

Review Article,

## Future Directions on Antibody-Drug Conjugates in Her-2 Negative Breast Cancer: A Comprehensive Review of New Agents

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### Abstract:

**Background:** Breast Cancer is the most frequent malignancy in women with a rising incidence. Antibody-drug conjugates targeting HER2 and TROP-2 are new tools already effective against breast cancer but ADCs targeting new transmembrane receptors are necessary to overcome the dependence on HER-2 pathway. New trials are emerging fast as new ADCs are under investigation.

**Methods:** We reviewed ClinicalTrials.gov and Pubmed for early clinical trials on new ADCs in clinical development for HER-2 negative breast cancer.

**Results:** Trials testing 12 new molecules were found. Pre-clinical studies, safety and results in phase I/II trials published of each new molecule were reviewed and described briefly. Side effects regarding ADCs with maytansine derivatives (DM1/4) or auristatins (MMAE/MMAF) payloads were described.

**Conclusion:** ADCs represent a practice-changing therapy for cancer treatment. New compounds against several new targets are expected to become part of clinical practice in the near future.

**Keywords:-** Breast Cancer, Antibody-Drug-Conjugate, HER-2 Negative Breast Cancer, Maytansine Derivatives, Auristatins

### Introduction:-

Breast Cancer (BC) is the most frequent malignancy in women with a rising incidence of 3,1% a year. In 2022 an additional 287.000 cases of breast cancer will occur in the United States [1]. This may be due to lifestyle, environmental factors, genetics and increasing use of hormone replacement therapy [2]. The biology of these tumors depend on their subtype and expression of hormonal receptors and HER-2. Breast cancer treatment has evolved through the years but still has a big impact in quality of life. There has been an urge to discover new and more effective drugs with less side effects and better tolerability profile. Overall survival is dependent on disease factors such as stage, tumor grade, estrogen receptor (ER), progesterone receptor (PR) and HER-2 expression. Since the 1990s there has been a decrease in breast cancer mortality and patients with this condition have been living longer due to

new treatment strategies developed in recent years [1,2]. Even in the metastatic setting the 5 year relative survival rate has increased from 75% (mid 1960-1970s) to 90% for those diagnosed from 2011 to 2017 and it is still rising [1,2].

A relatively new class of molecules has entered the list of drugs for treatment of BC: antibody-drug conjugates (ADCs). Here we review new ADCs in early clinical development for HER2-negative BC.

### **Structure and mechanisms of action of ADCs**

ADCs are a class of biopharmaceutical drugs highly effective against cancer cells. The idea behind these drugs was created over a century ago as an attempt to target cancer cells and spare healthy tissue [3]. These molecules carry a cytotoxic payload and target a pre-defined tumor surface antigen. When binded to this target, the compound is internalized and then releases the

payload interfering in the tumor cell cycle and inducing apoptosis.

ADCs are composed of three elemental parts (Figure 1): the monoclonal antibody agent, the linker and the cytotoxic payload. This tool has

been initially used against hematological cancers and recently has been adapted against solid tumors and breast cancer in particular [4].

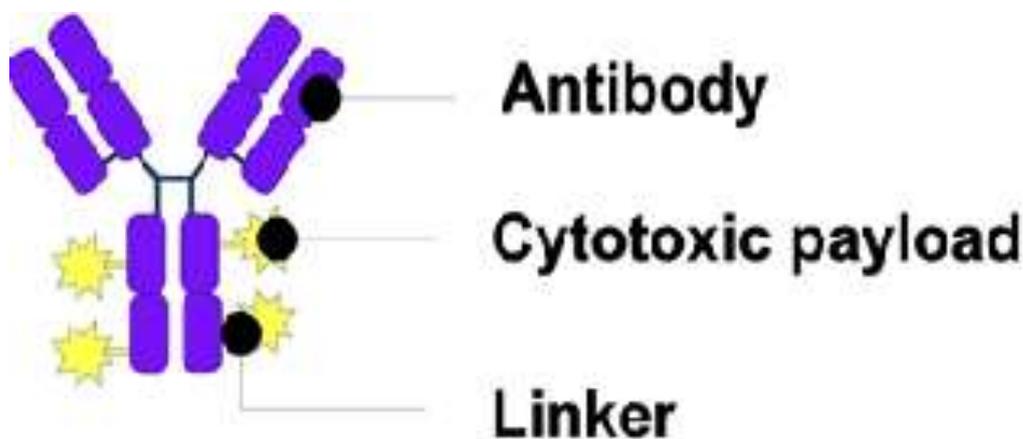


Figure 1. Structure of a Antibody Drug Conjugate

Initially a target antigen must be identified so that the drug can attach to it. This target must have a high expression in tumor cells but not in healthy cells, reducing side effects. In breast cancer, HER2 receptor has been the most studied target for the development of new monoclonal antibodies since it is 100-fold highly expressed in tumor cells when compared to healthy cells [5]. The target antigen should also be expressed on the outside part of the membrane cell, so it's possible to be recognized by the antibody.

After binding to the target the ADC is internalized and degraded by the lysosomal compartment, resulting in the releasing of the payload [5]. Currently most antibodies are developed with human immunoglobulin G (IGg) in order to minimize cross reactions [6].

The linker plays a central role in the operating mechanism of ADCs [4,6]. They are designed to attach the cytotoxic drug to the monoclonal antibody. It must be strong enough to keep the payload attached while in the blood circulation, avoiding the release of the drug outside the membrane cell, but sensible enough to recognize the trigger inside the cell to release the payload.

Linkers have been divided into non-cleavable or cleavable. The first was used in Trastuzumab-Emtansine (T-DM1) and exclusively releases the payload inside the cell's membrane. Cleavable linkers are currently more commonly used. They are more sensitive to environmental differences and can also function properly even if they are not internalized [3]. Tumoral microenvironment, changes in pH or expression of different immune cells around the tumor can activate the ADC and detach the drug outside the cells, conferring a greater effect. This mechanism is particularly important for solid tumors since they tend to have a heterogeneous expression of target antigen among cells (Figure 2) [7,8].

The third part of ADCs, the cytotoxic drug, also known as payload, is responsible for inducing apoptosis and tumor cell death. It may occur through different mechanisms (DNA damage, apoptosis induction or microtubules interference). It's also the cause of most of the ADC toxicity and side effects [9,10]. Most compounds currently being tested utilize maytansine derivatives (DM1/DM4) or auristatins (MMAE/MMAF).

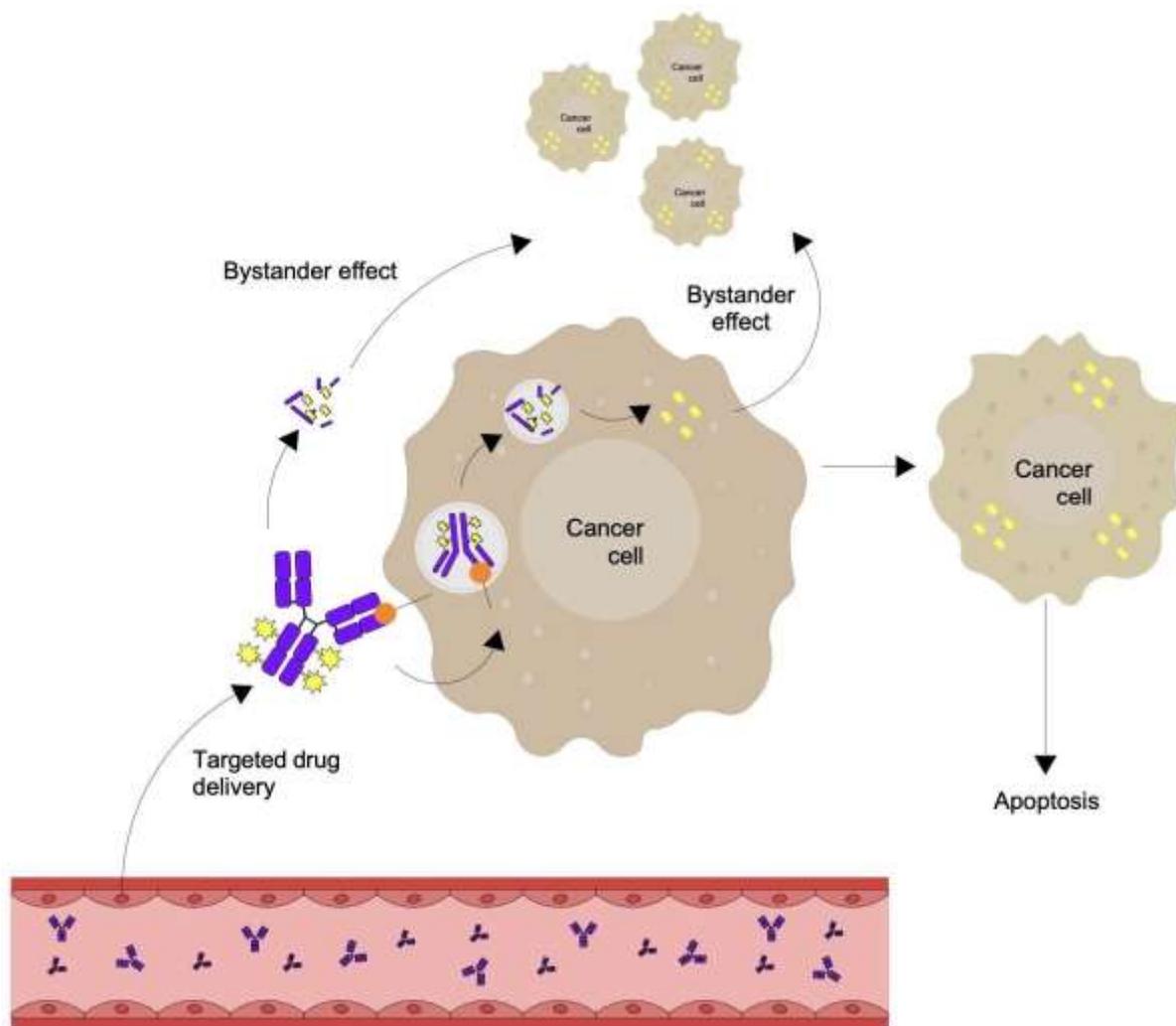


Figure 2: As the ADC attaches to the membrane receptor it is internalized and the payload is released inside the lysosome leading to cancer cell apoptosis through different mechanisms. The free payload inside the cell's membrane can be released to the microenvironment entering other cancer cells that can be antigen-negative through passive diffusion, endocytosis or specific transporters. This mechanism known as "bystander effect" allows a wider range of action and anti-tumor efficacy. In addition, when using

non-cleavable linkers, the payload can be released before reaching the cell membrane triggered by lower pH and contact with proteases from the tumor environment. This bystander effect overcomes tumoral heterogeneity of the ADC's target and increases the efficacy of this class of drugs. In clinical practice there are four ADCs currently approved by the FDA (Table 1): Sacituzumab-Govitecan, T-DM1, Trastuzumab-Deruxtecan (T-Dxd) and Trastuzumab Duocarmazine [3]

Table 1. Currently approved ADCs for breast cancer

.Medication	Target	Payload	Linker	Antibody	Approval
Trastuzumab-Emtansine(TDM1)	HER-2	maytansine (DM1)	Noncleavable	Humanized mAb	2013
Trastuzumab-Deruxtecan(TDXd)	HER-2	topoisomerase I inhibitor (DXd)	Cleavable	Humanized mAb	2019
Sacituzumab-Govitecan	TROP2	Topoisomerase II inhibitor irinotecan	Cleavable	Humanized mAb	2023

Trastuzumab Duocarmazine	HER-2	seco-DUBA	Cleavable	Humanized mAb	2023
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Trastuzumabe-Emtansine (T-DM1) was the first ADC approved for solid tumors. It was granted approval on the basis of EMILIA trial [11] for treatment of metastatic HER-2 positive breast cancer. Afterwards it was also approved for non-pCR patients after neoadjuvant therapy with early HER-2 positive breast cancer, on the basis of the KATHERINE trial [12]. When compared to capecitabine it showed a better toxicity profile being the grade 3 adverse event thrombocytopenia and elevation of serum concentrations of aspartate aminotransferase and alanine aminotransferase [12].

Trastuzumab-Deruxtecan (T-DXd) is a second approved HER-2 targeting ADC. It was designed to have a more potent payload and bystander effect, differently from T-DM1. It was approved for metastatic HER-2 positive breast cancer and for HER-2 low metastatic breast cancer [13,14]. Recently T-DXd is being tested for several different tumors with HER-2 expression. On Destiny Breast-03 T-DXd improved PFS when compared to T-DM1 becoming the standard second-line therapy for HER-2 positive metastatic breast cancer [13]. Interstitial lung Disease (ILD) is the biggest concern when using this drug. Patients with pre-existing pneumonitis should not be offered this drug.

Sacituzumab-Govitecan (Sac-Gov) is an ADC composed of an antigen that binds to TROP-2, a cell membrane receptor that is expressed in multiple tumor types including breast cancer. It was approved for both triple-negative and HR+/HER-2 negative metastatic breast cancer after the results of the ASCENT and TROPiCS-02 trials, respectively [15,16]. Sac-Gov demonstrated a 51% grade 3 neutropenia alongside with 10% grade 3 diarrhea in both ASCENT and TROPiCS-02 [15,16].

Trastuzumab Duocarmazine was FDA approved in 2022 for HER-2 expressing metastatic breast cancer after disease progression on multiple lines of therapy or progression on T-DM1. It Demonstrated a benefit in disease free survival compared to physician's choice of therapy [17]. More than a third of patients had to discontinue this medication due to eye or pulmonary disorders. The most observed eye-related events were conjunctivitis (38.2%) and keratitis (38.2%).

Fatigue (33.3%) and Interstitial lung disease/pneumonitis occurred in 7.6% of patients on the drug, including 2 deaths.

**Objective:**

The objective of this comprehensive review is to identify promising new ADCs for HER-2 negative breast cancer that may be incorporated in clinical practice in the future and shed light on ongoing trials involving this new class of drugs.

**Eligibility Criteria:**

We included only trials testing ADCs for HER2 negative (0+/3) or HER2 low (1+/3 or 2+/3 ISH Negative) breast cancer either early or metastatic. Trials recruiting patients or finished with unpublished results were included. Our search included English language trials only.

**Search Strategy and Selection Criteria:**

We searched Clinicaltrials.org at September 7th 2023 with no previous limit on date for ongoing or completed trials without published results using the terms "Breast Cancer" as Condition and "Antibody-Drug Conjugate" OR "ADC" OR "Antibody drug conjugate therapy" as Intervention/Treatment. We also searched PubMed on September 7th 2023 for articles published with ongoing trials limited to the last 5 years (September 2018). We used the Mesh Terms "Breast Cancer" AND "Antibody-Drug Conjugate" OR "Antibody drug conjugate therapy" filtering for Clinical Trials during this period. References from the articles retrieved by the search were reviewed by one reviewer who analyzed previous publications on the ADCs investigated. Prisma 2020 search methodology was used to guide the searching method [18].

**Results:-**

Among 137 trials on ClinicalTrials.org and 265 results on PubMed we found 12 Antibody Drug Conjugate drugs being tested that fulfilled the inclusion criteria. One trial (NCT05498597) was excluded for not having presented pre-clinical data or any published information on the ADC tested. The flow diagram for the review strategy is presented in Figure 3. The characteristics of each trial found are presented in Table 2.

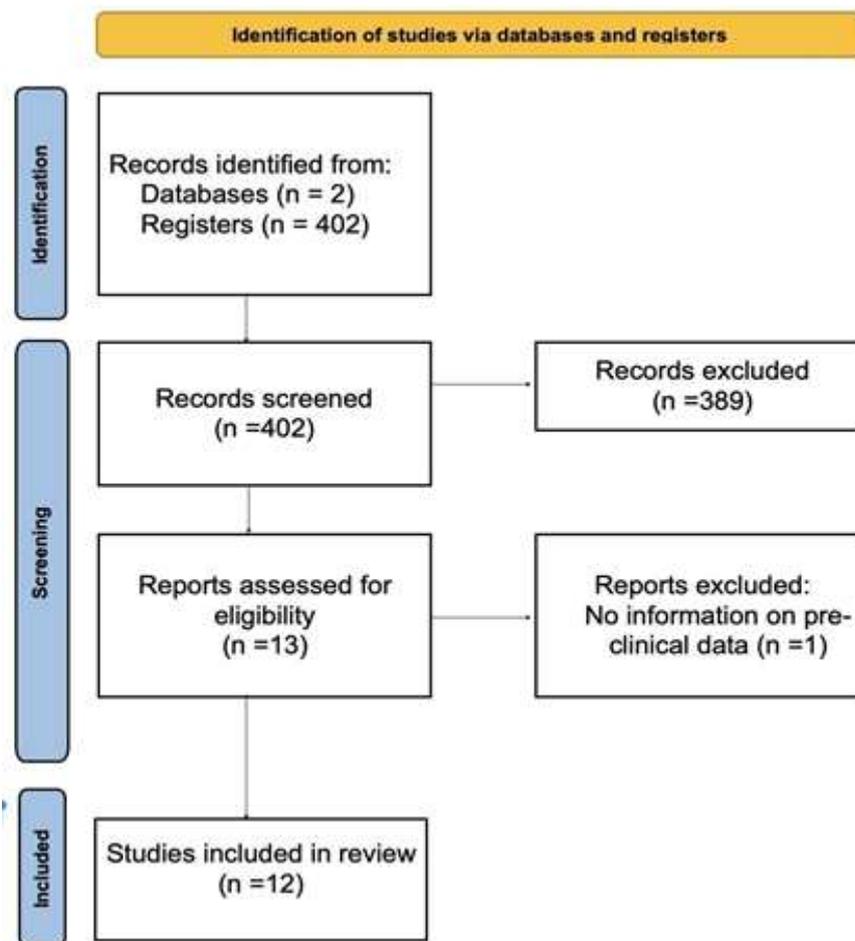


Figure 3: Flow diagram on the review strategy

Table 2. ADCs currently in clinical development for HER-2 negative Breast Cancer

Medication	Target	Payload	Linker	Antibody	No.	Countries recruiting	Intervention	Population	Activation Date	Phase trial
Patritumab Deruxtecan (NCT04965766)	HER3	Topoisomerase I inhibitor (DXd)	Cleavable	Humanized mAb	170	France	Patritumab Deruxtecan (U3-1402)	Metastatic Breast Cancer	May-11, 2021	Phase 2
MRG002 (NCT04742153)	HER-2	MMAE	Cleavable	Humanized mAb	66	China	MRG002	HER-2 low Metastatic Breast Cancer	May-13, 2021	Phase 2
Ladiratumumab vedotin (NCT03310957)	LIV-1	MMAE	Cleavable	Humanized mAb	186	United States, Germany, Republic of Korea, Spain	Ladiratumumab vedotin and Pembrolizumab	Triple-Negative Breast Cancer	February 27, 2018	Phase 1 Phase 2
AZD8205 (NCT05123482)	B7-H4	(topoisomerase I inhibitor)	Cleavable	Humanized mAb	125	United States, Germany, Republic of Korea, Spain	CX-2009 CX-072	Metastatic Breast Cancer	December 29, 2020	Phase 2

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Praluzatamab Ravtansine (NCT04596150)	CD 166	DM4	Cleavable	Humanized mAb	125	United States, Germany, Republic of Korea, Spain	CX-2009 CX 072	HR positive HER-2 negative Metastatic Breast Cancer	December 29, 2020	Phase 2
RC48-ADC (Disitamab-Vedotin) (NCT04400695)	HER-2	MMAE	Cleavable	Humanized mAb	366	China	RC48-ADC	HER-2 low metastatic Breast Cancer	September 29, 2020	Phase 3
XB002 (NCT04925284)	TF (tissue factor)	MMAE	Cleavable	Humanized mAb	561	United States, Spain	XB002	HR positive HER-2 negative Metastatic Breast Cancer	June 7, 2021	Phase 1
BA 3021 (NCT03504488)	ROR2	MMAE	Cleavable	Humanized mAb	420	United States, Germany, Greece, Hong Kong, Italy, Poland, Spain, Taiwan	CAB-ROR2-ADC	Triple Negative Breast Cancer	June 27, 2018	Phase 1 Phase 2
Trastuzumab Rezetecan (NCT05814354)	HER-2	SHR9265	Cleavable	Humanized mAb	530	China	SHR-A1811	HER-2 ultra-low and HER-2 negative	June 30, 2023	Phase 3
Datopotomab Deruxtecan (NCT05460273)	TROP-2	Deruxtecan	Cleavable	Humanized mAb	119	China	Datopotamab Deruxtecan (Dato-DXd)	Triple-negative Breast Cancer	July 11, 2022	Phase 1 Phase 2
Farletuzumab ecteribulin (MORAB-202) (NCT04300556)	Folate Receptor - $\alpha$	Eribulin	Cleavable	Humanized mAb	66	United States, France, United Kingdom	Farletuzumab ecteribulin	Triple-Negative Breast Cancer	August 6, 2020	Phase 1 Phase 2
Glembatumumab - Vedotim (CDX-011) (NCT01997333)	Glycoprotein non-metastaticB	Monomethyl auristatin E	Cleavable	Humanized mAb	327	United States, Australia, Belgium, Canada, France, Germany, Italy, Spain, United Kingdom	Glembatumumab-Vedotim	Triple-Negative Metastatic Breast Cancer	November, 2013	Phase 2

**Patritumab Deruxtecan (U3-1402)**

HER3 is part of a transmembrane tyrosine kinase family which is responsible for intracellular signaling, especially in PI3K/AKT and MAPK/ERK pathways. It is expressed in several types of cancer. The overexpression of HER3 has been associated with poor outcomes. Patritumab-deruxtecan (HER3-DXd) is a HER3-targeting antibody that binds and inhibits HER3 receptors [19]. In a phase I trial the medication was tested in metastatic non-small cell lung cancer with mutation on EGFR previously treated. After at least one dose this ADC showed an incidence of

37% partial responses and 33% stable disease was reported. There was a low rate of discontinuation for adverse events (9%) [20].

In a single-arm trial with early breast cancer patients exposed to HER3-DXd had significantly increased the number of CelTIL Score after a single dose administration. CelTIL is associated with increased immune recognition of cancer and has a hole in tumor response to oncology treatments [21].

HER3-DXd is being investigated under two different scenarios. First, it will be tested in a phase II trial with luminal or triple negative breast

cancer with intracranial metastasis with expectation to start in 2023 (NCT05865990). Second, the ICARUS-BREAST (NCT04965766) is investigating predictors of response and resistance to HER3-DXd in patients with HR+/HER2- advanced breast cancer. Results are still being analyzed.

#### **MRG002:**

This is a new ADC first designed for HER-2 hyperexpressed cells. The drug showed superiority in pre-clinical tests compared to T-DM1 and Trastuzumab. It is a new vcMMAE-based anti-HER2 ADC. This payload causes defects on the PTEN-PI3K/AKT pathway through microtubule inhibition. It showed significant anti-tumor activity in vitro and in vivo for breast and gastric cancer [22]. A phase I clinical trial is testing the efficacy of this new drug on different primary tumors, among them HER-2 low breast cancer [4] (NCT04742153).

#### **Glebatumumab Vedotin (CDX-011)**

Glebatumumab Vedotin (CDX-011, CR011-vcMMAE) is an ADC developed to target the extracellular domain of GPNMB (glycoprotein non-metastatic B) which is expressed in multiple malignancies including breast cancer. This expression is linked to aggressive phenotypes, angiogenesis and cellular growth. The payload is composed of MMAE.

Glebatumumab-Vedotin was tested in a phase I/II randomized trial [23]. Patients with triple-negative advanced breast cancer with overexpressing GPNMB were randomized to receive the ADC or capecitabine. The expression of GPNMB was tested upfront and was positive in up to 71% in triple-negative breast cancers. The result of the phase I/II trial was published recently and showed no benefit in PFS over capecitabine. At this time, there are no ongoing trials with this drug.

#### **AZD8205**

This ADC is composed by a human anti-B7-H4 antibody-conjugate connected to a cleavable linker and topoisomerase inhibitor. B7-H4 is a transmembrane protein that inhibits activated T cells which enhances tumor activity and is associated with worse prognosis. It's easily found in cholangiocarcinoma, breast, ovarian and endometrial cancers [24].

The ADC was tested alone and in combination with the PARP1-selective inhibitor AZD5305, in

preclinical models. It has already shown clinical activity in in vitro models. In preclinical trials it demonstrated an acceptable safety profile. AZD8205 is currently being tested on a phase I trial in monotherapy in pre-treated patients with cholangiocarcinoma, breast, ovarian and endometrial cancers. Expression of B7-H4 will be evaluated before, during and after the treatment (NCT05123482).

#### **Ladiratumumab vedotin (LV)**

LIV-1 is a transmembrane protein with zinc transporter and metalloproteinase activity and has a moderate/high level in most breast malignancies. It is attached to a MMAE payload. This ADC has been tested in a phase I trial with HR+/HER-2 negative patients previously exposed to more than one line of endocrine therapy in a metastatic setting or pre-exposed to chemotherapy in triple negative breast cancer. This trial showed that LIV-1 protein is expressed in most metastatic breast cancer patients. The trial reported a 32% objective response rate with a partial response rate of 21% in heavily pretreated MBC patients [25]. Another phase I trial is being developed in order to evaluate safety of the medication (NCT01969643). The combination of pembrolizumab and ladiratumumab in locally advanced / metastatic triple-negative breast cancer is being tested in a phase I/II trial (NCT03310957) . Results are expected by 2024.

#### **Praluzatamab Ravnansine (CX-2009)**

Praluzatamab Ravnansine is a probody drug conjugate (PDC) that targets CD 166. This antigen is largely expressed in both normal and tumor cells. It's a transmembrane protein that gives the tumor a greater invasive potential.

PDC is a new class of ADCs that are designed to be activated only with proteolytically substances inside the tumor cell, and remain inactivated in the circulation. This new mechanism was developed to avoid payload release outside the cell's membrane. This drug uses a potent microtubule inhibitor payload called S-Methyl (metabolite of DM4). A phase I/II study analyzed safety and tolerability and activity with this drug in monotherapy in patients pre-selected with high-expressing CD 166 identified by immunohistochemistry developed by a central lab of the study. This trial enrolled 99 patients with different primary tumors, among them HR+/HER-2 negative breast cancer (28 patients of the

cohort), HER2+ (6 patients) and triple-negative (11 patients). The study showed an acceptable safety profile with this ADC [26].

A new study is being developed to test this medication in a phase II trial with or without another in-test agent, CX-072, in advanced HER-2 negative, HR positive breast cancer (NCT04596150).

#### **RC48-ADC (Disitamab-Vedotin)**

Disitamab-Vedotin is another ADC composed of a MMAE payload and a HER-2 receptor targeting antibody. This drug was already tested in HER-2 gastric and urothelial cancers with promising efficacy [27]. It is now being tested in two different trials, one of them being a randomized phase III trial for HER2 low metastatic breast cancer previously treated with one or two lines of chemotherapy regimens (NCT04400695).

#### **XB002**

XB002 is an ADC composed of a high-affinity tissue factor (TF)-directed human monoclonal antibody conjugated to an auristatin payload. TF is overexpressed in different solid tumors and is associated with worse prognosis. XB002 demonstrated activity in xenograft models and solid tumors. This ADC was tested in a phase I trial, JEWEL-101, with different types of tumors, among them HR+/HER-2 negative breast cancer [28]. Initial safety results were published and ocular adverse events were experienced by 42% of patients. No grade 4 or 5 event was reported [29]. Its final results have not been published to date (NCT04925284).

#### **CAB-ROR2-ADC (BA3021)**

This ADC binds to ROR2 (tyrosine kinase orphan receptor 2), which in pre-clinical trials suggested to be useful as a target to anti-tumor medications, especially in NSCLC [30]. This novel ADC is built with a cleavable linker and MMAE payload. A phase I/II trial is recruiting patients with triple negative breast cancer among other types of neoplasms previously treated (CA-ROR2). Efficacy will also be tested combining this molecule with a PD-1 inhibitor or alone (NCT03504488)

#### **SHR-A1811 (Trastuzumab Rezetecan)**

This is a HER-2 targeting ADC composed of Trastuzumab, a cleavable linker and SHR9265, a topoisomerase I inhibitor payload. There is an ongoing Phase I/II trial that tries to test this ADC on ultra-low and non-expressing HER2 breast

cancers, while another phase III trial is recruiting for HER-2 positive metastatic breast cancer (NCT05814354). A phase I trial with HER-2 positive patients showed acceptable side effects and safety with the drug [31].

#### **Datopotomab-Deruxtecan**

This ADC is composed of a topoisomerase inhibitor (Deruxtecan). It binds to the trophoblast cell surface antigen 2 (TROP-2) membrane receptor which is highly expressed on several epithelial tumors and correlates with poor prognosis. TROP-2 is involved in the MAPK and PI3K/AKT pathway and stimulates cell proliferation, migration and invasion of cancer cells [32].

The TROPION-Breast01 was the first phase III trial designed with Dato-DXd for breast cancer. This study met its primary endpoint improving progression-free survival (PFS) compared with chemotherapy [33].

TROPION-Breast02 (NCT05374512) for advanced metastatic breast cancer and TROPION-Breast03 (NCT05629585), which combines Durvalumab with Dato-DXd for early triple-negative breast cancer, are under evaluation and results are waited.

An ongoing Phase I/II trial was also designed to evaluate the efficacy, safety, pharmacokinetic, and immunogenicity of Dato-DXd in Chinese participants with advanced or metastatic solid tumors, with a cohort including Triple-negative Breast Cancer (NCT05460273 -TROPION-PanTumor02).

#### **Farletuzumab ecteribulin (MORAB-202)**

This ADC targets anti-folate receptor alpha (FRA). Folate receptors are typically absent from normal tissues while in ovarian, lung and breast cancer it tends to be overexpressed. MORAB-202 carries a payload of eribulin (microtubule inhibitor) connected to a cleavable linker. A Phase I trial that tested this drug in unselected patients for FRA expression with advanced solid tumors reported an acceptable adverse events profile, with none grade 3 hematological event or grade 3 eye disorder [34]. Now, MORAB-202 is being tested in a phase I/II trial with solid tumors having triple negative breast cancer among them (NCT04300556).

### Discussion:-

Since the first FDA approval of an ADC, Gemtuzumab ozogamicin in 2000 for myeloid acute leukemia, over 100 ADC molecules have been studied. Recently ADCs have gained attention from scientists and pharmaceutical companies as possible candidates to substitute conventional chemotherapy for solid tumors [1]. Several ADCs have proved to be more effective than chemotherapy, but systemic toxicity is still a major concern. ADC's side effects depend mainly on the type of payload they carry [35]. Most compounds in clinical testing carry maytansine derivatives (DM1/4) or auristatins (MMAE/MMAF). These are both microtubule inhibitors that cause cell cycle arrest at G2/M phase [9]. ADCs were designed to concentrate the payload into or nearby cancer cells, reducing side effects, but this mechanism is still not perfect. MMAE ADC's demonstrated bone marrow toxicity with anemia, neutropenia and peripheral neuropathy. In vitro tests showed that proteases secreted by differentiating neutrophils interact with MMAE based ADCs, permitting the release of the payload nearby and causing neutrophils to decrease. Indeed, as seen in the Ascent trial [16], the incidence of any grade of neutropenia was 63% with Sac-Gov vs. 43% with chemotherapy. Anemia was also increased: 34% vs. 24% with chemotherapy. Sac-Gov for HR+/HER2-negative metastatic breast cancer showed more grade 3 adverse events when compared to chemotherapy: neutropenia (51% vs. 39%) and diarrhea (10% vs. 1%), respectively [36]. T-DXd also presents a similar incidence of myelotoxicity; on Destiny-03, 42,8% patients presented neutropenia being 19,1% of grade 3 or more [9,13,37]. With MMAF/DM4 payloads there's also a concern for ocular toxicity. Decreased visual acuity, keratitis and blurred vision were described in patients using these compounds. The mechanism is yet unknown since this phenomenon was seen with both cleavable and non-cleavable linkers. For instance, a phase I trial of an MMAF ADC targeting CD70 for non-Hodgkin lymphoma or metastatic renal cell carcinoma reported a 23% grade  $\geq 3$  eye disorder and 57% of any grade [38]. These uncommon side effects should raise concern on prescribers since DM4/MMAE compound payloads are common on ADCs. In this review, DM4/MMAE payload is used in 7 of 12 ADCs identified. As these compounds move further to clinical practice, clinicians will need to be familiar with these

toxicity profiles.

The linker also exhibits a central role on intensity of adverse reactions. Cleavable linkers are currently the most used since they have demonstrated superior efficacy especially attributed to the bystander effect, which is absent in non-cleavable compounds. This mechanism permits the free payload to diffuse to the local tumor environment, affecting tumor cells that do not necessarily express the receptor targeted by the ADC, which is known as tumor heterogeneity [3]. The easily-releasing mechanism also makes cleavable linkers more susceptible to plasma releases before reaching the cell, which increases toxicity [37]. This premature release is the cause of peripheral neuropathy associated with ADCs specially with MMAE and DM4 payloads and is an important cause of interruption of the drug [35,39].

As noted in this review there's still severe adverse reactions with ADCs. Finding the adequate receptor to internalize the payload and developing a linker that correctly releases the payload inside the cell's membrane seems to be the key to creating an "almost perfect" cancer bullet [40].

Few ADCs in our search have more mature results and are expected to become alternatives to cancer treatment in the future. Disitamab-Vedotin (RC48) is already under investigation in a phase III randomized trial. RC48 binds to HER-2 but in a different epitope than Trastuzumab, which is expected to have better molecular affinity [41]. Its activity was demonstrated in different scenarios: an 21% overall response rate (ORR) in HER2-low pretreated gastric cancer [42] and a 40% ORR in urothelial carcinoma [27]. In a phase II trial with 125 patients with pretreated HER-2 positive gastric tumors RC48 achieved a 24,8% ORR, a 4.7 months median duration of response (DOR) and a median progression free survival (PFS) of 4.1 months [43]. It's now being tested for HER-2 low metastatic breast cancer. Ladiratumumab Vedotin (LV), a different ADC targeting LIV-1 receptor also gained attention since pre-clinical data suggested that it increases immune response to the tumor and leads to immunogenic cell death [44]. Now LV is being combined to an anti-PD-L1 Pembrolizumab in an ongoing phase I/II trial for previously untreated metastatic triple-negative breast cancer (NCT03310957).

Similarly to Sac-Gov, Datopotumab-Deruxtecan, is a Trop-2 receptor targeting ADC, and has recently gained attention with first results of the

phase III TROPION-Breast01 trial [33]. Significant improvement in PFS compared to chemotherapy in patients with advanced HR+/HER2-low or negative pretreated breast cancer were shown. This ADC is also under investigation in several other scenarios.

HER-3 targeting also brought attention to scientists as the HER-2 pathway was studied [45]. ErbB/HER receptor tyrosine kinases are a group of transmembrane receptors that play a role in the development of tumors, with HER-2 being the most important representative. HER-3 is also a transmembrane receptor and is overexpressed in 42% of patients with solid tumors. It's associated with worse outcomes and indicates resistance to different oncological drugs [46]. Blocking the HER2 receptor is compensated by the increase of other receptors from the ErbB family, specially HER-3, in order to evade cell death [19]. Recently ADCs against HER3 were designed as a new method of cancer control. Pre-clinical trials showed that it is a safe and promising pathway to be explored [21]. As an example, Patritumab-Deruxtecan was tested in different heavily pretreated advanced breast cancer subtypes, including HR+/HER2-negative, triple-negative and HER2-positive breast cancer. A durable efficacy independently from HER-3 expression levels and a manageable safety profile was identified [47].

One of the challenges for clinicians will be to understand how to best sequence these compounds since many of them have similar targets and/or payloads [48]. Also the possibility of combining different ADCs with existing immunotherapies, targeted therapies and even between ADCs will bring a new wave of therapeutic research, and hopefully success, for patients.

### **Conclusion:-**

ADCs are a new tool for cancer treatment with an innovative mechanism of action. Their use in HER-2 overexpressed breast cancer has been a story of success. In HER-2 negative tumors, targets such as TROP-2 and HER-3 are being extensively investigated. Efficacy has been shown to improve with some ADCs, but uncommon and new side effects have been seen as well. As data emerges it seems inevitable that new ADCs will enter in our routine as treatment options for patients with HER-2 negative advanced breast cancer.

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### **Compliance with Ethical Standards**

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