Do it once, but do it right

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Do it once, but do it right
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Despite the widespread implementation of molecular tumour profiling for diagnostics, classification and finally treatment selection, there is still a debate as to whether tumour-normal sequencing is preferred or deemed unnecessarily costly compared to sequencing of only tumour material. In this issue, Newman et al., contributed a nice addition to this discussion and gave a clear and unequivocal answer, at least for children and young adults with haematological cancers.

Through retrospective analysis of nearly 1200 cases (0-35 years of age) with B-/- T-ALL, MDS/JMML or AML who underwent tumour-only (n=1034) or tumour-normal (n=156) gene panel testing, the authors confirmed 16 cases who had a proven cancer-predisposition syndrome (CPS). Ten cases were revealed from the much larger group of tumour-only panel sequencing and six cases came from the tumour-normal-sequencing group. The first matter that stands out is the high drop-out rate in the tumour-only group. Even if an underlying germline predisposition was suspected in 111 cases, only 29 of them underwent confirmatory gene testing. This low number might not come as a surprise for those involved in the daily clinical management of patients. Once tumour chemotherapy has started, discussions with children and parents about therapy response, prognosis as well as acute and long-term side effects occupy much of the attention of both doctors and family. Genetic germline testing might pose an additional psychological burden, leading to perpetual postponement. Another remarkable result of the study is the fact that all ten cases identified and confirmed in the tumour-only group would have had a clear indication for germline testing even without the suspicious tumour-only sequencing results. Specifically, a low-hypodiploid leukaemia karyotype in a child with B-ALL points towards Li-Fraumeni Syndrome and requires testing of the p53 gene. Furthermore, a child with overgrowth syndrome who was cured from neuroblastoma but presented thereafter with a secondary malignancy (T-lymphoblastic lymphoma) and all children with JMML or MDS would anyway require either panel or whole exome analysis. It is not surprising to clinicians that germline testing found an underlying CPS in all of those ten cases. In contrast, the six cases with CPS who had been detected in the much smaller tumour-normal sequencing group may have escaped their attention when only a phenotype-driven approach is applied. There are some subtle clinical and laboratory findings that may be indicative for a pathogenic germline ETV6, IKZF1 or RUNX1 variant but these are far less obvious or very unspecific 2 (see Table 1 from Newman et al., for details).

Finally, there are several inherent bioinformatic challenges when the results of tumour-only sequencing lead to the assumption of an underlying CPS. The main challenge bioinformaticians face is the ability of finding innovative strategies for accurate differentiation between germline and somatic hits. One approach is to use the expected variant allele frequency (VAF) of germline mutations, which typically falls between 40% and 60% or equals 100%, to differentiate them from somatic mutations. However, copy number variants (CNVs) such as the loss of the wild-type allele in the tumour sample or mutation amplification, complicate this differentiation process. Germline variants may become undetectable or could be mistakenly classified as somatic. 3-5 Another approach used by bioinformaticians involves characterizing and filtering germline variants using population databases. Yet, the problem here is that these databases do not reflect the genetic diversity of the population, particularly for under-represented ethnicities. This can lead to misclassification of variants and miscalculation of the overall tumour mutational burden (TMB). 6, 7 Taken together, the lack of matched
normal tissue complicates variant filtering. This can lead to inaccuracies and increase the rate of false negatives and positives.

All in all, these considerations raise the intriguing question of how many CPS were potentially overlooked in the tumour-only sequencing group. Furthermore, the identification of a cancer predisposing germline variant influences clinical care and long-term follow-up in what is termed as “personalized cancer surveillance and prevention”. The overall risk for the development of subsequent malignancies in long-term survivors of childhood cancer who are carriers of a cancer predisposing germline variant is at least three to four times higher compared to their non-carriers. 8

The other critical observation in this study comes from the fact that within the immediate family members of those 16 patients with a proven CPS, no early-onset cancer was diagnosed. Thus, the child with the haematological cancer became a red flag for the family. Out of the 33 that went for subsequent panel testing, a CPS for 12 family members was revealed. Although this obviously came as an unwelcome surprise, it also offers future strategies for surveillance and removes anxiety from all other family members. Of note, the de-novo mutation rate for many CPS genes is still unknown and family-based sequencing is required to fill this knowledge gap. 9

In an ideal world, both pre- and post-test genetic counselling should be offered not only to individuals whose germline DNA were molecularly profiled but also to those with tumour-only sequencing. However, in certain regions and communities the existing hurdles in accessing genetic counselling services need to be addressed. This is particularly the case for countries where the integration of genetic counselling into the healthcare systems remains a challenge.

In summary, even if cancers that arise in carriers of pathogenic germline alleles may not directly depend on or may even be unrelated to the specific germline variant, 10 the arguments for continuing with tumour-only sequencing are poor especially in light of recent cost-effective sequencing options.

References

Figure Legend

**Figure 1:** Comparison of tumour-only and paired tumour-normal sequencing for cancer-predisposition syndromes (CPS). Whereas paired tumour-normal sequencing enables a clear distinction of germline and somatic variants, tumour-only gene sequencing causes additional bioinformatic challenges.
Distinction between germline vs. somatic variants

**Tumour-only sequencing (n = 1034)**

- Tumour
- ACTC\textcolor{red}{ATS\textcolor{red}{G}}\textcolor{red}{TTATG} *Germline mutations* *Somatic mutations*

**Paired tumour-normal sequencing (n = 156)**

- Blood / fibroblasts
- ACTC\textcolor{red}{ATS\textcolor{red}{G}}\textcolor{red}{GCTATG} *Germline mutations* *Somatic mutations*

- Tumour
- ACTC\textcolor{red}{ATS\textcolor{red}{G}}\textcolor{red}{TTATG} *Germline mutations* *Somatic mutations*

**Genetic germline testing**

- Tumour-only sequencing: n = 10 CPS
- Paired tumour-normal sequencing: n = 6 CPS