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Review

**Glycogen synthase kinase 3 (GSK-3) inhibitors:
a patent update (2014-2015)**

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Abstract

Introduction: Glycogen synthase kinase (GSK-3) is a serine/threonine kinase that phosphorylates more than one hundred different sequences within proteins in a variety of different pathways. It is a key component of a remarkably large number of cellular processes and diseases. Imbalance of GSK-3 activity is involved in various prevalent pathological diseases, such as diabetes, neurodegenerative diseases and cancer. Understanding its role in different disorders has been central in the last several decades and there has been a significantly large development of GSK-3 inhibitors, some of which, show promising results for the treatment of these devastating diseases.

Areas covered: This review covers patent literature on GSK-3 inhibitors and their applications published and/or granted between 2014 and 2015.

Expert opinion: GSK-3 inhibitors have gained a prominent role in regenerative medicine based in their ability to modulate stem cells. Moreover, some allosteric modulators of GSK-3 emerge as safe compounds for chronic treatments.

Keywords: Alzheimer's disease, cancer, GSK-3 inhibitors, stem cells, regenerative medicine

1. Introduction

Glycogen synthase kinase 3 (GSK-3) is a serine/threonine kinase identified more than thirty years ago as the last enzyme involved in glycogen synthesis.[1] Some efforts were done those years looking for GSK-3 inhibitors able to modulate insulin signaling with potential therapeutic effects in pathologies involving glucose deficiencies and/or resistance.[2],[3]

During the last decade of the past century, a small revolution took place:[4] while seeking potential kinases involved in posttranslational modifications of the microtubule associated protein “tau”, tau protein kinases (TPK) I and II were isolated.[5],[6] It was only after cloning these apparently new kinases that the well-known GSK-3 and cyclin dependent kinase 5 (CDK-5) as TPKI and II, respectively, were discovered in brain.[7],[8] Consequently, both kinases emerged as promising targets for drug discovery in the field of neurodegenerative diseases and particularly in Alzheimer’s disease (AD). The search for specific GSK-3 inhibitors was then a prominent area both in academic and industrial environments leading to a great number of diverse compounds covered by different reviews and patent applications.[9] [10, 11] Such effort increased significantly the knowledge about GSK-3 and its inhibitors.[12] [13]

Today it is known that GSK-3 is involved in a great number of cellular pathways, phosphorylating more than hundred different substrates.[14] Its activity is crucial for life and several homologues have been identified in different organisms such as fungi, microorganisms, etc.[15],[16] discovering a new way to combat severe infectious diseases such as malaria, [17] aspergillosis, [18] sleep disorder, [19] etc. by targeting the specific GSK-3 homologues. In mammals, two different isoforms have been found, GSK-3 α and β that share more than 95% homology in the catalytic site. A splice variant, GSK-3 β 2 is also found in brain.[20] Human GSK-3 is highly regulated by phosphorylation, protein-protein interactions and different inhibitors.[14] It is

constitutively active, and specific phosphorylation transforms it to either inactive (Ser9 for GSK-3 β and Ser21 for GSK-3 α) or super active (Tyr216 or Tyr279, respectively) forms. Many pathologies such as neurodegenerative diseases, chronic inflammatory processes, diabetes type 2, mood disorders and certain types of cancer are developed by over-activity of GSK-3, which makes it an excellent target for therapeutic intervention.[21],[22],[23],[24], [25] Regarding AD, GSK-3 malfunction constitutes a comprehensive etiology hypothesis,[26] where GSK-3 would be the link between the two main hallmarks in this pathology: beta-amyloid formation and tau protein aggregation.[27]

Only two small molecules targeting GSK-3 have reached clinical trials for AD, AZD1080 and tideglusib (Figure 1). The indolyl derivative AZD1080, a potent and selective GSK-3 inhibitor with a K_i of 6.9 nM, decreased tau phosphorylation in cell cultures and restored synaptic plasticity on rat brain. It was tested in healthy volunteers in ascending dose studies. Suppression of GSK-3 activity observed in peripheral blood lymphocytes after oral administration confirmed human target engagement.[28] Its development was stopped however due to a poor therapeutic window. The safety concerns could be related to its chemical structure, the potent inhibition of the enzyme and/or the ATP-competitiveness in its binding to GSK-3.

Tideglusib is a thiadiazolidindione derivative that targets GSK-3 in a non ATP-competitive binding mode.[29] After a safe phase I study in young and elderly healthy volunteers,[30] it reached clinical trials phase II for cognitive disorders.[31] It is well tolerated by patients in two six months studies, performed with elderly people suffering progressive supranuclear palsy (PSP) and mild to moderate AD. Data obtained from these trials showed that tideglusib is able to cross the blood-brain barrier showing signs of efficacy. In PSP patients, it decreased brain atrophy in a dose dependent manner[32]

while in AD patients, levels of BACE-1 and phospho-tau in cerebral spinal fluid were diminished in the tideglusib treated group.[33] Currently, tideglusib is being assayed in adolescents with autism spectrum disorders with the main goal to improve their social behavior, [34] based on previously reported solid preclinical evidence. [35]

More recently, a third GSK-3 inhibitor reached clinical trials for cancer treatment,[36] an area where there are more than twenty protein kinases inhibitors on the market.[37]

LY2090314 is a potent inhibitor of GSK-3 with IC_{50} values of 1.5 nM and 0.9 nM for GSK-3 α and GSK-3 β respectively, and good activity in several melanoma cell lines.[38] Clinical phase I study was performed in advanced solid tumors patients where LY2090314 was intravenously administered in combination with cis-platino.[36] Although safety issues were not the problem, recent data from clinical trials phase II has shown that LY2090314 as a single agent has limited clinical benefit in patients with acute leukemia.[39]

Collectively, data from clinical and preclinical research confirms that GSK-3 is a validated target for therapeutic intervention in many severe and unmet human diseases. GSK-3 inhibitors therefore may have an important role in future pharmacological therapy.[40] However, it is necessary to consider the potential risks associated to target GSK-3 in a chronic treatment. In fact, the primary concern nowadays is developing GSK-3 inhibitors that are disease-effective but do not promote the up-regulation of oncogenes. Reducing the activity of GSK-3 without altering its role in β -catenin signaling constitutes the main challenge to be conquered by a developing GSK-3 inhibitor. [41] Inhibitors with moderate efficacy but specifically designed to target GSK-3 in an ATP non-competitive manner will have the better chances to reach the market. [42] The present short review summarizes new advances about GSK-3 and its inhibitors described in the last two years (2014-2015) in patent literature mainly to try

to understand the evolving value of these compounds. We have identified sixty different families of patents that can be classified in: new therapeutic uses of GSK-3 inhibitors, combinations of known drugs with GSK-3 inhibitors for therapy, use of GSK-3 inhibitors in regenerative medicine and discovery of new GSK-3 inhibitors.

2 New therapeutic uses of GSK-3 inhibitors

GSK-3 is frequently emerging as a key player in different diseases as the knowledge of their pathomolecular basis advances, making it a valuable target for their treatment. Consequently, new applications are being patented for known GSK-3 modulators at the same time that the therapeutic claims for newly discovered inhibitors grow.

Valproic acid is an histone deacetylase inhibitor used as antiepileptic drug in the clinic.[43] When administered *in vivo* it contributes to the inhibitory control of GSK-3.[44] Thus, valproic acid is recognized as an indirect GSK-3 modulator[45] that antagonizes seizures[46] and probably was the basis for the anticonvulsant activity claimed for indirubins. Concretely, different derivatives of indirubin such as compound BIO, well known GSK-3 inhibitor,[47] have shown anti-convulsant activity in Zebrafish and mouse models.[48]

More interesting is the recent discovery of GSK-3 as an upstream signaling molecule that controls immunosuppressive receptors transcription, such as programmed death (PD) 1, and expression of the transcriptional regulator of Th1, named Tbet, in immune cells.[49] Thus, GSK-3 promotes distinct expression of signature cytokines that play an important role in balancing pro- and anti-inflammatory cytokines. These findings show that it is possible to reprogram disease-causing cells through GSK-3 inhibition which makes it a relevant pathway in the context of autoimmune diseases.[50] The use of GSK-3 inhibitors, mainly the maleimides SB415286 and/or SB216763 (Figure 2), to

promote immunity in subjects with chronic conditions where the inhibition of PD-1 expression and/or blockade of Tbet up-regulation is therapeutically desirable such as cancer and infectious conditions, has recently been claimed.[51]

Moreover, GSK-3 inhibitors may be used to enhance antigen-specific immunotherapy (antigen-SIT) or the induction of tolerance to an antigen. Autoimmune diseases result from the loss of tolerance to self-antigens and the adaptive immune system must maintain the capacity to respond to an enormous variety of infectious agents while avoiding autoimmune attack of self-antigens contained within its own tissues. Thus, GSK-3 inhibitors may act as a “tolerogenic adjuvant” used alongside antigen-SIT increasing its efficacy. Compositions comprising a GSK-3 inhibitor, such as CHIR99021 and a peptide, may be used for treatment and/or prevention of diseases associated with pro-inflammatory T cells such allergies, autoimmune diseases and graft rejection.[52] This peptide may be myelin basic protein (MBP) to treat multiple sclerosis, oxidized LDL or heat shock protein to treat atherosclerosis, for example.

In the area of ovarian cancer it has recently been discovered that GSK-3 modulates the activity of FOXL2 transcription factor, which is significantly involved in the pathology of ovarian cancer. [53] More concretely, S33 phosphorylation of FOXL2 is proportional to the growth of ovarian granulosa cell tumors *in vivo*, thus making GSK-3 β inhibition a key target for the treatment of the disease.[54] As a result, the known GSK3 inhibitors SB216763 and AR-A014418 have been claimed for the treatment of these tumors, since they have shown a reduction of tumor growth *in vivo*. [55]

3 Combinations of known drugs with GSK-3 inhibitors

Cancer is one of the main causes of mortality nowadays. Therapy that combines drugs with different mechanisms of action constitutes an approach with great potential for the treatment of the disease since it decreases the probability of developing drug-resistant cancer cells while taking advantage of the synergistic effects that may be achieved using drug combination. There are currently more than 25 oncology drugs that target kinases clinically approved,[56] and GSK-3 inhibitors have shown hopeful results as the Wnt- β -catenin-GSK-3 pathway is involved in the formation and maintenance of cancer stem cells (CSC) responsible for tumor growth in several types of human malignancies.[57]

Malignant gliomas are one of the deadliest types of cancer, and the most common neoplasms in the central nervous system.[58] For the treatment of this disease an effective combination therapy would include a GSK-3 inhibitor with an autophagy modulating agent. This combination has shown promising results in killing LN18 glioblastoma cells *in vitro*. The GSK-3 inhibitors are BIO-acetoxime, TDZD-8 and L803mts (Figure 3) and/or the autophagy modulators are chloroquine, 3-methyladenine and/or bafilomycin.[59]

Another combination therapy for cancer comprising a GSK-3 inhibitor relates to the combined use with an anti-DR5 antibody. This antibody is an attractive target for tumor therapy that targets the tumor necrosis factor-related apoptosis-inducing receptor, and showed potent antitumor effects *in vivo* with no apparent toxicity.[60] It has been previously reported that monoclonal antibodies may cause synergistic effects in cancer patients, concretely using TRA-8, a monoclonal antibody targeting death receptor (DR5) and tamoxifen.[61] In the patent claimed by Daiichi Sankyo the combination of anti-DR5 and the GSK-3 inhibitor LY209314 shows increased antitumor activity.[62]

4 Use of GSK-3 inhibitors in regenerative medicine

Human embryonic stem cells (hESCs) have unlimited self-renewal and potential to differentiate into any cell comprising the three germ layers of the developing embryo, making them an attractive cell source for regenerative medicine and a fascinating model for early human development. Recently, GSK-3 inhibitors have been reported as valuable pharmacological agents to increase self-renewal and/or differentiation of stem cells, emerging as new useful agents for regenerative medicine (Figure 4).[63] Moreover recent publications describe the prominent role of GSK-3 inhibition *in vivo*, by treatment with specific inhibitors or with compounds able to increase the inactive GSK-3 form, in the enhancement of endogenous neurogenesis.[64], [65]

Incubation of hESCs with chemically diverse GSK-3 inhibitors such as the macrocycle 1M or the indirubin derivative BIO, has been claimed as an optimized method to induce differentiation of human embryonic stem cells to hepatocyte-like cells.[66] This effect is also observed using human induced pluripotent stem cells (hiPSC). Mature hepatocyte-like cells were obtained after their culture in media supplemented with BIO.[67] Additionally, maturation of these hepatocyte-like cells is achieved after their exposure to an activator of a retinoic acid responsive receptor such as retinoic acid in combination with an inhibitor of GSK-3 such as kenpaullone.[68]

GSK-3 inhibitors have also shown promising results in the generation of cardiovascular cells, which constitutes an important challenge for cardiovascular research and therapy. Cardiomyocyte progenitors or cardiomyocytes can be obtained from human pluripotent cells by temporal modulation of Wnt signaling under defined, growth factor-free conditions. [69] At the initial steps of differentiation, GSK-3 inhibitors activate Wnt/ β -catenin signaling providing the first population of cells. Cardiogenesis is induced from these first cells by blocking Wnt pathway under fully defined, growth factor free culture

conditions. In this case, the small molecules CHIR99021 and BIO targeting GSK-3 have been used for these experiments. The method is recently granted to the Wisconsin Alumni Research Foundation.[70] Functional human cardiomyocytes differentiated via these protocols may constitute a potential cell supply for heart disease modeling, drug screening and cell-based therapeutic applications.[71]

Neuronal phenotype can also be produced starting from somatic non-neuronal cells. The chemical cocktail used in these studies included GSK-3 inhibitors.[72] Shanghai Institute for Biological Sciences claimed for a composition comprising the GSK-3 inhibitor CHIR99021.[73] Further transplantation of induced somatic non-neuronal cells into the central nervous system may ameliorate the neurological condition of the patient. GSK-3 inhibitors are also able to induce the transition from astrocytes to neuronal cells that develop into mature functional neurons *in vivo*, which is interesting regarding this newly patented method.[74] Interestingly, these type of chemical cocktails have also been used to convert human fibroblasts into neuronal cells by passing through a neural progenitor stage. In that case, the human chemical-induced neuronal cells (hciNs) produced are similar in morphology, gene expression profiles, and electrophysiological properties to hiPSC-derived neurons providing a new strategy for modeling neurological diseases such AD using fibroblasts isolated directly from patients.[75]

5 Discovery of new GSK-3 inhibitors

As mentioned in the introduction, human GSK-3 is constitutively active, ubiquitous and essential for life. Thus, our organism has compensative mechanisms of action to counteract deficits in the expression and/or activity of this kinase.[76] However, in different diseases such as AD or diabetes, a tissue selective up-regulation of GSK-3 is produced.[77] In that pathological situation, our organism does not have alternative

mechanisms of action directed to endogenously decrease this over-activation. Consequently, a mild inhibition of GSK-3 able to restore the homeostasis on GSK-3 activity levels would be sufficient to produce therapeutic effect. The pleiotropic distribution of GSK-3 in the body would not be a problem for a chronic therapeutic treatment following this approach of inhibition. Enzymatic inhibition would be subtly modulated, decreased, producing the activation of compensatory mechanisms of action or the restoration of the GSK-3 activity balance, respectively, at those places needed. Recovering the homeostasis of GSK-3 activity emerges as the real therapeutic strategy for the treatment of pathologies where the up-regulation of GSK-3 is involved. In this manner, mild and selective GSK-3 inhibitors gain positions to be useful drug candidates.[40]

The thiadiazolidindione scaffold is the main chemical feature present in the first ATP non-competitive GSK-3 inhibitors reported in the literature.[29] TDZD-8 and tideglusib, a worldwide used pharmacological tool and a GSK-3 inhibitor in clinical development, respectively, share this heterocyclic core. These compounds are reversible covalent inhibitors of the enzyme and their binding is governed by electrostatic recognition followed by covalent interaction with cys199 located at the entrance of the active site.[78] The sulphur-sulphur bond formed produces the inactivation of the enzyme while the thiadiazolidinone core is broken into carbon monoxide and the corresponding *N,N'*-disubstituted urea. These two secondary products are excreted through breath and urine respectively. The residual GSK-3 activity of the urea derivatives is claimed by NeuroPharma and may contribute to the long lasting activity of these compounds.[79] Specifically, the *N*-naphthyl-*N'*-benzylurea **1** (Figure 5) corresponding to Tideglusib inhibits GSK-3 with an IC_{50} value of 17.1 μ M.[79] More recently the company Abbvie has also described potent GSK-3 inhibition of different

N,N' disubstituted ureas such as quinolinylpyrazinylureas **2** and **3** that are able to modulate β -catenin gene transcription in a new LEF/TCF reporter gene assay.[80]

New thiadiazolidindiones (**3-6**) were reported by the extinguished Noscira. These compounds were synthesized with the aim to improve some physicochemical properties of tideglusib, such the thermodynamic solubility.[81] Compounds **3-6** (Figure 5) showed greater solubility and plasma exposure after oral administration than tideglusib but no efficacy data or further development has been reported until now.

More interesting is the approach that looks for substrate competitive GSK-3 inhibitors,[82] that provide subtle and specific inhibition and has therapeutic value.[83] Small molecules and peptides are described covering this field.[84] Ramot at Tel Aviv University claim for different peptides acting as substrate competitive GSK-3 inhibitors. [85] [86] Different amino acid sequences that include a phosphorylated serine or threonine have shown to modulate the kinase targeting specifically the recognition site for some substrates such as glycogen synthase or tau protein. As a consequence, the peptide L803 has shown *in vivo* efficacy in different models of diabetes type II and CNS diseases[87],[88] and it is considered the lead compound in this new optimization peptide campaign. Now peptide L803R is reported with a 50% increase in GSK-3 inhibition regarding its previous lead.[86] Furthermore, the peptide described as L806mts has the advantage of being cell penetrable due to the addition of a fatty acid as myristic acid to the amino acid sequence.[85] This compound presents CNS target engagement, quantified as an increase of beta-catenin levels, a surrogate marker of GSK-3 inhibition, observed in brain after nasal administration to mice.

Recently different allosteric binding sites have been described in the GSK-3 surface.[89] Using these enzyme pockets as specific drug interaction sites, virtual

screening has been performed and the quinoline compound VP0.7 has been discovered as an allosteric modulator of GSK-3. Structure-activity relationships in those compounds revealed a polar heterocycle head (see VP0.7 and derivative **7**) with a long hydrophobic tail as the main pharmacophore. These compounds have recently been granted to Consejo Superior de Investigaciones Científicas (CSIC).[90] The *in vivo* activity of quinoline derivatives has been shown in preclinical models of fragile X[91] and multiple sclerosis.[92]

Additionally to the above mentioned allosteric GSK-3 inhibitors, in the last two years several heterocyclic compounds with new chemotypes have also been claimed as potential pharmacotherapy agents targeting GSK-3.[93] Thus, several quinolone derivatives containing a strained spirocycle have been proposed as candidates for the treatment of diabetes type 2. Spiroquinolone **8** has an IC_{50} of 36 nM and EC_{50} of 3.2 μ M, and showed a reducing effect of plasma glucose concentration in a dose dependent manner in mice.[94] Belonging to the same chemical family, tricyclic 5-quinolone compounds, such as derivative **9** have shown GSK-3 inhibition and present antitumor activity *in vitro* and *in vivo*. [95]

Another interesting finding comes from a family of oxadiazoles that, differently from any other ATP competitive inhibitor, shows selectivity for GSK-3 α and was claimed by Technische Universität Darmstadt. [96] The therapy of certain diseases, such as acute myeloid leukemia (AML), may require α -isoform specific targeting. A hit to lead optimization program guided to the “scorpion shaped” GSK-3 α selective inhibitors where representative compound **10** showed 92 fold selectivity for the alpha isoform.[97] Although these compounds showed no toxicity in a Zebrafish model, they suffered from insufficient aqueous solubility. A solubility-driven optimization of previous inhibitors

has provided improved compounds that has confirmed in cell cultures the potential of selective GSK-3 α inhibition in AML therapy.[98]

Among natural products, the citrus flavonoid luteolin has been reported as a submicromolar GSK-3 inhibitor that interestingly inhibits GSK-3 α indirectly as well. This inhibition results in a decrease of soluble A β levels and promotes the association between presenilin 1 and APP. Chronic treatment of Tg2576 mice with luteolin has also shown significant reduction of A β pathology and has reduced AD-like features after traumatic brain injury in mice.[99], [100], [101], [102] These results, however, deserve further research as luteolin, and other polyphenols are notorious pan assay interfering compounds due to their potent protein-crosslinking properties.[103]

Recently, a novel isonicotinamide family of GSK-3 inhibitors has been patented showing cellular p-tau lowering at nanomolar level.[104] The compounds bind to the rear of the ATP binding pocket and showed only GSK-3 inhibitory activity among a panel of 400 kinases. This selectivity may be explained considering that the ribose pocket is not highly conserved among different kinases and the fact that this is the pocket where luteolin is binding to. Compound **11** has shown good *in vivo* activity lowering p-tau levels in a triple transgenic AD mouse model.[105]

It has been pointed out that GSK-3 plays a significant role in mood disorders, and its inhibitors are promising agents for the treatment of this pathology.[106] In this field recent advances have been made with a family of inhibitors that bind the enzyme reversibly at the ATP binding pocket. In particular, compound **12** was rationally optimized considering ligand-enzyme interactions and nanomolar inhibitory activity was reached. Indazole **12** showed good preliminary pharmacokinetic profile *in vivo* and

showed a complete reversal of amphetamine hypermotility in a mouse model that mimics the hyperactivity component of bipolar disorder.[107],[108]

Very recently a family of pyrazolodihydropyridine was discovered from a high throughput screening (HTS) against GSK-3 with nanomolar activity binding at the ATP pocket.[109] Drug development efforts resulted in compound **13** that has an enhanced specificity among 311 kinases, improved stability, induces myeloid differentiation and neurogenesis and is a potential drug candidate for the treatment of neurological disorders.[110]

In the search for treatment of different types of cancer, multiple kinase inhibitors have been developed. In particular, compounds that comprise a central pyrazole and benzimidazole scaffold such as derivative **14**, inhibit or modulate the activity of GSK-3, Aurora and CDK kinases, have shown antiproliferative activity in a number of cell lines and have been claimed by Astex Therapeutics.[111] Moreover, Abbvie has recently extended its patent of indazoles derivatives such as compound **15** as potent and selective kinase inhibitors, comprising inhibition of GSK-3, ROCK, JAK, CDC7, AKT, PAK4, PLK, CK2, KDR, MK2, JNK1, aurora, pim 1 and nek 2.[112],[113]

Teijin Pharma,[114] Vertex[115],[116],[117] and Abbott [118],[119] have recently patented different heterocyclic small molecules targeting GSK-3 such as compound **16**, compounds **17-19** and derivatives **20-21** respectively. They have claimed their use in different diseases where GSK-3 upregulation is involved.

6 Conclusions

During these two last years, patent activity around GSK-3 inhibitors has provided several new small molecules able to target selectively this kinase, but also well-known

GSK-3 inhibitors have been extensively used to discover new therapeutic applications of these effective compounds.

Combination therapy is gaining momentum in the devastating disease of cancer where GSK-3 inhibitors are recently showing promising results together with other agents.

Furthermore and as our knowledge of new pathways where GSK-3 plays an active role, and more concretely where its dysregulation leads to pathology, has increased, it is reasonable that well known GSK-3 inhibitors are being tested in different pathologies showing remarkable results.

Regenerative medicine is one of the areas where outstanding results are being discovered. Production of hepatocytes, cardiomyocytes and neuronal phenotypes has been optimized *in vitro* using GSK-3 inhibitors, constituting a new technology with great applications for the treatment of different disorders.

In the field of new GSK-3 inhibitors there is a tendency to look for different binding modes such as targeting the substrate or other allosteric pockets. At the same time, high selectivity is sought out for those ATP competitive inhibitors targeting the ribose region of this pocket, or looking for selectivity between the α and β isoforms. Collectively there is an effort to provide safer drugs that could be used for chronic treatment of severe unmet diseases

7 Expert Opinion

Modulating GSK-3 is a key process of recognized importance for its therapeutic applications in diverse fields. Particularly, regenerative medicine is an area where GSK-3 inhibitors are providing fascinating results and it seems that in the coming years we will see an expansion of its therapeutic functions, due to the many possibilities and applications that it offers. GSK-3 inhibitors may show value not only to improve the ex

in vivo production of stem cells to different lineages and further transplantation but also they may act as enhancers of endogenous neurogenesis promoting the formation of new neurons in a damaged brain. Joined to the advances in regenerative medicine, the field of GSK-3 inhibitors may gain importance in the pharmaceutical sector in the next years. The idea that GSK-3 inhibitors might be also used in treatment of cancer seems rather counterintuitive since they are useful in regenerative medicine. However it is worthwhile mentioning that the neurogenic potential of GSK-3 able to increase the production of new neurons from neural stem cells, is completely different from stimulating cell division. Moreover, it is well-known that GSK-3 induces apoptosis of malignant cells but acts as neuroprotective agent in healthy cells. [120]

Among the new therapeutic applications discovered for GSK-3 inhibitors, immune system modulation is showing very interesting results. However, more research is needed to understand the role of GSK-3 and the effects of its inhibitors. Immunotherapy is in great expansion nowadays and only future research may provide the real value and contribution of GSK-3 inhibitors as coadjuvants in this promising therapeutic approach. Lastly, we still see a vast effort in the discovery of new inhibitors, looking for new candidates that may lead for future therapeutic treatments.

A great number of small molecules with very different chemical scaffolds targeting GSK-3 with different binding modes are under development. Due to the better drug-like profile of these compounds regarding any biological therapy, specific siRNAs[121] or antibodies[122] designed to block GSK-3 will offer very adequate and useful pharmacological tools for research but will not have many chances to be pharmacological treatments *per se*. It is worthwhile to mention that multitarget strategy for drug design is starting to be present in some new GSK-3 inhibitors, and the first examples reported chimeric compounds with dual activity on GSK-3 and BACE-1 have

been reported. [123],[124],[125] Synergy found in these compounds will also offer a promise for the treatment of Alzheimer's disease or other cognitive disorders where β -amyloid and GSK-3 are overexpressed.

Two kinds of human GSK-3 inhibitors can be envisaged in the future therapeutic field:

1.-potent inhibitors not necessarily very selective over other kinases and 2.-subtle selective modulators with a good drug-like profile including blood-brain barrier penetration. The first class may have good opportunities in different cancer treatments and as useful pharmacological tools to modulate *ex vivo* regenerative medicine. The latter group, which includes allosteric modulators of GSK-3 and ATP non-competitive inhibitors, may be good drug candidates to modulate different physiological disorders as diabetes type II, chronic inflammatory pathologies and neurological diseases. In fact the only GSK-3 inhibitor currently in clinical trials, tideglusib, is being assayed to improve social behaviour in young patients of autism disorder syndrome. When results become available we will be able to better evaluate the future prospects of these powerful drugs to restore the homeostasis of a key physiological player: GSK-3.

8 References

(** = of considerable interest; * = of interest)

1. Embi N, Rylatt DB, Cohen P. Glycogen synthase kinase-3 from rabbit skeletal muscle. *Eur J Biochem.* 1980;107:519-27.
2. Cohen P. The Croonian Lecture 1998. Identification of a protein kinase cascade of major importance in insulin signal transduction. *Philos Trans R Soc Lond B Biol Sci.* 1999;354:485-95.
3. Woodgett JR. Physiological Roles of Glycogen Synthase Kinase-3: Potential as a Therapeutic Target for Diabetes and Other Disorders. *Endocr Metab Immune Disord Drug Targets.* 2003;3:281-90.
*One of the first reviews dealing with the importance of GSK-3 in physiology and pathology
4. Cohen P, Frame S. The renaissance of GSK3. *Nat Rev Mol Cell Biol.* 2001;2:769-76.

5. Ishiguro K, Takamatsu M, Tomizawa K, et al. Tau protein kinase I converts normal tau protein into A68-like component of paired helical filaments. *J Biol Chem.* 1992;267:10897-901.
6. Arioka M, Tsukamoto M, Ishiguro K, et al. τ protein kinase II is involved in the regulation of the normal phosphorylation state of τ protein. *J Neurochem.* 1993;60:461-8.
7. Ishiguro K, Shiratsuchi A, Sato S, et al. Glycogen synthase kinase 3β is identical to tau protein kinase I generating several epitopes of paired helical filaments. *FEBS Lett.* 1993;325:167-72.
8. Uchida T, Ishiguro K, Ohnuma J, et al. Precursor of cdk5 activator, the 23 kDa subunit of tau protein kinase II: Its sequence and developmental change in brain. *FEBS Lett.* 1994;355:35-40.
9. Hui-Chuan H, Peter SK. Multiple roles for glycogen synthase kinase-3 as a drug target in alzheimers disease. *Curr Drug Targets.* 2006;7:1389-97.
10. Dorronsoro I, Castro A, Martinez A. Inhibitors of glycogen synthase kinase-3: future therapy for unmet medical needs? *Expert Opin Ther Pat.* 2002;12:1527-36.
11. Kypta RM. GSK-3 inhibitors and their potential in the treatment of Alzheimer's disease. *Expert Opin Ther Pat.* 2005;15:1315-31.
12. Cohen P, Goedert M. GSK3 inhibitors: development and therapeutic potential. *Nat Rev Drug Discov.* 2004;3:479-87.
13. Arfeen M, Bharatam PV. Design of glycogen synthase kinase-3 inhibitors: an overview on recent advancements. *Curr Pharm Des.* 2013;19:4755-75.
14. Beurel E, Grieco SF, Jope RS. Glycogen synthase kinase-3 (GSK3): Regulation, actions, and diseases. *Pharmacol Ther.* 2015;148:114-31.
- **General updated revision of GSK-3 and its involvement in different diseases.
15. Ojo KK, Arakaki TL, Napuli AJ, et al. Structure determination of glycogen synthase kinase-3 from *Leishmania major* and comparative inhibitor structure-activity relationships with *Trypanosoma brucei* GSK-3. *Mol Biochem Parasitol.* 2011;176:98-108.
16. Wang HH, Lamont RJ, Kumar A, et al. GSK3 β and the control of infectious bacterial diseases. *Trends Microbiol.* 2014;22:208-17.
17. Masch A, Kunick C. Selective inhibitors of *Plasmodium falciparum* glycogen synthase-3 (PfGSK-3): New antimalarial agents? *Biochim Biophys Acta.* 2015;1854:1644-9.
18. Sebastian V, Manoli MT, Perez DI, et al. New applications for known drugs: Human glycogen synthase kinase 3 inhibitors as modulators of *Aspergillus fumigatus* growth. *Eur J Med Chem.* 2016;116:281-9.
19. Swinney ZT, Haubrich BA, Xia S, et al. A Four-Point Screening Method for Assessing Molecular Mechanism of Action (MMOA) Identifies Tideglusib as a Time-Dependent Inhibitor of *Trypanosoma brucei* GSK3 β . *PLoS Negl Trop Dis.* 2016;10:e0004506.
20. Saeki K, Machida M, Kinoshita Y, et al. Glycogen synthase kinase-3 β 2 has lower phosphorylation activity to tau than glycogen synthase kinase-3 β 1. *Biol Pharm Bull.* 2011;34:146-9.
21. Gao C, Hölscher C, Liu Y, et al. GSK3: a key target for the development of novel treatments for type 2 diabetes mellitus and Alzheimer disease. *Rev Neurosci.* 2012;23:1.
22. McCubrey JA, Steelman LS, Bertrand FE, et al. GSK-3 as potential target for therapeutic intervention in cancer. *Oncotarget.* 2014;5:2881-911.

23. Golpich M, Amini E, Hemmati F, et al. Glycogen synthase kinase-3 beta (GSK-3 β) signaling: Implications for Parkinson's disease. *Pharmacol Res.* 2015;97:16-26.
24. Palomo V, Perez DI, Gil C, et al. The potential role of glycogen synthase kinase 3 inhibitors as amyotrophic lateral sclerosis pharmacological therapy. *Curr Med Chem.* 2011;18:3028-34.
25. Costemale-Lacoste JF, Guilloux JP, Gaillard R. The role of GSK-3 in treatment-resistant depression and links with the pharmacological effects of lithium and ketamine: A review of the literature. *L'Encephale.* 2016;42:156-64.
26. Hooper C, Killick R, Lovestone S. The GSK3 hypothesis of Alzheimer's disease. *J Neurochem.* 2008;104:1433-9.
- * An interesting review where the key role of GSK-3 in Alzheimer's disease is described.
27. Muyllaert D, Kremer A, Jaworski T, et al. Glycogen synthase kinase-3 β , or a link between amyloid and tau pathology? *Genes Brain Behav.* 2008;7:57-66.
28. Georgievska B, Sandin J, Doherty J, et al. AZD1080, a novel GSK3 inhibitor, rescues synaptic plasticity deficits in rodent brain and exhibits peripheral target engagement in humans. *J Neurochem.* 2013;125:446-56.
29. Martinez A, Alonso M, Castro A, et al. First Non-ATP Competitive Glycogen Synthase Kinase 3 β (GSK-3 β) Inhibitors: Thiadiazolidinones (TDZD) as Potential Drugs for the Treatment of Alzheimer's Disease. *J Med Chem.* 2002;45:1292-9.
30. del Ser T, Steinwachs KC, Gertz HJ, et al. Treatment of Alzheimer's Disease with the GSK-3 Inhibitor Tideglusib: A Pilot Study. *J Alzheimer's Dis.* 2013;33:205-15.
31. Tolosa E, Litvan I, Höglinger GU, et al. A phase 2 trial of the GSK-3 inhibitor tideglusib in progressive supranuclear palsy. *Mov Disord.* 2014;29:470-8.
32. Höglinger GU, Huppertz H-J, Wagenpfeil S, et al. Tideglusib reduces progression of brain atrophy in progressive supranuclear palsy in a randomized trial. *Mov Disord.* 2014;29:479-87.
33. Lovestone S, Boada M, Dubois B, et al. A phase II trial of tideglusib in alzheimer's disease. *J Alzheimers Dis.* 2015;45:75-88.
34. Tideglusib vs. Placebo in the treatment of adolescents with autism spectrum disorders. Clinical trials.gov. <https://clinicaltrials.gov/ct2/show/NCT02586935> [Last accessed 4 May 2016].
35. Mines MA, Yuskaitis CJ, King MK, et al. GSK3 influences social preference and anxiety-related behaviors during social interaction in a mouse model of fragile X syndrome and autism. *PloS one.* 2010;5:e9706. Epub 2010/03/20.
36. Gray JE, Infante JR, Brail LH, et al. A first-in-human phase I dose-escalation, pharmacokinetic, and pharmacodynamic evaluation of intravenous LY2090314, a glycogen synthase kinase 3 inhibitor, administered in combination with pemetrexed and carboplatin. *Investig New Drugs.* 2015;33:1187-96.
37. Wu P, Nielsen TE, Clausen MH. Small-molecule kinase inhibitors: an analysis of FDA-approved drugs. *Drug Discov Today.* 2016;21:5-10.
- * An updated revision of kinase inhibitors recently approved to be in the market
38. Atkinson JM, Rank KB, Zeng Y, et al. Activating the Wnt/ β -Catenin pathway for the treatment of melanoma – application of LY2090314, a novel selective inhibitor of glycogen synthase kinase-3. *PloS one.* 2015;10:e0125028.
39. Rizzieri DA, Cooley S, Odenike O, et al. An open-label phase 2 study of glycogen synthase kinase-3 inhibitor LY2090314 in patients with acute leukemia. *Leuk Lymphoma.* 2016;6:1-7.

40. Avrahami L, Licht-Murava A, Eisenstein M, et al. GSK-3 inhibition: Achieving moderate efficacy with high selectivity. *Biochim Biophys Acta, Proteins Proteomics*. 2013;1834:1410-4.
41. Stamos JL, Weis WI. The beta-catenin destruction complex. *Cold Spring Harbor perspectives in biology*. 2013;5:a007898.
42. Martinez A, Perez DI, Gil C. Lessons learnt from glycogen synthase kinase 3 inhibitors development for Alzheimer's disease. *Curr Top Med Chem*. 2013;13:1808-19.
- **Some strategic requirements for clinical development of a small molecule targeting GSK-3 are here discussed.
43. Loscher W. Basic pharmacology of valproate: a review after 35 years of clinical use for the treatment of epilepsy. *CNS Drugs*. 2002;16:669-94.
44. Joep RS, Roh M-S. Glycogen synthase kinase-3 (GSK3) in psychiatric diseases and therapeutic interventions. *Curr Drug Targets*. 2006;7:1421-34.
45. Leng Y, Liang MH, Ren M, et al. Synergistic neuroprotective effects of lithium and valproic acid or other histone deacetylase inhibitors in neurons: roles of glycogen synthase kinase-3 inhibition. *J Neurosci*. 2008;28:2576-88.
46. Abbott FS, Acheampong AA. Quantitative structure-anticonvulsant activity relationships of valproic acid, related carboxylic acids and tetrazoles. *Neuropharmacol*. 1988;27:287-94.
47. Leclerc S, Garnier M, Hoessel R, et al. Indirubins inhibit glycogen synthase kinase-3 beta and CDK5/p25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease. A property common to most cyclin-dependent kinase inhibitors? *J Biol Chem*. 2001;276:251-60.
48. De Witte PE, C; Crawford, A; Smolders, I; K U Leuven. Anticonvulsant activity of GSK-3 beta inhibitors. WO2014053580. 2014.
49. Taylor A, Harker JA, Chanthong K, et al. Glycogen Synthase Kinase 3 Inactivation Drives T-bet-Mediated Downregulation of Co-receptor PD-1 to Enhance CD8(+) Cytolytic T Cell Responses. *Immunity*. 2016;44:274-86.
- * Recent discoveries regarding GSK-3 inhibition and its application to immunotherapy.
50. Hill EV, Ng TH, Burton BR, et al. Glycogen synthase kinase-3 controls IL-10 expression in CD4(+) effector T-cell subsets through epigenetic modification of the IL-10 promoter. *Eur J Immunol*. 2015;45:1103-15.
51. Rudd CL, DC; Rothstein, DM; Lee, YM; Christopher Rudd. Use of GSK-3 inhibitors or activators which modulate pd-1 or t-bet expression to modulate t cell immunity. WO2015155738. 2016.
52. Wraith DH, E; U Bristol. Tolerisation- inducing composition. WO2013150284. 2013.
53. Jamieson S, Butzow R, Andersson N, et al. The FOXL2 C134W mutation is characteristic of adult granulosa cell tumors of the ovary. *Mod Pathol*. 2010;23:1477-85.
54. Kim JH, Kim YH, Kim HM, et al. FOXL2 posttranslational modifications mediated by GSK3 β determine the growth of granulosa cell tumours. *Nat Commun*. 2014;5:2936.
55. Bae JL, K; Kim, J; Chung-Ang University Industry-Academy Cooperation Foundation. Pharmaceutical composition for preventing or treating ovary granulosa cell tumors containing glycogen synthase kinase-3 beta inhibitor as active ingredient, and functional health food composition. EP2979696. 2016.
56. Gross S, Rahal R, Stransky N, et al. Targeting cancer with kinase inhibitors. *J Clin Invest*. 2015;125:1780-9.

57. Benoit YD, Guezguez B, Boyd AL, et al. Molecular pathways: epigenetic modulation of Wnt-glycogen synthase kinase-3 signaling to target human cancer stem cells. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 2014;20:5372-8.

*An interesting review covering the role of GSK-3 inhibition in the killing of cancer stem cells.

58. Maher EA, Furnari FB, Bachoo RM, et al. Malignant glioma: genetics and biology of a grave matter. *Genes Dev*. 2001;15:1311-33.

59. Korur SB, F; Wymann, M; Universitaet Basel. Combination of lysosomotropic or autophagy modulating agents and a GSK-3 inhibitor for treatment of cancer. WO2013182519. 2013.

60. Takeda K, Yamaguchi N, Akiba H, et al. Induction of Tumor-specific T Cell Immunity by Anti-DR5 Antibody Therapy. *J Exp Med*. 2004;199:437-48.

61. Kim H, Samuel SL, Zhai G, et al. Combination therapy with anti-DR5 antibody and tamoxifen for triple negative breast cancer. *Cancer Biol Ther*. 2014;15:1053-60.

62. Kenichi WN, S; Shiho, I; Daiichi Sankyo Comp. Combination of GSK3 inhibitor and anti-DR5 antibody. WO2014050779. 2014.

63. Li W, Sun W, Zhang Y, et al. Rapid induction and long-term self-renewal of primitive neural precursors from human embryonic stem cells by small molecule inhibitors. *Proc Natl Acad Sci U S A*. 2011;108:8299-304.

64. Hui J, Zhang J, Kim H, et al. Fluoxetine regulates neurogenesis in vitro through modulation of GSK-3 β / β -catenin signaling. *Int J Neuropsychopharmacol*. 2015;18.

65. Guo W, Murthy AC, Zhang L, et al. Inhibition of GSK3 β improves hippocampus-dependent learning and rescues neurogenesis in a mouse model of fragile X syndrome. *Hum Mol Genet*. 2012;21:681-91. Epub 2011/11/04.

66. Bone HT, D; Welham, MJ; Stem Cells for Safer Medicines. Composition and method for differentiation of human embryonic stem cells. WO2011064549. 2011.

67. Brolen GE, J; Cellartis Ab. Directed differentiation and maturation of pluripotent cells into hepatocyte like cells by modulation of wnt-signalling pathway. WO2011116930. 2011.

68. Koppers-Munther BE, J; Cellectis SA. Maturation of hepatocyte-like cells derived from human pluripotent stem cells. WO2014083132. 2014.

69. Lian X, Hsiao C, Wilson G, et al. Robust cardiomyocyte differentiation from human pluripotent stem cells via temporal modulation of canonical Wnt signaling. *Proc Natl Acad Sci U S A*. 2012;109:E1848-57.

** Interesting use of GSK-3 inhibitors in cardiovascular regenerative medicine.

70. Palecek SK, T; Lian, X; Wisconsin Alumni Research Found. Generation of cardiomyocytes from human pluripotent stem cells. US2015152389. 2015.

71. Lian X, Zhang J, Azarin SM, et al. Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating Wnt/ β -catenin signaling under fully defined conditions. *Nat Protoc*. 2013;8:162-75.

72. Cheng L, Hu W, Qiu B, et al. Generation of neural progenitor cells by chemical cocktails and hypoxia. *Cell Res*. 2014;24:665-79.

73. Pei GZ, J; Cheng, L; Hu, W; Qiu, B; Shanghai Institutes For Biological Sciences, Chinese Academy Of Sciences. Treatment of neurological conditions. WO2015131788. 2015.

74. Cheng L, Gao L, Guan W, et al. Direct conversion of astrocytes into neuronal cells by drug cocktail. *Cell Res*. 2015;25:1269-72.

75. Hu W, Qiu B, Guan W, et al. Direct conversion of normal and alzheimer's disease human fibroblasts into neuronal cells by small molecules. *Cell Stem Cell*. 2015;17:204-12.

**Conditions to produce iPSCs using only a cocktail of small molecules including a GSK-3 inhibitor.

76. Bouskila M, Hirshman MF, Jensen J, et al. Insulin promotes glycogen synthesis in the absence of GSK3 phosphorylation in skeletal muscle. *Am J Physiol Endocrinol Metab*. 2008;294:E28-35.

77. Martinez A, Gil C, Perez DI. Glycogen synthase kinase 3 inhibitors in the next horizon for Alzheimer's disease treatment. *International journal of Alzheimer's disease*. 2011;2011:280502.

78. Dominguez JM, Fuertes A, Orozco L, et al. Evidence for irreversible inhibition of glycogen synthase kinase-3beta by tideglusib. *J Biol Chem*. 2012;287:893-904.

79. Martinez AM, M; Alonso, M; Fuertes, A; Navarro, ML; Perez, MJ; Castro, A; Neuropharma, S.A. GSK-3 inhibitors. US08686042. 2014.

80. Turner SB, MH; Stewart, KD; Abbvie Inc. Heterocyclic compounds and their use as glycogen synthase kinase 3 inhibitors. US 8642598. 2014.

81. Medina MD, JM; De Cristobal, J; Fuertes, A; Sánchez-Quesada, J; Lopez, J; Herrero, S; Perez, MA; Martinez, O; Rodriguez, B; Francisco, N; Nosaira, S.A. Thiadiazolidinediones as GSK-3 inhibitors. WO2013124413. 2013.

82. Licht-Murava A, Plotkin B, Eisenstein M, et al. Elucidating substrate and inhibitor binding sites on the surface of GSK-3beta and the refinement of a competitive inhibitor. *J Mol Biol*. 2011;408:366-78.

83. Licht-Murava A, Eldar-Finkelman H. Exploiting substrate recognition for selective inhibition of protein kinases. *Curr Pharm Des*. 2012;18:2914-20.

*Importance of subtle GSK-3 modulation for chronic treatments are here reported.

84. Palomo V, Perez DI, Perez C, et al. 5-imino-1,2,4-thiadiazoles: first small molecules as substrate competitive inhibitors of glycogen synthase kinase 3. *J Med Chem*. 2012;55:1645-61.

85. Eldar-Finkelman HL-M, A; Plotkin, B; Ramot at Tel-Aviv U. Glycogen synthase kinase-3 inhibitors. US20130310303. 2013.

86. Eldar-Finkelman HE, M; Ramot at Tel-Aviv U. Glycogen synthase kinase-3 inhibitors. WO2012101601. 2013.

87. Kaidanovich-Beilin O, Eldar-Finkelman H. Long-term treatment with novel glycogen synthase kinase-3 inhibitor improves glucose homeostasis in ob/ob mice: molecular characterization in liver and muscle. *J Pharmacol Exp Ther*. 2006;316:17-24.

88. Shapira M, Licht A, Milman A, et al. Role of glycogen synthase kinase-3beta in early depressive behavior induced by mild traumatic brain injury. *Mol Cell Neurosci*. 2007;34:571-7.

89. Palomo V, Soteras I, Perez DI, et al. Exploring the binding sites of glycogen synthase kinase 3. Identification and characterization of allosteric modulation cavities. *J Med Chem*. 2011;54:8461-70.

*First allosteric modulator of GSK-3 described.

90. Martínez AG, C; Palomo, V; Perez, C; Perez, DI; Reyes, JA; Consejo Superior de Investigaciones Científicas (CSIC). Heterocyclic GSK-3 allosteric modulators. EP2769720. 2014.

91. Franklin AV, King MK, Palomo V, et al. Glycogen synthase kinase-3 inhibitors reverse deficits in long-term potentiation and cognition in fragile X mice. *Biol Psychiatry*. 2014;75:198-206.

92. Beurel E, Kaidanovich-Beilin O, Yeh WI, et al. Regulation of Th1 cells and experimental autoimmune encephalomyelitis by glycogen synthase kinase-3. *J Immunol.* 2013;190:5000-11.
93. Cociorva OF, Y; Kohno, Y; Li, B; Okada, K; Nakamura, A; Nomura, M; Seto, S; Szardenings, AK; Yumoto, K; Kyorin Pharmaceuticals Co., Ltd. Spirocyclic aminoquinolones as GSK-3 inhibitors. US20140005184. 2014.
94. Seto S, Yumoto K, Okada K, et al. Quinolone derivatives containing strained spirocycle as orally active glycogen synthase kinase 3beta (GSK-3beta) inhibitors for type 2 diabetics. *Bioorg Med Chem.* 2012;20:1188-200.
95. Kiyoshi NY, T; Noriyasu, H; Hiroyuki, N; Akiyoshi, M; Masanori, S; Takashi, O; Yoshihiro, S; Tomoyuki, T; Naomi, S; Daiichi Sankyo Comp. Tricyclic compounds. WO2014003098. 2014.
96. Schmidt BLM, F; Eldar-Finkelmann, H; Van Leuven, F; Kramer, T; Technische Universität Darmstadt. Derivate von 2-benzylsulfanyl[1,3,4]-oxadiazol und deren medizinische verwendung. WO2013007663. 2013.
97. Lo Monte F, Kramer T, Gu J, et al. Structure-based optimization of oxadiazole-based GSK-3 inhibitors. *Eur J Med Chem.* 2013;61:26-40. Epub 2012/07/04.
98. Neumann T, Benajiba L, Goring S, et al. Evaluation of Improved Glycogen Synthase Kinase-3alpha Inhibitors in Models of Acute Myeloid Leukemia. *J Med Chem.* 2015;58:8907-19.
99. Tan JS, D; Rezai-Zadeh, K; University of South Florida. Flavonoid treatment of glycogen synthase kinase-based disease. US8802638. 2014.
100. Rezai-Zadeh K, Douglas Shytte R, Bai Y, et al. Flavonoid-mediated presenilin-1 phosphorylation reduces Alzheimer's disease beta-amyloid production. *J Cell Mol Med.* 2009;13:574-88.
101. Johnson JL, Rupasinghe SG, Stefani F, et al. Citrus flavonoids luteolin, apigenin, and quercetin inhibit glycogen synthase kinase-3 β enzymatic activity by lowering the interaction energy within the binding cavity. *J Med Food.* 2011;14:325-33.
102. Sawmiller D, Li S, Shahaduzzaman M, et al. Luteolin reduces Alzheimer's disease pathologies induced by traumatic brain injury. *Int J Mol Sci.* 2014;15:895-904.
103. Glaser J, Holzgrabe U. Focus on PAINS: false friends in the quest for selective anti-protozoal lead structures from Nature? *MedChemComm.* 2016;7:214-23.
104. Luo GC, L; Dubowchik, GM; Jacutin-Porte, SE; Vrudhula, SV; Pan, S; Sivaprakasam, P; Macor, JE; Bristol Myers Co. GSK-3 Inhibitors. WO2015069594. 2015.
105. Luo G, Chen L, Burton CR, et al. Discovery of isonicotinamides as highly selective, brain penetrable, and orally active glycogen synthase kinase-3 inhibitors. *J Med Chem.* 2016;59:1041-51.
106. O'Leary O, Nolan Y. Glycogen synthase kinase-3 as a therapeutic target for cognitive dysfunction in neuropsychiatric disorders. *CNS Drugs.* 2015;29:1-15.
107. Alisi MC, N; Dragone, P; Furlotti, G; Maugeri, C; Ombrato, R; Mancini, F; Aziende Chimiche Riunite Ltd. 1H-Indazole-3-carboxamide compounds as glycogen synthase kinase 3 beta inhibitors. US20150057294. 2015.
108. Furlotti G, Alisi MA, Cazzolla N, et al. Hit optimization of 5-substituted-N-(piperidin-4-ylmethyl)-1H-indazole-3-carboxamides: potent glycogen synthase kinase-3 (GSK-3) inhibitors with in vivo activity in model of mood disorders. *J Med Chem.* 2015;58:8920-37.
109. Wagner FF, Bishop JA, Gale JP, et al. Inhibitors of Glycogen Synthase Kinase 3 with Exquisite Kinome-Wide Selectivity and Their Functional Effects. *ACS Chem Biol.* 2016;11:1952-63. Epub 2016/04/30.

110. Wagner FP, JQ; Dandapani, S; Germain, A; Holson, E; Munoz, B; Nag, PP; Weiwer, M; Lewis, MC; Haggarty, SJ; Bishop, JA; Stegmaier, K; Banerji, V; The Broad Institute Inc. GSK3 inhibitors and methods of use thereof. WO2014059383. 2014.
111. Berdini VC, MG; Gill, AL; Howard, S; Navarro, EF; Trewartha, G; Rees, DC; Vinkovic, M; Wyatt, PG; Astex Therapeutics, Ltd. Pyrazole compounds that modulate the activity of CDK, GSK and aurora kinases. US8778936. 2014.
112. Akritopoulou-Zanze I, Wakefield BD, Gasiecki A, et al. Scaffold oriented synthesis. Part 4: Design, synthesis and biological evaluation of novel 5-substituted indazoles as potent and selective kinase inhibitors employing heterocycle forming and multicomponent reactions. *Bioorg Med Chem Lett*. 2011;21:1480-3.
113. Akritopoulou-Zanze IW, BD; Mack, H; Turner, SC; Gasiecki, AF; Gracias, VJ; Sarris, KA; Kalvin, DM; Michmerhuizen, MJ; Shuai, Q; Abbvie Inc. 5-substituted indazoles as kinase inhibitors. US8648069. 2014.
114. Tsutsumi TS, S; Koga, M; Matsumoto, Y; Ishii, T; Nakano, A; Unoki, G; Sakai, Y; Takarada, R; Ogawa, H; Teijin Pharma. Pyrrolopyrimidine thion derivatives. US2005153992. 2005.
115. Green JG, R; Pierce, A; Vertex Pharmaceuticals Inc. Compositions useful as inhibitors of protein kinases. US8653088. 2014.
116. Bebbington DC, JD; Golec, J; Pierard, F; Vertex Pharmaceuticals Inc. Pyrazole compounds useful as protein kinase inhibitors. US8697698. 2014.
117. Charrier JL, P; Knegetel, R; Golec, JM; Bebbington, D; Binch, HM; Vertex Pharmaceuticals Inc. Triazole compounds useful as protein kinase inhibitors. US08633210. 2014.
118. Turner SM, H; Bakker, MH; Van Gaalen, M; Hoft, C; Hornberger, W; Abbott GmbH & Co. Heterocyclic compounds and their use as glycogen synthase kinase-3 inhibitors. US2012172376. 2012.
119. Turner SB, MH; Van Gaalen, M; Wolter, FE; Hornberger, W; Nijssen, M; Abbott GmbH & Co. Heterocyclic compounds and their use as glycogen synthase kinase-3 inhibitors. US2012077840. 2012.
120. Tejeda-Muñoz N, Robles-Flores M. Glycogen synthase kinase-3 in Wnt signaling and cancer. *IUBMB* 2015;67:914-922.
121. Sah DH, G; Alnylam Pharmaceuticals Inc. Compositions and methods for inhibiting expression of GSK-3 genes. US09029525. 2015.
122. Reed TA, AH; Intrexon Corp. GSK3 ligands and polynucleotides encoding GSK3 ligands. US20140273227. 2014.
123. Di Martino RM, De Simone A, Andrisano V, et al. Versatility of the Curcumin Scaffold: Discovery of Potent and Balanced Dual BACE-1 and GSK-3 β Inhibitors. *J Med Chem*. 2016;59:531-44.
124. Prati F, De Simone A, Armirotti A, et al. 3,4-Dihydro-1,3,5-triazin-2(1H)-ones as the First Dual BACE-1/GSK-3 β Fragment Hits against Alzheimer's Disease. *ACS Chem Neurosci*. 2015;6:1665-82.
125. Prati F, De Simone A, Bisignano P, et al. Multitarget drug discovery for Alzheimer's disease: triazinones as BACE-1 and GSK-3 β inhibitors. *Angew Chem Int Ed Engl*. 2015;54:1578-82.

Article Highlights box

- 1.- GSK-3 inhibitors have gained a prominent role in regenerative medicine
- 2.-Allosteric modulators of GSK-3 emerge as safe compounds for chronic treatments.
- 3.- Contribution of GSK-3 inhibitors as adjuvants in immunotherapy approaches is gaining relevance.
- 4.- GSK-3 inhibitors are showing promising results in different cancer combination therapies.
- 5.-New therapeutic uses for GSK-3 inhibitors are being discovered continuously

Figure 1. GSK-3 inhibitors that reached human clinical trials.

Figure 2.- Known GSK-3 inhibitors with new therapeutic applications.

Anticonvulsant activity

BIO

Immunity modulation

SB415286

SB216763

Tolerogenic adjuvant

CHIR99021

FOXL2 Modulation

AR-A014418

Figure 3. GSK-3 inhibitors used in combination with other therapeutic agents for cancer treatments.

Figure 4.-GSK-3 inhibitors used in regenerative medicine.

Figure 5. Chemical compounds recently patented as GSK-3 inhibitors.