Identification and clinical evaluation of the receptor for hyaluronic acid-mediated motility (RHAMM/CD 168) in AML patients

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Abstract

Purpose: this study was designated to detect RHAMM expression as an immunogenic antigen among Egyptian acute myeloid leukemia patients, regardless of their age, sex, concomitant cytogenetic abnormalities or FLT3-ITD status. RHAMM may be useful as an early diagnostic or prognostic marker and a potential target structure for cellular and antibody-based immunotherapies regardless its impact on the response to ttt. **Patients and Methods:** In the present study, RHAMM expression was tested in the peripheral blood samples of 40 AML patients as well as 20 healthy volunteers as a control group by RT-PCR. Patients were diagnosed according to the French-American-British cooperative group criteria. Their ages ranged between 12 and 71 years with a mean value of 37.4 ± 16.9 years. All patients were investigated at presentation prior to therapy. **Results:** The mRNA of RHAMM was detected in 24/40 (60%) of the patients while it was not detected in the control group. There were no statistically significant difference between RHAMM positive and negative patients as regard their clinical and laboratory data, although the highest remission rate was achieved in RHAMM positive patients while RHAMM negative patients had a higher rate of adverse treatment outcome (relapse and death during induction chemotherapy.
Conclusion: our findings are highly suggestive that the expression of RHAMM in newly diagnosed AML patients is a good prognostic marker as its expression is associated with favourable response to induction chemotherapy. Also being a tumour associated antigen, RHAMM represents a promising target for future immunotherapy.

Introduction

Acute myeloid leukaemia(AML) is a heterogeneous disease arising from clonal proliferation of neoplastic precursors in the bone marrow. A variety of prognostic factors have been identifed that predict for outcome, most notably, the presence of defined cytogenetic abnormalities. Intensive combination chemotherapy treatment for acute leukaemia results in excellent remission rates as defined by <5% blasts in the bone marrow ***(1)***. Unfortunately, many patients relapse from a state of minimal residual disease (MRD), particularly patients in poor-risk categories. Even in the best prognostic categories, patients with AML have overall survival (OS) rates of (50–55%). Allogeneic stem cell transplantation is associated with a lower risk of relapse (37–53%) compared with conventional chemotherapy, and results in improved disease-free survival rates in AML ***(2)***.

However, significant toxicity and transplant-related mortality limit the efficacy of this procedure and may abrogate the potential benefits of a lower relapse rate. The graft-vs-leukaemia effect mediated by donor cytotoxic T lymphocytes (CTLs) that potentially recognize and destroy the residual malignant cells is thought to be the mechanism by which disease control is achieved after allogeneic transplantation. This has led to the use of reduced-intensity conditioning allogeneic transplants and donor lymphocyte infusions (DLI) to utilize CTLs without the toxicity of conventional allogeneic transplantation. However, the use of allogeneic transplantation and DLI is still limited to a small number of suitable patients, and is complicated by the negative effects of alloreactive CTLs causing graft-vs-host disease due to their lack of specifcity for the malignant clone ***(1)***.

Immunotherapy for leukaemia patients, aiming at the generation of anti-leukaemic T cell responses, could provide a new therapeutic approach to eliminate minimal residual disease (MRD) cells in acute myeloid leukaemia (AML). Leukaemic blasts harbour several ways to escape the immune system including deficient MHC class II expression, low levels of co-stimulatory molecules and suppressive cytokines ***(3)***. The development of cancer vaccines directed against myeloid leukaemias has been a research area of intense interest in the past decade. Both human studies in vitro and mouse models in vivo have demonstrated that leukaemia-associated antigens (LAAs), such as the fusion protein BCR-ABL, Wilms' tumour protein and proteinase 3, may serve as effective targets for cellular immunotherapy. Pilot clinical trials have been initiated in chronic and acute myeloid leukaemia and other haematological malignancies, which include vaccination of patients with synthetic peptides derived from these LAAs ***(4)***. Among these leukemia-associated antigens (LAAs) that induce a humoral immune response in AML patients the receptor for hyaluronic acid-mediated motility (RHAMM) ***(5,6)***.

RHAMM/CD168 is a receptor for hyaluronan, a glycoaminoglycan that plays a fundamental role in cell growth, differentiation and motility. RHAMM is a cell-surface receptor and belongs to the group of extracellular matrix molecules. The gene for RHAMM is localized on the human chromosome band 5q33.2. The expression pattern of RHAMM is highly tissue restricted. mRNA expression

of RHAMM was found in testis, placenta , thymus tissue and endothelium cells involved in angiogenesis. No expression of RHAMM was described on non-activated normal B cells in blood, spleen, or lymph nodes ***(5,7).***

RHAMM is highly expressed in different tumor cell lines, also RHAMM mRNA and protein expression levels was detected in all leukemia cell lines. In non-haematological malignancies as melanomas, over-expression of RHAMM is essential for ras-mediated transformation and it is associated with the development of metastases. This demonstrates that RHAMM might be responsible for loss of control of the cell cycle and for the tendency of cancers to metastasize, which had been described earlier for other tumor entities as in melanomas ***(7).***

RHAMM is an antigen eliciting both humoral and cellular immune responses in patients with AML, myelodysplastic syndrome and multiple myeloma ***(8)***. ***Giles et al. (9)*** demonstrated by immunocytologic staining that RHAMM/CD168 is expressed both in the cytoplasm and the surface of leukemic blasts, opening new stratigies for potentially using RHAMM targeting antibody therapies.

The aim of this study is to detect RHAMM expression as an immunogenic antigen among Egyptian acute myeloid leukemia patients, regardless of their age, sex, concomitant cytogenetic abnormalities or FLT3-ITD status. RHAMM may be useful as an early diagnostic or prognostic marker and a potential target structure for cellular and antibody-based immunotherapies regardless its impact on the response to ttt.

Patients and Methods

The present study included 40 newly diagnosed AML patients. They were diagnosed at the departments of Medical Oncology and Clinical Pathology, Faculty of Medicine, Cairo University. Patients were diagnosed according to the French-American-British cooperative group criteria ***(10)***. Their ages ranged between 12 and 71 years with a mean value of 37.4 ± 16.9 years. All patients were investigated at presentation prior to therapy. Twenty age-sex matched healthy volunteers were included in this study as a control group. we didn't include more controls because RHAMM expression is tissue-restricted (testis, thymus and placenta) and is not normally detected in the peripheral blood mononuclear cells. All patients were subjected to the following:

- Full history taking, clinical examination with careful notation and assessment of clinical signs relevant to AML as: lymphadenopathy, hepatomegaly, splenomegaly, fever, fatigue, weight loss, jaundice, pallor, purpura, ecchymosis, easy bruising, recurrent infections and bone and joint pain.
- CT chest, abdomen and pelvis are done to assess lung, liver, spleen, lymph nodes and kidneys for possible pathological alterations.
- Cardiac examination including echo cardiography and ejection fraction to assess the cardiac condition of the patients that might be affected by anthracycline chemotherapy.
- Routine laboratory investigations as differential blood count (CBC), liver and kidney functions, serum uric acid, LDH and coagulation profile are done.
- In case of infection blood cultures and swabs are done.
- Bone marrow aspiration and cytochemical studies (including myeloperoxidase, non-specific esterase and dual esterase reactions).
- The immunophenotyping was done to establish the FAB subtyping.
- RHAMM gene expression in peripheral blood samples of patients and controls by conventional reverse transcriptase – polymerase chain reaction (RT-PCR).

Detection of RHAMM gene expression by RT-PCR as described by (7): Five ml of blood were withdrawn from every patient as well as the healthy voluntears in a sterile EDTA vaccutainer. The mononuclear cells are separated and preserved at - 20 °C. Total cellular RNA was extracted from the monnuclear cells using the QIA amp RNA blood Mini kit (QIAGEN, Catalogue number. 52304), followed by c-DNA preparation using Revert Aid™ First strand cDNA synthesis kit (Fermentas, K1621). A volume of 5 μl cDNA was added to a final PCR reaction mixture of 25 μl containing 12.5 μl Master Mix (Fermentas K0171 which contains TaqDNA polymerase in reaction buffer, $MgCl_2$ and dNTPs), 1 μl of 10 μM of each of the forward and reverse RHAMM specific primers and 1 μl of 10 μM of each of the forward and reverse primers of β-actin. For standardization, expression of RHAMM was correlated with the expression of the house keeping gene β-actin. The primers for RHAMM: forward primer: 5'- CAG GTC ACC CAA AGG AGT CTC G-3', reverse primer: 5'-CAA GCT CAT CCA GTG TTT GC-3'. For β-actin: forward primer: 5'-GCA TCG TGA TGG ACT CCG-3', reverse primer: 5'-GCT GGA AGG TGG ACA GCG A-3' (Fermentas™ – Germany). The following thermocycler program: initial denaturation at 95 °C for 1 minute, annealing at 60 °C for 1 minute, and extension at 72 °C for 1 minute. This was repeated for 36 cycles. The amplified products were separated on 2% agarose gel electrophoresis, stained with ethidium bromide. The electrophoretic pattern was visualized under UV light then photographed using a Polaroid camera with a red orange filter. The sample was considered positive when a clear sharp band was observed at the specific molecular weight; 661 bp for β-Actin and 565 bp for RHAMM.

Treatment and Assessment of the response to therapy:
Successful treatment of acute myeloid leukemia (AML) requires control of bone marrow, systemic disease and specific treatment of central nervous system (CNS) disease, if present. The cornerstone of this strategy includes systemically administered combination chemotherapy. Because only 5% of patients with AML develop CNS disease, prophylactic treatment is not indicated ***(11)***.

Treatment is divided into two phases: induction (to attain remission) and post-remission (to maintain remission). Maintenance therapy for AML was previously administered for several years but is not included in most current treatment clinical trials ***(12)***. Patients failing to respond to one or two cycles of such treatment are considered refractory. APL (FAB M3) induction chemotherapy included all-trans retinoic acid (ATRA) ***(13)***.

The induction chemotherapy regimen includes combination of mitoxantrone and Ara-C, in which Ara-C is given as 100 mg/m^2 IV by continuous infusion days 1-7 and mitoxantrone is given in a dose of 12 mg/m^2 intravenously daily for 3 days (*7 and 3 regimen*). Patients were admitted in the inpatient unit and they usually spend about one month in the hospital ***(14)***. Special consideration was given to induction therapy for acute promyelocytic leukemia (PML). Oral administration of all-trans-retinoic acid (ATRA) 45 mg/m^2/day PO until CR induces remission in 70% to 90% of patients with M3 AML. ATRA induces terminal differentiation of the leukemic cells followed by restoration of nonclonal hematopoiesis[18]. The consolidation chemotherapy regimen includes high doses of Ara-C, in which Ara-C is given as 2g/m^2 intravenous injections twice daily for 4 days. These were also given as inpatient treatments ***(15-17)***.
Adult acute myeloid leukemia (AML) in remission normalization of the

neutrophil count (>1500 /cm3), platelet count (>100.000/cm3), Cellular bone marrow with at least 20% cellularity, less than 5% blasts and no Auer rods, as well as absence of extramedullary infiltration. Resistence to induction is defined as more than 5% blasts in the bone marrow, lack of regeneration of normal haematopoiesis or evidence of extramedullary infiltration. Death during induction is defined as death during or after the first course of therapy with aplastic or hypoplastic marrow ***(18)***.

Statistical Analysis: Data were summarized and presented in the form of mean, range and standard deviation as descriptive statistics. Descriptive statistics and statistical comparison were performed using the statistical software program SPSS (version 15). Group comparison was done using either a 2-sampe test or analysis of variance (ANOVA test). Correlation analysis was evaluated using the Pearson coefficient. Odds ratio and 95% confidence intervals (95% CI) were done for detection of the response to therapy. For all of the above mentioned statistical tests done, the threshold of significance is fixed at 5% level (p-value). Probability value (p-value) of more than 0.05 was considered non-significant, while p-value less than 0.05 indicated a significant result.

Results

The present study included 40 de novo AML patients, 22 males and 18 females. The main clinical and laboratory characteristics of AML patients were summarized in **Table (1)**. By conventional RT-PCR, mRNA of RHAMM gene was detected in 24/40 (60%) of the patients while it was not detected in the peripheral blood mononuclear cells of the control group. Comparison between RHAMM positive and negative patients regarding their clinical and laboratory findings is presented in **Table (2)**. There were no statistically significant difference between RHAMM positive and negative patients as regard their clinical and laboratory data. As regard the response to induction chemotherapy, the highest remission rate was achieved in RHAMM positive patients while RHAMM negative patients had a higher rate of adverse treatment outcome (relapse and death during induction) OR 2.455 and 95% CI 0.954 – 6.313 (P = 0.035) **Table (3)**.

As acute prolymphocytic leukemia (FAB-M3) has been considered as a separate disease entity among AML, and the response to treatment with all-trans retinoic acid (ATRA) had dramatically improved its clinical outcome, we omitted FAB-M3 cases (6 cases) from the patients group before evaluating their response to induction therapy.

RHAMM was expresssed in 60% of our patients , and this high percentage should be taken in consideration for the importance of RHAMM peptide vaccine as an adjuvant therapy with chemotherapy or stem cell transplantation to eradicate residual leukemic cells and control minimal residual disease or augment graft versus leukemia effect after SCT.

Discussion

Treatment of patients with AML became more effective during the past decades, but a CR is often not durable and a high percentage of AML patients relapse. Therefore, complementary therapeutic approaches are under exploration for the prevention of relapse, and finding the reason for the unfavorable prognosis in AML patients. Specific immunotherapies for patients with acute myeloid leukemia (AML) using leukemia-associated antigens (LAA) as target structures might be a therapeutic option to enhance the graft-vs.-leukemia effect observed after allogeneic stem cell transplantation or to prolong a complete remission (CR) achieved by chemotherapy ***(19,20).***

Targeted immunotherapies require the identification and characterization of appropriate antigen structures. Initially, T-cell based cancer vaccines were designed for patients with solid tumors after the definition of suitable tumor-associated antigens. Several immunological and even clinical responses prompted researchers and clinicians to extend the spectrum of cancer vaccines towards hematologic malignancies such as acute myeloid leukemia (AML) ***(21)***. The identification of new immunogenic leukaemia-associated antigens (LAAs) is mandatory for the development of specific immunotherapies that selectively recognize and destroy leukaemia cells with the aim of reducing relapse rates without the need for allogeneic transplantation ***(1)***.

An ideal LAA that qualifies as a potential target for immunotherapies should be expressed preferentially in leukemic blasts, but neither on haematopoietic stem cells nor on normal tissues ***(22)***. Several leukemia-associated antigens (LAA) have been identified in patients with acute myeloid leukemia as BAGE, BCL-2, OFA-iLRP, FLT3-ITD, G250, hTERT, PRAME, proteinase 3, RHAMM, survivin, and WT-1 ***(23)***.

Excessive and detrimental vascularization occurs in neoplasia, promoting tumour growth and metastasis. Greater understanding of the mechanisms controlling the angiogenic process will provide mechanisms to inhibit neovascularization in tumours. The extracellular macromolecules, notably glycosaminoglycans (GAGs), are important mediators of angiogenesis. Hyaluronan (HA) is a large, non-sulphated GAG. Native high molecular weight HA (n-HA) is anti-angiogenic, whereas HA degradation products (o-HA; 3–10 disaccharides) stimulate endothelial cell (EC) proliferation, migration and tube formation following activation of specific HA receptors in particular, CD44 and Receptor for HA-Mediated Motility (RHAMM, CD168). Cell surface RHAMM and intracellular RHAMM are required for passage through G2/M phase of the cell cycle. Anti RHAMM antibodies blocked the migration of cells to the process of tissue injury and angiogenesis including EC, macrophages, smooth muscle cells and fibroblasts ***(23-26)***.

Greiner et al. (7) studied the mRNA expression of RHAMM in AML patients during their clinical course. After treatment of RHAMM positive patients with polychemotherapy, no mRNA expression of RHAMM was detectable. In all the peripheral blood mononuclear cells from the healthy volunteers, RHAMM was not expressed neither at the mRNA level nor the protein level.

In the present study, we focused on the influence of RHAMM expression on the response to induction chemotherapy of newly diagnosed AML patients, and also as a promising new target for future monovalent or polyvalent immunotherapeutic approaches. RHAMM expression was tested in the peripheral blood samples from 40 de novo AML patients as well as 15 healthy volunteers as a control group by conventional RT-PCR. All the control subjects were negative for RHAMM . This is in agreement with ***(5,7,19, 21,27)***.

Regarding the patient group, mRNA expression of RHAMM gene was detected in 24/40 (60%) of the patients. This is in accordance with ***(7,19,21)*** where RHAMM expression ranged between 60 to 70% of their patients. ***Greiner et al. (7)*** reoprted that RHAMM expression at the protien level was higher than the mRNA level (70% vs 60%). However, the percentage of RHAMM

positive patients was higher in the study of ***Greiner et al. (5)*** as RHAMM was expressed in more than 80% of their AML patients by RT-PCR and Western blot techniques. This may be attributed to the small patient group enrolled in this study (17 patients).

Comparing RHAMM positive with RHAMM negative patients in the present study, no statistically significant difference was found between the two groups regarding the age, the presenting symptoms or the incidence of hepatomegaly, splenomegaly or lymphadenopathy. RHAMM expression was more prominant among male patients however, the differnce between the two groups did not reach a statistically significant level (p= 0.072). This may be attributed to the relatively small number of patients under study. This is in agreement with ***(4,19)*** who reported that RHAMM expression did not correlate to age, sex or clinical presentation of the patients. On the other hand, ***Greiner et al. (27)*** reported that there was a significant association between ages more than 60 years and 'no TAAs' expression, as older age is known to be associated with adverse outcome.

No significant differences were detected as for the expression of RHAMM in the FAB subtypes M1 - M5. This is in accordance with ***(5,19,21)*** where no significant difference was noted in their studies as regards FAB subtypes M0 – M5. Although RHAMM expression was more prominant among FAB M1 patients yet it was statistically insignificant.

Correlation of RHAMM expression with the response to induction chemotherapy revealed that RHAMM positive patients reached a statistically significant higher complete remession rate (p=0.035). This is in accordance with ***(7,19,27)***. ***Greiner et al. (5)*** reported that most of the patients enrolled in their study showed complete remission of their disease following induction chemotherapy. Also, ***Greiner et al. (21)*** reported that RHAMM expression is a good prognostic marker, associated with favourable treatment outcome.

The expression of tumour associated antigens might play a critical role in the control of minimal residual disease, and therefore might be associated with the clinical outcome in AML. RHAMM, PRAME and G250 can induce strong antileukemic immune responses, possibly controlling MRD control. High expression of at least one of the three antigens, RHAMM, PRAME or G250 provided the strongest favourable prognostic effect. Thus, these tumour associated antigens represent interesting targets for polyvalent immunotheraputic approaches in AML ***(21)***.

The lack of RHAMM mRNA expression in CD34+ haematopoietic stem cells or in normal tissues (except for testis, placenta and thymus) renders it a potant immunologic target for the immune system to fight a residual tumour load following chemotherapy in AML ***(27)***. Immunotherapy targeting RHAMM might be an option to enhance such a specific antileukemia (GVL) effect after chemotherapy or allogeneic transplantation without aggravating graft-vs-host disease (GVHD). Moreover, the therapies might be a useful tool to prolong the duration of complete remission reached by induction chemotherapy. RHAMM is currently used in peptide vaccination trials for patients with haematological malignancies (RHAMM-R3 peptide vaccination) ***(8,28)***.

In conclusion, our findings are highly suggestive that the expression of RHAMM in newly diagnosed AML patients is a good prognostic marker as its expression is associated with favourable response to induction chemotherapy. Also being a tumour associated antigen, RHAMM represents a promising target for future vaccination trials to further augment immune responses relevant in AML therapy.

References

1. Caroline Duncan and Huw Roddie (2008): Dendritic cell vaccines in acute leukaemia. Best Practice & Research Clinical Haematology, Vol.21,No.3,pp.521–541.

2. Burnett A.K, Kell W.J, Goldstone A.H. et. al. (2006): The addition of gemtuzumabozogamic into inductionchemotherapy for AML improves disease free survival without extratoxicity: preliminary analysis of 1115 patients in the MRCAML 15 trial. ASHAnnual Meeting Abstracts; 108: 13.

3. van de Loosdrecht A.A, van den Ancker W, Houtenbos I, Ossenkoppele G.J, Westers T.M (2009): Dendritic cell-based immunotherapy in myeloid leukaemia: translating fundamental mechanisms into clinical applications. Handb Exp Pharmacol. ;(188):319-48.

4. Dao T and Scheinberg D.A. (2008): Peptide vaccines for myeloid leukaemias. Best Pract Res Clin Haematol. Sep;21(3):391-404.

5. Greiner J, Li L, Ringhoffer M, et. al. (2005): Identification and characterization of epitopes of the receptor for hyaluronic acid-mediated motility (RHAMM/ CD168) recognized by CD8_ T cells of HLA-A2-positive patients with acute myeloid leukemia. Blood;106:938-945.

6. Draghici S, Chatterjee M, Tainsky M.A. (2005): Epitomics: serum screening for the early detection of cancer on microarrays using complex panels of tumor antigens. Expert Rev Mol Diagn., 5:735–43.

7. Greiner J, Ringhoffer M, Taniguchi M, et. al. (2002): RHAMM is a new immunogenic tumor-associated antigen overexpressed in acute and chronic myeloid leukemia. Exp Hematol. 30: 1029-1035.

8. Schmitt M, Schmitt A, Rojewski M.T, Chen J, Giannopoulos K, Fei F, Yu Y, Götz M, Heyduk M, Ritter G, Speiser D.E, Gnjatic S, Guillaume P, Ringhoffer M, Schlenk R.F, Liebisch P, Bunjes D, Shiku H, Dohner H, Greiner J. (2008): RHAMM-R3 peptide vaccination in patients with acute myeloid leukemia, myelodysplastic syndrome, and multiple myeloma elicits immunologic and clinical responses. Blood. Feb 1;111(3):1357-65.

9. Giles F.J, Keating A, Goldstone A.H, Avivi I, Willman C.L, Kantarjian H.M (2003): Acute myeloid leukemia. Hematology (Am Soc Hematol Educ Program), 73-110.

10. Bennett J.M., Catvosky D. And Daniel M.T. (1985): Proposed revised criteria of the classification of acute myeloid leukemia. Ann Intern Med.; 103: 602-625.

11. Sheinberg D.A, Maslak P.G, Weiss M.A (2005): Acute leukemias. In: DeVita VT Jr, Hellman S, Rosenberg SA, eds.: Cancer: Principles and Practice of Oncology. 7th ed. Philadelphia, Pa: Lippincott Williams & Wilkins, pp 2088-116.

12. Cassileth P.A, Lynch E, Hines J.D, et. al. (1992): Varying intensity of postremission therapy in acute myeloid leukemia. Blood 79 (8): 1924-30.

13. Licht J.D, Chomienne C, Goy A, et. al. (1995): Clinical and molecular characterization of a rare syndrome of acute promyelocytic leukemia associated with translocation (11;17). Blood 85 (4): 1083-94.

14. MacCallum P.K, Rohatiner A, Davis C, Whelan J, Oza A.M, Lim J, Love S, Amess J.A, Leahy M, Gupta R.K, Price C.G and Lister T.A. (1995): Mitoxantrone and cytosine arabinoside as treatment for acute myeloblastic leukemia in older patients. Annals of Hematology. Volume 71, Number 1 / July.

15. Goldstone A.H, Burnett A.K, Wheatley K, et. al. (2001): Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: the results of the United Kingdom Medical Research Council AML11 trial. Blood 98 (5): 1302-11.

16. Cassileth P.A, Harrington D.P, Hines J.D, et. al. (1988): Maintenance chemotherapy prolongs remission duration in adult acute nonlymphocytic leukemia. J Clin Oncol 6 (4): 583-7.

17. Stone R.M, Berg D.T, George S.L, et. al. (2001): Postremission therapy in older patients with de novo acute myeloid leukemia: a randomized trial comparing mitoxantrone and intermediate-dose cytarabine with standard-dose cytarabine. Blood 98 (3): 548-53.

18. Bacher U, Kern W, Schoch C, et. al. (2006): Evaluation of complete disease remission in acute myeloid leukemia: a prospective study based on cytomorphology, interphase fluorescence in situ hybridization, and immunophenotyping during follow-up in patients with acute myeloid leukemia. Cancer 106 (4): 839-47.

19. Jochen Greiner, Mark Ringhoffer, Masanori Taniguchi, Li Li, Anita Schmitt, Hiroshi Shiku, Hartmut D¨Ohner and Michael Schmitt (2004): mRNA expression of leukemia-associated antigens in patients with acute myeloid leukemia for the developmentof specific immunotherapies. Int. J. Cancer: 108, 704–711.

20. Heslop H.E, Stevenson F.K, Molldrem J.J. (2003): Immunotherapy of hematologic malignancy. Hematology (Am Soc Hematol Educ Program), 331-349.

21. Jochen Greiner, Michael Schmitt, Li Li, Krzysztof Giannopoulos, Katrin Bosch, Anita Schmitt, Konstanze Dohner, Richard F. Schlenk, Jonathan R. Pollack, Hartmut Dohner, and Lars Bullinger (2006 -a): Expression of tumor-associated antigens in acute myeloid leukemia: implications for specific immunotherapeutic approaches. Blood, 108:4109-4117.

22. Jochen Greiner, Mark Ringhoffer, Masanori Taniguchi, Thomas Hauser, Anita Schmitt, Hartmut Dohner and Michael Schmitt (2003): Characterization of several leukemia associated antigens inducing humoral immune response in acute and chronic leukemia. Int.J.Cancer: 106, 224231.

23. Mark Slevin, Jurek Krupinski, John Gaffney, Sabine Matou, David West, Horace Delisser, Rashmin C. Savani, Shant Kumar (2007): Hyaluronan-mediated angiogenesis in vascular disease: Uncovering RHAMM and CD44 receptor signaling pathways. Matrix Biology, 26: 58–68.

24. Maxwell, C.A., Keats, J.J., Crainie,M., Sun, X., Yen, T., Shibuya, E., Hendzel,M., Chan, G., Pilarski, L.M (2003): Rhamm is a centrosomal protein that interacts with dynein and maintains spindle pole stability. Mol. Biol. Cell 14,2262–2276.

25. Noble P.W (2002): Hyaluronan and its catabolic products in tissue injury and repair. Matrix Biol. 21, 25–29.

26. West, D.C., Fan, T.-P.D. (2002): Hyaluronan as a regulator of angiogenesis in wound healing. In: Fan, T.-P.D., Kohn, E.C. (Eds.), The New Angiotherapy. Humana Press, pp. 177–188. Ch.11.

27. Greiner J, Döhner H, Schmitt M. (2006 –a): Cancer vaccines for patients with acute myeloid leukemia--definition of leukemia-associated antigens and current clinical protocols targeting these antigens. Haematologica. Dec;91(12):1653-61

28. Greiner J, Bullinger L, Guinn B.A, Döhner H, Schmitt M. (2008): Leukemia-associated antigens are critical for the proliferation of acute myeloid leukemia cells. Clin Cancer Res. Nov 15;14(22):7161-6.

Tables

Table 1: The main characteristics of AML patients included in the present study

	AML patient (Number = 40)	
Age (years)	12 – 71 (37.4 ± 16.9) years	
Sex (M/F)	Males= 22 / 40 (55%) Females= 18 / 40 (45%)	
Lymphadenopathy (number -%)	Absent	24/40 (60%)
	Present	16/40 (40%)
Hepatomegaly (number -%)	Absent	30/40 (75%)
	Present	10/40 (25%)
Splenomegaly (number -%)	Absent	18/40 (45%)
	Present	22/40 (55%)
Bleeding tendancy (number -%)	Absent	14/40 (35%)
	Present	26/40 (65%)
Fever (number -%)	Absent	13/40 (32.5%)
	Present	27/40 (67.5%)
Haemoglobin level (gm/dl) (range, mean ± SD)	5 – 11 (7.53 ± 1.28)	
Total leucocytic count ($X10^3$/cmm) (range, mean ± SD)	4 – 509 (58.35 ± 107.89)	
Platelet Count ($X10^3$/cmm) (range, mean ± SD)	8 – 91 (37 ± 27.41)	
Peripheral blood blasts (%) (Range, mean ± SD)	10 – 100% (53.5 ± 31.27)	
Bone marrow blasts (%) (Range, mean ± SD)	35 – 100% (76.35 ± 24.35)	
FAB classification (Number - %)	M1= 14 / 40 (35%) M2= 8 / 40 (20%) M3= 6 / 40 (15%) M4= 8 / 40 (20%) M5= 4 / 40 (10%)	
RHAMM expression by (RT-PCR)	Negative = 16 / 40 (40%)	
	Positive = 24 / 40 (60%)	
Response to induction chemotherapy	Complete remission = 15 / 40 (37.5%)	
	Relapsed = 3 / 40 (7.5%)	
	Death = 22 / 40 (55%)	

Table 2: Comparison between RHAMM positive and negative patients by RT-PCR regarding their clinical and laboratory data

			RHAMM Positive (n = 24)	RHAMM Negative (n = 16)	P value	Significance
Clinical Data	Sex: Male Female		16 / 24 (66.7%) 8 / 24 (33.3%)	6 / 16 (37.5%) 10 / 16 (62.5%)	0.072	NS
	Age (years) (range, mean ± SD)		12 – 63 (34 ± 16.65)	17 – 71 (42.43 ± 16.55)	0.13	NS
	Splenomegaly	-	12 /24 (50%)	6 / 16 (37.5%)	0.43	NS
		+	12 / 24 (50%)	10 / 16 (62.5%)		
	Hepatomegaly	-	18 / 24 (75%)	12 / 16 (75%)	1	NS
		+	6 / 24 (25%)	4 / 16 (25%)		
	Lymph-adenopathy	-	16 / 24 (66.7%)	8 / 16 (50%)	0.29	NS
		+	8 / 28 (33.3%)	8 / 16 (50%)		
	Fever	-	9 / 24 (37.5%)	4 / 16 (25%)	0.43	NS
		+	15 / 24 (62.5%)	12 / 16 (75%)		
	Bleeding tendency	-	8 / 24 (33.3%)	6 / 16 (37.5%)	0.78	NS
		+	16 / 24 (66.6%)	10 / 16 (62.5%)		
Laboratory Data (Mean ± SD)	Hb (g/dl)		7.7 ± 1.48	7.25 ± 0.88	0.26	NS
	WBC (x103/cm3)		73.7 ± 135.81	35.26 ± 32.17	0.269	NS
	Platelet (x103/cm3)		40.8 ± 30.9	31.25 ± 20.53	0.279	NS
	PB blast (%)		53.5 ± 30.9	53.6 ± 32.75	0.99	NS
	BM blasts (%)		75.3 ± 24.3	77.8 ± 25.04	0.74	NS
FAB Classification	M1		8 / 24 (33.3%)	6 / 16 (37.5%)	0.78	NS
	M2		4 / 24 (16.7%)	4 / 16 (25%)	0.52	
	M3		4 / 24 (16.7%)	2 / 16 (12.5%)	0.71	
	M4		6 / 24 (25%)	2 / 16 (12.5%)	0.32	
	M5		2 / 24 (8.3%)	2 / 16 (12.5%)	0.67	

- : Absent, + : Present
NS= Statistically not significant, S= Statistically significant.

Table 3: RHAMM gene expression in relation to the response to induction chemotherapy

Response to induction chemotherapy of non-M3 cases	RHAMM + (n=20)	RHAMM- (n=14)	P-value	significance
CR	10/20 (50%)	2/14 (14.2%)	0.043	significant
Failed induction	2/20 (10%)	1/14 (7.1%)		
Death during induction	8/20 (40%)	11/14 (78.5%)		

RHAMM + M3 cases = 4, all achieved CR.
RHAMM – M3 cases= 2, 1 died from DIC and 1 CR.
The highest remission rate was achieved in RHAMM + non-M3 (PML) cases.

Figures

Fig 1: RHAMM gene expression by RT-PC

-Upper panel: Amplification with RHAMM specific primers showing 565 bp PCR product. Lane 4,5,7 show +ve expression, Lane 2,3,6,8 show negative expression.
- Lower panel: the same RNA as in lanes given above amplified with β-actin specific primers (661 bp) serves as an internal control for the quality of RNA.
- Lane 1; 100- 1500 bp ladder size marker.