Patent highlights

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

WO/2014/101718

Methods and compositions for treating and/or preventing a disease or disorder associated with abnormally high level of mitochondria factor 4

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• cancer • mitochondria • transcriptional elongation factors

Elongation factor 4 (EF4, originally known as LepA) is one of the most conserved proteins across all known genomes. It back-translocates the elongating ribosome, causing the translation machinery to move one codon backward along the nascent mRNA. Its biological significance in mammals has not been fully elucidated, although it is expected that EF4 helps maintaining translation fidelity under stress conditions like in bacteria and yeast [1,2]. Eukaryotic homologs have a potential mitochondrial targeting signal in their N-termini, but it used to be unclear whether EF4 is needed there, and for what purpose. The answer seems to be respiratory function and cellular energy management. The inventors show that mtEF4 co-localizes with the mitochondrial respiratory chain complex IV subunit and while it maintains mitochondrial translation at catalytic concentrations, it is inhibitory at the stoichiometric level. They also show that mtEF4 is highly overexpressed in cancer cells. The anticancer agents of the invention can be antibodies, binding proteins or siRNAs that modify a nuclear factor that controls mtEF4 transcription or bind to its mitochondrial

target sequence. For a closely related peer review paper from this team, see [3].

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WO/2014/104224

PGC-1 β protein function regulator, mitochondria function regulator, anti-obesity agent, and screening method therefor

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• mitochondria • PPARGC1 • SYVN1 protein, human • transcription factors • ubiquitin-protein ligases

This patent application connects two factors that previously were not thought to be related – the peroxisome proliferator-activated receptor- γ coactivator 1 β (PGC-1 β ; a transcriptional regulator of cellular energy metabolism [4]) and the ubiquitin ligase synoviolin, which is highly expressed in rheumatoid synovial cells and is implicated in the endoplasmic-reticulum-associated degradation pathway [5]. The inventors have found that synoviolin negatively regulates PGC-1 β , and that blocking the interaction of these proteins (with small molecules) or reducing the expression of synoviolin (with siRNAs) promotes fatty acid β -oxidation in fat tissue mitochondria. One compound that is reported to be effective is LS-102 (Pharmacoceia, Inc.; a selective inhibitor for E3 ubiquitin ligase activity of synoviolin), which doubled PGC-1 β protein levels in the 3T3-L1 murine fat precursor cell line, although it did not alter its mRNA synthesis. LS-102 treatment (50 mg/kg) increased the

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volume, density and number of mitochondria in adipose tissue of C57BL/6J mice as well as β -oxidation of fatty acids. Other inhibitors are naphthalene derivatives, but quercetin is also effective. Note that inhibitors of small ubiquitin-like modifier E3 ligase activity suppress liver X receptor ligand-dependent transcriptional activation of the lipogenic program in hepatocytes [6], which points into the same direction.

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Metal-sensitive mutants of matrix metalloproteinases and uses thereof

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• calcium • fibrosis • matrix metalloproteinases

The extracellular matrix (ECM) provides structural support for cells and tissues; excessive deposition of ECM components, such as collagen, can lead to fibrotic disease. The inventors have modified ECM-degrading matrix metalloproteinases (MMPs) and hyaluronidases in such a way that they become fully active only at extracellular calcium concentrations much higher than the physiological levels of 1.0–1.3 mM. Thus, unlike wild-type MMP polypeptides, the activity of csMMP polypeptides can be regulated by controlling local concentrations of calcium so that uncontrolled or prolonged degradation of collagen and other ECM components – a side effect of treatment with bacterial collagenases – is avoided. The lyophilized mutant MMPs (MMP-1, which cleaves the most abundant skin proteins collagen type I and III, is preferred) can be reconstituted in a buffer containing 10 mM calcium. Following injection into the fibrotic tissue, the calcium steadily diffuses and the modified MMP becomes almost inactive. The MMPs of the invention typically contain one or more amino acid replacement at positions at or near the residues directly involved with zinc or calcium coordination. Efficacy was demonstrated with *in vivo* rat skin perfusion followed by western blotting, and by *in vitro* digestion of human keloidal collagen. The inventors claim utility for localized fibrotic conditions such as scars, Dupuytren's syndrome, Peyronie's disease, Ledderhose fibrosis or adhesive capsulitis ('frozen shoulder'). Note, however, that inhibition of MMP activity may be desired in preventing recurrent Dupuytren's contracture [7]. For information on metal-binding and catalytic sites of MMPs, see [8]. For the peer review companion paper, see [9].

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WO/2014/108520

Benzylguanidine derivatives and therapeutic use for the treatment of protein misfolding diseases

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• endoplasmic reticulum stress • eukaryotic initiation factor-2 • guanabenz • proteostasis deficiencies

Guanabenz, an $\alpha 2$ receptor agonist, is an old anti-hypertensive drug that nowadays finds only very limited use in its original indication but has some very interesting alternative potential applications, including activity against prions [10] and protection of cells against stress resulting from protein misfolding [11]. The inventors have designed guanabenz derivatives that lack the undesirable $\alpha 2$ receptor agonism but retain the capability to block stress-induced dephosphorylation of the α subunit of eukaryotic translation initiation factor 2 (eIF2 α). Earlier reports had indicated that the aryl group must be at least disubstituted in order for the compounds to exhibit useful pharmacological activity, but it is now evident that monosubstituted aryl derivatives are also active. Actually, 1-[(E)-[(2-chlorophenyl) methyldene]amino]-guanidine, which is guanabenz with one of the two chlorine substituents removed, is the most preferred compound. The inventors claim to have tested for accumulation of mutant huntingtin amino-terminal fragment (Htt48Q) associated with Huntington's disease and SOD1 mutant (A4V), associated with amyotrophic lateral sclerosis; of the P0S63del mutant causing Charcot-Marie-Tooth 1 B neuropathy; and for aggregation of rhodopsin P23H which is associated with retinitis pigmentosa. However, no data are disclosed. For other guanabenz derivatives modified for anti-prion activity, see [12].

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WO/2014/110036

SHIP1 modulators and methods related thereto

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• allergy and immunology • naphthalenes • phosphoric monoester hydrolases

SHIP1 (an endogenous inhibitor of the phosphoinositide-3-kinase pathway) differs from SHIP2 and the 3' inositol phosphatase phosphatase and tensin homolog in that its expression is essentially restricted to immune and hematopoietic cells. Diminished SHIP1 activity or expression has been observed in human inflammatory diseases and hematopoietic malignancies [13], but its involvement is broader, extending for example to bone metabolism [14]. This

application, which claims decahydronaphthalene derivatives and does not appear to cover the company's clinical-stage oral SHIP1 activator AQX-1125 (in development for asthma; [15–17]), continues from WO/2011/069118 which discloses tetracyclic SHIP1 modulators. Several compounds activated human SHIP1 *in vitro* by more than 65%, and also blocked Akt phosphorylation. Two of these – one of which is N-[(2R,3S,4S,4aS,8aS)-4-[(3,5-dihydroxyphenyl)carbonyl]-3,4a,8,8-tetramethyl-decahydronaphthalene-2-yl]acetamide – inhibited allergen-induced passive cutaneous anaphylaxis in mice by more than 50% at 20 mg/kg. The therapeutic claim (No. 23) is for autoimmune diseases, inflammation and neoplasms.

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WO/2014/112145

Therapeutic agent and therapeutic method relating to 1,25D3-MARRS for neurological disease such as Alzheimer's disease

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• Alzheimer disease • diosgenin • PDIA3 protein, human
• protein disulfide-isomerases • vitamin D

1,25D3-MARRS stands for '1,25 dihydroxyvitamin D3 membrane associated, rapid response steroid-binding' protein, an alternate receptor for vitamin D also referred to as ERp57, GRp58 and PDIA3 [18,19]. The inventors have shown that diosgenin, a plant-derived steroidal sapogenin – known to be neuroprotective in retroviral infections but originally presumed to act as an anti-oxidant [20] – directly activates 1,25D3-MARRS. The same is true for the related denosomin, which promotes axon regeneration in spinal cord injury models [21]. Data from novel object recognition tests in a transgenic mouse strain (5xFAD) harboring five familial Alzheimer-related mutations are presented. Although the mechanism of this alternate vitamin D pathway has not yet been fully elucidated – protein kinase C activation and nuclear translocation of the bound receptor protein are certainly involved – it could shed new light on how vitamin D and its analogs exert their effects against Alzheimer's disease, which might be protective rather than therapeutic because results from clinical trials had mostly negative results. The natural products diosgenin and denosomin could serve as lead structures to develop drugs that do not bind to the classical vitamin D receptor, and which have more immediate effects on cognition. For the companion peer review papers, see [22,23].

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WO/2014/113450

Decreasing inflammatory response in a blood vessel

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• angioplasty, balloon, laser-assisted • atherosclerosis
• radiation-sensitizing agents

Larger blood vessels can become acutely inflamed as a result of infection, or by damage to the endothelium (e.g., from balloon angioplasty). Chronic inflammation is prominently exemplified by atherosclerosis where the damage caused by the endothelial uptake of oxidized low-density lipoproteins triggers a cascade of immune responses, which eventually result in atheroma formation. The inventors have used 4-amino-1,8-naphthalimides (a group of compounds known to be chemically activated by radiation to form condensation products with proteins via their amino moiety) to treat such conditions. The core structure of these compounds is an inhibitor of poly(ADP-ribose) polymerase [24] and telomerase [25]. The delivery system contains fiber optics in its lumen to irradiate the target zone with blue light at low power while the vessel is perfused. With co-administration of the compounds and photoactivation in a balloon vessel injury model, rat femoral arteries developed little or no intimal thickening after 2 weeks. It is suggested that vascular compliance was also improved, but no data are presented in support. Some of the company's prior patents, which originated at PhotoBioMed Corp., claimed naphthalimide-labeled chitosan for photoangioplasty, tethering pharmaceutical agents to tissue surfaces to provide local drug delivery, and for chemically bonding skin care products (see, e.g., WO/2005/044309).

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WO/2014/114644

Use of Tungsten(VI) salts for the treatment of female infertility in non-diabetic mammals

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• anovulation • infertility, female • sodium tungstate(VI)

There are very few medical applications for tungsten salts, but Spanish researchers seem to have taken particular interest recently, as evidenced by WO/2014/096498 claiming tungstate salts as anti-platelet agents (reviewed in Pharm Patent Analyst 3(5)); earlier examples include WO/2002/098435 for obesity and EP-755681 for diabetes. It has been reported that sodium tungstate can reverse the loss of fertility in the standard rat model of streptozotocin-induced diabetes in male [26] as well as in female [27] animals. According to the data presented

here, the effect of tungsten on female fertility is independent of diabetes: in IRS2 knockout mice, whose females have reduced follicular development and only 9% of the wildtype's fertility but remain relatively euglycemic during youth, sodium tungstate (2 mg/ml in drinking water; about about 180 mg/kg/day) restored the normal estrous cycle. The question is, at what price? The major site of tungsten deposition in mice after 16 weeks of uptake in drinking water is bone, where it has a clear effect on DNA damage and pre-leukemic B-cell formation in bone marrow [28]. In humans, elevated tungsten concentrations are strongly associated with the prevalence of stroke [29]. However, none of the beneficial effects of tungsten salts that has been reported has found a proper explanation to date, which makes such reports extremely interesting.

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Substituted phthalamic acids as sortilin inhibitors

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• adaptor proteins • nerve degeneration • nerve growth factors • vesicular transport

Sortilin (NTR-3, GP95) is a widely expressed type I membrane receptor that was originally identified as a sorting receptor in *Saccharomyces cerevisiae*. It mediates pro-apoptotic effects of pro-neurotrophins, and is considered a key regulator of the balance between generally opposing effects of mature and pro-forms of neurotrophins [30]. Sortilin/P75-mediated apoptosis has been suggested as a neuroprotective target mechanism in neurodegenerative disorders. The compounds that are disclosed here are among the first small molecule inhibitors of sortilin interaction; they cover Lundbeck's current lead, AF38469 (N-(6-methyl-pyridin-2-yl)-5-trifluoromethyl-phthalamic acid; Example 1 in the application) [31] but not AF40431 which the company had described earlier [32]. The presented data show results from affinity assays based on the displacement of 3H-neurotensin from human sortilin and from homogeneous time-resolved fluorescence assays measuring inhibition of proNGF and progranulin binding to sortilin. The claims cover almost every known neurodegenerative disease; but how inhibition of neurotrophic factor binding to sortilin – which apparently is a node in several pathways – would affect essential mechanisms of neuronal maintenance and regeneration is not known yet.

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WO/2014/120569

Methods and compositions for human epididymis protein-4 (HE4)

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• fibrosis • glomerular filtration rate • kidney diseases

Most chronic renal damage, irrespective of etiology, leads to renal fibrosis, a self-perpetuating process of collagen deposition that is facilitated by the recruitment of activated fibroblasts and the progression of an inflammatory response. One of the most overexpressed proteins in fibrotic kidneys is the human epididymis protein-4 (HE4), a glycoprotein of unknown biological function that has recently been proven to be a reliable serum cancer biomarker especially for gynecological malignancies [33], and it probably is an anticancer agent [34]. The results presented here confirm that HE4 is a pan-serine protease inhibitor (as had been expected because of its four-disulfide WAP core domain repeats), specifically of matrix metalloproteinases 2 and 9, that prevents degradation of type I collagen. In fibrotic kidneys of aSMA-RFP transgenic mice subjected to unilateral ureteral obstruction, treatment with a neutralizing antibody to HE4 (described in U.S. Patent 7,846,692) improved not only renal histology but also functional parameters (blood urea nitrogen and urine albumin-to-creatinine ratio). Claims extend to non-antibody binding proteins, interfering RNA expression inhibitors, and other types of organ fibrosis. The invention also features HE4 and two specific serine proteases, Prss23 and Prss35, as a diagnostic marker for organ fibrosis or a risk of developing organ fibrosis. The nature of HE4 as a broad-spectrum protease inhibitor had been described before the patent application's priority date [35]. For the peer review companion paper, see [36].

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WO/2014/120885

Treatment of drug abuse by preventing GAPDH nitrosylation

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• glyceraldehyde-3-phosphate dehydrogenase (phosphorylating) • selegiline • ubiquitin-protein ligases

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key enzyme in glycolysis, can undergo S-nitrosylation, which facilitates its binding to the E3 ubiquitin ligase, Siah1. The GAPDH–Siah protein complex translocates to the nucleus and triggers apoptosis [37]. It was known that the neuroprotective effects of R(-) deprenyl, a monoamine oxidase B (MAO-B) inhibitor

used in early-stage Parkinson's disease, was at least in part attributable to its ability to prevent S-nitrosylation of GAPDH [38]. The inventors report that this same signaling cascade mediates at least some of the transcriptional and behavioral actions of cocaine, R-(–)-deprenyl, but particularly that of the related drug CGP3466B which is devoid of MAO-B inhibition. It prevents the enhancement of the cAMP response element-binding protein signaling system caused by lower, behavioral-stimulant doses of cocaine, and also the stimulation of the p53 cytotoxic system by higher, neurotoxic doses. CGP3466B (0.15 mg/kg i.p.) substantially reduced locomotor stimulation by cocaine in mice moving in an open field paradigm, without effect on basal locomotor activity. In contrast, the MAO-B inhibitors rasagiline, pargyline and lazabemide, when combined with cocaine, elicited greater locomotor stimulation than cocaine alone and did not prevent cocaine-induced neurotoxicity. Pretreatment with CGP3466B also reduced conditioned place preference, a measure of cocaine seeking behavior. This could open new avenues in the treatment of stimulant dependence. For the peer review companion paper, see [39].

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2,6,7 Substituted purines as HDM2 inhibitors

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• neoplasms • proto-oncogene proteins c-mdm2

Human double minute 2 (HDM2), an E3 ubiquitin-protein ligase, is a negative autoregulator of

the classical tumor suppressor protein, p53. In the absence of oncogenic stress signals, it binds to p53 and blocks its ability to activate transcription, while in the presence of such signals HDM2 lets p53 activity rise to induce cell cycle arrest and apoptosis [40]. Restoring the function of p53 in tumor cells by anti-HDM2 therapy should therefore result in slowing the tumor proliferation. Several classes of small-molecule inhibitors of HDM2-p53 interaction have been described, based on piperidines [41], spiro-oxindoles [42] and diphenylpyrroles [43]. Only two candidate drugs from Novartis, CGM097 and HDM201 have reached Phase I clinical trials. The present application introduces a new such class, 2,6,7 substituted purines. A large amount of synthesis and chemical characterization data is presented but not the slightest indication of biological data is shared, not even on a categorical scale. This underscores the competitiveness of this field. It is not to be expected that HDM2 inhibitors could become stand-alone oncology therapeutics; but at some point they might be useful to prevent cancer recurrence, or – in combination with cytotoxic drugs – slow metastasis.

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