ImmunoTools *special* Award 2015

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Biomarkers for a novel anti-cancer drug

Background

A new cytidine analog, fluorocyclopentenyl-cytosine (RX-3117), has shown promise as a new anti-cancer drug since it showed considerable anti-tumor activity in various xenograft models, including models resistant to gemcitabine, a drug predominantly used for treatment of patients with non-small cell lung cancer (NSCLC). The lack of cross resistance between these two drugs suggests a difference in mechanism of action or method by which they are metabolized in cells.

A previous study provided preliminary information on its mechanism of action and metabolism. Uptake of RX -3117 was shown to be mediated by human ENT1 (hENT1) and its cytotoxic activity exerted via its phosphorylated metabolites. This phosphorylation is performed by uridine-cytidine kinases (UCKs). Furthermore, this study showed that RX-3117, contrary to drugs such as gemcitabine, is not deaminated by cytidine deaminase (CDA) and that RX-3117 causes both inhibition of DNA and RNA synthesis, although the inhibition of the former is more pronounced. Apart from inhibiting synthesis of DNA and RNA, RX-3117 also targets DNA methyltransferase (DNMT) of which there are multiple variants. DNMT3A and DNMT3B establish *de novo* DNA methylation patterns in DNA, important during embryogenesis, while DNMT1 differs in that its role is to maintain the established DNA methylation pattern through cell division and thus DNA replication. In two previous studies a decrease in DNMT1 expression was found in cell lines treated with RX-3117, while this was not the case for DNMT3A. This suggests RX-3117 might be an efficient demethylating agent, comparable to Aza-CdR and Aza-CR.

Biomarker identification for RX-3117 activity

To overcome toxicity in patients, who will not profit from RX3117 treatment, biomarkers are desired. Biomarker analysis will be focused on RX-3117 target DNMT1. To test the possibility

of DNMT1 expression being valid biomarker, an appropriate and visible test should be developed to be used in clinic.

For this purpose human PBMCs will be analyzed by sensitive flow cytometry analysis. The idea is to identify DNMT1 subpopulations by flow cytometry and follow the DNMT1 expression pattern after incubation with RX-3117 in time. This will be done first in cell lines (because we know already from western blot analysis there are positive for DNMT1) and thereafter in human freshly obtained PBMCs.

DNMT1 positive subpopulation will be identified using cell surface markers and intracellular, nuclear localized DNMT1. When the appropriate subpopulation is found, it will be selected for the sterile cell sorting by flow cytometry. The identification procedure will consist of T cell marker/CD3, B cell/CD19, NK cell/CD56 and monocyte/CD14 and dendritic cell/CD/11c. The positivity of PBMC cells for CD34 should be analyzed as well. To this end, products from ImmunoTools listed below will be used including isotype control. A single cell sorting will be performed with subpopulation and after 24 and 48 hours of cell culture conditioned with RX3117, DNMTs expression will be examined. For DNMT1 staining streptavidin conjugated should be used to amplify the signal as it is very difficult target. In addition ICC of cell lines should be applied in line with flow cytometry data on DNMT1 expression. The intention is also to study apoptosis induction by RX-3117. The Annexin V staining is a tool for this purpose. In this way we hope to spare unnecessary side effects and provide patients with rational target directed treatment. Therefore we would like to use very nice initiative from ImmunoTools in order to accomplish this aim.

ImmunoTools *special* AWARD for **Dzjemma Sarkisjan** includes 25 reagents

FITC - conjugated anti-human CD3, CD14, CD19, CD56, Annexin V, Control-IgG2a, Control-IgG2b, Control-IgG1,

PE - conjugated anti-human CD11c, CD14, CD19, CD34, CD56, Annexin V, Control-IgG2a, Control-IgG2b, Control-IgG1,

PerCP - conjugated Control-IgG1

APC - conjugated anti-human CD3, CD11c, CD14, CD19, Annexin V, Control-IgG2a, Control-IgG2b, Control-IgG1 DETAILS more AWARDS