Commentary on Sandberg et al., "The *In Vivo* Chromosome Constitution of Marrow"

Bayard D. Clarkson

See related article by Sandberg et al., Cancer Res 1961;21:678-89.

German pathologists in the late nineteenth century appear to have been the first to publish observations of mitotic abnormalities in many types of human cancers and to suggest that they might have an important role in their pathogenesis (1). About two decades later, Theodore Boveri published his famous monograph for which he received the Nobel Prize (2). On the basis of his meticulous studies of mitotic abnormalities in sea urchins, Boveri hypothesized that mammalian tumors might be initiated by mitotic abnormalities, resulting in changes in the numbers of chromosomes in cells, called aneuploidy, that tumors might originate in a single cell, and that there might be causative submicroscopic genetic alterations not involving entire chromosomes. However, proof of these clairvoyant hypotheses had to wait several more decades for technological improvements in the resolution of chromosome analysis, including determining that the correct number of human chromosomes is 46 rather than 48 as had been proposed and widely accepted earlier (3, 4). The first human tumor clearly shown to have a consistent chromosomal clonal abnormality, a minute chromosome, was chronic myelogenous leukemia (CML; refs. 5–7). This discovery was first presented at an AACR meeting in 1961 in Atlantic City if I recall correctly, but the next year several other cytogeneticists reported that they could not find the minute chromosome in their CML patients. It was soon found that their negative results were probably largely because they had been using phytohemagglutinin (PHA) to stimulate mitoses; because PHA mainly stimulates lymphocytes that do not usually contain the minute chromosome, they failed to see it. Subsequently, many other cytogenetic experts confirmed that the minute chromosome is a highly consistent specific cytogenetic abnormality in CML. Tough and colleagues (8) suggested that the minute chromosome should be designated the "Philadelphia Chromosome" in accord with the common convention of naming an abnormal chromosome for the city in which it was discovered.

The cytogeneticists at Roswell Park led by Sandberg and Hauschka were among the foremost cytogeneticists in the world in the mid 1900s. In their 1961 article (9), they surveyed chromosome patterns in 34 patients with different types of leukemia and 60 nonleukemic "control" bone marrows, consisting of 10 normal and 50 individuals with various endocrinopathies or congenital anomalies, but not involving changes in the somatic diploid karyotype. Two of the leukemic patients were mongols, one with acute lymphoblastic leukemia (ALL) and the other with acute myelogenous leukemia (AML). The approximate 4-fold increased incidence of leukemia in mongols had been published earlier in 1956, and they speculated that the characteristic "trisomy G22 mutation" is probably a predisposing factor for development of leukemia. They also compared their findings with all the published cytogenetic reports then available. Overall, they found a higher incidence of aneuploidy (56.4%) in the leukemic marrow cells (56.4) than in the control (12.2%) marrows, with the highest incidence (77%) in ALL. Two of the treated aneuploid ALL patients who had marrow smears during hematologic remission had "almost normal" diploid chromosome counts, probably one of the first observations of cytogenetic remissions. They also observed in nine leukemic marrow samples processed both immediately after aspiration and after 10 hours of incubation that the percentage of diploid metaphases increased from 59% to 84%, suggesting that the presumably normal diploid cells resumed dividing before the "less adaptable" aneuploid, presumably leukemic cells. Almost nothing was known in 1960 about differences in the cytokinetic behavior of normal and leukemic cells, but their prescient observation that normal cells might enter mitosis faster than leukemic cells foreshadows later more extensive and detailed cytokinetic studies showing that acute leukemic stem/progenitor (S/P) cells almost always proliferate considerably slower than normal hematopoietic S/P cells (10).

In confirmation of other reports, 9 of 10 patients with AML studied at Roswell Park had "more or less pronounced normal modes at 46 chromosomes." At the time, there was a fair amount of controversy about whether chromosome changes had any role in initiation of tumors, although most observers acknowledged that changes might contribute to tumor progression. However, the Roswell Park scientists presciently noted that it was difficult to define where "initiation ends and progression begins." They also found no consistent karyotypic pattern in the different types of leukemia, treated or untreated or in remission or relapse, one of the earliest observations about the heterogeneity of human leukemias and other cancers. Interestingly, in the eight CML patients studied at Roswell Park, three were apparently in blastic phase and had aneuploid model karyotypes, whereas five apparently chronic phase CML patients had 46 chromosomes. Surprisingly, there is no mention of their observing the minute chromosome in the CML marrows that Nowell and Hungerford had consistently found in their seven CML patients and that was referenced in the Roswell Park article. This omission is probably because the authors were primarily focused on studying aneuploidy rather than paying attention to Nowell's key finding, a common fault if the sole focus is overly concentrated on a single parameter. (In a follow-up article in 1966, Sandberg did acknowledge the Ph1 chromosome's consistency and importance in CML; ref. 11).

Molecular Pharmacology and Chemical Biology Program, Sloan Kettering Institute, Memorial Sloan Kettering Cancer Center, New York, New York.

Corresponding Author: Bayard Clarkson, Memorial Sloan Kettering Cancer Center, 415 East 68th Street, 1275 York Ave, New York, NY 10065. Phone: 646-888-2080; Fax: 646-422-0247; E-mail: clarksob@mskcc.org

doi: 10.1158/0008-5472.CAN-16-0153

Consistent with Boveri's concept (2), they concluded that "the existence of leukemias without any recognizable departure from the diploid somatic constitution of the species clearly eliminates gross karyotypic changes as a necessary precondition of neoplasia," another observation that has proved to be correct.

These careful observations and conclusions reported in 1961 of the largest study and survey of leukemia cytogenetics then conducted of course may seem rather naïve and unsubstantial in today's light, but the experiments were performed with the best technology and procedures then available, and the authors could not possibly have foreseen all of the technological and conceptual advances that were going to take place in the evolution of cytogenetics and other discoveries during the next half century (12). It was another 13 to 24 years before Janet Rowley demonstrated that the Ph chromosome resulted from a translocation between chromosomes 9 and 22 (13, 14), and a few more years before the fusion genes were identified as the v-abl Abelson murine leukemia viral ongogene homolog (ABL) on chromosome 9 and the breakpoint cluster region (BCR) on chromosome 22 (15). The product of the BCR–ABL fusion gene was not shown to be a constitutively active tyrosine kinase (TK) responsible for the sustained hyperproliferation of Ph+ S/P cells until 1990 (16), and it then took another 10 years before the structural mechanism responsible for the specificity of imatinib's and other TK inhibitors' (TKI) inhibition of the tyrosine kinase domain of c-abl was demonstrated (17, 18). The clinical efficacy of imatinib in CML was first reported about the same time (19, 20). Whereas imatinib and subsequently even more potent inhibitors of BCR/ABL have greatly improved survival in the chronic phase CML and delayed or prevented blastic transformation, these TKIs are usually not curative and have only limited usefulness in blastic phase CML. An even more dramatic example of the importance of identifying specific chromosomal rearrangements was Rowley's discovery of the t(15;17) translocation in acute promyelocytic leukemia in 1977, which eventually led to discovery of the driving PML/RARα transcript and fusion protein and the near universally curative therapy with all trans-retinoic acid and arsenic trioxide, two simple, inexpensive, and unpatentable compounds (reviewed in Puccetti and Ruthardt, 2004; ref. 21).

Almost surely none of these or other similar major discoveries and remarkable therapeutic triumphs would have come about without the pioneering discoveries and conceptual advances of earlier visionary cytogeneticists who were convinced of the importance of their work, constantly trying to improve their experimental methods, and modifying their hypotheses and procedures in accordance with new experimental data. All multicellular organisms, including *Homo Sapiens*, have evolved over hundreds of millions of years and are so infinitely complex that highly consequential new biologic discoveries will ordinarily only take place very gradually in a stepwise fashion. The new discoveries are usually dependent largely on important new technological advances, meticulous experimental work, and often large doses of serendipity and luck, which, in turn, may eventually lead to inspired new and more correct concepts. So not only do the primary paramount visionaries in any given scientific realm such as Gregor Mendel and Theodore Boveri deserve to be universally lauded and revered, but the more common highly skilled pioneering workaday scientists such as the Roswell Park cytogeneticists also deserve considerable credit and recognition for their careful work in providing new technological discoveries and solid experimental evidence to confirm correct hypotheses, discard defective ones, and sometimes develop new concepts that are more accurately in accord with the ever-expanding experimental data.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The author thanks Ms. Lucinda Lewis for her help with typing the manuscript.

Grant Support

This work at MSKCC was supported in part by The Enid A Haupt Charitable Trust, The WestRock Foundation, and The E./S. Sindina Lymphoma Research Fund to B.D. Clarkson.

Received January 15, 2016; accepted January 15, 2016; published online March 1, 2016.

References

1. Von Hansemann C. Ueber asymmetrische Zelltheilung in Epithelhresbsen und deren biologische bedeutung. Virchows Arch A Pathol Anat 1890;119:299–326.
2. Boveri T. Zur Frage der Entstehung maligner Tumoren. Gustav Fisher: Jena, Germany; 1914. p. 64.
3. Tjio J, Levan A. The chromosome number of man. Hereditas 1956;42:1–6.
4. Ford C, Hamerton J. The chromosomes of man. Nature 1956;178:1020–3.
5. Nowell P, Hungerford D. A minute chromosome in human chronic granulocytic leukemia (Abstract). Science 1960;132:1497.
6. Nowell PC, Hungerford DA. Chromosome studies in human leukemia II. Chronic granulocytic leukemia. J Natl Cancer Inst 1961;27:1013.
7. Nowell P. Discovery of the Philadelphia chromosome: a personal perspective. J Clin Invest 2007;117:2033–5.
8. Tough I, Court Brown WM, Buckton KE. Cytogenetic studies in chronic leukemia and acute leukemia associated with mongolism. Lancet 1961;1:411.
9. Sandberg AA, Ishihara T, Miwa T, Hauschka TS. The *in vivo* chromosome constitution of marrow from 34 human leukemias and 60 nonleukemic controls. Cancer Res 1961;21:678–89.
10. Clarkson B, Strife A, Wisniewski D, Lambek CL, Liu C. Chronic myelogenous leukemia as a paradigm of early cancer and possibly curative strategies. Leukemia 2003;17:1211–62.
11. Sandberg A. The chromosomes and causation of human cancer and leukemia. Cancer Res 1966;269:2064–81.
12. Ferguson-Smith M. History and evolution of cytogenetics. Mol Cytogenet 2015;8:19.
13. Rowley JD. A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine, fluorescence and Giemsa staining. Nature 1973;243:290–3.
14. Rowley J. Ph1-positive leukaemia, including chronic myelogenous leukaemia. Clin Haematol 1980;9:55–86.
15. Groffen J, Stephenson JR, Heisterkamp N, de Klein A, Bartram CR, Grosveld G. Philadelphia chromosomal breakpoints are clustered within a limited region, bcr, on chromosome 22. Cell 1984;36:93–9.

16. Lugo T, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. Science 1990;247: 1079–82.
17. Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, Kuriyan J. Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. Science 2000;289:1938–42.
18. Nagar B, Bornmann WG, Pellicena P, Schindler T, Veach DR, Miller WT, et al. Crystal structure of the c-abl tyrosine kinase domain in complex with PD173955 and STI-571. Cancer Res 2002;62:4236–43.
19. Druker BJ, Talpaz M, Resta D, Peng B, Buchdunger E, Ford J, et al. Clinical efficacy and safety of an Abl specific tyrosine kinase inhibitor as targeted therapy for chronic myelogenous leukemia. ASH 1999. Blood 1999;94 (abstract) 1639.
20. Drucker B, Lydon N. Lessons learned from the development of an Abl tyrosine kinase inhibitor for chronic myelogenous leukemia. J Clin Invest 2000;105:3–7.
21. Puccetti E, Ruthardt M. Acute promyelocytic leukemia: PML/RARa and the leukemic stem cell. Leukemia 2004;18:1169–75.