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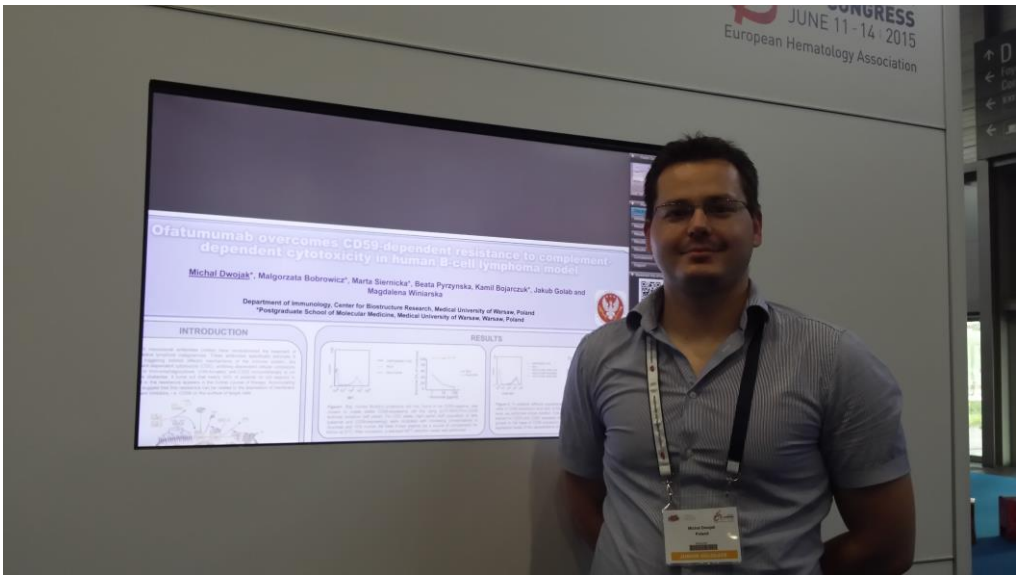
Report on active participation in 20th Congress of the European Hematology Association, Vienna, Austria, June 11-14, 2015

The annual Congress of the European Hematology Association held this year in Vienna was the most important European conference regarding latest advances in hematology. 20th EHA congress gathered around 10 000 participants – clinicians as well as scientists and corporate partners – from all over the world. I had pleasure to present the result of our studies entitled *Ofatumumab overcomes CD59-dependent resistance to complement-dependent cytotoxicity in human b-cell lymphoma model* in the e-poster session Non-Hodgkin & Hodgkin lymphoma – Biology. This conference was for me a unique chance to meet authors of many interesting abstracts related to our area of interests and to compare and discuss our results. Moreover, the conference was for me a great chance to listen to participate in many remarkable lectures and to establish future collaboration with other scientists.



Ofatumumab overcomes CD59-dependent resistance to complement-dependent cytotoxicity in human B-cell lymphoma model

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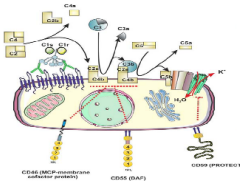
INTRODUCTION

Anti-CD20 monoclonal antibodies (mAbs) have revolutionized the treatment of CD20-positive lymphoid malignancies. These antibodies specifically eliminate B cells by triggering indirect effector mechanisms of the immune system, like complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), or immunophagocytosis. Unfortunately, anti-CD20 immunotherapy is not without its obstacles. It turns out that nearly 50% of patients do not respond to treatment or the resistance appears in the further course of therapy. Accumulating evidence suggest that this resistance can be related to the expression of membrane complement inhibitors, i.e. CD59 on the surface of target cells.



Activation of the complement cascade by anti-CD20 mAbs
(Winiarska M *et.al.* Front Biosci (Landmark Ed). 2011 Jan 1;16:277-306.)

AIM

The aim of this study was to evaluate the efficacy of the rituximab-or ofatumumab-triggered complement-dependent cytotoxicity (R-CDC or O-CDC) against CD59-overexpressing lymphoma cells and to determine the amount of CD59 and CD20 which allows anti-CD20 mAbs to effectively trigger CDC.

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RESULTS

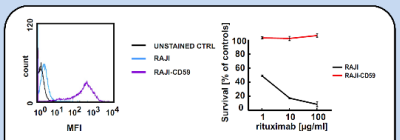

Figure1. Raji, human Burkitt's lymphoma cell line, found to be CD59-negative, was chosen to create stable CD59-expressing cell line using pLVX-IRES-Puro-CD59 lentiviral construct (left panel). For CDC assay (right panel) both population of cells (paternal and CD59-expressing) were incubated with increasing concentrations of rituximab and 10% human AB fresh frozen plasma (as a source of complement) for 60min at 37ºC. After incubation, a standard MTT reduction assay was performed.

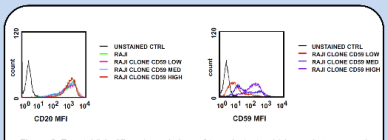

Figure 2. To establish different populations of transductants which vary between each other in CD59 expression level and, at the same time, do not vary in CD20 expression level, we performed clones isolation. One-cell-derived population (clones) were further stained for CD20 and CD59, assessed using flow cytometry, and divided into several group on the basis of CD59 expression level. The graphs shows CD20- and CD59-expression levels of the representative clones from each group.

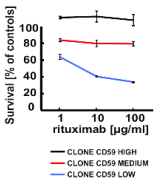

Figure 3. The representative clones from each obtained populations was further used in CDC assay to determine the influence of CD59 expression on rituximab activity. Since it is well established that CD20 level affects R-CDC our results clearly show that also CD59 expression, independently of CD20 level, has a critical influence on R-CDC and negatively correlates with the capability of rituximab to trigger the complement cascade. Of note, neither increasing the rituximab concentration up to 1000µm/ml, nor extension the incubation time could surmount this resistance (data not shown).

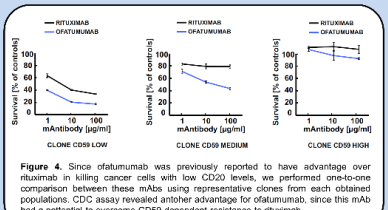

Figure 4. Since ofatumumab was previously reported to have advantage over rituximab in killing cancer cells with low CD20 levels, we performed one-to-one comparison between these mAbs using representative clones from each obtained populations. CDC assay revealed antoher advantage for ofatumumab, since this mAb had a pottential to overcome CD59-dependent resistance to rituximab.

CONCLUSIONS

- **CD59 expression level affects the efficacy of anti-CD20 monoclonal antibodies**
- **Evaluation of CD59 expression should be included in the diagnostic panels for leukemia/lymphoma immunophenotyping**
- **It is substantiated to include ofatumumab in therapeutic regimens for patients with high CD59 expression**