Targeting Notch signalling pathway of cancer stem cells

Vandana Venkatesh¹, Raghu Nataraj², Gopenath S. Thangaraj³, Murugesan Karthikeyan⁴, Ashok Gnanasekaran⁴, Shamukhappa B. Kaginelli⁵, Gobianand Kuppanna⁶, Chandrashekrappa Gowdru Kallappa⁷, Kanthesh M. Basalingappa¹

¹Division of Biochemistry, ²Division of Molecular Biology, ³Division of Biotechnology, Faculty of Life Sciences, JSS Academy of Higher Education and Research, (Deemed to be University), Mysuru, India; ⁴Senior Lecturer, Department of Microbiology, Faculty of Medicine, Quest International University Perak, Malaysia; ⁵Division of Medical Physics, Faculty of Life Sciences, JSS Academy of Higher Education and Research, (Deemed to be University), Mysuru, India; ⁶Department of Microbiology, Vivekanandha College of Arts and Sciences for Women, Elayampalayam, Tiruchengode, Tamil Nadu, India; ⁷Chairman, Faculty of Life Sciences, JSS Academy of Higher Education and Research, (Deemed to be University), Mysuru, India

Contributions: (I) Conception and design: V Venkatesh, SB Kaginelli, KM Basalingappa; (II) Administrative support: CG Kallappa, KM Basalingappa; (III) Provision of study material or patients: None; (IV) Collection and assembly of data: V Venkatesh, R Nataraj, GS Thangaraj, KM Basalingappa; (V) Data analysis and interpretation: None; (VI) Manuscript writing: All authors; (V) Final approval of manuscript: All authors.

Correspondence to: Kanthesh M. Basalingappa. Assistant Professor, Division of Molecular Biology, Faculty of Life Sciences, JSS Academy of Higher Education and Research, (Deemed to be University), SS Nagar, Mysuru-570015, India. Email: kanthu4001@gmail.com.

Abstract: Cancer stem cells (CSCs) have been defined as cells within tumor that possess the capacity to self-renew and to cause the heterogeneous lineages of cancer cells that comprise the tumor. CSCs have been increasingly identified in blood cancer, prostate, ovarian, lung, melanoma, pancreatic, colon, brain and many more malignancies. CSCs have slow growth rate and are resistant to chemotherapy and radiotherapy that lead to the failure of traditional current therapy. Eradicating the CSCs and recurrence, is promising aspect for the cure of cancer. The CSCs like any other stem cells activate the signal transduction pathways that involve the development and tissue homeostasis, which include Notch signaling pathway. The new treatment targets these pathway that control stem-cell replication, survival and differentiation that are under development. Notch inhibitors either single or in combination with chemotherapy drugs have been developed to treat cancer and its recurrence. This approach of targeting signaling pathway of CSCs represents a promising future direction for the therapeutic strategy to cure cancer.

Keywords: Cancer stem cells (CSC); Notch signaling pathway; γ-secretase inhibitors (GSIs); monoclonal antibodies (mAb); delta like ligand 4 (DLL-4); metastasis; angiogenesis; epithelial-mesenchymal transition (EMT); Notch intracellular domain (NICD)

Received: 24 November 2017; Accepted: 16 January 2018; Published: 12 March 2018.
doi: 10.21037/sci.2018.02.02
View this article at: http://dx.doi.org/10.21037/sci.2018.02.02

Introduction

Cancer stem cells (CSCs) are the undifferentiated cell of tumor cell with infinite proliferation, self-renew, differentiation and growth. These stem cells play a vital role in growth and development of the cancerous tumors. Many studies have shown that the CSCs are the progenitor of the tumor and are the reason for recurrence of cancer. CSCs being chemo and radio resistance have shown metastases several years after curative treatment of the tumor which leads to recurrence (1). Current cancer treatment has minimum survival rate as these conventional cancer therapies target neoplastic cells that are largely fast-growing in abnormally, suggesting that CSCs may survive due to their high resistance to drugs and slower proliferation rate which leads to treatment failure (2,3). There are various molecular mechanisms i.e. signaling pathways that control and regulate the development of stem cells, the abnormal activity of these pathways regulates the growth, differentiation and development of these CSCs that include
Notch which is vital to tumorigenicity of the CSCs (4,5). The blockage of development cellular pathway may be important as targeted therapy so as to obstruct the growth of the tumor cells. Herein, Notch signaling pathway is targeted which is juxtacrine signaling pathway by inhibitors that have undergone the clinical trials.

**Role of CSCs in metastasis**

CSCs are the cancer cells that possess characteristics associated with normal stem cells, specifically the ability to give rise to all cell types found in a particular cancer sample. CSCs also called as cancer initiating cells and tumor stem cells, they are the driving force of tumor development, initiation of invasion and metastasis as well as recurrence. CSCs differentiate and generate various tumor cells under the regulation of various signaling pathway that play a vital role in the development process (6,7).

CSCs might derive from a normal tissue stem cell that undergoes transformation as a result of oncogenic somatic mutations, under the influence of extrinsic micro environmental factors. Tumor cell progression towards metastasis is complex, multistage process, which classically acquire epithelial-mesenchymal transition (EMT) allowing them to disseminate from primary tumor and intravasate into the circulation as local invasion, then they have to survive in the circulation later they extravasate and colonize into foreign tissues, these cells adapt to the microenvironment and they form macro metastasis, i.e., CSC may arise from transformed epithelial metastasis, i.e., CSC may arise from transformed epithelial cells through an EMT process to acquire migratory and metastatic properties (7).

In various experiments conducted, CSCs seem to be more resistant to chemotherapy and radiotherapy than ‘differentiated’ tumor cells. Indeed, CSCs residing in micro environmental niches can escape from the effects of conventional cytotoxic treatments. Expansion of the remaining highly tumorigenic CSCs can resume after treatment, which is clinically relapsed or reoccurred. The tumorigenic capacity was studied by injecting the CSC in limiting dilutions into immunodeficient mice. On the basis of these theories and observations, numerous researchers hypothesize that treatments targeting the CSC population could be more effective than existing therapies, and could dramatically transform treatment outcomes in oncology (5).

*Figure 1* explains the evolving concept of MCSCs (metastatic CSCs), ICSCs (invasive CSCs) invading into the ECMs (extracellular matrix) and penetrating into blood vessels, DCSCs (disseminating CSC) surviving and disseminating in the circulation, MCSCs undergoing...
extravasations and forming colonization at the distant organs (7).

Various reports demonstrate that there are various CSC markers that play a crucial role in metastases and enhance their capabilities like CD44 and chemokine receptor CXCR4 expression by breast cancer tumor cells and CD44 expression in pancreatic tumor cells (8-11).

Signaling pathway such as Wnt, Notch and Hedgehog (Hh) are crucial for the regulation of EMT or metastasis and self-renew of CSCs in several cancers. This strategy of signaling pathway for the growth of tumor cells can be inhibited by inhibitors that block the signals for the growth and proliferation of the CSCs, which may act as a blockade for the metastasis, and recurrence that is effective therapy (7).

**Notch signalling pathway**

CSCs could self-renew and generate more differentiated cancer progenitor on replication, cancer progenitor cells have the capacity to dedifferentiate and acquire a stem-like phenotype by a series of mechanisms, such as the microenvironment, signaling pathways, molecular circuitries and epigenetic modifications.

Notch signaling pathway is highly conserved molecular cell signaling pathway that regulates a vital role in proliferation, stem cell maintenance, cell fate specification, differentiation, and homeostasis of multicellular organism and implicates angiogenesis. They play a key role in embryonic vasculature development (12). Notch signaling is one of the most activated pathways in cancer cells, which have been experimentally demonstrated that they contribute to the cancer metastasis. Notch pathway plays a critical role in the linkages between angiogenesis and CSCs self-renewal and is thus receiving increased attention as a target to eliminate CSCs. The inhibition of this pathway may contribute to the therapeutic strategy to cure cancer by completely eradicating the CSCs (8) (Figure 2).

**Targeting Notch signaling pathway**

Features of Notch signaling pathway has few unique relevance that has to be taken into consideration for targeting the pathway, Notch-ligand interaction not being enzymatic the ‘signal intensity’ can be modulated precisely by cellular regulatory mechanisms. Complete shutdown of the pathway is not necessary for the therapeutic purpose (13,14) (Table 1).

There are various data that suggests that the Notch pathway plays a fundamental role in the development and maintenance of the hematopoietic system by playing central role in cell fate in which they support the growth, proliferation and differentiation of the stem cells. There is also evidence that the Notch pathway affects cell fate choices at various stages of hematopoietic cell development, including the decisions of Hematopoietic stem cells of the cancer tumor to self-renew or differentiate and of common lymphoid precursors to undergo T- or B-cell differentiation (17,24) (Figure 3).

Notch signaling could be targeted by inhibiting the signaling pathway by two major classes of notch inhibitors that primarily focuses on the clinical development of promising agents that either obstruct Notch receptor cleavages such as γ-secretase inhibitors (GSIs) or interfere with the Notch ligand-receptor interaction by monoclonal antibodies (mAbs). Combining notch inhibitors with current cancer therapies can be effective method for treatment strategies that can give most promising results.

Targeting Notch signaling has the potential to affect multiple cell types within a tumor like CSCs to immune cells, the successful development of agents targeting the Notch pathway will require an understanding of the role of Notch signaling in specific cancers and ideally, the development and use of mechanism-based combination regimens. The main issues in the development of agents targeting Notch signaling in oncology include: choice of the most appropriate inhibitor for each patient, identification of pharmacodynamic biomarkers, selection of mechanism-based combination regimen and patient stratification according to recognized efficacy biomarkers. Antitumor activity has seen when both GSI and mAb are administered as a single agent in early stage of development of cancers like thyroid cancer, lung cancer, intracranial tumors, sarcoma, colorectal cancer, etc. (14).

In a study they confirmed that Notch1 inhibition delays tumorigenesis and effectively reduces CSC self-renewal of nude mice Head and Neck squamous cell carcinoma xenograft. The synergistic effect of Notch inhibitor and chemotherapy drug attenuated chemotherapy-enriched CSC population in vitro and in vivo, which provides the possibility to effectively eliminate head and neck CSCs.

**GSI**

γ-secretase is a large protease complex and is composed of catalytic and accessory subunits. The activation of Notch
Figure 2 Notch signaling: an emerging therapeutic target for cancer treatment. Diagram—Notch receptor activation and therapeutic target in clinical development. Notch signaling is initiated by ligand binding to Notch receptor, which undergoes a two-step proteolytic cleavage by ADAM family proteases and γ-secretase, releasing the Notch intracellular domain (NICD). The NICD translocates to the nucleus where it binds to CSL and converts the complex from a repressor to an activator of Notch target genes. Notch signaling could be inhibited by two major classes of Notch inhibitors: γ-secretase inhibitors and monoclonal antibodies directing against Notch receptors or ligands. Fragment—NEXT, Notch extracellular truncated; CSL, C protein binding factor 1/Suppressor of Hairless/Lag-1; NEC, Notch extracellular subunit; NTM, Notch transmembrane.

Table 1 Significance of Notch signalling pathway genes in cancer

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLL 1</td>
<td>Governing cell fate decisions and cell-to-cell communication</td>
<td>(15)</td>
</tr>
<tr>
<td>DLL 3</td>
<td>Suppressing cell growth by apoptosis induction</td>
<td>(13)</td>
</tr>
<tr>
<td>DLL 4</td>
<td>Activating NF-κB signalling to enhance VEGF; secretion and promote metastasis</td>
<td>(14,16)</td>
</tr>
<tr>
<td>JAG 1</td>
<td>Interaction with Notch2 to promote cell survival</td>
<td>(15)</td>
</tr>
<tr>
<td>JAG 2</td>
<td>Interaction with Notch2 to promote cell survival and proliferation</td>
<td>(12)</td>
</tr>
<tr>
<td>Receptors</td>
<td>Transmembrane receptor involved in cell proliferation, invasion and chemo resistance</td>
<td>(17-19)</td>
</tr>
<tr>
<td>Notch1</td>
<td>Constitutive Notch2 signalling induces hepatic tumours</td>
<td>(13)</td>
</tr>
<tr>
<td>Notch2</td>
<td>Promoting proliferation and migration, and modifying chemotherapy response</td>
<td>(21)</td>
</tr>
<tr>
<td>Notch3</td>
<td>Involved in endocrine therapy resistance and EMT in breast cancer</td>
<td>(13,22)</td>
</tr>
<tr>
<td>Hes1</td>
<td>Sequence-specific DNA binding transcriptional factor involved in cellular proliferation and differentiation</td>
<td>(23)</td>
</tr>
<tr>
<td>Hey1</td>
<td>Hes-related transcriptional factor involved in neoplastic vasculature development</td>
<td>(23)</td>
</tr>
</tbody>
</table>

VEGF, vascular endothelial growth factor; EMT, epithelial-mesenchymal transition.
signaling pathway mainly depends on the γ-secretase enzyme activity that helps in the proteolytic cleavage of the receptors that release the active intracellular fragment which is one of the most crucial steps. Thus, GSIs is a promising target for Notch inhibition. GSI were the first class of inhibitors that reached clinical development in oncology. There are more than 100 GSIs synthesized and they can be divided into three classes: peptide isosteres, azepines and sulfonamides. Azepines and sulfonamides are most popular and widely used (14).

In various preclinical studies these agents have shown strong anti-neoplastic activities, anti-angiogenesis, anti-CSC effects, anti-tumor growth and apoptosis especially in combination with chemotherapeutic or targeted drugs (25). Studies conducted showed that using multiple myeloma cell lines and primary multiple myeloma cells isolated from the bone marrow of patient found a strong synergistic effect of bortezomib in combination with one of the GSIs, which can be successfully used as anti-multiple myeloma drug which may enhance the mechanism inhibition of proteasome activity (18). This proves that GSIs can effectively repress the CSC that inhibit the recurrence of cancer by deregulating the angiogenesis and this triggers apoptosis of tumor cells by inhibiting proteasome activity and enhancing endoplasmic reticulum stress. This can be used as a promising therapy for the cure of multiple myeloma.

The well know toxicities or side effects of GSIs is associated with gastrointestinal tract, that cause the increase in goblet containing mucin in large and small intestine that leads to severe adverse diarrhea. So, they are dose limiting and require moderate, intermittent administration.

Studies demonstrated that Notch signaling inhibition using a GSI (GSI-IX, DAPT) decreased tumor burden in the mouse model after prophylactic treatment. In addition, flow cytometry analysis indicated that Notch signaling inhibition reduced the sub-population of myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and regulatory T cells (Tregs) in the circulation and in the tumor (20).

Oral GSI inhibits Notch pathway activity and expression of stem cell markers; this was proved by measuring the expression of established pathway targets in tumor-derived mRNA in three xenograft cohorts (21).

RO4929097 (RG-4733) is a novel gamma secretase inhibitor being studied as an anti-cancer drug, which is a key component of the enzymatic complex that cleaves and activates Notch (22). The drug was initially developed by Roche for the treatment of Alzheimer’s disease, but current research focuses on cancer. Over 35 phases I and II clinical trials have been performed but no phase III trials have yet commenced (23). Phase II studies have investigated the use of RO4929097 in ovarian cancer which when used as immunotherapy inhibits Notch pathway (26), renal cell carcinoma patients with solid tumor (27,28) metastatic pancreatic cancer (29), advanced brain tumors (30), and relapsed non-small cell lung cancer. The effects of RO4929097 on the oncogenic and stem cell properties of a panel of melanoma cell lines were tested both in vitro and in vivo, using xenograft models. Increased gene expression of Notch signaling components correlated with shorter post recurrence survival in metastatic melanoma cases (9). The most common severe adverse events were nausea, fatigue and anemia in patients with advanced cancer (31). Various research studies show that RO4929097 in phase 1 study can be safely used in combination with other agents like temsirolimus in patients with advanced solid tumors (32), cediranib which is a vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitor, showed antitumor activity with solid tumors (28), gemcitabine showed clinical antitumor activity and >4 months stable disease in pancreas, tracheal and primary breast cancer (29). Phase I study of RO4929097 in combination with bevacizumab in patients with recurrent malignant glioma showed anti-angiogenic effect with well tolerated synergic activity (30) (Table 2).

PF-03084014 is a small molecule reversible, non-competitive and selective GSI that inhibits growth of several T-acute lymphoblastic leukemia (T-ALL) cell lines by inducing cell cycle arrest at the G0–G1 phase and induction
of apoptosis in preclinical model (33).

An in vivo study shows that PF-03084014 when orally administrated in a single dose of 200 mg/kg causes maximal Notch intracellular domain (NICD) inhibition for ~80% in xenograft HPB-ALL tumors. PF-03084014 shows robust antitumor activity in this mode with a maximal tumor growth inhibition, cell growth inhibition with cell cycle arrest, apoptosis and they also caused significant reduction of NICD levels and dose dependent tumor growth inhibition in HPB-ALL tumors. The intermittent dosing reduced gastrointestinal toxicity (33).

Phase I study of the GSI PF-03084014 in combination with docetaxel in patients with advanced triple negative breast cancer, the maximum tolerated dose (MTD) was estimated to be 100 mg twice daily/docetaxel 75 mg/m². At this dose level, combination treatment was generally well tolerated. The most common, treatment-related adverse events in patients reported were neutropenia, fatigue, nausea, leukopenia, diarrhea, alopecia, anaemia and vomiting. No effect was observed on the pharmacokinetics of docetaxel when administered in combination with PF-03084014. They exhibited anti-tumor activity of this combination in xenograft models (34).

PF-03084014 (120 mg/kg) induces apoptosis, anti-angiogenesis, anti-proliferation, reduces tumor cell self-renewal ability, impairs tumor vasculature, and decreases metastasis activity in breast cancer HCC1599 tumor-bearing mice. PF-03084014 treatment displays significant antitumor activity in various types of the breast xenograft models with TGI (tumor growth inhibition) value of at least 50% in phase I clinical trials (35).

When PF-03084014 was combined with gemcitabine it resulted in tumor regression in 3 of 4 pancreatic cancer xenograft models via targeting malignant CSCs. The combined therapy showed enhanced efficacy in the inhibition of tumor cell proliferation and angiogenesis, attenuating (19) growth of primary tumor and controlling metastatic dissemination in a highly aggressive orthotopic model (36).

MK-0752, is a potent non-competitive oral inhibitor of γ-secretase which prevents cleavage of gamma-secretase substrates with a 50% inhibitory concentration (IC50) (35) (Table 3).

MK-0752 is well-tolerated and exhibits target inhibition in children with recurrent central nervous system (CNS)
malignancies supporting a rational application (44). MRK003 is structurally similar to MK0572 and is used in preclinical in vivo studies of both solid tumors and leukemia as it has superior pharmacokinetic properties in rodents (21).

Pre-treatment of pancreatic ductal adenocarcinoma (PDAC) cells with MRK-003 in cell culture significantly inhibits the subsequent engraftment in immunocompromised mice. MRK-003 monotherapy significantly blocked tumor growth in 5 of 9 (56%) PDAC xenografts. A combination of MRK-003 and gemcitabine showed enhanced antitumor effects compared to gemcitabine in 4 of 9 (44%) PDAC xenografts reduced tumor cell proliferation and induced both apoptosis and intra-tumoral necrosis (45). The patients with advanced solid tumors MK0572 GSI were administered weekly and this dosing was well tolerated and resulted in strong modulation of a Notch gene signature (37). Clinical benefit was observed, and rational combination trials are currently ongoing to maximize clinical benefit with this novel agent.

**mAb inhibitors**

These are mAbs tested in clinical, which recognize...
specific ligand (DLL-4) or receptors (Notch1–3) and they either prevent ligand/receptor interaction or the conformational change within the extracellular domain which is required to expose the TACE cleavage site (46). Delta-like ligand 4 (DLL-4) is an important component of NOTCH pathway that controls the proper growth, stem cell renewal and development. Any deletion in the gene that codes DLL-4 shows a lethal effect on the vasculature, i.e., abnormalities and over expression of DLL-4 is found in tumor vasculature and in tumor cells to activate Notch signaling for their further active growth (12,32,47). Various xenograft models showed that blockade of specific DLL-4 in combination with ionizing radiation retards the tumor growth activity of cancer cell lines by promoting nonproductive tumor angiogenesis and extensive tumor necrosis thereby reducing tumor-initiating cell frequency and delayed tumor recurrence (32,48,49). Data indicated that that DLL-4-mediated Notch signaling is crucial during active vascularisation, but less important for normal vessel maintenance and the antibodies against DLL-4 and antibodies against VEGF had distinct effects on tumor vasculature (32). Thus, it can be concluded that inhibiting these components one can successfully provide a promising therapeutic strategy to cure the cancer, targeting these components in CSCs. They have developed various mAbs such as trastuzumab, pertuzumab, rituximab, bevacizumab and others which have gained clinical acceptances part of combination regimens that can be targeted to the DLL-4 of the CSCs. Some have been proved to be effective and even with these agents resistance is observed (15,16,50).

Experimental evidence shows that notch inhibition by mAb against Notch1 or 2 alleviates the toxicities while inhibition of Notch1 and 2 leads to severe gastrointestinal toxicities (10). Two classes of blocking anti-Notch antibodies have been developed. One is directed to the extracellular negative regulator region (NRR) of notch blocking, the conformational change that allows the ADAM protease cleavage and the other is the ligand-competitors directed against the EGF-repeat region of notch receptors, blocking the ligand binding domain (LBD). Both NRR and LBD notch antibodies induce a strong and specific down regulation of notch 1 signaling. Studies using murine tumor models showed anti-angiogenic effect, reduce the blood circulation to the tumor cells and finally lead to retardation of tumor growth (32,43,51).

Development of Humanized mAbs OMP-59R5 (tarextumab) which alone or together with chemotherapeutic agents can inhibit Notch2 and Notch3 function. The antitumor effect of OMP-59R5 was observed on xenograft tumors representing different types of epithelial cancers like breast, small-cell lung, ovarian, and pancreatic was associated with down-regulation of Notch target genes in tumor cells and with suppression of Notch3, HeyL, and Rgs5, expression in tumor stroma and vasculature (52-54). OMP-59R5 inhibits xenograft tumor growth through reducing tumor cell proliferation and promotes differentiation this was accessed using passaged patients derived xenografts (PDX) models. They alone showed 40% of reduced proliferation. OMP-59R5 reduces CSC frequency and delays tumor recurrence after termination of chemotherapy. The antitumor effect of anti-Notch2/3 in combination with gemcitabine plus nab-paclitaxel was greater than the combination effect with gemcitabine alone. The MTDs of OMP-59R5 were observed to be 2.5 mg/kg weekly and 7.5 mg/kg every three weeks (55).

The mAbs REGN421 and SAR153192 (also called enoticumab) had a reasonable safety profile and demonstrated preliminary efficacy signals. REGN421 is a fully humanized IgG1 mAb that binds to human DLL-4 and disrupts Notch-mediated signaling (56). The most-common adverse effects were fatigue, headache, hypertension and nausea reversible severe adverse events included increased levels of the cardiac proteins natriuretic peptides B and troponin I and right and left ventricular dysfunctions. Partial responses and stable response were observed among the patients with ovarian cancer and other solid tumors who were treated with enoticumab (43,56,57) (Tables 4,5).

**Discussion**

Notch signaling pathway is a conserved cell fate determining factor in embryo development. Notch pathway plays an important role in linkage between angiogenesis and CSCs self-renewal. CSCs are population of cells that have stem cell properties and invasion capabilities that contribute to cancer metastasis. CSC escape from immune surveillance and they survive from radiation and chemotherapy later they result in reconstruction of cancer tissue. This concept of targeting notch signaling pathway as a therapeutic strategy for treating cancer has attracted increasing interest. Cell signaling pathway such as notch, they critically regulate the self-renewal and survival of CSC. Notch pathway is deregulated in CSC using inhibitors that inhibit the signaling for development of further tumor cells. CSC eradication is an important goal in curing cancer as CSCs.
### Table 4 Molecules moved from preclinical research to clinical development

<table>
<thead>
<tr>
<th>Class</th>
<th>Drug(s)</th>
<th>Target</th>
<th>Phase</th>
<th>Enrollment or estimated</th>
<th>Primary endpoint subjects</th>
<th>Trial status</th>
<th>Trial ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>γ-secretase inhibitors</td>
<td>MK0752</td>
<td>γ-secretase</td>
<td>I</td>
<td>5</td>
<td>Safety/efficacy; relapsed or refractory T-ALL/T-LL</td>
<td>Terminated</td>
<td>NCT00100152</td>
</tr>
<tr>
<td></td>
<td>MK0752</td>
<td>γ-secretase</td>
<td>I</td>
<td>103</td>
<td>Safety/MTD; advanced breast cancer/advanced solid tumors</td>
<td>Completed</td>
<td>NCT00106145</td>
</tr>
<tr>
<td></td>
<td>MK0752</td>
<td>γ-secretase</td>
<td>I</td>
<td>33</td>
<td>Safety; recurrent or refractory CNS malignancies</td>
<td>Terminated</td>
<td>NCT00572182</td>
</tr>
<tr>
<td></td>
<td>MK0752 + docetaxel</td>
<td>γ-secretase</td>
<td>II</td>
<td>30</td>
<td>DLT; locally advanced or metastatic breast cancer</td>
<td>Completed</td>
<td>NCT00645333</td>
</tr>
<tr>
<td>MK0752 + gemcitabine</td>
<td>MK0752</td>
<td>γ-secretase</td>
<td>I/Ia</td>
<td>60</td>
<td>Safety/MTD; stage IV pancreatic cancer</td>
<td>Recruiting</td>
<td>NCT01098344</td>
</tr>
<tr>
<td></td>
<td>MK0752</td>
<td>γ-secretase</td>
<td>I</td>
<td>30</td>
<td>Notch response signature; healthy young adults</td>
<td>Completed</td>
<td>NCT00803894</td>
</tr>
<tr>
<td></td>
<td>RO492909</td>
<td></td>
<td>Pre-surgical</td>
<td>20</td>
<td>Safety/tolerability; early stage ER-positive breast cancer</td>
<td>Unknown</td>
<td>NCT00756717</td>
</tr>
<tr>
<td></td>
<td>PF03084014</td>
<td></td>
<td>I</td>
<td>60</td>
<td>Safety/DLT; advanced solid Tumors/T-ALL/T-LL</td>
<td>Ongoing</td>
<td>NCT00878189</td>
</tr>
<tr>
<td></td>
<td>BMS-906024</td>
<td></td>
<td>I</td>
<td>95</td>
<td>Safety/tolerability; advanced solid tumors</td>
<td>Recruiting</td>
<td>NCT01292655</td>
</tr>
<tr>
<td></td>
<td>BMS-906024</td>
<td></td>
<td>I</td>
<td>42</td>
<td>Safety/tolerability; T-ALL/T-LL</td>
<td>Recruiting</td>
<td>NCT01363817</td>
</tr>
<tr>
<td></td>
<td>BMS906024 +</td>
<td></td>
<td>Ib</td>
<td>60</td>
<td>Safety; advanced or metastatic solid tumors</td>
<td>Recruiting</td>
<td>NCT01653470</td>
</tr>
<tr>
<td></td>
<td>chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LY3039478</td>
<td></td>
<td>I</td>
<td>80</td>
<td>DLTs; advanced or metastatic cancer</td>
<td>Recruiting</td>
<td>NCT01695005</td>
</tr>
<tr>
<td>mAbs to Notch receptors</td>
<td>OMP-59R5</td>
<td>Notch2/3</td>
<td>I</td>
<td>44</td>
<td>Safety; advanced solid tumors</td>
<td>Recruiting</td>
<td>NCT01277146</td>
</tr>
<tr>
<td>or ligands</td>
<td>OMP-59R5 + NgP &amp; gemcitabine</td>
<td>Notch2/3</td>
<td>Ib/Ii</td>
<td>154</td>
<td>DLT/MTD; first line for stage IV pancreatic cancer</td>
<td>Recruiting</td>
<td>NCT01647828</td>
</tr>
<tr>
<td></td>
<td>OMP-21M18</td>
<td>DLL4</td>
<td>I</td>
<td>30</td>
<td>Safety; advanced solid tumors</td>
<td>Recruiting</td>
<td>NCT00744562</td>
</tr>
<tr>
<td></td>
<td>OMP-21M18 + FOLFIRI</td>
<td>DLL4</td>
<td>Ib</td>
<td>32</td>
<td>MTD; first or second line for advanced colorectal cancer</td>
<td>Recruiting</td>
<td>NCT01189942</td>
</tr>
<tr>
<td></td>
<td>OMP-21M18 + CP</td>
<td>DLL4</td>
<td>Ib</td>
<td>32</td>
<td>MTD; first line for advanced non-squamous NSCLC</td>
<td>Recruiting</td>
<td>NCT01189968</td>
</tr>
<tr>
<td></td>
<td>OMP-21M18 + gemcitabine</td>
<td>DLL4</td>
<td>Ib</td>
<td>40</td>
<td>MTD; first line for advanced or metastatic pancreatic cancer</td>
<td>Recruiting</td>
<td>NCT01189929</td>
</tr>
<tr>
<td></td>
<td>REGN421</td>
<td>DLL4</td>
<td>I</td>
<td>80</td>
<td>Safety/tolerability; advanced solid tumors</td>
<td>Recruiting</td>
<td>NCT00871559</td>
</tr>
<tr>
<td></td>
<td>OMP52M51</td>
<td>Notch1</td>
<td>I</td>
<td>33</td>
<td>Safety; relapsed or refractory CNS</td>
<td>Recruiting</td>
<td>NCT01778439</td>
</tr>
</tbody>
</table>

*a, clinical trial registration and information at www.clinicaltrials.gov; b, clinical trials with RO4929097 are not shown due to the termination of its development; c, chemotherapy, weekly paclitaxel, FOLFIRI or carboplatin plus paclitaxel. FOLFIRI, 5-Fluorouracil (leucovorin), 5-Fluorouracil (FU) plus IRInotecan (irinotecan) or carboplatin plus paclitaxel; OP, carboplatin and pemetrexed; Nab-P, nab-paclitaxel; TTP, time to progression; MTD, maximum tolerated dose; NSCLC, non-small cell lung cancer; CNS, central nervous system; T-ALL, T-acute lymphoblastic leukemia; T-LL, T-cell lymphoblastic lymphoma.
result in reconstruction of cancer tissue even after successful conventional treatment, thus Notch Pathway is attractive target for treatment as they kill differentiated cancer cells but could also kill CSCs.

Since the activity of Notch is activated via gamma secretase, it is targeted and inhibited using GSI which effectively repress CSC and inhibit recurrence of cancer by retardation of angiogenesis and triggering apoptosis of tumor cells.

Another way to target Notch signaling pathway is by blocking DLL-4 that plays an important role in growth, renewal and development. Blockade of this DLL-4 using mAb in combination with either ionizing radiation or chemotherapy drugs retards tumor growth of CSC promoting tumor necrosis, reducing tumor initiating cell frequency, delayed tumor recurrence and nonfunctional tumor angiogenesis.

GSI and mAb cause adverse severe side effects. So, they are dose limiting and require moderate, intermittent administration.

In conclusion, Notch signaling inhibition of CSCs in tumor have opened a broad view for designing novel diagnostic and therapeutic strategies in order to detect and specifically target the cancer cells pathway that might bring future direction for the recurrence and cure of cancer.

Acknowledgements

None.

Table 5 Preliminary results of few agents

<table>
<thead>
<tr>
<th>Drug</th>
<th>Patients treated</th>
<th>Disease control rate</th>
<th>Major treatment-related toxicities</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MK0752</td>
<td>103</td>
<td>1 CR in anaplastic astrocytoma, 15 SDs in mostly GBM</td>
<td>GI disorders, fatigue, AST increase, rash</td>
<td>Krop et al., 2012 (38)</td>
</tr>
<tr>
<td>RO4929097</td>
<td>110</td>
<td>1 PR in colorectal adenocarcinoma with neuroendocrine features, 26 SDs in sarcoma, melanoma or ovarian cancer</td>
<td>GI events, fatigue, hypophosphatemia, rash</td>
<td>Tolcher et al., 2012 (58)</td>
</tr>
<tr>
<td>PF03084014</td>
<td>41</td>
<td>1 CR in papillary thyroid cancer, 4 PRs in desmoid tumors</td>
<td>Diarrhea, nausea, rash and fatigue</td>
<td>Messersmith et al., 2012 (59)</td>
</tr>
<tr>
<td>OMP-59R5</td>
<td>39</td>
<td>SDs in adenoid cystic-adenocarcinoma, sarcomas, rectal cancer, or triple-negative breast cancer</td>
<td>Diarrhea</td>
<td>Smith et al., 2012 (60)</td>
</tr>
<tr>
<td>REGN421</td>
<td>53</td>
<td>2 PRs in NSCLC and ovarian cancer, 16 SDs</td>
<td>Fatigue, headache, hypertension, nausea</td>
<td>Jimeno et al., 2013 (56)</td>
</tr>
</tbody>
</table>

*, disease control rate includes complete and partial responses, and stable disease. CR, complete response; SD, stable disease; PR, partial response; GBM, glioblastoma multiforme; AST, aspartate aminotransferase; GI, gastro intestinal; NSCLC, non-small cell lung cancer.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

9. Taichman RS, Cooper C, Keller ET, et al. Use of the


23. Available online: https://clinicaltrials.gov


doi: 10.21037/sci.2018.02.02