BRAF gene mutation analysis

BRAF gene encodes a serine/threonine kinase that functions within the Ras-Raf-MEK-MAPK pathway, which connects extracellular signals to transcriptional regulation. Activating mutations of BRAF are found in colorectal cancers (CRCs), melanomas, ovarian tumors, lung and thyroid cancers. The most frequent BRAF mutation is c.1799T>A (V600E), this mutation predisposes to apoptosis inhibition, increases invasiveness, and occurs during carcinogenesis.

Indications for mutation analysis:

1. BRAF mutation can be utilized to identify **sporadic MSI-H colorectal cancer** cases and exclude them from germline mismatch repair gene testing. BRAF V600E mutation accounts for over 90% of BRAF mutations in colorectal cancer and is found in 30–80% of sporadic MSI-H colorectal cancer cases. This mutation is extremely rare in tumours associated with Lynch syndrome. The presence of the BRAF V600E mutation is strongly associated with sporadic CRCs that exhibit MSI due to somatic inactivation of MLH1 protein expression. If either the BRAF V600E mutation or MLH1 promoter methylation is found in a MSI-H tumor, then the tumor is probably sporadic and further testing for Lynch syndrome may not be warranted.
2. BRAF mutation is an independent predictor for colorectal cancer patients' **responsiveness to EGFR inhibitors**. BRAF mutations cause resistance to anti-epidermal growth factor receptor therapy in colorectal cancer patients, therefore BRAF status is a useful biomarker for selecting patients suitable for anti-EGFR treatment.
3. BRAF mutation can be used to **guide therapy for melanoma patients**. BRAF mutations have been detected in 70% of primary melanomas. BRAF V600E kinase inhibitors are available for melanoma treatment, but their use is limited to patients with metastatic melanoma with a demonstrated BRAF V600E mutation.

Breast and Ovarian Cancer

Breast and ovarian cancers are most strongly associated with mutations of the BRCA1 and BRCA2 genes. Among women who have a clinically important BRCA gene mutation, the lifetime risk of developing breast and/or ovarian cancer can reach 80%. Cancer-predisposing mutations in the BRCA1 and BRCA2 genes are inherited in an autosomal dominant manner. The prognosis for breast cancer survival depends upon the stage at which breast cancer is diagnosed.

Indications for genetic testing:

1. Testing of individuals with early-age-onset of breast or ovarian cancer
2. Testing of individuals with family history of breast or ovarian cancer
3. Testing of at-risk family members for known mutations
3. Genetic counseling

Testing should be performed if there is a family history of breast or ovarian cancer, genetic alterations have been found in the family, or there is a history of breast cancer in males in the family.

Cancer Predisposition

Determination of cancer predisposition is vital for prevention and early detection of the disease. Early diagnosis of cancer will ensure the immediate start of treatment, which is a key to increasing the survival and recovery. Significant difference between the survival rates of early stage and advanced stage of cancer points out the need for risk assessment of the disease.

Identification of genetic susceptibility to **hereditary cancer syndromes** enables to implement risk-reduction strategies, estimate familial cancer risk and identify at-risk family members.

Cancer predisposition testing at Asper Biotech includes **two next generation sequencing based panels**, allowing to analyze multiple genes associated with an increased risk for a wide range of cancers.

Indications for genetic testing:

1. Testing of individuals with early-age-onset of cancer
2. Testing of individuals with multiple primary cancers
3. Testing of family members of the affected individuals
4. Testing individuals with family history suggesting inherited pattern of cancer but no genetic changes identified previously
5. Genetic counseling

Familial Adenomatous Polyposis – FAP

Familial adenomatous polyposis (FAP) is a colon cancer predisposition syndrome characterized by the early onset of hundreds to thousands of adenomatous polyps throughout the colon. By age 35, 95% of individuals with FAP have polyps. If left untreated, patients with this syndrome develop colon cancer by age 35-40. APC-associated polyposis conditions are inherited in an autosomal dominant manner.

Indications for mutation analysis:

1. Testing of individuals with adenomatous polyps
2. Testing of first degree relatives of the affected individuals
3. Genetic counseling

Glioblastoma

Glioblastomas are heterogeneous group of tumors originating from glial cells. Glioblastomas are most frequent in pediatric age. Approximately 60% from all the central nervous system tumors occur in childhood. Disease prevalence is 6-7:100 000 cases/year. Glioblastoma prognosis and treatment options depend on type and location of tumor, association to genetic disorders (e.g. neurofibromatosis) and tumor stage (localized or disseminated glioblastoma).

Indications for genetic testing:

1. Confirmation of clinical diagnosis
2. Determination of disease prognosis and recurrence risk
3. Prediction of treatment response
4. Genetic counseling

Lynch Syndrome/Hereditary Non-Polyposis Colon Cancer – HNPCC

Lynch syndrome, also called **hereditary non-polyposis colon cancer** (HNPCC), is characterized by an increased risk of colon cancer and other cancers (e.g., of the endometrium, ovary, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain, and skin). Lynch syndrome is inherited in an autosomal dominant manner and it is associated with germline mutations in the mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2. Mutation carriers have a lifetime risk of up to 80% for colorectal cancer, 20-60% risk of endometrial cancer, as well as other tumors. Lynch syndrome is associated with early onset of cancer, the average age of diagnosis is 45 years.

Referral for molecular testing of Lynch syndrome:

1. Tumour tissue analysis to evaluate the MMR proteins expression by immunohistochemical analysis (IHC) and DNA microsatellite instability (MSI) testing is suggested for the individuals meeting Amsterdam II/Bethesda criteria.
2. If absence of the MLH1/PMS2 proteins expression is observed by IHC, methylation analysis of the MLH1 gene promoter and/or testing of the somatic BRAF V600E mutation is recommended in order to exclude sporadic colorectal cancer cases.
3. If tumour with MMR deficiency and MSI high is detected, further mutation analysis from peripheral blood/normal tissue of the MMR genes is indicated.

Indications for mutation analysis:

1. Testing of individuals meeting Amsterdam II/Bethesda criteria
2. Testing of individuals with family history of colorectal cancer or other Lynch syndrome-related cancers
3. Testing of at-risk family members for known mutations

4. Genetic counseling

Lynch Syndrome and Polyposis Syndrome

Lynch syndrome is characterized by an increased risk of colon cancer and other cancers (e.g., of the endometrium, ovary, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain, and skin). Lynch syndrome is inherited in an autosomal dominant manner and most commonly it is associated with germline mutations in the mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2. Mutation carriers have a lifetime risk of up to 80% for colorectal cancer, 20-60% risk of endometrial cancer, as well as other tumors. Lynch syndrome is associated with early onset of cancer, the average age of diagnosis is 45 years.

In addition to Lynch syndrome the following syndromes are included in the testing: **Familial adenomatous polyposis** (FAP), **MUTYH-associated polyposis**, **BMPR1A-Related Juvenile Polyposis, SMAD4-Related Juvenile Polyposis, PTEN hamartoma tumor syndrome, Peutz-Jeghers Syndrome** and **other cancers**.

Indications for genetic testing:

1. Confirmation of clinical diagnosis
2. Testing of individuals meeting Amsterdam II/Bethesda criteria
3. Testing of individuals with family history of polyposis syndromes and Lynch syndrome-related cancers
4. Differentiation of FAP from MUTYH-associated polyposis
5. Differentiation of juvenile polyposis from other hamartomatous polyposis syndromes
6. Genetic counseling

Microsatellite instability – MSI

Microsatellite instability (**MSI**) is the mutational signature found in colorectal cancers that evolve as a result of inactivation of the DNA mismatch repair system. Defects in the genes involved in mismatch repair lead to an accumulation of somatic mutations in a cell, which may result in the cell becoming malignant. Germline mutations in mismatch repair genes are associated with developing hereditary non-polyposis colon cancer. Approximately 90% of colon cancers from families meeting Amsterdam criteria have MSI.

Indications for genetic testing:

1. Testing of individuals with colorectal or other Lynch-associated cancers to estimate the probability of mutations in mismatch repair genes
2. Determination of disease prognosis
3. Prediction of tumor response to chemotherapeutic agents
4. Genetic counseling

MUTYH-associated polyposis

MUTYH-associated polyposis (MAP) is an autosomal recessive disorder characterized by a variable number of colorectal adenomas with a high risk of developing colorectal cancer. MAP is caused by biallelic germline mutations in MUTYH gene, but there is also evidence that monoallelic mutation carriers have an increased risk for developing colorectal cancer. The clinical symptoms of MAP are often undistinguishable from that of familial adenomatous polyposis (FAP) or attenuated FAP (AFAP) caused by mutations in adenomatous polyposis coli (APC) gene, but the age of onset is usually later compared to FAP patients. The two most common mutations in Caucasians, accounting for about 80% of mutant MUTYH alleles, are p.Y179C and p.G396D (also known as Y165C and G382D).

Indications for genetic testing:

1. Testing of individuals with clinical symptoms similar to FAP or AFAP but in whom no APC gene mutation has been identified
2. Testing of first degree relatives of the affected individuals
3. Genetic counseling

Thiopurine S-Methyltransferase Deficiency – TPMT

Thiopurine drugs such as 6-mercaptopurine and azathioprine are used as chemotherapeutic agents and their active metabolites have both immunosuppressive as well as antiproliferative effects.**Thiopurine s-methyltransferase** (TPMT) is an enzyme that catalyzes the s-methylation of thiopurine-type compounds and mediates the formation of inactive metabolites. Genetic polymorphisms that affect this enzymatic activity can result in life-threatening toxicity. Drug-induced bone marrow toxicity may cause myelosuppression, anemia, bleeding tendency, leukopenia, and infection.

There are three main genetic changes in the TPMT gene that determine metabolization efficiency of the thiopurines (rs1800462, rs1800460, and rs16880254) in 80-95% of cases.

Indications for genetic testing:

Genetic testing of variations in the TPMT gene allows determining the activity of the TPMT enzyme, and makes it possible to choose an individual's optimal dose before starting treatment. In principal, this test should be done for all patients who are undergoing treatment with thiopurine drugs.