

CURRENT TREND AND STUDIES IN **HEALTH SCIENCES**



All Sciences Academy

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Editor

Prof. Dr. Fatih HATİPOĞLU





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Medication Use and Orthodontic Treatment: Biological Effects and Clinical Considerations

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ABSTRACT

Orthodontic treatment is carried out in a growing number of patients who use prescription drugs, over-the-counter analgesics, supplements, or long-term therapies for systemic disease. Because tooth movement depends on inflammatory signaling and coordinated bone remodeling, some medications can modify the biological response to orthodontic force or alter pain, periodontal tissues, gingival volume, root resorption, and post-treatment stability. This chapter reviews the medication groups most relevant to contemporary orthodontic practice, with emphasis on recent human studies, systematic reviews, and meta-analyses. Non-steroidal anti-inflammatory drugs are effective for pain relief, although repeated or prolonged exposure may attenuate prostaglandin-dependent remodeling. Bisphosphonates show the most consistent inhibitory effect on tooth movement, but human evidence remains limited and treatment planning must also consider medication-related osteonecrosis of the jaw. Small clinical trials suggest that vitamin D can increase movement rate, yet routine supplementation for orthodontic acceleration is not supported. Evidence for corticosteroids, statins, antidepressants, antiepileptic drugs, immunosuppressants, antidiabetic agents, and many cardiovascular medications is heterogeneous or largely preclinical. Drug-induced gingival enlargement, particularly with phenytoin, cyclosporine, and calcium-channel blockers, has direct implications for appliance therapy and periodontal care. In clinical practice, the drug indication, dose, route, duration, underlying disease, and planned orthodontic mechanics should be assessed together. Medically necessary therapy should not be changed solely to influence tooth movement without consultation with the prescribing clinician.

Keywords – Orthodontic tooth movement; medication use; analgesics; antiresorptive therapy; vitamin D; root resorption; relapse; gingival enlargement

INTRODUCTION

Orthodontic tooth movement is a biologically regulated response to mechanical loading. Force application changes the periodontal ligament, local vascular environment, extracellular matrix, and strain distribution, triggering mechanotransduction and a sterile inflammatory response. Periodontal ligament cells, osteocytes, osteoblast-lineage cells, osteoclast precursors, fibroblasts, endothelial cells, and immune cells act together to coordinate bone resorption and formation as the tooth changes position (Li et al., 2021; Maltha and Kuijpers-Jagtman, 2023).

Because this response depends on inflammatory mediators and bone turnover, medications can become clinically relevant even when they were prescribed for reasons unrelated to dentistry. Analgesics, antiresorptive drugs, corticosteroids, immunosuppressants, psychotropic agents, antiepileptic

drugs, statins, vitamin D preparations, and several cardiometabolic medications may influence tooth movement directly or alter periodontal health, gingival tissues, pain, wound healing, or the safety of adjunctive procedures. The effect of a drug cannot be summarized simply as acceleration or inhibition of tooth movement; indication, dose, duration, route, and the patient's underlying disease all matter (Giannini et al., 2026; Pacheco-Quito et al., 2026).

The evidence is uneven. Many proposed drug effects come from animal models in which exposure patterns differ substantially from routine human use, while well-controlled clinical studies remain uncommon. Recent systematic evidence continues to indicate low or very low certainty for many pharmacological interventions. For this reason, this chapter gives greatest weight to human studies and systematic reviews and uses preclinical work mainly to clarify mechanisms. The focus is practical: how medication use may affect tooth movement, pain control, periodontal tissues, root resorption, treatment duration, anchorage, and post-treatment stability (Giannini et al., 2026).

BIOLOGICAL BASIS OF DRUG EFFECTS ON ORTHODONTIC TOOTH MOVEMENT

Mechanical force is converted into a biological response through cellular sensing, inflammatory signaling, cell recruitment, and tissue remodeling. Within the periodontal ligament and alveolar bone, matrix deformation, fluid flow, local hypoxia, and cellular strain activate mechanosensitive pathways in periodontal ligament cells and osteocytes. These cells communicate with osteoblasts, osteoclast precursors, fibroblasts, endothelial cells, and immune cells through soluble mediators and direct cell signaling. Current mechanobiological models therefore describe orthodontic movement as a coordinated tissue response rather than a simple division into pressure and tension sides (Li et al., 2021; Maltha and Kuijpers-Jagtman, 2023).

Sterile inflammation is part of this response rather than merely a side effect of force application. Prostaglandins, cytokines, chemokines, nitric oxide, neuropeptides, and growth factors appear early after mechanical loading. Interleukin-1 beta, interleukin-6, tumor necrosis factor-alpha, and prostaglandin E2 contribute to the recruitment and activation of cells involved in bone resorption. Drugs that modify these pathways can therefore alter the early remodeling environment, although the magnitude of the clinical effect depends strongly on the intensity and duration of exposure (Giannini et al., 2026; Li et al., 2021).

Osteoclast formation is strongly influenced by the RANK/RANKL/osteoprotegerin system. RANKL produced by osteoblast-lineage cells, periodontal ligament cells, and osteocytes binds RANK on

osteoclast precursors, whereas osteoprotegerin limits this interaction by acting as a decoy receptor. This pathway helps explain why potent antiresorptive drugs can slow orthodontic movement and why statins and several experimental biologic agents have attracted interest as modifiers of bone remodeling (Amin et al., 2026; Crispino et al., 2026).

The prostaglandin pathway is equally relevant to daily practice. Cyclooxygenase activity contributes to PGE2 production, which supports osteoclastogenesis and bone resorption during the early phase of movement. NSAIDs reduce prostaglandin synthesis and are therefore capable, at least in principle, of attenuating this response. The opposite approach—local administration of prostaglandins to accelerate movement—has also been investigated, but repeated injections, discomfort, local inflammation, and limited clinical evidence have prevented routine use (Ciobotaru et al., 2024; Pacheco-Quito et al., 2026).

Exposure pattern is crucial when these mechanisms are translated to patients. A few analgesic doses around an appliance activation are not equivalent to daily anti-inflammatory treatment for months, and an experimental local injection cannot be assumed to reproduce the effect of a standard systemic prescription. Dose, route, tissue concentration, pharmacokinetics, duration, and the phase of orthodontic treatment should all be considered before attributing an observed clinical response to medication use.

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AND ORTHODONTIC PAIN CONTROL

Pain remains one of the most frequent short-term complaints during orthodontic treatment. It usually develops within hours of separator placement, initial archwire insertion, aligner change, or appliance activation, peaks during the first day, and then declines. The experience varies widely between patients and can affect eating, sleep, quality of life, and adherence, making effective analgesia an important part of treatment rather than a minor comfort issue (Cheng et al., 2020; Neves et al., 2026).

NSAIDs are effective because cyclooxygenase inhibition reduces prostaglandin synthesis. The same mechanism, however, has prompted concern about tooth movement because prostaglandins—particularly PGE2—participate in osteoclast recruitment and alveolar bone resorption. Experimental studies have repeatedly reported slower movement with several NSAIDs, but the findings vary with the agent, dose, duration, and model. The 2026 systematic review by Giannini et al. likewise concluded that NSAIDs relieve orthodontic pain but may reduce movement, with greater concern for repeated or sustained exposure than for occasional use (Giannini et al., 2026).

This distinction between episodic and chronic use is important. Many animal experiments administer NSAIDs continuously and at body-weight-adjusted doses that produce exposures unlike those of a patient taking a few tablets after an activation. It would be inappropriate to convert such findings into a blanket prohibition on ibuprofen or other NSAIDs. The medication history is more informative when it identifies patients who use these drugs regularly for chronic musculoskeletal or inflammatory conditions, because prolonged exposure is more likely to affect remodeling.

Clinical studies suggest that the mechanistic concerns regarding analgesic use should be interpreted cautiously. Several commonly used analgesics provide effective pain control during orthodontic treatment, and more recent evidence indicates that acetaminophen and ibuprofen achieve comparable reductions in pain at rest and during function in many clinical situations. These findings support acetaminophen as a practical alternative for orthodontic pain management when an additional anti-inflammatory effect is not clinically required (Cheng et al., 2020; Neves et al., 2026).

Acetaminophen is often preferred when the aim is to control pain while minimizing peripheral anti-inflammatory activity, provided that it is appropriate for the individual patient. That preference should not be treated as an absolute rule. Hepatic disease, alcohol use, cumulative daily dose, combination cold remedies, gastrointestinal or renal risk, cardiovascular disease, and concomitant medication may all change the safest choice. Analgesic selection should therefore follow the patient's medical profile rather than orthodontic biology alone.

CORTICOSTEROIDS AND IMMUNOSUPPRESSANTS

Systemic corticosteroids are used in respiratory, autoimmune, rheumatologic, hematologic, and other inflammatory disorders and may also form part of transplant therapy. Their effect on orthodontic biology is difficult to predict because they suppress inflammatory signaling while, with prolonged exposure, also reducing bone formation and altering osteoblast, osteocyte, and calcium metabolism. These effects can work in different directions depending on treatment duration and the patient's skeletal status.

Most evidence remains preclinical. Animal studies have shown marked heterogeneity between short- and long-term corticosteroid regimens, and the available findings do not indicate a single consistent direction of effect on orthodontic tooth movement. Reports of accelerated movement in corticosteroid-induced osteoporotic models and findings of reduced inflammatory remodeling should therefore not necessarily be regarded as contradictory, because they may reflect different biological states, doses, and exposure periods (Agrawal and Pandey, 2025).

In practice, chronic corticosteroid use is more useful as a marker of systemic complexity than as a predictor of a particular movement rate. Daily dose, duration, disease activity, bone density, fracture history, calcium and vitamin D status, periodontal condition, and infection risk deserve attention. Medical consultation is especially reasonable for patients receiving long-term or high-dose therapy, those with established osteoporosis, or those for whom extractions or other invasive procedures are planned.

Immunosuppressive agents add another layer of concern. Cyclosporine A, tacrolimus, sirolimus, and related therapies can affect immune regulation, bone turnover, wound healing, and susceptibility to infection. Cyclosporine is particularly important in orthodontics because gingival enlargement can be amplified by plaque accumulation around fixed appliances. In the 2026 systematic review and meta-analysis by Nibali et al., cyclosporine exposure was strongly associated with gingival enlargement, with risk differing between immunosuppressive regimens and with oral hygiene emerging as an important modifiable factor (Nibali et al., 2026).

These patients benefit from stable systemic disease, good plaque control, and careful soft-tissue follow-up before and during appliance therapy. Severe or persistent gingival enlargement may require intensified preventive care, periodontal treatment, and—when medically appropriate—discussion with the prescribing physician about alternative therapy. Medication changes should not be sought simply to make orthodontic treatment easier.

BISPHOSPHONATES AND OTHER ANTIRESORPTIVE AGENTS

Antiresorptive medications are especially relevant to orthodontists because they directly suppress the cellular process required for bone resorption. Bisphosphonates bind to mineralized bone and are taken up by osteoclasts during resorption; nitrogen-containing compounds interfere with the mevalonate pathway and impair osteoclast function. Their long skeletal retention distinguishes them from many other medications and means that previous exposure may remain relevant after treatment has stopped.

The expected consequence is slower or less predictable movement through alveolar bone. Available human evidence remains limited, but systemic bisphosphonate use has been associated with reduced tooth movement, prolonged treatment, difficulty in achieving some extraction-space objectives, and radiographic alterations. More recent umbrella-level evidence has also shown a consistent reduction in orthodontic tooth movement across seven systematic reviews, while emphasizing that six of these reviews relied predominantly on animal studies and that direct human evidence remains scarce. Overall, the direction of the effect appears relatively consistent, although the quality and clinical applicability of the evidence remain limited (Amin et al., 2026; Zymperdikas et al., 2020).

The medication history must distinguish osteoporosis therapy from oncology regimens. Low-dose oral treatment for osteoporosis is not comparable with high-dose intravenous or intensive antiresorptive therapy used in malignant disease. The planned mechanics matter as well. Large bodily movements, extensive extraction-space closure, or prolonged treatment may be more difficult when bone turnover is strongly suppressed, whereas limited tooth alignment may pose a different biological demand.

Medication-related osteonecrosis of the jaw (MRONJ) must be considered separately from the effect on tooth movement. The AAOMS position paper bases risk assessment on the drug, indication, route, duration, comorbidities, and the need for dentoalveolar surgery (Ruggiero et al., 2022). Orthodontic force itself is not established as a common trigger of MRONJ, but an orthodontic plan may include extractions, miniscrew placement, surgical exposure of impacted teeth, or periodontal surgery. Those procedures—not simply the presence of appliances—often determine the practical level of concern.

Denosumab requires its own assessment. It does not bind to bone mineral; instead, it suppresses osteoclast formation and activity through RANKL inhibition. Direct orthodontic evidence is scarce, so the magnitude of its effect should not be assumed to match that of bisphosphonates. At the same time, both altered bone turnover and MRONJ risk are relevant, and interruption of denosumab can have important systemic consequences, including rebound bone turnover. Decisions about timing or discontinuation belong with the prescribing clinician, not the orthodontist.

For patients treated for osteoporosis who have stable oral health and no major invasive procedures planned, orthodontic treatment may still be possible after individualized risk discussion. Conservative mechanics, realistic expectations about treatment speed, and careful periodontal and radiographic monitoring are sensible. Patients receiving high-dose antiresorptive therapy for cancer, those with previous MRONJ, or those requiring extensive surgery need a more cautious multidisciplinary assessment before elective orthodontic treatment is undertaken.

STATINS AND ORTHODONTIC TOOTH MOVEMENT

Statins inhibit HMG-CoA reductase and are widely used for dyslipidemia and cardiovascular risk reduction. Their effects extend beyond lipid lowering: experimental studies have described anti-inflammatory actions, changes in osteoblast activity, reduced osteoclastogenesis, and modulation of the RANKL/osteoprotegerin system. These observations led to interest in whether statins might alter active movement or help limit relapse.

The evidence remains predominantly experimental. An earlier systematic review found that statins often reduced tooth movement, but study quality and protocols varied considerably (Kommuri et al., 2020). A 2026 systematic review identified only seven eligible *in vivo* animal studies of active movement or relapse, using different statins, doses, routes, force systems, and observation periods. Some reported reduced movement or relapse with lower osteoclast activity or more mature alveolar bone; others found little or no effect (Crispino et al., 2026).

These findings are not a reason to alter cardiovascular therapy in an orthodontic patient. Local statin delivery and “pharmacological retention” remain research concepts, and no clinical protocol has established a predictable benefit. Retainers and sound finishing remain the basis of post-treatment stability (Crispino et al., 2026; Kaklamanos et al., 2021).

VITAMIN D, CALCITRIOL, AND MINERAL METABOLISM

Vitamin D influences calcium homeostasis, mineralization, immune regulation, and skeletal remodeling. Its active metabolite, calcitriol, acts through the vitamin D receptor and can affect RANKL expression, osteoclast formation, and osteoblast-lineage cells. For this reason, vitamin D has been studied both as a systemic factor and as a possible adjunct for accelerating tooth movement.

Compared with many experimental pharmacological agents, vitamin D is supported by a small but expanding body of human evidence. Current findings suggest that vitamin D may influence orthodontic tooth movement, external apical root resorption, bone biomarkers, and remodeling, although considerable heterogeneity remains across study designs and outcome measures (Ferrillo et al., 2024). Evidence from randomized clinical trials has also indicated a significant increase in the rate of orthodontic tooth movement following local or systemic vitamin D administration, but this conclusion is based on a limited number of studies (Tini et al., 2024).

The result is encouraging, but it does not establish vitamin D as a routine orthodontic accelerator. The trials were few, protocols differed, baseline vitamin D status was not uniform, and local and systemic administration were pooled across a limited evidence base. Data on root resorption were insufficient. More importantly, a faster movement rate over a short observation period does not necessarily shorten total treatment, which also depends on anchorage, appointments, finishing, patient cooperation, and retention.

The clinical distinction is between treating deficiency and prescribing supplementation solely to influence orthodontic movement. Vitamin D deficiency should be managed for appropriate health indications and, when

necessary, in coordination with the patient's physician. Supraphysiologic supplementation for orthodontic acceleration cannot be recommended on current evidence, particularly in patients at risk of hypercalcemia, renal disease, or drug interactions.

ANTIDEPRESSANTS, ANTIEPILEPTIC DRUGS, AND OTHER NEUROPSYCHIATRIC MEDICATIONS

Neuropsychiatric medications are common in both adolescent and adult orthodontic populations. Their relevance is broader than a possible effect on bone: xerostomia, bruxism, gingival changes, mineral metabolism, sedation, and adherence can all influence treatment. Among these drugs, selective serotonin reuptake inhibitors (SSRIs) and antiepileptic agents have received the most attention in orthodontic research.

Serotonin signaling is relevant to skeletal as well as central nervous system physiology, and long-term SSRI exposure has been associated with alterations in bone turnover in some populations. Fluoxetine is the most extensively studied SSRI in experimental orthodontics, but the available evidence remains limited. A recent systematic review and meta-analysis identified only four eligible animal studies; most showed no meaningful change in orthodontic tooth movement, the pooled effect was not statistically significant, and the certainty of evidence was rated as low or very low (Javed et al., 2025). Current evidence therefore does not support assuming a consistently faster or slower orthodontic response solely on the basis of SSRI use.

For antidepressants, the practical issues may be more immediate than any uncertain effect on bone. Xerostomia can increase caries risk and mucosal discomfort, and bruxism has been reported with some serotonergic drugs. Mood and anxiety disorders can also influence attendance and adherence. These considerations justify closer preventive care when needed, but not alteration of psychiatric medication for orthodontic purposes.

Antiepileptic therapy has clearer links with mineral and periodontal health. Long-term use of agents such as phenytoin, carbamazepine, or valproate can affect vitamin D metabolism, calcium homeostasis, or bone density, and phenytoin is a well-recognized cause of gingival enlargement. A 2026 retrospective cohort of 50 adolescents reported longer treatment duration and differences in craniofacial measurements among patients with more than three years of antiepileptic medication exposure compared with matched controls (Alam et al., 2026). The study is notable because human data are scarce, but its small, observational design does not establish causation or allow the findings to be generalized to all antiepileptic drugs.

Other neurologic and psychotropic agents have been studied mainly in animals. Diazepam, phenobarbital, lithium, and related medications have

produced variable results, with marked differences in dosing and experimental design (Makrygiannakis et al., 2018). Such findings are useful for hypothesis generation but are too weak to dictate orthodontic mechanics.

A useful clinical review therefore includes duration of therapy, bone-health risk, salivary symptoms, gingival enlargement, seizure control, possible analgesic interactions, and the patient's ability to maintain oral hygiene. Patients with epilepsy also require routine precautions related to seizure history and emergency preparedness. Stable medical treatment and prevention of oral complications take priority over any attempt to manipulate prescribed neuropsychiatric therapy.

ANTIDIABETIC AND CARDIOVASCULAR MEDICATIONS

Cardiometabolic medications are increasingly encountered in adult orthodontic practice. For many individual drugs, direct evidence on tooth movement is still sparse, and the underlying disease may be more important than the medication itself. The most useful clinical questions therefore concern metabolic control, periodontal health, gingival effects, wound healing, and the safety of planned adjunctive procedures.

Diabetes is a good example of this overlap between disease and treatment. Hyperglycemia can disturb periodontal healing, inflammatory signaling, and bone remodeling. A 2025 systematic review found less efficient movement and more adverse periodontal or pulpal responses in the available diabetic models and clinical literature, while also noting experimental evidence that insulin and metformin may attenuate some of these changes (García-Rios et al., 2025). These findings should not be interpreted as an orthodontic indication for metformin. Glycemic control and periodontal stability remain the priorities.

Calcium-channel blockers are clinically relevant in orthodontic patients primarily because of their association with drug-induced gingival enlargement. Agents such as nifedipine and amlodipine may increase gingival overgrowth in susceptible individuals, and recent systematic review and meta-analysis evidence supports an elevated risk among patients receiving this drug class (Nibali et al., 2026). Because fixed orthodontic appliances create additional plaque-retentive areas, inadequate plaque control may further exacerbate gingival inflammation and make soft-tissue management more difficult in these patients.

Newer cardiometabolic drugs, including GLP-1 receptor agonists used for diabetes and weight management, are now common enough to appear routinely in medical histories, yet direct orthodontic outcome data are lacking. There is no basis at present for changing force systems solely because a patient uses one of these agents. More immediate issues may include nutritional

intake, nausea or vomiting, hydration, glycemic status, and perioperative planning when sedation or surgery is contemplated. This is a clear example of clinical use advancing faster than orthodontic research.

HORMONAL AND LOCALLY DELIVERED BIOLOGICAL MODULATORS

Pharmacological acceleration of orthodontic movement has been explored for decades. Prostaglandins, parathyroid hormone (PTH), thyroid hormones, calcitriol, RANKL, growth factors, osteocalcin, relaxin, and other signaling molecules have all been tested with the aim of increasing local remodeling. Most of this work remains experimental, and the central challenge is to increase turnover where needed without creating unacceptable local or systemic effects.

Prostaglandins were among the earliest agents studied. Local PGE1 or PGE2 can increase osteoclast activity and has accelerated movement in experimental models and some small human studies. Clinical enthusiasm has been limited by the need for repeated administration, injection-related discomfort, local inflammation, and uncertain dosing. A 2024 systematic review of locally administered biofunctional molecules identified prostaglandins and calcitriol among the relatively few agents with both animal and human data but still judged the evidence insufficient for routine use (Ciobotaru et al., 2024).

PTH illustrates why route and exposure pattern matter. Intermittent systemic PTH can be anabolic, whereas sustained exposure can favor resorption. Experimental local delivery has increased tooth movement in animals, but PTH analogues are prescription drugs for selected metabolic bone conditions and should not be repurposed for orthodontic acceleration outside research. The same principle applies to thyroid hormone: altered thyroid status changes skeletal turnover, but there is too little direct clinical evidence to justify modifying endocrine treatment for orthodontic reasons.

Recent research has increasingly focused on local delivery systems, including hydrogels, microspheres, nanoparticles, and other carrier-based approaches designed to restrict pharmacological effects to specific sites and time periods. Although these strategies are biologically attractive, their clinical translation remains at an early stage, with topical delivery approaches still relatively underexplored and selective modulation of bone-remodeling pathways representing a promising but largely experimental direction (Ciobotaru et al., 2024; Danz and Degen, 2025).

A recurring problem is the choice of outcome. Many acceleration studies report millimeters of movement over a few weeks, whereas patients and clinicians are more concerned with total treatment time, comfort, anchorage,

root integrity, periodontal health, stability, and cost. An intervention that speeds one phase but complicates finishing or retention may offer little real benefit. Future studies of pharmacological adjuncts need to measure these broader outcomes before clinical adoption can be justified (Ciobotaru et al., 2024; Pacheco-Quito et al., 2026).

ROOT RESORPTION AND MEDICATION USE

Orthodontically induced inflammatory root resorption is a multifactorial complication in which clastic cells resorb cementum and, in more advanced lesions, dentin after damage to the protective root surface. Risk is influenced by treatment duration, force magnitude and continuity, intrusion, torque, apical displacement, root morphology, previous trauma, and individual susceptibility (Yassir et al., 2021).

Because resorption involves inflammatory and clastic pathways, a wide range of drugs has been tested as potential modifiers. Some animal studies report less root resorption with antiresorptives, NSAIDs, statins, or other agents, but the interpretation is difficult when the same intervention also reduces the intended tooth movement. Suppressing clastic activity may protect the root while simultaneously suppressing alveolar bone resorption. No systemic medication currently has an evidence-based indication for prevention of orthodontic root resorption.

Bisphosphonates illustrate this uncertainty well. Their mechanism could reduce clastic activity at the root surface, yet the 2026 umbrella review found contradictory evidence for root resorption across the included systematic reviews (Amin et al., 2026). Vitamin D trials are similarly inconclusive: although some human studies report faster movement, there are too few data to determine whether the risk of root resorption changes in parallel (Tini et al., 2024).

Clinical management therefore still rests on mechanics and monitoring. Patients with short or atypically shaped roots, previous trauma, pre-existing resorption, planned major intrusion or torque, or prolonged treatment need an individualized radiographic strategy. When progressive resorption is identified, reducing or temporarily suspending force and revisiting treatment goals are better supported than adding a pharmacological agent (Yassir et al., 2021).

ORTHODONTIC RELAPSE, RETENTION, AND MEDICATIONS

Post-treatment tooth movement reflects continued adaptation of periodontal fibers and alveolar bone together with occlusal forces, musculature, growth, aging, and patient-specific biology. Because remodeling

continues after active appliances are removed, several drugs have been tested experimentally as ways to reduce early relapse.

Animal reviews report reduced relapse with bisphosphonates, atorvastatin, tetracycline derivatives, osteoprotegerin, raloxifene, and strontium in some models, while results for simvastatin and relaxin are inconsistent. Species, force systems, relapse intervals, and drug protocols vary substantially, which limits comparison across studies (Kaklamanos et al., 2021). The 2026 statin review reached the same practical conclusion: the signal is biologically interesting, but it is not ready for human recommendations (Crispino et al., 2026).

None of this evidence changes present retention practice. Pharmacological retention cannot replace retainers, stable finishing, control of etiologic factors, and long-term follow-up. Even a successful local drug aimed at early bone remodeling would not address later changes related to growth, aging, periodontal adaptation, or function.

CLINICAL ASSESSMENT OF ORTHODONTIC PATIENTS USING MEDICATIONS

Medication history should be treated as part of diagnosis, not as a box to be checked on the medical form. The clinician needs more than the drug name: indication, dose, route, frequency, duration, prescriber, recent changes, and expected continuation may all affect interpretation. Patients should also be asked directly about over-the-counter analgesics, supplements, osteoporosis injections, psychiatric drugs, weight-management medication, antihistamines, and intermittent corticosteroid courses, which are often omitted when patients are asked only about “regular medicines.”

The underlying diagnosis should be considered alongside the prescription. Bisphosphonate use may indicate osteoporosis; cyclosporine may follow organ transplantation; chronic corticosteroids may accompany severe inflammatory disease; metformin usually signals diabetes or insulin resistance; antiepileptic therapy implies a seizure history; and calcium-channel blockers are prescribed for cardiovascular disease. In many situations the disease state contributes as much to orthodontic risk as the drug itself (Ardeshtna et al., 2024; Gupta et al., 2024).

A risk-based approach is more useful than a list of prohibited medications. Stable systemic disease, good periodontal health, modest planned movement, and drugs supported only by weak or preclinical orthodontic evidence usually allow conventional care with appropriate observation. Greater caution is warranted with potent antiresorptive therapy, previous MRONJ, uncontrolled diabetes, marked immunosuppression, severe drug-induced gingival enlargement, significant osteoporosis, or treatment plans that depend on

extractions or surgery. These situations may justify medical or dental specialist input before elective treatment.

When bone turnover is likely to be altered, increasing force in response to slow movement is not a sound strategy. Heavy force increases periodontal ischemia and hyalinization and may add root-resorption risk without overcoming pharmacological suppression of remodeling. Light, controlled forces, sufficient time for tissue response, and realistic movement goals are preferable. If treatment depends on extensive space closure or major root movement, the possibility of a longer or less predictable course should be discussed from the outset.

Periodontal review deserves particular attention in patients taking phenytoin, cyclosporine, or calcium-channel blockers. Baseline photographs and periodontal charting help separate pre-existing enlargement from appliance-related inflammation. Plaque control should be established before bonding, and some patients will benefit from more frequent professional cleaning and periodontal review. The 2026 gingival-enlargement meta-analysis supports oral-hygiene instruction and professional plaque removal as core elements of management (Nibali et al., 2026).

Antiresorptive therapy requires a more detailed history: drug name, indication, route, cumulative duration, and timing of the most recent dose. Patients may not think of an osteoporosis injection as a medication, so direct questioning is useful. The need for extractions, miniscrews, surgical exposure, or periodontal surgery should be identified early. When the risk is uncertain, discussion with the prescribing physician and, where appropriate, an oral surgeon or oral-medicine specialist is preferable to unilateral changes in medication. The orthodontist should not initiate a drug holiday (Ruggiero et al., 2022).

Communication with the patient should be equally measured. Statements that a medication will “stop the teeth from moving” or that a supplement will “make treatment faster” go beyond the evidence. A more accurate explanation is that certain drugs can influence bone or gingival biology, but the effect in an individual patient is often uncertain because much of the literature is preclinical or heterogeneous. This conveys the issue without encouraging patients to alter essential treatment on their own.

Table 1: Practical summary of medication classes relevant to orthodontic care

Medication/class	Main biological or oral effect	Current evidence	Practical orthodontic implication
NSAIDs / aspirin	Cyclooxygenase inhibition and reduced prostaglandin synthesis	Strong human evidence for analgesia; effect on movement is supported mainly by experimental and indirect clinical evidence	Use when indicated; distinguish occasional analgesia from chronic exposure. Do not alter antiplatelet therapy without the prescriber.
Acetaminophen	Analgesia with weak peripheral anti-inflammatory activity	Human meta-analysis supports pain control comparable with ibuprofen for several outcomes	Useful option when medically appropriate; consider hepatic risk and cumulative daily dose.
Bisphosphonates	Potent osteoclast inhibition with prolonged skeletal retention	Systematic reviews consistently report reduced movement; direct human evidence remains limited	Expect slower or less predictable movement; assess MRONJ risk and planned invasive procedures.
Denosumab / other antiresorptives	RANKL inhibition or other suppression of bone resorption	Direct orthodontic evidence is limited	Coordinate risk assessment with the prescribing clinician; do not initiate a drug holiday independently.
Corticosteroids	Anti-inflammatory action plus long-term effects on bone turnover	Conflicting and predominantly animal evidence	Interpret in relation to dose, duration, bone health, disease activity, and infection risk.
Statins	Mevalonate-pathway inhibition with osteogenic and anti-osteoclast effects	Predominantly animal evidence; 2026 systematic review remains inconclusive for clinical translation	Do not modify cardiovascular therapy; any effect on movement or relapse remains unproven clinically.
Vitamin D / calcitriol	Vitamin D receptor signaling, mineral metabolism, and bone turnover	Small human RCT meta-analysis suggests faster movement	Treat deficiency for health indications; routine orthodontic acceleration is not established.

Medication/class	Main biological or oral effect	Current evidence	Practical orthodontic implication
SSRIs	Serotonin signaling may influence bone metabolism	Fluoxetine meta-analysis based on animal studies found no significant pooled effect	No orthodontic indication to stop therapy; monitor xerostomia, bruxism, and general oral effects.
Antiepileptic drugs	Bone/mineral effects; phenytoin may cause gingival enlargement	Limited human orthodontic data; evidence for gingival enlargement is stronger	Prioritize periodontal prevention and seizure-safe care; coordinate with medical treatment.
Cyclosporine / immunosuppressants	Immune and bone effects; cyclosporine-related gingival enlargement	Clinically relevant evidence for gingival enlargement	Stabilize periodontal health; consider medical consultation if adverse oral effects are severe.
Calcium-channel blockers	Drug-related gingival fibroblast response	2026 meta-analysis supports increased risk of gingival enlargement	Intensify plaque control and periodontal surveillance; medication changes belong with the prescriber.
Metformin / other antidiabetics	May modify hyperglycemia-related inflammation and remodeling	Direct orthodontic drug evidence is limited; metabolic control is more important	Treat patients with stable diabetes and prioritize glycemic and periodontal control.
Local biological modulators	PGE, PTH, RANKL, growth factors, and targeted remodeling pathways	Promising but largely experimental	Not routine care; efficacy, safety, dose, and delivery require clinical validation.

LIMITATIONS OF THE EVIDENCE AND RESEARCH PRIORITIES

Research on drugs and orthodontics is mechanistically detailed but clinically uneven. The biological basis for some effects is strong—osteoclast suppression with bisphosphonates and prostaglandin inhibition with NSAIDs are obvious examples—but a plausible mechanism does not tell us how large the effect will be over a full course of treatment. Appliance design, type of movement, force level, age, periodontal phenotype, systemic disease, nutrition, adherence, and treatment decisions all contribute to the final outcome.

For many medications, animal models still provide most of the evidence. They are useful for identifying pathways and dose-response relationships, but

rodents differ from humans in bone turnover, periodontal anatomy, drug metabolism, and the time scale over which orthodontic forces are applied. Experimental doses and local injections may also create tissue exposures that have little resemblance to routine prescriptions. These limitations help explain why the 2026 systematic review by Giannini et al., despite including 64 studies, still judged certainty for many pharmacological effects as low or very low (Giannini et al., 2026).

Human studies face a different problem: confounding by indication. A patient taking corticosteroids is not simply a healthy patient exposed to a drug; the inflammatory disease itself may alter bone and periodontal biology. The same is true for osteoporosis in bisphosphonate users and diabetes in patients receiving metformin or insulin. Separating medication effects from disease effects requires larger cohorts, detailed exposure data, and appropriate multivariable adjustment, which are still uncommon in orthodontic research.

Future clinical studies should record medication exposure with the same care used for orthodontic mechanics. Dose, frequency, route, duration, indication, and adherence are more informative than a binary “user/non-user” label. Outcomes should also move beyond millimeters of short-term tooth movement to include total treatment duration, number of visits, anchorage loss, root resorption, periodontal outcomes, pain, patient-reported experience, and stability. Standardized outcome sets would make future comparisons and meta-analyses far more useful.

CONCLUSION

Medication use can influence orthodontic treatment because tooth movement depends on inflammation, osteoclast and osteoblast activity, periodontal adaptation, and bone remodeling. The clinical effect, however, is rarely determined by the drug alone. Dose, duration, route, the underlying disease, periodontal status, and the planned mechanics all shape the response.

The clearest current signals are practical rather than absolute. NSAIDs are effective for pain control, but repeated or prolonged exposure may attenuate prostaglandin-dependent remodeling. Bisphosphonates consistently reduce experimental tooth movement, although human evidence remains limited and MRONJ risk may affect treatment planning. Small human trials suggest that vitamin D can increase movement rate, but evidence is not sufficient to recommend supplementation solely for orthodontic acceleration. For corticosteroids, statins, antidepressants, antiepileptic drugs, immunosuppressants, antidiabetic agents, and many cardiovascular drugs, the evidence is heterogeneous or largely preclinical. Gingival enlargement with phenytoin, cyclosporine, and calcium-channel blockers remains one of the most immediate medication-related problems in routine care.

The practical message is to understand the medication rather than react to its name. A detailed history, recognition of the underlying disease, appropriate periodontal and skeletal assessment, controlled biomechanics, and medical consultation when indicated allow most decisions to be individualized. Medically necessary drugs should not be stopped or modified to improve orthodontic tooth movement without the prescribing clinician. Until stronger human evidence is available, tissue safety and predictable mechanics remain more important than attempts to pharmacologically accelerate treatment.

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Bromhexine: Pharmacological Properties and UV-Visible Spectrophotometric Analysis Method

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ABSTRACT

Excessive airway mucus secretion is a hallmark of chronic inflammatory respiratory disorders such as chronic obstructive pulmonary disease, bronchial asthma, bronchiectasis, and cystic fibrosis, and is closely linked to impaired mucociliary clearance, disease progression, and increased morbidity and mortality. Bromhexine hydrochloride, a synthetic benzylamine derivative inspired by the natural alkaloid vasicine, has been used since 1963 as a mucoactive agent that reduces mucus viscosity, stimulates serous secretion and pulmonary surfactant release, and enhances mucociliary transport. Following oral administration, bromhexine undergoes extensive hepatic first-pass metabolism to form its pharmacologically active metabolite, ambroxol, and is widely indicated in acute and chronic respiratory conditions characterized by thick, tenacious mucus. This chapter reviews the pharmacological properties of bromhexine and provides a comprehensive overview of the ultraviolet-visible (UV-Vis) spectrophotometric methods reported for its quantitative determination. Direct UV methods, derivative and zero-crossing techniques for combined formulations, visible-region approaches based on ion-pair and azo dye complexation (using reagents such as Tropaeolin OO, arsenazo III, and 4'-chloro-4-hydroxychalcone), and a recently developed green analytical method employing erythrosine B in an aqueous medium are discussed, together with their validation parameters (linearity, accuracy, precision, LOD, and LOQ). The comparative evaluation highlights that UV-Vis spectrophotometry remains a fast, economical, and reliable alternative or complement to chromatographic techniques, particularly valuable for routine quality control of bromhexine-containing pharmaceutical preparations in resource-limited laboratories.

Keywords – Bromhexine hydrochloride; Mucoactive drugs; UV-Visible spectrophotometry; Method validation; Green analytical chemistry; Ambroxol

INTRODUCTION

One of the important clinical and pathological features of airway diseases is excessive secretion of the airway mucosa, seen in chronic inflammatory conditions including chronic obstructive pulmonary disease (COPD), bronchial asthma, bronchiectasis, and cystic fibrosis. The prolonged and recurrent states of chronic inflammation give rise to a "secretory hyperresponsiveness" that causes metaplasia of goblet cells and hyperplasia and hypertrophy of submucosal glands, leading to impaired mucociliary clearance. As a result, excess mucus becomes more difficult to expel and accumulates to form mucus plugs in the airways; this worsens obstruction of the already narrowed airway and increases colonization by pathogenic bacteria. These events can lead to persistent inflammation, continuous

hypoxia, deterioration of the patient's condition, and even death (Evans and Koo, 2009; Voynow and Rubin, 2009; Zhang and Zhou, 2014).

The airway mucosa is a moist, soft, and protective layer of tissue that covers the inner surface of the entire respiratory system, from the nose to the smallest air sacs in the lungs. The airway mucosa is a heterogeneous mixture composed of glycoproteins, non-glycoprotein-like proteins, lipids, minerals, water, and other compounds (Evans and Koo, 2009; Zhang and Zhou, 2014). Although the components of the airway mucosa and their corresponding proportions vary considerably under different conditions, mucin, a highly glycosylated macromolecule, is always the main component and determines the hydrophilic and viscoelastic properties of airway mucus. During chronic airway inflammation, the apparent increased glycosylation and sulfation of mucus significantly increases the viscosity and acidity of airway mucus, immobilizing it in the airway and thereby making its clearance even more difficult, which further worsens airway obstruction. For this reason, excessive secretion of the airway mucosa plays an important role in the emergence and progression of chronic inflammatory airway diseases, and studies have shown that it is associated with the lung function, quality of life, hospitalization rate, and mortality of patients with chronic inflammatory airway diseases (Evans and Koo, 2009; Voynow and Rubin, 2009; Zhang and Zhou, 2014).

The airway mucosa is protected by ciliary activity and phagocytic activity, the primary defense mechanisms against inhaled particles, allergens, and pathogens. Together, these two prevent foreign substances from reaching the deep tissue of the lung and causing infections. Cilia are found on epithelial surfaces throughout the entire airway, from the pharynx down to the end of the respiratory bronchioles. Mucus-secreting epithelial cells and various glands are also found throughout the airway. Mucus is a fibrous gel composed of mucoproteins, mucopolysaccharides, pollen, and fat. Glycoproteins that polymerize via disulfide bonds give mucus its sticky property. Mucus warms and humidifies inhaled air and removes airborne foreign particles with the aid of ciliary movement. The cilia are in constant motion toward the pharynx. This structure can be likened to an escalator made of mucus. Thanks to this escalator, dust in the inhaled air adheres to the mucus and is carried toward the pharynx by the slow but continuous movement of the cilia; upon reaching the pharynx, it is either swallowed or expelled. This mucus escalator is essential for keeping the lungs clean. Ciliary activity can be inhibited by numerous harmful agents. For example, cigarette smoke and chemicals reduce ciliary movement and impair macrophage phagocytosis, causing mucus to accumulate in the lungs and leading to coughing (Fernandez-Blanco et al., 2018). The second protective mechanism is phagocytes. Found throughout the airway and alveoli, phagocytes engulf small inhaled particles and bacteria, preventing them from passing into other lung cells or the bloodstream (Katsnelson and Privalova, 1984).

Clinically available expectorant drugs aim to inhibit mucus production and secretion, reduce the viscoelasticity of mucus, restore the normal structure and function of the mucus layer, enhance mucociliary clearance, and accelerate mucus transport. The pharmacological drug class that affects the volume, viscosity, transport, and composition of sputum and mucosa — including expectorants, mucolytics, mucoregulators, and mucokinetics — is classified as mucoactive drugs (Balsamo, Lanata and Egan, 2010; Zhang and Zhou, 2014).

Mucoactive agents are compounds that facilitate the expectoration of mucus by altering the biophysical properties of airway secretions or by increasing ciliary clearance. Within this broad class, expectorants increase bronchial secretion volume, while mucokinetics enhance cough clearance and mucolytics reduce the viscoelasticity of mucus. The therapeutic efficacy of expectorants largely depends on systemic hydration status, since adequate fluid intake acts as a physiological expectorant that directly supports the dilution of secretions. Conversely, the pronounced anticholinergic effects of first-generation antihistamines, frequently found in combined antitussive/cold preparations, can antagonize the efficacy of expectorant agents by causing dryness of the bronchial mucosa and increased viscosity of secretions. In line with these pharmacodynamic properties, expectorants are indicated in cases of productive cough with impaired airway clearance rather than in non-productive (dry) cough (Rogers, 2007; Balsamo, Lanata and Egan, 2010; Gupta and Wadhwa, 2026).

1. Bromhexine, One of the Mucoactive Drugs

Bromhexine (bromhexine hydrochloride), since its introduction to the market in 1963, is a synthetic benzylamine derivative used in respiratory tract diseases to reduce mucus viscosity and enhance mucociliary clearance. It was developed by drawing inspiration from vasicine, a natural alkaloid derived from *Adhatoda vasica* (Zanasi, Mazzolini, and Kantar, 2017).

1.1. Mechanism of Action and Pharmacodynamics: Bromhexine is a versatile mucoactive agent that exhibits both mucolytic and mucokinetic properties (Zanasi, Mazzolini, and Kantar, 2017; Bhagat and Rachana, 2018):

- **Reduction of Mucus Viscosity:** It breaks down acid mucopolysaccharide fibers in bronchial secretions (particularly by triggering lysosomal activation and stimulating hydrolytic enzymes), liquefying the gel phase of mucus and thereby reducing its viscoelasticity.
- **Enhancement of Serous Secretion:** It stimulates serous gland cells in the tracheobronchial mucosa, promoting the production of more fluid, lower-viscosity secretions.
- **Stimulation of Surfactant Synthesis:** It induces the release of endogenous pulmonary surfactant from type II alveolar epithelial cells; this prevents mucus from adhering to the airway epithelium and facilitates ciliary movement.

- **Mucociliary Transport:** Through decreased mucus viscosity and increased ciliary beat frequency, it accelerates the transport of secretions to the proximal airways (i.e., cough clearance).

1.2. Pharmacokinetic Properties and Prodrug Nature (Padrid and Church, 2008; Weise, 2008; Duan et al., 2024):

- **Absorption:** It is rapidly and almost completely absorbed via the oral route; however, it undergoes extensive hepatic first-pass metabolism (bioavailability is approximately 20–25%).
- **Biotransformation (Relationship with Ambroxol):** Bromhexine is metabolized in the liver via cytochrome P450 enzymes. Its most important and therapeutically active principal metabolite is ambroxol (trans-4-(2-amino-3,5-dibromobenzylamino)cyclohexanol). A significant portion of bromhexine's clinical effects is attributable to its conversion into this active metabolite.
- **Distribution and Elimination:** It is distributed extensively into tissues (reaching particularly higher concentrations in bronchial tissue compared to plasma). Its plasma protein binding rate is high (90–99%). Its metabolites are eliminated primarily via the renal route.

1.3. Clinical Use and Indications (Padrid and Church, 2008; Zanasi, Mazzolini and Kantar, 2017): It is indicated in acute and chronic respiratory tract diseases characterized by excessive, tenacious, or thick mucus:

- Chronic obstructive pulmonary disease (COPD) and emphysema exacerbations
- Acute and chronic bronchitis, bronchiectasis
- Tracheobronchitis and productive cough conditions

1.4. Chemical Structure of Bromhexine

Bromhexine (IUPAC: 2-amino-3,5-dibromo-N-cyclohexyl-N-methylbenzylamine, Figure 1), a mucoactive agent, is a synthetic benzylamine derivative obtained through chemical modification of the alkaloid vasicine, isolated from the plant *Adhatoda vasica*. The compound ($C_{14}H_{20}Br_2N_2$), commonly present in formulations as its water-soluble hydrochloride salt, is composed of a tertiary amine core linking an aromatic dibromophenyl ring to a hydrophobic cyclohexyl group (Bhagat and Rachana, 2018). The bromine substitutions confer high lipophilicity on the molecule, facilitating its penetration into bronchopulmonary tissues. Following oral administration, bromhexine undergoes hepatic biotransformation involving hydroxylation of the cyclohexyl ring and N-demethylation to form its active metabolite, ambroxol; consequently, a significant portion of its clinical efficacy is exerted through this metabolic conversion.

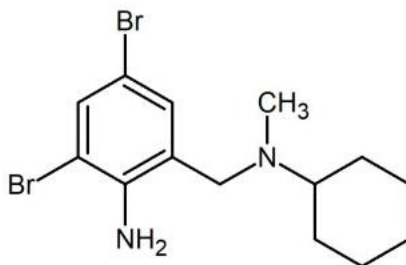


Figure 1. Chemical Structure of Bromhexine

2. Spectroscopic Analysis of Bromhexine

Numerous ultraviolet-visible (UV-Vis) spectrophotometric methods have been developed and validated in the literature for the quantitative determination of bromhexine hydrochloride. These methods are frequently preferred, particularly in quality control laboratories, as an alternative or complement to chromatographic techniques, owing to their speed, economy, ease of application, and the fact that they can be performed using widely available spectrophotometer instruments.

2.1. Fundamental Principle of UV-Vis. Spectrophotometry: The method is based on the Beer-Lambert law and operates on the principle that the amount of light absorbed by an analyte in a solution at a specific wavelength is directly proportional to its concentration. The dibromophenyl ring and conjugated aromatic system present in the structure of the bromhexine molecule (Figure 1) impart a characteristic absorption spectrum to the compound in the UV region; this property has enabled the development not only of simple methods based on direct measurement, but also of indirect methods based on the measurement, in the visible region, of colored derivatives formed with dyes or reagents (Alaboodi, Kadhim and Hussein, 2025).

2.2. Direct UV Spectrophotometric Methods: Direct determination of bromhexine hydrochloride in bulk substance and tablet formulations is possible without the need for any derivatization or extraction procedure. The compound has been reported to exhibit a characteristic maximum absorbance (λ_{max}) at approximately 248 nm in methanolic medium and around 415 nm in aqueous medium; calibration curves constructed at these wavelengths have been shown to conform to Beer's law with high linearity ($R^2 \approx 1.00$) (Lad, Jain and Raj, 2014). While the most important advantage of direct UV methods is

their speed and simplicity, selectivity may remain limited in combined formulations where other active ingredients absorb in the same region.

2.3. Derivative Spectrophotometry and Area-Under-the-Curve (AUC)

Methods: Mathematical derivatization-based spectrophotometric techniques are used to enhance selectivity in combined preparations in which bromhexine is formulated together with active ingredients such as ambroxol, terbutaline sulfate, or guaifenesin. Measurements performed at 283 nm and 218.6 nm in methanolic medium using the first-order derivative spectrum provided high linearity ($R^2 = 0.9998$); the zero-crossing point method was applied at 234 nm and 324 nm in a 50% methanol-phosphate buffer (pH 6.0) medium, yielding an R^2 value of 0.9999. The simultaneous equation method, on the other hand, was carried out using the absorbance values at 250 nm and 275 nm in methanolic medium (Lad, Jain and Raj, 2014).

2.4. Visible-Region (Vis) Spectrophotometry – Methods Based on Ion-Pair and Azo Dye Complexation:

The secondary amine group in the bromhexine molecule can form ion-pair or azo dye complexes with suitable dyes or reagents, producing colored products that can be measured in the visible region. This approach markedly enhances selectivity by minimizing the effect of matrix components that could interfere in the UV region.

Extractive Ion-Pair Method: Ion pairs formed in chloroform medium with the dyes Tropaeolin OO, Naphthalene Blue 12BR, and Azocarmine G were measured at 420 nm, 620 nm, and 540 nm, respectively; the methods were validated with linear ranges of 2.0–10 and 5.0–25 $\mu\text{g/mL}$, correlation coefficients of 0.9998–0.9999, recovery values of 99.59–100.92%, and relative standard deviation (RSD) values of 0.204–0.543% (Rao et al., 2005).

Cerium(III)–Arsenazo III Complex Method: In an indirect method developed based on the capacity of bromhexine to reduce cerium(IV) ions to cerium(III), the resulting cerium(III)-arsenazo III complex was measured at 651 nm in pH 3.4 formate buffer. The method was characterized by linearity ($R^2 = 0.9978$) in the range of 0.5–10 ppm, a molar absorptivity of $1.48 \times 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, a limit of detection (LOD) of 0.408 $\mu\text{g/mL}$, and a limit of quantification (LOQ) of 1.394 $\mu\text{g/mL}$ (Mohammed and Almukhtar, 2018).

Diazotization and Azo Dye Formation Method: The stable azo dye complex formed by diazotizing the primary aromatic amine group in the structure of bromhexine with sodium nitrite and coupling it with 4'-

chloro-4-hydroxychalcone under basic conditions was measured at 436 nm. The method was successfully applied to Solvodin® tablets with a linear range of 2–18 µg/mL, a molar absorptivity of 1.9063×10^4 L/mol·cm, an RSD below 0.985%, an LOD of 0.280 µg/mL, and a recovery of $\geq 99.4\%$ (Hamed, Hussein and Ahmed, 2026).

Hiba Abdul Salam Al-Hafidh and Rabee Mohammed Albadgari performed quantitative analysis for the spectrophotometric determination of bromhexine hydrochloride, in bulk form and in pharmaceutical formulations, using a diazotization-coupling reaction, in which bromhexine hydrochloride was diazotized and reacted with the organic reagent 4-chlororesorcinol, and the resulting azo dye was read at a wavelength of 455 nm. The LOD value of the method was determined to be 0.322 µg/mL and the LOQ value to be 1.073 µg/mL (Al-Hafidh and Albadgari, 2025).

2.5. Spectrophotometric Method with a Green Analytical Chemistry Approach: In a method developed in line with sustainable analytical chemistry principles, the dye erythrosine B was used as an ion-pairing agent, and water was preferred as the sole solvent. Measurements performed at 556 nm at pH 3.6 yielded linearity ($r = 0.9990$) in the range of 1.0–9.0 µg/mL, an LOD of 0.199 µg/mL, and an LOQ of 0.605 µg/mL; the method was successfully applied to Bisolvon® tablets, providing intra-day and inter-day accuracy/precision results in accordance with ICH guidelines. Having received a score of 89 on the Eco-Scale assessment, the method was classified as belonging to the "excellent green analysis" category due to its elimination of toxic organic solvent use (Mohamed, Nour-Eldeen and Omar, 2026).

2.6. Method Validation and Comparative Evaluation: All of the UV-Vis spectrophotometric methods summarized in this section have been validated in accordance with the ICH Q2(R1) guideline in terms of linearity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) parameters. While direct UV methods offer advantages in terms of speed and low cost, they may remain limited in terms of selectivity in combined formulations; derivative and zero-crossing techniques overcome this problem mathematically, whereas ion-pair- and azo dye-based visible-region methods offer high selectivity without requiring physical separation. Green analytical approaches, on the other hand, provide both environmental and operational advantages by eliminating the use of toxic organic solvents.

Compared to chromatographic methods (HPLC, HPTLC), UV-Vis spectrophotometry continues to be a preferred technique, particularly in quality control laboratories with limited resources, owing to its low cost, simple instrumentation requirements, and short analysis time.

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**Polyethylene succinate based polymeric
nanoparticles bearing azobenzene moieties
as potential hypoxia targeted cancer
treatment agent**

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ABSTRACT

Numerous studies have been conducted to develop effective methods to combat cancer and reduce the severity of the disease. One of the most promising approaches involves using powerful chemical drugs that have minimal impact on healthy cells. Additionally, scientists have been exploring the use of materials that can distinguish between healthy and cancer cells and produce a response. By utilizing these differences, researchers are working to create materials that can release antitumor drugs specifically at the site of tumors. One such difference is the variation in the amount of oxygen between healthy and cancerous tissues, leading to hypoxia. In this research project, a methoxy polyethylene glycol-azobenzene polyethylene succinate copolymer was synthesized using a one-pot method. To investigate the structure of the polymer nanoparticles, infrared spectroscopy analysis (FT-IR) and proton nuclear magnetic resonance ($^1\text{H-NMR}$) were employed. The results of the investigation indicate that these polymer nanoparticles can be used for drug release in hypoxic tumors. This is a promising development in the fight against cancer, as it offers a targeted approach that minimizes negative side effects on healthy cells. Through ongoing research and development, this innovative approach holds the potential to revolutionize cancer treatment by introducing more targeted and personalized therapies. These advancements could significantly improve patient outcomes and quality of life, offering new hope for those affected by cancer.

Keywords: synthesis, copolymer, hypoxia, drug delivery, tumor

INTRODUCTION

The advancement of knowledge and technology has impacted various scientific fields, including pharmaceutical industries [1]. Researchers are exploring multiple ways to combat cancer and reduce its severity [2]. However, hypoxia is a common symptom observed in more than 60% of solid tumors in microenvironments of tumor tissues [3,4]. The partial pressure of oxygen in healthy tissues is typically around 40-60 mm Hg, whereas it is less than 10 mm Hg in tumor tissues [5,6]. The reason behind this phenomenon is the increased oxygen consumption of cancer cells in the blood [7,8]. Hypoxic tumors often show an increase in tumor accumulation, a decrease in therapeutic effects, and poor diagnosis of chemotherapy [9,10]. Based on this, researchers have developed drugs activated by hypoxia, which can be activated by enzymes for the targeted treatment of hypoxic tumors [11,12]. Despite the advantages of hypoxia-activated drugs, the therapeutic effects are not satisfactory since small molecules have a short life in the circulatory system due to kidney filtration. As a result, the concentration of the drug in tumors is insufficient [13]. Moreover, hypoxia induced factors (HIF), that lead to tumor cells quickly

adapting to oxygen deficiency, affecting the regulation of the cell cycle, the preservation of stem cells, and increasing the likelihood of cancer returning in patients [14,15,16].

The combination of radiotherapy and chemotherapy, known as clinical chemotherapy, is the main treatment model for all types of cancers [17,18]. In recent years, chemotherapy based on nanocarriers has gained significant attention [19,20]. Optimized nanocarriers facilitate drug delivery to tumor tissues by effective targeting, ultimately increasing the therapeutic effect of radiation [21,22]. Currently, many nanoparticle-based systems have been developed for drug delivery and targeting with the primary objective of reducing drug degradation, preventing side effects, and enhancing drug availability and accumulation in the lesion site. By using drug-carrying nanoparticles, a diverse range of drugs, including hydrophilic, and hydrophobic drugs, proteins, vaccines, etc., can be delivered to the target tissues [1]. The aim of using nanoparticles in drug delivery is to deliver drugs to the target tissue in the shortest possible time and with the best therapeutic effects [23,24].

Also, Polymers are diverse and versatile materials that have gained much attention in the field of biomedicine. These materials are often engineered to create drug-carrying nanoparticles by combining different polymers or with other materials, resulting in desirable properties such as hydrophilicity, biocompatibility, and biodegradability [25,26]. Polymersomes are one type of double-layer polymer nanoparticles that are made from hydrophobic block copolymers. These nanoparticles are capable of encapsulating hydrophilic therapeutic agents such as proteins, anti-cancer drugs, and enzymes in their aqueous core. By incorporating polyethylene glycol (PEG) into their structure, polymersomes can release drugs slowly over a long period when released into the bloodstream. Researchers are particularly interested in using polymersomes for targeted drug delivery to hypoxic tumor sites [27].

Another type of polymer that has attracted attention in drug delivery is amphiphilic block copolymers. These polymers are created by polymerizing multiple types of monomers, typically a hydrophobic and a hydrophilic one. The resulting molecule is formed by interconnecting the aqueous and non-aqueous solvent parts. When used for drug delivery, amphiphilic block copolymers consist of biocompatible and biodegradable hydrophobic polymer blocks such as polyesters connected to a hydrophilic, biocompatible polymer block [28].

The release rate of a drug can be affected by its hydrophilicity, as more hydrophilic drugs are released faster due to water absorption. Biodegradable polymer nanocarriers can be used to control the release of drugs, with permeation being the mechanism that guides the process. The concentration gradient of the drug on the polymer matrix is the driving force behind drug permeation into the environment.

Factors that can affect drug permeability in polymersomes include the chemical composition of the copolymer, the thickness of the polymersome membrane, the permeability coefficient of the drug in the segmental copolymer, the hydrophilic and hydrophobic nature of the drug and charged molecules, and the uncharged nature of the drug molecule. Polyethylene glycol is often used as the hydrophilic part of two-part copolymers, as it increases solubility in aqueous medium. Polycaprolactone, poly(lactide), or poly(butadiene) are used as hydrophobic parts. By changing the percentage of each component in the final copolymer, the drug release rate can be adjusted [29]. Nitroaromatics and azo derivatives are often attached to amphiphilic copolymers to form hypoxia-responsive nano drug carriers through self-assembly [30,31]. These groups are sensitive to oxygen deficiency, and the physical and chemical changes of the drug carriers lead to the rapid release of the drug [7,32,33]. Hypoxia-responsive (HR) self-assembled amphiphilic polymeric nanoparticles exhibit high thermodynamic stability against oxygen deficiency and are expected to preferentially accumulate at the tumor site through enhanced permeation and persistence to rapidly release the drug [34].

Thambi et al. prepared hypoxia-responsive carboxymethyl dextran nanoparticles (CMD NPs) for the facile release of selective hydrophobic drugs in hypoxic tumor tissue. CMD was chosen as a drug carrier due to favorable properties such as hydrophilicity, water solubility, biocompatibility, biodegradability, and the ability to protect the drug from proteolytic absorption [8]. In another study, Danafara used mPEG-PCL copolymer to load the hydrophilic drug anapril maleate. The results obtained from TEM showed that the size of the obtained vesicles was about 80 nm and the encapsulation efficiency was 85% [35]. Ghorbanizamani et al. used PEO-PCL polymersome based on poly(ethylene oxide) and poly(caprolactone) loaded with glutathione (GSH) as a molecule for pH-responsive drug release for cancer inhibition and protein modification. The results showed that nanocomposites, apart from being non-toxic and capable of encapsulating and releasing GSH in response to acidic conditions, do not prevent the healing effects of its content on cancer cells and protein changes. This shows that polymer nanocarriers can be the basis of smart biomedical applications and theories in the future [36]. The goal of this article is to synthesize a copolymer of methoxy polyethylene glycol-azobenzene polyethylene succinate and evaluate its characteristics for use in drug delivery.

MATERIALS AND METHOD

Succinic anhydride, Ethylene glycol, and the required solvents, including Chloroform, N-hexane, Tetrahydrofuran (THF), Dimethylformamide (DMF), and Thionyl chloride (SOCl_2), were obtained from Merck, Germany. The binder of Azobenzene-4, 4-dicarboxylic acid was a product of Alfa Aesar, USA. Also, Polyethylene glycol monomethyl ether (m-PEG, 550 g/mol) was manufactured by

Sigma Aldrich, USA. As a part of this experiment, several devices were used. These include a universal centrifuge device manufactured by Raymand T.N.Co, Iran; a vacuum oven device manufactured by SHELDON LAB, an American company; a rotating evaporator (rotary) device manufactured by DRAGON LAB, China; and a stirring device magnet made by IKA, Germany.

During the research, the FT-IR spectrum of the synthesized materials was recorded using a Tensor 27 spectrophotometer made in Germany. The ^1H -NMR spectra were calculated using a 400 MHz Bruker NMR spectrometer.

II-1) Synthesis of polyethylene succinate polyester

To synthesize the desired polyester, 2 g of succinic acid was mixed with 1.2 g of ethylene glycol. The resulting product was heated, and a prepolymer with low molecular weight was formed. The water formed in the solution was separated using the Dean-Stark apparatus. Some THF solvent was added, and after dissolving the mixture, a small amount of n-hexane was added to form a relatively pasty deposit. The solvent was removed from the deposit in the oven, and a white powder was obtained. Finally, the precipitate was dried to obtain succinic polyethylene.

II-2) Chlorination of azobenzene-4, 4-dicarboxylic acid binder

To chlorinate the desired binder, 50 mg of azobenzene-4, 4-dicarboxylic acid was stirred with specific amounts of DMF and SOCl_2 for 72 h under ozone gas on a magnetic stirrer. After the synthesized substance was removed by rotary, the final product was obtained as an orange solid.

II-3) Synthesis of methoxy polyethylene glycol-azobenzene

The copolymer nanoparticles of methoxy polyethylene glycol-azobenzene polyethylene succinate were synthesized in one step and by a one-pot method. The process involved mixing the synthesized polyester with a few milligrams of polyethylene glycol mono-methyl ether and the above-chlorinated binder in THF. The mixture was stirred for 96 h on a magnetic stirrer, following which the reaction solvent was removed using a rotary evaporator. The final product was obtained as an orange solid.

RESULTS

III.1) Synthesis of methoxy polyethylene glycol-azobenzene polyethylene succinate polymer nanoparticles

The reaction mechanism of the synthesis of methoxy polyethylene glycol-azobenzene polyethylene succinate polymer nanoparticles is shown in Fig. 1.

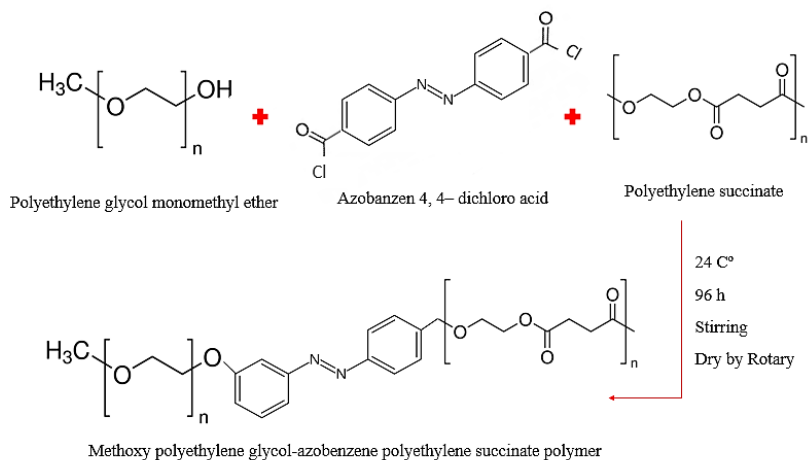


Fig.1. Synthesis mechanism of methoxy polyethylene glycol-azobenzene polyethylene succinate polymer nanoparticles

III.2) FT-IR spectrum of polyethylene succinate polyester

Fig. 2 shows the FT-IR spectrum of the obtained polyester.

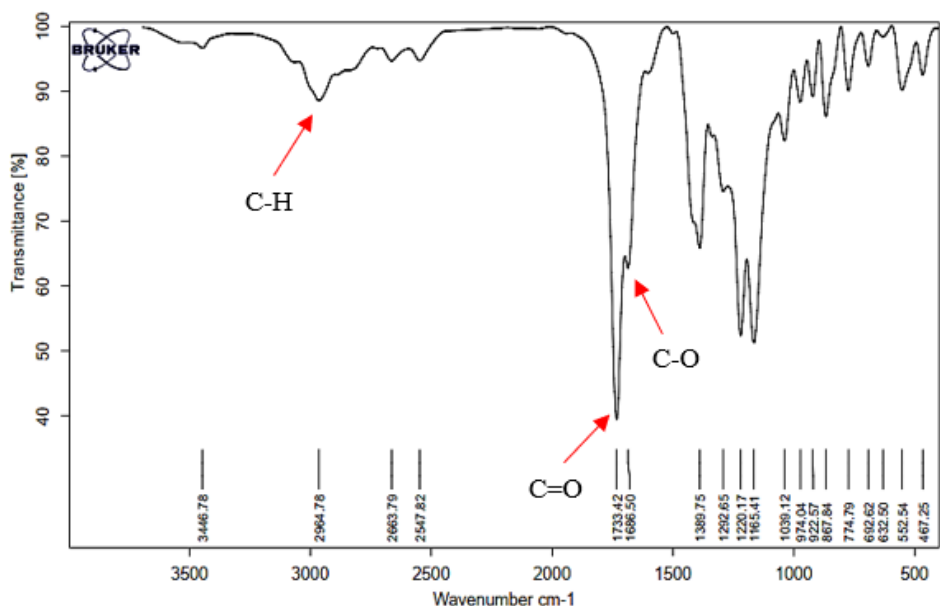


Fig. 2. FT-IR spectrum of polyethylene succinate polyester

III.3) FT-IR spectrum of methoxy polyethylene glycol-azobenzene polyethylene succinate polymer nanoparticles

After washing and purification, the FT-IR spectrum of methoxy polyethylene glycol-azobenzene polyethylene succinate polymer nanoparticles, which was synthesized by the one-pot method, was obtained as shown in Fig. 3.

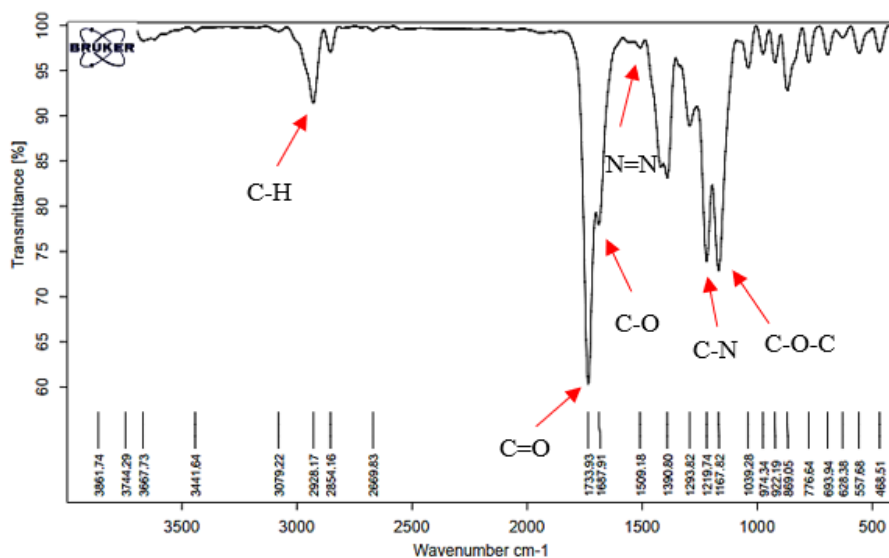


Fig.3. FT-IR spectrum of methoxy polyethylene glycol-azobenzene polyethylene succinate polymer nanoparticles

III.4) ¹H-NMR spectrum of methoxy polyethylene glycol-azobenzene polyethylene succinate polymer nanoparticles

¹H-NMR spectra of this compound in DMSO-d₆ solvent is shown in Fig. 4.

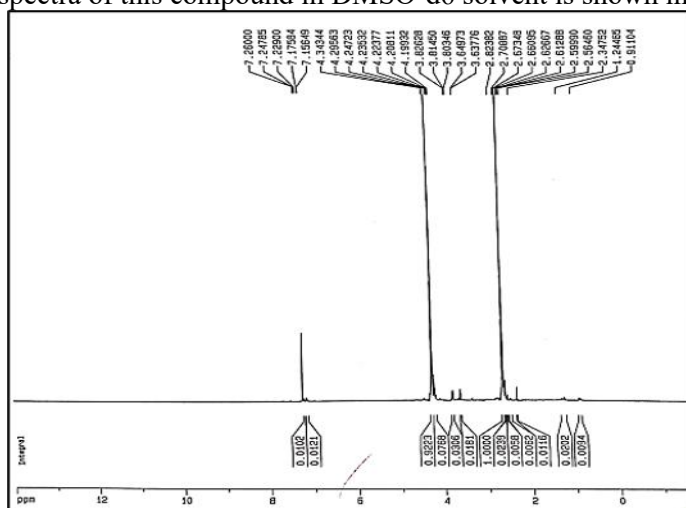


Fig.4. ¹H-NMR spectra of methoxy polyethylene glycol-azobenzene polyethylene succinate polymer nanoparticles

DISCUSSION

According to Fig. 2, The FT-IR spectrum of polyethylene succinate polyester reveals some important peaks. The peak at 3440 cm^{-1} wavelength shows O-H stretching vibrations. The peak at 2964 cm^{-1} wavelength corresponds to the stretching vibrations of the C-H group. Additionally, the peak observed at 1389 cm^{-1} wavelength is related to the C-H stretching vibrations of the methylene group. The ester C=O bond stretching vibrations are observed in the area of 1733 cm^{-1} . Moreover, the peak at 1686 cm^{-1} wavelength is related to the stretching vibrations of the C-O bond of the COOH group.

Based on Fig. 3, it can be inferred that the FT-IR spectrum of methoxy polyethylene glycol-azobenzene polyethylene succinate polymer nanoparticles shows O-H stretching vibrations at a wavelength of 3440 cm^{-1} . The peak at 2964 cm^{-1} corresponds to the stretching vibrations of the C-H group, while the peak at 1389 cm^{-1} is related to the C-H stretching vibrations of the methylene group. The 1733 cm^{-1} region shows the stretching vibrations of the ester C=O bond. The peak at 1686 cm^{-1} is related to the stretching vibrations of the C-O bond of the COOH group, which is associated with the polyester part of the synthesized polymer. Furthermore, the peak at 1167 cm^{-1} wavelength, which is related to C-O-C bond stretching vibration, and the peaks within the range of 2854 and 2928 cm^{-1} are related to C-H vibrations, indicating the presence of methoxy polyethylene glycol in the polymer. The presence of peaks corresponding to N=N vibrations at 1509 cm^{-1} and C-N stretching vibrations at 1220 cm^{-1} confirms the existence of azobenzene in polymer nanoparticles.

The compound was analyzed using the ^1H -NMR spectra in DMSO- d_6 solvent, as shown in Fig. 4. The results indicate that there is a seven-fold peak at 2.67 ppm with a coupling constant of 3.09 Hz, which is associated with the ester bond of methoxy polyethylene glycol-azobenzene polyethylene succinate polymer nanoparticles ($-\text{COO}-\text{CH}_2-\text{CH}_2-\text{COOH}$). Additionally, the methylene group of polymer nanoparticles shows a double peak at 3.66 ppm, and a triple band with a coupling constant of 0.17 Hz can be observed at 3.83 ppm. Another peak at 4.31 ppm, corresponding to ($-\text{COO}-\text{CH}_2-\text{CH}_2-\text{O}-\text{SA}$) with a coupling constant of 3 Hz, appears as a seven-fold peak.

Finally, a triple peak at 7.24 ppm is related to the CDCl_3 solvent of methoxy polyethylene glycol-azobenzene polyethylene succinate polymer nanoparticles.

CONCLUSION

In this research work, methoxy polyethylene glycol-azobenzene polyethylene succinate polymer nanoparticles were synthesized using the one-pot method and analyzed via FTIR and ¹H-NMR. Results showed successful synthesis of the polymer, which can be used for drug release in hypoxic tumors.

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Somatic Genetic Mosaicism in Human Disease: Molecular Origins, Genomic Detection, and Clinical Interpretation

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ABSTRACT

Somatic genetic mosaicism arises when a post-zygotic genetic alteration generates two or more genetically distinct cell populations within the same individual. Far from being a rare exception, mosaicism is increasingly recognized as a pervasive consequence of development, tissue maintenance, environmental exposure, aging, and clonal selection. Its biological significance depends not only on the molecular class of the variant but also on the developmental time at which it occurs, the lineage in which it is propagated, the fitness advantage or disadvantage conferred to affected cells, and the tissue in which the clone is ultimately represented. These variables create a continuum ranging from clinically silent low-level mosaicism to segmental developmental disorders, focal brain malformations, cancer predisposition, and age-related clonal hematopoiesis. Genomic detection remains technically challenging because pathogenic variants may be absent from peripheral blood, may exist at variant allele fractions below conventional reporting thresholds, or may be obscured by sequencing error and tissue admixture. High-depth next-generation sequencing, unique molecular identifiers, droplet digital PCR, and single-cell whole-genome approaches have substantially improved sensitivity and biological resolution. This chapter reviews the molecular origins and developmental architecture of somatic mosaicism, compares current genomic detection strategies, examines representative disease mechanisms, and discusses implications for clinical interpretation, recurrence-risk assessment, and precision medicine. Particular emphasis is placed on the principle that tissue distribution and clonal architecture are integral components of genotype-phenotype interpretation in mosaic disease.

Keywords – somatic mosaicism; post-zygotic variation; variant allele fraction; deep sequencing; single-cell genomics; clonal haematopoiesis; genetic counselling

INTRODUCTION

The traditional germline-centred model of human genetics assumes that nearly all nucleated cells of an individual carry the same inherited genome. Modern sequencing has shown that this approximation is incomplete. Every mitotic division creates an opportunity for replication error, DNA damage, imperfect repair, chromosome mis-segregation, or structural rearrangement. When such an event occurs after fertilisation and is transmitted to daughter cells, it generates a genetically distinct cellular lineage. The coexistence of genetically different cell populations derived from a single zygote is termed somatic genetic mosaicism (Biesecker and Spinner, 2013:307-320; Acuña-

Hidalgo et al., 2015:67-74; Waldvogel et al., 2024:698-714; Yu et al., 2024:548-562).

Mosaicism is not defined by a single variant type. Single-nucleotide variants (SNVs), small insertions and deletions (indels), copy-number changes, chromosomal gains or losses, copy-neutral loss of heterozygosity, mobile-element insertions, and complex structural variants can all occur in mosaic form (Biesecker and Spinner, 2013:307-320; Yu et al., 2024:548-562; Malumbres and Villarroya-Beltri, 2024:864-878). These consequences are determined by the timing of development, lineage allocation, mutational mechanism, tissue-specific selection and the functional impact of the altered gene. A variant generated in an early cleavage division may colonize a number of germ layers and a later one may be restricted to a single organ, anatomical segment or microscopic clone. This developmental logic accounts for the strikingly asymmetric or segmental phenotypes that pathogenic mosaic variants can produce, and the negative results of a blood-based genetic test in a clearly genetic disorder (Biesecker and Spinner, 2013:307-320; Acuña-Hidalgo et al., 2015:67-74; Waldvogel et al., 2024:698-714).

Mosaicism has been shown to be more than just a congenital disease by population-scale and single-cell studies. Somatic mutations accumulate with age in normal skin, esophageal epithelium, blood and brain and some of these variants are under positive selection and clonal expansion (Shao et al., 2025:761-774; Pan et al., 2026:2517-2528; Zhou and Walsh, 2026; Martincorena et al., 2015:880-886; Jaiswal and Ebert, 2019:eaa4673). Thus, mosaicism connects developmental genetics, cancer biology, ageing, neurological disease, and precision medicine. The clinical challenge is to determine which mosaic variants are causal, which are markers of clonal history, and which are biologically neutral passengers.

MOLECULAR ORIGINS AND DEVELOPMENTAL ARCHITECTURE OF SOMATIC MOSAICISM

Post-zygotic mutational mechanisms

Postzygotic variants are shaped by the same broad mutational processes that shape germline and cancer genomes but their phenotypic consequences are strongly conditioned by cellular context. Replication errors are of particular relevance in proliferative embryonic and adult stem-cell compartments, whereas oxidative damage, transcription-associated damage, defective DNA repair, and environmental exposures are potential contributors in long-lived or repeatedly renewed tissues (Yu et al., 2024:548-562; Pan et

al., 2026:2517-2528; Ju et al., 2017:714-718; Bae et al., 2018:550-555; Lodato et al., 2015:94-98; Lodato et al., 2018:555-559). Mosaic aneuploidies arise from errors in chromosome segregation, while copy-neutral loss of heterozygosity can result from mitotic recombination or chromosome-level repair. The spectrum of possible mosaic lesions ranges from one nucleotide change to large sections of the chromosomes (Biesecker and Spinner, 2013:307-320; Yu et al., 2024:548-562; Malumbres and Villarroya-Beltri, 2024:864-878).

Table 1: Major classes of somatic mosaic genetic variation and their biological consequences

Variant class	Typical mechanism	Potential consequence	Illustrative context
SNV / small indel	Replication error, DNA damage, imperfect repair	Altered protein function, splice disruption, regulatory change	Developmental disorders, brain mosaicism, clonal haematopoiesis
Copy-number variant	Mitotic recombination, replication stress, structural rearrangement	Dosage imbalance; segmental deletion or duplication	Developmental mosaicism and tissue-restricted disease
Aneuploidy	Mitotic chromosome mis-segregation	Cellular dosage imbalance, developmental impairment, clonal selection	Mosaic aneuploidy and chromosomal instability
Copy-neutral loss of heterozygosity	Mitotic recombination or chromosome repair	Unmasking of recessive allele; duplication of a selected allele	Age-related clonal mosaicism and haematopoietic selection
Complex structural change	DNA double-strand break and rearrangement	Gene disruption, fusion, altered chromatin topology	Rare developmental mosaicism and neoplastic evolution

Source: Prepared by the author based on Biesecker and Spinner (2013), Acuña-Hidalgo et al. (2015), Malumbres and Villarroya-Beltri (2024), and Loh et al. (2018).

Developmental timing, lineage allocation, and variant allele fraction

The timing of a postzygotic event is one of the most important factors in determining mosaic architecture. An early mutation in the embryo can generate a large clone contributing to several tissues, whereas a mutation after lineage restriction can be detected in a particular tissue or anatomical region (Waldvogel et al., 2024:698-714; Ju et al., 2017:714-718; Bae et al., 2018:550-555). Human embryonic lineage studies using somatic mutations as

naturally occurring barcodes have demonstrated that the first embryonic cell divisions can contribute asymmetrically to later tissues (Ju et al., 2017:714-718). Similarly, single-cell genomic studies of developing brain have shown that mutational rate and mutational mechanism change across developmental stages (Bae et al., 2018:550-555).

Variant allele fraction (VAF) is therefore a biological measurement rather than merely a technical output. In an ideal diploid sample composed of heterozygous mutant and wild-type cells, a VAF of 10% is roughly compatible with the variant being present in about 20% of sampled cells, but this approximation can fail because of local copy-number state, cell-type admixture, allelic imbalance, selection, sequencing bias, and differences in ploidy. Most importantly, VAF measured in one tissue cannot be assumed to represent the entire organism. A clinically relevant clone may be enriched in the affected tissue and nearly absent from blood or saliva. The relationship among mutation timing, lineage restriction, tissue distribution, and measurable VAF is summarized schematically in Figure 1.

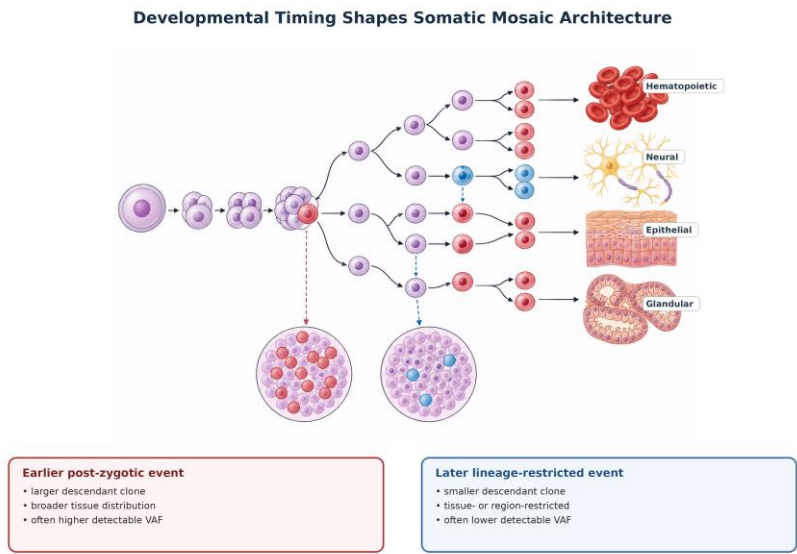


Figure 1: Developmental timing, lineage distribution, and detectable variant allele fraction in somatic mosaicism

Source: Author-generated schematic. Purple cells represent the founding lineage, red cells an early post-zygotic mutant clone, and blue cells a later lineage-restricted mutant clone.

Cellular selection and clonal expansion

Mosaic architecture is not fixed at the moment a mutation occurs. Cell competition and tissue-specific selection can expand or contract a clone over

time. A mutation that confers a proliferative or survival advantage may remain undetectable for years before occupying a substantial fraction of a tissue. Conversely, a deleterious mutation may be progressively depleted. This principle is evident in normal epithelia, where driver-gene mutations can form large clones without histological malignancy, and in blood, where mutations in genes such as DNMT3A, TET2, ASXL1, and spliceosome components can support clonal expansion (Martincorena et al., 2015:880-886; Martincorena et al., 2018:911-917; Lee-Six et al., 2018:473-478; Jaiswal and Ebert, 2019:eaan4673). Selection therefore modifies the relationship between chronological mutation age and present-day VAF.

GENOMIC DETECTION OF LOW-LEVEL MOSAICISM

Tissue selection as a component of the genetic test

The first analytical decision in suspected mosaic disease is often not which sequencing platform to use, but which tissue to test. Peripheral blood is convenient and appropriate for many constitutional disorders; however, it can be poorly informative for variants confined to skin, brain, vascular malformations, overgrown tissue, or other lineage-restricted lesions. Buccal epithelium, saliva, hair follicles, skin biopsy, surgically resected tissue, urinary epithelial cells, or cultured fibroblasts may provide higher diagnostic yield depending on phenotype. In tuberous sclerosis complex, for example, low-level mosaic pathogenic variants can vary substantially among tissues, and affected skin lesions may outperform blood for detection (Ye et al., 2022:1956-1969).

High-depth bulk sequencing and molecular error suppression

Conventional exome or genome sequencing at standard depth can miss low-VAF variants because the number of alternate reads approaches the background error rate. Targeted deep sequencing improves sampling, but depth alone does not remove polymerase and sequencing errors. Unique molecular identifiers (UMIs), duplex methods, replicated libraries, and orthogonal validation can differentiate true low frequency variants from recurrent technical noise. Computational methods designed specifically for mosaic calling, such as MosaicForecast, also use read-level features and phasing information rather than applying germline genotype assumptions to subclonal alleles (Dou et al., 2020:314-319).

Droplet digital PCR and targeted quantification

Droplet digital PCR (ddPCR) partitions a DNA sample into thousands of reactions and provides highly sensitive allele quantification when the candidate variant is already known (Hindson et al., 2011:8604-8610). It is particularly useful for confirming low-VAF calls, comparing variant fractions across tissues, and evaluating parental mosaicism. Its limitation is that it is a targeted method: it cannot discover unknown variants and each assay requires careful design and validation.

Single-cell DNA sequencing

Single-cell DNA sequencing can resolve clonal structure directly and distinguish variants shared by a lineage from private mutations present in individual cells. Early single-cell whole-genome amplification studies established the feasibility of detecting SNVs and copy-number variants at cellular resolution (Zong et al., 2012:1622-1626). Subsequent methods, including LIANTI, improved amplification linearity and variant detection (Chen et al., 2017:189-194). Single-cell analysis is nevertheless technically demanding because allelic dropout, amplification artefacts, uneven coverage, and cost can compromise sensitivity. Dedicated callers such as Monovar model single-cell specific error structures (Zafar et al., 2016:505-507). Single cell genomics is increasingly employed for lineage reconstruction of human development and somatic mutation histories (Shao et al., 2025:761-774).

Table 2: Comparison of major approaches for detecting somatic mosaic variants

Method	Strength	Principal limitation	Best use
Standard exome/genome sequencing	Genome-wide or exome-wide discovery	Limited sensitivity for low VAF; germline-oriented calling assumptions	Moderate/high-VAF mosaicism; broad discovery
Targeted ultra-deep sequencing	High depth at selected genes or loci	Restricted genomic scope; sequencing artefacts at very low VAF	Known disease genes; affected-tissue testing
UMI/error-corrected sequencing	Improved discrimination of true low-frequency alleles from technical errors	Higher library complexity and cost	Very low VAF detection and clonal monitoring
ddPCR	Sensitive quantitative validation of a known allele	Not a discovery method	Cross-tissue quantification; parental mosaicism

Method	Strength	Principal limitation	Best use
Single-cell DNA sequencing	Direct cellular resolution and lineage reconstruction	Allelic dropout, amplification bias, cost	Clonal architecture and cell-specific mutation burden
Mosaic-specific bioinformatics	Uses VAF, read features, phasing and context to improve calling	Performance depends on depth, platform and training assumptions	Bulk WGS/WES mosaic discovery

Source: Prepared by the author based on Shao et al. (2025), Dou et al. (2020), Zafar et al. (2016), Hindson et al. (2011), Zong et al. (2012), and Chen et al. (2017).

MOSAICISM IN DEVELOPMENTAL AND SEGMENTAL DISORDERS

PI3K-AKT-mTOR pathway disorders

The PI3K-AKT-mTOR pathway provides one of the clearest demonstrations of how post-zygotic mutation timing shapes phenotype. Somatic activating variants in PIK3CA, AKT3, and MTOR can produce localized or segmental overgrowth, megalencephaly, hemimegalencephaly, and focal cortical dysplasia. Brain-tissue sequencing identified mosaic PI3K-AKT3-mTOR pathway variants in hemimegalencephaly, with mutant alleles enriched in affected cerebral tissue (Lee et al., 2012:941-945). Related germline and post-zygotic variants in AKT3, PIK3R2, and PIK3CA define overlapping megalencephaly syndromes (Rivière et al., 2012:934-940), while mosaic PIK3CA variants are a major mechanism in CLOVES and the broader PIK3CA-related overgrowth spectrum (Kurek et al., 2012:1108-1115).

These disorders illustrate why variant classification cannot be separated from anatomical distribution. The same activated signalling pathway can yield distinct clinical syndromes according to developmental timing, clone size, and affected lineage. A mutation that would be incompatible with normal development in the germline may be viable in mosaic form because only a subset of cells carries the alteration.

AKT1, GNAQ, and RAS/MAPK mosaic disorders

Proteus syndrome is a paradigmatic mosaic overgrowth disorder caused by the recurrent activating AKT1 p.Glu17Lys variant, which is detectable in affected tissues but may be absent or present at very low levels in blood (Lindhurst et al., 2011:611-619). Sturge-Weber syndrome and many port-wine stains are associated with somatic activating GNAQ variants in affected vascular tissue (Shirley et al., 2013:1971-1979). Similarly, post-zygotic HRAS and KRAS variants underlie nevus sebaceous and Schimmelpenning

syndrome (Groesser et al., 2012:783-787). These examples demonstrate a recurring principle: clinically visible segmental lesions can serve as highly informative genomic specimens because they are enriched for the disease-causing clone.

Cerebral cortical malformations and focal epilepsy

The brain is particularly challenging for mosaic genetic diagnosis because the disease-relevant tissue is usually inaccessible unless surgery is clinically indicated. Deep sequencing of resected cerebral tissue has shown that somatic variants can cause focal cortical malformations and epilepsy even when blood testing is negative (Jamuar et al., 2014:733-743; Lim et al., 2015:395-400). In focal cortical dysplasia type II, brain-restricted MTOR variants provide a direct mechanistic link between low-level mosaicism, abnormal mTOR signalling, cortical dysplasia, and intractable epilepsy (Lim et al., 2015:395-400). These findings have shifted diagnostic strategies toward high-depth sequencing of resected tissue and toward analysis methods calibrated for low VAF.

Table 3: Representative disorders in which somatic mosaicism is central to genetic interpretation

Clinical context	Representative gene/pathway	Informative tissue principle	References
Hemimegalencephaly / megalencephaly spectrum	PIK3CA, AKT3, PIK3R2, MTOR	Affected brain or overgrown tissue can contain substantially higher VAF than blood	Lee et al. (2012); Rivière et al. (2012)
CLOVES / PIK3CA-related overgrowth	PIK3CA	Biopsy from affected overgrown or vascular tissue is often preferred	Kurek et al. (2012)
Proteus syndrome	AKT1	Segmental affected tissue enriches the pathogenic clone	Lindhurst et al. (2011)
Sturge-Weber syndrome / port-wine stain	GNAQ	Affected vascular/skin tissue is more informative than uninvolved tissue	Shirley et al. (2013)
Nevus sebaceous / Schimmelpenning syndrome	HRAS, KRAS	Lesional skin is the primary source for variant detection	Groesser et al. (2012)
Focal cortical dysplasia / cortical malformation	MTOR pathway and other neurodevelopmental genes	Resected brain tissue may be required for diagnosis	Jamuar et al. (2014); Lim et al. (2015)

Clinical context	Representative gene/pathway	Informative tissue principle	References
Tuberous sclerosis complex with negative routine testing	TSC1, TSC2	Multiple tissues and deep sequencing can reveal low-level mosaicism	Ye et al. (2022)

Source: Prepared by the author based on Lee et al. (2012), Rivière et al. (2012), Kurek et al. (2012), Lindhurst et al. (2011), Shirley et al. (2013), Groesser et al. (2012), Jamuar et al. (2014), Lim et al. (2015), and Ye et al. (2022).

SOMATIC MOSAICISM IN THE HUMAN BRAIN ACROSS THE LIFESPAN

Congenital malformations are not the only thing somatic mosaicism in the nervous system is found in. Single neuron sequencing has shown that individual neurons contain multiple somatic SNVs and that these variants can be used to reconstruct developmental lineage relationships (Lodato et al., 2015:94-98). In post-mitotic neurons, the mutational burden increases with age, which indicates that DNA damage and repair are drivers of genomic diversification even in cells that do not divide anymore (Lodato et al., 2018:555-559). Recent syntheses of developmental mosaicism, age-related mutation accumulation, cell-type-specific selection, and neurodegenerative disease in a single framework have presented the brain as a mosaic of related but non-identical genomes (Zhou and Walsh, 2026; Poduri et al., 2013:1237758).

The clinical interpretation of brain mosaicism requires caution. A somatic variant detected in neural tissue is not automatically pathogenic, because every brain contains private mutations. Causality is strengthened when the variant is enriched in anatomically affected tissue, disrupts a biologically plausible pathway, is supported by functional evidence, and correlates with lesion distribution. This is particularly relevant in neurodegenerative disease, where the mutational load may reflect both causal and downstream processes and so the distinction between a pathogenic clone and background age-related mutagenesis becomes important (Pan et al., 2026:2517-2528; Zhou and Walsh, 2026; Lodato et al., 2018:555-559).

AGE-RELATED CLONAL MOSAICISM IN NORMAL TISSUES

Epithelial clonal expansion

Genomic studies of histologically normal tissues have revealed widespread clonal expansion with age. While overt malignancy is absent, sun-

exposed skin contains many positively selected clones with mutations in cancer-associated genes (Martincorena et al., 2015:880-886). Similarly, there is a gradual replacement of mutant clones, including clones with NOTCH1 and TP53 alterations, in normal oesophageal epithelium (Martincorena et al., 2018:911-917; Yokoyama et al., 2019:312-317). These observations challenge a dichotomy of normal and neoplastic tissue: driver mutations may be prevalent in normal epithelia, while malignant transformation requires further evolutionary steps, ecological interactions and selective pressures.

Clonal haematopoiesis

The haematopoietic system is uniquely accessible for studying age-related mosaicism because blood can be sampled repeatedly. Large sequencing studies established that apparently healthy individuals can harbour expanded blood-cell clones carrying somatic mutations in genes recurrently altered in myeloid malignancies (Jaiswal et al., 2014:2488-2498; Genovese et al., 2014:2477-2487). This state, commonly termed clonal haematopoiesis of indeterminate potential when defined by recognized driver mutations in the appropriate clinical context, increases with age and is associated with elevated risk of haematologic malignancy and adverse outcomes (Jaiswal et al., 2014:2488-2498; Genovese et al., 2014:2477-2487; Jaiswal et al., 2017:111-121; Fabre et al., 2022:335-342; Mitchell et al., 2022:343-350; Jaiswal and Ebert, 2019:eaan4673).

Clonal haematopoiesis also illustrates the importance of non-malignant consequences of somatic variation. Associations with atherosclerotic cardiovascular disease suggest that mutant haematopoietic clones can influence systemic physiology through altered inflammatory signalling rather than solely through progression to leukaemia (Jaiswal et al., 2017:111-121; Jaiswal and Ebert, 2019:eaan4673). Mosaic chromosomal alterations represent another form of clonal blood-cell variation and can be detected at population scale (Loh et al., 2018:350-355). Longitudinal studies demonstrate that the growth rate of clones are highly variable according to the driver gene, and can be stable for many years, while sequencing of colonies derived from single cells demonstrates age-dependent changes in the architecture of human hematopoiesis (Fabre et al., 2022:335-342; Mitchell et al., 2022:343-350).

PARENTAL MOSAICISM AND RECURRENCE-RISK ASSESSMENT

Mosaicism has direct consequences for reproductive counselling. A variant classified as de novo in a child may actually be present at low level in

one parent, and the recurrence risk can therefore exceed the generic estimate traditionally assigned to apparently *de novo* disease. Deep sequencing of parents with children affected by neurodevelopmental disorders has demonstrated low-level parental mosaicism that would be missed by routine sequencing (Lecoquierre et al., 2024:5289). Sperm sequencing further shows that paternal gonadal mosaicism can sometimes be quantified directly and may refine recurrence-risk assessment for selected *de novo* variants (Breuss et al., 2020:143-150).

The relationship between somatic VAF and gonadal involvement is nevertheless imperfect. Blood, buccal cells, saliva, and sperm represent different developmental lineages, and absence of a variant in one tissue cannot prove absence from the germline. Counselling should therefore integrate the molecular mechanism, parental phenotype, tissues tested, analytical sensitivity, and developmental plausibility rather than converting a single negative blood test into a zero recurrence risk.

CLINICAL INTERPRETATION AND REPORTING OF MOSAIC VARIANTS

Clinical laboratories face a different interpretive problem for mosaic variants than for conventional heterozygous germline variants. Low VAF may reflect true mosaicism, contamination, technical artefact, clonal haematopoiesis, age-related background mutation, or a copy-number state that changes expected allelic balance. Confirmation should therefore consider read orientation, mapping quality, local sequence context, independent DNA extraction, orthogonal methodology, and testing of additional tissues where clinically justified.

The disease mechanism must also be interpreted in a tissue-aware manner. A pathogenic activating variant in a growth-signalling gene detected at low VAF in an affected lesion can be highly informative even if absent from blood. Conversely, a low-VAF variant in peripheral blood from an older adult may represent clonal haematopoiesis rather than constitutional mosaicism. This distinction is particularly important when blood sequencing is performed for hereditary cancer or cardiovascular indications, because age-related somatic variants can be mistaken for germline findings unless VAF, gene context, age, and secondary tissues are considered (Jaiswal et al., 2014:2488-2498; Genovese et al., 2014:2477-2487; Jaiswal et al., 2017:111-121; Fabre et al., 2022:335-342; Mitchell et al., 2022:343-350; Jaiswal and Ebert, 2019:eaan4673).

Laboratory reporting thresholds require similar nuance. A fixed 10% VAF cutoff can improve specificity but risks excluding clinically relevant low-level mosaicism. Deep sequencing studies of tuberous sclerosis complex demonstrate that pathogenic variants may be below conventional thresholds and that tissue selection makes a material difference to diagnostic yield (Ye et al., 2022:1956-1969). Reporting policies must thus be validated for the assay and clinical context for which they are intended, and that include clear statements about analytical sensitivity and the tissues tested.

FUTURE DIRECTIONS: FROM MOSAIC VARIANT DETECTION TO SPATIAL AND SINGLE-CELL GENOMICS

The next phase of mosaicism research is moving from variant detection toward spatially and functionally resolved clonal biology. Single-cell DNA sequencing can define lineage relationships, but integration with single-cell transcriptomics, chromatin accessibility, proteomics, and spatial profiling will be required to determine how a mosaic genotype alters cellular state within intact tissue. Such integration is particularly relevant when the same pathway mutation produces different phenotypes in different organs or when only a subset of mutant cells drives pathology.

Long-read and single-molecule sequencing may improve detection of structural variants, repeat-associated events, haplotype structure, and complex mosaic alleles that are incompletely resolved by short reads. Error-corrected sequencing will continue to extend detection toward lower VAF, but increasing analytical sensitivity must be matched by biological specificity: detecting ever smaller clones is useful only when their tissue origin, functional consequence, and clinical relevance can be interpreted. The key translational objective is therefore not maximal mutation detection alone, but accurate mapping of clone, tissue, time, and phenotype.

Mosaicism also creates opportunities for targeted therapy. Several developmental mosaic disorders involve hyperactive signalling pathways for which pharmacologic inhibitors already exist or are under clinical investigation. A molecular diagnosis can therefore influence treatment selection as well as prognosis. However, the therapeutic target is a cell population rather than a constitutional genotype, and response may depend on clone distribution, tissue penetration, developmental stage, and the relative fitness of mutant and wild-type cells.

CONCLUSION

Somatic genetic mosaicism is a fundamental source of within-individual genomic diversity. Its clinical significance emerges from the interaction of mutational mechanism, developmental timing, lineage allocation, tissue-specific selection, and clonal expansion. These principles explain why the same gene can produce different phenotypes in germline and mosaic states, why variant allele fraction varies across tissues, and why peripheral blood can be an inadequate specimen for some disorders. High-depth sequencing, molecular error suppression, ddPCR, mosaic-aware bioinformatics, and single-cell genomics have transformed the ability to detect and reconstruct mosaic clones, but analytical sensitivity must be accompanied by tissue-aware interpretation. In medical genetics, the most informative question is increasingly not only whether a pathogenic variant is present, but when it arose, which cells carry it, how the clone behaves, and whether that distribution is sufficient to explain the phenotype. Integrating these dimensions will strengthen diagnosis, recurrence-risk assessment, disease classification, and future precision therapies.

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