Osteogenesis imperfecta calls for caution

To the editor—In the March issue of *Nature Medicine*, Horwitz and colleagues presented results of bone marrow transplantation (BMT) in three children with osteogenesis imperfecta[1] (OI). Their results should be interpreted with caution.

Growth, bone density, histology and fracture number are important outcome parameters, but are not simple to measure accurately and reproducibly in children with severe OI. For example, using simple crown-to-heel measurements, it is difficult to distinguish increased length due to growth and increased length due to decreased contractures after physical therapy. Their patient 3 was followed independently at the NIH Clinical Center. Lumbar bone density (L1–L4) measured 4 months before and 9 months after BMT shows no significant change (0.133 gm/cm^2 to 0.115 gm/cm^2; –7.4 s.d. compared with age-matched controls).

The most troubling aspect of this article, however, is the contrast between the low level of osteoblast engraftment achieved and the dramatic changes reported in skeletal parameters. The iliac crest-derived osteoblasts for patients 1 and 2 were culture-expanded in my laboratory and tested at p1. Although I am confident of the 1.5% engraftment in patient 1 and of the mesenchymal type of the cells, I am doubtful that the presence of 1.5–2% normal osteoblasts could lead to a fourfold increase in osteoblast number, a 45–78% increase in bone mineral content and substantial growth.

Although osteoblast replacement is a valid approach for the treatment of OI, the level of engraftment required for clinical success is unknown. This assertion is based on data from mosaic carriers of OI. These individuals have a post-zygotic mutation, as evidenced by the occurrence of the collagen mutation in some but not all of their cells. As they are themselves clinically unaffected or have only very mild symptoms, they are usually recognized when they produce children with full expression of the mutation and a severe skeletal phenotype. The mosaic parents have a great deal to teach us about developmental patterns and the goals of gene therapy at the bone level. Although parental mosaicism is not rare in OI, molecular data on mosaic individuals is, having been reported in only 18 cases. Three of these have a high percent (approaching 100%) mutant cells in dermal fibroblasts, four have intermediate levels (50–75%) and five have undetectable levels[2–4]. Significantly, each mosaic individual has variation in the level of mutant cells in different tissues. Clearly, to determine the levels of normal cells required to affect skeletal phenotype, one must examine bone. However, neither bone tissue nor osteoblasts have been studied in any mosaicism cases. Thus, we lack the human context required before we can conclude what extent of osteoblast engraftment should be the goal of OI therapy. Bone data from mosaic parents can answer this important question.

Horwitz *et al.* suggest that normal collagen fibrils will be preferentially incorporated into and retained by bone matrix, allowing a small number of cells to dominate the composition of the bone matrix. The limited *in vitro* and *in vivo* data on bone matrix composition in OI reveals a complex structure with quantitative abnormalities of several noncollagenous proteins[5], as well as structurally abnormal collagen. Normal collagen may not have a selective survival advantage compared with mutant collagen at the bone matrix level. In several cases, mutant collagen was more efficiently incorporated into bone and into matrix deposited by osteoblasts in culture than into dermal matrix[6]. We have unpublished data showing that normal and mutant collagen 'chase' from cultured osteoblast matrix at the same rate (A. Forlino and J.C.M., unpublished data), consistent with the random assortment of collagen helices to form fibrils in the extracellular matrix.

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To the editor—Horwitz and colleagues are to be congratulated on the successful engraftment of mesenchymal cells from donor marrow[1]. However, the clinical changes they reported require careful evaluation, particularly when considering the potentially hazardous nature of the procedure and the availability of an effective alternative treatment such as bisphosphonates.

Bone biopsy in infants with osteogenesis imperfecta (OI) is technically difficult. The fragmentation of the pre-treatment biopsies may have prevented accurate assessment of osteoblast numbers, as the cells were identified by site and morphology rather than by osteoblast-specific stains.

Bone mineral content increased rapidly. Although weight is a factor that accounts for the most variation in bone mineral content in regression analyses, weight subsumes the contemporaneous effect of increasing length in normal children in such analyses. Bone densitometry in childhood is confounded by the nature of the measurement, such that larger children of the same age will have apparently increased bone mineral content and density. Thus, the increase in length of 5% in each child may have accounted for a proportion of the increase in bone mineral content.

The fracture rate in infancy is highest in severe OI between birth and six months of age, falling thereafter. The apparent decline may simply have been part of a continuing pattern, and a comparison with the fracture rate six months before transplant rather than from birth would give a clearer picture of the effect of the procedure on fracture rates.

Finally, I understood from Dr. Horwitz that more than three children were enrolled in this program. Would it be reasonable to ask whether the other recipients did as well as the three children described in the article?

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Horwitz replies—We fully agree that interpretation of clinical benefit in any pilot study must be made cautiously. In our study[1], we reported engraftment of marrow-derived mesenchymal cells in the bones of children with severe OI undergoing an allogeneic bone marrow transplant, and correlated this engraftment with evidence of improvement in the specified clinical parameters. Bishop correctly indicates the technical difficulty of performing bone biopsies in infants with OI. However, the specimens we obtained showed obvious differences in osteocyte arrangement with the formation of lamellar bone after transplant. Tetracycline labeling was smeared throughout the specimen before transplant, which we attribute to abnormal bone formation, and became much more crisp after the transplant. We agree that in an uncontrolled pilot study of this type, measurement of any single clinical parameter before and after transplant may produce misleading results. However, we found improvements in every parameter measured. In particular, fracture rates declined sharply compared with both