Mutational pathology in haematologic malignanies

As you know, molecular diagnostics is becoming more important in the haematopathology field. Developments here go fast, although only few tests really perpetrate into the routine of our daily practice. However, we would like to point out some recent additions to our portfolio:

MYD88 mutation analysis

Since a landmark New England paper from 2012, the L265P point mutation in the *MYD88* gene has been around as a great diagnostic test for lymphoplasmocytic lymphoma (LPL, formerly known as Waldenstroms disease). This LPL always was a difficult entity to confirm, since no specific immunhistochemic profile pointed to the diagnosis. With the *MYD88* alteration, we now finally have a sensitive and rather specific marker in place, distinguishing LPL from other small cell lymphomas. Be aware, some DLBCLs also have this alteration, but there the morphology is of course different. The *MYD88* alteration is not only a diagnostic but also a predictive marker, since the alteration needs to be in place for reimbursement of ibrutinib (a TKI inhibiting B-cell signaling) in this setting. In our lab, we offer *MYD88* mutation analysis by digital droplet PCR (ddPCR), to have an extremely sensitive assay in place for FFPE, blood and fresh bone marrow samples.

BCL6 FISH in DLBCL

Since 2016, we have a break-apart FISH detecting *BCL6*rearrangements. The *BCL6* rearrangement (often with an Ig gene partner) is associated with the non-GCB phenotype of DLBCL, and occurs in some follicular lymphomas and marginal zone lymphomas. Importantly, *BCL6* rearrangement is one of the translocations that can coexist with a MYC rearrangement and can constitute the ‘double-hit’ high-grade B-cell lymphoma- though most ‘double-hit’ lymphomas have BCL2 and MYC rearranged. In clinical practice, the threshold should be very low to test all DLBCLs (and this is one of the more common lymphomas) for these MYC, *BCL6*an BCL2 rearrangements, especially since trials are being established treating the double-hit subset of DLBCL differently.

MALT FISH in DLBCL

Additionally, we have added the FISH for the t(11;18)(q21;q21) to our portfolio. The translocation t(11;18) involves the *BIRC3* and *MALT1*genes. It is the most frequent translocation in gastric *MALT* (6-26%), intestinal *MALT* (12-56%) and pulmonary *MALT* (31-53%), but is rarely detected in other sites (such as ocular adnexa, salivary glands, skin or thyroid). Detecting

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the translocation can sometimes help in diagnosis and follow-up of *MALT*-lymphomas especially when too little tissue is available for B-cell clonality analysis (IgH rearrangement PCR).

Molecular Neuro-oncology

The primary molecular approach to classify gliomas in adults is to separate gliomas into *IDH*-wild type versus *IDH* mutant gliomas.

In patients aged > 54 years with a histologically classic glioblastoma, the probability that an alternative *IDH* mutation than the R132H-mutant *IDH1* variant that can be found be immunohistochemistry decreases to <1% except if it is a midline location or there is a history of a pre-existing lower-grade glioma (See *WHO-classification of tumours of the central nervous system*revised 4th ed. pag.28). Therefore, for such patients with a negative*IDH1* immunohistochemistry, our laboratory does not perform *IDH*mutation analysis, unless explicitly requested. In patients with glioblastoma aged ≤ 54 years, and without *IDH* R132H-mutant *IDH1*IHC, NGS is required to look at alternative *IDH1* or at *IDH*2 mutations.
Be aware that IDH1 R132H mutant IHC is not the easiest IHC staining; in case of doubt, NGS is advised.

Molecular pathology of lung cancer

Several studies showed benefit for patients with *BRAF* mutated lung cancer, treated with dabrafenib, preferentially in combination with the MEK inhibitor trametinib. Since this therapy is now available, it is mandatory to test also for *BRAF* mutations.

The *BRAF* mutation story is a complicated one. Only *BRAF* V600 mutions can be treated. Notice that it is not only V600E that matters (unlike in melanoma, where nearly all *BRAF* mutations are V600E). Half of the *BRAF* V600 mutations in NSCLC are not V600E mutations, but show also response to these inhibitors.

Molecular pathology of breast cancer

Almost all breast cancers that are initially hormone-dependent acquire anti-estrogen resistance after repeated endocrine therapies and eventually become hormone-independent.

The representative mutation is the *ESR1* ligand binding domain “hot spot” mutation that is confined to codons 537 and 538 in exon 8. Most of these mutations are acquired after long-term treatment with endocrine therapy (aromatase inhibition). Demonstrating an *ESR1*mutation can switch therapy to fulvestrant (or a fulvestrant containing regimen). In our lab, we offer *ESR1* mutation analysis by digital droplet PCR (ddPCR), to have an extremely sensitive assay in place for testing on FFPE and blood.

BAP1 and mesothelioma

Loss of BRCA-associated protein 1 (*BAP1*) protein expression permits differentiation of MPM from reactive mesothelial hyperplasia.*BAP1* is a tumor suppressor, meaning that it is loss of (nuclear) staining that is important. So, when tackling the problem reactive mesothelial hyperplasia versus mesothelioma, *BAP1* IHC is certainly very useful.

There are however some limitations: in epithelioid mesothelioma, loss of staining is seen in around 70% of cases (not 100% …) but this drops in sarcomatoid/biphasic mesothelioma to 30% or less.

When you see a malignant pleural tumor with loss of BAP staining, is it then mesothelioma? According to existing literature, this is nearly always the case. However, it is also important to know that clear cell renal cell carcinoma, intrahepatic cholangiocarcinoma and melanoma can harbor *BAP1* loss.

Targeting *NTRK*

NTRK gene rearrangements and mutations represent the molecular driver in a subset of solid tumors, including 3% of non-small-cell lung cancers. Also in colon cancers, brain tumors etc, these rearrangements have been demonstrated. One of the available drugs to target these rearrangements is Entrectinib (ongoing trialsavailable in Belgium). Entrectinib is an orally bioavailable multikinase inhibitor with activity against TRK receptors (coded by*NTRK* 1,2,3 gene) but also against ALK and ROS1 rearrangements.

We use a screening method with immunohistochemistry for TRK. Positivity has to be confirmed with NGS. We are sure *NTRK*mutations/rearrangements will become a new target in oncology.