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Nicolas STOUP[1], Maxime LIBERELLE[2], Nicolas LEBEGUE[2], Isabelle VAN SEUNINGEN[1*]

[1]Univ. Lille, CNRS, Inserm, CHU Lille, UMR9020-U1277 - CANTHER - Cancer - Heterogeneity Plasticity and Resistance to Therapies, F-59000 Lille, France.
[2]Univ. Lille, Inserm, CHU Lille, UMR-S 1172 –LiNC –Lille Neuroscience &Cognition, F-59000 Lille, France.
*Correspondence: isabelle.vanseuningen@inserm.fr; Phone: +33631529030

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ABSTRACT

The Epidermal Growth Factor Receptor family is a class of transmembrane proteins, highly regarded as anti-cancer targets due to their pivotal role in various malignancies. Standard cancer treatments targeting the ErbB receptors include tyrosine kinase inhibitors and monoclonal antibodies. Despite their substantial survival benefits, the achievement of curative outcomes is hindered by acquired resistance. Recent advancements in anti-ErbB approaches such as inhibitory peptides, nanobodies, targeted-protein degradation strategies and bi-specific antibodies aim at overcoming these resistances. More recently, emerging insights into the cell surface interactome of the ErbB family open new avenues for modulating ErbB signaling by targeting specific domains of ErbB partners. Here, we review recent progress in ErbB targeting and elucidate emerging paradigms that underscore the significance of EGF domain-containing proteins as new ErbB targeting pathways.

ErbB receptors targeting in cancers remain an open area for innovative approaches

The ErbB/HER family of receptor tyrosine kinases (RTK) comprises four distinct members: ErbB1/EGFR (HER1), ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4) that selectively bind diverse EGF ligands [1]. Upon ligand binding, ErbB receptors undergo conformational changes that allow homo- or heterodimerization, resulting in the transphosphorylation and activation of the dimerized ErbB receptors. This event initiates key downstream signaling effectors activation leading to cellular proliferation, differentiation and migration [1] with pivotal roles in tumor initiation and progression [2]. In numerous cancer types, ErbB receptors are frequently overexpressed or amplified, constituting oncogenic drivers that lead to excessive signaling and uncontrolled cellular growth and migration, well-established hallmarks of cancer [3]. Thus, ErbB receptors are compelling anti-cancer targets which have been subject to intensive drug research.

The first development and approval of selective ErbB inhibitors have led to exciting clinical improvement of ErbB-driven malignancies, sustained by impressive initial responses. Two strategies are currently the standard care for these cancers: Monoclonal antibodies (mAb), which enhance natural anti-cancer immune system to target ErbB ectodomains or antagonize ligand-receptor interaction; and tyrosine kinase inhibitors (TKIs), that bind to the ATP-binding pockets located within the ErbB catalytic kinase domain to prevent kinase domain phosphorylation [2]. Rapidly, the efficacy of these strategies has been offset by drug-resistant mutations and crosstalk activations, invariably condemning patients [2,4]. Hence, novel anti-ErbB therapies are in high demand to overcome these resistances mechanisms.

Recent advancements have led promising results in overcoming resistances, supported by multiple targets involvement, nanoscaling and click chemistry [5,6]. Here, we review the anti-ErbB drugs approved in cancer therapy, alongside the mechanisms of resistance which curtail their long-term therapeutic efficacy. We also provide an overview of emerging developments in ErbB-targeted approaches encompassing innovative strategies designed to overcome these resistances. Moreover, we highlight the importance of the ErbB interactome as an under-explored avenue for the discovery of novel anti-ErbB therapeutics for cancer patients.

Clinically approved ErbB targeted therapies in cancers

Anti-ErbB Monoclonal Antibodies

Several antibodies have been approved to efficiently target ErbB1, ErbB2, and more recently ErbB3 [7].

Currently, four primary mAbs targeting the ErbB1 receptor have been approved for clinical use: cetuximab, panitumumab, nimotuzumab, and necitumumab (Table 1). They impede downstream signaling [8] and activate immune component by involving natural killer cells and **antibody-dependent cellular cytotoxicity (ADCC)** (see Glossary) mechanisms [8,9]. Anti-ErbB1 mAbs are currently approved in non-small cell lung cancer (NSCLC), advanced colorectal cancers (CRC), head and neck cancer (HNSCC) and gastroesophageal cancers (GOC), in combination with radiotherapy or chemotherapy [8,10].

Several anti-ErbB2 mAbs are approved to treat breast (BC) and gastric cancers (Table 1). Trastuzumab binds to the extracellular domain IV of the ErbB2 receptor, impeding its dimerization with other ErbB members [11]. Pertuzumab, targets the extracellular domain II of ErbB2 to prevent ErbB2 heterodimerization, was developed to treat patients whose cancers had progressed during trastuzumab treatment [12]. Pertuzumab–trastuzumab–hyaluronidase-zzxf, a subcutaneous formulation of the trastuzumab – pertuzumab association was granted FDA approval for metastatic ErbB2+ BC [13]. ErbB2 **Antibody-Drug Conjugates (ADC)** also emerge as an attractive way to efficiently target ErbB2+ tumors. These ADC involve the association of ErbB2 mAbs with inhibitory molecules (tubulin inhibitors, topoisomerase I inhibitors, anti-mitotic and DNA alkylators – See Table 1) and have shown success to treat cancers [14-17]. Interestingly, trastuzumab-Deruxtecan (T-DXd), have been approved for ErbB2^{low} metastatic BC based on DESTINY-Breast04 trial, showing improved progression free survival (PFS) and overall survival (OS) of patients compared to chemotherapy. These remarkable results are due to enhanced efficacy and delivery of Deruxtecan compared to other ADC, and its bystander effect targeting intratumor ErbB2 heterogeneity. These results are impactful since they redefine the druggability of ErbB2-positive cancers, based on ErbB2 minimum expression [14-16].

Several next generation ADC formats are also developed to target ErbB2. ErbB2 immune-stimulating ADCs (ISAC) conjugate TLR7/TLR8 agonist or stimulator of

interferon genes (STING) to trastuzumab/pertuzumab and show high antitumor activity along with robust immune activation and cytokine release in mouse xenograft models of ErbB2+ BC [18]. More recently, click chemistry enables the development of dual-playload ADCs for cancer therapy *in vitro*, by involving anti-ErbB2 mAbs equipped with two different cytotoxic effectors (microtubule inhibitor MMAE + DNA damage induced-PBD SG3457 or microtubule inhibitor MMAF + topoisomerase II inhibitor PNU-15968). Despite exerting dual mechanisms of action, neither agent demonstrate an improvement in *in vitro* potency when compared with their corresponding single-drug ADCs. However, these different approaches offer promising capabilities for tackling drug resistance, tumor heterogeneity and treatment-related adverse effects [18]. Hence, anti-ErbB2 mAbs and ADC are currently approved or under clinical investigation in metastatic ErbB2+ BC, GOC or solid tumors, alone or combined with chemotherapy and kinase inhibitors [14,19,20] (Table 1).

More recently, mAbs targeting ErbB3 have been developed (Table 1), due to the emerging interest for ErbB3 in therapeutic resistance [7,21]. Patritumab and its Deruxtecan (DXd)-based ADC derivates have been extensively studied in various clinical and preclinical models of cancers, showing a reduction in cell proliferation, migration, and anchorage-independent growth [21,22]. These mAbs are also currently under advanced clinical investigations in various solid tumors, in combination with chemotherapy, kinase inhibitors or other mAbs [22,23,7].

The role of ErbB4 in cancers is still controversial, depending on cancer-subtype, cancer treatment and/or ErbB4 (iso)form, thus limiting its potential as a major ErbB target [24,25]. Particularly, studies show that the prognostic impact of ErbB4 does not only depend on its expression status but mainly depends on its subcellular localization, and more precisely, its cleavable intracellular domain (4ICD). Hence, the 4ICD regulates cell proliferation, differentiation, and cell death of epithelial cells [25,26]. P6-1 and Ab1479 mAbs that target the extracellular domain of ErbB4 and prevent NRG ligand binding show growth-inhibiting capacity of ErbB4-positive BC in 3D preclinical *in vitro* models (Table 1). Similarly, C6 mAb, which mimicks NRG1 effects and prevents translocation of 4ICD into the nucleus, mediates pro-apoptotic signaling via mitochondria impairment [26].

ErbB Tyrosine Kinase Inhibitors

TKI targeting ErbB have brought significant clinical advantages as anticancer therapies. However, despite primary responses, tumors rapidly develop resistance to these drugs [24, 27].

First-generation TKIs targeting ErbB1 (erlotinib, gefitinib, icotinib and lapatinib) or ErbB2 (lapatinib, tucatinib) are TKIs reversibly binding the ATP-binding pocket of ErbB receptors (Table 1). They have demonstrated high efficacy for patients with advanced lung cancer (LC) harboring ErbB mutations, as well as ErbB2-positive advanced CRC and BC. Consequently, PFS increases in all groups of patients treated with these TKIs, despite no statistical difference in OS. Since the initial treatment for these patients was platinum-based combination chemotherapy, these TKIs became second- and then first-line chemotherapeutic agents [27].

Second-generation TKIs are irreversible pan-ErbB (ErbB1, ErbB2, and ErbB4) inhibitors developed to overcome resistance mediated by the ErbB1^{T790M} mutation (Table 1) [24]. Despite promising pre-clinical data, the drug concentration could not reach the therapeutic range due to relatively severe adverse events. Afatinib and dacomitinib were approved as first-line treatments for patients with ErbB1-mutated NSCLC and neratinib was approved for the treatment of ErbB2-positive BC. Recent analysis of LUX-Lung 2/3/6 clinical studies indicate that afatinib is particularly effective against uncommon ErbB mutations (ErbB1^{G719X}, ErbB1^{S768I}, and ErbB1^{L861Q}) and less effective on classical ErbB1^{T790M} and ErbB1^{ins20} mutations [28]. Other second-generation TKIs targeting pan-ErbB (pyrotinib), ErbB3 (TX1-85-1), and ErbB4 (molecules I and II, compounds Ik and IIa, compounds A and B) are currently under preclinical and clinical evaluation for various advanced and mutated cancers [23, 26, 29, 30] (Table 1).

A pyrimidine-based third-generation TKI was subsequently developed to target ErbB1^{T790M} mutation as well as common ErbB1 mutations without inhibiting wild-type ErbB (Table 1). Osimertinib was initially approved as a second-line treatment in ErbB1^{T790M}-positive LC [24,31]. Thereafter, it was approved as a first-line treatment for advanced mutated LC [31, 32]. Lazertinib, rociletinib, olmutinib, naquotinib, avitinib, and nazartinib are currently under clinical evaluation in ErbB-mutated LC [24,32]. However, despite promising results, osimertinib resistances have also emerged [33].

Currently, fourth-generation TKI are under clinical investigations to counteract these resistances [24] (Table 1).

The development of ErbB-targeted therapies (mAbs and TKI) has greatly expanded treatment options in cancer therapeutics and spurred new avenues in the extensive and comprehensive research on ErbB-targeting improvement. However, despite considerable therapeutic progress, both primary and acquired resistances to these drugs have emerged, resulting in disease recurrence [2,4,27,33] (Box 1).

Anti-ErbB Innovative approaches: new perspectives to overcome resistances in cancers

Inhibitory Peptides

Several strategies, based on Protein-Protein Interaction (PPI) interfaces, have been employed to develop inhibitory peptides targeting ErbBs (Box 2).

The first approach is to target receptor-ligand interactions [24, 48] (Figure 1 b)). Among EGF-mimicking peptides, GE11 (YHWYGYTPQNVI) has demonstrated significant *in vitro* and *in vivo* anti-tumor properties in NSCLC and hepatocarcinoma cell lines. Subsequent research has further underscored its potential anticancer effects, indicating its progression toward clinical utility [52]. Pep11, a peptide designed on the ErbB1-cetuximab interface has also demonstrated significant *in vitro* anti-tumor properties in BC and skin cancer cells [53]. Interestingly, cyclic forms of receptor-ligand inhibitory peptides, such as cycloL1 (cycloKLARLLT) and cp23G, allow better stability and affinity for EGFR while enhancing anti-tumoral function *in vitro* [54,24].

The second approach is to disrupt dimerization of ErbBs [54,48] by designing peptides targeting the dimerization arm within domain II of ErbBs, thus preventing ErbB complexes formation. Proteolytically stable peptides mimicking the dimerization arm structure of ErbB1 or ErbB2, such as EDA2 (AhaYNPTTYQPg), Peptide 1 (CQTPYYMNTC) or **HSTC79**, show high efficacy to reduce survival in BC and LC [54] (Figure 1 b)). The use of nanoscaled particles fused to peptides can also improve the disruption of ErbB complexes. For example, TMP1 peptide (BP-FFVLK-YCDGFYACYMDV), which turns into nanofibers when it binds to Erb2 on cancer cells, shows impressive ErbB2/EGFR disruption and leads to BC cell apoptosis *in vitro* and *in vivo*. [55]

Peptides with intracellular inhibitory activity have also been developed to target ErbB1 (Figure 1 b)). Disruptin, a peptide featuring a motif similar to the intracellular segment of ErbB1, destabilizes ErbB1/ErbB2 dimerization, prevents receptor interaction and promotes receptor degradation [56]. Mimetic peptides (SAH-EJ1 stapled peptide and TE-64562) targeting the inner juxtamembrane domains of ErbB1 have demonstrated the formation of non-functional dimers, inhibiting the kinase activities of ErbBs while inducing cell death in various tumor models, including TKI-resistant LC [57]. Similarly, the study of intracellular regulatory proteins of ErbB family has yielded the development of inhibitory peptides. Mitogen-inducible gene 6 (Mig6) segment 1, a juxtamembrane protein that regulates ErbB kinase activity, was used to develop a pan-ErbB inhibitory peptide to treat resistant acquired mutated forms of LC [58].

Peptides can also be engineered to disrupt ErbB complex formation by anchoring to the plasma membrane, creating steric hindrance [59] (Figure 1 b)). This strategy has been employed to target ErbB1 and ErbB2 in diverse cancers, yielding the peptide PET1 and MTP-NeuT [60].

Similar screening strategies (computational design and phage display) have been applied to design inhibitory peptides targeting ErbB2 and ErbB3 receptors. These peptides impede receptor dimerization and activation, and ultimately promote receptor degradation, diminishing their oncogenic functions in BC and resistant pancreatic cancer models [61].

In addition to their potential as direct inhibitors or drug conjugates, peptides are used as diagnostic tools, including the radiolabeling of ErbB2 peptides such as A9 and AHNP [62,63], or the ErbB1-targeting peptide GE11 [52]. More recently, some of these peptides have found utility in **PhotoDynamic Therapies (PDT)** when employed as light-photosensitizers, specifically targeting ErbB-overexpressing cancers [64] (Figure 1 b)).

Despite their promising anti-tumor activities, none of these peptides are currently undergoing clinical trial development. However, building on the progress seen with GE11 peptide [52] coupled with the growing appeal of peptide inhibitor strategies in oncology [48], stable cyclic peptides and peptide-based nanocarrier efficiently targeting ErbB-overexpressing cancers should emerge in the coming years.

Bi-specific antibodies

Bi-specific antibodies (BsAbs) have emerged as a promising advancement in oncology to address drug resistance and enhance the efficacy and safety of mAbs, rendering them attractive for ErbBs therapeutics [65]. BsAbs possess a compelling attribute as they selectively recognize and bind two distinct antigenic epitopes simultaneously, holding potential for targeting compensatory mechanisms. They can be tailored to target tumor-associated antigens while minimizing damages to normal cells, thereby facilitating more precise and effective cancer treatment with reduced adverse effects [65]. This strategy is particularly enticing, facing the difficulty for tumors to develop two drug resistances at once. Consequently, BsAbs targeting ErbB mobilize the three primary mechanistic strategies of BsAb: bridging receptors, bridging tumor and immune cells, and bridging receptors and cytokines (Figure 1 a)) (Table 2).

Simultaneous inhibition of two tumor-associated proteins can lead to a more robust therapeutic effect by targeting multiple pathways involved in cancer progression and acquired bypass track resistances [66,67] (Box1) (Figure 1 a)) [65]. As LC exhibit ErbB amplification and c-Met mediated-bypass track resistance, the BsAb Amivantamab that targets both ErbB1 and c-Met has been used in cancer therapy [67,68]. It demonstrates *in vivo* antitumor activity, with an enhanced ErbB1/c-Met internalization and degradation [69], correlated to durable tumor regression in combination with third-generation TKI in NSCLC patients [70]. Interestingly, Amivantamab is effective against ErbB1 acquired mutations in clinical evaluations [71]. Likewise, ErbB3 is an emerging target in cancer resistance since it leads to compensatory activation when ErbB1/ErbB2 are targeted [7]. The BsAb Zenocutuzumab targets ErbB2 and ErbB3 receptors to mitigate this resistance and induce ADCC activity [72]. In the phase I/II "eNRGy" trial, a promising response rate of 35% was obtained in advanced pancreatic cancer [73]. This BsAb demonstrates particular efficacy in patients with *neuregulin-1* gene (NRG1) fusions, a genomic aberration found in less than 1% of solid tumors. *NRG1* fusion serve as potent oncogenic drivers, since NRG1 fusion-positive tumors rely on ligand-dependent activation of ErbB3 signaling cascades through paracrine loops, thereby limiting efficacy of therapies. Consequently, targeting the NRG1-fused ligand represents a rational approach for ErbB-directed interventions. Functionally, Zenocutuzumab employs a 'Dock & Block®' mechanism of action: initially the BsAb binds to the ErbB2 receptor at the cancer cell surface, and it subsequently impedes the interaction between NRG1-fused ligands and ErbB3 by obstructing the ErbB3

ectodomain. Consequently, this BsAb emerges as a promising therapeutic avenue to address the unmet clinical needs for patients harboring NRG1 fusions ligand dependency [72].

BsAbs can also bridge two receptors of the same type but located on different epitopes. For example, Anbenitamab (KN026) and Zanidatamab (ZW25) target two epitopes of ErbB2, combining the binding sites of trastuzumab and pertuzumab. They have demonstrated promising results in the treatment of ErbB2-positive BC, GO, and biliary tract cancers, limiting compensatory track resistances [74,75]. Several BsAbs have also been incorporated into ADC strategies, combining the high specificity of BsAb antibodies with the potent cytotoxicity of small molecules (Table 2). For instance, ZW49, a BsAb derived from ZW25, has exhibited favorable antitumor activity and safety in clinical trials [76]. To date, only Amivantamab (2021) and Zenocutuzumab (2023) have received approval.

The second strategy for BsAbs involves the bridging of tumor and immune cells, with particular emphasis on T-cell engaging bispecific antibodies (T-BsAbs or BiTE) [77] (Figure 1 a)). Importantly, **BiTE** antibodies overcome immune escape more effectively compared to traditional checkpoint inhibitors [78]. BiTE targeting ErbB2xCD3 or EGFRxCD27 induces tumor growth delay and increase T-cell mediated cytotoxicity in various ErbB-expressed cancer models [77,79]. These findings underscore the potential of BsAbs to overcome resistance to ErbB-targeted therapies. Targeting specific T-cell subsets with BsAb is a promising approach to enhance the efficacy and selectivity of BiTE therapies. BsAb 7D12-5 GS-6H4, which targets **Vγ9Vδ2 T cells** and ErbB1, has demonstrated activation of Vγ9Vδ2 T cells and apoptosis induction in CRC xenograft models [80].

The last strategy involves co-targeting of tumor antigens and pro-tumoral cytokines (Figure 1 a)). BCA101 consists of an anti-ErbB1 mAb linked to the extracellular domain of human TGFβRII to target soluble TGF-β. BCA101 combines the *in vitro* and *in vivo* effects of ErbB1 targeting, including the inhibition of cell proliferation and the activation of ADCC, with the functional neutralization of TGFβ, in PDX models of HNSCC [81].

Tri-/Tetra-specific antibodies

Trispecific (TsAbs) and tetraspecific (TvAbs) antibodies represent an advanced development beyond BsAbs, offering enhanced therapeutic potential. The ErbB2 x VEGFR2 x CD3 TsAb effectively inhibits tumor growth in BC and prostate cancer models resistant to trastuzumab [82]. ErbB2 x CD3 x CD28 TsAb resulted in effective tumor regression in a humanized BC model, even in patients who were not suitable for conventional anti-ErbB2 therapies [83]. GB263T, which recognizes ErbB1 and two different epitopes of Met, blocks their phosphorylation, activates enhanced ADCC function, and inhibits the proliferation of various tumor models harboring ErbB1 or Met mutations, highlighting its potential to counteract resistance to anti-ErbB therapies [84].

Despite the success of BsAbs in hematological malignancies and promising preclinical results in solid tumors, their therapeutic benefit in clinical trials, especially in the context of resistance to ErbB-targeted therapies, remains to be established. The limitations observed with BsAbs include mathematical models that do not consider the oversight of ADCC, internalization processes or the intricate dynamics of RTK signaling. The suppressive tumor microenvironment and the three-dimensional cellular organization (distribution of receptors, local steric hindrance) also hamper complex predictive modeling of BsAb efficacy [85, 86]. These mathematical models necessitate an integration of BsAbs' flexibility, as well as considerations of epitope density and stoichiometry, as these factors significantly influence BsAbs' **avidity**, thereby impacting their therapeutic benefits. This altered avidity arises from the multiplicity of binding specificities inherent in BsAbs, which are mediated by variations in antigen targeting proportions and the valency of BsAb arms [85,87]. Importantly, the sum of monovalent affinities does not necessarily reflect bispecific binding efficacy, especially in contexts where epitope proportions are imbalanced. Consequently, BsAbs often fail to show clinical improvement compared to mAbs. Thus, meticulous fine-tuning of both affinity and avidity is a prerequisite in the engineering of BsAbs to develop a new class of multivalent antibodies with tailored properties and enhanced functionalities [85]. Orthogonal experimental tests, such as immune system engagement assays (ADCC and complement activation), also need to be achieved during the screening process of BsAb to confirm their immunological superiority compared to monoclonal therapies [86].

Specific anti-ErbB BsAbs, such as Zenocutuzumab and Zanidatamab, have thus rapidly progressed toward late-stage clinical trials (Supplemental Table 1), indicating a promising future for this therapeutic approach. Optimizing the selection of anti-ErbB BsAbs, based on robust predictive models coupled with comparative immunological tests against control mAb, will be a prerequisite to the success of this approach in clinical trials [86].

Nanobodies

Despite exhibiting mixed clinical success, mAbs are associated with significant drawbacks, including limited tumor penetration and development of therapeutic resistance. Recent advancements in antibody-based therapeutics have led to the discovery of **nanobodies**, which have demonstrated substantial advantages and translational promise in both preclinical and clinical studies [88]. Their nanoscale dimensions enable efficient tumor penetration, minimize steric hindrance near targeted epitopes, and demonstrate reduced off-target accumulation [89].

Several nanobodies targeting ErbB1 [90], ErbB2 [91], and ErbB3 [92], have been developed to target various regions of ErbBs (tyrosine kinase domain, dimerization interface or ectodomains) [90] (Figure 1 c)) or other receptors involved in TKI resistance such as c-Met and overcome therapeutic resistance associated with mAbs. Recently, nanobodies have been designed to target soluble EGF in combination with osimertinib, thereby overcoming resistance in LC [93]. Interestingly, several anti-cancer BsAbs incorporate nanobodies, such as ErbB1xCD16 (7D12-C21), ErbB2xErbB2 (RR2-H-RR4), or EGFRxVγ9Vδ2-T (7D12-5GS-6H4) BsAbs [94].

In addition to their intrinsic therapeutic potential, nanobodies can enhance the efficacy of other cancer therapies, particularly in the context of the tumor microenvironment. Due to their nanoscale size, nanobodies have been investigated to circumvent the drawbacks encountered in Chimeric Antigen Receptor (CAR)-T cell management in solid tumors [95]. Recently, nanobody-CARs targeting ErbB1 have been developed, showing T cell activation, tumor regression and cytokine release in a BC model [96]. Studies have also explored the integration of nanobodies into the **universal CAR (UniCAR) system**, which offers a switchable CAR-T cell technology to rapidly mitigate CAR treatment-related adverse effects. Anti-ErbB nanobody-based UniCAR-T cells have induced degradation of ErbB1-expressing tumors. Based on cetuximab, bivalent

version of this UniCAR has been developed, further targeting low ErbB1-expressing tumors *in vivo* [97] (Figure 1 d)).

Extensive research has also unveiled the potential of nanobodies as carriers for drugs, toxins, radionuclides or nanoparticles [98,99]. (Figure 1 d)). For instance, fragment derived from the bacterial toxin *Pseudomonas exotoxin A* (PE24X7) have been fused to anti-ErbB2 nanobodies, enhancing cytotoxic effects in various ErbB overexpressing tumor models [100]. Another example shows that ferritin-based nanocage fused to nanobodies allow transport of therapeutic molecule within tumors. Encapsulation of manganese phthalocyanine to ferritin conjugated nanobodies (MnPc@Nb-Ftn) specifically induces cell death of EGFR-positive NSCLC *in vitro* [98].

Another promising application of nanobodies is in PDT where they offer a potent solution to overcome mAbs limitations (reduced PDT efficacy and extended patient photosensitivity) [64]. Anti-ErbB2 nanobodies conjugated to photosensitizers have effectively eliminated trastuzumab-resistant BC cells following a short laser treatment [101]. Similarly, anti-ErbB1 nanobody-PS conjugates have demonstrated targeted phototoxicity *in vitro* and *in vivo* in HNSCC and NSCLC [102] (Figure 1 d)).

In preclinical applications, nanobodies targeting ErbBs have shown promising anti-cancer effects, harnessing the therapeutic advantages of mAbs, and the targeting potential of nanoscale delivery systems. However, despite these substantial advantages, nanobodies exhibit a less immunogenic profile and are susceptible to rapid renal clearance, which restricts their therapeutic duration and can lead to renal toxicity, particularly with drug conjugates [88].

PROTACS / LYTACS

Targeted Protein Degradation (TPD) represents an innovative and rapidly advancing technology that exploits the protein degradation pathway [103]. Among the TPD strategies, Proteolysis-Targeting Chimeras (PROTACs) has emerged as an enticing approach [101]. PROTACs are heterobifunctional molecules that simultaneously engage the Protein of Interest (POI) and an E3 ubiquitin ligase, connecting them through a linker incorporated within the PROTAC structure. In ErbB-PROTACs, the selection of POI ligands derives from ErbB inhibitors, such as TKIs or their analogs (Table 1) (Figure 1 c)). This strategic choice preserves the specificity of the small molecule used as an ErbB ligand, especially when targeting ErbB mutated forms and

independently of the kinase inhibition activity. Consequently, ErbB-PROTACs overcome acquired resistance mechanisms [104-107]. Remarkably, the Dacomitinib-based PROTAC has been the first ErbB-PROTAC to show antitumor effects *in vivo*, demonstrating compelling degradation of $EGFR^{del19}$ mutated forms and promising antitumor activity in LC [107]. Fourth-generation inhibitors-based PROTACs have also been develop to target allosteric pockets on ErbB1. Fourth-generation EAI001 allosteric inhibitor as POI ligand demonstrates a selective *in vitro* inhibition of $EGFR^{L858R/T790M}$ mutated NSCLC proliferation. EAI001 in combination with osimertinib shows synergistically effects in Osimertinib-resistant cancer cell lines [108].
Click chemistry has shown the possibility of dual-ErbB PROTACs, enabling the simultaneous degradation of ErbB and another oncoprotein. Zheng *et al.* have incorporated gefitinib (ErbB1 TKI) and olaparib (PARP inhibitor) via click chemistry to efficiently degrade ErbB1 and PARP in cancer cells *in vitro* [109].

Antibody-based PROTAC (AbTAC) represents another emerging facet of TPD technology, capable of inducing the degradation of extracellular and membrane proteins [110]. Contrarily to POI ligands as in PROTACS, AbTAC strategy relies on BsAbs, with one arm designed to target a cell surface POI and the other arm directed at a transmembrane E3 ligase, such as RNF43 and ZNRF3 [111,112]. Recently, a cetuximab-based AbTAC was developed that induces significant apoptosis and inhibits cell proliferation in multicellular 3D spheroid assays [113].

AbTAC derivates, such as Degrader-Antibody based Conjugates (DAC), have also garnered significant attention in oncology due to the combination of ErbB antibody targeting and PROTAC technology (instead of the traditional cytotoxic payload). The mAb moiety recognizes an ErbB receptor, triggering internalization of the DAC–ErbB complex. The linker is degraded under proteolytic or acidic/reducing conditions, thus releasing the conjugated PROTAC to lead to POI degradation. In this way, ErbB2-DAC have been developed, coupling ErbB2 tumor-targeting (trastuzumab, pertuzumab) and PROTAC targeting the bromodomain-containing protein 4 (BRD4) - a crucial epigenetic regulator – or the G1 to S phase transition 1 (GSPT1) protein (ORM-5029). These DAC demonstrate interesting antitumor activity in mouse models of leukemia and BC respectively, with comparable activity with control ADC [18].

Similarly, **Lysosome-Targeting Chimeras (LYTAC)** have recently emerged as novel tools to induce the degradation of extracellular and membrane proteins via the endosome-lysosome pathway. LYTAC are BsAb simultaneously engaging the extracellular domain of a cell surface POI and a lysosome-targeting receptor. The formation of a ternary complex drives protein internalization through clathrin-mediated endocytosis, ultimately resulting in the degradation of the POI [114,115] (Figure 1 c)). Second-generation LYTACs featuring a triantennary N-acetylgalactosamine motif fused with cetuximab, have demonstrated comparable degradation of ErbB1 compared to first-generation LYTACs but with a powerful cell-type selectivity [115].

Although ErbB degraders have demonstrated impressive efficiency in target degradation and anti-tumor activity, they have not undergone comprehensive investigations regarding their potential off-target effects, toxicity profiles, and progression towards clinical development. Furthermore, the use of other E3 ligase ligands or lysosome-targeting ligands need further explorations. Optimizing TPD strategies necessitates a comprehensive analysis of the pivotal role played by the linker structure within the PROTAC and the development of BsAbs for the LYTAC approach. Undoubtedly, with progress in click chemistry and BsAbs optimization, TPDs hold the potential to enhance the selectivity, efficacy, and low resistance profiles to ErbB inhibitors.

Targeting of ErbB membrane partners: Seamy-side or promising approach?

ErbB interactome and EGF domains

The ErbB receptor family exhibits a complex interactome at the cell surface characterized by a growing number of co-receptors, probably underestimated, which play a pivotal role in promoting tumorigenic properties. For instance, ErbB1 interacts with MUC1 **transmembrane mucin** (TM), EphA2 receptor, and the glycoprotein CD44, thereby increasing oncogenic characteristics and resistances across various cancer types [116-118]. Within these membrane partners, a particular class of proteins garners substantial interest due to their possession of structural domains closely mimicking the natural ligands of ErbB receptors: the EGF-like domains (Box 3).

Recently, EGF domain-containing proteins (EDCP) have spurred interest due to their involvement in cancer resistance [122]. However, few EDCP have been so far identified to bind and activate ErbB1 or ErbB2 to promote cancer progression.

Studies have reported the tumorigenic properties of Laminin-332 γ2 chain (LAMC2), which encompasses EGF-like domains (Figure 2 a)). LAMC2 directly interacts with ErbB1 or is cleaved to release its EGF-like repeats which bind and activate ErbB1, leading to downstream MAPK activity in LC and HNSCC [123]. More recently, the EGF-repeats protein superfamily (EGFL) has been investigated for its pro-tumoral properties and its role in resistance mechanisms [124,125]. GC and CRC are mediated by ErbB1 activation in vitro, via the EGF-like domains of EGF-like proteins 6 (EGFL6) and EGFL. This oncogenic promotion is reverted when EGFL proteins are targeted by antibodies [124,125] (Figure 2 a)). EGF-like domains can also activate ErbB receptors invariably of the ErbB1 mutation status, such as tenascin-C EGF-like domains which bind and signal through both wild-type and mutated ErbB1 [126] (Figure 2 a)).

Several EDCP represent alternative activators of the ErbB receptors in cancers [123-127, 129]. These alternative activations underscore the role of specific domains from larger proteins to modulate biological pathways, supporting the overarching hypothesis that EGF-like domains hold potential as therapeutic drug targets [120,128]. (Figure 2 c)).

EGF domain-containing Transmembrane Mucins: MUC4 as ErbB unorthodox ligand

For several years now, TM have emerged as favored partners of ErbB receptors capable of establishing interactions with ErbB2 [129,130], ErbB3 [131], and ErbB1 [132]. Moreover, certain TM possess EGF-like domains [129] that can directly activate ErbBs thereby functioning as unconventional ligands and offering alternative ErbB targeting approaches [131].

Among TM harboring EGF-like domains, MUC4 has received particular attention, due to its overexpression in many aggressive cancers or cancers lacking efficient therapies [132,133]. Moreover, MUC4 EGF directly interact with ErbB2 via its EGF-like domains, providing oncogenic activity of the MUC4-ErbB2 complex in pancreatic cancer [129] (Figure 2 b)). As such the EGF domains of MUC4 are now considered as therapeutic targets in cancer (Figure 2 c)). Our team has designed the first peptide specifically targeting the EGF domains of MUC4. This peptide is able to disrupt the MUC4-ErbB2

complex by selectively targeting the EGF-like domains of MUC4, in order to reduce pancreatic tumor growth *in vitro*. This peptide also rescues the accessibility to ErbB2 epitopes for conventional targeted therapies (Trastuzumab) leading to pancreatic cancer cell sensitivity restoration to ErbB2 targeted therapy (unpublished data). These effects of the peptide represent one of the pioneering demonstrations that the strategy using peptide-based targeting of EDCP may open new avenues in ErbB targeting and therapeutic combinations (small inhibitory peptide + targeted therapy) to treat cancers overexpressing EDCP. Restoration of ErbB2 sensitivity to Trastuzumab in the presence of the inhibitory peptide suggests that targeting MUC4 EDCP holds the potential to mitigate mucin-mediated resistance of cancer cells [19, 134] (Figure 2 b)).

Concluding remarks and perspectives

Comprehensive analysis of the key determinants contributing to acquired resistance to conventional ErbB therapies (i.e., secondary mutations and bypass track) has catalysed the development of novel strategies targeting the ErbB receptors. These novel approaches encompass the use of smaller, highly specific molecular agents (nanobodies/peptides), adept at circumventing the biophysical constraints inherent to the tumor microenvironment. Furthermore, these approaches leverage a novel generation of antibodies (BsAbs) designed to enhance engagement with the anti-tumoral immune component and attenuate alternative signaling cascades. Recent innovations in this field also exploit protein degradation systems (PROTAC/LYTAC) and the targeting of PPI at the cancer cell surface (inhibitory peptides), thereby curtailing the potential for acquired resistance to ErbBs. These diverse approaches are supported by breakthrough technologies such as nanotechnology, click chemistry and computational design, which enable improved simultaneous delivery of anticancer molecules or complexify the targeting potential of PROTACs, peptides and antibodies (dual playload)/dual PROTAC/transformable peptide). [18, 135, 109, 55].

EDCP as alternative ligands of ErbBs represent a yet relatively unexplored field in deciphering novel targetable alternative signaling mechanisms within the ErbB interactome in cancers. However, it is important to note that not all EGF-like domains (ELD) within proteins operate as ligands for ErbB receptors, underscoring the diverse functional repertoire of these domains. Indeed, the “EGF-like domain” denomination lacks clear definition in the literature, referring to two subtypes: human epidermal

growth factor-like (hEGF), which are ligands for ErbB receptors, and complement C1r-EGF like (cEGF), unrelated to ErbBs and associated with integrin-like signaling [136,119,120]. In addition to this undifferentiated denomination, comprehensive molecular investigations elucidating (h)ELD implication in EDCP interactions are scarce, leading to a limited understanding of ELD precise binding modalities to ErbB receptors. For instance, ELDs of a same protein could exhibit differential affinities for ErbB receptors [137], as hypothesized by previous observations demonstrating preferential affinities between distinct ELDs of MUC4 (EGF1 or EGF2) and specific ErbB family members (ErbB2/ErbB3/ErbB1) [129]. Two EDCP can also exhibit differential affinity for the same ErbB receptor: MUC4 ELDs exhibit a high affinity for EGFR [129, 132], while tenascin-C (an extracellular matrix protein) ELD bind EGFR with low affinity [138]. However, despite molecular idiosyncrasies, ELDs that engage ErbB receptors trigger analogous activation of signaling pathways, involving the MAPK/AKT/mTOR/FAK axis [125,129,138,139]. These observations underscore the necessity for more in-depth molecular investigations to fine-tune the diverse interaction mechanisms and functional specificities of the ELDs.

Additionally, five TM mucins (MUC3, MUC4, MUC12, MUC13 and MUC17) possess ELDs [131], but most studies of their impact in cancers have been limited to the MUC4 ELDs. Only one study has implicated the EGF domains of MUC13 in the direct interaction with ErbB2 and the promotion of pancreatic cancer, resulting in the same mechanisms of activations as observed for MUC4 ELD [129,130]. There is no information regarding MUC12 ELD, while findings on MUC17 and MUC3 ELD lack precise molecular information concerning their direct interaction with ErbB receptors [140]. Considering the phylogenetic evidence regarding the origin of mucin ELD [131], we hypothesize that all mucins containing ELDs could potentially interact and activate ErbB receptors to facilitate cancer progression. Considering targeting approaches of MUC4, EGF-like domains (ELD) appear as new targets for mitigating compensatory ErbB receptor activation (ErbB2/ErB3) and resistance to anti-cancer therapies (mAbs) (outstanding questions).

Interestingly, certain TM mucins, such as MUC1, interact with EGFR despite lacking ELDs in their structure. MUC1-EGFR association is mediated by Galectin 3, which favor this interaction by cell surface polarization of MUC1 and EGFR. MUC1 mediates EGFR stabilization to promote its dimerization and prolonged activation [141].

Similarly, EDCPs, including TM and LAMC2, stabilize ErbB complexes at the cell surface via ELD interactions, preventing their degradation and sustaining their signaling activity [132,142]. In a context of ErbB resistance, mucin glycosylation may also impede the fixation of therapeutic antibodies [19,130,133]. Emerging literature also highlights the involvement of TM mucins, particularly MUC1, MUC13, and MUC4, in ErbB TKI resistance, showing their impact in upregulation of efflux pumps and activations/interactions with bypass RTKs [143,144]. In this way, global targeting of TM mucin family (dependently and independently of ELD) could be an interesting strategy to reduce progression of tumor harboring ErbB acquired resistances.

Although all these innovative strategies have yet to achieve widespread clinical implementation due to their relative novelty, promising preclinical investigations pertaining to their anti-tumor efficacy suggest their prospective emergence as impactful therapeutic modalities in forthcoming clinical trials.

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DECLARATION OF INTEREST

The authors declare no conflict of interest.

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GLOSSARY

Antibody-dependent cellular cytotoxicity (ADCC): Type of immune reaction in which immune cells bind antibodies fixed on cancer cells and release substances that kill the targeted-cancer cells.

Antibody-drug conjugates (ADC): Fusion between an antibody and a cytotoxic molecule. The antibody leads to a specific cell or target specificity, while the cytotoxic agent increases cancer therapy efficacy.

Avidity: Binding strength derived from the affinities of multiple individual interactions. It can be applied to antibody–antigen binding, clonal selection or effector functions.

BiTE: Bispecific T-cell Engagers are antibodies which simultaneously engage tumor-associated antigens and a receptor located on immune cells. This initiates a signaling cascade within T cells leading to target-dependent tumor cell lysis.

Gene fusion: Oncogenic gene fusions are hybrid genes that result from structural DNA rearrangements. These chromosome exchanges between two genes lead to the production of new specific fused-ligands, which can bind receptors to generate dysregulated oncogenic activities.

HCST79: Peptide which is part of herstatin antibody's sequence.

LYTAC: The first developed LYTAC molecule relies on the cation-independent mannose-6-phosphate receptor (CI-MPR) as the TLR, facilitating the intracellular trafficking of lysosomal enzymes. The low pH environment within late endosomes leads to the release of lysosomal enzymes from CI-MPR. While the POI is directed for lysosomal degradation, CI-MPR undergoes recycling at the cell surface.

Mimetic peptide: Peptide whose structure and/or aa sequence resembles the receptor original ligand without its detrimental functions. It can be utilized as antagonist of the natural ligand to limit receptor activation.

Nanobodies: Also referred as single-domain antibody, nanobodies are the smallest-known functional antibody fragment (2.5 nm in diameter and 4 nm in height) derived from a functional antibody termed heavy-chain antibody (HcAb) and discovered in camelid sera. Nanobodies consist of only a single small variable domain known as VHH, lacking light-chain polypeptides and the first constant domain (CH1).

Phage display technology: Technique which exploits protein-protein interaction, phage genomic and rules of evolution to rapidly generate huge screening libraries, bigger than those obtained by conventional drug discovery approaches, and allows identification of numerous new effective compounds.

Photodynamic therapy: Anti-cancer technology which utilizes light-activated photosensitizer (PS) to eliminate tumor cells. PS is applied to specifically target the tumor site, based on tumor-associated antigens and well-incorporated molecule in cancer cells. Then, PS sensitizes/enhances the impact of light (applied at a specific wavelength) to degrade tumor cells and recruit anti-cancer immune component in the tumor microenvironment.

Transmembrane Mucins: TM mucins are multi-modular O-glycoproteins involved in several pathophysiological processes. They play fundamental roles in epithelia homeostasis by protecting underlying epithelia and by modulating cell-cell and cell-matrix adhesion.

Trispecific and Tetraspecific antibodies: Antibodies which possess respectively three and four distinct antigen-binding sites, providing additional options for target selection.

UniCAR-T cells: UniCARs are CAR-T cells activated by externally-administered "Targeting Modules", linked to an antibody component recognizing the target antigen, thereby creating a "safety switch". This approach was developed to limit drawbacks of CAR-T cells technology, especially in terms of specificity, control of activation and toxicity.

Vγ9Vδ2 T cells: Specific T-cell subpopulation which possesses a conserved T-cell receptor capable of recognizing malignant cells without relying on major histocompatibility complex.

TEXT BOXES

Box 1 - Resistances to conventional anti-ErbB therapies: A multifaceted issue

Despite the availability of several ErbB-specific therapeutics, the long-term effectiveness of these drugs is limited by multiple resistance pathways. These resistances are primarily driven by both pre-existing on-target mutations (intrinsic resistance) and newly appeared mechanisms of resistance (acquired resistance) [24,27]. Acquired resistance encompass activation of compensatory pathways and secondary mutations appearing after first-line of treatment to limit tumor sensitivity to ErbB inhibitors. The most prevalent acquired resistance mechanism of first generation TKI involves the emergence of a secondary mutation (T790M) within the ErbB1 kinase domain [27]. This gatekeeper mutation induces drug resistance by enhancing ATP affinity. (Figure I). Second- and third-generation ErbB-specific TKIs, such as Osimertinib (2018), have been developed to irreversibly bind to the ATP-binding sites and specifically inhibit the ErbB1^{T790M} mutation [31]. However, recent data point new acquired resistances to this third-generation TKI, including C797X mutations that prevent covalent binding of the drug [24,33]. In contrast to TKIs, secondary mutations of specific binding site of antibody-based therapy are rare, but include the S492R ErbB ectodomain mutation [8]. Instead, major resistance mechanisms to anti-ErbB antibodies converge into two intrinsic pathways mutations: PIK3K/AKT and RAS/RAF/MAPK. Primary resistance in CRC to anti-ErbB1 antibodies commonly involves genomic alterations affecting downstream effectors, such as KRAS, NRAS, and PIK3CA mutations. Thus, patients with RAS mutations activate downstream pathways and establish a bypass survival route [34] (Figure I). Unfortunately, even responders develop secondary resistance in a few months, with less than 35% of CRC patients responding to the combination of chemotherapy and anti-ErbB therapies. This acquired resistance is driven by mutational or epigenetic activation of pathways downstream of ErbB receptors, including PTEN deletions or ErbB1/ErbB2 amplifications. These ErbB amplifications modify the availability of antibodies for their target and lead to the predominance and reactivation of the targeted receptor. Alterations in apoptosis pathways can also serve as a tumor-mediated survival mechanism.

In addition to mutational status, numerous cancers exhibit "bypass track" acquired resistances, by which the tumor maintains its oncogenic activity despite receptor targeting [24,34]. These alternative signaling pathways are driven by the activation of other RTK or changes in ErbB ligands abundance/diversity (Figure I). Hence, TKIs and antibodies resistances encompass bypass track activation due to Met amplification in LC and CRC [35] or insulin growth factor receptor (IGF) amplification in BC [36]. Overexpression of AXL [35] and hepatocyte growth factor (HGF) are also found in LC [37]. These alternative pathways also include a compensatory system among ErbB receptors, especially involving ErbB2 and ErbB3 [7].

The tumor microenvironment can also influence resistance to conventional therapies by secreting compounds that create physical barriers and limit antibody binding to their

targets. This biomechanical barrier includes tumor-related factors such as stiffness, porosity, and bioadhesiveness [38] as well as partners like MUC4 TM mucin and CD44, which may mask ErbB epitopes [19] (Figure I). Post-transcriptional regulation by long non-coding RNAs (lncRNAs) and exosomes which transport and remodel the stromal component of tumors, also contributes to the resistance against anti-ErbB therapies by modulating intracellular signaling [39,40].

Box 1. Figure I Intrinsic and acquired mechanisms of resistance to conventional ErbB-targeted therapies in cancers. *Mutations of ErbB receptors and/or downstream effectors, as well as activation of alternative pathways by ErbB partners and RTK bypass track lead to monoclonal antibody and tyrosine kinase inhibitor-resistant cancer cells.*

Box 2 - Protein-protein interfaces as attractive targets to design anti-ErbB small molecules

Protein-protein interactions (PPI) play a fundamental role in cellular and molecular processes, exerting regulatory control over various biochemical reactions, including signal transduction, metabolism and survival [41,42]. Given their pivotal role, modulating PPIs holds paramount importance in both fundamental and translational research, facilitating a deeper comprehension of biological processes and serving as a theoretical basis for the development of innovative therapeutic agents [43,44]. This led to the design of orthosteric ligands targeting the interaction interfaces, particularly the development of inhibitory peptides. These peptides typically mimic the secondary structure of the targeted interaction interfaces, disrupting the critical "interaction hotspots" identified within the PPI interface [45]. In this way, inhibitory peptides are engineered to exhibit a high degree of specificity for binding to and modulating a diverse range of targets implicated in various diseases [46].

Peptides are a type of pharmacological small molecules constituted of short-chain amino acids, usually <5000 Da, that are stabilized by disulfide bonds. Compared to conventional small molecules, therapeutic peptides offer several advantages, including enhanced flexibility, solubility, favorable pharmacokinetic profiles, and targeted tissue specific distribution patterns. They also demonstrate superior tissue penetration and membrane permeability compared to larger proteins and antibodies. However, they face problematics encompassing stability in sera, protease degradation and rapidity of clearance [46,47].

In cancers, membrane receptors participate in intricate interactomes, frequently driving the activation of oncoproteins. Consequently, the targeting of these receptors through peptide-based approaches, such as **phage-display technology** and computer-assisted design processes, has garnered significant attention, owing to the safety profile of peptide-based anticancer agents. Indeed, anti-cancer peptides exhibit notable efficacy, potency, target selectivity, low immunogenicity and low toxicity, making them a valuable therapeutic tool for oncological applications. Their reduced

toxicity is particularly advantageous in minimizing side effects, preventing tumorigenesis, and circumventing resistance to TKIs by targeting processes upstream of signaling pathways [48]. Therefore, therapeutic peptides represent a promising avenue for the development of anticancer agents and have been naturally used as a new strategy to target ErbB oncoreceptors [49]. In this context, peptide-based inhibitory strategies either focus on impeding the interaction between the ligand and the ErbB receptor or disrupting ErbB complexes or associated partners. The majority of these inhibitory peptides are designed to target ErbB1 and ErbB2 receptors, and can be categorized as "**mimetic peptides**" [48,50] or “de novo” synthesized peptides that specifically block portions of the receptor [46].

Box 3 - Biochemical features of EGF-like domains in membrane and secreted proteins: New ErbB activators?

There are several conventional ligands which selectively bind to specific ErbB receptors. Epidermal Growth Factor (EGF), Amphiregulin (AR), Transforming Growth Factor α (TGF-α), Betacellulin (BTC), Epiregulin (EPR), and Heparin binding-EGF (HB-EGF) bind ErbB1. ErbB3 is responsive to two ligands, Neuregulins 1 and 2 (NRG1 and NRG2), and ErbB4 interacts with seven ligands, including Neuregulins 1, 2, 3 and 4 (NRG3 and NRG4), BTC, HB-EGF, and EPR. Interestingly, only ErbB2 remains an orphan receptor with no known soluble ligands [1]. Among them, EGF is the ligand with the highest affinity for ErbB receptors and has been intensively identified to promote tumorigenesis [1,2]. However, alternative ligands of ErbB receptors have emerged from structural domains within extracellular and membrane proteins: EGF-like domains [119,120]. These domains are evolutionarily conserved protein domains derived from the EGF, whose nomenclature arises from their structural resemblance with the human EGF ligand. EGF-like domains are small domains (~5kDa) which possess 6 key cysteine residues that form three intramolecular disulfide bonds to fold the protein into a compact globular structure. Despite differences in aa sequences, these domains exhibit similar disulfide bridge arrangements, resulting in a shared 3D structure with soluble EGF (Figure I). Typically, EGF-like domains comprise approximately 30 to 40 aa residues and are present in approximately 30% of animal proteins [119,120]. Additionally, EGF-like domains often occur in multiple tandem copies within proteins, folding together to form a linear solenoid domain block that functions as a structural unit.

Given their prevalence in the extracellular compartment, EGF-like domains play pivotal roles in signaling pathways [120,121]. Functionally, they serve as sensors by regulating protein rigidity, or as spacers, positioning adjacent domains within proximity for interactions. However, their most intriguing modalities is their ability to directly participate in protein-protein interactions and receptor activation. In some cases, EGF-like domains are cleaved from their transmembrane proteins by extracellular proteases, resulting in long-range signaling events [121]. In the context of cancer

progression and resistances, these extracellular matrix proteins containing EGF-like domains represent an underexplored axis for activating ErbB family receptors.

Box 3. Figure I Putative 3D structure and electrostatic surfaces of oncomucin MUC4 EGF-like domains. *Spatial organization of MUC4 EGF-like domains, modelized with Chimera software, based on a homology model with the human EGF. EGF-like domains of MUC4 are particularly globular, conserving structural features with the native EGF ligand.*

TABLES

Table 1. Conventional therapies targeting ErbB receptors for cancer treatment. List of monoclonal antibodies, ADC and tyrosine kinase inhibitors (TKI) specifically targeting ErbB1/ErbB2/ErbB3/ErbB4 receptors, which were approved by FDA/EMA or being under clinical investigation. Anti-ErbB4 monoclonal antibodies and TKI targeting ErbB3 or ErbB4 are listed due to their first-in-class aspect, even if they are only under pre-clinical studies. Abbreviations: CRC: colorectal cancer; NSCLC: non-small cell lung cancer; HNSCC: head and neck squamous cell cancer; BC: breast cancer; GOC: gastroesophageal cancer.

Monoclonal antibodies targeting ErbB Receptors			
Therapeutic molecule	**ErbB targeted**	**Main indication**	**Clinical trial or date of approval or reference**
Cetuximab	ErbB1	Approved for Ras mutated CRC and metastatic HNSCC	2004
Panitumumab	ErbB1	Approved for Ras CRC	2006/2007
Nimotuzumab	ErbB1	Approved for gliomas and HNSCC	2014
Necitumumab	ErbB1	Approved for metastatic NSCLC	2015/2016
KL-140	ErbB1	Phase III trial for CRC	NCT03426371
CMAB-009	ErbB1	Phase II/III for metastatic CRC	NCT03206151
Imgatuzumab	ErbB1	Phase II for locally advances/metastatic EGFR+ solid tumors	NCT00721266
ABT-806	ErbB1	Phase II trial for advanced solid tumors	NCT01406119
CPGJ-602	ErbB1	Phase II trial for metastatic CRC	NCT04466254
Sym004	ErbB1	Phase II trial for solid tumors	NCT01955473
SCT-200	ErbB1	Phase I trial for metastatic HNSCC	NCT05552807
AMG-595	ErbB1	Phase I trial for recurrent gliomas	NCT01475006
HLX07	ErbB1	Phase I trial for advanced solid cancers	NCT02648490
Depatuxizumab mafodotin (ABT414)	ErbB1 + auristatin F (ADC)	Phase III trial for recurrent glioblastomas	NCT02343406
MRG-003	ErbB1 + auristatin E (ADC)	Phase II trials for advanced or metastatic EGFR+ biliary tract cancer, Nasopharyngeal carcinoma, HNSCC and NSCLC	NCT05126719 NCT04868162 NCT04838964 NCT04838548
Trastuzumab	ErbB2	Approved for ErbB2+ gastric and breast cancers	1998
Pertuzumab	ErbB2	Approved for ErbB2+ BC	2012
Margetuximab (MGHA22)	ErbB2	Approved for ErbB2+ metastatic BC	2020
MM-302	ErbB2	Phase II/III trial for ErbB2+ locally advanced/metastatic BC	NCT02213744
Inetetamab	ErbB2	Phase II trial for ErbB2+ metastatic BC	NCT05823623
Tastuzumab-emtansine	ErbB2 + microtubule inhibitor (ADC)	Approved for ErbB2+ metastatic BC	2013

Tastuzumab-Deruxtecan	ErbB2 + topoisomerase I inhibitor (ADC)	Approved for ErbB2+ metastatic BC	2022
Trastuzumab duocarmycin (SYD985)	ErbB2 + DNA alkylator derivate (ADC)	Phase III trial for ErbB2+metastatic BC	NCT03262935
Trastuzumab rezetecan (SHR-A1811)	ErbB2 + topoisomerase I inhibitor (ADC)	Phase II/III trial for ErbB2+ and ErbB2^{low} metastatic BC and ErbB2+ GOC	NCT06057610 NCT05769010 NCT06123494
ARX788	ErbB2 + anti-mitotic dolastatin (ADC)	Phase II/III trial for ErbB2+ BC	NCT05426486
Disitamab Vedotin (RC48)	ErbB2 + auristatin E (ADC)	Phase II/III trial for ErbB2+ and ErbB2^{low} metastatic BC and ErbB2+ gastric cancers	NCT03500380 NCT04714190 NCT04400695
MRG002	ErbB2 + auristatin E (ADC)	Phase II/III trial for ErbB2+ and ErbB2^{low} locally advanced/metastatic BC	NCT04924699 NCT04742153
BDC-1001	ErbB2 + immune stimulator TLR-7/8 agonist (ADC)	Phase II trial for ErbB2+ advanced solid tumors	NCT04278144
BAY2701439	ErbB2 + thorium-227 radionuclides (ADC)	Phase II trial for ErbB2+ advanced solid tumors	NCT04147819
SBT6050	ErbB2 + immune stimulator TLR-8 agonist (ADC)	Phase I trial for ErbB2+ solid tumors	NCT04460456
NJH395	ErbB2 + immune stimulator TLR-7 agonist (ADC)	Phase I trial for ErbB2+ advanced cancer	NCT03696771
ALT-P7	ErbB2 + auristatin E (ADC)	Phase I trial for ErbB2+ BC	NCT03281824
FS-1502/IKS014	ErbB2 + auristatin E (ADC)	Phase I trial for ErbB2+ advanced tumors and BC	NCT03944499
XMT-1522	ErbB2 + auristatin derivative AF-HPA (ADC)	Phase I trial for ErbB2+ advanced BC	NCT02952729
XMT-2056	ErbB2 + immune system activator (ADC)	Phase I for Advanced/Recurrent ErbB2+ Solid Tumors	NCT05514717
BB-1701	ErbB2 + microtubule inhibitor (ADC)	Phase I for ErbB2+ locally advanced/metastatic BC	NCT04257110
DB-1303	ErbB2 + topoisomerase I inhibitor (ADC)	Phase I trial for advanced and metastatic tumors	NCT05150691
A166	ErbB2 + microtubule inhibitor (ADC)	Phase I trial for refractory ErbB2+ solid tumors	NCT03602079
DX126-262	ErbB2 + microtubule inhibitor (ADC)	Phase I trial for refractory ErbB2+ advanced BC and/or Gastric cancers	CTR20191224
DHES0815A	ErbB2 + DNA alkylator/cross linker	Phase I trial for ErbB2+ BC	NCT03451162
ORM-5029	ErbB2 + PROTAC targeting cell cycle molecule GSTP1 (DAC)	Phase I trial for advanced-stage ErbB2+ solid tumours	NCT05511844

Patritumab (U3-1287)	ErbB3	Phase III trial for advanced/metastatic NSCLC	NCT02134015
Seribantumab (MM-121)	ErbB3	Phase II trial for ErbBs+ NSCLC and advanced solid tumors harboring NRG1 fusion	NCT02387216 NCT04383210
Barecetamab (ISU104)	ErbB3	Phase I trial for advanced solid tumors	NCT03552406
GSK2849330	ErbB3	Phase I trial for advanced ErbB3+ solid tumors	NCT01966445
AV-203 (CAN017)	ErbB3	Phase I trial for Metastatic or advanced solid tumors	NCT01603979
REGN1400	ErbB3	Phase I trial for advanced NSCLC, CRC or HNSCC	NCT01727869
HMBD-001	ErbB3	Phase I trial for solid tumors harboring an NRG1 Fusion, ErbB3+ solid tumor or cancers with ErbB3 Mutations	NCT05919537 NCT05057013
SIBP-03	ErbB3	Phase I trial for advanced malignant solid tumors	NCT05203601
Sym013	Pan-ErbB (mixture mAbs)	Phase I/II trial for epithelial malignancies	NCT02906670
Patritumab deruxtecan (U3-1402)	ErbB3 + topoisomerase I inhibitor (ADC)	Phase I trial for locally advanced or metastatic EGFR-mutated NSCLC	NCT04619004
YL202	ErbB3 + topoisomerase I inhibitor (ADC)	Phase I for locally advanced/metastatic NSCLC and BC	NCT05653752
DB-1310	ErbB3 + topoisomerase I inhibitor (ADC)	Phase I/II trial for advanced/metastatic solid tumors	NCT05785741
P6-1	ErbB4	Preclinical studies in 3D models of BC	[26]
C6	ErbB4	Preclinical studies in triple-negative BC cell lines (in vitro), metastatic BC cell lines and ovarian cancer cell lines (in vivo)	[26]
Ab1479	ErbB4	Preclinical studies in ErbB4+ BC cell lines and ER+ BC cell lines (in vitro)	[26]

1188

Tyrosine Kinase Inhibitors targeting ErbB Receptors			
Therapeutic molecule	**ErbB targeted**	**Main Indication**	**Clinical trial or date of approval or reference**
1st generation (reversible TKI – targeting Del19/L858R ErbB mutations)			
Erlotinib	ErbB1	Approved for metastatic NSCLC and metastatic pancreatic cancer	2004/2005
Gefitinib	ErbB1	Approved for metastatic NSCLC	2003
Lapatinib	ErbB2/ErbB1	Approved for ErbB2+ metastatic BC	2007
Tucatinib	ErbB2	Approved for ErbB2+ metastatic BC and ErbB2+ and CRC non Ras-mutated	2020
Icotinib	ErbB1	● Approved in China for advanced and metastatic NSCLC ● Phase II/III trial for late stage EGFR-mutated NSCLC	2011 (China) NCT02448797
BPI-15086	ErbB1	Phase I trial for EGFR-mutated NSCLC	NCT02914990
Theliatinib (HMPL-309)	ErbB1	Phase I trial for advanced solid tumors	NCT02601248
Epitinib (HMPL-813)	ErbB1	Phase I trial for advanced solid tumors	NCT02590952
Molecule Ik and IIa	ErbB4	Pre-clinical studies in metastatic BC cell lines	[26]
Compound A and B	ErbB4	Pre-clinical studies in metastatic BC cell lines	[29]
2nd generation (irreversible TKI – targeting Del19/L858R/T790M ex20ins ErbB mutations)			
Afatinib	Pan-ErbB	Approved for EGFR- mutated metastatic NSCLC	2013
Neratinib	ErbB2/ErbB1/ErbB4	● Approved for ErbB2+ breast cancer ● Phase II clinical studies in solid tumors with ErbB1 or ErbB2 mutations (NCT01953926)	2017
Dacomitinib	ErbB1/ErbB2/ErbB4	Approved for EGFR-mutated metastatic NSCLC	2018
Pyrotinib	ErbB1/ErbB2/ErbB4	● Approved in China for ErbB2+ breast cancer ● Phase II clinical studies for ErbB2+ advanced CRC ● Phase III trial for NSCLC Harboring a ErbB2 Exon 20 Mutation	2018 (China) NCT04380012 NCT04447118
Poziotinib	ErbB2/ErbB4/ ErbB1 (ex20ins)	Phase II trial for Exon 20 Mutant Advanced NSCLC	NCT03066206
Canertinib	ErbB1/ErbB2/ErbB4	Phase II trial for EGFR-mutated advanced NSCLC aborted due to toxicity and poor benefit/risk ratio	NCT00050830
Pelitinib	ErbB1	Phase I trial for solid tumors aborted due to toxicity and poor benefit/risk ratio	NCT00098501
TX1-85-1	ErbB3	Pre-clinical studies in NSCLC	[7]
Molecule I and II	ErbB4	Pre-clinical studies in several cancer cell lines	[30]
3rd generation (irreversible TKI – targeting Del19/L858R/T790M or ex20ins ErbB mutations)			
Osimertinib	ErbB1 /ErbB2/ ErbB3	Approved for EGFR-mutated advanced and metastatic NSCLC	2015
Lazertinib (YH25448)	ErbB1	Approved for EGFR-mutated advanced or metastatic NSCLC patients with previous EGFR TKI therapy and Phase III trial as first-line for EGFR-mutated advanced/metastatic NSCLC	2021 NCT04248829
Olmutinib	ErbB1	● Approved in South Korea for EGFR-mutated NSCLC ● Phase III trial for EGFR-mutated NSCLC	2016 (South Korea) NCT04035486
Mobocertinib (TAK-788)	ErbB1 (ex20ins)	Approved for EGFR-mutated ex20ins metastatic NSCLC	2021
Sunvozertinib (DZD9008)	ErbB1 (ex20ins)/ ErbB2	● Approved for locally advanced or metastatic NSCLC with EGFR exon20ins mutations ● Phase I/II trial for NSCLC with EGFR or ErbB2 mutations	2023 NCT03974022

Limertinib (ASK-120067)	ErbB1	Phase III trial for advanced or metastatic EGFR-mutated NSCLC	NCT04143607
Nazartinib (EGF816)	ErbB1	Phase III clinical studies in EGFR-mutated advanced or metastatic NSCLC	NCT03529084
Naquotinib (ASP8273)	ErbB1	Phase III trial for EGFR-mutated NSCLC	NCT02588261
Zipalertinib (CLN-081)	ErbB1 (ex20ins)	Phase III trial for advanced ex20ins EGFR-mutated NSCLC	NCT05973773
Tarloxotinib	Pan-ErbB	Phase II trial for NSCLC with EGFR Exon 20 Insertion, HER2-activating Mutations & Other Solid Tumors With NRG1/ERBB Gene Fusions	NCT03805841
Befotertinib (D-0316)	ErbB1	● Approved for advanced metastatic NSCLC who received EGFR TKI therapy ● Phase II trial for EGFR+ NSCLC	2023 (China) NCT03861156
Keynatinib	ErbB1	Phase II NSCLC and brain metastases	NCT04824079
SH-1028	ErbB1	Phase II trial for advanced or metastatic NSCLC	NCT04239833
Almonertinib (HS-10296)	ErbB1	Phase I/II trial for advanced or metastatic NSCLC	NCT02981108
Rezivertinib (BPI-7711)	ErbB1	Phase I/II trial for advanced or metastatic EGFR-mutated NSCLC	NCT03866499
olafertinib (CK-101/RK518)	ErbB1	Phase I/II trial for advanced NSCLC with EGFR mutations and other advanced malignancies	NCT02926768
TAS-121	ErbB1	Phase I trial for advanced NSCLC	JapicCTI-142651
FCN-411	ErbB1	Phase I for advanced EGFR-mutated NSCLC	NCT03420079
Rociletinib (CO-1686)	ErbB1	Phase I/II trial for advanced or metastatic EGFR-mutated NSCLC ○ Finally unapproved due to less anti-tumoral effects compared to osimertinib	NCT02580708
Alflutinib/Furmonertinib (AST2818)	ErbB1 (ex20ins)	● Approved in China for locally advanced/metastatic NSCLC with EGFR ex20ins ● Phase I for ex20ins EGFR-mutated NSCLC	2021 (China) NCT04858958
Simotinib (SIM-6802)	ErbB1	● Approved in China for solid tumor treatment ● Phase I trial for advanced NSCLC	2018 (China) NCT01772732
4th generation (TKI – targeting 3rd generation TKI resistance: targeting Del19/L858R/T790M/C797S or ex20ins ErbB mutations)			
BBT-176	ErbB1 (Reversible)	Phase I/II trial for advanced NSCLC with progression after EGFR TKI treatment	NCT04820023
JIN-A02	ErbB1	Phase I/II trial for advanced EGFR-mutated NSCLC	NCT05394831
BLU-701	ErbB1	Phase I/II trial for EGFR- mutated NSCLC	NCT05153408
HS-10375	ErbB1	Phase I/II trial for advanced or metastatic EGFR-mutated NSCLC	NCT05435248
WJ13405	ErbB1	Phase I/II trial for advanced or metastatic NSCLC	NCT05662670
BLU-945	ErbB1 (Reversible)	Phase I/II trial for the targeting of EGFR Resistance Mechanisms in NSCLC	NCT04862780
BPI-361175	ErbB1	Phase I/II for advanced Solid Tumors	NCT05329298
BAY2927088	ErbB1	Phase I/II trial for EGFR- mutated or ErbB2-mutated NSCLC	NCT05099172
BDTX-1535	ErbB1	Phase I/II trial for glioblastoma or NSCLC with EGFR mutations	NCT05256290
TBQ-3804	ErbB1	Phase I for advanced malignant tumors	NCT04128085
QLH11811	ErbB1	Phase I trial for advanced EGFR-mutated NSCLC	NCT05555212
Multi-kinase inhibitors			
Brigatinib	ErbB1/IGF1R/ALK/ ROS/ FLT3	Approved for refractory ALK-positive metastatic NSCLC	2017
Vandetanib	VEGFR2/RET/ ErbB1 T790M	Approved for locally advanced or metastatic disease	2011
Abivertinib / Avitinib (AC0010)	BTK/ErbB1 T790M	Phase II trial for advanced EGFR-mutated NSCLC	NCT03300115

Tesevatinib	ErbB1/ErbB2/VEGFR-2/VEGFR-3/ CSK/ EPHB4	Phase II trial for recurrent glioblastomas harboring EGFR amplifications	NCT02844439

1189

FIGURE LEGENDS

Figure 1. Innovative strategies targeting ErbB receptor to curtail therapeutic resistances in cancers.

(A) ErbB Bispecific antibodies strategies. ErbB-bispecific antibodies can either bridge two ErbB receptors or ErbB receptors and another RTK to reduce the RTK-mediated bypass track resistance (1). They can also bridge tumor cell and immune cell (2) or bridge ErbB receptors with cytokines to enhance anti-tumor immune response (3). **(B) Peptide-based targeted therapies.** Peptide can efficiently target the ErbB-ligand interaction or the ErbB dimerization to prevent ErbB activation. Juxtamembrane and intracellular peptides are also designed to destabilize the ErbB complex formation. They also act as drug carrier or ErbB-tumor specific photosensitizer for photodynamic therapy. **(C) PROTAC and LYTAC strategy targeting ErbB receptor for degradation.** PROTAC and LYTAC hijack proteasome and lysosome-endosome pathways respectively, to generate ErbB-targeted protein degradation. In ErbB-PROTACs, pomalidomide ligand (CRBN) and von Hippel-Lindau ligand (VHL) are commonly used E3 ligase ligands; TLR: Lysosome Targeting Receptor. **(D) ErbB-targeted nanobodies.** Nanobodies resume monoclonal antibodies functions with the targeting of ErbB epitopes, the capacity of carrying drugs and the ability to bind different epitopes as bispecific nanobodies. Due to their small size, they can easily penetrate tumors and minimize steric hindrance generated by tumor microenvironment. They can also be used for CAR/UniCAR-T cell and therapeutic stem cell strategies; TM: Targeting Module.

Figure 2. EGF-like domains containing proteins as ErbB alternative ligands.

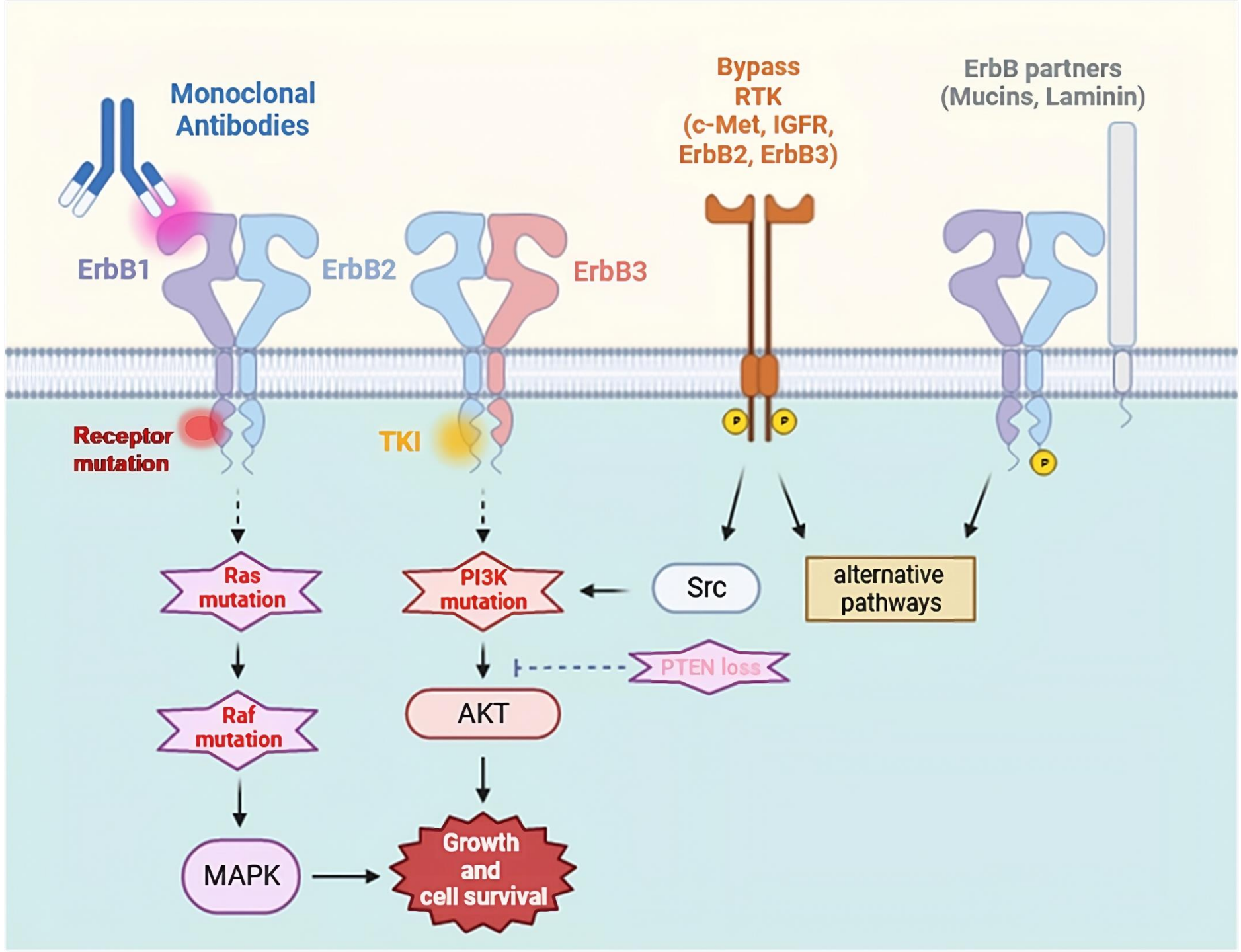
(A) Transmembrane proteins (1), or secreted proteins (2) encompassing regions with EGF-like domains can interact with the ErbB1 or ErbB2 receptors at the surface of tumor cells and constitute alternative activators/ligands of this receptor family. Matrix metalloprotease (MMP) can cleave and liberate EGF-like domains within secreted proteins (3) to activate ErbB receptor. **(B)** EGF-like domains of mucins, such as MUC4, interact with ErbB2 to activate signaling pathways and promote tumor progression. Mucin glycosylation may also limit accessibility to antibody-based targeted therapies

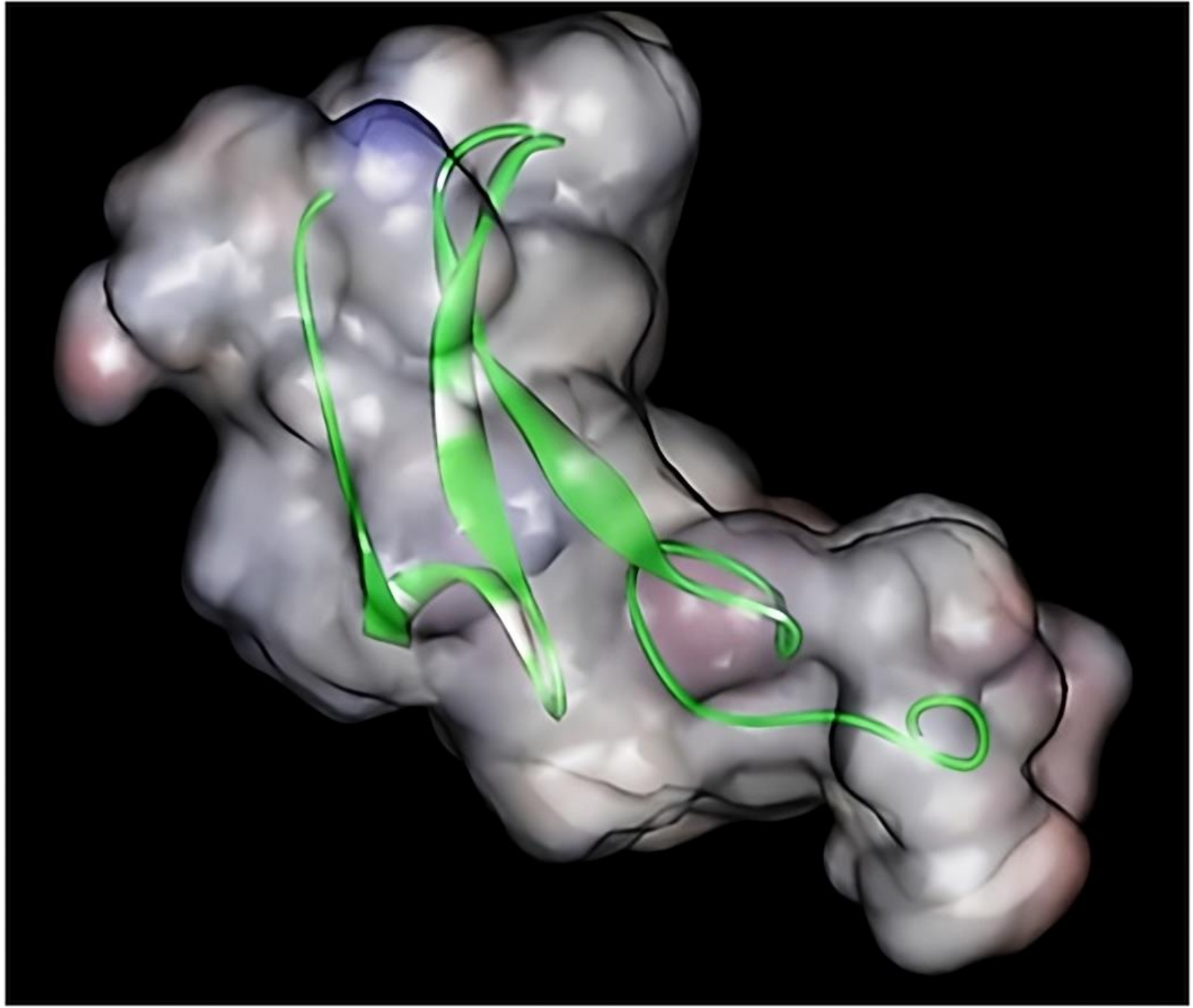
(ex: Trastuzumab). **(C)** EGF-like domains in extracellular matrix proteins or membrane proteins are targets to design therapeutic inhibitors (small molecules, peptides) of ErbB receptors.

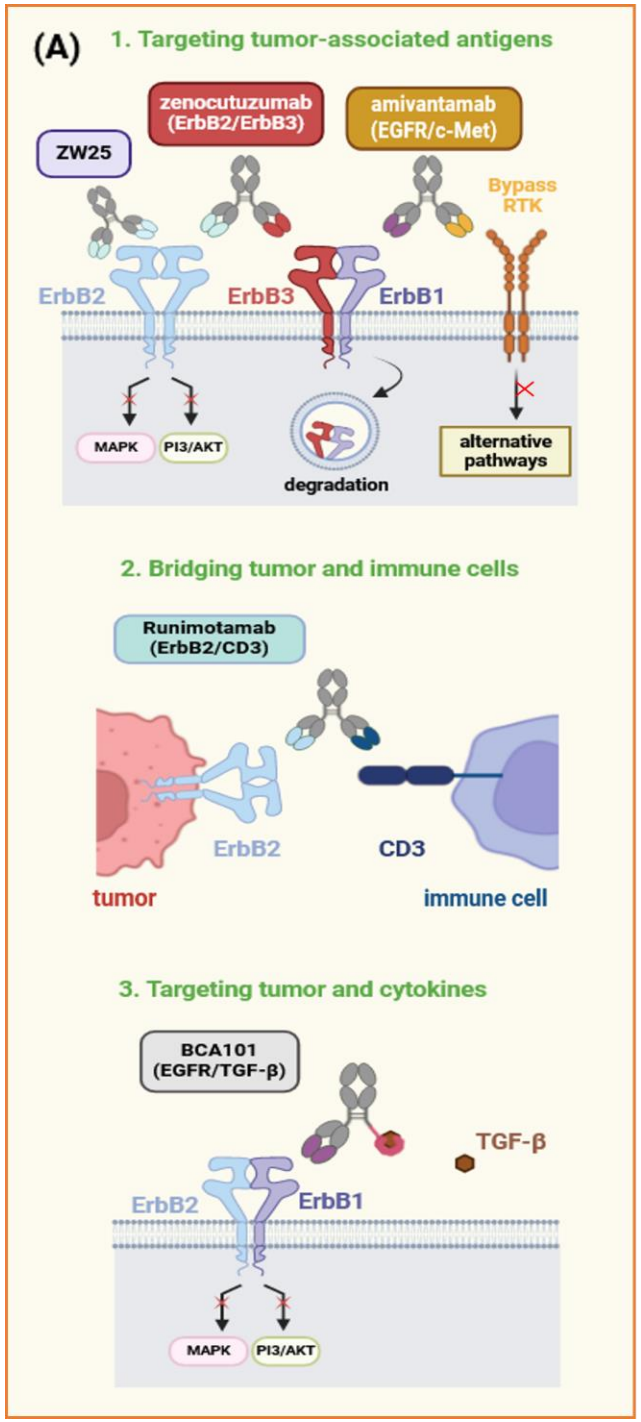
Bispecific antibodies targeting ErbB Receptors			
Therapeutic molecule	**Targets**	**Main indication**	**Clinical trial or date of approval or reference**
Bridging two receptors on tumor cells			
Amivantamab (JNJ61186372)	ErbB1/c-Met	Approved for NSCLC with EGFR mutation and MET amplification	2021
Bafisontamab (EMB-01)	ErbB1/c-Met	• Phase I/II trial for advanced solid tumors • Phase II trial for metastatic NSCLC with EGFR mutation and/or MET amplification	NCT03797391 NCT05498389
LY3164530	ErbB1/c-Met	Phase I trial for neoplasm	NCT02221882
Zenocutuzumab (MCLA-128)	ErbB2/ErbB3	eNRGy phase I/II trial in solid tumors harboring NRG1 fusion	NCT02912949
Izalontamab (SI-B001)	ErbB1/ErbB3	Phase II trial for locally advanced or metastatic EGFR/ALK wild-type NSCLC	NCT05020457
BL-B01D1	ErbB1/ErbB3	Phase I trial for locally advanced/metastatic gastrointestinal tumor and other solid Tumor	NCT05262491
M-1231	ErbB1/MUC1 + Hemiasterlin (ADC)	Phase I trial for advanced solid tumors	NCT04695847
Duligotuzumab (MEHD7945A)	ErbB3/ErbB1	Phase I trial for metastatic HNSCC	NCT01911598
Zanidatamab (ZW25)	ErbB2/ErbB2 (≠ epitopes)	Phase III trial for metastatic ErbB2+ GOC	NCT05152147
Zanidatamab Zovodotin (ZW49)	ErbB2/ErbB2 (≠ epitopes + N-acyl sulfonamide auristatin (ADC))	Phase I trial for ErbB2+ cancers	NCT03821233
Anbenitamab (KN026)	ErbB2/ErbB2 (biparatopic)	Phase II trial for locally advanced ErbB2+ solid tumors	NCT04521179
MEDI4276	ErbB2/ErbB2 (≠ epitopes + tubulysin microtubule inhibitor (ADC))	Phase I/II trial for ErbB2+ BC and gastric cancers	NCT02576548
MBS301	ErbB2/ErbB2 (≠ epitopes)	Phase I trial for ErbB2+ recurrent or metastatic malignant solid tumor	NCT03842085
BCD-147	ErbB2/ErbB2 (≠ epitopes)	Phase I trial for ErbB2+ solid tumors	NCT03912441
MP0274	ErbB2/ErbB2 (biparatopic)	Phase I trial for ErbB2+ solid tumors	NCT03084926
Istiratumab (MM-141)	ErbB3/IGFR	Phase II trial for locally advanced or metastatic pancreatic cancers	NCT02538627
AZD9592	ErbB1/c-Met + topoisomerase inhibitor (ADC)	Phase I trial for advanced solid tumors	NCT05647122
BL-B01D1 ADC	ErbB1/ErbB3 + topoisomerase inhibitor (ADC)	Phase I trial for unresecable or metastatic NSCLC	NCT05983432
Bridging tumor cells and immune cells			

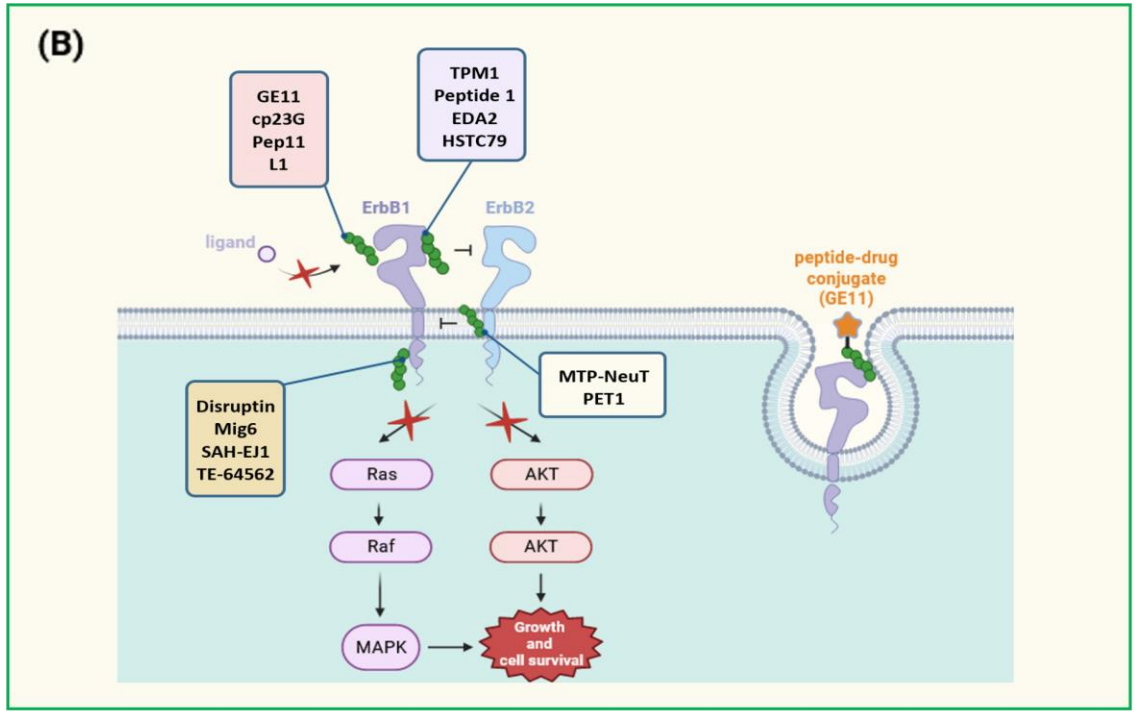
AFM24	ErbB1/CD3 ErbB1/CD16A	Phase I/II trial for advanced solid tumors	NCT04259450
Runimotamab (BTRC4017A)	ErbB2/CD3	Phase I trial for locally advanced or metastatic ErbB2+ Cancers	NCT03448042
HER2Bi ATC	ErbB2/CD3	Phase I trial for metastatic BC	NCT03272334
M802	ErbB2/CD3	Phase I trial for ErbB2+ advanced solid tumors	NCT04501770
IBI315	ErbB2/PD-L1	Phase I trial for ErbB2+ advanced solid tumors	NCT04162327
MCLA-158	ErbB1/LGR5	Phase I trial for advanced solid tumors	NCT03526835
DF1001	ErbB2/NK engager	Phase I/II trial for advanced solid tumors	NCT04143711
PRS-343	ErbB2/4-1BB	Phase I trial for ErbB2+ solid tumors	NCT03330561
Bridging tumor cells and cytokines			
BCA101	ErbB1/TGF-β	Phase I trial in EGFR-driven advanced solid tumors	NCT04429542

Table 2. Clinical development of Bispecific Antibodies targeting ErbB receptors. List of bispecific antibodies targeting one or several ErbB receptors, which were approved by FDA/EMA or being under clinical investigation. Abbreviations: CRC: colorectal cancer; NSCLC: non-small cell lung cancer; HNSCC: head and neck squamous cell cancer; BC: breast cancer.


Monoclonal Antibodies
Bypass RTK (c-Met, IGFR, ErbB2, ErbB3)
ErbB partners (Mucins, Laminin)
ErbB1
ErbB2
ErbB3
Receptor mutation
TKI
P
P
P
Ras mutation
PI3K mutation
Src
alternative pathways
PTEN loss
Raf mutation
AKT
MAPK
Growth and cell survival




(A)
1. Targeting tumor-associated antigens
zenocutuzumab (ErbB2/ErbB3)
amivantamab (EGFR/c-Met)
ZW25
Bypass RTK
ErbB2
ErbB3
ErbB1
MAPK
PI3/AKT
degradation
alternative pathways
2. Bridging tumor and immune cells
Runimotamab (ErbB2/CD3)
ErbB2
CD3
tumor
immune cell
3. Targeting tumor and cytokines
BCA101 (EGFR/TGF-β)
TGF-β
ErbB2
ErbB1
MAPK
PI3/AKT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GE11
cp23G
Pep11
L1
TPM1
Peptide 1
EDA2
HSTC79
ligand
ErbB1
ErbB2
peptide-drug conjugate (GE11)
Disruptin
Mig6
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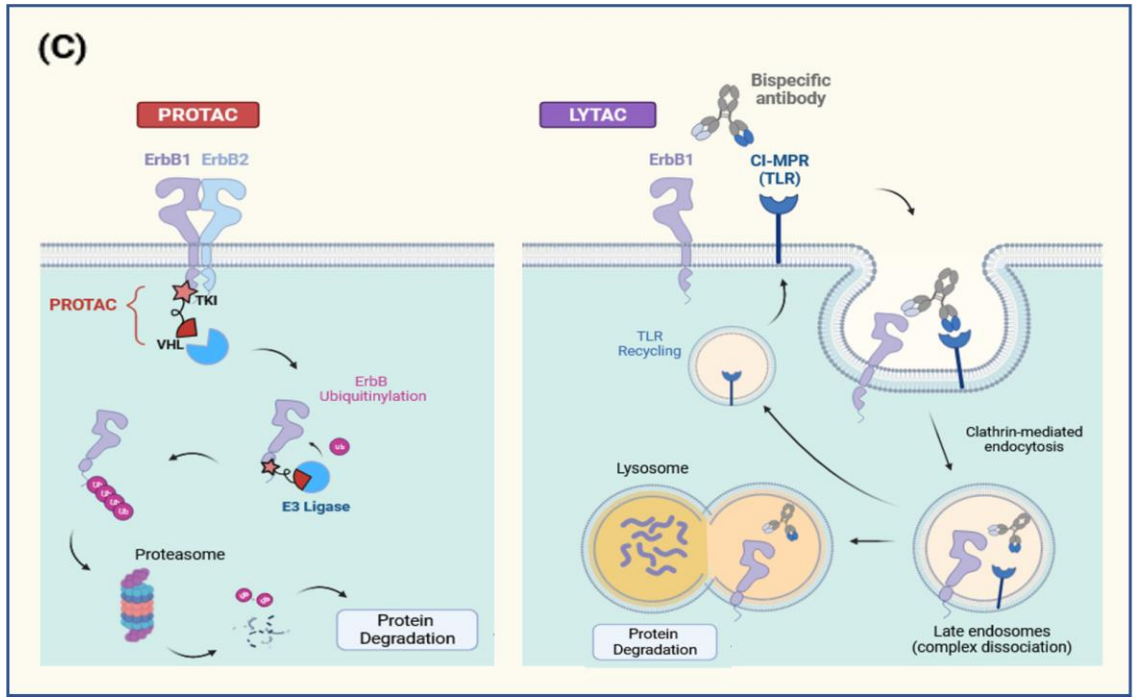
MTP-NeuT
PET1
Ras
Raf
MAPK
AKT
AKT
Growth and cell survival


(C)
PROTAC
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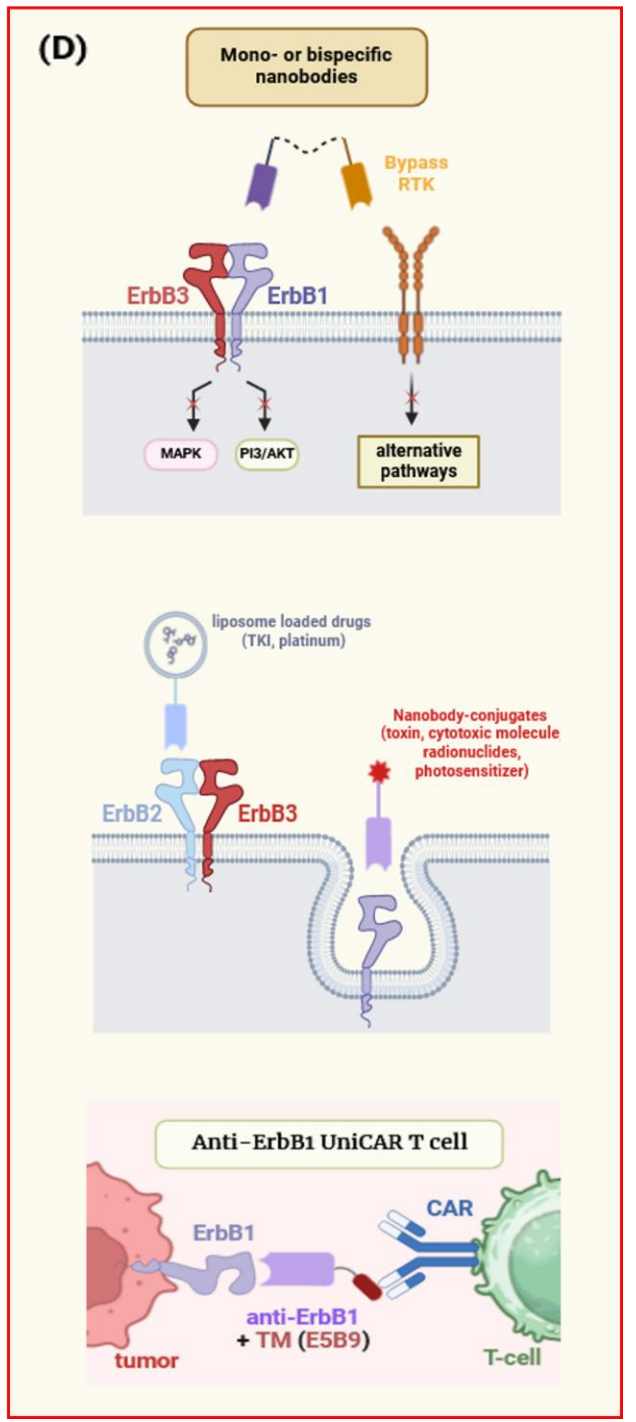
ErbB2
PROTAC
TKI
VHL
ErbB Ubiquitinylation
E3 Ligase
Proteasome
Protein Degradation
LYTAC
Bispecific antibody
ErbB1
CI-MPR (TLR)
TLR Recycling
Clathrin-mediated endocytosis
Lysosome
Protein Degradation
Late endosomes (complex dissociation)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ErbB3
ErbB1
MAPK
PI3/AKT
alternative pathways
liposome loaded drugs (TKI, platinum)
Nanobody-conjugates (toxin, cytotoxic molecule radionuclides, photosensitizer)
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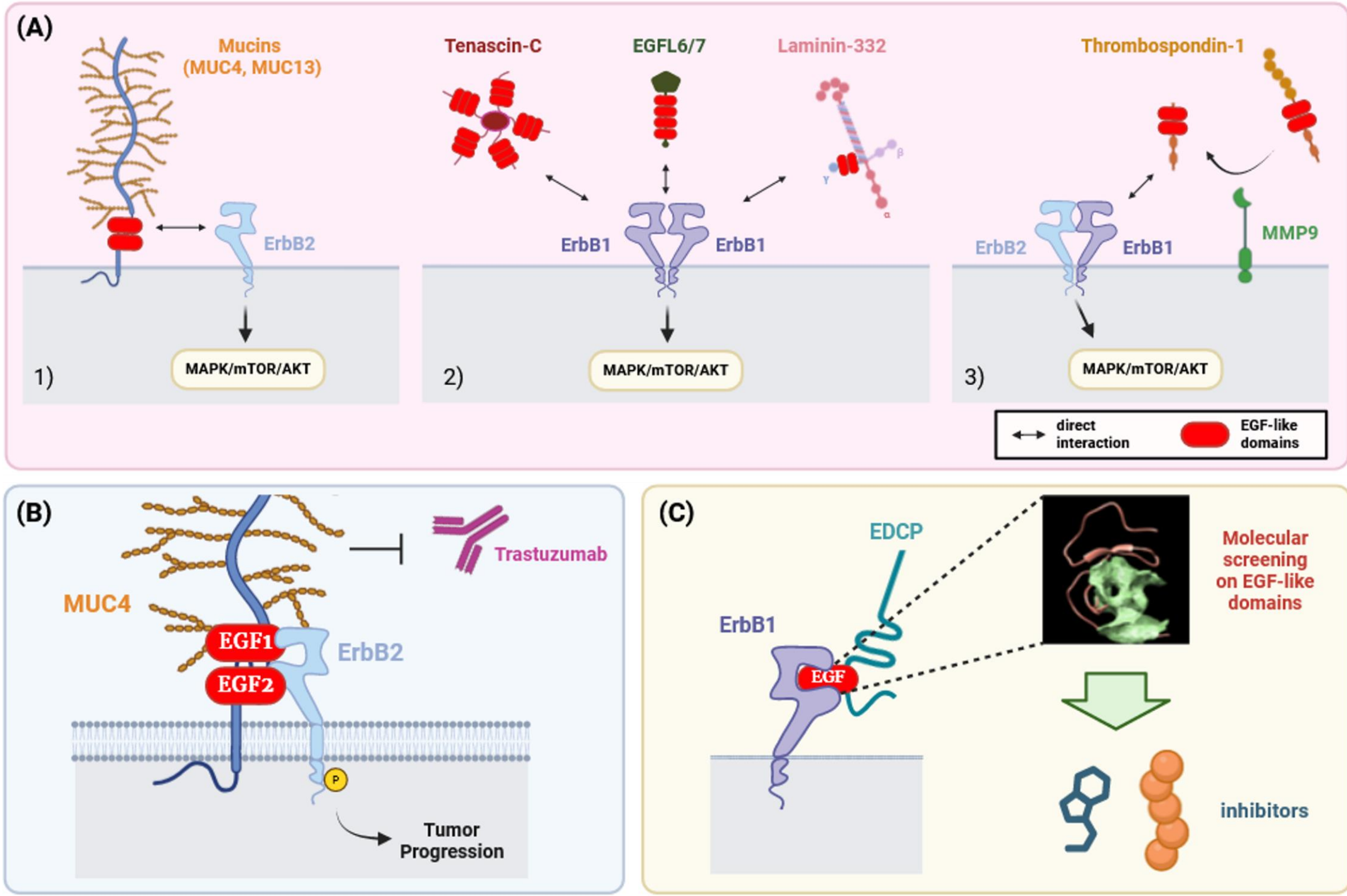
ErbB3
Anti-ErbB1 UniCAR T cell
CAR
ErbB1
anti-ErbB1 + TM (E5B9)
tumor
T-cell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(MUC4, MUC13)
ErbB2
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Tenascin-C
EGFL6/7
Laminin-332
ErbB1
ErbB1
2)
MAPK/mTOR/AKT
Thrombospondin-1
MMP9
ErbB2
ErbB1
3)
MAPK/mTOR/AKT
direct interaction
EGF-like domains
(B)
Trastuzumab
MUC4
EGF1
EGF2
ErbB2
P
Tumor Progression
(C)
EDCP
ErbB1
EGF
Molecular screening on EGF-like domains
inhibitors