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Research Article

Blockade of Integrin-α4-Mediated Adhesion of T-ALL Cells

Yao-Te Hsieh[1], Stephanie N. Shishido[1], Andrea Osborne[1], Colleen Garvey[1], Eun Ji Gang[1], Halvard Bönig[2] and Yong-Mi Kim[1]*

[1]Department of Pediatrics, University of Southern California Keck School of Medicine, USA

[2]German Red Cross Blood Service and Institute for Transfusion Medicine and Immunohematology, Goethe University, Germany

*Corresponding author

Yong-Mi Kim, Department of Pediatrics, Division of Hematology and Oncology, Children's Hospital Los Angeles, University of Southern California, 4650 Sunset Boulevard, Mailstop #57, Los Angeles, CA 90027, USA, Tel: 01-323-361-8544; Email: ymkim@chla.usc.edu

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Keywords

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Abstract

Objective: We showed previously that α4 blockade using a humanized monoclonal antibody against integrin α4, Natalizumab, antagonizes stromal adhesion of pre-B ALL cells and in combination with chemotherapy eliminates resistant pre-B-ALL in an MRD setting. Here we determined the effect of Natalizumab on adherence of patient-derived T-ALL cells in vitro and on survival prolongation of murine recipients of T-ALL cells, to explore the potential of Natalizumab as a novel T-ALL treatment strategy.

Methods: Adhesion to stromal matrix proteins in the presence/absence of Natalizumab and survival of murine recipients of primary T-ALL cells treated with/without Natalizumab were assessed, using previously described methods.

Results: Natalizumab inhibited adhesion of patient-derived T-ALL cells and attenuated leukemia progression, resulting in prolonged survival of recipient NOD/SCID IL2R gamma$^{-/-}$ mice of patient-derived T-ALL.

Conclusion: α4 blockade interferes with adhesion of T-ALL cells to its counter receptor and thus merits evaluation as a novel adjuvant strategy for T-ALL. Further study is needed to explore at the molecular level the effect of α4 blockade in T-ALL.

ABBREVIATIONS

BM: Bone Marrow; **IP:** Intraperitoneal; **MST**: Median Survival Time; **NZM**: Natalizumab; **Ig**: Immunoglobulin; **FD**: Found Dead; **hCD2**: Human CD2.

INTRODUCTION

Leukemia relapse still occurs despite the improvement in overall prognosis and survival of patients with acute lymphoblastic leukemia (ALL). ALL cell survival during exposure to chemotherapy is contributed in part to the cell-cell contact of bone marrow stromal and leukemia cells [1-3]. The integrin heterodimer VLA4 (Very Late Antigen-4) is composed of the integrin α4 (α4) chain in association with the integrin ß1 chain [4,5], which is capable of binding the counter receptors fibronectin, osteopontin (OPN), or VCAM-1[4-6]. VLA4 has been shown to regulate both homing and adhesion, as well as the engraftment of hematopoietic progenitors [7] and ALL cells [8] in BM. In acute myeloid leukemia (AML) cells VLA4 was shown to be a dominant adhesion molecule [9], suggesting that the expression of α4 may be an unfavorable risk factor in AML. It is important to note that alternative observations have been reported [10].We previously showed that α4 is a central molecule for chemoprotection of pre-B ALL and that α4 blockade can sensitize pre-B ALL to chemotherapy [11]. It has been shown that BM stromal cells contribute also to the survival of T-ALL cells, partially through adhesion signaling systems [12-14]. However, formal studies of the potential therapeutic effect of targeting α4 in T-ALL have not been performed. As T-cell-ALL occurs in 10%–15% of pediatric and in 25% of adult ALL cases [15,16], and since drug resistance remains as much of a problem as in pre-B ALL [17], novel therapies against T-ALL are needed. Here, we evaluate the effect of interference of α4-mediated adhesion of T-ALL cells using the humanized monoclonal anti-α4 antibody Natalizumab.

MATERIALS AND METHODS

Engraftment of primary T-ALL in a xenograft model

Bone marrow samples from T-ALL patients were obtained (LAX1R, SFO5, SFO6) in compliance with the Institutional

Review Board regulations of each institution. Informed consent was obtained from all human subjects. Under IACUC approved protocols, NOD/SCID/IL2Rγ$^{-/-}$ (NSG) mice of 5-10 weeks of age were conditioned with a single dose of 250 cGy of total body irradiation, followed by tail vein injection of fresh patient cells as described previously [11,18]. Animal care was in accordance with institutional guidelines.

Flow cytometry

Antibodies FITC human CD2 (G46-6) and PE human CD49d (9F10) as well as respective isotype controls were purchased from BD Biosciences.

CD2^{+} T cells

Mononuclear cells of peripheral blood from healthy donors were isolated by Ficoll and sorted by BD FACSAria II cell sorter (BD Biosciences).

Adhesion assay

T-ALL (LAX1R) cells were either pre-treated with Natalizumab (NZM) or corresponding control IgG4 (G17-4) (BD Biosciences), for 30 minutes and washed once with PBS. Cells were then loaded in triplicate onto 12-well plates coated with 10μg/ml human recombinant VCAM-1 (R&D Systems, Minneapolis, MN) [19]. After 2 hours of incubation, suspension cells in the supernatant were removed and the plate was washed once with PBS. The adhering cells were photographed by Olympus IX71 microscope with 100X magnification and then detached by pipetting 20 times. The cell count for adherent cells was assessed by trypan blue exclusion of dead cells.

Integrin α4 blockade by Natalizumab *in vivo*

LAX1R cells were lentivirally labeled with firefly luciferase as previously described [11] and injected into sub lethally irradiated NOD/SCID/IL2Rγ$^{-/-}$ mice (5x10^{4}cells/mouse). Control groups received control Ig. Natalizumab was given intraperitoneally (i.p.) (5mg/kg of mouse total body weight once per week) for 4 weeks. Leukemia progression was monitored by bioluminescent imaging.

RESULTS

Integrin α4 is highly expressed in primary T-ALL

Analysis of α4 expression on three cases of patient-derived T-ALL cells by flow cytometry indicated high expression in all three T-ALL samples (Figure 1, left panel),similar to CD2^{+} cells of healthy donors (Figure 1, right panel).

Integrin α4 blockade inhibits adhesion of primary T-ALL

We previously showed that α4 blockade using Natalizumab can inhibit adhesion of pre-B ALL cells to VCAM-1[13]. To determine the effects of integrin α4 antagonism on cellular adhesion of T-ALL cells, LAX1R cells were incubated withα4 blocking antibody, Natalizumab (NZM). Matched isotype antibody was used as control. NZM treatment led to a significant reduction in the number of VCAM-1 adherent cells when compared with control IgG4 exposure (3.67±1.2% vs. 72±4%; $p<0.05$) (Figure 2A and 2B).

Targeting Integrin α4 delays the progression of primary T-ALL cells *in vivo*

Luciferase-labeled LAX1R cells were injected into NOD/SCID/IL2Rγ$^{-/-}$ mice and subjected to 4 weekly injections of NZM (5mg/kg/mouse/day) or control Ig from Day 3 post-leukemia injection. Whole-body *in vivo* bioluminescent imaging 18 days after cell injection showed a marked decrease in T-ALL progression (Figure 3A). Overall, the integrin α4 blockade significantly prolonged survival of T-ALL recipient mice compared to control Ig-treated mice (MST=28 days vs. MST=17.5days; $p=0.008$; Figure 3B).

DISCUSSION

Integrins engage with cell surface ligands and extracellular matrix (ECM) components, such as fibronectin, collagen, and laminin. Outside the hematopoietic system they bear significant roles in embryogenesis, growth and repair, and haemostasis [20]. On blood cells, they are involved in a diverse number of leukocyte adhesion-dependent functions, with critical roles in inflammation and immune response and stem cell retention [21,22]. High α4 integrin expression has previously been reported for pre-B-ALL where it contributes to the survival of ALL cells in the presence of chemotherapy [11]. Antagonism of integrin α4 was proposed as a mobilizing strategy for hematopoietic stem cells [23-26]. We have shown that using humanized anti-α4 antibody, Natalizmab, as a novel de-adhering strategy against pre-B-ALL, leukemia cells were de-adhered from their counterreceptor VCAM-1 and sensitized to chemotherapy [11]. Integrins play also a role in protection of T-ALL cells against cytokine withdrawal, activation-induced cell death [13] and ligation of death receptors in endothelial cells [27,28]. A role of integrins has recently been implicated in poor-prognosis T-ALL patients [29]. Therefore,

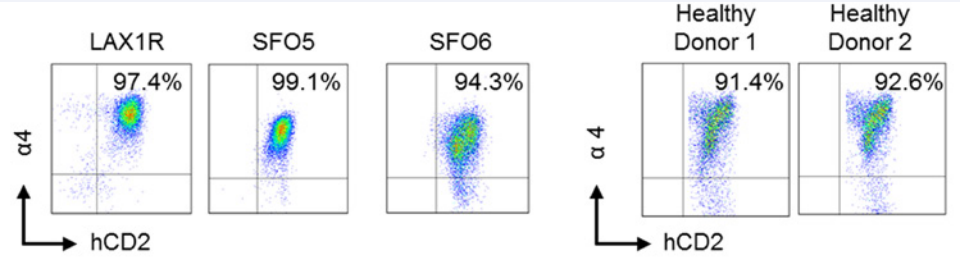

Figure 1 Integrin α4 is expressed highly in primary T-ALL. Dot plots of surface expression of α4 of primary T ALL samples (LAX1R, SFO5, SFO6) and two normal CD2^{+} donors determined by flow cytometry.

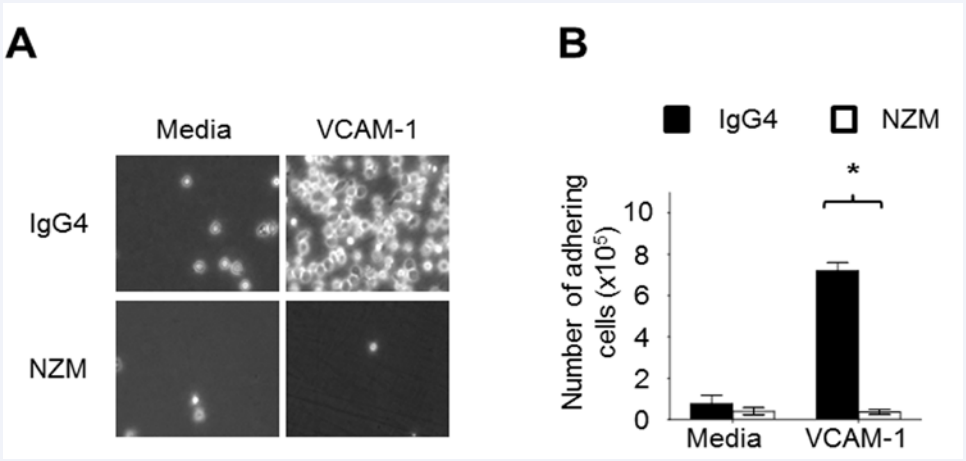

Figure 2 Integrin α4 blockade inhibits adhesion of primary T-ALL *in vitro*. Primary T-ALL#1 cells were pre-incubated with NZM or isotype control on plates coated with or without human VCAM-1. (A) Adhesion of ALL cells (400X) and (B) Number of adhering cells. *p<0.05, unpaired t-test, experiment was performed in triplicates.

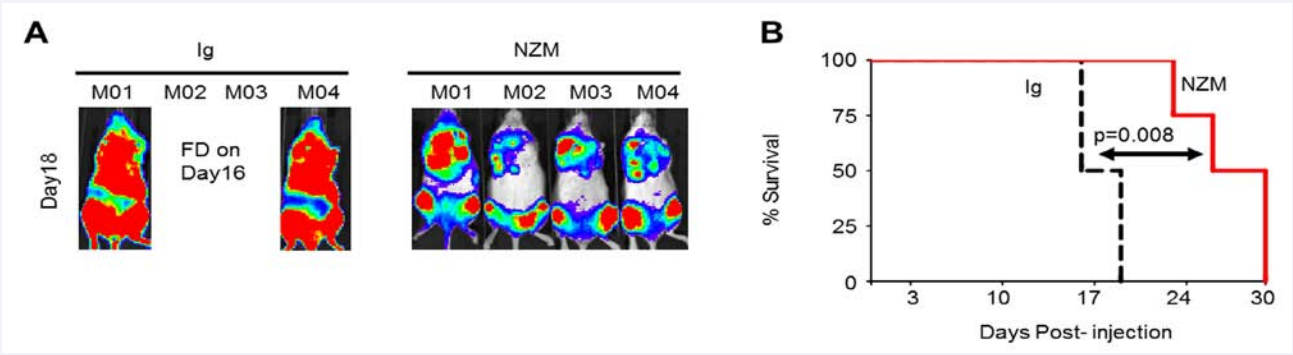

Figure 3 Targeting Integrin α4 delays the progression of primary T-ALL cells *in vivo*. (A) Bioluminescence imaging and **(B)** Kaplan-Meier survival curve was analyzed and MST for each group: Ig (n=4) (MST= 17.5 days) and NZM (n=4) (MST= 28 days). p-value (p=0.008) shown was determined from Log-rank Test. 2 mice in the control Ig group were found dead (FD) on Day16 post-injection.

antagonizing α4-mediated adhesion of T-ALL cells could be used as a novel strategy against T-ALL. So far, the underlying mechanism accounting for fibronectin/β1 integrin-dependent survival involves activation of the PI 3-kinase/AKT pathway or collagen-mediated activation of MAPK/ERK pathway [14,30]. Recently, a novel microtubule targeting compound, PBOX-15, has been shown to down-regulate β1-, β2- and α4-integrin expression and to disrupt integrin-mediated adhesion of a relapsed childhood T-ALL cell lines (CCRF-CEM) and a BCR-ABL positive adult B-ALL cell line (SD-1) [31], highlighting the promise of targeting integrins in T-ALL. We have shown that integrin α4 antagonism using a humanized monoclonal antibody, which is in clinical use against multiple sclerosis, de-adheres leukemia cells and that this monotreatment already leads to prolongation of murine recipients of T-ALL. Similar effects were observed in mice bearing primary pre-B ALL cells and, since in that model the combination with conventional chemotherapy led to leukemia eradication, leads us to propose similar effects of α4-blockade on T-ALL as on pre-B ALL treatment. Whether leukemia cell are mobilized to the periphery, as we have shown in a xenograft model of pre-B ALL treated with Natalizumab [11], and whether Natalizumab leads to apoptosis or otherwise sensitizes to chemotherapy remains to be determined.

CONCLUSION

Taken together, the results presented above demonstrate a role of integrin α4 in T-cell leukemia adhesion in vitro and in vivo. In conjunction with published data on pre-B ALL, these data suggest α4 blockade as a novel paradigm with broad anti-leukemic specificity, while further formal studies are warranted to evaluate α4 as a target for therapeutic intervention in T-ALL.

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REFERENCES

1. Manabe A, Coustan-Smith E, Behm FG, Raimondi SC, Campana D. Bone marrow-derived stromal cells prevent apoptotic cell death in B-lineage acute lymphoblastic leukemia. Blood. 1992; 79: 2370-2377.

2. Kumagai M, Manabe A, Pui CH, Behm FG, Raimondi SC, Hancock

ML, et al. Stroma-supported culture in childhood B-lineage acute lymphoblastic leukemia cells predicts treatment outcome. J Clin Invest. 1996; 97: 755-760.

3. Mudry RE, Fortney JE, York T, Hall BM, Gibson LF. Stromal cells regulate survival of B-lineage leukemic cells during chemotherapy. Blood. 2000; 96: 1926-1932.

4. Imai Y, Shimaoka M, Kurokawa M. Essential roles of VLA-4 in the hematopoietic system. Int J Hematol. 2010; 91: 569-575.

5. Barry ST, Ludbrook SB, Murrison E, Horgan CM. Analysis of the α4beta1 integrin-osteopontin interaction. Exp Cell Res. 2000; 258: 342-351.

6. Harima A, Nakaseko C, Yokota A, Kitagawa M, Morimoto C, Harigaya K, et al. Fibronectin promotes cell proliferation of human pre-B cell line via its interactions with VLA-4 and VLA-5. Hematology. 2008; 13: 236-243.

7. Scott LM, Priestley GV, Papayannopoulou T. Deletion of α4 integrins from adult hematopoietic cells reveals roles in homeostasis, regeneration, and homing. Mol Cell Biol. 2003; 23: 9349-9360.

8. Filshie R, Gottlieb D, Bradstock K. VLA-4 is involved in the engraftment of the human pre-B acute lymphoblastic leukaemia cell line NALM-6 in SCID mice. Br J Haematol. 1998; 102: 1292-1300.

9. Matsunaga T, Takemoto N, Sato T, Takimoto R, Tanaka I, Fujimi A, et al. Interaction between leukemic-cell VLA-4 and stromal fibronectin is a decisive factor for minimal residual disease of acute myelogenous leukemia. Nat Med. 2003; 9: 1158-1165.

10. Becker PS, Kopecky KJ, Wilks AN, Chien S, Harlan JM, Willman CL, et al. Very late antigen-4 function of myeloblasts correlates with improved overall survival for patients with acute myeloid leukemia. Blood. 2009; 113: 866-874.

11. Hsieh YT, Gang EJ, Geng H, Park E, Huantes S, Chudziak D, et al. Integrin α4 blockade sensitizes drug resistant pre-B acute lymphoblastic leukemia to chemotherapy. Blood. 2013; 121: 1814-1818.

12. Naci D, El Azreq MA, Chetoui N, Lauden L, Sigaux F, Charron D, et al. α2β1 integrin promotes chemoresistance against doxorubicin in cancer cells through extracellular signal-regulated kinase (ERK). J Biol Chem. 2012; 287: 17065-17076.

13. Aoudjit F, Vuori K. Engagement of the alpha2beta1 integrin inhibits Fas ligand expression and activation-induced cell death in T cells in a focal adhesion kinase-dependent manner. Blood. 2000; 95: 2044-2051.

14. Aoudjit F, Vuori K. Integrin signaling in cancer cell survival and chemoresistance. Chemother Res Pract. 2012; 2012: 283181.

15. Goldberg JM, Silverman LB, Levy DE, Dalton VK, Gelber RD, Lehmann L, et al. Childhood T-cell acute lymphoblastic leukemia: the Dana-Farber Cancer Institute acute lymphoblastic leukemia consortium experience. J Clin Oncol. 2003; 21: 3616-3622.

16. Chiaretti S, Foa R. T-cell acute lymphoblastic leukemia. Haematologica. 2009; 94: 160-162.

17. Pui CH, Pei D, Sandlund JT, Ribeiro RC, Rubnitz JE, Raimondi SC, et al. Long-term results of St Jude Total Therapy Studies 1, 12, 13A, 13B, and 14 for childhood acute lymphoblastic leukemia. Leukemia. 2010; 24: 371-382.

18. Park E, Gang EJ, Hsieh YT, Schaefer P, Chae S, Klemm L, et al. Targeting survivin overcomes drug resistance in acute lymphoblastic leukemia. Blood. 2011; 118: 2191-2199.

19. Rose DM, Cardarelli PM, Cobb RR, Ginsberg MH. Soluble VCAM-1 binding to α4 integrins is cell-type specific and activation dependent and is disrupted during apoptosis in T cells. Blood. 2000; 95: 602-609.

20. Larson RS, Springer TA. Structure and function of leukocyte integrins. Immunol Rev. 1990; 114: 181-217.

21. Kishimoto TK, Larson RS, Corbi AL, Dustin ML, Staunton DE, Springer TA. The leukocyte integrins. Adv Immunol. 1989; 46: 149-182.

22. Springer TA. Adhesion receptors of the immune system. Nature. 1990; 346: 425-434.

23. Ramirez P, Rettig MP, Uy GL, Deych E, Holt MS, Ritchey JK, et al. BIO5192, a small molecule inhibitor of VLA-4, mobilizes hematopoietic stem and progenitor cells. Blood. 2009; 114: 1340-1343.

24. Rettig MP, Ansstas G, DiPersio JF. Mobilization of hematopoietic stem and progenitor cells using inhibitors of CXCR4 and VLA-4. Leukemia. 2012; 26: 34-53.

25. Bonig H, Wundes A, Chang KH, Lucas S, Papayannopoulou T. Increased numbers of circulating hematopoietic stem/progenitor cells are chronically maintained in patients treated with the CD49d blocking antibody natalizumab. Blood. 2008; 111: 3439-3441.

26. Bonig H, Watts KL, Chang KH, Kiem HP, Papayannopoulou T. Concurrent blockade of α4-integrin and CXCR4 in hematopoietic stem/progenitor cell mobilization. Stem Cells. 2009; 27: 836-837.

27. Aoudjit F, Vuori K. Matrix attachment regulates Fas-induced apoptosis in endothelial cells: a role for c-flip and implications for anoikis. J Cell Biol. 2001; 152: 633-643.

28. Carlson TR, Hu H, Braren R, Kim YH, Wang RA. Cell-autonomous requirement for beta1 integrin in endothelial cell adhesion, migration and survival during angiogenesis in mice. Development. 2008;135: 2193-2202.

29. Cleaver AL, Beesley AH, Firth MJ, Sturges NC, O'Leary RA, Hunger SP, et al. Gene-based outcome prediction in multiple cohorts of pediatric T-cell acute lymphoblastic leukemia: a Children's Oncology Group study. Mol Cancer. 2010; 9: 105.

30. Gendron S, Couture J, Aoudjit F. Integrin alpha2beta1 inhibits Fas-mediated apoptosis in T lymphocytes by protein phosphatase 2A-dependent activation of the MAPK/ERK pathway. J Biol Chem. 2003; 278: 48633-48643.

31. Lysaght J, Verma NK, Maginn EN, Ryan JM, Campiani G, Zisterer DM, Williams DC. The microtubule targeting agent PBOX-15 inhibits integrin-mediated cell adhesion and induces apoptosis in acute lymphoblastic leukaemia cells. Int J Oncol. 2013; 42: 239-246.

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