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Title: The clinical application of rh-BMP7 for reconstruction of alveolar cleft; 10 years follow up

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Abstract:
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To report on a 10-year assessment following the application of rhBMP-7 for reconstruction of alveolar cleft.
Method:
This study was conducted as prospective phase II clinical trial on 9 unilateral and two bilateral alveolar clefts which received rhBMP-7 (Osigraft). The mean age of patients at surgery was 10.4 years. Six months postoperatively, occlusal radiographs were taken to evaluate bone formation at the cleft site. Patients were followed up within the routine cleft care pathway for up to 10 years to monitor the impact of BMP-7 on orthodontic treatments and maxillary growth. Radiographs were taken according to the standard cleft care protocol.
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The radiographic assessment of the UCLP cases suggested good bone formation with a Kindelan score at grade 1 or 2. The bilateral alveolar cleft cases had a score of grade 3 or 4 indicating failure or partial failure. The children with successful grafts underwent a routine orthodontics follow-up without incident. The maxillary growth appeared similar to that of cases grafted with autogenous bone. No long term complications or abnormal pattern of bone formation were detected.
Conclusions:
The study provides unique evidence on the long term safety of rhBMP-7 when applied at the area of skeletal immaturity for the reconstruction of alveolar clefts in children.

Cover Letter

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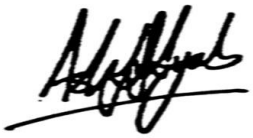
28th August 2018
The Editor JOMS

Dear Sir

Please find attached the revised manuscript titled: **The clinical application of BMP for reconstruction of alveolar cleft; 10 years follow up**

We found the reviewers comments valuable; we addressed the raised concerns which have been highlighted in the resubmitted manuscript.

Best regards



Ashraf F Ayoub

***Revision Notes**

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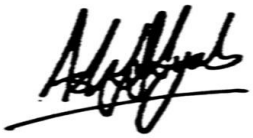
We carried out the required changes regarding the references and the figures; these were included in the revised version of the manuscript.

Best regards



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*Title Page

The clinical application of BMP for reconstruction of alveolar cleft; 10 years follow up

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The clinical application of rh-BMP-7 for reconstruction of alveolar cleft; 10 years follow up

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The radiographic assessment of the UCLP cases suggested good bone formation with a Kindelan score at grade 1 or 2. The bilateral alveolar cleft cases had a score of grade 3 or 4 indicating failure or partial failure. The children with successful grafts underwent a routine orthodontics follow-up without incident. The maxillary growth appeared similar to that of cases grafted with autogenous bone. No long term complications or abnormal pattern of bone formation were detected.

Conclusions:

The study provides unique evidence on the long term safety of rhBMP-7 when applied at the area of skeletal immaturity for the reconstruction of alveolar clefts in children.

Introduction:

Autogenous bone graft is the gold standard for the reconstruction of alveolar cleft which is associated with cleft lip and palate.[1] However, harvesting of bone graft is associated with well documented morbidities including pain, scarring, alteration in sensation and swelling.[2] Researchers worldwide are interested in discovering alternative treatment modalities, based on the recent advances in tissue bioengineering, to avoid these morbidities. The use of the osteoinductive growth factors including bone morphogenetic proteins (BMPs) has been considered for the reconstruction of the alveolar clefts. BMPs stimulate osteogenesis for the healing of skeletal fractures, the osteoinductive abilities of BMP-2 and BMP-7 are well documented.[3,4]

Bone morphogenetic protein 2 (BMP-2) has been used for the reconstruction of alveolar cleft defects over the last 10 years.[5-7] The quality and quantity of bone formation in alveolar cleft is dependent on the dose of the cytokines that is locally applied at the cleft site. BMP-2 at a low dose of 50Ug ml^{-1} failed to induce bone formation, whereas, up to 59% of the surgical defect was reconstructed with new bone formation when the concentration of BMP-2 was raised to 250ug ml^{-1}. However, this was associated with severe postoperative gingival swelling during the first week following surgery.[8] The development of a localized swelling following the application of BMP-2 is well documented.[9-11]

Comparative analysis between BMP-2 with autologous bone harvested from the iliac crest for the reconstruction of alveolar clefts has been carried out. The doses of the BMP-2 ranged from 1.05–1.5 mg ml^{-1} which was delivered by type I collagen sponge. In all the studies BMP-2 was successful in inducing bone formation comparable to the cases which received autogenous bone grafts.[5,7,8]

There is a concern of using BMP in the pediatric population and the possibility of induction of malignancy. The Food and Drug Administration has licensed the clinical application of recombinant BMP-7 (rhBMP-7) for the non-union of long bone fractures and spinal fusion.[12] The clinical applications of BMP-7 in the maxillofacial region are limited, but it has been utilized in some studies following the elevation of the maxillary sinus membrane for augmentation of the floor.[13]

We have conducted a series of pre-clinical studies on the application of BMP-7 for reconstruction of the jaw bones which have showed successful bone regeneration at the created surgical defects and minimal postoperative complications.[14-17]

Following these, we have conducted a clinical trial on the application of BMP-7 for the reconstruction of alveolar cleft which showed, radiographically, a satisfactory bone formation in the majority of cases with a successful eruption of the cleft canine through the site of reconstruction.[18] Similar to the preclinical studies, postoperative complications were minimal.

One of the major concerns regarding the application of cytokines including BMPs in growing children has always been their long-term impact on maxillary growth and the potential for enduring complications which include ectopic bone formation in the surrounding tissue and the ankylosis of the adjacent teeth. Most of the reported clinical manifestations and complications following the application of BMP in alveolar clefts are limited to the first year following surgery. Herford et al.,[5] reviewed the patients after 4 months, whereas Dickinson et al.,[7] and Alonso et al.,[8] reviewed the patients after 1 year. We could not find reports, in the English literature, on the long term effects of BMPs following the application in the maxillofacial region during skeletal immaturity of the childhood period.

Aim of the study:

The aim of this study is to provide the first 10-year postoperative assessment following the application of rhBMP-7 (Op1) for the reconstruction of alveolar cleft

Methods:

This study was reviewed and approved by West Glasgow Ethics Committee (EudraCT number: 2005-004392-38, REC reference number 05/S0703/1), the Medicines and Healthcare Products Regulatory Authority (MHRA) (CTA:27410/0001/001-0001, protocol number R050187), and the Research & Development (R&D Reference:R050187). This study was conducted as a prospective phase II clinical trial on 11 healthy cases, 9 unilateral and two bilateral alveolar clefts.[18] The mean age of patients at operation was 10.4 years ranging from 8.8 years to 11.6 years. In all the cases the alveolar cleft was reconstructed with rhBMP-7

(Osigraft, OP1, Stryker Biotech, UK). This was reviewed and approved by the local ethics committee, regulatory authority (MHRA) and research & development. For each case a vial of Osigraft® had 3.5mg of eptotermin alfa (a recombinant human osteogenic protein 1"Op-1") in bovine collagen (a bioresorbable scaffold) was applied at the alveolar defect. As explained in our previous publication,[18] The alveolar cleft was exposed following a standard surgical technique, the nasal mucosa was dissected, reflected upward and sutured before the application of the graft (Figure 1 a)~~.~~. A putty additive (Stryker Biotech) containing carboxymethyl cellulose was added to the Osigraft® and this mixture was reconstituted with 2 to 3 ml of sterile 9 mg/ml sodium chloride solution (0.9% w/v) prior to use. The graft was then packed locally into the cleft region. The reconstituted product was administered by direct surgical placement in the alveolar cleft site in contact with the bone surfaces (Figure 1b). Periosteal release was carried out to allow the stretch and the suturing of the mucoperiosteal flap without tension. Standard postoperative care was provided and patients were discharged from the hospital the day after surgery.

Patients were examined clinically every three days of the first 10 days and then on a weekly basis for three months, followed by another evaluation at 6 months and one year following surgery. The patients were reviewed on annual basis up to 10 years. The following clinical assessments were carried put:

1. Immediately following surgery the magnitude of facial swelling, discomfort and any signs of infection following surgery. Immediate post-operative radiographs were taken to assess the shape and appearance of the grafts and the position of the associated impacted. At three months the healing pattern and eruption of the adjacent teeth was assessed. Six months following surgery, the patients underwent a radiographic examination which was used for the initial assessment
2. The facial growth, residual facial asymmetries or abnormal growth, alteration in sensation, deformities related to the naso-maxillary complex, and the overall pattern of the jaw bones were assessed at each review appointment.
3. Assessment of the site of the graft and reporting in the healing process, bone over growth, exposure, dehiscence, or fistula formation Buccaly or palatally.
4. Eruption of the adjacent teeth the impacted canine at the graft site.

5. Assessment of the medio-lateral maxillo-mandibular occlusal relationship and recording of any cross bites
6. 3. Assessment of the vertical occlusal relationship and recording of any lateral open bites.
7. At each appointment the growth of the maxilla was evaluated.

In summary, patients were followed up within the routine care pathway for 10 years to monitor the impact of BMP-7 on the course of the orthodontic treatment, long term complications and the pattern of maxillary growth. Routine radiographs were taken according to the standard cleft care Scotland audit protocol for patients' management.

Results:

Surgical:

Clinically, the surgical site healed well, immediate postoperative swelling was similar to that of a routine dento-alveolar procedure. Patients were discharged from the hospital the following day after surgery. No critical incidents were reported. We have previously evaluated bone regeneration in the unilateral alveolar cleft using the Kindelan Scoring system as grade 1 (>75% in fill) in all the cases except one which scored grade II (50-70% bone infill). The trabeculation of the newly formed tissue was similar to the surrounding bone. None of these cases required further grafting. Both patients with bilateral clefts, at routine radiographic follow up, showed minimal bony infill (Figure 2) on at least one side and subsequently underwent further conventional grafting procedures. The results of the subsequent bone graft were successful. The Kindelan score, which was used for grading bone formation, suggested a successful outcome grade 1 or 2 (Figures 3b, 3c) for the children with UCLP. The details of these early findings have been published.[18]

Orthodontics

In all the UCLP cases, except one, the cleft canine erupted through the graft spontaneously. The children subsequently commenced orthodontic care aged between 9 yrs and 14 years. The duration of the subsequent orthodontic treatment ranged from 8 to 20 months. In the majority of the children the space was closed between the cleft canine and cleft central incisor.

Facial growth & long term complications

In the surgically managed UCLP cases the maxillary growth appeared similar to that of cases grafted with autogenous bone. A detailed radiograpahic analysis was not conducted in this study. Lateral radiographs are only taken where it is considered appropriate to aid the clinical orthodontic diagnosis. The analysed records when taken before puberty (Figures 4) and post puberty (Figure 5s) showed values consistent with those for a routine cleft population with unilateral or bilateral cleft lip and palate. Figure 6 shows the soft tissue analysis, and figure 7 shows the evaluation of the skeletal pattern of one of the UCLP cases.

We did not detect excessive bone fusion or excessive bony growth at the site where BMP was applied. None of the patient showed what would be considered an abnormal growth pattern for this cohort of patients. The symmetry of the face was monitored throughout the course of orthodontic treatment. None of the UCLP cases appeared to develop asymmetry secondary to the application of BMP-7 that would be considered different from those who receive conventional grafting procedures. Reviewing the patients' medical records did not reveal systemic or skeletal abnormalities that were of relevance to the application of BMP. The placement of the rhBMP- 7 into direct contact with the adjacent tissue did not cause ectopic bone formation or damage to the adjacent teeth.

Discussion:

In this study bone regeneration at the grafted sites was evaluated using Kindelan score which is widely used for the evaluation of alveolar bone grafts. It is a four point radiographic scale that assesses bone formation at the alveolar cleft independent to the eruption of the canine. Bilateral cleft lip and palate cases had a score of grade 3 or 4 for the grafted sites which is an indicative of graft failure. In both children the cleft canines failed to erupt through the graft site. The eruption of the cleft related canine in the UCLP cases occurred spontaneously in the bioengineered bone in all cases except one, which would be considered, despite the small numbers, a comparable level of spontaneous eruption of other studies using more conventional graft materials.[~~20~~19~~.~~,21] The duration of orthodontic treatment (8-20months) would be considered standard for children with cleft lip and palate and comparable with other cleft related studies. [19]

In the case of failed eruption, pre-operative radiographs suggest the cleft canine was in an unfavourable (horizontal) position pre-surgery and therefore unlikely to erupt through the graft site. Although this may have contributed to the failure, a recent study found that canine angulation prior to the graft appears to have no impact of its ability to erupt spontaneously.[22]

~~The age (9-14yrs) and duration (8-20months) of orthodontic treatment would be considered standard for children with cleft lip and palate and comparable with other cleft related studies.~~[19]

In the majority of the children the orthodontic plan was for space closure anteriorly between the cleft canine and cleft central incisor. The clinical decision as to whether to open or close the space orthodontically in the two cases that subsequently underwent space opening was based on the malocclusion rather than a lack of bone available for tooth movement on post-operative radiographs. However, that quality of bone graft analysed by radiographs six months post-graft appears to be only weakly correlated with whether space closure is possible long-term.[23]

In our experience the cohort showed a growth pattern similar (Figure 7) to those where autogenous bone graft has been used for the reconstruction of the alveolar defect. Direct comparison between the two groups (conventional bone graft versus BMP) was not considered part of this study as the sample size is too small for a robust evaluation. We do note however that orthodontic camouflage to mask the skeletal discrepancy was achievable in most of the cases which confirms a relatively mild nature of the skeletal discrepancy.

The successful manufacture of the recombinant BMP allowed its commercial production. [27–24] This facilitated a wide range of clinical studies to promote bone healing in spinal fusions and the management of the non-union of long bones.

BMP has the ability to induce the differentiation of mesenchymal stem cells to osteobalsts and also promote the proliferation of the commented cells toward the osteogenic lineage for bone regeneration. [25] However, the long term impact of the application of BMP for alveolar cleft reconstruction on the orthodontic tooth movements and bone growth is sparse in English literature.[26]

In this study, the morbidity associated with a donor site was eliminated by using rhMBP_7 for the reconstruction of the alveolar cleft. The intensity of the surgical procedure was reduced which facilitated postoperative recovery. The duration of the stay in hospital was one day which is shorter than that is in some surgical units, when iliac crest bone graft is harvested. Radiographic assessment gave an objective and reliable, although two dimensional indication of the quality and quantity of newly formed bone. These patients were treated prior to the routine use of CBCT for the evaluation of the volume of alveolar cleft and bone formation. In bilateral alveolar cleft cases, bone formation was limited, the premaxilla was not mobile but radiographs confirmed the failure of bone formation. We believe the main reason for the unsuccessful treatment of the bilateral alveolar clefts is the e small volume of the injected material. Increasing the dose of the applied BMP was not an option due to the hypothetical potential risk of excessive bone formation.

There is debate in the literature regarding the application of BMP in early years of life and a considerable concern regarding the implantation site, mainly in areas of skeletal

immaturity.[27] The findings of our study appear to confirm the safety of applying BMP-7 at immature skeletal sites. The clinical evaluation of the patients and the assessment of the lateral cephalographs whenever they were taken at various follow up intervals did not reveal adverse or unexpected effects that are not routinely seen in conventional iliac crest bone graft cases.

There is strong evidence in the literature suggesting that BMPs do not only affect bone, but are expressed in several tissues.[27] BMP-2 and -7 are expressed in prostate cancer,[28] breast cancer,[29] osteosarcomas,[30] and odontogenic tumors.[31]There is evidence that BMPs may play a role in the development and progression of malignant tumors, but the exact mechanism is not clear. The long term safety of the clinical application of BMPs for the bony reconstruction of alveolar cleft has not yet been fully investigated. In this study, the presented data provide some assurance as none of the cases which received BMP-7 developed tumors related to the maxillofacial region and we did not detect ectopic bone formation or ankylosis of the adjacent teeth.

Despite the satisfactory results in this study, one of the major obstacles of the routine application of BMP for the reconstruction of the alveolar cleft is its high cost which has to be counter balanced with the morbidities associated with the harvesting of iliac crest bone graft. Health authorities and policy makers should work closely with the industry to reduce the cost of the manufacturer of the rhBMP to facilitate its global utilisation. Another alternative to reduce the cost of the application of recombinant cytokines is their combination with osseoconductive materials. The outcomes of 15 patients grafted with autogenous bone were compared with 15 patients grafted with a combination of BMP-2 and Demineralised Bone Matrix (DBM). Occlusal radiographs and cone beam CTs were taken three months after surgery and evaluated using

Bergland, Chelsea, Long and Yen scales. The results showed equivalent successful outcomes.[32]

We are not aware of studies which directly compare between BMP2 and BMP7 for the reconstruction of the alveolar cleft, this worse considering in the future.

None of the patients in this study received dental implants. The cleft site was either closed orthodontically or the impacted canines, having erupted at the grafted site. Therefore, there was no opportunity to carry out histological assessment to evaluate the quality or the architectural characteristics of the regenerated bone.

The use of a collagen carrier in the presented cases allowed rhBMP-7 to be easily applied and directly injected at the alveolar cleft site. This facilitated access to the surgical site and is advantageous because it requires minimal exposure of the cleft which reduces the morbidity of the surgical procedure.

One of the objectives of the reconstruction of the alveolar cleft is the simultaneous augmentation of the bony foundation of the alar base to correct the associated nasal asymmetry, which is readily achievable with a mono-cortical block of cortico-cancelleous autogenous bone graft.[33] This cannot be achieved with the injectable rhBMP-7 due to its putty consistency. This could be accomplished in the future with the development of a block of biological scaffolding that is loaded with rhBMP or seeded with stem cells to induce bone formation. The collagen carrier used in this study is not ideal to achieve this particular objective.

This study suffers from the small sample size and the absence of the direct comparison with the UCLP cases which has autogenous bone graft for the reconstruction of the alveolar cleft. This requires further investigation.

Conclusions:

This 10 year follow up provides assurance regarding the safety of BMP-7 when applied at the area of skeletal immaturity. Patients did not develop clinical or radiographic manifestations of bony abnormalities. There appeared to be no additional alteration in growth pattern or development of tumours with the application of BMP-7 in children. Residual facial asymmetry was consistent with that of conventional iliac crest bone graft cases. Orthodontic movement of the teeth through the grafts site progressed in a similar manner to conventional cases. The high rate of success in this study with UCLP cases may encourage a larger scale clinical trial and facilitate phase III comparative studies in comparison with autogenous bone graft.

Legends of the figures:

Figures 1a:
Exposure of the right alveolar cleft
Figure 1b
The injection of rhBMP-7 in the alveolar cleft
~~Alveolar cleft, the clinical application of BMP-7 at the cleft site~~

Figure 2:
Ppostoperative occlusal radiograph showing failure of bone formation in one of the bilateral alveolar cleft cases

Figure 3 a:
Preoperative frontal view showing the characteristic features of UCLP on the left side.

Figure 3b:
Immediate preoperative occlusal radiogarph showing the alveolar cleft

Figure 3c:
Occlusal radiograph 6 months following the application of rhBMP-7 at the alveolar cleft, showing bone formation and the partial eruption of the impacted canine.

Figures 3d:
Postoperative palatal view of the UCLP cases showing good soft tissue healing and the eruption of the impacted canine 6 months after the application of rhBMP-7 at the left alveolar cleft.

~~Preoperative photographs and radiographs~~
~~Frontal view, anterior occlusal radiographs before & 6 months following the application of rhBMP-7 at the alveolar cleft h showing bone formation at the cleft site, intraoral photograph showing the eruption of the canine at the grafted cleft,~~ .

Figure 3e:
Lateral cephalograph at 6 month following the reconstruction of alveolar cleft using rhBMP-7.

Figures 4 a:
Frontal, profile and three-quarter facial photographs of the same patient at five years following the grafting of the alveolar cleft and before complete cessation of the growth

Figures 4b.
Intra-oral photographs ~~:~~ f~~F~~ive ~~years~~ years following alveolar cleft reconstruction with rhBMP-7 showing good healing, full eruption of the impacted canine and a satisfactory growth of the maxilla ~~postoperative views before complete cessation of growth~~

Figures 5a:

10 years postoperative facial views of the same patient showing a mild degree of maxillary hypoplasia, normal labial seal, and asymmetry of the left nostril.
~~Frontal view, left profile view, lateral cephalograph , submento-vertex view, dental occlusion.~~

Figures 5b:
10 years postoperative view of the dental arches showing mild cross bite at the left maxillary incisors, full eruption of the canine on the left cleft side, normal appearance & contour of the buccal mucosa of the grafted side and absence of overgrowth or clinical pathological findings.

Figure 5c:
10 years lateral cephalograph showing a satisfactory maxillary growth relative to the UCLP of the same case which was grafted with rhBMP-7

Figure 6: A: Soft tissue analysis to evaluate the antero-posterior growth of the maxilla
The TVL is constructed from the natural head position through subnasale.
Soft tissue A (stA) and soft tissue B (stB) points are projected to the TVL.

Figure 7: Skeletal and dental analysis of the lateral cephaolograph of one of the surgically managed UCLP cases.

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The clinical application of rh-BMP-7 for reconstruction of alveolar cleft; 10 years follow up

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Abstract:

Purpose:

To report on a 10-year assessment following the application of rhBMP-7 for reconstruction of alveolar cleft.

Method:

This study was conducted as prospective phase II clinical trial on 9 unilateral and two bilateral alveolar clefts which received rhBMP-7 (Osigraft). The mean age of patients at surgery was 10.4 years. Six months postoperatively, occlusal radiographs were taken to evaluate bone formation at the cleft site. Patients were followed up within the routine cleft care pathway for up to 10 years to monitor the impact of BMP-7 on orthodontic treatments and maxillary growth. Radiographs were taken according to the standard cleft care protocol.

Results:

The radiographic assessment of the UCLP cases suggested good bone formation with a Kindelan score at grade 1 or 2. The bilateral alveolar cleft cases had a score of grade 3 or 4 indicating failure or partial failure. The children with successful grafts underwent a routine orthodontics follow-up without incident. The maxillary growth appeared similar to that of cases grafted with autogenous bone. No long term complications or abnormal pattern of bone formation were detected.

Conclusions:

The study provides unique evidence on the long term safety of rhBMP-7 when applied at the area of skeletal immaturity for the reconstruction of alveolar clefts in children.

Introduction:

Autogenous bone graft is the gold standard for the reconstruction of alveolar cleft which is associated with cleft lip and palate.[1] However, harvesting of bone graft is associated with well documented morbidities including pain, scarring, alteration in sensation and swelling.[2] Researchers worldwide are interested in discovering alternative treatment modalities, based on the recent advances in tissue bioengineering, to avoid these morbidities. The use of the osteoinductive growth factors including bone morphogenetic proteins (BMPs) has been considered for the reconstruction of the alveolar clefts. BMPs stimulate osteogenesis for the healing of skeletal fractures, the osteoinductive abilities of BMP-2 and BMP-7 are well documented.[3,4]

Bone morphogenetic protein 2 (BMP-2) has been used for the reconstruction of alveolar cleft defects over the last 10 years.[5-7] The quality and quantity of bone formation in alveolar cleft is dependent on the dose of the cytokines that is locally applied at the cleft site. BMP-2 at a low dose of 50Ug ml^{-1} failed to induce bone formation, whereas, up to 59% of the surgical defect was reconstructed with new bone formation when the concentration of BMP-2 was raised to 250ug ml^{-1}. However, this was associated with severe postoperative gingival swelling during the first week following surgery.[8] The development of a localized swelling following the application of BMP-2 is well documented.[9-11]

Comparative analysis between BMP-2 with autologous bone harvested from the iliac crest for the reconstruction of alveolar clefts has been carried out. The doses of the BMP-2 ranged from 1.05–1.5 mg ml^{-1} which was delivered by type I collagen sponge. In all the studies BMP-2 was successful in inducing bone formation comparable to the cases which received autogenous bone grafts.[5,7,8]

There is a concern of using BMP in the pediatric population and the possibility of induction of malignancy. The Food and Drug Administration has licensed the clinical application of recombinant BMP-7 (rhBMP-7) for the non-union of long bone fractures and spinal fusion.[12] The clinical applications of BMP-7 in the maxillofacial region are limited, but it has been utilized in some studies following the elevation of the maxillary sinus membrane for augmentation of the floor.[13]

We have conducted a series of pre-clinical studies on the application of BMP-7 for reconstruction of the jaw bones which have showed successful bone regeneration at the created surgical defects and minimal postoperative complications.[14-17]

Following these, we have conducted a clinical trial on the application of BMP-7 for the reconstruction of alveolar cleft which showed, radiographically, a satisfactory bone formation in the majority of cases with a successful eruption of the cleft canine through the site of reconstruction.[18] Similar to the preclinical studies, postoperative complications were minimal.

One of the major concerns regarding the application of cytokines including BMPs in growing children has always been their long-term impact on maxillary growth and the potential for enduring complications which include ectopic bone formation in the surrounding tissue and the ankylosis of the adjacent teeth. Most of the reported clinical manifestations and complications following the application of BMP in alveolar clefts are limited to the first year following surgery. Herford et al.,[5] reviewed the patients after 4 months, whereas Dickinson et al.,[7] and Alonso et al.,[8] reviewed the patients after 1 year. We could not find reports, in the English literature, on the long term effects of BMPs following the application in the maxillofacial region during skeletal immaturity of the childhood period.

Aim of the study:

The aim of this study is to provide the first 10-year postoperative assessment following the application of rhBMP-7 (Op1) for the reconstruction of alveolar cleft

Methods:

This study was reviewed and approved by West Glasgow Ethics Committee (EudraCT number: 2005-004392-38, REC reference number 05/S0703/1), the Medicines and Healthcare Products Regulatory Authority (MHRA) (CTA:27410/0001/001-0001, protocol number R050187), and the Research & Development (R&D Reference:R050187). This study was conducted as a prospective phase II clinical trial on 11 healthy cases, 9 unilateral and two bilateral alveolar clefts.[18] The mean age of patients at operation was 10.4 years ranging from 8.8 years to 11.6 years. In all the cases the alveolar cleft was reconstructed with rhBMP-7

(Osigraft, OP1, Stryker Biotech, UK). This was reviewed and approved by the local ethics committee, regulatory authority (MHRA) and research & development. For each case a vial of Osigraft® had 3.5mg of eptotermin alfa (a recombinant human osteogenic protein 1"Op-1") in bovine collagen (a bioresorbable scaffold) was applied at the alveolar defect. As explained in our previous publication,[18] The alveolar cleft was exposed following a standard surgical technique, the nasal mucosa was dissected, reflected upward and sutured before the application of the graft (Figure 1 a). A putty additive (Stryker Biotech) containing carboxymethyl cellulose was added to the Osigraft® and this mixture was reconstituted with 2 to 3 ml of sterile 9 mg/ml sodium chloride solution (0.9% w/v) prior to use. The graft was then packed locally into the cleft region. The reconstituted product was administered by direct surgical placement in the alveolar cleft site in contact with the bone surfaces (Figure 1b). Periosteal release was carried out to allow the stretch and the suturing of the mucoperiosteal flap without tension. Standard postoperative care was provided and patients were discharged from the hospital the day after surgery.

Patients were examined clinically every three days of the first 10 days and then on a weekly basis for three months, followed by another evaluation at 6 months and one year following surgery. The patients were reviewed on annual basis up to 10 years. The following clinical assessments were carried put:

1. Immediately following surgery the magnitude of facial swelling, discomfort and any signs of infection following surgery. Immediate post-operative radiographs were taken to assess the shape and appearance of the grafts and the position of the associated impacted. At three months the healing pattern and eruption of the adjacent teeth was assessed. Six months following surgery, the patients underwent a radiographic examination which was used for the initial assessment
2. The facial growth, residual facial asymmetries or abnormal growth, alteration in sensation, deformities related to the naso-maxillary complex, and the overall pattern of the jaw bones were assessed at each review appointment.
3. Assessment of the site of the graft and reporting in the healing process, bone over growth, exposure, dehiscence, or fistula formation Buccaly or palatally.
4. Eruption of the adjacent teeth the impacted canine at the graft site.

5. Assessment of the medio-lateral maxillo-mandibular occlusal relationship and recording of any cross bites
6. 3. Assessment of the vertical occlusal relationship and recording of any lateral open bites.
7. At each appointment the growth of the maxilla was evaluated.

In summary, patients were followed up within the routine care pathway for 10 years to monitor the impact of BMP-7 on the course of the orthodontic treatment, long term complications and the pattern of maxillary growth. Routine radiographs were taken according to the standard cleft care Scotland audit protocol for patients' management.

Results:

Surgical:

Clinically, the surgical site healed well, immediate postoperative swelling was similar to that of a routine dento-alveolar procedure. Patients were discharged from the hospital the following day after surgery. No critical incidents were reported. We have previously evaluated bone regeneration in the unilateral alveolar cleft using the Kindelan Scoring system as grade 1 (>75% in fill) in all the cases except one which scored grade II (50-70% bone infill). The trabeculation of the newly formed tissue was similar to the surrounding bone. None of these cases required further grafting. Both patients with bilateral clefts, at routine radiographic follow up, showed minimal bony infill (Figure 2) on at least one side and subsequently underwent further conventional grafting procedures. The results of the subsequent bone graft were successful. The Kindelan score, which was used for grading bone formation, suggested a successful outcome grade 1 or 2 (Figures 3b, 3c) for the children with UCLP. The details of these early findings have been published.[18]

Orthodontics

In all the UCLP cases, except one, the cleft canine erupted through the graft spontaneously. The children subsequently commenced orthodontic care aged between 9 yrs and 14 years. The duration of the subsequent orthodontic treatment ranged from 8 to 20 months. In the majority of the children the space was closed between the cleft canine and cleft central incisor.

Facial growth & long term complications

In the surgically managed UCLP cases the maxillary growth appeared similar to that of cases grafted with autogenous bone. A detailed radiograpahic analysis was not conducted in this study. Lateral radiographs are only taken where it is considered appropriate to aid the clinical orthodontic diagnosis. The analysed records when taken before puberty (Figures 4) and post puberty (Figure 5s) showed values consistent with those for a routine cleft population with unilateral or bilateral cleft lip and palate. Figure 6 shows the soft tissue analysis, and figure 7 shows the evaluation of the skeletal pattern of one of the UCLP cases.

We did not detect excessive bone fusion or excessive bony growth at the site where BMP was applied. None of the patient showed what would be considered an abnormal growth pattern for this cohort of patients. The symmetry of the face was monitored throughout the course of orthodontic treatment. None of the UCLP cases appeared to develop asymmetry secondary to the application of BMP-7 that would be considered different from those who receive conventional grafting procedures. Reviewing the patients' medical records did not reveal systemic or skeletal abnormalities that were of relevance to the application of BMP. The placement of the rhBMP- 7 into direct contact with the adjacent tissue did not cause ectopic bone formation or damage to the adjacent teeth.

Discussion:

In this study bone regeneration at the grafted sites was evaluated using Kindelan score which is widely used for the evaluation of alveolar bone grafts. It is a four point radiographic scale that assesses bone formation at the alveolar cleft independent to the eruption of the canine. Bilateral cleft lip and palate cases had a score of grade 3 or 4 for the grafted sites which is an indicative of graft failure. In both children the cleft canines failed to erupt through the graft site. The eruption of the cleft related canine in the UCLP cases occurred spontaneously in the bioengineered bone in all cases except one, which would be considered, despite the small numbers, a comparable level of spontaneous eruption of other studies using more conventional graft materials.[19-,21] The duration of orthodontic treatment (8-20months) would be considered standard for children with cleft lip and palate and comparable with other cleft related studies. [19]

In the case of failed eruption, pre-operative radiographs suggest the cleft canine was in an unfavourable (horizontal) position pre-surgery and therefore unlikely to erupt through the graft site. Although this may have contributed to the failure, a recent study found that canine angulation prior to the graft appears to have no impact of its ability to erupt spontaneously.[22]

In the majority of the children the orthodontic plan was for space closure anteriorly between the cleft canine and cleft central incisor. The clinical decision as to whether to open or close the space orthodontically in the two cases that subsequently underwent space opening was based on the malocclusion rather than a lack of bone available for tooth movement on post-operative radiographs. However, that quality of bone graft analysed by radiographs six months post-graft appears to be only weakly correlated with whether space closure is possible long-term.[23]

In our experience the cohort showed a growth pattern similar (Figure 7) to those where autogenous bone graft has been used for the reconstruction of the alveolar defect. Direct comparison between the two groups (conventional bone graft versus BMP) was not considered part of this study as the sample size is too small for a robust evaluation. We do note however that orthodontic camouflage to mask the skeletal discrepancy was achievable in most of the cases which confirms a relatively mild nature of the skeletal discrepancy.

The successful manufacture of the recombinant BMP allowed its commercial production. [24] This facilitated a wide range of clinical studies to promote bone healing in spinal fusions and the management of the non-union of long bones.

BMP has the ability to induce the differentiation of mesenchymal stem cells to osteobalsts and also promote the proliferation of the commented cells toward the osteogenic lineage for bone regeneration. [25] However, the long term impact of the application of BMP for alveolar cleft reconstruction on the orthodontic tooth movements and bone growth is sparse in English literature.[26]

In this study, the morbidity associated with a donor site was eliminated by using rhMBP_7 for the reconstruction of the alveolar cleft. The intensity of the surgical procedure was reduced which facilitated postoperative recovery. The duration of the stay in hospital was one day which is shorter than that is in some surgical units, when iliac crest bone graft is harvested. Radiographic assessment gave an objective and reliable, although two dimensional indication of the quality and quantity of newly formed bone. These patients were treated prior to the routine use of CBCT for the evaluation of the volume of alveolar cleft and bone formation. In bilateral alveolar cleft cases, bone formation was limited, the premaxilla was not mobile but radiographs confirmed the failure of bone formation. We believe the main reason for the unsuccessful treatment of the bilateral alveolar clefts is the e small volume of the injected material. Increasing the dose of the applied BMP was not an option due to the hypothetical potential risk of excessive bone formation.

There is debate in the literature regarding the application of BMP in early years of life and a considerable concern regarding the implantation site, mainly in areas of skeletal immaturity.[27] The findings of our study appear to confirm the safety of applying BMP-7 at immature skeletal sites. The clinical evaluation of the patients and the

assessment of the lateral cephalographs whenever they were taken at various follow up intervals did not reveal adverse or unexpected effects that are not routinely seen in conventional iliac crest bone graft cases.

There is strong evidence in the literature suggesting that BMPs do not only affect bone, but are expressed in several tissues.[27] BMP-2 and -7 are expressed in prostate cancer,[28] breast cancer,[29] osteosarcomas,[30] and odontogenic tumors.[31]There is evidence that BMPs may play a role in the development and progression of malignant tumors, but the exact mechanism is not clear. The long term safety of the clinical application of BMPs for the bony reconstruction of alveolar cleft has not yet been fully investigated. In this study, the presented data provide some assurance as none of the cases which received BMP-7 developed tumors related to the maxillofacial region and we did not detect ectopic bone formation or ankylosis of the adjacent teeth.

Despite the satisfactory results in this study, one of the major obstacles of the routine application of BMP for the reconstruction of the alveolar cleft is its high cost which has to be counter balanced with the morbidities associated with the harvesting of iliac crest bone graft. Health authorities and policy makers should work closely with the industry to reduce the cost of the manufacturer of the rhBMP to facilitate its global utilisation. Another alternative to reduce the cost of the application of recombinant cytokines is their combination with osseoconductive materials. The outcomes of 15 patients grafted with autogenous bone were compared with 15 patients grafted with a combination of BMP-2 and Demineralised Bone Matrix (DBM). Occlusal radiographs and cone beam CTs were taken three months after surgery and evaluated using Bergland, Chelsea, Long and Yen scales. The results showed equivalent successful outcomes.[32]

We are not aware of studies which directly compare between BMP2 and BMP7 for the reconstruction of the alveolar cleft, this worse considering in the future.

None of the patients in this study received dental implants. The cleft site was either closed orthodontically or the impacted canines, having erupted at the grafted site. Therefore, there was no opportunity to carry out histological assessment to evaluate the quality or the architectural characteristics of the regenerated bone.

The use of a collagen carrier in the presented cases allowed rhBMP-7 to be easily applied and directly injected at the alveolar cleft site. This facilitated access to the surgical site and is advantageous because it requires minimal exposure of the cleft which reduces the morbidity of the surgical procedure.

One of the objectives of the reconstruction of the alveolar cleft is the simultaneous augmentation of the bony foundation of the alar base to correct the associated nasal asymmetry, which is readily achievable with a mono-cortical block of cortico-cancelleous autogenous bone graft.[33] This cannot be achieved with the injectable rhBMP-7 due to its putty consistency. This could be accomplished in the future with the development of a block of biological scaffolding that is loaded with rhBMP or seeded with stem cells to induce bone formation. The collagen carrier used in this study is not ideal to achieve this particular objective.

This study suffers from the small sample size and the absence of the direct comparison with the UCLP cases which has autogenous bone graft for the reconstruction of the alveolar cleft. This requires further investigation.

Conclusions:

This 10 year follow up provides assurance regarding the safety of BMP-7 when applied at the area of skeletal immaturity. Patients did not develop clinical or radiographic manifestations of bony abnormalities. There appeared to be no additional alteration in growth pattern or development of tumours with the application of BMP-7 in children. Residual facial asymmetry was consistent with that of conventional iliac crest bone graft cases. Orthodontic movement of the teeth through the grafts site progressed in a similar manner to conventional cases. The high rate of success in this study with UCLP cases may encourage a larger scale clinical trial and facilitate phase III comparative studies in comparison with autogenous bone graft.

Legends of the figures:

Figure 1a:
Exposure of the right alveolar cleft
Figure 1b
The injection of rhBMP-7 in the alveolar cleft

Figure 2:
Postoperative occlusal radiograph showing failure of bone formation in one of the bilateral alveolar cleft cases

Figure 3 a:
Preoperative frontal view showing the characteristic features of UCLP on the left side.

Figure 3b:
Immediate preoperative occlusal radiogarph showing the alveolar cleft

Figure 3c:
Occlusal radiograph 6 months following the application of rhBMP-7 at the alveolar cleft, showing bone formation and the partial eruption of the impacted canine.

Figures 3d:
Postoperative palatal view of the UCLP cases showing good soft tissue healing and the eruption of the impacted canine 6 months after the application of rhBMP-7 at the left alveolar cleft.

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Figure 3e:
Lateral cephalograph at 6 month following the reconstruction of alveolar cleft using rhBMP-7.

Figures 4 a:
Frontal, profile and three-quarter facial photographs of the same patient at five years following the grafting of the alveolar cleft and before complete cessation of the growth

Figures 4b.
Intra-oral photographs five years following alveolar cleft reconstruction with rhBMP-7 showing good healing, full eruption of the impacted canine and a satisfactory growth of the maxilla

Figures 5a:
10 years postoperative facial views of the same patient showing a mild degree of maxillary hypoplasia, normal labial seal, and asymmetry of the left nostril.

Figures 5b:
10 years postoperative view of the dental arches showing mild cross bite at the left maxillary incisors, full eruption of the canine on the left cleft side, normal appearance & contour of the buccal mucosa of the grafted side and absence of overgrowth or clinical pathological findings.

Figure 5c:
10 years lateral cephalograph showing a satisfactory maxillary growth relative to the UCLP of the same case which was grafted with rhBMP-7

Figure 6: A: Soft tissue analysis to evaluate the antero-posterior growth of the maxilla
The TVL is constructed from the natural head position through subnasale.
Soft tissue A (stA) and soft tissue B (stB) points are projected to the TVL.

Figure 7: Skeletal and dental analysis of the lateral cephaolograph of one of the surgically managed UCLP cases.

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Figure 1a

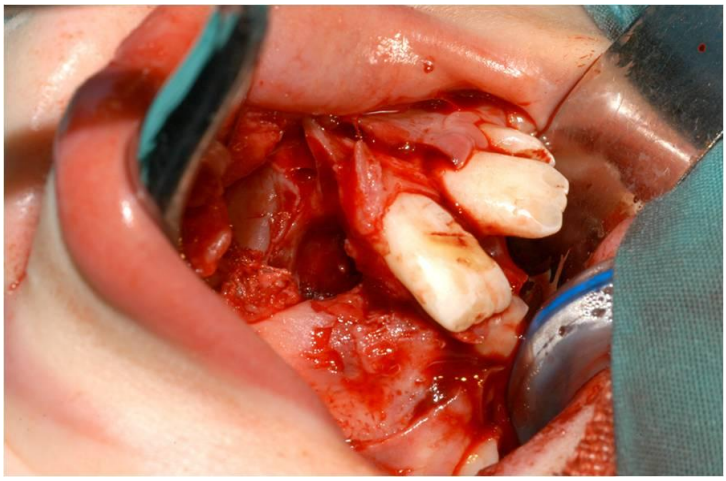


Figure 1b

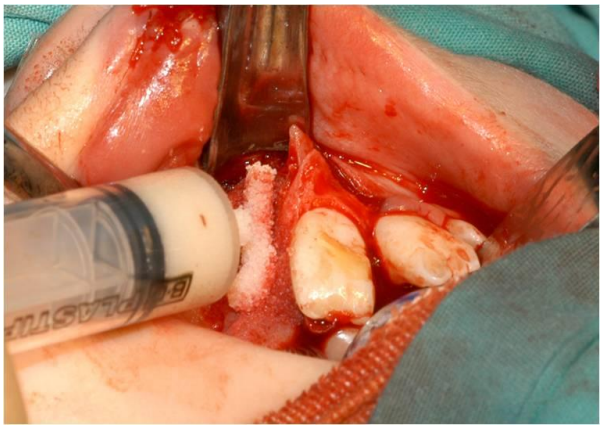


Figure 2

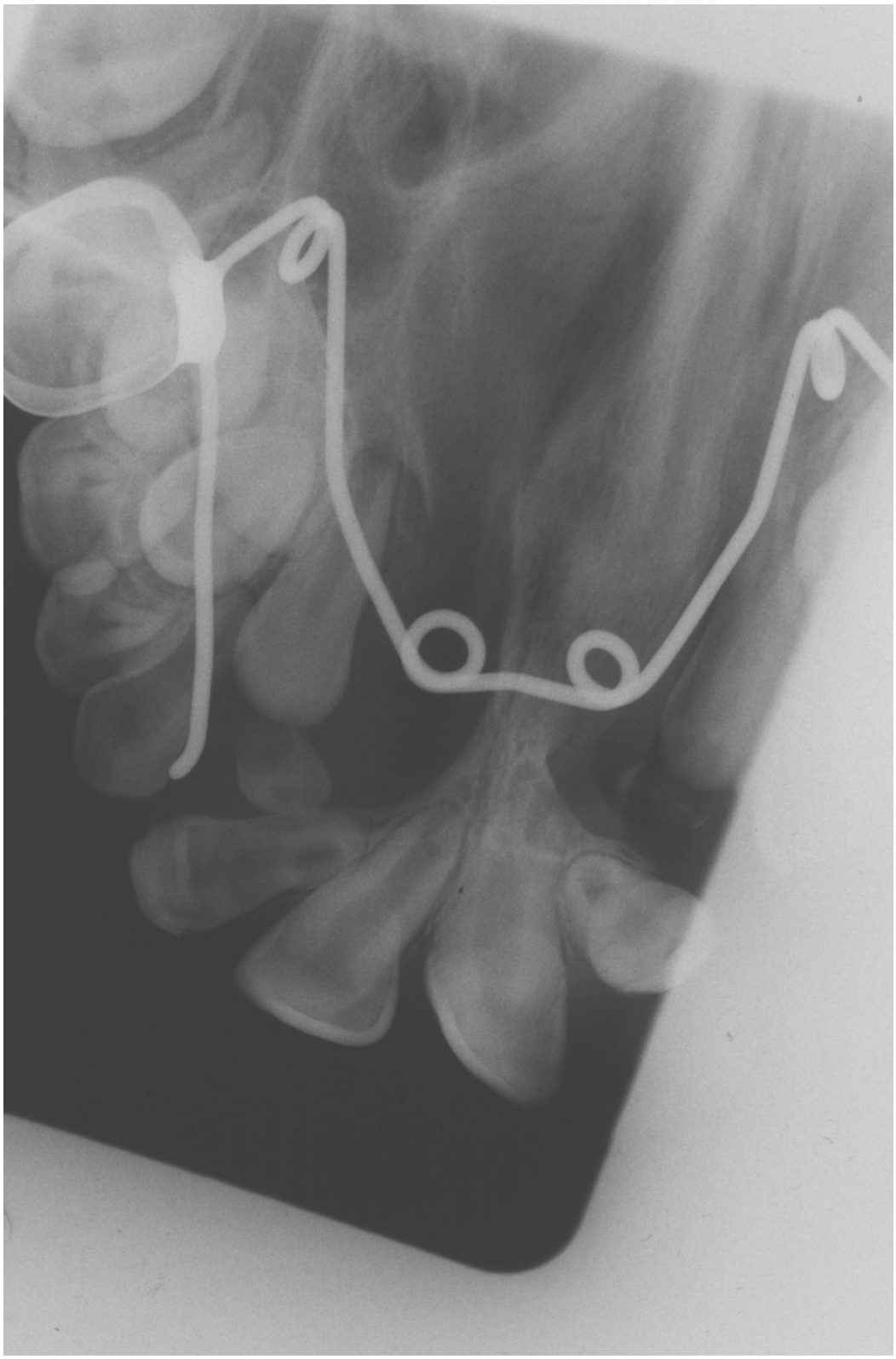


Figure 3a

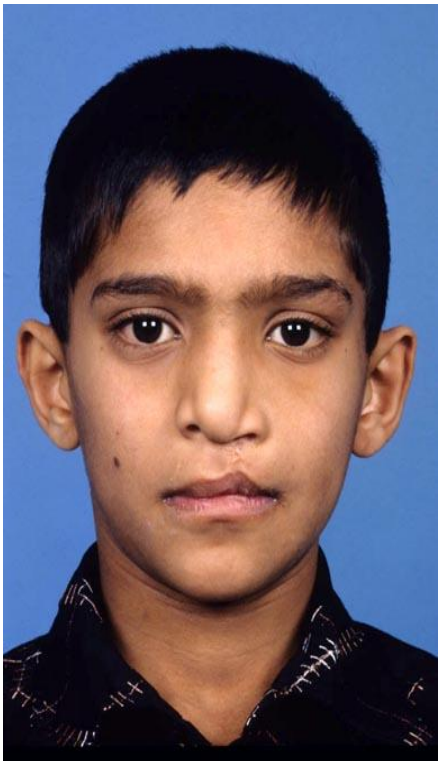


Figure 3b

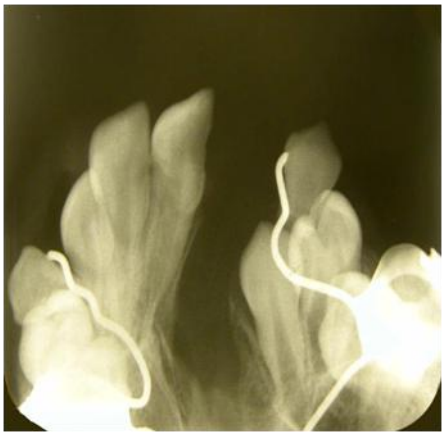


Figure 3c

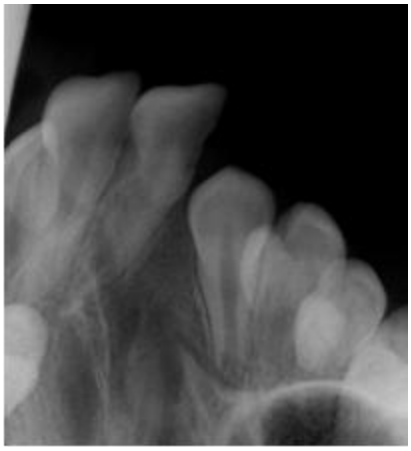
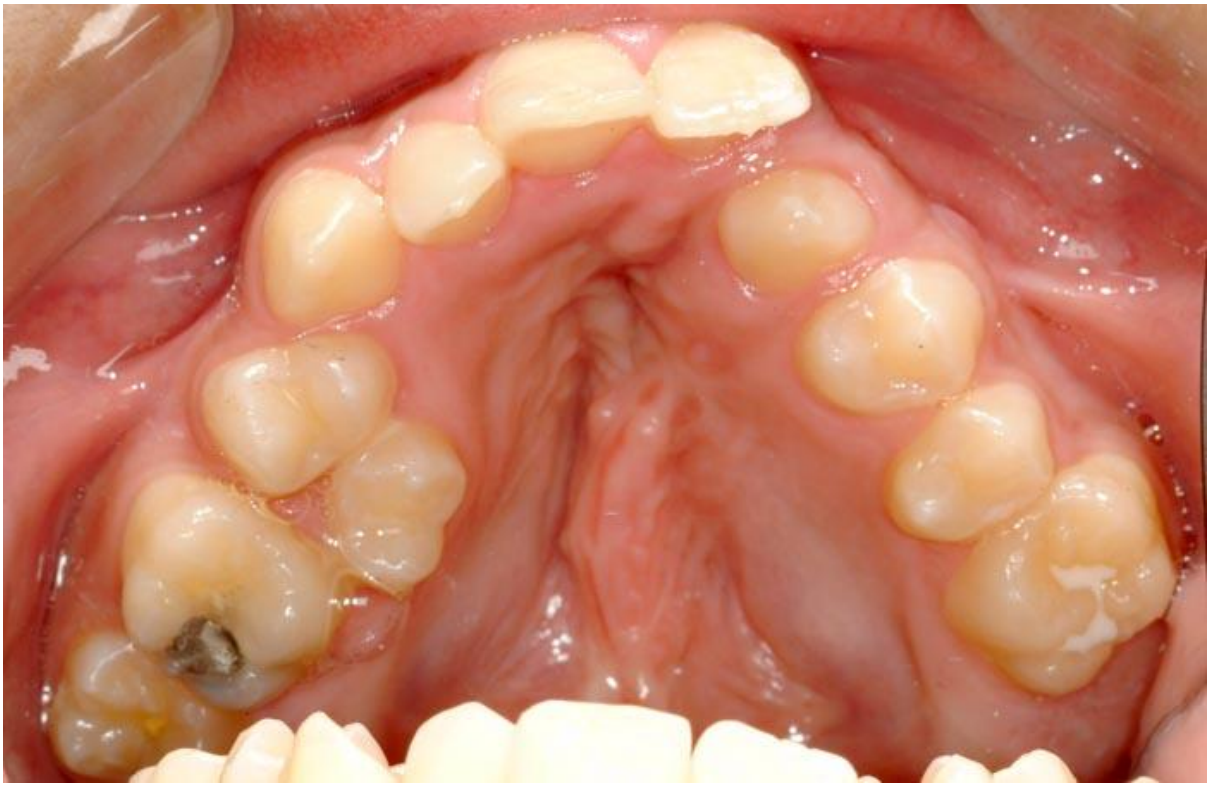


Figure 3d



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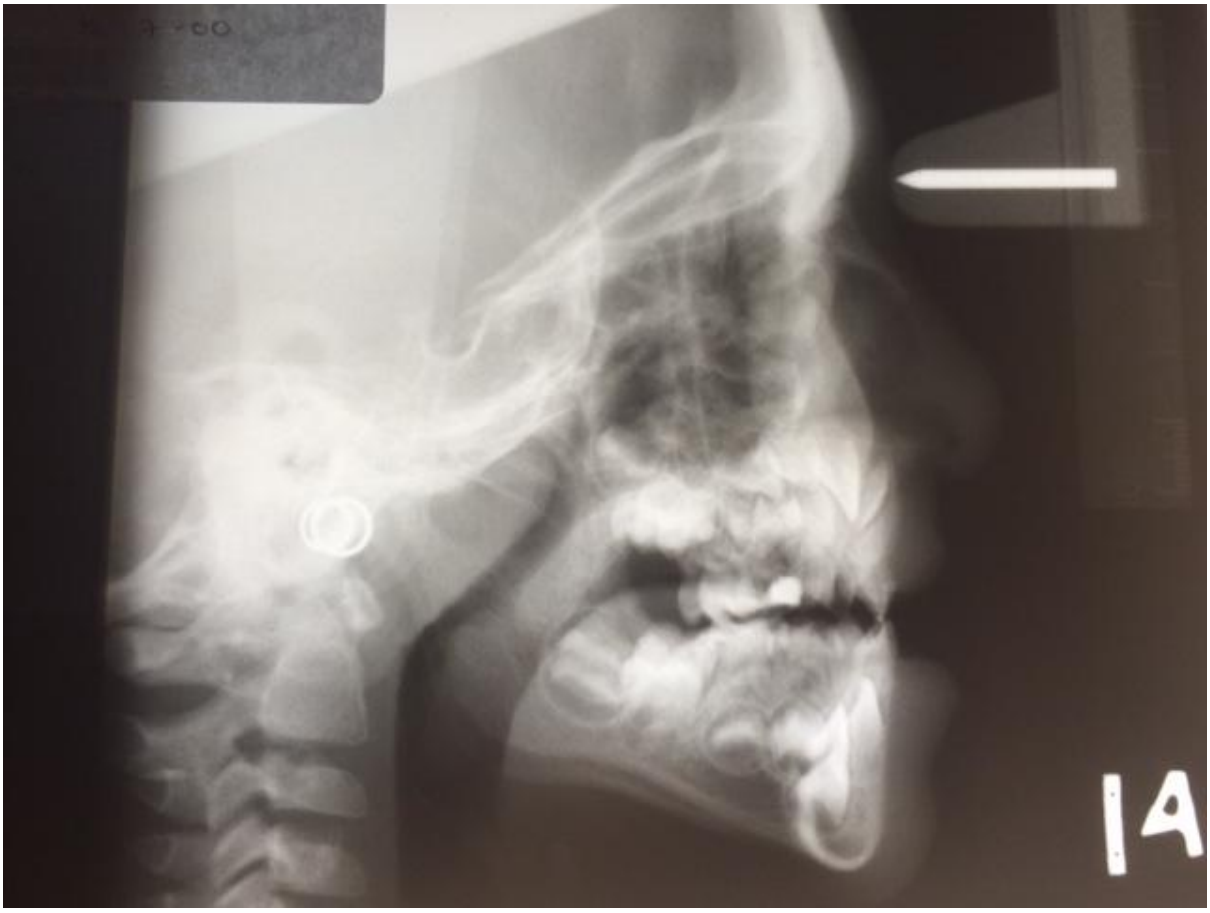

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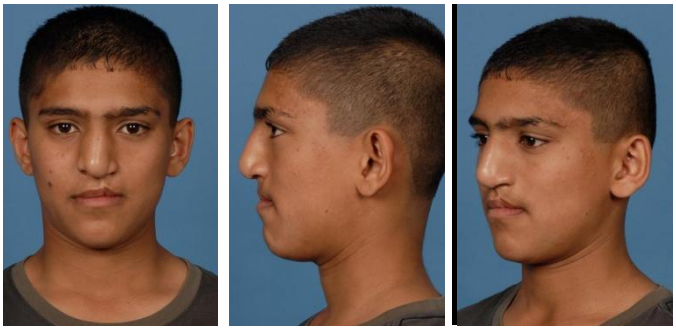
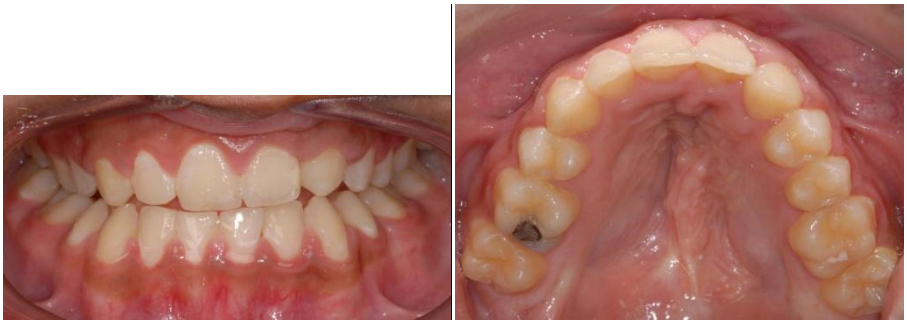


Figure 4b



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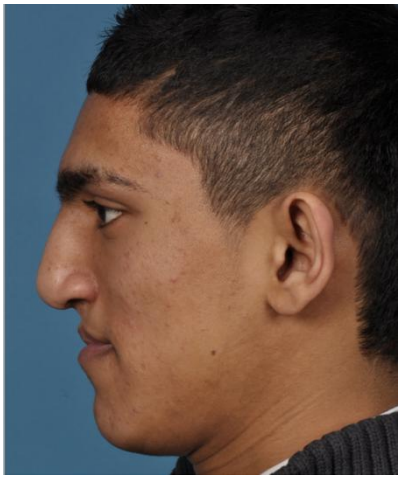

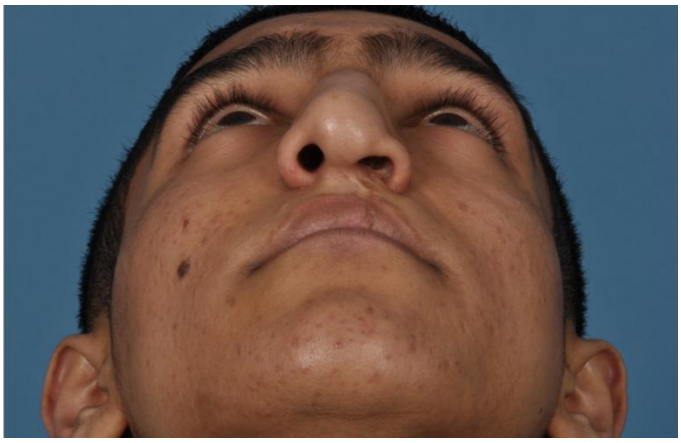

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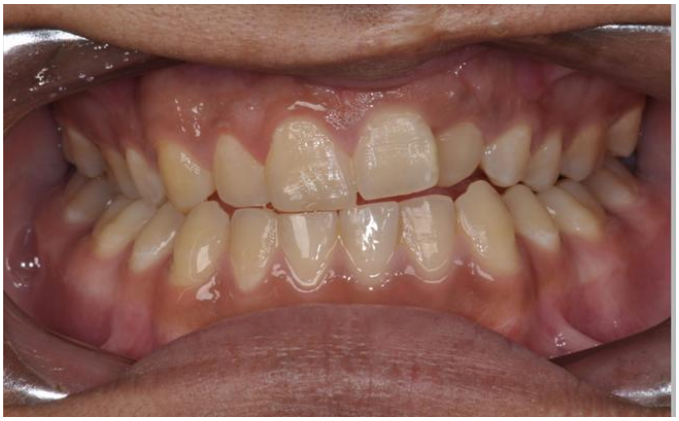

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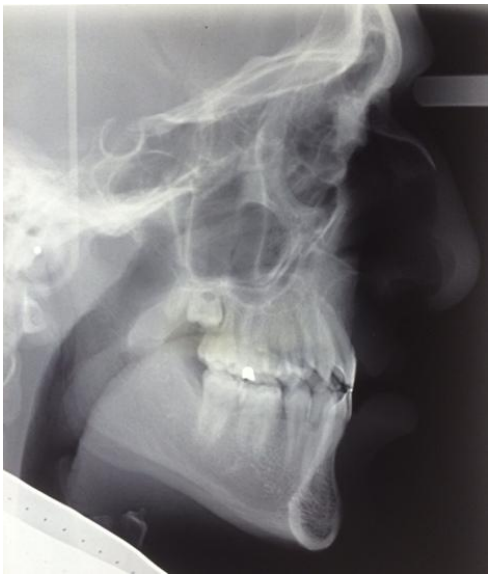

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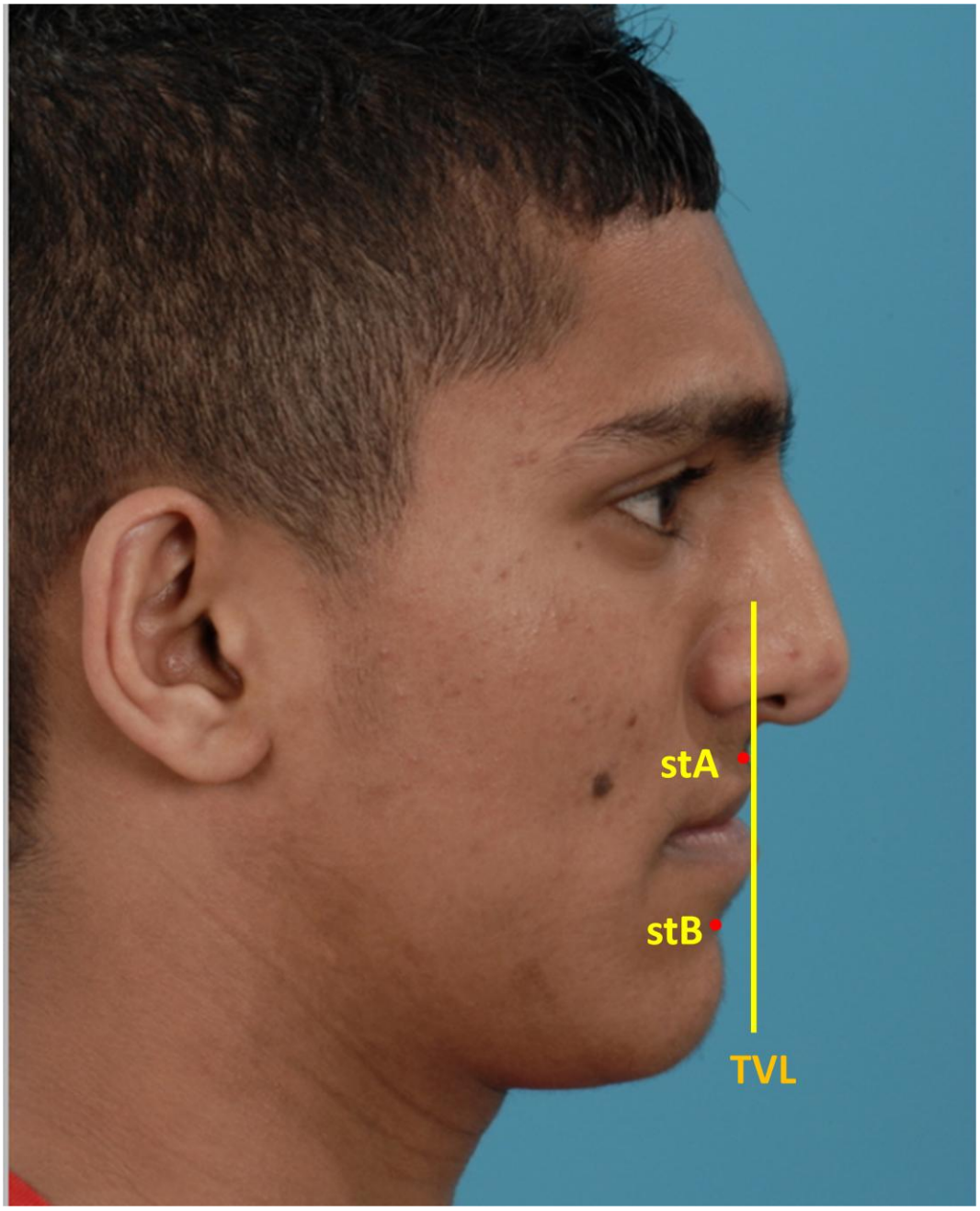
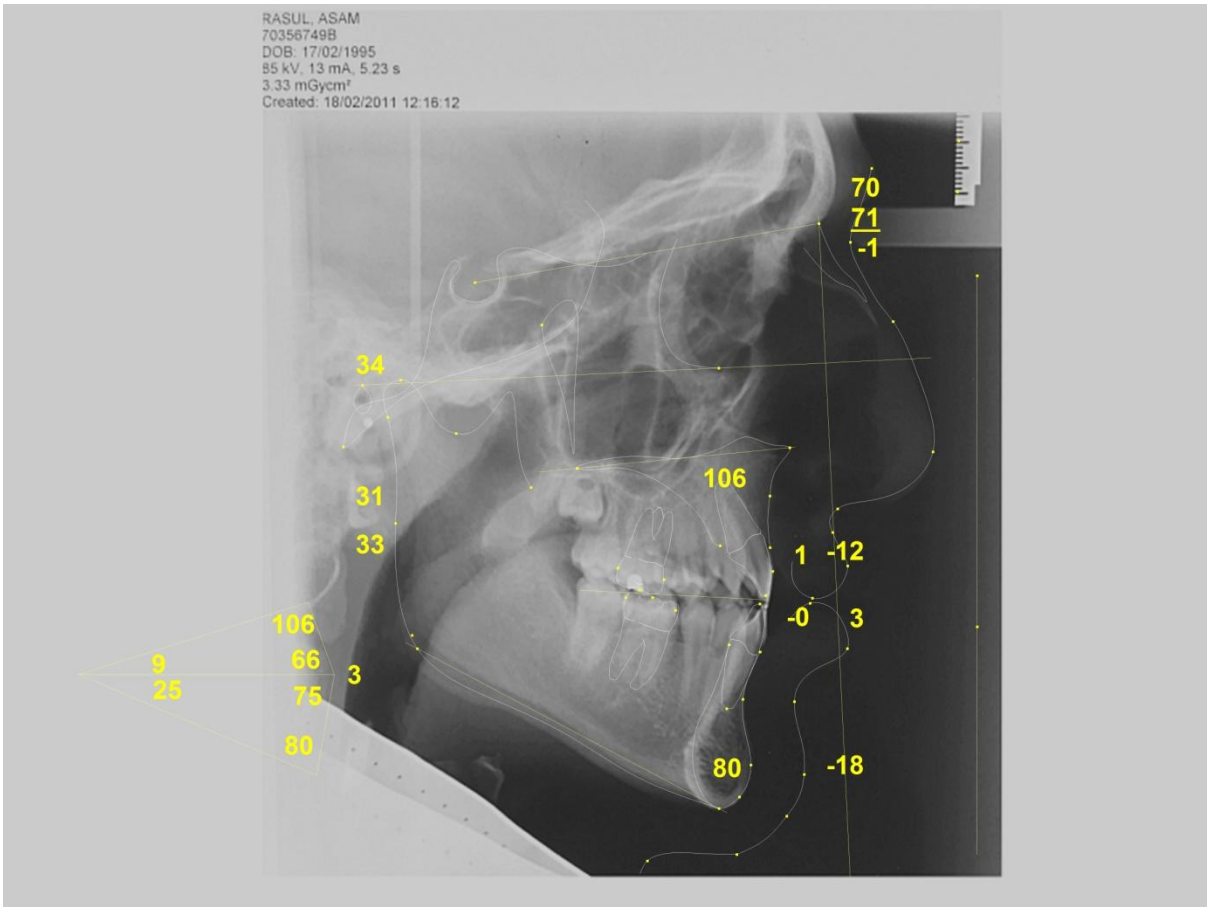


Figure 7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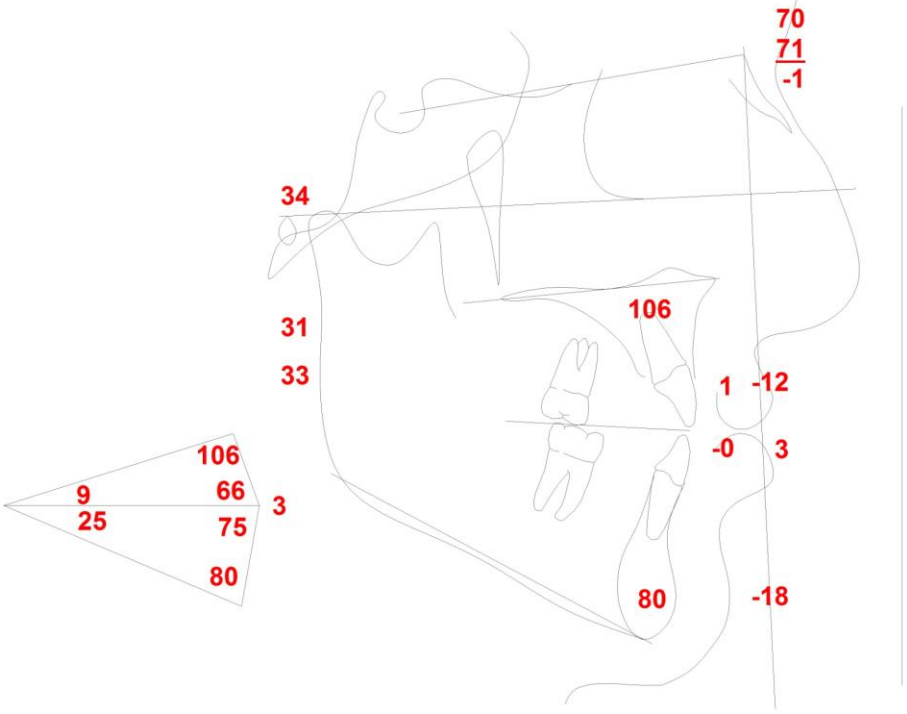
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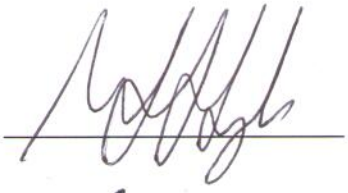
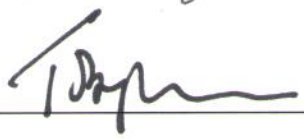
Ashraf Ayoub		20/9/17
Toby Gillgren		20/9/17

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Conflicts of Interest Statement

Manuscript title: The clinical application of BMP for reconstruction of alveolar cleft: 10 years follow up

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