1 General

Tumor DNA sequence information is essential for optimal cancer treatment selection. DNA sequence information complements the traditional clinical laboratory information from medical imaging, pathology report and clinical chemistry. The tumor has acquired several mutations in its DNA that are the root cause of the disease. The treating team of physicians need to have information on the basic biology of the disease and the available treatment modalities suggested by that biology to be able to make informed treatment decisions. Without the correct underlying information, choosing the best treatment is difficult or impossible.

2 Targeted DNA treatment test based on tissue biopsy sampling

The histologic subtype of the tumor is preferentially determined by pathologic examination of a tissue biopsy. Adequate tissue should be set aside for molecular testing, but this may not always be possible or desirable.

Cell free circulating tumor DNA (ctDNA) is a promising alternative sample type if the patient is medically unfit for invasive tissue sampling, or if there is insufficient accessible tissue material for sampling.

3 Targeted DNA treatment test based on blood samples

Tumor DNA can be extracted from blood samples, not only tissue biopsies. The tumor cells, like normal cells, not only grow but also break down. The breakdown fragments end up in the blood, together with the DNA breakdown fragments of normal cells. These DNA fragments in the peripheral circulation are found in the plasma (non-cell) fraction of the peripheral blood.

Tumor DNA can be recovered from the plasma fraction of peripheral blood by new technology and can be told apart from the normal DNA fragments that are also present, by large scale sequencing. The tumor DNA can subsequently be stitched together to reveal the essential features of the tumor DNA. For these new sequencing technologies to work with the required sensitivity and specificity it is necessary to use the patient's own individual normal DNA as a guide for identifying the specific DNA fragments that are unique to the tumor and that are not found in the patient's normal DNA. The DNA sampling test obviously works better the more tumor DNA breakdown fragments there are to analyze, i.e. the more tumor mass or the later the stage of the disease. Thus, the more the disease has progressed, the more technically appropriate the test.

The test can be used to broadly sample the biology of the individual patient's disease to search for new and experimental treatments or to narrowly focus on the currently established treatments. The treating physician must work with the patient to determine the most appropriate test in the individual case.

4 Clinical information

The following clinical information is recommended by the laboratory as a reference to the expected variant allele frequency in the blood plasma sample.

- Diagnosis (ICD-10)
- Clinical stage
- Tumor burden
- Mitotic index
- Tumor viability

Sample Requirements

Blood (min. 30ml) in an EDTA tube, to be prepared for plasma within 2 hours of sampling, according to a Standard Operating Procedure (SOP) and frozen.
Reference DNA blood sample (min. 10 ml) in an EDTA tube, kept at room temperature, or refrigerated for a maximum of 3 days according to Standard Operating Procedure (SOP).

5 DNA sequencing

The DNA samples are sequenced on an Illumina Sequencing System or similar, the best technology from the world's leader in NGS (Next-Generation Sequencing) systems.

Cancer treatment predictive gene list (22 genes)

The treatment modalities that are suggested by the oncogenic or likely oncogenic variants according to clinical guidelines in the below list of 22 genes will be presented, along with the evidence, in the clinical interpretation section of the referral report. The analyses are based on sequencing exomes, hotspots or whole coding regions of the following genes:

AKT1, ALK, BRAF, CTNNB1, EGFR (ERBB1), ERBB2 (HER-2, NEU), ERBB3, ESR1 (ERα), FOXL2, GNA11, GNAQ, IDH1, IDH2, KIT (CD117), KRAS, NRAS, MET, PDGFRA, PIK3CA (p110-alpha), RAF1, RET, TP53 (p53)

Cancer biology gene list (275 genes)

The disruptions of the biology of the individual patient that has resulted in cancer is mapped by DNA sequencing of the below 275 genes for oncogenic or likely oncogenic variants. The genes in this list continue to be revised by scientists, but already compromise a large sample of the estimated 700 genes in existence that can mutate to a cancer phenotype. Only a subset of the oncogenic or likely oncogenic variants have an associated suggested treatment modality based on established guidelines, but ongoing clinical trials will be accessed by searching clinicaltrials.gov for new and experimental treatment modalities. ClinicalTrials.gov provides patients, scientists and health care professionals with information on publicly and privately supported clinical studies. The website is maintained by the National Library of Medicine (NLM) at the National Institutes of Health (NIH).

TNFRSF14 , MTOR , SDHB , ID3 , ARID1A , CSF3R , MYCL , MPL , MUTYH , TAL1 , CDKN2C , JAK1 , FUBP1 , NRAS , FAM46C , NOTCH2 , MCL1 , RIT1 , NTRK1 , DDR2 , CDC73 , MDM4 , H3F3A , EGLN1 , FH , EXO1 , AKT3 , GATA3 , RET , PTEN , FAS , SUFU , SMC3 , FGFR2 , HRAS , FANCF , WT1 , MEN1 , CCND1 , FGF4 , EED , MRE11A , BIRC3 , ATM , KMT2A , CBL , CHEK1 , FGF6 , ETV6 , KRAS , ARID2 ,

KMT2D , ACVR1B , ERBB3 , CDK4 , MDM2 , PTPN11 , HNF1A , POLE , FLT3 , BRCA2 , RB1 , NFKBIA , NKX2-1 , TSHR , DICER1 , HSP90AA1 , TRAF3 , XRCC3 , AKT1 , GREM1 , RAD51 , B2M , MAP2K1 , NTRK3 , IDH2 , BLM , IGF1R , AXIN1 , TSC2 , CREBBP , GRIN2A , SOCS1 , PALB2 , CYLD , CTCF , CDH1 , FANCA , TP53 , AURKB , MAP2K4 , FLCN , NF1 , SUZ12 , CDK12 , ERBB2 , IKZF3 , STAT3 , BRCA1 , MAP3K14 , HOXB13 , SPOP , RNF43 , PPM1D , BRIP1 , CD79B , AXIN2 , PRKAR1A , SOX9 , SRSF2 , SETBP1 , SMAD2 , SMAD4 , BCL2 , STK11 , TCF3 , DOT1L , GNA11 , MAP2K2 , KEAP1 , DNM2 , SMARCA4 , CALR , NOTCH3 , JAK3 , PIK3R2 , MEF2B , CCNE1 , CEBPA , KMT2B , AKT2 , CD79A , CIC , CBLC , POLD1 , PPP2R1A , U2AF2 , AURKC , MYCN , GEN1 , DNMT3A , ALK , EPAS1 , MSH2 , MSH6 , XPO1 , CXCR4 , LRP1B , NFE2L2 , PMS1 , SF3B1 , IDH1 , ERBB4 , BCL2L1 , ASXL1 , SRC , PLCG1 , ZNF217 , AURKA , GNAS , RUNX1 , ERG , U2AF1 , MAPK1 , BCR , SMARCB1 , CHEK2 , NF2 , EP300 , FANCD2 , VHL , RAF1 , TGFBR2 , MLH1 , MYD88 , CTNNB1 , SETD2 , RHOA , BAP1 , PBRM1 , MITF , EPHA3 , CBLB , GATA2 , FOXL2 , ATR , PIK3CA , SOX2 , BCL6 , FGFR3 , WHSC1 , PDGFRA , KIT , KDR , EPHA5 , FAM175A , TET2 , FBXW7 , TERT , IL7R , MAP3K1 , PIK3R1 , APC , RAD50 , CTNNA1 , CSF1R , PDGFRB , NPM1 , FGFR4 , NSD1 , FLT4 , IRF4 , HIST1H3B , DAXX , FANCE , PIM1 , CCND3 , PRDM1 , ROS1 , TNFAIP3 , ESR1 , ARID1B , CARD11 , PMS2 , RAC1 , INHBA , IKZF1 , EGFR , HGF , CDK6 , CUX1 , MET , SMO , BRAF , PRSS1 , EZH2 , RHEB , KMT2C , XRCC2 , FGFR1 , KAT6A , PRKDC , RAD21 , MYC , JAK2 , CD274 , CDKN2A , CDKN2B , FANCG , PAX5 , GNAQ , NTRK2 , FANCC , PTCH1 , GALNT12 , ABL1 , TSC1 , NOTCH1 , CRLF2 , ZRSR2 , BCOR , KDM6A , ARAF , GATA1 , KDM5C , SMC1A , AMER1 , AR , MED12 , ATRX , BTK , PAK3 , STAG2 , BCORL1 , PHF6

6 Bioinformatics

The referral report includes a bioinformatics evaluation section of the somatic genetic defects that are unique to the tumor, and not found in the patient's normal DNA, and that are known to the scientific and clinical communities as pathogenic or likely pathogenic based on ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) at the National Center for Biotechnology Information (NCBI). ClinVar is an archive of scientific and clinical reports of the relationships between human genetic variations and phenotypes, i.e. disease signs and symptoms, with supporting evidence.

Clinical interpretation

The clinical utility, i.e. treatment modality prediction, of the bioinformatically identified somatic pathogenic or likely pathogenic variants is assigned based on the guidelines for treatment of cancer of the National Comprehensive Cancer Network® (NCCN®). The NCCN® is a not-for-profit alliance of 28 leading US cancer centers devoted to patient care, research, and education.