LATE BREAKING POSTERS

LBP-3

THE DETRIMENTAL SYNERGISTIC EFFECT OF HYPOXIA AND COMPLEMENT MASP-1 IN THE ATHEROSCLEROSIS-RELATED DISEASES, ON ENDOTHELIAL CELL MODEL.

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Abstract: Hypoxia and hypoxia/reoxygenation are direct pathogenic components in stroke and acute myocardial infarction (AMI) developing as a consequence of atherosclerosis. The complement lectin pathway (CLP) is known to play a crucial role both in atherosclerosis and in atherosclerosis-related diseases [1]. We previously demonstrated that Mannan-binding lectin-associated serine protease-1 (MASP-1), the most abundant enzyme of the CLP induces an inflammatory phenotype of endothelial cells by cleaving Protease Activated Receptors (PARs) [2].

There is no data whether hypoxia and MASP-1 can potentiate each other's effects, therefore, we aimed to investigate the possible synergism between them.

As a hypoxic endothel model we used $CoCl_2$ treated HUVECs. Adhesion molecules and cytokines were detected with cellular or sandwich ELISA. Signalization pathways were studied with immuno-fluorescence microscopy; Ca^{2+} mobilization with fluorescence microscopy using Fluo4-AM Ca-sensitive dye, PAR gene expression with qPCR and permeability changes with the XPerT method. Statistical analysis was performed by GraphPad Prism 7.0.

E-selectin was synergistically upregulated by $CoCl_2$ and MASP-1, whereas MASP-1 induced VCAM-1 expression could be inhibited by $CoCl_2$. $CoCl_2$ increased ICAM-1, while decreased ICAM-2 expression. The two agents synergistically elevated GROa production and increased IL-8 production and endothelial permeability without potentiating each other's effects. $CoCl_2$ pretreatment enhanced the Ca^{2+} mobilization in response to MASP-1. $CoCl_2$ and MASP-1 synergistically activated the CREB and NFκB signalization pathways. $CoCl_2$ time-dependently upregulated PAR2 gene expression.

Hypoxia potentiates the effect of MASP-1 on endothelial cells, at least partially, by increasing PAR2 expression. This results in the strong synergism between the two agents observed at the level of signalization pathways, adhesion molecules and cytokines, which may contribute to the development of the strong neutrophil infiltration well-known in the acute phase of stroke and AMI. This raises the role of MASP-1 as a drug target in the acute phase of atherosclerosis-related diseases.

Reference 1: *Collard, C.D., et al., Complement activation after oxidative stress: role of the lectin complement pathway. Am J Pathol, 2000. 156(5): p. 1549-56.*
Reference 2: *Megyeri, M., et al., Complement protease MASP-1 activates human endothelial cells: PAR4 activation is a link between complement and endothelial function. J Immunol, 2009. 183(5): p. 3409-16.*

LBP-5

EFFECTIVENSS OF MONTELUKAST IN OVALBUMIN-INDUCED FOOD ALLERGY MOUSE MODEL.

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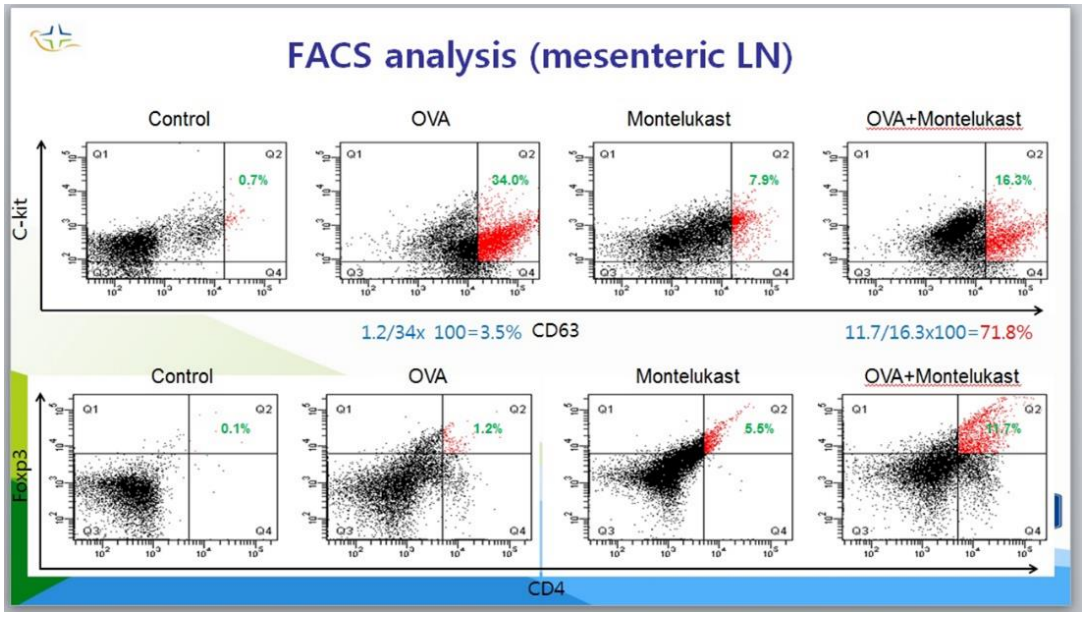
Background: Food allergy tends to increase in frequency with a prevalence of about 8% in children. However, there is no established treatment other than avoiding these foods. Leukotriene receptor antagonist (LTRA) is known as a treatment for asthma and allergic rhinitis, and has been tried in clinical practice as a treatment for eosinophilic gastroenteritis. Therefore, this researcher intends to investigate the effect and mechanism of montelukast in a mouse model of food allergy.

Materials and Methods: BALB/c mice were immunized intraperitoneally with 50ng of ovalbumin(OVA) and 2mg/ml of alum at intervals of 2 weeks, for a total of two inoculations. The mice were orally challenged with 50 mg OVA 6 times every 3 or 4 days for 16 days. Treatment group was challenged with OVA, and treated with montelukast (10mg/kg). Anaphylaxis were evaluated. In addition, ELISA for immunoglobulins (OVA-specific IgE) in serum sample, FACS (fluorescence activated cell sorter) analysis of CD4 and Foxp3 positive T cell from mesenteric lymph node (MLN), western blot for inflammatory marker in colon and histologic exam from colon were conducted.

Results: Any of the groups were not reached death by anaphylactic shock. Montelukast inhibited OVA-specific IgE in serum and eosinophilic infiltration in proximal colon increased by OVA. In FACS analysis, treatment group with montelukast showed that the fraction of Foxp3+ T cells to CD4+ T cells was significantly increased compared to the OVA group. Foxp3+ was detected only in treatment group in western blot.

Conclusions: This results suggest that montelukast can be a new alternative for prevention and treatment of food allergy by increasing Foxp3+ T cell.

Reference 1: *T Yamamoto, K Fujiwara and et al. Therapeutic effect of kakkonto in a mouse model of food allergy with gastrointestinal symptoms. Int Arch Allergy Immunol. 2009;148(3):175-85.*
Reference 2: *D Lee, HS Kim, E Shin, SG D, CK Lee, YM Kim, MB Lee and et al. Polysaccharide isolated from Aloe vera gel suppresses ovalbumin-induced food allergy through inhibition of Th2 immunity in mice. Biomed Pharmacother 2018 May;101:201-210.*



LBP-6

PHARMACOKINETIC AND PHARMACODYNAMIC CHARACTERIZATION OF CSL040.

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Abstract: Human Complement Receptor 1 (HuCR1) is a potent regulator of complement both *in vitro* and *in vivo*. It acts by interacting with its ligands C3b and C4b. In the present study, CSL040, a truncated version lacking the C-terminal long homologous repeat domain D (LHR-D), is introduced. The pharmacokinetic (PK) and pharmacodynamic (PD) properties are presented with a focus on the role of N-linked glycosylation. We demonstrate a relationship between the asialo content of CSL040 and its PK/PD properties in rats and non-human primates (NHPs), using recombinant CSL040 preparations with varying levels of asialylated glycans. Our data shows that the clearance mechanism likely involves the asialoglycoprotein receptor (ASGR), as the clearance rate of CSL040 with high asialo content was attenuated *in vivo* by co-administration of rats with asialofetuin, which saturates the ASGR. In addition, biodistribution studies were performed showing CSL040 to be localized in the liver following systemic administration. Our studies also uncovered striking effects on the PD parameters by CSL040showing an extended inhibition of the alternative pathway in both rats and NHPs. In contrast, no prolonged inhibition of the CP/LP was seen by CSL040. Overall, the extended effects on the AP were not correlated with the PK profile of CSL040. Dose-dependence of this effect was shown in additional *in vivo* studies with both, CSL040 and the full-length extracellular domain of HuCR1. Taken together, our data suggests that low asialo contents are favourable for developing CSL040 as a therapeutic candidate with improved PK properties, and that deletion of the LHR-D domain markedly improves its pharmacokinetics *in vivo*.

LBP-7

SAR443809: A POTENT AND SELECTIVE INHIBITOR OF THE ALTERNATIVE PATHWAY OF COMPLEMENT, TARGETING COMPLEMENT FACTOR BB

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Abstract:: Overactivation or dysregulation of the complement system is implicated in the onset or progression of a wide variety of diseases. Many complement inhibitors currently in development target nonactive pro-forms of complement proteins that are typically present at high concentrations in plasma. This results in increased clearance of the drug due to target-mediated drug disposition, necessitating frequent dosing to achieve the high drug levels required to sustain therapeutic inhibition. Furthermore, many complement inhibitors are aimed at inhibiting only terminal pathway activity, which leaves opsonin-mediated effector functions intact. We describe here the discovery of SAR443809, a monoclonal antibody that specifically inhibits the activated form of Factor B (Factor Bb). The antibody potently inhibits alternative pathway (AP) activity by blocking AP convertase cleavage of C3, leaving classical and lectin pathway activation unaffected. Experiments with erythrocytes isolated from human paroxysmal nocturnal hemoglobinuria (PNH) patients demonstrate that, while terminal pathway inhibition via C5 blockade can effectively inhibit hemolysis, proximal complement inhibition with SAR443809 potently inhibits both hemolysis and C3b deposition, abrogating the propensity for extravascular hemolysis. Finally, administration of the antibody to nonhuman primates shows sustained inhibition of AP activity following

a single injection. Overall, SAR443809 is a potent and selective inhibitor of Factor Bb and shows potential for treatment of AP-mediated disorders.

LBP-8

SAR445088, A MONOCLONAL ANTIBODY SPECIFIC FOR THE ACTIVE FORM OF C1S IS A POTENT INHIBITOR OF THE CLASSICAL PATHWAY OF COMPLEMENT.

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Abstract: A unique advantage of targeting only the classical pathway of complement (CP) for CP-mediated diseases is that the alternative and lectin pathways are left intact for immune surveillance. Sutimlimab, a monoclonal antibody specific for C1s, is the first FDA-approved, CP-specific complement inhibitor. SAR445088 is a next-generation, CP-specific complement therapeutic which, like sutimlimab, is a monoclonal antibody specific for C1s. However, SAR445088 is highly specific for the active form of C1s, in contrast to sutimlimab, which binds both the inactive and active forms of the protein. In this study, the ability of SAR445088 to inhibit CP activity was assessed. In both Wieslab® and hemolytic assays, SAR445088 and its murine precursor, TNT005, potently inhibited the CP. The two molecules were specific for the classical pathway and did not inhibit alternative or lectin pathway activity. Additionally, SAR445088 was tested in a ligand-binding assay using microwell plates coated with either the active or inactive form of C1s. SAR445088 demonstrated high specificity for the active form, with little to no binding to the inactive form. In conclusion, SAR445088 is a potent CP-specific complement inhibitor, with several potential advantages compared to its predecessor, sutimlimab, and is currently being evaluated in clinical trials.

LBP-9

RECOMBINANT HUMAN C1 ESTERASE INHIBITOR (CONESTAT ALFA) IN THE PREVENTION OF SEVERE SARS-COV-2 INFECTION IN HOSPITALIZED COVID-19 PATIENTS: A RANDOMIZED, OPEN-LABEL, MULTI-CENTER TRIAL.

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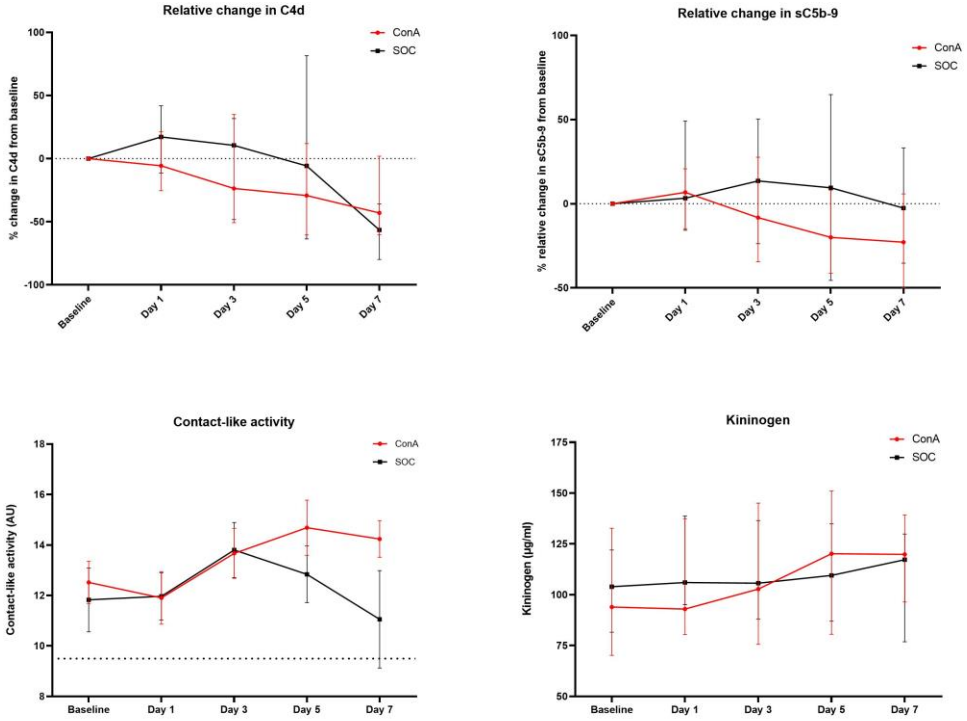
Background: Activation of the complement and the contact activation system drives thromboinflammation in COVID-19. We hypothesized that conestat alfa (ConA), a regulator of these plasmatic cascades, may prevent disease progression. The aim of the current study was to investigate the efficacy and safety of ConA in reducing disease severity in hospitalized, non-critically ill COVID-19 patients.

Methods: In this investigator-initiated, randomized, open-label, multi-center trial, patients hospitalized with COVID-19 pulmonary disease and at least one risk factor for disease progression were recruited at five centers and randomized (2:1) to receive ConA over a 72-hour period in addition to standard of care (SOC) or SOC only. The primary outcome was disease severity (WHO Ordinal Scale for Improvement) on day 7. This trial is registered with ClinicalTrials.gov, NCT04414631.

Results: The trial was stopped early after the second interim analysis and recruitment of a total of 83 patients. Baseline characteristics indicated more severe disease in the SOC group, e.g. 44/56 (79%) in the ConA group and 18/27 (67%) in the SOC group required supplemental oxygen at baseline. Dexamethasone and remdesivir were used in the majority of patients in both groups. There was no

difference in disease severity on day 7 (p=0.11), and median time to clinical improvement or discharge was 7 days in both groups. The number of patients with progression to mechanical ventilation within 7 days was similar (13% vs. 4%, p=0.26). Activation of the complement and contact activation system over time was similar in both groups (Figure 1). Drug-related serious adverse events were not observed.

Conclusions: These data do not support the use of the chosen ConA regimen in hospitalized COVID-19 patients. Earlier intervention, a higher dosage or longer treatment duration may be investigated in future trials.



LBP-10

DIFFERENCES BETWEEN AVACOPAN AND PREDNISONE FOR THE TREATMENT OF ANCA-ASSOCIATED VASCULITIS AT DIFFERENT THRESHOLDS OF GLUCOCORTICOID TOXICITY.

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Background: Treatment with glucocorticoids (GCs) for ANCA-associated vasculitis (AAV) is associated with substantial toxicity. The Glucocorticoid Toxicity Index (GTI), developed to measure change in GC toxicity over time, provides both an Aggregate Improvement Score (AIS) and a Cumulative Worsening Score (CWS).[1] The GTI was a pre-specified secondary outcome in ADVOCATE, a randomized, double-blind, placebo-controlled trial in patients with AAV that replaced a GC taper with avacopan, a complement C5a receptor inhibitor. Patients were randomized to either avacopan or a prednisone taper on a background of either cyclophosphamide/azathioprine or rituximab.[2] This study aimed to compare GC toxicity between the treatment groups at three different GC toxicity thresholds, beginning with the minimum clinically important difference (MCID) of the GTI.

Methods: The MCID for the GTI (10 points) was calculated in the validation phase of the instrument. We compared the two groups at GTI thresholds of 10, 20, and 30 points for CWS and AIS. The CWS comprises all GC-related toxicities that have occurred between baseline and 26 weeks. The AIS, in

contrast, allows toxicities to be added if they are new or subtracted if they improve. Higher CWS and AIS indicate greater severity of GC toxicity.

Results: The percentages of patients exceeding the specified AIS thresholds differentiated the avacopan from the prednisone group (48.2% vs. 60.4%, respectively, at the 10-point threshold, p=0.02; 29.5% vs. 45.1% at the 20-point threshold, p=0.003; and 18.1% vs. 33.5% at the 30-point threshold, p=0.001) (**Table**). The CWS differentiated avacopan from prednisone at the 20- and 30-point thresholds (57.8% vs 73.2%, respectively, at 20 points, p=0.002; 41.0% vs 55.5% at 30 points, p=0.007).

Conclusions: Among patients with AAV, treatment with avacopan was associated with lower GC toxicity across multiple GTI thresholds compared to treatment with prednisone, consistent with the substantial reduction in total GC exposure associated with avacopan.

Reference 1: *Stone JH, McDowell PJ, Jayne DRW et al. The Glucocorticoid Toxicity Index: Measuring changes in glucocorticoid toxicity over time. Semin Arthr Rheum 55 (2022): 152010.*

Reference 2: *Jayne DRW, Merkel PA, Schall TJ, et al. Avacopan for the Treatment of ANCA-Associated Vasculitis. N Engl J Med. 2021;384:599-609*

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Table. Percentages of Patients in ADVOCATE Exceeding Selected GTI Thresholds at Week 26

GTI threshold/study group	n (%) exceeding CWS threshold	p-value	n (%) exceeding AIS threshold	p-value
GTI worsening > 10 points				
Avacopan (N = 164)	138 (83.1%)	0.147	80 (48.2%)	0.022
Prednisone (N = 166)	144 (87.8%)		99 (60.4%)	
GTI worsening > 20 points				
Avacopan (N = 164)	96 (57.8%)	0.002	49 (29.5%)	0.003
Prednisone (N = 166)	120 (73.2%)		74 (45.1%)	
GTI worsening > 30 points				
Avacopan (N = 164)	68 (41.0%)	0.007	30 (18.1%)	0.001
Prednisone (N = 166)	91 (55.5%)		55 (33.5%)	

AIS, Aggregate Improvement Score; CWS, Cumulative Worsening Score; GTI, Glucocorticoid Toxicity Index

LBP-11

INSIGHTS FROM THE ADVOCATE STUDY: RESPIRATORY TRACT INVOLVEMENT IN PATIENTS WITH ANCA-ASSOCIATED VASCULITIS IN A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PHASE 3 TRIAL OF AVACOPAN.

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Background: Results from a trial evaluating avacopan, an orally administered selective C5aR inhibitor, in patients with ANCA-associated vasculitis have been reported previously. A subgroup analysis focused on lung and ENT (ear, nose, and throat) involvement is detailed here.

Methods: ADVOCATE was a phase 3, randomized, double-blind, controlled clinical study. Patients were randomized to receive prednisone taper or avacopan, on a background of either cyclophosphamide/azathioprine or rituximab. Primary efficacy endpoints were the percent of patients

achieving disease remission at Week 26 and sustained remission at Week 52. Remission was defined as a Birmingham Vasculitis Activity Score (BVAS) of 0 and no glucocorticoids (GCs) within 4 weeks prior to Week 26. Sustained remission was remission at Week 26 and Week 52 and no GCs use 4 weeks prior to Week 52. Lung and ENT involvement were defined as active vasculitis on BVAS.

Results: The avacopan group had an 86% median reduction in overall GC load, 26-week non-inferiority, and 52-week superiority compared to the prednisone group. Percent of patients with lung and ENT involvement at baseline for both groups are listed in the Table. In the avacopan group, lung involvement was present in 0.6% (1/166) and 0% (0/166) patients, at Weeks 26 and 52, respectively. In the prednisone group, lung involvement was present in 2.4% (4/164) and 1.8% (3/164) patients, at Weeks 26 and 52, respectively. In the avacopan group, ENT involvement was present in 1.2% (2/166) patients at both Weeks 26 and 52. In the prednisone group, ENT involvement was present in 3.7% (6/164) and 3.0% (5/164) patients, at Weeks 26 and 52, respectively (Table).

Conclusions: In ADVOCATE, the avacopan group achieved 52-week superiority in disease remission and numerically lower (but not statistically significant) rates in both lung and ENT involvement at Weeks 26 and 52 compared to the prednisone group.

Table. Effect of Avacopan and Prednisone Across Lung and ENT Involvement (based on BVAS)

Organ involvement	Sub-organ involvement	Avacopan (n=166)			Prednisone (n=164)		
		Baseline n (%)	Week 26 n (%)	Week 52 n (%)	Baseline n (%)	Week 26 n (%)	Week 52 n (%)
Lung	**Chest Overall**	**71 (42.8)**	**1 (0.6)**	**0 (0.0)**	**72 (42.9)**	**4 (2.4)**	**3 (1.8)**
	Endobronchial Involvement	6 (3.6)	0 (0.0)	0 (0.0)	9 (5.5)	1 (0.6)	0 (0.0)
	Infiltrate	29 (17.5)	0 (0.0)	0 (0.0)	38 (23.2)	2 (1.2)	2 (1.2)
	Massive Hemoptysis/ Alveolar hemorrhage	5 (3.0)	0 (0.0)	0 (0.0)	7 (4.3)	0 (0.0)	0 (0.0)
	Nodules or Cavities	38 (22.9)	1 (0.6)	0 (0.0)	35 (21.3)	1 (0.6)	2 (1.2)
	Other	1 (0.6)	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
	Pleural Effusion/Pleurisy	10 (6.0)	0 (0.0)	0 (0.0)	7 (4.3)	2 (1.2)	1 (0.6)
	Respiratory Failure	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Wheeze	11 (6.6)	0 (0.0)	0 (0.0)	10 (6.1)	0 (0.0)	0 (0.0)
ENT	**ENT Overall**	**75 (45.2)**	**2 (1.2)**	**2 (1.2)**	**69 (42.1)**	**6 (3.7)**	**5 (3.0)**
	Blood Nasal Disc/Crust/ Ulcer/Granulomata	51 (30.7)	1 (0.6)	1 (0.6)	47 (28.7)	5 (3.0)	4 (2.4)
	Conductive Hearing Loss	20 (12.0)	0 (0.0)	1 (0.6)	23 (14.0)	0 (0.0)	0 (0.0)
	Other	2 (1.2)	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
	Paranasal Sinus Involvement	36 (21.7)	1 (0.6)	0 (0.0)	30 (18.3)	1 (0.6)	1 (0.6)
	Sensorineural Hearing Loss	10 (6.0)	0 (0.0)	0 (0.0)	9 (5.5)	0 (0.0)	1 (0.6)
	Subglottic Stenosis	1 (0.6)	0 (0.0)	0 (0.0)	6 (3.7)	0 (0.0)	0 (0.0)

LBP-12

ROLES OF CYTOSKELETON PROTEINS ARP2/3 AND PHACTR4 IN COMPLEMENT MEDIATED PHAGOCYTOSIS.

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Abstract: There are several human diseases, termed "actinopathies" which result from genetic aberrations to actin cytoskeleton proteins leading to impaired clinical immunodeficiencies. Of these, several actinopathies are linked to dysregulated branched actin networks which are polymerized by the seven subunit Arp2/3 complex. While the function of Arp2/3 in the structural actin cytoskeleton has been well characterized, the mechanisms underlying heightened inflammatory phenotypes and immune

dysfunction in Arp2/3 deficient macrophages remain less well understood. We hypothesize that dysregulated complement biology and the role of the Arp2/3 complex in complement mediated phagocytosis contribute to these clinical manifestations. Arp2/3 deficient macrophages display differential phagocytic ability, where they develop phagocytic cups through the FcR pathway and internalize IgG-opsonized beads while iC3b-opsonized beads fail to be taken up. Unpublished data from our lab uncovered a novel Arp2/3 interacting protein called Phactr4, a relatively understudied protein which binds protein phosphatase 1 (PP1) as well as monomeric actin.

Preliminary data in WT macrophages suggests Phactr4 plays the same contrasting role in these phagocytosis contexts, where it is localized to the phagocytic cup in solely complement mediated phagocytosis. We hypothesize that Phactr4 is recruited to the cell surface through a CR3 integrin dependent localization, and in turn recruits the Arp2/3 complex for the generation of branched actin protrusions to form the phagocytic cup. Further investigating this interaction between Arp2/3 and Phactr4 will reveal novel molecular mechanisms in iC3b phagocytosis relevant to innate immunity as well as bring insight to how dysfunction in the complement system plays a role in human actinopathies.

Reference 1: *Rotty JD, Brighton HE, Craig SL, Asokan SB, Cheng N, Ting JP, Bear JE. Required for Macrophage Integrin Functions but Is Dispensable for FcR Phagocytosis and In Vivo Motility. Dev Cell. 2017. 42(5):498-513*

LBP-13

A VERY LITTLE KEY TO A VERY HEAVY DOOR: PHAGE DISPLAY DERIVED NOVEL PEPTIDE BINDERS SHOW SIGNIFICANT BINDING AFFINITY TO FACTOR-H RELATED PROTEIN-3 (FHR-3)

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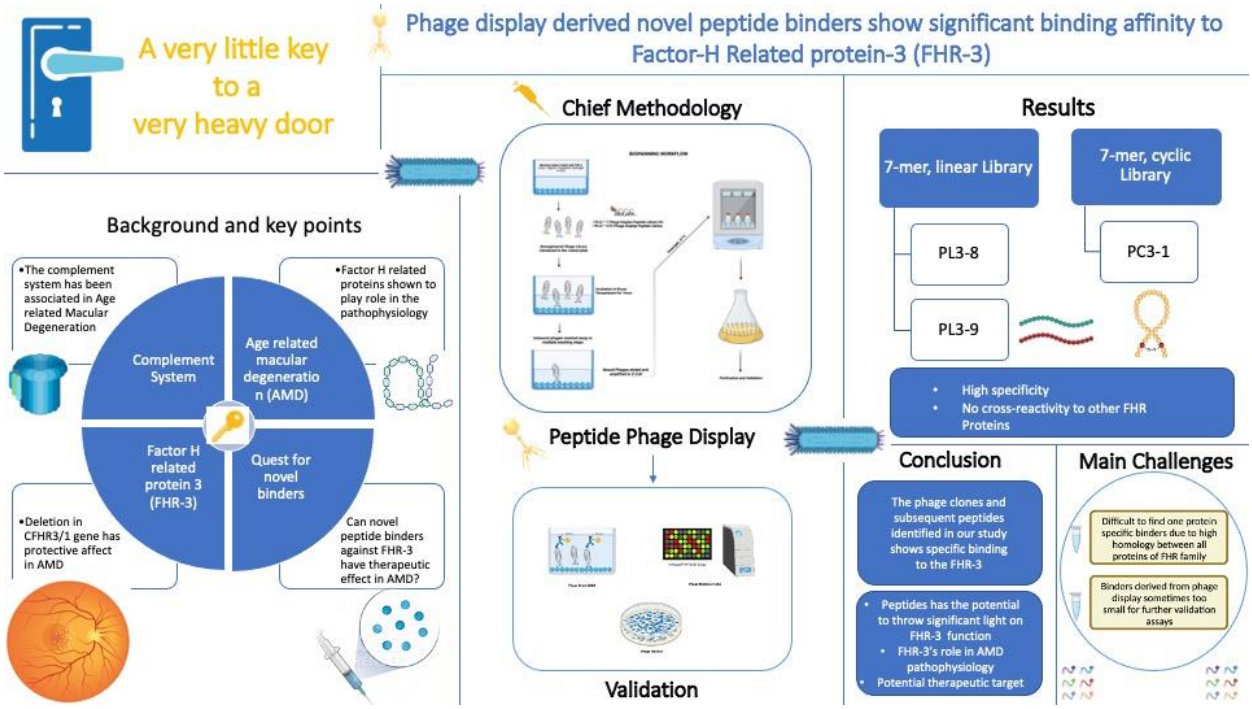
Background: Complement system dysregulation has long been implicated in age-related macular degeneration (AMD). The Factor H (FH) protein family have been recently described to be essential players in this dysregulation. Other than FH, the functions of the other highly homologous FH-related (FHR) proteins still remain elusive. Hence, to decipher their functions, the SciFiMed consortium (https://www.scifimed.eu/) was formed, with one of the primary goals to find novel peptide binders for each of the FHR proteins. Here, we report two novel FHR-3 binders that may prove to be instrumental in studying the function of FHR-3 and its role in the pathophysiology of AMD.

Methods: Ph.D.™-7 (linear) and Ph.D.™-C7C (cyclic) phage-displayed peptide libraries were used to screen for FHR-3 binders. After three rounds of panning, the phages were eluted, titrated and checked for binding specificity. An in-house developed, FHR protein multiplex assay was used to check the cross-reactivity and specificity of phage elutes and peptide binders. Sanger sequencing was performed from single, isolated colonies to obtain the binder sequences. These binders were custom-made and further validated by multiplexed ELISA. The binders were further used in C3b functional assays to study their role concerning FHR-3.

Results: Two FHR-3 positive phage clones from 7mer, linear library were found. They showed high specificity to FHR-3 and no cross-reactivity to the other FH-family proteins. One, amongst these binders, showed significant results on further specificity validations and C3b functional assays. The phage elutes from the cyclic peptide library also showed positive binding to FHR-3 and no cross-reactivity. The sequencing also yielded another peptide binder, which needs to be further validated.

Conclusions: The phage clones and subsequent peptides identified in our study show specific binding to the FHR-3. Hence has the potential to throw significant light on its function though further validation and more intensive peptide search is planned in later stages of the study.



LBP-14

IRON OXIDE NANOPARTICLE-INDUCED ENDOTHELIAL CELL ACTIVATION IS COUNTERACTED BY TARGETING COMPLEMENT C3 AND C5 IN A HUMAN WHOLE BLOOD AND ENDOTHELIAL CELL MODEL.

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Background: Nanoparticles (NPs) are particulate substances with at least one dimension smaller than 100 nm, which makes them attractive in various biomedical applications. Iron oxide nanoparticles (IONPs) are used clinically in diagnostics, magnetic resonance imaging, and drug delivery systems (1). However, like most artificial surfaces, IONPs can initiate an innate immune response when exposed to human whole blood, which may lower the clinical benefit and put the host at risk (2). Here, we combined human whole blood with microvascular endothelial cells to study the intravascular immune response from IONPs, focusing on the contribution from complement components C3, C5, and C5a receptor 1 (C5aR1).

Methods: Whole blood samples were collected from healthy volunteers (n=6) with lepirudin for anticoagulation and incubated for four hours with human lung microvascular endothelial cells (HLMVECs) with 100 µg/mL 10-30 nm IONPs and complement inhibitors; Cp40, eculizumab, and PMX53, targeting C3, C5, and C5aR1 respectively. The immune response from HLMVECs was analyzed by measuring ICAM-1 and E-selectin levels with flow cytometry. EDTA-treated plasma was isolated and analyzed for complement and cytokine markers by multiplex technology and ELISA.

Results: The addition of IONPs increased ICAM-1 ($p<0.0001$) and E-selectin ($p<0.05$) expression of HLMVECs by more than two-fold. Cp40 significantly inhibited both the ICAM-1 ($p<0.01$) and E-selectin ($p<0.05$) response and eculizumab significantly inhibited the E-selectin ($p<0.05$) response. No inhibition could be seen for PMX53. IONPs elevated the levels of C3bc ($p<0.05$) and TCC ($p<0.05$) and showed

higher levels of IL-1β, TNF, IL-6, and IL-8 ($p<0.05$). Cp40 inhibited the IL-8 and TNF responses ($p<0.01$), eculizumab inhibited the IL-6, IL-8, and TNF responses ($p<0.01$), and PMX53 inhibited the IL-8 response ($p<0.01$).

Conclusions: IONPs induced a strong immune response in the microvascular blood model. C3 inhibition reduced endothelial activation and cytokines. This inhibition was largely explained by inhibition of a subsequent C5 activation.

***Reference 1**: S. M. Dadfar et al., Iron oxide nanoparticles: Diagnostic, therapeutic and theranostic applications. Adv Drug Deliv Rev 138, 302-325 (2019).*

***Reference 2**: S. Wolf-Grosse, T. E. Mollnes, S. Ali, J. Stenvik, A. M. Nilsen, Iron oxide nanoparticles enhance Toll-like receptor-induced cytokines in a particle size- and actin-dependent manner in human blood. Nanomedicine (Lond) 13, 1773-1785 (2018).*

LBP-15

ACH50 TEST CAN HELP DETERMINE TREATMENT STRATEGY FOR DRUG-INDUCED TMA.

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Abstract: Atypical hemolytic uremic syndrome (aHUS) caused by abnormal complement regulation usually presents with thrombotic microangiopathy (TMA), and the anti-C5 antibody drugs have proven effective. Secondary TMAs are clasified as those caused by collagen disease, pregnancy, or drugs, but differential diagnosis from aHUS is difficult. In this study, we experienced two cases of severe TMA following gene therapy for spinal musclar atrophy. We report the complement titer ACH50 mesurement limited to the complement alternative pathway (AP), which may help determine the treatment strategy.

Neurogenic musclar atrophy, also known as spinal musclar atrophy (SMA), is caused by injuries to the spinal cord's anterior nucleus cells. SMA occurs through a mutation in the motor neuron survival (SMN) gene, which causes neuronal death. One of the most recently created medications for SMA, onasemnogen abeparvovec (OA), induces long-term expression of the SMN gene in nerve cells by incoporating the SMN gene into an adeno-associated virus serotype 9 vector.

Both one-year-old patients developed TMA one week after receiving OA. TMA was characterized by thrombocytopenia, raised LDH, and impaired renal function; both CH50 titers were below the measured sensitivity, while the ACH50 titer was only slightly reduced. Hematological recovery was attained in the first patient who received plasma exchange therapy (PE) for ten sessions. The second patient underwent fresh frozen plasma infusion rather than PE and fully recovered.

The clinical signs led to the first suspecion of aHUS in both patients who later acquired TMA. The results of the ACH50 titer indicated that there was no overactivation of AP. Anti-C5 antibody therapy was therefore expected to have only modest advantages. Although TMA after OA therapy is uncommon, some deathes have been documented in the past. Careful monitoring is required throughout OA treatment.

***Reference 1:** Chand DH, Zaidman C, Arya K, Millner R, Farrar MA, Mackie FE, et al. Thrombotic Microangiopathy Following Onasemnogene Abeparvovec for Spinal Muscular Atrophy: A Case Series. J Pediatrics. 2020;231:265–8.*

***Reference 2:** Yazaki K, Sakuma S, Hikita N, Fujimaru R, Hamazaki T. Child Neurology: Pathologically Confirmed Thrombotic Microangiopathy Caused by Onasemnogene Abeparvovec Treatment for SMA. Neurology. 2022;98(19):808–13.*

LBP-16

NEW ANTIBODIES TO MEASURE DIMERIZATION OF FACTOR H-RELATED PROTEIN 2

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Background: The main regulator of the alternative pathway (AP) of complement activation is factor H (FH). It is part of a family of seven circulating proteins which includes the five FH-related (FHR) proteins. The FHRs are hypothesized to be antagonists of FH thereby acting as de-regulators of the AP. Of the FHRs, FHR protein 2 (FHR-2) is the smallest and most elusive member. It mainly seems to circulate as heterodimer with the more abundant FHR protein 1 (FHR-1), with whom it also has a high overlap in sequence. FHR-2/2 homodimers are also present, but quantification is greatly hampered by the presence of FHR-1/2 heterodimers and lack of sufficiently sensitive and FHR-2 specifc reagents. Up until now, theoretical FHR-2/2 levels have been calculated based on FHR-1/1 and FHR-1/2 levels. This assumes unbiased formation and free exchange of hetero- and homodimers that results in a dimer equilibrium that solely depends on total FHR-1 and FHR-2 levels. With FHR-2 being associated with age-related macular degeneration, exact measurement of FHR-2/2 homodimers is now needed to confirm the inferred FHR-2 levels, the reported associations, and further elucidate the role of FHR-2 within the complement system.

Results: Here we describe newly generated mouse anti-human FHR-2 monoclonal antibodies (mAbs) obtained after immunisation with two relatively unique domains of FHR-2. Five promising mAbs were able to specifically bind both FHR-2 hetero- and homodimers in normal human serum. Furthermore, using different mAbs combinations both dimirisation forms and total levels of FHR-2 can be detected. Use of these novel mAbs permits assay development to further characterise and quantify FHR-2 homo- and heterodimers in health and disease.

LBP-17

EFFECT OF THE C5A RECEPTOR INHIBITOR AVACOPAN ON HEALTH-RELATED QUALITY OF LIFE IN ANCA-ASSOCIATED VASCULITIS.

An Pham[1], Vibeke Strand[2], Pirow Bekker[1], Huibin Yue[1], David R.W. Jayne[2], Peter Merkel[4]
[1]ChemoCentryx, [2]Stanford University, [3]University of Cambridge, [4]University of Pennsylvania

Background: Avacopan, an oral C5a receptor inhibitor, was evaluated in ANCA-associated vasculitis. Efficacy and safety results were reported previously. Health-related quality of life (HRQoL) improvements are reported here.

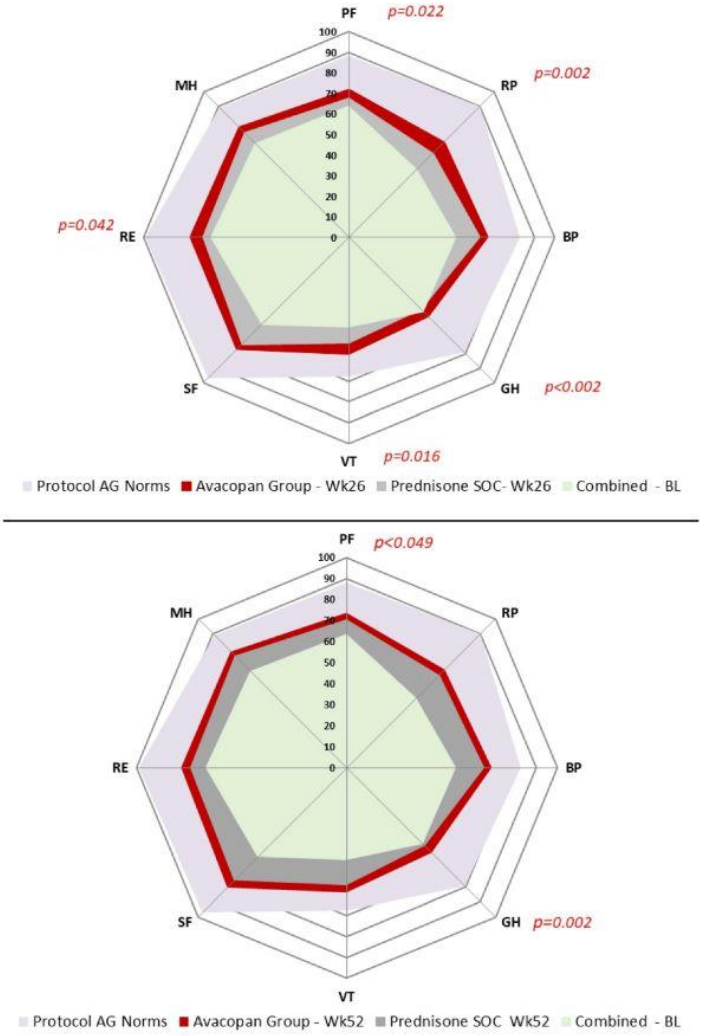
Methods: A 52-week blinded trial (ADVOCATE) randomized 331 patients with ANCA-associated vasculitis 1:1 to either daily oral prednisone with taper (Prednisone Standard of Care (SOC)) or avacopan without daily oral prednisone (Avacopan). Both received induction with rituximab or cyclophosphamide. HRQoL was assessed by Short Form-36 Health Survey version 2 (SF-36) and EuroQoL Group 5-Dimensions 5-Levels Questionnaire (EQ-5D-5L).

Results: Week 26 improvements in physical component summary (PCS) scores with Avacopan: 4.5 points (pts) vs Prednisone SOC: 1.3 pts (LSM for all analyses); statistically significant (p=0.002) and > minimum clinically important difference (MCID)=2.5 pts. Mental component summary scores: Avacopan: 4.9 pts; Prednisone SOC: 3.3 pts, >MCID in both groups. Week 26 improvements in physical function (PF), role physical (RP), general health (GH), vitality (VT) and role emotional (RE) domains with Avacopan were large (3.1 to 16.8 pts); statistically significant vs Prednisone SOC (p<0.002 to p=0.042), and >MCID of 5.0 pts in 4 domains **(Figure 1).**

Week 52 scores with Avacopan vs Prednisone SOC were maintained or improved: PCS scores were 5.0 vs 2.6 pts, clinically meaningful, and statistically significant (p=0.018). Improvements in PF and GH domains exceeded MCID and were statistically significant (p<0.049 and p=0.002). The health utility score, SF-6D, based on calculation across all 8 domains of SF-36, indicated broad improvements in patient-reported health status. Improvements at Weeks 26 and 52 were greater with Avacopan; > minimum important difference=0.041 and consistent with reported improvements in EQ-5D-5L utility score at week 52 (p=0.009).

Conclusions: There were significant improvements in HRQoL with Avacopan with less GC use vs Prednisone SOC. These findings have important clinical implications for treatment of patients with ANCA-associated vasculitis.

Figure 1. Spydergrams of SF-36 Domains vs Age and Gender (AG) Matched Norms – Baseline (BL) to Week 26 (top) and Week 52 (bottom)



PF: Physical Function; RP: Role Physical; BP: Bodily Pain; GH: General Health; VT: Vitality; SF: Social Function; RE: Role Emotional; MH: Mental Health

LBP-18

EFFECT OF AVACOPAN ON RELAPSE RATES AND RELAPSE-FREE TIME IN PATIENTS WITH ANCA-ASSOCIATED VASCULITIS, RESULTS OF THE PHASE 3 ADVOCATE STUDY.

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Background: For patients with ANCA-associated vasculitis, failure to achieve remission and subsequent relapse are associated with worse long-term outcomes. The phase 3 ADVOCATE study tested avacopan, a C5a receptor inhibitor, as a substitute for a standard oral glucocorticoid prednisone taper regimen.1 To further characterize efficacy, post-hoc analyses compared avacopan and prednisone groups regarding failure to achieve remission and subsequent relapse.

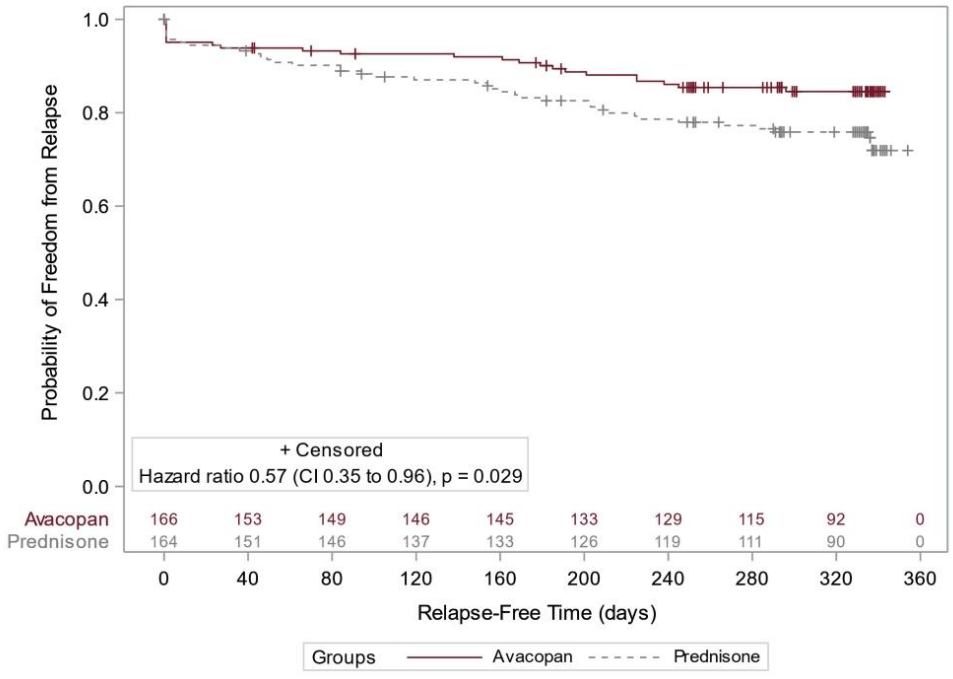
Methods: Remission was the first timepoint when a BVAS=0 was achieved. Relapse was worsening of disease after having previously achieved remission: 1+ major or 3+ minor items, or one or two minor items in the BVAS at two consecutive study visits. Two post-hoc sensitivity analyses examined: i) the proportion of patients who failed to achieve remission, or achieved remission and subsequently relapsed, and ii) relapse-free time. For patients who achieved remission, relapse-free time was the number of days from the initial remission to relapse. For patients who failed to achieve remission, relapse-free time was one day. Patients who did not relapse were censored at the last BVAS assessment.

Results: Time to achieve initial remission and the incidence of BVAS=0 at any point in the study were similar in both groups (158 avacopan vs. 157 prednisone). In the first sensitivity analysis, the avacopan group showed a reduced proportion of patients who relapsed after remission (including those who did not achieve remission), compared with the prednisone group (24 [14.5%] vs 40 [24.4%], p=0.011). In the second sensitivity analysis including all patients in the trial, the hazard ratio of relapse-free time for avacopan vs prednisone was 0.57, 95% CI (0.35, 0.96), p=0.029 (log-rank test) **(Figure 1).**

Conclusions: With avacopan therapy, more patients achieved remission, and fewer relapsed after achieving remission, compared to the prednisone group. Patients receiving avacopan had a longer relapse-free time compared to the prednisone group.

Reference: *Jayne D et al., Avacopan for the* Treatment *of ANCA-Associated Vasculitis. N Engl J Med. 2021. 384(7):599-609.*

Figure 1. Relapse-free time among patients with ANCA-associated vasculitis in ADVOCATE (intention-to-treat population)



LBP-19

C5A AND C5AR ARE PROMINENTLY ASSOCIATED WITH TUNNELS IN SEVERE HIDRADENITIS SUPPURATIVA.

Kathleen Sullivan[1], Chris Li[1], Carolyn Dunlap[1], Niky Zhao[1], James Kilgour[2], Aatman Shah[2], Karen Ebsworth[1], Israel Charo[1], Kavita Sarin[2], Thomas Schall[1]
[1]ChemoCentryx, [2]Stanford University

Background: Complement dysregulation and neutrophil activation have been implicated in the pathogenesis of hidradenitis suppurativa (HS). The presence of tunnels (subcutaneous cavities lined with squamous epithelium) is a feature of the moderate and severe forms of HS. The complement 5a (C5a) receptor (C5aR) is highly expressed on neutrophils and is a major pro-inflammatory mediator. Avacopan is a potent, specific inhibitor of human C5aR that was shown to be efficacious and well tolerated in patients with severe HS in a recent phase 2 clinical trial.

Results: Consistent with other reports, we found complement activation in HS skin lesions. In addition, we also observed elevated C5aR and C5a levels in severe HS skin lesions compared to mild/moderate HS lesions. C5aR-positive cells and C5a-positive cells are increased (>2 fold by semi-quantitative measurements) within and surrounding tunnel epithelium in severe HS lesions. The C5a-positive cells are more likely to be associated with tunnels that exhibit pseudo-psoriaform hyperplasia and have higher numbers of infiltrating cells. In the periphery, C5aR levels are not altered on circulating blood cells in HS patients and circulating C5/C5a levels are not associated with disease severity, suggesting that C5a within the skin lesions recruits C5aR-positive cells to the tunnels in severe HS. Furthermore, we observed that C5aR activation protects neutrophils from apoptosis, and that inhibition of C5aR with avacopan restores normal apoptosis.

Conclusions: These findings suggest that locally generated C5a/C5aR signaling, by promoting leukocyte recruitment, survival, and activation, is a major driver of tunnel development and disease progression in severe HS. The data provide a potential mechanistic basis for the clinical improvement seen with avacopan treatment in patients with severe HS.

LBP-20

COMPLEMENT C5A RECEPTOR IN MACROPHAGE-MEDIATED RENAL INFLAMMATION AND FIBROSIS IN LUPUS NEPHRITIS

Kathleen Sullivan[1], Chris Li[1], Carolyn Dunlap[1], Niky Zhao[1], Linda Ertl[1], Zhenhua Miao[1], Israel Charo[1], Thomas Schall[1]
[1]ChemoCentryx

Background: Lupus nephritis (LN) is caused by autoimmune responses in systemic lupus erythematosus, and end-stage renal disease is a major cause of mortality in patients. Complement activation, pro-inflammatory cytokine production, and the influx of macrophages have all been implicated in LN pathogenesis. The anaphylatoxin complement 5a (C5a) receptor 1 (C5aR) is a major pro-inflammatory mediator of complement activation. We examined the expression of C5aR in lupus nephritis kidney tissue and investigated the role of C5aR activation in controlling pro-fibrotic functions of macrophages.

Methods: C5aR expression, infiltrating immune cells, and fibrosis were examined by immunohistochemistry in LN patient kidney tissues. M2 macrophages derived from human peripheral blood monocytes were used in in vitro assays to examine the effect of C5a stimulation and avacopan, a specific C5aR inhibitor, on the secretion of cytokines and other factors.

Results: In LN kidney tissues, C5aR was expressed on macrophages, identified by CD68 staining, within areas of severe fibrosis. C5aR was also detected on distal tubules, but this expression was observed in both normal and lupus nephritis kidneys. In vitro, chemokines (MCP-3, MIP-1α, MIP-1β and MIP-3α), matrix metalloproteinases (MMP3 and MMP8), and pro-fibrotic growth factors (fibroblast activation protein, platelet-derived growth factor-AA) were strongly increased in M2 macrophages by C5a stimulation and blocked by addition of the C5aR inhibitor avacopan.

Conclusions: C5aR activation induced macrophage secretion of factors that are known to drive inflammation, fibroblast activation and tissue fibrosis; these pathological changes were blocked by avacopan. C5aR is expressed on macrophages in fibrotic regions of LN kidneys, where they may contribute to disease progression, suggesting that avacopan may provide therapeutic benefit to LN patients.

LBP-21

EFFECT OF AVACOPAN, A C5A RECEPTOR INHIBITOR, ON KIDNEY FUNCTION IN PATIENTS WITH ANCA-ASSOCIATED VASCULITIS

Henry Chen[1], David R.W. Jayne[2], Peter Merkel[3], Pirow Bekker[1], Annette Bruchfeld[4], Duvuru Geetha[5], Alexandre Karras[6], John Niles[7]

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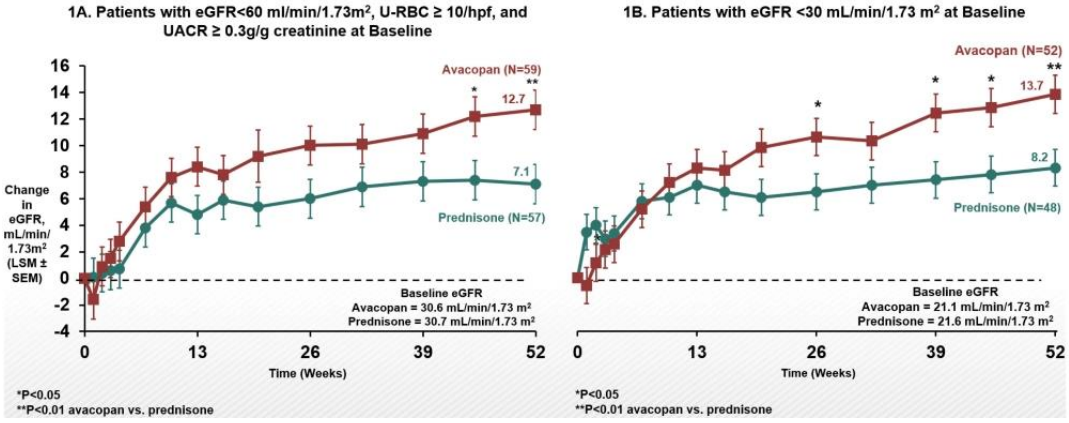
Background: Patients with anti-neutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis and renal disease have a poor prognosis. The effect of avacopan, an oral C5a receptor inhibitor, on renal function is described.

Methods: The ADVOCATE Phase 3 trial randomized patients to receive prednisone or avacopan (as a substitute for a prednisone taper). All patients in the trial also had background therapy of either cyclophosphamide (followed by azathioprine) or rituximab. Primary endpoints were remission at Week 26 and sustained remission at Week 52. Secondary objectives included evaluation of kidney function.

Results: At Week 52, the difference in estimated glomerular filtration rate (eGFR) recovery in patients with eGFR <60 mL/min/1.73 m2 and/or urinary abnormalities at baseline between avacopan and prednisone treatment groups was 5.5 mL/min/1.73 m^2 (95% CI [1.4, 9.6], p<0.01) (Figure 1A). Improvement in eGFR with avacopan was most prominent in patients with eGFR <30 mL/min/1.73 m^2 at baseline, when by week 52, the least squares mean (LSM) increase in eGFR was 13.7 (avacopan group) vs. 8.2 mL/min/1.73 m2 (prednisone group) (p<0.01) (Figure 1B). Avacopan was also associated with more rapid reduction in proteinuria and hematuria. Urinary albumin:creatinine ratio improved 40% within the first 4 weeks of treatment with avacopan vs. no change in the prednisone group (difference -40, 95% CI [-53, -22]).

Conclusions: In ADVOCATE, patients with ANCA-associated vasculitis in the avacopan group had greater recovery of kidney function compared to patients in the prednisone group, especially among patients with eGFR <30 mL/min/1.73 m^2, and <60 mL/min/1.73 m^2 and/or urinary abnormalities at baseline.

Figure 1. Change in Renal Function Through Week 52



LBP-22

C3A-MEDIATED ENDOTHELIAL BARRIER DISRUPTION IS ATTENUATED BY C5A IN NEUROMYELITIS OPTICA.

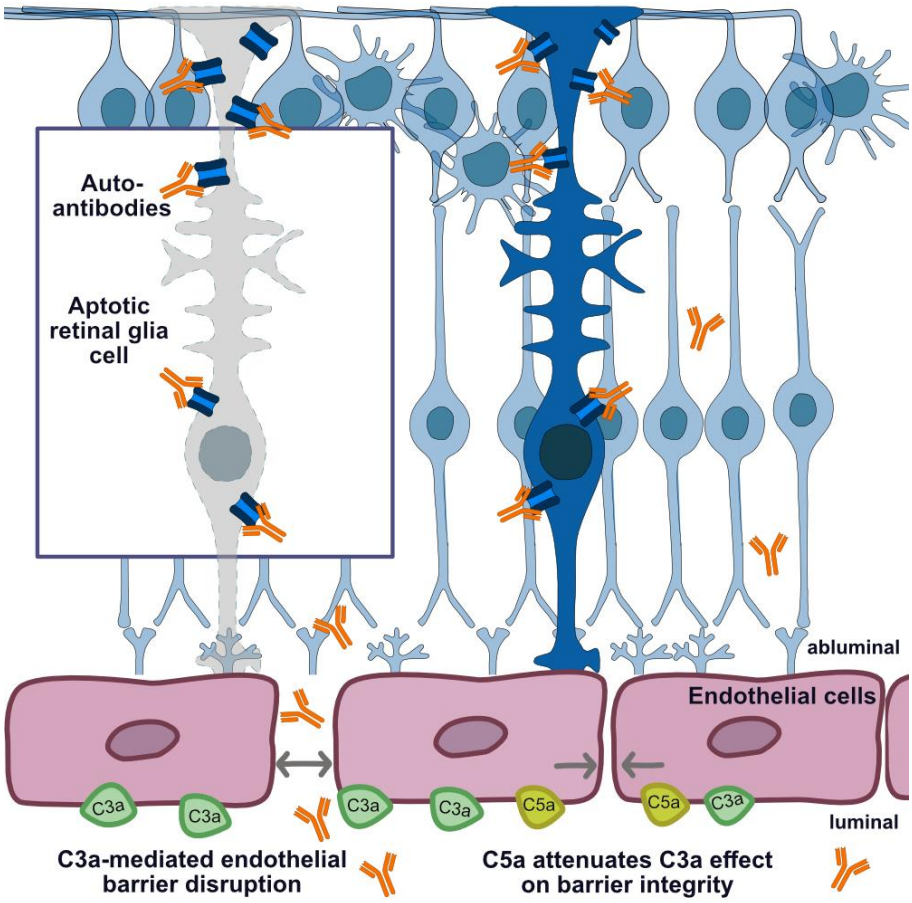
Hannah Wolf[1], Larissa Gümpelein[1], Joachim Havla[2], Diana Pauly[1]
[1]Department of Ophthalmology, University Marburg, Marburg, Germany, [2]Institute of Clinical Neuroimmunology, Ludwig-Maximilians-University Hospital, Munich, Germany

Background: Neuromyelitis optica spectrum disorders (NMOSD) is an autoantibody-mediated autoimmune disease affecting retina, optic nerve and spinal cord. The mechanism of autoantibody transmigration through blood-brain- and inner-blood-retinal-barrier is still under discussion. Here, we investigate the influence of anaphylatoxins C3a and C5a on barrier integrity of human brain microvascular endothelial cells (HBMEC).

Methods: Soluble complement proteins in culture supernatant and plasma samples were determined using ELISA. HBMEC cells were treated with H_2O_2 as well as recombinant human C3a and C5a. Expression of VE-Cadherin, CD31, C3, C3aR and C5aR was evaluated using immunocytochemistry. Impedance-based real-time cell analysis was performed over 3 days to measure cellular barrier function.

Results: Analysis of NMOSD patient plasma revealed increased systemic C3a and C5a levels compared to controls. Cultivated primary endothelial cells, HBMEC, exhibited (i) endothelial markers – VE-Cadherin, CD31 and (ii) complement proteins C3 as well as barrier-function relevant C3aR and C5aR shown on mRNA and protein level. HBMEC treated with H_2O_2 showed a concentration-dependent decrease of barrier function and an altered complement secretion profile. Treatment with recombinant C3a exacerbated a disruptive effect on barrier integrity, too. Additive C5a treatment rescued the C3a-mediated effect on barrier integrity.

Conclusions: Our data showed a local effect of increased C3a levels on endothelial cells, which was counteracted by C5a. These initial steps may shed light on the unexplained retinal thinning in NMOSD without optic neuritis and a possible mechanism on autoantibody transmigration.



LBP-23

COLEC11 SPLICE VARIANTS FOUND IN THE CIRCULATION ARE FUNCTIONALLY DISTINCT IN THEIR INTERACTION WITH CL-10 AND THE MASP-2.

Adrian Sutta, Nelia Nina Leemans, Anne Rosbjerg, Christian del Agua Villa, Adam S. Vincek, Tomás de Garay, Laura Pérez-Alós, Keith Rivera, Rafael Bayarri-Olmos

Abstract: Collectin-11 (CL-11), also known as collectin kidney 1- (CL-K1), is a pattern recognition molecule of the lectin pathway capable of interacting with collectin-10 (CL-10) and the MASPs and activating the complement cascade. Alternative splicing of the *COLEC11* gene gives rise to two different collectin isoforms present in serum (A and D). These isoforms vary in the length of their collagen-like region, involved in the stabilization of the trimeric subunit and the interaction with the MASPs. Here we aim at elucidating the biological differences of naturally occurring CL-11 isoforms A and D. We produced recombinant CL-11 (rCL-11) as independent isoforms (rCL-11A and rCL-11D) and together with rCL-10 (rCL-10/11A, rCL-10/11D). We analyzed oligomerization by size exclusion chromatography (SEC) and mass photometry; MASPs association by ELISA; and CL-10/11 heterocomplex formation by mass spectrometry, collagenase digestion, and nanoDSF. Although rCL-11 isoforms associated with rCL-10, rCL-11D does so to a lesser extent. We found that rCL-11A and rCL-11D are comparable in their ability to bind *MASP1* gene products (MASP-1, MASP-3, MAP-1).

However, CL-11D had a lower affinity towards MASP2 as determined by the ELISA. Immuno-precipitation of serum CL-11 and subsequent mass spectrometry analysis indicate that native CL-11 circulates in the form of CL-10/11 heterocomplexes that associate with MASP-1 and MASP-3, but not necessarily MASP-2. Collagenase digestion of rCL-10/11 heterocomplexes revealed that the trimeric subunit is composed of a mix of CL-10 and CL-11, as opposed to CL-10 and CL-11 trimers. Overall, our results suggest that naturally-occurring CL-11 isoforms may have different biological functions based on the difference in their association with MASP-2 and CL-10.

LBP-24

MONOZYGOTIC TWINS WITH A FACTOR B MUTATION AND DISCORDANT PRESENTATION OF ATYPICAL HEMOLYTIC UREMIC SYNDROME.

Sigridur Sunna Aradottir[1], Ann-Charlotte Kristoffersson[1], Brynjar Jensson[2], Patrick Sulem[2], Henning Gong[1], Runolfur Palsson[3,4], Diana Karpman[1]

[1]Department of Pediatrics, Clinical Sciences Lund, Lund University, Lund, Sweden, [2]deCODE genetics, Reykjavik, Iceland, [3]Landspitali - The National University Hospital of Iceland, Reykjavik, Iceland, [4]University of Iceland, Reykjavik, Iceland

Abstract: Factor B mutations have been reported in patients with atypical hemolytic uremic syndrome (aHUS). We have previously described the heterozygous gain-of-function factor B mutation D371G in aHUS leading to a hyperfunctional C3 convertase.[1] This study describes the investigation of this mutation in a large Icelandic family, in which three members developed aHUS. Four mutation carriers were unaffected and an additional 203 family members were found to be non-carriers. The mutant variant was not found in 45000 Icelandic donors. The mutation origin could thereby be traced to an ancestor most probably born in the late 1800s. No other complement mutations were found. One of the affected family members is a monozygotic twin. The affected twin presented with aHUS at 41 years of age while his twin brother remains unaffected for decades. Serum was available from 5 mutation carriers, including the twins, that exhibited normal levels of factor B but low levels of C3, indicating complement activation. Serum from the monozygotic twins was taken at three timepoints and exhibited markedly elevated levels of C3a, Ba, C5a and C5b-9 compared to normal sera.

There were no major differences between the twins except for serum Ba in the affected twin obtained after an acute infection. Serum from the affected twin induced more hemolysis of sheep erythrocytes and led to increased release of Ba in the supernatant of glomerular endothelial cells compared to serum from the unaffected twin ($P<0.0001$). This difference was, however, abolished when serum Ba levels were subtracted. Thus, the monozygotic twins with a factor B mutation exhibit discordance for the aHUS phenotype which may be associated with pre- and post-natal exposures, lifestyle[2] and/or epigenetic mechanisms that could contribute to complement activation.

Reference 1: *Aradottir SS, Kristoffersson AC, Roumenina LT, Bjerre A, Kashioulis P, Palsson R, Karpman D. Factor D Inhibition Blocks Complement Activation Induced by Mutant Factor B Associated With Atypical Hemolytic Uremic Syndrome and Membranoproliferative Glomerulonephritis. Front Immunol. 2021; 12: 690821.*

Reference 2: *Kaye S, Lokki AI, Hanttu A, Nissilä E, Heinonen S, Hakkarainen A, Lundbom J, Lundbom N, Saarinen L, Tynninen O, Muniandy M, Rissanen A, Kaprio J, Meri S, Pietiläinen KH. Upregulation of Early and Downregulation of Terminal Pathway Complement Genes in Subcutaneous Adipose Tissue and Adipocytes in Acquired Obesity. Front Immunol. 2017; 8: 545.*

LBP-26

PREDICTION OF RESPIRATORY FAILURE AND MORTALITY IN COVID-19 PATIENTS USING LONG PENTRAXIN PTX3.

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[1]Laboratory of Molecular Medicine, Department of Clinical Immunology, Section 7631, Rigshospitalet University Hospital, Copenhagen, Denmark, [2]Department of Infectious Diseases, Copenhagen University Hospital – Amager and Hvidovre, Hvidovre, Denmark, [3]Laboratory of Molecular Medicine, Department of Clinical Immunology, Section 7631, Rigshospitalet University Hospital, Copenhagen, Denmark, [4]Department of Clinical Biochemistry, Holbæk Hospital, Holbæk, Denmark

Background: The course of COVID-19 is unpredictable, ranging from asymptomatic to respiratory failure and death. Prognostic biomarkers are urgently needed. We hypothesized that long pentraxin PTX3 could be a valuable plasma biomarker due to its essential role in inflammatory processes.

Methods: : In a prospective hospitalized COVID-19 derivation cohort (n = 126) during the spring of 2020, we measured PTX3 within 4 days of admission. The predictive value of mechanical ventilation (MV) and 30-day mortality compared with clinical parameters and other markers of inflammation were assessed by logistic regression analysis and expressed as odds ratio (OR) with 95% confidence interval (CI). Analyses were repeated in a prospective validation cohort (n = 112) of hospitalized patients with COVID-19 treated with remdesivir and dexamethasone.

Results: Thirty-day mortality was 26.2% in the derivation cohort and 13.4% in the validation cohort. In patients who died, the median PTX3 concentration upon admission was 19.5 ng/mL (IQR: 12.5-33.3) versus 6.6 ng/mL (IQR 2.9-12.3) ($p < 0.0001$) in survivors in the derivation cohort. After adjustment for covariates, the odds of 30-day mortality increased two-fold for each doubling of PTX3 (OR 2.03 [95% CI: 1.23-3.34], $p = 0.006$), which was also observed in the validation cohort (OR 1.70 [95% CI: 1.09-2.67], $p = 0.02$). Similarly, PTX3 levels were associated with MV. After adjustment for covariates, OR of MV was 2.34 (95% CI: 1.33-4.12, $p = 0.003$) in the derivation cohort and 1.64 (95% CI: 1.03-2.62, $p = 0.04$) in the validation cohort.

Conclusions: PTX3 appears to be a useful clinical biomarker to predict 30-day respiratory failure and mortality risk in COVID-19 patients treated with and without remdesivir and dexamethasone.

LBP-27

DOUBLE-BLIND, PLACEBO-CONTROLLED RANDOMIZED PHASE 3 STUDY IN CRITICALLY ILL MECHANICALLY VENTILATED COVID-19 PATIENTS INVESTIGATING VILOBELIMAB'S EFFECT, AN ANTI-C5A MAB, ON 28-DAY ALL-CAUSE MORTALITY.

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Abstract: The complement anaphylatoxin, C5a, is elevated in severe COVID-19 patients.[1] Blocking the C5a-C5aR axis in these patients could improve outcomes by limiting myeloid cell infiltration in damaged organs and preventing excessive lung inflammation and endothelialitis. Vilobelimab, an anti-C5a monoclonal antibody that preserves the membrane attack complex (MAC),[2] was investigated in a randomized Phase 3 adaptively designed multicenter, double-blind, placebo-controlled study for survival in critically ill COVID-19 patients. COVID-19 pneumonia patients (N=368; Vilobelimab n=177, Placebo n=191) intubated within 48 hrs before first treatment received up to six, 800 mg infusions of Vilobelimab or Placebo on top of standard of care. Median (SD) C5a levels (European Region) were equivalent in both groups at baseline [Vilobelimab 130.25 (71.45) ng/mL, Placebo 125.15 (65.53) ng/mL] but by Day 8, C5a levels for Vilobelimab were significantly decreased [16.80 (9.15)) vs Placebo (129.81 (67.59)]. The primary outcome, 28-day all-cause mortality, was 31.7% Vilobelimab vs 41.6% Placebo (Kaplan-Meier estimates; Cox regression site stratified, HR 0.73; 95%CI:0.50-1.06; P=0.094) with a 23.8% relative mortality reduction with Vilobelimab. In predefined primary outcome analysis without site stratification, however, Vilobelimab significantly reduced 28-day mortality (HR 0.67; 95%CI:0.48-0.96; P=0.027) with a needed to treat number of 10 patients to save 1. Vilobelimab also significantly reduced 28-day mortality in more severe patients with baseline WHO ordinal scale score of 7 (n=237, HR 0.62; 95%CI:0.40-0.95; P=0.028) or severe ARDS/$PaO_2/FiO_2 \leq 100$ mmHg (n=98, HR 0.55; 95%CI:0.30-0.98; P=0.044) or eGFR<60 mL/min/1.73m^2 (n=108, HR 0.55; 95%CI:0.31-0.96; P=0.036). Treatment-emergent AEs were 90.9% Vilobelimab vs 91.0% Placebo.

Infections were comparable: Vilobelimab 62.9%, Placebo 59.3%. Serious AEs were 58.9% Vilobelimab, 63.5% Placebo. Vilobelimab significantly reduced mortality at 28 days in critically ill COVID-19 patients with no increase in infections suggesting the importance of targeting C5a while preserving MAC. Therefore, the efficacy and comparative safety is attributed to clearance of C5a by Vilobelimab.

Reference 1: *Carvelli J, Demaria O, Vély F, Batista L, Chouaki Benmansour N, Fares J, et al. Association of COVID-19 inflammation with activation of the C5a-C5aR1 axis. Nature. 2020;588(7836):146-150.*

Reference 2: *Vlaar APJ, Lim EHT, de Bruin S, Rückinger S, Pilz K, Brouwer MC, et al. The anti-C5a antibody vilobelimab efficiently inhibits C5a in patients with severe COVID-19. Clin Transl Sci. 2022;15(4):854-858.*

LBP-29

SC5B-9 AND BB FACTOR LEVELS AS POTENTIAL NOVEL BIOMARKERS IN CRESCENTIC IGA NEPHROPATHY.

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Background: IgA nephropathy (IgAN) is the most common primary glomerulonephritis worldwide and the first cause of end-stage kidney disease among primary glomerulonephritis. Local and systemic evidence of complement activation (CA) are prognostic markers of severe IgAN. Both the alternative and lectin pathway are responsible for CA in IgAN, they converge into the generation of C5 convertase, which forms the membrane attack complex (MAC), also called C5b-9. The C5b-9 complex perforates glomerular basement membranes, which may lead to crescent formation. A soluble form of C5b-9 complex can be detected in human plasma (SC5b-9). We hypothesize that SC5b-9 may be a novel biomarker in crescentic IgAN.

Aim of the study: To measure SC5b-9, and complement fragment Bb, a serum marker of complement alternative pathway activation, in patients with IgAN with crescents compared to patients with IgAN without crescents.

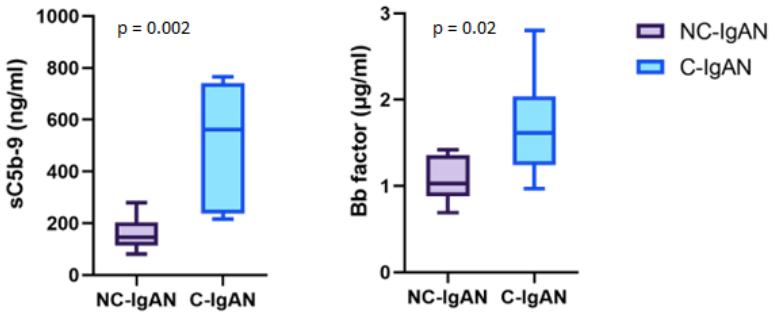
Results: Twenty-seven patients, with IgAN confirmed by kidney biopsy (KB), underwent complement analysis during three years in our institution. Seventeen patients were included in the study and gave informed consent to participate, of whom 6 patients had active crescents at the moment of KB. Creatinine, 24 hours proteinuria, albuminuria and systolic blood pressure were higher in the group with crescents. SC5b9 and Bb factor levels were markedly higher in the crescentic group (514 $\pm$ 236 vs 163 $\pm$ 61 ng/ml and 1.7 $\pm$ 0.6 vs 1.1 $\pm$ 0.3 µg/ml, p of 0.002 and 0.02, Figure 1). A serum C3 splitting activity was found giving rise to C3c in 4 patients with crescents.

Conclusion: In this pilot study, only patients with severe and crescentic forms of IgAN showed a strong CA (SC5b9), which appeared to be mediated also by the alternative pathway of complement (Bb factor). Thus, SC5b9 and factor Bb may represent novel plasma biomarkers, predictive of crescentic IgAN. A large multicentre trial is needed to confirm our preliminary findings.

Reference 1: *cite Itami H, Hara S, Samejima K, Tsushima H, Morimoto K, Okamoto K, Kosugi T, Kawano T, Fujiki K, Kitada H, Hatakeyama K, Tsuruya K, Ohbayashi C. Complement activation is associated with crescent formation in IgA nephropathy. Virchows Arch. 2020 Oct;477(4):565-572. doi: 10.1007/s00428-020-02800-0.*

Reference 2*: Mollnes TE. Early- and late-phase activation of complement evaluated by plasma levels of C3d,g and the terminal complement complex. Complement. 1985;2(2-3):156-64. doi: 10.1159/000467856.*

Figure 1. SC5b-9 and Bb factor levels compared between the non-crescentic (NC-IgAN) and the crescentic IgAN (C-IgAN) group



LBP-30

EXPLORING THE TARGET SELECTIVITY OF A LEECH-DERIVED SMALL PROTEIN TOWARDS COMPLEMENT SERINE PROTEASES USING COMPUTATIONAL METHODS.

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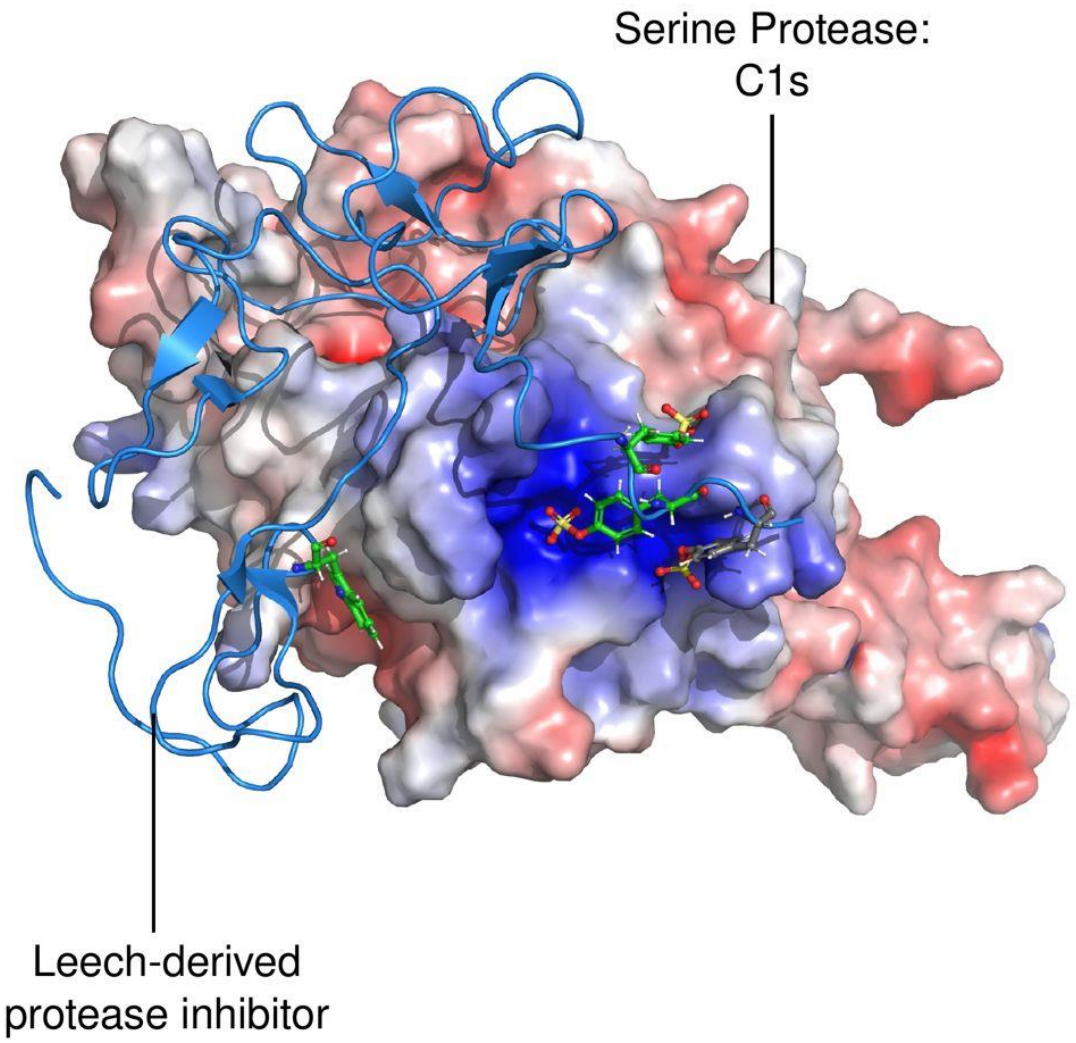
Abstract: Owing to the early-stage role of the classical and lectin pathways of complement activation in host defense, disease and therapy, knowledge about the function and modulation of associated serine proteases is of great medical importance. The high structural similarity of those proteases and the limited availability of natural or synthetic modulators with narrow and broad selectivity has rendered such efforts challenging.

In recent years, a small protein derived from the saliva of the giant Amazon leech was shown to inhibit both C1s and MASP-2, thereby simultaneously modulating the classical and lectin pathway. Starting from a published crystal structure, and employing advanced computational methods, we investigated the protease-modulator interactions to identify molecular determinants of target selectivity and affinity. In silico mutagenesis of the leech protein, followed by molecular dynamics simulations and free energy calculations, revealed key contacts between the inhibitor and its distinct targets. The impact of the identified residues on target binding could be validated by expressing mutated inhibitors and evaluating them using in vitro assays.

Several of the tested leech protein derivatives indeed showed the predicted effect on the target binding affinity and inhibitory activity for the classical and/or lectin pathway. This insight produced structural hypotheses on how the scaffold protein can be mutated to affect its selectivity profile for one or another structurally similar protease. Two exosites of the inhibitor seem to be pivotal for the target selectivity as they engage in intermolecular interactions with unique amino acids of the respective proteases without disturbing the core binding site.

The combination of computational methods and experimental validation provided novel insight into the ligand-binding profiles of complement-associated serine proteases and indicated that target selectivity properties of modulators may be tuned using protein engineering approaches.



LBP-31

A METHODICAL INVESTIGATION OF THE CROSSTALK BETWEEN THE LECTIN PATHWAY AND THE CONTACT/KALLIKREIN SYSTEM.

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Objectives: Unwarranted activation of thromboinflammation is prevalent in pathological conditions such as stroke, myocardial infarction and ischemia reperfusion. Modes of activation canonically involve the direct or indirect activation of the contact system (CS) or complement systems respectively. The ultimate result of thromboinflammation is a fibrin clot which is formed via the cleaving and crosslinking of fibrinogen by thrombin and FXa. Studies have suggested lectin pathway (LP) proteases MASP-1 and MASP-2 can replicate the activities of the aforementioned proteases. The aim is to expand the exploration of the interactions of complement and coagulation.

Methods: A multi technique approach was used to investigate the cross talk of the complement and CS. Thromboelastography (TEG) was used to see the effect of fibrin on the dynamics of clot formation while protease activity/cleavage was measured by the use of chromogenic assays and capillary electrophoresis (WES). Fibrin clots were also stained to detect the LP and CS constituents.

Results: Wes-capillary analysis showed FXII cleavage by LP proteases. Inhibitors of the LP were able to inhibit FXII cleavage in the presence of LP proteases and fibrin. LP and CS proteases were detected in plasma clots indicating that fibrin can be a scaffold to provide crosstalk between the two pathways. LP and CS proteases have substrate similarities indicated in a chromogenic assay where the systems may enhance each other's function. TEG analysis R-values were prolonged when LP inhibitors were used with fibrin activation.

Conclusions: The findings show an influence of the LP on the CS regarding thromboinflammation. Establishing a connection between the LP and CS via MASP-1 and 2 by activating FXII is a potential mode for upstream dampening of the intrinsic pathway of coagulation.

Reference 1: *The lectin complement pathway serine proteases (MASPs) represent a possible crossroad between the coagulation and complement systems in thromboinflammation. J Thromb Haemost. 2016 Mar;14(3):531-45. doi: 10.1111/jth.13208. Epub 2016 Feb 15. PMID: 26614707.*

LBP-32

DYSREGULATION OF THE COMPLEMENT ALTERNATIVE PATHWAY EXACERBATES RENAL DAMAGE IN AN EXPERIMENTAL MODEL OF MPO-ASSOCIATED VASCULITIS.

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Abstract: Complement alternative pathway (AP) activation has been involved in the pathogenesis of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), although the underlying molecular mechanism is still unclear. Our previous genetic studies in a Spanish AAV cohort with predominant ANCAs against myeloperoxidase (MPO) associated the *CfH*-H6 haplotype with risk to develop AAV *(OR* 3.33, 95% CI: 1.39-7.90) and the *CfB32Q/W* with protection (OR 0.585, 95% CI, 0.394-0.867). To gain insight into the role *CfH* and *CfB in vivo* we have developed an established experimental

autoimmune anti-MPO AAV model in mice with AP complement dysregulation: mice with a partial deficiency of FH (*CfH*$^{+/-}$) and mice with a heterozygous gain-of-function mutation in *CfB* (*CfB*$_{D279G}$$^{+/-}$).

These mice are characterized by C3 consumption in serum. To do so, wild-type, *CfH*$^{+/-}$ and *CfB*$_{D279G}$$^{+/-}$ mice were injected with human MPO (hMPO) (or BSA as a control) and nephrotoxic serum (NTS) as previously described by Ruth, AJ et al., 2006. Subsequently, sytemic immune response to MPO, complement studies and renal injury were assesed in these mice. As expected, all the mice injected with hMPO developed ANCAs against hMPO. No statistically significant differences were observed in plasma C3 levels between mice injected with MPO or BSA. Immunofluorescence studies on renal tissue demonstrated glomerular deposition of sheep IgG in all mice, consistent with the NTS injection. Additionally, an increase in C3 and mouse IgG glomerular deposition was observed in MPO-immunized mice compared with BSA controls. Renal histology studies evidenced the formation of glomerular crescents, the histological hallmark of AAV, in all mice, although MPO-immunized mice presented a higher degree compared with controls. Interestingly, *CfH*$^{+/-}$ and *CfB*$_{D279G}$$^{+/-}$ mice show higher percentage of glomerular crescents compared with the wild-type, and glomerular necrosis was higher in *CfH*$^{+/-}$. In conclusion, these data suggest that AP dysregulation could both predispose and exacerbate the AAV pathology.

Reference 1: *Anti-Neutrophil Cytoplasmic Antibodies and Effector CD4+ Cells Play Nonredundant Roles in Anti-Myeloperoxidase Crescentic Glomerulonephritis. Amanda-Jane Ruth, A. Richard Kitching, Rain Y.Q. Kwan, Dragana Odobasic, Joshua D.K. Ooi, Jennifer R. Timoshanko, Michael J. Hickey, and Stephen R. Holdsworth. Centre for Inflammatory Diseases, Monash Medical Centre, Clayton, Victoria, Australia*

LBP-33

THE C-TERMINUS OF FHR-5 INTERACTS WITH C3B AND 2,3´- SIALIC ACID SUGGESTING ITS POTENTIAL TO DE-REGULATE FH ON HOST CELL SURFACES.

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Abstract: Factor H (FH) is the main regulator of the alternative pathway both in fluid phase and on cell surfaces. The ability to regulate complement on cell surfaces relies on the capacity of the C-terminus of FH to simultaneously interact with deposited C3b and 2,3´sialic acid. We described that factor H-related protein 1 (FHR-1) does not bind sialic acid, preventing any competition with FH for the binding of C3b-deposited on host cell surfaces. Additionally, we showed that atypical hemolytic uremic syndrome (aHUS)-associated FHR-1 mutations are pathogenic because they acquire the capacity to interact with sialic acids. Here, we investigated the capacity of FHR-5 to bind sialic acids and to compete with FH for C3b-deposited on physiologically relevant surfaces. Molecular electrostatic potential surface analysis of FHR-5 SCR-9, identifies an interface for sialic acid binding that it is not present in FHR-1.Nuclear magnetic resonance experiments confirmed the interaction between FHR-5 and 2,3´-sialic acid containing glycans, but not with 2,6´sialic acid. Additionally, we observed by ELISA experiments that the binding of FHR-5 to C3b is stronger compared with FHR-1. We then interrogated if FHR-5 can compete the binding of FH to C3b by flow cytometry competition experiments using opsonized sheep erythrocytes. In contrast to FHR-1, FHR-5 compete the binding of FH to C3b only till certain extent, and this competition was lower compared with an aHUS-associated FHR-1 mutant (FHR-1$_{L290S,A296V}$).

To explore the impact of FHR-5 in complement activation, we performed hemolytic assays using sheep erythrocytes. Similar to the competition assay, FHR-5 increased the lysis only till certain extent and with a lower capacity compared with FHR-1$_{L290S,A296V}$, while FHR-1 did not have any effect. These data suggest that FHR-5 have a certain potential to interfere with the regulatory activity of FH on host cell surfaces, which might explain why FHR-5 is expressed at low levels.