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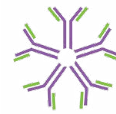

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Soluble Complement Receptor 1 Therapeutics

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ABSTRACT

Human Complement Receptor 1 (CR1/CD35) is a potent negative regulator of the complement system. Its mechanism of action is through interaction with the complement activation fragments, C3b and C4b to mediate decay acceleration of the C3 and C5 convertase complexes as well as cleavage of both ligands into inactive fragments via cofactor activity. The result is inhibition of the classical, lectin, and alternative complement pathways. This article will focus on recombinant soluble forms of CR1 that have been generated as potential therapeutics for complement-mediated disorders. Specifically, we will review and contrast the *in vitro* and *in vivo* properties of: sCR1 (BRL55730/TP10/CDX-1135), the soluble full-length extracellular domain of human CR1; sCR1-sLe^x (TP20), a glyco-engineered version of sCR1 additionally targeted to activated endothelium; APT070 (Mirocecept), a CR1 fragment conjugated to a myristoylated peptide to enhance tissue targeting; and CSL040, a soluble truncated version of the CR1 extracellular domain which exhibits altered potency and pharmacokinetic properties as compared to the parental molecule. The data obtained from studies on the effects of these CR1-based molecules in animal models of disease and their therapeutic applications will also be discussed.

Introduction

The complement cascade is an arm of the innate immune system that has evolved as a primary defence mechanism against pathogens and other deleterious processes. It consists of multiple proteins found both in plasma and at the surface of many cell types which work to target not only immune complexes and cells for phagocytosis via the process of opsonization, but also to drive inflammation via the formation of the anaphylatoxins C3a and C5a, and to initiate cell lysis directly through the generation of the membrane attack complex. These processes are initiated by the classical, lectin, and alternative complement pathways by a variety of activating factors such as immune complexes, endotoxin, neoepitopes, specific carbohydrate moieties, and by the spontaneous C3 'tick-over' mechanism[1-4]. To prevent unchecked activation of the complement cascade and damage to host cells or tissues, specific proteins have evolved as regulators of complement. Both transmembrane and soluble forms of these regulators exist, acting at all levels of the complement cascade. Examples include CD46[5], CD55[6], CD59[7], Factor H[8], Factor I[9], and the complement receptors CR1 – CR4[10].

Complement Receptor 1

Human CR1 (CD35) is a central regulator of the complement system, acting at the level of complement component C3 to inhibit the classical, lectin and alternative pathways[11]. It is primarily

a cell surface bound membrane protein expressed on erythrocytes (E-CR1) and immune cell types such as monocytes, macrophages, neutrophils, eosinophils, certain B and T cells, and glomerular podocytes[11-13]. A soluble version of CR1 exists; this is produced at very low levels (approximately 50 ng/mL) by proteolytic cleavage of the extracellular domain from the membrane, rather than produced constitutively[14,15]. CR1 belongs to a family of complement receptors that include CR2, CR3 and CR4[10]. It also belongs to a wider family of structurally related proteins referred to as the Regulators of Complement Activation. These include CD46, CD55, C4-binding protein, and Factor H, and are characterized by repetitive and highly homologous modules of approximately 60 amino acids known as short consensus repeat (SCR) domains[16]. The dominant allelic variant of human CR1 (Figure 1) is a 2039 amino acid protein with a 41 amino acid signal peptide, a 1930 amino acid extracellular domain, and a short cytoplasmic tail[16,17]. The extracellular domain of human CR1 is further comprised of 30 SCR domains which are themselves arranged into four long homologous repeat domains (LHR-A, -B, -C, -D), each composed of seven SCR domains[16,18,19]. Although SCR domains 29-30 are not technically part of LHR-D, they are often included for experimental purposes[20-22]. There is a high degree of homology (up to 99%) between SCR domains within CR1[16,23], such as the SCR8-10 and SCR15-17 domains located within LHR-B and LHR-C, respectively (Figure 1). Several allelic variants of human CR1 exist that contain fewer or additional LHR domains[16,17]. CR1 has a monomeric and highly flexible structure similar to other family members and adopts a "string of pearls" type conformation as suggested by its domain structure, negative stain microscopy, X-ray scattering and analytical ultracentrifugation[24-26]. CR1 is also highly glycosylated on multiple glycan sites found within its primary amino acid sequence, with its glycan structure determined to be exclusively N-linked[27].

The biological activity of human CR1 is mediated by binding to the ligands C3b and C4b, which are activated fragments of C3 and C4 zymogens, respectively[12,28]. Since they share similar binding sites on C3b and C4b, CR1 competitively displaces the Factor Bb and C2a catalytic fragments from the C3 convertases (C4bC2a, C3bBb) and C5 convertases (C4bC2aC3b, C3bBbC3b) which are formed upon complement activation. This mechanism of action is termed decay acceleration activity (DAA) and works to block further complement activation at the cell surface[29,30]. Human CR1 has a second function which is to act as a co-factor for complement Factor I, a serum protease that can cleave CR1-bound C3b and C4b via this co-factor activity (CFA) into the inactive forms iC3b and iC4b/C4c, to further inhibit complement activation[13,29-32]. Weak binding of human CR1 to iC3b and cleavage to further degradation products (C3c and C3dg) mediated by Factor I has also been reported[24,31,33].

Soluble Complement Receptor 1 Therapeutics

The structure of human CR1 as a type I membrane protein makes it particularly amenable to engineering to create soluble versions via removal of the transmembrane

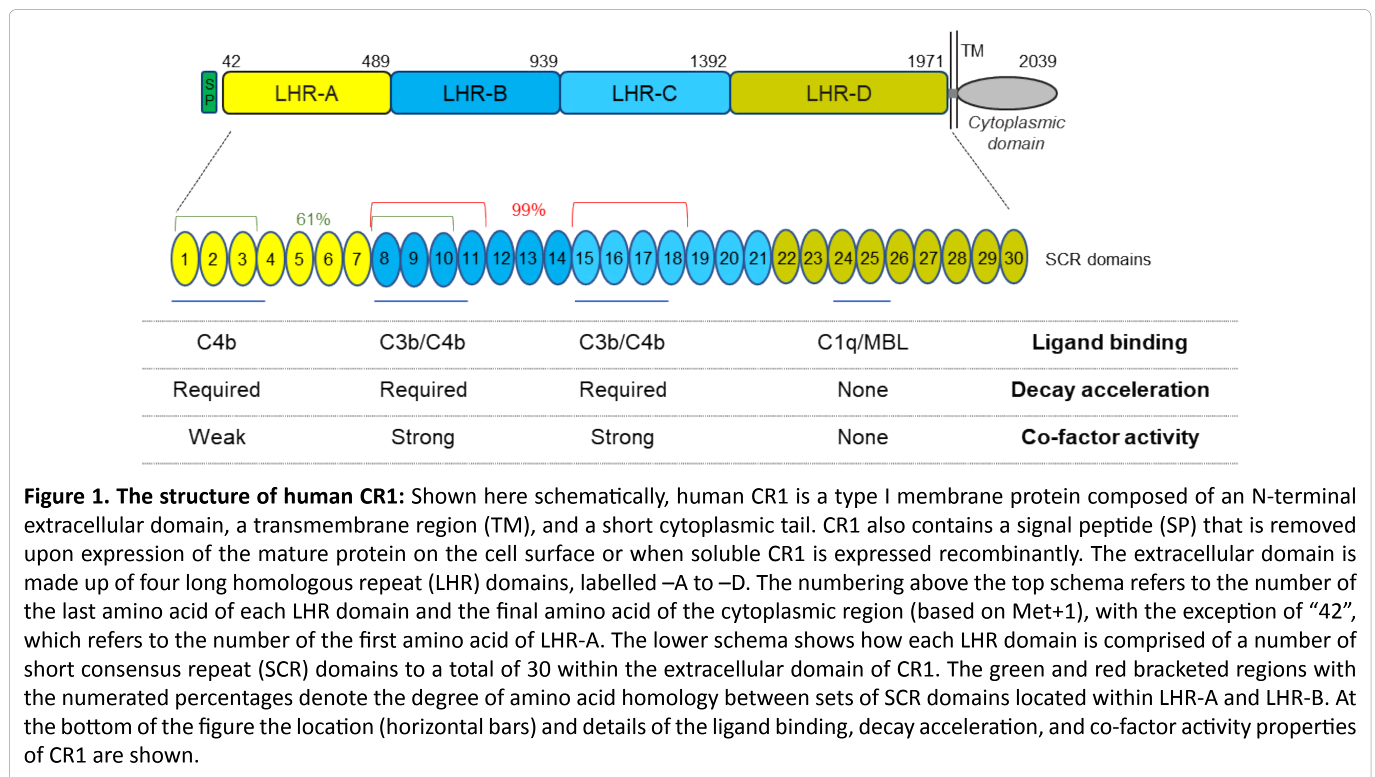

Figure 1. The structure of human CR1: Shown here schematically, human CR1 is a type I membrane protein composed of an N-terminal extracellular domain, a transmembrane region (TM), and a short cytoplasmic tail. CR1 also contains a signal peptide (SP) that is removed upon expression of the mature protein on the cell surface or when soluble CR1 is expressed recombinantly. The extracellular domain is made up of four long homologous repeat (LHR) domains, labelled –A to –D. The numbering above the top schema refers to the number of the last amino acid of each LHR domain and the final amino acid of the cytoplasmic region (based on Met+1), with the exception of "42", which refers to the number of the first amino acid of LHR-A. The lower schema shows how each LHR domain is comprised of a number of short consensus repeat (SCR) domains to a total of 30 within the extracellular domain of CR1. The green and red bracketed regions with the numerated percentages denote the degree of amino acid homology between sets of SCR domains located within LHR-A and LHR-B. At the bottom of the figure the location (horizontal bars) and details of the ligand binding, decay acceleration, and co-factor activity properties of CR1 are shown.

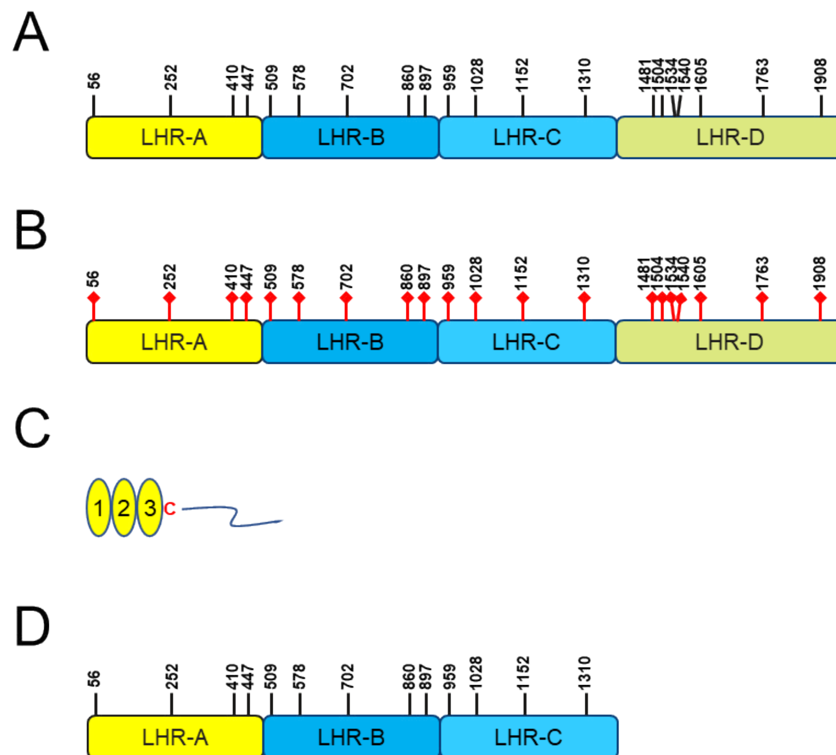

Figure 2. Therapeutic agents based on human CR1: Shown here schematically are the structures of four soluble CR1-based recombinant therapeutics: A) sCR1, also known as BRL55730, TP10, or CDX-1135; B) sCR1-sLe^x, also called TP20; C) APT070 (Mirocept); D) CSL040. Each LHR domain found within each molecule is indicated with the exception of APT070 which has its SCR domains denoted. The vertical lines and numbering above the schemas shown in A, B and D denote the position and amino acid numbering (based on Met+1) of N-linked glycosylation sites. The red vertical lines capped by diamonds in panel B represent the sialyl Lewis X glycans found on TP20. The red "C" at the C-terminal end of SCR3 in panel C denotes the presence of a C-terminal cysteine on APT070 to which is conjugated the myristoylated peptide (wavy line) needed for tissue targeting.

and cytoplasmic regions, plus any part of the extracellular domain deemed undesirable. Soluble CR1 proteins have been used for more than 30 years as negative regulators of the complement system in preclinical and clinical studies of the effects of complement inhibition in various disease and injury settings. Here we review four of them in detail: sCR1 (also known as BRL55730, TP10, and CDX-1135), sCR1-sLe^x (TP20), APT070 (Mirocept), and finally a recently identified and characterized molecule, CSL040 (Figure 2).

sCR1 (BRL55730 / TP10 / CDX-1135)

The first description of a soluble recombinant molecule designed as a potential therapeutic inhibitor of complement was published in 1990[24,34]. Using recently developed molecular biology techniques, it was possible to synthesize the cDNA encoding the entire extracellular domain of CR1 containing all four LHR domains (Figure 2A) and to express this protein recombinantly. Studies then showed that sCR1 retained all the biological functions of its parental molecule, such as ligand binding to C3b and C4b, Factor I-mediated CFA, C3 and C5 convertase DAA, and potent inhibition of all three complement pathways *in vitro*[20,22,24,34-36]. Broad cross-reactivity across multiple surrogate species also meant that sCR1 was particularly suitable for studies in animals to assess its pharmacokinetic and pharmacodynamic properties, as well as assessing its potency *in vivo* in animal models of disease or injury where it was hypothesized that complement played a role in the aetiology and/or progression of cellular or tissue damage. However, the *in vivo* half-life of sCR1 in rats was determined to be approximately 100 minutes[37,38]. This relatively short half-life limited its use to acute, rather than chronic settings.

This short half-life of sCR1 *in vivo* has not prevented its widespread use in a host of animal models for proof-of-concept testing, and we were able to identify over one hundred different studies in which it has been tested (Table 1). A breakdown of these studies is shown in Figure 3 where sCR1 has been applied to animal models across multiple therapeutic areas such as inflammation, tissue injury, neurology, auto-immunity and immune-complex mediated diseases, and infection, with a particular emphasis on ischemia-reperfusion injury (IRI) and transplantation (Figure 3A; Table 1). Several different animal species have been employed in these *in vivo* proof-of-concept studies with sCR1 (Figure 3B; Table 1), with most studies performed in rats as a surrogate species where the strength of the complement system is similar to that of humans[39]. Other

important information that has been gained from these animal studies has revolved around dosing and tolerability, which would have informed subsequent human studies. Soluble CR1 has been found to be safe and well tolerated across species at doses – both single and multiple – of up to 60 mg/kg, and various routes of administration such as intravenous, intraperitoneal, and even intrathecal have been safely employed (Table 1). Most importantly, sCR1 has been found to be protective in the vast majority of studies in which it has been tested (Figure 3C; Table 1), although it has been applied mostly prophylactically, rather than therapeutically, potentially limiting the translatability of data to human disease settings. *In vivo* studies weren't the only means to assess the suitability of sCR1 for clinical applications. Two early studies showed that sCR1 is a potent inhibitor of membrane-induced complement activation *ex vivo*[40,41], rendering it of potential clinical utility during haemodialysis.

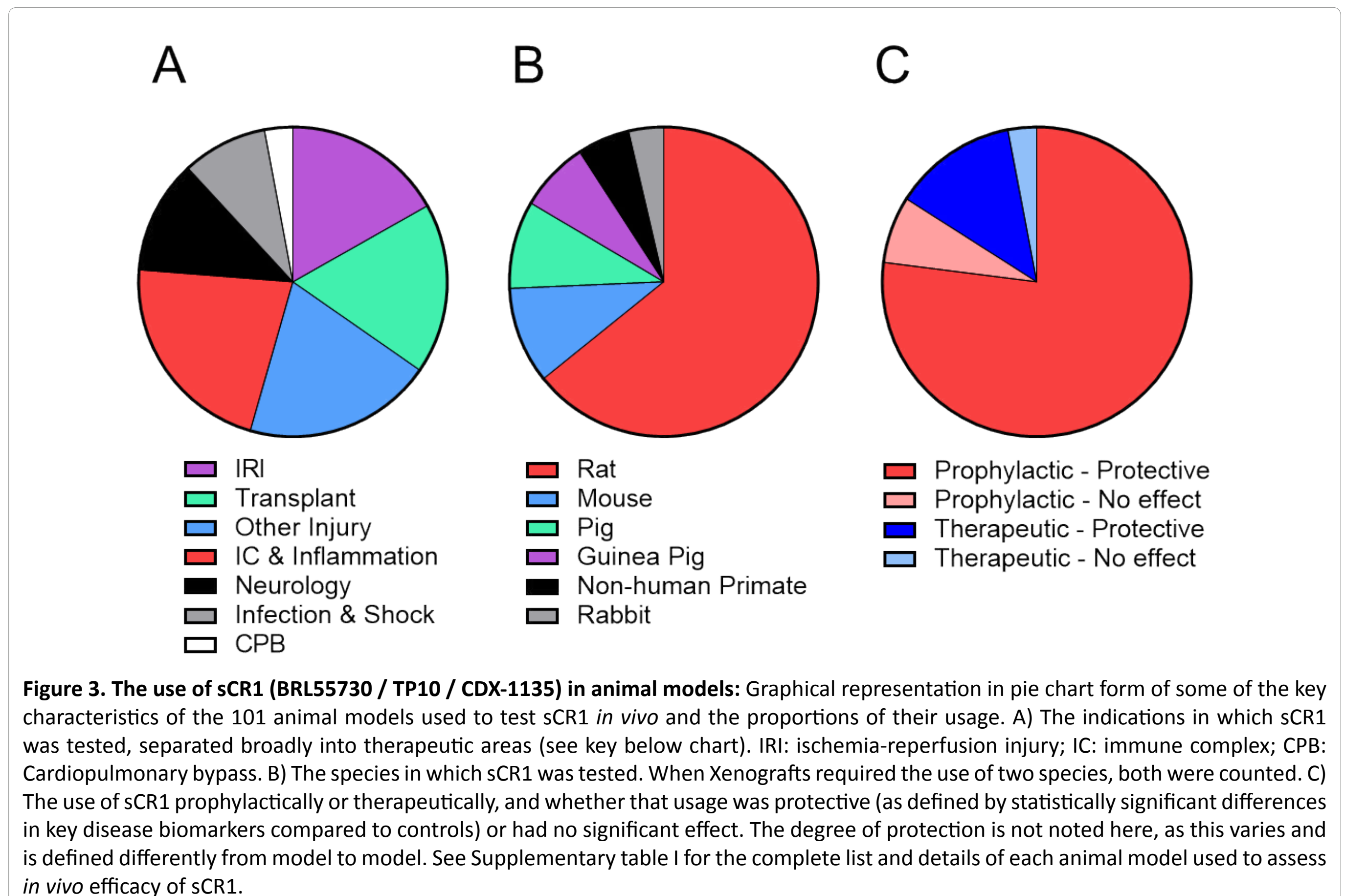

Figure 3. The use of sCR1 (BRL55730 / TP10 / CDX-1135) in animal models: Graphical representation in pie chart form of some of the key characteristics of the 101 animal models used to test sCR1 *in vivo* and the proportions of their usage. A) The indications in which sCR1 was tested, separated broadly into therapeutic areas (see key below chart). IRI: ischemia-reperfusion injury; IC: immune complex; CPB: Cardiopulmonary bypass. B) The species in which sCR1 was tested. When Xenografts required the use of two species, both were counted. C) The use of sCR1 prophylactically or therapeutically, and whether that usage was protective (as defined by statistically significant differences in key disease biomarkers compared to controls) or had no significant effect. The degree of protection is not noted here, as this varies and is defined differently from model to model. See Supplementary table I for the complete list and details of each animal model used to assess *in vivo* efficacy of sCR1.

Table 1: Use of sCR1 (BRL55730 / TP10 / CDX-1135) in vivo and ex vivo – animal models

Species	Experiment / model	Route(s) of administration	Dose(s)	Prophylactic or Therapeutic	Effect[a]	Reference
			Ischemia-Reperfusion Injury (IRI)			
Rat	Myocardial IRI	intravenous	1 mg/rat	Prophylactic	Protective	Weisman *et al* (1990a)[34] Weisman *et al* (1990b)[24]
Rat	Intestinal IRI	intravenous	3, 6 mg/rat x4	Prophylactic	Protective	Hill *et al* (1992)[42]
Rat	Hind Limb IRI	intravenous	1, 3, 6 mg/rat	Prophylactic	Protective	Lindsay *et al* (1992)[43]
Mouse	Skeletal (Cremaster) Muscle IRI	intravenous	100 ug/mouse + 100 ug/hr/mouse infusion	Prophylactic	Protective	Pemberton *et al* (1993)[44]
Rat[b]	Cardiac IRI	Perfusion	10 µg/mL	Prophylactic	Protective	Shandelya *et al* (1993)[45]
Rat	Myocardial IRI	intravenous	5 mg/kg	Prophylactic	Protective	Smith *et al* (1993)[46]
Rat	Liver IRI	intravenous	25 mg/kg x1, or 50 mg/kg x2	Prophylactic	Protective	Chavez-Cartaya *et al* (1995)[47]
Rat	Intestinal IRI	intravenous / Perfusion	20 mg/kg / 0.286 mg/mL	Prophylactic	Protective	Xiao *et al* (1997)[48]

Mouse	Intestinal IRI	intravenous	10 mg/kg	Prophylactic	Protective	Austen *et al* (1999)[49]
Rat	Intestinal IRI	intravenous	12 mg/kg x 2	Prophylactic	Protective	Eror *et al* (1999)[50]
Rat	Intestinal IRI	intravenous	12 mg/kg	Therapeutic	Protective	Eror *et al* (1999)[50]
Mouse	Intestinal IRI	intravenous	20 mg/kg	Prophylactic	Protective	Williams *et al* (1999)[51]
Rat	Acute Myocardial Infarction	intravenous	1, 5, 15 mg/kg	Prophylactic	Protective	Zacharowski *et al* (1999)[52]
Rat	Hepatic IRI	intravenous	15 mg/kg	Prophylactic	Protective	Lehmann *et al* (2001)[53]
Rat	Pancreatic IRI	intravenous	15 mg/kg	Prophylactic	Protective	von Dobschuetz *et al* (2004)[54]
Rat	Placental Ischemia	intravenous	15 mg/kg/day	Prophylactic	Protective	Lillegard *et al* (2013)[55] Regal *et al* (2019)[56]
Mouse	Renal IRI	intravenous	25 mg/kg	Prophylactic	Protective	Hameed *et al* (2020)[57]
Transplantation						
Guinea Pig to Rat	Cardiac Xenograft	intravenous	3, 5.9, 15, 60 mg/kg	Prophylactic	Protective	Pruitt *et al* (1991)[58]
Guinea Pig to Rat	Renal Xenograft	intravenous	50 mg/kg	Prophylactic	Protective	Chrupcala *et al* (1994)[59]
Guinea Pig to Rat	Cardiac Xenograft	intravenous	20 mg/kg	Prophylactic	Protective	Zehr *et al* (1994)[60]
Pig to Cyno	Cardiac Xenograft	intravenous	15 mg/kg	Prophylactic	Protective	Pruitt *et al* (1994)[61]
Guinea Pig to Rat	Cardiac Xenograft	intravenous	25 mg/kg + 20mg/kg/12hr (repeat), or 25mg/kg + 40 mg/kg/day (infusion)	Prophylactic	Protective	Candinas *et al* (1996)[62]
Pig to Cyno	Cardiac Xenograft	intravenous	25 mg/kg + 40 mg/kg/day	Prophylactic	Protective	Davis *et al* (1996)[63]
Rat	Lung Allograft	intravenous	25 mg/kg/day	Prophylactic	Protective	Pratt *et al* (1996a)[64] Pratt *et al* (1996b)[37] Pratt *et al* (1997)[65]
Guinea Pig to Rat	Cardiac Xenograft	intravenous	20 mg/kg	Prophylactic	Protective	Fujiwara *et al* (1997)[66]
Rat	Lung Allograft	intravenous	15 mg/kg	Prophylactic	Protective	Naka *et al* (1997)[67]
Pig to Cyno	Cardiac Xenograft	intravenous	25 mg/kg + 40 mg/kg/day	Prophylactic	Protective	Pruitt *et al* (1997)[68]
Rat	Liver Allograft	intravenous	15 mg/kg	Prophylactic	Protective	Lehmann *et al* (1998)[69]
Pig	Lung Allograft	intravenous	15 mg/kg	Prophylactic	Protective	Pierre *et al* (1998)[70]
Pig	Lung Allograft	intravenous	15 mg/kg	Prophylactic	Protective	Schmid *et al* (1998)[71]
Pig to Cyno	Intraportal Xenograft	intravenous	40 mg/kg	Prophylactic	Protective	Bennet *et al* (2000)[72] Lundgren *et al* (2001)[73]
Rat	Tracheal Allograft	intraperitoneal	20 mg/kg/day	Prophylactic	Protective	Kallio *et al* (2000)[74]
Rat	Lung allograft	intracardiac	10 mg/kg	Prophylactic	Protective	Stammberger *et al* (2000)[75] Schmid *et al* (2001)[76]
Pig to Cyno	Renal Xenograft	intravenous	40 mg/kg + (17-20 mg/kg Daily)	Prophylactic	No effect	Lam *et al* (2005)[77]
Rat	Renal Allograft	intravenous	25 mg/kg	Prophylactic	Protective	Damman *et al* (2011)[78]
Other Injury						
Rat	CVF-induced Lung Injury	intravenous	5, 10, 15, 20, 25 mg/kg	Prophylactic	Protective	Mulligan *et al* (1992)[79]
Rat	Thermal Injury	intravenous	5, 10, 15, 20, 25 mg/kg	Therapeutic	Protective	Mulligan *et al* (1992)[79]
Rat	LPS-induced Lung Injury	intravenous	1, 10 mg/kg	Prophylactic	Protective	Rabinovici *et al* (1992)[80]
Rabbit[b]	NHS-induced Cardiac Injury	Perfusion	20 nM	Prophylactic	Protective	Homeister *et al* (1993)[81]
Pig[b]	Human blood-induced Cardiac Injury	Perfusion	70, 300 μg/mL	Prophylactic	Protective	Pruitt *et al* (1994)[61]
Rat	IL2-induced Lung Injury	intravenous / intraperitoneal	10, 30 mg/kg (50% each route)	Prophylactic	Protective	Rabinovici *et al* (1994)[82]

Rabbit[b]	Human Plasma-induced Cardiac injury	Perfusion	20 mM	Prophylactic	Protective	Gralinski *et al* (1996)[83]
Rat	Acid-induced Lung Injury	intravenous	10 mg/kg	Prophylactic	Protective	Nishizawa *et al* (1996)[84]
Rat	Acid-induced Lung Injury	intravenous	10 mg/kg	Therapeutic	Protective	Nishizawa *et al* (1996)[84]
Mouse	Acid-induced Lung Injury	intravenous	20 mg/kg	Prophylactic	Protective	Weiser *et al* (1997)[85]
Guinea Pig	Porphyrin-induced Phototoxicity	intraperitoneal	60 + 20/40 mg/kg	Prophylactic	Protective	Nomura *et al* (1998)[86]
Rat	CVF-induced lung injury	intravenous	0.3, 1.5, 4.5 mg/rat	Prophylactic	Protective	Mulligan *et al* (1999)[87]
Rabbit[b]	NHS-induced Lung Injury	Perfusion	2.0 ug/mL	Prophylactic	Protective	Heller *et al* (2000)[88]
Rabbit[b]	NHS-induced Cardiac Injury	Perfusion	20 nM	Prophylactic	Protective	Tanhehco *et al* (2000)[89]
Mouse	Acid-induced Lung injury	intravenous	5, 10 mg/kg	Prophylactic	Protective	Kyriakides *et al* (2001)[90]
Mouse	Acid-induced Lung injury	intravenous	10 mg/kg	Therapeutic	Protective	Kyriakides *et al* (2001)[90]
Pig[b]	Human blood-induced Lung Injury	Perfusion	100 µg/mL	Prophylactic	No effect	Azimzadeh *et al* (2003)[91] Pfeiffer *et al* (2005)[92]
Mouse[b]	Human blood-induced Lung Injury	Perfusion	225 µg/mL	Prophylactic	Protective	Schroder *et al* (2003)[93]
Rat[c]	Hypertension and Renal Injury	intraperitoneal	15 mg/kg/day	Prophylactic	No effect	Regal *et al* (2018)[94]
Rat[c]	Hypertension and Renal Injury	intraperitoneal	15 mg/kg/day	Therapeutic	No effect	Regal *et al* (2018)[94]
			Immune complex & Inflammation			
Rat	Reverse Passive Arthus Reaction	intradermal	0.003, 0.03, 0.3, 1, 3, 30 µg/site	Prophylactic	Protective	Yeh *et al* (1991)[95]
Rat	Glycogen-induced Peritonitis	intravenous	7.5 mg/kg x2	Therapeutic	Protective	Mulligan *et al* (1992)[79]
Rat	IgG immune complex Alveolitis	intravenous	3.75 mg/kg x4	Prophylactic	Protective	Mulligan *et al* (1992)[79]
Rat	TNF- and Collagen-induced Arthritis	intraperitoneal	20 mg/kg/day	Prophylactic	Protective	Fava *et al* (1993)[96]
Rat	Anti-Thy1 Glomerulonephritis	intraperitoneal	60 mg/kg/day	Prophylactic	Protective	Couser *et al* (1995)[97]
Rat	Anti-Concavalin-A Glomerulonephritis	intraperitoneal	60 mg/kg/day	Prophylactic	Protective	Couser *et al* (1995)[97]
Rat	Passive Heymann Nephritis	intraperitoneal	60 mg/kg/day	Prophylactic	Protective	Couser *et al* (1995)[97]
Rat	Experimental Autoimmune Thyroiditis	intraperitoneal	10 mg/kg/day	Prophylactic	No effect	Metcalfe *et al* (1996)[98]
Rat	Cerulein-Induced Pancreatitis	intravenous	15 mg/kg/hour	Prophylactic	No effect	Weiser *et al* (1996)[99]
Rat	Cerulein-Induced Pancreatitis	intravenous	22.5 mg/kg	Prophylactic	Protective	Acioli *et al* (1997)[100]
Rat	Mono-articular Arthritis	intravenous and/or intra-articular	20 mg/kg/day and/or 200 µg/joint	Prophylactic	Protective	Goodfellow *et al* (1997)[101]
Rat	Mono-articular Arthritis	intra-articular	200 µg/joint	Therapeutic	No effect	Goodfellow *et al* (1997)[101]
Rat	Antigen-induced Pleural Inflammation	intravenous	10 mg/kg or 15 mg/kg x2	Prophylactic	Protective	Lima *et al* (1997)[102]
Rat	Muscle Inflammation	intraperitoneal	20 mg/kg + (10 mg/kg x 3)	Prophylactic	Protective	Frenette *et al* (2000)[103]
Rat	Collagen-induced Arthritis	intravenous	15 mg/kg twice daily	Prophylactic	Protective	Goodfellow *et al* (2000)[104]
Rat	Collagen-induced Arthritis	intravenous	15 mg/kg twice daily	Therapeutic	Protective	Goodfellow *et al* (2000)[104]
Rat	Acute Arthritis/Synovitis	intra-articular	0.5 mg/joint	Prophylactic	Protective	Mizuno *et al* (2000)[105]
Rat	Acute Arthritis/Synovitis	intravenous	20 mg/kg	Prophylactic	No effect	Mizuno *et al* (2000)[105]
Rat	Allergic Asthma	intraperitoneal	10 mg/kg	Prophylactic	Protective	Abe *et al* (2001)[106]
Rat	Thrombotic Glomerulonephritis	intravenous	20 mg/kg	Prophylactic	Protective	Kondo *et al* (2001)[107]
Rat	Severe Acute Pancreatitis	intravenous	12 mg/kg x 2	Prophylactic	Protective	Hartwig *et al* (2006)[108]
Mouse[d]	C3 Glomerulonephritis	intraperitoneal	25 or 50 mg/kg/day	Prophylactic	Protective	Zhang *et al* (2013)[109]

Cardiopulmonary Bypass						
Pig	Cardiopulmonary Bypass	intravenous	6 mg/kg x2	Prophylactic	Protective	Gillinov *et al* (1993)[110]
Pig	Cardiopulmonary Bypass	intravenous	10 mg/kg	Prophylactic	Protective	Lazar *et al* (1999)[111]
Pig	Cardiopulmonary Bypass	intravenous	10 mg/kg	Prophylactic	Protective	Chai *et al* (2000)[112]
Neurology						
Rat	Antibody-mediated demyelinating Experimental Allergic Encephalomyelitis	intraperitoneal	20 mg/kg/day	Prophylactic	Protective	Piddlesden *et al* (1994)[113]
Rat	Experimental Autoimmune Neuritis	intraperitoneal	30 mg/kg/day	Therapeutic	Protective	Jung *et al* (1995)[114]
Rat	Traumatic Brain Injury	intravenous / intraperitoneal	20 mg/kg + 15 mg/kg	Prophylactic	Protective	Kaczorowski *et al* (1995)[115]
Rat	Experimental Autoimmune Myasthenia Gravis	intraperitoneal	20 mg/kg/day	Prophylactic	Protective	Piddlesden *et al* (1996)[116]
Rat	Experimental Allergic Neuritis	intraperitoneal	60 mg/kg x 2	Therapeutic	No effect	Vriesendorp *et al* (1997)[117]
Mouse	Stroke (middle cerebral artery)	intravenous	15 mg/kg	Therapeutic	Protective	Huang *et al* (1999)[118]
Rat	Sciatic Inflammatory Neuropathy	intrathecal	50 μg/rat	Therapeutic	Protective	Twining *et al* (2005)[119]
Rat	Chronic Constriction Nerve Injury	intrathecal	50 μg/rat	Therapeutic	Protective	Twining *et al* (2005)[119]
Rat	gp120-induced Mechanical Allodynia	intrathecal	50 μg/rat	Therapeutic	Protective	Twining *et al* (2005)[119]
Baboon	Reperfused Stroke	intravenous	15 mg/kg	Prophylactic	No effect	Mocco *et al* (2006)[120]
Rat	Nerve Crush Injury	intraperitoneal	15 mg/kg/day	Prophylactic	Protective	Ramaglia *et al* (2008)[121] Ramaglia *et al* (2009)[122]
Rat	Spinal Cord Injury	intravenous	6 mg/kg/day	Therapeutic	Protective	Li *et al* (2010)[123]
Infection & Shock						
Guinea Pig	Anaphylaxis (Passive and active)	intravenous / intraperitoneal	15, 105 mg/kg[e]	Prophylactic	Protective	Regal *et al* (1993)[124]
Rat	Bacterial infection	intravenous	10, 25 mg/kg	Prophylactic	Protective	Swift *et al* (1994)[125]
Rat	Haemorrhage / Resuscitation	intravenous	15 mg/kg	Prophylactic	Protective	Fruchterman *et al* (1998)[126]
Rat	Haemorrhage / Resuscitation	intravenous	15 mg/kg	Prophylactic	Protective	Spain *et al* (1999)[127]
Guinea Pig	Forssman (pulmonary) Shock	intravenous	15 mg/kg	Prophylactic	Protective	Wagner *et al* (1999)[128]
Rat	Acute Shock	intravenous	20 mg/kg	Prophylactic	Protective	Mizuno *et al* (2002)[129]
Rat	Acute Shock	intravenous	20 mg/kg	Therapeutic	Protective	Mizuno *et al* (2002)[129]
Mouse	Red Blood Cell Transfusion	intravenous	1.5, 10 mg/kg	Prophylactic	Protective	Yazdanbakhsh *et al* (2003)[130]
Rat	Haemorrhagic Shock	intravenous	No dose provided	Prophylactic	Protective	Chen *et al* (2016)[131]

Listings are separated into broad therapeutic areas for ease of reading. Some animal models fit into more than one category; in these cases, the most appropriate was chosen. While every attempt has been made to capture all reported usage of sCR1 *in vivo* and *ex vivo* from extant literature, there may be examples that have been missed. [a]The degree of protection varies from model to model but must be statistically significant compared to controls to be deemed protective. [b]Experiment performed *ex vivo*. [c]Dahl Salt-sensitive (SS) rats. [d]Complement Factor H knock-out mice (a model of C3 Glomerulonephritis) with or without the transgene for human CR1. [e]15 mg/kg dose administered intravenously only; 105 mg/kg dose administered cumulatively both intravenously and intraperitoneally over a 24 hr dosing period, rather than as a single dose. IRI: Ischemia-reperfusion injury; CVF: Cobra Venom Factor; LPS: Lipopolysaccharide; TNF: Tumour Necrosis Factor; NHS: Normal Human Serum; Cyno: Cynomolgus monkey. The multipliers added to entries within the Dose(s) column refer to the number of doses used that were administered less frequently than daily.

The strength of the sCR1 *in vivo* data from animal models led to the initiation of human clinical trials as summarised in Table 2. A Phase I single ascending dose study[132] of sCR1 (TP10) in acute lung injury and acute respiratory distress syndrome patients demonstrated its safety and tolerability, with a pharmacokinetic/pharmacodynamic assessment showing an *in vivo* half-life of approximately 70 hours and complete inhibition of complement *ex vivo* in hemolytic assays at doses >1 mg/kg. Similar results were observed in a subsequent Phase

Table 2: Use of sCR1 (TP10 / CDX-1135) in vivo – human studies

Clinical Trial	Patient number / indication	Route of administration	Dose(s)	Main Outcomes	Reference
Phase I	24 Acute Lung Injury and ARDS	intravenous	0.1, 0.3, 1.0, 3.0, 10 mg/kg	Single ascending dose safe and well tolerated Half-life of 69 hours Doses >1 mg/kg inhibit Complement activity	Zimmerman *et al* (2000)[132]
Case Report	1 ABO-mismatched Lung Allograft	intravenous	15 mg/kg x 5 doses every 3-4 days	Reduction of anti-A antibody titer No humoral injury or cellular rejection Patient stable for 3 years post-transplant	Pierson *et al* (2002)[136]
Phase I/II	15 (infants <1 year old) Cardiopulmonary Bypass	intravenous	10 mg/kg plus 0.1 mg/mL to bypass circuit	All infants survived and no TP10-related adverse events Half-life of 71 hours TP10 may protect against increase in vascular permeability	Li *et al* (2004)[133]
Phase II	564 Cardiopumonary Bypass	intravenous	1, 3, 5, or 10 mg/kg	Elimination half-life of 55-57 hours; complement activity inhibited No effect on primary end point between TP10 and controls Significant improvement in endpoints in males only	Lazar *et al* (2004)[134]
Phase II	297 (Females) Cardiopulmonary Bypass	intravenous	10 mg/kg	TP10 well tolerated Effective inhibition of complement No effect on primary end points of death or myocardial infarction	Lazar *et al* (2007)[135]
Phase III	59 Lung Allograft	intravenous	10 mg/kg	Significant increase in patients undergoing early extubation Total time on ventilator and in intensive care shorter No difference in operative deaths, infection and rejection rates	Keshavjee *et al* (2005)[137]
Phase I	0 C3 Glomerulopathy	-	-	ClinicalTrials.gov Identifier: NCT02302755 Study withdrawn (2014) – no recruitment	-
Phase I	0 Dense Deposit Disease	-	-	ClinicalTrials.gov Identifier: NCT01791686 Study Terminated (2014) – due to slow enrolment, portfolio prioritization and issues with role of complement in indication	-

ARDS: Acute Respiratory Distress Syndrome; ABO: human blood groups.

I/II study in infants undergoing cardiopulmonary bypass (CPB), with evidence suggesting that sCR1 could confer some clinical benefit in this setting[133]. Given these tentative findings and previous data from pig models of CPB[110-112] , a Phase II clinical study with 564 patients undergoing CPB during cardiac surgery was then initiated to determine the efficacy of sCR1 (TP10) in this indication. Unfortunately, the outcome of this clinical trial reported no overall efficacy in this indication for sCR1[134] despite showing complete inhibition of complement. When the data from the study was assessed in more detail, there appeared to be a gender-specific effect, with males showing significant improvements in the primary endpoints compared to both females and controls[134]. A follow-on study of sCR1 (TP10) in female patients undergoing CPB and administered sCR1 (TP10) showed no effect[135]. Of equal interest is a study in a single patient who inadvertently received an ABO-mismatched lung allograft, where compassionate use of sCR1 (TP10) administration suggested some efficacy[136]. This led to a Phase II clinical study conducted in 59 patients undergoing lung transplantation, in which sCR1 (TP10) showed significant positive effects on extubation times and time of stay in intensive care, although no significant differences in other parameters such as death and graft rejection rates were found[137]. More recent attempts to use sCR1 clinically have been made (Table 2), but these failed for various reasons, and it appears that the entire program has been discontinued[138].

sCR1-sLe^x (TP20)

Glyco-engineering recombinant proteins to improve or modify their biological activity has been used for many years[139]. One such approach was used to generate a glyco-engineered variant of sCR1, denoted sCR1-sLe^x (TP20). Specific Sialyl-Lewis-X (sLe^x) tetra-saccharide carbohydrate motifs are found on the glycans of neutrophils and other leucocytes, and are made up of N-acetylglucosamine, galactose, neuraminic acid and fucose. These bind to specific ligands called selectins, particularly P-, L- and E-selectin, expressed on the surface of vascular endothelium[140,141].

Table 3: Use of sCR1-sLe^x (TP20) in vivo – animal models

Species	Experiment / model	Route of administration	Dose(s)	Prophylactic or Therapeutic	Effect[a]	References
Mouse	Stroke (middle cerebral artery)	intravenous	15 mg/kg	Prophylactic	Protective	Huang *et al* (1999)[118]
Mouse	Stroke (middle cerebral artery)	intravenous	15 mg/kg	Therapeutic	Protective	Huang *et al* (1999)[118]
Rat	CVF-induced lung injury	intravenous	0.3, 1.5, 4.5 mg/rat	Prophylactic	Protective	Mulligan *et al* (1999)[87]
Rat	Acute Myocardial Infarction	intravenous	1, 5, 15 mg/kg	Prophylactic	Protective	Zacharowski *et al* (1999)[52]
Rat	Lung allograft	intra-cardiac	10 mg/kg	Prophylactic	Protective	Stammberger *et a*l (2000)[75] Schmid et al (2001)[76]
Mouse	Acid-induced Lung injury	intravenous	5, 10 mg/kg	Prophylactic	Protective	Kyriakides *et al* (2001)[90]
Mouse	Acid-induced Lung injury	intravenous	10 mg/kg	Therapeutic	Protective	Kyriakides *et al* (2001)[90]
Baboon	Reperfused stroke	intravenous	15 mg/kg	Prophylactic	Worse outcome[b]	Ducruet *et al* (2007)[149]

[a]The degree of protection varies from model to model but must be statistically significant compared to controls to be deemed protective.
[b]Doubling of infarct volume measured 3 days post-operatively. Study terminated prematurely following an interim analysis. CVF: Cobra venom factor.

Since leucocyte migration mediated by selectin binding contributes to inflammation and tissue damage, and given that selectins can be upregulated during disease[142,143], it was thought that a bi-functional molecule (sCR1-sLe^x; Figure 2B) able to both inhibit complement and act as a selectin antagonist could be generated by decorating the N-Glycans of sCR1 with sLe^x motifs. There would be the added benefit of targeting sCR1 to the site of tissue damage[144]. Earlier studies have also demonstrated the ability of sLe^x to act as a stand-alone therapeutic, reducing neutrophil infiltration into tissue and protecting against damage[145,146].

Engineering soluble CR1 to specifically expresses the sLe^x motif on its N-glycans was not easily achieved, requiring the use of a Chinese Hamster Ovary (CHO) cell line – LEC11 – to express the α(1,3)-fucosyltransferase needed to add α(1,3)-fucose to the N-glycans present on sCR1[144,147,148]. The end-result, sCR1-sLe^x, was shown to have a 10:1 ratio of sLe^x to sCR1 with an increased sialic acid content as well as a doubling of fucose content[144]. *In vitro* assessment of sCR1-sLe^x demonstrated increased binding to CHO cell-expressed E-selectin relative to its unmodified counterpart, and a dose-dependent blockade of U937 cell adhesion to immobilized P-selectin-IgG. Unmodified sCR1 was ineffective in the latter assay. It should be noted that sCR1-sLe^x also exhibited a small (<2-fold) but statistically significant decrease in complement inhibitory activity compared to sCR1 alone[144].

Over the next few years, sCR1-sLe^x was tested in a number of animal disease models, several of which also employed sCR1 as a comparator, to determine its potency *in vivo* (See Table 3). The first model tested was a mouse model of middle cerebral artery occlusion (stroke) in which complement was shown to play a role[118]. In this model, sCR1 and sCR1-sLe^x were compared in both prophylactic and therapeutic settings, with equal doses of 15 mg/kg administered. In these experiments, sCR1-sLe^x was found to be more efficacious than sCR1, showing improvements in animal survival and additional reductions in infarct volume, neural deficit score and intracerebral hemorrhage. In an experimental rat model of cobra venom factor-induced lung injury, sCR1-sLe^x was almost twice as protective as sCR1, with dose-dependent reductions in both vascular leakage and lung neutrophil accumulation measured[87]. Of additional note in this study was the binding of sCR1-sLe^x to the lung vasculature, a phenomenon not observed with sCR1 alone. Addition of an anti-P-selectin antibody blocked this interaction, demonstrating the specificity of the binding of sCR1-sLe^x to P-selectin.

Zacharowski *et al*[52] tested sCR1-sLe^x in a rat model of myocardial IRI, showing reductions in both infarct size, cardiac troponin T release (a marker of cardiac tissue damage) and polymorphonuclear cell infiltrate at administered doses of 1 and 5 mg/kg, although a 15 mg/kg dose showed no further reduction of infarct size. In another study, a rat allogeneic single lung transplant model[75,76] was used to compare sCR1 and sCR1-sLe^x, both administered prophylactically at 10 mg/kg. Assessment of graft function post-transplant showed improvements in gas exchange for both molecules compared to vehicle controls (383 mmHg, sCR1-sLe^x; 243 mmHg, sCR1; 56 mmHg, vehicle) with a concomitant reduction in both neutrophil migration and lipid peroxidation. As in previous studies, sCR1-sLe^x out-performed sCR1 in its ability to protect against tissue damage. The final rodent model described was a mouse model of acid aspiration injury in which sCR1 and sCR1-sLe^x were compared in both prophylactic and therapeutic settings[90]. Significant decreases in measured lung permeability index and polymorphonuclear cell infiltrates were observed for both molecules in prophylactic and therapeutic settings, with sCR1-sLe^x again showing superior efficacy compared to sCR1. The therapeutic efficacy was reduced the longer administration was delayed following induction of lung injury, and at two hours no effect was observed compared to the vehicle control[90]. Lastly, sCR1-sLe^x was

tested in a NHP (baboon) model of reperfused stroke[149]. Although a previous study in the same NHP stroke model with unmodified sCR1 (as described above) showed no efficacy[120], other studies using sCR1-sLe^x in a mouse model of stroke[118] were successful and the authors hypothesized that adding sLe^x-mediated functionality to sCR1 would provide increased efficacy. Unfortunately, prophylactic administration of 15 mg/kg sCR1-sLe^x showed a worse outcome in NHPs than vehicle-treated animals, with a doubling of measured infarct volume at post-operative Day 3 and no change in neurological score, despite complete inhibition of complement activity for 12 hours following dosing[149]. The experiment was terminated following these interim results, and no further pre-clinical development of sCR1-sLe^x has occurred since then, given this was the last description of this molecule in the literature.

APT070 (Mirococept)

APT070 was first described[150] as an N-terminal fragment of human CR1 containing only the first three SCR domains found within LHR-A, fused to a myristoylated peptide designed to bind to cell surface lipid bilayers for targeted complement inhibition at the disease site (Figure 2C). Generation of unmodified SCR1-3 began several years prior to this, when Dodd *et al*[151] managed to successfully express and purify this protein from *E. coli* with yields of 6 – 15 mg/L. This and subsequent studies demonstrated that SCR1-3 was able to inhibit classical and alternative complement activity in hemolytic assays, display CFA for both C3b and C4b, and show similar DAA to the entire LHR-A domain[36,151,152]. However, purified SCR1-3 was still significantly less potent than soluble CR1 for both the classical/lectin and alternative pathways[36]. To overcome this deficiency in potency, a cysteine residue was added to the C-terminus of SCR1-3 (APT898) which allowed a membrane-localizing peptide (APT542) to be chemically coupled to it via disulphide bond formation, thereby generating APT070[153]. Comparative assessment of APT070 against its unmodified counterpart APT898 in human- and rat-specific assays showed cross-reactivity and significant improvements in potency (>100-fold) *in vitro*[150,153-155].

In order to assess its efficacy *in vivo*, APT070 was used in several animal models of disease where complement plays a role in the indication's pathophysiology (See Table 4 for a summary of all *in vivo* assessments for APT070). One

Table 4: Use of APT070 / Mirococept in vivo

Species	Experiment / model	Route(s) of administration	Dose(s)	Prophylactic or Therapeutic	Effect[a]	References
Rat	Antigen-induced Arthritis	intra-articular	90 & 250 µg/joint	Prophylactic	Protective	Linton *et al* (2000)[154]
Rat	Intravascular shock	intravenous	up to 5 mg/kg	Prophylactic	Protective	Smith and Smith (2001)[153] Smith (2002)[155]
Rat	Kidney Allograft	Perfusion	40 µg/mL	Prophylactic	Protective	Smith and Smith (2001)[153] Smith (2002)[155] Pratt *et al* (2003)[156]
Mouse	Miller-Fisher Syndrome	intravenous	580 µg/mouse	Prophylactic	Protective	Halstead *et al* (2005)[157]
Mouse	Miller-Fisher Syndrome	intravenous	580 µg/mouse	Therapeutic	Protective	Halstead *et al* (2005)[157]
Rat	Intestinal IRI – mild and severe	intravenous	1, 3, 10 mg/kg	Prophylactic	Protective	Souza *et al* (2005)[158]
Rat	Kidney Allograft	Perfusion	40 µg/mL	Prophylactic	Protective	Patel *et al* (2006)[159]
Pig	Acute Myocardial Infarction	intra-coronary	0.5 mg/kg	Prophylactic	Protective	Banz *et al* (2007)[160]
Pig to Baboon	Heart Xenograft	Perfusion & Intravenous	0.1 mg/mL (perfusion) & 3 mg/kg (2 doses i.v.)	Prophylactic	No effect	Wu *et al* (2007)[162]
Rat	Hind Limb IRI	intravenous	9 mg/kg	Prophylactic	No effect	Duehrkop *et al* (2013)[163]
Human to Mouse[b]	human islet Xenograft	in vitro	0.4 µM	Prophylactic	Protective	Xiao *et al* (2016)[161]
Human	Phase I healthy volunteers	intravenous	2, 5, 10, 20, 40, 70, 100 mg	N/A	safe and well tolerated	Smith (2002)[155] Smith *et al* (2007)[165] Kassimatis *et al* (2017)[166]
Human	Phase IIa Kidney transplant	Perfusion	10 mg	Prophylactic	No effect	Kassimatis *et al* (2017)[166]
Human	Phase IIb	Perfusion	10 mg[c]	Prophylactic	No effect	Kassimatis *et al* (2017)[166] Kassimatis *et al* (2021)[167]
Pig	Dose finding	Perfusion	20, 40, 80, 160 mg	N/A	80mg dose suitable	Kassimatis *et al* (2021)[167]

[a]The degree of protection varies from model to model but must be statistically significant compared to controls to be deemed protective. IRI: Ischemia Reperfusion Injury. [b]Humanized mice; [c]EMPIRIKAL study plan was designed with an initial 10 mg dose for Cohort 1 followed by with doses ranging from 5 – 25 mg for subsequent cohorts. However, study was terminated following administration of 10 mg dose due to lack of efficacy. i.v.: intravenous.

of the earliest studies was in a rat model of complement-dependent acute intravascular shock, where intravenous prophylactic administration of APT070 up to 5 mg/kg showed a protective effect[153,155]. This was followed by a study in a model of antigen-induced arthritis[154] where APT070 was administered directly to the relevant joint of rats at a 90 µg dose, resulting in a reduction in joint swelling and mean histological score compared to both vehicle control and APT898. A higher (250 µg) subsequent dose resulted in an even greater beneficial effect. A third study involved a rat kidney transplant model in which APT070 was shown to bind both glomerular endothelial and tubular epithelial cells[156]. Addition of APT070 to the perfusate applied to Donor's kidneys prior to syngeneic renal transplantation led to improved graft function for up to 20 weeks. At 24 hours post-transplant, reduced acute tubular necrosis, neutrophil activity, complement deposition and blood urea nitrogen levels were observed[156]. Very little PK data in rats for APT070 is known with the exception of a single report stating a terminal half-life of 1 hour[155], also limiting its pre-clinical use to acute settings.

APT070 then gained an additional name, Mirococept, following commercial acquisition, and was then used prophylactically in several additional animal models of disease: a mouse model of Miller-Fisher syndrome (a variant of Guillain-Barre syndrome)[157]; mild and severe rat intestinal IRI models[158]; another rat renal transplant model[159]; a closed-chest pig model of acute myocardial infarction[160]; and a transplant model whereby human pancreatic islets exposed to allogeneic whole blood were transplanted to the kidney capsule of humanized mice[161]. In all cases, APT070 was found to attenuate disease. APT070 was also found to be effective when administered in a therapeutic, rather than prophylactic setting. In the mouse model of Miller-Fisher syndrome, therapeutic administration of APT070 also resulted in a neuro-protective effect, although this was not as strong as observed in the prophylactic setting[157]. Not all animal models where APT070 was tested showed a beneficial effect. APT070 showed no effect on graft survival in a xenotransplantation model where baboons received hearts from transgenic pigs, even when administered both to the perfusate and separately to the donor itself both prior to and after reperfusion[162]. Similarly, in a rat model of hind limb IRI, APT070 administration had no effect on edema formation and other parameters of tissue damage[163]. This was in contrast to a comparator, C1-inhibitor, where a protective effect was shown. *In vitro*, APT070 was shown to inhibit complement activation in a cardiopulmonary bypass circuit model, and a reduction in the neutrophil activation marker CD11b was also observed[164].

In humans, early reports of a Phase I trial of APT070 indicated that intravenous administration to healthy volunteers was safe and well-tolerated[155,165]. Additional information was provided in a later paper describing the design of the Phase IIb (EMPIRIKAL) trial, where 7 doses of 2, 5, 10, 20, 40, 70 and 100 mg were reported to have been administered[166]. Adverse events were described for the highest (100 mg) dose cohort, along with data showing the pharmacokinetics of APT070 (a plasma elimination half-life of 3 hours) and a lack of complement inhibition at doses below 10 mg[166]. The outcome of a pilot Phase IIa study in twelve patients was also described, where 10 mg APT070 perfused into donor kidneys pre-transplant was well tolerated with 80% of drug retained in the grafted kidney, but with no systemic complement inhibition reported and only a "trend to lower creatinine in the Mirocept group" [166]. In the Phase IIb EMPIRIKAL trial which was aimed at reducing delayed graft function in transplanted kidneys, a similar (10 mg) dose of APT070 showed no efficacy, resulting in the premature termination of the study before additional doses could be tested[167]. In an attempt to determine a more suitable and efficacious dose of APT070 for further assessment in humans, the authors have recently conducted a dose-finding study in pigs at doses ranging from 20 to 160 mg, selecting a 80 mg dose (equivalent in humans to 120 mg) indicated as safe and sufficiently potent for further study[167].

CSL040

In devising our own therapeutic candidate based on CR1, we were conscious of ensuring that any new molecule had two key benefits over its predecessors: increased potency, and improved pharmacokinetics and pharmacodynamic profiles. We were aware that there was a limit to which soluble CR1 could be truncated to create any new molecule, based on the comparatively weak complement inhibition profile of SCR1-3 compared to sCR1[36] and existing knowledge on the roles of the LHR domains, as described above. The strategy of adding dual functionality such as tissue/membrane targeting was not pursued, given the negative outcome of sCR1-sLe^x in the previously described NHP model of stroke[149]. So, relatively straightforward approach was devised involving the construction of a series of basic N- and C-terminal truncation variants of sCR1[22], which were expressed using mammalian cells and purified. Comparative assessment in complement inhibition assays quickly identified one variant, truncated at amino acid 1392 and containing the LHR-A, -B and -C domains (designated as CSL040; Figure 2D) which exhibited significantly greater *in vitro* potency for all three complement pathways than sCR1. Why removal of the LHR-D domain of sCR1 would produce this effect in CSL040 is not fully understood, but it is likely that a combination of increased stability and affinity for ligand, decreased steric hindrance along with the removal of interaction sites located within the LHR-D domain for C1q, MBL and certain Ficolins might all play a role[22,168,169].

CSL040 was demonstrated to be a more potent inhibitor of the alternative complement pathway compared to that of the classical and lectin pathways[22]. This differential pathway activity can be explained mechanistically. In terms of the relative binding affinities of CR1 to C3b and C4b, 10-fold more sCR1 is required to inhibit the binding of C4b to erythrocytes compared to C3b[34]; a later study showed a 20-fold weaker affinity for the C4b-CR1 interaction compared to C3b-CR1[170]. This clearly has an effect on the DAA of CR1, with 5-10-fold more sCR1 needed to inhibit classical pathway convertase formation *in vitro* compared to alternative pathway convertase formation[35]. Other studies[171,172] also showed differences in CFA for C3b compared to C4. C4b cleavage mediated by CR1 with Factor I is slower than that for C3b, with a preference for C3b if both ligands are present with Factor I. Mossakowska *et al*[36] confirmed this finding, showing co-factor IC_{50} values for sCR1 to C3b of 0.8 nM compared to 15 nM for sCR1 to C4b.

Comparative assessment of CSL040 and sCR1 in a series of pharmacokinetic/ pharmacodynamic studies in both mice and rats was performed next[22,173]. In these studies, we took steps to ensure that the asialo-N-glycan levels of both molecules tested in these studies were similar, since previous studies showed the importance of protein sialylation for protein clearance[174,175]. In both species, CSL040 displayed a superior PK profile compared to sCR1. As the only point of difference between the two molecules tested, the LHR-D domain must be responsible for the faster clearance of sCR1 compared to CSL040; it was hypothesized that suboptimal glycosylation of the glycans present within LHR-D might contribute to more rapid clearance via clearance receptors such as the asialoglycoprotein or mannose receptors[173]. A series of additional experiments determined a relationship between the levels of CSL040 sialylation and *in vivo* clearance[173], making this a critical quality attribute for any future *in vivo* studies. These experiments also demonstrated that CSL040 was safe and well tolerated at single doses of up to 90 mg/kg in both rats and non-human primates.

An analysis of the pharmacodynamic properties of CSL040 revealed an extended duration of alternative pathway inhibition, relative to the duration of the classical/ lectin pathway response. An extended alternative pathway response *in vivo* was anticipated, given the increased *in vitro* potency of CSL040 for this pathway, but the extent of the response relative to the other pathways was not, since the studies performed with sCR1, sCR1-sLe^x, and APT070 discussed above typically only showed classical pathway activity following *in vivo* administration. Our data suggests some scope to potentially widen indication selection for CSL040 to chronic indications primarily involving the alternative complement pathway, rather than restricting development to acute indications.

The final studies performed to date with CSL040 have been proof-of-concept experiments in animal models of disease to evaluate *in vivo* efficacy. The vast body of literature for sCR1 in surrogate species (Table 1) informed decisions around indication and model selection for our own *in vivo* efficacy studies, and two mouse models were selected in which to test CSL040 (Table 5). The first model was a previously described[176] model of immune-complex mediated kidney disease, the attenuated passive anti-glomerular basement membrane antibody-induced glomerulonephritis model, in which we found that CSL040 was able to significantly attenuate kidney damage (as measured by urine albumin output) at single 20 and 60 mg/kg doses administered prophylactically[22]. The second model in which CSL040 was tested was a mouse model of warm kidney IRI[177]. Here, we also used sCR1 (generated in-house) as a comparator, again ensuring that it retained similar asialo-N-glycan levels to that of CSL040. While two doses of 60 mg/kg CSL040 were able to significantly attenuate kidney damage, equimolar doses of 85.2 mg/kg sCR1 showed no significant effect[177]. This difference in relative *in vivo* potency is likely explained by both the 3-fold increased potency of CSL040 *in vitro* compared to sCR1, as well its significantly improved pharmacokinetic and pharmacodynamic properties[22,173]. We are now looking to expand our assessment of CSL040 to additional relevant *in vivo* animal models, and in settings in which CSL040 can be tested therapeutically, rather than prophylactically.

Summary

It has now been more than three decades since a soluble regulator of complement and potential therapeutic based on CR1 has been described. As is clear from this review, the various forms of soluble CR1 that have been engineered and developed as therapeutics since that time have shown great promise in multiple disease indications with protective effects shown in a wide variety of animal

Table 5: Use of CSL040 in vivo in animal models of disease

Species	Experiment / model	Route of administration	Dose(s)	Prophylactic or Therapeutic	Effect[a]	References
Mouse	Glomerulonephritis[b]	intraperitoneal	5, 20, or 60 mg/kg	Prophylactic	Protective	Wymann *et al* (2021)[22]
Mouse	Kidney IRI	intraperitoneal	15, 30, or 60 mg/kg x 2 doses each	Prophylactic	Protective	Bongoni *et al* (2021)[177]

[a]The degree of protection varies from model to model but must be statistically significant compared to controls to be deemed protective.
[b]The Glomerulonephritis model used is abbreviation of its full name, the attenuated passive anti-glomerular basement membrane antibody-induced glomerulonephritis model.

models of specific indications where complement plays a role in mediating the pathophysiology and/or progression of disease or injury. Unfortunately, it also seems clear that translating efficacy from animal models to humans for the CR1-based molecules we have reviewed has been challenging, with perhaps an over-reliance of prophylactic, rather than therapeutic animal models from which to select indications for non-human primate and human clinical studies. It is hoped that with CSL040, we can learn from past knowledge and with informed indication selection achieve sustained success in the clinic.

Supporting information

None

Conflict of interest statement

M.P.H, T.R. and S.W. are listed as inventors on International Patent Publication number WO2019/218009. All authors are CSL shareholders.

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References

1. Lachmann PJ. Biological functions of the complement system. *Biochem Soc Trans*. 1990; 18(6): 1143-1145.
2. Ricklin D, Hajishengallis G, Yang K, et al. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol*. 2010; 11(9): 785-797.
3. Kolev M, Le Friec G, Kemper C. Complement--tapping into new sites and effector systems. *Nat Rev Immunol*. 2014; 14(12): 811-820.
4. Lachmann PJ. The amplification loop of the complement pathways. *Adv Immunol*. 2009; 104: 115-149.
5. Yamamoto H, Fara AF, Dasgupta P, et al. CD46: the 'multitasker' of complement proteins. *Int J Biochem Cell Biol*. 2013; 45(12): 2808-2820.
6. Dho SH, Lim JC, Kim LK. Beyond the Role of CD55 as a Complement Component. *Immune Netw*. 2018; 18(1): e11.
7. Davies A, Simmons DL, Hale G, et al. CD59, an LY-6-like protein expressed in human lymphoid cells, regulates the action of the complement membrane attack complex on homologous cells. *J Exp Med*. 1989; 170(3): 637-654.
8. Ferreira VP, Pangburn MK, Cortes C. Complement control protein factor H: the good, the bad, and the inadequate. *Mol Immunol*. 2010; 47(13): 2187-2197.
9. Lachmann PJ. The story of complement factor I. *Immunobiology*. 2019; 224(4): 511-517.
10. Liu D, Niu ZX. The structure, genetic polymorphisms, expression and biological functions of complement receptor type 1 (CR1/CD35). *Immunopharmacol Immunotoxicol*. 2009; 31(4): 524-535.
11. Ahearn JM, Fearon DT. Structure and function of the complement receptors, CR1 (CD35) and CR2 (CD21). *Adv Immunol*. 1989; 46: 183-219.
12. Fearon DT. Identification of the membrane glycoprotein that is the C3b receptor of the human erythrocyte, polymorphonuclear leukocyte, B lymphocyte, and monocyte. *J Exp Med*. 1980; 152(1): 20-30.
13. Ross GD, Lambris JD. Identification of a C3bi-specific membrane complement receptor that is expressed on lymphocytes, monocytes, neutrophils, and erythrocytes. *J Exp Med*. 1982; 155(1): 96-110.
14. Karthikeyan G, Baalasubramanian S, Seth S, et al. Low levels of plasma soluble complement receptor type 1 in patients receiving thrombolytic therapy for acute myocardial infarction. *J Thromb Thrombolysis*. 2007; 23(2): 115-120.
15. Hamer I, Paccaud JP, Belin D, et al. Soluble form of complement C3b/C4b receptor (CR1) results from a proteolytic cleavage in the C-terminal region of CR1 transmembrane domain. *Biochem J*. 1998; 329 (Pt 1): 183-190.
16. Krych-Goldberg M, Atkinson JP. Structure-function relationships of complement receptor type 1. *Immunol Rev*. 2001; 180: 112-122.
17. Torvell M, Carpanini SM, Daskoulidou N, et al. Genetic Insights into the Impact of Complement in Alzheimer's Disease. *Genes (Basel)*. 2021; 12(12).
18. Weis JH, Morton CC, Bruns GA, et al. A complement receptor locus: genes encoding C3b/C4b receptor and C3d/Epstein-Barr virus receptor map to 1q32. *J Immunol*. 1987; 138(1): 312-315.
19. Klickstein LB, Wong WW, Smith JA, et al. Human C3b/C4b receptor (CR1). Demonstration of long homologous repeating domains that are composed of the short consensus repeats characteristics of C3/C4 binding proteins. *J Exp Med*. 1987; 165(4): 1095-1112.
20. Kalli KR, Hsu PH, Bartow TJ, et al. Mapping of the C3b-binding site of CR1 and construction of a (CR1)2-F(ab')2 chimeric complement inhibitor. *J Exp Med*. 1991; 174(6): 1451-1460.
21. Mqadmi A, Abdullah Y, Yazdanbakhsh K. Characterization of complement receptor 1 domains for prevention of complement-mediated red cell destruction. *Transfusion*. 2005; 45(2): 234-244.
22. Wymann S, Dai Y, Nair AG, et al. A novel soluble complement receptor 1 fragment with enhanced therapeutic potential. *J Biol Chem*. 2021; 296: 100200.
23. Kirkitadze MD, Barlow PN. Structure and flexibility of the multiple domain proteins that regulate complement activation. *Immunol Rev*. 2001; 180: 146-161.
24. Weisman HF, Bartow T, Leppo MK, et al. Recombinant soluble CR1 suppressed complement activation, inflammation, and necrosis associated with reperfusion of ischemic myocardium. *Trans Assoc Am Physicians*. 1990; 103: 64-72.
25. Gilbert HE, Asokan R, Holers VM, et al. The 15 SCR flexible extracellular domains of human complement receptor type 2 can mediate multiple ligand and antigen interactions. *J Mol Biol*. 2006; 362(5): 1132-1147.
26. Furtado PB, Huang CY, Ihyembe D, et al. The partly folded back solution structure arrangement of the 30 SCR domains in human complement receptor type 1 (CR1) permits access to its C3b and C4b ligands. *J Mol Biol*. 2008; 375(1): 102-118.
27. Lublin DM, Griffith RC, Atkinson JP. Influence of glycosylation on allelic and cell-specific Mr variation, receptor processing, and ligand binding of the human complement C3b/C4b receptor. *J Biol Chem*. 1986; 261(13): 5736-5744.
28. Ross GD. Analysis of the different types of leukocyte membrane complement receptors and their interaction with the complement system. *J Immunol Methods*. 1980; 37(3-4): 197-211.
29. Fearon DT. Regulation of the amplification C3 convertase of human complement by an inhibitory protein isolated from human erythrocyte membrane. *Proc Natl Acad Sci U S A*. 1979; 76(11): 5867-5871.
30. Iida K, Nussenzweig V. Complement receptor is an inhibitor of the complement cascade. *J Exp Med*. 1981; 153(5): 1138-1150.
31. Medof ME, Iida K, Mold C, et al. Unique role of the complement receptor CR1 in the degradation of C3b associated with immune complexes. *J Exp Med*. 1982; 156(6): 1739-1754.

32. Ross GD, Lambris JD, Cain JA, et al. Generation of three different fragments of bound C3 with purified factor I or serum. I. Requirements for factor H vs CR1 cofactor activity. *J Immunol*. 1982; 129(5): 2051-2060.

33. Alcorlo M, Martinez-Barricarte R, Fernandez FJ, et al. Unique structure of iC3b resolved at a resolution of 24 A by 3D-electron microscopy. *Proc Natl Acad Sci U S A*. 2011; 108(32): 13236-13240.

34. Weisman HF, Bartow T, Leppo MK, et al. Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science*. 1990; 249(4965): 146-151.

35. Wong WW, Farrell SA. Proposed structure of the F' allotype of human CR1. Loss of a C3b binding site may be associated with altered function. *J Immunol*. 1991; 146(2): 656-662.

36. Mossakowska D, Dodd I, Pindar W, et al. Structure-activity relationships within the N-terminal short consensus repeats (SCR) of human CR1 (C3b/C4b receptor, CD35): SCR 3 plays a critical role in inhibition of the classical and alternative pathways of complement activation. *Eur J Immunol*. 1999; 29(6): 1955-1965.

37. Pratt JR, Hibbs MJ, Laver AJ, et al. Effects of complement inhibition with soluble complement receptor-1 on vascular injury and inflammation during renal allograft rejection in the rat. *Am J Pathol*. 1996; 149(6): 2055-2066.

38. Makrides SC, Nygren PA, Andrews B, et al. Extended in vivo half-life of human soluble complement receptor type 1 fused to a serum albumin-binding receptor. *J Pharmacol Exp Ther*. 1996; 277(1): 534-542.

39. Ong GL, Mattes MJ. Mouse strains with typical mammalian levels of complement activity. *J Immunol Methods*. 1989; 125(1-2): 147-158.

40. Cheung AK, Parker CJ, Hohnholt M. Soluble complement receptor type 1 inhibits complement activation induced by hemodialysis membranes in vitro. *Kidney Int*. 1994; 46(6): 1680-1687.

41. Himmelfarb J, McMonagle E, Holbrook D, et al. Soluble complement receptor 1 inhibits both complement and granulocyte activation during ex vivo hemodialysis. *J Lab Clin Med*. 1995; 126(4): 392-400.

42. Hill J, Lindsay TF, Ortiz F, et al. Soluble complement receptor type 1 ameliorates the local and remote organ injury after intestinal ischemia-reperfusion in the rat. *J Immunol*. 1992; 149(5): 1723-1728.

43. Lindsay TF, Hill J, Ortiz F, et al. Blockade of complement activation prevents local and pulmonary albumin leak after lower torso ischemia-reperfusion. *Ann Surg*. 1992; 216(6): 677-683.

44. Pemberton M, Anderson G, Vetvicka V, et al. Microvascular effects of complement blockade with soluble recombinant CR1 on ischemia/reperfusion injury of skeletal muscle. *J Immunol*. 1993; 150(11): 5104-5113.

45. Shandelya SM, Kuppusamy P, Herskowitz A, et al. Soluble complement receptor type 1 inhibits the complement pathway and prevents contractile failure in the postischemic heart. Evidence that complement activation is required for neutrophil-mediated reperfusion injury. *Circulation*. 1993; 88(6): 2812-2826.

46. Smith EF, 3rd, Griswold DE, Egan JW, et al. Reduction of myocardial reperfusion injury with human soluble complement receptor type 1 (BRL 55730). *Eur J Pharmacol*. 1993; 236(3): 477-481.

47. Chavez-Cartaya RE, DeSola GP, Wright L, et al. Regulation of the complement cascade by soluble complement receptor type 1. Protective effect in experimental liver ischemia and reperfusion. *Transplantation*. 1995; 59(7): 1047-1052.

48. Xiao F, Eppihimer MJ, Willis BH, et al. Complement-mediated lung injury and neutrophil retention after intestinal ischemia-reperfusion. *J Appl Physiol (1985)*. 1997; 82(5): 1459-1465.

49. Austen WG, Jr., Kyriakides C, Favuzza J, et al. Intestinal ischemia-reperfusion injury is mediated by the membrane attack complex. *Surgery*. 1999; 126(2): 343-348.

50. Eror AT, Stojadinovic A, Starnes BW, et al. Antiinflammatory effects of soluble complement receptor type 1 promote rapid recovery of ischemia/reperfusion injury in rat small intestine. *Clin Immunol*. 1999; 90(2): 266-275.

51. Williams JP, Pechet TT, Weiser MR, et al. Intestinal reperfusion injury is mediated by IgM and complement. *J Appl Physiol (1985)*. 1999; 86(3): 938-942.

52. Zacharowski K, Otto M, Hafner G, et al. Thiemermann C. Reduction of myocardial infarct size with sCR1sLe(x), an alternatively glycosylated form of human soluble complement receptor type 1 (sCR1), possessing sialyl Lewis x. *Br J Pharmacol*. 1999; 128(5): 945-952.

53. Lehmann TG, Koeppel TA, Munch S, et al. Impact of inhibition of complement by sCR1 on hepatic microcirculation after warm ischemia. *Microvasc Res*. 2001; 62(3): 284-292.

54. von Dobschuetz E, Bleiziffer O, Pahernik S, et al. Soluble complement receptor 1 preserves endothelial barrier function and microcirculation in postischemic pancreatitis in the rat. *Am J Physiol Gastrointest Liver Physiol*. 2004; 286(5): G791-796.

55. Lillegard KE, Johnson AC, Lojovich SJ, et al. Complement activation is critical for placental ischemia-induced hypertension in the rat. *Mol Immunol*. 2013; 56(1-2): 91-97.

56. Regal JF, Lund JM, Wing CR, et al. Interactions between the complement and endothelin systems in normal pregnancy and following placental ischemia. *Mol Immunol*. 2019; 114: 10-18.

57. Hameed AM, Lu DB, Burns H, et al. Pharmacologic targeting of renal ischemia-reperfusion injury using a normothermic machine perfusion platform. *Sci Rep*. 2020; 10(1): 6930.

58. Pruitt SK, Baldwin WM, 3rd, Marsh HC, Jr., et al. The effect of soluble complement receptor type 1 on hyperacute xenograft rejection. *Transplantation*. 1991; 52(5): 868-873.

59. Chrupcala M, Pomer S, Staehler G, et al. Prolongation of discordant renal xenograft survival by depletion of complement. Comparative effects of systemically administered cobra venom factor and soluble complement receptor type 1 in a guinea-pig to rat model. *Transpl Int*. 1994; 7 Suppl 1: S650-653.

60. Zehr KJ, Herskowitz A, Lee PC, et al. Neutrophil adhesion and complement inhibition prolongs survival of cardiac xenografts in discordant species. *Transplantation*. 1994; 57(6): 900-906.

61. Pruitt SK, Kirk AD, Bollinger RR, et al. The effect of soluble complement receptor type 1 on hyperacute rejection of porcine xenografts. *Transplantation*. 1994; 57(3): 363-370.

62. Candinas D, Lesnikoski BA, Robson SC, et al. Effect of repetitive high-dose treatment with soluble complement receptor type 1 and cobra venom factor on discordant xenograft survival. *Transplantation*. 1996; 62(3): 336-342.

63. Davis EA, Pruitt SK, Greene PS, et al. Inhibition of complement, evoked antibody, and cellular response prevents rejection of pig-to-primate cardiac xenografts. *Transplantation*. 1996; 62(7): 1018-1023.

64. Pratt JR, Hibbs MJ, Laver AJ, et al. Allograft immune response with sCR1 intervention. *Transpl Immunol*. 1996; 4(1): 72-75.

65. Pratt JR, Harmer AW, Levin J, et al. Influence of complement on the allospecific antibody response to a primary vascularized organ graft. *Eur J Immunol*. 1997; 27(11): 2848-2853.

66. Fujiwara I, Nakajima H, Akioka K, et al. Soluble complement receptor type 1 and antithrombin-III combination therapy prolongs xenograft survival: the role of thrombin and prostacyclin in hyperacute rejection. *Transplant Proc*. 1997; 29(1-2): 935-937.

67. Naka Y, Marsh HC, Scesney SM, et al. Complement activation as a cause for primary graft failure in an isogeneic rat model of hypothermic lung preservation and transplantation. *Transplantation*. 1997; 64(9): 1248-1255.

68. Pruitt SK, Bollinger RR, Collins BH, et al. Effect of continuous complement inhibition using soluble complement receptor type 1 on survival of pig-to-primate cardiac xenografts. *Transplantation*. 1997; 63(6): 900-902.

69. Lehmann TG, Koeppel TA, Kirschfink M, et al. Complement inhibition by soluble complement receptor type 1 improves microcirculation after rat liver transplantation. *Transplantation*. 1998; 66(6): 717-722.

70. Pierre AF, Xavier AM, Liu M, et al. Effect of complement inhibition with soluble complement receptor 1 on pig allotransplant lung function. *Transplantation*. 1998; 66(6): 723-732.

71. Schmid RA, Zollinger A, Singer T, et al. Effect of soluble complement receptor type 1 on reperfusion edema and neutrophil migration after lung allotransplantation in swine. *J Thorac Cardiovasc Surg*. 1998; 116(1): 90-97.

72. Bennet W, Sundberg B, Lundgren T, et al. Damage to porcine islets of Langerhans after exposure to human blood in vitro, or after intraportal transplantation to cynomologus monkeys: protective effects of sCR1 and heparin. *Transplantation*. 2000; 69(5): 711-719.

73. Lundgren T, Bennet W, Tibell A, et al. Soluble complement receptor 1 (TP10) preserves adult porcine islet morphology after intraportal transplantation into cynomolgus monkeys. *Transplant Proc*. 2001; 33(1-2): 725.

74. Kallio EA, Lemstrom KB, Hayry PJ, et al. Blockade of complement inhibits obliterative bronchiolitis in rat tracheal allografts. *Am J Respir Crit Care Med*. 2000; 161(4 Pt 1): 1332-1339.

75. Stammberger U, Hamacher J, Hillinger S, et al. sCR1sLe ameliorates ischemia/reperfusion injury in experimental lung transplantation. *J Thorac Cardiovasc Surg*. 2000; 120(6): 1078-1084.

76. Schmid RA, Hillinger S, Hamacher J, et al. TP20 is superior to TP10 in reducing ischemia/reperfusion injury in rat lung grafts. *Transplant Proc*. 2001; 33(1-2): 948-949.

77. Lam TT, Hausen B, Hook L, et al. The effect of soluble complement receptor type 1 on acute humoral xenograft rejection in hDAF-transgenic pig-to-primate life-supporting kidney xenografts. *Xenotransplantation*. 2005; 12(1): 20-29.

78. Damman J, Hoeger S, Boneschansker L, et al. Targeting complement activation in brain-dead donors improves renal function after transplantation. *Transpl Immunol*. 2011; 24(4): 233-237.

79. Mulligan MS, Yeh CG, Rudolph AR, et al. Protective effects of soluble CR1 in complement- and neutrophil-mediated tissue injury. *J Immunol*. 1992; 148(5): 1479-1485.

80. Rabinovici R, Yeh CG, Hillegass LM, et al. Role of complement in endotoxin/platelet-activating factor-induced lung injury. *J Immunol*. 1992; 149(5): 1744-1750.

81. Homeister JW, Satoh PS, Kilgore KS, et al. Soluble complement receptor type 1 prevents human complement-mediated damage of the rabbit isolated heart. *J Immunol*. 1993; 150(3): 1055-1064.

82. Rabinovici R, Sofronski MD, Borboroglu P, et al. Interleukin-2-induced lung injury. The role of complement. *Circ Res*. 1994; 74(2): 329-335.

83. Gralinski MR, Wiater BC, Assenmacher AN, et al. Selective inhibition of the alternative complement pathway by sCR1[desLHR-A] protects the rabbit isolated heart from human complement-mediated damage. *Immunopharmacology*. 1996; 34(2-3): 79-88.

84. Nishizawa H, Yamada H, Miyazaki H, et al. Soluble complement receptor type 1 inhibited the systemic organ injury caused by acid instillation into a lung. *Anesthesiology*. 1996; 85(5): 1120-1128.

85. Weiser MR, Pechet TT, Williams JP, et al. Experimental murine acid aspiration injury is mediated by neutrophils and the alternative complement pathway. *J Appl Physiol (1985)*. 1997; 83(4): 1090-1095.

86. Nomura N, Lim HW, Levin JL, et al. Effect of soluble complement receptor type 1 on porphyrin-induced phototoxicity in guinea pigs. *J Photochem Photobiol B*. 1998; 42(1): 28-31.

87. Mulligan MS, Warner RL, Rittershaus CW, et al. Endothelial targeting and enhanced antiinflammatory effects of complement inhibitors possessing sialyl Lewisx moieties. *J Immunol*. 1999; 162(8): 4952-4959.

88. Heller A, Kunz M, Samakas A, et al. The complement regulators C1 inhibitor and soluble complement receptor 1 attenuate acute lung injury in rabbits. *Shock*. 2000; 13(4): 285-290.

89. Tanhehco EJ, Lee H, Lucchesi BR. Sublytic complement attack reduces infarct size in rabbit isolated hearts: evidence for C5a-mediated cardioprotection. *Immunopharmacology*. 2000; 49(3): 391-399.

90. Kyriakides C, Wang Y, Austen WG, Jr., et al. Sialyl Lewis(x) hybridized complement receptor type 1 moderates acid aspiration injury. *Am J Physiol Lung Cell Mol Physiol*. 2001; 281(6): L1494-1499.

91. Azimzadeh A, Zorn GL, 3rd, Blair KS, et al. Hyperacute lung rejection in the pig-to-human model. 2. Synergy between soluble and membrane complement inhibition. *Xenotransplantation*. 2003; 10(2): 120-131.

92. Pfeiffer S, Zorn GL, 3rd, Zhang JP, et al. Hyperacute lung rejection in the pig-to-human model. III. Platelet receptor inhibitors synergistically modulate complement activation and lung injury. *Transplantation*. 2003; 75(7): 953-959.

93. Schroder C, Wu GS, Price E, et al. Hyperacute rejection of mouse lung by human blood: characterization of the model and the role of complement. *Transplantation*. 2003; 76(5): 755-760.

94. Regal JF, Laule CF, McCutcheon L, et al. The complement system in hypertension and renal damage in the Dahl SS rat. *Physiol Rep*. 2018; 6(6): e13655.

95. Yeh CG, Marsh HC, Jr., Carson GR, et al. Recombinant soluble human complement receptor type 1 inhibits inflammation in the reversed passive arthus reaction in rats. *J Immunol*. 1991; 146(1): 250-256.

96. Fava RA, Gates C, Townes AS. Critical role of peripheral blood phagocytes and the involvement of complement in tumour necrosis factor enhancement of passive collagen-arthritis. *Clin Exp Immunol*. 1993; 94(2): 261-266.

97. Couser WG, Johnson RJ, Young BA, et al. The effects of soluble recombinant complement receptor 1 on complement-mediated experimental glomerulonephritis. *J Am Soc Nephrol*. 1995; 5(11): 1888-1894.

98. Metcalfe RA, McIntosh RS, Morgan BP, et al. The effect of soluble complement receptor 1 (sCR1) and human thyroid antibodies on the course of experimental autoimmune thyroiditis in rats. *Autoimmunity*. 1996; 23(1): 1-8.

99. Weiser MR, Gibbs SA, Moore FD, Jr., et al. Complement inhibition by soluble complement receptor type 1 fails to moderate cerulein-induced pancreatitis in the rat. *Int J Pancreatol*. 1996; 19(2): 129-134.

100. Acioli JM, Isobe M, Kawasaki S. Early complement system activation and neutrophil priming in acute pancreatitis: participation of trypsin. *Surgery*. 1997; 122(5): 909-917.

101. Goodfellow RM, Williams AS, Levin JL, et al. Local therapy with soluble complement receptor 1 (sCR1) suppresses inflammation in rat mono-articular arthritis. *Clin Exp Immunol*. 1997; 110(1): 45-52.

102. Lima MC, Prouvost-Danon A, e Silva PM, et al. Studies on the mechanisms involved in antigen-evoked pleural inflammation in rats: contribution of IgE and complement. *J Leukoc Biol*. 1997; 61(3): 286-292.

103. Frenette J, Cai B, Tidball JG. Complement activation promotes muscle inflammation during modified muscle use. *Am J Pathol*. 2000; 156(6): 2103-2110.

104. Goodfellow RM, Williams AS, Levin JL, et al. Soluble complement receptor one (sCR1) inhibits the development and progression of rat collagen-induced arthritis. *Clin Exp Immunol*. 2000; 119(1): 210-216.

105. Mizuno M, Nishikawa K, Morgan BP, et al. Comparison of the suppressive effects of soluble CR1 and C5a receptor antagonist in acute arthritis induced in rats by blocking of CD59. *Clin Exp Immunol*. 2000; 119(2): 368-375.

106. Abe M, Shibata K, Akatsu H, et al. Contribution of anaphylatoxin C5a to late airway responses after repeated exposure of antigen to allergic rats. *J Immunol*. 2001; 167(8): 4651-4660.

107. Kondo C, Mizuno M, Nishikawa K, et al. The role of C5a in the development of thrombotic glomerulonephritis in rats. *Clin Exp Immunol*. 2001; 124(2): 323-329.

108. Hartwig W, Klafs M, Kirschfink M, et al. Interaction of complement and leukocytes in severe acute pancreatitis: potential for therapeutic intervention. *Am J Physiol Gastrointest Liver Physiol*. 2006; 291(5): G844-850.

109. Zhang Y, Nester CM, Holanda DG, et al. Soluble CR1 therapy improves complement regulation in C3 glomerulopathy. *J Am Soc Nephrol*. 2013; 24(11): 1820-1829.

110. Gillinov AM, DeValeria PA, Winkelstein JA, et al. Complement inhibition with soluble complement receptor type 1 in cardiopulmonary bypass. *Ann Thorac Surg*. 1993; 55(3): 619-624.

111. Lazar HL, Bao Y, Gaudiani J, et al. Total complement inhibition: an effective strategy to limit ischemic injury during coronary revascularization on cardiopulmonary bypass. *Circulation*. 1999; 100(13): 1438-1442.

112. Chai PJ, Nassar R, Oakeley AE, et al. Soluble complement receptor-1 protects heart, lung, and cardiac myofilament function from cardiopulmonary bypass damage. *Circulation*. 2000; 101(5): 541-546.

113. Piddlesden SJ, Storch MK, Hibbs M, et al. Soluble recombinant complement receptor 1 inhibits inflammation and demyelination in antibody-mediated demyelinating experimental allergic encephalomyelitis. *J Immunol*. 1994; 152(11): 5477-5484.

114. Jung S, Toyka KV, Hartung HP. Soluble complement receptor type 1 inhibits experimental autoimmune neuritis in Lewis rats. *Neurosci Lett*. 1995; 200(3): 167-170.

115. Kaczorowski SL, Schiding JK, Toth CA, et al. Effect of soluble complement receptor-1 on neutrophil accumulation after traumatic brain injury in rats. *J Cereb Blood Flow Metab*. 1995; 15(5): 860-864.

116. Piddlesden SJ, Jiang S, Levin JL, et al. Soluble complement receptor 1 (sCR1) protects against experimental autoimmune myasthenia gravis. *J Neuroimmunol*. 1996; 71(1-2): 173-177.

117. Vriesendorp FJ, Flynn RE, Pappolla MA, et al. Soluble complement receptor 1 (sCR1) is not as effective as cobra venom factor in the treatment of experimental allergic neuritis. *Int J Neurosci*. 1997; 92(3-4): 287-298.

118. Huang J, Kim LJ, Mealey R, et al. Neuronal protection in stroke by an sLex-glycosylated complement inhibitory protein. *Science*. 1999; 285(5427): 595-599.

119. Twining CM, Sloane EM, Schoeniger DK, et al. Activation of the spinal cord complement cascade might contribute to mechanical allodynia induced by three animal models of spinal sensitization. *J Pain*. 2005; 6(3): 174-183.

120. Mocco J, Mack WJ, Ducruet AF, et al. Preclinical evaluation of the neuroprotective effect of soluble complement receptor type 1 in a nonhuman primate model of reperfused stroke. *J Neurosurg*. 2006; 105(4): 595-601.

121. Ramaglia V, Wolterman R, de Kok M, et al. Soluble complement receptor 1 protects the peripheral nerve from early axon loss after injury. *Am J Pathol*. 2008; 172(4): 1043-1052.

122. Ramaglia V, Tannemaat MR, de Kok M, et al. Complement inhibition accelerates regeneration in a model of peripheral nerve injury. *Mol Immunol*. 2009; 47(2-3): 302-309.

123. Li LM, Li JB, Zhu Y, et al. Soluble complement receptor type 1 inhibits complement system activation and improves motor function in acute spinal cord injury. *Spinal Cord*. 2010; 48(2): 105-111.

124. Regal JF, Fraser DG, Toth CA. Role of the complement system in antigen-induced bronchoconstriction and changes in blood pressure in the guinea pig. *J Pharmacol Exp Ther*. 1993; 267(2): 979-988.

125. Swift AJ, Collins TS, Bugelski P, et al. Soluble human complement receptor type 1 inhibits complement-mediated host defense. *Clin Diagn Lab Immunol*. 1994; 1(5): 585-589.

126. Fruchterman TM, Spain DA, Wilson MA, et al. Complement inhibition prevents gut ischemia and endothelial cell dysfunction after hemorrhage/resuscitation. *Surgery*. 1998; 124(4): 782-791; discussion 791-782.

127. Spain DA, Fruchterman TM, Matheson PJ, et al. Complement activation mediates intestinal injury after resuscitation from hemorrhagic shock. *J Trauma*. 1999; 46(2): 224-233.

128. Wagner E, Platt JL, Howell DN, et al. IgG and complement-mediated tissue damage in the absence of C2: evidence of a functionally active C2-bypass pathway in a guinea pig model. *J Immunol*. 1999; 163(6): 3549-3558.

129. Mizuno M, Nishikawa K, Okada N, et al. Soluble complement receptor type 1 protects rats from lethal shock induced by anti-Crry antibody following lipopolysaccharide priming. *Int Arch Allergy Immunol*. 2002; 127(1): 55-62.

130. Yazdanbakhsh K, Kang S, Tamasauskas D, et al. Complement receptor 1 inhibitors for prevention of immune-mediated red cell destruction: potential use in transfusion therapy. *Blood*. 2003; 101(12): 5046-5052.

131. Chen D, Song MQ, Liu YJ, et al. Inhibition of complement C3 might rescue vascular hyporeactivity in a conscious hemorrhagic shock rat model. *Microvasc Res*. 2016; 105: 23-29.

132. Zimmerman JL, Dellinger RP, Straube RC, et al. Phase I trial of the recombinant soluble complement receptor 1 in acute lung injury and acute respiratory distress syndrome. *Crit Care Med*. 2000; 28(9): 3149-3154.

133. Li JS, Sanders SP, Perry AE, et al. Pharmacokinetics and safety of TP10, soluble complement receptor 1, in infants undergoing cardiopulmonary bypass. *Am Heart J*. 2004; 147(1): 173-180.

134. Lazar HL, Bokesch PM, van Lenta F, et al. Soluble human complement receptor 1 limits ischemic damage in cardiac surgery patients at high risk requiring cardiopulmonary bypass. *Circulation*. 2004; 110(11 Suppl 1): II274-279.

135. Lazar HL, Keilani T, Fitzgerald CA, et al. Beneficial effects of complement inhibition with soluble complement receptor 1 (TP10) during cardiac surgery: is there a gender difference? *Circulation*. 2007; 116(11 Suppl): I83-88.

136. Pierson RN, 3rd, Loyd JE, Goodwin A, et al. Successful management of an ABO-mismatched lung allograft using antigen-specific immunoadsorption, complement inhibition, and immunomodulatory therapy. *Transplantation*. 2002; 74(1): 79-84.

137. Keshavjee S, Davis RD, Zamora MR, et al. A randomized, placebo-controlled trial of complement inhibition in ischemia-reperfusion injury after lung transplantation in human beings. *J Thorac Cardiovasc Surg*. 2005; 129(2): 423-428.

138. Ricklin D, Mastellos DC, Reis ES, et al. The renaissance of complement therapeutics. *Nat Rev Nephrol*. 2018; 14(1): 26-47.

139. Dicker M, Strasser R. Using glyco-engineering to produce therapeutic proteins. *Expert Opin Biol Ther*. 2015; 15(10): 1501-1516.

140. Rosen SD, Bertozzi CR. The selectins and their ligands. *Curr Opin Cell Biol*. 1994; 6(5): 663-673.

141. Foxall C, Watson SR, Dowbenko D, et al. The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis(x) oligosaccharide. *J Cell Biol*. 1992; 117(4): 895-902.

142. Lasky LA. Selectins: interpreters of cell-specific carbohydrate information during inflammation. *Science*. 1992; 258(5084): 964-969.

143. Gonzalez-Amaro R, Diaz-Gonzalez F, Sanchez-Madrid F. Adhesion molecules in inflammatory diseases. *Drugs*. 1998; 56(6): 977-988.

144. Rittershaus CW, Thomas LJ, Miller DP, et al. Recombinant glycoproteins that inhibit complement activation and also bind the selectin adhesion molecules. *J Biol Chem*. 1999; 274(16): 11237-11244.

145. Mulligan MS, Lowe JB, Larsen RD, et al. Protective effects of sialylated oligosaccharides in immune complex-induced acute lung injury. *J Exp Med*. 1993; 178(2): 623-631.

146. Mulligan MS, Paulson JC, De Frees S, et al. Protective effects of oligosaccharides in P-selectin-dependent lung injury. *Nature*. 1993; 364(6433): 149-151.

147. Stanley P, Atkinson PH. The LEC11 Chinese hamster ovary mutant synthesizes N-linked carbohydrates containing sialylated, fucosylated lactosamine units. Analysis by one- and two-dimensional 1H NMR spectroscopy. *J Biol Chem*. 1988; 263(23): 11374-11381.

148. Picard MD, Pettey CL, Marsh HC, et al. Characterization of N-linked oligosaccharides bearing sialyl lewis x moieties on an alternatively glycosylated form of soluble complement receptor type 1 (sCR1). *Biotechnol Appl Biochem*. 2000; 31(1): 5-13.

149. Ducruet AF, Mocco J, Mack WJ, et al. Pre-clinical evaluation of an sLe x-glycosylated complement inhibitory protein in a non-human primate model of reperfused stroke. *J Med Primatol*. 2007; 36(6): 375-380.

150. Dong J, Pratt JR, Smith RA, et al. Strategies for targeting complement inhibitors in ischaemia/reperfusion injury. *Mol Immunol*. 1999; 36(13-14): 957-963.

151. Dodd I, Mossakowska DE, Camilleri P, et al. Overexpression in Escherichia coli, folding, purification, and characterization of the first three short consensus repeat modules of human complement receptor type 1. *Protein Expr Purif*. 1995; 6(6): 727-736.

152. Krych-Goldberg M, Hauhart RE, Subramanian VB, et al. Decay accelerating activity of complement receptor type 1 (CD35). Two active sites are required for dissociating C5 convertases. *J Biol Chem*. 1999; 274(44): 31160-31168.

153. Smith GP, Smith RA. Membrane-targeted complement inhibitors. *Mol Immunol*. 2001; 38(2-3): 249-255.

154. Linton SM, Williams AS, Dodd I, et al. Therapeutic efficacy of a novel membrane-targeted complement regulator in antigen-induced arthritis in the rat. *Arthritis Rheum*. 2000; 43(11): 2590-2597.

155. Smith RA. Targeting anticomplement agents. *Biochem Soc Trans*. 2002; 30(Pt 6): 1037-1041.

156. Pratt JR, Jones ME, Dong J, et al. Nontransgenic hyperexpression of a complement regulator in donor kidney modulates transplant ischemia/reperfusion damage, acute rejection, and chronic nephropathy. *Am J Pathol*. 2003; 163(4): 1457-1465.

157. Halstead SK, Humphreys PD, Goodfellow JA, et al. Complement inhibition abrogates nerve terminal injury in Miller Fisher syndrome. *Ann Neurol*. 2005; 58(2): 203-210.

158. Souza DG, Esser D, Bradford R, et al. APT070 (Mirococept), a membrane-localised complement inhibitor, inhibits inflammatory responses that follow intestinal ischaemia and reperfusion injury. *Br J Pharmacol*. 2005; 145(8): 1027-1034.

159. Patel H, Smith RA, Sacks SH, et al. Therapeutic strategy with a membrane-localizing complement regulator to increase the number of usable donor organs after prolonged cold storage. *J Am Soc Nephrol*. 2006; 17(4): 1102-1111.

160. Banz Y, Hess OM, Robson SC, et al. Attenuation of myocardial reperfusion injury in pigs by Mirococept, a membrane-targeted complement inhibitor derived from human CR1. *Cardiovasc Res*. 2007; 76(3): 482-493.

161. Xiao F, Ma L, Zhao M, et al. APT070 (mirococept), a membrane-localizing C3 convertase inhibitor, attenuates early human islet allograft damage in vitro and in vivo in a humanized mouse model. *Br J Pharmacol*. 2016; 173(3): 575-587.

162. Wu G, Pfeiffer S, Schroder C, et al. Coagulation cascade activation triggers early failure of pig hearts expressing human complement regulatory genes. *Xenotransplantation*. 2007; 14(1): 34-47.

163. Duehrkop C, Banz Y, Spirig R, et al. C1 esterase inhibitor reduces lower extremity ischemia/reperfusion injury and associated lung damage. *PLoS One*. 2013; 8(8): e72059.

164. De Silva RJ, Vuylsteke A, Fritchley SJ, et al. APT070 inhibits complement activation during in vitro cardiopulmonary bypass. *Eur J Cardiothorac Surg*. 2006; 30(1): 72-76.

165. Proceedings and abstracts of the XIth European Meeting on Complement in Human Disease. *Mol Immunol*. 2007; 44(16): 3809-3994.

166. Kassimatis T, Qasem A, Douiri A, et al. A double-blind randomised controlled investigation into the efficacy of Mirococept (APT070) for preventing ischaemia reperfusion injury in the kidney allograft (EMPIRIKAL): study protocol for a randomised controlled trial. *Trials*. 2017; 18(1): 255.

167. Kassimatis T, Greenlaw R, Hunter JP, et al. Ex vivo delivery of Mirococept: A dose-finding study in pig kidney after showing a low dose is insufficient to reduce delayed graft function in human kidney. *Am J Transplant*. 2021; 21(3): 1012-1026.

168. Klickstein LB, Barbashov SF, Liu T, et al. Complement receptor type 1 (CR1, CD35) is a receptor for C1q. *Immunity*. 1997; 7(3): 345-355.

169. Jacquet M, Lacroix M, Ancelet S, et al. Deciphering complement receptor type 1 interactions with recognition proteins of the lectin complement pathway. *J Immunol*. 2013; 190(7): 3721-3731.

170. Reilly BD, Makrides SC, Ford PJ, et al. Quantitative analysis of C4b dimer binding to distinct sites on the C3b/C4b receptor (CR1). *J Biol Chem*. 1994; 269(10): 7696-7701.

171. Medof ME, Nussenzweig V. Control of the function of substrate-bound C4b-C3b by the complement receptor Cr1. *J Exp Med*. 1984; 159(6): 1669-1685.

172. Krych M, Clemenza L, Howdeshell D, et al. Analysis of the functional domains of complement receptor type 1 (C3b/C4b receptor; CD35) by substitution mutagenesis. *J Biol Chem*. 1994; 269(18): 13273-13278.

173. Wymann S, Mischnik M, Leong D, et al. Sialylation-dependent pharmacokinetics and differential complement pathway inhibition are hallmarks of CR1 activity in vivo. *Biochem J*. 2022; 479(9): 1007-1030.

174. Morell AG, Gregoriadis G, Scheinberg IH, et al. The role of sialic acid in determining the survival of glycoproteins in the circulation. *J Biol Chem*. 1971; 246(5): 1461-1467.

175. Park EI, Manzella SM, Baenziger JU. Rapid clearance of sialylated glycoproteins by the asialoglycoprotein receptor. *J Biol Chem*. 2003; 278(7): 4597-4602.

176. Otten MA, Groeneveld TW, Flierman R, et al. Both complement and IgG fc receptors are required for development of attenuated antiglomerular basement membrane nephritis in mice. *J Immunol*. 2009; 183(6): 3980-3988.

177. Bongoni AK, Vikstrom IB, McRae JL, et al. A potent truncated form of human soluble CR1 is protective in a mouse model of renal ischemia-reperfusion injury. *Sci Rep*. 2021; 11(1): 21873.