Report from 18th Congress of the European Hematology Association – 12-17th June 2013, Stockholm, Sweden –Magdalena Winiarska (active participation – co-author of two posters presented during poster session)

Annual Congress of EHA attracts every year hematologists from all over the world and creates a nonesuch opportunity to present own findings, share scientific ideas and create scientific networks.

A. Poster session Non-Hodgkin Lymphoma – Biology (poster walk moderator Philippe Gaulard) – presenting author

INFLUENCE OF BCR SIGNALING PATHWAYS ON CD20 LEVELS IN TUMOR CELLS AND ACTIVITY OF ANTI-CD20 MONOCLONAL ANTIBODIES

P296

M.Winiarska[1], K.Bojarczuk[1], M. Wanczyk[1], M. Dwojak[1], P. Zapala[1], N.Miazek[1], M. Siernicka[1], J. Golab[1,2]

1. Department of Immunology, Center of Biostructure Research, Medical University of Warsaw 2.Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland

INTRODUCTION

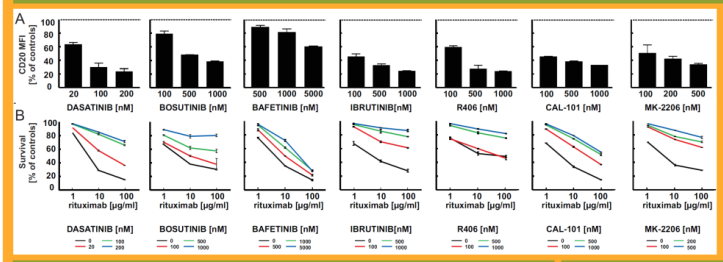
Anti-CD20 monoclonal antibodies (mAbs) are widely used in the treatment of non-Hodgkin's lymphomas (NHL) and chronic lymphocytic leukemia (CLL). Combining new agents with already used anti-CD20 mAbs seems to be a reasonable approach to further improve current therapeutic options. It seems that signaling via the aberrantly activated B-cell receptor (BCR) plays a key role in the pathogenesis of B-cell tumors. Blocking BCR signaling complex network holds a great therapeutic potential in both NHL and CLL. Several trials are currently being conducted to investigate the effects of combination of BCR- targeting agents with anti-CD20 mAbs–based therapies. To improve these therapeutic approaches in a planned manner it will be utterly important to decipher actual mechanisms of interactions between BCR-targeted therapies and anti-CD20 mAbs in established *in vitro* models.

OBJECTIVES

The aim of this study is to elucidate role of BCR signaling pathways in regulation of CD20 levels in tumor cells and antitumor activity of anti-CD20 mAbs.

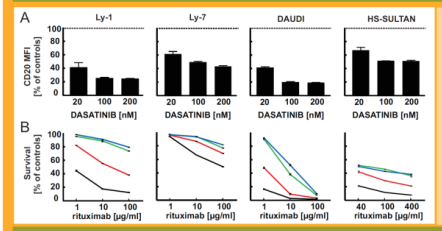
RESULTS

BCR inhibitors down-regulate surface CD20 and impair rituximab-mediated complement-dependent cytotoxicity (R-CDC) in Raji cell line



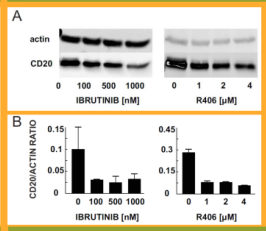
Raji cells were pretreated for 48h with dasatinib (BCR/ABL and Src inhibitor), bosutinib (BCR/ABL and Src inhibitor), bafetinib (BCR/ABL and Lyn inhibitor), ibrutinib (BTK inhibitor), R406 (SYK inhibitor), CAL-101 (PI3K inhibitor) or MK-2206 (Akt inhibitor).
(A) CD20 surface levels were determined with FITC-conjugated anti-CD20 mAb and flow cytometry.
(B) Cells were washed and incubated for 60 min with increasing concentrations of rituximab and 10% human AB serum. Rituximab-mediated complement -dependent cytotoxicity (R-CDC) was measured with PI staining using flow cytometry.

Dasatinib down-regulates surface CD20 and impairs R-CDC in various cell lines



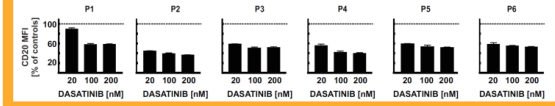
DLBCL cell lines (Ly-1 and Ly-7) and Burkitt's lymphoma cell lines (Daudi and HS-Sultan) were pretreated for 48h with dasatinib. CD20 levels (A) and R-CDC (B) were measured as described above.

BCR inhibitors down-regulate total CD20 protein and CD20 mRNA levels



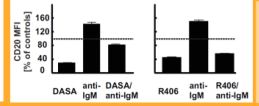
Raji cells were incubated for 48h with ibrutinib or R406.
(A) Total protein lysates were separated in a polyacrylamide gel and analysed for CD20 expression and actin by Western blotting with specific antibodies.
(B) cDNA was used for quantitative real-time PCR (qRT-PCR) amplification with SYBR Green Master Mix .

Dasatinib down-regulates surface CD20 in CLL primary cell lines



CLL primary cells (P1-P6) were pretreated for 48h with dasatinib. CD20 surface levels were determined with FITC-conjugated anti-CD20 mAb and flow cytometry.

BCR stimulation reverses the effect of selected BCR inhibitors



Raji cells were incubated for 48h with dasatinib or R406 and anti-IgM antibody.
Anti-IgM antibody was used to stimulate BCR signaling pathways.

CD20 surface levels were determined with FITC-conjugated anti-CD20 mAb and flow cytometry.

CONCLUSIONS

Blocking BCR complex network on nearly every step of signal initiation and propagation considerably down-regulates CD20 levels what might have extremely important consequences for the anti-cancer therapy that is based on the use of anti-CD20 mAbs. In light of our experiments therapeutic combinations of BCR inhibitors and anti-CD20 mAbs-based modalities should be rationally and consciously introduced into clinic in optimized therapeutic schemes.

The research was supported by Polish Ministry of Science and Higher Education [grants 1M19/3DG4/12 (M. Wan.) and IP2011 060271 (M. Win.)] and by a grant from the European Commission 7th Framework Programme: FP7-REGPOT-2012-CT2012-316254-BASTION. All authors declared no relevant financial relationship to disclose as well as no off-label drug usage to be included into presented data.

B. Novel therapeutics, targeted therapies and gene therapy (poster walk moderator Hubert Serve) – senior author

INFLUENCE OF HISTONE DEACETYLASE INHIBITORS (HDACi) ON CD20 LEVEL AND EFFICACY OF ANTI-CD20 MONOCLONAL ANTIBODIES

P990

Malgorzata Wanczyk[1], Mieszko Kozikowski[1], Michal Dwojak[1], Kamil Bojarczuk[1], Jakub Golab[1,2], Magdalena Winiarska[1]

1. Department of Immunology, Center of Biostructure Research, Medical University of Warsaw **2**.Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland

Introduction

Anti-CD20 monoclonal antibodies (mAbs) have considerably improved the outcomes of patients with B-cell malignancies and reveal promising therapeutic activity in some autoimmune diseases. Accumulating evidence indicates that CD20 can be modulated at several levels, both transcriptional and posttranscriptional and its up-regulation would result in increased efficacy of anti-CD20 mAbs. CD20 antigen has been reported to be regulated epigenetically e.g. by blocking the activity of histone deacetylases (HDACs). Such observations has been made in B-cell lymphoma cells with very low basal CD20 level. The use of non-selective pan-inhibitors of HDACs (HDACi) gives promising results both *in vitro* and *in vivo* in several tumor models, including hematological malignancies. The results of our preliminary experiments show that use of HDACi leads to up-regulation of CD20 protein in B-cell lymphoma independently of basal CD20 levels and subsequent increase of the efficacy of therapy with anti-CD20 mAbs.

Objectives

The aim of this study was to understand which HDAC isoforms are responsible for the observed effect of CD20 up-regulation. Determination of a specific isoform influencing CD20 expression could help us decipher the molecular mechanism in which HDAC inhibition increases CD20 expression in human B-cell tumors.

Results

HDAC pan-inhibitors up-regulate CD20 level and increase R-CDC

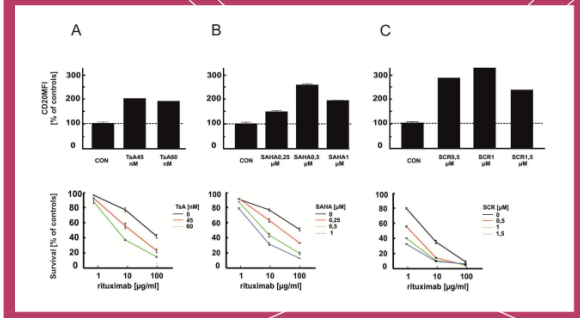


Fig. 1 Raji cells, pretreated for 48 hours with HDAC pan-inhibitors – (A) Trichostatin A (TsA), (B) – suberoylanilide hydroxamic acid (SAHA) and (C) Scriptaid (SCR), were incubated with FITC-anti-CD20 mAb. Binding of mAb was determined with flow cytometry. The efficacy od rituximab-mediated CDC was assessed with PI staining using flow cytometry after 1h incubation with serial dilutions of rituximab in the presence of 10% human AB serum as a source of complement.

HDAC6 inhibition up-regulates CD20 and increases R-CDC in lymphoma cell lines

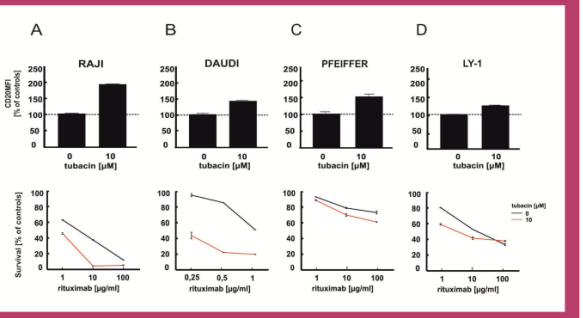


Fig. 2 Raji (A), Daudi (B), Pfeiffer (C) and LY-1 (D) cells pretreated for 48 hours with specific HDAC6 inhibitor – tubacin were incubated with FITC-anti-CD20 mAb. Binding of mAb was determined with flow cytometry.The efficacy od rituximab-mediated CDC was assessed with PI staining using flow cytometry after 1h incubation with serial dilutions of rituximab in the presence of 10% human AB serum as a source of complement.

Up-regulation of CD20 by HDACi correlates with tubulin acetylation

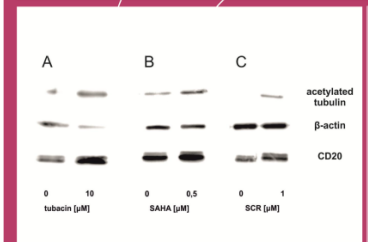


Fig. 3 Raji cells pretreated for 48 hours with tubacin (A), SAHA (B) and SCR (C) were analysed for CD20 expression by Western blotting. The level of acetylated tubulin – a hallmark of HDAC6 inhibition was analysed using specific antibody.

Use of pan-HDACi affects CD20 transcription, while HDAC6 inhibition does not

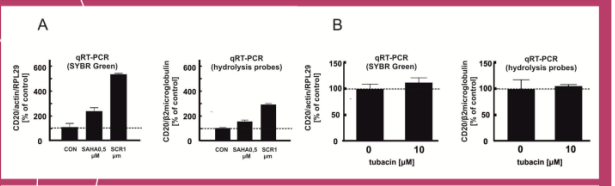


Fig.4 Raji cells pretreated for 24 hours with HDAC pan-inhibitors (A) and tubacin (B) were analysed for CD20 expression qRT-PCR with SYBR Green and hydrolysis probes.

Conclusions

Our experiments indicate that selective inhibition of HDAC6 is sufficient for up-regulation of CD20 level and may have potential clinical application in hematological malignancies. This observed regulation does not seem to involve transcriptional mechanism. However, the molecular mechanisms of the observed phenomenon need to be elucidated. Extensive experiments aiming at determining what factors are engaged in the regulation of CD20 by HDAC6 will be performed.

The research was supported by Polish Ministry of Science and Higher Education [grants 1M19/3DG4/12 (M. Wan.) and IP2011 060271 (M. Win.)]. This work was also supported by a grant from the European Commission 7th Framework Programme: FP7-REGPOT-2012-CT2012-316254-BASTION. All authors declared no relevant financial relationship to disclose as well as no off-label drug usage to be included into presented data.

Discussion during poster walk moderated by Philippe Gaulard

Travel Grant Winner - conference fee and travel costs covered by EHA

TRAVEL GRANT WINNERS

For this Congress 95 travel grants have been awarded to junior members of EHA, based on the mean score of their abstracts. Of these travel grants 15 have been reserved for abstract authors from countries with lower- and lower/middle income economies.

EHA congratulates the following persons with their travel grants:

Aalbers A, *the Netherlands*
Abolhasani B, *Germany*
Al L, *China*
Alpar D, *Hungary*
Ballakas P, *Greece*
Baran Y, *Turkey*
Bazeos A, *United Kingdom*
Benedetti D, *Italy*
Bolli N, *United Kingdom*
Bonina S, *Italy*
Bosmann M, *Germany*
Bray S, *Australia*
Brioll A, *United Kingdom*
Burin S, *Brazil*
Castagnetti F, *Italy*
Chiappella A, *Italy*
Chilton L, *United Kingdom*
Cimino M, *Italy*
Coelho V, *United Kingdom*
Davids M, *USA*
De Angelis B, *Italy*
Degryse S, *Belgium*
Denk D, *Austria*
Dickinson M, *Australia*
Dinmohamed A, *the Netherlands*
Doñate C, *Switzerland*
Dovey O, *United Kingdom*
Eda H, *USA*
El Chaar T, *France*
Elmaomly S, *Egypt*
Fedor M, *Slovakia*
Giammarco S, *Italy*
Goossens S, *Belgium*
Gorantla S, *Germany*
Gugliotta G, *Italy*
Guitart A, *United Kingdom*
Iqbal Z, *Saudi Arabia*
Jilg S, *Germany*
Jones A, *United Kingdom*
Jutzi J, *Germany*
Kapp-Schwoerer S, *Germany*
Kato T, *Japan*
Kenderian S, *USA*
Khan A, *India*
Klingeberg C, *Germany*
Koszarska M, *Hungary*
Kumiawan A, *Indonesia*
Kuznetsova I, *Germany*
Lauw M, *the Netherlands*
Lekovic D, *Serbia*
Leo E, *Italy*
Lesteven E, *France*
Loke C, *United Kingdom*
Malques A, *Spain*
Malso P, *USA*
Malato A, *Italy*
Malcikova J, *Czech Republic*
Mansur M, *Brazil*
Martello M, *Italy*
Mensen A, *Germany*
Mitrovic M, *Serbia*
Mraz M, *Czech Republic*
Mukherjee L, *United Kingdom*
Nagata Y, *Japan*
Niederwieser C, *USA*
Pallis F, *Brazil*
Papaemmanuil E, *United Kingdom*
Parry M, *United Kingdom*
Pede V, *Belgium*
Pereira M, *Portugal*
Pimenova M, *Russian Federation*
Pugliese N, *Italy*
Quintarelli C, *Italy*
Rampal R, *USA*
Rheinemann L, *Germany*
Rumi E, *Italy*
Russo R, *Italy*
Sanchez-Aguilera A, *Spain*
Schimmel M, *the Netherlands*
Schwind S, *Germany*
Shlyakhto V, *Germany*
Sinclair A, *United Kingdom*
Sins J, *the Netherlands*
Sportoletti P, *Italy*
Stephens D, *USA*
Strati P, *USA*
Tissino E, *Italy*
Torun D, *Turkey*
Trentin L, *Germany*
Vegi M, *Germany*
Walker C, *USA*
Wanczyk M, *Poland*
Williamson H, *United Kingdom*
Winiarska M, *Poland*

Travel Grant Supported By Giuseppe Bigi Association
Messina M, *Italy*