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

the preceding 6 months and the first 6 months of life.

Marini is concerned that there was no measurable increase in lumbar bone density. However, bone mineralization is not uniform throughout the skeleton. Furthermore, bone density measurements may not be the most appropriate means to express bone mineralization in pediatrics[7]. For this reason, we chose to measure changes in total bone mineral content with dual energy X-ray absorptiometry. This is a more sensitive assay of overall bone mineralization than measurements of the spine, which is only a small portion of the skeleton. Marini also confirms the presence of mesenchymal engraftment, but questions how so low a level of engraftment can produce the profound changes seen. We agree that this is puzzling, and in our article and the accompanying *News & Views* article[8], several possible mechanisms were proposed. Marini discounts many of these based on the fact that they are not consistent with predictions made after studying mosaic carriers of OI. It is an equally valid argument, however, that mosaic carriers of OI simply fail to accurately model the effects of normal osteoblasts in children with severe disease.

Finally, Bishop correctly states that other children with HLA-compatible siblings have been enrolled subsequently in our protocol. There are two such additional children and both demonstrated histologic and clinical findings similar to the reported patients, corroborating our initial results. We emphasize that our work represents only the 'proof of principle' of mesenchymal cell engraftment, and encourages future investigations of both cell and gene therapy of mesenchymal disorders. Only extensive study of carefully selected patients will demonstrate whether or not such therapies are the optimal treatment for children with severe osteogenesis imperfecta.

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Adeno-associated virus 2 co-receptors?

To the editor—We were surprised to read two papers in the January issue of *Nature Medicine* claiming both human fibroblast growth factor receptor[1] (FGFR) and αVβ5 integrin[2] as co-receptors for adeno-associated virus 2 (AAV2) infection.

FGFR is the high-affinity receptor for basic fibroblast growth factor[3] (bFGF), although it has been shown that low-affinity binding to heparan sulfate molecules is prerequisite for the binding of bFGF with FGFR (ref. 4). This interaction between FGFR and heparan sulfate led to the proposal that herpes simplex virus, which uses heparan sulfate as an attachment molecule[5], may use FGFR as its cellular receptor[6]. Qing *et al.* made an analogous suggestion[1] about the binding of AAV2 to FGFR. The data on the relationship between HSV/FGFR and AAV2/FGFR were almost identical: bFGF was able to compete the viral binding and infection, and transfection of FGFR cDNA was capable of increasing viral binding and infection in 'nonpermissive' cells. However, subsequent research has shown that FGFR is not required (e.g., ref. 7) for HSV infection. Part of the viral-inhibiting properties of the bFGF is due to competition with binding to cell surface heparan sulfate, and cell lines such as Hep-2 and A431 that do not have FGFR on their cell surface are fully susceptible to HSV. We have done similar experiments for AAV2 (Table). Flow cytometry confirmed the absence of FGFR on Hep-2 or A431 cells[7], yet they were readily transduced by rAAV2, showing that FGFR is also not required for AAV2 infection.

In the paper by Summerford *et al.*[2], αVβ5 integrin was identified as a co-receptor for AAV, mainly on the basis of a viral overlay reported to show binding to denatured β5. Using the same viral overlay technique, we found a strongly binding, 150-kDa AAV2 binding protein[8]. We have also identified a weaker band at about 110 kDa, and have identified the protein as human nucleolin, based on peptide sequencing and antibody detection[9]. However, using viral overlay, we were unable to detect any binding of AAV2 to purified or recombinant β5 integrin. In addition, despite the title of the Summerford paper ("αVβ5 integrin..."), the gels seem to have been truncated so that binding to αV (150 kDa) could not be seen. This is surprising, because for adenovirus, most of the viral interaction is through the αV motif, and any β subunit can substitute (although typically αVβ3 is used for attachment and internalization)(ref. 10). In addition, adenovirus binding seems to depend on conformation and RGD motif and seems to be not detected by viral overlay. Finally, the transfection of β5 cDNA only increased by 260% the transduction of AAV2 infection, with significant transduction in CS cells (which lack β5). As in the Qing paper[1], the ability to significantly infect or transduce cells that do not have the putative receptor suggests that these molecules are not essential in initiation of AAV2 infection.

Table FGFR and AAV2

Cell line	FGFR (mAb VBS1)	% rAAV2 transduction
HeLa S3	++	60
Hep-2	-	40
A431	-	10
U937	-	0
Mo7e	+	0
CHO-K1	++	70

Analysis of FGFR on cell surfaces, using flow cytometry with monoclonal VBS1 (Chemicon, Temecula, California). AAV2 transduction was done by infection with rAAV-LacZ (6.5×10^5 genome copies/cell); β-galactosidase positive cells were counted 2 days later.

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