Patent highlights August–September 2020

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

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WO/2020/157334**Methods of treating castrate-resistant prostate cancer***Inventors: Alajati A, d'Ambrosio M, Alimonti A**Assignee: Fondazione per l'Istituto Oncologica di Ricerca (IOR), Bellinzona (Italy)*

In the majority of metastatic prostate cancers the Pi3k signaling pathway is activated through loss or mutations of *PTEN*. The inventors have found that Cdcpl, a Cub domain-containing protein that acts as a substrate for Src family tyrosine kinases [1], is upregulated during therapy with androgen receptor antagonists. In transgenic mouse models, conditional overexpression of Cdcpl promotes prostate tumorigenesis by synergizing with *PTEN*-loss and overcoming *PTEN*-loss induced cellular senescence, thereby driving full malignancy and eventually contributing to castration resistance and metastasis through the Src/MAPK pathway. In patients under androgen deprivation therapy Cdcpl levels were significantly increased as their cancers progressed. That the expression level and extent of N-glycosylation of Cdcpl correlates with metastatic status of prostate cancer has been reported years ago [2]; monoclonal antibodies and small molecules that bind extracellular epitopes of Cdcpl and inhibit phosphorylation of Cdcpl by Src kinase, and/or the interaction between the C2 domain of PKC δ and phosphorylated Cdcpl, are already known (see He *et al.* [3] and, for example, the heavy chain sequences disclosed in WO-2015082446 and WO-2011023389). These agents could provide a new means to counteract development of castration resistance. For the peer review companion paper see Alajati *et al.* [4].

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MeSH keywords: CDCP1 protein, human • prostatic neoplasms • castration-resistant • src-family kinases

WO/2020/160333**Enpp1 inhibitors & methods of modulating immune response***Inventors: Li L, Smith M, Carozza JA, Boehnert V**Assignee: The Board of Trustees of the Leland Stanford Junior University, CA, USA*

Cyclic guanosine monophosphate-adenosine monophosphate (cGAMP) acts as a second messenger that activates the STING-dependent type I interferon response in the presence of cytoplasmic DNA, a situation occurring during infections, cancer or autoimmune disorders [5]. Inhibiting Enpp1, a type II transmembrane glycoprotein which hydrolytically degrades cGAMP (along with several other substrates) could be beneficial under these conditions: Enpp1 expression levels correlate with drug resistance in human breast cancers [6], bone metastases [7] and poor prognosis [8]. The inventors have designed such inhibitors and importantly, they have also shown that cancer cell lines continuously export cGAMP; even Enpp1 inhibitors that do not penetrate cancer cells can therefore exert benefits (in synergy with ionizing radiation and anti-CTLA-4 treatment) by preventing the abrogation of cGAMP signaling. Therefore, Enpp1 serves as an innate immune checkpoint. The preferred compound is dimethyl (2-(1-(6,7-dimethoxyquinazolin-4-yl)piperidin-4-yl)ethyl)phosphonic acid (STF-1084). Also see the inventors' inhibitors disclosed in WO/2019/051269 and the quinoline and quinazoline inhibitors claimed by Stingray Therapeutics in WO/2020/190912. For the peer review companion paper see Carozza *et al.* [9].

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MeSH keywords: cyclic guanosine monophosphate-adenosine monophosphate • ectonucleotide pyrophosphatase phosphodiesterase 1 • neoplasms • STING protein • human

WO/2020/163778

Tyk2 pseudokinase ligands

Inventors: Mohan R, Nuss J, Harris J, Yuan S

Assignee: Ventyx Biosciences, Inc., CA, USA

The Janus kinase family consists of the three Jak kinases and the tyrosine kinase Tyr2. In each of these enzymes the catalytic domain (JH1) is preceded by a pseudokinase domain (JH2) to which most disease-associated Jak mutations map. The Tyk2 JH2 lacks phosphotransferase activity, but nevertheless binds ATP, thereby stabilizing the inactivated state of the kinase without inducing major conformational changes [10,11]. Small-molecule ligands such as BMS-986165 can stabilize this auto-inhibitory conformation, thereby preventing kinase function in an allosteric manner, and provide strong protection against common auto-immune diseases without induction of immunodeficiency [12]. Many of the inventors' pyrazolopyrimidines have IC₅₀ values in the mid-nanomolar range, and potentially provide useful additions to the chemical space covering this type of activity.

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MeSH keywords: autoimmunity • TYK2 kinase

WO/2020/165907

Spt5 inhibitors & uses thereof

Inventors: Dikstein R, Bahat A

Assignee: Yeda Research & Development Co. Ltd, Rehovot, Israel

Spt5 promotes proximal promoter pausing, promoter escape, elongation and miRNA processing. Through complex interactions with other protein factors it can function as a positive or a negative transcription elongation factor [13,14]. It plays specific roles in transcription of genes involved in inflammation and stress response, amyotrophic lateral sclerosis, frontotemporal dementia, and also in tri-nucleotide expansion diseases such as Huntington's disease. To date, no Spt5 inhibitor has been identified, a situation which the inventors attempted to remedy using a high-throughput split-Renilla luciferase complementation assay to detect Spt5 interaction with Pol II. About 100,000 pure natural plant compounds from the MEGxp library were screened. Two simple compounds, SPI-21 and SPI-18, were further validated for their direct interaction with either Spt5 or the Rpb1 CC domain of Pol II. SPI-21 was found to primarily bind the Rpb1 CC domain in a dose-dependent manner with an IC₅₀ of 23 μM while SPI-18 binds to both proteins (IC₅₀ 40 μM for Spt5 and 76 μM for Pol II). Neither compound disrupts Spt5-Pol II binding but both seem to induce conformational changes that diminish function of the complex, as a multitude of *in vitro* and *in vivo* experiments revealed. Most notable, both compounds inhibit transcription of mutant Huntingtin's genes. For the peer review companion paper see Bahat *et al.* [15].

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MeSH keywords: RNA polymerase II • SPT5 transcriptional elongation factor • trinucleotide repeat expansion

WO/2020/168241

X-reactivation modulators & use thereof

Inventors: Stebbins NW, Portney BA, Valeur EB

Assignee: Flagship Pioneering Inc., MA, USA

The X-inactive-specific transcript (Xist noncoding RNA) silences functional genes on the X-chromosome to maintain homeostasis. This is initiated early in the development (and maintained throughout the lifetime of a cell in a heterozygous female) by recruiting the Xist silencing complex, which comprises a multitude of biomolecules [16]. However, Xist has also been implicated in epigenetic irregular X-chromosome silencing that leads to the various X-linked genetic disorders. Reactivation of the silenced genes, even to a small degree, can be beneficial to treat X-linked diseases with a defective allele. This invention provides a method for treating an X-linked genetic disorder (for example, Rett syndrome, adrenoleukodystrophy or spinal muscular atrophy) with modulators of Hdac3, Dnmt1, Pi3k or a combination thereof, with the intent to increase the expression of the affected Xist-inactivated gene. Many experimental details are presented for this extremely interesting approach, including a model system of Rett syndrome neurons generated from the T158M-iPSC cell line that harbors a heterozygous T158M missense

mutation in MECP2; but no results are disclosed. For recent related research in Rett syndrome see Przanowski *et al.* [17].

Published: 20 August 2020

MeSH keywords: adrenoleukodystrophy • mental retardation • X-linked • Rett syndrome • XIST noncoding RNA

WO/2020/169593

Antibacterials based on monocyclic fragments coupled to aminopiperidine naphthyridine scaffold

Inventors: Kolaric A, Minovski N, Anderluh M, Hrast M

Assignee: Univerza v Ljubljani, Ljubljana, Slovenia

The aminopiperidine naphthyridine scaffold, substituted with bicyclic moieties, has been used in antibacterials for some time, and is still considered promising in the development of the ‘novel bacterial topoisomerase inhibitor’ class of antibiotics [18,19]. By replacing the bicyclic moiety of the existing antibacterials with monocyclic entities, the inventors developed compounds with antibacterial activity and potency that outperform known antibacterial agents from the DNA gyrase and topoisomerase inhibitor class. In terms of minimal inhibitory concentration determined after 20 h of incubation, N-(4-bromobenzyl)-1-(2-[6-methoxy-1,5-naphthyridin-4-yl]ethyl)piperidin-4-amine was most effective against methicillin-resistant *Staphylococcus* species and strains, with minimally inhibitory concentrations in the mid-ng/ml range and little cytotoxicity toward HUVEC and HepG2 cell lines. Its IC₅₀ toward DNA gyrase from *S. aureus* and *E. coli* was 7 and 570 nM, respectively.

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MeSH keywords: antibacterial agents • naphthyridines • piperidines • topoisomerase inhibitors

WO/2020/172635

Optimized vaccine compositions & methods for making same

Inventors: Glanville J, Ives S, Izahy M, Youssef S, Bürkert J-P

Assignee: Distributed Bio, Inc., CA, USA

In designing vaccines against pathogens with rapid antigenic drift, one strategy is to shift the abundance distribution of antigenic epitopes to favor responses to antigens that are more conserved across various species or clades of microbes. This approach should be particularly useful with influenza virus [20] or trypanosoma vaccines. Disclosed are vaccines comprising a set of microbial antigens wherein the smallest pairwise edit distance between two of the antigens is at least 10% of the average size of the antigens and the largest pairwise edit distance is no more than 98% of the average size of the antigens; this makes the set representative of at least 60% of fifth order clades of a microbe. On this rationale the inventors developed vaccines that contain at least six (but up to 30) antigens, each in a low amount (for example, 50 ng) per dose. While this allows for a relatively low amount of total antigen content and produces a broad immunogenic response, the response elicited against shared sites on the antigens will effectively be up to 30-fold higher. Pigs were inoculated up to three-times with several influenza vaccine compositions that were prepared along these ‘swarm effect’ lines. Neutralization titers proved that such a low-dose ensemble can elicit a potentially protective immune response even though individual components cannot, and that a conservation-concentration coupling approach can focus the immune response toward highly conserved epitopes on the hemagglutinin coat protein. This is the scientific backing for Distributed Bio’s Centivax™ technology which aims at providing coverage for future strains and clades of influenza [21].

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MeSH keywords: antigens • influenza vaccines

WO/2020/176863

Thiazole derivatives as protein secretion inhibitors

Inventors: McMinn D, Rao M

Assignee: Kezar Life Sciences, CA, USA

Protein secretion is a basic process which can theoretically be interfered with on several levels to treat inflammation and cancer. Protein import across the endoplasmic reticulum membrane is the first step in this process. Many inhibitors of varying specificity are known, although none have been developed as drugs. They interact with the signal peptide, chaperones, or the Sec61 channel (a part of the translocon complex) [22,23] to block substrate binding

or to prevent the required conformational changes. All known Sec61 inhibitors lack selectivity and/or are chemically highly complex (for example, mycolactone). The inventors attempt to change both drawbacks with a broad range of amidothiazoles. Complex doxycycline-induced cell culture assays were used to test efficacy, mostly yielding IC₅₀ values in the high-nanomolar to low-micromolar range. Animal data cannot be expected at this early stage but would most likely be from cancer models: the protein transport mechanism is too ubiquitous, and too essential, to block in any condition that does not involve very rapidly growing cells, or perhaps cytokine storms as in septic shock [24] and influenza or COVID-19. However, it has to be noted that Sec61 blockade is immunosuppressive [25].

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MeSH Keywords: Endoplasmic reticulum stress • neoplasms • SEC translocation channels

WO/2020/180356

Istaroxime-containing intravenous formulation for the treatment of acute heart failure

Inventors: Bianchi G, Ferrari P, Ferrandi M, Barrassi P

Assignee: Windtree Therapeutics, Inc., FL, USA

Istaroxime, an inotropic androstane 6,17-dione inhibitor of the Na⁺/K⁺ pump and an activator of the sarcoplasmic reticulum ATPase Serca2a (by inhibiting its inhibitor phospholamban), is under clinical development for the treatment of acute heart failure syndromes [26,27]. This dual activity inhibits calcium extrusion from myocardial cells while also improving the efficacy by which calcium release from the sarcoplasmic reticulum is triggered. Serca2a inhibition should be particularly useful for improving diastolic function in heart failure with (relatively) preserved ejection fraction, a condition for which no approved therapy exists [28]. In the HORIZON study, where istaroxime had been infused for 6 h, there was no clear separation between the echocardiographic indexes of relaxation and those of contraction when increasing the doses of istaroxime; therefore, the potential beneficial effect due to the Serca2a activation could not be separated from the potential detrimental effect due to the Na⁺/K⁺ pump inhibition. The present document provides an analysis of four independent sets of data confirming that istaroxime infusion for more than 6 h provides a prevailing lusitropic effect (i.e., improvement of myocardial relaxation) at constant istaroxime plasma levels. These claims are remarkable because HORIZON results have been published in 2009, including the lusitropic effect [29].

Published: 10 September 2020

MeSH keywords: ATP2A2 protein • human • cardiotonic agents • heart failure • diastolic • istaroxime

WO/2020/181035

Muscarinic agonists as nonsteroidal & nonopioid analgesics & methods of use thereof

Inventors: Dobbelaere A, Vieau S, Nelson G, Barrison F

Assignee: Barry University, FL, USA

Although the antinociceptive and analgesic effects of muscarinic receptor ligands in human and nonhuman species have been evident for more than half a century, the specific targeting of M₂ and M₄ muscarinic receptors with selective agonists to alleviate pain is a relatively novel approach, and a very promising one: analgesia induced by such cholinergic agonists can be comparable with that observed with morphine [30,31]. Similar to the analgesia mediated by opioid receptors, activation of M₂ receptors leads to activation of G_{i/o} G-proteins and a consequent decrease in cAMP, which causes attenuation in the activity of tetrodotoxin-resistant voltage-gated sodium channels. The novel muscarinic agonists described herein are the first to activate only the α₁ subunit of G proteins and hence, are strongly biased at a specific signaling pathway. A 1-(thiophen-2-ylmethyl)-1,2,3,6-tetrahydropyridin-1-ium core structure was substituted at the nitrogen and at positions 5 and 4 of the thiophene ring. To exclude the possibility that activation of the nonpreferential G_s pathway was obscured by activation of the preferential G_{i/o} pathway, accumulation of cAMP was measured in CHO cells expressing M₂ or M₄ receptors while G_{i/o} G-proteins were inactivated by pertussis toxin. 1-([Thiophen-2-yl]methyl)-1,2,3,6-tetrahydropyridin-1-ium chloride and 1-methyl-1-([thiophen-2-yl]methyl)-1,2,3,6-tetrahydropyridin-1-ium iodide were two compounds that did not activate G_s nor G_q signaling pathways at all. In dissipated native rat tissues they only inhibited cAMP synthesis, in some cases with efficacies approaching that of the full agonist carbachol.

Published: 10 September 2020

MeSH keywords: analgesia • GTP-binding protein alpha subunits, Gs • muscarinic agonists

WO/2020/185675**Compositions & methods for modulating viral infections by regulating glucosylceramides***Inventors: Kester M, Drews KC**Assignee: University of Virginia Patent Foundation, VA, USA*

The membrane of influenza virus contains sphingolipids, a class of signaling molecules that has been shown to play diverse roles in virus-host interactions [32]. At the hub of sphingolipid metabolism is ceramide, which is converted to glucosylceramide by glucosylceramide synthase. The glucose group can be catabolically removed by glucosylceramidase, a lysosomal enzyme well known for its mutations that cause Gaucher disease but not yet investigated in the context of the initiation of viral infection (only for its maintenance [33]). The inventors used CRISPR/Cas9 to knock out glucosylceramidase in HEK 293 human embryonic kidney and A549 epithelial lung carcinoma cell lines, and demonstrated reduced influenza A virus trafficking to late endosomes and a consequent reduction of viral fusion, entry and infection. This suggests that this enzyme, and by extension an optimal level of its substrate glucosylceramide, is critical for maintaining the influenza virus life cycle in host cells. Also provided is evidence that glucosylceramidase is required for the entry of other viruses that enter cells by endocytosis as well as for the proper trafficking and disposition of normal cellular vesicular cargos destined for late endosomes including EGF and its receptor. Examples of glucosylceramidase inhibitors include conduritol b epoxide (a mixture of 1,2-anhydro-myo-inositol isomers), D-threo-1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol (also referred to as P4; see US Patent no 8,168,587), castanospermine, isofagomine and validamine. Glucosylceramide synthase inhibitors (e.g., miglustat) can also be used. That an intact sphingomyelin biosynthetic pathway is essential for intracellular transport of influenza virus glycoproteins is well known [34] but the pivotal findings in this patent application might still be considered novel.

Published: 17 September 2020

MeSH keywords: castanospermine • glucosylceramidase • glucosylceramides • miglustat • sphingolipids

WO/2020/190935**Benzodiazepine derivatives as RSV inhibitors***Inventors: Zhu K, McGrath K, Negretti-Emmanuelli S, Szymaniak A, Yu J, Kim I-J et al.**Assignee: Enanta Pharmaceuticals, Inc., NY, USA*

Respiratory syncytial virus (RSV) is the leading cause of acute lower respiratory tract infections, especially in infants [35], and in persons who are immunosuppressed or suffer from cardiorespiratory impairments. Infection induces incomplete immune protection, no approved vaccine exists, and although many inhibitors (mostly targeting the viral N and L proteins) have been reported in patents and in the literature, the monoclonal antibody palivizumab and ribavirin are the only options for prevention and treatment [36]. The inventors' RSV inhibitors are aminoheteroaryl-substituted benzodiazepines carrying 1,3,4-oxadiazole and pyrimidine ring systems, with high-nanomolar EC₅₀ values against replication and/or shedding of both RSV-A 'long' and RSV-B genotype B strain 9320 cultured in Hep-2 or A549 cell lines. The target of the molecules is not disclosed, but benzodiazepines have been common among many RSV inhibitors reported so far (e.g., RSV604) [37,38].

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MeSH keywords: respiratory syncytial virus infections

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