

10th R. Gorbacheva Memorial Symposium "Hematopoietic Stem Cell Transplantation. Gene and Cellular Therapy": an overview

Boris V. Afanasyev

Professor Boris V. Afanasyev, R.Gorbacheva Memorial Research Institute of Children Oncology, Hematology and Transplantation, First State I. Pavlov Medical University, L. Tolstoy St 6-8, 197022, St. Petersburg, Russia.

Phone: +7(812) 338 6265

E-mail: bvafan@gmail.com

Recent advances in clinical hematopoietic transplantation

The X. Jubilee Symposium was introduced by Prof. Boris V. Afanasyev who has presented a short introduction into history and current activities of R.Gorbacheva Memorial Institute of Children Oncology, Hematology and Transplantation. Among recent advances, he mentioned novel approaches to treatment of refractory/relapsed lymphomas, large studies in haploidentical hematopoietic stem cell transplantation (HSCT), and special features of lymphoma therapy in HIV-infected patients.

Professor Rüdiger Hehlmann has opened his memorial lecture with deep regrets on Prof. Thomas Buechner who passed away quite recently. For the last three decades, Prof Buechner was closely involved into key programs of the European LeukemiaNet and other networks aimed for better knowledge and therapy of acute leukemias. Another part of his lecture was dedicated to current alternatives in treatment of chronic myeloid leukemia (CML cure: HCT or drug treatment?). His message was that, in the era of tyrosine kinase inhibitors, allo-HSCT remains a valid option for some clinical situations. Disease and transplant risks should be considered in these cases.

An extensive review "What is the impact of targeted therapy with TKI and immunotherapy on SCT in adult ALL" was presented by Professor Dieter Hölzer (Frankfurt a.M., Germany). He discussed different therapies applying tyrosine kinase inhibitors, including second-generation TKIs. Another class of targeted drugs is represented by monoclonal antibodies which are effective and well tolerated even in elderly patients. CAR T cells is another promising option for the future immunotherapy. However, a lot of questions still remain with monoclonal antibodies and their combinations with chemotherapy, especially for different disease status (e.g., MRD+ versus MRD- patients)

Our colleagues from Belarus have reported their data on HSCT in pediatric patients (Olga Aleinikova: Relapse after alloHCT in children in Acute Lymphoblastic Leukemia. How to manage?). Relapse is a major cause of treatment failure in children with acute leukemia after SCT. About 1/3 of patients are eligible for 2nd SCT (n=75 cases). Event-free survival after 2nd SCT was 40%, as reported by this group. Major factors predicting better survival are absence of minimal residual disease pre-HSCT and presence of aGvHD post-transplant. In conclusion, pediatric ALL relapse after SCT may be effectively cured by a repeated SCT which presents the only curative option in this cohort.

Dr. Michael Maschan (Moscow) held a lecture Haploidentical transplantation in pediatric acute leukemia, being focused, mainly, on immunological aspects of HSCT. As based on their experience of HSCT in ALL and AML patients (a total of 184 cases), the speaker suggests that ALL and other acute leukemias may be treated with haploidentical transplants rather than matched unrelated HSCT. Moscow experience with CD45RA depleted donor lymphocytes was reported, as well as assessment of viral infection risks, along with immune recovery after TCR alpha/beta- and CD19-depleted grafting.

Several reports were dedicated to post-transplant usage of cyclophosphamide which is highly discussed and is regarded now as a promising option for aGvHD prevention. Present experience and future perspectives in this field were analyzed by Professor Andrea Bacigalupo (Rome, Italy), in his report Haploidentical transplantation and post-transplant cyclophosphamide. Good preventive effect of cyclophosphamide against aGvHD was shown in such different clinical settings as Hodgkin disease and AML. Some novel drugs (e.g., Ipilimumab in AML) were also discussed. In summary, Prof.Bacigalupo concluded on high efficiency of haploidentical transplantation with posttransplant cyclophosphamide even in such disorders as myelodysplastic syndrome and myelofibrosis.

Dr. Ivan S. Moiseev presented a report: "Prospective trials of novel graft-versus-host disease prophylaxis" where different approaches to aGvHD prevention are discussed, including classical schedules with CsA+Mtx±ATG, and recent studies with post-transplant cyclophosphamide applications. Appropriate studies are performed within several clinical trials which are now under evaluation.

Dr. Maxim Kucher (St. Petersburg) has shown some interesting results showing improved stem cell harvest from peripheral blood: effectiveness and safety of plerixafor containing regimens for mobilization of hematopoietic stem cells in poor mobilizers for autologous graft preparation. «G-CSF + Plerixafor» combination proved to be a safe and effective schedule (90% efficiency) for CD34+ cell mobilization for autologous HSCT in patients who were considered «poor mobilizers», both children and adults. Among rare adverse effects, diarrhea, and hypocalcaemia-induced paresthesia were observed.

Chronic myeloid leukemia (CML) is a traditional item discussed at our Symposium. This time, Professor Jane Apperley presented a report: "Challenges in CML: a case based discussion." This instructive presentation was based on several clinical cases of CML. Attention was focused on the initial patient status, decision making for therapy with Imatinib or other TKI's, 2nd line therapy and HSCT (if necessary). Some important questions still remain, e.g.: How deep should the molecular response be to allow stopping of the treatment? What is the place of HSCT in cases of developing blast crisis?

Professor Andrey Zaritskey (St. Petersburg, Russia) presented a comprehensive review concerning CML treatment in blast crisis: Transplantation in CML BP. This difficult condition is uncommon now, in Imatinib era. Different chemotherapy schedules combined with TKI's are proposed for remission induction, or as a bridge therapy for allo-HSCT. Allotransplants proved to be superior to chemotherapy, and haplo-identical donors may be a good alternative for allogeneic HSCT, as demonstrated by their own data on HSCT in CML blast phase.

Current attitudes for biological treatment methods were analyzed in comprehensive review by Professor Hans J. Kolb "The Role of Allogeneic Transplantation in a Changing World of Immunotherapies". He described relative role of immune cell populations as potential tools for leukemia treatment, and clinical perspectives of donor lymphocyte infusions (e.g., in CML and AML treatment). More targeted approaches includes treatment with T cells armed against Epstein-Barr virus, or CAR-T cells, in potential combination with specific antibodies directed against B cells, being potentially effective in CLL and other lymphoid neoplasias.

Professor Belinda Simoes from São Paulo (Brazil) has reported a summarized Brazilian experience in HSCT for systemic sclerosis, multiple sclerosis and type I diabetes mellitus (Stem Cell Transplantation in Autoimmune Disease). Longitudinal EBMT data show increased auto-transplant activity for MS treatment which, generally proved to be safe and effective method. A concept of "immunological reset" is suggested as a biological basis for auto-HSCT in autoimmune disorders.

Dr. Alexander D. Kulagin (St. Petersburg, Russia) discussed potential options for HSCT in severe anemias: Bridging therapy with eculizumab and allogeneic hematopoietic stem cell transplantation for hemolytic paroxysmal nocturnal hemoglobinuria/severe aplastic anemia. Among other treatments, eculizumab could be a promising option for bridge therapy preceding allo-HSCT. No specific complications (i.e. thrombosis, VOD) were observed after HSCT in paroxysmal nocturnal hemoglobinuria.

International protocols and networks

Implications of international protocols in Russian clinics were well described by Professor Alexander G. Roumyantsev (Moscow) who has reminded the main stages of cooperative studies of Russian and West European clinics on cancer treatment. He pointed to overall better treatment results along with reduction of toxicity, decreased hospital staying and lowering costs for medical care. The Speaker also pointed to well-functioning Reference Center in Moscow coordinating efforts of Russian oncohematologists and accumulating appropriate clinical data from pediatric oncological clinics throughout Russia. Recent advances are connected with arrangement of HSCT donor Registry and rehabilitation programs for HSCT recipients.

Professor Dietger Niederwieser (Leipzig, Germany) presented a competent overview of a world-wide BMT networking in his lecture WBMT Global Survey 2013. This international effort is aiming to follow annual trends of different HSCT modalities, being applied for therapy of distinct malignant disorders. Indeed, total number of stem cell transplantation increases, with predominance of allo-HSCT over autologous grafting in both malignant and non-malignant diseases. What concerns alternative donors, the haploidentical HSCT becomes more popular, exceeding cord blood transplantation. There are, however, great differences for availability of compatible unrelated donors for different regions and countries.

Trends and attitudes for haplo-HSCT in multicentric studies were discussed in the lecture presented by Professor Arnon Nagler (Israel, Tel Hashomer, Israel): "Haploidentical Stem Cell Transplantation for AML – Update from the ALWP of the EBMT". A sufficient increase in haplo-SCTs for leukemia treatment is registered worldwide. A tolerance for haploidentical graft may be explained by activity of Treg cells and other biological factors. With respect to efficiency of haplo-HSCT, no gross differences in clinical outcomes are reported between the T-replete haplo SCT and HLA-compatible transplants from unrelated donors. HLA-DRB1 locus matching is not an independent prognostic factor for acute GvHD grade II-IV associated with haplo-HSCT.

Professor Alexander Karachunskiy (Moscow) has reviewed results of induction leukemia treatment protocols: "Prospective randomized ALL-MB studies by children and adolescents in Russia". He traced evolution of the protocols, from ALL-MBM-2002 adapted for Russian conditions, to current modifi-

cations. Some aspects of dose optimization for glucocorticoids, asparaginase, and other components are discussed. Enhanced therapy schedules are suggested for refractory leukemia cases. Meanwhile, results of frontline therapy are again declared a significant predictive factor for the patients' survival.

Professor Norbert Schmitz (Hamburg, Germany) has followed evolution in Hodgkin and non-Hodgkin lymphoma treatment from 2002 to present in his lecture: "Autologous and allogeneic transplantation in lymphoma in the context of new drugs". Current data from EBMT registries show good efficiency of currently used protocols. Auto- and allo-HSCT remain important modalities to treat NHL. Meanwhile, new targeted drugs and cellular therapies in lymphomas (e.g., CAR-T) will impact further developments, with respect to indications (competitor vs bridge therapy), subsequent HCT toxicity, aGvHD risk, and relapse risk.

Dr. Kirill Kirgizov (Moscow) presented a Report of autologous hematopoietic stem cell transplantation for pediatric patients with multiple sclerosis: It was a retrospective multicenter study by the EBMT Autoimmune Diseases Working Party based on a Registry of >2000 hematopoietic transplants performed in patients with multiple sclerosis (MS), connective tissue disorders, severe arthritis, inflammatory bowel diseases, etc., with participation of 251 centers from 41 countries. Autologous HSCT in MS proved aHSCT for pediatric MS appears to be safe and effective way to reduce autoimmune inflammation, being accompanied by, at least, stabilization of MS symptoms by EDSS scale.

Cellular mechanisms and diagnostics

A lecture by Dr. Magne Børset Attacking intracellular signalling pathways in myeloma cells concerned multiple molecular mechanisms of multiple myeloma. In view of complex regulatory changes, a positive role of next-generation sequencing is considered. A distinct group of genes is shown to be mutated in different myeloma patients. However, neither of specific gene mutations still cannot be regarded as a key factor for genesis of myeloma. The same seems to be true for proteomic analysis, however a possible role of PRL-3 (the phosphatase of regenerating liver-3) is suspected now. Such intracellular signalling molecules could be good targets for therapy.

An experimental report by Prof. Valery G. Savchenko (National Center of Hematology, Moscow) concerned modification of stromal microenvironment and T-cell subpopulations imbalance in patients with leukemia before and after allo-HSCT. He discussed functional role and composition of the bone marrow niche, especially, mesenchymal stem cells (MSC). A hypothesis on MSC suppression by leukemic cells was considered. MSCs from leukemia patients showed different changes in growth rates. Some specific gene signatures were revealed when analyzing their gene expression profile before and after HSCT. Changes in T cell profiles and recovery are considered in view of aGvHD risk posttransplant.

An interesting approach to diagnosis and studies of leukemia

was presented by Claudia Lange (Hamburg, Germany). The item was described as The potential of extracellular vesicles (EV) in oncology, a class of microparticles shedding from the surface of normal and leukemic cells. A variety of structural and signalling proteins, nucleic acids provides a lot of opportunities for intercellular communications and RNA transfer, thus suggesting their sufficient role in tumor biology. E.g., EV derived from mesenchymal stem cells may be applied in cancer immunotherapy. Radiation protection of MSC-derived extracellular vesicles was shown in animal models.

A quite original study was presented by Dr. Orit Collet (Rehovot, Israel) in her report Metabolic regulation of blood and bone forming stem cells: the role of ROS and nitric oxide. According to her data, the nitrogen monoxide (NO) levels produced by stromal and endothelial cells may regulate hematopoietic stem cells (HSC), with EPCR playing a sufficient role in HSC homing. Dynamic permeability of BM endothelium and MSCs regulate HSC maintenance in bone marrow, probably, by secretion of ROS and NO. In this respect, HSC are shown to transfer mitochondria and ROS to BM MSC, and, vice versa, leukemic cells are also able to transfer mitochondria and ROS to BM MSC, thus producing a potential regulatory circuit within the marrow niche.

Professor Tapani Ruutu (Helsinki, Finland) has reported about Progress in the diagnosis and treatment of veno-occlusive disease/ sinusoidal obstruction syndrome of the liver. Prediction, detection and treatment of this common post-transplant condition present a difficult task. Obstruction of liver sinusoids may be confused with other complications, due to absence of specific symptoms and markers. Recent severity criteria are presented. Defibrotide is effective for the treatment of severe VOD.

Among molecular markers of minimal residual disease, "WT1 expression is among the most searched markers. Professor Andrea Bacigalupo (Rome, Italy), with his lecture: WT1 as a marker of minimal residual disease post transplant in AML, and pre-emptive therapy of relapse". Generally, there was a good correlation with disease-free survival and relapse risk in AML.

Experimental therapy

Prof. G. Wagemaker from Erasmus University Rotterdam, The Netherlands has made a comprehensive overview of prospective in gene therapy of genetic diseases (Progress in Hematopoietic Stem Cell Gene Therapy in Inherited Disorders). Current symptomatic or replacement therapies are costly and require lifelong treatment. Modern gene therapy is now developed for some genetic defects, e.g., inherited immune deficiencies, lysosomal storage disorders, sickle cell anemia, thalassemia, hemophilia A. In 2000's stem cell gene therapy based on lentiviral vectors was applied for Wiskott-Aldrich syndrome, X-linked SCID, and some enzyme deficiencies. General protocol for HSCT of autologous gene-modified cells is presented. Comparative characteristics of different gene constructs, transfection efficiency, in vitro expansion of modified cells and their clinical efficiency are also reviewed.

Dr. Polina Stepenska (Hadassah Center, Israel) shared her experience in a lecture: “Genetic diagnosis in Pediatric Stem Cell Transplantation”. She provided an overview of whole-exome sequencing (WES) techniques, and considered it an effective, and currently, economic approach to precise diagnostics of known and freshly detected mutations in patients and their relatives. Diagnostic experience with WES for >70 children treated at Hadassah Center allowed to assess a definitive genetic diagnosis in 70 % (familial immunodeficiencies, osteopetrosis etc.), and to perform HSCT with good results in 50 cases. Additional family members with same defect could be also diagnosed in this trial.

Molecular engineering and its applications for gene editing for neuromuscular diseases were briefly described by I.A. Yakovlev, MD (Human Stem Cells Institute PJSC) and exemplified by the in vitro DYSF mutation correction performed by means of CRIPSR\Cas9 nuclease system.

Professor J.Glover is an expert in cell propagation in different culture systems. His report was The iPS cell technology – Promise and Limitations. The iPS (induced pluripotent stem cells) may be obtained by means of genetic manipulations thus being artificial cell products. Therefore, they may be used, mainly, for in vitro disease modeling, high-throughput drug screening, or tissue/organ reconstruction. Autologous or allogeneic cell therapy in patients may be started in long-range perspective, after coping with some safety issues.

Genome editing workshop (Day 1, Athens)

A lecture by Prof. Boris Fehse (Hamburg) “Manipulating the genome for research and therapy” concerned quite recent opportunities of introducing targeted changes into genome of living cells, mostly, by means of viral vectors. For many years, these approaches were used for induction of gene knockout in laboratory animals. Now, however, the molecular interventions have become more accurate, thus blazing new trails for targeted therapy on the genome level. I.e., novel viral vectors allow to safely introduce a wild-type gene material into cells of the patients with inherited diseases, thus correcting their phenotype. Several new nuclease-based systems, especially, RNA-based CRISP/CAS9 designer nucleases allow highly efficient and precise gene editing of mutated genes, i.e., gene replacement, or ‘knock-in’ of desired gene material leaving the target cells alive. The gene editing may cause revolutionary changes in transplantation, since one may modify hematopoietic cells from, e.g., a patient with genetic enzyme deficiency, and then return them back, hoping for correction of the gene defect.

A special review by Mikhael Samsonov (R-Pharm, St.Petersburg) “Human gene editing: a strategy of development and registration” beared on design, testing and law regulations of prospective gene modification therapies. Current clinical trials with TALEN- and CRISPR/Cas9-modified human cells are underway, including T cell modifications for leukemia treatment. Among main concerns, the off-target activity of these agents is considered, thus rising concerns on their

safety for patients. Among promising therapeutic approaches to immunotherapy of leukemia and lymphoma, a series of CART-producing systems seem to arrive at the market in sooner time.

Cristina Khodova from Skolkovo Foundation presented even more general view to the problems in her lecture: “Games of Genomes: market challenges, investment opportunities & patent battles”. The aspects of gene therapy, still being in cradle, are now abundantly supported from venture capitals. A NIH approval for treatment of leukemia by CRISP-modified T cells seems to be an early bird in this respect.

Finally, the audience has a discussion (per Skype, but with much interest) with Fyodor Urnov, a California-based molecular biologist, an enthusiast of gene editing. His brisk speech was devoted to current state of gene editing experiments and their bright perspectives for human medicine.

Nursery in transplantation clinics

A novel section Nursery in hemato-oncological patients has gathered a large audience of physicians and medical nurses.

A keynote lecture was held by our Israeli colleague Natan Gorelik, the Head Nurse of Pediatric Oncology at the Hadassah Medical Center (Jerusalem). He presented main options, techniques and infection control of central and peripheral venous catheters. Some aspects of catheter-associated blood clots and infectious hazards were described, like as their control which is in competence of the hospital nurses. Other complications of vein catheterization were considered. The quality of life (QoL) issues were also in scope.

Extensive data on daily life of medical nurses at intensive care unit (ICU) #3 () were presented by E.A.Goncharov and colleagues from The R.Gorbacheva Memorial Institute. They noted multifaceted duties of the ICU nurses including daily general care, control of mucositis, prophylaxis of respiratory and urinary infections, monitoring main physiological parameters, e.g., water and salt balance, etc. Communications with relatives of the patient is an important task as well.

Anna A. Apostolova discussed clinical significance of body weight in patients on chemotherapy and after HSCT, and its dependence on nutritional status, aGVHD severity and infectious complications in the patients.

Technical issues of blood drawing from different CVC ports, as exemplified by Tacrolimus assays were described by Anastasia D. Nikiforova.

A team from Moscow (O.A. Voroshilina et al.) presented their experience of nutritional support for hemato-oncological patients admitted to aseptic boxed department at the Rogachev Center of Children Hematology, Oncology and Immunology. Appropriate tasks for the nurses include monitoring of nutritional state and consulting with specialists in clinical nutrition, concerning modes and volumes of required nutritional support.

Clinical psychologists at the RICOHT clinics (Svetlana I. Oleshko et al.) reported their data on Prevention of burn-out syndrome in BMT nursing staff. A number of preventive measures are proposed, in order to increase psychological resistance of medical nurses employed with severe patients and to prevent potential conflicts with colleagues, patients and their relatives, e.g., individual consulting and group colloquia for discussion of stressful points.

In this connection, an interesting report was made by a psychologist Olga V. Ushakova who showed a significance of the patient's commitment for treatment and role of competent information on the issues relevant to therapy and its effects, also taking into account personal psychological traits of the patient

Keywords

Symposium, jubilee, St. Petersburg, oncohematology, overview.

Обзор докладов на 10 симпозиуме «Трансплантация гемопоэтических стволовых клеток у детей и взрослых» памяти Р. М. Горбачевой

Борис В. Афанасьев

НИИ детской онкологии, гематологии и трансплантологии им. Р.М. Горбачевой, Первый Санкт-Петербургский государственный медицинский университет им. И.П.Павлова, Санкт-Петербург, Российская Федерация

Резюме

Основная тематика 10-го юбилейного симпозиума памяти Р. М. Горбачевой была традиционно посвящена вопросам терапии онкогематологических заболеваний и смежным проблемам. Участники симпозиума из России, СНГ и европейских стран представили обширные данные об успехах и проблемах в области трансплантации гемопоэтических стволовых клеток (ТГСК). В частности, обсуждались вопросы оптимизации и комплексного лечения хронического миелоидного лейкоза и острых лейкозов с применением ТГСК в сочетании с таргетными препаратами, такими, как ингибиторы тирозинкиназ, моноклональные антитела. Эти результаты во многом основаны на данных мультицентрических исследований, проведенных как в Европе, так и в клиниках России и Белоруссии. Особое внимание привлечено к повышению клинических эффектов при лечении продвинутых клинических форм заболеваний (например – рефрактерных форм и рецидивов).

В некоторых докладах также рассматривались вопросы клеточной иммунотерапии (в частности – использования CAR-T клеток), усиливающей противоопухолевые эффекты и расширяющей возрастные лимиты проведения эффективной терапии злокачественных заболеваний. В ряде выступлений обсуждалась клиническая эффективность гаплоидентичной ТГСК в онкогематологии, и показана ее применимость в целом ряде клинических ситуаций. На симпозиуме также был ряд важных докладов о применении ТГСК в лечении генетических заболеваний и апластических анемий. Остается актуальной также тема ТГСК в терапии аутоиммунных заболеваний.

Профилактика реакции «трансплантат против хозяина» также была предметом ряда сообщений, и показана достаточная эффективность применения с этой целью циклофосфида после ТГСК. Актуальной остается и проблема вено-окклюзионной болезни после трансплантации. Как обычно, поиск маркеров минимальной остаточной болезни являлся предметом дискуссий на симпозиуме.

Среди экспериментальных работ представляли интерес исследования клеточной биологии множественной миеломы, роль мезенхимных стромальных клеток в кроветворении, вопросы межклеточного сигналинга и метаболических взаимодействий в системе гемопоэза.

Специальная сессия касалась методик генного редактирования клеток для их аутологичной трансплантации, прежде всего – пациентам с ВИЧ-инфекцией и врожденными иммунодефицитами пациентам, а также перспективам их клинического внедрения и легально-этическим проблемам применения таких клеточных продуктов.

Отдельное заседание было посвящено особенностям работы медицинских сестер в отделениях трансплантации костного мозга и специфике их функциональных задач.

Ключевые слова

Симпозиум, юбилейный, Санкт-Петербург, онкогематология, обзор.