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Review

Pharmacological Targeting of EphA2: Advancing Precision Therapeutics in Cancer Treatment

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Abstract

EphA2, an oncogenic receptor tyrosine kinase overexpressed in various malignancies, represents a compelling therapeutic target in oncology. The role of this factor in the growth of tumors, angiogenesis, and cancer metastasis has prompted the emergence of pharmacological interventions, including small-molecule inhibitors, therapeutic monoclonal antibodies, antibody-drug conjugates (ADCs), and novel modalities like proteolysis-targeting chimeras (PROTACs). While preclinical studies have demonstrated potent anticancer effects, clinical translation remains challenging due to tumor heterogeneity, suboptimal pharmacokinetics, and toxicity profiles. Current strategies focus on improving drug delivery using EphA2-targeted nanoparticles and bicycle toxin conjugates, which enhance specificity and reduce off-target effects. Immune-based approaches, such as EphA2-specific CAR-T cells and dendritic cell vaccines, are being explored for synergistic combination therapies to overcome immune resistance. Despite limited success in trials, ongoing innovations in delivery systems and biomarker development aims to address these barriers. This review emphasizes the pharmacological potential of EphA2-targeted therapies and their integration into precision oncology, highlighting critical challenges and emerging solutions for advancing these agents into clinical practice.

Keywords:

EphA2 receptor, Antineoplastic agents, Immunotherapy, Targeted drug delivery systems, Drug resistance

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1. Introduction

Receptor tyrosine kinases (RTKs) represent an essential class of enzymes responsible for facilitating signal transduction between cells [1]. In humans, this family includes 58 distinct RTKs, which are classified into 20 subfamilies according to the structure of their external regions. These receptors are essential for cell functions, including growth, differentiation, cell survival, and migration. The dysregulation of RTKs by mutations, deletions, amplifications, or overexpression is significantly linked to the onset and progression of various cancer types [2]. Consequently, targeted therapies, including tyrosine kinase inhibitors (TKIs), monoclonal antibodies, and ligand-blocking agents, have transformed the landscape of personalized cancer treatment [3]. Prominent examples include HER2 inhibitors in breast cancer, BCR-ABL inhibitors in chronic myelogenous leukemia, and EGFR-targeted therapies in colorectal cancer [4].

Within the RTK family, the ephrin (Eph) receptor group stands out as the largest, consisting of 14 receptors and 8 membrane-bound ligands [5]. Initially discovered in 1987 with the identification of EphA1 in liver cancer cells, Eph receptors have since been recognized for their critical roles in embryonic development, including tissue patterning, neuronal targeting, and blood vessel formation [6].

In addition to their physiological functions, Eph receptors have been linked to a variety of diseases, notably cardiovascular conditions, infections caused by viruses, and neurological disorders [7]. EphA2 has been identified as a significant factor in cancer biology. Overexpression is commonly observed in solid tumors, notably carcinomas and sarcomas, where it facilitates essential processes like tumor initiation, progression, and metastasis. Notably, EphA2 exhibits a dual role in cancer, acting as either a promoter or suppressor of tumorigenesis depending on the cellular context and the availability of its ligands [8]. Ligand-independent activation of EphA2 typically promotes oncogenic signaling pathways, enhancing cell proliferation, migration, and angiogenesis [9]. Conversely, ligand-dependent activation can inhibit tumor progression by facilitating cell adhesion and suppressing metastatic behavior [10].

Given its significant role in cancer, EphA2 has garnered attention as a promising target for therapeutic intervention. Anti-EphA2 strategies, including monoclonal antibodies, TKIs, and ligand mimetics, are under development to selectively inhibit its oncogenic activity while preserving its tumor-suppressive functions [11]. However, the molecular complexity of EphA2 presents substantial challenges, including the risk of acquired drug resistance, the absence of reliable biomarkers for therapeutic response, and the involvement of non-kinase signaling pathways [12].

This review examines the multifaceted role of EphA2 in cancer biology, examining its contribution to oncogenesis and tumor progression, as well as its potential as a therapeutic target. By addressing the challenges associated with EphA2-targeted therapies, this review aims to provide insights into developing more effective strategies for leveraging this receptor in cancer treatment.

2. Receptor Tyrosine Kinases

RTKs constitute a crucial category of transmembrane proteins that mediate cellular communication and signal transduction. They play an essential role in regulating key biological processes, including cell proliferation, differentiation, survival, and migration [13]. Structurally, RTKs comprise an extracellular ligand-binding domain, a single transmembrane segment, and an intracellular tyrosine kinase domain. Ligand binding triggers receptor dimerization and autophosphorylation, initiating intracellular signaling cascades that regulate a wide range of cellular functions [1].

Due to their central role in cellular regulation, RTK dysregulation is frequently implicated in cancer, often resulting from mutations, overexpression, or amplification. This aberrant activation leads to uncontrolled cell growth, enhanced survival, and metastasis, making RTKs key therapeutic targets in oncology [14]. Targeted treatments, including monoclonal antibodies and tyrosine kinase inhibitors, have been developed to precisely modulate RTK activity across different types of malignancies [1].

RTKs are classified into 20 subfamilies, each defined by unique structural and functional characteristics. These subfamilies regulate a diverse range of cellular activities, including growth factor signaling, metabolic regulation, angiogenesis, and cell adhesion. Several RTK families, such as EGFR, FGFR, PDGFR, VEGFR, MET, and Eph receptors, play crucial roles in normal physiology and are frequently implicated in oncogenesis [13]. As a result, they have been extensively studied as therapeutic targets, with multiple inhibitors and monoclonal antibodies developed to regulate their activity in cancer treatment. Table 1 provides an overview of the major RTK subfamilies, their key members, and their primary biological functions [15].

The Eph receptor subfamily, comprising 14 members classified as EphA and EphB, is the largest RTK family and distinct from others due to its membrane-bound ligand activation mechanism. Unlike classical RTKs, which bind soluble growth factors, Eph receptors require cell-cell interactions via ephrin ligands, enabling bidirectional signaling that influences cell adhesion, tissue organization, and neuronal guidance [20].

EphA2, a key member of this family, exhibits a dual role in cancer, acting as both an oncogene and a tumor suppressor, depending on ligand availability. In ligand-independent conditions, overexpressed EphA2 interacts with integrins and

growth factor receptors, stimulating PI3K/Akt and Ras/MAPK signaling, which enhances tumor growth, invasion, and angiogenesis [24]. Conversely, ligand-dependent activation promotes receptor degradation, stabilizes cell adhesion, and suppresses metastasis. This context-dependent function complicates EphA2-targeted therapy, requiring strategies that selectively inhibit its oncogenic effects while preserving tumor-suppressive functions [25].

Furthermore, unlike EGFR and HER2, which have established biomarkers guiding therapy selection, EphA2 lacks clinically validated predictive markers, making patient stratification and therapeutic targeting challenging. Emerging therapeutic strategies focus on monoclonal antibodies, small-molecule inhibitors, and combination therapies to optimize EphA2-targeted interventions [26].

Table 1. Classification of Receptor Tyrosine Kinases.

RTK Subfamily	Notable Members	Primary Functions	Ref.
Class I: Epidermal Growth Factor Receptor (EGFR) Family	EGFR (ErbB1), HER2 (ErbB2), HER3 (ErbB3), HER4 (ErbB4)	Regulate cell growth, survival, proliferation, and differentiation; implicated in various cancers.	[16]
Class II: Insulin Receptor Family	Insulin Receptor (IR), Insulin-like Growth Factor 1 Receptor (IGF-1R)	Control glucose uptake, metabolism, and growth; essential for metabolic homeostasis.	[17]
Class III: Platelet-Derived Growth Factor Receptor (PDGFR) Family	PDGFRα, PDGFRβ, c-Kit, FLT3	Involved in development, cell proliferation, and survival; mutations linked to cancers and developmental disorders.	[18]
Class IV: Vascular Endothelial Growth Factor Receptor (VEGFR) Family	VEGFR1, VEGFR2, VEGFR3	Key regulators of angiogenesis and lymphangiogenesis; targets for anti-angiogenic cancer therapies.	[19]
Class V: Fibroblast Growth Factor Receptor (FGFR) Family	FGFR1, FGFR2, FGFR3, FGFR4	Influence cell differentiation, growth, and tissue repair; mutations associated with skeletal disorders and cancers.	[20]
Class VI: Receptor Tyrosine Kinase-like Orphan Receptors (ROR) Family	ROR1, ROR2	Play roles in skeletal and neuronal development; aberrant expression linked to certain cancers.	[21]
Class VII: Neurotrophin Receptor (Trk) Family	TrkA, TrkB, TrkC	Mediate neuronal survival, development, and function; involved in neurodegenerative diseases and cancers.	[22]
Class VIII: Eph Receptor Family	EphA1–A8, EphA10, EphB1–B4, EphB6	Largest RTK family; regulate cell positioning, shape, and mobility; implicated in developmental processes and cancer progression.	[23]

3. Signaling Mechanisms of the Eph Receptors

Eph receptors, a prominent subgroup of RTKs, are extensively present among numerous types of cells across both developmental and mature tissues [13,27]. These receptors are structurally conserved and classified into two main classes, EphA and EphB, based on homology in their external regions and unique ligand-binding preferences. The human genome represents nine receptors for EphA, including (A1–8, A10) and five receptors for EphB (B1–4, B6), as well as eight corresponding ligands, comprising Ephrin-A1–5 and Ephrin-B1–3 [28,29]. Despite some exceptions, for instance, the binding of the EphA4 to both the Ephrin-B2 and Ephrin-B3, as well as EphB2 interacting with Ephrin-A5 [30].

These receptors are transmembrane proteins. Their extracellular region facilitates ligand interaction and includes two fibronectin (FN) domains, a ligand-binding domain (LBD), and a cysteine-rich domain with Sushi and EGF-like motifs. The internal section comprises a transmembrane segment, a tyrosine kinase domain, a sterile alpha motif, and a PDZ-binding domain. Conversely, ephrin ligands consist of a conserved receptor-binding domain (RBD) and are membrane-bound. Although Ephrin-Bs are transmembrane proteins with an interior PDZ-binding domain, Ephrin-As are attached to the cell membrane by glycosylphosphatidylinositol (GPI) anchors [28,29].

Bidirectional signaling is triggered by the interaction of Eph receptors with their ligands, which affects cells that express both the receptor and the ligand. This signaling can be categorized as forward (from Ephrin to Eph) or reverse (from Eph to Ephrin), depending on the direction of signal propagation [31,32]. Forward signaling typically operates through the receptor, driving cellular processes such as migration, adhesion, and repulsion, often relying on the receptor's kinase activity. Conversely, reverse signaling is mediated through the ligand and depends on kinases like Fyn, a member of the Src family, which regulates cytoskeletal dynamics and cellular communication [31,32]. Furthermore, Eph-Ephrin complexes can transmit signals either in parallel (same direction) or antiparallel (opposite directions), depending on their functional context [27,33].

Proteolytic cleavage plays a critical regulatory role in Eph-Ephrin signaling. Metalloproteases such as MMPs and ADAMs can cleave Ephrin ligands, releasing soluble fragments that enable paracrine or endocrine signaling. For

instance, soluble Ephrin-A1 fragments can activate EphA receptors in distant cells, disrupt cell adhesion, and increase vascular permeability, facilitating tumor metastasis. Similarly, cleaved Ephrin-B ligands have been linked to pathological processes such as fibrosis and cancer progression (Figure 1) [34-36].

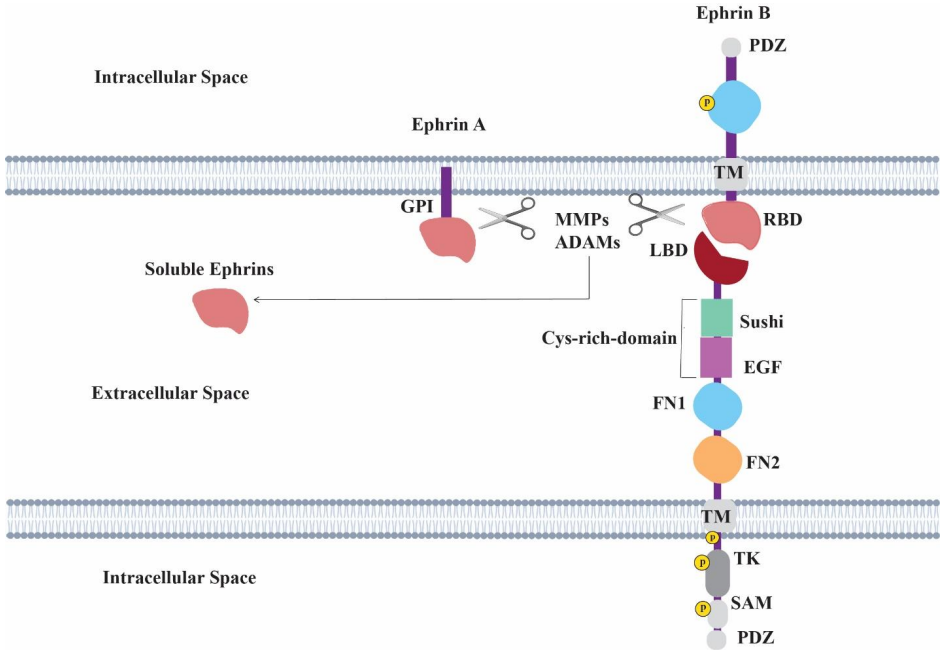

Figure 1. Structural and functional features of Eph receptors and their ligands. Eph receptors are single-pass transmembrane proteins with distinct extracellular and intracellular domains. The extracellular part comprises a ligand-binding domain (LBD), a cysteine-rich region that includes Sushi and Epidermal Growth Factor (EGF)-like motifs, and two fibronectin type III (FN1 and FN2) domains. The intracellular region consists of a transmembrane (TM) segment, a tyrosine kinase (TK) domain, a Sterile Alpha Motif (SAM), and a PDZ-binding domain. Ephrin ligands have a receptor-binding domain (RBD). Class A ephrins are anchored to the cell membrane through glycosylphosphatidylinositol (GPI), whereas class B ephrins contain a transmembrane domain and an intracellular tail ending in a PDZ-binding motif. Proteases such as matrix metalloproteinases (MMPs) and ADAMs can enzymatically cleave ephrins from the cell surface, facilitating paracrine activation of Eph receptors.

4. EphA2 - an Oncofetal Protein

Several tumor forms have a notable overexpression of EphA2, a crucial member of the Eph receptor family. The 976 amino acids that make up this 130 kDa transmembrane glycoprotein are encoded by the EPHA2 gene on chromosome 1p36. As a fetal oncoprotein, it plays a crucial role in the development of several areas, including kidney growth, mammary gland morphology, bone homeostasis, and lens and inner ear formation. A variety of solid cancers have been linked to incorrect EphA2 activation, emphasizing the pathogenic relevance of this protein [37-39].

In normal physiological situations, EphA2 predominantly interacts with Ephrin-A1 as a TNF-α-inducible ligand. This interaction facilitates forward and reverse signaling, maintaining cellular organization and suppressing proliferation pathways through regulated mechanisms. However, in tumor environments, EphA2 becomes aberrantly activated, deviating from its canonical pathways and contributing to oncogenesis (Figure 2).

In cancer, EphA2 drives tumor progression through several mechanisms. Initially, it forms dimers that bind multiple RTKs, including EGFR, HER2, FGFRs, and VEGFRs, or engages with adhesive molecules, E-cadherin and integrins. These interactions enhance cell adhesion, migration, and metastatic potential [40-42]. Additionally, EphA2 attaches to Platelet-Derived Growth Factor A (PDGFA), activating oncogenic pathways and promoting tumor survival [43].

Another critical mechanism involves the phosphorylation of Ser897 within EphA2's intracellular domain by oncogenic kinases such as ERK, Akt, and RSK. This modification enables EphA2 to activate the Rho G-Akt signaling pathway, which promotes resistance to anoikis—a form of apoptosis triggered by loss of cell adhesion. This resistance supports the survival and dissemination of cancer cells within the extracellular matrix [44-46].

EphA2's role extends to integrin-mediated adhesion and migration. It interacts with FAK to facilitate cellular motility, but this activity can be disrupted by Ephrin-A1-induced tyrosine phosphorylation. In prostate cancer cells, such disruption leads to dephosphorylation of FAK and reduced migration. However, overexpression of LMW-PTP reverses this effect, destabilizing cell adhesion via a RhoA-dependent pathway and enhancing cell detachment and motility [8,47,48].

EphA2's involvement in cancers such as melanoma, glioblastoma, and carcinomas of the lung, breast, stomach, and colon underscores its potential as both a biomarker for poor prognosis and a therapeutic target. Moreover, its interactions with the tumor microenvironment, particularly with cancer-associated fibroblasts (CAFs), further amplify

its tumorigenic role. EphA2-PI3K signaling, for example, facilitates vascular mimicry, allowing cancer cells to form endothelial-free blood vessels and promote angiogenesis through interactions with Caveolin-1 and bFGF production (Figure 3) [8,49-52].

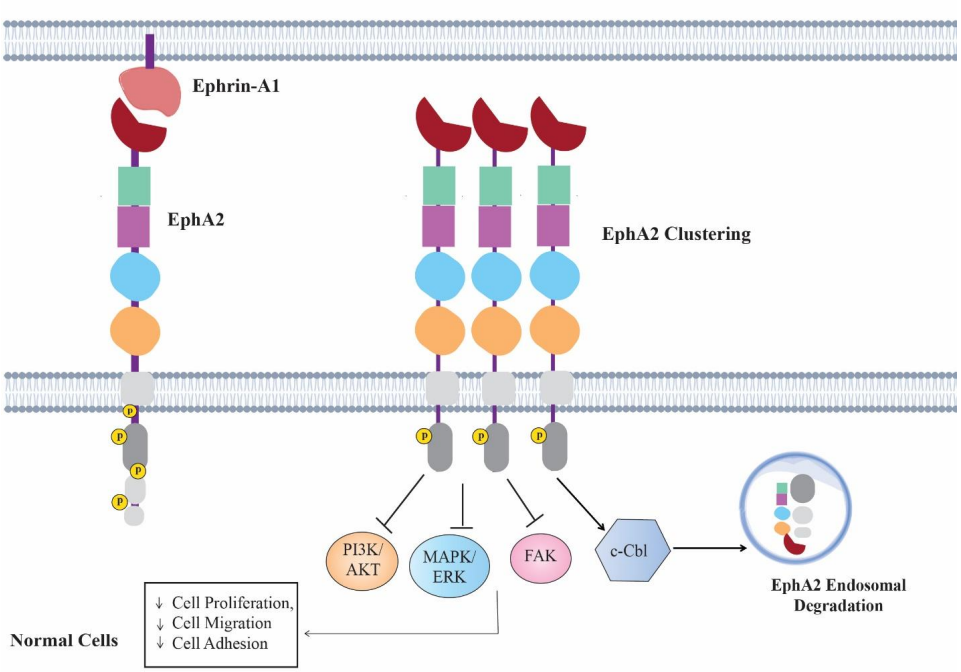

Figure 2. Ligand-dependent and ligand-independent signaling of EphA2 in normal cells. In typical cellular environments, EphA2 signaling is predominantly activated by ligand binding, resulting in phosphorylation of specific Tyrosine (Y) and Serine (S) residues. This phosphorylation facilitates clustering of EphA2, which subsequently inhibits signaling pathways involved in cell proliferation, survival, and migration, such as ERK, Akt, and FAK. As a result, key cellular processes including cell growth, resistance to apoptosis, and migration are effectively restrained. Furthermore, the c-Cbl protein mediates endosomal degradation and recycling of EphA2, maintaining the receptor's regulated activity within the cell.

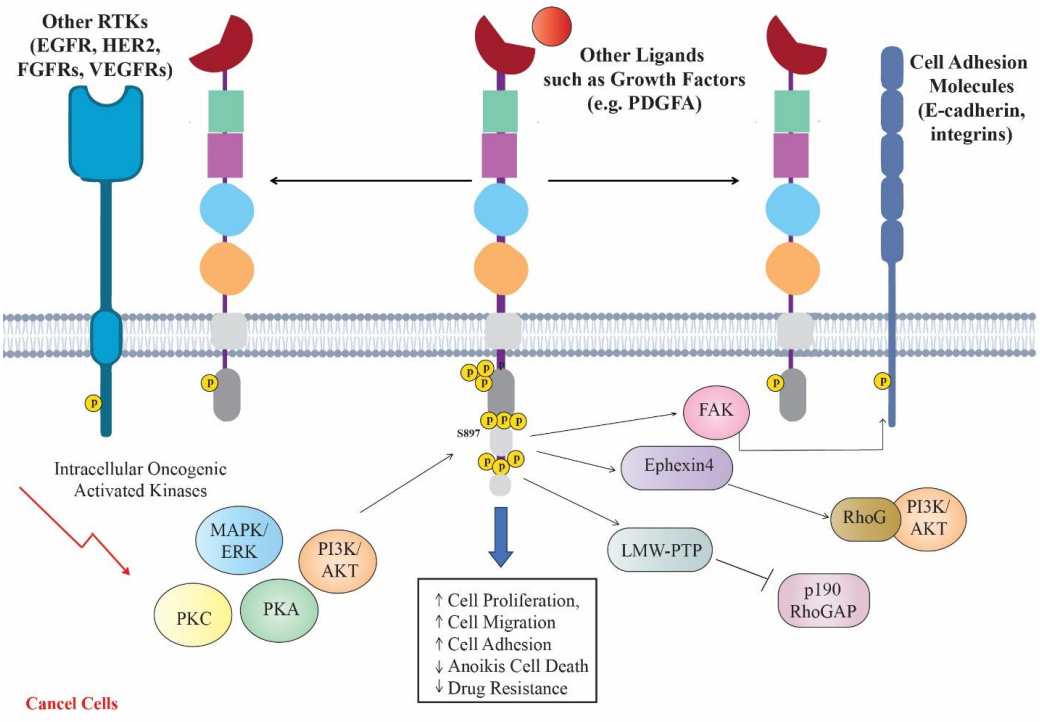

Figure 3. Ligand-dependent versus ligand-independent EphA2 signaling in cancer cells. In cancerous cells, EphA2 signaling shifts to a ligand-independent and non-canonical mode. This type of activation can occur via dimerization with other receptor tyrosine kinases (RTKs) such as EGFR, HER2, FGFRs, and VEGFRs, or with adhesion molecules like E-cadherin and integrins. Alternatively, it may result from direct interactions with other ligands, such as platelet-derived growth factor A (PDGFA), or through the activation of intracellular oncogenic kinases, including Akt, PKA, PKC, and ERK. This signaling pathway leads to phosphorylation of Ser897, which enhances processes like cell proliferation, adhesion, migration, therapy resistance, and protection against apoptosis and anoikis.

5. Targeting EphA2 in Cancer

Preclinical and translational research has demonstrated EphA2's pivotal involvement in cancer development, prompting the development and evaluation of therapies that inhibit its activity in tumor cells. However, targeting EphA2 requires an in-depth knowledge of its function and intricate signaling networks. The subsequent sections provide an overview of current anti-EphA2 therapeutic strategies, outlining their respective strengths and limitations.

5.1 Mechanism-Based Therapies for EphA2

5.1.1 EphA2 Inhibition by Small Molecule

A variety of small molecules have been designed to target EphA2. These molecules are typically classified into two main categories. The first category includes inhibitors that target the EphA2 ligand-binding domain (LBD). They interfere with the binding process between ephrin ligands and EphA2, affecting EphA2 phosphorylation [53]. The second class comprises kinase domain blockers that interfere with subsequent signaling processes and hinder EphA2 kinase activity, such as ATP-mimicking compounds [54].

The molecular structures of PPI inhibitors vary, spanning bile acid derivatives, salicylic acids, and other repurposing substances intended to inhibit EphA2. These inhibitors can function as either agonists, which mimic EphA2/ligand interactions to restore tumor-suppressing activity, or antagonists, which block these interactions [55]. Despite their potential, designing effective PPI inhibitors remains challenging due to the extensive interaction surface between EphA2 and its ligands. Among the agonists, doxazosin has shown promising preclinical outcomes, demonstrating the ability to inhibit AKT and ERK kinase activity in an EphA2-dependent manner [48]. It also promotes EphA2 internalization, reduces cell migration in prostate, breast, and glioma cancers, limits metastasis in prostate cancer, and improves survival in mouse models [56]. Additionally, doxazosin has the ability to inhibit vasculogenic mimicry (VM) by downregulating the expression of genes related to VM, including VEGF-A, MMP-2, and VE-cadherin, and by suppressing the EphA2/AKT/mTOR/MMP/Laminin-5γ2 route, which is located further downstream [12,57]. Subsequently improving the efficiency of doxazosin resulted in the discovery of more potent substances exhibiting improved permeability across the blood-brain barrier and enhanced pharmacological activity [58,59]. Despite these advances, further refinement is needed before these agents can be evaluated in clinical trials.

Various compounds, including Farnesoid X Receptor (FXR) agonists like GW4064, cilofexor, and vonafexor, have been repurposed as EphA2 antagonists. Although these agents showed promising preclinical outcomes in cancer models, particularly prostate cancer [60], no clinical trials have evaluated their effectiveness in EphA2-positive malignant cells mainly. In order to replicate the antitumor effects observed with ephrin A1 binding to EphA2, synthetic agents, notably chimeric proteins, including soluble EphA2 or ephrin-A1, as well as peptides, have also demonstrated promise in targeting the EphA2-ephrin system [61,62].

Inhibitors that specifically target the EphA2 kinase domain are currently not abundant, as many exhibit a lack of specificity and also inhibit other kinases. Nanomolar inhibitors such as ALW-II-41-27 and GLPG1790 (50) are examples of the chemicals classified within this category. These compounds include catechol and quinazoline derivatives [63]. ALW-II-41-27, which is frequently utilized in preclinical models for bone sarcomas, slows cell development in multiple types of cancers; however, it exhibits cross-activity against kinases such as BRAF, CSF1R, DDR1, and DDR2, which raises concerns regarding off-target effects [12,64]. On the other hand, dasatinib, initially intended for targeting BCR/ABL and Src family kinases, demonstrates a considerable affinity for EphA2 and demonstrates preclinical effectiveness in cancers that contain large quantities of S897-phosphorylated EphA2 [65]. In endometrial cancer patients, a clinical trial that combined dasatinib with paclitaxel and carboplatin found positive outcomes with toxicity that was under control [66].

Furthermore, antiviral drugs such as ledipasvir (LDV) and daclatasvir (DCV) suppress AKT phosphorylation by disrupting the Src/EphA2 complex [67]. These substances may be used to treat Src-associated tumors, as preclinical research showed that they efficiently prevent triple-negative MDA-MB-231 breast cancer cells, SRC-transduced SW620 colon cancer cells, and SRC-transduced NIH3T3 fibroblasts from proliferating, invading, and colonizing [67]. The effectiveness of these antiviral agents in targeting Src-associated malignancies highlights their potential as therapeutic options beyond traditional antiviral applications.

5.1.2 Using Monoclonal Antibodies to Target EphA2

Preclinical investigations have revealed that monoclonal antibodies (mAbs) targeting EphA2 may treat various malignancies. EphA2-targeting mAbs have demonstrated two functions: they can imitate the ligand ephrin A1 to initiate tumor-suppressive EphA2 signaling and cause the receptor to internalize and degrade, hence preventing its activity [68]. Initial instances of anti-EphA2 mAbs, including EA1.2, EA2, and B233, demonstrated effectiveness in preclinical experiments by reducing cancer cell proliferation in both in vitro and in vivo models [69,70]. SHM16 [71], DS-8895a [71], and MEDI-547 [72] are examples of additional monoclonal antibodies that have demonstrated promising preclinical results across a wide range of cancer types. These mAbs achieve high specificity by precisely targeting the EphA2 receptor, thereby minimizing off-target effects and enhancing therapeutic efficacy [73]. Compared to small-

molecule inhibitors, mAbs offer greater specificity for EphA2. Phase I clinical trials testing anti-EphA2 mAbs like DS-8895a for progressive EphA2-positive malignancies showed limited efficacy and significant toxicity. Although DS-8895a was well-tolerated, it showed low therapeutic effectiveness. The negligible tumor response was likely due to poor uptake in tumor tissues, possibly caused by EphA2 expression heterogeneity or reduced expression in metastatic lesions [74,75]. This underscores the importance of combined approaches over relying solely on single therapies. For example, DS-8895a reduced PD-L1 expression, suggesting potential benefits when combined with immunological checkpoint inhibitors (ICIs) [74].

An antibody-drug conjugate (ADC), MEDI-547, was designed for direct delivery of cytotoxic agents to EphA2-expressing tumors. It is made up of a human IgG1 mAb that targets EphA2 (1C1) and is conjugated to an auristatin derivative. The conjugation of auristatin derivatives, known for their potent microtubule-disrupting activity, enhances the therapeutic potential of MEDI-547 by selectively targeting tumor cells while sparing normal tissues [76]. Preclinical studies revealed that MEDI-547 induces EphA2 degradation and internalization, suppresses proliferation, increases apoptosis, and exhibits antiangiogenic effects in endometrial and prostate cancer models [72,77]. A phase I clinical trial of MEDI-547 in patients with relapsing or refractory cancers was stopped because of toxicity and disease progression, despite promising preclinical evidence. Adverse effects included bleeding, coagulation disorders, elevated liver enzymes, and anemia, with serious events such as conjunctival hemorrhage and liver dysfunction reported in a minority of patients [78]. More recently, the auristatin-based hSD5-vedotin ADC has been tested in preclinical models of pancreatic cancer. This ADC effectively triggered EphA2 internalization, inhibited tumor growth, and induced apoptosis in xenograft models [79]. However, its clinical safety profile remains to be established.

5.2 Innovative Delivery Systems

Recent advancements in cancer therapy have emphasized the importance of novel drug delivery systems, which aim to enhance the specificity, stability, and efficacy of treatments while minimizing side effects. These innovative delivery methods have emerged as promising strategies to overcome the limitations of traditional therapies, such as chemotherapy and monoclonal antibodies. By improving targeted drug delivery and utilizing cutting-edge technologies like nanoparticles, bicyclic peptides, and protein degradation systems, these approaches are revolutionizing the way cancer treatments are designed and administered. Below, we discuss some of the most promising innovative delivery systems in cancer therapy [80].

5.2.1 EphA2 Bicycle Treatment

Bicyclic peptides are developing as a novel strategy for tackling tumors that exhibit resistance to traditional therapies, including chemotherapy and monoclonal antibodies. These peptides are small proteins featuring a bicyclic structure, which offers increased stability, elevated target selectivity, and robust attraction to targets [81]. One notable example is Bicycle Toxin Conjugates (BTCs), which, due to their small size, penetrate tumors more effectively and rapidly than traditional antibody-drug conjugates (ADCs) (Figure 3). Unbound conjugates are quickly cleared from the system, reducing toxicity to healthy tissues [81].

Recent screening investigations have led to the discovery of BT5528, a BTC that targets EphA2. This BTC attaches to the ligand-binding domain of EphA2 at low nanomolar concentrations through a cleavable linker [80]. In preclinical rat models, BT5528 showed great dose tolerability, and clinical trials are now underway to evaluate its efficacy in progressive EphA2-overexpressing solid tumors (NCT04180371) [82].

5.2.2 EphA2-Based Nanoparticles in Cancer Therapy

Nanoparticle-based drug delivery involves the utilization of nanoscale carriers, typically within the 1 to 100 nanometer range, to facilitate the targeted transport of therapeutic agents - including chemotherapeutic drugs, RNA-based treatments, and molecular inhibitors - directly to pathological tissues [83]. This strategy aims to enhance treatment efficacy while modifying systemic toxicity. In contrast to conventional drug administration methods, which frequently suffer from low bioavailability, non-specific distribution, and dose-limiting adverse effects, nanoparticle-based delivery systems offer significant advantages, including improved solubility of therapeutics, protection from enzymatic degradation, and precise, controlled release at tumor sites [84].

Among the molecular targets investigated for nanomedicine-based interventions, EphA2 has garnered substantial interest due to its aberrant overexpression in multiple aggressive malignancies, such as glioblastoma, lung, prostate, breast, and pancreatic cancers [84-89]. Elevated levels of EphA2 contribute to tumor cell proliferation, enhanced metastatic potential, and increased invasive capabilities, underscoring its relevance as a target in precision oncology. The use of nanoparticles targeting EphA2 has gained significant attention in preclinical and clinical studies due to their potential to improve drug delivery efficacy, specificity, and safety. Most EphA2-targeted nanoparticles are either liposomes, spherical vesicles that deliver siRNAs, pharmaceutical products, or nanoparticles of polymeric material that are produced from biodegradable substances [90-92].

Therapies based on RNA interference (RNAi), which utilize shRNAs or siRNAs, have the ability to precisely silence the EphA2 gene, thereby lowering the amount of protein that it produces. For example, siRNA-mediated inhibition of

EphA2 in glioma cells significantly reduced tumor cell proliferation and induced apoptosis [93]. However, in vivo delivery of siRNAs remains a major challenge, which nanoparticles aim to address. Nanoparticles, such as liposome-based systems, can enhance the stability and uptake of siRNAs [94]. EPHARNA, a liposome-incorporated siRNA targeting EphA2, showed efficacy in orthotopic tumor models and was well-tolerated in Rhesus monkeys without significant toxicity, paving the way for a phase I clinical trial (NCT01591356) [95,96].

Cationic solid lipid nanoparticles (cSLNs) have also been developed for delivering anti-EphA2 siRNAs in prostate cancer, addressing the limitations of siRNA stability and uptake [97,98]. EphA2-targeted nanoliposomes containing doxorubicin (DOX) and siRNAs directed against JNK-interacting protein 1 (JIP1) were effective in restoring chemosensitivity in osteosarcoma cells exhibiting elevated EphA2 expression, addressing the issues of inadequate cellular uptake and plasma instability [99].

Recent advancements include microvesicles (MVs) coated with EphA2-targeting peptides and surface-modified with superparamagnetic nanoparticles (SPIONs). These YSA-SPION-MV/MTX constructs demonstrated superior drug delivery to osteosarcoma tumors in murine models with reduced toxicity compared to standard methotrexate treatment [100]. A nanotherapeutic targeting EphA2, encapsulating a docetaxel (EphA2-ILs-DTXp), was established for bladder cancer treatment, showing greater effectiveness in patient-derived xenograft (PDX) models compared to docetaxel monotherapy. Combining this therapy with gentamicin further amplified its tumor-suppressive effects [101].

In lung cancer, pegylated nanoparticles coated with EphA2-targeting peptides successfully delivered dual therapeutic agents, exhibiting high affinity for EphA2-expressing lung tumor cells. These nanoparticles achieved superior tumor penetration, improved anticancer efficacy, and decreased systemic adverse effects [102,103]. Furthermore, the targeted nanoparticle system facilitated sustained release of the therapeutic agents, ensuring prolonged efficacy and reducing the frequency of dosing [104]. In breast cancer, doxorubicin was delivered to EphA2-overexpressing cells selectively using mesoporous silica nanoparticles (MSNs) combined with YSA peptides, improving therapeutic efficacy while minimizing toxicity [105].

5.2.3 Targeted Protein Degradation (TPD)

Regulating protein stability offers a promising therapeutic approach for countering oncogenic proteins in cancer [106,107]. Strategies targeting the ubiquitin-proteasome system (UPS), such as inhibitors of E3 ligases and proteasomes, are already FDA-approved for cancer treatment [108].

Recent advancements focus on targeted degradation of proteins by molecules like proteolysis-targeting chimeras (PROTACs), which enhance the interaction between proteins of interest and E3 ubiquitin ligases, resulting in the proteasomal cleavage of oncogenic targets [109]. By regulating its ubiquitination and degradation in an ephrin A1-dependent manner, the E3 ubiquitin ligase c-Cbl negatively regulates EphA2 [110,111]. Additionally, the c-Cbl-EphA2 axis plays a pivotal role in modulating downstream signaling pathways that influence cell proliferation and migration [12]. By inhibiting the connection between c-Cbl and EphA2, for example, through Annexin A1 (ANXA1), EphA2 is stabilized, which in turn increases the growth of tumors and the spread of metastases in nasopharyngeal cancer models. ANXA1-derived peptide (A11), on the other hand, enhances c-Cbl-mediated degradation of EphA2, which in turn reduces tumor development and metastasis both in vitro and in vivo (87). This peptide prevents ANXA1 from binding to EphA2, which increases the efficiency of the degradation process [111]. Similarly, RNF5, another E3 ligase, interacts with EphA2 in HER2-negative breast cancer cells, promoting its ubiquitination and degradation and thereby inhibiting tumor-suppressive functions of EphA2 in this subtype [112]. RNF5 inhibitors, such as FX12, have been developed to degrade RNF5 via the proteasome, potentially counteracting EphA2-associated tumor growth in cancers where EphA2 exerts tumor-suppressive effects [113]. In addition to RNF5 inhibitors, PROTACs (Proteolysis Targeting Chimeras) offer a novel strategy for targeted protein degradation by simultaneously binding to the target protein and an E3 ubiquitin ligase, thereby facilitating the selective degradation of proteins like EphA2 [12].

PROTACs represent a next-generation approach in targeted degradation of proteins. An E3 ligase-recruiting moiety, a flexible linker, and a particular target-binding component are all meticulously comprised in these heterobifunctional molecules, each one of which is essential to their overall functionality. This precise arrangement enables PROTACs to effectively harness the cell's ubiquitin-proteasome system, ensuring the targeted degradation of specific proteins [114]. PROTACs can selectively degrade proteins lacking kinase domains or target specific protein isoforms, overcoming drug resistance associated with traditional therapies [115]. For example, PROTAC2, which uses foretinib as its target-binding component and cereblon as the E3 ligase recruiter, demonstrated high affinity for EphA2 and c-MET and successfully degraded both proteins in vitro [116]. Although these findings are preliminary, they highlight the potential of PROTAC-based therapies for targeting EphA2 and overcoming limitations of conventional treatments.

Unlike conventional small-molecule inhibitors, TPD provides a more sustained therapeutic effect by eliminating oncogenic proteins rather than merely inhibiting their function. This approach prevents protein re-accumulation, minimizing resistance development and ensuring prolonged suppression of oncogenic signaling [117]. Additionally, TPD allows for selective degradation of proteins traditionally considered undruggable, such as transcription factors and scaffolding proteins, offering broader therapeutic potential [118]. By degrading specific oncogenic proteins while preserving homologous non-pathogenic variants, TPD enhances target specificity, reduces off-target toxicity, and

complements other cancer treatment modalities, including immunotherapy and combination treatments [119]. Given these advantages, TPD is emerging as a highly promising strategy to overcome limitations associated with traditional drug development in oncology.

5.3 Immune-Mediated Approaches

Immunotherapy has established itself as a fundamental strategy in cancer treatment, harnessing the immune system's natural ability to detect and eliminate malignant cells with greater specificity and efficiency. Unlike conventional treatments that directly target tumor cells, immunotherapeutic strategies modulate immune mechanisms to potentiate antitumor responses [120,121]. These therapeutic approaches include monoclonal antibodies, immune checkpoint inhibitors, cancer vaccines, and adoptive cell therapies, such as chimeric antigen receptor (CAR)-T cell therapy, all of which are designed to enhance the immune system's ability to recognize and eliminate cancer cells. Each modality employs distinct immunomodulatory mechanisms, from disrupting immune checkpoint signaling to augmenting antigen-specific T-cell activation [122]. While immune checkpoint inhibitors have demonstrated durable responses in a subset of malignancies, their efficacy remains influenced by factors such as tumor immunogenicity and the immune composition of the tumor microenvironment. Concurrently, adoptive cellular therapies and antigen-directed vaccines provide alternative strategies to reinforce tumor-specific immunity [123].

Given its dysregulated expression in highly aggressive cancers and its involvement in immune evasion, EphA2 has garnered attention as a potential immunotherapeutic target. The following sections explore EphA2-directed immunotherapy, including cancer vaccines and CAR-T cell therapies, discussing their mechanisms of action, therapeutic implications, and translational challenges.

5.3.1 Cancer Vaccines

Cancer vaccines aim to activate the patient's immune system to recognize and attack EphA2-expressing tumor cells. Innovations in adjuvant technologies have enhanced the ability of these vaccines to provoke stronger immune responses, improving their immunogenicity [124]. One notable strategy employs dendritic cell (DC)-based vaccines. DCs are a subset of white blood cells capable of activating both cytotoxic T lymphocytes (CTLs) and helper T cells [125]. Enhancing the maturation and activation processes of DCs has been demonstrated to increase their effectiveness in generating precise immune responses [126]. Vaccines that utilize DCs loaded with EphA2 peptides have shown significant antitumor immune activity in mouse models of colorectal cancer [127]. The combination of DC-based vaccines with immune checkpoint inhibitors (ICIs) has shown promise in boosting the immune response. Currently, two preliminary clinical trials are enrolling patients with advanced solid tumors or relapsed/refractory lymphomas to assess the safety and effectiveness of EphA2-DC-based vaccines in conjunction with ICIs (NCT05631886; NCT05631899) [12,128].

5.3.2 CAR-T Cell Treatment

Another potential strategy is chimeric antigen receptor (CAR) T cell therapy. This approach involves genetically modifying T cells to target specific tumor antigens. EphA2-CAR-T cells, for instance, have been tested in glioblastoma models, where they successfully recognized and suppressed EphA2-positive glioblastoma cells, significantly reducing tumor growth in vivo [129,130]. Models of esophageal squamous cell carcinoma and non-small cell lung cancer have shown comparable outcomes [131,132].

Clinical trials have also explored EphA2-CAR-T cells in patients with malignant gliomas. A trial (NCT03423992) involving recurrent glioblastoma patients showed limited efficacy, with disease progression in two participants and toxicity affecting multiple organs [133]. New studies continue to investigate the potential of EphA2-CAR-T cells in other cancers, such as prostate and breast cancer. EphA2-CAR-T cells, for example, limited tumor formation in prostate cancer models and showed promising results in Her-2-enriched and triple-negative breast cancer subgroups [87,134]. Furthermore, advancements in CAR-T cell engineering have enhanced their persistence and efficacy within the tumor microenvironment, contributing to improved therapeutic outcomes. However, the use of CAR-T cell therapy in treating solid tumors is impeded by several significant issues, including harmful effects on healthy tissues, reduced effectiveness, instability of the therapy, and the presence of immunosuppressive conditions within the tumor microenvironment. Recent studies have attempted to address these issues. For example, dual CAR-T cells targeting different EphA2 epitopes demonstrated strong antitumor activity in glioblastoma models [135,136]. However, overexpression of interferon-γ (IFN-γ) in CAR-T cells led to upregulation of PD-L1, suppressing T cell activity. Adding PD-1 blockade significantly improved CAR-T cell efficacy in these models, suggesting that combining EphA2-CAR-T therapy with ICIs could overcome immunosuppressive barriers and improve outcomes (Table 2) [135].

Table 2. Overview of EphA2-Targeted Therapies: Categories, Mechanisms, and Outcomes

Category	Therapy type	Examples	Mechanism	Preclinical/Clinical outcomes	Ref.
Mechanism-based therapies	PPI inhibitors	Doxazosin, FXR agonists (e.g., GW4064, cilofexor), synthetic agents (ephrin-A1)	Disrupt EphA2-ephrin interactions, inhibit AKT/ERK signaling, promote EphA2 internalization	Inhibit metastasis, reduce migration, downregulate VM genes	[12,53,55-59]
	Kinase domain inhibitors	ALW-II-41-27, GLPG1790, Dasatinib, Ledipasvir, Daclatasvir	Block EphA2 kinase activity, inhibit downstream signaling pathways	Promising results in various cancers, potential off-target effects	[12,54,64-68]
	Monoclonal antibodies	DS-8895a, MEDI-547, hSD5-vedotin	Trigger tumor-suppressive EphA2 signaling, internalization, and degradation	Limited efficacy in clinical trials, toxicity challenges	[68-72,74,75,137]
Innovative delivery systems	Bicyclic peptides (BTCs)	BT5528	Bind EphA2 with high specificity, reduced toxicity compared to ADCs	Enhanced tumor penetration, ongoing clinical trials (e.g., NCT04180371)	[80-82]
	Nanoparticles	EPHARNA, cSLNs, YSA-SPION-MV/MTX, EphA2-ILs-DTXp	Deliver siRNAs or drugs, enhance stability and uptake, reduce systemic toxicity	Improved chemosensitivity, reduced toxicity in murine and xenograft models	[90,91,93-97,99-101]
	Protein degradation	PROTACs (e.g., Foretinib-based), RNF5 inhibitors (FX12)	Target ubiquitination and degradation of EphA2	Effective in vitro/in vivo degradation of EphA2, potential to overcome resistance	[110-113,116]
Immune-mediated approaches	Cancer vaccines	DC-based vaccines (EphA2 peptides)	Stimulate immune response against EphA2-expressing tumor cells	Strong antitumor immune response, potential combination with ICIs	[12,125,127,128]
	CAR-T cell therapy	EphA2-CAR-T cells	Genetically modify T cells to target EphA2 on tumor cells	Significant tumor suppression, challenges with stability and toxicity in solid tumors	[87,129-136]

6. EphA2-Targeted Combination Therapies

Preclinical studies have extensively explored the potential of EphA2-based combination therapies across different cancer types, aiming to enhance therapeutic efficacy and overcome resistance mechanisms. In glioblastoma, UniPR1331 has demonstrated the ability to significantly enhance the effects of bevacizumab, leading to greater tumor suppression in mouse xenograft models [138]. Similarly, in glioma, siRNA-mediated EphA2 knockdown has exhibited comparable inhibitory effects on cell proliferation to standard chemotherapeutics such as cisplatin, etoposide, and nimustine hydrochloride. When combined with these agents, siRNA EphA2 further enhanced their cytotoxic effects, suggesting a promising combinatorial strategy [93].

In colorectal cancer, studies have shown that cetuximab-resistant cells exhibit increased EphA2 activation compared to their cetuximab-sensitive counterparts. The administration of ALW-II-41-27 in combination with cetuximab successfully reversed both primary and acquired resistance, leading to suppressed cell proliferation and enhanced apoptosis. In vivo models further confirmed that this combination significantly reduced tumor growth compared to cetuximab alone [52].

In breast cancer treatment, the combination of the small-molecule inhibitor ALW-II-41-27 with WW437, a histone deacetylase inhibitor, has demonstrated superior suppression of cell viability and migration compared to monotherapy with either agent. This enhanced efficacy is attributed to the dual inhibition of EphA2 phosphorylation and expression, highlighting its potential in targeted cancer therapy [139]. Additionally, in a murine breast cancer model, the co-administration of MM-310 with PD-1 inhibitors (anti-mouse PD-1 antibody J43 and anti-PD-L1 antibody MPL3280) yielded a striking 60% complete response rate. This combination therapy achieved a tumor growth inhibition (TGI) rate of 93%, significantly surpassing the effects of MM-310 alone (81% TGI) or PD-1 inhibitors as monotherapies (54% TGI) [140]. In gastric cancer, a study demonstrated that cisplatin at a dose of 10 mg/kg failed to effectively suppress tumor growth when administered alone. However, its combination with DS-8895a resulted in a substantial therapeutic benefit, further validating the potential of EphA2-targeted therapeutic strategies [137].

Collectively, these findings emphasize the promise of EphA2-based combination regimens in enhancing treatment efficacy across multiple malignancies, particularly in overcoming drug resistance and improving tumor suppression.

7. Development Status of EphA2-Targeted Therapies: Clinical Stages, Challenges, and Preclinical Insights

EphA2 has garnered considerable attention as a therapeutic target due to its overexpression in aggressive tumor types, serving as a key regulator in cancer progression [52,75,141]. Several therapeutic strategies have been investigated, including monoclonal antibodies, antibody-drug conjugates, small-molecule inhibitors, RNA interference-based approaches, and immunotherapies. Despite early setbacks, ongoing clinical research continues to explore refined strategies aimed at effectively targeting EphA2 while mitigating off-target effects [142].

Among early efforts to exploit EphA2 as a therapeutic target, monoclonal antibody-based drug conjugates were developed to facilitate selective tumor cell killing. One such candidate, MEDI-547, was designed by conjugating a human monoclonal antibody (IC1) specific to EphA2 with the cytotoxic agent monomethyl auristatin phenylalanine (MMAP). Preclinical evaluations demonstrated that binding of IC1 induced receptor internalization, leading to degradation and enhanced antitumor activity in vitro and in xenograft models. However, when tested in a Phase I clinical trial (NCT00796055) for patients with relapsed and refractory solid tumors, severe adverse effects emerged. High rates of hemorrhagic and coagulation-related toxicities led to premature termination of the trial, suggesting that either unexpected cross-reactivity with other targets or an on-target effect on EphA2-expressing endothelial cells contributed to these complications [77,78].

To address concerns surrounding adverse vascular effects, a second-generation anti-EphA2 antibody, DS-8895a, was developed. Unlike its predecessor, which exhibited agonistic activity upon receptor binding, DS-8895a functioned as an antagonist, preventing EphA2 phosphorylation while simultaneously triggering antibody-dependent cellular cytotoxicity (ADCC). Preclinical assessments revealed strong anticancer activity across gastric and breast cancer models, prompting its advancement to human trials. Two Phase I trials (NCT02004717, NCT02252211) evaluated its efficacy in solid tumors, including EphA2-positive esophageal and gastric cancers. Among 37 enrolled patients, 14 demonstrated disease stabilization or partial response, and notably, no severe coagulation-related toxicities were reported. However, some patients developed grade ≥ 3 cytopenia, warranting further refinement of this therapeutic strategy [74,137].

Building upon these earlier experiences, newer drug conjugates employing bicyclic peptides rather than full-length antibodies have been developed to minimize adverse hematologic effects while preserving tumor specificity. One such example, BT5528, was designed as a bicyclic peptide-toxin conjugate with a high affinity for EphA2 while demonstrating reduced off-target interactions. In preclinical tumor models, this compound exhibited robust tumor suppression in an EphA2 expression-dependent manner without significant hematologic toxicity. A Phase I/II clinical trial (NCT04180371) is currently ongoing to assess the safety and efficacy of BT5528 in patients with advanced solid tumors, with early findings suggesting manageable toxicity and preliminary signals of clinical efficacy [93,94].

Beyond monoclonal antibody-based therapies, several small-molecule inhibitors have been explored for their potential to block EphA2-mediated oncogenic signaling. Among these, dasatinib, an ATP-competitive tyrosine kinase inhibitor originally developed for BCR-ABL-positive leukemia, has been evaluated in EphA2-positive malignancies, including breast cancer, lung cancer, and pancreatic adenocarcinoma. Various clinical studies have examined its efficacy in combination with other agents. A Phase I/II trial (NCT00566618) investigated dasatinib in patients with metastatic breast cancer with bone involvement. While tolerability was acceptable, overall response rates remained modest [143]. Similarly, in triple-negative breast cancer (TNBC), a Phase II study (CA180059) reported limited single-agent activity, suggesting that dasatinib alone may be insufficient in unselected patient populations [100]. Another Phase I/II study (NCT02954523) evaluated dasatinib in combination with osimertinib in patients with advanced non-small cell lung cancer (NSCLC), demonstrating some clinical benefit in a subset of patients [144]. Despite dasatinib's broad kinase inhibition, its ability to suppress EphA2-driven oncogenesis may be inherently constrained by its mechanism of action. Unlike direct EphA2 inhibitors, which aim to block both ligand-dependent and ligand-independent oncogenic signaling, dasatinib primarily targets kinase-dependent activity. Given that EphA2 signaling in cancer frequently operates independently of its kinase domain, alternative inhibitors with greater specificity for EphA2's oncogenic pathways may be necessary to enhance clinical benefit [145]. As a result, ongoing trials such as NCT03878524 and NCT02465060 are investigating biomarker-driven patient selection approaches to optimize therapeutic efficacy [65].

In addition to small-molecule inhibitors, RNA interference (RNAi)-based therapeutics have been investigated to silence EphA2 expression at the transcriptional level. One of the most notable efforts, EPHARNA, utilizes DOPC-liposomal siRNA encapsulation to facilitate targeted EphA2 gene silencing. Preclinical studies demonstrated significant tumor growth suppression and enhanced chemosensitivity when EphA2 siRNA was administered in combination with chemotherapy. A Phase I clinical trial (NCT01591356) was conducted in patients with advanced solid tumors to evaluate its safety and efficacy. While results from this study are yet to be fully disclosed, early findings suggest potential clinical utility [146].

Beyond conventional pharmacologic approaches, EphA2 has also been explored as an immunotherapeutic target due to its tumor-specific overexpression and potential role in immune evasion. One of the most promising strategies involves the use of chimeric antigen receptor (CAR)-T cell therapy to redirect cytotoxic T lymphocytes toward EphA2-

expressing tumor cells. A Phase I trial (NCT03423992) is currently underway to assess the feasibility and safety of EphA2-directed CAR-T therapy in malignant gliomas. Preclinical investigations demonstrated potent tumor regression following administration of EphA2-targeted CAR-T cells. However, the therapeutic efficacy of CAR-T therapy in solid tumors remains a challenge due to tumor heterogeneity and the immunosuppressive tumor microenvironment [147].

Given the complexity of EphA2 signaling and its broad expression across multiple tumor types, the development of precision medicine-based strategies is crucial for optimizing clinical outcomes. Several ongoing clinical trials are now integrating genomic and molecular profiling approaches to identify patient subgroups most likely to benefit from EphA2-targeted interventions. A more personalized approach to patient selection may help improve therapeutic efficacy and mitigate previous challenges associated with heterogeneous tumor responses [65]. While initial efforts to target EphA2 in cancer therapy faced considerable hurdles, newer therapeutic strategies-including next-generation ADCs, peptide-drug conjugates, small-molecule inhibitors, RNAi therapies, and CAR-T cell approaches-continue to advance in clinical trials. Future research should focus on refining patient selection criteria, optimizing drug formulations, and exploring combination treatment approaches to enhance clinical benefit while minimizing toxicity. Given EphA2's central role in tumor growth, metastasis, and immune regulation, its continued investigation remains an important avenue for the development of novel cancer therapies.

8. Conclusion and Future Perspectives

EphA2 has emerged as a promising target in cancer therapy due to its crucial role in tumor progression, metastasis, and resistance to conventional treatments. Its selective overexpression in multiple malignancies, while being limited in normal tissues, highlights its therapeutic potential. However, translating EphA2-targeted strategies into clinical success has proven challenging. Trials involving agents such as MEDI-547 and DS-8895a have faced safety concerns and limited efficacy, underscoring the complexity of EphA2-targeting approaches. The dual nature of EphA2 signaling—acting as both an oncogene and a tumor suppressor depending on cellular context—adds another layer of difficulty in designing effective therapies.

To advance EphA2-based treatments, several key areas require further exploration. First, a deeper understanding of the molecular mechanisms governing EphA2's ligand-dependent and ligand-independent activities is critical. This knowledge will aid in designing therapeutic agents that selectively inhibit oncogenic EphA2 signaling while preserving its tumor-suppressive functions. Recent advances in structural biology and computational modeling could facilitate the development of highly selective inhibitors with improved efficacy.

Second, enhancing drug delivery strategies is crucial for optimizing the therapeutic potential of EphA2-targeting agents. Innovations such as nanoparticle-based delivery systems, bicyclic peptides, and antibody-drug conjugates with enhanced stability and tumor specificity can significantly improve drug uptake while minimizing off-target effects. Personalized delivery platforms tailored to individual tumor profiles, such as siRNA-loaded nanocarriers, represent a promising avenue to maximize therapeutic efficiency.

Third, combinatorial treatment approaches incorporating EphA2-targeted therapies with existing clinical interventions may offer superior outcomes. For instance, pairing EphA2 inhibitors with immune checkpoint inhibitors (ICIs) like anti-PD-1/PD-L1 therapies could enhance antitumor immune responses. Similarly, combining EphA2-targeted therapies with chemotherapy or radiotherapy may exploit vulnerabilities in EphA2-overexpressing tumors, leading to improved clinical outcomes. Ongoing research into synergistic drug combinations will be essential for overcoming resistance mechanisms and expanding the therapeutic utility of EphA2 inhibitors.

Beyond these therapeutic strategies, patient stratification remains a critical factor in the clinical success of EphA2-targeted interventions. Tumor heterogeneity and EphA2's variable expression across different cancers necessitates biomarker-driven approaches to identify patient subgroups most likely to respond to these treatments. Advances in genomic profiling and imaging techniques could improve patient selection, leading to more tailored and effective therapies. Moreover, emerging therapeutic technologies such as PROTACs and molecular glues offer new avenues for targeting EphA2-driven malignancies. These approaches enable selective degradation of EphA2 and its oncogenic signaling complexes, providing a potential breakthrough for overcoming resistance mechanisms seen with traditional inhibitors.

While preclinical studies have demonstrated the potential of EphA2-targeted therapies, their clinical integration requires a strategic approach that aligns with existing standard-of-care treatments. EphA2-based therapies should not be viewed as standalone treatments but rather as part of a multi-faceted strategy that complements established oncology therapies. Given its involvement in multiple oncogenic pathways, EphA2 represents a valuable target for combination regimens. For example, targeting EphA2 alongside other well-established clinical targets, such as HER2, EGFR, or VEGFR, could enhance treatment efficacy in resistant tumors. Similarly, integrating EphA2 inhibitors into personalized treatment regimens guided by molecular profiling could improve therapeutic responses by selecting patients based on EphA2 expression levels and related oncogenic signatures.

Furthermore, EphA2's unique role in modulating the tumor microenvironment and immune evasion suggests that its inhibition could enhance the efficacy of immunotherapies. EphA2-targeted CAR-T cell therapies, dendritic cell vaccines,

and antibody-drug conjugates could be optimized in combination with immune checkpoint blockade to induce a more robust and sustained immune response. Despite the challenges associated with targeting EphA2, ongoing research continues to refine its therapeutic potential. The integration of novel drug formulations, optimized delivery mechanisms, combinatorial strategies, and precision medicine approaches will be critical for achieving meaningful clinical outcomes. By addressing these challenges, EphA2-targeted therapies have the potential to redefine cancer treatment paradigms and provide durable, personalized therapeutic solutions for patients with advanced malignancies.

Abbreviations

ADAMs: A Disintegrin and Metalloproteinase

ADCs: Antibody-Drug Conjugates

CAR-T: Chimeric Antigen Receptor T Cell

DCs: Dendritic Cells

EGFR: Epidermal Growth Factor Receptor

FXR: Farnesoid X Receptor

GPI: Glycosylphosphatidylinositol

ICIs: Immune Checkpoint Inhibitors

mAbs: Monoclonal Antibodies

MMPs: Matrix Metalloproteinases

MSNs: Mesoporous Silica Nanoparticles

PDGFA: Platelet-Derived Growth Factor A

PD-L1: Programmed Death-Ligand 1

PI3K: Phosphoinositide 3-Kinase

PKA: Protein Kinase A

PKC: Protein Kinase C

PROTACs: Proteolysis-Targeting Chimeras

RTKs: Receptor Tyrosine Kinases

S897: Serine 897

Competing Interests

The author declares no competing interests

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