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REVIEW

Advances in systemic delivery of anti-cancer agents for the treatment of metastatic cancer

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ABSTRACT

Introduction: The successful treatment of metastatic cancer is refractory to strategies employed to treat confined, primary lesions, such as surgical resection and radiation therapy, and thus must be addressed by systemic delivery of anti-cancer agents. Conventional systemically administered chemotherapeutics are often ineffective and come with severe dose-limiting toxicities.

Areas covered: This review focuses on the recent developments in systemic therapy for metastatic cancer. Firstly, the strategies employed to improve the efficacy of conventional chemotherapeutics by 'passively' and 'actively' targeting them at tumors are discussed. Secondly, recent advances in the use of biologics to better target cancer and to instigate anti-tumor immunity are reviewed. Under the label of 'biologics', antibody-therapies, T-cell engaging therapies, oncolytic virotherapies and cell-based therapies are examined and evaluated.

Expert opinion: Improving specificity of action, and engaging the immune system appear to be key goals in the development of novel or reformulated anti-cancer agents for the treatment of metastatic cancer. One of the largest areas of opportunity in this field will be the identification of robust predictive biomarkers for use in conjunction with these agents. Treatment regimens that combine an agent to elicit an immune response (such as an oncolytic virus), and an agent to potentiate/mediate that immune response (such as immune checkpoint inhibitors) are predicted to be more effective than treatment with either agent alone.

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AQ3 1. Introduction

Metastases present a formidable challenge to successful cancer therapy and are the leading cause of cancer-related deaths.[1] Indeed, the WHO reports that the majority of the 8.2 million cancer deaths in 2012 were due to metastases.[2] Many treatment options, such as surgical resection or radiation therapy, that can effectively control earlier stage cancer at a primary site, are ineffective for the treatment of more diffuse and systemically spread metastatic lesions.[1] Thus, the treatment of metastatic cancer is heavily reliant upon systemically administered therapeutics. Systemic delivery of conventional chemotherapeutics to metastases, however, is hindered by a variety of factors. Poor pharmacokinetics (PK) reduce the effectiveness of many conventionally used chemotherapeutics, with a low proportion of the dose achieving delivery to tumor deposits and high levels accumulating in off-target healthy tissue instead. In addition to this lack of selectivity in deposition, conventional chemotherapeutic agents also lack selectivity in mechanism of action. Whether they act as antimetabolites, make direct modification to DNA, inhibit replication enzymes, or block cytokinesis, these agents all rely on a raised rate of cellular division which is also essential to the normal function of several vital organ systems.[3]

This lack of specificity of action means they instigate a range of deleterious off-target effects, which ultimately limits the dose that can be administered systemically. For example, the severe cardiotoxicity of doxorubicin can lead to congestive heart failure and therefore limits the lifetime dose a patient can receive.[4] In addition to the danger posed by the anticancer drug itself, many conventional chemotherapeutics have very low solubility in water and must thus be administered in solvents that can themselves be associated with serious toxicities. Paclitaxel (an anti-microtubule agent), for example, is marketed as Taxol – a formulation containing paclitaxel dissolved in Cremophor and dehydrated ethanol USP.[5] AQ4 Cremophor is not inert, and its use has been linked to a range of serious toxicities, including severe to fatal hypersensitivity reactions.[6]

In addition to poor circulation kinetics, limited specificity, and dose-limiting toxicities, the efficacy of systemically delivered anticancer agents is limited by poor intratumoral (i.t.) penetration. Abnormal microvasculature, lymphatics, and extracellular matrix composition in tumors lead to poor drug deposition and distribution throughout tumors (reviewed in [7]). In murine tumors, as well as in human cancer xenografts in mice, when doxorubicin is administered with a single intravenous (i.v.) injection, many tumor cells (particularly those in regions of hypoxia and therefore implicated as the location of epithelial-to-mesenchymal transition [8]), are not exposed to the drug. Indeed, concentration of drug decreases exponentially with distance from blood vessels.[9]

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Article Highlights

- Treatment of metastatic cancer (the leading cause of cancer-related deaths) requires systemic administration of therapeutics. Conventionally used chemotherapeutics have limited efficacy largely due to lack of selectivity in deposition and mechanism of action.
- Conventional chemotherapeutics can be reformulated to improve delivery and selectivity of uptake. Nanoparticulate drug formulations can be 'passively' targeted to tumors by exploiting the EPR effect. 'Active' targeting can be accomplished through use of targeting ligands specific for structures overexpressed by tumor cells or by tumor vasculature.
- Antibodies can exert antitumor activity by modifying cancer cell signaling, inducing apoptosis, or engaging the immune system. Antibodies can be also be conjugated to drugs and impart a specificity which allows for the administration of more potent cytotoxic drugs.
- BiTEs and ImmTACs are biologic therapeutics that engage autologous T cells *in situ* and redirect an immune response against tumors cells displaying their target surface protein, or intracellularly processed protein presented in the context of MHC, respectively.
- Oncolytic viruses can selectively self-amplify within tumor deposits, can be 'armed' with therapeutic protein, and can elicit an antitumor immune response. Currently, oncolytic virotherapy is largely limited to administration via i.t. injection, which is unsuitable for treatment of metastatic disease. Recent work addressing this limitation suggests that virotherapy will soon be more applicable in treating metastatic cancer.
- Cell-based therapies can be used to facilitate oncolytic virotherapy, or directly to instigate anticancer immunity. All approaches require *ex vivo* manipulation of autologous cells, suggesting that the ultimate utility of this approach could be limited by inherently costly and laborious protocols.

Finally, it is also notable that many conventional chemotherapeutics ultimately rely on triggering apoptotic death mechanisms to kill cancer cells. Such a strategy may ultimately be limited in cancer cells, which are often defined by a loss of such mechanisms.[10]

This review is not intended to provide exhaustive coverage of all current systemically administered anticancer agents, but rather to highlight emerging and novel approaches to improving systemic treatment of metastatic cancer. Specifically, this review will focus on (1) approaches to modifying and targeting existing small-molecule drugs (rather than the generation of new small-molecule drugs) and (2) the development of new targeted biological agents.

2. Particulate delivery of existing drugs

Whilst the selectivity of action of many conventional chemotherapeutic agents cannot be easily improved, much research has focused on trying to achieve improved selectivity of deposition. The approach often taken is to repackage the drugs within macromolecular carrier constructs so that they benefit from the enhanced permeability and retention (EPR) effect.

2.1. EPR effect

Although tumor physiology poses a formidable challenge to drug delivery, its unique characteristics also provide opportunities for passive targeting. Leaky vasculature and poor lymphatic drainage allows for passive accumulation of long-circulating macromolecules within tumors; this is known as the 'EPR effect.'[11] The EPR effect depends on size, surface charge, biocompatibility, and PK of the drug, as well as the specific physiology of the tumor being treated. In particular, if a carrier system is above the size of the gaps between endothelial cells in normal tissue (2–10 nm) and liver (100 nm) but below the size in tumors (typically reported as up to 500 nm) then a window of opportunity is created where accumulation into nontarget tissue can be avoided, providing the extended circulation required to allow passive diffusion into all metastatic tumor deposits.[12]

Though the EPR effect has a demonstrable influence on drug uptake into tumors, it is not yet fully understood and has certain limitations. First, though most tumors possess the characteristics conducive to the EPR effect (leaky vasculature and lack of lymphatic drainage), these characteristics are heterogeneous, both within and between tumors, with the levels of perfusion and interstitial fluid pressure (IFP) varying considerably even between different nodules within the same patient.[13] Furthermore, the true scale of the EPR effect – as well as its impact on i.t. distribution – warrants further scrutiny. Analysis of EPR enhanced delivery shows perivascular distribution of deposited drug, rather than homogenous distribution throughout the tumor. Hence, a paradox is created where increasing the size of an agent enhances its EPR-assisted accumulation into the perivascular space, but decreases its diffusion-dependent transfer from this site and into the tumor.[14]

2.2. Passive targeting

Conventional anticancer drugs typically have low molecular weights and do not have any tumor selectivity due to the EPR effect.[15] Many existing chemotherapeutics are being reformulated on the nanoscale, in part to capitalize on EPR-mediated passive accumulation within tumors. Nanoparticulate drug formulations can be fine-tuned to achieve a size and surface charge which prevents sieving through hepatic sinusoidal endothelia and capture by the mononuclear phagocytic system (MPS), whilst still allowing EPR-assisted tumor accumulation. Furthermore, nanoparticulate formulations can allow for control of release kinetics of active drug payload, can protect drug from degradation, and can therefore mitigate certain deleterious off-target effects seen with administration of free drug.

Polyethylene glycol (PEG)ylated liposomal doxorubicin (Doxil® or Caelyx® in Europe), for example, measures ~100 nm in size, which provides improved PK and allows levels of EPR-mediated tumor accumulation unattainable by the free drug.[16] The composition of lipids used in PEGylated liposomal doxorubicin – particularly a DSPE lipid with a PEG head, which provides a biocompatible polymer coating to the liposome – reduces sequestration by the MPS and plasma components, leading to an elimination half-life of ~2–3 days in humans. Free doxorubicin by comparison, is cleared from the plasma with a half-life on the order of hours.[17] In addition to improved PK, PEGylated liposomal doxorubicin has an improved safety profile with no cardiotoxicity and only the occasional reports of mucositis and hand–foot syndrome.[18] Despite these advantages, and the fact that it is approved for

use in ovarian cancer, multiple myeloma, and Kaposi's sarcoma, it is only the latter of these indications where it seems to have gained clinical traction. This may relate to an inefficiency of payload release once within the tumor, a problem which has been addressed by altering the formulation to provide sensitivity to external targetable stimuli such as heat or cavitation.[19,20] Other liposomal formulations with current FDA approval include liposomal daunorubicin (DaunoXome®), and liposomal vincristine (Marqibo®). A non-PEGylated liposomal formulation of doxorubicin (Myocet®) is approved for use in Europe. In contrast to Doxil®, these three liposomal formulations do not have a coating of biocompatible PEG and are therefore sequestered by the MPS and thus released slowly into circulation.[21]

Paclitaxel has also been reformulated, and FDA approved, as a nanoparticle therapeutic. Albumin-bound paclitaxel (Abraxane®), measuring ~120 nm in size is approved for treatment of breast, pancreatic, and lung cancers. Though the size of albumin-bound paclitaxel imparts nanoparticle-associated delivery benefits (improved circulation time and EPR effect), the main clinical benefit of albumin-bound paclitaxel versus conventional paclitaxel is most likely due the removal of Cremophor as a solvent.[22]

Though liposomal formulations currently dominate the market-approved nano-therapeutic stage, a variety of different nanoscale drug formulations are in the clinical testing pipeline. Examples of platforms under current investigation include polymeric micelles (e.g. Genexol-PM®, with paclitaxel as the active ingredient) and polymer–drug conjugates (e.g. paclitaxel poliglumex).[23]

2.3. Active targeting

Unlike the EPR effect, which can be used to 'passively' target drugs, 'actively' targeted therapeutics aim to exploit various hallmarks of cancer by employing ligands targeted to structures typically overexpressed in tumors (see Figure 1). Peptides or antibodies can be used in isolation as active agents, or to decorate the surface of drugs in order to improve target-cell recognition and subsequent uptake, consequently reducing uptake of cytotoxic drugs into nontarget cells. The use of antibodies as actively targeted agents will be discussed thoroughly in Sections 3.1 and 3.2, while the rationale and strategies for active targeting, and its limitations, are described here.

Examples of actively targeted therapeutic agents under investigation for cancer therapy include CALAA-01 (a polymer-based nanoparticle containing siRNA and targeted to human transferrin receptor),[24] folate-targeted liposomal doxorubicin,[25] trastuzumab (a monoclonal antibody [mAb] targeted to the HER2/neu receptor),[26] and ibritumomab tiuxetan (an antibody–radionuclide conjugate).[27]

In practice, active targeting has certain limitations. In order for ligands to bind to cellular targets, they must be brought in contact with the cells themselves. In tissues, where penetration of molecules is already limited, this represents a substantial challenge. Hence, agents such as the mAb trastuzumab require high and repeated dosing for uniform distribution of drug, while cells located within hypoxic regions of tumors are still often not exposed to drug.[28,29] Moreover, (and independent of any effects due to high IFP and dense tumor interstitium), binding to cellular targets perivascularly can limit the spread of actively targeted therapeutics into tumors; this effect is known as the 'binding site barrier.'[30]

Generally, the benefit of 'active' targeting is more efficient drug uptake into target cells (and reduced off-target uptake), rather than an absolute increase in drug dose reaching all regions of the tumor. This is because the main mechanism for drug accumulation within tumors is still EPR-mediated extravasation, regardless of the presence of a targeting ligand. For certain therapeutics, such as siRNA, this advantage can be considerable; but for other therapeutics which enter cancer cells readily, it is unclear whether 'active' targeting confers a definite advantage or not.[23,31]

In response to the difficulty faced in trying to deliver into and throughout the tumor, therapies that target the tumor vasculature rather than the tumor cells themselves, have gained clinical traction. For example, bevacizumab, a mAb targeting vascular endothelial growth factor (VEGF), which plays a significant role in mediating tumor angiogenesis,[32] is approved by the FDA for the first and second-line treatment of a range of cancers. Additionally, preclinical studies have targeted tumor vasculature utilizing the decoration of liposomal carrier systems with ligands against tumor endothelial markers.[33] The rationale for such vasculature targeting (targeting of the endothelial cells lining angiogenic tumor blood vessels) is clear. First, it should permit drug to more readily contact its target and so avoids the challenge of permeation against the IFP and the 'binding site barrier.' Second, nontransformed vasculature is less capable of mutating away from the mechanism of drug action, and finally, the destruction of just a few endothelial cells should have an amplified effect on the many tumor cells they support. However, it is now clear that this rather simplistic interpretation fails to consider the degree of control needed to prevent the epithelial–mesenchymal transition which results from vascular inhibition and ultimately causes tumors to become more invasive.[8] Moreover, clinical results of treatment of solid tumors with anti-VEGF agents (such as bevacizumab) as a monotherapy have been underwhelming, while trials with anti-VEGF therapy coadministered with conventional chemotherapy have shown to be significantly more effective than chemotherapy alone.[34] These results support the hypothesis that vascular-targeting approaches will have the most clinical utility when used sparingly to 'normalize' tumor vasculature and allow for better delivery of subsequently systemically administered drugs with direct anticancer cell activity.[35,36] However, there is heterogeneity in the scale and duration of such normalization, making it difficult to predict and control. In addition to direct vasculature-targeting approaches such as anti-VEGF, it has been shown that drug-loaded nanoparticles (particularly liposomes) can also exert a normalizing effect on tumor vasculature, likely through the action of loaded drug on tumor vasculature, as drug is initially deposited perivascularly after i.v. delivery of long-circulating nanoparticle drug carriers.[37,38] Preclinical models have indicated that this nanoparticle-mediated effect is dynamic, and so scheduling of drug administration after pretreatment with vasculature-

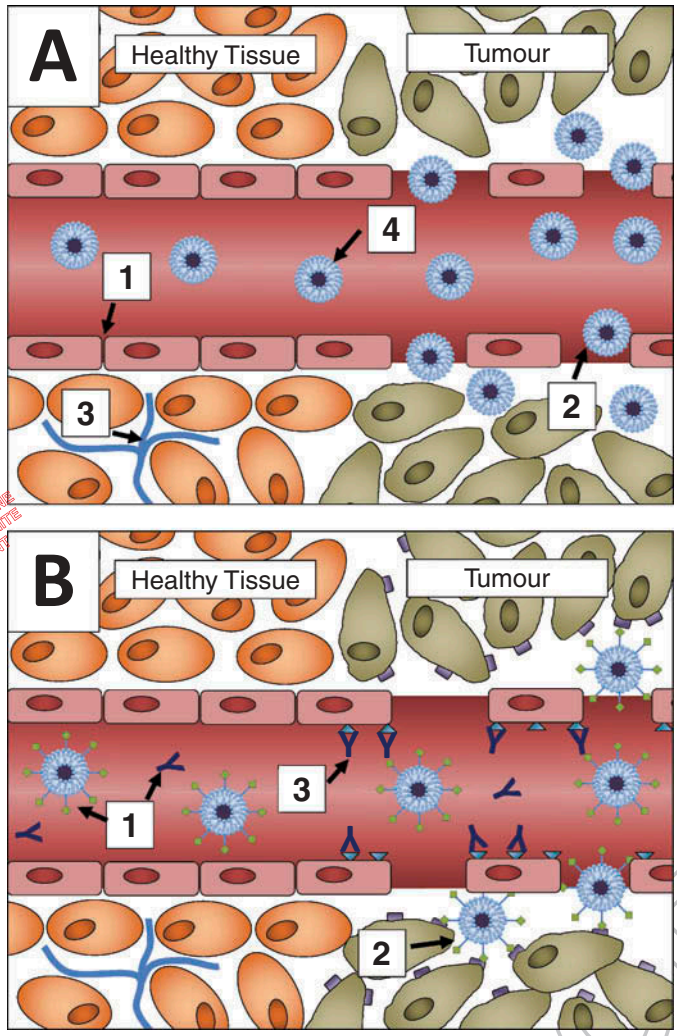

Figure 1. Passive (A) and active (B) targeting of therapeutic agents to tumours. (A) In healthy tissue, tight endothelial junctions (A1) prevent extravasation of therapeutics greater than approximately 10 nm in size. Conversely, in tumours, gaps between endothelial cells can be up to 500 nm in size, allowing for extravasation of nanoparticulate drug formulations, such as liposomes (A2). This leaky vasculature, in combination with a lack of lymphatic drainage as is present in healthy tissue (A3), cause macromolecules to extravasate into and be retained within tumour deposits: this is known as the 'enhanced permeability and retention' (EPR) effect. The EPR effect underpins passive targeting of long-circulating nanosized drugs (A4) to tumours. (B) Active targeting employs targeting ligands (or naturally selective therapeutics, such as monoclonal antibodies and their derivatives) specific for structures overexpressed in tumours (and tumour vasculature) (B1). Drugs can be modified to directly target surface antigens on cancer cells (B2), or can target the endothelial cells of tumour vasculature (B3). Active targeting can be limited by the 'binding site barrier', whereby extravasation of targeted drug deep into tumours is prevent by drug binding to target antigen displayed on perivascular cells.

normalizing nanoparticles is likely to play a key role in the effectiveness of this therapeutic approach.[38]

3. Biologics

3.1. Antibodies

Although cancer cells cannot, in general, be distinguished by completely unique surface-expressed proteins (as this would lead to rapid immune clearance), they often express or overexpress proteins otherwise downregulated in healthy somatic cells, a prime example being HER2 overexpression in breast cancer.[39] Antibodies against cell-surface-expressed antigens can be administered systemically and will achieve extended circulation (as compared to small molecule drugs) allowing them to exert an effect either exclusively or preferentially on all metastatic tumor deposits displaying the target antigen. Antibodies can exert an antitumor effect through a range of modes of action, as outlined in Table 1. First, mAb therapies can act directly on cancer cells by acting antagonistically (or agonistically) on receptors involved in crucial cancer cell signaling pathways, or by binding to cytotoxic receptors and thereby triggering apoptotic pathways. The FDA-approved mAb cetuximab targets the epidermal growth factor receptor (EGFR), and when bound to its target, blocks growth factor binding, and can induce internalization of the receptor, as well as downregulation of cell expression of EGFR, ultimately resulting in inhibited cell proliferation.[40] Antibodies can indirectly affect tumor cells by targeting elements of the tumor microenvironment that drive uncontrolled growth. The previously mentioned mAb, bevacizumab, employs this approach by targeting VEGF expressed by endothelial cells in the tumor vasculature in order to suppress pathophysiological neoangiogenesis.[32] Moreover, mAbs can exert an antitumor effect indirectly through immune-mediated mechanisms. Namely, mAbs targeted to tumor-associated cell-surface antigens can initiate and mediate complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC), via the clustering of their Fc regions on the target cell surface. For examples, rituximab, a mAb directed toward CD20 (expressed on the surface of B cells), has been shown to instigate both CDC and ADCC (in addition to direct cancer-cell apoptosis).[41] Additionally, mAbs can be targeted to indirectly regulate T-cell function by interfering with 'immune checkpoint' signaling. Notably, antibody (Ab) therapies are not limited to only one mode of action; cetuximab for instance has been shown to also initiate ADCC by activating cellular components of immunity such as natural-killer cells, dendritic cells, and indirectly, T cells.[42,43] The exact balance of activity will depend on the mutation profile and immune status of the particular tumor.

Systemically administered antibody therapies targeted to tumors and their microenvironments have shown in general, improved safety and lower toxicity as compared to conventional chemotherapeutics.[69] It is important to note, however, that off-target effects can and do occur with antibody therapies and can be due to off-target action of the therapeutic through its intended mechanism. For instance, cardiotoxicity of trastuzumab has been proposed to be related to binding of HER2 receptors in cardiac tissue.[55,70] Additionally, the improved specificity of these agents has the downside of reducing how widespread their use can be, with their use and efficacy being restricted by heterogeneous expression of target antigen within and across patients. Studies on the use of cetuximab in colorectal cancer have shown that patients with tumors bearing a specific mutation (in the *K-ras* gene) do not benefit from the treatment, while those with the wild-type gene do benefit from improved overall survival and progression-free survival (as compared to current best supportive care).[71] As a result, cetuximab was the one of the first therapeutics to be approved for use with a

Table 1. Antitumor mechanisms of action for current clinical antibody-based therapies for cancer.

Target	Examples	Target antigen	Mechanisms of action	References
Cancer cells	Alemtuzumab	CD52	Induction of apoptosis, ADCC, CDC	[44–46]
	Cetuximab	EGFR	Interference with signaling pathways, ADCC	[40,42,43,47]
	Daratumumab	CD38	Induction of apoptosis, induction of phagocytosis, ADCC, CDC	[48,49]
	Dinutuximab	GD2	ADCC, CDC	[50]
	Elotuzumab	SLAMF7 (CS1)	Inhibition of cancer cell adhesion to stromal cells, ADCC, direct NK cell activation	[51,52]
	Necitumumab	EGFR	Interference with signaling pathways, ADCC	[47]
	Obinutuzumab	CD20	Induction of apoptosis, ADCC, CDC, induction of phagocytosis	[53]
	Ofatumumab	CD20	ADCC, CDC, induction of phagocytosis	[53]
	Panitumumab	EGFR	Interference with signaling pathways	[47]
	Pertuzumab	HER2	Interference with signaling pathways, ADCC	[54]
	Rituximab	CD20	Induction of apoptosis, ADCC, CDC, induction of phagocytosis	[41,44,46,53]
	Trastuzumab	HER2	Interference with signaling pathways, ADCC	[26,54,55]
	Brentuximab vedotin	CD30	Delivery of conjugated cytotoxic drug	[56,57]
	Ibritumomab tiuxetan	CD20	Delivery of conjugated radionuclide	[58]
	Trastuzumab emtansine	HER2	Delivery of conjugated cytotoxic drug	[59–62]
Tumor microenvironment	Bevacizumab	VEGF	Inhibition of angiogenic signaling	[32]
	Denosumab	RANKL	Inhibition of osteoclast differentiation factor (to prevent tumor-associated bone lysis)	[63,64]
	Ramucirumab	VEGFR-2	Inhibition of angiogenic signaling	[65]
Immune Cells	Ipilimumab	CTLA-4	Blockade of immune checkpoints	[66]
	Nivolumab	PD-1	Blockade of immune checkpoints	[67]
	Pembrolizumab	PD-1	Blockade of immune checkpoints	[68]

ADCC, Antibody-dependent cell-mediated cytotoxicity; CDC, complement-dependent cytotoxicity.

biomarker diagnostic test, which verifies *K-ras* wild type status prior to treatment.

3.1.1. Immune checkpoint inhibitors

Numerous studies have shown that the presence of tumor-infiltrating lymphocytes (TILs) is positively correlated with an improved prognosis.[72,73] While mAbs can indirectly elicit or potentiate antitumor responses by immune cells including T cells, tumors develop mechanisms to evade such immune responses. One such mechanism is to co-opt 'immune checkpoint' pathways.[74] Briefly, immune checkpoints refer to inhibitory pathways employed by cells to avoid destruction by any self-reactive immune cells. These pathways are key to the maintenance of 'self-tolerance,' but can be misappropriated by cancer cells to avoid the immune system's natural response to tumor-associated antigens (reviewed in [74–76]). Many of the immune-checkpoint pathways identified so far involve receptor–ligand interactions, and so the blockade of immune-checkpoint receptors or ligands has been identified as a strategy to enhance antitumor immunity. Thus far, clinical research into immune-checkpoint blockade has focused primarily on two pathways: that of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), and that of programmed cell death protein 1 (PD1).[74] Although both pathways act on T cells, they function at different levels of the cellular immune response. CTLA-4 is a receptor expressed on the surface of T cells, where it exerts a regulatory effect on the early stages of T-cell activation primarily in the lymph nodes.[77] In contrast the PD1, receptor is expressed after T-cell activation and modulates T-cell activity locally within tissues.[78]

Recent clinical trials have shown blockade of the CTLA-4 pathway, or the PD-1–programmed cell-death protein 1 ligand (PD-L1) interaction, to be highly effective in a certain proportion of patients.[66–68,79] These results have generated a great deal of interest and led to the FDA approval of ipilimumab, nivolumab, and pembrolizumab. Although use of these agents has been demonstrated to provide survival benefits compared to a variety of other therapies, their use in the clinic has also presented a range of challenges unlike those faced by other more conventional therapies.[76]

Of particular interest is the time-course of treatment with these checkpoint inhibitors. In many patients, progression of disease is observed in the beginning months of treatment, followed ultimately by a robust and long-lasting therapeutic response. The initial progression of disease (according to standard RECIST/WHO criteria) is thought to be due to tumor infiltration by immune cells and has led to the creation of a novel criteria system for evaluating studies with CTLA-4 therapy, the 'immune-related response criteria.'[80] This development underscores the importance of re-evaluating traditional practices and paradigms in the face of emerging novel therapies that confront cancer in new ways.

Moreover, as immune checkpoints are key elements of 'self-tolerance,' blockade of immune checkpoints can produce a range of immune-related adverse effects due to nonspecific activation of the immune system (reviewed in [76]). Notably, blockade of CTLA-4 has resulted in more severe/common immune-related adverse effects than blockade of the PD-1 pathway.[74] This is most likely due to the differences in mechanisms of action, with anti-CTLA-4 acting on inactivated T cells in the lymph nodes to produce a more systemic response, while anti-PD-1 and anti-PD-L1 antibodies work on activated T cells in the periphery and may therefore work in a more tumor-selective way.[74] This different site of action may also reveal differing potential limitations, with anti-PD-1 and anti-PD-L1 antibodies requiring passage deep into tumors to contact resident T cells, whereas anti-CTLA4 antibodies may not face this restriction.

Similar to the other forms of antibody therapy outlined above, treatment by inhibition of immune checkpoints will only be effective to a subsection of cancer patients – those who have tumors which use immune checkpoints as a mechanism to escape immune eradication. As such, predictive biomarkers of clinical outcome with immune checkpoint therapy are being actively investigated. Thus far, a correlation between expression of PD-L1 and response rate to anti-PD-1/anti-PD-L1 therapy has been shown.[81] No clear predictive biomarkers for the success of anti-CTLA-4 therapy have yet been elucidated, however, certain pharmacodynamic markers in the peripheral blood such as absolute lymphocyte count (and lymphocyte increase), have been shown to relate to improved survival.[82]

3.2. Antibody–drug conjugates

In addition to their role as active therapeutics discussed in Section 3.1, antibodies can also play a role in metastatic cancer therapy as targeting agents for other therapeutics, such as radioisotopes and cytotoxic drugs. Therapeutic mode of action and efficacy can be altered/enhanced by conjugating a mAb to a cytotoxic drug. Linking a cytotoxic drug to a mAb should allow for administration of more potent cytotoxic drugs than are currently used in systemic chemotherapy, as the mAb must bind to its target antigen to facilitate uptake of drug, and thus uptake is restricted to cells expressing target antigen. This specificity of action is largely dependent on the linker between drug and mAb. On the one hand, the linker must be stable in circulation to prevent off-target release of drug and to allow for long circulation of drug. The linker must also, however, be able to release drug efficiently once internalized into a target cell. Moreover, the method of attachment of drug (and amount of drug attached) can affect the internalization efficiency of the antibody–drug conjugate (ADC), and must therefore be carefully controlled.[83] Two ADCs with current FDA approval, brentuximab vedotin and trastuzumab emtansine, employ different linking strategies and have both shown considerable clinical efficacy thus far.[56,59] Brentuximab vedotin comprised a mAb specific for CD30 (often overexpressed in Hodgkin lymphoma and anaplastic large-cell lymphoma) and a potent cytotoxic drug, monomethylauristatin E (MMAE – an auristatin). These two entities are linked via a protease-sensitive covalent dipeptide linker, which allows for release of MMAE within lysosomes.[57] Trastuzomab emtasine comprised an anti-HER2 mAb linked to mertansine (DM1 – a maytansinoid) via a non-cleavable thioether linker.[60] Non-cleavable linkers are extremely stable in circulation, and ADCs, employing this type of link, rely on intracellular digestion of mAb in order to release the potent cytotoxic agent.[84]

ADCs combine the specificity of a mAb and the cytotoxicity of an anticancer drug (often too cytotoxic to be administered alone), in theory creating a more targeted, safe, and efficacious therapeutic. This concept has been demonstrated clinically with trastuzumab emtansine. This ADC has been shown to be efficacious against metastatic breast cancers that were previously treated with the mAb trastuzumab [61] and in a trial comparing the action of the ADC to that of the mAb trastuzumab coadministered with conventional chemotherapy (docetaxel), trastuzumab emtansine showed an improved safety profile.[62] In addition to having strong scientific rationale and some promising clinical data, the ADC approach also benefits from providing a continued income stream for mAbs reaching their patent expiry. In the face of the rise of generic biosimilars, this provides a strong commercial imperative for the development of ADCs.

3.3. BiTEs (bi-specific T-cell engagers)

In the past two decades, novel bi- or multivalent antibody constructs have been engineered to harness the power of T cells in destruction of cancer cells. This approach is exemplified by bi-specific T-cell engaging antibodies (BiTEs), composed of two single-chain variable fragments (scFv) linked to form a peptide of approximately 55 kDa. These BiTEs have been shown to be effective and precise in instigating and mediating T-cell destruction of cancer cells, both preclinically and in patients.[85,86] In common with mAbs, or ADCs, BiTEs are specific for target antigens expressed on the surface of cancer cells. Rather than relying on indirect stimulation of the immune system, however, BiTEs incite T cells directly to attack cancer cells, by also binding CD3 (a signaling component of the T-cell receptor complex). As many cancers have evolved to avoid normal immune surveillance (through a variety of methods including immune editing and MHC downregulation),[87] the ingenuity of the BiTE approach is that T cells can be activated irrespective of their original specificity and in the absence of MHC presentation.[88,89] BiTE-induced T-cell activation should occur only in conjunction with cells bearing the antigen targeted by the BiTEs,[89] thus mitigating the safety risk posed by polyclonal activation of T cells.

BiTEs have been shown to induce a potent and accelerated form of T-cell-mediated cell lysis [90] and are effective even at concentrations orders of magnitude lower than comparable mAb therapeutics.[91,92] BiTE-redirected T cells form cytolytic synapses with cells bearing target antigens, instigating the formation of perforin pores and the release of cytolytic granule contents. The effectiveness of this redirected T-cell-mediated cell lysis has been demonstrated in the clinic with blinatunomab, a bi-specific antibody targeting CD3 and CD19 (CD19 is expressed on the surface of B cells, and while its expression is not increased in cancerous B cells versus normal cells, the transient destruction of healthy B cells expressing CD19 appears to be tolerable). Blinatumomab has shown clinical efficacy against hematological cancer in both disseminated blood and bone marrow deposits of infiltrating disease.[92–94] For instance, in a phase II trial for patients with minimal residual disease (MRD – a leading cause of hematologic relapse following chemotherapy) in B-lineage acute lymphoblastic leukemia (B-cell ALL), treatment with blinatumomab resulted in elimination of MRD in an impressive 80% of patients.[93] Blinatumomab was approved by the FDA in 2014 for the treatment of Philadelphia chromosome-negative precursor B-cell ALL. Though blinatumomab is currently the only market-approved BiTE, many BiTE formulations directed toward other target proteins have been investigated clinically (e.g. EpCAM, CEA, PSMA – reviewed in [95]) and preclinically (e.g. Her2/neu, EGFR, CD33 [85]).

Despite early clinical success, BiTE technology is limited by several factors. Clearly, BiTEs face the same challenges as mAbs or ADCs in terms of choosing appropriate target antigens and the same general delivery limitations of any actively targeted therapeutic. Moreover, BiTEs do not contain any Fc regions, which both reduces their recycling and their size, allowing them to be cleared through the kidneys more rapidly than conventional mAbs, and thus their serum half-life is on the order of a couple of hours.[85,96] This short serum half-life necessitates the administration of BiTEs by continuous i.v. infusion using portable mini-pumps, for periods of 4–8 weeks. However, a size of 55 kDa is still large enough to substantially impede transport deep into solid tumors, especially to the regions rich in T_L cells with suppressed activity. Finally, though the hallmark of BiTE therapy is a *targeted* redirection of T-cell lysis, blinatumomab has shown clinically important adverse side effects, notably including cytokine release syndrome and central nervous system (CNS) disorders.[92–94] Though the CNS events reported in trials were reversible, they are troubling, as the underlying mechanism behind these events remains unclear.

Similar to BiTEs, bi-specific antibodies containing an Fc region, allowing for engagement of Fc receptors on immune cells other than T_L cells, have been investigated clinically, and catumaxomab (a bi-specific tri-functional antibody directed toward EpCAM and CD3) is approved in Europe for the treatment of malignant ascites.[97]

3.4. ImmTAC (immune mobilizing monoclonal T-cell receptors against cancer)

In an approach similar to the antibody-based T-cell engagers, ImmTACs (immune mobilizing monoclonal T-cell receptors [mTCRs] against cancer) work to redirect autologous T_L cells to lyse cells presenting specific antigens associated with cancer. Antibody-based therapeutics have been largely restricted by the need for antigen to be surface expressed, with work only recently demonstrating the possibility of designing BiTEs to target specific intracellular peptides presented in the context of MHC.[98] In contrast, the development of ImmTACs has been based on the principle of targeting cells using affinity-enhanced mTCRs and can thus target the range of endogenously processed tumor-associated antigens. TCRs natural affinity for cognate antigen is typically orders of magnitude less than the affinity of antibodies for their target peptides (and affinity for self-derived tumor-associated antigens is even poorer due to mechanisms of T-cell selection and central tolerance [99]), and so soluble mTCRs, targeting specific peptides presented in the context of human MHC (pMHC) at greatly enhanced affinities, have been engineered and fused with anti-CD3 single-chain antibody fragments to create a novel class of bi-specific therapeutics, ImmTACs.[100] The potency of ImmTACs directed against a range of target antigens has been demonstrated *in vitro* and *in vivo*, with ImmTACs inciting and redirecting a target-specific T-cell response, despite low levels of target pMHC presentation on cells tested.[100,101] Furthermore, ImmTAC redirected cancer cell lysis (specifically with an IMCgp100 – an ImmTAC specific to the melanocyte differentiation antigen gp100) was shown to lead to antigen cross-presentation by dendritic cells, a process whereby polyclonal T_L cells can become activated either within the tumor or within draining lymph nodes. Moreover, cross-presentation in the presence of the ImmTAC was shown to enhance the activation of T_L cells naturally specific for tumor-associated antigens. This exciting finding is significant as antigen cross-presentation and consequent activation of T_L cells specific for a wider range of tumor-associated epitopes could foster a more robust and self-sustaining immune response against cancer.[102]

Currently, IMCgp100 is undergoing clinical testing in a phase II trial for late-stage melanoma, having been well tolerated in a phase I trial to determine maximum-tolerated dose and toxicity following i.v. delivery.[103] Though the targeting of intracellular antigens gives rise to a larger pool of possible cancer-specific targets (as compared to surface expressed targets), targeting through MHC restricts the patient population that stands to benefit from therapy with a particular ImmTAC (as MHC alleles vary across individuals, and each ImmTAC is targeted to one allele group).[104] As with any immunotherapy, the potential for off-target effects (either by destruction of healthy cells presenting target antigen, or by unpredicted off-target activity) necessitates thorough preclinical testing. This process is, however, considerably more difficult for a therapeutic targeting, specifically human proteins (human MHC), as in the case of ImmTACs.[104] The precise location where ImmTAC (and BiTE) molecules interact with T_L cells has yet to be fully characterized or reported. However, the work of Brischwein et al.[89] suggests that close context of CD3 on T_L cells and target ligands on cancer cells is required for the multivalent stimulation of CD3 needed for activation. It is likely therefore that effective delivery into and throughout the tumor will be required for these molecules to be fully effective against solid, poorly perfused tumors.

3.5. Viruses

Oncolytic viruses are emerging as powerful therapeutics, unrivalled in their capacity for selective self-amplification. Certain viruses are naturally cancer selective, or have become so through many rounds of *in vitro* culture. In most cases, however, oncolytic viruses are engineered to be cancer specific.[105] This can be accomplished by exploiting the adaptability of the viral genome and the strategies employed by tumor cells to avoid normal mechanisms of cell death and immune destruction.[105,106] In general, engineering cancer-specific viruses can be achieved in three ways. First, virus–cell entry can be controlled by engineering pathways of virus entry and infection to depend on receptors or proteins with raised or exclusive expression in tumors.[107,108] Second, as the indefinite division of cancer cells is reliant on upregulation or suppression of many gene products otherwise dormant or active in healthy differentiated somatic tissue, viruses can be engineered to be cancer specific by placing transcription of genes essential to their replication under the control of tumor-specific promoter(s).[109–111] Third, specificity can be achieved by regulation through attenuation of virulence in healthy tissue, that is viruses can be made cancer selective by attenuating their

action in healthy tissue (e.g. engineering virus to be more susceptible to normal mechanisms of immune clearance), while retaining close to wild-type virulence in cancer cells. [112,113] A variety of viruses have been designed to target a wide range of cancers, and those in current clinical development are summarized in [114].

In addition to direct cell lysis, oncolytic viruses can have an important therapeutic effect by potentiating or eliciting an antitumor immune response (reviewed in [115]). Studies have demonstrated that efficacy of reovirus, for instance, depends critically on an antitumor immune response.[116] Viruses tend to induce immunogenic cell death; lysed cells release danger signals relating to the viral infection, as well as a range of tumor-associated antigens. These factors displayed in concert by antigen presenting cells can initiate an adaptive immune response against the tumor cells.[117,118] The benefits of this virus-induced anti-tumor immunity are elegantly demonstrated by experiments in bilateral syngeneic tumor models in immunocompetent mice, where i.t. injection of an oncolytic virus into one tumor causes a regression of the un-injected lesion. No effect is observed in un-injected lesions of athymic mice, indicating that the effects in untreated lesions are immune-mediated.[119] These effects have also been observed in humans, in trials for late-stage melanoma, where regression of untreated metastatic nodes was observed after treatment of separate lesions with a modified herpes simplex virus (talimogene laherparepvec [T-Vec]).[120]

To increase their efficacy, oncolytic viruses can be 'armed' with transgenes encoding a variety of useful proteins.[121] Notable examples include transgenes of prodrug converting enzymes,[122] immune-stimulating proteins,[120] extracellular matrix degrading enzymes,[123] syncytia forming proteins, [124] and antiangiogenic proteins.[125] Once infected by an 'armed' oncolytic virus, cancer cells will produce the encoded transgene, in addition to progeny virus.

Currently, the only market-approved oncolytic viruses are Oncorine (H101), a recombinant type 5 human adenovirus, [126] and T-Vec, a transcriptionally regulated modified herpes simplex virus encoding the gene sequence for human granulocyte-macrophage colony-stimulating factor (GM-CSF, an inflammatory cytokine).[120] Chinese authorities approved Oncorine in 2005, while T-Vec was approved by the FDA and EMA in late 2015. Other oncolytic viruses in current clinical development include pelareorep (Reolysin®, a wild-type reovirus) and pexastimogene devacirepvec (Pexa-Vec, a modified vaccinia virus encoding GM-CSF).[114] While oncolytic virotherapy has shown considerable promise in the clinic and has been so far quite safe and well tolerated, the clinical efficacy has, in general, not matched that observed in many preclinical studies.[121] This clinical translation deficit is a consequence of the factors discussed below.

Although oncolytic virotherapy has the potential to elicit a powerful antitumor immune response, there is a balance between the benefits of an antitumor immune response and the detriment of an immune response to the efficacy of viral spread (reviewed in [118]). Hence, despite tumors being, in general, immune-suppressed environments, studies have shown that infection of cancer cells by oncolytic viruses can still activate immune responses which act rapidly to prevent spread of the virus, thereby reducing any therapeutic effect due to viral oncolysis.[127,128] Administration of immunosuppressive agents has been investigated in an effort to circumvent the immune barrier to viral spread. For instance, treatment of glioma in an immunocompetent rat model with an oncolytic herpes simplex virus can be enhanced by pretreatment with cyclophosphamide, an immunosuppressive agent.[129] A virus-induced immune response thus appears to be a double-edged sword. The immune system is a barrier to viral replication and spread within a tumor, but once infection has occurred and tumor-associated antigens are presented in the context of danger signals due to viral infection, a powerful antitumor response can potentiate tumor debulking.[118]

Additionally, although the size of most viruses is ideal for passive targeting via the EPR effect,[15] the circulation time required for tumoral accumulation poses a formidable challenge, as the immune system rapidly clears pathogens such as viruses. Systemically administered viruses are coated with antibodies (neutralizing and non-neutralizing), complement, and other serum factors before being sequestered in the liver and spleen by the MPS.[130] This has the added complexity that such clearance is often not predicted by preclinical models.[131] The efficacy of these clearance mechanisms means that most oncolytic viruses have very poor circulation half-lives. Of particular, concern are neutralizing antibodies in serum of preexposed individuals (or in individuals requiring repeated administration), as they can present a significant barrier to systemic delivery and contribute to variation in treatment results across individuals. [132,133] It is important to note, however, that this limitation varies across virus types – the utility of certain serotypes of adenovirus, for instance, is notably hampered by a high prevalence of preexisting neutralizing antibodies within the general population.[134]

In the case of adenovirus serotype 5 specifically, complex interactions between blood coagulation factors and systemically administered virus have also been shown (largely in preclinical models) to impact on delivery efficacy. Coagulation factor X (FX) has been shown to play a key role in hepatocyte transduction – a barrier to efficient biodistribution of adenovirus – though it is currently unclear whether the main role of FX is as a direct bridge between adenovirus and hepatocytes,[135] or if FX works to 'protect' virus neutralization via natural IgM antibodies and complement.[136] Studies on adenovirus infectivity conducted in human blood or sera suggest, however, that although the interaction between coagulation factors and adenovirus is important within the context of preclinical models, it may be secondary to the effects of neutralizing antibodies in the serum of preexposed individuals. [134,137]

Numerous strategies to circumvent these barriers have been investigated. Examples include as follows: immunosuppression,[133,138] serotype switching (applicable to viruses with more than one serotype),[139] engineering of novel serotypes (for monotypic viruses),[140] and

chemical modification or 'stealthing.'[141] Recent work with the modified vaccinia virus, Pexa-Vec, has shown in rats, macaques, and human blood that pretreatment with a transient complement inhibitor prevents neutralization of i.v. administered virus and thus increases infectious dose available to tumors. This strategy holds tremendous promise for improving systemic delivery of vaccinia viruses (as a large portion of current cancer patients have been immunized against smallpox, and thus have preexisting immunity to treatment), but could also be potentially applied to other viruses susceptible to complement-dependent neutralization.[133] There is therefore reason to hope that these powerful agents, which are currently restricted to use in approaches using direct i.t. administration, will soon be more applicable in treating metastatic disease.

Finally, even if a significant proportion of administered virus were to accumulate intratumorally, distribution subsequent to delivery has been shown to greatly impact the ultimate efficacy of an oncolytic virus. In a recent study, i.t. spread of an oncolytic vesicular stomatitis virus following i.v. injection was tracked using NIS expression, and results showed a heterogeneous distribution of infection within tumors and across animals receiving uniform treatments.[142]

In response to the challenge of i.v. delivery, many studies have delivered virus via i.t. injection to try to circumvent the barrier posed by the bloodstream and the limitation of poor i.t. distribution.[143] However, i.t. injection is not optimal [144] or even feasible for many tumor locations and is unacceptable for treatment of metastatic cancer, thus impacting the clinical utility of this approach.[145]

Physical approaches such as the use of ultrasound-generated inertial cavitation have offered a means of improving i.t. spread by providing a controllable stimuli to propel viruses deep into tumors.[146] These technologies have provided >100-fold increases in activity of i.v. delivered adenovirus vectors to murine xenograft tumors.[147] The challenge will be to see how this technology can scale and translate from preclinical models to humans.

The tremendous promise oncolytic viruses have shown in the treatment of cancer both preclinically, and in clinical trials,[114] means it is likely that work to overcome the delivery challenge will gather pace. The capacity of these agents to selectively self-amplify within tumors and turn cancer cells into factories for the production encoded therapeutic protein is unmatched, and it is this functionality which makes them an exciting candidate for combination with the range of biologic agents currently being developed. In particular, recent studies showing that efficacy of a measles virus expressing anti-PD-L1 can surpass that of the antibody alone, and match that of the virus (not expressing anti-PD-L1) used in combination with a separately delivered antibody,[148] gives an indication of where the field of oncology is heading. Indeed, trials combining oncolytics and biologic checkpoint inhibitors are underway, and although commercial imperatives may initially restrict their use to combination studies, scientific rationale and the need for new parties to carve out freedom to operate are likely to lead to the use of viruses expressing checkpoint inhibitor.

3.6. Cell-based therapies

A more recent approach to achieving viral delivery, without instigating vector-induced immunity, is to use cells as carriers to 'hide' virus from barriers to circulation and systemic delivery. Generally speaking, the cell-carrier-based approach involves the *ex vivo* loading of virus into (or onto) the chosen cell carrier, circulation of the loaded carrier cells, and ultimately virus delivery at the tumor site (reviewed in [149]). The success of this complex process is reliant upon an in depth knowledge of the chosen oncolytic virus and cell carrier, and their interactions. A range of cell types has been tested for the carriage of a range of viruses, including autologous T cells [150,151] and dendritic cells,[152,153] cytokine-induced killer cells,[154] tumor cells,[155,156] and mesenchymal stem cells.[157,158] The choice of cell carrier rests on a number of factors, notably the tropism (if any) of the cell for metastatic tumors, and the mechanism through which the cell will transport the virus; T cells, for example, have been reported to transport surface-bound reovirus, while dendritic cells will internalize the same reovirus, thereby providing a greater protection from neutralizing antibodies.[152]

Using a cell carrier permissive to viral replication allows for shielding of virus within the cell and for amplification of dose between loading and delivery stages.[159] However, timing of *ex vivo* loading and for carrier cells to reach (and ideally target) tumors must be measured against the life cycle of the chosen oncolytic virus within that particular cell type, if the cell is to effectively prevent immune recognition of virus.[149]

Other cell-based treatments focus on the use of adoptively transferred immune cells (natural or genetically engineered) to treat metastatic cancer (reviewed in [160]). In one approach, T cells are taken from a patient, and those with an antitumor specificity are isolated, expanded, then re-introduced into the patient. Though this method of treatment has had impressive clinical results [161] and is currently one of the best available for patients with metastatic melanoma, clinical utility has been dampened by stringent patient-screening requirements and labor-intensive protocols for the selection and isolation of appropriate T cells.[162]

Treatments using adoptively transferred genetically engineered T cells share much mechanistic similarity with BiTE and ImmTAC technology. T cells have been isolated and genetically modified to present chimeric antigen receptors (CAR) on their surface, which recognize specific surface antigen; like BiTEs, the specificity of CAR T cells is derived from antibodies.[163] Alternatively, T cells can be engineered to present a modified T-cell receptor, which will instead recognize intracellularly processed protein in the context of MHC.[164] Though the use of genetically engineered T cells removes the need to find autologous tumor-specific T cells to isolate and expand, the resulting engineered T cells have only monoclonal specificity and are still labor-intensive to produce.

Though cell-based therapies show promise, as facilitators to virotherapy, or directly as instigators of cancer immunotherapy, all approaches require extensive manipulation of cells *ex vivo* prior to treatment. It thus stands to reason that the inherently complicated, labor intensive, and costly process involved in cell-based therapy could preclude its widespread clinical adoption.

4. Conclusion

In response to the failures of more conventional therapies (surgical resection, radiotherapy, and chemotherapy with low-molecular-weight drugs), a wide range of novel approaches to treating metastatic cancer have recently emerged. The possibilities for passive targeting of nanoscale drugs via the EPR effect are being exploited by the reformulation of existing anticancer drugs into nanoparticulate formulations. In addition, active targeting is being investigated clinically, with targeting ligands specific for cancer cells, or the tumor microenvironment providing a specificity of action that allows for the systemic administration of more potent cytotoxic drugs. Biologics, such as monoclonal antibodies, can be used as targeting ligands for these potent cytotoxic drugs, or can exert antitumor activity directly through effects on cancer-cell signaling or induction of apoptosis. Antibodies can also have an indirect effect by engaging various components of the immune system, or by inhibiting the action of immune checkpoint ligands secreted by cancer cells. Other novel therapeutics that have been shown to successfully engage the immune system include BiTEs and immune mobilizing mTCRs against cancer. Oncolytic viruses are gaining traction in the clinic and are distinguished by their ability to self-replicate and produce encoded and potentially toxic therapeutic protein specifically from within the tumor. Finally, cell-based therapies (in combination with oncolytic virotherapy or alone as immunotherapeutics) present innovative approaches to challenges in treatment of metastatic cancer, but their widespread use may ultimately be limited by costly and complicated protocol requirements.

5. Expert opinion

The two main themes emerging from the recent approaches for systemic treatment of metastatic cancer are (1) the need for improved specificity of action and (2) the tremendous potential of the immune system in mounting a robust, durable, and specific and systemic antitumor response.

Work done on nanoscale reformulation of existing cancer drugs has shown exciting results in terms of decreased toxicity and improved tumoral accumulation of drug. The addition of a targeting ligand can, in some cases, further provide an effective targeting of drug. Although antibodies can be used as targeting ligands to provide that specificity, the more interesting – and perhaps more clinically relevant – mode of action of antibody therapies is the engagement with the immune system.

As many studies have shown a link between positive patient prognosis, and the presence of infiltrating immune cells within tumors,[72,73] it is clear that the immune system has an important role to play, likely not only in the debulking of tumors, but also in a robust, long-duration, body-wide anticancer immunity. With this in mind, it is not surprising that the number of different therapies that act to directly elicit or enhance an antitumor immune response is growing rapidly (see Figure 2). BiTEs, ImmTACs, and adoptively transferred T cells are appealing due to the specific nature of their immune stimulation, while immune checkpoint inhibitors provide less specificity (and thus potentially a less favorable safety profile), but have to date been demonstrably potent against a wider range of indications.

One of the largest challenges facing immunotherapy (and targeted therapy in general) in cancer is the identification of predictive biomarkers of clinical outcome. Cancer-targeted therapies like mAbs, BiTE, and ImmTAC require identification of patient populations expressing the appropriate target antigen. In the case of immune-checkpoint inhibitors, a few potential biomarkers have been identified, but further research is required in order to maximize the potential of this therapy. Moreover, as BiTEs, ImmTACs, and many checkpoint inhibitors may engage with T cells directly within the tumor itself, it is likely that the tumoral delivery efficiency and the presence of TILs will also play a key role in determining the success of these therapies. Going forward, one strategy holding potential benefit for treatment in combination with these therapies is the use of some sort of immune adjuvant to increase the number of TILs.

As viruses can be engineered to selectively replicate in and lyse cancers cells (in a particularly immunogenic fashion) oncolytic virotherapy can be used to initiate an antitumor immune response which could potentiate the benefits of an immune therapy, such as immune checkpoint blockade. Early trials have already been conducted comparing the use of ipilimumab or pembrolizumab as monotherapies, versus pretreatment with the oncolytic virus T-Vec followed by treatment with the respective immune-checkpoint inhibitors.[165,166] Preliminary results from a phase 1b trial with i.v. ipilimumab with and without i.t. T-Vec pretreatment indicate that the combination regimen is well tolerated and is more efficacious than monotherapy with either agent.[165] These exciting findings underscore a key limitation, but also an area of opportunity, for immunotherapy of cancer; though agents such as immune checkpoint inhibitors, BiTEs, and ImmTACs are extremely effective at (re-)directing antitumor immune responses, their efficacy is contingent upon the presence of immune cells (particularly T cells) within the tumor microenvironment. This inherent presupposition is likely erroneous for many tumors, and thus an important role for synergistic therapeutics capable of *eliciting* (rather than directing) an antitumor immune response emerges. Oncolytic viruses are an appealing choice, as they are uniquely capable of selective self-amplification, their mechanism of action is conducive to combination with a wide range of therapies, and they actually benefit from an initial dearth of tumor-infiltrating immune cells. Moreover, as viruses can encode therapeutic protein, they can be engineered to encode for immune-stimulating therapies, such as immune-checkpoint-inhibiting antibodies. Not only would this approach benefit from the logistical simplicity of administering one therapeutic rather than a combination, but also it would allow for the therapeutic to be produced *in situ*, potentially improving the safety profile of therapeutics prone to causing immune-related adverse effects. Preclinical models have provided proof-of-concept for the encoding of immune checkpoint inhibiting antibodies into oncolytic viruses. These studies have indicated that although local expression of antibodies can reduce toxicity, this approach will likely be more effective for targeting of the PD-1 pathway, as its activity is at the level of the tumor, while the CTLA-4 pathway is more

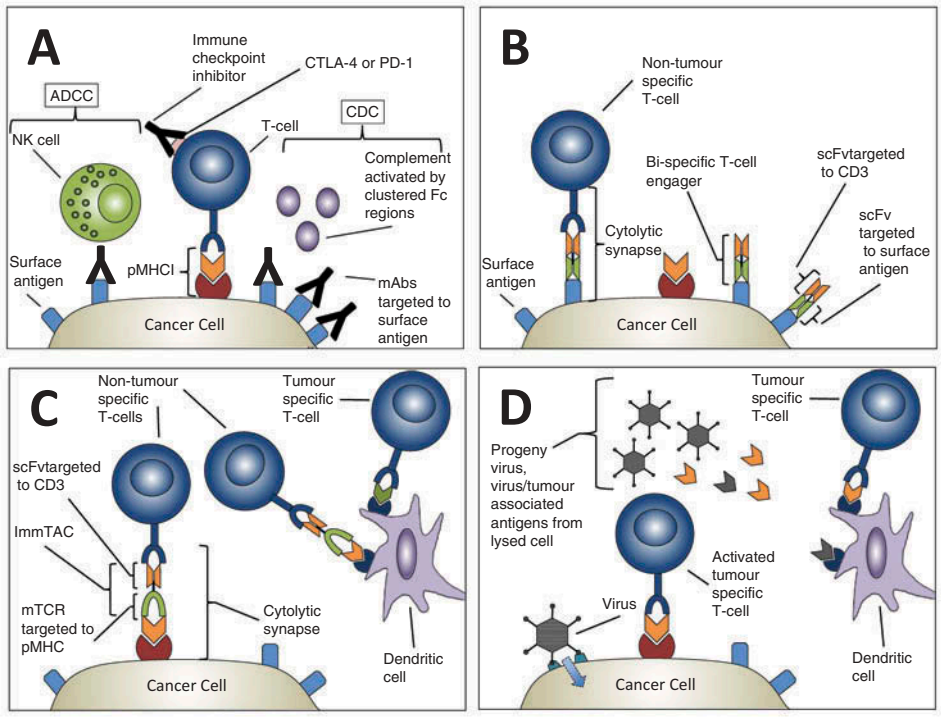

Figure 2. Immune-mediated tumour cell killing by four different types of therapeutic agents. (A) Antibodies can engage with the immune system via antibody-dependent cell-mediated cytoxicity (ADCC), complement-dependent cytoxicity (CDC), or by directly targeting immune checkpoint receptors on the surface of T-cells. (B) Bi-specific T-cell engagers target both cancer cell surface antigen and CD3 within the T-cell receptor complex. Simultaneous binding of a non-specific T-cell to the scFv targeted to CD3, and the cancer cell to the scFv targeted to surface antigen induces T-cell activation and formation of a cytolytic synapse. (C) Immune mobilizing monoclonal T-cell receptors against cancer (ImmTACs) target and bind to endogenously processed protein expressed in the context of MHC (pMHC) through an affinity enhanced monoclonal T-cell receptor (mTCR). ImmTACs also bind to CD3 within the T-cell receptor complex, and when bound to both targets, ImmTACs induce T-cell activation and the formation of a cytolytic synapse. Additionally, ImmTACs can enhance cross-presentation of antigen by dendritic cells, leading to activation of a wider range of T-cells (epitope spreading). (D) Oncolytic viruses infect and replicate within cancer cells, eventually lysing the cells and releasing progeny virus in addition to antigen (both virus-associated and tumour-associated antigen). Antigen is taken up and presented by dendritic cells to T-cells, which then become activated and kill other tumour cells presenting tumour-associated (and virus-associated) antigen.

important at the level of the lymph nodes and would thus likely require a more systemic treatment.[148,167] Clearly, a more comprehensive understanding of these pathways (and any other potential checkpoint pathways) will help guide further studies on the potentially impressive combination of viro- and immunotherapy.

Declaration of Interest

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