EDITORIAL

Do it once, but do it right

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Despite the widespread implementation of molecular tumor profiling for diagnostics, classification and, finally, treatment selection, there is still a debate as to whether tumor-normal sequencing is preferred or deemed unnecessarily costly compared to sequencing of only tumor material. In this issue of Haematologica, Newman *et al.*[1] have contributed a nice addition to this discussion, and gave a clear and unequivocal answer, at least for children and young adults with hematologic cancers.

Through a retrospective analysis of nearly 1,200 cases (0-35 years of age) with B-/T-cell acute lymphoblastic lymphoma (ALL), myelodysplastic syndromes (MDS) / juvenile myelomonocytic leukemia (JMML) or acute myeloid leukemia (AML) who underwent tumor-only (N=1,034) or tumor-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 tumor-only panel sequencing and 6 cases came from the tumor-normal sequencing group (Figure 1). The first thing that stands out is the high drop-out rate in the tumor-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 tumor chemotherapy has started, discussions with children and parents about therapy response and prognosis, as well as acute and long-term side effects occupy much of the attention of both doctors and the 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 10 cases identified and confirmed in the tumor-only group would have had a clear indication for germline testing even without the suspicious tumor-only sequencing results. Specifically, a low-hypodiploid leukemia karyotype in a child with B-ALL points towards Li-Fraumeni Syndrome and requires testing of the *TP53* 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 require either panel or whole exome analysis anyway. It is not surprising to clinicians that germline testing found an underlying CPS in all of those 10 cases. In contrast, the 6 cases with CPS who had been detected in the much smaller tumor-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 tumor-only sequencing lead to the assumption of an underlying CPS. The main challenge bioinformaticians face is the ability to find 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 (CNV), such as the loss of the wild-type allele in the tumor 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 tumor mutational burden (TMB).[6,7] Taken together, the lack of matched normal tissue complicates variant filtering. This can lead to inaccuracies, and increases 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 tumor-only sequencing group. Furthermore, the identification of a cancer predisposing germline variant influences clinical

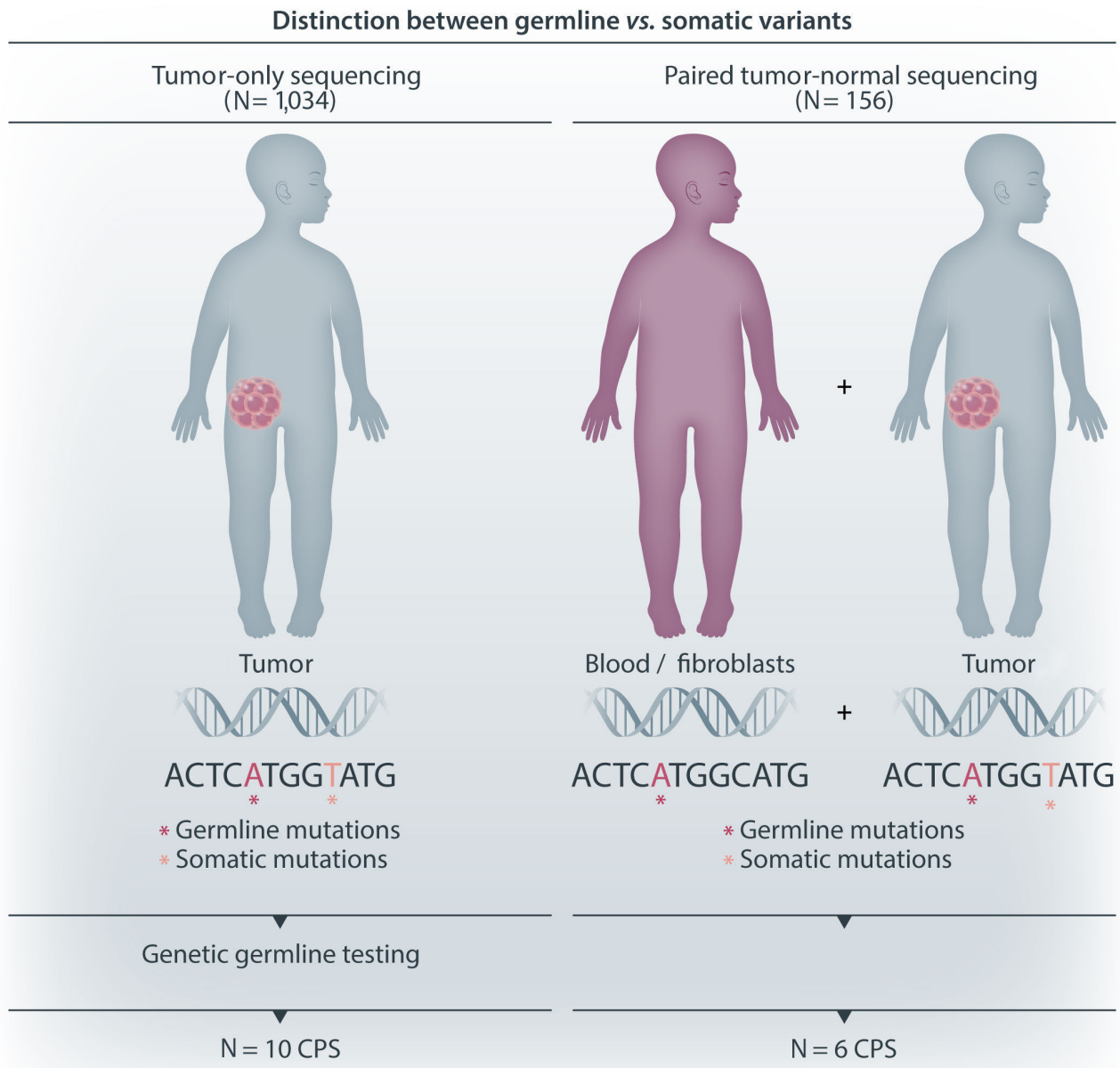

Figure 1. Comparison of tumor-only and paired tumor-normal sequencing for cancer-predisposition syndromes. Whereas paired tumor-normal sequencing enables a clear distinction of germline and somatic variants, tumor-only gene sequencing causes additional bioinformatic challenges. CPS: cancer-predisposition syndrome.

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 3 to 4 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 hematologic 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 counseling should be offered not only to individuals whose germline DNA were molecularly profiled, but also to those with tumor-only sequencing. However, in certain regions and communities, the existing hurdles in accessing genetic counseling services need to be addressed. This is particularly the case for countries where the integration of genetic counseling 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 tumor-only sequencing are poor, especially in the light of recent cost-effective sequencing options.

Disclosures

No conflicts of interest to disclosure.

Contributions

Both authors wrote the editorial.

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