blood

Prepublished online January 14, 2013;
doi:10.1182/blood-2012-01-406272

Integrin alpha4 blockade sensitizes drug resistant pre-B acute lymphoblastic leukemia to chemotherapy

Yao-Te Hsieh, Eun Ji Gang, Huimin Geng, Eugene Park, Sandra Huantes, Doreen Chudziak, Katrin Dauber, Paul Schaefer, Carlton Scharman, Hiroyuki Shimada, Seyedmehdi Shojaee, Lars Klemm, Reshmi Parameswaran, Mignon Loh, Eun-Suk Kang, Hong Hoe Koo, Wolf-Karsten Hoffman, Jacob Andrade, Gay M. Crooks, Cheryl L. Willman, Markus Müschen, Thalia Papayannopoulou, Nora Heisterkamp, Halvard Bönig and Yong-Mi Kim

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.

Integrin alpha4 blockade sensitizes drug resistant pre-B acute lymphoblastic leukemia to chemotherapy

Yao-Te Hsieh[1]*, Eun Ji Gang[1]*, Huimin Geng[2,] Eugene Park[1], Sandra Huantes[1], Doreen Chudziak[3], Katrin Dauber[3], Paul Schaefer[1], Carlton Scharman[1], Hiroyuki Shimada[4], Seyedmehdi Shojaee[2], Lars Klemm[2], Reshmi Parameswaran[1], Mignon Loh[5], Eun-Suk Kang[6], Hong Hoe Koo[7], Wolf-Karsten Hofmann[8], Jacob Andrade[9], Gay M Crooks[9], Cheryl L.Willman[10], Markus Müschen[2], Thalia Papayannopoulou[11], Nora Heisterkamp[1], Halvard Bönig[3,11]*, Yong-Mi Kim[1]*.

[1]Department of Pediatrics, Division of Hematology and Oncology, Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles; [2]Department of Laboratory Medicine, University of California San Francisco, San Francisco; [3]German Red Cross Blood Service and Institute for Transfusion Medicine and Immunohematology, Goethe University, Frankfurt, Germany; [4]Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles; [5]Department of Pediatrics, University of California San Francisco, San Francisco; [6]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [7]Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [8]Department of Hematology and Oncology, University Hospital Mannheim, Mannheim, Germany; [9]Department of Pathology & Laboratory Medicine, University of California Los Angeles, Los Angeles; [10]University of New Mexico Cancer Center and Departments of Pathology, Internal Medicine, Mathematics and Statistics, and Physics and Astronomy, University of New Mexico, Albuquerque; [11]Department of Medicine/Hematology, University of Washington, Seattle.

*: Both authors contributed equally

Running title: Integrin alpha4 blockade for treatment of ALL

Correspondence:

Yong-Mi Kim, MD MPH
Division of Hematology and Oncology
Children's Hospital Los Angeles, University of Southern California
4650 Sunset Boulevard, Mailstop #57
Los Angeles, CA 90027
Phone number: (323) 361-8544
Email: ymkim@chla.usc.edu

Key Points

1. We evaluated interference with integrin alpha4-mediated stromal adhesion as a new acute lymphoblastic leukemia treatment.

2. Integrin alpha4-blockade using Natalizumab in combination with chemotherapy sensitizes pre-B acute lymphoblastic leukemia to chemotherapy.

Abstract

The bone marrow (BM) provides chemoprotection for acute lymphoblastic leukemia (ALL) cells thus contributing to the lack of efficacy of current therapies. Integrin alpha4 (alpha4) mediates adhesion of normal and malignant B-cell precursors in BM, and, according to gene expression analyses from 207 children with high-risk pre-B ALL with minimal residual disease, is particularly highly expressed in patients with the poorest outcome. Therefore, we tested whether interference with alpha4-mediated stromal adhesion might be a new ALL treatment. For this purpose, two models of leukemia were used, one genetic (conditional alpha4 ablation of BCR-ABL1 ($p210^{+}$)-induced murine leukemia) and one pharmacological (anti-functional alpha4 antibody treatment of primary pre-B ALL). Conditional deletion of alpha4 sensitized leukemia cell to Nilotinib. Adhesion of primary pre-B ALL cells was alpha4-dependent and alpha4 blockade sensitized primary ALL cells towards chemotherapy. Combination of chemotherapy with an anti-integrin alpha4 antibody, Natalizumab, prolonged survival of NOD/SCID recipients of primary ALL suggesting adjuvant integrin alpha4 inhibition as a novel strategy for pre-B ALL.

Introduction

Although the overall prognosis of pediatric acute lymphoblastic leukemia (ALL) has improved, relapse, originating from leukemia cells which have evaded chemotherapy, continues to occur. Contact to bone marrow stromal cells is required for survival of ALL cells in the presence of chemotherapy.[1-3] The integrin alpha4 (alpha4) chain associates with the integrin ß1 chain to form Very Late Antigen-4 (VLA4)[4], which binds to its counter receptors VCAM-1, fibronectin, or osteopontin (OPN),[5;6] and regulates homing, adhesion, and engraftment of hematopoietic progenitors in BM[7] and engraftment of ALL cells.[8] VLA4 was shown to be a dominant adhesion molecule for acute myeloid leukemia (AML) cells,[9] indicating that alpha4 expression might be an unfavorable risk factor in AML, but alternative observations have also been reported.[10] Formal studies of the role of alpha4 as a potential therapeutic target in ALL have not been performed. Using genetic and pharmacological models of alpha4 modulation, we tested whether alpha4 blockade can overcome drug resistance in pre-B ALL.

Methods

Correlation of integrin alpha4 gene expression on leukemic blasts with clinical outcomes of pre-B ALL patients

Patient clinical and outcome data were obtained from the National Cancer Institute TARGET Data Matrix of the Children's Oncology Group (COG) Clinical Trial P9906.[11] Analysis is described in the Supplementary Methods. All studies have been approved by IRB or IACUC. Human studies were conducted in accordance with the Declaration of Helsinki.

In vitro and *in vivo* studies with integrin alpha4$^{fl/fl}$ cells transduced with BCR/ABL1 (p210)

Bone marrow from integrin alpha4$^{fl/fl}$ knockout mice[6] was processed as indicated in the Supplementary Methods. [12]

Quantitative RT-PCR, PCR and flow cytometry

Information is listed in the Supplementary Methods and Tables.[13-15]

Patient ALL samples and cell lines

Primary pre-B ALL samples were used for *in vivo* and *in vitro* as described in the Supplementary Methods.[12]

Pharmacological integrin alpha4 blockade

A detailed protocol for *in vivo* and *in vitro* assays can be found in the Supplementary Methods.

Results and Discussion

Integrin alpha4 expression on pre-B ALL cells inversely correlates with clinical outcome of patients with ALL

To determine the role of alpha4 in ALL, expression of alpha4 mRNA (ITGA4) in 207 ALL patients uniformly treated with the COG P9906 clinical trial[11] was correlated with outcome. Overall survival (OS) of minimal residual disease positive patients (MRD^{+}) (n=67) was analyzed further by alpha4 expression and could be separated into MRD^{+} alpha4^{high} (ITGA4

expression ≥ mean; n=34) and MRD$^+$ alpha4^{low} expressing cases (ITGA4 expression < mean, n=33) (**Figure 1A,B**). Alpha4^{high} leukemias were associated with inferior outcome (**Supplementary Figure S1A-C**) indicating the potential use of alpha4 as a therapeutic target, as it is expressed especially highly in the prognostically poorest cases.

Integrin alpha4 deletion sensitizes murine BCR-ABL1 (p210)$^+$ leukemia to chemotherapy

First, we compared the course of alpha4 competent and alpha4 deficient murine leukemia. We used bone marrow cells (BMC) from alpha4$^{fl/fl}$ mice[6;16;17] and retrovirally transduced them *in vitro* using BCR-ABL1(p210)[12;18;19] to generate B220$^+$/CD19$^+$ murine leukemia (**Supplementary Figure S2A**). Subsequently, leukemia cells were transduced with either Empty-ER^{T2} control or Cre-ER^{T2} vector to delete alpha4 (**Supplementary Figure S2B,C and S3A-C**). Deletion of alpha4 in transduced cells was efficient, as demonstrated by genetic (**Supplementary Figure S3D**) and flow cytometric analyses (**Figure 1C**). Alpha4-deficient murine leukemia cells adhered to mVCAM-1-coated plates with markedly reduced efficiency compared to Empty-ER^{T2} (alpha4 competent) control cells ($p<0.05$) (**Supplementary Figure S3E**) and alpha4-ablation sensitized murine leukemia cells to chemotherapy (**Figure 1D, Supplementary Figure S3F and Supplementary Table S1**). Moreover, alpha4-deletion was associated with loss of colony forming units (CFU) of BCR/ABL1$^+$ cells in primary and secondary platings (**Figure 1E and Supplementary Table S1**). When these *in vitro* alpha4 pre-deleted and undeleted murine leukemia cells were injected into sublethally irradiated mice, Nilotinib treatment led to prolonged survival of alpha4-deleted recipients as opposed to recipients of alpha4-competent cells (**Supplementary Figure S3G,H**). To account for potential differences in engraftment of deleted leukemia cells, alpha4 deletion was induced *in vivo* 3 days after transfer of alpha4 competent alpha4-Empty-ER^{T2} or Cre-ER^{T2} cells to sublethally irradiated C57/BL6 Ly5.1$^+$

recipient mice. Both cell types showed similar viability and proliferation rate (data not shown). After engraftment, all animals were treated with Tamoxifen to induce alpha4 deletion in Cre-ER leukemia cells *in vivo*. Kaplan-Meier survival analysis revealed prolonged survival of the group receiving alpha4-Cre-ER^{T2} *in vivo* ablated leukemia cells compared with the Empty-ER^{T2} group (MST = 31 days vs. MST = 15 days; p=0.0008; **Figure 1F**). Flow cytometric analyses confirmed complete *in vivo* alpha4-deletion in sacrificed animals (**Supplementary Figure S4A,B**). Importantly, animals receiving alpha4-Cre-ER^{T2} murine leukemia cells plus Nilotinib survived until the end of follow-up, compared to animals receiving alpha4-Empty-ER^{T2} (i.e. non-ablated) murine leukemia cells plus Nilotinib (MST= Undefined vs. MST= 45 days; p=0.002). Similar results were obtained with intrafemoral injection of leukemia cells (**Supplementary Figure S4C,D**). We furthermore determined that mice died of leukemia-associated anemia and thromobocytopenia as assessed by blood count analysis (**Supplementary Figure S4E**), and also determined that chemotherapy treatment of alpha4-deficient mice did not result in excessive hematopoietic toxicity against normal cells **(Supplementary Figure S5**).

Pharmacological integrin alpha4 blockade sensitizes resistant human leukemia blasts to ALL chemotherapy *in vitro*, and its addition to standard ALL chemotherapy prolongs survival of NOD/SCID recipients of human pre-B ALL cells *in vivo*

To corroborate our observations in an alternative model, we next tested in a pharmacological model whether alpha4 blockade with anti-functional antibodies can overcome drug resistance of primary human precursor B-ALL cells. The humanized anti-alpha4 monoclonal antibody (mAb) Natalizumab (NZM), licensed as a disease modifying treatment for autoimmune diseases, including multiple sclerosis,[20;21] was previously shown to mobilize HSC into circulation in

humans and monkeys.[22;23] Chemoresistant LAX7R pre-B ALL cells from a patient with normal karyotype (**Supplementary Figure S6A, B**), who had relapsed despite treatment with chemotherapy, were used for subsequent studies. Anti-alpha4 antibody inhibited adhesion to human VCAM-1 (hVCAM-1) compared to control Ig-treatment (**Figure 2A, Supplementary Figure S7A,B and Supplementary Table S2**). Marked changes in viability after alpha4-mAb treatment were not observed (**Figure 2B).** Matched isotypic Ab served as control. This may not constitute an ideal control antibody, because it does not bind to the ALL cells at a similar density and affinity as the anti-alpha4 antibody, but has been routinely used for similar studies. Alpha4 antibody-dependent cell-mediated cytotoxicity (ADCC) was specifically excluded by demonstrating that NK cells ($CD56^{+}CD3^{-}$) of healthy donors do not impact lysis of primary ALL cells treated with Ig or NZM as determined by a Calcein-AM release assay **(Supplementary Figure S7C,D)**. Viability of normal pre-B cells was unaffected by alpha4 mAb treatment **(Supplementary Figure S8A,B**). However, anti-alpha4-mediated de-adhesion significantly sensitized LAX7R cells to chemotherapy (Vincristine, Dexamethasone and L-Asparaginase, VDL) (**Figure 2C and Supplementary Table S2**). When 6 different primary B-ALL cases were pre-incubated with function-blocking anti-alpha4 antibodies, we observed that pharmacological alpha4-blockade prior to *in vivo* transfer significantly prolonged survival of recipient mice (**Supplementary Figure S9A,B**). We next injected luciferase-labeled LAX7R cells into NOD/SCID mice and subjected them to whole-body *in vivo* bioluminescent imaging (**Supplementary Figure S2D and S10A,B**). Three days after leukemia cell injection leukemia cell-bearing mice received four weekly injections of function-blocking alpha4 mAb (NZM, a humanized IgG4) or control Immunoglobulin (Ig) ± VDL (**Figure 2D**). Human and murine ALL cells were detected on Day 3 after leukemia cell injection by immunohistochemistry

(**Supplementary Figure 11A,B**) or by detecting a chromosomal translocation by real-time PCR (**Supplementary Figure S11C,D**), evidence that at least partial bone marrow engraftment had been achieved. Mice treated with NZM survived modestly longer than control antibody-treated mice. Chemotherapy-treated mice relapsed shortly after the end of the four-week treatment and rapidly succumbed to leukemia, as evidenced by almost complete replacement of normal murine hematopoiesis by human ALL blasts (**Figure 2G**). In marked contrast, mice treated with chemotherapy plus NZM survived disease-free until day 151 ($p<0.0001$) (**Figure 2E)**, when the animals were sacrificed and the absence of human leukemia cells was confirmed using sensitive techniques (**Figure 2F-H**). These data were confirmed with a repeat experiment with NOD/SCID IL2R$\gamma^{-/-}$ hosts (**Supplementary Figure S12**) as well as with two additional primary leukemia cases (**Supplementary Figure S13 and S14**). Furthermore, we repeated the experiment starting NZM ± chemotherapy only on Day 6 after leukemia cell injection, to allow for more time for *in vivo* expansion of leukemia cells (**Supplementary Figure S15A-D**). Again, NZM ± chemotherapy markedly prolonged survival of leukemia-bearing mice (MST=75 days vs. 147 days). Blood count analysis showed that the death of the animals was due to leukemia-associated anemia and thrombocytopenia (**Supplementary Figure S15E**). We also determined the role of alpha4 in homing and mobilization of primary ALL cells. Anti-alpha4 blockade inhibits homing of primary ALL cells to bone marrow, spleen, and liver; instead, cells were increased in lung compared to control Ig as assessed 18 hours post-leukemia injection by CFU counts (**Figure 2I,J**). Primary ALL cells were mobilized by a one-time *in vivo* treatment with NZM out of the bone marrow, spleen and liver and into the lung and the peripheral blood while proliferation in the bone marrow was unaffected (**Supplementary Figure S16A-G**).

Taken together, we demonstrated that alpha4-blockade in combination with chemotherapy sensitizes drug resistant pre-B acute lymphoblastic leukemia to chemotherapy, proposing alpha4-blockade as a novel therapy to existing chemotherapy.

Acknowledgements

Supported by grants from the Nautica Triathlon Foundation, Hyundai Hope on Wheels Foundation and the St. Baldrick's Foundation Scholar Award (YMK). HB acknowledges support from LOEWE OSF TP5a and Deutsche Forschungsgemeinschaft BO3553/1-1. MM acknowledges support from the R01CA137060, R01CA139032, R01CA157644 and Scholar of Leukemia and Lymphoma Society. We thank Dr. Donald Kohn, (University of California, Los Angeles), for sharing the pCCL-MNDU3-LUC lentiviral vector. We thank Dr. Fernandez Esteban of the Imaging Core of the Saban Research Institute for his expert assistance.

Authorship

Contributions: Y.H and E.G. designed and performed research, collected data, performed analysis, and wrote the manuscript. H.G., E.P., S.H., D.C., K.D., P.S, C.S. and H.S. performed research and collected data. M.L., E.S.K., H.H.K., W.K.H., J.A., G.C., W.L.C., C.L.W., M.M., C.H., S.S., R.P., NH and T.P. contributed vital new reagents, analytical tools or patient samples and interpreted the data. H.B. and Y.M.K. designed, analyzed and interpreted data, and wrote the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence:
Yong-Mi Kim, MD MPH

Division of Hematology and Oncology
Children's Hospital Los Angeles, University of Southern California
4650 Sunset Boulevard, Mailstop #57
Los Angeles, CA 90027
Phone number: (323) 361-8544
Email: ymkim@chla.usc.edu

Reference List

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 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. Guo W, Giancotti FG. Integrin signalling during tumour progression. Nat.Rev.Mol.Cell Biol. 2004;5:816-826.

5. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell 2002;110:673-687.

6. Scott LM, Priestley GV, Papayannopoulou T. Deletion of alpha4 integrins from adult hematopoietic cells reveals roles in homeostasis, regeneration, and homing. Mol.Cell Biol. 2003;23:9349-9360.

7. Priestley GV, Ulyanova T, Papayannopoulou T. Sustained alterations in biodistribution of stem/progenitor cells in Tie2Cre+ alpha4(f/f) mice are hematopoietic cell autonomous. Blood 2007;109:109-111.

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 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 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. Kang H, Chen IM, Wilson CS et al. Gene expression classifiers for relapse-free survival and minimal residual disease improve risk classification and outcome prediction in pediatric B-precursor acute lymphoblastic leukemia. Blood 2010;115:1394-1405.

12. Park E, Gang EJ, Hsieh YT et al. Targeting survivin overcomes drug resistance in acute lymphoblastic leukemia. Blood 2011;118:2191-2199.

13. Trageser D, Iacobucci I, Nahar R et al. Pre-B cell receptor-mediated cell cycle arrest in Philadelphia chromosome-positive acute lymphoblastic leukemia requires IKAROS function. J.Exp.Med. 2009;206:1739-1753.

14. Siraj AK, Ozbek U, Sazawal S et al. Preclinical validation of a monochrome real-time multiplex assay for translocations in childhood acute lymphoblastic leukemia. Clin.Cancer Res. 2002;8:3832-3840.

15. Lavau C, Luo RT, Du C, Thirman MJ. Retrovirus-mediated gene transfer of MLL-ELL transforms primary myeloid progenitors and causes acute myeloid leukemias in mice. Proc.Natl.Acad.Sci.U.S.A 2000;97:10984-10989.

16. Priestley GV, Scott LM, Ulyanova T, Papayannopoulou T. Lack of alpha4 integrin expression in stem cells restricts competitive function and self-renewal activity. Blood 2006;107:2959-2967.

17. Bonig H, Watts KL, Chang KH, Kiem HP, Papayannopoulou T. Concurrent blockade of alpha4-integrin and CXCR4 in hematopoietic stem/progenitor cell mobilization. Stem Cells 2009;27:836-837.

18. Duy C, Hurtz C, Shojaee S et al. BCL6 enables Ph+ acute lymphoblastic leukaemia cells to survive BCR-ABL1 kinase inhibition. Nature 2011;473:384-388.

19. Daley GQ, Van Etten RA, Baltimore D. Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science 1990;247:824-830.

20. Steinman L. Blocking adhesion molecules as therapy for multiple sclerosis: natalizumab. Nat.Rev.Drug Discov. 2005;4:510-518.

21. Steinman L, Merrill JT, McInnes IB, Peakman M. Optimization of current and future therapy for autoimmune diseases. Nat.Med. 2012;18:59-65.

22. 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.

23. Zohren F, Toutzaris D, Klarner V et al. The monoclonal anti-VLA-4 antibody natalizumab mobilizes CD34+ hematopoietic progenitor cells in humans. Blood 2008;111:3893-3895.

24. Tai YT, Li X, Tong X, et al. *Human anti-CD40 antagonist antibody triggers significant antitumor activity against human multiple myeloma.* Cancer Res. 2005; 1;65(13):5898-906.

25. Li R, Heydon K, Hammond ME, et al. *Ki-67 staining index predicts distant metastasis and survival in locally advanced prostate cancer treated with radiotherapy: an analysis of patients in radiation therapy oncology group protocol 86-10.* Clin Cancer Res. 2004;10(12 Pt 1):4118-4124.

Figure Legends

Figure 1. Integrin alpha4 expression inversely correlates with clinical outcomes of pre-B ALL patients and mediates adhesion-dependent chemoprotection in leukemia cells.

(A) Kaplan-Meier estimates of overall survival (OS) for ALL patients negative (black) or positive (red) with minimal residual disease (MRD) at the end of the induction chemotherapy cycle of flow cytometry (day 29).[11] **(B)** Analysis of the overall survival (OS) of minimal residual disease positive patients (MRD^+) (n=67) and alpha4 expression (205885_at) separates MRD^+ Integrin $alpha4^{high}$ (alpha4 expression ≥ mean; n=34) and MRD^+ $alpha4^{low}$ expressing cases (alpha4 expression < mean, n=33) (p=0.0175, logrank test). **(C)** Deletion of alpha4 induced by Tamoxifen was confirmed by flow cytometry 6 days after treatment. **(D)** Alpha4 deleted cells ($CreER^{T2}$: Red) and non-deleted control cells ($EmptyER^{T2}$: Black) were cultured with mVCAM-1(+) or without mVCAM-1 (Control). Cells were then treated with standard chemotherapy VDL (0.005 µM Vincristine, 0.05 nM Dexamethasone, 0.005 IU/ml L-Asparaginase) for 4 days. Cell viability relative to the initial viability on Day 0 was assessed by trypan blue exclusion of dead cells. *p<0.05, mean± s.d., unpaired t-test, three independent experiments performed in triplicate. NS= non-significant. **(E)** Colony forming ability in primary and secondary platings**.** *p<0.05, mean± s.d., unpaired t-test, three independent experiments performed in triplicate. **(F)** Kaplan-Meier survival curve of alpha4-$CreER^{T2}$ and $EmptyER^{T2}$ cells injected C57/BL6 Ly5.1+ recipient mice treated with or without nilotinib (NTB). MST was calculated for each group by Log-rank Test.

Figure 2. Integrin alpha4 blockade sensitizes primary pre-B ALL cells to chemotherapy.

(A) LAX7R cells were plated on BSA as control (Control) or humanVCAM-1 and treated with control IgG4 or anti-alpha4 mAb (Natalizumab, NZM). Numbers of viable adhering cells were counted after 48 hours. **(B)** Cell viability was determined by trypan blue exclusion of dead cells. NS = non-significant ($p>0.05$). **(C)** LAX7R cells were plated for 3 days on BSA as control or VCAM-1+ and treated with control Ig or anti-alpha4 mAb (Natalizumab, NZM) with or without Vincristine, Dexamethasone and L-Asparaginase (VDL). Depicted is the cell viability by trypan blue exclusion. *$p=0.0001$ for IgG4+VDL versus NZM+VDL, incubated on VCAM-1-coated plates, mean±s.d., unpaired two-tailed t-test, three independent experiments performed in triplicates. **(D)** Bioluminescent imaging of mice transplanted with LAX7R cells and treated with Ig (n=4), Natalizumab (NZM) (n=4), VDL+Ig (n=9) or VDL+Natalizumab (NZM) (n=9) on Day 34, Day 57, and Day 71 after leukemia cell transfer. A mouse with no leukemia injection treated only with luciferin at time of imaging was included as background control (Ctrl). **(E)** Kaplan-Meier survival curve was analyzed and MST was calculated for each group: Ig (MST = 38 days), NZM (MST = 52 days), VDL+Ig (MST = 74 days), VDL+Natalizumab (sacrificed at the end of follow-up, Day 151 post leukemia injection). **(F)** The absence of human LAX7R cells in spleen (SPC) and bone marrow (BM) of the VDL+Natalizumab group was determined by flow cytometry using an anti-human CD45 Ab. **(G)** Tissues, including SPC, BM, liver, and lung from two groups were stained with anti-human CD45 antibody by immunohistochemistry (brown). **(H)** The presence of murine and human DNA in spleen (SPC) and bone marrow (BM) was evaluated using genomic PCR for murine HPRT and human GAPDH, respectively. **(I)** Homing of ALL cells to tissues was assessed by CFU assay. **(J)** Quantified number of colonies. *$p<0.05$, mean±s.d. (unpaired two-tailed t-test). NS= non-significant ($p>0.05$)

Figure 1.

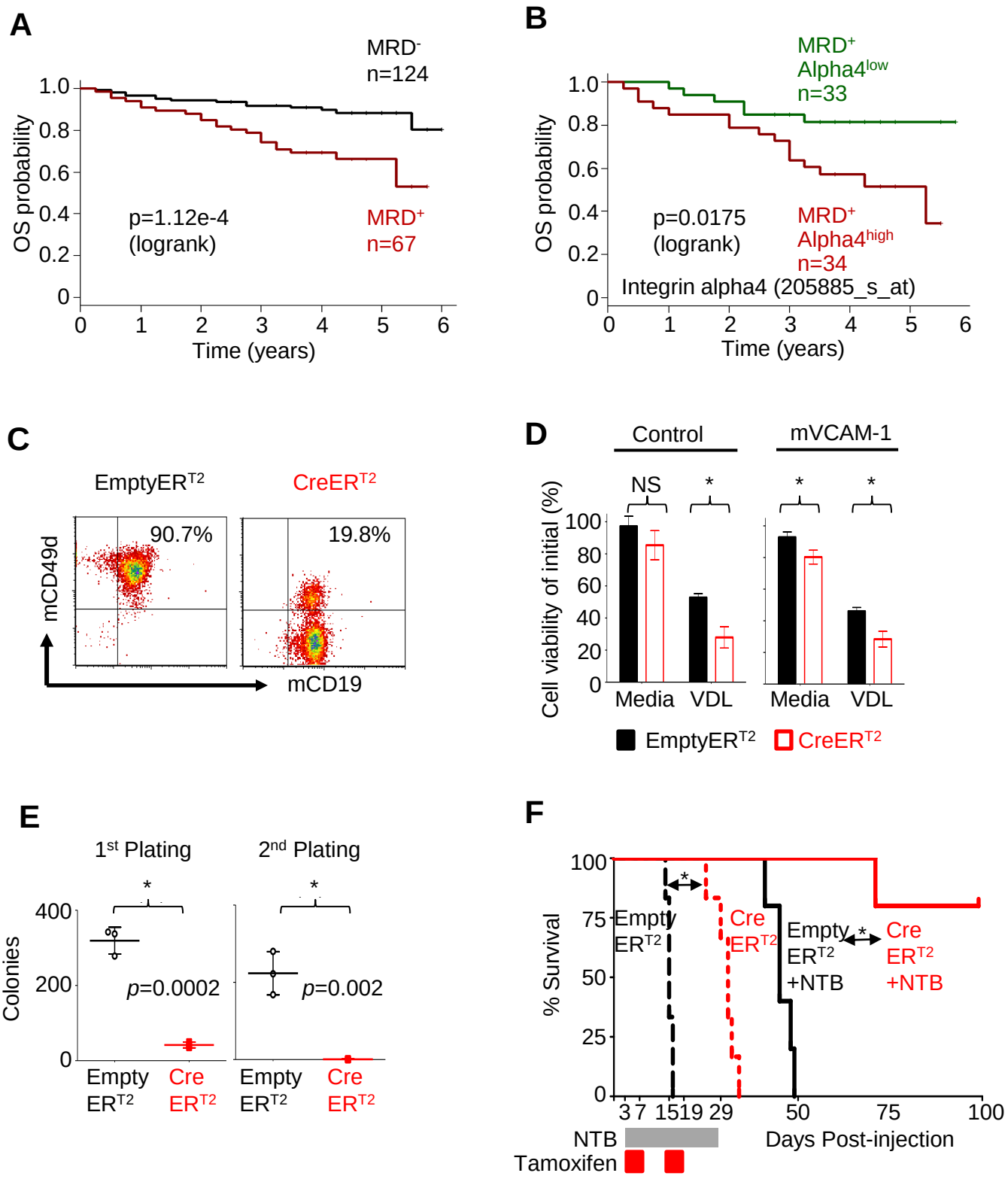
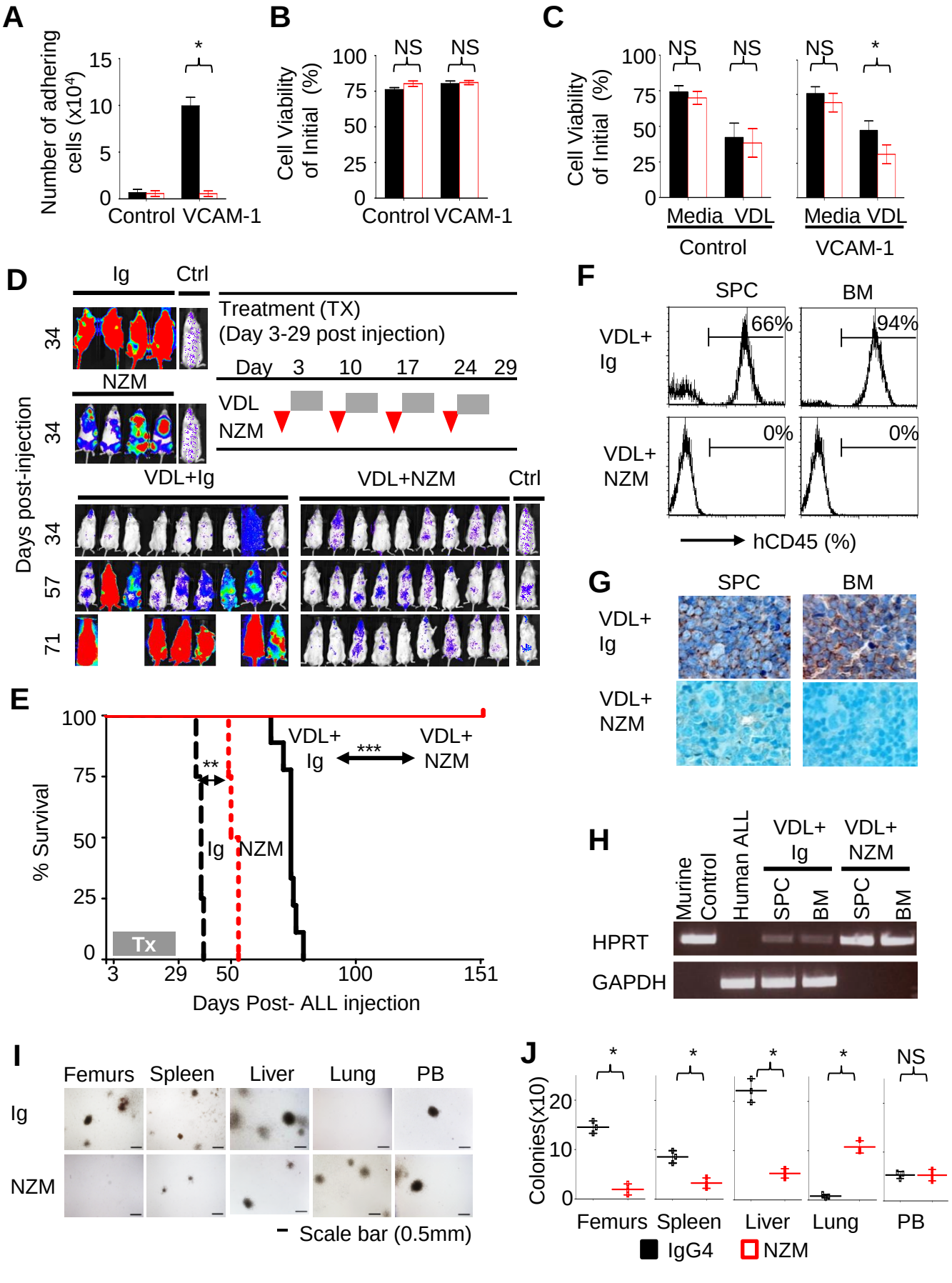

A
MRD-
n=124
OS probability
1.0
0.8
0.6
0.4
0.2
0
p=1.12e-4
(logrank)
MRD+
n=67
0 1 2 3 4 5 6
Time (years)
B
MRD+
Alpha4low
n=33
OS probability
p=0.0175
(logrank)
MRD+
Alpha4high
n=34
Integrin alpha4 (205885_s_at)
Time (years)
C
EmptyERT2
CreERT2
90.7%
19.8%
mCD49d
mCD19
D
Control
mVCAM-1
NS
*
Cell viability of initial (%)
100
80
60
40
20
0
Media
VDL
EmptyERT2
CreERT2
E
1st Plating
2nd Plating
Colonies
400
200
0
p=0.0002
p=0.002
Empty ERT2
Cre ERT2
F
% Survival
100
75
50
25
0
Empty ERT2
Cre ERT2
Empty ERT2 +NTB
Cre ERT2 +NTB
3 7 15 19 29 50 75 100
Days Post-injection
NTB
Tamoxifen

Figure 2.


A
Number of adhering cells (x10⁴)
15
10
5
0
*
Control VCAM-1
B
Cell Viability of Initial (%)
100
75
50
25
0
NS NS
Control VCAM-1
C
Cell Viability of Initial (%)
100
75
50
25
0
NS NS NS *
Media VDL Media VDL
Control VCAM-1
D
Ig Ctrl
Treatment (TX)
(Day 3-29 post injection)
Day 3 10 17 24 29
VDL
NZM
NZM
Days post-injection
34
34
VDL+Ig
VDL+NZM
Ctrl
34
57
71
E
% Survival
100
75
50
25
0
**
***
VDL+ Ig
VDL+ NZM
Ig
NZM
Tx
3 29 50 100 151
Days Post- ALL injection
F
SPC BM
VDL+ Ig
66% 94%
VDL+ NZM
0% 0%
hCD45 (%)
G
SPC BM
VDL+ Ig
VDL+ NZM
H
Murine Control
Human ALL
VDL+ Ig
VDL+ NZM
SPC BM SPC BM
HPRT
GAPDH
I
Femurs Spleen Liver Lung PB
Ig
NZM
Scale bar (0.5mm)
J
Colonies(x10)
20
10
0
* * * * NS
Femurs Spleen Liver Lung PB
IgG4
NZM