Patent Highlights

A snapshot of recent key developments in the patent literature of relevance to the advancement of pharmaceutical and medical R&D.

WO/2014/032755

DDR2 inhibitors for the treatment of osteoarthritis

Wucherer-Plietker M, Werkmann D, Gigout A *et al.*
Merck Patent GmbH, Darmstadt, Germany

• discoidin receptors • fibrillar collagens • neoplasms
• osteoarthritis • receptor protein-tyrosine kinases

The discoidin domain-containing receptor 2 (DDR2; also CCK-2, tyro-10, or CD167b) is a receptor tyrosine kinase that is activated upon collagen binding in a delayed but sustained fashion. It is an important regulator of neutrophil chemotaxis in 3D collagen matrices [1]. In osteoarthritis, joint cartilage injury or loss of growth factor sensitivity of articular chondrocytes results in a breakdown of the pericellular collagen VI-rich matrix, exposing the DDR2 receptor on chondrocytes to collagen II fibers or fragments and activating it, which results in the release of cytokines and degradation by proteases. Compounds of the present invention bearing phenyl or hetero-aromatic rings in the R3 position are strong and selective inhibitors of DDR2. Its expression is also associated with invasiveness of carcinomas and inferior prognosis [2], and patenting for this target has focused on solid tumors. Related ureidomethyl-phenoxy-pyridines and phenyl-3-[4-(pyridin-4-yloxy)-benzyl]-ureas have been disclosed as Tie-2 and cRaf kinase inhibitors in WO/2004/037789 and WO/2006/042599, and as Aurora and RON kinase inhibitors in WO/2011/017142; all in the context of cancer. Note that actinomycin D is also an inhibitor of DDR2

activation by type I collagen; however, its IC_{50} in HEK-293 human embryonic kidney cells, 9 μ M [3], is 1–2 orders of magnitude higher than that for the compounds presented here.

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Hermann AM Mucke

H.M. Pharma Consultancy e.U.,
Enenkelstrasse 28/32, A-1160 Wien,
Austria
h.mucke@hmpharmacon.com

WO/2014/036198

Compositions and methods for specific inhibition of *Leishmania* receptor for activated C kinase

Qvit N, Mochly-Rosen D,
Schlechtmann D & Churchill E
The Board of Trustees of the Leland
Stanford Junior University, Stanford,
CA, USA

• antigens • cell surface • *Leishmania* • protozoan
• receptors • *Trypanosoma*

The Receptor for Activated C-Kinase 1 (RACK1) is an ubiquitous and highly conserved scaffold protein that interacts with several isoforms of protein kinase C, regulating cell growth, survival, differentiation, shape and protein translation. The RACK1 ortholog in *Leishmania* ssp. LACK (*Leishmania* homolog of receptors for activated C kinase) also interacts with multiple signaling enzymes in these parasites, is present in a multiprotein complex in the kinetoplast, and is under investigation as a vaccine target [4,5]. A similar situation exists with the *Trypanosoma* homolog, *Trypanosoma*-activated C kinase (TRACK) [6]. This invention discloses rationally designed modulatory peptides of 5–20 amino acid residues' length (e.g., CVSLAHATD, RNGQCQRK, or

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TPDGAKPSE; designed using five domains with low levels of amino acid conservation as templates) that were conjugated to cargo peptides derived from the human immunodeficiency virus TAT protein. They inhibit interactions of LACK or TRACK with their cognate target proteins. *In vitro*, *L. amazonensis* promastigote death was achieved at low to medium micromolar concentrations, while 20–90% inhibition of macrophage infection required 200 μ M. This approach might be preferable over attempting to inhibit parasite enzymes, which is more prone to side effects in the host.

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Tetrabranched peptides & analogues as a new class of antimicrobials and their preparation thereof

Beuerman RW, Liu S, Rajamani L *et al.*
Agency of Science, Technology, and Research, Singapore, Singapore Health Services PTE Ltd., Singapore

• antifungal agents • eye infections • fungal • keratitis • peptides

Several types of peptides, including the defensins, which are part of the first-line immune defense, are known to have antifungal activity. This invention pursues a relatively new concept in the design of peptides with activity against eukaryotic pathogens – branched peptides [7]. Central to the invention is peptide B4010, which consists of four copies of the sequence RGRKVVRR that are linked through a branched lysine core, and might be particularly useful to treat fungal keratitis. This construct exhibited a minimal inhibitory concentration of 0.34 μ M for two *Candida albicans* clinical isolates (as opposed to 15 μ M for natamycin, the only US FDA approved drug for fungal infections of the eye). The monomer and the linear retrodimer, RGRKWRRKKRRWKRRGR, displayed only weak activity against both *C. albicans* and *Fusarium* strains. Circular dichroism spectropolarimetry revealed strong minima around 200 nm for B4010, suggesting an unordered conformation. Scrambling the sequence increased MI values two- to four-fold, yet retained significant potency. Time-kill kinetics showed complete killing within 1 h. The peptide did not inhibit corneal wound healing in rabbits at 22 μ M, a concentration where tear fluid did not diminish its antifungal activity – an important

result because corneal epithelial wound healing re-establishes a critical component of innate immunity for the eye. Also, B4010 had no hemolytic activity at antimicrobial concentrations, in contrast to natamycin. For the companion peer review paper, see [8]. For B2088, a related antibacterial peptide from this group, see [9].

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Magnetodynamic activation of ^{13}C -acyl isoniazid and ethionamide derivatives

Timmins G & Choi SW
STC.UNM, Albuquerque, NM, USA

• carbon isotopes • isoniazid • thioamides • tuberculosis

Isoniazid, the oldest drug to treat tuberculosis, is a prodrug that is activated by the catalase peroxidase of the etiologic agent, *Mycobacterium tuberculosis* to produce isonicotinoyl radicals; these form covalent adducts with nicotine adenine dinucleotide that are inhibitors of mycobacterial enoyl reductase (InhA) and mycolate synthesis [10]. The inventors have found that the isonicotinoyl intermediates also decompose to release carbon monoxide, which makes them unable to form adducts while the liberated carbon monoxide induces dormancy in mycobacteria [11], which supports persistent infection. Hypothesizing that decarbonylation could be reduced by a magnetic isotope effect, they substituted the isoniazid acyl carbon with the ^{13}C isotope, which increased its antimicrobial activity. ‘Heavy’ isoniazide was significantly more active than the normal molecule in 28-day monotherapy of mice that were aerosol-infected with *M. tuberculosis* strain H37Rv. Because ^{13}C exhibits paramagnetism, efficacy is further increased when the culture (and presumably, the patient) is placed in a static magnetic field. When H37Rv was treated with heavy isoniazid at 0.05 $\mu\text{g}/\text{ml}$ under various magnetic fields for 5 days, enhancement was optimal at field strengths around 14 mT, and tapered out at above 80 mT. The structurally related thioamide antimycobacterial drugs ethionamide and prothionamide are activated by a flavin-dependent monooxygenase, but their activation and adduct formation chemistry is very similar to that of isoniazid [12]; replacing their thioamide carbon with ^{13}C has a similar enhancing effect.

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WO/2014/043223**iRhom2 inhibition for the treatment of complement-mediated disorders**

Issuree P, Maretzky T, McIlwail DR *et al.*
Hospital for Special Surgery, New York, NY,
USA; University Health Network, Toronto,
Ontario, Canada

• ADAM proteins • arthritis • human • RHBDF2 protein • rheumatoid

Tumor necrosis factor α (TNF α), the pro-inflammatory cytokine which plays a central role in rheumatoid arthritis, is synthesized as a membrane-anchored precursor and is released from cells by the TNF α convertase (TACE; also known as A Disintegrin And Metalloproteinase-17, ADAM17) [13]. TACE inhibitors are effective in preventing inflammatory arthritis in animal models, but so far all drug candidates (most prominently, Bristol Myers Squibb's BMS-561392) have failed in clinical trials because TACE blockade upregulates other cytokines, and their metalloproteinase inhibition causes pronounced side effects [14,15]. TACE also protects the skin and intestinal barrier by activating epidermal growth factor receptor signaling. The present invention shows that an alternative to direct TACE inhibition is to target its complexation with its maturation factor and positive regulator, inactive rhomboid protein 2 (iRhom2) [16]. Inactivation of the encoding gene, *Rhbdf2*, selectively prevents TACE maturation in myeloid immune cells after stimulation by complement anaphylatoxin C5a or immune complexes, without affecting its homeostatic functions in other tissues. Since TACE in myeloid cells is crucial for the initiation and progression of arthritis, and since iRhom2 knockout mice lack mature TACE in immune cells (but not in other tissues), and are healthy with no spontaneous pathological phenotypes, targeting iRhom2 may offer a unique opportunity for cell type-specific blockade of TNF α shedding. Antibody fragments or polypeptide decoys that correspond to the key interaction regions between iRhom2 and TACE on their respective extracellular loops are the preferred agents. For the companion peer review paper, see [17].

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WO/2014/045126**Treatment of pain by inhibition of USP5 de-ubiquitinase**

Garcia-Caballero A, Gadotti V, Weiss N
& Zamponi GW

UTI Limited Partnership, Calgary, Alberta, Canada

• calcium channels • chronic pain • T-type • ubiquitin isopeptidase
• ubiquitin-protein ligases

A substantial body of evidence exists in the literature that describes ubiquitination of neuronal ion channel component proteins by E3 ubiquitin ligases and the relation of this modification to sensory perception and pain, but T-type calcium channels are rarely mentioned in this context. The inventors have investigated the Cav3.2 T-channel, which contributes to the development of hyperalgesia and allodynia by enhancing excitability of nociceptors, especially in painful diabetic neuropathy [18], from this perspective. They have found that Cav3.2 is regulated by ubiquitin-specific cysteine protease 5 (USP5, also known as isopeptidase T) [19] that binds to and regulates low-voltage gated calcium channel activity, in an interplay of the WW domain-containing E3 ubiquitin protein ligase 1 (WWP1) [20] and USP5. This occurs at the intracellular domain III–IV linker and fine tunes the stability of T-type channel proteins in the plasma membrane. Mice that were treated via intrathecal injection of USP5 short hairpin RNA (12.5 μ g) 1 day prior to formalin pain testing had a reduced nocifensive response time by $47 \pm 7\%$ in the initial phase, and $57 \pm 5\%$ in the second (inflammatory) phase. In the sciatic nerve constriction model of neuropathic pain, the same dose prevented development of mechanical and thermal hyperalgesia for several days. Similar observations were made when USP5 was uncoupled from its binding site on the channel via delivery of Tat-3.2-III-IV linker peptides. Importantly USP5-mediated regulation of T-type channels occurs only during chronic pain states but not under basal conditions. Neither USP5 nor WWP1 have so far been described in the context of pain.

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WO/2014/046125**Prevention/treatment agent for pulmonary hypertension comprising thrombin receptor antagonist as active component**

Hirano K, Abe K & Hirano M
Eisai R&D Management Co., Ltd, Tokyo,
Japan; Kyushu University, National University
Cooperation, Fukuoka, Japan

• hypertension • PAR-1 • pulmonary • receptor • thrombin

The platelet protease-activated receptor-1 (PAR-1), a receptor for thrombin, has a key role in mediating

coagulation at low concentrations of thrombin [21]. Its activation triggers thromboxane A2 production, release of adenosine diphosphate and serotonin, activation of P-selectin, and finally platelet activation. Its involvement in the pathology of the thrombotic form of pulmonary hypertension, where a localized hypercoagulant state exists and thrombi block the lumen of the pulmonary artery, diminishing lung perfusion and increasing pressure load on the right heart ventricle, is also known [22]. The inventors have used atopaxar (E5555; an orally active PAR-1 inhibitor developed by Eisai to treat myocardial infarction) at 30 mg/kg/day subcutaneously to demonstrate that PAR-1 inhibition is also effective in the rat model of monocrotaline-induced pulmonary hypertension, which does not involve thrombosis at all. No rationale is presented; but studying the literature yields clues concerning the potential mechanism. Thrombin-induced signaling occurs in the endothelium and, although incompletely understood, definitely involves PAR-1 which is expressed in human pulmonary arteries, where it has a biphasic effect [23]. More recently it has been shown that thrombin and Forkhead box-O (FoxO) factors functionally interact through PAR-1 activated PI3K/Akt-dependent FoxO phosphorylation, leading to expression of cell cycle regulating genes and ultimately proliferation of vascular smooth muscle cells [24].

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WO/2014/052611

Generation of new pancreatic β -cells

Levetan C

Individual inventor, affiliated with the Division of Endocrinology, Chestnut Hill Hospital, Philadelphia, PA, USA

• diabetes mellitus • human • insulin-secreting cells • REG1A protein

A better approach to the treatment of diabetes mellitus than to increase insulin sensitivity or to stimulate the remaining β -cells would be to generate them *de novo* – especially in Type 1 diabetes. This has not yet been achieved in clinical practice even when the immune attack is blocked. Regenerating (Reg) proteins are anti-inflammatory, antiapoptotic and mitogenic agents in multiple physiologic and disease contexts, including diabetes [25]. Islets of Goto–Kakizaki rats, a model of spontaneous Type 2 diabetes, overexpress Reg1, 3 α , 3 β and 3 γ [26]. These C-type lectin-like proteins are secreted by the exocrine pancreas, which has been associated with β -cell regenera-

tion in rodents. Intermittent hypoxia upregulates the respective genes, and causes β -cell proliferation [27]. The pertinent invention identifies or confirms peptide sequences – not previously described for their utility in β -cell generation – that are highly conserved and 100% identical within the human Reg1 α , Reg1 β , Reg3 α and Reg 4 proteins and shows their direct binding to a specific 20-amino acid site within the 919-amino acid Reg receptor on pancreatic extra-islet ductal tissue. This results in the translocation of the Reg receptor to the nucleus of extra-islet exocrine tissue inclusive of ducts, acinar cells and progenitor cells contained within these cells, triggering β -cell growth, acceleration and turnover. Regenerative bioactive peptide sequences that are referred to as ‘Perle Peptides’ are claimed, including the peptide NVWIGLHDP within human Reg1. Also see the concurrently published application WO/2014/048788 assigned to INSERM *et al.* claiming methods for inducing the conversion of pancreatic alpha cells to β -cells.

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WO/2014/055624

Methods relating to DNA-sensing pathway related conditions

Lee MN & Hacohen N

The General Hospital Corp., d/b/a Massachusetts General Hospital, Boston, MA, USA

• ATP-binding cassette transporters • autoimmune diseases • HSP90 heat-shock proteins • retroviridae infections • three prime repair exonuclease 1

The innate immune system detects viral infection primarily by recognizing viral DNA inside an infected cell. In addition to the membrane-bound type DNA-sensing Toll-like receptor TLR9, there are cytosolic DNA receptors that can also evoke these responses [28]. If the endoplasmic reticulum-associated three prime repair exonuclease-1 (TREX1) is present, it can degrade DNA that is reverse-transcribed from retroviral RNA genomes before the DNA sensing reaction is triggered [29]. Conversely, deficiency in TREX1 leads to the accumulation of endogenous retroelements and genomic DNA in the cytoplasm, causing aberrant over-activation of the DNA-sensing pathway and initiation of rare autoimmune diseases such as Aicardi–Goutieres syndrome, familial chilblain lupus [30] and retinal vasculopathy with cerebral leukodystrophy. Using quantitative proteomics, loss-of-function screening of candidates and network analysis, the

inventors have identified the ATP-binding cassette sub-family F member 1 (ABCF1; a translational regulator of the inflammatory cytokine pathway), the molecular chaperone heat-shock protein 90 (Hsp90) and its essential co-chaperone Cdc37 as previously unknown components of the DNA-sensing pathway that interact with TREX1. Known inhibitors for these proteins can be used to upregulate or downregulate DNA-sensing. Because Cdc37 is believed to interact with TANK-binding kinase 1 (TBK1; a non-canonical I κ B protein kinase that phosphorylates transcription factors controlling interferon production), TBK1 inhibitors are also claimed.

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Novel approaches for individualized therapy of pancreatic ductal adenocarcinoma

Eisen CT, Trumpp A & Sprick MR
HI-STEM gGmbH, Heidelberg, Germany

• hepatocyte nuclear factors • pancreatic neoplasms • protein kinase inhibitors • src-family kinases

Adenocarcinoma of the pancreatic duct – the most frequent pancreatic cancer and the fourth cause of cancer death in the United States and Europe – is still categorized and treated as a single cancer entity, although at least two additional subforms (quasi-mesenchymal and exocrine-like) seem to exist besides the classical one; these three forms have distinct transcriptional profiles [31], suggesting the possibility of tailored therapy. Cell cultures, which allow the preparation of pure tumor RNA, do not represent the exocrine-like subtype. The inventors have established *in vitro* and xenograft models that faithfully represent the human disease and allow for molecular classification and biomarker characterization of the classical subtype, and a certain part of the quasi-mesenchymal tumor subtype. Keratin 81 and vimentin are markers for ductal carcinoma cells of the quasi-mesenchymal subtype; the hepatocyte nuclear factors HNF-1A, HNF-1 B, FOXA2 (HNF3B), FOXA3 (HNF3G), HNF4G and Onecut1 (HNF6) are markers for cells of the exocrine-like subtype, whereas tumors of the classical subtype are characterized by the absence of keratin 81 and/or vimentin, and HNF-1 A and/or HNF-1 B. This allowed to establish gene expression signatures predicting sensitivity to Src kinase inhibitors (Bristol-Myers Squibb's dasatinib, Pfizer's bosutinib and AstraZeneca's saracatinib) by means

of gene-set enrichment analysis [32]. The differential response of tumors to dasatinib was further explored in a subtype-specific mouse model. The exocrine-like tumor subtype is resistant to Src inhibitors, as are some of the quasi-mesenchymal cases, while all classical and some quasi-mesenchymal tumors are sensitive to Src inhibitors. When dasatinib was used in combination with gemcitabine, a decrease in tumor size was observed only in the tumors of the classical subtype while the combination had no greater effect on the exocrine-like subtype than gemcitabine alone, or even showed an increased tumor growth.

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WO/2014/057455

Influenza virus type 1 & Type 1 diabetes

Piemonti L & Capua I
Ospedale San Raffaele s.r.l., Milano, Italy;
Istituto Zooprofilattico Sperimentale delle
Venezie, Padova, Italy

• diabetes mellitus • influenza A virus • pancreatitis • Type 1

That transgenic expression of influenza virus hemagglutinin in pancreatic β -cells triggers autoimmune diabetes has been reported as early as 1990 [33], and the suspicion that viral infection might play a role in Type 1 diabetes has been documented in the scientific literature 40 years ago [34]. Epidemiological evidence has mounted since the H1N1 influenza epidemic [35]. The inventors claim priority for identifying a causal link between influenza A virus infection and Type 1 diabetes, as well as pancreatitis. Turkeys challenged with H7N1 or H7N3 tended to show an increase in plasma lipase levels (hazard ratio: 2.51; 95% CI: 0.92–96.81; $p = 0.07$), which normalized within a few days. Hyperglycemia developed in 25% of the animals, but only in those whose lipase levels have strongly increased between days 3 and 9 postinfection; the median time between onset of hyperlipasemia and hyperglycemia was 4.5 days. Histopathology revealed acute pancreatitis with necrotic acinar cells and massive inflammatory infiltration at days 4–8 postinfection, which was gradually replaced by lymphocytic infiltration and mild fibroplasia. Immunohistological staining showed a specific reaction to influenza virus nucleoprotein antibody in degenerating and necrotic acinar cells. The inventors also demonstrated growth of H1N1 and H3N2 influenza viruses in human pancreatic islet cells, with enhanced production of inflammatory cytokines and

chemokines. The claims cover only influenza vaccination and diagnosis of influenza infection; they do not extend to agents that could break the link between influenza and Type 1 diabetes or pancreatitis. Nevertheless, current concepts of influenza sequels would need to be broadened if these findings are independently replicated. For the peer review companion paper, see [36].

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Conjugated knottin mini-proteins containing non-natural amino acids

Cochran JR, Kim JW & Cochran FV
The Board of Trustees of the Leland Stanford Junior University, Palo Alto, CA, USA

• cystine-knot miniproteins • integrins

Cystine-knot ('knottin') peptides are characterized by three intramolecular disulfide bonds that often produce a triple-stranded β -sheet scaffold combining high chemical and biological stability with substantial potency and selectivity. They occur naturally, especially as toxins, but rational protein design and directed evolution technologies have meanwhile produced many engineered variants [37]. In the case of the present invention, site-specific oxime conjugation chemistry, in particular incorporating a non-natural amino acid containing an aminooxy side chain to which a linker can be attached, has been employed to produce knottin peptides with loops that have high binding affinity for $\alpha v \beta 3$, $\alpha v \beta 5$ and $\alpha 5 \beta 1$ integrins. The *Ecballium elaterium* trypsin inhibitor (EETI) and the Agouti-related protein (AgRP) were used as scaffolds that were site-selectively dimerized with a polyether linker. The EETI 2.5F dimer was more effective by two orders of magnitude (IC_{50} , 0.036 vs 1.57 nM) in preventing adhesion of U87MG human glioblastoma cells [38] compared with the monomer, and could be conjugated with doxorubicin. Growth inhibition assays with MDA231 cells showed that the location of the aminooxy group within the knottin mattered, but the crosslinker length did not. There are numerous conceivable applications in all diseases that involve cell invasion, but also in tissue regeneration and interactions of the body with implants. For earlier work from this group, see [39].

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Cancer cell trap

Tang L & Nevriy DJ

Board of Regents, The University of Texas System, Austin, TX, USA

• chemokines • hydrogels • neoplasm metastasis

This invention elaborates on an unconventional approach to the prevention of metastasis that has been brought up only recently – attracting floating or migrating cancer cells into a scaffold that immobilizes, isolates and optionally kills them. In the present case, the 'cancer trap' scaffold (pre-formed as microparticles or semi-solidified *in situ* as a self-forming gel implant) releases small amounts of interleukines, chemokines, growth factors, histamine, or erythropoietin [40] – all of which can recruit migratory cancer cells. In some embodiments, the inflammatory response caused by the injection of the scaffold into subcutaneous tissue is sufficiently intense to serve as attractor. Real-time *in vivo* imaging in a mouse model showed intraperitoneally transplanted B16F10 melanoma cells (near-infrared dye labeled, or transduced by Ad5 virus expressing green fluorescent protein) migrating toward polylactic acid microbead implants. The CXCR4/CXCL12 chemokine pathway [41], but not the CCR7/CCL21 pathway, was shown to be essential to cell immigration into the subcutaneous implantation site, which is the exact opposite of the situation defining melanoma migration through the lymphatic system. C57 mice were transplanted with fluorescein isothiocyanate-labeled melanoma or LLC leukemia cells via intravenous injection, and thermogelling dispersions of polymer nanoparticles loaded with erythropoietin (600 IU/ml) in the presence or absence of doxorubicin (300 μ g/ml) and paclitaxel (30 mg/ml) showed that such cancer cell traps can eradicate circulating cancer cells at the implant sites (>95% elimination in 7 days). Gel microspheres have been described in the literature in the context of local delivery of chemotherapy drugs [42], but not as semi-active cell traps.

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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.

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