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Comment on “Tumor Growth Need Not Be Driven by Rare Cancer Stem Cells”

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Kelly *et al.* (Brevia, 20 July 2007, p. 337) questioned xenotransplant experiments supporting the cancer stem cell (CSC) hypothesis because they found a high frequency of leukemia-initiating cells (L-IC) in some transgenic mouse models. However, the CSC hypothesis depends on prospective purification of cells with tumor-initiating capacity, irrespective of frequency. Moreover, we found similar L-IC frequencies in genetically comparable leukemias using syngeneic or xenogeneic models.

Kelly *et al.* (1) observed that more than 10% of the bulk tumor cells in several transgenic mouse models of leukemia and lymphoma were capable of initiating malignant growth upon transplantation into histocompatible mice. These values clearly contrast with the leukemia-initiating cell (L-IC) frequency on the order of 1 in 10⁴ to 10⁷ that has been reported for primary human acute myeloid leukemia (AML) after intravenous transplantation of bulk blast cells at limiting doses into severe combined immunodeficient (SCID) or nonobese diabetic (NOD)/SCID mice (2–4). Kelly *et al.* proposed that this quantitative difference arises from an inability of the human cells to grow efficiently in the murine bone marrow (BM) microenvironment and leads to a routine underestimation of the frequency of tumor-initiating cells in xenotransplantation assays. Based on these observations, the authors called into question the main conclusion of these and subsequent studies, namely that both human leukemias and solid tumors are hierarchically organized and sustained by a population of biologically distinct cancer stem cells (CSC) (2, 3, 5). Kelly *et al.* clearly demonstrated that in some experimental cancer models, tumorigenic growth need not be driven by rare CSCs; however, their criticism of xenotransplantation assays and their challenge of the generality of the CSC hypothesis merit some discussion.

Undoubtedly, human cell engraftment in xenotransplant models is limited by residual elements of the recipient immune system, the absence of cross-species reactivity of some cytokines, and

other components of the murine microenvironment. The first-generation models used to perform the aforementioned calculations of L-IC frequency presented substantial barriers to human cell engraftment. Since these initial reports, improvements including depletion of residual immune activity, direct injection of cells into bone cavities, and transgenic expression of human cytokines have increased the sensitivity of detection of normal human hematopoietic stem cells, warranting a reassessment of L-IC frequency in primary human leukemias (6–8). Our unpublished work and that of others (8) suggests that optimized assays increase L-IC detection by 1 to 2 orders of magnitude; nevertheless, L-ICs in primary human AML remain relatively rare, with high variation in frequency from sample to sample.

Using these improved xenograft assays, we recently developed a genetically induced model of human B cell acute lymphoblastic leukemia (B-ALL) by transplanting primary human umbilical cord blood stem/progenitor cells expressing the *mixed lineage leukemia–eleven-nineteen leukemia (MLL-ENL)* oncogene into immunodeficient mice (9). To determine L-IC frequency in this model, limiting numbers of BM cells from primary leukemic mice were transplanted into secondary recipients. As summarized in Table 1, the L-IC frequency in the bulk leukemic blast

population was on the order of 1%. Interestingly, our L-IC frequency was comparable to that reported for a murine retroviral transduction/transplantation model of MLL-AF9–induced AML (1 in 150) (10). Furthermore, purification of human leukemic cells from the BM of primary mice on the basis of CD19 and CD34 expression showed that L-ICs were enriched in the CD19⁺CD34⁺ fraction compared with the CD19⁺CD34[−] fraction and were absent in the CD19[−]CD34[−] fraction (table S1). Thus, these data prove the existence of discrete L-IC in this model and demonstrate that these cells can be detected at high frequencies in xenograft systems despite barriers to human cell growth. Furthermore, these studies indicate that when genetically comparable leukemia models are studied, syngeneic and xenogeneic approaches can yield similar calculations of L-IC frequency.

In addition to Kelly *et al.* (1) and the studies discussed above, demonstration of functional heterogeneity and assessment of L-IC frequencies have been reported in the literature for many decades. For example, in 1963, Bruce and van der Gaag (11) reported that the frequency of clonogenic lymphoma cells from spontaneously occurring murine lymphomas ranged from 0.001% to 1% in syngeneic recipients. In a MOZ-TIF retroviral transduction/transplantation model, the L-IC frequency was on the order of 1 in 10⁴ (12), whereas it was 1 in 6 × 10⁵ in a *Pten* deletion model of AML (13). Thus, it is evident that L-IC frequencies can vary widely between different cancers regardless of whether they are quantified using xeno- or syngeneic transplant assays. We hypothesize that different cancers will exhibit variable degrees of functional heterogeneity as a consequence of the specific oncogenic pathways operating within the neoplasm, resulting in different CSC frequencies. However, we must emphasize that the fundamental concept underlying the CSC hypothesis is not related to the absolute frequency of these cells; instead, this model proposes that the basis of the functional heterogeneity within tumors is the presence of a distinct population of cells that

Table 1. Limiting dilution analysis of *MLL-ENL* B-precursor ALL. Leukemia was defined as ≥20% CD19⁺CD20[−] blasts in the BM. The data from leukemia 1 have appeared previously (9).

	Cell dose	Frequency of leukemia	Median disease latency
MLL-ENL leukemia 1	250,000	2/2	77
	50,000	2/2	85
	10,000	5/5	85
	2,000	4/4	104
	400	0/3	n/a
MLL-ENL leukemia 2	125,000	2/2	57
	25,000	2/2	64
	5,000	5/5	85
	1,000	4/4	92
	200	3/4	125

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can be prospectively isolated and can initiate malignant growth in vivo while the remaining cells cannot. The therapeutic implications of CSCs remain the same regardless of their absolute frequency: These cells, which may have growth or therapeutic resistance properties that differ from those of the bulk tumor, must be effectively targeted to achieve definitive curative benefits.

Kelly *et al.* (1) raise the important point that some experimental cancer models may not follow the CSC hypothesis. Accordingly, some human cancers may be found that also do not adhere to this model. However, it is important to consider that, similar to many cell lines that have lost the hierarchical structure of the primary leukemia from which they originated, some experimental mouse models may not accurately reflect spontaneously occurring human malignancies. For example, one could argue

that some of the models used by Kelly *et al.* (i.e., the *N-Ras* lymphoma) have limited human antecedents.

Finally, Kelly *et al.* state that the term “cancer stem cell” designates an origin from normal tissue stem cells, a point that has long been a source of confusion in the literature. Recently, an AACR panel made up of experts in the stem cell field agreed that the term “cancer stem cell” does not speak to the cell of origin (the normal cell type that becomes transformed and gives rise to the cancer). Instead, this term, albeit imperfect, encompasses the notion that the cell type that sustains the growth of many cancers possesses stem cell properties, such as a capacity for self-renewal, and lies at the pinnacle of a neoplastic hierarchy, giving rise to “differentiated” progeny that lack these same properties (14).

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