Volume 30, Supplement 2, April 2006 ISSN 0145-2126

ELSEVIER

Leukemia Research

CLINICAL AND LABORATORY STUDIES

Abstracts of the 4th International Symposium on MDS in Childhood
April 24-26, Freiburg, Germany

severely decreased (RC versus other MDS: p = 0.000628, Mann–Whitney). Decrease of CD34pos cells in the RC group was less prominent (n.s.). Level of B cells was significantly higher in patients with JMML and JMML/Noonan compared to other subtypes (Kruskal–Wallis p = 0.035, Mann–Whitney p = 0.0056). Development of B cells is often asynchronous in JMML patients (4/6 cases) when analysed expression of CD10, CD20 and CD45 compared to normal regenerating bone marrow. These immature B cells can be found even in peripheral blood and may mimic BCP ALL cells (2/4 cases). The percentage of CD34pos38neg cells was analysed in a limited cohort of patients, percentage ranged from 0.04% to 10%, mean 1.4%, increased values were observed in JMML, RAEB-t and MDR-AML patients. Increased expression of thrombocytic antigens (≥10% at least one of CD41, CD42b and/or CD61) analysed by single color FC was found in 12/21 patients. All investigated patients with monosomy 7 (6 pts) were positive (chi-square p = 0.012).

Conclusion: Our interim findings prompt for investigation of BM and peripheral blood by a standardized panel of mAbs. This panel should focus at myeloid and B lineages. Number of B cells is typically increased (often with asynchronies in CD10, CD20 and/or CD45) in JMML and JMML-like/Noonan sy compared to other MDS patients. In patients with monosomy 7 increased expression of thrombocytic antigens was found. A significant decrease of CD117pos precursors was found in RC patients.

Supported by VZ MZO 00064203.

7. Advanced MDS: Molecular Changes and Therapy-Related Disease

7.1 Case Forum: CAPILLARY LEAK SYNDROME – AN UNUSUAL COMPLICATION OF MDS-RAEB?

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We report 8 years old girl who was diagnosed with MDS-RAEB. She presented with 3 months history of weakness, fatigue and weight loss. She was pancytopenic with 1% of blasts in her peripheral blood count. Bone marrow aspirates were markedly hypoplastic with blast count ranging from 5 to 10%. Bone marrow histology was hypercellular with fibrosis and blast count above 30%. She had trisomy of chromosome 11 and X in bone marrow cytogenetics. The diagnosis of MDS-RAEB has been confirmed in EWOG-MDS reference center in Freiburg. We found markedly elevated IgG level and moderately increased lactate dehydrogenase (LDH). No organomegaly was present. She was left untreated and search for an unrelated donor was activated. Six weeks after the initial diagnosis she suddenly developed short episode of high grade fever with normal level of C reactive protein. At the same time painful hepatomegaly, peripheral oedema and weight gain were observed. Sparse, bilateral infiltrates were detected on chest X ray. Despite symptomatic and antimicrobial treatment, she progressed to fully developed capillary leak syndrome with ARDS, pseudotumour cerebri, coagulopathy and elevation of LDH. She needed very aggressive ventilation support, and treatment with high dose steroids did not improve the situation. We started salvage AML-like chemotherapy with cytosine arabinosid and anthracyclin. We added one dose of rituximab on day +3 because of PCR-EBV positivity in peripheral blood. This positivity has not been confirmed afterwards. Within 48 hours after the dose of rituximab, her ventilation parameters got better and coagulopathy resolved. The forthcoming aplasia lasted for 24 days requiring granulocyte concentrates for fungal pneumonia. After a total of 44 days she was extubated. At that time she had normal peripheral blood count with slightly hypocellular marrow without blasts. Her IgG and LDH level were normal. At present time, two months later, she is on 6-MP awaiting allogeneic bone marrow transplantation (MUD) which is scheduled for the end of February 2006. Her Karnofsky score is 70%.

We conclude that our patient suffered from capillary leak syndrome due to a degranulation of myeloblasts. The possible extramedullar hematopoeisis in liver is a matter of discussion. Searching the literature, we did not find any similar case except for Sweet's syndrome which has been described exclusively in adults.

7.2 Invited Lecture: GTP-BINDING PROTEINS OF THE RAS SUBFAMILY AND DISEASES

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The function of Ras-like GTP-binding proteins is to switch between the GTP-bound active and the GDP-bound inactive state. They also act as timers, since the duration of the signal is inversely proportional to the GTPase reaction rate. Therefore the GTPase reaction is of fundamental importance for the function of Ras and other GTP-binding proteins such as Rho, Rap and RheB. Blockage of the GTPase reaction by oncogenic point mutations of Ras leads to tumor formation in many types of human neoplasia. Other diseases such as Neurofibromatosis type I and Tuberous Sclerosis are caused by mutations in or deletion of GTPase-activating proteins neurofibromin and tuberin, which activate the GTPase reaction of Rap and RheB. Recently a number of developmental diseases caused by mutations in the three different Ras genes were described.

All of these will be considered in light of the biochemistry and structure of these proteins.

7.3 THERAPY-RELATED MYELODYSPLASTIC SYNDROMES IN CHILDHOOD: REPORT ON A DIAGNOSTIC SURVEILLANCE PROTOCOL IN A SINGLE INSTITUTION

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Therapy-related MDS (t-MDS) are emerging diseases in children previously treated with chemotherapy (especially high doses of alkylating drugs and epipodophyllotoxins) and/or radiotherapy; the diagnosis is often difficult, particularly in the early phases, and may require prolonged observation and repeated bone marrow biopsies. t-MDS are at high risk of evolution to AML and SCT is, at present, the only effective therapeutic choice.

We report on a diagnostic surveillance protocol applied in our Institution since 2000 for early detection of t-MDS in patients (pt) at high risk: children during chemo and/or radiotherapy or in off-therapy follow-up for CNS tumors, acute leukemias, lymphomas, at the time of unexplained cytopenia.

We performed morphological analysis on peripheral blood, marrow aspirate and biopsy; immunophenotypic clustering by flow cytometry; standard cytogenetic test; FISH and biomolecular analysis (AML translocations, number of copies of WT1 gene); clonogenic hemopoietic progenitors assays in standard conditions in the absence of stimula, with increasing concentration of growth factors; GPI-linked proteins. Since 2002 circulating CD34+ cell count and apoptotic rate were evaluated by flow cytometry (CD34 and annexin V expression, absolute count evaluated by an ISHAGE-derived method).

We diagnosed 5 t-MDS (4 RAEB, 1 CMML) among 478 consecutive pt diagnosed between 1996–2005 (1%). CMML with monosomy 7 was identified 36 months after a diagnosis of AML-M3 t(15;17); 1 RAEB was diagnosed during maintenance phase of ALL; 2 RAEB in pt previously treated for CNS tumor rapidly developed to AML; 1 RAEB followed Hodgkin disease. All pt underwent SCT: 2 died for progression of disease, 3 are alive and in complete remission (follow-up 18 to 55 months).

Two out of 5 pt showed a normal karyotype, 1 had monosomy 7, 1 t(3;11) and 1 a complex karyotype. In all evaluable pt a high peripheral blood CD34+ cell count with a low apoptotic rate has been found.

In our experience the association of cytogenetic tests, bone marrow biopsy and simultaneous evaluation of circulating CD34+ cell count and apoptotic rate allowed a timely diagnosis of t-MDS.

The employment of more intensive therapy protocols in pediatric malignancies improved their overall survival, but at the same time predisposes them to an increased risk of secondary neoplasms including MDS; thus a diagnostic surveillance protocol is recommended in selected high risk population.

7.4 CHARACTERISTICS AND OUTCOME OF TREATMENT-RELATED MDS AFTER CHILDHOOD CANCER: THE EWOG-MDS EXPERIENCE

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Treatment-related MDS (tMDS) is a serious complication of therapy of childhood cancer. Here we report on 59 patients (pts; 35 males, 24 females) with tMDS enrolled in the prospective study EWOG-MDS 98. Prior malignancy was ALL in 21 pts, AML in 5, lymphoma in 6, CNS tumors in 12, and other solid tumors in 15. Median age at diagnosis of tMDS was 11.2 yrs (range 1.2–23.2), and median time between first malignancy and tMDS 3.3 yrs (range 0.5–11.1). Disease was classified as RC in 15 pts, RAEB in 30 and RAEB-t in 14. Karyotype at diagnosis was abnormal in 77%, including 38% with complex aberrations, and 38% with monosomy 7. Progression of disease was noted in 26 of 52 evaluable pts at a median time of 2.5 mo (range 0.3–16) from diagnosis. After a median follow-up time of 20 mo (range 3–69) 28 pts were alive with an estimated 5-year overall survival of 32% (95% CI 20–51). No child survived beyond 15 mo from diagnosis without receiving allogeneic HSCT. HSCT was performed in 45 pts. Source of stem cells was bone marrow in 24 pts, peripheral blood in 20 and cord blood in 1. The donor was an HLA-identical relative (MFD) in 18 pts, while 26 pts were transplanted from an HLA-matched or 1-antigen/allele disparate unrelated donor (UD) and 1 pt from a 2-antigen disparate parent. Preparative regimen included busulfan 16 mg/kg, cyclophosphamide 120 mg/kg and melphalan 140 mg/m^2 in 32 pts. Prophylaxis of GVHD generally consisted of cyclosporine A for MFD, combined with methotrexate and ALG for UD. Two patients had graft failure. The cumulative incidences of grade II–IV acute GVHD and chronic GVHD were 32% and 24%, respectively. Twelve pts suffered transplant-related mortality (TRM), the cumulative incidence of TRM in pts transplanted from a MFD or UD being 12% and 39%, respectively ($P = 0.058$). Prior therapy with platinum compounds was associated with an increased risk of TRM. Fifteen pts relapsed at a median time of 9 mo (range 2–29) after HSCT, the 5-year cumulative incidence of relapse being 44%. Of the 45 pts transplanted, 21 were alive and 19 disease-free. The estimated 5-year EFS after HSCT was 27% (95% CI 15–47). Prior therapy with platinum compounds, a WBC > 4 G/L at diagnosis, and a female donor predicted inferior EFS, while HSCT from an UD versus MFD resulted in similar outcome (5-year EFS, 21% and 39%, respectively). AML-type therapy prior to HSCT did not improve survival. Innovative stragegies for improving the outcome of these patients are warranted.

7.5 Invited Lecture: LOW-DOSE DNA DEMETHYLATING AGENTS AS NONINTENSIVE TREATMENT OPTION IN MDS

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The regulated expression of differentiation-associated genes during normal hematopoiesis is governed by the epigenetic process of coordinate gene activation by promoter demethylation, e.g. during the activation of the myeloperoxidase (MPO) during myeloid differentiation Therefore DNA methylation has provided a target for inducing differentiation by pharmacological inhibition of this process to achieve cellular differentiation. More recently, the aberrant hypermethylation of many growth-regulatory and several proapoptotic genes has led to further acknowledgement of hypermethylation as a molecular target for novel treatment approaches in hematologic neoplasias and hemoglobinopathies. DNA methylation inhibition is achieved by the azanucleosides 5-azacytidine (Vidaza®) and 5-aza-2′-deoxycytidine (Decitabine, Dacogen®). With the more recent establishment of epigenetic silencing as a major step during carcinogenesis, development of these drugs has been spurred.

The development of low-dose 5-azacytidine in the USA led to the pivotal phase III study by the CALGB demonstrating that a treatment with subcutaneous 5-azacytidine given on 7 consecutive days over at least 4 months results in prolonged time to AML