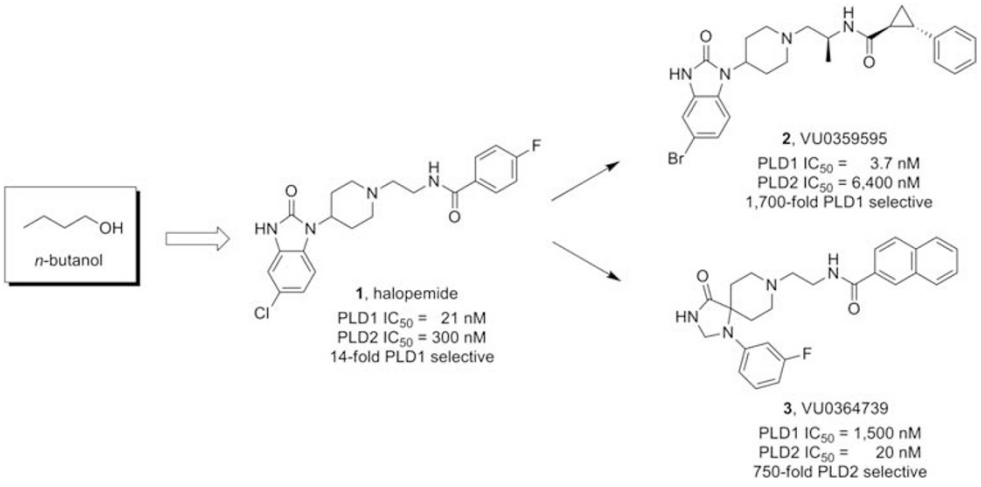

Figure 1. Evolution of small molecule, isoform-selective PLD inhibitors.

n-butanol, a small molecule that is not a PLD inhibitor, rather *n*-butanol blocks PLD-catalyzed phosphatidic acid production by competing with water as a nucleophile, thereby generating phosphatidylbutanol in a competitive transphosphatidylation reaction. A renaissance in the PLD inhibitor field began in 2007 when halopemide (**1**), a psychotropic agent discovered in the late 1970s, was shown to be a dual PLD1/2 inhibitor (Scott *et al*, 2009). More importantly, **1** has been in humans in five clinical trials and was shown to be safe and effective; thus inhibition of PLD with a small molecule is a viable therapeutic approach, a finding also noted in the PLD KO mice. Using **1** as a lead, a diversity-oriented synthesis campaign was pursued by the Brown and Lindsley labs, where ~1000 analogs of **1** were synthesized and evaluated in cell-based and biochemical PLD1 and PLD2 assays (Scott *et al*, 2009). From this effort, isoform-selective PLD1 (**2**) and PLD2 (**3**) inhibitors were developed with low nanomolar potencies, unprecedented PLD isoform selectivity and DMPK profiles to allow *in vivo* target validation studies to be pursued (Lavieri *et al*, 2010; Figure 1).

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DISCLOSURE

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Transferrin Antibodies into the Brain

Opening the central nervous system (CNS) to antibody therapies would substantially improve our ability to selectively target neurological disease. However, brain uptake of antibodies is limited by the presence of the blood–brain barrier (BBB). Over the past 20 years, progress has been made in designing methods to improve uptake of antibodies via molecular engineering, with most attention being placed on utilizing the BBB's endogenous mechanisms to transport proteins into the brain, known as receptor-mediated transcytosis (RMT; Jones and Shusta, 2007). Nevertheless, challenges in both understanding the biology of BBB transport and in engineering antibodies to optimally cross the BBB remain. In particular, the majority of studies assessing RMT pathways at the BBB have relied on radiolabeled proteins. However, from a drug development standpoint, success is only achieved if antibody is delivered to the brain in sufficient

quantities to be therapeutically beneficial. Thus, this experimental approach may be misleading, as trace doses do not assess the therapeutic capacity of a particular RMT pathway.

Transferrin, insulin, Apo-proteins, IGF-1, and leptin, are among an ever-increasing list of proteins that have been proposed to undergo RMT at the BBB. The transferrin/transferrin receptor (TfR) system, which mediates cellular uptake of iron, has been of particular interest as a pathway to increase uptake of biologics into the brain. Early studies with anti-TfR antibodies showed promise for the TfR pathway (Friden *et al*, 1991). Nevertheless, subsequent studies questioned how effective the TfR pathway is in driving CNS uptake of Tf itself (Crowe and Morgan, 1992), and also questioned the ability of anti-TfR antibodies to traverse the BBB and distribute throughout the brain (Moos and Morgan 2001). These, and subsequent studies, showed that anti-TfR antibodies largely remained trapped in the BBB vasculature and cast doubt on the TfR pathway as a route to transport therapeutic antibodies into the brain.

An additional limitation to understanding antibody uptake in brain has been the lack of robust and acute readouts of antibody activity. Pharmacodynamic measures of antibody function allow for the establishment of a relationship between drug levels and drug activity, termed the pharmacokinetic/pharmacodynamic (PKPD) relationship. We recently developed an antibody that would allow us to address the PKPD relationship in brain, by targeting the enzyme β-secretase (*BACE1*), an Alzheimer's disease drug target, which is required for the production of β-amyloid (Abeta; Atwal *et al*, 2011). Using this antibody, we were able to show a direct relationship between drug levels and activity (Abeta reduction) in brain. Furthermore, it was confirmed that normal antibody uptake in brain is both limited and dose-dependent, with the steady state concentrations in brain ranging from 0.05–0.2% of injected dose.

In search of a solution to increase the penetration of anti-BACE1 in brain, we turned to the most studied RMT pathway, TfR, and generated antibodies to evaluate uptake in brain in both trace and therapeutic dosing paradigms (Yu *et al*, 2011). Initial studies with our high-affinity anti-TfR antibody matched those of others; despite a substantial increase in initial drug levels as measured by trace dosing, therapeutic dosing resulted in limited uptake and was almost completely localized to the BBB vasculature. To solve this problem, we engineered anti-TfR antibodies with lower affinity for TfR, and observed an inverse relationship: reduced uptake in trace dosing paradigms and increased uptake in therapeutic dosing paradigms. More importantly, the engineered low-affinity anti-TfR antibodies distributed broadly throughout the brain, allowing us to combine anti-TfR and anti-BACE1 as a bispecific antibody to improve penetration and activity in brain. We therefore propose that RMT is indeed a viable route to the brain; however, translating these findings to humans through testing bispecifics in higher species, including safety studies, is an important next step for the anti-TfR/BACE1 program, and this approach in general.

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DISCLOSURE

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Endogenous Opioids as Physiological Antidepressants: Complementary Role of Delta Receptors and Dopamine

Major depressive disorder was shown to be associated with a reduction in response to rewarding stimuli in the dopaminergic mesolimbic pathway in a recent neuroimaging study (Forbes *et al*, 2009). This neuronal network is modulated by opioids at the level of dopamine (DA) neurons and afferent structures, typically by activation of mu- and delta-opioid receptors (MORs and DORs, respectively) enhancing reward- and motivation-related processes. Therefore, a deficit in endogenous opioids, mainly enkephalins (ENKs), in the nucleus accumbens and ventral tegmental area, may lead to a decrease in the neurobiological control of mood states and reward. To develop fast-acting therapeutics for severe depression, particularly in adolescents, MORs and DORs have been investigated for potential antidepressant activity, using: (i) exogenous agonists and antagonists for both receptors and (ii) ENKs protected from their inactivating enzymes by dual inhibitors such as RB101 (N-(R,S)-2-benzyl-3-((S)(2-amino-4-methyl-thio)-butyl-dithio)-1-oxopropyl)-1-phenylalanine benzyl ester) (Noble and Roques, 2007), which induced a synaptic