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Detection of Minimal Residual Disease in Bone Marrow During or After Therapy as a Prognostic Marker for High-Risk Neuroblastoma

For most of the 20th century, the outlook for patients with high-risk neuroblastoma (stage 4 and older than age 1 year at diagnosis and/or stage 3 tumors with *MYCN* gene amplification) has been dismal (1). Myeloablative therapy (2-4), especially if followed-up by 13-*cis*-retinoic acid (13-*cis*-RA) (4), has improved event-free survival (EFS) to approximately 40% at 4 years from diagnosis. Developing a means to distinguish those high-risk neuroblastoma patients for whom current therapy achieves long-term EFS from those in whom progressive disease will develop may facilitate interpretation of ongoing and future clinical trials. Markers that accurately predict a good outcome can be used to identify a subset of patients in whom future trials may explore a decrease in the intensity of cytotoxic therapy. Especially valuable would be reliable markers that predict failures with current therapy because such a group of patients could then be offered new (experimental) treatment modalities.

Therapy failures in high-risk neuroblastoma patients are likely caused by tumor cells developing resistance to chemotherapy (5). Most stage 4 neuroblastoma patients have marrow metastases at diagnosis (6), and marrow is a frequent site of recurrent disease (7). Therefore, sensitive (and, ideally, quantitative) detection of small numbers of neuroblastoma cells in bone marrow offers a means of assessing the efficacy of chemotherapy across a wider dynamic range than is possible with routine clinical methods. Recently, three different groups of investigators have reported that sensitive detection of neuroblastoma cells in bone marrow

during or after therapy correlated with a significantly worse EFS (8-10). Two of these studies examined bone marrow during therapy, one study by the Children's Cancer Group used immunocytology (8), whereas a study from Japan used detection of tyrosine hydroxylase (*TH*) gene expression by reverse-transcription polymerase chain reaction (RT-PCR) (10). Both of these studies showed that persistence of detectable tumor in marrow after completion of induction chemotherapy was associated with a lower EFS rate. A third study showed that detection of *GAGE* expression by RT-PCR in bone marrow 24 months after completion of intensive chemotherapy combined with anti-*GD2* antibody therapy correlated with poor EFS (9).

Given that three different studies (using different methodologies) showed a correlation between detection of neuroblastoma in bone marrow and EFS, one might be tempted to begin using detection of tumor in bone marrow during or after therapy as a prognostic factor to identify subsets of patients. However, current and future therapies not used for many of the patients in the previous three studies could likely influence results. In the Children's Cancer Group study (4), patients received one of four possible different therapeutic combinations (chemotherapy only, chemotherapy plus 13-*cis*-RA, autologous bone marrow transplantation only, and autologous bone marrow transplantation plus 13-*cis*-RA). Currently, most patients with stage 4 neuroblastoma receive myeloablative therapy followed-up by 13-*cis*-RA and, in some cases, immunotherapy as well. It is possible that developing more effective therapy could decrease the significance of minimal residual disease (MRD) detected before completion of therapy; this is especially true for therapies used after the MRD is measured. Indeed, in the Children's Cancer Group study, MRD detection in marrow harvested for autologous bone marrow transplantation in patients who were randomized to not receive 13-*cis*-RA appeared to correlate with a poor EFS, but this was not the case for patients randomized to receive 13-*cis*-RA (8). In the study reported by Fukuda et al., patients did not receive postmyeloablative therapy, which could decrease the adverse prognostic impact of detecting persistent tumor earlier in therapy (10). Other differences in therapeutic approach (such as purging of tumor cells from marrow or peripheral blood stem cells) could also influence the impact on EFS of MRD that persists in bone marrow during induction therapy.

In contrast to measuring MRD during therapy, detection of tumor cells in bone marrow 2 years after completion of therapy (9) would seem to be less influenced by different therapeutic strategies used since the initial study. However, detection of MRD with RT-PCR in patients treated with a differentiation inducer (such as 13-*cis*-RA) could mean something quite different from detection of a neuroblastoma-associated gene expression (*GAGE* or *TH*) in the bone marrow of patients who were not treated with a retinoid. The mechanism of action for 13-*cis*-RA is induction of tumor cell differentiation and sustained arrest of tumor cell

proliferation (11). Although neuroblastoma cells that respond to 13-*cis*-RA in the bone marrow do eventually show undetectable tumor by routine morphology or immunocytology (11,12), it is possible that small numbers of viable (but terminally differentiated and thus, nonproliferating) tumor cells could persist in bone marrow for months to years after therapy, without leading to progressive disease. Conversely, the persistence of such detectable cells may identify those patients in whom late relapses will develop.

The recent reports linking detection of MRD in bone marrow to outcome in high-risk neuroblastoma are provocative, but additional studies are required to truly define this approach to prognostication. Except in the context of clinical trials, subjecting neuroblastoma patients to bone marrow aspirations solely for the purpose of evaluating MRD should not yet become a routine part of care for children with high-risk neuroblastoma. For patients in whom consideration is being given to treatment with experimental approaches aimed at eradicating MRD, detection of MRD would be useful in deciding on such experimental therapy. However, for the reasons outlined previously, detection of MRD in bone marrow after completion of therapy is not yet a clear indication for additional therapy. Moreover, there are currently no treatments proven in a randomized trial to be effective in preventing or delaying progressive disease in such a setting other than those already used for all patients, i.e., myeloablative therapy and 13-*cis*-retinoic acid.

A careful evaluation of the impact of MRD in bone marrow and blood before, during, and after therapy is planned as an integral part of the new high-risk neuroblastoma phase III trials in the Children's Oncology Group. Detection of MRD in the Children's Oncology Group studies will be performed at various times during therapy and will be performed by both immunocytology (8) and RT-PCR for multiple gene products (9,13–15). The large number of uniformly treated patients that will be studied in the Children's Oncology Group phase III trials will provide the statistical power needed to clearly define the prognostic value of detecting persistent MRD in marrow during therapy and after completion of therapy.

If persistent MRD in bone marrow is confirmed to portend a poor outcome, then a logical next question will be what should be performed to improve therapy? A number of novel approaches to treating neuroblastoma are being tested in clinical and preclinical studies (16–24). Developing markers to identify those stage 4 neuroblastoma patients older than age 1 year at diagnosis who are at high-risk for progressive disease with current therapy will facilitate design of clinical trials aimed at testing such new approaches. Indeed, the use of MRD detection in bone marrow may not only allow stratifying patients for such studies but also will provide a means for assessing the effect of novel therapies against MRD, without having to wait for the larger tumor burden necessary to quantify tumor by routine clinical methods.

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