Campagne 2020 Contrats Doctoraux Instituts/Initiatives

Proposition de Projet de Recherche Doctoral (PRD)

Appel à projet ISVI - Intitiative Sces du vivant ses interfaces 2020

Intitulé du Projet de Recherche Doctoral : Designing novel KCC2 enhancers for epilepsy and related disorders: Molecular modeling and virtual screening for drug discovery

Directeur de Thèse porteur du projet (titulaire d'une HDR) :

NOM : **PONCER** Prénom : **Jean Christophe**
Titre : Directeur de Recherche ou
e-mail : jean-christophe.poncer@inserm.fr
Adresse professionnelle : Institut du Fer à Moulin, UMR-S 1270, 17 rue du Fer à Moulin,
(site, adresse, bât., bureau) 75005 Paris

Unité de Recherche :
Intitulé : Institut du Fer à Moulin
Code *(ex. UMR xxxx)* : UMR_S 1270

Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant : **ED158-Cerveau, cognition, comportem**

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 1, F Donneger, Oct. 2018, à 100%

Co-encadrant :

NOM : **ACHER** Prénom : **Francine**
Titre : Directeur de Recherche ou HDR ☒
e-mail : francine.acher@parisdescartes.fr

Unité de Recherche :
Intitulé : Lab. Chimie et Biochimie Pharmacologiques et Toxicologiques
Code *(ex. UMR xxxx)* : UMR_S 8601

Ecole Doctorale de rattachement : **Choisissez un élément :**
Ou si ED non Alliance SU : **ED436-MTCI**

Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) : 3, N Cristiano Oct 2017, A Cabayé, Juil 2018, H Xiong, Sept 2019, tous à 50% (taux encadrement effectif: 1.5)

Cotutelle internationale : ☒ Non ☐ Oui, précisez Pays et Université :

Description du projet de recherche doctoral *(en français ou en anglais)*

3 pages maximum – interligne simple – Ce texte sera diffusé en ligne

Détailler le contexte, l'objectif scientifique, la justification de l'approche scientifique ainsi que l'adéquation à l'initiative/l'Institut.
Le cas échéant, préciser le rôle de chaque encadrant ainsi que les compétences scientifiques apportées. Indiquer les publications/productions des encadrants en lien avec le projet.

i-Bio PhD project (Neuroscience & Chemistry)

Designing novel *KCC2* enhancers for epilepsy and related disorders: Molecular modeling and virtual screening for drug discovery

Context and objectives

Alterations in neuronal chloride regulation is emerging as a hallmark of neurological disorders[1], including epilepsy[2-6], autism spectrum disorders[7-9] and neuropathic pain[10-12]. Perturbed expression and/or function of the cation-chloride cotransporters (CCCs) KCC2 and NKCC1 are reported in these conditions and expected to impair fast inhibitory signalling mediated by the chloride-permeable GABAA receptor (GABAAR)[1]. Thus, while NKCC1 imports Cl^- and K^+ inside neurons, KCC2 usually contributes to maintain low intraneuronal [Cl^-], thereby promoting the inhibitory effect of GABAAR activation[13]. Reduced KCC2/NKCC1 functional ratio in the pathology may then depolarize the reversal potential of GABAAR currents and even promote excitatory actions of GABA[7,10,14-16]. This in turn may contribute to anomalous neuronal activities underlying the pathology. Moreover, several recent studies revealed a variety of effects of KCC2 downregulation, independent of ion transport, including altered glutamatergic synaptic transmission[17] and plasticity[18] as well as enhanced neuronal excitability[19].

Strategies targeting CCC function or expression have therefore been tested in animal models of neurological disorders, including epilepsy. For instance, intracerebral infusion of the NKCC1 antagonist bumetanide was shown to prevent epileptogenesis in an experimental model of temporal lobe epilepsy (TLE)[20]. However, bumetanide has numerous side-effects - as NKCC1 is also widely expressed in non-neuronal cells - and hardly crosses the blood/brain barrier, making it a poor candidate in CNS disorders[21]. Instead, promoting KCC2 function or expression holds many promises, as KCC2 is almost exclusively expressed in neurons. Thus, viral-based KCC2 overexpression in cortex was recently shown to prevent seizures in mice[3]. Screening of drug-like compound libraries also identified small molecules suggested to act as *KCC2 enhancers*[11,22,23]. Despite their well-documented therapeutic benefit in neuropathic pain and other disorders[11,22-24], their effect in epilepsy, however, has been overlooked[25]. In addition, their mechanism(s) of action and specificity remain unknown and even controversial[26,27]. **An advantageous alternative to the screening of chemical compound libraries consists in specifically designing ligands to target effectors by molecular modelling and virtual screening**[28-30]. Considerable progress has been made recently in understanding the molecular mechanisms regulating KCC2 function and membrane stability[31-34] as well as in deciphering the 3D structure of CCCs[6,35,36] and some of their key regulators[37-39]. This knowledge may then now be used to implement novel approaches of computational chemistry to design specific KCC2 ligands to promote its membrane expression and function.

We therefore propose that **acting to promote KCC2 function represents a highly promising therapeutic strategy in epilepsy and related disorders**. Our preliminary data demonstrate the effectiveness of existing KCC2 enhancers in preventing epileptiform activity *in vitro* and *in vivo*. However, their specificity is limited and other adverse effects are also observed. Therefore, our project aims at i) designing new molecules with improved specificity and bioavailability and ii) evaluating and comparing their therapeutic potential in the context of epilepsy. To this end, an **interdisciplinary approach** will be implemented, combining **advanced computational chemistry and virtual ligand screening** with **biochemical and super-resolution imaging techniques** as well as **electrophysiological recordings** from human brain tissue and animal models.

Specific aims

The project will comprise 3 main complementary tasks.

Task 1. To model the carboxy-terminal domain of KCC2 and identify new candidate KCC2 enhancers by virtual screening

Our modeling approach will aim at designing new molecules to more selectively enhance KCC2 function and stability than existing compounds. KCC2 membrane stability and function are primarily regulated through posttranslational modifications, in particular phosphorylation of residues of its carboxy-terminal domain[31-34]. These sites therefore represent targets of choice for designing specific KCC2 enhancers. We will focus on $Thr^{906/1007}$ residues in the carboxy-terminal domain (CTD) that we and others have shown reduce KCC2

membrane stability and function upon phosphorylation by the SPAK and OSR1 kinases[31-34]. In order to avoid the lack of selectivity inherent to the highly conserved ATP-binding site of the kinases, we will use molecular modelling and virtual screening to design ligands targeting specific allosteric sites of the kinase or substrate. The recently resolved crystal structure of NKCC1[36] allows us to build a homology model of KCC2 and reveals the NKCC1 residues homologous to KCC2 Thr$^{906/1007}$ to be in a solvent-exposed position. Based on available structures and homology models of SPAK and OSR1[38,39], we propose to dock these structures to the KCC2-CTD 3D-model in order to define selective contact hotspots. These will first be validated by mutagenesis and chloride-imaging based functional assays. We will then search for small molecules/peptides with high affinity for these hotspots by virtual high-throughput screening[29] using Discovery Studio (Dassault Systemes BIOVIA), routinely used by F. Acher. Curated libraries of commercial small compounds/peptides will be virtually docked at the specific sites of the 3D structures and scored. Top-ranked molecules will then be used for pharmacological evaluation. The validated hits will be then chemically optimized for *in vivo* delivery and stability.

Task 2. <u>To compare the specificity and modes of action of existing *vs*. novel KCC2 enhancers in hippocampal neurons *in vitro*</u>

We will compare the efficacy and specificity of compounds obtained from Task 1 with those of existing, putative KCC2 enhancers[11,22,40] and characterize their effects on KCC2 expression/function as well as GABA signaling in cultured rat hippocampal neurons. Specifically, we will test i) KCC2 membrane expression (surface biotinylation), ii) KCC2 phosphorylation state (phosphosite-specific antibodies[41]), iii) KCC2 membrane dynamics (single particle tracking[31,41]) and iv) KCC2 function (focal GABA uncaging in whole-cell and gramicidin perforated patch recordings[18,19,41]). Preliminary results from JC. Poncer suggest CLP257 and piperazine derivatives may act similarly to promote KCC2 function through reduced phosphorylation at Thr$^{906/1007}$ and enhanced clustering. We will also explore the specificity of these effects, in particular with respect to NKCC1 and GABAAR function[26].

Task 3. <u>To evaluate and compare the antiepileptic potential of KCC2 enhancers in human epileptic tissue *in vitro* as well as in animal models of temporal lobe epilepsy</u>

Human cerebral tissue resected from patients with pharmaco-resistant epilepsy represent an invaluable model for exploring the effect of candidate antiepileptic drugs *in vitro*. Using multielectrode array recordings[42], we will compare the effect of novel KCC2 enhancers on neuronal activity and spontaneous interictal discharges (IIDs) from slices of human epileptic hippocampus. Our preliminary results, in collaboration with the Neurosurgery department (F. Chassoux and B. Devaux) of Sainte-Anne Hospital (Paris) suggest CLP257 may prevent IIDs in human epileptic hippocampus. In parallel, we will test these compounds in the pilocarpine mouse model of TLE, recently optimized by Poncer. This will let us test the effect of KCC2 enhancers both on epileptogenesis (chronic injection during 8 days post *status epilepticus* (SE)) and during the chronic phase (from day 35 post SE). Using telemetric ECoG and video recordings, we will monitor and quantify seizure frequency and duration as well as IIDs in saline *vs.* KCC2 enhancer-injected mice. This task based on assays routinely used in the Poncer lab will be performed in collaboration with trained electrophysiologists.

This project gathers two groups with **complementary expertise in medicinal chemistry and cellular neurobiology**. It is based on solid preliminary data and expertise from both partners and aims to produce **the first proof-of-concept preclinical evidence for using KCC2 enhancers in epilepsy**. It is also innovative and ambitious as it proposes to develop a **totally novel approach to designing KCC2 enhancers** with improved specificity and bioavailability, using state-of-the-art molecular modeling and virtual screening of allosteric antagonists KCC2 phosphorylation. We trust this approach, which has been successfully applied to other receptors, transporters and kinases[28-30], should let us **identify the most promising molecules for optimal translation to clinical studies**, both in epilepsy and other neurological disorders associated with CCC dysfunction[1]. It will also produce invaluable data on KCC2 structure and the understanding of molecular interactions between KCC2 and its regulatory partners, with a predicted impact beyond the field of epilepsy research and beyond.

Co-supervisor 1: **Jean Christophe Poncer**, PhD. Research Director, INSERM. Institut du Fer à Moulin (UMR 1270, INSERM/Sorbonne University, Paris).
His group combines state-of-the-art super-resolution imaging (Lévi/Renner) and electrophysiological approaches, both *in vitro* (patch clamp, multi-electrode arrays) and *in vivo* (telemetric EEG and intracerebral silicon probes). His lab has made important discoveries in the field of cation chloride cotransporters, revealing in particular i) a variety of KCC2 physiological functions independent of ion transport (*PNAS* 2011, *JNeurosci* 2015, *Cell Rep* 2019) and ii) mechanisms of KCC2 activity-dependent regulation through phosphorylation-induced changes in membrane dynamics (*JNeurosci* 2013, *Nat Comm* 2017).
Publications: 46 articles in peer-reviewed journals. H-index 27. 3940 citations.

Co-supervisor 2: **Francine Acher**, PhD. Research Director, CNRS. Laboratoire de Chimie et Biochimie pharmacologiques et toxicologiques (LCBPT, UMR 8601 CNRS/Univ. de Paris).
Her group has a very solid background in medicinal chemistry. F Acher has been involved in the design and synthesis of non-natural amino acids and the investigation of their roles in various biological systems. Her interdisciplinary research was made possible by close interaction with expert collaborators in pharmacology, molecular biology and behaviour. She discovered potent ligands using virtual HTS of homology models of GPCRs and membrane transporters (*Neuron* 2008, *J Med Chem* 2005/2010, *JBC* 2012). Several of these hits, once chemically optimized, showed beneficial effects in animal models of Parkinson's disease, chronic pain, schizophrenia, addiction and epilepsy (*Faseb J* 2012, *JNeurosci* 2013, *Psychopharm* 2012, *Neuropharmacol* 2018, *Neurobiol Dis* 2019). She will be in charge of supervising all the molecular modelling approach and chemical synthesis.
Publications: 128 articles in peer reviewed journals. H-index 33. 11 international patents. 3638 citations.

The candidate should have a background in synthetic chemistry or pharmaceutical chemistry as well as notions of cellular biology and biochemistry, and be fluent with programming in Matlab or equivalent. Additional training in cellular and molecular biology as well as electrophysiology will be provided through the *i-Bio* training program. Specific training in molecular modelling and virtual screening tools (Discovery Studio; Dassault Systemes BIOVIA) will be provided through an active collaboration of F. Acher with Dassault Systemes.

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