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Response to Comment on "Tumor Growth Need Not Be Driven by Rare Cancer Stem Cells"

Jerry M. Adams,^{1*} Priscilla N. Kelly,^{1,2} Aleksandar Dakic,^{1,2} Stephen L. Nutt,¹ Andreas Strasser^{1*}

A critical issue for cancer biology and therapy is whether most tumor cells or only rare "cancer stem cells" sustain tumor growth. Although the latter model seems supported by the minute proportion of human leukemia cells that can grow in immunodeficient mice, evidence that more than 10% of cells in many mouse leukemias and lymphomas are transplantable challenges its generality.

A key unresolved issue for cancer biology and therapy is whether the relentless growth of a tumor is driven by most of its cells or, as proposed by the cancer stem cell hypothesis, exclusively by a minor subpopulation capable of self-renewal, akin to the numerically rare normal stem cells that maintain tissues (1). The impetus for that hypothesis has come primarily from experiments in which human tumor cells are transplanted at limit dilution into immunodeficient mice. The most widely cited evidence is that, in human acute myelogenous leukemia (AML), only a minute proportion of the cells (10^{-4} to 10^{-7}) could seed leukemia growth in immunodeficient nonobese diabetic (NOD)/severe combined immunodeficient (SCID) mice (2). Xenotransplantation is problematic, however, because the incompatibility of many cytokine-receptor interactions between mouse and man may prevent critical relationships with the microenvironment. In striking contrast to human AML, with three types of primary monoclonal mouse tumors from genetically modified mice (B lymphomas, T lymphomas, and AML), we found that >10% of the cells readily seeded tumor growth in nonconditioned congenic recipients, and three of eight single-cell transfers attempted with a B lymphoma succeeded (3).

Other mouse leukemia models (albeit not all) have yielded similar results. Pertinently, in mouse AML induced by the *MLL-AF9* translocation gene, the leukemia growth-sustaining cells represented 25% of all myeloid cells (4). Similarly, as few as 20 BCR-ABL-transduced Arf-deficient pre-B cells could rapidly induce acute lymphocytic leukemia (5). Notably, in all these studies the cells that seeded leukemia resembled relatively mature cell types and not hematopoietic stem cells or primitive progenitors (3–5). Although an early study of an AKR mouse thymoma cited by Kennedy *et al.* (6) reported that <1% of cells

were tumorigenic (7), it used a less sensitive, indirect assay (spleen colony formation rather than tumor growth), and the retroviral etiology of AKR tumors may well have caused their immunological rejection. In more relevant classical studies with both myeloid and lymphoid tumors, the leukemia growth-sustaining cells have typically ranged from >1 in 100 to the majority of cells, and some single-cell transfers have succeeded (8, 9).

Thus, many mouse lymphomas and leukemias, including ones closely modeling human counterparts and involving equivalent genetic changes and stochastic onset, seem to be sustained by a substantial proportion of their cells (probably at least 1 to 30%). Hence, xenotransplantation may reveal only a minute fraction of the human AML cells that drive those leukemias. Supporting that view, 50% of human AML samples did not engraft NOD/SCID mice even when 10^7 or 10^8 cells were introduced (10). The new data from Kennedy *et al.* (6) showing that 10^{-2} to 10^{-3} of the cells in a human B cell acute lymphoblastic leukemia (B-ALL) model can initiate tumors in secondary transfers does not answer our major reservation about xenotransplantation, because the initial generation of these tumors within mice may have allowed selection for growth in that milieu. Also, the evidence that those tumors are maintained by a defined subpopulation of cells appears limited.

Kennedy *et al.* and other cancer stem cell advocates argue that the proof of the model is that cell populations prospectively isolated from human AML samples by surface markers initiate leukemia in mice, whereas the cell population lacking those markers does not (1, 2). Two considerations question this argument. First, a human tumor cell population may fail to propagate in mice not from lack of self-renewal capacity but because of the lack of a receptor responsive to murine growth factors or the inability to home to a nurturing microenvironment. Second, rather than possessing unique self-renewal capacity, the NOD/SCID-transplantable population in human AML may have inadvertently acquired addressing molecules that target them

to favorable niches in the mouse. With some types of mouse leukemias, no functionally distinct tumorigenic subpopulations have been discerned (3–5). Nevertheless, with other types, subpopulations enriched for leukemogenic cells have been identified (11–13), so the cancer stem cell model may well hold for some types of leukemia but not others. Interestingly, however, the reported phenotypes of the mouse leukemogenic subpopulations are variable and more similar to relatively mature cells than hematopoietic stem cells (11–13).

The evidence for cancer stem cells in solid tumors is less advanced than for AML (1) and is subject to the same reservations regarding xenotransplantation. The cancer-propagating cells are often found within subpopulations (e.g., CD133⁺) that can contain up to 20% of the cells within a tumor (14, 15). In some instances, the transplantable population might also contain essential support cells. For example, co-transfer of CD133⁺ support cells might explain the puzzle that the colon cancer CD133⁺ population appeared to contain 20 times as many cancer growth-sustaining cells as the unfractionated population (16). Much of the heterogeneity in tumors may well result from the subclonal genetic and epigenetic variation produced during tumor evolution, without the need to invoke a strict hierarchical relationship between subpopulations.

We agree with Kennedy *et al.* (6) that tumors are likely to fall on a spectrum in which the tumor-propagating cells range from infrequent to the dominant population. However, the marked disparities between most transplant results with human and mouse leukemias suggests that current xenotransplantation systems seriously underestimate the frequency of cells that can maintain the growth of human tumors. Several mouse tumor models challenge the generality of the cancer stem cell hypothesis, and more compelling tests with human tumors presumably will require transfer into mice installed with all the requisite human support cells and support factors. Much of the excitement about the cancer stem cell hypothesis arises from the possibility that the putative stem cell population will prove to be uniquely responsible for the relapses that so frequently follow conventional therapy (1). On the available evidence, however, we suggest that curative therapy will require targeting all the tumor subpopulations.

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¹Walter and Eliza Hall Institute of Medical Research, Melbourne 3050, Australia. ²Department of Medical Biology, University of Melbourne, Melbourne 3050, Australia.

*To whom correspondence should be addressed. E-mail: adams@wehi.edu.au (J.M.A.); strasser@wehi.edu.au (A.S.)

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11 September 2007; accepted 13 November 2007
10.1126/science.1149672