Review

Modulation of Oncogenic NOTCH Signaling in Highly Aggressive Neoplasia by Targeting the G-Secretase Complex. A Systematic Review

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Modulation of Oncogenic NOTCH Signaling in Highly Aggressive Neoplasia by Targeting the G-Secretase Complex. A Systematic Review

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Abstract

Background. NOTCH receptors play a pivotal role in carcinogenesis. Upon ligand binding, a cascade of proteolytic cleavages mediated by ADAM proteases and γ-secretase complex activates the receptor, culminating in the release of the NOTCH intracellular domain (NICD). NICD translocates to the nucleus, where it modulates gene expression. γ-secretase inhibitors (GSIs) and ADAM secretases inhibitors have emerged as promising anticancer agents, with preclinical studies demonstrating their potential to disrupt tumor progression, cancer stem cell maintenance, and resistance to conventional therapies. **Methods**. A systematic search was made of the ISIWeb of Science (http://www.webofknowledge.com) for the relevant works published from 2015 to October 2025 in the scientific cancer field according to the Preferred Reporting Items for Systematic Review (PRISMA) guidelines and by using appropriated search terms and the exclusion/inclusion strategy. **Results**. We evaluated the therapeutic advances achieved through GSIs in highly aggressive cancers where NOTCH signaling is oncogenic: pancreatic ductal adenocarcinoma (PDAC), gastric adenocarcinoma (GC), non-small cell lung cancer (NSCLC), metastatic melanoma, and triple-negative breast cancer (TNBC). Although GSIs have entered clinical trials for PDAC, metastatic melanoma, and TNBC, their efficacy remains limited. However, combinatorial strategies involving GSIs, ADAM secretase inhibitors and other antitumor agents have shown promise in enhancing treatment outcomes and reducing side effects. **Discussion and Conclusions.** Future research should focus on identifying the specific NOTCH receptor(s) involved in each tumor type and tailoring therapies, including consideration of gender-based prevalence differences. Moreover, nanoparticle-based delivery systems and synergistic drug combinations may further improve the therapeutic index of GSIs and ADAM inhibitors while minimizing adverse effects.

Keywords: NOTCH; GSIs; PDAC; GC; NSCLC; TNBC; metastatic melanoma

1. Introduction

1.1. Global Cancer Epidemiology Overview

Cancer remains one of the leading causes of death worldwide. According to estimates from the International Agency for Research on Cancer (IARC – International Agency for Research on Cancer), based on the most reliable data available across countries in 2022, the global burden of cancer

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continues to grow. That year, there were approximately 20 million new cancer cases and 9.7 million cancer-related deaths (Global cancer burden growing, amidst mounting need for services). Looking ahead, the projected number of new cancer cases worldwide between 2022 and 2040, across both sexes, is expected to reach 29.9 million. During the same period, the estimated number of cancer-related deaths is anticipated to rise to 15.3 million.

According to the World Health Organization (World Health Organization (WHO)), and excluding non-melanoma skin cancers, the most frequently diagnosed tumors worldwide are breast cancer, followed by lung, colorectal, prostate, and stomach cancers [Available in Cancer]. In terms of mortality, the most lethal cancers, ranked in descending order, are lung, colorectal, liver, stomach, breast and pancreas cancer [Available in Cancer Today and Global Cancer Observatory] (Figure 1).

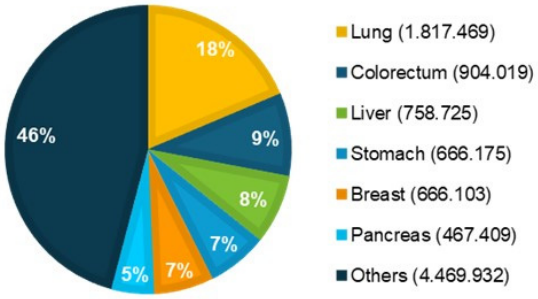

Figure 1. Absolute numbers of cancer-related deaths worldwide in 2022, for both sexes and all age groups. Source: Global Cancer (Global Cancer Observatory).

The molecular and cellular mechanisms driving cancer development across different tissues and organs are highly complex. Intensive global research efforts, encompassing both basic and clinical studies, are focused on elucidating these mechanisms. Such investigations are essential for the development of novel therapeutic strategies that are not only more effective but also tailored to individual patients, with reduced adverse effects.

Among the many intracellular signaling pathways implicated in cancer, the NOTCH receptor signaling pathway stands out due to its pivotal role in the initiation and progression of various neoplasms. Aberrant NOTCH signaling contributes to several hallmark features of cancer and is particularly associated with the emergence of highly aggressive tumors with poor prognosis [1]. Moreover, the NOTCH pathway forms an intricate network of interactions with other key signaling pathways involved in oncogenesis, further driving tumor development and progression in multiple malignancies.

1.2. Structure of NOTCH Receptors and Their Ligands

NOTCH receptors and their ligands constitute a highly conserved protein family across evolution. From a biological standpoint, the NOTCH receptor signaling pathway acts as a key regulator in cell fate decisions, controlling cellular proliferation and differentiation, senescence, or apoptosis of progenitor and stem cells, among other biological processes [2]. Its function is critical in numerous cellular contexts such as neurogenesis, angiogenesis, hematopoiesis, epithelial morphogenesis, and the specification of cell lineages in organs like the digestive system, lungs, skin, immune system, and in cancer [2,3]. The NOTCH signaling pathway also plays a central role in maintaining the identity and plasticity of adult stem cells and in establishing spatial and temporal patterns of cell differentiation. Furthermore, its involvement in phenomena such as epithelial-mesenchymal transition (EMT) and its interaction with other intracellular signaling pathways reinforces its importance in many complex biological processes in both healthy and pathological states, such as cancer [3,4]. In mammals, the main members of this protein family, both receptors and ligands, are differentially expressed depending on the cell type, physiological context, or tumor microenvironment, participating in essential cell-to-cell interactions that modulate cell fate [5–7].

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In mammals, unlike in Drosophila melanogaster, where the first Notch gene was discovered [8–10], there are four NOTCH receptors: NOTCH1, NOTCH2, NOTCH3, and NOTCH4 (Figure 2) [2,8,11–13].

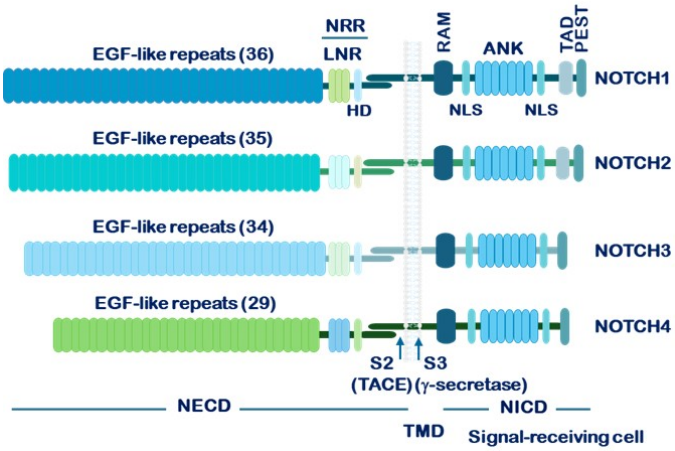

Figure 2. Structure of NOTCH receptors in mammals. The four NOTCH receptors differ primarily in the number of EGF-like repeats, with NOTCH1 and NOTCH2 containing the highest number (36 and 35 repeats, respectively). The EGF-like repeats 11–12 of NOTCH receptors interact with the DSL (Delta/Serrate/LAG2) domain of canonical activating ligands. The Negative Regulatory Region (NRR) consists of three LNR repeats and a heterodimerization (HD) domain. The NOTCH intracellular domain (NICD) includes the RAM domain, ankyrin repeats (ANK), nuclear localization signals (NLS), and the PEST domain. The S2 (Ala1710- Val1711) and S3 (Gly1753-Val1754) sites represent the proteolytic cleavage points involved in receptor processing and activation at the plasma membrane upon ligand interaction.

NOTCH1 and NOTCH2 are broadly expressed across adult tissues, while NOTCH3 is predominantly found in vascular smooth muscle, and NOTCH4 is mainly expressed in endothelial cells [2,5–8,13]. NOTCH receptors are heterodimeric transmembrane proteins composed of three main regions: an extracellular domain (NECD), a transmembrane domain (TMD), and an intracellular domain (NICD) [4,14–16]. The NECD contains multiple EGF-like repeats (epidermal growth factor-like), the number of which varies depending on the receptor subtype. Adjacent to the transmembrane domain, the NECD also includes a segment known as the negative regulatory region (NRR), composed of Lin12/NOTCH repeats. The active NICD region includes several functional domains: a RAM domain for protein binding, seven ankyrin repeat domains, nuclear localization signals, a transcriptional activation domain (TAD), and a PEST domain involved in protein degradation. There are five canonical activating ligands of NOTCH receptors: Delta-Like 1 (DLL1), Delta-Like 3 (DLL3), and Delta-Like 4 (DLL4), three homologs of the Drosophila Delta ligand, and Jagged1 (JAG1) and Jagged2 (JAG2), which are homologs of the Drosophila Serrate ligand [13,17–21] (Supplementary Figure 1). These ligands are transmembrane proteins, and their interaction with NOTCH receptors requires direct intercellular contact. Specifically, the binding occurs between specific EGF-like repeats on the NOTCH receptors and the DSL (Delta/Serrate/LAG2) domain present in the canonical ligands. In addition to canonical activating ligands, several non-canonical ligands with inhibitory functions have been identified, including DLK1 and DLK2 (Supplementary Figure 2), as well as DNER and EGFL7 [22–28]. DLK1 and DLK2 proteins possess six EGF-like repeat sequences in their extracellular region, a transmembrane region, and a short intracellular region [22,29]. The extracellular region of DLK1 is recognized by the TACE protease, which processes DLK1 and releases a soluble form containing the extracellular region [30–33]. DLK2 might be processed similarly to DLK1, although there is no evidence to confirm or refute this possibility. Despite lacking the DSL domain that canonical ligands possess at the N-terminal end, DLK1 and DLK2 can interact with NOTCH receptors through their N-terminal DOS domains, and function as non-canonical inhibitory ligands, competing with canonical ligands [23,34–36]. Regarding their tissue distribution, DLK proteins also show

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differences. DLK1 expression in adults is limited to the adrenal glands, while DLK2 has a broader expression in adults, being mainly expressed in the skin, prostate, esophagus, brain, and salivary glands, although its expression is not remarkably high [37,38].

1.3. Mechanism of NOTCH Receptor Activation and Downstream Signaling

To understand the functional dynamics of NOTCH receptors, it is essential to recognize that these receptors autonomously initiate a signaling cascade that culminates in the regulation of gene expression within the nucleus [1]. Activation of NOTCH receptors involves three sequential proteolytic cleavages at conserved sites known as S1, S2, and S3–S4 [4,7,39–41]. The first cleavage occurs during receptor maturation in the Golgi apparatus, where the enzyme Furin processes the receptor at the S1 site. This event generates a heterodimer composed of an extracellular domain, a transmembrane, and an intracellular domain, held together by non-covalent interactions [42,43]. Once the receptor is transported to the plasma membrane, it remains inactive due to the presence of the Negative Regulatory Region (NRR).

Activation is triggered by the interaction of a canonical ligand, expressed on the membrane of a neighboring cell, with the NOTCH receptor. This ligand–receptor binding exposes the S2 cleavage site, allowing ADAM10 or ADAM17 secretases to cleave the receptor. As a result, most of the extracellular domain is endocytosed by the ligand-expressing cell [42,43] (Figure 3). This S2 cleavage is the only ligand-dependent step and represents a critical event in the canonical NOTCH signaling pathway. Following S2 cleavage, the remaining membrane-bound fragment, known as NEXT (NOTCH extracellular truncation), undergoes further processing at the S3–S4 sites by the γ-secretase complex. This final cleavage releases the active NOTCH intracellular domain (NICD), which translocates to the nucleus [7,44–47].

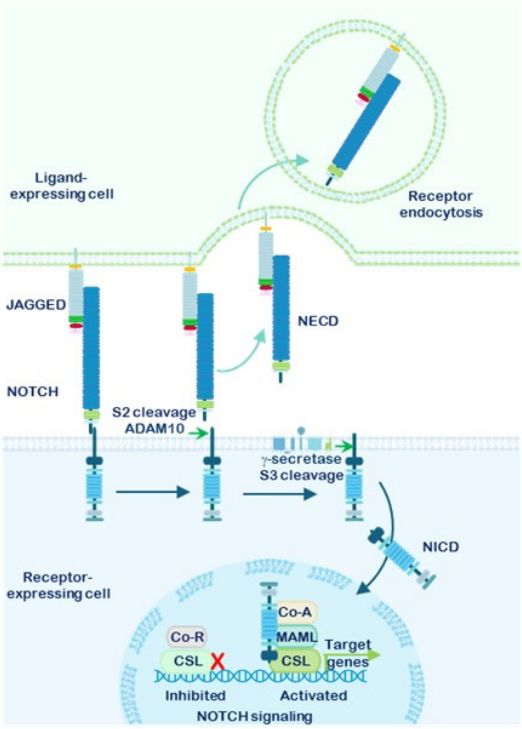

Figure 3. Canonical NOTCH receptors signaling pathway. This figure depicts the sequential proteolytic processing of a NOTCH receptor at cleavage sites S2 and S3–S4, culminating in the release of the active form, NICD (NOTCH intracellular domain). Once released, NICD translocates to the nucleus, where it interacts with transcriptional regulators to activate gene expression. Abbreviations: Co-R: co-repressor; Co-A: co-activator; CSL: CSL/RBPJκ transcription factor (CBF1/Suppressor of hairless/Lag1/Recombination signal binding protein-J); MAML: Mastermind-like proteins.

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Within the nucleus, NICD binds to the transcription factor CSL/RBPJκ (C-promoter-binding factor, CBF1 (in mammals; also known as Recombination Signal Binding Protein Jκ, RBPJκ)/Suppressor of hairless (in *Drosophila melanogaster*) /Lag1 (in *Caenorhabditis elegans*)) along with Mastermind-like (MAML) proteins and other cofactors. This complex displaces transcriptional repressors and forms the NOTCH Transcriptional Complex (NTC), which activates the expression of target genes, including members of the *Hes* and *Hey* families (Hairy and Enhancer-of-Split) [4,39,48–50]. These transcriptional responses define the canonical NOTCH signaling pathway.

In addition to the canonical pathway, NOTCH signaling can also proceed via a non-canonical route, in which the NICD interacts with other intracellular proteins such as NF-κB, the serine-protein kinase ATM, or RAC1 (RAS-related C3 botulinum toxin substrate 1), among many others [51].

1.4. Role of NOTCH Receptors and NOTCH Ligands in Carcinogenesis

Abnormalities in the NOTCH receptor signaling pathway are well-established contributors to carcinogenesis. Depending on the context, aberrant

NOTCH activation can promote tumor initiation and progression, functioning as an oncogene, or conversely, its inactivation can lead to tumor development, acting as a tumor suppressor [1,51–53]. The dual role of NOTCH signaling is highly dependent on the specific receptor involved and the tumor type. For instance, NOTCH1 may act as an oncogene in certain hematological malignancies, while serving a tumor-suppressive role in skin cancers. Despite extensive research, the molecular mechanisms underlying these opposing effects remain poorly understood. Elucidating these mechanisms is critical for the development of targeted anticancer therapies. Such therapies would aim to selectively inhibit the NOTCH receptor exhibiting oncogenic activity in a specific tumor type or counteract its role in mediating resistance to conventional chemotherapeutic agents.

NOTCH receptors can contribute to neoplastic transformation through three distinct mutational patterns [1]. The first mechanism involves chromosomal translocations that eliminate the Negative Regulatory Region (NRR), leading to constitutive activation of the receptor. This mechanism has been identified in triple-negative breast cancer. The second pattern consists of mutations in the PEST domain, which impair NICD degradation and prolong its activity. The third pattern includes mutations in the N-terminal region of the receptor, commonly observed in squamous cell carcinomas such as esophageal and lung cancers [3,52,54].

Beyond point mutations, two additional mechanisms can contribute to NOTCH receptor dysregulation: gene amplification and chromosomal rearrangements [1]. Gain-of-function alterations in NOTCH signaling have been associated with several cancers, including breast cancer and non-small cell lung cancer (NSCLC) [1]. Conversely, loss-of-function mutations, where NOTCH acts as a tumor suppressor, are primarily linked to squamous cell carcinomas of the esophagus, and lung [51] (Figure 4). In triple-negative breast cancer, activation of NOTCH1 has been associated with increased tumor aggressiveness, in contrast to NOTCH2–4, which may play more protective roles [55]. In highly aggressive cancers, the *Int3* oncogene, a truncated form of the NOTCH4 receptor, has also been implicated in tumorigenesis [3,56].

Beyond its role as an oncogene or tumor suppressor, NOTCH signaling plays a critical role in different processes that lead to the generation and development of the tumors (Supplementary Figure 3). NOTCH signaling plays a role in the tumor microenvironment, particularly in the maintenance of cancer stem cells [1]. For example, in gastric cancer, overactivation of NOTCH signaling has been observed in tumor stem cell populations [57]. Increasing evidence has shown that the activation of the γ-secretase/NOTCH pathway is a key driver of drug resistance development. In breast cancer, NOTCH signaling induces cell cycle arrest in stem cells, promoting the emergence of chemoresistance [1]. NOTCH activity has also been linked to protection against drugs targeting the estrogen receptor or the MAPK pathway in certain melanomas and breast cancers [3].

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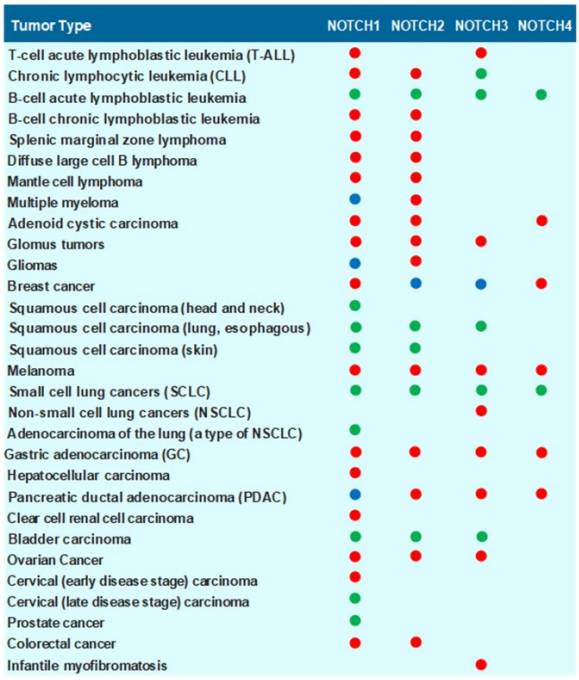

Figure 4. NOTCH signaling in human cancer. Various cancer types are classified based on whether NOTCH functions predominantly as an oncogene or as a tumor suppressor.

NOTCH signaling is also involved in tumor infiltration and metastasis, particularly through its role in the epithelial–mesenchymal transition (EMT). During EMT, NOTCH activation leads to the downregulation of cell adhesion proteins such as E-cadherin, facilitating tumor cell migration and invasion [1]. Additionally, NOTCH receptors contribute to immune evasion, metabolic reprogramming, apoptosis inhibition, and tumor-associated inflammation [58].

In the context of angiogenesis, NOTCH ligands exhibit opposing roles. The canonical ligand DLL4 suppresses angiogenic sprouting, whereas JAG1 promotes angiogenesis, tumor growth, and the maintenance of cancer stem cells. In pancreatic ductal adenocarcinoma (PDAC), NOTCH overactivation has been linked to enhanced angiogenic sprouting [59]. Elevated JAG1 expression has also been reported in breast, lung, and pancreatic cancers [60].

Among non-canonical NOTCH ligands, DLK1 (Delta-like homolog 1) is minimally expressed in adult tissues but exhibits elevated levels in various neoplasms, where it appears to contribute to the maintenance of cancer stem cells [61]. In metastatic melanoma, NOTCH signaling plays an oncogenic role, and both DLK1 and DLK2 have been shown to promote tumor formation in nude mice in a dose-dependent manner by inhibiting NOTCH signaling [62]. In triple-negative breast cancer, high DLK1 expression has been associated with reduced tumor progression, whereas low DLK1 levels correlate with increased tumor size *in vivo*. Conversely, low DLK2 expression enhances tumor aggressiveness, while high DLK2 levels have been shown to prevent tumor formation in nude mice [63,64]. These findings suggest that DLK1 and DLK2 exert context-dependent regulatory effects on NOTCH signaling and tumor behavior, highlighting their potential as therapeutic targets in specific cancer subtypes.

1.5. Strategies for the Inhibition of NOTCH Receptor Signaling

Extensive evidence supports the hypothesis that NOTCH signaling is one of the most promising therapeutic targets in cancer treatment. In recent years, various pharmacological strategies have been developed to inhibit this pathway [65–67]. Key approaches to disrupting NOTCH receptor signaling include monoclonal antibodies (mAb) that block the receptors or their canonical and non-canonical ligands; peptides that interfere with the transcriptional activation complex; inhibitors of the α-secretases ADAM10 and ADAM17; and γ-secretase inhibitors (GSIs), which prevent the final

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proteolytic processing of NOTCH receptors. GSIs specifically target the γ-secretase complex, thereby blocking the release of the active NOTCH intracellular domain (NICD) (Figure 5).

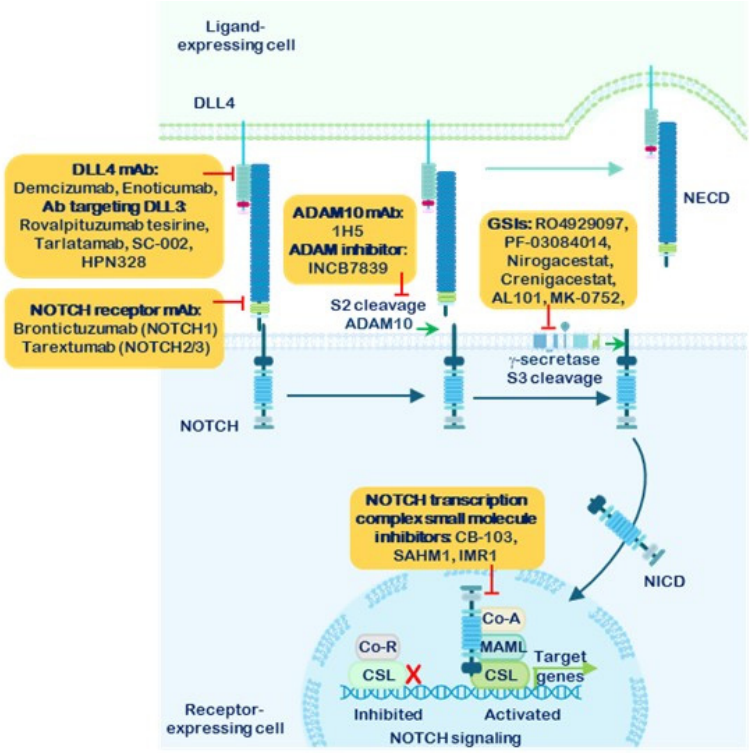

Figure 5. Strategies for Inhibiting NOTCH Receptor Signaling. Figure 5 illustrates several therapeutic strategies designed to modulate NOTCH signaling. These include immunological approaches using antibodies that target NOTCH receptors and their ligands, chemical inhibition of ADAM proteases and the γ-secretase complex, and disruption of transcriptional regulators such as CSL (CBF1/Suppressor of Hairless/LAG-1, also known as RBP-Jκ) and Mastermind-like proteins. Collectively, these interventions aim to suppress the transcription of NOTCH target genes.

1.6. The γ-Secretase Complex and γ-Secretase Complex Inhibitors (GSIs)

The γ-secretase complex (GSC) is a member of the intramembrane-cleaving proteases (I-CLiPs) family [45]. It plays a central role in the proteolytic processing of several substrates, including NOTCH receptors, the β-amyloid precursor protein, whose cleavage generates the β-amyloid peptide implicated in the formation of senile plaques in Alzheimer's disease [65], and E-cadherin, a key molecule in cell adhesion [68]. Beyond its proteolytic functions, the GSC also participates in non-proteolytic processes such as calcium homeostasis, autophagy, and apoptosis [69], earning it the nickname "Transmembrane Cellular Proteasome".

The GSC is composed of several transmembrane subunits (Figure 6): Presenilin (PS), with two isoforms (PS1 and PS2), is responsible for the catalytic activity of the complex [69,70]; Nicastrin (NCT) acts as a scaffolding protein that recognizes and binds substrates targeted for cleavage; Anterior pharynx defective 1 (APH-1), which exists in multiple isoforms, contributes to complex stabilization [71]; and Presenilin enhancer 2 (PEN-2) is involved in the heterodimerization of presenilin and the maturation of nicastrin.

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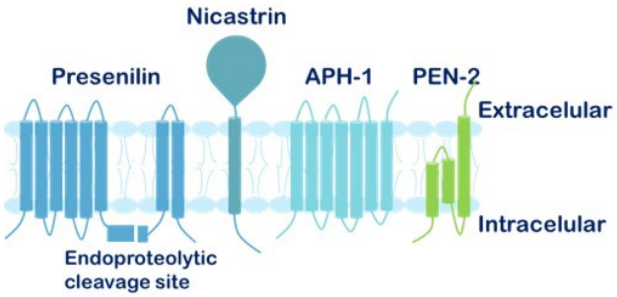

Figure 6. Schematic representation of the γ-secretase complex (GSC). This diagram illustrates the structural organization of the γ-secretase complex, with each component traversing the membrane multiple times. Presenilin, the catalytic core of the complex, is depicted as comprising two subunits. Each subunit contains a critical aspartate residue (indicated by stars), which is essential for the proteolytic activity of the enzyme.

γ-secretase inhibitors (GSIs) were initially developed as therapeutic agents for Alzheimer's disease [65]. However, their clinical use was discontinued due to limited efficacy and significant adverse effects, including the emergence of non-melanoma skin tumors. Interest in GSIs was later rekindled in the field of oncology, driven by the role of NOTCH receptors in tumorigenesis. Currently, extensive research is underway to evaluate GSIs as potential anti-cancer agents. Both preclinical and clinical studies have explored their ability to suppress tumor progression by targeting NOTCH signaling pathways *in vitro* and *in vivo*. Moreover, GSIs may hold promise in overcoming chemotherapy resistance by modulating the γ-secretase/NOTCH axis. In addition, GSIs are being investigated as candidates for therapies targeting cancer stem cells (CSCs), which are characterized by slow proliferation and resistance to conventional chemotherapy and radiotherapy, factors that contribute to treatment failure and disease recurrence. Eradicating CSCs is considered a key strategy for achieving long-term cancer remission. Studies have shown that GSIs, when combined with other anticancer agents, exert a stronger inhibitory effect on CSCs [72].

It is important to note that GSIs exhibit differential inhibition profiles across various NOTCH substrates. In some cases, GSIs enhance the cleavage of certain NOTCH substrates at concentrations that inhibit NOTCH1 cleavage [73]. This paradoxical effect may result from the direct action of low GSI concentrations on γ-secretase itself. Nonetheless, numerous side effects have been reported in clinical trials, largely due to GSIs' broad activity across multiple substrates and biological processes.

2. Methods

A systematic review was carried out according to the Preferred Reporting Items for Systematic Review (PRISMA) guidelines [74]. The systematic search was made of the ISIWeb of Science (http://www.webofknowledge.com) for the relevant works published until October 2025 in the scientific cancer field. The keywords used for the search included: "GSI", "NOTCH signaling", "Gamma-secretase complex", "DLK1", "DLK2", "Cancer", "therapy", "Pancreatic adenocarcinoma (PDAC)", "Triple Negative Breast Cancer (TNBC)", "Metastatic melanoma", "Non-small cell lung cancer (NSCLC)", and "Gastric Cancer (GC)". After completing the search, the articles were filtered based on publication date and relevance to the topic, following the inclusion and exclusion criteria outlined by the PRISMA methodology.

Regarding the inclusion criteria, selected articles had to be preclinical studies conducted *in vitro* or *in vivo* using mice or other animal models, or clinical trials in humans investigating the use of GSIs as monotherapy or in combination with other antitumor drugs targeting the cancers under study. As for the exclusion criteria, articles published before 2015 were discarded. However, we include articles and reviews published before 2015 related to Introduction Section contents. Articles not originally written in English were also excluded. In terms of thematic relevance, studies focusing on the use of GSIs as therapy for Alzheimer's disease or other non-cancer pathologies were excluded, as were those

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investigating alternative therapeutic targets within the NOTCH signaling pathway that did not involve GSIs directly (Figure 7).

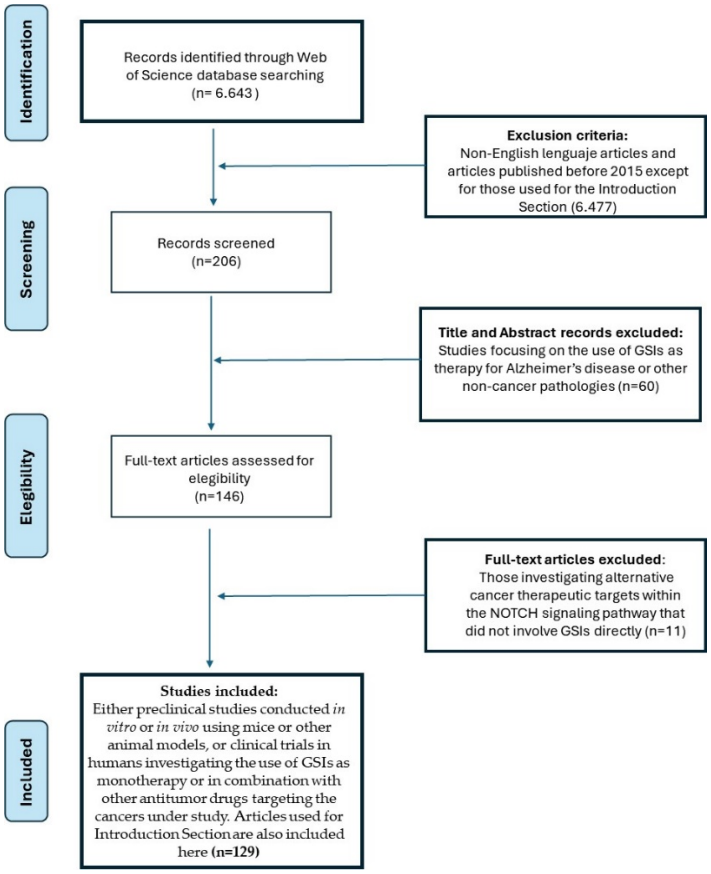

Figure 7. Flow chart diagram. Visualization of the database searches, number of publications identified and screened, and final full texts included in the present systematic review. Exclusion and inclusion criteria are indicated.

3. Results

The following sections of this review highlight recent progress in GSI-based therapies for aggressive malignancies with poor prognosis and limited survival rates, such as pancreatic and gastric cancers, metastatic melanoma, triple-negative breast cancer, and non-small cell lung cancer. The revision includes findings from preclinical studies using cell lines and animal models, as well as clinical trials involving human participants. Furthermore, it explores combination strategies in which GSIs are paired with established anti-tumor agents to enhance therapeutic efficacy and mitigate adverse effects stemming from their non-specific mechanisms of action.

3.1. The Combination of GSIs with Other Therapeutic Agents Has Demonstrated Efficacy in Reducing Pancreatic Ductal Adenocarcinoma (PDAC) Progression in Preclinical Studies

The development of pancreatic ductal adenocarcinoma (PDAC) is known to involve the progression of a precursor lesion termed pancreatic intraepithelial neoplasia (PanIN). A critical genetic alteration frequently identified in PanIN is the mutation of the KRAS gene, which appears to be indispensable for both the malignant transformation of pancreatic epithelial cells within PanIN and their subsequent progression to PDAC [75].

Regarding NOTCH signaling, current evidence indicates that it may exert dual functions, either tumor-suppressive or oncogenic, depending on the stage of tumor development [76]. Studies using mouse models have shown that the coactivation of KRAS and NOTCH1 markedly increases the number of PanIN lesions [76,77]. These findings support the hypothesis that NOTCH signaling may

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inhibit PanIN formation during the early phases of tumorigenesis, while facilitating its progression in later stages.

Cook and colleagues evaluated the γ-secretase inhibitor MRK003 in pancreatic ductal adenocarcinoma (PDAC) xenograft models, both as a monotherapy and in combination with gemcitabine. Their results indicated that MRK003 alone did not elicit significant therapeutic effects; however, its combination with gemcitabine led to a substantial improvement in mouse survival [59]. Treatment with MRK003 was associated with increased necrosis of neoplastic tissue, enhanced apoptosis, and reduced cellular proliferation. One of the principal mechanisms attributed to MRK003 was its capacity to inhibit intratumoral vascular proliferation, observed both as a standalone treatment and in combination therapy. This vascular suppression induced hypoxic conditions within the tumor microenvironment, thereby potentiating the efficacy of the therapeutic agents. In a separate study, Mizuma and colleagues similarly demonstrated that the combination of MRK003 and gemcitabine effectively impeded tumor progression in PDAC mouse models [78].

Palagani and colleagues explored the therapeutic potential of combining GSI-IX with AG-490, a Janus Kinase 2 (JAK2) inhibitor that blocks activation of STAT3, signaling molecules implicated in PDAC pathogenesis. Using mouse models exhibiting pancreatic intraepithelial neoplasia (PanIN) and acinar-to-ductal metaplasia (ADM), they tested each compound as monotherapy. After six weeks, small tumors and microscopic foci of PDAC were observed. Remarkably, in the group treated with the combination of GSI-IX and AG-490, none of the five mice developed visible tumors, indicating a synergistic therapeutic effect [79].

The γ-secretase inhibitor PF-03084014 has shown promising results in a phase III clinical trial for desmoid tumors (aggressive fibromatosis), rare, non-malignant connective tissue growths that are locally invasive and prone to recurrence [80]. In the context of PDAC, Yabuuchi and colleagues assessed PF-03084014 in various xenograft mouse models, both as monotherapy and in combination with gemcitabine [77]. While PF-03084014 alone did not significantly inhibit tumor proliferation, its combination with gemcitabine resulted in notable antiproliferative effects and tumor regression. Flow cytometry analyses revealed that gemcitabine alone failed to eliminate tumor stem cells and even appeared to increase their prevalence. In contrast, PF-03084014, both as monotherapy and in combination, reduced the population of tumor stem cells. Moreover, the combination therapy significantly decreased distant metastasis, suggesting a broader impact on tumor aggressiveness and dissemination [77].

It is worth highlighting the development of the γ-secretase inhibitor (GSI) MRK-0752, which was evaluated by Cook and colleagues in a phase I clinical trial in combination with gemcitabine in patients with stage IV pancreatic ductal adenocarcinoma (PDAC). In this study, 14 out of 44 patients achieved disease stabilization. However, the outcomes were comparable to those observed with gemcitabine monotherapy [81]. Notably, several adverse effects associated with GSI treatment were reported, including gastrointestinal disturbances, thrombocytopenia, and anemia.

Finally, RO4929097 GSI was used in a clinical trial phase II but the trial could not be completed because GSI synthesis was discontinued [82].

3.2. Treatment Resistance in Non-Small Cell Lung Cancer (NSCLC) Can Be Mitigated Through the Application of γ-Secretase Inhibitors Alone and Combined with Other Drugs

NOTCH signaling plays a critical role in the pathogenesis of non-small cell lung cancer (NSCLC). NOTCH3 overexpression has been reported in approximately 40–50% of cases (23), while elevated NOTCH1 levels are associated with poor prognosis and tumor initiation, particularly in KRAS-driven models [83]. Interestingly, in the squamous subtype of NSCLC, NOTCH signaling appears to be suppressed, suggesting a potential tumor-suppressive function in this context [84,85].

Liu and colleagues demonstrated *in vitro* that cisplatin treatment of NSCLC cell lines led to the enrichment of $CD133^+$ and $ALDH^+$ cells, markers of lung cancer stem cells (LCSCs) linked to chemoresistance [86]. Pre-treatment with the γ-secretase inhibitor DAPT significantly inhibited the selection of these stem-like cells and reduced cisplatin resistance. Treatment with GSI-34 of a

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CD166^{+}Lin^{-} subpopulation with LCSC characteristics and intrinsic resistance to cisplatin in xenograft mouse models sensitized these cells to cisplatin, resulting in reduced tumor size, with the most pronounced effect observed when both agents were combined [83].

Xie and colleagues investigated strategies to overcome resistance to gefitinib, an EGFR inhibitor, in NSCLC. Using gefitinib-resistant cell lines, they applied GSI BMS-708163 and found that high doses reversed resistance. In 3D cultures, treated cells formed significantly smaller colonies, with enhanced effects when combined with gefitinib. *In vivo* experiments in xenograft models confirmed that the combination significantly inhibited tumor growth [87].

BMS-906024 GSI was analyzed both as monotherapy and in combination with radiotherapy (RT). While no significant reduction in cell proliferation was observed in 2D cultures, combining BMS-906024 with paclitaxel or crizotinib, either with or without RT, led to a marked decrease in proliferation. In 3D cultures, BMS-906024 alone reduced spheroidal growth, with even greater effects when combined with crizotinib or RT. The most pronounced reduction was achieved when all three treatments were used together [85]. The use of BMS-906024 GSI in xenograft models demonstrated also enhanced cytotoxicity when combined with paclitaxel, particularly in tumors harboring KRAS or BRAF mutations. This effect was linked to mutant or null p53 status, contrasting with other studies that associated NOTCH1 activity with p53 expression [84,88].

Mizugaki and colleagues found that NSCLC cell lines exposed to RT exhibited increased expression of NOTCH1 and NOTCH3 at 48 hours. Treatment with GSI-I or GSI-XX in combination with RT led to higher levels of apoptosis compared to RT alone. In xenograft models, the combination of RT and GSI-XX significantly delayed tumor growth relative to either treatment alone [89].

Recently, evodiamine (EVO), a low toxicity natural alkaloid, has emerged as a potential antitumor agent for NSCLC [90]. EVO reduced cell proliferation and metastasis and lowered expression of NOTCH3 and GSC. Although EVO did not bind directly to NOTCH3, it showed strong affinity for GSC, comparable to that of GSIs.

3.3. The Use of GSIs, ADAM Inhibitors and Other Combined Therapies Have Contributed to Elucidating the Role of NOTCH Signaling in Gastric Cancer (GC)

NOTCH signaling has been firmly established as a key regulator of gastric stem cell proliferation, differentiation, and maintenance. Moreover, it plays a role in glandular fission, a process in which a glandular unit is divided into two due to elevated cell proliferation, thereby contributing to tumor development. NOTCH signaling has also been implicated in the initiation of oncogenesis through crosstalk with other pathways and is associated with increased levels of CD44^{+} and CD133^{+} cells, which are recognized markers of gastric cancer stem cells (GCSCs) [57,91].

CD133^{+} gastric cancer stem cells (GCSCs) exhibited low RECK protein expression, a cysteine-rich protein with Kazal motifs known for its protease activity that inhibits metastasis and angiogenesis, and high NOTCH1 levels. RECK suppress ADAM-mediated NOTCH1 activation. Moreover, by using the γ-secretase inhibitor DAPT, they demonstrated that NOTCH signaling was oncogenic in these tumors, with DAPT reducing the formation of GCSC-rich spheres by 25% compared to the control group [92].

CD44^{+} GCSCs, which showed elevated NOTCH1 expression and increased resistance to 5-fluorouracil (5-FU) were compared to CD44^{-} cells. Treatment with DAPT selectively affected CD44^{+} cells, leading to reduced self-renewal, diminished tumor initiation and migration, and enhanced sensitivity to 5-FU. In xenograft models, intraperitoneal administration of DAPT significantly inhibited tumor growth and epithelial–mesenchymal transition (EMT) [93].

Barat and colleagues also studied CD44^{+} cells, hypothesizing a functional link between NOTCH1 and the WNT/β-catenin pathway, another key signaling axis in gastric carcinoma. Treatment with GSI-IX produced dose- and time-dependent reductions in cell proliferation, migration, invasion, and tumor sphere size, along with increased apoptosis. Treated cells showed decreased levels of active NOTCH intracellular domain 1 (NICD1) and WNT signaling components. Similar results were

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observed in xenograft mice, with reduced tumor growth and increased necrosis in the GSI-IX-treated group [94].

NOTCH signaling and PTEN, a tumor suppressor gene frequently inactivated in gastric cancer (GC), seem to be related. Treatment of GC cells with GSI-I led to reduced tumor activity and increased PTEN expression compared to controls [95]. In xenograft models, the combination of GSI-I and paclitaxel resulted in significantly greater tumor growth inhibition than either agent alone. Lee and colleagues further evaluated GSI-I in GC cell lines and xenograft mice, confirming its efficacy and lack of significant side effects. When combined with 5-FU, both *in vitro* and *in vivo*, the dual treatment produced markedly greater tumor suppression than monotherapy [96].

Yao and colleagues observed that DAPT treatment increased ERK1/2 phosphorylation in GC cells, suggesting that NOTCH signaling may suppress this oncogenic kinase. Combining DAPT with PD98059, an ERK1/2 MAPK inhibitor, led to reduced tumor growth and increased apoptosis *in vitro*, with similar synergistic effects observed in xenograft models [97].

Finally, a combination of DAPT and an anti-DLL4 antibody was tested in GC cell lines and xenograft mice. This dual approach significantly enhanced apoptosis, reduced cell invasion, and decreased tumor size compared to control treatments [98].

3.4. GSIs Enhance the Efficacy of Targeted Therapies in Phase II Clinical Trials for Metastatic Melanoma

NOTCH signaling has also been implicated in the oncogenic potential of melanoma stem cells (MSCs). Kumar and colleagues associated $CD133^+$ melanoma cells with MSCs and demonstrated, both *in vitro* and *in vivo*, enhanced proliferation, angiogenesis, epithelial–mesenchymal transition (EMT), metastasis, and chemoresistance. These cells exhibited overactivation of NOTCH1, and treatment with GSIs (GSI-IX and GSI-X) significantly reduced the number of $CD133^+$ MSCs, leading to decreased migration and interaction with vascular endothelium [99].

Approximately half of metastatic melanomas harbor mutations in the BRAF gene, for which targeted therapies using BRAF inhibitors (BRAFi) and MEK inhibitors (MEKi) are available. Zhu and colleagues explored the role of NOTCH signaling in acquired resistance to BRAFi. They found that combining DAPT with BRAFi reversed resistance in melanoma cell lines, suggesting that NOTCH signaling enables BRAFi-treated cells to escape senescence, a state that was re-induced by DAPT treatment [100]. Porcelli and colleagues tested the combination of MEKi and the GSI nirogacestat (PF-03084014), observing enhanced inhibition of cell proliferation and migration *in vitro* compared to monotherapy [101].

NOTCH signaling also appears to contribute to resistance against other targeted therapies. Krepler and colleagues combined the GSI RO4929097 with an ERK inhibitor (ERKi), finding that in ERKi-resistant cell lines, the combination significantly reduced cell viability and increased apoptosis compared to either agent alone. In xenograft models, the combination also produced superior tumor growth inhibition [102].

The combination of GSI-I with BCL-2 inhibitors (BCL2i) demonstrated greater efficacy than monotherapy in both melanoma cell lines and xenograft mice. The treatment increased apoptosis and reduced the population of $ALDH^+$ MSCs [103].

Nueda and colleagues studied the dose-dependent effects of DAPT on metastatic melanoma cell lines. High doses reduced proliferation, while low doses paradoxically increased it [104]. Nueda and colleagues investigated the combination of DAPT with high expression levels of DLK1 or DLK2, finding synergistic inhibition of NOTCH signaling and reduced proliferation of metastatic melanoma cells. Keyghobadi and colleagues further observed that prolonged DAPT treatment led to increased tumor growth *in vitro* and *in vivo* [105].

Among GSIs, RO4929097 is the only one to have entered clinical trials for metastatic melanoma. Tolcher and colleagues conducted a phase I trial in patients with metastatic or locally advanced tumors to determine the maximum tolerated dose (MTD), adverse effects, and preliminary efficacy. A total of 110 patients were enrolled and divided into three dosage groups (A, B, and C). Drug levels in all patients exceeded the threshold for antitumor activity. The most common adverse effects were

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gastrointestinal, with some cases of hypophosphatemia (Figure 9). Notably, one patient with melanoma showed a minor response, and 33% and 41% of patients in groups A and B, respectively, achieved disease stabilization [106,107].

Building on earlier findings, Lee and colleagues conducted a phase II clinical trial to assess the efficacy and tolerability of RO4929097 GSI in patients with metastatic melanoma [107]. Among the 32 patients enrolled, most adverse effects were grade 1 or 2, with only six patients experiencing grade 3 toxicities, including hypophosphatemia. In terms of clinical response, one patient achieved a partial response lasting seven months and survived for over 28 months. Additionally, eight patients reached a stable disease state. However, overall efficacy was limited: the disease control rate at 12 weeks was 31%, the median progression-free survival (PFS) was 1.5 months, and the 6-month PFS rate was 9%. The 1-year survival rate was 50%, with a confidence interval of 23%–66%. The authors attributed the modest outcomes to subtherapeutic drug levels.

More recently, Jayaprakash and colleagues revisited the use of RO4929097 in melanoma, identifying a potential synergistic effect at low doses when combined with radiotherapy (RT). *In vitro* studies also showed reduced cell migration with this combination [108].

3.5. Various Clinical Studies Explore the Use of GSIs as Monotherapy and in Combination Therapies for Triple-Negative Breast Cancer (TNBC)

As in other cancer types, tumor stem cells play a critical role in the tumorigenesis of triple-negative breast cancer (TNBC) [109]. Azzam and colleagues identified two subpopulations in TNBC cell lines, $CD44^{+}CD24low$ (hereafter CD24low) and $CD44^{+}CD24^{-}$ (hereafter $CD24^{-}$), both exhibiting characteristics of breast cancer stem cells (BCSCs). Treatment with the GSI RO4929097 inhibited sphere formation in CD24low cells and significantly slowed tumor growth and metastasis in xenograft models, effects not observed in $CD24^{-}$ cells. Treatment of BCSC cell lines with DAPT decreased cell proliferation, increased apoptosis, reduced invasion, and diminished sphere formation. In xenograft mice, DAPT delayed tumor onset and slowed subsequent tumor growth [110].

Stoeck and colleagues explored the relationship between NOTCH gene mutations and sensitivity to the GSI MRK003 in TNBC models. They found that therapeutic response was more closely linked to levels of active NOTCH intracellular domain (NICD) than to the presence of NOTCH mutations. In xenograft mice with elevated NICD levels, MRK003 was more effective, and *in vitro*, its combination with paclitaxel showed significant antitumor activity [111].

The combined use of the GSI MK-0752 and the MET inhibitor (METi) SU11274 in two TNBC cell lines with differing NOTCH1 expression was also analyzed in other work. MK-0752 alone did not inhibit cell growth, whereas SU11274 was effective, and its efficacy was enhanced when combined with MK-0752. Interestingly, MK-0752 was more effective than SU11274 in blocking colony formation, and the combination yielded the strongest inhibitory effect [112].

Schott and colleagues were the first to evaluate a GSI as a therapeutic agent for TNBC in humans. They studied chemotherapy-resistant BCSCs models treated with MK-0752 and found that, while tumors formed in 50% of control mice, none developed in the treated group. These findings led to a phase I clinical trial combining MK-0752 with docetaxel. Among 24 patients, one experienced grade 5 pneumonitis (likely due to docetaxel), while 11 had partial responses, 9 achieved stable disease, and 3 showed progression. Serial biopsies from six patients revealed a reduction in BCSCs populations [113].

A phase I trial combining the GSI PF-03084014 with docetaxel in 29 women with TNBC was also conducted. The study aimed to determine the maximum tolerated dose (MTD). Severe adverse events were reported, including one death from septic shock following febrile neutropenia. Grade 4 neutropenia occurred in 24 of the 29 patients, and hypophosphatemia was also observed (Figure 10). Treatment efficacy was limited [114].

In another phase I study, the MTD of RO4929097 in combination with paclitaxel and carboplatin in 14 patients with triple-negative breast cancer (TNBC) was analyzed. Similar to the previous study,

 doi:10.20944/preprints202511.0585.v1

several grade 4 adverse events were reported, the most significant being neutropenia and thrombocytopenia. In terms of clinical response, 5 patients showed a partial response, 4 achieved disease stabilization, and 5 exhibited residual disease [115]. RO4929097 has also been investigated in a phase Ib clinical trial for metastatic estrogen receptor-positive breast cancer (EPBCm). Means-Powell and colleagues administered RO4929097 alongside exemestane, an aromatase inhibitor, to 15 patients with EPBCm [116]. One dose-limiting grade 4 adverse event was observed, and grade 3 hypophosphatemia occurred in 13% of patients. Regarding efficacy, 7 patients demonstrated a partial response, and 7 maintained stable disease among the 14 evaluated.

Although clinical trial outcomes have been limited, GSIs are increasingly being explored in preclinical studies in combination with novel therapies for TNBC. Wan and colleagues developed a nanoparticle formulation containing erlotinib, DAPT, and a tumor-targeting peptide, aiming to reduce adverse effects. This nanoparticle significantly inhibited cell migration and tumor growth in xenograft mouse models [117].

Another innovative strategy was proposed by Paroni and colleagues [118]. All-trans retinoic acid (ATRA), an unconventional therapy for breast cancer, is often ineffective in TNBC. However, their study revealed a correlation between ATRA sensitivity and elevated levels of active NICD1 in certain TNBC subtypes. In various TNBC cell lines, the combination of DAPT and ATRA proved more effective in suppressing tumor growth than either agent alone. Similar results were obtained in TNBC xenograft mice treated with PF-03084014 and ATRA.

Recent literature highlights suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, as a promising therapeutic candidate for TNBC [68]. However, SAHA may promote epithelial–mesenchymal transition (EMT), potentially due to NOTCH pathway overactivation [119]. They combined SAHA with the GSI LY411575 in TNBC cell lines. This combination enhanced apoptosis, increased reactive oxygen species, induced mitochondrial depolarization, reduced EMT marker expression, and diminished breast cancer stem cell (BCSC) characteristics. Sen and colleagues also used a multi-targeting TACE/ADAM17 and gamma-secretase of NOTCH signaling pathway in TNBC via drug repurpose approach using lomitapide [120].

The limited success of GSIs in clinical trials is largely attributed to their intestinal toxicity and potential immunological side effects, given the critical role of NOTCH signaling in T-cell activation, including CD8+ T cells within tumors. To overcome these limitations, Hossain and colleagues explored alternative agents that lack systemic toxicity and preserve tumor immunity [121]. They identified sulindac sulfide (SS), the active metabolite of the FDA-approved NSAID sulindac, as a promising GSI substitute. SS significantly inhibited nanosphere formation across human and murine TNBC models *in vivo*, *in vitro*, and *ex vivo*. In a transplantable TNBC mouse model (C0321), SS demonstrated potent single-agent antitumor activity and effectively suppressed NOTCH1 protein expression in tumors [121].

The following two tables summarize the most relevant data reviewed in this work (Tables 1 and 2).

Table 1. Summary of the effect of different GSIs *in vitro* studies and xenograft mice in the cancer types under study in this review. PDAC: pancreatic ductal adenocarcinoma. TNBC: triple-negative breast cancer. GC: gastric carcinoma. NSCLC: non-small cell lung cancer.

GSI	Type of cancer	Type of study	Main results	Reference
MRK003	**PDAC**	*In vivo* (xenograft) +/- gemcitabine	The combination blocked tumor progression	[59,77]
	TNBC	*In vitro e in vivo* + placitaxel	Greater antitumor activity of the combination in cells with higher NICD levels	[110]
GSI-IX	**PDAC**	*In vivo* (xenograft) + AG-490	Mice treated with the combination showed no visible tumors	[78]
	GC	*In vitro*, in $CD44^+$ cells	Smaller tumor spheres and increased apoptosis	[93]

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		In vivo (xenograft)	Reduced tumor growth and increased necrosis	
GSI-X	**METASTATIC MELANOMA**	*In vitro*	GSI decreased CD133+ cells (MSCs)	[98]
PF-03084014 (Nirogacestat)	**PDAC**	*In vivo* (xenograft) +/- gemcitabine	Only in combination did it show antiproliferative activity and reduce cancer stem cells	[76,79]
	METASTATIC MELANOMA	*In vitro* + MEKi	The combination was more effective in stopping proliferation and migration	[99]
DAPT	**NSCLC**	*In vitro* + cisplatin	Decrease in the appearance of CD133+, ALDH+ LCSC cells, with lower resistance to cisplatin	[85]
	GC	*In vitro*	Inhibited the formation of GCSC-rich spheres by 25%	91
		In vitro, in $CD44^+$ and $CD44^-$ cells	CD44+ cells, behaving as GCSCs, showed greater antitumor response to GSI	[92]
		In vivo (xenograft)	Significant inhibition of tumor growth and EMT	
		In vitro +/- PD98059 *In vivo* (xenograft) +/- PD98059	Reduced tumor growth and increased apoptosis in combination	[96]
		In vitro +/- anti-DLL4	Significant increase in apoptosis and reduced invasion and tumor size	[97]
	METASTATIC MELANOMA	*In vivo* (xenograft) +/- BRAFi	Reversal of melanoma cell resistance to BRAFi	[100]
		In vitro +/- DLK1 and/or DLK2 levels	Dose-dependent effect of DAPT: decreased proliferation at high doses, increased at low doses. The combination reduced cell proliferation	[103]
		In vitro e *In vivo* (Xenograft)	Long-term use of DAPT increased tumor growth	[104]
	TNBC	*In vitro, in* BCSCs cells	Reduced proliferation and increased apoptosis	[109]
		In vivo (xenograft)	Delay in tumor formation and reduced subsequent growth	
		In vivo (xenograft) + Erlotinib + Director peptide	The nanoparticle reduced tumor growth and cell migration	[116]
		In vitro + ATRA	The combination was more effective in inhibiting tumor growth	[117]
GSI-34	**NSCLC**	*In vivo* (xenograft) with $CD166^+Lin^-$ LCSC cells +/- cisplatin	CD166+Lin- showed intrinsic resistance to cisplatin, which was reversed with GSI. The combination effectively reduced tumor size	[82]
BMS-708163	**NSCLC**	*In vitro, in* NSCLC-gefitinb resistant cells + gefitinib	High doses of GSI reversed resistance to gefitinib and formed smaller colonies	[86]
		In vivo (xenograft) + gefitinib	The combination produced considerable inhibition of tumor growth	
BMS-906024		*In vitro,* in NSCLC cells + RT +/- placitaxel y crizotinib	Monotherapy + RT did not show significant reduction. It was observed with the combination	[84]
		In vivo (xenograft) + placitaxel	The combination enhanced the cytotoxic effect of paclitaxel	[83,87]
GSI-XX		*In vivo* (xenograft) + RT	The combination caused a significant delay in tumor growth	[88]
GSI-I		*In vitro* + RT	Higher level of apoptosis than isolated RT	[88]

 doi:10.20944/preprints202511.0585.v1

	GC	*In vitro and in vivo* (xenograft) + placitaxel or 5-FU	Increased activity of PTEN, a tumor suppressor gene. Both combinations were more effective than monotherapy	[94]
	METASTATIC MELANOMA	*In vivo* (xenofraft) and *in vitro* + BCL2i	The combination was more effective than monotherapy	[102]
RO4929097		*In vitro* + ERKi	Sensitization to ERKi in cell lines that did not respond to it in monotherapy	[101]
		In vivo (xenograft) +ERKi	The combination was more effective than monotherapy	
		In vitro + RT	Synergism at low doses in combination	[107]
	TNBC	*In vitro,* in CD24 low and CD24$^-$ (BCSCs) cells	Inhibition of CD24low sphere growth	[64,114]
		In vivo (xenograft) with CD24 low and CD24$^-$ (BCSCs) cells	Halted tumor growth and metastasis in CD24 low models	
MK-0752		*In vitro. Different levels of* NOTCH expression + METi	The combination showed synergism in halting cell growth	[111]
		In vivo (xenograft)	Injecting BCSC cells treated with GSI did not reproduce the tumor	[112]
LY411575		*In vitro* + SAHA	SAHA in monotherapy was seen to promote EMT. The combination reduced EMT and increased apoptosis	[68]
OTHERS (Evodiamina)	**NSCLC**	*In vitro*	Not a GSI but behaves like one. It reduced cell proliferation and metastasis	[89]
OTHERS (NSAID sulindac (SS))	**TNBC**	*In vitro, in vivo and ex vivo*	Significantly inhibited nanosphere growth in all human and murine TNBC models. Eliminated NOTCH1 protein expression in tumors	[120]
OTHERS (Lomitapide)		*In vitro*	Multi-targeting TACE/ADAM17 and gamma-secretase complex of NOTCH signaling pathway	[119]

Table 2. Summary of the different clinical trials conducted with GSIs in the cancer types under study in this review. PDAC: pancreatic ductal adenocarcinoma. TNBC: triple-negative breast cancer. Table created by the author. EPBCm: metastatic estrogen receptor-positive breast cancer.

Type of cancer	GSI	Phase	Results	Reference
PDAC	MK-0752 + gemcitabine	I	14 out of 44 patients reached a stable condition, in both monotherapy and combination therapy. Gastrointestinal disorders and anemia were observed	[80]
	RO4929097	II	The trial could not be completed because GSI synthesis was discontinued	[81]
METASTATIC MELANOMA	RO4929097	I	In two groups of 110 patients, 33% and 41% reached a stable condition. Hypophosphatemia was noted	[105]
	RO4929097	II	Of 32 evaluated patients, 1 had a partial response and 8 reached a stable condition. Hypophosphatemia was also observed	[106]
TNBC	MK-0752 + docetaxel	I	Among 24 patients, 11 had a partial response, 9 reached a stable condition, and 3 showed tumor progression. There was one case of severe pneumonitis.	[112]
	PF-03084014 + docetaxel	I	29 women showed limited treatment efficacy, with severe hematologic and infectious reactions	[113]

 doi:10.20944/preprints202511.0585.v1

	RO4929097 + placitaxel + carboplatin	I	Of 14 evaluated patients, 5 had a partial response, 4 reached a stable condition, and 5 had residual disease. *Neutropenia* was reported	[64,114]
EPBCm	RO4929097 + exemestane	Ib	Among 14 evaluated patients, 7 had a partial response and 7 reached a stable condition	[115]

4. Discussion and Conclusions

The use of γ-secretase inhibitors *in vitro* and in mouse xenograft models has emerged as a promising therapeutic strategy. These preclinical studies have shown encouraging results, particularly when GSIs are combined with other treatments. GSIs have demonstrated efficacy in enhancing the effects of various chemotherapeutic agents, for example, gemcitabine in pancreatic ductal adenocarcinoma (PDAC) [59,122], paclitaxel in non-small cell lung cancer (NSCLC) [88], and 5-FU in gastric cancer (GC) [95]. Additionally, combinations with radiotherapy (RT) have shown effectiveness in NSCLC and metastatic melanoma [85,89,108]. The synergistic potential of GSIs with other drugs and their ability to sensitize tumors to treatment has been observed across all five cancer types reviewed, with particular emphasis on NSCLC and melanoma [85,87,97,98,101,102,104,112,116,123–125].

Research has also focused on the role of NOTCH signaling in cancer stem cells and its inhibition to overcome resistance to therapy and improve prognosis. A significant relationship has been identified between NOTCH signaling and gastric cancer stem cells (GCSCs), NSCLC, metastatic melanoma, and TNBC, suggesting promising therapeutic applications [83,86,91,93,94,99,103,109,110,126]. Another key area of investigation is the potential of GSIs to block epithelial-mesenchymal transition (EMT), a process implicated in GC, metastatic melanoma, and TNBC [93,99,119,127].

Recent studies have increasingly focused on combining GSIs with innovative therapeutic agents, yielding promising results. For instance, in TNBC, GSIs have been successfully combined with SAHA and ATRA [118,120,127]. Table 1 classifies the data analyzed by GSI used, cancer type, study model (*in vitro* or xenograft), and outcomes. DAPT was the most frequently used GSI, followed by RO4929097.

GSIs have also entered clinical trials, although results remain limited (Table 2). Despite the promising preclinical data, clinical trials have yet to meet expectations. In a phase I trial involving stage IV PDAC patients, combining GSI with gemcitabine did not yield superior outcomes compared to gemcitabine alone [81]. Another phase II trial in metastatic PDAC was discontinued due to the discontinuation of the GSI under investigation [82]. In metastatic melanoma, a phase I trial of GSI monotherapy showed promising results, prompting a phase II trial. However, the latter produced limited outcomes, likely due to subtherapeutic dosing. Overall tolerability was acceptable, although severe hypophosphatemia was reported as a significant adverse event [106,107].

TNBC has been the cancer type with the highest number of clinical trials involving GSIs. Three phase I trials tested different GSIs in combination with various chemotherapeutic agents, revealing several severe hematological and infectious adverse reactions. Although partial responses were observed in some patients, overall clinical efficacy was limited. A GSI was also tested in EPBCm with similar results [102,109,113–115]. Table 1 presents a classification of clinical trial data by cancer type and outcomes, showing RO4929097 as the most frequently tested GSI.

5. Challenges and Future Directions

Despite promising preclinical findings, clinical trials have not yet delivered the expected results. One possible explanation is the reliance on cell lines with overactivated NOTCH signaling, which may not accurately represent the heterogeneity of human tumors. These cell lines were treated with GSIs and used to generate xenograft models [87,97,99,128]. However, human tumors consist of diverse cell populations with varying levels of NOTCH receptor expression and activation. A specific

 doi:10.20944/preprints202511.0585.v1

GSI may only affect certain cell types, and its efficacy may depend more on activation levels than expression levels.

Moreover, many studies have not accounted for the specificity of GSIs toward different NOTCH receptors. This non-selective inhibition may lead to adverse effects, such as increased incidence of non-melanoma skin neoplasms. For example, indiscriminate inhibition of all NOTCH receptors may suppress tumor-suppressive pathways, as seen with NOTCH2 in breast cancer [55]. Additionally, low levels of NOTCH inhibition may paradoxically increase cell proliferation, as observed by Nueda and colleagues, Naranjo and Colleagues and others [63,104,129].

To enhance GSI efficacy and minimize adverse effects in human clinical trials, combined treatments of GSIs and other current drugs should be studied and drug delivery methods should be optimized. Nanoparticle-based delivery systems offer a promising avenue. Although some studies have explored GSI-loaded nanoparticles in non-cancer contexts, their application in oncology remains limited. Wan and co-workers used pH-sensitive peptide-functionalized nanoparticles to co-deliver erlotinib and DAPT, effectively restricting TNBC progression [117]. Zhou and coworkers employed DT7-modified lecithin nanoparticles loaded with a GSI and dexamethasone, achieving effective inhibition of T-cell acute lymphoblastic leukemia while reducing gastrointestinal toxicity [130].

Future research should aim to identify the specific NOTCH receptors or ligands responsible for oncogenic signaling and develop targeted therapies accordingly, either through receptor-specific GSIs or alternative inhibitory molecules or natural inhibitors of NOTCH signaling such as DLK proteins. Clinical trials should incorporate patient stratification based on NOTCH receptor and ligand expression, and more importantly, NOTCH activation levels. Integrating these approaches with big data analytics and artificial intelligence could enable personalized medicine, including consideration of gender differences in cancer prevalence [51].

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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Abbreviations

The following abbreviations are used in this manuscript:

- ADAM: A Disintegrin And Metalloproteinase
- ALDH: aldehyde dehydrogenase
- ATRA: All-trans retinoic acid.
- BCL2i: BLC-2 inhibitors.
- BCSCs: Breast cancer stem cells.
- BRAFi: BRAF inhibitor.
- CD44: Cell Surface Glycoprotein CD44
- CD133: Transmembrane glycoprotein CD133
- CSCs: cancer stem cells
- CSL: CBF1/Suppressor of Hairless/LAG-1, also known as RBP-Jκ
- DAPT: GSI-IX
- DLK1: Delta like homolog 1.
- DLK2: Delta like homolog 2.
- DLL1: Canonical Delta-Like1 ligand.
- DLL3: Canonical Delta-Like3 ligand.
- DLL4: Canonical Delta-Like4 ligand.
- DOS: Delta and OSM-11 Motif.
- DSL: Delta/Serrate/LAG-2 domain
- EGF: Epidermal growth factor.
- EMT: Epithelial-Mesenchymal Transition.
- EPBCm: Estrogen receptor-positive metastatic breast cancer.
- ERK: Extracellular Signal-Regulated Kinase
- ERKi: ERK MAPK inhibitor.
- EVO: Evodiamine.
- 5-FU: 5-fluorouracil
- GC: Gastric cancer.
- GCSCs: Gastric cancer stem cells.
- GSC: γ-Secretase complex
- GSI: γ-Secretase inhibitor.
- JAG1: Canonical Jagged 1 ligand.
- JAG2: Canonical Jagged 2 ligand.
- KRAS: Kirsten rat Sarcoma
- LCSCs: Lung cancer stem cells.
- mAb: monoclonal antibodies
- MAML: Mastermind-like protein.
- MAPK: Mitogen-Activated Protein Kinase
- MEK: Mitogen-Activated Protein Kinase 1 (MAP2K1)
- MEKi: MEK inhibitor.
- MET: Mesenchymal Epithelial Transition receptor tyrosine kinase
- METi: MET inhibitor.
- MTD: maximum tolerated dose
- MSC: Melanoma stem cells.
- NECD: NOTCH extracellular domain
- NICD: NOTCH intracellular domain.
- NRR: Negative regulatory region.
- NSCLC: Non-small cell lung cancer.
- PDAC: Pancreatic ductal adenocarcinoma.
- PEST: proline, glutamic acid, serine, and threonine domain

 doi:10.20944/preprints202511.0585.v1

- PFS: Progression-free survival.
- RBP-Jκ: Recombination signal binding protein for immunoglobulin kappa J region
- RECK: Reversion-inducing cysteine-rich protein with Kazal motifs.
- RT: Radiotherapy.
- SAHA: Suberoylanilide hydroxamic acid.
- SS: Sulindac sulfide.
- TACE: Tumor necrosis factor (TNF)-converting enzyme
- TMD: transmembrane domain.
- TNBC: Triple-negative breast cancer.
- WNT: Wingless and Int-1.
- 2D: Two dimensions.
- 3D: Three dimensions.
- WHO: World Health Organization.

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