The effect of cyclical intravenous pamidronate in children and adolescents with osteogenesis imperfecta Type V

Leonid Zeitlin, Frank Rauch⁎, Rose Travers, Craig Munns, Francis H. Glorieux

Genetics Unit, Shriners Hospital for Children and McGill University, 1529 Cedar Avenue, Montréal, Québec, Canada H3G 1A6

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Abstract

Intravenous treatment with pamidronate is beneficial in children and adolescents with moderate to severe forms of osteogenesis imperfecta (OI) types I, III and IV, but there is little information on the effects of this treatment on the newly described OI type V. Here, we describe the results of 2 years of pamidronate treatment in 11 children and adolescents with OI type V (age at start of therapy 1.8 to 15.0 years; 6 girls). Pamidronate was given in intravenous cycles at a cumulative yearly dose of 9 mg/kg. The first infusion cycle was associated with fever and mild hypocalcemia in most patients, but no other short-term side effects were noted. Two years of pamidronate treatment led to a decrease in the urinary excretion of N-terminal telopeptide of type I collagen to 50% of baseline levels. Both the size and volumetric bone mineral density of lumbar vertebrae increased compared to age- and sex-matched reference data (\(P_{\text{b}} < 0.05\) in both cases). Histomorphometry of transiliac bone samples showed an average increase of 86% in cortical thickness (\(N = 7\); \(P = 0.005\)). No significant changes with treatment were observed in the age-related \(z\) scores of isometric maximal grip force and height. Fracture incidence decreased from 1.5 fractures per year before treatment to 0.5 fractures per year during the first 2 years of treatment. Ambulation status improved in four patients and remained unchanged in the others. In conclusion, the intravenous pamidronate therapy has a similar effect in OI type V as it has in the other OI types.

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Introduction

Osteogenesis imperfecta (OI) type V is a newly described form of brittle bone disease [1]. Similar to other types of OI, type V disease is characterized by low bone mass, bone fragility and deformities. Clinical features that set it apart as a distinct entity are a predisposition to develop hypertrophic callus after fractures, calcification of the interosseous membrane at the forearm, hyperdense metaphyseal bands and a lack of tooth involvement [1]. On the histologic level, there is a peculiar abnormality in bone lamellation, which is irregular and appears mesh-like. OI type V is inherited in an autosomal-dominant fashion, but mutations affecting collagen type I are absent. Disease severity may vary from mild non-deforming to progressively deforming [1]. The majority of patients fall into the category of moderately severe OI, comparable to OI type IV.

It has been reported by a number of investigators that cyclical intravenous therapy with the bisphosphonate pamidronate has a beneficial effect in children and adolescents with OI types III, IV and severe type I (reviewed in [2]). Pamidronate therapy was observed to increase muscle force, vertebral bone mass and cortical thickness, but also to suppress bone turnover.

Published information on the effects of intravenous pamidronate treatment in children and adolescents with OI type V is presently limited to the case report of a 9-year old girl whose fracture frequency decreased after pamidronate was started [3]. In the present study, we therefore assessed the effects of pamidronate treatment in children and adolescents with OI type V. The results were compared to...
those of patients with OI types I, III and IV who had a similar severity of the disease.

**Patients and methods**

**Patients**

The study group comprised children and adolescents with OI type V who received cyclic intravenous pamidronate therapy at the Shriners Hospital for Children in Montreal, Canada. The diagnosis of OI type V was based on the criteria as described [1]: calcification of interosseous membrane of forearms; tendency to develop hypertrophic callus formation after fractures; “mesh-like” pattern of bone lamellation on polarized light microscopy; negative results of molecular diagnosis for collagen I mutations. Patients were eligible for pamidronate treatment if they had long-bone deformities or had suffered at least two fractures per year (including vertebrae) during the previous 2 years [4,5].

Among the 16 patients who were diagnosed with OI type V at our institution during the past 10 years, 13 fulfilled the criteria for treatment with pamidronate. One of these is receiving treatment at another hospital and in one patient the clinical picture is compounded by severe cerebral palsy. The remaining eleven patients with OI type V were included in the present analysis. All of them completed at least 2 years of pamidronate treatment.

The patients of the OI control group were selected from the population of children with OI types I, III and IV who had received pamidronate treatment at this hospital for at least 2 years. One OI control patient was selected for each patient with OI type V, based on age and criteria reflecting disease severity at the start of pamidronate therapy (height z score and bone densitometric results). Nine subjects in the OI control group had OI type IV, one had OI type I and one was diagnosed with OI type III. The type of OI was assigned using the Sillence criteria [6]. However, the type IV group did not include patients who fulfilled Sillence criteria for this type, but could be further classified as having OI type V, VI or VII on the basis of our expanded classification [2].

**Treatment**

Pamidronate was administered intravenously on three consecutive days in all patients. As described earlier [5], the clinical effect of the infusions (suppression of bone pain, sense of well-being) was more short-lived in younger children. Therefore, the timing and dosage of these 3-day cycles varied with age. Children below 2 years of age received 0.25 mg/kg on the first day of the first cycle, 0.5 mg/kg on days 2 and 3 of the first cycle and 0.5 mg/kg daily on all 3 days in subsequent cycles. Cycles were repeated every 12 weeks. Above 3 years of age, the first 3-day cycle consisted of a dose of 0.5 mg/kg on the first day and 1 mg/kg on days 2 and 3. In subsequent cycles the dose was 1 mg/kg daily for 3 days. Cycles were repeated every 16 weeks. Thus, the yearly dose of the drug was the same at all ages. Each dose was diluted in 0.9% saline solution and administered slowly over 4 h, as described [4,5].

Calcium and vitamin D intake was maintained as adequate according to the recommended daily allowance in all patients. All patients underwent physiotherapy and occupational therapy evaluation and support, including exercises and design of special devices for transportation and sitting.

Most of OI patients with long bone deformities undergo corrective osteotomies with insertion of metal rods into long bones of upper or lower extremities [7]. One patient in the OI type V group and five patients of the OI control group underwent rodding surgery of lower limbs during the first 2 years of pamidronate treatment.

**Clinical evaluation**

Height was measured with a Harpenden stadiometer. Height measurements were converted to age- and sex-specific z scores on the basis of reference data published by the Centers for Disease Control and Prevention [8].

Maximal isometric grip force of the nondominant hand was determined with a standard adjustable-handle Jamar dynamometer (Preston, Jackson, MI, USA), as described [9]. The maximal value of three trials was noted.

Information about skeletal fractures, including vertebral compressions, was retrieved from medical charts. Only fractures confirmed by X-ray s were counted. The incidence of fractures during 2 years before and 2 years after the beginning of treatment was annualized.

Ambulation was evaluated for each child by physiotherapists experienced in the care of children with bone diseases. A 5-point score was used: 0 (bed-wheelchair-bound), 1 (walking with aids possible, but non functional), 2 (household walker, with/without aids), 3 (neighborhood walker with/without aids) and 4 (independent walker) [10].

**Bone densitometry**

Bone densitometry was performed in the antero-posterior direction at the lumbar spine (L1–L4) using a Hologic QDR 2000W or 4500A device (Hologic Inc., Waltham, MA). Volumetric bone mineral density (vBMD) was calculated as the ratio between bone mineral content and the extrapolated external volume of the measured bones. This was done as described by Carter et al. [11] using the formula:

\[ \text{vBMD} = \frac{\text{bone mineral content}}{\text{projection area}}^{1.5} \]

Age- and sex-specific mean values for projection area, bone volume and vBMD in healthy children were calculated from published densitometric reference material for BMC.
and areal BMD [12,13]. These reference data cover the age ranges from birth to 25 months of age [12] and from 8 to 17 years [13], respectively. The reference mean values for children between the ages of 25 months and 8 years were derived by linear interpolation between the two data sets. Results for bone mineral content, bone volume and vBMD in OI patients were expressed as a percentage of these mean values for healthy children.

Biochemical measurements

Serum total calcium, phosphate and alkaline phosphatase activity were measured using colorimetric methods (Monarch®; Instrumentation Laboratories Inc., Lexington, MA, USA). Serum parathyroid hormone concentrations (fragments 39–84) were determined by radioimmunoassay [14]. 25-OH vitamin D and 1,25-DH vitamin D were measured with radioimmunoassays (25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D Osteo SP; Incstar Corp., Stillwater, MN, USA). Urinary calcium and creatinine (uCr) were quantified colorimetrically. The bone resorption marker urinary cross-linked N-telopeptide of type I collagen (uNTX) was quantified by enzyme-linked immunoabsorbent assay (Osteomark®; Ostex, Seattle, WA) on the second void sample of the morning. Results for uNTX/Cr ratios were compared with published reference data [15]. Patients were fasting at the time of blood and urine sampling.

Histomorphometry

Full-thickness transiliac bone biopsy specimens were obtained at baseline and after 2 to 4 years of treatment. A Bordier trephine (5 or 6 mm core diameter) was used under general anesthesia, from a site located 2 cm below and behind the anterior superior iliac spine. Transiliac bone samples were collected on days 4 or 5 after dual labeling with demeclocycline (15 samples were collected on days 4 or 5 after dual labeling behind the anterior superior iliac spine. Transiliac bone biopsy specimens were obtained at baseline and after 2 to 4 years of treatment. A Bordier trephine (5 or 6 mm core diameter) was used under general anesthesia, from a site located 2 cm below and behind the anterior superior iliac spine. Transiliac bone samples were collected on days 4 or 5 after dual labeling with demeclocycline (15–20 mg/kg per day taken orally during two 2-day periods separated by a 10-day free interval). Biopsy preparation and histomorphometric analyses were performed using procedures that have been described previously [16]. Measurements were carried out using a digitizing table with Osteomeasure® software (Osteometrics Inc., Atlanta, GA). Nomenclature and abbreviations follow the recommendations of the American Society for Bone and Mineral Research [17].

In four patients, one of the two paired biopsy specimens was not sufficiently preserved for histomorphometric analysis. In one patient, only the external cortex of the pretreatment sample could be analyzed. In an additional three patients, the cancellous compartment was not entirely preserved in one of the two paired specimens.

Statistical analyses

Differences between OI type V and OI-control groups were tested for significance using unpaired t test or Mann–Whitney U test, as appropriate. Paired t test or Wilcoxon test was used to analyze changes during treatment. The chi-square test was used to evaluate differences in frequency of clinical signs between the groups. All tests were two-tailed, and throughout the study, \( P < 0.05 \) was considered significant.

Results

Baseline clinical characteristics of the OI type V and OI control groups are presented in Table 1. Calcification of the forearm interosseous membrane as well as dislocation of radial heads caused limited rotation of forearms in all subjects with OI type V. Mutations in collagen I genes were found in 10/11 (91%) patients of the OI control group but in none of the patients with OI type V.

Mineral metabolism

At baseline all children with OI type V had serum levels of calcium, phosphorus, 25-OH vitamin D and parathyroid hormone within the reference range, similar to the OI control group.

During the first 3 days of the initial infusion cycle, total serum calcium dropped by 0.44 ± 0.05 mmol/l (mean ± SE) to reach a nadir of 1.92 ± 0.04 mmol/l. Serum calcium had returned to baseline values by the time the patients were readmitted for the second infusion cycle 2 to 4 months later. Fluctuations in serum calcium were smaller during subsequent treatment cycles (decreases of 0.30 ± 0.03 mmol/l and 0.18 ± 0.04 mmol/l during the second and third cycles,

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics of type V OI and OI control groups at the start of pamidronate therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type V OI</td>
</tr>
<tr>
<td>N (m/f)</td>
<td>11 (5/6)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>8.7 (1.8 to 15.0)</td>
</tr>
<tr>
<td>Height (z score)</td>
<td>−2.6 (−6.0 to 0.7)</td>
</tr>
<tr>
<td>vBMD (% of healthy mean value)</td>
<td>54 (18 to 81)</td>
</tr>
<tr>
<td>Long bone deformities (n [%])</td>
<td>10 (90)</td>
</tr>
<tr>
<td>Vertebral compressions (n [%])</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Scoliosis (n [%])</td>
<td>8 (73)</td>
</tr>
<tr>
<td>Dentinogenesis</td>
<td>0</td>
</tr>
<tr>
<td>Imperfecta (n [%])</td>
<td>7 (64)</td>
</tr>
<tr>
<td>Hypertrophic callus (n [%])</td>
<td>11 (100)</td>
</tr>
<tr>
<td>Calcification of forearm interosseous membrane (n [%])</td>
<td>4 (36)</td>
</tr>
<tr>
<td>Dislocation of radial head (n [%])</td>
<td>11 (100)</td>
</tr>
</tbody>
</table>

\( P \) values represent the significance of difference between the two groups (Mann–Whitney U test or chi square test, as appropriate).
respectively). Hypocalcemia was not associated with clinical symptoms and was treated with oral calcium supplements. In no case was intravenous calcium therapy required.

The decrease in serum calcium was associated with an increase in serum parathyroid hormone levels, reaching twice the upper limit of the reference range on day 3 of infusion cycle one. However, levels consistently returned to pre-treatment results by the time of the next treatment cycle. The transient rise in parathyroid hormone levels was associated with the expected transient decrease in serum phosphorus (by 0.64 ± 0.09 mmol/l) and a tripling in 1,25(OH)2 vitamin D concentrations (from 72 ± 14 pmol/l to 225 ± 22 pmol/l; n = 7). These short-term fluctuations in mineral metabolism were similar to those in the OI control group (data not shown).

The long-term effects of pamidronate on biochemical results were evaluated in samples that were obtained immediately before a treatment cycle. During the first 2 years of pamidronate therapy, serum levels of calcium, phosphate and 25-OH vitamin D, as well as urinary excretion of calcium remained stable. However, serum parathyroid hormone levels tended to increase (by 50%, range −27% to 166%, P = 0.06). The OI control group showed a similar rise of parathyroid hormone (by 31%; range −23% to 350%, P = 0.02) that was associated with stable measures of mineral metabolism.

Bone metabolism

At baseline, uNTX/Cr ratios were 43 ± 14% (P = 0.02) above the mean for age- and sex-matched healthy children. The average serum alkaline phosphatase activity was above the upper limit of the reference range (300 U/l) at 436 ± 160 U/l.

Pamidronate had a marked short-term effect on uNTX/Cr. By day 3 of the first treatment cycle, values had dropped to 22 ± 4% of the baseline value (P < 0.001). When patients returned for the second infusion cycle three to four months later, uNTX/Cr had re-increased to 68 ± 10% of the pretreatment level (Fig. 1). Analysis of samples that were obtained immediately before a treatment cycle revealed a constant decrease in uNTX/Cr during the first 2 years of treatment (Fig. 1). Alkaline phosphatase levels changed less than those of uNTX/Cr, but were significantly below baseline values after 2 years of therapy (Fig. 1).

The OI control group had similar results for uNTX/Cr, both before and during pamidronate therapy. Alkaline phosphatase activity was lower than in OI type V at baseline and remained so during the 2 years of therapy. However, the relative changes in alkaline phosphatase activity during treatment were similar in the OI type V and control groups.

Iliac bone histomorphometry

Cortical thickness was low at baseline, but increased by 86% during therapy (Table 2; Fig. 3). Cancellous bone volume also appeared to increase, but this was based on only three sample pairs.

Regarding bone formation markers, all bone-surface related measures (osteoid surface, osteoblast surface, mineralizing surface, bone formation rate) decreased markedly during pamidronate treatment. In particular, bone formation rate dropped to about 10% of the pretreatment level. Mineral apposition rate did not change significantly. Mineralization lag time was prolonged during pamidronate therapy, but osteoid thickness did not change. None of the patients had signs of osteomalacia (which is defined as an increase in both osteoid thickness and mineralization lag time).

Bone resorption parameters varied over a wide range, both before and during pamidronate treatment. No significant changes were found with therapy.

Grip force

Before the start of pamidronate therapy, all OI type V patients had a grip force below the age- and sex-specific mean value for healthy children (z score: median −2.7; range −5.4 to −1.6). No significant changes were noted after 2 years of treatment (z score median −2.8; range −4.6 to −1.9).
Grip force tended to increase in the OI-control group (from a pretreatment median z score of −2.1 [range −7.0 to −0.2] to −0.6 [range −8.1 to 0.1]; \( P = 0.13 \)).

**Effect on growth**

Most patients had a height below the 5th percentile for healthy children, but growth rates appeared to be close to normal during pamidronate therapy (Fig. 4). Therefore, the median height z score did not change significantly (\( P = 0.66 \)). A trend towards higher height z scores was noted in the control group (\( P = 0.07 \)).

**Clinical response**

The majority of patients underwent the well-known acute phase reaction after the first infusion, with short-term elevation of body temperature up to 38.5°C [4,5,18]. No other side effects were noted. Bone pain decreased markedly after the first pamidronate cycle, as had also been observed in other types of OI [4,5,18]. Fracture incidence decreased from 1.5 fractures per year before treatment to 0.5 fractures per year during the first 2 years of treatment (Table 3). Fracture rates also decreased in five of the seven children who remained prepubertal.

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>Pre-treatment</th>
<th>During treatment</th>
<th>( P )</th>
<th>n</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone structure</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cortical width (μm)</td>
<td>7</td>
<td>495 ± 196</td>
<td>921 ± 396</td>
<td>0.005</td>
<td>6</td>
<td>758 ± 241</td>
</tr>
<tr>
<td>Bone volume per tissue volume (%)</td>
<td>3</td>
<td>4.4 ± 0.9</td>
<td>20.5 ± 2.7</td>
<td>0.01</td>
<td>6</td>
<td>21.7 ± 3.9</td>
</tr>
<tr>
<td>Bone formation</td>
<td></td>
<td></td>
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<tr>
<td>Osteoid thickness (μm)</td>
<td>6</td>
<td>4.2 (2.8–13.6)</td>
<td>4.0 (2.9–4.4)</td>
<td>0.46</td>
<td>6</td>
<td>6.3 (4.4–8.5)</td>
</tr>
<tr>
<td>Osteoid surface per bone surface (%)</td>
<td>6</td>
<td>53 ± 18</td>
<td>20 ± 12</td>
<td>0.001</td>
<td>6</td>
<td>29 ± 11</td>
</tr>
<tr>
<td>Osteoblast surface per bone surface (%)</td>
<td>5</td>
<td>12 (5–75)</td>
<td>3 (0–6)</td>
<td>0.04</td>
<td>6</td>
<td>9 (3–15)</td>
</tr>
<tr>
<td>Mineralizing surface per bone surface (%)</td>
<td>6</td>
<td>20 (8–81)</td>
<td>2 (1–4)</td>
<td>0.03</td>
<td>5</td>
<td>13 (6–21)</td>
</tr>
<tr>
<td>Mineral apposition rate (μm/day)</td>
<td>6</td>
<td>0.98 ± 0.33</td>
<td>0.74 ± 0.15</td>
<td>0.21</td>
<td>5</td>
<td>0.93 ± 0.08</td>
</tr>
<tr>
<td>Mineralization lag time (day)</td>
<td>6</td>
<td>12 (9–26)</td>
<td>62 (5–216)</td>
<td>0.12</td>
<td>5</td>
<td>16 (9–27)</td>
</tr>
<tr>
<td>BFR per bone surface (μm² × μm² × year⁻¹)</td>
<td>6</td>
<td>61 (21–444)</td>
<td>6 (1–14)</td>
<td>0.03</td>
<td>5</td>
<td>44 (29–78)</td>
</tr>
<tr>
<td>Bone resorption</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Eroded surface per bone surface (%)</td>
<td>6</td>
<td>18 ± 8</td>
<td>17 ± 10</td>
<td>0.87</td>
<td>6</td>
<td>18 ± 7</td>
</tr>
<tr>
<td>Osteoclast surface per bone surface (%)</td>
<td>4</td>
<td>1.0 ± 0.6</td>
<td>0.6 ± 0.4</td>
<td>0.46</td>
<td>6</td>
<td>1.3 ± 0.7</td>
</tr>
</tbody>
</table>

Values are mean ± SD or median (range). \( P \) values represent the significance of difference between results before and during treatment (paired \( t \) test or Wilcoxon test, as appropriate). BFR: Bone formation rate.
throughout the study period. Fracture incidence similarly decreased in the control group.

During the study period, ambulation score improved in four patients with OI type V and remained unchanged in one patient (Table 3). One patient was not assessed because of young age. The other five patients were independent walkers (grade 4) before pamidronate treatment was started and remained so during the observation interval. In the control group, four patients gained one to three grades and in two patients no progress was noted. The other five patients were independent walkers before and after 2 years of treatment.

### Discussion

This study provides evidence that 2 years of cyclical therapy with intravenous pamidronate has a beneficial effect in patients with OI type V. Although OI type V is a distinctive entity with specific clinical, histological and molecular features, the response to pamidronate treatment is generally similar to that of other OI patients with comparable disease severity.

As to safety, no major side effects were noted within the 2 years of follow up. The most common adverse event was the acute-phase reaction that affects the majority of children.
during the first treatment cycle [4]. Similar to the other OI types, hypocalcemic episodes after infusions were mild in OI type V and induced the expected conterregulatory increase in parathyroid hormone and 1,25 (OH)2 vitamin D levels [19]. These transient increases in parathyroid hormone levels conceivably may contribute to the therapeutic effect of the treatment regimen, as intermittent injections of parathyroid hormone increase bone mass, at least in adults with postmenopausal osteoporosis [20]. None of the patients developed radiographic or histological signs of a mineralization defect and linear growth was not affected. However, pamidronate therapy was associated with a substantial decrease in cancellous bone remodeling activity, as shown by both biochemical bone markers and iliac bone histomorphometry. This is a possible cause of concern, as the long-term consequences of chronically suppressed bone turnover in children are unknown at present.

Regarding the therapeutic effects of pamidronate treatment, bone mass increased almost two-fold during 2 years of pamidronate therapy. This was due to an increase in both bone size and in vBMD. The increase in vBMD may partly be due to cortical thickening, as shown by the histomorphometric data. Higher trabecular bone volume during pamidronate treatment may also have contributed to increase vBMD, although the number of informative biopsy samples was insufficient to gain certainty on this point. Radiographic observations suggest that the density of vertebral bodies increases most at locations that are close to the growth cartilage (end plates) [4]. These sclerosing changes will also contribute to increase vBMD at the lumbar spine.

Although higher bone mass and density cannot be simply equated to improved bone strength, it is likely that these changes contributed to decrease fracture incidence during pamidronate therapy. It must be recognized, however, that fracture rates may decrease also in patients who do not receive medical therapy, especially after puberty [21]. In any case, fracture incidence may be a rather weak outcome parameter in children with OI, as more opportunities for accidents and fractures arise with improving mobility.

Ambulation scores improved in 4 of the 5 previously non-ambulating OI type V patients. In an observational study such as the present one, it is not possible to prove that better mobility is due to pamidronate treatment. Indeed, some improvement in ambulation can also be achieved in patients who receive rehabilitation and orthotic care only [22]. However, it is reasonable to assume that rehabilitation programs are more effective when pain and fracture risk are lower under pamidronate therapy.

Thus, OI type V patients generally responded similar to pamidronate therapy as patients with other OI types. Pamidronate treatment appears to be beneficial in these disorders regardless of the differences in disease etiology. However, grip force z scores increased less with therapy in OI type V patients than in the OI control group. This difference might be explained by the limited range of forearm motion in the OI type V patients which results from the calcification of the interosseous membrane and dislocation of radial heads.

In conclusion, bone mineral mass markedly increased and fracture rate decreased in children and adolescents with OI type V during 2 years of pamidronate treatment. These changes were similar to changes observed in other types of OI with similar disease severity. There were no significant side effects during the time of follow-up. The long-term effect of low bone remodeling needs to be further evaluated.

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References


