

Targeting γ -secretase in breast cancer

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Abstract: γ -secretase complexes are multisubunit protease complexes that perform the intramembrane cleavage of more than 60 type-I transmembrane proteins, including Notch receptors. Since dysregulated Notch signaling has been implicated in the tumorigenesis and progression of breast cancer, small molecule γ -secretase inhibitors (GSIs) are being tested for their therapeutic potential in breast cancer treatment in several clinical trials. Here, the structure of γ -secretase complex and the development of GSIs are briefly reviewed, the roles of Notch and several other γ -secretase substrates in breast cancer are discussed, and the difference between γ -secretase inhibition and Notch inhibition, as well as the side effects associated with GSIs, are described. A better understanding of molecular mechanisms that affect the responsiveness of breast cancer to GSI might help to develop strategies to enhance the antitumor activity and, at the same time, alleviate the side effects of GSI.

Keywords: γ -secretase, GSI, Notch, breast cancer

Overview of γ -secretase complexes

γ -secretases are intramembrane multisubunit protease complexes that are composed of four core components (presenilin, nicastrin, presenilin enhancer 2 [Pen2], and anterior pharynx-defective 1 [Aph1]) and several associated proteins such as TMP21, CD147, and Rer1p.¹⁻³ Two *presenilin* genes and two *Aph1* genes have been identified in the human genome. Given that the *Aph1a* gene can be expressed in two isoforms due to alternative splicing,⁴ there could be at least six different γ -secretase complexes. It has been shown that γ -secretase complexes containing different presenilin or Aph1 could exhibit distinct activities.^{5,6}

The catalytic subunit presenilins are multipass transmembrane proteins with nine transmembrane domains. The catalytic active site lies between their sixth and seventh transmembrane domains, with two aspartates (D257 and D385 of presenilin 1) conferring the aspartate protease activity to the γ -secretase complex.³ Nicastrin was originally proposed to be the “gatekeeper” of γ -secretase complex,⁷ but a recent study suggested that it functions to stabilize γ -secretase complexes.⁸ Pen2 is believed to be required for endoproteolytic processing of inactive full-length presenilin into the catalytically active heterodimer.⁹ Aph1 was recently shown to associate with γ -secretase substrates even in the absence of intact γ -secretase complex, suggesting that Aph1 may be the substrate receptor for the protease complex.¹⁰ The functions of other associated proteins are even less clear.

Substrates of γ -secretases are a growing family of single-pass type I transmembrane proteins with more than 60 identified members.¹ Notably, these substrates do not

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share a consensus sequence. The only known prerequisite for γ -secretase-mediated cleavage of these substrates is a short (<40 amino acids) extracellular domain. Therefore, a proteolytic processing within the ectodomain performed by a disintegrin and metalloproteinase (ADAM) or other sheddases at a site close to the transmembrane domain usually precedes the γ -secretase-mediated cleavage. While the cleavage of some substrates produces functional free ectodomain or intracellular domain (ICD), the γ -secretase-mediated intramembrane cleavage of other substrates might just serve as a clearance mechanism to regulate the abundance of these transmembrane proteins.^{2,3}

γ -secretase inhibitor (GSI)

Due to their therapeutic potential in the treatment of Alzheimer's disease where γ -secretase-mediated processing of β -amyloid precursor protein generates amyloid- β peptide, the key component of amyloid plaques in Alzheimer's disease,¹¹ considerable effort has been made to develop potent and selective GSIs. As a result, more than 100 GSIs have been synthesized.^{12,13} They can be classified into transition-state analogs and non-transition-state analog inhibitors based on their structure and binding sites. The transition-state analog GSIs are peptide isosteres that mimic the transition state of substrate cleavage by γ -secretase and bind to the catalytic active site of presenilins. The best characterized first generation GSI of this type is L-685458.¹⁴ On the other hand, the binding site for the non-transition-state analog GSIs has yet to be better defined, although a report suggested that they bind to the interface of a γ -secretase complex dimer.¹⁵ The two most widely used first generation non-transition-state analog GSIs are N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT)¹⁶ and Compound E.¹⁷

Recently, new generations of GSIs with enhanced potency and/or improved drug-like properties (such as pharmacokinetics) have been developed and tested in animal studies and clinical trials.¹³ The first potent GSI with drug-like properties is LY-411575, which is structurally similar to – but 100-fold more potent than – DAPT.^{18,19} However, LY-411575 has not been tested in clinical trials although it has been used in several animal studies.^{20,21} In contrast, two GSIs with a structural core derived from LY411575, LY-450139 (semagacestat)^{22,23} and RO4929097,²⁴ have been or are being tested in several clinical trials. In addition to these azepine-containing GSIs, two sulfonamide-containing non-transition-state analog GSIs, MRK003 and MK0752, have been developed by Merck & Co., Inc. (Whitehouse Station, NJ) and the latter is being tested in several clinical trials.

It should be noted that information regarding the specificity of most GSIs is not publicly available. It has been shown that the cytotoxicity of Z-LLNle-CHO, commonly referred as GSI-I from Calbiochem® (EMD Millipore, Billerica, MA), in breast cancer cells is due to proteasome inhibition, but not γ -secretase inhibition.²⁵ Another commonly used GSI from Calbiochem, Z-IL-CHO (GSI-XII), can also suppress the activity of proteasome at concentrations that show cytotoxicity in breast cancer cells (authors' unpublished observation). Although it is unlikely that the new generation non-transition-state analog GSIs that are tested in animal studies and clinical trials have proteasome-inhibitory activity, it has been reported that both L-685458 and LY411575 – but not DAPT – can inhibit the activity of signal peptide peptidases (SPPs), a family of aspartyl proteases that is closely related to γ -secretase complex.²⁶ Fewer than ten SPP substrates have been identified with the significance of SPP-mediated processing largely unknown.²⁷ However, a recent study reported that SPP-like 2-mediated tumor necrosis factor- α signal peptide clearance could modulate the response of breast cancer cells to tumor necrosis factor- α signaling.²⁸ Therefore, observations from treatment with GSIs with unknown specificity should be interpreted cautiously.

In addition to GSIs, γ -secretase activity has also been downregulated by using ribonucleic acid (RNA) interference to knock down nicastrin or by using neutralizing antibody against nicastrin in vitro (Figure 1).^{29,30} These approaches can avoid affecting the activity of γ -secretase-related proteases such as SPP and, therefore, are good complementary methods to examine the outcome of γ -secretase inhibition. Nonetheless, GSI might still be the first choice when it comes to in vivo studies and eventually clinical use due to its convenience to administer.

Notch as γ -secretase substrates in breast cancer

To date, the interest in targeting γ -secretase for cancer treatment stems almost exclusively from the fact that Notch receptors are γ -secretase substrates. Notch receptors are a family of evolutionarily conserved transmembrane proteins and humans have four Notch homologs, Notch1–4. They share similar structural domains, activation process, and mechanism of target gene regulation. Humans also have five classical Notch ligands: Jagged 1, Jagged 2, Delta-like 1 (Dll1), Dll3, and Dll4. In the absence of ligand engagement, cytomembrane-anchored Notch receptors adopt a conformation that masks its ADAM cleavage site. Upon ligand binding, physical force generated from

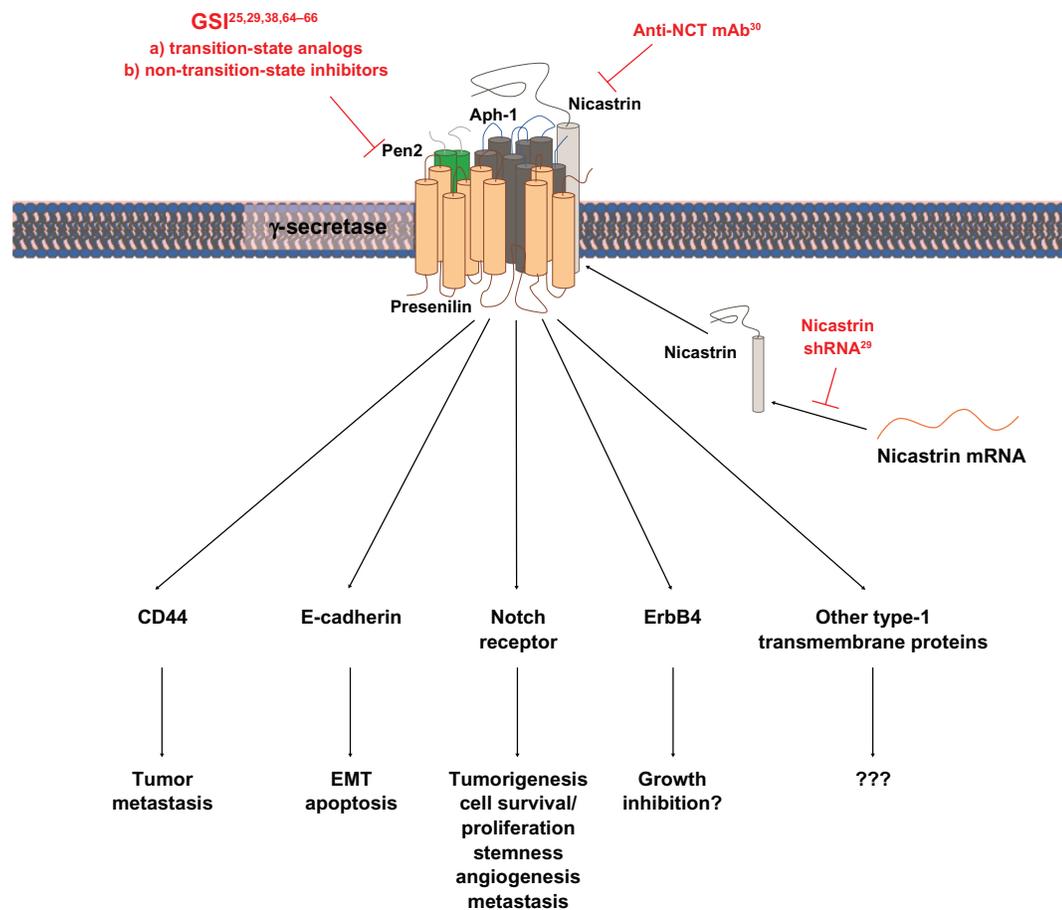


Figure 1 Schematic description of approaches that have been used to suppress γ -secretase activity in breast cancer studies and of signaling pathways that are possibly affected by γ -secretase inhibition.

Note: The reference numbers of relevant studies are indicated.

Abbreviations: Aph-1, anterior pharynx-defective 1; EMT, epithelial-mesenchymal transition; GSI, γ -secretase inhibitor; mAb, monoclonal antibody; mRNA, messenger ribonucleic acid; NCT, nicastrin; Pen2, presenilin enhancer 2; shRNA, small hairpin ribonucleic acid.

ligand endocytosis exposes the ADAM cleavage site. The C-terminal product of the ADAM-mediated cleavage is a constitutive substrate of γ -secretase complex, which releases the active Notch ICD from the cell membrane. Free Notch ICD translocates into the nucleus to interact with the sequence-specific DNA binding protein, CSL (named after C-promoter binding factor 1, Su[H], and Lag-1), mastermind or mastermind-like proteins, and other transcription coactivators to activate the transcription of Notch target genes.³¹ In addition to the well-characterized canonical Notch signaling pathway, noncanonical Notch activities have also been reported.³²

Notch signaling has been implicated in the tumorigenesis and progression of breast cancer and, consequently, has been proposed to be a promising therapeutic target for breast cancer treatment.^{33–36} However, discrepancies among key observations raise concerns over the therapeutic potential of

blocking Notch signaling, especially through GSIs, in breast cancer.³⁷ Encouragingly, a recent study provided compelling evidence that the Notch pathway is dysregulated in a small subset of breast cancers and these cancers are responsive to γ -secretase inhibition.³⁸ Using paired-end transcriptome sequencing, the authors detected *Notch1* or *Notch2* involving chromosomal translocation in three out of 38 breast cancer tissues and five out of 41 breast cancer cell lines. Among these eight translocations, three found in cell lines and one found in breast cancer tissues are expected to result in dysregulated Notch activity. Indeed, all three cell lines showed much higher Notch transcriptional activity. Most importantly, DAPT treatment inhibited the proliferation of the two cell lines that express truncated Notch1 with short extracellular domains C-terminal to the ADAM cleavage site, but not that of the cell line that expresses a truncated Notch2 with no γ -secretase cleavage site as expected.

In addition to regulating cancer cell survival/proliferation cell-autonomously, Notch signaling could also affect the tumor phenotypes through non-cell autonomous mechanisms. The best known example is through angiogenesis, where excessive but nonfunctional angiogenesis triggered by Notch inhibition can suppress tumor growth.^{39,40} A recent study provided another example of non-cell autonomous effects of Notch signaling on breast cancer.⁴¹ Sethi et al showed that tumor-expressed Jagged 1 could promote breast cancer bone metastasis by activating Notch receptors expressed in osteoblasts and osteoclasts. Higher Notch activity in osteoblasts stimulated the release of interleukin-6 from osteoblasts, which in turn promoted the growth of metastatic cancer cells. On the other hand, Notch activation in osteoclasts caused severe osteolysis and consequently provided space for metastatic breast cancer cells to proliferate.⁴¹ This inspiring study raises an intriguing possibility that tumor-expressed Notch ligands might also activate Notch signaling in other tumor stromal cells, such as fibroblasts and immune cells, which in turn promote the survival and proliferation of breast cancer cells. Although this hypothesis remains to be rigorously tested, it is consistent with the observation that the expression level of Notch ligands rather than that of Notch receptors in the breast cancer tissues correlates with clinical outcomes.^{41,42}

Other γ -secretase substrates in breast cancer

In addition to Notch receptors and ligands, more than 50 transmembrane proteins have been identified as substrates for γ -secretase.¹ Among them, several proteins such as ErbB4, E-cadherin, and CD44 are known to have important functions in breast cancer (Figure 1).

ErbB4 receptor tyrosine kinase is a member of epidermal growth factor receptor (EGFR) family. In contrast to the well-documented proproliferative effects of other members of EGFR family, the functions of ErbB4 in breast cancer are open to debate.^{43,44} In some breast cancer cell lines, activation of ErbB4 by its ligand heregulin resulted in its intramembrane cleavage and concomitant growth inhibition.^{45,46} The intramembrane cleavage of ErbB4 is performed by γ -secretase as the expression of dominant-negative presenilin or treatment with Compound E could prevent the generation of free ErbB4 ICD and reverse the growth inhibition induced by heregulin.⁴⁶ The significance of γ -secretase in ErbB4 signaling was further confirmed by later studies.^{47,48} By expressing a mutant ErbB4 (ErbB4-V673I) that does not undergo γ -secretase-mediated cleavage, it was

found that some of the ErbB4-induced gene expression and proapoptotic activity were substantially abolished.⁴⁷ However, it should be noted that ErbB4-V673I still retained some of the ErbB4 activities,⁴⁷ which might even be enhanced due to a possibly higher level of membrane-anchored ErbB4 when its cleavage is blocked. In addition, the abundance of cell surface ErbB4 might affect the signaling of other members of EGFR family by changing their dimeric association with available EGFR family members. Therefore, it cannot yet be concluded whether γ -secretase inhibition is therapeutically disadvantageous from the ErbB4 perspective.

E-cadherin, a cell-cell adhesion molecule, is also a γ -secretase substrate.⁴⁹ As a potent tumor suppressor, partial or complete loss of E-cadherin expression has been associated with the onset of metastasis and poor prognosis in breast cancer patients.⁵⁰ The γ -secretase-mediated cleavage of E-cadherin between Leu731 and Arg732 promotes the disassembly of adherens junctions and increases the level of free cytosolic β -catenin, with the former possibly promoting epithelial-mesenchymal transition and the latter enhancing the Wnt signaling activity.⁴⁹ Significantly, a later study found that C-terminal fragment 2 of E-cadherin generated from γ -secretase-mediated cleavage could also translocate into the nucleus where it modulates the p120/Kaiso-mediated gene regulation and protects cells from staurosporine-induced apoptosis.⁵¹ Furthermore, it was found in another study that exposure of breast cancer T47D cells to cadmium (a toxic heavy metal) induced γ -secretase-mediated E-cadherin cleavage.⁵² Interestingly, this cadmium-induced γ -secretase-mediated E-cadherin cleavage can be blocked by N-acetylcysteine (an antioxidant), suggesting that other reactive oxygen species-producing reagents might also be able to induce γ -secretase-mediated E-cadherin cleavage. In the later study, it was also observed that cadmium exposure enhanced T47D cell motility and migration, an effect that could be inhibited by DAPT. Taking all these observations together, it appears that blocking γ -secretase activity is therapeutically advantageous from the E-cadherin perspective.

CD44 is another γ -secretase substrate that has been associated with breast cancer tumorigenesis and progression.^{53,54} As a major cell adhesion molecule for binding hyaluronan in the extracellular matrix, CD44 has been implicated in cell proliferation, differentiation, and migration in both physiological and pathological conditions.⁵⁵ In breast cancer, CD44 is generally believed to promote tumor metastasis, although the opposite has also been observed.⁵⁶ Although there is no report on the functional consequence of γ -secretase-mediated CD44 cleavage in breast cancer

cells yet, the free ICD (CD44 ICD) generated from such cleavage has been shown to translocate into the nucleus to regulate gene expression,^{54,57} and be capable of transforming rat fibroblast cells.⁵⁸ These observations are corroborated by a recent study, which reported that CD44 ICD enhanced cyclic adenosine monophosphate response element-binding-mediated *cyclin D1* transcription and, consequently, cell proliferation in thyroid tumor cells.⁵⁹ Most importantly, γ -secretase inhibition suppressed the proliferation of thyroid cancer cells, which could be reversed by ectopic expression of CD44 ICD. If these results could be replicated in breast cancer cells, it will undoubtedly provide a strong argument for using GSIs to achieve therapeutic benefits.

In addition to the three proteins discussed above, γ -secretase also regulates many other molecules that might have potential roles in tumorigenesis and disease progression. For example, the ICD of amyloid precursor protein has been shown to bind directly to the promoter of *EGFR*, a receptor tyrosine kinase that is involved in the tumorigenesis and progression of breast cancer, and to suppress its expression.⁶⁰ In addition, cleavage of low-density lipoprotein receptor-related protein 1B by γ -secretase was shown to suppress the anchorage-independent growth of glioma cells,⁶¹ and cleavage of voltage-gated sodium channel β 2-subunit by γ -secretase was reported to regulate cell adhesion and migration.⁶² Although the functional consequences of γ -secretase-mediated cleavage of these substrates in breast cancer cells have not been reported, they should be taken into consideration when developing therapeutics that target γ -secretase.

Targeting γ -secretase versus targeting Notch

Given that several γ -secretase substrates other than Notch receptors also play important roles in the survival, proliferation, and migration of breast cancer cells, it will not be surprising to see that the outcomes of γ -secretase inhibition in breast cancer cells might be different from that of down-regulating Notch activity. Indeed, RNA interference-mediated knockdown of individual Notch receptors or ligands produced stronger cytotoxicity/proliferation inhibition in a large collection of breast cancer cell lines than GSIs,^{25,29,38,63–66} suggesting that blocking the cleavage of some γ -secretase substrates neutralized the tumor-suppressing effect of Notch inhibition. If this is true, identification of this/these substrate(s) may help to improve the therapeutic potential of GSI. However, before this/these γ -secretase substrate(s) can begin to be identified, it is imperative to verify that the difference between Notch inhibition and γ -secretase inhibition is real, as RNA

interference-mediated knockdown often suffers from off-target effects.^{67,68} This can be addressed by testing whether breast cancer cells can be rescued from small interfering RNA-induced proliferation inhibition with expression of small interfering RNA-resistant complementary DNA.

A good start to search for the γ -secretase substrates that functionally antagonize prosurvival Notch signaling, if it turns out to be necessary, is to examine the status of γ -secretase substrates in the two breast cancer cell lines that harbor Notch1 involving translocation.³⁸ Since these two cell lines are responsive to GSI treatment, the expression and/or activity of the γ -secretase substrates, whose reduced cleavage by GSI treatment provides prosurvival/proliferation signal in the GSI nonresponsive breast cancer cell lines, should be significantly downregulated prior to GSI treatment so that its reduced processing by GSI would no longer result in sufficient prosurvival/proliferation signal in these two cell lines. One noticeable feature of these two cell lines is that they grow as weakly adherent culture or in suspension, a sign of reduced expression of cell–cell and/or cell–matrix adhesion molecules. Therefore, it will be interesting to see whether the expression levels of E-cadherin and CD44 are reduced in these two cells.

Side effects from γ -secretase inhibition

Most of the side effects associated with GSI treatment in animal studies or in clinical trials to date can be attributed to reduced Notch activity. Diarrhea was one of the earliest observed side effects of systemic GSI administration in mice.¹⁸ Notch activity is essential for maintaining the population of undifferentiated intestinal stem cells and their differentiation into absorptive enterocytes.⁶⁹ As a result, Notch inhibition resulted in a rapid increase in the number of secretory goblet cells at the expense of absorptive cells, and consequently diarrhea. Consistent with animal studies, gastrointestinal tract disorders were also a prominent side effect observed in a phase II clinical trial of semagacestat in Alzheimer's patients.⁷⁰ Encouragingly, recent studies have demonstrated that the occurrence of diarrhea could be greatly reduced by intermittent dosing or coadministration of glucocorticoid while the antitumor effect was still maintained in T-cell acute lymphoblastic leukemia mouse models.^{71,72} Therefore, it is hopeful that gastrointestinal tract disorders would not be a big barrier to using GSI in patients.

Notch plays multiple roles in regulating immune system development and function, including T-cell lineage commitment, marginal zone B-cell development, and activation

of peripheral T helper 2 cells.⁷³ Therefore, it is expected that blocking Notch signaling will adversely affect the immune defense. However, although GSI treatment caused several immune-related side effects in mice,¹⁸ no increase in the upper respiratory tract and urinary tract infection or seasonal allergy was reported in the phase II clinical trial mentioned above.⁷⁰ Nonetheless, the frequency of immune system disorders in patients receiving GSI treatment should be closely monitored as immunosuppression might help the spread of tumor cells.

The critical role Notch plays in angiogenesis and vascular homeostasis not only can be exploited for therapeutic benefits, but can also cause severe side effects when Notch signaling is blocked for a prolonged time. Dou et al showed in a mouse model that loss of Notch activity accelerated choroidal neovascularization, a common symptom of wet age-related macular degeneration that results in vision loss in elderly people.⁷⁴ Additionally, chronic treatment with Dll4 antibody or gradual loss of Notch1 activity through loss of heterogeneity in mouse models triggered development of vascular tumors and lethal hemorrhage.^{75,76} Of note, vision deterioration occurred in two out of 36 patients receiving semagacestat while none was reported in patients receiving placebo in the phase II clinical trial mentioned above,⁷⁰ although whether vision deterioration was due to abnormal angiogenesis in retina was not reported.

An even more problematic side effect of blocking Notch signaling for cancer treatment is paradoxically an increase in the risk of developing cancers. It has been well established that loss of Notch activity could trigger skin squamous cell carcinoma (SCC) development through creating a tumor-promoting microenvironment.^{77,78} Consistent with mouse studies, an increase in the risk of developing skin cancers was observed in two prematurely terminated phase III clinical trials that tested semagacestat in more than 2600 patients.⁷⁹ In addition to skin SCC, blocking Notch signaling might also increase the risk of developing lung SCC as a recent study suggested that loss-of-function Notch receptor mutation might also contribute to a small population of lung SCC.⁸⁰

Another notable side effect of semagacestat in the failed clinical trials of Alzheimer's disease is the worsening of cognition.⁷⁹ Whether this side effect is specific to Alzheimer's patients or to the general population is not known, but warrants close monitoring. γ -secretase substrates other than Notch might be responsible for this side effect as Notch activity has not been associated with cognitive symptoms in genetic studies before.

Future direction

A recent publication reported by far the most encouraging therapeutic effects of GSI treatment.⁸¹ The authors found that in vitro exposure of primary mammosphere cultures from mammary carcinomas that developed in a transgenic mouse model to MRK003 for 4 days resulted in a dose-dependent decrease in the frequency and the sizes of tumors formed when these primary cultures were injected subcutaneously into syngeneic immune-competent mice. Notably, in cases when a mammosphere that was exposed to a higher dose of MRK003 did develop a tumor, cells prepared from such tumors failed to produce secondary tumors when injected into new hosts, indicating that exposure to MRK003 for 4 days in vitro completely eliminated the long-term tumor initiating cells. More encouragingly, when mice bearing subcutaneous tumors that developed from injected nontreated primary mammosphere culture were treated with MRK003 for two cycles (3 days on followed by 4 days off), tumors in most treated mice completely regressed without recurrence for up to 1 year while the tumors in control mice continued to grow. Although two previous animal studies did not observe such a strong therapeutic effect of MRK003,^{82,83} this recent success indicates that GSI can be effective if the cases are properly selected, and that some yet-to-be-identified factors affect the responsiveness of breast cancer to GSI treatment and need to be investigated. Through identifying – and modulating the activities of – these contributing factors, it is hopeful that the antitumor activity of GSIs can be potentiated, and its side effects alleviated, so a good therapeutic window can be found for its use in breast cancer patients.

Disclosure

The authors report no conflicts of interest in this work.

References

1. McCarthy JV, Twomey C, Wujek P. Presenilin-dependent regulated intramembrane proteolysis and gamma-secretase activity. *Cell Mol Life Sci.* 2009;66(9):1534–1555.
2. De Strooper B, Annaert W. Novel research horizons for presenilins and γ -secretases in cell biology and disease. *Annu Rev Cell Dev Biol.* 2010;26:235–260.
3. Jorissen E, De Strooper B. Gamma-secretase and the intramembrane proteolysis of Notch. *Curr Top Dev Biol.* 2010;92:201–230.
4. Gu Y, Chen F, Sanjo N, et al. APH-1 interacts with mature and immature forms of presenilins and nicastrin and may play a role in maturation of presenilin, nicastrin complexes. *J Biol Chem.* 2003;278(9):7374–7380.
5. Lai MT, Chen E, Crouthamel MC, et al. Presenilin-1 and presenilin-2 exhibit distinct yet overlapping gamma-secretase activities. *J Biol Chem.* 2003;278(25):22475–22481.
6. Serneels L, Van Biervliet J, Craessaerts K, et al. Gamma-secretase heterogeneity in the Aph1 subunit: relevance for Alzheimer's disease. *Science.* 2009;324(5927):639–642.

7. Shah S, Lee SF, Tabuchi K, et al. Nicastrin functions as a gamma-secretase-substrate receptor. *Cell*. 2005;122(3):435–447.
8. Zhao G, Liu Z, Ilagan MX, Kopan R. Gamma-secretase composed of PS1/Pen2/Aph1a can cleave notch and amyloid precursor protein in the absence of nicastrin. *J Neurosci*. 2010;30(5):1648–1656.
9. Takasugi N, Tomita T, Hayashi I, et al. The role of presenilin cofactors in the gamma-secretase complex. *Nature*. 2003;422(6930):438–441.
10. Chen AC, Guo LY, Ostaszewski BL, Selkoe DJ, LaVoie MJ. Aph-1 associates directly with full-length and C-terminal fragments of gamma-secretase substrates. *J Biol Chem*. 2010;285(15):11378–11391.
11. Haass C, Steiner H. Alzheimer disease gamma-secretase: a complex story of GxGD-type presenilin proteases. *Trends Cell Biol*. 2002;12(12):556–562.
12. Olson RE, Albright CF. Recent progress in the medicinal chemistry of gamma-secretase inhibitors. *Curr Top Med Chem*. 2008;8(1):17–33.
13. Kreft AF, Martone R, Porte A. Recent advances in the identification of gamma-secretase inhibitors to clinically test the Abeta oligomer hypothesis of Alzheimer's disease. *J Med Chem*. 2009;52(20):6169–6188.
14. Shearman MS, Behr D, Clarke EE, et al. L-685,458, an aspartyl protease transition state mimic, is a potent inhibitor of amyloid beta-protein precursor gamma-secretase activity. *Biochemistry*. 2000;39(30):8698–8704.
15. Clarke EE, Churcher I, Ellis S, et al. Intra- or intercomplex binding to the gamma-secretase enzyme. A model to differentiate inhibitor classes. *J Biol Chem*. 2006;281(42):31279–31289.
16. Dovey HF, John V, Anderson JP, et al. Functional gamma-secretase inhibitors reduce beta-amyloid peptide levels in brain. *J Neurochem*. 2001;76(1):173–181.
17. Seiffert D, Bradley JD, Rominger CM, et al. Presenilin-1 and -2 are molecular targets for gamma-secretase inhibitors. *J Biol Chem*. 2000;275(44):34086–34091.
18. Wong GT, Manfra D, Poulet FM, et al. Chronic treatment with the gamma-secretase inhibitor LY-411,575 inhibits beta-amyloid peptide production and alters lymphopoiesis and intestinal cell differentiation. *J Biol Chem*. 2004;279(13):12876–12882.
19. Lewis HD, Perez Revuelta BI, Nadin A, et al. Catalytic site-directed gamma-secretase complex inhibitors do not discriminate pharmacologically between Notch S3 and beta-APP cleavages. *Biochemistry*. 2003;42(24):7580–7586.
20. Lanz TA, Hosley JD, Adams WJ, Merchant KM. Studies of Abeta pharmacodynamics in the brain, cerebrospinal fluid, and plasma in young (plaque-free) Tg2576 mice using the gamma-secretase inhibitor N2-[(2S)-2-(3,5-difluorophenyl)-2-hydroxyethanoyl]-N1-[(7S)-5-methyl-6-oxo-6,7-di hydro-5H-dibenzo[b,d]azepin-7-yl]-L-alaninamide (LY-411575). *J Pharmacol Exp Ther*. 2004;309(1):49–55.
21. Hyde LA, McHugh NA, Chen J, et al. Studies to investigate the in vivo therapeutic window of the gamma-secretase inhibitor N2-[(2S)-2-(3,5-difluorophenyl)-2-hydroxyethanoyl]-N1-[(7S)-5-methyl-6-oxo-6,7-di hydro-5H-dibenzo[b,d]azepin-7-yl]-L-alaninamide (LY-411,575) in the CRND8 mouse. *J Pharmacol Exp Ther*. 2006;319(3):1133–1143.
22. Siemers E, Skinner M, Dean RA, et al. Safety, tolerability, and changes in amyloid beta concentrations after administration of a gamma-secretase inhibitor in volunteers. *Clin Neuropharmacol*. 2005;28(3):126–132.
23. Henley DB, May PC, Dean RA, Siemers ER. Development of semagacestat (LY450139), a functional gamma-secretase inhibitor, for the treatment of Alzheimer's disease. *Expert Opin Pharmacother*. 2009;10(10):1657–1664.
24. Luistro L, He W, Smith M, et al. Preclinical profile of a potent gamma-secretase inhibitor targeting notch signaling with in vivo efficacy and pharmacodynamic properties. *Cancer Res*. 2009;69(19):7672–7680.
25. Han J, Ma I, Hendzel MJ, Allalunis-Turner J. The cytotoxicity of gamma-secretase inhibitor I to breast cancer cells is mediated by proteasome inhibition, not by gamma-secretase inhibition. *Breast Cancer Res*. 2009;11(4):R57.
26. Weihofen A, Lemberg MK, Friedmann E, et al. Targeting presenilin-type aspartic protease signal peptide peptidase with gamma-secretase inhibitors. *J Biol Chem*. 2003;278(19):16528–16533.
27. Golde TE, Wolfe MS, Greenbaum DC. Signal peptide peptidases: a family of intramembrane-cleaving proteases that cleave type 2 transmembrane proteins. *Semin Cell Dev Biol*. 2009;20(2):225–230.
28. Yan D, Qin N, Zhang H, et al. Expression of TNF-alpha leader sequence renders MCF-7 tumor cells resistant to the cytotoxicity of soluble TNF-alpha. *Breast Cancer Res Treat*. 2009;116(1):91–102.
29. Dong Y, Li A, Wang J, Weber JD, Michel LS. Synthetic lethality through combined Notch-epidermal growth factor receptor pathway inhibition in basal-like breast cancer. *Cancer Res*. 2010;70(13):5465–5474.
30. Filipovic A, Gronau JH, Green AR, et al. Biological and clinical implications of nicastrin expression in invasive breast cancer. *Breast Cancer Res Treat*. 2011;125(1):43–53.
31. Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell*. 2009;137(2):216–233.
32. Heitzler P. Biodiversity and noncanonical Notch signaling. *Curr Top Dev Biol*. 2010;92:457–481.
33. Al-Hussaini H, Subramanyam D, Reedijk M, Sridhar SS. Notch signaling pathway as a therapeutic target in breast cancer. *Mol Cancer Ther*. 2011;10(1):9–15.
34. Rizzo P, Osipo C, Foreman K, Golde T, Osborne B, Miele L. Rational targeting of Notch signaling in cancer. *Oncogene*. 2008;27(38):5124–5131.
35. Shi W, Harris AL. Notch signaling in breast cancer and tumor angiogenesis: cross-talk and therapeutic potentials. *J Mammary Gland Biol Neoplasia*. 2006;11(1):41–52.
36. Harrison H, Farnie G, Brennan KR, Clarke RB. Breast cancer stem cells: something out of notching? *Cancer Res*. 2010;70(22):8973–8976.
37. Han J, Hendzel MJ, Allalunis-Turner J. Notch signaling as a therapeutic target for breast cancer treatment? *Breast Cancer Res*. 2011;13(3):210.
38. Robinson DR, Kalyana-Sundaram S, Wu YM, et al. Functionally recurrent rearrangements of the MAST kinase and Notch gene families in breast cancer. *Nat Med*. 2011;17(12):1646–1651.
39. Ridgway J, Zhang G, Wu Y, et al. Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. *Nature*. 2006;444(7122):1083–1087.
40. Noguera-Troise I, Daly C, Papadopoulos NJ, et al. Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. *Nature*. 2006;444(7122):1032–1037.
41. Sethi N, Dai X, Winter CG, Kang Y. Tumor-derived JAGGED1 promotes osteolytic bone metastasis of breast cancer by engaging notch signaling in bone cells. *Cancer Cell*. 2011;19(2):192–205.
42. Xing F, Okuda H, Watabe M, et al. Hypoxia-induced Jagged2 promotes breast cancer metastasis and self-renewal of cancer stem-like cells. *Oncogene*. 2011;30(39):4075–4086.
43. Sundvall M, Iljin K, Kilpinen S, Sara H, Kallioniemi OP, Elenius K. Role of ErbB4 in breast cancer. *J Mammary Gland Biol Neoplasia*. 2008;13(2):259–268.
44. Chuu CP, Chen RY, Barking JL, Ciaccio MF, Jones RB. Systems-level analysis of ErbB4 signaling in breast cancer: a laboratory to clinical perspective. *Mol Cancer Res*. 2008;6(6):885–891.
45. Sartor CI, Zhou H, Kozlowska E, et al. Her4 mediates ligand-dependent antiproliferative and differentiation responses in human breast cancer cells. *Mol Cell Biol*. 2001;21(13):4265–4275.
46. Ni CY, Murphy MP, Golde TE, Carpenter G. Gamma-secretase cleavage and nuclear localization of ErbB-4 receptor tyrosine kinase. *Science*. 2001;294(5549):2179–2181.
47. Vidal GA, Naresh A, Marrero L, Jones FE. Presenilin-dependent gamma-secretase processing regulates multiple ERBB4/HER4 activities. *J Biol Chem*. 2005;280(20):19777–19783.
48. Feng SM, Sartor CI, Hunter D, et al. The HER4 cytoplasmic domain, but not its C terminus, inhibits mammary cell proliferation. *Mol Endocrinol*. 2007;21(8):1861–1876.
49. Marambaud P, Shioi J, Serban G, et al. A presenilin-1/gamma-secretase cleavage releases the E-cadherin intracellular domain and regulates disassembly of adherens junctions. *EMBO J*. 2002;21(8):1948–1956.

50. Berx G, Van Roy F. The E-cadherin/catenin complex: an important gatekeeper in breast cancer tumorigenesis and malignant progression. *Breast Cancer Res.* 2001;3(5):289–293.
51. Ferber EC, Kajita M, Wadlow A, et al. A role for the cleaved cytoplasmic domain of E-cadherin in the nucleus. *J Biol Chem.* 2008;283(19):12691–12700.
52. Park CS, Kim OS, Yun SM, Jo SA, Jo I, Koh YH. Presenilin 1/gamma-secretase is associated with cadmium-induced E-cadherin cleavage and COX-2 gene expression in T47D breast cancer cells. *Toxicol Sci.* 2008;106(2):413–422.
53. Lammich S, Okochi M, Takeda M, et al. Presenilin-dependent intramembrane proteolysis of CD44 leads to the liberation of its intracellular domain and the secretion of an Abeta-like peptide. *J Biol Chem.* 2002;277(47):44754–44759.
54. Murakami D, Okamoto I, Nagano O, et al. Presenilin-dependent gamma-secretase activity mediates the intramembranous cleavage of CD44. *Oncogene.* 2003;22(10):1511–1516.
55. Marhaba R, Zoller M. CD44 in cancer progression: adhesion, migration and growth regulation. *J Mol Histol.* 2004;35(3):211–231.
56. Louderbough JM, Schroeder JA. Understanding the dual nature of CD44 in breast cancer progression. *Mol Cancer Res.* 2011;9(12):1573–1586.
57. Okamoto I, Kawano Y, Murakami D, et al. Proteolytic release of CD44 intracellular domain and its role in the CD44 signaling pathway. *J Cell Biol.* 2001;155(5):755–762.
58. Pelletier L, Guillaumot P, Freche B, et al. Gamma-secretase-dependent proteolysis of CD44 promotes neoplastic transformation of rat fibroblastic cells. *Cancer Res.* 2006;66(7):3681–3687.
59. De Falco V, Tamburrino A, Ventre S, et al. CD44 proteolysis increases CREB phosphorylation and sustains proliferation of thyroid cancer cells. *Cancer Res.* 2012;72(6):1449–1458.
60. Zhang YW, Wang R, Liu Q, Zhang H, Liao FF, Xu H. Presenilin/gamma-secretase-dependent processing of beta-amyloid precursor protein regulates EGF receptor expression. *Proc Natl Acad Sci U S A.* 2007;104(25):10613–10618.
61. Liu CX, Ranganathan S, Robinson S, Strickland DK. Gamma-secretase-mediated release of the low density lipoprotein receptor-related protein 1B intracellular domain suppresses anchorage-independent growth of neuroglioma cells. *J Biol Chem.* 2007;282(10):7504–7511.
62. Kim DY, Ingano LA, Carey BW, Pettingell WH, Kovacs DM. Presenilin/gamma-secretase-mediated cleavage of the voltage-gated sodium channel beta2-subunit regulates cell adhesion and migration. *J Biol Chem.* 2005;280(24):23251–23261.
63. Yamaguchi N, Oyama T, Ito E, et al. NOTCH3 signaling pathway plays crucial roles in the proliferation of ErbB2-negative human breast cancer cells. *Cancer Res.* 2008;68(6):1881–1888.
64. Rizzo P, Miao H, D'Souza G, et al. Cross-talk between notch and the estrogen receptor in breast cancer suggests novel therapeutic approaches. *Cancer Res.* 2008;68(13):5226–5235.
65. Osipo C, Patel P, Rizzo P, et al. ErbB-2 inhibition activates Notch-1 and sensitizes breast cancer cells to a gamma-secretase inhibitor. *Oncogene.* 2008;27(37):5019–5032.
66. Yong T, Sun A, Henry MD, Meyers S, Davis JN. Down regulation of CSL activity inhibits cell proliferation in prostate and breast cancer cells. *J Cell Biochem.* 2011;112(9):2340–2351.
67. Jackson AL, Bartz SR, Schelter J, et al. Expression profiling reveals off-target gene regulation by RNAi. *Nat Biotechnol.* 2003;21(6):635–637.
68. Scacheri PC, Rozenblatt-Rosen O, Caplen NJ, et al. Short interfering RNAs can induce unexpected and divergent changes in the levels of untargeted proteins in mammalian cells. *Proc Natl Acad Sci U S A.* 2004;101(7):1892–1897.
69. van Es JH, van Gijn ME, Riccio O, et al. Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature.* 2005;435(7044):959–963.
70. Fleisher AS, Raman R, Siemers ER, et al. Phase 2 safety trial targeting amyloid beta production with a gamma-secretase inhibitor in Alzheimer disease. *Arch Neurol.* 2008;65(8):1031–1038.
71. Tammam J, Ware C, Efferson C, et al. Down-regulation of the Notch pathway mediated by a gamma-secretase inhibitor induces anti-tumour effects in mouse models of T-cell leukaemia. *Br J Pharmacol.* 2009;158(5):1183–1195.
72. Real PJ, Tosello V, Palomero T, et al. Gamma-secretase inhibitors reverse glucocorticoid resistance in T cell acute lymphoblastic leukemia. *Nat Med.* 2009;15(1):50–58.
73. Radtke F, Fasnacht N, Macdonald HR. Notch signaling in the immune system. *Immunity.* 2010;32(1):14–27.
74. Dou GR, Wang YC, Hu XB, et al. RBP-J, the transcription factor downstream of Notch receptors, is essential for the maintenance of vascular homeostasis in adult mice. *FASEB J.* 2008;22(5):1606–1617.
75. Yan M, Callahan CA, Beyer JC, et al. Chronic DLL4 blockade induces vascular neoplasms. *Nature.* 2010;463(7282):E6–E7.
76. Liu Z, Turkoz A, Jackson EN, et al. Notch1 loss of heterozygosity causes vascular tumors and lethal hemorrhage in mice. *J Clin Invest.* 2011;121(2):800–808.
77. Demehri S, Turkoz A, Kopan R. Epidermal Notch1 loss promotes skin tumorigenesis by impacting the stromal microenvironment. *Cancer Cell.* 2009;16(1):55–66.
78. Nicolas M, Wolfer A, Raj K, et al. Notch1 functions as a tumor suppressor in mouse skin. *Nat Genet.* 2003;33(3):416–421.
79. Extnance A. Alzheimer's failure raises questions about disease-modifying strategies. *Nat Rev Drug Discov.* 2010;9(10):749–751.
80. Wang NJ, Sanborn Z, Arnett KL, et al. Loss-of-function mutations in Notch receptors in cutaneous and lung squamous cell carcinoma. *Proc Natl Acad Sci U S A.* 2011;108(43):17761–17766.
81. Kondratyev M, Kreso A, Hallett RM, et al. Gamma-secretase inhibitors target tumor-initiating cells in a mouse model of ERBB2 breast cancer. *Oncogene.* 2012;31(1):93–103.
82. Efferson CL, Winkelmann CT, Ware C, et al. Downregulation of Notch pathway by a gamma-secretase inhibitor attenuates AKT/mammalian target of rapamycin signaling and glucose uptake in an ERBB2 transgenic breast cancer model. *Cancer Res.* 2010;70(6):2476–2484.
83. Watters JW, Cheng C, Majumder PK, et al. De novo discovery of a gamma-secretase inhibitor response signature using a novel in vivo breast tumor model. *Cancer Res.* 2009;69(23):8949–8957.

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