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REVIEW ARTICLE

EpCAM-targeting cancer immunotherapies: Evidence from clinical studies and the way forward

Dennis Makafui Dogbey[1], Ursula-Claire Andong-Koung-Edzidzi[1], Gomolemo Atlegang Molope[1], Jesmika Singh[1], Tatenda Lovemore Bvudzijena[1], Krupa Naran[1], and Stefan Barth[1,2*]

[1]Medical Biotechnology and Immunotherapy Research Unit, Institute of Infectious Diseases and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

[2]Department of Integrative Biomedical Sciences, South African Research Chair in Cancer Biotechnology, Division of Chemical and Systems Biology, University of Cape Town, Cape Town, South Africa

Abstract

Overexpressed cell-surface receptors play a crucial role as biomarkers in immunodiagnostics and immunotherapy. Epithelial cell adhesion molecule (EpCAM) is a 40 kDa cell-membrane glycoprotein associated with cell differentiation, survival, migration, and proliferation. The structure, biological functions, and role of EpCAM in tumorigenesis have been extensively studied, including its involvement in metastasis, tumor cellular signaling, and pro-proliferating pathways. It is differentially overexpressed on the surface of several tumor types, with substantial evidence suggesting a positive correlation with poor disease prognosis and overall survival. Consequently, it has emerged as a significant target for immunotherapy development, with some of them already entering clinical evaluation and one having been approved by the U.S. Food and Drug Administration to date. Pre-clinical and clinical studies evaluating anti-EpCAM immunotherapies have reported diverse outcomes necessitating critical assessment in considering future therapy development. EpCAM-targeting immunotherapies including monoclonal antibodies, adoptive cell transfer therapy, immunocytokines, recombinant immunotoxin, and bispecific T-cell engagers have been studied as monotherapy or in combination with other treatments resulting in improved outcomes. Depending on disease status, EpCAM-targeting therapies are studied in adjuvant and neoadjuvant settings by integrating the clinical features of patients and treatment intent. However, the wide range of adverse events reported in various unsuccessful clinical studies has implications for future clinical trial designs, patient stratification, and endpoint criteria measures. This review examines the efficacy and toxicity profiles of anti-EpCAM immunotherapeutic approaches that have undergone clinical investigations for the treatment of antigen-positive malignancies and provides perspectives to guide future research and development strategies toward precision medicine.

Keywords: Epithelial cell adhesion molecule; Immunotherapy; Clinical research; Solid tumors; Clinical trial

***Corresponding author:**
Stefan Barth
(stefan.barth@uct.ac.za)

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1. Introduction

Tumor cell-surface receptors (CSR) play a vital role as overexpressed biomarkers and are regarded as pivotal targets for cancer immuno-diagnosis and -therapy. A significant example is the epithelial cell adhesion molecule (EpCAM) or CD326. Interestingly, the structural similarities between the human EpCAM antigen and those found in mice or other surface markers in the GA733 family present challenges in developing fully humanized, high-affinity targeting agents against this antigen.[1] Numerous clinical studies on anti-EpCAM immunotherapeutics have yielded inconclusive outcomes, creating uncertainties for future therapy developments aimed at targeting this differentially overexpressed CSR.

1.1. Brief biology and function of EpCAM

EpCAM is a cell surface type 1 transmembrane glycoprotein belonging to the unique GA733 protein family. It is encoded by the *TACSTD1* gene and functionally mediates cell-to-cell adhesion, resulting in intracellular dimerization cascades of major pro-proliferative signaling pathways.[2,3] EpCAM is expressed on epithelial cells and during the embryonic states in vertebrates, where it is located at tight junctions. In addition, it is responsible for the differentiation and survival of parietal trophoblast giant cells, the normal development of the placental labyrinth, and the establishment of competent maternal-fetal circulation *in vivo*. Gene-knockout mice, which died *in utero*, were significantly small-for-age and displayed extensive placental abnormalities, highlighting its involvement in the embryogenesis of vital organs and body structures, such as ear, upper and lower extremities, and abdominal organs of vertebrates, signifying the cardinal role of the molecule in the physiological processes of fetal development.[4] Structurally, EpCAM (38 kDa) consists of an extracellular cell surface-exposed part (EpEX, 27 kDa), transmembrane and intracellular components. The intracellular domain, known as the EpIC (3 kDa), is involved in ligand-dependent dimerization. The polypeptide chains of the EpEX are structurally arranged into three distinctive domains: N-domains, thyroglobulin type 1A, and C-domains, known for mediating signal transductions.[5] EpCAM is cleaved mainly at residues Arg80 – Arg81 by proteolytic substrates.[6] However, detailed structural analysis showed that its exposed basolateral positions are covered with glycan side chains, which are involved in N-linked glycosylation at three sites and harbor epitopes that serve as the binding pockets for an affinity ligand.[7] Recent functional studies have implicated it as a crucial mediator of immune homeostasis of the intestinal tract by upregulating the polymeric immunoglobulin receptor within the intestinal epithelium.[8] In addition, there is a positive correlation between EpCAM co-expression and pro-neovascularization growth factors.[9]

2. Approved and clinical studies of anti-EpCAM immunotherapies

Almost all types of EpCAM-targeting immunotherapies have demonstrated remarkable pre-clinical efficacy, and a significant number of these treatments have transitioned to clinical trials. Figure 1 shows the mechanism of action of common EpCAM-specific antibody-based immunotherapies that have entered clinical evaluation. Regarding armed recombinant antibodies, the toxic (cytolytic) domain fused to the EpCAM-targeting ligands can be derived from bacterial, human, or plant origin. The selection of these sources depends on various factors, including immunogenicity, potency, and host of expression. Upon release through endosomal escape, the effector domains cleave their specific substrates, resulting in the intracellular induction of cell death.

3. Monoclonal antibodies

This type of monoclonal antibody induces cancer cell death by direct immune-related toxicity and disruptions of pathophysiological pathways of malignant cells resulting in antibody-dependent cellular cytotoxicity. Thus, the EpCAM-specific antibody (IgG1) interacts with the

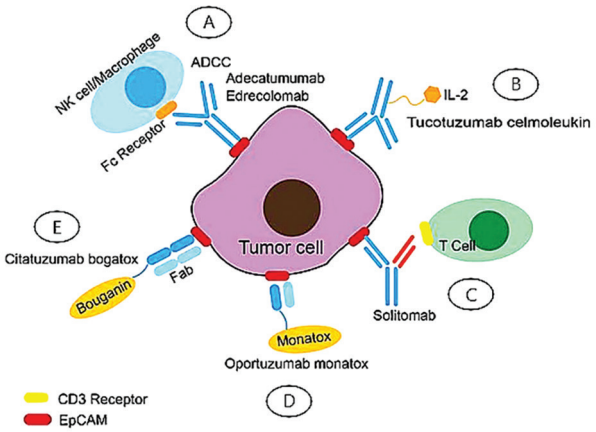


Figure 1. Mechanism of action of selected EpCAM-specific antibody-based immunotherapies. The anti-EpCAM immunotherapy types consist of a full antibody or antibody fragment at one end that is specific to the EpCAM antigen on cancer cells (red) and binds through the Fc receptor and recruits immune effector cells (A), immunomodulatory cytokine (B), secondary antibody fragment that binds to T cells through CD3 receptor (C), bacterial (D) or plant toxin as the cytolytic domain (E). Adapted from Creative Biolabs available at https://www.creativebiolabs.net/adecatumumab-overview.htm.

Abbreviations: ADCC: Antibody-dependent cellular cytotoxicity; EpCAM: Epithelial cell adhesion molecule; IL: Interleukin; NK cell: Natural killer cell.

antigen, leading to the disruption of the EpCAM-signaling pathways and the recruitment of immune effector cells to close proximity to tumor cells. Over the years, there have been numerous clinical trials using antibodies targeting EpCAM; some trials have shown excellent results and were approved, whereas others require further improvements to achieve success.

One of the very first clinical studies involved an EpCAM-specific monoclonal antibody called 17-A or edrecolomab to treat patients with colorectal cancer in Dukes' stage C (stage III), who had undergone curative surgery without residual disease.[10] While this mouse-derived monoclonal antibody extended survival and delayed disease remission with adjuvant treatment, it also caused several toxicities in patients. To enhance its effectiveness, combination therapy including edrecolomab with established anticancer treatment options was explored in a multicenter randomized trial, involving 27 countries, led by Punt *et al.*[11] The study included 2761 stage III colon cancer patients administered with edrecolomab as neoadjuvant therapy in combination with fluorouracil and folinic acid. Patients were randomly assigned to receive combination therapy (n = 912), chemotherapy plus folinic acid (n = 927), or edrecolomab alone (n = 922). Besides aiming for successful treatment, the study also assessed the efficacy of combined treatments on overall survival compared to other options. In addition, it compared edrecolomab's effectiveness alone versus chemotherapy on disease-free survival. With a median follow-up of 26 months, the study found no significant difference in 3-year overall survival between patients receiving combination therapy (74.7%) and those on chemotherapy plus folinic acid (76.1%). However, disease-free survival was notably lower for patients on edrecolomab alone compared to those on chemotherapy (53.0% vs. 65.5%). Due to hypersensitivity reactions experienced by some patients (25%), 4% chose to discontinue treatment. The combination therapy did not worsen the adverse events compared to previous trials. Thus, edrecolomab monotherapy was associated with significantly shorter overall and disease-free survival compared to treatment with fluorouracil and folinic acid, indicating it as a less effective option.[11]

With the limited success of edrecolomab therapy, adecatumumab (MT201) – a human recombinant IgG1 monoclonal antibody – was approved for clinical trial studies. This antibody was known to bind with a low affinity to EpCAM compared to edrecolomab, so its pharmacokinetics (PK) requires to be closely assessed. In phase I trial evaluation of adecatumumab led by Oberneder *et al.*,[12] 20 patients were treated with two intravenous (IV) infusions on days 0 and 14 up to a maximum dose of 262 mg/m^2. This dose escalation study was observed to have induced an increase in serum level of tumor necrosis factor-alpha (TNFα).[12] In a subsequent open-label phase II clinical trial, 109 patients were categorized into two groups: EpCAM^{high} and EpCAM^{low} metastatic breast cancer. The patients were randomly subjected to either 6 mg/kg or 2 mg/kg monotherapy every 2 weeks. Notable findings after study completion included reduced tumor progression in EpCAM^{high} subset of patients. In addition, only three out of 18 patients with high expression levels of EpCAM (14 out of 29) developed new metastases within 6 weeks post-treatment. Significant similar side effects that were observed in both groups were primarily mild to moderate such as chills, nausea, diarrhea, and fatigue. Interestingly, this study found that adecatumumab reduced peripheral natural killer (NK) cells, increased serum levels of TNFα at the highest dose, and induced no detectable antidrug immune response.[12]

4. Antibody-drug conjugates (ADCs)

Information on EpCAM-specific ADCs under clinical evaluation is limited. However, at the 2023 World ADC Conference, CytomX Therapeutics announced the pre-clinical data for CX-2051, which is developed based on the Probody therapeutics technology consisting of topoisomerase-1 inhibitor payload and CX-801 (a conditionally activated interferon [IFN] alpha-2b). The adoption of probody technology is to deliver a proteolytically activated antibody prodrug and to localize the antitumor activities of the ADC to the tumor microenvironment.[13] These compelling pre-clinical data revealed a satisfactory therapeutic index of CX-2051 against many EpCAM-positive carcinomas, becoming the first publicly announced EpCAM-specific ADC (having received investigational new drug approval) transitioning to clinical evaluation. CX-2051 received U.S. Food and Drug Administration clearance to commence phase I dose-escalation clinical studies scheduled to begin in the first quarter of 2024 in patients with solid tumors including colorectal, melanoma, and head and neck squamous cell carcinomas.

Earlier in 2024, ClinicalTrials.gov (through the study identifier NCT06265688) announced the initiation of the clinical evaluation of CX-2051 as a monotherapy. The ongoing recruitment process, which began in March 2024, included the following eligibility criteria: adults (≥18 years) with advanced-stage solid malignancies as determined by Response evaluation criteria in solid tumours (RECIST) criteria, and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 – 1. The study aimed to evaluate the safety and tolerability profiles of the ADC within 44 months from the date of administration with an expected enrollment duration of 5 years.

5. Immunocytokines

Immunocytokines are antibody-cytokine recombinant fusion proteins, with the potential to selectively localize at tumor sites and to stimulate localized and systemic anticancer immunity.[14,15] To date, one anti-EpCAM immunocytokine has undergone clinical evaluation. Tucotuzumab celmoleukin-interleukin-2 (huKS-IL2) is an anti-EpCAM fusion protein consisting of a humanized antibody specific for EpCAM linked at its Fc end to two molecules of IL2.[16,17] A phase 1b clinical study assessed the safety and unraveled the maximum tolerated dose (MTD) of huKS-IL2 following a single low-dose of cyclophosphamide in patients with EpCAM-expressing advanced solid cancers.[17] The investigators evaluated the immunogenicity, antitumor, biological activity, and PK profiles of the fusion protein. The study recruited 22 patients aged >18 years, who had advanced or recurrent EpCAM-positive (25% or higher) solid tumors. The patients underwent up to six cycles of treatment. The treatment was 300 mg/m^2 cyclophosphamide on day 1 and then increasing doses of 0.5 – 4.0 mg/m^2 intravenous continuous infusion of huKS-IL2 over 4 h the following day and for 3 days within the 21-day cycle. The most common adverse reaction was transient lymphopenia (which is considered grade 3 and 4 adverse reactions),[18] whereas nausea, pyrexia, rash, chills, diarrhea, fatigue, headache, vomiting, hypophosphatemia, increased serum creatinine, and hypotension were the frequently observed minor adverse effects. In addition, 88.5% of patients had a transient immune response to huKS-IL2. The authors established that the MTD was 3.0 mg/m^2 for huKS-IL2 when combined with 300 mg/m^2 cyclophosphamide, with dose-limiting myelosuppression noted at higher dose concentrations. The dose-limiting toxicity was attributed to non-specific activities of the cyclophosphamide because the huKS-IL2 exposure was not dose-proportional but dose-dependent with negligible off-target accumulation. A transient immune response to huKS-IL2 was noted for most patients (mainly anti-idiotype or antilinker antibody induction) without an established association of these responses to the neutralizing effect of the immunocytokine. The best response was stable disease which lasted for more than four cycles in three out of 22 patients. The combination of huKS-IL2 and cyclophosphamide was well-tolerated without beneficial clinical endpoints. This study showed that there is a potential for huKS-IL2 as an immunotherapeutic in combination with low-dose cyclophosphamide. The combination of these drugs in patients with advanced tumors was deemed to be a feasible and safe treatment strategy.

6. Recombinant immunotoxins (rITs)

rITs are an emerging class of anticancer therapeutics involving the genetic fusion of antigen-specific antibodies or cytokines to plant or bacterial toxins.[19] As a subclass of anticancer protein therapeutics, rITs specifically target and cleave a designated substrate, ultimately inducing apoptosis. The development of EpCAM-targeting rITs has reached various degrees of clinical success. An example is opportuzumab monatox (VB4-845), which was evaluated in patients with non-muscle-invasive bladder cancer (NMIBC) refractory to or intolerant of Bacillus Calmette–Guerin (BCG). VB4-845 is a recombinant fusion protein that targets EpCAM-positive cancer cells. It is made up of an anti-EpCAM humanized single-chain variable fragment (scFv) linked to a truncated form of *Pseudomonas* exotoxin A (ETA252 – 608) that lacks the cell-binding domain.[20] Regarding bacterial-based rITs, the mode of action of VB4-845 is through antigen-specific binding and endocytosis into the cytosol, followed by the release of the toxin through escape from endosomal cleavage, leading to inhibition of EF1 protein synthesis and cell death.[21] A phase I dose-escalation clinical study was conducted to investigate the MTD of VB4-845. As a secondary aim of the clinical study, the authors investigated the immunogenicity, safety, efficacy, tolerability, and PKs profile of VB4-845.[22,23] The study recruited 64 participants (>18 years old), with early-stage transitional cell carcinoma or *in situ* carcinoma, grade 2/3, who were refractory and intolerant to BCG therapy. Other inclusion criteria of the study were patients with adequate renal, hepatic, and hematological function. Second, the recruitment of patients of childbearing age was expected to undergo contraceptives before and during the study. The rIT was administered intravesically for 6 consecutive weeks, in increasing weekly doses (from 0.1 to 30.16 mg) through an indwelling urinary catheter. The study participants were tracked for 4 – 6 weeks following treatment and assessed at the 12th week. After the study, 64% of patients had adverse reactions, with 33% facing pharyngolaryngeal pain, bladder spasm, urinary tract infection, incontinence, and nocturia. These reactions were believed to have resulted from concomitant medications. In addition, a third of patients experienced mild-to-moderate adverse reactions that were believed to be caused directly by rIT including dysuria and hematuria, fatigue, loss of appetite, fever and chills, myalgia, nausea, and dizziness. However, these reactions did not increase in frequency with dose. Following the last dose of VB4-845, one patient died from cardiac failure, and his demise was unrelated to the effects of the rIT. Overall, the adverse reactions were mild and adequately managed. The authors were unable to determine an MTD for VB4-845, and the apparent lack of relationship between the adverse reactions and the

dose indicated a satisfactory safety profile. VB4-845 was shown to not be systemically absorbed but remained in the bladder until elimination, probably due to the molecular size of the drug and cellular integrity of the bladder. In addition, VB4-845 was found to be specific to antigen-positive tumors. Although it induced an immunogenic response, this expected outcome was attributed to the origin of the antibody fragment and the bacterial toxin. The authors proposed that tumor response may be saturated at higher doses due to the dose range of administered VB4-845 exceeding the drug concentration required to kill more than 99% of cells in EpCAM-positive bladder tumor cell lines. Thus, VB4-845 can safely inhibit the growth of tumors in high-grade EpCAM-positive NMIBC patients in a dose-dependent manner.

Another rIT evaluated against EpCAM-positive cancers is MOC31PE consisting of the full murine MOC31 IgG1 monoclonal anti-EpCAM antibody covalently linked to the complete *Pseudomonas* exotoxin A (PE) was investigated in metastatic colorectal cancer patients in a phase I clinical study.[24] Because the main limitation of rITs is immunogenicity, MOC31PE was administered intravenously with and without the immunosuppressant cyclosporine. The aim was to minimize the generation of systemic antidrug immunogenic responses. The administration of MOC31 as monotherapy demonstrated improved overall survival (12.7 versus 6.2 months) in a cohort of colorectal cancer patients compared to combination therapy with immunosuppressant.[25] Second, another study investigated the safety and maximum tolerable doses in patients diagnosed with EpCAM-positive tumors in combination with immunosuppression.[24] The study included 63 patients divided into three groups: 34 received MOC31PE alone, 23 received MOC31PE in conjunction with IV cyclosporine, and the remaining six were administered MOC31PE and cyclosporine orally. Inclusion criteria include adults 18 years or older, ECOG performance status of 0 – 2, and evidence of at least 10% EpCAM-positive metastatic carcinoma. In addition, conventional treatments were discontinued 4 weeks before and during the study. MOC31PE was administered up to 4 times every 2 weeks, while cyclosporine was given 1 day before MOC31PE and continued for four days post-initial administration. At the end of the study, the authors observed that patients undergoing monotherapy had better therapeutic outcomes compared to those receiving combination therapy. Conceivably, the efficacy of the rIT was compromised by the coadministration of the immunosuppressant in the combination therapy group, as in previous studies.[25,26] indicated that MOC31PE can induce an unexplained antitumor immune response that can provide additional antitumor activities. These findings challenge the notion that existing neutralizing antibodies are a primary cause of immunogenicity to bacteria-based rITs in humans.[27] Furthermore, a clinical study of MOC31PE was conducted with the aim of establishing the efficacy of the rIT in the context of intraperitoneal administration in late-stage colorectal cancer patients with confirmed peritoneal metastasis.[28] The study was conducted to analyze the local and systemic cytokine response in these patient populations undergoing cytoreductive surgery and hyperthermic intraperitoneal chemotherapy with or without intraperitoneal treatment with MOC31PE. The rIT activated local and systemic cytokines, an added advantage that can potentiate its antitumor activities. The investigators enrolled 21 and 26 adult patients who were administered with or without MOC31PE, respectively. A tumor debulking cytoreductive surgery baseline treatment was performed on all patients before receiving the rIT as a single rapid intraperitoneal instillation through two abdominal drainage catheters. To assess cytokine responses, the authors analyzed 27 growth factors, chemokines, IFN, and pro- and anti-inflammatory interleukins in serum and intraperitoneal fluids. Higher levels of pro-inflammatory cytokines were observed in the MOC31PE group compared to patients who did not receive the rIT, with time-dependent variability. In addition, elevated levels of innate proinflammatory cytokines, TNF, and a time-response curve for the strong T-cell stimulator IFN and its associated chemokine IFNγ-induced protein/chemokine (C-X-C motif) ligand 10 (IP-10) were noted in the serum of patient who received the rITs. Inferentially, elevated pro-inflammatory cytokines are associated with the induction of immunogenic cell death and have the potential to overturn "cold" tumors into immunologically "hot."[29] The study concluded that the overall survival in approximately 78% of patients was 3 years, with 53% achieving an overall survival rate of up to 5 years. In contrast, an intensified local inflammatory response was not found to adversely affect long-term outcomes. The authors reported a compartmentalized inflammatory response in the peritoneal cavity that correlates with the localized response, which was further enhanced by the peritoneal infusion of MOC31PE.

7. Bi-specific T-cell engagers (BiTEs)

BiTEs are a relatively new class of monoclonal antibodies that drive T-cells to cancer cells by binding to T-cells specific antigen (usually CD3) and tumor-specific surface antigens simultaneously.[30] For EpCAM-specific BiTEs, one arm of the antibody binds to T-cells, whereas the others recognize and bind to EpCAM. Catumaxomab is a chimeric (rat-murine) bispecific and trifunctional antibody that combines the qualities of bispecific molecules and traditional

monoclonal antibodies. Specifically, the antibody binds to three different cell types: dendritic cells, macrophages, and NK cells – the immune system's accessory cells which are activated when the Fc region binds to type I, IIa, and III Fcγ receptors on these cells. In 2010, a pivotal clinical study led by Heiss *et al.*[31] was conducted to investigate the efficacy of catumaxomab in treating malignant ascites, a common occurrence in aggressive cancers. A randomized phase II/III clinical trial was conducted in patients with EpCAM-positive tumors.[31] In the trial, cancer patients (n = 258) with recurrent symptomatic malignant ascites resistant to conventional treatment were randomly assigned to receive either paracentesis abdominis plus catumaxomab (experimental group) or to undergo paracentesis alone (control group). The catumaxomab treatment involved intraperitoneal administration on days 0, 3, 7, and 10 at doses of 10, 20, 50, and 150 μg, respectively. The results demonstrated that patients in the experimental group had significantly increased puncture-free survival time following paracentesis compared to the control group, with durations of 46 days versus 11 days and 77 days versus 13 days, respectively. In addition, patients treated with catumaxomab exhibited fewer signs and symptoms of ascites compared to the control group, with an overall survival of 77 days versus 44 days. The reported side effects were generally reversible and primarily associated with the immunologic mode of action. Overall, catumaxomab demonstrated a clear therapeutic benefit with an acceptable safety profile in patients with malignant ascites caused by epithelial malignancies, particularly in gastric cancer. This finding contradicts the results of a similar study in which a subset of patients with malignant pleural effusion who were administered with catumaxomab developed severe dose-limiting toxicities, including fatal sepsis, grade 3 erythema, and hepatobiliary tract disorders.[32] This further raises concerns regarding patient selection and the mechanism of action of catumaxomab. In this context, successive evaluations of catumaxomab have concentrated on its use for palliative intents for patients with late-stage diseases characterized by significant tumor burden associated with malignant ascites.[33,34] Further evaluation of catumaxomab was conducted to compare intravenous to intraperitoneal routes of administration. This is based on the premise that there is a positive correlation in the increased accumulation of fluid in the peritoneal cavity of patients with EpCAM-positive tumors.[35]

A multicenter, prospective, randomized, open-label phase II study consisting of 31 patients (≥18 years), with 15 patients receiving catumaxomab and chemotherapy with 5-fluorouracil, leucovorin, oxaliplatin, and docetaxel (FLOT), and 16 patients receiving only FLOT, was conducted.[36] A full set of catumaxomab consisted of four doses, and 12 patients in the study received the full dose. All patients had a histologically confirmed diagnosis of gastric cancer, including metastatic esophagogastric junction cancer types II and III, macroscopic peritoneal carcinomatosis (stages P1–4 according to Gilly's classification), and an ECOG performance status of 0 or 1. Patients were also required to have a life expectancy of at least 12 weeks, be medically fit to undergo gastrectomy after primary systemic and intraperitoneal treatment and have adequate organ function. Regarding exclusion criteria, patients with clinically significant cardiovascular disease within the past year, a history of human immunodeficiency virus infection, chronic hepatitis B or C, active and clinically serious infection, pre-existing neuropathy higher than grade 1, or distant metastasis other than peritoneal carcinomatosis were deemed ineligible for enrolment. In this study design, catumaxomab was administered every 3 days over a 10-day cycle using a constant infusion pump system to control the delivery of the drug over a period of 3 h. Dosage escalation from 10 to 150 μg was initiated before the administration of catumaxomab and the intravenous injection of 1 g of paracetamol as prophylaxis for cytokine-release-associated symptoms. After 7 days and the final infusion of catumaxomab, six cycles of FLOT chemotherapy were administered. The regimen consisted of 5-fluorouracil (2600 mg/m² as a 24-h infusion on day 1), leucovorin (200 mg/m² on day 1), oxaliplatin (85 mg/m² on day 1), and docetaxel (50 mg/m² on day 1), delivered every 2 weeks. The median follow-up period for patients was 52 months. Observed side effects (grade 3 – 4) associated with catumaxomab included nausea, elevated levels of liver enzymes and bilirubin, and abdominal pain. The authors did not observe any significant differences in median progression-free survival and overall survival between the various treatment strategies. The addition of catumaxomab to chemotherapy was well tolerated and feasible for advanced gastric cancer. Although the study did not demonstrate an improvement in the macroscopic complete remission rate of peritoneal carcinomatosis, catumaxomab exhibited an acceptable safety profile, and the addition of chemotherapy did not result in unexpected adverse reactions.[38] Another anti-EpCAM BiTE, known as solitomab, MT110, or AMG 110, was developed by Amgen. In a recent clinical trial, the bispecific single-chain antibody (scFv) construct/bispecific T-cell-engaging solitomab demonstrated efficacy in patients with refractory solid tumors.[37,38] This multicenter phase I study enrolled 65 patients who received varying doses (ranging between 1 and 96 μg/day) of solitomab via continuous intravenous infusion over a period of approximately 28 days. Following treatment, 15 patients experienced dose-limiting toxicities, whereas eight patients exhibited transient abnormal

liver parameters shortly after infusion initiation or dose escalation (grade 3, $n = 4$; grade 4, $n = 4$). In addition, one patient experienced grade 3 supraventricular tachycardia resulting in treatment discontinuation in all affected patients. Furthermore, six patients experienced dose-limiting toxicity in the form of diarrhea. Among these six patients, four (grade 3, $n = 3$; grade 4, $n = 1$) experienced resolution, one (grade 3) still experienced symptoms at the time of treatment-unrelated death, and one (grade 3) progressed to grade 5 after cessation of therapy. The MTD was determined to be 24 μg/day. Diarrhea, elevated liver parameters, and high lipase levels were the most frequently observed grade ≥3 treatment-related adverse events, occurring in 95% of patients. However, solitomab exhibited a half-life of 4.5 h, and serum levels reached a plateau within 1 day. The treatment of relapsed/refractory EpCAM-positive solid tumors with solitomab, a BiTE® antibody construct targeting solid tumors, resulted in dose-limiting toxicities, including severe diarrhea and elevated liver enzymes, thereby impeding dose escalation to potential therapeutic levels. Despite achieving the key endpoints of assessing the frequency and severity of adverse events, as well as understanding its PKs and antitumor activity, further investigation and exploration are required.[37] Table 1 presents a general overview of antibody-based targeted immunotherapies, which have either received approval or are currently in various stages of clinical evaluation.

8. Adoptive cell therapy (ACT)

Clinical trials of ACT-based immunotherapy against EpCAM-positive cancers have expanded within the last 5 years based on promising pre-clinical data. For example, an anti-EpCAM nanobody Chimeric antigen receptor T-cells (CAR T) with dectin-1 costimulatory domain demonstrated higher T-cell activation and less exhaustive phenotypes in xenograph mouse models, a finding that led to the initiation of clinical evaluation.[39] In the study, the authors conducted a phase I clinical investigation by enrolling 12 patients who were diagnosed with EpCAM-positive epithelial tumors (NCT02915445). During the study, 50% of patients experienced grade 1/2 toxicities. A total of 384 enrolled patients with multiple advanced-stage cancer types have been administered with anti-EpCAM CAR T.[42] In addition, an anti-EpCAM (scFv) CAR T therapy, indicated for antigen-positive colon, esophagus, pancreas, and prostate tumors, concluded its Phase I/II recruiting 60 patients (NCT03013712) concluded in 2020 without further information on study outcome (https://clinicaltrials.gov/study/NCT03013712). In the study, a novel CAR consisting of an anti-EpCAM(scFv) fused to two intracellular co-stimulatory signaling domains derived from CD3-zeta and CD28 was administered at the infusion dose of $1 - 10 \times 10^6$ modified T cells/kg.[40] Table 2 provides a list of the ongoing and completed anti-EpCAM CAR T clinical studies, with the majority still in early phases. Out of the 10 ongoing clinical trials, five are clinical

Table 1. Selected anti-EpCAM antibody-based immunotherapies that have been approved or currently undergoing clinical evaluation

Product name/Clinical trial #	Status	Indications	Reported adverse effects
Catumaxomab	Approved	• EpCAM-positive tumors • Malignant Ascites	Fever, nausea, vomiting, abdominal pain, and elevated liver enzymes
Edrecolomab (17-1A)	Phase III	• Mucinous adenocarcinoma of the colon • Adenocarcinoma of colon • Stage IIA – IIC colon cancer	High immunogenicity, short serum half-life
Adecatumumab (MT201)	Phase II	• Metastatic breast cancer	Chills, nausea, fatigue, and diarrhea
M701	Phase I	• Malignant ascites • Advanced solid tumors	Grade 1 adverse drug events
EMD 273066	Phase I	• EpCAM-positive ovarian and colorectal carcinomas • Non-small-cell lung cancer • Prostate cancer	Unknown
ING-1	Phase I	• Advanced adenocarcinomas	Acute pancreatitis, asthenia
Citatuzumab bogatox	Phase I	• Advanced epithelial tumors	Fever, hypotension, and hypoalbuminemia
Opportuzumab monatox	Phase I and II	• Squamous cell carcinoma of the head and neck • Non-muscle invasive bladder cancer • *In situ* urothelial carcinoma of the bladder	Pain at the site of injection, mild-to-moderate adverse effects
MOC31PE	Phase I	• Advanced adenocarcinomas	Reversible liver toxicity
Solitomab (MT110)	Phase 1	• Relapse/refractory tumors	Elevated hepatic enzymes, and diarrhea

Abbreviation: EpCAM: Epithelial cell adhesion molecule.

Table 2. Selected ongoing and clinically approved anti-EpCAM ACTs clinical trials (Clinicaltrial.gov)

Trial identifier	Phases	Year	Indications	No. of enrolments	Study location
NCT02729493	NA	2019	Hepatocellular carcinoma	25	China
NCT02725125	NA	2015 – 2019	Stomach neoplasms	19	China
NCT02915445	Phase I	2016 – 2023	Malignant neoplasms of nasopharynx, breast, and stomach cancer with metastasis	30	China
NCT03563326	Phase I	2018 – 2022	Advanced-stage gastric cancer with peritoneal metastasis	40	China
NCT03013712	Phase I and II	2017 – 2020	Multiple cancer types	60	China
NCT04151186	NA	2019 – 2021	Advanced solid tumor	72	China
NCT05703815	NA	2023 – ongoing	Invasive breast cancer with EpCAM and p53 expressions	40	Egypt
NCT05576519	NA	2017 – 2022	Ovarian carcinoma	50	Egypt
NCT05028933	Phase I	2021 – 2024	Advanced-stage hepatocellular carcinoma, as well as colorectal, gastric and pancreatic cancers	48	China

Abbreviations: ACT: Adoptive cell therapy; EpCAM: Epithelial cell adhesion molecule.

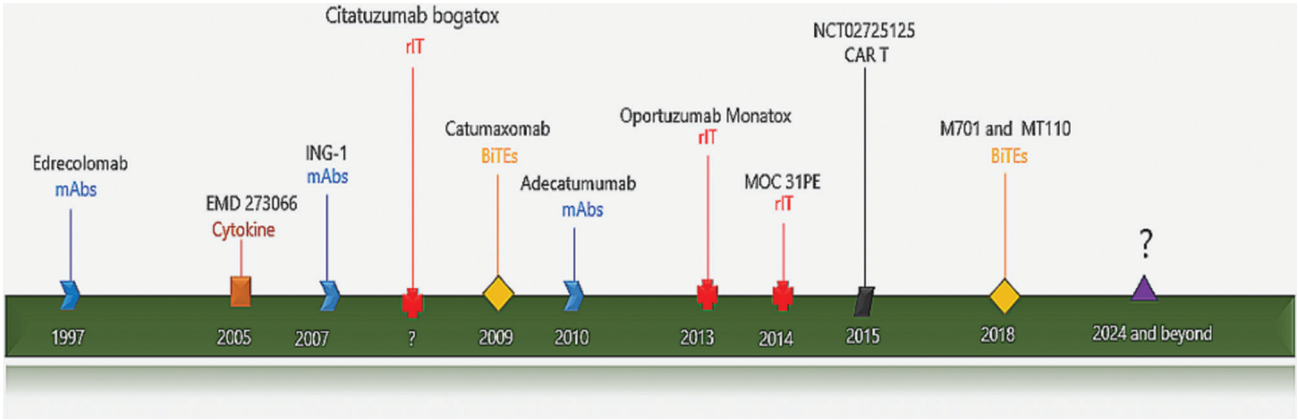


Figure 2. Timelines for the initial clinical evaluation of selected anti-EpCAM immunotherapies. The schematic is derived from pooled study evidence pertaining to the initial year in which the clinical studies were conducted (https://clinicaltrials.gov).
Abbreviation: CAR T: Chimeric antigen receptor T-cells.

observational studies. Taken together, it appears that CAR T therapy targeting EpCAM-overexpressing cancers is primarily indicated for advanced-stage solid malignancies.

The anti-EpCAM therapy landscape has expanded to cover almost all aspects of cancer immunotherapy. Figure 2 represents the timelines for the initial clinical evaluation of selected anti-EpCAM immunotherapies (https://clinicaltrials.gov). The estimated year of the evaluation was based on the reported year of publication of the first clinical study results.

9. Concluding remarks and perspectives

Precision medicine seeks to provide customized solutions for disease management by stratifying patients into subpopulations based on disease-specific profiles,[41] allowing for the identification of patients with the highest probabilities of responding positively to targeted therapies. However, tumor classification based on routine diagnostic assays undermines the precision medicine paradigm due to its limitations: (i) variability in therapeutic response and clinical outcomes, even among tumors with similar clinicopathological features; and (ii) neglect of the biological significance and molecular pathways that differentiate tumors into distinct subtypes and stages.

In breast cancer, for example, variability in tumor responses and drug-induced adverse events is associated with the transcriptional profiles of CSRs in breast tumors and normal tissues. Clinical trials designed to evaluate the efficacy of CSR-targeting treatments often fail due to dose-limiting toxicity and other undesirable side effects.[42,43] The toxicity and side effects of CSR-targeting drugs are at least partially attributable to the expression of the targeted CSR in healthy tissues.[44,45] Therefore,

treatment success rates could be improved, and associated off-target toxicity limited, by targeting CSRs that are both overexpressed in cancer cells and under-expressed in proximal and distal healthy tissues. In light of this, statistical methods, machine learning, and predictive modeling have been applied to determine which CSRs in breast cancer subtypes, if targeted by therapeutics, would have the lowest likelihood of off-target toxicity.[46] However, the varied outcomes in pre-/clinical settings when targeting EpCAM can be attributed to the substantial heterogeneity of EpCAM expression, both within tumor subtypes and between diseased and healthy tissues suggesting that EpCAM may not be an "easy" therapeutic target.[42,43] Although monoclonal antibody-based immunotherapy was the first to undergo clinical testing against EpCAM-positive cancers, an increasing focus on armed antibody-based targeted therapies, specifically rIT, has been subjected to clinical evaluation in recent decades. This trend may be attributed to the marginal successes achieved by relying on monoclonal antibodies as monotherapy. Researchers have recognized the importance of actively inducing cytotoxicity in EpCAM-positive tumor cells by arming antibodies with bacterial or plant-derived toxins. Nevertheless, adverse effects associated with earlier generations of rITs have primarily emerged from dose-limiting antidrug immune responses and vascular leak syndromes, which have led to severe clinical complications.[47,48] Furthermore, immunotherapy strategies such as BiTEs, cytokines, ACTs targeting EpCAM overexpression, and recruitment of cytotoxic T cells to tumor sites have made significant strides in clinical studies, despite some minor limitations. For instance, the favorable clinical outcomes of anti-EpCAM BiTEs were only observed in combination with chemotherapy. This is similar to the limited clinical trial reports on ACTs (which mainly rely on T-cell mediated cytotoxicity) and therefore may require active immunotherapy or chemoradiation combination for improved efficacy against EpCAM-positive solid cancers.[49] Because clinical endpoints of early-phase clinical testing are measured with the toxicity and efficacy profiles, there is a need to strike a balance in designing future EpCAM-specific clinical trials. In this regard, EpCAM-specific immunotherapy may be enhanced by adopting a more recent innovative approach such as the delivery of therapeutic payloads by envelope-targeted vectors.[50]

Furthermore, EpCAM overexpression mediates the development of resistance to several anticancer therapies in different tumor types.[51-53] This induced tumor plasticity post-therapy observed in pre-clinical models partly contributes to the challenges affecting the smooth translation of anti-EpCAM therapies into clinical practice. This concept may be considered in future therapy development and clinical trial designs, including patient selection and measurement of baseline characteristics. In addition, the development of new therapies may require an improved understanding of the biology and signaling pathways associated with EpCAM such that their selective efficacy is dependent on the affinity of the receptor-targeting domain, which drives the toxic domains to the target in the case of armed antibody therapies. Importantly, the high specificity of the EpCAM-targeting ligands can contribute to limiting off-target toxicities and interference with signaling pathways involving similar surface receptors through crosstalk signaling. However, it is important to strike the balance between the binding affinity of anti-EpCAM ligands and on- and off-target toxicities. In this context, our group and others have demonstrated the potential of recombinant antibodies fused to the N-terminus of bacteria and human-based toxins for the generation of rITs and cytolytic fusion proteins targeting multiple cancers.[54,55] In addition to these advances, other immunotherapeutics such as CAR T, cytokines, therapeutic nucleic acids, and oncolytic viruses for the therapy of EpCAM-positive cancers are under development, harboring the potential to transition into clinical evaluations.[55-58] Furthermore, arming EpCAM-targeting antibodies with humanized toxin moieties is a promising approach for reducing antitoxin immune responses. In this regard, supercomputing and protein engineering techniques aimed at identifying and ablating reactive epitopes while retaining the cytolytic properties of the attached toxin remain the gold standard that needs to be explored.[59] These approaches, together with the development of less immunogenic and humanized toxin domains and the activation of antitumor immune effectors, are the desired anti-EpCAM-targeted therapeutic strategies awaiting future applications. Finally, high-throughput analysis of spatiotemporal distribution, identification, and capitalization on significant tumor vulnerabilities, as well as the density of EpCAM overexpression in heterogeneous tumor lesions remain important factors to be explored.[60-62] Taken together, these suggestions may guide the design of future clinical trials allowing for the potential approval of EpCAM-specific immunotherapies.

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Conflict of interest

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Author contributions

Conceptualization: Dennis Makafui Dogbey, Stefan Barth
Writing–original draft: Dennis Makafui Dogbey, Ursula-Claire Andong-Koung-Edzidzi, Gomolemo Atlegang Molope, Jesmika Singh, Tatenda Lovemore Bvudzijena
Writing–review & editing: Krupa Naran, Stefan Barth

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