Expert Opinion

Targeted drug delivery strategies to treat lung metastasis

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Background: Most cancer patients die of metastatic disease, and in a high proportion of cases, from lung metastasis. Methods to target therapy to metastatic disease in general and specifically to lung metastasis are required. *Objective:* To describe the current and potential tools for the treatment of lung metastasis. *Methods:* Literature search tools were used with no predefined limitations to encompass the main tumor targeting methods. Methods in standard clinical use, in clinical trials and in preclinical development are reviewed. Data about treatment of lung metastasis and solid tumors are emphasized. *Results:* Physically targeting therapies to lung metastasis is feasible by aerosol-carried agents, magnetic targeting and intravascular devices. Biological targeting includes methods such as polymers and liposomes, which are based on the principle of enhanced permeability and retention of large molecules in tumor vascular field. Ligand-targeted treatments depend on cancer-specific antibodies or receptors. Few of these methods are in clinical trials or in standard clinical use. However, promising techniques are in advanced preclinical or early clinical studies. The authors believe that targeted treatments will be one the major anticancer tools in the near future.

Keywords: aerosol, antibody-conjugated, enhanced permeability and retention, ligand-targeted therapy, liposomes, magnetic targeting, nanoparticle, polymer conjugates, vascular targeting agents

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1. Introduction

1.1 Cancer metastasis to lung

A primary malignant tumor, if diagnosed early enough, can be removed by surgery or be radically treated by radiotherapy. However, a hallmark of cancer is its ability to metastasize. Migratory and invasive properties, as well as an aptitude to survive changing microenvironments, endow a cancer cell this ability. One of the common sites of cancer metastasis is the lungs. The reason is thought to be a combination of physical and biological factors. The lungs form a vascular mesh that all of the body's blood flows through. Most lymphatic fluid is filtered by the lungs right after entering the venous system. Adhesion molecules and extracellular matrix proteins specific to the lungs probably mediate attachment and survival of cancer cells in this microenvironment. Indeed, cancer-related death is inflicted most often by metastasis and in many cases by lung metastasis. In the vast majority of cases, once metastasis has evolved, cancer is incurable. The possible exceptions to this rule are discussed in this review.

An autopsy case series reported an incidence of 30 – 50% of lung metastasis in cancer patients who died of extrathoracic malignancy. Of these, 10 – 20% had metastatic disease limited to the lungs [1]. Overall, isolated and potentially resectable lung metastasis is found in ~ 1 – 2% of cancer patients. Lung metastasis is the

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sole cause of treatment failure in 50 – 80% of patients with osteogenic sarcoma and in 30 – 50% of patients with soft tissue sarcoma. Malignant melanoma patients develop lung metastasis as the only affected organ in 11% of metastatic cases [2]. Colorectal cancer recurs most commonly as liver metastasis, but lung metastasis is common and sometimes the only site of disease. Traditionally, those types of cancer are the main candidates for lung metastasectomy, the only method that thus far has demonstrated a real potential to cure metastatic patients.

Lung metastasis may occur hematogenously or through lymphatic channels. Hematogenous spread originates from blood-borne cancer cells that survive the immune system cells and blood turbulence, adhere to lung endothelial capillaries, invade the wall of these vessels and proliferate there. Lymphatic spread occurs by retrograde dissemination of cancer cells from involved extrathoracic lymph nodes, through lymphatic vessels in the pleura, diaphragm, or the thoracic duct, to mediastinal or hilar lymph nodes. From there disease can spread to lung lymph capillaries, causing lymphangitic carcinomatosis. Rarely, tumor cell clumps create tumor emboli, or endobronchial metastasis.

Just as with extrathoracic malignancy, lung cancer can also seed lung metastasis. The biological phenomenon is essentially similar to lung metastasis originating from an extrathoracic cancer. Clinically, metastatic nodes in the same lobe as the original tumor, other ipsilateral lobes, or contralateral lung lobe metastasis each have very different prognostic implication [3]. However, the underlying biological processes are probably similar for all of them.

1.2 The curative potential of treating lung metastasis

A patient harboring lung metastasis has a small but real potential for cure. An international registry for lung metastasectomy was set up in 1991, and a report summarizing data of > 5000 patients from 18 thoracic surgery departments in Europe and North America has been published. Prognostic factors have been identified, including a single or multiple metastases, complete respectability, disease-free interval and the type of the original tumor [4]. Patients who had a complete resection of a single metastasis after a disease-free interval of 3 years or more had a 61-months median survival. Importantly, patients who underwent a complete resection had a 30% survival at 5 years and 22% survival at 15 years, suggesting a significant chance of cure [5]. This data set obviously represents a highly selected set of patients, whose primary disease is under control and is in good general condition, with no significant co-morbidities. Most cancer patients with lung metastasis are not candidates for surgical resection of the metastasis, and an alternative route of treatment must be followed. Local surgical, radiotherapeutic and ablative approaches are discussed briefly below, followed by a review of the available ways to target systemic therapy to metastatic disease, with emphasis on the available data regarding lung metastasis.

2. The treatment of lung metastasis

2.1 Non-pharmacological treatment of lung metastasis

Surgical lung metastasectomy represents a chance for cure when the extrathoracic disease is under control, the metastasis amendable to complete resection, and the general status of the patients renders them fit for this operation [5,6]. Alternative, non-surgical, non-pharmacological methods of targeting lung lesions are radiotherapy, stereotactic body radiotherapy and radiofrequency ablation (RFA). Radiotherapy treatment involves using megavoltage linear accelerators, delivering high-energy photons to the tumor tissue. Stereotactic body radiotherapy targets the tumors with more precision. Radiofrequency ablation is a procedure done percutaneously, usually under computerized tomography guidance. The treatment probe is advanced into the middle of the tumor mass, and high-frequency alternating current is applied through it. Tissue impedance produces heat and coagulative necrosis results. **Table 1** summarizes all available methods for the treatment of lung metastasis.

2.2 Physically directing chemotherapeutic agents to tumor

2.2.1 Surgical methods of drug delivery

Isolated lung perfusion has been developed as a method of delivering therapeutic agents directly to the blood vessels that perfuse the lungs. This method offers the promise of high-dose treatment to the relevant organ, with minimal systemic exposure. The agents perfused can be chemotherapeutic agents, biologic modifiers such as tumor necrosis factor (TNF), or other agents. A thoracotomy is required, followed by isolation and cannulation of pulmonary arteries and veins, as well as installation of an extracorporeal cardiovascular system. In an animal model, it was demonstrated to achieve higher levels of the perfused agent and lower systemic levels compared with intravenous treatment [7]. Phase I studies of isolated lung perfusion using melphalan were carried out, combined with hyperthermia in some of the cases, followed by metastasectomy. Lung toxicity was dose limiting [8]. The dose of delivered drug correlated with lung drug concentrations, although not with tumor drug concentrations [9]. This procedure has yet to gain acceptance.

2.2.2 Percutaneus, endovascular approaches

Chemoembolization involves selective perfusion of a chemotherapeutic agent to the tumor vasculature, followed by embolizing and blocking further blood flow in the relevant blood vessels. It reduces the systemic exposure of the perfused drug while increasing and prolonging its local retention. Methods for embolizing blood vessels include microspheres that block small vessels [10] and gelfoam blockage of larger vessels. In an animal model of lung metastasis, microsphere embolization was better than intravenous chemotherapy, and as effective as isolated lung perfusion in terms of tumor control [11]. In patients with non-resectable lung metastasis,

Table 1. A summary of all available methods for the treatment of lung metastasis.

Targeting principle	Method	Route of administration	Effector	Advantages	Disadvantages	Current clinical use (organ)
Physical removal of tumor	Surgery	Invasive, surgery	operation	Removal of tumor	Risks of operative procedure, micrometastases are not treated	Common (lung and more) [1]
Radiation damage to tumor	Radiotherapy, stereotactic body radiotherapy	External beam radiation	Linear accelerator	Local killing of tumor cells	Damage to normal tissues in radiated field, micrometastases are not treated	Common (brain, lung) [2]
Hyperthermia	Alternating electrical current	Invasive, through thorax wall	RFA	Local killing of tumor cells	Limited size of target, invasive procedure, micrometastases are not treated	Common (lung, liver) [3]
Physical enhancement of local therapeutic agent delivery	Surgical intravascular	Invasive, surgery	Vascular cannulation	Maximal local delivery	Operative procedure, micrometastases are not treated	Clinical trials reported (lung) [4]
	Percutaneous intravascular chemoembolization	Catheterization through blood vessels	Vascular cannulation, microspheres, lipiodol (mithomycin C)	Non-operative, less systemic distribution	Invasive procedure, micrometastases are not treated	Preliminary data (lung) [5]
	Aerosol	Upper airways	Airborne droplets (adriamycin, gemcitabine, camptothecin, liposomes, DNA)	Lung-specific	Toxicity to all airways, systemic micrometastases not treated	Phase I/II (lung) [6]
	Magnetic targeting	Intravascular	Magnetic beads (adriamycin, mitomycin C, external/implanted magnet, alternating field magnet)	Simple physical targeting	Requires conjugation of therapeutic agent to magnetic particle, micrometastases not treated	Phase I/II/III, (liver, lung) [7]
	Magnetic localization of aerosol	Upper airways	Airborne droplets containing magnetic particles	Does not require conjugation to magnetic particle, lung-specific	Micrometastases not treated	Preclinical [8]
Enhanced permeability and retention	Liposomes	Intravenous	Liposomal-encapsulated chemotherapeutic agents	Local retention at sites of abnormal vasulcature and lymphatic system	Specificity is relative, potentially significant damage to normal tissues, potential RES uptake	Common; Doxil (breast, Kaposi's sarcoma), Myocyt (breast), DaunoXome (Kaposi's sarcoma) [9]

RES: Reticuloendothelial system; RFA: Radiofrequency ablation.

Table 1. A summary of all available methods for the treatment of lung metastasis (continued).

Targeting principle	Method	Route of administration	Effector	Advantages	Disadvantages	Current clinical use (organ)
	Polymer conjugation	Intravenous	Large molecular complex of polymer and therapeutic agent	Local retention at sites of abnormal vasculature and lymphatic system	Specificity is relative, potentially significant damage to normal tissues, potential RES uptake	Phase III reported (Xyotax) [10]
	Protein conjugation	Intravenous	Molecular complex of protein and therapeutic agent	Local retention at sites of abnormal vasculature and lymphatic system, biochemically simpler than liposomes and polymer conjugates	Specificity is relative, potentially significant damage to normal tissues, potential RES uptake	Abraxane (breast cancer) [11]
Release of toxin by cancer-specific factors	Polymer conjugation	Intravenous	Chemotherapeutic agent conjugated to polymer, released by cancer-specific protease, or acidic pH	Targeting specific biological cancer phenotype	Dependent on protease expression by tumor cells, or on lysosomal uptake	Phase III reported (Xyotax) [10]
	pH controlled-release liposome	Intravenous	Release of liposome content in an acidic milieu	Targeting specific biological cancer phenotype	Dependent on acidic pH of tumor environment	Preclinical [12]
	Reducing environment controlled-release liposome	Intravenous	Release of liposome content in a reducing milieu	Targeting specific biological cancer phenotype	Dependent on reducing properties of tumor environment	Preclinical [13]
Sustained release	Biodegradable polymers conjugation	Intravenous	Continuous release of therapeutic agent	Sustained active systemic concentration	No cancer-specific targeting	Preclinical [14]
Epitope-targeting	Immuno-liposomes	Intravenous	Therapeutic agent loaded liposomes conjugated to antibody targeted to a cancer-specific epitope	Targeting a specific cancer epitope	Dependent on cancer expression of a specific epitope	Preclinical [15]
	Radioisotope-conjugated antibodies	Intravenous	Radioactive isotope conjugated to a tumor-specific antibody	Targeting a specific cancer epitope	Dependent on cancer expression of a specific epitope	^{90}Y anti-CD20, hematological malignancies [16]
	Toxin-conjugated antibodies	Intravenous	Cytotoxic agent conjugated to a tumor-specific antibody	Targeting a specific cancer epitope	Dependent on cancer expression of a specific epitope	Gemtuzumab ozogamicin, hemtologic malignancies [17]
Receptor targeting	Liposomes carrying cancer-specific ligand	Intravenous	Therapeutic agent loaded liposomes conjugated to a ligand targeted to a cancer-specific receptor	Targeting a specific cancer receptor	Dependent on cancer expression of a specific receptor	Preclinical [18]

RES: Reticuloendothelial system; RFA: Radiofrequency ablation.

Table 1. A summary of all available methods for the treatment of lung metastasis (continued).

Targeting principle	Method	Route of administration	Effector	Advantages	Disadvantages	Current clinical use (organ)
	Copolymers including a targeting moiety	Intravenous	Therapeutic agent conjugated to a polymer targeted to a cancer-specific receptor	Targeting a specific cancer receptor	Dependent on cancer expression of a specific receptor	Phase I reported (PK2, hepatocellular carcinoma) [19]
Targeting biologic processes	Targeting osteoblastic bone activity	Intravenous	Radioisotope targeted to areas of active bone formation	Targeting osteoblastic activity typical of bone metastasis	Dependent on osteoblastic activity, only for bone metastasis	^{89}Sr, bone pain palliation for patients with painful bone metastasis [20]
Targeting tumor vasculature	Vascular targeted therapy	Intravenous	Therapeutic agent targeted to a cancer vasculature-specific epitope	More accessible targets compared with tumor cell targets	Indirect damage to cancer cells	Phase I reported (^{111}In- labeled mAbJ591) [21]

RES: Reticuloendothelial system; RFA: Radiofrequency ablation.

mithomycin C was administered followed by iodized oil (Lipiodol) and microsphere particles, achieving disease regression in 8 of 23 patients treated. No significant side effects or complications were noted, proving the feasibility of this method [12].

2.2.3 *Aerosol-based treatments*

Using aerosol, drugs can be delivered directly to the lung tissue. Airborne microscopic droplets are inhaled through the airways to the lung alveoli during aerosol administration. Manipulations of the method of administration can provide some control over this process, favoring central airways or lung periphery deposition [13]. Aerosol droplets can incorporate water-soluble chemotherapy, liposome formulations, DNA for potential gene therapy and more [14]. An aerosolized liposomal formulation of camptothecin was administered to patients with lung metastasis or primary lung cancer in a Phase II study. Dose limiting toxicities were chemical pharyngitis and fatigue. Some responses were observed [15]. A recent Phase I study demonstrated the feasibility of adriamycin administration by inhalation to 53 patients with lung metastasis or primary lung cancer, with a pulmonary dose limiting toxicity. A partial response was noted in a patient with osteosarcoma resistant to conventional chemotherapy [16].

In mice models, gene therapy vectors were delivered in aerosol, together with polyethyleneimine (PEI) or lipofectin, cationic agents that reversibly bind DNA. Aerosol-mediated gene therapy achieved a longer lung half-life and less distribution to other organs compared with intravenous delivery [17-20].

Targeting aerosol treatment to specific foci of lung disease was achieved recently by the use of magnetic nanoparticles [21]. In a mouse model, droplets that contain a magnetized component together with the targeted agent allowed their co-targeting by the magnetic field, thus conjugation to the magnetic particles was not required. Magnetic fields *in vitro* also enhance uptake of DNA or RNA into cells, suggesting that magnetic targeting of DNA by magnetized aerosols might be a future useful approach for gene therapy [22].

2.2.4 *Magnetic targeting*

More than 10 years ago, epirubicin conjugated to magnetic particles was administered intravenously to patients with metastatic solid tumors in a Phase I trial, targeted by an externally applied magnetic field. Almost half of these patients had thoracic metastasis [23]. The treatment was well tolerated, and some responses were noted. Targeting adriamycin to primary liver cancer using an external magnetic field has failed in a recent clinical trial [24,25]. Intravenous treatment by magnetic fluid-loaded liposome is being explored at present [26,27] for targeting chemotherapy to the liver [28]. Targeting agents to the lung in this manner is a valid option, but no study exploring it has been reported so far.

2.3 Biologically targeting tumor cells

2.3.1 Enhanced permeability and retention effect

Many cancer-targeted agents depend on the enhanced permeability and retention (EPR) properties of tumor blood vessels. Several factors contribute to this phenomenon. Tumors commonly demonstrate active angiogenesis, resulting in a high vascular density. The blood vessels of tumors are leakier than normal blood vessels. Tumor expression of permeability factors such as VEGF, bradikinin and nitric oxide might enhance extravasation of large molecules from these vessels. Unlike in normal or inflamed tissues, the lymphatic outflow from tumor interstitium is impaired. These conditions allow for macromolecules to accumulate preferentially in tumor interstitium [29]. The EPR effect can be seen when molecules > 50 kDa, liposomes and some lipids are administered systemically to tumor-bearing experimental animals. Larger molecules extravasate preferentially from vessels with enhanced permeability. For example, polymeric biocompatible compounds have been shown to reach tumor concentration levels > 10 times the levels in non-cancerous tissue [30]. To enhance the EPR effect, drugs need to have prolonged half-life in the circulation. Conjugation of small molecules to large polymers increases their half-life and, consequently, the EPR effect. In addition, a negative or neutral charge of molecules would increase their circulation half-life by reducing the binding to endothelium, as the luminal surface of blood vessels is mostly negatively charged [30,31]. The selective accumulation of macromolecules in tumor might be augmented by modulating blood flow and blood pressures. Angiotensin II infusion caused increased systemic blood pressure and reduced the accumulation of large molecules in the kidneys and bone marrow, owing to vasoconstriction in these organs. By contrast, blood flow and drug accumulation increased in the tumors, where homeostatic autoregulation of blood flow is abnormal. It can be speculated that increasing pulmonary arterial pressure would have a similar affect on lung perfusion: a reduction in blood flow to normal lung areas and an enhancement of blood flow to cancer blood vessels. To the best of the authors' knowledge, that hypothesis was never suggested or put to the test. **Table 2** summarizes the components of therapies biologically targeting tumor cells.

2.3.2 Nanoparticles and ligand-targeted treatments

Various types of nanometer-sized, molecular formulation exist and are being developed, aiming for a more efficient and more specific drug delivery to cancer. They include liposomes and other lipid-based formulations, polymer–drug conjugates, and microspheres, whose cancer-specific delivery depends on the EPR principle. A variety of ligand-targeted treatments (LTT) are also being developed, aiming for cancer-specific antigens. Nanoparticles using both EPR and LTT principles are assayed frequently.

LTT require an antigen that is specific for the tumor tissue. Ideally it is a membrane epitope, to allow access of the targeting moiety. The target has to be present in sufficient numbers on the cell membrane for targeting to be effective [32]. Her2/neu, an orphan receptor present in high numbers on the surface of ~ 20% of breast cancers, is an example of such a target [33]. Antibodies or fragments of monoclonal antibodies are a common targeting tool to a cancer-specific antigen. Complexes including antigen-binding fragments (Fab′) have been demonstrated to have longer circulation half-life compared with those with whole monoclonal antibodies, possibly because of the lack the immunogenic Fc domain [34]. Ligands of cancer-specific receptors are also important means of targeting cancer cells. Already part of standard clinical practice, antibodies targeted against tumor ligands such as rituxamab (targeting CD20) and targeting receptors such Herceptin (targeting Her2/neu) and cetuximab (targeting EGFR) are beyond the scope of this review.

For successful drug delivery into the cell, the target should undergo internalization eventually [35]. This requirement is not essential for therapeutic agents that can exert their effect from outside the cells, such as radionucleotide–antibody conjugates.

2.3.3 Radionucleotide/toxin antibody conjugates

A radioemitting isotope conjugated to a cancer-targeted antibody is a type of LTT. It utilizes the relative-cancer-specific gamma radiation of the isotope and the epitope specificity of the antibody. ^{90}Yttrium ibritumomab tiuxetan (^{90}Y Zevalin™, Cell therapeutics, Seattle, WA, USA) is one such agent targeting ^{90}yttrium to cells expressing CD20, in clinical use for hematological malignancies [36]. Gemtuzumab ozogamicin (Mylotarg®, Wyeth, NJ, USA) is an anti-CD33 monoclonal antibody conjugated to calicheamicin, a cytotoxic agent active in hematologic malignancies [37]. Bone-structure-based targeting of radionucleotides to sites of osteoblastic bone metastasis of prostate and breast cancers is used for palliation of pain from bone metastasis [38].

2.3.4 Drug–protein conjugates

The simplest nanoparticle type is a therapeutic agent conjugated to a protein in order to improve its stability, prolong its circulation half-life and enhance its tumor delivery. Such a chemotherapy-delivering nanoparticle that has entered clinical practice is albumin-conjugated paclitaxel (a microtubule-toxin) (Abraxane®, Abraxis Oncology, NJ, USA). The compound is exploiting the property of albumin to function as a ubiquitous carrier of lipophilic molecules. Tumor uptake is enhanced by an albumin transport mechanism. Abraxane can be administered to patients without the solvents that must be given with the free paclitaxel, thus eliminating the common hypersensitivity reactions of this drug. Compared with free paclitaxel, Abraxane increased time to tumor progression and had fewer side effects in metastatic breast cancer patients [39]. In about a third of the 453 patients on this trial the lungs were the major site of metastatic disease.

2.3.5 Polymers

Polymers have an important role in several drug delivery nanopaticles. These are versatile molecules that can be designed

Table 2. The components of therapies biologically targeting tumor cells.

Targeting moiety
Antibody for a cancer-specific antigen
Ligand to a cancer-specific receptor
Large molecule/complex (liposome, polymer, protein) targeting by EPR
Acid-dependent release of conjugated drug
Reducing-environment-dependent release of conjugated drug
Biologic process (biphophonates for osteoblastic lesions)
Tumor-vasculature targeting agent
Packing or linkage of targeting moiety to therapeutic agent
Peptidyl linker
Disulfide bonds
Biodegradable linkage and sustained release
Embedding of therapeutic agent in a targeting polymer
Liposome/micelle embedment, conjugation to polymer or protein, solubilizes and stabilizes therapeutic agent
Polycation electrostatic binding of nucleic acid-based therapy
Therapeutic agent
Chemotherapy agent
Radioisotope
Toxin
Cancer-specific pathway inhibitor
DNA (gene-therapy expression vector)
Short-inhibitory RNA gene therapy

EPR: Enhanced permeability and retention.

to address specific needs. Covalent or non-covalent conjugation of a drug to a polymer can resolve problems such as stability and solubility of the drug. Conjugation of a drug to a polymer creates a large molecule that would not be cleared by renal ultrafiltration and would accumulate in tumor tissue through the EPR mechanism. Polymers can be designed to include a specific targeting moiety. In addition, polymers might participate in microenvironment-dependent reactions, allowing the polymer itself to have therapeutic applications, and also to function as a controlled-release vehicle of the conjugated drug [40]. To utilize polymer–drug conjugate, the drug has to be active in its conjugated form, or must be released from the polymer within the tumor tissue or cells. Drugs delivered by a polymer are not necessarily conjugated to it but rather can be embedded in the polymer sieve. The slow degradation of the polymer, spontaneously or by enzymes, releases the drug in a sustained manner.

An example of a polymer–drug conjugate entering clinical use is paclitaxel-polioglumex (Xyotax™, Cell therapeutics, Seattle, WA, USA). Paclitaxel is linked in this case to a biodegradable polymer, poly-L-glutamic acid, thus markedly reducing its toxicity. The conjugate was shown to accumulate in tumor tissue, mainly because of the EPR phenomenon. Further studies have shown that the conjugate can enter tumor cells, where it undergoes proteolytic cleavage to mono and di-glutamile-paclitaxel products. The cleavage of the polymer is essential to its cytotoxic activity. Cathepsin B was shown to be one of the proteases that are required for the cleavage of paclitaxel-poly-glutamate [41]. Cathepsin B and additional proteases are upregulated in the microenvironment of many tumor types. Paclitaxel-polioglumex is being tested in advanced non-small-cell lung cancer patients, and was shown recently to be less toxic and more efficacious than free paclitaxel. Interestingly, a benefit was demonstrated in women with pre-menopausal blood levels of estrogen [42]. This might be related to the finding that following exposure of lung cancer cells to estrogen, the *cathepsin B* gene is induced [43]. These results might be implemented in the future for the treatment of lung metastasis in young female cancer patients.

Poly-DL-lactic acid (PLA) is one of the first biodegradable polymers to be put to use for sustained release of drugs. Varying the polymer length and structure allows control over its physical properties. Proteins embedded in PLA or poly-(lactic/glycolic acid) (PLGA) microspheres are released at a rate dependent on the polymer properties and composition. Using its versatility, release of different proteins at different rates can be achieved [44]. A PLA-encapsulated CXCR4 antagonist prevented lung metastatic spread of melanoma cells expressing CXCR4 in a mouse model [45]. Interleukin-1 receptor antagonist (IL-1Ra) encapsulated in PLGA biodegradable microspheres had a similar effect [46].

Polycationic polymers can be used to stabilize DNA or short inhibitory RNA (siRNA) and enhance the intracellular delivery of gene therapy vectors. Polycation-DNA or RNA structures are called polyplexes, or lipoplexes if the polycations are lipids. Poly-*N*-vinyl pyrrolidone injected intramuscularly with expression vectors of anti-angiogenic factors allowed for enhanced levels of these factors in the injected mice sera, and prevented metastasis development [47]. Polycation delivery of siRNA designed to downregulate the EWS-FLI1 oncogenic translocation product inhibited metastasis in a mouse model, including lung metastasis [48]. In another model of lung metastasis, cationic peptide encapsulated in a cationic liposome allowed the targeted delivery of a mixture of siRNAs designed to silence Mdm2, Myc and VEGF [49].

One of the first polymers to be used for drug delivery was *N*-(2-hydroxypropyl)methacrylamide (HPMA), a linear, water-soluble, non-degradable copolymer. Preferential tumor accumulation is dependent on its molecular mass [50]. A nanoparticle containing HPMA, adriamycin and glucosamine (PK2) was assayed in a Phase I trial. Glucosamine was used as a targeting moiety, aiming the adriamycin complex to the liver asialoglycoprotein receptor [51]. Another complex polymer assayed was composed of PEI as a DNA delivery polymer and the $\alpha v\beta 3/\alpha v\beta 5$ integrin-binding RGD peptide as an endothelial targeting moiety. A hydrophilic polyethylene glycol (PEG) spacer was included, producing a PEI-g-PEG-RGD polymer. This polymer was co-administered with DNA encoding the *interleukin-2* and soluble *VEGFR* expression vectors, and inhibited lung metastasis formation in a mouse model of

renal cell carcinoma [52]. These examples illustrate the above-mentioned versatility of polymer chemistry, including both targeting and carrying moieties in the same molecule. Another level of complexity can be achieved using polymers that deliver two types of therapeutic agents conjugated to the same polymer. The feasibility of delivering both an endocrine therapeutic agent and a chemotherapeutic agent has been demonstrated [53].

Poly-oxypropylene and poly-oxyethylene copolymer pluronic L61 have the capacity to modulate cellular uptake, multi-drug resistance (MDR)-mediated efflux and cellular distribution of drugs [54]. Cells exposed to this copolymer demonstrated enhanced uptake of adriamycin, release of the drug from intracellular endosomes and increased nuclear localization. Adriamycin incorporated into micelles was co-administered with such polymers to patients in a Phase I study. Although no sustained responses were noted, one patient with Ewing's sarcoma and lung metastasis had a non-sustained response, suggesting the feasibility of this copolymer for the treatment of lung metastasis [55]. Polymer-conjugated agents might allow bypass of the MDR-mediated mechanism of resistance of cancer cells, possibly owing to endocytic uptake and lysosomal compartmentalization of the conjugates [53].

Polymers can enhance tumor delivery of any conjugated drug. TNP-470, an anti-angiogenic agent not used in the clinic because of neurotoxicity, was conjugated to HPMA. In a mouse model it had an improved therapeutic window, thanks to tumor-specific, EPR-mediated localization. In addition, the large polymer prevented blood–brain barrier crossing, minimizing neurotoxicity [56].

An interesting new approach using polymer-conjugated agents is a two-agent treatment, called the polymer-enzyme-polymer-prodrug therapy (PDEPT). It involves administration of a drug conjugated to a polymer, and the administration of another polymer carrying the enzyme that releases the drug from the other polymer [57]. Similarly, polymer phospholipases can be used to modulate drug liberation from liposomes (discussed below), an approach called polymer-enzyme liposome therapy (PELT) [53].

Conjugation of a drug to a polymer carrier may also enhance its toxicity. Adriamycin conjugated to dextran was examined in Phase I clinical trial, and the dose equivalent to a standard dose of free adriamycin brought about significant bone marrow and liver toxicity. This was probably caused by uptake of the conjugated complex by the reticuloendothelial system (RES), thus targeting it to the liver and bone marrow cells [58]. Means for avoiding such a problem are discussed below.

The appropriate use of polymer conjugation requires complex decisions about choices of the polymers, the drugs and the manner of conjugation. The linking bond must be labile enough to allow release of the drug in tumor tissue, and not too labile to prevent its pre-mature release in the circulation. A MAG (a water-soluble copolymer)–camptothecin conjugate had no antitumor effect, and Phase I studies were stopped because of significant bladder toxicity, probably related to kidney proteolytic release of the drug from the MAG polymer [59]. Camptothecin was conjugated to the MAG polymer by an ester linkage. A probably better and recently more commonly chosen linker is degradable peptidyl bond, cleaved by lysosomal proteases. Alternative linkers are spontaneously cleaved in an acidic pH, which brings drug release in a tumoral-acidic microenvironment. For example, poly(β-amino ester) rapidly releases its content at a pH of 6.5 or lower [60]. Hydrazone linker, on the other hand, would release a drug at a lower pH, typical of the lysosomal compartment [61,62]. Reducing environment-triggered release can be achieved by using disulfide bonds, which are cleaved inside cells owing to the high gluthatione content [63].

Besides the linear-type polymers described above, complex branched structures called dendrimers and dendronized polymers are now being explored. They are promising structures because of the possibility of integrating within them a variety of physical properties. For a comprehensive review of the use of polymers in nanomedicine, see [61].

2.3.6 *Liposomal formulations*

Liposomes are vesicular structures composed of lipid-based molecules. They can be produced to be unilayered, including a single monolayer or bi-layer phospholipid membrane, or multilayered, structured as an onion. Liposomes can be used to deliver hydrophilic drugs carried in the center of such structures, or hydrophobic molecules that are incorporated in their lipid membrane. Stabilized formulations of chemotherapy-containing liposomes enhance targeting of the chemotherapeutic agent to the cancer cells, probably through the EPR effect.

A specific type of liposomes is micelles composed of block copolymers (formed of two types of polymers linked together), which can be used for drug delivery. Such copolymers have a hydrophilic domain and a hydrophobic domain, and above a certain concentration form a monolayered micelle, which could contain a hydrophobic therapeutic molecule in its center. Polymeric micelles have the delivery advantages of liposomes and design flexibility of polymers (see below). The following discussion of liposomes relates also to polymeric micelles.

Liposomes that have a simple phopholipid external membrane are rapidly cleared from the circulation by macrophage phagocytosis. Such liposomes are called non-stealth liposomes, and can be used when the RES is the target. PEG polymer incorporation into the external membrane creates 'stealth liposomes', masking them from immune system uptake, preventing opsonization and allowing for prolonged circulation and eventual delivery to tumors.

Compared with free adriamycin, PEG-coated adriamycin-liposomes (Doxil®, Ortho Biotech, NJ, USA) stayed longer in the circulation. As assayed in malignant exudates, it accumulated to higher concentration compared with free adriamycin [64]. Non-peylated liposomes are also in clinical use, including liposomal adriamycin (D-99, Myocet™; Elan Pharmaceuticals, NJ, USA) and liposomal daunorubicin

(DaunoXome®, Diatos, Paris, France). Both of these non-pegylated liposomal formulations have a shorter plasma half-life and less tumor targeting than pegylated liposomal adriamycin [65]. However, non-pegylated liposomal adriamycin is equivalent to free adriamycin in the treatment of metastatic breast cancer, but is less toxic. Liposomal daunorubicin was shown to be equivalent to a combination of free doxorubicin, bleomycin and vincristine in the treatment of AIDS-related Kaposi's sarcoma, with a different toxicity spectrum [66]. The use of liposomal camptothecin as a lung-targeted inhaled treatment was mentioned above, and another liposomal formulation of a Topoisomerase-I inhibitor is in clinical trials (lurtotecan, OSI-211, OSI Pharmaceuticals, NY, USA) [67]. Cisplatin is a DNA crosslinking agent, active against a variety of tumors, including lung cancer. A sterically stabilized liposomal cisplatin (SPI-77, Alza Pharmaceuticals, CA, USA) has also been developed, and although promising in the preclinical stages, overall response rate was 4.5% in a Phase II study of lung cancer patients [68].

An interesting use of the liposomal packaging is to administer two types of therapeutic agent in the same liposome. Such packaging would assure both the cancer-specific delivery of both agents (owing to EPR of cancer tissue) and that each affected cell receives a dose of each of the drugs [69]. Those are examples of the large potential of liposomal formulations to function as targeting agents for various types of metastatic cancer.

2.3.7 *Release of liposome contents*

Molecules that are trapped inside targeted liposomes are delivered to cells by endocytosis [70], followed by lysosomal release. Non-targeted liposome contents are commonly released in the extracellular compartment, or sometimes by fusion of the liposome membrane with the cellular plasma membrane of cells [71]. Liposome packaging of charged molecules in a pH-dependent manner can be done. As protons diffuse relatively freely through thinner liposome membranes, pH changes in the microenvironment would be reflected inside the liposome. Some drugs would become neutrally charged in acidic pH, diffusing out of the liposome in an acidic microenvironment. In addition, using various components of the core micelle, pH-dependent release of micelle content has been demonstrated [72]. Extracellular pH-dependent release could enhance tumor-specific drug delivery to the usually mildly acidic (pH ~ 6.5) cancer microenvironment. Therapeutic agents that could be delivered to cancer in this way have to be active without necessarily entering the cell. Examples of such agents include radioactive isotopes and cellular toxins. Alternatively, intralysosomal release of liposomal (pH 4 – 5) contents can be achieved by tailoring it to a lower pH-triggered mechanism, and would enhance intracellular targeting. In addition, disulfide bonds can be used in the assembly of lipids structures. Such liposomes would release their contents in the intracellular reducing environment [73]. For a comprehensive review of pH or reducing triggered release of liposome contents, see [62,74].

Liposomes designed to disperse on exposure to light or temperature have also been described [74]. Such targeting agents would release their cytotoxic contents in an organ volume defined by exogenous heat or light stimuli. In the setting of lung metastases that are not amendable to surgical or radiotherapeutic treatment, the utility of such liposome targeting seems limited.

2.3.8 *Immunoliposomes*

Liposome targeting can be achieved by their attachment to tumor-specific antibodies, forming immunoliposomes, a type of LTT. Such liposomes could contain any possible therapeutic agent, including chemotherapy, or nucleic acids as a potential gene therapy vector [33].

Immunoliposomes were constructed to deliver not only chemotherapeutic agents such as adriamycin or gene therapy vectors, but also specific kinase inhibitors such as Glivec [75]. Thus, even specific kinase inhibitors, targeted therapies by design, can be physically directed to sites of tumor deposits by this technique. It remains to be seen whether such approaches will improve the efficacy or reduce the toxicity of targeted therapies.

2.3.9 *Receptor targeting*

Cancer-specific receptors are another way of allowing therapeutic agents to be targeted to cancer. The use of glucosamine to target the liver-specific asialoglycoprotein receptor was mentioned above [51]. Fibroblast-growth factor receptors are overexpressed on many types of cancer, whereas their expression on normal adult cells is restricted. Fibroblast growth factor receptors (FGFRs) have been suggested to be a potential tumor-homing tag [76]. FGF2 linked to a Fab′ fragment against the adenovirus knob region was added to cells to enhance the infectivity by recombinant adenovirus. Cell lines that overexpress FGFR-1 were infected more efficiently by adenovirus in the presence of the FGF2-Fab′. Herpes simplex tymidine kinase gene expression by the adenovirus allowed killing of the infected cells by Ganciclovir [77].

Folate receptor is overexpressed by a large number of tumor cells, whereas its expression is limited in normal tissues. Therefore, it is another potential tumor-specific target. Folate attached to the outer end of PEG molecules that are anchored in a drug-carrying liposome increased drug delivery to tumor cells expressing the folate receptor [78].

Incorporation of polymers including RGD peptides targets liposomes to cells expressing specific integrins [79]. This approach might be more specific to tumor-endothelial cells than to cancer cells (see below).

2.3.10 *Targeting the vasculature*

Targeting cancer vasculature-specific antigens using vascular targeted agents (VTAs) is an interesting cancer therapeutic approach. Apparently more accessible than most tumor cells

are, the tumor vasculature is a convenient target. The feasibility and potential value of this method were demonstrated 15 years ago in a mouse experimental system [80]. VTAs typically cause necrosis of most of the targeted tumor, but a rim of viable cells remains and is the culprit of tumor regrowth. VTA complements anti-angiogenesis treatments that aim for developing nascent blood vessels, as well as conventional chemotherapy or radiotherapy treatments that target rapidly dividing cells.

VTA can be produced by inhibiting biological processes that are essential for tumor angiogenesis, such as rapid proliferation. Regarding the topic of this review, VTA can be targeted specifically to tumor blood vessels [81]. Antibodies or small peptides can be used for this purpose. Proteins expressed specifically on tumor blood vessels must be recognized in order to utilize this approach. These include specific integrins, endoglin, and prostate-specific membrane antigen (PSMA).

PSMA is one molecule claimed to be specifically expressed by neovascular endothelium and not by normal vasculature. This is a membrane-located protein, initially characterized in prostate cancer. J591 is an antibody targeted against the extracellular domain of PSMA. Immunohistochemistry using J591 showed strong staining of the tumor vessel's endothelium. A proof-of-concept Phase I study demonstrated targeting of this antibody to metastasis of various tumors, including lung, breast, colon, kidney and bladder and melanoma. In more than half of the patients, this agent targeted lung metastasis [82]. Radiolabeled J591 was shown to localize to known metastatic sites and to have acceptable toxicity. It induced a minor rate of stable disease, and no objective responses [82]. However, VTAs might not induce real responses by themselves, but rather stabilize disease, as might be true for most anti-angiogenic agents.

A method for detecting tumor-specific antigens, and a potentially targeting moiety, is the use of phage display in animal model systems [83]. This involves repeated selection of a phage-expressed peptide library for retention in a specific organ of the model animal. Importantly, phage libraries produce small peptides whose molecular mass allows them better penetration into tumor mass, and potentially better efficacy.

Various integrins, as well as other endothelial cell-adhesion molecules, are expressed in an organ-specific manner. Heterotypic interactions between adhesion molecules expressed by cancer cells and the adhesion molecules expressed by the various endothelial cell beds are the first step in the process of adhesion and extravasation of tumor cells in the future metastatic site. The spectrum of metastatic sites that is typical of each cancer is partly explained by this mechanism. This same mechanism can thus be exploited to target the therapeutic agent to the relevant vascular bed. Targeting of integrins that are relatively specific for cancer endothelial cells such as αV [84,85] is attempted by RGD-based peptide ligands [79]. A phage library-selected peptide was shown to bind to the endothelium of various cancer xenografts in several mouse models. This peptide bound lung endothelial structures specifically in human lung cancer specimens while not binding endothelium of normal lung tissue. Furthermore, when linked to an adriamycin-carrying liposome, this peptide improved the efficacy of treatment of lung and oral human cancer xenografts in nude mice when compared with the naked liposome [86]. Targeting tumor vasculature by specific intergrin-binding peptides could be an important route of anticancer treatment.

Targeting tissue factor to tumor vasculature has also been shown to be a potential tool. In several mouse models, it has been demonstrated to cause active coagulation in those vessels, leading to massive intratumoral necrosis [81,87].

Another potential VTA is based on the use of growth factor receptor specifically by tumor endothelium, principally VEGF receptor. A diphtheria toxin or Gelonin toxin conjugated to VEGF-A inhibited endothelial cell proliferation and *in vivo* angiogenesis in a mouse model [88,89].

3. Conclusion

Cancer metastasis is the most common cause of death of cancer patients. Among them, lung metastasis is one of the more frequent. In the vast majority of cases, the presence of metastatic disease implicates the presence of multiple micrometastatic or macrometastatic deposits throughout the body, and entails an uncurable condition. However, there are known exceptions to this rule. Hopefully, with the progress of the treatments outlined in this review, those exceptions will be expanded to give more patients the hope of prolonged survival.

There is a variety of approaches to the treatment of lung metastasis. They can be broadly divided into those targeting the clinically evident lung disease and those targeting micrometastatic disease, which is assumed to be present throughout the body.

The most efficient manner of targeting lung disease that is clinically evident is its total removal by surgery. However, sometimes the extent of disease, the general medical condition of the patient, or other considerations, preclude the use of this option. Other therapeutic options exist, which can offer significant long-term palliation. The most commonly used are radiotherapy (either standard or stereotactic) and radiofrequency ablation. Both of these methods deliver high energy to the tumor, either by ionizing radiation or by heat, in an attempt to sterilize it. These approaches are limited by their applicability to relatively small localized and peripheral tumors. Alternatively, chemotherapy can be used. Attempting to avoid the toxicity of systemic chemotherapy, various methods are being developed to deliver chemotherapy and other therapeutic agents specifically to tumor sites. Those methods involve intravascular catheter-mediated methods, aerosol-mediated methods and magnetic targeting. They have been tested in human patients, and appear to be of potential benefit. However, none of these is in routine use.

Considering the usually systemic nature of metastatic disease, targeting therapeutic agents to the evident location of disease might not be the best approach. Methods that target therapy

to cancer cells wherever they might be offer hope for better control of the disease. Several properties of cancer cells and their microenvironment have been exploited as targeting agents. They include the acidic pH of tumor microenvironment, tumor vasculature, tumor-specific antigens and receptors overexpressed by cancer cells. Ligand-targeted therapy is dependent on the identification of cancer-specific ligands, either growth factor receptors or other cell surface-expressed proteins. They are promising methods, some of them assayed on human patients, but none is in routine use in the clinic. Agents already in clinical practice utilize EPR of cancer tissues, through extravasation and retention of larger molecules, polymers and liposomes preferentially in the cancer.

4. Expert opinion

Lung metastasis can be an isolated, localized disease, or systemically disseminated. It is imperative to differentiate between those two states of disease. Beyond the scope of this review, molecular imaging would hopefully allow accurate visualization of all cancer deposits. This information would allow better treatment decisions. If all metastatic deposits are in the lung, radical local approaches, some of which have been discussed in this review, should be advocated. However, even a single extrathoracic metastasis would render any lung-localized treatment just palliative. Molecular imaging is an expanding field of research. Progress in this field is imperative for better diagnosis and treatment of cancer patients. Importantly, molecular imaging could offer visual evidence of tumor-specific expression of potential therapeutic targets.

Targeting therapy to cancer-specific environments or to cancer-specific molecules can bypass the problem presented by the potential of extrathoracic malignancy. Targeting the cancer cells wherever they are is probably the best way to treat metastatic cancer. In fact, this is the reason systemic chemotherapy is usually offered to patients with metastatic disease not amendable to surgical resection. To the vast majority of patients, this is the only valid therapeutic approach today. However, the relatively narrow therapeutic window of systemic chemotherapy implicates dose limiting toxicity resulting from damage to normal cells. Selectivity of the therapeutic agents that would spare normal cells is a critical requirement, as it would allow dose increases and potentially an augmented tumor killing. This goal requires a cancer-specific target, such as the cancer vasculature by the EPR effect. However, EPR allows only for relative specificity; when visualized by a gamma camera, only mild tumor uptake was demonstrated in a copolymer conjugate study [90]. Therefore, better targeting is required, such as to a specific molecule that is expressed only by cancer cells. Genetic and proteomic analysis of cancer tissues in comparison with normal tissues could provide tumor-specific molecular targets. Fresh-frozen tumor specimen banking, available for approved studies, should be a standard procedure in academic cancer centers to allow for more research aimed at elucidation of tumor tissue molecular components.

The most validated molecular tool for targeting specific molecules is monoclonal antibodies. Several examples of antibodies used as anticancer therapeutic agents have been mentioned in this review, although their full discussion is beyond its scope. When administered as a therapeutic agent, antibodies can affect tumor cell death by inhibition of the activity of their target, by antibody-directed cell cytotoxicity (ADCC), or by other means. Monoclonal antibodies can be used as the targeting moiety in a complex including an extra therapeutic molecule, such as standard chemotherapy, a radioisotope, or a cancer-specific pathway inhibitor. The apparent downside to antibody-based treatment is the relative large size, compared with small molecule tyrosine kinase inhibitors. However, considering the EPR effect, which probably enhances the specificity of all large molecule therapeutics, this disadvantage might actually turn out to be an advantage. Fab′ or single-chain variable fragments of antibodies might have some advantages over whole antibodies [34].

Gene therapy is another potential anticancer tool that has not lived up to its expectations so far. The major problem hindering the progress of this methodology is the method of specific delivery of large nucleic acids agents. Targeting tools mentioned in this review, specifically polycationic polymers, could be the solution to this problem. Exciting progress is expected in the coming years in this field.

Declaration of interest

R Herbst declares that he has received research grants, and has served as a consultant to Genetech, AstraZeneca, ImClone/Bristol-Myers Squibb and Pfizer. No potential conflicts of interests are reported by J Bar and A Onn.

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