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In Reponse: Drs. Viprey and Burchill assert superiority of their marker discovery strategy where candidate genes have absolute negativity in blood or marrow, when based on National Center for Biotechnology Information expressed sequence tag (EST) database and expression arrays of blood from healthy volunteers (1). This contrasts with our approach using differential display between high-risk metastatic-stage neuroblastoma and patient remission marrows.

The ideal tumor marker (abundant in tumor and totally absent in blood or marrow) rarely exists. For example, serum prostate-specific antigen is measurable in normal men. Although suboptimal for screening the normal population, its utility as a tumor marker in patients with prostate cancer is well established. *CCND1* transcript is another example, which, despite low expression in remission marrows, correlated strongly with survival in patients with neuroblastoma (2), Ewing's family of tumors (3), and mantle cell lymphoma (4). We therefore do not exclude markers even if they are present at low levels in marrow, as long as they are abundant in neuroblastoma.

A careful examination of the tissue source classification in the EST database showed that nearly all of the claimed positive EST hits in marrow or blood from the gene list were in fact derived from JURKAT cell line, leukemia, excised breast tumors, and cultured marrow cells. Because cytokines and mitogens can stimulate illegitimate expression of many markers (even *PHOX2B*) in white cells, using cultured cells as "normals" can be misleading. When EST database is misused for the purposes claimed (1), potential markers will be discarded prematurely.

The use of adult volunteer blood as normals may also be inappropriate when studying a pediatric cancer (1), where patient marrow is the appropriate context for studying disseminated (distinguished from circulating) tumor cells (5). Our algorithm of marker discovery was intentionally inclusive (e.g., *CCND1*, *MEG3*, and *MLLT11*) while being discriminative (based on sensitivity and survival prognostic tests). *MEG3* was not excluded upfront because its other Affymetrix probes were trending similarly. We did exclude cytoskeletal genes that were ubiquitous in marrows and tissues.

Before any marker can be considered promising, its utility in established disease and in minimal disease using retrospective samples is critical (6). Conclusions derived from strategies without validating with patient samples should be viewed with caution (1). The strength of our approach is comparative Kaplan-Meier analyses of candidate markers using marrow samples from patients treated on a standardized treatment protocol. Further testing of these markers in a prospective clinical trial is ongoing.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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