head and neck (7%), lung (4%), thyroid (3%), prostate (2%) and pancreatic (2%) cancers (http://cancer.sanger.ac.uk/cosmic).[42]

Role in disease: More than 80% of PIK3CA mutations occur in exon 9 (E545K, E542K, E545G, E545K/ D549H, Q546K), which codes for the p110 subunit of PI3K and exon 20 (H1047R, H1047L, G1049R, M1043V, M1043I), which codes for the kinase domain. All mutations in PIK3CA are activating and result in and activated PI3 kinase protein that may result in abnormal cell proliferation (Greenman et al., 2007; 17344846, Tabernero et al., 2015; 26184520, Dawson et al., 2013; 23484797). PIK3CA mutations are not mutually exclusive with EGFR or KRAS or BRAF mutations, and are associated with increased PI3K signaling and increased activation of Akt in lung cancers, and occur more frequently in lung squamous cell carcinoma than in lung adenocarcinoma (COSMIC, cBioPortal for Cancer Genomics, May 2014) (Yamamoto et al., 2008; 18757405, Janku et al., 2011; 21829508, An et al., 2012; 22768234, Scarpa et al., 2013; 24236184, Stjernström et al., 2014; 24500884). A study of 1394 early stage breast cancer samples reported that positive p110-alpha expression was associated with higher tumor grade, larger tumor size, nodal involvement, and vascular invasion. Higher p110-alpha expression was associated with basal-like breast cancer, Her2 positive breast cancer, and triple negative non-basal tumors (Aleskandarany et al., 2010; 19701705).

PIK3CA in Breast Cancer

Somatic mutations in PIK3CA have been found in a substantial fraction of breast cancers (see Table). PIK3CA mutations are positive prognostic factors in breast cancer (Cizkova et al. 2012; Janku et al. 2012).

Gene Mutation	Invasive Breast Cancer	Hormone Receptor Positive (ER+ and/or PR+) Invasive Breast Cancer	HER2 positive Invasive Breast Cancer	Triple-negative Invasive Breast Cancer
PIK3CA	26% (Saal et al. 2005; Stemke-Hale et al. 2008; O'Brien et al. 2010)	34.5% (Saal et al. 2005; Stemke-Hale et al. 2008)	22–31% (Saal et al. 2005; Stemke-Hale et al. 2008)	8.3% (Stemke-Hale et al. 2008)

These mutations usually occur within two "hotspot" areas within exon 9 (the helical domain) and exon 20 (the kinase domain).
PIK3CA mutations have been reported in 26% (2778/10672) of breast carcinoma samples analyzed

in COSMIC, including in 15% (56/382) of samples included that were specified to be triple negative breast carcinoma specimens (Jun 2015). Scientific studies have reported PIK3CA mutations in 8-17% of triple negative breast cancer samples analyzed (Tilch et al., 2014; 24318467, Santarpia et al., 2012; 22538770, Gonzalez-Angulo et al., 2009; 19276248, Castaneda et al., 2014; 25467032).

PIK3CA in Non-Small Cell Lung Cancer (NSCLC)

Somatic mutations in *PIK3CA* have been found in 1–3% of all NSCLC (COSMIC; Kawano et al. 2006; Samuels et al. 2004). These mutations usually occur within two "hotspot" areas within exon 9 (the helical domain) and exon 20 (the kinase domain). *PIK3CA* mutations appear to be more common in squamous cell histology compared to adenocarcinoma (Kawano et al. 2006) and occur in both never smokers and ever smokers. *PIK3CA* mutations can co-occur with *EGFR* mutations (Kawano et al. 2006; Sun et al. 2010). In addition, *PIK3CA* mutations have been detected in a small percentage (~5%) of *EGFR*-mutated lung cancers with acquired resistance to EGFR TKI therapy (Sequist et al. 2011).

PIK3CA in Colorectal Cancer

Somatic mutations in PIK3CA have been found in 10–30% of colorectal cancers (COSMIC; Samuels et al. 2004).
These mutations usually occur within two "hotspot" areas within exon 9 (the helical domain) and exon 20 (the kinase domain).

PIK3CA in Ovarian Cancer

Somatic alterations in PIK3CA have been found in a substantial fraction of ovarian cancers (Samuels et al. 2004; COSMIC). Both genetic and biochemical data suggest that activation of the PI3K/AKT survival pathway contributes to ovarian cancer development and tumorigenesis.
PIK3CA amplifications are more common in type II high grade serous ovarian tumors (TCGA 2011). PTEN loss is more common in type I ovarian tumors (Kurman and Shih 2011). The impact of these alterations on the virulence of ovarian cancer and patient outcome is still under investigation. Prospective studies to confirm these findings are in progress. Preclinical evidence exists for inhibitors of the PI3K pathway and novel PI3K inhibitors are currently in clinical development. Frequencies of PIK3CA mutations in subtypes of ovarian cancer are shown in table 1, and frequencies of specific PIK3CA mutations in ovarian cancer are shown in table 2.
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 ovarian cancer. A recent phase I study showed that ovarian cancer patients treated with PI3K/AKT/mTOR inhibitors were more sensitive to treatment than those without PIK3CA mutations (Janku et al. 2012).

Table. Frequency of Somatic PIK3CA Gene Mutations in Epithelial Ovarian Cancer (EOC)

	EOC Overall	Type I				Type II
Gene Mutation		**Low Grade Serous**	**Clear Cell**	**Endometrioid**	**Mucinous**	**High Grade Serous**
PIK3CA	6.7% (Campbell, Russell, and Phillips 2005; Levine et al. 2005; Wang et al. 2005)	5% (Nakayama et al. 2006)	20–33% (Campbell et al. 2004; Kuo et al. 2009)	20% (Campbell et al. 2004)	Rare	<1% (TCGA 2011)

Effect on drug sensitivity: Activating PIK3CA mutations may predict sensitivity to PI3K or Akt inhibitors, which are under investigation in clinical trials, or to mTOR inhibitors, which are approved in some tumor types and in clinical trials for other solid tumors (Janku et al., 2011; 21216929, Loi et al., 2013; 23301057, Mackay et al., 2014; 24166148, Deming et al., 2013; 23593290, Massacesi et al., 2013; 23551097). Inhibitors of PI3K and Akt, alone or in combination with other therapies, are currently in clinical trials in solid tumors. Several inhibitors designed to target both the mTORC1/Raptor and

mTORC2/Rictor complexes are being tested in early phase clinical trials for advanced solid tumors (Grunt and Mariani, 2013; 23215720). The mTOR inhibitors everolimus and temsirolimus, which have been approved by the FDA in some tumor types, as well as other mTOR inhibitors, are being tested in clinical trials in a variety of solid tumors. The mTOR inhibitor everolimus, in combination with exemestane, has been approved by the FDA for treatment of advanced hormone receptor-positive, Her2-negative breast cancer after failure of treatment with letrozole or anastrozole (Baselga et al., 2012; 22149876).
Studies have reported that patients with PIK3CA-mutant colorectal cancer are more likely to benefit from aspirin therapy than patients with wild-type PIK3CA (Liao et al., 2012; 23094721, Domingo et al., 2013; 24062397).

PI3K inhibitor buparlisib, which inhibits the four isoforms of PI3K protein, has shown some promising results in treating PIK3CA mutant-positive tumors.[55] PIK3CA genotype along with wild type KRAS, wild type BRAF and NRAS is required for positive response to anti-EGFR therapy.[55]

Effect on drug resistance: PIK3CA mutations and activation of the PI3K pathway may play a role in resistance to hormonal therapy in ER+ breast cancers (Fox et al., 2012; 23087906). PI3K pathway activation, as evidenced by the presence of activating PIK3CA mutations or decreased expression of PTEN, has also been associated with resistance to Her2-targeted therapies in some clinical studies, though in other studies no association was found (Jensen et al., 2012; 22172323, O'Brien et al., 2010; 20501798, Wang et al., 2011; 21676217, Barbareschi et al., 2012; 22744290, Loibl et al., 2013; AACR 2013, Abstract S4-06, Razis et al., 2011; 21594665).

RET

The *RET* gene (rearranged during transfection; Takahashi, Ritz, and Cooper 1985), located on chromosome 10, encodes a receptor tyrosine kinase (RTK) belonging to the *RET* family of RTKs. This gene plays a crucial role in neural crest development. Binding of its ligands, the glial cell line derived neurotrophic factor (GDNF) family of extracellular signaling molecules (Airaksinen, Titievsky, and Saarma 1999), induces receptor phosphorylation and activation. Activated *RET* then phosphorylates its substrates, resulting in activation of multiple downstream cellular pathways (Figure 1; Phay and Shah 2010).
Genomic alterations in *RET* are found in several different types of cancer. Activating point mutations in *RET* can give rise to the hereditary cancer syndrome, multiple endocrine neoplasia 2 (MEN2; Salvatore et al. 2000). Somatic point mutations in *RET* are also associated with sporadic medullary thyroid cancer (Ciampi and Nikiforov 2007; Salvatore et al. 2000). Oncogenic kinase fusions involving the *RET* gene are found in ~1% of non-small cell lung cancers (Pao and Hutchinson 2012).

RET in Lung Cancer

Approximately 1.3% of lung tumors evaluated have chromosomal changes which lead to *RET* fusion genes (Ju et al. 2012; Kohno et al. 2012; Takeuchi et al. 2012; Lipson et al. 2012). These gene rearrangements appear to occur almost entirely in adenocarcinoma histology tumors. Histology has not been thoroughly evaluated, but all of the reported lung tumors with RET fusions have been adenocarcinomas (more than 400 lung cancers with histologies other than adenocarcinoma have been tested). Where overlap was evaluated, RET fusions have been shown to occur in tumors without other common driver oncogenes (e.g., *EGFR*, *KRAS*, *ALK*). The three reported fusion genes are *CCDC6-RET*, *KIF5B-RET* and *TRIM33-RET*.
RET fusions were initially identified by RT-PCR, immunohistochemistry, and next-generation sequencing. There is no current standard test for identification of RET fusions in patient samples, but fluorescence in situ hybridization (FISH) or targeted capture/next-generation sequencing are potential methods.
While the functional consequences of RET fusion proteins in lung adenocarcinoma are not fully understood, RET fusions are oncogenic in vitro and in vivo. In in vitro models, RET fusion products may be sensitive to multi-targeted kinase inhibitors such as vandetanib, sorafenib, and sunitinib (Kohno et al. 2012; Lipson et al. 2012).

The clinical significance of RET fusions is not fully understood. There is limited retrospective or prospective data that link presence of RET fusions to response to any particular therapy. However, this is an area of active investigation with prospective clinical trial research currently ongoing (Drilon et al. 2013).
Currently, an inhibitor specific only for RET is not available, but trials of kinase inhibitors with anti-RET activity have been conducted in NSCLC (Table 1). RET testing was not conducted in any of the completed clinical trials listed in table 1; therefore, only limited information is available about the performance of these therapies in patients whose tumors possess RET fusions.
Multi-kinase inhibitors with RET activity include:

- Vandetanib, which has activity against VEGFR 2/3, EGFR, and RET.
- Sorafenib, which has activity against VEGFR 1/2, KIT, RET, CRAF, and BRAF.
- Sunitinib, which has activity against VEGFR 2, KIT, RET, and PDGFRa.
- Cabozantinib, which has activity against VEGFR 2, KIT, RET, MET, FLT-1/3/4, TIE-2 and AXL.

TP53

Biological function: The TP53 (Tumor protein p53) gene (OMIM 191170) encodes a protein, p53, which acts as a tumor suppressor.[41] The p53 protein regulates cell division by preventing DNA damage by agents like toxic chemicals, radiation and UV rays. Mutations in TP53 render the protein non-functional and results in uncontrolled cell division (Greenman et al., 2007; 17344846, Madic et al., 2015; 25307450, Hamakawa et al., 2015; 25490524).

Incidence in cancer: Somatic TP53 gene mutations have been identified in ovarian (46%), colorectal (45%), head and neck (38%), pancreatic (36%), gastric (33%), lung cancers (34%), hepatocellular carcinoma (28%), glioma (27%), osteosarcoma (24%), breast (23%), endometrial (17%), prostate cancers (14%), as well as melanoma (12%), thyroid (6%) and testicular cancers (5%) (http://cancer.sanger.ac.uk/cosmic).[42] Using ctDNA analysis, TP53 somatic mutations were detected in bladder, breast, colorectal, prostate, hepatocellular cancers and gastric carcinoma.[56-59] In gastric carcinoma, TP53 mutations correlate with treatment response. There was an increase in TP53-mutant DNA in patients with no response or recurrence.[59] In triple negative breast cancer patients, 81% concordance was observed between tumor and plasma TP53- mutation analysis.[57] At present there are no FDA approved drugs to target TP53 gene mutations for the treatment of cancer, although several MDM2 inhibitors are in clinical trials.
TP53 is one of the most commonly mutated genes in triple negative breast cancer; TP53 mutations have been reported in approximately 44-80% of triple negative breast carcinomas analyzed (COSMIC, Aug 2015) (Lehmann and Pietenpol, 2014; 24114677, Shah et al., 2012; 22495314, Grob et al., 2012; 22610646, Carey et al., 2006; 16757721, Kriegsmann et al., 2014; 25296970, Millis et al., 2015; 26051240).
TP53 is one of the most commonly mutated genes in lung cancer; scientific studies have reported TP53 mutations to be present in 29-42% of non-small cell lung cancers (NSCLC) analyzed and specifically in 45% of lung adenocarcinoma samples (Mogi and Kuwano, 2011; 21331359, Tekpli et al., 2013; 23011884, Vignot et al., 2013; 23630207, Ma et al., 2014; 24495481, Maeng et al., 2013; 24222160, Molina-Vila et al., 2014; 24696321). TP53 alterations are believed to be early events in lung adenocarcinoma, preceding lymph node metastasis (Chang et al., 2011; 20811949).

Role in disease: Loss of tumor suppressor p53, which is encoded by the TP53 gene, is common in aggressive advanced cancers (Brown et al., 2009; 19935675). Carriers of a germline mutation in TP53 have Li-Fraumeni Syndrome, an inherited cancer syndrome resulting in multiple tumors in early adulthood, including breast cancer, brain tumors, and leukemias (Malkin et al., 1990; 1978757, Srivastava et al., 1991; 2259385, Santibáñez-Koref et al., 1991; 1683921). Estimates for the prevalence of germline TP53 mutations in the general population range from 1:5.000 to 1:20.000, and in the appropriate clinical context, germline testing of TP53 is recommended.
Expression of p53 in normal cells is low; however, TP53 alterations, including those that result in loss of p53 tumor suppressor function, may lead to stabilization and increased expression of p53, particularly in the nucleus, and several studies have shown that it may have oncogenic gain-of-function effects (Wang et

al., 2005; 15625370, Koga et al., 2001; 11400116, Kato et al., 2003; 12826609, Houben et al., 2011; 21760960, Olivier et al., 2009; 18802452). A preclinical study has reported that the knockdown of TP53 and PTEN expression in human breast cells promotes formation of cancer stem cells and a gene expression profile resembling that of basal-like triple negative breast tumors (Kim et al., 2014; 24531711).

Effect on drug sensitivity: At present, there are no approved therapies targeting TP53 alterations, despite their high prevalence in cancer. Therapeutic approaches under investigation include gene therapy for TP53 and (dendritic cell-based) TP53 vaccines (Schuler et al., 2014; 24583792, Vermeij et al., 2011; 21541192, Saito et al., 2014; 24982341).
Inhibition of components of the DNA damage checkpoint, including Checkpoint Kinase 1 (Chk1) and Wee1, has been reported to enhance the activity of DNA-damaging agents in preclinical cancer models with deficiency of p53 function (Ma et al., 2011; 21087899, Hirai et al., 2010; 20107315, Bridges et al., 2011; 21799033). Clinical trials of the Wee1 inhibitor AZD1775 are currently underway for patients with solid tumors. Combination of AZD1775 with paclitaxel and carboplatin achieved significantly longer progression-free survival than paclitaxel and carboplatin alone in patients with TP53-mutant ovarian cancer (Oza et al., 2015; ASCO Abstract 5506). Furthermore, AZD1775 in combination with carboplatin achieved a 27% (6/22) response rate and 41% (9/22) stable disease rate in patients with TP53-mutant ovarian cancer refractory or resistant to carboplatin plus paclitaxel (Leijen et al., 2015; ASCO Abstract 2507).
Chk1 inhibitors in combination with chemotherapy are also under investigation in clinical trials. In a Phase 1 clinical trial, 8 of 11 evaluable patients receiving SGT-53 as a single agent exhibited stable disease78. Clinical trials of SGT-53 in combination with chemotherapy are underway. Additionally, the combination of a CHK1 inhibitor and irinotecan reportedly reduced tumor growth and prolonged survival in a TP53 mutant, but not TP53 wild-type, breast cancer xenotransplant mouse model.
Kevetrin (thioureidobutyronitrile) is a novel molecule currently under clinical investigation, which has been reported to have anti-tumorigenic effects in preclinical models. It has been suggested to act upon several tumor-associated pathways, with its activities including activation of wild-type p53 and selective destabilization of mutant p53 (cellceutix.com) (Kumar et al., 2012; AACR 2012, Abstract 2874). ALT-801 is a fusion of IL-2 with a T-cell receptor domain that recognizes a p53-derived peptide in the context of a specific HLA haplotype; this molecule is expected to target cells that express p53 containing the peptide epitope (aa 264-272), and would not be relevant in the context of TP53 alterations that are not expressed (Fishman et al., 2011; 21994418). Studies have reported Aurora kinase A to be activated in cells harboring TP53 mutation, and Aurora kinase A inhibitors have been reported to activate p53 in cancer cells (Vilgelm et al., 2015; 25398437, Li et al., 2015; 25512615, Katayama and Sen, 2011; 21761334, Tentler et al., 2015; 25758253). Thus, cancer cells harboring TP53 alterations may be sensitive to Aurora kinase A inhibitors, several of which are in preclinical and clinical development (Malumbres and Pérez, 2014; 25200357). A preclinical study reported that suppressing Tlr4 expression via siRNA or the pharmacologic inhibitor TAK242 inhibited cell growth in breast cancer cell lines with mutant TP53 and stimulated cell growth in breast cancer cell lines with wild-type TP53. In contrast, activating Tlr4 signaling promoted growth in breast cancer cell lines with mutant TP53 and inhibited growth in breast cancer cell lines with wild-type TP53 (Haricharan and Brown, 2015; 26063617).

Effect on drug resistance: Mutations in TP53 may increase resistance to ionizing radiation therapy (El-Deiry, 2003; 14576853, Miyasaka et al., 2015; 25913131).

REFERENCES

1. Greenman, C., et al., Patterns of somatic mutation in human cancer genomes. Nature, 2007. 446(7132): p.153-8.
2. Stephens, P.J., et al., The landscape of cancer genes and mutational processes in breast cancer. Nature, 2012. 486(7403): p. 400-4.
3. Diaz, L.A., Jr. and A. Bardelli, Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol, 2014. 32(6): p. 579-86.
4. Heitzer, E., P. Ulz, and J.B. Geigl, Circulating tumor DNA as a liquid biopsy for cancer. Clin Chem, 2015. 61(1): p. 112-23.
5. Lebofsky, R., et al., Circulating tumor DNA as a non-invasive substitute to metastasis biopsy for tumor genotyping and personalized medicine in a prospective trial across all tumor types. Mol Oncol, 2015. 9(4): p. 783-90.
6. Esposito, A., et al., Monitoring tumor-derived cell-free DNA in patients with solid tumors: clinical perspectives and research opportunities. Cancer Treat Rev, 2014. 40(5): p. 648-55.
7. Newman, A.M., et al., An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med, 2014. 20(5): p. 548-54.
8. Kidess, E., et al., Mutation profiling of tumor DNA from plasma and tumor tissue of colorectal cancer patients with a novel, high-sensitivity multiplexed mutation detection platform. Oncotarget, 2015. 6(4): p. 2549-61.
9. Forshew, T., et al., Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Sci Transl Med, 2012. 4(136): p. 136ra68.
10. Siegel, R.L., K.D. Miller, and A. Jemal, Cancer statistics, 2015. CA Cancer J Clin, 2015. 65(1): p. 5-29.
11. Davies, H., et al., Mutations of the BRAF gene in human cancer. Nature, 2002. 417(6892): p. 949-54.
12. Romero, A., et al., Identification of E545k mutation in plasma from a PIK3CA wild-type metastatic breast cancer patient by array-based digital polymerase chain reaction: Circulating-free DNA a powerful tool for biomarker testing in advance disease. Transl Res, 2015.
13. Ascierto, P.A., et al., Phase II trial (BREAK-2) of the BRAF inhibitor dabrafenib (GSK2118436) in patients with metastatic melanoma. J Clin Oncol, 2013. 31(26): p. 3205-11.
14. Rothschild, S.I., Targeted Therapies in Non-Small Cell Lung Cancer-Beyond EGFR and ALK. Cancers (Basel), 2015. 7(2): p. 930-49.
15. Heidary, M., et al., The dynamic range of circulating tumor DNA in metastatic breast cancer. Breast Cancer Res, 2014. 16(4): p. 421.
16. Zill, O.A., et al., Cell-Free DNA Next-Generation Sequencing in Pancreatobiliary Carcinomas. Cancer Discov, 2015.
17. Janku, F., et al., Actionable mutations in plasma cell-free DNA in patients with advanced cancers referred for experimental targeted therapies. Oncotarget, 2015. 6(14): p. 12809-21.
18. Tabernero, J., et al., Analysis of circulating DNA and protein biomarkers to predict the clinical activity of regorafenib and assess prognosis in patients with metastatic colorectal cancer: a retrospective, exploratory analysis of the CORRECT trial. Lancet Oncol, 2015.
19. Forbes, S.A., et al., COSMIC: exploring the world's knowledge of somatic mutations in human cancer. Nucleic Acids Res, 2015. 43(Database issue): p. D805-11.
20. Shah, S.P., et al., Mutation of FOXL2 in granulosa-cell tumors of the ovary. N Engl J Med, 2009. 360(26): p.2719-29.
21. Schirripa, M., et al., Role of NRAS mutations as prognostic and predictive markers in metastatic colorectal cancer. Int J Cancer, 2015. 136(1): p. 83-90.
22. Janku, F., et al., PIK3CA mutations frequently coexist with RAS and BRAF mutations in patients with advanced cancers. PLoS One, 2011. 6(7): p. e22769.
23. Kalfa, N., et al., Activating mutations of Gsalpha in kidney cancer. J Urol, 2006. 176(3): p. 891-5.
24. Fecteau, R.E., et al., GNAS mutations identify a set of right-sided, RAS mutant, villous colon cancers. PLoS One, 2014. 9(1): p. e87966.
25. Sparks, A.B., et al., Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer. Cancer Res, 1998. 58(6): p. 1130-4.
26. Bettegowda, C., et al., Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med, 2014. 6(224): p. 224ra24.
27. Oshiro, C., et al., PIK3CA mutations in serum DNA are predictive of recurrence in primary breast cancer patients. Breast Cancer Res Treat, 2015. 150(2): p. 299-307.
28. Beaver, J.A., et al., Detection of cancer DNA in plasma of patients with early-stage breast cancer. Clin Cancer Res, 2014. 20(10): p. 2643-50.
29. Dawson, S.J., et al., Analysis of circulating tumor DNA to monitor metastatic breast cancer. N Engl J Med, 2013. 368(13): p. 1199-209.
30. Ignatiadis, M. and S.J. Dawson, Circulating tumor cells and circulating tumor DNA for precision medicine: dream or reality? Ann Oncol, 2014. 25(12): p. 2304-13.
31. Murtaza, M., et al., Non-invasive analysis of acquired resistance to cancer therapy by sequencing of plasma DNA. Nature, 2013. 497(7447): p. 108-12.
32. Mohamed Suhaimi, N.A., et al., Non-invasive sensitive detection of KRAS and BRAF mutation in circulating tumor cells of colorectal cancer patients. Mol Oncol, 2015.
33. Sanmamed, M.F., et al., Quantitative cell-free circulating BRAFV600E mutation analysis by use of droplet digital PCR in the follow-up of patients with melanoma being treated with BRAF inhibitors. Clin Chem, 2015. 61(1): p. 297-304.
34. Siravegna, G., et al., Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. Nat Med, 2015.
35. Roschewski, M., et al., Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. Lancet Oncol, 2015.
36. Heitzer E, Ulz P, Geigl JB. Circulating tumor DNA as a liquid biopsy for cancer. Clinical chemistry. 2015;61:112-23.
37. Lebofsky R, Decraene C, Bernard V, et al. Circulating tumor DNA as a non-invasive substitute to metastasis biopsy for tumor genotyping and personalized medicine in a prospective trial across all tumor types. Molecular oncology. 2015;9:783-90.
38. Esposito A, Bardelli A, Criscitiello C, et al. Monitoring tumor-derived cell-free DNA in patients with solid tumors: clinical perspectives and research opportunities. Cancer treatment reviews. 2014;40:648-55.
39. Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2014;32:579-86.
40. Perrone F, Lampis A, Bertan C, et al. Circulating free DNA in a screening program for early colorectal cancer detection. Tumori. 2014;100:115-21.

41. Amberger JS, Bocchini CA, Schiettecatte F, Scott AF, Hamosh A. OMIM.org: Online Mendelian Inheritance in Man (OMIM®), an online catalog of human genes and genetic disorders. Nucleic acids research. 2015;43:D789-98.
42. Forbes SA, Beare D, Gunasekaran P, et al. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. Nucleic acids research. 2015;43:D805-11.
43. Ascierto PA, Minor D, Ribas A, et al. Phase II trial (BREAK-2) of the BRAF inhibitor dabrafenib (GSK2118436) in patients with metastatic melanoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2013;31:3205-11.
44. Smalley KS, Xiao M, Villanueva J, et al. CRAF inhibition induces apoptosis in melanoma cells with non-V600E BRAF mutations. Oncogene. 2009;28:85-94.
45. Homet Moreno B, Ribas A. Anti-programmed cell death protein-1/ligand-1 therapy in different cancers. British journal of cancer. 2015;112:1421-7.
46. Morelli MP, Overman MJ, Dasari A, et al. Characterizing the patterns of clonal selection in circulating tumor DNA from patients with colorectal cancer refractory to anti-EGFR treatment. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2015;26:731-6.
47. Zill OA, Greene C, Sebisanovic D, et al. Cell-Free DNA Next-Generation Sequencing in Pancreatobiliary Carcinomas. Cancer discovery. 2015;
48. Thress KS, Paweletz CP, Felip E, et al. Acquired EGFR C797S mutation mediates resistance to AZD9291 in non- small cell lung cancer harboring EGFR T790M. Nature medicine. 2015;21:560-2.
49. Karachaliou N, Mayo-de Las Casas C, Queralt C, et al. Association of EGFR L858R Mutation in Circulating Free DNA With Survival in the EURTAC Trial. JAMA oncology. 2015;1:149-57.
50. Shah SP, Köbel M, Senz J, et al. Mutation of FOXL2 in granulosa-cell tumors of the ovary. The New England journal of medicine. 2009;360:2719-29.
51. De Stefano A, Carlomagno C. Beyond KRAS: Predictive factors of the efficacy of anti-EGFR monoclonal antibodies in the treatment of metastatic colorectal cancer. World journal of gastroenterology : WJG. 2014;20:9732-43.
52. Siravegna G, Mussolin B, Buscarino M, et al. Clonal evolution and resistance to EGFR blockade in the blood of colorectal cancer patients. Nature medicine. 2015;21:795-801.
53. Schirripa M, Cremolini C, Loupakis F, et al. Role of NRAS mutations as prognostic and predictive markers in metastatic colorectal cancer. International journal of cancer. Journal international du cancer. 2015;136:83-90.
54. Wong AL, Lim JS, Sinha A, et al. Tumour pharmacodynamics and circulating cell free DNA in patients with refractory colorectal carcinoma treated with regorafenib. Journal of translational medicine. 2015;13:57.
55. Rodon J, Braña I, Siu LL, et al. Phase I dose-escalation and -expansion study of buparlisib (BKM120), an oral pan- Class I PI3K inhibitor, in patients with advanced solid tumors. Investigational new drugs. 2014;32:670-81.
56. Forshew T, Murtaza M, Parkinson C, et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. Science translational medicine. 2012;4:136ra68.
57. Madic J, Kiialainen A, Bidard FC, et al. Circulating tumor DNA and circulating tumor cells in metastatic triple negative breast cancer patients. International journal of cancer. Journal international du cancer. 2015;136:2158-65.
58. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. Science translational medicine. 2014;6:224ra24.
59. NCCN Clinical Practice Guidelines in Oncology (www.nccn.org/professionals/physician_gls/f_guidelines.asp#site) Accessed 15 June 2015.
60. Van Cutsem E, Cervantes A, Nordlinger B, Arnold D; ESMO Guidelines Working Group. Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2014;25 Suppl 3:iii1-9.
61. Higgins MJ, Baselga J (2011) Targeted therapies for breast cancer. J Clin Invest 121(10):3797-803.
62. Cancer Genome Atlas Research Network (2014) Comprehensive molecular profiling of lung adenocarcinoma. Nature 511(7511):543-50.
63. Swanton C, Futreal A, Eisen T (2006) Her2-targeted therapies in non-small cell lung cancer. Clin Cancer Res 12(14 Pt 2):4377s- 4383s.
64. Nakamura H, Kawasaki N, Taguchi M, et al. (2005) Association of HER-2 overexpression with prognosis in nonsmall cell lung carcinoma: a metaanalysis. Cancer 103(9):1865-73.
65. Tan D, Deeb G, Wang J, et al. (2003) HER-2/neu protein expression and gene alteration in stage I-IIIA non-small-cell lung cancer: a study of 140 cases using a combination of high throughput tissue microarray, immunohistochemistry, and fluorescent in situ hybridization. Diagn Mol Pathol 12(4):201-11.
66. Slamon DJ, Leyland-Jones B, Shak S, et al. (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344(11):783-92.
67. Bang YJ, Van Cutsem E, Feyereislova A, et al. (2010) Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 376(9742):687-97.
68. Chumsri S, Weidler J, Ali S, et al. (2015) Prolonged Response to Trastuzumab in a Patient With HER2-Nonamplified Breast Cancer With Elevated HER2 Dimerization Harboring an ERBB2 S310F Mutation. J Natl Compr Canc Netw 13(9):1066-70.
69. Cappuzzo F, Bemis L, Varella-Garcia M (2006) HER2 mutation and response to trastuzumab therapy in non-small-cell lung cancer.
70. N Engl J Med 354(24):2619-21.
71. Falchook GS, Janku F, Tsao AS, et al. (2013) Non-small-cell lung cancer with HER2 exon 20 mutation: regression with dual HER2 inhibition and anti-VEGF combination treatment. J Thorac Oncol 8(2):e19-20.
72. Mazières J, Peters S, Lepage B, et al. (2013) Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives. J Clin Oncol 31(16):1997-2003.
73. Baselga J, Cortés J, Kim SB, et al. (2012) Pertuzumab plus trastuzumab plus docetaxel for metastatic breast cancer. N Engl J Med 366(2):109-19.

74. Swain SM, Baselga J, Kim SB, et al. (2015) Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. N Engl J Med 372(8):724-34.
75. Verma S, Miles D, Gianni L, et al. (2012) Trastuzumab emtansine for HER2-positive advanced breast cancer. N Engl J Med 367(19):1783-91.
76. Cameron D, Casey M, Oliva C, et al. (2010) Lapatinib plus capecitabine in women with HER-2-positive advanced breast cancer: final survival analysis of a phase III randomized trial. Oncologist 15(9):924-34.
77. Geyer CE, Forster J, Lindquist D, et al. (2006) Lapatinib plus capecitabine for HER2-positive advanced breast cancer. N Engl J Med 355(26):2733-43.
78. Serra V, Vivancos A, Puente XS, et al. (2013) Clinical response to a lapatinib-based therapy for a Li-Fraumeni syndrome patient with a novel HER2V659E mutation. Cancer Discov 3(11):1238-44.
79. Ali SM, Alpaugh RK, Downing SR, et al. (2014) Response of an ERBB2-Mutated Inflammatory Breast Carcinoma to Human Epidermal Growth Factor Receptor 2-Targeted Therapy. J Clin Oncol ePub Feb 2014.
80. Lin NU, Winer EP, Wheatley D, et al. (2012) A phase II study of afatinib (BIBW 2992), an irreversible ErbB family blocker, in patients with HER2-positive metastatic breast cancer progressing after trastuzumab. Breast Cancer Res Treat 133(3):1057-65.
81. Schwab CL, Bellone S, English DP, et al. (2014) Afatinib demonstrates remarkable activity against HER2-amplified uterine serous endometrial cancer in vitro and in vivo. Br J Cancer 111(9):1750-6.
82. De Grève J, Teugels E, Geers C, et al. (2012) Clinical activity of afatinib (BIBW 2992) in patients with lung adenocarcinoma with mutations in the kinase domain of HER2/neu. Lung Cancer 76(1):123-7.
83. De Grève J, Moran T, Graas MP, et al. (2015) Phase II study of afatinib, an irreversible ErbB family blocker, in demographically and genotypically defined lung adenocarcinoma. Lung Cancer 88(1):63-9.
84. Gandhi L, Bahleda R, Tolaney SM, et al. (2014) Phase I study of neratinib in combination with temsirolimus in patients with human epidermal growth factor receptor 2-dependent and other solid tumors. J Clin Oncol 32(2):68-75.
85. Ben-Baruch NE, Bose R, Kavuri SM, et al. (2015) HER2-Mutated Breast Cancer Responds to Treatment With Single-Agent Neratinib, a Second-Generation HER2/EGFR Tyrosine Kinase Inhibitor. J Natl Compr Canc Netw 13(9):1061-4.
86. Kris MG, Camidge DR, Giaccone G, et al. (2015) Targeting HER2 aberrations as actionable drivers in lung cancers: phase II trial of the pan-HER tyrosine kinase inhibitor dacomitinib in patients with HER2-mutant or amplified tumors. Ann Oncol ePub Apr 2015.
87. Takada M, Higuchi T, Tozuka K, et al. (2013) Alterations of the genes involved in the PI3K and estrogen-receptor pathways influence outcome in human epidermal growth factor receptor 2-positive and hormone receptor-positive breast cancer patients treated with trastuzumab-containing neoadjuvant chemotherapy. BMC Cancer 13:241.
88. Jensen JD, Knoop A, Laenkholm AV, et al. (2012) PIK3CA mutations, PTEN, and pHER2 expression and impact on outcome in HER2-positive early-stage breast cancer patients treated with adjuvant chemotherapy and trastuzumab. Ann Oncol 23(8):2034- 42.
89. Berns K, Horlings HM, Hennessy BT, et al. (2007) A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. Cancer Cell 12(4):395-402.
90. Dave B, Migliaccio I, Gutierrez MC, et al. (2011) Loss of phosphatase and tensin homolog or phosphoinositol-3 kinase activation and response to trastuzumab or lapatinib in human epidermal growth factor receptor 2-overexpressing locally advanced breast cancers. J Clin Oncol 29(2):166-73.
91. Loibl S, von Minckwitz G, Schneeweiss A, et al. (2014) PIK3CA Mutations Are Associated With Lower Rates of Pathologic Complete Response to Anti-Human Epidermal Growth Factor Receptor 2 (HER2) Therapy in Primary HER2-Overexpressing Breast Cancer. J Clin Oncol ePub Sep 2014.
92. Barbareschi M, Cuorvo LV, Girlando S, et al. (2012) PI3KCA mutations and/or PTEN loss in Her2-positive breast carcinomas treated with trastuzumab are not related to resistance to anti-Her2 therapy. Virchows Arch 461(2):129-39.
93. Guarneri V, Generali DG, Frassoldati A, et al. (2014) Double-blind, placebo-controlled, multicenter, randomized, phase IIb neoadjuvant study of letrozole-lapatinib in postmenopausal hormone receptor-positive, human epidermal growth factor receptor 2-negative, operable breast cancer. J Clin Oncol 32(10):1050-7.
94. Jones KL, Buzdar AU (2009) Evolving novel anti-HER2 strategies. Lancet Oncol 10(12):1179-87.
95. Zagouri F, Sergentanis TN, Chrysikos D, et al. (2013) Hsp90 inhibitors in breast cancer: a systematic review. Breast 22(5):569-78.