CLX-105

CLX-105: A NOVEL MOLECULAR CONJUGATE OF VALPROIC ACID AND EICOSAPENTAENOIC ACID FOR THE TREATMENT OF EPILEPSY, BIPOLAR DISORDERS AND CANCER

- A novel pro-drug conjugate of Valproic acid (VPA) and Eicosapentaenoic acid (EPA)
- Designed for slow sustained release of the actives
- Host enzyme mediated release of the actives
- VPA and EPA can have beneficial synergistic pharmacological effects
- Expected to be more efficacious than VPA for approved indications
- Expected to have better hepatic and gastric safety profile than VPA
- Potential opportunity in oncology considering histone deacetylase inhibitory activity of VPA
- Preclinical studies are ongoing
- Robust patent estate

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1. Opportunity Overview

CLX-105 is a novel molecular conjugate of Valproic acid (VPA) with Eicosapentaenoic acid (EPA). In this conjugate, VPA is chemically linked with EPA utilizing CellixBio's proprietary patented Synergix platform technology. VPA is linked with EPA through a novel linker moiety using simple ester linkages. The ester bonds undergo hydrolytic cleavage by the host hydrolytic enzymes present in the small intestinal fluid, intestinal epithelium and liver microsomes. The hydrolytic cleavage of ester bonds in the molecule is expected to control the release profile of VPA and EPA in the intestine and ensure a slow and sustained release and systemic absorption of the active moieties.

CLX-105 is designed to provide superior pharmacokinetic, pharmacodynamic and safety advantages over the currently approved products of VPA in the treatment of epilepsy and bipolar disorders. EPA is approved by US FDA as an adjunct to diet to reduce triglyceride and apolipoprotein B levels in patients with very high (≥500 mg/dL) triglycerides with proven clinical safety for chronic use. In addition to its effect in controlling TG levels, EPA is reported to have several beneficial biological effects that include anti-inflammatory, modulation of ion channels, growth and differentiation, controlling oxidative stress, stabilization of neuronal membranes, modulation of gene expression, neurotransmission and learning and memory (Taha A.Y. *et al*., 2008 and 2010; Larsson S.C. *et al*., 2004), which are expected to provide synergistic pharmacological effect with VPA as well as minimize the hepatic adverse effects of VPA. EPA is also reported to be effective in various in-vitro models as well as in controlling seizures in patients with epilepsy (Taha A.Y. *et al*., 2010). Similarly the efficacy of EPA in bipolar disorders has also been shown in several randomized controlled clinical trials (Grosso G *et al*., 2014).

CLX-105 also presents potential new opportunities in the treatment of cancer. In a large series of preclinical studies, exposure to VPA resulted in dose-dependent reversible cell cycle arrest and cell growth inhibition as well as chromatin decondensation and cellular differentiation in several neoplastic cell models (Duenas-Gonzalez A *et al,* 2008*).* Several

phase I and II studies in adults with hematologic and solid malignancies showed that VPA treatment, either as a monotherapy or combined with other agents, was well tolerated and resulted in encouraging tumour responses (Chavez-Blanco A. *et al*., 2005; Munster P. *et al*., 2009; Krauze AV *et al.,* 2015; Avallone A *et al*., 2014;).

So far, we have completed biopharmaceutical assessment and a preliminary pharmacokinetic study in the rats. Studies that are ongoing / planned include: clinical formulation development, pharmacokinetic studies in large animal models, efficacy studies in animal models of disease such as drug resistant epilepsy and anticancer models and preclinical safety studies. The expected target product profile/differentiation opportunities of CLX-105 include:

- ✓ Oral route of administration
- ✓ Low potential to cause upper GI irritation due to delayed release of VPA
- ✓ Low potential for hepatic adverse effects due to protection by EPA
- ✓ Proven chronic clinical safety of VPA and EPA as individual entities
- ✓ Superior efficacy than VPA in approved indications
- ✓ Potential for new indications such as disease resistant epilepsy and oncology.

2. Market Description

The global market for epilepsy yielded $4.2 billion in sales during 2012 and $4.5 billion in 2014. The introduction of novel antiepileptic drugs and increased access to therapies is expected to drive market growth. By 2022, global sales are expected to rise to $5.4 billion, with more than 50%, or $2.7 billion of those sales in the U.S. Although there are many generic drugs for the treatment of epilepsy, the current focus is to develop safe adjunctive therapies for refractory patients (Gohil K., *et al*., 2014).

Bipolar disorder affects approximately 5.7 million adult Americans, or about 2.6% of the U.S. population age 18 and older every year. The median age of onset for bipolar disorder is 25 years (National Institute of Mental Health), although the illness can start in early

childhood or as late as the 40's and 50's and it is the sixth leading cause of disability in the world (World Health Organization). Sales of drugs used to treat bipolar disorder were at the value of $6.3 billion in 2011. The Depakote ER and its generic counterparts had combined U.S. sales of approximately $194 million for the 12-month period that ended in June, 2013.

The current market size in the treatment of all solid tumors is USD 10 billion (Estimation by Bionomics, Roth Conference, 2014). Global sales for top-20 drugs cancer drugs was USD 55 billion in 2014 (Reported by First World Pharma, 2015).

3.Unmet Medical Need

Epilepsy:

Even though seizures are well-controlled with currently available anti-epileptic drugs (AEDs), seizures persist in a substantial proportion (approximately 30%) of epilepsy patients, who do not respond to any of two to three first line AEDs, despite administration in an optimally monitored regimen (Remy S and Beck H., 2006). In addition, Pharmaco-resistance also reported to occur frequently in patients with partial seizures. It is apparent that there are more than 7.5 million people in the world are with refractory complex partial seizures (Treiman D.M., 2011). Overall, Seizures in approximately, 30% to 40% of patients with epilepsy fail to respond to antiepileptic drugs or other treatments. While much has been made of the risks of new drug therapies, not enough attention has been given to the risks of uncontrolled and progressive epilepsy (Laxer *et al*., 2014). CLX-105 could potentially offer to reduce the incidence of drug resistance in epilepsy due to its synergistic pharmacological effects with EPA for patients who are on VPA or combination with other drugs.

Bipolar disorders:

Despite the number of treatment options for bipolar depression has increased over the last decade, there is a substantial unmet need for new interventions that are efficacious and effective, and that have a low side effect burden (Frye *et al*., 2014). Valproic acid is one of the approved drugs for acute mania in bipolar disorder; but not indicated for bipolar

depression. In a systemic review and meta-anlaysis of four trials (with a total sample size of 142 patients with bipolar depression) indicates that the relative risks of response and remission were significantly greater for divalproex than placebo. Mean response rates were 39.3% for divalproex and 17.5% for placebo, and mean remission rates were 40.6% and 24.3%, respectively. These results provide preliminary evidence that divalproex is efficacious in the treatment of BD depression (Bond D.J *et al*., 2010). Eicosapentaenoic acid in several clinical trials had shown to be more effective and safer in bipolar depression (Frangou S., *et al*, 2006; Osher Y and Belmaker RH., 2009). Significant effect of omega-3 fatty acids were reported in ameliorating depressive symptoms in adults with bipolar disorder (Grosso G., et al 2014). CLX-105 could be a potentially differentiating and disease modifying drug, effective in both in bipolar mania and depression and also could offer better safety profile than valproic aid.

Oncology:

There is an ongoing need for the development of new cancer therapies that can effectively target tumor cells and to reduce significant morbidity and mortality without harming normal cells or tissue. There are several HDAC inhibitors currently in various stages of the clinical development and two molecules were approved by USFDA for the treatment of cutaneous T-cell lymphoma and peripheral T-cell lymphoma (Vorinostat and Romidepsin). The class related adverse reactions reported were neutropenia, lymphopenia, thrombocytopenia, GI events (nausea, fatigue, vomiting and anorexia), anaemia and ECG T wave changes. Valproic acid is a HDAC inhibitor with long history of human use for chronic indications and is clinically well tolerated. Additionally, several clinical studies are on-going with VPA in combination with standard of care in various tumour types. CLX-105 could offer several advantages that include sustained exposure, effective delivery to tumor tissues and highly improved safety.

4. Scientific Rationale

Epilepsy/bipolar disorders:

VPA is a short-chain fatty acid and FDA approved for epilepsy/bipolar mania/migraine. VPA acts by multiple mechanism of action that include inhibition of fast and persistent Na+ current, T-type Ca+ channels, increase GABA turn over, inhibit HDAC activity there by modulate gene expression. EPA is shown to modulate ion channels, inhibit chemical/electrical excitatory signals in rat hippocampal slices, inhibit CA3 evoked AP in brain slices, stabilize the neuronal membranes and suppress Na+ and Ca+ channels. Mixed results are reported in efficacy studies animal models of epilepsy. Recent clinical studies with omega-3 fatty acids show strong POC in drug resistant epilepsy (DeGiorgio CM *et al*, 2015). Significant effect of omega-3 fatty acids were reported in ameliorating depressive symptoms in bipolar disorder (Grosso G., et al 2014). Based on this Cellix believes that combining VPA with EPA could offer significant beneficial synergistic pharmacological effects that could translate into improved efficacy in epilepsy and bipolar disorders in relation to VPA alone.

Oncology:

VPA has been shown to inhibit histone deacetylases (HDACs). HDACs regulate the acetylation of a variety of histone and non-histone proteins, controlling the transcription and regulation of genes involved in cell cycle control, proliferation, survival, DNA repair and differentiation. Histone Deacetylase inhibitors (HDACi) represent a new class of antitumor agents based on the function of the epigenetic enzymes they regulate, multiple genes and pathways. Several HDACi are currently in clinical development as anticancer agents and two (Vorinostat and Romidepsin) have been approved by the US FDA for the treatment of cutaneous T-cell lymphoma.

VPA has been considered a good candidate for anticancer therapy, due the HDAC inhibiting activity and the safety record of chronic human use. In preclinical studies, exposure to VPA results in dose-dependent reversible cell cycle arrest and cell growth inhibition as well as

chromatin decondensation and cellular differentiation in several neoplastic cell models. The ability of VPA to inhibit deacetylase activity in solid tumors has been demonstrated in monotherapy at oral doses between 20 and 60 mg/kg (Chavez-Blanco A *et al.*, 2005). In a phase I/II trial of VPA in combination with Epirubicin or in combination with 5-Fluorouracil, Epirubicin, and Cyclophosphamide (FEC100) for patients with solid tumors, 44 patients received escalating doses of valproate with a fixed dose of Epirubicin. The maximum tolerated dose (MTD) was 140 mg/kg/day with nine patients achieving a partial response. During the second part of the study, a disease-specific cohort of 15 breast cancer patients were treated with 120 mg/kg/day Valproate and the combination regimen FEC100. With nine out of 14 patients responding to therapy; Overall, somnolence was the most noted adverse effect related to VPA and the acetylation levels measured in peripheral blood mononuclear cell (PBMC) correlated with VPA serum levels (Munster P *et al.,* 2009*).* The common adverse effects associated with HDAC inhibitors include thrombocytopenia, neutropenia, diarrhoea, nausea, vomiting and fatigue. Toxicity reported were class-specific and has been observed with all HDAC inhibitors. However, differently from other HDAC inhibitors, VPA showed a good safety profile. CLX-105 could be of significant value add in improving the chronic tolerability as a standalone as well as in combination regimens with standard of care (SOC) anticancer drugs in cancer patients.

Hepatic safety:

VPA is reported to cause two types of hepatic adverse effects in humans. Type-I is an acute and rare form that is irreversible and idiosyncratic. This is characterized by micro-vesicular steatosis and necrosis, histological characteristics similar to Reye syndrome. Children under the age of 2 years, on a multidrug antiepileptic regimen are primarily at risk (1/500–1/800). The incidence in general population is 1/20,000. Type II hepatic adverse effects are reported mainly in a chronic regimen. This is milder, reversible, dose related and is seen mainly seen at the start of treatment. Approximately 44% of patients have elevated levels of liver enzymes without clinical symptoms.

Mitochondria are proposed as the key target for the hepatic toxicity caused by VPA. The key features includes, inhibition of fatty acid oxidation, Reye-like syndrome in liver toxicity, dicarboxylic aciduria in patients, decrease in plasma ketone bodies and formation of reactive metabolites, oxidative stress and drug-induced coenzyme A/Carnitine deficiency.

Omega 3 FAs that include EPA/DHA are known to protect mitochondria as well as liver toxicity induced by VPA (Jeng JY, *et al*., 2009; Abdel-Dayem MA *et al*., 2014; El-Mowafy AM *et al*., 2011). The mechanism of protection could be by stimulating beta oxidation, protection from oxidative stress and inhibiting the inflammation through GPR120 receptors (Nobili V *et al.*, 2014).

5. Preclinical Summary

Several preclinical investigations were carried out with CLX-105 in order to assess the biopharmaceutical and pharmacokinetic properties.

The biopharmaceutical properties of CLX-105 (solubility, physico-chemical properties and stability in simulated intestinal fluids) are suitable for developing a delayed and extended release oral dosage form for optimal delivery. Additionally, in-vitro stability studies showed that VPA-EPA conjugate was stable in simulated gastric fluid while undergoes cleavage in the simulated intestinal fluids. This condition is ideal for developing a delayed and extended release formulation that could potentially improve the GI tolerability of VPA. Additionally, CLX-105 undergoes rapid cleavage in liver microsomes of rats, dog and humans, thereby minimizing the systemic exposure to the conjugate per se.

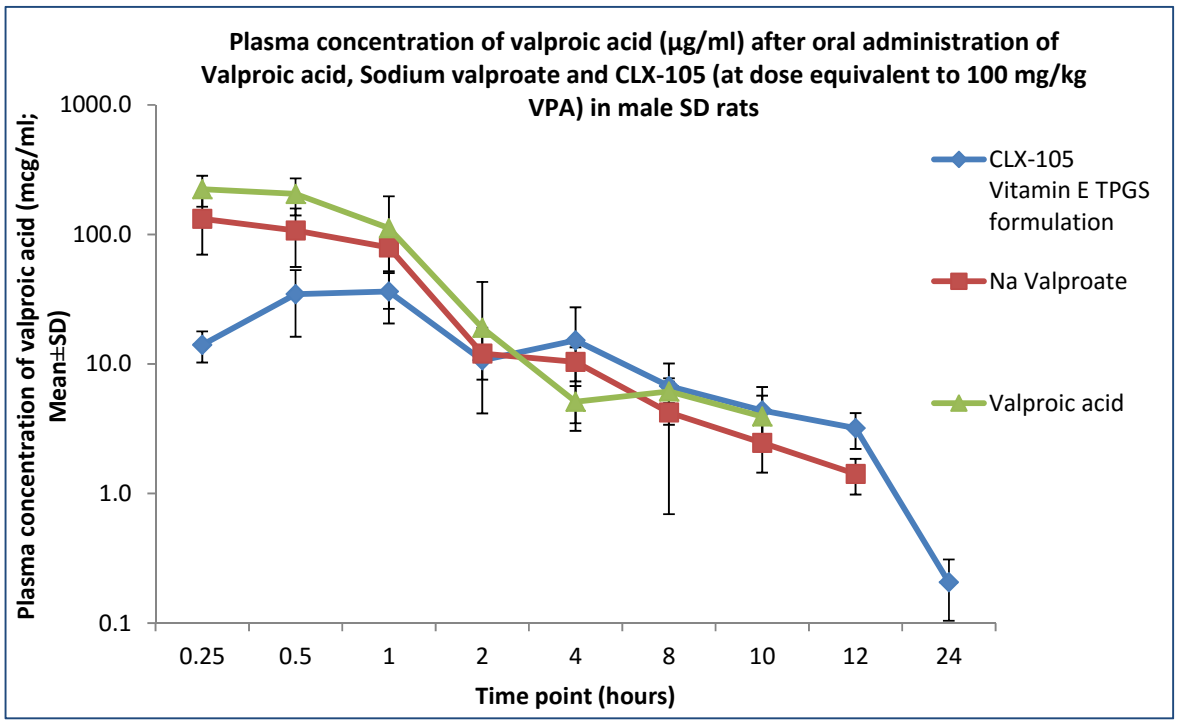
Oral (gavage) pharmacokinetics of VPA-EPA conjugate was studied in rats (at equivalent dose of 100 mg/kg valproic acid) using a Vitamin E TPGS emulsion formulation. The key results are summarized below.

- A substantial reduction in C_{max} in comparison with the valproic acid and sodium valproate administration

- Prolonged T_{max} in comparison with the valproic acid and sodium valproate administration
- Relative bioavailability was approximately 54% in comparison with valproic acid and 76% in comparison with sodium valproate.

  Significant plasma levels of VPA seen at 12 hours and 24 hours with VPA-EPA conjugate in comparison with the Na-VPA administration suggestive of extended absorption from the GI tract.

Analyte: Valproic acid			
Compound	**C_{max} (µg/mL)**	**T_{max} (h)**	**AUC_{0-t} (µg.h/mL)**
Valproic acid (VPA) 100 mg/kg	231 ± 51.0	0.33 ± 0.13	283 ± 119
Sodium valproate (Equivalent to 100 mg/kg VPA)	113 ± 59.5	0.40 ± 0.34	201 ± 49.3
CLX-105 (Equivalent to 100 mg/kg VPA) CLX-105:Vitamin E- TPGS emulsion formulation	44.7 ± 8.20	0.75 ± 0.29	153 ± 55.8



Further studies that being planned/on-going:

- *Development of a prototype clinical drug product*
- *Relative BA study of clinical drug product in dogs*
- *Comparative efficacy study in a drug resistant epilepsy model*
- *Antitumor activity as mono and combination (with standard of care) in an appropriate tumor model*
- *Comparative liver toxicity study of CLX-105 with VPA in rats*

INTELLECTUAL PROPERTY

- PCT number: PCT/14/572,812 (US patent grant: 9,206,111 B1)
- PCT number: PCT/IB2013/050801 (US patent under examination)
- Filed with ten national patent offices
- The patentability report from the international search authority (Chapter 1) confirms novelty of the invention, non-obviousness and eligibility for patentability.

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