EXPERT OPINION

Epithelial cell adhesion molecule-targeted drug delivery for cancer therapy

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Introduction: The epithelial cell adhesion molecule (EpCAM) is abundantly expressed in epithelial tumors, on cancer stem cells and circulating tumor cells. Together with its role in oncogenic signaling, this has sparked interest in its potential for tumor targeting with antibodies and drug conjugates for safe and effective cancer therapy. Recent advances in protein engineering, linker design and drug formulations have provided a multitude of EpCAM-targeting anticancer agents, several of them with good perspectives for clinical development.

Areas covered: This article reviews the biological, therapeutic and technical aspects of EpCAM-targeted drug delivery for cancer therapy. The authors discuss seminal findings, which distinguish EpCAM as a target with oncogenic function and abundant expression in epithelial tumors. Moreover, recent trends in engineering improved anti-EpCAM antibodies, binding proteins that are not derived from immunoglobulins and drug conjugates derived from them are highlighted and their therapeutic potential based on reported preclinical and clinical data, originality of design and perspectives are critically assessed.

Expert opinion: EpCAM has shown promise for safe and efficient targeting of solid tumors using antibodies, alternative binding molecules and novel drug conjugates. Among the myriad of EpCAM-targeting drug delivery systems investigated so far, several could demonstrate therapeutic benefit, other formulations engineered to become tailor-made missiles are on the brink.

Keywords: antibody drug conjugates, cancer therapy, EpCAM, novel binding proteins, tumor targeting

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1. Introduction

Tumor-targeted anticancer agents with a high destructive potential yet designed to better discriminate between malignant and normal tissues, compared to standard chemotherapy, have become the focus of drug development programs worldwide. Particularly, antibodies – either as whole immunoglobulin G (IgGs) or their fragments – now constitute a clinically important class of protein therapeutics. Their Fc portions can be further engineered not only for improved tumor targeting but also for optimizing their effector functions, that is, their interactions with Fc receptors. Furthermore, numerous antibody drug conjugates (ADCs) have been described, including conjugates of small cytotoxic drugs, protein toxins and enzymes, cytokines and oligonucleotides. Finally, antibodies have been coupled to nanoparticles and viral vectors [1-4].

Many technologies are now available for generating human antibodies, which results in reduced immunogenicity [5]. More recently, non-IgG protein scaffolds

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Article highlights.

- EpCAM is a signaling molecule with oncogenic potential and abundantly expressed in epithelial tumors.
- Its recent identification on cancer stem cells and circulating tumor cells has raised further interest in its use for tumor targeting and therapy.
- Engineering of anti-EpCAM antibodies with improved clinical activity and of alternative binding scaffolds with high stability and new opportunities for engineering offer new therapeutic options.
- Various types of EpCAM-targeted drug conjugates have been developed and investigated in preclinical and clinical studies with different success.
- The use of state-of-the-art knowledge in protein engineering, linker design and drug activity has provided new generations of drug conjugates as cancer therapeutics with high specificity and efficacy.

This box summarizes key points contained in the article.

have become available [6] and also RNA-based aptamers selected from large synthetic libraries [7-9] have been introduced as binding moieties for drug delivery. These formats may even outperform new antibodies (or other agents) in terms of tumor-targeting capacity and efficacy, due to their high stability and opportunities for engineering of new formats.

Despite undeniable advances in design of both the targeting reagent as well as the payload, for any tumor targeting, the best binder, however, is only as good as the molecular target to which it binds, that is, its tumor-specific expression and thus limited binding to normal tissues and its role in oncogenic signaling. The epithelial cell adhesion molecule (EpCAM, CD326) is abundantly expressed in primary tumors and metastases of many epithelial tumors [10,11]. Ligand binding to overexpressed EpCAM on tumor cells by homotypic adhesion triggers oncogenic signaling involving the Wnt pathway and various cell cycle regulators [12-17]. Its recent discovery on cancer stem cells (CSCs) [13,18,19] and circulating tumor cells [20,21] has further sparked interest in its biological function and potential for tumor targeting. Although EpCAM is also expressed in several normal epithelia [22], evidence for its better accessibility on tumor cells and the promising data from tumor targeting studies have nevertheless raised enthusiasm to use EpCAM as a target for drug delivery with a reasonably high therapeutic index.

Here, we focus on those antibodies, novel scaffold proteins and drug conjugates that so far have been investigated for EpCAM-targeted cancer therapy. The various formats are depicted in **Figures 1** and **2**; **Table 1** summarizes the various EpCAM binders and drug conjugates, including their development stage, which represent state-of-the-art in the field. We discuss their use in preclinical and clinical studies and critically assess their innovative potential and future perspectives as cancer therapeutics with improved efficacy. Some of the strategies which have been employed for EpCAM-targeted cancer therapy are depicted in **Figure 3**.

2. EpCAM structure

EpCAM was initially identified as a tumor-associated antigen overexpressed in colon carcinoma [23]. Since it was independently discovered by several groups, it was synonymously named 17-1A, HEA125, MK-1, GA733-2, EGP-2, EGP34, KSA, TROP-1, ESA and KS1/4 [24]. EpCAM is a 30 – 40 kDa type I transmembrane glycoprotein of 291 amino acids which is expressed in normal and malignant epithelia and functions as a homotypic cell adhesion molecule [12,24-26]. The largest part is formed by the extracellular domain, which contains a structural unit with no convincing homology to known folds – some authors have prematurely proposed an EGF-like fold – and a second thyroglobulin type-1 domain [26,27]. EpCAM is commonly co-expressed and forms complexes with claudin-7, CO-029 and CD44v6 in the cell membrane [28]. It is thus not surprising that the majority of antibodies which were raised against EpCAM on tumor cells share a limited number of epitopes accessible on the cell surface and which are not blocked by other proteins in the complex. These are located in the N-terminal sequence of EpCAM encoded by exon 2 [29]. An exception is the monoclonal antibody adecatumumab (MT201) [30] which recognizes a more membrane-proximal epitope encoded by exon 5 [31]. If purified soluble EpCAM is used to screen synthetic libraries, the diversity of binders substantially increases but with the restriction that a large fraction is unable to contact its epitope on cells [32].

3. EpCAM expression on tumor cells, CSCs and normal tissues

EpCAM is abundantly expressed in primary tumors and metastases of most epithelial malignancies, particularly on adenocarcinoma, certain squamous cell carcinoma hepatocellular carcinoma and retinoblastoma [10,11]. Of further clinical relevance is its expression on CSCs of various histotypes [13,18,19,33]. CSCs are capable of unregulated asymmetric division, which is responsible for self-renewal and generation of a diverse population of differentiated progenitor cells that make up a heterogeneous tumor [34-36]. Thus, this subgroup of cells initiates tumor growth and spread, and it was found to be particularly resistant to cytotoxic therapy. In addition, EpCAM is expressed on circulating tumor cells in the blood which have a high propensity to form hematogenous metastases [20], and their presence is an independent predictor of poor prognosis [21]. The possibility to eradicate these two populations of devastating cells with EpCAM-targeted therapies is appealing. In one study, EpCAM expression was found to be upregulated in G2/M arrested cells, probably due to inhibition of normal cycles of endocytosis [37], suggesting its targeting to be particularly efficient in combination with spindle-disrupting anticancer agents.

On the other hand, EpCAM is also expressed in the corresponding normal epithelia, albeit more variable and at levels below that found in tumors [10,22]. Targeting with cytotoxic

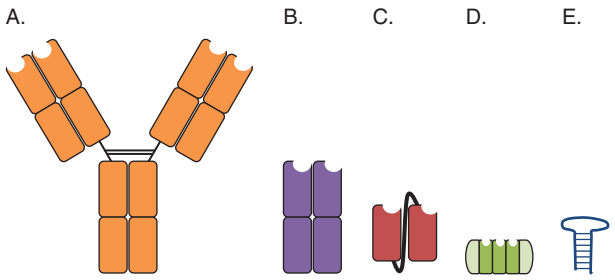

Figure 1. Schematic overview of EpCAM-targeting binding molecules. (**A**) IgG, (**B**) Fab fragment, (**C**) scFv, (**D**) DARPin and (**E**) RNA aptamer.

antibodies or drug conjugates would nevertheless be possible, provided that antigen expression is restricted to non-vital tissues for which damage can be tolerated on a temporary basis. For example, in early clinical trials with the well-tolerated anti-EpCAM antibody adecatumumab (see Section 6.1 below) dose-limiting adverse effects were limited to reversible gastrointestinal disorders. Moreover, cross-reactivity with normal tissues may impair tumor targeting by sequestration of binding ligands. In this context, investigations in EpCAM's distribution on the cell surface unveiled differential patterns with a more accessible and homogeneous orientation on tumor cells, while mainly a basolateral location with sequestration in intercellular boundaries is found in normal epithelia [38,39]. Although still remaining to be demonstrated, it is hoped that the differential subcellular distribution is a factor that is responsible of increasing the therapeutic window by sparing normal cells from binding by anti-EpCAM antibodies delivered via the blood stream and subsequent destruction by cytotoxic mechanisms. Results with antibodies of different affinities have been interpreted by some investigators to suggest that low affinity binders are indeed better tolerated [31].

4. Oncogenic potential of EpCAM

In contrast to receptor tyrosine kinases, which have attracted the attention of cell biologists and oncologists for many years [40-42], EpCAM's biological function has been just recently partly deciphered. In addition to its role as adhesion molecule [38], more important functions comprise cellular signaling to regulate various biological responses, such as proliferation, differentiation and migration. Upregulation of EpCAM during carcinogenesis is consequently associated with a gain of function due to increased homotypic adhesion [14,15]. On adequate cell–cell contact, the extracellular domain is cleaved by intramembrane proteolysis and released, while the intracellular domain translocates to the nucleus in combination with four-and-a-half LIM domains protein 2 (FHL2) and β-catenin to drive tumor and stem cell proliferation. Crosstalk with the Wnt pathway is suggested at the level of β-catenin and Lef-1 [13,15]. Stimulation of the cell cycle was found to result from inhibition of retinoblastoma protein and cyclin D1, E and A [14-17]. Moreover, EpCAM expression can influence the JNK/AP-1 signal transduction pathway, suggesting that modulation of the activity of transcription factor AP-1 contributes to EpCAM-dependent invasion [43]. How activation of the EpCAM signaling cascade can be reconciled with the unregulated asymmetric division of CSCs remains to be investigated.

Evidence for EpCAM as a promising target on tumor cells thus not only comes from immunotherapy studies but also from its biological function. Indeed, RNA interference (RNAi)-mediated knockdown of EpCAM was shown to suppress proliferation and clonogenic growth of lung cancer cells, yet without induction of cell cycle arrest, and apoptosis was induced in cancer cells, but to a much lesser extent in normal bronchial epithelial cells [44]. Similarly, in breast cancer cells EpCAM-targeted RNAi reduced the promalignant effects associated with EpCAM signaling described above [45]. Among the clinically tested antibodies, only adecatumumab was found to reduce tumor cell proliferation without affecting normal epithelia [31]. Although these data suggest that EpCAM-targeted molecular intervention can diminish its oncogenic signaling, it is unlikely that this strategy alone is sufficient to meet with clinical success. Moreover, the picture of EpCAM's oncogenic function is not black and white, as was recently shown in lung cancer cells, where EpCAM-targeted RNAi may instead relieve the suppression of metastasis induced by epigenetic silencing [46].

5. EpCAM shedding and internalization

Shedding of the target antigen from tumor cells into the circulation unfavorably facilitates formation of immune complexes with therapeutic antibodies and ADCs. This may increase systemic toxicity, accelerate clearance and impair tumor localization. EpCAM seems to be cleaved at areas of cell-to-cell contact and the ectodomain is then shed to form soluble EpEX, which acts as a ligand in EpCAM signaling on other cells [15]. To what extent this occurs in solid tumors and whether EpEX enters the circulation in significant amounts is unclear. The excellent tumor localization of anti-EpCAM antibodies investigated in preclinical and clinical studies, however, suggests that this process is less relevant. EpCAM is efficiently internalized by receptor-mediated endocytosis [32,47-49], thereby also internalizing antibodies or other bound ligands, indicating that there is still abundant full length receptor present on the cell surface, despite its postulated cleavage at cell–cell contact sites. These properties highlight EpCAM as a particularly efficient carrier for intracellular delivery of payloads acting on intracellular targets, even though the payload still has to cross the endosomal membrane.

6. EpCAM-targeted therapies

From a simplistic point of view, the oncogenic function of EpCAM and its expression on CSCs suggest that its

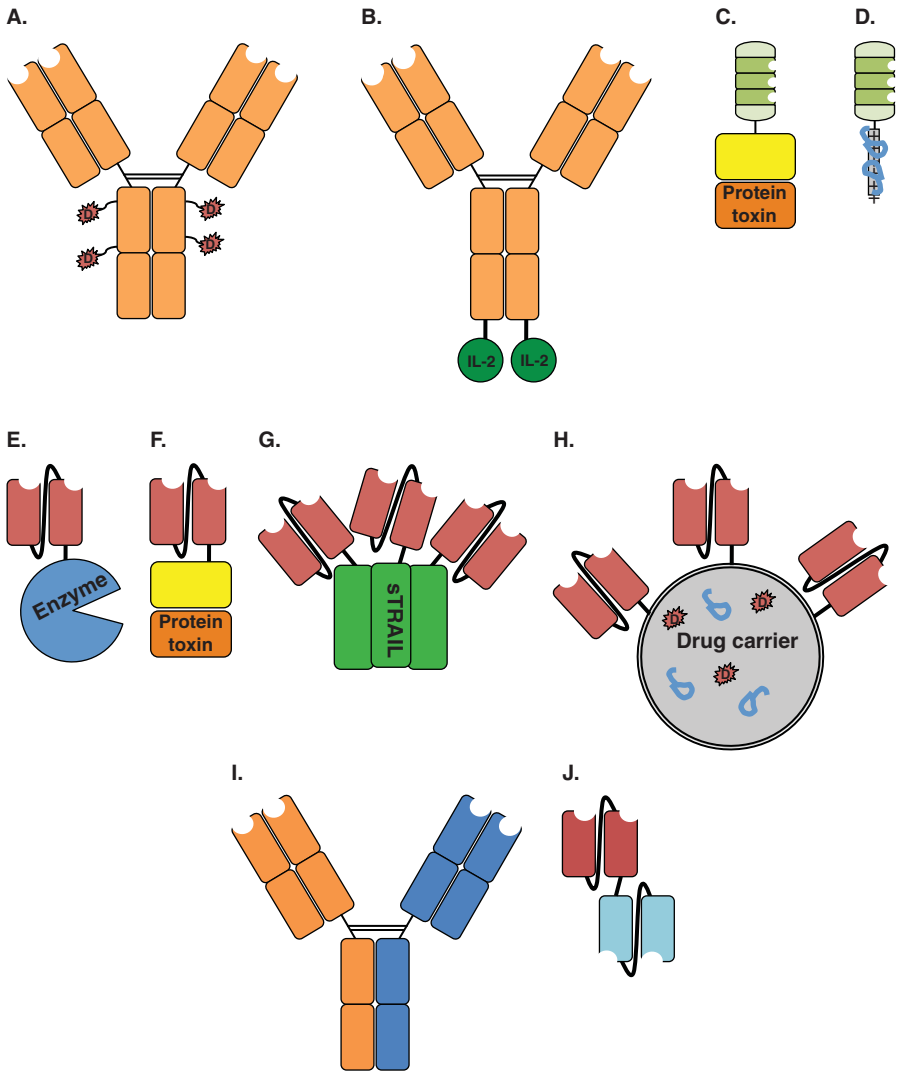

Figure 2. Schematic overview of EpCAM-targeting drug delivery systems and bispecific binding constructs. (**A**) ADCs, (**B**) immunocytokines, (**C**) DARPin–toxin fusion proteins, (**D**) DARPin–protamine fusion proteins (**E**) scFv–enzyme fusion proteins for ADEPT, (**F**) scFv–sTRAIL, (**G**) scFv–toxin fusion protein, (**H**) scFv-coated nanocarriers, (**I**) bispecific hybrid IgG for CD3 targeting and (**J**) bispecific scFv for CD3 or adenovirus (re)targeting.

overexpression in tumors might be a marker of poor prognosis. Indeed, this hypothesis of bad news brought by EpCAM overexpression is supported by several findings [50-52]. On the other hand, there are opposite reports, for example, in patients with renal cell carcinoma and in patients with pancreatic cancer receiving curative resection, that EpCAM was found to be an independent predictor of improved survival [53,54]. The latter study, however, used an experimentally generated cell model by transfection-induced EpCAM overexpression without further demonstrating oncogenic signaling. Moreover, the prognostic value in this study was limited to patients without lymph node involvement and additional markers of 'stemness' were not examined, such as CD133, CD44 and CXCR4, which are also expressed on metastatic pancreatic cancer cells [55]. In summary, there is convincing evidence that destruction of EpCAM-positive tumor cells has therapeutic benefit, provided that side effects on normal epithelia are tolerated.

The identification of EpCAM on CSCs and circulating tumor cells led to an enthusiasm for selectively targeting these highly tumorigenic, metastatic and often drug-resistant cell populations. In contrast to receptor tyrosine kinases, such as members of the EGFR family, where antibodies inhibit proproliferative signaling by blocking receptor dimerization and ligand binding, antibodies binding to EpCAM on tumor cells have not demonstrated significant growth inhibition, with the possible exception of adecatumumab. Since in general interference with EpCAM signaling is not sufficiently efficacious, efforts have been directed toward recruiting immune effector functions via the antibody Fc part and by generating ADCs

Table 1. Summary of selected anti-EpCAM binders and drug conjugates.

Generic name	Binder format	Effector moiety	Type of linkage	Stage of development	Described in section	Refs.
MOC31-ETA	IgG	Truncated *Pseudomonas* exotoxin A	Chemical	Preclinical	6.3.3	[117]
4D5MOCB-ETA, VB4-845	scFv	Truncated *Pseudomonas* exotoxin A	Fusion	Phase II/III	6.3.3	[76,119]
Ec4-ETA"	DARPin	Truncated *Pseudomonas* exotoxin A	Fusion	Preclinical	6.3.3	[122]
VB6-845-C_H	scFv	T-cell epitope-depleted bouganin	Fusion	Phase I	6.1.2 6.3.3	[75,120]
scFvC54:sTRAIL	scFv	Human soluble TRAIL	Fusion	Preclinical	6.3.3	[123,124]
Adecatumumab	IgG	Fc optimized		Phase II	6.1.1	[30]
Catumaxomab	IgG	Anti-CD3, Fc optimized	Mouse-rat hybrid	EU approved	6.1.3	[81]
MT110	Tandem scFv	Anti-CD3	Fusion	Phase I	6.1.3	[82]
MOC31-Dox	IgG	Doxorubicin	Chemical	Preclinical	6.3.1	[98]
B38.1-DM1	IgG	Maytansinoid DM1	Chemical	Preclinical	6.3.1	[105]
chiHEA125	IgG	α-Amanitin	Chemical	Preclinical	6.3.1	[106]
323/A3-mGUS	IgG	β-Glucuronidase	chemical	preclinical	6.3.2	[108]
C28-GUSh	scFv	β-Glucuronidase	Fusion	Preclinical	6.3.2	[109]
C28-sCE2	scFv	Carboxylesterase	Fusion	Preclinical	6.3.2	[110]
Onc-SS-HSA-4D5MOCB	scFv	Onconase, serum albumin	Fusion	Preclinical	6.3.4	[78]
KS-IL2	IgG	Interleukin 2	Fusion	Phase I	6.3.5	[135,136]
SIL-Dox	scFv	Immunoliposomal doxorubicin	Chemical	Preclinical	6.3.6	[74]
C9-P	DARPin	Protamine-anti bcl-2/bcl-xL siRNA	Fusion	Preclinical	6.3.6	[143]
Ep-DT3	Aptamer	Fluorophore	-	Cellular studies	6.2.2	[47]
Ad-FZ33	IgG	Adenovirus carrying the *UPRT* gene	Protein A adapter	Preclinical	6.3.7	[146]

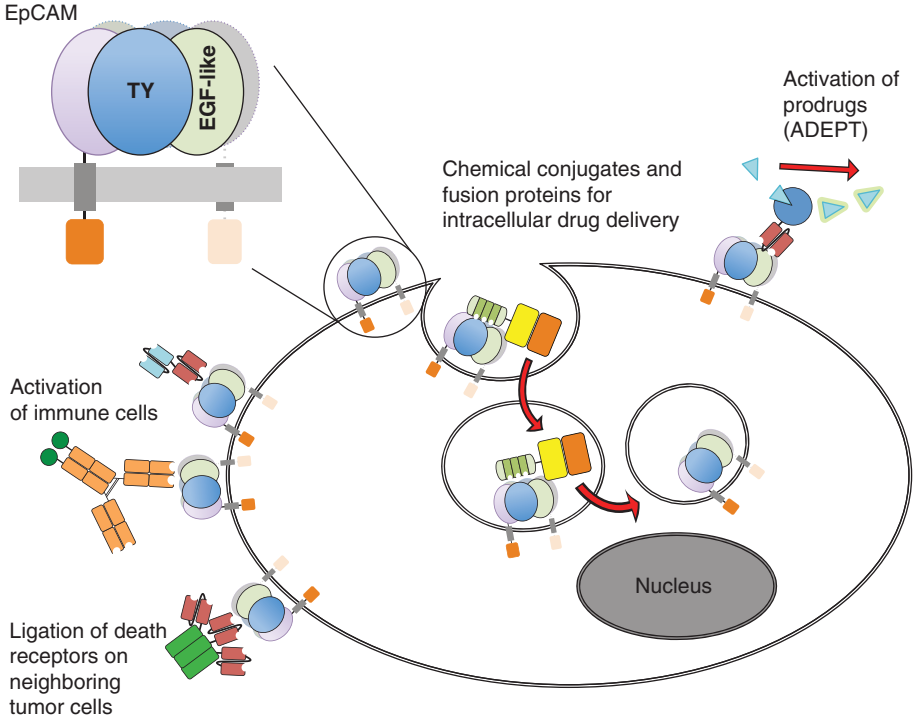

Figure 3. Schematic overview of selected EpCAM-targeting strategies in cancer therapy discussed in this review. EpCAM-specific binding molecules can be payloaded with various effector functions for tumor targeting; some of the constructs shown in Figure 2 are presented as examples. They include immunocytokines and bispecific antibodies for immune cell activation, TRAIL–scFv fusion proteins for apoptosis induction in neighboring tumor cells, ADEPT and a variety of constructs which act on intracellular targets and require internalization by receptor-mediated endocytosis.

and drug conjugates made with alternative binding molecules. The various formats of EpCAM binders and their drug conjugates are depicted in **Figures 1** and **2**.

6.1 EpCAM-targeted antibodies

Recent advances in protein design and engineering provided versatile platforms for the generation of functionally improved antibodies, from conventional murine to chimeric, humanized and fully human IgGs, and with different affinities [5]. Promising and not mutually exclusive strategies to enhance the antitumor activity include the payloading of antibodies with anticancer agents and Fc optimization to recruit accessory immune functions. Isotype selection is crucial for this, as only the IgG1 format is fully capable of activating antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) [1,2].

Reviewing the considerable quantity of preclinical and clinical studies with EpCAM-targeted monoclonal antibodies and bispecific antibody formats recruiting immune effector cells is beyond the scope of this article and discussed elsewhere [31,56,57]. Nevertheless, several important milestones with anti-EpCAM monoclonal antibodies should not be ignored here as they have also provided valuable information for the design and clinical development of ADCs.

6.1.1 *Monoclonal antibodies*

The monoclonal antibody edrecolomab (Mab 17-1A, Panorex®) is a chimeric hybridoma-derived murine IgG2a, the mouse equivalent of human IgG1, which binds EpCAM with low affinity [58,59]. It is thought to destroy tumor cells by activating ADCC/ADCP and possibly also by CDC and a host anti-idiotypic antibody response [60]. Edrecolomab was approved in Germany in 1995 as a well-tolerated adjuvant treatment for advanced colorectal cancer [61,62]. The initial approval, however, was finally withdrawn in 2000, because it failed to confirm any advantage over chemotherapy in a subsequent larger study [63,64]. Likely reasons for this failure were low affinity, insufficient activation of ADCC/ADCP, neutralization by human anti-mouse antibodies (HAMA) and a short half-life [65].

Thereafter, IgGs with lower immunogenicity, longer half-life, higher affinity and improved ADCC/ADCP recruiting capacity were engineered which all share the Fcγ1 portion. As shown in clinical Phase I studies with the humanized 3622W94 [66] and ING-1 antibodies [67], however, engineering for high affinity and efficacy, unfortunately, came with the price of a low maximum tolerated dose (MTD) of only 1 mg/kg, with acute pancreatitis as dose-limiting toxicity [31]. This disappointing outcome with high affinity antibodies suggests binding to normal vital tissues and challenges the optimistic hypothesis of limited accessibility of EpCAM on normal cells described above.

Much better tolerated is adecatumumab, a fully human IgG of intermediate affinity, which is currently under clinical investigation in patients with prostate [68] or breast cancer, where it is showing particularly promising results in patients with high levels of EpCAM and in combination with taxotere [69,70]. Interestingly, epitope mapping by flow cytometry and expression of human and chimeric EpCAM from other species unveiled that, in contrast to most other antibodies recognizing an N-terminal sequence of EpCAM encoded by exon 2 [29], adecatumumab binds to an exon 5-encoded sequence. If this epitope is engaged in homotypic EpCAM adhesion and signaling, this might explain the ADCC/ADCP- and CDC-independent antitumor effect of adecatumumab [31].

6.1.2 *Antibody fragments*

Antibody fragments, particularly small recombinant scFv fragments, have also been used for EpCAM targeting (**Figure 1**). Since they lack an Fc portion, they must be payloaded for tumor therapy and are therefore discussed under drug conjugates below. Due to their convenient production and expression in *E. coli* and many opportunities for further engineering, recombinant antibody scFv fragments may be better suited for assembling drug delivery systems in which the Fc part is dispensable.

The first anti-EpCAM scFv was generated by phage display cloning from the MOC31 hybridoma first described elsewhere [71]. However, despite EpCAM-specific binding to tumor cells *in vitro*, this mouse-derived scFv was too unstable *in vivo* and thus failed to target tumor xenografts in mice [72]. Based on an analysis of the structural basis of the stability problems of the murine scFv, a second-generation scFv was engineered by grafting the hypervariable loops of MOC31 onto the humanized stable framework of the Her-2 binder 4D5, followed by structure-guided replacement of several amino acids [72], thereby also humanizing it as a side effect. The resulting scFv 4D5MOCB showed high thermal stability, good folding and favorable EpCAM-targeting properties *in vivo*. It was subsequently used for tumor targeting in various drug delivery systems [73-78] (see Section 6.3 below).

6.1.3 *Bispecific antibodies*

Catumaxomab (Removab®) is a mouse–rat hybrid anti-EpCAM × anti-CD3 bispecific mAb, which – since it still contains an Fc part – has been termed 'trifunctional'. It, thus, should engage T cells and FcR-positive accessory cells to contact EpCAM-positive tumor cells [79,80]. In 2009, catumaxomab received European market approval for the treatment of malignant ascites in patients with EpCAM-positive carcinomas and intraperitoneal spread by intraperitoneal infusion. Not unexpectedly, in a Phase II pharmacokinetic study, all patients developed antibodies against the hybrid protein before the last infusion [81]. Tandem scFv constructs (bispecific T-cell engaging antibodies) have also been generated with anti-EpCAM/anti-CD3 specificity. In a recent preclinical study, the bispecific binder MT110 [82] was capable of

eliminating stem cells from primary pancreatic tumors and established cell lines [83].

6.2 Alternative non-IgG scaffolds for EpCAM-targeting

For delivering a biologically active payload, the binding unit must not necessarily be derived from an antibody. Instead, by choosing scaffolds with very good biophysical properties, such new targeting proteins can expand the range of applications of the resulting construct. The new targeting proteins also need to distinguish themselves by showing much less aggregation than many antibody constructs. This is especially relevant for fusion proteins and multimeric constructs. For the chemical coupling to toxins or nanoparticles, it is advantageous to make use of an engineered unique cysteine, and thus the protein should not contain other cysteine residues. If such proteins express well in bacteria, not only can new prototypes be developed very rapidly but also the costs of the final product remain manageable.

6.2.1 *Designed ankyrin repeat proteins*

By extracting sequence and structural information from natural ankyrin repeats, consensus amino acid sequence motifs encoding repeat modules were designed, comprising fixed and variable positions [84]. The variable positions mainly reflect non-conserved surface-exposed residues on the concave site which can be engaged in interactions with the target, as known from crystal structures of natural ankyrin complexes. Using such a designed repeat module with randomized surface residues, proteins can be built by stacking several modules together to form one contiguous protein domain, in one chain. Such designed ankyrin repeat protein (DARPin) molecules show high solubility and thermodynamic stability, and can be expressed in soluble form in the cytoplasm of *E. coli* to very high yields [84,85]. DARPins against various targets, including EpCAM, have been isolated from large libraries by ribosome or phage display [32,86]. They have unique properties which make them particularly suitable for tumor targeting. One advantage is the variable format, which can be easily switched from monomeric to oligomeric, resulting in the generation of multispecific binding molecules. Another benefit is the robustness of the DARPin scaffold and other unique features, such as the lack of cysteines. This offers intriguing engineering possibilities for conjugation with different types of effector molecules, such as by site-specific modification at an artificially introduced cysteine or bio-orthogonal conjugation with click chemistry [87,88]. Finally, owing to its ease of manufacture, the large-scale production of therapeutics derived from DARPins is more cost effective.

Using a DARPin library with three randomized repeats (between two capping repeats), anti-EpCAM binders with affinities in the low nanomolar and picomolar range were selected recognizing two distinct EpCAM epitopes. They were expressed in soluble form in the cytoplasm of *E. coli* at amounts of up to 140 mg/L in shake flasks, which was 100-fold higher than a previously generated anti-EpCAM scFv antibody [32]. These binders have been used for the generation of EpCAM-targeted drug conjugates as described below.

6.2.2 *Aptamers*

Aptamers are single-stranded RNA or DNA molecules of about 40 nucleotides in length with unique conformations based on hairpin formation and additional three-dimensional interactions, resulting in target recognition abilities. They are isolated from large libraries containing 10^{13} – 10^{16} random nucleic acid sequences through systematic evolution of ligands by exponential enrichment (SELEX) [7,8]. Aptamers represent another class of binding molecules of small molecular size, high stability and tolerance to chemical and physical modifications. However, their high negative charge presents restrictions to which epitopes on the cell surface and on the target molecule can be targeted, and at least RNA-based aptamers must be chemically modified to prevent enzymatic digestion, precluding enzymatic synthesis and requiring manufacture by total synthesis [89]. For tumor targeting, they can also be directly payloaded with chemotherapeutic agents, therapeutic oligonucleotides or conjugated to drug-loaded nanoparticles [89-91].

An anti-EpCAM RNA aptamer isolated from a random oligonucleotide library and further truncated from 40 to 19 nucleotides was recently tested on a panel of human tumor cell lines of various histotypes *in vitro* [47]. On binding to EpCAM, the aptamer was well internalized into the cytoplasm despite its high negative charge, probably by clathrin-mediated endocytosis, showing some potential for EpCAM-targeted drug delivery. Due to the small size of the 19-nt RNA with a MW of only 6 kDa, however, rapid renal clearance and consequently low tumor localization must be expected. This is probably addressable by polyethylene glycol conjugation (PEGylation), but the RNA-based molecule would also have to be converted to less digestible analogs for *in vivo* use.

6.3 EpCAM-targeted drug conjugates

A wide range of studies have been performed to investigate whether the therapeutic efficacy of antibodies and alternative binding proteins can be augmented by payloading them with anticancer agents (**Figure 2**). The various conjugation strategies have been reviewed elsewhere [1,2,92,93]. The ability of such conjugates to discriminate between tumor and normal tissues allows the use of even extraordinarily potent drugs, which cannot otherwise be administered to patients in free form, due to inacceptable toxicity. As a rule, the more specific and efficient the delivery system, the higher the MTD and hence efficacy of the treatment. Since the drug conjugate is exposed to different conditions, from the injection site and the blood stream to its molecular target in the tumor, pharmacological properties and therapeutic efficacy also strongly depend on the stability of the linker. For a clinically useful delivery system, it must be designed to prevent premature release of the drug in the circulation to minimize targeting of normal tissues and support tumor localization.

On the other hand, many drugs that act in the cytoplasm must first be detached from the protein, either by degradation of the protein [94] or the linker must be cleaved within the endosome [95]. This allows a (hydrophobic) small molecule to pass through the endosomal membrane and, furthermore, the protein would not sterically block its function.

Here we focus on those payloads which have been examined for EpCAM-targeted cancer therapy in the form of chemical conjugates or fusion proteins (**Table 1**). They include chemotherapeutic agents, toxins of plant, fungal and bacterial origin, an arginine-rich polypeptide for oligonucleotide complexation, immunostimulatory cytokines, RNase, enzymes for antibody-directed enzyme prodrug therapy (ADEPT), the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), liposomes, nanoparticles and adenoviral vectors (**Figure 2**). **Figure 3** illustrates several strategies which have been employed in EpCAM-targeted cancer therapy. The properties and perspectives of these various conjugates are discussed in the following sections. Radioimmunoconjugates, on the other hand, are not a subject of this review, as there was only one reported Fab fragment conjugated to ^{186}Re (NR-LU-10, NeoRx/Poniard Pharmaceuticals Inc.), which was tested in a clinical Phase I trial in 1998 but was not further mentioned thereafter.

6.3.1 Chemically linked ADCs

In early studies methotrexate was covalently conjugated to the anti-EpCAM murine antibody KS1/4 using a heterobifunctional linker to achieve an antibody-to-drug ratio of 1:50. The conjugate was investigated in a clinical Phase I study in patients with advanced non-small-cell lung cancer [96,97]. Almost all patients developed a HAMA immune response and showed signs of considerable toxicity, whereas the clinical response was poor. Based on current knowledge, this failure was likely due to the low drug potency and inefficient drug release by the linker, inhomogeneity and poor quality of the ADC as a result of random chemical modification, short half-life of the antibody and neutralization by HAMA.

Subsequently, a better designed acid-cleavable hydrazone linker was used to attach doxorubicin to the murine antibody MOC31. A conjugation ratio of seven doxorubicin molecules per IgG was achieved and the linker enabled drug release under mildly acidic conditions [98]. Although promising results were shown in preclinical tumor models, the work was discontinued because at the same time a very similar ADC (BR96-doxorubicin) targeting the Lewis-Y antigen failed to show clinical efficacy, despite good antitumor effects in preclinical models [99]. Possibilities to overcome this limited efficacy would be the delivery of larger amounts of cytotoxic payload, for example, in the form of immunoliposomes (see below) or the use of new drug generations which are at least two orders of magnitude more potent [95,100].

Maytansinoids represent a new generation of spindle inhibitors, interfering with spindle assembly by binding to tubulin at or near the vinblastine binding site and with potencies up to two to three orders of magnitude higher than standard chemotherapeutic agents [101]. Recently, a synthetic derivate of maytansine (emtansine) was linked to the humanized anti-Her-2 binder trastuzumab. The conjugate T-DM1 showed substantial antitumor activity in clinical trials in patients with Her-2-positive trastuzumab-refractory breast cancer [102,103] and is close to FDA approval [104]. For EpCAM-targeting, the maytansinoid DM1 was conjugated to the murine antibody B38.1 using a maleimide-based hydrophilic PEG_4 linker [105]. On internalization, the ADC was processed to a hydrophilic cytotoxic PEG metabolite, which, in contrast to the hydrophobic metabolites released from a conjugate prepared with a non-polar SMCC linker, could overcome drug resistance in MDR1-expressing cells. Thus, it appears that the polar metabolite cannot be transported out of the cell. The advantage of the hydrophilic linker was also shown in tumor xenograft models in mice by the higher antitumor activity of the respective ADC.

Another strategy to generate ADCs with high potency is the use of cytotoxic peptides. Recently, the chimeric antibody chiHEA125 was conjugated with α-amanitin, a potent cyclic octapeptide derived from basidiomycetes mushrooms which binds to eukaryotic RNA-polymerase II and inhibits DNA transcription resulting in apoptosis [106,107]. The α-amanitin peptide was conjugated to the antibody via a protease-cleavable glutarate linker, expected to release the toxic peptide inside the cell on contact with esterases or proteases. The drug:IgG ratio varied from 4:1 to 8:1 and the ADC inhibited tumor cell proliferation with a cell line-dependent therapeutic index of up to five orders of magnitude, as calculated in comparison to a non-targeted ADC and free α-amanitin. A single intraperitoneal injection of the conjugate in mice inhibited the growth of pancreatic tumor xenografts which could be completely eradicated by a repeated administration [106]. However, more analytical data, additional *in vivo* efficacy experiments in other tumor models and pharmacological examinations are warranted to assess the true therapeutic potential of this conjugate.

6.3.2 Antibody-directed enzyme prodrug therapy

In ADEPT, an antibody is used to target an enzyme to tumors, which then catalytically generates a cytotoxic drug from a separately administered prodrug [4]. To generate an enzyme immunoconjugate for EpCAM-targeted ADEPT, the murine anti-EpCAM antibody 323/A3 was chemically linked via a stable thioether bond to β-glucuronidase. Using a glucuronylated prodrug of doxorubicin (DOX-GA3) as substrate, where glucuronylation reduces its toxicity, significant growth inhibition of ovarian cancer xenografts in mice was observed at a 60-fold higher MTD compared to native doxorubicin [108]. In a subsequent study, the human antibody scFv C28 was genetically fused to β-glucuronidase via a flexible $(Gly_4Ser)_2$ linker to generate a human enzyme immunoconjugate converting DOX-GA3 to fully active doxorubicin [109]. Similarly, other constructs with the C28 scFv were then

prepared later on, for example, a fusion protein with carboxylesterase-2 for conversion of the prodrug irinotecan (CPT-11) to its active form SN-38 [110], and an engineered EpCAM-binding adenoviral vector for gene-directed enzyme prodrug therapy [111]. All constructs converted CPT-11 to its active form and displayed antiproliferative effects on EpCAM-positive tumor cells *in vitro*. Despite these results, follow-up studies were not reported. While ADEPT is conceptually elegant, several challenges remain: many prodrugs only partially diminish systemic toxicity, the antibody–enzyme conjugate (like any targeting agent) only partially localizes to the tumor, the turnover rates for the artificial substrates (the prodrugs) are often not very high for the given enzyme, and all non-human enzymes are highly immunogenic.

6.3.3 Chemical immunotoxins and fusion toxins

Protein toxins constitute another class of cytotoxic agents which have been conjugated or fused to antibodies for cancer therapy. The most frequently used are diphtheria toxin (DT) and *Pseudomonas aeruginosa* exotoxin A (termed PE or ETA) that exert their destructive effect inside the cell by shutting down protein synthesis. A significant downside of bacterial toxins, however, is their high immunogenicity in humans. Attempts to reduce the formation of neutralizing antibodies by surface-masking with PEG or deletion of putative T-cell and B-cell epitopes have met with limited success [112-114]. PE/ETA immunotoxins have been used in numerous preclinical and clinical studies and are reviewed elsewhere [114,115]. A major feature of this toxin is the intrinsic ability of domain II to release and translocate the enzymatically active domain III into the cytosol, and the modularity of the protein, allowing the easy exchange of the targeting unit.

In early studies, full length PE/ETA and a truncated form lacking the cell-binding domain I ($ETA_{252-613}$) were conjugated to the anti-EpCAM antibody MOC31 via a stable thioether linkage [116,117]. In both toxin constructs, the native ER retention sequence at the C terminus was replaced by a KDEL motif for enhanced activity in mammalian cells. Selective cytotoxic effects were reported using various carcinoma cell lines *in vitro*, including drug-resistant variants. In tumor xenograft models *in vivo*, full length MOC31-PE prevented the development of metastases from a breast cancer cell line and MOC31-$ETA_{252-613}$ demonstrated strong antitumor effects on small xenografts, whereas its effect on the growth of larger tumors was limited. This was explained with the relatively large size of the immunotoxin (> 190 kDa), which likely could not sufficiently penetrate the tumor tissue [117]. Supporting evidence for this comes from a recent study where it was shown that on intravenous injection in mice bearing established HT29 tumor xenografts, the anti-EpCAM antibody MOC31 bound only to tumor cells in the vicinity of perfused blood vessels [118]. The authors found that the tumor blood vessels were largely immature and more prone for fluid flux out into the interstitial space. This consequently resulted in increased interstitial fluid pressure, which likely impeded intratumoral diffusion and distribution of the antibody. Later studies therefore focused on fully recombinant fusion toxins made with smaller anti-EpCAM scFv antibody fragments or DARPins as binding moiety to maintain a lower molecular weight (see below).

To generate a second-generation anti-EpCAM immunotoxin, the humanized scFv 4D5MOCB [72] was at its C terminus fused to truncated PE/ETA ($ETA_{252-608}$) [76]. Owing to its higher stability and improved tumor penetration, the fusion toxin 4D5MOCB-$ETA_{252-608}$ demonstrated strong antitumor activity in tumor xenograft models in mice on systemic administration. It was later licensed by Viventia Biotechnologies Inc. under the name VB4-845, and a codon-optimized variant was generated which yielded ~ 300 mg/L pure protein in bacterial cultures by fermentation [119]. Under the name Proxinium™ it has been investigated in clinical Phase II/III trials with intratumoral injection in patients with EpCAM-positive squamous cell carcinoma of the head and neck, and in a different formulation under the name Vicinium™ in patients with noninvasive urothelial carcinoma, where it is administered through the urethra (www.viventia.com).

The anti-EpCAM scFv 4D5MOCB [72] was also used as a basis to generate a Fab fragment for fusion to a T-cell epitope-depleted variant of the type I ribosome inactivating enzyme bouganin (debouganin) [120]. The immunotoxin VB6-845-C_H killed EpCAM-positive tumor cells at concentrations in the nanomolar range, which is, however, almost three orders of magnitude less potent than VB4-845, and also displayed antitumor effects on tumor xenografts in mice. Further safety studies in rats and monkeys revealed the reduced immunogenicity of debouganin and its good tolerability, which prompted initiation of an exploratory Phase I trial in patients with EpCAM-positive tumors [75].

The next step to obtain further improved third-generation fusion toxins for EpCAM-targeting was the use of DARPins as highly stable binding scaffolds to overcome the inherent practical limitations of antibody fragments in such fusion proteins. These limitations mostly includes poor expression yield and high aggregation tendency of the scFv–toxin fusions [72,121]. DARPins with affinities in the subnanomolar range were fused to $ETA_{252-608}$ (denoted as ETA") and the fusion toxin was expressed in soluble form at high yields up to 40 mg/L *E. coli* culture in shake flasks [32,122]. It could be shown that the disulfide bonds within the toxin spontaneously formed almost quantitatively. Most other fusion toxins with antibody scFv fragments must be expressed in inclusion bodies and then refolded to achieve reasonable high yields.

Despite the relatively short circulation half-life of the fusion protein of only 11 min, strong antitumor effects were observed in preclinical tumor models *in vitro* and *in vivo*. The flexibility and modularity of the DARPin platform, which, for example, allows further regiospecific functionalization by bio-orthogonal conjugation [87], permit engineering for longer serum half-life of the fusion toxins and to design prodrug versions thereof.

Another type of fusion protein for specific tumor cell killing was generated by linking TRAIL to the human anti-EpCAM scFv C54 [123,124]. TRAIL is a type II transmembrane protein expressed on immune effector cells. It triggers apoptosis in target cells by engagement of the TRAIL receptor, a member of the TNF receptor family, which recruits the intracellular Fas-associated death domain and activates the caspase cascade. Soluble TRAIL (sTRAIL) released from the cell membrane of effector cells does not induce apoptosis because crosslinking of the receptor is required. The scFv54:sTRAIL fusion protein binds to EpCAM on tumor cells and is thought to mimic the membrane-bound form by its deposition on the cell surface. The construct showed a bystander effect on EpCAM-negative tumor cells, did not induce hepatotoxicity and its fully human nature minimized immunogenicity [125,126]. On the other hand, the sensitivity of solid tumors to TRAIL is often low [127] and premature binding to blood cells cells upon intravenous administration may impede tumor targeting and lead to dose-limiting toxicity.

6.3.4 Fusion proteins with onconase

Onconase is a cytotoxic ribonuclease derived from oocytes of the northern leopard frog (*Rana pipiens*) [128]. It is closely related to human RNase A but has a distribution of positive surface charges allowing it to interact with anionic glycans [129], and it is not a target of the potent cytosolic ribonuclease inhibitor protein [130]. It inhibits protein synthesis in glioma cells with an IC_{50} in the mid-micromolar range [131]. Onconase is endocytosed after adsorption to cells – without cell-type specificity – and escapes, at least to some extend, into the cytoplasm to achieve RNA degradation [132]. In free form, onconase failed to demonstrate efficacy in a clinical Phase III trial, probably, among other reasons, due to its small size of 12 kDa and thus rapid renal clearance [133]. To increase specific uptake by tumor cells, it was therefore conjugated to a fusion protein consisting of the anti-EpCAM scFv 4D5MOCB [72] and human serum albumin, both proteins being made by artificial genes, by gene fusion or using a cleavable disulfide linker [78]. On EpCAM binding on HT29 colon cancer cells and rapid internalization, only the cleavable linker enabled sufficient endosomal escape and cytoplasmic translocation of the RNase to achieve significant cell killing. Despite the known stability problems of sterically non-hindered disulfides in the circulation, this conjugate displayed a measurable but marginal growth inhibitory effect on very small (< 20 mm^3) HT29 xenografts in mice. Besides other inconsistencies in the experimental part of the study it remains unclear why human serum albumin and not the mouse homolog was used for testing the conjugates in a mouse model, as this difference may significantly affect pharmacology and efficacy [134].

6.3.5 Immunocytokines

Immunocytokines act as modulators for enhancing tumor-specific cellular immune responses against tumor cells and are intended to be used to overcome the immunosuppressive conditions often found in patients with advanced malignancies and after cytotoxic therapy. As a potent activator of NK cells, interleukin-2 (IL-2) was extensively investigated for cancer therapy by systemic administration, but mainly with disappointing results, due to dose-limiting adverse effects and lack of efficacy [2]. To avoid side effects associated with high-dose IL-2 and to specifically stimulate the immune response at the tumor site and improve ADCC of Fc-optimized IgGs, IL-2 can be conjugated to antibodies to generate immunocytokines. In one study, IL-2 was fused to the humanized anti-EpCAM antibody huKS and evaluated *ex vivo* on ovarian carcinoma cells [135]. The presence of KS-IL2 in tumor cell cultures activated co-cultured effector cells isolated from patients and facilitated tumor cell lysis. A subsequent clinical Phase I trial in patients with androgen-independent prostate cancer demonstrated good tolerability at biologically active doses [136], and later preclinical investigations focused on combination treatment with radiofrequency ablation [137].

6.3.6 Immunoliposomes and nanoparticles

Antibodies have also been widely used for conjugation to liposomes and nanoparticles which can be loaded with high amounts of anticancer agents and have shown increased therapeutic indices, compared to the anti-cancer drugs themselves due to improved tumor targeting and controlled drug release [138]. On the other hand, safety issues, which have satisfactorily been clarified only for liposomal carriers and some biodegradable nanomaterials, and low cost-effectiveness resulting from laborious manufacturing have impeded their broader clinical application to date.

To generate immunoliposomes, the anti-EpCAM scFv 4D5MOCB [72] was directly linked to the PEG coat of liposomes loaded with the chemotherapeutic agent doxorubicin using thiol chemistry [73]. In mice bearing HT29 colon cancer xenografts, the sterically stabilized liposomes demonstrated favorable pharmacokinetic properties with a serum half-life of 11 h and high tumor localization (13% injected dose/g tissue). Growth of the tumor xenografts was delayed by almost 40 days with a 7.5 mg/kg dose. Interestingly, non-targeted control liposomes also displayed significant tumor localization and improved efficacy compared to free doxorubicin, which was likely due to passive targeting by the enhanced permeability and retention (EPR) effect [139]. Moreover, the EPR effect also resulted in almost equal antitumor activity compared to targeted liposomes, but only if the highest drug dose close to the MTD was used [73]. For molecules in the size range of liposomes, it was predicted that antigen targeting will not significantly increase tumor uptake relative to untargeted molecules [140]. Thus, targeting itself may be limited to facilitating cell-specific uptake by binding of the scFv to EpCAM, while the actual body distribution is dominated by the EPR effect [138,139].

Cationic sterically stabilized (PEGylated) liposomes surface-modified with 4D5MOCB [72] were also used for

tumor-targeted delivery of bispecific antisense oligonucleotides hybridizing to the bcl-2 and bcl-xL mRNA [74]. *In vitro* studies demonstrated efficient downregulation of target gene expression in EpCAM-positive cells and consequently facilitated apoptosis induced by doxorubicin. Larger DNA molecules, for example, plasmids coding for whole genes, can be targeted to tumor cells by encapsulation in surface-modified lipoplexes. In one study, the DNA coding for green fluorescent protein was combined with a cationic lipid (pyridinium amphiphiles, Synthetic Amphiphile INTeraction [SAINT]) coupled to the MOC31 antibody or its $F(ab')_2$ fragment, and the gene delivery system was tested for transfection efficiency on EpCAM-transfected mouse melanoma cells and on HT29 colon cancer cells [141]. Improved efficiency of the EpCAM-targeted lipoplexes, however, was only found with HT29 cells showing high EpCAM expression, emphasizing the necessity to further optimize this first-generation gene delivery system.

Alternative to encapsulation in liposomes or cationic lipids, anionic nucleic acids can be complexed with positively charged proteins like arginine-rich protamine. The ability of a protamine peptide fused to an antibody fragment to achieve cytoplasmic delivery of siRNA molecules into HIV-infected T cells was described [142]. To adopt this concept for tumor targeting, the human protamine-1 sequence was fused to an anti-EpCAM DARPin and nanocomplexes were generated by charge-directed payloading with siRNA designed to downregulate expression of the apoptosis inhibitor bcl-2 [143]. Four to five siRNA molecules could be attached to the fusion protein, and the complex was rapidly internalized in MCF-7 breast cancer cells by receptor-mediated endocytosis and inhibited bcl-2 expression. Transfection efficiency and proapoptotic effect correlated with the affinity of the binding moiety, as demonstrated with affinity-improved dimeric formats, which closely matched the potency of unspecific Lipofectamine™, a commonly used *in vitro* transfection reagent.

EpCAM-targeting nanoparticles were also prepared with the biodegradable polymer poly-lactic-*co*-glycolic acid (PLGA) and loaded with paclitaxel for treating EpCAM-positive retinoblastoma cells [48]. The drug was encapsulated using the oil-in-water single emulsion method resulting in a loading efficiency of 80%. The nanoparticles were then conjugated with a commercial anti-EpCAM full-length antibody using a random NHS/EDC-based conjugation strategy. The final product had an average diameter of 272 and 313 nm for non-targeted and targeted nanoparticles, respectively, and the drug was released in a time-dependent manner as a result of biodegradation of the PLGA carrier. In cell proliferation assays, the EpCAM-targeting nanoparticles demonstrated higher specificity for the retinoblastoma cells compared to non-targeted particles and free drug after longer incubation times. However, compared to non-targeted particles, cellular uptake of the targeted counterparts was only twofold higher and only slightly above the nonspecific uptake in EpCAM-negative control cells. This moderate internalization might result from steric hindrance of EpCAM binding by the relatively large particles or decreased binding capacity of the antibody as a result of random conjugation. Detailed analytical examinations and *in vivo* studies were not reported.

6.3.7 *Viral vectors for gene delivery*

The favorable expression profile of EpCAM in epithelial tumors, its rapid internalization on ligand binding and the promising results with antibodies and ADCs have also stimulated the development of viral vectors for EpCAM-targeted gene delivery. A retargeting strategy to render an adenovirus specific for EpCAM was reported for the first time in 1999 [144]. The concept was based on a bispecific antibody adapter which binds with one arm to the fiber-knob protein of the virus and competes with binding to the coxsackievirus and adenovirus receptor (CAR) and with the other arm to EpCAM for retargeting the viral vector to tumor cells. As shown in a luciferase reporter assay, the gene transduction efficiency of the engineered adenovirus correlated with EpCAM expression levels on tumor cells and was fourfold higher compared to the native virus in cells expressing very high levels of EpCAM. In very similar follow-up studies, the same group reported on several other adenoviral constructs ablated for native tropism and engineered for EpCAM binding [77]. Beyond these early proof-of-concept studies in *in vitro* models, more disease-relevant *in vivo* data have yet not been reported and safety concerns still represent the main obstacle for the clinical development of viral vectors. It should be pointed out that in the meantime the adapter strategy to retarget adenovirus has been significantly improved by using extremely tight binding, trimeric anti-knob binding DARPins, leading to a much higher increase in transduction efficiency [145].

As an alternative strategy, an IgG Fc-binding motif from *Staphylococcus* protein A (Z33) was introduced in an adenoviral vector as an adapter by fusion to the fiber protein, and it did not inhibit the inherent trimer formation of the native fiber-knob [146]. The protein A-containing adenovirus Ad-FZ33 was equipped with an anti-EpCAM antibody by binding to its Fc part and demonstrated significant antigen-specific transduction of tumor cells. The vector was used to deliver the uracil phosphoribosyl transferase (*UPRT*) gene into cells to enhance the toxicity of 5-fluoruracil (5-FU) by a factor of five to ten. At least this study reported positive *in vivo* efficacy data by combined treatment with Ad-FZ33 and 5-FU in tumor xenograft models [146]. Yet, the exchange of the bound antibody by any serum IgG molecule during *in vivo* applications might be a cause for concern.

In conclusion, although the reported findings on EpCAM-targeted viral gene delivery are preliminary, adenovirus retargeting may still be further engineered, and it will be crucial to see future *in vivo* data in this field.

7. Conclusions

EpCAM has been extensively investigated as target for the safe and effective delivery of anticancer agents to epithelial

malignancies. It is attractive for both its overexpression in solid tumors as well as its expression on CSCs and circulating tumor cells. Almost every possible targeting system and effector or drug conjugation concept has been tested on EpCAM, a summary of selected binding molecules and drug conjugates is given in **Table 1**. These were based on either optimized antibody formats or other binding scaffolds. Clinically most advanced is the phage display-derived human IgG adecatumumab, which was well tolerated and showed considerable efficacy in early clinical trials in patients with prostate or breast cancer. The rat–mouse bispecific anti-EpCAM/anti-CD3 IgG catumaxomab (termed 'trifunctional', since the authors also count the Fc part) should also be mentioned here, as it received European market approval, as an intraperitoneal infusion, for the treatment of patients with malignant ascites.

On the other hand, most of the EpCAM-targeting drug conjugates are still in preclinical development. One exception is the fusion toxin VB4-845, which is in clinical Phase II trials for treating patients with noninvasive urothelial carcinoma *in situ*. Nonetheless, despite remarkable progress in engineering antibodies and alternative anti-EpCAM binding molecules, much remains to be done on the effector and linker site of drug conjugates to translate technical advancement and intelligent drug design into clinical efficacy. Patients with EpCAM-positive metastatic disease and high CSC load will likely derive the largest benefit from these developments.

8. Expert opinion

EpCAM is abundantly expressed in epithelial tumors, whereas its expression in normal epithelia is limited. In tumors of various histotypes high EpCAM expression is associated with poor prognosis and is an independent marker for reduced patient survival. The recent finding of EpCAM on CSCs and circulating tumor cells with a high drug-resistant and metastatic potential has considerably increased its importance for tumor targeting. Moreover, recent insights into its role as an oncogenic signal transducer suggest antiproliferative effects by targeted molecular intervention with selected binding molecules, albeit the use of armed antibodies and more complex ADCs remains imperative to achieve substantial therapeutic benefit.

Since EpCAM is efficiently internalized on ligand binding by receptor-mediated endocytosis, it is particularly well suited for intracellular drug delivery, even though the transfer across the endosomal membrane is an independent problem. This awareness has motivated scientists from various biomedical disciplines to develop almost all possible antibody–drug combinations which can be expected to show any kind of antitumor activity predictable from its biological function. The result was an enormous armamentarium of EpCAM-targeting ADC candidates of which only few survived rigorous preclinical testing.

Recent advances in antibody engineering have provided a number of functionally optimized anti-EpCAM antibodies and ADCs. Improved antibody formats, such as adecatumumab, could indeed meet with clinical success, and novel non-IgG binding scaffolds, such as aptamers and DARPins, designed to be more stable and easier to engineer for tumor targeting and drug delivery, for example, using bioorthogonal click chemistry, have become available. Such progress will definitely expand our arsenal of tumor-destructive missiles. Together with improved drug formulations and linker constructs capable of releasing the payload on demand under defined conditions in the tumor microenvironment to achieve maximum destruction, this provides a platform of new EpCAM-targeting drug generations, which are better tolerated and equipped with higher potency.

Despite reasonable enthusiasm and good perspectives, so far only the mouse–rat bispecific antibody catumaxomab, but none of the investigative anti-EpCAM ADCs has been awarded with approval. History repeatedly unveiled that cross-reactivity of the target-binding domain with normal tissues or unfavorable linker design, resulting in premature drug release, may reduce the therapeutic index due to dose-limiting side effects. This is particularly evident for ADCs with high potency and cell-binding capacity. Future studies are warranted to better tackle this dilemma by more contemporary drug design and well-engineered potency-to-specificity ratios. The prodrug concept is one example how this could be realized.

In summary, EpCAM has become an established target for safe and effective drug delivery to solid tumors. Among the myriad of EpCAM-targeting antibodies and ADCs investigated so far, several could demonstrate potential therapeutic benefit and other formulations engineered to tailor-made powerful missiles are on the brink.

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Declaration of interest

AP is a cofounder and shareholder of Molecular Partners AG, commercializing the DARPin technology. AP and UZW are co-inventors on a patent underlying VB4-845, which is developed by Viventia Biotechnologies, Inc. The other authors declare no conflict of interest.

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