Send Orders for Reprints to reprints@benthamscience.net

Current Drug Targets, XXXX, *XX*, 1-10

REVIEW ARTICLE

Gamma Secretase Inhibitor: Therapeutic Target *via* NOTCH Signaling in T Cell Acute Lymphoblastic Leukemia

Bhupendra Kaushik[1], Dilipkumar Pal[2,*] and Supriyo Saha[3]

[1]*New Product Development (R&D) Leader, 42, Kenneth road, RM66LL London, UK;* [2]*Department of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya (A Central University), Bilaspur, C.G., 495 009, India;* [3]*School of Pharmaceutical Sciences & Technology, Sardar Bhagwan Singh University, Dehradun-248161, Uttarakhand, India*

Abstract: ***Background*:** T-cell acute lymphoblastic leukemia (T-ALL) is a diseased condition of bone marrow and lymphoblast, mainly expressed on T-cell immune phenotype. Diagnosis of T-ALL patients shows the burden of a large tumour and leukemia cells in the peripheral blood vessel which often infiltrates into the central nervous system.

***Objective*:** Chemotherapy is considered the primary mode of treatment for this disease, but recent advancements in the molecular understanding of the disease, including NOTCH1 signaling, could offer some alternatives. NOTCH signaling undergoes a non-regulated mutation at NOTCH1 in most T-ALL. Gamma-secretase (GS) plays a key role in blocking of proteolytic activation of NOTCH receptors, which could potentially be a new targeted therapy for this type of leukaemia. This study mainly aims to outline the role of γ-secretase inhibitors *via* NOTCH signaling in T-ALL.

***Results*:** The role of GSI (γ-secretase inhibitor) in most T-ALL cell lines has been linked to the targeting pathway of NOTCH signaling. Mutation at NOTCH1 has however not served as a predictor of γ-secretase inhibitor sensitivity because of several factors, including gene expression of NOTCH pathway activity. Therefore, despite the promising outcome of this approach in NOTCH-1 activated T-ALL, not all patients with this condition are expected to respond.

***Conclusion*:** Long-term therapeutic success against cancerous cells is rarely achieved with monotherapy, and even targeting investigational pathways such as NOTCH may require a combination regimen. Ultimately, the optimised use of these new therapeutic agents may become the next tool in our search for an effective 'individualized medicine'.

ARTICLE HISTORY

Received: August 24, 2020
Revised: December 11, 2020
Accepted: December 18, 2020

DOI:
10.2174/1389450122666210203192752

Keywords: γ-secretase inhibitor, T-ALL, NOTCH1, mutation, cancer, targeted therapy.

1. INTRODUCTION

T-cell acute lymphoblastic leukemia (T-ALL) is a condition that can be attributed to the genetic makeup of an individual [1]. The condition has been described as an extremely aggressive hematologic malignancy, often resulting in infiltration of bone marrow with immature lymphoblasts that express as a T-cell immune phenotype [2]. The condition is normally very common among the paediatric population, and mainly characterized by tumor burden. The disease has reported to exhibit leukemic cells circulating in large numbers in the bloodstream [3]. Inflammation of the central nervous system and multiplication of tumor cells have also been associated with the condition [4]. Several studies have been undertaken to determine the most effective treatment methods for this disease [5]. The studies range from the early stages of diagnosis to treatment trials that have been conducted to establish the biological control of the tumor cells [6]. Studies conducted on treatment methods of the disease have shown that a combination of chemotherapies and other methods exhibits a high risk of relapse, primarily because of its mechanism of improving the survival rates of B-precursor cells. These studies displayed about 10% of cure level after treatment [7]. Other studies have revealed that molecular targeting of specifics signals along with chemotherapy has the potential for improving patient recovery by 75% in children and 50% in adults [8]. Historically, to develop more effective anti-leukemia drugs, researchers mainly focused on reducing the toxicity levels associated with chemotherapy [9]. Some researchers have, however, also focused on the identification of anti-NOTCH1 therapy that could be evaluated for the treatment. This study offers a review on the role of γ-secretase inhibitor (GSI) *via* NOTCH signaling in T-ALL [10].

2. γ-SECRETASE (GS)

γ-secretase has been described as a protease enzyme that attaches to the transmembrane domain of a substrate and

*Address correspondence to this author at the Department of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya (A Central University), Bilaspur, C.G., 495 009, India; Tel: (+91) 7389263761;
E-mail: drdilip2003@yahoo.com

1389-4501/XX $65.00+.00

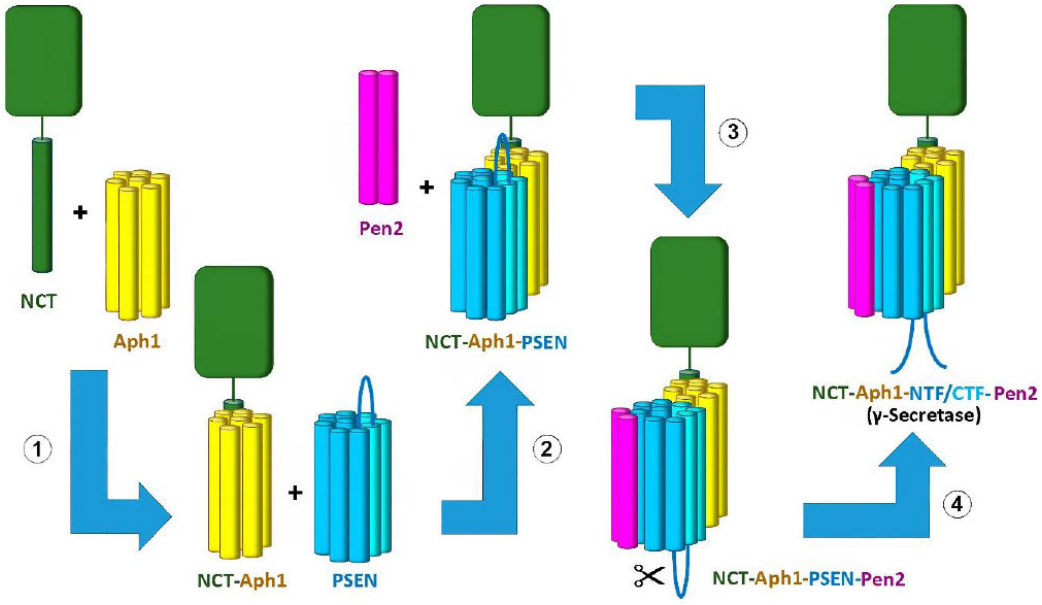

Fig. (1). Assembly and activation of the γ-secretase complex [Copyright permission granted from American Chemical Society]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

then forms multiple subunits. The enzyme is part of a membrane structure, which mainly comprises the following proteins: presenilin, nicastrin, APH-1 and PEN-2 [11]. Presenilin is known for its major role in catalyzing the mutagenesis in genes as well as induction of cell activity. It is worth noting that γ-secretase complex has proteins that often undergo proteolysis at N and C terminals [12]. Studies on the activity of γ-secretase have also shown that the enzyme can interact with γ-secretase activated proteins to form a cleavage called amyloid precursor protein [13]. The enzyme also promotes apoptosis within mitochondria and proteolysis in the endoplasmic reticulum (Fig. **1**) [14, 15]. The cleavage in the amyloid beta amino acid complex transformed the amyloid plaques into fibres (Fig. **2**) [16]. The enzyme is also critical for catalysis of other integral membrane proteins, which include NOTCH, B2 and cluster of differentiation (CD)44 [17, 18]. The main function of the enzyme γ-secretase is the activation of NOTCH1 during the signaling process (Fig. **3**).

3. NOTCH SIGNALING PATHWAY

This is an activation process whereby a ligand binds with a NOTCH receptor to complete signaling pathway for transmission [19, 20]. Studies have shown that mammals have four types of NOTCH receptors which include NOTCH (1-4) and five ligands. The ligands associated with the NOTCH receptor include delta-like ligand 1 (DLL1), delta-like ligand 3 (DLL3), delta-like ligand 4 (DLL4), Jagged-1 (JAG1) and Jagged-2 (JAG2) ligands [21]. NOTCH signaling occurs through a signaling pathway which normally comprises three components [22]. The first component comprises Delta-like 1, 3 and 4 ligands and Jagged 1 and 2 ligands [23] (Fig. **4**). It is essential to note that these ligands are membrane proteins and normally occur at surfaces of signaling cells [24]. Another component of NOTCH signaling is the NOTCH1 receptor, while the third component includes the C-protein binding factor 1 (CBF1) and lag1 DNA binding protein. NOTCH pathway shows that NOTCH1 receptor is activated because of its association with Delta-like and jagged ligands. The ligand-receptor interaction leads to a change in NOTCH1 Lin-12/NOTCH repeat (LNR) and domain dissociation [25, 26]. The receptor is split by γ-secretase complex with other activators of NOTCH1 target genes [27]. NOTCH1 has been found to have a number of epithelial growth factors responsible for ligands receptor interaction. NOTCH1 also consists of a negative regulatory LNR region and a heterodimerization (HD) domain. The HD domain comprises an extracellular subunit receptor and a C-terminal component, which lies on transmembrane and intracellular subunits of NOTCH1 [28, 29]. The role of LRN-HD is to hold these two subunits of the receptor and keep NOTCH1 in a resting place [30]. LRN-HD also plays the role of folding over the HD domain in the absence of a ligand to form a molecular lock which helps to stabilize the interactions between the subunits. During this process, NOTCH1 undergoes a conformational change which destabilizes the interface of the subunits uncovering the C-terminal HD subunit, hence exposing it as a substrate for ADAM10. ADAM10 is a metalloprotease which is normally expressed on the cell surface, resulting in the cleavage of the extracellular (S2) part of the receptor. S2 is composed of 12 to 13 amino acids, which is located outside the plasma membrane. The cleavage of S2 induces the processing of NOTCH1 by the γ-secretase complex. γ-secretase has been described earlier as an enzyme which catalyzes the proteolysis of final endomembrane (S3) cleavage [31]. γ-secretase inhibitor cleaves the intracellular domain of NOTCH1 (ICN1) and translocates to the nuclear forming complexes, such as

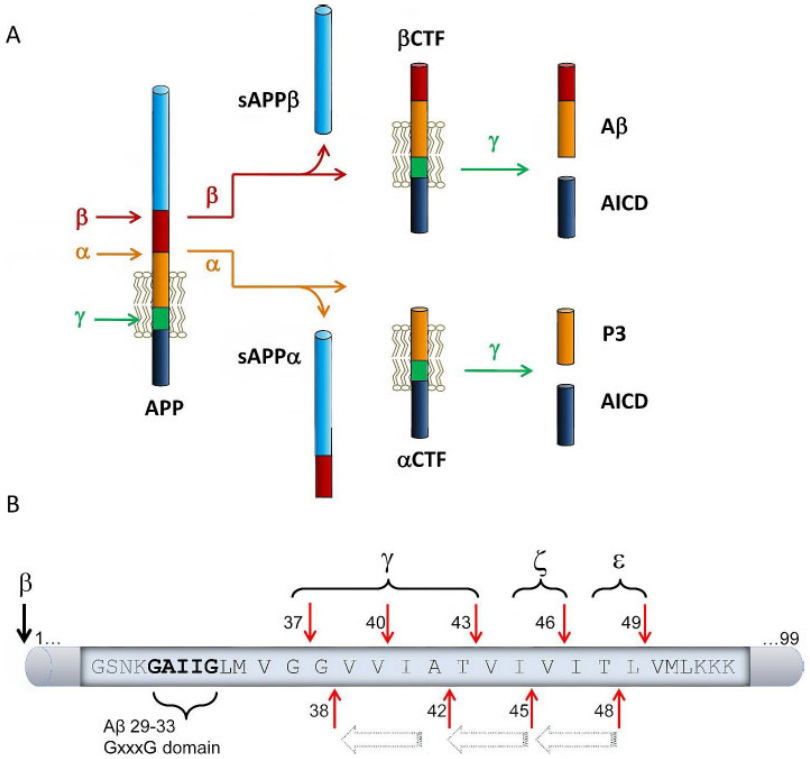

Fig. (2). (**A, B**) Illustration of APP processing by α-, β-, and γ-secretases, and the corresponding products assay [Copyright permission granted from American Chemical Society]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

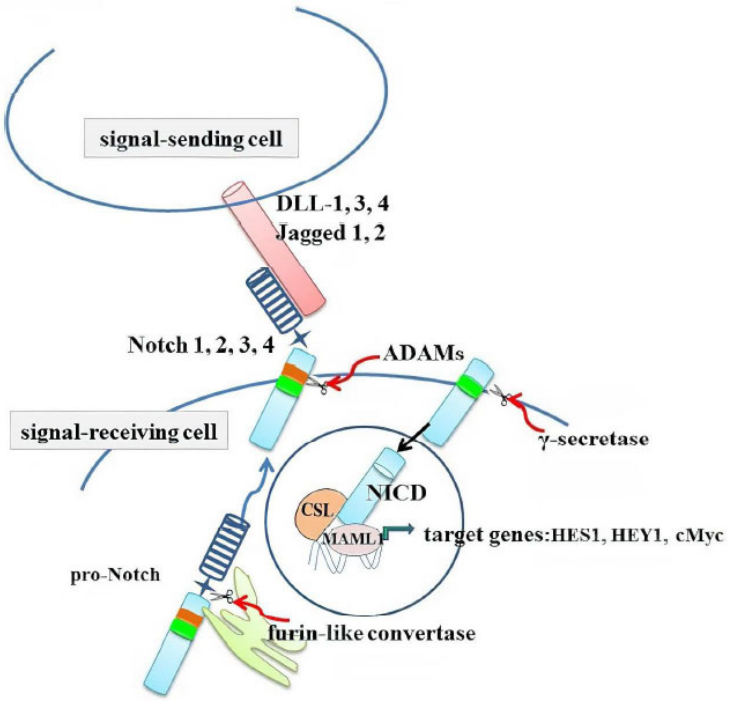

Fig. (3). Notch receptors and ligands: canonical activation and signal transduction pathways [Copyright permission granted from American Chemical Society]. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

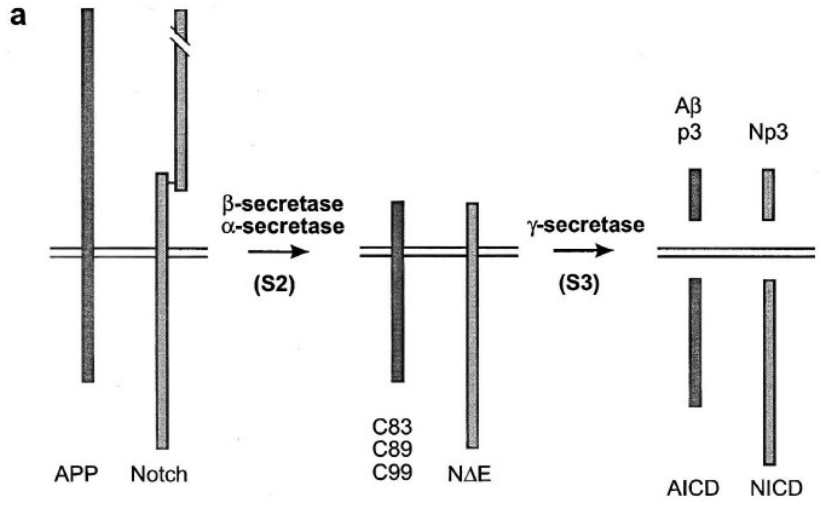

Fig. (4). Notch proteolysis and optimization of an *in vitro* gamma-secretase activity assay. [Copyright permission granted from American Chemical Society].

CBF-1, suppressor of hairless, Lag-2 (CSL) DNA binding protein. NOTCH1 CBF-1, suppressor of hairless, Lag-2 (CSL) target gene are normally activated by histone deacetylase and other transcriptional co-repressors (Fig. **3**). It is worth noting that for every transcriptional activation, a termination of NOTCH1 signaling through proteasomal degradation of the intracellular domain of NOTCH1 (ICN1) occurs [32]. It is also vital to observe that the NOTCH signalling pathway is significant for specific hematopoietic progenitors linked to T-cell lineage. That is why studies conducted on immunodeficient mice transplanted with precursor cells show the expression of an active form of NOTCH1 in T-cell, which is unable to form B-Cells. Studies also show that inactivation of NOTCH1 in bone marrow always leads to blockage of the development of T-cell but promotes their differentiation towards B-cell lineage. NOTCH1 signaling processes play a significant role in the proliferation and survival of thymocytes that form lineage between αβ and γδ T-cell. NOTCH signaling equally plays a key role in the regulation and development of stem cells. As a result, there is a loss of B-cells that reduces intestinal stem cell growth with increased multiplication of goblet cells. Findings also show that inhibition of the NOTCH signaling pathway could lead to an increase in the differentiation of somatic cells followed by the formation of pluripotent factor lack human stem cells [33].

4. MUTATIONS IN THE NOTCH1 GENE

Mutation is defined as a change in the structure of a gene or a chromosome that may result in a genetic disorder. NOTCH1 activation in T-cell is linked with oncogenesis in human cells, which leads to leukaemia. Several studies have demonstrated NOTCH1 signaling in human T-ALL that is always defined by the translocation of these cells. However, recent studies show that the disease is caused by mutations of the NOTCH1 gene, which affects the T-ALL cells. An analysis of several T-ALL cell samples found a mutation of NOTCH1 which results from the expression of transcription factor oncogenes like transcription factors 1 and 2. These mutations are mainly associated with activation of NOTCH1, which plays a vital part in T-ALL oncogenesis [34].

The above-mentioned diagram shows that mutation on the NOTCH1 HD domain increases ICN1 levels through the induction of ligand through the NOTCH1 signaling process. It is also important to understand the process resulting in the oncogenesis of NOTCH1 signaling in T-ALL, which will help to describe how inhibition of these cells is affected by GS (Table **1**).

Table 1. Oncogenic events leading to NOTCH1 signaling in T-ALL.

Mutation	Mechanism of Action	Prevalence in T-ALL
NOTCH 1 GENES	NOTCH1 truncation along with translocation to the TCR locus. Overexpression combined with ligand-independent activation	1%
NOTCH1 HD class I	Ligand hypersensitivity or the independent activation	40–45%
NOTCH1 HD class II	Ligand hypersensitivity	1%
NOTCH1 JME	Ligand hypersensitivity or independent activation	3%
NOTCH1 ΔPEST	Activated NOTCH1 protein stability increases	10–20%
NOTCH 1 mutations	NOTCH1, c-MYC, Cyclin E, JUN along with mTOR protein stability increase	15%

Studies on NOTCH1 mutation at a specific location of T-cell have shown that it negatively affects the regulation of protein. These include amino acid substitutions, insertions plus deletions of exons 26 and 27 associated with the encoding of the N and C-terminals of the heterodimerization do-

main. The mutation of HD results from the activation of disrupted ligand, but at rare occasions, frame insertions are observed at 3′ region of exon 27. This type of mutation is believed to uncover the S2 processing site of ADAM cleavage by displacement outside the HD-LNR complex [35]. Other forms of mutation in T-ALL also affect the extracellular juxtamembrane of the receptor, resulting in the displacement of the HD-LNR domain located outside the plasma membrane, which finally increases the cleavage of S2. The mutations are also associated with premature stop codons and insertions as well as deletions of the gene structure.

5. ROLE OF NOTCH1 IN T-ALL

Several reports have linked the pathogenesis of T-ALL with the NOTCH1 signaling pathway. Analysis of T-cell translocation and recurrent chromosomal abnormality in several human T-ALL cells directly linked these with the NOTCH1 signaling [36]. The chromosomal mutations are mainly related to the truncation of the NOTCH1 gene. These mutations in the adjacent cells are directly linked with the increased number of truncated cells. Various studies on the oncogenic activity of NOTCH1 signaling inside T-cell have shown that when a transplanted cell with hematopoietic progenitors is transduced with viruses, ICN1 is observed. Several other studies have also confirmed that expression of ICN1 in hematopoietic progenitor cells or in immature thymocytes leads to the formation of T-cell tumors along with activation of NOTCH1 in retrovirus-induced T-cell neoplasm in mice. Studies have also shown that T-cell tumors generated through mutagenesis result in NOTCH1 transcription. Transduction of bone marrow progenitors deficient in pre-TCR signaling process with an active form of NOTCH1 showed a direct link with the inability to induce leukaemia. There is a clear indication that aberrant NOTCH1 signaling cannot induce leukemia unless signal transduction pathways are available to exert its leukemogenic potential. Therefore, an important correlation exists between mutations of the NOTCH1 gene and T-ALL [37]. Despite that several studies have been done to evaluate how NOTCH1 signaling process affects T-cell transformation, further studies are required to establish the exact molecular mechanism.

6. TARGETING NOTCH1 SIGNALLING IN T-CELL ACUTE LYMPHOBLASTS

The role of NOTCH1 signalling in leukemogenesis was first reported in the 1990s by Ellisen who identified a unique chromosomal translocation comprising of the NOTCH1 gene in human and T-ALL. The prime role of NOTCH1 signaling in the pathogenesis of T-ALL, however, was first demonstrated in the year 2004; after that, over 60% of mutations have been attributed to NOTCH1 activation. These mutations are mainly observed due to ligand-independent activation of receptors and prolonged activation of the NOTCH1 gene. The existence of activating mutations in NOTCH1 has shown that the cells rely on the receptor, and hence, not all NOTCH mutated cell lines are sensitive to γ-secretase inhibitor. In a normal T-cell, NOTCH1 genes encode the single-pass transmembrane receptors to behave like a signaling pathway. These receptors bind with ligands, undergo proteolytic cleavage in the cell which releases intracellular domain from the membranes, and allow the translocation of the gene into the nucleus. Studies show that oncogenic receptors are normally encoded by NOTCH1 mutant alleles, which are reliant on S3 and γ-secretase-mediated cleavage of the receptor for activation [38]. In these studies, presenilin γ-secretase complex has been shown to be the best therapeutic target for lymphoblast. This is because the complex plays a critical part in the production of pathogenic amyloid deposits in the brain of Alzheimer's patients. Other studies have also recommended to study γ-secretase inhibitors as a potential treatment therapy for T-ALL. Inhibition of NOTCH1 signaling pathway with γ-secretase inhibitors in T-ALL has been noted to result in speedy clearance of ICN1 along with transcriptional downregulation of NOTCH1 target genes. These alterations have also been associated with reduced growth along with proliferation, as depicted by the G0/G1 cell cycle arrest and reduction in cell size in some cell lines [39]. Non-randomised clinical trials have been conducted to establish the effectiveness of this molecular therapy, and have shown that there are few mutations on genes that result in the anti--tumour effects on cells. Several challenges were however experienced during the trials focused on the anti-NOTCH1 signaling treatment for T-ALL. These include high levels of apoptosis during experimental trials and high cytostatic effects of γ-secretase inhibitors on T-ALL. Other challenges include high levels of NOTCH1 inhibition and the presence of very sensitive intestinal epithelium related to systemic inhibition of NOTCH signaling along with γ-secretase inhibitor treatment. This impact pertains to dose-limiting gastrointestinal toxicity that results from the build-up of mucus-secreting goblet cells in the gut. In a normal T-cell development, NOTCH1 signaling plays a critical part in supporting cell growth, proliferation along with thymocyte development. Studies have also shown that conditional recombination signal binding protein for immunoglobulin kappa J region (RBPJ) inactivated T-cells results in the blockage of T-cell development. This is also a confirmation that NOTCH1 regulates T lymphoid and B lymphoid cells. NOTCH1 equally plays a role in the early growth of DN1, 2 and 3 as well as regulation of TCR arrangement. A relationship has also been established between oncogenic NOTCH1 signaling and transcriptional control of cell growth along with metabolism. The relationship involves the ability of NOTCH1 to control genes responsible for anabolic pathway as well as cell growth promotion through the direct transcriptional up-regulation process. T-cell transformation occurs through NOTCH1, promoting oncogenic G1/S cell growth along with regulation of cyclin-dependent kinase (CDK)4 and (CDK)6. NOTCH1 also activates nuclear factor-κB (NF-κB) signaling process through induction of T-cell transformation. Hairy and Enhancer of Split-1 (HES1) gene is transcribed through a repressor downstream for NOTCH1 which activates inhibitor of NF-κB kinase (IKK) in T-ALL by repressing the CYLD deubiquitinase [40]. NOTCH1 signaling also controls the epigenetic modulation of gene expression which results in the loss of repressive mark: Lys27 trimethylation of histone 3 in NOTCH1 target genes. NOTCH1, thus, antag-

onizes the function of the polycomb repressive complex 2 which is accountable for the gene mark, which causes a mutational loss of key components of the gene complex that are normally present in T-ALL. Other studies have also focused on the role of microRNA regulation in NOTCH1 induced T-ALL and noted that RNA can speed up the development of NOTCH1 induced T-ALL cells. NOTCH1 also degrades tumor suppressor cells resulting in T-cell transformation in Leukemia. Analysis of NOTCH1 mutation on the treatment of T-ALL has revealed that the presence of this mutation is directly correlated to a good prednisone response. Studies have also shown that a combination of γ-secretase inhibitor with a CDK4 inhibitor could cause potent cell cycle arrest and death. This is because of the synthesis of CDK4 inhibitor resulting in sensitizing T-ALL cells to γ-secretase inhibitor by strengthening the retinoblastoma (RB) pathway. γ-secretase inhibitor shows its activity against leukemia through T-cell transmembrane receptors, but several safety concerns have been raised regarding the developed drugs. These range from the effects of dexamethasone that have been noted to be very lethal to gut toxicity induced by the γ-secretase inhibitor. For this reason, the treatment has been conducted in association with glucocorticoids for providing a more effective and safer treatment. Experts also argue that a targeted combination of these types of medications could improve the treatment of T-ALL. It has been noted that the combination therapy induced apoptosis in T-cell in a more advanced manner compared to the sole effect of dexamethasone or the γ-secretase inhibitor. This combination has also been recommended for the treatment of childhood leukemia which accounts for 15% of the disease, as well as 25% of adults who remained uncured by the current chemotherapy treatment mechanism [41].

NOTCH1 cellular transformation is thought to be induced by the upregulation of proliferation-promoting oncogene MYC. MYC has an inability to perform its role in T-ALL inhibition with the utilization of supportive genes. This has been further confirmed by the role of activated NOTCH1 in the maintenance of human malignant cells in a transformed state. Studies also suggest that if T-ALL cell lines activated by NOTCH1 are resistant to glucocorticoid-induced apoptosis, then cells go through a minimal viability loss after they are treated with dexamethasone or γ-secretase inhibitor only. The cells become very sensitive when they undergo treatment with a combination of these two drugs. This sensitivity is linked to inhibitory transcription factor HES1 which targets the activated NOTCH1 and binding with the promoter gene NR3C1 (Nuclear Receptor Subfamily 3 Group C Member 1), encoding the glucocorticoid receptor inhibiting its expression. Cells achieve apoptosis through the treatment of inhibitor with the proapoptotic gene BCL2L11 (B-cell lymphoma 2 like 11) [42].

Dexamethasone has the ability to reverse γ-secretase inhibitor-induced intestinal toxicity which contributes to research on T-ALL therapies. γ-secretase inhibitor causes intestinal secretory metaplasia because of a marked surge in goblet cell differentiation with a cell cycle arrest in intestinal crypts. Goblet cells differentiation is normally regulated by tumor suppressor gene KLF4 (Kruppel Like Factor 4). Studies on Dexamethasone-induction have shown that the drug can protect goblet cells from metaplasia. Despite the role played by dexamethasone in the treatment of T-ALL, its toxicity could result in osteopenia, hypertension, and muscle atrophy. Several trials have also been conducted on the role of γ-secretase inhibitors in T-ALL treatment due to their adverse effects on the gastrointestinal system of participants. It is therefore recommended that before application of a combination of γ-secretase inhibitor and dexamethasone therapy to treat T-ALL, analysis on its effects and safety dosage for recipients needs to be undertaken. One trial of this combination has, however, established a more detrimental effect on cells such as enhanced lymphoid atrophy in the thymus and spleen [43].

The application of activated NOTCH1 in T-ALL has also faced challenges in treating patients with the disease due to numerous factors. These include various mutations in cells such as homozygous deletion of FBW7 (F-box and WD repeat domain-containing 7) gene that encrypts ubiquitin ligase responsible for NOTCH1 ICN1 turnover and resistance of T-ALL cell mutations to γ-secretase inhibitor. NOTCH1 signaling has been found to be involved in several cancers. Studies on NOTCH1 targeting cancer cells have, however, shown that a NOTCH ligand interaction rarely incorporates an enzymatic amplification process. This is an advantage fpr the therapy that enzyme signal intensity modulates to act on a particular cell mechanism. NOTCH1 activation is a dose-independent pathway, so complete shutdown of this pathway may not result in a therapeutic effect on cells [44].

The mechanism of NOTCH1 signaling has the potential as a therapeutic mechanism in T-ALL and other cancer cells because of the short intracellular half-life of NOTCH upon activation. This feature presents NOTCH signaling as a quick pulse of gene regulation that promotes intermittent inhibition rather than sustained inhibition. NOTCH target gene signaling has also been used in T-ALL because of its content-dependent effects on cells. This implies that NOTCH1 signaling could achieve different tasks in different cells without affecting the neighboring cells. The effects of inhibiting NOTCH signaling in particular cell lines are significant, as they can be easily monitored without preconceived assumptions. Inhibition of NOTCH signaling pathway can be achieved *via* several ways, such as interference of NOTCH ligand interactions by employing receptor decoys, blocking ligand ubiquitination/trans-endocytosis and NOTCH receptor fucosylation. Inhibition may also be achieved through the interference of receptor activation by preventing ligand-induced conformational modifications in NOTCH receptors.

NOTCH signaling can also be inhibited through disruption of protein-protein interactions engaged in NOTCH-dependent nuclear actions. This affects the assembly of co-activators with the NOTCH transcriptional complex (NTC) and the formation of higher-order DNA-bound complexes which result in disorganized angiogenesis.

Table 2. Current and future GSIs under clinical studies.

Name	Target	Type of study	Trial ID (As per clinicaltrials.gov data)
Individual Therapy			
MK-0752	Metastatic or locally advanced breast cancer	Phase 1	NCT00106145
PF03084014	Advanced solid tumors	Phase 1	NCT00878189
RO-4929097	Advance or metastatic breast cancer or recurrent triple-negative breast cancer	Phase 2	
Combination Therapy			
MK-0752+ Docetaxel	Locally advanced or metastatic breast cancer	Phase 1/2	NCT00645333
MK-0752+ Tamoxifen/Letrozole	Early stage breast cancer	Pilot study	NCT00756717
Ridaforolimus+MK-0752/MK-2206	Advanced solid tumor	Phase 1	NCT01295632
RO-4929097+Capecitabine	Refractory solid tumor	Phase 1	NCT01158274
RO-4929097+Cediranic maleate	Advanced solid tumor	Phase 1	NCT01131234
RO-4929097+Letrozole	Post-menopausal estrogen/progesterone receptor related breast cancer	Phase 1b	NCT01208441
RO-4929097+Vismodegib	Metastatic breast cancer	Phase 1	NCT01071564
RO-4929097+Paclitaxel+ Carboplatin	Stage 2 or 3 triple negative breast cancer	Phase 1	NCT01238133

The advantage of these approaches includes ease of γ-secretase inhibitor administration and oral bioavailability. This approach is quite interesting due to the short half-life of the molecules. The approach is also of great interest since a single agent can block the activation of all the four NOTCH homologs in a cell [45].

Reports on NOTCH inhibition in cancer therapy have shown that NOTCH helps to maintain certain stem cell populations. Expression of tyrosine kinase is regulated by NOTCH through epidermal growth factor receptor along with vascular endothelial growth factor receptor-1 [46]. It has been reported that some oncogenic pathways boost NOTCH activities, thus triggering apoptosis in cancer cells. NOTCH inhibition equally slows down cancer cell proliferation, resulting in blockage of cell senescence by NOTCH inhibitor and chemotherapeutic drugs [47]. Inhibition of NOTCH signaling participates in hampering the cancer-supporting process called angiogenesis [48].

7. ROLE OF γ-SECRETASE INHIBITORS

These are enzyme inhibitors whose major role is to prevent a process within an organism. They achieve cell growth inhibition by interfering with the communication pathway [49].

Clinical trials for the treatment of Alzheimer's disease through the application of γ-secretase inhibitors are currently under an advanced development stage [50]. γ-secretase inhibitors have been believed to operate through reduction of NOTCH1 level on cells and deregulation of NOTCH1 target genes in T-cell during the transcription process [51]. The effectiveness correlates with the anti-leukemic effects of γ-secretase inhibitors through compound E in which specific γ-secretase inhibitor blocks the NOTCH1 signaling process [52]. The inhibitor reduced T-ALL cell proliferation with the activation of the cell cycle arrest [53] (Table **2**).

8. DISCUSSION

Targeting the NOTCH signaling process, especially NOTCH1 mutations, can be the prime target in the treatment for leukaemia [54]. This hypothesis has also been tested in a study conducted on effects of GSI on target gene, which always inhibits NOTCH1 and NOTCH2 in intestinal progenitor cells. NOTCH1 and NOTCH2 play a critical role in absorptive cell fate in intestinal stem cells [55]. This causes genetic and pharmacologic inhibitions of NOTCH1 and NOTCH2 signals that further result in intestinal secretory cell metaplasia accumulating in mucus-secreting goblet cells [56]. We have also noted that inhibition of NOTCH1 signaling with GSIs can reverse glucocorticoid resistance in T-ALL and abrogate the gastrointestinal toxicity caused by GSI [57]. This could result in synergistic effects that may cause γ-secretase inhibitor-induced gut toxicity. New parenteral drug formulations also aim to avoid the toxic effects of NOTCH signal inhibitors in the gut [58].

Other studies have also tried to combine chemotherapy and GSI as an alternative in antileukemic treatment by reducing the dose of γ-secretase inhibitor with minimal γ-secretase inhibitor-induced gut toxicity [59]. In this method, the drug always targets the NF-kB signaling and other signaling pathways. It is, however, worth noting that despite all the clinical trials that have been conducted on NOTCH1 signaling and γ-secretase inhibitors, T-ALL cells offer resistance to NOTCH inhibition [60].

9. AUTHORS' INSIGHT ON THE TOPIC

This manuscript has evaluated the role of the γ-secretase enzyme in the mutation of NOTCH signaling pathway observed during T-cell gated acute lymphoblastic lymphoma. γ-secretase inhibitor controls cancer cell proliferation and the progression of Alzheimer's disease by interfering with amyloid plaque formation. Improved strategies for clinical

application of γ-secretase inhibitor and NOTCH inhibition may use therapies that will increase the antileukemic effects of GSI with a better therapeutic window. Combination therapy might also be beneficial for solid tumors with aberrant NOTCH1 signaling.

CONCLUSION

NOTCH1 signaling in T-cell lymphoblast is considered a better molecular targeting mechanism; if successfully developed and evaluated, it can be applied in the treatment of T-ALL. However, further research is required on the oncogenic mechanisms controlled by the NOTCH1 gene and the pathways mediated by the effects of GSI on the intestinal epithelium of leukemia patients. This review has outlined the importance of γ-secretase inhibitors and how this enzyme plays a crucial role in the treatment of the disease. The study outlines that genetic mutations aided by NOTCH1 in T-ALL mainly occur on genes that take part in the cellular metabolism. We acknowledge that these genes play a crucial role in cell signaling. Effective utilization of biomarkers to predict the interaction between T-ALL tumor cells and γ-secretase inhibitor is noted as a valuable feature.

This study has also outlined the prime role of NOTCH1 as a cell growth and metabolism regulator in T-ALL and its prospective therapeutic strategies to augment the activity of γ-secretase inhibitors *via* inhibition of NOTCH1 pathways. NOTCH signaling plays a significant role in carcinogenesis combined with tumor progression in T- cell leukemia and breast cancer. Therefore, NOTCH inhibitors along with chemotherapy or radiotherapy are considered to be a very promising cancer control strategy. NOTCH signaling controls embryonic and adult stem cell self-renewal, stem cell quiescence, cell fate and differentiation, cell survival, apoptosis and tumorigenesis. Studies on NOTCH signaling in cancer cells indicate that NOTCH receptor along with ligands is both oncogenic and tumour-suppressive in nature. NOTCH plays an important role in tumor therapy by gaining mutation of a cell and activating a ligand-mediated cell down-regulation. NOTCH tumorigenicity has also been noted to be organ-dependent, hence it plays a role in cell growth and metabolism in T-ALL. These organs may include skin, intestine, and bone marrow, which aid a number of NOTCH interactive pathways. This study has thus shown the role played by the interactions of NOTCH specific to organs in oncogenesis. The study also establishes that NOTCH signaling pathways constitute an important therapeutic challenge to most cancer treatments, including T-ALL. Enhanced observations of the oncogenic mechanisms regulated by NOTCH1 genes and pathways mediating the effects of γ-secretase inhibitors in the leukemic cells and the intestinal epithelium are needed to surpass the difficulties in targeting NOTCH1 signaling in T-cell lymphoblasts. Although the approach of NOTCH-1 activated T-ALL is promising, not all patients with this condition would be expected to respond. Long-term therapeutic success in cancer is rarely achieved with monotherapy, and even targeted developmental pathways such as NOTCH are likely required for the development of combination regimens. Eventually, this therapeutic targeted agent may become the next tool for 'individualized medicine'.

LIST OF ABBREVIATIONS

CBF1	=	C promoter Binding Factor 1
CD44	=	Cell-surface glycoprotein by the CD44 gene on Chromosome 11
CDK	=	Cyclin-Dependent Kinase
CSL	=	Suppressor of Hairless, Lag-2" after its mammalian, Drosophila, and Caenorhabditis elegans orthologues
CYLD	=	CYLD Lysine 63 Deubiquitinase.
FBW7	=	F-box and WD repeat domain-containing 7
HES1	=	Hairy and Enhancer of Split-1
ICN1	=	Intra Cellular portion of NOTCH1
IKK	=	Inhibitor of nuclear factor Kappa-B Kinase
KLF4	=	Kruppel Like Factor 4
LNR	=	Lin-12/Notch Repeats
NF-κB	=	Nuclear Factor kappa-light-chain-enhancer of activated B cells
NR3C1	=	Nuclear Receptor Subfamily 3 Group C Member 1
RB	=	Retinoblastoma
RBPJ	=	Recombination Signal Binding Protein For Immunoglobulin Kappa J Region
TCR	=	T-Cell Receptor

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Lau A, Weng A. Notch signalling in T-cell acute lymphoblastic leukaemia. J Pathol 2018; 4: 511-9.

[2] Winter G, Liberator D, Kohl N. Inhibition of NOTCH signalling by gamma secretase inhibitor engages the rb pathway and elicits cell cycle exit in t-cell acute lymphoblastic leukemia cells. Cancer Res J (N Y N Y) 2018; 69: 3060-8.

[3] Ferrando A, Palomero T. Therapeutic targeting of notch1 signaling in t-cell acute lymphoblastic leukemia. Clin Lymphoma Myeloma 2012; 3: 205-22.

[4] Tosello V, Ferrando AA. The NOTCH signaling pathway: role in the pathogenesis of T-cell acute lymphoblastic leukemia and implication for therapy. Ther Adv Hematol 2013; 4(3): 199-210. http://dx.doi.org/10.1177/2040620712471368 PMID: 23730497

[5] Martinelli G, Garcia-Manero GG, Papayannidis C. A Phase 1 study of the novel gamma-secretase inhibitor PF-03084014 in patients with T-cell acute lymphoblastic leukaemia and T-cell lymphoblastic lymphoma. Blood Cancer J 2015; 5: 71-9.

[6] Schumich A, Prchal-Murphy M, Maurer-Granofszky M, *et al.* Phospho-profiling linking biology and clinics in pediatric acute myeloid leukemia. HemaSphere 2019; 4(1): e312. http://dx.doi.org/10.1097/HS9.0000000000000312 PMID: 32072137

[7] Zhu YM, Zhao WL, Fu JF, *et al.* NOTCH1 mutations in T-cell acute lymphoblastic leukemia: prognostic significance and implication in multifactorial leukemogenesis. Clin Cancer Res 2006; 12(10): 3043-9. http://dx.doi.org/10.1158/1078-0432.CCR-05-2832 PMID: 16707600

[8] Weng AP, Millholland JM, Yashiro-Ohtani Y, *et al.* c-Myc is an important direct target of Notch1 in T-cell acute lymphoblastic leukemia/lymphoma. Genes Dev 2006; 20(15): 2096-109. http://dx.doi.org/10.1101/gad.1450406 PMID: 16847353

[9] Weng AP, Ferrando AA, Lee W, *et al.* Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 2004; 306(5694): 269-71. http://dx.doi.org/10.1126/science.1102160 PMID: 15472075

[10] Saha S, Pal DK, Kumar S. Hydroxyacetamide derivatives: cytotoxicity, genotoxicity, antioxidative and metal chelating studies. Indian J Exp Biol 2017; 55: 831-7.

[11] Weng AP, Nam Y, Wolfe MS, *et al.* Growth suppression of pre-T acute lymphoblastic leukemia cells by inhibition of notch signaling. Mol Cell Biol 2003; 23(2): 655-64. http://dx.doi.org/10.1128/MCB.23.2.655-664.2003 PMID: 12509463

[12] Sharma VM, Calvo JA, Draheim KM, *et al.* Notch1 contributes to mouse T-cell leukemia by directly inducing the expression of c-myc. Mol Cell Biol 2006; 26(21): 8022-31. http://dx.doi.org/10.1128/MCB.01091-06 PMID: 16954387

[13] Shah NP, Kasap C, Weier C, *et al.* Transient potent BCR-ABL inhibition is sufficient to commit chronic myeloid leukemia cells irreversibly to apoptosis. Cancer Cell 2008; 14(6): 485-93. http://dx.doi.org/10.1016/j.ccr.2008.11.001 PMID: 19061839

[14] Breit S, Stanulla M, Flohr T, *et al.* Activating NOTCH1 mutations predict favorable early treatment response and long-term outcome in childhood precursor T-cell lymphoblastic leukemia. Blood 2006; 108(4): 1151-7. http://dx.doi.org/10.1182/blood-2005-12-4956 PMID: 16614245

[15] Chan SM, Weng AP, Tibshirani R, Aster JC, Utz PJ. Notch signals positively regulate activity of the mTOR pathway in T-cell acute lymphoblastic leukemia. Blood 2007; 110(1): 278-86. http://dx.doi.org/10.1182/blood-2006-08-039883 PMID: 17363738

[16] Cullion K, Draheim KM, Hermance N, *et al.* Targeting the Notch1 and mTOR pathways in a mouse T-ALL model. Blood 2009; 113(24): 6172-81. http://dx.doi.org/10.1182/blood-2008-02-136762 PMID: 19246562

[17] Crump CJ, Johnson DS, Li YM. Development and mechanism of γ-secretase modulators for Alzheimer's disease. Biochemistry 2013; 52(19): 3197-216. http://dx.doi.org/10.1021/bi400377p PMID: 23614767

[18] Cai T, Tomita T. Structure-activity relationship of presenilin in γ-secretase-mediated intramembrane cleavage. Semin Cell Dev Biol 2020; 105: 102-9. http://dx.doi.org/10.1016/j.semcdb.2020.02.006 PMID: 32171519

[19] Escamilla-Ayala A, Wouters R, Sannerud R, Annaert W. Contribution of the Presenilins in the cell biology, structure and function of γ-secretase. Semin Cell Dev Biol 2020; 105: 12-26. http://dx.doi.org/10.1016/j.semcdb.2020.02.005 PMID: 32146031

[20] 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): 959-63. http://dx.doi.org/10.1038/nature03659 PMID: 15959515

[21] Haruki N, Kawaguchi KS, Eichenberger S, *et al.* Dominant-negative Notch3 receptor inhibits mitogen-activated protein kinase pathway and the growth of human lung cancers. Cancer Res 2005; 65(9): 3555-61. http://dx.doi.org/10.1158/0008-5472.CAN-04-3132 PMID: 15867348

[22] Pal DK, Kumar S, Saha S. Antihyperglycemic activity of phenyl and ortho-hydroxy phenyl linked imidazolyl triazolo hydroxamic acid derivatives. Int J Pharm Pharm Sci 2017; 9(12): 247-51. http://dx.doi.org/10.22159/ijpps.2017v9i12.22086

[23] Pal DK, Saha S. Chondroitin: a natural biomarker with immense biomedical applications. RSC Adv 2019; 9(48): 28061-77. http://dx.doi.org/10.1039/C9RA05546K

[24] Sorensen EB, Conner SD. γ-secretase-dependent cleavage initiates notch signaling from the plasma membrane. Traffic 2010; 11(9): 1234-45. http://dx.doi.org/10.1111/j.1600-0854.2010.01090.x PMID: 20573067

[25] Radtke F, Wilson A, Stark G, *et al.* Deficient T cell fate specification in mice with an induced inactivation of Notch1. Immunity 1999; 10(5): 547-58. http://dx.doi.org/10.1016/S1074-7613(00)80054-0 PMID: 10367900

[26] Palomero T, McKenna K, O-Neil J, *et al.* Activating mutations in NOTCH1 in acute myeloid leukemia and lineage switch leukemias. Leukemia 2006; 20(11): 1963-6. http://dx.doi.org/10.1038/sj.leu.2404409 PMID: 17008890

[27] Wouters BJ, Jordà MA, Keeshan K, *et al.* Distinct gene expression profiles of acute myeloid/T-lymphoid leukemia with silenced CEBPA and mutations in NOTCH1. Blood 2007; 110(10): 3706-14. http://dx.doi.org/10.1182/blood-2007-02-073486 PMID: 17671232

[28] Wolfe MS. Structure and function of the γ-secretase complex. Biochemistry 2019; 58(27): 2953-66. http://dx.doi.org/10.1021/acs.biochem.9b00401 PMID: 31198028

[29] Hasserjian RP, Aster JC, Davi F, Weinberg DS, Sklar J. Modulated expression of notch1 during thymocyte development. Blood 1996; 88(3): 970-6. http://dx.doi.org/10.1182/blood.V88.3.970.970 PMID: 8704256

[30] Rao SS, O'Neil J, Liberator CD, *et al.* Inhibition of NOTCH signaling by gamma secretase inhibitor engages the RB pathway and elicits cell cycle exit in T-cell acute lymphoblastic leukemia cells. Cancer Res 2009; 69(7): 3060-8. http://dx.doi.org/10.1158/0008-5472.CAN-08-4295 PMID: 19318552

[31] Jundt F, Acikgöz O, Kwon SH, *et al.* Aberrant expression of Notch1 interferes with the B-lymphoid phenotype of neoplastic B cells in classical Hodgkin lymphoma. Leukemia 2008; 22(8): 1587-94. http://dx.doi.org/10.1038/leu.2008.101 PMID: 18449208

[32] Liu S, Breit S, Danckwardt S, Muckenthaler MU, Kulozik AE. Downregulation of Notch signaling by gamma-secretase inhibition can abrogate chemotherapy-induced apoptosis in T-ALL cell lines. Ann Hematol 2009; 88(7): 613-21. http://dx.doi.org/10.1007/s00277-008-0646-x PMID: 19057901

[33] Rania Gawdat M, Shereen M, Darwish D, Sheir E, El-Nabwey M. Overexpression of the giant FAT1 cadherin gene and its prognostic significance in de novo acute leukaemia patients. Comp Clin Pathol 2017; 3: 505-12. http://dx.doi.org/10.1007/s00580-017-2409-3

[34] Kimberly WT, Esler WP, Ye W, *et al.* Notch and the amyloid precursor protein are cleaved by similar γ-secretase(s). Biochemistry 2003; 42(1): 137-44. http://dx.doi.org/10.1021/bi026888g PMID: 12515548

[35] Johansson B, Mertens F, Mitelman F. Clinical and biological importance of cytogenetic abnormalities in childhood and adult acute lymphoblastic leukemia. Ann Med 2004; 36(7): 492-503. http://dx.doi.org/10.1080/07853890410018808 PMID: 15513300

[36] Li K, Li Y, Wu W, *et al.* Modulation of Notch signaling by antibodies specific for the extracellular negative regulatory region of NOTCH3. J Biol Chem 2008; 283(12): 8046-54.

http://dx.doi.org/10.1074/jbc.M800170200 PMID: 18182388
[37] Hubmann R, Schwarzmeier JD, Shehata M, *et al.* Notch2 is involved in the overexpression of CD23 in B-cell chronic lymphocytic leukemia. Blood 2002; 99(10): 3742-7. http://dx.doi.org/10.1182/blood.V99.10.3742 PMID: 11986231
[38] Saha S, Pal DK, Kumar S. Design, synthesis and antiproliferative activity of hydroxyacetamide derivatives against HeLa cervical carcinoma cell and breast cancer cell line. Trop J Pharm Res 2016; 15(7): 1319-26. http://dx.doi.org/10.4314/tjpr.v15i7.8
[39] Saha S, Pal DK, Kumar S. Antifungal and Antibacterial Activities of Phenyl and Ortho-Hydroxy Phenyl Linked Imidazolyl Triazolo Hydroxamic Acid Derivatives. Inventi Rapid: Med Chem 2017; 2017(2): 42-9.
[40] Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. N Engl J Med 2005; 352(8): 804-15. http://dx.doi.org/10.1056/NEJMra041720 PMID: 15728813
[41] Goldberg JM, Silverman LB, Levy DE, *et al.* Childhood T-cell acute lymphoblastic leukemia: the Dana-Farber Cancer Institute acute lymphoblastic leukemia consortium experience. J Clin Oncol 2003; 21(19): 3616-22. http://dx.doi.org/10.1200/JCO.2003.10.116 PMID: 14512392
[42] Duechler M, Shehata M, Schwarzmeier JD, Hoelbl A, Hilgarth M, Hubmann R. Induction of apoptosis by proteasome inhibitors in B-CLL cells is associated with downregulation of CD23 and inactivation of Notch2. Leukemia 2005; 19(2): 260-7. http://dx.doi.org/10.1038/sj.leu.2403592 PMID: 15565166
[43] Grabher C, von Boehmer H, Look AT. Notch 1 activation in the molecular pathogenesis of T-cell acute lymphoblastic leukaemia. Nat Rev Cancer 2006; 6(5): 347-59. http://dx.doi.org/10.1038/nrc1880 PMID: 16612405
[44] Pear WS, Aster JC. T cell acute lymphoblastic leukemia/lymphoma: a human cancer commonly associated with aberrant NOTCH1 signaling. Curr Opin Hematol 2004; 11(6): 426-33. http://dx.doi.org/10.1097/01.moh.0000143965.90813.70 PMID: 15548998
[45] Kumar R, Juillerat-Jeanneret L, Golshayan D. Notch antagonists: potential modulators of cancer and inflammatory diseases. J Med Chem 2016; 59(17): 7719-37. http://dx.doi.org/10.1021/acs.jmedchem.5b01516 PMID: 27045975
[46] Uckun FM, Sensel MG, Sun L, *et al.* Biology and treatment of childhood T-lineage acute lymphoblastic leukemia. Blood 1998; 91(3): 735-46. PMID: 9446631
[47] Fortini ME, Artavanis-Tsakonas S. The suppressor of hairless protein participates in notch receptor signaling. Cell 1994; 79(2): 273-82. http://dx.doi.org/10.1016/0092-8674(94)90196-1 PMID: 7954795
[48] Schwarzmeier JD, Hubmann R, Düchler M, Jäger U, Shehata M. Regulation of CD23 expression by Notch2 in B-cell chronic lymphocytic leukemia. Leuk Lymphoma 2005; 46(2): 157-65. http://dx.doi.org/10.1080/10428190400010742 PMID: 15621797
[49] Saha S, Pal D, Log P. Encyclopedia of Physical Organic Chemistry. (First Edition.). NewYork: Wiley Interscience 2017; pp. 1-22.
[50] Pal D, Nayak AK, Saha S. Interpenetrating Polymer Network Hydrogels of Chitosan: Applications in Controlling Drug Release.Cellulose-Based Superabsorbent Hydrogels Polymers and Polymeric Composites: A Reference Series. Springer: Cham 2018.
[51] Swelem S, Tahoun M, El-Kaffash MNE, Hassab MAF, Zeid A. TPMT gene polymorphism detection by conventional PCR in pediatric acute lymphoblastic leukemia and its toxic effect. Egyptian Journal of Haematology 2014; 86: 456-75. http://dx.doi.org/10.4103/1110-1067.139775
[52] Harrison CJ, Foroni L. Cytogenetics and molecular genetics of acute lymphoblastic leukemia. Rev Clin Exp Hematol 2002; 6(2): 91-113. http://dx.doi.org/10.1046/j.1468-0734.2002.00069.x PMID: 12196211
[53] Ye Y, Lukinova N, Fortini ME. Neurogenic phenotypes and altered Notch processing in Drosophila Presenilin mutants. Nature 1999; 398(6727): 525-9. http://dx.doi.org/10.1038/19096 PMID: 10206647
[54] Shih IeM, Wang TL. Notch signaling, γ-secretase inhibitors, and cancer therapy. Cancer Res 2007; 67(5): 1879-82. http://dx.doi.org/10.1158/0008-5472.CAN-06-3958 PMID: 17332312
[55] Pine SR. Rethinking Gamma-secretase Inhibitors for Treatment of Non-small-Cell Lung Cancer: Is Notch the Target? Clin Cancer Res 2018; 24(24): 6136-41. http://dx.doi.org/10.1158/1078-0432.CCR-18-1635 PMID: 30104200
[56] Struhl G, Greenwald I. Presenilin is required for activity and nuclear access of Notch in Drosophila. Nature 1999; 398(6727): 522-5. http://dx.doi.org/10.1038/19091 PMID: 10206646
[57] Pal D, Saha S. Current Status and Prospects of Chitosan–Metal Nanoparticles and Their Applications as Nanotheranostic Agents.-Nanotheranostics. Switzerland AG 2019; pp. 79-114.
[58] Saha S, Pal D. Gymnemic Acids: Sources, Properties, and Biotechnological Production.Plant-derived Bioactives. Singapore: Springer 2020; pp. 177-93. http://dx.doi.org/10.1007/978-981-15-1761-7_7
[59] Pal D, Nayak AK, Saha S. Cellulose Based Hydrogel.Natural Bioactive Compounds. Singapore: Springer Nature 2019; pp. 285-332. http://dx.doi.org/10.1007/978-981-13-7154-7_10
[60] De Keersmaecker K, Lahortiga I, Mentens N, *et al.* In vitro validation of gamma-secretase inhibitors alone or in combination with other anti-cancer drugs for the treatment of T-cell acute lymphoblastic leukemia. Haematologica 2008; 93(4): 533-42. http://dx.doi.org/10.3324/haematol.11894 PMID: 18322257