Response

We appreciate the opportunity to address the issues raised by our comparison of the prognostic utility of the Glucksberg grading system of acute graft-versus-host disease (GVHD) with the International Bone Marrow Transplant Registry (IBMTR) index.1 Because the report in question was not published in Blood, for the benefit of readers we should point out the purpose of the IBMTR analysis was to determine if there was heterogeneity of outcome within each Glucksberg grade and, if so, to define more homogeneous grades of GVHD so that outcome for an individual patient could be estimated with greater accuracy. We sought, additionally, to use the objective Glucksberg staging criteria for skin, liver, and gut involvement without relying on the subjective assessment of performance score. Therefore, the IBMTR is a refinement rather than an abandonment of the Glucksberg system.

Although we welcome the review of our analysis by Martin et al, we perceive some methodological flaws both in their interpretation of our analysis and in the analysis of their own data. As they correctly note, the average standard errors for the IBMTR index model are larger than those for the Glucksberg grade model. This fact does not imply that the Glucksberg model fits the data better, but rather is an artifact of the IBMTR model having more parameters (4) than the model based on the Glucksberg grading (3 parameters). It is well known that the standard errors of parameter estimates do not depend on the strength of the relationship between the independent and dependent variables. The magnitude of the standard errors is affected by the total sample size and the number of parameters in the model. Interestingly, Martin and colleagues do not provide any standard errors for their own data.

The correct measure of how well different models fit is some type of information criterion. For regular regression, the standard measure is the square of the correlation, $R^2$. For the Cox model, the Akaike information criterion (AIC)2,3 can be used to compare information in non-nested models. This criterion is defined as $\text{AIC} = -2 \ln(L) + 2p$ where $p$ is the number of parameters in the model and $L$ is the value of the maximized partial likelihood for the fitted model. When comparing two models, the model with smaller AIC provides a better, more parsimonious fit to the data. For the model in Table 3 (Glucksberg Grade) the AIC is 5924.781 while for the data in Table 7 (IBMTR index) the AIC is 5813.258. This strongly suggests that the model in Table 7 provides a superior fit to the data.

Additionally, to compare the prognostic ability solely on the basis of Kaplan-Meier curves is misleading. The computation of a Kaplan-Meier curve, as depicted in the figures, requires that the GVHD grade of every patient be known at the time of transplantation or that a left truncated Kaplan-Meier4 estimator be used. This is clearly not the case for the patients in Martin et al’s cohort, especially for grade IV patients who must die before being assigned to this group. With violations of this assumption, the basic statistical theory5,6 used to derive the curves breaks down. In our analysis we analyzed the prognostic ability of grades of GVHD using a series of time-dependent covariates and presented survival curves as summary statistics to illustrate our findings. Outcome (ie, death) was not used to assign grade.

Martin et al comment that early deaths before occurrence of acute GVHD may be affecting interpretation of their data. We agree. We purposefully only included patients surviving $\geq 21$ days with engraftment in our study to aid in interpreting the effect of acute GVHD on outcomes.

Heterogeneity within Glucksberg grade is clearly seen in Table 1 of the letter from Martin et al. Glucksberg grade II patients can be in either IBMTR Index B or C, groups with distinctly different outcomes. As was shown in our study this is largely due to the adverse prognosis of developing stage 3 skin GVHD, important information not considered in the Glucksberg grade.

Ongoing research in this area requires a grading system based on objective data that can be used prospectively to accurately assess risk. Useful prognostic systems predict outcome rather than describing it afterward, thereby allowing high-risk patients to be identified and targeted for intervention. Such a system would benefit both design and conduct of GVHD treatment trials as well as immediate patient care. Whether the IBMTR Index fulfills these requirements awaits the results from prospective validation studies, as is currently being undertaken by the French Society of Bone Marrow Transplantation in collaboration with the IBMTR Statistical Center.

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REFERENCES


EHT, a New Member of the MTG8/ETO Gene Family, Maps on 20q11 Region and Is Deleted in Acute Myeloid Leukemias

To the Editor:

In an attempt to identify potential new genes homologous to ETO,1,2 we screened the dbEST database (http://www.ncbi.nlm.nih.gov/dbEST/index.html) using the entire ETO cDNA sequence as a probe (EMBL accession no. X79990). Among the ESTs identified, we selected two overlapping clones (clone IDs 43629 and 274508) and sequenced them to completion. A putative translation initiation site was identified by the presence of a strong Kozak consensus sequence,3 followed by a 1,725-bp open reading frame (ORF) coding for a putative protein of 575
amino acids (aa) (GenBank accession no. AF068266). We named this gene EHT (ETO Homologous on chromosome Twenty; see mapping data below). The putative EHT protein is closely homologous to ETO/MITG8 (~65% identity) and Nervy, an ETO Drosophila homolog (~24% identity) showing the four conserved domains found in MITG8 and Nervy. To find EST clones that could represent the EHT 5' UTR, we screened the dbEST data bank with the 5' end of clone 274508, and identified an EST clone (GenBank accession no. AA635096) encompassing the EHT ATG codon, preceded by an in-frame stop codon 129 bp upstream (Fig 1, top). Therefore, we considered this sequence the putative 5' UTR of EHT gene. While this work was in progress, the cloning of a similar ORF, named MTGR1 (myeloid translocation gene-related protein 1) was reported; it presents an ATG codon, immediately preceded by a stop codon and with no clear Kozak sequence, upstream to and in-frame with the EHT ATG (see Fig 1, top), leading to the coding of additional 29 aa with respect to EHT ORF. Interestingly, Calabi and Cilli have recently deposited in the dbEST data bank a 5' UTR MTGR1 sequence (accession no. AF052212) that is identical to the putative EHT 5' UTR (GenBank clone AA635096). Overall, these data suggest the presence of two alternative 5' ends in the MTGR1/EHT gene, similar to ETO/MITG8 gene. The existence in nature of EHT ORF was confirmed by direct sequencing of a reverse transcriptase-polymerase chain reaction (RT-PCR) amplified fragment encompassing the coding sequence from HL60 mRNA (data not shown).

To map the EHT gene, we generated by PCR a probe from the specific 3' UTR of the EHT gene and screened a human placenta cosmid library (Clontech, Palo Alto, CA), isolating a specific cosmid clone subsequently used as probe in fluorescence in situ hybridization (FISH) analysis on normal human metaphase spreads. By FISH we mapped the gene on chromosome 20q11 (Fig 1, bottom); the mapping was further refined by PCR of DNA from somatic cell hybrids (kindly provided by Mariano Rocchi, University of Bari, Italy) on 20q11.2-20q11.3 (data not shown).

Cytogenetic studies have shown that this region is deleted in ~10% cases of polycythemia vera (PV),~5% myelodysplastic syndromes (MDS), and in ~3% of acute myeloid leukemias (AML).~11 We performed Southern blot analysis on 40 cases of AML (9 M0, 8 M1, 6 M2, 9 M4, 8 M5) and found gene deletion apparently in four cases (1 M0, 1 M1, 2 M4) (10%) (Fig 2A), a frequency higher than that found by conventional cytogenetics. However, the assessment of the deletion frequency needs the analysis of a more representative series. Furthermore, as no RNA was available from hemizygous deleted patients, we were not able to test whether EHT may be a candidate tumor suppressor gene of the region by mutation analysis of the retained allele.

We investigated the expression pattern of EHT by means of Northern blot analysis using the 3' UTR specific probe. Two transcripts of ~9.5 and 7.5 kb (Fig 2B) were detected; the difference in size of the two transcripts may be due to the use of different polyadenylation signals and/or alternative 5' UTR regions, as in the case of the MTGR8 gene. In normal fetal and adult human tissues, the two transcripts were expressed ubiquitously, although at different levels (Fig 2B); in tumoral cell lines, low levels of expression were detected in hematopoietic cell lines of lymphoid and myeloid origin and melanoma cells, while higher levels of expression were found in the SW480 carcinoma cell line, but
apparently absent in the A549 lung carcinoma cell line (data not shown).

As far as normal MTGR1/EHT function is concerned, Kitabayashi et al. showed the direct interaction of MTGR1 and the AML1-MTG8 fusion protein, leading to an enhancement of cell proliferation mediated by granulocyte colony-stimulating factor (G-CSF) in a murine myeloid model (L-G cell line). This suggests that MTGR1 has an oncogenic rather than a tumor suppressor activity. Nevertheless, when MTGR1 is transfected alone in L-G cells, the proliferative response to G-CSF was lower than in the normal control, thus suggesting a possible negative growth-control in normal cells.

In conclusion, the data presented here and those previously reported suggest that the MTGR1/EHT gene may represent a new candidate for the tumor suppressor gene supposed to be involved in the deletion of the 20q11 region in myeloid tumors. Further studies are necessary to rule out this hypothesis.

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A partial cDNA sequence of the EHT gene was deposited by us in the GenBank database under accession number AF039200, on 18-DEC-1997, before the MTGR1 delivery date (January 22, 1998). This work was supported by a grant from Associazione Italiana Ricerca sul Cancro (AIRC) to A.N.

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Rapid Regeneration of Normally Functional Naive CD4+ T Cells After Bone Marrow Transplantation From Unrelated Donors for Combined Immunodeficiency

To the Editor:

Bone marrow transplantation (BMT) is the only curative treatment for children with (severe) combined immunodeficiency (SCID). In the absence of HLA-identical siblings suitable as donors, alternative sources, such as HLA-identical marrow unrelated donors (MUD), should be used. Among the many factors that may influence the speed of T-cell repopulation, the source of the donor may be relevant. However, the majority of data available in the literature on the recovery of the T-cell number and function after BMT deal with transplantation from HLA-identical siblings and only one report described the immunological reconstitution in a series of children, mainly affected by malignancies, receiving T-cell-depleted BMT either from MUD or HLA-haploidentical family donors.

Therefore, we have studied the reconstitution of T-cell number and function after BMT from MUD in eight children affected by (S)CID. Main clinical data are presented in Table 1. No patient developed chronic graft-versus-host disease (GVHD). Phenotypic analysis and proliferative response were evaluated every 1 to 2 months until month +6, then every 6 to 12 months. In vitro T-cell depletion with Campath-1M was performed only in patient GRMA transplanted from an MUD mismatched for one locus HLA-A. Immunological reconstitution in this patient was similar to that observed in other children; data concerning this patient are presented here pooled together with the others, and compared with those from 10 healthy children of similar age, studied as controls.

In the first months after BMT, the striking predominance of CD4+ cells coexpressed the CD45RO molecule, associated with a primed/activated phenotype, whereas naive CD4+CD45RA+ cells were initially rare. On the other hand, CD45RA+ and CD45RO+ subsets were almost equivalent among CD8+ cells (Fig 1). The absolute number of CD45RA+ cells progressively increased, reaching normal levels at month +8 after BMT, whereas that of CD45RO+ cells remained fairly constant (Fig 1). These changes led to a normalization of the proportion of these subsets within 1 year (Fig 1).

A high proportion of activated T cells (CD3+ HLA-DR+) was also observed in the first months after BMT (months 1-4 after BMT: median: 30% [25th-75th percentile: 12-42] vs 3% [2-8] in healthy controls; P < .05), which progressively decreased (r = -.43; P < .02) with normalization of values after month +5.

Proliferative response to phytohemagglutinin (PHA) was reduced in the first months after BMT, and increased progressively (r = .68; P < .001), reaching normal values after month +8 (94,050 cpm [60,650 to 158,300] vs 96,100 [59,150 to 122,600]; P = not significant [NS]). Similar data were observed in cultures stimulated with CD3 monoclonal antibody. The level of proliferative response was significantly correlated with the proportion of CD4+CD45RA+ cells among lymphocytes (r = .61; P = .001), but not with that of CD8+CD45RA+ cells (r = .26; P = NS).

Taken together, these data suggest that the defective proliferative response observed in the first months after BMT is linked to the presence of primed/activated T cells and recovers in parallel with the regeneration of naive CD4+ T cells. Defective lymphocyte proliferation might be caused by increased cell death during the culture: in fact, peripheral blood lymphocyte regenerating after BMT are highly susceptible to spontaneous or “programmed” cell death, as a consequence of defective production of interleukin-2 (IL-2) and downregulation of Bcl-2, and to activation-induced cell death after restimulation with mitogens, strictly correlated with a high level of CD95/Fas expression. Therefore, the T-cell hyperactivated status accounts for their susceptibility to apoptosis and impaired ability to mount a proliferative response, contributing to the genesis of immunodeficiency that follows BMT.

However, our data show a fast regeneration of naive, normally functioning, CD4+ T cells after BMT from MUD and indicate that the lack of host/donor HLA diversity and the possibility to avoid the process of T-cell depletion in this setting allow a full T-cell reconstitution within 8 months from BMT in children affected by (S)CID. Moreover, these observations are in agreement with studies reporting that the ability to regenerate naive CD4+ T-cell number after allogeneic BMT or after intensive chemotheraphy is optimal in children and inversely correlated with age. This can be explained by the essential role of the thymic-dependent pathway, still operating in the first years of postnatal life, but limited with advancing age, in the process of T-cell regeneration.

Our observations are reflected clinically in a recent report of the European registry: overall survival of children with primary immunodeficiency.
EHT, a New Member of the MTG8/ETO Gene Family, Maps on 20q11 Region and Is Deleted in Acute Myeloid Leukemias

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