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USAG-1 as a Regenerative Switch: Current Evidence and Future Directions for Third-Dentition-Based Tooth Renewal

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Abstract

Background:
Tooth agenesis is a common developmental anomaly that affects mastication, esthetics, and psychosocial well-being. Conventional treatments such as dentures, bridges, and implants can restore function but cannot regenerate living dental tissues. USAG-1, a dual antagonist of BMP and Wnt pathways, has emerged as a key inhibitory regulator of tooth number, making it a promising molecular target for activating dormant third-dentition potential. This review aims to summarize current experimental evidence on USAG-1 inhibition as a biological approach to tooth regeneration.

Methods:
A systematic search of PubMed, ScienceDirect, and Google Scholar (2019–2025) identified in vitro, in vivo, and preclinical studies evaluating USAG-1 modulation through gene knockout, siRNA delivery, or monoclonal antibodies. Studies lacking primary data or unrelated to odontogenesis were excluded.

Results:
Across major investigations, USAG-1 inhibition consistently restored tooth development in congenital agenesis models. Gene knockout animals produced supernumerary teeth due to enhanced odontogenic signaling. Local delivery of USAG-1 siRNA reactivated arrested tooth germs, demonstrating effective site-specific regeneration. Neutralizing antibodies further induced new teeth and rescued agenesis in multiple mammalian models, producing enamel–dentin structures similar to natural dentition. Genetic evidence supports USAG-1 as a central molecular switch coordinating key odontogenic pathways.

Conclusion:
Current findings establish USAG-1 inhibition as a promising strategy for activating third-dentition tooth formation. The development of humanized anti-USAG-1 antibodies represents a significant translational step toward future clinical applications in regenerative dentistry.

Keywords: USAG-1, tooth regeneration, third dentition, BMP/Wnt signaling, congenital tooth agenesis.

Introduction

Tooth agenesis, defined as the congenital absence of one or more permanent teeth (excluding third molars), is among the most frequent developmental anomalies of the dentition. Hypodontia, referring to the absence of up to five teeth, affects approximately 3–10% of the population, whereas oligodontia (≥6 missing teeth) is far less common, with a prevalence estimated at 0.1–0.5%. Cases may arise as isolated defects or as part of syndromic conditions and show a slight female predominance. The clinical consequences of agenesis extend beyond oral function, impairing mastication, speech, occlusion, and facial esthetics, and can lead to considerable psychosocial distress. In contrast, hyperdontia or supernumerary teeth occurs in roughly 1% of individuals[(1)].

Beyond congenital absence, tooth loss is highly prevalent in adults due to caries, periodontal disease, and trauma, frequently progressing to partial or complete edentulism. Complete edentulism affects predominantly older populations and remains a global public health concern, with an estimated 4.4% of the world's population completely edentulous in 2021. In the United State, about 1 in 6 older adults (17%) had lost all of their teeth during 2011-2016. Meanwhile, the mean number of permanent teeth retained among adults 20 to 64 years was 25.5 teeth and 2.2% of them have no remaining teeth. The functional burden is substantial, contributing to malnutrition, speech difficulties, and diminished quality of life, alongside psychological sequelae such as low self-esteem and social withdrawal. Current rehabilitative options such as removable prostheses, fixed bridges, and osseointegrated implants can restore appearance and function but do not reconstitute living, self-maintaining dental tissues[(2-4)].

These limitations have stimulated growing interest in biological strategies that aim not merely to replace but to regenerate teeth. Although tissue engineering approaches using bioengineered tooth germs or stem cell constructs have produced teeth in experimental animals they remain technically complex and are not yet translatable to routine clinical practice. Consequently attention has shifted toward molecular approaches that revive intrinsic odontogenic programs. One promising direction is the activation of latent third dentition tooth germs by modulating key inhibitory regulators of tooth number particularly USAG 1 which governs the balance of odontogenic signaling and has emerged as a tractable target for therapeutic tooth regeneration[(5)].

USAG-1 also known as (uterine sensitization-associated gene-1, also called SOSTDC1 or WISE) is a secreted antagonist of both BMP and Wnt signalling which are essential pathways for early tooth morphogenesis. By binding BMP ligands and the Wnt co receptor LRP5/6, USAG 1 functions as a molecular brake that constrains tooth number formation. Genetic deletion of USAG 1 in mice consistently leads to the formation of supernumerary teeth supporting its role as a negative regulator of odontogenesis. Conversely targeted inactivation through antibodies or siRNA can restore congenitally missing teeth in multiple animal models indicating that suppression of USAG 1 is sufficient to unlock dormant odontogenic potential. These findings position USAG 1 not only as a mechanistic determinant of tooth number but also as an actionable therapeutic target. The objective of this paper is to review current evidence on modulating USAG 1 to induce third dentition and to evaluate its prospect challenges and translational relevance for future regenerative dentistry[(5,6)].

Methods

A systematic literature search was conducted in PubMed, ScienceDirect, and Google Scholar to identify studies published between 2019 and 2025 using the Boolean strategy: ("USAG-1" OR "USAG1" OR "gene" OR "genetics") AND ("third dentition" OR "teeth" OR "dental" OR "odontogenesis" OR "tooth development") AND ("morphogenesis" OR "development" OR "formation" OR "growth"). Titles and abstracts were screened to identify studies examining the role or modulation of USAG-1 in tooth development or regeneration. Eligible full-text articles, including in vitro, in vivo, and preclinical animal studies, were retrieved and critically assessed. Studies were included if they evaluated USAG-1 function or inhibition and reported outcomes related to tooth number, morphogenesis, or regenerative potential. Exclusion criteria included non-English articles, reviews without original data, and studies unrelated to odontogenic development or dentition biology.

Result and Discussion

Table 1. Table of Key Methodology

Authors	Year	Country	Research Method	Brief Result
Murashima-Suginami *et al.*	2021	Japan	In vivo neutralization of USAG-1 using monoclonal antibody in mouse and ferret agenesis models	Blocking USAG-1 rescued congenitally missing teeth and induced third dentition by enhancing BMP signaling. The regenerated teeth exhibited histologically normal enamel and dentin layers, confirming functional odontogenesis.
Mishima *et al.*	2021	Japan	Local delivery of USAG-1 siRNA with gelatin hydrogel in Runx2–/– mice	Silencing USAG-1 locally restored tooth development in a model with arrested odontogenesis. The siRNA treatment reactivated tooth germ progression without systemic intervention, demonstrating feasibility of localized molecular therapy.
Takashi *et al.*	2020	Japan	Application of anti-USAG-1 neutralizing antibodies in mice, ferrets, and TOYO beagle models of congenital tooth agenesis.	Neutralizing antibodies against USAG-1 successfully restored missing teeth together with alveolar bone in animal models of congenital tooth agenesis, demonstrating strong regenerative potential, enabling the formation of third dentition like teeth, and supporting the advancement of the humanized antibody.

Ravi *et al.*	2023	Japan	Genetics analysis of tooth development genes (MSX1, PAX9, WNT10A, EDA, USAG-1)	Antibody based inhibition of USAG-1 activates the third-dentition pathway and drives functional tooth regeneration in congenital agnesis models, offering a natural and more accessible alternative to implants or dentures.

USAG-1 is a secreted antagonist expressed in odontogenic tissues. It binds both bone morphogenetic proteins (BMPs) and the Wnt co-receptor LRP5/6, inhibiting these essential signaling pathways during tooth development. In normal development, USAG-1 limits tooth formation by suppressing extra tooth germs. Consistent with this role, mice lacking Usag-1 develop multiple supernumerary teeth due to unchecked BMP/Wnt signaling. These findings imply that USAG-1 normally suppresses a latent tooth-forming field (the so-called third dentition). Accordingly, blocking USAG-1 activity can release this inhibition and promote new tooth growth. In other words, USAG-1 is a compelling target for regenerative therapy aimed at growing additional teeth[6,7].

Humans are diphyodont (having two sets of teeth), and a full third dentition normally never erupts. However, vestiges of a third dentition exist in early development. Imaging studies have shown that most human supernumerary teeth originate from this embryonic third set. For example, a computed-tomography study of 78 patients with hyperdontia found that their extra premolars and molars derived from third-dentition tooth germs. Thus, although the third dentition usually regresses, it retains the capacity to form whole teeth if appropriately stimulated. This suggests a therapeutic strategy: by activating the third dentition via molecular cues, missing teeth might be regenerated. Since USAG-1 normally inhibits third-dentition development, its inhibition could free these dormant germs to mature. In fact, in EDA1-deficient mice (another tooth-agenesis model), systemic USAG-1 antibody treatment was shown to activate third-dentition tooth formation[8].

Proof-of-principle for USAG-1 targeting has come from mouse experiments. Mishima et al. applied USAG-1–targeted siRNA to the embryonic jaw area of Runx2-knockout mice (which otherwise have arrested tooth development). They found that this topical USAG-1 knockdown significantly increased tooth formation and partly rescued the missing-molar phenotype. The most effective siRNA construct (#304) restored tooth germs in both cultured mandible explants and in vivo in Runx2-null mice. Notably, the siRNA was delivered via a cationic gelatin hydrogel carrier, demonstrating a feasible drug-delivery approach. In summary, locally delivered USAG-1 siRNA successfully engaged the latent tooth-regeneration pathway, producing new tooth structures without genetic modification of the animal[8,9].

Antibody-based USAG-1 inhibition has also been effective. Murashima-Suginami et al. generated a neutralizing monoclonal antibody against USAG-1 and tested it in various mouse models of agenesis. A single systemic dose of this antibody given to neonatal mice restored normal tooth development in models of MSX1 or EDA1 deficiency. In essence, the antibody blocks USAG-1's binding to

BMPs (but not to Wnt components), selectively boosting BMP signaling required for odontogenesis. The treated mice showed rescued tooth formation (no missing teeth), and importantly no adverse side effects were observed. These results demonstrate that neutralizing USAG-1 protein alone is sufficient to trigger extra tooth growth in vivo. Thus, an injectable biologic agent (anti-USAG-1) can activate dormant tooth germs in mammals[6].

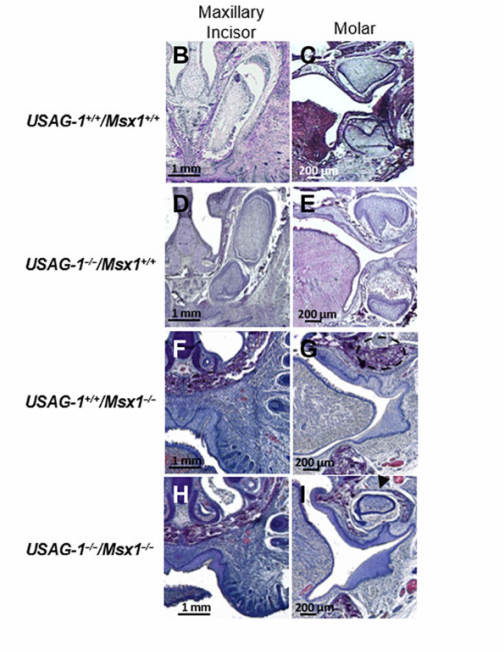

Figure 1. (B to I) Frontal hematoxylin and eosin–stained sections of the left maxillary incisor and third molar (M3) in USAG-$1^{-/-}$/$Msx1^{-/-}$ mice immediately after birth[6].

Refers to the research by Suginami *et al*., 2021, the experiment compared four genetic groups: wild-type mice with intact USAG-1 and Msx1, single knockouts lacking either USAG-1 or Msx1, and a double-knockout line lacking both USAG-1 and Msx1. $Msx1^{-/-}$ mice normally show arrested tooth development because Msx1 is required for continuation of the odontogenic program, whereas USAG-$1^{-/-}$ mice develop extra teeth because USAG-1 acts as a brake on BMP and Wnt signaling. By creating the USAG-$1^{-/-}$/$Msx1^{-/-}$ double knockout, the study tested whether removing the inhibitory USAG-1 signal could compensate for the Msx1-dependent block and restore tooth formation. The histological panel compares tooth morphology in the maxillary incisor and third molar across four genotypes to demonstrate the functional interaction between USAG-1 and MSX1, with panels B–C showing normal tooth histology in wild-type controls, D–E and F–G illustrating the distinct phenotypes of USAG-1 knockout and Msx1 knockout animals respectively, and H–I showing that simultaneous loss of USAG-1 in the $Msx1^{-/-}$ background restores tooth formation. In particular, the USAG-$1^{-/-}$/$Msx1^{-/-}$ sections (H–I) display well-formed tooth structures with organized enamel- and dentin-like tissues where $Msx1^{-/-}$ alone (F–G) exhibits arrested or abnormal development, indicating that ablation of USAG-1 can rescue the developmental block caused by Msx1 deficiency. The images therefore provide direct morphological evidence that USAG-1 acts as a negative regulator of odontogenesis and that its removal permits progression of tooth morphogenesis even in a genetic context that otherwise produces agenesis, consistent with the proposed mechanism that USAG-1 limits BMP/Wnt signaling during tooth development. These results support the concept that targeted inhibition of USAG-1 can reactivate latent tooth germs and underlie the rationale for therapeutic strategies aimed at inducing third dentition[6,7].

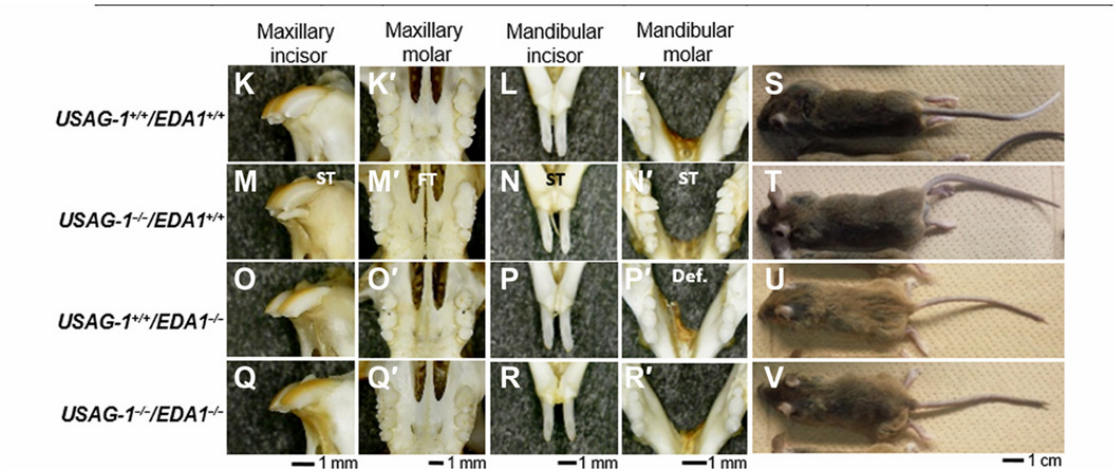

Figure 2. (K to R') Representative tooth phenotypes in dry skulls of 8-month-old F2 generation EDA1/USAG-1 double-mutant mice[6].

The composite panel compares oral and whole-animal phenotypes across four genotypes to illustrate the genetic interaction between USAG-1 and EDA1 in tooth development. Columns show the maxillary incisor and molar, and mandibular incisor and molar, while rows correspond to wild type, USAG-1 knockout, EDA1 knockout, and the USAG-1/EDA1 double knockout, with adjacent whole-animal images for gross phenotype comparison. Wild-type specimens present normal dentition, whereas USAG-1–/– animals display supernumerary teeth in multiple regions, reflecting derepression of tooth-forming signals. By contrast EDA1–/– mice show hypodontia or defective tooth formation in the affected positions, consistent with congenital agenesis. Crucially the USAG-1–/–/EDA1–/– double mutants exhibit restoration of tooth structures in locations that are missing in EDA1 single knockouts, demonstrating that loss of USAG-1 can compensate for the developmental block caused by EDA1 deficiency. These panels therefore provide clear phenotypic evidence that antagonism of USAG-1 releases latent odontogenic potential and can rescue genetically induced tooth agenesis[6].

Following these preclinical successes, efforts are turning toward translation. A humanized anti–USAG-1 antibody has been created and is now being prepared for clinical trials in patients with congenital tooth agenesis. Complementary strategies are also under study (for example, targeting other pathways like EDA or Wnt, or improving delivery of siRNA). Patient stratification will be important: genetic or imaging biomarkers could identify individuals who retain third-dentition germs and are most likely to benefit. Indeed, Ravi et al. emphasize that understanding USAG-1 variants and their partners will aid development of predictive biomarkers for tooth-regeneration therapy. As an example of progress beyond rodents, systemic USAG-1 antibody in ferrets (whose dentition is similar to humans) produced an extra tooth resembling a permanent premolar. These large-animal results are encouraging, but further safety and efficacy validation will be needed before human application[8,9].

Modifying USAG-1 represents a novel strategy to regenerate teeth by harnessing the body's own developmental programs. Multiple studies show that interfering with USAG-1 (via gene knockout,

siRNA or antibody) can unleash a tooth-forming program (the latent third dentition) in mammalian jaws. This approach merges developmental biology with targeted molecular therapy: instead of implanting cells or prosthetics, one "drugs" the genome to regrow teeth. Remaining challenges include precisely controlling the size, number and position of regenerated teeth and ensuring proper integration with jaw bone. If these hurdles can be overcome, USAG-1–based therapies could revolutionize treatment of tooth loss, potentially regenerating new teeth in place of missing ones. Ongoing research and eventual clinical trials will determine whether this gene-targeted approach can fulfill the long-held dream of true tooth regeneration.

Conclusion

Current research demonstrates that targeting USAG-1 is a promising biological strategy for stimulating the latent third dentition and restoring tooth formation in cases of congenital or acquired tooth loss. Evidence from genetic models, siRNA delivery systems, and monoclonal antibody therapies consistently shows that suppression of USAG-1 can stimulate odontogenic activity in agenesis phenotypes and induce the formation of new tooth structures, supporting its role as a central regulator of odontogenic signaling. The recent development of humanized anti-USAG-1 antibodies represents a critical milestone toward clinical translation, indicating the feasibility of future therapeutic application in humans.

Although these findings are encouraging, further studies are required to validate long-term safety, optimize delivery and dosing, control the morphology and positioning of regenerated teeth, and identify ideal patient candidates. USAG-1-based intervention therefore holds substantial potential to shift dental treatment paradigms from prosthetic replacement toward biologically driven regeneration. Continued interdisciplinary research and well-designed clinical trials will be essential to determine whether this approach can be safely and effectively translated into routine regenerative dentistry practice.

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