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Application Note

"In-Cell Western"- A novel approach to detect gross changes in protein expression and protein phosphorylation

A group from the Department of Medicine in Virginia Commonwealth University at Richmond, VA, USA has recently published an article about Rationally Repurposing Ruxolitinib (Jakafi®) as a Solid Tumor Therapeutic on ***Frontiers in Oncology*** journal.

In this paper the group, under the leadership of Prof. Paul Dent, describes a novel approach for the detection of Cell Viability, Protein Expression, and Protein Phosphorylation by Immuno-Fluorescence Using *IDEA Bio-Medical*'s Hermes WiScan imaging system.

For the vast majority of the in vitro analyses and the present set of studies in these manuscripts, the group used a Hermes WiScan wide field microscope, set at 10X magnification, in a 96-well plate format.

Ruxolitinib and ERBB receptor inhibitors are known to modulate the functions and activities of many intracellular signal transduction pathways, and the group explored the impact that ruxolitinib based drug combination had on cell signaling processes.

In BT474 cells as judged by western immunoblotting lapatinib and ruxolitinib in combination caused prolonged inhibition of the phosphorylation of ERBB1, ERBB2, ERK1/2, AKT, mTOR, STAT3, STAT5, and the phosphorylation of p65 NFκB (Figure 2A; effects all >50%, $p < 0.05$). Very similar data were obtained in triple-negative SUM149 cells when the changes in cell signaling parameters were measured using in situ immuno-fluorescence on native proteins in a Hermes WiScan machine at 10× magnification (Figures 2B,C).

The group found that gross assessments of protein expression/phosphorylation can be made, i.e., an "in-cell western", using unbiased pre-programed electronic data acquisition, much as has previously been performed for the last 45 years using SDS-PAGE and western immunoblotting.

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In their manuscript, they compare the work with Hermes Wiscan imaging system as compared to conventional Western Blot technics, saying "*the Hermes system permits 96 samples to be measured simultaneously in up to three fluorescent color channels in contrast to our Odyssey infra-red machine for western blotting that has two channels and is limited to ~20 samples per gel*".

They add that "*Using the Hermes system has an additional benefit: while western immunoblotting often "loses" proteins that denature and form detergent insoluble complexes that do not electrophorese down the gel, using the Hermes system all cellular proteins have the potential of being detected due to its in situ non-denatured methodology . The machine also has in-built software to assess changes in fluorescent intensity thereby making the quantitation of signal intensities straight-forward*".

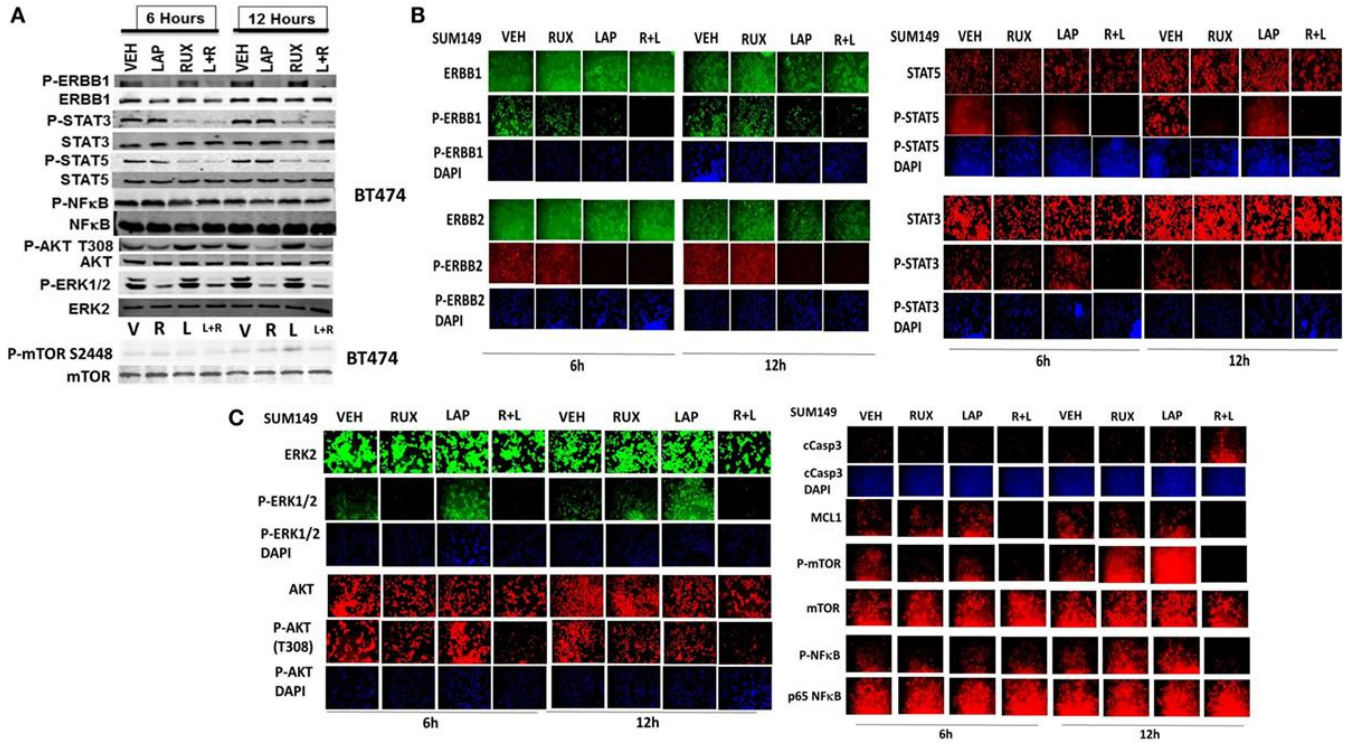

Figure 2. Lapatinib and ruxolitinib interact to inactivate AKT, mTOR, STAT3, STAT5, and ERK1/2 as judged by western blotting. (A) BT474 cells were treated with vehicle control, ruxolitinib (1.0 μM), lapatinib (1.0 μM) or the drugs in combination for 6 h and for 12 h. At each time point cells were lysed with RIPA buffer and clarified by centrifugation. Ten milligram of protein from each lysate was subjected to SDS-PAGE on 10% gels. Proteins were transferred to 0.2 μm nitrocellulose and probed with antibodies generated against the indicated proteins and phospho-proteins. Bands were imaged using a first generation Odyssey Infra-Red imager at 300 dpi. (n = 3 ± SEM). (B,C) SUM149 cells were treated with vehicle control, ruxolitinib (1.0 μM), lapatinib (1.0 μM), or the drugs in combination for 6 h and for 12 h. At each time point, cells were fixed in place and permeabilized using 0.5% Triton X100. Immuno-fluorescence was performed on native proteins to detect the total expression and phosphorylation levels of ERBB1, ERBB2, STAT3, STAT5, ERK1/2, AKT T308, mTOR S2448; NFκB p65 S536, MCL-1, and cleaved caspase 3 (cCasp3) (n = 3 ± SEM).

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