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Gamma Secretase Inhibitors in Cancer: A Current Perspective on Clinical Performance

Tyler R. McCaw,[a] Evelyn Inga,[a] Herbert Chen,[b] Renata Jaskula-Sztul,[b] Vikas Dudeja,[a] James A. Bibb,[c] Bin Ren,[d] J. Bart Rose[a]

Divisions of [a]Surgical Oncology, [b]Breast & Endocrine Surgery, [c]Gastrointestinal Surgery, and [d]Vascular Surgery & Endovascular Therapy, Department of Surgery, The University of Alabama at Birmingham, Birmingham, Alabama, USA

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Abstract

Gamma secretase inhibitors (GSIs), initially developed as Alzheimer's therapies, have been repurposed as anticancer agents given their inhibition of Notch receptor cleavage. The success of GSIs in preclinical models has been ascribed to induction of cancer stem-like cell differentiation and apoptosis, while also impairing epithelial-to-mesenchymal transition and sensitizing cells to traditional chemoradiotherapies. The promise of these agents has yet to be realized in the clinic, however, as GSIs have failed to demonstrate clinical benefit in most solid tumors with the notable exceptions of CNS malignancies and desmoid tumors. Disappointing clinical performance to date reflects important questions that remain to be answered. For example, what is the net impact of these agents on antitumor immune responses, and will they require concurrent targeting of tumor-intrinsic compensatory pathways? Addressing these limitations in our current understanding of GSI mechanisms will undoubtedly facilitate their rational incorporation into combinatorial strategies and provide a valuable tool with which to combat Notch-dependent cancers. In the present review, we provide a current understanding of GSI mechanisms, discuss clinical performance to date, and suggest areas for future investigation that might maximize the utility of these agents. ***The Oncologist*** 2021;26:e608–e621

Implications for Practice: The performance of gamma secretase inhibitors (GSIs) in clinical trials generally has not reflected their encouraging performance in preclinical studies. This review provides a current perspective on the clinical performance of GSIs across various solid tumor types alongside putative mechanisms of antitumor activity. Through exploration of outstanding gaps in knowledge as well as reasons for success in certain cancer types, the authors identify areas for future investigation that will likely enable incorporation of GSIs into rational combinatorial strategies for superior tumor control and patient outcomes.

Development of Gamma Secretase Inhibitors

Efforts to curtail amyloid beta production in treatment of Alzheimer's disease led to development of gamma secretase inhibitors (GSIs) [1], which advanced to phase III trials [2]. Adverse events and lack of efficacy limited the utility of these agents [3, 4], but a shared proteolytic processing pathway between amyloid beta and the Notch family has been implicated in off-target effects [5]. As a result, these agents have been repurposed for their ability to broadly inhibit the Notch pathway. In the current review, we discuss gamma secretase (GS) inhibition as a potential point of intervention to broadly target dysregulated Notch signaling in cancer.

Gamma Secretase Inhibitors and the Notch Pathway

Notch is an evolutionarily conserved pathway that transmutes extracellular information, through cell–cell interactions, into regulatory events guiding cell fate decisions. One of four Notch surface protein receptors (Notch 1–4 paralogs) binds to one of three delta-like ligands (Dll) or one of two

Correspondence: J. Bart Rose, M.D., M.A.S., Division of Surgical Oncology, Pancreatobiliary Disease Center, The University of Alabama at Birmingham, 1808 7th Avenue South, Building 583, Birmingham, Alabama 35233-3411, USA. Telephone: 205-975-5000; e-mail: jbrose@uabmc.edu Received July 29, 2020; accepted for publication November 13, 2020; published Online First on January 2, 2021. http://dx.doi.org/10.1002/onco.13627

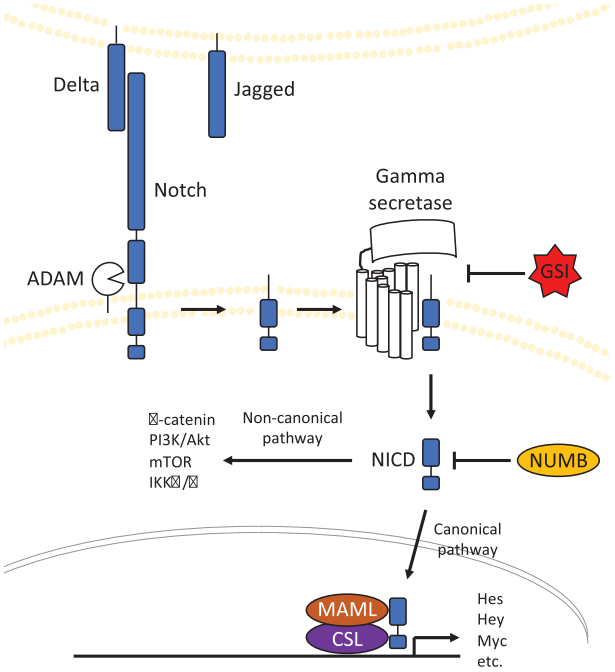

Figure 1. Schematic representation of Notch signaling pathway. A Notch receptor binds to cell-bound or soluble Delta/Jagged ligands. Bound Notch is first cleaved by ADAM to release the extracellular portion. Gamma secretase then catalyzes a second cleavage event, liberating NICD into the intracellular space. NICD can interact with other pathways independent of transcriptional activity through noncanonical signaling or translocate to the nucleus, associate with CSL and MAML, and promote expression of target genes through canonical signaling.
Abbreviations: GSI, gamma secretase inhibitor; MAML, mastermind-like family; NICD, Notch intracellular domain.

jagged ligands on itself (cis-activation) or a neighboring cell (transactivation). Receptor-ligand binding triggers sequential cleavage events by a disintegrin and metalloprotease (ADAM) and GS that culminate in release of the Notch intracellular domain (NICD) and translocation to the nucleus. Interaction with the RBP-J (or CSL) transcription factor and recruitment of a transcriptional coactivator, mastermind-like family (MAML), then enact changes in gene expression (Fig. 1) [6]. Many layers of complexity help explain this pathway's pleiotropic roles in embryonic development and cellular homeostasis, including variable ligand glycosylation [7] and dynamic ligand binding patterns that contribute to activation of distinct target genes [8]. Moreover, Notch signaling can block cell differentiation to preserve progenitor cells and maintain these pools through symmetric or asymmetric division [9]. In this manner, coordinated Notch signaling is integral to muscle, vasculature, cardiac, hematopoietic, nervous system, and pancreatic development [10].

Adaption of this pathway in malignant transformation was first recognized in T-cell acute lymphoblastic leukemia (T-ALL), wherein a t(7;9) chromosomal translocation positions a truncated Notch1 protein, similar to the NICD1, under control of the T-cell receptor β locus [11]. In fact, activating mutations in Notch-1 are present in the majority of T-ALL cases [12]. Contribution of Notch signaling to tumorigenesis, however, is somewhat more nuanced, as coexpression of additional oncogenic alterations are typically required for transformation [13]. For example, truncated Notch1 can transform HC11 mouse mammary epithelial cells in vitro [13], whereas in transgenic mice Notch1 cooperates with c-neu/erbB2 and c-myc to induce transformation in vivo [13, 14]. Likewise, transformation of baby rat kidney cells in vivo required the presence of an additional oncogene, E1A [15]. NICD1 signaling was not sufficient to induce cancer initiation but could cooperate with AKT, Myc, and Ras/Raf/MAPK to drive formation of prostate adenocarcinoma in mice and promote more aggressive phenotypes in human prostate cancer cells [16]. Accordingly, although mutations in Notch regulatory sequences or domains (e.g., PEST [17]) may be sufficient to drive tumorigenesis in some instances, the majority of solid tumors with oncogenic Notch signaling might instead harbor aberrations in regulatory proteins (e.g., NUMB [18], TBC1D15 [19]), signaling partners, or relative dosage [20]. Thus, the role of Notch in tumorigenesis may be context dependent, acting most convincingly in cells already poised for transformation.

Contributions of Notch signaling to cancer progression extend beyond its roles in tumorigenesis. Cancer stem(-like) cell proliferation and renewal are integral to disease progression and are promoted by Notch ligand expression in niche cells (in human glioblastoma [21], breast cancer [22], and colorectal cancer [23]) and broadly by dysregulated intracellular pathway signaling [24]. Activation of Notch promotes epithelial-to-mesenchymal transition (EMT), which then facilitates disease invasion and metastatic spread [25]. Further complicating its role in tumor progression, juxtacrine and paracrine Notch signaling help shape the tumor microenvironment (TME) [26]. Notch also participates as a coregulator of other tumorigenic pathways. For example, noncanonical Notch signaling, independent of its transcription factor function, titrates intracellular levels of active Wnt/β-catenin in a context dependent manner [27]. Through these processes, the Notch pathway can instigate cancer resistance to traditional therapeutic strategies, rendering it a critical oncologic target.

Gamma Secretase: Structure and Function

Gamma secretase is a large heterotetrameric transmembrane protein complex composed of presenilin (PS), nicastrin (Nct), anterior pharynx defective-1 (Aph-1), and presenilin enhancer-2 in an equimolar ratio. Two PS isoforms (PS1 or PS2) and three different Aph-1 isoforms (Aph-1aS, Aph-1aL, Aph-1ab) can be incorporated into the quaternary protein structure, each with tissue-specific expression patterns [28]. Collectively, these proteins form a 19-pass transmembrane disk structure organized around a central cleft with the large ectodomain of Nct located directly above. Following an initial cleavage event, termed "ectodomain shedding," substrates are recognized and positioned within the intermembrane cleft of GS by Nct, adjacent to the catalytic site of PS [29]. In the case of Notch proteins, this enables cleavage of the intracellular domain, translocation to the nucleus, and initiation of transcriptional changes. The activity of GS extends well beyond Notch, however, to encompass more than 90 substrates [30], including half of the human receptor tyrosine kinases [31].

Complexity is introduced to this process through several mechanisms. First, the specific combination of PS and Aph-1 isoforms likely dictate distinct substrate and cleavage site preferences, influenced by tissue of origin and intracellular isoform equilibria. Activity of assembled complexes is then further regulated by GS modulators, nonessential proteins that interact with the GS quaternary structure [32].

Gamma Secretase Inhibitors and Cancer

GS cleaves Notch receptors 1–4; however, pharmacologic inhibition with GSIs does not block signaling of each Notch receptor equally. In fact, clinical GSIs are pharmacologically distinct, each with a discrete profile of Notch inhibition. Moreover, low concentrations of GSIs can actually potentiate cleavage of select Notch receptors [33]. It is logical to extrapolate these findings to infer that unique properties of individual GSIs likely influence utility for specific clinical indications. Thus far, observational bias has precluded a greater mechanistic understanding of GSIs in that many investigations have been limited to readouts of Notch and/or amyloid precursor protein (APP) cleavage [34]. Common GSIs having reached various stages of development with corresponding half-maximal inhibitory concentration (IC_{50}) values for different Notch receptors and APP are provided in Table 1 [35–45].

Mechanisms of Gamma Secretase Inhibition in Cancer

Targeting the Notch pathway with GSIs has led to impaired cancer cell growth and tumor progression across a number of models, reflecting the common upregulation of this pathway. For example, GSI-I and LY-411,575 demonstrated ability to directly induce apoptosis in Kaposi sarcoma cells in vitro and in vivo, an effect that could be rescued by transfection and enforced expression of NICD [46]. Increased apoptosis following GSIs has also been suggested to be mediated by proteosomal inhibition. The combination of a GSI (GSI-XII) plus the proteasome inhibitor bortezomib induced apoptosis in multiple myeloma cell lines and primary patient samples in vitro that could not be rescued by NICD overexpression [47]. However, restoring proteasome function with edaravone effectively abrogated apoptosis [47], a finding similarly observed in human breast cancer cell lines [48]. Interestingly, treatment of glioblastoma tumor-initiating cells with GSI-I–induced apoptosis. This was not the case with selective Notch inhibitors, suggesting a predominant contribution of proteasome inhibition relative to Notch inhibition in this model [49].

As mentioned, Notch is an important target of GS activity. It is a cell fate sensor and acts as a receptor to a variety of canonical and noncanonical ligands including the Delta, Jagged, Lag2 class of cell surface proteins [50]. Upon ligand binding, Notch isoforms are dependent upon cleavage by ADAM metalloproteases and GS. This allows the NICD to translocate to the nucleus, where it forms a transcriptional activation complex with CSL and coactivators of the MAML family [51]. One important downstream effector whose expression is activated by Notch is Hes1, a transcriptional repressor that prevents irreversible cell cycle exit [52]. Upregulated Notch signaling, therefore, might contribute to maintenance of cancer cell proliferative potential. Supporting this hypothesis, pancreatic adenocarcinoma (PDAC) cell lines downregulated Hes1 following treatment with the GSI MRK-003 in vitro. Similarly, pretreatment of PDAC xenografts reduced tumor growth in nude mice by decreasing the number of tumor-initiating cells [53]. GSI treatment also decreased Hes1 expression and tumor sphere formation in human ovarian cancer (SKOV3 and HO8910) [54] and a panel of melanoma cells [55] following treatment in vitro. Treatment of primary melanoma xenografts with the GSI RO4929097 led to decreased expression of stem cell markers and decreased tumor formation in serial xenograft transplants despite no additional treatment cycles [55]. Brief treatment of ERBB2 murine mammary tumor cells with MRK-003 was sufficient to drive histologic changes and sustained impairment of tumor formation in syngeneic mice [56]. A durable impairment of proliferation following GS inhibitor exposure has also been observed in T-cell acute lymphocytic leukemia [57], non-small cell lung cancer [58], and prostate cancer [59]. These findings suggest that blocking Notch signaling with GSIs induces irreversible differentiation and cell cycle exit of tumor-initiating cells.

Overcoming Treatment Resistance with Gamma Secretase Inhibitors

Although GS inhibitors can impair tumor growth through multiple mechanisms, combination with traditional therapeutic modalities might mitigate resistance mediated through Notch upregulation. Sequential treatment of A2780 and OVCAR3 human ovarian cancer cells with cisplatin followed by the GSI MK-0725 significantly reduced growth in vitro (compared with concurrent administration or the reverse order) and in vivo [60]. Similarly, sequential administration of radiation followed by GSI in human NSCLC cells was concluded to more effectively impair Notch-1 or Notch-3 upregulation (pending cell line used), delaying tumor growth [61, 62]. In contrast, pretreatment with the GSI DAPT resensitized platinum resistant A2780/CP70 and OV2008/C13 cell lines to subsequent cisplatin treatment [63]. These discordant findings may reflect different Notch signaling equilibria at baseline, as resistant cell lines likely upregulated Notch signaling during acquisition of platinum resistance [64, 65].

More generally, concurrent treatment with GSIs and traditional chemotherapeutics has demonstrated superior tumor control in a variety of models. In prostate xenografts, PF-0308014 plus docetaxel cotreatment significantly reduced tumor growth [59]. Likewise, coculture of human head and neck squamous cell carcinoma with DAPT or AG490 (impairs STAT3 activation) increased sensitivity to cisplatin in vitro [66]. Efficacy of these combinations was postulated to be a function PI3K/Akt pathway downregulation, potentially through disrupted Notch crosstalk, in preclinical models of human retinoblastoma [67], lung [68], gastric [69], colon [70], triple-negative breast [71], and ERBB2-driven murine breast [72] cancers. A somewhat disparate result was found in a study of multiple myeloma and non-Hodgkin's lymphoma cell lines and patient samples, which nearly homogeneously expressed Notch-1 and Notch-2. MRK-003 downregulated Notch targets and induced apoptosis in vitro but modestly increased pAkt; MRK-003 and Akt inhibitor cotreatment then drove

Table 1. Common GSIs at various stages of (pre-)clinical development. IC_{50} values of each compound for different Notch receptors as well as those for APP (inhibition of amyloid beta production) are provided

GSI	Names	IC_{50} (nM)					Clinical status	Reference
		Notch1	Notch2	Notch3	Notch4	APP		
BMS-906024	AL101	0.29–1.6	0.14–0.7	0.32–3.4	0.71–2.9		II	Gavai, 2015 [35]; Ran, 2017 [33]; Lessard, 2019 [36]
BMS-708163	Avagacestat	20.6–58				0.30–0.79	II	Mitani, 2012 [38]; Gillman, 2010 [37]
BMS-986115							I	
DAPT	GSI-IX	4.9	85.22	552.4–623.5	51.5	29	preclinical	Ran, 2017 [33]; Lessard, 2019 [36]; Martone, 2009 [39]
MK-0752		55–87.44	46.62	146.3–370	191	5	II	Ran, 2017 [33]; Deangelo, 2006 [41]; Lessard, 2019 [36]; Cook, 2010 [40]
PF-03084014	Nirogacestat	0.6–13.3	0.002–0.01	1.21–15.07	0.81–10.77	1.2–6.2	III	Wei, 2010 [43]; Ran 2017 [33]; Lessard, 2019 [36]; Lanz, 2010 [42]
RO4929097		0.46–4	2.24	19.8–28.31	3.4	14	II	Ran, 2017 [33]; Luistro, 2009 [47]; Lessard, 2019 [36]; Gu, 2017 [44]
GSI-953	Begacestat					8	I	Martone, 2009 [39]
LY-450139	Semagacestat	7.02–14.1	38.7	390.9–523.3	45.98	10.9–38	III	Ran, 2017 [33]; Mitani, 2012 [38]; Lessard, 2019 [36]; Martone, 2009 [39]
LY-411,575		0.129				0.119	preclinical	Lewis, 2003 [45]
LY-900009		0.27					I	Pant, 2012 [109]

Abbreviations: APP, amyloid precursor protein; GSI, gamma secretase inhibitor; IC50, half-maximal inhibitory concentration.

impressive apoptosis [73]. An observation that illustrates potential for compensatory pathways to subvert GSI monotherapy.

Enhanced efficacy of GSIs in combinatorial strategies is likely due, in part, to impairment of EMT, which commonly involves acquisition of stem cell features and upregulation of multidrug resistance transporters [74]. Specifically, upregulation of SNAI1/2, ZEB1/2, and TWIST1/2 regulates this process [75]. In the human hepatocellular carcinoma cell line MHCC97H, combination of sorafenib plus PF-03084014 significantly reduced expression of SNAI1/2, Nanog, Oct4, and the multidrug resistance transporter ABCG2 [76]. Sensitization to chemotherapy was also demonstrated using MKN45 gastric cancer cells. Specifically, CD44+ cells, which strongly upregulated Notch1 signaling relative to CD44–, became sensitive to 5-fluorouracil (5-FU) by combination with DAPT. This correlated with loss of Snail, vimentin, and N-cadherin protein expression alongside decreased migration and invasion in vitro [77]. In the case of ovarian cancer, a disease characterized by early and diffuse intraperitoneal metastases, EMT is driven by positive feedback between TGFβ/Smad and Notch signaling, a process in which each of the Notch1–4 receptors have been implicated [78–80]. GSI blockade of Notch signaling impaired TGFβ-induced EMT in multiple cell lines, downregulating SNAI1/2, TWIST, and ZEB1 as well as migration and invasiveness [79–81]. Thus, GS inhibition of EMT might stymie acquisition of drug resistance and limit metastatic capacity of established tumors.

Clinical Trials with Gamma Secretase Inhibitors

Ability to drive tumor cell differentiation, reduce cancer stem cell burden, and sensitize tumors to traditional chemoradiotherapy strategies through multiple mechanisms in preclinical studies has spurred clinical investigation of GSIs in multiple cancer types (Table 2).

Lung Cancer

A meta-analysis encompassing over 3,600 patients from 19 studies revealed a significant correlation between overexpression of Notch1 and Notch3 receptors, as well as the Notch pathway components DLL3 and HES1, and poor outcome in patients with NSCLC [82]. Further suggesting a role for targeting Notch in NSCLC, GSIs can sensitize NSCLC cells to traditional chemotherapeutics and impair development of resistance [64, 83]. A small cohort of patients with lung cancer have been included in phase I trials using MK-0752 or PF-03084014 monotherapy to treat advanced-stage solid tumors refractory to traditional measures; however, no evidence of clinical activity in patients with lung cancer was reported [84, 85]. In an effort to combat inevitable resistance to erlotinib, a phase I/II trial treated 16 patients with

Table 2. Clinical trials using gamma secretase inhibitors (GSIs) organized by cancer type of participating patients. Specific GSI used, and potential combinatorial agents, are provided alongside trial phase, number of patients with indicated cancer type, and trial outcomes

Cancer and study cohort	GSI	Combination	Phase	Patients	*n*	Outcome	Reference
Ovarian							
EOC: platinum resistant	RO4929097		II	Recurrent or metastatic	45	PFS: 1.3 mo	Diaz-Padilla et al. 2015 [112]
Advanced solid tumors	RO4929097		I	Refractory to standard therapy	9	3 clinical benefit	Tolcher et al. 2012 [93]
Advanced solid tumors	RO4929097	Gemcitibine	I	Refractory to standard therapy	2	safe combination	Richter et al. 2014 [91]
Advanced solid tumors	RO4929097	Temsirolimus	Ib	Refractory to standard therapy	1	safe combination	Diaz-Padilla et al. 2013 [110]
Advanced solid tumors	RO4929097	Cediranib	I	Refractory to standard therapy		safe combination	Sahebjam et al. 2013 [111]
Advanced solid tumors	LY900009		I	Refractory to standard therapy	11	MTD 30 mg 3×/wk	Pant et al. 2016 [109]
Advanced solid tumors	MK-0752		I	Refractory to standard therapy	3	Inhibited Notch, minimal clinical activity as monotherapy	Krop et al. 2012 [84]
Colorectal							
Advanced solid tumors	MK-0752	Dalotuzumab	I	Refractory to standard therapy	9	Safe combination	Brana et al. 2014 [122]
Advanced solid tumors	RO4929097	Cediranib	I	Refractory to standard therapy	6	Safe combination	Sahebjam et al. 2013 [111]
Advanced solid tumors	PF-03084014		I	Refractory to standard therapy	11	Safe, response in desmoid tumors, overall PFS 1.6 mo	Messersmith et al. 2015 [85]
Stage IV, metastatic	RO4929097		II	Refractory to standard therapy	37	Safe, PFS 1.8 mo, dose/schedule have minimal activity	Strosberg et al. 2012 [121]
Advanced solid tumors	RO4929097	Capecitabine	I	Refractory to standard therapy	18	Safe, promising activity in colon cancer	LoConte et al. 2015 [123]
Advanced solid tumors	RO4929097		I	Refractory to standard therapy	12	3 clinical benefit	Tolcher et al. 2012 [93]
Advanced solid tumors	LY900009		I	Refractory to standard therapy	5	MTD 30 mg 3×/wk	Pant et al. 2016 [109]
Advanced solid tumors	MK-0752		I	Refractory to standard therapy	16	Inhibited Notch, minimal clinical activity as monotherapy	Krop et al. 2012 [88]
Advanced solid tumors	BMS-986115		I	Refractory to standard therapy	5	Intermittent schedule likely preferable, better tolerated	Aung et al. 2018 [120]

(continued)

Table 2. (continued)

Cancer and study cohort	GSI	Combination	Phase	Patients	*n*	Outcome	Reference
Desmoid							
Advanced solid tumors	BMS-986115		I	Refractory to standard therapy	1	Intermittent schedule likely preferable/better tolerated	Aung et al. 2018 [120]
Advanced solid tumors	PF-03084014		I	Refractory to standard therapy	9	Safe, response in desmoid tumors, overall PFS 1.6 mo	Messersmith et al. 2015 [85]
Unresectable	PF-03084014		II	Progressive disease after more than one line	17	Promising strategy for desmoid tumors	Chen et al. 2016 [60]
Breast							
Advanced breast tumors	MK-0752	Docetaxel	I	Refractory to first-line chemotherapy	30	Preliminary evidence of clinical efficacy	Schott et al. 2013 [107]
Advanced TNBC	PF-03084014	Docetaxel	Ib	Refractory to standard therapy	29	Median PFS 4.1 mo, 6 mo PFS 17.1%	Locatelli et al. 2017 [106]
Metastatic ER+ breast cancer	RO4929097	Exemestane	Ib	Refractory to standard therapy	15	Safe combination, preliminary evidence of stable disease	Means-Powell et al. 2012 [105]
Advanced solid tumors	MK-0752		I	Refractory to standard therapy	24	Inhibited Notch, minimal clinical activity as monotherapy	Krop et al. 2012 [84]
Advanced solid tumors	PF-03084014		I	Refractory to standard therapy	7	Safe combination, overall PFS 1.6 mo	Messersmith et al. 2015 [85]
Advanced solid tumors	RO4929097	Gemcitibine	I	Refractory to standard therapy	5	Safe combination	Richter et al. 2014 [91]
Advanced solid tumors	RO4929097		I	Refractory to standard therapy	10	Safe combination	Tolcher et al. 2012 [93]
Glioma							
Advanced solid tumors	MK-0752		I	Refractory to standard therapy	42	Modest level of clinical activity in gliomas	Krop et al. 2012 [84]
CNS malignancies	MK-0752		I	Refractory pediatric tumors	4	Well tolerated, trial not completed	Hoffman et al. 2015 [99]
CNS malignancies	MK-0752		I	Refractory pediatric tumors	9	Well tolerated	Fouladi et al. 2011 [98]
Malignant gliomas	RO4929097	Bevacizumab	I	Refractory to standard therapy	13	Well tolerated, PFS 3.7 mo	Pan et al. 2016 [100]
Glioblastoma, grade III AA	RO4929097	Temozolomide, RT	I	Newly diagnosed	21	Well tolerated, glioblastoma median PFS 13 mo	Xu et al. 2016 [101]

(continued)

Table 2. (continued)

Cancer and study cohort	GSI	Combination	Phase	Patients	*n*	Outcome	Reference
Melanoma							
Advanced solid tumors	MK-0752		I	Refractory to standard therapy	3	Inhibited Notch, minimal clinical activity as monotherapy	Krop et al. 2012 [84]
Advanced solid tumors	RO4929097		I	Refractory to standard therapy	24	Safe combination	Tolcher et al. 2012 [93]
Metastatic melanoma	RO4929097		II	Chemotherapy naïve	36	Safe combination, PFS: 1.5 mo	Lee et al. 2015 [69]
Pancreatic							
Metastatic PDAC	RO4929097		II	Refractory to standard therapy	18	Well-tolerated, PFS: 1.5 mo	De Jesus-Acosta et al. 2014 [89]
Advanced solid tumors	RO4929097	Gemcitibine	I	Refractory to standard therapy	3	Safe combination	Richter et al. 2014 [91]
Advanced solid tumors	PF-03084014		I	Refractory to standard therapy	3	Safe combination, overall PFS 1.6mo	Messersmith et al. 2015 [85]
Advanced solid tumors	MK-0752		I	Refractory to standard therapy	2	Inhibited Notch, minimal clinical activity as monotherapy	Krop et al. 2012 [84]
Stage III/IV PDAC	MK-0752	Gemcitibine	I	Prior therapy acceptable	44	Well tolerated, PFS: 5.6 mo	Cook et al. 2018 [92]
NSCLC							
Advanced solid tumors	MK-0752		I	Refractory to standard therapy	3	Inhibited Notch, minimal clinical activity as monotherapy	Krop et al. 2012 [84]
Advanced solid tumors	PF-03084014		I	Refractory to standard therapy	5	Safe combination, overall PFS 1.6 mo	Messersmith et al. 2015 [85]
NSCLC	RO4929097	Erlotinib	I/II	Refractory to standard therapy	16	Safe combination, does not merit further eval	Gold et al. 2013 [86]

Abbreviations: AA, anaplastic astrocytoma; CNS, central nervous system; EOC, epithelial ovarian cancer; ER, estrogen receptor; MTD, maximum tolerated dose; NSCLC, non-small cell lung cancer; PDAC, pancreatic adenocarcinoma; PFS, progression-free survival; TNBC, triple-negative breast cancer.

NSCLC with RO4929097 plus erlotinib. One patient had a partial response, four experienced stable disease at 6 weeks, and median progression-free survival (PFS) was 42 days (64 days in patients with prior progression on erlotinib) [86]. Enrollment of heavily pretreated patients likely selected for aggressive disease and contributed to a lack of notable efficacy in these trials.

Pancreatic Cancer

Interestingly, constituent proteins of the GS tertiary complex, specifically APH1A, are upregulated in malignant relative to normal pancreatic tissue and higher expression may correlate with worse prognosis [87]. Correspondingly, Notch receptors 1–4 and the downstream target Hes1 are significantly increased in human malignant pancreatic tissue [88]. Significantly increased expression in PDAC and known contribution to tumor progression served as rationale for clinical investigation. Eighteen patients with metastatic refractory PDAC were treated with RO4929097 monotherapy in a phase II trial. Among 12 evaluable patients, three experienced stable disease as best response, and the median PFS was 1.5 months. A trend toward decreased HeyL expression was observed on pre- and post-treatment biopsies [89]. Patients with pancreatic cancer included in phase I trials of advanced solid tumors treating with PF-03084014 [85] or MK-0752 [84] similarly did not experience clinical efficacy. Preclinical studies have suggested that antimetastatic activity of GSIs might be improved by combination with gemcitabine, providing rationale for further investigation of this combination in PDAC [90]. One of three patients with pancreatic cancer experienced prolonged stable disease in a phase I trial of RO4929097 plus gemcitabine [91]. However, in a phase I trial of exclusively stage III or IV pancreatic ductal adenocarcinoma, the clinical activity seen with MK-0752 and gemcitabine combination was similar to what would be expected for gemcitabine alone. This led the authors conclude that the combination used does not warrant further evaluation [92].

Melanoma

Twenty-four patients with melanoma were enrolled in a phase I trial of RO4929097 for those with advanced-stage refractory solid tumors. Melanoma was among the most frequently benefited cancers, with one near complete response (by fluorodeoxyglucose–positron emission tomography) and one minor response (by RECIST). A total of four of 24 patients with melanoma were reported to have seen clinical benefit [93]. Following encouraging results, a phase II trial was initiated treating chemotherapy naïve patients with metastatic melanoma with RO4929097 monotherapy. One partial response and eight instances of stable disease were observed in 32 evaluable patients, with a median PFS of 1.5 months. Relative to patients with melanoma treated in the phase I trial, those in the phase II trial received a lower dose of RO4929097, which the authors postulate might contribute to poorer responses [94].

Gliomas

Notch signaling is frequently activated in human gliomas and maintains self-renewal capacity of glioma stem cells [95]. It follows that expression of Notch-1 predicts poor patient survival in proneural and classic glioblastomas [96]. Importantly, enrichment of Notch signaling components correlates with response to GSIs in glioma tumor-initiating cells [97]. These findings served as the basis for clinical development of GSIs in patients with gliomas and other central nervous system (CNS) tumors. In a phase I trial of pediatric patients with CNS tumors refractory to solid tumors, MK-0752 was found to be well tolerated; however, among the nine study patients with gliomas, no objective responses and one prolonged stable disease were observed. The study did identify consistent Hes1 and Hes5 staining across all tumors, suggesting Notch signaling is broadly activated in pediatric brain tumors [98]. A similar phase I trial treating pediatric patients with refractory CNS tumors found that, although MK-0752 decreased NICD1 expression from baseline, the GSI had limited clinical activity. Ultimately, the sponsor withdrew support prior to trial completion [99]. Somewhat more encouraging results were observed when adult patients with gliomas were treated with MK-0752 monotherapy. Here, among 42 patients with gliomas, a complete response of greater than a year was seen in anaplastic astrocytoma, as was stable disease greater than 1 year in glioblastoma multiform. A total of 10 patients with gliomas (24% of study patients with gliomas) experienced stable disease [84].

Rational combination of GSIs with existing treatment strategies has led to improved clinical outcomes. For instance, RO4929097 was used in combination with bevacizumab in an effort to preempt outgrowth of aggressive disease seen in bevacizumab refractory cases. Thirteen patients with malignant gliomas were treated with both agents in a phase I trial and, of 12 evaluable patients, 2 had radiographic responses, a complete response and a partial response. Median overall survival was 10.9 months, with a median PFS of 3.7 months [100]. Alternatively, combination of RO4929097 plus temozolomide and radiotherapy demonstrated a trend toward decreased intratumoral Ki67 staining and significantly decreased NICD1-positive cells, the degree of which correlated with overall survival. GSI alone led to decreased glioma perfusion on dynamic contrast enhanced magnetic resonance imaging and significantly decreased CD133+ cells in tumor explants [101]. Reduced Notch signaling in these trials indicates GSIs can effectively cross the blood brain barrier and reach therapeutic concentrations. As such, further clinical investigations of GSIs in rational combinatorial strategies are warranted in malignant CNS tumors.

Breast Cancer

Oncogenic Notch signaling is also implicated in breast cancer, which represents approximately 30% of all new cancer diagnoses in female patients within the U.S. [102]. Here, increased expression of Notch-1 and Jagged1 have been found to negatively correlate with overall survival [103]. Association of Notch signaling with disease progression led to the investigation of GSIs in clinical trials. A phase I trial of MK-0752 in patients with advanced solid tumors, including 24 patients with breast cancer, demonstrated minimal clinical activity of this GSI in breast cancer [84]. Absence of significant clinical efficacy was also reported in phase I trials treating advanced solid tumors with PF-03084014 [85] and

RO4929097 [82]. Although GSIs are ineffective as a monotherapy, preclinical ability of GSIs to reduce cancer stem cell proliferative capacity and curtail chemoresistance has led to investigation of clinical combinatorial strategies [104]. A phase Ib trial of RO4929097 plus exemestane in 15 patients with estrogen receptor–positive metastatic breast cancer showed limited efficacy with one partial response, six stable disease, and seven progressive disease [105]. Combination of RO4929097 plus gemcitabine has also been investigated in a phase I trial of patients with advanced solid tumors, including five with breast cancer, although efficacy in this cohort was not reported [91]. A phase Ib clinical trial combining PF-03084014 and docetaxel observed moderate clinical efficacy in patients with advanced triple-negative breast cancer, with four partial responses (of 25 evaluable patients) and a median PFS of 4.1 months [106]. Encouragingly, a similar phase I clinical trial of MK-0752 plus docetaxel combination therapy in patients with breast cancer resulted in 11 partial responses, 9 patients with stable disease, and 3 with progressive disease out of 24 evaluable. Combination therapy also reduced breast cancer stem cells and mammosphere-forming efficacy in a subcohort of evaluable patients [107]. Although overall response rates have thus far been rather low, appropriate patient selection and mechanistic guided combinatorial strategies may guide future efficacy of GSIs in breast cancer.

Ovarian Cancer

Cytoplasmic NICD1 is highly expressed in human ovarian cancer and was found to be a poor prognostic factor for overall survival [108]. Following positive results in preclinical studies demonstrating prolonged response to cyclical GS inhibition, similar schedules have been adopted in multiple phase I clinical trials including patients with advanced-stage ovarian cancer resistant to standard therapies. These include therapeutic intervention using RO4929097 [93], LY900009 [109], and MK-0752 [84] alone or with RO4929097 in combination with gemcitabine [91], temsirolimus (mTOR inhibitor) [110], or cediranib (vascular endothelial growth factor inhibitor) [111]. These studies concluded these agents alone and in combination had an acceptable safety profile. Although three of nine patients with advanced ovarian cancer saw clinical benefit from RO4929097 monotherapy [93], the majority in these trials had brief stable or progressive disease. In a phase II study of 45 patients with recurrent or metastatic platinum resistant ovarian cancer, no objective responses were observed, and median progression-free survival was 1.3 months on RO4929097, leading the authors to conclude this GSI monotherapy is not effective in ovarian cancer [112]. Interestingly, inhibition of Notch signaling with enoticumab, a fully human immunoglobulin G1 specific for Dll4, produced one partial response, six with stable disease, and two with reductions in cancer antigen-125 that met response criteria among patients with ovarian cancer [113]. This suggests more specific targeting of Notch signaling, alone or in combination, might be a more favorable approach in this disease.

Colorectal Cancer

NICD1 and NICD3 signaling, denoted by nuclear localization on immunohistochemistry, correlates with recurrence and worse outcomes in patients with stage II and III colon cancer [114], whereas Notch 2 signaling might confer some degree of protection [115]. Downstream of Notch receptors, increased expression of Notch targets Hes1, Hey1, and Sox9 is associated with chemoresistance to 5-FU, upregulated Wnt pathway signaling, metastases at the time of diagnosis, and worse overall survival [116–118]. In support of clinical investigation, the patient-derived xenografts sensitive to PF-03084014 were those with increased Notch and Wnt pathway signaling [119]. In a phase I trial of advanced solid tumors including 11 colorectal cancers, PF-03084014 monotherapy was found to consistently reduce Hes4 expression but gave a median PFS of only 1.6 months [85]. Minimal antitumor activity was also observed in phase I trials of RO4929097 [93], LY900009 [109], MK-0752 [84], and BMS-986115 [120]. RO4929097 monotherapy in patients with metastatic, refractory colorectal cancer in a phase II study found no objective responses, 6 with stable disease, and 21 with progressive disease (a median PFS of 1.8 months), leading investigators to conclude minimal activity of this intervention [121]. Failure as a monotherapy has spurred combinatorial regimens. The combination of RO4929097 plus cediranib in a phase I trial of patients with advanced solid tumors, including 6 colorectal cancers of 20 total participants, found one partial response and prolonged disease stabilization in 11 patients [111]. An investigation of blocking Insulin-like growth factor 1 receptor with dalotuzumab plus MK-0752 was unsuccessful in the colorectal cancer cohort, as all evaluable patients on this combination had disease progression at the first radiologic evaluation [122]. Combination of RO4929097 with capecitabine proved somewhat more encouraging in a phase I evaluation of 30 advanced-stage patients, 18 with colorectal cancer, as patients with colorectal cancer accounted for two of three observed partial responses. The investigators concluded this combination might be promising in fluoropyrimidine-resistant metastatic colorectal cancer [123].

Desmoid Tumors

Although pathogenesis of desmoid tumors is frequently cited to involve perturbations in the Wnt pathway [124], upregulated Notch signaling is increasingly recognized as a contributor to disease progression [125]. In a phase I trial using PF-03084014 for advanced-stage solid tumors refractory to standard therapy, including nine patients with desmoid tumors (seven were evaluable), five patients had partial responses (by RECIST criteria) and two had prolonged disease stabilization [85]. On follow-up of these same patients, all those that achieved a partial response continued to maintain duration of response for 47.9 to 73.6 months, and only one of seven had progressed. The mean clinical benefit of PF-03084014 was 64 months compared with 13 for all prior interventions [126]. Encouraging results were also observed for a single patient with a desmoid tumor when treated with BMS-986115 in a phase I trial [120]. Building upon these results, a phase II trial treating 17 patients with unresectable desmoid tumors having progressed on multiple lines of therapy PF-03084014 was initiated. Of the 16 patients evaluable, 5 (29%) achieved a partial response (by RECIST criteria) and 11 patients experienced stable disease, with no cases of disease progression [127]. Success of the GSI PF-03084014 (Nirogacestat) has spurred the phase III trial in adult patients with desmoid tumors (NCT03785964), recent breakthrough

designation by the U.S. Food and Drug Administration, and orphan drug designation by the European Commission. Unravelling of mechanistic changes imposed by GSIs in these tumors may help translate their promise to other malignancies.

Current Limitations

A substantial limitation to our current understanding of these agents is the paucity of immune competent models used in their study. Although Notch inhibition may impede tumor progression, GSIs might conversely impair the antitumor immune response. CD8 T cells, the primary effectors of antitumor immunity, require Notch signaling for expression of canonical effector molecules, including interferon-γ and granzyme B [128]. Lower levels of Notch-1/2 in murine tumor-infiltrating CD8 T cells then correlated with reduced cytotoxicity, whereas enforced Notch-1 signaling increased cytotoxicity and led to superior tumor control [129]. Furthermore, activation of human T cells [94] and proliferation of murine CD8 T cells can be inhibited by GSIs in a dose-dependent fashion [130]. Regulatory T cells (Tregs), a significant suppressive population within the TME, might also represent a substantial challenge. At baseline, Notch signaling destabilizes the Treg program; therefore, GSIs might promote Treg-mediated suppressive functions and compromise antitumor immunity [131].

Elevated serum levels of interleukin (IL)-6 and IL-8, both signaling through STAT3, correlated with less-favorable patient responses to RO4929097 [132]. Overexpressing these cytokines rendered a sensitive xenograft resistant to GSIs, possibly through abrogated Hes1 downregulation, an effect partially reversed by blocking IL-8 [132]. Cytokine-mediated resistance may be a factor of increased cancer stem cell activation (or formation) and proliferation. In a metastatic model of hormone therapy resistant human breast cancer, upregulation of IL-6 increased pSTAT3 and Notch-3 to increase CD133+ CD44− cancer stem cell renewal. Blockade of IL-6 signaling effectively reversed the stem-like features induced through this signaling cascade [133]. Stromal cells in the TME can also promote this resistance mechanism. Cancer-associated fibroblasts increase cancer stem(-like) cells through induction of IL-6/pSTAT3/Notch signaling in human hepatocellular carcinoma. Nonselective Notch inhibition with RO4929097 or selective Notch-1 inhibition with small interfering RNA impaired cancer-associated fibroblast–induced stemness, as did inhibition of STAT3 phosphorylation [133]. Myeloid derived suppressor cells similarly induced stemness in human breast cancer xenografts, through IL-6 and Notch-2/3–driven STAT3 activation. Pathway inhibition with GSI-I or pSTAT3 inhibition each partially reversed stemness, whereas the combination completely impaired stem cell formation [135]. Although apparently variable, which Notch receptor predominates likely depends on many factors including tissue of origin and Notch equilibria at baseline. Interestingly, in Notch3-expressing breast cancer cells, MK-0752 or RO4929097 inhibited Notch-3, and thereby Hey2, significantly inducing IL-6 and leading to increased numbers of breast cancer stem cells, an effect reversed by coadministration of GS inhibitor and anti–IL-6R [136]. These findings seem to reiterate convergence of IL-6, Notch, and STAT3 signaling; impairment of IL-6 or Notch alone might be able to be rescued by upregulation of the other, whereas blocking both dramatically reduces stemness and tumor proliferation. Rational combination of GS inhibitors with other therapeutic modalities may preempt upregulation of such pathways responsible for eventual tumor resistance. More work is needed to explore the feasibility of incorporating these compounds into clinical treatment strategies as adjuvants.

Future Perspectives

Given the diversity of pathways in which GS participates, nonselective inhibition of this enzyme complex invites off-target toxicity, including disruption of the gastrointestinal epithelium and abnormalities in lymphoid tissues in rodent models [137, 138] and humans [139]. It is also important to consider that certain GSIs might actually promote oncogenic transformation in the skin [140]. It might be that implementation of GSIs requires targeted administration. Specific tumor targeting with liposomes might improve overall utility, and brief cyclical dosing might mitigate impediments to antitumor immunity. Additionally, the structure of GS might lend itself to tailorable inhibition, as the Nct substrate binding site is located approximately 60Å from the catalytic site [29], suggesting potential inhibition at one or both sites. Targeting specific PS isoforms incorporated might then afford a semiselective inhibition of target pathways [141]. Optimization of GSIs should also include investigation of dosage de-escalation, specific treatment schedules, and rational combinatorial strategies [34].

It is important to develop a profile by which to stratify tumors more or less likely to respond to GS inhibition, as treatment of resistant cell lines might actually increase the number of tumor-initiating cells [53]. Although Notch-1 expression alone does not correlate with response to GS inhibition, expression of Notch target genes (including Hes1, Hes4, Hes5, Hey2, HeyL, DTX1, and c-Myc) correlated with response in T-ALL cells [57]. Given this association, neuroendocrine tumors (NETs) may represent a type of cancers in which GSIs are well-suited, as NETs derived from select tissues express Notch components frequently (pancreatic) or uniformly (rectal) [142]. Evaluation of NETs in clinical trials treating with GSIs thus far, however, has been minimal. Ten patients have been included in trials of advanced solid tumors treating with RO4929097 alone or in combination with some encouraging clinical responses [93, 110, 123].

Conclusion

There are many gaps in our knowledge that need to be answered to advance development of GSIs [143]. These agents have repeatedly demonstrated promising preclinical control of tumor progression; however, with the exception of desmoid tumors, this potential has not yet been harnessed clinically. Refinement of tumor Notch expression profiles and further mechanistic understanding of GSIs will necessarily assist in appropriate patient selection. Additionally, rational design of combinatoria'l strategies will maximize the potential of these agents by sensitizing tumors to traditional chemotherapeutics, while also compromising tumor ability to engage treatment resistance programs.

Author Contributions

Conception/design: Tyler R. McCaw, Evelyn Inga, Evelyn Inga, Renata Jaskula-Sztul, Vikas Dudeja, James A. Bibb, Bin Ren, J. Bart Rose
Provision of study material or patients:
Collection and/or assembly of data: Tyler R. McCaw, Evelyn Inga, J. Bart Rose
Data analysis and interpretation:
Manuscript writing: Tyler R. McCaw, Evelyn Inga, Evelyn Inga, Renata Jaskula-Sztul, Vikas Dudeja, James A. Bibb, Bin Ren, J. Bart Rose
Final approval of manuscript: Tyler R. McCaw, Evelyn Inga, Herbert Chen, Renata Jaskula-Sztul, Vikas Dudeja, James A. Bibb, Bin Ren, J. Bart Rose

Disclosures

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