# Pharmacological IRS1/2 Inhibition Reduces Cell Viability in BCR-ABL1 Positive Cells

Scopim-Ribeiro, R.[1]; Machado-Neto, J. A.[1]; de Melo Campos, P.[2]; Duarte, A. S. S.[2]; Scheucher, P. S.[1]; Góes, A.[1]; Olalla Saad, S. T.[2]; Simões, B. P.[1]; Rego, E. M.[1]; Traina, F.[1]

[1]Department of Internal Medicine, University of São Paulo at Ribeirão Preto Medical School, Ribeirão Preto, São Paulo, Brazil.
[2]Hematology and Hemotherapy Center-University of Campinas/Hemocentro-Unicamp, Instituto Nacional de Ciência e Tecnologia do Sangue, Campinas, São Paulo, Brazil.

**INTRODUCTION**: Chronic myeloid leukemia (CML) is associated with the BCR-ABL1 fusion gene, which activates multiple signaling pathways and is targetable with tyrosine kinase inhibitors (TKI). Acquired drug resistance limits TKI efficacy in a significant subset of patients, warranting efforts to identify additional crucial proteins involved in BCR-ABL1 signaling. We have previously identified IRS1 as a substrate of BCR-ABL1 in K562 cells. Pharmacological IRS1/2 inhibitor (NT157) has been developed and has shown promising results in preclinical studies on solid tumors. **OBJECTIVES**: To investigate *IRS1* and *IRS2* expression and the effects of IRS1/2 inhibition in BCR-ABL1+ and normal hematopoietic cells. **MATERIALS AND METHODS**: Healthy donors (n=12) and CML patients at the time of diagnosis (n=25) were included. K562 (BCR-ABL+) cells were submitted to IRS1 and IRS2 silencing, or NT157 treatment, and submitted for cell viability, proliferation, apoptosis and protein expression/activation assays. NT157 effects were analyzed in primary CML (n=3) and normal cord blood (n=2) cells. Mann-Whitney or ANOVA test were used. **DISCUSSION AND RESULTS**: *IRS1* mRNA expression was reduced in CML group ($p$=0.02), but *IRS2* was similar between normal donors and CML samples. NT157 treatment reduced K562 cell viability and proliferation, and induced apoptosis in a time and dose-dependent manner (IC50: 0.6 µM). Upon NT157 treatment, colony formation of CML primary cells was inhibited, with a reduction predominance in granulomonocytic colonies for all patients. NT157 treatment did not inhibit colony formation of committed normal cord blood cells. IRS1 silencing, but not IRS2, significantly decreased K562 cell viability. **CONCLUSION**: Pharmacological IRS1/2 inhibition (*i*) reduces colony formation in primary CML, but not in normal cells, (*ii*) decreases cell viability and proliferation, and (*iii*) increases apoptosis of K562 cells in a time and dose-dependent manner. IRS1 inhibition may be the main mechanism by which NT157 exerts its effects on BCR-ABL1 positive cells. NT157 IRS1/2-targeting may optimize the anti-CML approaches.

**Key words:** BCR-ABL1, Chronic myeloid leukemia, Insulin Receptor Substrate

Supported by FAPESP and CNPq.