HHS Public Access
Author manuscript
Circ Res. Author manuscript; available in PMC 2024 April 28.

Published in final edited form as:
Circ Res. 2023 April 28; 132(9): 1185–1202. doi:10.1161/CIRCRESAHA.123.321673.

Clinical Potential of Adrenomedullin Signaling in the Cardiovascular System

László Bálint, PhD[1], **Nathan Nelson-Maney**[1], **Yanna Tian, PhD**[1], **D. Stephen Serafin**[1], **Kathleen M. Caron, PhD**[1]

[1.]Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill; 111 Mason Farm Road, Chapel Hill, North Carolina, USA 27599

Abstract

Numerous clinical studies have revealed the utility of circulating adrenomedullin (AM) or mid-regional pro-adrenomedullin (MR proAM) as an effective prognostic and diagnostic biomarker for a variety of cardiovascular-related pathophysiologies. Thus, there is strong supporting evidence encouraging the exploration of the AM- calcitonin receptor like receptor (CLR) signaling pathway as a therapeutic target. This is further bolstered because several drugs targeting the shared calcitonin gene-related peptide (CGRP) - CLR pathway are already FDA approved and on the market for the treatment of migraine. In this review, we summarize the AM – CLR signaling pathway as well as its modulatory mechanisms and provide an overview of the current understanding of the physiological and pathological roles of AM – CLR signaling and the yet untapped potentials of AM as a biomarker or therapeutic target in cardiac and vascular diseases and provide an outlook on the recently emerged strategies that may provide further boost to the possible clinical applications of AM signaling.

Keywords

Adrenomedullin; Cardiovascular Disease; Biomarker; Therapeutic Target; Clinical Trials

CELLULAR PHYSIOLOGY AND CLINICAL POTENTIAL OF ADRENOMEDULLIN

Biosynthesis

Adrenomedullin (Gene = *Adm*; Protein = AM) is a 52 amino acid peptide hormone that is a member of the calcitonin family of peptides alongside calcitonin gene-related peptide (CGRP), amylin, and adrenomedullin 2/intermedin. AM was first isolated from the adrenal medulla[1], but secreted in high amounts by endothelial cells (EC) and vascular smooth muscle cells[2]. Similarly to many other hormones, during AM biosynthesis, first preproadrenomedullin, a large precursor protein is synthetized, which is then cleaved

Corresponding Author: Kathleen M. Caron, PhD, 111 Mason Farm Rd., 6312 MBRB, CB 7545, Department of Cell Biology and Physiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA 27599 kathleen_caron@med.unc.edu.

Disclosures: None.

into 4 peptides (namely, proAM N-terminal 20 peptide (PAMP)-Gly, mid-regional pro-adrenomedullin 45-92 (MR pro-AM), AM-Gly, and C-terminal pro-AM[3]. Active AM is produced by the cleavage of C-terminal Gly from AM-Gly. Cytokines[4], lipopolysaccharide (LPS)[5], pressure overload[6], vascular shear stress[7], and hypoxia[8] have been shown to stimulate AM synthesis and release.

AM has a half-life of 20-25 minutes in the blood circulation[9], limiting its application as a biomarker. In contrast, the biologically inactive MR-proADM, cleaved from the precursor protein of AM, has been found to be a stable and reliable molecule acting as a surrogate of AM peptide, independent of its degradation[10]. In cases where real time assessment of instantaneous AM levels is needed, biologically active AM concentrations might be preferable, but given time constraints in most clinical scenarios, in almost all pathologies, MR proAM has more potential as a biomarker.

AM's short half-life also impairs its therapeutic potential. In order to reach adequate plasma concentrations of AM, continuous intravenous administration is required. When infusion is halted plasma concentrations drop precipitously, again reflecting the short half-life of AM[9]. Adrecizumab is a humanized non-neutralizing monoclonal antibody targeting AM, leading to a significant increase in plasma AM levels without changing MR proAM concentrations[11], effectively increasing the amount of bioactive AM in the plasma. Adrecizumab presumably functions by preventing the extravascularization or slowing the degradation of AM in the plasma (Table 1), thereby increasing effective plasma levels of AM and elongating the timeframe of its action.

Receptors and downstream signaling mechanisms

AM predominantly acts by binding to its class B G protein coupled receptor, calcitonin-receptor like receptor (Gene = *Calcrl*; Protein = CLR) (Figure 1). G protein-coupled receptors (GPCRs) are 7 transmembrane receptors that play critical roles in many physiological processes through signal initiation and propagation, thereby making them ideal drug targets[12]. To establish biologically active receptors for the members of the calcitonin family of peptides, CLR must form a heterodimer with one of three receptor activity-modifying proteins (RAMPs1-3); the CGRP receptor (CLR/ RAMP1), the AM_1 receptor (CLR/ RAMP2), and the AM_2 receptor (CLR/ RAMP3). RAMPs, which were first discovered and characterized due to their relationship with CLR, are single pass transmembrane proteins that interact and allosterically regulate GPCRs. While originally thought to only interact with a select group of GPCRs, there is an ever-expanding list of known RAMP-interacting GPCRs among all GPCR classes[13]. RAMPs regulate GPCRs in a RAMP-GPCR dependent manner; therefore, the specific CLR-RAMP pairings have broad pharmacological and physiological consequences. Not only does CLR require interaction with RAMPs to be chaperoned to the plasma membrane, the three CLR-RAMP endogenous ligands have differential affinity for the receptor-complex dependent on which RAMP is present. That is, CGRP preferentially binds the CLR/ RAMP1 complex, while AM preferentially binds the CLR/ RAMP2 dimer, followed by the CLR/ RAMP3 coupling. The CLR/RAMP3 heterodimer is also the preferred receptor complex of AM2/intermedin[14,15].

There has been exciting advancement in our understanding of CLR-RAMP signal initiation, propagation, and desensitization over the last several years. It is well established that ligand (CGRP, AM, AM2) binding to CLR-RAMP complexes results in intracellular cAMP accumulation and calcium mobilization. That being said, using modified yeast strains and HEK-293 cells, Weston et al. show that CLR-RAMP complexes can couple with multiple types of Gα subunits and demonstrate that coupling to these alternative Gα subunits alters the ligand binding potencies of the CLR-RAMP receptor complexes[16]. After agonist-stimulation, besides activation of Gα mediated signaling pathways, β-arrestins-1 and −2 are also recruited to ligand-bound CLR receptor complexes, and are required for the rapid internalization, endosomal sorting, and desensitization of these receptor complexes. However, while full agonism does not require β-arrestins-1 and −2 recruitment, their recruitment may initiate signaling cascades independent of $G\alpha_s$[17]. Excitingly, CLR-RAMP complexes are among a growing list of GPCRs shown to continue to signal from endosomes and not solely just at the plasma membrane. For example, Yarwood et al. demonstrate that CLR-RAMP1 signaling endosomes promote migraine pain transmission[18]. Downstream intracellular consequences of cyclic adenosine monophosphate (cAMP) production, increase in $[Ca^{2+}]_{IC}$ levels, β-arrestins-1 and −2-mediated recruitment and internalization, and endosomal signaling result in the activation of downstream effectors such as mitogen-activated protein kinase (MAPK) cascade as well as the phosphorylation of AKT and cAMP response element-binding protein (CREB) to initiate cellular responses and nitrogen monoxide (NO) production[15]. Recent articles found that AM-CLR signaling preferably signals through $G\alpha_s$-mediated signaling mechanisms over $G\alpha_q$ in Cos7 and HEK293s cell lines transfected with CLR-RAMP receptor constructs [19,20]. On the other hand, Clark et al. demonstrated that AM and AM2-dependent $[Ca^{2+}]_{IC}$ mobilization can be impeded by co-treatment with an anti- $G\alpha_{q/11/14}$ inhibitor in human umbilical vein endothelial cells (HUVECs) [15]. Moreover, HUVECs and human umbilical arterial endothelial cells (HUAECs) show different downstream signaling bias[15], highlighting the importance of the cell type for CLR signaling. It has been also demonstrated that AM signaling can lead to a ligand-independent transactivation of the vascular endothelial growth factor receptor 2 (VEGFR2) in HUVECs and immortalized endothelial cell lines[21], and VEGFR3 in lymphatic endothelial cells (LECs) [22], respectively. Therefore, besides $G\alpha_s$ signaling, other alternative intracellular mechanisms might also contribute to AM-dependent downstream cAMP production in endothelial cells. Further studies elucidating the downstream signaling preference of AM on different cell types and unveiling the underlying molecular mechanisms may help us understand the cell type-specific properties of AM signaling.

As discussed more in detail below, both increased and decreased AM may lead to pathology. Therefore, a tight regulation of the biological levels of AM peptide by ACKR3 is essential for proper AM function. Atypical chemokine receptor 3 (ACKR3, other common aliases: CXCR7, RDC-1, GPR159) is a 7 transmembrane receptor, with the highest expression in fibroblasts and ECs[23], proposing its importance during tissue repair and in vascular physiology. Currently, known ligands of ACKR3 include chemokine x ligand 11 (CXCL11, also known as I-TAC), CXCL12 (also known as SDF-1)[24], macrophage migration-inhibitory factor [25], virus-encoded chemokine vCCL2/vMIP-II[26], endogenous opioid peptides[27,28], PAMPs [29], and AM[30]. Upon ligand binding, ACKR3 recruits G protein-coupled receptor kinases that phosphorylate the C-terminal tail of ACKR3 recruiting arrestin which in turn promotes ligand internalization and degradation[31-34]. Through this mechanism, ACKR3 eliminates the bound AM molecules and thereby regulates its function[35].

Current therapeutic applications

Because of their crucial physiological and pathological roles and pharmacological tractability, GPCR signaling pathways are intensively studied drug targets. Approximately 1/3 of the FDA-approved drugs act on GPCRs[12]. Currently a highly successful clinical approach targeting the AM receptor CLR is being applied for the inhibition of the AM-related peptide CGRP in migraine patients. CGRP is a neuropeptide that is produced by Aδ and C nerve fibers and functions in pain sensation and vasodilation[36-38]. Given these functions, it is a compelling biomarker for various human pathophysiologies, the foremost being migraine[39]. In 1988 CGRPs role in activation of the trigeminovascular system was described by Dr. Lars Edvinsson[40] and in 2003 elevated CGRP was identified in the cerebrospinal fluid in migraine patients[41]. A later study demonstrated CGRP elevation in jugular venous blood of patients with migraines[42], suggesting it as a potential biomarker for migraine. Additionally, a recent meta-analysis of genome wide association studies examining risk loci in migraine has identified polymorphisms in the gene encoding CGRP (*Calca*/*Calcb*) as a novel risk locus in migraine[43]. Migraine is extremely responsive to anti-CGRP targeting medications such as monoclonal antibodies targeting a shared epitope between CGRP/RAMP1 (erenumab)[44] or soluble CGRP (fremanezumab, galcanezumab, and eptinezumab) or small molecule receptor antagonists (‘gepant class medications)[45]. Since 2018, four CGRP-inhibitor monoclonal antibodies and three small molecule inhibitors have been approved to date.

The successful therapeutic application of agents targeting CLR/RAMP1 signaling demonstrates that CLR signaling is a clinically targetable pathway with a high clinical efficacy. Thus, it stands to reason that CLR/RAMP2 and CLR/RAMP3 heterodimers may also be exploited as therapeutic targets for manipulating AM signaling. Therefore, understanding the physiological functions of AM and its roles in disease helps us to evaluate the clinical potential of AM and to determine future directions for its use as a biomarker and therapeutical target.

VASCULAR AND CARDIAC FUNCTIONS OF ADRENOMEDULLIN

Vasoactive functions

AM peptide is best known for its vasoactive functions. As a part of its intracellular signaling cascades, AM increases intracellular cAMP and Ca2+ levels in endothelial cells, leading to endothelial nitric oxide synthase (eNOS) activation and NO formation[7,15,46]. Endothelial NO then diffuses to the surrounding vascular smooth muscle (VSM) cells, promoting smooth muscle relaxation and vasodilation. Other preclinical results demonstrated that AM also acts on VSM cells directly, and promotes VSM relaxation through increasing cAMP levels and inhibiting endothelin production[47]. A clinical study demonstrated that intravenous administration of 50 ng·kg−1·min−1 AM is efficient to decrease mean arterial blood pressure of healthy subjects[48], and the authors suggested that this AM-mediated vasodilation mechanism contributes to basal vascular tone regulation. A recent article also demonstrated this role of AM signaling in basal blood pressure control [7]. Regardless, because AM has many known functions, it is unlikely to be useful as a specific therapeutic agent in the management of hypertension.

Vascular development and remodeling

Besides regulating basal vascular tone, AM also contributes to numerous other cardiovascular mechanisms. *Adm* null and *Calcrl* null mouse embryos do not display abnormal vascular expansion, but studies reported reduction in arterial wall thickness and VSM coverage and abnormal basal membrane structure in the larger vessels. These findings suggest a role for AM in vascular remodeling and maintaining vascular barrier integrity, but not in embryonic angiogenesis[49-51].

Cardiac development

The role of the AM/CLR/RAMP pathway in cardiac development has been extensively studied. AM deficient mouse embryos die at mid-gestation due to hydrops fetalis and cardiovascular defects, including overdeveloped ventricular trabeculae, underdeveloped arterial walls with reduced myocardial proliferation and increased apoptosis[49]. Deletion of AM receptors *Calcrl*, or *Ramp2* also leads to embryonic lethality characterized by hydrops fetalis, thin vascular smooth muscle walls, and small disorganized hearts[51]. Additionally, *Ramp2* null embryos display systemic and cardiac edema, small hearts, and vascular defects including vascular wall structure disruption, diminished junctional protein expression, increased vascular permeability, and diminished neovascularization[52,53]. Thus, the embryological phenotypes of these deficient mouse lines is conserved, supporting a requisite role for this signaling pathway in cardiac and vascular development.

In contrast, $Adm^{hi/hi}$ mice overexpressing AM have enlarged hearts due to hyperplasia, which can be reversed with reduction of AM levels[54]. Similarly, mouse embryos lacking the AM decoy receptor ACKR3 develop enlarged hearts due to cardiomyocyte hyperplasia and die by postnatal day 1 due to cardiac valve defects[55,56]. Similar to $Adm^{hi/hi}$ mice, genetic reduction of AM in the ACKR3 null mice is sufficient to reverse the embryonic cardiac hyperplasia[35].

Lymphatic development

The lymphatic vasculature is an open-ended vessel system that functions primarily to drain excess interstitial fluid, traffic leukocytes to immune organs, and facilitate dietary lipid absorption, among many other tissue-specific roles[57]. In mice, development of the lymphatic system starts around embryonic day 10.5, when lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) / prospero homeobox protein 1 (PROX1) double positive endothelial cells first appear in the cardinal vein. These cells bud from the cardinal vein and form the jugular lymph sac, the first lymphatic structure[58]. Eventually, a mature lymphatic vessel network derived from both venous and non-venous LEC precursors develops to enmesh most vascularized organs[59].

PROX1 is a key transcription factor for the determination and maintenance of lymphatic identity and function and is continually expressed by lymphatic endothelial cells. Overexpression of PROX1 in LECs has been shown to induce *Calcrl* expression levels. Because Prox1 is a key transcription factor for maintaining lymphatic cell fate, it is not surprising that expression levels of *Adm*, *Calcrl* and *Ramp2* are significantly higher in cultured LECs than blood endothelial cells (BECs)[52]. Not surprisingly, $Adm^{-/-}$, $Calcrl^{-/-}$ and $Ramp2^{-/-}$ mouse embryos all display lymph sac hypoplasia at E13.5[52], and hydrops fetalis[49,51,60], revealing the importance of AM signaling in lymphatic development. The relevance of the AM/CLR/RAMP2 signaling pathway for human lymphatic development and survival was also uncovered from whole exome sequencing of a 4-generation family pedigree, revealing a recessive in frame mutation in the *CALCRL* gene that was homozygous in two infants with nonimmune hydrops fetalis. Biochemical characterization of the receptor mutation showed reduced association with RAMP2 protein, thereby precluding receptor presentation to the cell surface[60].

Other studies have shown that AM treatment stabilizes LEC intercellular junctions *in vitro*, and AM administration improves lymphatic barrier function and decreases lymphatic permeability *in vivo*[61]. This suggests that disruption of the AM/CLR/RAMP2 signaling axis leads to systemic edema, at least partially due to impaired lymphangiogenesis and altered lymphatic vessel permeability. Of note, ACKR3 deficient mouse embryos however, had blood-filled, enlarged lymph sacs interstitial edema, and dilated and aberrant lymphatic vasculature, emphasizing that precise regulation of AM levels and signaling is crucial for lymphatic vessel function[35].

In adult mice, inducible postnatal global deletion of CLR results in disorganized subcellular organization of the junctional proteins vascular endothelial cadherin (VE-Cadherin) and zona occludens 1 (ZO-1), leading to disrupted lymphatic vessel permeability and impaired dietary lipid absorption[62-64], highlighting the central role of AM signaling in maintaining proper lymphatic function in adulthood. Because VE-Cadherin and ZO-1 are also important junctional proteins in the blood vasculature, it is likely that AM promotes blood vascular barrier integrity through a similar molecular mechanism. Furthermore, VE-Cadherin has been shown to be essential for maintaining pro-lymphangiogenic VEGFR3 signaling nodes at the plasma membrane in LECs[22], providing additional mechanisms through which AM signaling exerts its pro-lymphangiogenic effects.

In summary, AM plays important roles in cardiac development, lymphatic growth, and vascular permeability. It is likely that upregulation of AM promotes cardiac tissue regeneration, regulates immune responses, helps resolve cardiac edema after injury, and reduces fibrosis following injury by the mechanisms discussed above in detail. In Figure 2, we summarize these diverse biological functions of AM, and mark which of these functions contribute to cardiovascular pathologies or to diseases with significant cardiovascular involvement as discussed below.

PATHOPHYSIOLOGICAL ROLES AND POTENTIAL THERAPEUTIC APPLICATIONS OF AM IN CARDIOVASCULAR DISEASE

AM has potent cardiovascular activity. As a potent vasodilator, it reduces arterial blood pressure, decreases peripheral vascular resistance, thereby increasing heart rate and cardiac output[65]. Moreover, it is also essential for maintaining vascular integrity[66] and promotes angiogenesis under hypoxic conditions[67]. Given these functions of AM in regulating cardiovascular physiology and development, it is not surprising that AM also plays a role in numerous cardiovascular diseases. Here, we provide a brief summary of those conditions where AM has emerged as a biomarker or therapeutic target due to its cardiovascular functions.

Heart failure

Currently, natriuretic peptides, such as atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) and their prohormones proANP and proBNP are commonly used prognostic biomarkers in patients with heart failure. Natriuretic peptides are secreted from the atria and ventricles and provide an accurate prediction on heart failure (HF) mortality and hospitalization[68]. Clinically however, ANP/BNP utility is limited by high variability and these biomarkers of heart failure are not sufficient to guide management of HF alone. Therefore, additional predictive molecular markers could help to improve both the specificity and sensitivity of HF related clinical testing.

Shortly after the discovery of AM, studies reported a significant increase in plasma AM levels in heart failure patients[69,70], proposing AM as a potential prognostic biomarker of HF. Clinical studies have reported high levels of MR-proAM and bioactive AM as independent prognostic biomarkers of hemodynamic impairment, tissue congestion, organ dysfunction and to predict mortality risk of HF patients (Table 2)[69,71-74]. While some researchers proposed MR-proAM 45-92 as a more accurate prognostic marker of HF than the natriuretic peptides[71,75-77], other studies questioned the additional prognostic value of MR-proADM[78-80]. As discussed in detail in this manuscript, AM is involved in numerous physiological or pathophysiological processes. Therefore, multiple underlying mechanisms might be responsible for the high levels of plasma AM or MR proAM. Combination of MR proAM with additional biomarkers will improve the specificity of AM as a biomarker.

The clinical potential of AM in heart failure is not limited to its application as a biomarker. An early ex vivo study revealed the positive inotropic effect of AM[81], by which AM can increase stroke volume and therefore cardiac output and systolic blood pressure[82]. On the other hand, however, vasodilatory effects of AM combined with its positive inotropic properties may promote the development of hyperdynamic circulation (Figure 2). An ongoing phase II clinical study is aiming to evaluate safety and tolerability of an Adrecizumab in patients with acute HF. This suggests an alternative approach for targeting AM in HF patients.

Myocardial Infarction

Heart disease is the leading cause of death and over 800,000 people have myocardial infarction (MI) annually in the United States only [83]. Though MI is generally treated by coronary artery catheterization or with thrombolytic agents, novel diagnostic and therapeutic approaches are desirable in order to improve long term outcomes and provide additional information about end organ damage. Early studies reported an immediate elevation in plasma AM levels following myocardial infarction[84]. Recent clinical data have also indicated that MR-proAM is a reliable predictive marker to predict long-term mortality, volume overload, and congestion during recovery from MI, but similar to HF, the additional value to the currently used biomarkers is questioned (Table 2) [85,86].

In addition to ECs and smooth muscle cells, mesenchymal stem cells have also been reported to contribute to AM expression after myocardial infarction [87]. An early study reported elevated ANP secretion in AM-treated cultured rat myocytes, and reduced collagen production in AM-treated cultured fibroblasts[88]. Preclinical studies found that AM administration after myocardial infarction reduced pathologic tissue remodeling and fibrosis formation post injury, suggesting AM as a possible therapeutic target after MI[89,90]. Similarly, cardioprotective effects of AM administration were shown in preclinical HF models[91,92]. Pilot clinical studies have suggested intravenous infusion of AM improves cardiac outcomes in patients recovering from acute MI[93] and that co-treatment with ANP as a potential therapeutic approach for patients with acute decompensated HF[94].

AM overexpressing $Adm^{hi/hi}$ mice have a more robust cardiac lymphangiogenic response following MI which was correlated with improved cardiac function and reduced cardiac edema and tissue remodeling when compared to wild type controls[95]. Moreover, a recent study suggested that lymphatic vessels promote embryonic heart development as well as MI recovery mediated by reelin secretion[96]. These findings suggest that the cardioprotective effects of AM might be partly related to its pro-lymphangiogenic function. The currently available experimental results suggest a reactivation of embryonic epicardial gene expression program upon cardiac injury, including the epicardial expression of AM[97].

Furthermore, the current body of literature suggests that AM plays an important role in cardiac function and recovery following insult, indicating a potential therapeutic role for AM in numerous cardiac diseases. AM's short half-life significantly decreases its therapeutic potential. Therefore, pharmaceutical strategies to increase the half-life of AM might improve its usefulness as a therapeutic agent.

Pulmonary hypertension and congestion

Plasma AM and MR pro-AM levels have been suggested as reliable prognostic markers in patients with pulmonary hypertension[98,99]. Moreover, inhalation of aerosolized AM was shown to improve survival as well as reduce pulmonary arterial pressure and pulmonary vascular resistance in rats with monocrotaline-induced pulmonary hypertension (Figure 2)[100]. Although clinical pilot studies have proposed AM as a promising therapeutic target to treat pulmonary hypertension[101,102], its short half-life is once again a significant obstacle in AM's therapeutic potential. Though pulmonary congestion is associated with higher mortality in HF patients[103,104], assessment and staging of pulmonary congestion remains challenging and lacks standardization[105-107]. A recent study found AM to be the strongest clinical predictor of pulmonary congestion[74], suggesting that despite the debate over the added value of AM testing to predict mortality risk in HF patients, AM levels may be a valuable tool in determining therapeutic approach.

Importantly, pulmonary hypertension and congestion are not the only pulmonary vascular conditions where clinical application of AM have emerged. A promising diagnostic approach takes advantage of the fact that CLR expression is abundant in the alveolar endothelium[108]. PulmoBind, a radiolabeled AM derivative that can be used to detect pulmonary microcirculatory occlusions and abnormalities with single-photon emission computerized tomography (SPECT) has passed phase I and II clinical trials[109,110]. This represents a promising tool for the early detection of pulmonary embolism and of other pathophysiologies that alter circulation through the pulmonary vasculature.

Stroke

Under pathophysiological circumstances, such as ischemia, preclinical studies demonstrated that AM promotes blood and lymphatic vessel expansion[111-113], suggesting AM as a potential biomarker and therapeutic target for ischemic diseases. Moreover, a preclinical study found anti-apoptotic effects of AM infusion on neuronal cells and transplanted mesenchymal stem cells [114], showing that direct vascular and non-vascular effects of AM might improve stroke outcomes. An earlier human study found elevated AM levels in the cerebrospinal fluid (CSF) but not in the plasma of patients with cerebral vasospasm after hemorrhage[115]. Other researchers reported elevated plasma AM and mid-regional pro-AM levels in both ischemic and acute hemorrhagic stroke patients[116-118]. These findings suggest AM as a potential diagnostic biomarker of stroke. Moreover, its neuroprotective properties, such as reducing neuron apoptosis and promoting oligodendrocyte differentiation[119-121], make AM an even more promising therapeutic target in stroke (Figure 2). A current phase II study aims to use AM as a treatment option for ischemic stroke[122].

Sepsis

Sepsis is a life threatening extreme immune reaction of the body to disseminated infection. AM and MR pro-AM levels both are increased in patients with sepsis, and correlate with increased disease severity and mortality risk[123-126]. The use of AM as a morbidity-independent sepsis biomarker would help predict illness severity and inform personalized therapy decisions for septic patients. The therapeutic effect of increasing AM as a treatment strategy in sepsis has also been intensively studied. Administration of AM exerts anti-inflammatory, antimicrobial, and protective effects on endothelial barrier function during sepsis[127]. On the other hand, as AM has both vasodilatory and positive inotropic roles, elevated AM can lead to hyperdynamic circulation, which may contribute to sepsis severity and push the patient toward developing septic shock (Figure 2). Adrecizumab has been shown to the improve vascular barrier maintenance function of AM but does not promote AM detrimental vasoactive effects[128], which might be beneficial for septic patients. Of note, it is currently it is not known if Adrecizumab also alters other functions of AM. A phase II clinical trial of 301 patients found that both 2 and 4 mg/BWkg adrecizumab is well tolerated and organ failure scores were significantly improved compared to placebo (Table 2). Additionally, 28-day mortality trended lower in adrecizumab treated septic patients than placebo controls, but was not significant (Table 1)[129].

Other diagnostic potentials of AM in cardiovascular diseases

Adrenomedullin has recently been implicated in a variety of cardiovascular diseases, expanding its diagnostic and therapeutic potential. Interestingly, in contrast to the low AM expression levels in adult heart tissue under physiological conditions, increased AM immunoreactivity was found to be correlated with myocyte hypertrophy in the endomyocardium of transplanted hearts in contrast to the low levels of AM expression in adult, non-hypertrophic cardiac tissue under physiological conditions[130]. MR pro-AM has recently emerged as a promising prognostic biomarker of aortic stenosis and severity and to predict mortality risk after transcatheter aortic valve replacement[131,132], which is probably also related to AM's vasoactive function.

Lymphedema

The lymphatic system plays an important role in maintaining tissue fluid homeostasis. Failure or impaired function of the lymphatic vascular system results in disfiguring, disabling, and sometimes life-threatening swelling of the affected tissues, called lymphedema[133]. Lymphedema occurs as a result of a hereditary genetic mutations in the genes involved in lymphatic development and function (primary lymphedema), or after surgical removal of lymph nodes, radiation therapy, or parasite infections (secondary lymphedema)[134]. Despite its high incidence and serious effects on the patients' quality of life, no effective treatment is available for lymphedema currently. As AM signaling has important roles in promoting lymphangiogenesis and modulating lymphatic function, it is a promising therapeutic target to treat patients with lymphedema.

In addition to the severe edema reported in AM, CLR and RAMP2 deficient mouse embryos, other studies also support the anti-edematous effects of AM signaling. In addition, mice lacking RAMP3, although do not show embryonic edematous phenotype, have impaired lymphatic drainage. Moreover, in a mouse model of lymphedema, RAMP3 deficient mice represent more severe edema and display defective lymphatic cell migration when challenged with scratch wound assay[135]. This suggests the importance of CLR/RAMP3 mediated signaling in lymphedema in addition to CLR/RAMP2 signaling. Accordingly, dilated dermal lymphatics were reported after induced global deletion of CLR. CLR deleted mice developed a much more severe local edema after hind paw injection of complete Freund's adjuvant[62]. AM haploinsufficient mice, but not wild type controls developed a lymphedematous phenotype following hind limb skin incision, which could be restored by exogenous AM administration[136]. Following hind limb skin incision elevated AM expression was found in both wild type and AM haploinsufficient mice compared to baseline, indicating that AM signaling was triggered by the surgery. In addition, another research group reported that continuous AM administration promoted the angiogenic and lymphangiogenic response and resolved lymphedema severity in a mouse model of lymphedema (Figure 2)[112].

These preclinical studies suggest that AM is a potent therapeutic target to treat lymphedema. For its therapeutic use in patients with lymphedema however, a reliable delivery method that provides long lasting local AM signal is required, which is currently not available.

CARDIOVASCUALR CORRELATES IN OTHER PREVALENT DISEASES

Tumors

There is a large and ever-expanding amount of evidence linking AM as well as the AM receptors (AM_1, AM_2, and ACKR3) to cancer pathogenesis and metastasis. Vázquez et al. 2021 have provided a comprehensive review highlighting much of this research, including preclinical studies assessing the efficacy of anti-AM therapies in neoplasia (ie. AM-neutralizing antibodies)[137]. Overall, the pro-tumorigenic properties of AM are not surprising given that AM has known roles in lymphangiogenesis and angiogenesis, cellular proliferation, and cell survival[138]. A variety of solid tumors as well as other stromal cell-types present within the tumor microenvironment, such as endothelial cells, CAFs and TAMs, express the AM and its receptors (CLR/RAMP1, CLR/RAMP2, CLR/RAMP3, ACKR3)[137,139-141]. AM signaling promotes tumor cell growth, proliferation, and survival[139]. Second, AM secreted within the tumor microenvironment facilitates tumor-associated angiogenesis and -lymphangiogenesis, thereby providing the tumor microenvironment with nutrients and oxygen to promote continued survival and growth and contributing to metastasis formation. [142,143] (Figure 2). Importantly, AM and CLR overexpression is correlated with a worse prognosis, disease severity, and relapse for a variety of cancers such as in acute myeloid leukemia (AML)[144-149]. However, these findings are context- and cancer-dependent whereby AM is associated with a more favorable prognosis for other cancer types such as triple-negative breast cancer [150]. That being said, the contribution of AM and the AM receptors to cancer pathogenesis is undeniable, sparking a new wave of anti-AM therapies that are currently being assessed for efficacy in various clinical trials[137].

Pneumonia

Besides hypoxia, pro-inflammatory cytokines and LPS also promote AM release, so that infections and inflammatory responses trigger AM secretion. Broadly, the inflammatory condition of pulmonary pneumonia infection, resulting in tissue damage and alveolar fluid accumulation, has been closely associated with pathophysiological levels of AM peptide, which, given its vascular roles, might increase local blood flow and promote a lymphangiogenic response, supporting local immune responses and edema resolution. A study found increased proAM levels in community-acquired pneumonia (CAP) patients who died during follow-up compared with survivors. The researchers found that pneumonia severity index (PSI) combined with plasma proAM levels more accurately predicted mortality than PSI alone[151], indicating adrenomedullin levels are a useful metric in determining the severity of pneumonia. The prognostic accuracy of proAM to predict the outcome of CAP was also confirmed by additional studies[152,153], and its usefulness was not affected by various etiologies of pneumonia infection[154]. A recent study reported that serum AM levels are also a promising predictive marker in the assessment of ventilator associated pneumonia (VAP) diagnosis[155].

Of note, it has long been demonstrated that AM also has an antimicrobial role, participating in the prevention of local infection acting synergistically with the immune response (Figure 2)[156]. Interestingly, antimicrobial effects of AM seem to be independent from its classical signaling pathway. Instead, AM was demonstrated to disrupt and permeabilize cell walls of *E. Coli* and *S. Aureus*[157]. These antimicrobial effects of AM, in combination with its vascular functions, such as improving lymphatic function, suggest that in addition to being a valuable prognostic biomarker, AM might also be a potential antimicrobial agent for infectious diseases like pneumonia.

Glaucoma

Glaucoma is an ocular disease primarily mediated by elevated intraocular pressure, eventually leading to vision loss due to damage of the optic nerve. An early pilot clinical study found higher AM levels in the aqueous humor of patients with primary open-angle glaucoma compared to neovascular glaucoma[158]. Accordingly, a pre-clinical study reported that CLR overexpression in the pupillary sphincter muscle led to increased intra-ocular pressure which was reversed when crossed to AM haploinsufficient mice[159]. AM and its receptors have both been found to be expressed in the retina[160], and genetic mutations in the human RAMP2 gene have been associated with primary open-angle glaucoma[161]. An interesting study demonstrated reduced pore formation in the ECs of the hybrid vessel Schlemm's canal in patients with glaucoma[162]. AM signaling is known to play an important role in regulating intercellular junctions in ECs and modulate their barrier function. Increased AM signaling might enhance the barrier function, reducing the uptake capability of Schlemm's canal leading to decreased intra-ocular fluid drainage (Figure 2). Further studies are needed to understand the role of AM signaling in glaucoma and to unveil whether it can be as a diagnostic marker or therapeutic target in glaucoma.

Inflammatory Bowel Disease

AM is also widely expressed throughout the highly vascularized system of the gastrointestinal (GI) tract. AM producing cells are spread throughout the GI endocrine system, including the mucosal epithelium, glandular duct cells, enteroendocrine cells, and smooth muscle cells, indicating its important roles in digestion and nutrient absorption[163,164]. In addition to its various functions in non-endothelial cells of the GI tract [165-167], AM was reported to regulate lipid transport through lacteal LECs. Induced deletion of CLR in PROX1 positive cells led to lymphatic insufficiency and lymphangiectasia[63], as well as disrupted lipid trafficking and enteric nerve patterning in HFD fed mice[64]. Similarly to its role in ECs, AM is also critical in maintaining intestinal epithelial barrier integrity (Figure 2). Lack of endogenous AM accelerates the development and severity of colitis induced by rectal instillation of 3 mg 2,4,6-trinitrobenzenesulfonic acid (TNBS) in mice mitigates the expression levels of junctional adhesion molecule-A (JAM-A) and e-cadherin[168]. Moreover, reduced AM expression resulted in altered colonic microbiota with a lower proportion of beneficial bacteria in mice, such as *Lactobacillus gasser*i and *Bifidobacterium choerinum*, which was correlated with significantly worse colitis[169]. The broad functions of AM in the GI tract propose multiple possible applications of AM in GI disorders. Currently, AM is considered as a potential therapeutic approach for inflammatory bowel disease[170-173]. Two phase II studies have suggested [174,175] the clinical potential of AM for treatment of inflammatory bowel disease (IBD) patients.

BOOSTING THE CLINICAL POTENTCY OF AM-TARGETING APPROACHES

As demonstrated above in detail, AM has important roles in cardiovascular physiology and pathophysiology. Given these functions, AM has been proposed as a promising predictive biomarker and therapeutic target for multiple diseases. On the other hand, the broad, systemic roles of AM could also lead to off target effects of AM. Despite the promising clinical potential of AM, there are currently no diagnostic or therapeutic approaches that exploit AM signaling in clinical practice. However, recent advances have provided numerous tools which may help us to leverage the key roles of AM signaling in this wide spectrum of diseases. These include the diagnostic use of MR proAM and the therapeutic application of adrecizumab, which both help to overcome the limitation of the short half-life of AM. In this section, we summarize other current approaches for developing AM-targeting pharmaceuticals that may facilitate or improve the clinical usefulness of AM signaling.

Improving pharmacologic abilities of AM by peptide modifications

Besides reducing the degradation of AM by non-neutralizing antibodies, such as Adrecizumab, another approach to increase the half-life of AM is biochemical modification of the peptide by methods such as PEGylation, acylation, or additional modifications to the peptide structure. These biochemical modifications often decrease the potency or the affinity of the signal for its receptor[176,177]. Encouragingly, an acylated α-CGRP analogue has been demonstrated to reduce end organ damage in a model of murine hypertension[178]. The Pioszak laboratory has developed CLR/Ramp agonists for both AM and CGRP that have improved receptor affinities down to the picomolar range and have receptor dwell times ranging from 5 to 10 hours[179]. This group has also designed sustained signaling AM (ss-AM) and CGRP (ss-CGRP) that increase cAMP levels in stimulated cells after washout of CLR/Ramp agonist[179]. Currently, the only clinically approved therapeutics that target CLR/Ramps are CGRP antagonists for migraine, these modified proteins represent exciting therapeutic avenues for CLR/Ramp agonists in the context of heart and vascular disease.

The Hay laboratory has developed small molecule agonists of the CLR receptor that are capable of upregulating cAMP in vascular endothelial cells [180]. Hsu and colleagues have also successfully developed CLR/RAMP receptor agonists and antagonists that were up to 100-fold more selective to their respective receptor complexes as the natural ligands[181]. In their later studies, the researchers could evoke a sustained activation of CLR/RAMP1 and CLR/RAMP2 receptor signaling pathways using palmitoylated AM and AM2/IMD analogues[182]. Moreover, the selected AM analogue was demonstrated to have gel-like characteristics, limiting its spatial spreading. Therefore, application of these gel-forming AM analogues would also provide a predominantly local stimulation of AM signaling pathways. It is yet to be investigated whether these analogues bear the same intracellular signaling bias as AM in the different cell types, or act through a distinct signaling preference.

Therapeutic Approaches to Increase Plasma AM levels

Sacubitril/valsartan is an FDA- and EMA-approved angiotensin receptor - neprilysin inhibitor agent to treat patients with chronic heart failure with reduced ejection fraction (HFrEF). A recent clinical study aimed at characterizing the changes in natriuretic peptide levels after neprilysin-inhibition treatment of patients with HFrEF. Surprisingly however, Pavo and colleagues found that neprilysin-inhibition evoked a modest change in plasma BNP levels, but instead led to a more robust elevation in plasma bio-AM and MR proAM[183]. Their results also suggest that neprilysin-inhibition probably not only mitigates AM degradation, but also promotes AM production by a yet unknown mechanism. These data propose administration of neprilysin inhibitors as a possible alternative indirect approach to increase plasma levels of biologically active AM in those patients where its use is not contraindicated.

As demonstrated above in this review, another known mechanism that is responsible for the biodegradation of AM is through its decoy receptor ACKR3. Diphenylacetamides were recently discovered to inhibit ACKR3 signaling[184]. Administration of these pharmacological agents may be a suitable way to evoke increased AM levels. Besides small molecule inhibition of neprilysin and ACKR3, biological targeting of these molecules is also a promising option to target AM signaling.

Potential Approaches for Targeted AM Treatments

As AM receptors are expressed in various cell types all around the body, and AM binding to CLR can evoke different intracellular signaling pathways, systemic administration of AM might lead to undesired effects, as seen in sepsis. Discovering molecular mechanisms that determine which intracellular signaling pathway is activated by AM binding is essential for developing more targeted therapeutic approaches. Development of engineered AM variants or AM agonists that evoke only a distinct downstream mechanism out of the many know AM signaling pathways may be a possible approach to mitigate off-target AM effects. Developing specific AM or CLR inhibitors that mitigate undesirable AM effects without affecting the desired intracellular mechanism might also improve the clinical potential of AM signaling. Developing an administration technique enabling tissue-specific delivery of AM, such as a cell type-specific antibody labeled coating of the bioactive peptide, would help reducing off-target effects of AM. Encapsulation of AM into an enzymatically degradable coating might also help titrate desired AM concentrations locally and control the dynamics of AM administration.

CONCLUDING REMARKS

Adrenomedullin, predominantly known for its vasoactive effects exerts numerous additional biological functions, especially in the cardiovascular and lymphatic vascular systems. In addition to regulating vascular flow by promoting vasodilation, it is indispensable for maintaining endothelial barrier function of blood and lymphatic vessels. AM signaling is essential for cardiac and lymphatic development and under pathological conditions, it may promote adult angiogenesis and lymphangiogenesis and cardiac regeneration after injury. This wide diversity in its physiological and pathophysiological roles elevates AM as a promising biomarker and therapeutic target in numerous cardiovascular diseases, and consequently multiple therapeutic approaches targeting AM signaling are under clinical testing or trials. Recent advances in the understanding of AM-mediated pathways and development of novel approaches, such as AM-specific antibodies increasing its high-life and modifying its intracellular signaling bias or engineered AM-analogues enabling a more specific and sustained activation of AM receptors may provide us with possible tools to tackle the two main limitations of AM in the clinic, namely its short half-life and the wide variety of biological functions. These novel advances further boost the clinical potential of AM signaling pathways. Further studies are required to fully understand the mechanisms that determine which intracellular signaling pathway is activated upon AM binding by its receptor complex. Understanding these mechanisms may further improve the application of AM as a predictive biomarker and development of tissue specific AM-targeted therapeutic approaches.

Acknowledgements:

The authors thank members of the Caron Lab for helpful discussions and critical reading of the manuscript. We gratefully and humbly acknowledge all members of the scientific community who have made impactful and significant contributions to the study of AM/CLR/RAMP signaling and regret that we were unable to discuss or cite all studies in this review. This work was supported by the following grants: NIH/NHLBI HL1290986 and NIH/NIDDK DK119145 to KMC, an American Heart Association Postdoctoral Fellowship 23POST1022945 to LB, an American Heart Association Predoctoral Fellowship 909016 to NPNM, and an NIH/NHLBI F31 Fellowship F31HL163885 to DSS.

Non-standard Abbreviations and Acronyms

ACKR3	atypical chemokine receptor 3
AM	adrenomedullin (protein; gene: Adm)
ANP	atrial natriuretic peptide
BEC	blood endothelial cell
BNP	B-type natriuretic peptide
cAMP	cyclic adenosine monophosphate
CAP	community-acquired pneumonia
CGRP	calcitonin gene-related peptide (protein; gene: Calca/Calcb)
CLR	calcitonin-receptor like receptor (protein; gene: Calcrl)
CREB	cAMP response element-binding protein
CSF	cerebrospinal fluid
CXCL	chemokine x ligand
EC	endothelial cell

eNOS	endothelial nitric oxide synthase
GPCR	G protein-coupled receptor
HF	heart failure
HFrEF.	heart failure with reduced ejection fraction
HUAEC	human umbilical arterial endothelial cells
HUVEC	human umbilical vein endothelial cells
IBD	inflammatory bowel disease
JAM-A	junctional adhesion molecule-A
LEC	lymphatic endothelial cells
LPS	lipopolysaccharide
LYVE1	lymphatic vessel endothelial hyaluronan receptor 1
MAPK	mitogen-activated protein kinase
MI	myocardial infarction
MR pro-ADM	pro-adrenomedullin 45-92
NO	nitric oxide
PAMP	proAM N-terminal 20 peptide
Pro-AM	pro-adrenomedullin
PROX1	prospero homeobox protein 1
PSI	pneumonia severity index
RAMP	receptor activity-modifying protein
SPECT	single-photon emission computerized tomography
ss-AM	sustained signaling AM
TNBS	2,4,6-trinitrobenzenesulfonic acid
VAP	ventilator associated pneumonia
VE-Cadherin	vascular endothelial cadherin
VEGFR	vascularar endothelial growth factor receptor
ZO-1	zona occludens 1

References

1. Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, Eto T. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. Biochem Biophys Res Commun. 1993;192:553–560. doi: 10.1006/bbrc.1993.1451 [PubMed: 8387282]
2. Isumi Y, Shoji H, Sugo S, Tochimoto T, Yoshioka M, Kangawa K, Matsuo H, Minamino N. Regulation of adrenomedullin production in rat endothelial cells. Endocrinology. 1998;139:838–846. doi: 10.1210/endo.139.3.5789 [PubMed: 9492011]
3. Kitamura K, Sakata J, Kangawa K, Kojima M, Matsuo H, Eto T. Cloning and characterization of cDNA encoding a precursor for human adrenomedullin. Biochem Biophys Res Commun. 1993;194:720–725. doi: 10.1006/bbrc.1993.1881 [PubMed: 7688224]
4. Sugo S, Minamino N, Shoji H, Kangawa K, Kitamura K, Eto T, Matsuo H. Production and secretion of adrenomedullin from vascular smooth muscle cells: augmented production by tumor necrosis factor-alpha. Biochem Biophys Res Commun. 1994;203:719–726. doi: 10.1006/bbrc.1994.2241 [PubMed: 8074727]
5. Shoji H, Minamino N, Kangawa K, Matsuo H. Endotoxin markedly elevates plasma concentration and gene transcription of adrenomedullin in rat. Biochem Biophys Res Commun. 1995;215:531–537. doi: 10.1006/bbrc.1995.2497 [PubMed: 7487988]
6. Morimoto A, Nishikimi T, Yoshihara F, Horio T, Nagaya N, Matsuo H, Dohi K, Kangawa K. Ventricular adrenomedullin levels correlate with the extent of cardiac hypertrophy in rats. Hypertension. 1999;33:1146–1152. doi: 10.1161/01.hyp.33.5.1146 [PubMed: 10334802]
7. Iring A, Jin YJ, Albarran-Juarez J, Siragusa M, Wang S, Dancs PT, Nakayama A, Tonack S, Chen M, Kunne C, et al. Shear stress-induced endothelial adrenomedullin signaling regulates vascular tone and blood pressure. J Clin Invest. 2019;129:2775–2791. doi: 10.1172/JCI123825 [PubMed: 31205027]
8. Nguyen SV, Claycomb WC. Hypoxia regulates the expression of the adrenomedullin and HIF-1 genes in cultured HL-1 cardiomyocytes. Biochem Biophys Res Commun. 1999;265:382–386. doi: 10.1006/bbrc.1999.1674 [PubMed: 10558876]
9. Meeran K, O'Shea D, Upton PD, Small CJ, Ghatei MA, Byfield PH, Bloom SR. Circulating adrenomedullin does not regulate systemic blood pressure but increases plasma prolactin after intravenous infusion in humans: a pharmacokinetic study. J Clin Endocrinol Metab. 1997;82:95–100. doi: 10.1210/jcem.82.1.3656 [PubMed: 8989240]
10. Struck J, Tao C, Morgenthaler NG, Bergmann A. Identification of an Adrenomedullin precursor fragment in plasma of sepsis patients. Peptides. 2004;25:1369–1372. doi: 10.1016/j.peptides.2004.06.019 [PubMed: 15350706]
11. Geven C, van Lier D, Blet A, Peelen R, Ten Elzen B, Mebazaa A, Kox M, Pickkers P. Safety, tolerability and pharmacokinetics/pharmacodynamics of the adrenomedullin antibody adrecizumab in a first-in-human study and during experimental human endotoxaemia in healthy subjects. Br J Clin Pharmacol. 2018;84:2129–2141. doi: 10.1111/bcp.13655 [PubMed: 29856470]
12. Hauser AS, Attwood MM, Rask-Andersen M, Schioth HB, Gloriam DE. Trends in GPCR drug discovery: new agents, targets and indications. Nat Rev Drug Discov. 2017;16:829–842. doi: 10.1038/nrd.2017.178 [PubMed: 29075003]
13. Serafin DS, Harris NR, Nielsen NR, Mackie DI, Caron KM. Dawn of a New RAMPage. Trends Pharmacol Sci. 2020;41:249–265. doi: 10.1016/j.tips.2020.01.009 [PubMed: 32115276]
14. Hay DL, Garelja ML, Poyner DR, Walker CS. Update on the pharmacology of calcitonin/CGRP family of peptides: IUPHAR Review 25. Br J Pharmacol. 2018;175:3–17. doi: 10.1111/bph.14075 [PubMed: 29059473]
15. Clark AJ, Mullooly N, Safitri D, Harris M, de Vries T, MaassenVanDenBrink A, Poyner DR, Gianni D, Wigglesworth M, Ladds G. CGRP, adrenomedullin and adrenomedullin 2 display endogenous GPCR agonist bias in primary human cardiovascular cells. Commun Biol. 2021;4:776. doi: 10.1038/s42003-021-02293-w [PubMed: 34163006]
16. Weston C, Winfield I, Harris M, Hodgson R, Shah A, Dowell SJ, Mobarec JC, Woodlock DA, Reynolds CA, Poyner DR, et al. Receptor Activity-modifying Protein-directed G Protein

Signaling Specificity for the Calcitonin Gene-related Peptide Family of Receptors. J Biol Chem. 2016;291:21925–21944. doi: 10.1074/jbc.M116.751362 [PubMed: 27566546]

17. Pearce A, Redfern-Nichols T, Harris M, Poyner DR, Wigglesworth M, Ladds G. Determining the Effects of Differential Expression of GRKs and beta-arrestins on CLR-RAMP Agonist Bias. Front Physiol. 2022;13:840763. doi: 10.3389/fphys.2022.840763 [PubMed: 35422711]
18. Yarwood RE, Imlach WL, Lieu T, Veldhuis NA, Jensen DD, Klein Herenbrink C, Aurelio L, Cai Z, Christie MJ, Poole DP, et al. Endosomal signaling of the receptor for calcitonin gene-related peptide mediates pain transmission. Proc Natl Acad Sci U S A. 2017;114:12309–12314. doi: 10.1073/pnas.1706656114 [PubMed: 29087309]
19. Garelja ML, Au M, Brimble MA, Gingell JJ, Hendrikse ER, Lovell A, Prodan N, Sexton PM, Siow A, Walker CS, et al. Molecular Mechanisms of Class B GPCR Activation: Insights from Adrenomedullin Receptors. ACS Pharmacol Transl Sci. 2020;3:246–262. doi: 10.1021/acsptsci.9b00083 [PubMed: 32296766]
20. Roehrkasse AM, Warner ML, Booe JM, Pioszak AA. Biochemical characterization of G protein coupling to calcitonin gene-related peptide and adrenomedullin receptors using a native PAGE assay. J Biol Chem. 2020;295:9736–9751. doi: 10.1074/jbc.RA120.013854 [PubMed: 32487746]
21. Guidolin D, Albertin G, Spinazzi R, Sorato E, Mascarin A, Cavallo D, Antonello M, Ribatti D. Adrenomedullin stimulates angiogenic response in cultured human vascular endothelial cells: involvement of the vascular endothelial growth factor receptor 2. Peptides. 2008;29:2013–2023. doi: 10.1016/j.peptides.2008.07.009 [PubMed: 18692535]
22. Harris NR, Nielsen NR, Pawlak JB, Aghajanian A, Rangarajan K, Serafin DS, Farber G, Dy DM, Nelson-Maney NP, Xu W, et al. VE-Cadherin Is Required for Cardiac Lymphatic Maintenance and Signaling. Circ Res. 2022;130:5–23. doi: 10.1161/CIRCRESAHA.121.318852 [PubMed: 34789016]
23. Karlsson M, Zhang C, Mear L, Zhong W, Digre A, Katona B, Sjostedt E, Butler L, Odeberg J, Dusart P, et al. A single-cell type transcriptomics map of human tissues. Sci Adv. 2021;7. doi: 10.1126/sciadv.abh2169
24. Burns JM, Summers BC, Wang Y, Melikian A, Berahovich R, Miao Z, Penfold ME, Sunshine MJ, Littman DR, Kuo CJ, et al. A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. J Exp Med. 2006;203:2201–2213. doi: 10.1084/jem.20052144 [PubMed: 16940167]
25. Alampour-Rajabi S, El Bounkari O, Rot A, Muller-Newen G, Bachelerie F, Gawaz M, Weber C, Schober A, Bernhagen J. MIF interacts with CXCR7 to promote receptor internalization, ERK1/2 and ZAP-70 signaling, and lymphocyte chemotaxis. FASEB J. 2015;29:4497–4511. doi: 10.1096/fj.15-273904 [PubMed: 26139098]
26. Szpakowska M, Dupuis N, Baragli A, Counson M, Hanson J, Piette J, Chevigne A. Human herpesvirus 8-encoded chemokine vCCL2/vMIP-II is an agonist of the atypical chemokine receptor ACKR3/CXCR7. Biochem Pharmacol. 2016;114:14–21. doi: 10.1016/j.bcp.2016.05.012 [PubMed: 27238288]
27. Meyrath M, Szpakowska M, Zeiner J, Massotte L, Merz MP, Benkel T, Simon K, Ohnmacht J, Turner JD, Kruger R, et al. The atypical chemokine receptor ACKR3/CXCR7 is a broad-spectrum scavenger for opioid peptides. Nat Commun. 2020;11:3033. doi: 10.1038/s41467-020-16664-0 [PubMed: 32561830]
28. Ikeda Y, Kumagai H, Skach A, Sato M, Yanagisawa M. Modulation of circadian glucocorticoid oscillation via adrenal opioid-CXCR7 signaling alters emotional behavior. Cell. 2013;155:1323–1336. doi: 10.1016/j.cell.2013.10.052 [PubMed: 24315101]
29. Meyrath M, Palmer CB, Reynders N, Vanderplasschen A, Ollert M, Bouvier M, Szpakowska M, Chevigne A. Proadrenomedullin N-Terminal 20 Peptides (PAMPs) Are Agonists of the Chemokine Scavenger Receptor ACKR3/CXCR7. ACS Pharmacol Transl Sci. 2021;4:813–823. doi: 10.1021/acsptsci.1c00006 [PubMed: 33860204]
30. Kapas S, Clark AJ. Identification of an orphan receptor gene as a type 1 calcitonin gene-related peptide receptor. Biochem Biophys Res Commun. 1995;217:832–838. doi: 10.1006/bbrc.1995.2847 [PubMed: 8554605]

31. Boldajipour B, Mahabaleshwar H, Kardash E, Reichman-Fried M, Blaser H, Minina S, Wilson D, Xu Q, Raz E. Control of chemokine-guided cell migration by ligand sequestration. Cell. 2008;132:463–473. doi: 10.1016/j.cell.2007.12.034 [PubMed: 18267076]
32. Rajagopal S, Kim J, Ahn S, Craig S, Lam CM, Gerard NP, Gerard C, Lefkowitz RJ. Beta-arrestin- but not G protein-mediated signaling by the "decoy" receptor CXCR7. Proc Natl Acad Sci U S A. 2010;107:628–632. doi: 10.1073/pnas.0912852107 [PubMed: 20018651]
33. Naumann U, Cameroni E, Pruenster M, Mahabaleshwar H, Raz E, Zerwes HG, Rot A, Thelen M. CXCR7 functions as a scavenger for CXCL12 and CXCL11. PLoS One. 2010;5:e9175. doi: 10.1371/journal.pone.0009175 [PubMed: 20161793]
34. Zarca A, Perez C, van den Bor J, Bebelman JP, Heuninck J, de Jonker RJF, Durroux T, Vischer HF, Siderius M, Smit MJ. Differential Involvement of ACKR3 C-Tail in beta-Arrestin Recruitment, Trafficking and Internalization. Cells. 2021;10. doi: 10.3390/cells10030618
35. Klein KR, Karpinich NO, Espenschied ST, Willcockson HH, Dunworth WP, Hoopes SL, Kushner EJ, Bautch VL, Caron KM. Decoy receptor CXCR7 modulates adrenomedullin-mediated cardiac and lymphatic vascular development. Dev Cell. 2014;30:528–540. doi: 10.1016/j.devcel.2014.07.012 [PubMed: 25203207]
36. Rosenfeld MG, Mermod JJ, Amara SG, Swanson LW, Sawchenko PE, Rivier J, Vale WW, Evans RM. Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature. 1983;304:129–135. doi: 10.1038/304129a0 [PubMed: 6346105]
37. Maggi CA. Tachykinins and calcitonin gene-related peptide (CGRP) as co-transmitters released from peripheral endings of sensory nerves. Prog Neurobiol. 1995;45:1–98. doi: 10.1016/0301-0082(94)e0017-b [PubMed: 7716258]
38. Iyengar S, Ossipov MH, Johnson KW. The role of calcitonin gene-related peptide in peripheral and central pain mechanisms including migraine. Pain. 2017;158:543–559. doi: 10.1097/j.pain.0000000000000831 [PubMed: 28301400]
39. Ashina M, Terwindt GM, Al-Karagholi MA, de Boer I, Lee MJ, Hay DL, Schulte LH, Hadjikhani N, Sinclair AJ, Ashina H, et al. Migraine: disease characterisation, biomarkers, and precision medicine. Lancet. 2021;397:1496–1504. doi: 10.1016/S0140-6736(20)32162-0 [PubMed: 33773610]
40. Goadsby PJ, Edvinsson L, Ekman R. Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigeminovascular system. Ann Neurol. 1988;23:193–196. doi: 10.1002/ana.410230214 [PubMed: 2454066]
41. Gallai V, Alberti A, Gallai B, Coppola F, Floridi A, Sarchielli P. Glutamate and nitric oxide pathway in chronic daily headache: evidence from cerebrospinal fluid. Cephalalgia. 2003;23:166–174. doi: 10.1046/j.1468-2982.2003.00552.x [PubMed: 12662182]
42. Fan PC, Kuo PH, Lee MT, Chang SH, Chiou LC. Plasma Calcitonin Gene-Related Peptide: A Potential Biomarker for Diagnosis and Therapeutic Responses in Pediatric Migraine. Front Neurol. 2019;10:10. doi: 10.3389/fneur.2019.00010 [PubMed: 30733702]
43. Hautakangas H, Winsvold BS, Ruotsalainen SE, Bjornsdottir G, Harder AVE, Kogelman LJA, Thomas LF, Noordam R, Benner C, Gormley P, et al. Genome-wide analysis of 102,084 migraine cases identifies 123 risk loci and subtype-specific risk alleles. Nat Genet. 2022;54:152–160. doi: 10.1038/s41588-021-00990-0 [PubMed: 35115687]
44. Sherman ML, Shafman TD, Colman MS, Kufe DW. Tiazofurin induction of mouse erythroleukemia cell hemoglobin production in the absence of commitment or changes in protooncogene expression. Blood. 1989;73:431–434. [PubMed: 2917182]
45. Robbins MS. Diagnosis and Management of Headache: A Review. JAMA. 2021;325:1874–1885. doi: 10.1001/jama.2021.1640 [PubMed: 33974014]
46. Shimekake Y, Nagata K, Ohta S, Kambayashi Y, Teraoka H, Kitamura K, Eto T, Kangawa K, Matsuo H. Adrenomedullin stimulates two signal transduction pathways, cAMP accumulation and Ca2+ mobilization, in bovine aortic endothelial cells. J Biol Chem. 1995;270:4412–4417. doi: 10.1074/jbc.270.9.4412 [PubMed: 7876206]
47. Kohno M, Kano H, Horio T, Yokokawa K, Yasunari K, Takeda T. Inhibition of endothelin production by adrenomedullin in vascular smooth muscle cells. Hypertension. 1995;25:1185–1190. doi: 10.1161/01.hyp.25.6.1185 [PubMed: 7768561]

48. Nagaya N, Satoh T, Nishikimi T, Uematsu M, Furuichi S, Sakamaki F, Oya H, Kyotani S, Nakanishi N, Goto Y, et al. Hemodynamic, renal, and hormonal effects of adrenomedullin infusion in patients with congestive heart failure. Circulation. 2000;101:498–503. doi: 10.1161/01.cir.101.5.498 [PubMed: 10662746]
49. Caron KM, Smithies O. Extreme hydrops fetalis and cardiovascular abnormalities in mice lacking a functional Adrenomedullin gene. Proc Natl Acad Sci U S A. 2001;98:615–619. doi: 10.1073/pnas.98.2.615 [PubMed: 11149956]
50. Shindo T, Kurihara Y, Nishimatsu H, Moriyama N, Kakoki M, Wang Y, Imai Y, Ebihara A, Kuwaki T, Ju KH, et al. Vascular abnormalities and elevated blood pressure in mice lacking adrenomedullin gene. Circulation. 2001;104:1964–1971. doi: 10.1161/hc4101.097111 [PubMed: 11602502]
51. Dackor RT, Fritz-Six K, Dunworth WP, Gibbons CL, Smithies O, Caron KM. Hydrops fetalis, cardiovascular defects, and embryonic lethality in mice lacking the calcitonin receptor-like receptor gene. Mol Cell Biol. 2006;26:2511–2518. doi: 10.1128/MCB.26.7.2511-2518.2006 [PubMed: 16537897]
52. Fritz-Six KL, Dunworth WP, Li M, Caron KM. Adrenomedullin signaling is necessary for murine lymphatic vascular development. J Clin Invest. 2008;118:40–50. doi: 10.1172/JCI33302 [PubMed: 18097475]
53. Ichikawa-Shindo Y, Sakurai T, Kamiyoshi A, Kawate H, Iinuma N, Yoshizawa T, Koyama T, Fukuchi J, Iimuro S, Moriyama N, et al. The GPCR modulator protein RAMP2 is essential for angiogenesis and vascular integrity. J Clin Invest. 2008;118:29–39. doi: 10.1172/JCI33022 [PubMed: 18097473]
54. Wetzel-Strong SE, Li M, Klein KR, Nishikimi T, Caron KM. Epicardial-derived adrenomedullin drives cardiac hyperplasia during embryogenesis. Dev Dyn. 2014;243:243–256. doi: 10.1002/dvdy.24065 [PubMed: 24123312]
55. Yu S, Crawford D, Tsuchihashi T, Behrens TW, Srivastava D. The chemokine receptor CXCR7 functions to regulate cardiac valve remodeling. Dev Dyn. 2011;240:384–393. doi: 10.1002/dvdy.22549 [PubMed: 21246655]
56. Gerrits H, van Ingen Schenau DS, Bakker NE, van Disseldorp AJ, Strik A, Hermens LS, Koenen TB, Krajnc-Franken MA, Gossen JA. Early postnatal lethality and cardiovascular defects in CXCR7-deficient mice. Genesis. 2008;46:235–245. doi: 10.1002/dvg.20387 [PubMed: 18442043]
57. Oliver G, Kipnis J, Randolph GJ, Harvey NL. The Lymphatic Vasculature in the 21(st) Century: Novel Functional Roles in Homeostasis and Disease. Cell. 2020;182:270–296. doi: 10.1016/j.cell.2020.06.039 [PubMed: 32707093]
58. Wigle JT, Harvey N, Detmar M, Lagutina I, Grosveld G, Gunn MD, Jackson DG, Oliver G. An essential role for Prox1 in the induction of the lymphatic endothelial cell phenotype. EMBO J. 2002;21:1505–1513. doi: 10.1093/emboj/21.7.1505 [PubMed: 11927535]
59. Gonzalez-Loyola A, Petrova TV. Development and aging of the lymphatic vascular system. Adv Drug Deliv Rev. 2021;169:63–78. doi: 10.1016/j.addr.2020.12.005 [PubMed: 33316347]
60. Mackie DI, Al Mutairi F, Davis RB, Kechele DO, Nielsen NR, Snyder JC, Caron MG, Kliman HJ, Berg JS, Simms J, et al. hCALCRL mutation causes autosomal recessive nonimmune hydrops fetalis with lymphatic dysplasia. J Exp Med. 2018;215:2339–2353. doi: 10.1084/jem.20180528 [PubMed: 30115739]
61. Dunworth WP, Fritz-Six KL, Caron KM. Adrenomedullin stabilizes the lymphatic endothelial barrier in vitro and in vivo. Peptides. 2008;29:2243–2249. doi: 10.1016/j.peptides.2008.09.009 [PubMed: 18929609]
62. Hoopes SL, Willcockson HH, Caron KM. Characteristics of multi-organ lymphangiectasia resulting from temporal deletion of calcitonin receptor-like receptor in adult mice. PLoS One. 2012;7:e45261. doi: 10.1371/journal.pone.0045261 [PubMed: 23028890]
63. Davis RB, Kechele DO, Blakeney ES, Pawlak JB, Caron KM. Lymphatic deletion of calcitonin receptor-like receptor exacerbates intestinal inflammation. JCI Insight. 2017;2:e92465. doi: 10.1172/jci.insight.92465 [PubMed: 28352669]

64. Davis RB, Ding S, Nielsen NR, Pawlak JB, Blakeney ES, Caron KM. Calcitonin-Receptor-Like Receptor Signaling Governs Intestinal Lymphatic Innervation and Lipid Uptake. ACS Pharmacol Transl Sci. 2019;2:114–121. doi: 10.1021/acsptsci.8b00061 [PubMed: 32219216]
65. Nicholls MG. Hemodynamic and hormonal actions of adrenomedullin. Braz J Med Biol Res. 2004;37:1247–1253. doi: 10.1590/s0100-879x2004000800016 [PubMed: 15273827]
66. Koyama T, Ochoa-Callejero L, Sakurai T, Kamiyoshi A, Ichikawa-Shindo Y, Iinuma N, Arai T, Yoshizawa T, Iesato Y, Lei Y, et al. Vascular endothelial adrenomedullin-RAMP2 system is essential for vascular integrity and organ homeostasis. Circulation. 2013;127:842–853. doi: 10.1161/CIRCULATIONAHA.112.000756 [PubMed: 23355623]
67. Martinez A. A new family of angiogenic factors. Cancer Lett. 2006;236:157–163. doi: 10.1016/j.canlet.2005.04.008 [PubMed: 15927357]
68. Mueller C, McDonald K, de Boer RA, Maisel A, Cleland JGF, Kozhuharov N, Coats AJS, Metra M, Mebazaa A, Ruschitzka F, et al. Heart Failure Association of the European Society of Cardiology practical guidance on the use of natriuretic peptide concentrations. Eur J Heart Fail. 2019;21:715–731. doi: 10.1002/ejhf.1494 [PubMed: 31222929]
69. Jougasaki M, Wei CM, McKinley LJ, Burnett JC Jr. Elevation of circulating and ventricular adrenomedullin in human congestive heart failure. Circulation. 1995;92:286–289. doi: 10.1161/01.cir.92.3.286 [PubMed: 7634439]
70. Nishikimi T, Saito Y, Kitamura K, Ishimitsu T, Eto T, Kangawa K, Matsuo H, Omae T, Matsuoka H. Increased plasma levels of adrenomedullin in patients with heart failure. J Am Coll Cardiol. 1995;26:1424–1431. doi: 10.1016/0735-1097(95)00338-X [PubMed: 7594065]
71. Maisel A, Mueller C, Nowak R, Peacock WF, Landsberg JW, Ponikowski P, Mockel M, Hogan C, Wu AH, Richards M, et al. Mid-region pro-hormone markers for diagnosis and prognosis in acute dyspnea: results from the BACH (Biomarkers in Acute Heart Failure) trial. J Am Coll Cardiol. 2010;55:2062–2076. doi: 10.1016/j.jacc.2010.02.025 [PubMed: 20447528]
72. Self WH, Storrow AB, Hartmann O, Barrett TW, Fermann GJ, Maisel AS, Struck J, Bergmann A, Collins SP. Plasma bioactive adrenomedullin as a prognostic biomarker in acute heart failure. Am J Emerg Med. 2016;34:257–262. doi: 10.1016/j.ajem.2015.10.033 [PubMed: 26577429]
73. Tolppanen H, Rivas-Lasarte M, Lassus J, Sans-Rosello J, Hartmann O, Lindholm M, Arrigo M, Tarvasmaki T, Kober L, Thiele H, et al. Adrenomedullin: a marker of impaired hemodynamics, organ dysfunction, and poor prognosis in cardiogenic shock. Ann Intensive Care. 2017;7:6. doi: 10.1186/s13613-016-0229-2 [PubMed: 28050899]
74. Ter Maaten JM, Kremer D, Demissei BG, Struck J, Bergmann A, Anker SD, Ng LL, Dickstein K, Metra M, Samani NJ, et al. Bio-adrenomedullin as a marker of congestion in patients with new-onset and worsening heart failure. Eur J Heart Fail. 2019;21:732–743. doi: 10.1002/ejhf.1437 [PubMed: 30843353]
75. Morbach C, Marx A, Kaspar M, Guder G, Brenner S, Feldmann C, Stork S, Vollert JO, Ertl G, Angermann CE, et al. Prognostic potential of midregional pro-adrenomedullin following decompensation for systolic heart failure: comparison with cardiac natriuretic peptides. Eur J Heart Fail. 2017;19:1166–1175. doi: 10.1002/ejhf.859 [PubMed: 28516504]
76. Kuan WS, Ibrahim I, Chan SP, Li Z, Liew OW, Frampton C, Troughton R, Pemberton CJ, Chong JPC, Tan LL, et al. Mid-regional pro-adrenomedullin outperforms N-terminal pro-B-type natriuretic peptide for the diagnosis of acute heart failure in the presence of atrial fibrillation. Eur J Heart Fail. 2020;22:692–700. doi: 10.1002/ejhf.1660 [PubMed: 31808279]
77. Feng Z, Akinrimisi OP, Gornbein JA, Truong QA, Das S, Singh JP, Ajijola O. Combination Biomarkers for Risk Stratification in Patients With Chronic Heart Failure Biomarkers Prognostication in HF. J Card Fail. 2021;27:1321–1327. doi: 10.1016/j.cardfail.2021.05.028 [PubMed: 34153460]
78. Dungen HD, Tscholl V, Obradovic D, Radenovic S, Matic D, Musial Bright L, Tahirovic E, Marx A, Inkrot S, Hashemi D, et al. Prognostic performance of serial in-hospital measurements of copeptin and multiple novel biomarkers among patients with worsening heart failure: results from the MOLITOR study. ESC Heart Fail. 2018;5:288–296. doi: 10.1002/ehf2.12231 [PubMed: 29476612]
79. Fraty M, Velho G, Gand E, Fumeron F, Ragot S, Sosner P, Mohammedi K, Gellen B, Saulnier PJ, Halimi JM, et al. Prognostic value of plasma MR-proADM vs NT-proBNP for heart failure in

people with type 2 diabetes: the SURDIAGENE prospective study. Diabetologia. 2018;61:2643–2653. doi: 10.1007/s00125-018-4727-7 [PubMed: 30232509]

80. Welsh P, Kou L, Yu C, Anand I, van Veldhuisen DJ, Maggioni AP, Desai AS, Solomon SD, Pfeffer MA, Cheng S, et al. Prognostic importance of emerging cardiac, inflammatory, and renal biomarkers in chronic heart failure patients with reduced ejection fraction and anaemia: RED-HF study. Eur J Heart Fail. 2018;20:268–277. doi: 10.1002/ejhf.988 [PubMed: 28960777]
81. Szokodi I, Kinnunen P, Tavi P, Weckstrom M, Toth M, Ruskoaho H. Evidence for cAMP-independent mechanisms mediating the effects of adrenomedullin, a new inotropic peptide. Circulation. 1998;97:1062–1070. doi: 10.1161/01.cir.97.11.1062 [PubMed: 9531253]
82. Wang P, Ba ZF, Cioffi WG, Bland KI, Chaudry IH. The pivotal role of adrenomedullin in producing hyperdynamic circulation during the early stage of sepsis. Arch Surg. 1998;133:1298–1304. doi: 10.1001/archsurg.133.12.1298 [PubMed: 9865646]
83. Tsao CW, Aday AW, Almarzooq ZI, Alonso A, Beaton AZ, Bittencourt MS, Boehme AK, Buxton AE, Carson AP, Commodore-Mensah Y, et al. Heart Disease and Stroke Statistics-2022 Update: A Report From the American Heart Association. Circulation. 2022;145:e153–e639. doi: 10.1161/CIR.0000000000001052 [PubMed: 35078371]
84. Kobayashi K, Kitamura K, Hirayama N, Date H, Kashiwagi T, Ikushima I, Hanada Y, Nagatomo Y, Takenaga M, Ishikawa T, et al. Increased plasma adrenomedullin in acute myocardial infarction. Am Heart J. 1996;131:676–680. doi: 10.1016/s0002-8703(96)90270-7 [PubMed: 8721638]
85. Falkentoft AC, Rorth R, Iversen K, Hofsten DE, Kelbaek H, Holmvang L, Frydland M, Schoos MM, Helqvist S, Axelsson A, et al. MR-proADM as a Prognostic Marker in Patients With ST-Segment-Elevation Myocardial Infarction-DANAMI-3 (a Danish Study of Optimal Acute Treatment of Patients With STEMI) Substudy. J Am Heart Assoc. 2018;7. doi: 10.1161/JAHA.117.008123
86. Horiuchi Y, Wettersten N, Patel MP, Mueller C, Neath SX, Christenson RH, Morgenthaler NG, McCord J, Nowak RM, Vilke GM, et al. Biomarkers Enhance Discrimination and Prognosis of Type 2 Myocardial Infarction. Circulation. 2020;142:1532–1544. doi: 10.1161/CIRCULATIONAHA.120.046682 [PubMed: 32820656]
87. Li L, Zhang S, Zhang Y, Yu B, Xu Y, Guan Z. Paracrine action mediate the antifibrotic effect of transplanted mesenchymal stem cells in a rat model of global heart failure. Mol Biol Rep. 2009;36:725–731. doi: 10.1007/s11033-008-9235-2 [PubMed: 18368514]
88. Horio T, Nishikimi T, Yoshihara F, Matsuo H, Takishita S, Kangawa K. Effects of adrenomedullin on cultured rat cardiac myocytes and fibroblasts. Eur J Pharmacol. 1999;382:1–9. doi: 10.1016/s0014-2999(99)00559-2 [PubMed: 10556498]
89. Nakamura R, Kato J, Kitamura K, Onitsuka H, Imamura T, Marutsuka K, Asada Y, Kangawa K, Eto T. Beneficial effects of adrenomedullin on left ventricular remodeling after myocardial infarction in rats. Cardiovasc Res. 2002;56:373–380. doi: 10.1016/s0008-6363(02)00594-1 [PubMed: 12445878]
90. Okumura H, Nagaya N, Kangawa K. Adrenomedullin infusion during ischemia/reperfusion attenuates left ventricular remodeling and myocardial fibrosis in rats. Hypertens Res. 2003;26 Suppl:S99–104. doi: 10.1291/hypres.26.s99 [PubMed: 12630818]
91. Nishikimi T, Yoshihara F, Horinaka S, Kobayashi N, Mori Y, Tadokoro K, Akimoto K, Minamino N, Kangawa K, Matsuoka H. Chronic administration of adrenomedullin attenuates transition from left ventricular hypertrophy to heart failure in rats. Hypertension. 2003;42:1034–1041. doi: 10.1161/01.HYP.0000097604.64716.D2 [PubMed: 14568998]
92. Yoshizawa T, Takizawa S, Shimada S, Tokudome T, Shindo T, Matsumoto K. Effects of Adrenomedullin on Doxorubicin-Induced Cardiac Damage in Mice. Biol Pharm Bull. 2016;39:737–746. doi: 10.1248/bpb.b15-00832 [PubMed: 26902282]
93. Kataoka Y, Miyazaki S, Yasuda S, Nagaya N, Noguchi T, Yamada N, Morii I, Kawamura A, Doi K, Miyatake K, et al. The first clinical pilot study of intravenous adrenomedullin administration in patients with acute myocardial infarction. J Cardiovasc Pharmacol. 2010;56:413–419. doi: 10.1097/FJC.0b013e3181f15b45 [PubMed: 20930593]
94. Nishikimi T, Karasawa T, Inaba C, Ishimura K, Tadokoro K, Koshikawa S, Yoshihara F, Nagaya N, Sakio H, Kangawa K, et al. Effects of long-term intravenous administration of adrenomedullin

(AM) plus hANP therapy in acute decompensated heart failure: a pilot study. Circ J. 2009;73:892–898. doi: 10.1253/circj.cj-08-0487 [PubMed: 19346663]

95. Trincot CE, Xu W, Zhang H, Kulikauskas MR, Caranasos TG, Jensen BC, Sabine A, Petrova TV, Caron KM. Adrenomedullin Induces Cardiac Lymphangiogenesis After Myocardial Infarction and Regulates Cardiac Edema Via Connexin 43. Circ Res. 2019;124:101–113. doi: 10.1161/CIRCRESAHA.118.313835 [PubMed: 30582443]
96. Liu X, De la Cruz E, Gu X, Balint L, Oxendine-Burns M, Terrones T, Ma W, Kuo HH, Lantz C, Bansal T, et al. Lymphoangiocrine signals promote cardiac growth and repair. Nature. 2020;588:705–711. doi: 10.1038/s41586-020-2998-x [PubMed: 33299187]
97. Harris NR, Balint L, Dy DM, Nielsen NR, Mendez HG, Aghajanian A, Caron KM. The ebb and flow of cardiac lymphatics: a tidal wave of new discoveries. Physiol Rev. 2023;103:391–432. doi: 10.1152/physrev.00052.2021 [PubMed: 35953269]
98. Kolditz M, Seyfarth HJ, Wilkens H, Ewert R, Bollmann T, Dinter C, Hertel S, Klose H, Opitz C, Grunig E, et al. MR-proADM Predicts Exercise Capacity and Survival Superior to Other Biomarkers in PH. Lung. 2015;193:901–910. doi: 10.1007/s00408-015-9802-y [PubMed: 26363916]
99. Bouzina H, Radegran G. Plasma adrenomedullin peptides and precursor levels in pulmonary arterial hypertension disease severity and risk stratification. Pulm Circ. 2020;10:2045894020931317. doi: 10.1177/2045894020931317 [PubMed: 32595932]
100. Nagaya N, Okumura H, Uematsu M, Shimizu W, Ono F, Shirai M, Mori H, Miyatake K, Kangawa K. Repeated inhalation of adrenomedullin ameliorates pulmonary hypertension and survival in monocrotaline rats. Am J Physiol Heart Circ Physiol. 2003;285:H2125–2131. doi: 10.1152/ajpheart.00548.2002 [PubMed: 14561683]
101. Nagaya N, Miyatake K, Kyotani S, Nishikimi T, Nakanishi N, Kangawa K. Pulmonary vasodilator response to adrenomedullin in patients with pulmonary hypertension. Hypertens Res. 2003;26 Suppl:S141–146. doi: 10.1291/hypres.26.s141 [PubMed: 12630825]
102. Nagaya N, Kyotani S, Uematsu M, Ueno K, Oya H, Nakanishi N, Shirai M, Mori H, Miyatake K, Kangawa K. Effects of adrenomedullin inhalation on hemodynamics and exercise capacity in patients with idiopathic pulmonary arterial hypertension. Circulation. 2004;109:351–356. doi: 10.1161/01.CIR.0000109493.05849.14 [PubMed: 14718403]
103. Melenovsky V, Andersen MJ, Andress K, Reddy YN, Borlaug BA. Lung congestion in chronic heart failure: haemodynamic, clinical, and prognostic implications. Eur J Heart Fail. 2015;17:1161–1171. doi: 10.1002/ejhf.417 [PubMed: 26467180]
104. Ambrosy AP, Pang PS, Khan S, Konstam MA, Fonarow GC, Traver B, Maggioni AP, Cook T, Swedberg K, Burnett JC Jr., et al. Clinical course and predictive value of congestion during hospitalization in patients admitted for worsening signs and symptoms of heart failure with reduced ejection fraction: findings from the EVEREST trial. Eur Heart J. 2013;34:835–843. doi: 10.1093/eurheartj/ehs444 [PubMed: 23293303]
105. Van Aelst LNL, Arrigo M, Placido R, Akiyama E, Girerd N, Zannad F, Manivet P, Rossignol P, Badoz M, Sadoune M, et al. Acutely decompensated heart failure with preserved and reduced ejection fraction present with comparable haemodynamic congestion. Eur J Heart Fail. 2018;20:738–747. doi: 10.1002/ejhf.1050 [PubMed: 29251818]
106. Gheorghiade M, Follath F, Ponikowski P, Barsuk JH, Blair JE, Cleland JG, Dickstein K, Drazner MH, Fonarow GC, Jaarsma T, et al. Assessing and grading congestion in acute heart failure: a scientific statement from the acute heart failure committee of the heart failure association of the European Society of Cardiology and endorsed by the European Society of Intensive Care Medicine. Eur J Heart Fail. 2010;12:423–433. doi: 10.1093/eurjhf/hfq045 [PubMed: 20354029]
107. Voors AA, Ter Maaten JM. Tackling Early Heart Failure Deaths and Readmissions by Estimating Congestion. JACC Heart Fail. 2015;3:894–895. doi: 10.1016/j.jchf.2015.06.013 [PubMed: 26541788]
108. Hagner S, Stahl U, Knoblauch B, McGregor GP, Lang RE. Calcitonin receptor-like receptor: identification and distribution in human peripheral tissues. Cell Tissue Res. 2002;310:41–50. doi: 10.1007/s00441-002-0616-x [PubMed: 12242482]
109. Harel F, Levac X, Nguyen QT, Letourneau M, Marcil S, Finnerty V, Cossette M, Fournier A, Dupuis J. Molecular Imaging of the Human Pulmonary Vascular Endothelium

Using an Adrenomedullin Receptor Ligand. Mol Imaging. 2015;14:7290201500003. doi: 10.2310/7290.2015.00003 [PubMed: 28654347]

110. Harel F, Langleben D, Provencher S, Fournier A, Finnerty V, Nguyen QT, Letourneau M, Levac X, Abikhzer G, Guimond J, et al. Molecular imaging of the human pulmonary vascular endothelium in pulmonary hypertension: a phase II safety and proof of principle trial. Eur J Nucl Med Mol Imaging. 2017;44:1136–1144. doi: 10.1007/s00259-017-3655-y [PubMed: 28236024]

111. Iimuro S, Shindo T, Moriyama N, Amaki T, Niu P, Takeda N, Iwata H, Zhang Y, Ebihara A, Nagai R. Angiogenic effects of adrenomedullin in ischemia and tumor growth. Circ Res. 2004;95:415–423. doi: 10.1161/01.RES.0000138018.61065.d1 [PubMed: 15242974]

112. Jin D, Harada K, Ohnishi S, Yamahara K, Kangawa K, Nagaya N. Adrenomedullin induces lymphangiogenesis and ameliorates secondary lymphoedema. Cardiovasc Res. 2008;80:339–345. doi: 10.1093/cvr/cvn228 [PubMed: 18708640]

113. Maki T, Ihara M, Fujita Y, Nambu T, Miyashita K, Yamada M, Washida K, Nishio K, Ito H, Harada H, et al. Angiogenic and vasoprotective effects of adrenomedullin on prevention of cognitive decline after chronic cerebral hypoperfusion in mice. Stroke. 2011;42:1122–1128. doi: 10.1161/STROKEAHA.110.603399 [PubMed: 21393586]

114. Hanabusa K, Nagaya N, Iwase T, Itoh T, Murakami S, Shimizu Y, Taki W, Miyatake K, Kangawa K. Adrenomedullin enhances therapeutic potency of mesenchymal stem cells after experimental stroke in rats. Stroke. 2005;36:853–858. doi: 10.1161/01.STR.0000157661.69482.76 [PubMed: 15746464]

115. Fujioka M, Nishio K, Sakaki T, Minamino N, Kitamura K. Adrenomedullin in patients with cerebral vasospasm after aneurysmal subarachnoid hemorrhage. Stroke. 2000;31:3079–3083. doi: 10.1161/01.str.31.12.3079-d

116. Seifert-Held T, Pekar T, Gattringer T, Simmet NE, Scharnagl H, Bocksrucker C, Lampl C, Storch MK, Stojakovic T, Fazekas F. Plasma midregional pro-adrenomedullin improves prediction of functional outcome in ischemic stroke. PLoS One. 2013;8:e68768. doi: 10.1371/journal.pone.0068768 [PubMed: 23894342]

117. Zhang H, Tang B, Yin CG, Chen Y, Meng QL, Jiang L, Wang WP, Niu GZ. Plasma adrenomedullin levels are associated with long-term outcomes of acute ischemic stroke. Peptides. 2014;52:44–48. doi: 10.1016/j.peptides.2013.11.025 [PubMed: 24333654]

118. Julian-Villaverde FJ, Ochoa-Callejero L, Siles E, Martinez-Lara E, Martinez A. Adrenomedullin Is a Diagnostic and Prognostic Biomarker for Acute Intracerebral Hemorrhage. Curr Issues Mol Biol. 2021;43:324–334. doi: 10.3390/cimb43010027 [PubMed: 34208106]

119. Xia CF, Yin H, Borlongan CV, Chao J, Chao L. Postischemic infusion of adrenomedullin protects against ischemic stroke by inhibiting apoptosis and promoting angiogenesis. Exp Neurol. 2006;197:521–530. doi: 10.1016/j.expneurol.2005.10.027 [PubMed: 16343485]

120. Chaung WW, Wu R, Ji Y, Wang Z, Dong W, Cheyuo C, Qi L, Qiang X, Wang H, Wang P. Peripheral administration of human adrenomedullin and its binding protein attenuates stroke-induced apoptosis and brain injury in rats. Mol Med. 2011;17:1075–1083. doi: 10.2119/molmed.2010.00104 [PubMed: 21695352]

121. Maki T, Takahashi Y, Miyamoto N, Liang AC, Ihara M, Lo EH, Arai K. Adrenomedullin promotes differentiation of oligodendrocyte precursor cells into myelin-basic-protein expressing oligodendrocytes under pathological conditions in vitro. Stem Cell Res. 2015;15:68–74. doi: 10.1016/j.scr.2015.05.001 [PubMed: 26002630]

122. Yoshimoto T, Saito S, Omae K, Hattori Y, Fukuma K, Kitamura K, Kakuta R, Kita T, Maruyama H, Yamamoto H, et al. Study Protocol for a Randomized, Double-Blind, Placebo-Controlled, Phase-II Trial: AdrenoMedullin for Ischemic Stroke Study. J Stroke Cerebrovasc Dis. 2021;30:105761. doi: 10.1016/j.jstrokecerebrovasdis.2021.105761 [PubMed: 33813084]

123. Ueda S, Nishio K, Minamino N, Kubo A, Akai Y, Kangawa K, Matsuo H, Fujimura Y, Yoshioka A, Masui K, et al. Increased plasma levels of adrenomedullin in patients with systemic inflammatory response syndrome. Am J Respir Crit Care Med. 1999;160:132–136. doi: 10.1164/ajrccm.160.1.9810006 [PubMed: 10390390]

124. Guignant C, Voirin N, Venet F, Poitevin F, Malcus C, Bohe J, Lepape A, Monneret G. Assessment of pro-vasopressin and pro-adrenomedullin as predictors of 28-day mortality in septic shock

patients. Intensive Care Med. 2009;35:1859–1867. doi: 10.1007/s00134-009-1610-5 [PubMed: 19662382]

125. Marino R, Struck J, Maisel AS, Magrini L, Bergmann A, Di Somma S. Plasma adrenomedullin is associated with short-term mortality and vasopressor requirement in patients admitted with sepsis. Crit Care. 2014;18:R34. doi: 10.1186/cc13731 [PubMed: 24533868]

126. Lundberg OHM, Lengquist M, Spangfors M, Annborn M, Bergmann D, Schulte J, Levin H, Melander O, Frigyesi A, Friberg H. Circulating bioactive adrenomedullin as a marker of sepsis, septic shock and critical illness. Crit Care. 2020;24:636. doi: 10.1186/s13054-020-03351-1 [PubMed: 33148300]

127. Temmesfeld-Wollbruck B, Brell B, David I, Dorenberg M, Adolphs J, Schmeck B, Suttorp N, Hippenstiel S. Adrenomedullin reduces vascular hyperpermeability and improves survival in rat septic shock. Intensive Care Med. 2007;33:703–710. doi: 10.1007/s00134-007-0561-y [PubMed: 17318497]

128. Geven C, Bergmann A, Kox M, Pickkers P. Vascular Effects of Adrenomedullin and the Anti-Adrenomedullin Antibody Adrecizumab in Sepsis. Shock. 2018;50:132–140. doi: 10.1097/SHK.0000000000001103 [PubMed: 29324626]

129. Laterre PF, Pickkers P, Marx G, Wittebole X, Meziani F, Dugernier T, Huberlant V, Schuerholz T, Francois B, Lascarrou JB, et al. Safety and tolerability of non-neutralizing adrenomedullin antibody adrecizumab (HAM8101) in septic shock patients: the AdrenOSS-2 phase 2a biomarker-guided trial. Intensive Care Med. 2021;47:1284–1294. doi: 10.1007/s00134-021-06537-5 [PubMed: 34605947]

130. Tsuruda T, Jougasaki M, Boerrigter G, Costello-Boerrigter LC, Cataliotti A, Lee SC, Salz-Gilman L, Nordstrom LJ, McGregor CG, Burnett JC. Ventricular adrenomedullin is associated with myocyte hypertrophy in human transplanted heart. Regul Pept. 2003;112:161–166. doi: 10.1016/s0167-0115(03)00035-1 [PubMed: 12667638]

131. Csordas A, Nietlispach F, Schuetz P, Huber A, Muller B, Maisano F, Taramasso M, Moarof I, Obeid S, Stahli BE, et al. Midregional Proadrenomedullin Improves Risk Stratification beyond Surgical Risk Scores in Patients Undergoing Transcatheter Aortic Valve Replacement. PLoS One. 2015;10:e0143761. doi: 10.1371/journal.pone.0143761 [PubMed: 26630012]

132. Tan ESJ, Oon YY, Chan SP, Liew OW, Chong JPC, Tay E, Soo WM, Yip JWL, Gong L, Lunaria JB, et al. Novel predictive role for mid-regional proadrenomedullin in moderate to severe aortic stenosis. Heart. 2022;108:1319–1327. doi: 10.1136/heartjnl-2021-320707 [PubMed: 35332049]

133. Witte MH, Bernas MJ, Martin CP, Witte CL. Lymphangiogenesis and lymphangiodysplasia: from molecular to clinical lymphology. Microsc Res Tech. 2001;55:122–145. doi: 10.1002/jemt.1163 [PubMed: 11596157]

134. Karkkainen MJ, Alitalo K. Lymphatic endothelial regulation, lymphoedema, and lymph node metastasis. Semin Cell Dev Biol. 2002;13:9–18. doi: 10.1006/scdb.2001.0286 [PubMed: 11969367]

135. Yamauchi A, Sakurai T, Kamiyoshi A, Ichikawa-Shindo Y, Kawate H, Igarashi K, Toriyama Y, Tanaka M, Liu T, Xian X, et al. Functional differentiation of RAMP2 and RAMP3 in their regulation of the vascular system. J Mol Cell Cardiol. 2014;77:73–85. doi: 10.1016/j.yjmcc.2014.09.017 [PubMed: 25264174]

136. Nikitenko LL, Shimosawa T, Henderson S, Makinen T, Shimosawa H, Qureshi U, Pedley RB, Rees MC, Fujita T, Boshoff C. Adrenomedullin haploinsufficiency predisposes to secondary lymphedema. J Invest Dermatol. 2013;133:1768–1776. doi: 10.1038/jid.2013.47 [PubMed: 23364478]

137. Vazquez R, Riveiro ME, Berenguer-Daize C, O'Kane A, Gormley J, Touzelet O, Rezai K, Bekradda M, Ouafik L. Targeting Adrenomedullin in Oncology: A Feasible Strategy With Potential as Much More Than an Alternative Anti-Angiogenic Therapy. Front Oncol. 2020;10:589218. doi: 10.3389/fonc.2020.589218 [PubMed: 33489885]

138. Wu XY, Hao CP, Ling M, Guo CH, Ma W. Hypoxia-induced apoptosis is blocked by adrenomedullin via upregulation of Bcl-2 in human osteosarcoma cells. Oncol Rep. 2015;34:787–794. doi: 10.3892/or.2015.4011 [PubMed: 26035796]

139. Benyahia Z, Dussault N, Cayol M, Sigaud R, Berenguer-Daize C, Delfino C, Tounsi A, Garcia S, Martin PM, Mabrouk K, et al. Stromal fibroblasts present in breast carcinomas promote tumor

growth and angiogenesis through adrenomedullin secretion. Oncotarget. 2017;8:15744–15762. doi: 10.18632/oncotarget.14999 [PubMed: 28178651]

140. Chen P, Huang Y, Bong R, Ding Y, Song N, Wang X, Song X, Luo Y. Tumor-associated macrophages promote angiogenesis and melanoma growth via adrenomedullin in a paracrine and autocrine manner. Clin Cancer Res. 2011;17:7230–7239. doi: 10.1158/1078-0432.CCR-11-1354 [PubMed: 21994414]

141. Zhao Q, Zhang P, Qin G, Ren F, Zheng Y, Qiao Y, Sun T, Zhang Y. Role of CXCR7 as a Common Predictor for Prognosis in Solid Tumors: a Meta-Analysis. J Cancer. 2018;9:3138–3148. doi: 10.7150/jca.25377 [PubMed: 30210637]

142. Karpinich NO, Kechele DO, Espenschied ST, Willcockson HH, Fedoriw Y, Caron KM. Adrenomedullin gene dosage correlates with tumor and lymph node lymphangiogenesis. FASEB J. 2013;27:590–600. doi: 10.1096/fj.12-214080 [PubMed: 23099649]

143. Karpinich NO, Caron KM. Gap junction coupling is required for tumor cell migration through lymphatic endothelium. Arterioscler Thromb Vasc Biol. 2015;35:1147–1155. doi: 10.1161/ATVBAHA.114.304752 [PubMed: 25792452]

144. Dai X, Ma W, He XJ, Jha RK. Elevated expression of adrenomedullin is correlated with prognosis and disease severity in osteosarcoma. Med Oncol. 2013;30:347. doi: 10.1007/s12032-012-0347-0 [PubMed: 23269582]

145. Angenendt L, Bormann E, Pabst C, Alla V, Gorlich D, Braun L, Dohlich K, Schwoppe C, Bohlander SK, Arteaga MF, et al. The neuropeptide receptor calcitonin receptor-like (CALCRL) is a potential therapeutic target in acute myeloid leukemia. Leukemia. 2019;33:2830–2841. doi: 10.1038/s41375-019-0505-x [PubMed: 31182782]

146. Larrue C, Guiraud N, Mouchel PL, Dubois M, Farge T, Gotanegre M, Bosc C, Saland E, Nicolau-Travers ML, Sabatier M, et al. Adrenomedullin-CALCRL axis controls relapse-initiating drug tolerant acute myeloid leukemia cells. Nat Commun. 2021;12:422. doi: 10.1038/s41467-020-20717-9 [PubMed: 33462236]

147. Angenendt L, Woste M, Mikesch JH, Arteaga MF, Angenendt A, Sandmann S, Berdel WE, Lenz G, Dugas M, Meshinchi S, et al. Calcitonin receptor-like (CALCRL) is a marker of stemness and an independent predictor of outcome in pediatric AML. Blood Adv. 2021;5:4413–4421. doi: 10.1182/bloodadvances.2021005236 [PubMed: 34559198]

148. Huang Z, Zhang H, Xing C, Zhang L, Zhu H, Deng Z, Yin L, Dong E, Wang C, Peng H. Identification and validation of CALCRL-associated prognostic genes in acute myeloid leukemia. Gene. 2022;809:146009. doi: 10.1016/j.gene.2021.146009 [PubMed: 34655717]

149. Wang R, Li M, Bai Y, Jiao Y, Qi X. CALCRL Gene is a Suitable Prognostic Factor in AML/ETO(+) AML Patients. J Oncol. 2022;2022:3024360. doi: 10.1155/2022/3024360 [PubMed: 35342399]

150. Liu LL, Chen SL, Huang YH, Yang X, Wang CH, He JH, Yun JP, Luo RZ. Adrenomedullin inhibits tumor metastasis and is associated with good prognosis in triple-negative breast cancer patients. Am J Transl Res. 2020;12:773–786. [PubMed: 32269711]

151. Christ-Crain M, Morgenthaler NG, Stolz D, Muller C, Bingisser R, Harbarth S, Tamm M, Struck J, Bergmann A, Muller B. Pro-adrenomedullin to predict severity and outcome in community-acquired pneumonia [ISRCTN04176397]. Crit Care. 2006;10:R96. doi: 10.1186/cc4955 [PubMed: 16805922]

152. Espana PP, Capelastegui A, Mar C, Bilbao A, Quintana JM, Diez R, Esteban C, Bereciartua E, Unanue U, Uranga A. Performance of pro-adrenomedullin for identifying adverse outcomes in community-acquired pneumonia. J Infect. 2015;70:457–466. doi: 10.1016/j.jinf.2014.12.003 [PubMed: 25499199]

153. Legramante JM, Mastropasqua M, Susi B, Porzio O, Mazza M, Miranda Agrippino G, C DA, Brandi A, Giovagnoli G, Di Lecce VN, et al. Prognostic performance of MR-pro-adrenomedullin in patients with community acquired pneumonia in the Emergency Department compared to clinical severity scores PSI and CURB. PLoS One. 2017;12:e0187702. doi: 10.1371/journal.pone.0187702 [PubMed: 29161297]

154. Bello S, Lasierra AB, Minchole E, Fandos S, Ruiz MA, Vera E, de Pablo F, Ferrer M, Menendez R, Torres A. Prognostic power of proadrenomedullin in community-acquired pneumonia is

independent of aetiology. Eur Respir J. 2012;39:1144–1155. doi: 10.1183/09031936.00080411 [PubMed: 22075489]

155. Helmy TA, Tammam HH, Leuis ME, Beshey BN. Prognostic Role of Serum Adrenomedullin in Patients with Ventilator Associated Pneumonia. Adv Respir Med. 2022;90:349–359. doi: 10.3390/arm90040044 [PubMed: 36004964]

156. Allaker RP, Zihni C, Kapas S. An investigation into the antimicrobial effects of adrenomedullin on members of the skin, oral, respiratory tract and gut microflora. FEMS Immunol Med Microbiol. 1999;23:289–293. doi: 10.1111/j.1574-695X.1999.tb01250.x [PubMed: 10225288]

157. Allaker RP, Grosvenor PW, McAnerney DC, Sheehan BE, Srikanta BH, Pell K, Kapas S. Mechanisms of adrenomedullin antimicrobial action. Peptides. 2006;27:661–666. doi: 10.1016/j.peptides.2005.09.003 [PubMed: 16226342]

158. Evereklioglu C, Doganay S, Er H, Yurekli M. Aqueous humor adrenomedullin levels differ in patients with different types of glaucoma. Jpn J Ophthalmol. 2002;46:203–208. doi: 10.1016/s0021-5155(01)00501-9 [PubMed: 12062228]

159. Ittner LM, Schwerdtfeger K, Kunz TH, Muff R, Husmann K, Grimm C, Hafezi F, Lang KS, Kurrer MO, Gotz J, et al. Transgenic mice with ocular overexpression of an adrenomedullin receptor reflect human acute angle-closure glaucoma. Clin Sci (Lond). 2008;114:49–58. doi: 10.1042/CS20070163 [PubMed: 17608625]

160. Blom J, Giove TJ, Pong WW, Blute TA, Eldred WD. Evidence for a functional adrenomedullin signaling pathway in the mouse retina. Mol Vis. 2012;18:1339–1353. [PubMed: 22690112]

161. Gong B, Zhang H, Huang L, Chen Y, Shi Y, Tam PO, Zhu X, Huang Y, Lei B, Sundaresan P, et al. Mutant RAMP2 causes primary open-angle glaucoma via the CRLR-cAMP axis. Genet Med. 2019;21:2345–2354. doi: 10.1038/s41436-019-0507-0 [PubMed: 31000793]

162. Overby DR, Zhou EH, Vargas-Pinto R, Pedrigi RM, Fuchshofer R, Braakman ST, Gupta R, Perkumas KM, Sherwood JM, Vahabikashi A, et al. Altered mechanobiology of Schlemm's canal endothelial cells in glaucoma. Proc Natl Acad Sci U S A. 2014;111:13876–13881. doi: 10.1073/pnas.1410602111 [PubMed: 25201985]

163. Sakata J, Asada Y, Shimokubo T, Kitani M, Inatsu H, Kitamura K, Kangawa K, Matsuo H, Sumiyoshi A, Eto T. Adrenomedullin in the gastrointestinal tract. Distribution and gene expression in rat and augmented gastric adrenomedullin after fasting. J Gastroenterol. 1998;33:828–834. doi: 10.1007/s005350050183 [PubMed: 9853555]

164. Martinez-Herrero S, Martinez A. Adrenomedullin regulates intestinal physiology and pathophysiology. Domest Anim Endocrinol. 2016;56 Suppl:S66–83. doi: 10.1016/j.domaniend.2016.02.004 [PubMed: 27345325]

165. Fukuda K, Tsukada H, Oya M, Onomura M, Kodama M, Nakamura H, Hosokawa M, Seino Y. Adrenomedullin promotes epithelial restitution of rat and human gastric mucosa in vitro. Peptides. 1999;20:127–132. doi: 10.1016/s0196-9781(98)00146-6 [PubMed: 10098633]

166. Fernandez de Arcaya I, Lostao MP, Martinez A, Berjon A, Barber A. Effect of adrenomedullin and proadrenomedullin N-terminal 20 peptide on sugar transport in the rat intestine. Regul Pept. 2005;129:147–154. doi: 10.1016/j.regpep.2005.02.001 [PubMed: 15927710]

167. Fukuda K, Tsukada H, Onomura M, Saito T, Kodama M, Nakamura H, Taniguchi T, Tominaga M, Hosokawa M, Seino Y. Effect of adrenomedullin on ion transport and muscle contraction in rat distal colon. Peptides. 1998;19:1043–1047. doi: 10.1016/s0196-9781(98)00043-6 [PubMed: 9700753]

168. Martinez-Herrero S, Larrayoz IM, Narro-Iniguez J, Rubio-Mediavilla S, Martinez A. Lack of Adrenomedullin Aggravates Acute TNBS-Induced Colitis Symptoms in Mice, Especially in Females. Front Physiol. 2017;8:1058. doi: 10.3389/fphys.2017.01058 [PubMed: 29311984]

169. Martinez-Herrero S, Larrayoz IM, Narro-Iniguez J, Villanueva-Millan MJ, Recio-Fernandez E, Perez-Matute P, Oteo JA, Martinez A. Lack of Adrenomedullin Results in Microbiota Changes and Aggravates Azoxymethane and Dextran Sulfate Sodium-Induced Colitis in Mice. Front Physiol. 2016;7:595. doi: 10.3389/fphys.2016.00595 [PubMed: 27965594]

170. Ashizuka S, Inagaki-Ohara K, Kuwasako K, Kato J, Inatsu H, Kitamura K. Adrenomedullin treatment reduces intestinal inflammation and maintains epithelial barrier function in mice

administered dextran sulphate sodium. Microbiol Immunol. 2009;53:573–581. doi: 10.1111/j.1348-0421.2009.00159.x [PubMed: 19780971]

171. Ashizuka S, Ishikawa N, Kato J, Yamaga J, Inatsu H, Eto T, Kitamura K. Effect of adrenomedullin administration on acetic acid-induced colitis in rats. Peptides. 2005;26:2610–2615. doi: 10.1016/j.peptides.2005.05.007 [PubMed: 15978699]

172. Ashizuka S, Kita T, Inatsu H, Kitamura K. Adrenomedullin: a novel therapy for intractable ulcerative colitis. Inflamm Bowel Dis. 2013;19:E26–27. doi: 10.1002/ibd.22891 [PubMed: 22294498]

173. Ashizuka S, Inatsu H, Kita T, Kitamura K. Adrenomedullin Therapy in Patients with Refractory Ulcerative Colitis: A Case Series. Dig Dis Sci. 2016;61:872–880. doi: 10.1007/s10620-015-3917-0 [PubMed: 26470867]

174. Kita T, Ashizuka S, Ohmiya N, Yamamoto T, Kanai T, Motoya S, Hirai F, Nakase H, Moriyama T, Nakamura M, et al. Adrenomedullin for steroid-resistant ulcerative colitis: a randomized, double-blind, placebo-controlled phase-2a clinical trial. J Gastroenterol. 2021;56:147–157. doi: 10.1007/s00535-020-01741-4 [PubMed: 33140199]

175. Kita T, Ashizuka S, Takeda T, Matsumoto T, Ohmiya N, Nakase H, Motoya S, Ohi H, Mitsuyama K, Hisamatsu T, et al. Adrenomedullin for biologic-resistant Crohn's disease: A randomized, double-blind, placebo-controlled phase 2a clinical trial. J Gastroenterol Hepatol. 2022;37:2051–2059. doi: 10.1111/jgh.15945 [PubMed: 35840351]

176. Kubo K, Tokashiki M, Kuwasako K, Tamura M, Tsuda S, Kubo S, Yoshizawa-Kumagaye K, Kato J, Kitamura K. Biological properties of adrenomedullin conjugated with polyethylene glycol. Peptides. 2014;57:118–121. doi: 10.1016/j.peptides.2014.05.005 [PubMed: 24874704]

177. Nilsson C, Hansen TK, Rosenquist C, Hartmann B, Kodra JT, Lau JF, Clausen TR, Raun K, Sams A. Long acting analogue of the calcitonin gene-related peptide induces positive metabolic effects and secretion of the glucagon-like peptide-1. Eur J Pharmacol. 2016;773:24–31. doi: 10.1016/j.ejphar.2016.01.003 [PubMed: 26808305]

178. Aubdool AA, Thakore P, Argunhan F, Smillie SJ, Schnelle M, Srivastava S, Alawi KM, Wilde E, Mitchell J, Farrell-Dillon K, et al. A Novel alpha-Calcitonin Gene-Related Peptide Analogue Protects Against End-Organ Damage in Experimental Hypertension, Cardiac Hypertrophy, and Heart Failure. Circulation. 2017;136:367–383. doi: 10.1161/CIRCULATIONAHA.117.028388 [PubMed: 28446517]

179. Booe JM, Warner ML, Pioszak AA. Picomolar Affinity Antagonist and Sustained Signaling Agonist Peptide Ligands for the Adrenomedullin and Calcitonin Gene-Related Peptide Receptors. ACS Pharmacol Transl Sci. 2020;3:759–772. doi: 10.1021/acsptsci.0c00031 [PubMed: 32832875]

180. Hendrikse ER, Liew LP, Bower RL, Bonnet M, Jamaluddin MA, Prodan N, Richards KD, Walker CS, Pairaudeau G, Smith DM, et al. Identification of Small-Molecule Positive Modulators of Calcitonin-like Receptor-Based Receptors. ACS Pharmacol Transl Sci. 2020;3:305–320. doi: 10.1021/acsptsci.9b00108 [PubMed: 32296770]

181. Chang CL, Hsu SYT. Development of chimeric and bifunctional antagonists for CLR/RAMP receptors. PLoS One. 2019;14:e0216996. doi: 10.1371/journal.pone.0216996 [PubMed: 31150417]

182. Chang CL, Cai Z, Hsu SYT. Sustained Activation of CLR/RAMP Receptors by Gel-Forming Agonists. Int J Mol Sci. 2022;23. doi: 10.3390/ijms232113408

183. Arfsten H, Goliasch G, Bartko PE, Prausmuller S, Spinka G, Cho A, Novak J, Haslacher H, Strunk G, Struck J, et al. Increased concentrations of bioactive adrenomedullin subsequently to angiotensin-receptor/neprilysin-inhibitor treatment in chronic systolic heart failure. Br J Clin Pharmacol. 2021;87:916–924. doi: 10.1111/bcp.14442 [PubMed: 32598074]

184. Menhaji-Klotz E, Ward J, Brown JA, Loria PM, Tan C, Hesp KD, Riccardi KA, Litchfield J, Boehm M. Discovery of Diphenylacetamides as CXCR7 Inhibitors with Novel beta-Arrestin Antagonist Activity. ACS Med Chem Lett. 2020;11:1330–1334. doi: 10.1021/acsmedchemlett.0c00163 [PubMed: 32551020]

185. Karakas M, Akin I, Burdelski C, Clemmensen P, Grahn H, Jarczak D, Kessler M, Kirchhof P, Landmesser U, Lezius S, et al. Single-dose of adrecizumab versus placebo in acute cardiogenic shock (ACCOST-HH): an investigator-initiated, randomised, double-blinded, placebo-controlled,

multicentre trial. Lancet Respir Med. 2022;10:247–254. doi: 10.1016/S2213-2600(21)00439-2 [PubMed: 34895483]

186. Simonavicius J, Mikalauskas A, Cerlinskaite K, Gayat E, Juknevicius V, Paleviciute E, Alitoit-Marrote I, Kablucko D, Bagdonaite L, Balciunas M, et al. Biologically Active Adrenomedullin (bio-ADM) is of Potential Value in Identifying Congestion and Selecting Patients for Neurohormonal Blockade in Acute Dyspnea. Am J Med. 2022;135:e165–e181. doi: 10.1016/j.amjmed.2022.02.006 [PubMed: 35245495]

187. Maisel A, Mueller C, Nowak RM, Peacock WF, Ponikowski P, Mockel M, Hogan C, Wu AH, Richards M, Clopton P, et al. Midregion prohormone adrenomedullin and prognosis in patients presenting with acute dyspnea: results from the BACH (Biomarkers in Acute Heart Failure) trial. J Am Coll Cardiol. 2011;58:1057–1067. doi: 10.1016/j.jacc.2011.06.006 [PubMed: 21867843]

188. Miguel D, Prieto B, Costa M, Coto D, Alvarez FV. Cord blood plasma reference intervals for potential sepsis markers: pro-adrenomedullin, pro-endothelin, and pro-atrial natriuretic peptide. Clin Biochem. 2011;44:337–341. doi: 10.1016/j.clinbiochem.2010.12.012 [PubMed: 21211519]

189. Mebazaa A, Geven C, Hollinger A, Wittebole X, Chousterman BG, Blet A, Gayat E, Hartmann O, Scigalla P, Struck J, et al. Circulating adrenomedullin estimates survival and reversibility of organ failure in sepsis: the prospective observational multinational Adrenomedullin and Outcome in Sepsis and Septic Shock-1 (AdrenOSS-1) study. Crit Care. 2018;22:354. doi: 10.1186/s13054-018-2243-2 [PubMed: 30583748]

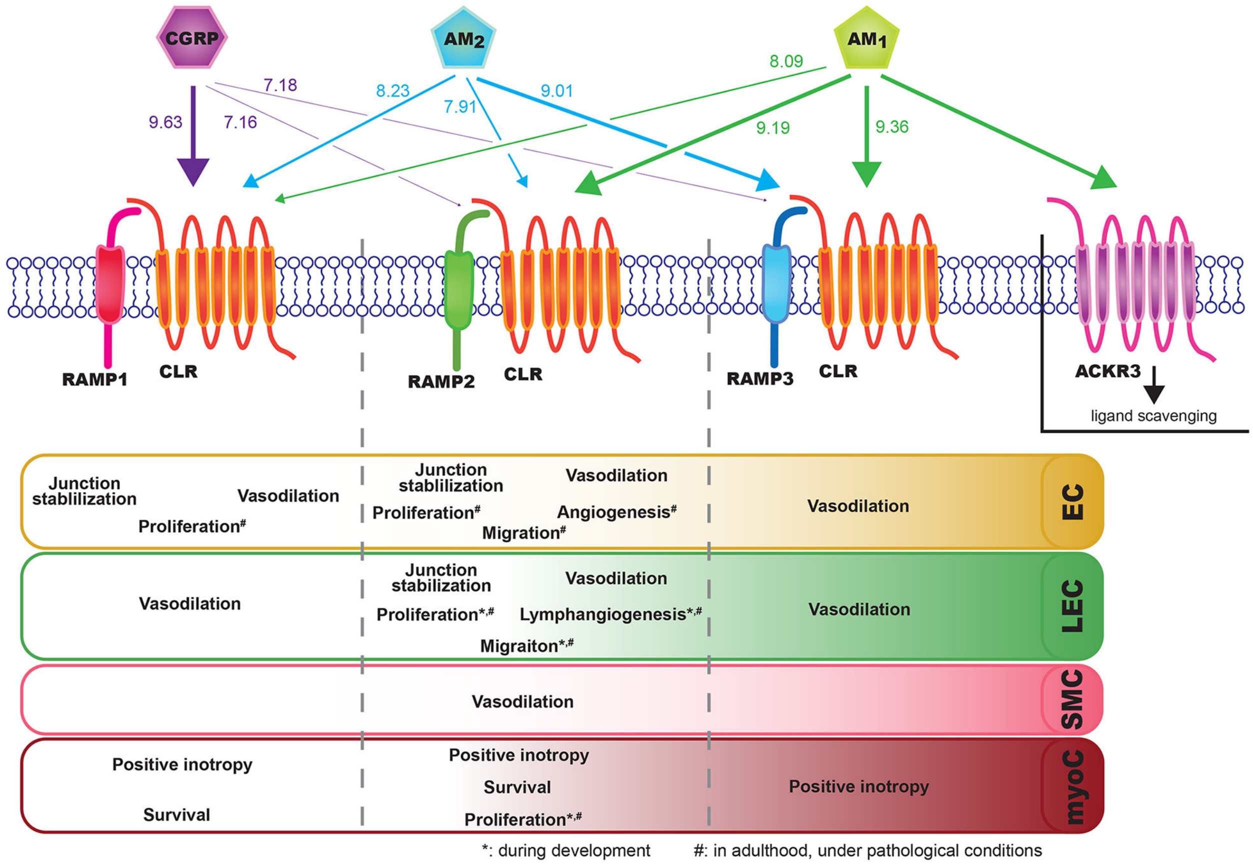

Figure 1: Schematic summarization of the intracellular signaling pathways activated by the binding of AM, CGRP and AM2.

Adrenomedullin acts through CLR/RAMP2 and CLR/RAMP3 receptor complexes and activates G-protein mediated intracellular pathways, which lead to cell-type specific cellular effects. ACKR3 degrades AM by recruiting G protein-coupled receptor kinases and β-arrestin.

Human pEC_{50} values for cAMP production are shown, where applicable (data from Hay et al. 2017[14]). Arrow thickness represent the corresponding pEC_{50} value. Known cellular effects in blood endothelial cells (BEC), lymphatic endothelial cells (LEC), vascular smooth muscle cells (SMC) and myocardial cells (myoC) are indicated.

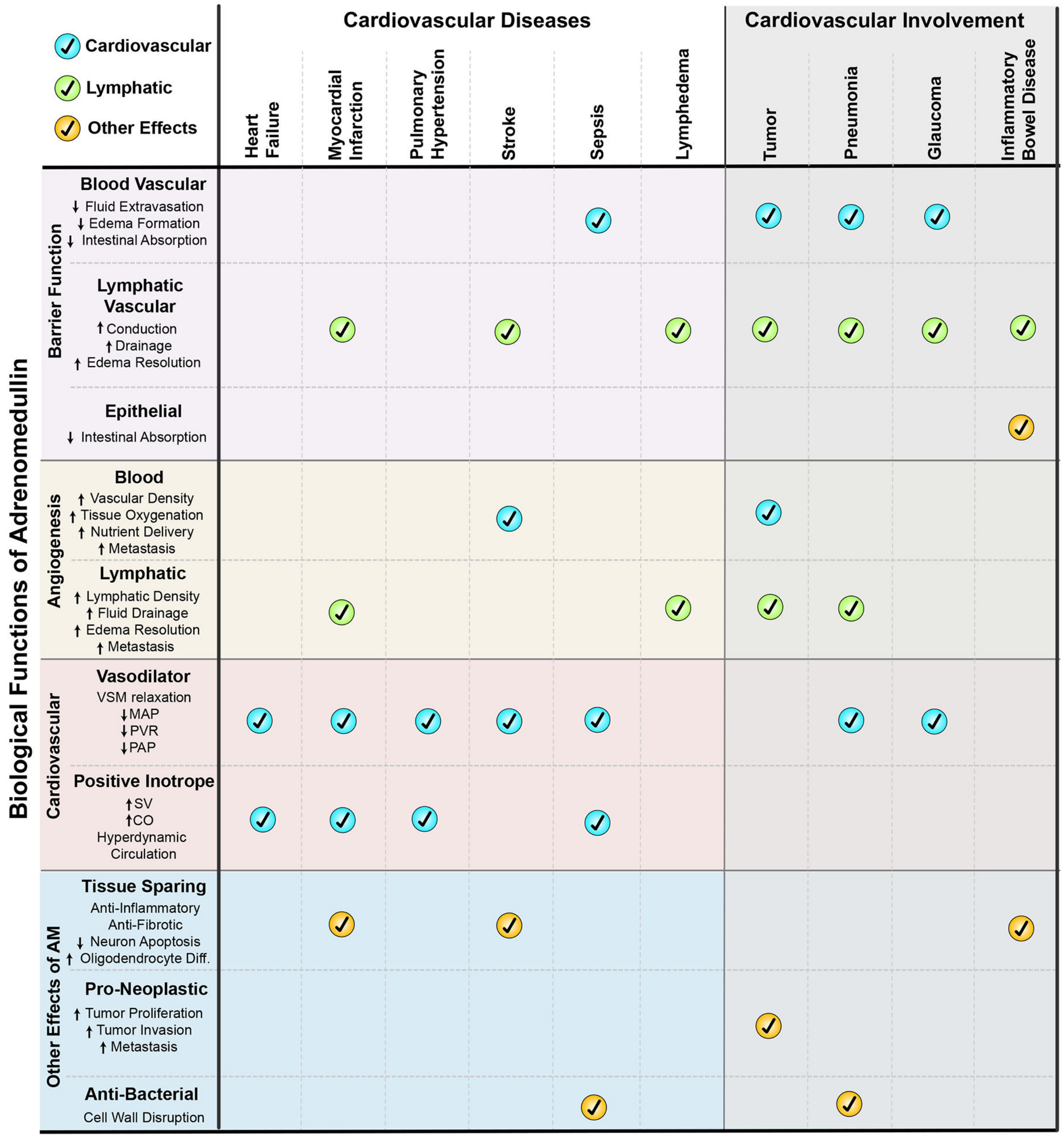

Figure 2: Involvement of AM-related biological functions in cardiovascular diseases.
Adrenomedullin acts on a diverse range of tissues generally favoring reduced cellular barrier permeability, angiogenesis, and has significant impact on cardiovascular parameters. Background colors distinguish the different types of AM's function (modulating barrier function, promoting angiogenesis, vasoactive and cardiac functions and non-vascular functions). The involvement of these AM-mediated mechanisms in cardiovascular related diseases is summarized above and grouped by physiologic role of AM. Checkmarks mark which AM functions contribute to each disease. Color of the checkmarks label whether that given function of AM is associated with blood endothelial, lymphatic endothelial cells or other cell types.

Table 1:

Clinical trials of therapeutic approaches targeting AM-signaling in cardiovascular diseases.

Disease	Clinical trial title	Type, status	Disease condition	Agent	Arms	Outcomes
Healthy	Adrecizumab Phase 1 Trial (NCT02991508)	Phase I completed	24 healthy male participants	Adrecizumab	• single infusion over 1 hour adrecizumab 0.5 mg/kg • single infusion over 1 hour adrecizumab 2 mg/kg • single infusion over 1 hour adrecizumab 8 mg/kg • Single dose i.v. placebo	• Adrecizumab showed an excellent safety profile. • Adrecizumab increased the maximum plasma concentration, a small volume of distribution, a low clearance rate and a terminal half-life of ~14 days. • Adrecizumab elicited a pronounced increase in plasma AM levels, but not mid-regional pro-adrenomedullin levels. [11]
Healthy	Adrecizumab-LPS Study (NCT03083171)	Phase I completed	24 healthy male participants i.v. 0 1 ng/kg E. Coli type O113 lipopolysaccharide followed by 1 ng/kg/hour for 3 hours.	Adrecizumab	• single infusion over 1 hour adrecizumab 0.5 mg/kg • single infusion over 1 hour adrecizumab 2 mg/kg • single infusion over 1 hour adrecizumab 8 mg/kg • Single dose i.v. placebo	• No effects of adrecizumab on cytokine clearance were observed. • Adrecizumab resolved endotoxin-induced flu-like symptoms more rapidly. [11]
Sepsis	AdrenOSS-2 (NCT03085758)	Phase IIa completed	301 patients with adrenomedullin above 70 pg/mL, at early stage of septic shock	Adrecizumab	• Single dose i.v. adrecizumab 2mg/kg b.w. • Single dose i.v. adrecizumab 4mg/kg b.w. • Single dose i.v. placebo	• Adrecizumab was well tolerated and showed a favorable safety profile • Adrecizumab demonstrated significant and fast improvement of organ function and a substantial reduction on short-term mortality (45% relative reduction at day 14) • Adrecizumab rapidly increased plasma levels of bioactive Adrenomedullin. [11,129]
Cardiogenic shock	Adrecizumabin Cardiogenic Shock	Phase II, completed	150 patients with cardiogenic shock	Adrecizumab	• Single dose i.v. adrecizumab 8mg/kg b.w.	• Adrecizumab was well tolerated in patients with cardiogenic shock.

Disease	Clinical trial title	Type, status	Disease condition	Agent	Arms	Outcomes
	(ACCOST-HH) (NCT03989531)				• Single dose i.v. placebo	• Adrecizumab did not reduce the need for cardiovascular organ support or improve survival at days 30 and 90.[185]
Heart Failure	Adrecizumab Dose Escalation Safety and Tolerability Evaluation (NCT04252937)	Phase II, recruiting	est. 40 patients with acute heart failure (NYHA class II-IV), within 48h from hospital admission	Adrecizumab	• 0.5mg/kg b.w. adrecizumab • 2mg/kg b.w. adrecizumab • 8mg/kg b.w. adrecizumab • standard of care treatment	
Inflammatory Bowel Disease	Assessment of the Safety, Tolerability, and Pharmacokinetic of HM201 (NCT05088369)	Phase I, recruiting	est. 68 healthy male subjects	HM201 (Pegylated Human Adreniomedullin)	SAD cohorts: • Single dose i.v. 0.01mg/kg b.w. HM201 • Single dose i.v. 0.03mg/kg b.w. HM201 • Single dose i.v. 0.06mg/kg b.w. HM201 • Single dose i.v. 0.12mg/kg b.w. HM201 • Single dose i.v. placebo MAD cohorts: • i.v. 0.01mg/kg b.w. HM201 once a week for 4 weeks. • i.v. 0.03mg/kg b.w. HM201 once a week for 4 weeks • i.v. 0.06mg/kg b.w. HM201 once a week for 4 weeks • i.v. 0.12mg/kg b.w. HM201 once a week for 4 weeks • i.v. placebo once a week for 4 weeks	
Ulcerative colitis (UC)	Adrenomedullin for steroid-resistant ulcerative colitis (Japic CTI-205255 [200410115290])	Phase IIa completed	28 patients with steroid-resistant UC	Adrenomedullin	• i.v. 5 ng/kg/min of AM for 8 h per day for 14 days • i.v. 10 ng/kg/min of AM for 8 h per day for 14 days • i.v. 15 ng/kg/min of AM for 8 h per day for 14 days	• Mayo score at 8 weeks was decreased in the 15 ng/kg/min AM group compared with the placebo group.

Disease	Clinical trial title	Type, status	Disease condition	Agent	Arms	Outcomes
					• i.v. placebo for 8 h per day for 14 days	• Complete remission at 8 weeks in patients receiving 15 ng/kg/min AM. • Mild but no serious adverse events caused by the vasodilatory effect of AM. [174]
Crohn's disease (CD)	Adrenomedullin for biologic-resistant Crohn's disease (JAPIC CTI-183947 [200612912663])	Phase IIa completed	24 patients with biologic-resistant CD	Adrenomedullin	• i.v. 10 of adrenomedullin for 8 h per day for 7 days • i.v. 15 ng/kg/min of adrenomedullin or placebo for 8 h per day for 7 days • i.v. placebo for 8 h per day for 7 days	• A 24-weeks decrease in CD activity index in the adrenomedullin-treated groups. [175]

Table 2:

Clinical trials investigating the diagnostic use of AM-signaling in cardiovascular diseases.

Disease	Clinical trial title	Type, status	Disease condition	Investigated biomarkers	Outcomes
Acute heart failure	Lithuanian Echocardiography Study of Dyspnea in Acute Settings (LEDA) (NCT03048032)	Completed	643 patients with acute heart failure, dyspnea, and 545 control subjects	Bio-AM NT-proBNP Troponin T CRP	• Bio-AM concentration was higher in patients with peripheral edema • Bio-AM reflects the presence and the degree of pulmonary, peripheral, and intravascular volume overload. • Bio-AM is strongly related to 90-day mortality in acute dyspnea. [186]
Acute Heart Failure	Biomarkers in Acute Heart Failure (BACH) (NCT00537628)	Completed	568 patients with acute heart failure, Dyspnea	MR proAM Copeptin BNP NT-proBNP MR proANP PCT Troponin T Troponin I	• MR-proADM identifies patients with high 90-day mortality and adds prognostic value to natriuretic peptides in patients presenting with acute shortness of breath. [71,187]
Sepsis	Pro-adrenomedullin as a Prognostic Marker in Neonatal Sepsis (NCT01362504)	Completed	73 newborn infants with neonatal sepsis and 194 control subjects	MR-proAM CT-proET-1 MR-proANP (PCT).	• The median values (reference interval) of CT-proET-1, MR-pro-ADM, and MR-proANP measured in cord blood plasma were 72 pmol/L (39–115), 0.84 nmol/L (0.5–1.38), and 163 pmol/L (76–389), respectively. • These reference intervals can be used for further clinical investigations aimed to elucidate the potential prognostic/ diagnostic value of these markers in neonatal sepsis management. [188]
Sepsis	Adrenomedullin and Outcome in Severe Sepsis and Septic Shock (AdrenOSS) (NCT02393781)	Completed	583 patiens with severe sepsis, septic shock	Bio- AM BNP NT-proBNP PCT Troponin T Troponin I	• Marked associations between bio-ADM upon admission and 28-day mortality, adjusted HR 1.6 and between bio-ADM levels and SOFA score ($p < 0.0001$). • Need of vasopressor, renal replacement therapy, and positive fluid balance were more prevalent in patients with a bio-ADM > 70 pg/ml. • persistently elevated bio-ADM at day 2 was associated with prolonged organ dysfunction and high 28-day mortality. [189]
Pulmonary hypertension	PulmoBind (NCT02216279)	Phase II, completed	30 patients with Pulmonary arterial Hypertension (PAH, n=23), or chronic thromboembolic Pulmonary Hypertension (PH, n=7), 15 healthy controls after injection	AM	• PulmoBind injection was well tolerated. • Segmental defects compatible with segmental defects were present in 7/7 CTEPH patients and in 2/23 PAH patients.

Disease	Clinical trial title	Type, status	Disease condition	Investigated biomarkers	Outcomes
			of 15 mCi 99mTc-PulmoBind in supine position		• Pulmobind activity distribution index was elevated in PH vs. controls (65% +/− 28% vs. 41% +/− 13%, p=0.0003).[110]
Pancreatic cancer	Tumour Regulatory Molecules in Early Pancreatic Cancer Detection (TEM-PAC) (NCT03536793)	Recriuiting	est. 50 patients with pancreatic cysts, 50 patients with pancreas cancer, and 80 healthy controls	Bio-AM TF	