Intratumoral heterogeneity: Biological basis, clinical impact and the emerging theory of tumor plasticity (Review)

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Received September 3, 2026; Accepted October 2, 2026

DOI: 10.3892/mco.2026.2983

Abstract. Intratumoral heterogeneity (ITH) is a central feature of cancer biology and a major obstacle to effective long-term treatment; it encompasses the presence of diverse cellular subpopulations within a single tumor or across metastatic sites, differing at the genetic, epigenetic, transcriptional, metabolic and phenotypic levels. This diversity fuels tumor evolution, promotes immune evasion, facilitates metastasis and drives resistance to therapy. Recent technological advances, including next-generation sequencing, single-cell multi-omics, spatial transcriptomics and liquid biopsies, have revealed tumors as dynamic and adaptive ecosystems shaped by clonal competition and interactions with the tumor microenvironment. In addition to clonal evolution, tumor plasticity has emerged as a key mechanism, allowing cancer cells to reversibly shift between functional states (such as stem-like, proliferative, invasive, mesenchymal and drug-tolerant) without requiring new genetic mutations. This adaptability notably contributes to treatment failure and disease recurrence. The biological basis of ITH involves genomic instability, epigenetic alterations, cancer stem cells and microenvironmental influences. Both spatial and temporal heterogeneity further complicate diagnosis and treatment, as tumor composition evolves and varies across regions. Current evidence suggests that integrating comprehensive molecular profiling with longitudinal monitoring could enable adaptive, personalized therapeutic strategies.

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Key words: intratumoral heterogeneity, tumor plasticity, cancer evolution, clonal selection, cancer stem cells, drug resistance, precision oncology, tumor microenvironment

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1. Introduction

Cancer has traditionally been classified according to tissue origin and histopathological appearance. However, increasing molecular evidence has demonstrated that tumors with similar morphology often display profound biological differences, leading to variable clinical behavior and treatment responses (1). One of the most important contributors to this complexity is intratumoral heterogeneity (ITH), defined as the presence of multiple distinct cellular populations within the same tumor or between primary and metastatic lesions in one patient (2,3).

ITH occurs at several levels, including genomic mutations, copy number variation, epigenetic regulation, transcriptional states, metabolic programs and interactions with stromal and immune cells (4). This diversity enables subclonal populations to survive selective pressures such as hypoxia, immune surveillance, chemotherapy, radiotherapy and targeted therapy. As a consequence, ITH has been strongly associated with disease progression, metastasis, relapse and poor survival outcomes (5).

Historically, tumor evolution has been explained by Darwinian clonal selection, where genetically advantageous subclones progressively dominate the tumor population (Fig. 1A) (6). While this model remains fundamental, previous studies suggest that genetic mutation alone does not fully explain rapid adaptation to treatment (7-9). Numerous tumors

can switch cellular identity reversibly through transcriptional reprogramming, epigenetic remodeling and microenvironmental signaling. This phenomenon has been conceptualized as tumor plasticity (7).

Tumor plasticity allows malignant cells to move between differentiated and stem-like states, epithelial and mesenchymal phenotypes, proliferative and quiescent programs or sensitive and drug-tolerant persister states (Fig. 1B). Such transitions may occur without acquisition of new mutations and can arise within days of therapy exposure (8). Therefore, understanding ITH now requires integration of both clonal evolution and non-genetic adaptation. The present literature review aims to summarize the biological basis of ITH, current evidence regarding its clinical significance, modern methods for its assessment and the emerging role of plasticity theory in cancer progression and therapeutic resistance.

2. Literature search strategy

The present literature review was conducted using a structured narrative approach. Electronic databases including PubMed (https://pubmed.ncbi.nlm.nih.gov/), Scopus (https://www.scopus.com/), Web of Science (https://www.webofscience.com/) and Google Scholar (https://scholar.google.com/) were searched for relevant English-language studies published between January 2010 and March 2026. Search terms included: 'Intratumoral heterogeneity', 'tumor heterogeneity', 'cancer plasticity', 'clonal evolution', 'single-cell sequencing', 'drug resistance in cancer' and 'tumor microenvironment'.

Primary studies, systematic reviews, translational studies and landmark conceptual papers were prioritized. Studies involving solid tumors and hematological malignancies were considered when relevant to heterogeneity mechanisms. Duplicate records, conference abstracts lacking adequate data and non-English publications were excluded. Earlier landmark studies were also included when required for biological context.

3. Biological basis of ITH

ITH emerges through the interaction of genetic instability and environmental selection. Chromosomal instability, defective DNA repair pathways, replication stress and mutagen exposure generate continuous genomic diversification (10). Common mechanisms include point mutations, copy number alterations, chromothripsis, gene amplification and structural rearrangements. Cancer cells carrying advantageous mutations may gain proliferative capacity, metastatic potential or treatment resistance. However, not all diversity is genetic. Epigenetic changes such as DNA methylation, histone modification and chromatin remodeling can create persistent yet reversible phenotypic states (11). Examples include DNA methylation differences among colorectal cancer lineages (12), histone demethylase-dependent drug tolerance in cancer cell lines (8) and a poised chromatin configuration at the ZEB1 promoter enabling breast cancer cell plasticity (13).

4. Spatial and temporal heterogeneity

Spatial heterogeneity refers to molecular differences between separate regions of the same tumor or between primary and metastatic lesions. Multi-region sequencing studies in renal cell carcinoma (14), lung cancer (15) and glioblastoma (16) have shown that a number of driver mutations are region-specific rather than universally shared across all tumor cells.

Temporal heterogeneity refers to changes in tumor composition over time, particularly under therapeutic pressure. For example, serial biopsies from patients with lung cancer treated with epidermal growth factor receptor (EGFR) inhibitors have demonstrated the emergence of resistance clones, including acquisition of the T790M mutation and activation of alternative signaling pathways (17). Similar temporal evolutionary patterns have been observed in melanoma (18), colorectal cancer (19) and breast cancer (20).

5. Cancer stem cells (CSCs) and hierarchical diversity

The CSC model proposes that a subset of tumor cells possesses self-renewal capacity and can regenerate heterogeneous tumor populations. CSCs are relatively resistant to chemotherapy and radiotherapy due to efficient DNA repair mechanisms, drug efflux pumps, quiescence and anti-apoptotic signaling pathways (21).

Markers such as CD133, CD44, aldehyde dehydrogenase 1 (ALDH1), epithelial cell adhesion molecule (EpCAM) and leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5) have been associated with stem-like cellular populations across multiple malignancies and are frequently used to identify CSCs with enhanced capacities for self-renewal, tumor initiation, metastatic dissemination and therapeutic resistance (21-27). Notably, these markers rarely function in isolation; rather, their co-expression often reflects overlapping biological programs related to stemness, epithelial plasticity and survival signaling (21,28). For example, CD44 is a cell-adhesion receptor that mediates interactions with the extracellular matrix and participates in Wnt/β-catenin signaling (29). CD44 can be co-expressed with LGR5, a well-established Wnt target gene and stem cell marker (particularly in colorectal cancer) (27,30,31). Their concurrent expression has been observed in colorectal cancer specimens, while LGR5/CD44/EpCAM-positive cells showed enhanced clonogenic and tumor-initiating capacity in experimental models (30,31).

Similarly, EpCAM, an epithelial adhesion molecule, is commonly co-expressed with CD44 in epithelial-derived tumors, including colorectal, pancreatic and breast cancer (24,26,32). In this context, EpCAM may reflect epithelial lineage identity, whereas CD44 and ALDH1 are more closely associated with functional stemness and therapeutic resistance. ALDH1, an enzyme involved in aldehyde detoxification and retinoic acid metabolism, has been strongly linked to chemoresistance and cellular survival under stress, and its activity can complement CD44/CD24 phenotyping to enrich for tumorigenic breast cancer cells (25,33). Meanwhile, CD133, one of the most extensively studied CSC markers, is frequently used in glioblastoma and colorectal cancer, where CD133-positive cells have demonstrated enhanced tumor-initiating capacity in xenograft assays (22,23). Table I summarizes commonly used CSC markers, the malignancies in which they have been studied and their reported functional associations.

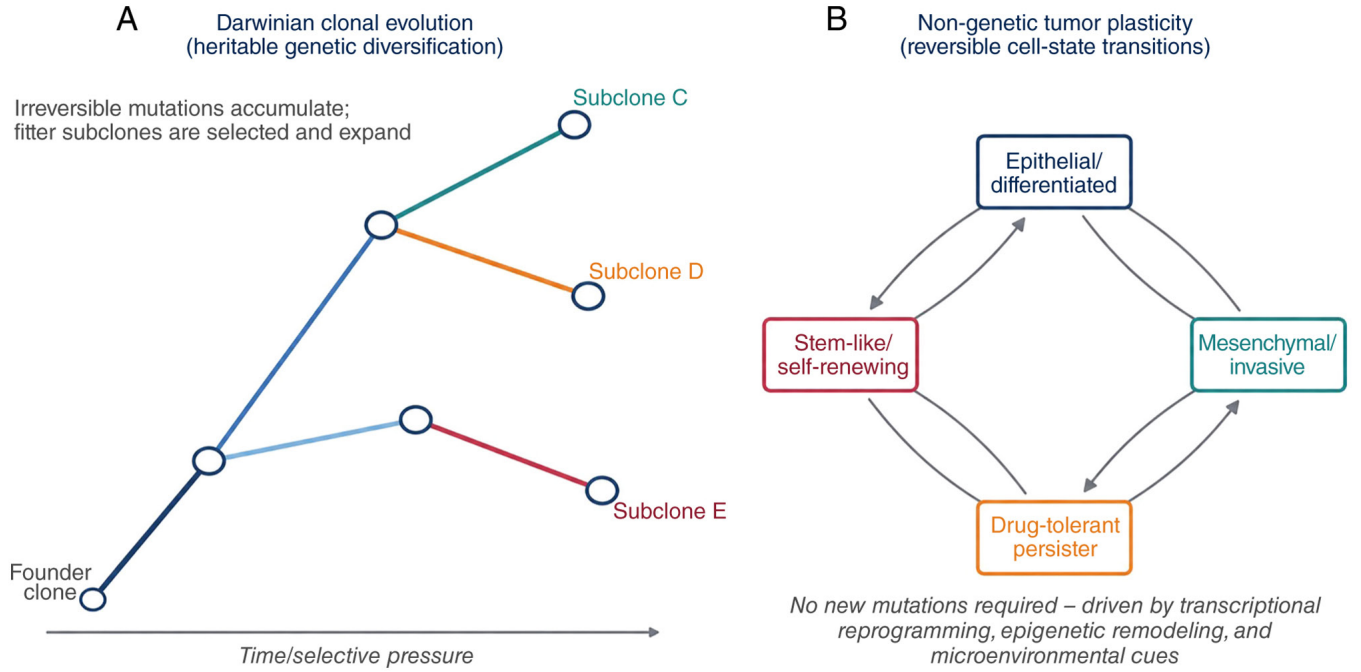


Figure 1. Schematic of two complementary models of intratumoral heterogeneity. (A) Darwinian clonal evolution, in which heritable genetic mutations accumulate irreversibly and fitter subclones are selected and expand over time under therapeutic and microenvironmental pressure. (B) Non-genetic tumor plasticity, in which cancer cells reversibly transition between epithelial/differentiated, mesenchymal/invasive, stem-like/self-renewing and drug-tolerant persister states without acquiring new mutations, driven instead by transcriptional reprogramming, epigenetic remodeling and microenvironmental signaling. Both mechanisms operate in parallel and jointly shape tumor evolution, therapeutic resistance and relapse.

6. Tumor microenvironment as a driver of heterogeneity

Tumors function as complex ecosystems composed of malignant cells, fibroblasts, endothelial cells, extracellular matrix components, macrophages, lymphocytes and various soluble mediators. Within this ecosystem, hypoxia serves as a major driver of heterogeneity by activating hypoxia-inducible factor-dependent pathways that promote angiogenesis, glycolytic metabolism, cancer stemness and epithelial-mesenchymal transition (EMT) (28). For example, stromal hepatocyte growth factor secretion induced resistance to RAF inhibition in melanoma co-culture models and was associated with innate resistance in patient samples (34). In metastatic urothelial cancer, fibroblast-associated TGF-β signaling was linked to T-cell exclusion and lack of response to atezolizumab; combined TGF-β and programmed death-ligand 1 blockade restored T-cell infiltration and tumor regression in a mouse model (35). In colorectal cancer, multiplexed imaging identified distinct immune-cell neighborhoods associated with clinical outcomes, illustrating spatial variation in the local microenvironment (36).

7. Tumor plasticity

Tumor plasticity refers to the capacity of cancer cells to reversibly alter their phenotype in response to intrinsic regulatory programs or environmental stress without necessarily acquiring new genetic mutations. This concept extends beyond the traditional model of clonal evolution by recognizing dynamic cellular state transitions as a major contributor to ITH (37).

Common manifestations of tumor plasticity involve reversible cellular state transitions that facilitate cancer adaptation under environmental and therapeutic pressures. One of the most extensively studied forms is EMT, during which epithelial tumor cells lose polarity and cell-cell adhesion while acquiring migratory and invasive mesenchymal characteristics (38-40). Examples include EMT-associated stem-like properties in cultured mammary epithelial cells (38), epithelial, hybrid and mesenchymal states in mouse skin and mammary tumors (39) and changing epithelial and mesenchymal composition of circulating tumor cells in patients with breast cancer (40). The reverse process, mesenchymal-epithelial transition, may promote metastatic colonization at distant sites (41). In a mouse squamous cell carcinoma model, turning off Twist1 allowed disseminated cells to revert toward an epithelial state and form metastases (41). Tumor cells may also undergo dedifferentiation or acquire stem cell-like properties, allowing differentiated cancer cells to regain self-renewal capacity, therapeutic resistance and tumor-initiating potential. For example, differentiated keratin 20-positive cells replenished the LGR5-positive CSC pool after its ablation in human colorectal cancer organoid-derived tumors (27). These experimental findings support plasticity but do not establish that every differentiated cancer cell can regain stemness.

8. Technologies for characterizing ITH

The past decade has witnessed a technological transformation in how ITH is characterized, moving from bulk, single-specimen assays toward high-resolution platforms capable of resolving heterogeneity at the single-cell and subcellular level. Next-generation sequencing approaches, including whole-exome and whole-genome sequencing, initially enabled detection of the genomic instability and mutational

Table I. Commonly used CSC markers and their reported functional associations[a] across malignancies.

Marker	Malignancies commonly studied	Functional significance	(Refs.)
CD133 (PROM1)	Glioblastoma and colorectal cancer	Widely used tumor-initiating cell marker; $CD133^+$ cells show enhanced tumor-initiating capacity in xenograft assays.	(22,23)
CD44	Colorectal and breast cancer	Cell-adhesion receptor mediating extracellular-matrix interactions; participates in Wnt/β-catenin signaling; co-expression with LGR5 and EpCAM has been associated with enhanced clonogenic and tumor-initiating capacity in colorectal cancer models.	(24,26,29-31)
ALDH1	Breast, pancreatic and colorectal cancer	Aldehyde-detoxifying enzyme involved in retinoic acid metabolism; linked to chemoresistance and survival under stress; ALDH activity can complement CD44/CD24 phenotyping to enrich for tumorigenic breast cancer cells.	(25,33,67)
EpCAM	Colorectal, pancreatic and breast cancer (epithelial-derived tumors)	Epithelial adhesion molecule reflecting epithelial lineage identity; frequently co-expressed with CD44.	(24,26,32)
LGR5	Colorectal cancer	Canonical Wnt target gene and intestinal stem cell marker; co-expression with CD44 and EpCAM has been associated with enhanced clonogenic and tumor-initiating capacity in colorectal cancer models.	(27,30,31)

[a]Markers rarely function in isolation; their co-expression frequently reflects overlapping biological programs related to stemness, epithelial plasticity and survival signaling. CSC, cancer stem cell; ALDH1, aldehyde dehydrogenase 1; LGR5, leucine-rich repeat-containing G-protein-coupled receptor 5; EpCAM, epithelial cell adhesion molecule.

diversification that underlie clonal heterogeneity (10). However, a single specimen still captures only a fraction of the true biological complexity of a tumor.

Single-cell RNA sequencing and single-cell multi-omics platforms, which jointly profile the transcriptome alongside the genome, epigenome or proteome of the same cell, have substantially advanced the resolution at which ITH can be studied, revealing rare subclones, transitional cell states and transcriptional programs that are diluted or entirely missed by bulk sequencing (12,42). Building on this, spatial transcriptomic techniques preserve the anatomical context of gene expression within intact tissue sections, allowing investigators to map how heterogeneous cell states are organized relative to the tumor microenvironment, vasculature and immune infiltrate (43,44). Complementing these single-cell platforms, multi-region bulk sequencing captures the branched clonal architecture of a tumor by sampling multiple spatially separated regions of the same lesion, exposing region-specific driver alterations that a single biopsy would otherwise miss (14).

Multiplexed tissue imaging technologies, such as co-detection by indexing and cyclic immunofluorescence, extend spatial resolution to the protein level, enabling simultaneous visualization of dozens of markers within a single tissue section and revealing how neighborhoods of interacting cell types shape local heterogeneity (36,45,46). Together with liquid biopsy-based approaches such as circulating tumor DNA (ctDNA) monitoring (discussed in the following section), these technologies collectively provide a multi-scale view of ITH spanning the genomic, transcriptomic, proteomic and spatial dimensions. Integrating these complementary data types remains computationally demanding and is not yet standardized for routine clinical use, but their combined application is rapidly redefining how ITH is measured and interpreted in both research and clinical settings (Table II).

9. Clinical implications and discussion

ITH provides a compelling biological explanation for why a number of patients with initially responsive malignancies ultimately experience disease relapse despite receiving therapies that appear effective at the outset. Conventional tissue biopsy, although still considered the diagnostic standard in numerous settings, samples only a limited portion of a tumor at a single time point. Since tumors are spatially and temporally dynamic, a single specimen may fail to capture resistant subclones, rare stem-like populations, region-specific driver alterations or compensatory signaling pathways that later dominate under therapeutic pressure (47). As a result, treatment decisions based solely on baseline biopsy findings may underestimate the true biological complexity of the disease and contribute to premature treatment failure (48,49).

The clinical significance of ITH extends across nearly all major cancer types. In non-small cell lung cancer, heterogeneous EGFR, MET, KRAS or TP53 alterations can coexist within the same patient, leading to mixed responses to targeted therapies (15,17). In breast cancer, intratumoral variation in HER2 expression, hormone receptor status or proliferative signaling may contribute to variable treatment responses (47,50). In glioblastoma, extensive regional heterogeneity involving EGFR amplification, PTEN loss, hypoxia-associated programs

Table II. Overview of technologies used to characterize intratumoral heterogeneity, the molecular layer and resolution each provides and their principal application in ITH research.

Technology	Molecular layer and resolution	Key application in ITH research	(Refs.)
Multi-region bulk sequencing	Genomic DNA; tumor-region resolution	Reconstructs clonal phylogenies; detects region-specific driver mutations missed by single biopsies.	(14)
Single-cell RNA sequencing	Transcriptome; single-cell resolution	Identifies heterogeneous transcriptional programs and transcriptional/plasticity states within a tumor.	(51)
Single-cell multi-omics	Genome/epigenome/transcriptome; single-cell resolution	Jointly links genotype to phenotype within the same cell.	(12)
Spatial transcriptomics	Transcriptome; multicellular-spot resolution with spatial coordinates	Maps heterogeneous cell states relative to the microenvironment, vasculature and immune infiltrate.	(44)
Multiplexed tissue imaging (CODEX and cyclic IF)	Protein; single-cell resolution with spatial coordinates	Visualizes dozens of markers simultaneously and defines cellular neighborhoods.	(45,46)

ITH, intratumoral heterogeneity; CODEX, co-detection by indexing; cyclic IF, cyclic immunofluorescence.

and stem-like niches contributes to profound resistance to radiotherapy and chemotherapy (16,51). Similar patterns have been described in renal cell carcinoma (14), melanoma (18), colorectal cancer (19) and hematological malignancies such as acute myeloid leukemia (52). These observations emphasize that ITH is not an isolated phenomenon but a common biological principle of malignant progression.

The coexistence of clonal evolution and tumor plasticity suggests that cancer adapts through at least two complementary systems. The first is Darwinian genetic evolution, in which permanent heritable mutations confer survival advantages and allow resistant clones to expand. The second is non-genetic plasticity, in which cells rapidly and reversibly enter protective phenotypic states without requiring new mutations. For example, a small population of drug-tolerant persister cells may survive therapy through transient quiescence, altered metabolism, epigenetic rewiring or lineage switching (8,53). These persister cells can later reacquire proliferative capacity or accumulate stable genomic resistance mechanisms (53). Thus, plasticity may function as an early survival bridge that precedes and facilitates long-term clonal resistance.

This dual model has important implications for precision oncology. Current treatment paradigms often focus on identifying a dominant actionable mutation and matching it with a targeted drug. While effective in selected settings, this approach may be insufficient when multiple resistant populations coexist or when tumor cells can rapidly switch state under treatment pressure (54,55). Future management strategies may require iterative molecular profiling, dynamic risk stratification and adaptive combination therapy rather than static one-time genomic decisions. Precision oncology may therefore evolve from 'mutation matching' toward 'ecosystem management', where clinicians monitor and suppress multiple evolutionary escape routes simultaneously (56).

Liquid biopsy technologies, particularly ctDNA, are highly promising in this context. ctDNA monitoring can detect emerging resistant clones months before radiographic progression becomes clinically evident (19,57). Serial ctDNA analysis may reveal subclonal expansion, minimal residual disease or new actionable mutations during therapy, enabling earlier intervention. Compared with tissue biopsy, liquid biopsy is less invasive, more repeatable and potentially more representative of total disease burden across multiple metastatic sites. However, limitations remain, including low-shedding tumors that release insufficient ctDNA for detection and may therefore yield false-negative results, assay sensitivity and challenges in distinguishing tumor-derived signals from clonal hematopoiesis (58,59).

Beyond genomic monitoring, therapeutic strategies targeting non-genetic adaptation are increasingly relevant. Drugs directed at chromatin remodeling, histone deacetylases, bromodomain proteins, DNA methylation, EMT pathways, CSC signaling and tumor microenvironmental support may suppress phenotypic escape routes. Inhibitors of TGF-β, Wnt/β-catenin, Notch, Hedgehog, PI3K/Akt and inflammatory cytokine networks are under investigation as adjuncts to standard therapies (7,35,60-63). Such approaches may be particularly valuable when used in rational combinations with chemotherapy, immunotherapy or targeted agents to prevent the emergence of persister states (20,60).

The relationship between ITH and immunotherapy deserves particular attention. Tumors with high mutational burden may generate more neoantigens and become more immunogenic, yet heterogeneous antigen expression can also enable immune escape. Distinct tumor regions may differ substantially in programmed death-ligand 1 expression, interferon signaling, T-cell infiltration or antigen presentation machinery. Consequently, some clones remain susceptible to immune attack while others evade recognition (64). This may partly explain why some patients experience mixed responses, pseudoprogression or late relapse after checkpoint inhibitor therapy. Future immuno-oncology strategies may need to consider not only total mutation burden but also clonal neoantigen architecture and immune microenvironment heterogeneity.

Despite substantial progress in characterizing the genetic, transcriptional and spatial organization of tumors, several

challenges remain. Single-cell sequencing, spatial transcriptomics and multiplex imaging technologies have transformed the understanding of ITH, but they are expensive, computationally intensive and not yet universally standardized for routine clinical use (65,66). Definitions of clinically meaningful heterogeneity also vary across studies, making cross-comparison difficult. Some degree of diversity may be biologically neutral, whereas other forms strongly predict relapse or metastasis. Distinguishing actionable heterogeneity from incidental variability remains a major translational challenge.

Another unresolved question is whether all forms of plasticity are therapeutically targetable without unacceptable toxicity. Numerous plasticity pathways overlap with normal tissue regeneration, wound healing, immune regulation or stem cell maintenance. Broad inhibition may therefore produce adverse effects. For example, WNT974, a Wnt pathway inhibitor, is associated with dysgeusia (61); demcizumab, targeting the Notch ligand Delta-like ligand 4, is associated with hypertension and congestive heart failure during prolonged exposure (62); and the Hedgehog inhibitor vismodegib is associated with muscle spasms, alopecia and taste disturbance (63). A more practical strategy may involve transient or context-specific targeting during vulnerable treatment windows, rather than continuous suppression of developmental programs. Nevertheless, the convergence of genomics with phenotypic state analysis is likely to redefine oncology practice over the next decade. Rather than viewing tumors as uniform static masses, modern oncology increasingly recognizes cancer as a changing adaptive ecosystem. Therapeutic success will likely depend not only on eliminating dominant clones but also on anticipating evolutionary escape, suppressing plastic transitions and continuously adapting treatment strategies over time.

10. Conclusion

ITH is a central hallmark of cancer progression and a major cause of therapeutic failure; it arises through genetic diversification, epigenetic remodeling, CSC dynamics and tumor microenvironmental selection. Beyond classical clonal evolution, tumor plasticity has emerged as a critical mechanism enabling rapid, reversible adaptation to treatment stress. Understanding both permanent genomic alterations and transient cellular states is essential for modern precision oncology. Future therapeutic strategies should combine molecular targeting, dynamic monitoring and interventions against plasticity pathways to improve durable cancer control.

Acknowledgements

Not applicable.

Funding

This study was partially supported by the Indonesian Society of Hematology and Medical Oncology (ISHMO), Semarang Branch, Indonesia.

Availability of data and materials

Not applicable.

Authors' contributions

DNEA conceptualized the review, developed the overall framework and scope of the manuscript, performed the literature search and interpretation of the relevant literature and drafted the original manuscript. DR contributed to the conceptualization of the review, interpretation of the literature and critical revision of the manuscript for important intellectual content. KT contributed to the literature search, synthesis and interpretation of the evidence, manuscript preparation and critical revision of the manuscript. DS contributed to the interpretation of the literature and critically reviewed the manuscript for important intellectual content. EAP contributed to the conceptual development of the review, interpretation of the evidence and critical revision of the manuscript. BS contributed to the conceptualization and intellectual development of the review and critically revised the manuscript. CS provided senior intellectual input, contributed to the interpretation of the literature and critically reviewed the manuscript for important intellectual content. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the artificial intelligence tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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