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FROM THE EDITOR-IN-CHIEF

Distinguished authors and readers of our medical journal! I am proud to say that over the course of 18 years of our presence we have managed to form our own audience — scientists, doctors and researchers. These people are truly passionate about tacking the cancer problems in the area of immunodiagnostics, immunotherapy with a focus on bone marrow, as well as its role in the emergence and progression of cancer.

As usual, we commemorate publication of our journal to the next conference to be held in Istanbul, October 5, 2022. The main topic is minimal residual disease in hematopoietic and lymphoid tumors, but surely not limited to it.

This issue of our journal was contributed by a substantial number of luminaries in oncology and oncohematology: such as Robert Gale, Dieter Hölzer, Vera Donnenberg, Jean-François Rossi, Vladimir Jurisic, the leading Russian oncologists, haematologists, transplantologists such as Kapitolina Melkova and Zhanna Sharoyan, Alexandra Palladina, Mary Shervashidze and Timur Valiyev. A reader will find a fascinating wave of opinions and insights into the possibilities to eradicate cancer.

In this particular issue, Mikhail Davydov takes a critical look at different approaches (including cancer surgery, to which he has devoted more than 50 years) considering it from the perspective of cancer eradication.

Dear friends! We are looking forward to meet you at the conference and do hope that materials published in this journal will be beneficial in practical sense, as well as in terms of igniting the fruitful discussions targeted to defeat cancer.

Editor-in-chief,
Head of the Hematopoiesis Immunology Lab
Federal State Budgetary Institute "N.N. Blokhin National Medical Research Centre for Oncology" of the Ministry of Health of Russia
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CANCER. POSSIBLE WAYS TO TACKLE

M. Davydov

Our previous paper "There is no cancer immunology" published 5 years ago, laid out the reasons why it makes no sense to rely on the body's immune system in a cancer patient.

The main therapy for cancer patients in the early disease stages is surgery.

Having worked as a surgical oncologist for over 50 years who has performed tens of thousands of most complex operations, I have come to the disappointing conclusion: surgery is not at all a cure for cancer. Sure, there can be exceptions. Of course, surgery can save lives for millions of patients. But it's not always the case that cancer can be cured by surgery. After all, tumors are dark-minded: individual seedings, micro-metastases in various organs, especially in the bone marrow, simply cannot be removed surgically. And they can reveal themselves many years later — 10, 20 years or even more — and cause death of the patient.

But there is a chemotherapy available, you might say. Yes, the success of chemotherapy is difficult to overestimate. There have been great success stories in controlling metastases and extending lives of the patients. The problems are the following: on one hand, chemotherapy is a non-selective method of treating the tumor and therefore we observe multiple complications, sometimes very serious, up to the fatal cases; on the other hand, chemotherapeutic agents may not be effective on minimal cancers due to the simple reason that disseminated tumor cells are generally dormant and non-sensitive to chemotherapeutic effects. Having detected a few disseminated tumor cells in the patient's bone marrow, we can destroy the entire hematopoiesis, while the tumor cells will remain alive and, under certain conditions, can become awaken and metastasize to the brain or other vital organs. The so-called target therapy represents an undeniable step forward. This is a therapy that targets specific target structures in the tumor cells. The drawbacks of this approach are associated with the fact that as a rule, these targets are not strictly specific for tumor cells and, moreover, are not present in some of the tumor cells. Nevertheless, it is certainly an important step towards development of the selective therapy enabling to overcome resistance of dormant cancer cells.

Today's immunotherapy offers the broad-based range of tumor treatments. From attempts to target the tumor in a specific manner to all kinds of methods meant to overcome tumor's resistance towards immune rejection. This approach has become the most prevalent nowadays and is based on formation of monoclonal antibodies blocking the checkpoints of immune response resistance. There are success stories, but much is still to be proven; there is no specificity of effect. There are no clear indications to prescribe relevant drugs and prevent complications, either. Perhaps,

the most importantly, there are neither rationales, nor evidences for feasibility to eradicate cancer by the checkpoint inhibitors.

All these arguments speak to the point that currently in the world there is no single program or system of approaches to eradicate cancer. The reason being is that once a cancer cell manages to evade the body's defense powers, it begins to develop according to its own rules of life, changing its phenotype, preferences and priorities. To catch it becomes increasingly difficult, sometimes purely impossible.

Sounds like a quite pessimistic story. But there is beacon of hope. We just need to turn to the natural defense powers against cancer, the very defense that exists in a human body which makes it very rare for the tumors to succeed in their development compared to the incidence of potentially cancerous cells.

It is not my intention to discuss such defense mechanisms here. There are multiple papers and reviews devoted to it. In a nutshell, it means lipo-apoptosis, i.e. death of tumor cells, so to speak, caused by obesity, which is nothing but accumulation of lipids in cytoplasm in the amount that is fatal. And the lipids can be delivered there by the natural pentameric IgM specific to the tumor-associated glycans (sugars).

The immune response to these sugars declines over the years and weakens under the influence of carcinogens. Antibodies stop operating as protectors and miss out the cancer cells, failing to kill them. The real challenge is to correct the relevant immunodeficiencies and find ways to either stimulate the innate humoral anti-tumor immunity or replace it with full-scale specific antibodies. This is the main idea behind the pathogenetic immunoprophylaxis of cancer.

August, 2022
Academician M. Davydov

WILL IMMUNE THERAPY REPLACE HAEMATOPOIETIC CELL TRANSPLANTS?

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ABSTRACT

There are important advances in the use of immune therapy to treat haematological cancers including monoclonal antibodies and cell therapies. Almost all successful immune therapies are in B-cell cancers including acute lymphoblastic leukaemia (ALL), lymphomas and plasma cell myeloma (PCM). Immune therapy of myeloid cancers including acute and chronic myeloid leukaemias (AML and CML) and myelo-proliferative neoplasms (MPNs) is less successful and there are no approved immune therapies for these cancers. (An exception is gemtuzumab in AML but which is rarely used). In some lymphomas immune therapy may replace autotransplants as initial therapy of chemotherapy failures. Sometimes, immune therapy is combined with haematopoietic cell transplants. How to best use or combine these therapies is unknown.

Introduction

Hematopoietic cells transplants including auto- and allotransplants are used to treat several haematological cancers including acute leukaemias (acute lympho-

blastic [ALL] and acute myeloid leukaemia [AML]), diverse lymphomas (diffuse large B-cell lymphoma [DLBCL], Hodgkin lymphoma [HL], mantle cell lymphoma [MCL] and follicular lymphoma [FL]) and plasma cells myeloma (PCM). Table 1 displays annual US transplants for these cancers and Table 2, an analysis by transplant type. Immune therapies including diverse monoclonal antibody constructs and cells (T- and natural-killer [NK]-cells) are also used to treat haematological cancers almost all of which are B-cell cancers including ALL, lymphomas and PCM. This raises the question whether immune therapy will replace haematopoietic cell transplants in some or all of these cancers, whether different therapies will be used in different cancers, persons and/or disease states or whether they will be combined.

Table 1.
Annual transplant rates in the US

PCM	8,000
AML	3,000
NHL	2,500
MDS/MPN	2,000
ALL	1,500

Table 2.
Types and numbers of transplants

	Allotransplants	**Autotransplants**
AML	3,000	–
MDS/MPN	2,000	–
ALL	1,500	–
NHL	500	2,500
HD	–	1,000
PCM	–	8,000

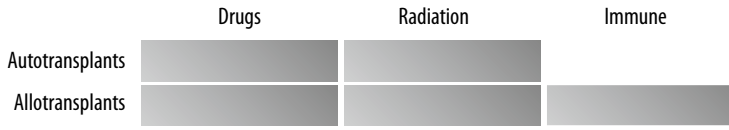

Fig. 1. Modes of action of auto- and allotransplants

Transplants

Different types of transplants operate by different mechanisms displayed in Fig. 1. The important point is immune therapy is an immune-mediated anti-cancer effect operates in allo- but not autotransplants. In this regard the distinction between allotransplants and immune therapy is semantic. Whether in the setting of allotransplants there is an anti-cancer effect distinct from graft-*versus*-host disease (GvHD) is controversial and unproved.

Immune therapy

Current types of immune therapies are displayed in Table 3. Most current interest is in various types of monoclonal antibodies and chimeric antigen receptor (CAR)-T-cells. Almost all of these therapies are directed against B-cell lineage specific antigens such as CD19, CD20 and B-cell maturation antigen (BCMA). Importantly, none target a cancer specific antigen.

Table 3.
Types of Immune therapies

Monoclonal antibodies
Vaccination
Donor lymphocyte infusions (antigen specific)
Checkpoint inhibitors
Innate immune cells
T-cell re-directing antibodies
Gene-modified T-cells
CAR-T-cells
TCR gene-modified T-cells

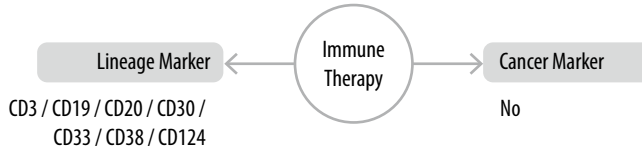

Fig. 2. Targets of immune therapy

Lineage- *versus* cancer-specific targets

The distinction between targeting a lineage-specific *versus* a cancer-specific antigen is central to understanding the safety and efficacy of immune therapy in B-cell *versus* myeloid cancers (Fig. 2). Humans can survive destruction of normal B-cells but not of myeloid cells which is predominately why successful immune therapies are directed against lineage-specific B-cell antigens. T-cell antigen targets are also problematic for immune therapy as these interventions would eliminate effector cells.

Some results of immune therapy

There are several examples of successful immune therapy of haematological cancers such as an advantage of blinatumomab over chemotherapy in adults with advanced ALL, CAR-T-cells in children with advanced ALL and CAR-T-cells in advanced DLBCL[1,2]. However, in most instances in advanced cancers there is ultimately a high failure rate despite a potential advantage over conventional therapies.

Which therapy is better?

Table 4 displays cancers where transplants and immune therapies are available and Fig. 3, mechanisms of action. Notably, none of these modalities is cancer-specific. As discussed above, discordances reflect primarily with B-cell *versus* myeloid cancers. There are few randomized clinical trials comparing autotransplants to immune therapy, mostly in DLBCL in persons failing soon after initial chemotherapy. 3 trials addressed this question (reviewed in 3). Two showed an advantage for CAR-T-cells and 1 a disadvantage. This discordance likely reflects subject selection biases, different protocol structures, different CAR-T-cell efficacies and other known and unknown co-variates. Other uncontrolled data suggest an advantage for CAR-T-cells over conventional therapy in young persons with advanced FL[4] .

Table 4.
Comparison of transplants and cancers where there are immune therapies

	Transplants	Immune Therapy
PCM	8,000	Some
AML	3,000	No
NHL	2,500	Yes
MDS/MPN	2,000	No
ALL	1,500	Yes

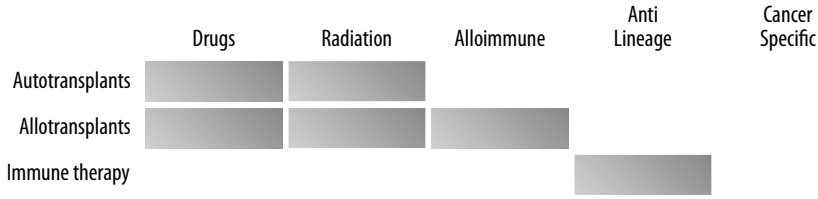

Fig. 3. Modes of action of transplants *versus* immune therapy

Is immune therapy a bridge-to-transplant?

This notion immune therapy is a bridge-to-transplant is, to a large extent, based on the unproved notion someone must in in complete remission before receiving a transplant. Although people in complete remission have at transplant have better outcomes compared with those not in complete remission this likely reflects a selection bias. Namely, persons achieving a complete remission with r immune therapy likely have more responsive cancers compared with those not responding. Furthermore, there are no convincing data the magnitude of benefit conferred by a transplant in persons transplanted in complete remission exceeds the benefit of those transplanted not in complete remission. These concepts are often mis-understood.

Conclusion

Immune therapy and transplants (a form of immune therapy) have roles in treating haematological cancers. Most of the competition and controversy relates to B-cell

lymphomas and PCM. There is no convincing role for immune therapy of myeloid cancers such as AML, CML or MPNs. The major advantage of immune therapy is avoidance of adverse effects from high-dose drugs and radiations and acute and chronic GvHD. However, some immune therapies also have adverse effects cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS).

A fundamental limitation of both therapies is a high rate of cancer recurrence. As such it's perhaps wise to consider where these therapies are best combined. An example is combining blinatumomab with an allotransplant in children with ALL5. More studies of combination therapy, especially with CAR-T-cells and transplants are needed.

Remaining questions about the role of immune therapy and transplant are summarized in Table 5, There is great progress in developing safe and effective immune therapies of haematological cancers. As with any new therapy the challenges is optimizing integration into current therapies. This will take time. Presently, it is more likely immune therapies will complement rather than replace transplants.

Table 5.
Remaining Questions

Who should receive which therapy and when?
Immune therapy for transplant failures?
Transplants for immune therapy successes, failures or both?
Can we accurately predict who should receive either therapy or both?
Can someone not a transplant candidate receive immune therapy and the reverse?
Will results of immune therapy and of transplants improve further?
Can we have the best of both therapies?

Conflict of interest. RPG is a consultant to: NexImmune Inc., Annexa Pharma, Ascentage Pharm Group, Antengene Biotech LLC. Medical Director: FFF Enterprises Inc. Partner: AZAC Inc. Board of Directors: Russian Foundation for Cancer Research Support; Scientific Advisory Board: StemRad Ltd, Nanexa AB.

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References

1. Kantarjian H., Stein A., Gökbuget N., et al. Blinatumomab versus Chemotherapy for Advanced Acute Lymphoblastic Leukemia. N Engl J Med. 2017;

376(9): 836–47. doi: 10.1056/NEJMoa1609783. PMID: 28249141; PMCID: PMC5881572.

2. Nastoupil L.J., Jain M.D., Feng L., et al. Standard-of-Care Axicabtagene Ciloleucel for Relapsed or Refractory Large B-Cell Lymphoma: Results From the US Lymphoma CAR T Consortium. J Clin Oncol. 2020; 38(27): 3119–28. doi: 10.1200/JCO.19.02104. Epub 2020 May 13. PMID: 32401634; PMCID: PMC7499611.
3. Roschewski M., Longo D.L., Wilson W.H. CAR T-Cell Therapy for Large B-Cell Lymphoma — Who, When, and How? N Engl J Med. 2022; 386(7): 692–6.
4. Jacobson C.A., Chavez J.C., Sehgal AR, et al. Axicabtagene ciloleucel in relapsed or refractory indolent non-Hodgkin lymphoma (ZUMA-5): a single-arm, multicentre, phase 2 trial. Lancet Oncol. 2022; 23(1): 91–103. doi: 10.1016/S1470-2045(21)00591-X. Epub 2021 Dec 8. PMID: 34895487.
5. Locatelli F., Zugmaier G., Rizzari C., et al. Effect of Blinatumomab vs Chemotherapy on Event-Free Survival Among Children With High-risk First-Relapse B-Cell Acute Lymphoblastic Leukemia: A Randomized Clinical Trial. JAMA. 2021 Mar 2; 325(9): 843–54. doi: 10.1001 / jama.2021.0987. PMID: 33651091; PMCID: PMC7926287.

HOW THE EVALUATION OF RESIDUAL DISEASE MAY INFLUENCE A MEDICAL DECISION IN HEMATOLOGICAL MALIGNANCIES

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Running title: Clinical impact of minimal residual disease evaluation

ABSTRACT

Currently, due to extraordinary advances in biology and therapeutics, achieving maximal tumor mass reduction has become the clinical goal, corresponding to negative minimal residual disease (MRD) correlated to survival in hematological malignancies. MRD monitoring is highly recommended at different times of the therapy, not only as strong independent post-diagnosis prognostic factor, but also as the best tool to define risk-based therapeutic strategies. Several questions arise concerning the choice of treatments, according to the directed or adapted risk, the level of MRD impacting the survival, the eventual curability, the need for MRD control treatment (commonly called maintenance) or the modulation of the therapies according to the MRD kinetics and the best time to do it. The advent of multiplex analyses opens up new possibilities incorporating new combinations or sequencing of therapies. Real dynamic multifactorial personalized medicine is on the way with its new assisted decision criteria.

The concept of minimal residual diseases (MRD)

As mentioned by USA National Cancer Institute (NCI), MRD is “a term used to describe a very small number of cancer cells that remain in the body during or after treatment. Minimal residual disease can be found only by highly sensitive laboratory methods that are able to find one cancer cell among one million normal cells.

Checking to see if there is minimal residual disease may help plan treatment, find out how well treatment is working or if cancer has come back, or make a prognosis. Minimal residual disease testing is used mostly for blood cancers such as lymphoma and leukemia» [1].

The concept of residual disease is clearly linked to the evaluation of response in cancer. Till now, the question of whether there is a correlation between the depth of tumor mass reduction and the duration of response remained one of the key questions in oncology (Fig. 1). Historically, morphological complete remission which was represented by less than 5 % of blasts cells in the bone marrow observed by light microscopy has been considered of key importance in the long-term prognostication of patients with acute myeloid leukemia (AML) [2]. Since then, the extraordinary development of the technique allowed to evaluate residual disease deeper than morphological aspects. From the 1980s, patients in complete response (CR) after morphological analysis were declared in MRD if more sensitive technologies did not detect leukemia cells [3]. Since the 80s, any technique with a sensitivity beyond that of immunohistochemistry-assisted light micros-

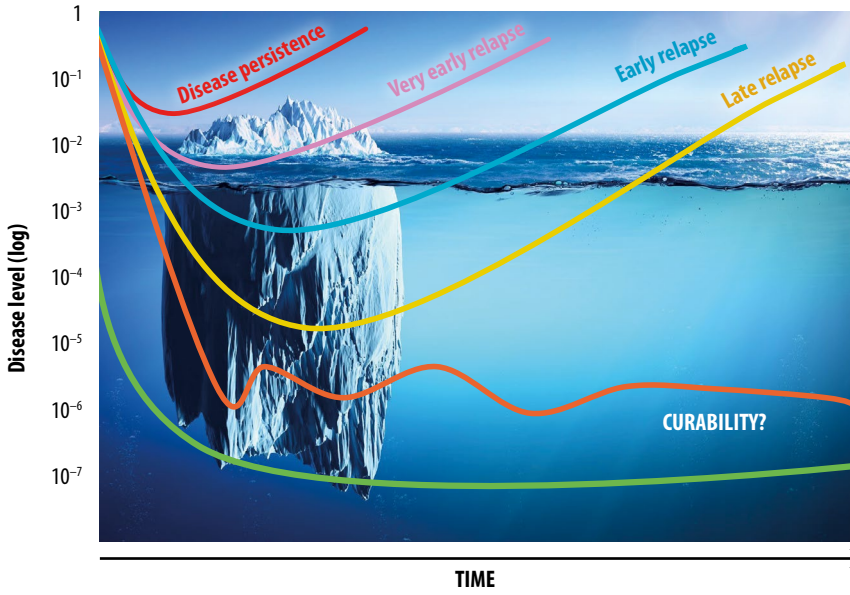

Fig. 1. Correlation of the level of residual disease (log) and the risk of relapse in hematological malignancies

copy can be used as an MRD detection technique in the traditional definition [4]. Initially, the notion of MRD was associated to the response analyses until the demonstration of its impact on prognosis was clearly observed, thus becoming a therapeutic decision guide. This dichotomy of response status was observed in the definition of response criteria in early 2000s [5]. The first demonstration of clinical impact of MRD analysis in targeted/precision medicine was observed in chronic myeloid leukemia (CML) with tyrosine kinase inhibitors (TKI) and *BCR/ABL* translocation monitoring [6].

Several questions arise, including the recognition of the cancer cells and their stem cell subpopulations by phenotypic analysis, the tumor features by genomic analysis, as well as the level of tumor mass detection and the risk of progression or relapse.

How to evaluate MRD

To recognize cancer cells

The quality of the biological tools used is defined by its ability to detect the smallest level of cancer cells or cancer-associated biological features. Evaluation of residual leukemic cells in the bone marrow is known to influence prognosis since several decades. This evaluation is clearly associated to the technique employed, from 10^{-2} by morphology and by fluorescence in situ hybridization (FISH), to 10^{-4} to 10^{-5} by multiparameter flow cytometry (MFC) and to 10^{-3} to 10^{-5} by next generation sequencing (NGS) [7]. The advantages of MFC are the wide applicability with single sample interpretable, the relative quick result obtention, the high specificity when a panel of markers could be used, with the possibility to detect leukemia stem-cell phenotype. Clearly, the sensitivity depends on the antibody panel used with the identification of leukemia-associated immune phenotypes (LAIPs) [8] that differ from most normal hematopoietic cells and the other approach entails identification of different-from-normal patterns [9], leading to a limited harmonization and standardization across laboratories. In addition, the leukemic phenotype is not necessarily stable over time and particularly after therapies. The main aberrancies are cross-lineage expression of antigens, antigen overexpression, lack of antigen expression, and asynchronous expression of antigens. The number of different LAIPs have reported to be very large and requires the use a large panel of biomarkers, with 8–12 color channels [10]. Aberrant patterns of differentiation are based on the recognition of LAIPs, particularly at diagnosis, with quantifiable aberrant cell populations using a standard fixed antibody panel to recognize leukemic cells based on their difference with normal hematopoietic cells at all stages of disease/treatment [8]. Comparatively to hema-

tological malignancies (HM), technological progress has made possible to detect rare cells by MFC in solid cancers, such as circulating tumoral cells (CTC), after their isolation by using biological methods, e.g., capturing them by Ep-CAM expression, or physical methods by microfiltration, density gradient centrifugation, electrophoresis, or any other methods [11]. Detection of such circulating residual cells has been correlated to prognosis and particularly to the risk of metastasis. Additionally, PDL-1 positive CTCs have been associated with shorter progression-free survival (PFS) [12].

To recognize genomic alterations associated to cancer

NGS is easily to realize with a good sensitivity, but with limited standardization of the technique. Some mutated genes can be detected in healthy people at low concentration (0–100 ng/mL, median ≈ 10) in different situations, notably increasing with age, i.e., clonal hematopoiesis of indeterminate potential (CHIP) which may represent a biological noise in such analysis [13]. The ratio between circulating tumor DNA (ctDNA) and the cell-free DNA (cfDNA) is variable in solid cancer as well as in HM, ranging from 0 to 90 %. CtDNA is quickly cleared by organs, including liver, kidney, spleen, leading to a short and variable half-life ranging from 16 min to 2.5 hours [14]. This effect has direct implications on the collection timing following cancer treatment when ctDNA is used as a surrogate biomarker of tumor reduction.

RT-qPCR could be used to evaluate MRD. It is associated with high sensitivity and well standardized technology. However, this technique has some limitations, including its expendability, its time-consuming and requiring high-level expertise. In addition, it requires the prior identification of molecular targets, generally observed in 30 % of elderly population to 50 % for a younger population and associated with possible clonal evolution. Thus, these techniques have better clarified the prognosis and make it possible to adapt the therapeutic strategy, in particular for a decision on allogenic stem cell transplantation in patients suffering from acute leukemias.

It is difficult to define a threshold correlated with the prognosis to classify the positivity or the negativity of the MRD, certain patients being in MRD+ and still in CR. These “false positivity and false negativity” could be explained by the sensitivity of the assay, the phenotypic shifts, the quality of the aspirate or the frequencies of leukemic stem cell including the identification of molecular event that are not clearly associated with leukemia but mostly observed in CHIP.

Moreover, the reappearance of AML is dependent on the molecular type with different kinetics described which can help in the therapeutic decision to control the recurrent disease if a preemptive treatment is not chosen [15].

Multiplexed analysis

After studying thousands or even millions of individual cells from cancer, it is now possible to obtain analysis at the single-cell level of resolution [16]. This high-dimensional, multi-faceted characterization of tumoral and/or associated immune/stromal cell characteristics allows for the dissection of cancer heterogeneity, micro-environment interactions, and details of the evolutionary trajectory of each tumor. Multiplexing analysis of immunohistochemistry has already demonstrated clinical interest in predicting response or resistance to immunotherapy [17]. Thus, different transcriptional programs of resistance have been defined, such as an increased expression of CDK4 with concomitant reductions in the expression of genes encoding proteins involved in antigen processing and presentation including *B2M, HLA-A, HLA-B* and *HLA-E*, interferon signaling or components of the complement system [17]. Single-cell analysis of T-cell receptor analysis enables the identification of paired α and β TCR subunits, which is a crucial step in determining the specificity of infiltrating T cells [18]. Combining single-cell RNA-sequencing (scRNA-seq) with single-cell TCR sequencing (scTCR-seq) links T cell phenotypes, such as activation, memory, and exhaustion, with the specific TCR clonotypes of individual T cells [19–21]. These high-dimensional transcriptomics and proteomics approaches for cancer profiling are currently available to understand cellular composition and intercellular interactions in cancer. The success of this multi-disciplinary approach requires coordinated teams to process samples and high-level of computational analysis [22].

Alternative biomarkers are being analyzed, such as miRNAs and mitochondrial DNA (mtDNA) mutations that have been described in human solid and hematological cancers and are in the monitoring of discontinuation of targeted therapy [23, 24].

Trogocytosis, to recognize immune cells in contact with cancer cells

Trogocytosis is the intercellular transfer of membrane and membrane-associated molecules with contact-dependent requiring actin cytoskeletal rearrangement. Trogocytosis is performed by multiple immune cell types, including basophils, macrophages, dendritic cells, neutrophils, natural killer cells, B cells, γδ T cells, and $CD4^+$ and $CD8^+$ αβ T cells. It has been described in a great variety of cells including other types of cellular interactions, such as microglial presynaptic remodeling [25] or stromal cell interactions with cancer cells [26]. Presentation by trogocytosis-positive effector $CD4^+$ T cells vs. trogocytosis-positive Treg results in differential regulation of CD4-mediated immune responses, to enhance or to reduce an immune response [27]. The presence of trogocytosed molecules has the potential to significantly impact the biological functions of cells, as observed on NK cells

with the trogocytosed CCR7 conferring to the cells some additional functions [28]. While performing trogocytosis, both Natural Killer (NK) and $CD8^+$ T cells acquire the checkpoint receptor PD-1 from leukemia cells, revealing the role of such mechanism in the immune regulation [29]. The presence of cancer-sculpted NK populations may be of great interest in defining or predicting different clinical situations in cancer with presence of CD45RARO when activated by HM [30, 31].

How undetectable MRD became a clinical goal and how MRD monitoring modulates risk- adapted or -directed therapies

Due to the correlation with survival, achieving an undetectable MRD represents the new clinical goal in HM.

In chronic myeloid leukemia (CML)

With its hallmark molecular event seen in the majority of patients, the prognosis of CML has been changed by using targeted therapy against TK. Furthermore, CML remains the first and the best example of piloting a therapeutic strategy on the kinetics of tumor reduction until a negative MRD is obtained. CML MRD monitoring is based on RNA, DNA and protein analysis. Detection of the *BCR-ABL1* transcript by the quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) is the reference method, but other systems based on digital PCR or on GeneXpert technology have been developed [23, 24]. RQ-PCR for *BCR-ABL1* mRNA reflects a composite of circulating leukemic cell count and the *BCR-ABL1* transcripts per cell. *BCR-ABL1* genomic DNA only reflects the number of leukemia cells [32]. In the first line imatinib TIDEL-II study, analysis of both mRNA and DNA *BCR-ABL1* showed the rapid decline of *BCR-ABL1* mRNA during the first three months of treatment, which was due to a reduction in cell number and transcript level per cell, whereas beyond three months, the fall in *BCR-ABL1* mRNA levels is proportional to the depletion of leukemic cells [33]. The level of MRD at 3-, 6- and 12-months has been shown to influence therapy with ITKs. Transcript levels greater than 9.84 % at 3 months were shown to have significantly lower 8-year overall survival (OS) probabilities (56.9 % v 93.3 %; $p < .001$), progression-free survival (PFS), cumulative incidence of cytogenetic complete response, and molecular complete response, than those with higher transcript levels [33]. Similarly, transcript levels of more than 1.67 % at 6 months and more than 0.53 % at 12 months identified high-risk patients. The transcript level observed at 3 months was the most predictive for the various outcomes [33]. TKIs induces a chronicisation of the disease and the question concerns the sustainability of their long-term therapy in terms of compliance, toxicity, and costs. The ability of TKIs to cure the

disease has not been demonstrated with any of the TKIs, considering treatment-free remission (TFR) as the goal of CML treatment [34, 35]. Thus, treatment discontinuation is only discussed in patients after achieving a deep molecular response (≥ MR4.0, defined as 4-log decrease in *BCR-ABL1* transcript levels from the standardized baseline) and durable (2 or 3 years). Nevertheless, only 40 % of them maintain TFR, while the remaining patients losing major molecular response (MMR or MR3.0) can safely regain the previous status by reassuming the daily therapy, without any risk of disease progression [36]. Several strategies are discussed, including dose reduction with the search for the minimum effective dose, intermittent TKI therapy and supplemental immune therapy, particularly on the specific role of lymphocyte subpopulations such as NK cells [37].

In Chronic Lymphocytic Leukemia (CLL)

The monitoring of MRD in the daily practice of patients with CLL has been well recognized since the European Medicines Agency (EMA) included the assessment of MRD as an interim endpoint of phase III clinical trials for the approval of new drugs in CLL [38]. MRD levels are now set to 10^{-4} to 10^{-5} and referred to as MRD4 or MRD5. MRD4 has been shown to impact survival with significant improvements in both PFS and OS per log reduction in tumor burden [39]. The appearance of new and increasingly effective drugs has been concomitant with technological development for the diagnosis of MRD. MRD techniques include MFC and of immunoglobulin heavy chain variable gene PCR [40]. Bruton's tyrosine kinase (Btk) inhibitors such as ibrutinib are generally associated with low levels of MRD negativity, approximately 6 % in peripheral blood after 4 years of treatment [41]. MRD monitoring helps to understand the disease characteristics and kinetics of CLL and to define the optimal duration of treatment in clinical studies. In addition, it can optimize therapeutic strategies, pespecially in high-risk patients when the MRD is positive, by adding venetoclax to ibrutinib or vice versa, after 1 year if MRD remains positive (Phase II IMPROVE study, 42) or using new therapies such as CAR T-cells or bispecific monoclonal antibodies (moAbs). MRD monitoring can guide the duration of a treatment. Indeed, in a retrospective analysis, patients treated with the association of fludarabine, cyclophosphamide and rituximab (FCR), some of them stopped treatment after 3 instead of 6 cycles due to toxicity, however they had a similar PFS and OS when MRD was negative in bone marrow after 3 cycles [43].

In Acute Lymphoblastic Leukemia (ALL)

Patients with ALL, who achieved MRD negative response by MFC, had superior event-free survival (EFS) and OS. The 10-year EFS rate for MRD-negative

versus MRD-positive patients was 77 % vs 32 % in children and 64 % vs 21 % in adults [44]. Additionally, the time to achieve MRD negativity as well as the depth of response are clinically significant [45].

MRD monitoring can further improve risk stratification and influence treatment decision in some emergent situations, especially when MRD is positive after induction therapy, after relapse or to avoid allogenic stem cell transplantation due to availability of highly effective agents for eradication of MRD, such as blinatumomab, inotuzumab ozogamicin with or without autologous stem cell transplantation (ASCT), and/or chimeric antigen receptor (CAR) T-cell therapies [45]. Additionally, the choice to suppress total body irradiation because of its side effects, in the ASCT conditioning regimen, may be guided by MRD analysis as demonstrated in children [46].

It is time to consider the risk-benefit balance, and ALL represents the best example to explore new innovative therapeutic strategies based on the monitoring of MRD and the post-chemotherapy use of the highly effective blinatumomab and inotuzumab followed by CAR T-cells as explored by the MD Anderson team [45, 47, 48]. Fig. 2 (*A*, *B*) illustrates such a discussion from a case report, with proposed standard treatment and best therapeutic management based on MRD follow-up.

In Acute Myeloid Leukemia (AML)

The analysis of risk factors at the time of diagnosis influences the therapeutic strategy. Thereafter, the time of MRD monitoring is debatable, based on its impact on decision-making, especially for intermediate-risk patients, for allogenic stem cell transplantation. Early assessment of MRD may allow treatment to be intensified or modified by adding gemtuzumab-ozogamicin [49]. It has been claimed that MRD negativity prior to allogenic transplantation may impact the decision on the conditioning regimen, to determine optimal donor type [51] or to use donor lymphocyte infusion (DLI) [50, 51]. The question of treating patients with relapsed MRD with hypomethylating agents, venetoclax, targeted therapy or immune therapy remains open [52].

In non-Hodgkin's lymphoma (NHL)

In NHL, ctDNA is a promising and emerging biomarker for disease diagnosis, prognosis, and monitoring. However, it is too early to define a therapeutic strategy guided on MRD, and ^{18}F-Fluorodeoxyglucose Positron Emission Tomography analysis remains the dominant tool for this. The questions relate to sensitivity, the lack of standardization of preclinical techniques, the need for standardization and technological harmonization, consensus on threshold nominating elevated ctDNA levels in pretreatment samples, and MRD negativity during / after treatment [53].

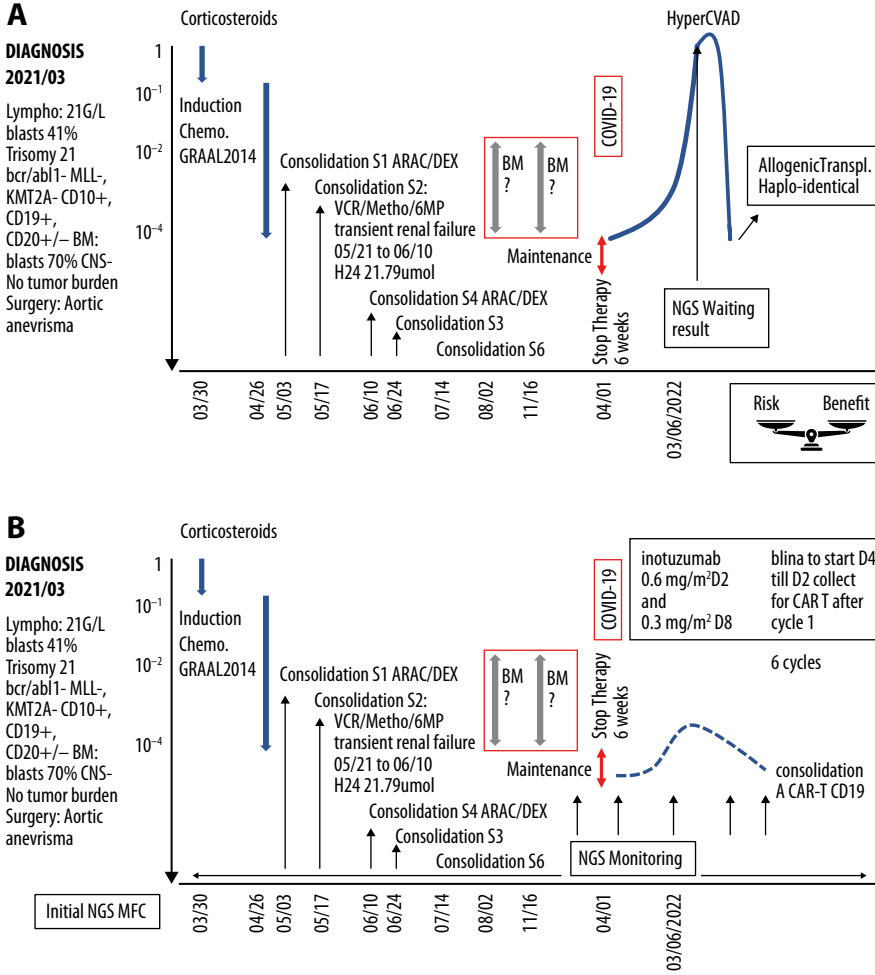

Fig. 2. Case report: 55 years-old male, with acute lymphoblastic leukemia (ALL), B EGIL, Philadelphia negative; *A*: with partial follow-up of MRD, particularly absent during COVID-19, without treatment and followed by macroscopic relapse. Allogenic stem cell transplantation is discussed in this high-risk patient; *B*: with MRD follow-up, an early relapse in the positive MRD could have been diagnosed with the therapeutic proposal including bi-specific antibodies followed by CAR T-cells in consolidation

Table 1.
Multiparametric flow cytometry (MFC) for analysis of the minmal residual disease (MRD) in hematological malignancies. ARAC: Cytarabine or Ara-C; Dex: dexamethasone; VCR: vincristine; Metho: methotrexate; 6MP: 6-mercaptopurine; BM: bone marrow, lympho: lymphocytes; NGS: next generation sequencing.

	B-ALL	**T-ALL**	**AML**	**MM**	**CLL**
Sensitivity	10^{-4} to 10^{-5}	10^{-4} to 10^{-5}	10^{-3} to 10^{-5}	10^{-5} to 10^{-6}	10^{-4} to 10^{-5}
Standard panel	CD34, CD19, CD10, CD20, CD38, CD45	CD1a, CD2, CD3, CD5, CD4, CD8, CD7, CD34, CD45, CD99	CD34, CD117, CD45, CD13, CD15, CD7	CD38, CD138, CD45, CD56, CD19, CD27, CD28, CD11, κ/λ, CD81, CD200	CD19, CD20, CD5, CD79b, CD43, CD81
Additional biomarkers	CD22, CD66c, CD81, CD123, CD73, CD304	CD10, CD38, CD56, TdT	CD14, CD64, HLA-DR, CD4, CD11b, CD38, CD90, CD123, CD133	CD33, CD54, CD229, CD307, CD319, CD150, VS38	CD200, CD23, CD160, ROR1

In Multiple Myeloma (MM)

Achieving a negative MRD has recently been shown to correlate with survival in MM [54]. The timing of monitoring treatment based on MRD tracking is the next step underway in different therapeutic windows of opportunity. Due to the cytokine burst in the early period after autologous stem cell transplantation (ASCT), we have demonstrated that this period is a particularly opportunistic early period for immune

therapy or anti-interleukin 6 (IL-6) therapy. This early period is conducive to further control of residual disease due to the release of hematopoietic niches which are also plasma cell niches. Indeed, there may exist early relapses after ASCT due to the rapid emergence of chemo-resistant clones in a favorable immunocompromised environment with the presence of IL-6 [55].

How is the future of multiplexed precision therapy

With technological progress, the level of detection of MRD becomes lower and lower, thus revealing the notion of monitoring dynamics MRD, the time and the type of treatment to be implemented. As shown in Fig. 3, therapeutic options based on MRD monitoring are discussed. The first situation observed is the progression of MRD untill macroscopic relapse. Then, it is a question of quickly defining the therapeutic option, based on the prognostic biomarkers of NGS and for the near future, on the complementary immune status, the trogocytosed immune cells or the miRNA. Immune therapies can include moAbs, bi-specific/tri-specific killer engagers (BiKEs / TriKEs), and directed cellular therapies such as CAR-T cells or chimeric antigen receptor-NKs (CAR-NKs) [56].

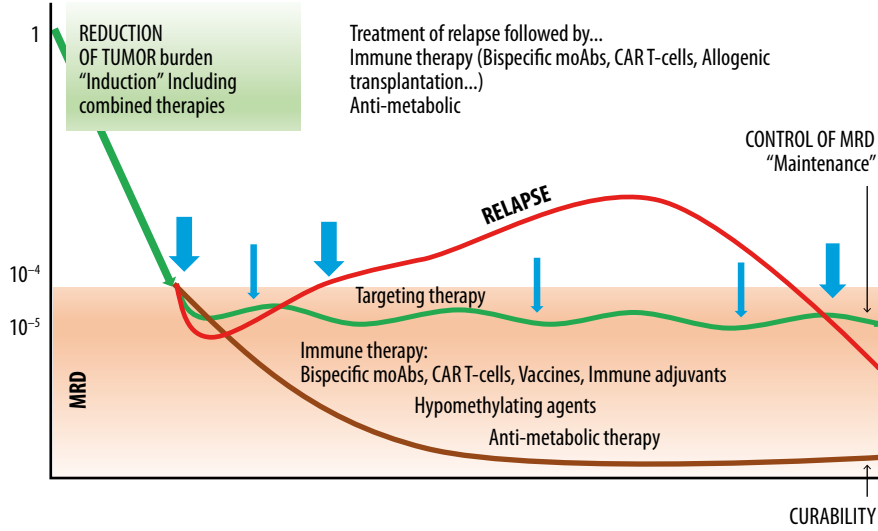

Fig. 3. Therapeutic possibilities (in green) under MRD monitoring

The second situation, probably more often seen in specific targeted therapies like CML, is signal fluctuation around detectability. In this situation, the question of complementary therapies arises, in particular to improve the control of MRD, generally with immune therapies, including immune adjuvants such as azoximer bromide (AZB), cancer vaccines or combined therapies. The biological activity of AZB is mainly observed in the bridging inflammatory responses the activation of phagocytic cells, T- and NK-cells, maturation of dendritic cell, the modulation of the synthesis of cytokines such as interferons -alpha and -gamma, antitoxic activities and membrane stabilizing effect [57, 58]. AZB has been widely used, having a good safety profile throughout clinical development and post-marketing surveillance [59]. Immune cells undergo significant metabolic reprogramming during immune response [60].

Anti-metabolic agents represent a novel therapeutic route to modulate both cancer cells and immune cells in the vicinity of cancer cells [61].

In conclusion, the demonstration of a correlation between survival and depth of response was only associated to the level of the residual disease. This correlation was linked to technological progress, in particular due to the detection of prognostic biomarkers and their interest in delimiting optimal therapy. The widening of the choice of effective therapeutic tools has paved the way for a medicine closer to the patient that some call personalized medicine to which should be added the notion of dynamic evaluation.

References

1. https://www.cancer.gov/publications/dictionaries/cancer-terms/def/minimal-residual-disease.
2. Freireich E., Gehan E., Sulman D., et al. The effect of chemotherapy on acute leukemia in the human. J. Chronic. Dis. 1961; 14: 593–608.
3. Campana D., Pui C. Detection of minimal residual disease in acute leukemia: methodologic advances and clinical significance. Blood 1995; 85: 1416–34.
4. Ommen H.B. Monitoring minimal residual disease in acute myeloid leukaemia: a review of the current evolving strategies. Ther. Adv. Hematol. 2016; 7: 3–16. doi: 10.1177/ 2040620715614529.
5. Cheson B., Bennett J., Kopecky K., et al. Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J. Clin. Oncol. 2003; 21: 4642–9. doi: 10.1200/JCO.2003.04.036.
6. Shah K., Parikh S., Rawal R. Tyrosine kinase inhibitors in Ph+ chronic myeloid leukemia therapy: A review. Asian Pac. J. Cancer Prev. 2016; 17: 3025–33.

7. Ravandi F., Walter R.B., Freeman S.D. Evaluating measurable residual disease in acute myeloid leukemia. Blood Adv. 2018; 2: 1356–66. doi: 10.1182/bloodadvances.2018016378.
8. Ossenkoppele G.J., van de Loosdrecht A.A., Schuurhuis G.J. Review of the relevance of aberrant antigen expression by flow cytometry in myeloid neoplasms. Br J. Haematol. 2011; 153: 421–43. doi: 10.1111/j.1365-2141.2011.08595.
9. Loken M.R., Alonzo T.A., Pardo L., et al. Residual disease detected by multidimensional flow cytometry signifies high relapse risk in patients with de novo acute myeloid leukemia: a report from Children's Oncology Group. Blood 2012; 120: 1581–8. doi: 10.1182/blood-2012-02-408336.
10. Terwijn M., van Putten W.L., Kelder A., et al. High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: data from the HOVON/SAKK AML 42A study. J. Clin. Oncol. 2013; 31: 3889-3897. doi: 10.1200/JCO.2012.45.9628.
11. Zhong Z., Zang H., Zhu Y., et al. Circulating tumor cells in cancer patients: developments and clinical applications for immunotherapy. Mol. Cancer 2020; 19: 15. doi: 10.1186/s12943-020-1141-9
12. Kulasinghe A., Kapeleris J., Kimberley R., et al. The prognostic significance of circulating tumor cells in head and neck and non-small-cell lung cancer. Cancer Med. 2018; 7: 5910-5919. doi: 10.1002/cam4.1832
13. Steensma D.P., Bejar R., Jaiswal S., et al. Clonal hematopoiesis and its distinction from myelodysplastic syndromes. Blood 2015; 126: 9–16. doi: 10.1182/blood-2015-03-631747.
14. Diehl F., Schmidt K., Choti M.A., et al. Circulating mutant DNA to assess tumor dynamics. Nat. Med. 2008; 14: 985–90. doi: 10.1038/nm.1789.
15. Ommen H.B. Monitoring minimal residual disease in acute myeloid leukemia: a review of the current evolving strategies. Ther. Adv. Hematol. 2016; 7: 3–16. doi: 10.1177/2040620715614529.
16. Svensson V., Vento-Tormo R., Teichmann S.A. Exponential scaling of single-cell RNA-seq in the past decade. Nat. Protoc. 2018; 13: 599–604. doi: 10.1038/nprot.2017.149.
17. Lu S., Stein J.E., Rimm D.L., et al. Comparison of biomarker modalities for predicting response to PD-1/PD-L1 checkpoint blockade: a systematic review and meta-analysis. JAMA Oncol. 2019; 5: 1195–204. doi: 10.1001/jamaoncol.2019.1549.
18. Jerby-Arnon L., Shah P., Cuoco M.S., et al. A cancer cell program promotes T cell exclusion and resistance to checkpoint blockade. Cell. 2018; 175: 984–997.e24.
19. Gohil S.H., Iorgulescu J.B., Braun D.A., et al. Applying high-dimensional single-cell technologies to the analysis of cancer immunotherapy. Nat. Rev. Clin. Oncol. 2021; 18: 244–56. doi: 10.1038/s41571-020-00449.

20. Kula T., Dezfulian M.H., Wang C.I., Abdelfattah N.S., et al. T-Scan: a genome-wide method for the systematic discovery of T cell epitopes. Cell. 2019; 178: 1016-1028.e13. doi: 10.1016/j.cell.2019.07.009.
21. Zhang S-Q., Ma K-Y., Schonnesen A., et al. High-throughput determination of the antigen specificities of T cell receptors in single cells. Nat. Biotechnol. 2018; 36: 1156–9. doi: 10.1038/nbt.4282.
22. Davis-Marcisak E.F., Deshpande A., Stein-O'Brien G.L., et al. From bench to bedside: single-cell analysis for cancer immunotherapy. Cancer Cell. 2021; 39: 1062–80. doi: 10.1016/j.ccell.2021.07.004.
23. Pagani I.S., Kok C.H., Saunders V.A., et al. A method for next-generation sequencing of paired diagnostic and remission samples to detect mitochondrial DNA mutations associated with leukemia. J. Mol. Diagn. 2017; 19: 711–21. doi: 10.1016/j.jmoldx.2017.05.009.
24. Cumbo C., Anelli L., Specchia G., et al. Monitoring of Minimal Residual Disease (MRD) in Chronic Myeloid Leukemia: Recent Advances. Cancer Manag. Res. 2020; 12: 3175–89. doi: 10.2147/CMAR.S232752.
25. Weinhard L., Di Bartolomei G., Bolasco G., et al. Microglia remodel synapses by presynaptic trogocytosis and spine head filopodia induction. Nat. Commun. 2018; 9: 1228. doi: 10.1038/s41467-018-03566-5.
26. Lis R., Capdet J., Mirshahi P., et al. Oncologic trogocytosis with hospicells induces the expression of N-cadherin by breast cancer cells. Int. J. Oncol. 2010; 37: 1453–61. doi: 10.3892/ijo_00000797.
27. Reed J., Reichelt M., Wetzel S.A. Lymphocytes and trogocytosis-mediated signaling. Cells 2021; 10: 1478. doi: 10.3390/cells10061478.
28. Somanchi S.S., Somanchi A., Cooper L.J., et al. Engineering lymph node homing of ex vivo — expanded human natural killer cells via trogocytosis of the chemokine receptor CCR7. Blood. 2012; 119: 5164–72. doi: 10.1182/blood-2011-11-389924.
29. Hasim M.S., Marotel M., Hodgins J.J., et al. When killers become thieves: Trogocytosed PD-1 inhibits NK cells in cancer. Sci Adv. 2022; 8: eabj3286. doi: 10.1126/sciadv.abj3286.
30. Vo D.N., Constantinides M., Allende-Vega N., et al. Dissecting the NK cell population in hematological cancers confirms the presence of tumor cells and their impact on NK population function. Vaccines (Basel). 2020; 8: 727. doi: 10.3390 / vaccines8040727.
31. Krzywinska E., Allende-Vega N., Cornillon A., et al. Identification of anti-tumor cells carrying Natural Killer (NK) cell antigens in patients with hematological cancers. EBioMedicine 2015; 2: 1364–76. doi: 10.1016/j.ebiom.2015.08.021.
32. Pagani I.S., Dang P., Kommers I.O., et al. BCR-ABL1 genomic DNA PCR response kinetics during first-line imatinib treatment of chronic my-

eloid leukemia. Haematologica 2018; 103: 2026–32. doi: 10.3324/haematol.2018.189787.
33. Marin D., Ibrahim A.R., Lucas C., et al. Assessment of BCR-ABL1 transcript levels at 3 months is the only requirement for predicting outcome for patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors. J. Clin. Oncol. 2012; 30: 232–8. doi: 10.1200/JCO.2011.38.6565.
34. Muselli F., Peyron J.F., Mary D. Druggable biochemical pathways and potential therapeutic alternatives to target leukemic stem cells and eliminate the residual disease in chronic myeloid leukemia. Int. J. Mol. Sci. 2019; 20: 5616. doi: 10.3390/ijms20225616
35. Cortes J., Rea D., Lipton J.H. Treatment-free remission with first- and second-generation tyrosine kinase inhibitors. Am. J. Hematol. 2019; 94: 346–57. doi: 10.1002/ajh.25342.
36. Russo D., Garcia-Gutierrez J.V., Soverini S., et al. Chronic myeloid leukemia prognosis and therapy: Criticisms and perspectives. J. Clin. Med. 2020; 9: 1709. doi: 10.3390/jcm9061709.
37. Carlsten M., Järås M. Natural Killer cells in myeloid malignancies: Immune surveillance, NK cell dysfunction, and pharmacological opportunities to bolster the endogenous NK cells. Front. Immunol. 2019; 10: 2357. doi: 10.3389 / fimmu.2019.02357.
38. European Medicines Agency Appendix 4 to the Guidelines on the Evaluation of Anticancer Medicinal Products in Man. [(accessed on 27 June 2021)]. Available online: https://www.ema.europa.eu/en/appendix-4-guideline-evaluation-anticancer-medicinal-products-man-condition-specific-guidance.
39. Riva G., Nasillo V., Ottomano A.M., et al. Multiparametric Flow Cytometry for MRD Monitoring in Hematologic Malignancies: Clinical Applications and New Challenges. Cancers (Basel) 2021; 13: 4582. doi: 10.3390/cancers13184582.
40. Böttcher S., Hallek M., Ritgen M., et al. The role of minimal residual disease measurements in the therapy for CLL: is it ready for prime time? Hematol. Oncol. Clin. North Am. 2013; 27: 267–88. doi: 10.1016/j.hoc.2013.01.005.
41. Ahn I.E., Farooqui M.Z.H., Tian X., et al. Depth and durability of response to ibrutinib in CLL: 5-year follow-up of a phase II study. Blood 2018; 131: 2357–66. doi: 10.1182/blood-2017-12-820910.
42. Scarfo L., Heltai S., Albi E., et al. Minimal residual disease-driventreatment intensification with sequential addition of ibrutinib to venetoclax in R/R CLL. Blood. 2022 Aug 3: blood.2022016901. doi: 10.1182/blood.2022016901.
43. Al-Sawaf O., Seymour J.F., Kater A.P., et al. Should undetectable minimal residual disease be the goal of chronic lymphocytic leukemia therapy? Hematol. Oncol. Clin. North Am. 2021; 35: 775–91. doi: 10.1016/j.hoc.2021.03.007.

44. Berry D.A., Zhou S., Higley H., et al. Association of minimal residual disease with clinical outcome in pediatric and adult acute lymphoblastic leukemia: a meta-analysis. JAMA Oncol. 2017; 3(7): e170580–e170580. doi: 10.1001/jamaoncol.2017.0580.
45. Hein K., Short N., Jabbour E., et al. Clinical value of measurable residual disease in acute lymphoblastic leukemia. Blood Lymphat. Cancer. 2022; 12: 7–16. doi: 10.2147/BLCTT.S270134.
46. Friend BD, Bailey-Olson M, Melton A, et al. The impact of total body irradiation-based regimens on outcomes in children and young adults with acute lymphoblastic leukemia undergoing allogeneic hematopoietic stem cell transplantation. Pediatr. Blood Cancer 2020; 67: e28079. doi: 10.1002/pbc.28079.
47. https://clinicaltrials.gov/ct2/show/NCT02458014?term=philadelphia+negative&cond=ALL%2C+Adult&cntry=US&draw=3&rank=12.
48. https://clinicaltrials.gov/ct2/show/NCT03488225?term=philadelphia+negative&cond=ALL%2C+Adult&cntry=US&draw=2&rank=24
49. O'Hear C., Inaba H., Pounds S., et al. Gemtuzumab ozogamicin can reduce minimal residual disease in patients with childhood acute myeloid leukemia. Cancer. 2013; 119: 4036–43. doi: 10.1002/cncr.28334.
50. Freeman S., Craddock C. Less is not necessarily more: Toward a rational selection of the conditioning regimen in acute myeloid leukemia. J. Clin. Oncol. 2020; 38: 1249–51. doi: 10.1200/JCO.19.03161.
51. Yu S., Huang F., Wang Y., et al. Haploidentical transplantation might have superior graft-versus-leukemia effect than HLA-matched sibling transplantation for high-risk acute myeloid leukemia in first complete remission: A prospective multicentre cohort study. Leukemia. 2020; 34: 1433–43. doi: 10.1038/s41375-019-0686-3.
52. Azenkot T., Jonas B.A. Clinical impact of measurable residual disease in acute myeloid leukemia. Cancers (Basel). 2022; 14: 3634. doi: 10.3390/cancers14153634.
53. Pirosa M.C., Borchmann S., Jardin F., et al. Controversies in the interpretation of liquid biopsy data in lymphoma. Hemasphere. 2022; 6: e727. doi: 10.1097/HS9.0000000000000727. eCollection 2022 Jun.
54. Daniele P, Mamolo C, Cappelleri JC, et al. Response rates and minimal residual disease outcomes as potential surrogates for progression-free survival in newly diagnosed multiple myeloma. PLoS One. 2022; 17(5): e0267979. doi: 10.1371/journal.pone.0267979. eCollection 2022.
55. Condomines M., Veyrune J.L., Larroque M., et al. Increased plasma-immune cytokines throughout the high-dose melphalan-induced lymphodepletion in patients with multiple myeloma: a window for adoptive immunotherapy. J. Immunol. 2010; 184: 1079–84. doi: 10.4049/jimmunol.0804159.

56. Grivtsova L.Y., Falaleeva N.A., Tupitsyn N.N. Azoximer Bromide: Mystery, serendipity, and promise. Front Oncol. 2021; 11: 699546. doi: 10.3389/fonc.2021.699546.
57. Allison M., Mathews J., Gilliland T., Mathew S.O. Natural Killer cell-mediated immunotherapy for leukemia. Cancers (Basel). 2022; 14: 843. doi: 10.3390/cancers14030843.
58. Alexia C., Cren M., Louis-Plence P., Vo D.N., et al. Polyoxidonium® Activates Cytotoxic Lymphocyte Responses Through Dendritic Cell Maturation: Clinical Effects in Breast Cancer. Front Immunol. 2019; 10: 2693. doi: 10.3389/fimmu.2019.02693.
59. Pružinec P., Chirun N., Sveikata A. The safety profile of Polyoxidonium in daily practice: results from postauthorization safety study in Slovakia. Immunotherapy 2018; 10: 131–7. doi: 10.2217/imt-2017-0116.
60. Britt E., John S.V., Locasale J.W., et al. Metabolic regulation of epigenetic remodeling in immune cells. Curr. Opin. Biotech. 2020; 63: 111–17. doi:10.1016/j.copbio.2019.12.008.
61. Margetis A.T. Metabolic targeting of malignant tumors: a need for systemic approach. J Cancer Res Clin Oncol. 2022 Aug 4. doi: 10.1007/s00432-022-04212-w.

LOCALIZED INTRA-PLEURAL CONDITIONING TO BLOCK INFLAMMATION, REVERSE MESENCHYMAL TUMOR STATE, AND SUPPORT ANTI-TUMOR IMMUNITY

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Background

The pleura are a common site for metastatic malignancy and are frequently accompanied by pleural effusions. Malignant pleural effusions (MPE) are highly symptomatic complications of cancer, and frequently are the ultimate cause of cancer mortality [1–3]. There are limited treatment options for the management of malignant pleural effusions. To meet the need for novel therapeutic approaches to malignant pleural effusions, characterization of the pleural and peritoneal humoral and cellular immune environment represents an opportunity to identify targets for current and novel immunotherapies. Several immunotherapy approaches have been studied for the treatment and palliation of malignant pleural effusions [5, 6]. In addition, peritoneal fluid cytokine levels have been studied as diagnostic and prognostic biomarkers. Specifically, increased levels of interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), tumor necrosis factor alpha (TNF-α) have been correlated with the severity of pleural metastasis [10]. Our published studies of the pleural secretome in non-small cell lung cancer, mesothelioma [1], and ovarian cancer [2] as well as extensive secretomic data in MPE from other cancers, indicate that the IL-6/IL-6Rα axis is prominent in all pleural effusions and drives the epithelial to mesenchymal transition (EMT) [3, 4], promoting disease progression and metastasis.

Malignant pleural effusions have a US incidence of more than 150,000 cases per year [5] and a life expectancy measured in months [6]. A 2020 study [7] reported that in 2014 MPE accounted for more than 1.6 billion dollars in hospitalization charges alone. Despite significant clinical progress in immuno-oncology, there has been almost no change in survival or quality of life for patients with malignant pleural effusions. Using data obtained from the National Inpatient Sample of the

healthcare and cost research project we estimate that 13 % of MPE patients die in-hospital and are a major source of cancer-related healthcare cost ($20,000 per case, Fig. 1). With the broader use of expensive immune-oncology drugs, the cost is likely to continue rising.

Pleura as a bioreactor

The pleural space is lined with mesothelial cells joined by tight junctions [8], presenting barriers to passive diffusion of large molecules, and sequestering secreted factors in a fluid-phase tumor environment. Given the palette of tumor-promoting and immunosuppressive cytokines present in malignant effusions, this space can be thought of as a bioreactor in which cancer and immune cells are redirected toward an aggressive EMT phenotype. We propose that the rich cellular infiltrate of the malignant pleural macro-environment be re-programmed into a more conducive pleural environment capable of supporting efficacious adoptive cellular therapy products [1, 9–13].

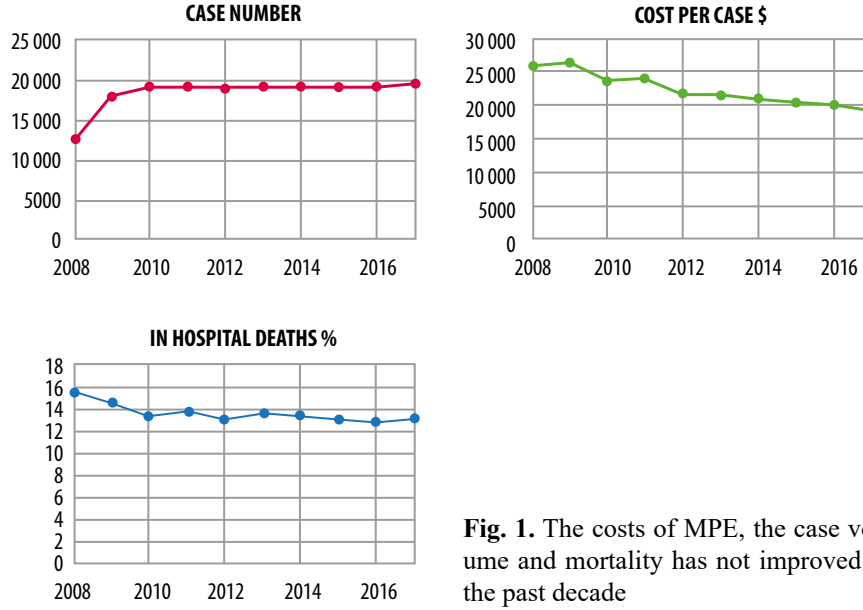

Fig. 1. The costs of MPE, the case volume and mortality has not improved in the past decade

Intrapleural conditioning to reverse tumor emt and support antitumor immunity

We have reported that IL-6 and IL-6Rα are present at ng/mL concentration in non-small cell lung cancer and benign effusions [1], and have extended these findings to other cancers metastatic to the pleura. The central role of IL-6 trans-signaling upstream of pathologic processes mediated by multi-cytokine cascades is supported by the efficacy of the anti-IL-6Rα antibody tocilizumab in the treatment of rheumatoid arthritis [14] and cytokine release syndrome (CRS) induced by administration of therapeutic chimeric antigen receptor T cells (CAR-T) (15) [16]. Although long-term antagonism of IL-6Rα is immunosuppressive [17], single dose exposure can break the cytokine storm associated with CRS or Severe Acute Respiratory Syndrome without compromising effector responses [18] or incurring serious adverse events [19]. Intrapleural administration of anti-IL-6 or anti-IL-6Rα may likewise be expected to exert profound effects on the pleural environment without incurring immunosuppression. Similarly, a proportion of pleural T cells express PD-1 and therefore may require protection from PD-L1 ligation by localized administration of immune checkpoints.

Case for localized intra-pleural therapy

Unlike chemotherapeutics, high molecular weight immuno-oncology drugs remain concentrated when administered directly to the pleura [20–22]. Further, the routine use of indwelling catheters, and periodic drainage facilitates monitoring and personalized drug dosing, as well as real time assessment of pleural T-cell quality and state. We argue for local rather than systemic administration of protein-based therapies, since systemic administration may not achieve the necessary therapeutic levels in the pleura [13].

Conclusions

Despite many significant medical and surgical advances, malignant pleural effusions are only palliated, not treated. Although malignant pleural effusions cause a great deal of morbidity the prevailing thought is that an MPE is an end stage event and not that different from other extra-pleural metastases such as brain or bone or liver metastases. Therefore, most patients with malignant pleural effusions are considered to be failing because of systemic progression, and the MPE is just a manifestation of this progression. However, if we are to make real prog-

Table 1.
SWOT Analysis of Intrapleural conditioning and therapy

STRENGTHS:
- Multiple combinations including protein therapeutics with narrow therapeutic index (Tl) when given systemically
- High Tl: Protein therapeutics will be sequestered, high target occupancy
- Low systemic exposure
- Low toxicity: low adverse effect occurrence, low on-target off-tumor effects
- Abscopal effects (lymphocyte and antigen-presenting cell trafficking)
- Can be given In combination with current systemic immune checkpoint molecules

WEAKNESSES:
- Logistics: surgical intervention, repeated administration, dosing schedules
- Difficult to determine best combination of therapeutics
- Varied formulations of therapeutics
- Challenging logistics: radiology/image guided drug-delivery
- Only “accessible” tumors can be injected
- Drug retention at intratumoral injection sites

OPPORTUNITIES:
- All immuno-oncology drugs and combinationscan be administered locally
- Easy monitoring: a minimum anticipated biological effect level can be established in real time via therapeutic drainage
- PK/PD in real time
- Innovative combinations that include cancer vaccines, radiation, and localized conditioning, cytokine mRNA, can be explored
- Innovative tool development: industry partnerships
- Wide range of tumor types/indications: locally overexpressed molecules can be useful targets even if not-tumor specific
- Unmasking additional tumor-antigens by a variety of modalities
- Harvest and store of in vivo expanded tumor-specific T cells

THREATS:
- Access and monitoring of injected and non-injected sites may be limited
- **Dedicated surgical personnel**
- **Dedicated research personnel and facilities**
- Development of RECIST-like response assessment tools
- Unexpected delayed toxicities and adverse events as the result of localized immune-over-activation

ress in metastatic cancer treatment, we need to start by understanding malignant pleural effusions, treating MPE locally, training the local pleural immunity to recognize tumor associated antigens and assuring that immune responses will propagate systemically. Despite the many potential strengths and opportunities associated with localized intra-pleural therapy, there are only few studies investigating the potential of intrapleural therapies, most focusing on intrapleural delivery of mesothelin-specific CAR T cells [12]. Table 1 summarizes the strengths and the potential problems associated with intra-pleural therapies [2]. Probably the most important aspect of intrapleural administration is to determine the most efficacious combination of therapeutics as well as to work out the logistics associated with intra-pleural and intra-tumoral administration. There is a broad range of potential conditioning and therapeutic modalities that can be locally delivered. The varied formulations and compatibilities of potentially useful therapeutics must be considered if they are to be co-administered through indwelling catheters or by video-assisted thoracoscopic surgery (VATS). Drug retention at tumor injection sites also poses potential difficulty and needs to be addressed by choosing appropriate agents and vehicles. Probably the most serious aspect to localized administration of therapeutics is the potential unexpected toxicities resulting from localized immune hyper-responsiveness, or interference with normal tissue maintenance especially if these serious adverse effects are delayed. Therefore a new objective criteria for quantification of responses similar to the RECIST [23] score for solid tumors will need to be developed. Lastly, the technical challenges to implementation of intra-pleural therapy include the need for dedicated personnel and facilities, including those required for image guided drug delivery. For drug delivery protocols requiring general anesthesia, the ability to administer repeated doses will be limited. That said, we argue that combination therapies designed to condition the malignant pleural environment, reverse mesenchymal tumor state, and stimulate persistent anti-tumor immunity locally and systemically will succeed and therefore must be tested to determine the safest and most effective therapeutic combinations for this intractable and deadly disease.

References

1. Donnenberg A.D., Luketich J.D., Donnenberg V.S. Secretome of pleural effusions associated with non-small cell lung cancer (NSCLC) and malignant mesothelioma: therapeutic implications. Oncotarget. 2019; 10: 6456–65.
2. Donnenberg V.S., Wagner P.L., Luketich J.D., et al. Localized Intra-Cavitary Therapy to Drive Systemic Anti-Tumor Immunity. Frontiers in immunology In Press. 2022.

3. Mehta K., Moravcikova E., McFall D., et al. The Mesenchymal State Predicts Poor Disease-Free Survival in Resectable Non-Small Cell Lung Cancer. Ann Thorac Surg. 2017; 104: 321–8.
4. Lu H., Clauser K.R., Tam W.L., et al. A breast cancer stem cell niche supported by juxtacrine signalling from monocytes and macrophages. Nature cell biology. 2014; 16: 1105–17.
5. Directors A. T. S. B. o. Management of malignant pleural effusions. Am J Respir Crit Care Med. 2000; 162: 1987–2001.
6. Clive A.O., Kahan B.C., Hooper C.E., et al. Predicting survival in malignant pleural effusion: development and validation of the LENT prognostic score. Thorax. 2014; 69: 1098–104.
7. Shafiq M. Ma X., Taghizadeh N., Kharrazi H., et al. Healthcare Costs and Utilization among Patients Hospitalized for Malignant Pleural Effusion. Respiration; international review of thoracic diseases. 2020; 99: 257–63.
8. Amasheh S., Markov A.G., Volgin G.N., et al. Barrier function of human pleura mesothelium is constituted by tight junctions. The FASEB Journal. 2011; 25: 1036.1033–1036.1033.
9. Mehta G.U., Malekzadeh P., Shelton T., et al. Outcomes of Adoptive Cell Transfer With Tumor-infiltrating Lymphocytes for Metastatic Melanoma Patients With and Without Brain Metastases. Journal of Immunotherapy. 2018; 41: 241–7.
10. Chandran S.S., Somerville R.P.T., Yang J.C., et al. Treatment of metastatic uveal melanoma with adoptive transfer of tumour-infiltrating lymphocytes: a single-centre, two-stage, single-arm, phase 2 study. The Lancet Oncology. 2017; 18, 792–802.
11. Kiesgen S., Chicaybam L., Chintala N.K., Adusumilli P.S. Chimeric Antigen Receptor (CAR) T-Cell Therapy for Thoracic Malignancies. Journal of Thoracic Oncology. 2018; 13, 16–26.
12. Adusumilli P.S., Zauderer M.G., Rusch V.W., et al. A phase I clinical trial of malignant pleural disease treated with regionally delivered autologous mesothelin-targeted CAR T cells: Safety and efficacy. In AACR Annual Meeting p. CT036, American Association for Cancer Research, Altanta, GA. 2019.
13. Donnenberg A.D., Luketich J.D., Dhupar R., Donnenberg V.S. Treatment of malignant pleural effusions: the case for localized immunotherapy. Journal for ImmunoTherapy of Cancer. 2019; 7: 110.
14. Smolen J.S., Beaulieu A., Rubbert-Roth A., et al. Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. The Lancet. 2008; 371: 987–97.

15. Maude S.L., Barrett D., Teachey D.T., Grupp S.A. Managing Cytokine Release Syndrome Associated With Novel T Cell-Engaging Therapies. The Cancer Journal. 2014; 20: 119–22.
16. Maude S.L., Frey N., Shaw P.A., et al. Chimeric Antigen Receptor T Cells for Sustained Remissions in Leukemia. New England Journal of Medicine. 2014; 371: 1507–17.
17. Pawar A., Desai R.J., Solomon D.H., et al. Risk of serious infections in tocilizumab versus other biologic drugs in patients with rheumatoid arthritis: a multidatabase cohort study. Annals of the rheumatic diseases. 2019; 78: 456.
18. Davila M.L., Riviere I., Wang X., et al. Efficacy and toxicity management of 19–28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Science translational medicine. 2014; 6: 224ra225–224ra225.
19. Xu X., Han M., Li T., et al. Effective Treatment of Severe COVID-19 Patients with Tocilizumab. ChinaXiv. 2020; 00026v1.
20. Goey S.H., Eggermont A.M., Punt C.J., et al. Intrapleural administration of interleukin 2 in pleural mesothelioma: a phase I-II study. Br J Cancer. 1995; 72: 1283–8.
21. Ruf P., Kluge M., Jager M., et al. Pharmacokinetics, immunogenicity and bioactivity of the therapeutic antibody catumaxomab intraperitoneally administered to cancer patients. Br J Clin Pharmacol. 2010; 69: 617–25.
22. Sebastian M., Kiewe P., Schuette W., et al. Treatment of Malignant Pleural Effusion With the Trifunctional Antibody Catumaxomab (Removab) (Anti-EpCAM×Anti-CD3): Results of a Phase 1/2 Study. Journal of Immunotherapy. 2009; 32: 195–202.
23. Eisenhauer E.A., Therasse P., Bogaerts J., et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009; 45, 228–47.

MULTIOMIC CYTOKINE ANALYSIS IN HEMATO-IMMUNOLOGY

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ABSTRACT

Cytokines are a diverse group of peptides produced by many cells of immune system but also from tumor cells. They show a complex interaction in the regulation of cell differentiation, inflammation, tumor proliferation, regulation of tumor growth as well as tumor stroma interaction. Multiomics approach regarding cytokines estimations involves analyses by the various methods depending on the cell sample on different cellular level. In this paper, we will focus on the possibilities of cytokine analysis in different cells isolated from patients with hematological malignancies, and particularly from multiple myeloma. Modern methods of immunology, molecular biology, and complex technologies have, in addition to serum cytokine analysis, today enabled cytokine analysis in separated cells, analysis of cytokine production from individual cell populations, as well as gene regulation of cytokine production and cytokine receptor analysis. Analysis of cytokines from separated tumor cells in hematological malignancies and multiple myeloma provided a better understanding of the response between immune system cells, stroma, and tumor proliferation. The results showed that the production of pro-inflammatory cytokines, IL-6, TNF-α, as well as TGF-β was mainly increased and that they could correlate with the degree of immune-suppression of effector cytotoxic lymphocytes or NK cells. Understanding the complex immune-hematology problems and the role of cytokines in these mechanisms, especially on different cellular levels based on a multiomics approach, gives a really hope how all these new findings and discoveries may help to better patients treatment in the future.

Keywords: cytokines, tecnology, assay, flow cytometry, hematology, tumor-stro, a interaction, multiomics

Introduction

Cytokines were discovered more than 50 years ago and are produced by various cells of the immune system but also from tumor cells [1, 2]. According to their

structure, they are designated as small glycoproteins and show effects on other cells, thus providing communication between different cells [3]. Cytokines regulate many cellular functions, including cell stimulation, cell activation, cell growth, and cell differentiation [4–13]. Before the appearance and use of the term cytokines, other names such as monokines or lymphokines were often used to explain how these mediators originate from lymphocytes [14]. Interleukins is a name that was previously used to explain the occurrence of communication between leukocytes [15, 16]. The term chemokines refers to those mediators that play an important role in inflammation and hematology and refers to mediators that help in adhesion, ie the attachment of various cells to the endothelium of blood vessels or to other cells [17]. The term interferons, which is still used today, refers to those mediators that are primarily secreted from host cells, mainly after viral infections [1, 16, 18].

Cytokine research is still an interesting field in science, as well as the discovery of new cytokines that contribute to the modification and classification of certain groups of cytokines [19]. Interest in the study of cytokines is so great today in all fields of basic science and medicine that there is a special group of scientists gathered in the International Cytokine and Interferone Society [20–24]. This group brings together scientists in various fields of study, both the structure and function of cytokines in basic sciences and their importance in medicine and various diseases [25–28, 30, 31].

Multiomics principles of studying cytokines in hematology and oncology imply a multiple and connected analysis of cytokine receptors and signals, which is the field of proteomics, enzyme reactivity, modulation of receptor activity labeled as metabolomics, gene expression of cytokines labeled as genomics, regulation of gene expression labeled as epigenomics, and the effect of therapy depending on the gene expression marked as pharmacogenomics [17, 21, 22, 32, 33]. All these modern principles of study try to give a better answer and understanding of the complex reaction of cytokines.

Principles of cytokine action

Cytokines achieve their effect primarily by showing effects on specific cytokine receptors [6, 34–38]. If the receptor is located on its own cell, then such an effect is called an autocrine effect; if the receptors are located on the surrounding cells, then it is designated as a paracrine effect, and if they exert effects on distant receptors and are transmitted through the blood, then it is a distant effect [3, 38–43]. However, cytokines can also act on similar receptors and simultaneously activate several types of cells, so that in addition to a specific effect, they also have a so-called pleiotronic effect, which leads to complexity [44–46]. Recently, therefore, there is more and more talk about the existence of a cytokine network that manifests complex effects

of cytokines [9, 46]. But individual cells do not secrete only one cytokine, but several types of cytokines, so the cells are then divided according to the type of cytokine they produce [14–16].

Cytokines are usually produced after some stimulation of the cell, then the cytokines are secreted from the cell and bind to the appropriate receptors, and perform the function of cell communication (Fig. 1).

Fig. 1 shows the mechanism of action of cytokines.

Thanks to modern knowledge, today cytokines are classified into several groups [32] and several classifications of cytokines are used. Cytokines can be divided according to the function they perform towards other cells of the immune system [32, 47–51]. Thus, some of them are labeled as stimulating cytokines, where interleukin-2 and interleukin 12 (IL-2, IL-12) are typical examples [12, 26, 52]. Another group of cytokines with opposite effects would be described such as inhibitory cytokines which include; Interleukin-4 (IL-4) and interleukin-10 (IL-10), IL-23, Interleukin-35 (IL-35) and transforming growth factor-β (TGF-β) [7, 16, 53–55].

IL-2 stimulates the proliferation and expansion of T cells, as well as B cells and NK cells [12]. IL-12 is interesting because it can activate NK cells that further produce other cytokines including IFN-γ, granzymes, and perforin to exert cytotoxic activity [6, 26, 56, 57]. Cytokines that initiate inflammation as well as those associated with chronic inflammation include IL-1, IL-6, and TNF-α and are associated with the role of macrophages and dendritic cells [21, 34]. Between them, TNF-α plays a significant role in various inflammatory processes and induces necrosis and apoptosis in the corresponding cells [8, 27–30].

Cytokines usually act specifically on individual receptors [36, 52]. However, bearing in mind that the family of cytokine receptors is really complex, individual cytokines can act on multiple receptors and individual receptors and can transmit signals between similar cytokines, so that pleiotropic effects of individual cytokines are obtained [58]. For this reason, the new term cytokine network is being intro-

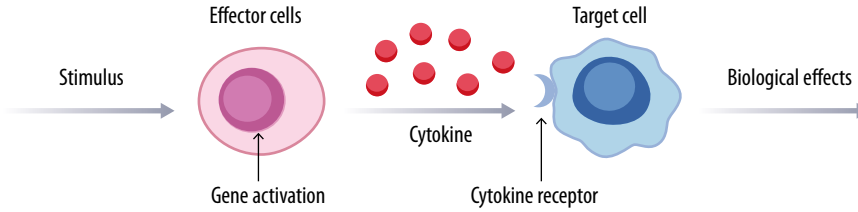

Fig. 1. Principle of cytokine action

duced today, in order to understand the complex action of cytokines [3, 9, 57, 59]. Recently, a group of immunoregulatory cytokines was identified, which have the ability to modulate the immune response depending on the need or state of the immune system [57]. At the same time, it was observed that individual cell populations do not produce only one cytokine but a group of cytokines, and then cytokines were divided according to the types of cells that produce cytokines and according to groups of cytokines such as Th1 and Th2 immune response [57, 59].

In hemato-oncology it is of interest to study groups of cytokines that play complex roles in the tumor-host relationship [7, 36, 46, 49, 60]. Some of these cytokines greatly help the processes of tumor proliferation, as well as the development of blood vessels and help in the vascularization of tumors and are included. Sometimes they are designated as negative or unfavorable regulators of tumor growth [8, 36, 37]. Likewise, tumor cells produce cytokines that act on the surrounding tissue, but also on the cells of the immune system, thus creating a complex interaction of the tumor tissue microenvironment [5, 41].

Significant cytokines in hematoimmunology

Growth factors

Growth factors are a special group of cytokines, which includes various molecules that have the ability to carry out proliferation, differentiation or migration of cells. Here we would discuss only some of the importance for immune-hematology [19, 38, 40, 49, 61].

Vascular endothelial growth factor (VEGF) is a cytokine that plays a significant role in the formation of new blood vessels and enables cell proliferation, discovered as early as 1980 [6, 38]. VEGF comprises a superfamily of cytokines and growth factors that are heterodimers and designated as VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E, while VEGF-F binds the heparin sulfate binding domain [37, 38, 44]. VEGF enables proliferation and migration of leukemic and multiple myeloma cells [9, 36, 44]. Its role and connection with the activity of osteoclast cells and with osteolysis in these tumors has been shown.

Members of the epidermal growth factor (EFR) family are cytokines that mainly exert effects on the proliferation of mesenchymal and epithelial cells [38]. The activity of EGF family members is mediated by the EGF R/ErbB receptor tyrosine kinases.

Hepatocyte growth factor (HGF) as multifactorial protein that is involved in the regulation of cell proliferation and survival, activation of osteoblast function, but also has an anti-apoptotic effect on myeloma cells [35, 42, 58, 61]. It achieves its effects by binding to its specific receptor c-MET, which is distributed on various tissues, including tumors of the hematopoietic system.

Insulin-like growth factor type 1 (IGF-1). Numerous studies have shown that the insulin-like growth factor receptor (IGF-IR) and its primary ligand IGF-I play an important role in tumor formation and progression, most likely by inhibiting apoptosis [62, 63]. IGF-IR is also thought to assist malignant cells in achieving cell detachment from the tumor matrix as well as aiding the process of tumor dissemination [64–66].

Fibroblast growth factor (FGF). The fibroblast growth factor (FGF) family consists of a number of FGF polypeptides that are potent mitogens [19, 67]. FGFs exert their mitogenic activity by stimulating the growth of fibroblasts, endothelium and many cancer cells including myeloma. These mediators play an important role during the embryonic development of organs, and in adults they enable the regeneration of tissue injuries and the maintenance of the functioning of various organ systems as well as the regulation of hematopoiesis [67–69]. In myeloma, FGF exhibits a mitogenic role together with other cytokines, mainly IL-6, based on the fact that FGF receptors (FGFRs) are expressed in myeloma cell lines, patient-derived multiple myeloma cells, and within the BM milieu. Among many growth factors, FGF2 is an important regulator of cell growth and differentiation under physiological and pathological conditions and it has been shown as a useful marker for tumor progression [19, 69].

The platelet-derived growth factor (PDGF) family consists of four structurally related polypeptides namely: PDGF-AA, PDGF-AB, PDGF-BB, and PDGF-CC with significant role in angiogenesis [19, 60]. Among them, angiogenin (ANG) has been reported to be a potent stimulator of angiogenesis by activating capillary endothelial cells after binding to action and consequently induced complex interaction with laminin (angiogenin) [70, 71]. This interaction induced destruction of basal membrane enabling endothelial cell migration during neovascularisation [71].

Methods for determination of cytokines

The discovery of cytokines in various fields of research was based first on the characterization, isolation and detailed description of their structure [72]. Later, with the development of methods of molecular biology and immunology, interest in the study of cytokines in many medical diseases, including oncology and hematology, increased significantly [73, 74].

First of all, tests were carried out in the blood and available fluids in order to study the clinical significance of certain cytokines, primarily in diagnostics because it was shown that cytokines mediate during the progression of the disease in the blood, serum, of bone marrow aspirate (Fig. 2). Certain cytokines, such as proinflammatory cytokines, increase their values during tumor progression and this has been used to monitor tumor development or therapy application including transplantation [75–80].

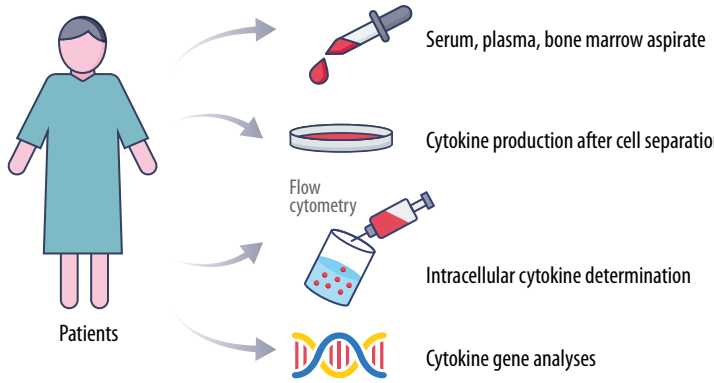

Fig. 2. Possibility of cytokine determination in hemato-immunology

It was interesting to see and predict the prognostic significance of certain cytokines [24, 80, 81]. It has been shown that the cytokine TNF is elevated in serum in multiple myeloma, although also in other hematological malignancies, and that it correlates well with the advanced stage of the disease, LDH and proinflammatory markers [24, 31]. During the progression of the disease in most hematological malignancies, with an increase in TNF in the serum, there is a decrease in NK cell function, cell destruction, as well as an increase in inhibitory receptors and a decrease in activation receptors on NK cells which indicate that cytokine values are associated with the regulation of non-specific immunity [82, 83].

Today, it is possible that in clinical practice, due to new techniques and methods, certain cytokines are regularly used and determined in daily work, which was undetectable a few years ago [68, 74, 76]. Thus, in addition to classic biochemical markers, cytokines are also determined to confirm the clinical stage and degree of tumor progression [32].

Scientific works have shown that the measured values in the serum can vary and depend on tumor localization, tumor size and certainly the type of tumor [24, 58]. It is very important for the cytokine values in the serum to have direct contact with the blood vessels because well-vascularized tumors as well as those with tissue necrosis show higher concentrations in the blood [31, 84].

In many scientific papers, the values were always compared and analyzed in relation to a healthy control group and then in relation to disease stages, clinical stage and the presence of other comorbidities or other inflammatory conditions [24].

Enzyme immunosorbent assay (ELISA) has been the gold standard in immunology for a long time and with the help of this test certain cytokines are specifically

determined with the help of standards as control values [24, 25, 32]. This test requires a classic biochemical spectrophotometer and antibodies that react with a specific cytokine as a standard. The principle of reading is to determine the changes in absorbance after the binding reaction of a specific antibody with the corresponding cytokine and to determine the concentration of the cytokine at a precisely determined wavelength based on the color change after the addition of the substrate [31].

ELISpot is a similar method to ELISA, but it does not quantitatively determine the amount of cytokines, but determines the number of positive cells for the investigated cytokine [85].

Determination of multiple cytokines simultaneously

The Luminex Texnology is a type of immunoassay that accurately measures multiple cytokines in a single sample (Fig. 3). Luminex is an modified immunoassay based on the use of beads that enables multiplex detection of up to 100 analytes simultaneously (86). Color-coded microspheres, or beads, are internally colored with varying proportions of red and infrared fluorophores that match the bead's precise spectrum. Quantification of multiple cytokines in a sample provides significant information about the mutual relationship of cytokines much more than the determination of only one cytokine in the sample [31].

Antibodies specific for the desired cytokine bind to a unique region of the beads and are incubated together with the sample. After washing to remove excess antibody, samples are further incubated with a mixture of biotinylated detection antibodies and streptavidin-phycoerythrin (PE) label. In order to obtain a result, a Luminex instrument with two lasers is required. One laser is for excitation and the other determines the output signal which is proportional to the amount of bound antibody [87].

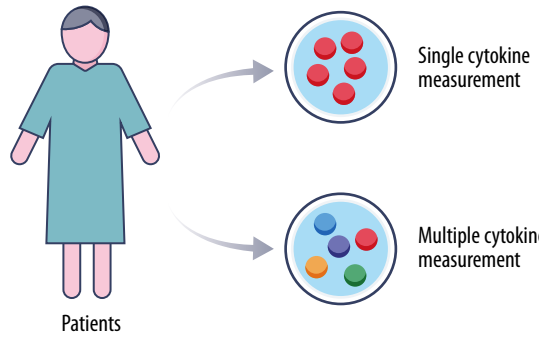

Fig. 3. Single and multiple cytokine detection

Flow cytomety for investigation of cytokine

Simultaneous determination of cytokines in serum, supernatant of cultured cells in vitro, or bone marrow aspirate obtained from hematological malignancies is possible with the help of a flow cytometer (see Fig. 2).

These bead-based immunoassays are similar in principle to sandwich ELISAs, but each bead population is conjugated to a specific capture antibody that binds to the cytokine of interest in the sample [88]. The amount of bound sample is detected by means of a biotinylated antibody against the secondary epitope of the protein, and the final step is the streptavidin-R-phycoerythrin reaction. The fluorescence intensity of R-phycoerythrin on the beads is quantified on a flow cytometer.

Intracellular cytokine determination

It is very important that cytokines are determined within the cells [32]. After the process of separating cells from the blood, or after isolating purified populations of lymphocytes using any technique, or from individual tumor cells, the intracellular amount of cytokines is very often determined [12]. This can be done with a special technique and the use of flow cytometry, as well as with the help of fluorescent monoclonal antibodies that bind to intracellular molecules [7, 18]. For intravellular cytokine determination, first of all, it is necessary to permeabilize the cell, that is, for antibodies to penetrate to the internal structure and bind to intracellular cytokines there. In practice, usually for hematological malignancies or for any other cell population, a combination of antibodies that mark a particular type of cell by binding to the membrane in combination with antibodies that penetrate inside the cell is used. In this way, we obtain the percentage of positive cell populations positive for cytokine determination. For example, we first label lymphocytes with an antibody for the membrane with the help of anti-CD3+ antibodies and then with the help of another cytokine antibody to determine some of the cytokines inside them. Thus, we can compare cytokine values between various cell populations of interest or, for example, before and after some kind of stimulation [89]. In this way, we can observe whether the cells respond to a certain stimulus, which in fact tests the functionality of the cells [16, 33]. It is very often used in hematology when we want to examine the functional ability of a cell population to see if cell differentiation or maturation is taking place [19, 90].

Cytokine gene analyses

Gene analysis of cytokines is important in hematology to understand signaling pathway disorders that exist in various hematopoietic cells with incorrect division, or during the application of certain therapies including target therapy [92, 93]. Quan-

titative PCR (Q-PCR) or "real-time PCR" methods are most often used techniques for these analyses. Cells can have various alterations in the expression of cytokines, cytokine receptors or cytokine production, which is essential for understanding the signaling process [3, 33, 35, 37, 49, 94]. In addition, the are attempts to treat signal transduction disturbance with appropriated blockers of these altered signaling pathways. Another possibility is to block the disturbed receptor expression. Today, these principles are designated as new directions in treatment, i.e. immunomodulation or immunomodulatory gene therapy [95].

Methods for data analyses

With the discovery of a large number of cytokines, a better understanding of the process of signal transmission through the cell, gene regulation of cytokines, there was a large amount of data that needed to be analyzed and properly interpreted [96–98]. That's why, with the development of technology, mathematical programs for data enrichment were also developed. Today, not only simple statistical tests are used that show the difference in the value of cytokines in certain processes, but complex mathematical models are used that can compare data from various databases, predict analyzes and integrate a large number of data [97]. All of this requires super-fast computers, servers where data is stored and processed. Some Institutions also develop their own programs and mathematical models, but then the problem is how to harmonize the various models. Some programs analyze data directly online by entering data into the existing database and the programs provide results. There are numerous statistical programs, among which are Oligo (Bioconductor package for data processing), Biobase (which contained a standardized data structure), array QualityMetrics and others. For example, ESTIMATE (Estimation of STromal and Immune cells in Malignant Tumor tissues using Expression data) is a tool between many other programs for predicting presence of infiltrating stromal/immune cells in tumor tissues generated by MD Anderson Center in USA [97].

Concusion

In the current development of science and medicine, cytokines are one of the very important parameters for the study of the immune system, but with the development of new technologies, an even better understanding of the complex effects on genes and their application in immunotherapy in many diseases of hematopoiesis is expected in the future.

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Reference

1. Isaacs A., Lindenmann J.J. Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci. 1957; 147(927): 258–67.
2. Cannon J.G., van der Meer J.W., Kwiatkowski D., et al. Interleukin-1 beta in human plasma: optimization of blood collection, plasma extraction, and radioimmunoassay methods. Lymphokine Res. 1988; 7: 457–67.
3. Kawano M., Hirano T., Matsuda T., et al. Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas. Nature. 1988; 332: 83–5.
4. Van der Meide P.H., Schellekens H. Cytokines and the immune response. Biotherapy. 1996; 8: 243–9.
5. Konjević G.M., Vuletić A.M., Mirjačić Martinović K.M., et al. The role of cytokines in the regulation of NK cells in the tumor environment. Cytokine. 2019; 117: 30–40. doi: 10.1016/j.cyto.2019.02.001.
6. Zumkeller W., Schofield P.N. Growth factors, cytokines and soluble forms of receptor molecules in cancer patients. Anticancer Res. 1995; 15: 344–48.
7. Castriconi R., Cantoni C., Della Chiesa M., et al. Transforming Growth Factor beta 1 Inhibits Expression of NKp30 and NKG2D Receptors: Consequences for the NK-mediated Killing of Dendritic Cells. Proc Natl Acad Sci U S A. 2003; 100(7): 4120–5.
8. Jurišić V., Spužić I., Konjević G. A comparison of the NK cell cytotoxicity with effects of TNF-α against K-562 cells, determined by LDH release assay. Cancer letters. 1999; 138(1–2): 67–72.
9. Klein B., Bataille R. Cytokine network in human multiple myeloma. Hematol Oncol Clin North Am. 1992; 6: 273–84.
10. Bergui L., Schena M., Gaidano G., et al. Interleukin 3 and interleukin 6 synergistically promote the proliferation and differentiation of malignant plasma cell precursors in multiple myeloma. J Exp Med. 1989; 170: 613–8.
11. Kodama H., Nose M., Niida S., Yamasaki A. Essential role of macrophage colony-stimulating factor in the osteoclast differentiation supported by stromal cells. J Exp Med. 1991; 173: 1291–4.
12. Mirjačić Martinović K., Babović N.L., Džodić R.R., et al. Beneficial in-vitro effects of interleukin-2, interleukin-12, and their combination on functional and receptor characteristics of natural killer cells in metastatic melanoma patients with normal serum lactate dehydrogenase levels. Melanoma Res. 2016; 26: 551–64.
13. Vuletić A., Jovanić I., Jurišić V., et al. IL-2 And IL-15 Induced NKG2D, CD158a and CD158b Expression on T, NKT- like and NK Cell Lymphocyte Subsets from Regional Lymph Nodes of Melanoma Patients. Pathol Oncol Res. 2020; 26(1): 223–31.

14. Dumonde D.C., Wolstencroft R.A., Panayi G.S., et al. Lymphokines: non-antibody mediators of cellular immunity generated by lymphocyte activation. Nature. 1969; 224 (5214): 38–42.
15. Buchbinder E.I., Mc Dermott D.F. Interferon, interleukin-2, and other cytokines. Hematol Oncol Clin North Am. 2014; 28: 571–83.
16. Voorzanger N., Touitou R., Garcia E., et al. Interleukin (IL)-10 and IL-6 are produced in vivo by non-Hodgkins Lymphoma Cells and act as cooperative growth factors. Cancer Res. 1996; 56: 5499–505.
17. Zlotnik A., Burkhardt A.M., Homey B. Homeostatic chemokine receptors and organ-specific metastasis. Nat Rev Immunol. 2011; 11(9): 597–606.
18. Wheelock E.F. Interferon-Like Virus-Inhibitor Induced in Human Leukocytes by Phytohemagglutinin. Science. 1965; 149(3681): 310–11.
19. Bayer A., Wijaya B., Rademacher F., et al. Platelet-Released Growth Factors Induce Genes Involved in Extracellular Matrix Formation in Human Fibroblasts. Int J Mol Sci. 2021; 22(19): 10536. doi: 10.3390/ijms221910536.
20. Jain M.D., Zhao H., Wang X., et al. Tumor interferon signaling and suppressive myeloid cells associate with CAR T cell failure in large B cell lymphoma. Blood. 2021; 137(19): 2621–33. doi: 10.1182/blood.2020007445.
21. Giavridis T., van der Stegen S.J.C., Eyquem J., et al. CAR T Cell-Induced Cytokine Release Syndrome is Mediated by Macrophages and Abated by IL-1 Blockade. Nat Med. 2018; 24(6): 731–8. doi: 10.1038/s41591-018-0041-7.
22. Rath T., Billmeier U., Waldner M.J., et al. From physiology to disease and targeted therapy: interleukin-6 in inflammation and inflammation-associated carcinogenesis. Arch Toxicol. 2015; 89: 541–54.
23. Jurisić V., Bogdanovic G., Srdic T., et al. Modulation of TNF-alpha activity in tumor PC cells using anti-CD45 and anti-CD95 monoclonal antibodies. Modulation of TNF-alpha activity in tumor PC cells using anti-CD45 and anti-CD95 monoclonal antibodies. Cancer Lett. 2004; 214: 55–61.
24. Jurisić V., Colović M. Correlation of sera TNF-alpha with percentage of bone marrow plasma cells, LDH, beta2-microglobulin, and clinical stage in multiple myeloma. Med Oncol. 2002; 19(3): 133–9. doi: 10.1385/MO:19:3:133.
25. Jurisic V., Terzic T., Colic S., Jurisic M. The concentration of TNF-alpha correlate with number of inflammatory cells and degree of vascularization in radicular cysts. Oral Dis. 2008; 14(7): 600–5.
26. Vuletić A., Jovanić A., Jurišić V., et al. In-vitro activation of natural killer cells from regional lymph nodes of melanoma patients with interleukin-2 and interleukin-15. Melanoma Res. 2015; 25: 22–34.
27. Jurisic V., Bogdanovic G., Kojic V., et al. Effect of TNF-alpha on Raji cells at different cellular levels estimated by various methods. Ann Hematol. 2006; 85(2): 86–94. doi: 10.1007/s00277-005-0010-3.

28. Jurisic V., Bumbasirevic V., Konjevic G., et al. TNF-alpha induces changes in LDH isotype profile following triggering of apoptosis in PBL of non-Hodgkin's lymphomas. Ann Hematol. 2004; 83(2): 84–91. doi: 10.1007/s00277-003-0731-0.
29. Jurisic V., Srdic-Rajic T., Konjevic G., et al. TNF-α induced apoptosis is accompanied with rapid CD30 and slower CD45 shedding from K-562 cells. J Membr Biol. 2011; 239(3): 115–22. doi: 10.1007/s00232-010-9309-7.
30. Jurisic V., Srdic-Rajic T., Konjevic G., et al. TNF-α induced apoptosis is accompanied with rapid CD30 and slower CD45 shedding from K-562 cells. J Membr Biol. 2011; 239: 115–22.
31. Jurisic V., Terzic T., Pavlovic S., et al. Elevated TNF-alpha and LDH without parathormone disturbance is associated with diffuse osteolytic lesions in leukemic transformation of myelofibrosis. Pathol Res Pract. 2008; 204(2): 129–32.
32. Jurisic V. Multiomic analysis of cytokines in immuno-oncology. Expert Rev Proteomics. 2020; 17(9): 663–74.
33. Lieksche G.J., Burgess A.W. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. N Engl J Med. 1992; 327: 28–34.
34. Mc Shane L.M., Polley M.Y. Development of omics-based clinical tests for prognosis and therapy selection: the challenge of achieving statistical robustness and clinical utility. Clin Trials. 2013; 10: 653–65.
35. Nemunaitis J., Andrews F., Mochizuki D., et al. Human marrow stromal cells: response to interleukin-6 (IL-6) and control of IL-6 expression. Blood. 1989; 74: 1929–36.
36. Borset M., Hjorth-Hansen H., Seidel C., et al. Hepatocyte growth factor and its receptor c-met in multiple myeloma. Blood. 1996; 88: 3998–4004.
37. Masood R., Cai J., Zheng T., et al. Vascular endothelial growth factor (VEGF) is an autocrine growth factor for VEGF receptor-positive human tumors. Blood. 2001; 98: 1904–13.
38. Karamysheva A.F., Shushanov S.S., Adelaide J., et al. Expression of the FLT4/VEGFR3 receptor tyrosine kinase encoding gene in hepatic tumors. Int J Oncol. 1996; 8: 921–24.
39. Larsen A.K., Ouaret D., El Ouadrani K., et al. Targeting EGFR and VEGF(R) pathway cross-talk in tumor survival and angiogenesis. Pharmacol Ther. 2011; 131: 80–90.
40. Colombo M., Galletti S., Bulfamante G., et al. Multiple myeloma-derived Jagged ligands increases autocrine and paracrine interleukin-6 expression in bone marrow niche. Oncotarget. 2016; 7(35): 56013–29.
41. Bellamy W.T., Richter L., Sirjani D., et al. Vascular endothelial cell growth factor is an autocrine promoter of abnormal localized immature myeloid pre-

cursors and leukemia progenitor formation in myelodysplastic syndromes. Blood. 2001; 97: 1427–34.
42. Klein B., Zhang X.G., Jourdan M., et al. Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by interleukin-6. Blood. 1989; 73: 517–26.
43. Derksen P.W.B., De Gorter D.J.J., Meijer H.P., et al. The hepatocyte growth factor/Met pathway controls proliferation and apoptosis in multiple myeloma. Leukemia. 2003; 17: 764–74.
44. Frassanito M.A., Cusmai A., Iodice G., Dammacco F. Autocrine interleukin-6 production and highly malignant multiple myeloma: relation with resistance to drug-induced apoptosis. Blood. 2001; 97: 483–9.
45. Keyt B.A., Nguyen H.V., Berleau L.T., et al. Identification of vascular endothelial growth factor determinants for binding KDR and FLT-1 receptors. Generation of receptor-selective VEGF variants by site-directed mutagenesis. J Biol Chem. 1996; 271: 5638–46.
46. Negaard H.F., Iversen N., Bowitz-Lothe I.M., et al. Increased bone marrow microvascular density in haematological malignancies is associated with differential regulation of angiogenic factors. Leukemia. 2009; 23: 162–9.
47. Reynaud D., Pietras E., Barry-Holson K., et al. IL6 controls leukemic multipotent progenitor cell fate and contributes to chronic myelogenous leukemia development. Cancer Cell. 2011; 20(5): 661–73.
48. Liu Z., Yuan X., Luo Y., et al. Evaluating the effects of immunosuppressants on human immunity using cytokine profiles of whole blood. Cytokine. 2009; 45: 141–7.
49. Li M.O., Wan Y.Y., Sanjabi S., et al. Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol. 2006; 24: 99–146.
50. Ebbo M., Crinier A., Vély F., Vivier E. Innate lymphoid cells: major players in inflammatory diseases. Nat Rev Immunol. 2017; 17(11): 665–78.
51. Blobe G.C., Schiemann W.P., Lodish H.F. Role of transforming growth factor beta in human disease. N Engl J Med. 2000; 342: 1350–8.
52. Lieksche G.J., Burgess A.W. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. N Engl J Med. 1992; 327: 28–34.
53. Depper J.M., Leonard W.J., Drogula C., et al. Interleukin2 (IL-2) augments transcription of the IL2 receptor gene. Proc Natl Acad Sci. U S A. 1985; 82: 4230–4.
54. Vuletić A.M., Konjević G.M., Larsen A.K., et al. Interleukin-4-induced natural killer cell antitumor activity in metastatic melanoma patients. Eur Cytokine Netw. 2020. doi: 10.1684/ecn.2020.0449.
55. Castellani M.L., Anogeianaki A., Felaco P., et al. IL-35, an anti-inflammatory cytokine which expands CD4+CD25+ Treg Cells. J Biol Regul Homeost Agents. 2010; 24: 131–5.

56. Guo L., Junttila I.S., Paul W.E. Cytokine-induced cytokine production by conventional and innate lymphoid cells. Trends Immunol. 2012; 33: 598.
57. Fort M.M., Cheung J., Yen D., et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity. 2001; 15: 985.
58. Kara I.O., Sahin B., Günesacar R., Unsal C. Clinical significance of hepatocyte growth factor, platelet-derived growth factor-AB, and transforming growth factor-α in bone marrow and peripheral blood of patients with multiple myeloma. Adv Ther. 2006; 23(4): 635–45.
59. Moro K., Yamada T., Tanabe M. et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. Nature. 2010; 463: 540.
60. Andrae J., Gallini R., Betsholtz C. Role of platelet-derived growth factors in physiology and medicine. Genes Dev. 2008; 15(22): 1276–312.
61. Borset M., Hjorth H.H., Seidel C., et al. Hepatocyte growth factor in patients with multiple myeloma. The Nordic Myeloma Study Group. Blood. 1988;91:806–12.
62. Li W., Hyun T., Heller M., et al. Activation of insulin-like growth factor I receptor signaling pathway is critical for mouse plasma cell tumor growth. Cancer Res. 2000; 60: 3909–15.
63. Resnicoff M., Abraham D., Yutanawiboonchai W., et al. The insulin-like growth factor I receptor protects tumor cells from apoptosis in vivo. Cancer Res. 1995; 55: 2463–9.
64. Pollak M. The insulin and insulin-like growth factor receptor family in neoplasia: an update. Nat Rev Cancer. 2012; 12: 159–69.
65. Bieghs L., Johnsen H.E., Maes K., et al. The insulin-like growth factor system in multiple myeloma: diagnostic and therapeutic potential. Oncotarget. 2016; 7(30): 48732–52. doi: 10.18632/oncotarget.8982.
66. Sprynski A.C., Hose D., Caillot L., et al. The role of IGF-1 as a major growth factor for myeloma cell lines and the prognostic relevance of the expression of its receptor. Blood. 2009; 113(19): 4614–26.
67. Bisping G., Leo R., Wenning D., et al. Paracrine interactions of basic fibroblast growth factor and interleukin-6 in multiple myeloma. Blood. 2003;101: 2775–83.
68. Sato N., Hattori Y., Wenlin D., et al. Elevated level of plasma basic fibroblast growth factor in multiple myeloma correlates with increased disease activity. Jpn J Cancer Res. 2002;93: 459–66.
69. Bisping G., Leo R., Wenning D., et al. Paracrine interactions of basic fibroblast growth factor and interleukin-6 in multiple myeloma. Blood. 2003;101: 2775–83.
70. Alexandrakis M.G., Passam F.H., Sfiridaki A., et al. Elevated serum angiogenin in multiple myeloma. Hematol J. 2003;4: 454–5.

71. Verstovsek S., Kantarjian H., Aguayo A., et al. Significance of angiogenin in plasma concentrations in patients with acute myeloid leukaemia and advanced myelodysplastic syndrome. Br J Haematol. 2001;114: 290–5.
72. Shi M., Zhu J., Wang R., et al. Latent TGF-β structure and activation. Nature. 2011;474(7351): 343–9.
73. House R.V. Theory and practice of cytokines assessment in immunotoxicology. Methods. 1999;19: 17–27.
74. Yeung D., Ciotti S., Purushothama S., et al. Evaluation of highly sensitive immunoassay technologies for quantitative measurements of sub-pg/ml levels of cytokines in human serum. J Immunol Methods. 2016;437: 53–63.
75. Jain M.D., Zhao H., Wang X., et al. Tumor interferon signaling and suppressive myeloid cells associate with CAR T cell failure in large B cell lymphoma. Blood. 2021;137(19): 2621–33.
76. Pirogova O.V., Moiseev I.S., Surkova E.A., et al. Profiles of proinflammatory cytokines in allogenic stem cell transplantation with post-transplantation cyclophosphamide. Cytokine. 2017;99: 148–153.
77. Kaya Z., Yüce D., Kirkiz S., et al. Prognostic role of serum cytokines and soluble HLA-G levels in children with leukemia who undergo allogeneic stem cell transplantation. Cytokine. 2022; 153: 155869. doi: 10.1016/j.cyto.2022.155869.
78. Schots R., Kaufman L., Van Riet I., et al. Proinflammatory cytokines and their role in the development of major transplant-related complications in the early phase after allogeneic bone marrow transplantation. Leukemia. 2003; 17(6): 1150–6.
79. Guillaume T., Sekhavat M., Rubinstein D.B., et al. Defective cytokine production following autologous stem cell transplantation for solid tumors and hematologic malignancies regardless of bone marrow or peripheral origin and lack of evidence for a role for interleukine-10 in delayed immune reconstitution. Cancer Res. 1994; 54: 3800.
80. Kučera J., Strnadová K., Dvořánková B., et al. Serum proteomic analysis of melanoma patients with immunohistochemical profiling of primary melanomas and cultured cells: Pilot study. Oncol Rep. 2019; 42(5): 1793–804.
81. Kupsa T., Vasatova M., Karesova I., et al. Baseline serum levels of multiple cytokines and adhesion molecules in patients with acute miyeloid leukemia: results of a pivotal trial. Exp Oncol. 2014; 36: 252–7.
82. Jurisic V., Srdic T., Konjevic G., et al. Clinical stage-depending decrease of NK cell activity in multiple myeloma patients. Med Oncol. 2007; 24(3): 312–7.
83. Konjević G., Vuletić A., Mirjačić Martinović K., et al. Decreased CD161 activating and increased CD158a inhibitory receptor expression on NK

cells underlies impaired NK cell cytotoxicity in patients with multiple myeloma. J Clin Pathol. 2016: jclinpath-2016-203614. doi: 10.1136/jclinpath-2016-203614.
84. Riches P., Gooding R., Millar B.C., Rowbottom A.W. Influence of collection and separation of blood samples on plasma IL-1, IL-6 and TNF-alpha concentrations. J Immunol Methods. 1992; 153: 125–31.
85. Fujihashi K., McGhee J.R., Beagley K.W., et al. Cytokine-specific ELISPOT assay. Single cell analysis of IL-2, IL-4 and IL-6 producing cells. J Immunol Methods. 1993; 160(2): 181–9.
86. Robak P., Węgłowska E., Dróżdż I., et al. Cytokine and Chemokine Profile in Patients with Multiple Myeloma Treated with Bortezomib. Mediators Inflamm. 2020; 2020: 1835836. doi: 10.1155/2020/1835836.
87. Jani I.V., Janossy G., Brown D.W., Mandy F. Multiplexed immunoassays by flow cytometry for diagnosis and surveillance of infectious diseases in resource-poor settings. Lancet Infect Dis. 2002; 2: 243–50.
88. Schuerwegh A.J., de Clerck L.S., Bridts C.H., Stevens W.J. Comparison of intracellular cytokine production with extracellular cytokine levels using two flow cytometric techniques. Cytometry B Clin Cytom. 2003; 55: 52–8.
89. Damle R.N., Advani S.H., Gangal S.G. Impairment in proliferation, lymphokine production and frequency distribution of mitogen-responsive and interleukin-2-producing cells in Hodgkin's disease. Cancer Immunol Immunother. 1991; 34: 205–10.
90. Wang K.S., Frank D.A., Ritz J. Interleukin-2 enhances the response of natural killer cells to interleukin-12 through up-regulation of the interleukin-12 receptor and STAT4. Blood. 2000; 95: 3183–90.
91. Mangan B.A., Dunne M.R., O'Reilly V.P., et al. Cutting edge: CD1d restriction and Th1/Th2/ Th17 cytokine secretion by human Vdelta3 T cells. J Immunol. 2013; 191(1): 30–4.
92. Hata H., Xiao H., Petrucci M.T., et al. Interleukin-6 gene expression in multiple myeloma: a characteristic of immature tumor cells. Blood. 1993; 81: 3357–64.
93. Petersen S.L., Madsen H.O., Ryder L.P., et al. Cytokine gene expression in peripheral blood mononuclear cells and alloreactivity in hematopoietic cell transplantation with nonmyeloablative conditioning, Biol Blood Marrow Transplant. 2006; 12: 48–60.
94. Yagi R., Zhong C., Northrup D.L., et al. The transcription factor GATA3 is critical for the development of all IL-7Rα expressing innate lymphoid cells. Immunity. 2014; 40: 378.
95. Mikulski D., Robak P., Perdas E., et al. Pretreatment Serum Levels of IL-1 Receptor Antagonist and IL-4 Are Predictors of Overall Survival in Multiple

Myeloma Patients Treated with Bortezomib. J Clin Med. 2021; 11(1): 112. doi: 10.3390/jcm11010112.
96. Huang D.W., Sherman B.T., Lempicki R.A. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009; 37: 1–13.
97. Perez De Souza L., Alseekh S., Brotman Y., et al, Expert Rev Proteomics. Network-based strategies in metabolomics data analysis and interpretation: from molecular networking to biological interpretation. 2020; 17(4): 243–55.
98. Siegel R.L., Miller K.D., Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015; 65(1): 5–29.

MINIMAL RESIDUAL DISEASE IN ACUTE MYELOID LEUKEMIA IN CHILDREN

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ABSTRACT

Acute myeloid leukemia (AML) is a heterogeneous group of neoplastic clonal diseases of the hematopoietic system, each of which is characterized by a block in the differentiation of myeloid precursors, their uncontrolled proliferation and accumulation in the bone marrow, blood, liver, spleen, and, less commonly, in other organs. In the context of modern methods of treatment, the assessment of minimal residual disease (MRD) in the bone marrow by flow cytometry is becoming increasingly important. The article discusses modern approaches to the definition of MRD and presents our own data.

Introduction

Acute myeloid leukemia (AML) of childhood is one of the most complex groups of hematological tumors, which still does not have reliable cures. This is an extremely heterogeneous group of diseases: the classification of AML began in 1976, when the first FAB classification was published, which included 6 morphocytochemical variants of AML [1]. When revising the classification in 1985, 8 variants were identified [2, 3]. To date, in connection with the division of AML into subgroups of diseases with different prognostic and pathogenetic features, about 20 variants of AML have been identified [4]. Some of these variants are rare, others are more common, but in general AML is much less common in children than acute lymphoblastic leukemias (approximately 15–20 % of acute leukemias), and this, combined with the heterogeneity of the disease, makes the search for diagnostic and therapeutic agents more complex [4]. It is important to note that significant progress has been achieved in the treatment of AML in children, which is due to both the

emergence of new therapeutic regimens and improvement of supportive care, and to the individualization of therapy depending on the risk groups.

To date, some of the risk factors that were previously used in determining the tactics of AML treatment have lost their significance. Relevant prognostic factors are the presence of hyper leukocytosis at diagnosis, cytogenetic and molecular aberrations of the tumor clone, and the response to the first induction course. Failure to achieve remission at the end of the induction course of treatment is an important predictor of poor prognosis even if complete remission is achieved with subsequent treatment [5–7]. The bone marrow remission is documented based on the presence of less than 5 % of blast cells in the bone marrow. At the same time, 20–41 % of patients under 18 years of age who achieve bone marrow remission after induction subsequently develop a relapse [8]. Currently, there are methods that can significantly improve the detection threshold for malignant myeloblasts in the bone marrow. This is, first of all, flow cytometry, which makes it possible to detect one malignant cell per 100,000 or more myelokaryocytes. Moreover, the flow cytometry method allows to accurately monitor the dynamics of the tumor process in the bone marrow, to monitor the presence of a residual leukemic population. Such in-depth studies are absolutely necessary, since it has been proven that the presence of minimal residual disease is a powerful predictor of relapse, and timely modification of treatment, including its intensification using high-dose chemotherapy, targeted drugs, and allogeneic hematopoietic stem cell transplantation, in some cases, can stop the disease and prolong the patient's life. In this regard, the search for immunological methods for AML malignant cells detection is one of the most urgent tasks of pediatric oncohematology.

Morphological enumeration of bone marrow cells remains the standard approach to assessing response to treatment, both in routine practice and in clinical trials. The results of the morphology study allow one to determine the tactics of further treatment. However, the accuracy of counting the blast population using light microscopy is hampered by the limited sensitivity of the method and its relative subjectivity. Thus, a recent study conducted on a large cohort of AML in children (n = 203) showed significant inconsistencies in the assessment of remission between the morphological method and flow cytometry. Some patients had less than 5 % blast cells with morphology, however, according to flow cytometry, more than 5 % of blast cells remained in their bone marrow. On the contrary, 67 % of patients who were morphologically regarded as having a partial response (from 5 to 15 % of blasts), and 26 % of those who were found to be primarily resistant (more than 15 % of blast cells), according to flow cytometry, had a complete response, minimal residual disease was not determined [9, 10]. Several other studies have also shown similar inconsistencies between the morphological method and flow cytometry, with the latter method having a greater impact on prognosis [11, 12].

Modern intensive regimens in most cases lead to a rapid decrease in leukemia cells, and the tumor becomes unrecognizable by light microscopy. Flow-cytometric determination of the number of residual aberrant cells makes it possible to assess the dynamics of the tumor load in the setting of bone marrow remission. The determination of MRD at this stage of treatment is sometimes the only indicator of the dynamics of tumor cell clearance. To date, most studies are devoted to the determination of MRD in the early stages of specific therapy [11, 13–16]. Many foreign clinical studies have shown that MRD-negative status (in part of the works - undetectable MRD) is a favorable prognostic factor [17–19].

In acute myeloid leukemia, the criterion for flow cytometric MRD status has only recently been introduced into treatment guidelines [20]. The determination of MRD status in AML is more difficult than in acute lymphoblastic leukemia and B-cell chronic lymphocytic leukemia due to the high heterogeneity of the immunological characteristics of myeloid blast cells. Tumor cells in AML differ from normal myeloid progenitors; these differences can be detected using flow cytometry — when looking for signs of an aberrant immunophenotype. These include co-expression of antigens that are not characteristic of the myeloid line of differentiation; the intensity of expression of myeloid lineage antigens, which is different from that characteristic of normal myeloblasts; combination of antigens of early stages of differentiation with antigens of mature granulocytes on tumor cells. An example of the first type of aberration would be CD56 co-expression. Expression on myeloblasts of markers of mature granulocytes, such as CD11b, CD15, absence of CD34, HLA-DR are also signs of aberration in particular cases. The assessment of minimal residual disease in AML can be based on one of the principles: the first is the search for cells with an individual leukemia-associated immunophenotype (LAIF) corresponding to the immunophenotype at diagnosis, and the second is the "different from normal" method, or counting all immunologically different myeloid cells [11, 20, 21]. The application of the first principle requires a detailed study of the tumor immunophenotype at the onset of the disease. Its limitation is the phenomenon of "immunological shif": according to some studies, the immunophenotype of AML in relapse often does not coincide with that at diagnosis [22, 23]. Thus, it can be assumed that the assessment of MRD using the first method is most applicable at the early stages of the treatment of myeloid leukemia. The limitation of the second method is the possibility of the appearance of small populations of myeloid progenitors with aberrant immunophenotype in the bone marrow [24–26]. That may be associated with cytostatic, targeted therapy, as well as with the use of colony-stimulating factors. Also, the appearance of cells with an aberrant immunophenotype is a characteristic of dysplastic changes and myelodysplastic syndrome [27]; thus, it becomes difficult to distinguish spontaneously occurring abnormalities of the myeloblast immunophenotype from those characterizing the tumor population.

Materials and methods

In our work, minimal residual disease was analyzed in 28 children with AML. The group included 12 boys and 16 girls aged 5 months to 17 years (median age 5 years) who received treatment for newly diagnosed acute myeloid leukemia in the department of chemotherapy for haemoblastoses of the Research Institute of Pediatric Oncology and Hematology of the Federal State Budgetary Institution "N.N. Blokhin National Medical Research Center of Oncology" of the Ministry of Health of the Russian Federation (26 patients) and Morozov Children's City Clinical Hospital (2 patients) in the period from 2014 to 2021 according to the protocol of the Research Institute for NII DOG AML 2012. 2 patients had Down syndrome. In 2 patients, acute myeloid leukemia was associated with previous chemotherapy for other malignant neoplasms: the first diagnosis of a 10-year-old patient was T2N0M0 nephroblastoma, the first diagnosis of a 6-year-old patient was small round cell sarcoma with lesions of the posterior segments of X, XI ribs and pleura on the right, posterior sections of the left orbit. 6 children of the analyzed group underwent allogeneic transplantation. In all patients of the group, at the time of determining the minimum residual disease, bone marrow remission was achieved. At the same time, some patients showed incomplete recovery of blood parameters (neutropenia $< 1 \times 10^9$/l, thrombocytopenia $< 100 \times 10^9$/l). In most patients of the group, the level of MRD was assessed before the start of the 2nd course of chemotherapy with epigenetic drugs; in 5 patients, MRD was assessed at a later date. When determining the minimal residual disease, the panel of antibodies was compiled individually for each case according to the primary immunophenotype of the patient's blast cells. In each case, aberrations or features of the immunophenotype were noted, which made it possible to distinguish between normal myeloid precursors and tumor ones. 2 million events were analyzed. By sequential gating, a population of cells with immunological aberration corresponding to that in the primary study was isolated. The assessment of MRD status in the work was carried out relative to the value of 0.1 % of myelokaryocytes (with the number of aberrant myeloid cells of more than 0.1 % of myelokaryocytes — MRD-positive status), which corresponds to the strategy of most research groups [20, 28–30].

Results

We evaluated the relationship of clinical, haematological and immunophenotypic factors with relapse-free (RFS) and overall (OS) survival of patients with AML. Overall and relapse-free survival were assessed in 28 patients of the group. There were no relationships between the gender of patients with OS (log-rank $p = 0.52$) and RFS (log-rank $p = 0.67$). The age of the patients in the group ranged from

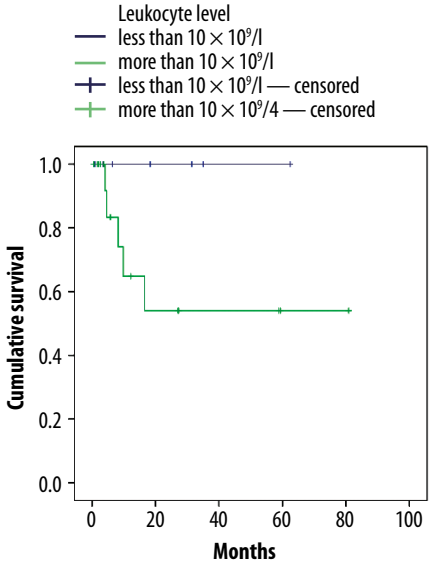

Fig. 1. Relapse-free survival of patients depending on the level of leukocytes at diagnosis:
blue line — < 10 × 10^9/*n*, *n* = 7; green line — > 10 × 10^9/*n*, *n* = 18

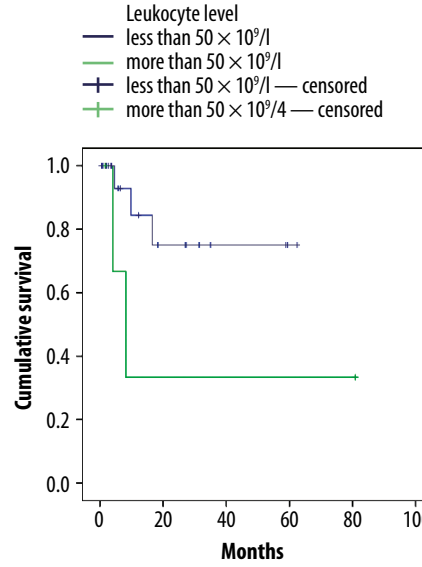

Fig. 2. Relapse-free survival depending on the presence of hyperleukocytosis at diagnosis:
blue line — < 50 × 10^9/*n*, *n* = 21; green line — > 50 × 10^9/*n*, *n* = 4

5 months to 17 years, 2 patients were younger than 1 year. The relationship of age less than and more than 1 year and less than and more than 5 years children with the duration of OS and RFS was not found (for 1 year RFS: log-rank $p = 0.43$, OS: log-rank $p = 0.62$, and for 5 years RFS: log-rank $p = 0.51$, OS: log-rank $p = 0{,}69$). The level of leukocytes at diagnosis ranged from 4.0 × 10^9/l to 133 × 10^9/l (median 19.2 × 10^9/l), the level of leukocytes more than 10 × 10^9/l was observed in 18 out of 26 patients, hyperleukocytosis above 50 × 10^9/l was noted in 4 out of 26 patients. When analyzing the relationship between the level of leukocytes at the diagnosis of more and less than 10 × 10^9/l, differences in OS (log-rank $p = 0.92$) were not revealed. The relationship between the level of leukocytes and the duration of RFS was near to significant (log-rank $p = 0.11$) (Fig. 1).

When analyzing the relationship between the levels of leukocytes at the diagnosis of more and less than 50 × 10^9/l, there were close to significant differences between the groups in terms of the duration of the RFS (log-rank $p = 0.067$) (Fig. 2).

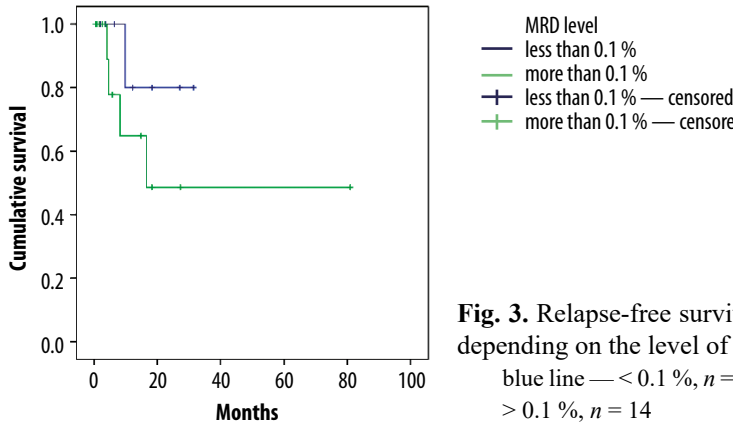

Fig. 3. Relapse-free survival of patients depending on the level of MRD:
blue line — < 0.1 %, $n = 9$; green line — > 0.1 %, $n = 14$

At the same time, hyperleukocytosis above 50 × 10^9/l did not have a significant effect on overall survival: log-rank $p = 0.38$. We also assessed the relationship between MRD levels greater than and less than 0.1 % and RFS among those patients who had the level of minimal residual disease determined simultaneously with the first bone marrow remission, $n = 23$ (log-rank $p = 0.157$) (Fig. 3).

OS was almost the same in groups of patients with MRD levels of more and less than 0.1 % of myelokaryocytes, log-rank $p = 0.55$. Allogeneic HSCT was performed in 6 patients of the group due to unfavorable prognosis factors; in each of these patients, the level of MRD during the first remission was more than 0.1 %. It is interesting to note that at the initial stages of the work, we evaluated 5 patients for the presence of MRD at a later time after the start of treatment (8 months, 2, 6, 4, 5 months from remission achievement date). The evaluation was carried out during the period of bone marrow remission. Two of these patients were MRD-positive. It is clear that after the end of induction therapy in these patients, the MRD status was positive. Evaluation of survival for the entire group of patients showed interesting results. Patients with MRD had significantly lower disease-free survival rates (log-rank $p = 0.09$). Thus, the persistence of MRD in combination with other factors of poor prognosis may be a criterion for restratification of patients into a higher risk group.

Conclusion

Assessment of minimal residual disease in acute myeloid leukemia in children is a rather difficult task. This is due to the complexity and heterogeneity of the im-

munophenotypes of acute myeloid leukemia, which actually includes 8 different morphocytochemical variants of leukemia. We used a comprehensive and standardized approach to the immunological diagnosis of AML, based on the concept of Euroflow, which includes the use of immunological markers for the diagnosis of all types of leukemia (including megakaryoblastic, erythroblastic, and also blast plasmacytoid dendritic cells neoplasm, which is not included into the FAB classification). We used markers of immature cells, general myeloid markers, markers of various lines of myelopoiesis, and antigens that are not characteristic of myeloid cells, which can be considered aberrant and used later in the diagnosis of MRD. Undoubtedly, the use of a larger panel of markers in the diagnosis and treatment of AML increases sensitivity, reliability, and provides grounds for a wider introduction of MRD studies in children, which is important in improving the prognosis for this disease. The applied approach can be considered successful, since it made it possible already at the stage of AML diagnosis to determine which set of markers can be used in each specific case for the diagnosis of MRD.

References

1. Bennett J.M., Catovsky D., Daniel M.T., et al. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol. 1976;33(4):451–8. doi: 10.1111/j.1365-2141.1976.tb03563.x.
2. Bennett J.M., Catovsky D., Daniel M.T., et al. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French–American–British Group. Ann Intern Med. 1985;103:620–5.
3. Bennett J.M., Catovsky D., Daniel M.T., et al. Proposal for the recognition of minimally differentiated acute myeloid leukaemia (AML-MO). Br J Haematol. 1991;78:325–9.
4. WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. Eds. S.H. Swerdlow, E. Campo, N.L. Harris, et al. 4th ed. Lyon: IARC, 2017.
5. Maschan A.A., Maschan M.A., Novichkova G.A., et al. Clinical guidelines "Acute myeloid leukemia". Ministry of Health of the Russian Federation, 2020.
6. Rubnitz J.E. Current Management of Childhood Acute Myeloid Leukemia. Pediatric Drugs. 2017;19(1):1–10. doi: 10.1007/s40272-016-0200-6.
7. Wheatley K., Burnett A.K., Goldstone A.H., et al. A simple, robust, validated and highly predictive index for the determination of risk-directed therapy in acute myeloid leukaemia derived from the MRC AML 10 trial. United Kingdom Medical Research Council's Adult and Childhood Leukaemia Working Parties. Br J Haematol. 1999;107:69–79.

8. Buldini B., Maurer-Granofszky M., Varotto E., et al. Flow-Cytometric Monitoring of Minimal Residual Disease in 121 Pediatric Patients with Acute Myeloid Leukemia: Recent Advances and Future Strategies. Front Pediatrician. 2019;117:412. doi: 10.3389/fped.2019.00412.
9. Inaba H., Coustan-Smith E., Cao X., et al. Comparative analysis of different approaches to measure treatment response in acute myeloid leukemia. J Clin Oncol. 2012;30(29):3625–32. doi: 10.1200/JCO.2011.41.5323.
10. Ouyang J., Goswami M., Tang G., et al. The clinical significance of negative flow cytometry immunophenotypic results in a morphologically scored positive bone marrow in 127 patients following treatment for acute myeloid leukemia. Am J Hematol. 2015;90(6):504–10. doi: 10.1002/ajh.23988.
11. Loken M.R., Alonzo T.A., Pardo L., et al. Residual disease detected by multidimensional flow cytometry signifies high relapse risk in patients with de novo acute myeloid leukemia: a report from Children's Oncology Group. Blood. 2012;120(8):1581–8. doi: 10.1182/blood-2012-02-408336.
12. Döhner H., Estey E., Grimwade D., et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424–47.
13. San Miguel J.F., Vidriales M.B., Lopez-Berges C., et al. Early immunophenotypical evaluation of minimal residual disease in acute myeloid leukemia identifies different patient risk groups and may contribute to postinduction treatment stratification. Blood. 2001;98:1746–51.
14. Kern W., Voskova D., Schoch C., et al. Prognostic impact of early response to induction therapy as 126 assessed by multiparameter flow cytometry in acute myeloid leukemia. Haematologica. 2004;89(5):528–40.
15. Köhnke T., Sauter D., Ringel K., et al. Early assessment of minimal residual disease in AML by flow cytometry during aplasia identifies patients at increased risk of relapse. Leukemia. 2015;29(2):377–86. doi: 10.1038/leu.2014.186.
16. Wood B. Principles of Minimal Residual Disease Detection for 135 Hematopoietic Neoplasms by Flow Cytometry. Cytometry B Clin Cytom. 2016;90(1):47–53. doi: 10.1002/cyto.b.21239.
17. Hourigan C.S., Gale R.P., Gormley N.J., et al. Measurable residual disease testing in acute myeloid 122 leukaemia. Leukemia. 2017;31(7):1482–90. doi: 10.1038/leu.2017.113.
18. Buckley S.A., Wood B.L., Othus M., et al. Minimal residual disease prior to allogeneic hematopoietic cell transplantation in acute myeloid leukemia: a meta-analysis. Haematologica. 2017;102(5):865–73. doi: 10.3324/haematol.2016.159343.
19. Walter R.B., Buckley S.A., Pagel J.M., et al. Significance of minimal residual disease before myeloablative allogeneic hematopoietic cell transplantation

for AML in first and second complete remission. Blood. 2013;122(10):1813–21. doi: 10.1182/blood-2013-06-506725.
20. Schuurhuis G.J., Heuser M., Freeman S., et al. Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. Blood. 2018;131(12):1275–91. doi: 10.1182/blood-2017-09-801498.
21. Grimwade D., Freeman S.D. Defining minimal residual disease in acute myeloid leukemia: which platforms are ready for "prime time"? Blood. 2014;124(23):3345–55. doi: 10.1182/blood-2014-05-577593.
22. Baer M.R., Stewart C.C., Dodge R.K., et al. High frequency of immunophenotype changes in acute myeloid leukemia at relapse: implications for residual disease detection (Cancer and Leukemia Group B Study 8361). Blood. 2001;97:3574–80.
23. Langebrake C., Brinkmann I., Teigler-Schlegel A., et al. Immunophenotypic differences between diagnosis and relapse in childhood AML: implications for MRD monitoring. Cytometry B Clin Cytom. 2005;63:1–9.
24. Hanekamp D., Bachas C., van de Loosdrecht A., et al. Myeloblasts in normal bone marrows expressing leukaemia-associated immunophenotypes. Pathology. 2020;52(2):289–91.
25. Wood B. Principles of Minimal Residual Disease Detection for 135 Hematopoietic Neoplasms by Flow Cytometry. Cytometry B Clin Cytom. 2016;90(1):47–53. doi: 10.1002/cyto.b.21239.
26. Ngai L., Kelder A., Janssen J., et al. MRD Tailored Therapy in AML: What We Have Learned So Far. Front Oncol. 2021;10:1–14. doi: 10.3389/fonc.2020.603636.
27. Valent P., Orazi A., Steensma D.P., et al. Proposed minimal diagnostic criteria for myelodysplastic syndromes (MDS) and potential pre-MDS conditions. Oncotarget. 2017;8(43):73483–500. doi: 10.18632/oncotarget.19008.
28. Langebrake C., Creutzig U., Dworzak M., et al. Residual disease monitoring in childhood acute myeloid leukemia by multiparameter flow cytometry: the MRD-AML-BFM Study Group. J Clin Oncol. 2006;24(22):3686–92. doi: 10.1200/JCO.2005.05.4312.
29. Rubnitz J.E., Inaba H., Dahl G., et al. Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial. Lancet Oncol. 2010;11(6):543–52. doi: 10.1016/S1470-2045(10)70090-5.
30. Tierens A., Bjørklund E., Siitonen S., et al. Residual disease detected by flow cytometry is an independent predictor of survival in childhood acute myeloid leukaemia; results of the NOPHOAML 2004 study. Br J Haematol. 2016;174(4):600–9. doi: 10.1111/bjh.14093.

UMBILICAL CORD BLOOD AND BONE MARROW AS A SOURCE OF NATURAL KILLERS FOR KIR-ALLOREACTIVE ADOPTIVE IMMUNOTHERAPY

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ABSTRACT

Chemotherapy, including dose escalation, has reached its limit in cancer treatment. One possible cause is quiescent and slow growth of cancer stem cells. These cells escape from chemotherapy and radiotherapy and make a risk of treatment failure. Remission of disease is maintained as long as the cancer stem cells are under the control of the patient's immune system. In cases of dysfunction or lack of anticancer immune response cancer progression occurs. Thereby immunotherapy with modulation of immune response opens up new prospects of effective cancer treatment. In the article we present the results of KIR-alloreactive adoptive immunotherapy with umbilical cord blood and bone marrow as a source.

Immunotherapy is a type of treatment that modulates the immune response (stimulate or suppress) to help the body fight cancer, infection and other diseases. Unlike chemotherapy, which acts directly on cancer fast-growing cells, immunotherapy treats patients by boosting the person's immune response. In some cases, the immune system doesn't see the cancer cells as foreign. In other cases, it recognizes the cancer cells, but the response might not be strong enough to destroy the cancer. In addition, cancer cells themselves can produce substances that keep the immune system from finding and attacking them. **Now we have two fundamentally different types of immunotherapies:** drug-mediated (DMI) and adoptive (cellular, AI).

In the late 1800s William Coley lost his very first patient with bone sarcoma. A young women died in 2,5 mo after amputation of her right arm below the elbow. Shaken by this failure, Coley started to look for any approaches to the treatment of sarcoma. In the historical literature and hospital records he found a numerous of cases with spontaneous tumor regression associated with acute infections. Trying to simulate an acute infection with system immune response, he developed "Coley's toxins" — vaccine containing two killed bacteria (Streptococcus pyogenes

and Serratia marcescens) [1, 2]. The vaccine was used as a treatment in inoperable cases and as prophylactic after surgical removing of sarcoma of any region. The 5-year survival for inoperable microscopically verified sarcomas was 13–79 % (the range varying with tumor subtype). According to the retrospective study patients receiving modern therapies have similar 10-year survival rates with patients treated with Coley's vaccine (data from the Surveillance Epidemiology End Result cancer registry) [3]. But despite good treatment outcomes, Coley's toxins were criticized by colleagues. And after his death the vaccine disappeared from use, especially considering rapidly developing of radiotherapy and chemotherapy. Today clinical cases with spontaneous tumor regression became less common (probable causes: sterile surgery, wild use drugs suppressing immune response — antibiotics, antipyretics, highly immunosuppressive chemotherapy). One of the last cases of spontaneous regression was a couple of years ago after COVID-19 infection [4].

Modern drug-mediated immunotherapy is quite diverse. It includes monoclonal antibodies, immune checkpoint inhibitors, cytokines etc. Drug-mediated immunotherapy is widely used in various types of tumors, indications for DMI continue to expand.

Adoptive cell therapy, also known as cellular immunotherapy, is a form of treatment that uses the cells of immune system to eliminate cancer. For this treatment own (autologous) or donor (allogeneic) cells can be used. In case of autoAI the immunological effect can be achieved only after laboratory manipulation of cells. In case of alloAI the effect can be achieved after using both manipulated or non-manipulated cells.

The first information about non-manipulated allogenic cells transfusion further back to antiquity, peripheral blood cells were used for rejuvenation and for recovery. In the last century repeated cases of childhood acute lymphoblastic leukemia remission after non-irradiated blood transfusion without chemotherapy were described. In the 1980s similar remissions after blood transfusion without any additional treatment have also been described. At that time there were no chemotherapy of osteogenic sarcoma. Therefore, transfusion of allogeneic non-manipulated bone marrow from a random donor before radical surgery was performed as a treatment. Such transfusion significantly reduced the incidence of lung metastases [5].

The most famous example of the cellular modulation of immune response is allogeneic bone marrow transplantation (alloBMT). Therapeutic success of alloBMT is based on the graft-versus-tumor effect due to which tumor cells are under the lifelong control of the donor's immune system [6–8]. But despite the advantages, there are a number of serious limitations for widespread use of alloBMT: side effects, risks related to alloBMT, long period of immune recovery and development of anti-tumor effect. These limitations formed the basis of the modern AI, which takes the benefits of alloBMT without the side effects.

The history of modern alloAI with manipulated cells is associated with S. Rosenberg. In the early 1980s he studied lymphokine-activated killers (LAK) and tumor-infiltrating lymphocytes (TIL). His research had shown high efficiency of LAKs and TILs as a treatment of melanoma [9]. But that results were not very reproducible, probably because of different modifications for cells culture and various other technical details. Therefore, other options for immunotherapy with manipulated immune cells began to study. In 1987 the first chimeric antigen receptor (CAR) was created; in 2002 CAR-T cells demonstrated the first successful ex vivo targeting against prostate cancer antigen. And in 2012 after the first successful outcomes in the treatment children with refractory and relapsed B-cell acute lymphoblastic leukemia, CAR-T therapy began to seem a breakthrough. But now it's obvious that cancer cells find the way of immune escape from targeted attack and then rapid relapse occurs. Long-term remissions after CAR-T therapy probably occur in cases when transfusion in some reason modifies patient's own immune system and it takes long control of the disease. Similar modification of the immune response can be achieved after alloAI. Over the past 30 years, the importance of the immune system in cancer preventing, regulation of tumor growth and metastasis became obvious [10, 11]. But so far, we have little information about mechanisms of immune regulation and its interaction with tumor cells. It is known that during such interaction cancer cells suppress body's antitumor immune system and try to escape the immune recognition and destruction [12, 13]. This can be achieved by producing environment that suppress immune cells and by increasing of intratumor heterogeneity with selection of treatment- resistant clones [14].

In 2018 James P. Allison and Tasuku Honjo awarded Nobel Prize in Physiology or Medicine for their discovery of cancer therapy by inhibition of negative immune regulation. This treatment became an alternative for many patients, especially taking into account that chemotherapy, including dose increase, have reached their limit in cancer treatment. Moreover, chemotherapy and radiotherapy not only doesn't use any benefits of person's antitumor immunity, but instead lead to additional immunosuppression. Important to note that according to one of the heterogeneity theories, all tumor cells (both oncogenic and non-oncogenic cells of the tumor environment) develop from cancer stem cells (CSCs). CSCs escape to chemotherapy because of quiescent and slow growth, so there is always a risk for tumor recurrence/metastasis. Remission of disease is maintained as long as the CSCs are under the control of the patient's immune system. CSCs eradication is essential for successful treatment. The possibility of destroying CSCs by immunotherapy opens up new prospects of effective cancer treatment [15–17].

In the early 1970s natural killer (NK) cells were discovered. They have ability to rapidly recognize and exhibit cytotoxicity toward tumor and virus infected cells in a HLA-independent manner without any priming or prior activation. At the

beginning that cells were called like null-cells because of lack the common characteristic surface markers that can be found in mature B- and T-cells [18]. Although they are classified as cells of innate immune system, we have evidence that NK can develop a long and highly specific memory for various antigens which is associated with an adaptive immune response [19–21].

Since the discovery of NK cells, studies of NK cytotoxic activity have been initiated in both healthy people and cancer patients. It was shown that a lack of or low function of NK cells predicts an increased risk of developing both a primary tumor and its recurrence [22]. Thereby NK deficiency or dysfunction may play a crucial role in tumor progression.

Since the discovery of NK cells, studies of NK cytotoxic activity have been initiated in both healthy people and cancer patients. it was shown that a lack or low function of NK cells predicts an increased risk of developing both a primary tumor and its recurrence [23, 24]. Thereby NK deficiency or dysfunction may play a crucial role in tumor progression. That's why a personal

selection of KIR-alloreactive donor with high NK activity is so important for successful alloAI [25].

In Russia, a systematic discussion of new approaches to cancer treatment was initiated by MD, DSc, professor Dolgopolov I.S. in October 2018 at the VII Congress of Pediatric Oncologists of Russia. Futher at the 1st Adoptive Immunotherapy workshop in January 27, 2019 (Empire Tower, Moscow City) participants discussed practical issues of AI, the place and perspective for cellular immunotherapy in the era of DMI. Based on the meeting results, data from medical literature, international studies and trials, the clinical protocol for AI with allogeneic non-manipulated KIR-alloreactive cells (KIR-AI) was developed.

The participants also agreed that the indications for alloAI today are much wider than just "salvage therapy" in refractory patients. Expansion of indications to alloAI is possible due to the potential ability to destroy CSCs by immune cells in general, ant by NK in particular [26]. AI seems promising treatment because it uses advantages of NK and take into account the role of immune system in persistence of minimal residual disease and cancer progression. So as the next promising step for anticancer treatment can be considered KIR-AI.

According to the workshop protocol the indication for KIR-AI are salvage therapy, MRD persistence, high risk of recurrence after treatment, non-compliance with treatment standards (ECOG, comorbidities etc). At first bone marrow from personal selected KIR-alloreactive donor as a source of unmanipulated allogenic cells was used. To select donor for KIR-AI we investigate med 3 samples (range 3–8). Lymphodepletion included Cy/Flu (up to 3 d).

During the study we also evaluate and compare key characteristics between NK cells from different sources (bone marrow, umbilical cord blood and peripheral

blood of healthy donors and patients with long-term remission of cancer > 5 years). We study number of NK-cells and balance of activating and inhibitory receptors. Receptors were evaluated by flow cytometry (CD3, CD7, CD16, CD56, CD94, NKG2A), KIR were investigated by PCR. In cord blood samples NK-cells amount ranged from 5 % to 56 % of the total number of lymphocytes (mean 21 %, median 16 %). Mathematical model for evaluation the balance of activating and inhibitory receptors (Rp) as an index of cytotoxic activity (ICA) of mature NK was developed. We didn't find significant ICA differences on donor's sex, age, source of cells, but ICA depends on depression and virus. For patients no ICA difference by type of cancer, germinal mutations, but strong correlation with nearest outcome of cancer, follow up med 9 mo (2–52).

Considering the similar profile of NK-cells in different sources, umbilical cord blood (UCB) was used as an alternative to BM for KIR-AI of malignant neoplasms (breast, gastric cancer, high grade gliomas, metachronous cancers, hematologic malignancies). The indication to AI with UCB was salvage therapy (ECOG > 3). Quick availability of UCB from a KIR-typed UCB register is an indisputable advantage. Overall survival of 11 pts (14 cord blood transfusions) is from 2 + to 10 mth, median 6 mth (follow up 2–52 mth, median 9 mth) and OS is comparable to historical controls, where bone marrow as a source of cells was used. AI outcomes depend on the intensity of lymphodepletion and donor cells ICA.

Considering acceptable toxicity of lymphodepletion and good KIR-AI tolerability the indications for cellular anticancer treatment can be expanded. We start pilot using UCB for KIR-AI for overcome chemoresistance and to achieve remission after treatment completion in solid tumors and for MRD-eradication in hematology.

References

1. Coley W.B. Contribution to the Knowledge of Sarcoma. Ann Surg. 1891;14(3):199–220. doi: 10.1097/00000658-189112000-00015.
2. Hoption Cann S.A., van Netten J.P., van Netten C. Dr William Coley and tumour regression: a place in history or in the future. Postgrad Med J. 2003;79(938):672–80.
3. Richardson M.A., Ramirez T., Russell N.C., et al. Coley toxins immunotherapy: a retrospective review. Altern Ther Health Med. 1999;5:42–7.
4. Challenor S., Tucker D. SARS-CoV-2-induced remission of Hodgkin lymphoma. Br J Haematol. 2021;192(3):415. doi: 10.1111/bjh.17116.
5. Esartia P.T., Deichman G.I., Trapeznikov N.N. Suppression of lung metastases development after radical surgery for osteogenic sarcoma by single transfusion of normal allogenic bone marrow. Clin Invest. 1993.

6. Weiden P.L., Flournoy N., Thomas E.D., et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. N Engl J Med. 1979;300;1068–73. doi: 10.1056/NEJM197905103001902.
7. Weiden P.L., Sullivan K.M., Flournoy N., et al. Antileukemic effect of chronic graft-versus-host disease: Contribution to improved survival after allogeneic marrow transplantation. N Engl J Med. 1981;304:1529–33.
8. Baron F., Maris M.B., Sandmaier B.M. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. J Clin Oncol. 2005;23(9):1993–2003. doi: 10.1200/JCO.2005.08.136.
9. Terry W.D., Rosenberg S.A. Immunotherapy of human cancer. New York: Elsevier/North Holland, 1982.
10. Robertson M.J., Ritz J. Biology and clinical relevance of human natural killer cells. Blood. 1990;76:2421–38.
11. López-Soto Al., Gonzalez S. Control of Metastasis by NK Cells. Cancer Cell. 2017;32(2):135–54. doi: 10.1016/j.ccell.2017.06.009.
12. Vinay D.S., Ryan E.P., Pawelec G., et al. Immune evasion in cancer: Mechanistic basis and therapeutic strategies. Semin Cancer Biol. 2015;35:185–98. doi: 10.1016/j.semcancer.2015.03.004.
13. Langers I., Renoux V.M., Thiry M., et al. Natural killer cells: role in local tumor growth and metastasis. Biol Targ Ther. 2012;6:73–82. doi: 10.2147/BTT.S23976.
14. Shackleton M., Quintana E., Fearon E.R., et al. Heterogeneity in cancer: cancer stem cells versus clonal evolution. Cell. 2009;138:822–9. doi: 10.1016/j.cell.2009.08.017.
15. Zhang D., Tang D.G., Rycaj K. Cancer stem cells: Regulation programs, immunological properties and immunotherapy. Semin Cancer Biol. 2018;52(2):94–106. doi: 10.1016/j.semcancer.2018.05.001.
16. Badrinath N., Yoo S.Y. Recent advances in cancer stem cell-targeted immunotherapy. Cancers (Basel). 2019;11(3):310. doi: 10.3390/cancers11030310.
17. Luna J.I., Grossenbacher S.K., Murphy W.J., et al. Targeting cancer stem cells with natural killer cell immunotherapy. Expert Opin Biol. 2016;17:313–24.
18. Kannan G.S., Aquino-Lopez A., Lee D.A. Natural killer cells in malignant hematology: A primer for the non-immunologist. Blood Rev. 2017;31(2):1–10. doi: 10.1016/j.blre.2016.08.007.
19. Paust S., von Andrian U.H. NK-cell memory. Nat Immunol. 2011;12(6):500–8. doi: 10.1038/ni.2032.
20. Marcus A., Raulet D.H. Evidence for natural killer cell memory. Curr Biol. 2013;23(17):817–20. doi: 10.1016/j.cub.2013.07.015.
21. O'Sullivan T.E., Sun J.C., Lanier L.L. Natural killer cell memory. Immunity. 2015;43(4):634–45. doi: 10.1016/j.immuni.2015.09.013.

22. Whiteside T.L., Herberman R.B. Role of human natural killer cells in health and disease. Clin Diagn Lab Immunol. 1994;1(2):125–33. doi: 10.1128/cdli.1.2.125–133.1994.
23. Berrien-Elliot M.M., Romee R., Fehniger T.A. Improving natural killer cell cancer immunotherapy. Curr Opin Organ Transplant. 2015;20(6):671–80. doi: 10.1097/MOT.0000000000000243.
24. Imai K., Matsuyama S., Miyake S., et al. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet. 2000;356(9244):1795–9. doi: 10.1016/S0140-6736(00)03231-1.
25. McCurdy S.R., Fuchs E.J. Selecting the best haploidentical donor. Semin Hematol. 2016;53(4):246–51. doi: 10.1053/j.seminhematol.2016.08.001.
26. Smyth M.J., Hayakawa Y., Takeda K., et al. New aspects of natural-killer-cell surveillance and therapy of cancer. Nat Rev Cancer. 2002;2(11):850–61. doi: 10.1038/nrc928.

PROGNOSTIC VALUE OF MINIMAL RESIDUAL DISEASE ON THE POST-INDUCTION PHASE OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA THERAPY: PRELIMINARY DATA

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ABSTRACT

Detection of minimal residual disease (MRD) has become an integral part of the comprehensive definition of the antitumor therapy effectiveness in case of acute lymphoblastic leukemia (ALL). There is no doubt that it is necessary to estimate MRD during the period of remission induction and before allogeneic hematopoietic stem cell transplantation. However, the prognostic value of MRD at the post-induction phase of treatment is still being actively studied.

Aim. To estimate the prognostic value of MRD at the post-induction phase of pediatric ALL treatment.

Materials and methods. 135 pediatric patients with a primary diagnosed B-precursor ALL since October 2010 to March 2022 were included in the study. The treatment was carried out according to the protocol ALL-IC BFM 2009.

Results. 6-year OS for patients with MRD-positive status at the post-induction phase of treatment (on day 78) was 90.8 ± 4.0 %, with MRD-negative — 90.4 ± 6.5 % (p = 0.3). RFS with MRD-positive status — 66.3 ± 11.8 %, MRD-negative — 88.5 ± 4.5 % (p = 0.09). EFS with maintaining MRD-positive status on day 78 of therapy was 66.3 ± 11.8 %, MRD-negative — 90.4 ± 6.5 % (p = 0.09).

Conclusion. Nowadays MRD levels during the phase of remission induction are an important risk-stratifying factor for pediatric ALL. However, it is necessary to study the prognostic value of MRD in the post-induction period. The results of our study show increasing frequency of relapses to 22 % in the group of patients with MRD-positive status on the 78th day of therapy. The rate of the patients' OS does not differ according to the level of MRD. Consequently, it is necessary to continue the study.

Keywords: acute lymphoblastic leukemia, minimal residual disease, treatment, children

Introduction

Modern pediatric acute lymphoblastic leukemia therapy protocols (ALL) are based on differentiated approach to the treatment according to the prognostic factors of the disease. The age of the patient (up to 1 year and older than 6 years), initial hyperleukocytosis, translocations t (4;11) and t (9;22), number of blast cells in blood on day 8 of prednisolone therapy (more than 1000/μl), as well as insufficient bone marrow response on days 15 and 33 (more than 5 % of blast cells during cytological examination of the bone marrow) are factors associated with lower survival rates due to the protocol ALL-IC BFM 2002.

Invention of sensitive methods for detection of a residual population of blast cells (by polymerase chain reaction, flow cytofluorimetry) formed the concept of "minimal residual disease". It is a number of blast cells with an aberrant immunophenotype that is beyond the sensitivity of the light-optical diagnostic level. Since the 1990s, MRD has become a proven prognostic indicator that significantly predicts the survival of ALL patients. Now immunological criteria of the aberrant immunophenotype have been established for the most frequent subvariant ALL — B-precursor ALL (B-ALL). The use of MRD levels as a tool for stratification and treatment optimization has become the basis of modern ALL therapy programs [1]. In the UKALL 97/99 study there was shown that the MRD level more than 0.01 % at the end of remission induction phase ($p = 0.207$), at the beginning of consolidation ($p = 0.019$) and by the beginning of intensification ($p = 0.0104$) was associated with relapse of the disease. In addition, patients with MRD more than 0.1 % at the initiation phase of maintenance therapy ($p = 0.001$) had significantly more frequent relapses too [2]. According to the COG group, MRD level ≥ 0.01 % at week 12 of treatment (consolidation phase) was significantly associated with a poor response to therapy: patients with MRD-positive status had a 5-year relapse-free survival rate of 39 % ± 7 %, compared with 79 % ± 5 % with MRD-negative status ($p < 0.0001$) [3].

There are several periods of program treatment of pediatric ALL. MRD level is the most important factor at remission induction and pretransplantation phases. The values of MRD after induction therapy and the effect of MRD status on patients' survival require more careful study. There is an information about the correlation between MRD level at the post-induction phase and the frequency of relapses in several scientific publications. Thus, patients with T-precursor ALL (T-ALL) and MRD-positive status on the 42d day of therapy has statistically significant increasing of relapse frequency for next 4.5 months. However, there were no cases of relapse in patients with MRD-negative status for more than 20 months [4].

Modern ideas about the significance of MRD levels during the post-induction phase of therapy indicate the importance of a comprehensive assessment of combination between MRD and already known prognostic factors.

The aim of the study was to determine the prognostic value of MRD at the post-induction phase of pediatric ALL treatment.

Materials and methods

135 patients with a primary diagnosed B-ALL since October 2010 to March 2022 were included in the study. The average age of patients was 5.4 years (from 1 to 17 years). There were 62 boys (45.9 %) and 73 girls (54.1 %).

The inclusion of patients with B-ALL in the study was due to the presence of optimal immunological panels and proven model for determining MRD.

All patients received an induction therapy according to the protocol ALL-IC BFM 2009. MRD determination was carried out on the 15th, 33d and 78th days of treatment. If a residual population of leukemic cells was in the amount of ≥ 0.01 % among myelocariocytes, it had been considered positive status; if < 0.01 %, negative one.

Patients' stratification into prognostic risk groups according to MRD status was based on the criteria of the protocol ALL-IC BFM 2009. Children were divided into standard, intermediate and high risk groups.

Statistical evaluation of the obtained parametric data was carried out by comparing the average values using the Student's coefficient. Nonparametric data were compared by constructing conjugacy tables according to Pearson's χ^2 criterion and Fisher's exact criterion for comparing groups with small samples. The difference in the groups was considered significant at $p < 0.05$. The survival rate was assessed by constructing curves using the Kaplan—Meyer method. In this study, the assessment of relapse-free (RFS), event-free (EFS) and overall survival (OS) was carried out. The long-rank method was used to compare survival curves. The difference between the curves was considered significant at $p < 0.05$. RFS was calculated from the moment of achieving remission to the moment of relapse, EFS — from the beginning of treatment to the moment of cessation of remission, regardless of the cause (cases of progression, relapse and death were attributed to the events) and OS — from the beginning of treatment to the death of the patient. The survival rate was assessed on the 15 of March 2022.

Results

Gradual decrease of MRD level was noted during it determination in dynamics on 15th, 33d and 78th days of therapy. On day 15 only 13.3 % of patients reached MRD-negative status, on day 78 this number was already 50.4 % ($n = 68$) (Fig. 1).

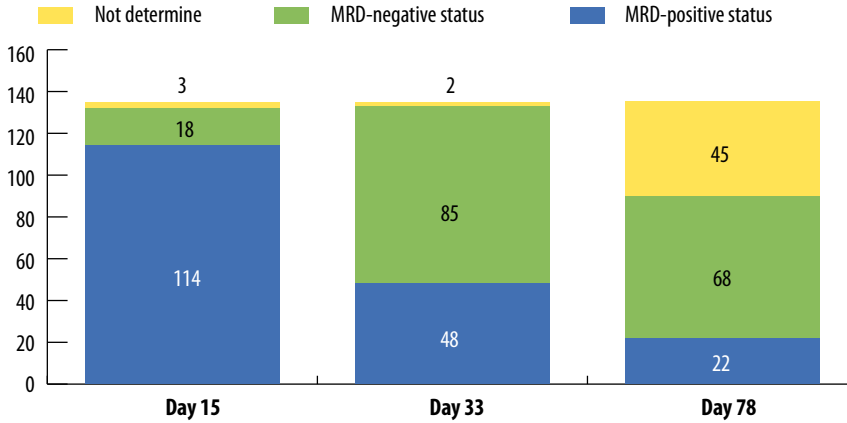

Fig. 1. Distribution of ALL patients by MRD status on the 15th, 33d and 78th days of therapy

On the 33d day of the therapy all patients were examined for achieving cytological remission. According to the results of this analysis, 128 (94.8 %) patients had a remission (the number of blast cells in the bone marrow < 5 %), and 7 (5.2 %) patients did not have. There was death during induction phase in 1.4 %. All patients without remission on day 33 had an MRD-positive status.

Analysis on the survival rate of patients regardless of the prognostic risk group based only on MRD status on the 78th day of therapy showed the 6-year OS for patients with MRD-positive status was 90.8 ± 4.0 %, with MRD-negative — 90.4 ± 6.5 % (p = 0.3). RFS with MRD-positive status was 66.3 ± 11.8 %, MRD-negative — 88.5 ± 4.5 % (p = 0.09). EFS with persistence of MRD-positive status on day 78 of therapy was 66.3 ± 11.8 %, MRD-negative — 90.4 ± 6.5 % (p = 0.09) (Fig. 2–4).

Thus, patients' OS did not depend on the MRD status on the 78th day of therapy because of the effectiveness of anti-relapse treatment protocols and transplantation technologies. However, the relapse rate was 22 % higher in the group of patients with MRD-positive status by the 78th day of treatment. The study will be continued to obtain reliable data.

Conclusion

Determination of MRD level during the induction remission phase of treatment and before allogeneic hematopoietic stem cell transplantation is the standard

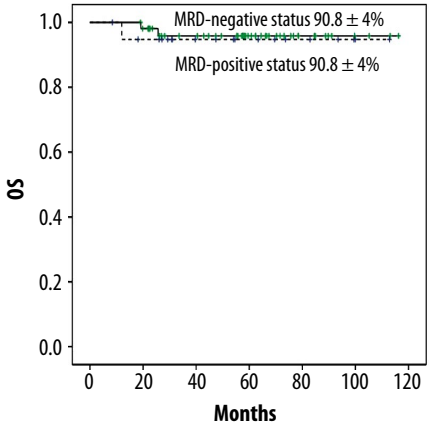

Fig. 2. ALL patients' OS depending on the MRD level on the 78th day of therapy

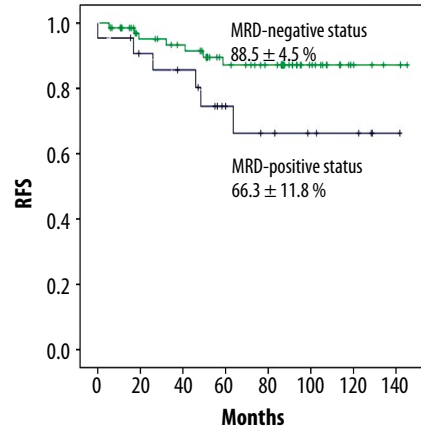

Fig. 3. ALL patients' RFS depending on the MRD level on the 78th day of therapy

for evaluating the effectiveness of ALL therapy. It is necessary to study additionally the persistence MRD in the post-induction period. As a result of our study there is a correlation between MRD-positive status on the 78th day of therapy and increasing the relapse incidence (p = 0.09). The patients› OS does not depend on the MRD status at the post-induction stage of treatment.

Both continuing study of the MRD prognostic effect on the relapses frequency and an increasing the number of patients in the analyzed group may allow us to obtain a reliable correlation between the level of MRD during the post-induction phase of therapy and RFS. The data obtained will make it possible to intensify treatment, for example, to use a "block" program, targeted drugs (blinatumomab) and to determine additional indications for allo-HSCT [5, 6].

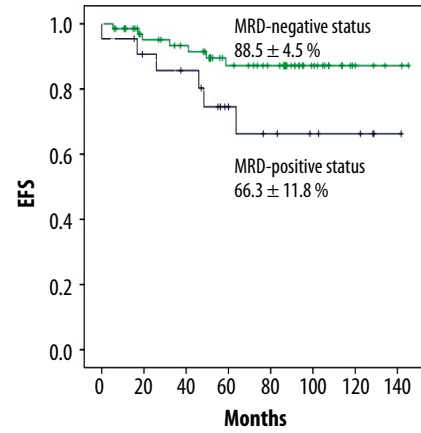

Fig. 4. EFS of ALL patients depending on the MRD level the 78th day of therapy

References

1. Campana D., Pui C.H. Minimal residual disease–guided therapy in childhood acute lymphoblastic leukemia. Blood. 2017; 129(14): 1913–18. doi: 10.1182/blood-2016-12-725804.
2. Ryan J., Quinn F., Meunier A., et al. Minimal residual disease detection in childhood acute lymphoblastic leukemia patients at multiple time-points reveals high levels of concordance between molecular an immunophenotypic approaches. Br J Haematol. 2009; 144(1): 107–15. doi: 10.1111/j.1365–2141.2008.07429.x.
3. Borowitz M.J., Wood B.L., Devidas M., et al. Prognostic significance of minimal residual disease in high risk B-ALL: a report from Children's Oncology Group study AALL0232. Blood. 2015; 126(8): 964–71. doi: 10.1182/blood-2015-03-633685.
4. Kandeel E., Madney Y., Amin R., Kamel A. Role of Minimal Residual Disease in the Clinical Course of T cell Acute Lymphoblastic Leukemia in Pediatric Patients. J Leuk. 2019; 7: 256. doi: 10.35248/2329-6917.19.07.256.
5. Maloney K.W., Devidas M., Wang C., et al. Outcome in Children With Standard-Risk B-Cell Acute Lymphoblastic Leukemia: Results of Children's Oncology Group Trial AALL0331. J Clin Oncol. 2020; 38(6): 602–12. doi: 10.1200/JCO.19.01086. Epub 2019 Dec 11. PMID: 31825704; PMCID: PMC7030893.
6. Meraj F., Jabbar N., Nadeem K., et al. Minimal residual disease in childhood B Lymphoblastic Leukemia and its correlation with other risk factors. Pakistan journal of medical sciences. 2020; 36(1): S20–S26. doi: 10.12669/pjms.36.ICON-Suppl.1721.

ОБЪЕДИНЕННАЯ КОНФЕРЕНЦИЯ

«Иммунология гемопоэза» & EHOG «Диагностика и лечение минимальной остаточной болезни (МОБ) при опухолях кроветворной и лимфоидной тканей»

JOINT CONFERENCE

Haematopoiesis Immunology & EHOG "MRD diagnosis and treatment in tumors of haematopoietic and lymphoid tissues"

OCTOBER 5, 2022. ISTANBUL

9:00–9:15	Opening Ceremony Professor George Janossy (UK) Professor Robert Gale (USA) Professor Nikolai Tupitsyn (Russia) Professor Irina Poddubnaya (Russia) — President of Russian oncohaematology Society
	Chairmen: Professor George Janossy (UK) Professor Robert Gale (USA) Professor Dieter Hoelzer (Germany) Professor Nikolai Tupitsyn
9:15–9:35	Professor Robert Gale (USA). "Should MRD be an endpoint of CLL therapy?"
9:35–9:55	Professor Jean-Francois Rossi (France). "How the evaluation of minimal residual disease may influence a clinical decision?"
9:55–10:15	Professor Dieter Hoelzer (Germany). "Modern possibilities of MRD eradication in oncohematology. Which acute leukemia may be treated immunologically w/o chemotherapy?"
10:15–10:35	Vera S. Donnenberg (USA). "Localized conditioning of pleural environment"

10:35–10:55	Alexandra Palladina (Russia). “General principles of MRD monitoring in AML”
10:55–11:10	Coffee break
11:10–11:30	Mary Shervashidze (co-authored with Alexandra Palladina, T.T. Valiev) (Russia). “Prognostic significance of MRD in children B-All at post-induction period”
11:30–11:50	Kapitolina Melkova (co-authored with Zhanna Sharoyan) (Russia). “Umbilical cord blood NK-cells”
11:50–12:10	Professor Nikolai Tupitsyn. “Modern approaches to hematogeneous metastasis prevention and cancer immune prophylaxis”
12:10–12:30	Vladimir Jurisic (Serbia). “Multiomic cytokine analysis in hemato-immunology”
12:30–13:00	General discussion