Baier *et al.*: *Intrinsic Activity*, 2022; **10**(Suppl. 2):A1.5
doi:10.25006/IA.10.S2-A1.5
published online: 16 September 2022

INTRINSIC ACTIVITY

26th Scientific Symposium of the Austrian Pharmacological Society

Graz, 23–24 September 2022

MEETING ABSTRACT

A1.5

The anti-Warburg ruthenium compound BOLD-100 targets the cellular lipid metabolism and impacts on histone acetylation

Dina BAIER[1,2,3], Theresa MENDRINA[1,2,3], Beatrix SCHOENHACKER-ALTE[1,2,3], Máté RUSZ[1,3,4], Helena A. HERRMANN[4], Christine PIRKER[2,3], Thomas MOHR[2], Samuel MEIER-MENCHES[1,4,5], Benedikt REGNER[6], Gerhard ZEITLER[1,2], Karin NOWIKOVSKY[6,7], Jürgen ZANGHELLINI[4], Gunda KÖLLENSPERGER[4], Petra HEFFETER[2,3], Bernhard K. KEPPLER[1,3], Walter BERGER[2,3,*]

[1]*Institute of Inorganic Chemistry, University of Vienna, Austria;* [2]*Center for Cancer Research and Comprehensive Cancer Center, Medical University Vienna, Austria;* [3]*Research Cluster "Translational Cancer Therapy Research" Vienna, Austria;* [4]*Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Austria;* [5]*Joint Metabolome Facility, Faculty of Chemistry, University of Vienna, Austria;* [6]*Anna Spiegel Center of Translational Research, Department of Medicine I, Medical University Vienna, Austria;* [7]*Unit of Physiology and Biophysics, Department of Biomedical Sciences, University of Veterinary Medicine Vienna, Austria*

Background: The ruthenium anticancer complex sodium *trans*-tetrachloro-[bis(1*H*-indazole)ruthenate(III)] (BOLD-100) currently undergoes multicentre clinical phase II evaluation in combination with the FOLFOX regimen against several solid tumor types. Systemically, BOLD-100 hitchhikes serum albumin and, thereby, accumulates in the malignant tissue where it is 'activated by reduction'. Mechanistically, BOLD-100 is an ER stress inducer via the inhibition of the chaperone glucose-regulated protein 78 (GRP78) leading to unfolded protein response and caspase-dependent apoptosis. A major limitation for effective cancer therapy represents the acquisition of resistance. Thus, the dissection of underlying mechanisms is necessary already during (pre)clinical development.
Methods: In this study, colon cancer HCT 116 and pancreatic cancer Capan-1 cells were selected for BOLD-100 resistance. Cell/molecular biological as well as analytical chemistry methods and omics approaches—including, amongst others, metabolomics, transcriptomics, Seahorse XF analyses, western blotting, cell viability assays, mass spectrometric (MS) and nuclear magnetic resonance (NMR) analyses—were used to dissect molecular mechanisms underlying acquired BOLD-100 resistance.
Results: BOLD-100 was identified as anti-Warburg drug reducing lactate and pyruvate levels of parental HCT 116 cells. Computational network analysis of gene expression and metabolomics data revealed a clear upregulation of glycolysis in BOLD-100-resistant HCT 116 (HCTR) cells associated with increased pyruvate and citrate levels creating a vulnerability towards glucose deprivation by 2-deoxy-D-glucose (2-DG). The enhanced glycolytic activity fueled into cellular lipid enrichment. Accordingly, increased lipid droplet (LD) levels in HCTR cells were associated with an altered *de novo* lipid synthesis. Hence, pharmacological targeting of the lipid phenotype with diverse lipid metabolism inhibitors, such as triacsin C or the β-oxidation inhibitor etomoxir, exposed the lipid metabolism as Achilles' heel of cells and xenograft models with acquired BOLD-100 resistance. Coenzyme A (CoA), as key metabolite, connects glycolysis via the tricarboxylic acid (TCA) cycle with the lipid metabolism and, consequently, histone acetylation. BOLD-100 treatment reduced histone acetylation only in parental HCT 116 cells while it enhanced this parameter in HCTR cells. Thus, a potential interaction between BOLD-100 and CoA was postulated. Consequently, BOLD-100 and CoA were co-incubated under cell-free conditions and, indeed, MS and NMR analyses revealed the formation of a BOLD-100-CoA thioester. Upon combination of BOLD-100 with another CoA-binding compound, namely 4-phenylbutyric acid (PBA), synergistic anticancer activity in several tested cancer models and, furthermore, the reversal of acquired BOLD-100 resistance were observed.
Discussion: In summary, the strong metabolic activity of BOLD-100 identified in this work presents a novel mode of action of the ruthenium complex. This interference with several hubs of the complex onco-metabolism network bears huge potential for broad therapeutic applicability. Current work focusses on further in-depth investigation of the specific crosstalk between diverse cellular metabolic routes and the identification of synergism-associated mechanisms of reasonable therapeutic combination partners.
Acknowledgements: We wish to thank the Austrian Science Fund FWF, the Fellinger Cancer Research Fund and the Mahlke-Obermann Foundation for their financial support.
Keywords: BOLD-100 – ruthenium-based compounds – cancer chemotherapy resistance – glycolysis – lipid metabolism – histone acetylation

*Corresponding author e-mail: walter.berger@meduniwien.ac.at