THERAPEUTIC APPROACES FOR CNS DISORDERS

Room:	**Jan Willem Schaapfoyer**
Time:	**Tuesday 13:30 - 15:00**
Chair(s):	**Elizabeth de Lange, Martina Schmidt**
Organizing FIGON-partner(s):	**NVF, GRIP, NVT, ZonMw**

Invited lectures:

13:30 – 13:55 **Targeting Gut4Brain in Autism Spectrum Disorders**
Prof. Aletta Kraneveld, Utrecht University

13:55 – 14:15 **From smart in vivo data to predictive models for CNS drug distribution in health and disease**
Prof. Elisabeth de Lange, Leiden University

Selected abstracts:

14:15 – 14:30 **Targeting NAMPT in glioblastoma by photo-activated ruthenium complex**
S. Abyar – CHDR, Leiden University

14:30 – 14:45 **Effects of sodium channel blockers on nerve excitability threshold tracking**
Titia Ruijs – CHDR, Leiden University

14:45 – 15:00 **Safety, Pharmacokinetics and Target Engagement of novel RIPK1 inhibitor SAR443060 (DNL747) in Patients with Amyotrophic Lateral Sclerosis**
Maurits F J M Vissers – Leiden University

Indicated speaker time includes 5 minutes for discussion

NVF Dutch Pharmacological Society

groningen research institute of pharmacy

NVT TOXICOLOGIE

ZonMw

Targeting NAMPT in glioblastoma by photo-activated ruthenium complex

S. Abyar[1], Sylvia. E Le Dévédec[2], S.Bonnnet[1]

[1]Leiden Institute of Chemistry, Gorlaeus Laboratories, Einsteinweg 55, P.O. Box 9502, 2300 RA Leiden, The Netherlands
[2] Division of Toxicology, Leiden Academic Center for Drug Research, Leiden University, Leiden 2333CC, the Netherlands.

Aim

Nicotinamide adenine dinucleotide (NAD+) is a central molecule in health and disease, as it serves as substrate or cofactor for several cellular bio-energetic functions. NAMPT is a main enzyme in the production of this substrate inside cells, and expression of this enzyme is dependent on the cell type. In this project we aim at inhibition of this enzyme with a light-activated drug to stop unlimited cell growth in glioblastoma and ultimately achieve lower side effects during brain cancer treatment.

Methods

The SRB cell viability assay was use to measure half-maximal effective concentrations (EC50) of a ruthenium-based photoactivated chemotherapy compounds in different cell lines. RNA sequencing and Western blotting were used for checking NAMPT's RNA and protein expression. HLIC-MS method was used for checking metabolites amount including NAD+ levels.

Result/conclusion

Our result showed that U87 (glioblastoma cancer cell line) has higher expression of NAMPT compared to cell lines originating from skin (A431), lung (A549), or liver (HepG2) cancer. The cytotoxicity in both normoxic and hypoxic U87 cells of the known NAMPT inhibitor STF31, and of its photocaged analogue Ru-STF31 shows that Ru-STF31+light is significantly more cytotoxic than freely administered STF31 in normoxic glioblastoma cell line. Normoxic U87 cells treated with Ru-STF31+light disclosed diminished NAD+ levels which is a strong evidenced that cytotoxicity is at least partly due to NAMPT inhibition. Synergistic effects (combination index CI<1) between photoactivated ruthenium cage and free STF31 was found, which confirms the additional toxicity generated by the ruthenium fragment obtained after light activation of Ru-STF31. Higher toxicity of unactivated Ru-STF31 in comparison to that of STF31 in hypoxia, emphasizes different mechanism of toxicity of Ru-STF31 at low dioxygen concentrations. This was confirmed by a rescue experiment: addition of NAD+ to cells treated with Ru-STF31+light had strong protective effect in normoxic cells and almost no effect in hypoxic cells.
Targeting NAMPT with photo activated caged STF31, diminished NAD+ level in U87, cell line. As NAD+ is one of the foremost substrates of central enzymes inside the cells, therefore photo activated caged STF31 has potential anticancer effect in glioblstoma.

References:

1. Lameijer, L. N., Ernst, D., Hopkins, S. L., Meijer, M. S., Askes, S. H., Le Dévédec, S. E., & Bonnet, S. (2017). A red-light-activated ruthenium-caged NAMPT inhibitor remains phototoxic in hypoxic cancer cells. Angewandte Chemie, 129(38), 11707-11711.
2. Kraus, D., Reckenbeil, J., Veit, N., Kuerpig, S., Meisenheimer, M., Beier, I., ... & Probstmeier, R. (2018). Targeting glucose transport and the NAD pathway in tumor cells with STF-31: a re-evaluation. Cellular Oncology, 41(5), 485-494.

EFFECTS OF SODIUM CHANNEL BLOCKERS ON NERVE EXCITABILITY THRESHOLD TRACKING

Titia Ruijs[1,2], Jules Heuberger[1], Ingrid Koopmans[1,2], Marieke de Kam[1], Michiel van Esdonk[1], Geert Jan Groeneveld[1,2].
[1]Centre for Human Drug Research, Leiden, [2]Leiden University Medical Centre, Leiden.

Introduction

Selective voltage gated sodium channel blockers are of growing interest as treatment for pain. For drug development of such compounds, it would be critical to have a biomarker that can be used for proof-of-mechanism.

Aims

We aimed to evaluate whether drug-induced changes in sodium conductance can be detected using nerve excitability threshold tracking in 18 healthy subjects.

Methods

In a randomized, double-blind, three-way crossover study, effects of single doses of mexiletine and lacosamide were compared to placebo. On each study visit, motor- and sensory nerve excitability measurements of the median nerve were performed (pre-dose; 3- and 6-hours post-dose). Stimulation was guided by QTRAC-S. Treatment effects were calculated using an ANCOVA, with baseline as covariate.

Results

Mexiletine and lacosamide had significant effects on a multitude of motor- and sensory nerve excitability parameters. In motor nerves, TEd 40-60ms was significantly decreased when compared to placebo, with an estimated difference -1.37% (95%CI:-2.20,-0.55;p=0.002) after mexiletine and -1.27% (95%CI:-2.0968,-0.4430; p=0.004) after lacosamide. Moreover, mexiletine and lacosamide significantly increased superexcitability in motor nerves, with an estimated difference of 1.74% (95%CI:0.61,2.87; p=0.004) and 1.47% (95%CI:0.34, 2.60;p=0.013), respectively. The strength-duration time constant decreased after lacosamide in both motor nerves -0.03ms (95%CI:-0.06,-0.01;p=0.005) and sensory nerves -0.08ms (95%CI:-0.12,-0.05;p<0.001).

Conclusion

Mexiletine and lacosamide significantly decrease excitability of motor and sensory nerves, in line with the mechanism of action. This study shows that threshold tracking can be an effective biomarker in pharmacological studies. The method would therefore be a valuable tool in drug development, to help identifying target engagement in healthy subjects.

Safety, Pharmacokinetics and Target Engagement of novel RIPK1 inhibitor SAR443060 (DNL747) in Patients with Amyotrophic Lateral Sclerosis

Maurits F J M Vissers[1,2], Jules A A C Heuberger[1], R Tsai[3], R Erickson[3], A Cruz-Herrranz[3], J Harris[3], F Huang[3], V Tong[3], Y Zhu[3], K Scearce-Levie[3], J Hsiao-Nakamoto[3], X Tang[3], C McKenna[3], C Ho[3], M Troyer[3], R.J. Pomponio[4], M. Alonso-Alonso[4], M. Zilberstein[4], N. Atassi[4], Geert Jan Groeneveld[1,2]

[1] CHDR, Leiden, The Netherlands; [2] LUMC, Leiden, The Netherlands; [3] Denali Therapeutics Inc., South San Francisco, USA; [4] Sanofi Genzyme, Cambridge, USA.

Introduction

Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) is a master regulator of inflammatory signaling and cell death and increased RIPK1 activity is observed in human diseases characterized by excess cell death and inflammation, including in amyotrophic lateral sclerosis (ALS).[1] RIPK1 inhibition has been shown to protect against pathology and cell death in a range of preclinical cellular and animal models of neurodegenerative diseases.[2] SAR443060 (DNL747) is a selective, orally bioavailable, CNS–penetrant, small-molecule, reversible inhibitor of RIPK1.

Objectives

The goal of this study was to evaluate the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of 28 days of SAR443060 50 mg twice-daily (BID) dosing in 16 patients with ALS.

Methods

This multicenter, randomized, double-blind, placebo-controlled phase 1B study (NCT03757351), used a cross-over design consisting of two 28-day treatment periods, separated by 14 days of wash-out. Vital signs, ECG, standard laboratory safety assessments, physical and neurological examinations, and adverse events were recorded to monitor safety and tolerability. Drug concentrations were determined in blood and cerebrospinal fluid (CSF) samples for PK, and target engagement was measured via phosphorylation of RIPK1 at serine 166 (pRIPK1) in human peripheral blood mononuclear cells (PBMCs).

Results

RIPK1 inhibition was generally safe and well tolerated for 28 days in patients with ALS (n=15). SAR443060 distributed into CSF after oral administration and demonstrated peripheral target engagement as measured by a reduction in pRIPK1 in stimulated PBMCs compared to baseline. The maximal mean inhibition after SAR44306 treatment was -66.0 (± 21.6, n=8) percent change from baseline in the placebo/SAR443060 treatment sequence and -75.7% (± 15.0%, n=6) in the SAR443060/placebo treatment sequence (**Figure 1**).

Conclusion

This study demonstrates short term safety, CNS penetration, and peripheral target engagement of SAR443060 in patients with ALS.

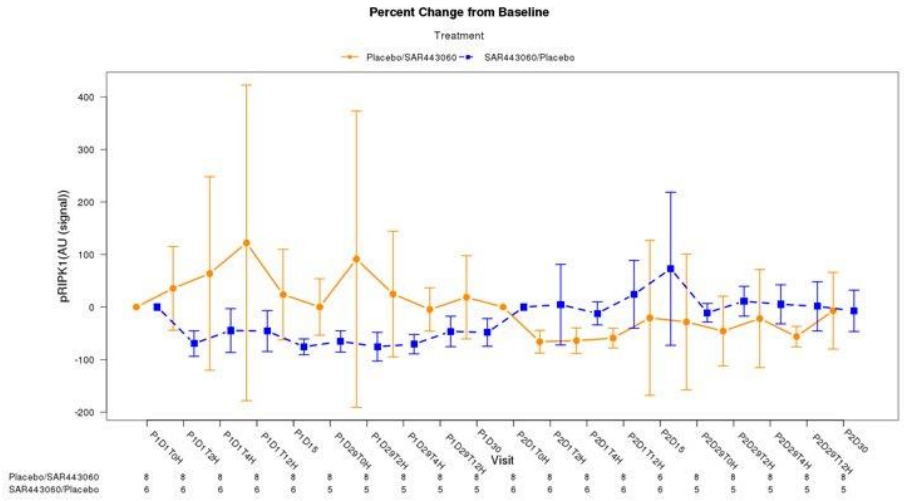

Figure 1. Mean (± SD) percentage pRIPK1 inhibition (AU(signal)) compared to baseline after SAR443060 and placebo administration in ALS patients by treatment sequence

References:

1. Ito Y, Ofengeim D, Najafov A, et al. RIPK1 mediates axonal degeneration by promoting inflammation and necroptosis in ALS. Science. 2016;353(6299):603-608. doi:10.1126/science.aaf6803
2. Mifflin L, Ofengeim D, Yuan J. Receptor-interacting protein kinase 1 (RIPK1) as a therapeutic target. Nature Reviews Drug Discovery. Published online July 15, 2020. doi:10.1038/s41573-020-0071-y