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Review

Anti-USAG-1 therapy for teeth agenesis and biological tooth regeneration: review of current evidence and future directions

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ABSTRACT

Hereditary and acquired tooth loss poses a significant challenge for dental patients worldwide. This has led to a lot of research into methods that have alternative teeth loss solutions. One promising area of research is focusing on molecular pathways related to tooth development. Tooth agenesis or the failure to develop teeth is a common developmental dental abnormality. It can manifest as anodontia, where no teeth develop, oligodontia, where a few teeth are missing, or hypodontia, where one or two teeth are missing. This condition can be part of a syndrome or occur on its own due to genetic abnormalities related to tooth development. Recent studies have identified Uterine Sensitization-Associated Gene-1 *(USAG-1/SOSTDC1)* as a regulator of tooth development. It does this by inhibiting Wnt signaling pathways and Bone Morphogenetic Protein (BMP). The goal of this review is to provide an overview of the role of the Wnt signalling pathways and *USAG-1*-mediated tooth agenesis, with a focus on their implications for tooth regeneration. We looked at published research on *USAG-1* biology, tooth agenesis genetics and animal studies well as new clinical trials. The evidence shows that tooth agenesis is often caused by mutations in genes, including *MSX1, PAX9, WNT10A, AXIN2, EDA etc…* Experimental studies have found that inhibiting *USAG-1* can awaken tooth germs and improve BMP signaling. Several monoclonal antibodies that target *USAG-1* have been discussed. One, antibody #37 showed the most promise in treating hypodontia in animal models. In mice and ferrets, anti-USAG-1 treatment promoted the development of additional or regenerated tooth-like structures and stimulated odontogenic growth. This suggests that BMP signaling plays a role in controlling tooth number. The use of *USAG-1* treatment has advanced to Phase I clinical trials in Japan to evaluate its safety and pharmacokinetics. Importantly, current preclinical evidence of anti-USAG-1 mediated tooth regeneration is mainly based on animal models of congenital tooth agenesis, such as hypodontia and oligodontia. However, evidence supporting regeneration after acquired tooth loss due to trauma or disease is still limited. The potential application of anti-USAG-1 therapy to acquired tooth loss, therefore, should be viewed as a translational hypothesis rather than an experimentally validated therapeutic outcome. Further studies in suitable models of acquired tooth loss are necessary to determine if this approach can be effective in inducing tooth regeneration in this context.

1. Introduction and background

The human dentition is fundamental not only to masticatory function and nutritional intake but also to craniofacial integrity, speech articulation and psychosocial well-being. Despite advancements in preventive dentistry, tooth loss remains a global health burden. According to Borg-Bartolo, R., et al., 22% of adults over 65 are completely edentate (Borg-Bartolo et al., 2022). While acquired tooth loss affects a vast majority of the adult population, congenital tooth agenesis represents a distinct and complex developmental challenge. Congenital tooth agenesis is defined as the developmental absence of one or more teeth due to failure of odontogenic initiation in the dental lamina. It can affect either the

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primary dentition or the permanent dentition and can present as a syndromic or non-syndromic condition (Meade and Dreyer, 2023). The clinical manifestation of congenital tooth agenesis is categorized based on the severity of the deficiency into hypodontia, oligodontia or anodontia. Hypodontia is characterized by the absence of one to five permanent teeth (excluding third molar). It represents the most prevalent form where *meta*-analyses suggest a global prevalence of approximately 3% to 10% in the general population, with higher frequencies noted in females (Khalaf et al., 2014). Oligodontia, a more severe phenotype involving the absence of six or more permanent teeth, is significantly rare, it affects roughly 0.1% to 0.5% of the population, and is often associated with specific genetic mutations such as *MSX1, PAX9 or AXIN2* (Yang et al., 2020; Safari et al., 2020; Ren et al., 2023). Anodontia, the most extreme and rarest form, defined by the total absence of all teeth within the primary or permanent dentition. This is typically observed as a component of a multi-systemic syndromes like hypo-hidrotic ectodermal dysplasia (Klupś et al., 2022).

Dental implants are considered the current gold standard due to their high success rates and ability to preserve alveolar bone. However, they possess a fundamental biological flaw which is the absence of the periodontal ligament. Natural teeth are held by periodontal ligaments, which contains mechanoreceptors that provide sensory feedback during mastication. Implants on the other hand are ankylosed to the alveolar bone leading to a lack of tactile sensitivity which can result in occlusal overloading (González-Gil et al., 2022).

Unlike natural teeth, which have a complex biological seal, the interface between an implant and the gingiva is more susceptible to aggressive inflammatory bone resorption (Ivanovski and Lee, 2000. 2018). More importantly, implants cannot be placed in developing children such as in cases of oligodontia because they do not move with the developing jaw which represent a pediatric limitation (Cherian et al., 2023). Fixed partial dentures on the other hand, require the permanent reduction of healthy adjacent teeth to support the prosthetic crown. This increases the risk of pulpitis, secondary caries and eventual tooth loss of the abutment itself. Also, prosthetic bridges do not provide alveolar bone stimulation in the edentulous gap. Overtime, the alveolar ridge undergoes significant resorption creating ''dark triangles'' and aesthetic failure (Vlăduțu et al., 2026). Fig. 1.

The field of dental regeneration has transitioned from structural prosthetics to advanced molecular therapeutics. Initial regenerative efforts focused on scaffold-mediated tissue engineering, which utilized the seeding of multipotent stem cells onto synthetic, biodegradable polymers. Research demonstrated that dental pulp stem cells could be harvested and expanded *in-vitro* to generate rudimentary odontogenic structures (Young et al., 2002). While this proved the regenerative capacity of dental pulp stem cells, the primary challenge remained the inability to replicate the complex, organized morphology and hard-tissue integration of a natural tooth (Olaru et al., 2021).

Subsequently the field pivoted toward developmental bioengineering, specifically the organ germ method. Rather than using

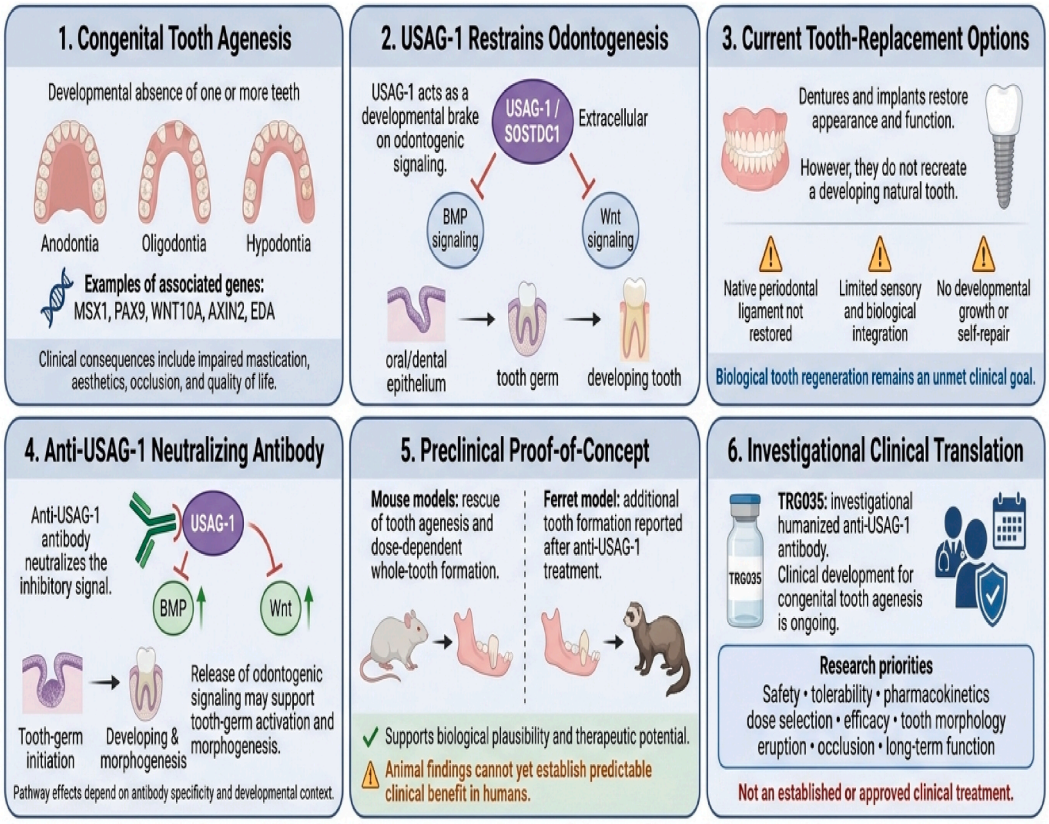


Fig. 1. USAG-1 in tooth development and regeneration: molecular mechanisms and therapeutic potential of anti-USAG-1 antibodies. Tooth agenesis is associated with dysregulation of those genes that are involved in tooth formation, such as MSX1, PAX9, WNT10A, AXIN2, and EDA. Toothlessness or lack of full set of teeth can also be caused by such genetic defects. USAG-1, which is alternatively called SOSTDC1, acts as a negative regulator in the tooth development process by blocking Wnt and bone morphogenetic protein (BMP) signaling. Preventing USAG-1 activity through the use of monoclonal antibodies could increase odontogenic signaling, reactivate dormant tooth germs, and ultimately lead to tooth formation. Both mouse and ferret studies have confirmed effective tooth regeneration results with the method whereas safety of the intervention, pharmacokinetics, dosing, and regenerative efficacy in humans are being clinically investigated. In addition, tooth replacement using prosthetics and implants are shown in the figure as limited options. The graphics were drawn with help from AI model, Google Gemini, for the conceptual layout and visual arrangement. The authors reviewed, corrected, and finalized all the scientific content, terminology, references, and artwork; hence they bear full responsibility for the accuracy and reliability of the material presented.

synthetic molds, researchers focused on the reciprocal induction between epithelial and mesenchymal cell populations (Jamal, 2016).

By recombining these cell populations onto a bioengineered tooth germ, scientists successfully developed fully functional teeth that exhibited physiological eruption and occlusal loading when transplanted into adult jawbones (Ikeda et al., 2009). However, the reliance on embryonic or highly specific cell sources created significant barriers to widespread clinical application.

The current frontier is defined by the modulation of endogenous regenerative pathways via molecular switches. This approach centers on *USAG-1* (Uterine Sensitization Associated Gene-1), a glycoprotein that acts as a dual antagonist to Bone Morphogenetic Protein (*BMP*) and Wnt signaling pathways which are both critical for odontogenic initiation and patterning (Moradi et al., 2026). In experimental models, *USAG-1* suppresses the development of additional tooth germs; whether an equivalent regulatory mechanism operates in humans remains uncertain. The third dentition is tooth-like structures appeared after the permanent dentition in animal models. In humans, a normally occurring and complete third dentition has not been established. Therefore, references to a possible human third dentition should be regarded as a hypothesis rather than a demonstrated biological process (Kiso et al., 2019).

By shifting from invasive cellular transplantation to targeted pharmacological intervention, hypothesized *USAG-1* therapy represents a paradigm shift toward non-surgical, ligand-based dental regeneration.

USAG-1 has been identified as a potent antagonist of odontogenesis. It is a bifunctional glycoprotein that serves as a critical regulator in limiting the number of teeth an individual develops by simultaneously antagonizing two essential biological pathways; Bone Morphogenetic Protein (BMP) and Wnt/B-catenin signaling pathways (Ahn et al., 2010).

The identification of *USAG-1* as a therapeutic target for dental regeneration originated from the clinical observation of hypodontia. Research led by Dr. Katsu Takahashi and his team at Kyoto University demonstrated that mice deficient in the *USAG-1* null murine models exhibited supernumerary dentition, indicating that the protein's primary physiological function is the suppression of tertiary odontogenic progeny (Takahashi et al., 2020). By neutralizing this protein, the dormant regenerative potential of the dental lamina can be reactivated.

The therapeutic breakthrough came with the development of monoclonal antibodies specifically designed to interfere with *USAG-1*/BMP molecular interface. In contrast to the canonical Wnt signaling pathway, which regulates diverse homeostatic systems, the BMP-specific interaction of *USAG-1* displays higher tissue specificity within the dental mesenchyme. In phenotypic models of tooth agenesis, the administration of these antibodies successfully rescued arrested odontogenic buds, leading to the formation of morphologically complete, functional teeth (Murashima-Suginami et al., 2021).

USAG-1 became a leading preclinical candidate for odontogenic regeneration research focus because of its molecular tractability and the efficacy observed in animal models; however, human genetic causality and clinical efficacy remain unestablished. By targeting a specific inhibitory ligand rather than employing cellular transplantation, the modality shifts from a surgical intervention to a targeted pharmacological therapy. As clinical trials transition to human cohorts, *USAG-1* modulation represents the first viable molecular therapy for treating anodontia and hypodontia by leveraging endogenous regenerative blueprints. This molecular disinhibition strategy avoids the ethical and technical hurdles of stem cell sourcing, positioning *USAG-1* as the cornerstone of 21st century regenerative dentistry (Moradi et al., 2026).

2. Ethical concerns of Anti-USAG-1

The therapeutic exploitation of Anti-*USAG-1* not only ethical concern but also scientific caution. To begin with, augmenting *BMP* signal might not be limited to the targeted tooth germ only. Overactivation or inappropriate activation of the pathway might, in principle, lead to increased tooth number, morphological modification, ectopic mineralization or disturbed root development as well as other unintended effects on alveolar bone and other tissues. It has been actually demonstrated experimentally that *USAG-1* gene ablation may result in supernumerary teeth, therefore highlighting the importance of the careful regulation of the intensity, period, and spatial distribution of the treatment (Ravi et al., 2023).

Although systemic administration of an Anti-*USAG-1* antibody does not permanently alter the host genome, it may still induce sustained biological effects, immune-mediated responses, off-target consequences, or unpredicted complications. *USAG-1* is not only produced by dental tissues but also participates in signaling pathways that control skeletal and organ development.

In addition, systemic neutralization of a developmental signaling antagonist warrants specific attention to on-target but off-organ toxicities. Wnt signaling is essential for bone homeostasis and adult tissue maintenance, and clinical experience with upstream Wnt-pathway inhibitors has shown bone related adverse events, including fractures and even early trial interruptions, illustrating how pathway-directed interventions can produce mechanism-based toxicity outside the intended tissue (Nachef et al., 2026; Madan et al., 2018). Because Wnt signaling also supports regeneration in tissues with high cellular turnover, pathway perturbation has been associated more broadly with gastrointestinal, hematologic, immune, and neurologic adverse effects in clinical development programs (Nachef et al., 2026). Although Anti-*USAG-1* is biologically distinct from these agents, these data justify caution that systemic manipulation of the BMP/Wnt axis could affect alveolar bone. Skeletal remodeling, and non-dental organs beyond the oral cavity (Nachef et al., 2026; Madan et al., 2018).

Another significant issue is that the limited application of present findings in human health may be due to the fact that most research has been done using genetically modified animals or other preclinical models which are not directly applicable to humans. A key translational limitation is that current preclinical models do not yet establish predictable root maturation, durable periodontal ligament attachment, long-term functional stability, or controlled eruption kinetics of regenerated teeth, and it remains uncertain wether latent or vestigial odontogenic buds persist in adult human jaws in a therapeutically targetable form comparable to those described in experimental animals (Ikeda et al., 2009; Jin et al., 2026; Furquim et al., 2022; Vaquette et al., 2019; Dangaria et al., 2011; Nuñez et al., 2000. 2019,; Calabrese et al., 2024; Sui et al., 2023). It could be that human tooth development, craniofacial morphology, immune responses, pharmacokinetics, and the ability to regenerate tissue are very different from those of experimental animals (Takahashi et al., 2024). The first requirement of future clinical trials should therefore be to demonstrate not merely radiographic tooth presence, but the regeneration of a fully functional tooth unit with appropriate crown morphology, root length, periodontal attachment, pulp-dentin organization, vascularization, sensory function, occlusal integration, and long-term resistance to caries and periodontal breakdown.

A further ethical issue is therapeutic misconception, particularly in pediatric and adolescent candidates with tooth agenesis and in their caregivers. Therapeutic misconception arises when research participation is confused with individualized treatment, and this confusion can undermine the validity of consent (Ceruti, 2021; Woods et al., 2014; de Melo-Martín and Ho, 2008). In pediatric settings, vulnerability is heightened because parental hope, trust in clinician-investigators, and emotionally charged translational narratives can foster unrealistic expectations of direct benefit (Woods et al., 2014; de Melo-Martín and Ho, 2008). This concern is especially relevant in regenerative dentistry, where public and media portrayals of ''growing a third set of human teeth'' may encourage promissory hype and a collective overestimation of clinical readiness (Woods et al., 2014; Woods, 2018; Langford and Foong, 2024).

The research paper published by Verma et al. 2025 showcases the

difference between the hope and hype of cell free based teeth regeneration: while 82% of dental professionals surveyed thought that *USAG-1*-based therapy will significantly impact clinical dentistry, only 36.4% were confident that they could explain the mechanism to the patient, 56.4% recognized potential ethical dilemmas, and 65.7% thought it was too soon to trust humans with *USAG-1*-based therapy (Verma et al., 2025). These results indicate that optimism among dental professionals appears to exceed explanatory confidence and ethical certainty, reinforcing the need for careful expectation management.

Many potential participants may be children or teenagers who can provide only age-appropriate assent and therefor require additional protections. Ethical guidance consistently holds that children should be involved in the decision-making process in a manner adapted to their age, maturity, language ability, and understanding, while comprehensive information must also be given to parents or legal representatives (Lepola et al., 2022; Lombardi et al., 2018). A child or adolescent capable of forming a view should provide assent, and potential dissent should be actively elicited and respected rather than treated as a mere formality (Lepola et al., 2022; Ren et al., 2026). Contemporary ethics literature further emphasizes that assent should be understood as an ongoing process, not a signature event, and that meaningful participation is improved by clear, empathetic, developmentally appropriate communication and youth-friendly formats such as visual or multimedia materials (Ren et al., 2026; Luengo et al., 2025; McMillan, 2022).

For further phase II/III trials, consent procedures should therefore clearly distinguish research from clinical care, explain uncertainty regarding benefit, and foreground foreseeable risks, adverse effects, and post-trial provisions, since these are among the items that children and parents rate as most important in pediatric trial information (Koonrungsesomboon et al., 2022). Strict risk–benefit evaluation remains essential. Ethical frameworks support children's inclusion in trials, but only alongside protection of their rights, shared decision-making, and a best-interest standard that accounts for both short and long-term burdens (Langford and Foong, 2024; Nepi, 2019). Special situations, including varying maturity, re-assent in long studies, and context-specific consent complexities, should be anticipated prospectively in trial protocols and publications (Lepola et al., 2022).

Concluding ethical concerns, an efficient translation will rely upon access to the treatment, reasonably priced drug, regulatory approval, monitoring post-treatment and collaboration from a multidisciplinary team of dental clinicians, developmental biologists, pharmacologists, bioethicists and patients.

Until the problems are solved through human trials of sufficient scale.

Anti-*USAG-1* has to be considered a promising investigational regenerative approach rather than a clinically confirmed treatment.

The primary objective of this review is to evaluate the clinical viability of anti-*USAG-1* monoclonal antibodies as a pharmacological modality for de novo odontogenesis. Specifically, this paper aims to elucidate the molecular interplay by which the targeted inhibition of *USAG-1* facilitates the disinhibition of odontogenic buds.

The significance of this research lies in its potential to catalyze a paradigm shift in the management of congenital tooth agenesis and, ultimately acquired tooth loss. For decades, the prosthodontic ''gold standard'' has relied upon alloplastic implants or removal prostheses-interventions that lack biomimetic integration, proprioceptive feedback, and the capacity for functional remodeling within the alveolar bone.

Anti-*USAG-1* therapy represents a departure from traditional tissue engineering; it is the inaugural pharmacotherapeutic agent that leverages endogenous morphogenetic pathways rather than exogenous synthetic scaffolds. By reactivating tertiary dentition, this therapy offers a permanent biological restoration of the entire odontogenic unit, including the periodontal ligament and the surrounding alveolar process.

This review focuses strictly on the biochemical role of *USAG-1* as a dual-pathway antagonist within the BMP and Wnt signaling cascades. While acknowledging prior advancements in mesenchymal stem cell research and scaffold-based tissue engineering, the scope is primarily delimited to literature published between the initial identification of *USAG-1* in odontogenesis and the current trial updates. Priority is given to high-impact studies involving monoclonal antibodies and molecularly targeted ligands, excluding unrelated dental biomaterials or conventional osteoconductive grafting techniques.

3. USAG-1 protein characterization

3.1. Structural organization and disulfide-bond architecture

Uterine sensitization-associated gene-1 (USAG-1), also known as sclerostin domain-containing protein 1 (SOSTDC1), ectodin, or Wise, is a secreted glycoprotein of the sclerostin family. The canonical human SOSTDC1 precursor (universal protein resource: UniProtKB Q6X4U4) comprises 206 amino acids, including an N-terminal signal peptide at residues 1–23 and a mature secreted chain at residues 24–206. Its principal structured region is a C-terminal cystine-knot (CTCK) domain spanning residues 75–170, annotated by PROSITE-ProRule PRU00039. Two predicted N-linked glycosylation sites are annotated at Asn47 and Asn173 (Fig. 2) (Vaquette et al., 2019; Dangaria et al., 2011; Nuñez et al., 2000. 2019).

The CTCK domain contains eight cysteine residues that form four annotated intramolecular disulfide bonds: Cys75–Cys133, Cys89–Cys147, Cys100–Cys163, and Cys104–Cys165. These disulfide bonds stabilize the cystine-knot-like fold; none should be described as an intermolecular linkage or as individually responsible for dimerization. Biochemical and biophysical analyses instead indicate that SOSTDC1 forms a stable non-disulfide-linked dimer in solution. The sequence-derived molecular mass of the 206-residue precursor is approximately 23 kDa, whereas the mature glycosylated protein has been reported to migrate at approximately 28–32 kDa (Dangaria et al., 2011). SOSTDC1 functionally associates with BMP ligands and the Wnt co-receptors LRP5/6; these associations are shown schematically in Fig. 2 and are not intended to represent precisely mapped residue-level binding sites



Fig. 2. AlphaFold3-predicted structure of full-length human SOSTDC1/USAG-1 rendered in ChimeraX. The model for human USAG-1 [UniProt Q6X4U4, 206 aa] was generated with AlphaFold3 via AlphaFold Server [file: fold_sostdc1_model_0.cif] and manually visualized in UCSF ChimeraX in cartoon representation. The central cystine-knot domain [CTCK, PRU00039, residues ~90-180] forms the structured core with two beta-strands and conserved cysteines. Flanking N-terminal residues 1–89, which include the signal peptide 1–23, and C-terminal residues 181–206 are predicted with low confidence [pLDDT < 50] and appear as extended disordered loops and a short helical segment at lower left. High-confidence region pLDDT > 70 corresponds to the CTCK domain. The structure is a prediction, not experimentally determined. (UCSF ChimeraX, xxxx).

(Ahn et al., 2010; Furquim et al., 2022).

3.2. Spatiotemporal expression during odontogenesis

During craniofacial morphogenesis, *Sostdc1/USAG-1* expression is detected in odontogenic epithelial and mesenchymal compartments in stage and region dependent patterns. In developing tooth germs, its expression is associated with the dental lamina, enamel-organ-associated tissues, and surrounding dental mesenchyme, where it contributes to the spatial regulation of *BMP* and Wnt signaling. Through this local inhibitory activity, *SOSTDC1* helps delimit signaling domains involved in tooth initiation, enamel-knot organization, cusp patterning, and tooth-number determination (Calabrese et al., 2024; Sui et al., 2023).

3.3. Bifunctional antagonism

USAG-1 holds a unique antagonism property among secreted modulators, which works on both BMP and Wnt/beta-catenin signaling (Zhu et al., 2024). In cell-based assays, *USAG-1* binds directly to multiple BMP ligands (BMP 2, 4, 6 and 7), consequently avoiding their binding to type I and type II BMP receptors (Liu et al., 2022). The increase of production of USAG-1 concentrations progressively elevates the suppression rate of BMP-induced *Smad* 1/5 phosphorylation and downstream transcriptional responses (Liu et al., 2023). This means that at tooth development, *USAG-1* can restrain BMP signals that would otherwise drive differentiation and proliferation with odontogenic epithelium and mesenchyme (Rudresh, et al., 2022).

Not only does *USAG-1* antagonize BMP signals, but it also inhibits the Wnt pathway, acting at the level of *LRP 5,6* co-receptors. *USAG-1* contains a conserved molecular plug protein *NXI* motif that mirrors the *LRP6*-binding sequence in sclerostin (SOST). For instance, it recognizes the same E1 domain on the extracellular portion of *LRP5, 6* signals.

Ahn, Sanderson et al. 2010 employed a combination of cell-based reporter assays and co-expression experiments in murine and human cell lines to demonstrate that Wise/*USAG-1* may physically and functionally interact with the extracellular domain of LRP6 (Ahn et al., 2010). In their study, they transiently transfected cells with Wnt ligands, *LRP6* and Wise *(SOSTDC1)*, and then measured canonical Wnt/beta-catenin activity using a T-cell factor/lymphoid enhancer factor (TCF/LEF) –driven luciferase plasmid reporter (TOPFlash), observing that adding Wise caused a clear drop in reporter signal, which indicated that Wise dampens Wnt signaling at the level of the co-receptor (Ahn et al., 2017). Co-immunoprecipitation binding assays were employed, wherein *LRP6* and *Wise* were jointly expressed, and one protein was isolated using a particular antibody in order to validate that *Wise* can form a complex with the *LRP5/6* ectodomain *in-vitro*, thereby substantiating the hypothesis that direct binding contributes to the reduction in Wnt activity (Ahn et al., 2010).

Murashima-Suginami et al., 2021 advanced this research by producing monoclonal antibodies targeting *USAG-1* and evaluating their efficacy in binding and reporter experiments with recombinant mouse *USAG-1* and *LRP6 E1–E2* segments (Murashima-Suginami et al., 2021). Their methodology combines the use of *USAG-1* antibodies by ELISA-type plate binding assay to analyze the mode of how *USAG-1* binds to immobilized LRP-6 E1- E2 segments. They found that some clones completely disabled this interaction. Concurrently, they employed a TOPFlash dual-luciferase reporter system in cultured cells to investigate the impact of these antibodies on Wnt/β-catenin signaling in the presence of recombinant *USAG-1*, demonstrating that the same antibodies that interfered with *USAG*-1–*LRP6* binding also mitigated the inhibitory effect of *USAG-1* on Wnt signaling.

Based on these in vitro findings, they deduced that *USAG-1* inhibits canonical Wnt signaling by directly binding to the *LRP5/6* ectodomain, and that the blockade of this interaction by specific neutralizing antibodies allows for the restoration of Wnt signaling, thereby illustrating that co-receptor engagement is crucial for the Wnt-antagonist function of *USAG-1*.

3.4. Role in regulation of odontogenesis number

The process of teeth number regulation during development relies on a number of meticulous processes with proper morphogen activation and inhibition. Consequently, *USAG-1* becomes an important regulatory factor mediating the process of tooth development (Bodensteiner RO, Culler S. Tooth Regeneration: Communicating Novel Dental Research Responsibly, 2025). During the early tooth formation stage, *USAG-1* expression is detected around the normally regressing primitive incisor buds, where it overlaps with BMP-7 expression domains in the upper incisor region. Furthermore, *USAG-1* shares in the process of apoptosis in specialized ectomesenchymal cells, blocking their continuity to division (Kiso et al., 2014).

To validate the hypothesis of altering tooth number by releasing BMP signaling during early odontogenesis, Murashima-Suginami Aal., 2008 formulated USAG-1 knockout mice by its loss. They used in situ hybridization and immunostaining for BMP target receptors, namely, phospho-*Smad 1/5, Msx1, and Dlx2*, examining it by micro-CT imaging assays and postnatal and embryonic jaw histology in primitive tooth germs. They found supernumerary upper incisors and paramolars in the *USAG-1*-depleted mice group, these extra teeth arose from primitive buds that would normally regress but instead survived in the mutated background. They concluded that *USAG-1* normally inhibits BMP activity to promote programmed cell death of latent tooth buds, thereby preventing the formation of excess teeth (Verma et al., 2025).

Kiso, Takahashi et al. 2014 confirmed this conclusion by observing *USAG-1* and *BMP-7* interaction in the number regulation of maxillary incisors. They aimed to describe if *BMP-7* was the critical linker that *USAG-1* can counteract to inactivate the deciduous tooth-like incisor germ. Their methodology included that in situ hybridization and for BMP7 and *USAG-1*, in addition to TUNEL assay for apoptosis and tissue culture of maxillary incisor tooth germ E15 followed by subrenal capsule transplantation. Their results showed that early tooth germs, after culturing and transplantation of different genotypes, induced or deprived from exogenous BMP-7, did not affect the loss of USAG-1, and high BMP-7 signals produced supernumerary maxillary incisors; however, double mutations lacking both USAG-1 and BMP-7 lost these extra teeth (Kiso et al., 2014).

Murashima-Suginami, Kiso et al. 2021 wanted to complete their clarification of whether blocking USAG-1 will help in preventing hypodontia and inducing extra teeth, therefore establishing its role in tooth number regulation, especially in congenitally missing teeth cases (Murashima-Suginami et al., 2021). Using in vitro reporter assays to clarify monoclonal characterized antibodies through binding to BMP and LRP 5,6 signaling against USAG-1, classifying them as dual BMP and Wnt blockage. These selected antibodies were injected into animal models, including neonatal mice, pregnant mice, and wild-type tooth agenesis. Outcome measures of tooth shape, root form, and number were assessed by histology and micro-CT. They found that antibodies focusing on disabling BMP-*USAG-1* interactions in early development dental tissue have a stronger effect on producing tooth number than antibodies focused on *LRP 5,6* interface in hypodontia animal models restoring their missing teeth. The antagonist effect of *USAG-1* on BMP signaling can stimulate latent tooth germs towards full development.

Arıkan et al. (2018) investigated coding variation in the human SOSTDC1 gene in children with non-syndromic supernumerary teeth using PCR and Sanger sequencing of the coding exons, followed by in-silico variant assessment. They identified several rare or private sequence variants, including changes in conserved residues; however, the study did not establish a statistically or functionally validated genotype/phenotype relationship, and no single variant explained the observed supernumerary-tooth phenotypes. Accordingly, these findings should be interpreted as evidence that SOSTDC1 variation may

contribute to susceptibility within a broader developmental network, rather than as proof of a monogenic cause. Importantly, the available human evidence does not establish firmly pathogenic germline SOSTDC1 mutations as a common cause of non-syndromic tooth agenesis; many reported variants remain variants of uncertain significance. This contrasts with the reproducible supernumerary-tooth phenotypes observed after SOSTDC1 loss in mice, which demonstrate biological plausibility but cannot by themselves establish causality in humans. Therefore, *USAG-1/SOSTDC1* should be regarded as a promising regulatory and therapeutic target, not as an established common genetic driver of congenital missing teeth (Calabrese et al., 2024; Ding et al., 2025; Bourhis et al., 2011).

4. Genetic basis of tooth agenesis

4.1. Genetic architecture and developmental signaling

Tooth agenesis results from disruption of reciprocal signaling between the oral epithelium and neural-crest-derived dental mesenchyme. Its genetic architecture is heterogeneous: some families show autosomal dominant, autosomal recessive, or X-linked inheritance, whereas others exhibit variable expressivity, incomplete penetrance, and possible multilocal contributions. A sequence variant in a candidate gene does not, by itself, establish causality; segregation, population frequency, phenotype consistency, and functional evidence must be considered. Key interacting pathways include canonical *Wnt/β-catenin, BMP/TGF-β, FGF, SHH, and EDA/EDAR/NF-κB* (Koonrungsesomboon et al., 2022; Ding et al., 2025; Bourhis et al., 2011; Matoba et al., 2017).

4.2. Genetic heterogeneity and regulatory architecture

Tooth agenesis is a genetically heterogeneous developmental disorder that results from disruption of reciprocal signaling between the oral epithelium and the neural-crest-derived dental mesenchyme. Although variants in *MSX1, PAX9, WNT10A, AXIN2, and EDA* are among the most consistently associated genetic findings, additional genes, including WNT10B, LRP6, BMP4, *EDAR, EDARADD, GREM2, LTBP3, SMOC2, PITX2, and FGFR1* have also been implicated. The phenotypic effect of these variants may differ according to the affected tooth, developmental stage, allele type, genetic background, and interaction with other signaling pathways (Fallea et al., 2025; Borges et al., 2025).

Recent evidence indicates that tooth agenesis should not be considered exclusively a single-gene disorder. In addition to pathogenic coding variants, its genetic architecture may include common susceptibility variants, copy-number changes, chromosomal abnormalities, regulatory variants, epigenetic alterations, and non-coding RNAs. Genome-wide association studies have identified susceptibility loci involving *WNT10A, EDAR, BMP7, MSX2, PRICKLE2*, and other genes involved in craniofacial development. These findings support a multifactorial and polygenic model in which combinations of genetic variants may influence tooth number, position, morphology, and eruption (Fallea et al., 2025).

The severity of tooth agenesis may also reflect the functional impact of the affected variant. Variants that severely disrupt the function of key developmental regulators, such as *PAX9, MSX1, or WNT10A*, may produce oligodontia or syndromic phenotypes, whereas hypomorphic or regulatory variants may be associated with milder and more localized hypodontia. However, genotype/phenotype relationships are not always predictable, and incomplete penetrance, variable expressivity, and environmental influences may contribute to substantial variation among individuals carrying variants in the same gene (Fallea et al., 2025; Borges et al., 2025).

In addition to genetic variation, epigenetic mechanisms may influence odontogenesis. DNA methylation, histone modification, and non-coding RNA-mediated regulation can alter the expression of genes involved in epithelial/mesenchymal signaling without changing the underlying DNA sequence. These regulatory mechanisms may help explain why individuals with similar genetic variants can exhibit different numbers, locations, or patterns of missing teeth (Borges et al., 2025).

4.3. Wnt signaling

At initiation, epithelial *Wnt* signals help establish the odontogenic field and sustain epithelial–mesenchymal communication. Wnt10A and Wnt10B encode Wnt ligands, while *LRP6* is a co-receptor required for canonical Wnt signaling, while *AXIN2* is a negative-feedback regulator. Pathogenic variation in Wnt10A is among the best-supported causes of isolated tooth agenesis, with severity influenced by allelic dosage and genetic background. Variants in *LRP6, Wnt10B, and AXIN2* can also be associated with missing teeth. Because germline pathogenic *AXIN2* variants may confer colorectal neoplasia predisposition, a clinically confirmed *AXIN2* diagnosis has implications beyond dental management (Ding et al., 2025; Bourhis et al., 2011; Matoba et al., 2017).

4.4. Mesenchymal transcription factors and BMP

PAX9 and *MSX1* are mesenchymal transcription factors that cooperate in regulating *BMP4* expression and progression beyond the tooth-bud stage. Disruption of this regulatory module may arrest tooth-germ development; *PAX9*-associated agenesis often affects posterior teeth, although patterns overlap and are not diagnostic on their own. BMP/TGF-β signaling regulates proliferation, differentiation, and tooth-number control. BMP antagonists, including *GREM2*, have also been reported as candidate contributors; the strength of human genetic evidence varies among genes. *USAG-1/SOSTDC1* modulates BMP and WNT signaling, but mouse knockout phenotypes should not be taken as proof that pathogenic germline *SOSTDC1* variants commonly cause human non-syndromic tooth agenesis (Ahn et al., 2010; Calabrese et al., 2024; Verma et al., 2025; Ding et al., 2025; Bourhis et al., 2011; Matoba et al., 2017).

4.5. Ectodysplasin A (EDA), Fibroblast growth factor (FGF), and sonic-Hedgehog (SHH) network

EDA binding to *EDAR* recruits *EDARADD* and activates *NF-κB* signaling in the dental epithelium, supporting epithelial signaling centers, tooth number, and crown morphogenesis. Variants in *EDA, EDAR, or EDARADD* may occur in isolated tooth agenesis or in ectodermal-dysplasia phenotypes; assessment of hair, skin, nails, and sweating helps distinguish these presentations. *FGF* signals, including *FGF3 and FGF10* acting through *FGF* receptors, contribute to epithelial–mesenchymal growth and signaling, while *SHH* helps coordinate placode development and subsequent tooth-germ patterning. These pathways interact rather than acting as independent linear switches (Bodensteiner RO, Culler S. Tooth Regeneration: Communicating Novel Dental Research Responsibly, 2025; Kiso et al., 2014; Ding et al., 2025; Bourhis et al., 2011; Matoba et al., 2017).

4.6. Integrated mechanistic interpretation

In a simplified model, Wnt activity specifies and maintains an odontogenic epithelial domain; epithelial signals engage mesenchymal *PAX9/MSX1–BMP4 and FGF* feedback; *EDA/EDAR/NF-κB and SHH* support signaling-center organization and morphogenesis. Pathogenic changes that reduce signaling below a stage- and site-dependent developmental threshold can cause a tooth germ to fail to initiate or progress, producing agenesis. Variation in allele function, interacting loci, and developmental context may explain differences in the number and position of missing teeth among affected relatives. This is a conceptual synthesis, not a deterministic genotype-tooth map. In particular, experimental rescue of tooth agenesis by anti-*USAG-1* antibodies in

animals does not imply that *SOSTDC1* mutation is a common cause of human congenital missing teeth (Ahn et al., 2010; Ding et al., 2025; Bourhis et al., 2011; Matoba et al., 2017).

4.7. Non-syndromic tooth agenesis

There is strong evidence that the pathophysiology of non-syndromic tooth agenesis is significantly influenced by genetic variables (Gao et al., 2023). According to Galluccio, Castellano et al. 2012, the prevalence of tooth agenesis ranges from 0.9% to 0.4% in the European population, however it is approximately 8% worldwide. The hereditary basis of this condition is confirmed by this variation amongst nations. Furthermore, autosomal dominant, autosomal recessive, or X-linked inheritance patterns for non-syndromic tooth agenesis have been documented (Galluccio et al., 2012), demonstrating the genetic and allelic variability of this defect (Yu et al., 2019).

Many mutations in various genes have been identified in individuals with non-syndromic TA since an *MSX1* mutation was found in patients with selective tooth agenesis. Tooth agenesis has already been linked to a number of genes, including *MSX1, PAX9, WNT10A, AXIN2*, and *EDA* (Yu et al., 2019). The genes *MSX1* and *PAX9*, which co-express in the mesenchyme during tooth formation, are located on chromosomes 4 and 14 and have two and five exons, respectively. In the same mesenchyme, they encode transcription factors that are essential for sustaining the expression of other target genes and proteins, such as BMP4 (Galluccio et al., 2012).

Additionally, *AXIN2* inhibits Wnt/catenin signalling. *AXIN2 and WNT10A* have 14 and 6 exons, respectively, and are found on chromosomes 17 and 2. The identification of an AXIN2 gene mutation in an oligodontia family was the first indication of the genetic connection between tooth agenesis and the Wnt pathway (Yu et al., 2019). The X chromosome contains the EDA gene, which is made up of 13 exons and codes for TNF ligand proteins. The receptor-binding TNF homology domain is only present in the isoforms *EDA1 and EDA2* proteins, and 80% of these mutations are linked to non-syndromic tooth agenesis (Gao et al., 2023).

Boutahari N et al., 2024, conducted a study including 636 permanent teeth in relation to tooth agenesis. According to earlier published systematic research on non-syndromic dental agenesis patterns, the most often missing tooth was the lower second premolar, followed by the upper lateral incisors (Boutahari et al., 2024; Rakhshan, 2015).

In a clinical series examining non-syndromic tooth agenesis, 636 permanent teeth were assessed (Nepi, 2019). Consistent with a previous systematic review, mandibular second premolars were the most frequently absent teeth, followed by maxillary lateral incisors (Zhu et al., 2024). These observations describe the distribution of missing teeth in the studied population and should not be interpreted as evidence that tooth agenesis is invariably isolated or that its inheritance follows a single pattern.

Additionally, this study described autosomal dominant, autosomal recessive, and complicated inheritance patterns. These results are consistent with prior research that describes an additional X-linked inheritance pattern (Letra et al., 2021), indicating a sex-related relationship with dental agenesis. In this regard, 91 patients with non-syndromic tooth agenesis were included in our study; 48 of them were female and 43 were male, with no discernible sex predominance.

Others, however, not only maintain that sex has a role in tooth agenesis, but they also connect sex to other elements like the kind of teeth involved, the number of dental agenesis, its symmetry, and its mandibular or maxillary position (Kanchanasevee et al., 2023).

Advances in genetic analysis techniques have improved the understanding of this condition. Traditional methods such as linkage analysis and Sanger sequencing have been largely complemented by **Next-Generation Sequencing (NGS)**, which allows rapid, high-throughput analysis of entire genomes or specific regions. NGS involves DNA fragmentation, library preparation, sequencing, and bioinformatic analysis (Kim et al., 2020). The use of NGS has enhanced the identification of genetic mutations associated with tooth agenesis and offers important clinical benefits, including early diagnosis and improved treatment planning, such as timely prosthetic rehabilitation (Prasad et al., 2016).

Tooth agenesis can also form part of a broader neurodevelopmental or chromosomal syndrome. For example, SATB2-associated syndrome combines intellectual disability and marked speech impairment with palatal and dental abnormalities, which may include congenitally missing teeth. SATB2 encodes a transcriptional regulator involved in craniofacial and neuronal development; disruption of this shared developmental program offers a biologically plausible explanation for co-occurring dental and neurodevelopmental findings. Nevertheless, missing teeth are not obligatory in every affected individual, and delayed mineralization or eruption must not be mistaken for true agenesis without age-appropriate radiographic assessment (Weidauer et al., 2009; Lintern et al., 2009). In Down syndrome, caused by full or partial trisomy 21, hypodontia is a recognized component of the variable dental phenotype rather than an independent non-syndromic diagnosis. One clinical study reported hypodontia in 56% of participants when third molars were excluded, compared with 92% when they were included; these estimates are sample-specific and illustrate the importance of defining which teeth are counted. Although altered dosage of chromosome 21 genes and downstream effects on craniofacial and odontogenic development are plausible, a single causal pathway for hypodontia in Down syndrome has not been established (Li et al., 2017). Thus, intellectual disability alone is not a cause or diagnostic marker of tooth agenesis: the combination warrants evaluation for a shared syndromic or chromosomal diagnosis when accompanied by other clinical features. See Fig. 3.

4.8. EDA/EDAR/NF-κB signalling pathway

The tumour necrosis factor (TNF) ligand superfamily includes the EDA-encoded ligand. From the epithelial thickening until the bud stage of tooth development, EDA is continually expressed in the dental epithelium. It is subsequently released into the mesenchyme, where it is expressed until the cap stage is finished (Tucker et al., 2000). It has been demonstrated that EDA promotes communication between various epithelial compartments and regulates the development of enamel knots, which function as dental signalling centres (Laurikkala et al., 2001).

Because of alternative splicing, EDA contains eight isoforms (Fan et al., 2008). Nevertheless, among all the isoforms, only EDA-A1 (a protein with 391 amino acids) and EDA-A2 (a protein with 389 amino acids) have a receptor-binding domain. The N-terminal intracellular domain, furin protease recognition sequence, collagen-like repeat domain, and C-terminal TNF homology domain are the four functional domains of the type II transmembrane protein EDA-A1 (Andreoni et al., 2021). At the furin consensus site, the C-terminal segment that contains the collagen and TNF homology domains cleaves off, releasing homotrimers that can attach to the specific receptor EDAR. However, TA does not entail EDA-A2 interaction with its receptor, X-linked ectodysplasin A (XEDAR), which is also encoded by EDAR (Newton et al., 2004; Mues et al., 2009; 149a(9):2037–41.). The inhibited EDA/EDAR/NF-κB signalling pathway cannot be replaced by an undamaged EDA-A2/XEDAR signalling pathway, despite the fact that XEDAR can also recruit TRAF3 and TRAF6 to activate the NF-κB pathway (Verhelst et al., 2015).

Three EDA, one EDAR, and two WNT10A mutations were found in two patients with X-Linked Hypohidrotic Ectodermal Dysplasia (XLHED) and four patients with non-syndromic tooth agenesis, according to a recent study. The first reports of EDA mutation c.1051G > T (p. Val351Phe) and EDAR mutation c.73C > T (p. Arg25*). These findings add to our understanding of the morbidity associated with mutations in the EDAR and WNT10A genes. Specifically, mutations in XLHED cause EDA expression to completely disappear, which stops the signalling cascade. Conversely, mutations in dental agenesis result in poor

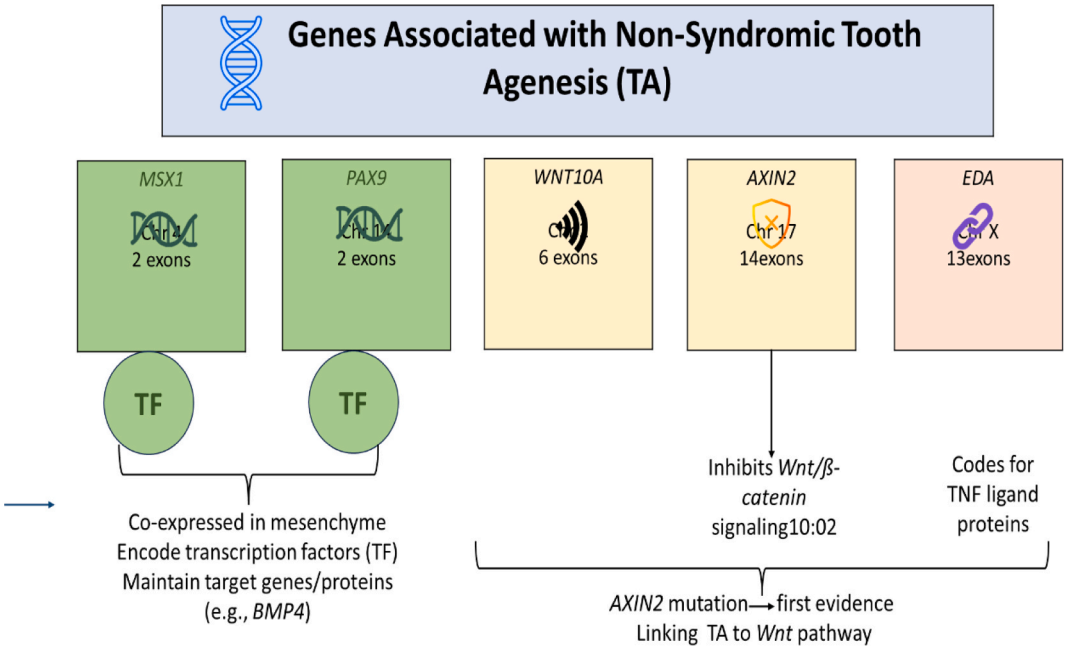


Fig. 3. Schematic figure Genes associated with nonsyndromic tooth agenesis (TA). MSX1 and PAX9 encode transcription factors that are co-expressed in the dental mesenchyme and regulate target genes involved in tooth development, including BMP4. WNT10A encodes a ligand involved in Wnt signaling, whereas AXIN2 functions as a negative regulator of the Wnt/β-catenin pathway. Mutations in AXIN2 provided early evidence linking nonsyndromic TA to dysregulation of Wnt signaling. EDA, located on the X chromosome, encodes a tumor necrosis factor (TNF) ligand-family protein involved in ectodermal development. The figure also presents the chromosomal locations and exon numbers of the genes shown.

signalling activity and decreased EDA affinity for their target receptors. A link between familial non-syndromic tooth agenesis and nucleotide changes in the TNF homology domain of the EDA may exist (Zeng et al., 2017). See Fig. 4.

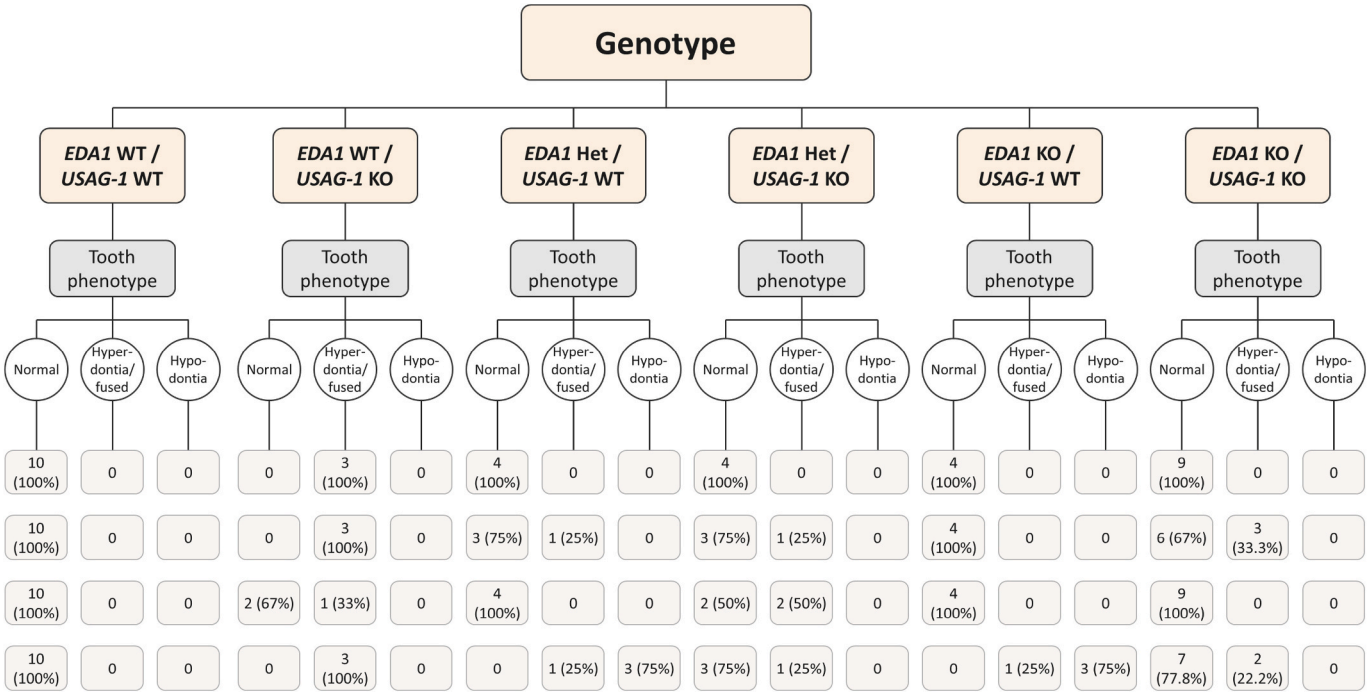


Fig. 4. Tooth phenotypes in mice with different EDA1 and USAG-1 genotypes. The diagram summarizes the relationship between genotype and tooth phenotype in mice carrying wild-type (WT), heterozygous (Het), or knockout (KO) alleles of EDA1 and USAG-1. Six genotype groups are shown: EDA1 WT/USAG-1 wt, EDA1 WT/USAG-1 KO, EDA1 Het/USAG-1 wt, EDA1 Het/USAG-1 KO, EDA1 KO/USAG-1 wt, and EDA1 KO/USAG-1 KO. Each genotype is classified according to the observed dental phenotype—normal dentition, hyperdontia or tooth fusion, and hypodontia. The values beneath each phenotype indicate the number of animals and, where applicable, the percentage within the corresponding genotype group. The data demonstrate that loss of EDA1 is associated with tooth agenesis, whereas loss of USAG-1 can rescue the tooth-deficient phenotype and may result in normal or additional/fused teeth. These findings support the role of USAG-1 as a negative regulator of tooth development and suggest that its inhibition can enhance odontogenic signaling in EDA-related tooth agenesis.

4.9. Syndromic tooth agenesis

Tooth aberrations are linked to over 60 syndromes listed in On-line Mendelian Inheritance in Man (OMIM), suggesting that similar molecular processes underlie the development of teeth and other organs. Agenesis of several teeth is frequently linked to certain syndromes or systemic disorders, especially ectodermal dysplasia (Hamosh et al., 2021). Tooth agenesis in conjunction with other anomalies can result from somatic diseases like syphilis, scarlet fever, rickets, or nutritional disturbances during pregnancy or infancy, in addition to hereditary defects. Additionally, early cerebral irradiation might result in dental abnormalities and glandular malfunction (Koch et al., 2017). Marie and Sainton initially referred to cleidocranial dysplasia as cleidocranial dysostosis. One person out of every million is thought to have CCD. Many cases of CCD seem to be sporadic; however, family cases are inherited in an autosomal dominant manner with significant phenotypic variety (Yoshida et al., 2002).

Ectodermal Dysplasia Syndrome CLPED-1, an autosomal recessive CLP–ED syndrome, has been associated with a homozygous loss-of-function mutation in the PVRL1 gene. Scanty eyebrows, thin and dry hair, syndactyly of the fingers and toes, CL/CLP, nail dysplasia, hypodontia of the upper central incisors, and aberrant tooth crown size and shape are all characteristics of CLPED-1 patients.

4.10. Syndromes of ectodermal dysplasia

A constellation of abnormalities comprising a main deficiency of the skin, teeth, and appendageal structures, such as hair, nails, exocrine glands, and sebaceous glands, are together referred to as ED. The mutation or deletion of specific genes on various chromosomes causes the various forms of ED (Yoshida et al., 2015). An uncommon multisystem syndrome with extra visual and central neurological abnormalities, incontinentia pigmenti (IP) is categorized as an ED condition. Because of mutations in the NEMO gene, IP is an X-linked dominant illness. Males with the mutation typically do not live through gestation, hence females are primarily impacted. Oligodontia, microdontia, supernumerary cusps in the posterior permanent teeth, delayed tooth eruption, and/or taurodontia are associated in over 90% of IP patients. An X-linked recessive trait that is allelic to IP segregates as a unique variant of HED with immunodeficiency. The protein under investigation, NEMO, is necessary for the activation of NF-κB, a transcription factor that transduces TNF signals (Puel et al., 2006).

A number of oral clefting syndromes, specifically P63 syndromes, have been linked to P63 gene mutations. Split hands and feet, ED, and CLP are the hallmarks of the uncommon autosomal dominantly inherited Ectrodactyly–ED–clefting (EEC) syndrome. Lacrimal duct abnormalities, urogenital problems, conductive hearing loss, persistent respiratory infections, cardiac cardiomyopathy, and developmental delay are other characteristics of EEC. EEC-like disorders that are allelic to the EEC syndrome include ankyloblepharon ectodermal abnormalities, CL/CLP syndrome, limb mammary syndrome, acro-dermato-ungual-lacrimal-tooth syndrome, and Rapp-Hodgkin syndrome. In these families, a number of distinct P63 gene mutations have been identified (Hassan et al., 2024).

Skeletal abnormalities include hypoplastic clavicles, delayed closure of the cranial sutures, small height, and various dental abnormalities, such as the presence of supernumerary teeth and delayed eruption of permanent teeth, are characteristics of CCD. When permanent teeth replacement is delayed or the permanent dentition does not erupt, the maxilla does not expand vertically, which causes the mandible to overgrow horizontally. Dental treatment for CCD patients should begin as soon as possible in order to achieve appropriate occlusion (Weise et al., 2022).

Tooth agenesis and rare syndromic conditions. Rare diseases like Rieger, Williams, Wolf-Hirschhorn, Kabuki, and others can also cause multiple tooth bud agenesis. Bloch and Sulzberger, Van der Woude, Ellis Van Creveld, Laurence-Moon-Bied-Bardet, Goltz, Schopf-Schulz-Passarge, Franceschetti-Jadassohn, and Rapp-Hodgkin.

These syndromes' traits become apparent during the prenatal or neonatal stages. A notable characteristic of the disorders listed above is the agenesis of several dental buds, which is strongly linked to the diagnosis of a particular disease (Klupś et al., 2022).

Multidisciplinary care is necessary for patients with uncommon syndromes. Because the major clinical signs of a particular disease entity manifest during the neonatal or infancy period, a neonatologist or pediatrician is typically the primary care physician who makes the diagnosis. Patients with hypodontia should have specific dental treatment in the future since they have numerous functional and cosmetic issues. X-ray confirmation is always necessary for the clinical diagnosis of dental agenesis. It is advised to take a pantomographic X-ray, which is crucial for the final diagnosis and enables the exclusion of potential tooth impaction (Goswami et al., 2012). Children who have been diagnosed with missing tooth buds should receive comprehensive care from dentists who specialize in orthodontics, prosthodontics, maxillofacial surgery, or dentistry (Klupś et al., 2022).

A child's normal growth is seriously hindered by the loss of one or more teeth since tooth agenesis has an impact on both craniofacial and psychosocial development of a patient who is still developing. Malocclusion, speech, and aesthetic impairments result from it. This may improve patient satisfaction from a dental perspective and is crucial for our comprehension of how dental agenesis affects children's and their parents' quality of life (Yu et al., 2019).

4.11. Development of monoclonal anti-USAG-1 antibodies

Uterine sensitization–associated gene-1 (USAG-1) also known as Sclerostin domain containing 1 (SOSTDC1) is a protein that inhibits both BMP and Wnt signaling pathways, both of which are critical for tooth development (Takahashi et al., 2020; Thesleff, 2006). Although the key signaling pathway in determining tooth number is yet unknown, prior research has shown that regulation of these pathways promotes the production of supernumerary teeth. Inhibition of USAG-1 can restore halted tooth growth, according to genetic research employing models of knockout mice, underscoring its crucial involvement in congenital tooth agenesis (Murashima-Suginami et al., 2021). This study desired to produce a monoclonal anti-USAG-1 antibody as a therapeutic approach to locally restore tooth formation and to further explore whether BMP or Wnt signaling predominates in tooth morphogenesis, given the increasing clinical use of monoclonal antibody based targeted therapeutics (Murashima-Suginami et al., 2021).

In order to determine whether congenital tooth agenesis could be therapeutically saved by functional inhibition of USAG-1, five monoclonal anti-USAG-1 antibodies were produced in mice (#12, #16, #37, #48 and #57). USAG-1-/- mice were used to develop these antibodies using bioactive recombinant human USAG-1 protein as the antigen (Murashima-Suginami et al., 2021).

4.12. Functional classification of antibodies

Since USAG-1 directly binds to BMP ligand and the Wnt coreceptor LRP5/6, the antibodies were functionally classified based on their capacity to disrupt the BMP and Wnt signaling pathways (Murashima-Suginami et al., 2021).

Antibody #57 suppressed both BMP and Wnt signaling, antibodies #12 and #37 both selectively disrupted BMP signaling, and antibodies #16 and #48 selectively inhibited Wnt signaling.

Although antibodies #16 and #48 showed somewhat reduced affinity, all five antibodies showed binding affinity to both human and mouse recombinant USAG-1 proteins. Further research into the relative roles of BMP and Wnt signaling in tooth number determination was made possible by this classification (Murashima-Suginami et al., 2021).

4.13. In vivo therapeutic evaluation

To evaluate each monoclonal antibody's capacity to prevent congenital tooth agenesis, pregnant Ectodysplasin A1 (EDA-1) deficient mice were given it systemically. The EDA gene, which is found on chromosome Xq12-13.1, encodes the protein EDA-1. It is a member of the tumor necrosis factor (TNF) superfamily and is essential for the development of the embryonic ectoderm, especially for the production of teeth (Ding et al., 2025). In comparison to control groups, treatment with antibodies #16, #37, #48, and #57 effectively restored mandibular molar development in EDA1-/- mice. Antibody #37 showed the strongest efficacy among them, reversing hypodontia in a dose-dependent manner at a high rate. Furthermore, administering antibodies #12, #16, #37, and #57 to EDA1 deleted or heterozygous mice's maxillary incisors, mandibular incisors, and molars induced the formation of extra teeth. See Table 1.

These results underscore USAG-1′s potential as a targeted molecular therapy for tooth regeneration by showing the monoclonal antibody-mediated neutralization of the protein can repair lost teeth and promote full tooth formation (Murashima-Suginami et al., 2021).

Surprisingly, antibody #57 also frequently and dose-dependently caused the maxillary incisors, mandibular incisors, and molars in wild-type mice to develop extra teeth. Instead of separate supernumerary teeth, merged molars were seen in the maxillary molar region (Murashima-Suginami et al., 2021).

These antibodies neutralized USAG-1′s BMP antagonistic action, according to in vitro investigations. Together, these results showed that BMP signaling is essential for determining the number of teeth in mice and show that a single systemic injection of monoclonal antibody that neutralize USAG-1 can promote the development of a whole tooth (Murashima-Suginami et al., 2021).

Table 1
Comparison of anti-USAG-1 monoclonal antibodies.

Antibody	Pathway selectivity	Epitope	In vivo efficacy	Adverse or unintended phenotypes
#12	BMP-selective	Not mapped	Did not restore mandibular molars	Extra teeth in EDA1 KO/ heterozygous mice; lower birth and survival rates
#16	Wnt-selective; reduced affinity; nearly fully blocked USAG-1-LRP6	Not mapped	Restored mandibular molar	Extra teeth in EDA1 KO/ heterozygous mice; lower birth and survival rates
#37	BMP-selective;	Linear; peptides D16-D21 (Q129-Q148, core VNDKTRTQRI); edge strand of the central β-sheet, distant from NXI motif	Restored mandibular molars; strongest effect, dose-dependent, high rate	Extra teeth in EDA1 KO/ heterozygous mice; no SOST signal
#48	Wnt-selective; reduced affinity; partial Wnt inhibition	Not mapped	Restored mandibular molars	lower birth and survival rates
#57	Dual (BMP and Wnt) in signaling assays; no effect on Wnt antagonism or LRP6 binding	Conformational (no linear peptide binding)	Restored mandibular molars	Extra teeth in EDA1 KO/ heterozygous and wild type mice (dose dependent); sur maxillary molars; faint SOST signal

By binding to the "E1" extracellular domain of the LRP6 co-receptor, the natural Wnt-pathway inhibitor sclerostin (SOST) inhibits signaling (Bourhis et al., 2011). Researchers postulated that USAG-1 binds the same E1 region because it contains the conserved NXI motif that mediates LRP6 binding. Recombinant USAG-1 was incubated with a panel of human LRP6 ectodomain fragments of different lengths in order to evaluate this. The key binding site is located inside the E1 module, as demonstrated by quantitative experiments that showed a stoichiometric (1:1) interaction between USAG-1 and the segment containing the E1-E2 domains. Neither the shorter E3-E4 fragment nor the longer E1-E4 construct showed any binding (Murashima-Suginami et al., 2021). Steric occlusion was used to explain why there was no binding to the E1-E4 fragment even though it contained E1. In the context of the whole ectodomain, the protein's highly curved "C-shape" shown in electron microscope pictures obscures the NXI-binding surface of E1 (Matoba et al., 2017).

The five anti-USAG-1 monoclonal antibodies were tested to see if they could prevent USAG-1 from interacting with the extracellular domain of LRP6. USAG-1 binding to LRP6-E1-E2 was nearly entirely inhibited by antibody #16, although USAG-1 mediated Wnt signaling was only somewhat inhibited by antibody #48. On the other hand, the USAG-1-LRP6 connection was not affected by antibodies #12, #37, and #57, indicating that they are unable to block USAG-1′s Wnt-modulating function (Murashima-Suginami et al., 2021). These results promoted the authors to infer that the most noticeable phenotypic rescue (tooth recovery or whole-tooth creation) is obtained by neutralizing USAG-1′s BMP antagonism rather that its Wnt activity (Murashima-Suginami et al., 2021).

The researchers investigated how USAG-1 neutralizing antibodies #37 and #57 recognize their target by conducting an extensive epitope mapping experiment that screened 169 overlapping linear peptides, each 20 residues long, across the USAG-1 sequence. Antibody #37 displayed selective binding to a series of six contiguous peptides (designated D16-D21) that together span the amino acid stretch Q^{129}*EWRCVNDKTRTQ- RIQLQCQ*^{148}, pinpointing the critical binding region to a central ten-residue motif that includes *VNDK-TRTQRI* (Murashima-Suginami et al., 2021). Because the three-dimensional structure of USAG-1 has not been experimentally resolved, the team leveraged the high sequence similarity between USAG-1 and BMP antagonist sclerostin (SOST), a member of the same DAN protein family, to generate a homology model of the mouse USAG-1 protein using the nuclear-magnetic-resonance structure of SOST as a template (Weidauer et al., 2009).

The structural study revealed that the binding site for the neutralizing monoclonal antibody #37 is located on an outward-facing strand at the periphery of the core β-sheet of the USAG-1 protein, elucidating why #37 can interact with the native, correctly folded conformation of USAG-1. This epitope is significantly distant from the conserved *NXI* motif, which facilitates the interaction between USAG-1 and the Wnt co-receptor LRP5/6; therefore, antibody #37 does not impede USAG-1′s ability to bind LRP5/6 nor does it reduce USAG-1′s capacity to antagonize Wnt1 signaling, as demonstrated in functional assays (Murashima-Suginami et al., 2021; Lintern et al., 2009).

Antibody #57 demonstrated no detectable binding to the panel of overlapping linear peptides derived from USAG-1, suggesting that #57 recognizes a conformational, three-dimensional epitope exclusively presented on the intact protein surface, rather than on linear sequences (Murashima-Suginami et al., 2021).

Because antibody #37 leaves the Wnt-antagonizing activity of USAG-1 unaffected (Murashima-Suginami et al., 2021), the findings below bear the effects of sparing Wnt-pathway inhibition. In EDA1 deficient mice, antibodies that targeted only BMP signaling rescued tooth agenesis without changing the other phenotypes of that line

(Murashima-Suginami et al., 2021). By contrast, Wnt signaling acts in tissues beyond the dentition. Modulating Wnt corrected the cleft palate in *Pax9* mutant mice (Li et al., 2017; Jia et al., 2017). *AXIN2* variants that are expected to overactivate Wnt signalling cause familial tooth agenesis and predispose carriers to colorectal cancer, so authors concluded that both under- and overactivation of Wnt signalling may lead to tooth agenesis (Lammi et al., 2004). Consistent with these systemic risks, mice given Wnt-inhibiting antibodies (#12, #16, and #48) showed lower birth and survival rates. However, the original authors noted that the involvement of Wnt in tooth formation cannot be excluded because several mice were not born or did not survive and that further experiments are needed (Murashima-Suginami et al., 2021).

A 2025 systemic review f 20 studies (91 patients, 34 AXIN2 variants) found colorectal cancer and polyps to be the most common malignancies, and nonsense variants were linked to the most severe agenesis, averaging 12.9 missing teeth. Evidence outside these genetic subgroups is weaker. A 2026 review advises considering counseling and referral for several oligodontia, especially in families with history of colorectal polyps or cancer. Severe oligodontia is a plausible early target population for the use of anti USAG 1, hence genetic testing and counseling should be respectively considered with patient selection and safety monitoring. Germline AXIN2 variants don't show that USAG-1 blockade raises cancer risk (Ratanasereeprasert et al., 2025; Kuc et al., 2026).

Cross-reactivity with other DAN-family proteins was examined to clarify the functional differences between #37 and #57. On *SOST-expressing HEK293* cells, #57 produced a faint signal while #37 showed none, most likely because *SOST and USAG-1* have similar three-dimensional structure (Murashima-Suginami et al., 2021; Weidauer et al., 2009).

In USAG-1-deficient mice, systemic administration of an antibody cocktail comprising #12, #16, #37, #48, and #57 increased both the number of supernumerary teeth and the size of fused mandibular teeth. These findings suggest that #57 may disturb the genetic redundancy responsible for formation of supernumerary teeth, through an effect on SOST which is a BMP antagonist, and as described earlier, #57 also induced extra teeth in wild-type mice and fused molars in the maxillary molar region (Murashima-Suginami et al., 2021).

Lastly, in post-natal ferrets, systemic administration of antibody #37 (five-fold higher concentration, three injections, immunosuppression) resulted in an extra maxillary incisor that resembled a “third dentition” (similar shape, shorter root) and displayed phosphorylated Smad-positive cells within its pulp, confirming activation of BMP signaling in the newly formed tooth (Murashima-Suginami et al., 2021; Kiso et al., 2019).

4.14. Wnt, BMP and anti-USAG-1 intermolecular interactions

A complex series of interactions between the epithelial and mesenchymal layers that is regulated by many signaling pathways control tooth development. Wnt Canonical and BMPs signaling are two of the most important essential signaling pathways for tooth development (Murashima-Suginami et al., 2021). Wnt Ligands are present in early stages of tooth formation within the dental lamina’s epithelium where they signal predominantly (Ding et al., 2025). A critical role is played by Wnt signaling during tooth development specially during initiation and morphogenesis which is evidently clear during dental cell differentiation (Balic et al., 2015). Studies and experiments involving animal models, specifically mice, have proved that Wnt signaling forced activation within the oral epithelium of both embryonic and adult mice resulted in new teeth continuous initiation (Sui et al., 2023). Another important signaling pathway in the regulation of tooth development is *BMPs*, they directly affect the tooth number, cell proliferation and differentiation (Murashima-Suginami et al., 2021). Since both Wnt and BMPs directly affect the process by which teeth are formed, affecting the signaling pathways will affect tooth formation. *USAG-1* functions as an antagonist that inhibits the process by which BMPs molecules affect tooth formation by binding to *BMPs. USAG-1* also binds to Wnt co receptors that negatively regulates the pathways necessary for tooth formation (Murashima-Suginami et al., 2021). Inhibition of *USAG-1* activity improves BMP signaling resulting in the formation of new teeth, this has been supported by studies done on animal studies (Murashima-Suginami et al., 2021). Local inhibition of *USAG-1* using *RNAi* or in the form of antibodies has shown to result in the promotion of tooth regeneration in mice that were genetically modified (Kiso et al., 2019). The balance between Wnt signaling, Inhibitory activity of *USAG-1*, and *BMP* signaling is crucial for tooth number and development regulation (Murashima-Suginami et al., 2021).

5. Animal models

Mice are frequently used as animal models in biomedical and dental research because they provide several important advantages for experimental studies. Their genome has been extensively mapped, and they can be easily genetically modified, allowing scientists to study the roles of specific genes involved in tooth development and regeneration. In addition, mice reproduce quickly and have short life spans, which makes them practical for laboratory research. Many of the biological pathways that control odontogenesis, such as the BMP and Wnt signaling pathways, are also highly conserved between mice and humans, making mouse models useful for investigating mechanisms relevant to human dental biology (Domínguez-Oliva et al., 2023).

Using USAG-1 protein in mice with a neutralizing antibody activates dormant tooth buds, which may lead to the formation of supernumerary teeth. These teeth developed normal tissues including enamel, dentin and developing roots. This indicates that the suppression of USAG-1 can result in the reactivation of odontogenic signaling pathways during tooth development (Ravi et al., 2023).

The administration was done in pregnant mice given one injection through the intraperitoneal route during the embryonic development (around the 13th embryonic day) of anti USAG-1 at a dose of 16 Âµg per gram body weight which resulted in the formation of supernumerary teeth in the carried offspring, by enhancing the BMP signalling in tooth development (Murashima-Suginami et al., 2021; Ravi et al., 2023).

In other animal studies using mice, a different experimental design was used, using a similar antibody dose ranging from 8 to 48 mg per kilogram that has been determined to show optimal efficacy after being evaluated in promoting tooth development (Ravi et al., 2023).

Despite that, like most mammals, ferrets develop two teeth dentitions only. However, after the administration of anti USAG-1 to ferrets regenerated teeth have been observed. The new teeth erupting that can be referred to as a third dentition were most commonly present in the maxillary arch incisor region with normal anatomical characteristics to teeth of the second dentition including having well-formed enamel tissue and dentin tissue. Moreover, these teeth had the capability to form proper roots that allowed the teeth to erupt in the oral cavity and function as permanent teeth (Ravi et al., 2023).

The role of USAG-1 inhibiting signaling pathways in tooth regeneration has been supported by topical experiments using locally applied USAG-1 siRNA to tissues related to the mandible in mice that pointed towards partial restoration of tooth formation leading to further confirmation of the effect of anti USAG-1 on tooth development (Mishima et al., 2021).

6. Clinical trials

6.1. Clinical trial design and objectives

Phase I of the clinical trials was initiated in Japan through a collaboration between Kyoto University Hospital and Kitano Medical Research Institute Hospital (Institute KMR, 2024). As this agent represents a first-in-class therapeutic approach, prior consultation was conducted with the Pharmaceuticals and Medical Devices Agency to define

the safety, pharmacokinetics, and pharmacological assessments required for trial initiation (Institute KMR, 2024). The primary objective of the Phase I study is to evaluate the safety, tolerability, and pharmacokinetics of a single dose of TRG035 in healthy adult males; tooth-regeneration efficacy is not an endpoint of this study (jRCT, 2024). The investigational drug was designated as *TRG035* (Institute KMR, 2024).

The efficacy of the drug on humans has not yet been demonstrated. Although the drug is effective in animal studies, its efficacy on humans still needs to be confirmed.

6.2. Study population and Eligibility criteria

The trial enrolled adult males aged 30–65 years who met predefined anthropometric and dental criteria, including a body mass index between 18.5–25 kg/m^2, body weight ranging from 40 kg to < 80 kg, and the absence of one or more molar teeth (jRCT, 2024). All participants provided written informed consent prior to enrollment. Exclusion criteria encompassed the presence or history of clinically significant systemic diseases such as hepatic, renal, cardiovascular, circulatory, respiratory, gastrointestinal, hematologic, malignant, or autoimmune disorders (jRCT, 2024). Neurological conditions associated with an increased risk of seizures were also exclusionary (jRCT, 2024). Individuals with known allergic predisposition, or hypersensitivity to foods, drugs, metals, antibody-based products, or any component of the investigational agent were excluded (jRCT, 2024). Additional exclusions included alcohol or substance dependence, prolonged QTc interval (>450 ms) on screening electrocardiography, molar-region dental implants, or clinically relevant abnormalities identified during physical examination or laboratory investigations (jRCT, 2024). Participants were also ineligible if they had recently received investigational or systemic medications, donated blood beyond defined thresholds, or been administered live or inactivated vaccines within specified washout periods (jRCT, 2024). Subjects unwilling to use appropriate contraception during the study period, or otherwise judged unsuitable by the investigator, were also excluded (jRCT, 2024).

6.3. Intervention and dose escalation strategy

The intervention consisted of a single intravenous administration of the investigational agent, *TRG035*, or placebo, delivered using a stepwise dose-escalation design. Participants received one dose according to predefined cohorts, starting at 0.4 mg/kg and subsequently increasing to 1.2, 4.0, 12.0 and 24.0 mg/kg. Each dose level was administered as a single infusion, allowing assessment of safety, tolerability, and pharmacokinetics across ascending exposure levels (jRCT, 2024).

6.4. Discontinuation and early termination criteria

Participants were discontinued from the study under several circumstances (jRCT, 2024). Discontinuation was warranted if the investigator or another responsible party determined that continued administration of the investigational drug was unsafe due to adverse events, or if the participant personally requested to stop treatment (jRCT, 2024). Other reasons included the discovery of serious protocol violations, ineligibility after enrollment, death, inability to initiate the investigational drug on the day following enrollment, or assignment as a reserve participant (jRCT, 2024). Additionally, continuation of the trial could be halted if the investigator deemed it inappropriate for other reasons (jRCT, 2024). The study also allowed for early termination or suspension if post-enrollment data revealed significant safety or efficacy concerns regarding the investigational treatment, or if the Efficacy and Safety Assessment Committee recommended discontinuation, in which case the investigator made a final determination based on these recommendations (jRCT, 2024).

6.5. Future Directions and translational perspective

Following completion of the first-in-human study, a Phase IIa clinical trial is planned at Kitano Hospital targeting patients with congenital edentulism characterized by six or more congenitally missing teeth (oligodontia), a rare condition with substantial unmet clinical need (Institute KMR, 2024). Upon confirmation of efficacy in this population, subsequent expansion is anticipated to include individuals with milder forms of congenital partial edentulism involving one to five missing teeth (hypodontia), a more prevalent condition thought to arise predominantly from environmental rather than genetic factors. Beyond congenital indications, TRG035 is also being explored for the treatment of acquired tooth loss, particularly in Japan's aging population, where tooth loss remains highly prevalent (Institute KMR, 2024). Future strategies include local administration approaches aimed at regeneration of so-called "third dentition" following permanent tooth loss. The local administration of the drug is more preferable as USAG-1 has an important regulatory role in controlling the BMPs and WNT signals (Murashima-Suginami et al., 2021). BMPs is the key regulator for a diverse array of many physiological and pathological processes (Zhu et al., 2022), while WNT signaling is one of the key regulators of the embryonic development, cell proliferation, differentiation and migration (Tamura and Nemoto, 2016). Introducing a systemic drug that affect USAG-1 may therefore result in unintended effects in tissues beyond the target site due to the broad involvement of BMPs and WNT signaling in physiological processes. A concentration of the therapeutic effect at the intended site and reducing the risk of off-target effects may potentially be reached by limiting systemic exposure through local administration.

In parallel, increasing emphasis on preventive and preemptive medicine has underscored the role of dentition in maintaining oral function and mitigating oral frailty, a recognized precursor of systemic frailty in older adults. Accordingly, ongoing research efforts seek to enable functional tooth regeneration in cases of tooth loss due to caries, periodontal disease, trauma, or aging, with the broader goal of preserving masticatory function and improving quality of life. A dedicated research and development framework has been established in Japan to support clinical translation, including early-stage subject identification for forthcoming trials. Anti-USAG-1 therapy should currently be regarded as a promising investigational approach rather than a clinically confirmed treatment.

On the other hand, several clinical questions remain unresolved before teeth regeneration can be translated into routine dental practice. Would it better to take the drug in the childhood or the adult? The therapeutic time-window is important and need to be tested to detect the optimal time-window. The dosing regimen is currently not clear, what dosing regimen produces a clinically meaningful tooth regeneration? Would the regenerated teeth survive for a long time? Would it resist the normal masticatory forces? Would it have less susceptibility to caries and periodontal diseases? The durability of the regenerated teeth needs to be confirmed.

CRediT authorship contribution statement

Ahmed Mohamed Tawfik: Writing – original draft, Conceptualization. **Ahmed Mohamed Aboulyazeed:** Writing – review & editing. **Hazem Elazizi:** Writing – original draft. **Mennatullah Hazem:** Writing – original draft. **Mahmoud Amer:** Writing – original draft. **Bassant Ashraf Badr:** Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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