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Investigational Notch and Hedgehog Inhibitors – Therapies for Cardiovascular disease

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Abstract

Importance to the field—During the past decade a variety of Notch and Hedgehog pathway inhibitors have been developed for the treatment of several cancers. An emerging paradigm suggests that these same gene regulatory networks are often recapitulated in the context of cardiovascular disease and may now offer an attractive target for therapeutic intervention.

Areas Covered—This article briefly reviews the profile of Notch and Hedgehog inhibitors that have reached the pre-clinic and clinic for cancer treatment and discusses the clinical issues surrounding targeted use of these inhibitors in the treatment of vascular disorders.

Expert Opinion—Pre-clinical and clinical data using pan-Notch inhibitors (γ -secretase inhibitors) and selective antibodies to preferentially target notch receptors and ligands has proven successful but concerns remain over normal organ homeostasis and significant pathology in multiple organs. In contrast, the Hedgehog based drug pipeline is rich with more than a dozen Smoothed (SMO) inhibitors at various stages of development. Overall, refined strategies will be necessary to harness these pathways safely as a powerful tool to disrupt angiogenesis and vascular proliferative phenomena without causing prohibitive side effects already seen with cancer models and patients.

1. Introduction

According to the World Health Organization (WHO) cardiovascular disease (CVD) is the number one cause of death globally; more people die annually from CVD than from cancer, respiratory diseases and accidents combined. By 2030, almost 23.6 million people/year will die from CVD mainly from heart disease and stroke.

One of the standing paradigms in cardiovascular biology is that signaling and transcription factor pathways important for cardiac and vascular development are often recapitulated in adults following disease or injury¹. Much of the support for this contention comes from findings that demonstrate developmental gene regulatory networks and embryonic isoforms of vascular and cardiac specific genes are re-expressed after vascular injury, whereas the adult isoforms are down-regulated^{2, 3}. Several important signaling pathways have been

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shown to regulate cardiac and vascular development including bone morphogenetic protein (BMP), Hedgehogs (Hh), Wnt, and Notch. Of these, Notch and Hedgehog signaling plays a critical role in a variety of cellular processes including cell fate changes in proliferation, and differentiation⁴.

The cellular and molecular signatures for Notch and Hedgehog gene regulatory networks have been extensively studied in *Drosophila*, mice and numerous cancer, haemopoietic and vascular cell systems and have provided fundamental insights into the recapitulation of these pathways in disease development and progression.⁵⁻⁸ As a result intense efforts have been made to develop novel therapies that target these pathways individually⁹⁻¹¹.

2. Notch Signaling Pathway

Thomas Morgan first discovered a Notch mutation in 1917 in the fruit fly *Drosophila melanogaster*, with an adult phenotype consisting of 'notches' at the wing margin. Both loss-of-function and gain-of-function *Notch* mutations are dominant in *Drosophila*, where loss and gain of a single gene copy is sufficient to mimic hypomorphic and hypermorphic mutations¹². Thus, the *Notch* expression level is likely to be critical to ensure the subtle balance between neuroblast and epidermal cell fate decision during *Drosophila* development.

Notch receptor-ligand interactions are a highly conserved mechanism that regulate intercellular communication and directs individual cell fate decisions⁴ [Figure 1]. The four mammalian Notch receptors (Notch 1–4) and five ligands (Jagged1 and -2; Delta-like1, -3, and -4) all contain transmembrane domains such that ligand-receptor signaling occurs between adjacent cells. Ligand-receptor binding triggers two cleavage events that release the intracellular domain of Notch to the nucleus and facilitate an association with the transcription factor CBF-1 (also known as RBP-Jκ or CSL). The subsequent recruitment of the co-activator, Mastermind-like (MAML) protein¹³, promotes the transcriptional activation of downstream effectors. Established vascular target genes of the Notch cascade are the *Hes* and *Hey* [*Hey1*, *Hey 2* and *Hey L*] gene families, the latter also known as *Hesr*, *Herp*, *Hrt*, *Chf* or *gridlock*¹⁴. Recent reviews provide a more thorough description of the subject¹⁵⁻¹⁷.

While a detailed understanding of how Notch selects between CBF-1/RBP-Jκ-dependent (canonical) and -independent (noncanonical) pathways is under intense investigation, Notch exists at the cell surface in a heterodimeric form (cleaved by furin in the trans-Golgi) or as an intact (colinear) protein. Canonical and noncanonical signaling pathways are activated downstream of these two physically distinct Notch receptors in response to ligand binding¹⁸. In general, association between Notch ligands and receptors occurs between cells (homotypic or heterotypic) resulting in trans-signaling events. However, binding to receptors of the plasma membrane of the same cell may also occur^{18, 19}. Specificity between the ligands and receptors has not been fully delineated, and recent experimental evidence suggests that not all receptor/ligand interactions result in downstream signaling²⁰. Moreover, downstream Basic helix-loop-helix (bHLH) transcriptional activity can occur independent of Notch signaling²¹.

The engagement of Notch by ligand results in extracellular processing of the Notch receptor by a disintegrin-metalloprotease, thought to be transforming growth factor alpha (TNFα) converting enzyme (TACE/ADAM17)²². Further cleavage takes place within the transmembrane domain by the γ-secretase, which is dependent on the presence of presenilins. These cleavage events release the intracellular domain of Notch (NICD), thereby allowing translocation to the nucleus mediated by nuclear translocation signals (NLS). Because, in most cases, Notch function requires ligand-dependent cleavage of the

intracellular domain (NICD), enforced expression of NICD provides a constitutively active signaling form of the receptor and has been successfully utilized to examine the role of Notch in differentiation, proliferation and more recently apoptotic pathways of several mammalian cell types^{23, 24}. This cleavage-dependent pathway involving γ -secretase has been the focus of much work in identifying novel inhibitors of canonical Notch signaling in several cell systems and in preclinical and clinical Phase I and II studies.

In mammals, Notch receptors are activated by five type I transmembrane ligands, three Delta-like (Dll1, Dll3 and Dll4) and two Serrate/Jagged (Jag1 and Jag2). All contain a cysteine-rich 'Delta, Serrate, Lag' (DSL) motif found in respective *Drosophila* orthologs Delta and Serrate/Jagged and in *Caenorhabditis elegans* Lag2. Numbers of EGF repeats vary between Dll and Jag ligands (6-8 and 15-16, respectively). Epidermal growth factor-like domain 7 (EGFL7) has been identified as a soluble antagonist of Notch signaling. Recently, a previously unknown Notch ligand in *Drosophila* was identified that when deleted causes cardiomyopathy²⁵.

An additional ligand-dependent cleavage event at extracellular site S2 leads to the release of a soluble form of Notch named Notch extracellular truncation (NEXT)²⁶. Further, a non-canonical CBF-1/RBP-J κ -independent and Deltex-dependent alternative pathway has been described in humans and in *Drosophila*^{27, 28}. Together with this observation, in T helper (Th) cells, Jagged induces Th2 cell differentiation by triggering the CBF-1/RBP-J κ -dependent canonical pathway, while Delta-like instructs Th1 commitment through a RBP-J κ -independent alternative pathway, presumably Deltex-dependent²⁹. Physical interactions between Notch target gene products HES1 and HEY1 with signal transducers and activators of transcription-3 (Stat-3) point to crosstalks between Notch and Stat3-activating pathways such as Gp130/Jak2/stat3 and Sonic hedgehog (Shh)^{30, 31}. In parallel, Shh is also capable of stimulating *HES1* transcription²¹. In addition, β -catenin has been shown to interact with Notch and CBF-1/RBP-J κ to induce *HES1* transcription, indicating crosstalk between the Wnt and Notch pathways^{32,33}.

In humans, *Notch* mutations have been associated with dominant developmental disorders and diseases that include brain/neurological, cardiovascular and/or kidney defects. Mutations in *Notch1* in aortic valve disease³⁴; in *Notch2* in Alagille syndrome³⁵; in *Notch3* in CADASIL syndrome³⁶ and possibly in *Notch4* in schizophrenia³⁷. In mice, global knockout of *Notch1* or *Notch2* are embryonic and perinatal lethal with vascular and kidney defects³⁸. Surprisingly, *Notch3* and *Notch4* null mice show normal development, viability and fertility. Although *Notch1/Notch4* double mutants had more severe defects in angiogenic vascular remodeling, there is no evidence of a genetic interaction between *Notch1* and *Notch3*. Hemizygosity of *Dll4* as well as *Jag1* and RBP-J κ knockouts consistently result in embryonic death due to vascular defects³⁹. The fact that inactivation of Notch signaling results in constant defects in angiogenesis demonstrates its pivotal role in vascular morphogenesis, remodeling during embryonic development and homeostasis of adult self-renewing organs^{5, 8, 33} and points to a potential involvement of Notch signaling in vascular disease and tumor neovasculature. It is therefore unsurprising that perturbation of Notch signaling may often lead to aberrant growth of vessels and cells in adults (arterial remodeling and tumorigenesis).

(a) Notch Signaling and Cancer

Disruption of Notch signaling has been implicated in a variety of hematological and solid cancers. The best-studied example is the link between mutations of Notch1 and T-cell acute lymphoblastic leukemia and lymphoma (TALL) with activating mutations in Notch1 (independent of the translocation) have been found in more than 50% of human T-ALL cases⁴⁰. This results in a truncated Notch1 protein, which is constitutively active and

aberrantly expressed. Abnormal Notch signaling has also been reported in solid tumors, including cancers of the breast, kidney, pancreas, prostate, cervix, endometrium, brain, intestine, lung and skin. Without evidence of genetic lesions, however, Notch may play either an oncogenic or a tumor-suppressive role⁴¹, depending on the cancer type, other signaling pathways present and the identity of the Notch receptor activated^{11, 16}. However, in a large majority of cases including breast cancer, Notch signaling promotes tumor growth.

One overriding mechanism for the oncogenic role of Notch may derive from its ability to prevent differentiation and maintain the stem cell phenotype⁴². Stem cells and tumor cells share common characteristics, such as unlimited proliferation and un-differentiation. The role of Notch signaling in stem cells versus cancer cells has been elucidated in the intestine and breast⁴³. Notch signaling may also interact with the hypoxia-sensing pathway via HIF1 α to synergistically promote the stem cell phenotype⁴⁴. In neuronal stem cells and muscle precursors, hypoxia activated Notch downstream target genes and promoted the undifferentiated state in a Notch-dependent manner⁴⁴. This interaction may have implications for tumor growth since the hypoxia response up-regulates a number of pathways conducive for tumor survival, such as angiogenesis, cell survival, glucose metabolism and invasion⁴⁵.

The Notch pathway also crosstalks with other signaling pathways involved in tumorigenesis including vascular endothelial growth factor (VEGF)⁴⁶, Ras oncogenes, transforming growth factor beta (TGF β)⁴⁷ and Wnt⁴⁸ signaling. The relative importance and the oncogenic mechanisms of developmental pathways vary with the tumour type, the stage of the disease as well as the interaction with the tumour microenvironment, thus highlighting the complexity of cellular signaling strategies employed during tumorigenesis. Collectively, it is clear that Notch signaling antagonizes or synergizes with signaling pathways leading to tumor growth in most cases.

(b) Notch Signaling and Vascular Disease

Notch signaling plays a myriad of roles during vascular development. These roles include the regulation of arteriovenous specification and differentiation in both endothelial cells and vascular smooth muscle cells, regulation of blood vessel sprouting and branching during normal and pathological angiogenesis, and the physiological responses of vascular smooth muscle cells. Defects in Notch signaling cause inherited vascular diseases, such as the degenerative vascular disorder cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)⁴⁹. Proper development of the vasculature requires heterogeneity in the response of endothelial cells to angiogenic cues provided by other tissues and organs. The pathogenesis of vascular diseases results from genetic mutations in pathways that provide these cues and in signals that coordinate endothelial heterogeneity during blood vessel formation, namely Hedgehog, VEGF, bone morphogenetic protein (BMP), and the Notch/Delta/Jagged pathways. Hedgehogs are critical in determining arterial and venous specification⁵⁰. The VEGF pathway is critical for the initiation and spatial coordination of angiogenic sprouting and endothelial proliferation, BMP signaling appears to act in a context-dependent manner to promote angiogenic expansion and remodeling, and the Notch pathway is a critical integrator of endothelial cell phenotype and heterogeneity³⁵¹.

Occlusive vasculopathies such as coronary artery, carotid and peripheral artery disease, are a consequence of atherosclerosis-related neointimal formation involving maladapted migration and proliferation of vSMC. Vascular SMCs are not terminally differentiated and exhibit substantial plasticity in phenotypic modulation in response to various stimuli²⁴. Several pre-clinical studies have since shown that Notch signaling is recapitulated in adult vascular tissue following injury⁵²⁵³ and that selective smooth muscle Notch1 receptor

knockdown reduces neointimal formation in these vessels⁵⁴. Moreover, inhibition of Notch with soluble jagged1 attenuates neointima formation after balloon injury by decreasing vSMC proliferation and migration through interference with Notch-Herp2 signaling⁵⁵. Results from recent studies in zebrafish also suggest that activation of Notch signaling by the Sonic hedgehog (Shh) and VEGF pathways is essential for arterial specification during development, and that these pathways may control Notch signaling and vSMC growth (balance of proliferation and apoptosis) *in vitro* following injury^{31, 52}. Collectively, these findings suggest that aberrant Notch signaling can lead to vascular abnormalities and, consequently, that appropriate regulation of Notch signaling in a context-dependent manner is critical both during vascular development and during vascular remodeling in adults following vascular injury⁴.

The putative role of Notch in smaller diameter vessel disease has also been highlighted by the seminal work on CADASIL and using various Notch deficient mouse models⁵⁶. Studies combining Notch 3^{-/-} mice⁵⁷ and transgenic mice expressing representative CADASIL-associated mutant NOTCH3⁵⁶ support novel pathogenic roles for mutant Notch3 receptors, rather than compromised function, as the primary determinant of the CADASIL disease. However, the possibility that loss of function of Notch 3 may contribute to some aspects of this phenotype remains an open question that may well be addressed in the future using the PAC-R169C transgenic mouse⁵⁸. The data also suggest that Notch3 is necessary for the adaptive response of the vasculature to vasoactive systems. A deficiency in the expression of Notch3 could have important physiopathological consequences in the adaptation of the cardiac and renal function to chronic increase of blood pressure⁵⁷.

3. Notch Inhibitors

The Notch pathway emerged in the 1990s as an attractive target for eliminating the stem cell-like tumor progenitor cells thought to drive many endothelial and lymphatic cancers⁵⁹. Starting with a 1997 licensing deal on Notch targets between Exelixis Inc. and Yale University, companies have been trying to inhibit various components of the pathway (with e.g., gamma-secretase inhibitors (GSIs), monoclonal antibodies (mAbs) against receptors, MAb against ligands and stapled peptides) in hopes of blocking both tumor growth and vascularization. One of the most promising targets was delta-like ligand 4 (DLL4), a ligand that activates all four mammalian Notch receptors. However, a Genentech team last year reported that antibodies against DLL4 induced liver and vascular endothelial cell proliferation in mice, rats and monkeys⁶⁰. Genentech subsequently reoriented its program toward monoclonal antibodies (mAbs) that selectively block individual receptors reporting that such antibodies block tumor vascularization and growth without eliciting intestinal endothelial tumors⁶¹.

(a) Gamma-Secretase Inhibitors (GSI's)

Efforts to antagonize the Notch pathway have primarily relied on blocking the generation of NICD using small-molecule inhibitors of the γ -secretase complex (GSIs). Gamma-secretase is an intramembrane-cleaving protease complex comprising at least four essential components, presenilin (PS1 or PS2), presenilin enhancer 2 (PEN2), nicastrin and anterior pharynx defective 1 (Aph1)⁶². Presenilin is an aspartyl protease and is the catalytic subunit of γ -secretase. It is activated by endoproteolysis into amino- and carboxyl-terminal fragments (PS-NTF and PS-CTF), which interact with each other to form stable biologically active heterodimers. Each fragment contains one of the two transmembrane aspartates and photoreactive aspartyl protease transition-state inhibitors have been shown to label both presenilin fragments suggesting that the active site is at the interface of the NTF-CTF heterodimer⁶³. γ -Secretase cleaves many type I membrane proteins, including amyloid

precursor protein (APP) and Notch⁶⁴ and therefore, targeting γ -secretase has become a principal therapeutic strategy aimed at modulating Notch activation.

The increasing evidence that Notch signaling is aberrant in several human malignancies has fuelled interest in Notch receptors as attractive targets for selective killing of malignant cells⁹⁶⁵. Targeted inhibition of Notch signaling has the potential to affect tumor angiogenesis, tumor cell differentiation, and survival of tumor stem cells. Compared with proliferating normal cells, tumor cells are characterized by markedly enhanced levels of activated Notch-1 receptor⁶⁵. Using small molecule GSI's consisting of a tripeptide aldehyde, N-benzyloxycarbonyl-Leu-Leu-Nle-CHO, which can block processing and activation of all four different Notch receptors, specific apoptotic vulnerability in tumor cells has been reported⁶⁵. Growing preclinical evidence shows that inactivation of the Notch pathway by targeting γ -secretase may be a viable strategy for the treatment of cancer with several studies demonstrating potent inhibition of tumorigenesis⁹.

Early studies on the activity of GSIs as an anti-Notch therapy for acute lymphoblastic leukemia (T-ALL) showed that treatment of T-ALL cell lines with these drugs resulted in rapid clearance of activated Notch1 protein and effective down-regulation of Notch1 target genes⁶⁶. Most notably, Notch inhibition reduced growth and proliferation by inducing G1 cell cycle arrest and decreasing cell size. Subsequently, the Dana-Farber Cancer Institute performed a phase I clinical trial testing MK-0752, an oral GSI developed by Merck for the treatment of Alzheimer's disease, in T-ALL patients. The most common dose-limiting toxicity was grade 3/4 diarrhea, revealing an unfavorable gastrointestinal toxicity (GI) profile most probably related to inhibition of Notch signaling in the gut. The development of GI toxicity in the context of GSI therapy was not completely unanticipated and has emerged as a significant obstacle for the clinical development of these drugs. Notch1 and Notch2 play an important role in the intestinal epithelium, where they are involved in the control of cell proliferation and differentiation, and as noted above, GSIs are pan-Notch inhibitors that cause a systemic block of all 4 Notch receptors.

Genetic inhibition of Notch signaling in the gut using animal models via deletion of the *CBF-1/RBP-J κ* gene or in the context of double *Notch1/Notch2* conditional knockouts induces cell cycle arrest and differentiation to secretory cell lineages at the expense of the absorptive epithelium; a phenotype that is recapitulated upon pharmacologic inhibition of the Notch pathway with GSIs. Overall, these results strongly suggest that alternative strategies with an improved therapeutic window may be needed for the successful implementation of GSIs as anti-Notch therapies. In this regard, a recent report from Merck has shown that three days of > 70% Notch inhibition with a GSI is sufficient to induce effective antileukemic responses in T-ALL xenograft models and is well-tolerated⁶⁷. A similar intermittent dosing approach reduced the toxicity associated with PF-03084014, a GSI developed by Pfizer⁶⁸. These results illustrate that secretory metaplasia induced by GSIs is time- and dose-dependent and can be avoided using intermittent dosing schemes. RO4929097 is another potent and selective γ -secretase small-molecule inhibitor targeting the Notch signaling pathway resulting in decreased NICD production and mRNA expression of the Notch target gene, *Hes1*, in tumor cells. Addition of RO4929097 to tumor-derived cell lines produced a flattened, less transformed phenotype as well as a reduced capability for soft-agar growth⁶⁵. RO4929097 produced efficacy when dosed orally in 7 of 8 xenograft models, which was sustained in the absence of further dosing and was not associated with significant body weight loss in mice.

An alternative approach to improve the safety and efficacy of anti-Notch GSI therapies for cancer results from the combined use of GSIs with chemotherapy or other molecularly targeted drugs⁶⁶. The idea is to use GSIs at high doses for short periods of time to avoid the

development of gastrointestinal toxicity while using drug combinations that increase their antileukemic efficacy. Combination therapies of GSIs with cyclin-dependent kinase (CDK) inhibitors, drugs targeting NF κ B signaling, or small molecule inhibitors of CK2 and the PI3K–AKT–mTOR pathway have been shown to increase the antileukemic effects of these pan-Notch inhibitors⁶⁶.

The effect of GSIs on tumor vasculature has not been conclusively determined. Compound X (CX), a GSI previously reported to potentially inhibit Notch signaling in vitro and in vivo, promotes angiogenic sprouting in vitro and during developmental angiogenesis in mice⁶⁹. GSI significantly attenuated growth factor-induced EC proliferation and migration as well as c-fos promoter activity in a dose-dependent manner in EC where γ -secretase activity was up-regulated under hypoxia or the treatment with VEGF. GSI also attenuated VEGF-induced tube formation and inhibited FGF-2-induced angiogenesis on matrigel in mice as quantified by FITC-lectin staining of EC. In vSMC, treatment with GSI significantly attenuated growth factor-induced VEGF and fibroblast growth factor-2 (FGF-2) expression⁷⁰.

Within the normal vasculature, modulating Notch signaling enhances neovascularization and perfusion recovery in diabetic mice suffering from ischemia, suggesting this approach could have utility for human diabetics⁷¹. Dll4/Notch interaction is essential for proper reparative angiogenesis. Moreover, Dll4/Notch signaling regulates sprouting angiogenesis and coordinates the interaction between inflammation and angiogenesis under ischemic conditions⁷². Notch receptor signaling is implicated in controlling vSMC proliferation and in maintaining vSMC in an undifferentiated state. Administration of N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT), a γ -secretase inhibitor that blocks activation of Notch3 in vSMC demonstrates a mechanistic link from Notch3 receptor signaling through the Hairy and enhancer of Split-5 (HES-5) that is crucial for the development of pulmonary arterial hypertension and may provide a target pathway for wider therapeutic intervention⁷³. A unidirectional effect of Jagged1 on both EC and vSMC that contributes to inhibition of arterial lesions after vascular injury has also been reported. The data indicate that Jagged1 may be a novel therapeutic target for aging-related vascular diseases⁷⁴.

(b) Parologue-specific antagonists

Although γ -secretase inhibitors (GSIs) have progressed into the clinic, GSIs fail to distinguish individual Notch receptors, inhibit other signaling pathways and cause intestinal toxicity attributed to dual inhibition of Notch1 and 2. Researchers from Roche's Genentech Inc. and Exelixis Inc., and a separate team at Aveo Pharmaceuticals Inc., have designed antibodies that are selective for cancer-associated subtypes of the Notch receptor. Both groups expect that hitting only the Notch receptors involved in cancer will offer improved safety compared with that for γ -secretase inhibitors and other molecules that target all Notch receptors⁶¹.

To elucidate the discrete functions of Notch1 and Notch2 and develop clinically relevant inhibitors that reduce intestinal toxicity, phage display technology was utilized to generate highly specialized antibodies that specifically antagonize each receptor paralogue and yet cross-react with the human and mouse sequences, enabling the discrimination of Notch1 versus Notch2 function in human patients and rodent models⁷⁵. Selective blocking of Notch1 inhibited tumour growth in pre-clinical models through two mechanisms: inhibition of cancer cell growth and deregulation of angiogenesis. Whereas inhibition of Notch1 plus Notch2 causes severe intestinal toxicity, inhibition of either receptor alone reduces or avoids this effect, demonstrating a clear advantage over pan-Notch inhibitors (GSI's). Despite the positive data, many have argued that the new antibodies are unlikely to surpass the other approaches to inhibiting Notch. Indeed, it may be necessary and beneficial to target multiple

Notch receptors to get a therapeutic effect by modifying the dosing regimens of pan-Notch blockade with GSI's to lower toxicity without impairing efficacy. Stemline Therapeutics Inc has two γ -secretase inhibitors— SL-301 and SL-302—in preclinical development to treat solid cancers. OncoMed's OMP-21M18, an antibody targeting Notch ligand delta-like ligand 4 (DLL4), is in Phase I testing to treat advanced solid tumors and is partnered with GlaxoSmithKline plc. In an in vivo tumor growth assay, the antibody alone or in combination with chemotherapy reduced cancer stem cell frequency and decreased rates of breast tumor recurrence compared with chemotherapy alone⁷⁶.

In terms of safety, researchers have developed dosing regimens that are helping circumvent the GI toxicity associated with γ -secretase inhibitors. The Dana-Farber Cancer Institute are running a Phase I trial of MK-0752, a γ -secretase inhibitor from Merck & Co. Inc., in combination with docetaxel to treat advanced breast cancer. Merck researchers published animal data showing that three days of >70% Notch pathway inhibition using a γ -secretase inhibitor was sufficient to provide an antitumor effect which was well tolerated⁶⁷. Stemline Therapeutics Inc's other approach is combining a γ -secretase inhibitor with glucocorticoids to reduce GI toxicity without impairing efficacy in a mouse model of T-ALL⁷⁷.

While preclinical studies from Roche's Genentech Inc. unit and Aveo Pharmaceuticals Inc minimized the adverse effects of pan-Notch inhibition by selectively targeting Notch 1, a recent new study suggests that Notch 1 inhibition may also cause tumors⁷⁸ thereby necessitating further work on these inhibitors.

(c) Stapled Peptides

Direct inhibition of transcription factor complexes remains a central challenge for drug discovery. In general terms, these proteins lack surface involutions suitable for high-affinity binding by small molecules. However, the design of synthetic, cell-permeable, stabilized α -helical peptides that target a critical protein-protein interface in the Notch transactivation complex has been reported⁷⁹. Direct, high-affinity binding of the hydrocarbon-‘stapled peptide’ mimetic, SAHM1 prevented assembly of the active Notch transcriptional complex. SAHM1 slots in where the MAML1 protein should be, potently inhibiting Notch signaling. The peptides are taken up by an active transport mechanism into compartments called endosomes, from which they can reach their target. The treatment of leukaemic cells with SAHM1 results in genome-wide suppression of Notch-activated genes. Direct antagonism of the Notch transcriptional program causes potent, Notch-specific anti-proliferative effects in cultured cells and in a mouse model of Notch1-driven tumorigenesis. Because stapled peptides target the very last step before the Notch genes are turned on transcriptionally, they are unlikely to interfere with other cell processes and therefore reduced toxicity should ensue.

(d) MicroRNA's

MicroRNAs (miRNAs) are integral elements in the post-transcriptional control of gene expression. As such, miRNAs serve as nodes of signaling networks that ensure homeostasis and regulate cancer, metastasis, fibrosis and stem cell biology. After the identification of hundreds of miRNAs, the challenge is now to understand their specific biological function. Signaling pathways are ideal candidates for miRNA-mediated regulation owing to the sharp dose-sensitive nature of their effects. Indeed, emerging evidence suggests that miRNAs affect the responsiveness of cells to signaling molecules such as TGF β , Wnt and Notch⁸⁰ that may result in specific cancer therapies.

Recent studies show that microRNA (miRNA)-143/145 regulates vSMC phenotype. Using miRNA arrays, miR-143/145 is induced following expression of a constitutively active

Notch1 NICD and represents a novel transcriptional target of Jag-1/Notch signaling in vSMC⁸¹. Multiple pathways converging on miRNA-143/145 provide potential strategies for the fine-tuning or amplification of Notch-based therapies in vSMC by antisense inhibition or following re-expression with suitable mimetics of these or other relevant microRNAs.

4. Expert Opinion Notch Inhibitors

Aberrant Notch signaling has been strongly implicated in the etiology of several types of cancer and in vascular disease. Therefore, components of the Notch signaling pathway are considered highly ‘druggable’ and present as important targets for the treatment of these disease conditions. To date, pan-Notch receptor inhibition using GSI's is associated with numerous complications. This is not surprising as γ -secretase is a promiscuous protease enzyme complex with up to 60 potential substrates in addition to Notch. As a result, RO4929097 and other reported γ -secretase inhibitors are likely to carry additional inhibitory activities that preclude a complete understanding of Notch function in tumor and vascular biology at this time. Differences in substrate selectivity can explain some differences in biology reported for other γ -secretase inhibitors such as cell cycle inhibition. It is clear there are many remaining uncertainties that must be addressed experimentally if inhibition of Notch signaling is to be exploited as a treatment approach. Nonetheless, targeted Notch inhibition, in combination with other therapies is a promising avenue for future management of various cancers, in particular breast tumors. In this context, aberrant Notch4 activity can induce mammary gland carcinoma in the absence of CBF-1/RBP-Jk fuelling the need for a better understanding of the components of CBF-1-independent oncogenic Notch signaling pathways and their contribution to Notch-induced tumorigenesis. Collectively, accumulating data provides experimental support for proceeding into clinical development using intermittent dosing regimes, which may allow for recovery in normal tissue from Notch inhibition while maintaining anti-tumor efficacy.

The realization that the lung carcinoma H460a cell line appears to be refractory to the effects of the GSI, RO4929097 highlights the molecular defects driving the resistance of the H460a cell line and may thus provide a deeper understanding of the Notch signaling pathway in cancer thereby laying the groundwork for an appropriate patient selection molecular profile. It is clear that further evaluation of the key drivers of efficacy will provide important information on the types of tumors responsive to treatment and appropriate selection of patients who are most likely to derive clinical benefit from GSI's such as RO4929097⁶⁵. Recent evidence suggests that the Notch signaling pathway is one of the most important in drug-resistant tumor cells⁷⁵. Moreover, down-regulation of Notch signaling could induce drug sensitivity, leading to increased inhibition of cancer cell growth, invasion, and metastasis. In this context, much effort has been invested in supporting the roles of Notch in drug resistance and how targeting Notch by “natural agents” could become a novel and safer approach for the improvement of tumor treatment by overcoming drug resistance.

In terms of selective receptor inhibition, initial studies have shown minimal side effects with short-term blockade of either Notch1 or its ligand Delta-like 4, but long-term side effects were not investigated. Using mouse models to demonstrate the consequence of long-term Notch1 inhibition produced evidence indicating that this results in vascular tumors in the liver and decreased survival, suggesting that specific Notch1 therapies should be reevaluated. That being said, anti-Notch1 and 2 antibodies stand among the strongest therapeutic candidates for treating indications linked to aberrant Notch signaling, particularly in cancer, immunology and vascular regenerative medicine. Compared to pan-Notch inhibitors such as GSIs or stapled peptides⁷⁹, antibodies offer the advantages of an improved safety profile, paralogue-specific inhibition and a clinically established drug format. Anti-Notch1 holds the promise of simultaneously targeting cancers by directly

inhibiting cancer cell growth and disrupting tumour angiogenesis. As anti-DLL4 blocking antibodies are entering the clinic because of their anti-angiogenic effects, Notch1 inhibitors may also directly affect tumour cell viability in Notch1-driven cancers and block both ligand-dependent and -independent activation. Similarly, Notch2 has been implicated in many cancers and anti-Notch2 mAbs stand as candidates for treating melanomas and some B-cell leukaemias.

As hereditary mutations of Notch components are associated with congenital defects of the cardiovascular system in humans such as Alagille syndrome³⁸ and CADASIL³⁹, the case for using Notch inhibitors to treat vascular disease is strong. The characterizations of anti-Notch1 mAbs and the development of anti-Notch3 mAbs make a compelling case for adding these Notch receptor-specific antibodies to prevent the aberrant signaling that prevails following vascular injury and pulmonary hypertension. Notch ligands have distinct functions in vascular development and disease. However, given that the effects of Notch pathway activation on endothelial cells are context-dependent, many questions remain to be answered. First, the upstream signaling pathways that control the expression of Notch ligands in blood vessels remain largely unknown; VEGF induces Dll4 expression in endothelial cells but Jagged1 is absent in tip cells where Dll4 is highly expressed, which suggests that the two ligands are regulated differently. Second, the selective activation of Notch in vascular endothelium remains unclear; for example, Notch signaling is not activated in arteries of *Dll1* mutant mice, despite the presence of Jagged1 and Dll4. Third, the role of non-canonical Notch ligands, such as microfibril-associated glycoprotein (MAGP)-2, is poorly understood. MAGP-2 binds to Jagged1, Jagged2, Dll1, and Notch1 and is known to modulate Notch signaling in sprouting angiogenesis, but the mechanistic basis for the function of MAGP-2 in ligand-dependent Notch activation has yet to be elucidated⁸². Finally, given that Dll4 and Jagged1 have opposing effects on angiogenesis, experiments that specifically inhibit each ligand with selective neutralizing antibodies may be important not only for understanding how Notch is activated in the vasculature, but also for the development of therapeutic strategies designed to control angiogenesis by targeting Notch signaling.

Vascular SMC are integral to the formation and progression of vascular lesions in atherosclerosis, restenosis and arteriosclerosis through their migration and proliferation⁵⁴ and thus present themselves as a target cell for therapeutic interventions. More importantly, as vSMC proliferation and migration is associated with enhanced Notch1 signaling both *in vitro*^{4, 24} and *in vivo*⁵² Notch1 inhibitors are a potentially important therapeutic option. The transition of progenitor Sca1⁺ stem cells within adventitial layer to intimal and medial vSMC following injury⁵² and their potential regulation by a Hh stimulated Notch pathway offers further possibilities for targeted intervention. Moreover, manipulation of Notch1 proteosomal degradation using glycogen synthase kinase 3 beta (GSK-3 β) inhibitors *in vitro*⁵³ may present further opportunities for regulation of Notch1 levels *in vivo*.

5. Hedgehog Signaling Pathway

The Hedgehog gene was identified in genetic screens aimed to provide an understanding of body segmentation in *Drosophila*⁸³. Loss of the secreted Hedgehog signaling protein was found to cause *Drosophila* embryos to develop as spiny balls reminiscent of hedgehogs. Hedgehog signaling has since been shown to play a role in many processes during embryonic development and remains active in the adult where it is involved in the maintenance of stem cell populations where aberrant Hedgehog signaling in some cases can lead to certain forms of cancer.

Hh ligands are soluble, lipid-modified morphogens that may be secreted in two different forms: a short-range acting (poorly diffusible) type, and a second form for long-range transport, “packed” in membranous structures that dictate the angiogenic profile of several endothelial cell types^{84,85}. Hh proteins are able to interact with Patched (Ptc), a membrane-spanning receptor on the surface of Hh-responsive cells⁸⁶. In *Drosophila*, Hedgehog signaling is initiated by the binding of Hedgehog ligand to Patched (Ptc) which is a 12-transmembrane protein receptor^{68,7}. Ptc acts as an inhibitor of Smoothened (SMO), a 7-transmembrane protein related to the Frizzled family of Wnt receptors and to other 7-transmembrane G protein-coupled receptors. Downstream of SMO is a multi-protein complex known as the Hedgehog signaling complex (HSC), which comprises the transcription factor Cubitus interruptus (Ci), the serine/threonine kinase Fused (Fu), the kinesin-like molecule Costal 2 (Cos2) and Suppressor of fused (Sufu). Cos2 also binds to protein kinase A (PKA), protein kinase CK1 (formerly casein kinase 1) and glycogen synthase kinase 3 beta (GSK-3 β), which are other kinases that are implicated in the Hedgehog signaling pathway [Figure 2].

In the absence of ligand, Ptc represses SMO preventing the activation of Hedgehog signaling. The HSC is bound to microtubules/membranes and associates with SMO through Cos2. The full-length form of Ci is prevented from nuclear translocation through interactions with Sufu and Cos2. A portion of full length Ci is proteolytically cleaved to produce a repressor form of Ci, which enters the nucleus leading to the inhibition of Hedgehog target gene expression. In the presence of Hh, the inhibitory effects of Ptc on SMO are relieved and the HSC is freed from microtubules and membranes. SMO becomes phosphorylated by PKA and CK1 and PKA, CK1 and GSK-3 β are released from Cos2, precluding the generation of the repressor form of Ci. Full length Ci is no longer inhibited by Sufu and is therefore free to enter the nucleus to induce the transcription of Hedgehog target genes such as *engrailed*, *Ptc* and *decapentaplegic* (encodes Bone Morphogenetic Proteins in vertebrates)⁸⁸.

Hedgehog ligand is synthesized as a precursor, which undergoes an autoproteolytic cleavage to liberate a 19 kDa N-terminal fragment (N-Hh), which displays all known signaling properties and a slightly larger C-terminal peptide fragment that has no apparent function other than to catalyze cleavage⁸⁸. The auto-processing reaction provides a trigger for the addition of a cholesterol moiety to the C-terminal of N-Hh. The N terminus is modified via the addition of a palmitate molecule by the acyltransferase Skinny Hedgehog (Skn) - also known as Central missing (Cmn), Rasp and Sightless. Membrane tethered Hedgehog proteins initiate signaling in the nearby vicinity of the producing cell or Hh proteins form multimeric complexes in which the hydrophobic moieties cluster together in an inner core allowing diffusion of ligand and long range signaling⁸⁸. Dispatched (Disp) and Toutvelu (Ttv) mediate Hh release and diffusion. Disp is required for release of membrane anchored Hh protein and Ttv regulates the synthesis of proteoglycans enabling the movement of Hedgehog ligands thereby facilitating long range signaling.

The Hedgehog signaling pathway in vertebrates shares many common features with *Drosophila*, although distinct differences are also apparent⁸⁹. In mammals there are three Hedgehog genes, Sonic, Indian and Desert Hedgehog. There are also two Ptc genes (Ptc 1 and Ptc 2) as well as three Ci homologues known as Gli 1, Gli 2 and Gli 3. Gli1 and Gli2 are transcriptional activators, whereas Gli 3 functions as a transcriptional repressor. Regulators of Hh signaling in vertebrates include megalin, which is a member of the low-density lipoprotein receptor related family and binds Hh⁹⁰ and SIL which functions downstream of Ptc⁹¹. Missing in metastasis (MIM or BEG4) is an actin-binding protein that regulates Gli-dependent transcriptional activation in vertebrates, thereby modulating Hh signaling⁹².

(a) Hedgehog and Cancer

Aberrations of the Hh pathway in human tumors derives from observations in patients with inactivating germline mutations of the Hh receptor Ptch that develop Gorlin's syndrome, also known as nevoid basal cell carcinoma (BCC)⁹³. The repercussions of these mutations include skeletal and dental abnormalities due to developmental patterning defects. In addition to a high incidence of sporadic BCC, patients with the mutation also exhibited an increased incidence of medulloblastoma, ovarian cysts, and ovarian carcinoma (reviewed⁹). Moreover, somatic mutations in Ptch⁹⁴ and SMO⁹⁵ that trigger constitutive and cell-autonomous pathway activation have been found in 20 % of pediatric medulloblastomas and in more than 70% of sporadic BCC⁹⁶. Additionally, tumor types such as small-cell lung cancer, as well as gastric, pancreatic, and prostate cancer, have been reported to display abnormal activation of the Hh pathway in the absence of known mutations⁹.

Recent evidence has emerged that Hh signaling, in a similar manner to Notch, is also important for driving the self-renewal of cancer stem cells (CSC), a small subset of cells in a tumor that are able to initiate tumor spread and which are typically resistant to chemotherapy, possibly contributing to relapse⁹⁷. Elimination of these cancer stem cells by targeting the Hh pathway in combination with chemotherapy has been shown to increase therapeutic efficacy in animal models of pancreatic cancer⁹⁷. In the last few years, cross-talk during carcinogenesis has also been established between the Hh signaling pathway and several other key molecular signaling pathways such as Notch, Wnt and growth factors, which will have implications for the treatment of Hh-dependent cancer⁹⁷.

Several issues in the precise role of the Hh signaling pathway in cancer remain unresolved, including the precise mechanisms of signal transduction, the exact mode of signaling between tumor cells and the microenvironment, and the role of signaling in the regulation of CSCs⁹⁸. However, it is clear that the Hh pathway can play an important role in tumor cell growth and survival, and these functions are likely to be broadly applicable across a variety of malignancies. As several novel inhibitors have entered clinical testing, precise correlative studies may allow many of these conflicts to be resolved within the most relevant system, the patients themselves.

(b) Hedgehog and Vascular Disease

The Hedgehog signaling pathway controls pattern formation, cellular proliferation, and differentiation of a number of different cell types including vascular cells during vertebrate embryogenesis⁵¹. The appearance of molecular differences between an arterial and venous cell before circulation suggested that genetic factors may determine these cell types. Indeed, VEGF acts downstream of Hh's and upstream of the Notch pathway to determine arterial cell fate during development⁵⁰. In adults, a similar hierarchy exists since Shh stimulates Notch target gene expression via VEGF-A in mature vSMC⁵².

As Hh ligands are packed in membraneous structures, the transfer of Hh signals by microparticles and microvesicles has been utilized to determine the effect of Hh signaling on adult vascular cells. Myofibroblastic hepatic stellate cells and cholangiocytes release exosome-enriched microvesicles containing biologically-active Hh ligands that induced changes in angiogenic gene expression of hepatic sinusoidal endothelial cells⁸⁴. Also, *in vitro* treatment of human umbilical vein endothelial cells with microparticles harboring Shh induced proangiogenic changes in these cells⁸⁵. Moreover, *in vivo* treatment of hindlimb ischemic mice with Hh microparticles improved blood flow in the ischemic leg⁹⁹.

Given that Shh can stimulate cell proliferation during development and cancer, it was not surprising that Shh might function to induce vascular proliferate phenomena typical of vascular disease. Hh signaling is recapitulated postnatally in adult vascular tissues and

induces neovascularization of ischemic tissue¹⁰⁰ and arteriogenesis in diabetic nephropathy¹⁰¹. The Hh pathway is also required for retinal angiogenesis and its inhibition represents a potential therapeutic strategy targeting ocular neovascular disease¹⁰².

Further studies have demonstrated that Shh/Gli2 signaling induces arterial vSMC proliferation³¹⁵² via the Rb/E2F pathway underscoring the potential importance of Shh during intimal hyperplasia¹⁰³. Indeed, Shh receptors Ptc1 are differentially expressed within the adventitial medial boundary layer of normal vessels only to spread to intimal and medial cells vSMC following injury⁵². Interestingly, the adventitial medial layer is where a Sca-1⁺ adventitial progenitor cell niche is resident raising the possibility that Hh signaling may control the differentiation of vascular stem cells analogous to cancer stem cells during tumor initiation and progression¹⁰⁴.

Finally, while aberrant proliferation and migration of vascular cells may derive in part from the recapitulation of Hh signaling in adults following vascular injury (and thus represent a potentially important target for Hh inhibitors), it is equally important to consider that Hh pathway activation can also be beneficial under ischemic conditions where micro- and/or nano-particle delivery of Hh agonists may present as a novel targeted therapeutic option¹⁰⁵.

(c) Hedgehog Inhibitors

In 1998, Beachy and his colleagues first showed that a compound derived from the corn lily plant called cyclopamine worked by blocking the Hedgehog pathway¹⁰⁶. Four years later, his team identified four small molecules that inhibit Hedgehog activity but are structurally distinct from cyclopamine¹⁰⁷. The Hedgehog signaling pathway has since emerged as an important target for anticancer drugs, with several compounds in clinical trials. The initial focus was on cyclopamine - the first compound discovered that could inhibit the Hedgehog pathway - and selected cyclopamine analogues, including the development of IPI-926. In addition, a number of other compounds have been developed or are in clinical development (Vismodegib [GDC-0449], a small molecule inhibitor of SMO) along with combination chemotherapy - incorporating a Hedgehog pathway inhibitor as well as another drug from the perspective of drug resistance and effects on cancer stem cells¹⁰⁸. A number of tumor types rely on overexpression of Hh ligands to activate the pathway in a paracrine manner from the tumor to the surrounding stroma. Alternatively, Hh ligands may act on cancer stem cells in some hematopoietic cancers, such as chronic myelogenous leukemia¹⁰⁹. However, the role of the Hh pathway is best established in tumors, such as basal cell carcinoma and medulloblastoma, where the pathway is activated via mutations¹⁰⁹. Understanding the contribution of Hh signaling in these various tumor types will be critical to the development and use of agents targeting this pathway in the clinic.

GDC-0449 was the first systemic SMO-inhibitor entering clinical trials with Genentech¹¹⁰. It was successfully tested in a phase-I clinical trial demonstrating good pharmacodynamic (PD) and pharmacokinetic (PK) properties and showing objective response and clinical benefit in several patients with basal cell carcinoma (BCC)¹¹⁰. In a phase 2 study of 41 people with BCC, a team led by Ervin Epstein from the Children's Hospital Oakland Research Institute in California found that participants taking GDC-0449 developed only four new tumors on average over the course of a year, compared to 24 in subjects on placebo. Researchers from a handful of other drug companies, including Pfizer, Takeda, Novartis and Infinity, have all generated phase 1 or preclinical data from their own experimental Hh inhibitor drugs, all of which target SMO. Eli Lilly's preclinical data showing that its drug, called LY2940680, inhibits cancer growth in cell lines containing a mutation in the gene encoding SMO in a patient with cancer who developed resistance to vismodegib. The drug is currently being tested in phase 1 trials for people with a range of solid tumors. Similarly, Infinity Pharmaceuticals is advancing its own derivative of

cyclopamine, known as IPI-926, with improved potency and stability. After completing a successful phase 1 trial of the drug in people with advanced solid tumors, including those with BCC, they have launched separate phase 2 trials in people with pancreatic cancer and with chondrosarcoma bone cancer. IPI-926 changes the blood vessel support of the tumor to improve the delivery of other chemotherapeutic drugs. Although there are no Hedgehog pathway mutations seen in chondrosarcoma, no drugs exist to treat this type of cancer.

6. Expert Opinion Hedgehog Inhibitors

Specific Hh pathway inhibition has become an attractive strategy in anticancer therapy. To date, several targets within the pathway have been identified and specific assays have been developed to detect small molecules capable of altering the activity of these targets. This strategy targets either the tumor cells directly, or in surrounding nonmalignant stromal cells that supply growth-promoting factors to the tumor. Small-molecule modulators of Hh signaling have been the subject of recent reviews^{6, 11197} and the last few years have brought a tremendous increase in reports of novel inhibitors¹¹². Recent results from clinical trials using topical and systemic administration of Hh pathway inhibitors to BCC patients provide the first evidence of the therapeutic benefit resulting from inhibition of this signaling pathway¹¹³. There are many components involved in the regulation of Hh signaling and this diversity of regulatory interactions reflects the myriad of cellular responses in which Hh signaling is implicated. Indeed, most of the components described here (as with Notch signaling) function in a context-dependent manner. Regulating their expression or activity can modulate Hh signaling and therefore the Hh-dependent cell response. Even when no Hh mutations are present, aberrant Hedgehog signaling still drives solid tumor formation and growth by supporting the blood vessels that fuel their growth. In this context, Genentech is currently testing its Hh inhibitor GDC-0449 for numerous types of cancer. The phase 1 data from a range of solid tumors, published last month, look promising¹⁰⁵. Accordingly, many companies are now developing Hedgehog inhibitors because Hh pathway components are highly 'druggable' once cyclopamine was identified. Based on the amount of data accumulated to date on Hh inhibitors, it is highly likely that the GDC-0449 will reach market first as it is better characterized than its competitors and may be ready for regulatory approval before the end of 2011¹¹⁴.

While the primary focus of research and the target of most drug screens to date has been aimed at the level of SMO or further downstream, a greater understanding of the mechanisms of Hh secretion and spread should lead to new targets for drug discovery. However, several outstanding issues remain surrounding the secretion, spread and reception of Hh. Lipid rafts and their components probably play a central role in Hh secretion and release, but it is unclear at which step of the process these membrane microdomains are required. Lipophorins also appear to be of prime importance in controlling Hh secretion and spread; however, little is known about the function of lipophorins in Hh-producing cells, and important questions remain unanswered¹¹⁵. Lipoprotein particles (such as LDL and VLDL) circulate in the bloodstream to transport lipids, raising the possibility that Hh might be present in the blood to provide an unknown function. Inhibition of such a function might also lead to adverse effects of such a therapy. The discovery of interference hedgehog (iHog), and its orthologs also increases the complexity of Hh reception, and sophisticated genetic approaches will be required for complete identification of many components of Hh reception. Because of the technical difficulties in isolating membrane proteins without disrupting their structural conformation, studying the biochemistry of membrane-associated proteins will not be a simple task. Nevertheless, genetic screens [either in cell culture with RNAi high-throughput screens, or *in vivo* using *C. elegans* or *Drosophila*] will help in the identification of new components involved in Hh secretion, release, spread and reception.

Moreover innovative technologies and new selection criteria will be needed in order avoid past failures (e.g., low efficiency of RNAi treatments, gene redundancy).

Next to its importance in cancer biology, Hh plays an essential role in the development of the cardiovascular system. Indeed, Hh signaling has been found essential for the developing vasculature, proper looping of the heart tube and the formation of the aortic arches. A substantial body of evidence now suggests that the Hh pathway plays an important role in tissue salvage during ischemia in adults¹⁰⁰. Therapeutic strategies targeting the Hh pathway thus seem to be interesting avenue to pursue where it seems likely that specific SMO agonists might prove useful in a clinical setting. Hh agonists for the treatment of ischemia have been proposed. Strategies under consideration for targeting include DNA coding for Shh that will probably not yield a feasible strategy in humans due to obvious regulatory obstacles despite initial positive results. An important issue to consider, however, is whether a Shh-based treatment strategy is the best option. It is important to fully understand the underlying mechanism by which exogenous Hh plays its role in limiting ischemia-induced tissue injury as that would allow targeting specific signaling molecules that limit tissue injury but which are not carcinogenic.

Importantly, Hh might exert a dualistic action in cardiac ischemia in which high exogenous levels are able to foster tissue repair, whereas endogenous Hh seems to aggravate coronary disease. In this context, it might be an attractive strategy to prevent endogenous Hh production. However, the requirement for an active Hh pathway in adult tissue (as known for some stem cell compartments in the brain) places limitations on long-term use of SMO inhibitors. Further investigation into the role of aberrant Hh signaling within the vasculature³¹ in particular within adventitial Sca1⁺ stem cell progenitors¹⁰⁴ and their permissive role in vascular proliferative disorders, is required⁵². Thereafter, the underlying mechanism by which Hh pathway antagonists might limit vascular injury would allow for the further identification of therapeutic targets that are not important to such a wide array of biological processes as the Hh pathway itself.

7. Conclusions

While pan-Notch antagonists are associated with GI toxicity in animal models of disease and cancer patients, pre-clinical and clinical data using selective antibody strategies to preferentially target Notch receptors and ligands has proven less toxic but concerns remain over normal organ homeostasis and significant pathology in multiple organs. In contrast, the Hh-based drug pipeline has been more fruitful with greater than a dozen smoothened (SMO) Hh inhibitors at various stage of development. Cutting-edge chemical biology is also unveiling new targets within the Hh pathway. Overall, refined strategies will be necessary to harness these pathways safely as a powerful tool to disrupt angiogenesis and vascular proliferative phenomena without causing prohibitive side effects.

Notch and Hh pathways have important functions in the development and maintenance of the cardiovascular system, and are significantly recapitulated in vascular tissue following injury. These developmental pathways offer exciting new therapeutic avenues for the treatment of various vascular conditions. The expansion of the target list for components of each pathway in cancer and tools to approach them is moving extraordinarily rapidly to clinical trials. This effort is also propelling biological discoveries in cardiovascular research. The combined efforts of cardiovascular and cancer biologists, along with clinical investigators in these fields, will be needed to understand how to safely exploit these efforts. However, it remains to be determined whether specific targeting of components of the Notch and Hh pathway pathways for cardiovascular disease will be successful in the clinic. Finally, mechanistic insights will determine the timing of drug administration, thereby minimizing

the severity of toxicity and lead to an important discovery platform for the mechanistic regulation of the vascular system following injury and may well be an important strategy to improve clinical outcomes for cardiovascular patients in the future.

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Article highlights

- Inhibition of Notch and Hedgehog pathways has offered an effective strategy for treating several cancers
- The use of more than one drug affecting these pathways may increase their respective efficiency and conversely might decrease their toxicity.
- The sensitivity of cancer cells to inhibitors of the Notch and Hedgehog pathways suggests that combining them may overcome the poor sensitivity of selective cancers to current therapies.
- Up-regulation of Notch and Hedgehog also occurs during vascular development and is recapitulated in adults following vascular injury.
- The sensitivity of vascular cells to inhibitors of the Notch and Hedgehog pathways suggests that their use in certain vascular proliferative disorders should enhance current therapies

Fig 1.
Notch signaling components demonstrating current therapeutic target sites for intervention.

Fig 2.
Hedgehog signaling components demonstrating current therapeutic target sites for intervention