Right at your fingertips!

Kathy Chun

Department of Paediatric Laboratory Medicine, The Hospital for Sick Children and Department of Laboratory Medicine and Pathobiology, The University of Toronto, Toronto, Canada

Correspondence: K. Chun
kathy.chun@sickkids.ca

Received: April 26, 2024.
Accepted: May 3, 2024.
Early view: May 16, 2024.

https://doi.org/10.3324/haematol.2024.285371

Records of the detection and management of cancer date as far back as 3000 BC.[1] It wasn't for another ~5,000 years that a link of cancer to a genetic etiology was established with the discovery of the Philadelphia chromosome in 1960.[2,3] The pace of discovery has been astronomical since 1960, and advances in sequencing technologies have played a critical role in significantly improving the diagnosis and management of cancer patients (Figure 1). Although sequencing of tumor and germline DNA has traditionally been performed separately, paired tumor:normal analysis by next-generation sequencing is emerging as a strategy that is faster and more efficient in distinguishing germline variants from acquired somatic changes in the tumor, thus enabling more accurate analysis of the variants.[4-7] The source of germline DNA is most often peripheral blood, as it is easily obtainable; however, it is not suitable in the analysis of hematologic malignancies, as the blood may be contaminated by tumor cells, which could result in inaccurate distinction between germline and tumor variants. Other sources of germline DNA, such as buccal swabs, saliva and direct skin biopsies, can also be contaminated by tumor cells. Furthermore, the time required to culture skin fibroblasts to eliminate possible blood tumor contamination leads to significant delays in receiving the results, possibly impacting patient management. Hence, an alternative source of germline DNA is required in the analysis of hematologic cancers.

In this issue of *Haematologica*, Krystel-Whittemore *et al.*[8] report on one institution's extensive experience in using cell-free DNA (cfDNA) obtained from nail clippings as a source of normal control DNA for paired tumor:normal genomic analysis of hematologic malignancies. In addition, they describe a rapid protocol for extraction of cfDNA from nail clippings, and discuss the benefits and disadvantages, as well as highlight some interesting findings, using cfDNA obtained from nail.

In their study, 2,610 diagnostic tumor samples (blood, bone marrow, tumor biopsies), with nail as the normal control, underwent molecular profiling using MSK-IMPACT, a custom 400-gene next-generation sequencing panel used for the detection of somatic mutations and copy number changes.[9] Analysis of these tumor:normal pairs yielded 10,942 somatic variants, of which 4,640 were in myeloid and 6,302 in lymphoid disease categories. Mutations in the nail were detected in 13.9% of the patients tested. Interestingly, the majority of these mutations were found in patients with myeloid neoplasms (20.5%) as compared to lymphoid neoplasms (5.4%).

While the average variant allele frequency (VAF) of the tumor variants was 26.7%, the average VAF of the nail variants was significantly less at 4.4%. However, in 19 patients (0.7%), the nail VAF was surprisingly close to and even slightly higher than the corresponding tumor VAF, suggesting a high tumor contamination in the nail. Why would this be? The authors attribute three of these cases to gaps in collection. In other words, these cases correlated with nails collected at the time of greatest disease burden, but tumor collected after interim therapy, a time of very low disease burden. Despite this high level of contamination, the authors were readily able to distinguish germline from somatic variants in all cases, except in one unique case.

Mutations in nails were significantly biased towards genes frequently altered in myeloproliferative neoplasms and myelodysplastic syndrome. While the VAF observed in nail were generally <5%, those observed at VAF >5% (92 patients) were associated with the presence of marked bone marrow fibrosis and osteosclerosis (33%) and myeloid neoplasms with monocytic features (13%). Loss of heterozygosity was observed for those mutations with the highest VAF. In stark contrast, among patients with lymphoid and plasma cell neoplasms, mutations in genes recurrently associated with these neoplasms were absent in nail. Furthermore, in a select group, generally with nail mutation VAF >3%, only mutations of myeloid origin were identified in the nail. Interestingly, these patients were determined to have an emerging or coexisting clonal myeloid process.

Krystel-Whittemore *et al.*[8] looked at a subset of 50 patients with nails collected after allogeneic stem cell transplantation. Donor DNA could be identified in 22% of the nails.

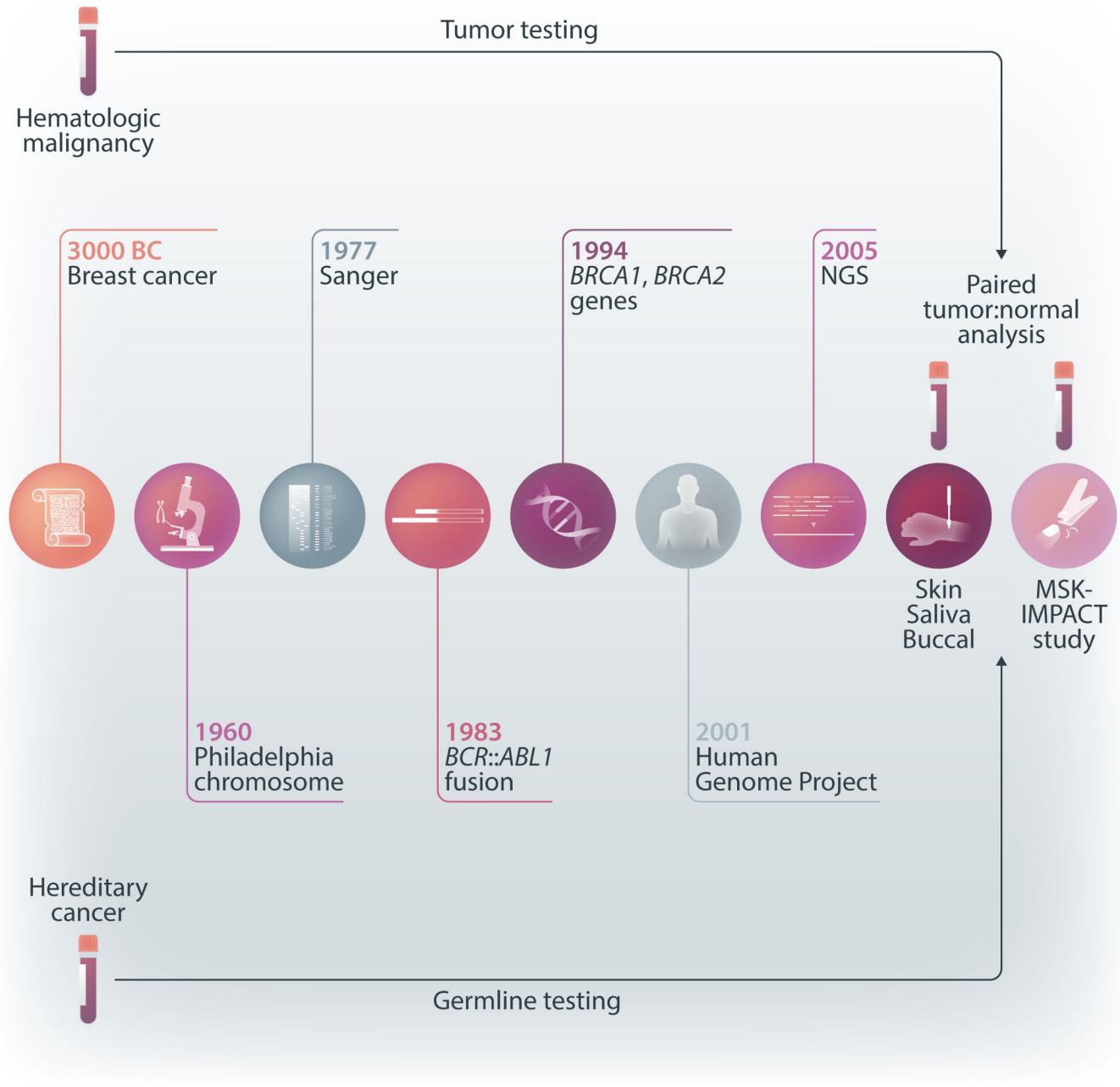

Figure 1. Evolution of cancer diagnostics. The earliest written record of human cancer, a breast cancer, was documented in ~3000 BC.[1] Chronic myelogenous leukemia was the first cancer linked to a genetic etiology upon discovery of the Philadelphia chromosome in 1960.[2,3] The exact molecular mechanism of the Philadelphia chromosome rearrangement, fusion of the *BCR* gene with the *ABL1* gene, was not elucidated until 1980,[13] after Sanger had developed a reliable method of sequencing DNA.[14] Advances in molecular technologies led to the discovery of the first hereditary cancer genes in 1994,[15,16] followed by the initial completion of the Human Genome Project in 2001.[17] While traditionally tumor and germline DNA have been sequenced separately, the development of next-generation sequencing,[18] which allowed for the massively parallel sequencing of large amounts of DNA, revolutionized cancer diagnostics such that tumor and germline DNA could be paired and sequenced/analyzed together.[4-9] The study by Krystel-Whittemore *et al.*[8] confirms that cell-free DNA extracted from nail clippings is a robust and reliable source of germline DNA in the genomic analysis of hematologic malignancies. NGS: next-generation sequencing; MSK-IMPACT®: Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets.

The authors determined that a history of graft-*versus*-host-disease was significantly more frequent in patients with donor DNA in their nails (63.6%) than in those without donor DNA in their nails (15.4%). They suggest graft-*versus*-host-disease as a potential cause of donor cells in the nail, among other causes that have been documented in the literature. To use nail as a normal control, it will be important to determine the presence and level of chimerism in the nail. In the presence of minimal or no donor contamination, the authors found that sequencing both the nail and donor DNA was extremely useful in the analysis of difficult post-transplant samples.

In order to be able to use nail routinely as a source of normal DNA in the clinical setting, it must be possible to extract high quality DNA efficiently from nail. Using a commercial kit, Krystel-Whittemore *et al.*[8] compared two different nail fragmentation methods on 20 validation samples. The first used cut-up nail fragments and overnight (sometimes several nights) proteinase K digestion. With the second method, nail clippings were pulverized using a tissue homogenizer. The authors found that method 2 had a significantly shorter procedure time with improved results. Specifically, method 2 produced larger fragment sizes, a higher yield of DNA and greater targeted coverage

as compared to method 1. Furthermore, nail cfDNA was found to be high-performing for hybrid-capture-based next-generation sequencing assays, as well as for short amplicon polymerase chain reaction-based assays, thus confirming the clinical utility of cfDNA extracted from nail. In addition to using nail cfDNA in paired tumor:normal genomic analyses, it can also be used in identifying hereditary predisposition in individuals with hematologic malignancies, as illustrated by Ceyhan-Birsoy *et al.*[10] in an accompanying paper in this issue of *Haematologica*. Using nail cfDNA for germline testing of patients with hematologic malignancies, they found that only 0.08% of patients tested had somatic contamination in nail at levels that could potentially make unequivocal distinction of germline and somatic variants difficult in the absence of a matched tumor sample. One of these patients had a myeloproliferative neoplasm and fibrotic bone marrow while the second had myelodysplastic syndrome, reminiscent of the results obtained by Krystel-Whittemore *et al.*[8] Hence, Ceyhan-Birsoy *et al.*[10] show that nail cfDNA is a reliable source of germline DNA for the testing of patients with hematologic malignancies. The impressive work by Krystel-Whittemore *et al.*,[8] supported by the study of Ceyhan-Birsoy *et al.*,[10] demonstrates that nail cfDNA is a robust source of germline DNA in the genomic analysis of patients with hematologic disorders. These results would have a particularly significant impact in the pediatric population, in whom hematologic neoplasms are the most commonly occurring cancer.[11,12] Parents would be less hesitant to provide nail clippings as the source of germline control, whereas an invasive skin biopsy procedure would add considerable anxiety to an already devastating and stressful situation. Furthermore, since acute lymphoblastic leukemia is the most common cancer in children,[12] one would not expect lymphoid tumor contamination in the nail cfDNA of these patients if the findings of this current study can be extrapolated to the pediatric population, making nail cfDNA preferable. In summary, Krystel-Whittemore *et al.*[8] show that there can be tumor contamination in nail cfDNA, notably in patients with myeloid neoplasms with marked fibrosis and in patients following allogeneic stem-cell transplantation, but that this problem is readily resolved. Tumor contamination in nail could also provide important diagnostic information for the patient. Despite some potential confounding factors, nail cfDNA should be considered a reliable and robust source of germline DNA for patients with hematologic malignancies.

Disclosures

No conflicts of interest to disclose.

References

1. Hajdu SI. A note from history: landmarks in history of cancer, part 1. Cancer. 2011;117(5):1097-1102.
2. Nowell PC, Hungerford DA. A minute chromosome in human chronic granulocytic leukemia. Science. 1960;132:1497.
3. Nowell PC, Hungerford DA. Chromosome studies on normal and leukemic human leukocytes. J Natl Cancer Inst. 1960;25:85-109.
4. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. Nat Med. 2017;23(6):703-713.
5. Priestley P, Baber J, Lolkema MP, et al. Pan-cancer whole-genome analyses of metastatic solid tumours. Nature. 2019;575(7781):210-216.
6. Villani A, Davidson S, Kanwar N, et al. The clinical utility of integrative genomics in childhood cancer extends beyond targetable mutations. Nat Cancer. 2023;4(2):203-221.
7. Newman H, Clark ME, Wong D, et al. Genomic profiling of pediatric hematologic malignancies and diagnosis of cancer predisposition syndromes: tumor-only versus paired tumor-normal sequencing. Haematologica. 2024; Feb 22. doi: 10.3324/haematol.2023.284855 [Epub ahead of print].
8. Krystel-Whittemore M, Petrova-Drus K, Ptashkin RN, et al. Cell-free DNA from nail clippings as source of normal control for genomic studies in hematologic malignancies. Haematologica. 2024;109(10):3269-3281.
9. Ptashkin RN, Ewalt MD, Jayakumaran G, et al. Enhanced clinical assessment of hematologic malignancies through routine paired tumor and normal sequencing. Nature Commun. 2023;14(1):6895.
10. Ceyhan-Birsoy O, Fiala E, Rana S, et al. Universal germline genetic testing in patients with hematologic malignancies using DNA isolated from nail clippings. Haematologica. 2024;109(10):3383-3390.
11. Steliarova-Foucher E, Colombet M, Ries LAG, et al. International incidence of childhood cancer, 2001-10: a population-based registry study. Lancet Oncol. 2017;18(6):719-731.
12. Linabery AM, Ross JA. Trends in childhood cancer incidence in the U.S. (1992-2004). Cancer. 2008;112(2):416-432.
13. Heisterkamp N, Stephenson JR, Groffen J, et al. Localization of the c-abl oncogene adjacent to a translocation break point in chronic myelocytic leukaemia. Nature. 1983;306(5940):239-242.
14. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA. 1977;74(12):5463-5467.
15. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science. 1994;266(5182):66-71.
16. Wooster R, Neuhausen SL, Mangion J, et al. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science. 1994;265(5181):2088-2090.
17. Lander ES, Linton LM, Birren B, et al. Initial sequencing and analysis of the human genome. Nature. 2001;409(6822):860-921.
18. Margulies M, Egholm M, Altman WE, et al. Genome sequencing in microfabricated high-density picolitre reactors. Nature. 2005;437(7057):376-380.