



Deposited via The University of Sheffield.

White Rose Research Online URL for this paper:

<https://eprints.whiterose.ac.uk/id/eprint/190521/>

Version: Published Version

---

**Article:**

Jailani, A.B.A., Bigos, K.J.A., Avgoustou, P. et al. (2022) Targeting the adrenomedullin-2 receptor for the discovery and development of novel anti-cancer agents. *Expert Opinion on Drug Discovery*, 17 (8). pp. 839-848. ISSN: 1746-0441

<https://doi.org/10.1080/17460441.2022.2090541>

---

**Reuse**

This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here:

<https://creativecommons.org/licenses/>

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.

# Targeting the adrenomedullin-2 receptor for the discovery and development of novel anti-cancer agents

Ameera B. A. Jailani, Kamilla J. A. Bigos, Paris Avgoustou, Joseph L. Egan, Robert A. Hathway, Timothy M. Skerry & Gareth O. Richards

To cite this article: Ameera B. A. Jailani, Kamilla J. A. Bigos, Paris Avgoustou, Joseph L. Egan, Robert A. Hathway, Timothy M. Skerry & Gareth O. Richards (2022) Targeting the adrenomedullin-2 receptor for the discovery and development of novel anti-cancer agents, Expert Opinion on Drug Discovery, 17:8, 839-848, DOI: [10.1080/17460441.2022.2090541](https://doi.org/10.1080/17460441.2022.2090541)

To link to this article: <https://doi.org/10.1080/17460441.2022.2090541>



© 2022 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



Published online: 22 Jun 2022.



Submit your article to this journal [↗](#)



Article views: 995










View related articles [↗](#)



View Crossmark data [↗](#)

## Targeting the adrenomedullin-2 receptor for the discovery and development of novel anti-cancer agents

Ameera B. A. Jailani <sup>a,†</sup>, Kamilla J. A. Bigos <sup>a,†</sup>, Paris Avgoustou <sup>a,†</sup>, Joseph L. Egan <sup>b</sup>, Robert A. Hathway <sup>b</sup>, Timothy M. Skerry <sup>a,†</sup> and Gareth O. Richards <sup>a,†</sup>

<sup>a</sup>Department of Oncology and Metabolism, University of Sheffield, Sheffield, UK; <sup>b</sup>Department of Chemistry, University of Sheffield, Sheffield, UK

### ABSTRACT

**Introduction:** Adrenomedullin (AM) is a peptide responsible for many physiological processes including vascular health and hormone regulation. Dysregulation of AM signaling can stimulate cancers by promoting proliferation, angiogenesis and metastasis. Two AM receptors contribute to tumor progression in different ways. Adrenomedullin-1 receptor (AM<sub>1</sub>R) regulates blood pressure and blocking AM signaling via AM<sub>1</sub>R would be clinically unacceptable. Therefore, antagonizing adrenomedullin-2 receptor (AM<sub>2</sub>R) presents as an avenue for anti-cancer drug development.

**Areas covered:** We review the literature to highlight AM's role in cancer as well as delineating the specific roles AM<sub>1</sub>R and AM<sub>2</sub>R mediate in the development of a pro-tumoral microenvironment. We highlight the importance of exploring the residue differences between the receptors that led to the development of first-in-class selective AM<sub>2</sub>R small molecule antagonists. We also summarize the current approaches targeting AM and its receptors, their anti-tumor effects and their limitations.

**Expert opinion:** As tool compounds, AM<sub>2</sub>R antagonists will allow the dissection of the functions of CGRPR (calcitonin gene-related peptide receptor), AM<sub>1</sub>R and AM<sub>2</sub>R, and has considerable potential as a first-in-class oncology therapy. Furthermore, the lack of detectable side effects and good drug-like pharmacokinetic properties of these AM<sub>2</sub>R antagonists support the promise of this class of compounds as potential anti-cancer therapeutics.

### ARTICLE HISTORY

Received 8 February 2022  
Accepted 13 June 2022

### KEYWORDS

Adrenomedullin; angiogenesis; antagonists; cancer; calcitonin receptor-like receptor; receptor activity-modifying protein; therapeutic target; G protein-coupled receptor; hypoxia; metastasis

## 1. Introduction

Adrenomedullin (AM) is a multifunctional peptide belonging to the calcitonin gene-related peptide (CGRP) superfamily of peptide hormones. Originally identified as a hypotensive agent, AM is also a bronchodilator, hormone regulator and neurotransmitter [1]. AM has a closely related peptide called intermedin (IMD or adrenomedullin-2) with similar physiological effects and tissue expression [2].

AM is mainly secreted by endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) as important sources of circulating AM [3]. Serum levels of AM in humans normally range between 10 and 30 pg/mL [4–6]. An elevated serum AM level is associated with many diseases including heart failure (~50 pg/mL) [5] and sepsis (~33–500 pg/mL) [6,7], warranting investigations into AM being a potential biomarker [8,9].

AM mediates its activity through receptor complexes comprising a G protein-coupled receptor (GPCR) – calcitonin receptor-like receptor (CLR) – together with one of three accessory proteins known as receptor activity-modifying protein (RAMPs; Figure 1). RAMPs aid in GPCR trafficking, ligand selectivity and downstream signaling [10]. There are two AM receptors – adrenomedullin-1 receptor (AM<sub>1</sub>R) and adrenomedullin-2 receptor (AM<sub>2</sub>R) – which are formed by CLR/RAMP2

and CLR/RAMP3 heteromers respectively. CLR/RAMP1 forms the CGRP receptor (CGRPR) which AM binds to less potently compared to AM<sub>1</sub>R and AM<sub>2</sub>R. AM<sub>1</sub>R regulates physiological roles including fetal development and cardiovascular health [11,12]. However, dysregulation of AM signaling via both AM<sub>1</sub>R and AM<sub>2</sub>R can mediate different types of pro-tumoral signaling within the tumor microenvironment.

## 2. AM in cancer

AM and its receptor components are expressed in many cancers and is usually associated with poorer prognosis (Table 1). AM and its receptors are secreted and expressed not only by cancer cells but also stromal cells including cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs) and mast cells [13–17]. Bidirectional AM signaling between cancer and stromal cells can result in various pro-tumoral effects within the microenvironment (Figure 2).

Overexpression and exogenous treatment of AM has been shown to increase proliferation of some cancer cells *in vitro* and *in vivo* [33–35] by promoting signaling pathways including ERK/MAPK and JNK/AP-1 [33,36]. Furthermore, AM also enables cancer cells to evade apoptosis by up-regulating NF-

### Article highlights

- Tumor hypoxia up-regulates many proangiogenic targets, one of which is adrenomedullin (AM).
- AM is secreted by cancer and stromal cells to mediate and sustain pro-tumoral processes including angiogenesis, metastasis and immune evasion.
- Overexpression of AM and its receptors is correlated with poorer prognosis in a variety of cancers.
- AM signals through two receptors which contribute to tumor progression and development in distinct ways.
- AM receptors are each comprised of the same G protein-coupled receptor coupled with a different accessory protein - receptor activity-modifying proteins (RAMPs).
- Current methods targeting AM and its receptors (CLR or RAMPs individually) show promising anti-cancer activity, but do not target the receptor complexes as a whole.
- To reduce off-target effects, new therapies should target the adrenomedullin-2 receptor complex, not the individual components.
- Key residue differences between AM receptors allow selective small molecule antagonists against adrenomedullin-2 receptor complex to be developed as potential anti-cancer agents.

kB and Bcl-2 and downregulating caspases-3 and -8 [35,37–39]. AM has also been shown to activate molecules associated with invasion and migration in several cancers, including up-regulating integrin  $\alpha 5\beta 1$  and phosphorylation of FAK and paxillin [37,40,41]. These signals can modify various functions of cancer cells including their shape and motility, encouraging them to migrate and invade neighboring tissues to eventually metastasize to distant sites.

The role of AM in angiogenesis has been extensively researched due to its expression in ECs and VSMCs. AM is one of several proangiogenic targets up-regulated in hypoxia to maintain tumor growth and progression [42]. Transcription factor HIF-1 $\alpha$  synthesizes AM through hypoxic response elements [43,44]. Multiple studies have shown that AM treatment or overexpression in cancer cells resulted in increased capillary density and tumor weight *in vivo* [33,36,45–49]. AM is not only secreted by cancer cells, but also CAFs, TAMs and mast cells to

induce and maintain angiogenesis within the tumor microenvironment [13–17].

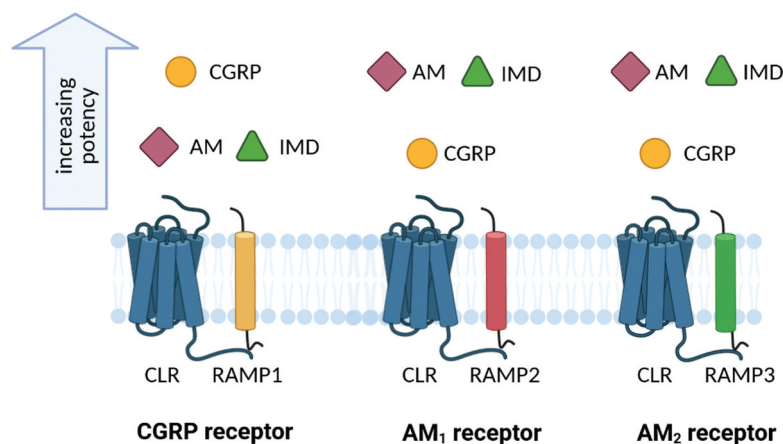
AM can protect tumors from immune surveillance by dampening pro-inflammatory cytokine signals [50]. Tumor-derived AM increases infiltration of pro-tumoral immune cells (myeloid-derived suppressor cells, mast cells, and M2 TAMs) [51]. AM also mediates macrophage polarization into M2 TAMs [52] which are not just immunosuppressive but also promote tumor growth and metastasis. Plasma and tissue AM expression are also correlated with neuroendocrine differentiation in tumors, which is associated with poorer prognosis [53].

Although there is a body of evidence for the role of AM in mediating pro-tumoral processes, it is less clear which AM receptors mediate each function.

### 3. AM receptors in cancer

Receptor components for both AM receptors are expressed in many cancers (Table 1), making it difficult to distinguish which AM receptor is responsible for pro-tumorigenic effects. Whilst CLR, RAMP2 and RAMP3 are all expressed in tumors, RAMP3 expression is particularly associated with stromal cells such as infiltrating endothelial and immune cells [37,51,54] as well as CAFs [14].

Knockout mouse studies have provided an insight into the specific roles of AM<sub>1</sub>R and AM<sub>2</sub>R in cancer. As complete RAMP2 knockout mice result in embryo lethality due to defects including lymphatic vascular development [55], tissue-specific RAMP2 knockout [56] or heterozygous mouse models [57] have been used to interrogate the role of RAMP2 in cancer. Tanaka *et al.* have shown that in mice with tamoxifen-inducible endothelial-specific RAMP2 knockout (DI-E-RAMP2<sup>-/-</sup>), sarcoma and melanoma *in vivo* tumor growth and angiogenesis were decreased compared with wild-type mice [56]. However, lung metastasis was increased in DI-E-RAMP2<sup>-/-</sup> mice because pulmonary ECs allowed infiltrating immune cells to express chemotactic factors,



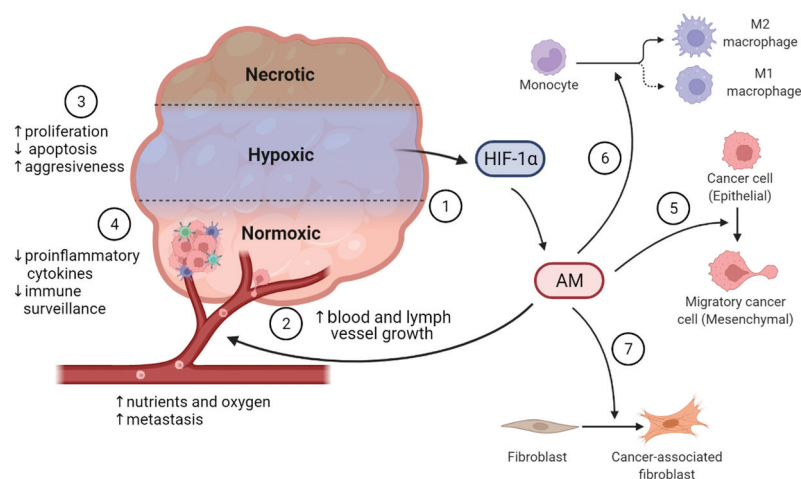
AM: adrenomedullin; CGRP: calcitonin gene-related peptide; CLR: calcitonin receptor-like receptor; IMD: intermedin; RAMP: receptor activity-modifying protein

**Figure 1.** Calcitonin receptor-like receptor (CLR) and receptor activity-modifying protein (RAMP) receptor complexes. Interaction of CLR with one of three RAMPs leads to the formation of three CLR/RAMP receptor complexes with distinct pharmacology, trafficking and signaling consequences. Each CLR/RAMP receptor favors the calcitonin family of peptides at different potencies. Image created in Biorender.com.

**Table 1.** Expression of AM and its receptor components (CLR, RAMP2 and RAMP3) in different cancers.

Cancer type	AM	CLR	RAMP2	RAMP3	Clinical implication	References
Acute myeloid leukemia	+(m)(p)	+(m)(p)	+(m)(p)	+(m)(p)	↑ AM ↑ CLR → ↓ OS and DFS	[18,60]
Breast	+(m)(p)	-	-	+(m)	↑ AM → ↑ lymph node mets	[19,20,101]
Colorectal	+(m)(p)	+(p)	+(p)	+(p)	↑ AM, CLR, RAMP2, RAMP3 → ↑ lymph node and distant mets ↑ AM → ↓ DFS	[21,34]
Glioblastoma	+(m)(p)	+(m)	+(m)	+(m)	No prognostic data	[98]
Renal	+(m)(p)	+(m)(p)	+(m)(p)	+(m)(p)	↑ AM ↑ CLR → tumor vs. healthy tissues ↑ AM → ↑ risk of relapse following nephrectomy ↑ CLR → ↑ tumor grade	[22,23,37]
Liver	+(m)(p)	+(m)	+(m)	+(m)	↑ AM → ↑ intrahepatic mets	[24,25]
Lung	+(m)(p)	+(m)(p)	+(m)(p)	+(m)(p)	No correlation between AM expression, tumor stage and OS	[26,92]
Melanoma	+(p)	+(p)	+(p)	+(p)	No prognostic data	[13,27]
Osteosarcoma	+(m)(p)	+(m)	+(m)	+(m)	↑ AM → ↑ mets	[28,29]
Ovarian	+(m)(p)	+(m)	+(m)	+(m)	↑ AM → ↑ tumor stage	[30,40]
Pancreas	+(m)(p)	+(m)(p)	+(m)(p)	+(m)	↑ AM → ↓ DFS	[31,90]
Prostate	+(m)(p)	+(m)(p)	+(m)(p)	+(m)(p)	↑ AM → ↑ Gleason score	[32,33]

All results presented were obtained from human cancer cell lines or tissues. +: positive expression; -: undetected; m: mRNA expression determined by endpoint or real-time PCR; p: protein expression determined by western blotting or immunohistochemistry; mets: metastasis; DFS: disease-free survival; OS: overall survival



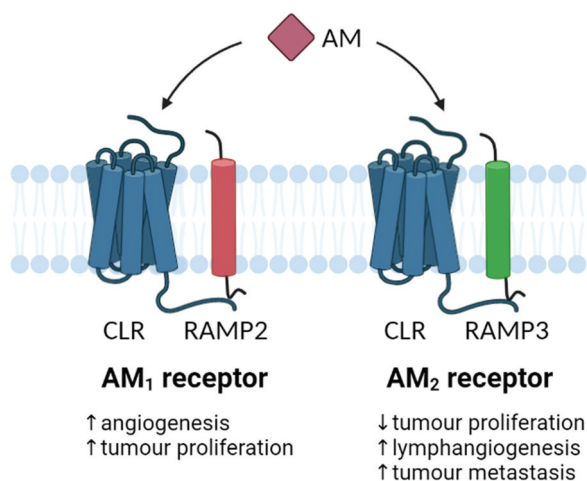
**Figure 2.** Bidirectional AM signaling between cancer and stromal cells can result in various pro-tumoral effects within the microenvironment. (1) Hypoxic tumors up-regulate transcription factor HIF-1 $\alpha$  to increase angiogenesis for more oxygen supply to the area. Aside from VEGF, another of HIF-1 $\alpha$ 's targets is AM, which not only regulates blood vessel growth into the tumor, but also lymph vasculature (2). (3) By increasing nutrient and oxygen availability to the once hypoxic tumor, AM mediates cancer cells within the microenvironment to increase proliferation, evade apoptosis, become more aggressive (neuroendocrine phenotype). (4) AM also enables pro-tumoral immune cells to infiltrate the tumor and dampen the pro-inflammatory signals, protecting tumors from immune surveillance. (5) AM can induce cancer cells to undergo epithelial-to-mesenchymal transformation, becoming more migratory and metastatic. Aside from affecting cancer cells themselves, AM also promotes pro-tumoral phenotype in other stromal cells. AM promotes infiltrating monocytes to polarize into M2 tumor-associated macrophages (TAMs, 6) as well as activate normal fibroblasts into cancer-associated fibroblasts (CAFs, 7). TAMs and CAFs themselves secrete AM and other factors into the microenvironment to sustain cancer growth and development. Image created in Biorender.com.

forming a pre-metastatic niche. This shows how important the AM/RAMP2 system is for vascular integrity and to inhibit tumor metastasis.

The group also investigated the roles of both RAMP2 and RAMP3 in pancreatic tumors, using DI-E-RAMP2 $^{-/-}$  mice and RAMP3 $^{-/-}$  mice [58]. Overall, the study found that selective activation of AM/RAMP2 and inhibition of AM/RAMP3 suppresses both tumor growth and metastasis. As in the previous study, metastasis was increased in DI-E-RAMP2 $^{-/-}$  mice. However, liver metastasis was decreased in RAMP3 $^{-/-}$  mice, correlating with reduced infiltrating podoplanin-expressing CAFs. This subtype of CAFs has been demonstrated to be associated with poorer prognosis in cancer patients [59]. This is one of the first studies to show the significant role of AM<sub>2</sub>R in cancer.

Recently, elevated expression of AM and CLR was shown to correlate with adverse outcomes in acute myeloid leukemia (AML) [60]. AM/CLR axis is required for cell growth and survival of AML blasts. Most interestingly, the study revealed a critical role of AM/CLR in maintaining resistant stem cell populations that persist after chemotherapy. As a whole, these studies suggest that AM and its receptors not only mediate tumor growth and metastasis, but also drug resistance (Figure 3).

The field is still limited in available tools to clearly delineate the roles of each AM receptor. Many of these studies target individual receptor components (CLR, RAMP2 or RAMP3) by using peptide antagonists, small molecules, antibodies or genetic manipulation. However, those methods do not allow for targeting of CLR/RAMP receptors as heteromeric complexes and it is imperative to develop compounds that do



AM: adrenomedullin; CLR: calcitonin receptor-like receptor; RAMP: receptor activity-modifying protein

**Figure 3.** AM and its receptors contribute to tumor growth and development by different functions. AM<sub>1</sub> receptor is associated with increased tumor proliferation and regulation of endothelial cells for angiogenesis. AM<sub>2</sub> receptor, whilst it is linked with inhibition of tumor proliferation, regulates cancer-associated fibroblasts to promote lymphangiogenesis and induce metastasis. Image created in Biorender.com.

because heteromeric receptors comprising either the CLR or the RAMPs associated with other partners make interpretation of such studies complex. Selective antagonists of specific receptor/RAMP complexes provide more specific ability to explore such questions.

#### 4. Differences between CLR/RAMP receptor complexes

Published crystal structures of CGRPR and AM<sub>1</sub>R bound to truncated peptide antagonists CGRP<sub>27–37</sub> and AM<sub>35–52</sub> respectively, give insights into the association of ligands with CLR/RAMP receptors [61,62]. Different research groups have shown independently the presence of a hydrophobic patch and pocket separated by the Trp72 shelf (W72 bulge), which largely form the binding pocket of the heteromers [61,62]. Furthermore, it was demonstrated that a  $\beta$ -turn on both CGRP and AM peptides enables them to occupy their binding pockets and modulate their interactions with CLR and RAMP residues [61].

CGRP interacts with CLR residues G71 and W72 as well as RAMP1 residue W84 via its F37 phenyl ring [62]. As described by ter Haar and coworkers, CGRP binds almost entirely to the CLR domain and makes only one critical contact with RAMP1 (residue W84) [62]. Also, hydrogen bonds are formed between CGRP residue V32 and CLR's W72 bulge and a main-chain to side-chain connection is made between CGRP T30 and CLR loop 3 D94 residues [61].

Similarly, AM residues Y52 and K46 interact with residues R97, E101 and E105 on RAMP2. An extension of a single helical turn allows AM K64 to contact the W72 bulge. It is worth mentioning that the equivalent residue on RAMP1 (W74) was unable to interact with AM. This was explained by the lack of a glutamine residue at position 74 of RAMP1 that discourages AM interaction [63].

Recent studies, including the published cryo-EM structures of CGRPR, AM<sub>1</sub>R, and AM<sub>2</sub>R, have given a deeper understanding to these receptor complexes and their mechanisms of activation [64–66]. An alanine substitution study on the AM<sub>15–52</sub> identified several residues important for receptor signaling. More specifically, substitution of AM residues F18, T20, L26, and I30 showed a significant decrease in activity in all the three CLR/RAMP receptors [66]. Interestingly, alanine substitution on AM<sub>15–52</sub> residue G19 showed an increase in activity in all pathways tested and in all receptors. More importantly, this substitution revealed a more CGRP-like profile to AM response whereby AM could activate IP1 production which was otherwise only restricted to CGRP [66].

#### 5. Development of molecules targeting CLR/RAMP receptors

A notable example of pharmacological targeting of GPCR complexes and more importantly CLR/RAMP complexes (Table 2) is the modulation of CGRPR for migraine treatment [67]. Evidence of CGRP's key role in migraine [68,69] have been accumulating since its discovery [70], leading to the initiation of many drug development programs against the receptor complex. This led to the development of several small-molecule antagonists against CGRPR, collectively known as 'gepants' with promising preclinical and clinical indications [67].

Although many members of the family, including telcagepant [71] and olcegepant [72], have been shown to reduce migraine symptoms without the cardiovascular side-effects (such as myocardial infarction, cardiovascular death and ischemic heart disease) of existing treatments (i.e. triptans and DHE), they were later discontinued due to modest elevations of liver enzymes indicating potential liver toxicity [73]. Other gepants such as ubrogepant [74] and rimegepant [75] have not been associated with liver toxicity, leading to their FDA approval for acute migraine treatment in 2019 [76] and 2020 [77], respectively. Even though gepants have significant differences in their chemical structures, they act in a similar manner by blocking the CLR/RAMP1 interface preventing CGRP binding [62], indicating a distinct chemophore that can be utilized for targeting this receptor complex. This was further supported by structure–activity relationship studies that showed the presence of three important interactive regions on CGRPR antagonists that bind to CLR/RAMP heteromers that facilitate their binding and selectivity: 1) CLR-binding region; 2) interface region that binds close to the Trp72 bulge; 3) CLR/RAMP1 binding region that interacts with the CLR/RAMP1 hydrophobic patch [78].

Similar mode of action was shown by erenumab [79], the first FDA-approved monoclonal antibody which targets the ligand binding site of the CGRPR [80]. Three other recently approved human CGRP antibodies (fremanezumab [81], galcanezumab [82] and eptinezumab [83]), target the CGRP ligand itself, preventing it from accessing the CGRPR binding pocket.

The importance of the distinct chemophore described earlier, was further supported by the recent development of the first-in-class small molecule antagonist against AM<sub>2</sub>R [84]. As the three receptors (CGRPR, AM<sub>1</sub>R and AM<sub>2</sub>R) comprise the

same receptor but a different RAMP, it is important to investigate the differences between RAMPs when developing potent and selective antagonists. Avgoustou *et al.* highlighted four key residues in the vicinity of the small molecule ligand-binding pocket that are different between each RAMP (Figure 4) [84]. By inspecting the different RAMP sequences and how these interact with known small molecule CGRPR antagonists, the authors were able to utilize specific interactions with RAMP3 that provided a drastic increase in potency for AM<sub>2</sub>R (nanomolar range) with significant selectivity (1000-fold) over the AM<sub>1</sub>R. These molecules, although still in the preclinical stage, have shown very promising anti-tumor effects against both pancreatic [84] and breast [85] cancers. These small molecules are the first selective AM<sub>2</sub>R antagonists.

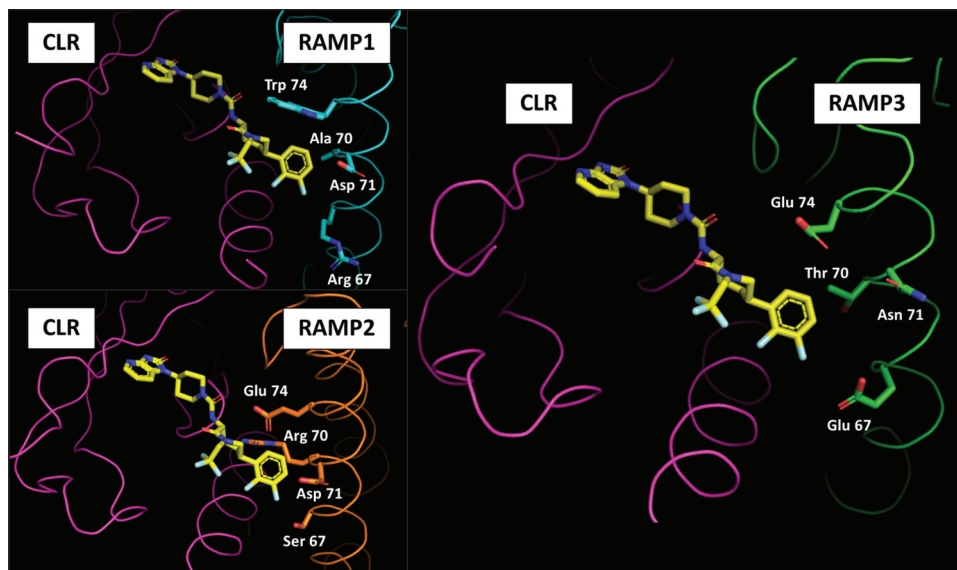
## 6. Targeting AM and its receptors in cancer

Multiple approaches have been used to target the AM system in cancer: 1) neutralizing the AM ligand itself; 2) antagonizing AM receptor complexes (i.e. CLR/RAMP2 or CLR/RAMP3); 3) antagonizing individual components of the AM receptors (i.e. CLR, RAMP2 or RAMP3). To date, several peptides, antibodies and small molecules have been developed to antagonize either AM or its receptors. Their therapeutic effects have not only been shown against various cancers but also in other disease models including sepsis and heart failure [86,87]. The potential of targeting AM in oncology has been reviewed elsewhere [88]. This review will focus on how these molecules and antibodies (Table 2) may specifically target the human AM<sub>2</sub>R in cancer.

Table 2. Drugs targeting CLR family of receptors.

Name	Target	Discovered by	Developed by	Developmental status	Disease indication	Ref
<i>Small molecule antagonists</i>						
Telcagepant	CGRPR	Merck & Co	N/A	Failed due to hepatic toxicity [73]	Prophylaxis of episodic migraine	[71]
Olcegepant	CGRPR	Boehringer Ingelheim	N/A	Failed due to hepatic toxicity [73]	Migraine treatment	[72]
Ubrogepant	CGRPR	Merck & Co	Allergan USA, Inc.	FDA approved, Ubrovelvy [76]	Acute and preventative treatment of migraine	[74]
Rimegepant	CGRPR	Bristol-Myers Squibb	Biohaven Pharmaceuticals	FDA approved, NURTEC [77]	Acute and preventative treatment of migraine	[75]
NSC 16311	AM ligand	Siclari <i>et al.</i>	N/A	N/A	Anti-tumor and osteolytic effects in <i>in vitro</i> breast cancer model	[101]
NSC 37133	AM ligand	Fang <i>et al.</i>	N/A	N/A	Neutralizes lymphatic endothelial cell tube formation <i>in vitro</i>	[102]
2,2-dimethyl-N-[[2-(methylaminomethyl)phenyl]methyl]-N-[2-oxo-2-[[[(2 R)-2'-oxospiro[1,3-dihydroindene-2,3'-1 H-pyrrolo[2,3-b]pyridine]-5-yl]amino]ethyl]propanamide	AM <sub>2</sub> R	University of Sheffield	N/A	Preclinical	Anti-tumor effects in breast and pancreatic cancer preclinical models	[84,85]
<i>Antibodies</i>						
Erenumab	CGRPR	Amgen Inc.	Amgen Inc. & Novartis	FDA approved, Aimovig [80]	Preventative treatment of migraine	[79]
Fremanezumab	CGRP ligand	Rinat Neuroscience/Pfizer	Rinat Neuroscience/Pfizer & Teva	FDA approved, Ajovy [81]	Preventative treatment of migraine	[81]
Galcanezumab	CGRP ligand	Eli Lilly and Company	Eli Lilly and Company	FDA approved, Emgality [82]	Preventative treatment of migraine and cluster headaches treatment	[82]
Eptinezumab	CGRP ligand	Lundbeck Seattle BioPharmaceuticals	Lundbeck Seattle BioPharmaceuticals	FDA approved, Vyepti [83]	Preventative treatment of migraine	[83]
Cocktail of antibodies against CLR, RAMP2 and RAMP3	Individual AM receptor components	Kaafarani <i>et al.</i> Oufik <i>et al.</i>	N/A	N/A	Anti-tumor effects in preclinical cancer models of glioblastoma, lung, colon and mesothelioma	[54,98]
<i>Peptide antagonists</i>						
AM <sub>22-52</sub>	AM <sub>1</sub> R	Eguchi <i>et al.</i>	N/A	N/A	Anti-tumor effects in preclinical cancer models including melanoma, pancreatic, breast, ovarian, renal and mesothelioma	[89]
CGRP <sub>8-37</sub>	CGRPR	Chiba <i>et al.</i>	N/A	N/A	Anti-cancer effect in <i>in vitro</i> prostate cancer model	[95]
C7	AM <sub>1</sub> R & AM <sub>2</sub> R	Robinson <i>et al.</i>	N/A	N/A	N/A	[93]
Acylated truncated AM/intermedin analogs	CGRPR & AM <sub>1</sub> R	Chang <i>et al.</i>	N/A	N/A	N/A	[97]

AM: adrenomedullin; AM<sub>1</sub>R: adrenomedullin-1 receptor (CLR/RAMP2); AM<sub>2</sub>R: adrenomedullin-2 receptor (CLR/RAMP3); CGRP: calcitonin gene-related peptide; CGRPR: calcitonin gene-related peptide receptor (CLR/RAMP1); N/A not applicable. Legends:



**Figure 4.** Models of CLR/RAMP complexes with telcagepant, based on crystal structures of CGRP and AM<sub>1</sub> receptors (PDB codes: 3N7R and 3AQF, respectively). For the AM<sub>2</sub> receptor, a hybrid model combining information from the published crystal structures of the CLR domains of the CGRP and AM<sub>1</sub> receptors with a predicted structure for the RAMP3 domain was used. CLR is rendered in magenta, RAMP1 in cyan, RAMP2 in orange and RAMP3 in green. RAMP residues (at telcagepant binding site) that differ across three RAMPs are labeled.

### 6.1. Peptide antagonists

AM<sub>22-52</sub>, also known as AM antagonist (AMA), is a truncated version of AM developed to compete with AM for its receptors [89]. AM<sub>22-52</sub> has been used to antagonize AM in many cancer models including melanoma, pancreatic, breast, ovarian, renal and mesothelioma [13,47,51,52,90–92]. AM<sub>22-52</sub> has been shown to exert its anti-tumor effects by decreasing tumor cell proliferation, angiogenesis [47,51,91,92], lymphangiogenesis [92], pro-tumoral macrophage differentiation [13,52] and recruitment of myelomonocytic cells [51]. AM<sub>22-52</sub> is slightly selective for AM<sub>1</sub>R ( $pA_2$  7.34 ± 0.14) over AM<sub>2</sub>R ( $pA_2$  6.73 ± 0.14) [93,94] making it hard to distinguish its effect in antagonizing either of these AM receptors in disease models.

CGRP<sub>8-37</sub> is a truncated version of CGRP, and was initially developed as a selective antagonist for CGRPR [95]. Interestingly, CGRP<sub>8-37</sub> is slightly more selective for AM<sub>2</sub>R than AM<sub>22-52</sub>, although it appears to antagonize both AM receptors equally [94]. While CGRP<sub>8-37</sub> has not been as extensively used as an AM antagonist compared to AM<sub>22-52</sub>, CGRP<sub>8-37</sub> has been shown to exert anti-tumor effects in prostate cancer *in vitro* models [96]. CGRP<sub>8-37</sub> was slightly more effective in inhibiting proliferation and enhancing apoptosis in DU-145 prostate cancer cell line *in vitro*, as compared to AM<sub>22-52</sub>, leading the authors to conclude that AM's tumor-promoting effect in prostate cancer is primarily mediated by AM<sub>2</sub>R. However, due to CGRP<sub>8-37</sub>'s limited selectivity between the AM receptors, it is again difficult to discriminate between its effects on either receptor. CGRP<sub>8-37</sub>'s anti-tumor effect has also not been reported in more complex cancer models.

Peptide chimeras were also developed using fragments of AM and its related ligands (intermedin and CGRP) to identify molecules with a higher affinity for AM receptors compared to the aforementioned truncated peptides [93]. While the peptide 'C7' had a similar affinity to AM<sub>22-52</sub> in inhibiting both AM<sub>1</sub>R and AM<sub>2</sub>R (~100 nM), 'C7' showed a higher selectivity for

AM<sub>2</sub>R ( $pA_2$  7.81 ± 0.20) compared to AM<sub>1</sub>R ( $pA_2$  7.25 ± 0.13), albeit modest. Unfortunately, the therapeutic potential of these peptide chimeras in cancer or other applications has not been reported.

Most recently, chimeric and bifunctional antagonists have also been developed against CLR/RAMP receptors [97]. These include acylated truncated AM/intermedin analogs with potent antagonistic activity against CGRPR and AM<sub>1</sub>R and chimeric analogs (comprising a somatostatin analog and AM antagonist) which exhibit dual antagonistic activities on both somatostatin and CLR/RAMP receptors (specifically CGRPR and AM<sub>1</sub>R). However, the potency and affinity of these compounds against AM<sub>2</sub>R, as well as their therapeutic potential, were not reported and are therefore currently unknown.

### 6.2. Antibodies

Ouafik's group developed the first antibody-based method to target AM receptors involving a combination of antibodies ( $\alpha$ AMR) against individual AM receptor components (i.e. CLR, RAMP2 and RAMP3) [54,98]. This method is limited in its selectivity in targeting specific AM receptors as it may also antagonize other receptors that bind to these individual components including CGRPR, amylin receptors and other RAMP-interacting receptors. However, despite there being several FDA-approved CGRPR antibodies for migraine treatment, there are currently no antibodies developed to bind to AM receptor complexes. Regardless,  $\alpha$ AMR treatment has been used to demonstrate anti-tumor activity in preclinical cancer models of glioblastoma, lung, colon and mesothelioma by suppressing not only tumor burden but also disrupting tumor vasculature [34,54,92,98]. However, due to the aforementioned limitation of  $\alpha$ AMR possibly antagonizing other related receptors and lack of pharmacology characterization

data, it is unclear whether the observed anti-tumor effects can be attributed directly to antagonism of only the AM receptors.

### 6.3. Small molecules

Chemical library screening identified some small molecule AM antagonists including NSC 16311 and NSC 37133 [99]. NSC 16311 has been shown *in vivo* to efficiently inhibit tobacco-induced lung cancer growth [100] and may also be effective against breast cancer metastasis by blocking AM's pro-tumoral and osteolytic effects [101]. NSC 16311 is also able to neutralize AM-induced tube formation of lymphatic ECs *in vitro*, suggesting it may have possible therapeutic applications in edema and metastatic diseases [102]. It is worth noting that these compounds bind to AM and not the receptors. To date, there are no clinical trials using small molecule AM antagonists.

In 2020, the development of first-in-class potent and selective small molecule antagonists of AM<sub>2</sub>R was reported [84]. Interestingly, these molecules have anti-tumor effects *in vitro* and *in vivo* in both breast and pancreatic cancer models [84,85]. Immunohistochemical analysis of pancreatic cancer xenografts from AM<sub>2</sub>R antagonist-treated animals revealed significant decreases in markers of proliferation, blood vasculature and CAFs [84], suggesting that AM<sub>2</sub>R antagonists exert anti-tumor effects on both cancer and stromal cells within the tumor microenvironment. The good selectivity of these antagonists over the AM<sub>1</sub>R (1000-fold) is also of particular importance as AM<sub>1</sub>R is essential for physiological processes such as cardiovascular health [57]. It is worth noting that the current class of AM<sub>2</sub>R antagonists are equipotent in inhibiting CGRPR. However, the anti-proliferative actions were shown to be mediated through the inhibition of the AM<sub>2</sub>R, by use of selective CGRPR antagonist control which showed no anti-proliferative effect. The lack of detectable side effects and good drug-like pharmacokinetic properties of these AM<sub>2</sub>R antagonists support the promise of this class of compounds as potential anti-cancer therapeutics.

## 7. Expert opinion

This review has addressed specifically the developments and strength of evidence for AM<sub>2</sub>R's roles in physiology and as potential therapeutic targets. The article has two broad implications. First, it is now recognized that AM<sub>2</sub>R has important roles in both physiology and pathology that can be distinguished from that of AM<sub>1</sub>R. This comes from studies across a range of disciplines, so that structural information combined with pharmacological studies [65,66], knockout mouse research [55–57] and the development of selective antagonists for CLR/RAMP receptors [84,85] provide a compelling (but as yet incomplete) explanation of the way AM mediates its functions.

The second implication is broader. The development of selective antagonists for GPCR/RAMP receptor complexes began with the CGRPR antagonists, as a search for agents to prevent or treat migraines. However, since those programmes which started over 20 years ago, progress had slowed until we demonstrated the ability to design selective AM<sub>2</sub>R antagonists capable of discriminating between AM receptors (albeit with small discrimination over CGRPR) [84,85]. Development of small molecule AM<sub>1</sub>R antagonists appears to be more

challenging, although progress is being made [103]. AM<sub>2</sub>R antagonists are likely to provide the tools to aid in the understanding of AM biology in both physiology and disease, as well as a potential therapeutic intervention in diseases involving AM<sub>2</sub>R, including cancer.

There is growing knowledge of other GPCRs that associate with RAMPs and other accessory proteins. RAMP interactions have historically been associated Class B GPCRs, but emerging data suggest that GPCR/RAMP interactions are far more common, with new partner GPCRs being discovered [104]. A greater understanding of the consequences of RAMP-interactions on signaling and cellular events [105] appears certain to increase importance of GPCR/RAMP interactions as potential drug targets.

Development of highly selective compounds against specific CLR/RAMP heteromers is tractable and may spark interest in development of compounds against the wider GPCR/RAMP interactions previously considered either undruggable or too challenging to be considered worthwhile targets. Like most science, each step forward that we hope will answer questions often raises more uncertainties. However, we believe it is likely that RAMP-interacting GPCR biology will see an upturn in interest and activity. Exciting times are ahead.

### Acknowledgments

The authors thank Professor JPA Harrity of The University of Sheffield for reviewing drafts of this manuscript.

### Funding

P Avgoustou, A Jailani, T Skerry and G Richards were funded by s University of Sheffield. R Hathaway was funded by a studentship from the EPSRC while J Egan was funded by an MRC DiMeN Doctoral Training Partnership Studentship. The authors were also supported by Prostate Cancer UK [via grant: PA12-12] and the Wellcome Trust [through grants: 104046/Z/14/1 and 205291/Z/16/Z]

### Declaration of Interest

A Jailani, P Avgoustou, T Skerry (Founder director) and G Richards (Founder director) have financial interests (shareholdings) in Modulus Oncology, a University of Sheffield spinout company involved in the development of AM<sub>2</sub>R antagonists for the treatment of cancer. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

### Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

### ORCID

Ameera B. A. Jailani  <http://orcid.org/0000-0001-9837-6062>  
 Kamilla J. A. Bigos  <http://orcid.org/0000-0002-7824-6479>  
 Paris Avgoustou  <http://orcid.org/0000-0002-1642-1083>  
 Joseph L. Egan  <http://orcid.org/0000-0003-2812-0074>  
 Robert A. Hathaway  <http://orcid.org/0000-0002-1948-6472>  
 Timothy M. Skerry  <http://orcid.org/0000-0003-1319-5575>  
 Gareth O. Richards  <http://orcid.org/0000-0001-7984-6882>

## References

Papers of special note have been highlighted as either of interest (\*) or of considerable interest (\*\*\*) to readers.

1. Fischer JP, Els-Heindl S, Beck-Sickingler AG. Adrenomedullin - current perspective on a peptide hormone with significant therapeutic potential. *Peptides*. 2020;09;131:170347.
2. Hong Y, Hay DL, Quirion R, et al. The pharmacology of adrenomedullin 2/intermedin. *Br J Pharmacol*. 2012;May;166(1):110–120.
3. Sugo S, Minamino N, Shoji H, et al. Production and secretion of adrenomedullin from vascular smooth muscle cells: augmented production by tumor necrosis factor- $\alpha$ . *Biochem Biophys Res Commun*. 1994;Aug 30;203(1):719–726.
4. Kohno M, Hanehira T, Kano H, et al. Plasma adrenomedullin concentrations in essential hypertension. *Hypertension*. 1996;Jan;27(1):102–107.
5. Jougasaki M, Wei CM, McKinley LJ, et al. Elevation of circulating and ventricular adrenomedullin in human congestive heart failure. *Circulation*. 1995;Aug 01;92(3):286–289.
6. Chen YX, Li CS. Prognostic value of adrenomedullin in septic patients in the ED. *Am J Emerg Med*. 2013;Jul;31(7):1017–1021.
7. Daga MK, Kumar L, Mawari G, et al. Adrenomedullin and its possible role in improved survival in female patients with sepsis: a study in the south east Asian region. *Indian J Crit Care Med*. 2020;Dec;24(12):1180–1184.
8. Lundberg OHM, Lengquist M, Spångfors M, et al. Circulating bioactive adrenomedullin as a marker of sepsis, septic shock and critical illness. *Crit Care*. 2020;April 11;24(1):636.
9. Nishikimi T, Nakagawa Y. Adrenomedullin as a biomarker of heart failure. *Heart Fail Clin*. 2018;Jan;14(1):49–55.
10. McLatchie LM, Fraser NJ, Main MJ, et al. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature*. 1998;May;393(6683):333–339.
- **First report of GPCR/RAMP interaction**
11. Kitamura K, Kangawa K, Kawamoto M, et al. Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem Biophys Res Commun*. 1993;Apr;192(2):553–560.
- **First report of AM peptide**
12. Ichikawa-Shindo Y, Sakurai T, Kamiyoshi A, et al. The GPCR modulator protein RAMP2 is essential for angiogenesis and vascular integrity. *J Clin Invest*. 2008;Jan;118(1):29–39.
13. Chen P, Huang Y, Bong R, et al. Tumor-associated macrophages promote angiogenesis and melanoma growth via adrenomedullin in a paracrine and autocrine manner. *Clin Cancer Res*. 2011;Dec;17(23):7230–7239.
14. Benyahia Z, Dussault N, Cayol M, et al. Stromal fibroblasts present in breast carcinomas promote tumor growth and angiogenesis through adrenomedullin secretion. *Oncotarget*. 2017;Feb 28;8(9):15744–15762.
15. Fujita Y, Mimata H, Nasu N, et al. Involvement of adrenomedullin induced by hypoxia in angiogenesis in human renal cell carcinoma. *Int J Urol*. 2002;Jun;9(6):285–295.
16. Zhang Y, Xu Y, Ma J, et al. Adrenomedullin promotes angiogenesis in epithelial ovarian cancer through upregulating hypoxia-inducible factor-1 $\alpha$  and vascular endothelial growth factor. *Sci Rep*. 2017;Jan 16;7:40524.
17. Zudaire E, Martínez A, Garayoa M, et al. Adrenomedullin is a cross-talk molecule that regulates tumor and mast cell function during human carcinogenesis. *Am J Pathol*. 2006;Jan;168(1):280–291.
18. Gluexam T, Grandits AM, Schlerka A, et al. CGRP signaling via CALCRL increases chemotherapy resistance and stem cell properties in acute myeloid leukemia. *Int J Mol Sci*. 2019;Nov 20;20(23):5826.
19. Brekhman V, Lugassie J, Zaffryar-Eilout S, et al. Receptor activity modifying protein-3 mediates the protumorigenic activity of lysyl oxidase-like protein-2. *FASEB J*. 2011;Jan;25(1):55–65.
20. Oehler MK, Fischer DC, Orłowska-Volk M, et al. Tissue and plasma expression of the angiogenic peptide adrenomedullin in breast cancer. *Br J Cancer*. 2003;Nov;89(10):1927–1933.
21. Uemura M, Yamamoto H, Takemasa I, et al. Hypoxia-inducible adrenomedullin in colorectal cancer. *Anticancer Res*. 2011;Feb;31(2):507–514.
22. Michelsen J, Thieson H, Walter S, et al. Tissue expression and plasma levels of adrenomedullin in renal cancer patients. *Clin Sci (Lond)*. 2006;Jul;111(1):61–70.
23. Nikitenko LL, Leek R, Henderson S, et al. The G-protein-coupled receptor CLR is upregulated in an autocrine loop with adrenomedullin in clear cell renal cell carcinoma and associated with poor prognosis. *Clin Cancer Res*. 2013;Oct;19(20):5740–5748.
24. Park SC, Yoon JH, Lee JH, et al. Hypoxia-inducible adrenomedullin accelerates hepatocellular carcinoma cell growth. *Cancer Lett*. 2008;Nov;271(2):314–322.
25. Nakata T, Seki N, Miwa S, et al. Identification of genes associated with multiple nodules in hepatocellular carcinoma using cDNA microarray: multicentric occurrence or intrahepatic metastasis? *Hepatogastroenterology*. 2008;May-Jun;55(84):865–872.
26. Buyukberber S, Sari I, Camci C, et al. Adrenomedullin expression does not correlate with survival in lung cancer. *Med Oncol*. 2007;24(2):245–249.
27. Martínez A, Elsasser TH, Muro-Cacho C, et al. Expression of adrenomedullin and its receptor in normal and malignant human skin: a potential pluripotent role in the integument. *Endocrinology*. 1997;Dec;138(12):5597–5604.
28. Dai X, Ma W, He XJ, et al. Elevated expression of adrenomedullin is correlated with prognosis and disease severity in osteosarcoma. *Med Oncol*. 2013;Mar;30(1):347.
29. Wu XY, Hao CP, Ling M, et al. Hypoxia-induced apoptosis is blocked by adrenomedullin via upregulation of Bcl-2 in human osteosarcoma cells. *Oncol Rep*. 2015;Aug;34(2):787–794.
30. Giacalone PL, Vuaroqueaux V, Daurés JP, et al. Expression of adrenomedullin in human ovaries, ovarian cysts and cancers Correlation with estrogens receptor status. *Eur J Obstet Gynecol Reprod Biol*. 2003;Oct;110(2):224–229.
31. Keleg S, Kayed H, Jiang X, et al. Adrenomedullin is induced by hypoxia and enhances pancreatic cancer cell invasion. *Int J Cancer*. 2007;Jul;121(1):21–32.
32. Rocchi P, Boudouresque F, Zamora AJ, et al. Expression of adrenomedullin and peptide amidation activity in human prostate cancer and in human prostate cancer cell lines. *Cancer Res*. 2001;Feb;61(3):1196–1206.
33. Berenguer-Daizé C, Boudouresque F, Bastide C, et al. Adrenomedullin blockade suppresses growth of human hormone-independent prostate tumor xenograft in mice. *Clin Cancer Res*. 2013;Nov;19(22):6138–6150.
34. Nouguerède E, Berenguer C, Garcia S, et al. Expression of adrenomedullin in human colorectal tumors and its role in cell growth and invasion in vitro and in xenograft growth in vivo. *Cancer Med*. 2013;Apr;2(2):196–207.
35. Martínez A, Vos M, Guédez L, et al. The effects of adrenomedullin overexpression in breast tumor cells. *J Natl Cancer Inst*. 2002;Aug;94(16):1226–1237.
36. Chen Q, Chen P, Pang X, et al. Adrenomedullin up-regulates the expression of vascular endothelial growth factor in epithelial ovarian carcinoma cells via JNK/AP-1 pathway. *Int J Gynecol Cancer*. 2015;Jul;25(6):953–960.
37. Deville JL, Bartoli C, Berenguer C, et al. Expression and role of adrenomedullin in renal tumors and value of its mRNA levels as prognostic factor in clear-cell renal carcinoma. *Int J Cancer*. 2009;Nov;125(10):2307–2315.
38. Liu AG, Zhang XZ, Li FB, et al. RNA interference targeting adrenomedullin induces apoptosis and reduces the growth of human bladder urothelial cell carcinoma. *Med Oncol*. 2013;30(3):616.
39. Li Z, Takeuchi S, Ohara N, et al. Paradoxically abundant expression of Bcl-2 and adrenomedullin in invasive cervical squamous carcinoma. *Int J Clin Oncol*. 2003;Apr;8(2):83–89.
40. Deng B, Zhang S, Miao Y, et al. Adrenomedullin expression in epithelial ovarian cancers and promotes HO8910 cell migration associated with upregulating integrin  $\alpha 5 \beta 1$  and phosphorylating FAK and paxillin. *J Exp Clin Cancer Res*. 2012;31:19.

41. Oulidi A, Bokhobza A, Gkika D, et al. TRPV2 mediates adrenomedullin stimulation of prostate and urothelial cancer cell adhesion, migration and invasion. *PLoS One*. 2013;8(5):e64885.
42. Wang X, Yue TL, Barone FC, et al. Discovery of adrenomedullin in rat ischemic cortex and evidence for its role in exacerbating focal brain ischemic damage. *Proc Natl Acad Sci U S A*. 1995;Dec 05;92(25):11480–11484.
43. Garayoa M, Martínez A, Lee S, et al. Hypoxia-inducible factor-1 (HIF-1) up-regulates adrenomedullin expression in human tumor cell lines during oxygen deprivation: a possible promotion mechanism of carcinogenesis. *Mol Endocrinol*. 2000;Jun;14(6):848–862.
44. Yao L, Wang Y, Ma W, et al. Downregulation of adrenomedullin leads to the inhibition of the tumorigenesis via VEGF pathway in human and nude mice osteosarcoma models. *Arch Med Res*. 2019;01;50(1):47–57.
45. Imuro S, Shindo T, Moriyama N, et al. Angiogenic effects of adrenomedullin in ischemia and tumor growth. *Circ Res*. 2004;Aug;95(4):415–423.
46. Oehler MK, Hague S, Rees MC, et al. Adrenomedullin promotes formation of xenografted endometrial tumors by stimulation of autocrine growth and angiogenesis. *Oncogene*. 2002;Apr;21(18):2815–2821.
47. Tsuchiya K, Hida K, Hida Y, et al. Adrenomedullin antagonist suppresses tumor formation in renal cell carcinoma through inhibitory effects on tumor endothelial cells and endothelial progenitor mobilization. *Int J Oncol*. 2010;Jun;36(6):1379–1386.
48. Berenguer C, Boudouresque F, Dussert C, et al. Adrenomedullin, an autocrine/paracrine factor induced by androgen withdrawal, stimulates 'neuroendocrine phenotype' in LNCaP prostate tumor cells. *Oncogene*. 2008;Jan;27(4):506–518.
49. Kocemba KA, van Andel H, de Haan-Kramer A, et al. The hypoxia target adrenomedullin is aberrantly expressed in multiple myeloma and promotes angiogenesis. *Leukemia*. 2013;Aug;27(8):1729–1737.
50. Zudaire E, Portal-Núñez S, Cuttitta F. The central role of adrenomedullin in host defense. *J Leukoc Biol*. 2006;Aug;80(2):237–244.
51. Xu M, Qi F, Zhang S, et al. Adrenomedullin promotes the growth of pancreatic ductal adenocarcinoma through recruitment of myelomonocytic cells. *Oncotarget*. 2016;Aug 23;7(34):55043–55056.
52. Pang X, Shang H, Deng B, et al. The interaction of adrenomedullin and macrophages induces ovarian cancer cell migration via activation of RhoA signaling pathway. *Int J Mol Sci*. 2013;Jan 29;14(2):2774–2787.
53. Pavel ME, Hoppe S, Papadopoulos T, et al. Adrenomedullin is a novel marker of tumor progression in neuroendocrine carcinomas. *Horm Metab Res*. 2006;Feb;38(2):112–118.
54. Kaafarani I, Fernandez-Sauze S, Berenguer C, et al. Targeting adrenomedullin receptors with systemic delivery of neutralizing antibodies inhibits tumor angiogenesis and suppresses growth of human tumor xenografts in mice. *FASEB J*. 2009;Oct;23(10):3424–3435.
55. Fritz-Six KL, Dunworth WP, Li M, et al. Adrenomedullin signaling is necessary for murine lymphatic vascular development. *J Clin Invest*. 2008;Jan;118(1):40–50.
56. Tanaka M, Koyama T, Sakurai T, et al. The endothelial adrenomedullin-RAMP2 system regulates vascular integrity and suppresses tumour metastasis. *Cardiovasc Res*. 2016;09;111(4):398–409.
57. Dackor R, Fritz-Six K, Smithies O, et al. Receptor activity-modifying proteins 2 and 3 have distinct physiological functions from embryogenesis to old age. *J Biol Chem*. 2007;Jun;282(25):18094–18099.
- **Knockout mice studies showing the distinct physiological differences mediated by RAMPs**
58. Dai K, Tanaka M, Kamiyoshi A, et al. Deficiency of the adrenomedullin-RAMP3 system suppresses metastasis through the modification of cancer-associated fibroblasts. *Oncogene*. 2020;Feb;39(9):1914–1930.
- **Knockout mice studies showing mechanistic evidence of AM/RAMP3 in cancer**
59. Shindo K, Aishima S, Ohuchida K, et al. Podoplanin expression in cancer-associated fibroblasts enhances tumor progression of invasive ductal carcinoma of the pancreas. *Mol Cancer*. 2013;Dec 20;12(1):168.
60. Larrue C, Guiraud N, Mouchel PL, et al. Adrenomedullin-CALCRL axis controls relapse-initiating drug tolerant acute myeloid leukemia cells. *Nat Commun*. 2021;January 18;12(1):422.
61. Booe JM, Walker CS, Barwell J, et al. Structural basis for receptor activity-modifying protein-dependent selective peptide recognition by a G protein-coupled receptor. *Mol Cell*. 2015;Jun 18;58(6):1040–1052.
62. ter Haar E, Koth CM, Abdul-Manan N, et al. Crystal structure of the ectodomain complex of the CGRP receptor, a class-B GPCR, reveals the site of drug antagonism [article]. *Structure*. 2010;Sep;18(9):1083–1093.
- **First paper highlighting the ligand binding pocket of CGRP receptor**
63. Hay DL, Walker CS, Gingell JJ, et al. Receptor activity-modifying proteins; multifunctional G protein-coupled receptor accessory proteins. *Biochem Soc Trans*. 2016;44:568–573.
64. Liang Y-L, Khoshouei M, Deganutti G, et al. Cryo-EM structure of the active, G(s)- protein complexed, human CGRP receptor. *Nature*. 2018;Sep 27;561(7724):492–497.
65. Liang Y-L, Belousoff MJ, Fletcher MM, et al. Structure and dynamics of adrenomedullin receptors AM1 and AM2 reveal key mechanisms in the control of receptor phenotype by receptor activity-modifying proteins. *ACS Pharmacol Transl Sci*. 2020;Apr 10;3(2):263–284.
66. Garelja ML, Au M, Brimble MA, et al. Molecular mechanisms of class B GPCR activation: insights from adrenomedullin receptors. *ACS Pharmacol Transl Sci*. 2020;Apr 10;3(2):246–262.
67. Edvinsson L, Haanes KA, Warfvinge K, et al. CGRP as the target of new migraine therapies - successful translation from bench to clinic. *Nat Rev Neurol*. 2018;Jun;14(6):338–350.
68. Lassen LH, Haderslev P, Jacobsen VB, et al. CGRP may play a causative role in migraine. *Cephalalgia*. 2002;Feb;22(1):54–61.
69. Hansen JM, Hauge AW, Olesen J, et al. Calcitonin gene-related peptide triggers migraine-like attacks in patients with migraine with aura. *Cephalalgia*. 2010;Oct;30(10):1179–1186.
70. Amara SG, Jonas V, Rosenfeld MG, et al. Alternative RNA processing in calcitonin gene-expression generates messenger-RNAs encoding different polypeptide products. *Nature*. 1982;298(5871):240–244.
71. Ho TW, Ferrari MD, Dodick DW, et al. Efficacy and tolerability of MK-0974 (telcagepant), a new oral antagonist of calcitonin gene-related peptide receptor, compared with zolmitriptan for acute migraine: a randomised, placebo-controlled, parallel-treatment trial. *Lancet*. 2008;Dec-Jan;372(9656):2115–2123.
72. Doods H, Hallermayer G, Wu DM, et al. Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. *Br J Pharmacol*. 2000;Feb;129(3):420–423.
73. Schuster NM, Rapoport AM. Calcitonin gene-related peptide-targeted therapies for migraine and cluster headache: a review. *Clin Neuropharmacol*. 2017;Jul-Aug;40(4):169–174.
74. Voss T, Lipton RB, Dodick DW, et al. A phase IIb randomized, double-blind, placebo-controlled trial of ubrogepant for the acute treatment of migraine. *Cephalalgia*. 2016;Aug;36(9):887–898.
75. Croop R, Goadsby PJ, Stock DA, et al. Efficacy, safety, and tolerability of rimegepant orally disintegrating tablet for the acute treatment of migraine: a randomised, phase 3, double-blind, placebo-controlled trial. *Lancet*. 2019;Aug 31;394(10200):737–745.
76. Scott LJ. Ubrogapant: first approval. *Drugs*. 2020;Feb;80(3):323–328.
77. Scott LJ. Rimegepant: first approval. *Drugs*. 2020;May;80(7):741–746.
78. Archbold JK, Flanagan JU, Watkins HA, et al. Structural insights into RAMP modification of secretin family G protein-coupled receptors: implications for drug development. *Trends Pharmacol Sci*. 2011;Oct;32(10):591–600.
79. Shi LC, Lehto SG, Zhu DXD, et al. Pharmacologic characterization of AMG 334, a potent and selective human monoclonal antibody against the calcitonin gene-related peptide receptor. *J Pharmacol Exp Ther*. 2016;Jan;356(1):223–231.

80. Markham A. Erenumab: first global approval. *Drugs*. 2018;Jul;78(11):1157–1161.
81. Hoy SM. Fremanezumab: first global approval. *Drugs*. 2018;Nov;78(17):1829–1834.
82. Lamb YN. Galcanezumab: first global approval. *Drugs*. 2018;Nov;78(16):1769–1775.
83. Dhillon S. Eptinezumab: first approval. *Drugs*. 2020;May;80(7):733–739.
84. Avgoustou P, Jailani ABA, Zirimwabagabo JO, et al. Discovery of a first-in-class potent small molecule antagonist against the adrenomedullin-2 receptor. *ACS Pharmacol Transl Sci*. 2020;Aug 14;3(4):706–719.
85. Zirimwabagabo JO, Jailani ABA, Avgoustou P, et al. Discovery of a first-in-class small molecule antagonist against the adrenomedullin-2 receptor: structure-activity relationships and optimization. *J Med Chem*. 2021;March 25;64(6):3299–3319.
86. Geven C, Kox M, Pickkers P. Adrenomedullin and adrenomedullin-targeted therapy as treatment strategies relevant for sepsis. *Front Immunol*. 2018;9:292.
87. Voors AA, Kremer D, Geven C, et al. Adrenomedullin in heart failure: pathophysiology and therapeutic application. *Eur J Heart Fail*. 2019;02;21(2):163–171.
88. Vázquez R, Riveiro ME, Berenguer-Daizé C, et al. Targeting adrenomedullin in oncology: a feasible strategy with potential as much more than an alternative anti-angiogenic therapy. *Front Oncol*. 2020;10:589218.
89. Eguchi S, Hirata Y, Iwasaki H, et al. Structure-activity relationship of adrenomedullin, a novel vasodilatory peptide, in cultured rat vascular smooth muscle cells. *Endocrinology*. 1994;Dec;135(6):2454–2458.
90. Ishikawa T, Chen J, Wang J, et al. Adrenomedullin antagonist suppresses in vivo growth of human pancreatic cancer cells in SCID mice by suppressing angiogenesis. *Oncogene*. 2003;Feb;22(8):1238–1242.
91. Miseki T, Kawakami H, Natsuzaka M, et al. Suppression of tumor growth by intra-muscular transfer of naked DNA encoding adrenomedullin antagonist. *Cancer Gene Ther*. 2007;Jan;14(1):39–44.
92. Greillier L, Tounsi A, Berenguer-Daizé C, et al. Functional analysis of the adrenomedullin pathway in malignant pleural mesothelioma. *J Thorac Oncol*. 2016;Jan;11(1):94–107.
93. Robinson SD, Aitken JF, Bailey RJ, et al. Novel peptide antagonists of adrenomedullin and calcitonin gene-related peptide receptors: identification, pharmacological characterization, and interactions with position 74 in receptor activity-modifying protein 1/3. *J Pharmacol Exp Ther*. 2009;Nov;331(2):513–521.
94. Hay DL, Howitt SG, Conner AC, et al. CL/RAMP2 and CL/RAMP3 produce pharmacologically distinct adrenomedullin receptors: a comparison of effects of adrenomedullin22-52, CGRP8-37 and BIBN4096BS. *Br J Pharmacol*. 2003;Oct;140(3):477–486.
95. Chiba T, Yamaguchi A, Yamatani T, et al. Calcitonin gene-related peptide receptor antagonist human CGRP-(8-37). *Am J Physiol*. 1989;Feb;256(2 Pt 1):E331–5.
96. Mazzocchi G, Malendowicz LK, Ziolkowska A, et al. Adrenomedullin (AM) and AM receptor type 2 expression is up-regulated in prostate carcinomas (PC), and AM stimulates in vitro growth of a PC-derived cell line by enhancing proliferation and decreasing apoptosis rates. *Int J Oncol*. 2004;Dec;25(6):1781–1787.
97. Chang CL, Hsu SYT. Development of chimeric and bifunctional antagonists for CLR/RAMP receptors. *PLoS One*. 2019;14(5):e0216996.
98. Ouafik L, Sauze S, Boudouresque F, et al. Neutralization of adrenomedullin inhibits the growth of human glioblastoma cell lines in vitro and suppresses tumor xenograft growth in vivo. *Am J Pathol*. 2002;Apr;160(4):1279–1292.
- **First approach to target individual components of AM receptors in cancer**
99. Martínez A, Julián M, Bregonzio C, et al. Identification of vasoactive nonpeptidic positive and negative modulators of adrenomedullin using a neutralizing antibody-based screening strategy. *Endocrinology*. 2004;Aug;145(8):3858–3865.
100. Portal-Nuñez S, Shankavaram UT, Rao M, et al. Aryl hydrocarbon receptor-induced adrenomedullin mediates cigarette smoke carcinogenicity in humans and mice. *Cancer Res*. 2012;Nov;72(22):5790–5800.
101. Siclari VA, Mohammad KS, Tompkins DR, et al. Tumor-expressed adrenomedullin accelerates breast cancer bone metastasis. *Breast Cancer Res*. 2014;16(6):458.
102. Fang C, Miguel MA, Avis I, et al. Non-peptide small molecule regulators of lymphangiogenesis. *Lymphat Res Biol*. 2009;Dec;7(4):189–196.
103. Hendrikse ER, Liew LP, Bower RL, et al. Identification of small-molecule positive modulators of calcitonin-like receptor-based receptors. *ACS Pharmacol Transl Sci*. 2020;Apr 10;3(2):305–320.
104. McGlone ER, Manchanda Y, Jones B, et al. Receptor activity-modifying protein 2 (RAMP2) alters glucagon receptor trafficking in hepatocytes with functional effects on receptor signalling. *Mol Metab*. 2021;11;53:101296.
105. Clark AJ, Mullooly N, Safitri D, et al. CGRP, adrenomedullin and adrenomedullin 2 display endogenous GPCR agonist bias in primary human cardiovascular cells. *Commun Biol*. 2021;June 23;4(1):776.