Mutational analysis has moved to the Next-generation sequencing platform

As most of you already know, we got ISO15189 accreditation for our Next Generation Sequencing (NGS) platform (MiSeq) in June 2015. Over the past few months we have moved forward to perform almost all our mutation analysis testing (detection of single nucleotide variants and small insertions and deletions) on the NGS platform. This means that the High-Resolution Melting (HRM) technique and the Sanger sequencing is not routinely performed in our laboratory anymore for standard analysis. Changing to NGS gives us the advantage of multiplexing, enabling analysis of multiple genes simultaneously, with consequent advantages in workflow and in reporting. Obviously, the NGS technique- just as the HRM- still needs enough tissue input, so please select blocks with a high percentage and amount of tumor cells and a good fixation.

Sometimes, obtaining sufficient tumor cells or cell percentage can be really difficult in daily practice. For lung carcinoma, we do offer digital droplet analysis on formalin-fixed tissues for *EGFR* analysis, as well as offering this technology on plasma- the 'liquid biopsy' story. So it can happen that your lab requests NGS lung (with a main purpose of analysing *EGFR*), but that we decide to do ddPCR in place. This is only when the tissue is really too little to make the NGS successful. Additionally, we work on expanding our portfolio of ddPCR analysis for other genes. In fact, we already documented an EGFR TKI resistance due to MET amplification via liquid biopsy. Our aim now is to detect ALK rearrangements via liquid biopsy.

In conclusion, NGS is the main technique we know perform for mutation analysis, with the following panels:
Lung carcinoma: *ALK, BRAF, CDKN2A, DDR2, EGFR, ERBB2, KRAS, MET, PIK3CA, RET and ROS1*
Colon carcinoma *BRAF, CTNNB1, HRAS, KRAS, NRAS and PIK3CA*
Melanoma: *BRAF, KIT and NRAS*
GIST: *BRAF, KIT and PDGFRA*
Extended solid tumor panel (eg for Phase I trial inclusion): ClearSeq Cancer panel: *ABL1, AKT1, ALK, AR, ATM, BRAF, CDKN2A, CSF1R, EGFR, ERBB2, ERBB4, FANCA, FANCC, FANCF, FANCG, FGFR1, FGFR2, FGFR3, FLT3, HRAS, IDH1, IDH2, JAK2, JAK3, KIT, KRAS, MAP2K1, MAP2K2, MAP2K4, MET, NOTCH1, NPM1,*

Voor meer informatie kan u steeds terecht bij ons secretariaat (03/8213753 of secr.anapat@uza.be).

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

NRAS, PDGFRA, PIK3CA, PIK3CA, PIK3R1, PTEN, RET, RUNX1, SMAD4, SMO, SRC, STK11, TP53, VHL, WT1

A word of caution; some people believe that by asking for NGS lung, ALK rearrangements can be detected. This is not the case. ALK rearrangements are looked for by immunohistochemistry, and confirmed by FISH. The mutations we detect with NGS are mostly acquired resistance mutations (resistance to crizotinib). Knowledge of these mutations can guide further therapy. Second generation inhibitors (alectinib and ceritinib) have different activities against different resistance mutations.

We are also particularly pleased to detect MET exon 14 skipping variants. These tumors “skipped” exon 14. Exon 14 is an important region in the MET protein. For downregulatory MET, the protein can be ubiquitinated. This can only happen when exon 14 is present. If not, the MET protein cannot be ubiquitinated, meaning it cannot be endocytosed and destroyed and remains active on the cell membrane. It comes to no surprise that in this case, you can see a beautiful, strong MET membranous expression on the cell membrane by immunohistochemistry.

BRCA testing

We are particularly pleased to announce that we are BELAC accredited for BRCA testing. Furthermore, we passed the BRCA ring trial organized by AstraZeneca. This ring trial used FFPE material. We were the only Belgian lab participating in this ring trial…

A lot of confusion exists about BRCA testing. The problem is that people don’t make a clear distinction between germline mutations and somatic mutations. When you are dealing with a (metastatic) high grade serous ovarian tumor, and you want to treat the patient with olaparib, then you need BRCA testing. If the patients has already been tested for germline mutations, and is negative, then we will look for somatic mutations on the FFPE material from the tumor. You will have a response within 14 days. Unfortunately, some rare huge deletions are not easily detected. This takes more time. So, the first result we give is conditional. But if we already found a mutation, then the patient can be treated. If germline testing has not been done, it is advisable to send a blood sample.

According to the law, BRCA testing is coordinated by the centers of human genetics. So, the testing material should be send to our Center of Human Genetics, Prins Boudewijnlaan 43, 2650 Edegem.

ROS1 FISH

Due to the excellent response to crizotinib in ROS1 rearranged adenocarcinomas, guidelines start to implement ROS1 FISH testing in NSCLC. The gold standard remains FISH, but also IHC is in development. The problem here is that ROS1 positive cases are even rarer than ALK positive cases, so central material is scarce. As in the ALK story, ROS IHC cannot be used as a screening test if not properly validated.

BRAF mutations

One thing we learned about BRAF, is that treating BRAF mutations with the currently available inhibitors (vemurafenib, dabrafenib) is a tricky business. We learned from cellular biology that you have to make a clear distinction between the V600 (and 599) mutants and other mutants. The classical V600 and 599 mutants are active as monomers, whereas others still need a dimerisation partner for activation. If you give a BRAF TKi to this last category of mutants, you accelerate tumor development.

ALK IHC in melanoma

An exciting finding is that up to 11% of melanomas show ALK expression, duo to the presence of an aberrant splicing variant. Responses to crizotinib have already been documented. This means that probably ALK immunohistochemistry could join the list of actionable aberrations to look for in melanoma. Of course, the hunt for ALK splicing variants in other tumors is going on…

PDL1

Immunotherapy is now a “hype”. Unfortunately, most patients do not respond to this therapy and there is an urgent need for biomarkers. PDL1 immunohistochemistry can be performed, but this is not the best biomarker in use. One of the best markers for prediction of response to antibodies such as nivolumab or pembroluzumab is the presence of CD8 cytotoxic T-cells in the tumor, in particular at the invasion front. Active CD8 positive T-cells can induce PDL1 expression (via secretion of interference). So, in the best of worlds, CD8 positive T-cells and PDL1 positive tumor cells are present when you want to start therapy targeting the PD1/PDL1 axis. The more presence of PDL1 on tumor cells, is in my opinion not a guarantee for success. PDL1 immunohistochemistry has been called “the best of the worst biomarkers we have”. Immune-oncology is a rapidly evolving, exciting field with consequences for the practicizing pathologist. For this reason, we will organize a meeting about immune-oncology for pathologists.