Chapter

New Perspectives in Personalization of Therapy for Hematological Cancers

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Abstract

A progress in treatment of hematological cancers was achieved. Unfortunately, some youngsters, because of rare genetic alterations that are not easy to detect, as well as heavily pretreated old patients, because of coexisting diseases that lead to changes in patient metabolism, do not respond to therapy. Moreover, sometimes familiar diversities and alterations on genetic or epigenetic level that could be transferred on diversities in metabolism or cell signaling might be a reason why patients do not respond to therapy. Interestingly, for older patients a resistance to therapy could also occur as a reason of drug cross-reactivity. For designing of effective anticancer therapy for patient with chronic lymphocytic leukemia before drug administration, patient's leukemic cell response to anticancer drug(s) should be checked. Moreover, for patient response to treatment, also drugs prescribed previously by other medical doctors or even patients' diet could be important for achieving therapeutic success of therapy. Therefore it is important to choose the effective drugs before their administration to patient that will improve treatment efficacy and exclude resistance to therapy. It must be stated that the special attention for personalized therapy tests should be focused on patients previously resistant to therapy, more sensitive to drugs or heavily pretreated.

Keywords: personalization of therapy, resistance to treatment, anticancer drugs

1. Introduction

Several studies around the world are focus on molecular aspects related with developing of cancer. It seems to be not easy, because of high complexity of carcinogenesis and a difference between patients in disease progressing based on genetic, epigenetic or even environmental alteration. It is possible that in our bodies expression of proteins or external factors regulate hormone expression. Moreover, hormones could affect cell signal transduction and metabolism. It looks like we have some logical plan that coordinates gene expression, but because of potential involvement of many factors and familiar, sometimes even personal, predispositions for particular type of disease, diagnostic is not easy. The personal differences in disease progression activity and response to treatment reveal that even now cancers are the leading cause of death. The best way to avoid carcinogenesis is a health prophylactic since childhood. It is more likely that cancer or other diseases related with some disturbances in metabolism, except genetically related

documented cases, will develop in the group of overweight people with metabolic or hormonal disturbances and without physical exercises. For good health condition since childhood, we need to supplement probiotic bacteria to have bacterial flora in intestines that could cooperate somehow and save people against most infections and inflammation [1]. Moreover, it is very important what we consume. Food processing should be ecological, without unhealthy chemical additives [2, 3]. It must be underlined that health prophylactic might decrease the risk of getting ill or even save us from many diseases, including cancers. Proper screening tests that are able to find health problems in not advanced phase of disease are very important for prophylactic. It is extremely important to discover disease in early stage of development so we can effectively help patient to cure the disease, achieving success. It is always better and also cheaper to prevent than to cure diseases.

Before we had enough knowledge to cure successive cancers, the most effective in the war against cancers seems to be a prophylactic. The prophylactic tests should be designed for each person individually based on personal or genetic predispositions, work-related conditions, weight, or diet. Prophylactic test program should be organized by the Ministry of Health and paid from money directed to health service from our taxes.

The suitable prophylactic for professions, and increasing knowledge about disease development, could enlarge human life and decrease number of cases of cardiovascular diseases (e.g. stroke or heart attack). Therefore, we must remember that the special diet and physical exercises are able to regulate our hormone level, metabolism, and cell signaling leading to homeostasis [4, 5]. The disturbances in homeostasis could accompany the development of many diseases, including cancers.

There are several different types of lymphoproliferative disorders, characterized by disturbances in cell cycle and/or signal transduction that lead to fast proliferation of cancer cells (acute form) [6]. The other chronic type of neoplasm is caused, for example, by abrogation in apoptosis induction leading to accumulation of leukemic cells in peripheral blood. It was previously reported that in patient blood a quiescent and cycling cells were found that could be a problem in diagnostics and treatment [7–9].

Chronic lymphocytic leukemia (CLL), except bone marrow transplantation, for some patients is incurable. CLL usually occurs in older individuals but in youngsters could transform into a more aggressive form with fast developing disease [8].

Interestingly, a high heterogeneity in disease development, the characteristic accumulation in patient blood of mainly resting B lymphocytes, was observed. Leukemic cells accumulate in patient peripheral blood, because of inhibition in induction of apoptosis.

Several factors, even patient diet, could affect disease development and transformation into active form. It could lead to disturbances in cell cycle leading to increased cancer cell proliferation index. It could also induce changes in cell sensitivity to drug(s) [10]. The results of experiments directed toward personalization of therapy for CLL patients revealed that it is important to analyze before drug(s) administration if drug(s) will be active for this patient curing [10–14]. Moreover, during leukemic cell incubation with drugs, we are able to check also patients' cell sensitivity to in vitro incubation conditions. If in control untreated cells, cell viability decreases similarly like in treated ones, it is an indication that patient might need a reduction in drug dose in vivo [15]. Moreover, we need more knowledge on this topic to be able precisely to transform drug doses obtained in patient's blood into in vitro conditions. It must be underlined that for some patients more sensitive to drug(s) than others using above tests in vitro we are able to choose the

proper drug(s) dose to optimize drug(s) doses administration in vivo. For patients responding to treatment in nonstandard way and for patients more sensitive than others to drugs, a personalized therapy test could be a chance to choose effective treatment for curing to avoid drug resistance, patient fatigue or sometimes also secondary cancers. Moreover, the ineffective treatment might reduce patients' life longevity. Therefore it is very important to check before administration if drug will be active to patient or in the case of resistance to search for other potentially effective drug.

The special value of personalized therapy tests is presented in **Figure 1**. The representative combined results obtained for CLL patient by tests such as cell viability (flow cytometry; Vybrant Apoptosis Assay), analyses of thermal profiles of nuclei (differential scanning calorimetry) and PARP expression/proteolytic cleavage (Western blot) for patient confirm the value for such test in choosing effective therapy for CLL patient (**Figure 1**). The representative results for CLL patient confirm that leukemic cells were more sensitive to the combination of cladribine and cyclophosphamide/mafosfamide than the combination of fludarabine and cyclophosphamide/mafosfamide. For this patient adding rituximab does not change anything in cells' reactivity to drugs (compare CM and Rit20CM results). The combination of cladribine and cyclophosphamide/mafosfamide will be more effective for this patient curing than the combination including fludarabine. Moreover, the addition of monoclonal antibody to CM combination did not change anything when we analyze all results obtained for patient.

In **Figure 2** there is an explanation why we should analyze each patient results separately. It must be stated that in the median results of cell viability presented in **Figure 2** (Section A), there are also results of both patients presented in Sections B (better responder to CM than FM) and C (resistant to therapy). The median results of cell viability for patients B and C could be misleading and suggest that both patients should be administered with fludarabine and cyclophosphamide. For both patients (see **Figure 2B** and **C**) fludarabine will not be an optimal drug for curing.

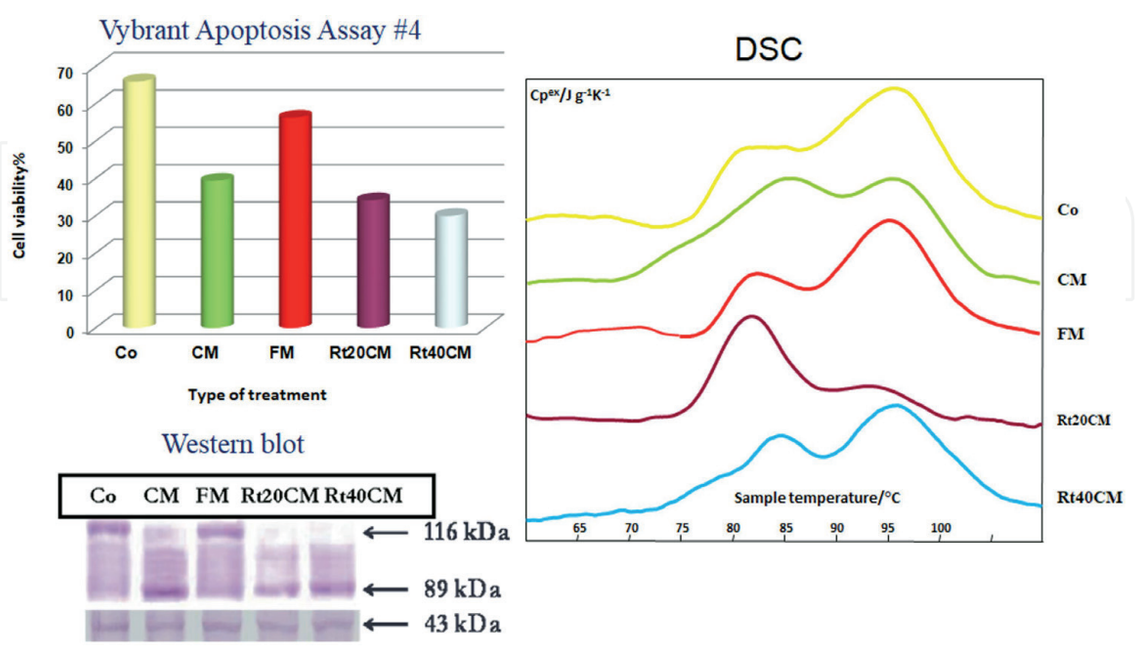

Figure 1.
Personalized therapy for CLL patient. Results of cell sensitivity with anticancer agents. Cell viability of chronic lymphocytic leukemia cells incubated for 48 h without Co and with anticancer drugs; Co, controlled untreated CLL cells; CM, cladribine + mafosfamide; FM, fludarabine + mafosfamide; RtCM (20 or 40 μM), rituximab + CM. Differences in thermal profiles were analyzed by differential scanning calorimetry (DSC). Analysis of protein expression related to apoptosis (PARP cleavage; 89 kDa) or actin (43 kDa) was studied by Western blot.

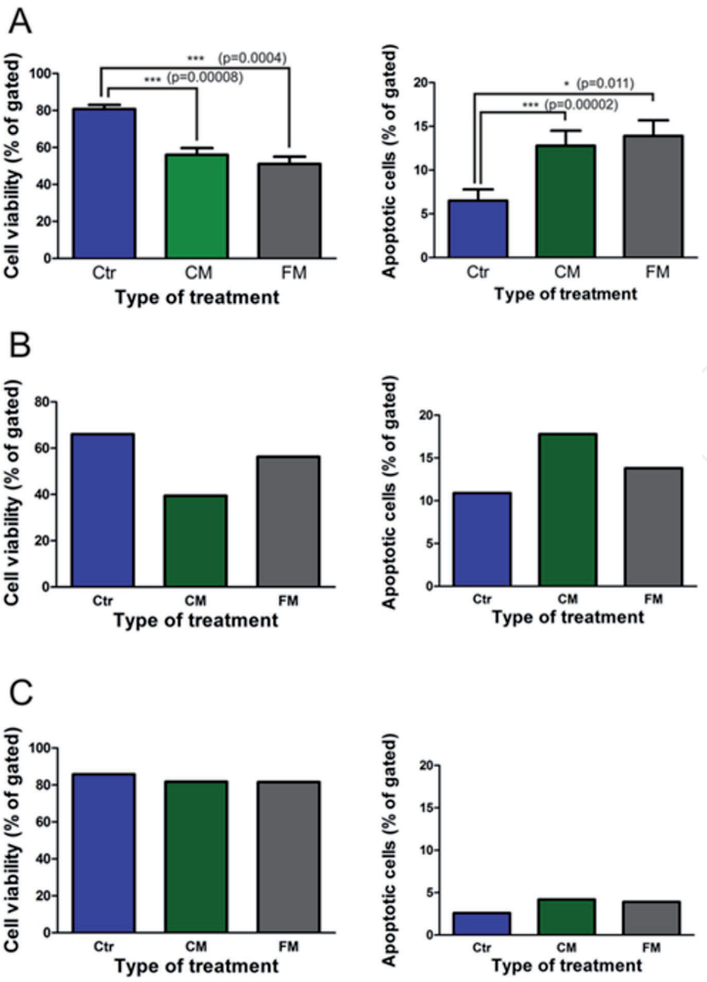

Figure 2.
Cell viability of chronic lymphocytic leukemia cells incubated for 48 h without Ctr and with anticancer drugs; CM, cladribine + mafosfamide; FM, fludarabine + mafosfamide; ***A****, median value of cell viability;* ***B****, results obtained for cells of CLL patient (better responded to CM than to FM);* ***C****, results obtained for cells of CLL patient resistant to treatment.*

Using above tests we can also monitor the differences in drug sensitivity during disease development (**Figure 3**) [10]. As presented in **Figure 3** results of cell sensitivity to anticancer agents could change during disease development. In active form of disease (year 2013), a higher leukocytosis than 18 months earlier results (year 2011) was noticed. For the same patient, we can observe differences in leukemic cell sensitivity to the same anticancer agents, i.e., combinations of cladribine + mafosfamide (cyclophosphamide), CM; fludarabine + mafosfamide (cyclophosphamide), FM; rituximab + CM (RCM); kinetin riboside (RK), that confirm the usefulness of personalized therapy tests also in monitoring of disease development and the changes in drug sensitivity [10].

Results of cell viability of chronic lymphocytic leukemia cells incubated for 48 h (24, 48 h) without Ctr and with anticancer drugs; CM, cladribine + mafosfamide; FM, fludarabine + mafosfamide; RCM, rituximab + CM; Rit, rituximab; RK, kinetin riboside [10].

The existing data confirm that in the development of CLL, some disturbances in cell signaling [16], apoptosis inhibition, or changes on epigenetic level [17–20] are observed. Moreover, the results of studies confirm that there are several molecules generated from encoded sequences of genes, characterized as miRNA that could affect gene expression, mainly by silencing. It could also be involved in regulation of protein synthesis important for cell cycle.

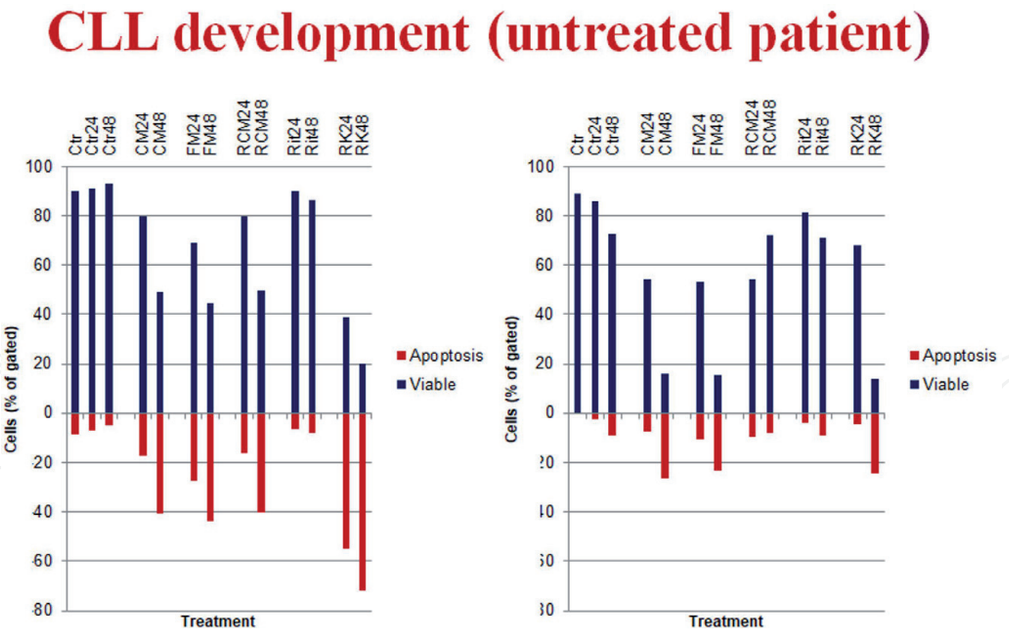

Figure 3.
Differences in drug sensitivity during disease development for the same CLL patient (results from year 2011 and 2013).

The background of chemoresistance includes one or more of the following mechanisms: induction of DNA repair, silencing of gene expression, some alterations in metabolism or in drug target structure, modifications in cell membrane or microenvironment composition, elevated expression of drug efflux pumps, and inhibition of apoptosis [21–24].

Everything is very complex, and on the one hand familiar diversities could affect cell signaling and metabolism; on the other hand also environmental factors could be important for final reaction of our cells and bodies to drugs, even human diet.

At the moment it is better to analyze the total effect of drug activity on leukemic cells before drug administration to CLL patient, preventing resistance to treatment.

Diagnostics for CLL are still based on the analysis of expression of characteristic clusters of differentiation (CD) as well as the presence of cytogenetic alterations (chromosomal aberrations) [25–27].

The currently used drugs in hematological clinics for CLL treatment are usually directed toward inhibition of Bcl-2 gene expression (venotoclax), inhibition of pathways related with signal transduction, for example, involved in inhibition of competitive binding of ATP to Bruton kinase (ibrutinib), or inhibition of PI3K signaling (idelalisib) [27–31]. Both kinases are involved in B-cell receptor signaling.

Venotoclax (VEN, ABT-199) is a selective inhibitor of antiapoptotic protein Bcl-2 expression. It is a BH3-mimetic molecule targeting BCL-2. VEN binds to BCL-2 and could activate BIM and induce apoptosis signaling. VEN demonstrates an activity in patients with poor prognostic, 17p-deleted chronic lymphocytic leukemia (CLL) [28]. VEN shows clinical activity on many hematological malignancies, lymphomas, acute myeloid leukemia, and early T-cell precursor ALL.

Ibrutinib directly works as an inhibitor of Bruton tyrosine kinase (BTK). Interestingly, there is crosstalk between Bruton tyrosine kinase signaling and bioenergetic stress responses. In primary chronic lymphocytic leukemia cells, a pharmacological interference between mitochondrial ATP synthesis and glucose metabolism could affect BTK activity. Moreover, ibrutinib could induce bioenergetic stress responses [32] that might affect for its resistance. Therefore ibrutinib activity could be regulated by glucose level, and patients with hyperglycemia might

be resistant to ibrutinib treatment in TP53 deficient chronic lymphocytic leukemia (CLL) lymphocytes [28].

Idelalisib is an inhibitor of the delta isoform of the phosphatidylinositol 3-kinase (PI3K). Drug could be active on cell proliferation, survival, or even induction of apoptosis. Moreover the strong heterogeneity of CLL feature and involvement in PI3K signaling could be a reason of differences between patients in clinical development of CLL and diversities in response to therapy. Idelalisib could induce several possible side effects, including hepatotoxicity, diarrhea, colitis, pneumonitis, or intestinal perforation. A special importance for personalize therapy is a chance to avoid resistance to treatment and reduce the development of secondary cancer (melanoma, head and neck, prostate, breast, or lung) [16].

Drugs usually for some patients lead to disease remission that could last for many years. It is also possible that for the group of patients, drugs could be ineffective and cause resistance to therapy [31]. For CLL, drugs based on higher generations of monoclonal antibodies could usually cause cytotoxicity in B-cells and fast reductions in the number of B lymphocytes from peripheral blood, decreasing patient's immunological strength. To increase drug activity, the combined therapy based on few drugs could in theory increase patients' response to therapy. Based on our experience with leukemic cell incubations with anticancer drug(s), sometimes it does work this way; for other patients, for example, the addition of second drug will not change cell response to anticancer agents (**Figure 1**). It could prove that sometimes one drug will be enough instead of combined therapy that will not be more effective, will cause patient's weakness, and will not improve the final effect of treatment. For the group of patients, who do not react at standard way to anticancer drugs or are resistant to treatment, choosing the optimal way of treatment for patient seems to be very important for his curing.

There are several molecules, for example, generated from encoded sequences of genes (pre-mRNA), characterized as miRNA, that could affect gene expression, mainly by their silencing, and could decrease or block protein expression. Because of a high complexity of metabolic reactions and several metabolic pathways included inside the cells that could be activated by drugs, hormonal regulation of human body metabolism and several different factors, previously taken medicaments or even diet, could affect patient's response to anticancer treatment [19, 21, 23, 32, 33]. Several potential targets for anticancer agents met in the human body are a reason of drug's side effect. Moreover, some familiar or personal diversities could also change patient reactivity to drugs. Drug reactivity could also be affected by environmental factors or even patient's diet.

At the moment it is better to analyze the total effect of drug activity on leukemic cells before drug administration to patient to avoid potential resistance to treatment.

2. Conclusion

The best way to prevent diseases is a prophylactic. The well-balanced healthy diet, physical exercises, supplementation of probiotic bacteria, or screening tests showing our health condition are the best way to prevent diseases and increase life expectancy. We have to start thinking about our health since childhood, and several wrong decisions could shorten our life. Because of a high complexity in cells and human body function and not enough knowledge related to disease etiology, currently it is better to analyze in vitro the apoptosis induction potential of leukemic cells incubated for 48 h with anticancer drugs before drug administration to CLL patient to exclude resistance to treatment. The resistance to treatment is usually confirmed in CLL cells incubated for 48 h with anticancer drug(s).

While, after 24 h of cell incubation in vitro with anticancer drug(s), the necessity of dose modification usually for lower values in the case of CLL patients' more sensitive cells to drug(s) was noticed. It must be underlined that sometimes the addition of drug (rituximab) as presented in **Figure 1** does not change anything in CLL cell reactivity to drugs (CM and Rit20CM).

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