"SQUALAMINE: PLAYS A MAJOR ROLE IN THE TREATMENT OF TUMOR AND RETINAL DISEASES"

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ABSTRACT: Squalamine, a natural cationic steroid isolated and extracted from different species of dogfish shark (*Squalus acanthias*) and later identified in the circulating white blood cells of Sea lamprey (*Petromyzon marinus*). This compound is used as a novel approach in the treatment of various tumors by targeting angiogenesis-associated tumors, which plays a crucial role in the formation of metastati. The chemical representation of squalamine is 7,24 dihydroxylated 24-sulphate steroid conjugated with spermidine at position C-3, which possesses strong anti-angiogenic properties and causes tumour proliferation, invasion and migration disruption. Its mechanism of action includes, when it binds to the cell membrane, inhibits the sodium hydrogen exchanger membrane and also functions as a calmodulin chaperone. Various theories of the mechanism of action of squalamine have been covered in this review paper, highlighting the role of squalamine in the treatment of various types of tumors and retinal diseases, despite the advancement of cancer therapy. Comparative analysis of squalamine with other anti-angiogenic steroids is covered despite of lacking glucocorticoids and mineralocortiocoids showing its anti-angiogenic properties without a much side effects because it is a natural derived product and when combined with chemotherapy agents, its anti-angiogenic properties have been improved in many clinical and pre-clinical studies.

Keywords: Squalamine, Ovarian Cancer, Lung Cancer.

INTRODUCTION: Squalamine, a natural steroidal compound isolated and extracted from the various species of dogfish shark (*Squalus acanthias*) and later identified within the circulating white blood cells of Sea lamprey (*Petromyzon marinus*)(15). This compound when enter the cell causes the changes in vascular endothelial cell shape and has been recognised to possessed significant antiangiogenic activity in models of lung,breast,brain and ovarian cancer. In the shark,squalamine is found primarily in sites of bile synthesis such as liver and gall bladder,but the aminosterol compound also occurs in small amounts in the spleen,testes,stomach and small intestine(15)(4). In the laboratory, squalamine was originally found to have bactericidal activity against gram negative and gram-positive bacteria as well as some fungicidal qualities(4). The chemical structure of squalamine is a 7,24 dihydroxylated 24-sulfated cholestane steroid conjugated to a spermidine at position C-3(15). Squalamine has been demonstrated to be an angiostatic steroid by virtue of its inhibition of growth of vascular endothelial cells in culture,activity in the chick embryo chorioallantoic membrane assay and a rabbit corneal micropocket assay,as well as growth inhibition of gliomas and lung cancers in vivo. Squalamine induces angiogenesis by inhibiting endothelial cell proliferation and migration induced by a wide variety of growth factors including basic fibroblasts growth factor(bFGF) and vascular endothelial growth factor (VEGF). The

interaction between squalamine and vascular endothelial cell results in blocking membrane sodium hydrogen exchanger and act as a calmodulin chaperone. These primary actions further promote inhibiton of several vital steps in angiogenesis such as blockade of mitogen-induced actin polymerization, cell-cell adhesion and cell migration leading to suppression of endothelial cell proliferation(3).

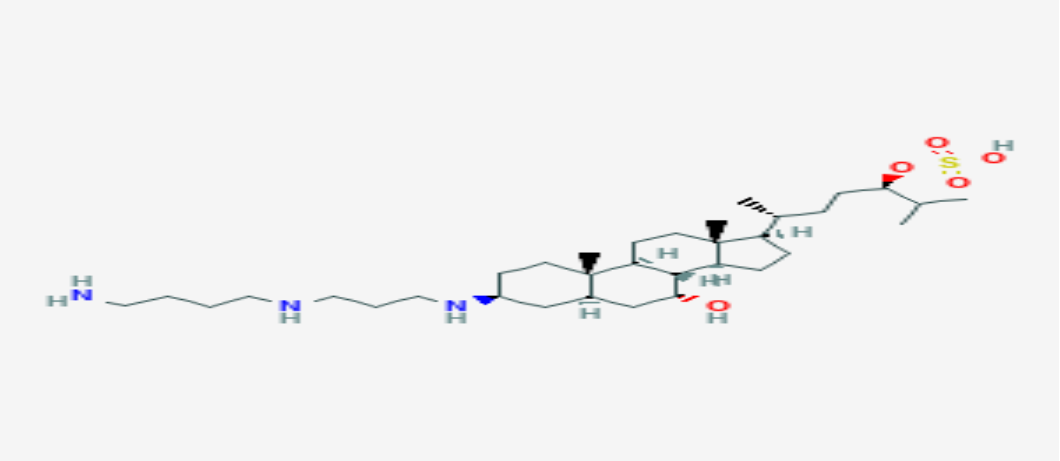

Figure 1: Chemical structure of Squalamine.

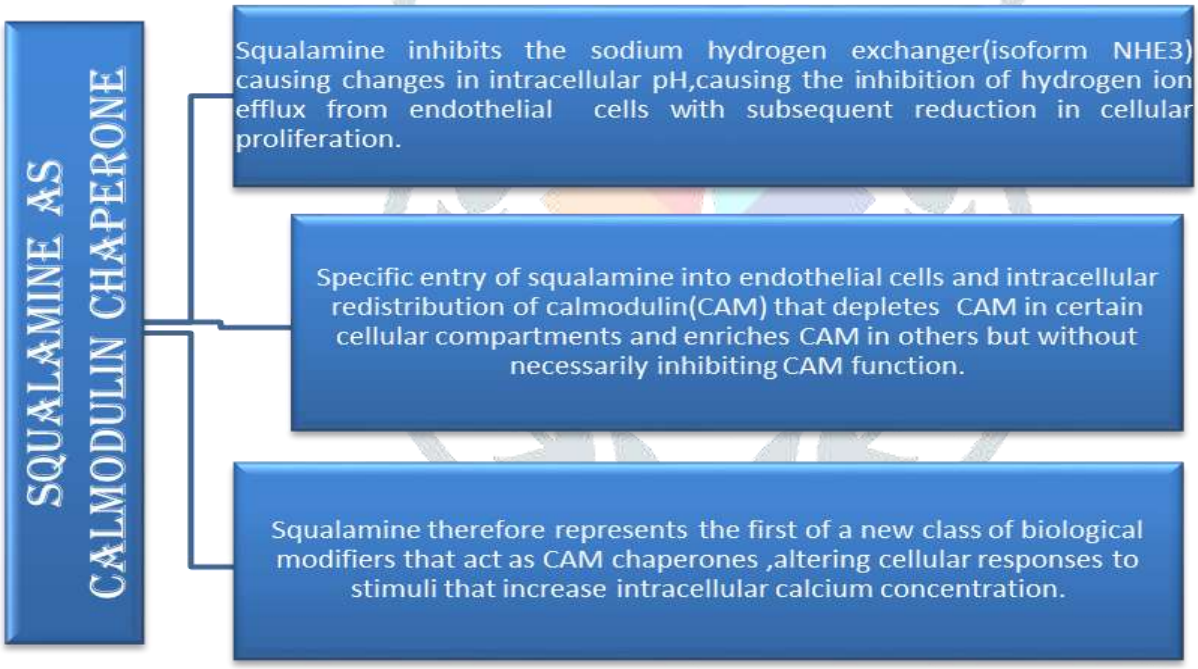

Figure 1.1: Squalamine as a calmodulin chaperone.

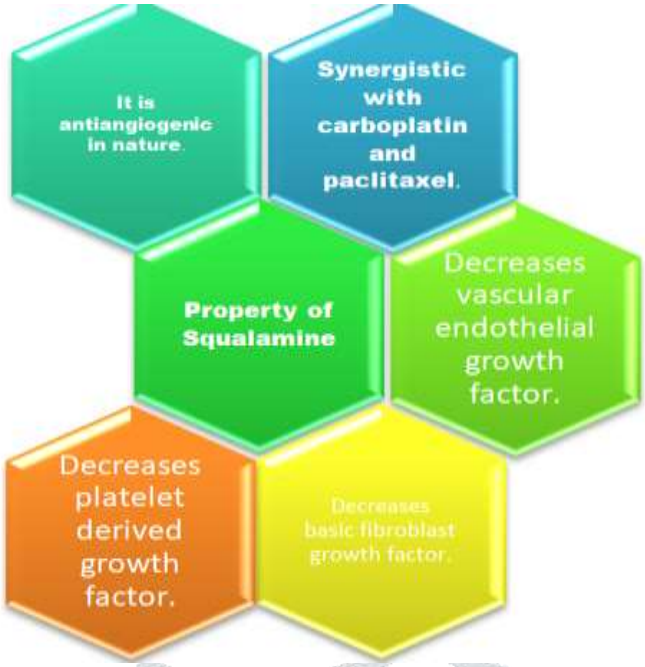

Figure 1.3: Property of squalamine by which it acts on endothelial cells of tumor.

COMPARATIVE ANALYSIS OF SQUALAMINE WITH OTHER ANTIANGIOGENIC STEROIDS: Squalamine is exclusive among most presently out there and undergoing development antiangiogenic steroids as a result of it inhibits epithelium cell proliferation and migration elicited by a large form of issue proteins together with basic embryonic cell protein and tube-shaped structure epithelium growth factor.Like alternative antiangiogenic steroids,squalamine has important structural variations and doesn't act with hormone or corticoid receptors(3).

Antiangiogenic steroids	**Methods used**	**Mechanism of action**
Progestin,medroxyprogesterone acetate and glucocorticoids such as dexamethasone and cortisone	Chick embryo allantoic membrane assay system for angiogenesis.	Effect on tumor associated angiogenesis,inhibition of endothelial cell proliferation,inhibition of collagenolysis and plasminogen activator production as well as direct antitumor activity.
11α-Hydrocortisone and tetrahydrocortisone(potent antiangiogenic steroids)	Chick embryo allantoic membrane assay	Capillary regression in the assay inspite of lacking mineralocorticoid and glucocorticoid.
some steroids given in combination with heparin or heparin like molecule	Using tumor model	Enhanced antiangiogenic activity

2-methoxyestradiol (endogenous metabolite of estrogen)	In vivo and in vitro tumor model	Inhibit proliferation,migration and invasion of endothelial cells in tumor models but the mechanism action of these compound is not fully elucidated,it initiate apoptosis in both vascular endothelial and solid tumors.

MECHANISM OF ACTION OF SQUALAMINE: Squalamine could be a cholestane steroid conjugated with spermidine at position-3. but it doesn't have glucocortiocoid and adrenal cortical steroid effects, squalamine binds to cell membranes and inhibits the NHE3 atomic number 11 membrane money handler, inflicting alteration of animate thing hydrogen ion concentration and interruption of animate thing signal elicited by angiogenic growth factors. Squalamine changes the shape and reduces the degree of epithelium cells in embryonic tube beds, inflicting narrowing of the lumen and occluding blood flow. These activities impede many key steps in ontogenesis, together with mitogen-induced simple protein chemical action, cell adhesion, and cell migration, eventually inhibiting epithelium cell proliferation. Squalamine blocks downstream signal pathways of VEGF,including VEGF-induced phosphorylation of p44/p42 MAP enzyme in tube epithelium cells,disrupts F-actin fibers,and induces internalization of tube endothelial-cadherin from the membrane into the animate thing compartment. It decreases retinal neovascularization that's thought to profit devolution. Squalamine is an amphipathic compound that interacts with varied membrane glycerophospholipids at distinctive affinities. it's the next killing rate of gram-positive bacterium than gram-negative bacterium. It conjointly improves the toxicity of therapy medicine by encouraging tumour cell caspase-mediated cell death and decreasing ontogenesis. Its antiangiogenic effects arr because of inhibition of epithelium cell proliferation(9).

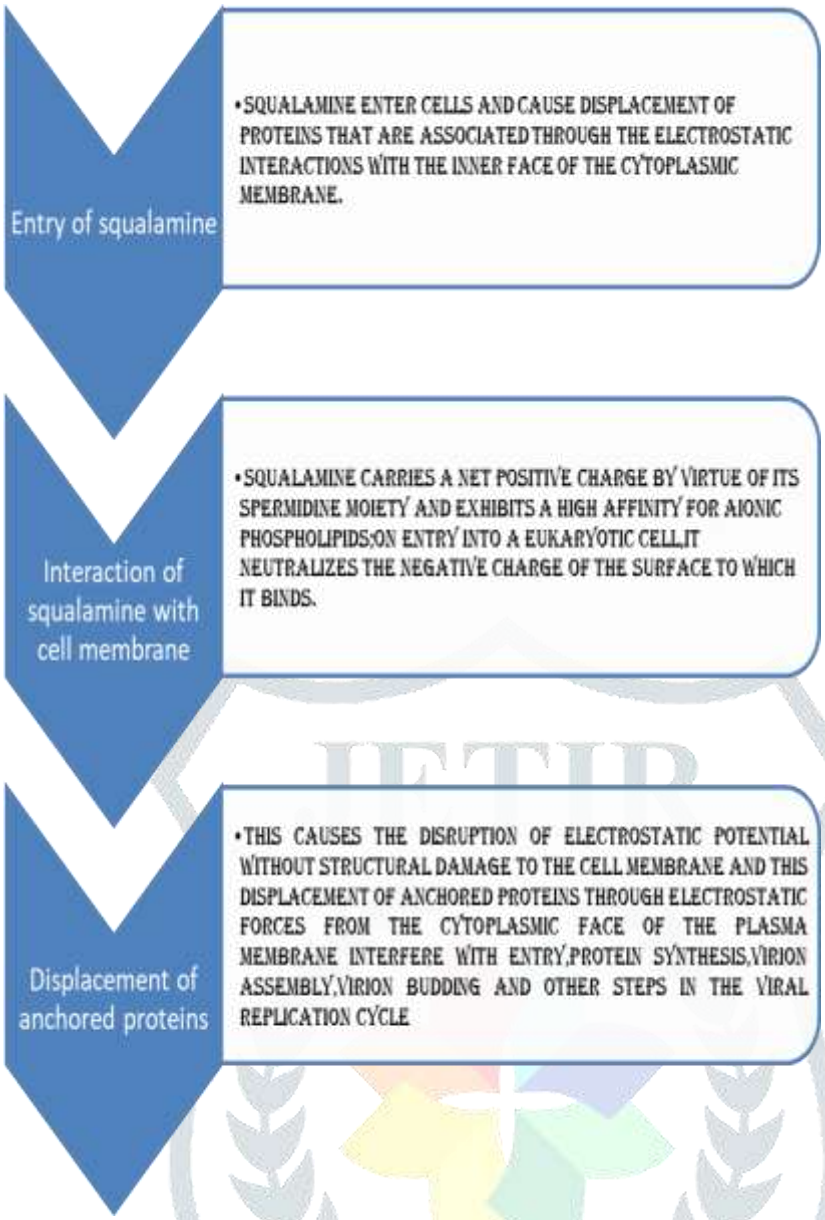

Figure 2: Pharmacological action of squalamine on tumor cells (Schematic representation).

DIFFERENT THEORIES ABOUT THE MECHANISM OF ACTION OF SQUALAMINE:

THEORIES PROPOSED BY	**EFFECTS OF SQUALAMINE**
"Akhter et al"	In this study, the action of squalamine is based upon the inhibition of mammalian brush border sodium-hydrogen exchanger and he found that the squalamine show inhibitory effects on sodium hydrogen exchanger activity which is dependent both on time and concentration and reversible also,since this exchanger is responsible for regulating cell volume and shape changes.

"Chen et al"proposed squalamine as calmodulin chaperone.	This mechanism resides upon the specific entry of squalamine into endothelial cells,resulting in intracellular redistribution of calmodulin without calmodulin function inhibition.
"Sills et all"assessed the biologic actions of squalamine.	Squalamine was able to inhibit endothelial cell proliferation of rat brain and induces migration by mitogens such asVEGF,bFGF,PDGF and scatter hepatocyte growth factor and this study also reveals the direct application of squalamine to the 4-day old chick embryo vasculature(3).
"Williams et al"proposed the mechanism by which squalamine inhibits tumor growth at molecular and cellular level.	In this study,the effects of squalamine was evaluated on the endothelial cell actin cytoskeleton and transmembrane cell-cell adhesion elements which concludes that squalamine perturbs both actin polymerization and cell-cell adhesion in endothelial cells making it clear that squalamine interferes with the ability of endothelial cells movement,growth and communication leading to decreased formation of blood vessels,their nourishment and tumor progression(3).
"Li at al"conducted the action of squalamine on ovarian tumor.	This study claims that squalamine was taken at low doses due to its inability to decrease VEGF secretion by tumor cells and show no direct growth inhibition of ovarian cells invitro .At low doses squalamine inhibits human vascular endothelial cells and squalamine was able to induced blockade of the rapid VEGF-stimulation phosphorylation of p44/p42 MAP kinase in endothelial cells,which is an early response to active proliferation(3).

ANGIOGENESIS: Angiogenesis is an essential event in many of the physiological processes such as wound repair, ovulation and embryogenesis. Angiogenesis is also known as Neovascularization and is a key component in many of the physiological and pathological processes such as inflammation, glaucoma, diabetic and retinopathies, myocardial ischemia, rheumatoid arthritis, psoriasis and tumor formation. Two major processes involved in the formation of blood vessel in vascular system; Vasculogenic and Angiogenesis. Vasculogenic occurs in the embryo and refers to the formation of de novo blood vessels by in situ differentiation of the mesoderm derived angioblasts and endothelial precursors. Angiogenesis is the formation of new capillaries from pre-existing vessels and circulating endothelial precursors. Angiogenesis is a tightly controlled dynamic physiological process that occur in tissues that undergo active remodelling in response to stress and hypoxia(5).

Figure 3:Flow chart showing how angiogenesis process is a hallmark for cancer.

REGULATION OF ANGIOGENESIS: Vascular biology and angiogenic switch: tumor growth depends on angiogenesis, the formation of new blood vessels from pre-existing beds in order to receive an adequate supply of oxygen and nutrients. Genetic instability and local tumor hypoxia lead to the secretion of soluble angiogenic factors that trigger complex interactions between different cells, the basal membrane and soluble pro-and anti-angiogenic factors. Angiogenesis is based on the fundamental principles of activation, growth and migration of endothelial cells in adjacent blood vessels. Activated endothelial cells lose their interendothelial cell bond, leading to a breakdown of the surrounding basement membrane and extracellular matrix by secreting proteases. Matrix proteins contain and sequester a variety of angiogenic factors, such as VEGF, which are released after matrix degradation and further stimulate endothelial cells. This has many effects: it increases endothelium permeability and allows endothelium to release more MMPs (Matrix metallic proteins) and UPAs (Urokinase plasminogen activator) to further degrade the extracellular matrix. These proliferate and migrate, involve different integrins during migration, and finally assemble into a tubular structure. Subsequently, the formation of a lumen contributes to the formation of a new blood vessel. Components for the new basal membrane and extracellular matrix are secreted and pericytes (supportive smooth muscle cells) are recycled. Pericytes are involved in the stabilization and maturation of newly formed vessels. Tumor vessels differ in many aspects from normal vessels due to the deregulation of signaling pathways in the angiogenesis process and the alteration of gene expression of angiogenic factors. Depending on the normal blod vessels, tumor vessels differ on a structural and functional basis which is abnormal. Tumor vessels usually have an irregular diameter and abnormal vascular branch pattern, lack a full basal membrane, are totally exposed by pericytes and are leaky in nature. In addition, mosaic vessels in tumors are lined with endothelial cells, but they have interspersed tumor cells that can make up to 25% of the luminal surface in certain tumor cells. Tumor vasculature as compared to normal vasculature differs not only in terms of architecture but also in terms of molecular expression and regulation. Acquiring the capacity to stimulate angiogenesis by changing the balance between the stimulatory and inhibitory factors of angiogenesis towards the pro-angiogenic factors known as angiogenic turn is a rate limiting step in tumor formation. The secretion of various tumor factors includes vascular endothelial growth factor (VEGF), basic fibroblast growth factors (BFGF), angiopoietins platelet derived growth factors (PDGF), placental growth factor (PLGF), transforming growth factor (TGF) beta and other factors that promote the germination of new vessels from nearby existing vesselsHypoxia in the center of the growing tumor leads to intracellular stabilization of the hypoxia inducible factor (HIF)-I Alpha, the key transcription factors in hypoxic tissues, and induces the expression of several hypoxia response genes, such as VEGF. On the other contrary, hypoxia decreases antiangiogenic factors like thromospondin. Additionally, genetic alterations in tumour suppression genes (loss of function) and oncogenes (gain of function) like p53, ras,myc,c-jun and others can upregulate pro-angiogenic factors. VEGF is one of the strongest stimulators angiogenic factors in most human tumors. The effects of VEGF, which is a gene family of glycoproteins consisting of VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor (PLGF) are mainly mediated by 2 cell surface tyrosine kinase receptors. Fit-1 or VEGFR-1 and KDR/FIK-1(or VEGFR-2)(8).

VASCULAR BASEMENT MEMBRANE: The growth of neoplasms and also the construction of personal tumor vessels need the breakdown of the encircling basal membrane and living thing matrix i.e extracellular matrix.Matrix proteins square measure shaped by traditional cells like epithelium cells and neoplasm cells and secretion, self-assemble to make an extremely cross linked,insoluble network.Relaxation of this network needs the intervention of multiple enzymes like cathepsins,urokinase,tissue proteolytic enzyme and matrix metalloproteinases(MMPs) and causes the loss of angiogenic factors embedded within the matrix(8).

ENDOTHELIUM AND ENDOTHELIAL PRO GENITOR CELLS: The angiogenic switch stimulates local endothelial cells, which proliferate, transfer from pre-existing vascular beds to the tumor tissue and form new microvessels, raising the endothelial turnover time to 5 days and increasing the expression of different endothelial surface markers. Recent evidence indicates that circulating endothelial cells and haematopoietic stem cells derived from bone marrow also contribute to tumor angiogenesis. Thereby, tumor-derived systemic circulating factors such as VEGF and other chemokines recruit endothelial progenitor cells and haematopoietic bone marrow stem cells after MMP-9 releases ligand kits from bone marrow stromal cells. Thereby, tumor-derived systemic circulating factors such as VEGF and other chemokines recruit endothelial progenitor cells and haematopoietic bone marrow stem cells after MMP-9 releases ligand kits from bone marrow stromal cells(8).

PERICYTES: These are potential antiangiogenic therapy and functionally important cell type in the tumor vasculature and act as supporting vascular smooth muscle cells, which are important for the function and survival of endothelial cells. Pericytes help in the development of functional vessels, making them stable during vascular development. Tumor vessels with no pericytes are more dependent on VEGF and more vulnerable to antiangiogenic therapi These are potential antiangiogenic therapy and functionally important cell type in the tumor vasculature and act as supporting vascular smooth muscle cells, which are important for the function and survival of endothelial cells. Pericytes help in the development of functional vessels, making them stable during vascular development. Tumor vessels with no pericytes are more dependent on VEGF and more vulnerable to antiangiogenic therapies(8).

Figure 4: The angiogenic switch represented as imbalance between pro- and anti-angionic factors and Antiangiogenic factors with subsequent activation of angiogenesis.

EFFECTS OF SQUALAMINE ON VARIOUS TYPES OF TUMOURS: During ontogeny, cancer cells secrete hormones that lead to the creation of recent blood vessels from neighboring traditional blood vessels to provide gas and nutrition to cancer cells. once squalamine reaches the blood vessels close the growth, it fully inhibits the event of recent blood vessels. The result's growth gangrene. Squalamine is thought to inhibit growth factor-dependent pathways in epithelial tissue cells and has since been studied as a treatment for age-related devolution and cancer. Injection type has been reported to be tolerated in age-related devolution and cancer-related patients in part one and II trials. additionally, squalamine doesn't have important direct effects on primary growth growth in animal models once administered as one agent, magnified antineoplastic responses are discovered by squalamine together with cytotoxic chemotherapeutical agents compared with cytotoxic agents used alone(12).

CANCER: Cancer is the most common cause of death and the second most deadly disease in the world. Cancer requires several different types of uncontrolled cell development. Clusters of cancer cells can invade and kill the surrounding organs, impairing physiological function and leading to death. Antiangiogenic substances are intended to prevent the development of new blood vessels and thus suppress tumor growth. Cancer patients are usually treated with a combination of surgery, radiation therapy, and chemotherapy. Surgery and radiation therapy can be especially successful in patients who have not yet spread the disease to other tissues or organs. Chemotherapy is the main treatment for tumors that have spread from primary to secondary or metastatic sites. Since chemotherapeutic agents typically target rapidly dividing cells indistinctly, destroying both normal and cancer cells, patients with chemotherapy often experience severe side effects. In addition, chemotherapy resistance also occurs over time In different xenograft models, squalamine has been shown to greatly improve the antitumor activity of cytotoxic agents against primary lung tumors, chemoresistant human non-small cell lung carcinoma, breast [, brain and ovarian tumors. It is therefore not shocking to know that the most remarkable effect is shown in preclinical tumors. Cisplatin,Carboplatin,Cyclophosphamide and 5-Fluorouracil(12).

LUNG CANCER: Squalamine has been shown to avoid lung metastases in the murine model of Lewis lung carcinoma, both as a single agent and in conjunction with various other chemotherapeutics. The antitumor effects of squalamine tend to be the result of inhibiting mitogen-induced proliferation and endothelial cell migration. In addition, squalamine does not have substantial direct effects on primary tumor growth in animal models when administered as a single agent. Enhanced antitumor reactions are observed when squalamine is administered in combination with cytotoxic chemotherapeutic agents compared to cytotoxic agents used alone. Thus the combination of squalamine with paclitaxel and carboplatin reduced the formation of CD31 vessels by 25 per cent compared to controls, squalamine alone or cisplatin alone. In this setting, the production of squalamine as a first-line therapy for non-small cell lung cancer would depend on the number and quality of treatment responses from the Phase IIb clinical trial and survival results(12).

OVARIAN CANCER: Ovarian cancer is the most prevalent cause of gynecological malignancy. HER-2 (a growth factor receptor tyrosine kinase) gene overexpression is commonly thought to contribute to tumor production through its effects on the promotion of uncontrolled cancer cell growth. However, recent findings suggest that HER-2 may also regulate cell survival functions, such as angiogenesis, by encouraging tumor production of the endothelial growth factor (VEGF). Potential antitumor effects of squalamine in murine xenograft tumors with or without concomitant platinum therapy have been evaluated. Immunohistochemical assessment of tumors revealed decreased microvessel density and increased apoptosis. In a clinical trial conducted in 26 patients, 35 per cent of evaluable patients had an objective response to the study drug regimen of squalamine and carboplatin with five full responses and four partial responses. In vitro, squalamine has been shown to have no direct effect on the spread of ovarian cancer cells, although VEGF-induced activation of mitogen-activated protein kinase (MAPK) and cell proliferation in human endothelial vascular cells has been blocked. These findings indicate that squalamine is antiangiogenic to xenografts of ovarian cancer and improves the cytotoxic effects of cisplatin chemotherapy with HER-2 tumor expression status.

AGE RELATED MACULAR DEGENERATION: Squalamine received the FDA fast track status in 2005 for the treatment of "wet" age-related macular degeneration. Age induced macular degeneration (AMD) is a disorder in which there is a gradual loss of visual acuity and degenerative eye diseases. It is responsible for legal blindness in patients 50 years of age or older in the Western world. There are two forms of AMD: Dry and Wet, which are the most extreme and need urgent care. Wet AMD is caused by the development of abnormal blood vessels in macula. Squalamine lactate is an investigational medication used for this treatment(12).

NOVEL TREATMENT FOR DIABETIC RETINOPATHY: Diabetic retinopathy is the main factor responsible for the loss of vision in people's working age. It is distinguished by the presence of retinal neovascularisation, the formation of microneurysms, the existence of protein exudates in vitreous humour, which contributes to a progressive deterioration in the visual acuity of patients suffering from it. Clinical pathophysiology of diabetic retinopathy involves blood retinal barrier dysfunction and blood constituents leak into the retinal neuropillar due to disruption to the blood retinal barrier leading to diabetic macular edema (DMO). The thickening of the vascular basement membrane is also seen in diabetic retinopathy and often results from the upregulation of fibronectin, collagen and laminin, leading to changes in microenvironmental factors that facilitate the growth, survival and function of pericytes and endothelial cellsPericytes is responsible for normal retinal function i.e. the differentiation, migration and proliferation of endothelial angiogenic cells and the loss of pericyte and the presence of pericyte ghosts are key characteristics of histopathological diabetic retinopathy. Molecular pathophysiology is complex and the presence of diabetes leads to hyperglycemic episodes affecting five essential biochemical pathways: activation of the polyol pathway, production of advanced glycation end products (AGEs), activation of the protein kinase c(PKC), activation of the hexosamine pathway and upregulation of poly(ADP-RIBOSE)polymerase. This leads to oxidative stress that causes mitochondrial dysfunction, deregulation of proinflammatory mediators and eventually hypoxia, and causes apoptosis of vascular and neuronal cells and increased regulation of VEGP expression leading to neurovascular dysfunction and hypermeable blood vessels. In collaboration with these, angiotensin aldosterone system is equally responsible for neuronal disruption. Treatment for these conditions is performed by Glycemic control with insulin injections, laser photocoagulation without visual effects, by RAAS blockade, Glucocorticoid therapy with the aid of IVTA (a long acting non-soluble hormone) which reduces macular edema and wet age related macular deneration, PKC inhibitors,eg. Ruboxistan, Fenofibrate by eliminating triglycerides from blood plasma in patients with diabetes II, anti VEGF therapies. There are also novel treatments that include mediators of angiopoietin signaling axes, immunosuppressants, NSAIDS, oxidative stress inhibitors and vitriol viscosity inhibitors (VVIs) and gene therapy can be a significant approach to their treatment(10).

A research revealed within the Proceedings of the National Academy of Sciences states that a compound in sharks has the flexibility to be used as a "broad spectrum" medicament in humans. The compound is thought as squalamine. Dr. Michael Zasloff was the lead author within the discovery of this compound and named this compound incorporates a "remarkable property" and is inflicting a revolution within the medicative product. He isolated this compound from the tissues of the dogfish shark in 1993. in line with his analysis, he was pleased to grasp that the system of sharks is immune to viruses and may be utilized in the treatment of assorted severe tumour conditions. He found that gristle may be a natural supply of fabric with sturdy antiangiogenic activity(4).

This makes him question what makes the shark’s system totally different from people at large and the way the compound extracted from this supply may be wont to treat cancer.According to his analysis,Squalamine’s virus killing talents square measure attributed to its electric charge,when squalamine enters a cell,it adheres to the cell’s charged inner membrane.In the method,squalamine knocks off charged proteins that hold tight the inner membrane.While squalamine action hinders viruses,it doesn't hurt the cells integrity.Since viruses need proteins on the inner membrane so as to breed in cells.He additionally noted this can be a stimulating property of those

compound and there's no alternative compound famous to science having this property and this makes the opposite scientists curious to explore this compound on varied aspects and judge out the opposite properties of squalamine.On additional investigations and analysis trials by alternative scientists,more advanced property of those compound have inherit existence.To check the antiangiogenic and growth impact of squalamine,various diagnosing and clinical studies were conducted and squalamine with success qualify it with less toxicity and facet effects.In this review paper,the main aim is that why we elect this compound for cancer treatment,it was a good discovery by Dr.Zasloff as a result of there have been several natural merchandise offered within the atmosphere which might be wont to treat cancer proved in diagnosing and clinical trials,is there one thing distinctive in squalamine structure that creates it totally different from alternative natural derived merchandise.Despite the advancement within the early detection of tumors and within the surgery,radiation and chemical for unwellness management,the worldwide mortality from human cancer remains intolerably high and has magnified within the previous couple of years.Although advances within the early detection of tumors and within the use of therapy and surgery for unwellness management have helped to reinforce the general survival of afflicted patients,major enhancements for cancer treatments square measure desperately needed.There is a maxim that "Prevention is often higher then cure"so the aim of any treatment ought to be supported preventing instead of hardening.In general,the additional longer someone survives,the additional seemingly he/she can develop cancer.Therefore,prevention can produce a large impact on dominant prices and deaths in returning decades.According to survey,cancer is that the second leading reason for death within the western world.The interference and medical aid of cancer might like introduction of latest treatments derived from natural merchandise.Many pharmaceutical merchandise approved for human unwellness treatment square measure derived from natural sources.The discovery of efficacious compounds for cancer management can like new understanding of the molecular and cellular pathways that regulate tumour proliferation and progression. Patients are getting acutely tuned in to the choice approaches. 9 studies performed worldwide among cancer patients showed that forty one.2% used complementary and medicine throughout their treatment. In the majority cases of treatment failure, the patient develops distant metastases. whereas surgery, radiation therapy and che-motherapy square measure all offered to eradicate regional diseases, they're of very little worth as compared to distant metastases. For such distant metastases, therapy is that the counseled approach, however effectiveness is proscribed by deadly side-effects at high doses and lack of specificity.It is well proved that each radiation therapy and therapy invariably injury or weaken the patients system,which may be already broken by the cancer itself.A new awareness has been developed in cancer medical aid regarding the importance of the patients system.Biological Response Modifiers(BRMs) have currently evolved because the fourth technique of cancer treatment additionally to surgery ,radiotherapy and therapy.Such treatments with BRMs square measure thought-about additional biological than directly cytotoxic.Squalamine was one amongst the biological molecule to be used as AN agent in cancer treatment.Cartilage may be a natural supply for squalamine with sturdy antiangiogenic activity,because their entire skeletal system consists of animal tissue,sharks square measure thought-about to be a perfect supply of angiogenic and tumour growth inhibitors and their animal tissue extract has shown antiangiogenic and growth activities in animals and humanbeings.It has been seen that oral administration of animal tissue extract is efficacious in decreasing maturation.It is a coffee mass aminosterol

and once it's combined with chemotherapeutical substance,their growth activity is increased.It is AN antiangiogenic molecule that possess distinctive mechanism of action that involves obstruction of epithelial tissue cell,migration and proliferation by inhibiting varied growth factors.The angiogenic tissue matter of metalloprotease 3(TIMP-3) and tumour suppressor protein(snm 23)genes from shark animal tissue were cloned and evaluated.Squalamine has been verified to be antiangiogenic in nature in varied clinical trials,individually additionally together with chemotherapeutical agents.The mechanism of action of squalamine is principally thanks to specific entry of squalamine through membrane invaginations called caveolae and this action has 3 principle antiangiogenic effects on epithelial tissue cell;1.blockage of cell signals from multiple growth factors together with VEGF,altering activation and division of cell,2.reduced expression of surface integrin alpha-v-beta-3,destabilising cell-cell interactions(15)(9).

Dr.Michael Zasloff claimed this compound has "Remarkable property"which is used as a potent medicament.On his note and proof performed by him on sharks tissue,other scientists and researchers were optimistic and curious to figure on this compound to place into a famous classification i.e broad spectrum general medicament on their performance on varied species and analysis parameters.Dr.Zasloff solely found the existence of squalamine in dogfish shark however later it had been conjointly known inside the current white blood cells of ocean lamprey(Petromyzon marinus).They dispensed the the in vitro studies by exposing the human microvascular epithelial tissue cells(HMEC-1) with dandy fever virus.With varied concentrations of squalamine they predict the hypothesis of squalamine inhibiting varied crucial steps like protein inducer inhibition,actin dependent responses in epithelial tissue cells,cell migration,cell division and tube-shaped structure tube formation during a 3D matrix conjointly,also determined its potency on human infectious disease virus,(HBV) and human infectious disease infectious disease virus(HDV) and for in vivo activity 3 well characterised animal models of infection were selected:Yellow fever within the golden hamster,eastern cephalitis virus and murine herpes virus within the BALB/c mouse.This studies reveals that the antiviral property of squalamine must be explored.

A section I/IIA was designed to assess the security,clinical response and materia medica of squalamine once administered as a five day continuous infusion together with customary therapy medication each three weeks in patients with stage IIIB(pleural effusion) or stage IV non-small cell carcinoma.The beginning dose of squalamine was a hundred mg/m2/day and escalated to four hundred mg/m2/day had dose limiting that enclosed grade ¾ pain,myalgia and leukopenia.The combination of squalamine given daily for five days,with paclitaxel and carboplatin given on day one is well tolerated.Patient survival information and also the safety profile of this drug combination suggests that the utilization of squalamine given at its most tolerated dose with cytotoxic therapy ought to be explored more as a probably effective therapeutic strategy for patients with stage III B or IV non-small carcinoma. At the counseled dose of phase II of five hundred mg/m2/day, squalamine is well tolerated associate degreed ends up in plasma a minimum of an order of magnitude more than those needed for distinguished antiangiogenic effects in diagnosis studies(3).

In clinical trials, squalamine has been granted Orphan Drug designation for the treat- ment of female internal reproductive organ cancer by the U.S. Food and Drug Ad- relief (FDA). Squalamine has been proved in a very therapeutic run to own positive results against non-small cell carcinoma (NSCLC). An- giogenesis ensuing from

age-related macular dege- neration (AMD) is that the leading reason behind legal visual defect among adults age fifty or older within the Western world. VEGF-A medical aid has revolutionized the treatment. The drug binds to a "chaperones" calmodulin to associate intra- cellular compartment and blocks development at many levels. VEGF-A has been concerned in recent years because the major issue accountable for neovascular and exudative malady of the attention. AMD seems to return in 2 types: the "dry" kind and therefore the additional severe "wet" kind. Dry AMD, the additional common and milder variety of AMD, accounts for eighty fifth to ninetieth of all cases. Dry AMD leads to varied types of sight loss and will or might not eventually grow to be the wet kind. though the wet variety of AMD accounts for under ten - fifteen percent of all AMD, the possibility for severe sight loss is larger. it's accountable for ninety percent of severe vision loss related to AMD. Squalamine was found to exhibit very little general toxicity and was usually well tolerated by treated patients with varied solid growth malignancies, together with female internal reproductive organ, non-small cell respiratory organ and carcinoma(1).

Xenograft growth shrinkage was seen for the MV-522 growth together treatments together with Squalamine; whereas, no growth shrinkage was seen once squalamine was omitted from the treatment plan. Squalamine treatment was found to retard 2 cellular events necessary for development causation disorganization of F- simple protein stress fibers and inflicting a concomitant reduction of detectable cell the surface molecular epithelial tissue cadherin (VE-cadherin). we tend to propose that the augmentation by squalamine of toxicity from platinum-based therapies is owing to interference by squalamine with the power of stimuli to market epithelial tissue cell movement and cell-cell communication necessary for growth of recent blood vessels in xenografts when therapy injury to the growth(3).

Several categories of agents that currently exist target the various steps concerned in development. medicine like squalamine, celecoxib, ZD6126, TNP-470 and people targeting the integrins are being evaluated in carcinoma(7).

Squalamine could be a natural antiangiogenic alcohol, and its potential role in treatment of female internal reproductive organ cancers with or while not normal cisplatin therapy was assessed. Since HER-2 cistron overexpression is related to cisplatin resistance in vitro and promotion of growth development in vivo, the response of female internal reproductive organ cancer cells with or while not HER-2 cistron overexpression to squa- lamine and cisplatin was evaluated each in growth transplant models and in tissue culture. In in vitro studies, we tend to found that squalamine doesn't directly have an effect on proliferation of female internal reproductive organ cells. However, squalamine considerably blocked VEGF-induced activation of MAP enzyme and cell proliferation in human tube epithelial tissue cells.

The progressive growth and unfold of the many solid tumors depends, in part, on the formation of associate adequate blood provide and growth development has been rumored to own prognostic significance in many human cancers. medical aid directed toward the vasculature of solid growths is currently being pursued as a very important new direction in cancer treatment as a result of avascular tumors exhibit solely restricted growth and tumor aggressiveness, and pathologic process potential ordinarily correlates with growth property. tube

epithelial tissue protein (VEGF) is created by most solid tumors and elicits a mitogenic impact on tumor-associated epithelial tissue cells. VEGF binding to receptor aminoalkanoic acid kinases triggers activation of down- stream communication enzymes, together with MAP kinases, that successively, regulate organic phenomenon and specific epithelial tissue cell responses together with proliferation, migration and caspase-mediated cell death. many studies have urged that VEGF plays a very important role within the progression of the many cancers. protein pathways, like those captivated with EGF and HER-2 receptors, seem to upregulate VEGF production in solid tumors. Since EGF and HER family receptors ar activated and/or overexpressed in important numbers of human cancers, these protein receptor pathways could play a job in promoting any growth of human malignancy by increasing VEGF- dependent growth development(3)(1).

Akhter, et al. (1999) planned one mechanism of action of squalamine to involve inhibition of the class brush-border Na+/H+ money dealer isoform NHE3. The Na+/H+ money dealer could be a transport supermolecule that's famed to control changes in cell volume or cell form. Squalamine was found to inhibit rat brain epithelial tissue cell proliferation and migration elicited by mitogens, like VEGF, bFGF, thrombocyte Derived protein (PDGF) and scatter factor/hepatocyte protein. within the absence of those mitogens, squalamine was found to own no direct impact on survival or proliferation of epithelial tissue cells. additionally, squalamine was conjointly found to inhibit nucleon secretion by mitogenstimulated epithelial tissue cells, a finding per results rumored by Akhter, et al. a motivating finding of this study concerned the direct application of squalamine to 4-day-old chick embryo vasculature. when solely twenty min, squalamine evoked constriction of the tiniest capillaries throughout the food sac, with denial of red cells. This acute transforming method resulted in narrowed tube segments and blocked blood cell movement and was con- firmed by microscopic anatomy examination of treated and un- treated food sacs. Since these new vessels ar composed alone of epithelial tissue cells, the phenobarbitone narrowing was finished to flow from to squalamine-induced changes within the form or volume of epithelial tissue cells. Immunohistochemical analyses of those tumors when treatment with squalamine discovered important reductions in tumor- associated blood-vessel density(3).

Li, et al. conducted studies of human sex gland tumor- associated growing. sex gland cells were found to secrete vital levels of VEGF, a right away matter of growing, however squalamine failed to cut back VEGF secretion by growth cells, and it induced no direct growth inhibition of sex gland cells in vitro. However, squalamine at doses as low as one hundred sixty nM did halt the proliferation of human tube epithelial tissue cells and markedly reduced VEGF-induced capillary tube-like formations by tube epithelial tissue cells growing in Matrigel culture. Squalamine interference with these downstream signal pathways in tube epithelial tissue cells could also be important in disrupting the method of tumor-associated growing(3).

Studies by Teicher, et al. noted that squalamine as one agent contains a modest result on neoplasm growth delay on rat 13762 exocrine gland malignant neoplastic disease, with squalamine dosing at forty mg/kg. Moreover, it absolutely was found that the amount of respiratory organ metastases faded once mice were treated with squalamine Specifically, by day 20, the numbers of metastases were reduced to half those gifts in controls. Since

respiratory organ metastases are actively implanting and growing exploitation new blood vessels, this result of squalamine suggests that it's robust antian- giogenic efficiency(3).

Previous studies have urged that VEGF plays a crucial role in progression of sex gland cancer. sex gland cancer is that the most threatening medical specialty malignancy.Although advances in therapy and surgery have helped to enhance the general survival of afflicted patients, 5-year survival rates from sex gland cancer remained regarding four hundred and forty yards within the early a part of this decade. By the time several patients square measure diagnosed with sex gland cancer, serous membrane dissemination of the tumour has typically occurred. This growth and unfold of sex gland cancers depends, in part, on formation of associate adequate blood offer. Tumor- associated maturation is crucial for growth of most solid tumors, and neovascularization has additionally been shown to own prognostic significance in animal tissue sex gland cancer. Administration of squalamine together with cisplatin semiconductor diode to increased levels of programmed cell death in many sex gland tumour cells assessed in vivo(14).

On the premise of sturdy proof of antiangiogenic and antineoplastic properties of squalamine, it had been elected for clinical development as a therapeutic agent for treatment of human malignancies. The investigators recruited nineteen patients with associate jap Cooperative medical specialty cluster (ECOG) performance standing of two with advanced non- leukemic cancers. Squalamine was administered as an eternal endovenous infusion over a hundred and twenty h, with repeat dosing each fourteen days. The best-tolerated dose of squalamine was found to be 192 mg/m2/day, though a dose of 384 mg/m2/day additionally seemed to be well-tolerated in patients while not previous exposure to squalamine(14).

In one in every of the recent review paper,an effective squalamine spinoff has been discovered that is additional efficacious than squalamine.The name of the squalamine analogue is NV669 obtained from low cost and obtainable precursors through an artless metal subtractive amination reaction, that is employed as Associate in Nursing agent for exocrine gland and viscus cancer,which powerfully inhibits the proliferation of those cancer cells.To know the potency of this spinoff was, each invitro and invivo studies was conducted within which it induces cell cycle arrest in G2/M pre-mitotic section and caspase-mediated cell death.In vitro NV669 inhibits PTB1B(it could be a non-transmembrane supermolecule aminoalkanoic acid enzyme that regulates metablosim,inhibiting internal secretion and leptin) activity and FAK expressions.NV669 impacts on the expression of adhesion molecules DCH-1,-2 and -3 in BxPC3 and Huh seven lines that kind cell monolayers.Consecutively,NV669 induces cell detachment.This reveals that NV669 induces cell detachment and caspase-mediated cell death by inhibiting PTP1B.In vivo study shown that ,there was decrease of BxPC3 and HepG2 growth growth in nude mice xenografts upon NV669 treatment.The results of NV669 discovery have shown that the antitumoral result of NV669 was terribly completely different from squalamine,from that NV669 was derived.Indeed,squalamine blocks proliferation of HUVEC IN vitro,prevents growth of recent blood vessels in chick chorioallantois and rabbit tissue layer and impairs neoangiogenesis in xenografts tumors in mice originating from differing types of cancer(2).

In summary, squalamine has shown to be helpful for the treatment of vital diseases like cancers (lung, ovarian, brain, and others), age-related macular degen- eration (AMD) and therefore the management of weight in man. Squalamine causes changes in tube-shaped structure epithelium cell form and has been rumored to possess important antiangiogenic activity. Squalamine is somewhat distinctive among most current anti-angiogenic agents in development as a result of it inhibits epithelium cell proliferation and migration elicited by a good style of growth factors, as well as basic formative cell protein (BFGF) and VEGF. This broad antiangiogenic activity of squalamine might result from its inhibition of surface metal nucleon exchangers (thus neutering animate thing hydrogen ion concentration and thereby preventive animate thing signal by many growth factors) and alternative downstream signal pathways in epithelium cells .The squalamine spinoff aminosterol compound NV669 with chemicals synthesized for clinical applications and possess robust antiangiogenic activity in invitro and invivo reveals a contradictory study by claiming to be additional efficacious than squalamine and show its action by a unique mechanism on exocrine gland and viscus cancer(1)(4)(7)(13)(14).

MATERIALS AND METHODS: For any compound to study and carry out its mechanism of action,to know its antiangiogenic property on various tumor models, firstly, it is isolated and extracted from the particular species,identified and purified with various solutions and the original structure was determine by NMR spectroscopy.

PURIFICATION OF SQUALAMINE: Squalus acanthias sharks were captured off the coast of New England. Shark stomach tissue (400 g) was frozen immediately after dissecting, pulverized in liquid nitrogen, and extracted at 5 vol 60 per cent (vol/vol) of acetonitrile in 1% trifluoroacetic acid. After centifugation, the supernatant was lyophilized and resuspended in 250 ml of 0.1 per cent trifluoroacetic acid. Next the sample was extracted by a modification of the Folk method. The aqueous phase was recovered, lyophilized, resuspended in 30 ml of H20 and then loaded onto a 45 x 5 cm Bio-Gel P-30 (Bio-Rad) gel-filtration column of 0.1 per cent trifluoroacetic acid/20 per cent acetonitrile. The fractions were dried under the vacuum, resuspended in water, and assayed. Active fraction containing low molecular weight molecules (Mr = 500-1000) was pooled and applied to the C18 reverse-phase HPLC column. After extensive flushing of the column with buffer A (0.1 per cent trifluoroacetic acid in H20) and allowing the absorbance to return to baseline, the material was eluted with a linear gradient of 0-60 % buffer B (0.08 per cent trifluoroacetic acid in acetonitrile) in buffer A at a flow rate of 1 ml/min in 45 min. Fractions were dried, resuspended in water (100 µl) and tested. Fractions containing the main peak of activity were pooled and then loaded onto a strong cation-exchange column of HPLC . After loading the sample, the column was thoroughly washed with buffer C (5 mM KH2PO4, pH 3/25% acetonitrile) and the absorbance was allowed to return to the baseline. The column was eluted with a gradient of 0-60 % buffer D (5 mM KH2PO4, pH 3/25 % acetonitrile/1 M NaCl) in buffer C at 1 ml/min in 45 min. The fractions have been dried and assayed as above. The fractions comprising the main peak of operation were then pooled and loaded onto the C4 reverse-phase HPLC column. The column C4 was built with the same buffers used for the column C18 (buffers A and B). The column was washed with buffer A until no absorbance was detected and then developed with a gradient t of 0-30 buffer B in buffer A in 20 min, followed by 30-50 % buffer B in

buffer A in 30 min. Other Squalus acanthia tissues (=50 g of liver, gallbladder, spleen, testes, gill, and intestine) were extracted as mentioned above and purified through the C18 HPLC level. Antimicrobial activity was detected in each tissue and an inverted fraction of each tissue was analyzed by rapid atom bombardment (FAB) mass spectroscopy. The performance of the stomach was determined by spotting multiple dilutions on the TLC plate and comparing the ninhydrin staining to the typical spermidine curve. An estimate of the yield of squalamine from other tissues was made by comparing the antimicrobial activity of the reversed phase fractions with the activity of the stomach(15).

NMR Spectroscopy: Spectra was recorded on the Bruker AM 400 and 600 instruments both equipped with the Aspect 3000 data system. Proton and carbon spectra were developed with a 5 mm dual probe on the AM 400 and a 5 mm inverse probe on the AM 600. Proton and carbon chemical shifts in dimethylsulfoxide (DMSO) solutions are reported relative to tetramethylsilane (TMS) with DMSO residual signals serving as secondary reference at 2.5 ppm for proton and 39.5 ppm for carbon spectra. In water, proton and carbon chemical shifts relative to sodium 3-(trimethylsilyl) propionate-d4 (TSP) are reported. An external capillary of TSP solution in D20 (0 ppm) was used for reference of proton spectra and a capillary of pure dioxane (67.5 ppm) was used for reference of carbon spectra in water. ~H spectra at 400 MHz were recorded with a spectral width (SW) of 4 kHz, a 45° pulse flip angle (PW), a size (SI) of 32 K data points, and a 3.9 second repeat time (Tr). Acquisition conditions for composite decoupled pulse (CPD) 17 13C spectra at 100.62 MHz in DMSO or water were as follows: SW = 11.1 kHz, SI = 16 K, PW = 60 °, and Tr = 0.7 seconds. ~H spectra at 600 MHz were recorded with SW—6.02 kHz, PW = 30°, SI = 16K, and Tr = 2.5 seconds. CPD decoupled ~3C spectra at 150.9 MHz with PW = 30 °, SW = 13.5 kHz, SI = 16K, and Tr = 0.6 seconds. Conventional DEPT-135 spectra ~8'~9 were acquired with a digital resolution similar to the CPD decoupled carbon spectra but with a Tr of 1.7 seconds. The following two-dimensional (2D) experiments were carried out at 600 MHz and data were collected in phase-sensitive mode using time-proportional phase increment (TPPI).Homonuclear correlated spectra with double quantum filter (DQF COSY) were acquired under the following conditions: SW = 2.5 kHz, SI = 2K, 32 scans, 1.5 second relaxation delay (RD), 512 spectra with evolution time. Prior to the Fourier transformation, the data were zero-filled to 2K x 2K and the sinebell functions shifted to 30 ° and 90 ° were applied to the first and second dimensions, respectively. Total correlated spectroscopy (TOCSY) experiments were performed using Bruker's reverse electronics. Isotropic mixing of 62.9 ms was achieved by an MLEV sequence using a 90° pulse of 20.7 microseconds and an RD of 1.6 seconds. The data set included 512 spectra (SW = 5.681 kHz, SI = 2K, 8 scans and 2 dummy scans) with t1 times increased from 44 microseconds to 45 milliseconds. The final size of the matrix after zero filling was 1K × 1K and the Gaussian window was applied in both dimensions. For the 2D Nuclear Overhauser Spectroscopy (NOESY), the data set included 512 spectra (SW2 = 2.5 kHz, SI = 1K, 48 scans, RD = 1.5 s, and 2 dummy scans) with a mixing time of 300 ms randomly variated by 10% to suppress J cross-sectional peaks. T1 time increased from 5 microseconds to 100 milliseconds. Data were zero-filled to a final size of 1K x 1K and a Gaussian window was applied in both dimensions. Heteronuclear chemical shift correlation spectra (HMQC) were collected in inverse mode with globally optimized alternating rectangular pulse (GARP) decoupling during acquisition. The data set included 256 spectra (SW = 2.3 kHz, SI = 2K, 512 scans, RD = 0.5 seconds and 2 dummy scans) with delay A l = 1/21Jcrt of 3.91 ms. The final matrix size was

1K × IK and FIDs in the first and second dimensions were multiplied by sinebell functions shifted by 90° and 60°, respectively. Proton-detected carbon-proton multiple bond correlation spectra (HMBC) was recorded in magnitude mode without carbon decoupling during acquisition (SW = 3 kHz, SI = 2K, 512 scans, RD = 0.7 s, and 4 dummy scans). A delay A t = 1/21Jcn of 3.91 ms was used for the low pass J-filter. The long range IH-~3C couplings were allowed to evolve during a delay A 2 of 50 ms. The data set contained 256 spectra with the the time varying from 3 microseconds to 20 milliseconds (zerofilling and Gaussian windows as for HMQC) with a delay A l = 1/21Jcrt of 3.91 ms. The final matrix size was 1K × IK and FIDs in the first and the second dimension were multiplied by sinebell functions shifted by 90 ° and 60 °, respec- tively. Proton-detected carbon-proton multiple bond correlation spectra (HMBC) 28 were recorded in the magnitude mode without carbon decoupling during acquisition (SW = 3 kHz, SI = 2K, 512 scans, RD = 0.7 s, and 4 dummy scans). The data set contained 256 spectra with the tl time varying from 3 microseconds to 20 milliseconds (zerofilling and Gaussian windows as for HMQC) (11).

METHODS FOR EVALUATING THE ROLE OF SQUALAMINE ON VARIOUS TUMOR CELLS: Squalamine ,a novel aminosterol compound inhibits angiogenesis and tumor growth in multiple animal models and this effect is mediated by blocking mitogen-induced proliferation and migration of endothelial cells,thus preventing neovascularization of the tumor.Due to its activity on both mitogen and tumor induced angiogenesis and also due to its steroid characteristics led us to explore its activity as an inhibitor of angiogenesis and tumor growth in multiple invitro and invivo models.The following table shows the antiangiogenic property of squalamine on various tumor models to observe the action of squalamine on vital steps of angiogenesis i.e endothelial cell proliferation,endothelial cell migration,angiogenesis and solid tumor growth,capillary ingrowth.

METHODS(M)	ASSAYS	APPROACHES
M1	Endothelial cell proliferation assay.	In this method,the action of squalamine on endothelial cell proliferation was assayed with mitogen and without mitogen stimulation.Rat brain endothelial cells of the RBE-4 was cloned,immortalized and maintained at 37°Cin 5% CO2 in fibronectin-coated plates in DMEM containing 10% fetal bovine serum, 1% L-glutamine, 10 mM HEPES, and geneticin (complete media) with basic fibroblastic growth factor at 1 ng/ml. During experimentation,

		RBE-4 cells (10,000 cells/well) was plated in fibronectin-coated plates and allowed to attach overnight in complete media without growth factors. The medium was then removed and replaced with either fresh complete medium or medium supplemented with the mitogen to be studied. Mitogens used were VEGF3 (20 ng/ml), bFGF (10 ng/ml), PDGF (10 ng/ml), scatter factor/hepatocyte growth factor (5 ng/ml), tumor conditioned medium from the 9L rat glioma (9LCM), and human hemangioblastoma cyst fluid. 9LCM was generated by growing 9L cells in supplemented MEM for 3 days, followed by centrifugation and resuspension of the pellet in the RBE-4 complete media (50% dilution) without additional mitogens. Human hemangioblastoma cyst fluid was obtained from an intraoperative specimen of a cyst associated with an intracranial hemangioblastoma. This fluid was diluted (1% dilution) into complete RBE-4 media (no additional mitogen) for proliferation studies.

		Squalamine to create a final concentration of 10, 30, or 50 µg/ml or buffer alone was added simultaneously with mitogen. Cell proliferation was determined by Coulter counter analysis at days 1, 3, and 6 in the presence and absence of squalamine. All assays were performed in triplicate, and data were analyzed using an ANOVA method for multiple comparisons.(3)
M2	Endothelial cell migration assay..	The effect of squalamine on the mitogen-stimulated movement of RBE-4 endothelial cells across "wounded" monolayers was quantified. RBE-4 cells were maintained and stimulated with mitogens . Cells were plated on 5-cm-diameter tissue culture plates with imprinted grid markings. After overnight attachment in complete media without added growth factors, the confluent monolayer was wounded with a sterile straight edge, and all adherent cells to one side of the wound were removed by mechanical stripping under microscopic guidance. The remaining adherent cells were rinsed and replenished with complete media containing purified

		mitogens with or without squalamine at 10 or 50 µg/ml. Cultures were incubated at 37°Cto allow cells to migrate out from the wounded monolayer. At 24 h, cultures were fixed in 3.7% formaldehyde and treated with Wright's stain. Migration was then analyzed by a blinded observer using a digital analysis program with Macintosh computer. The total area of RBE-4 migration across the wound was calculated and expressed in mm3 for each plate. Four assays were run for each squalamine concentration using each mitogen, and comparisons were made using ANOVA.(3)
M3	Effects of squalamine on endothelial cell proton secretion.	Studies were conducted to assess the effects of squalamine on the cellular metabolism of both resting and mitogen-stimulated endothelial cells. Effects of squalamine and related substances on in vitro endothelial cell proton secretion after VEGF stimulation were measured using a Cytosensor microphysiometer. Human microvascular endothelial cells were maintained in

		supplemented EBM media. Cells were used at a density of 200.000-300.000 cells per Capsule Cap and were serum starved for 24 h prior to the assay to deplete any growth factors. Cells were maintained at 37°C on the Cytosensor system in low-buffered balanced salt solution (138 mM NaCl,5 mM KC1, 1.3 mM CaCl,. 0.5 mM MgCl2, 0.81 mM K2HPO4, 0.11 mM KH2PO4, and 10 mM glucose) with a flow rate of 120 ml/min. After equilibration on the system for 30 min, cells were exposed to squalamine, 1436 (another natural aminosterol isolated from the shark that differs from squalamine in that the polyamine moiety is spermine rather than spermidine. which exhibits no effect on endothelial cell proliferation), or methylisobutyl amiloride (a potent inhibitor of the NHE isoform) each at a concentration of 10 mM for 1 h, followed immediately by exposure to 20 ng/ml of VEGF. Extracellular acidification rates were measured every 2 min through out the duration of the experiment.(3)

M4	Angiogenesis and solid tumor growth assays in the rabbit cornea.	To determine whether squalamine might have therapeutic value in vivo in the treatment of solid tumor-induced angiogenesis, it was incorporated into a sustained release polymer for testing in the rabbit cornea model. Squalamine was incorporated at a 20% (w/w) loading into ethylene vinyl acetate copolymer which provides local sustained release Final polymer shape was a disc with a diameter and height of 0.5 mm. Sustained first-order drug release for 14 days was quantified in vitro by placing a squalamine polymer into a vial containing 5% dextrose in water at 37°Cand then replacing the solution daily for analysis of the amount of drug present. The VX2 carcinoma, a tumor syngeneic to the New Zealand White rabbit was propagated by serial transplantation in the flank of a carrier animal. To stimulate angiogenesis, a 1-mm3 solid piece of the VX2 tumor was inserted into a corneal micropocket of an anesthetized rabbit 3 mm from the limbus . For efficacy testing, either a squalamine loaded or blank polymer (no drug) was inserted into the

		pocket just distal to the tumor piece between the limbus (the source of new vessels) and the tumor (the angiogenic stimulus). For each animal, one eye received squalamine polymer, whereas the contralateral eye received a blank polymer so that each animal served as its own control. Because the cornea is normally avascular, the ingrowth of new vessels from the limbus toward an angiogenic stimulus was easily quantified by slit lamp stereomicroscopic examination at 7, 14, and 21 days after implantation by two observers blinded to the treatment. An angiogenesis index was calculated for each cornea at each time point. For biocompatibility testing, only a squalamine-containing polymer was inserted into the corneal pocket (no tu mor), and the corneal reaction was assessed. Three separate experiments were performed with a total of 50 corneas studied. Statistical analysis was performed by ANOVA with ranked data and a nonparametric method. With cell culture methodology identical to that used for 9L glioma cells , squalamine (up

		to 30 µg/ml) was tested for cytotoxicity against VX2 cells in vitro.(3)
M5	Capillary in growth into matrigel plugs in the mouse flank.	To determine the efficacy of systemic administration of squalamine as an angiogenesis inhibitor in vivo, bFGF-impregnated Matrigel pellets were implanted s.c. in the ventral abdominal wall of mice, and vessel ingrowth was quantified. Human recombinant bFGF was incorporated at 150 ng/ml into liquid Matrigel. Plugs of 0.5 ml were injected into the ventral abdominal wall of C57BL/6J mice . The bFGF released from the plug served as an angiogenic stimulus for ingrowth of vessels. Animals were treated with twice-daily injections of either sterile water or squalamine (given s.c. at a distal site at 50 mg/kg/day) for 7 consecutive days beginning on the day of plug injection. All animals were sacrificed 7 days after plug placement. The plug and adjacent peritoneal wall were removed en bloc and fixed in 10% formalin. Sections (4 µm) were cut and stained with either Masson's trichrome for standard histology or with an antibody

		directed against the endothelial cell integrin CD34 for immunohistochemistry. Vessel density was quantified by a blinded observer examining the sections at high power (X630) and counting the number of vessels in 10 consecutive fields in the zone of tissue adjacent to the Matrigel plug.(3)
M6	Assay of tumor growth and vessel density(in vivo).	The effect of systemically administered squalamine on the growth of solid tumors in vivo was evaluated in the rat flank 9L glioma model. 9L rat glioma was propagated in the flank of a carrier animal. At the time of tumor implantation, the tumor was excised, and solid tumor pieces measuring 1 mm3 were cut using the operating microscope. A single piece was then implanted s.c. into the flank of Fischer 344 rats. Treatment was begun 5 days later with a twice-daily dose of either i.p. saline or squalamine (20 mg/kg/day). A separate group of animals received a single dose of l,3-bis(2-chloroethyl)-l-nitrosourea (14 mg/kg) given 5 days after tumor implantation. Animals were

		sacrificed 25 days after tumor implantation, and the tumor mass was exposed and measured with calipers. Tumor volume was estimated, and the tumor was weighed and processed for staining with H&E. Immunohistochemistry against the endothelial cell integrin, CD34, was also done. Microvessel density was then quantified by two blinded observers using a double-headed microscope to review the most vascularized areas from the middle of the tumor in the CD34-stained sections at X400 magnification as described . Results were expressed as number of microvessels per X400 field (mean A±SD) for each sample, and comparisons were made between treatment groups by an ANOVA. Using the methodology for culture of 9L glioma cells , squalamine (30 mg/ml) was tested in vitro for toxic and antiproliferative effects on 9L cells. The effect of squalamine on VEGF production by 9L cells was measured by ELISA.(3)
M7	Effect of squalamine on chick embryo vasculature.	Effects of squalamine on the yolk sac vessels of the 4-day chick embryo were investi

		gated. Two-day-old chick embryos were purchased from a local hatchery and maintained at 37°C.Shell caps were removed at day 4, exposing the embryos and their extraembryonic vasculature. Dulbecco's PBS (without magnesium and calcium; 0.3 ml) containing 30% (w/w) Ficoll 400 with or without 0.1 µg/mlsqualamine was applied directly over the entire vascular embryonic structure. The vasculature was studied under a Zeiss stereomicroscope and photographed at various times after initial treatment. One group of yolk sac membranes exposed to 0.1 µg/ml squalamine for l h was fixed overnight in situ by direct application of 10% phosphate-buffered formalin onto the exposed surface. The egg contents were emptied into a dish, and the yolk sac was carefully removed, embedded in paraffin, sectioned, and stained with H&E for microscopic examinationof vessel morphology(3)

METHODS FOR EVALUATING THE EFFECTS OF SQUALAMINE ON RETINAL NEOVASCULARIZATION

Squalamine has been efficacious in preventing or inhibiting tumor growth or metastasis in human cancers,it is also useful as a potential treatment to prevent human retinal neovascularization to block angiogenesis in a non-cancerous model of mouse oxygen induced retinopathy(OIR)model despite of lacking mineralocorticoid or glucocorticoid. The methods include Mouse model of oxygen induced, squaramide was administered, retinal perfusions was performed, Quantification of Extraretinal neovascularization, animal and organ were weighed, statistical analysis was performed using various test.

RETINAL PERFUSION	Fluorescein-conjugated dextran perfusion of retinal vessels was performed using high molecular weight fluorescein conjugated dextran in 4 per cent of paraformaldehyde in phosphate-buffered saline (PBS). Briefly, animals received a lethal dose of pentobarbital sodium (120 mg/kg) and median sternotomy was performed when deep anesthesia was obtained. The left ventricle was identified and 1 ml of fluorescein-conjugated dextran solution of 50 mg/ml was injected. Eyes were enucleated and put in 4 per cent of paraformaldehyde for 3 to 24 hours. The retinas were dissected using light microscopy and placed flat. The following numbers of animals were used in 5-day squalamine treatment experiments: room air (control) party, N = 19 out of 4 litters (16 killed at P17 and 3 killed at P19); room air + squalamine group, n = 17 out of 4 litters (9 killed at P17 and 8 killed at P19); oxygen group, n = 20 out of 4 litters (17 killed at P17, 2 in P18 and 2 in P19); and oxygen + squalamine group, n = 25 out of 8 litters (25 killed at P17).(10)

QUANTIFICATION OF EXTRARETINAL NEOVASCULARIZATION	After perfusion of animals with 4% paraformaldehyde, eyes were removed, placed in optimal cutting temperature embedding compound, and frozen at 270°C. Serial sections (7–10 mm) were cut through the cornea parallel to the optic disc using a cryostat. Tissue sections were stained with periodic acid–Schiff stain and hematoxylin. For the 5-day treatment with squalamine, there were the following numbers of animals: room air (control) group, n = 7 from 5 litters (6 killed at P17 and 1 at P18); room air +squalamine group, n = 6 from 2 litters (all 6 killed at P17); oxygen group, n = 8 from 4 litters (4 killed at P17, 2 at P18, and 2 at P19); and oxygen + squalamine, n = 5 from 2 litters (all five killed at P17). In the single-dose squalamine experiments, the animal numbers were as follows: the room air (control) group, n = 10 from 6 litters (3 killed at P17, 1 at P19, and 6 at P20); room air + squalamine group, n = 8 from 4 litters (4 killed at P17, 2 at P19, and 2 at P20); oxygen group, n = 15 from 11 litters from 11 litters (1 killed at P17, 8 at P18, 5 at P19, and 1 at P20); and oxygen + squalamine group, n = 9 from 6 litters (1 killed at P18, 7 at P19, and 1 at P21). Multiple sections were scored in a masked fashion by counting the number of nuclei extending beyond the inner limiting membrane into the vitreous. A minimum of eight sections at least 50 μm apart over a minimum distance of 450 μm were counted and averaged for each eye. The average number for each eye was pooled and averaged across replicates for each treatment condition, and these averages were used in the statistical data analysis (10).

ANIMAL AND ORGAN WEIGHTS	Animals and individual organs were weighed on a standard laboratory balance. In addition, a log of animal death was kept throughout the course of the experiments (10).
STATISTICAL ANALYSIS	Analysis of variance using the Kruskal–Wallis test was performed to test for differences between the treatment groups. The Mann–Whitney test was used to compare the total retinopathy scores and retinopathy subscores between individual groups. Student's t-tests assuming unequal variance were performed formed to compare the number of nuclei in the retinal sections, weights, and organ-to-body weight ratios. Significance was defined as $P<0.005$.(10)

[1]RESULTS AND DISCUSSION:

The results of the methods i.e effect of squalamine on various tumor models show that strong inhibitory effects of of squalamine on angiogenesis pathways by acting on various growth factors,squalamine inhibits in invitro endothelial cell proliferation and migration induced by multiple mitogens,including those produced in combination by more angiogenic tumors,it reduces angiogenesis and VX2 tumor growth in the rabbit cornea model,in the mouse it inhibited the ingrowth of vessels into bFGF-impregnated Matrigel plugs and in the rat ,it inhibited the growth of solid tumors with an associated reduction of tumor vessel density and it was analysed by ANOVA or ELISA technique. Based on this method,Phase I clinical trials are initiated to evaluate the feasibility of Squalamine in the treatment of cancer.The results of higher than strategies show that squalamine significantly reduces retinal neovascularization in a very mouse model of oxygen-induced proliferative retinopathy. general squalamine given either throughout the five days from P12 through P16 or as one dose on P12 considerably ablated retinopathy. There was less neovascularization once measured by vessel tuft formation, ERNV, and vessel tortuousness within the squalamine + oxygen–treated retinas when put next with the oxygen-only–treated retinas. Thus, squalamine is ready to inhibit or decrease the neovascular response seen when exposure of the baby mice to seventy fifth gas. additionally no hurtful general aspect effects of squalamine like weight loss or impaired organ growth were found during this methodology.Despite the advancement in the technology of cancer treatment like chemotherapy,radiotherapy and surgery for disease management,what makes a squalamine different from these agents.We have seen that squalamine is chemically synthesized for its chemical applications and known to have strong anti-angiogenic activity in invitro and invivo and it was confirmed in various tumor xenograft model.Squalamine efficiently inhibited the growth of tumors of

lung,breast,brain,ovaries and in retinal neovascularization and prostate implanted in nude mice and it was assessed in phase I and phase II of clinical trials on lung cancer.Squalamine has been placed in different categories based on their activity i.e activity against bacteria,Broad spectrum antimicrobial agent and activity against virus,Broad spectrum antiviral agent,as an antifungal agent which induces osmotic lysis of protozoa,synthetic derivatives were also studied due to low availability of squalamine in animal sources and sophisticated procedures.Squalamine has got a name that it can be Polyvalent drug of the future based on its antimicrobial and antiangiogenic activity useful in the treatment of cancers(lung,ovarian,brain)age related macular degeneration.Squalamine was also combined with other chemotherapeutic agents to enhance its antitumor tumor and antiangiogenic properties.There are many reasons to called Squalamine :A polyvalent drug of the future and can be used in the treatment of retinal diseases and cancerous tumor.

1. It is a natural derived steroid easily accessible from the animal source and also the technique which is used for its extraction is invasive.
2. The treatment which is used is a novel approach because it directly targets the tumor angiogenesis without much affecting the surrounding tissue.
3. It exhibits little systemic toxicity and usually tolerated by treated patients with various solid tumor malignancies including ovarian,non small cell lung and breast cancer proved in preclinical and clinical trials.
4. The US Food and Drug Administration has designated squalamine as an “orphan drug”for development as a new antitumor agent to treat ovarian cancer.
5. This compound has also received FDA fast track status for the treatment of “wet”age related macular degeneration.

CONCLUSION:

According to the aim of this review,we have seen the property of squalamine as antibiotic agent,antiviral agent,how squalamine has acted on a hallmark for tumor formation process of angiogenesis,various theories for the action of squalamine on tumor cells proposed by scientists,their approval in preclinical and clinical trials,A squalamine derivative NV669 which is obtained by simple titanium reductive amination reaction but this compound was only specific to pancreatic and hepatic cancer but it doesn’t cover its action on other tumors like ovarian,lung,retinal diseases etc.Since it is chemically synthesized product,toxicity will be one of the factors which will be considered for patient safety drug profile and compliance which was still needed to be studied,after being more efficacious than squalamine and if we consider squalamine as a drug,it requires more study and also the way squalamine capture cell and intracellular signaling pathways activated by this drug remain unclear.

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