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Breast cancer: key clinical advancements

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Review

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Chimeric antigen receptor T-cell therapy for breast cancer

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One of the main reasons that researchers pay enormous attention to immunotherapy is that, despite significant advances in conventional therapy approaches, breast cancer remains the leading cause of death from malignant tumors among women. Genetically modifying T cells with chimeric antigen receptors (CAR) is one of the novel methods that has exhibited encouraging activity with relative safety, further urging investigators to develop several CAR T cells to target overexpressed antigens in breast tumors. This article is aimed not only to present such CAR T cells and discuss their remarkable results but also indicates their shortcomings with the hope of achieving possible strategies for improving therapeutic response.

Lay abstract: Breast cancer is the most dangerous and fatal malignancy among women worldwide. This disease has a heterogeneous behavior, that is, it can present different characteristics in various penitents. Consequently, treatments such as chemotherapies could not have the same satisfactory outcomes in all patients. The researchers are putting a huge amount of effort to discover treatments in a more specialized way for each individual. Cancer cells express specific antigens not present in normal cells, and this characteristic could be used to specialize breast cancer treatment. This feature is used in a novel method termed immunotherapy, through which human body immune cells are genetically engineered and enhanced in function to target the antigen-expressing cancer cells. CAR T-cell therapy is a new strategy in immunotherapy that harnesses the aforementioned technique. The results of such treatments were unprecedented in laboratory experiments; however, to use this method widely in humans, further investigation is necessary. In this review, comprehensive information about the CAR T-cell therapy as well as its laboratory and clinical results are discussed.

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Breast cancer is the most frequently diagnosed malignancy in women worldwide, with more than 2 million new cases reported in 2018. Additionally, it is a major cause of death in patients with advanced diseases in both developed and less developed countries [1].

Due to the disappointing results of conventional cytotoxic approaches in advanced stages of cancer, recurrence of this malignancy poses a great challenge [2]. Hence, novel approaches for breast cancer treatment are urgently needed. Immunotherapy in cancer has shown extremely significant advancement in recent years and was lauded by the journal *Science* in 2013 as the "Breakthrough of the Year" [3].

Immune cells have an elemental role in the human body defense mechanism [3]. Considerable evidence indicates that the body's immune system responds to cancer tumors and may generate long-term memory to any disease; however, it is apparent that the T cells generally do not create immune responses potent enough to eliminate established tumors or prevent metastasis. This incapability is because cancer cells can "hide" from immune cells by

down-regulation of tumor-specific antigens or major histocompatibility complex (MHC) molecules [4]. Nowadays, one of the most promising approaches focuses on harnessing an individual's own immune system through which the cancer cells are targeted again in a specific manner. Furthermore, it could hamper the spread of evolving malignant cells, triggering complete control over cancer, which poses the profit of staying executable even when conventional methods fail. To date, several adoptive T-cell immunotherapeutic strategies hold a valuable position among various immunotherapeutic approaches [3].

T-cell-based immune therapies that harness the immune system against cancer are generally based on two approaches. The first is tumor-infiltrating lymphocytes (TILs) that contains the separation of antitumor activated T lymphocytes from malignant tissues. In another method, unique T cells are developed to eliminate tumors via gene-therapy-based techniques [5]. There are two strategies for gene manipulation, T-cell receptor (TCR) and CAR gene rearrangements, that could provide applicable tools in the development of polyclonal T cells that target desired antigens [5]. The T cells are redirected through an antigen-specific TCR expression on the cell's surface; further, it would provide a recognition signal for T cells followed by a costimulatory signal controlling the T-cell behavior against tumor cells [6]. A TCR could target intracellular as well as extracellular antigen in the context of significant MHC presentation [7]. Chimeric antigen receptors or CARs may also bring several rewards in cancer immunotherapy field [8].

- The main advantage is that the single-chain Fv fragment variable (scFv) antibody fragment can be provided with higher affinities than TCRs [9];
- Because CAR T cells can be induced through generally activated polyclonal T cells, they could overcome the complication of preparation processes of tumor CD4+ and CD8+ T cells from isolation until reinjection into the patient's body [10];
- Although TCRs are limited to MHC-dependent peptide presentation to identify antigens, CARs overcome this restriction because the scFvs possess the capability of cell surface proteins recognition in their intact mode [11]. With the assistance of this feature, CARs can pass the mechanism, downregulating MHC-I molecule, through which tumors evade immune detection [12];
- CAR T cells aimed to target tumor-associated antigens may hold an advantage compared with mAbs because they infiltrate the tumor tissue and persist in the periphery over long periods [13,14];
- CAR T cells may cross the blood–brain barrier [15]. This characteristic is completely valuable when treating malignant lesions that affect the central nervous system.

In this review, based on clinical remarkable efficacy in treating solid tumors including breast cancer [16–21], we attempted to overview the to date experiments of breast cancer-targeted CAR T cells. As CAR-T-cell strategy comes to be satisfying, in the not-too-distant future, these kinds of therapeutics will be part of the usual treatment for breast cancer patients.

Clinical experience

The majority of preclinical studies in the CAR T-cell therapeutics field have focused on hematologic malignancies, but breast cancer has also been a potent section of the investigation since the early 2000s. CAR T cells which are designed to target multiple antigens involved in breast cancer progression, have approached clinical trials. Table 1 summarizes ongoing and completed clinical trials.

Antigen targets for CAR T-cell therapy of breast cancer

CAR T cells confront various complications in the breast tumor areas. To execute a satisfactory clinical consequence, CAR T cells have to overcome a bunch of progressively arduous challenges. First, they should be designed specifically for an antigen that makes a distinction between tumor and normal tissue. Then they could penetrate the tumor stroma. Once within the tumor, they must stand harsh tumor microenvironment and mediate cytotoxicity. Many studies have been performed on different targets with several structures and generations of CAR T cells (Figure 1) to measure their effect on breast cancer cells, which we discuss below.

CAR T-cell generations

1. The first generation of CARs was developed from scFv and only a single signaling domain derived from CD3ζ [22]. Receptor ligation of a first-generation CAR induces the release of antigen-specific cytokine and

Table 1. Summary of CAR T-cell clinical trials for breast cancer.

Target	Identifier	Phase	Status	Disease	Comments	Location
EpCAM	NCT02915445	1	Recruiting	• Malignant neoplasm of nasopharynx TNM staging distant metastasis (M) • Breast cancer – recurrent	CAR T cells recognizing EpCAM	• West China Hospital, Sichuan University, Chengdu, Sichuan, China
MET	NCT03060356	Early phase 1	Terminated	• Malignant melanoma • Breast cancer	T cells modified with RNA anti-cMET CAR	• University of Pennsylvania, Philadelphia, PA, USA
HER2	NCT03696030	1	Recruiting	• Malignant neoplasm • Metastatic malignant neoplasm in the brain • Metastatic malignant Neoplasm in the Leptomeninges • Breast cancer • HER2-positive breast cancer	Chimeric antigen receptor T-cell therapy	• City of Hope Medical Center, Duarte, CA, USA
MUC1	NCT02587689	1/2	Unknown status	• Hepatocellular carcinoma • Non-small-cell lung cancer • Pancreatic carcinoma • Triple-negative invasive breast carcinoma	Anti-MUC1 CAR T cells	• PersonGen Biomedicine (Suzhou) Co., Ltd. Suzhou, Jiangsu, China
MESO	NCT02580747	1	Unknown status	• Malignant mesothelioma • Pancreatic cancer • Ovarian tumor • Triple negative breast cancer • endometrial other • other mesothelin Positive tumors	Anti-meso-CAR vector transduced T cells	• Biotherapeutic Department and Pediatrics Department of Chinese PLA General Hospital Beijing, Beijing, China
TnMUC1	NCT04025216	1	Recruiting	• Non-small-cell lung cancer • Ovarian cancer • Fallopian tube cancer • Triple negative breast cancer • Multiple myeloma • Pancreatic ductal adenocarcinoma	• Biological: CART-TnMUC1 • Drug: Cyclophosphamide • Drug: Fludarabine	• The Angeles Clinic and Research Institute, Los Angeles, CA, USA Hospital of the University of Pennsylvania, Philadelphia, PA, USA • Sarah Cannon Research Institute, Nashville, TN, USA
C7R-GD2	NCT03635632	1	Recruiting	• Relapsed neuroblastoma • Refractory Neuroblastoma • Relapsed osteosarcoma • Relapsed Ewing sarcoma • Relapsed Rhabdomyosarcoma • Uveal melanoma • Phyllodes breast tumor	Genetic: C7R-GD2.CART cells • Drug: Cyclophosphamide • Drug: Fludarabine	• Houston Methodist Hospital, Houston, TX, USA Texas Children's Hospital, Houston, TX, USA
CEA	NCT04348643	1/2	Recruiting	• Solid tumor • Lung cancer • Colorectal cancer • Liver cancer • Pancreatic cancer • Gastric cancer • Breast cancer	CEA CAR-T cells	• Chongqing University Cancer Hospital, Chongqing, Chongqing, China
CEA	NCT02349724	1	Unknown status	• Lung cancer • Colorectal cancer • Gastric cancer • Breast cancer • Pancreatic cancer	Anti-CEA-CAR T cells	• Southwest Hospital of Third Military Medical University Chongqing, Chongqing, China
HER2	NCT02713984	1/2	Withdrawn (Reform CAR structure due to safety consideration	• Breast cancer • Ovarian cancer • Lung cancer • Gastric cancer • Colorectal cancer • Glioma • Pancreatic cancer	Anti-HER2 CAR-T cells	• Southwest Hospital of Third Military Medical University Chongqing, Chongqing, China

Data taken from search results from www.clinicaltrials.gov.

Table 1. Summary of CAR T-cell clinical trials for breast cancer (cont.).

Target	Identifier	Phase	Status	Disease	Comments	Location
CEA	NCT03682744	1	Active, not recruiting	• Peritoneal carcinomatosis • Peritoneal metastases colorectal cancer • Gastric cancer • Breast cancer • Pancreas cancer	Anti-CEA CAR-T cells	• Rutgers Cancer Institute of New Jersey New Brunswick, NJ, United States • Roger Williams Medical Center Providence, RI, USA
HER2	NCT03740256	1	Not yet recruiting	• Bladder cancer • Head and neck squamous cell carcinoma • Cancer of the salivary gland • Lung cancer • Breast cancer • Gastric cancer • Esophageal cancer • Colorectal cancer • Pancreatic adenocarcinoma	CAdVEC	-
CD133	NCT02541370	1/2	Completed	• Liver cancer • Pancreatic cancer • Brain tumor • Breast cancer • Ovarian tumor • Colorectal cancer • Acute myeloid and lymphoid leukemias	anti-CD133-CAR vector-transduced T cells	• Biotherapeutic Department and Pediatrics Department of Chinese PLA General Hospital Beijing, Beijing, China
huMNC2-CAR44	NCT04020575	1	Recruiting	• Metastatic breast cancer	Biological: huMNC2-CAR44 CAR T cells	• Fred Hutchinson Cancer Research Center Seattle, WA, USA
HER2	NCT02547961	1/2	Withdrawn (project terminated due to revision of local regulations)	• Breast cancer	HER-2-targeting CAR T-cell infusion	• Central laboratory in Fuda Cancer Hospital Guangzhou, Guangdong, China
CEA	NCT02416466	1	Completed	• Liver metastases	Biological: anti-CEA CAR T-cell device: Sir-Spheres	• Roger Williams Medical Center Providence, RI, USA
CEA	NCT02850536	1	Active, not recruiting	• Liver metastases	Biological: anti-CEA CAR T cells	• University of Colorado Hospital Aurora, CO, USA • Roger Williams Medical Center Providence, RI, USA
MET	NCT01837602	1	Completed	• Metastatic breast cancer • Triple negative breast cancer	Biological: cMet RNA CAR T cells	• Abramson Cancer Center of the University of Pennsylvania Philadelphia, PA, USA
MSLN	NCT02792114	1	Recruiting	• Breast cancer • Metastatic HER2-negative breast cancer	Drug: cyclophosphamide Biological: mesothelin-targeted T cells Drug: AP1903	• Memorial Sloan Kettering Cancer Center (Consent and follow-up only) Basking Ridge, NJ, USA • Memorial Sloan Kettering Monmouth (Consent and follow-up only) Middletown, NJ, USA • Memorial Sloan Kettering Bergen (Consent and follow-up only) Montvale, NJ, USA • (and 4 more)
NKG2DL	NCT04107142	1	Not yet recruiting	• Colorectal cancer • Triple negative breast cancer • Sarcoma • Nasopharyngeal carcinoma • Prostate cancer • Gastric cancer	Adoptive cell transfer of NKG2DL-targeting chimeric antigen receptor-grafted Gamma delta T cell	• Landmark Medical Centre Johor Bahru, Johor, Malaysia

Data taken from search results from www.clinicaltrials.gov.

Table 1. Summary of CAR T-cell clinical trials for breast cancer (cont.).

Target	Identifier	Phase	Status	Disease	Comments	Location
ROR1	NCT02706392	1	Recruiting	• Estrogen receptor negative • HER2/neu negative • Progesterone receptor negative • Recurrent adult acute Lymphoblastic leukemia • Recurrent mantle cell lymphoma • Refractory chronic lymphocytic leukemia • Stage IV breast cancer • Non-small-cell lung cancer • Triple-negative breast carcinoma	Other: laboratory biomarker analysis biological: ROR1 CAR-specific autologous T lymphocytes	• Fred Hutch/University of Washington Cancer Consortium Seattle, WA, USA

Data taken from search results from www.clinicaltrials.gov.

cytolytic function *in vitro;* nonetheless, T-cell proliferation and survival could not be sustained; therefore, to activate T cells entirely and prevent the apoptosis, the use of a costimulatory signal is of great importance.

2. Due to the completion of the CAR T-cell activation signal, second-generation CAR T cells were constructed comprising two or three costimulatory signal domains, for instance, CD28 and/or 4-1BB. Moreover, signaling domains from other costimulatory molecules, including CD27, CD134 (OX40), CD154 (CD40L), CD278 (ICOS) and CD244 (2B4) have also been successfully evaluated [23]. It has been indicated that human and mouse T cells modified with second-generation CARs (Figure 2) are notably more active and efficient when tested *in vitro* and in murine models [24–33].
3. The third-generation CARs provide the CAR T cells with a superior function, proliferation, suppression resistance or survival, for example, that were described as TRUCK CAR T cells [34,35]. TRUCK is a method by which the CAR T cells are directed through a transgenic product, attacking tumors invisible to them. Not only would they improve the T-cell activation, they would also engage other immune cells to target antigen-negative cancer cells invisible to CAR T cells; proposing an advantage for solid tumors treatment [36].

ERBB2

The ERBB2 overexpression/amplification in breast cancer patients has a negative effect on chemotherapy and endocrine therapy process leading to treatment failure with earlier relapse [37]. CARs targeting ErbB2 have been well studied in both *in vitro* and *in vivo* models [38–42]. Nevertheless, several boundaries have to be broken before using ErbB2 directed CAR T cells in the clinical level. ErbB2 CAR-modified T cells are composed of a scFv from murine mAb. Moreover, inclusion of a costimulatory signal through the TCR/CD3 part is essential for effective activation of T cells, both CD3ζ and CD28. As was demonstrated in a study by Hu *et al.*, the CD3 ζ-CD28 complex is required for IL-2 secretion [43].

Wang *et al.* designed a CAR that stimulates antitumor immunity in syngeneic mice; IFN-γ and perforin have a fundamental effect on a complete antitumor response, highlighting the essential role of cytotoxicity and cytokine secretion in tumor elimination. However, after 200 days, the gene-modified T cells did not persevere. The substitute mice memory response protects the host against tumor rechallenge [8].

Munisvaradass *et al.* developed a second-generation CAR (ERBB2 scFv-CD8+-CD28-CD3-ζ). In other words, they manipulated a heterogeneous cell complex consisting CD8+ and CD4+ T cells to augment cytolysis [37]. Then, CAR T-cell functionality was studied by viability and apoptosis assays in SKBR3 cells. The results indicated that CAR T cells, not non-transduced T cells, were able to show remarkable cell death in SKBR3 cancer cells after 72 h [43,44]. High CD69 and CD25 expression, the production of the cytokine IFN-γ by CD4+ Th1 helper cells, as well as CD8+ cytotoxic T cells were evident. The ability to recognize antigen (ErbB2) in a MHC-independent manner assists CAR T cells to have an efficient cytolysis of target cells and produce remarkable levels of IFN-γ [37].

In one study, Zhao *et al.* [45] constructed a CAR against ErbB2 via linking a single-chain Ab derived from the humanized mAb, 4D5 Herceptin (trastuzumab) T cell signaling domains derived from CD28 and the CD3ζ, named as MSGV-4D5-28Z (4D5-28Z). The results of the investigation showed that ErbB2 expression was observed in breast tumor lines easily and 4D5-28Z-transduced human peripheral blood lymphocytes (PBL) had the potential

to specifically eliminate ErbB2 positive tumor cells. Unfortunately, they found a gradual transgene decrease for PBLs transferred with this 4D5 CAR related to activation-induced cell death (AICD) of transduced T cells with low levels of ErbB2 expression. Loss of CAR expression was not observed when the CD3ζ signaling domain of the CAR was mutated, proposing that the CD3ζ signaling led to transgene decrease. To enhance the efficacy of this CAR T cell, they considered using 4-1BB domain. The cytoplasmic domain of human 4-1BB could be placed between the CD28 and CD3ζ (construct 28BBZ) or after CD3ζ (construct 28ZBB). The inclusion of the 4-1BB intracellular signaling domain could increase CAR efficacy due to the T-cell proliferation, cytokine secretion, upregulation of anti-apoptotic genes and prevention of AICD [46–49]. Furthermore, PBLs transduced with 4D5-CD8-28BBZ displayed a higher functionality compared to PBLs transduced with 4D5-28Z, considering the remarkable smaller tumor size after treatment.

In the previous study, although the use of 4-1BB was considered beneficial, the severe effects of them are also of concern since ERBB2 has the lowest expression in normal cells, hence, precise measures must be considered [50] to lower the off-target reactivity.

With the use of 4-1BB, Morgan *et al.* [51] developed third-generation CAR against ERBB2-positive tumor cells and elucidated notable CAR T-cell apoptotic activity and high levels of T-cell activation; however, clinical respiratory failure was induced as a result of the physiological levels of expressed ERBB2 on the normal lung epithelium. Nevertheless, because ERBB2 targeting second-generation CAR T cells lack the 4-1BB motif, these side effects may be less observable. In the work of Munisvaradass *et al.*, to decrease the potential chronic cytotoxicity by CAR T cells, T-cell costimulatory 4-1BB sequence was not included. Additionally, it was proposed that the sensitivity of ErbB2 CAR T-cell recognition could be adjusted by changing the ErbB2-specific scFv binding affinity. To investigate the efficacy of CAR T cells with different affinities, Liu *et al.* [52] assembled tumor lines with several levels of ErbB2 expression. A group of ErbB2 CARs was developed using scFvs derived from the published mutations of the 4D5-8 antibody. Various scFvs were then linked to the CD8 alpha hinge, the 4-1BB, and CD3 zeta intracellular signaling domains. CAR T cells with lower affinity (4D5-5 and 4D5-3) were reactive against tumors with amplified ErbB2 expression and represented an unnoticeable reactivity against tumors. Furthermore, they produced more cytokines when attacking cells with high levels of antigens. Conversely, higher-affinity CAR T cells (4D5 and 4D5-7) indicated powerful antitumor activity against tumor lines with both high and low levels of ErbB2 expression, secreting higher amounts of IFN-γ and IL2 when targeting cells with low levels of ErbB2. Overall, their findings indicated enhanced safety and clinical results of CARs that target various antigens, embracing several affinities in their structures.

MUC1

MUC1 is a potential biomarker for immunotherapy as it has been correlated with tumor invasiveness and metastatis [53]. Herein, a brief review about anti MUC1 CAR T cells is provided.

In Wilkie *et al.* [54] study, they have constructed a panel of CARs that target MUC1 positive tumor cells. MUC1 utilizing a SM3 scFv displayed unsatisfactory activity due to steric hindrance and glycosylation-related epitope heterogeneity, namely, MUC1 ST. Binding to MUC1 ST is poor since it is extremely enriched in tumor cells [55,56]. To overcome this problem, they focused on better mobility and reach of CAR binding arms. The significance of Ab mobility has become remarkably valued in elucidating why naive B cells coexpress cell surface IgM (which lacks a hinge) and IgD (whose elongated monomeric hinge is the longest of all Ab isotypes) [57]. Thus, IgD can assist antigens to engage in any kind of orientation [58]. The IgD hinge was fused to S28z, leading to a significant enhancement in MUC1-dependent T-cell proliferation and IFN-γ secretion. The efficacy of S28z was improved through successive stimulation with MUC1 positive tumor cells, alongside Ag-mediated selection of increased expression of SD28z CAR. However, this method could prove to be highly cumbersome in clinical protocols, necessitating another optimization of MUC1 CAR T cells. An HMFG2 scFv was cloned to enhance binding power for MUC1 positive tumor cells. Compared with SM3, HMFG2 offered improved affinity for unglycosylated MUC1. In another attempt, insertion of the IgD hinge in combination with IgG1 Fc led to the production of HDF28z CAR. This new CAR was highly expressed on the cell surface retargeting, newly transduced T-cell cytolytic activity against various MUC1-positive tumor cells. Next, OX40 intracellular domain (aa 241–277) or 4-1BB (aa 214–255) was fused between the CD28 and CD3ζ in HDF28z. No improved function was observed after 4-1BB inclusion, and changes in CD4/CD8 distribution were not observable because of the fusion of either the OX40 or 4-1BB component. Nonetheless, CARs containing OX40 (HOX) showed higher levels of IFN-γ production when targeting tumor cells with MUC1 intermediate expression levels.

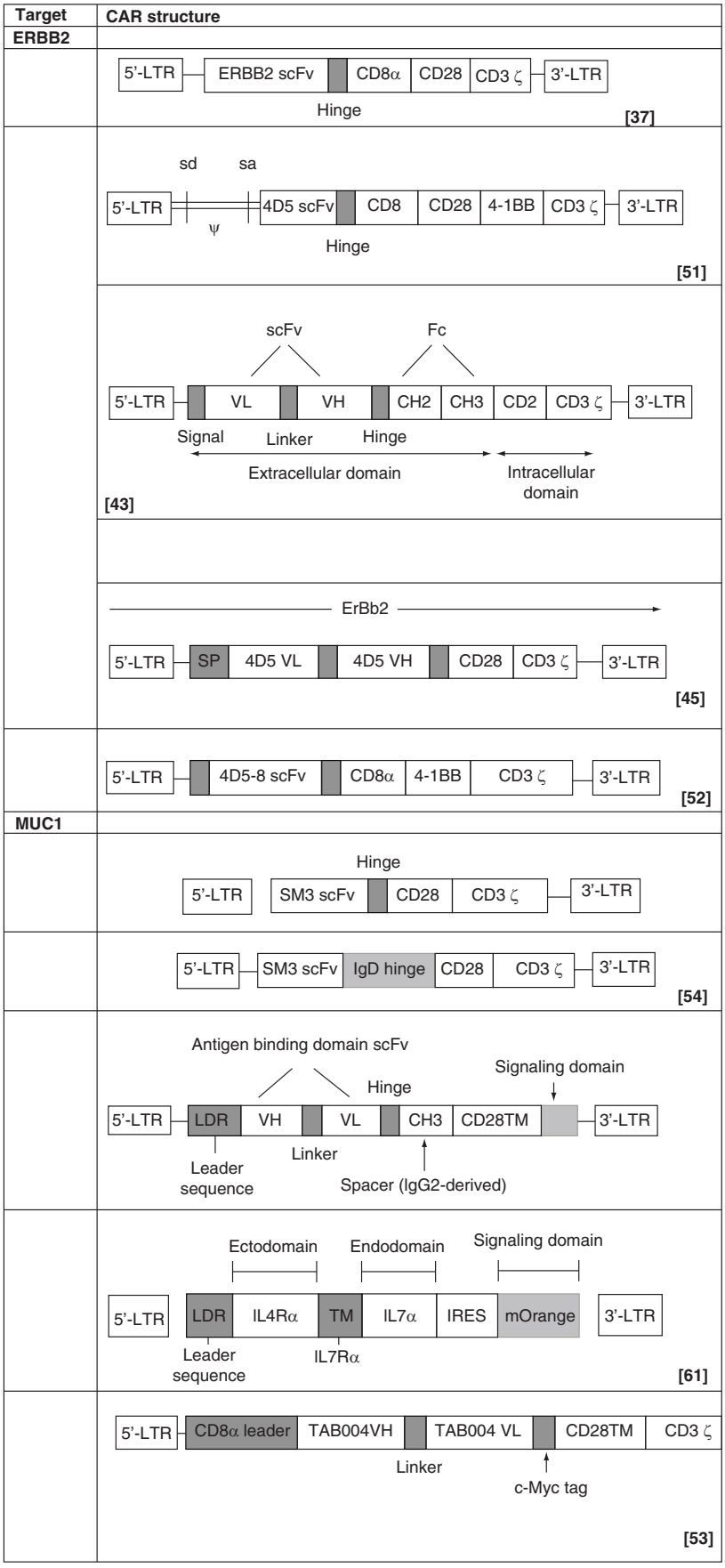

Figure 1. CAR T-cell structures used in breast cancer therapeutics.
CAR: Chimeric antigen receptor.

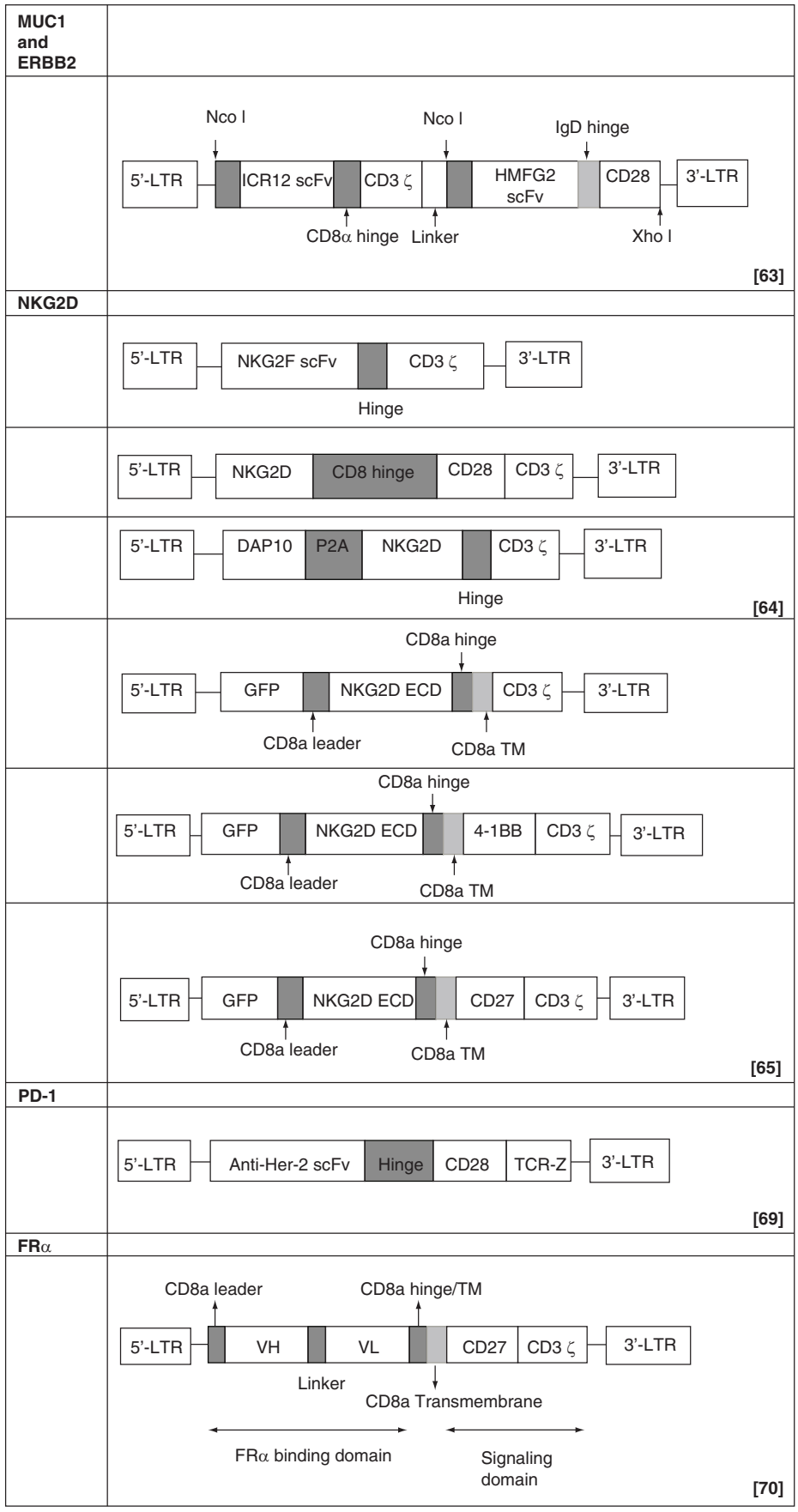

Figure 1. CAR T-cell structures used in breast cancer therapeutics (cont.).
CAR: Chimeric antigen receptor.

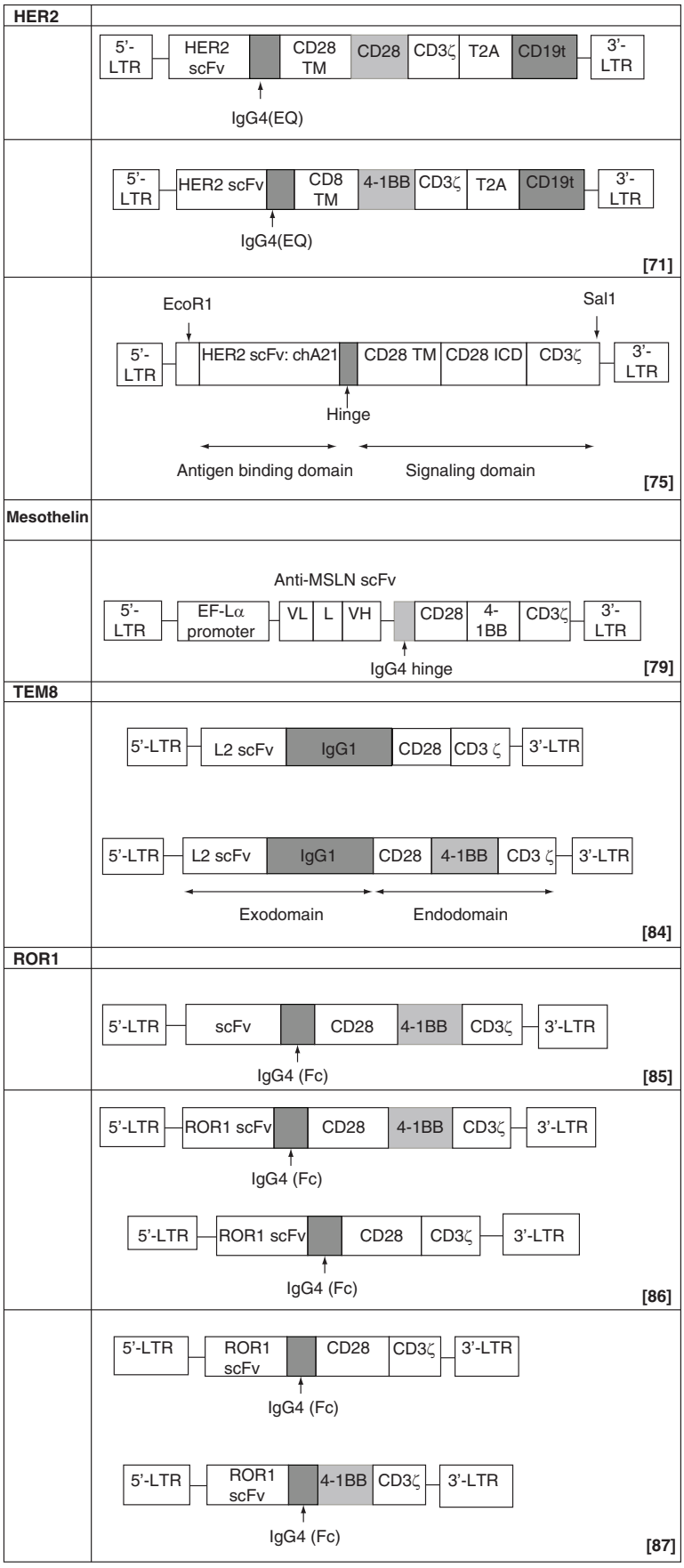

Figure 1. CAR T-cell structures used in breast cancer therapeutics (cont.).
CAR: Chimeric antigen receptor.

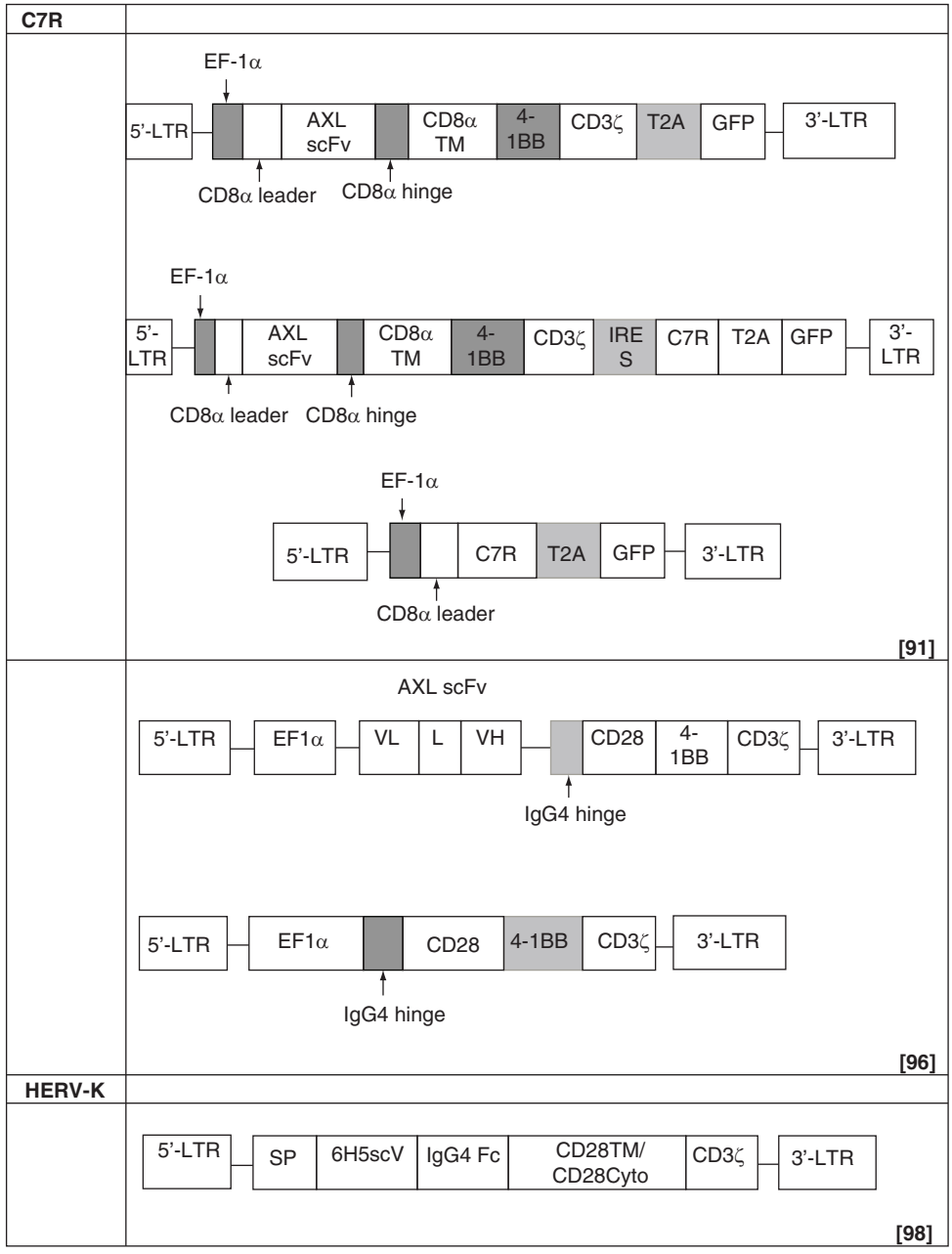

Figure 1. CAR T-cell structures used in breast cancer therapeutics (cont.).
CAR: Chimeric antigen receptor.

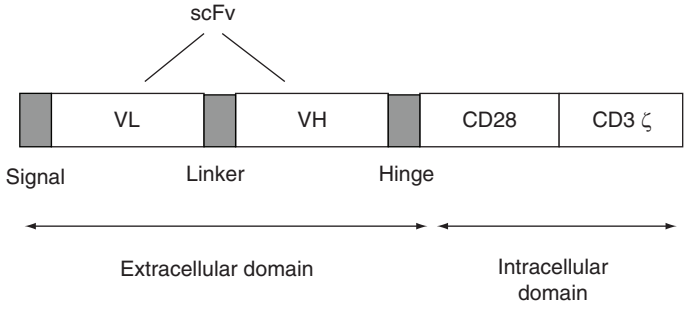

Figure 2. Sample of second-generation CAR T cell.
CAR: Chimeric antigen receptor.

T cells have to be able to remain active in the suppressive tumor environment. This highlights the vital issue of offering T cells with signals that repeat TCR signaling to ensure persistency and memory formation. IL-4 is produced in both malignant and normal cells; thus, it is a prevailing element in the tumor microenvironment [59,60]. Physiologic TCR signaling should therefore be constructed carefully when designing CAR T cells that target various antigens, as such MUC1. Bajgain *et al.* [61] assessed the effect of IL-4 in various MUC CAR T cells in their study. Initially, to target breast cancer cells, they constructed a first-generation anti-MUC1 CAR utilizing the HMFG2 scFv, which was cloned with the IgG2-CH3 domain and the zeta (ζ) chain of the TCR CD3 complex [62]. This molecule may be expressed on activated T cells, allowing CAR T cells to eliminate MUC1-positive tumors without targeting MUC1-negative cells (293T). However, because of the elevated levels of IL-4 in the breast cancer tumor microenvironment, CAR T cells demonstrated poor efficacy. To confirm that MUC1 CAR T cells continue to be functional in the tumor environment, an inverted chimeric cytokine receptor, 4/7ICR, was expressed along with the first-generation CAR to reverse the IL-4 (produced by tumor cells) inhibitory effects. The cytolytic potential of first-generation T cells was notably lower than first-generation 4/7ICR T cells when they were exposed to IL-4. The 41BB costimulatory endodomain was fused to the first-generation structure to develop the second-generation CAR. T cells with two signals (2GCAR.MUC1) were dysfunctional and did not display tumor control. Nevertheless, T cells with all three signals (cytokine support as signal 3; offered via the 4/7ICR) could provide sustained T-cell responses.

Coexpressing 4/7ICR with the CAR resulted in protection of transgenic cells from IL-4 inhibitory effects, increased proliferation of T cells at the tumor site and enhanced activation against transgenic cells both *in vitro* and *in vivo*. On the other hand, antigen specificity of CARs was not changed and antigen or cytokine withdrawal led to quick T-cell contraction; although highly unlikely, this treatment does not show the possibility of immune escape because of mutation or loss of the target molecules. However, to impede this, the 4/7ICR should be combined with multiple tumor-targeted CARs.

Another MUC1-targeting monoclonal antibody is TAB004, which recognizes all types of breast cancer but not normal tissues; The use of TAB004 in CAR T-cell construction was investigated in Zhou *et al.* [53] study. A CAR including scFv of TAB004 attached to CD28 and CD3z, termed MUC28z, was transduced with human T cells to investigate the efficacy of T cells. When MUC28z was injected into human triple negative breast cancer (TNBC) mice models, TAB004 proliferated only in the tumor cells, not in normal cells. Thus, TAB004 targets MUC1 in an extremely specific manner, resulting in minimal destruction to normal breast epithelial cells. Furthermore, TNBC tumor growth was remarkably decreased by injection of a single dose of MUC28z CAR T cells.

MUC1 & ERBB2

To enhance the efficiency of CAR-based immunotherapy, T cells with dual antigen specificity have been used; in this method, two supplementary CARs are expressed at the same time in the same T cells, each targeting a different tumor antigen. This setting not only optimizes T-cell homing and tumor specificity but also reduces toxic effects. Additionally, it may enhance tumor survival. This concept was investigated by Wilkie *et al.* by coexpressing an ErbB2- and MUC1-specific CAR utilizing CD3ζ and CD28 [63]. It was proposed that "dual-targeted" T cells eliminate ErbB2-positive tumor cells and proliferate in a way that necessitates the both expression of MUC1 and ErbB2 in tumor cells. However, this method was accompanied by limited IL-2 secretion compared with control CAR T cells, in which signaling is modulated by CD28+CD3ζ endodomain.

While additional investigations are essential to test this type of treatment, these findings indicate that dual targeting could be attained via genetically engineered T cells.

NKG2D

NKG2D ligands (NKG2DLs) overexpression in tumor cells turned them into a proper target for immunotherapy. To evaluate the efficacy of NKG2D ligands targeting T cells, VanSeggelen *et al.* [64] constructed two murine NKG2D targeting CAR T cells: an NKG2D receptor attached to the CD3ζ chain (NKz) and a second-generation CAR, in which the extracellular domain of NKG2D was attached to CD28 and CD3ζ (NK28z). Further, they constructed T cells to coexpress DAP10 (NKz10) to increase the CAR surface expression. Although using a NKG2D targeting CAR that is composed of CD3ζ TCR signaling domain assists T cells to recognize any natural NKG2DL receptor, it is considered a costimulatory receptor on activated CD8+ T cells. Therefore, DAP10 adaptor protein is responsible for signaling through the NKG2D receptor, which acts similarly to the CD28 T-cell costimulatory molecule. Mice models with breast tumors were used to investigate the efficacy of NKG2D-CAR T cells *in vivo*.

Although all three NKG2D-CARs prompt the elimination of murine breast tumor cells (NKz-CAR T cells displayed the same function), the inclusion of DAP10 in CARs significantly elevates the T-cell functionality after adoptive transfer. NKG2D-CAR T-cell adoptive transfer, both *in vivo* and *in vitro*, resulting in significant clinical toxicity that was not similar between the CAR types and resembles their *in vitro* NKG2D surface expression; the greatest expression, as well as most drastic toxicities, were seen with NKz10-CAR T cells, whereas the NKz-CAR T cell showed the lowest one. Additionally, the toxicity was intensified when T cells were combined with chemotherapeutic lymphodepletion. Despite these severe toxicities, all mice survived this therapy and recovered. Thus, although NKG2D-based CART cells could be useful for immunotherapy, they could be toxic when delivered systemically, highlighting the need to investigate related therapies with caution.

In addition, Han *et al.* [65] had also studied several CAR T cells targeting NKG2D on human TNBCs. The NKG2D CAR constructs had the extracellular domain of human NKG2D connected to a CD8a hinge and transmembrane region that are linked to a CD3ζ signaling moiety alone (NKG2D-z) or with the 4-1BB or CD27 intracellular signaling motif [66,67].

While NKG2D CAR T cells secreted IFN-γ and indicated probable cytolytic potential *in vitro* against tumor cells, NKG2D-z CAR T cells that did not have costimulatory domains were less potent, emphasizing the vital necessity for costimulatory signals. Not surprisingly, two infusions of 4-1BB or CD27 costimulated NKG2D CAR T cells particularly increased NKG2D CAR surface expression, T-cell persistence and the eradication of a highly developed invasive TNBC model in comparison with the first-generation NKG2D CAR T cells. Overall, increased activity against tumors and persistence of CARs with these configurations indicated that the inclusion of both 4-1BB and CD27 costimulatory domains to CAR structure promotes T-cell survival and persistency.

PD-1

PD-1 attaches to two ligands: PD-L1, the dominant mediator of immunosuppression, and PD-L2, which has an exclusive expression on macrophages and dendritic cells [68]. To increase the CAR T-cell efficiency in the tumor environment, John *et al.* [69] investigated whether the use of a PD-1 blocking antibody would enhance the CAR T-cell activity against two various Her-2 tumors. They used two Her-2 tumor targets, as well as anti-Her2 alone and anti-PD-1 alone therapy to evaluate antitumor effectuality as well as the safety of CAR T cells and anti-PD-1 antibody combination therapy. It was observed that mice injected with either anti-HER2 alone or anti-PD-1 alone did not have satisfying results. However, John *et al.* presented that the strongest and long-term survival of anti-HER2 CARs is observable when accompanied by anti-PD-1 antibody; moreover, the indicators of activation and proliferation were enhanced in anti-Her-2 T cells when the anti-PD-1 antibody was present. There was also a remarkable improvement in growth inhibition and regression of the established of two Her-2 tumors. Importantly, in normal Her-2-positive cells, escalated effects against tumors were not observed with any autoimmune pathology.

All in all, the results demonstrated that PD-1 immunosuppression blocking could increase vital functional parameters in CAR T cells.

FRα

FRα is overexpressed in breast cancer tumors representing a potential marker for CAR T-cell therapy in patients with TNBC. Song *et al.* [70] generated an FRα-specific CAR with a MOv19 scFv intracellular domain linked to a CD8a hinge and transmembrane region that is attached to a CD3ζ and CD27 intracellular signaling domain. They assessed the therapeutic potency of them both in vitro and in vivo.

FRα protein is expressed in three high, intermediate, or low levels in TNBC patients. In the current study, FRα-CARs identified all human TNBC cell lines expressing different levels of FRα. Although FRα-CAR T cells eliminated TNBC in an antigen specific manner, they expressed a moderate efficiency compared with the same CAR T cells used in ovarian tumor models with higher FRα expression. Hence, these CARs are more potent when applied in patients with high levels of FRα expression in TNBC tumors, highlighting the vital need for pre-selection of patients in future clinical trials.

HER2

Considering that metastasis form breast cancer to the brain stays a huge clinical problem and because of potential harm due to "on-target off-tumor" activity, the optimal configuration of CAR T cell must be a priority when treating this malignancy.

Priceman *et al.* [71] had optimized two types of second-generation CARs with different intracellular costimulatory domains, such as 4-1BB (HER2-BBz) and CD28 (HER2-28z), targeting HER2 receptors that are overexpressed in the breast to brain metastases patients; additionally, because CAR T-cell treatment encounters the problem of "on-target off-tumor" toxicity, Priceman *et al.* presented a HER2-CAR T-cell treatment method; they were the pioneers of clinically investigating two ways of administration for brain tumors: local intratumoral and regional intraventricular delivery.

Compared with HER2-CARs that comprises the CD28 costimulatory domain, HER2-CARs having the 4-1BB costimulatory domain displayed an enhanced antitumor activity with prolonged T-cell survival time. local intratumoral and regional intraventricular delivery of HER2-CAR T cells showed comparable antitumor efficacy and robust antitumor responses, while HER2-CAR T-cell i.v. delivery showed incomplete antitumor activities, even at higher doses. Thus, regional intraventricular delivery in the brain could be a promising treatment for multifocal brain metastases and leptomeningeal disease, while possibly inhibiting systemic T-cell proliferation. Probable toxicity challenges caused by the intracellular costimulatory signaling domain were also addressed in this study [51,72–76]. As described in previous studies, 4-1BB costimulation, compared with CD28, enhances T-cell proliferation [77,78]. This study indicated a notable decrease in cytokine secretion, as well as a need for higher levels of HER2 expression for cytokine production by HER2-BBz compared with HER2-28z CAR T cells. Therefore, 4-1BB costimulation, with the new method of HER2-CAR T-cell administration, could present a safe and efficient treatment for HER2-positive breast cancer patients with brain metastases. Sun *et al.* [75] also developed a new and optimized HER2 CAR comprising chA21 scFv and CD28 and CD3ζ intracellular signaling chains (chA21-28z CAR) and evaluated their antitumor activity through tumor cell lines. The outcomes revealed that these HER2-specific CAR T cells not only targeted HER2-positive breast and ovarian cancer cells *ex vivo* but also dramatically inhibited tumor growth.

Mesothelin

The immunosuppressive TME is a vital element attenuating the effectiveness of CAR T-cell treatment. However, Li *et al.* [79] formerly constructed an oncolytic adenovirus (OAds) TGF-β signaling (rAd.sT), which is an essential factor influencing immunosuppression [80–82]. OAds may eliminate tumor cells and induce host antitumor immune responses b secreting a huge amount of antigens [83]. Therefore, to improve the efficiency of Meso-CAR T cells, Li et al. investigated the combination therapy of Meso-CAR T cells and OAds in TNBC models.

Both rAd.sT and meso-CAR T cells represented activity against tumors. They indicated that rAd.sT may straightly eradicate tumor cells and have remarkable responses against tumors at the primary stages, nonetheless, at the final stages the anti-tumor activity was reduced; however, at late stages, CAR T-cell therapy had a stronger response against tumors. Moreover, combined therapy of rAd.sT and CAR T cells displayed the strongest inhibitory effects, demonstrated more remarkable antitumor activities, and it may enhance large numbers of cytokine secretion, namely interleukin IL-6 and IL-12. Thus, rAd.sT is an encouraging treatment to improve the meso-CAR T-cell antitumor efficacy.

TEM8

Byrd *et al.* [84] constructed a CAR T-cell targeting TEM8 (L2 CAR T cells), a marker overexpressed in TNBC patients. This marker is responsible for both tumor establishment and progression, in addition to metastasis and resistance to conventional chemotherapy and radiotherapy.

Results indicated that L2 CAR T cells robustly released IFN-γ and IL-2 when targeting TEM8, prompted xenograft tumors regression, eliminated tumor endothelial cells and TEM8-expressing TNBC cells and obliterated tumor neovasculature. Furthermore, the TEM8 CAR T cells attacked breast cancer stem-like cells, compensating for the development of mammospheres related to nontransduced T cells. Additionally, because the L2 scFv antibody fragment was isolated from a human Fab antibody, it would avoid human anti-mouse antibody induction and immune system clearance. However, compared with L2, third-generation (3G) CAR T cells were more effectual because L2 3G CAR T cells showed an enhanced cytokine release, expansion and preservation of better central memory phenotype. Sadly, in both CAR T-cell groups, tumor lesions ultimately progressed.

In conclusion, their study proposes TEM8 as an attractive marker antigen for CAR-TNBC patient's treatment.

ROR1

ROR1 is expressed in tumor cells but not in normal cells, making it an interesting antigen for CAR T-cell therapy.

Hudecek *et al.* [85] developed ROR1-CARs from scFVs with various affinities that are composed of extracellular IgG4-Fc spacer domains with several lengths. They investigated the ability of this CAR to recognize the ROR1 molecule *in vitro*. Their data indicated for the first time that optimized ROR1-CAR properly targeted epithelial cancers *in vitro*, secreted cytokine, and induced T-cell proliferation, although lacking the CD80/86 costimulatory ligand. However, ROR1-CARs with a short hinge extracellular spacer showed a better eradication of ROR1-positive tumor cells compared with CARs with long "hinge-CH2-CH3" spacers. Overall, they demonstrated that CAR T cells with the best configuration have *in vitro* antitumor activity.

In an attempt, Wallstabe *et al.* [86] developed microphysiologic 3D lung and breast cancer models and investigated the antitumor efficacy of ROR1-CAR T cells. Although simplistic, the use of 2D culture does not reflect the particular problems encountered in the tumor microenvironment. In the current study, they are investigating the efficacy of ROR1-CAR T cells in microphysiologic 3D tumor models, providing more realistic models and an alternative to the use of animal models.

ROR1-CAR T-cell treatment presented potential effects against tumors because CAR T cells promptly proliferated in the tumor lesions and destroyed numerous tumor cell layers. Additionally, Wallstabe *et al.* illustrated that CD8+ T cells expressing ROR1-CARs have the potential for cytolysis of tumor cells in 2D coculture as well [85]. Therefore, the use of these specific types of 3D models could be a significant asset before the clinical trial of several treatments.

Because the tumor microenvironment has an immunosuppressive element, such as TGF-β, prohibiting the body's immune response, it would cause a potential problem for CAR T-cell efficacy. Stüber *et al.* [87] investigated whether ROR1-CAR T cells (CD8+ and CD4+ ROR1-CAR T cells were used to construct this CAR T cell) are affected by TGF-β inhibition or not; they also assessed the TGF-β-receptor signaling blockade as a method for offsetting its prohibitory effects.

It was represented that ROR1-CAR T-cell cytolytic activity and proliferation were significantly inhibited when TGF-β was present; additionally, the ROR1-CAR T-cell survival time decreased. Blockade of TGF-β-receptor signaling using the specific kinase inhibitor SD-208 not only secured the CD8+ and CD4+ ROR1-CAR T cells from TGF-β but also preserved their efficacy against tumors. Furthermore, the use of SD-208 blockade resulted in prolonged survival time and decreased the expression of PD-1 on ROR1-CAR T cells. Overall, combinatorial treatment regimens of CAR T-cell therapy and TGF-β-receptor blockade is clinically practical.

C7R

The unsatisfactory outcomes of CAR T cells are due to the CAR T cells' short survival time in tumor lesions [88,89]. It was demonstrated that the IL-7 could prolong the CAR T-cell survival time *in vivo* [90]. For TNBC treatment, in Zhao *et al.* [91] study, activated IL-7 receptor (C7R) was fused to CAR T cells.

The results of *in vitro* and *in vivo* experiments indicated that the coexpressed C7R may significantly enhance the efficacy of CAR T cells, especially in TNBC xenograft model where the CAR T cells' survival time was prolonged. Hence, these outcomes present CAR T cells with C7R coexpression as a novel therapeutic strategy for TNBC.

In AXL, a receptor tyrosine kinase (RTK), improper expression is related to low survival rate [92]. It has been discovered that AXL is highly expressed in breast cancer cell membrane compared with normal breast tissues [92,93], suggesting a potential marker for cancer treatment [94,95]. Zhao *et al.* designed AXL-CAR T cells coexpressing C7R to evaluate the efficacy of enhanced CAR T cells on TNBC patients.

Zhao *et al.* demonstrated that, compared with AXL-CAR T cells, CAR T cells with C7R expression showed elevated activity against tumors. Additionally, CAR T cells extended survival time *in vivo* and thus could reduce tumor recurrence. Interestingly, CAR T cells are activated by C7R, avoiding the cytotoxicity of cytokines to untargeted cells.

In summary, Zhao *et al.* demonstrated that although AXL-CAR T cells expressing C7R displayed promising results, their investigations showed a few restrictions to the method: first, in the AXL-CAR T-cell group, the mice that had early-stage tumors, treatment resulted in the entire eradication of tumors; however, the optimized AXL-CAR T cells did not show remarkable efficacy. Second, CART cells that only target AXL antigen may also present the danger of off-targeting. Third, a mouse model survival curve was not accomplished, necessitating further study of these novel CAR T cells.

In addition, Jing *et al.* [96] developed CAR T cells composed of an anti-AXL scFv and CD28 and CD137 (4/1BB) costimulatory intracellular signaling domain. In TNBC xenograft model, AXL-CAR T cells exhibited efficacy and persistency against tumors, representing a promising approach in AXL positive TNBCs treatment.

HERV-K

HERV-K envelope (env) protein antibodies show antitumor activity against cancer. A HERV-K env protein specific CAR T cell (K-CAR) was developed by Zhou *et al.* [97] using anti-HERVK mAb.

The growth of breast tumor cells was halted by K-CAR T cells. They also reduced tumor growth and tumor weight, enhanced IL-2 production, tumor metastasis was stopped and multiple cytokines were secreted, including IFN-γ and TNF-α, exhibiting the remarkable cytotoxicity only against tumor cells. In the case of control T cells, antitumor activity was relevantly impeded.

Furthermore, it was demonstrated that HERV-K env protein has the potency to activate T-cell as well as B-cell responses in patients with breast cancer [98]. HERV-K env protein, as an oncoprotein, could have a vital impact on the immunotherapy of BC.

Conclusion

based on different reports the administration of CAR T-cells which targets various targets can consider as a promising approche not only in breast but also in other cancer types. Also the combination of CAR T-cells increases the effectiveness o fimmunotherapy against a number of cancers that are resistant to classic chemotherapy and radiotherapy treatments.

Future perspective

Although the significant results of several studies hold promise in CAR T-cell therapy for breast cancer patients, the limitations of such approaches must be considered as well. As mentioned previously, many of the evaluations still need to address potential shortcomings of this method, such as the toxicity of CAR T cells in the human body that may lead to severe side effects. Furthermore, when the CAR T cells are transferred to the human body rather than deficient mouse models used for CAR T-cell efficacy evaluation, they could have an effect on the human immune system that enhance additional immune response against tumor lesions. Overall, to implement this novel technique widely in breast cancer treatment, numerous investigations remain to be conducted.

Executive summary

- Considering the heterogeneous behavior of breast cancer, most of the novel methods presented here are focused on specializing the treatment of this malignancy.
- CAR T cells could have a revolutionary impact on breast cancer treatment because of their ability to treat this malignancy in a specific manner.
- CAR T-cell configurations were improved by developing several generations (the third being the most effective), adding several domains to recognize and destroy cancerous cells more efficiently.
- Most of the laboratory results were of CAR T cells were favorable because they robustly eliminated specific-antigen-expressing cancer cells.
- However, many shortcomings still need to be addressed as they hamper the progress of CAR T-cell therapy for breast cancer patients. 'Off-targeting' behavior and high toxicity in some cases highlight the fact that CAR T-cell therapy for breast cancer is still in its initial stages.
- Moreover, although CAR T cells have also undergone clinical evaluations, patients with metastatic breast cancer eventually died.
- To conclude, this review provides detailed information regarding the investigations of CAR T-cell therapy in breast cancer and may assist researchers in improving theissues related to this treatment so that it is feasible to use this method widely in the future.

Author contributions

MS Gharghani, M Simonian and F Bakhtiari drafted the manuscript. MH Ghaffari, G Fazli, AA Bayat and M Simonian discussed and revised the manuscript. B Negahdari and M S Gharghani designed the research and drafted the manuscript. All authors read and approved final manuscript.

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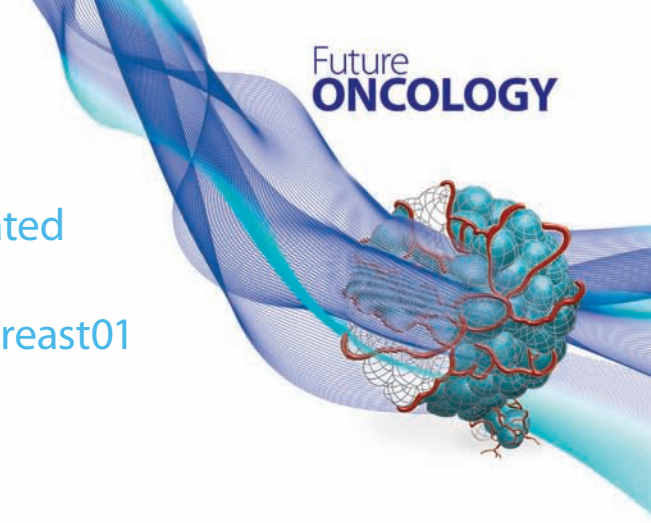
Plain Language Summary of Publication



Trastuzumab deruxtecan in previously treated HER2-positive metastatic breast cancer: plain language summary of the DESTINY-Breast01 study

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Summary

This is a summary of the article discussing the results of the DESTINY-Breast01 study originally published in the *New England Journal of Medicine*. The DESTINY-Breast01 study is a clinical study in participants with a type of breast cancer called HER2-positive breast cancer. The participants in the study received a treatment called trastuzumab deruxtecan, also known as T-DXd. The purpose of this summary is to help you understand the results of the DESTINY-Breast01 study. T-DXd is currently available as a treatment for adults with HER2-positive breast cancer that cannot be removed by surgery, also called unresectable, or that has spread, also called metastatic. In the DESTINY-Breast01 study, all the participants had HER2-positive breast cancer that was metastatic or unresectable. All participants were required to have had previous treatment for their HER2-positive breast cancer with another treatment, called trastuzumab emtansine or T-DM1. All the participants received T-DXd every 3 weeks. Part 1 was done to learn how T-DXd acted in the body, and to choose a dose to give to all the participants in Part 2. In Part 2, 184 participants received T-DXd at 5.4 mg/kg and the results showed that T-DXd reduced tumor growth. Up to 60.9% of the participants had their tumors shrink or disappear, with a treatment response that lasted for nearly 15 months on average. The participants lived with their cancer for around 16 months before it got worse. During the study, 183 out of 184 participants had side effects, known as adverse events. The most common adverse event was nausea. There were 42 participants (22.8%) who had serious adverse events, including lung toxicity. These results suggest that T-DXd could be a treatment option for people with metastatic HER2-positive breast cancer who have already been treated with T-DM1. Additional studies will provide more information and results about T-DXd.

How to say (double click to play sound)...

- **Trastuzumab:** tras-tuh-ZUH-mab
- **Deruxtecan:** der-UHX-teh-can

What is HER2-positive breast cancer?

Proteins called human epidermal growth factor receptor 2, also called HER2, are found on the surface of breast cells and help control each cell's normal growth. In people with HER2-postive breast cancer, too many HER2 proteins cause breast cells to grow and multiply in an uncontrolled way, forming tumors. About 15% to 20% of breast cancers are HER2-positive breast cancers.

Scientists determine if someone has HER2-positive breast cancer by looking at their breast cancer cells collected during a biopsy. Scientists do certain tests on the cells to look at the HER2 proteins under a microscope. This way, the scientists can count the HER2 proteins to see if there are too many.

Who should read this article?

This summary may be helpful for patients with HER2-positive breast cancer and their family members or caregivers. It may also be helpful for patient advocates and healthcare professionals. This includes those who are looking for treatment options for patients with HER2-positive metastatic breast cancer.

Future Medicine part of fsg

What is HER2-positive breast cancer?

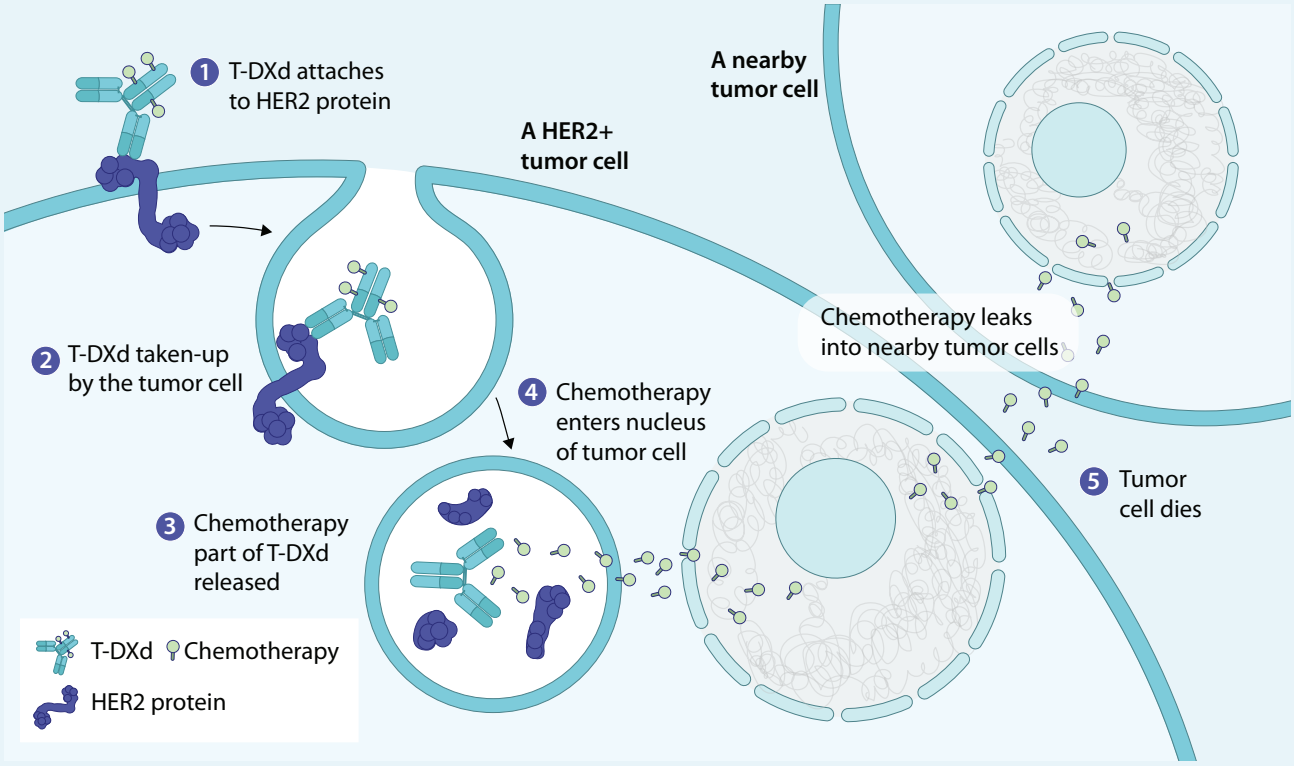
HER2-positive breast cancers can grow quickly compared to other breast cancers. They are also more likely to either come back after treatment or become metastatic. Metastatic means that the cancer has grown beyond the organ where it started and has spread to other parts of the body.

The participants in the DESTINY-Breast01 study had HER2-positive breast cancer that was metastatic or unresectable.

What is trastuzumab deruxtecan?

In this summary, trastuzumab deruxtecan will be referred to as T-DXd. T-DXd consists of a chemotherapy drug linked to a manmade antibody. Chemotherapy drugs work by attacking cancer cells before they can divide and grow. Normally, antibodies are made by the immune system to fight off infections. But, researchers can also make antibodies in a laboratory and use them to treat certain conditions or diseases. The antibody in T-DXd is a protein that specifically targets and attaches to the HER2 protein on tumor cells.

When the antibody attaches to the HER2 protein, T-DXd is taken up into the tumor cell. Once inside, the chemotherapy part of T-DXd is released and the chemotherapy kills the tumor cell. Some of the chemotherapy can spread into nearby tumor cells to kill these too, even if they do not have many HER2 proteins on the surface.



T-DXd works in a similar way to another treatment for HER2-positive breast cancer called trastuzumab emtansine, also called T-DM1. But, T-DXd uses a different chemotherapy drug. Each T-DXd drug has on average 8 of the chemotherapy particles per antibody, while T-DM1 has on average 3–4 chemotherapy particles per antibody.

Why was the clinical study needed?

Treatments for metastatic HER2-positive breast cancer are designed to control the growth of tumors. This can help improve patients' quality of life and help them live longer.

There are standard treatments that doctors usually use first for patients with HER2-positive breast cancer. These treatments are known as first-line therapies. First-line therapies can stop working over time, so doctors give patients other treatments to help control tumor growth. These are known as second-line or third-line therapies, and so on. In general, many tumors are affected less by second-line and third-line therapies compared with first-line therapies.

In patients with cancer, researchers measure the amount of time between the start of treatment and the cancer spreading or tumors growing. This is known as progression-free survival. This may only be 3 to 6 months after starting third-line therapy.

So, more treatment options are needed for people with metastatic HER2-positive breast cancer that can help control the growth or spread of their tumors, and to lengthen the time before their cancer gets worse or returns.

What was the purpose of the clinical study?

The main questions the researchers wanted to answer in the DESTINY-Breast01 study were:

- Did the participants' tumors shrink or disappear after receiving T-DXd?
- For how long did the participants' tumors shrink or disappear before growing again?
- For how long did the participants live with their cancer before it got worse?
- What were the most common adverse events during treatment with T-DXd? An adverse event is any sign or symptom that participants have during a study.

For a full list of the questions that the researchers in this clinical study wanted to answer, please refer to the websites listed at the end of this summary.

Who took part in the clinical study?

The DESTINY-Breast01 study included **253 women** aged 18 and older.

All of the participants:

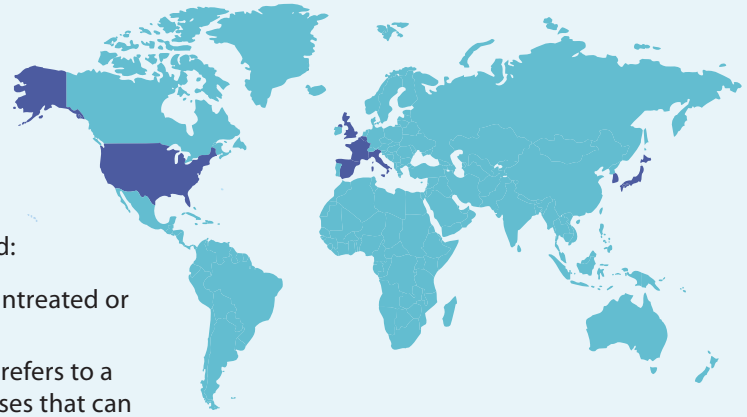
- ✓ Had HER2-positive breast cancer that was metastatic or unresectable
- ✓ Had previous treatment with TDM-1
- ✓ Were able to move around easily or were fully active

People were not able to join the study if they had:

- ✗ Metastatic cancer in the brain that had been untreated or was causing symptoms
- ✗ Interstitial lung disease, also called ILD, which refers to a number of different non-infectious lung diseases that can cause scarring and stiffness of the lungs
- ✗ Pneumonitis, which is inflammation of the lung tissue

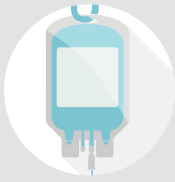
The study included participants from:

Belgium	Italy	South Korea	UK
France	Japan	Spain	USA

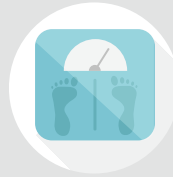


What happened during the clinical study?

The study had two parts. Both parts were open label. This means the participants, researchers, study doctors, and other study staff knew what each participant was receiving.



All of the study participants received T-DXd every 3 weeks through a needle put into the veins, also known as an IV infusion.



The dose each participant received was based on their body weight. The doses were measured in milligrams per kilogram of body weight, also called mg/kg.

Part 1 was done to find out how T-DXd acted in the body, and to choose a dose to give to all of the participants in Part 2. To do this, the doctors gave different doses to the participants in Part 1 and took blood samples at different times during the study. In Part 1, the researchers used a computer program to randomly choose which dose of T-DXd each participant received. This helped make sure the doses were chosen fairly and comparing the results of the doses was as accurate as possible.

The chart below shows how the study was done:

Part 1

Part 1a: finding out how T-DXd doses behave in the body

- 22 participants got 5.4 mg/kg of T-DXd
- 22 participants got 6.4 mg/kg of T-DXd
- 21 participants got 7.4 mg/kg of T-DXd

Part 1b: finding out how well two chosen T-DXd doses work

- 28 participants got 5.4 mg/kg of T-DXd
- 26 participants got 6.4 mg/kg of T-DXd

Part 2

Finding out how well T-DXd works at the chosen dose of 5.4 mg per kg

184 participants got T-DXd at the chosen dose of 5.4 mg/kg

- 50 participants who had already got 5.4 mg/kg of T-DXd in Part 1a or Part 1b
- 4 participants who had stopped taking TDM-1 for reasons other than their cancer worsening
- 130 participants who had already tried TDM-1 but their tumors were still growing

What were the overall results of the clinical study?

Below is a summary of the main results of this clinical study. A full report of the study results can be found on the websites listed at end of this summary. This summary and the full report do not have each participant's individual results.

Researchers look at the results of many clinical studies to decide which treatments work best and are safest. Additional studies will provide more information and results about trastuzumab deruxtecan.

The results below include information for the 184 participants in Part 2, who received T-DXd at the chosen dose of 5.4 mg/kg.

Did the participants' tumors shrink or disappear after receiving T-DXd?

To answer this question, the researchers measured the overall response of the participants. This was the main question that the researchers wanted to answer. To calculate the overall response, the researchers counted how many participants:

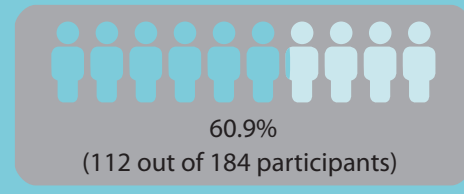
- had their tumors shrink by more than 30.0%, called a **partial response**

- had their tumors disappear completely, called a **complete response**

The researchers measured the participants' tumors using a computerized tomography scan, also called a CT scan. Then, they analyzed the scans using a set of rules or criteria called Response Evaluation Criteria in Solid Tumors, also called RECIST. These rules define what it means for a tumor to decrease in size (respond), stay the same (stabilize), or increase in size or spread (progress) during treatment.

Based on the results, the researchers calculated the response rate for the participants. To do this, they used a type of average called a median. In a set of numbers, the median is the middle number between the lowest and highest numbers. The researchers found that:

- After receiving T-DXd, the median response rate was **60.9%**. This means that 112 out of 184 participants had a treatment response, based on the RECIST criteria.



Some other questions the researchers wanted to answer were about the overall responses in different subgroups of participants. They found similar results in these subgroups, including in participants with tumors that had spread to the brain. These participants had been treated for their tumors in the brain before coming onto the study and the tumors in their brain were stable and not growing.

For how long did the participants' tumors shrink or disappear before growing again?

To answer this question, the researchers calculated the median number of months that the participants had no tumor growth after their tumors had shrunk or disappeared. This is called the response duration. This was calculated for all of the participants who had a response to treatment during the study.

- The researchers found that the participants' median response duration was **14.8 months**.

For how long did the participants live with their cancer before it got worse?

The researchers calculated the median number of months after starting the study that the participants lived with their cancer before it got worse. This is called progression-free survival. This was calculated for all of the participants who took part in the study.

- The researchers found that the participants' median progression-free survival was **16.4 months**.

What were the most common adverse events during treatment with T-DXd?

The doctors kept track of the adverse events that the participants had during the clinical study. An adverse event is any sign or symptom that participants have during a study. An adverse event is considered serious when it is life-threatening, causes lasting problems, or the participant needs hospital care.

Adverse events may or may not be caused by the treatments in the study. A lot of research is needed to know whether a treatment causes an adverse event.

The results below include information for the 184 participants in Part 2 who had received T-DXd at the chosen dose of 5.4 mg/kg.

How many participants had adverse events?

99.5%
(183 out of 184 participants)

How many participants had serious adverse events?

22.8%
(42 out of 184 participants)

How many participants died because of adverse events?

4.9%
(9 out of 184 participants)

How many participants stopped getting study treatment because of adverse events?

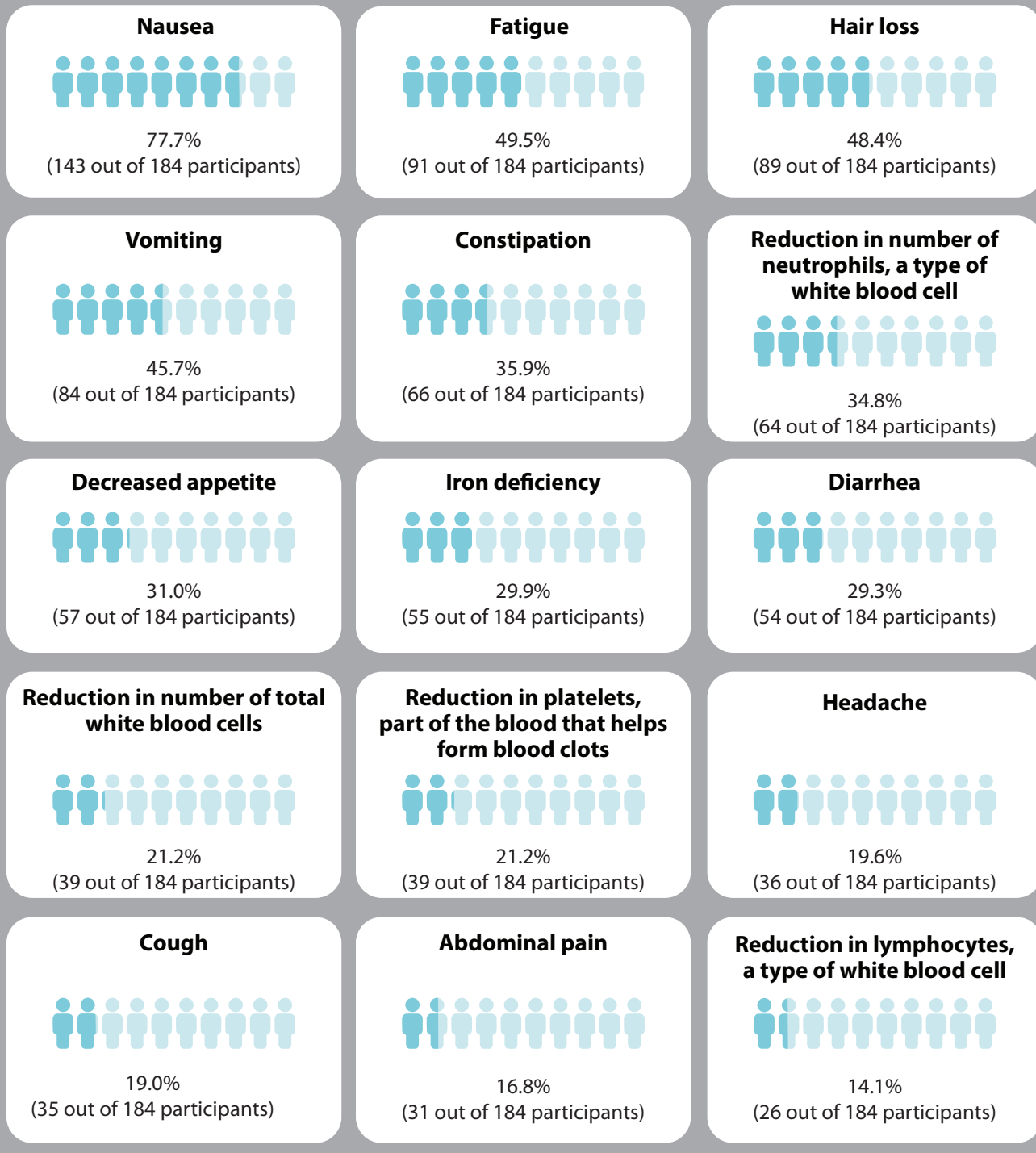
15.2%
(28 out of 184 participants)

Details on the adverse events that led to death include a case each of general deterioration in physical health (0.5%), pneumonia (0.5%), organ failure (0.5%), shut down of the body due to loss of blood (0.5%). There were two cases of interstitial lung disease (1.1%), which were considered to be related to the study treatment.

What were the most common adverse events?

The most common adverse event during the clinical study was nausea.

The results below show the adverse events that happened in at least 14.0% of participants during Part 2. There were other adverse events, but these happened in fewer participants. Some participants may have had more than one adverse event.



What were the most common adverse events? (continued)

In other clinical studies with T-DXd, the participants had certain adverse events. The researchers wanted to learn if the participants in this study also had these same adverse events. These are called adverse events of special interest.

One of these adverse events of special interest is interstitial lung disease, also called ILD. This term refers to different non-infectious lung diseases that cause scarring of the lungs, also called fibrosis. This scarring causes stiffness in the lungs which makes it difficult to breathe and to get oxygen to the bloodstream. ILD can also include pneumonitis, which is when the lungs become inflamed.

During the study, any participants with signs and symptoms of ILD were required to stop receiving T-DXd right away and be treated with steroids.

The signs and symptoms of ILD include fever, cough, or shortness of breath. Patients and their doctors should carefully monitor for these signs and symptoms so that ILD can be found early and treated.

The researchers found that during Part 2 of the study:

- ILD happened in 13.6% of participants. This was 25 out of 184 participants.
- Most ILD cases (20 out of 184) were mild or moderate in severity.
- There were 4 participants who died because of ILD.
- At the end of the study, 19 of the participants had recovered or were receiving treatment.

What do the results of this clinical study mean?

The results from this study showed that overall, T-DXd reduced tumor growth in participants who had already received treatment with TDM-1 for their metastatic or unresectable HER2-positive breast cancer.

The result showed that up to 60.9% of the participants had their tumors shrink or disappear, with a response duration of nearly 15 months. The participants lived with their cancer for around 16 months before it got worse.

The results also showed that people receiving T-DXd may have an increased risk for ILD. Patients and their doctors should carefully monitor for the signs and symptoms of ILD and be open to discussing these, so it can be found early and treated.

The results from this clinical study suggest that T-DXd could be a treatment option for people with metastatic or unresectable HER2-positive breast cancer who have already been treated with T-DM1. Patients should always talk to a doctor before making any decisions about their treatment.

Who sponsored the clinical study?

Daiichi Sankyo Co., Ltd., and AstraZeneca funded this study. The study was designed and led by Daiichi Sankyo Co., Ltd., for data collection and analysis, and was approved by the institutional review board at each participating site. In March 2019, AstraZeneca entered into a collaboration agreement with Daiichi Sankyo Co., Ltd., for trastuzumab deruxtecan. Both Daiichi Sankyo Co., Ltd., and AstraZeneca were involved in study oversight and data collection. All authors and sponsors assisted in data interpretation, writing the report, and reviewing the manuscript. All authors had full access to all data in the study and provided final approval to submit the manuscript for publication.

Where can readers find more information on this clinical study?

The full title of the original publication in the *New England Journal of Medicine* is:
Trastuzumab Deruxtecan in Previously Treated HER2-Positive Breast Cancer.

You can read the abstract of the original publication at:
https://www.nejm.org/doi/full/10.1056/nejmoa1914510.

You can read more about the DESTINY-Breast01 study on the following websites:

- Enter the study number NCT03248492 into the search field at www.clinicaltrials.gov
- Enter the EudraCT identifier 2019-001512-34 in the search field at www.clinicaltrialsregister.eu

If you were a study participant and have questions about the results of this study, please speak with the doctor or staff at your study center.

Educational resources

- Read more about metastatic HER2-positive breast cancer on the Cancer.Net website at: https://www.cancer.net/cancer-types/breast-cancer-metastatic/types-treatment
- Learn about the National Comprehensive Cancer Network (or NCCN) clinical practice guidelines for treatment of breast cancer. These guidelines assist physicians in determining the best treatment for their patients. Read these guidelines at: https://www2.tri-kobe.org/nccn/guideline/breast/english/breast.pdf
- Read the NCCN patient guidelines for the treatment of metastatic breast cancer at: https://www.nccn.org/patients/guidelines/content/PDF/stage_iv_breast-patient.pdf

Additional resources for breast cancer patients:

- American Cancer Society: www.cancer.org
- Living Beyond Breast Cancer: www.lbbc.org
- Metavivor: www.metavivor.org
- Share Cancer Support: www.sharecancersupport.org
- Susan G. Komen: www.komen.org

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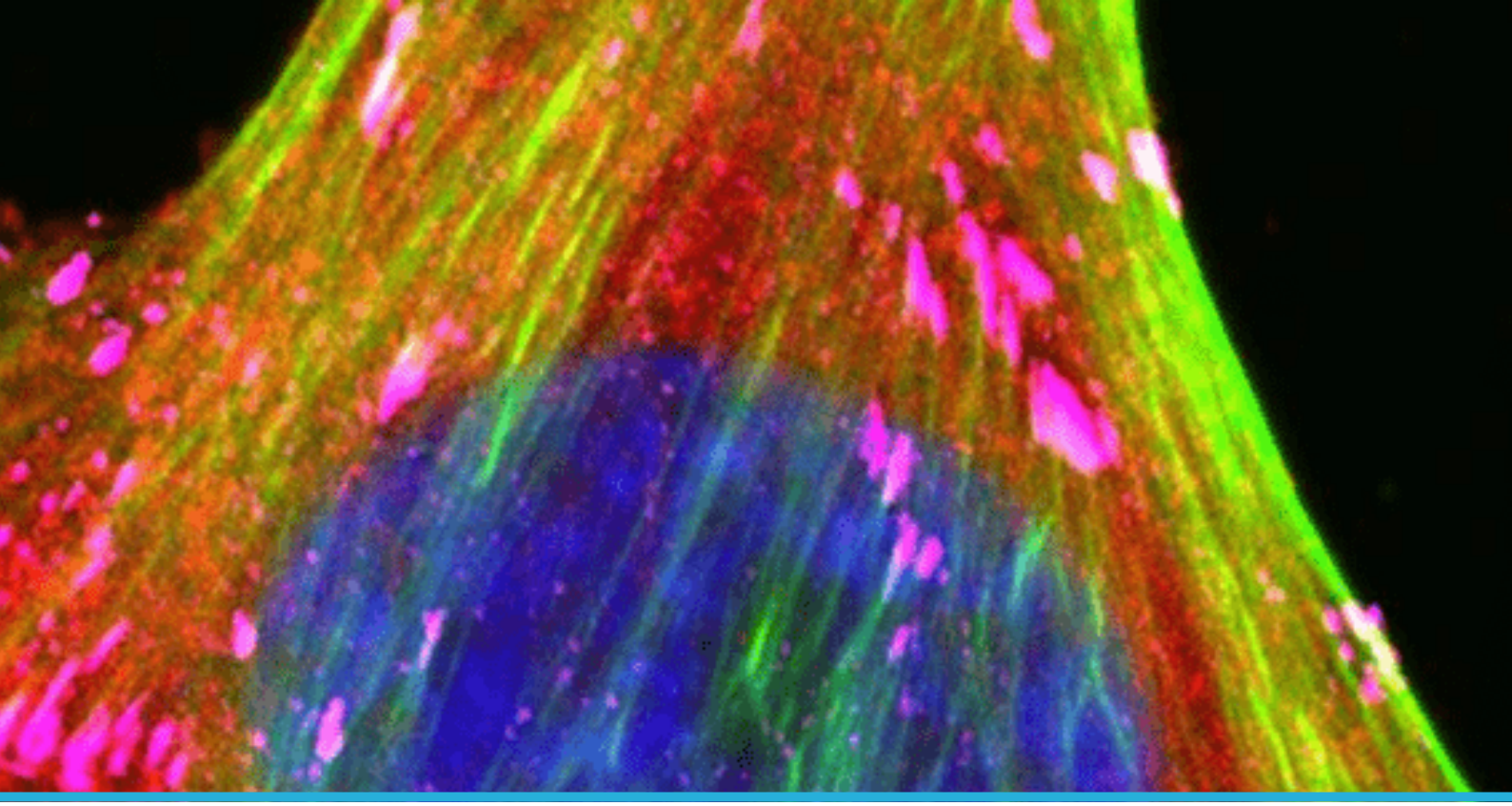

What tucatinib means for oncologists and their patients: an interview with Erika Hamilton

Please introduce yourself and tell us about your career to date.

I'm Erika Hamilton and I lead Breast Cancer Research at Sarah Cannon Research Institute at Tennessee Oncology in Nashville (TN, USA). I am also a partner within Tennessee Oncology, PLLC. I joined Sarah Cannon in 2013 and focus on breast cancer and gynecologic cancer. I care for patients receiving standard therapies and am the primary investigator on many of our late Phase breast cancer studies and in our Phase I Drug Development Unit. I am passionate about improving the care for people facing breast cancer and bringing new and exciting therapies to the communities we serve.

How has the treatment landscape for locally advanced or metastatic HER2-positive breast cancer evolved over the last 5 years?

The biggest story in HER-2-positive advanced disease is around two drugs approved approximately a year and a half ago – trastuzumab deruxtecan and tucatinib. They are very different drugs but are both highly active in patients facing HER-2 positive disease. Our algorithm of treatment was highly scripted in first and second line (with first-line TCHP and second-line T-DM1), but third line and beyond was less clear with no single standard of care.

Oncology Central

What tucatinib means for oncologists and their patients: an interview with Erika Hamilton

Trastuzumab deruxtecan is a novel antibody–drug conjugate and was approved in December 2019 for patients with HER-2-positive metastatic breast cancer (MBC) and showed an impressive response rate and duration of response, which has only improved with even further follow-up. On the other hand, tucatinib, which was approved in early 2020, is a tyrosine kinase inhibitor that is specific to HER-2 (sparing EGFR) and given in combination with capecitabine and trastuzumab for patients with second line and beyond HER-2-positive MBC. A unique aspect of tucatinib is its excellent central nervous system (CNS) activity, having activity in not only those with stable brain metastases, but also those that are untreated or progressing.

What hurdles need to be overcome to continue the advancement HER2-positive breast cancer treatment?

It is exciting to have two novel agents in this space, but we continue to need more for our patients. We know patients whose cancers are driven off the HER-2 protein often do well for some time with continued targeting of the HER-2 pathway, so I remain excited about a variety of drugs in development from novel antibody-drug conjugates, to bispecific antibodies, to tyrosine kinase inhibitors.

Brain metastases are also an area that continues to be problematic. Up to 50% of patients with HER-2 metastatic disease will have to face this complication over their disease course and unfortunately many of our drugs are not as active in the CNS as they are in the rest of the body. Drug discovery in this space will be critical.

Finally, we continue to grow in our recognition that not all cancers (even those that are HER-2) are the same. Our treatments, therefore, should not be one-size-fits-all either. Tumors that express hormone receptors in addition to HER-2, for example, may respond to therapies in the endocrine pathway, such as CDK4/6 inhibitors in addition to HER-2 agents. We continue to strive for additional therapies with fewer side effects for our patients.

Tucatinib has recently been approved by the European Commission for the treatment of patients with locally advanced or metastatic HER2-positive breast cancer. Could you tell us more about the use of tucatinib in HER2-positive breast cancer?

HER2CLIMB tested capecitabine and trastuzumab with or without tucatinib in a large, randomized trial. The addition of tucatinib not only improved progression free survival (PFS) and overall survival (OS), but also PFS and OS in patients with brain metastases. Most of the side effects of the regimen come from the capecitabine backbone with known hand-foot syndrome, GI disturbances and fatigue. The addition of tucatinib also increases a small rate of reversible liver function test abnormalities. Based on this data, tucatinib was approved for second line and beyond HER-2 positive MBC.

What tucatinib means for oncologists and their patients: an interview with Erika Hamilton

What will this approval mean in the clinic and for patients with HER2-positive breast cancer?

Not only does this tucatinib regimen provide a great option for patients with brain metastases, but there is also benefit in OS for patients without brain metastases. Therefore, the tucatinib regimen is a good option for all regardless of brain metastases. I am also hopeful that perhaps as we use this CNS-effective drug earlier in the disease course, that we could start to see fewer brain metastases. This 'prevention' possibility is something that still needs to be tested.

In your opinion, how will the treatment landscape for HER2-positive breast cancer continue to evolve over the next 5 years?

I think we will see less use of 'naked' chemotherapy and increasing use of therapies like antibody–drug conjugates, bispecific antibodies and truly targeted drugs. This enables us to target the tumor specifically, which often translates to a better side effect profile for patients. I also think we will see some of our known agents moving earlier in the disease course, such as trastuzumab deruxtecan and tucatinib – maybe even into the adjuvant curative setting.

Breast cancer: signs, symptoms & risk factors

Discover the signs, symptoms of breast cancer as well as its incidence and risk factors in this video transcript with Macmillan Consultant Nurse Victoria Harmer (Imperial College Health Care NHS Trust, UK).

Hi, I'm Vickki Harmer, a Macmillan Consultant Nurse in Breast Care at Imperial College Healthcare NHS trust.

We've decided to do a video with Oncology Central to support Breast Cancer Awareness Month and this video will hopefully enlighten you on the NHS breast screening program and also how to be a bit more breast aware.

The breast is a mixture of breast tissue and fat, which lies on the muscle. Inside the breast, there are about twelve to fourteen lobules which go down through the ducts and come out at the nipple.

There are just under half a million new diagnosis of breast cancer each year in Europe and the estimated number of people that die are 143,000.

In the UK breast cancer is the most commonly diagnosed cancer with over 55,000 women and about 400 men diagnosed each year.

So that translates to about one in eight women in the UK who will be diagnosed with breast cancer at some point in their life. However, more women than ever surviving breast cancer and that's down to better awareness, better screening and better treatments.

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Breast cancer: signs, symptoms & risk factors

The breast awareness five-point code is really important, and it's as follows:

1. Know what is normal for you
2. Look and feel
3. Know what changes to look out for
4. Report any changes without delay
5. Get screened if you're aged 50 years or over

So what should you be looking out for?

- You need to focus on any change in shape or size of one or both breasts
- Look out for changes in one or both nipples and that includes the appearance, the direction that it points and if there's any discharge
- Check to see if there's any dimpling or puckering in the skin of either of the breasts and also if there are any lumps
- Dry skin, eczema, thickening of the breasts or around the armpit and constant pain would also be worth reporting

There are a number of risk factors for breast cancer, the main two are being a woman and increasing age. Using HRT for over 10 years can also increase your risk of breast cancer as can a family history. There are also factors such as alcohol intake or a high BMI.

So breast cancer risk factors can be modifiable. For example the diet that you take, your weight, any exercise you choose to take or not, if you choose to smoke, drink alcohol or use estrogen and non-modifiable such as genetic disposition, increasing age and being a woman.

If you do find a lump and report it to your GP, they will refer you to the breast service. There you will see a clinician who will examine you and examine your breasts and then order a mammogram and/or an ultrasound. If there is a lump they will also take a biopsy and it's the pairing up of all of these tests that allow us to make a diagnosis of cancer.

The other route where breast cancer is diagnosed is through the NHS breast screening program. This was set up in 1988 with the aim of reducing mortality by 25% in the population screened. Women aged 50–70 are invited every 3 years for a mammogram and if you're over 70 then you can certainly ask to be referred by your GP or the nearest screening centre to you.

Oncology Central

Breast cancer: signs, symptoms & risk factors

Over 2 million women are screened annually and 15,000 breast cancers are diagnosed through the screening program in the UK each year. That translates to an estimation of saving 1300 lives per year.

It is important to attend your breast screening appointment when invited and we usually find that women who are basically concerned about their health are more likely to get screened. We know that partner gender is not associated barriers to screening and that there's a strong association between academic women and breast screening. Vulnerable groups, individuals with the learning disabilities as they're less likely to attend NHS breast screening and those in residential care are even less likely to. Black, Asian and minority ethnic women, there is a lower uptake of breast screening reported as well.

So it's really important to get to these vulnerable populations and cohorts of people.

40–45% of cancers can be avoided by lifestyle such as stopping smoking, exercising, keeping a healthy weight, reducing your alcohol and this also improves mood. These activities can also decrease the risk of recurrence if somebody has had breast cancer.

The food we eat can also affect the risk of developing cancer. We could reduce the red and processed meats that we eat and also salt and saturated fats.
So in summary I'd like to encourage you to be healthy. Try to have a healthy weight, limit your alcohol, limit your energy dense food, eat more food from a plant origin and take regular physical exercise.

For Breast Cancer Awareness Month, and thank you for watching this video, it's really time to get involved to have fun, maybe think of something in the workplace or something that charities are doing and promote breast awareness and the breast screening program.

Thank you very much for watching.

How I treat:
HER2-positive breast cancer

Oncology Central
In focus

Including:
- Current treatment landscape
- Overcoming challenges
- Case studies

Panel discussion: how I treat metastatic HER2-positive breast cancer

OncologyCentral

Gain an understanding of how oncologists are treating metastatic HER2-positive breast cancer in Oncology Central's latest panel discussion, featuring experts from international institutions including Hans-Christian Kolberg (HCK) (Marienhospital Bottrop, Germany), Franklin Castillero (FC) (National Oncology Institute of Panama) and Max Mano (MM) (Hospital Sírio-Libanês, São Paulo, Brazil).

Contents

Question 1: How has treatment for HER2-positive metastatic breast cancer evolved over the past decade?

HCK: At the start of the last decade, we had two targeted agents for the treatment of HER2-positive metastatic breast cancer in our hands, the monoclonal antibody, trastuzumab, and the tyrosine kinase inhibitor, lapatinib. The limitation on two biologicals led to concepts of 'treatment beyond progression'. A key year for the treatment of HER2-positive metastatic breast cancer in Europe was 2013. In 2013, the EMA approved pertuzumab in combination with trastuzumab and docetaxel for firstline therapy in metastatic disease and T-DM1 for the secondline. This changed options and outcomes dramatically. In the registration study for the dual blockade, the CLEOPATRA study (NCT00567190), median overall survival was 57 months in the interventional cohort compared with 41 months in the trastuzumab cohort. The registration study for T-DM1, EMILIA (NCT00829166), demonstrated a median overall survival of 31 months compared with 25 months for the former secondline standard lapatinib and capecitabine. The two new drugs did not only improve outcomes, but they also represented new ideas. The combination of trastuzumab and pertuzumab targeted the same receptor with two antibodies.

This paradigm change led to combinations of biologicals that were investigated after EMILIA being given other terms, because the term 'dual blockade' was then reserved for the combination of trastuzumab and pertuzumab. T-DM1 was a first-in-class drug – an antibody-drug-conjugate. For the first time it was possible to link a cytotoxic payload to an antibody delivering the payload to the tumor cell. This was what Paul Ehrlich had in mind when he developed the concept of a 'magic bullet' in 1900.
The past decade also saw the development of tyrosine kinase inhibitors from lapatinib over neratinib to tucatinib, consequently reducing toxicity not only without compromising, but even increasing efficacy. The concept of antibody-drug-conjugates was improved, resulting in the development of agents like trastuzumab deruxtecan, an antibody-drug-conjugate with an antibody-drug ratio of almost eight compared to about two for T-DM1 and thus an increased efficacy and bystander effect.

FC: Therapeutic strategies for HER2-overexpressed breast cancer have been improving since the discovery of trastuzumab. During the last decade, we have evolved from dual blockade using pertuzumab/trastuzumab (NCT00567190) to antibody–drug conjugates (ADC). The general concept has been to combine the therapeutic effect of trastuzumab with chemotherapeutics that can be delivered safely to the tumoral intracellular compartment, transforming chemotherapy and targeted therapy, reaching a higher clinical benefit without classical chemotherapy side effects. Trastuzumab–emtansine was the first ADC approved by the US FDA based on the EMILIA trial (NCT00829166).

FC continued: Combining trastuzumab with an anti-tubulin chemotherapeutic, emtansine, as second-line therapy for HER-2 metastatic breast cancer, demonstrated a high overall response rate of 43% and a long overall survival of 30.9 months. Lately, trastuzumab in combination with a topoisomerase I inhibitor, deruxtecan, showed impressive response rates (60.9%), even in a heavily treated population (Destiny–Breast01: NCT03248492).

MM: The introduction of trastuzumab – now an outdated option for the treatment of metastatic breast cancer (MBC) – was considered a major achievement in the late 1990s (of note, it is still the only available option in many low- and middle-income countries). In 2012, CLEOPATRA (NCT00567190) – one of the most successful trials ever conducted in metastatic breast cancer – firmly established pertuzumab-based dual blockade as a new standard of care in the first-line setting. At the end of study analysis, after a 100-month follow-up of the trial, a clinically relevant overall survival gain was still apparent, with 37% of the pertuzumab-treated patients still alive and 59 patients still in complete remission. In the second-line setting, the immunoconjugate trastuzumab emtansine (T-DM1) became standard of care treatment based on two successful randomized clinical trials. Beyond second-line, several options were available, with no single agent or schedule being considered a standard of care.

However, over the past 2 years, the tyrosine kinase inhibitor, tucatinib (in combination with capecitabine and trastuzumab), and the immunoconjugate trastuzumab-deruxtecan have both become preferred treatment options after failure of dual-blockade and T-DM1 – with the former also showing impressive activity against brain metastases.

However, along with this phenomenally successful anti-HER2 drug development story, it is important to highlight the contribution of other ‘non-drug’ approaches, such as stereotactic body radiation therapy (SBRT) to treat brain metastases (a major cause of death and morbidity in this population) and occasional foci of oligometastatic systemic disease.

Question 2: What treatments and treatment sequencing do you currently use to treat HER2-positive metastatic breast cancer?

HCK: The first-line standard is dual blockade with trastuzumab and pertuzumab, in combination with a taxane. An exception is made for cases progressing within less than 6 months after termination of the dual blockade in the adjuvant setting. In hormone receptor-positive disease we usually replace the taxane with an endocrine therapy after 18 weeks. Second-line therapy usually consists of T-DM1 irrespective of hormone receptor status. For third-line therapy, new options like tucatinib, capecitabine and trastuzumab or trastuzumab deruxtecan have replaced the former third-line standard lapatinib and capecitabine. Another option in higher lines is the combination of neratinib and capecitabine. A chemotherapy-free combination also used in later lines of therapy is trastuzumab and lapatinib.

FC: I work as a Medical Oncologist at The Instituto Oncologico Nacional in Panama so we don't have access to many of these newer treatments. Therefore, we currently start with taxanes – trastuzumab as first-line therapy. Dual pertuzumab/trastuzumab plus chemotherapy is not approved in our center. After a response, we frequently start trastuzumab maintenance. An approach with endocrine therapy – trastuzumab in HR-positive patients – is also frequently used. As second-line therapy, we have access to T-DM1, which is usually discussed on a multidisciplinary board to optimize patient selection. Further lines include lapatinib, capecitabine, gemcitabine, trastuzumab, vinorelbine, trastuzumab and other chemotherapeutics such as anthracyclines.

MM: I would usually employ pertuzumab-based dual-blockade with a taxane as a first-line option – though selected patients with HR+ disease can be treated with dual-blockade and endocrine therapy. My natural second-line choice is T-DM1, though occasional patients experiencing long-term control on dual-blockade can be re-challenged with chemotherapy before switching to T-DM1. In countries where tucatinib (with capecitabine and trastuzumab) and trastuzumab-deruxtecan are available, they might be the optimal next treatment choice (probably in this order). Due to its exceptional activity against brain metastases, earlier use of tucatinib may be considered in patients with life-threatening brain metastases. Beyond this point, further treatment options include lapatinib, neratinib, margetuximab and pyrotinib (in China) – though none will match the efficacy of earlier line therapies.
Of note, I also make frequent use of SBRT (and occasional surgery) to treat residual or active foci of oligometastatic and/or locoregional disease.

Question 3: What are the major challenges for HER2-positive breast cancer treatment? How do you think they can be overcome?

HCK: One of the major challenges in the era of a variety of HER2-positive-directed agents is the right choice for the individual patient. Especially in higher lines of therapy, head-to-head comparisons never include all options and therefore optimal sequencing is a challenge. Real-world data from prospective registries like the PRAEGNANT-study (NCT02338167) have provided and will provide data that will help decide the best agent or combination for the individual patient.

FC: The main challenge is the high costs of these treatments. The inequity in access to cancer therapies has been a reality in developing countries for many years. From my point of view, as a general oncologist, this challenge can be partially overcome by applying a pharmacoeconomic analysis for treatment selection, using parameters for value-based pricing like quality-adjusted life years and cost-effectiveness ratios. Additionally, optimizing the patient selection (for which a multidisciplinary board is really helpful), is essential. Finally, it is important to expand the access of clinical trials to cancer centers in developing countries, which have the potential to contribute to cancer research in many tumoral models.

MM: The median survival of patients with HER2-positive metastatic breast cancer is now well above 5 years – which is an impressive achievement. However, the more these powerful drugs prolong survival, the greatest challenge becomes central nervous system (CNS) disease control. New agents should ideally have meaningful activity against CNS disease – because SBRT and whole brain radiotherapy will eventually fail. In this aspect, tucatinib represents major progress.

Furthermore, anti-HER2 treatments employed in the adjuvant setting (pertuzumab, trastuzumab and T-DM1) have shown limited ability in preventing CNS recurrence – in contrast with their impressive performance in terms of preventing systemic recurrences.

In summary, there is a pressing need for the development of new treatments or techniques that truly prevent CNS disease recurrence or progression. Clinical trials that incorporate CNS-active anti-HER2 agents (such as tucatinib) into the early-stage setting are a promising approach to achieve this goal.

Question 4:
What promising treatment developments in HER2-positive metastatic breast cancer do you think will be implemented into clinical practice?

HCK: There are ongoing studies trying to improve the combined endocrine and HER2-directed therapy in triple-positive (hormone receptor and HER2-positive) metastatic breast cancer either after de-escalation from chemotherapy to endocrine therapy like the PATINA-study (NCT02947685), or initially offering a chemotherapy-free regimen like the DETECT V-study (NCT02344472). Both studies combined dual blockade with trastuzumab and pertuzumab with endocrine-based therapy and an endocrine agent plus a CDK4/6 inhibitor. The concept of combining a CDK4/6 inhibitor and HER2-directed therapy has already demonstrated efficacy in the monarcHER-study (NCT02675231) for single blockade with trastuzumab. The concept of combining dual blockade with CDK4/6 inhibition is further developing this principle and will hopefully lead to chemotherapy-free options for patients with triple-positive metastatic breast cancer.

FC: Over the next few years, we are looking forward to good oncological results of other types of new drugs, now applied to specific populations. Tailoring the cell cycle could be a really encouraging strategy in HR-positive/HER2-overexpressed patients, given the results of CDK 4/6 inhibitors in HR-positive metastatic breast cancer. The SOLTI–1303 PATRICIA trial (NCT02448420) has recently reported good progression-free survival at 6 months in a pretreated (two lines) population, opening the door for a Phase III trial. From my point of view, this treatment has a high probability of being implemented into clinical practice over the next few years.

Another potential target in HER2-overexpressed breast cancer is the immune evasion. Although there is no approved second-line or further treatment in this area, there is a trial comparing dual-HER therapy plus taxanes plus atezolizumab versus dual-HER2 therapy plus taxanes plus placebo. In my opinion, this approach probably won't be able to clinically impact the entire population. Potentially, the clinical benefit could be increased by tailoring patient selection with predictive biomarkers.

Additionally, there are more ADCs under development, including bi-specific antibodies that are directed to both HER2 and specific T-cell epitopes, redirecting these cells to attack breast cancer cells. There are also T-cell dependent antibodies under preclinical investigation. Therefore, a higher clinical benefit could be expected with this therapeutic strategy.

I think that we can expect trastuzumab–deruxtecan and tucatinib being implemented into routine clinical practice over the next few years, particularly in patients with CNS disease.

MM: It is likely that agents with powerful activity against brain metastases will increasingly become major players in the treatment of HER2-positive breast cancer – especially when employed in earlier treatment lines. It is also possible that new technologies, such as liquid cfDNA analyses, as recently demonstrated in a study in triple-negative breast cancer, will help identify patients at high risk for CNS recurrence/progression who might need the addition of a CNS-active agent or true prophylactic treatment (such as whole brain radiotherapy).

However, in my opinion, it looks like the future of anti-HER2 therapy belongs to immunoconjugates. Two of these agents are already commercially available, and others will likely follow. This class of drugs has unique advantages – such as a high level of clinical efficacy and a favorable tolerability profile when compared to chemotherapy.

Furthermore, part of the progress in the management of HER2+ MBC will be inevitably attributable to non-drug therapies. This is probably the case of SBRT for oligometastatic disease, which has an evolving role in breast and other cancers. As suggested by the 59 patients (7–8%) still in complete remission at 8 years of follow-up in the CLEOPATRA trial, some of these patients might be potentially curable. In line with this argument, I still think that the role of surgical resection of the primary tumor – especially those showing incomplete response to induction systemic therapy – has not been properly addressed in HER2-positive metastatic breast cancer (in the ECOG E2108 trial, for instance, few patients were HER2-positive).

Finally, there is a small group of patients whose tumors show incomplete expression of HER2 – this is the case of 'heterogeneous HER2 expression' and (maybe) some tumors with IHC 1+ or 2+ and FISH positive. These tumors tend to show lower responses to anti-HER2 therapies and may be more dependent on chemotherapy. Trastuzumab-deruxtecan – a novel immunoconjugate – shows promising activity against these tumors and larger studies are now ongoing. Nevertheless, a lot of work must be done to better characterize these tumors in terms of biology and response to treatment.

Case studies

Case 1 (HCK):

I am presenting one of my favorite cases of HER2-positive breast cancer, a patient who is surviving now for over 13 years on first-line therapy. At the time of writing, the patient is a 76-year-old woman who was diagnosed with HER2-positive hormone receptor-positive breast cancer with pulmonary metastases at primary diagnosis at the age of 63. We performed a CT-guided core cut biopsy of one of the pulmonary metastases and found the same tumor biology in the lung as in the breast. She started on trastuzumab and paclitaxel. After 18 weeks (6 cycles) we de-escalated the therapy to trastuzumab and anastrozol. The choice of the aromatase inhibitor was driven by the results of the TANDEM-trial (NCT03517540). After 52 weeks of therapy with trastuzumab the lung CT showed no evidence of disease and after 30 more weeks the breast ultrasound and MRI demonstrated a clinical complete remission also in the breast. In this situation, we added denosumab (60 mg q6m) because we realized that this patient could possibly stay on an aromatase inhibitor for long enough to develop aromatase inhibitor-induced bone loss. The patient has an excellent quality of life, works in her garden and takes care of her grandchildren. Every six months we discuss if maybe after so many years of complete remission the patient wants a drug holiday but she always refuses and begs not to take away her life insurance.

HCK continues: I am presenting this case because it illustrates how far we have come even in the aggressive subtype of HER2-positive breast cancer by the implementation of targeted therapies.

FC comments: This case illustrates the impact of achieving a ‘No Evidence of Disease’ state after systemic therapy for HER2 metastatic breast cancer. There is retrospective evidence that suggests this population (about 13% of metastatic HER2 breast cancer) has unique clinical characteristics, usually oligometastasic disease, with a 10-year overall survival of almost 100%, in contrast with 4% of those patients who do not achieve this state. Regarding the trastuzumab–anastrozole maintenance, there is also evidence suggesting an interaction between both the HER2 and estrogen receptors in the resistance development, so this approach is usually used in current clinical practice. In this case I would suggest continuing the same therapeutic regimen until toxicity, taking in consideration the potential heart toxicity of trastuzumab at this point of the treatment.

Case studies

MM comments: Long-term disease control has become quite common in HER2-positive breast cancer. In the CLEOPATRA trial, 59 patients are still in complete remission at the end of study analysis (100 months of follow-up). Every single oncologist who treats breast cancer will have seen a couple of such cases, although in this case it was achieved with trastuzumab alone, they are becoming more and more common with the availability of modern HER2-targeting therapies.

Curiously, after many years on treatment and usually enjoying a normal life, these patients become so reassured that they 'forget' the initial scenario of 'an incurable disease with a dismal prognosis' and instead, start to develop treatment fatigue. However, few oncologists currently feel reassured to recommend treatment pause or discontinuation because of scarce clinical data to support this approach. Nevertheless, it is a relevant question for patients and for the sustainability of healthcare systems. I remain hopeful that clinical data will be eventually produced and shed some light into this misty area. In the meantime, I would personally try to persuade this patient to remain on treatment.

Case studies

Case 2 (FC):

A 44-year-old patient, who presented with stage II triple-negative breast cancer in 2011. She received a mastectomy and adjuvant adriamycin–cyclophosphamide followed by radiation therapy. In a follow-up visit in 2016, a 2.5 cm tumor on the right breast was detected. The biopsy confirmed a new primary, with a negative hormone receptor and HER2 overexpression. Now she received quadrantectomy and trastuzumab for 1 year. In January 2019, bone and liver metastases were detected. They received capecitabine–trastuzumab as first-line therapy, completing 8 cycles. However, in October 2019, they progressed on the right breast. They were presented on a multidisciplinary board and T-DM1 was recommended. During T-DM1, a partial response was documented (including liver lesions), but 6 months after, they progressed in the right breast and axilla, so we changed therapy and started paclitaxel–trastuzumab. They received further lines, including gemcitabine–trastuzumab and doxorubicin, but finally succumbed on March 2021.

This case represents the usual therapeutic strategies that are used in routine clinical practice in our center (considered a National Cancer Center), where we don't currently have access to dual HER2 therapy in the metastatic setting. This patient did not receive because it wasn't approved at the time.

FC continues: When the recurrence was detected, an IV chemotherapy was proposed, but the patient preferred an oral route administration, so capecitabine–trastuzumab was used as first-line therapy (and controlled the disease as expected for 8 months).

Besides that, T-DM1 was considered a good option and, therefore, a partial response was reached.

In an ideal scenario, this patient should have been treated with a sequence including a dual HER2 blockade plus taxanes as first-line therapy, TDM-1 as second-line, capecitabine–tucatinib–trastuzumab as third-line (also capecitabine–lapatinib is a good option) and further lines should include a HER2 therapy.

HCK comments: I completely agree with the colleagues regarding the ideal scenario and would only add the options of trastuzumab deruxtecan and neratinib in higher lines. In our center the patient would have received the TCH as a neoadjuvant therapy and a taxane with trastuzumab and pertuzumab as first-line therapy. Second-line would then have been T-DM1 and after that in the time before the approval of trastuzumab deruxtecan, neratinib and tucatinib the combination of lapatinib and capecitabine or lapatinib and trastuzumab. Our first choice would of course in any line be therapy in a clinical trial.

MM: Limitations in terms of access to novel therapies may soon become the greatest challenge in HER2-positive breast cancer; worldwide inequalities are already impressive. Over the past 15 years, this has been a major issue for patients from the public sector in my own country (currently covering 75% of the population).

Case studies

MM continues: Facing such challenges, physicians may sometimes make use of strategies exploring local treatments – such as surgical resection (or radiation) of a local / axillary disease progression to avoid 'wasting' lines of systemic therapy that will be desperately needed later. This could have been an option in her first (right breast) and second (breast and axilla) disease progression events – depending on the suitability of a local therapy, of course. We also try to have as many active clinical trials as possible, especially in the public sector where limitations are more striking. However, I acknowledge that these strategies have limitations, and socially driven disparities in survival expectations for patients with HER2+ MBC are likely to skyrocket over the next few years.

Case studies

Case 3 (MM):

A 50-year-old patient presented in June 2014 with a right breast mass and enlarged axillary lymph nodes. A breast biopsy revealed a HER2 3+, ER 100%, PR-negative breast cancer. They were staged with a PET-CT that, in addition to extensive locoregional disease, showed a small lytic lesion in the right ischium (SUV 4,7). A CT-guided biopsy of this lesion confirmed MBC. Clinical stage was T3N2M1.

The patient was started on first-line chemotherapy (docetaxel), pertuzumab and trastuzumab to which she had a complete metabolic response. Chemotherapy was discontinued after 8 cycles and she was kept on maintenance dual-blockade and endocrine therapy.

In March 2015, because of apparent residual disease on an MRI of the right breast and based on the limited evidence available at the time, we proposed a right mastectomy with axillary dissection (ypTisN0), followed by radiotherapy to the chest wall and regional lymph nodes. We performed SBRT treatment of the single (now fully sclerotic) bone lesion at the same time.
As of June 2021, the patient remains in complete remission – still on dual-blockade and endocrine therapy.

MM continues: In summary, we employed a strategy in this case that involved generous use of surgery and SBRT as adjuncts to the optimal systemic therapy. Though it is difficult to estimate the relative contribution of these measures to the long-term disease control experienced by this patient, such a strategy may at least contribute to avoid wasting lines of systemic treatment to address events of oligometastatic or locoregional tumor progression.

HCK comments: Before ASCO 2020, we also would have recommended local therapy of the breast with the aim of leaving no residual tumor behind. Although randomized Phase III data from trial E2108 demonstrated no benefit for this strategy (NCT01242800). The case reported here suggests that in selected cases it could still be an option. This may especially be the case in oligometastatic disease, a situation with a growing body of evidence regarding the benefit of local therapy, especially radiation to the metastatic sites. The case reported here is an impressive example that the long-term survival demonstrated in recent prospective trials and retrospective reports is not only a hypothesis but is really happening in clinical practice.

FC comments: This is a typical case of oligometastatic breast cancer, in which a metastasis – directed local therapy has been integrated to systemic approach, reaching a clinical benefit to the patient. Since the conception of oligometastatic disease as an intermediate stage between local and metastatic disease, some prospective trials have demonstrated that this multidisciplinary approach impact on disease control and overall survival.

Case studies

FC continues: Besides this, there is no prospective trial evaluating the role of metastasis – directed therapy among oligometastatic breast cancer. The ongoing Phase II/III NRG BR002 (NCT02364557) trial seeks to provide the first randomized data to determine whether MDT in oligometastatic breast cancer improves outcomes; however, it's reasonable to indicate this approach in those patients with good disease control, preferably with bone metastasis or few lung/liver metastasis, given the potential impact in delay progression or, in some cases, to achieve a cure.

The opinions expressed in this panel discussion are those of the author and do not necessarily reflect the views of Oncology Central or Future Science Group.

Hans-Christen Kolberg

Priv-Doz Dr med Hans-Christian Kolberg is Head of Department of Gynecology and Obstetrics at Marienhospital Bottrop, an Academic Teaching Hospital of the University of Duisburg-Essen, in Germany, a position he has held since 2005. Before that he served as senior consultant and Head of the Breast Cancer Center at the University Hospital of Schleswig-Holstein, Campus Lübeck.

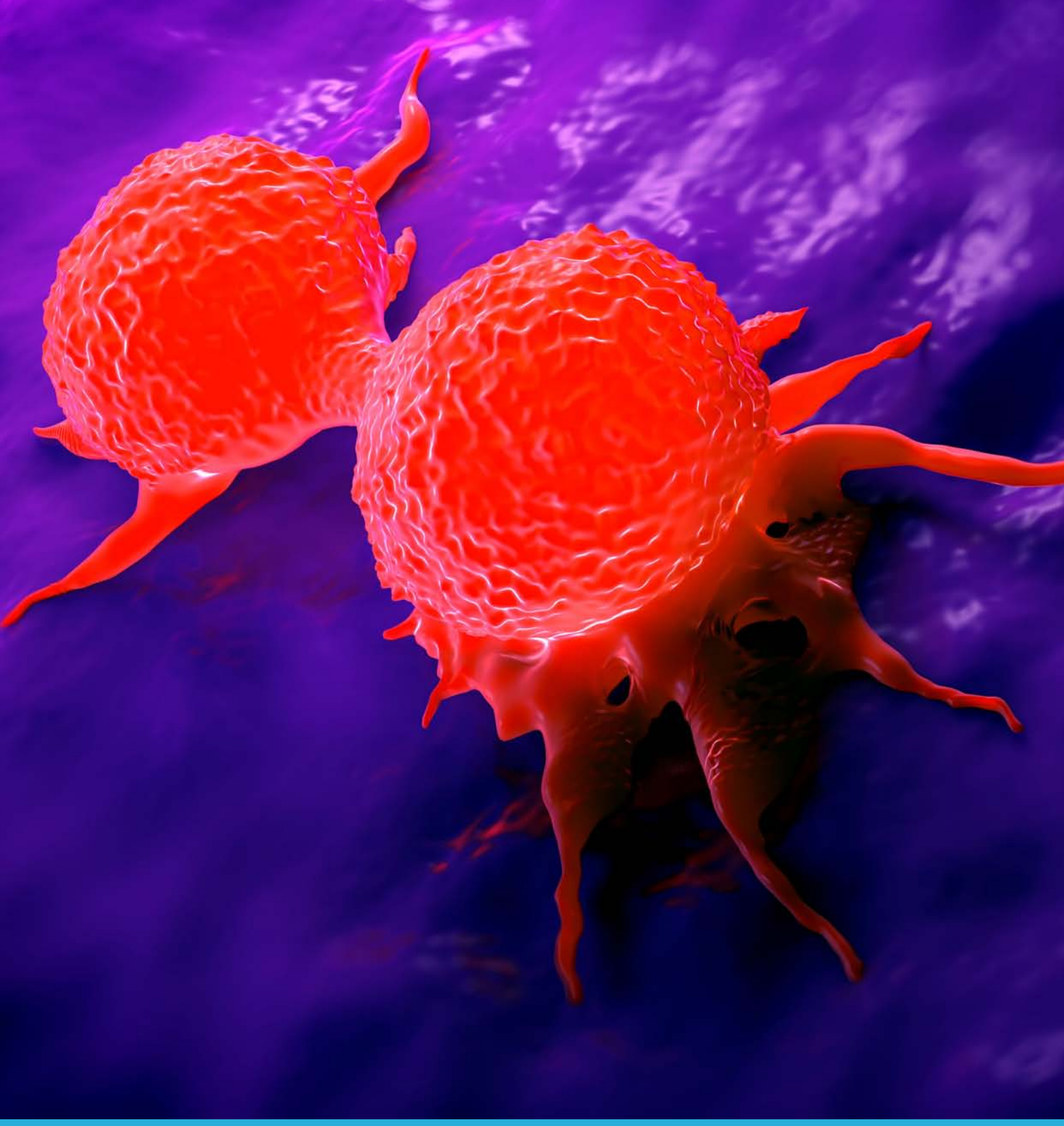
Franklin Castillero

Dr Franklin Castillero is a Medical Oncologist at National Oncology Institute of Panama and also educational coordinator of the residence program in Medical Oncology. He finished Medical School at University of Panama on 2012, then completed an Internal Medicine Residency in Hospital Doctor Arnulfo Arias Madrid (Panama) on May 2017 and a Residency in Medical Oncology at the National Oncology Institute of Panama in December 2020. He also has an International Diploma on Molecular Biology applied to Medical Oncology from Liege University (Belgium).

Max Mano

Dr Mano graduated in 1996 and was trained in internal medicine and clinical oncology at Hospital Nossa Senhora da Conceicao (Porto Alegre, Brazil) from 1997–2000. He engaged in a fellowship programme at Institut Jules Bordet (Brussels, Belgium) from 2001–2003 and has a PhD in Medical Sciences at the Federal University of Rio Grande do Sul (UFRGS; Porto Alegre, Brazil). In 2018, he accomplished a Master of Advanced Studies in Medical Leadership programme at the Academy of Leadership Sciences Switzerland.

Dr Mano has practiced as a consultant oncologist at Beatson Oncology Centre (Glasgow, UK), Institut Jules Bordet (Brussels, Belgium), Cliniques Universitaires St Luc (Brussels, Belgium), Instituto do Câncer do Estado de São Paulo (Brazil) and has been an Assistant Professor of Oncology at the University of São Paulo (Brazil). He has worked part-time as a clinical oncologist at Hospital Sirio Libanes (São Paulo, Brazil) and since 2020 has been at Grupo Oncoclínicas (São Paulo, Brazil). Since 2018, Dr Mano has also been Faculty at the Academy of Leadership Sciences Switzerland. Dr Mano is currently a member of the European Society of Medical Oncology (ESMO) and the Brazilian Society of Clinical Oncology (SBOC) Breast Cancer Groups.



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