**Abstract**

**BACKGROUND:** Metastases are simply known as cancers spread to another part of the body, and often be responsible for the severity of cancer prognosis. Somehow, the complex mechanisms of metastases are not fully understood yet.

**CONTENT:** The characteristic of cancer is akin to a never-healing wound. Cancer cells are plastic and dynamic as they build their niches and developed into metastases, even when they seem dormant. Therefore, cancer cells can survive the immune system. Recent research has shown the distinct biology of metastasis-initiating cell, which leads to tumor development in distant organs, immune surveillance evasion, and co-option of metastatic micro-environments. Effective cancer therapies must consider the regenerative states of metastatic malignancies and have careful observation of patient phenotypes.

**SUMMARY:** This review aimed to provide an insight on genesis and characteristics of metastases, starting from its seeding and dormancy, until the advance phase. Thus, developing therapy for cancer metastases should not start as it grows, but even as earlier strategies since the primary tumor was detected.

**KEYWORDS:** cancer metastasis, DTC, CTC, CSC, dormancy, pre-metastatic niche, plasticity

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dormant seeds in distant organs. These cells need time to penetrate the organ barriers, generate the lesions and finally dominate them.(6) The majority of the disseminated cancer cells in their early stage fail to colonize the distant organ and instead perish to numerous stressors.(7,8) To produce metastases, cancer cells must navigate a series of stages known as the 'metastatic cascade', each of which needs a certain function.(5,6)

This review aimed to provide an insight on genesis and characteristics of metastases, starting from its seeding and dormancy, until the advance phase. Thus, developing therapy for cancer metastases should not started as it grows but even as earlier strategies since the primary tumor was detected.

**Emerging Biology of Metastasis Cascade**

The first step of the metastatic cascade is known as invasion, where the malignant cells disseminate to another distant organ. Host interactions, genetic, and epigenetic modification of primary tumor cells were needed for this metastasis.(9) The metastatic dissemination can occur in two manners, the linear progression model where the metastasis-competent clone emerges late in carcinogenesis and disseminates shortly before clinical identification of the initial lesion, and the parallel progression model where the primary tumor and the metastasis develop together at one time but create a different genetic character between the primary and metastatic lesions.(10) Both theories imply that the main tumor and its metastases are clonally linked and stem from the same ancestor cell. The two models are separated primarily by the predicted genetic divergence between the primary tumor and its metastases, and the relative time of the formation of the metastatic precursor population in the primary tumor. Figure 1 describe how metastases can happens in lymph node by single clones (LN1) or multiple (LN2). The metastases also can be spread directly by the primary tumor (M3,M4,M5) or can be a cascade from one metastatic site to generate others (M3,M6,M7). It can also happens in parallel manner and then evolve (M8,M9,M10).(1) (Adapted with permission from the American Association for the Advancement of Science).

All pro-metastatic behaviours in the metastatic cascades are facilitated by several genes produced by cancer cells in the initial tumor, preparing them for metastasis when they are spread to specific organs. In their process of adaptation to the host microenvironment, the disseminated cells express obvious metastasis-promoting genes, which actually can be a potential target for therapy. For example, breast cancer and melanoma express secreted protein, acidic, cysteine-rich/osteonectin (SPARC), tumor growth factor (TGF)-inducible factor angiopoietin-like 4 (ANGPTL4), and secreted C-terminal fibrinogen-like domain of angiopoietin-like 4 (cANGPTL4). Some of them are now undergoing clinical trials.(11) However, there is other facets of tumor growth and metastasis such as how primary tumors disrupt inter-immune-cell communication, both locally and systemically, generating a persistent inflammatory but immunosuppressive state that aids immune evasion and metastasis formation. Tumor cell dispersion can happen at any time during primary tumor growth, even if metastatic outgrowth doesn't happen for years, and immune cell

![Figure 1. Metastatic spread can take place through multiple routes and in different directions. Metastases can happens in lymph node by single clones (LN1) or multiple (LN2). The metastases also can be spread directly by the primary tumor (M3,M4,M5) or can be a cascade from one metastatic site to generate others (M3,M6,M7). It can also happens in parallel manner and then evolve (M8,M9,M10).](image)
interaction, both locally and systemically, is crucial in determining if outgrowth is conceivable.(12)

The innate/adaptive immune crosstalk needs to be subverted to deflect the immune attack at the primary tumor site and metastasis site. In their evolving process, tumors have many opportunities to subvert the generation and/or efficacy of antitumor immunity such as escape from cytotoxic T lymphocyte (CTL)-mediated killing by preventing the T cell priming or infiltration and suppressing the T cell effector functions. The immune system depends so much on these aberrant innate and adaptive immune cells crosstalk for tumor evasion, therefore disease progression and metastasis.

The clinical and preclinical studies suggest that tumors may avoid immune system destruction by disrupting immunological crosstalk, allowing disease progression. Systemic inflammation is associated with disease development in many malignancies, and higher myeloid cell counts in the blood are employed as a surrogate for diagnosing systemic inflammation in the clinic. A high neutrophil-to-lymphocyte ratio (NLR) in the blood is closely linked to a poor prognosis in cancer patients. (13) Furthermore, preclinical investigations have shown that systemic neutrophil mobilization aided metastatic dissemination.(14)

The classic forward-time perspective from metastatic phenotypes (e.g., invasion, dissemination, and colonization) think that metastasis occurs after the first cancer-initiating mutation started as a linear progression model, and is described as a late event.(1) This is unquestionably true if the clock begins ticking at tumor onset. Indeed, carcinogenesis is understood to be a multi-step process that takes several years (perhaps decades) to complete, with driver mutations occurring years before the initial tumor is diagnosed. Inference of metastatic timing from cancer genetic data, on the other hand, implies that this can happen years before clinical discovery. Genomic evidence also suggests that a main mutant clone in the original tumor is frequently used to seed metastases in many malignancies. These findings contribute to resolving the debate over early vs late metastatic seeding and its clinical consequences.(7)

Breast cancer, gastrointestinal tumors, renal carcinomas, melanoma, several forms of sarcoma, and lung cancer have a high rate of lung metastasis.(15) Endothelial cells fill lung capillaries, which are bordered by a basement membrane and neighboring alveolar cells. (16) The extravasation of tumor cells in the lung is aided by the dissociation of cell-cell connections between endothelial cells caused by the production of mediators to penetrate lung capillaries and seed the metastatic cells, including the epidermal growth factor (EGF) receptor ligand epiregulin, the prostaglandin synthase cytochrome c oxidase polypeptide II (COX2), and the matrix metalloproteinases (MMP)1 and MMP2.(17) All of these mediators are upregulated in breast tumors, and their expression predicts lung metastasis (17-19), suggesting that indeed the traits for metastatic cascades are started early with the primary tumor, although have a different role.

Bone metastases are seen in 60-85 percent of individuals with metastatic breast and prostate cancer, leading to pathological fractures, persistent pain, and neurological compression syndromes.(20) The sinusoids, which are tiny blood arteries in the bone marrow, are coated with fenestrated endothelia to allow hematopoietic cells to pass through. Circulating tumor cells (CTCs) are more likely to thrive in bone marrow sinusoids than in other kinds of capillaries. Furthermore, bone matrix cells, such as osteoblasts, release several chemoattractive molecules (such as chemokine C-X-C motif ligand (CXCL)-12, receptor activator of nuclear factor kappa-B ligand (RANKL), osteopontin (OPN), or bone morphogenetic proteins (BMPs)) that attract cancer cells to the bone marrow.(20,21)

The brain parenchyma and leptomeninges are the primary sites of metastasis in the central nervous system (CNS), and it has a very dismal prognosis with substantial morbidity and death. Patients with brain metastases have a median survival time of months, and there are few effective therapies available right now.(22) Lung adenocarcinoma causes more than half of brain metastases, followed by breast cancer and melanoma.(15) Cancer cells must pass across the blood-brain barrier, to penetrate the brain parenchyma (23), and this process is mediated by some molecules like the acetylgalactosaminide sialyltransferase, COX2, heparin-binding EGF-like growth factor (HBEGF), matrix metalloproteinase MMP2, Mir-105, and the protease cathepsin S.(24)

The liver is the most common organ as metastatic target. As a result, the liver presents an abundance of opportunities for cancer cells to arrest, extravasate, and invade the hepatic parenchyma.(25) Lung and breast cancers are two other primary tumors that can metastasize to the liver.(15,26) Uveal (ocular) melanoma nearly always recurs in the liver, indicating that, in addition to circulation patterns, metastatic cell compatibility with the host stroma plays a role in organ-specific metastasis.(27) Each step in the metastatic cascade exposes cancer cells to inherent vulnerabilities that might be exploited to avoid overt metastasis and improve the prognosis of individuals with metastatic disease.
CTCs are cells that are lost into the bloodstream from primary tumors and metastatic deposits. CTCs have the potential to significantly increase our understanding of the stages involved in the metastatic cascade, from tumor cell intravasation into the bloodstream to the creation of clinically detectable metastases.

Tumor cancer cells spread by infiltrating blood and lymphatic arteries. Extravascular migratory metastasis is a condition where cutaneous melanoma cells migrate on the abluminal surface of lymphatic veins to spread.(28) Cancer cells migrating along nerves have also been shown to invade the perineural space.(29) Hematogenous dispersion, on the other hand, is thought to be the most common form of metastasis to distant organs. CTCs have progenitor and Epithelial-mesenchymal transition (EMT) markers, indicating that they are primed to become metastatic tumor cells.(30) Different from CTCs which are still in circulation, disseminated tumor cells (DTCs) refer to cancer cells that have already discharged from a primary tumor and resided in another permissive organ, such as bone marrow.(6,31)

EMT, or the loss of epithelial phenotype and promotion of mesenchymal features, is linked to tumor cells' enhanced ability to move and infiltrate other organs. (31) Downregulation of the cell adhesion protein E-cadherin is a common feature of EMT, which can let cancer cells escape from the main tumor, enter the circulation, and spread widely. Tumor cells have been characterized as going through a reversal process, called mesenchymalto-epithelial transition (MET), to form metastases at metastatic locations.(31)

Cancer cells can be released from tumors as single cells or clusters, and the cooperative behavior between different cancer-cell increases their mutual survival and spreading capacity (32), and polyclonal clusters of CTCs produce metastases more rapidly than single cells (33). Circulating cancer cells are subjected to high shear pressures, the innate immune system, and oxidative damage. They connect with platelets to protect themselves during transit and undergo reversible metabolic alterations that improve their capacity to survive oxidative stress.(34,35) Melanoma cells, for example, have a high reliance on the folate system's nicotinamide adenine dinucleotide phosphate (NADPH)-generating enzymes, so inhibiting this route inhibits the metastasis.(36)

The timing of when CTCs enter circulation is important. Regarding their early development, there are arguments saying that the primary tumor can release the cells, but they are not mature enough to initiate metastasis.(37) Therefore, it is important to check the CTCs and DTCs character in the original tumor, especially the bone marrow as DTCs have mostly been found and defined there, and are easier to be identified.(38) DTC genome analysis revealed that cancer cells may diffuse early and follow a 'parallel path' to the formation of overt metastases.(10) Another crucial question is whether cancer cells actively invade or are passively shed from their source tumor. EMT is important for the processes causing tumor cell intravasation since ET has that motile mesenchymal-like state.(3) EMT can occur when tumor cells penetrate blood arteries and are coated by platelets, and the migratory potential is boosted.(34)

CTCs should contain cells with the capability to initiate metastatic growth in distant organs, and the CTC count has been linked to a worse progression-free and overall survival rate in patients with breast, prostate, lung, and colorectal cancer.(39) Recently, it was shown that possibly metastatic CTCs had a distinct protein profile which was correlated with their approach to seeding metastasis.(30,40) CTCs have a short half-life in circulation and mainly vanish when the original tumor is removed. Most of CTCs are destroyed and do not develop into metastases. The persistence or reappearance of certain CTCs after the main tumor has been removed most likely indicates the presence of active metastases. As a result, treating metastatic cells that have already established residency in distant organs will continue to be necessary.(41)

The advances in CTC and tumor cell-free DNA (cfDNA) detection technologies are allowing for early diagnosis and may help guide early treatment of metastatic recurrence.(42) Patients with advanced metastatic cancer, on the other hand, generally have larger CTC and cfDNA loads than can be identified with existing tests, which are useful for repeated non-invasive 'liquid biopsy' monitoring of medication response and the formation of resistant tumor subclones.(39,43,44) Metabolic pathways in CTCs with high metastatic potential may also be altered to fulfill the particular energy requirements of invasive and metastatic CTCs. Indeed, metastatic CTCs with enhanced mitochondrial respiration/oxidative phosphorylation shas recently been discovered to rely on Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) to drive mitochondrial biogenesis and oxidative phosphorylation. As a result, inducing mitochondrial biogenesis and respiration appears to be critical for cancer cell motility and metastasis.(45-48)
The cancer stem cell (CSC) theory believes that only some cells have the potential to originate and spread cancers (49,50), for example, leukemia and intestinal tumors (51), as well as oral (52) and breast cancer (53,54). Therefore, CSCs may become metastases initiating cells (MICs) without losing their stem cell characteristics by activating an extra set of metastasis-promoting genes.

According to the CSC theory, malignancies in tumor development were stimulated only by a few hidden CSC. That is why after any effective treatments such as chemotherapy and radiotherapy, we still find tumor recurrence, tumor dormancy, and metastasis. The comprehensive therapy needs to eradicate CSC too and not only shrink the tumor mass. There are four hierarchies of CSCs. First, a significant portion of the cellular heterogeneity found in tumors is due to its hierarchical arrangement, which is typically but not always, evocative of the tissue of origin hierarchy. Second, tumor hierarchies are fueled by a small number of self-renewing, that is typically dormant, CSCs, whereas the majority of the tumor is made up of non-CSCs, which can only proliferate for a short time and so do not contribute to long-term development. Third, non-CSCs seldom generate tumors in xenograft tests, indicating that CSC identification is hardwired. As a result, the tumor hierarchy has limited adaptability. Finally, CSCs are resistant to traditional chemotherapy and radiation treatments, which primarily target non-CSCs, which explains treatment relapse.(51,55)

The concept of CSCs supports the epigenetic's contribution to the phenotypic variety of different subpopulations of cancer cells within a tumor (49,56-60). The epigenetics contribute to the tumor's CSC to generate new CSCs and also nonself-renewing offspring to make up the bulk of the tumor. The key idea that continuing tumor development, metastasis formation, and recurrence after therapy are mostly due to the relatively uncommon subpopulations of CSCs inside individual tumors is implicit in this paradigm.(61,62) The surviving CSCs are competent to function as the antecedents of new tumor masses, ultimately leading to clinical recurrence, due to their tumor-initiating potential.

The distinction between CSCs and non-CSCs in carcinomas is considered to be mostly due to the cell biological program known as EMT.(63,64) EMT uses epigenetic modifications to give heritable phenotypic changes to cancer cells without delivering new genetic mutations. When EMT is activated, cancer cells lose many of their epithelial properties, such as epithelial cell junctions and apical-basal polarity, and instead gain mesenchymal traits, such as an elongated, fibroblast-like appearance and greater motility and invasion potential.(65) Only neoplastic cells within the CSC enriched fraction demonstrate elements of EMT program activation in a variety of carcinomas. (66) Forced activation of an EMT program in epithelial tumor cells significantly boosts their ability for tumor initiation in several experimental models of carcinoma.(67) Furthermore, activation of the EMT program imparts these tumor cells resistance to a variety of therapeutic treatments, which is another essential characteristic of CSCs.(68,69)

CSCs should be able to establish metastatic colonies after propagating to external tissues because of their unique ability to seed new tumors.(70) EMT program activation is needed for EMT-CSC connection, which is to open the entry into the CSC state and create the foundation for metastatic colonies, physical dissemination of carcinoma cells to distant tissues, and colonization in the distant tissues. EMT program activation suggests that lung metastasis grows to happens and skips the early phases of the invasion-metastasis cascade. (71) Furthermore, as previously indicated, disseminated carcinoma cells frequently show evidence of EMT activation prior to the development of macrometastases.(72,73) These findings support the idea that carcinoma cells can function as metastatic colony founders when the EMT program is activated. Figure 2 shows the mechanistic link between the EMT and CSC. When EMT was not activated, cells have very few or even no filopodium-like protrusions (FLPs), and become quiescence. EMT activation formed abundant FLPs, followed by integrin-extracellular matrix (ECM) connections formation, contributes to the development of mature adhesion plaques, thereby enabling the potent activation of focal adhesion kinase (FAK) signalling. FLP extension will enable metastases seeding efficiently, and enhanced metastasis-seeding ability of carcinoma cells (Figure 2A). On the other side, EMT-activated cells will induce the autocrine signaling and contribute to the CSC properties of cells with an active EMT programme (Figure 2B).(74)

The development of medication resistance frequently limits the efficacy of anticancer therapy. Intratumoral heterogeneity, or the phenotypic variety of cancer cells coexisting in the same tumor mass, has a role in acquired resistance. The CSC idea, which proposes the existence of small subpopulations of CSCs capable of seeding new tumors, has offered a framework for understanding one component of intratumoural heterogeneity.(74) Understanding the epigenetic mechanisms in tumors’ CSCs
Leader Cells In Collective Cancer Invasion

Leader cells are defined as cells in charge of route generation. Leader cells assist numerous elements of the collective invasion process, such as detecting the physicochemical microenvironment, guiding the invasion direction, generating a low-resistance invasion route, and coordinating with follower cells, which are located at the invading front. The metastatic cascade is increasingly recognizing collective cancer invasion with the leader-follower organization as a prominent mechanism. Leader cells aid cancer invasion by forming invasion tracks, perceiving environmental signals, and biochemically and biomechanically cooperating with follower cells.

Four major categories of leader cells including Mesenchymal and hybrid epithelial-mesenchymal (EM), basal, cancer-associated fibroblast (CAF) and tumour-associated macrophage (TAM). Matrix remodelling, cell mechanics and cell signalling, and cell reprogramming are all methods used by leader cells to build migratory pathways and performs their key functions as describe in Figure 3. Leader cells lead and coordinate the follower cells direction through biochemical and biomechanical mechanisms. The interaction can involve direct effects on the motility machinery, but also influence factors like metabolism that support a particular leader or follower cell activities. When all of these skills are combined, removing the leaders reduces the collective invasion. Leader cells can develop from tumor cells directly, or from stromal cells. Tumor cells-derived leader cells have better survival and tumor spreading due to their stemness and resistance to treatments, for example, mesenchymal or hybrid epithelial-mesenchymal (EM) and basal leader cells.

Basal cells are often thought to be solely epithelial, and their movement is unaffected by any sort of EMT. The leader cells express E-cadherin and don’t express mesenchymal markers such as TWIST1, SLUG (also known as SNAI2), or vimentin, which is consistent with this supposed epithelial phenotype. Smooth muscle actin (SMA) and calponin 1 were also absent from the leader cells, indicating that they were myoepithelial cells.

Stromal cells, in addition to tumor cells, can act as leader cells. Fibroblasts play a vital role in matrix remodeling and wound healing in normal tissue. Some alterations in extracellular matrix (ECM), DNA damage, oxidative stress, redox imbalance, and cell signaling, as well as microenvironment variant, can activate stromal fibroblasts during cancer growth. Through soluble factor production, metabolic consequences, immunological interaction, and matrix remodeling, these activated CAFs contribute to cancer development. For instance, in neck and lung cancer, the CAF leader cells both in vivo and in vitro express mesenchymal markers such as N-cadherin and vimentin, and EMT-related transcription factors, similar to mesenchymal leader cells.

Figure 2. The mechanistic link between the epithelial-to-mesenchymal transition (EMT) program and cancer stem cell (CSC) status. A: Interactions with ECM; B: Autocrine signalling loops. (Adapted with permission from Macmillan Publisher Limited).

Figure 3. Leader cell categories and key functions. (Adapted with permission from Springer Nature Limited).
Smooth muscle actin (SMA) and fibroblast activation protein (FAP) expression are also markers of CAFs. It's worth noting that CAFs' matrix remodeling capacity allows them to create tracks for cancer cells to spread from the initial tumor, allowing them to act as leader cells. TAMs, which are stroma-derived leader cells, can also help in the collective invasion. Macrophages are recognized to impact the development, regeneration, and repair of various tissue types under normal physiological settings, and TAMs usually contain macrophages. Originally, there is no single source of cells that can operate as cancer metastasis leader cells. Any cells of varied origins and morphologies are capable of performing the duties required for successful leadership with certain mutations (in non-clonal populations) and/or epigenetic modifications to permit leadership. When forced into the role of leader, however, a wide range of cells perform well.

Leader cells change the ECM to make it easier for follower cells to spread. Follower cells might have strong or weak adherence to the leader cells. Leader cells use a combination of techniques to modify 3D matrices, including matrix deposition, force-mediated matrix deformation, and pericellular proteolysis. Leader cells may biomechanically coordinate with follower cells and interact directly with the surrounding matrix. The exact nature of these mechanical interactions will be determined by the ECM's characteristics with cell movement. The development of coherently moving groupings of cells involving cell-ECM interactions, actomyosin contractility, and intercellular junctions is the result of this coordination. Various molecular pathways guarantee that the cells can create the necessary traction, transit through the ECM, and stay together to meet these demands. Different collectives respond to these demands in different ways, which is an intriguing sort of phenotypic development.

Cancer cells can reprogram to provide them with tumor-initiating ability, treatment resistance, and altered metabolic activities. In a 3D invasion study, cancer stem-like cells can act as leader cells and are capable to invade front and lead collective invasion. For these leader cells, this would be consistent with hybrid EM characteristics. However, reprogramming to a more stem-like state might be more widespread, as cancer stem-like markers like CD44 positive, CD24 negative, and Nanog expression have been seen in mesenchymal and basal leader cells in breast cancer. The metastatic process also is affected by the tumor type, the leader cell type such as tumor cell-derived or stromal cell-derived, including the stem-like leader cells inherent features.
proliferation and cell death to keep tumor mass constant in size; immune-mediated dormancy, when the immune system keeps proliferating tumor cells unchanged, as well as maintain the equilibrium between cell death and proliferation mostly via the cytotoxic activity of immune cells; and cellular dormancy, where the cell arrested in G0-G1 of the cell cycle thus become inactive, however not dead.(103)

Recent research focusing on cellular dormancy and its connection with immune cells suggests that quiescent DTCs are escaping identification and clearance by CD8+ T cells and natural killer cells (NK cells).(104,105) As they proliferate, immune cytotoxic responses may keep them in check, demonstrating how these two processes can be complementary. Single solitary DTCs or tiny DTC clusters of 10-20 cells make up dormant cancer-cell populations. These DTCs may enter a reversible growth-arrest or quiescence state, which supports the theory that solitary dormant cancer cells have this ability.(106) Indeed, one of the most excellent characteristics of dormant DTCs is their capacity to retain a high level of epigenetic and transcriptional flexibility while reactivating several developmental programs to stop growing and survive.(103,107) As a result, two crucial aspects appear to be required for a better understanding of DTC dormancy. One is their capability to resemble quiescence, senescence, or differentiation, and the second is their interaction to keep the angiogenic dormancy and immune-mediated homeostasis. The second is to determine whether the models' programs are functionally relevant in patients.(107,108)

Observations of niche-dependent chemoprotection of dormant cancer cells are independent of the cell cycle with the following mechanisms. First, dormant cells entered the G0-G1 cell cycle, making them less susceptible to cytotoxic chemotherapy drugs that target rapidly reproducing cells. (97) Second, the dormant cancer cells switch on Hypoxia-inducible factors (HIFs), endoplasmic reticulum stress pathways, and autophagy to downregulate pro-apoptotic and upregulate anti-apoptotic B-cell lymphoma (BCl)-2 family members, facilitating drug toxicity resistance. (109) Lastly, the dormant cancer cells upregulate Major Histocompatibility Complex (mHC)-II131 and programmed cell death 1 ligand 1 (PDl-1), which may interact with co-inhibitory receptors on natural killer (NK) and T cells and contribute to adaptive immunological resistance to immune checkpoint inhibitor treatment.(110) So, we can conclude the characteristics of dormant cancer cells as niche dependents, cell cycle arrested, drug resistance, immune evasion, and metastatic relapse as described in Figure 4.(111)

**Figure 4. Dormant cancer cell life cycle and the hallmarks of cancer cell dormancy.**(111) (Adapted with permission from Crown)

These elusive cells can disperse early and hide in specialized niches in distant organs before reactivating and causing disease recurrence after the initial tumor has been successfully treated. Despite their relevance, we have yet to utilize the promise of targeting dormant cancer cells to prevent disease relapse in the clinic using the knowledge gained from experimental models. As a result, advancements in technologies that combine geographical information with single-cell transcriptomics, epigenomics, proteomics, and metabolomics will drive future discoveries. Using this new understanding, it is expected that latent cancer cells will be eradicated and illness recurrence will be avoided.(111)

### Principle of Drug Resistance in Cancer

Drug resistance is the most significant impediment to cancer patients attaining cures. Polychemotherapy, or the combination administration of agents having non-overlapping modes of action, was the first response to the problem of single-agent chemotherapy resistance. Polychemotherapy's accomplishments had essentially plateaued by the turn of the century, some 50 years after its commencement. Many tumor forms were incurable after surgery, radiation, and polychemotherapy. As a result, novel treatment techniques aimed at addressing the essential enabling traits and acquired capacities that turn normal cells and tissues into cancers began to emerge. The development of medicines that disturbed these distinguishing characteristics, including targeted therapy, was a significant step forward.(3,4)
Oncological therapy has progressed significantly in recent years, thanks to the use of immunological techniques to detect and combat cancer. Anti-CTLA4 and anti-PD-1/PD-L1 monoclonal antibodies that inhibit adaptive immune system negative regulators, or checkpoints, have shown significant anti-tumor activity—and even cures—in a variety of tumor types.(112) Nonetheless, much like with traditional chemotherapy, eventual resistance to targeted and immunological treatments is the norm. The parallels between cancer and infectious illnesses may begin to fade at this point: Combination therapy frequently results in illness being undetectable in HIV or cure in TB, although this is the exception rather than the rule in metastatic malignancies. (113) Seems cancer is more complicated than that.

Generally, the clinical response of cancer therapy depends on three factors: a pharmacological feature of the therapy, immune responses of the cancer cell population target; and a specific host environment. These factors also involved tumor resistance. Thus, the resistance problem may be controlled by focusing both cancer and therapeutic science on targeting each variable separately. Through several mutational events, cancer cells acquire genomic changes, resulting in geographic and temporal genetic diversity.(113,114) The genomic changes can happen either at a slow rate as compensation for the aging process but can be dramatic and catastrophic in a short time due to genomic or chromosomal instability, and demand even earlier intervention.(115)

Another factor that mediates resistance is the niche or tumor microenvironment. This surrounding space is made up of immune cells, stroma, and blood vessels. Those can prevent the immune system from clearing tumor cells, obstructing drug absorption, and stimulating paracrine growth factors signal to support cancer growth.(116) The lack of 2-microglobulin (which hampers tumoral antigen presentation) and JAK1 or JAK2 are two mechanisms of checkpoint blockade resistance that have been identified. (117-119) Even with state-of-the-art, high-resolution imaging methods, solitary tumor cells disseminated into the bloodstream in individuals with early-stage cancer are frequently undetectable. However, DTCs that have moved to distant organs via the vascular or lymphatic system might survive treatment and induce tumor regrowth, leading to disease recurrence and metastasis.(31,120)

It's worth noting that the immune system might play a crucial 'gatekeeper' function in these processes. Immune escape or suppression has long been thought to be a crucial stage in the creation and spread of tumors.(121) The term "cancer immunoediting" refers to a variety of complicated biological processes involving a variety of cell types and molecular mechanisms.(122) Elimination, equilibrium, and escape are the three critical steps of cancer immunoediting. (123) The first step, elimination, depicts the innate and adaptive immune cells' confinement and destruction of neoplastic cell microcolonies. After neoplastic cells have survived their initial contact with the immune system, the second phase is the equilibrium of tumor and immune cells, and a selective pressure will be exerted on to the genetically unstable and highly diverse cancer cells, causing the tumor cell subpopulations to become more refined.(124) Although the immune systems engaged in the equilibrium phase are largely meant to prevent tumor formation, they can also support tumor proliferation through clonal selection, leading to the third stage, which is immune escape. The chosen cancer cell clones that have acquired immune evasion and/or suppression mechanisms obtain the capacity to thrive and multiply in an immunocompetent environment during the escape phase.

The CTCs will leave the primary tumor with an immunosuppressive microenvironment and reach the non-cancer tissues' active immunosurveillance systems. The higher number of tumor-immune cells and lyse the CTCs. As a result, cancer cells may find the circulatory system to be a hostile environment. The fact that primary tumors are expected to release millions of cells into circulation every day, but only a tiny number of them have the potential to spread to distant metastases, supports this hypothesis. Nonetheless, research has shown additional routes by which CTCs may dodge or survive immune cell interactions.(125)

**Lineage Plasticity in Cancer**

In contrast to these genetic pathways of drug resistance, new research suggests that non-mutational processes play an important role in therapeutic evasion.(126) Small populations of cancer cells typically survive medication therapy, even with the most potent cancer medicines known as a MRD, which can result in a partial or full response.(127) These leftover cells are also regarded to be a reservoir of slow-cycling cells that might eventually develop permanent genetic changes, resulting in medication resistance.(128)

Phenotype switching, also known as cell plasticity, is a crucial mechanism that occurs during development, as well as after injury and illness, and allows cells to adopt different In contrast to these genetic pathways of drug resistance, new research suggests that non-mutational processes play an important role in therapeutic evasion,
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Phenotype switching, also known as cell plasticity, is a crucial mechanism that occurs during development, as well as after injury and illness, and allows cells to adopt different phenotypes to adapt to changes in their environment. (129) Cancer cell plasticity is described as a cell's capacity to significantly alter its identity and adopt a new phenotype that more closely mimics a unique developmental lineage. Under the threatened condition of targeted anticancer therapy, tumor cells adapt and survive through lineage plasticity, resulting in drug resistance and metastasis. (130,131) This survival of pluripotent progenitors increases tumor cell variety and yields a wide complement of different cell phenotypes, either in a well-defined canonical lineage or even a very new hybrid lineage. (132)

Cell plasticity allows cancer cells to revert to a cell identity that is not dependent on the drug-targeted pathway. Targeting cell plasticity opens up new possibilities for preventing drug-resistant cell states and allowing particularly targeted medicines to reach deeper responses. (133) For example, the occurrences of non small-cell lung cancer (NSCLC) transformation into small-cell lung cancer (SCLC) after therapy with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs). (134) Surprisingly, after a medication break, several of these individuals recovered sensitivity to EGFR-TKI therapy showing the reversibility of drug sensitivity points to non-mutational drug escape mechanisms. (135)

EMT program is known to increase plasticity. In the NSCLC cell line, MET induces EGFR-TKI resistance by dual histone deacetylase (HDAC), 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor JMF3086, and the E-cadherin, vimentin, E3 ubiquitin-protein ligase Hakai knockdown. (136,137) Then post-treatment biopsy samples from patients who developed resistance to EGFR-TKIs revealed that certain tumors had increased vimentin expression while losing E-cadherin expression when compared to pre-treatment samples. These suggested that EMT is an acquired resistance mechanism and shows a strong link between plasticity, stemness, and metastasis. (134,138)

Along with well-known genetic changes, cell plasticity has lately emerged as a key factor in therapeutic resistance. Cells that survive cancer medication therapy can undergo additional reprogramming to become drug tolerant. This reprogramming has been linked to epigenetic and transcriptional alterations, which is consistent with the reported phenotypic flipping reversibility. Furthermore, the tumor microenvironment and cancer cell of origin may play a role in the formation of new cell identity in response to medication therapy. Upon drug exposure, tumour cells first go through a quiescent drug-tolerant state which are in the G0/G1 cell-cycle phase, before further reprogramming into a drug-resistant identity. These dormant, or slow-cycling cells that can either regain drug sensitivity when treatment is discontinued, or acquire permanent resistance to therapy and drive relapse. Crosstalk with the tumour niches, via secretion of cytokines including CAFs, TGF-β, hepatocyte growth factor (HGF) or interleukin (IL)-6 or tumour necrosis factor (TNF) can control cell plasticity. Prolonged therapy will lead residual cells to resume proliferation, by further epigenetic reprogramming or genetic mutation and become permanently resistant (Figure 5). (139)

### Metastatic Niche Functions and Therapeutic Opportunities

Cancer biologists and clinical oncologists are both interested in how cancer cells migrating from a tumor into the bloodstream manage to infiltrate distant organs and commence metastatic development. Recent discoveries have begun to characterize the origins, phenotypic features, and how cancer cells acquire survival, self-renewal, dormancy, hosting niches, initiate metastasis and reactivate them. Hope metastasis can be prevented when we reach an understanding of these. (140)

Cancer cells create a local tumor microenvironment (TME) or metastatic niche during the early stages of metastatic development. It is distinct from normal tissue structure and essential for metastatic outgrowth. It is difficult to differentiate these metastatic-niche cells within the bulk tissue, especially in the early stages, which hampers the identification of cells while they still colonize in low numbers, and the metastases grow. (141) The organs of potential metastasis are not passive recipients of circulating tumor cells, but rather are selectively and actively changed by the source tumor before metastatic dissemination. Activity of tumor-secreted proteins and tumor-shed extracellular vesicles, that enable the ‘soil’ at distant metastatic locations to support the expansion of arriving cancer cells, is required to sow the ‘seeds’ of metastasis. (142)
‘Pre-metastatic niches’ (PMNs) are pre-determined microenvironments that facilitate future metastatic tumor growth.(143) The first step in this sequence is vascular leakiness, continued by local resident cells changes, then non-resident cells attraction including bone marrow-derived cells (BMDCs) to PMNs, which then attract CTCs.(144-147) Although it is consistent with both Paget’s and Ewing’s theories, the PMN posits that the original tumor uses tumor-derived substances to prepare certain organ locations for future metastatic illness that is prior to CTC arrival. In contrast to the metastatic niche, which is established and molded following the entrance of cancer cells, the PMN is an aberrant, tumor-growth-promoting milieu devoid of cancer cells.

PMN formation can be induced by tumor-secreted factors and extracellular vesicles (EVs) which are secreted by primary tumors, and non-tumor factors which alter the microenvironment to be sufficiently receptive to colonization by CTCs such as ageing, infection, or post-surgery effect in on or more target organ, prepare for metastasis seeding.(74) Due to the existence of a pro-tumorigenic, inflammatory milieu caused by tumor-secreted proteins, PMNs are likely sites of immunological dysregulation, resulting in immunosuppression and coagulation problems.(148) Thus, PMNs are the combination product of systemic tumor-secreted factors and tumor-shed EVs that support a temporal sequence of events during PMN development. Specific subclones derived from the primary tumour will yield different PMN for distant organ, as described in Figure 6.

Many efforts have been developed to increase the accuracy of metastasis detection. One of those is by identifying PMN structural changes using biomarkers such as vascular endothelial growth factor receptor (VEGFR)-1, BMDCs, or CD68+ myeloid cells, genetic profiling, high-resolution imaging, and tissue density.(149,150) Importantly, the activation of the sphingosine 1-phosphate receptor 1 (S1PR1)–signal transducer and activator of transcription 3 (STAT3) signaling pathway in CD68+ myeloid cells is required for their recruitment to PMNs and subsequent metastatic expansion.(151-153) In mice, inhibiting VEGFR1 with a new peptide antagonist called iVR1 totally stopped the production of hepatic PMNs and the spread of colorectal cancer cells to the liver.(154) Other molecules including dickkopf 1 (DKK1), VEGFA, VEGFD, and HIF-1 might be used as PMN biomarkers in human LNs to predict future metastasis in cancer patients.(155)

EVs might be one of the most promising indicators for predicting tumor development and metastatic risk because of their accessibility, abundance, and stability in circulation. The sensitivity of detecting tumor EV-associated biomarkers in cancer patient's blood can be improved by developing strategies to enrich for tumor-derived EVs and characterize their specific cargo.(156,157) Tumor organoids might also be utilized to develop tumor-specific biomarkers that can be validated in the patient plasma due to technical difficulties, such as acquiring fresh specimens and limited survival of tumor-derived explants, in identifying tumor-
secreted substances that are implicated in PMN production. Exosomal miR-105 produced from primary tumors from breast cancer patients whose tumor cells showed high levels of miR-105 may be found in patient blood before clinical identification of metastases.(24)

Metastatic habitats can arise either as DTCs arrive in the recipient tissues (158), or before DTCs are seeded, under the impact of secreted factors and/or exosomes produced by the source tumor, also known as the pre-metastatic niche. (159) Many investigations have shown PMN in many organs, including lymph nodes, the lung, the liver, the bone, and, to a lesser extent, the brain. (158) DTCs can potentially create metastatic niches by occupying pre-existing resident stem cell niches. This phenomenon has been reported in bone metastases (160), but its significance in other organs has yet to be determined.

The metastatic niche has a variety of roles that support disease development and can change during the metastatic initiation phase. The microenvironment is needed to support the DTCs recovery process by protecting the cancer stem cell phenotype and keeping the cellular plasticity from differentiation signals. The prolongation of this first phase might lead to metastatic dormancy, which can last for a long time depending on the interaction between the niche and the DTCs' MIC capacities. By providing supporting growth signals and promoting cell flexibility and immune evasion, the niche can promote metastatic expansion even further.

The PMN and early metastatic niches play an important role in cancer cell landing, providing anchoring while avoiding anoikis and apoptosis. Extravasated cancer cells, for example, stay adhered to the exterior artery walls in the perivascular niche, seeking physical support for recovery while remaining inactive. (160,161) This process is aided by mesenchymal stem cells, which operate as pericytes around the vasculature. (162) Primary tumors secrete EVs and cytokines to enhance endothelial FAK/E-selectin-mediated permeability and generate angiogenesis, lymphangiogenesis, and vascular leakiness which helps DTCs. (162) Pro-inflammatory consequences of vascular leakiness and tumor exosomes include increased expression of the chemoattractant S100A8/9 (163), which attracts CTCs to the pre-metastatic lung niche. Chemoattractants serve as homing receptors in metastatic organs, for example, CXCL12 and CCL21 for CXCR4 or CCR7 in breast cancer and melanoma cells. (164)

Recent results claiming that EMT is not essential for metastasis might be explained by this severe mesenchymal differentiation. (165) Metastatic cancer cells in partial EMT or MET phases, on the other hand, are extremely plastic and have improved metastasis-initiation skills. (166) Metastatic niches may thus play a role in facilitating epithelial and mesenchymal state plasticity interconversion.

Because a metastatic niche is required, it is possible to prevent or limit metastasis by therapeutically blocking its molecular and cellular connections with DTCs. Astrocytes were found interacting with DTCs through gap junctions in the brain metastatic niche to support their survival. (167) Tonabersat and meclofenamate are two FDA-approved gap junction inhibitors to target brain metastasis which showed encouraging results in their clinical trials.
Targeting Metastatic Cancer Therapies

Patients with overt metastatic illness are diagnosed as having stage IV cancer or have a distant recurrence after the initial tumor has been removed. Cross-sectional imaging can identify metastases of at least 1 cm³ in these individuals. Systemic treatment may be able to control such overt metastases clinically, but it is usually incurable. Systemic treatment can be delivered for early-stage cancer with palpable tumors, but no visible metastasis though micrometastasis is suspected. Adjuvants were needed for patients with stage II or III, and significantly prolong overall life as well as decrease recurrence rates, showing that adjuvants and neoadjuvant therapy can eradicate the DTCs or their metastasis-initiating capacity. The majority of treatment efforts, on the other hand, have been directed towards the last stages of the metastatic cascade: colonization of distant organs. New medications are usually tested in clinical trials on patients with advanced, therapy-resistant metastatic illness, partly because there are no viable mainstream therapies for these individuals. This gives an ethical justification for testing novel drugs in these individuals who have never been exposed to them before. Second, given the high pace of growth of advanced metastatic illness, the success of therapy may be assessed in a relatively short period of a few months.(171) While actually for more successful therapy, it is not only about early treatment to prevent metastasis but also paying attention to the micrometastatic cells. Primary tumor disseminate cancer cells via blood of lymphatic system to migrate, circulate and extravasate while adapting to a number of stress. The survive cells can enter dormancy, or retain the ability to regenerate tumor during the cryptic phase of metastasis in distant organs, so-called MICs. Most resistance and relapse are driven by the plasticity and persistence of MIC states within macrometastases (Figure 7).(41)

Current therapies only target the macrometastatic cells. Even in early-stage cancer patients, surgery alone is done without any adjuvant/neoadjuvant anti-metastatic therapy. Furthermore, because several years of follow-up of large patient cohorts are required to prove treatment effectiveness, the expense and administrative burden of doing adjuvant/neoadjuvant research are significantly higher than those of conducting a trial in the advanced metastatic scenario.(8) Three systemic treatment approach for treating micrometastatic and macrometastatic disease including chemotherapy, targeted therapy, and immunotherapy. While
Cytotoxic chemotherapy is still the gold standard for treating metastasis and is the sole therapeutic choice for many cancer subtypes, medications that target tumor-driving oncoproteins, or ‘targeted therapy,’ are improving results in many malignancies. Somehow, tumor subclones with drug-resistance mutation need additional treatment specific for the mutation. The treatments targeting genetic or pharmacological mutated cells allow cancer cells to be selectively killed while normal cells are spared, for example, malignancies with DNA damage repair deficiencies can be treated with poly ADP-ribose polymerase (PARP) inhibitors, while BRCA positive breast cancer is treated with cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors with estrogen receptor antagonists. Other experiments use nanoparticle delivery systems, antibody or bispecific antibodies conjugates to deliver higher doses of drugs targeting specifically to the cancer cells with minimal effect on normal cells so the therapeutic window can be maximized.

Immune checkpoint inhibition (ICI) has revolutionized the treatment of metastasis during the last decade, and has a potential value of targeting specific components of the tumor stroma (T cells) to treat metastasis (Figure 8). Dormant and micrometastatic are the cryptic phase is the potent target for effective ICI strategy. Antibodies that inhibit the receptor-ligand interactions of CTLA4 and PD-1, unlike other medications for metastatic cancer, can provide long-term, persistent responses, including full tumor regression in certain individuals. Those are metastatic cancers that have a high tumor mutational load, resulting in more mutated peptides being processed, displayed on tumor cell-surface MHC class I molecules, and identified by the immune system as foreign 'neoantigens'. Not all types of metastatic cancers respond to anticancer ICI responses. A combination of multikinase inhibitor treatment with ICI in metastatic tumors that were previously immune-resistant is expected to be a potential of this strategy.

While most anti-metastatic medications are systemic, targeting organ-specific metastasis mediators has the potential to significantly enhance patient outcomes. Some tumors metastasize to a single organ and remain there for the rest of the patient's life, while others spread to other organs over a long period. Such characteristics provide the possibility of improving survival through localized treatment. A typical example is CRC metastatic to the liver. The hepatic portal vein receives all blood from the intestines and transports it to the liver. Thus liver becomes the most often metastasis target compared to other organ, especially from intestinal malignancy which must first pass via the hepatic sinusoids, then usually stuck there. Hepatic artery infusion chemotherapy, radiofrequency ablation, and embolization are all examples of liver metastasis-directed treatments that are becoming more popular.

The discovery of medications that target the processes underpinning cellular plasticity and epigenetics might lead to deeper and more persistent responses, given its significance in the formation of drug tolerance and resistance. There are three primary kinds of cell plasticity strategies:
avoiding tumor cell plasticity, deleting emerging cell identity, and reversing phenotypic flipping. By inhibiting tumor cell plasticity, targeted medicines may have a longer therapeutic response. The molecular processes that allow slow-cycling cells to survive, such as chromatin landscape remodeling and transcriptional adaptability, can be blocked, for example. Interfering with numerous signaling pathways, such as WNT and STAT3, as well as cellular compartments that make up the tumor microenvironment, such as fibroblasts and macrophages, is another intriguing option for therapeutic development. The evolving drug-tolerant cell state might include weaknesses that could be exploited. The drug-tolerant mesenchymal cells would be killed if their pharmacological blockage was successful. Because phenotypic flipping is mostly controlled by epigenetic changes, it's fair to believe that it can be reversed to re-sensitize cells to treatment.

Targeting the evolving drug-tolerant cell identity is another technique to target cell plasticity. This might be accomplished by utilizing known medicines or by identifying new vulnerabilities. Ongoing research is being done to see if tumors that have undergone neuroendocrine differentiation have treatment sensitivity profiles that are comparable to de novo small-cell histology. In contrast to non-transformed EGFR-mutant adenocarcinomas, transformed SCLC lose EGFR expression, which explains their resistance to EGFR inhibitors.(180) Transformed SCLC, on the other hand, is more like de novo SCLC in that it is transiently susceptible to platinum-etoposide. (181) However, transformed SCLC showed greater response rates to taxanes than de novo SCLC in a recent retrospective analysis of 67 patients with NSCLC but did not react to checkpoint inhibitor treatment, which is similar to typical EGFR-mutant adenocarcinoma.(182) In a phase II study with the anti-PD-1 pembrolizumab, a subgroup of patients with enzalutamide-refractory prostate tumors were identified as remarkable responders.(183)

### Adjuvant Trial Design

Adjuvant therapy is additional therapy given after primary therapy has been given, to lessen the chance of recurrence and enhance patient survival.(184) Adjuvant chemotherapy, for example, improves absolute overall survival by 4% in NSCLC patients after resection after 5 years.(185) Adjuvant clinical studies, somehow, may require a large number of data to have a significant beneficial result. The APHINITY (NCT/01358877) involved 4,805 breast cancer patients and only showed a 0.9% advantage.(186)

Early detection and therapy in cancer such as ctDNA analysis, increase a good percentage of survival. There are two types of studies that have been done so far to identify ctDNA in the adjuvant context and identify the "molecular relapse" which is the lead time before clinical relapse. Multiple approaches for detecting CTCs have been developed; however, the FDA-approved Cell Search system, which positively selects for EpCAM-expressing cells, is the most extensively used method in clinical research.(187) Some researchers have looked at the capacity of CTCs to identify MRD after final therapy. In 21.5% of individuals with early breast cancer, CTCs were discovered after surgery but before adjuvant treatment. CTC detection, on the other hand, was only marginally related to a shorter disease-free survival in this patient group (hazard ratio = 2.1). Although having a larger number of CTCs (>5 CTCs) was associated with a worse disease-free survival (hazard ratio = 4.5), this was only detected in about 3% of individuals.(188) When directly compared to CTCs in the context of metastatic illness, ctDNA has been demonstrated to have greater sensitivity and to capture a broad range of a patient's diverse mutational profile.(189)

These approaches are particularly positioned to guide targeted therapy in the adjuvant scenario due to the substantial genetic information provided by tumor-derived material from blood analysis. A non-randomized strategy, similar to the clinical trial design proposed above, may be viable if ctDNA is first established as a surrogate endpoint. Due to intratumoral heterogeneity or tumor evolution, targeted therapy may be directed to the genomes of the residual illness, which may differ from the primary due to intratumoral heterogeneity or tumor progression.(190) ctDNA analysis in advanced EGFR-mutated NSCLC might detect the emergence of resistance mutations during adjuvant targeted treatment to trigger an appropriate adjustment in therapy.(191) Furthermore, adjuvant treatment with sequential analysis of ctDNA may offer rich genetic information on mechanisms of response and resistance, allowing therapy to be tailored before disease development. As a result, biomarkers like ctDNA might be used as surrogate endpoints in adjuvant studies, allowing for faster progress in the field of adjuvant medicines by allowing for earlier endpoints than disease-free survival or overall survival to show treatment benefits. To verify the suggested novel surrogate endpoint, rigorous review and analysis will be necessary.(192)
All cancer treatments, sometimes add with adjuvant therapies were aimed to excised the primary tumors and avoid recurrence, and enhance patients survival. To create effective therapies, a deeper knowledge of the foundation for metastatic colonization, their niches, the characteristics, any genetic and epigenetic pathways involved in different organs, and its latent period is required to treat both micro, and macrometastasis.

Conclusion

AM drafted and wrote the manuscript, NMD edited the manuscript, AW proposed the manuscript topic, supervised, and edited the manuscript.

References


vesicle-MaNiA: extracellular vesicles in liquid

Human prostate cancer metastases target the hematopoietic

Primary tumor-secreted lymphangiogenic

Prognostic

Powerful anti-tumor and anti-angiogenic


