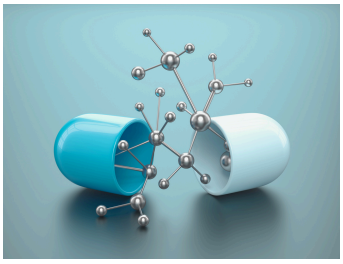

SCIENCE BREAKTHROUGH

MOLECULAR MEDICINE FOR THE GENERAL ONCOLOGIST

Through our BSMO newsletters and flashes, we aim to improve our knowledge of molecular biology and NGS by publishing a series of small articles that will update or improve your understanding of this very hot topic. We will put a specific focus on practical important aspects for daily oncological practice.
Like NGS, this effort is multidisciplinary and, involves several experts in the field : Dr Philippe Aftimos (medical oncologist at Institut Jules Bordet), Dr Brigitte Maes (pathologist at Jessa Ziekenhuis), Dr Vasiliki Siozopoulou (pathologist at Cliniques universitaires Saint-Luc), Dr Léon Van Kempen (pathologist at UZ Antwerp), coordinated by Dr Cédric Van Marcke (medical oncologist at Cliniques universitaires Saint-Luc).

Clinical interpretation of next generation sequencing data

As discussed in previous letters, the introduction of next generation sequencing allowed for the assessment of mutations in a growing number of genes at the same time. Whereas initially a handful of genes could be analysed for mutations in well-known hotspots, the transition towards ***profiling of the complete coding sequence of hundreds of genes simultaneously requires guidelines for the interpretation of the variants****, regarding both the* ***expected effect on the function of the protein and clinical actionability.***

Language matters

When a Parisian asks you 'Savez-vous où je peux garer ma voiture?' and a Quebecer asks 'Savez-vous où je peux parker mon char ?' they both ask you where to find a parking spot for their vehicle … albeit in slightly different French.

To avoid interpretation problems in molecular diagnostics, it is imperative that ***we speak the same language****. For each gene, multiple reference sequences are available, often referring to slightly* ***different alternative transcripts****, that may result in a different numbering of the variant at the DNA and protein level.*

When reporting gene variants, it is therefore important to know the reference sequence that was used to map the variant*. The Human Genome Gene Nomenclature Committee (HGNC, www.genenames.org) has recreated a gene database in which the reference that should be used for reporting variants are listed, i.e. MANE gene reference number.* ***Without reporting to which reference gene a variant was matched, one could misinterpret two identical mutations as distinct whereas they are actually the same****. For example, MET (NM_00245.4) c.2888A>T and MET(NM_001127500.3) c.2942A>T may appear to be two different mutations but are the same because two different NM gene reference numbers were used to describe the same classic MET exon 14 skipping mutation.*

Similarly, the description of variant must adhere to 'grammatical' rules *(https://hgvs-nomenclature.org/stable/) in which the position at the DNA sequence is indicated with c. and the deduced variant at the protein level as p.(..). For example, BRAF (NM_004333.6): c.1799T>A p.(Val600Glu), to be preferred over the one letter code p.(V600E), although the latter is better recognized as the classic BRAF activating mutation.*

Reading comprehension

Pathogenicity

When a variant is detected, one needs to ***understand the consequence on protein function and clinical relevance.***
Guidelines for assessment of pathogenicity of somatic variants have been established [1], which resulted in a ***5-tier classification****:*

5- pathogenic (P), 4 - likely pathogenic (LP),
3 - variant of unknown significance (VUS),
2 - likely benign (LB) and 1 - benign (B).

The assessment is based on a scoring algorithm in which, among others, known or anticipated effect on protein function, prevalence of the variant in the population and in cancers and clinical evidence are included.

Online knowledge databases [2], often publicly available, are precious resources for the assessment of pathogenicity*. These include OncoKB (https://www.oncokb.org/) , CKB-JAX (https://ckb.jax.org/) and Genome Nexus (https://www.genomenexus.org/).*

Pathogenic and likely pathogenic variants must be reported, whereas likely benign and benign variants should not *(Figure 1). Variants of unknown significance should only be reported and clearly indicated as VUS, if some arguments point towards pathogenic potential, but no data is registered in curated databases.* ***In the vast majority of cases, VUSs should not be reported, to prevent misinterpretation of their clinical relevance.***

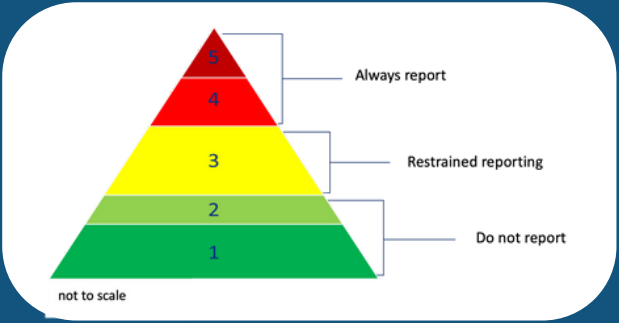

Figure 1: Variant classification and reporting.

5 - pathogenic
4 - likely pathogenic
3 - variant of unknown significance
2 - likely benign
1 – benign

Clinical actionability

(Likely) pathogenic is not synonymous with actionable. *A few algorithms have been developed by AMP/CAP/ASCO/ACMG [3], ESMO [4] and NCT [5] to determine actionability. There is large overlap in these structured methodologies to rank* ***evidence-levels for biomarker-drug response associations****. This is based on the assessment of effective drug (combinations) in this clinical situation or in a different disease, targeting the impacted protein or the biological pathway. Available preclinical data can give rise to new study hypotheses.*

When a reported variant is not a validated biomarker for first or later lines of registered (targeted) therapy, the variant(s) should be discussed in a multidisciplinary team meeting in which at least oncologists, pathologists and clinical molecular biologists participate to weigh the current evidence of actionability in the disease-specific context. *This prevents overinterpretation of variants, especially when variants of unknown significance are reported. Furthermore, these teams have developed a deep understanding of molecular pathways to discriminate actionable pathogenic drivers from passenger mutations that may not be critical for tumor growth, as well as identifying possible innate or acquired resistance mechanism.*

Importantly, a multidisciplinary discussion helps with the rapid identification of clinical studies that the patient may be eligible for. *The website www.cancertrials.be was established by the Belgian Society of Medical Oncology (BSMO) with participation of the Belgian Group of Digestive Oncology (BGDO) to support rapid identification of biomarker-driven studies.*

Reading a molecular pathology report

A molecular pathology report should contain all details of the analyses (how was the test performed, what are the limitations of the test, which genes were analysed) or link to a website where this critical information can be found readily. A full description of the (likely) pathogenic variants that were detected should be presented as part of the molecular results [6]. It is highly recommended to report on all the genes clinically relevant for the indication, also when they are not mutated, e.g. that no KRAS, NRAS or BRAF mutations were detected in a colorectal cancer samples in the context of anti-EGFR therapy.

However, this may often be not legible for non-experts. As such, the conclusion of the molecular analysis must be an unambiguous and legible summary of the results. It is recommended to include a clinical interpretation of the results in general term to support the oncologist with the translation towards a treatment plan, including possible eligibility for a clinical study, and to stimulate the discussion of the result in a multidisciplinary (molecular) tumor board.

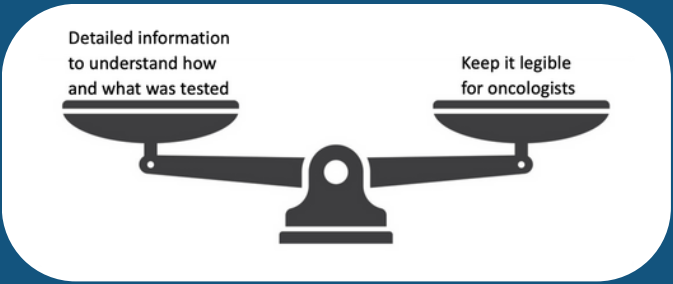

Figure 2: Seeking a balance between detailed information in a molecular pathology report and presentation of the clinically relevant result to the oncologist.

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