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

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Abstract
In the pathogenesis of chronic lymphocytic leukemia (CLL) the microenvironment plays an important role, as it produces survival signals and mediates drug resistance. Lenalidomide, which has immunomodulatory effect, can enhance the activation of T-, NK-cells and endothelial cells, however there are no data available whether it can modulate bone marrow stromal cells (BMSCs). In our study, we investigated the effects of lenalidomide on BMSCs and CLL cells. CLL cells were cultured alone or with BMSCs and were treated with lenalidomide. Apoptosis, immunophenotype, and cytokine secretion of BMSCs and CLL cells were determined by flow cytometry. Lenalidomide slightly increased the apoptosis of CLL cells and abrogated the anti-apoptotic effect of BMSCs on CLL cells. Lenalidomide treatment decreased the expression of antigens on CLL cells, which mediate the interactions with the microenvironment. Interestingly, lenalidomide enhanced the expression of IRF4 and the co-stimulatory molecule CD86. The secretion of several cytokines was not changed significantly by lenalidomide. CD49d-negative CLL cases were more sensitive to lenalidomide treatment. Our results suggest that lenalidomide has a limited effect on BMSCs, but it renders CLL cells more immunogenic and unresponsive to survival signals provided by BMSCs.

Keywords
bone marrow stromal cells, CLL, IMIDs, lenalidomide, microenvironment

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is one of the most frequent haematological malignancies, in which the pathologic B-lymphocytes infiltrate the secondary lymphoid organs and appear in the bloodstream and bone marrow. In the pathogenesis of CLL the microenvironment, which producing anti-apoptotic signals and promoting proliferation, plays an important role in proliferation centers in lymph nodes and bone marrow.1 Due to high proliferation rate and the increased survival of CLL cells in these proliferation centers, mutations occur frequently, leading to the appearance of new tumour cell clones and, ultimately, to disease progression.2

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Besides genetic factors, the microenvironment mediates resistance to conventional chemo-immunotherapy (e.g., fludarabine, cyclophosphamide, rituximab) or new targeted therapies (e.g., venetoclax, ibrutinib). Lenalidomide (Revlimid, CC-5013), a second-generation immunomodulatory drug (IMiD) – which is now recommended as maintenance therapy for CLL\(^5\) according to the 2019 NCCN guideline-exerts immunomodulatory and anti-proliferative effect.\(^6\) The known direct molecular target of lenalidomide is Cereblon. Lenalidomide causes selective ubiquitination and degradation of two lymphoid transcription factors, IKZF1 and IKZF3, and decreases the expression of interferon regulatory factor 4 (IRF4).\(^7\) Lenalidomide enhances the activation of T- and NK-cells\(^8\) and regulates the endothelium.\(^9\) However, there are no data whether it can modulate bone marrow stromal cells (BMSCs), which provide several survival factors for CLL cells.\(^1\)

In our study, we investigated whether lenalidomide influences the bone marrow stromal cells regarding the protection from apoptosis and the cytokine production. We showed that lenalidomide abrogated the anti-apoptotic and immunophenotype alteration effect of BMSCs on CLL cells. Furthermore, lenalidomide treatment increased the level of IRF4 in CLL cells. Interestingly, the secretion of several cytokines was not changed significantly by lenalidomide. Our results suggest that lenalidomide has a limited effect on BMSCs, but renders CLL cells unresponsive to survival signals provided by BMSCs.

### 2. Material and Methods

#### 2.1 Patients, samples and drugs

Samples of 21 CLL patients (mean age 73.1 years (54–85), female/male ratio (8/13)) were used in the study. The diagnoses were established according to the World Health Organization (WHO) classification of tumours of lymphoid tissues.\(^10\) The patients were not previously treated or had not received treatment in the preceding six months. From the 21 studied cases only three cases showed 11q deletion, one case with 11q and 13p deletion and one case had TP53 mutation. Written informed consent was obtained from all participants, and the study was conducted in accordance with the Declaration of Helsinki and has been approved by the local Ethics Committee in Semmelweis University (199/2015). Lenalidomide (provided by Celgene) was used in 10 μM concentrations as used before in an in vitro study.\(^11\)

#### 2.2 Cell isolation and cell culture

CLL cells were isolated from peripheral blood by Ficoll density gradient centrifugation \((n = 13)\) or by EasySep immunomagnetic positive selection \((n = 8)\). After Ficoll (Histopaque-1077, Sigma Aldrich, USA) density gradient centrifugation the CLL cell ratio was above 90% among peripheral blood mononuclear cells (PBMCs). We performed B-cell isolation by EasySep CD19 Positive Selection Kit II (Stemcell\(^\text{TM}\) technologies, Canada) in order to achieve the maximal CLL cell ratio in our samples. Consequently, the CLL cell ratio was proved to be above 99% (Suppl. Figure S1). We performed the positive selection according to the manufacturer’s instructions.

The cells were cultured as previously described.\(^12\) Briefly, 2 × 10\(^6\)/ml CLL cells were cultured in RPMI-1640 (Sigma) supplemented with 10% FBS (Biosera, Philippines) medium alone or in co-culture with BMSCs for 7 days. Lenalidomide was added on the first day. The BMSC cultures, isolated from bone marrow aspirates of healthy individuals or ITP patients without abnormal cells (as determined by flow cytometry), were prepared as described previously.\(^13\) BMSCs were cultured in 24-well plates maintained at a concentration of 2 × 10\(^4\) cells/ml in Dulbecco’s modified Eagle’s medium (DMEM; Sigma) supplemented with 20% FBS (Biosera). In co-culture experiments, the DMEM medium of the BMSC monolayers was replaced with CLL cell suspension (1 ml of RPMI medium containing 2 × 10\(^6\) CLL cells were added to each well).

#### 2.3 Immunophenotyping of CLL cells by flow cytometry

CLL cells were stained with fluorochrome-conjugated monoclonal antibodies (see in Suppl. Table S1). The measurements were carried out using a FACSCalibur (BD Biosciences, USA) (BD) flow cytometer and analysed by CellQuest Pro software (BD). Twenty thousand events were acquired from every sample. We used isotype controls to check the aspecific binding and autofluorescence change in various culture conditions. Where the determination of positivity was required, we gated on lymphocytes, and the double (e.g. CD19\(^−\)/CD49d\(^−\)) negative population was used as an internal control. In the case of markers where the determination of fluorescence intensity was required, we gated on CD19-positive cells. Data were presented as geometric mean fluorescence intensity (MFI) or as the percentage of positive cells. After culturing, the immunophenotype was determined by gating on living cells. The cut-off value between the CD49d-positive and CD49d-negative samples was determined at a 30% presence of positive cells.\(^14\)

#### 2.4 Intracellular staining of IRF4 and Mcl-1

Firstly, CLL cells were stained with fluorochrome-conjugated monoclonal antibodies specific for surface antigens: CD49d PE (BD), CD19 APC (BC). After incubation with surface antibodies, fixation and permeabilization were performed by Intrastain Kit (DAKO, Denmark). IRF4 PE-Cy7 (Biolegend, USA), Mcl-1 Alexa 647 (Invitrogen, USA) and matched isotype control was used for intracellular staining. Thirty thousand events were collected from every sample, and the samples were measured by a Navios einigt color flow cytometer (Beckman Coulter, USA) (BC). Data were analysed by Kaluza software (BC). The difference of median fluorescence intensity (MFI) of isotype controls and IRF4-stained samples were calculated and
presented. In case of Mcl-1 staining, results of the isotype control were controversial, therefore we used the simple MFI values. The antibody was validated with an Mcl-1 positive cell line.

2.5 | Determination of apoptosis by subG1 measurement

SubG1 apoptosis measurements were performed as described by Gong et al. 15 5 × 10^5 cells were fixed in ice-cold ethanol (70%), followed by alkaline extraction (200 mM Na2HPO4, pH 7.4), then stained with propidium-iodide (Sigma). Ten thousand events were measured with FACS caliber (BD) and analysed by Kaluza software (BC). The SubG1 population was defined as apoptotic cells.

2.6 | Cytokine detection by CBA assay

The concentration of cytokines was determined by cytokine bead assays (CBA immunoassay) (Suppl Table S2). We cultured CLL cells with or without stromal cells for 7 days and treated them with lenalidomide. We cultured BMSCs without CLL cells to check the cytokine production of them and the effect of lenalidomide on them. We collected supernatant from all of the samples. The assay was carried out according to the manufacturer’s instructions. The samples were acquired by a FACSCalibur (BD) flow cytometer and analysed by FCAP Array software (BD). The results were presented as relative cytokine levels. Cytokine levels were normalized to the value measured in the medium of the untreated CLL cells cultured alone.

2.7 | Statistical analysis

All variables were tested for normal distribution to select the appropriate parametric or non-parametric statistical procedure. Paired t-test, Wilcoxon paired test and Mann–Whitney U-test were used for statistical evaluation using the SPSS statistics software, version 25.0 (SPSS, USA). Statistical significance was set at p < 0.05.

3 | RESULTS

3.1 | Lenalidomide inhibits the anti-apoptotic effect of BMSCs on CLL cells

In our study, we investigated the effect of lenalidomide on CLL cells cultured alone or in co-culture with BMSCs for 7 days. We found that 53.8% (SEM = 5.35) of the CLL cells showed apoptotic DNA fragmentation after 7 days of culturing in medium (n = 21) (Figure 1). 10 μM lenalidomide (Len) increased the ratio of the apoptotic cells by 5.3 percentage points compared to CLL cells cultured in medium (p = 0.042). BMSCs protected CLL cells from apoptosis and reduced the apoptotic CLL cell ratio by 9.9 percentage points compared to CLL cells cultured in medium (p = 0.001). Lenalidomide inhibited this anti-apoptotic effect and elevated the ratio of apoptotic CLL cells by 10.4 percentage points compared to CLL and BMSC co-culture samples (p = 0.001) (Figure 1A). Cases with genetical alteration did not show different behavior to lenalidomide treatment. Furthermore, spontaneous and lenalidomide-induced apoptotic rates of Ficoll and magnetic bead separated cases did not show significant differences (Suppl. Figure S2).

Since CD49d is an important factor in the communication of CLL cells and microenvironment, we compared the survival of CD49d-positive (n = 10) and CD49d-negative (n = 11) samples (Figure 1B). We showed earlier that the in vitro apoptosis rate was lower in CD49d-negative cases. 15 Here we found that the CD49d-negative CLL cells were more sensitive to the direct apoptotic effect of lenalidomide than CD49d-positive ones. At the end of the 7-days cultivation, 37.8% (SEM = 5.9) of the CD49d-negative CLL cells were apoptotic, and 10 μM lenalidomide increased this ratio by 8.5 percentage points (p = 0.011). Co-culture with BMSCs significantly decreased the ratio of apoptotic CD49d-negative CLL cells to 30.5% (SEM = 4.45) (p = 0.032). Nevertheless, lenalidomide further elevated the level of apoptotic CLL cells by 9.5 percentage points (SEM = 5.6) (p = 0.012) compared with CLL cells co-culture with BMSCs.

In CD49d-positive cases lenalidomide did not show significant direct apoptotic effect on CLL cells cultured alone, but it was able to overcome the protective effect of BMSCs by increasing the apoptotic ratio from 58.5% (SEM = 5.7) to 70.2% (SEM = 5.0, p = 0.024) (Figure 1B). To clarify the origin of this difference, we compared the expression of IRF4 - which mediates the effect of IMiDs 16 - in CLL cells (n = 16). The level of IRF4 was almost two times higher in CD49d-negative cases than in CD49d-positive ones (p = 0.016) (Figure 2A).

IRF4 influences the expression of several proteins, among others the anti-apoptotic protein, Mcl-1 which is important in the development of CLL. 17 Therefore, we measured the IRF4 and the Mcl-1 levels after lenalidomide treatment. Lenalidomide treatment increased IRF4 level, especially in CLL cells co-cultured with BMSCs (p = 0.036) (Figure 2B). Lenalidomide did not alter Mcl-1 level in CLL cells.

3.2 | The effect of lenalidomide on the cytokine production of stromal cells

It was shown that lenalidomide alters the cytokine production of T-cells and NK-cells in the microenvironment of CLL cells. 8,9 Here, we investigated the effect of lenalidomide on the cytokine production of BMSCs. We measured the level of IL-6, IL-8, IL-1β, human bFGF, sCD54 (ICAM1), sCD154 (CD40L) and TNFα. The level of IL-6, bFGF and IL-8 were elevated in the media of CLL-BMSCs co-culture compared with CLL alone (IL-6: CLL:1, CLL + str:125.4 ± 57.2, (p = 0.009); bFGF:CLL:1, CLL + str:1.98 ± 0.42, (p = 0.007); IL-8: CLL:1, CLL + str:5.38 ± 2.4, (p = 0.047)) (mean ± SEM MFI values)
Lenalidomide did not influence the production of bFGF but diminished the secretion of IL-6 and IL-8 in co-culture condition (IL-6: 125.4 ± 57.2; CD5+ str + Len: 112.8 ± 48.3; IL-8: 142.4 ± 24.0, CD5+ str + Len: 4.47 ± 1.6) (mean ± SEM), although these did not reach statistical significance. The level of other cytokines was not influenced by lenalidomide (Suppl. Figure S3).

3.3 | Lenalidomide alters the immunophenotype of CLL cells

The immunophenotype of CLL cells changed during co-culturing with BMSCs as showed in our previous work. Here, we analysed the effect of lenalidomide on the immunophenotype of CLL cells. Lenalidomide decreased the expression of CD5, CD19, CD44 and increased the expression of CD86. Furthermore, it hindered the stimulating effect of BMSCs on CD5, CD19, CD49d and CD44 expression (Figure 4).

We also investigated how lenalidomide affects the immunophenotype of CLL cells in CD49d-positive and CD49d-negative samples. In CD49d-negative samples, the level of CD5 was more than two times higher than in CD49d-positive samples. BMSCs elevated the CD5 expression of CLL cells in both groups. Lenalidomide significantly decreased the CD5 expression in CD49d-negative CLL cells cultured alone or with BMSCs (the MFI decreased from 142.4 to 103.4 [p = 0.037] and from 163.7 to 114.1 [p = 0.007], respectively), which are a 27.4% and a 30% decrease compared to untreated and co-cultured CLL cells, respectively. In the CD49d-positive group, lenalidomide only slightly decreased the CD5 expression of CLL cells cultured alone, but in co-culture it inhibited the CD5 expression increasing the effect of BMSCs (p = 0.046) (Figure 5A).

We also investigated whether lenalidomide treatment or co-cultivation with BMSCs have any effects on the CD49d expression of CLL cells. We found that in the case of CD49d-positive samples, stromal cells increased the level of CD49d (with 44%, MFI changed from 25.8 to 37.2, p = 0.028), but lenalidomide was able to decrease
the CD49d expression below the original level of the CLL cells even in mono- (from 25.8 to 22.5, p = 0.028) or in co-culture with BMSCs (from 37.2 to 25.8, p = 0.028) (Figure 5B).

Regarding other markers, lenalidomide had no significant effect on the marker expression neither in CD49d-positive, nor in CD49d-negative samples (data not shown).

4 | DISCUSSION

Lenalidomide, a second-generation derivative of thalidomide, is approved for the treatment of multiple myeloma,\(^{18}\) myelodysplastic syndromes and mantle cell lymphoma.\(^{19,20}\) In these diseases, it has direct cytotoxic and immunomodulatory effects\(^{21}\) on pathologic cells. The drug also has a significant effect in CLL, which is mediated by the inhibition of the proliferation of CLL cells, enhanced immune responses, and the reduction of survival factors from the microenvironment.\(^{6,8}\) In the treatment of CLL, lenalidomide was optimally applied in combination with other drugs.\(^{22-24}\) The direct cytotoxic effects of lenalidomide in CLL have been investigated in a few studies,\(^{6,25}\) and no notable apoptosis rate could be detected after 48 hours. However, Acebes-Huerta A. et al. observed a significant apoptosis rate after 7 days.\(^{8}\) We cultured isolated CLL cells in the presence or absence of BMSCs and treated them with lenalidomide for 7 days and we also detected significant but not very high level apoptosis induced by lenalidomide. Furthermore, it inhibited the anti-apoptotic effect of BMSCs. Similarly, the group of Maffei R. showed that lenalidomide was able to inhibit the survival effect of endothelial cells and myeloid nurse-like cells (NLCS).\(^{7,26}\) The apoptotic effect of lenalidomide was stronger in CD49d-negative cells compared to CD49d-positive samples. These results suggest that CLL cells without CD49d expression are more sensitive to the cytotoxic effect of lenalidomide. In myeloma cases, lenalidomide downregulated the level of IRF4 via cerebrol and induced apoptosis with high efficiency.\(^{27}\) Therefore, we measured the level of IRF4 in CLL cases, and revealed that CD49d-negative cases expressed a higher level of IRF4 than CD49d-positive cases. This result is corroborated by the study of Shukla V. et al. or with the study of Fiorcari S. et al. who found that IRF4-positive CLL cells were negative for CD38 or for CD49d.\(^{28,29}\) Furthermore, there are various studies, in which IRF4-negative cells were positive for Notch2 and for Mcl-1, which are essential for the development of CLL.\(^{17,26}\) These
results are consistent with that CD49d-positive CLL cases - with lower IRF4 expression - also have poor prognosis. We have investigated whether lenalidomide is able to influence the level of IRF4 or Mcl-1. We found that lenalidomide elevated IRF4 level mainly in co-culture conditions independently of CD49d level. Chen Ch. et al. also detected the elevation of IRF4 after 48 hours exposure of lenalidomide in B-cells of 3 patients with CLL. The CD49d status of these patients is unknown.30

Patients who responded to lenalidomide therapy showed a more pronounced decrease of IL-6, IL-8, VEGF and bFGF than non-responders.31 Lenalidomide decreased the level of CCL2, CXCL12, and HGF1 produced by NLcs. We supposed that lenalidomide also decreases the cytokine production of BMSCs. We confirmed the former results that IL-6, IL-8, and bFGF levels increased in the co-culture of CLL cells and BMSCs.13,32 Interestingly, although the secretion of IL-6, IL-832 was slightly decreased by lenalidomide in accordance with a previous work,31 the levels of other cytokines (human bFGF, sCD54, sCD154, and TNF) were not altered by lenalidomide treatment.

The clinical effects of lenalidomide treatment were associated with the upregulation of CD40, CD80, CD86, CD154 on CLL cells.25,33,34 Lenalidomide also influenced the expression of CD20, however, the results are controversial: Lapalombella R. et al. showed the downregulation,35 while Acebes-Huerta A. et al. detected the upregulation of CD20 after lenalidomide treatment.8 We investigated the effect of lenalidomide on the immunophenotype of CLL cells. Here we show that lenalidomide decreased the expression of markers mediating microenvironmental interactions, such as CD5, CD49d and CD19, and increased the expression of CD86 on CLL cells. Currently, a correlation between the expression of IRF4 and CD86 was shown in CLL and the poor prognostic role of low IRF4 expression was demonstrated due to enhanced tumour immune evasion.35 Lenalidomide also decreased the level of CD44, which is responsible for cell adhesion-mediated drug resistance in lenalidomide-refractory myeloma cells.36 Lenalidomide counteracted the stimulating effect of BMSCs in case of CD5, CD19, CD49d and enhanced further the expression of CD86. We compared the two prognostic groups – CD49d-positive and CD49d-negative CLL samples – in terms of how they respond to lenalidomide treatment. We found differences in CD5 and CD49d expression: lenalidomide decreased the CD5 expression on CD49d-negative samples and inhibited the stimulating effect of BMSCs on these samples. This process may be due to anti-proliferative effect of lenalidomide,6 because CLL cells with high CD5 expression have a higher proliferation rate as shown in Calissano’s study.37 Furthermore, in CD49d-expressing CLL cells, lenalidomide was able to decrease the CD49d level, and BMSCs had no effect on its expression.

Our results suggest that lenalidomide gives rise to apoptosis of CLL cells, but exert an only minimal effect on BMSCs. Lenalidomide alters the immunophenotype of CLL cells rendering them more immunogenic and inhibiting the protective effect of the bone marrow stroma. Here, we prove for the first time that CD49d-negative cells are more sensitive to the pro-apoptotic and immunomodulatory effects of lenalidomide. Thus, such cases may respond better to lenalidomide treatment and are the optimal candidate for this therapy.

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**AUTHORS CONTRIBUTIONS**

Csilla Kriston, Márk Hernádföi, Ferenc Takács and Ágnes Czeti performed the research, analysed the data and revised the paper. Orsolya Szabó performed research and analysed data, Márk Plander, Gábor Szalóki, András Matolcsy and Gábor Barna designed research and wrote the paper.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**DATA AVAILABILITY STATEMENT**

Data that support the study are available at the corresponding author, Gábor Barna.

**REFERENCES**

1. Burger JA, Gribben JG. The microenvironment in chronic lymphocytic leukemia (CLL) and other B cell malignancies: insight into disease biology and new targeted therapies. Seminars cancer Biol. 2014;24:71-81.
2. Szurija K, Csala I, Marosvári D, et al. EZH2 is upregulated in the proliferation centers of CLL/SLL lymph nodes. Exp Mol pathology. 2018;105(2):161-165.
3. Thijsen R, Slinger E, Wellers K, et al. Resistance to ABT-199 induced by microenvironmental signals in chronic lymphocytic leukemia can be counteracted by CD20 antibodies or kinase inhibitors. Haematologica. 2015;100(8):e302-e306.
4. Woyach JA, Furman RR, Liu T-M, et al. Resistance mechanisms for the Bruton’s tyrosine kinase inhibitor ibrutinib. N Engl J Med. 2014;370(24):2286-2294.
5. Fink AM, Bahlo J, Robrecht S, et al. Lenalidomide maintenance after first-line therapy for high-risk chronic lymphocytic leukaemia (CLLM1): final results from a randomised, double-blind, phase 3 study. Lancet Haematol. 2017;4(10):e475-e486.
6. Fecteau J-F, Corral LG, Ghia EM, et al. Lenalidomide inhibits the proliferation of CLL cells via a cereblon/p21WAF1/Cip1-dependent mechanism independent of functional p53. Blood. 2014;124(10):1637-1644.
7. Kronke J, Udeshi ND, Narla A, et al. Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. Science. 2014;343(6168):301-305.
8. Acebes-Huerta A, Huergo-Zapico L, Gonzalez-Rodriguez AP, et al. Lenalidomide induces immunomodulation in chronic lymphocytic leukemia and enhances antitumor immune responses mediated by NK and CD4 T cells. BioMed Res Int. 2014;2014:265840.
9. Maffei R, Fiorcari S, Bulgarelli J, et al. Endothelium-mediated survival of leukemic cells and angiogenesis-related factors are affected by lenalidomide treatment in chronic lymphocytic leukemia. Exp Hematol. 2014;42(2):126-136e1.
10. Jaffe ES, Harris NL. SHaVJW. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissue. IARC Press; 2001. 168-170 p.
11. Lopez-Millan B, Diaz de la Guardia R, Roca-Ho H, et al. IMiDs mobilize acute myeloid leukemia blasts to peripheral blood through downregulation of CXCR4 but fail to potentiate AraC/Idarubicin activity in preclinical models of non del5q/5q: AML. OncolImmunology. 2018;7(9):e1477460.
12. Kriston C, Plander M, Mark A, et al. In contrast to high CD49d, low CXCR4 expression indicates the dependency of chronic lymphocytic leukemia (CLL) cells on the microenvironment. Ann Hematol. 2018;97(11):2145-2152.
13. Plander M, Ugocsai P, Seegers S, et al. Chronic lymphocytic leukemia cells induce anti-apoptotic effects of bone marrow stroma. Ann Hematol. 2011;90(12):1381-1390.
14. Gattei V, Bulian P, Del Principe MI, et al. Relevance of CD49d protein expression as overall survival and progressive disease prognosticator in chronic lymphocytic leukemia. Blood. 2008;111(2):865-873.
15. Gong JP, Traganos F, Darzynkiewicz Z. A selective procedure for DNA extraction from apoptotic cells applicable for gel electrophoresis and flow cytometry. Anal Biochem. 1994;218(2):314-319.

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**TRANSPARENT PEER REVIEW**

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16. Kritharis A, Coyle M, Sharma J, Evens AM. Lenalidomide in non-Hodgkin lymphoma: biological perspectives and therapeutic opportunities. Blood. 2015;125(16):2471-2476.
17. Shukla V, Ma S, Hardy RR, Joshi SS, Lu R. A role for IRF4 in the development of CLL. Blood. 2013;122(16):2848-2855.
18. Rajkumar SV, Hayman SR, Lacy MQ, et al. Combination therapy with lenalidomide plus dexamethasone (Rev/Dex) for newly diagnosed myeloma. Blood. 2005;106(13):4050-4053.
19. Hagner PR, Chiu H, Ortiz M, et al. Activity of lenalidomide in mantle cell lymphoma can be explained by NK cell-mediated cytotoxicity. Br J Haematol. 2017;179(3):399-409.
20. Kim H, Lee JH, Lee WS, et al. Lenalidomide as a second-line therapy after failure of hypomethylating agents in patients with myelodysplastic syndrome. Br J Haematol. 2019;186(5):e151-e155.
21. Chang XB, Stewart AK. What is the functional role of the thalidomide binding protein cereblon? Int J Biochem Mol Biol. 2011;2(3):287-294.
22. Kater AP, van Oers MHJ, van Norden Y, et al. Feasibility and efficacy of addition of individualized-dose lenalidomide to chlorambucil and rituximab as first-line treatment in elderly and FCR-unfit patients with advanced chronic lymphocytic leukemia. Haematol. 2019;104(1):147-154.
23. Vitale C, Falchi L, Ten Hacken E, et al. Ofatumumab and lenalidomide interfere with the development of chronic lymphocytic leukemia: correlation between responses and immune characteristics. Clin Canc Res. 2016;22(10):2359-2367.
24. Ichaki G, Brown JR. Lenalidomide in the treatment of chronic lymphocytic leukemia. Expet Open Invest Drugs. 2017;26(5):633-650.
25. Chanan-Khan AA, Chitta K, Ersing N, et al. Biological effects and clinical significance of lenalidomide-induced tumor flare reaction in patients with chronic lymphocytic leukaemia: in vivo evidence of immune activation and antitumour response. Br J Haematol. 2011;155(4):457-467.
26. Fiorcari S, Martinelli S, Bulgarelli J, et al. Lenalidomide interferes with tumor-promoting properties of nurse-like cells in chronic lymphocytic leukemia. Haematol. 2015;100(2):253-262.
27. Shaffer AL, Emre NCT, Lamy L, et al. IRF4 addiction in multiple myeloma. Nature. 2008;454(7201):226-231.
28. Shukla V, Shukla A, Joshi SS, Lu R. Interferon regulatory factor 4 attenuates Notch signaling to suppress the development of chronic lymphocytic leukemia. Onco Targets. 2016;7(27):41081-41094.
29. Fiorcari S, Benatti S, Zucchetta A, et al. Overexpression of CD49d in trisomy 12 chronic lymphocytic leukemia patients is mediated by IRF4 through induction of IKAROS. Leukemia. 2019;33(5):1278-1302.
30. Chen CI, Paul H, Smitzler S, et al. A phase 2 study of lenalidomide and dexamethasone in previously untreated patients with chronic lymphocytic leukemia (CLL). Leukemia Lymphoma. 2019;60(4):980-989.
31. Ferrajoli A, Lee B-N, Schlette EJ, et al. Lenalidomide induces complete and partial remissions in patients with relapsed and refractory chronic lymphocytic leukemia. Blood. 2008;111(11):5291-5297.
32. Kay NE. Angiogenesis revisited in CLL. Leukemia Res. 2007;31(11):1459-1460.
33. Andritsos LA, Johnson AJ, Lozanski G, et al. Higher doses of lenalidomide are associated with unacceptable toxicity including life-threatening tumor flare in patients with chronic lymphocytic leukemia. Jco. 2008;26(15):2519-2525.
34. Lapalombella R, Andritsos L, Liu Q, et al. Lenalidomide treatment promotes CD154 expression on CLL cells and enhances production of antibodies by normal B cells through a PI3-kinase-dependent pathway. Blood. 2010;115(13):2619-2629.
35. Asslaber D, Qi Y, Maeding N, et al. B-cell-specific IRF4 deletion accelerates chronic lymphocytic leukemia development by enhanced tumor immune evasion. Blood. 2019;134(20):1717-1729.
36. Bjorklund CC, Baladandayuthapani V, Lin HY, et al. Evidence of a role for CD44 and cell adhesion in mediating resistance to lenalidomide in multiple myeloma: therapeutic implications. Leukemia. 2014;28(2):373-383.
37. Calissano C, Damlé RN, Marsilio S, et al. Intracranial complexity in chronic lymphocytic leukemia: fractions enriched in recently born/divided and older/quiescent cells. Mol Med [Cambi]. 2011;17(11-12):1374-1382.

SUPPORTING INFORMATION

Additional supporting information may be found in the Supporting Information section at the end of this article.

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