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Personalized therapy versus targeted therapy, differences in the meaning

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ABSTRACT

The diagnostics and treatment options for numerous cancers are improving and giving the opportunity for increase disease curing efficacy. Unfortunately, the etiology of cancers' transformation is still under investigations. Because of a high number of diverse factors involved in cellular signal trafficking, as well as several metabolic pathways and their potential simultaneous cross-reactivity, choosing the proper type of therapy based on expression of some common changes in marker profile characteristics for a particular type of cancer could not be fully informative, especially for some patients who are resistant to the treatment. There is no doubt that there is a need to transfer from trial-and-error medicine into directed and even more precise towards personalized medicine based on analysis of parameters for a single patient in vivo and in vitro for checking before administration of the potentially effective type of drug(s) to avoid application of ineffective therapy. In this review, we compare the differences between directed and personalized therapy based on our previous studies on chronic lymphocytic leukemia cells versus chronic myelocytic leukemia treatment analysis related to aberrant activity of Bcr-Abl kinase, which is an indication to tyrosine kinase inhibitor – Gleevec application.

KEYWORDS : Leukemias, personalized-therapy, cytotoxicity, apoptotic markers, differential scanning calorimetry

Introduction

The last decade has witnessed a substantial advance in diagnostics and treatment options for many cancers, as well as for various hematological malignancies. A large number of cancer treatments succeed as a result of highly precise diagnostics and therapy. There has been a spectacular increase in the number of clinical trials. For example, up to December 2014, over 1,000 records on clinical studies for B-cell malignancies were registered (www. clinicaltrials.gov). Moreover, the growing number of new anticancer agents in clinical trials reflects the extensive therapeutic options directed toward the increase of drug-treatment efficacy, and reduce the side effects of drugs. The cancer molecular profiling analysis on the human genome project compared genomic differences in the sequence of various genes. These studies were carried out in numerous laboratories around the world and finally confirmed a high DNA sequence compatibility between people.

Recently, there has been a progressive change in medical practice from traditional trial-and-error medicine, based on drug dosage related to a patient's weight, to targeted therapy, which is a step forward into personalized medicine. Cancer diagnostics have been modified by analysis of alterations in DNA of cancer cells and related to studies using microarray system for expression of genes related to cancerogenesis, cell cycle or apoptosis regulation. Personalized anti-cancer therapy is an evidence-based medicine directed to the individual patient that delivers the proper care to the cancer patient at the right time, resulting in spectacular improvement in outcomes, improving the patient's quality of life, and reducing health care expenses. The importance for personalized medicine underlines the right biomarker application.

It is generally accepted that cancer cells typically indicate an acquired ability to evade apoptosis process. We hope that in the near future using achievements based on different modern technologies, it might be possible to scan patient's unique parameters related to cancer development and monitor anticancer drug proapoptotic activity by evaluation of cancer cell viability using simple tests. Therefore, we would be able to tailor optimal therapy for cancers, or at least for some of them [1-4].

The tremendous scientific progress involving modern technologies and ideas has allowed for advances in cancer marker diagnostics, especially those appearing in serum, urine or tissue specimens.

The difference between targeted and personalized therapy

The diagnostics of cancers and subsequent treatment decisions in clinical practice are usually based on the tests which are able to distinguish the level of specific biomarkers. At present, oncologists divide these biomarkers into those with predictive, prognostic, and early response activity [5].

A good example for targeted therapy is a monoclonal antibody - Herceptin application for Her2 positive breast cancer patients, as well as tyrosine kinase inhibitors when mutation in the EGFR gene occurs [1]. In 2007 it was reported by the American Society of Clinical Oncology that in a group of 31 breast cancer patients who received the tyrosine kinase inhibitor - Iressa (Astra Zeneca), 71% of patients survived 12 months, in comparison to 15% of those who were administered with traditional chemotherapy regimens. There is no doubt that for these 31 patients with breast cancer and mutated *EGFR* status, Irresa was more effective in the majority of cases (22 patients having a positive reaction; 71%) and more effective than traditionally used regimens [1].

Moreover, mutation in *EGFR* could be a reason of gefitinib unsuccessful activity for patients with non-small-cell lung cancer [6]. For increased probability of therapeutic success in cancer, searching for gene alterations involved in carcinogenesis are in progress [7-9]. However, some patients did not respond to anticancer therapy. There is no doubt that analysis of mutation status of genes involved in cancer development increases the probability of therapeutic success for patient. The other example of directed therapy is panitumumab used in colorectal cancer treatment for patients with *Kras* mutation [9]. However, for other reasons which we do not know, sometimes, for heavy pretreated or patients who are resistant to therapy, personalized therapy protocols based on anticancer drug efficacy *in vitro* monitoring should be introduced.

It must be underlined that before and/or during personalized therapy procedure, the analysis should be focused on each patient for whom treatment is developed. The broad spectrum of assays should be done separately for choosing the most effective type of treatment.

The clinical results of randomized multicenter trials of 229 previously untreated chronic lymphocytic leukemia (CLL) patients cured with chlorambucil (12 mg/m^2/day, 7 days), combined with prednisone (30 mg/m^2/day, 7 days) or cladribine (2-CdA; 0.12 mg/kg), or in com-

bination with prednisone, revealed the differences in the patients' response to therapy [10]. The overall response (OR) of patients who received cladribine+prednisone, versus chlorambucil+prednisone was 87 vs 57 ($p<0.001$), respectively.

In the other study, the efficacy and toxicity of cladribine combined with cyclophosphamide, among 20 CLL patients with 17p13.1/P53 deletion, were compared. Interestingly, 80% of the above patients yielded a significant overall response rate. A complete response (CR) was achieved by 50%, and a partial response (PR) by 30% [11].

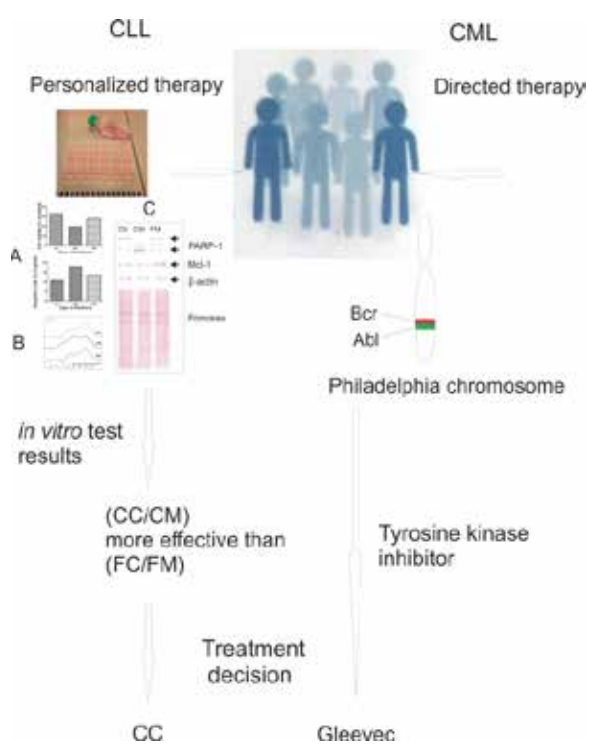
The goal of this report is to compare the differences between targeted and personalized therapy as new treatment approaches in CLL and other leukemia therapeutic options. In both types of therapy trials, it is important to use such drug(s) as monoclonal antibodies, tyrosine kinase inhibitors, and proteasome inhibitors, which attack the cells involved in carcinogenesis and are less harmful to normal ones [12]. Actually, a large number of new agents reflected anticancer potency are under clinical or preclinical investigations [13-15]. Some new therapeutic approaches could in the future improve the opportunity for oncologic curing of patients. We hope that in cases reflected anticancer resistance *in vitro* in the future searching for effective anticancer treatment with a high impact of reactivity towards common cancer cells will be a standard procedure. Emerging personalized therapy points towards a total anticancer effect provides prognosis of drug efficacy for a single patient [4, 7, 13, 14, 16-23]. In personalized therapy, the optimal type of treatment should be chosen by applying the prognostic and predictive biomarkers, supplemented by the *in vitro* exposure for the patient's cancer cells to the planned drugs, to confirm their cytotoxicity and proapoptotic potential before patients' administration *in vivo*. Moreover, the activity of drugs against cancer cells by the evaluation of apoptosis induction potential should be evaluated before the treatment as well during drug administration *in vivo* [21, 22].

For several years, the chemotherapy standards have been evolving towards the search for new anticancer agents directed for modified polypeptides, expressed specially in different tumor types, which are involved in cellular signal trafficking (eg. tyrosine kinase inhibitors). In this fashion the genetic alterations could be helpful to identify which group of patients are likely to benefit from this kind of treatment. The rapid scientific progress provides the new comprehensive technologies to analyze alterations in gene expression. Such approaches allow improvement in clinical response to the drugs used, but it will not reduce resistance level to anticancer agents [23]. The changes in patient responses, eg. the mutations in kinase *BRAF* appeared in over 50% melanomas, in *EGFR* in about 15% of non-small lung cancers (NSCLC), and *ERBB2* - 15-20% breast cancers were reported. These alterations could result in aberrant protein biosynthesis which disturb cellular signal trafficking and predestine the importance in cancer cell survival. The search for molecules which could harbor or inhibit target molecules important in cellular signal transduction was an idea to increase clinical therapy response. Several small molecular agents with tyrosine kinase inhibitor activities were introduced to induce responses in cancers with particular mutations (for example melanomas with *BRAF* mutation), deletion/insertion in *EGFR*, amplification of *ERBB2*. Technological advances allow for the application of knowledge gained from the human genome project for development of several tests, for instance – microarrays, which could be helpful in expression of genes involved in cell metabolism and maintenance. The age of microarrays starts in clinics, and could improve patients' diagnostics, but because of several personal differences between patients, this idea will not be fully successful for all patients' anticancer treatment efficacy prediction. The differences in protein expression/modifications in cellular networking, which we potentially do not know at the moment, could make this aspect more complicated.

In standardization of clinical diagnostics of cancers, the modern version of tests engaged the changes in DNA, mRNA or protein sequence as mainly used microarrays with microchip technology allowing us to analyse expression of genes involved in carcinogenesis, cell cycle or apoptosis. It must be underlined that targeted therapy is usually chosen as the result of gene mutation study or expression of gene on mRNA or protein level [16, 24]. These alterations could imply for important signaling cross-talk in signalization pathways.

Why is targeted therapy not effective for some patients?

A high compatibility in DNA code between individuals reported by the Human Genome Project opens new options for searching the differences in DNA sequences or even changes in genetic material remodeling that could imply cell signaling or metabolism. In several cancers, gene polymorphisms were found [25-27]. These molecular diversities would display the straight dependence into the elevation of susceptibility to particular type of cancer or could be associated with disease progression or unfavorable disease prognosis. Moreover, in some cancers, for instance the mutations in *BRCA1* and *BRCA2* in breast cancer [28], the disturbance or deletion on 17p [24, 29] could be translated on cancer development/aggressiveness or prognosis. We still do not know why the majority of CLL patients with unfavorable deletion of 17p or mutations of *P53* display reduced susceptibility to chemotherapy [24]. The reasons for patients' differing reactions to treatment might be personal differences in the response to applied drugs, even when chemotherapy is combined with immunotherapy [30]. The personal diversities in the expression of proteins involved in cellular metabolism, signal transduction paths, drug metabolism, or high expression level of proteins related to antiapoptotic functions, reflect a cell response to therapy. The strong impact on the response could be associated with personal differences in a proportion of some events, eg. the properties of natural killer (NK) or dendritic cells, important in immunological interactions. We do not know why, with a high compatibility of DNA sequence between patients, people respond so diversely to the therapy. Several variances in genetic information reading paths, related to the coding or uncoding parts of DNA, crossing over, as well as familiar predispositions to epigenetically-related conformational changes in regulation of gene expression, could be the reason for patients' different responses to anticancer therapy on cellular level. On the other hand, the special character of DNA conformation could also affect patients' disease development. Because of some rare sequence or conformational alterations of genetic material observed in a small group of patients, even prospective studies on the mutational profile carried out for numerous cancers could not be fully successful. Therefore, for several reasons the way of patient perception ought to be transferred from the present manner, based on "trial and error medicine" or even analysis of known mutation of genes based on patients' diagnostics according to standards accepted for the treatment of a particular type of disease, into personalized medicine which in case of necessity directs attention for patient diagnostics towards choosing potentially effective treatment for individual patients. A good example for directed targeted therapy is the appearance of Philadelphia chromosome in most cases of chronic myelocytic leukemia (CML) which is an indication for tyrosine kinase inhibitor – Gleevec application. Gleevec (Imatinib; STI571) blocks the ATP-dependent domain of Bcr/Abl tyrosine kinase [12, 31]. However, there is well-estimated data of Gleevec resistance requirement among the patients cured with it [29]. In Figure 1 the example displaying the differences between directed and personalized therapy, i.e. CLL and CML is given, respectively.



In personalized therapy fashion, the scheme of procedure is suggested. CLL

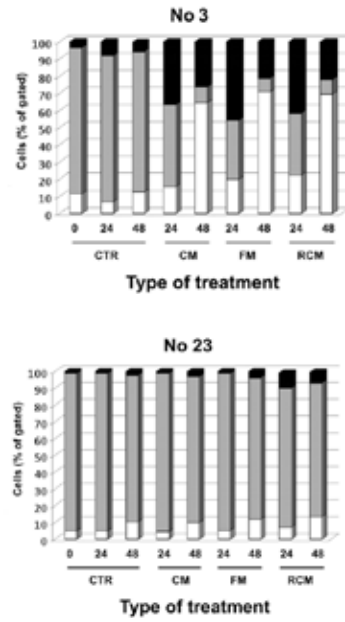
cells should be incubated with drug(s), for example CM (cladribine+mafosfamide; mafosfamide is *in vitro* active form of cyclophosphamide) or FM (fludarabibe+mafosfamide) for estimation of drug toxicity and proapoptotic potential, before their in vivo application, to avoid administration of ineffective drug(s) (Fig.1). Taking into account combined *in vivo* and *in vitro* results on CLL treatment efficacy by combined purine analogs with cyclophosphamide/mafosfamide, the *in vitro* evaluation of drug potency against CLL cells before drug administration reflects special importance [1, 4, 18, 19, 22]. The *in vitro* results of complementary tests, i.e. cytotoxicity, apoptosis rate, differential scanning calorimetry (DSC) and apoptosis-related protein expression in CLL cells exposed to the above drug combinations, seem to indicate their predictive anti-leukemic potential (see Fig.1, left). The data obtained revealed that for an exemplary patient, his leukemic cells exposed to cladribine + mafosfamide (CM), responded by more effective cell elimination, intensive PARP-1 cleavage and DSC profile change at 95 ± 5^0C in comparison with the cell samples treated with FM or control cells (Fig.1, left). Importantly, this patient after 6 cycles of chemotherapy based on cladribine +cyclophosphamide (CC) reached complete remission (CR).

The experimental data suggest that a small, or lack of sensitivity *in vitro* of CLL cells to the planned drugs, should lead researchers to search for other agents which are more effective in the eradication of leukemic cells by apoptosis. In Fig. 1 (right) the basis of applied targeted therapy in patients with CML is shown. The molecular target in this case is tyrosine kinase Bcr/Abl - the fusion protein coded by gene *Bcr/Abl* located on Philadelphia chromosome, which appeared in about 95% of CML patients and some of the patients with acute lymphocytic leukemia (ALL) [31, 32]. The Bcr/Abl kinase stimulates multiple signaling pathways responsible for protection from apoptosis transformation, and also for the resistance to therapy. It was stated that Gleevec blocks Bcr/Abl kinase activity with significant efficacy. However, it has also been reported that as a single drug may not be sufficient to eliminate leukemic stem cells [33]. Extensive research based on the second generation of new tyrosine kinase inhibitors (eg. dasatinib) or combination of tyrosine kinase inhibitors with special microenvironment condition was developed [32, 33]. In this context anti-leukemic therapeutic option therapy with supplementation of microenvironment – an important barrier towards effective eradication of leukemic cells should be undertaken, and such studies are in progress [33].

The comparative results of anti-leukemic drug potential based on analysis of CLL cell viability, apoptosis-rate, changes in DSC profile, and expression/proteolysis of apoptotic marker polymerase PARP-1 could be helpful in choosing effective anticancer treatment before its application to patient [Fig. 1 left]. The standardization of the above techniques was previously published [4, 18, 19, 22].

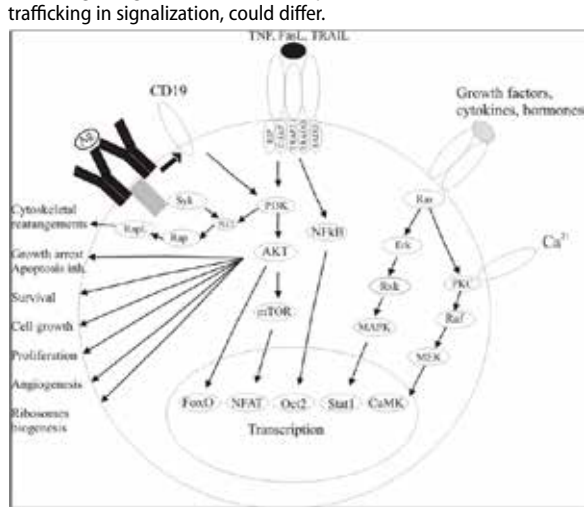
DSC is a relatively simple technique, which could evaluate and compare the changes in chromatin conformation of cell nuclei obtained from peripheral PBMCs from the blood of healthy individuals and CLL patients incubated with anticancer agents. In this matter the conformation of chromatin could reveal different condensation status in control nuclear samples and those treated with drugs. This technique allows for analysis of heat induced conformational changes of nuclear compounds i.e. thermodynamic parameters of constrained or relaxed DNA, protein-nucleic acid interactions and structures scaffolding [34-36]. In the majority of CLL patients in advanced stage of disease in nuclear preparations, the additional transition at about 93 °C was observed [36]. It must be underlined that in the cells sensitive to anticancer drug(s), this transition usually decreased or even disappeared after 48h of cell exposure to drugs (see Fig. 1 CLL panel B). The results presented for the example patient show that PBMCs displayed potential fludarabine + cyclophosphamide resistance *in vitro*. Interestingly, the clinical response of this patient to *in vivo* treatment obtained after 6 courses of therapy also confirmed patient resistance to fludarabine + cyclophosphamide *in vivo* therapy [22, 36]. The compilation of applied techniques (cell viability test/apoptosis rate; Fig. 1A), changes in DSC thermal profiles (Fig. 1B), apoptosis- related protein expression analysis (PARP, Mcl-1; Fig. 1C) could be helpful in predicting the potentially active drug treatment for the patient, which could be helpful in reducing ineffective patient therapy [23]. The published data confirms the high importance of such tests, because of potential resistance of leukemic cells, for example to fludarabine to further courses with other purine analogs [37].

Importantly, during CLL cell exposure to anticancer drug combinations i.e. CM and FM, or RCM (immunochemotherapy - rituximab+CM), it is possible to monitor the sensitivity of model cells to the drugs used. In Fig. 2 the results of two example patients' cell viability with opposite cell responses are shown. PBMCs cells from the blood of patient No 23 after drug exposure responded very weakly to drugs *in vitro* and also he did not respond to cladribine+cyclophosphamide (CC) *in vivo*. While PBMCs samples from the blood of patient No 3, who reached complete response (CR) *in vivo* to CC administration were characterized by a significant drop of cell viability, as well as necrotic and apoptotic cell levels (Fig. 2).



It seems that estimation *in vitro* of individual CLL sample sensitivity to drug(s) may facilitate the choice of CLL treatment to avoid unwanted inflammation and better therapy effects. Therefore, for some CLL patients' leukemic cells, especially to those characterized by a high sensitivity to *in vitro* culture condition [38, 39], the comparative analysis directed towards special dose choosing, to avoid necrosis or *in vivo* unwanted inflammation to applied therapy should be recommended.

In Fig. 3 the compilation of signaling pathways in leukemic cell is illustrated. Signaling cascade, because of personal diversities in molecules trafficking in signalization, could differ.



As shown in Fig.3. cell signal trafficking has some common parts (eg. PKB/kinase Akt) that are involved in many processes, i.e. cell survival and growth, proliferation and angiogenesis, as well as growth arrest or apoptosis inhibition. Therefore, overexpression of Akt is observed in many types of cancers. The other example oncogenic protein - Ras involvement in genes transcription (signal transducer and activator of transcription 1; Stat1, or calmodulin modulate kinase CaMK). The involvement of several signal ways found in

cells makes also signal cross-talk more complicated and could be a reason for diverse response to therapy. Moreover, there are not the same proteins involved in signaling pathways and thus personalized medicine for patients is needed. Moreover, missing or changes in expression of some factors important in signaling trafficking could be a cause of personal differences between humans. The differences may have an impact on cellular pathways and influence on responses to anticancer treatment efficacy *in vitro* or *in vivo*. Very recent publications confirm the involvement of microRNAs in several cellular functions, also in cellular potential resistance for anticancer treatment [40-42]. It is highly probable that some new factors which are currently unknown might be important in cellular trafficking. Because of that, projected models of test based on genetic alteration assays (directed to particular pathways) [24, 42], comparing the differences in DNA sequence, as well as on mRNA level could not be always valuable as diagnostic tests, because of epigenetic modifications [43].

Barriers in personalized therapy

A few years ago M.G. Aspinall and R.C. Hamermesh [1] paid attention to the barriers that obstruct the transition from trial-and-error medicine to personalized therapy in the United States and also in other countries. One of them is the **pharmaceutical industry's** model which could fear personalized medicine. The pharmaceutical concerns have a particular interest in large scale drug sales, instead of small profits when the drug(s) and clinical trials of new substances but too small for evaluation of its efficacy and safety. The next barrier represents a **regulatory environment** connected with too long period to be devoted on administrative and clinical trials of new substances but too small for evaluation of its efficacy and safety.

The other one is the strange **dysfunctional payment** system which rewards physicians mainly for administrative activity (taking care of procedures, issuing prescriptions). Finally, the other big problem is to change **physicians' behavior** to use old standard type of treatment, instead of individual patient approaches, taking into account of unfavorable diagnostic markers, potentially related to drug resistance.

Concluding remarks

For many types of cancers, there are numerous studies concerning mainly targeted cancer therapy [8, 32]. Some of the targeted applications directed towards alterations in gene expression studied on DNA, RNA or protein level are under investigations in numerous cancers [2, 43, 44]. The changes in health system directed towards single patients are needed. Patients ought to be placed at the centre of attention and for health and economic reasons, cancer cells' anticancer potency of drug(s) should be tested. Moreover, the personalized therapy reflects the special importance for the subset of patients resistant to commonly used therapy options, as well as for heavily pretreated or weaker patients. The approaches involved in personalized medicine may well play a part in avoiding months of ineffective treatment. In this regard, our preliminary results suggest that the tests used for choosing optimal therapy for CLL patients could be also useful in anticancer therapy efficacy monitoring.

It must be underlined that changes in the health system are needed and could provide reductions (when therapy is successful) in patient's hospital time and overall costs. It is also very important that it should have a positive effect on the treatment of non-cancer patients too. The differences between directed and personalized therapy are debatable and are usually interpreted diversely by specialists in different fields.

Legend to figures

Fig.1. Directed versus personalized therapy of CLL and CML. The presence of Philadelphia chromosome, a marker for CML – indication for Gleevec application (targeted therapy). For estimation of patient's response to therapy the leukemic cells were incubated with drugs for 48h. After cell incubation their anti-leukemic potency was estimated by determination of cell viability, apoptosis rate, DSC profiling and expression of PARP-1 and Mcl-1 (C). Polimerase PARP-1 cleavage is a known apoptotic marker.

Fig. 2. Cell viability/apoptosis or necrosis level from two patients with chronic lymphocytic leukemia (A and B) with various leukemic cell responses to anticancer drugs *in vitro*. The detail explanation is given in the text.

Fig.3. Cell trafficking in leukemic cell involved in cell survival, apoptosis, and signaling molecules important for cancer transformation.

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