REVIEW

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# Safety of Repeated Mesenchymal Stem Cell Transplantation in Solid Tumors: Evidence from an Immunocompetent EGFR-Mutant Lung Cancer Model

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## ABSTRACT

**Background:** Mesenchymal stem cells (MSCs) have therapeutic promise, but their safety in repeated intravenous administration remains debated, especially regarding solid tumor promotion. Most prior studies used immunodeficient models, limiting clinical translation. This review evaluates safety evidence from an immunocompetent EGFR-mutant lung cancer model..

**Methods:** A structured literature review was performed on PubMed, Scopus, ScienceDirect, and Google Scholar up to 2026 using MSC, safety, repeated transplantation, and EGFR-mutant lung cancer keywords. Relevant English publications were synthesized narratively.

**Results:** In an immunocompetent EGFR-mutant lung adenocarcinoma model, repeated human umbilical cord MSC (hUC-MSC) administration did not accelerate tumor occurrence or progression. Intravenously infused MSCs underwent rapid pulmonary entrapment and clearance without cumulative toxicity, organ damage, or distant metastasis. Molecular analysis revealed that repeated MSC treatment significantly downregulated pro-tumorigenic cytokines including IL-6 and IL-8, preserved the physiological M1/M2 macrophage balance, and upregulated IFN-γ expression. This IFN-γ elevation correlated with expanded CD8+ cytotoxic T-cell infiltration into the tumor microenvironment, indicating sustained immune surveillance rather than immunosuppression. No pro-angiogenic shift (VEGF-A, VEGF-C, VEGF-D) was observed. Ki67 proliferation indices in alveolar epithelial cells remained unchanged compared to controls.

**Conclusion:** Repeated intravenous MSC transplantation does not promote tumorigenesis in an immunocompetent EGFR-mutant lung cancer model, supporting its systemic safety even under pre-existing oncogenic conditions.

**Keywords:** Mesenchymal stem cells, repeated transplantation, EGFR-mutant lung cancer; immunocompetent model, tumor safety.

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## INTRODUCTION

Mesenchymal stem (or stromal) cells (MSCs) have become one of the most intensively investigated cellular products in regenerative and immunomodulatory medicine, with their therapeutic appeal resting on multipotency, abundant secretion of trophic growth factors and cytokines, and a capacity to reprogramme local and systemic immune responses through paracrine and phagocytosis-dependent mechanisms. Among the available tissue sources, umbilical cord-derived MSCs (UC-MSCs) have attracted particular interest because they can be obtained non-invasively from perinatal tissue, expanded to clinical scale with comparative genetic stability, and exhibit low immunogenicity owing to minimal expression of HLA-DR and MHC class I and an absence of MHC class II.[1]

In clinical practice, intravascular infusion is the most frequently used route of MSC delivery because it is technically straightforward and broadly applicable.[1] A defining pharmacokinetic feature of this route is that the great majority of intravenously administered MSCs do not persist in the systemic circulation; instead they are mechanically trapped within the pulmonary capillary bed and subsequently cleared by resident macrophages over a period of days.[1] This "pulmonary first-pass" phenomenon places the lung at the centre of any safety consideration for systemically delivered MSCs, since the organ most exposed to the infused product is also the site of the most commonly diagnosed and most lethal malignancy worldwide.[2]

This intersection raises a biologically coherent safety concern. The same properties that render MSCs therapeutically useful immunosuppression, promotion of M2 macrophage polarisation, and proangiogenic signalling overlap mechanistically with the immune-permissive, vascularised conditions that characterise a tumour-supportive microenvironment.[1] Conceptually, MSCs have therefore been described as a "double-edged sword," capable of both restraining and promoting tumour behaviour depending on cell source, dose, tumour type, and host context.[3] Although there is at present no robust clinical signal indicating that MSC therapy induces de novo carcinogenesis, the theoretical risk of facilitating tumour initiation or early progression particularly in the lung, and particularly with the repeated dosing regimens increasingly favoured for their superior efficacy has not been definitively resolved.[1,3]

A central limitation of the existing preclinical literature is the nature of the experimental models employed. Most prior studies addressing the tumorigenic safety of MSCs were conducted in immunodeficient mice bearing xenografts of established cancer cell lines of heterogeneous type and origin, yielding inconsistent and sometimes contradictory conclusions.[1] Such models, by design, lack the intact adaptive immune compartment through which MSCs exert much of their biological effect, and they model the growth of pre-existing tumours rather than the de novo *occurrence* of carcinoma within a physiologically relevant immune microenvironment.[1] It is against this background that a genetically engineered, immunocompetent model of epidermal growth factor receptor (EGFR)-mutant lung adenocarcinoma offers a uniquely informative testbed. EGFR-activating mutations represent the most common targetable driver alteration in non-small cell lung cancer (NSCLC), and inducible *EGFR*-mutant transgenic mice recapitulate the stepwise natural history of human lung adenocarcinogenesis within a normal immune system.[4] A recent study using repeated intravenous transplantation of human UC-MSCs into such mice provides the most direct evidence to date that frequent UC-MSC dosing neither accelerates the occurrence and early progression of lung cancer nor establishes a pro-tumour immune microenvironment.[1] The present review synthesises the relevant biology of EGFR-mutant lung cancer and of MSC–tumour interactions, critically appraises the preclinical and clinical safety evidence, and positions this immunocompetent model within the broader question of whether repeated MSC transplantation is safe with respect to solid-tumour risk.

## METHODS

This review was conducted as a structured literature review to provide an integrative, mechanistically oriented synthesis of the biology of EGFR-mutant lung cancer, the behaviour of MSCs within the tumour microenvironment, and the safety of repeated MSC transplantation.

### Information Sources

A comprehensive search of the published literature was performed across four electronic databases PubMed/MEDLINE, Scopus, ScienceDirect, and Google Scholar covering publications up to 2026. Reference lists of key articles and relevant reviews were additionally hand-searched to identify further eligible sources.

### Search Strategy

A search strategy was developed by decomposing the review topic into its principal conceptual domains (i) mesenchymal stem cells and their safety, (ii) repeated/intravenous administration, (iii) EGFR-mutant lung cancer, (iv) the tumour microenvironment and immune interaction, and (v) the experimental model context and translating each domain into controlled vocabulary (e.g., MeSH terms) and free-text keywords. Within each domain, synonymous terms were combined using the Boolean operator **OR**, and the domains were then intersected using the Boolean operator **AND**; truncation and phrase searching were applied where supported. The principal search strings were structured as follows:

1. **String 1 (core safety question):** ("mesenchymal stem cells" OR "mesenchymal stromal cells" OR "MSC" OR "umbilical cord mesenchymal stem cells" OR "UC-MSC") **AND** ("safety" OR "tumorigenicity" OR "tumorigenesis" OR "carcinogenesis" OR "tumor promotion") **AND** ("repeated transplantation" OR "repeated administration" OR "intravenous infusion" OR "cell therapy")
2. **String 2 (lung cancer and molecular biology):** ("non-small cell lung cancer" OR "NSCLC" OR "lung adenocarcinoma") **AND** ("EGFR" OR "epidermal growth factor receptor") **AND** ("exon 19 deletion" OR "L858R" OR "tyrosine kinase" OR "MAPK" OR "PI3K/Akt/mTOR" OR "signaling pathway")
3. **String 3 (immune interaction and model context):** ("mesenchymal stem cells" OR "MSC") **AND** ("tumor microenvironment" OR "immunomodulation" OR "macrophage polarization" OR "regulatory T cells" OR "PGE2" OR "IDO" OR "TGF-β") **AND** ("immunocompetent" OR "immunodeficient" OR "xenograft" OR "transgenic mouse model")

### Selection and Synthesis

Searches were restricted to English-language publications. Sources were selected on the basis of topical relevance, methodological quality, and contribution to the central question of MSC tumorigenic safety in the lung, with priority given to internationally recognised epidemiological datasets, consensus position statements from professional societies, and mechanistic or safety studies of direct bearing on the theme. Extracted information was organised thematically and integrated in narrative form into the structured review that follows.

## LITERATURE REVIEW

### EGFR-Mutant Lung Cancer Clinical Characteristics and Epidemiology of NSCLC

Non-small cell lung cancer (NSCLC) is the most prevalent form of lung malignancy, accounting for approximately 85% of all lung cancer cases worldwide. As a class, NSCLC is an epithelial lung cancer that excludes small cell lung cancer (SCLC) and encompasses several histological subtypes

with distinct clinical and molecular characteristics. Lung cancer remains the leading cause of cancer-related mortality in the United States, with an estimated 226,650 new cases and 124,730 deaths projected for 2025. The 5-year relative survival rate from 2014 to 2020 for patients with lung cancer was 27%, which varies markedly by stage at diagnosis: 64% for localized disease, 36% for regional spread, and only 9% for distant metastasis.[5]

The World Health Organization (WHO) and the International Association for the Study of Lung Cancer (IASLC) classify malignant non-small cell epithelial tumors of the lung into several subtypes. The three main subtypes are adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. **Table 1** below summarizes the key characteristics of each major subtype.[5,6]

**Table 1. Histological Classification and Characteristics of Non-Small Cell Lung Cancer Subtypes[7]**

| Subtype | Prevalence | Pathological features | Typical location | Key associations |
|---|---|---|---|---|
| Adeno-carcinoma (LUAD) | 40-50% | Glandular differentiation; mucin production; lepidic, papillary, or acinar growth patterns | Peripheral lung regions | Most common in non-smokers, women, and younger patients; frequently associated with EGFR, KRAS, ALK mutations |
| Squamous cell carcinoma (LUSC) | 25-30% | Keratinization; intercellular bridges; polygonal cells | Central bronchial origin | Strongly associated with smoking; alterations in FGFR1, DDR2, PIK3CA |
| Large cell carcinoma (LCLC) | 10-15% | Undifferentiated morphology; large nuclei; prominent nucleoli; abundant cytoplasm | Variable | Aggressive behavior; often diagnosed at advanced stage |
| Other variants | <5% | Mixed histology or poorly differentiated features | Variable | Includes adenosquamous, sarcomatoid, neuroendocrine NSCLC; often aggressive and treatment-resistant |

The epidemiology of NSCLC reveals important demographic patterns. A retrospective cohort study from Palestine including 102 patients with metastatic NSCLC reported that 80.4% were male, 40.2% were current smokers, 42.2% were ex-smokers, and 17.6% were never-smokers. The majority of patients (86.35%) were diagnosed with adenocarcinoma, and 77.5% presented with stage IV disease at diagnosis. Data from a large cohort of 1,550 patients showed that adenocarcinoma is the predominant subtype in both early-stage (78.4%) and advanced-stage (78.6%) NSCLC. Cigarette smoking remains the leading cause of lung cancer, with an estimated 85% of lung cancer deaths attributable to smoking. However, lung cancer can also occur in individuals who have never smoked. Other significant risk factors include exposure to radon gas, asbestos, air pollution, and diesel exhaust. Inherited genetic mutations may increase risk, though they account for a minority of cases.[8]

The molecular landscape of NSCLC is characterized by several actionable driver mutations that have transformed treatment paradigms. EGFR mutations are present in approximately 10-20% of NSCLC cases, occurring more frequently among never-smokers, females, and individuals of Asian descent. KRAS mutations are associated with 20-25% of NSCLC cases and are more common in smokers and former smokers. ALK gene rearrangements are found in approximately 5% of NSCLC cases in never-smokers, with studies reporting ALK mutation rates of 14.2% in younger patient populations. ROS1 mutations occur in 1-2% of NSCLC cases, and BRAF mutations in approximately 5% of cases. PD-L1 expression is an important biomarker for immunotherapy response, with studies reporting PD-L1 expression ≥10% in 56.9% of patients. Non-small cell lung cancer is frequently undiagnosed until late stages due to the insidious onset of symptoms. Coughing is the most common symptom, occurring in 50-75% of patients, followed by hemoptysis (coughing up blood), chest pain, and dyspnea (shortness of breath). Other less common manifestations include laboratory abnormalities or paraneoplastic syndromes. Diagnosis requires histological confirmation through biopsy procedures including bronchoscopy, computed tomography-guided biopsy, or more invasive methods. Staging is critical for determining prognosis and treatment options and follows the TNM classification system based on primary tumor characteristics (T), lymph node involvement (N), and presence of metastasis (M).[5,6]

## Molecular Pathway of EGFR

The structural organization and activation dynamics of the epidermal growth factor receptor, also designated as HER1 or ErbB1, initiate at the cell membrane where this 170 kDa glycoprotein undergoes homodimerization or heterodimerization with partner receptors like HER2/ErbB2 upon binding extracellular ligands such as EGF, TGF-α, or Amphiregulin. This dimeric orientation triggers a critical conformational shift that induces tyrosine autophosphorylation within its intracellular kinase domain, specifically targeting residues pY992, pY1068, and pY1173.[9,10] As illustrated in **Figure 1**, these phosphorylated residues serve as docking sites for various SH2 adaptors and signaling proteins, which simultaneously launch four principal downstream signaling cascades. The first major cascade is the classic MAPK pathway, where the recruitment of GRB2/SHC adaptors engages SOS to act as a guanine nucleotide exchange factor, converting inactive RAS-GDP to active RAS-GTP. This molecular switch activates the serine/threonine kinase RAF, which sequentially phosphorylates MEK1/2 and ERK1/2, enabling ERK1/2 to translocate into the nucleus and turn on immediate-

early transcription factors like c-Fos, c-Jun, and Myc to drive robust cellular proliferation via Cyclin D1 and CDK4/6. Concurrently, the second cascade, the PI3K/Akt axis, is recruited through GAB1 to activate the p85/p110 catalytic complex, transforming PIP2 to PIP3. This lipid secondary messenger recruits PDK1 to phosphorylate AKT/PKB at T308/S473, which subsequently suppresses TSC1/2 to release mTORC1 signaling, upregulating S6K and 4E-BP1 protein synthesis while downregulating pro-apoptotic factors like BAD and FOXO to facilitate structural cell survival.[11]

Parallel to these systems, the third and fourth signaling branches establish vital pathways for tumor progression, cell migration, and transcriptional auto-regulation. The third pathway involves phospholipase C-gamma 1, which binds specifically to the pY992 residue to hydrolyze PIP2 into the twin secondary messengers IP3 and DAG. Increased IP3 prompts an immediate release of $Ca^{2+}$ from the endoplasmic reticulum to stimulate CaMK and NFAT transcription, while localized DAG activates downstream PKC isoforms, collectively stimulating the NF-kB and AP-1 networks to generate pro-survival cytokines and direct focal adhesion molecules like FAK, Paxillin, and MMPs toward active cell migration and tissue invasion.[11] The fourth branch is governed by the JAK2/STAT3 tyrosine kinase axis, wherein activated JAK2 induces STAT3 phosphorylation at the Y705 residue, triggering its dimerization and nuclear translocation to upregulate Cyclin D1, Bcl-2, iNOS, Myc, and VEGF. Intriguigenly, as displayed in Figure 1, internalizing EGFR can translocate directly to the nucleus to function as a physical co-activator for STAT3, further driving the expression of oncogenic targets like B-Myb, iNOS, and COX-2. When driver mutations such as exon 19 deletions or L858R lock this entire multi-pathway machinery into a state of constitutional hyperactivation, it results in profound oncogenesis across diverse malignancies like non-small cell lung cancer.[11,12] To restrict this continuous hyperactivation, the cell attempts signal termination via endocytosis and lysosomal degradation,

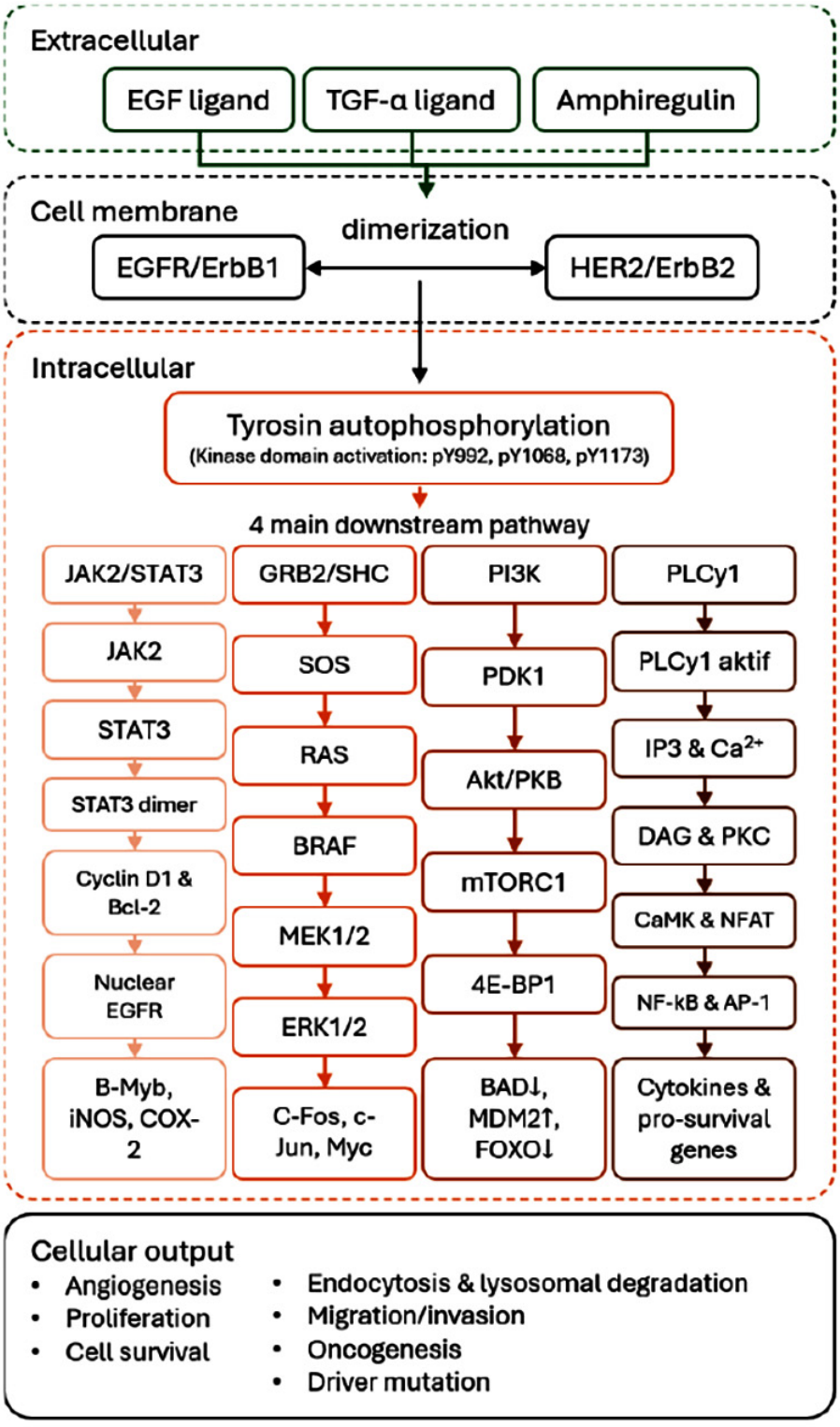


**Figure 1.** Molecular Pathway of EGFR-Mutant Lung Cancer

though clinical interventions ultimately require targeted tyrosine kinase inhibitors or monoclonal antibodies to disrupt this complex biochemical network.[13]

## Mesenchymal Stem Cells (MSCs) in Cancer Treatment

### Biological Characteristics and Sources of MSCs

Mesenchymal stem cells (MSCs) are multipotent stromal cells characterized by their capacity to differentiate into various mesodermal lineages, robust self-renewal potential, and an extensive paracrine secretome comprising a rich cocktail of growth factors and immunomodulatory cytokines. While bone marrow has historically served as the conventional gold-standard source for clinical trials, umbilical cord-derived MSCs (UC-MSCs) have recently gained significant traction in translational medicine due to distinct biological advantages. Extracted noninvasively from perinatal tissues, UC-MSCs possess a highly favorable surface marker profile, demonstrating low expression of human leukocyte antigen-DR (HLA-DR) and major histocompatibility complex class I (MHC-I), alongside a total absence of MHC-II molecules. This specific immunophenotype grants them exceptionally low immunogenicity, minimizing the risks of acute allogeneic graft rejection and making them highly suitable as a standardized, off-the-shelf therapeutic product.[14]

Furthermore, MSCs exhibit a unique physiological trait known as tumor-homing, which is a directed migration toward sites of chronic inflammation and active tissue remodeling. This migratory behavior is primarily governed by strong chemokine gradients established within injured tissues or developing tumor niches, where specialized receptors on the MSC surface interact with localized inflammatory signals. Consequently, intravascularly administered MSCs naturally navigate toward these physiological disruptions, a characteristic that makes them attractive vehicles for targeted drug delivery but simultaneously demands a rigorous baseline evaluation of how their presence alters the local tissue dynamics.[14,15]

### Paradox of MSCs in Tumor Microenvironment (TME)

The functional integration of MSCs into the tumor microenvironment presents a profound biological paradox in modern oncology, as these cells display a dual capability to either suppress or facilitate malignancy depending on the experimental context. On one hand, several in vitro co-culture frameworks and in vivo models have shown that MSCs can inhibit cancer cell proliferation, block invasion pathways, and trigger cellular apoptosis through the targeted secretion of tumor-suppressive factors. These anti-tumorigenic outcomes suggest a potential therapeutic utility in stabilizing or reducing established tumor burdens under specific clinical conditions.[16]

Conversely, an equally substantial body of literature warns that the natural immunomodulatory and tissue-repair activities of MSCs can be hijacked by a developing neoplasm. When exposed to sustained inflammatory signaling within the TME, MSCs can undergo a functional transition into cancer-associated fibroblasts (CAFs), which actively expand the local fibrovascular network and support structural tumor progression.[17] Furthermore, their theoretical capacity to promote immune escape via the secretion of extracellular vesicles or the suppression of systemic anti-tumor leukocyte responses has driven intense academic scrutiny. This deep-seated controversy regarding whether MSCs act as structural inhibitors or accidental promoters of tumorigenesis highlights the urgent need to evaluate their safety profiles within realistic, immunologically complete in situ models.[18]

## Immunological Interactions of MSCs in an Immunocompetent Model

### Significance of Immunocompetent vs. Immunocompromised in MSC-related Studies

The historical literature examining the tumorigenic safety of mesenchymal stem cells (MSCs) is heavily saturated with varied and highly controversial conclusions. While several groups have reported that MSCs can actively suppress tumor proliferation and induce apoptosis in vitro, others have observed that MSC administration promotes subcutaneous tumor growth and accelerates distant metastasis in vivo. A critical methodological critique of these past decades of preclinical data reveals that the vast majority of these conflicting studies were modeled in immunodeficient or immunocompromised mice, such as athymic nude or SCID mice, utilizing xenografts of established human cancer cell lines. Although immunodeficient models are highly convenient for bypassing species-specific graft rejection, they inherently fail to mimic the true physiological paradigm of de novo carcinogenesis because they lack a fully functional, intact host immune system.[15]

The use of immunocompromised animal models in preclinical cell therapy studies introduces a profound biological bias, particularly when evaluating cells with potential immunomodulatory mechanisms. In an immunocompromised host, the complex network of adaptive immunity (including T cells, NK cells, and regulatory T cells) is non-functional, meaning that critical interactions involving T-cell activation, cytotoxic cell recruitment, and regulatory T-cell differentiation cannot be captured. This structural limitation becomes critically important when assessing the safety of Mesenchymal Stromal Cells (MSCs). A study by Marodin et al. (2022) explicitly demonstrates this issue: although human MSC products with clonal trisomy 5 (a chromosomal aberration that theoretically increases neoplastic risk) were subcutaneously injected into immunodeficient mice, no tumors formed during a six-month follow-up period. In other words, a model lacking a functional adaptive immune system erroneously suggests that even MSCs with genetic abnormalities have "poor tumor initiating capability." Consequently, preclinical safety data derived from these immunocompromised systems tend to underestimate the potential pro-tumorigenic risks of MSCs, as such models cannot simulate the host's natural immune surveillance and editing mechanisms that would normally constrain aberrant cell growth in a biologically intact environment.[16]

Furthermore, the immunological

background of the host model directly dictates the biological half-life and clearance kinetics of transplanted MSCs. In immunocompromised mice, xenogeneic or allogeneic MSCs may exhibit prolonged survival within host tissues due to the absence of active immune-mediated rejection. In contrast, within an immunocompetent host possessing a normal immune system, the half-life of intravascularly administered MSCs is drastically shortened to a matter of days. Upon systemic injection into an immunologically intact system, entrapped MSCs are rapidly recognized and cleared by host monocytic cells and macrophages through efferocytosis. Mechanistic insights from Ankrum et al. (2014) confirm that MSCs are immune evasive, not immune privileged. The authors demonstrate that although MSCs can temporarily evade immune detection, they remain susceptible to clearance by an intact innate immune system. This rapid clearance fundamentally alters their paracrine output, transforming them from long-lived cellular factories into short-lived signaling vehicles operating via a "hit and run" mechanism. Consequently, this rapid clearance profile means that the biological exposure window of MSCs in an immunocompetent model is self-limiting, preventing the long-term, continuous exposure that often drives the artificial tumor progression observed in immunodeficient models.[19]

Moreover, MSC-tumor microenvironment interactions strictly depend on the host's baseline inflammatory status. As Galipeau and Sensébé (2018) comprehensively review, the presence of functional host immune cells such as interferon-gamma secreting T-cells is mandatory to "license" or activate the full immunomodulatory potential of MSCs. Without this cellular crosstalk, which is absent in immunocompromised models, MSCs may exhibit unpredictable phenotypes that do not translate clinically.[20] Recognizing these profound limitations, modern oncological research underscores the necessity of utilizing immunocompetent, inducible transgenic models to evaluate cellular therapies. The transgenic model deployed by Zhang et al. (2025) represents a major methodological advancement by tracking repeated transplantation of human UC-MSCs in mice that maintain a fully functional immune system while concurrently developing spontaneous lung adenocarcinoma via an inducible mutated EGFR gene. By allowing xenogeneic stem cells to interact naturally with an intact host immune network during the earliest stages of in situ carcinogenesis, including the crucial interferon-gamma licensing pathway, such models provide a vastly more authentic and clinically translatable baseline for establishing the true safety profile of repeated MSC therapies in oncology.[15]

## Biochemical Modulation of Immune System by MSCs

Mesenchymal stem cells (MSCs) exert their therapeutic and immunomodulatory effects predominantly through paracrine signaling and secretion of biochemical mediators rather than long-term tissue engraftment. When transplanted into an immunocompetent macroenvironment, MSCs actively interact with the host's innate immune cell populations. They secrete an array of soluble factors including transforming growth factor-beta (TGF-β) and prostaglandin E2, which modulate the local inflammatory tone. These factors can influence macrophage behavior, traditionally characterized by a shift from a classic pro-inflammatory M1 phenotype toward a tissue-repairing, anti-inflammatory M2 phenotype. In the context of early lung oncogenesis, this specific biochemical modulation is a major source of safety concerns. An over-activation of anti-inflammatory pathways could inadvertently shield nascent cancer cells from early host detection. This immune evasion mechanism may accelerate tumor progression by preventing the innate immune system from recognizing and eliminating abnormal cells during the critical early stages of carcinogenesis.[15,21]

However, recent empirical evidence from an inducible EGFR-mutant lung cancer model demonstrates that this biochemical modulation does not automatically drive a pro-tumorigenic trajectory. Zhang et al. (2025) showed that during the early dysplastic stages of lung adenocarcinoma, repeated hUC-MSC administrations did not cause a malignant shift in the local cytokine profile. Instead, quantitative assessments revealed a sustained upregulation of local interferon-gamma (IFN-γ) mRNA expression, which serves an essential role in early tumor surveillance by maintaining active host immune defenses. Concurrently, crucial pro-tumorigenic drivers such as interleukin-6 (IL-6) and the human functional homolog interleukin-8 (CXCL8) were significantly downregulated in the MSC-treated microenvironment. Rather than triggering a generalized immunosuppression that facilitates cancer growth, the repeated introduction of hUC-MSCs maintains a tightly regulated biochemical balance. This balance permits functional immune surveillance through sustained Ifnγ signaling while suppressing specific cytokines associated with accelerated tumor progression, including Il6 and CXCL8. These findings challenge the assumption that MSC-induced M2 polarization inevitably promotes lung carcinogenesis.[15]

## Safety Profile of Repeated MSC Transplantation

### Evaluation of Systemic Toxicity and Tumorigenicity

The systemic safety and potential tumorigenic risks of intravascular mesenchymal stem cell (MSC) therapy remain highly debated due to the cells' inherent immunosuppressive and proangiogenic capabilities. A primary physiological event during intravascular administration is the phenomenon of pulmonary entrapment. Mechanistically, due to the mismatch between the physical diameter of MSCs, which is typically 15 to 30 , and the microvascular lumen of pulmonary capillaries, which is around 5 to 8 , the vast majority of intravenously injected MSCs are mechanically filtered and trapped within the lungs almost immediately after infusion.[22,23]

Under normal physiological conditions, classic tracing studies by Eggenhofer et al. (2012) have established that these entrapped cells are short-lived, do not migrate significantly beyond the pulmonary vasculature, and

are rapidly cleared by host monocytic cells within a few days. This clearing process, known as efferocytosis, induces a systemic immunomodulatory cascade as host macrophages phagocytose the dying or apoptotic MSCs, a mechanism further detailed by Galleu et al. (2017) to show that recipient-mediated apoptosis is essential to initiate MSC-induced immunosuppression. However, in patients with a pre-existing oncogenic background, such as latent epidermal growth factor receptor (EGFR) mutations, there is a long-standing concern that this dense, localized accumulation of MSCs in the lungs could accidentally remodel the tumor microenvironment (TME), promoting an immune escape niche that accelerates early tumor initiation.[15,22,23]

Theoretically, concerns regarding the tumorigenic potential of mesenchymal stem cells (MSCs) extend beyond their physical retention within the pulmonary microvasculature to encompass their potent paracrine signaling capabilities. MSCs are known to secrete a cocktail of bioactive molecules, including vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), and interleukin-6 (IL-6), which are critical regulators of the tumor microenvironment (TME). In the context of an established oncogenic background, such as an EGFR mutation, these secreted factors could hypothetically create a pro-tumorigenic niche. Specifically, these signals have been shown to induce epithelial-mesenchymal transition (EMT), enhance local angiogenesis, and recruit tumor-associated macrophages (TAMs) that exhibit an immunosuppressive, pro-tumorigenic phenotype. For instance, IL-6 derived from MSCs can activate STAT3 signaling in cancer cells, promoting proliferation and survival, while MSC-secreted VEGF can stimulate neovascularization to support tumor growth.[24]

However, it is crucial to note that the majority of this mechanistic evidence originates from xenograft models or co-culture systems using established tumor cell lines, where the immune system is either compromised or absent. A systematic review highlighted that the effect of MSCs on tumors is highly context-dependent, with outcomes varying based on the MSC source, the cancer type, and crucially, the immunocompetence of the host model.[25] Consequently, findings from these conventional models may not accurately reflect the complex, multi-stage process of de novo carcinogenesis in an immunocompetent host, underscoring the necessity of studies like the one by Zhang et al. (2025) that utilize immunocompetent, inducible transgenic models of early lung cancer.[15,26]

To definitively evaluate this risk under rigorous conditions, Zhang et al. (2025) utilized an inducible, immunologically intact transgenic mouse model, specifically TetO-EGFR (Del19); CC10-rtTA mice. By monitoring the longitudinal dynamics of human umbilical cord MSCs (hUC-MSCs) via live bioluminescence imaging, the study confirmed that while the majority of cells accumulated in the lungs during active alveolar carcinogenesis, they decreased dramatically and were completely cleared within a few days. Crucially, this rapid pulmonary clearance dynamic remained unaffected even when mice were subjected to a highly frequent, repeated regimen of twice-weekly tail vein injections over a six-to-nine-week period. This demonstrates that repeated administration does not cause cumulative cellular clogging or persistent survival of xenogeneic MSCs in the lung tissue.[15]

Complementing the clearance analysis, extensive post-mortem histopathological screening was conducted to monitor for systemic toxicity and distal tumorigenic acceleration. Comprehensive evaluation of major vital organs, specifically the heart, liver, and kidneys, showed no morphological abnormalities, tissue necrosis, or microscopic evidence of secondary tumor cell infiltration or metastasis. Longitudinal micro-computed tomography (micro-CT) imaging and localized hematoxylin and eosin (H&E) staining further proved that frequent hUC-MSC exposure did not accelerate precancerous atypical adenomatous hyperplasia (AAH) or aggravate diffuse bronchiolar alveolar carcinoma (BAC) progression.[15]

Immunohistochemical quantification of Ki67 confirmed that the proliferative rate of mutated alveolar epithelial cells remained statistically unchanged compared to control groups. Aside from a transient, clinically manageable splenic enlargement and a minor decline in peripheral platelets, which represents the physical workload of the spleen in clearing cellular debris, all other peripheral blood parameters, including red blood cells, white blood cells, neutrophils, lymphocytes, and hemoglobin, remained entirely within normal physiological ranges. Taken together, these findings provide robust evidence that the transient pulmonary entrapment of repeatedly transplanted UC-MSCs does not exert systemic toxicity or promote tumorigenesis within an immunocompetent EGFR-mutant model.[15]

## Molecular Parameters in MSCs Safety Profile

Beyond anatomical and macro-histopathological evaluations, a comprehensive assessment of mesenchymal stem cell (MSC) safety requires rigorous interrogation of molecular parameters within the tumor microenvironment (TME). Theoretically, the primary molecular concern stems from the well-documented capacity of MSCs to secrete a panel of immunosuppressive and pro-angiogenic soluble factors, including indoleamine 2,3-dioxygenase (IDO), prostaglandin E2 (PGE2), transforming growth factor-beta (TGF-β), and interleukin-10 (IL-10).[27] In the context of early lung oncogenesis driven by an EGFR mutation, repeated exposure to exogenous MSCs could hypothetically induce a persistent biochemical shift from an acute, self-limited inflammatory response toward a chronic, pro-tumorigenic molecular signature.[28]

Such a shift might manifest as sustained downregulation of Th1-type cytokines (e.g., interferon-gamma [IFN-γ]), upregulation of pro-angiogenic factors (e.g., vascular endothelial growth factor [VEGF]), or preferential recruitment of M2-polarized tumor-associated macrophages (TAMs) via chemokine axes such as CCL2-CCR2. Importantly, these theoretical risks are not merely speculative; they are grounded in evidence from co-culture systems and xenograft models where MSCs have been shown to promote tumor growth through paracrine activation of STAT3, AKT, and NF-**κB** pathways in malignant epithelial cells.[24,29] However, whether these molecular perturbations occur in

an in vivo, immunocompetent model of de novo carcinogenesis, where the immune system remains fully intact and capable of rejecting or modulating MSC signals, remained unclear prior to the study by Zhang et al. (2025). Therefore, the following molecular parameters were evaluated to determine whether repeated intravascular MSC administration actively remodels the pulmonary TME toward a pro-tumorigenic or pro-angiogenic state.[15]

To rigorously test these molecular dynamics, Zhang et al. (2025) performed extensive quantitative PCR (Q-PCR) and flow cytometry analyses across different progressive stages of lung adenocarcinoma, spanning from early atypical adenomatous hyperplasia to advanced solid micro-adenocarcinomas. The transcriptional profiling of inflammatory mediators in lung tissues revealed that a highly frequent, twice-weekly hUC-MSC regimen did not provoke a malignant molecular remodeling of the local niche. During the early precancerous AAH stage, the transcription of the majority of inflammatory factors and chemokines, including interleukin-1 beta (IL-1β), interleukin-2 (IL-2), and interleukin-6 (IL-6), exhibited no significant deviations from the control groups. This baseline transcriptional stability indicates that the active clearing of entrapped MSCs by host phagocytes does not trigger an acute, hyper-inflammatory cytokine storm or launch a sustained immunosuppressive feedback loop within the lung parenchyma. Of note, this finding aligns with the mechanistic model proposed by Eggenhofer et al. (2012) and Galleu et al. (2017), wherein rapid pulmonary clearance and apoptotic cell engulfment (efferocytosis) lead to a regulated immunomodulatory response rather than pathological inflammation.[22,23]

As the pathology progressed to the bronchiolar alveolar carcinoma dysplasia stage at six weeks of induction, the local molecular landscape exhibited variable alterations that, interestingly, favored tumor restriction rather than acceleration. A key molecular finding was the continuous upregulation of interferon-gamma (IFN-γ) mRNA expression in the MSC-treated cohort. Functionally, elevated IFN-γ plays a bidirectional yet predominantly anti-tumorigenic role in early-stage tumor surveillance by enhancing the cytotoxic

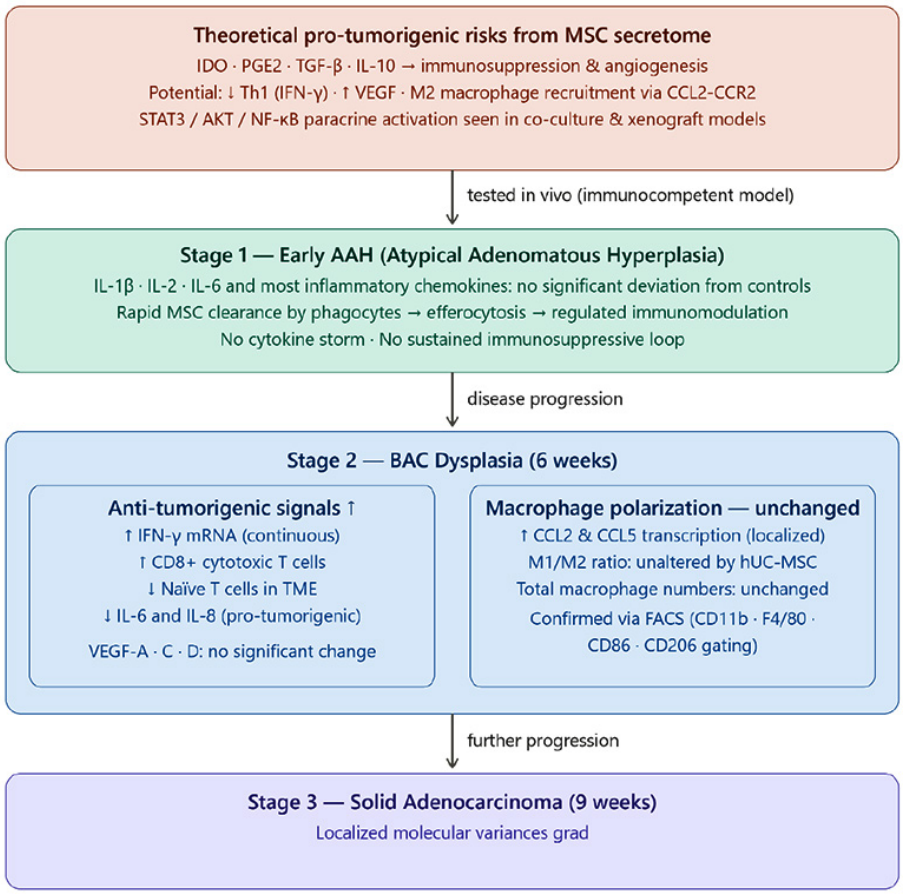


**Figure 2.** Molecular Parameters in hUC-MSC Safety.[15]

capacity of localized immune cells.[28] This molecular upregulation directly correlated with a verified shift in the adaptive immune cell subpopulation, where flow cytometry confirmed an expansion of $CD8^+$ cytotoxic effector T cells and a concomitant reduction in naïve T cell populations within the tumor microenvironment. Conversely, classic pro-tumorigenic drivers such as IL-6 and IL-8, which are typically associated with accelerated cancer cell proliferation and survival through downstream pathways, were significantly downregulated in the MSC-treated microenvironment during this critical dysplastic window.[24]

The study also tracked key chemokines involved in myeloid cell recruitment, noting localized increases in chemokine ligand 2 (CCL2) and chemokine ligand 5 (CCL5) transcription. While these chemokines possess the theoretical capacity to recruit immunosuppressive tumor-associated macrophages, high-resolution fluorescence activated cell sorting (FACS) utilizing CD11b, $F4/80^{high}$, CD86, and CD206 gating demonstrated that the total numbers and, crucially, the polarization ratios of M1 versus M2 macrophage subgroups remained entirely unaltered by the hUC-MSC transplantation. This finding is particularly notable given that previous reports have shown MSC-mediated M2b polarization in other experimental contexts.[24,29] Furthermore, molecular markers governing local angiogenesis, specifically vascular endothelial growth factor A (VEGF-A), VEGF-C, and VEGF-D, showed minor to no significant changes at the transcription level across all evaluated stages, confirming that repeated hUC-MSC exposure does not activate angiogenic signaling cascades to support tumor expansion. As the disease progressed to the formal solid adenocarcinoma stage at nine weeks, these localized molecular variances gradually diminished, resulting in equivalent cytokine profiles between the MSC and

control groups. Taken together, these molecular parameters demonstrate that a repeated hUC-MSC regimen maintains strict genetic and biochemical stability within the lung microenvironment, effectively refuting the hypothesis that intravascular MSC delivery drives a pro-tumorigenic or pro-angiogenic molecular transformation in an immunocompetent host.[24]

## CONCLUSION

In conclusion, this review refutes the paradigm that intravascular mesenchymal stem cell (MSC) transplantation inherently accelerates solid tumor progression, exposing the biological biases of past immunodeficient models. Synthesized evidence from an immunologically intact, inducible EGFR-mutant lung cancer model demonstrates that highly frequent, repeated human umbilical cord MSC (hUC-MSC) administration induces neither systemic toxicity nor localized oncogenesis. Despite initial pulmonary first-pass entrapment, the cells remain short-lived and undergo rapid host-mediated clearance without causing macrovascular clogging, organ damage, or distal metastasis. Mechanistically, repeated hUC-MSC exposure does not drive an immunosuppressive or pro-angiogenic microenvironmental transformation. Instead, it maintains strict biochemical stability by downregulating oncogenic cytokines like IL-6 and IL-8, preserving baseline M1/M2 macrophage ratios, and upregulating IFN-γ expression to sustain robust $CD8^+$ cytotoxic effector T-cell surveillance. Ultimately, these findings highlight the necessity of using immunocompetent transgenic systems for cellular therapy evaluation, providing critical, clinically translatable evidence that confirms the systemic safety of repeated MSC therapies under pre-existing oncogenic backgrounds.

## CONFLICT OF INTEREST

None.

## FUNDING

None.

## AUTHOR CONTRIBUTIONS

GADD, IKWAK, IGAPS, IGGAWK and IGPS designed the study framework. GADD, IKWAK, IGAPS, and IGGAWK conducted the comprehensive literature search, screened the databases, and synthesized the initial manuscript draft. IGPS supervised the project, critically reviewed and revised the manuscript for intellectual content, and finalized the formatting. All authors read and approved the final version of the manuscript.

## ETHICAL ASPECT

As this study is a systematic literature review and did not involve direct interventions, sample collections, or experimental procedures on human participants or live animals by the authors, formal institutional ethical review and approval were not required.

## AI DECLARATION

During the preparation of this manuscript, the authors utilized an AI tool solely to refine grammatical structure, improve sentence flow, and assist in technical formatting. The authors maintain full accountability for the scientific integrity, accuracy, data interpretation, and final literature synthesis presented in this work.

## REFERENCES

1. Zhang Z, Xu A, Zhou Q, Wen F, Chen F, Chen H, et al. Repeated intravenous transplantation of human umbilical cord mesenchymal stem cells does not promote tumorigenesis in EGFR-mutated lung cancer mice. Stem Cells Transl Med. 2025;14(8).
2. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229–63.
3. Liang W, Chen X, Zhang S, Fang J, Chen M, Xu Y, et al. Mesenchymal stem cells as a double-edged sword in tumor growth: focusing on MSC-derived cytokines. Vol. 26, Cellular and Molecular Biology Letters. BioMed Central Ltd; 2021.
4. Politi K, Zakowski MF, Fan PD, Schonfeld EA, Pao W, Varmus HE. Lung adenocarcinomas induced in mice by mutant EGF receptors found in human lung cancers respond to a tyrosine kinase inhibitor or to down-regulation of the receptors. Genes Dev. 2006;20(11):1496–510.
5. Clark SB, Alsubait S. Non–Small Cell Lung Cancer [Internet]. StatPearls. 2026. Available from: http://www.ncbi.nlm.nih.gov/pubmed/31912902
6. Akbulu H. Non-small cell lung cancer and its treatment. Demiroglu Science University Florence Nightingale Transplantation Journal. 2020;4(1–2):23–40. Available from: https://www.journaltxdbu.com/full-text/33
7. Ho C, Tong KM, Ramsden K, Ionescu DN, Laskin J. Histologic classification of non-small-cell lung cancer over time: Reducing the rates of not-otherwise-specified. Current Oncology. 2015;22(3):e164–70.
8. Abukhalil AD, Mansour K, Alhaj W, Salah I, Sahoury Y, Al-Shami N, et al. Non-small cell lung cancer (NSCLC): characteristics, risk factors, molecular profile patterns, and treatment — a retrospective cohort study from Palestine. J Egypt Natl Canc Inst. 2025;37(1).
9. Murphrey MB, Quaim L, Rahimi N, Varacallo MA. Biochemistry, Epidermal Growth Factor Receptor [Internet]. StatPearls. 2026. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30975211
10. Gao C, Wang W, Liu T, Li X, Yu Y, Wu J. Annual review of EGFR inhibitors in 2024. Vol. 292, European Journal of Medicinal Chemistry. Elsevier Masson s.r.l.; 2025.
11. Wee P, Wang Z. Epidermal growth factor receptor cell proliferation signaling pathways. Vol. 9, Cancers. MDPI AG; 2017.
12. Lo HW, Hung MC. Nuclear EGFR signalling network in cancers: Linking EGFR pathway to cell cycle progression, nitric oxide pathway and patient survival. Vol. 94, British Journal of Cancer. 2006. p. 184–8.
13. Levantini E, Maroni G, Del Re M, Tenen DG. EGFR signaling pathway as therapeutic target in human cancers. Vol. 85, Seminars in Cancer Biology. Academic Press; 2022. p. 253–75.
14. Kim JH, Jo CH, Kim HR, Hwang Y Il. Comparison of immunological characteristics of mesenchymal stem cells from the periodontal ligament, umbilical cord, and adipose tissue. Stem Cells Int. 2018;2018.
15. Zhang Z, Xu A, Zhou Q, Wen F, Chen F, Chen H, et al. Repeated intravenous transplantation of human umbilical cord mesenchymal stem cells does not promote tumorigenesis in EGFR-mutated lung cancer mice. Stem Cells Transl Med. 2025;14(8).
16. Marodin MSJ, Godoy JA, Alves-Paiva RM, Alvarez K, Mitsugi TG, Krepischi ACV, et al. Preclinical Evaluation of the Tumorigenic and Immunomodulatory Properties of Human Bone Marrow Mesenchymal Stromal Cell Populations with Clonal Trisomy 5. Stem Cells Int. 2022;2022.
17. Xu J, Fang S, Dong X, Liang C, Yang R, Zhao Y, et al. Hypoxia-induced upregulation of HIF1A-AS3 promotes MSC transition to cancer-associated fibroblasts and confers drug resistance in gastric cancer. Drug Resistance Updates. 2025;82.
18. Wang XS, Yu XJ, Wei K, Wang SX, Liu QK, Wang YG, et al. Mesenchymal stem cells shuttling miR-503 via extracellular vesicles enhance

glioma immune escape. Oncoimmunology. 2022;11(1).
19. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: Immune evasive, not immune privileged. Vol. 32, Nature Biotechnology. Nature Publishing Group; 2014. p. 252–60.
20. Galipeau J, Sensébé L. Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities. Vol. 22, Cell Stem Cell. Cell Press; 2018. p. 824–33.
21. Li H, Wang T, Yang C, Dong W, Wu J, Zhang J, et al. Mesenchymal stem cells regulating macrophage polarization. Vol. 168, International immunopharmacology. 2026. p. 115801.
22. Galleu A, Riffo-Vasquez Y, Trento C, Lomas C, Dolcetti L, Cheung TS, et al. Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. Sci Transl Med. 2017;9(416).
23. Eggenhofer E, Benseler V, Kroemer A, Popp FC, Geissler EK, Schlitt HJ, et al. Mesenchymal stem cells are short-lived and do not migrate beyond the lungs after intravenous infusion. Front Immunol. 2012;3(SEP).
24. Antoon R, Overdevest N, Saleh AH, Keating A. Mesenchymal stromal cells as cancer promoters. Vol. 43, Oncogene. Springer Nature; 2024. p. 3545–55.
25. Oloyo AK, Ambele MA, Pepper MS. Contrasting views on the role of mesenchymal stromal/stem cells in tumour growth: A systematic review of experimental design. In: Advances in Experimental Medicine and Biology. Springer New York LLC; 2018. p. 103–24.
26. Zong C, Zhang H, Yang X, Gao L, Hou J, Ye F, et al. The distinct roles of mesenchymal stem cells in the initial and progressive stage of hepatocarcinoma article. Cell Death Dis. 2018;9(3).
27. Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases. Vol. 14, Nature Reviews Nephrology. Nature Publishing Group; 2018. p. 493–507.
28. Liu R, Wei S, Chen J, Xu S. Mesenchymal stem cells in lung cancer tumor microenvironment: Their biological properties, influence on tumor growth and therapeutic implications. Vol. 353, Cancer Letters. Elsevier Ireland Ltd; 2014. p. 145–52.
29. Kudlik G, Hegyi B, Czibula Á, Monostori É, Buday L, Uher F. Mesenchymal stem cells promote macrophage polarization toward M2b-like cells. Exp Cell Res. 2016;348(1):36–45.