Cell Cycle 10:10, 1540-1544; May 15, 2011; © 2011 Landes Bioscience

Explaining the in vitro and in vivo differences in leukemia therapy

Tom Lenaerts,[1,2] Fausto Castagnetti,[3] Arne Traulsen,[4] Jorge M. Pacheco,[5,6] Gianantonio Rosti[3] and David Dingli[7,*]

[1]Machine Learning Group; Département d'Informatique; Université Libre de Bruxelles; Brussels, Belgium [2]AI-Lab; Computer Science Department; Vrije Universiteit Brussel; Brussels, Belgium; [3]Department of Hematology and Oncology; "L. and A. Seràgnoli" St. Orsola University Hospital; Bologna, Italy; [4]Evolutionary Theory Group; Max-Planck-Institute for Evolutionary Biology; Plön, Germany; [5]Departamento de Matematica e Aplicações; Universidade do Minho; Braga, Portugal; [6]ATP-Group; Centro de Matemática e Aplicações Fundamentais; Complexo Interdisciplinar; Lisboa, Portugal; [7]Division of Hematology; Mayo Clinic College of Medicine; Rochester, MN USA

The majority of patients with chronic myeloid leukemia in early chronic phase (CML-ECP) who are treated with imatinib achieve a complete cytogenetic response with a significant reduction in the risk of progression to advanced phases. Recent studies show that therapy of CML-ECP with nilotinib leads to a faster and deeper response compared to imatinib. However, in vitro data indicates that there is no detectable difference in inhibition of signaling downstream of *Bcr-Abl* between the two agents, and that neither drug induces apoptosis of CML CD34$^+$ cells. We use a computational model of hematopoiesis and CML combined with serial quantitative data of disease burden under imatinib and nilotinib therapy to explain this apparent disconnect between in vivo and in vitro responses. We show how a subtle difference in the differentiation rate of CML cells under therapy with either agent, with marginal impact onto the in vitro studies, translates into a significantly different reproductive fitness of treated cells in vivo, providing a sizeable difference, hence providing an explanation for the superior response observed with nilotinib.

Key words: hematopoiesis, self-renewal, selection, evolution, reproductive fitness

Submitted: 01/19/11

Accepted: 03/18/11

DOI: 10.4161/cc.10.10.15518

*Correspondence to: David Dingli; Email: dingli.david@mayo.edu

Introduction

The advent of tyrosine kinase inhibitors (TKI) has brought a paradigm shift in the therapy of chronic myeloid leukemia (CML). Imatinib is the current standard of care for patients in early chronic phase (ECP); most patients respond to therapy, and the risk of treatment failure is low.[1] However, some patients develop resistance to imatinib due to a variety of mechanisms, including point mutations in the *Abl* kinase and overexpression of *Bcr-Abl*.[2] Imatinib is not the cause of these mutations but simply selects for the resistant clones that may be present even at diagnosis and before exposure to the drug[3,4] or that may emerge during therapy. Therefore, novel TKI agents have been developed that can inhibit *Bcr-Abl* even in the presence of many imatinib resistant mutations.[5] In vitro, nilotinib has a higher affinity for *Bcr-Abl* compared to imatinib, but it does not lead to enhanced inhibition of signaling downstream of *Bcr-Abl*.[6,7] Neither imatinib nor nilotinib increase apoptosis of CML-derived CD34$^+$ cells.[6] However, therapy of CML-ECP with nilotinib leads to a faster and deeper reduction in tumor burden compared with imatinib.[8,9] In the following, we provide a potential explanation for this apparent disconnect between the in vitro and in vivo results.

Results

In order to understand the differences observed in the response dynamics of CML-ECP under either imatinib or nilotinib therapy, we fitted our model of hematopoiesis[10] to clinical data derived from two patient cohorts treated with either of these agents (**Fig. 1A**).[8,11] The data from the IRIS trial[11] was truncated to 2 years to make the comparison with the more limited data on nilotinib

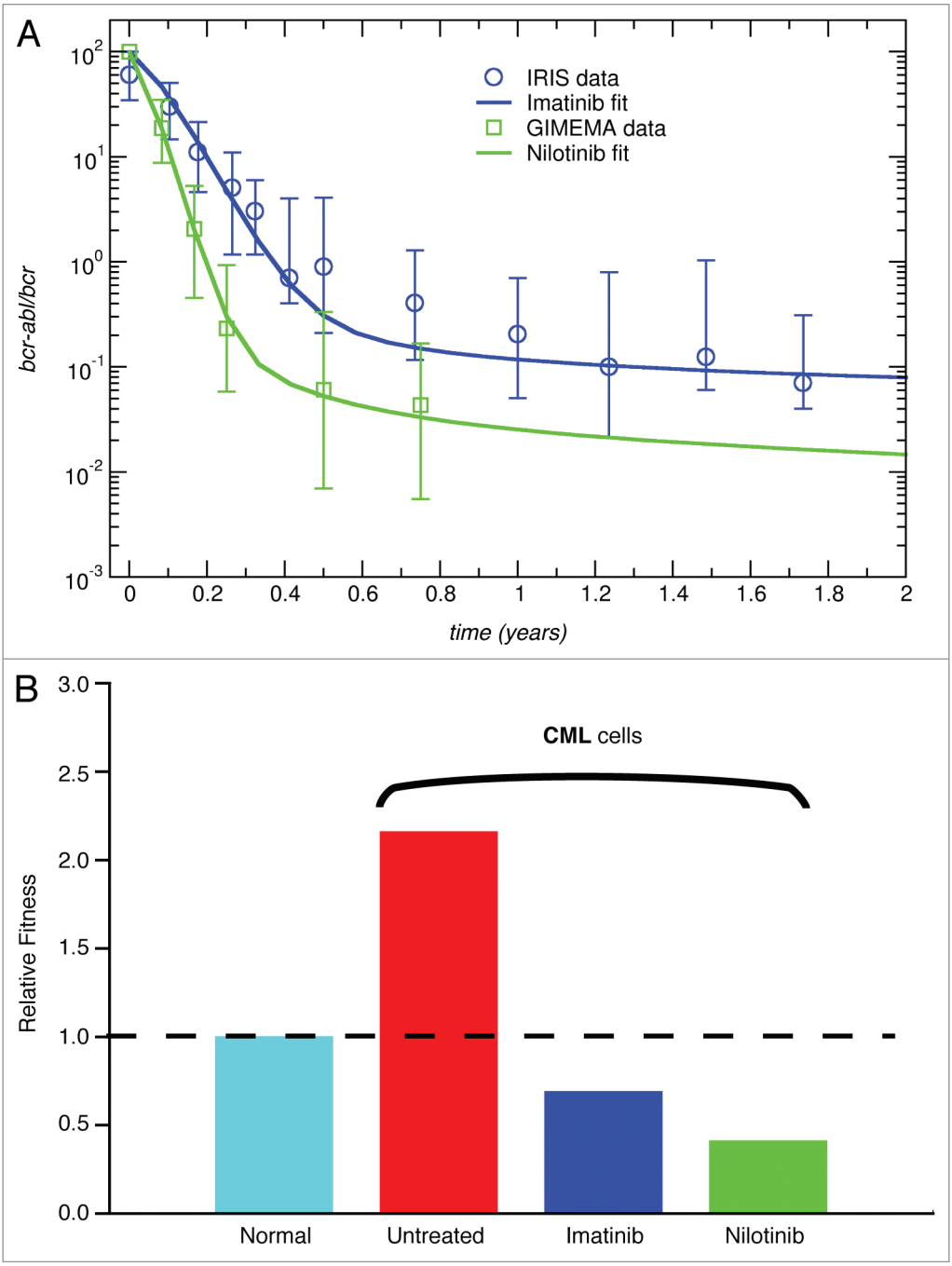

Figure 1. (A) Dynamics of response in patients with early chronic phase CML treated with imatinib or nilotinib.[8,11] Nilotinib results in a faster and deeper response compared to imatinib. (B) The difference in response is due to a more effective reduction in reproductive fitness of CML cells with nilotinib compared with imatinib. Normal hematopoietic cells have relative fitness 1.

easier to visualize. This truncation had no impact on the parameter estimation. We determined (1) the fraction of CML cells responding to treatment with either agent (z_{IMAT} and z_{NIL}) and (2) the impact of therapy on the differentiation probability of each type of treated cells (ε_{IMAT} and ε_{NIL}),[12,13] given that neither imatinib nor nilotinib enhance apoptosis of CD34$^+$ cells.[6] Our results show that only a small fraction of CML cells are responding to therapy (z) at any time:

z_{NIL} = 0.083 (0.083–0.084), while the corresponding fraction for imatinib is z_{IMAT} = 0.046 (0.046–0.047).[10] The difference between the two agents suggests that nilotinib has a higher affinity for *Bcr-Abl*, leading to a faster response, in accord with lab data.[6,14] Yet, this difference alone cannot explain the deeper reduction in disease burden observed with nilotinib (see **Fig. 1A**). Our fitting further reveals that nilotinib increases the probability of differentiation of CML progenitors ε_{NIL}= 0.932 (0.907–0.946) compared to imatinib, ε_{IMAT} = 0.889 (0.881–0.893).[10]

Discussion

Clonal expansion is an evolutionary process driven by differences in cell division. Cells with a probability of differentiation larger than normal effectively have a reduced fitness compared to normal cells.[15] Thus, a drug that increases the differentiation rate imposes a relative fitness disadvantage on that clone. Such a clone will eventually be washed out. The relative fitness of any type of cancer cells (f_C) compared to the fitness of normal cells ($f_N = 1$) can be estimated by

$$f_C = \frac{1-\varepsilon_C}{1-\varepsilon_N}\frac{\varepsilon_N}{\varepsilon_C}$$

where C defines the specific therapy given to the cell.[16] Without therapy, the relative fitness advantage of CML progenitors is f_{CML} = 2.16 (2.02–2.45).[16] The fitness of CML cells under therapy using ε_{NIL} and ε_{IMAT} and estimated above yields f_{NIL} = 0.41 (0.317–0.57) and f_{IMAT} = 0.69 (0.67–0.75), respectively (**Fig. 1B**). Therefore, therapy reduces the relative fitness of CML cells compared to both untreated and normal cells. Moreover, this effect is stronger with nilotinib than with imatinib by as much as ~40%, providing an explanation for the deeper response obtained with nilotinib. It may sound surprising that a drug inhibiting an oncogene can reduce the impact of that gene to levels below that of normal cells. However, available experimental data confirms this possibility; imatinib effectively reduces the amplification (self-renewal) of CML-derived CFU-GM to subnormal levels compared to Ph' negative CFU-GM cells in vitro[17] and is an example of "oncogene addiction."[18,19]

At a cellular level, the change in ε_C is less than 5% (ε_{NIL} = 0.932; ε_{IMAT} = 0.889), explaining perhaps the difficulty in observing differences in cell behavior in vitro.[7] However, when such cellular properties are placed in the proper evolutionary context (that is, in competition with other cell lineages), the full impact of various therapies on CML dynamics becomes clear. Minor variations in cell differentiation as a result of therapy can have an important impact on the evolutionary dynamics of the population and therapeutic outcome. It is the environment that selects for or against a clone and this environment is very difficult to reproduce in vitro, illustrating the challenges of extrapolating from in vitro studies to the in vivo situation. Although nilotinib therapy could suppress pre-existing imatinib resistant clones and the fraction of cells responding to therapy is slightly higher, these features cannot account for the observed differences in response dynamics.

Based on recent experimental evidence that suggests that *Bcr-Abl* expression does not give a fitness advantage to CML stem cells,[20] our modeling approach assumes that this population of cells follows neutral drift.[16,21,22] This in part explains why the population of CML stem cells is small and is compatible with the view that, although the disease is derived from a hematopoietic stem cell, it is driven by progenitor cells.[23] Cell dynamics under neutral drift (a consequence of the lack of a fitness advantage of the CML stem cell compared to normal hematopoietic stem cells) also influences CML therapy, since in the absence of acquired resistance, there is a fair chance that the CML stem cell clone will be stochastically eliminated.[10] Recent observations show that with continued therapy, patients lose the population of CML stem cells,[24] and perhaps they can be cured even in the absence of a therapy that directly affects the CML stem cell. Given the superiority of nilotinib compared to imatinib, we expect a faster elimination of the CML progenitors.

In conclusion, we provide evidence that a small difference in the differentiation probability of CML cells under nilotinib versus imatinib therapy can be the main reason for the deeper responses observed with the former agent. Such small differences may be difficult to detect in vitro. Yet, as shown here, they have significant implications on the reproductive fitness of treated cells compared to normal cells. Given that the risk of acquired resistance and transformation is proportional to the population of progenitor cells at risk, and the main source of blast crisis is the CFU-GM population,[25] from an evolutionary perspective, our model suggests that it makes sense to utilize nilotinib as a first line agent. More rapid reduction in disease burden together with a deeper response should further reduce the probability of acquired resistance and progression to blast crisis as reported in the ENESTnd study.[9]

Methods

Clinical data. The results of a Phase 2 trial of nilotinib as primary therapy of patients with CML-ECP were reported recently in reference 8. Expression of *BCR-ABL/ABL* was serially determined by Q-RT-PCR for each patient after 1, 2, 3, 6, 9, 12 months and every 3 months thereafter, starting at the time of diagnosis.

Mathematical modeling. We resort to our mathematical description of hematopoiesis,[26] which has been applied to understand CML dynamics before and during imatinib therapy.[10,21] Briefly, hematopoiesis is described as a hierarchical branching process where cells divide and differentiate. These two processes are coupled stochastic events. In order to keep track of all levels of cell differentiation, cells in different stages of differentiation are considered to occupy hypothetical compartments that are coupled together, such that, under normal hematopoiesis, each compartment on average has a constant number of cells. Compartments are identified by an index k, such that $k = 0$ corresponds to the stem cell compartment. When a cell in compartment $k > 0$ (downstream of the hematopoietic stem cell pool) is selected for replication with probability ε, both daughter cells differentiate and move to next downstream compartment ($k + 1$), while with probability $1 - \varepsilon$, the cell self renews and hence remains in the same compartment. Differentiation leads to the net loss of one cell from the compartment, while self renewal increases the number of cells by one in the original compartment. Since differentiation is more likely than self-renewal ($\varepsilon > 0.5$), there is net cell loss from one compartment, and the lost cells are replaced by transfer of cells from the next upstream compartment ($k - 1$). This process continues all the way to the HSC pool that maintains hematopoiesis ($k = 0$). Cells in a specific compartment replicate at rate r_k. All cell behavior is stochastic, compatible with the current view of hematopoiesis,[27,28] although for large cell populations, the dynamics can be well

approximated by differential equations.[10] The end result is that hematopoiesis is described by a coupled chain of cell compartments whose size grows exponentially, such that in each compartment, cells have limited self-renewal potential and generally differentiate. Using data for normal granulocyte and erythrocyte kinetics, we have estimated that $\varepsilon_N \approx 0.85$,[29-31] although at the level of the stem cells ($k = 0$) $\varepsilon_N = 0.50$, compatible with the "asymmetric" replication of these cells. Circulating blood cells naturally die, with neutrophils (the main cells responsible for increased bone marrow output in CML) having a circulating half-life of less than a day. TKI therapy has no impact on these cells. The introduction of therapy with TKI requires that we consider a fraction of cells (z) in each compartment that is responding to therapy. Therefore in any compartment we have three populations: normal cells, CML cells and CML cells that are responding to therapy.

Data fitting and constraints. Different values of the model parameters may lead to very different disease histories of CML dynamics under treatment. Therefore, the model parameters were fitted against serial *Bcr-Abl/Abl* data for patients under nilotinib or imatinib therapy using a non-linear least squares approach. As modeling constraints, we imposed that (1) at least one leukemic stem cell is present,[11,16] and (2) the time between the appearance of the first leukemic stem cell and diagnosis (defined as hematopoietic output > 10^{12} cells per day[32]) is between 3.6–6 years.[33] The parameters of interest extracted from the fits are (1) the fraction of CML cells responding to therapy (z) and (2) the impact of therapy on the differentiation probability of CML progenitor cells (ε_{CML}, ε_{NIL} and ε_{IMAT}). The differentiation probability of CML progenitors in the absence of therapy (ε_{CML}) has been estimated previously in reference 10 and 21. We consider that *Bcr-Abl* expression gives no reproductive fitness advantage to the CML stem cell compared to normal hematopoietic stem cells.[20,34] This is compatible with the view that, although CML starts in a hematopoietic stem cell, the main drivers of the disease are CML progenitor cells downstream of the stem cells.[23] As we have shown previously, the lack of any effect of TKI therapy on the leukemic stem cells is compatible with the continued response to TKI therapy, and, therefore, there is no need to assume or impose that TKI therapy affects the leukemic stem cell pool directly.[11] The scatter in both parameters was determined by allowing the time to diagnosis to vary by 25% in either direction (to cover 3.54–5.90 years), considering a mean time to diagnosis of 4.7 years[33] and accomplished by varying ε_{CML} between 0.69–0.73.

Acknowledgements

T.L. is supported by the FNRS and VUB research council. A.T. is supported by DFG and DAAD (Project 0813008); J.M.P. by FCT Portugal and DAAD, while D.D. is supported by Mayo Foundation and the Minnesota Partnership for Biotechnology and Medical Genomics.

The authors thank all investigators within the GIMEMA CML Working Party for recruitment of patients to the CML/0307 study (ClinicalTrials.gov: NCT00481052).

Authorship Contributions

T.L.: Concept, design of mathematical model, data analysis, critical review of paper

F.C.: Provided clinical data, critical review of paper

A.T.: Concept, design of mathematical model, critical review of paper

J.M.P.: Concept, design of mathematical model, wrote the paper

G.R.: Provided clinical data, critical review of paper

D.D.: Concept, design of mathematical model, analysis of data, wrote the paper.

References

1. Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med 2006; 355:2408-17.
2. Kantarjian HM, Talpaz M, Giles F, O'Brien S, Cortes J. New insights into the pathophysiology of chronic myeloid leukemia and imatinib resistance. Ann Intern Med 2006; 145:913-23.
3. Luzzatto L, Melo JV. Acquired resistance to imatinib mesylate: selection for pre-existing mutant cells. Blood 2002; 100:1105.
4. Roche-Lestienne C, Soenen-Cornu V, Grardel-Duflos N, Lai JL, Philippe N, Facon T, et al. Several types of mutations of the Abl gene can be found in chronic myeloid leukemia patients resistant to STI571, and they can pre-exist to the onset of treatment. Blood 2002; 100:1014-8.
5. Weisberg E, Manley PW, Cowan-Jacob SW, Hochhaus A, Griffin JD. Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia. Nat Rev 2007; 7:345-56.
6. Jorgensen HG, Allan EK, Jordanides NE, Mountford JC, Holyoake TL. Nilotinib exerts equipotent anti-proliferative effects to imatinib and does not induce apoptosis in $CD34^+$ CML cells. Blood 2007.
7. Konig H, Holtz M, Modi H, Manley P, Holyoake TL, Forman SJ, et al. Enhanced BCR-ABL kinase inhibition does not result in increased inhibition of downstream signaling pathways or increased growth suppression in CML progenitors. Leukemia 2008; 22:748-55.
8. Rosti G, Palandri F, Castagnetti F, Breccia M, Levato L, Gugliotta G, et al. Nilotinib for the frontline treatment of Ph(+) chronic myeloid leukemia. Blood 2009; 114:4933-8.
9. Saglio G, Kim DW, Issaragrisil S, le Coutre P, Etienne G, Lobo C, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. N Engl J Med 2010.
10. Lenaerts T, Pacheco JM, Traulsen A, Dingli D. Tyrosine kinase inhibitor therapy can cure chronic myeloid leukemia without hitting leukemic stem cells. Haematologica 2010; 95:900-7.
11. Roeder I, Horn M, Glauche I, Hochhaus A, Mueller MC, Loeffler M. Dynamic modeling of imatinib-treated chronic myeloid leukemia: functional insights and clinical implications. Nat Med 2006; 12:1181-4.
12. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med 1996; 2:561-6.
13. Marley SB, Davidson RJ, Goldman JM, Gordon MY. Effects of combinations of therapeutic agents on the proliferation of progenitor cells in chronic myeloid leukaemia. Br J Haematol 2002; 116:162-5.
14. Shah NP, Nicoll JM, Nagar B, Gorre ME, Paquette RL, Kuriyan J, et al. Multiple BCR-ABL kinase domain mutations confer polyclonal resistance to the tyrosine kinase inhibitor imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. Cancer Cell 2002; 2:117-25.
15. Dingli D, Traulsen A, Michor F. (A)Symmetric stem cell replication and cancer. PLoS Comput Biol 2007; 3:53.
16. Traulsen A, Pacheco JM, Dingli D. Reproductive fitness advantage of BCR-ABL expressing leukemia cells. Cancer Lett 2010; 294:43-8.
17. Marley SB, Deininger MW, Davidson RJ, Goldman JM, Gordon MY. The tyrosine kinase inhibitor STI571, like interferon-alpha, preferentially reduces the capacity for amplification of granulocyte-macrophage progenitors from patients with chronic myeloid leukemia. Exp Hematol 2000; 28:551-7.
18. Chen R, Gandhi V, Plunkett W. A sequential blockade strategy for the design of combination therapies to overcome oncogene addiction in chronic myelogenous leukemia. Cancer Res 2006; 66:10959-66.
19. Sharma SV, Gajowniczek P, Way IP, Lee DY, Jiang J, Yuza Y, et al. A common signaling cascade may underlie "addiction" to the Src, BCR-ABL and EGF receptor oncogenes. Cancer Cell 2006; 10:425-35.
20. Schemionek M, Elling C, Steidl U, Baumer N, Hamilton A, Spieker T, et al. BCR-ABL enhances differentiation of long-term repopulating hematopoietic stem cells. Blood 2010; 115:3185-95.
21. Dingli D, Traulsen A, Pacheco JM. Chronic myeloid leukemia: origin, development, response to therapy and relapse. Clinical Leukemia 2008; 2:133-9.
22. Dingli D, Traulsen A, Lenaerts T, Pacheco JM. Evolutionary dynamics of chronic myeloid leukemia. Genes Cancer 2010; 1:309-15.
23. Marley SB, Gordon MY. Chronic myeloid leukaemia: stem cell derived but progenitor cell driven. Clin Sci 2005; 109:13-25.

24. Sobrinho-Simoes M, Wilczek V, Score J, Cross NC, Apperley JF, Melo JV. In search of the original leukemic clone in chronic myeloid leukemia patients in complete molecular remission after stem cell transplantation or imatinib. Blood 2010.
25. Jamieson CH, Ailles LE, Dylla SJ, Muijtjens M, Jones C, Zehnder JL, et al. Granulocyte-macrophage progenitors as candidate leukemic stem cells in blast-crisis CML. E Enlg J Med 2004; 351:657-67.
26. Dingli D, Traulsen A, Pacheco JM. Compartmental architecture and dynamics of hematopoiesis. PLoS ONE 2007; 2:345.
27. Gordon MY, Blackett NM. Routes to repopulation—a unification of the stochastic model and separation of stem-cell subpopulations. Leukemia 1994; 8:1068-72.
28. Abkowitz JL, Catlin SN, Guttorp P. Evidence that hematopoiesis may be a stochastic process in vivo. Nat Med 1996; 2:190-7.
29. Donohue DM, Reiff RH, Hanson ML, Betson Y, Finch CA. Quantitative measurement of the erythrocytic and granulocytic cells of the marrow and blood. J Clin Invest 1958; 37:1571-6.
30. Cronkite EP, Fliedner TM. Granulocytopoiesis. The New England journal of medicine 1964; 270:1347 52.
31. Finch CA, Harker LA, Cook JD. Kinetics of the formed elements of human blood. Blood 1977; 50:699-707.
32. Holyoake TL, Jiang X, Drummond MW, Eaves AC, Eaves CJ. Elucidating critical mechanisms of deregulated stem cell turnover in the chronic phase of chronic myeloid leukemia. Leukemia 2002; 16:549-58.
33. Ichimaru M, Ishimaru T, Mikami M, Yamada Y, Ohkita T. Incidence of leukemia in a fixed cohort of atomic bomb survivors and controls, Hiroshima and Nagasaki October 1950–December 1978: Technical Report RERF TR 13–81. Hiroshima: Radiation Effects Research Foundation 1981.
34. Jaras M, Johnels P, Hansen N, Agerstam H, Tsapogas P, Rissler M, et al. Isolation and killing of candidate chronic myeloid leukemia stem cells by antibody targeting of IL-1 receptor accessory protein. Proc Natl Acad Sci USA 2010; 107:16280-5.