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Activity-Based Protein Profiling Delivers Selective Drug Candidate ABX-1431, a Monoacylglycerol Lipase Inhibitor, To Control Lipid Metabolism in Neurological Disorders

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ABSTRACT: Monoacylglycerol lipase (MGLL or MAGL) is a critical point of regulation of both endocannabinoid and eicosanoid signaling pathways in the brain, thereby providing novel therapeutic opportunities for neurological and neurodegenerative diseases. In this issue Cisar et al. disclose the discovery, optimization, and initial preclinical profiling of ABX-1431, a covalent, irreversible MGLL inhibitor. Activity-based protein profiling was key to the discovery of ABX-1431. ABX-1431 is a first-in-class experimental drug that was well-tolerated and safe in phase 1 clinical studies. Data from an exploratory phase 1b study indicate that it has the potential to treat symptoms of adult patients with syndrome of Gilles de la Tourette. ABX-1431 is currently entering clinical phase 2 studies for this neurological disorder as well as for other indications, such as neuromyeltis optica and multiple sclerosis.

Drug discovery for disorders of the central nervous system (CNS) is hard. Several factors contribute to the daunting task to discover novel therapies for brain diseases. First and foremost, there is a lack of validated therapeutic targets largely because of our limited understanding of the function of the brain in health and disease. Once a potential suitable target has been identified, the optimization of small molecules into drug candidates is complicated by the strict physicochemical properties required to pass the blood−brain barrier. Furthermore, the determination of the target-interaction landscape (i.e., its selectivity profile) of the drug in human brain is essential to avoid disasters as recently witnessed with fatal phase 1 clinical trial of BIA 10-2474. A volunteer died due to an overdosis of BIA 10-2474.[1] Thus, studies enabling target and off-target engagement in the brain are essential to guide drug discovery and development.[2,3] In this issue of *J. Med. Chem.* Cisar and colleagues report the discovery, optimization, and profiling of ABX-1431 (Figure 1), a first-in-class experimental drug of monoacylglycerol lipase (MGLL, also termed as MAG lipase), using activity-based protein profiling for the treatment of neurological disorders, including neuropathic pain and syndrome of Gilles de la Tourette.[4]

MGLL is a membrane-bound enzyme that belongs to the family of serine hydrolases.[5,6] It is the principal metabolic enzyme that controls the levels of 2-arachidonoylglycerol (2-AG) in the brain.[7] 2-AG acts as an endogenous agonist of the cannabinoid CB_1 and CB_2 receptors. MGLL catalyzes the hydrolysis of the ester bond in 2-AG, thereby terminating the 2-AG-mediated signaling of the CB_1/CB_2 receptor and producing arachidonic acid and glycerol (Figure 1). 2-AG serves as an important source of arachidonic acid, the precursor of proinflammatory prostaglandins, in the brain. In vivo studies have shown that inhibition of MGLL leads to CB receptor dependent antinociceptive effects in mouse models of inflammatory and neuropathic pain. MGLL inhibitors exert also anxiolytic and anti-inflammatory effects. In various animal models of neurodegeneration, including Parkinson's disease, Alzheimer's disease, and acute brain injury, MGLL inhibition exerted neuroprotective effects by reducing proinflammatory prostanoid and cytokine signaling independent of the CB_1 receptor. Thus, emerging data suggest that MGLL is a critical point of regulation of both endocannabinoid and eicosanoid signaling pathways in the brain, thereby providing novel therapeutic opportunities.

To this end, several academic groups and pharmaceutical companies have developed MGLL inhibitors that have a reversible or irreversible mode-of-action.[8] Irreversible inhibitors that covalently interact with the catalytic serine (Ser-122) of MGLL, may achieve higher potency and sustained inactivation of the enzyme, thereby putting less demand on the pharmacokinetic properties. Determination of the selectivity profile of mechanism-based covalent inhibitors is, however, essential because other proteins from the same enzyme family of the primary target may also react with the warhead of the experimental drug in the same fashion. This could lead to unwanted side effects or toxicity. BIA 10-2474, for instance, is a mechanism-based covalent fatty acid amide hydrolase inhibitor that reacted with several lipases and disrupted the metabolic profile of human cortical neurons.[3] Thus, assessment of the interaction profile of the covalent inhibitor in human cells and brain is important.

Cisar et al. used activitity-based protein profiling (ABPP) as the central technology for the discovery, optimization, and profiling of their clinical MGLL inhibitor ABX-1431.[4] Competitive ABPP is an efficient chemical biology approach to study target engagement and interaction-landscape of covalent irreversible inhibitors in living systems.[2,3] It makes use of broad-spectrum chemical probes that report on the abundance of active enzymes in lysates, (human) cells, or even intact animals. The interaction of a small molecule with endogenously expressed enzymes, including all post-transla-

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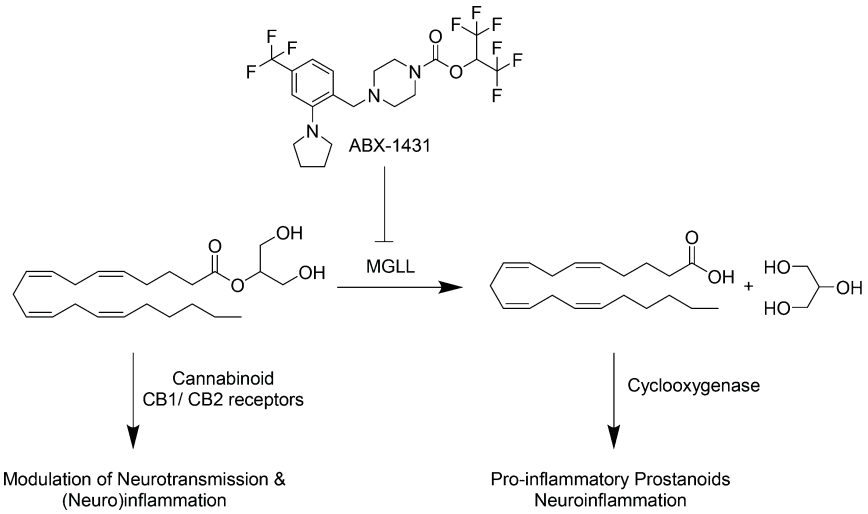

Figure 1. Clinical candidate ABX-1431 is a monoacylglycerol lipase (MGLL) inhibitor that prevents the hydrolysis of the endocannabinoid 2-arachidonoylglycerol in the human brain. It prolongs the action of 2-arachidonoylglycerol on the cannabinoid CB1 and CB2 receptors and reduces the formation of arachidonic acid, the substrate of proinflammatory prostanoids, thereby alleviating neurological symptoms and reducing neuroinflammation.

tional modifications, protein−protein interactions in the presence of endogenous substrates, can be assessed in one single experiment. ABPP makes use of activity-based probes consisting of warhead, recognition element, and reporter group. A fluorescent reporter group is used for gel-based ABPP, whereas a biotin reporter allows mass spectrometry (MS)-based identification of the interacting proteins.

Cisar et al. used the prototypical fluorophosphonate (FP)-based probes to assess the interaction of their MGLL inhibitors on the serine hydrolase family.[4] JZL184 and KML29 were used as a starting point for the rational design of novel MGLL inhibitors. Careful optimization of the activity and selectivity using gel-based ABPP with multiple human proteomes and rodent brain homogenates led to the discovery of ABX-1431 (1,1,1,3,3,3-hexafluoropropan-2-yl-4-(2-(pyrrolidin-1-yl)-4-(trifluoromethyl)benzyl)piperazine-1-carboxylate), which was selected as the lead compound for clinical evaluation. ABX-1431 is a potent human MGLL inhibitor with an average IC_{50} of 14 nM that only cross-reacted to a minor extent with α,β-hydrolase domain containing protein 6 (ABHD6), PLA2G7, and some carboxylesterases. The compound maintained activity and selectivity in human cellular assays and in human prefrontal cortex proteomes as determined by MS-based ABPP. ABX-1431 is a lipophilic molecule and has a basic amine, yet it has only weak hERG channel activity with an IC_{20} of 7 μM. The compound did not display any significant activity against a panel of common off-targets and has low propensity to CYP-inhibition. ABX-1431 demonstrated acceptable pharmacokinetics in rodents and dogs. It inhibited MGLL activity with an ED_{50} of 0.5−1.4 mg/kg (po) and dose-dependently increased brain 2-AG levels in mouse brain. A rat inflammatory pain model was used to assess the pharmacodynamics effect. ABX-1431 demonstrated potent antinociceptive effects in a formalin paw test at a dose that produced near complete MGLL inhibition and maximal elevation of 2-AG. Other pharmacological effects were not (yet) described.

Currently, ABX-1431 is being tested in at least five different clinical trials (www.clinicaltrials.gov). Notably, it has successfully completed phase 1 clinical trials.[10] The compound was generally well-tolerated and safe. The most commonly observed adverse effects were headache, somnolence, and fatigue. It inhibited MGLL in the brain in a dose-dependent manner as demonstrated with a PET study. Importantly, in a randomized, double-blind, placebo-controlled crossover, exploratory phase 1b study, ABX-1431 was able to show a positive impact on key measures of symptoms in adult patients with the syndrome of Gilles de la Tourette.[10] It is now entering phase 2 clinical trial for this indication (NCT03625453). The compound will also be tested in neuromyeltis optica, multiple sclerosis and as an add-on therapy in patients suffering from central neuropathic pain (NCT03138421).

In summary, ABX-1431 is a first-in-class MGLL inhibitor that was discovered and optimized using ABPP. ABX-1431 has entered phase 2 clinical trials and shows promising preliminary results in patients suffering from a neurological disease. It will be interesting to see whether MGLL inhibitors mimic some of the psychoactive effects of cannabinoid CB_1 receptor agonists, such as Δ^9-THC, the psychoactive component in marijuana, or whether chronic dosing leads to functional antagonism of the CB_1 receptor.[5,9] Finally, the study presented by Cisar et al. emphasizes the crucial role of ABPP in drug discovery and development of covalent irreversible inhibitors and will spur the field of MGLL inhibitors. It is hoped that MGLL inhibitors, such as ABX-1431, may provide a new treatment option for Tourette's syndrome and patients with neuroinflammatory conditions.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

MGLL or MAGL, monoacylglycerol lipase; 2-AG, 2-arachidonoylglycerol; CB_1R, type 1 cannabinoid receptor; CB_2R,

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type 2 cannabinoid receptor; ABPP, activity-based protein profiling; FP, fluorophosphonate; ABHD6, α,β-hydrolase domain containing protein 6

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