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ORIGINAL ARTICLE

Identification of a novel resistance mechanism in venetoclax treatment and its prediction in chronic lymphocytic leukemia

Ferenc Takács[a], Gábor Mikala[b], Noémi Nagy[a], Andrea Reszegi[a], Ágnes Czeti[a], Gábor Szalóki[a] and Gábor Barna[a]

[a]1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary; [b]South-Pest Central Hospital – National Institute for Hematology and Infectious Diseases, Department of Hematology and Stem Cell Transplantation, Budapest, Hungary

ABSTRACT

Background: The Bcl-2 inhibitor venetoclax has been recently introduced into the treatment of chronic lymphocytic leukemia. Venetoclax is a highly effective drug, however acquired resistance may make long-term treatment challenging. In our study, we present potential novel resistance mechanisms and prognostic markers that are potentially able to predict the early appearance of the resistance.

Material and methods: Repeated complete blood counts, flow cytometric measurements, and physical examinations were performed during the patient follow-up. Clinical and laboratory parameters showed that the patient developed clinical resistance to venetoclax on day 450 of therapy. Resistance mutation analysis (D103Y) and apoptosis arrays from samples at the time of resistance were done.

Results: We were able to identify the resistance mutations just a very low variant allele frequency level from the resistant samples. Furthermore we detected increased Bcl-2 expression in peripheral blood (PB), and XIAP overexpression in bone marrow (BM) that could lead to venetoclax resistance. We examined the immunophenotype of CLL cells and recognized that while the expression of CD86 did not change until day 270 of the treatment, since then its expression steadily increased. Moreover, we compared the expression of CD86 in the resistant PB and BM samples and did not find a notable difference between the compartments.

Conclusion: Our results imply that CLL cells may try to avoid the apoptotic effect of venetoclax through increased CD86 expression by activating antiapoptotic mechanisms. Confirmatory experiments are still required to unequivocally prove that CD86 is a prognostic marker, however, its predictive property during the venetoclax treatment is promising.

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Chronic lymphocytic leukemia; drug resistance; flow cytometry; targeted therapy

Background

CLL is an indolent and incurable, biologically heterogeneous disease [1]. In recent years, several innovative drugs were introduced in the therapeutic repertoire of CLL including the selective Bcl-2 inhibitor venetoclax (VEN). Venetoclax, by selectively binding to Bcl-2, suspends the antiapoptotic effect of Bcl-2, which causes apoptosis in the tumor cells [2]. Since continuous treatment is required to maintain remission, the emergence of drug resistance may make long-term treatment difficult. Numerous resistance mechanisms of venetoclax have been described in the literature. Resistance can develop either due to the appearance of resistance mutations in the BCL2 gene or because of the compensatory overexpression of other antiapoptotic molecules (e.g., Bcl-X_L, MCL-1) [3–7]. In our study, we found Bcl-2 overexpression in peripheral blood and XIAP overexpression in the bone marrow which could lead to venetoclax resistance. Besides this, the expression of surface CD86 might predict the development of resistance during venetoclax treatment.

Material and methods

Patient history

A 76-year-old caucasian man was diagnosed with CLL in October 2013. Following standard chemoimmunotherapies (rituximab-cyclophosphamide-vincristine-prednisone, rituximab-fludarabine-cyclophosphamide, rituximab-bendamustine) the disease progressed, and venetoclax salvage therapy was started with 400 mg daily dose (after standard step-up dose titration) in April 2018. The lymphadenomegaly regressed, no tumor lysis syndrome was detected, however, minimal residual disease negativity was not achieved. With good disease control under continuous therapy, the patient has been followed-up. Relapse occurred after 450 days of starting the VEN treatment. The patient had 51.23 G/l white

CONTACT Gábor Barna barna.gabor@med.semmelweis-univ.hu 1st Department of Pathology and Experimental Cancer Research, Faculty of Medicine, Semmelweis University, Üllői út 26, Budapest, H-1085, Hungary

Supplemental data for this article can be accessed here.

blood cell count, and the lymphadenomegaly was again reappered. Bone marrow sampling was performed to exclude possible disease transformation, but Richter transformation was not histologically confirmed. Ibrutinib salvage treatment was introduced that resulted in the regression of the lymph nodes and clinical stabilization of the patient.

Samples and methods

In this study, peripheral blood samples were examined by flow cytometry on days 0, 180, 270, 360, and 450 of venetoclax monotherapy. The samples were measured by using a Navios Flow cytometer (Beckman Coulter (BC), Brea, USA) and data were analyzed with Kaluza 2.1 (BC) analysis software. The proportion of CLL cells was determined by using CD45, CD19, CD5, and CD3 antibodies (antibody clones and fluorochromes are in Supplementary Table 1). The expression of the CD86 molecule was examined among CD19 positive cells. We used CD19 negative lymphocytes as an internal control. The CD86 median fluorescence intensity (MFI) value was determined by subtracting the CD19 negative cells MFI value from the CD19 positive cells MFI value. The gating strategy is shown in Supplementary Figure 1 and 2. A bone marrow sample was available on day 450 and the phenotype of CLL cells was also determined in this sample. According to the current literature, the role of the G101V *Bcl-2* mutation seems controversial in venetoclax resistance [8,9]. Therefore the presence of the *Bcl-2* D103Y resistance mutation was tested in the resistant peripheral blood and bone marrow samples by high-sensitivity digital droplet PCR (Bio-Rad Laboratories Hercules, USA, Analysis QuantaSoft software 1.7 version) [8]. Apoptosis array (R&D Systems Minneapolis, USA, Human apoptosis antibody array kit) was performed from the mononuclear cell fraction of the samples according to the manufacturer's instructions (Analysis ImageJ software version 1.50d, NIH, USA) [10,11]. The study was conducted following the Declaration of Helsinki and has been approved by the local ethics committee (number of ethical permission: TUKEB 7/2006). The patient had understood the details of study and he agreed by freely given consent in writing.

Results and discussion

During the venetoclax monotherapy, the phenotype and the ratio of CLL cells were monitored by flow cytometry (Figure 1). Until day 270 of the VEN treatment, the CLL ratio among the white blood cells were decreased, but it started to increase indicating the appearance of acquired drug resistance (Figure. 1). The D103Y resistance mutation was identified just on a very low variant allele frequency level from bone marrow (0.19%) and peripheral blood (0.53%) samples at the time of resistance (on day 450 of VEN treatment). Therefore this mutation of *Bcl-2* cannot explain the resistance. We examined the expression of critical proteins involved in the apoptotic cascade to explore alternative resistance mechanisms. In apoptosis arrays, 33 apoptotic protein levels were determined on patient samples before the VEN treatment (day 0) and on samples at the time of clinical resistance (day 450). We compared the expression pattern of these proteins in the peripheral blood and bone marrow samples to detect potential compartmental differences. We did not find any notable difference in the expression of the antiapoptotic Bcl-X_L (day 0 PB 176.66 vs day 450 PB 148.36 vs day 450 BM 219.78 pixel density), but elevated Bcl-2 expression (2-fold in blood and 1.6-fold in bone marrow) was detected in the resistant samples (day 0 PB 1797.82 vs day 450 PB 3566.64 vs day 450 BM 2943.55 pixel density) (Figure 2). There was a small difference between peripheral blood and bone marrow samples on the level of proapoptotic BAD (0.88-fold in blood and 0.85-fold in bone marrow) (day 0 PB 589.51 vs day 450 PB 521.89 vs day 450 BM 498.39 pixel density), and BAX (0.84-fold in blood and 0.99-fold in bone marrow) (day 0 PB 850.70 vs day 450 PB 716.08 vs day 450 BM 843.28 pixel density). Interestingly, the expression of antiapoptotic XIAP (day 0 PB 2411.33 vs day 450 BM 3687.27 pixel density) was increased (1.5-fold) in bone marrow compared to the initial peripheral blood sample. Moreover, we experienced that there was a 1.9-fold difference in expression of XIAP between the resistant bone marrow and peripheral blood samples (day 450 PB 1940.43 vs day 450 BM 3687.27 pixel density). It is known that the elevated XIAP level in CLL can be a result of constitutive AKT activation, however, its biological role is poorly investigated in CLL [12]. In this case, the

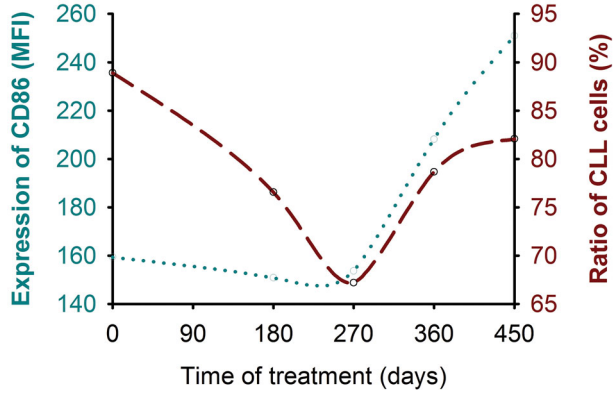

Figure 1. Change of CLL cell ratio during venetoclax treatment and change of CD86 expression during venetoclax treatment measured by flow cytometry.

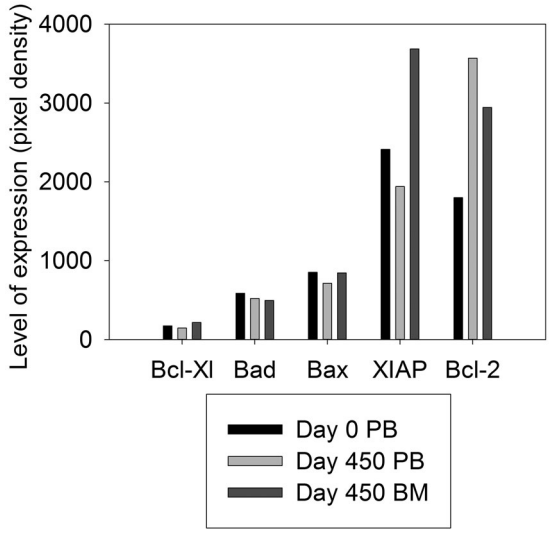

Figure 2. Change of protein expression during venetoclax treatment measured by apoptosis arrays.

expression of Bcl-2 and XIAP were increased at the time of the resistance and showed compartmental differences. (Figure 2). This suggests that CLL may develop different resistance mechanisms in different compartments and besides resistance mutations overexpression of antiapoptotic proteins may have led to resistance. An earlier study suggested that detecting Bcl-2 expression may be useful before the initiation of venetoclax therapy but it is currently not part of the routine diagnostic process [13]. Our results indicate that monitoring of the expression of antiapoptotic molecules (i.e., Bcl-2, XIAP) could be helpful not only at the beginning of the therapy but also during the treatment period.

It is well known that the detection of intracellular proteins by flow cytometry presents difficulty. Thus, it would be useful to find cell surface markers that can predict the emergence of resistance. We monitored various surface markers (see Supplementary Table 1) during the treatment and only the change of CD86 expression was notable (Figure 1). Until day 270 of the treatment, the expression of CD86 did not change (day 0 159.38, day 180 150.85, day 270 153.65 MFI value) since then the expression of CD86 steadily increased (day 360 208.07, day 450 250.83 MFI value). We compared CD86 expression in the resistant peripheral blood and bone marrow samples and did not find notable expression differences between the two compartments (PB 250.83 vs BM 253.26 MFI value). The prognostic role of CD86 in CLL has been mentioned in the literature before [14,15]. Suvas et al. showed that increased expression of antiapoptotic proteins such as Bcl-X_L was associated with increased CD86 expression in B-cells and lymphoma cells [16]. Our results raise the possibility that increased CD86 expression indicates the elevation of levels of antiapoptotic proteins.

Summary

Overall, in this study, we wanted to draw attention to a potentially new venetoclax resistance mechanism in CLL by presenting our case. We found that CLL cells may develop different resistance mechanisms in different compartments. Furthermore, the expression of CD86 molecule can be associated with disease progression during the venetoclax treatment, and its expression in the bone marrow and peripheral blood is closely correlated with each other. Several confirmatory experiments are still required to establish CD86 as a prognostic marker, however, its predictive property during the venetoclax treatment is promising.

Disclosure statement

The authors report no conflicts of interest.

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ORCID

Ferenc Takács http://orcid.org/0000-0001-8686-0295
Noémi Nagy http://orcid.org/0000-0002-7848-3466
Andrea Reszegi http://orcid.org/0000-0001-6902-7883
Gábor Szalóki http://orcid.org/0000-0001-6190-9128
Gábor Barna http://orcid.org/0000-0002-8895-3062

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