Seeking for the possible prognostic and predictive factors in chronic lymphocytic leukaemia (CLL) focusing on the main role of the CD86 molecule

PhD Thesis

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Budapest

2023

1. Introduction

CLL is an indolent and incurable disease, but the life expectancy of patients has increased dramatically in the recent years due to the development of more accurate diagnostics and novel therapeutics. Newer treatments such as the Bruton's tyrosine kinase inhibitor ibrutinib (IBR) and the Bcl-2 inhibitor venetoclax (VEN) have emerged and the use of these agents has dramatically increased the life expectancy of patients with CLL. However, not all patients respond equally to treatment and drug resistance can occur even during treatment with ibrutinib or venetoclax. Since, it is difficult to find the right treatment for the right patient without predicting disease progression, the search for novel prognostic or predictive factors has recently become an increasingly attractive topic in haematological research, especially in CLL. Molecular biology methods have provided many potential prognostic or predictive factors, but their detection can sometimes be very time consuming and expensive, thus there is still a need to find these kind of factors in CLL, which can be detected in a less expensive and less time consuming way. Therefore, we wanted to look for novel

factors in our studies, focusing on the important role of CD86, which is a very promising candidate.

2. Objectives

Against this background, we wanted to address the following questions:

The prognostic value of the CD86 molecule in CLL

- Is there any connection between CD86 expression of CLL cells and disease outcome?

The influence of the venetoclax resistance on the phenotype of CLL cells

- Is there any connection between the venetoclax treatment and the phenotype of CLL cells?
- Is there any connection among the resistance mechanisms of CLL cells and their immunophenotype?

The effect of the ibrutinib and its resistance on the phenotype of CLL cells

- Is there any connection between the microenvironment related surface markers of CLL cells and the ibrutinib treatment?
- Is there any correlation between the expression of the microenvironment related surface markers on

the CLL cells and the presence of the BTK^{C481S} mutation of the CLL cells?

3. Methods

Patients' Clinical Characteristics:

In our first study, we collected peripheral blood (PB) samples from 49 patients with CLL. The female/male ratio was 22/27 with a median age of 67 years (40–87). Patients were divided into $CD86^{high}$ and $CD86^{low}$ groups based on their CD86 levels measured by flow cytometry. For the analysis of the time to treatment values, clinical data were available for only 18 patients (7 from the $CD86^{low}$ group and 11 from the $CD86^{high}$ group).

In our second study, we collected PB samples from CLL patients who were treated with VEN monotherapy. We enrolled 4 patients in our study (referred to as V1-V4). The V1, V3, and V4 patients were MRD negative during the study. Patients V2 did not achieve MRD negativity and attended regular follow-up visits, and his samples were analysed by flow cytometry on days 0, 180, 270, 360, and 450 of the treatment. Patient V2 lost his clinical response to VEN at month 15 of treatment. A BM sample was collected at this time. PB samples from patient V2 were also used later when this patient was treated with ibrutinib.

In our third study, PB samples were collected from treatment-naïve (Co) (n=10), ibrutinib-sensitive (IS) (n=7) and clinically ibrutinib-resistant (IR) (n=11) CLL patients. All patients treated with ibrutinib received the drug as a single agent at a daily dose of 420 mg. Patients in the IS cohort were treated with IBR for exactly one year, and PB samples were collected after one year of IBR treatment. All these patients were found to be negative for the BTK^{C481S} mutation. Patients in the IR group were treated with ibrutinib for at least 4 months (median 28.5 months, range: 4-57 months) and the PB samples were collected when they were considered to be clinicaly ibrutinib resistant.

Measurement of the surface markers expression by flow cytometry:

During our studies we determined the ROR1, CD69. CD44, CD49d, CD184, CD40, CD5, CD45, CD27, CD38, CD185, CD3, CD19, CD86, CD197 surface expression by flow cytometry. And for the MRD measurement the following panel was used: Syto40, CD5, CD3, CD81, ROR1, CD79b, CD19, CD43 and CD20.

Apoptosis array:

In our second study, we performed an apoptosis array using a human apoptosis antibody array kit containing 46 proteins (i.e. XIAP, Bcl-2, Bcl-XL, BAD, BAX etc.).

Molecular methods:

The presence of the Bcl-2^{D103Y} resistance mutation in the venetoclax-resistant peripheral blood and bone marrow samples of patient V2 and the *BTK*^{C481S} resistance mutation in the IS and IR samples was detected by high-sensitivity digital droplet PCR (ddPCR).

Statistical analysis:

SigmaPlot 12.5 was used for plotting and statistical analysis. Differences were considered statistically significant at $p<0.05$.

4. Results

The results of our first study suggested that the increased CD86 expression on CLL cells may lead to an unfavourable disease outcome, as the high CD86 group had a shorter time to treatment volume. However, our result was not statistically significant, thus further investigation is needed to draw a conclusion.

The results of our second study showed that CD86 expression in CLL cells is decreased by venetoclax treatment, whereas it is increased in the presence of venetoclax resistance associated with Bcl-2^{D103Y} resistance mutation. Our second study also showed that the levels of the anti-apoptotic proteins Bcl-2 and XIAP were increased in the resistant PB and BM samples compared to their levels at the start of the venetoclax treatment. Furthermore, we found a compartmental difference in the expression of these markers, namely the expression of XIAP was elevated in the resistant BM samples, whereas the expression of Bcl-2 was elevated in PB.

Our third study showed that the expression of CD27 and CD86 is reduced by ibrutinib treatment, and that the expression of both markers increases in the presence of

ibrutinib resistance. Furthermore, the expression of CD86 and CD27 was significantly higher in the IR cohort than in the IS cohort. In addition, we found an association between the ibrutinib resistance mutation (*BTK*^{C481S}) and the expression of CD86 and CD27 on CLL cells; namely, their expression is increased in the presence of *BTK*^{C481S}.

In conclusion, CD86 appears to be a suitable predictive marker in CLL, as its increased expression may predict potential treatment failure with ibrutinib and venetoclax. Furthermore, measuring its expression during ibrutinib or venetoclax treatment may be a surrogate method to detect resistance in the absence of appropriate molecular tests.

5. Conclusions

As a conclusion, we can make the following new statements:

1. The expression of CD86 is increased in the case of venetoclax resistance.
2. There is connection between the increased Bcl-2 protein level and increased CD86 expression of the venetoclax resistant CLL cells.
3. The expression of CD27 and CD86 is decreased due to ibrutinib but both expressions are increased in the case of ibrutinib resistance.
4. The expression of CD27 and CD86 is increased in case of the occurring of BTK^{C481S} resistance mutation in CLL cells.
5. CD86 seems to be a suitable predictive marker of ibrutinib and venetoclax treatment in CLL, since its increased expression can foreshadow a potential ibrutinib and venetoclax treatment failure. Moreover, measuring its expression during ibrutinib or venetoclax treatment can be a surrogate method of

revealing the resistance in case of the lack of the appropriate molecular tests.

6. Bibliography of the candidate's publications

Thesis is based on the following publication:

Revealing a phenotypical appearance of Ibrutinib Resistance in Patients with Chronic Lymphocytic Leukaemia by Flow Cytometry

***Ferenc Takács**, Lili Kotmayer, Ágnes Czeti, Gábor Szalóki, Tamás László, Gábor Mikala, Ágnes Márk, András Masszi, Péter Farkas, Márk Plander, Júlia Weisinger, Judit Demeter, Sándor Fekete, László Szerafin, Beáta Margit Deák, Erika Szaleczky, Adrienn Sulák, Zita Borbényi, Gábor Barna*

Pathology & Oncology Research DOI: 10.3389/pore.2022.1610659 (2022)

IF:2.8

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

***Ferenc Takács**, Gábor Mikala, Noémi Nagy, Andrea Reszegi, Ágnes Czeti, Gábor Szalóki, Gábor Barna*

Acta Oncologica DOI: 10.1080/0284186X.2021.1878388 (2021)

IF: 4.311

The role of minimal residual disease in chronic lymphocytic leukemia - a review (Hungarian)

***Ferenc Takács**, Ilona Kardos, Ágnes Czeti, Dóra Aczél, Sarolta Illés, Alexandra Balogh, Júlia Gaál-Weisinger, Gábor Szalóki, Gábor Barna*

Hematológia és Transzfuziológia DOI:10.1556/2068.2020.53.1.4 (2020)

The Effect of CD86 Expression on the Proliferation and the Survival of CLL Cells

***Ferenc Takács**, Csilla Tolnai-Kriston, Márk Hernádfői, Orsolya Szabó, Gábor Szalóki, Ágota Szepesi, Ágnes Czeti, András Matolcsy, Gábor Barna*

Pathology & Oncology Research DOI: 10.1007/s12253-018-0512-7 (2019)

IF: 2.826

Thesis non-related publications:

Successful thrombolytic therapy is associated with increased granulocyte CD15 expression and reduced stroke-induced immunosupression

Katalin Anna Béres-Molnár, Ágnes Czeti, ***Ferenc Takács****, Gábor Barna, Dániel Kis, Gabriella Róka, András Folyovich, Gergely Toldi*

Brain and Behavior DOI: 10.1002/brb3.2732 (2022)

IF: 3.1

First-in-human study of WT1 recombinant protein vaccination in elderly patients with AML in remission: a single-center experience

Stefanie Kreutmair, Dietmar Pfeifer, Miguel Waterhouse, ***Ferenc Takács****, Linda Graessel, Konstanze Döhner, Justus Duyster, Anna Lena Illert, Anna-Verena Frey, Michael Schmitt, Michael Lübbert*

Cancer Immunology, Immunotherapy DOI: 10.1007/s00262-022-03202-8 (2022)

IF: 5.8

Limitations of VS38c labeling in the detection of plasma cell myeloma by flow cytometry

Ágnes Czeti, Gábor Szalóki, Gergely Varga, Virág Réka Szita, Zsolt István Komlósi, ***Ferenc Takács****, Ágnes Márk, Botond Timár, András Matolcsy, Gábor Barna*

Cytometry part A DOI: 10.1002/cyto.a.24488 (2021)

IF: 3.7

Lenalidomide abrogates the survival effect of bone marrow stromal cells in chronic lymphocytic leukemia

Csilla Kriston,Márk Hernádfői,Márk Plander, Ágnes Márk, ***Ferenc Takács****, Ágnes Czeti, Gábor Szalóki, Orsolya Szabó, András Matolcsy, Gábor Barna*

Hematological Oncology DOI: 10.1002/hon.2888 (2021)

IF: 4.850

Screening and monitoring of BTK C481S mutation in a real-world cohort of patients with relapsed/refractory chronic lymphocytic leukemia during ibrutinib therapy

Csaba Bödör, Lili Kotmayer, Tamas Laszlo, ***Ferenc Takacs****, Gabor Barna, Richard Kiss, Endre Sebestyen,Tibor Nagy, Lajos Laszlo Hegyi, Gabor Mikala, Sándor Fekete, Péter Farkas, Alexandra Balogh,Tamás Masszi, Judit Demeter, Júlia Weisinger, Hussain Alizadeh, Béla Kajtár, Zoltán Kohl, Róbert Szász, Lajos Gergely, Timea Gurbity Pálfi, Adrienn Sulák, Balázs Kollár, Miklós Egyed, Márk Plander, László Rejtő, László Szerafin, Péter Ilonczai, Péter Tamáska, Piroska*

Pettendi, Dóra Lévai, Tamás Schneider, Anna Sebestyén, Péter Csermely, András Matolcsy, Zoltán Mátrai, Donát Alpár

British Journal of Hematology DOI: 10.1111/bjh.17502 (2021)

IF: 8.615