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Warming-up the immune cell engagers (ICEs) era in breast cancer: state of the art and future directions

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ABSTRACT

The advent of immune checkpoint inhibitors (ICIs) has deeply reshaped the therapeutic algorithm of triple-negative breast cancer (TNBC). However, there is considerable scope for better engagement of the immune system in other BC subtypes. ICIs have paved the way for investigations into emerging immunotherapeutic strategies, such as immune cell engagers (ICEs) that work by promoting efficient tumor cell killing through the redirection of immune system against cancer cells. Most ICEs are bispecific antibodies that simultaneously recognize and bind to both cancer and immune cells generating an artificial synapse. Major side effects are cytokine release syndrome, hepatotoxicity, and neurotoxicity related to inappropriate immune system activation. Here, we provide a comprehensive overview of this compounds, the available preclinical and clinical evidence supporting their investigation and development in BC also highlighting the challenges that have prevented their widespread use in oncology. Finally, major strategies are explored to broaden their use in BC.

1. Introduction

Immunotherapy was a major breakthrough in the treatment of several tumour types (Sun et al., 2023). However, its role in breast cancer (BC) is not uniform because it depends on the tumour subtype and its immunogenicity (Dieci et al., 2016). Indeed, the interaction between tumour cells and the immune milieu plays a significant role in the tumorigenesis of triple negative (TN) and human epidermal growth factor receptor-2 -positive (HER2+) BC subtypes, whereas hormone-receptor positive (HR+) BC progression seems to marginally rely on the tumour-immune system interaction (Aghapour et al., 2024; Dieci et al., 2016). As such, evidence about immune biomarkers in BC are heterogeneous. Several data highlighted the prognostic value of tumour-infiltrating lymphocytes (TILs) in both TN and HER2+ BC patients (Denkert et al., 2018; Loi et al., 2019a; Luen et al., 2017), while no consistent data on a relevant clinical role of TILs, and other immune biomarkers, in HR+ BC have been published to date (Dieci et al., 2016).

To date, immune checkpoint inhibitors (ICIs), including the programmed death-1 (PD-1) inhibitor pembrolizumab and the programmed death–ligand 1 inhibitor (PD-L1) atezolizumab have significantly transformed the therapeutic algorithms for TNBC in both early (Schmid et al., 2020) and advanced stages (Cortes et al., 2022; Emens et al.,

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2021) and are currently the only immunotherapeutic agents approved for the treatment of this disease in these settings, whereas ICIs have been tested and are still under investigation in HER2+ (Emens et al., 2020; Loi et al., 2019b) and HR+ BC subtypes (Cardoso et al., 2023; Loi et al., 2023). Despite ICIs activity, primary and acquired resistance may hamper the efficacy of these drugs. Several resistance mechanisms have been described, including loss of function mutation in the major histocompatibility complex (MHC) components (Gettinger et al., 2017) and disruption of interferon-gamma signalling in cancer cells (Sucker et al., 2017).

Hence, while immunotherapy reshaped the treatment of TNBC, investigations are underway either to broaden the indication of these treatments to the other BC subtypes and to overcome resistance.

In this context, a new class of immunotherapeutic agents, immune cell engagers (ICEs), is gaining momentum in solid malignancies. Despite these molecules are still under clinical development, they might have a role in BC treatment, broadening the current indication and improving the efficacy of immunotherapy in this disease.

Here we will review the main pharmacological properties of ICEs, also focusing on their safety profile as well as on the potential strategies to implement their use in the practice. Lastly, we will explore the emerging role of ICEs in BC by reporting the available data about the compounds that are under investigation.

2. ICEs general structure and classification

Immune cell engagers are an emerging class of immunotherapeutic agents that enhance tumour cell killing by redirecting immune effector cells against cancer cells (Fenis et al., 2024; Fucà et al., 2021).

The capability to bind two or more epitopes simultaneously differentiates ICEs from other antibody-based therapies such as monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs) (Labrijn et al., 2019). Compared to mAbs, ICEs have several advantages, both in terms of tumour-cell selectivity and reduction of resistance which improves therapeutic efficacy (Mazor et al., 2015).

These drugs are classified based on their "functionality", i.e., the number of cells targeted simultaneously, and on their "specificity", i.e., the number of antigens or epitopes recognized.

Thus, bi-, tri- or even tetravalent ICEs can contact up to four different cells, while bi-, tri- or multispecific ICEs combine the binding site of two or more epitopes.

Most ICEs, are transbinding bispecific antibodies (bsAbs) that simultaneously recognize and contact both cancer cells and cells involved in immune response in order to create an immune cytolytic synapse, which ultimately leads to tumour cell death (Thiery et al., 2011). (Fig. 1)

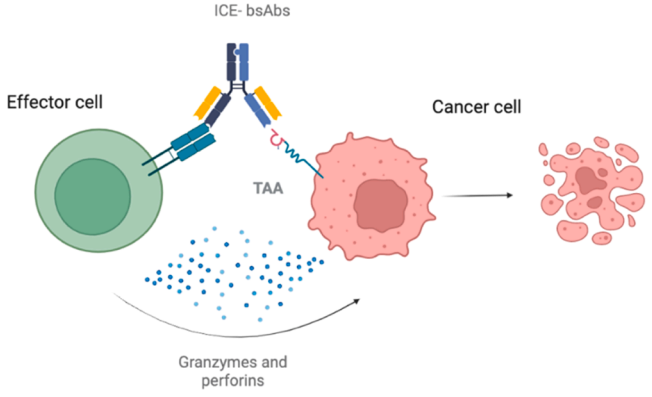

Fig. 1. Formation of the artificial immune cytolytic synapse between effector T cells and cancer cells via immune cell engagers. After the engagement between the immune effector cell and the cancer cell, granzymes and perforins released by T cells lead to cancer cell death. Abbreviations: TAA: tumour-associated antigens; ICE: immune cell engagers; bsAbs: bispecific antibodies. (Created with BioRender.com).

In cancer treatment, bsAbs are used to stimulate a strong immune response by poorly immunogenic tumours. In fact, bsAbs promote the interaction between tumour-associated antigens (TAAs), which are mainly expressed by cancer cells, and receptors or proteins expressed by effector cells of the immune system. This unique mechanism of action leverages immune cell priming and activation and results in lower on-target/off- tumour toxicity (Rudolph et al., 2006; Trabolsi et al., 2019).

The manufacturing processes of bsAbs are very complex and have evolved over the last decades (Wang et al., 2019). Recently, thanks to advances in antibody and protein engineering, various platforms have been created for the production of different types of bsAbs. Currently, bsAbs are produced via different and sophisticated techniques whose discussion goes beyond the scope of this review. It is worth mentioning that orthogonal Fab interfaces, DuoBody, XmAb, CrossMab and knobs-into-holes are the most widely used and well-known techniques for bsAbs development (Ma et al., 2021a; Wang et al., 2019).

Structurally, natural IgG antibodies consist of two identical light chains (LCs) and heavy chains (HCs). Disulfide bonds link together each domain generating three zones: two antigen-binding fragments (Fabs) that are responsible for antigen recognition, and one crystallizable fragment (Fc). Bispecific antibody-based ICEs can be classified more precisely based on their structural affinity to IgG. There are two main categories: fragment variable (Fv)-based ICEs, also referred to as non-IgG-like bsAbs, and immunoglobulin G (IgG)-based ICEs (Spiess et al., 2015). Fv-based ICEs consist of two single-chain fragment variables (scFvs) derived from two different monoclonal antibodies.

Fv-based ICEs are minimalist forms of a functional antibody that result from the fusion of variable domains of the IgG heavy and light chain via a flexible polypeptide linkage (Wang et al., 2019). One scFvs can recognize a specific TAA, while the other contacts a specific molecule expressed on effector cells of the immune system (Choi et al., 2011).

IgG-based ICEs, on the other hand, consist of two different Fvs or Fab fused to a Fc region in an IgG-like molecule (Labrijn et al., 2019).

Fragment variable-based ICEs are easier to produce and exert less immunogenic activity. Their relatively low molecular weight, which is due to the absence of an Fc region, ensures deeper penetration into tissue along with a rapid renal elimination (Spiess et al., 2015). Conversely, IgG-based ICEs are more stable, exhibit higher plasma solubility, has stronger antigen affinity and a longer half-life with lower renal clearance which affects dosing and toxicity (Kontermann and Brinkmann, 2015; Ma et al., 2021a; Shin et al., 2022). Moreover, the presence of the Fc region is also required to trigger Fc-mediated effector functions, namely, ADCC and complement fixation (CDC) (Griguolo et al., 2019). By contrast, the activity of Fv-based ICEs relies only on their antigen-binding ability (Ma et al., 2021a).The biological differences between these antibodies are likely to influence their clinical development and eventually their use in clinical practice (Shin et al., 2022) (Fig. 2).

3. ICEs mechanism of action

Various immune effector cells can be engaged in the synapses complex created by ICEs, including: T cells, natural killer (NK) cells and cytotoxic-phagocytic cells (Gleason et al., 2012; Kamakura et al., 2021; Schweizer et al., 2002) (Fig. 3).

The so-called bispecific T cell engagers (BiTEs), consist of two interconnected scFv that simultaneously target a TAA on a neoplastic cell and a component of the T cell receptor (TCR), mainly CD3 on T cell (Kamakura et al., 2021).Normally, the TCR recognizes TAAs presented by MHC molecules on the cancer cell itself or on antigen-presenting cells (APCs). Indeed, the loss or depletion of MHC, especially MHC-I on cancer cells, is a mechanism by which tumours elude immune surveillance (Aptsiauri et al., 2018). In this scenario, BiTEs can redirect T cells against TAAs without the intervention of the MHC-TCR complex, thus

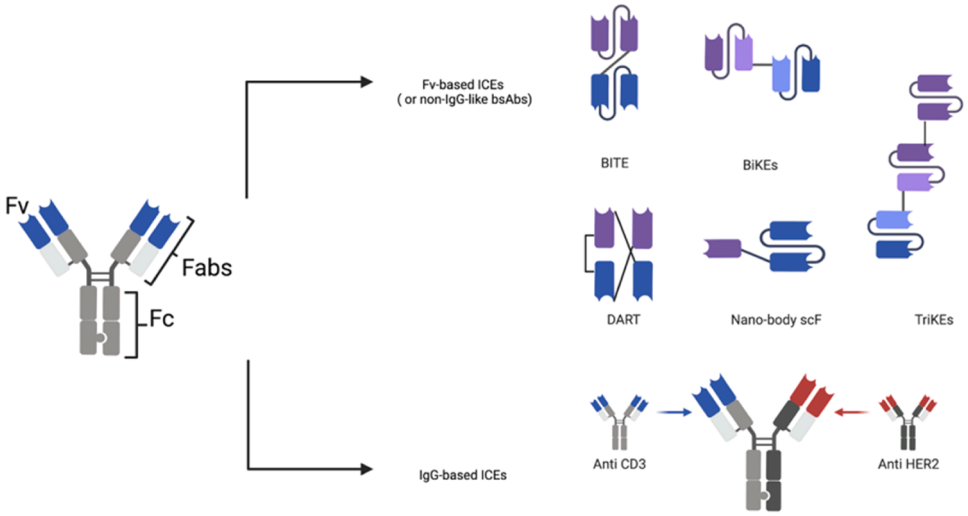

Fig. 2. Some current multivalent antibody structures under study divided into two main categories: Fv-based ICEs (or non IgG-like) and IgG-based ICEs. Abbreviations: Fv: fragment variables; Fc crystallizable fragment; Fabs: antigen-binding fragments; BiTE: bispecific T cell engagers; BiKEs: bispecific NK engagers; DART: dual affinity retargeting antibodies; TriKEs: trispecific NK-cell engagers; Nano-body scF: nano-body single chain fragment (Created with BioRender.com).

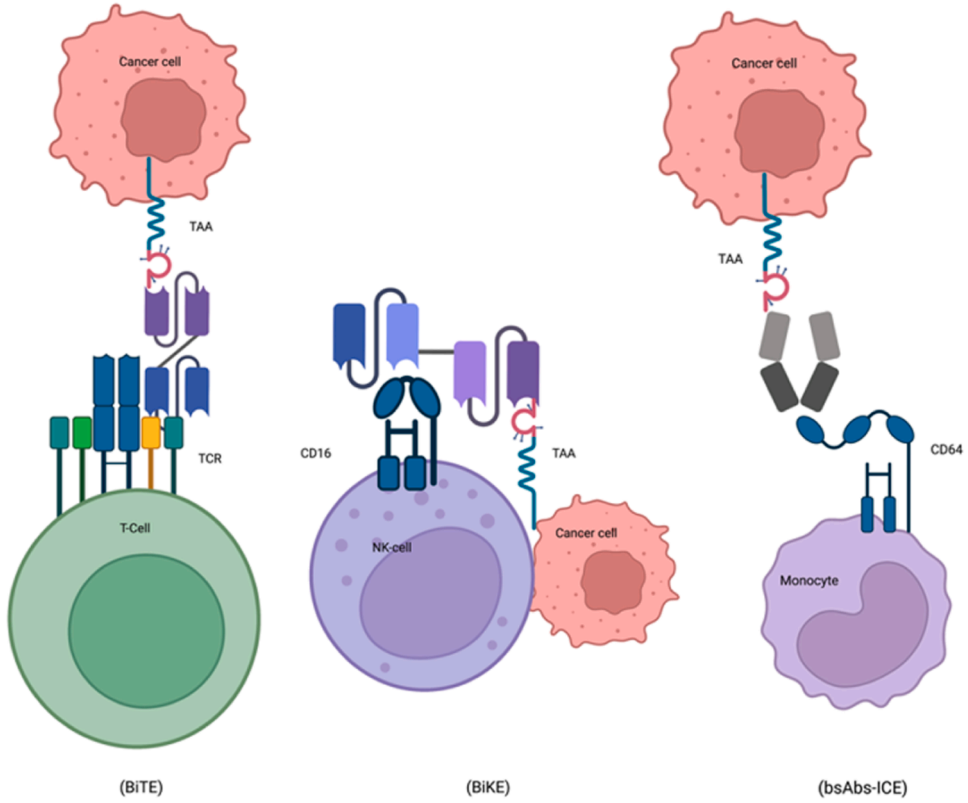

Fig. 3. Immune cell engagers and their main targets in cancer immunotherapy. The figure shows the main effector cells (T cell, NK-cell and monocyte) of both the innate and adaptive immune system involved in artificial immune synapses with cancer cells and their tumor associated antigens (TAAs) via different immune cell engagers (BiTE, BiKE and other ICEs). Abbreviations: BiTE: bispecific T cell engagers; BiKEs: bispecific NK engagers; TAAs: tumour-associated antigens. (Created with BioRender.com).

overcoming the aforementioned mechanism of resistance. Moreover, the creation of an artificial immune synapse allows for the activation of T cell functions that consist, on the one hand, in the secretion of enzymes such as perforins and granzymes and, on the other hand, in the release of cytokines. Perforins are responsible for the formation of pores on the surface of cancer cells, while granzymes initiate proteolysis of cancer cell proteins. Moreover, T cell activation promotes their self-sustenance, which is pivotal to maintain a durable antitumor response (Huehls et al., 2015).

Bispecific natural killer engagers (BiKEs) are another class of ICEs that exploit the presence of the CD16 antigen on the surface of NK cells to exert their therapeutic effects. This antigen, together with CD56, is physiologically responsible for NK cell functioning by recognizing and eliminating infected or transformed cells (Shimasaki et al., 2020).

Indeed, CD16-directed engagers induce NK cell activation and tumour cell specific cytotoxicity through the release of cytokines and chemokines as well as the perforins and granzymes (Gleason et al., 2012). According to several studies the downregulation of NK activity in BC is a biomarker of poor response to chemotherapy, thereby confirming that this subset of immune effectors play an important role (Piroozmand and Hassan, 2010). Indeed, low infiltration of NK cells in BC TME plays both a prognostic and a predictive negative role in metastatic BC patients (Garcia-Chagollan et al., 2018).

Finally, other cytotoxic-phagocytic immune cells such as macrophages, DC, monocytes, and neutrophils, can also be targeted by antibodies that bind to CD64, the high-affinity receptor for immunoglobulin G (FCγRI), triggering ADCC (Schweizer et al., 2002).

The relationship between these myeloid immune cells and BC biology and prognosis is less clear, although some preclinical data suggest that there is a bidirectional cross-talk between cancer cells and myeloid immune cells that likely prevents tumour growth and metastatic spread (Hagerling et al., 2019).

4. ICEs toxicity profile

ICE-related toxicities are mainly due to off-target binding of bsAbs to antigens or receptors expressed by normal tissues. For instance, the clinical development of catumaxomab, a trifunctional bsABs antibody developed for the intraperitoneal treatment of malignant ascites in adult patients with EpCAM-positive carcinoma (Heiss et al., 2010), was limited by off-target liver toxicity. Indeed, catumaxomab simultaneously interacts with EpCAM, a TAA, CD3 and the Fcγ receptor on NK-cells, thereby leading to the destruction of EpCAM-positive cells without the intervention of co-stimulatory signals and MHC restriction (Seimetz et al., 2010).

Unfortunately, Kupffer cells in the liver and enterocytes in the colon also express the Fcy receptor on their surface and when catumaxomab was infused intravenously, the release of cytokines and T cell-mediated hepato-enteric toxicity resulted in different degrees of hepatotoxicity and enterotoxicity, as confirmed by the elevation of serum liver enzymes, bilirubin, and γGT, and the development of diarrhoea (Borlak et al., 2016).

ICEs-related side effect can also be attributed to massive release of cytokines. Bivalent or multivalent anti-CD3 BiTEs, whose scFv have affinity for more than one CD3 epitope, could be associated with excessive activation of immune effectors, leading to decreased efficacy in cancer cells and increased toxicity toward CD3-rich tissues such as spleen and lymph nodes, where the drug is redistributed due to the high density of the antigen (Leong et al., 2017; Mandikian et al., 2018a).

Cytokine release syndrome (CRS) consists of a constellation of inflammatory symptoms due to an abnormal cytokine elevation associated with immune cell engagement, activation, and proliferation (Maude et al., 2014). Most patients diagnosed with CRS experienced only mild symptoms ranging from flu-like syndrome, chills, and fevers to arthro-myalgias, but life-threatening CRS, with severe cardiovascular and respiratory distress, can also occur (Stein et al., 2019).

Neurotoxicity is a less studied, but equally relevant, potential consequence of ICEs therapy. Its pathogenesis has been largely attributed to the adhesion of T cells to endothelial cells of the blood brain barrier (BBB). This adhesion leads to endothelial cells activation and T cell transmigration through brain micro vessels. Neurological adverse events (AE) may also be due to the release of cytokine by T cells. Preclinical data have shown that antiadhesive agents can attenuate and mitigate this phenomenon. Heparinoid inhibits the adhesion molecule P-selectin expressed on blood vessels whereas the tetracycline antibiotic minocycline impairs the binding of T cell to intercellular adhesion molecule-1 (ICAM-1), expressed on the endothelial surface, by calcium chelation (Klinger et al., 2020). Neurotoxicity can range from personality changes, tremor, dizziness, confusion, and focal neurologic symptoms to encephalopathy, ataxia, convulsions, and delirium. Dexamethasone and antiepileptic drugs such as levetiracetam and phenytoin can be used to successfully manage neurological symptoms (Stein et al., 2019).

5. ICEs implementation in solid tumours: pitfalls and potential solutions

ICEs have been successfully developed in haematologic malignancies where they have shown impressive and encouraging results. Blinatumomab was the first BiTE approved by the Food and Drug Administration (FDA) for the treatment of CD19 positive relapsed or refractory acute lymphoblastic leukaemia (ALL) in adults and children (Przepiorka et al., 2015). Blinatumomab is a bsAbs ICE that binds simultaneously to CD19 on ALL cells and to CD3 on T cells, and its efficacy has been demonstrated in several clinical trials, some of which are still ongoing (Labrijn et al., 2019).

However, obtaining similar results in solid malignancies is challenging due to tumour and drug-related features, ultimately resulting in lower efficacy and increased toxicity.

The heterogeneity of TME in solid tumours may hamper and compromise immunotherapy efficacy. Indeed, regulatory T cells (T-regs) infiltration and immune-checkpoints activation may create a hostile environment for the antitumor immune response (Argilés et al., 2017; Kebenko et al., 2018; Przepiorka et al., 2015; Wermke et al., 2018a).

After administration and localization to the tumour site, ICEs encounter a highly variable tumour microenvironment (TME) that may exhibit immunosuppressive properties, depending on the composition of infiltrating cells and local signalling molecules. In many solid tumours, the TME contains immunosuppressive cells, such as regulatory T cells (T-reg),tumor-associated macrophages (TAMs), and myeloid-derived suppressor cells (MSCs) all of which emit inhibitory signals that can reduce ICE efficacy. The effectiveness of ICEs is thus potentially limited by the density and distribution of these immunosuppressive elements, which vary widely between tumours and even within regions of the same tumour. To address this, agent capable of reprogramming TAMs towards a pro-inflammatory, anti- tumor phenotype (M1) can be used to promote an immune-supportive environment. Additionally, selective depletion or inhibition of T-regs and MDSCs within the TME may further enhance ICEs performance (Kenkel et al., 2022). Moreover, the implementation of emerging immunotherapy in solid cancers might also be hindered by obstacles in tumour trafficking and penetration, as well as by the development of anti-ICEs antibodies, as demonstrated in a study using a CEAxCD3 BiTE (Pishvaian et al., 2016a).

Another significant barrier is the formation of antidrug antibodies (ADAs) following ICEs administration. ADA production, triggered by CD4 T lymphocytes, memory B cells, and plasma cells activation can alter pharmacokinetics and pharmacodynamics of ICEs reducing their efficacy and increasing toxicity (Penny et al., 2023). Factors influencing ADAs formations include the immunogenicity of the ICE itself, the presence of foreign sequences, the dose regimen, the route of administration, and individual patients' immune responsiveness. One promising approach to mitigate ADA formation involves the co-administration of anti-CD20 antibodies which reduce B-cell activation, ADAs secretion, enhancing ICE stability and therapeutic effect (de Miguel et al., 2021; Penny et al., 2023).

Some pharmacokinetic characteristics may also limit the efficacy and clinical implementation of ICEs (Wang et al., 2019). Due to their short-half life, Fv-based ICEs require continuous intravenous administration to maintain therapeutic concentrations. To overcome the rapid renal excretion of these compounds, newer molecules are being engineered in an IgG-like or Fc-fused form, characterised by a higher molecular weight and a more favourable excretion profile. In addition, dual affinity retargeting (DART) proteins have been developed that are more stable in the bloodstream and, when fused to an Fc domain, have a longer half-life, resulting in more sustainable intermittent administration rather than continuous (Moore et al., 2018).

The PK profile of ICEs can be optimized to improve tumour penetration. Indeed, recent advancements including the modification of the Fc region of the molecule to reduce clearance and extend half-life, as seen with FC engineering techniques, have demonstrated to prevent rapid renal filtration. Moreover, polyethylene glycol (PEG)- conjugated ICEs (Cheng et al., 2022)or ICEs engineered utilizing albumin-binding domains(Mandrup et al., 2021) show improved pharmacokinetics properties characterized by a more sustained exposure to the tumour and a more suitable toxicity profile. Therefore, inclusion of human albumin sequences into ICEs design could represent a valuable strategy to potentially increase treatment efficacy and guarantee lower side effects and a better patient compliance (Mandrup et al., 2021).

In addition, ICEs may exhibit limited tissue penetration within solid tumors. The dense extracellular matrix and elevated interstitial fluid pressure restrict the distribution of ICEs, often confining their action to the tumor periphery. Strategies to overcome these barriers include the development of smaller ICE molecules or the use of tumor-penetrating peptides that facilitate deeper diffusion within the TME (Geiger et al., 2020). Another innovative approach is the design of "peptide-masked" ICEs that are selectively activated by proteases enriched in the TME, improving both tissue penetration and specificity while minimizing off-target effects (Geiger et al., 2020). The issue of on-target, off-tumor toxicity remains a concern, particularly when ICEs target antigens expressed on both tumor and healthy cells. Conditional activation strategies have been developed to address this limitation, such as engineering ICEs with tumor-specific protease-cleavable linkers or designing masked binding domains that are only exposed in the tumor microenvironment. These approaches allow ICEs to remain inactive in healthy tissues, thereby reducing systemic toxicity and enhancing the therapeutic index. Lastly, ICEs as previously mentioned can induce CRS, resulting from excessive immune activation (Mandikian et al., 2018; Maude et al., 2014). CRS poses a particular safety risk in solid tumors, where a large tumor burden and extensive immune cell infiltration can amplify inflammatory responses. Approaches to mitigate CRS include modifying the affinity of ICEs for their immune targets, optimizing overall potency to avoid overstimulation, and engineering Fc modifications that modulate immune cell activation (Mandikian et al., 2018). Such adjustments aim to control the extent of immune activation, allowing for safer application of ICEs in solid tumor settings.

A major challenge in the development of ICEs is their toxicity. In solid tumours, the lack of tumour-specific antigens (TSAs) led to on-target off-tumour adverse events, ultimately causing the premature discontinuation of several ICEs development (Argilés et al., 2017; Kebenko et al., 2018; Przepiorka et al., 2015; Wermke et al., 2018b).

Reducing the affinity between anti-CD3 antibodies and T cells results in a better distribution of anti-CD3 antibodies in cancer tissue along with a relative sparing of normal tissue containing T cells, such as spleen and lymph nodes (Mandikian et al., 2018a). Indeed, while maximising antigen binding affinity is highly desirable, higher T cell epitope binding affinity may weaken the distribution of ICEs in the TME (Mandikian et al., 2018a). This not only results in lower systemic exposure to these drugs, but also has important implications for efficacy and safety.

The presence of the Fc region in the structure of ICEs is another source of unwanted toxicity. Although the Fc region is beneficial from a pharmacokinetic standpoint, it enhances the immunotoxicity of ICEs since its interaction with Fcγ receptors on innate and adaptive immune cells promotes an inflammatory state. Therefore, several strategies have been explored to reduce this phenomenon, such as the introduction of mutations in the genes encoding the Fc component (Labrijn et al., 2019; Schlothauer et al., 2016).

Lastly, in preliminary data suggest that switching from intravenous (iv) to subcutaneous (sc) administration not only minimise systemic toxicities such as CRS, but also improve patient compliance and is economically advantageous (Matasar et al., 2020).

6. ICEs in BC

Triple negative and HER2+ BC are the two BC subtypes where ICEs are mostly studied mainly due to the expression of specific antigens and epitopes on tumor cell surface.

Incorporating ICEs in the treatment of HR+ tumours is far more complex due to the cytoplasmatic localization of oestrogen (ER) and progesterone receptors (PgR).

Table 1 reports a list of selected trials testing ICEs in BC patients, along with main characteristics and results, when available. A detailed list of ongoing trials of ICEs in BC is provided in the Supplementary material.

6.1. *ICEs in HER2+ BC*

HER2 is an oncoprotein known to be overexpressed in up to 20 % of BC tumours (Krystel-Whittemore and Wen, 2022) and is responsible for tumour proliferation and survival (Korkaya et al., 2008). The possibility to redirect immune effector cells against this TAA makes HER2+ BC a good candidate for the use of ICEs (Mandó et al., 2021).Therefore, many ICEs targeting HER2 have been developed since the early 90 s. Nevertheless, several limitations occurred in the transition from *in-vivo* or *ex-vivo* assays to clinical trials. Hereinafter, we report several examples of ICEs targeting HER2.

A primordial candidate drug investigated in advanced HER2+ BC as an immune engager was 2B1. 2B1 is a bispecific monoclonal antibody (bsmAb) whose Fab binds the extracellular domain of HER2 on the surface of BC cells and whose Fc contacts the FcγRIII isoform A expressed on NK cells and mononuclear phagocytes. Although it cannot truly be defined as an ICE, 2B1 represents one of the first attempts to redirect innate immunity against cancer cells. In E3194, a phase Ib/II study (Borghaei et al., 2007), 20 women with metastatic BC (mBC) previously treated with chemotherapy were enrolled and recruited to receive 2B1. After administration of the first dose, dose-limiting toxicities (DLT) requiring dose reduction occurred in three out of eight patients. The extent of these toxicities required an adjustment of the dose from 2·5 mg/m^2/day to 1 mg/m^2/day in the remaining 12 patients. Although 2B1 exhibited ADCC activity that directed the adaptive immune response to both the intracellular and extracellular domains, no objective antitumor responses were reported. Additionally, the safety profile of this drug was unfavourable, with fever, vomiting, diarrhoea, and nausea among the most common toxicities.

Ertumaxomab is a trifunctional bispecific antibody that targets HER2 on BC cells, CD3 on T cells, and the FcγRI, IIa, and III on accessory immune cells, resulting in a three-cell complex and artificial synapse responsible for cancer cells killing.

The first encouraging application of this approach dates back to 2006, when a phase I study (Kiewe et al., 2006) evaluated the safety and activity of ertumaxomab in heavily pre-treated HER2+ mBC. The study demonstrated an acceptable safety profile and clinical benefit in five out of 15 evaluable patients (Kiewe et al., 2006).

Fever, elevated liver enzymes, headache, gastrointestinal disorders and lymphocytopenia were the most common and expected AEs but all of them all were reversible.

Two patients experienced life-threatening but nonfatal toxicities. The first developed severe hypotension and respiratory distress syndrome, while the other showed systemic inflammatory syndrome and acute renal failure (Kiewe et al., 2006).

A subsequent phase II trial in 2007 in patients with HER2+ mBC that progressed on trastuzumab was terminated early due to a change in development plan of the drug. Although no efficacy data are available due to the early termination of the trial, no new safety concerns have been reported (US National Library of Medicine., 2007).

More recently, in 2016, ertumaxomab was evaluated in a phase I trial (Haense et al., 2016) recruiting patients whose tumours expressed HER2 (1+/SISH positive, 2+ and 3+) and progressed to standard treatment for

Table 1
Selected trials with ICEs in clinical development in breast and other solid malignancies. In the table above available data and information about ICEs in clinical investigation are provided. Abbreviations: NA: not available; PR: partial response, SD: stable disease, CR: complete response; PD: progression disease; AE: adverse event; SIRS: severe immune-related syndrome; ARDS: acute respiratory distress syndrome; AKI: acute kidney impairment; IRR: infusion-related reactions; CRS: cytokine release syndrome; ORR: objective response rate, DCR: disease control rate; CBR: clinical benefit rate; DLT: drug limiting toxicity; mOS: median overall survival; MTD: maximum tolerated dose.

Trial Identifier	Drug	Phase	Engagement	Cancer type	Main AEs	Outcomes	Trial status
NCT00351858	Ertumaxomab	I	HER2xCD3	HER2+ BC	G3 Lymphocytopenia (76 %) G3 Elevation of liver enzymes (47 %) 1 severe hypotension and ARDS 1 SIRS and AKI	1/15 CR 2/15 PR 2/15 SD	Terminated
NCT01569412	Ertumaxomab	I/II	HER2xCD3	HER2+ advanced solid tumors	G3 Fatigue (43 %) G3 Fever (14 %) G3 Pain (21 %) 1 Allergic reaction 1 SIRS	1/11 PR 2/11 SD	Terminated
NCT03330561	PRS−343	I	HER2x4–1BB	HER2+ solid tumors	IRR (25 %) Nausea (7 %) Arthralgia (5 %)	12 % ORR 52 % DCR	Completed
NCT03922204	MCLA−145	I	PD-L1x4–1BB	Advanced solid tumors	G3 febrile neutropenia ALT/AST elevation Fatigue Myositis	NA	Recruiting
NCT04128423	AMV564 +/-Pembrolizumab	I	CD33xCD3	Advanced solid tumors	Pyrexia, injection site reactions, fatigue, anemia, hypotension, pruritis, chills, and nausea G2 CRS	1/20 CR	Active, not Recruiting
NCT02324257	RO6958688	I	CD3xCEA	CEA+ advanced solid tumours	G3 IRR (16.3 %) G3 diarrhea (5 %)	2/36 PR	Completed
NCT02650713	RO6958688 + Atezolizumab	I	CD3xCEA	CEA+ Advanced solid tumours	IRR, Diarrhea, G3 dyspnea, G3 hypoxia G4 colitis G5 respiaratory failure	2/10 PR	Completed
NCT04501744	M701	I	CD3xEpCAM	EpCAM+ tumor cells in ascites	Hypoproteinemia anemi hypokalemia hyponatremia	ORR 62.5 %; DCR 100 %;	Recruiting
NCT04143711	DF1001	I/II	HER2xCD3xCD16	HER2+ advanced solid tumors	infusion related reactions (26 %) asthenia (15 %) fatigue (12 %),	5 PR 22 SD CBR 39.7 %	Recruiting

metastatic disease.

Fourteen patients with breast, rectal, or gastric cancer were enrolled. Consistently with previous evidence, most AEs were transient and reversible. Here, only two severe adverse events (SAEs) were attributed to the experimental drug: one patient experienced an allergic reaction, and the other experienced fever and gastrointestinal disturbances requiring hospitalisation. Both SAEs reverted after treatment discontinuation and supportive management.

Regarding efficacy, one partial response (PR) and two disease stabilizations (SD) were observed after the first treatment cycle (Haense et al., 2016).

Another bsAb that simultaneously binds HER2 on BC cells and the FcγRI (CD64) expressed on myeloid progenitors, monocytes, macrophages, and polymorphonuclear (PMN) cells is MDX-H210.

Preclinical studies (Stockmeyer et al., 1997) demonstrated the ability of MDX-H210 to bind FcγRI on PMN primed with granulocyte-colony-stimulating factor (G-CSF) and its cytotoxic effect against HER2-overexpressing cells.

Two phase I trials (Pullarkat et al., 1999; Repp et al., 2003) of MDX-H210 in combination with G-CSF enrolled patients with HER2+ mBC after failure of at least two standard chemotherapy and/or hormonal treatments.

The primary objective of both trials was to evaluate the safety profile of MDX-H210, determine the maximum tolerated dose (MTD), and the biologically active dose, while the secondary objective was to define the clinical response.

As with ertumaxomab, the main toxicity events were: fever, nausea, vomiting and diarrhoea with no evidence of grade 4 AEs.

In terms of efficacy, no objective response was reported, with stable disease as best response (Repp et al., 2003).

A phase Ia/Ib trial (Valone et al., 1995) of MDX-H210 in patients with either breast or ovarian cancer yielded similar results.

M802 is a bsAb composed of two units. The first unit contains a heavy chain and a light chain that bind HER2 similarly to trastuzumab. The second unit is a single chain binding CD3. In preclinical (Yu et al., 2019) models, M802 combined the ability to bind HER2, down-regulating its intracellular signalling pathway, while retaining a moderate binding capacity to CD3 on T cells. This property allowed M802 to exert sufficient cytotoxicity while reducing the potential side effects of CRS.

In these preclinical models, M802 showed superior efficacy compared to trastuzumab in both HER2-expressing and trastuzumab-resistant cell lines and overcame multiple anti-HER2 resistance mechanisms (Yu et al., 2019).

These data led to M802 being tested in an ongoing dose-escalation clinical trial in HER2+ advanced BC patients (US National Library of Medicine, 2021b).

PRS-343, also known as cinrebafusp alfa, is a bispecific fusion protein that targets HER2 on tumour cells and the costimulatory immune receptor 4–1BB on T cells.

A phase I dose-escalation trial evaluated the activity and safety profile of this drug in 78 patients with HER2+ solid tumours (Ku et al.,

2020) 16 (20.51 %) with advanced BC.

After drug dose evaluation, efficacy was initially tested in 33 patients, who had an overall response rate (ORR) of 12 % and a disease control rate (DCR) of 52 % without SAEs. Most common AEs were infusion-related reactions, nausea, arthralgia, vomiting, chills, and fatigue.

A dose-escalation to 8 mg/kg increased the ORR and the DCR to 40 % and 70 %, respectively without affecting treatment tolerance. After drug administration, an increase in CD8+ T cells was detected suggesting a role for PRS-343 in increasing the immune infiltrate in the TME.

An update analysis of the same trial conducted in 2024, confirmed the efficacy of cinrebafusp alfa in forty evaluable patients with 5 patients showing an antitumor response, resulting in an ORR of 12.5 % and a DCR of 52.5 %(Piha-Paul et al., 2024). Clinical activity was observed at two different dose (8 and 18 mg/kg dose levels), with confirmed ORR of 28.6 % and 25.0 %, respectively, in heavily pre-treated patients with a median of 4 previous lines in the advanced setting (Piha-Paul et al., 2024).

In terms of safety and toxicity, Cinrebafusp alfa was safe and tolerable, with grade $\leq$2 infusion-related

reactions being the most frequent treatment-related adverse event and no MTD reached during the study(Piha-Paul et al., 2024).

More interestingly the drug demonstrates promising activity in patients who have progressed on prior HER2-targeting regimens suggesting its utility for patients not responding to the most efficacious existing HER2-directed therapies widening the therapeutic arsenal also in HER2+ BC.

In terms of ADA, although preexisting ADAs were detected in only 5 % of patients, 32 % of patients become positive on Cycle 1, Day 15, suggesting a high immunogenicity for the compound in B-cell activation. To reduce ADA formation, an exploratory cohort with obinutuzumab, an anti-CD20, pretreatment was initiated to assess the consequence of B-cell depletion on ADA frequency and exposure. Although safe, it was not deemed to be necessary and therefore was dismissed. Based on these results, Cinrebafusp alfa is being investigated in a phase 2 trial with a more homogeneous patient population is required(Piha-Paul et al., 2024).

The combination of this novel agent with atezolizumab, an anti PD-L1 antibody, is being investigated in a phase I trial (US National Library of Medicine, 2021a).

IBI315 is a first-in-class anti-HER2xPD-1 bsAb that has been investigated in a phase Ia study in patients with advanced HER2-expressing tumours. Preliminary data presented at the 2021 Chinese Society of Clinical Oncology (CSCO) Annual Meeting showed an ORR of 20 % (Innovent Biologics. Innovent Releases Preliminary Results of the Phase Ia Dose-Escalation study of IBI315 (Anti-Her2/PD-1 Bispecific Antibody) in Patients with Advanced Solid Tumors at CSCO Annual Meeting, 2021. PR Newswire https://www.prnewswire.com/news-releases/innovent-releases-preliminary-results-of-the-phase-ia-dose-escalation-study-of-ibi315-anti-her2pd-1-bispecific-antibody-in-patients-with-advanced-solid-tumors-at-csco-annual-meeting-2021–301386697.html, n.d.). Additionally, no DLT was observed and the MTD was not reached, suggesting a manageable safety profile. Following these promising results, additional studies have been initiated to evaluate the efficacy of IBI315 both as monotherapy and in combination with chemotherapy in HER2 + positive and HER2-low tumours.

GBR 1302 is a novel HER2xCD3 BITE designed to leverage T cell activity. It has been investigated in HER2-expressing tumours in a first-in-human study involving adult patients with solid tumours and not available standard treatment (Wermke et al., 2018b). Its therapeutic window ensures a relatively high sparing of cells expressing normal levels of HER2, while harming both HER2+ and HER2-equivocal cancer cells. A preliminary analysis in 19 evaluable patients, safety, tolerability, and initial efficacy of GBR 1302 were assessed. The toxicity profile was consistent with that of previously developed T cell engagers. In fact, CRS was the most frequently observed SAE in patients who received a dose of $\geq$100 ng/kg. Only two patients had DLT: one experienced an asymptomatic reduction in left-ventricular ejection fraction that resolved spontaneously after treatment discontinuation, the other one had grade 4 CRS requiring hospitalization. No documented radiological response were reported, but one patient with gastroesophageal adenocarcinoma and another one with BC experienced prolonged stabilisation of disease lasting $\geq$ four months (Wermke et al., 2018b).

It is widely recognized that HER2-negative (HER2-) tumour expressing low to moderate amounts of HER2 protein on their surface, derive little to no benefit from conventional anti-HER2 therapies (Burris et al., 2011; Fehrenbacher et al., 2020). Consequently, there is an unmet need in oncology to extend the benefit of other anti-HER2 therapies beyond ADCs to this group of malignancies (von Arx et al., 2023). Trispecific antibodies (tsAbs) consisting of three distinct binding sites for HER2, CD3, and CD28, respectively, have been developed to target, activate and prolong survival of T cells against cancer cells (Seung et al., 2022; Sha et al., 2021). In preclinical trials, two first-in-class agents, have been shown to both stimulate T cell activation by increasing the expression of granzyme in CD8+ T cells, and inhibit tumour growth in HER2-depleted, namely HER2-low, BC cell lines (Seung et al., 2022; Sha et al., 2021). Although trispecific antibodies show promise as a multi-targeting immune intervention, they have yet to be studied in humans. Experiments in mice showed an acceptable safety profile (Seung et al., 2022; Sha et al., 2021).

DF1001 is a first in class trispecific NK cell engager developed to redirect both NK and CD8 T cells against HER2 expressing tumour (Safran et al., 2023). The safety profile and efficacy of DF1001 is currently under investigation in an ongoing phase I/II trial in patients with advanced and/or refractory solid tumours (US National Library of Medicine, 2022b).

Preliminary data suggests that DF1001 is safe and active among a wide range of solid malignancies (Safran et al., 2023). In fact, treatment related AEs, reported in 79 % of the 124 enrolled patients, were mainly grade 1 and 2 and rarely led to treatment discontinuation. Infusion related reaction, asthenia and fatigue were the most common toxicities, with no DLT detected.

In terms of activity, treatment with DF1001 resulted in a global clinical benefit rate (CBR) of approximately 40 % with five and 22 patients achieving partial response (PR) and stable disease (SD) as their best response, respectively (Safran et al., 2023).

In this trial, 38 % of the enrolled patients had BC.

Biomarker analysis showed an increase in inflammatory cytokines and chemokines in responders patients consistently with an increase in both NK and CD8 population in their TME (Safran et al., 2023).

This data supports further exploration of DF1001, and other trispecific engagers, either alone or in combination with other compounds.

6.2. ICEs in TNBC

The absence of distinctive biomarkers has historically limited the development of targeted therapies for TNBC. However, due to its immunogenic properties, TNBC is a good candidate for the use of immunotherapies and is the only subtype with approved indications for anti-PD-(L)1 agents (Cortes et al., 2020; Emens et al., 2021; Luo et al., 2022; Schmid et al., 2020). Several ICEs are currently under preclinical investigation or in early clinical development in this BC subtypes.

Carcinoembryonic antigen (CEA) is a cell surface-anchored glycoprotein involved in cell adhesion and is normally expressed on foetal gastrointestinal stem cells (Gold and Freedman, 1965). In addition to gastrointestinal tumours, it is also frequently overexpressed on TNBC cells and is implicated in the development of liver metastases (Thomas et al., 2011). CEAxCD3 ICEs have been tested in gastrointestinal tumour (GI), but future studies may clarify whether these drugs can also have a role in TNBC.

MEDI-565, a bsAb targeting CD3 and CEA, was tested in a dose-

escalation study in advanced GI adenocarcinomas with no evidence of objective response (Pishvaian et al., 2016a). Similarly, RO6958688 was evaluated, alone or in combination with atezolizumab, in two different phase I trials enrolling patients with advanced CEA-positive solid tumours, including BC. Partial response was achieved in 5 % (2/36) and 20 % (2/10) of patients, respectively (Pishvaian et al., 2016b; Tabernero et al., 2017). In both studies, RO6958688 had a manageable safety profile, with infusion-related reactions and diarrhea as common SAEs (Tabernero et al., 2017).

To overcome dose-limiting toxicities and improve outcomes, sequential administration of CEAxCD3 bsAb, NILK2301, and CEAxCD28, NILK3301, although in non-BC preclinical studies, have reduced drug dose and cytokine release while improving activity (Seckinger et al., 2022).

In TNBC, evidence are rather sparse, scarce and limited although preclinical data have confirmed that CD3xCEA bsAbs can significantly inhibit TNBC spheroids growth (Chang et al., 2017a).

Trophoblast cell surface antigen 2 (TROP2) is a calcium signal transducer that promotes cell growth and proliferation and is overexpressed in several solid malignancies, including TNBC (Stepan et al., 2011).

Its expression has been associated with a dismal prognosis (Bardia et al., 2021; Trerotola et al., 2013),and several efforts have been made to target this surface protein (Bardia et al., 2021).

In a preclinical study, F7AK3, a bsAb directed against CD3 and TROP2, was able to induce T cell infiltration and activation in tumour tissues obtained from TNBC patients (Liu et al., 2021).

As mentioned earlier, F7AK3 was designed with a higher binding capacity to TROP2 than to CD3 to prevent undesired T cell activation.

Immune cell engager can also be combined with other types of immunotherapies (Chang et al., 2017a). Preclinical data suggest that combining T cell redirecting bsAbs with antagonists or agonists that attenuate T cell inhibition, such as PD-1 and PD-L1 inhibitors, especially in TNBC, has the potential to improve immunotherapy efficacy (Chang et al., 2017a).

Proof of concept comes from (E1)-3s, a bsAbs targeting Trop-2-expressing BC cells, which has shown a highly potent effect on TNBC tumour cells (Chang et al., 2017a). In the same study, the addition of cPD-1, a novel ICI, was shown to enhance the killing of T cell mediated by (E1)-3s (Chang et al., 2017a).

7. Future perspectives

Several strategies are currently being explored to broaden the development and use of ICEs in breast cancer, including the search for new potential targets.

Preclinical data suggest that ephrin receptor A10 (EphA10), epithelial cell adhesion molecule (EpCAM), P-cadherin, epidermal growth factor receptor (EGFR), mesothelin (MSLN), receptor tyrosine kinase-like orphan receptor 1 (ROR1) are suitable targets for the development of novel ICEs in TNBC (Del Bano et al., 2019; Chang et al., 2017b; Fisher et al., 2018; Kamada et al., 2015; KUBO et al., 2018; Taki et al., 2015) (Fig. 4).

An open-label phase I dose-escalation study is evaluating the safety profile of PF-06671008 in patients with TNBC expressing P-cadherin (US National Library of Medicine, 2022a).

The development of bsAbs directed against BC epitopes that are minimally expressed or undetectable in normal tissues represent a promising strategy that merits further investigation.

The prolactin receptor (PRLR) is a type I cytokine receptor known to be highly expressed in various BC cells with lower expression on normal cells (Touraine et al., 1998). Upon its activation, PRLR stimulates downstream signalling pathways leading to cell proliferation. Indeed, blocking PRLR has demonstrated, *in-vitro* and *in-vivo*, to be both safe and effective against BC cells (Agarwal et al., 2016). Previous experience has elucidated that this receptor can be targeted with conventional

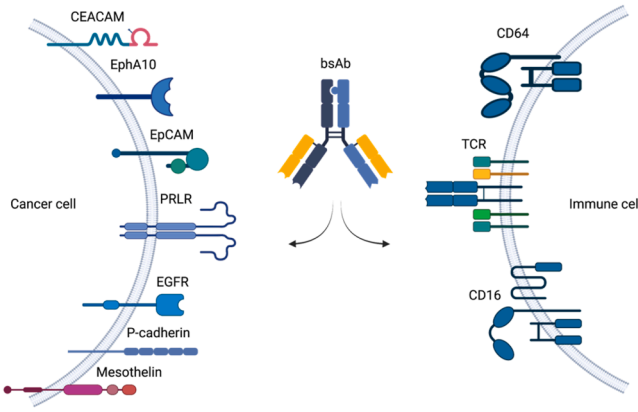

Fig. 4. Novel antigens expressed on cancer cells that are under investigation for the development of new ICEs in BC immunotherapy. Abbreviations: CEACAM: EphA10: ephrin receptor A10; EpCAM: epithelial cell adhesion molecule; PRLR: Prolactin receptor; EGFR: epidermal growth factor receptor; TCR: T-cell receptor; bsAb: bispecific antibodies; (Created with BioRender.com).

antibodies, but their efficacy was disappointing in a phase I trial (Damiano et al., 2013). More recently, PRLR-DbsAb, a novel ICE targeting this BC-associated antigen, has been developed with increased cytotoxicity and better activity compared with the PRLR monoclonal antibody (Zhou et al., 2020). Hence, PRLR-DbsAb could be considered for further development (Zhou et al., 2020).

An even more selective epitope in BC is p95HER2. This protein arises from the alternative initiation of translation of the transcript encoding the full-length HER2 receptor, as a result of resistance mechanisms experienced during or after anti-HER2 treatments (Gajria and Chandarlapaty, 2011). As a consequence, p95HER2 is only detectable in cancer cells, differently from HER2, that can be encountered also on normal cells. Of note, p95HER2xCD3, a bsAb, has shown in vitro and in vivo clear antitumor effect, also confirmed when treated intracranially implanted cells (Rius Ruiz et al., 2018).

A major pitfall of bsAbs directed against a single cancer cell epitope is the potential emergence of escaping clones, especially in case of TAAs that are scarcely specific for transformed cell (Ellerman, 2019).

In order to overcome this issue, trispecific antibodies (tsAbs) with the ability of establishing an efficient anticancer response for dual-TAAs have been investigated in vitro and in vivo. Some encouraging preclinical data are available for HER2xVEGFR2xCD3, a tsAbs, targeting both HER2 and vascular endothelial growth factor receptor 2 (VEGFR2) that showed significant inhibition of BC tumour growth also in models pretreated with anti-HER2 and anti-VEGFR2 drugs (Liu et al., 2022).

Mesothelin (MSLN) is expressed as a glycophosphatidylinositol-linked cell surface glycoprotein implicated in cell adhesion without a precise physiologic function in humans (Tozbikian et al., 2014). The overexpression of MSLN in TNBC correlates with poor prognosis (Tozbikian et al., 2014), making it as an attractive target for MSLN-directed therapeutics whom investigation and development have been hindered due to the expression of MSLN on normal mesothelial cells, potentially leading to dose-limiting toxicities (Thongchot et al., 2024) or tumor-induced MSLN shedding (Chakraborty et al., 2024) potentially blocking Ab-based MSLN-targeting drugs from killing cancer cells.

HPN536 (MSLNxCD3) (Molloy et al., 2021) is a Trispecific T-cell–Activating Construct (TriTAC) made up of a single polypeptide chain containing three humanized antibody derived binding domains: a single-domain antibody (sdAb) directed to a TAA, a specific serum albumin-sdAb to extend its half-life, and a scFv specific for CD3.

Another MSLNxCD3 bsAb acting as ICE is 15B6 which have proven to be active also against MSLN-shedding cancer(Chakraborty et al., 2024).

Both HPN536 and 15B6 specifically activate and redirect T cells for

potent lysis of MSLN-expressing cancer cells, and are two of the more promising MSLN-directed ICEs currently in clinical investigation in solid malignancies (NTC03872206) (Chakraborty et al., 2024).

ROR1 is a TSA expressed in multiple hematological and solid malignancies including TNBC (Fultang et al., 2020). A novel monovalent asymmetrical tandem Fab (MAT-Fab) antibody, named EMB-07, composed of 2 different Fab fragments targeting ROR1 and CD3 has shown promising *in vivo* activity both alone and in combination with ICIs in PD-1 resistant tumor xenograft mice models with an acceptable toxicity profile (Wu et al., 2024).

Another way to harness the immune system against BC could be the generation of patients-derived activated T cells (ATCs). Activated T cells derive from patients polyclonal T cell population. These cells are expanded, and activated *ex-vivo*, and can be armed with an HER2xCD3 bsAb. Preliminary efficacy results in patients with metastatic BC can be derived from a phase I trial (Lum et al., 2015) that evaluated HER2xCD3 bsAb armed ATCs in 23 advanced patients. In this small trial 13 out of 22 evaluable patients had stable disease after 14.5 weeks from enrolment while the other nine had progressive disease. The median OS was 32·2 months for all patients. When the mOS was stratified according to the HER2 status; patients with HER2 3+ BC had an higher mOS if compared with HER2 0 patients (57·4 vs 27·4 months, respectively) (Lum et al., 2015).

Interestingly, the combination of ICIs and ICEs has proved to be effective at least in preclinical models.

T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT) and its ligand poliovirus receptor (PVR) have emerged as novel and promising target in cancer immunotherapy (Rousseau et al., 2023).

Poliovirus receptor is highly expressed on TNBC cells and its levels correlates with a dismal prognosis and poor outcomes (Boissière-Michot et al., 2022).

Consequently, anti-TIGIT have been developed to block the biological interaction between PVR and TIGIT to enhance cell killing by the immune system (Boissière-Michot et al., 2022). Indeed, upon binding to PVR, TIGIT suppresses the immune response against cancer cell. Additionally, anti-TIGIT has proved to increase the cytotoxic activity of a CD3xEGFR BiTE in patients with EGFR-expressing TNBC (Stamm et al., 2019).

As previously mentioned, TME can play a crucial role in breast cancer progression and resistance to conventional immunotherapy (Gruosso et al., 2019). In fact, tumour microenvironment is influenced by the interplay between cancer and immune cells and among the latter, TAMs represent a prominent subpopulation that largely participate in the immune response since they can comprise over 50 % of the total infiltrating immune cell (Qiu et al., 2018).

Tumour associated macrophages can be functionally divided into M1 and M2 with the first implicated in tumour-killing functions and the latter in tumour promoting functions (Choi et al., 2018).

Classically, during tumour progression, cancer cell polarize TAMs differentiation towards the M2 phenotype that becomes dominant in the TME and enhance tumour cell invasion, proliferation, angiogenesis, and cancer stemness (Choi et al., 2018).

In this scenario, BDC-3042, a novel antibody directed against dectin-2 (CLEC6A), a receptor expressed on TAMs and involved in antigen processing and antifungal immune responses(Aghaei et al., 2024), has proven to efficiently re-direct and activate them against cancerous cell promoting the repolarization of M2 into M1 macrophages and the secretion of proinflammatory mediators (eg. TNFa, IL-1b, and CCL3/4) involved in tumour cell killing(Kenkel et al., 2022).

A phase I/II trial is now investigating BDC-3042 role, also in combination with pembrolizumab, in leverage the power of immune cells in stimulating cancer cell killing in TNBC by engaging M2 TAMs and tumour cells and promoting cytokine release in the TME (US National Library of Medicine, 2023).

Lastly, in order to improve ICEs tolerability, a novel class of ICEs that are specifically activated within the TME, have being developed.

The technology behind these new molecules relies on the insertion of a peptide connected to the antigen recognition region of ICEs via a protease cleavable linker. This peptide masks the antigen recognition region until the ICE does not reach cancer cells, preventing its activation in the blood stream and/or in healthy tissue that share the same TAAs. When this peptide-masked ICEs reaches the tumour, proteases in TME cut the linker releasing the drug (Geiger et al., 2020).

8. Conclusion

Breast cancer has historically been considered poorly immunogenic compared to other hematologic and non-hematologic malignancies (Dieci et al., 2016; Jiang and Shapiro, 2014). However, several studies have demonstrated that a subset of patients, especially those with TNBC, may derive considerable benefits when treated with ICIs both in early and advanced settings (Cortes et al., 2020; Emens et al., 2021; Schmid et al., 2020). Despite that, immunotherapy has a large room for improvement in BC. Immune cell engagers represented a major innovation for the treatment of some haematologic malignancies but many questions are still unanswered about their use in solid tumours (Fucà et al., 2021; Shin et al., 2022). Although proof of principle for bispecific antibodies exists in establishing and promoting an effective engagement between cancer cells and immune effectors (Huehls et al., 2015; Kontermann and Brinkmann, 2015; Ma et al., 2021b; Trabolsi et al., 2019), several factors have limited so far their implementation in the clinical practice (Ellerman, 2019; Labrijn et al., 2019; Mandikian et al., 2018b; Mazor et al., 2015; Spiess et al., 2015; Wang et al., 2019). Challenges that remain to be solved concern both on-target/off-tumour effects and pharmacological limitations.

These variables, that affect the safety and efficacy of first-generation ICEs, should prompt further research to optimise immune system-cancer engagement.

The immunosuppressive TME represents another challenge for ICEs development in solid tumours, including BC (Gruosso et al., 2019). Combination treatment with conventional chemotherapy, ICIs and other treatments could be required to convert the immune desert microenvironment into an inflamed one (Argilés et al., 2017; Chang et al., 2017b, 2017a; KUBO et al., 2018; Pullarkat et al., 1999; Repp et al., 2003; Stockmeyer et al., 1997; Tabernero et al., 2017).

By exploiting technological advances, it could be possible to refine ICE features, in order to overcome the pitfalls that have limited their use in BC treatment thus far. Whether these improvements will be implemented, ICEs could represent a game-changer in the treatment of BC, hopefully establishing the same revolution as in haematologic malignancies.

Critical view section

In this original review, we present and discuss a novel class of immunotherapeutics known as immune cell engagers (ICEs), focusing on their investigation and development in the field of breast cancer. To the best of our knowledge, this work represents the first comprehensive review of the literature that describes both the mechanisms of action and the preclinical and clinical data of ICEs development, with a dedicated analysis of their efficacy and safety profiles in breast cancer patients.

Our review aims to inform the breast oncology community about the potential role of this innovative class of agents in breast cancer therapy, depicting their potential strengths and pitfalls. Indeed, in our work, we outline the current state of these novel compounds and critically examine their limitations. Additionally, we address the challenges associated with their development and application, including potential adverse effects and the need for optimized delivery mechanisms.

In conclusion, our analysis provides a thorough and balanced view of immune cell engagers, emphasizing their potential impact on breast cancer treatment. We hope this work will highlight the therapeutic potential of ICEs, thus stimulating further research and clinical trials in this rapidly evolving field.

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