Patent Highlights

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

WO/2014/155301

Substituted bicyclic compounds as inhibitors of EZH2

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• epigenomics • histone-lysine N-methyltransferase • neoplasms • polycomb repressive complex 2

For most scientists who are not deeply involved with cancer therapy the enhancer of zeste homolog 2 (EZH2) might not be the first target that comes to mind. However, this histone-lysine N-methyltransferase (the catalytic subunit of the polycomb repressive complex 2, which blocks gene expression by methylating lysine 27 of histone H3), not only regulates embryonic development but is also a key factor for the self-renewal of cancer stem cells. Increased expression of EZH2 contributes to cancer aggressiveness and metastasis; for example, it epigenetically promotes prostate cancer by coordinating the silencing of proapoptotic microRNAs [1]. Recently, gain-of-function EZH2 mutations have been reported in non-Hodgkin's lymphoma [2], with S-adenosylmethionine-competitive small molecule inhibitors of EZH2 as potential therapeutics [3]. Judged from the *in vitro* inhibition assay that the inventors have used to characterize the compounds (some of which have nanomolar IC₅₀ values), this is also the mechanism for these new molecules. For the companion peer-reviewed paper see [4]. Also see GlaxoSmithKline's indazole derivative GSK-343 [5], and Epizyme's 6,5-fused bicyclic heteroaryls disclosed in WO2012/118812.

Published 2 October 2014

WO/2014/160409

RBP4 antagonists for the treatment of age-related macular degeneration & Stargardt disease

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• lipofuscin • macular degeneration • photoreceptor cells • retinaldehyde

The 'wet' form of age-related macular degeneration (AMD), caused by subretinal edema and choroidal neovascularization, has become a treatable condition. However, there is still no therapy for the 'dry' (atrophic) form of AMD or the juvenile form, Stargardt Disease, which are characterized by excessive deposition of cytotoxic lipid-protein-retinoid aggregates (lipofuscin) in the retinal pigment epithelium that supports the photoreceptor layer. The mayor component of lipofuscin is a pyridinium bisretinoid known as A2E, a nonenzymatic condensation product of all-*trans* retinaldehyde with phosphatidyl-ethanolamine that is generated during the visual cycle that regenerates the light-sensing pigments in the dark [6]. Even with all the consequences that this entails for the biochemistry of vision, it might be preferable to partially inhibit the visual cycle to forestall further deposition of its 'escape products.' The inventors present 2-(4-(2-(trifluoromethyl)phenyl)piperidine-1-carboxamido)benzoic acid (A1120), a high affinity (K_i = 8.3 nM) non-retinoid ligand for retinol-binding protein 4 which disrupts its interaction with trans-thyretin. A1120 has been reported before in this mechanistic context [7], but this had not been applied to the treatment dry AMD or Stargardt Disease. In the Abca4(-/-) mouse

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model that manifests accelerated accumulation of lipofuscin in the retinal pigment epithelium [8], the levels of A2E and other bisretinoids were reduced by approximately 50%.

Published 2 October 2014

WO/2014/165746

Methods & compositions using ADAM10 inhibitors to treat bacterial infections

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• ADAM proteins • bacterial infections • hemolysin proteins
• methicillin-resistant *Staphylococcus aureus*

The widely expressed transmembrane proteins of the 'A Disintegrin And Metalloprotease' (ADAM) class are also known as 'shedases' because their intrinsically disordered extracellular domains can induce shedding of many important cell surface proteins resulting in the release of biologically active soluble factors; they also act as integrin ligands [9]. ADAM10 has attracted particular attention because of its involvement in cancer. The inventors have found ADAM10 to also be a key factor in invasive infection with *Staphylococcus aureus*. This human commensal must breach the innate epithelial barrier defense to cause invasive disease, and it does so by exploiting the fact that ADAM10 expressed on epithelial cells is a receptor for its pore-forming cytotoxin α -hemolysin (as well as for streptolysin O and pneumolysin). Novel inhibitors were designed and synthesized based on the structure of the known ADAM10 inhibitor, (R)-N-((S)-3,3-dimethyl-1-(methylamino)-1-oxobutan-2-yl)-2-((S)-1-(N-hydroxyformamido)-ethyl)-5-phenylpentanamide (GI254023X) [10], which has an IC_{50} of 5 μ M and is poorly soluble. Assays used lactate dehydrogenase release from the A549 cell line as a measure for inhibition. This extends earlier reports on blood infection by methicillin-resistant *S. aureus* [11]. For the companion peer-reviewed paper see [12].

Published 9 October 2014

WO/2014/167091

Novel hemoglobin-derived peptide based pharmaceutical compositions

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• fetal hemoglobin • immunity, innate • lipopeptides
• Toll-like receptors

It has long been known that low concentrations of bacterial lipopolysaccharide (LPS) endotoxins can trigger nonspecific immunostimulating effects that

are beneficial in the treatment of tumors, infections and allergies and for treating certain age-related immune imbalances. That Toll-like receptors (TLRs) mediate this by acting as sensors that activate innate immune responses after detecting certain generic pathogen-associated molecular patterns is a recent insight [13]. This invention comprises at least one TLR-activating amphiphilic bacterial lipopeptide (preferably, a mono-, di- or triacylated lipopeptide having a S-(2,3-dihydroxypropyl)-cysteine backbone) and at least one synthetic peptide derived from human fetal hemoglobin that synergizes with the LPS-triggered activity. Examples of such lipopeptides are fibroblast-stimulating lipopeptide-1 (FSL-1), and the macrophage-activating lipopeptide from *Mycoplasma fermentans* (MALP2). The hemoglobin-derived peptides are built around the VTVLAIHFGKEFTPE-VQASW core structural element. X-ray small-angle scattering and atomic force microscopy showed that these alpha-helical peptides intercalate with monophosphoryl lipid A (a prototypical LPS fragment), converting the biologically inactive multilamellar lipopeptide structure into a highly active disaggregated one with cubic symmetry. This should be much more practical than using complete fetal hemoglobin as was proposed earlier [14,15].

Published 16 October 2014

WO/2014/167230

Composition including the HIP/PAP protein or one of the derivatives thereof for treating insulin resistance

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• diabetes mellitus • lectins, C-type • metabolic syndrome X

The human hepatocarcinoma-intestine-pancreas/pancreatic-associated protein (HIP/PAP), a secreted C-type lectin, is known as a paracrine hepatic growth factor and has been suggested as a target for the treatment of liver failure [16,17]. Using the ob/ob mouse model of Type II diabetes and the murine high-fat diet model the inventors have demonstrated that HIP/PAP also specifically reduces insulin resistance in prediabetes, presumably by increasing glucose uptake by skeletal muscles. In addition the protein has a significant effect on dyslipidemia, and even in subjects that are not overweight it causes a significant increase in lean body mass associated with a tendency to decrease fat mass, without overall weight loss. The extensive data,

which include functional imaging, were generated with ALF5755 which is a recombinant *Escherichia coli*-produced HIP/PAP with methionine added at the N-terminal position. In humans, the minimal effective dose with parenteral administration is said to be about 40 µg/kg, but no clinical data are presented.

Published 16 October 2014

WO/2014/170892

Inhibition of RIP kinases for treating lysosomal storage diseases

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- genetic diseases, inborn • lysosomal storage diseases
- receptor-interacting protein serine-threonine kinases

Mice deficient for receptor-interacting protein (RIP) kinase, particularly RIP3 kinase, were significantly less sensitive to the glucocerebrosidase inhibitor conduritol β-epoxide (CBE) in comparison to RIP3-expressing mice that exhibited symptoms of Gaucher disease upon administration of CBE: this is the discovery reported in this invention. It is unexpected because the known roles of RIP kinases are in various infectious and sterile inflammatory diseases as well as in necrosis [18]. That RIPK3 contributes to organ damage in sepsis by promoting cytokine release and neutrophil infiltration [19] illustrates the connection with Gaucher disease. Data were obtained from *Gba(flox/flox);Nestin-Cre* mice (a mouse model with downregulation of macroautophagy in neuronal precursors) and their respective littermate controls, and from C57BL/6 mice that were injected intraperitoneally with either 50 mg/kg/day CBE or phosphate-buffered saline starting at 8 days of age. With small-molecule RIP kinase inhibitor development based on necrostatin-1 still in its infancy [20], the best current option seems to be reducing *RIPK3* gene expression with RNA interference. This could offer new therapeutic hope especially for patients with the neuro-pathic Gaucher disease types 2 and 3 because the recombinant glucocerebrosidase that is currently used for enzyme replacement therapy does not penetrate into the brain, and reducing glucosylceramide influx (as in type 1 disease) is also not an option. For the companion peer-reviewed paper see [21].

Published 23 October 2014

WO/2014/173859

PAR-4 antagonists for use in the treatment or prevention of influenza virus type A infections

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- influenza A virus • pneumonia • receptors, thrombin

Endogenous proteases in the respiratory tract play a role both in influenza virus replication, and also in the 'cytokine storm' that the virus can trigger. These inflammatory processes are mediated by protease-activated receptors (PARs) of which four (activated by different proteases) have been characterized. While it is known that the PAR-1 and PAR-2 pathways contribute to the innate immune response during single-stranded RNA viral infection [22,23], a specific role for PAR4, which is activated by thrombin cleavage to expose an N-terminal tethered ligand, has not been reported beyond the fact that PAR-4 modulates airway smooth muscle tone in influenza A virus-infected mice [24]. The inventors have found that interfering with PAR4 activation by inhibiting thrombin-induced platelet activation with pepducins (modified peptides that combine an intracellular loop of a G protein coupled receptor and a cell-penetrating or membrane-tethering moiety), or by reducing PAR4 expression with RNA interference, can protect against influenza infection, or can reduce pulmonary inflammation if such an infection has occurred. Mice infected with 40 or 60% lethal doses of influenza A/PR/8/34 (H1N1) showed higher survival rates and reduced weight loss when treated with 10 µg pepducin PAR4 antagonists N-palmitoyl-SGRRYGHALR-NH2 (P4pal-10) or trans-cinnamoyl-YPGKF-NH2 (tcY-NH2).

Published 30 October 2014

WO/2014/175287

Medicinal composition for inhibiting formation &/or enlargement of cerebral aneurysm or shrinking same

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- intracranial aneurysm • intracranial hemorrhages
- lysophingolipid receptors

Receptors for the lipid mediator sphingosine-1-phosphate (S1P), of which five subtypes are currently known, play an important role in angiogenesis, vascular stability and immune cell trafficking through vessel walls [25]. Their role in the weakening of arterial walls that precedes formation of aneurysms is differentiated; for example, the S1P2 receptor is understood to be a key regulator of the proinflammatory phenotype of the endothelium [26], but in human abdominal

aortic aneurysms its expression is downregulated while S1P3 expression increases [27]. In this invention, S1P1 agonists such as Astellas' ASP4058, which has been suggested as an agent to treat multiple sclerosis [28], are used to counteract the mechanisms that promote cerebral aneurysms. At 30 µg/kg p.o. once daily, this benzimidazole was effective in the rat cerebral artery bifurcation model. It also reduced the number of lymphocytes in a concentration-dependent manner in the peripheral blood. There seems to be no peer-reviewed literature report on the role of S1P receptor agonists in cerebral aneurysms, a condition found in 2–5% of the general population [29].

Published 30 October 2014

WO/2014/179793

Blood substitute composition & method of use

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• blood substitutes • hemoglobins • nanoparticles
• transfusion medicine

Plasma volume expanders that counteract the shock triggered by acute blood loss have been available for decades, and are now very safe and effective. However, full artificial blood – which would be extremely desirable given the supply shortages of donated blood, and because such a biocompatible synthetic oxygen carrier would not be burdened with the concerns of donor matching or blood-borne infections – has remained elusive [30]: the physiological O₂/CO₂ binding and exchange characteristics of hemoglobin in erythrocytes have never been fully matched by any chemical substitute. The present disclosure takes a step toward artificial erythrocytes by providing disc-shaped bi-concave nanoparticles comprising an aqueous core containing an oxygen-carrying agent (hemoglobin, 20–60% w/v), an allosteric effector (such as 2,3-diphosphoglycerate, to modulate oxygen affinity in a pH-dependent fashion) and a reducing agent (leucomethylene blue); and a shell consisting of an amphiphilic branched polymer (e.g., polyethyleneimine) conjugated to a lipid, such as palmitic acid or C24-pentacosadiynoic acid. The particles can be stored lyophilized for extended periods at ambient conditions. In a rat hemorrhagic shock model (25% blood volume removal under anesthesia, followed by resuscitation with an equal volume of the blood substitute) tissue oxygen levels and hemodynamics were partially reconstituted. For related work see [31,32].

Published 6 November 2014

WO/2014/182277

FGF modulation of *in vivo* antibody production & humoral immunity

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• B-lymphocytes • fibroblast growth factor 2 • immunity, humoral

Fibroblast growth factor-2 (FGF2 or bFGF, 'basic FGF') was the first identified member of this extremely diverse family of extracellular regulators that controls the physiology and development of virtually all higher vertebrate tissues, and it remains the best-studied one [33]. The inventor has shown that FGF2 deficient mice have an altered humoral immune response: they produce more antibody to a Type I independent antigen while FGF2 overexpressing mice show suppressed antibody production to the same pathogenic stimulus. In addition, germinal center formation is compromised in the absence of FGF2. Changes in both antibody production and germinal center formation are observed in mice lacking a single copy of *FGF2*, demonstrating that lymphocytes are particularly sensitive to gene dosage. These studies provide the first evidence that FGF signaling is a crucial negative regulator of mature B cell function. Recombinant FGF2 and FGF2 agonists are readily available, which should make this initial report (which has no peer-reviewed counterpart yet) straightforward to reproduce and extend, especially toward applications in autoimmune disorders. On the other hand, small-molecule FGF2 antagonists and traps (such as the soluble parts of its receptor), as developed for cancer therapy, could improve immune responses. Interestingly, bFGF adsorbed to mannan-modified nanoparticles has been used as a vaccine adjuvant to improve humoral immunity [34].

Published 13 November 2014

WO/2014/186755

Myocyte-derived flow assist device: extravasal sheaths of rhythmically contracting myocytes aiding flow of biological fluids

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• lymph node excision • lymphedema • tissue engineering
• tissue scaffolds • venous insufficiency

The cardiovascular system relies on extra-cardiac mechanisms to maintain a proper circulation of blood and lymph. When the skeletal muscle pump mechanism fails (either due to lack of leg muscle activity, chronic distention of veins, failure of venous valves or a combination of the above), it leads to chronic venous insufficiency, potentially resulting in phlebitis and leg

ulcers [35]. Lymphedema may be inherited, but its secondary forms are among the common complications of breast cancer surgery, resulting from the dissection of lymph nodes and radiation therapy [36,37]. This invention provides a potential solution for both conditions: the affected vein or lymph vessel is wrapped in prefabricated scaffold sheets covered with layers of myocytes (e.g., cardiomyocytes) engineered from stem cells, or in thin layers of engineered muscle tissue containing a spontaneously and rhythmically active mixture of pacemaker and contractile cells (e.g., heart tissue); or the vessel is surrounded with a mesh of muscle fibers (e.g., cardiac fibers), or with a single fiber wrapped in a spiral fashion. Such tubular biological 'mini pumps' resemble the simplest kinds of hearts seen in lower invertebrates and/or during human embryonic development. Even if such directional flow-assist devices are not strong enough to fully combat hydrostatic pressure upon standing, their rhythmic contraction can support the pulsatile shear stress within the vein which enhances secretion of cytokines by endothelial cells and counteracts hypercoagulability.

Published 20 November 2014

WO/2014/190157

TMPRSS6 compositions & methods of use thereof

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• iron overload • RNA interference • serine endopeptidases

Hereditary iron deficiency anemia that is refractory to oral iron therapy can be caused by mutations in *TMPRSS6*, which encodes a type II transmembrane serine protease produced mostly by hepatocytes. This enzyme (also known as matriptase-2) regulates

the expression of the systemic iron regulatory hormone hepcidin [38], a 25-amino acid polypeptide which reduces iron absorption from the intestine by binding to the only known cellular iron exporter, ferroportin [39,40]. What is causal for anemia through a loss of function can be expected to be beneficial in disorders associated with iron overload – hereditary hemochromatosis, β -thalassemia or erythropoietic porphyria. This is the rationale for the inventors' design of double stranded RNAi agents of 15–30 modified nucleotide pairs that inhibit the expression of matriptase-2. In 6-week-old thalassemic Th3/+ mice fed on low-iron (3–5 ppm) diets, such an RNAi agent synergized with the iron chelator, deferiprone (250 mg/kg/day) to reduce the chronically elevated non-heme iron concentrations in liver and spleen. – Like the other members of its family, matriptase-2 is multifunctional; in addition to its serine protease domain it contains LDL receptor class A domains, a scavenger receptor cysteine-rich domain and a bone morphogenetic protein domain. Permanently repressing it might not be an optimal solution, but the established intermittent therapies with iron chelators might be substantially aided by these nucleic acid agents.

Published 27 November 2014

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.

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