Since our last newsletter, new techniques have been introduced in our molecular lab. With this newsletter we are happy to give you an update on these new developments in our lab.

Next generation sequencing platform changed from MiSeq to Ion S5

Since 2015 mutation analysis is performed in our lab using Next Generation Sequencing on the Illumina MiSeq platform with Haloplex target enrichment. Recently our pathology department invested in an Ion S5 platform. This additional platform is now "up and running" and we are proud to announce that we got our ISO15189 accreditation for mutation analysis by AmpliSeq detection on this platform. The introduction of this technique and extra platform has consequent advantages and changes to our workflow, which are listed below.

Oncomine Focus Assay

The Oncomine Focus Assay uses the AmpliSeq technique to detect Single Nucleotide Variants (SNVs) and small insertions and deletions in the following genes:*AKT1, ALK, AR, BRAF, CDK4, CTNNB1, DDR2, EGFR, ERBB2, ERBB3, ERBB4, FGFR2, FGFR3, GNA11, GNAQ, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, PDGFRA, PIK3CA, RAF1, RET, ROS1 and SMO.* Also fusions in *MET* (MET exon 14 skipping) are detected. This panel will be used for mutation analysis testing of lung carcinomas, colorectal carcinomas, melanoma tumours and GIST. Obviously, as with all molecular techniques, this technique will also be most successful with blocks with a high percentage (>10%) and amount of tumor cells and a good fixation (formalin fixed between 6-72h). However, in contrast to the Haloplex method, the Oncomine Focus Assay can be performed when only small tumor amounts are present (5 mm^2 on H&E).

Gynecological tumors (breast, ovarium, endometrium)

Besides the Oncomine Focus Assay, we developed an AmpliSeq custom panel for mutation analysis in breast-, ovarian- and endometrial carcinomas. With this panel we can detect SNVs and small insertions and deletions in the exones of the following genes: *AKT1, ATM, ATR, CDK12, CHEK2, DICER1, ERBB2, ESR1, FOXL2, MLH1, MSH2, MSH6, PALB2, PIK3CA, PMS2, POLE, PPP2R1A, RAD51C, RAD51C, RAD51D, TP53 and TP53BP1.* With this panel, we aim to have an answer to the expanding demand of mutation analysis in predictive biomarkers implied in PARP inhibition in ovarian cancer and triple-negative breast cancers. Furthermore, the panel contains prognostic markers, such as *POLE* endometrial carcinomas and resistance markers, such as *ESR1* in hormone resistance breast carcinomas. Last but not least, the panel contains several diagnostic markers for epithelial and non-epithelial ovarian carcinomas.

Other NGS developments

Wil u ook onze andere nieuwsbrieven lezen, neem dan een kijkje op de **labogids**

Wil u ook onze andere nieuwsbrieven lezen, neem dan een kijkje op de **labogids**

In addition to the tests above we are working on expanding our portfolio of NGS with a panel specific for glioma and meningioma/medulloblastoma as well as a thyroid panel and mutation analysis with NGS in liquid biopsy samples.

MLH1 hypermethylation

When loss of MLH1 expression is observed, this can be due to a somatic hypermethylation of the MLH1 promoter region or due to an inherited mutation.

In colorectal cancer, when loss of MLH1 expression is observed together with a BRAF V600E mutation, we know that the microsatellite instability is sporadic and MLH1 hypermethylation testing is not needed to distinguish Lynch syndrome from sporadic MMR. Still 40% of the sporadic microsatellite instable CRC cases do not have a BRAF mutation. Consequently, when loss of MLH1 expression is observed without a BRAF mutation, MLH1 hypermethylation testing is needed to evaluate whether the patient should be referred to a clinical geneticist for counseling.

In other cancer types (such as. endometrial cancer, ovarian cancer, …) displaying MLH1 loss, BRAF analysis is not useful to distinguish sporadic from genetic reasons for the microsatellite instability phenotype. In these cancer types, MLH1 promoter hypermethylation is always needed to evaluate whether the patients should be referred to a clinical geneticist for further analysis.