Patent Highlights October–November 2016

A snapshot of noteworthy recent developments in the patent literature of relevance to pharmaceutical and medical research and development.

First draft submitted: 13 January 2017; Accepted for publication: 13 January 2017;
Published online: 1 March 2017

Hermann AM Mucke
H.M. Pharma Consultancy, A-1160 Wien
(Vienna), Austria
h.mucke@hmpharmacon.com

WO/2016/154749

Indole analogs as 5-oxo-ETE receptor antagonists and methods of use thereof

Powell WS, Rokach J

The Roxal Institution for the Advancement of Learning/McGill University, Montreal, Québec, Canada

MeSH keywords: alcohol

- oxidoreductases
- allergy and immunology
- chemotactic factors
- eicosanoid receptors

fold, had identified racemic compounds with IC_{50} values below 30 nM, widely disparate between the stereoisomers [5]. In the present patent application, the limits have been pushed much further: (*S*)-5-(5-chloro-2-(6-(3-chlorophenyl)hexyl)-1-methyl-1*H*-indol-3-yl)-3-methyl-5-oxopentanoic acid has an IC_{50} of 8 pM. When administered to cynomolgus monkeys at a dose of either 5 mg/kg or 2×5 mg/kg 8 h apart, it was found converted to a single polar metabolite that also showed potent OXE antagonistic activity.

Published: 6 October 2016

WO/2016/161429

Compounds and methods for modulating Tmprss6 expression

Guo S, Aghajan M, Swayze EE

Ionis Pharmaceuticals, Inc., Carlsbad, CA, USA

MeSH keywords: hepcidins • human

TMPRSS6 protein • iron metabolism disorders • oligonucleotides • antisense

Metabolism of arachidonic acid by the 5-lipoxygenase pathway leads to the formation of leukotrienes including 5(*S*)-hydroxyeicosatetraenoic acid (5-HETE), which is oxidized by 5-hydroxyeicosanoid dehydrogenase to give 5-oxo-EETE (OXE), a potent chemoattractant primarily for eosinophils and also for neutrophils. The OXE receptor [1] is also expressed on monocytes, as well as on prostate tumor cells; antagonists might be useful as therapeutic or prophylactic agents for asthma [2], allergic rhinitis, chronic obstructive pulmonary disease, atopic dermatitis, psoriasis and certain cancers [3]. The inventors, who have published extensively on the subject, had reported 5-(6-chloro-2-hexyl-1*H*-indol-1-yl)-5-oxo-valeric acid as an OXE antagonist lead compound with an IC₅₀ of 400 nM [4]. Further refinements using a structure-based approach in which substituents mimicking the essential polar (C1–C5) and hydrophobic (C15–C20) regions of OXE were incorporated in an indole scaffold

Matriptase-2 (TMPRSS6), a type-2 transmembrane serine protease, is an important negative expression modulator of the hepatic peptide hormone, hepcidin which is a master regulator of iron homeostasis [6]. This gives TMPRSS6 therapeutic potential in disorders of iron metabolism [7,8]. In iron-refractory microcytic anemia, loss-of-function mutations in *TMPRSS6* lead to overproduction of hepcidin, which, in turn, compromises iron absorption and utilization [9]. Vice versa, interfering with

TMPRSS6 expression or function could offer exciting perspectives for the treatment of iron overload disorders, especially genetic ones such as hemochromatosis and β -thalassemia [10]. The invention provides modified 12–30mer oligonucleotides that are complementary to positions 3162 to 3184 of *TMPRSS6* over at least eight sequential nucleobases. The antisense oligonucleotide can contain a carbohydrate moiety, such as 5'-tris(2-hydroxyethyl)amino-(THA)-C6 GalNAc3, and can be double-stranded or branched. About 2200 antisense compounds with a phosphorothioate gapmer motif or a cEt-containing motif-targeting human *TMPRSS6* were designed and screened in Hep3B cells for their effect on human *TMPRSS6* mRNA after administering a single dose to the cells. Of these, about 100 were chosen for testing in dose-dependent inhibition studies, and about 77 were further selected, based on their IC_{50} values, for study in CD-1 mice to determine tolerability. Sprague-Dawley rats and transgenic huTMPRSS6 tg mice provided further filters, additional chemical modifications were undertaken for potency and finally eight GalNAc-conjugated antisense nucleotides were tested in cynomolgus monkeys. High-dose groups (30 mg/kg subcutaneously every other day for the first week, then once weekly for 11 weeks) assessed toxicity; low-dose (5 mg/kg) groups were compared with a corresponding unconjugated parent sequence to assess activity. Reduction of monkey liver *TMPRSS6* mRNA ranged from 61 to 82% while serum hepcidin levels increased, and serum iron levels and transferrin saturation were reduced at day 86 in treated groups compared with controls.

Published: 6 October 2016

WO/2016/164295

Fused pyridines as kinase inhibitors

Nara SJ, Dzierba CD, Macor JE *et al.*

Bristol-Myers Squibb Co., Princeton, NJ, USA

MeSH keywords: human AAK1 protein

• neuropathology • nociceptive pain • schizophrenia

AAK1 (short for adaptor-associated kinase) is a member of the Prk/Ark family of serine/threonine kinases, enriched at presynaptic terminals, whereas in non-neuronal cells it colocalizes with clathrin and AP-2 (a heterotetramer which links receptor cargo to the clathrin coat) in clathrin-coated pits and at the leading edge of migrating cells. AAK1 phosphorylates the $\mu 2$ subunit of AP-2, which promotes the binding of $\mu 2$ to tyrosine containing sorting motifs on cargo recep-

tors [11,12]. This kinase is an inhibitor of neuregulin-1/ ErbB4 signaling, and a relevant target for K252a [13]. Mouse models defective in this pathway have morphological changes and behavioral phenotypes that are relevant for schizophrenia. In rodent models of amyotrophic lateral sclerosis AAK1 is mislocated into aggregates that contain mutant SOD1s and neurofilament proteins [14]. In familial Parkinson's disease *AAK1* is a susceptibility gene associated with the age of onset [15]. BMS – essentially the only patentee in this field – is continuing its series of AAK1 inhibitor patent applications that started in 2013. AAK1 knockout mice and their wild-type littermates were tested using the formalin paw test in order to assess their nociceptive responses. IC_{50} values for most compounds presented here were mostly in the low nanomolar range and sometimes, as, for example, with (S)-8-((2-amino-2,4-dimethylpentyl)oxy)-7-chloro-6H-isochromeno[3,4-c]pyridin-2-amine, even lower (IC_{50} = 0.41 nM).

Published: 13 October 2016

WO/2016/164593

Compositions and methods for modulating hydroxylation of ACC2 by PHD3

Haigis MC, German N

President & Fellows of Harvard College, Cambridge, MA, USA

MeSH keywords: acetyl-CoA carboxylase

• hypoxia-inducible factor-proline dioxygenases • neoplasms • precision medicine

It has been reported that increased expression of PHD3 under hypoxia enhances cell cycle progression and survival of carcinoma cells [16,17]. The present disclosure is based on the discovery that this prolyl hydroxylase can repress long-chain fatty acid oxidation by hydroxylating acetyl-CoA carboxylase 2 (ACC2). Cancer cells can amplify fatty acid catabolism through PHD3 repression to promote survival by serving as a source of ATP or NADPH, in preference over glucose and glutamine utilization. While the details of this discovery have recently been published in a peer-review companion paper [18], the patent application focuses on novel personalized cancer treatment strategies. For PHD3-underexpressing cancers, this can be achieved by inhibitors of fatty acid oxidation. CPT-I inhibitors (etomoxir, oxfenicine, perhexiline), 3-KAT inhibitors (trimetazidine, ranolazine) and mitochondrial thiolase inhibitors, such as 4-bromocrotonic acid, should be useful. On the other hand, glycolytic pathway blockers (inhibitors of hexo-

kinase, transketolase, phosphofructokinase, GAPDH or of the glucose transporter) should be beneficial in PHD3-overexpressing cancers. Inhibitors of PHD3 would sensitize such cancers to inhibition of fatty acid metabolism; proline analogs would come to mind [19], along with interfering nucleic acids. The decision would depend on the level of ACC2 hydroxylation at proline 450. Anti-ACC2 antibodies, or any other agents that discriminate between the ACC2 hydroxylation states, can be employed as diagnostics in cancer tissues.

Published: 13 October 2016

WO/2016/168658

Compositions and methods for Xi reactivation
Green MR, Bhatnagar S

University of Massachusetts, Boston, MA, USA

MeSH keywords: methyl-CpG-binding protein 2 • Rett syndrome • X chromosome inactivation • X-linked genetic diseases

To equalize X-chromosomal gene dosage between the sexes, one of the two X-chromosomes is rendered transcriptionally silent in females. X-chromosome inactivation (XCI) occurs concomitant with upregulation of the 17 kb noncoding RNA, Xist, on the future inactive X-chromosome [20] that continues to express it even in fully differentiated somatic cells. Understanding of the factors and mechanisms involved is directly relevant to dominant X-linked diseases such as Rett syndrome where loss-of-function mutations in the *MECP2* gene occur, which codes for X-linked methyl-CpG-binding protein 2. Most Rett patients are females who are heterozygous for *MECP2* deficiency due to random X-chromosome inactivation [21]. The inventors propose treating such diseases with inhibitors of a broad range of known XCI inactivation factors. Specific pairings of inactivators and their small-molecule inhibitors include PI3K that is inhibited by GNE-317 or LY-29400; PDK1 inhibited by OSU-03012 or BX912; AURKA inhibited by VX680, CD532 or MLN8237; SGK1/2 inhibited by GSK-650394; and ACVR1 inhibited by dorso-morphin, K02288 or LDN193189. In some embodiments, the therapeutic inhibitor (i.e., X-chromosome reactivator) is a selective mTOR inhibitor such as rapamycin, KU-0063794 or everolimus. Many of these targets are downstream substrates of PDK1, a phosphoinositide protein kinase that regulates PI3K/AKT signaling, and is involved in cancer progression [22]. Extensive biological data show that all these combinations reactivate expression of *MECP2*

in vitro and in mouse models. For confirmation, a hypoxanthine-aminopterin-thymidine dual selection assay was performed which requires activation of the Xi-linked *HPRT* gene by an inhibitor with sufficiently low cytotoxicity to allow cellular proliferation and survival.

Published: 20 October 2016

WO/2016/170022

Therapeutic methods, products and composition inhibiting ZNF555

Pirozhkova I, Ogryzko V

Institut Gustave Roussy, Villejuif, France

MeSH keywords: adenine nucleotide translocator 1 • facioscapulohumeral muscular dystrophies • human ZNF555 protein • muscular diseases

This invention reveals the largely uncharacterized zinc finger endogenous transcription factor, ZNF555 as a new therapeutic target for the prevention or treatment of the diseases related to deregulation of the *SLC25A4* adenine nucleotide translocator gene. Typically, this is a musculoskeletal disease such as a myopathy, but could also be a cancer. The inventors also describe the functional role of 4qA β -satellite repeats (BSR) in facioscapulohumeral dystrophy (FSHD) [23,24]. *SLC25A4* is implicated in muscle function protecting the cells from apoptosis and mitochondrial membrane instability [25,26], and is related to the hypersensitivity of FSHD muscles to oxidative stress. An individual BSR acts as an active unit participating in the transcriptional regulation of genes at locus 4q35. ZNF555, which is highly expressed in myogenic cells, specifically binds the BSR 4qA enhancer, which regulates the transcriptional activity of the *SLC25A4* promoter. Inhibition of ZNF555 by other proteins (e.g., ADAM12 or FGF20), or antibodies, was able to correct the abnormal *SLC25A4* levels in subjects with musculoskeletal disease. The intrinsic sequence differences between BSRs reveal a variable transcriptional activity and can interact with different protein partners. The inventors propose a working model of the functional role of the 4qA allele in transcriptional control of *SLC25A4* in FSHD patients, where the cooperative BSR binding with ZNF555 could be a critical step in the formation of a transcriptionally productive complex. This opens the theoretical possibility of treating FSHD with vaccination; however, we do not know anything about other – perhaps essential – roles of ZNF555.

Published: 27 October 2016

WO/2016/172330

Selective androgen receptor degrader (SARD) ligands and methods of use thereof

Narayanan R, Miller DD, Ponnusamy T et al.

GTX, Inc., Memphis, TN, USA;

University of Tennessee Research Knoxville, TN, USA

MeSH keywords: androgen receptor antagonists • prostatic neoplasms

Castration-resistant prostate carcinoma remains dependent on the androgen-receptor-signaling axis [27]. Intracrine androgen synthesis, amplifications of the androgen-receptor gene within the tumor and emergence of modified androgen receptors (splice variants that lack the ligand-binding domain, or mutants that are not sensitive to inhibition by antagonists such as abiraterone or enzalutamide or even convert antagonist activity to agonism) allow continued growth [28,29], resulting in a median survival of 12–18 months. This invention describes novel 3-amino-propanamide androgen-receptor antagonists that are not only highly potent and selective but also reduce the expression of their target – including the splice-variant receptors whose growth is driven by the N-terminal ligand-binding domain. The last-mentioned capability is an important improvement over the known selective androgen-receptor degraders apalutamide (ARN-509), AZD-3514 and ASC-J9. Several of the novel compounds exhibited degradation activity at concentrations comparable to their binding and antagonistic activity. Two molecules – (5)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-((6-cyano-[1,1'-biphenyl]-3-yl)amino)-2-hydroxy-2-methylpropanamide and (5)-N-(4-cyano-3-(trifluoromethyl)phenyl)-3-((6-cyano-[1,1'-biphenyl]-3-yl)(methyl)amino)-2-hydroxy-2-methylpropanamide – antagonized the R1881-stimulated wild-type androgen-receptor transcriptional activity at 77 and 95 nM, compared with 420 and 489 nM for bicalutamide and enzalutamide, respectively. In microsomal metabolism assays, both had half-lives between 5 and 10 min, and high clearance. Both did not inhibit transactivation of other receptors at concentrations below 10 μ M. Besides offering new options for the treatment of castration-resistant prostate carcinoma, topical application of such compounds could treat androgen-dependent skin conditions such as severe acne, seborrheic dermatitis or male pattern baldness without exerting any systemic antiandrogenism. An alternate strategy, androgen-receptor-signaling inhibitors, which block the nuclear translocation of the receptor protein, is exemplified by darolutamide (ODM-201; Bayer/Orion Pharmaceuticals) [30].

Published: 27 October 2016

WO/2016/179194

LILRA3 and method of using the same
Phennicie R, Wong J

Jounce Therapeutics, Inc., Cambridge, MA, USA

MeSH keywords: cytokines • immunologic receptors • neoplasms • Th1–Th2 balance

The TIM family of proteins regulates T-cell activation and tolerance. They contain a characteristic N-terminal immunoglobulin-V-like domain, a mucin domain with O-linked glycosylation sites, membrane proximal N-linked glycosylation sites, a single transmembrane domain and (in most cases) a cytoplasmic region with a tyrosine kinase phosphorylation motif [31]. TIM-3 is hypothesized to have paradoxical roles in modulating immune responses by providing costimulatory or coinhibitory signals depending on its binding to different receptors and/or its spatial expression on different immune cells; it has been implicated in either promoting or terminating Th1 immunity [32]. The inventors have found that the secretion of myeloid-associated cytokines (IL-12, TNF- α , IL-1 β , GM-CSF or IL-6) can be stimulated by interaction of TIM-3 with the leukocyte immunoglobulin-like receptor-A3 (LILRA3), which is known to be expressed only by monocytes and not macrophages or dendritic cells [33]. Bioinformatic data were used to examine genes whose expression correlates with TIM-3 expression in human tumor samples. The list of expressed proteins was limited to surface receptors that could serve as a ligand for TIM-3. Of these candidates, LILRB2 bound TIM-3 with an affinity of approximately 30 nM (see Jounce Therapeutics' U.S. Patent Application 2016/0200815). From these results, other members of the LILRB family were investigated. LILRA3, which had not previously been reported as a counter receptor for TIM-3, bound with an affinity of approximately 10 nM. Antibodies directed against TIM-3 can modulate LILRA3 binding positively as well as negatively, with corresponding increase or decrease in cytokine production. In particular, expression of IL-10, CCL2, CCL3, CCL4 and CCL5 can be downregulated by inhibitors of LILRA3/TIM-3 binding. The most promising applications are in cancer and septic shock.

Published: 10 November 2016

WO/2016/179645

4H-Pyran compounds as insulin-regulated aminopeptidase (IRAP) inhibitors

Chai SY, Thompson P, Mountford S, Parker MW

Monash University, Clayton, Australia;

The Florey Institute of Neuroscience & Mental Health, Melbourne, Australia

MeSH keywords: angiotensin receptors • cognition disorders • cystinyl aminopeptidase

Angiotensin IV (AT₄), its more stable analogs, and the structurally distinct hemoglobin-derived peptide VV-hemorphin 7 [34] all bind, with high affinity and relative specificity, to a type II integral membrane glycoprotein with a large C-terminal extracellular tail which contains a catalytic site, although it does not cleave its ligands. The AT₄ receptor, as the protein was therefore tentatively named, is widely distributed in many peripheral tissues and organs, and also in brain regions involved in cognitive function where it is co-expressed in neurons with the glucose transporter, GLUT4. Later, it was shown that the AT₄ receptor actually is an insulin-regulated aminopeptidase (IRAP [35]; not to be confused with IL-1 receptor antagonist protein). Inhibiting it enhances memory and learning in normal animals, and reverses memory deficits induced by muscarinic antagonists or neurosurgery, likely by potentiating glucose uptake into neurons and/or cleavage of neuropeptides involved in memory processing. Remaining controversies over the nature of AT₄/IRAP notwithstanding, brain-penetrating inhibitors could be beneficial in Alzheimer's disease [36,37]. For that purpose, the inventors have developed 4H-pyrans, some of which also have neurogenic potential: 4-(3-acetyl-6-amino-5-cyano-2-methyl-4H-pyran-4-yl)benzoic acid ammonium salt, when applied to rat primary hippocampal cell cultures at 10 μM on days 14, 17 and 20, resulted in statistically significant increases in dendritic spine densities – which indicates actin reorganization, a requirement for stabilization of memory. This is a useful addition to the various other chemical classes of IRAP inhibitors that have been reported [38,39].

Published: 17 November 2016

WO/2016/180770

Functional cure of retroviral infection

Christ F, Debyser Z, Gijsbergs R, Vranckx L

Katholieke Universiteit Leuven, Leuven, Belgium

MeSH keywords: anti-HIV agents • HIV integrase • transcriptional co-activator p75

LEDGF/p75 is recruited into HIV-1 particles through direct interaction with the integrase (or Pol polyprotein) and is a substrate for HIV-1 protease [40]. LEDGINs are molecules that bind the LEDGF/p75-binding pocket in the retroviral integrase. They augment integrase multimerization during HIV virion assembly or in the released viral particles, cause a severe replication defect and severely hamper the infectivity of progeny virions produced in their presence [41]. Therefore, such drugs already reduce the buildup of a viral reservoir that could be reactivated, and this was seen as a potential synergy with more conventional anti-HIV drugs that are given as a chronic suppression treatment. According to the present invention, the LEDGINs are administered for a limited time (1–24 weeks), until the functional proviral load (as assessed by a qPCR method or an outgrowth assay) has been reduced to levels where the host is resistant to reactivation. LEDGINs such as CX014442, described earlier [42], were used to show that they retarget HIV integration into safer locations, in other words, away from oncogenes, miRNA-encoding regions, transcription units and ultraconserved regions, leading to quiescence of the residual integrants both in SupT1 and primary CD4⁺ T-cells. For the companion peer-review paper, see Vranckx *et al.* [43]. For a general discussion, see Mespiède and Weinberg [44].

Published: 17 November 2016

WO/2016/183280

Treatment of β-thalassemia using ActRII ligand traps

Attie KM, Laadem A, Chopra R, Backstrom J

Celgene Corp., Summit, NJ, USA;

Acceleron Pharma, Inc., Cambridge, MA, USA

MeSH Keywords: β-thalassemia • growth differentiation factors • recombinant fusion proteins

Sotatercept (formerly ACE-011), an activin ligand trap antibody consisting of the extracellular domain of activin-receptor type IIB (ActRIIB) and the Fc region of human IgG1, is being developed by the applicants for various anemia conditions; this is part of a broader

industry effort to lessen the dependency on erythropoietin. This includes clinical trials for treatment of subjects with β -thalassemia [45]. The rationale is that such signaling inhibitors reduce expression of GDF11, an ActRIIA ligand that depends on reactive oxygen species, suggesting the existence of an autocrine amplification loop in β -thalassemia. GDF11 inactivation also corrects the abnormal ratio of immature/mature erythroblasts by inducing apoptosis of immature erythroblasts through the Fas–Fas ligand pathway [46]. Besides disclosing many technical details, this patent application offers a detailed discussion of the Phase II results as well as the protocol of the Phase III study evaluating sotatercept plus luspatercept (in Phase III trials for β -thalassemia since May 2016, also by Acceleron and Celgene) against placebo plus luspatercept in adults with β -thalassemia who require regular red blood cell transfusion.

Published: 17 November 2016

WO/2016/185041

Antisense oligonucleotides to treat dystrophic epidermolysis bullosa

Haisma EM, Potman M, Plattenburg GJ

ProQR Therapeutics II B.V., Leiden, The Netherlands

MeSH Keywords: antisense oligonucleotides

• collagen type VII • epidermolysis bullosa dystrophica

The dystrophic form of epidermolysis bullosa (the ‘butterfly children’ disease) accounts for about 25% of the patients suffering from this genetic dermatological condition. Dominantly and recessively inherited cases are almost equally represented, the recessive ones being more severe [47]. Mutations in *COL7A1*, which encodes the $\alpha 1$ chain of collagen VII, are responsible [48]. Defective $\alpha 1$ chains cannot properly assemble the collagen VII fibrils which anchor the upper part of the dermis to the lamina densa, which is part of the basement membrane. Because the majority of the *COL7A1* exons are in-frame, exon skipping is potentially a viable strategy to eliminate exons with premature termination codon mutations, allowing expression of a slightly shorter spliced protein that is functional [49]. INSERM’s WO/2013/053819 had disclosed two 24mer antisense oligonucleotides of which 22 nucleotides that are complementary to exon 80 (the most frequently affected one in recessive disease) and extend to the first two nucleotides that are complementary to the upstream intron; this is supposed to cause the entire exon to be skipped from the mRNA. The present inventors tested the prior art oligos in human

primary fibroblasts and in HeLa cells, and found their skipping efficiency to be less than 50%. They designed oligos that are shorter in the part that is complementary to a 3'-sequence of exon 80 and its splice site (11–14 nucleotides are optimal), and additionally target the 5' part of the downstream intron, while not hybridizing to the upstream intron. Constructs that are complementary only to exon 80 and not to either of its flanking introns are also effective, and can be any length up to the 36nt length of the whole exon. All ribose moieties can be 2'-O-methylated, and substantially all internucleosidic linkages can be phosphorothioates. The best variants achieve 70% exon 80 exclusion in lab-on-a-chip assays. This is a very competitive field; for closely related work from a French group, see Turczynski *et al.* [50]. Researchers from The Netherlands and Germany have shown that skipping exon 105 achieves a similar effect [51].

Published: 24 November 2016

WO/2016/185279

Benzoimidazole derivatives as PAD4 inhibitors

Amans D, Atkinson SJ, Barker MD *et al.*

GlaxoSmithKline Intellectual Property Development Ltd., Brentford, UK

MeSH Keywords: citrulline • epigenomics

• extracellular traps • neutrophils • peptidylarginine deiminase IV • rheumatoid arthritis

PAD4 is a member of the peptidylarginine deiminase family of enzymes that is responsible for the deimination or citrullination of a variety of proteins [52]. It has been detected in synovial tissue, and a weak and inconsistent association between PAD4 polymorphisms and susceptibility to rheumatoid arthritis has been observed in a number of population studies. Deimination of proteins might lead to a break of tolerance to, and initiation of immune responses to, citrullinated substrates such as fibrinogen, vimentin and collagen in the joints of rheumatoid arthritis patients [53]. PAD4 inhibitors may also reduce pathological neutrophil activity in other inflammatory diseases, as well as in cancer, because formation of neutrophil extracellular traps is associated with histone citrullination [54,55]. By converting histone arginine residues to citrulline, PAD4 might modulate epigenetic regulation via depletion of arginine residues that are available for methylation [56]. In a PAD4 enzyme assay, the bezimidazole compounds that are presented here had pIC_{50} values ranging from 6.4 to 7.4, the most active inhibitor being (*R*)-(3-aminopiperidin-1-yl)(2-(1-(cyclopropylmethyl)-1*H*-indol-2-yl)-7-methoxy-1-methyl-1*H*-benzo[d]imidazol-5-yl)

methanone. PAD4 is attracting a considerable amount of attention in the patenting scenery; see the Boston Children's Medical Center's WO/2016/118476 for wound healing, and Yokohama University's WO/2016/143753 and the Danish Rigshospitalet's WO/2016/155745 for anti-PAD4 antibodies. There are also multiple efforts ongoing to develop small-molecule inhibitors.

Published: 24 November 2016

Financial & competing interests disclosure

The author has no 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. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

References

- Powell WS, Rokach J. The eosinophil chemoattractant 5-oxo-ETE and the OXE receptor. *Prog. Lipid Res.* 52(4), 651–665 (2013).
- Jones CE. The OXE receptor: a new therapeutic approach for asthma? *Trends Mol. Med.* 11(6), 266–270 (2005).
- O'Flaherty JT, Rogers LC, Paumi CM *et al.* 5-Oxo-ETE analogs and the proliferation of cancer cells. *Biochim. Biophys. Acta* 1736(3), 228–236 (2005).
- Gore V, Patel P, Chang CT *et al.* 5-Oxo-ETE receptor antagonists. *J. Med. Chem.* 56(9), 3725–3732 (2013).
- Gore V, Gravel S, Cossette C *et al.* Inhibition of 5-oxo-6,8,11,14-eicosatetraenoic acid-induced activation of neutrophils and eosinophils by novel indole OXE receptor antagonists. *J. Med. Chem.* 57(2), 364–377 (2014).
- Vyoral D, Petrak J. Therapeutic potential of hepcidin - the master regulator of iron metabolism. *Pharmacol. Res.* 115, 242–254 (2016).
- Ramsay AJ, Hooper JD, Folgueras AR, Velasco G, López-Otín C. Matriptase-2 (TMPRSS6), a proteolytic regulator of iron homeostasis. *Haematologica* 94(6), 840–849 (2009).
- Du X, She E, Gelbart T *et al.* The serine protease TMPRSS6 is required to sense iron deficiency. *Science* 320(5879), 1088–1092 (2008).
- Melis MA, Cau M, Congiu R *et al.* A mutation in the TMPRSS6 gene, encoding a transmembrane serine protease that suppresses hepcidin production, in familial iron deficiency anemia refractory to oral iron. *Haematologica* 93(10), 1473–1479 (2008).
- Schmidt PJ, Fleming MD. Modulation of hepcidin as therapy for primary and secondary iron overload disorders: preclinical models and approaches. *Hematol. Oncol. Clin. North Am.* 28(2), 387–401 (2014).
- Conner SD, Schmid SL. Identification of an adaptor-associated kinase, AAK1, as a regulator of clathrin-mediated endocytosis. *J. Cell. Biol.* 156(5), 921–929 (2002).
- Ricotta D, Conner SD, Schmid SL, von Figura K, Honing S. Phosphorylation of the AP2 mu subunit by AAK1 mediates high affinity binding to membrane protein sorting signals. *J. Cell. Biol.* 156(5), 791–795 (2002).
- Kuai L, Ong SE, Madison JM *et al.* AAK1 identified as an inhibitor of neuregulin-1/ErbB4-dependent neurotrophic factor signaling using integrative chemical genomics and proteomics. *Chem. Biol.* 18(7), 891–906 (2011).
- Shi B, Conner SD, Liu J. Dysfunction of endocytic kinase AAK1 in ALS. *Int. J. Mol. Sci.* 15(12), 22918–22932 (2014).
- Latourelle JC, Pankratz N, Dumitriu A *et al.* Genomewide association study for onset age in Parkinson disease. *BMC Med. Genet.* 10, 98 (2009).
- Högel H, Rantanen K, Jokilehto T, Grenman R, Jaakkola PM. Prolyl hydroxylase PHD3 enhances the hypoxic survival and G1 to S transition of carcinoma cells. *PLoS ONE* 6(11), e27112 (2011).
- Högel H, Miikkulainen P, Bino L, Jaakkola PM. Hypoxia inducible prolyl hydroxylase PHD3 maintains carcinoma cell growth by decreasing the stability of p27. *Mol. Cancer* 14, 143 (2015).
- German NJ, Yoon H, Yusuf RZ *et al.* PHD3 loss in cancer enables metabolic reliance on fatty acid oxidation via deactivation of ACC2. *Mol. Cell* 63(6), 1006–1020 (2016).
- Ma X, Wang X, Cao J, Geng Z, Wang Z. Effect of proline analogues on activity of human prolyl hydroxylase and the regulation of HIF signal transduction pathway. *PLoS ONE* 9(4), e95692 (2014).
- Goodrich L, Panning B, Leung KN. Activators and repressors: a balancing act for X-inactivation. *Semin. Cell. Dev. Biol.* 56, 3–8 (2016).
- Ehrhart F, Coort SL, Cirillo E, Smeets E, Evelo CT, Curfs LM. Rett syndrome - biological pathways leading from MECP2 to disorder phenotypes. *Orphanet. J. Rare Dis.* 11(1), 158 (2016).
- Chinen Y, Kuroda J, Shimura Y *et al.* Phosphoinositide protein kinase PDK1 is a crucial cell signaling mediator in multiple myeloma. *Cancer Res.* 74(24), 7418–7429 (2014).
- Wang LH, Tawil R. Facioscapulohumeral dystrophy. *Curr. Neurol. Neurosci. Rep.* 16(7), 66 (2016).
- Pirozhkova I, Petrov A, Dmitriev P, Laoudj D, Lipinski M, Vassetzky Y. A functional role for 4qA/B in the structural rearrangement of the 4q35 region and in the regulation of FRG1 and ANT1 in facioscapulohumeral dystrophy. *PLoS ONE* 3(10), e3389 (2008).
- Barca E, Aguenouz M, Mazzeo A *et al.* ANT1 is reduced in sporadic inclusion body myositis. *Neurol. Sci.* 34(2), 217–224 (2013).
- Kawamata H, Tiranti V, Magrané J, Chinopoulos C, Manfredi G. adPEO mutations in ANT1 impair ADP-ATP translocation in muscle mitochondria. *Hum. Mol. Genet.* 20(15), 2964–2974 (2011).

- 27 Jentzmik F, Azoitei A, Zengerling F, Damjanoski I, Cronauer MV. Androgen receptor aberrations in the era of abiraterone and enzalutamide. *World J. Urol.* 34(3), 297–303 (2016).
- 28 Beltran H, Prandi D, Mosquera JM *et al.* Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. *Nat. Med.* 22(3), 298–305 (2016).
- 29 Bánfi G, Teleki I, Nyirády P *et al.* Changes of protein expression in prostate cancer having lost its androgen sensitivity. *Int. Urol. Nephrol.* 47(7), 1149–1154 (2015).
- 30 Moilanen AM, Riikonen R, Oksala R *et al.* Discovery of ODM-201, a new-generation androgen receptor inhibitor targeting resistance mechanisms to androgen signaling-directed prostate cancer therapies. *Sci. Rep.* 5, 12007 (2015).
- 31 Freeman GJ, Casasnovas JM, Umetsu DT, DeKruyff RH. TIM genes: a family of cell surface phosphatidylserine receptors that regulate innate and adaptive immunity. *Immunol. Rev.* 235(1), 172–189 (2010).
- 32 Han G, Chen G, Shen B, Li Y. Tim-3: an activation marker and activation limiter of innate immune cells. *Front. Immunol.* 4, 449 (2013).
- 33 Ryu M, Chen Y, Qi J *et al.* LILRA3 binds both classical and non-classical HLA class I molecules but with reduced affinities compared with LILRB1/LILRB2: structural evidence. *PLoS ONE* 6(4), e19245 (2011).
- 34 Szikra J, Benyhe S, Orosz G *et al.* Radioligand binding properties of VV-hemorphin 7, an atypical opioid peptide. *Biochem. Biophys. Res. Commun.* 281(3), 670–677 (2001).
- 35 Lew RA, Mustafa T, Ye S, McDowall SG, Chai SY, Albiston AL. Angiotensin AT4 ligands are potent, competitive inhibitors of insulin regulated aminopeptidase (IRAP). *J. Neurochem.* 86(2), 344–350 (2003).
- 36 Albiston AL, Fernando R, Ye S, Peck GR, Chai SY. Alzheimer's, angiotensin IV and an aminopeptidase. *Biol. Pharm. Bull.* 27(6), 765–767 (2004).
- 37 Wright JW, Kawas LH, Harding JW. The development of small molecule angiotensin IV analogs to treat Alzheimer's and Parkinson's diseases. *Prog. Neurobiol.* 125, 26–46 (2015).
- 38 Borhade SR, Rosenström U, Sävmarker J *et al.* Inhibition of insulin-regulated aminopeptidase (IRAP) by arylsulfonamides. *ChemistryOpen* 3(6), 256–263 (2014).
- 39 Albiston AL, Diwakarla S, Fernando RN *et al.* Identification and development of specific inhibitors for insulin-regulated aminopeptidase as a new class of cognitive enhancers. *Br. J. Pharmacol.* 164(1), 37–47 (2011).
- 40 Desimmie BA, Weydert C, Schrijvers R *et al.* HIV-1 IN/Pol recruits LEDGF/p75 into viral particles. *Retrovirology* 12, 16 (2015).
- 41 Desimmie BA, Schrijvers R, Demeulemeester J *et al.* LEDGINs inhibit late stage HIV-1 replication by modulating integrase multimerization in the virions. *Retrovirology* 10, 57 (2013).
- 42 Christ F, Voet A, Marchand A *et al.* Rational design of small-molecule inhibitors of the LEDGF/p75-integrase interaction and HIV replication. *Nat. Chem. Biol.* 6(6), 442–448 (2010).
- 43 Vranckx LS, Demeulemeester J, Saleh S *et al.* LEDGIN-mediated inhibition of integrase-LEDGF/p75 interaction reduces reactivation of residual latent HIV. *EBioMedicine* 8, 248–264 (2016).
- 44 Mesplède T, Wainberg MA. Will LEDGIN molecules be able to play a role in a cure for HIV infection? *EBioMedicine* 8, 14–15 (2016).
- 45 Walpurgis K, Thomas A, Vogel M *et al.* Testing for the erythropoiesis-stimulating agent Sotatercept/ACE-011 (ActRIIA-Fc) in serum by means of Western blotting and LC-HRMS. *Drug Test Anal.* 8, 11–12 (2016).
- 46 Dussiot M, Maciel TT, Fricot A *et al.* An activin receptor IIA ligand trap corrects ineffective erythropoiesis in β -thalassemia. *Nat. Med.* 20(4), 398–407 (2014).
- 47 Soro L, Bartus C, Purcell S. Recessive dystrophic epidermolysis bullosa: a review of disease pathogenesis and update on future therapies. *J. Clin. Aesthet. Dermatol.* 8(5), 41–46 (2015).
- 48 Dang N, Murrell DF. Mutation analysis and characterization of COL7A1 mutations in dystrophic epidermolysis bullosa. *Exp. Dermatol.* 17(7), 553–568 (2008).
- 49 Goto M, Sawamura D, Nishie W *et al.* Targeted skipping of a single exon harboring a premature termination codon mutation: implications and potential for gene correction therapy for selective dystrophic epidermolysis bullosa patients. *J. Invest. Dermatol.* 126(12), 2614–2620 (2006).
- 50 Turczynski S, Titeux M, Tonasso L, Décha A, Ishida-Yamamoto A, Hovnanian A. Targeted exon skipping restores Type VII collagen expression and anchoring fibril formation in an in vivo RDEB model. *J. Invest. Dermatol.* 136(12), 2387–2395 (2016).
- 51 Bremer J, Bornert O, Nyström A *et al.* Antisense oligonucleotide-mediated exon skipping as a systemic therapeutic approach for recessive dystrophic epidermolysis bullosa. *Mol. Ther. Nucleic Acids* 5(10), e379 (2016).
- 52 Jones JE, Causey CP, Knuckley B, Slack-Noyes JL, Thompson PR. Protein arginine deiminase 4 (PAD4), Current understanding and future therapeutic potential. *Curr. Opin. Drug Discov. Dev.* 12(5), 616–627 (2009).
- 53 Badillo-Soto MA, Rodríguez-Rodríguez M, Pérez-Pérez ME *et al.* Potential protein targets of the peptidylarginine deiminase 2 and peptidylarginine deiminase 4 enzymes in rheumatoid synovial tissue and its possible meaning. *Eur. J. Rheumatol.* 3(2), 44–49 (2016).
- 54 Kusunoki Y, Nakazawa D, Shida H *et al.* Peptidylarginine deiminase inhibitor suppresses neutrophil extracellular trap formation and MPO-ANCA production. *Front. Immunol.* 7, 227 (2016).
- 55 Lewis HD, Liddle J, Coote JE *et al.* Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation. *Nat. Chem. Biol.* 11(3), 189–191 (2015).
- 56 Slack JL, Causey CP, Thompson PR. Protein arginine deiminase 4: a target for an epigenetic cancer therapy. *Cell. Mol. Life Sci.* 68(4), 709–720 (2011).