Application of a quantitative systems pharmacology (QSP) model to evaluate xCT inhibition as a target for central nervous system diseases.

CM Friedrich[1], CM Witt[1], MM Pryor[1], T Rooney[2], A Genevois-Borella[2], MC Obinu[2], A Guerreiro[2], J Konop[2], MJ Reed[1], Z Bocskei[2]

[1]Rosa&Co, CA, USA [2]Sanofi R&D, Chilly-Mazarin, FRANCE (funding source)

Drug Development Advisors

Driving Scientific Innovation Since 2002

Introduction

- Neuronal excitotoxicity, often mediated by glutamate, has been implicated as a factor in a number of central nervous system (CNS) diseases.
- In order to improve the understanding of the role of the cysteine-glutamate transporter (xCT) in excitotoxicity and CNS diseases and the potential utility of xCT inhibitors, Sanofi and Rosa collaborated in the development of a CNS PhysioPD™ Research Platform, a QSP model that supported hypothesis generation and testing.

Objectives

- Evaluate the degree of xCT inhibition required to reduce CNS glutamate levels below neurotoxic levels and retain normal microglial phagocytic function.
- Provide guidance on the CNS indications most likely to respond to this mechanism of action.

Methods

The CNS PhysioPD™ Research Platform is an ordinary differential equation-based Platform that represents:

- Cell dynamics of microglial activation, neuronal stress and death
- Synthesis/expression and metabolism of mediators and surface markers
- xCT function (amino acid transport) and dysfunction in processes associated with multiple sclerosis (MS) and Alzheimer's disease (AD)
- Glutathione (GSH) metabolism in healthy white and gray matter
- Effects of treatment by xCT inhibitors

Publicly available literature were analyzed to provide guidance for the representation of white and gray matter in health, multiple sclerosis (MS) and Alzheimer's disease (AD) (References 1-11)

Properties of xCT inhibitor compounds were provided by Sanofi for representation in the Platform.

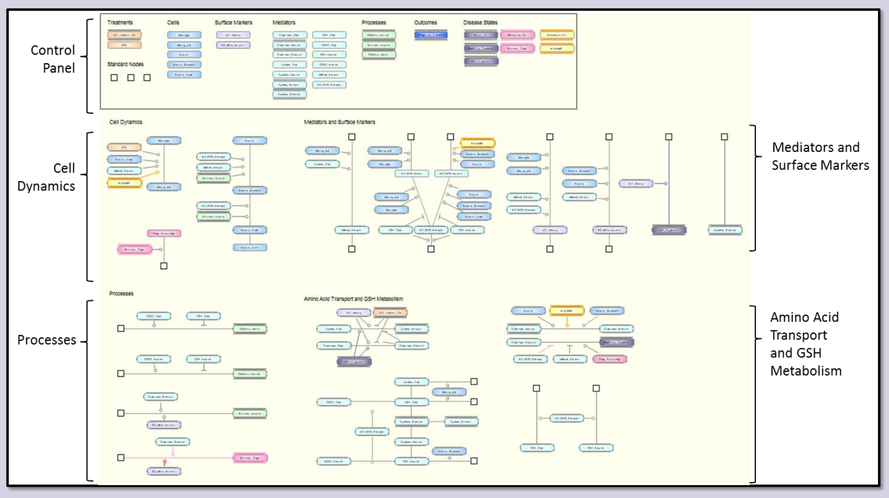

Figure 1. The CNS PhysioPD Platform is an explicit graphical and mathematical representation of mechanisms relevant to xCT inhibitor effects in the CNS.

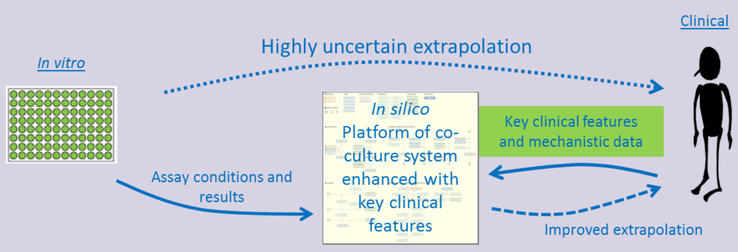

Figure 2. The *in silico* CNS Platform supported focused extrapolation from *in silico* assays to clinical scenarios for CNS disease.

Results

- The CNS Platform reproduced *in vitro* results, supporting current understanding of biological mechanisms (Figure 3, Figure 4)
- Focused extension of the Platform enabled guidance for CNS disease applications
- Simulation research informed key recommendations:
  - *In vitro* experiments to resolve material uncertainties and reduce risk
  - Prioritization of MS as lead therapeutic indication

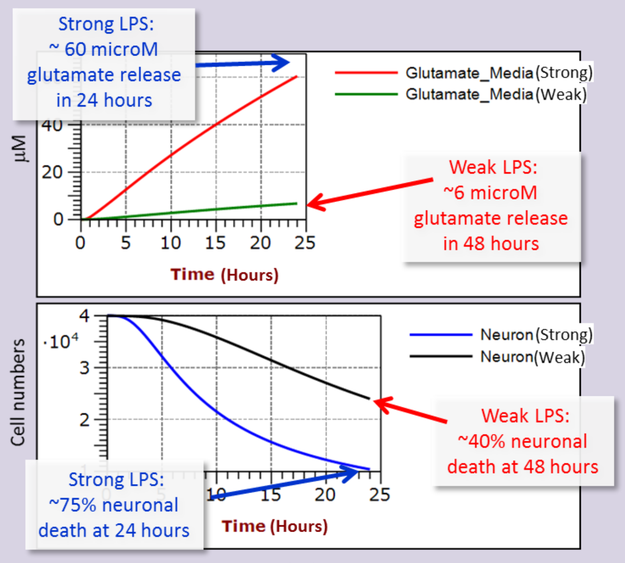

Figure 3. Time course of glutamate concentration (top) and neuronal survival (bottom) simulation results given LPS in microglia/neuron co-culture assay.

<u>Comparison to Key Sanofi *In Vitro* Data</u>

Sanofi's microglia/neuron co-culture experiments were simulated in the Platform

1. Strong or weak LPS stimulation was simulated for 24h
   - Simulated results for glutamate production and neuronal death matched Sanofi data (Figure 3)
2. Dose response for Sanofi compounds given in combination with LPS was also simulated
   - Simulated results for glutamate production and neuronal death matched Sanofi's dose response data (Figure 4)
3. Additional simulation results with weak LPS stimulation and for additional compounds were also consistent with reported data (not shown).

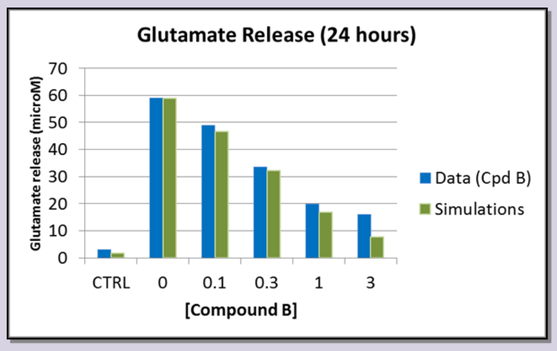

Figure 4. Dose response for Sanofi xCT inhibitor compound effects on glutamate release (top) and neuronal survival (bottom) in response to strong LPS stimulation.

Results

<u>*In Vivo* Simulation Results</u>

Different versions of the Platform were created to represent *in vivo* white matter (relevant for MS) and *in vivo* gray matter (relevant for AD). Administration of Sanofi's xCT inhibitor compounds was simulated to assess potential *in vivo* efficacy.

- MS simulations (white matter) of xCT inhibition suggested dose-dependent reductions in glutamate release, axonal injury, and death of oligodendrocytes (Figure 5).
- AD simulations (gray matter) of xCT inhibition suggested limited benefits on glutamate release and neuronal death (Figure 6).
- Decreased glutamate release and GSH production in response to xCT inhibition increased oxidative stress (Figure 7). Similar results were seen in MS simulations (not shown).

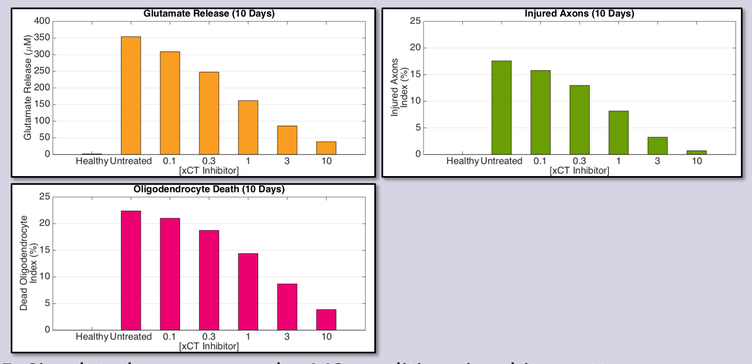

Figure 5. Simulated response under MS conditions in white matter to administration of xCT inhibitor compound.

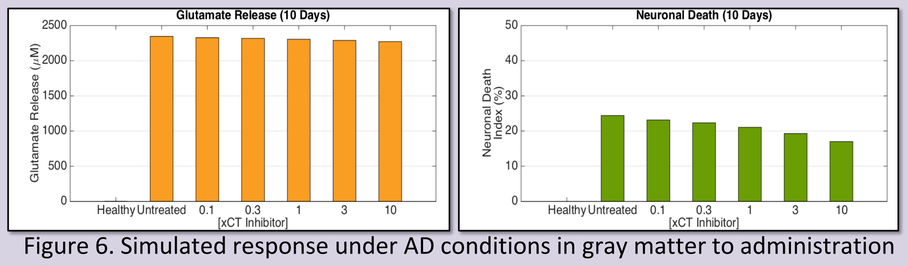

Figure 6. Simulated response under AD conditions in gray matter to administration of xCT inhibitor compound.

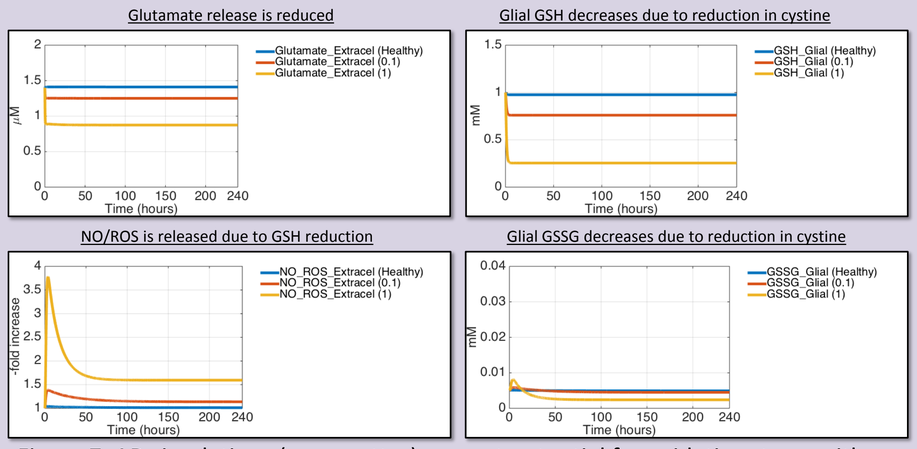

Figure 7. AD simulations (gray matter) suggest potential for oxidative stress with xCT inhibition

Conclusions

- Results from simulated xCT inhibition in the CNS PhysioPD Research Platform supported the prioritization of MS over AD as the lead therapeutic indication.
  - In MS, glutamate is increased and leads to excitotoxicity of oligodendrocytes and axonal damage, and xCT is likely to contribute significantly to extracellular glutamate concentration.
  - For AD, the therapeutic rationale is mixed. While glutamate is locally and transiently increased, xCT contribution appears to be initially limited.
- Simulation research and insights led to focused recommendations for *in vitro* experiments to resolve material uncertainties in the understanding of xCT function and to reduce risk in the development program.
- The research illustrates the key role that QSP modeling can play in providing quantitative integration of internal and external knowledge, mechanistic insight and decision support in early stage drug discovery.

References

1. Albrecht, P., J. Lewerenz, et al. (2010). "Mechanisms of oxidative glutamate toxicity: the glutamate/cystine antiporter system xc- as a neuroprotective drug target." CNS Neurol Disord Drug Targets 9(3): 373-382.
2. Azevedo, F. A., L. R. Carvalho, et al. (2009). "Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain." J Comp Neurol 513(5): 532-541.
3. Baker, D. A., Z. X. Xi, et al. (2002). "The origin and neuronal function of in vivo nonsynaptic glutamate." J Neurosci 22(20): 9134-9141.
4. Cui, Q. L., T. Kuhlmann, et al. (2013). "Oligodendrocyte progenitor cell susceptibility to injury in multiple sclerosis." Am J Pathol 183(2): 516-525.
5. Lewerenz, J., S. J. Hewett, et al. (2013). "The cystine/glutamate antiporter system x(c)(-) in health and disease: from molecular mechanisms to novel therapeutic opportunities." Antioxid Redox Signal 18(5): 522-555.
6. Lucchinetti, C., W. Bruck, et al. (1999). "A quantitative analysis of oligodendrocytes in multiple sclerosis lesions. A study of 113 cases." Brain 122 (Pt 12): 2279-2295.
7. Moussawi, K., A. Riegel, et al. (2011). "Extracellular glutamate: functional compartments operate in different concentration ranges." Front Syst Neurosci 5: 94.
8. Pelvig, D. P., H. Pakkenberg, et al. (2008). "Neocortical glial cell numbers in human brains." Neurobiol Aging 29(11): 1754-1762.
9. Pitt, D., P. Werner, et al. (2000). "Glutamate excitotoxicity in a model of multiple sclerosis." Nat Med 6(1): 67-70.
10. Slivka, A., M. B. Spina, et al. (1987). "Reduced and oxidized glutathione in human and monkey brain." Neurosci Lett 74(1): 112-118.
11. Wang, X. F. and M. S. Cynader (2000). "Astrocytes provide cysteine to neurons by releasing glutathione." J Neurochem 74(4): 1434-1442.

For more information about this work, please contact:

Mike Reed
Rosa & Co LLC
(650) 393-3738
mreed@rosaandco.com