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1 Review

2 New therapies of neovascular AMD – Beyond anti VEGFs

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Abstract

Neovascular age-related macular degeneration (nAMD) accounts for one of the leading causes of blindness among the aging population. The current treatment options for nAMD include intravitreal injections of anti-vascular endothelial growth factor (anti-VEGF). However, standardised frequent administration of anti-VEGF injections only improves the vision in approximately 30%-40% of nAMD patients. Current therapies targeting nAMD pose a significant risk of retinal fibrosis and geographic atrophy (GA) development in nAMD patients. A need exists to develop new therapies to treat nAMD with effective and long-term anti-angiogenic effects. Recent research on nAMD has discovered novel therapeutic targets and angiogenic signalling mechanisms involved in its pathogenesis. For example, tissue factor, human intravenous immune globulin, interferon-β signalling, cyclooxygenase-2 (COX-2) and cytochrome P450 monooxygenase lipid metabolites have been identified as key players in the development of angiogenesis in AMD disease models. Furthermore, novel therapies such as NLRP3 inflammasome inhibition, targeted intraceptor nanoparticle therapy, inhibitors of integrins and tissue factor are currently being tested at the level of clinical trials to treat nAMD. The aim of this review is to discuss the scope for alternative therapies proposed to anti-VEGFs for the treatment of nAMD.

Key words: Neovascular AMD; new therapies; anti-VEGFs; AMD signalling

1. Introduction

Age related macular degeneration (AMD) is the most common cause of irreversible blindness among the elderly population [1, 2]. Current global prevalence of AMD stands at 170 million and with aging as a major risk factor, it is expected to increase to 288 million by the year 2040 [3]. AMD can be classified into early, intermediate and advanced forms depending on the severity of the symptoms [4, 5]. Early and intermediate AMD, also referred to as dry AMD (non-exudative), is characterised by the accumulation of the yellowish-lipid rich protein content known as drusen between the retinal pigment epithelium (RPE) and Bruch's membrane resulting in the functional loss of the retinal photoreceptors [4]. Drusen deposition is considered as the hallmark of AMD [4, 5]. The advanced form of AMD is known as geographic atrophy (GA) and is characterised by the loss of RPE and choroid near the macular region leading to the loss of photoreceptors and central vision [4, 5]. The severe form of AMD (exudative) presents with the growth of abnormal blood vessels from the choroid extending into the avascular RPE and sub-retinal regions. This phenomenon is known as choroidal neo-vascularization (CNV) and the form of AMD with CNV is termed as nAMD [4, 5]. nAMD accounts for the majority of the blindness in AMD patients.

The formation of neovascularization in AMD is a complex process involving multiple signalling pathways mediated by VEGF, platelet derived growth factor, fibroblast growth factor, transforming growth factor, the Wnt pathway, the NLRP3 inflammasome, MAPK signalling, interleukins and chemokines [6-10]. VEGF-A, a potent pro-angiogenic factor has been implicated in the pathogenesis of nAMD through CNV.[11] RPE produce VEGF-A via two major pathways: complement activation and oxidative stress [12, 13]. Over production of VEGF-A leads to the breakdown of the blood-retinal barrier and formation

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of new blood vessels into the retina. Leakage of blood from these abnormal vessels results in oedema and loss of vision if untreated [9]. Immune cells such as microglia (resident macrophages in the retina) along with chemokines such as CCL2 are known to contribute to CNV and retinal inflammation in AMD pathogenesis [14, 15]. Inflammation and its role in AMD has been discussed in the previous reviews [10, 16]. The focus of the current review is to emphasize novel treatment modalities of nAMD beyond anti-VEGFs.

2. Current Treatment Modalities of nAMD

2.a Anti-VEGF injections

Injection of VEGF inhibitors into the vitreous is considered as the standard treatment for nAMD [17]. However intravitreal injection of VEGF inhibitors will not cure AMD completely but only slows down the progression of the disease [18, 19]. Agents such as ranibizumab, bevacizumab, pegaptanib, aflibercept have been approved by Food and Drug Administration (FDA) of the USA for the treatment of nAMD [18]. Ranibizumab and bevacizumab are humanized monoclonal antibodies that inhibit all isoforms of VEGF-A [18]. Pegaptanib is the first anti-VEGF agent that binds and inhibits the activation of VEGF-A [18, 20, 21]. Aflibercept is a human recombinant protein that acts as a VEGF decoy to prevent VEGF production [18, 22]. Other anti-VEGF agents considered for nAMD treatment include brolucizumab, abicipar pegol, angiopoietin, RG7716 and nesvacumab that are currently being tested in phase I, II and III clinical trials [23-27].

2.b Photodynamic therapy

Photodynamic therapy (PDT) for nAMD involves intravenous injection of verteporfin, approved by the FDA [28, 29]. Injected verteporfin binds to abnormal blood vessels to exert its anti-angiogenic effects [28, 29]. However, treatment with anti-VEGF agents is

considered superior as PDT damage has been reported to cause damage to the endothelial cells, and result in thrombosis and secondary platelet adhesion [18].

3. Rationale for Developing New Therapies

Current treatment strategies for nAMD require repeated, frequent intravitreal injections [18]. Long term administration of intravitreal anti-VEGF injections is associated with increased risk of developing retinal scaring and geographic atrophy in nAMD patients 2 to 5 years after initiating treatment [30, 31]. Furthermore, recent reports from multiple studies suggest that intravitreal injections of anti-VEGF drugs could result in complications such as vitreous and subconjunctival haemorrhage, fluid accumulation under the fovea, increased intra-ocular pressure, endophthalmitis and ocular inflammation [30, 32-35]. Results from the multicentral SEVEN-UP study show that only one third of the patients enrolled in the ANCHOR and MARINA trials had an improved visual outcome, leaving the other third with poor outcomes after 7 years of ranibizumab therapy [36]. Considering that the current therapies for nAMD are associated with multiple adverse events, there is a clear need to develop novel therapies to treat nAMD.

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4. New Therapies for nAMD – Thinking Beyond Anti-VEGFs

4.a Intravenous injection of immune globulin

Intravenous immune globulin (IVIg) is pooled plasma from thousands of healthy donors with a diverse antibody repertoire and specificity [37]. IVIg has been approved by the FDA for the treatment of primary immunodeficiency diseases [38, 39]. The first record of IVIg use dates to the year 1881 for the treatment of thrombocytopenic purpura in children [40]. Bogdanovich *et al*, reported that human IgG1 is a potent anti-angiogenic factor and achieves this via Fc mediated signalling through the FcγR1 receptor, a strong receptor for IgG1 [41]. Based on these facts, Yasuma *et al*, tested the anti-angiogenic properties of IVIg which is composed of approximately 60% IgG1 in five different humanized mouse models of angiogenesis [42]. Intravenous and intravitreal administration of IVIg in nAMD mice supressed angiogenesis effectively and attenuated macrophage infiltration, a key factor in angiogenesis development [42]. Most importantly, IVIg inhibited neovascularization via the activation of the FcγR1 receptor, a VEGF independent pathway [42]. As Intravenous administration of IVIg effectively suppressed CNV equivalent to intravitreal injections, this could provide an alternative mode of treatment to repeated intravitreal injection of anti-VEGFs in nAMD patients [42].

4.b Targeting the Cytochrome P450 Monooxygenase Pathway

Cytochrome P450 (CYP) is a class of enzymes that can synthesize fatty acid metabolites [43]. CYP monooxygenase is a CYP enzyme that plays a vital role in the metabolism of long-chain polyunsaturated fatty acids (LCPUFAs) into epoxydocosapentaenoic acids (EDPs) and epoxyeicosatetraenoic acids (EEQs) ultimately regulating vascular function (Figure 1) [43]. Previous studies have shown that LCPUFAs derived CYP monooxygenase metabolites, 17,18-EEQ and 19, 20-EDP are associated with the regulation of CNV in

mouse models (Figure 1) [44]. The intake of diet enriched with eicosapentaenonic acid (EPA) and docosahexaenonic acid (DHA) reduced the severity of nAMD in mice by increaseing the plasma levels of EEQs and EDPs [44]. Furthermore, direct treatment of mice with intraperitoneal injections of 17,18-EEQ and 19, 20-EDP reduced CNV [44]. CYP2C8 is a potent monooxygenase that converts EPA to 17,18-EEQ and DHA to 19,20 EDP [43]. Overexpression of CYP2C8, 17,18-EEQ and 19,20-EDP enriched diet significantly inhibited CNV in nAMD mice [45]. 17, 18-EEQ and 19, 20-EDP inhibited CNV by downregulating the expression of cell adhesion molecules, intracellular adhesion molecule-1 (ICAM-1) and E-selectin [45]. ICAM-1 and E-selectin contribute to the formation of CNV by recruiting leukocytes into the tissue [46]. All this evidence suggests that CYP monooxygenase plays a vital role in inhibiting CNV via LCPUFAs metabolites. In a separate study by Fu *et al*, oral supplements of ω3 and ω6-LCPUFAs to CNV induced mice corelated with reduced risk of nAMD development [47]. Oral or dietary ω3, ω6-LCPUFAs, 17,18-EEQ and 19,20-EDP could serve as a non-invasive treatment modality for nAMD patients [45, 47].

4.c Interferon-β Therapy

Immune cells such as microglia and mononuclear phagocytes play important roles in angiogenesis [48, 49]. Microglia, the resident macrophages in the retina are attracted to choroid and RPE during CNV [14]. Inhibition of monocyte (precursors for macrophages) migration into the retina reduced CNV in a laser induced mouse model suggesting that microglia and monocyte derived macrophages may be pro-angiogenic [48, 50]. Targeting the signalling pathways involving macrophage migration could be of therapeutic benefit in neovascular diseases such as AMD [51]. Interferon-beta (IFN-β), via interferon-alpha/beta receptor (IFNAR) signalling, has been identified as a critical pathway in regulating autoimmunity and monocyte/microglia influx in the central nervous system (CNS) [52, 53].

Anika Luckoff *et al*, investigated the role of IFN-β and its receptor IFNAR in a laser-induced CNV mouse model and reported that IFN-β and IFNAR play a pivotal role in retinal microglia/macrophage activation and infiltration [54]. IFNAR knock-out (KO) mice presented with increased microglia/macrophage activation and promoted CNV. Delivering IFN-β to CNV induced wild-type mice significantly attenuated CNV formation, vascular leakage and microglia/macrophage infiltration suggesting that systemic IFN-β therapy could be a promising treatment option for nAMD patients. Systemic treatment could reduce the complications resulting from intravitreal injections. Since IFN-β therapy is a well-established treatment option for multiple sclerosis and autoimmune encephalomyelitis, it could have a great potential for treating neovascular diseases such as AMD [55-57].

4.d Interleukin-33 Therapy

Interleukin-33 (IL-33), a pro-inflammatory cytokine, is a member of type-2 IL-1 family [58]. Once activated, IL-33 signals via its receptor, ST2 and the IL-1R accessory protein [59]. In humans, IL-33 is expressed in epithelia cells, endothelial cells, fibroblasts, and in rodents its expression has been detected in RPE, te inner retina and choroid along with lymph nodes, spleen and CNS [58, 60, 61]. In mouse experimental autoimmune uveitis, systemic administration of IL-33 attenuated the disease severity [61]. Similarly, Theodoropoulou *et al*, reported a protective role of IL-33 in a laser-induced CNV mouse model [62]. Intravitreal injection of recombinant IL-33 inhibited the development of CNV in mice via inhibition of ST2 expressing fibroblasts and endothelial cells but did not alter the levels of VEGF [62]. This study discovered a novel mechanism involved in attenuating CNV independent to VEGF signalling suggesting that recombinant IL-33 therapy could serve as an alternative treatment for nAMD patients that do not respond to conventional anti-VEGF treatments.

4.e Semaphorin 3F

Semaphorins were initially discovered as molecules that contribute to the embryonic development of the nervous system [63]. Semaphorin 3F (Sema3F) is a member of class 3 semaphorin proteins and is expressed in the outer retina under normal conditions and in inner retina under hypoxia [64]. Previously, Sema3F was reported to be protective against subretinal vascularization in mouse models [65]. Sun *et al*, investigated the anti-angiogenic role of Sema3F in two different mouse models mimicking human nAMD and found that intravitreal injection of recombinant Sema3F effectively inhibited the subretinal neovascularization and CNV in both models (Figure 1) [66]. Considering this data, Sema3F could be a potential target to design novel therapies for nAMD.

4.f Targeting MyD88 Pathway and DICER 1

Geographic atrophy (GA) is an advanced form of AMD for which there is no current effective treatment [9]. GA could occur in patients after repeated administration of anti-VEGFs over time. So, it is important to understand the molecular mechanism involved in the pathogenesis of GA to identify novel therapeutic targets. RPE degeneration leading to loss of photoreceptors function is commonly seen in the patients with GA [9, 67]. This RPE degeneration is associated with the accumulation of *Alu* RNA which was previously shown to cause RPE cell death (Figure 1) [67]. Tarallo *et al*, recently discovered that *Alu* RNA accumulates in the RPE of GA patients due to a deficiency of the enzyme DICER 1 that functions to cleave *Alu* RNA [8]. The accumulated *Alu* RNA activated the NLRP3 inflammasome (Figure 1) and triggered IL-18 dependent MyD88 signalling in the RPE [8]. The NLRP3 inflammasome is an intracellular NOD-like receptor that operates in innate immunity [68]. Upon activation it cleaves pro-IL-1β and pro-IL-18 into their biologically active forms via caspase-1 [68]. Pharmacological inhibition of the NLRP3 inflammasome,

MyD88 or IL-18 in mouse models and human RPE cell cultures prevented the RPE cell death resulting from DICER 1 deficiency [67]. Furthermore, activation of caspase-11 (caspase-4 in humans) in mice has been implicated in the pathogenesis of GA [69]. This activation was mediated by cyclic GMP-AMP synthase leading to IFN-β production and gasdermin D-dependent IL-18 secretion (Figure 1) [69]. Elevated levels of gasdermin D, IFN-β, caspase-4 and cGAS has also been observed in the RPE of human eyes with GA [69]. Discovery of these series of events from DICER 1 deficiency in RPE to cGAS is a breakthrough in understanding the pathogenesis of GA and opens new platforms for novel therapies to treat GA [67, 69].

4.g Inhibitors of Integrins

Integrins are transmembrane proteins that bind to extracellular matrix proteins such as laminin, fibronectin and collagens [70]. Integrins are localised on the surface of RPE and mediate the process of phagocytosis of the outer segment particles of the photoreceptors by RPE [71, 72]. Members of the integrin family α5β1, αvβ3 and αvβ5 are expressed during CNV and their antagonists could possibly have a therapeutic role in inhibiting CNV in AMD patients (Figure 1) [73-75].

Integrin α5β1 is a fibronectin receptor which is linked to endothelial cell migration and proliferation. JSM6427 is an inhibitor of integrin α5β1, intravitreal injection of JSM6427 significantly attenuated the vascularization in mice [76]. In a phase I clinical trial (ClinicalTrials.gov Identifier: NCT00536016) on 36 patients evaluated the pharmacological efficacy and safety of JSM6427 as intravitreal injection. The study ended in 2009 with promising results however, to date no further studies have been undertaken to investigate JSM6427.

Volciximab is a monoclonal antibody (Ophthotech Corporation, Princeton, NY, USA) that inhibits the binding of fibronectin to integrin α5β [77]. A phase I clinical trial (ClinicalTrials.gov identifier: NCT00782093) which evaluated the safety of Volciximab in combination with ranibizumab reported positive results [77].

ALG-1001 is a synthetic oligopeptide (Allegro Ophthalmics, San Juan Capistrano, CA, USA) that attenuates α5β1, αvβ3 and αvβ5 integrin mediated blood vessel growth [73]. A phase I clinical trial (ClinicalTrials.gov Identifier: NCT01749891) reported that ALG-1001 was safe to administer as intravitreal injections with improvement in the visual acuity of nAMD patients [73].

Tenascin-C is an extracellular glycoprotein which is mainly expressed during developmental stages and its levels are upregulated in inflammatory conditions [78]. Tenascin-C has been found in the CNV membranes of AMD patients (Figure 1) [79]. Tenascin-C promotes retinal neovascularization in proliferative diabetic retinopathy patients [80]. Kobayashi *et al*, reported that tenascin-C was co-localised with integrin αvβ3 in the CNV membranes of AMD patients and laser-induced CNV mice [81]. Furthermore, tenascin-C promoted CNV in mice by binding to integrin αvβ3. Tenascin-C knock out and tenascin-C mRNA silenced (intravitreal injection of siRNA) mice showed a significant reduction in CNV formation, suggesting that tenascin-C mediated integrin αvβ3 could be a potential target for nAMD [81].

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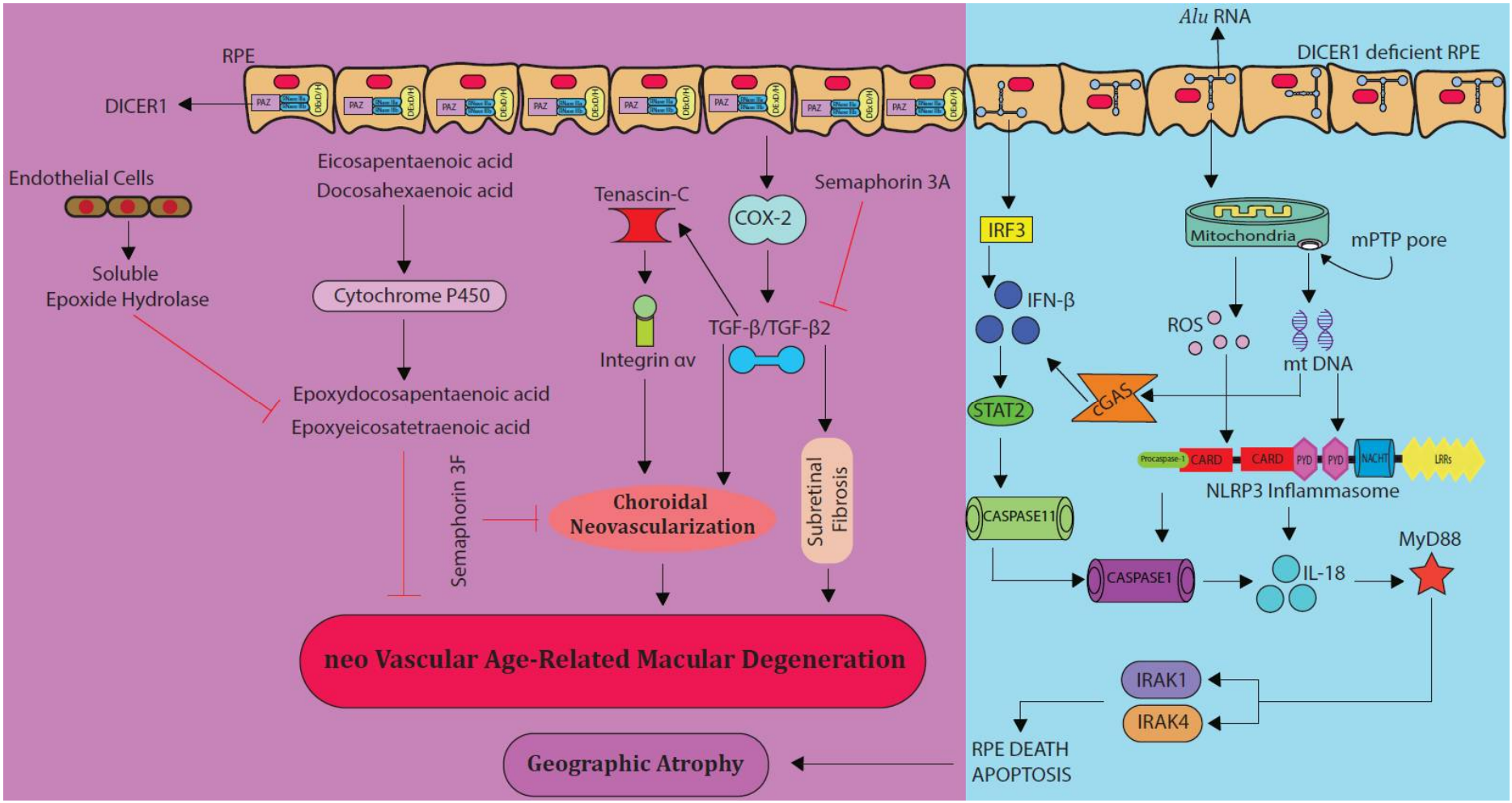

Figure 1. Major signalling pathways involved in the development of nAMD other than VEGF (purple panel on the left-hand side). Cytochrome P450, COX-2 and TGF-β pathways play critical role in CNV leading to nAMD. On the other hand, semaphorins (3A, 3F) and cytochrome P450 metabolites naturally inhibit the formation of CNV. DICER 1 enzyme plays crucial role in *Alu* RNA breakdown preventing GA. In DICER 1 deficient RPE (blue panel on the right-hand side) *Alu* RNA deposits in RPE activating the NLRP3 inflammasome and cGAS activates the noncanonical inflammasome ultimately leading to apoptosis of RPE and GA development.

4.h Inhibition of CCR3

CCR3 (also known as CD193) is a cell surface chemokine receptor that is expressed by eosinophils, Th2 cells, basophils and mast cells [82]. It's expression by choroidal endothelial cells was recently discovered in CNV membranes excised from nAMD patients [83]. Inhibition of CCR3 using intravitreal injection of anti-CCR3 antibody, a small molecule CCR3 antagonist or by using CCR3 knock out mice significantly attenuated the formation of CNV in mice [83]. Furthermore, a comparison of CCR3 neutralization versus anti-VEGF treatments in mice reported that CCR3 inhibition was superior to anti-VEGF treatment suggesting CCR3 as a potential target to treat nAMD [83].

4.i COX-2 Inhibitors

Cyclooxygenases (COX) are a group of enzymes that are involved in inflammatory immune responses required for the conversion of arachidonic acid to prostaglandins [84]. Out of the three COX isoforms (COX-1, COX-2 and COX-3), COX-2 mediates inflammation and is induced by pathological stimuli [84]. In mice, COX-2 involvement has been implicated in CNV and subretinal fibrosis of the retina (Figure 1). Subretinal fibrosis was associated with upregulation of transforming growth factor-β2 (TGF-β) by COX-2 in AMD (Figure 1) [85, 86]. The study reported the expression of COX-2 in CNV, and that the inhibition of COX-2 using NS-398 significantly attenuated CNV and subretinal fibrosis via inhibition of macrophage infiltration, TGF-β2 and VEGF [87].

4.j Tissue Factor Inhibition:

Tissue factor (TF) is a transmembrane receptor for plasma coagulation factor VII. Excluding its involvement in thrombosis, studies have reported that TF is one of the key mediators in pathological neovascularization [88]. Under normal physiological conditions

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TF is not expressed by cells, however vascular endothelial cells, monocytes and macrophage express TF in response to inflammation [89]. Increased expression of TF has been observed in the RPE of nAMD patients compared to non-AMD retinas [88, 90]. Intravitreal injection of anti-TF monoclonal antibody contributed to the reduction of CNV in a mouse model [91]. With this evidence, TF has been identified as a novel target to treat nAMD by developing hI-con1. hI-con1, a synthetic molecule coupled with factor VII conjugated to the Fc region of an antibody, selectively binds to TF and destroys pathological vessels [92]. hI-con1 is being tested in a multicentric phase II clinical trial, with pending results (ClinicalTrials.gov identifier: NCT02358889) [92].

4.k Targeted Intraceptor Nanoparticle Therapy

Targeted intraceptor nanoparticle therapy is a three-component system that consist of 1) plasmids expressing *Flt23k* intraceptors, 2) PLGA biodegradable nanoparticles, and 3) tripeptide adhesion motif Arg-Gly-Asp (RGD) [93]. Flt23k intraceptors are composed of VEGF-binding domains 2-3 of *Flt*, a high affinity VEGF receptor and RGD facilitates the selective localization of nanoparticles to CNV after intravenous injection [93]. The *Flt23k* component inhibited CNV, and the RGD component suppressed fibrosis in mice and primates [93]. Although this is an anti-VEGF strategy to inhibit CNV, it has advantages over the conventional treatment for nAMD, (intravitreal injections) that is associated with pain, retinal detachment and scarring [93]. Targeted intraceptor nanoparticle therapy is administered as intravenous injections, can cross the blood-retinal barrier which is a major challenge for intravenous delivery and was proven to be nontoxic in *vivo* suggesting that this could provide a means for alternative drug delivery route to treat nAMD [93, 94].

5. Targeting Signalling Pathways with Partial Involvement of VEGF

A Number of other pathways and novel agents that primarily focus on targets other than VEGF and partially on VEGF were recently implicated in the pathogenesis of nAMD. Such mechanisms/agents include but are not limited to the complement pathway, BMP9/ALK1 signalling, Erythropoietin Signalling, long non-coding RNAs, STAT3 activation, neuropilin 1, platelet activating factor, mTOR signalling, and Yes-associated protein (YAP) inhibitors. A brief mechanism of action of all these signalling pathways partially involving VEGF and their inhibitors with possible therapeutic benefits is listed in the table 1.

289 Table.1 List of potential targets for nAMD treatment partially involving VEGF

	SIGNALING/ INHIBITOR	KEY MOLECULES/ PROTEINS INVOLVED	FINDINGS	REFERENCE
1	Complement pathway	C3a, C5a, monocyte chemoattractant protein-1 (MCP-1), VEGF, and MG4 domain, IL-17, γδ T-cells	Antibody mediated neutralization of C3a, C5a, MG4 domain of β chain or pharmacological inhibition of their receptors inhibited CNV in mouse nAMD	Jo *et al*, 2017 [95]; Nozaki *et al*, 2006 [12]; Tan *et al*, 2015 [96]; Coughlin *et al*, 2015 [97]; Robrer *et al*, 2009 [98]
2	BMP9/Alk1 signalling	BMP9, Alk1, VEGF, and VEGFR2	Activating Alk1 signalling inhibited growth of blood vessels in nAMD mouse model	Ntumba *et al*, 2016 [99]
3	Erythropoietin signalling	Erythropoietin, macrophages, CCL2, CXCL10, CCL22, IL-6, and IL-10	Increased erythropoietin signalling is associated with increased CNV in mice	Bretz *et al*, 2018 [100]
4	Long non-coding RNAs	MAPK signaling, Vax2osl, and Vax2os2	326 or 51 long non-coding RNAs that play role in human nAMD were identified and their dysregulation could provide novel insights into nAMD treatments	Xu *et al*, 2014 [101]
5	Neuropilin 1 (Nrp1)	Nrp1, and VEGF	Reduced CNV was seen in Nrp1 KO mice	Fernandez-Robredo *et al*, 2017
6	Platelet-activating factor (PFA)	PFA, PFA-receptor (PFA-R), macrophages, VEGF, MCP-1, and IL-6	WEB2086, a novel PAF-R antagonist inhibited CNV and experimentally induced subretinal fibrosis in mice	Zhang *et al*, 2013 [102]
7	Nucleoside reverse transcriptase inhibitors (NRTIs)	VEGF-A, and P2X7 receptor	Intravitreal injection of NRTIs, lamivudine, zidovudine, abacavir and P2X7 antagonist A438079 reduced CNV in mice	Mizutani *et al*, 2015 [103]
8	RG7716 antibody	VEGF, and angiopoietin 2	Phase II clinical trial underway. Phase I results indicated improvement in visual acuity in patients and RG7716 was safe	Chakravarthy *et al*, 2017 [104]

9	STAT3 signalling	Monocytes, macrophages, CX3CR1, HLA-DR, STAT3, VEGF, Suppressor of Cytokine Signalling 3	Inhibition of STAT3 activation using LLL12 attenuated CNV in mice and intermediate monocytes ($CD14^+$ $CD16^+$) are activated in nAMD patients	Chen *et al*, 2016 [105]
10	TGF-β signalling	TGF-β, Smad2/3, VEGF, and TNF-α	Inhibition of TGF-β using a synthetic inhibitor, LY2157299 or Decorin a natural TGF-β inhibitor significantly inhibited CNV in mice	Wang *et al*, 2017 [106]
11	Yes-associated protein (YAP) signalling	YAP, proliferating cell nuclear antigen (PCNA), CD31, VEGF	YAP siRNA and ranibizumab treatment reduced VEGF, PCNA, reduced endothelial cell proliferation and CNV formation in mice	Yan *et al*, 2018 [107]
12	Adeno-associated virus-mediated gene therapy with cartilage oligomeric matrix protein angiopoietin-1 (AAV2.COMP-Ang1)	VEGF, and hypoxia-inducible factor (HIF) -α	Subretinal injection of AAV2.COMP-Ang1 reduced VEGF levels and inhibited CNV in mice	Lambert *et al*, 2016 [108]
13	Fenofibric acid (Feno-FA) signalling	Feno-FA, VEGF, TNF-α, ICAM-1, and peroxisome proliferator–activated receptor-alpha (PPARα)	Feno-FA injections in mice supressed neovascularization	Qiu *et al*, 2017 [109]
14	mTOR signalling	hypoxia-inducible gene *RTP801*, VEGF,	A phase II clinical trial reported that the use of siRNA, PF-04523655 in combination with ranibizumab compared to ranibizumab alone improved vision in nAMD patients	Nguyen *et al*, 2012 [110]
15	Connective growth factor (CTGF)	CTGF, and ERK signalling	RXI-109 an inhibitor of CTGF designed to reduce retinal fibrosis in nAMD patients. Phase I clinical trial is currently underway	Kothary *et al*, 2010 [111]; ClinicalTrials.gov identifier: NCT02599064

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6. Concluding Remarks

The current review presents insights into a new era of treatment modalities for nAMD contrary to anti-VEGF agents. Intravitreal injection of anti-VEGFs has been the standard mode of treatment for nAMD since many years. Despite the success of anti-VEGFs there is no improvement in the vision of a third of nAMD patients and their long-term use is associated with adverse events such as the development of GA and retinal fibrosis to name a few. There exists a need to develop ideal strategies to reduce the frequency of injections and better clinical outcome. Recent research has reported many molecular targets other than anti-VEGFs and alternate drug delivery methods which are currently being tested at the level of clinical trials opening new avenues to treat nAMD. It could also be postulated that employing multiple target approach to treat nAMD could yield better results than single approach, specially to treat nAMD and subretinal fibrosis at the same time.

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Conflict of interest

The author declares no conflict of interest

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