Since our last newsletter, a lot of new techniques have been introduced in our molecular lab. We are sure that with those new acquisitions, we can ultimately help our oncology patients better than before.

**Next generation sequencing platform**
We are proud to announce that we got our ISO15189 accreditation for our Next Generation Sequencing (NGS) platform last June. The NGS MiSeq is now "up and running" in our pathology department. The following genes can be screened for genomic Single Nucleotide Variants (SNVs) and small insertions and deletions: *ALK, BRAF, BRCA1, BRCA2, CDKN2A, CTNNB1, DDR2, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, HRAS, IDH1, IDH2, JAK2, KIT, KRAS, MET, NRAS, PDGFRA, PIK3CA, RET, ROS1 and TP53.*
Detection of mutations in other clinically relevant genes, such as AKT1, MAP2K1, GNA11, and GNAQ is currently being developed.

**Idylla platform/*BRAF***
Most of you know this platform. It is (very) user-friendly to handle (no separate DNA extraction needed) and gives you a test result within two hours.

*BRAF* and *KRAS* testing can be done, but *EGFR* and *NRAS* testing will follow soon. We were involved in the development of the tests (together with -amongst others- the Charité Hospital in Berlin) and we compared the test results with the results of pyrosequencing PCR. Tests with conflicting results between pyrosequencing PCR and the Idylla platform were again performed on the NGS; it turned out that the Idylla platform was right.

Most of the *BRAF* testing on the Idylla is done in melanomas, but in papillary thyroid carcinoma it is also useful. It seems that *BRAF*positive papillary thyroid carcinomas are more aggressive than*BRAF* wild type carcinomas, and some even advocate cervical lymph node dissection in those cases.

One can also use *BRAF* testing in colorectal carcinomas, particularly in deciding if microsatellite instability (MSI) is hereditary (Lynch syndrome) or sporadic. When a *BRAF* (V600E) mutation is present in MSI positive cases then you are dealing with a sporadic case. Some people use *BRAF* testing in colon cancer as a prognostic marker. *BRAF* mutated colorectal carcinomas have a bad prognosis, but this is only true if there is no MSI. Finding a*BRAF* mutation in an MSI positive tumour does not mean that the

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

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

tumour will behave aggressively; on the contrary, the prognosis for MSI positive tumours is better than for MSI negative tumours. This contradiction is now explained by the fact that MSI provokes a marked inflammatory reaction, with the presence of a lot of tumour infiltrating lymphocytes. It seems that this immune attack is the reason for the better prognosis in MSI positive tumours. *BRAF*mutations do not to seem be predictive for the (lack of) response to cetuximab/panitumumab treatment.

By the way, new data have been published on *BRAF* in lung cancer by the European EURAF cohort (Journal of Thoracic Oncology, online, august 2015). A clinically relevant antitumor activity of vemurafenib and dabrafenib was seen, in particular when dealing with a V600E mutation.

Patients with a non-V600E mutation had a shorter overall survival. Probably you already know that *BRAF* mutations can also be found in hairy-cell leukemia (100%) and in Langerhans cell histiocytosis (60%).

One of the most important issues in the Idylla story is that you get results very fast. And sometimes the determination of the *BRAF*status of a melanoma patient is really urgent…

**The "liquid biopsy" digital droplet PCR story**
Tumours shed tumoural DNA in the circulation, in particular by apoptosis. The problem is that we (hopefully normal persons) have also DNA circulating in our blood. Apoptosis is omnipresent in our body (consider for example dying, aged lymphocytes). You need very sensitive techniques to detect tumoural DNA in the circulation (depending of course also on the stage of the disease).

In our pathology lab, we introduced the "digital droplet PCR" system (BIORAD). This system divides a plasma sample in thousands of droplets. In each droplet, the necessary material is present for a PCR reaction. The machine will tell you how many droplets are empty and how many droplets contain DNA. Also, the test will tell you how many DNA molecules contain a mutation, for example EGFR. By partitioning (ie creating droplets) you have now a very sensitive tool to look for mutations in plasma. We use this technique when it is difficult to obtain tumour tissue, but also to monitor patients. For example, in NSCLC patients with activating EGFR mutations, resistance to TKI is due to a T790M mutation in at least half of the cases. Currently AstraZeneca is developing a new TKI, targeting this mutation (AZD9291). Although this drug is not routinely available yet, we are sure that it will be tomorrow, so to say. You can find all practical information on our website.

**HPV Xpert**
For the detection of High-risk HPV in cervical cytology samples, our lab switched to the Cepheid Xpert HPV assay. This is a new, qualitative, real-time PCR assay for the detection of hrHPV DNA in a single-use GeneXpert test cartridge. Xpert HPV specifically calls out types 16 and 18/45 in separate detection channels, with 11 other high-risk types detected in combined channels.

As the Idylla platform for *BRAF* analysis, this is a user-friendly system (no need for DNA extraction) that gives a result within 1 hour. As the samples are immediately processed one by one, there is no need to pool samples, with a reduced risk of contamination and a faster result as a consequence. Also, the Xpert HPV targets the E6/E7 region in contrast to the Roche Cobas HPV tests that targets the L1 region. Since integration of viral HPV will result in a

lost L1 expression, a L1 developed test looking for only L1 expression will miss this (pre)cancer, while the Xpert HPV E6/E7 test not.

**HPV detection in FFPE samples**

For the detection of High and Low risk HPV in FFPE samples, our lab has validated the Sacace HPV Real-TM assay. This kit is a Real-time amplification test for the detection and genotyping of 14 High-risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) and the two low-risk genotypes most commonly associated with genital warts (6 and 11), also in the E6/E7 region.

**Additional tests**

a) We are now performing FISH for PDGFB (Platelet-Devided Growth Factor B). In dermatofibrosarcoma protuberans (DFSP); there is a fusion between (part of) COL1A1 and PDGFB.
The strong promoter activity of COL1A1 results in an overexpression of PDGFB. Because of the many different exons that can be donated by COL1A, RT-PCR primer design is challenging and requires multiplexed amplification reactions. That is why we use a break-apart FISH probe. Interpretation can be a bit challenging, because the centromeric probes can be amplified, sometimes prominently in ring chromosomes. But this is our problem.
As we all know, making a diagnosis of DFSP is not always that easy, particularly when dealing with (small) biopsies, or with a fibrosarcomatous variant. In these situations, our PDGFB FISH test is very useful. Although DFSP is usually a surgical disease, tumours not amenable to resection can be treated with imatinib (Gleevec) and in this setting, demonstration of the fusion event is desirable. Imatinib blocks the kinase function of the PDGF receptor-B and thus inhibitor the function of the overproduced ligand, PDGFB. Don't mess up with the PDGFRA story: here we are dealing with a mutation (PCR test) in our beloved GIST's.

b) On the immunohistochemistry front, we also can perform PD1/PDL1 staining. Here we are dealing with immunomodulatory therapy.
As you know, cancer cells should be attacked by our immune system because they express antigens that are foreign to our body ("neo-antigens"). The more mutations in a tumour, the more aberrant proteins will be expressed. But cancer cells are clever. Every time our immune cells attack something foreign (for example a virus-infected cell), our immune cells produces also molecules that damper this reaction, in order to prevent collateral damage (or even the development of auto-immune diseases). These molecules block the immune effector function, so to say. Now cancer cells can produce the same molecules and so are preventive sabotaging an effective immune attack. One of the important players in the field is the PD1 receptor and its ligand (PDL1). We can block this axis (PD1-PDL1) with antibodies, such as nivolumab and pembrolizumab (anti-PD1 antibodies) so that our immune system can make a more successful attack. Following this, demonstrating the presence of PD1/PDL1 in tumours and/or tumour infiltrating lymphocytes should be a good biomarker for predicting response to this kind of therapy. Oncologists start to ask for this test on all kind of tumours, but a lot of questions are open. What particularly worries me, is that too many patients with a negative IHC still respond to this therapy. Probably, we don't have a very reliable biomarker yet.

**Belgian working group of molecular pathology**
It is with pleasure that we can announce that a Molecular Pathology Working Group has been created, thanks to the Belgian Society of Pathology (and in particular to the initiative of the president of the society, Prof. dr. Anne Mourin). Although not being present in the meeting, Prof. Pauwels was elected “chair”. This is a real honour to him, and he promised to do his best to help you in the exciting field of molecular pathology.

**Molecular pathology conferences**
We organize a symposium in Antwerp, the 3th of October. Probably you all received an invitation. If not, you can find a copy in attachment. The main topics are on next generation sequencing and on the liquid biopsy story. An update on molecular testing in lymphomas will also be presented.

I would also like to draw your attention on a “Human Cancer Immunology Course” on October 9, 2015 organised by the institute Jules Bordet. I am sure that we have to refresh our knowledge about immunology and cancer!

**New pathologist in our lab**
Since the 1st of May 2015 a new pathologist, Amélie Dendooven, has joined our team. After her medical studies in Ghent, sherecently graduated as a pathologist at UMC Utrecht, where she alsoobtained her PhD. Her areas of interest are nephropathology,hematopathology and molecular pathology. In addition, she will be co-responsible for the Next-Generation Sequencing at our department.