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Adding nanotechnology to metastasis treatment arsenal

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Abstract

Metastasis is a major culprit behind cancer-related mortality as it accounts for 90 % of cancer deaths. The explosive growth of research on cancer biology has revealed new mechanistic networks information and pathways that promote metastasis. Consequently, a large number of anti-tumor agents have been developed and tested for their anti-metastatic efficacy. Despite their exciting cytotoxic effect on tumor cells *in vitro* and anti-tumor activities in preclinical studies *in vivo*, only a few of them showed potent anti-metastatic activities in clinical trials. This review provides a brief overview of current anti-metastatic strategies that show clinical efficacy and reviews nanotechnology-based approaches that are currently being incorporated into these therapies to mitigate challenges associated in treating cancer metastasis.

Cancer metastasis

Cancer metastasis is a modern epidemic, whose cures are yet to be found. It is the process of spreading and growth of cancer to different parts of the body from the primary tumor site where the cancer started. Almost 90 % of the mortality associated with cancer is due to the metastasis and not from the primary tumor [1]. Mechanistically, cancer metastasis consists of a series of processes where cancer cells (i) dissociate from the extracellular matrix (ECM) and survive cell death by a process known as **anoikis (see Glossary)**, (ii) invade through the ECM, (iii) intravasate into the lymph nodes or blood vessels and spread to distant organ sites, (iv) extravasate and arrest in distant tissues, (v) survive against the local immune response from the organ microenvironment to colonize and form micrometastases, and (vi) induce proliferative signals and **angiogenesis** processes to stimulate growth and enlargement to form macrometastases (Figure 1, Key Figure). Continuous growth of metastases ultimately interferes with the distant organ's physiological functions. Only a few cancer cells from a primary tumor can successfully complete all these steps to form secondary lesions, and cells that fail to complete any step of this process, die and are eliminated [2]. Tumor cell entry into the circulation is common, and more than a million cells per gram of primary tumor can be shed daily [3]. However, although circulating tumor cells are often detected in cancer patients their mere presence cannot definitely predict that metastasis will occur [4]. Most of the circulating tumor cells are rapidly killed and destroyed in the circulation and those

that survive are trapped by the capillary bed of distant organs. Moreover, tumor cells can also remain inactive for many years in distant organs [5].

Although prevention of metastasis in experimental settings has been shown [6], the molecular complexity of this process is a serious problem in effective cancer treatment. Therefore, in the clinic, the current therapeutic regimens focus on treating established metastases and normally do not try to prevent formation of new metastases. Anti-metastatic therapeutics mostly use agents that selectively target signaling pathways, which are active in the primary tumor, and their effect on the metastases are evaluated mostly via parameters such as **progression-free survival (PFS)** and **overall survival (OS)** only. However, metastatic lesions usually show different heterogeneous subpopulations of cells than the original primary tumor cells, with differential gene expression patterns, growth properties, cell surface proteins, and enzyme/protein functions [7,8] and the molecular and cellular signatures vary both within single metastases and among different metastases [9]. Therefore, these often exhibit less sensitivity to a drug which targets the primary tumor. Metastatic lesions also show increased genetic instability that gives rise to mutations which confer drug resistance. This genomic instability and heterogeneity in the cell population makes it difficult to identify reliable targets for therapy [9]. However, several drugs targeting one or multiple steps of metastasis have been developed in the last 40 years. These drugs show an incredible cytotoxic effect in cells *in vitro* and anti-tumor activities *in vivo* in preclinical mouse model settings, but only a small percentage of these drugs were able to go to clinical trials for the treatment of advanced cancer. Here we briefly review the current strategies that are used to treat metastasis and highlight the opportunities to integrate nanotechnology-based approaches to facilitate and mitigate current challenges associated to treat cancer metastasis.

Anti-metastatic strategies and agents

Chemotherapeutic agents

Chemotherapeutic agents are traditionally first applied to treat a primary tumor and later, with better understanding of their molecular mechanism, their use is extended for the treatment of metastatic neoplasms [10]. A variety of chemotherapeutic agents targeting different pathways associated with cancer cell proliferation and survival has been developed for the treatment of cancer metastasis [10] and are broadly classified based on their mode of action that include tumor cell invasion, tumor cell extravasation, angiogenesis and tumor growth. These drugs show clinical

efficacy in increasing PFS and OS of patients with metastatic diseases. However, they show broad range anti-tumor activities targeting multiple oncogenic pathways and are not deemed as selective agents. For example, cisplatin, a widely-tested **alkylating agent** is used to treat metastatic testicular cancer [10] and patients with metastasized ovarian cancer who do not respond to other chemotherapy treatments [11]. Gemcitabine, a nucleoside analog agent, is a Food and Drug Administration (FDA)-approved drug that is currently in a phase II clinical trial (Clinical Trial Number- NCT01028495) to evaluate its efficacy for the treatment of metastatic pancreatic cancer. Eribulin mesylate, a non-taxane microtubule dynamics inhibitor that induces tumor cell mitotic arrest, is FDA-approved for the treatment with patients suffering from metastatic liposarcoma after a clinical study (Clinical Trial Number- NCT01327885) showed that this drug increased median OS to 15.6 months. However, while a single agent kills the drug-sensitive cancer cells, often it leaves behind a higher proportion of drug-resistant cells in a tumor. To overcome such resistance, combination chemotherapy involving multiple drugs have been evaluated and approved by FDA to treat metastatic cancer. For example, combining gemcitabine with cisplatin significantly increased PFS and OS of patients suffering from metastatic pancreatic cancer [12] than gemcitabine treatment alone.

Targeting tumor invasion and cancer cell extravasation

A. Matrix metalloproteinase (MMP) inhibitors

One of the hallmarks of metastatic cancer is the ability of tumor cells to achieve a journey from the primary site to different parts of the body through the circulatory system. Tumor cells migrate and invade through the ECM with the help of classes of enzymes collectively known as matrix metalloproteinases (MMPs). They are expressed in almost every type of human cancer and often correlate with disease progression and poor survival [13,14]. One of their major roles is to catalytically degrade several components of the ECM and the basement membrane that allows migration and invasion of tumor cells. Over a 100 natural or chemically modified MMP inhibitors have been developed and many clinically tested [15]. However, despite showing anti-metastatic potency in preclinical studies, the MMP inhibitors have failed in clinical trials due to severe side effects observed in patients administered with the inhibitors [16]. Only a few are currently being evaluated in combination with chemotherapy as a treatment regimen for metastatic cancers. One

of these, genistein, which blocks MMP-2 and -9, was evaluated in combination with a standard chemotherapy treatment in a phase I/II clinical trial of metastatic colorectal cancer (Clinical Trial Number- NCT01985763) and results are currently awaited.

B. Integrin antagonists

Modulation of integrin expression has been associated with tumor cell motility and intravasation due to the involvement of integrins in ECM - cytoskeleton interactions. This in turn influences the epithelial–mesenchymal transition (EMT) and anoikis, which are critical players in tumor invasion and metastasis [17]. The $\alpha 5 \beta 1$, $\alpha v \beta 3$ and $\alpha v \beta 5$ integrins are widely expressed in different cancers and the tripeptide Arg-Gly-Asp (RGD) motif recognised by integrins is present in several ECM proteins [18]. Several classes of integrin inhibitors have been validated in clinical phase I, II and III trials for cancers that span from colorectal, ovarian, melanoma and renal, hepatocellular carcinoma, breast, pancreatic to prostate cancer [19,20]. However, there is no currently FDA approved integrin agonist available for metastatic cancer treatment. The first small molecule integrin antagonist developed was cilengitide [Cyclo-L-Arg-Gly-L-Asp-D-Phe-N (Me) L-Val], which is a cyclic peptide belonging to the RGD family. Cilengitide, prevents interaction between integrins and endogenous ECM ligands by binding to the integrin β chain [19] (Table 1A). It has been evaluated in clinical trials for the stage IV metastatic prostate cancer (Clinical Trial Number- NCT00103337) and high-grade progressive glioma (Clinical Trial Number- NCT00679354). However, in both cases, very less therapeutic benefit was observed. The $\alpha 5 \beta 1$ integrin antagonist ATN-161, which inhibits metastasis via interaction with fibronectin [21] has also been evaluated in clinical trials with no dose-limiting toxicities reported in patients suffering from advanced solid tumors [22,23]. Further, monoclonal antibodies like CTNO 95 targeting $\alpha v \beta 3$ and $\alpha v \beta 5$ have been examined in phase I clinical trials with reports of prolonged response [24]. Another antibody, vitaxin targeting $v \beta 3 \alpha$ integrin has been tested in clinical trials on patients diagnosed with progressive colon, breast, ovarian, sarcoma tumors with stage IV disease [25,26]. Furthermore, volociximab (M200), a chimeric monoclonal antibody that acts as an inhibitor of $\alpha 5 \beta 1$ integrin has demonstrated anti-metastatic activity against a number of advanced solid tumors [19]. Table 1A lists these integrin inhibitors along with details of the clinical trials in which they are currently involved.

Targeting Angiogenesis

Following extravasation to distant organs, tumor cells need to evade host immune response for their early survival. After the initial transformation and growth of cells, angiogenesis is needed for metastatic foci that exceed 1 mm in diameter to survive and propagate [27,28]. Angiogenesis is regulated by a complex combination of pro-angiogenic and anti-angiogenic signaling pathways where tumor cells mainly synthesize and secrete several pro-angiogenic molecules that induces endothelial cells present in the host microenvironment to establish a capillary network [27,28]. Although several pro-angiogenic molecules have been shown to initiate and maintain blood vessel formation, vascular endothelial growth factor (VEGF) has been studied most [29]. VEGF is induced by tumor cell hypoxia which also stimulates VEGF secretion. Secreted VEGF acts to (i) induce formation of blood vessels and (ii) promote the survival of new vessels formed in tumors. VEGF diffuses to nearby existing blood vessels, where it binds to heparan sulfate proteoglycans through its heparin binding domain [29]. VEGF binds to and activates VEGFR-2 in endothelial cells (ECs), leading to degradation of the ECM, releasing more VEGF. The ECs will then sprout, migrate, divide and finally grow tubes towards the VEGF gradient [29,30].

A. Anti-VEGF agents

A number of anti-VEGF agents have shown efficacy in preclinical and early clinical studies for the treatment of primary tumors. However, only two are currently approved by FDA for treatment against metastasis. These are bevacizumab and ziv-aflibercept [28,31,32]. Bevacizumab in combination with interferon alfa is under investigation for the treatment of patients with metastatic renal cell carcinoma [32]. Moreover, bevacizumab is currently used in the clinic in combination therapy for the treatment of metastatic cervical cancer. [32]. Survival is significantly improved in patients who receive bevacizumab and chemotherapy compared to those who receive chemotherapy alone. Bevacizumab is also approved by the FDA for use singly or in combination with fluoropyrimidine–irinotecan- or fluoropyrimidine–oxaliplatin-based chemotherapy for the treatment of metastatic colorectal cancer patients [33]. Further, a phase II clinical study shows that a combination treatment of bevacizumab+carboplatin+paclitaxel significantly increases the PFS and this treatment modality is approved by the FDA [32]. Ziv-aflibercept approved by FDA in August 2012 for the treatment of metastatic colorectal cancer that shows chemotherapy resistance [31], is a fusion protein specifically designed to bind all forms of VEGF-A and placental

growth factor (PlGF). Patients treated with ziv-aflibercept show ~ 2 month increase in median OS [32].

B. Small molecule receptor tyrosine kinase inhibitors (RTKI)

Receptor tyrosine kinases (RTKs) such as VEGFR, EGFR (epidermal growth factor receptor), PDGFR (platelet-derived growth factor receptor), c-kit and FLT-3 (fms-like tyrosine kinase 3) play central roles in angiogenesis, cell survival, proliferation, differentiation, migration, survival, cell death and invasion [34,35]. They have been linked with tumorigenesis in several adult and pediatric cancers [34,35]. RTKs become unregulated in cancer cells due to mutation(s) and autocrine, paracrine stimulation and become causal to cancer metastasis [34,35]. The FDA has approved multiple RTKIs for the treatment of metastases of several cancers [32]. Sorafenib was the first FDA-approved small molecule RTKI to treat metastatic kidney cancer (Table 1B) [32]. It blocks the activity of several kinases such as Raf kinase, VEGFR2, c-Kit, FLT-3, and PDGFR- β . Sunitinib is FDA approved for the treatment of both gastrointestinal stromal tumors (GISTs) and renal cell carcinoma [32], and is currently used in the clinic for metastatic pancreatic neuroendocrine tumors and advanced kidney cancer after findings from a clinical trial (Clinical Trial Number-NCT00428597) in 2011 [36]. Furthermore, treatment of GISTs and metastatic renal cell carcinoma has also been enforced by imatinib and pazopanib, respectively[37,38]. Vandetanib inhibits both VEGFR and EGFR, and is currently in clinical trials (Clinical Trial Number-NCT00410761) for metastatic medullary thyroid cancer where it increases the PFS of these patients [39]. Three other RTKIs, erlotinib, gefitinib[40] and afatinib, have been approved by the FDA for the treatment of locally advanced or metastatic NSCLC. These drugs target mutated EGFR which is known to be linked to NSCLC tumorigenesis[41]. It is interesting to note that erlotinib was initially approved by FDA for the treatment of patients with locally advanced or metastatic NSCLC after failure of at least one prior chemotherapy regimen [42]. Later, it was also approved for maintenance treatment of NSCLC where the disease has not progressed after four cycles of platinum-based first-line chemotherapy and, for first-line treatment of patients with metastatic NSCLC with an EGFR mutation [43].

However, many RTKIs have failed to show anti-tumor activities in clinical trials[44,45]. Numerous patients who benefited initially from the therapy showed lack of responses later due to the tumor's acquired resistance due to mutations in RTK genes.

C. Small molecule non-receptor tyrosine kinase inhibitors

Non-receptor tyrosine kinases are cytosolic enzymes that are implicated in tumorigenesis [46]. Currently there are a few small molecule non-receptor inhibitors targeting tyrosine kinase available in clinic [32]. These are listed in Table 1B and discussed here. Crizotinib is used to treat patients with metastatic NSCLC that contain mutated anaplastic lymphoma kinase (ALK) protein (Table 1B) [47]. Alectinib is another kinase inhibitor that is clinically used for the treatment of metastatic NSCLC patients who shows no response to crizotinib [48]. However, both of them can have life-threatening side effects, including liver problems and severe inflammation of lungs [47,48]. Cabozantinib is a pan-tyrosine kinase inhibitor and is an FDA-approved drug for the treatment of metastatic medullary thyroid carcinoma [49]. Further, since tumor progression has been linked with aberrant activation of two or more cytosolic kinases, therapeutic approaches to block multiple kinases have shown merit [50]. For example, combination of trametinib and dabrafenib, inhibitors of mitogen-activated protein kinase (MEK) is used clinically to treat metastatic melanoma with BRAF (B-raf proto-oncogene, serine/threonine kinase) V600E (valine600-glutamic acid) or V600K (valine-600-lysine) mutations [51].

D. Immune-checkpoint inhibitors

Immune checkpoints, such as cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed death ligand 1 (PD-L1) or its receptor, programmed death 1 (PD-1), are Achilles' heels for multiple tumor types such as melanoma, breast cancer, NSCLC, squamous cell carcinoma [52,53]. Both CTLA-4 and PD-1 signaling play essential roles in modulating immune responses. CTLA-4 inhibits immune responses by blocking naïve and memory T cell activation [52] whereas PD-L1, PD-1 signaling allows tumor cells to escape from local immune responses inducing apoptosis in activated T cells [53]. A typical cancer therapy normally includes a targeted antibody that can bind to CTLA-4 or PD1 receptors, thereby preventing them from interacting with tumor cell ligands. Immune checkpoint inhibitors provide more months of survival advantage to patients than other anti-metastatic therapies such as anti-VEGF drugs or RTKs. Due to their efficacy, at least five types of immune checkpoint inhibitor antibodies (nivolumab, pembrolizumab, MEDI4736, MPDL-3280A and ipilimumab) are currently under investigation in clinical trials for several metastatic cancers that have shown no response against chemotherapy or anti-VEGF therapy. The details of these immune checkpoint inhibitors are given in Table 1C.

Use of nanotechnology in metastatic cancer therapy

Nanoparticles (NPs) have already found range of applications in medicine due to several advantages that include cell selective response [54,55], ability to perform diagnosis and therapy in parallel [56] and the versatility of delivering various therapeutics combination to accommodate individual differences in drug responses (personalized medicine) [57]. These have been investigated for the targeted delivery of drugs in cancer treatment [56,58,59] including in dual roles as a drug carrier and drug itself [58,60]. The targeted delivery of anticancer nanomedicine is possible due to **enhanced permeability and retention (EPR) effect** [61]. Over the past decade, the architecture of the nanoparticle has been improved to enhance their utility in metastatic cancer therapy (see **Box 1**). This has resulted in approval of multiple nanomedicine in different cancer therapies. Table 2A lists these nanomedicines and the cancers that each treat based on information provided by drug manufacturers and literature [62,63].

The initial purpose of utilizing NPs to treat cancer was to protect drugs from premature clearance and prolonging their circulation time, thus increasing their chance of passing through the diseased site [64]. The first FDA approved anticancer nanomedicine Doxil[®] (Table 2A) was a liposome composed of polyethylene glycol (PEG)-modified phosphatidylethanolamine and cholesterol that encapsulated doxorubicin (an anti-cancer drug that blocks the growth of cancer cells) [65]. It has been shown to be more efficacious than doxorubicin [65] and is currently used to treat ovarian cancer, AIDS-related Kaposi's sarcoma, breast cancer and other solid tumors [66]. A non-PEGylated liposome-encapsulated doxorubicin sold as Myocet[®] is approved in Canada and Europe for treatment of metastatic breast cancer [67]. DaunoXome, a non-PEGylated liposomal daunorubicin, is approved for treating HIV-associated Kaposi's sarcoma [68]. Other clinically approved liposomal anti-cancer nanomedicines include DepoCyt[®] [69], Mepact[®] [70], Marqibo[®] [71], and Onivyde[®] [72] (see details in Table 2A). Nab-paclitaxel, known as Abraxane[®] is an albumin-bound colloidal suspension form of paclitaxel indicated for the treatment of breast cancer, NSCLC and pancreatic cancer [73]. Similarly, Genexol[®]PM, a micellar paclitaxel, is approved in Korea and Europe for treating various cancers [74]. Other clinically approved anti-cancer nanomedicines include Ontak[®] (cutaneous T-cell lymphoma) [75], Eligard[®] (prostate cancer) [76], Oncaspar[®] (acute lymphoblastic leukemia) [77], and NanoTherm[®] (glioblastoma). All of the aforementioned nanomedicines are summarized in Table 2A in a chronological order. NPs have been modified with targeting ligands (e.g., amino acid antibodies, antibody fragments, small

peptides, aptamers or small molecules) [55,78] that would selectively interact with overexpressed tumor cell surface receptors [79] in efforts to increase drug targeting specificity. Although such formulation is yet to be approved, it has been extensively investigated in clinical trials. For instance, BIND-014, a polymeric nanoparticle formulation targeting the prostate-specific membrane antigen, has been examined in phase II clinical trial (see details in Table 2B).

To control the specific release of the anti-cancer drugs and enhance their specificity, various external stimuli (e.g. temperature, ultraviolet (UV) light, ultrasound, magnetic and electric fields) are being investigated [62,80]. For example, NanoTherm[®] (Table 2A) is a novel procedure where magnetic NPs are introduced to the tumor and subsequently heated through alternating magnetic field [81]. The NPs in the therapy are made of iron oxide and coated with aminosilane [81]. NanoTherm[®] is primarily used for focal treatment of solid tumors and was recently approved in Europe for treatment against glioblastoma [81–83]. Similarly, ThermoDox[®] is a nanomedicine that utilizes lysolipid thermally sensitive liposome to encapsulate doxorubicin. When heated to 40°C-45°C, the liposome becomes porous and doxorubicin is directly released into the targeted tumor [84]. ThermoDox is currently in phase III clinical trials for the treatment of hepatocellular carcinoma (HCC), in phase I clinical trial for metastatic tumour of the liver and in phase I/II clinical trial for the treatment of breast cancer recurrence at the chest wall (See Table 2B for details)(Clinical Trial Number - NCT00826085).

Ongoing NP based clinical trials for metastasis therapy

Multiple ongoing clinical trials are directed towards nanomedicine-based strategies exploring anti-metastatic capability of the nanoagent as well as combination therapy with other drugs [85]. However, there are also numerous trials with new drug formulations towards cancer treatment. CRLX101 (Table 2B) is a promising nanomedicine currently in phase II clinical trial for advanced NSCLC and ovarian cancer [86]. A promising pilot trial has been completed with advanced or metastatic stomach, gastroesophageal, or esophageal cancer, those that are not removable by surgery Clinical Trial Number - NCT01612546. In mouse models of triple-negative breast tumor xenografts, it has been shown that administration of CRLX101 leads to tumor regressions, reduced metastasis and extended survival of mice with breast cancer [87]. . CRLX101 is comprised of camptothecin, a known topoisomerase-I inhibitor that is covalently conjugated through a glycine to a linear cyclodextrin-polyethylene glycol (CD-PEG) co-polymer and was

designed to overcome the challenges associated with camptothecin lability, insolubility and to augment its efficacy [88].

Hafnium oxide (HfO_2) based nanomedicine NBTXR3 works as an excitable radiosensitizer. Once accumulated in the tumor cells, subsequent radiation causes HfO_2 particles to generate free radicals that cause DNA damage and tumor cell death [89]. Phase I/II trials are expected to be initiated soon for NBTXR3 in combination with immune checkpoint inhibitor anti-PD-1 antibody in patients with advanced head and neck squamous cell carcinoma and NSCLC (Clinical Trial number- NCT03589339). NU-0129 is a first of its kind advanced nanomedicine platform that has been developed using spherical nucleic acid (SNA)[90]. It comprises of short interfering RNA (siRNAs) targeting the Bcl-2-like protein 12 (BCL2L12) arranged on the surface of gold NPs and is in clinical trial for recurrent glioblastoma and gliosarcoma (Clinical Trial Number- NCT03020017). While some of the examples of promising nanomedicines are described briefly here, readers are referred to Table 2B and literature[62,63,91,92] for details on more of these.

Conclusions and Future Perspectives

Treating metastasis is the ultimate challenge in reducing global cancer-associated mortality (see **Outstanding Questions**). A number of anti-metastatic inhibitors have been tested as therapeutic regimens and are shown to improve PFS [93]. Targeting several important regulatory molecules implicated in invasion of metastatic cells, cell survival and tumor growth has shown promise in clinical development and multiple inhibitors have been approved for clinical treatment of advanced tumors. However, many of such therapeutic drugs often result in only few months' longer survival period for patients with metastatic disease. Many of the anti-cancer agents that are used to treat metastasis are targeted to the distinct stages of the process that include invasion by tumor cells, extravasation to the distant organ and growth and maintenance of the metastatic lesion in the distant organ. These agents are briefly reviewed above that attempt to provide an overview of the current landscape of anti-metastatic strategies (**Figure 1**). Further, we review the emerging arsenal for treatment of metastasis- use of NPs. This comprises one of the main directions in cancer treatment, in particular towards improvement of the effectiveness and safety of available chemotherapeutics. Various nanostructure of NPs that provide specific and controlled drug delivery to metastatic cancer cells are being developed and are briefly reviewed above. Further,

apart from delivering anti-proliferation/anti-growth chemotherapeutic agents and immune checkpoint inhibitors for treating metastases, nanomedicine is also being extensively investigated as a tool for inhibition of angiogenesis, a process that is often associated with metastasis [94].

The concept of incorporating nanotechnology-based strategies has already demonstrated many encouraging outcomes. Recent studies have shown that immune cells can act as transporters to mediate the delivery of drugs across blood barrier and thus enhance transport of nanodrugs to tumor sites. Some very promising results were obtained on neutrophils [95] macrophages [96] and monocytes [97], which were all shown to facilitate delivery of nanoparticles into tumor sites. The utility of nanotechnology for the treatment of metastasis cancer can potentially be increased by use of programmable [85] combination therapy (Figure 2) where we can envision a nanosystem containing multiple therapeutics designed to target the different metastasis specific events and/or targets. Such nanosystems can potentially help achieve high targeting and drug efficacy, increase drug accumulation at the desired site and reduce systemic side effect.

However, while some of the nanotechnology-based strategies have been clinically implemented, others remain in pre-clinical or clinical phases. Although many of these preclinical studies show improved effectiveness of the nano formulation compared to “standard” chemotherapeutics, difficulties with translation of *in vivo* animal studies into human body as well as heterogeneous nature (cellular complexity) of tumors is the limiting factor in the search for efficient NPs [98]. Other issues that impede the effectiveness of nano-assisted drugs include considering EPR effect and physicochemical-dependent nanoparticle transport through the tumor stroma [98]. Selection of drug combinations and optimization of their spatiotemporal doses and selective anti-metastatic effects are needed to discover the most effective therapeutic strategy. Ultimately, integration of this knowledge leading to better development and therapeutic strategies for metastasis-oriented cancer drugs will be needed to produce clinically effective drugs to treat and cure metastatic disease.

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DISCLOSURES

The authors declare no competing interests.

RESOURCES

- i) <https://reference.medscape.com/drug/ontak-denileukin-342205>
- ii) <https://investor.celsion.com>
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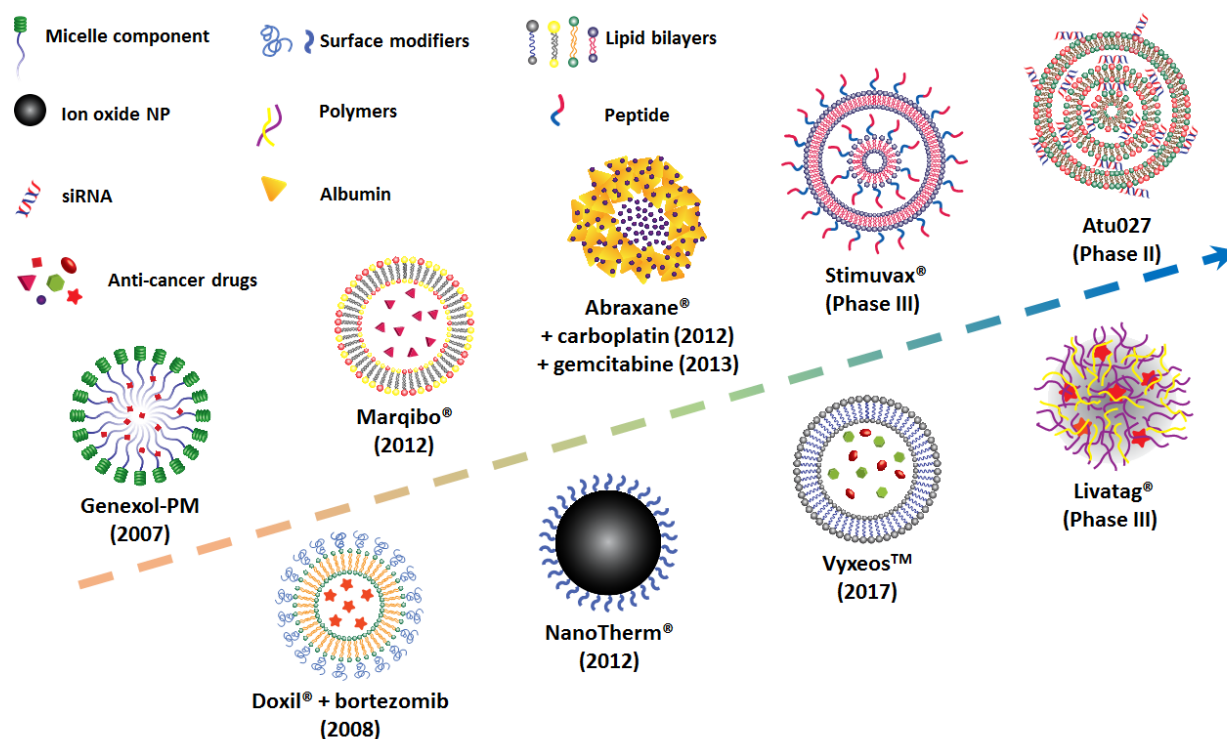
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TEXT BOX

Box 1: Evolution of the nanoarchitecture towards custom-fit requirement in cancer therapy.



Box 1, Figure I: Evolution of the nanoarchitecture towards custom-fit use in cancer therapy: The earlier generation of nanomedicines had simple structures, such as micellar (Genexol-PM) and liposomal (Doxil®, Marqibo®) formulation. As the field has progressed, more advanced nanoparticles were prepared starting with NanoTherm® to Abraxane® and systems that can deliver multiple therapeutics emerged (Vyxeos™). More recently, multi-layered liposomal (Stimuvax® & Atu027) and multi-polymer (Livatag®) nanoparticles have been developed. Further, the payload (drugs) being delivered have expanded from small molecule anti-cancer drugs to peptide (Stimuvax®) and siRNA (Atu027). Abbreviations used- NP: nanoparticle.

Anti-cancer nanomedicines initially started with simpler nanoarchitecture for delivering cargo (such as Genexol-PM, 2007, Box 1, Figure I). Since then, NP-based anti-cancer drug formulations have evolved over the years with wider range of materials and increasing level of complexity to accommodate various therapeutic needs. Initially, most of the nanomedicine are made with micellar (Genexol-PM) and liposomal (Doxil®, Marqibo®) formulations [71,74,99]. The sole purpose of these nanomedicines was to encapsulate therapeutic drugs. Later, inorganic materials were investigated (NanoTherm®) towards utilizing their unique physical properties (hyperthermia under alternating magnetic field) to treat cancer [100]. Further, biomaterials such as albumin was

also used to fabricate nanomedicine (Abraxane®) [101], and systems that can deliver multiple therapeutics emerged (Vyxeos™) [102]. In more recent effort, nanoparticles with more complicated structures, including multi-layered liposome (Stimuvax® and Atu027 [103]) and multi-polymer nanoparticles (Livatag®) [104] have been developed. The therapeutics of interest in the nanomedicines have now expanded from small molecule anti-cancer drugs to peptide (Stimuvax®) and siRNA (Atu027). Atu027, in combination with gemcitabine, is currently in Phase II clinical trial (Clinical Trial Number- NCT01808638 for metastatic pancreatic cancer) [103]. The evolution of structure and architecture of the nanomedicines has enabled them to accommodate multiple functions in a single nanomedicine delivery route that improves and meets mitigate the clinical requirements of treating a metastatic cancer.

FIGURES

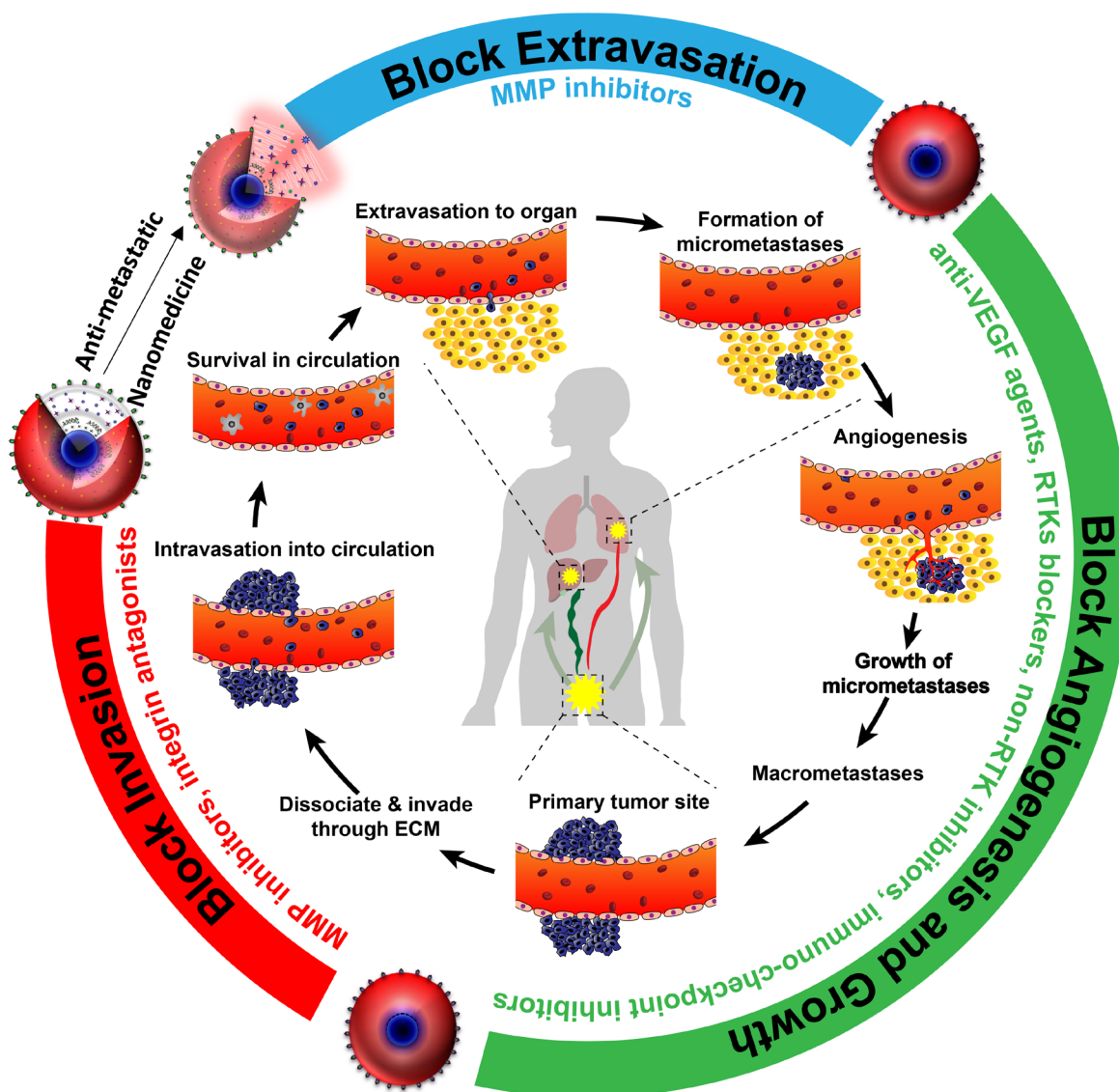


Figure 1, Key Figure: Steps in cancer metastasis and associated therapeutic strategies. The innermost circle around the schematic of the human figure shows the detailed steps of cancer metastasis. The outer circle broadly lists the stages of the metastatic process that can be targeted therapeutically. These include: invasion, extravasation and angiogenesis. The different therapeutic strategies for each stage are listed underneath it.

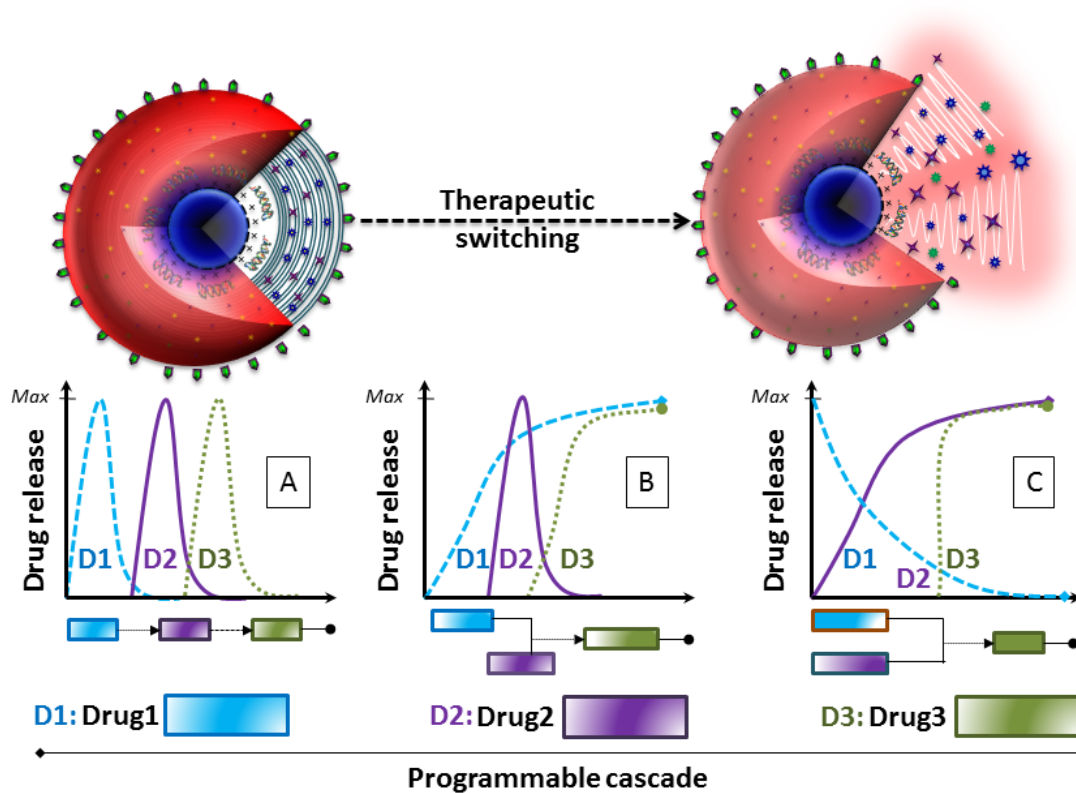


Figure 2: Potential nanotechnology-based programmable combination therapy for metastases treatment. Schematic overview of a potential nanosystem (upper panel) that can respond to metastasis specific events. The nanosystem could contain multiple drugs (D1 – D3) with programmable release profiles (shown in A - C) towards addressing different targets during metastasis. A shows a simulated profile for sequential release of 3 drug payloads at different time points, B shows a simulated profile for sustained release of D1 followed by release of D2 and another sustained release of D3 and, C shows a simulated profile for simultaneous burst release of D1 and sustained release of D2 followed by sustained release of D3.

TABLES

Table1: Table listing various integrin inhibitors, tyrosine kinase inhibitors and immune-checkpoint inhibitors that are currently in use or being tested in selected clinical trials for treatment of cancer metastasis.

Table 1A: Integrin inhibitors in clinical trials

Inhibitor	Mechanism of action	Metastatic disease, clinical trial phase and identifier
Cilengitide (cyclic RGD)	Peptide, selectively blocks $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins	Lung cancer (Phase I, NCT00884598) Melanoma (Phase II, NCT00082875) Breast cancer (Phase I, NCT01276496) Squamous cell cancer (Phase I, II, NCT00705016)
ATN-161	Non-RGD-based integrin binding peptide	Renal cell cancer (Phase II, NCT00131651)
CNT095	Monoclonal antibody, targets αv integrin	Prostate cancer (Phase II, NCT00537381)
Vitaxin	Monoclonal antibody, targets $v\beta 3\alpha$	Colon cancer (Phase I, II, NCT 00027729) Small intestine cancer (Phase I, NCT00049712) Lymphoma (Phase I, NCT00049712), Melanoma (Phase I, NCT00111696) Renal cell cancer (Phase I, II, NCT00684996)
Volociximab	Chimeric monoclonal antibody, targets $\alpha 5\beta 1$	Melanoma (Phase II, NCT00369395) Epithelial ovarian cancer (Phase II, NCT00516841) Renal cell carcinoma (Phase II, NCT00100685) Pancreatic cancer (Phase II, NCT00401570)

Table 1B: Selected FDA-approved small molecule RTK inhibitors

Drug	Target	Clinical Application	Current Clinical trial (phase and identifier)
Sorafenib	VEGFR, PDGFR, c-Kit, FLT3, PDGFR- β	Metastatic thyroid cancer [105] Metastatic renal cell carcinoma [106]	Metastatic or locally advanced medullary thyroid cancer (Phase II, NCT00390325) Advanced or metastatic liver cancer (Phase II, NCT03211416)
Sunitinib	VEGFR, PDGFR, c-Kit, FLT3, CSF-1R	Adjuvant treatment of adult patients at high risk of recurrent renal cell carcinoma [107] Metastatic pancreatic tumors [108] Gastrointestinal stromal tumors and advanced renal cell carcinoma [109]	Metastatic renal cell cancer (Phase II, NCT02060370) Metastatic endometrial cancer (Phase II, NCT00478426)
Imatinib	c-Kit, PDGFR	Metastatic malignant gastrointestinal stromal tumors (GISTs) [38]	Metastatic melanoma (Phase I, NCT01738139)
Pazopanib	VEGFR, PDGFR, c-Kit	Metastatic renal cell carcinoma [37,110]	Metastatic renal cell cancer (Phase IV, NCT02555748)
Vandetanib	VEGFR, EGFR	Metastatic medullary thyroid cancer[39]	Metastatic medullary thyroid (Phase III, NCT00410761)
Erlotinib	EGFR exon 19 deletion or exon 21 (L858R) substitution	Locally advanced or metastatic NSCLC [111]	Metastatic head and neck squamous cell cancer (Phase III, NCT01856478)
Gefitinib	EGFR	Metastatic NSCLC[40]	Metastatic neuroendocrine tumors (phase II, NCT00075439)
Afatinib	EGFR exon 19 deletion or exon 21 (L858R) substitution	Metastatic NSCLC [112]	Advanced NSCLC with EGFR mutation (Phase III, NCT01853826)
Crizotinib	Mutated ALK	Metastatic NSCLC with mutated ALK [47]	Metastatic urothelial cancer (Phase II, NCT02612194)
Alectinib	Mutated ALK	Metastatic NSCLC [48]	Locally advanced or metastatic NSCLC with ALK positive (Early phase, NCT03271554)
Cabozantinib	Pan kinase inhibitor	Metastatic medullary thyroid cancer [49]	Metastatic merkel cell cancer (Phase II, NCT02036476)
Trametinib + Dabrafenib	MEK	Metastatic melanoma with BRAF V600E and V600K mutation [51]	Stage 4 NSCLC with BRAF V600E mutation (Phase II, NCT01336634) Metastatic thyroid cancer (Phase II, NCT03244956)

Table 1C: Immune checkpoint inhibitors

Drug	Mode Of Action	Clinical Application	Current Clinical Trials (phase and identifier)
Nivolumab	Blocks interaction between PD-L1 and PD-L2	Metastatic non-small cell lung cancer [113] Metastatic melanoma [32] Metastatic small cell lung cancer [32] Squamous cell carcinoma [113] Urothelial cancer [32]	Metastatic renal cell carcinoma (Phase II, NCT03126331) Breast cancer brain metastasis (Phase I, NCT03807765) Advanced NSCLC (Phase II, NCT03121417) Advanced germ cell tumors (Phase II, NCT03726281) Metastatic melanoma (Phase IV, NCT02626065)
Pembrolizumab	Blocks interaction between PD-L1 and PPD-L2	Metastatic melanoma [114] Metastatic cervical cancer[114] Merkel cell cancer [32] Squamous cell carcinoma [114] Urothelial cancer [32]	Metastatic anal cancer (Phase II, NCT02919969) Metastatic prostate cancer castration resistant (Phase II, NCT03506997) Metastatic skin cancer (Phase II, NCT02964559) Metastatic melanoma (Phase IV, NCT03715205) Advanced breast cancer (Phase III, NCT02555657)
MEDI4736 (Durvalumab)	Inhibits binding to PD-1 and CD80		Metastatic NSCLC (Phase III, NCT02352948) Metastatic squamous cell cancer (Phase II, NCT02207530) Metastatic pancreatic ductal cancer (Phase I, II, NCT02583477)
MPDL-3280A (Atezolizumab)	Inhibits binding to PD-1 and CD80	Metastatic NSCLC [32] Metastatic urothelial cancer [32]	Locally advanced or metastatic NSCLC (Phase IV, NCT03285763) Metastatic bladder cancer (Phase II, NCT02951767)
Ipilimumab	CTLA-4	Metastatic Melanoma [114] Metastatic renal cell cancer [32]	Metastatic NSCLC (Phase III, NCT03302234) Untreated advanced or metastatic renal cell cancer (Phase IV, NCT02982954) Metastatic uveal melanoma (Phase II, NCT02626962) Metastatic urothelial cancer (phase III, NCT03036098)

Table 2: Table listing selected approved anti-metastatic nanomedicines along with those in clinical trials

Table 2A: Approved anti-cancer nanomedicine in the clinic

Name	Nanoagent	Drug	Mode of action	Clinical application	Year of approval/ Agency and details
Doxil (U.S.) /Caelyx (E.U.)	PEGylated liposome	Doxorubicin	Doxorubicin-chemotherapy drug (cytotoxic anthracycline antibiotic) used to treat many different types of cancer	Ovarian cancer, metastatic breast cancer, myeloma [65,66]	1995 FDA (NDA: 050718), 1996 EMA (EMA/H/C/000089)
DaunoXome	Non-PEGylated liposome	Daunorubicine	Daunorubicin - chemotherapy drug (cytotoxic anthracycline antibiotic), commonly used to treat acute leukaemias	HIV-associated Kaposi's sarcoma [68]	1996 FDA (NDA: 050704)
DepoCyt	Liposome	Cytarabine	Cytarabine - antineoplastic agent that inhibits the synthesis of DNA	Lymphomatous meningitis [69]	1999 FDA (NDA: 21041)
Ontak	Engineered protein combining IL-2 and diphtheria toxin	Denileukin diftitox	Denileukin diftitox selectively delivers the cell-killing activity of the diphtheria toxin to targeted cells.	Cutaneous T-cell lymphoma [75]	1999 FDA (BLA: 103767) (In 2014 Ontak was discontinued in the U.S.) ⁱ
Myocet	Non-PEGylated liposome	Doxorubicin	Doxorubicin - chemotherapy drug (cytotoxic anthracycline antibiotic) used to treat many different types of cancer	Metastatic breast cancer [115]	2000 EMA (EMA/H/C/000297)
Eligard	Poly (DL-lactide-co-glycolide) (PLGH) dissolved in N-methyl-2-pyrrolidone	Leuprolide acetate	Leuprolide reduces levels of testosterone	Prostate cancer [76]	2004 FDA (NDA: 21731)

Abraxane	Albumin-bound paclitaxel NPs	Paclitaxel	Paclitaxel- a mitotic inhibitor used in cancer chemotherapy	Metastatic breast cancer, non-small cell lung cancer, pancreatic cancer [116]	2005 FDA (NDA: 201660), 2008 EMA (EMA/H/C/000778)
Oncaspar (pegaspargase)	PEGylated Lasparaginase	L-Asparaginase	Asparaginase- enzyme used to treat acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and non-Hodgkin's lymphoma	Acute lymphoblastic leukemia [77]	2006 FDA (BLA 103411)
Genexol-PM (Korea) Cynviloq (E.U.)	Paclitaxel-loaded polymeric micelle	Paclitaxel		Non-small cell lung cancer, breast cancer, ovarian cancer [92]	2007 South Korea
Mepact	Non-PEGylated liposome	Mifamurtide	Mifamurtide- an immuno modulator with antitumor effects	Osteosarcoma [70]	2009 EMA (EMA/H/C/802)
NanoTherm	Iron oxide NPs with an aminosilane coating	Iron oxide NPs	Magnetic NPs are introduced directly into a tumor and heated with an alternating magnetic field	Glioblastoma [117]	2010 EMA
Marqibo	Non-PEGylated liposome	Vincristine	Vincristine - a chemotherapy drug (natural alkaloid isolated from the Vinca rosea Linn plant) used to treat various cancers	Acute lymphoblastic leukemia [71]	2012 FDA (NDA: 202497)
Onivyde	PEGylated liposome	Irinotecan	Irinotecan - a semisynthetic derivative of camptothecin, topoisomerase I inhibitor.	Metastatic pancreatic cancer [72]	2015 FDA (NDA: 207793) 2016 EMA (EMA/H/C/004125)

This table includes information from drug manufacturers and summarized literature [62,63]. NDA: New Drug Application. This six digit number is assigned by FDA staff to each application for approval to market a new drug in the United States; BLA: Biologics License Application (for FDA); EMA: European Medicines Agency

Table 2B: Selected nanotechnology-based anti-cancer systems in clinical trials

Name	Nanoagent	Active compound	Description / mode of action	Application	Clinical trial phase and identifier
BIND-014	Polymeric nanoparticle (PEG-polylactic acid)	Docetaxel	Prostate-specific membrane antigen targeted nanoparticle	Metastatic prostate cancer, NSCLC	Phase II, NCT01812746 Phase II, NCT01792479 Phase II, NCT02283320
ThermoDox	Liposome	Doxorubicin	When heated, liposome changes its structure and release doxorubicin into targeted tumor	HCC, metastatic tumor of liver, breast cancer recurrence at chest wall	Phase III, NCT02112656 Phase I, NCT00441376 Phase I/II, NCT00826085 ThermoDox was granted orphan drug designation for primary liver cancer in U.S. (2009) and Europe (2011) ⁱⁱ
CRLX101	Cyclodextrin	Camptothecin	Sugar molecule cyclodextrin linked to a camptothecin - DNA topoisomerase I inhibitor	Solid tumors, small cell lung carcinoma, NSCLC Ovarian cancer	Phase I/ II, NCT02769962 Phase II, NCT01652079
NBTXR3	Functionalized hafnium oxide NPs	Hafnium oxide	Radiosensitizer, enhances the effectiveness of radiotherapy	Head and neck, liver, prostate cancers	Phase I/II, NCT03589339 NCT02721056 NCT02805894
NU-0129	Gold NPs nanoparticles	Small interfering RNA (siRNAs)	siRNAs targeting the Bcl-2-like protein 12 (BCL2L12) conjugated to gold NPs	Recurrent glioblastoma / gliosarcoma	Phase I, NCT03020017
CPX-351	Liposome	Cytarabine and daunorubicin	Cytarabine - antineoplastic agent that inhibits the synthesis of DNA Daunorubicin - a chemotherapy drug commonly used to treat acute leukaemias	Acute myeloid leukemia	Phase III, NCT02272478
SGT-53	Liposome	p53 gene	p53 is a human tumor suppressor gene. Loss of p53 suppressor function is present in the majority of human cancers	Glioblastoma, metastatic pancreatic cancer, solid tumors	Phase II, NCT02340156
NC-6004	Polymeric micelle (PEG-polyaspartate)	Gemcitabine Cetuximab/5-FU Pembrolizumab	Gemcitabine inhibits thymidylate synthase. Cetuximab is an epidermal growth factor receptor inhibitor; 5-FU inhibits thymidylate synthase. Pembrolizumab is a humanized antibody which blocks the protective mechanism of	Advanced solid tumors Squamous Cell of Head and Neck	Phase I/II, NCT02240238, NCT03109158, NCT03771820

			cancer cells from immune system.		
NK-105	Polymeric micelle (PEG-polyaspartate)	Paclitaxel		Breast cancer	Phase III, NCT01644890
NK-012	Polymeric micelle	SN-38	SN-38- an antineoplastic drug, active metabolite of irinotecan. Shows 1000 times higher activity than irinotecan	Breast cancer, small cell lung cancer	Phase II NCT00951613, NCT00951054 (In 2016 it received orphan drug designation from FDA) ⁱⁱⁱ
LEP-ETU	Liposome	Paclitaxel		Ovarian cancer, metastatic breast cancer, lung cancer	Phase I/II/III/IV, NCT02996214 (Received orphan drug designation from FDA for ovarian cancer treatment) ^{iv}
Rexin-G	Retrovector	Cytocidal cyclin G1 construct	Cyclin G1 - protooncogene involved in cell cycle regulation	Sarcoma, osteosarcoma, pancreatic cancer	Phase I/II, NCT00505713, NCT00572130, NCT00504998
Lipoplatin	Liposome	Cisplatin		Non-small cell lung cancer, pancreatic cancer	Phase III, 2011-003601-25
Paclical	Polymeric micelles	Paclitaxel		Ovarian cancer	Phase III, NCT00989131 /approved in Kazakhstan ^v
LipoCURC	Liposome	Curcumin	Curcumin -natural anti-inflammatory and anti-cancer agent	Various advanced cancers	Phase I/II, NCT02138955
Promitil	PEGylated liposome	Mitomycin-C	Mitomycin-C - antitumor antibiotic that inhibits DNA synthesis and halts cell replication	Solid tumors Metastatic Disease	Phase I/II, NCT03823989

This table includes information from web resources including <https://clinicaltrials.gov/> and summarized literature [62,63,91,92].

GLOSSARY

Alkylating agents, inhibit DNA synthesis and cell division, ultimately leading to cell death. They are used to treat different cancers including lymphoma, leukemia, lung, and breast sarcoma. Several types of alkylating agents are available including nitrogen mustards, ethyleneimine, alkyl sulfonates, nitrosoureas triazenes, and platinum complexes. Some popular chemotherapeutic agents belonging to this class are cisplatin, satraplatin, picoplatin, altretamine, procarbazine, dacarbazine, carmustine, lomustine and streptozocin.

Angiogenesis, a process of formation of new blood vessels, which is induced by tumors at an early stage of growth to support nutrient supply and growth.

Anoikis, a type of programmed cell death that occurs in anchorage-dependent cells when they detach from the surrounding extracellular matrix (ECM). Cancer cells very often escape this process and survive to invade the ECM to enter the circulation via lymph nodes or blood vessels.

Enhanced permeability and retention effect (EPR), the phenomenon in which molecules under certain size are more likely to accumulate in tumor tissue, due to the increased permeability of neovasculature and the lack of lymphatic drainage in the tumor microenvironment.

Overall survival (OS), the length of time from either the date of diagnosis or the start of treatment for a disease, such as cancer, that patients diagnosed with the disease are still alive.

Progression free survival (PFS), the length of time during and after the treatment of a disease, such as cancer, that a patient lives with the disease but it does not get worse.