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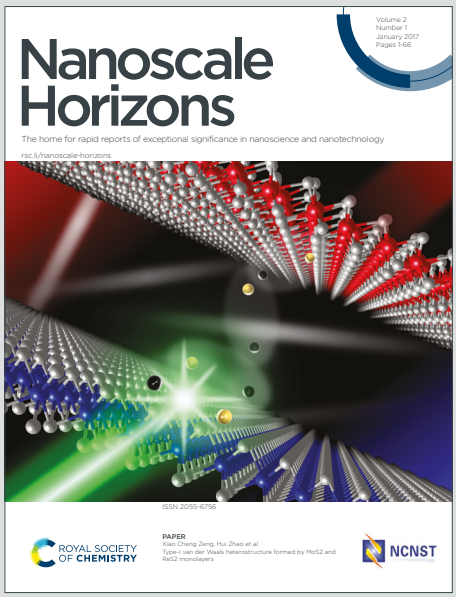

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Disrupting the metastatic cascade: Nanoparticle-based innovations in antimetastatic therapy

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Abstract

Antimetastatic therapy represents a transformative shift in oncology, addressing one of the greatest challenges in cancer care: the prevention and management of metastasis. By targeting the critical steps of the metastatic cascade and integrating them into multimodal treatment strategies, these therapies have the potential to greatly improve patient survival, enhance quality of life, and advance cancer care worldwide. Each stage of the metastatic cascade presents unique therapeutic vulnerabilities and opportunities for targeted intervention. Nanoparticle-based therapies have emerged as a promising frontier for disrupting the process of metastasis due to their ability to deliver therapeutic agents with high precision, reduce off-target effects of treatment, and overcome biological barriers. This review explores key targets suitable for antimetastatic therapy and recent advances in strategies using these modalities, highlighting their potential to improve treatment specificity and efficacy. By examining the integration of nanotechnology into antimetastatic therapy, we aim to underscore its transformative potential in combating metastatic cancer and improving patient outcomes.

Keywords: antimetastatic treatment, nanoparticles, migrastatics, metastasis

1. Metastatic cascade

To this day, secondary tumor foci – metastases - remain the leading cause of cancer-related morbidity and mortality.[1] To colonize distant sites, cancer cells have to undergo several fundamental events collectively termed "the metastatic cascade", wherein such activation of

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invasion and metastasis is specifically recognized as a hallmark of cancer.[2] The process is initiated by the invasion of primary tumor cells into their local surroundings, followed by intravasation into the bloodstream or lymphatic system. Circulating tumor cells (CTCs) must then survive in blood circulation and lymphatic system, extravasate, and proliferate in distant organs. The initial switch of the metastatic process triggering the tumor dissemination arises from a set of morphological, metabolic, and functional changes cancer cells undergo, collectively termed epithelial-mesenchymal transition (EMT).[3] This process is generally employed in cell development, during embryogenesis, and wound healing.[4] However, in this case, it is hijacked by cancer cells, enabling them to lose epithelial properties and obtain certain mesenchymal features such as increased motility or invasiveness, and resistance to anoikis.[5] These changes are critical for successful cancer progression and play a key role at every stage of metastatic cascade including invasion, intravasation, and extravasation. During EMT, epithelial cells lose cell-cell junctions, reorganize their cytoskeleton, and alter their gene expression patterns leading to a transition from polarized, cuboidal epithelial cells to mesenchymal spindle cells.

EMT is often triggered by microenvironment changes such as inflammation, hypoxia, and exposure to cytokines and growth factors.[6] Many studies report the "partial EMT" or cells residing in a spectrum of intermediate states between epithelial and mesenchymal phenotypes leading to a re-evaluation of how EMT is defined and understood in the context of cancer progression.[7],[8] Metastasis exhibit organ-specific distribution. The "Seed and soil" hypothesis suggests that tumor spreading is guided by the cooperation of the malignant cells ("seed") and host organ environment ("soil") and therefore, proceeds in a specific manner.[9] Cancer stem cells (CSCs) possessing tumor initiation and phenotypic plasticity properties also contribute to the tumorigenic and malignant potential of tumors.[10] In general, the metastatic progression can follow either linear or parallel model.[11] While the linear model suggests a high genetic similarity in primary tumor and subsequently evolved metastases, in the parallel model the early disseminated tumor cells acquire metastatic capabilities independently of the primary tumor. Together these models underscore the complexity of the cancer metastasis process including the phenotypic and genetic diversity between primary and metastatic tumors.

1.1.Local invasion

At certain critical point, the tumor size and progression are severely limited by the amount of oxygen and nutrients supplied by blood vessels.[12] To access the distant sites, tumor cells must successfully invade the stromal and vasculature surroundings of their primary tumor.

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Establishing the tumor's own neo-vasculature beyond preexisting blood vessels is therefore a decisive step in the evolution of metastasis.[13] Not only it provides crucial nutrients and oxygen for the progression of the primary tumor, it also offers a route for the dissemination of tumor cells into hematogenous circulation and to colonize distant anatomic sites. This transition is referred to as "angiogenic switch".[14] This component of tumor development is initiated by a disturbance of the equilibrium between pro- and antiangiogenic factors.[15] While the prevalence of antiangiogenic aspects is associated with tumor dormancy, inclining the balance towards pro-angiogenic factors results in tumor angiogenesis and overall tumor proliferation. The angiogenic switch is triggered by a complex interplay of factors, including the release of pro-angiogenic molecules as vascular endothelial growth factor (VEGF) by cancer cells, the protease degradation of the extracellular matrix and recruitment of endothelial cells to the tumor site. Once this switch is turned on, the formation of new vasculature begins and supplies the tumor with oxygen and nutrients, allowing it to grow and spread. Therefore, the angiogenic switch is a crucial therapeutic target and inhibiting angiogenesis can prevent further tumor development.[16] Several anti-angiogenic drugs are currently in use, including bevacizumab (Avastin®) and sorafenib (Nexavar®).[17] Bevacizumab is a monoclonal antibody that specifically targets VEGF and prevents it from binding to endothelial cell receptors and thereby inhibiting angiogenesis. Sorafenib is a kinase inhibitor that blocks angiogenesis-promoting signaling pathways by targeting several receptors involved in angiogenesis, including VEGF receptors, platelet-derived growth factor (PDGF) receptors, and Raf kinase. However, there are also challenges associated with anti-angiogenic therapy, including resistance and many side effects often limiting their use in certain patient populations.[16]

Another process also observed in primary tumors is lymphangiogenesis. In cancer, lymphangiogenesis can promote the spread of tumor cells to nearby lymph nodes and distant organ sites, a process known as lymphatic metastasis.[18] Therefore, the presence of metastasis in lymph node often serves as an early prognostic indicator of metastatic dissemination and tumor invasiveness, particularly in carcinomas or melanomas.[19] Additionally, changes in lymphatic endothelial cell (LEC) characteristics play a role in the formation of premetastatic niche. A signaling pathway involving VEGF-C and PI3Kα activates integrin α4β1 on LECs, promoting expansion of lymphatic endothelium and enhancing the capture of metastatic cells expressing vascular cell adhesion molecule-1 (VCAM-1).[20] Furthermore, cancer-associated fibroblasts (CAFs) producing high levels of periostin can compromise the lymphatic endothelial barrier by activating the integrin-FAK/Src-VE-cadherin pathway in LECs, therefore promoting

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lymph node metastasis.[21] Integrin αIIb is also upregulated in LECs of tumor-draining lymph nodes, enhancing their ability to bind fibrinogen, which supports the adherence and survival of metastatic tumor cells.[22]

1.2.Intravasation and survival

Successful intravasation of tumor cells provides a route for circulating tumor cells (CTCs) that are shed into the bloodstream by primary tumors and can be found in the peripheral blood of cancer patients.[23] CTCs can move either as individual cells or as clumps of cells that can survive circulatory stresses until they encounter microvessels in distant sites.[24] Nowadays, studying their molecular and functional characteristics represents an important area of cancer research of highly lethal cancers. CTCs are difficult to detect as they only represent a small fraction of the total cells in the blood.[25] CTCs analysis can be used to identify specific mutations or biomarkers associated with drug resistance or other clinical outcomes and can help with overall treatment decisions.[26],[27] Moreover, by studying CTCs we can also gain insights into the mechanisms of cancer metastasis, providing opportunities for the development of new targeted therapies.

The transport and survival of CTCs is a complex process that involves several factors. CTCs need to evade the immune system[28], withstand shear forces[29], and adhere to blood vessel endothelium[30] in order to extravasate and form metastasis. Shear forces of the bloodstream cause huge mechanical stress on CTCs, leading to their rupture or deformation. Some CTCs can withstand such forces by altering their cell membrane proteins and cytoskeleton.[31] Evading the immune system is possible by downregulating the expression of MHC class I molecules that are recognized by immune cells, reducing their visibility to the immune system.[32] Additionally, CTCs can secrete factors that suppress immune cells, such as TGF-beta (transforming growth factor beta).[33] During transport, CTCs also interact with other blood cells, especially platelets, which is mediated by the expression of integrins and selectins.[34] When platelets encounter CTCs, they can adhere to them and form complexes called circulating-tumor cell-platelet aggregates (CTC-PAs). CTC-PAs then travel through hematogenous circulation, providing CTCs with many benefits, such as forming a protective shield around them, shielding them from immune cells, and preventing their destruction. These adhesions enable cancer cells to evade natural killer cells due to the upregulation of CD155 inhibitory checkpoint.[35],[36] Moreover, platelets can assist in the extravasation of CTCs into nearby tissues and promote adhesion to the endothelial cells that line blood vessels.[37],[38] Specifically, P-selectin responsible for the cell arrest at activated endothelium is being

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investigated nowadays as a potential nanotherapeutic target due to its high expression in tumor tissue compared to healthy tissue.[39] Further, platelets promotes the formation of small blood clots or microthrombi that create a favorable environment for establishing new tumor sites. Understanding the precise mechanisms of these interactions as well as identifying selective pathways for inhibiting them, may translate into establishment of promising antimetastatic therapies. Neutrophil extracellular networks (NETs) have also emerged as major factor facilitating cancer metastasis. CTCs typically invade DNA- and protein-rich NETs, which generates protective particles that hide CTCs from immune responses, hematogenous and mechanical stresses, and facilitate CTCs adhesion to endothelial cells.[40] In addition, components of NETs, including histones, proteases, and antimicrobial peptides, can degrade extracellular matrix (ECM) components, increasing the invasive ability of tumor cells.[41] NETs may provide pro-tumorigenic pathways by sustaining tumor-induced inflammation conducive to metastatic colonization and growth.

Another mechanism involved in the survival of cancer cells in circulation is adhesive support. Loss of attachment to the extracellular matrix or neighboring cells often leads to anoikis, a type of programmed cell death. Resistance to anoikis is a key mechanism that allows survival of cancer cells since the bloodstream represents a hostile environment to CTCs with a lack of extracellular matrix.[42],[43] The resistance mechanisms involve alterations in gene expression or the formation of the above-mentioned CTC-PAs.[44] Research has shown that CTCs resisting anoikis are likely to successfully extravasate and establish new tumors, subsequently evade immune surveillance, and resist chemotherapy and other treatments of cancer.[45] For this reason, targeting anoikis in cancer therapy may further improve the prevention of cancer metastasis.

1.3.Extravasation

Another challenge for tumor cells disseminated from primary tumors and surviving stresses in the bloodstream is their invasion through the endothelial vasculature into secondary tissues, a process known as transendothelial migration (TEM). The complex process of homing disseminated cancer cells into the secondary organs is still not fully understood. However, interactions between cancer cells and the target organ microenvironment, involving signaling molecules providing cues for CTCs to adhere, invade, and establish a metastasis, are crucial. Key steps and actions of successful extravasation include the adhesion of CTCs to the endothelial cells lining the blood vessels of target organs via surface adhesion molecules such as integrins and selectins, subsequent rolling of CTCs along the walls of blood vessels allowing

finding the optimal extravasation site,[46] activation of endothelial cells by CTCs to create gaps in the endothelial layer to facilitate their extravasation accomplished through the secretion of chemokines, cytokines,[47] then diapedesis or transmigration of CTCs through endothelial gaps and final extravasation in the target organ and establishment of a new tumor.[48] One of the proteins involved in the homing process is metadherin (MTDH). MTDH was shown to promote the attachment of CTCs to the extracellular matrix components of the target site and increase their invasive potential by promoting invadopodia formation.[49] Furthermore, MTDH can modulate the activity of various key signaling pathways including PI3K/AKT and NK-κB pathways, further supporting metastatic progression.[50]

TEM, however, is not always present as arrested CTCs have been found to proliferate directly in blood vessel lumina leading to their rupture by large intraluminal cancer colonies.[51] Finally, carcinoma cells that successfully managed to extravasate seem to be sentenced to either their elimination from the parenchyma of the tissue or to enter the state of dormancy in which they outlast as single CTCs or micrometastatic clusters for extended periods of time before they advance into detectable metastases. A summary of the metastatic process is shown in **Figure 1.**

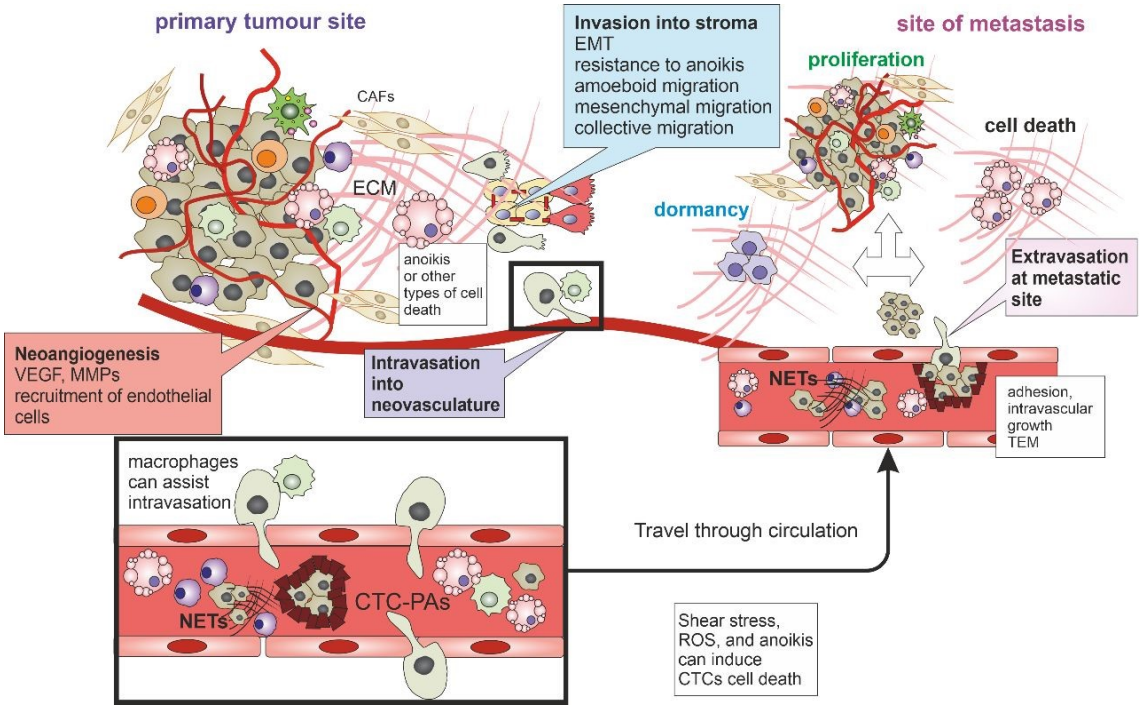

Figure 1: Scheme of the metastatic process. *The sequential stages involved in the tumor metastasis process start at the primary tumor location and lead to the formation of secondary metastatic lesions. At the primary tumor location, cancer cells engage with the ECM and cancer-associated fibroblasts (CAFs) promoting tumor expansion and angiogenesis via*

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elements like VEGF and matrix metalloproteinases (MMPs). Tumor cells undergo epithelial-to-mesenchymal transition (EMT) to acquire invasive potential, allowing them to infiltrate the stroma and resist anoikis. In the stroma, cancer cells invade the newly developed blood vessels. Circulating tumor cells (CTCs) face shear stress, reactive oxygen species (ROS), and various obstacles while travelling through the bloodstream, where they can create platelet aggregates (CTC-PAs) or engage with neutrophil extracellular traps (NETs) to improve their survival. At distant sites, CTCs extravasate into the new microenvironment, propelled by adhesion and transendothelial migration (TEM). These spread tumor cells can enter a dormant state, may undergo cell death, or begin to proliferate and form secondary tumors.

1.4.Forming pro-metastatic microenvironment at secondary sites

Previous studies suggest that the vast majority of disseminated tumor cells (DTCs) do not survive circulation and only less than 0.2% of them form metastasis.[52] The final destination of CTCs is determined by several factors. The main phenomenon describing the preferential migration or colonization is termed organ-specific tropism. Such selectivity is driven by many cellular and molecular mechanisms, including communication between the cancer cells and the secondary tumor site. This communication is governed by specific chemokine signaling pathways, the most well-known being CXCR4 receptors on CTCs and CXCL12 located in the bone marrow, or the CCR5-CCL5 axis facilitating lung metastasis.[53],[54] Other strong indicators are adhesion molecules guiding the whole CTC transport, mainly α4β1 (breast CTCs adhesion to the endothelium of the bone marrow) and α5β1 (liver adhesion) integrins[55] and E-/P- selectins facilitating the rolling of CTCs along the blood vessel walls.[56] The secondary tumor site is also being primed in advance to ease the extravasation and the formation of metastases. This process of priming on a specific site creates a pre-metastatic niche. One of the most studied driving forces of this priming are nowadays extracellular vesicles (EVs). EVs encapsulating many molecular signals are sent from the primary tumor to alter the secondary microenvironment.[57] The pro-tumorigenic pre-metastatic niche is characterized by modulated ECM, collagen crosslinking, local inflammation supporting dual functions (both anti- and pro-tumorigenic) of immune cells, and growth factors facilitating cancer cell survival and growth. Recent studies revealed other factors in this dynamic complex priming including the synergy of B cells and T cells creating an immunosuppressive environment[58] and tissue remodeling by neutrophils[59] highlighting the dual role of immune factors. Potential therapies targeting the pre-metastatic niche and disrupting the primary tumor-secondary site communication are nowadays investigated as an additive novel therapeutic avenue to tumor eradication.[60]

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The new tissue microenvironment also presents a challenge since it is devoid of the familiar ECM constituents, growth factors, and stromal cells of the primary site. Poor adaptation often results in dormancy, a prolonged state of growth arrest that reflects a failure of tumor cells to adapt and proliferate in secondary tissues.[61] There are several types of dormant states including cellular dormancy,[62] angiogenic dormancy,[62] immune-mediated dormancy,[63] microenvironment-mediated dormancy,[64] or therapy-induced dormancy.[65] During cellular dormancy, the cell cycle of DTCs is arrested and their metabolic activity is reduced. Angiogenic dormancy occurs before the angiogenic switch, in which DTCs depend only on the pre-existing vasculature and oxygen supply. Immunosurveillance composed mainly of cytotoxic T-cells and natural killer cells can eliminate DTCs and maintain them in an immune-mediated dormancy state. Microenvironment-mediated dormancy depends on microenvironmental signals inhibiting proliferation and promoting dormancy. Such dormancy states can also emerge because of targeted therapy or chemotherapy, however, there is a high risk of developing resistance to treatment and subsequent tumor relapse. The switch from dormancy to active proliferation may be provoked by a range of environmental factors.

2. Intrinsic and extrinsic factors in the metastatic setting

The formation of metastases is a complex process dependent on intrinsic factors inside cancer cells and extrinsic factors of the surrounding tumor microenvironment. The spread of cancer is driven by numerous genetic mutations and alterations in cellular metabolism enabling cancer cells to acquire the capability to colonize distant sites. The total measure of genetic alterations present in tumor DNA is termed tumor mutational burden (TMB) and is often expressed as the number of mutations per megabase (Mb) of DNA.[66] High TMB indicates a significant accumulation of genomic instabilities, leading to the production of aberrant proteins and at the same time promoting the generation of neoantigens for immune recognition.[67] Therefore, high TMB is often associated with elevated responsiveness to immunotherapies.[68] On the other hand, the higher the number of mutations, the higher the tumor cell population diversity, enabling convenient subclones to initiate metastases.[69] Mutations providing a selective growth advantage to tumor cells directly contributing to the progression of the disease are referred to as driver mutations.[70] Their identification is crucial for the development of targeted treatments. *KRAS* as well as *TP53* mutations, found in NSCLC, colorectal, and pancreatic cancer, have been linked to increased risk of metastasis.[71],[72] Currently, targeted inhibitors against *EGFR* and *BRAF* mutations have been successfully used in the treatment of metastatic cancers characterized by these genetic alterations.[73],[74] Nevertheless, their sustained efficacy can be

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limited by the development of resistance. Gene expression patterns in metastatic sites are influenced also by epigenetic alterations. In this context, potential epigenetic markers have been identified in metastatic breast cancer,[75] DNA methylation has been linked to treatment resistance in metastatic prostate cancer,[76] the role of lncRNAs has been discussed in metastatic melanoma[77] as well as chromatin remodeling in metastatic ovarian cancer[78] and many more. Given that epigenetic changes are reversible, targeted therapies aimed at modifying or completely reversing these changes are being developed, making them an appealing target in cancer therapy.

The highly dynamic and adaptable nature of cancer cells is facilitated by their exceptional phenotypic plasticity. This feature allows reversible switching between cellular states, contributing to the tumor heterogeneity. Genetic and epigenetic alterations and programs mentioned above define specific cellular states and their biological properties, increasing phenotypic plasticity. These switches, interchangeable over time, lead to the phenotypic versatility of cancer cells promoting metastatic progression and having profound implications for the course of the disease. The most extensively studied example of phenotypic plasticity is the epithelial-mesenchymal / mesenchymal-epithelial (EMT-MET) process. However, hybrid state cancer cells, also referred to as "quasi-mesenchymal cells", are considered the most aggressive cell phenotype.[79],[80] This cellular shift in the epithelial–mesenchymal spectrum, primarily characterized by the expression of CD51, CD61, and CD106 proteins, allows them to adapt to many different environments, ultimately leading to the evolution of cells with the highest metastatic potential.[79] Cancer cells undergoing EMT are also less responsive to cancer therapies, underscoring the significant role of phenotypic plasticity in contributing to the development of drug resistance.[81] Furthermore, cancer cells exhibit dynamic transitions between cancer stem cells (CSCs) and differentiated cancer cells.[82] The interplay of CSCs, shifting between their mesenchymal phenotype with enhanced invasive metastatic properties and their epithelial phenotype, which facilitates the formation of secondary sites, is a critical determinant of colonization.

Throughout the metastatic cascade, cancer cells encounter diverse environments with variations of oxygen availability, nutrient resources, and metabolites, exerting substantial external pressures on their metabolic states. This pressure affects both enzymatic machinery and substrate utilization. The metabolic plasticity during metastatic colonization allows cancer cells to adapt to the microenvironment of the secondary organs and their prevailing nutrient profile. For instance, in a mouse model of breast cancer lung metastasis, there is an abundance

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of pyruvate in lung interstitial fluid.[83] Therefore, pyruvate uptake, mainly fueling pyruvate-dependent anaplerosis, is a crucial factor supporting the successful colonization and outgrowth of lung metastasis. In another study, fatty acid metabolism mediated by FABP-5, was found to notably support lymph node metastases in a mouse model of cervical cancer.[84] Overall, these findings collectively illustrate that in each distant metastatic site cancer cells undergoing the metastatic cascade exhibit distinct metabolic phenotypes, underscoring their remarkable metabolic plasticity.

The major components of TME contributing to tumor metastasis are cancer-associated fibroblasts (CAFs). Their most powerful cancer-promoting mechanisms include ECM remodeling by secretion and modification of collagen, laminin, and fibronectin, production of MMPs allowing cancer cells to migrate through ECM, and production of VEGF, which directly stimulates angiogenesis. Additionally, CAFs secrete many pro-tumorigenic factors, including chemokines that attract immunosuppressive cells. CAFs, therefore, represent a promising target for both anticancer or even antimetastatic therapies due to their critical role in tumor progression and immune modulation. These potential therapies aim to reduce their pro-tumorigenic functions, induce phenotypic switch and lock CAFs in the tumor-restrictive state, block their differentiation, or target specific $CD10^{+}GPR77^{+}$ CAF-subset and eliminate them by targeted ablation.[85] Recently, metastasis-associated fibroblasts (MAFs) were identified as another subgroup contributing mainly to cancer metastasis, establishing metastatic niches and mediating resistance to therapy.[86] Unlike CAFs, MAFs emerge specifically at the metastatic sites, including brain metastases, in which fibroblasts are usually absent under normal physiological conditions.[87] Another ECM component promoting metastasis are tumor-associated macrophages (TAMs). TAMs are key cell subsets producing chemokines, cytokines and growth factors, contributing to the establishment of an immunosuppressive TME. Moreover, TAMs are abundantly present in EMT hotpots, therefore their activity is directly linked to the EMT process.[88] They are also critical aspects of the angiogenic switch. It was shown that the absence of TAMs leads to a 40% reduction of the blood vessel density.[89],[90] CAFs, MAFs, and TAMs altogether facilitate secondary lesion formation by promoting pre-metastatic niche. TAMs help to recruit CAFs to the secondary site, both modulate immune responses and provide structural support for metastatic formation.[91] MAFs arise in response to communication between fibroblasts and macrophages.[92] Altogether they provide complex machinery to ease extravasation and formation of metastasis. The main targetable intrinsic and extrinsic factors involved in the metastatic setting are shown in **Figure 2.**

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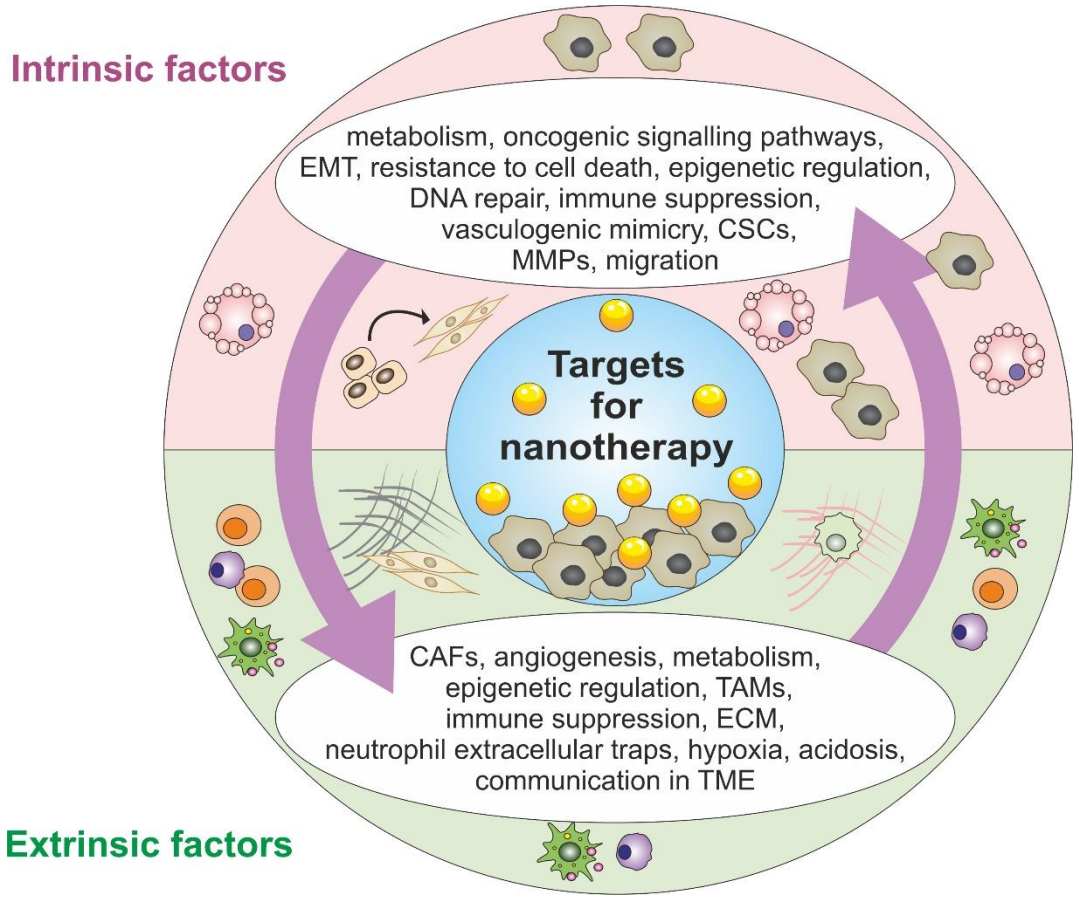

Figure 2: Overview of targetable intrinsic and extrinsic factors in the metastatic setting. *The interplay between intrinsic molecular alterations and external influences of the tumor microenvironment defines the metastatic potential of cancer cells. Abbreviations: EMT – epithelial-mesenchymal transition, CSCs – cancer stem cells, MMPs – matrix metalloproteinases, CAFs – cancer-associated fibroblasts, TAMs - tumor-associated macrophages, ECM- extracellular matrix, TME – tumor microenvironment.*

The migration of cancer cells is ensured by substantial cytoskeletal reorganization.[93] Cytoskeleton responds to external signals orchestrating complex behavior such as the formation of lamellipodia, pseudopodia, and, principally, the process of migration. The migration modes depend also on the stiffness and composition of the extracellular matrix.[94] The motility of cancer cells is mainly enabled by the rearrangement of actin and intermediate filaments, microtubule dynamics, and focal adhesions. Contractile forces generated by actin and myosin interactions aid tumor cells to squeeze through tight gaps in the ECM.[95] These forces are particularly challenging for the nucleus of the cell, which is the stiffest organelle determining the extent of possible compression of the whole cell. However, this extensive compression of

the cell and the nucleus threatens genome stability and often leads to nuclear rupture. This rupture can be prevented by nucleus-centrosome linkage, which senses the tension and facilitates endosomal vesicles trafficking to the cell leading edge, delivering membrane type 1 matrix metalloprotease (MT1-MMP).[96] However, if the nucleus indeed breaks open, this rupture causes severe DNA damage and genomic instability which, surprisingly, may further promote cancer progression.[97] For example, it was shown that DNA-destroying enzyme TREX1 vastly adds to the invasiveness of the cancer cell.[98] On the other hand, this phenomenon may lead to the development of novel antimetastatic drugs by specifically targeting these cancer cells and preventing them from recovery. Microtubules establish cell polarity, an essential factor in guiding controlled and directed cancer cell invasion.[99] During EMT, the expression of vimentin, as an intermediate filament protein, is upregulated in tumor cells, providing mesenchymal cells with structural support, ensuring anchorage of organelles during migration,[100] and facilitating cell adhesion to a substrate.[101] Focal adhesions, which serve as the cell anchor points, are formed by cell surface integrins binding to ECM components and enabling cells to apply force and relocate.[102] Therapeutic strategies aimed at disruption of cytoskeletal dynamics are actively under investigation as potential therapeutic approaches limiting the progression of metastases.[103],[104]

3. Antimetastatic treatments

Targeting metastatic cancer involves a broad spectrum of approaches interfering with the ongoing metastatic formation. Micrometastases refer to small clusters of cancer cells in secondary sites that are hardly detectable due to their size, therefore, micrometastases are often only assumed to be present and their treatment is mostly preventive. On the contrary, macrometastases are detectable through physical examination or imaging and are the main focus of treatment efforts. Predominant systemic approaches for both micro- as well as macrometastasis are chemotherapy, immunotherapy, and targeted therapy or combinations thereof. Eliminating metastasis in the vicinity of primary tumor can be performed via radiation, whereas distant metastases are generally managed by chemotherapy. In some cases, such as metastatic breast cancer afflicted axillary lymph nodes, surgical resection is also an option.[105] Chemotherapy remains the standard of care across a broad spectrum of malignant tumors. However, recent studies suggest that chemotherapy can even promote metastasis through multiple mechanisms, such as promoting cells with highly metastatic phenotypes, activation of EMT, or induction of cancer stem cells populations.[106],[107] Moreover, chemotherapeutics

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greatly affect the hematological system as well as immune responses, inflammatory responses and overall ECM of tumor tissue.[108],[109] For example, paclitaxel increases invasive behavior by inducing invadopodia and at the same time facilitates vascular alterations such as blood vessel leakage or overall vascular permeability leading to angiogenesis.[110] Current cytotoxic chemotherapies lack complementary approaches targeting metastatic cascade. Therefore, in metastatic settings, combination therapies of broad-spectrum cytotoxic chemotherapies together with antimetastatic treatments are crucial for higher survival rates and for overall reduction of metastatic burden.

The early therapeutic target in the TME was tumor neovasculature. The clinically approved angiogenesis inhibitor bevacizumab has shown efficiency in metastatic settings by binding vascular endothelial growth factor (VEGF).[111] This leads to the inhibition of angiogenesis and overall restriction of tumor growth by depriving it of the blood supply. Moreover, the addition of bevacizumab to combination fluorouracil-based chemotherapy termed FOLFOX significantly improved the survival of metastatic colorectal cancer patients.[112] Matrix metalloproteinase (MMP) inhibitors emerged as another promising therapeutic target of metastatic cancers since dysregulation and overexpression of MMPs are associated with aggressive disease and poor outcomes.[113],[114] Unfortunately, to this day no MMP inhibitor was approved for clinical use due to their severe disruption of healthy tissue homeostasis.[115],[116] Clinical failure of MMP inhibitors may also be related to the ability of tumor cells to switch between MMP-dependent mesenchymal movement, and MMP-independent ameboid motility. This switch provides cancer cells with an alternative invasion pathway in cases when MMP activity is inhibited.[117] Overexpressed CXCR-4 (C-X-C chemokine receptor type 4) is also highly involved in cell migration and metastasis.[118] The interaction of CXCR-4 and its ligand CXCL-12 plays a pivotal role in the recruitment of tumor cells in CXCL-12-rich distant sites such as lymph nodes or bone marrow.[119] CXCR-4 inhibitors are blocking the CXCR-4/CXCL-12 axis and inhibiting metastatic formation. Many preclinical investigations and clinical trials in this context have investigated CXCR4 antagonist AMD3100 (Plexifor) in the context of metastatic breast cancer,[120] metastatic colon cancer,[121] and hematological cancers[122]. Other potential CXCR4 inhibitors, such as BL-8040 (Motixafortide), have been evaluated for non-small lung cancer[123] and pancreatic cancer metastasis.[124] A clinically approved class of antimetastatic drugs are tyrosine-kinase inhibitors (TKIs). TKIs are interfering with metastasis driving signaling pathways, thereby halting or slowing down the disease progression. TKIs specifically target abnormal signaling pathways that drive cancer progression by inhibiting tyrosine kinase phosphorylating tyrosine residues.

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This disruption prevents downstream signaling, thereby inhibiting tumor expansion and metastasis.[125] TKIs clinically approved for the treatment of metastatic cancers are, for example, sunitinib (brand name Sutent)[126] for the treatment of metastatic renal cell carcinoma, lapatinib for metastatic HER2-positive breast cancer[127], and regorafenib for metastatic colorectal cancer.[128] These treatments are often used in combination with chemotherapy, radiation therapy, or immunotherapy.

Historically, cancer treatment has primarily focused on the intrinsic properties of tumor cells. However, the advent of immunotherapy has underlined the undeniable significance of the tumor microenvironment in the treatment of metastasis. Immune checkpoint inhibitors (ICIs) revolutionized the treatment of metastases by their ability to block proteins inhibiting the recognition of cancer cells by the immune system and their subsequent attack.[129] The most well-known checkpoint proteins muting the immune system are programmed cell death protein (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4).[130] Metastatic cancers that respond to ICIs, such as metastatic melanoma, usually have a high TMB. These tumors generate many mutated peptides displayed on tumor major histocompatibility complex (MHC) and are identified by the immune system as foreign "neoantigens".[131] Another therapeutic strategy demonstrating promising results especially in hematological malignancies is chimeric antigen receptor T-cell therapy (CAR-T). In this process, the autologous T cells are reprogrammed to target antigens specific for cancer cells. In B-cell malignancies, the T cells used for CAR-T are mostly designed to target CD19.[132] Another immunotherapy involving patient-derived T cells is tumor-infiltrating lymphocyte (TIL) therapy, in which autologous T cells isolated from the patient's tumor are expanded *ex vivo* in the lab and then reinfused into the patient. TIL therapy resulted in long-term remission of patients with metastatic melanoma.[133],[134]

The novel term "migrastatics" has been introduced for drugs interfering with the ability of cells to metastasize.[135],[136] Among them, pentamethinium salts, initially designed as mitochondrial probes, and curcumin derivatives, derived from turmeric, emerged as very promising therapeutics in preclinical research. Pentamethinium salts demonstrated a significant impact on mitochondria, effectively inhibiting cancer cell proliferation and migration *in vitro*. This mechanism is likely attributed to the inhibition of dihydroorotate dehydrogenase (DHODH)-dependent respiration. Moreover, pentamethinium salts have been shown to induce oxidative stress, alter mitochondrial distribution, and decrease mitochondria mass.[137] Curcuminoids or curcumin derivatives are also associated with inhibition of cancer cell migration and invasion. Curcumin has been identified as an inhibitor of NF-κB signaling as well as many other signaling pathways linked to cancer cell motility.[138] Additionally, curcumin

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reduces levels of hypoxia-induced factor 1 (HIF-1) subsequently reducing the VEGF production.[139] Curcumin also downregulates antiapoptotic protein BcI-xL, therefore enhances cancer cell susceptibility to apoptosis.[140] The combined anticarcinogenic, anti-inflammatory, and antioxidative effects of curcumin and its derivatives highlight their potential as promising anticancer therapeutics. Another group of potential migrastatics are multikinase inhibitors designed to inhibit Rho-associated protein kinase (ROCK) and myotonic dystrophy kinase-related Cdc-42 binding kinase (MRCK), both involved in cytoskeletal dynamics and motility of cancer cells.[141] In general, combining migrastatics targeting invasiveness of the cells with antiproliferative therapies could result in highly effective synergic approach for treating metastatic cancers. Optimal migrastatic targets are likely those essential to all cell migration forms, such as cytoskeletal reorganization, ATP production, mitochondrial metabolism, and cellular contractility.[142] Moreover, halting metastatic dissemination by migrastatics can also decrease the need for cytotoxic therapies in high doses.

Nowadays, many investigational approaches aim to develop advanced combination therapies. By simultaneously targeting two distinct cellular targets, these bispecific therapies aim to leverage dual-target recognition to achieve synergistic effects, enhanced selectivity, and reduced off-target toxicity compared to mono-specific approaches. This is achieved for example by using bispecific antibodies simultaneously targeting cancer cell antigen and an immune cell receptor, or by employing nanoparticle delivery systems designed to deliver agents to the TME.

4. Nanotechnology-based approaches for metastatic cancer treatment

The application of nanotechnology in medicine, known as nanomedicine, has emerged as a breakthrough approach for more precise and early disease diagnosis, including cancer, while also serving as a powerful tool for effective therapy. To this day, several nano-based carriers have been described in the medical field, including liposomes, polymeric micelles, quantum dots, golden nanoshells, and many more. Moreover, the coupling of nano-drugs with targeted nanoscale delivery systems has led to the development of advanced nanoscale devices - nanorobots - engineered to perform precise therapeutic actions. Considering their tunable physical and chemical characteristics, which allow for a wide spectrum of therapeutic functions, these nanomachines can serve as both detection agents for specific biomarkers or disease locations and targeting agents delivering therapies to targeted sites. Further, nanotechnology revolutionized personalized medicine in terms of reducing severe side effects of conventionally used chemotherapy. This was achieved through the modulation of biodistribution, thereby improving the selective delivery and overall balance between drug efficiency and toxicity.

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These advances may enable the overcoming of drug resistance and improve biocompatibility. For instance, golden nanoparticles embedded in hydrogel silencing MRP1 gene were reported to prevent the efflux of the chemotherapeutic 5-fluorouracil and thereby overcoming 5-FU treatment resistance.[143] Additionally, nanotherapeutics have the potential to overcome biological barriers, such as the blood-brain barrier (BBB), dense extracellular matrix, and high tumor interstitial fluid pressure. In recent years, nanotechnology has reintroduced the concept of theranostics, which combines diagnostic procedures with therapeutic interventions.

The term "nanotechnology" inherently refers to the most significant feature of such a delivery system, its nanoscale size. Nanoparticles (NPs) typically range from 1 to 100 nm in size. The efficiency of NPs delivery can be adapted by the modulation of the size and surface characteristics of NPs. However, a significant drawback of nanocarriers is their early removal of NPs from circulation through reticuloendothelial system (RES) systems. Nevertheless, this limitation may be resolved using various strategies e.g., by employing highly hydrophilic polymeric material.[144] After being altered by hydrophilic polymers, NPs can remain in circulation for prolonged time which increases the possibility for their interaction with cancer cells.[145]

Anticancer drugs are mostly delivered to tumor sites by injection into the tumor parenchyma or systemic circulation. Administration of anticancer drugs into the systemic circulation is considered easier and more acceptable to patients. However, since targeting specific areas throughout the distribution by systemic circulation remains challenging and possible side effects often occur, this technique is not always effective. Moreover, medications often accumulate at the tumor periphery in low concentrations, failing to effectively penetrate and eradicate the tumor. This can result in tumor recurrence or metastasis.[146] Furthermore, while being transported, anticancer drugs may be metabolized, bind non-specifically to proteins or other tissue components. The strategies used to enhance the specificity of nanoparticles (NPs) for targeting tumor sites include both active and passive targeting mechanisms. Both are essential for increasing the potency of treatments while reducing the adverse effects. For passive targeting, the key aspect is termed the Enhanced Permeability and Retention (EPR) effect.[147] EPR effect depends on specific features of tumor vasculature, specifically, its leaky and irregular nature. This tissue characteristic allows NPs to passively accumulate in tumor tissue. The ideal size range of NPs for effective passive targeting and leaking out of the vasculature is in the range of 10 to 200 nm. Also, the extent of NPs accumulation in tumor site is increased with their prolonged circulation in the bloodstream. Nevertheless, the EPR effect is variable, as

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it is influenced by heterogeneity among tumor types. Small metastases, with size below 100 mm^3, with poor vascularization cannot be accessed by EPR.[148] By passive tumor targeting the NPs are unable to directly target malignant cells.[149] Several clinically approved nanoformulations of traditional anticancer drugs reply on passive targeting such as daunorubicin (DaunoXome®)[150] or paclitaxel (PTX) (Genexol PM®, Abraxane®)[151]. On the other hand, active targeting depends on the functionalization of nanoparticle surface with specific antibodies or ligands. These components interact with cancer cell surface receptors and after the NP binding, the therapeutic cargo is released into the cell. These specifically designed ligands therefore improve the targeting specificity of NPs which allows for the generation of novel innovative therapeutic and diagnostic agents. Since endocytosis absorbs NPs selectively after their attachment, selective binding improves the drug penetration while attenuating off-target toxicity.[152] However, as stated before, the efficacy of active targeting can also vary. This variability can be attributed to the tumor heterogeneity and variability of receptors' expression. A specific example of modified nano-delivery systems that utilize active targeting principles are those modified with arginine-glycine-aspartic acid (RGD) peptides.[153] RGD motifs recognize integrin receptors.[154] These are overexpressed on tumor cells and are highly involved in the process of cancer invasion and metastasis.[155] RDG-recognizing integrins are the most common integrins in terms of cell adherence to the ECM. The expression of RDG-binding integrins, specifically $\alpha_5\beta_1$ and αv, in tumor vasculature inspired the initial attempts to develop specific agents using RGD for reducing tumor angiogenesis and metastasis.[156] Unfortunately, despite promising preclinical results, these findings have not yet been consistently replicated in clinical trials. The intense research continues to explore the potential of RGD ligands to inhibit adhesion, migration, and survival of cancer cells. Another receptor widely overexpressed on cancer cell membranes is the transferrin receptor. Therefore, NPs with ligands targeting transferrin receptors preferentially accumulate in transferrin overexpressing tumor cells.[157] Another customized NP delivery systems involve glycoproteins, folate receptors, or EGFR.[158] The multistructural glycoprotein CD44 found on the cell surface is primarily involved in inflammation, cellular motility, and cell-to-cell interactions.[159] The active targeting of the CD44 receptor using nanocarriers has been therefore widely studied.[160] The CD44 receptors have shown selective binding to hyaluronic acid and chondroitin sulphate functioning as substrate. Once the CD44 receptor binds to hyaluronic acid,[161],[162] it is activated and leads to increased tumor growth. An innovative NP platform for the treatment of highly invasive triple-negative breast cancer (TNBC) overexpressing CD44 integrates a CD44-mediated therapy combined with JAK/STAT inhibitors synergistically disrupting CD44

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signaling and blocking JAK/STAT-mediated pro-tumor inflammation, both key drivers of cancer resistance. Polymeric NPs loaded with CD44-targeted NPs significantly reduced cell viability with association to high dose reduction index (DRI), quantifying the fold reduction in drug dose required to achieve the same efficacy when used synergistically.[163]

To this day, NPs of various nature have shown promising results in both metastatic cancer as well as carcinoma *in situ*. The first nanotechnology-based drugs that gained FDA approval were Doxil (1995),[164] a pegylated liposomal doxorubicin, and Abraxane (2005), a protein-bound paclitaxel formulation.[165] In recent article, it was found that also drug-free polystyrene NPs alone attach tightly to two different types of ECM components: retraction fibers (RFs) and migrasomes.[166] These structures are typically located at the posterior region of tumor cells during migration. Cell adhesion, motion range restriction, and morphology seem to be affected by this interaction. Through mechanical interaction with the lipid raft/caveolae substructures, NPs coat and form a hard shell on the surface of migrasomes and retraction fibers. Consequently, NPs prevent migrasomes from being recognized, endocytosed, and removed by the surrounding cancer cells or other cells within the TME. As a result, NPs disrupt the connection between cells and ECM, therefore interfering with the role of migrasomes in the process of cancer cell migration. Moreover, NPs alter the expression of proteins involved in cytoskeleton organization and cell-to-cell adhesion, further limiting cell migration.

In recent years, many studies have been conducted on the improvement of efficacy and delivery of many conventionally used therapeutics targeting various aspects of metastatic pathways by addressing their poor water solubility or low bioavailability. Synergistic therapy of NPs containing sorafenib and ursolic acid has been developed for potential advanced treatment of hepatocellular carcinoma.[167] Since sorafenib is poorly soluble in the water and is extensively metabolized by CYP3A4 its bioavailability is very low. To improve the bioavailability of sorafenib, in another study were generated sorafenib polymeric NPs exhibiting a significant impact on lung and breast cancer *CDKN1A* and *STMN1* gene expression, as well as an increase in DNA damage in model cancer cell lines.[168] The delivery of another previously mentioned drug bevacizumab was enhanced by PEGylated cationic liposomes, PEGylated NPs,[169] as well as poly(D,L-lactic-*co*-glycolic acid-PLGA) NPs. In *in vivo* glioblastoma model, these PLGA NPs demonstrated higher anticancer activity and tumor reduction in comparison to free bevacizumab.[170] Similarly, sunitinib malate activity was boosted by lipid polymer hybrid NPs. In *in vitro* breast cancer model sunitinib loaded NPs exhibited significant cytotoxic activity, as evidenced by increased p53 and caspase-3/9

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activities.[171] Lapatinib, another tyrosine kinase inhibitor, exhibited enhanced effectivity in breast cancer was encapsulated into PEGylated lipid layer NPs containing polymeric core. This nanoparticle design allowed for enhanced cellular internalization and effective induction of apoptosis.[172] In another study, Lapatinib/pseudolaric acid B@Ferritin NPs were designed for the therapy of triple-negative breast cancer sensitive to ferroptosis. In both, *in vitro* and *in vivo* these structures showed a more potent tumor suppression accompanied by the lipid peroxidation.[173]

Immune checkpoint inhibitors are also being conjugated to several nanostructures. For instance, a CXCR4 inhibitor Plerixafor together with PD-L1 inhibitor BMS-1 were co-loaded on Cu_2MoS_4 (CMS)/PEG NPs for combined immunotherapy of pancreatic ductal adenocarcinoma. This combination enhanced CXCR and PD-L1 inhibition relative to the free inhibitors while remodeling the TME by increasing tumor infiltration of CD4+/CD8+ T cells, and at the same time reducing immunosuppressive cells.[174]

Latest advances in cancer nanotechnology have led to the categorization of its applications into several focused strategies designed to reduce metastatic spreading. These include targeting the process of tumor cell invasion and intravasation, targeting dissemination and migration, targeting micrometastasis and pre-metastatic niche, and targeting macrometastasis.

Targeting the process of tumor intravasation is widely investigated as a metastatic treatment strategy. For example, nano-formulated quinacrine (NQC), originally an anti-malarial drug, combined with ADAM-17 inhibitor (GW280264) was shown to decrease the invasion rates in cervical cancer stem cells. This combination therapy targets the increased expression of Nectin-4, protein linked to the Notch signaling pathway and possibly responsible for 5-FU resistance in metastatic models, by blocking its nuclear translocation.[175] Further, the co-prodrug-loaded micelles (CLMs) containing cathepsin B/pH dual-sensitive block copolymer conjugated with doxorubicin and loaded with nifuroxazide were synthetized and explored as potential breast cancer metastasis nanomedicine. CLMs were shown to inhibit migration and invasion *in vitro* and in *in vivo* orthotopic and lung metastasis breast cancer mice models demonstrated significant antimetastatic effects.[176] In another study, an intravenous folate-conjugated nanocarrier loaded with miR-125b-5p plasmid effectively inhibited the invasiveness of hepatocellular carcinoma via the Wnt/β-Catenin pathway.[177]

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Migration disruption could be another approach to halt metastatic cascade. Dp44mT (Di-2-pyridylketone-4,4-dimethyl-3-thiosemicarbazone) and cisplatin-coloaded NPs have been shown to suppress VEGF, HIF-1, vimentin, and MMP2 expression. Dp44mT is an iron chelator inhibiting cell migration. In *in vivo* model, this nanoplatform significantly inhibited lung metastasis as well as overall tumor growth.[178] This nanoformulation is another example of combining conventional functional drugs together with antimetastatic approach encapsulated in nanocarrier for enhanced tumor intracellular drug accumulation and therefore, reduction of side effects. In another example, etoposide, a topoisomerase II inhibitor, was combined with a layered double hydroxide (LDH) carrier, which significantly inhibited cancer migration and invasion *in vitro*.[179]

The strategy of targeting the pre-metastatic niche was also demonstrated as promising. As mentioned earlier, exosomes play a crucial role in establishing a pre-metastatic niche. CBSA/siS100A4@Exosome nanoparticles (cationic bovine serum albumin conjugated siS100A4 and exosome membrane coated nanoparticles) exhibited potent gene-silencing effects inhibiting malignant breast cancer cells and demonstrating strong affinity for lung metastasis.[180]

Macrometastases remain the primary focus of the majority of nano-antimetastatic studies. In a recent study, NPs loaded with docetaxel and sonic hedgehog protein (SHH) siRNA were used for bone metastasis targeting in prostate cancer. After reaching the bone microenvironment, they inhibited cancer cell proliferation and migration by SHH autocrine/paracrine inhibition, increasing the anticancer effects of docetaxel.[181] Bone-targeted nanodelivery system was also demonstrated in breast cancer bone metastasis. Gold nanorods inside silica nanoparticles combined with zoledronic acid showed specific bone-targeting *in vivo* as well as reducing VEGF levels and inducing apoptosis of cancer cells.[182] Previously mentioned AMD3100, a CXCR4 antagonist, was also conjugated with NPs and *proMel,* artificial gene encoding prodrug form of melittin releasing cytolytic melittin once activated by MMP-2 present in tumors. In a breast cancer brain metastases mouse model, poly(lactone-*co*-β-amino ester)-AMID3100-*proMel* nanoparticles effectively inhibited tumor progression, establishing novel promelittin-mediated gene therapy.[183]

Nanotherapeutics can also target components of the TME. Several NP platforms are actively targeting MAFs. In liver cancer, the equivalent to CAFs or MAFs are hepatic stellate cells (HSC)/myofibroblasts presenting a great target for potential stroma-based therapies. Surface-modified nanocarriers with cyclic peptide binding to the PDGFRβ or with mannose-6-

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phosphate binding to the IGFRII successfully directed NPs loaded with drugs to HSC/CAF *in vivo*.[184]

In clinical practice, a variety of tumor ablation techniques are currently used to treat cancer, including thermal, cryo-, microwave, and ultrasound ablation. These techniques are valued for their reduced trauma to the affected area, site-specific tumor cell killing, and controllability. Apart from the immediate lethal impact, the eventual death of tumor cells following tumor ablation serves as a possible source of antigens to initiate an immune reaction.[185] Both primary and metastatic cancers can be treated with thermal ablation by inducing an immune response.[186] This concept is also relevant and has been adapted to the nanotechnology-based approaches. Specifically, nanoparticle-based photothermal therapy (PTT) therapy can trigger specific immune responses, such as immunogenic cell death (ICD), release of damage-associated molecular patterns (DAMPs), activation of antigen-presenting cells (APCs), release of cytokines or stimulation of tumor-infiltrating lymphocytes (TILs).[187] However, several recent studies have shown that PTT alone may not be sufficient to limit the growth of primary tumors or distant metastases.[188] The symbiotic interaction between tumor cells and the host immune system introduces additional complexity to cancer immunotherapy. Tumor cells may exhibit tolerance to immune-mediated elimination, or in some cases, even exploit the immune system components to promote their progression. In addition to cell apoptosis and necrosis induced by heat exhaustion, PTT may have additional anticancer effects. PTT, for instance, showed exceptional superiority in overcoming drug resistance. It can eradicate both drug-sensitive and multidrug-resistant (MDR) cancer cells and has shown no discernible signs of resistance in preclinical tumor treatment models.[189] It is well accepted that the overexpression of drug-efflux transporters, p-glycoprotein (p-gp), and multidrug resistance-associated protein 1 (MRP1) contributes to the MDR of various cancers.[190] According to a recent study, drug resistance in A549R cells, a drug-resistant variant of the A549 cell line, may be reversed by inhibiting MRP1 expression using cyanine dye-loaded nanoparticle-based PTT.[191] According to another study, PTT based on carbon and gold nanoparticles can help overcome doxorubicin (DOX) resistance by promoting the activation of heat-shock factor trimers, which in turn downregulate p-gp.[192] Additionally, PTT may enhance the chemotherapy effectiveness by rupturing the integrity of the cell membrane, leading to higher drug accumulation within the tumor.[193] Recently, scientists have created a novel active-targeted drug delivery nanoplatform based on copper sulfide nanoparticles (CuS NPs) functionalized with RGD ligand. This system has been designed for laser irradiation capable of

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deep tissue penetration. It provides an alternating therapy approach combining chemotherapy and PTT resulting in a synergistic 3.53-fold increase in therapeutic effect *in vivo*.[194] This approach also addressed the tumor metastasis spread caused by hypoxia. The nanoplatform demonstrated high efficiency photothermal conversion, deep tissue heat penetration, and DOX release from nanostructures during laser irradiation. This can be attributed to the simple structure of the platform and the ability to target cells. Additionally, the alternating chemo/PTT technique may prevent tumor liver metastasis by reducing tumor hypoxia, indicating that this nano-platform may enhance cancer treatment and improve prognosis in preclinical models.[195]

Another approach, photodynamic therapy (PDT), is nowadays also studied in the context of nanotechnology and treatment of metastatic cancer. The treatment was demonstrated in a recent study focusing on uveal melanoma (UM), a rare ocular malignancy. Here, a rho-kinase (ROCK) inhibitor was used in combination with PDT based on Ce6-embedded nanophotosensitizers (FIC-PDT) and was further combined with PD-1//PDL-1 blockage. FICs were utilized to boost the effectiveness of PDT, and their application has been explored for their ability to induce ICD. One of the main challenges in UM therapy is metastasis, particularly spreading to the liver, which can be potentially inhibited by a mechanism known as immunogenic clearance — a process by which signals release by dying tumor cells activate a broader immune response to eliminate tumor cells. This combination therapeutic approach stimulated the immunological response and led to the trafficking of CD8+ T cells into the initial tumor site. Consequently, anti-PD-L1 antibody plus immunogenic clearance brought on by FIC-PDT and ROCK inhibitor could be a powerful immunotherapeutic approach for UM.[196]

Among various nanotechnology applications in the treatment of cancer, NPs coupled with RNA molecules have the potential to greatly upregulate the expression of target tumor suppressor genes or to suppress the expression of target oncogenes. RNA-based tumor therapy is known for its low toxicity and for lack of contribution to drug resistance of tumors. However, due to RNases and exonucleases comprised in the human immune system, such therapeutic molecules degrade rapidly. Therefore, RNA encapsulation in NPs (lipid, inorganic, polymer, or biomimetic NPs) significantly improves cancer therapy effectiveness, particularly for metastatic cancer, where targeted delivery is crucial for reaching distant sites and overcoming treatment resistance. For instance, in melanoma patients, RNA lipoplexes (DNA-carrying nonviral cationic liposomes) comprising 1,2-dioleoyl-sn-glycerol-3-phosphatidylethanolamine (DOPE) and N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) lipid carriers were shown to induce T-cell immune response, and interestingly, mediate

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powerful interferon α-dependent metastatic tumor rejection.[197] In another example, the overexpression of TWIST-related protein 1 in metastatic carcinomas was successfully suppressed using mesoporous silica nanoparticles (MSNs) modified with hyaluronic acid (HA). HA-modified MSN designed to deliver siRNA-419 (siTWIST) target CD44 overexpressed on cancer cells. Combined treatment of siTWIST-HA and cisplatin led to noticeably smaller tumors and fewer metastases in mice.[198]

Another contribution in anticancer nanotechnology has been triggered by quantum dots. Due to their broad biocompatibility, high water solubility, and tunable surface functionalities, quantum dots have emerged as a promising platform for medical applications. A recent study has shown that downregulation of matrix metalloproteinase-2 and -9 (MMP-2/9) and vascular endothelial growth factor receptor 2 (VEGFR2) expression *in vitro* by carbon-based quantum dots (CQDs)/Cu_2O can reduce migration and angiogenesis and thereby inhibit tumor progression.[199] The particle internalization significantly reduced the growth and invasiveness of B16F10 melanoma cells. Furthermore, B16F10 cells labeled with quantum dots-GSH can detect the early distribution and migration of B16F10 cells *in vivo* in C57BL/6 mice.[200] In another study, tangeretin-zinc oxide (Tan-ZnO) quantum dots, a type of ultra-small quantum dots with dimensions ranging from 5–4 nm, were shown to significantly boost cytotoxicity and morphological damage in H358 metastatic human lung carcinoma cells. When compared to the untreated control treatment of H358 cells with Tan-ZnO QDs resulted in reduced cell proliferation, cell cycle arrest in the G2/M phase cells, increased nuclear fragmentation and apoptosis, while significantly inhibiting migration and invasion, both crucial components of metastasis.[201]

Ongoing nanomedicine research holds a great promise for developing highly specific antimetastatic treatments with many of therapeutical approaches targeting metastases advancing to clinical translation. Inhibitors of crucial migrastatic pathways as Rho/ROCK, CXCR4, integrins as well as RNA-based approaches aiming at EMT drivers are now nearing clinical applications. As we continue to unravel the complexity of tumor microenvironment and metastatic pathways, the potential for nanotherapeutics to redefine the treatment of metastatic cancer is becoming increasingly evident. However, further advancements in nanomedicines remain necessary to fully utilize its clinical potential.

Table 1: Overview of Nanotherapies

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Drug name	Nanocarrier	Cargo	Cancer type	Development stage	Reference
Doxil/Caelyx	PEGylated liposome	Doxorubicin	Metastatic breast cancer	FDA- and EMA-approved for Europe as Caelyx)	[164]
Genexol PM®	Polymeric micelles	Paclitaxel	Metastatic breast cancer	Approved in South Korea	[202]
Abraxane®	Albumin-bound NP	Paclitaxel	Metastatic breast cancer, metastatic pancreatic cancer	FDA-and EMA-approved	[165]
CD44 targeted polymeric nanoparticles	Polymeric NPs	Momelotinib + CFM.4.16	TNBC	In vitro/in vivo (preclinical)	[163]
US NPs	-	Sorafenib + Ursulonic acid	Hepatocellular carcinoma	In vitro/In vivo (preclinical)	[167]
Sorafenib NPs (SFB-PNs)	Polymeric NP	Sorafenib	Lung cancer, breast cancer	In vitro/In vivo (preclinical)	[168]

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PLGA NPs	PLGA NPs	Bevacizumab	Glioblastoma	In vitro/In vivo (preclinical)	[170]
Lipid polymer hybrid NPs (LPHNPs)	Chitosan-based lipid–polymer hybrid NP	Sunitinib malate	Breast cancer	In vitro/In vivo (preclinical)	[171]
PEGylated lipid layer NPs containing polymeric core	Core-shell NP (PEGylated lipid layer + polymeric core)	Lapatinib	Breast cancer	In vitro/In vivo (preclinical)	[172]
Lapatinib/pseudolaric acid B@Ferritin NPs	Protein-based NP (ferritin nanocage)	Lapatinib + Pseudolaric acid	TNBC	In vitro (preclinical)	[173]
Cu_2MoS_4 (CMS)/PEG NPs	Hollow mesoporous NP (PEGylated)	BMS-1 + Plerixafor	Pancreatic ductal adenocarcinoma	In vitro/In vivo (preclinical)	[174]
Nano-formulated quinacrine (NQC),	PLGA based NPs	Quinacrine	Cervical cancer	In vitro (preclinical)	[175]
CLMs containing cathepsin B/pH dual-sensitive	Polymeric micelles	Doxorubicin + Nifuroxazide	Metastatic breast cancer	In vitro/In vivo (preclinical)	[176]

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Folate-conjugated nanocarrier loaded with miR-125b-5p plasmid	Fa-PEG-g-PEI-SPION (FaPPS)	miR-125b-5b	Hepatocellular carcinoma	In vitro/In vivo (preclinical)	[177]
Dp44mT (Di-2-pyridylketone-4,4-dimethyl-3-thiosemicarbazone) and cisplatin-coloaded NPs	Polymeric NP (PEG-PLGA)	Dp44mT + platinum(IV) prodrug (cisplatin)	Metastatic breast cancer	In vitro/In vivo (preclinical)	[178]
VP16-LDH	Inorganic layered double hydroxide (LDH) NPs	Etoposide	Lung cancer	In vitro/In vivo (preclinical)	[179]
CBSA/siS100A4@Exosome NPs	Exosome-based NP	siS100A4 siRNA	Metastatic breast cancer	In vitro/In vivo (preclinical)	[180]
NPs loaded with docetaxel and sonic hedgehog protein (Shh) siRNA	Lipid-polymeric hybrid NP	Docetaxel + siRNA targetting Sonic Hedgehog gene	Metastatic prostate cancer	In vitro/In vivo (preclinical)	[181]
Gold nanorods inside silica nanoparticles (Au@MSNs)	Gold nanorod-silica NP (photothermal)	Zoledronic acid	Metastatic breast cancer	In vitro/In vivo (preclinical)	[182]

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Poly(lactone-*co*-β-amino ester)-AMID3100-*proMel*	Peptide-based NPs	AMID3100 + pro-Melittin	Metastatic breast cancer	In vitro/In vivo (preclinical)	[183]
PDGFRβ-targeted polymeric nanoparticle (cyclic peptide-modified)	Surface-modified polymeric NP	siRNA (CAF-targeting)	Hepatocellular carcinoma	In vitro/In vivo (preclinical)	[184]
Cyanine dye-loaded nanoparticle-based PTT	Micelles (photothermal)	Pt(IV) prodrug + cyanine dye (Cypate)	Lung cancer	In vitro/In vivo (preclinical)	[191]
RGD-functionalized CuS NPs	Copper sulfide NP	None (PTT agent)	Metastatic breast cancer	In vitro/In vivo (preclinical)	[194]
FIC-PDT	Photosensitizer NP (PDT therapy)	Rho-kinase (ROCK) inhibitor + anti-PD-L1 antibody	Uveal melanoma	In vitro/In vivo (preclinical)	[196]
HA-modified MSN	HA-modified mesoporous silica NP (MSN-HAs)	siRNA against TWIST1	Ovarian cancer	In vitro/In vivo (preclinical)	[198]

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Carbon-based quantum dots (CQDs)/Cu_2O	Carbon quantum dot / Cu_2O hybrid NP	None	Ovarian cancer	In vitro (preclinical)	[203]
(Tan-ZnO) quantum dots	tangeretin-zinc oxide quantum dots	Tangeretin	Metastatic lung cancer	In vitro/In vivo (preclinical)	[201]

736 **Table 2.: Clinically approved nanomedicines for metastatic cancer treatment**

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Drug name	Drug	Nanocarrier	Metastatic Cancer Type	Regulatory status	Reference
Doxil/Caelyx	Doxorubicin	PEGylated liposome	Metastatic breast cancer	FDA-(Doxil) and EMA-(Caelyx) approved	[204], [205], [206]
Myocet	Doxorubicin	Liposomes	Metastatic breast cancer	EMA-approved	[207], [208]
Lipusu	Paclitaxel	Liposomes	Advanced gastric cancer*	NMPA-approved	[209]
Genexol PM	Paclitaxel	Polymeric micelle	Metastatic breast cancer	approved in South Korea	[210], [211]
Onivyde	Irinotecan	PEGylated liposomes	Metastatic pancreatic adenocarcinoma	FDA- and EMA-approved	[212], [213]
Pazenir	Paclitaxel	Polymeric micelle	Metastatic breast cancer, metastatic pancreatic cancer	approved in South Korea	[214], [215]
Abraxane	Paclitaxel	Albumin	Metastatic adenocarcinoma of the pancreas, metastatic breast cancer	FDA-and EMA-approved	[216]
DaunoXome	Daunorubicin	Liposomes	Advanced Kaposi´s sarcoma*	FDA-approved	[217]

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DepoCyt	Cytarabine	Liposomes	Lymphomatous meningitis[#]	FDA-approved, EMA-approved (withdrawn)	[218]

[*]Metastatic disease not explicitly specified in regulatory approval.

[#]Lymphomatous meningitis represents CNS dissemination of hematological malignancies and is functionally analogous to leptomeningeal metastases in solid tumors; therefore, it is included as a clinically relevant model of disseminated (metastasis-like) disease.

Drug uptake

Improving the therapeutic efficacy of nanodrugs remains a pressing issue. Overcoming several biological barriers along the route to the tumor site, as well as achieving efficient extravasation and infiltration in the tumor itself, are major unresolved obstacles. Only a small fraction of injected nanoparticles (often less than 1%) actually reaches solid tumors, calling into question the traditional reliance on the EPR effect. The EPR effect is limited because most human tumors lack the vascular leakiness and tissue conditions necessary for passive nanoparticle accumulation.[219] The acronym "CAPIR" cascade summarizes the main sequential barriers NPs have to cross along the way into the tumor: circulation, accumulation, penetration, internalization, and release.

The most common route for nanotherapeutics to reach the tumor site is through endocytosis, yet it presents a significant bottleneck for delivery efficiency. Cellular internalization occurs via clathrin-, and caveolae-dependent endocytosis, or via micropinocytosis. However, following internalization, the NPs often became trapped in the endosomal-lysosomal stage, in which the acidic environment often degrades or destabilizes NPs. Endocytic uptake also often leads to perivascular accumulation, very limited tissue penetration, and, in general, heterogeneous intratumoral distribution of the drug. Efficient cargo release from endosomes into the cytosol remains the main limiting step in intracellular drug delivery and a critical barrier blocking the NPs treatment efficiency. Transcytosis can overcome the limits of endocytic uptake by actively transporting nanoparticles across endothelial cells, preventing perivascular trapping and enabling deeper, more uniform penetration into tumor tissue. By transporting particles through cells rather than leaving them stuck near vessels, transcytosis overcomes poor vascular permeability and dense stroma that limit endocytic

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delivery. Transcytosis is an ATP-dependent, active form of transport that is independent of vascular leakiness. It starts with endocytosis via adsorptive-mediated transcytosis (AMT) proposed by electrostatic interaction between anionic plasma membrane and cationic molecules, or receptor-mediated transcytosis (RMT) initiated by binding of the ligands to target receptors (**Fig. 3**). One of the next-generation strategies is the induction of transcytosis in cancer cells or epithelial cells to enable active extravasation and infiltration into the tumor, resulting in increased anticancer efficacy.[219] Several studies have demonstrated the potential of transcytosis-based nanoparticle strategies for deep tumor penetration. Cationized polymer–drug NPs were shown to possess strong active transcytosis ability, leading to effective suppression of established tumors reaching approximately 500 mm^3 in volume.[220] Similarly, another study demonstrated that positively charged poly(L-lysine)-functionalized upconversion NPs could be transported into deep tissues through transcytosis.[221] Despite these advances, strategies that actively promote transcytosis within solid tumors remain largely unexplored.

Zwitterionic polymer-drug conjugate PBEAGA-CPT exhibits an overall neutral surface charge and therefore circulates through blood without significant clearance. Upon contact with endothelial / cancer cells, the overexpressed γ-glutamyl transpeptidase (GGT) removes the γ-glutamyls from PPBEAGA, generating primary amines and therefore resulting in a positive surface charge. This cationization allows membrane adsorption, rapid caveolae-mediated endocytosis, and subsequent transcytosis, enabling transendothelial extravasation and intra-tumoral infiltration. Consequently, enhanced antitumor activity and eradication of approximately 100 mm^3 tumors as well as and larger, exponentially growing tumors was observed.[222] In another study, enamine N-oxides-modified nanoparticles were developed, encapsulating a stearic acid-modified gemcitabine prodrug (GemC18) and a pSMAD2/3 inhibitor, galunisertib, for the treatment of pancreatic ductal adenocarcinoma. Upon reaching the tumor site, particles respond to the acidic TME and hypoxia, resulting in surface charge conversion to positive state, promoting tumor penetration and nanoparticle disintegration, releasing the cargo.[223] In a recent study, the RMT strategy of active transcytosis was demonstrated by metallic iron-oxide NPs aiming at overexpressed glucose transporters, mainly GLUT1, on glioblastoma endothelial cells. These glucuronic acid-functionalized NPs enhance GLUT-mediated transcytosis, which enables highly precise targeting of glioblastoma and overcoming the blood-brain barrier.[224]

Another approach involves cell-mediated nanoparticle delivery systems, which utilize living cells, such as immune cells, stem cells, or engineered cell carriers, to transport therapeutic

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nanoparticles directly to tumors. Because these cells naturally home to sites of inflammation, hypoxia, and cancer, they can overcome biological barriers that synthetic nanoparticles often fail to penetrate. This approach enhances targeted delivery, improves penetration into deep tumor regions, and reduces off-target toxicity. By combining the programmable behavior of cells with the tunable properties of nanoparticles, cell-mediated delivery represents a highly adaptable platform for precision nanomedicine, enabling more selective, efficient, and personalized cancer therapy.[225]

The physicochemical properties of NPs, mainly the shape, size, and stiffness, influence the specific uptake pathway involved in internalization. While clathrin- and caveolae-mediated endocytosis occur at comparable levels in cancer and healthy cells, macropinocytosis is markedly upregulated in cancer. This increase is driven by oncogenic alterations, most notably KRAS mutations, and is particularly prominent in highly aggressive malignancies such as breast, lung, and pancreatic cancers, where it supports enhanced nutrient uptake. This suggests that more malignant types of cancer exhibit elevated activity in micropinocytosis to sustain their biosynthetic and energetic needs of rapid proliferation and invasion. This uptake preference can be exploited in the design of nanotherapeutics that selectively target micropinocytosis. Moreover, given that cancer cells exhibit an overall negatively charged plasma membrane and are softer, charge-switching NPs have been developed to target the negatively charged membrane by switching from negative charge to positive to specifically target cancer cells and increase intracellular internalization.[226]

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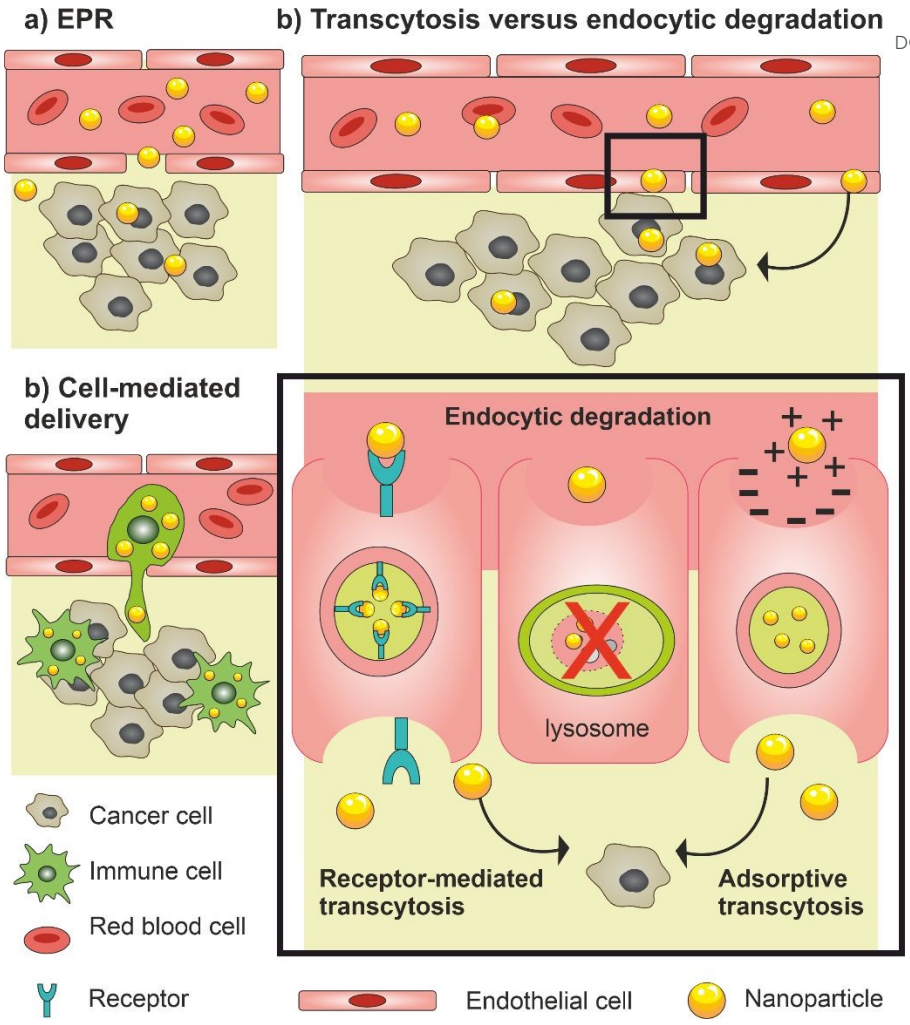

Figure 3: ***Mechanisms for in vivo nanoparticle delivery****. a) Passive nanoparticle accumulation via the enhanced permeability and retention (EPR) effect, driven by leaky or fenestrated tumor vasculature and impaired lymphatic drainage; b) Cell-mediated delivery, whereby engineered immune cells internalize nanoparticles and actively migrate into tumors; c) Active transcytosis, in which nanoparticles are internalized by endothelial cells, trafficked across the cytoplasm, and released on the abluminal side, enabling transport across the vascular barrier while avoiding lysosomal endocytic degradation and perivascular retention. Adsorptive-mediated transcytosis (AMT) is driven by nonspecific electrostatic interactions between cationic nanoparticles and the anionic endothelial plasma membrane, resulting in relatively high uptake but limited selectivity and potential off-target transport. In contrast, receptor-mediated transcytosis (RMT) is initiated by specific ligand–receptor interactions, enabling controlled, cell-type-selective transport across the endothelium with improved targeting efficiency and reduced nonspecific uptake. Adapted from Pandit et al.*[219]

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The physicochemical properties favoring micropinocytosis include NPs size typically larger than 200 nm, since these are too big to be internalized by the clathrin- or caveolae-dependent pathway, rod-shaped like NPs, due to increase in membrane contact area and induction of asymmetric membrane wrapping, and lipoprotein-coated NPs that mimic nutrient-like cargo. These findings suggest that tuning NPs for intracellular uptake via transcytosis or macropinocytotic endocytosis may present a promising strategy to enhance transport across biological barriers and improve selective targeting of tumor cells.[227], [221]

Cargo-free nanotherapeutics

In nanomedicine, the nanotherapeutics can be either formulated as a nanocarrier containing a conventional or novel drug as cargo to transport the treatment directly to the tumor site, or the nanoformulation itself can be an anticancer therapeutic. Drug-free unmodified gold nanoparticles (AuNPs) were found to suppress tumor cell proliferation through inhibition of MAPK signaling while also reversing EMT by disrupting the function of heparin-binding growth factors (HB-GFs), which are essential for angiogenesis and EMT progression. The underlying mechanisms included upregulation of E-cadherin expression and reduced secretion of EMT-associated proteins such as Snail, N-cadherin, and vimentin. These observations were validated in two distinct orthotopic mouse models of ovarian cancer.[228] Another types of metal NPs are non-persistent gold/copper ultrasmall-in-nano architectures (NAs). Evaluation of NAs in the chorioallantoic membrane (CAM) model of pancreatic ductal adenocarcinoma revealed their ability to modulate antimetastatic behavior, mainly through alterations in EMT-related protein and gene expression.[229] Poly(ethyleneglycol)-functionalized dendrimers have shown to deplete bioavailable copper and therefore inhibit angiogenesis in vivo. In comparison to conventional chemotherapeutics, the dendrimer exhibited antitumor activity in a non-cytotoxic way, yet resulting in suppressed tumor proliferation and metastasis.[230] In another study, mesoporous silica NPs (MSN-PEG/TA 25) have shown antimetastatic activity in breast cancer mouse models. Combined treatment with MSN-PEG/TA 25 and liposomal doxorubicin significantly improved survival in mice compared with treatment using liposomal doxorubicin alone. This approach may represent a more effective strategy to inhibit metastasis by targeting focal adhesion kinase (FAK) rather than EMT directly, as it suppresses cancer cell migration and interferes with several stages of the metastatic cascade, including invasion and colonization. [231] Other cargo-free nanotherapeutics with antimetastatic activity and potent synergic effect with conventional drugs are carbon nanotubes mimicking cytoskeletal filaments. They showed intrinsic anticancer activity and inhibition of metastasis via VEGF receptor targeting. In a

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murine metastatic melanoma model, treatment reduced pulmonary metastases by more than 80%. When combined with paclitaxel, this antimetastatic effect increased to approximately 90%.[232] In immunocompetent 4T1 mice, intragastric administration of citrate- and PEG-coated silver nanoparticles significantly reduced lung metastases without affecting primary tumor growth or EMT-related gene expression. The observed effects instead correlated with modulation of inflammation-related pathways.[233] A very recent study demonstrated that boron-incorporated alginate carbon nanogels significantly reduced lung metastases by over 85%. These particles selectively target TNBC cells by interaction with sialic acid on the cancer cells surface and result in disruption of F-actin and ROS-mediated cell cycle arrest.[234] CXCR4-targeted metallo-nanodrugs were designed to treat breast cancer metastasis by inducing in situ oxidative stress enhanced by hypothermia and by blocking the CXCR4/CXCL12 signaling axis. This approach inhibited invasion and migration of 4T1 cancer cells. In a BALB/c mouse mammary tumor model, these nanostructures suppressed both primary tumor growth and lung metastases with minimal side effects, demonstrating strong potential for precision nanomedicine.[235] Targeting cancer metastasis does not necessarily need to focus solely on cancer cells but can also involve modulation of the complex tumor microenvironment. For example, gold-core silver-shell nanoparticles (Au@Ag) have been reported to affect cancer-promoting activity of cancer-associated fibroblasts (CAFs) by altering their communication with tumor cells. Transcriptomic analysis revealed alterations in the secretory profiles of nanoparticle-exposed CAFs, especially downregulation of *Spp1* expression observed in the 4T1 in vivo model following Au@Ag treatment. Elevated expression of this protein has been associated to poor survival in breast cancer patients, suggesting that it may represent a promising target for future therapeutic strategies.[231] Overall, these findings demonstrate that rationally engineered nanoparticles can function as standalone antimetastatic agents or synergize with conventional drugs by modulating metastasis-related pathways or directly inhibiting tumor progression.

Challenges and future perspectives

Owing to their structural complexity and the need for multifunctional design, including optimized drug encapsulation, controlled release, tissue penetration, biodistribution, metabolism, and clearance, the development and comprehensive evaluation of nanotherapeutics remain highly challenging. Many factors complicate formulation design, pharmacokinetic, as well as pharmacodynamic characterization, and hinder the accurate assessment of therapeutic

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efficacy and safety. In addition, nanomedicine delivery is impeded by barriers within the tumor microenvironment, which constitutes a highly complex, heterogeneous, and patient-specific network of interactions. At the same time, nanotherapeutics themselves may elicit toxicity or unintended immune responses within the local tissue microenvironment. Taken together, these challenges underscore the need for more rational nanotherapeutic design, improved preclinical models, and integrative evaluation strategies. A major factor contributing to impaired nanomedicine delivery is the compromised EPR effect within the tumor microenvironment. The EPR effect varies substantially depending on the xenograft mouse model used and shows pronounced heterogeneity across patients, limiting its reliability for effective nanoparticle accumulation.[236], [237] The EPR effect also exhibits substantial variability between xenograft mouse models and patients. Human tissues display markedly higher blood flow rates than rodent tissues, resulting in greater shear forces and faster vascular washout. This may partially explain the superior accumulation of nanomedicines in rodent tumors and pose a significant challenge to the clinical translatability of preclinical nanomedicine findings.[147] Furthermore, differing tumor vasculature and desmoplasia restrain the proper delivery of nanotherapeutics to tumor sites. On top of this, in clinical practice, nanotherapeutics are frequently administered in later treatment lines or in combination with other anticancer agents, and prior or concomitant therapies may significantly remodel tumor vasculature and stromal architecture, thereby further modulating the EPR effect. In metastasis, the EPR effect differs even more.[238] In macrometastasis, which are highly evolved and angiogenic, the EPR effect may be present; however, micrometastasis may lack any vasculature. Depending on the secondary site, each tissue where metastasis is present constitutes another barrier for nanotherapeutics, often different from the one present in the primary tumor. In brain metastases, the blood-brain barrier is highly restrictive and has very limited permeability. In bone metastasis, the complex dense matrix limits the diffusion of NPs into lesions, and hydroxyapatite can bind charged NPs non-specifically. In the liver, NPs must sustain clearance by Kupfer cells and avoid accumulation in sinusoidal spaces. One of the solutions may be the stratification of patients before the nanotherapeutic delivery by EPR-predictive biomarkers or by imaging agents.[239] Patients with dense, non-leaky tumor vasculature may benefit from active or bypass delivery strategies, including targeting of the tumor vasculature, locoregional administration, integrin-RGD-mediated targeting, or cell-mediated delivery approaches. However, each of these potential therapies comes with many other obstacles as well. The phenomenon known as “biomolecular corona” is a significant obstacle occurring when nanoparticles meet biological fluids. During

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this interaction, biomolecules adsorb onto the nanoparticle surface, forming a corona that can mask targeting ligands and alter the physicochemical properties of the nanocarrier. In addition, the biomolecular corona may trigger immune recognition, leading to rapid clearance by the mononuclear phagocyte system before the nanoparticles reach their intended target.[240], [241] When the NPs successfully reach near the tumor site, another physiological barrier is present called interstitial fluid pressure (IFP). Whereas in normal tissues the IFP is low, in solid primary tumors and macrometastases, there is a significant elevation in IFP due to leaky vessels and dense matrix.[242] This IFP elevation causes limited extravasation of NPs and their accumulation on the tumor site periphery. So even if the EPR effect favors the successful transport of NPs into the tumor site, the high IFP reduces it dramatically. As well as EPR, the IFP varies through different stages of metastatic formation. While micrometastases show almost none IFP, the fully developed macrometastases mimic the solid primary tumor IFP. Some nanodrugs were also found to be accumulating in the body, mainly in the liver and spleen, for a long time.[243], [244] Their improper elimination then results in physical damage of the affected tissue, resulting in inflammation and toxic reactions, including the production of ROS and elevation in pro-inflammatory cytokines.[245], [246] Therefore, there is a strong interest in the development of biodegradable or biocompatible nanocarriers. RGD-, CD44-, and transferrin-targeted nanotherapies, as mentioned above, represent promising treatment strategies for targeting cancer cells; however, their efficacy in a metastasis setting is heavily dependent on intra- and inter-metastatic heterogeneity.[247], [248], [249] The expression of these target molecules is highly variable across different stages of the disease and metastatic organ sites and also within the same lesion itself. Expression of RGD-binding integrins is often reduced in dormant and poorly angiogenic micrometastases. CD44 expression exhibits temporal and spatial heterogeneity across metastatic lesions and undergoes switching of isoforms (CD44v ↔ CD44s) during EMT.[250] Transferrin receptor expression is generally higher in rapidly growing tumor sites due to increased iron demand; however in slow-growing and dormant lesions the expression is lower, contributing to the overall heterogeneity between primary and metastatic disease. The translational phase of nanomedicine also presents additional challenges due to a lack of validated analytical methods for biodistribution and pharmacokinetics, and a lack of predictive biological models. Because of these translational challenges, the current approval rate of nanotherapeutics in oncology is estimated to be below 10%, and in the Phase III trials, the failure rate is up to 86%.[251] However, these percentages are broadly comparable to those of conventional cancer therapies, reflecting the overall complexity of cancer drug development. In recent years, the rapid advancement of artificial intelligence (AI) has introduced powerful

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tools for nanomedicine development, ranging from the rational design of nanoparticles with improved delivery efficiency to decision-support systems that help identify which nanotherapeutics are most likely to succeed in clinical trials. The gap between early-stage nanoparticle development and large-scale translation processes could be bridged by these novel tools, facilitating the successful clinical translation of nanoparticle therapeutics.[252]

Table 3.: Nanoparticle-based therapies undergoing clinical evaluation for metastatic indications

Drug/System Name	Cargo	Nanocarrier	Metastatic Cancer Type	Phase	NCT Identifier
AGuIX (NH TherAguix)	NA	Gadolinium-chelated polysiloxane based NPs	Advanced/unresectable pancreatic cancer*	Phase I/II	NCT04789486
AGuIX (NH TherAguix)	NA	Gadolinium-chelated polysiloxane based NPs	Multiple Brain Metastases	Phase II	NCT03818386
BIND-014	Docetaxel	PEG-PLGA NP	Metastatic castration-resistant prostate cancer	Phase II	NCT01812746
NanoTherm	None	Aminosilane-coated iron oxide NPs	Glioblastoma (reccurent)*	NA	NCT06271421
Lipo-MERIT vaccine	Tumor-associated antigen mRNA (immunotherapy)	Cationic lipoplex (DOTMA/DOPE)	Advanced melanoma*	Phase I	NCT02410733
CriPec®, CPC634	Docetaxel	PEG-polymeric NP	Advanced solid malignancies*	Phase I	NCT02442531
64Cu-MM-302	Doxorubicin	PEGylated liposome	HER2 positive metastatic breast cancer	Phase I	NCT01304797

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PRECIOUS-01	Threitolceramide-6 + NY-ESO-1 cancer-testis antigen peptides	Poly(lactic-co-glycolic acid) (PLGA) NPs	Advanced NY-ESO-1-positive cancers*	Phase I	NCT04751786
ThermoDOX®	Doxorubicin	Lyso-thermosensitive Liposomes	Liver metastases	Phase I	NCT02181075
Docetaxel-PM	Docetaxel	Polymeric micelle	Metastatic esophageal squamous cell carcinoma	Phase II	NCT03585673
Docetaxel-PNP	Docetaxel	Liposome	Advanced solid malignancies*	Phase I	NCT01103791
LipoVNB	Vinorelbine tartrate	Liposomes	Advanced malignancy*	Phase I, II	NCT02925000
iExosomes	KRAS G12D siRNA	Exosomes	Metastatic pancreas cancer with KrasG12D mutation	Phase I, II	NCT03608631
Sarah Nanotechnology System	NA	Iron oxide nanoparticles	Advanced metastatic solid tumors (stage 4)	NA	NCT07224464
NBTXR3 (Hensify)	Anti-PD-1	Hafnium oxide nanoparticles	Metastatic non-small cell lung cancer, liver metastases	Phase I	NCT03589339
NBTXR3 (Hensify)	Anti-PD-1	Hafnium oxide nanoparticles	Advanced solid malignancies*	Phase I, II	NCT05039632
NBTXR3 (Hensify)	Anti-PD-1	Hafnium oxide nanoparticles	Advanced or borderline-	Phase I	NCT04484909

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			resectable pancreatic cancer*		
E-EDV-D682	PNU-159682	EnGeneIC Dream Vector (EDV) nanocell	Metastatic pancreatic cancer	Phase I, II	NCT07049055
Carbon Nanoparticle-Loaded Iron [CNSI-Fe(II)]	Ferrous iron (Fe^{2+})	Carbon-based nanoparticles	Advanced solid tumors*	Phase I	NCT06048367
CPX-1	Irinotecan floxuridine	Liposomes	Advanced/metastatic colorectal cancer	Phase II	NCT00361842
Liposomal irinotecan	Irinotecan	Liposomes	Metastatic Pancreatic, Colorectal, Gastroesophageal, or Biliary Cancer	Phase I, II	NCT03337087
FF-10850	Topotecan	Liposomes	Advanced solid cancers*	Phase I	NCT04047251

*Metastatic disease not explicitly specified in regulatory approval.

Conclusions

While primary tumors can often be removed surgically, metastatic disease presents a far greater challenge. Once cancer has spread to distant organs, it can become incurable, significantly reducing patient survival rates. Recent advances in antimetastatic approaches, such as migrastatic therapies, nanomedicine, and targeted drug delivery, offer promising opportunities to improve patient outcomes. These therapies focus on disrupting the key mechanisms that drive metastasis, such as neovascularisation, cancer cell motility, metabolic and phenotypic plasticity, and immune evasion, providing a more precise and effective approach to cancer treatment. By targeting the critical steps of the metastatic cascade, modifying the tumor microenvironment, and integrating them into multimodal treatment strategies, antimetastatic therapies may improve patient survival, quality of life, and overall cancer care for patients worldwide. NPs have emerged as a powerful tool in cancer therapy,

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offering unique physicochemical properties and versatile functionalities. NPs are particularly promising due to their ability to target metastatic processes at the cellular and molecular levels. The versatility of NPs enables precise delivery of therapeutic agents, reduction of systemic toxicity, and modulation of the tumor microenvironment. Additionally, NPs can also be integrated into photodynamic therapy (PDT) and photothermal therapy (PTT) strategies. While preclinical studies demonstrate the potential of NPs in antimetastatic therapy, challenges remain in translating these findings into clinical practice. Key barriers include large-scale production, long-term biocompatibility, and precise targeting. Ongoing research aims to address these limitations, with advances in NP design, surface functionalization, and safety profiles bringing nanoparticle-based antimetastatic treatments closer to clinical reality.

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