ADDENDUM - ERRATUM

Abstrakt- page 10

Correction on the page 11, the seventh line from the top:

Objevili jsme, že hladina CRBN jako proteinu u pacientů s 5q- syndromem předpovídá rychlejší odpověď na LEN terapii než hladina CRBN mRNA.

List of abbreviations- page 12

Correction on the page 14, the second line from the top:

HO 1- heme oxygenase

1.4.1. 5q- syndrome- page 22

Correction on the page 25, Fig.1:

Figure 1 represents pathological cell with del(5q31).

1.8.2. Lenalidomide- page 35

Correction on the page 35, the fifteenth line from the top:

Unfortunately, thalidomide affected human fetus and babies were then born with malformations (limb deformations- dysmelia, stunted limb growth, including congenital heart disease, ear, and eye damage, internal organ damage), or increased the incidence of miscarriage [Short et al., 2013; Lu et al., 2014; Ruchelman et al., 2013, Gao et al., 2020].

3. MATERIALS AND METHODS- page 46

3.2.2. Real-time quantitative PCR (polymerase chain reaction)- page 47

Correction on the page 49. The new text is added to the end of the existing text (before chapter 3.2.3. DNA sequencing):

The level of CRBN mRNA was related to GAPDH mRNA and healthy controls (healthy persons of the same age as used MDS patients). The Ct value for GAPDH was around 13-16, and the Ct value for CRBN was around 18- 22. For calculating of the final result (relative fold of CRBN mRNA expression) was used the mathematical formula $2^{-\Delta\Delta Ct}$. The first step was to average the Ct values for the replicates of each sample. The next step was to calculate delta Ct (ΔCt) for each sample using calculated average Ct values.

The formula to calculate delta Ct is:

$\Delta Ct_{patient}$ = Ct (gene of interest /CRBN/)$_{patient}$ – Ct (housekeeping gene /GAPDH/)$_{patient}$

The average value of Ct for the control was used as reference Ct when calculating the delta delta Ct (ΔΔCt) values for all patient samples.

The formula to calculate reference samples:

$\Delta Ct_{average\ healthy\ control}$ = Ct (gene of interest /CRBN/)$_{healthy\ control}$ – Ct (housekeeping gene /GAPDH/)$_{healthy\ control}$

The next step was to calculate ΔΔCt values:

ΔΔCt= ΔCt (patient´s sample) - ΔCt (average healthy control)

The last step was to calculate the relative fold gene expression values by mathematical formula:

Relative fold change of CRBN mRNA level = 2^-(ΔΔCt)

3.2.4. Immunoblotting- page 51

Correction on the page 53. The new text is added before - The list of antibodies used for immune detection:

The lower average obtained concentration of protein in lysate was around 240 µg per 150µl sample, and the higher average concentration of protein lysate was 450 µg/150µl sample. The necessary amount of the patient´s protein in lysate sample was 20 µg and higher in 15 µl for the detection of bands on the membrane by Ponceau S.

3.4. Cells culture- page 54- the corrected title of this chapter

Correction on the page 54. The new text is added to the end of the existing text (before 3.5. Statistical analysis)

We used MDS-L and SKM-1 cell cultures, which have a complex karyotype and SKM1 cells originally from patient with MDS who progressed to AML. There is no available cell culture model that can be used to research low-risk MDS patients. The low-risk MDS patients have a lower concentration of cells than the high-risk MDS. Because of this, it is better to use mouse models for research of low-risk MDS (Li W, Li M, Yang X, Zhang W, Cao L, Gao R. Summary of animal models of myelodysplastic syndrome. Animal Models Exp Med 2021; 4(1): 71-76).

4. RESULTS- page 56

4.1. Statistical analysis obtained MDS patients- page 56

Correction on the page 56, the fourth line from the top.

The obtained MDS group had 14 patients, of which were four women and ten men.

4.2. Comparison of the CRBN mRNA level in chosen groups- page 57- the corrected title

Corrections from the page 58 to the page 59 and on the page 61. The new text is added to the end of the existing text (before Fig.9):

The number of MDS patients with isolated del(5q) and patients with del(5q) with trisomy 8 analysed to detect the expression of CRBN mRNA in mononuclear cells of peripheral blood (n=84 and n=20) in Fig.9 was different from the number of these patients on lenalidomide therapy because not all these patients were treated by lenalidomide.The level of CRBN mRNA in patient´s mononuclear cells was related to GAPDH mRNA level in patient´s mononuclear cells and to values obtained from mononuclear cells of healthy controls.

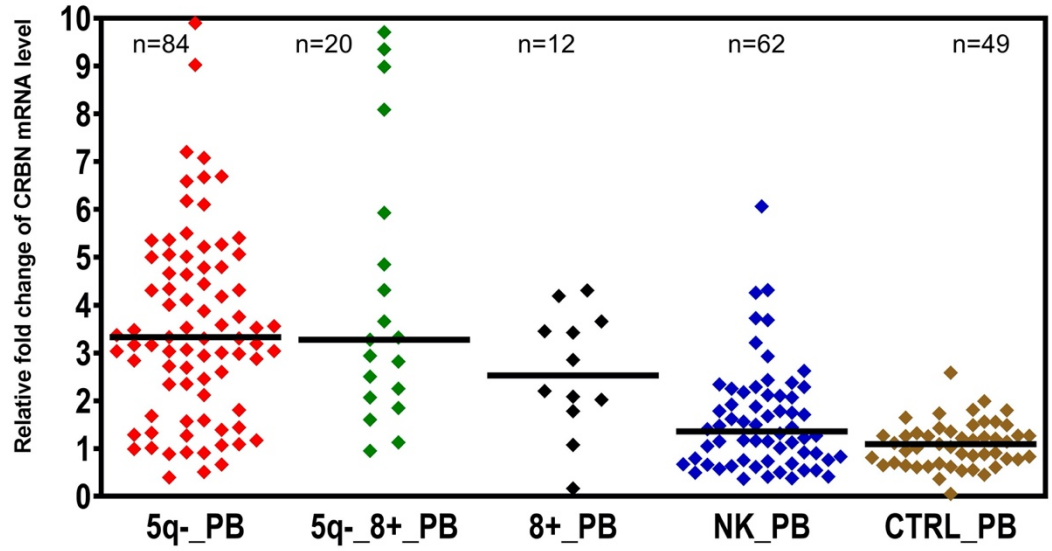


Fig.9: CRBN mRNA levels in peripheral blood (PB) mononuclear cells of chosen groups (MDS patients with isolated del(5q), MDS patients with combination of del(5q) and trisomy 8, MDS patients with isolated trisomy 8, MDS patients with normal karyotype (NK), and healthy control group). CRBN gene expression was measured using TaqMan assay Hs00372271_m1 (primers in exons 8 and 10).

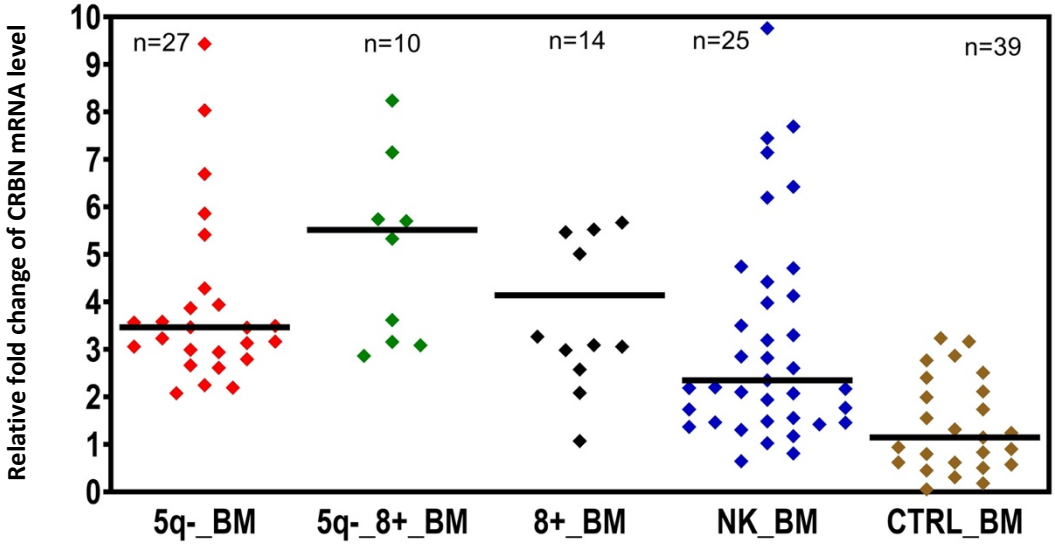


Fig.10: CRBN mRNA levels in bone marrow (BM) mononuclear cells of chosen groups (MDS patients with isolated del(5q), MDS patients with combination of del(5q) and trisomy 8, MDS patients with isolated trisomy 8, MDS patients with normal karyotype (NK), and healthy control group). CRBN gene expression was measured using TaqMan assay Hs00372271_m1 (primers in exons 8 and 10).

Page 60

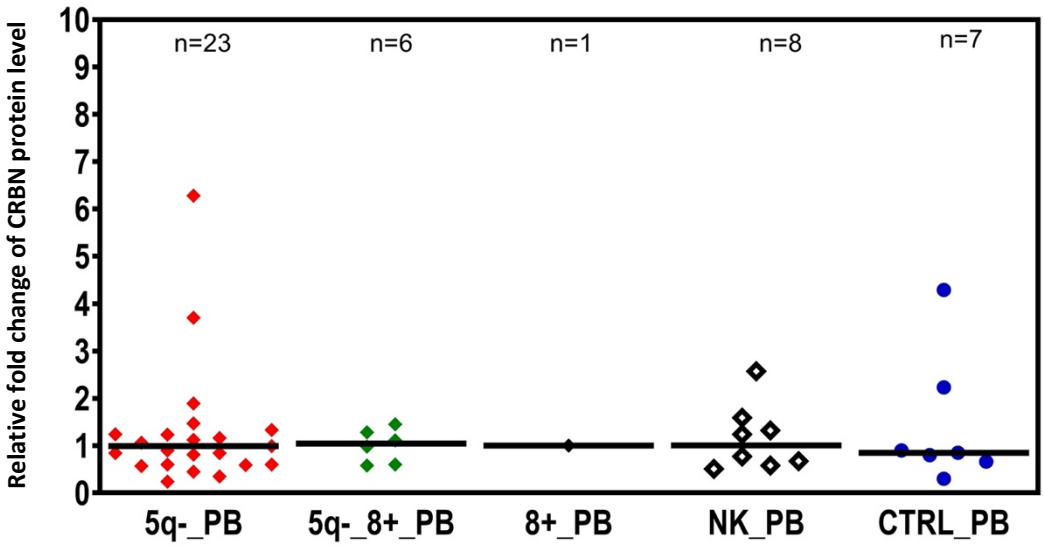


Fig.11: The level of CRBN protein in peripheral blood (PB) lysate of chosen groups (MDS patients with isolated del(5q), MDS patients with combination of del(5q) and trisomy 8, MDS patients with isolated trisomy 8, MDS patients with normal karyotype (NK) and healthy control group). The level of CRBN protein$_{patient}$ was related to GAPDH level$_{patient}$ and to values obtained from PB mononuclear cells lysate of healthy controls.

Page 61

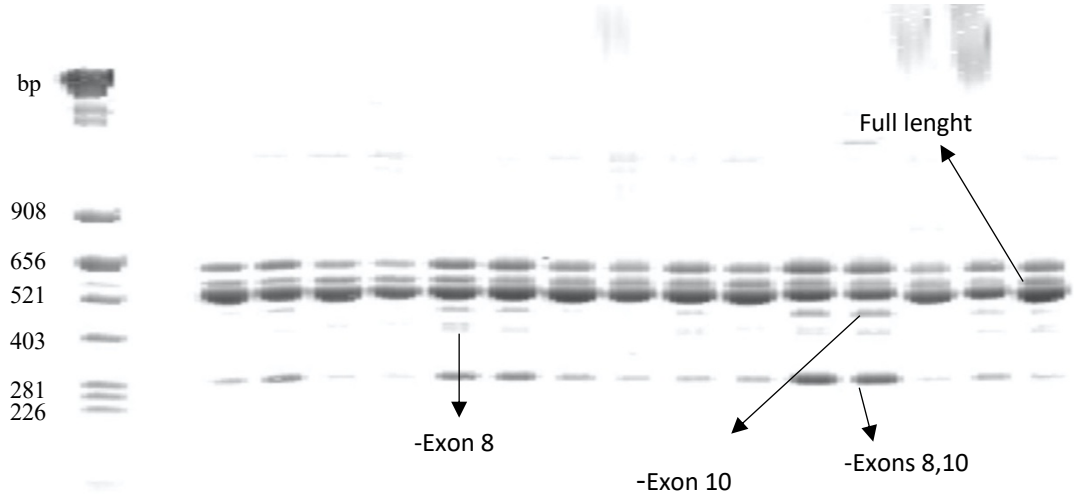


Fig.13: Representation of full- length CRBN mRNA and CRBN mRNA splice variants with deleted exon 8, exon 10 or both exons 8 and 10. Oligonucleotide primers FCRB7ex and RCRB11ex (see the list of primers on pages 50-51) were used for amplification of CRBN cDNA. PCR products were electrophoresed on agarose gels, electroluated from the pieces of gel, purified and sequenced using Genome Lab DTCS Quick Start Kit and Beckman Coulter CEQ 3000 DNA sequencer. GenBank Accession NM_016302.3 (Homo sapiens CRBN transcript variant 1 mRNA) was used for evaluation of obtained sequences.

4.3. Cereblon as a prognostic factor for the success of lenalidomide therapy- page 61

Corrections for pages 62 – 67 (corrections of legends to figures). The new text is added to the end of the existing text (before Fig.14A).

The level of CRBN $mRNA_{patient\ mononuclear\ cells}$ was related to GAPDH $mRNA_{patient\ mononuclear\ cells}$ and to values obtained from mononuclear cells of healthy controls.

A.)

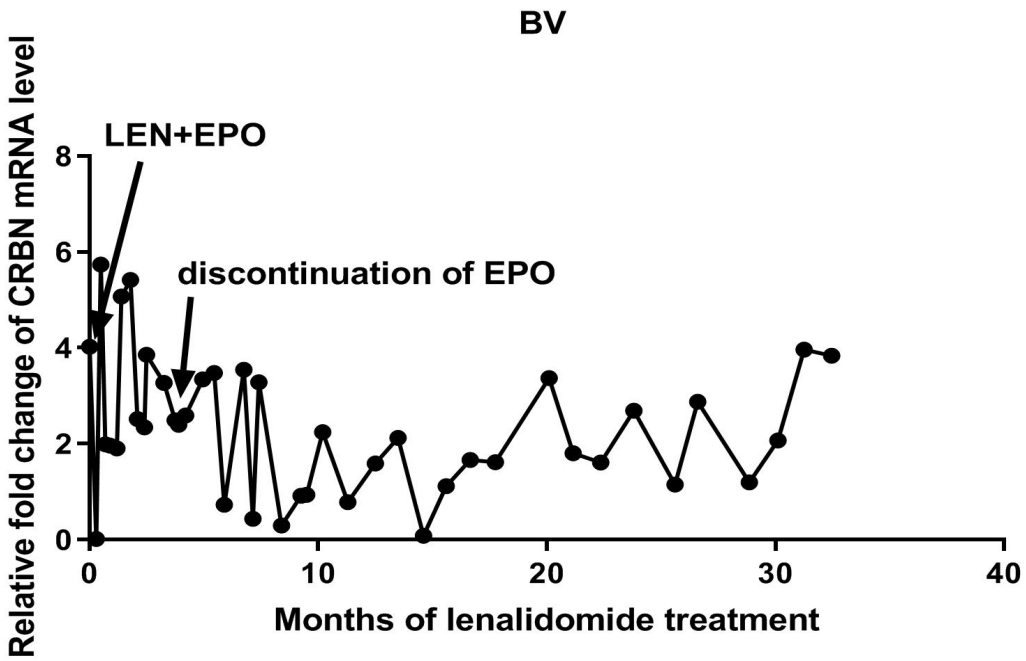


Fig.14A: Relative fold change of CRBN mRNA level in peripheral blood (PB) mononuclear cells of female MDS patient with isolated del(5q) before and during

lenalidomide (LEN) treatment. This patient responded to therapy. Before LEN, this patient was treated by erythropoietin (EPO). This patient was then treated for five months with the combination of EPO and LEN and the therapy continued only with LEN.

B.)

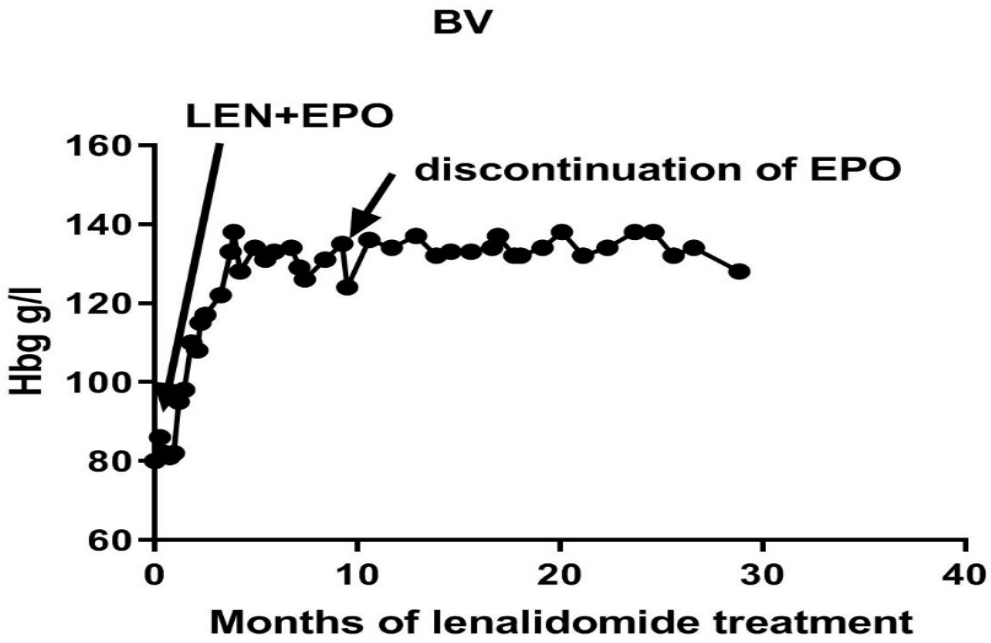


Fig.14B: Values of hemoglobin level (g/l) are shown as function of time during lenalidomide (LEN) treatment of female MDS patient with isolated del(5q). Before LEN, this patient was treated by erythropoietin (EPO). This patient was then treated for five months with the combination of EPO and LEN and the therapy continued only with LEN.

A.)

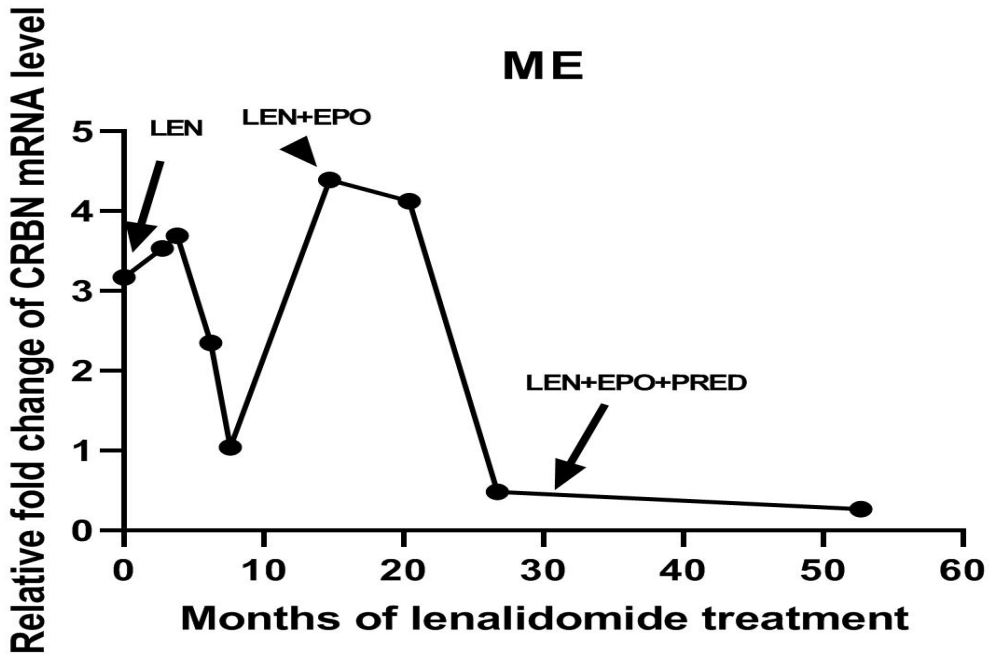


Fig.15A: Relative fold change of CRBN mRNA level in peripheral blood (PB) mononuclear cells of female MDS patient with isolated del(5q) before and during lenalidomide (LEN) treatment. This patient did not respond to therapy. The patient was treated for 15 months with lenalidomide (LEN). Erythropoietin (EPO) was then added but without success and therefore combination of LEN, EPO and prednisone (PRED) was finally used but also without success.

B.)

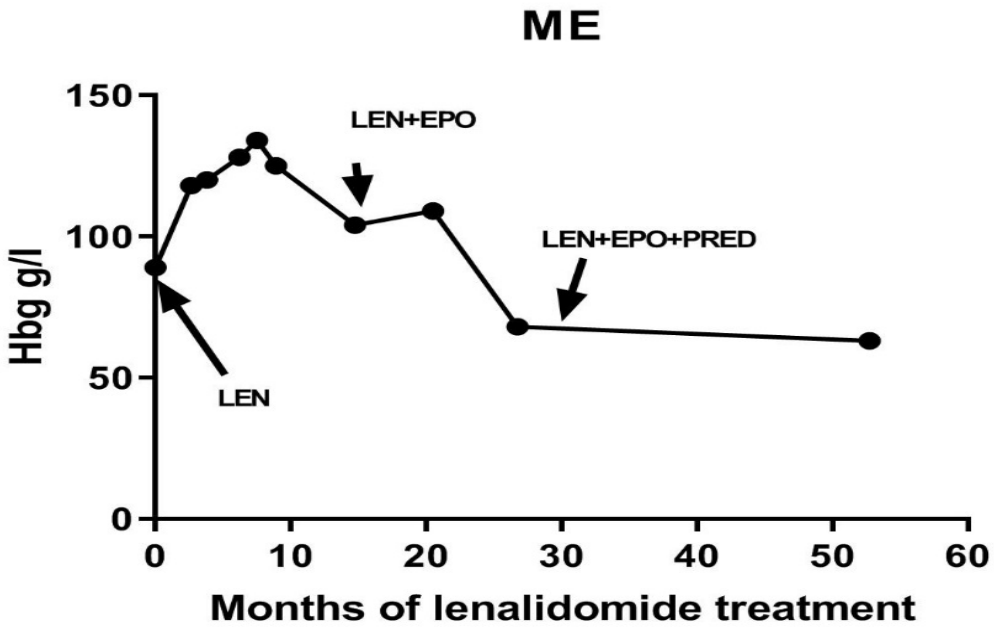


Fig.15B: Values of hemoglobin level (g/l) are shown as function of time during lenalidomide (LEN) treatment of female MDS patient with isolated del(5q). The patient was treated for 15 months with lenalidomide (LEN). Erythropoietin (EPO) was then added but without success and therefore combination of LEN, EPO and prednisone (PRED) was finally used but also without success.

A.)

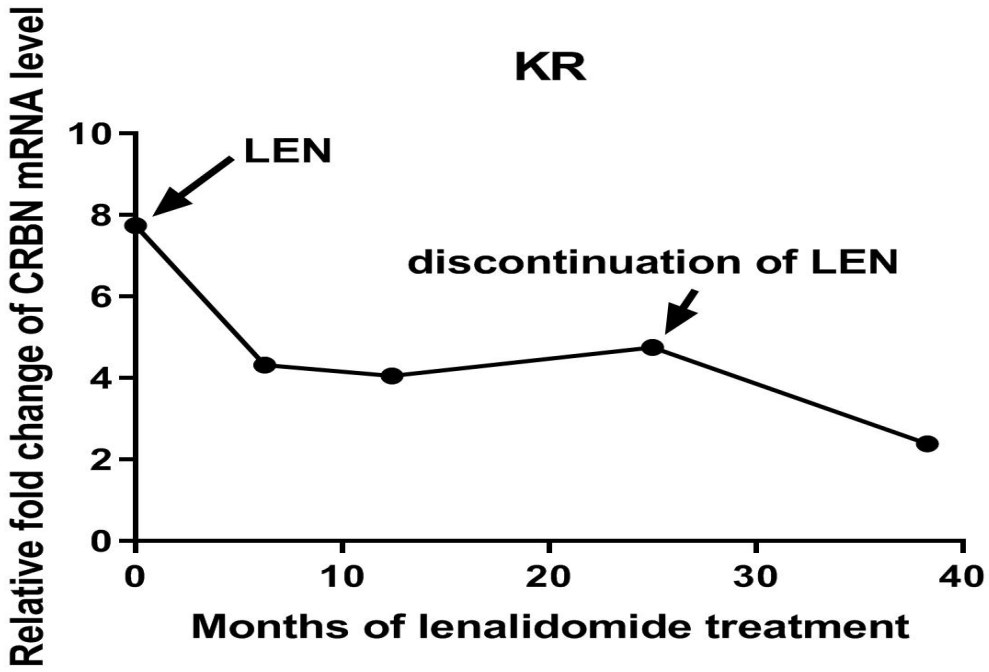


Fig.16A: Relative fold change of CRBN mRNA level in peripheral blood (PB) mononuclear cells of female MDS patient with del(5q) and trisomy 8 in different clones during lenalidomide (LEN) therapy. The patient reached complete hematologic and cytogenetic remission after 25 months of LEN treatment. After one year of discontinuation of LEN, we have observed decreased level of CRBN mRNA. After three years without treatment, the patient relapsed and returned to LEN treatment.

B.)

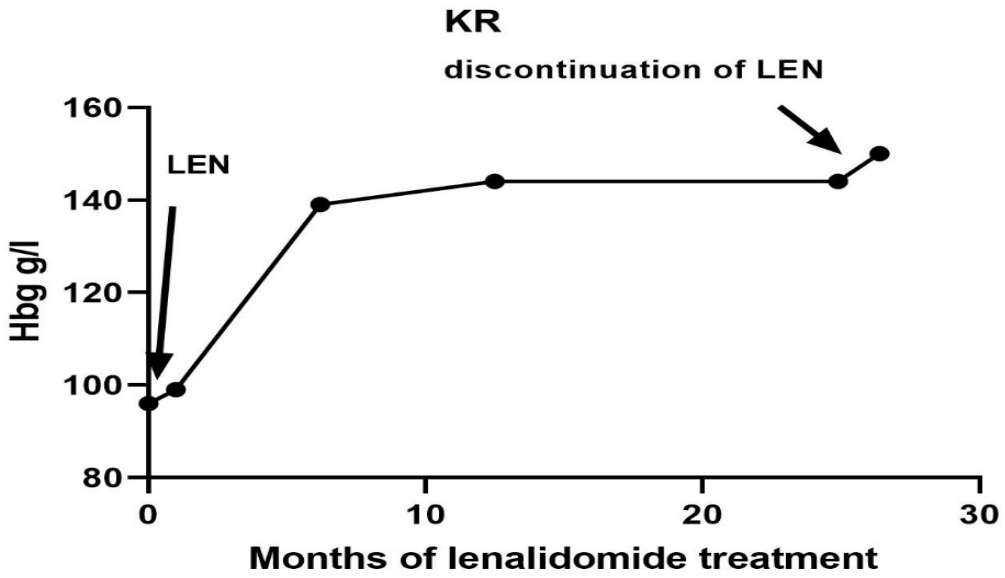


Fig.16B: Values of hemoglobin level (g/l) are shown as function of time during lenalidomide (LEN) treatment of female MDS patient del(5q) and trisomy 8 in different clones. The patient reached complete hematologic and cytogenetic remission after 25 months of LEN treatment. After one year of discontinuation of LEN, we have observed decreased level of CRBN mRNA. After three years without treatment, the patient relapsed and returned to LEN treatment.

A.)

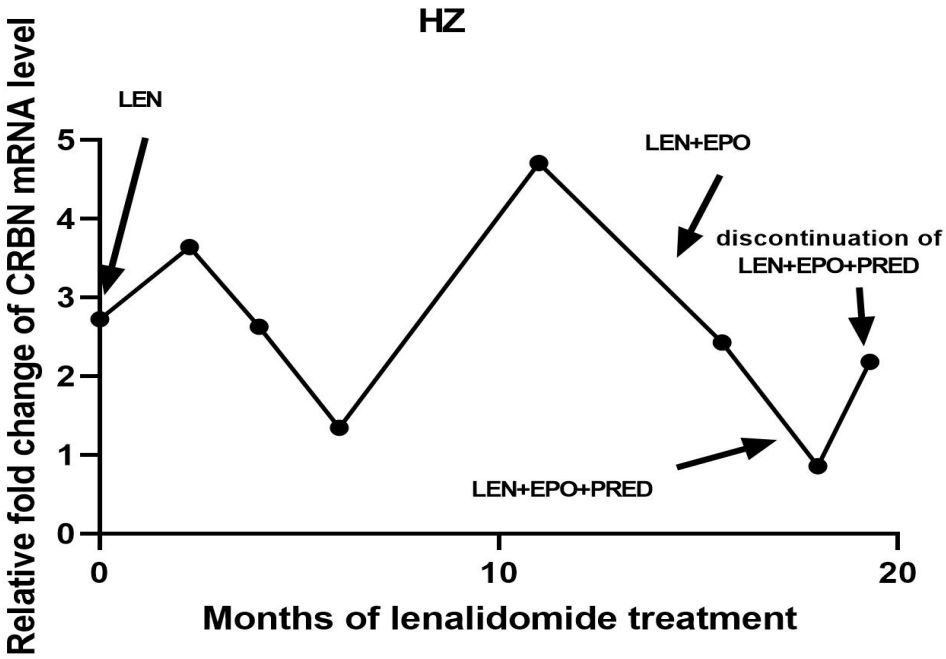


Fig.17A: Relative fold change of CRBN mRNA level in peripheral blood (PB) mononuclear cells of MDS-RCMD patient with del(5) (13q33,q31) during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) therapy, and at time of the last LEN combination discontinuation. The patient transformed to MDS-EB-1 according the analysis of bone marrow (BM) biopsy at time of last LEN combination discontinuation.

B.)

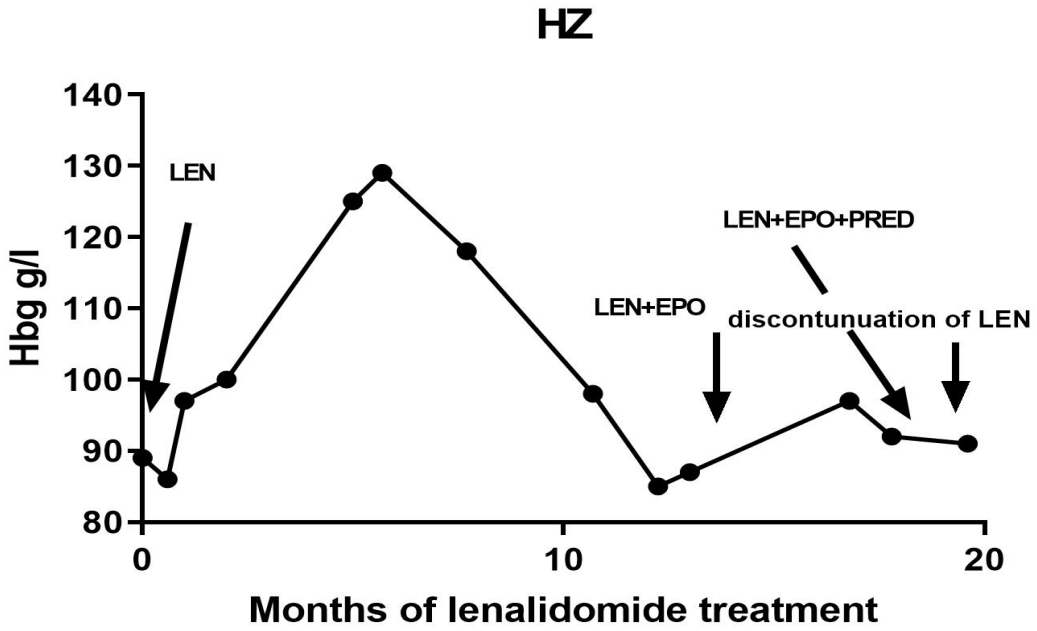


Fig.17B: Values of hemoglobin level (g/l) are shown as function of time during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) therapy of MDS-RCMD patient with del(5) (13q33,q31), and at time of the last LEN combination discontinuation. The patient transformed to MDS-EB-1 according the analysis of bone marrow (BM) biopsy at time of the last LEN combination discontinuation.

4.4. Effect of combination lenalidomide, erythropoietin, prednisone in MDS patients with del(5q) or combination del(5q) and trisomy 8 in the various clones- page 71

Corrections from page 72 to page 76.

A.)

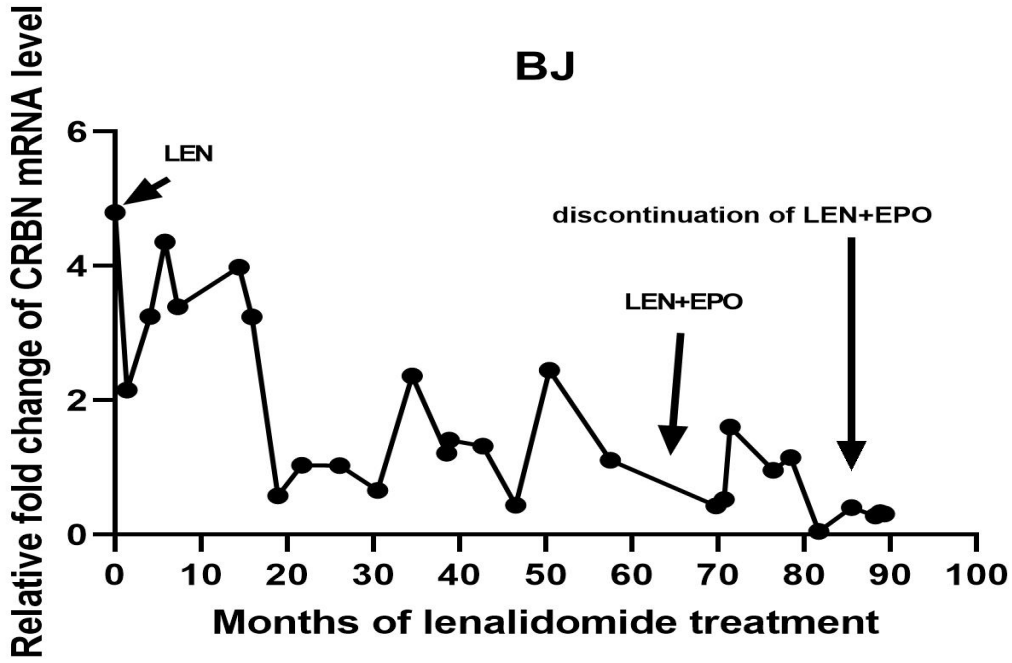


Fig.22A: Relative fold change of CRBN mRNA level in peripheral blood (PB) mononuclear cells of the female MDS patient with del(5q) who was treated with lenalidomide (LEN) and with the combination of LEN and erythropoietin (EPO) after the decrease of hemoglobin level to 80 g/l. In most cases, the response of EPO after adding to LEN is temporal. This patient transformed to MDS-EB-1 and then to AML.

B.)

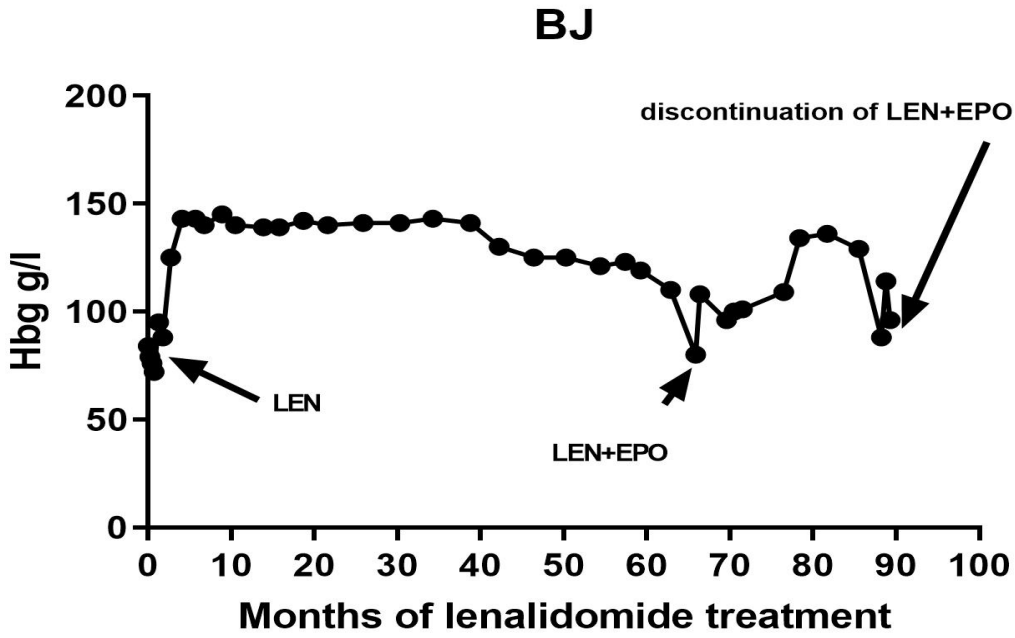


Fig.22B: Values of hemoglobin level (g/l) are shown as function of time during lenalidomide (LEN) and the combination of LEN and erythropoietin (EPO) treatment of the patient with del(5q). This patient transformed to MDS-EB-1 and then to AML.

A.)

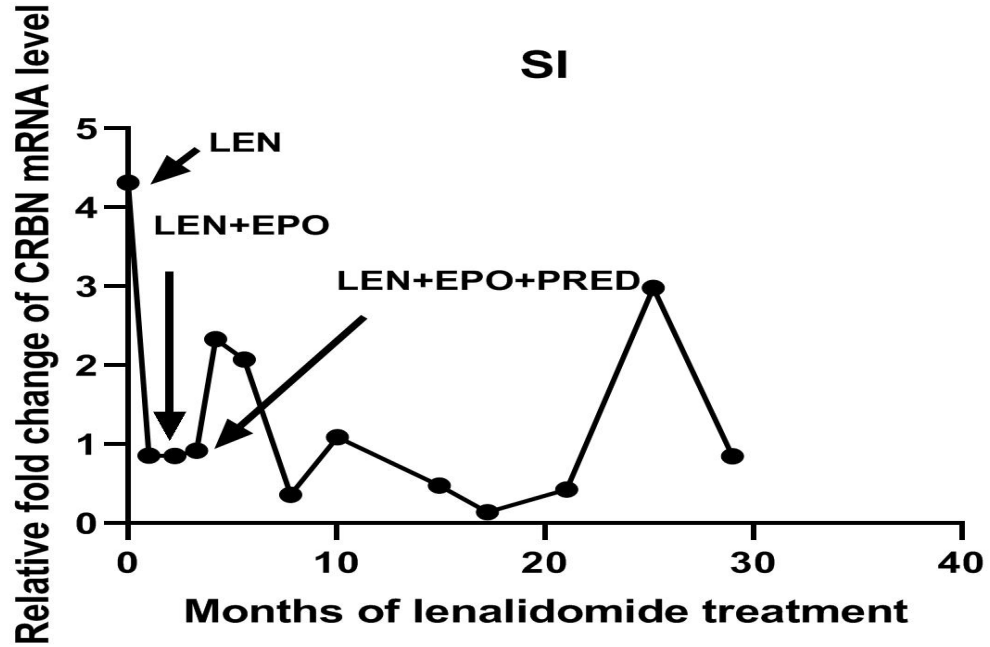


Fig.23A: Relative fold change of CRBN mRNA level in peripheral blood (PB) mononuclear cells of female MDS-RCMD patient with del(5q) and trisomy 8 in different clones during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) therapy. The effect of LEN combinations was only temporary and this patient did not respond to therapy.

B.)

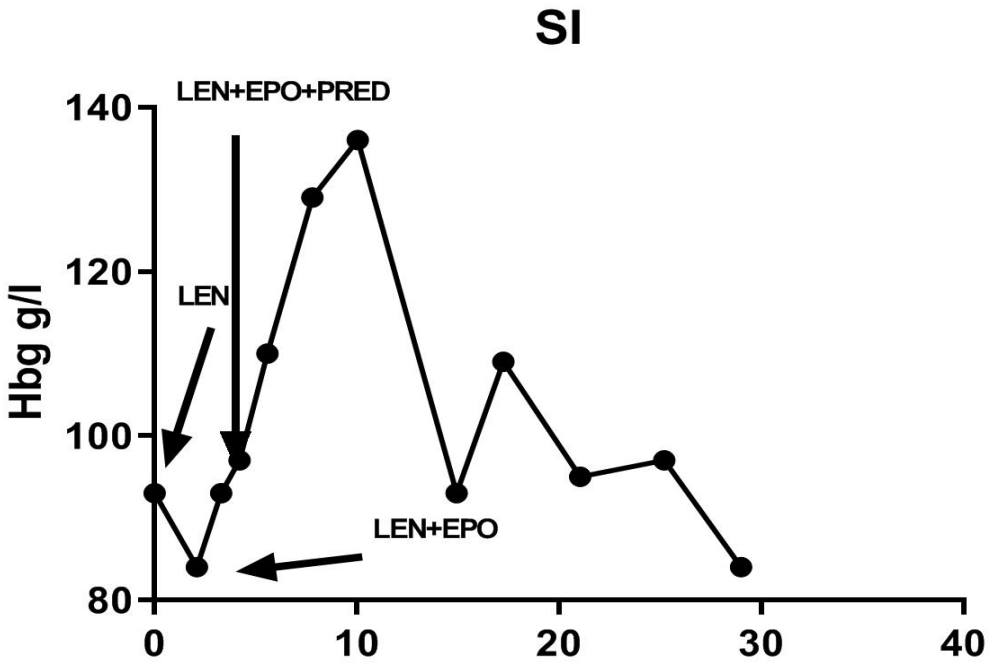


Fig.23B: Values of hemoglobin level (g/l) are shown as function of time during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment of female MDS-RCMD patient with del(5q) and trisomy 8 in different clones. The effect of LEN combinations was only temporary and this patient did not respond to therapy.

A.)

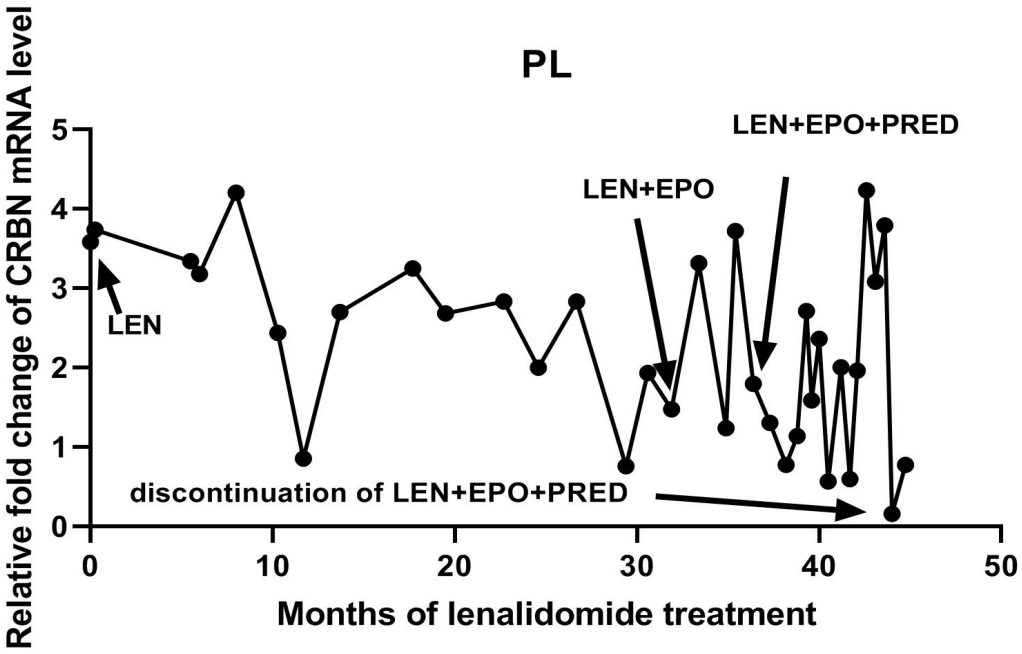


Fig.24A: Relative fold change of CRBN mRNA level in peripheral blood (PB) mononuclear cells of female MDS patient with del(5q) during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment. The effect of LEN combinations was only temporary and this patient was then treated with azacytidine (VIDAZA).

B.)

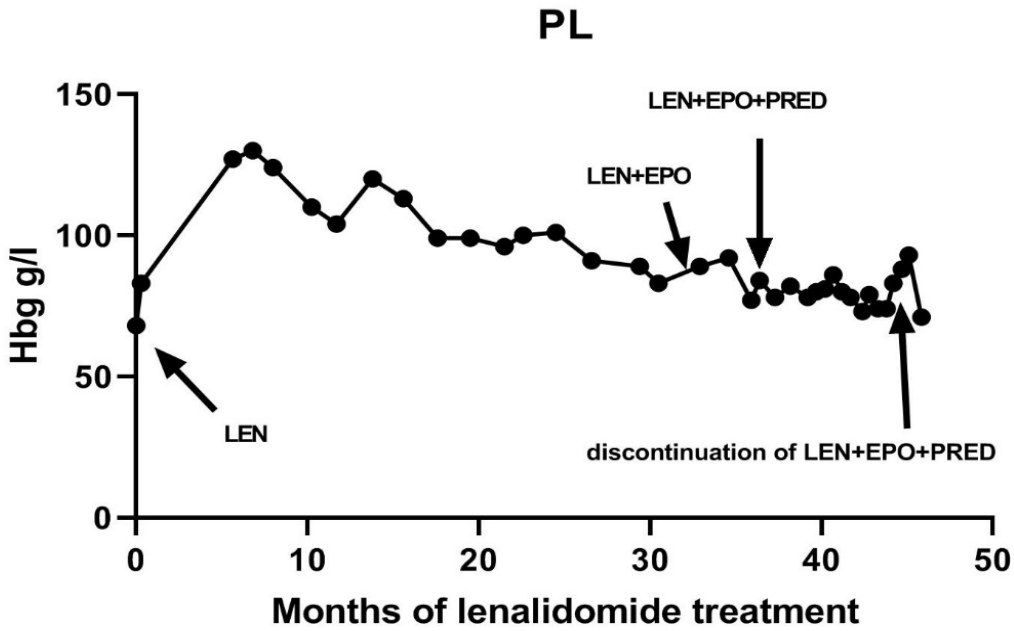


Fig.24B: Values of hemoglobin level (g/l) are shown as function of time during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment of female MDS patient with del(5q). The effect of LEN combinations was only temporary and this patient was then treated with the hypomethylating agent azacytidine (VIDAZA).

A.)

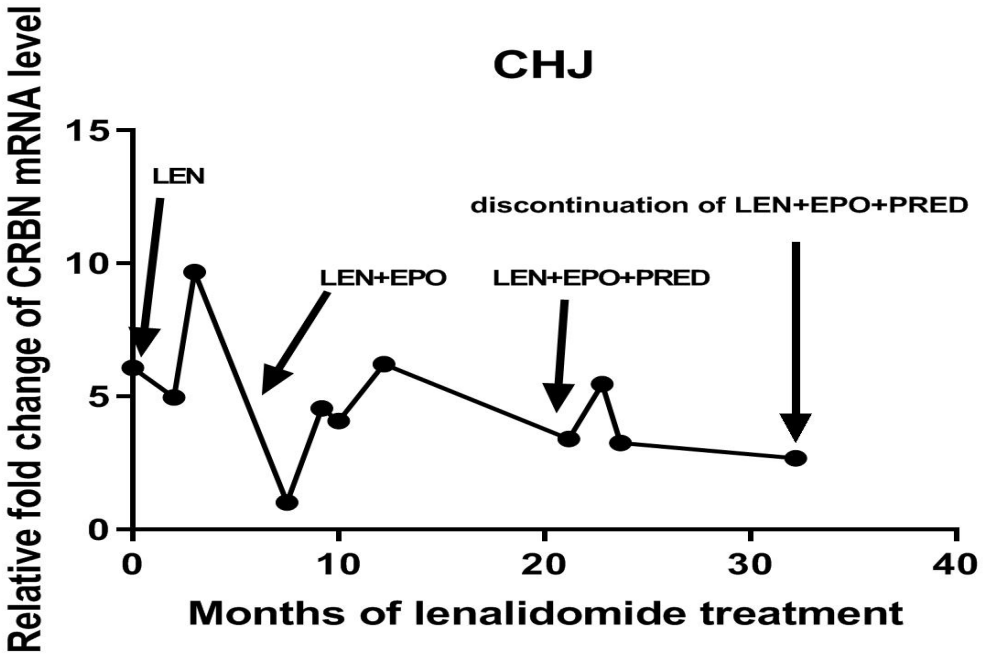


Fig.25A: Relative fold change of CRBN mRNA level in peripheral blood (PB) mononuclear cells of female MDS/MPD- RARS-T patient with normal karyotype during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment with only partial response. Treatment was ended after transformation to MDS-EB-2.

B.)

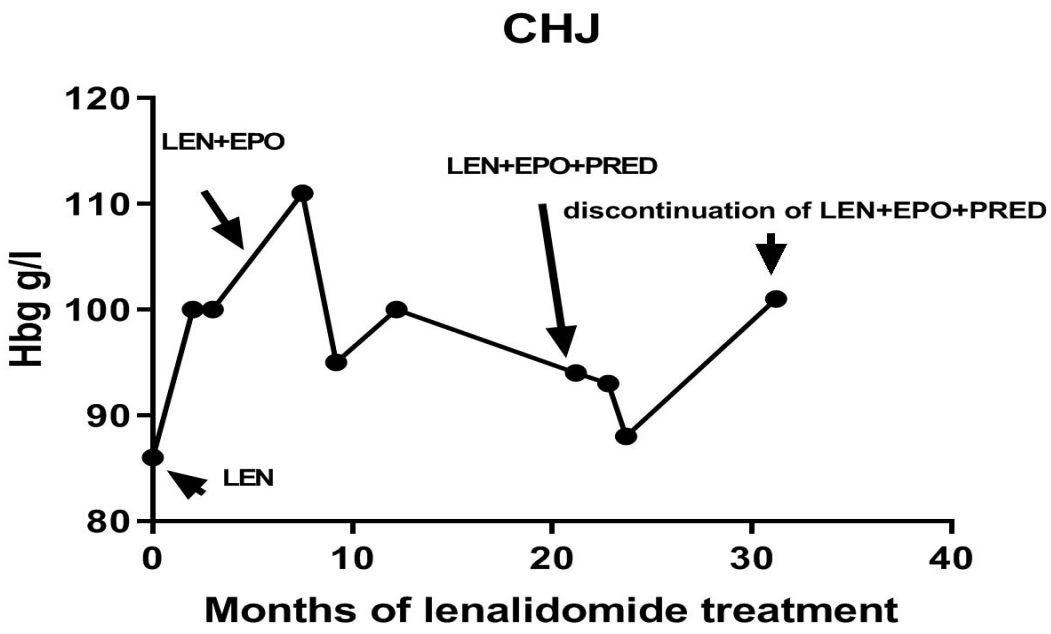
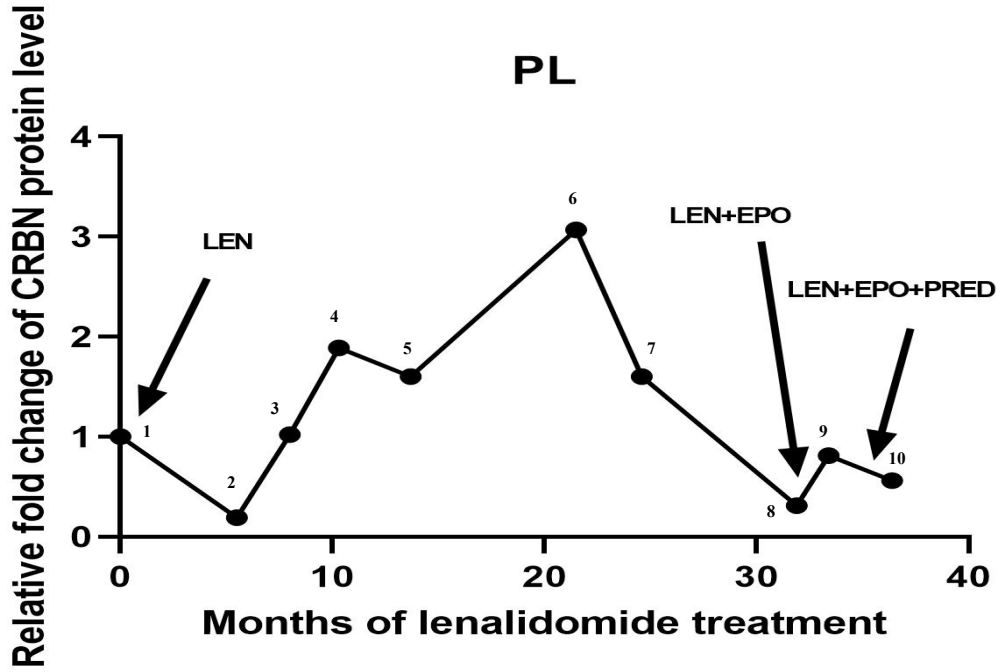


Fig.25B: Values of hemoglobin level (g/l) are shown as function of time during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment of female MDS/MPD- RARS-T patient with normal karyotype during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment with only partial response. Treatment was ended after transformation to MDS-EB-2.

4.5. Comparison of CRBN mRNA and CRBN protein levels- page 76- the corrected title of this chapter

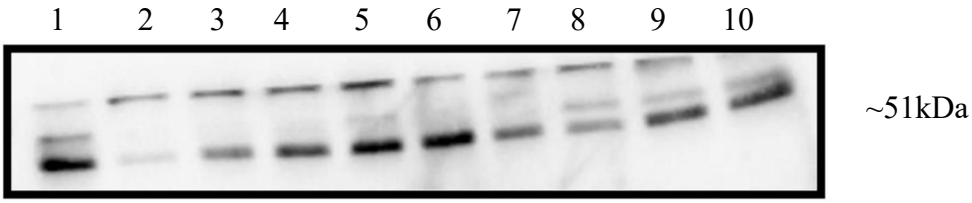
Corrections from page 77 to page 90.

A.)



B.)

The level of CRBN protein during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment



The level of GAPDH protein during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment

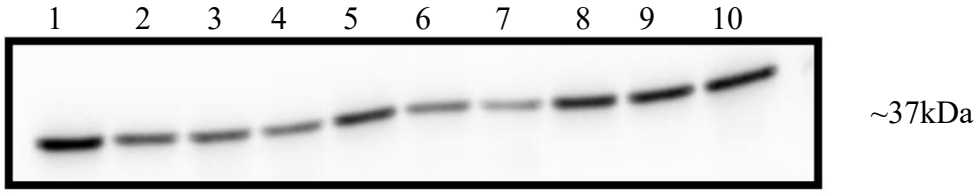
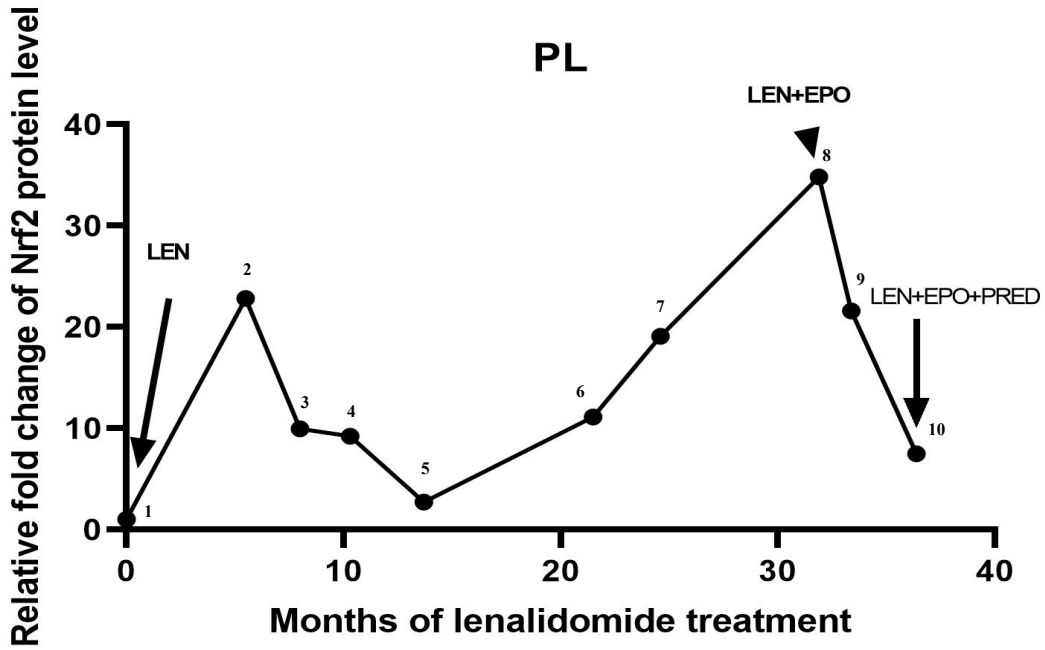


Fig.26A.: Relative fold change of CRBN protein level in lysates of peripheral blood mononuclear cells of female patient with 5q- syndrome during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment.

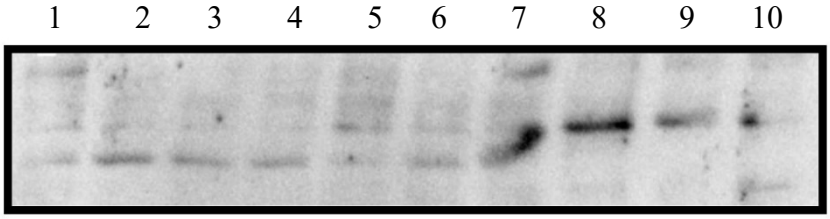
CRBN protein level was related to GAPDH protein level and to relative CRBN level in healthy control. This patient responded to LEN therapy around two years but progressed to MDS-EB-1. Then to LEN was added EPO, and after a couple of months followed PRED. The treatment was unsuccessful and patient was treated with the hypomethylating drug azacytidine (VIDAZA). Cereblon protein levels in this figure can be compared with CRBN mRNA levels shown in Fig. 24A or Fig.26D.

B.: Bands of CRBN protein with a molecular weight of ~51kDa and GAPDH protein with a molecular weight of ~37kDa before and during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment (in times shown on Fig. 26A). The first band 1 is before LEN and the rest bands are during the treatment. The analysis was done by Western blot.

C.)



The level of Nrf2 protein during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment



The level of GAPDH protein during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment

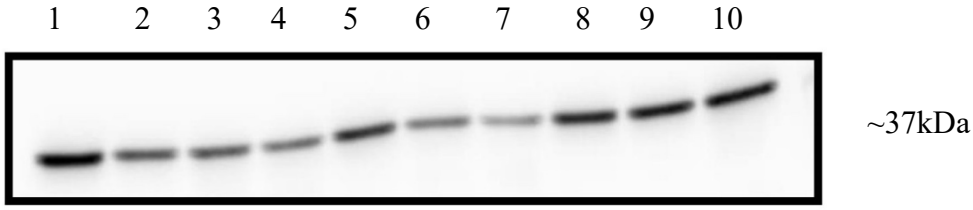
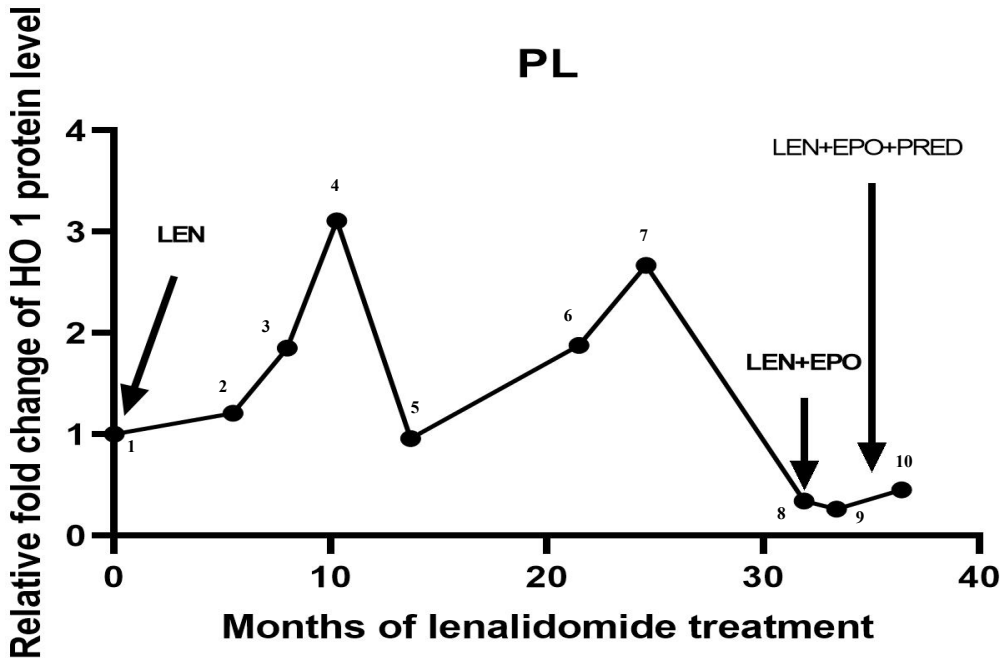
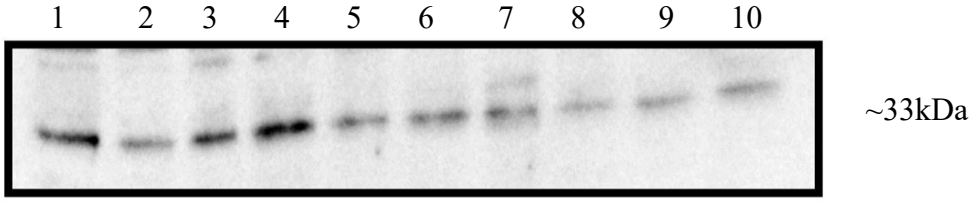


Fig.26C.: Relative fold change of Nrf2 (nuclear factor erythroid 2-related factor 2) protein level in lysates of peripheral blood mononuclear cells of female patient with 5q- syndrome during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment. Bands of Nrf2 protein with a molecular weight of ~62kDa and GAPDH protein with a molecular weight of ~37kDa before and during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment. The first band 1 is before LEN and the rest bands are during the treatment. The female patient with 5q- syndrome responded to LEN therapy around two years but progressed to MDS-EB-1. Then to LEN was added EPO, and after a couple of months followed PRED. The treatment was unsuccessful and patient was then treated with the hypomethylating drug azacytidine (VIDAZA).

D.)



The level of HO 1 protein during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment



The level of GAPDH protein during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment

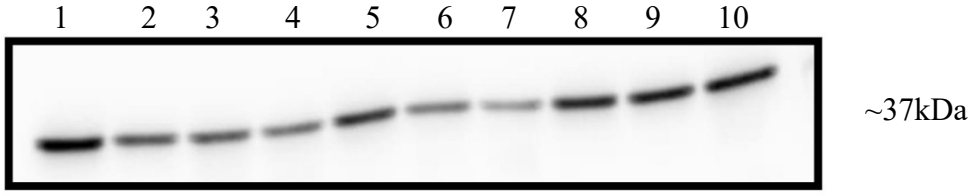


Fig.26D.: Relative fold change of heme oxygenase 1 (HO 1) protein level in lysates of peripheral blood mononuclear cells of female patient with 5q- syndrome during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and

prednisone (PRED) treatment. CRBN protein level was related to GAPDH protein level and to relative CRBN level in healthy control. This patient responded to LEN therapy around two years but progressed to MDS-EB-1. Then to LEN was added EPO, and after a couple of months followed PRED. The treatment was unsuccessful and patient was then treated with the hypomethylating drug azacytidine (VIDAZA). The first band is before LEN and the rest bands are during the treatment. Bands of HO 1 protein with a molecular weight of ~33kDa and bands of GAPDH with a molecular weight of ~37kDa are shown.

E.)

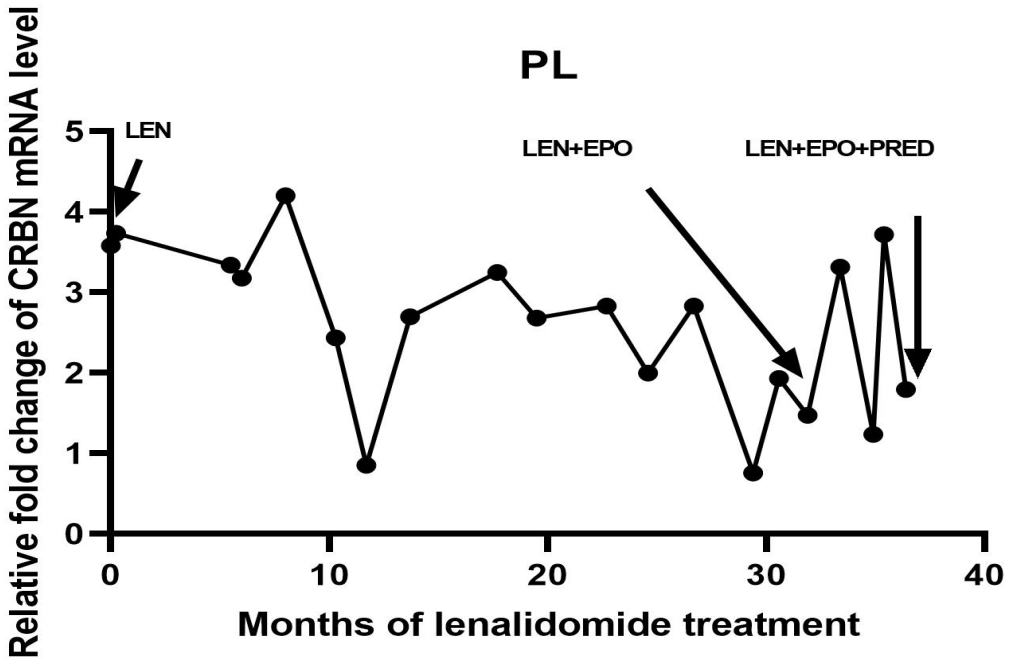


Fig.26E: Relative fold change of CRBN mRNA level in peripheral blood (PB) mononuclear cells of female MDS patient with del(5q) during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment. The effect of LEN combinations was only temporary and this patient was then treated with azacytidine (VIDAZA).

F.)

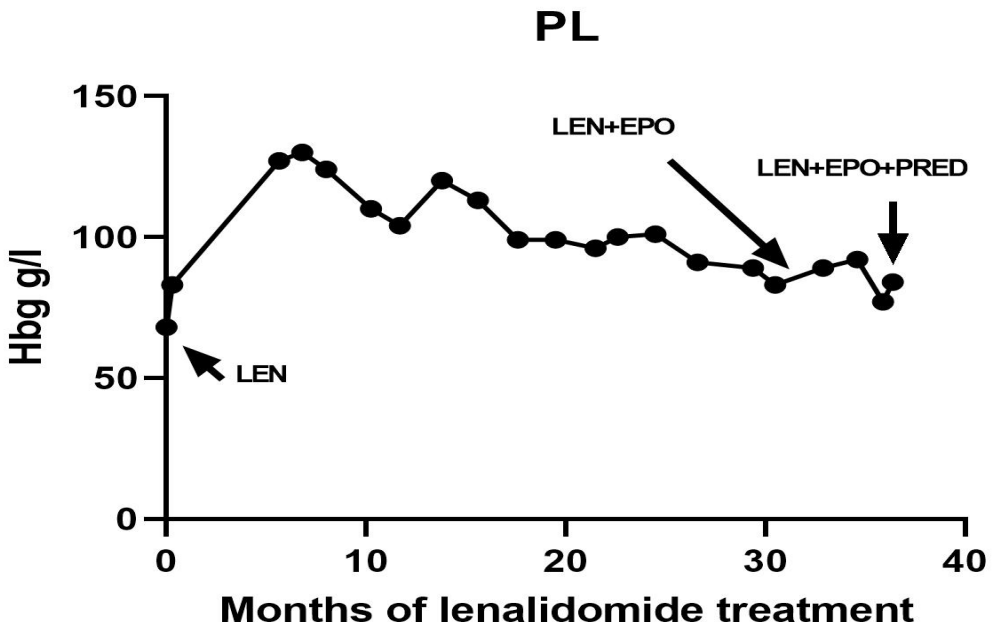


Fig.26F: Values of hemoglobin level (g/l) are shown as function of time during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment of female MDS patient with del(5q). The effect of LEN combinations was only temporary and this patient was then treated with the hypomethylating agent azacytidine (VIDAZA).

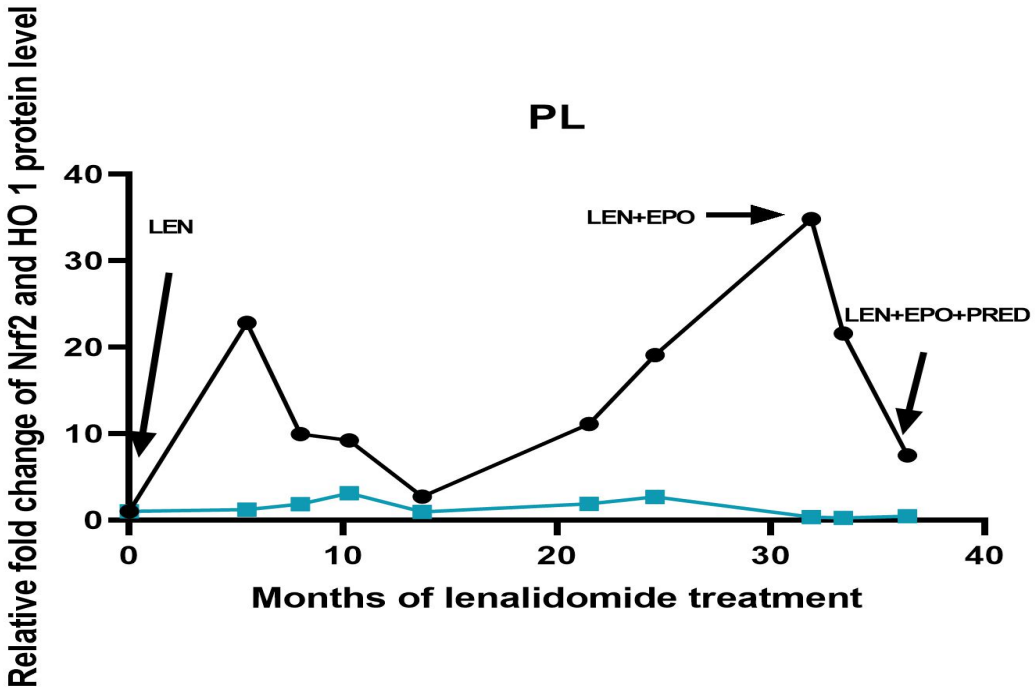
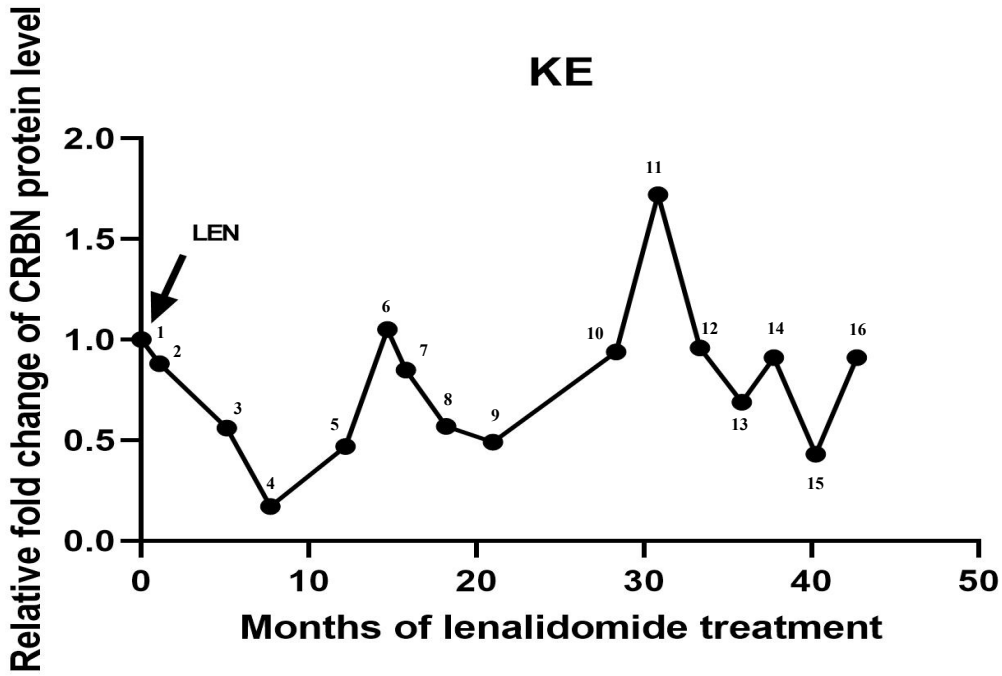


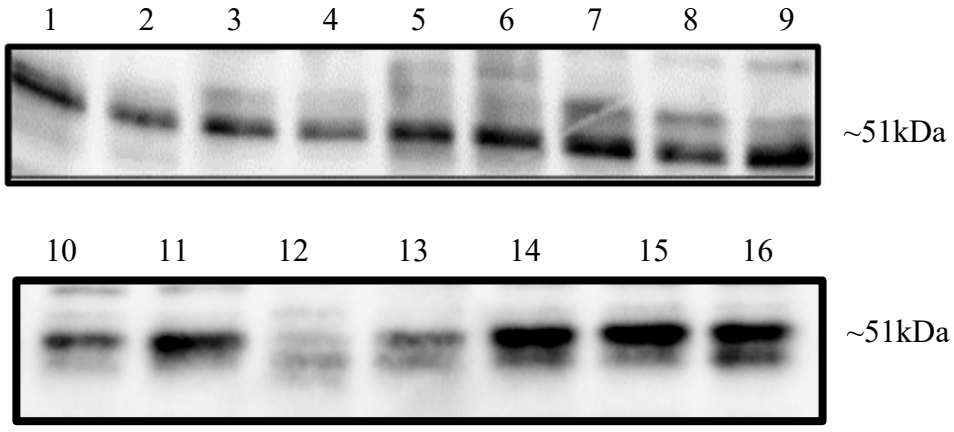
Fig.27: The comparison of relative fold change of Nrf2 (nuclear factor erythroid 2-related factor 2) protein level and relative fold change of HO 1 (heme oxygenase 1) in lysates of peripheral blood mononuclear cells of female patient with 5q- syndrome during lenalidomide (LEN), LEN and erythropoietin (EPO), and LEN plus EPO and prednisone (PRED) treatment. Blue color represents relative fold change of HO 1 protein and black color represents relative fold change of Nrf2 protein.

A.)

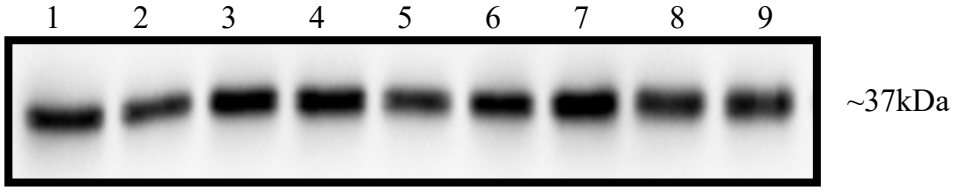


B.)

The level of CRBN protein during lenalidomide therapy



The level of GAPDH protein during lenalidomide therapy



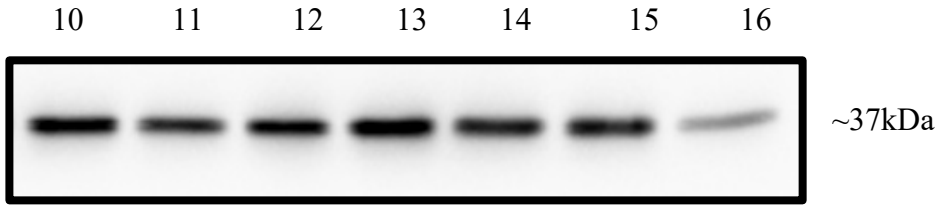


Fig.28A.: Relative fold change of CRBN protein level in lysates of peripheral blood mononuclear cells of female patient with del(5q) and trisomy 8 in different clones during lenalidomide (LEN) therapy. This patient has been successfully treated with LEN for five years.

B. Bands of CRBN protein with a molecular weight of ~51kDa and GAPDH protein with a molecular weight of ~37kDa before and during lenalidomide (LEN) therapy analysed by Western blot.

C.)

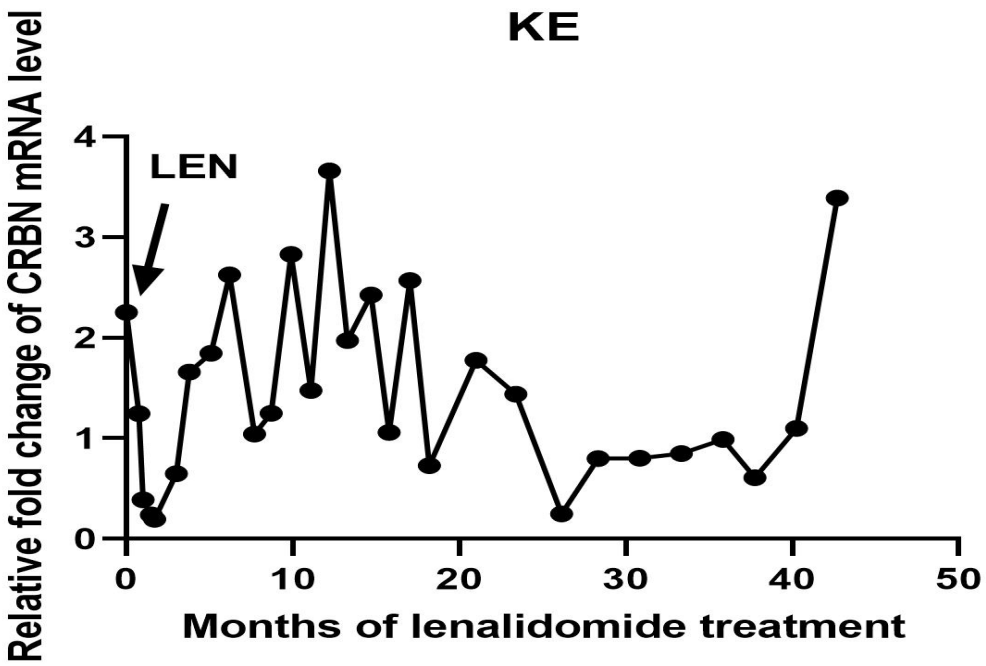


Fig.28C: Relative fold change of CRBN mRNA level in peripheral blood (PB) mononuclear cells of female patient with with del(5q) and trisomy 8 in different clones during lenalidomide (LEN) therapy. This patient has been successfully treated with LEN for five years.

D.)

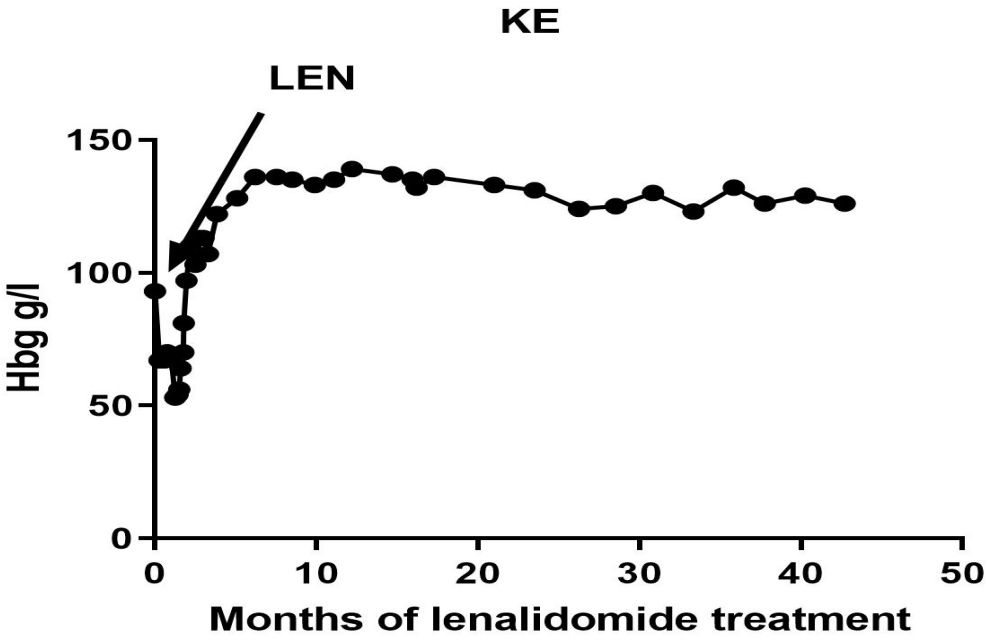
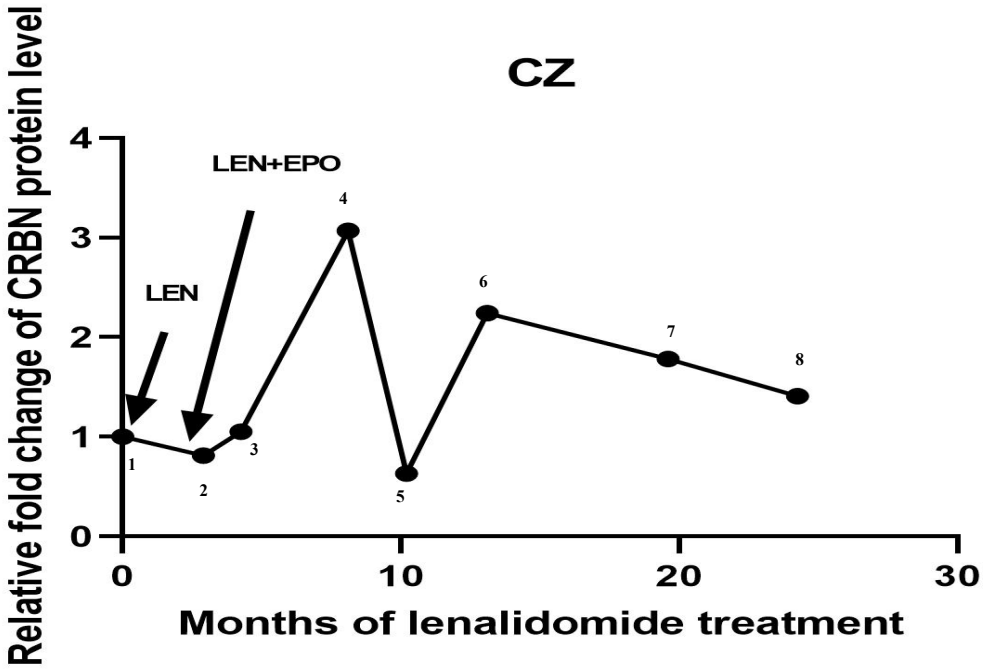


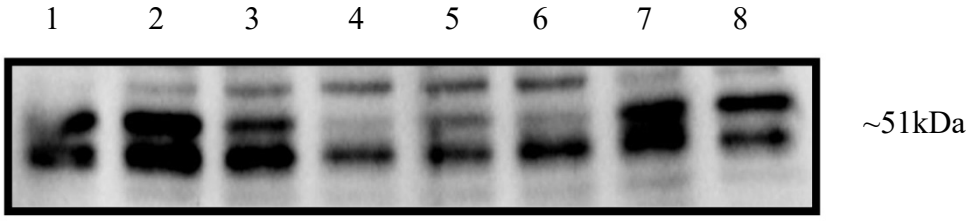
Fig.28D: Values of hemoglobin level (g/l) are shown as function of time during lenalidomide (LEN) treatment of the female patient with del(5q) and trisomy 8 with successful therapy for five years.

A.)



B.)

The level of CRBN protein during lenalidomide and lenalidomide plus erythropoietin therapy



The level of GAPDH protein during lenalidomide and lenalidomide plus erythropoietin therapy

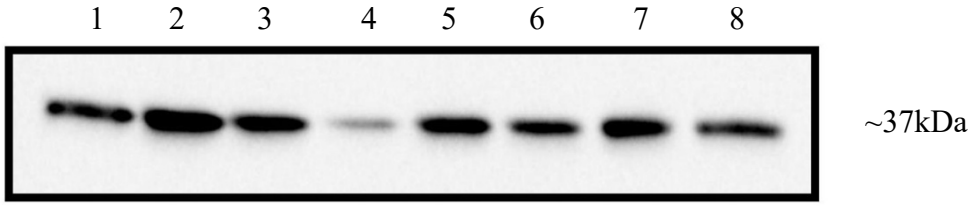
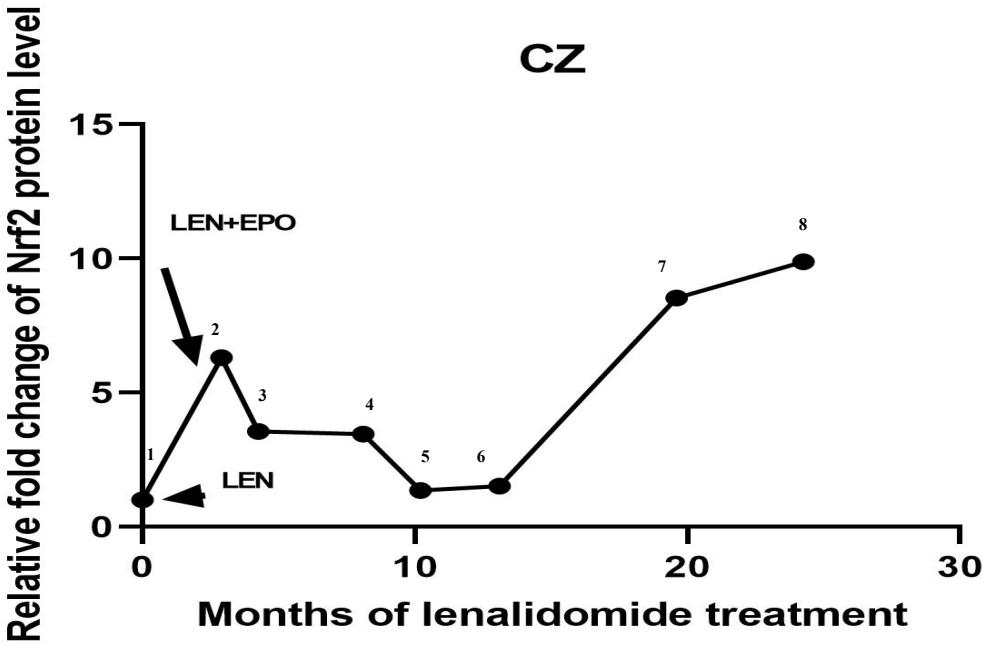
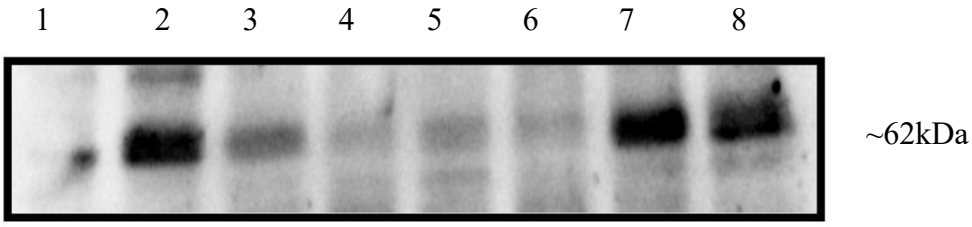


Fig.29A: Relative fold change of CRBN protein level in lysates of peripheral blood (PB) mononuclear cells of female patient with del(5q) during lenalidomide (LEN) and LEN plus erythropoietin (EPO) therapy. The patient achieved a complete remission.
B. Bands of CRBN protein with a molecular weight of ~51kDa and GAPDH protein with a molecular weight of ~37kDa before and during lenalidomide and lenalidomide plus erythropoietin therapy obtained by Western blot analysis.

C.)



The level of Nrf2 protein during lenalidomide (LEN) and LEN plus erythropoietin (EPO) therapy



The level of GAPDH protein during lenalidomide (LEN) and LEN plus erythropoietin (EPO) therapy

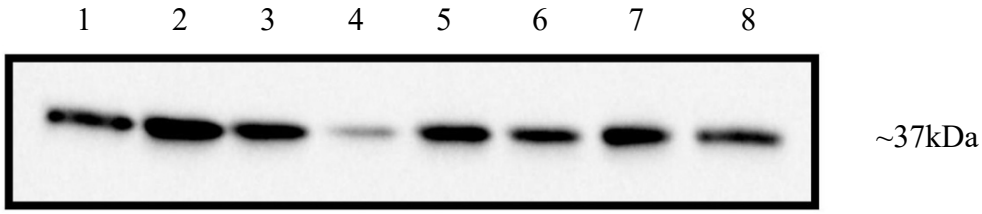
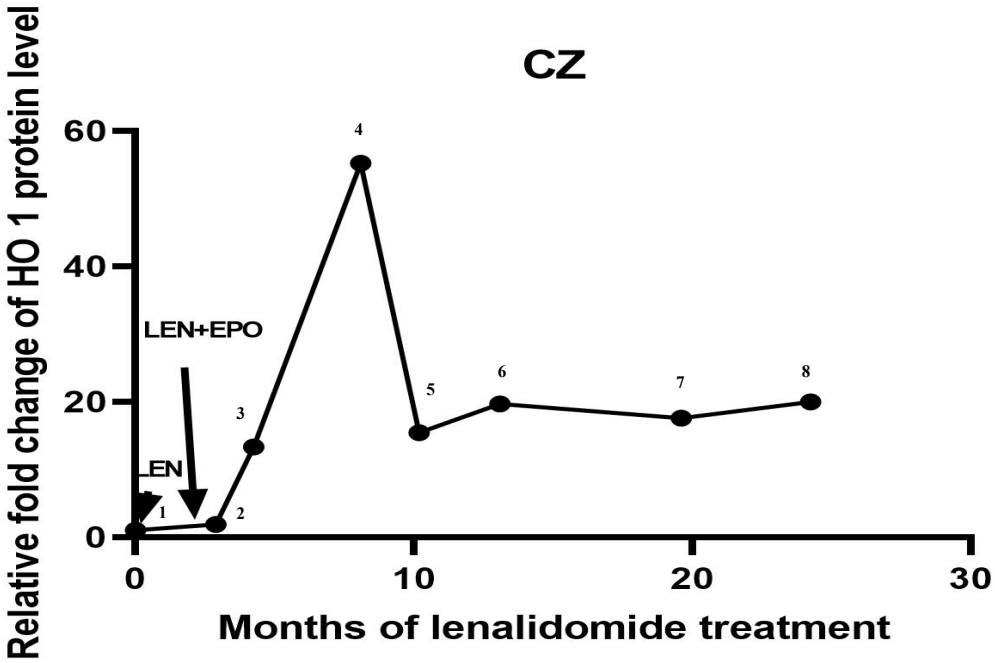


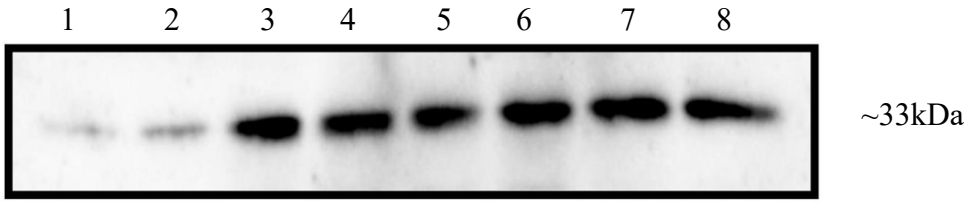
Fig.29C: Relative fold change of Nrf2 protein with del(5q) in lysates of peripheral blood (PB) mononuclear cells during lenalidomide (LEN) and LEN plus erythropoietin (EPO) therapy. The patient achieved a complete response to combined therapy. Bands

of Nrf2 protein with a molecular weight of ~62kDa and GAPDH protein with a molecular weight of ~37kDa before and during lenalidomide (LEN) and LEN plus erythropoietin (EPO) therapy.

D.)



The level of HO 1 protein during lenalidomide (LEN) and LEN plus erythropoietin (EPO) therapy



The level of GAPDH protein during lenalidomide (LEN) and LEN plus erythropoietin (EPO) therapy

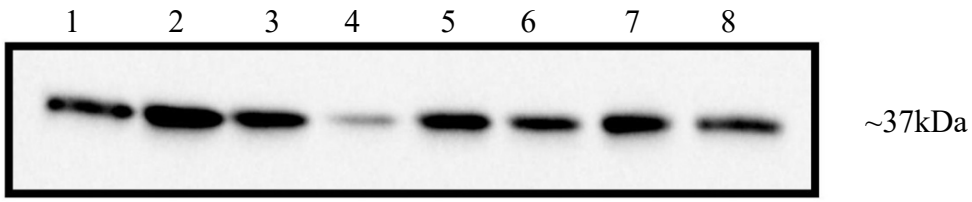


Fig.29D: Relative fold change of HO 1 protein with del(5q) in lysates of peripheral blood mononuclear cells during lenalidomide (LEN) and LEN plus erythropoietin (EPO) therapy. The patient achieved a complete response to combined therapy. Bands of HO 1 protein with a molecular weight of ~33kDa and bands of GAPDH with a molecular weight of ~37kDa are shown.

E.)

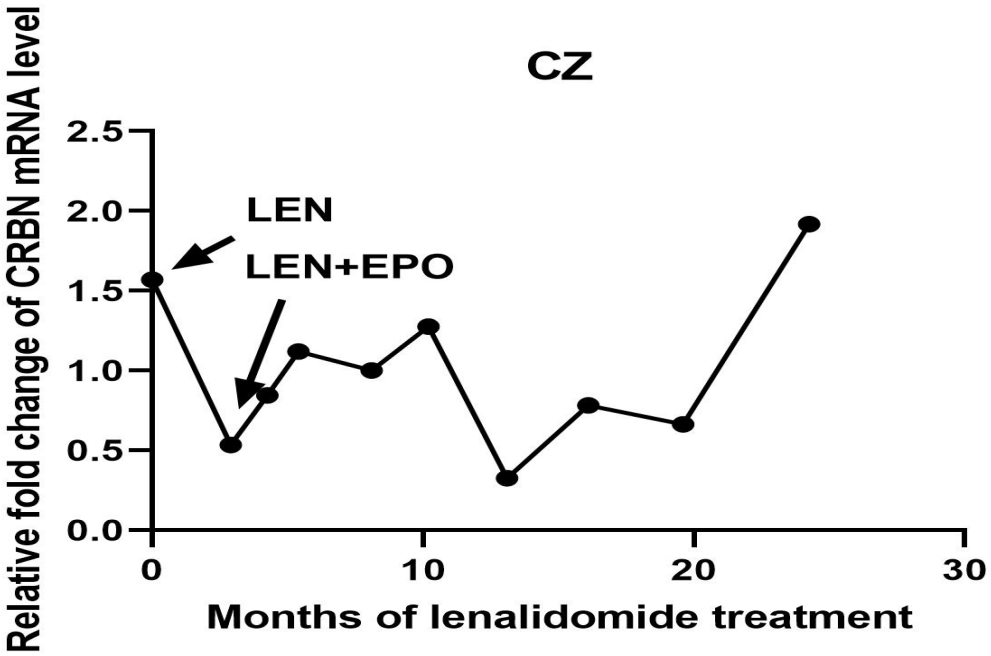


Fig.29E: Relative fold change of CRBN mRNA with del(5q) in peripheral blood (PB) mononuclear cells during lenalidomide (LEN)+ erythropoietin (EPO) therapy. The patient achieved a complete remission.

F.)

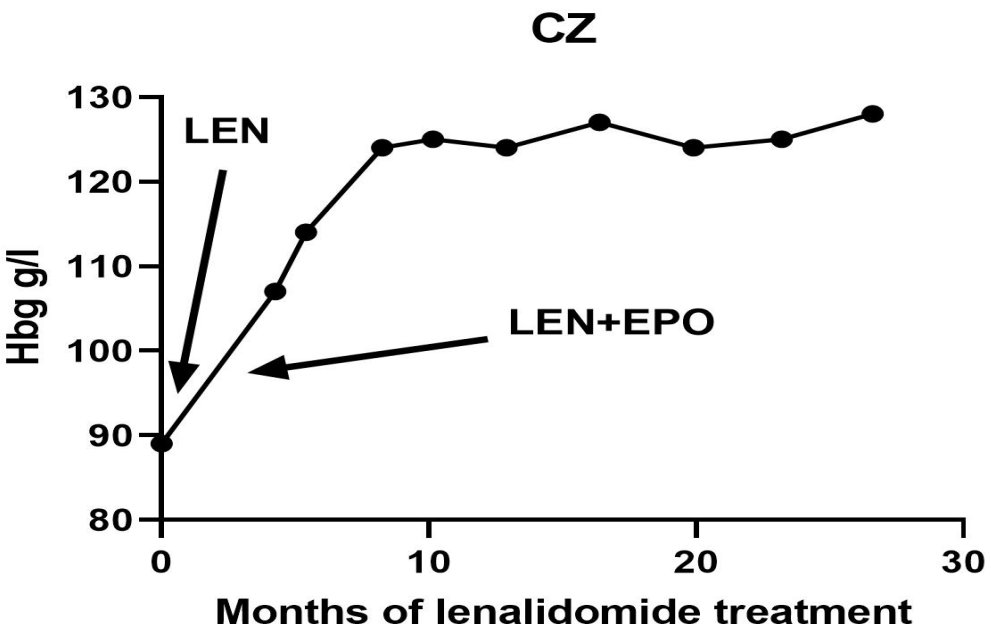


Fig.29F: Values of hemoglobin level (g/l) are shown as function of time during lenalidomide (LEN) and LEN plus erythropoietin (EPO) therapy of female patient with del(5q). The patient achieved a complete remission.

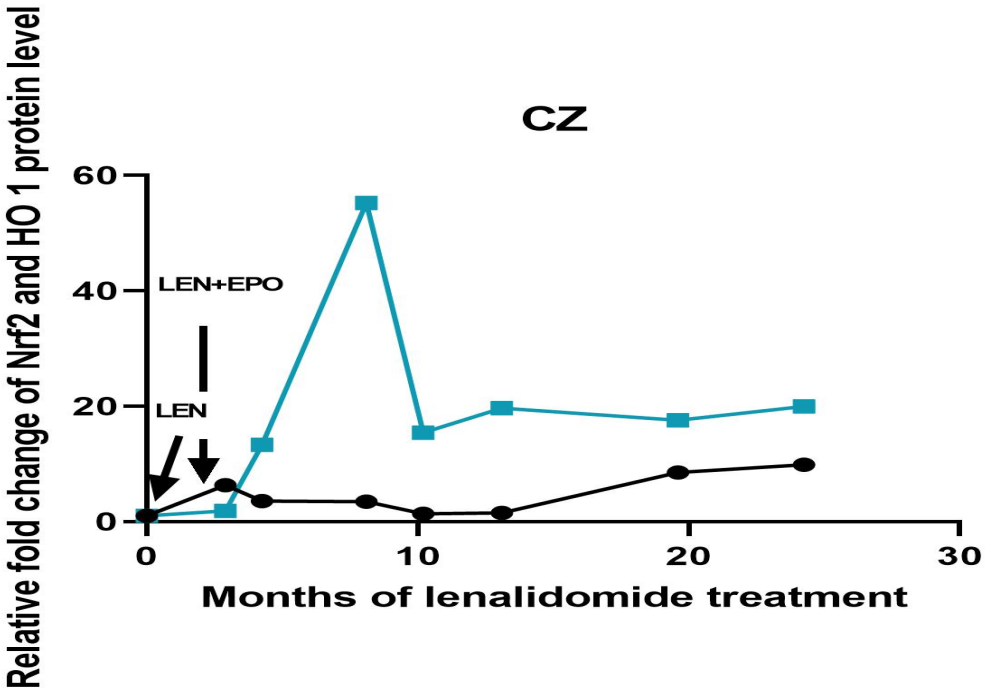
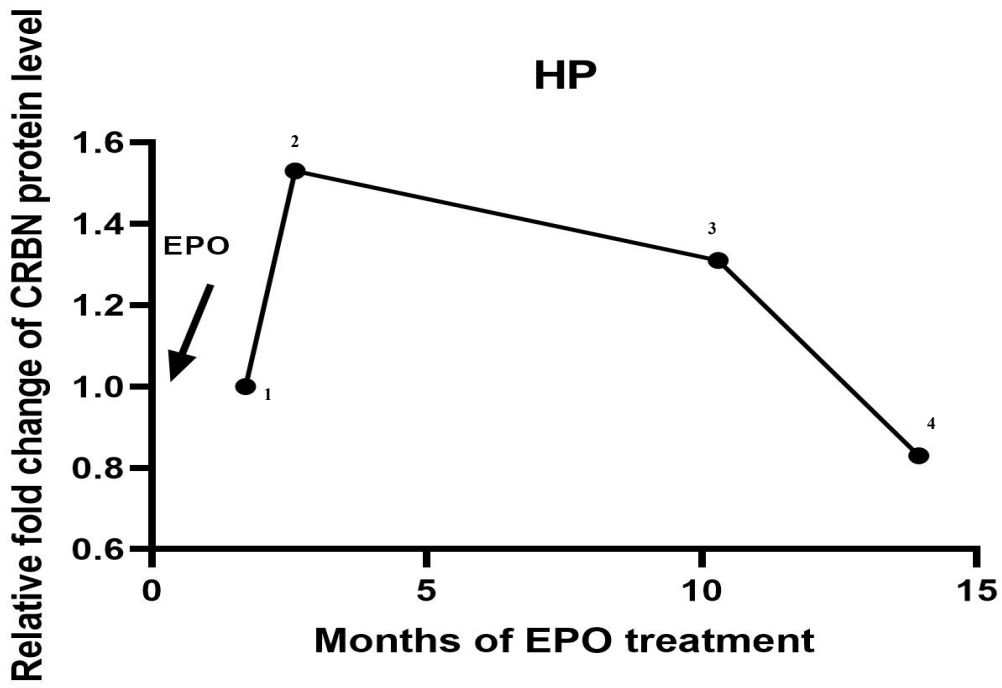


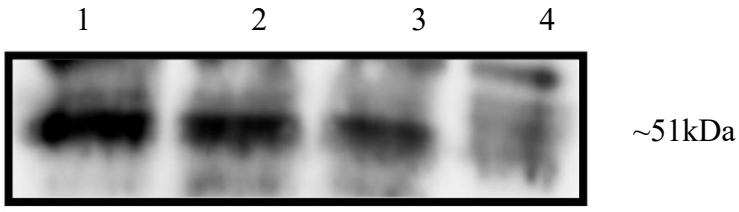
Fig.30: The comparison of relative fold change of Nrf2 (nuclear factor erythroid 2-related factor 2) protein level and relative fold change of HO-1 (heme oxygenase 1) in lysates of peripheral blood (PB) mononuclear cells of female patient with 5q- syndrome during lenalidomide (LEN) and LEN plus erythropoietin (EPO) therapy. Blue color represents relative fold change of HO 1 protein and black color represents relative fold change of Nrf2 protein.

A.)



B.)

The level of CRBN protein during erythropoietin (EPO) therapy



The level of GAPDH protein during erythropoietin (EPO) therapy

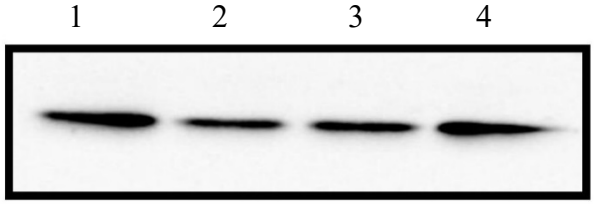
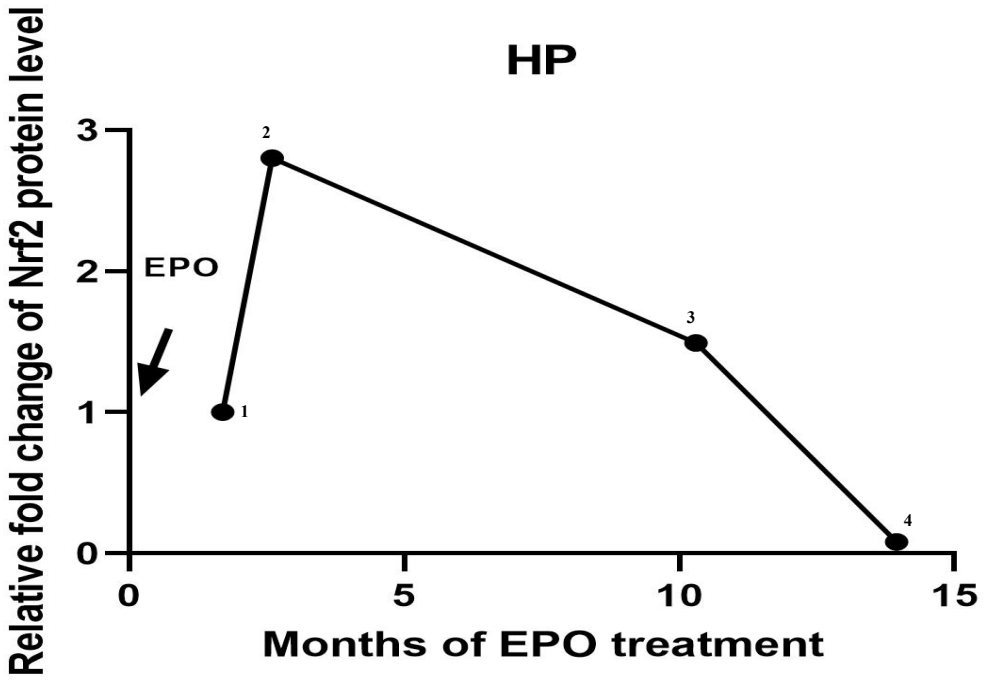


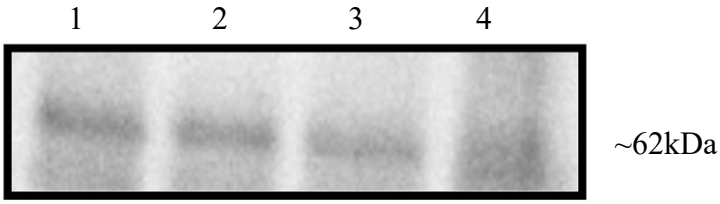
Fig.31A: Relative fold change of CRBN protein of female MDS patient with normal karyotype in lysates of peripheral blood mononuclear cells during erythropoietin (EPO) therapy. The patient did not respond to therapy. The molecular weight of CRBN protein is ~51kDa. The molecular weight of GAPDH protein is ~37kDa.

B.: Bands of CRBN protein with a molecular weight of ~51kDa and GAPDH protein with a molecular weight of ~37kDa during erythropoietin (EPO) treatment.

C.)



The level of Nrf2 protein during erythropoietin (EPO) therapy



The level of GAPDH protein during erythropoietin (EPO) therapy

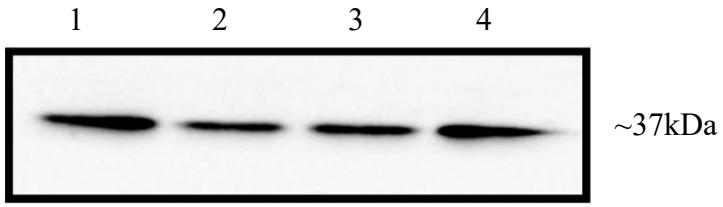
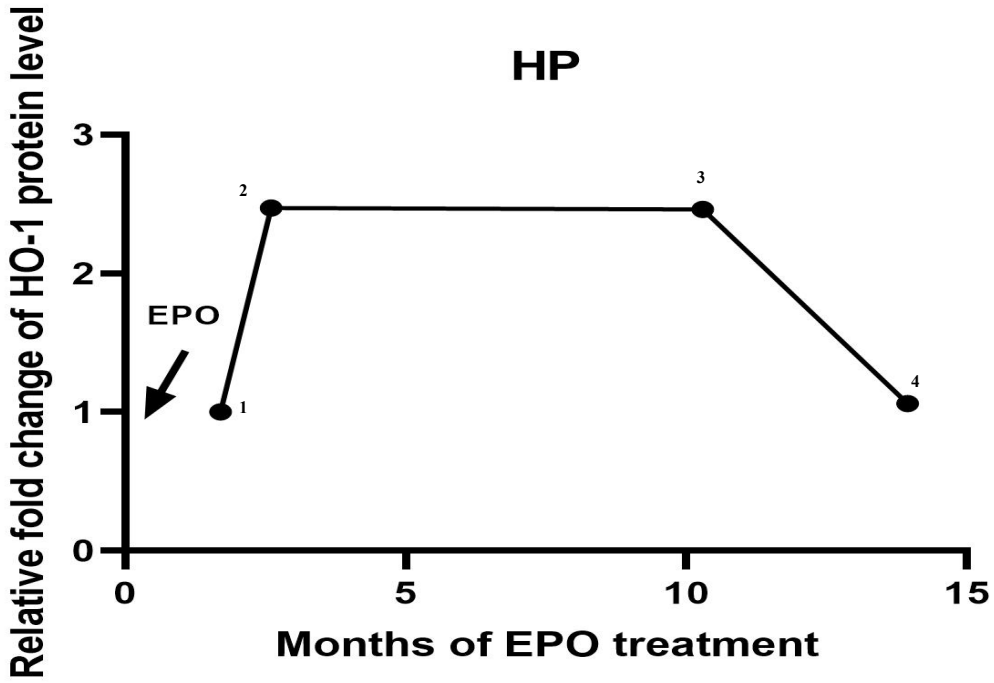
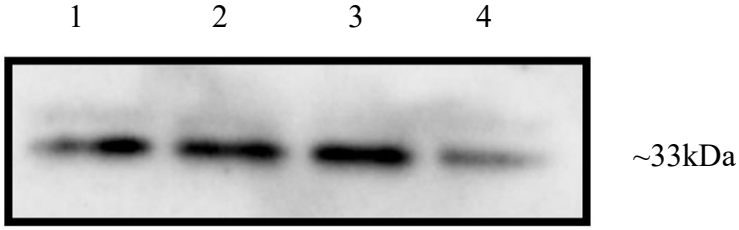


Fig.31C: Relative fold change of Nrf2 (nuclear factor erythroid 2-related factor 2) protein of female MDS patient with normal karyotype in lysates of mononuclear cells during erythropoietin (EPO) therapy. The patient did not respond to therapy. Bands of Nrf2 protein with a molecular weight of ~62kDa and GAPDH protein with a molecular weight of ~37kDa during erythropoietin (EPO) treatment.

D.)



The level of HO 1 protein during erythropoietin (EPO) therapy



The level of GAPDH protein during erythropoietin (EPO) therapy

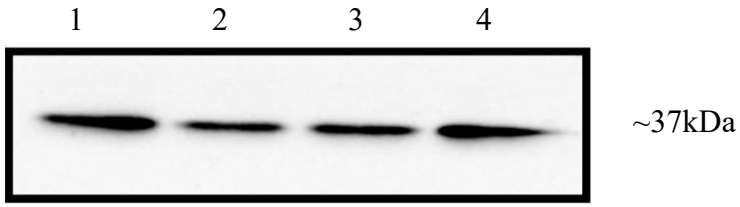


Fig.31D: Relative fold change of heme oxygenase 1 (HO 1) protein of female MDS patient with normal karyotype in lysates of mononuclear cells during erythropoietin (EPO) therapy. The patient did not respond to therapy. Bands of HO 1 protein with a molecular weight of ~33kDa and bands of GAPDH with a molecular weight of ~37kDa are shown.

E.)

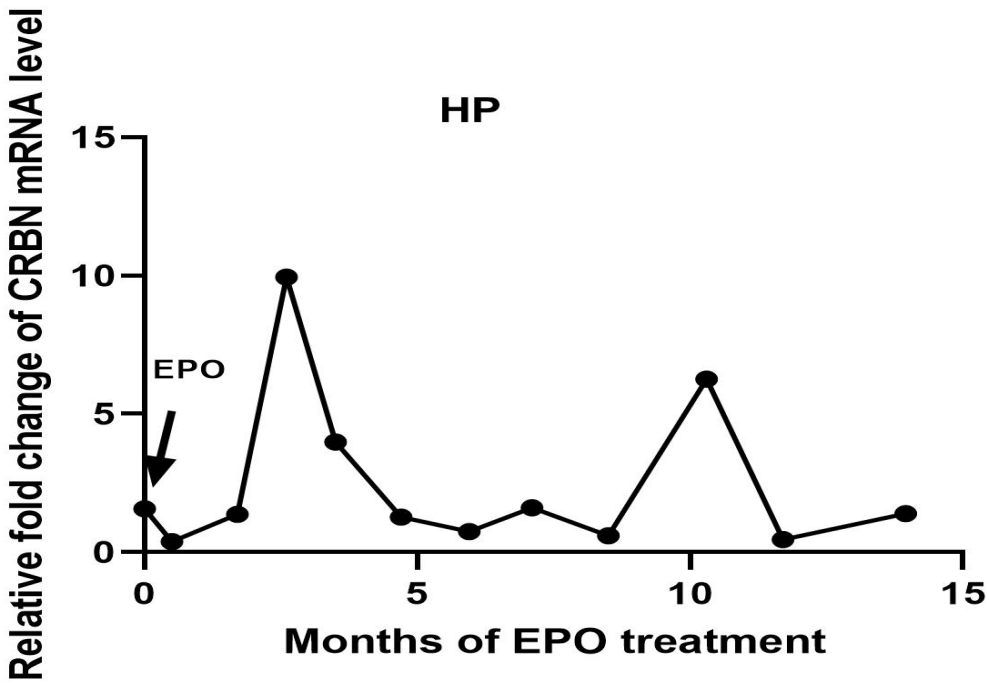


Fig.31E: Relative fold change of CRBN mRNA of female MDS patient with normal karyotype in peripheral blood mononuclear cells during erythropoietin (EPO) therapy. The patient did not respond to therapy.

F.)

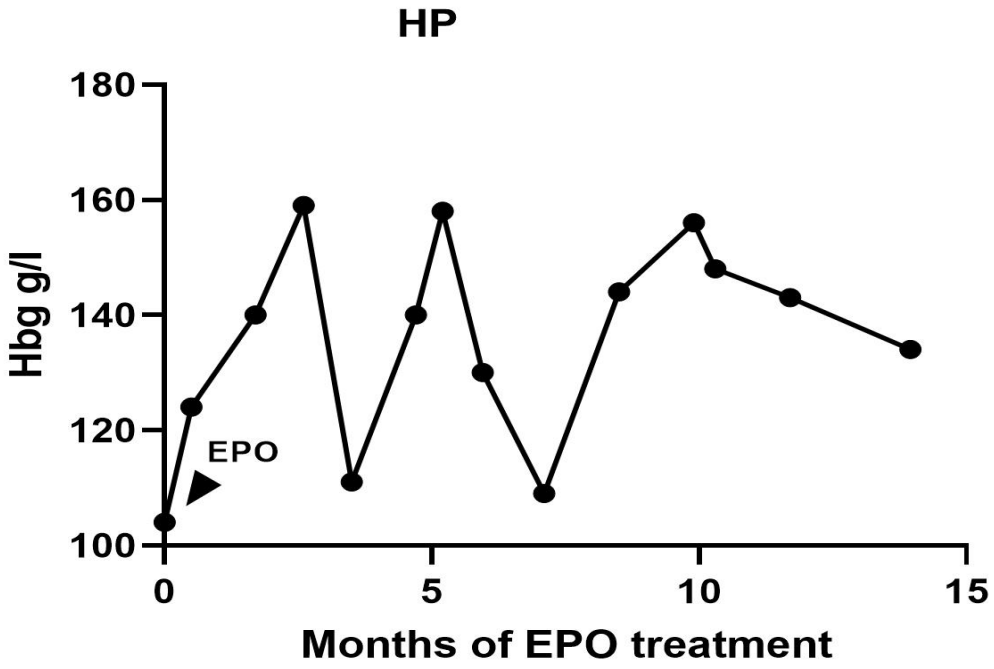


Fig.31F: Values of hemoglobin level (g/l) are shown as function of time during erythropoietin (EPO) treatment of female MDS patient with normal karyotype. The patient did not respond to therapy.

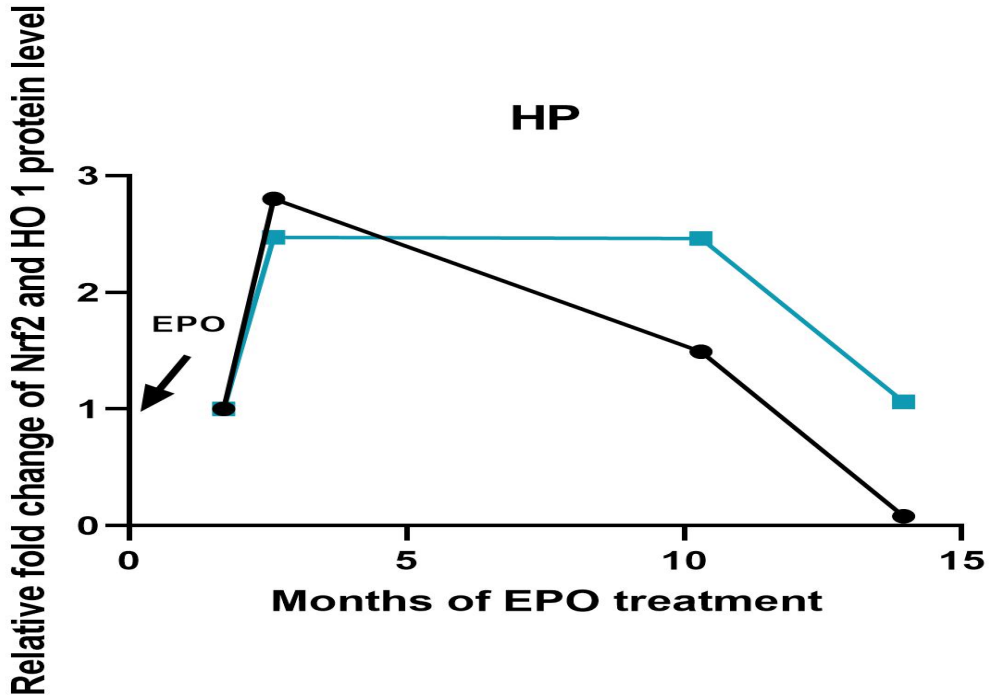


Fig.32: The comparison of relative fold change of Nrf2 (nuclear factor erythroid 2-related factor 2) protein level and relative fold change of HO-1 (heme oxygenase 1) protein level in lysates of peripheral blood (PB) mononuclear cells of female patient with normal karyotype during erythropoietin (EPO treatment. Blue color represents relative fold change of HO 1 protein and black color represents relative fold change of Nrf2 protein.

4.6. The effect of arsenic trioxide with LEN + EPO +PRED combination in MDS-L and SKM-1 cells- page 90

Corrections from page 91 to 98.

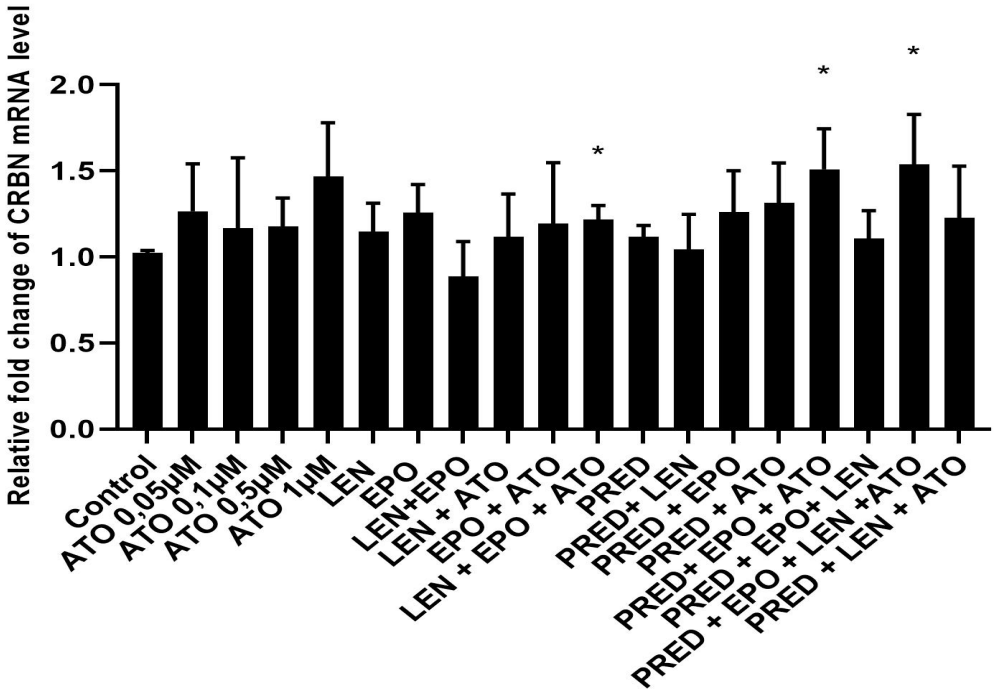


Fig.33: The graph shows the relative fold change of CRBN mRNA level in triplicates of MDS-L cells treated in culture with listed agents. Cell cultures were incubated for 24 hours, including control with the same addition of DMSO (dimethyl sulfoxide) as is in all groups. Aberrations: ATO-arsenic trioxide, LEN-lenalidomide, EPO-erythropoietin, PRED- prednisone). CRBN mRNA level was related to GAPDH and to values for CRBN mRNA level and GAPDH mRNA level in control group.

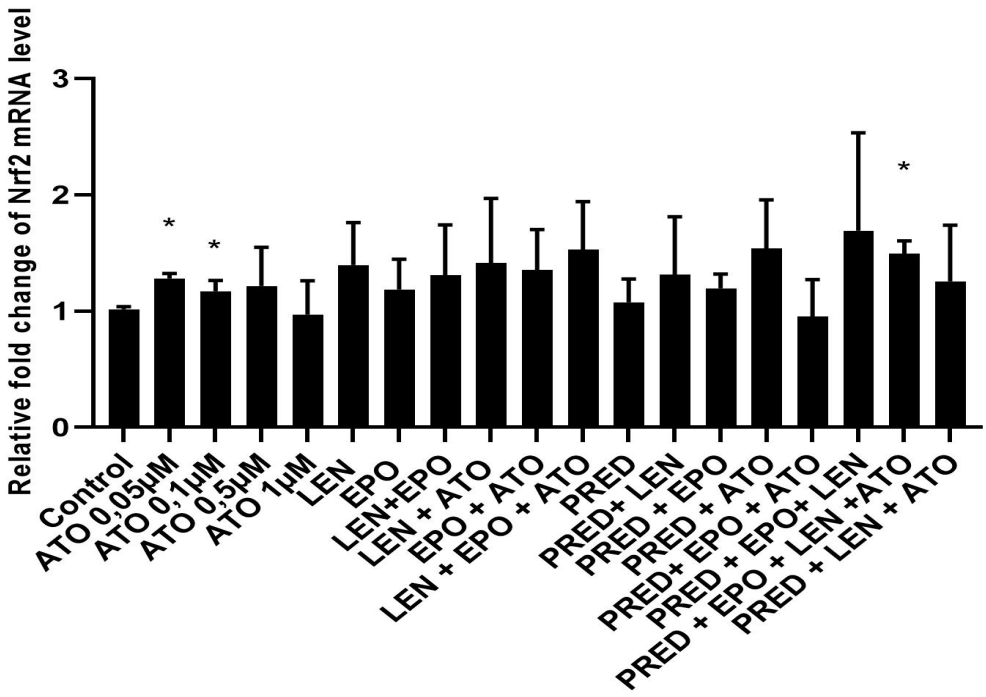


Fig.34: The graph shows the relative fold change of Nrf2 mRNA level in triplicates of MDS-L cells treated in culture with listed agents. Cell cultures were incubated for 24 hours, including control with the same addition of DMSO (dimethyl sulfoxide) as is in all groups. Aberrations: ATO-arsenic trioxide, LEN-lenalidomide, EPO-erythropoietin, PRED- prednisone). Nrf2 mRNA level was related to GAPDH and to values for Nrf2 mRNA level and GAPDH mRNA level in control group.

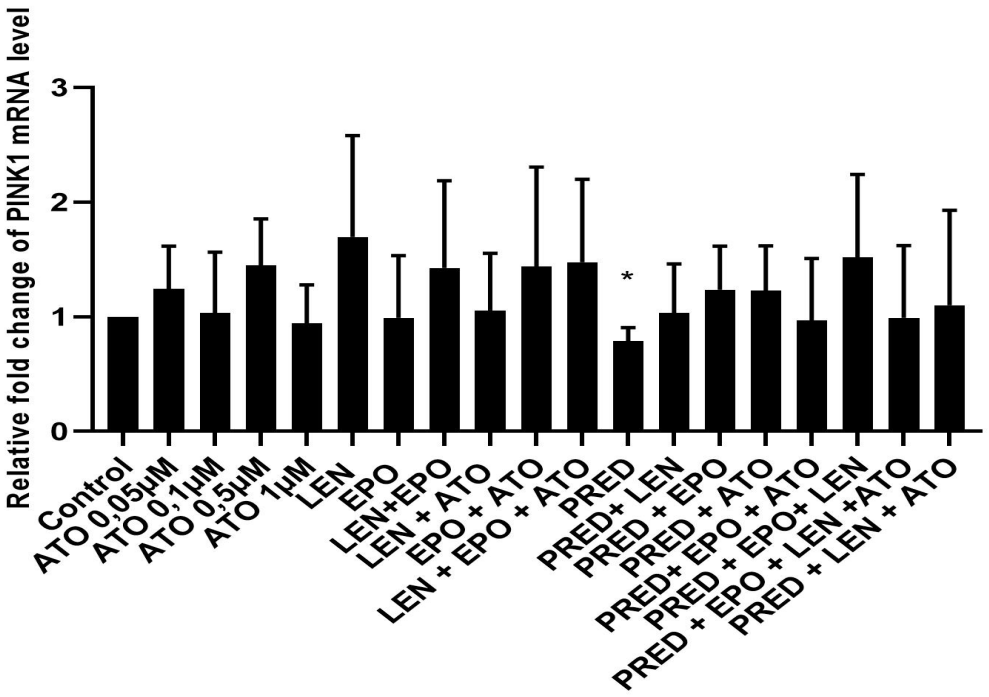
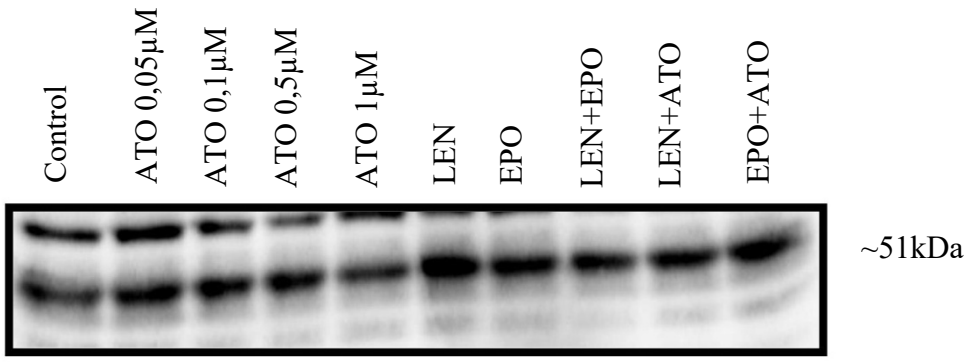


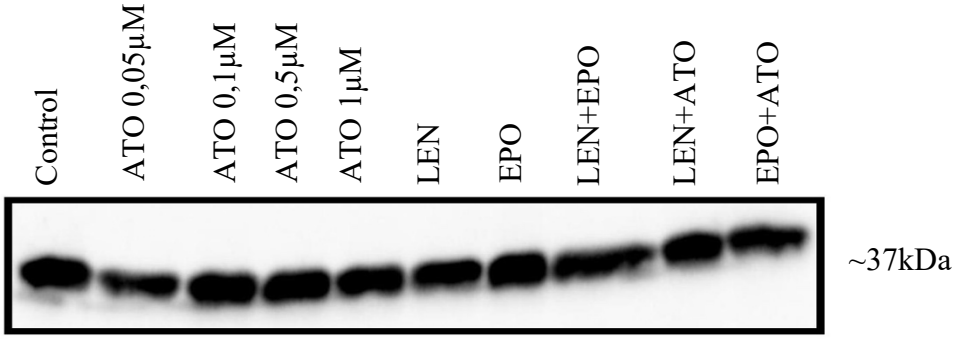
Fig.35: The graph shows the relative fold change of PINK1 mRNA level in triplicates of MDS-L cells treated in culture with listed agents. Cell cultures were incubated for 24 hours including control with the same addition of DMSO (dimethyl sulfoxide) as is in all groups. Aberrations: ATO-arsenic trioxide, LEN-lenalidomide, EPO-erythropoietin, PRED- prednisone). PINK1 mRNA level was related to GAPDH and to values for PINK1 mRNA level and GAPDH mRNA level in control group.

A.)

The level of CRBN protein in MDS-L cell cultures

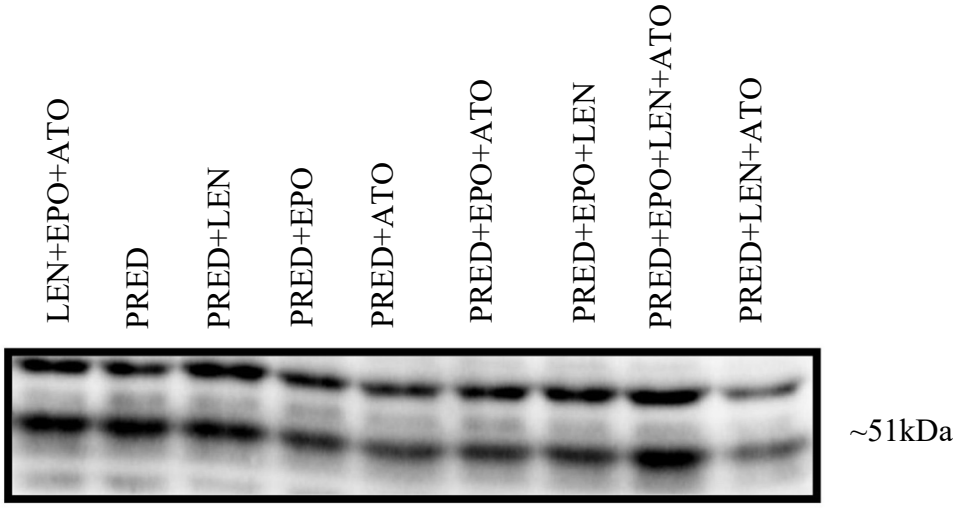


The level of GAPDH protein in MDS-L cell cultures



B.)

The level of CRBN protein in MDS-L cell cultures



The level of GAPDH protein in MDS-L cell cultures

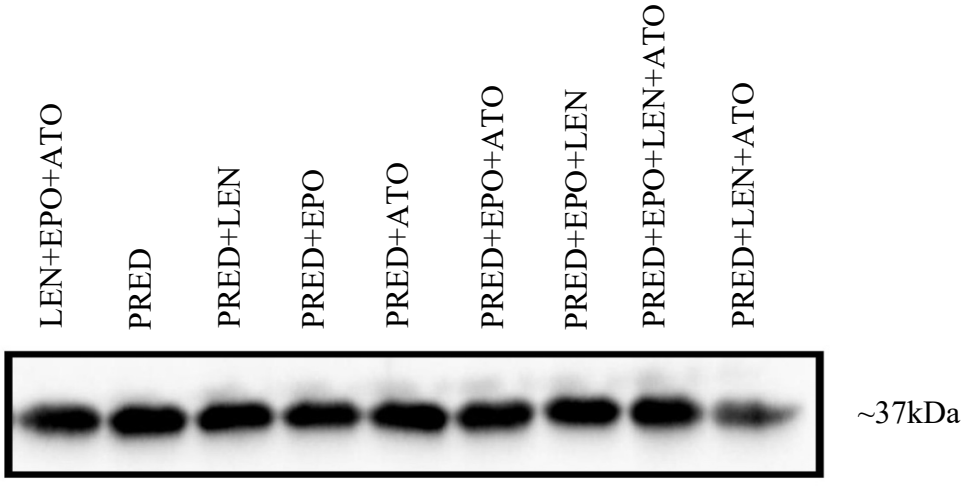


Fig.36: The graph represents the level of CRBN protein in triplicates of MDS-L cells treated in culture with listed agents. Cell cultures were incubated for 24 hours, including control with the same addition of DMSO (dimethyl sulfoxide) as in all groups. Aberrations: ATO-arsenic trioxide, LEN-lenalidomide, EPO- erythropoietin, PRED- prednisone). The first picture is CRBN protein with a molecular weight of ~51kDa. The second picture is GAPDH with a molecular weight of ~37kDa.

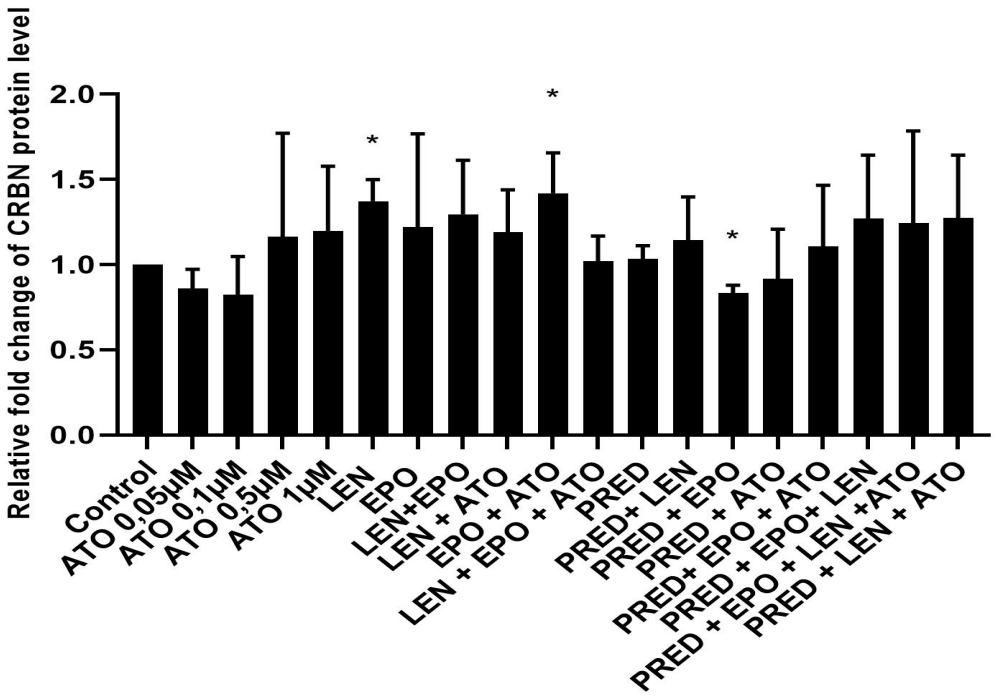


Fig.37: The graph represents the level of CRBN protein in triplicates of MDS-L cells treated in culture with listed agents. Cell cultures were incubated for 24 hours, including control with the same addition of DMSO (dimethyl sulfoxide) as in all groups. Aberrations: ATO-arsenic trioxide, LEN-lenalidomide, EPO- erythropoietin, PRED- prednisone).

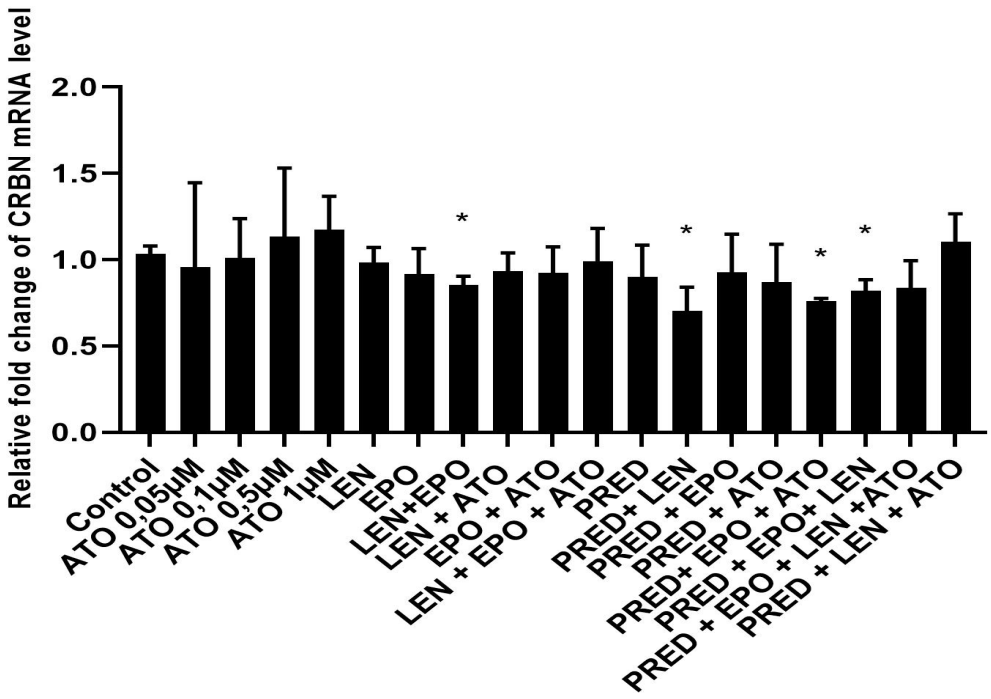


Fig.38: The graph describes the level of CRBN mRNA in triplicates of SKM-1 cells treated in culture with listed agents. Cell cultures were incubated for 24 hours, including control with the same addition of DMSO (dimethyl sulfoxide) as in all groups. Aberrations: ATO-arsenic trioxide, LEN-lenalidomide, EPO- erythropoietin, PRED- prednisone).

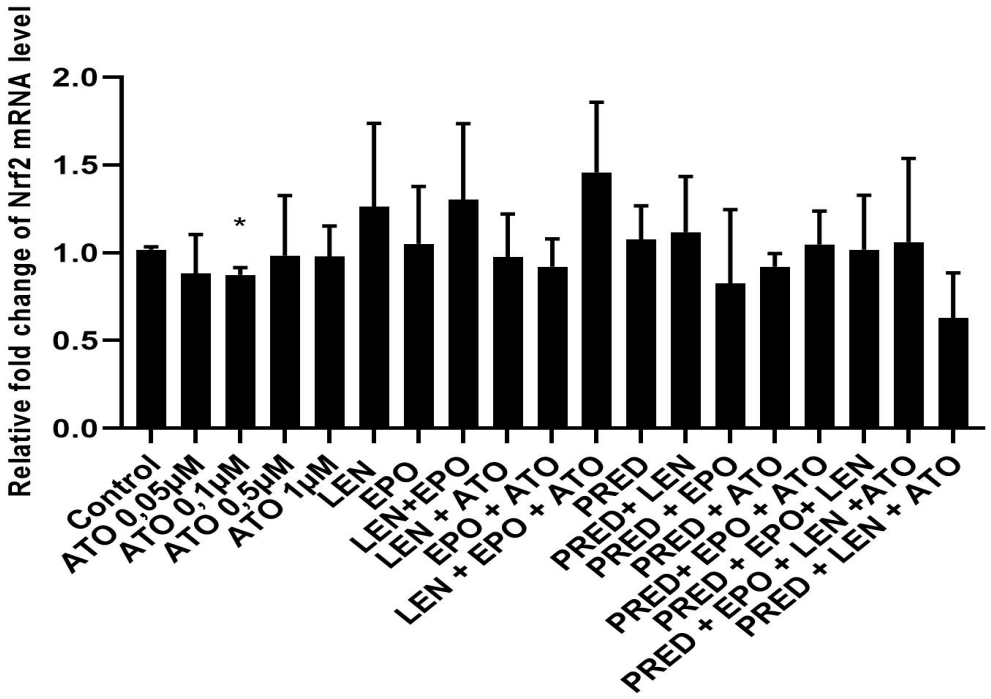


Fig.39: The graph describes the level of Nrf2 mRNA in triplicates of SKM-1 cells treated in culture with listed agents. Cell cultures were incubated for 24 hours, including control with the same addition of DMSO (dimethyl sulfoxide) as is in all groups. Aberrations: ATO-arsenic trioxide, LEN-lenalidomide, EPO- erythropoietin, PRED- prednisone).

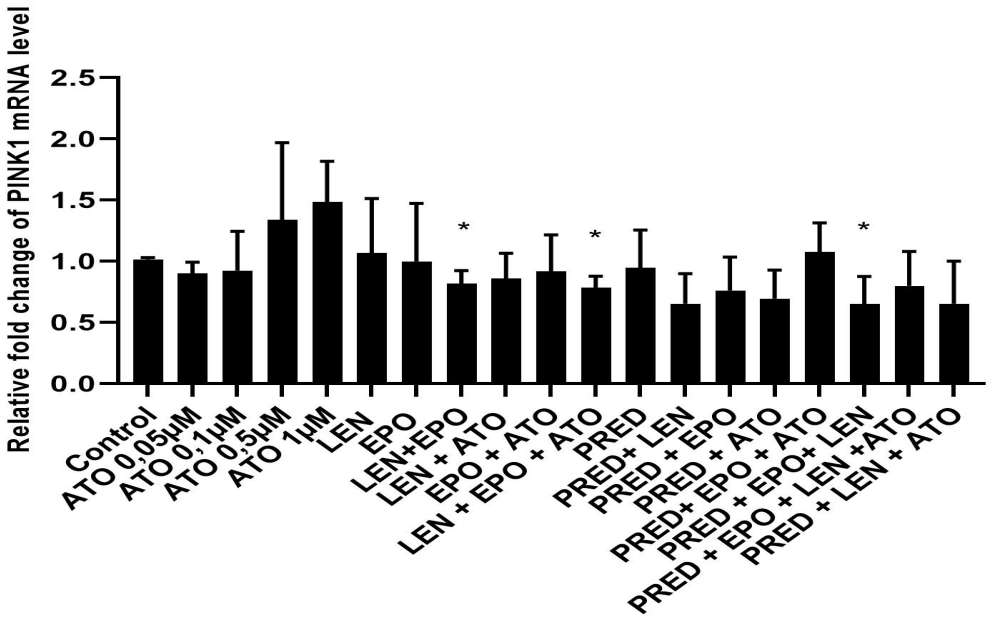
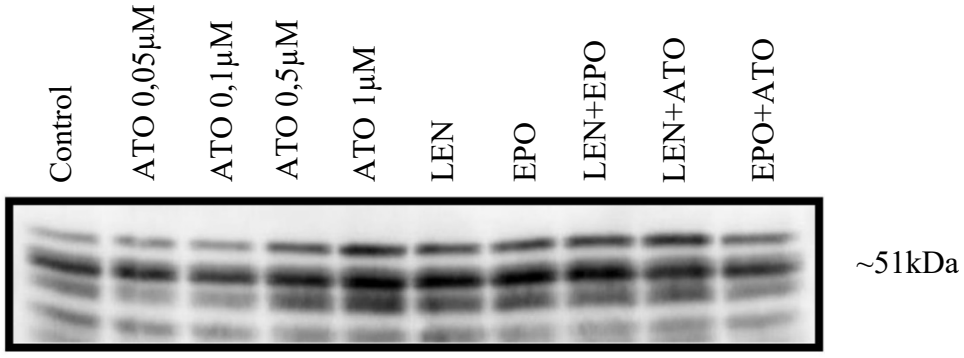


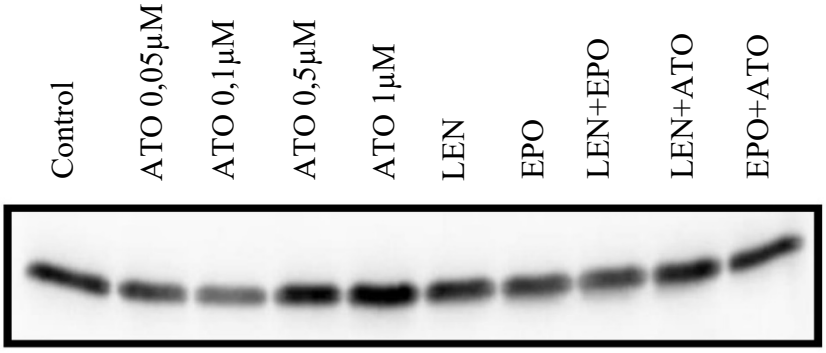
Fig.40 The graph describes the level of PINK1 mRNA in triplicates of SKM-1 cells treated in culture with listed agents. Cell cultures were incubated for 24 hours, including control with the same addition of DMSO (dimethyl sulfoxide) as in all groups. Aberrations: ATO-arsenic trioxide, LEN-lenalidomide, EPO- erythropoietin, PRED- prednisone).

A.)

The level of CRBN protein in SKM-1 cell cultures

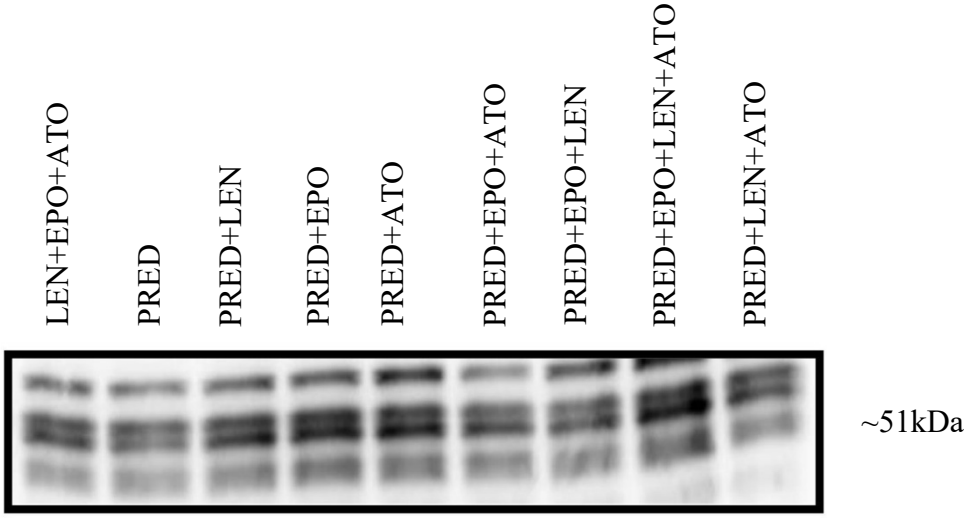


The level of GAPDH protein in SKM-1 cell cultures



B.)

The level of CRBN protein in SKM-1 cell cultures



The level of GAPDH protein in SKM-1 cell cultures

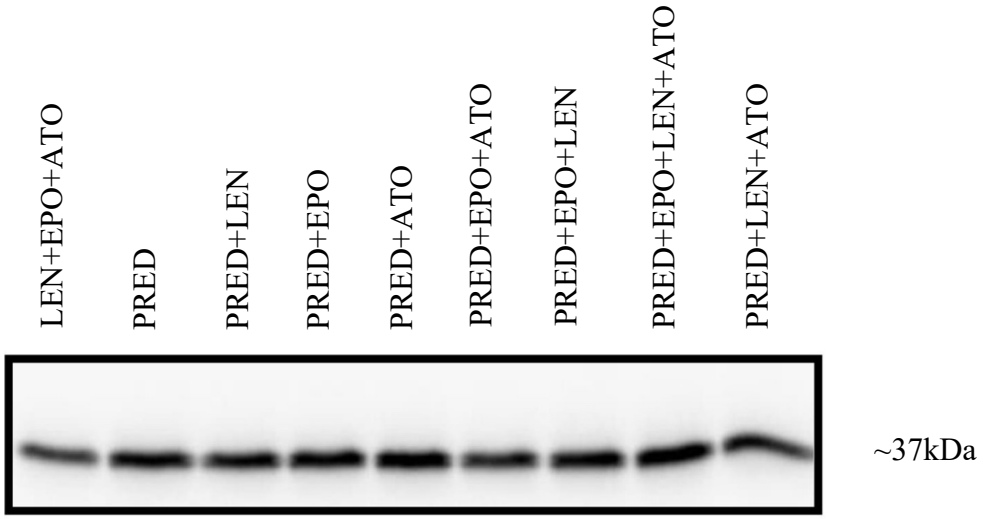


Fig.41: The graph represents the relative fold change of CRBN protein level in triplicates of SKM-1 cells treated in culture with listed agents. Cell cultures were incubated for 24 hours, including control with the same addition of DMSO (dimethyl sulfoxide) as in all groups. Aberrations: ATO-arsenic trioxide, LEN-lenalidomide, EPO- erythropoietin, PRED- prednisone). The first picture is CRBN protein with a molecular weight of ~51kDa. The second picture is GAPDH with a molecular weight of ~37kDa.

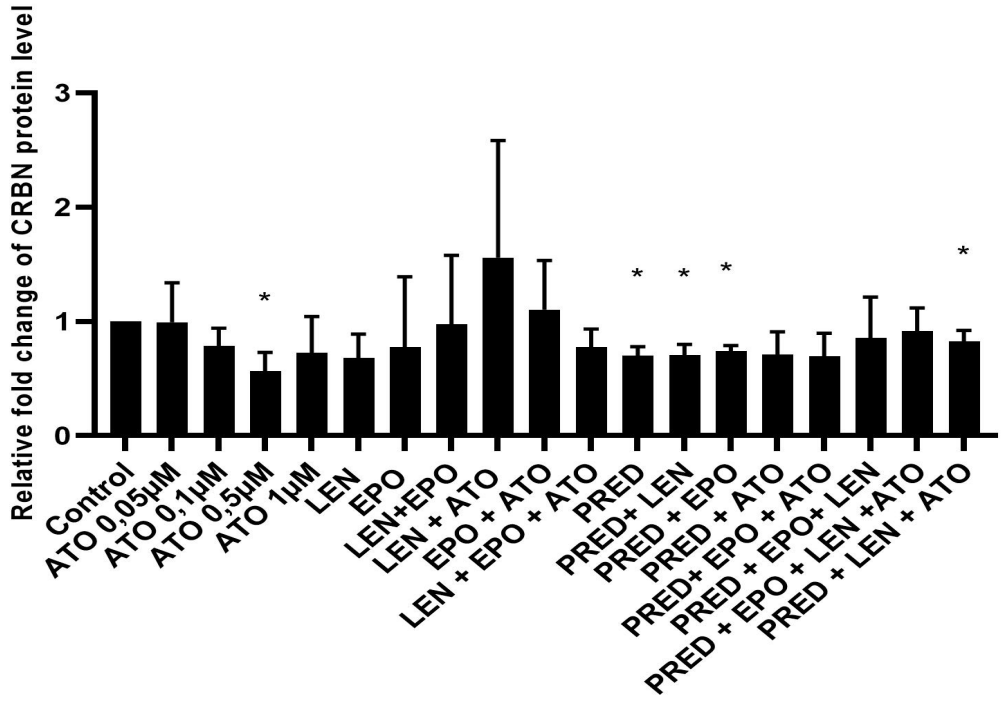


Fig.42: The graph represents the level of CRBN protein in triplicates of SKM-1 cells culture treated with listed agents. Cell cultures were incubated for 24 hours, including control with the same addition of DMSO (dimethyl sulfoxide) as in all groups. Aberrations: ATO-arsenic trioxide, LEN-lenalidomide, EPO- erythropoietin, PRED-prednisone).

DISCUSSION- page 99

Correction on page 101 - The new text is added to the sixth line from the top.

As the relative cut off value of CRBN mRNA was chosen the value 1, which is the same as the result for healthy control. Under this value patients stopped to respond to lenalidomide.When the started relative fold CRBN mRNA value before lenalidomide (LEN) therapy was high (above 5), the sharp decrease of this value under 1 is associated with the progression of disease to higher risk MDS or AML.

Correction on page 102 - Correction of the thirteenth line from the top

Outside of the clinical trial, there were four MDS patients without del(5q) from the General Faculty Hospital whom the State Institute for Drug Control (SÚKL) approved lenalidomide treatment.

Correction on page 104. The new text is added to first line from the bottom.

We did not use CRBN antibody to detect the non-specificity of this antibody on the whole membrane, because we did not have enough antibodies. The lower average obtained concentration of protein lysate was around 240 µg per 150µl sample, and the higher average concentration of protein lysate was 450 µg/150µl sample. The ammount of protein in patient sample of protein lysate was 20 µg and higher in 15 µl necessary for detection on the membrane by Ponceau S. The volume 15 µl could be maximally used for the sample path in our electrophoresis apparatus.

Correction on page 105. Correction of the tenth line from the bottom.

In the total ammount of 14 patients on EPO treatment, we detrmined only two cases (14,29 %) where Nrf2 mRNA expression did not follow CRBN mRNA expression.