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Mathieu Charvériat[a,*], **Vincent Lafon**[b], **Franck Mouthon**[a], **Luc Zimmer**[c,d]

[a] *Theranexus, 69008 Lyon, France*
[b] *Insiliance, 75014 Paris, France*
[c] *Université de Lyon, 69675 Lyon, France*
[d] *Hospices civils de Lyon, 69500 Bron, France*

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Summary Central nervous system disorders remain the leading causes of mortality and morbidity worldwide, affecting more than 1 billion patients. This therapeutic area suffers from high unmet medical needs and the search for innovative approaches to identify therapeutic strategies is urgent in the field. This review will first cover the challenges and opportunities of drug discovery in neurology, and then as well as the new models, such as human model stem cells and animal models under development in the field. In addition, innovative and translational neuroimaging techniques will be discussed, as the use of big data and artificial intelligence to discovery new drugs in neurological and neuropsychiatric disorders.

Abbreviations

AD	Alzheimer's disease
AI	artificial intelligence
ALS	amyotrophic lateral sclerosis
BOLD	blood-oxygen-level-dependent
CMap	connectivity map
CNS	central nervous system
CT	computed tomography
FD	familial dysautonomia
fUS	functional ultrasound
FXS	fragile X syndrome
HER	electronic health records
IBI	International Brain Initiative
iPSC	induced pluripotent stem cell
LID	levodopa-induced dyskinesia
MND	motor neuron disease
MRC	Medical Research Council
MRI	magnetic resonance imaging
NDD	neurodegenerative disorders

* Corresponding author. Theranexus, 60, avenue Rockefeller, 69008 Lyon, France.
E-mail address: mathieu.charveriat@theranexus.com (M. Charvériat).

NIH	National Institute of Health
NPC	Niemann-pick disease type C
PD	Parkinson's disease
PET	positron emission tomography
PK-PD	pharmacokinetics-pharmacodynamic
QSP	quantitative systems polypharmacy
SMA	spinal muscular atrophy
STAIR	group Stroke Therapy Academic Industry Roundtable
TBI	traumatic brain injury

Introduction: challenges and opportunities in CNS field

Important and exciting advances have been realized over the past decades in the understanding of neurochemical mechanisms of brain disorders, dysregulation of circuitry, discovery of genetic loci or innovative biomarkers. However, those disorders still remain the leading causes of mortality and morbidity [1], and globally affect more than one billion people worldwide [2]. The global cost of those diseases is projected to markedly increase to $6.5 trillion in 2030 [3], with more than two thirds of the patients in the USA and in Europe still untreated [4].

Despite high unmet medical needs, many companies have retreated from the development of new drugs in central nervous system (CNS) disorders [5]. Indeed, this field is endowed with the lowest probability of success—only 7% of the CNS drugs reach the market place compared to 15% in other therapeutic areas. Approved drugs took an average of 12.6 years in development and regulatory approval compared with 6.3 years and 7.5 years for cardiovascular and gastrointestinal indications [6]. Those limitations are notably due to the complexity of the brain, the lack of solid biological data demonstrating the clear role of the molecular target in the pathophysiology, the poor predictive validity of animal models, the difficulty of drugs to pass through the blood-brain-barrier, the poor availability of relevant biomarkers. Additionally, adequate target occupancy is hard to reach, correlatively to the difficulty of dose selection [6].

A large pharmaceutical company recently summarized its conceptual framework based on the five most important technical reasons of success for drug projects, (i) the right target, (ii) the right patient, (iii) the right tissue, (iv) the right safety, (v) the right commercial potential [7]. This review will focus on what innovative technologies and tools may bring to this framework applied to the CNS drug discovery process, and how they can help better understanding of the central mechanisms of disease, identifying and validating targets and biomarkers and designing more predictive models, as key challenges.

Biomarker-driven discovery of CNS drugs

Biomarkers are important clinical tools to improve diagnosis, monitor drug activity, and therapeutic response. Preclinical biomarkers have also a place in the development of new therapies as indicators of efficacy or toxicity and as tools for selecting the most promising drug candidates.

This multiplicity of applications explains why biomarker terminology is frequently used in different contexts. This also explains why government drug agencies and academic societies have carefully defined the term. A biomarker is a biological substance or a physiological parameter (cellular, biochemical, molecular, genetic, protein, metabolite, specific post-translational modification or physiological or biophysical sign) that can be measured and evaluated objectively as an indicator of a normal biological processes, a pathogenic processes and their progression or pharmacological responses to therapeutic intervention [8]. In general, biomarkers are classified as molecular, imaging, and psychometric (e.g., memory loss to predict Alzheimer's disease [AD]) biomarkers. The discovery, validation, qualification, and use of biomarkers adapted to a variety of drug development and regulatory decision-making purposes are areas of tremendous interest and need (Fig. 1) [9].

While different types of biomarkers have had an impact in drug discovery and development, the process of identifying and validating disease-specific biomarkers has been quite difficult [10]. Recent advances in multiple ''omics'' (multi-omics) approaches (e.g. genomics, transcriptomics, proteomics, metabolomics, cytometry and imaging) in combination with bioinformatics and biostatistics have accelerated the discovery and development of specific biomarkers for complex chronic diseases [11]. Although many challenges remain, current efforts in the discovery and development of disease-specific biomarkers will contribute to optimal decision-making throughout drug development and improve our understanding of disease processes.

A broad reflection has been initiated in the field of CNS drug discovery with a central positioning of biomarkers [12]. There is a growing need for biomarkers of CNS pathologies for proof of mechanisms which are measurable in both preclinical and clinical paradigm, with an exposure—response relationship amenable to pharmacokinetics-pharmacodynamic (PK-PD) modeling, demonstrating target engagement in CNS and a pharmacological activity, potentially correlated with clinical efficacy, usable as a treatment-monitoring tool. The whole issue is the effective translation of preclinical biomarkers into the clinic [13]. The notion of biomarker is now the central concept that guides the drug discovery process, as it will be developed through the technologies and approaches described below.

Drug discovery at cellular scale using human stem cells

Recent groundbreaking studies have led to the possibility to convert patient-specific cell (typically fibroblasts) into pluripotent cell, called induced pluripotent cells (iPSCs) [14], cells that can be furtherly differentiated into active neurons [15], with characteristics consistent with forebrain, dopaminergic, glutamatergic, or motoneuronal phenotypes [16]. Meanwhile, those cells have also been differentiated into brain non-neuronal cells, notably astrocytes, oligodendrocytes, microglia [17], as those cells increasingly considered as new targets in drug discovery [17,18].

Studies on iPSCs have notably nourished the interesting concept of ''*disease-in-a-dish*'' [19], with favorable

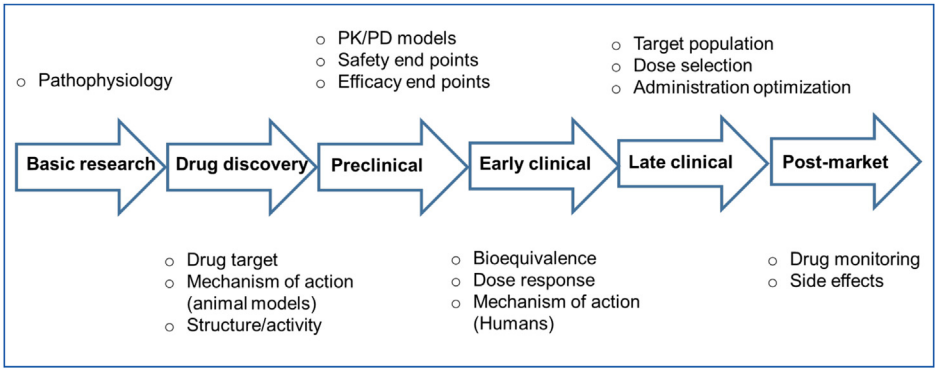

Figure 1. Diverse biomarkers at various stages from drug discovery to safety.

phenotypic modeling of Rett syndrome [20], familial dysautonomia (FD) [21], schizophrenia [22], bipolar disorders [23], spinal muscular atrophy (SMA) [24] or neurodegenerative disorders such as Alzheimer's (AD), Parkinson's and Huntington's disease [25]. Hence, iPSCs represent a unique material for CNS drug discovery, as they are an unlimited source of human tissue with no cross-species issues, using patient-derived cell, with a disease-associated phenotype [26].

iPSCs hence provide a unique platform for phenotype-based drug screening, in the cell type that is to be targeted in the chosen indication [27]. Those assays led to the discovery of drugs that can reverse the disease-associated phenotype, with no particular prerequisite on their molecular targets (Table 1) [28—36]. As an example, FD is a rare fatal genetic disorder affecting neural crest lineages; 6912 small molecules were screened in neural crest precursors derived from iPSCs and lead to the discovery of eight drugs able to rescue the expression of the gene responsible for this disorder [21]. In another indication, amyotrophic lateral sclerosis (ALS), the screenings of 1757 [28] and 5000 [29] compounds on iPSC-derived motor neurons help identifying new potent drugs. iPSC-derived neurons were also screened using a high-content, image-based approach focused on neurite growth with 4421 bioactive small molecules, leading to new drugs and targets [30]. Other drug screening assays have been validated in Parkinson's disease [31], fragile X syndrome (FXS) [32], Niemann-pick disease type C [33] and AD [34]. In parallel to the identification of active drugs, iPSCs are currently envisioned to test potential CNS adverse effects—notably epileptogenic activity of drugs in development or risk of cognitive impairment or neurotoxicity—as new safety and toxicology tools [37].

The concept of ''*disease-in-a-dish*'' has evolved and that of ''*clinical-trial-in-a-dish*'' recently emerged, based on potential to use large cohorts of disease-bearing iPSCs to cellular assays as clinical trials [38]. As a proof of concept, iPSCs from patients with SMA were used to corroborate the clinical responsiveness of those patients to valproic acid with levels of expression of markers in their neuron-derived cells [39], furtherly demonstrating the potential role to select potential responders in clinical trials. In perspective, the idea of ''*pharmaco-iPSCellomic*'' has recently been introduced [40], notably in neurodegenerative disorders [41], based on the person-specific potential of iPSC technology in drug discovery in personalized medicine.

More generally, differentiated cells from iPSC has the potency to address the first four barriers from AstraZeneca's rule, the right target, patient, tissue and safety. However, this huge potential still faces many challenges and limitations. Genetic variability, epigenetic variations or genetic stability and variable differentiation efficiency between different patients or healthy volunteers, as well as between clones from the same individual can generate new phenotypes or mask the disease phenotypes. Other difficulties often encountered with iPSC are reproducibility, end-point maturation, large-scale production and heterogeneity at all stages [38]. Such impact can be reduced thanks to new gene editing tools such as CRISPR/Cas or using large biobanks of patient-derived iPSC lines and clones [26].

New exciting developments have been achieved in the field of iPSCs, with the use of organoids, which are more representative of brain architecture than traditional 2D cultures. Those 3D cultures recapitulate key features of human cortical development, including progenitor zone organization, neurogenesis, gene expression and human-specific glia cell layers [25,42]. Those new innovation have help to model microcephalia [43], autism spectrum disorders [44]. However, 2D and 3D cell culture still lack vascularization, patterning cues and complex cell—cell interactions, animal models should still continue to be necessary for drug evaluation [25]. Alternatively, ''*Organ-on-chips*'', a combined use of iPSC and microfluidics, have very recently been developed, and successfully help modeling familial AD and motor neuron diseases (MND) [42].

The need for new and adequate animal models in CNS disorders

From an historical perspective, non-clinical models have led to the discovery of numerous widely used psychoactive drugs, in major neurological and psychiatric diseases [45]. It is now commonly assumed that current high failure rate in CNS drug development is tightly linked to the poor predictive validity (*i.e.* the prediction of human efficacy) of animal models, despite having face validity (i.e. the same phenotype or the same mechanism than the targeted disease). This is all the more relevant in stroke, traumatic brain injury (TBI), chronic and neuropathic pain

Table 1 Selected examples of compound screening campaigns using iPSCs in CNS indications.

Disorder	Cells	Compounds	Outcomes	Ref.
AD	Neurons from healthy iPSCs	Several hundreds	$A\beta_{1-42}$-induced cellular toxicity	[34]
ALS	Motor neurons derived from ALS patient iPSCs	1,757 compounds	TDP-43 aggregation	[28]
ALS	Motor neurons derived from ALS patient iPSCs	~ 5000 compounds	Neuronal survival	[29]
BD	Neuronal progenitors derived from healthy iPSCs	~ 1500 compounds	Modulation of Wnt/β-catenin signaling	[35]
FD	Neural crest precursors from FD patient iPSCs	6912 compounds	Rescued expression of IKBKAP	[36]
FXS	Neuronal progenitors derived from FXS patient iPSCs	~ 50,000 compounds	Reactivation of the silenced *Fmr1* gene	[32]
NDD	Neurons from healthy iPSCs	4421 compounds	Neurite outgrowth	[30]
NPC	Neural stem cells derived from NPC patient iPSCs	Undisclosed	Lysosomal cholesterol accumulation	[33]
PD	Neurons from PD patient iPSCs	~ 2000 compounds	MEF2 C activity	[31]

AD: Alzheimer's disease; ALS: amyotrophic lateral sclerosis; BD: bipolar disorder; FD: familial dysautonomia; FXS: fragile X syndrome; NDD: neurodegenerative disorders; NPC: Niemann-pick disease type C; PD: Parkinson's disease.

and most neurodegenerative disorders, as those indications are endowed with very limited number of drug approvals [46].

As an example, stroke is a field often perceived by industries as too risky for investing in drug discovery, with numerous large and expensive clinical failures, despite the fact that stroke is the third leading cause of death [6]. The group Stroke Therapy Academic Industry Roundtable (STAIR) was hence formed [47]. This consortium analyzed failed stroke clinical trials and draw conclusions about the reasons of failure, such as the use of insufficiently stringent preclinical models or inadequate timing or dose of administration of tested drugs. STAIR group published non-clinical guidelines to improve probability of success of drugs before clinical development; it was notably recommended to study the impact of candidates on at least two rodent models and one non-human primate mode, with dose-response and time-course evaluation on infract size, histopathology and functional outcomes. NYX-059 was the first drug to reach phase III trials in stroke based on those recommendations, however the trials failed to demonstrate efficacy of the compound. Further proposals are hence required to reduce attrition rates in stroke, as proposed by the STAIR consortium and other groups [48,49].

More generally, STAIR initiative has been followed by other groups in ALS and MND [50], TBI, [51], pain [52] or spinal cord injury [53], often with scientists both from academic and industrial laboratories. Those public—private partnerships are also linked to the open-access of clinical-stage drugs to scientists, thanks to initiatives from the European College of Neuropharmacology, AstraZeneca, the National Institute of Health (NIH) or the Medical Research Council (MRC) in the UK [54], towards better exchanges for higher success rates of CNS drugs development.

Although tremendous efforts have been managed to develop new animal models, it is still considered that they not adequately predict human efficacy. One of the hypotheses has been formulated under the quote *''Treating the biomarker may not treat the disease''* [46], as experienced in AD with bapineuzumab that failed in late-stage clinical trials on clinical outcome but that was clearly efficient to reduce plaque, β-amyloid and phosphorylated tau protein deposition as classical biomarkers of AD [55]. Once again, lessons have to be drawn from those failures to increase success of new drugs [56].

Towards new translational neuroimaging techniques

Imaging of animal models has emerged as an important component of preclinical biomedical research. Small-animal imaging provides a non-invasive approach of exploring biological structures and functions *in vivo* on normal and diseased tissues [57].

The success of imaging is explained by its non-invasive nature, allowing longitudinal studies in animal models, from diagnosis to progression of the pathology, and monitoring of pharmacological effects. Importantly, studies in living animals take into account all the interacting physiological factors, especially in an organ as complex as the brain. Intact whole-animal models accessible to imaging thus facilitate investigation of systemic and integrated aspects of disease, which are almost impossible to fully replicate in *in vitro* or *ex vivo* systems.

In the context of drug development, many imaging studies aim to provide translatable data that will be used in the clinical development process, from preliminary proof of concept to clinical trials. Imaging biomarkers for the drug development (''pharmaco-imaging'') have become more widely used in recent years [58]. Several characteristics of imaging biomarkers favor their use in preclinical

and clinical pharmacological tests to explore the drug's binding localization and to determine its brain transport efficiency, target occupancy or functional effect. This provides an advantage over the classical pharmacological approach because of the opportunity to explore brain neurochemistry *in vivo*, with shorter experimental duration thanks to longitudinal studies. Consequently, imaging can accelerate the drug development process from preclinical studies in animal models to clinical imaging. This explains that the largest pharmaceutical companies have bought or developed their own preclinical imaging centers, or outsourced their biomarker activity to academic centers.

Another key advantage of *in vivo* imaging is the ability to perform multiple types of studies in the same animal. Because each animal can serve as its own control, the number of experimental animals required for a particular study is minimized, on the contrary to classical approaches implying sacrificing the animal for histological and biochemical analysis. Imaging thus provides additional ethical benefit in reducing the number of animals, respecting the 3Rs principle of animal use (replacement, reduction and refinement).

In this context, the choice of the right modality for brain imaging is crucial. There has been recently a rapid increase in the variety of *in vivo* imaging technologies accompanied by the development of specialized hardware dedicated to small-animal imaging, particularly to overcome the limitations of spatial resolution and sensitivity associated with clinical scanners.

The animal models used to study the CNS have their own specificities, two of which in particular have to be considered when embarking on an imaging approach. The first is the complex nature of the brain. In addition to its 3-dimensional structure, it contains several substructures i.e., brain regions, nuclei, etc. The second specificity is that the brain is a particularly well protected organ, first by the skull and then by the surrounding blood-brain barrier, complicating access to brain tissue for diagnostic and therapeutic purposes.

Therefore, the advent of sophisticated technologies for imaging small animals offers an opportunity to learn to manage some of the characteristics mentioned above. Several techniques for molecular imaging are now available for animal studies, from simplistic planar imaging to isotopic imaging and non-radioactive optical imaging. Although the recently developed bioluminescence method is more sensitive than fluorescence for small-animal imaging, these techniques still represent a challenge for cerebral imaging because of heavy scattering and attenuation at the skull interface. Functional ultrasound (fUS) is a recently developed neuroimaging modality designed to monitor whole brain activity during pharmacological stimulations, through the recording of cerebral blood volume dynamics [59]. Despite recent significant progress through pharmaco-fUS [60,61], this technique requires thinning of the cranial bone (in the rat model) and therefore cannot be properly considered as a non-invasive technique.

In contrast, computed tomography (CT) does not suffer from a lack of penetration into deep brain areas. Most X-ray techniques known from clinical medicine have been back-translated to small-animal imaging, from 2D projection imaging (radiography, angiography) to the more advanced 3D CT imaging techniques. However, due to the poor contrast of X-rays in soft tissue, CT applications in brain exploration are restricted to angiography and brain infarct measurement with few applications in neuropharmacology.

Magnetic resonance imaging (MRI) and positron emission tomography (PET) remain the technologies of choice in brain exploration, both for their translational aspect and for the range of brain functions that can be explored within the scope of drug discovery.

MRI has outstanding spatial resolution and the wide range of soft-tissue contrast accounts for its widespread success in brain imaging [62]. This technology allows complementary explorations. MR spectroscopy provides a non-invasive window onto metabolism and can be viewed as an additional contrast beside standard anatomical MRI examination. The concentration of the several metabolites (e.g., creatine, lactate, etc.) can be measured in vivo through a proton spectrum of the rodent brain. Different MRI sequences allows among others the acquisition of anatomical images (T1, T2, T2*), images reflecting the CNS neuronal fiber tracts (diffusion-weighted imaging), images quantifying blood flow and blood volume (perfusion-weighted imaging), images relying on blood-oxygen-level-dependent (BOLD) contrast and highlighting changes in brain activity during a pharmacological stimulation. The MR image contrast or sensitivity can be further enhanced or modified by a variety of contrast agents e.g., gadolinium chelates, metal-based nanoparticles, etc.

Complementarily, PET is a nuclear medicine technology, based on the use of radiopharmaceuticals [63]. PET shows exquisite sensitivity and the ability to provide quantitative in vivo measurements of physiology, metabolic pathways and molecular targets inside tissue [64]. The most common approach is to use the radiotracer [^{18}F]2-Fluoro-2-deoxy-D-glucose ([^{18}F]FDG) is a fluorine-18-labeled glucose analog, which is transported into the cell, phosphorylated, and trapped within the cytoplasm. Thus, changes in the rate of glucose metabolism, reflected by the [^{18}F]FDG PET signal correspond to changes in neuronal and glial functions. Other PET radioligands are radiolabeled with a high specific activity that allows a very small amount to be injected. These so-called 'tracers' enable visualization and quantification of brain receptors in areas where they concentrate at picomolar levels. According to the experimental protocols, the outcome measures of neuroreceptor imaging are receptor/transporter density, binding affinity to the target of interest, quantification of receptor occupancy by a drug, which may be correlated to therapeutic effect and, more rarely, dynamic measure of neurotransmission by measuring acute variations in extracellular neurotransmitter concentrations in the living brain. All of these paradigms can be applied to the exploration of drug candidates at different stages of their development [65].

Big data, artificial intelligence and identification of new CNS drugs

Due to the availability of big data and to its diversity—results of internal projects, scientific literature, patents, ''omics'' data, real world evidence, images, videos, etc. —, the medico-scientific and pharmaceutical community relies

Table 2 Selected examples of repurposed drugs in CNS indications using in silico approaches.

Drug	Initial indication	New indication	Methodology	Ref.
Aliretinoin	Cancer	ALS	Polypharmacology for targeting multiple etiopathogenic pathways Repositioning analysis based on systems biology approach (TPMS technology)	[92]
Benperidol	Schizophrenia	AD	Computational method based on ligand-protein interaction Five major protein targets (AChE, BuChE, BACE-1, MAO and NMDA)	[93]
Fasudil	Stroke	NDD	Building of gene expression networks Identification of new autophagy inducers	[91]
Phenoxybenzamine	Hypertension	Pain	Identification of differentially expressed genes in dorsal root ganglia in a rat pain model Use of the CMap approach to reverse this signature	[90]
Pridopidine	HD	LID	IBM Watson ranked pridopidine 59/3527 candidate compounds (top 1.6%) representing a strong prediction of anti-dyskinetic efficacy	[94]

AD: Alzheimer's disease; ALS: amyotrophic lateral sclerosis; HD: Huntington's disease; LID: levodopa-induced dyskinesia; NDD: neurodegenerative disorders.

more and more on artificial intelligence (AI) to increase the speed and the precision of data mining and data analysis to improve decision-making processes applied to drug discovery [66—71].

Computer-aided drug discovery has hence been widely used over the past two decades, with increasing application in the field of CNS disorders. Target-based discovery approaches are typically based on physicochemical rules or quantitative structure — activity relationships; in this field deep learning approaches have successfully defined new chemical entities in Alzheimer's dementia, with an computer-backed chemical evolution of an acetylcholinesterase inhibitor drug into brain-penetrable ligands with either specific polypharmacology or selectivity profiles for G-protein-coupled receptors; new drugs in PD were also designed using structure- and ligand-based *in silico* approaches [72]. Target-based approaches have also been linked to PK-modeling for better design of CNS-accessible drugs [73]. On the other end of the spectrum, computer-assisted research uses systems biology to discovery new drug in CNS disorders. As an example, quantitative systems pharmacology (QSP) is based on mathematical complex and clinically calibrated models of human neuronal circuitry, used to better identify and characterize efficient drugs in neurological disorders [74]; based on this modeling new polypharmacological approaches have been proposed in cognitive impairment in schizophrenia [75]. New deep learning algorithms are now increasingly powerful and quicker than machine learning tools or more classical procedures. A recent study described how those algorithms help produced totally new and highly active chemical entities in less than 21 days and valid *in vitro* and *in vivo* in just 46 days, notably opening broad avenues in CNS drug discovery [76].

A drastically different approach can also be followed. Drug repositioning refers to the use of a drug for the treatment of a new indication different from the drug's intended one. Several examples are often presented, in excessive daytime sleepiness [77—80], PD [81], neuropathic pain [82] or AD [83,84]. Those successes are relying on ''*activity-based*'' repositioning activities, using drug screening procedures, case reports, animal studies, etc. Chemical libraries of already approved drugs are already available and often screened in cell cultures [85], typically Prestwick Chemical Library with 1280 compounds [86,87], or SelleckChem Library with 2572 drugs [88]. In this review, we focused on ''*in silico-based*'' repositioning processes, referring to the use of public databases and bioinformatics tools to identify new drugs and targets [89] (Table 2) [90—94].

Different approaches are commonly used, the most frequently used is based on the comparison of transcriptomic signatures, lists of over- and under-expressed genes modulated (i) in the indication and (ii) after treatment of a biological material (cell line, tissue, animal, blood, etc.) with marketed drugs. The connectivity map (CMap) is an database widely used to repurpose drugs [95], successfully applied in pain with a non-CNS drug [90] or in neurodegenerative disorders (NDDs) [91] or to repurpose a CNS drug, topiramate [96]. The concept of network-based algorithms based on the use of big data has then evolved and is more and more used in the field [91]; other recent approaches potentially compliant with CNS context have been nicely reviewed by March-Vila and colleagues [97]. Recently, the use of social media or electronic health records (EHR) of the patients, providing medications details along with patient history, have also been taken as new sources of information for drug discovery; those records have been mined in epilepsy [98], multiple sclerosis [99] or to characterize the profile of antipsychotics [100].

Conclusion and perspectives

Over the last years, the discovery of new treatments in CNS has benefited from new breakthroughs. In 2019, for the

first-time, a gene therapy in a CNS disorder got approved by the FDA, with an adeno-associated viral vector developed by AveXis, for pediatric patients suffering from SMA [101]. In parallel the Human Brain Project, a large European flagship project, at the interface between neuroscience, computing, informatics, and brain-inspired technologies also opens new opportunities in the field [102]. Even larger initiatives, such as the International Brain Initiative (IBI) have been established to coordinate efforts across national and regional brain initiatives [103], further widening the horizon in CNS drug discovery.

Disclosure of interest

MC and FM are employees of Theranexus; VL is an employee of Insiliance. MC and LZ are members of the SFPT, Société Française de Pharmacologie et de Thérapeutique.

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