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RESEARCH ARTICLE

PROGNOSTIC IMPACT OF FAT1 CADHERIN EXPRESSION IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

Mahira I Elmougy[1] & Ghada M. ElGohary[2]

1. Clinical Pathology Department Ain Shams University Hospital

2. Clinical Adult Hematology/Internal Medicine Department, Faculty Of Medicine Ain Shams University Hospital

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Corresponding Author

Mahira I Elmougy

Abstract

Background: Improved survival of adult patients with acute lymphoblastic leukemia (ALL) has emerged from identifying new prognostic markers. Overexpression of FAT1 cadherin has been implicated in leukemogenesis and predicts disease outcome.

The aim of this study is to detect the expression of FAT1 cadherin in adult patients with ALL and its impact on prognosis and disease outcome.

Subjects and Methods: Fifty two adult patients with ALL as well as ten hematologically normal subjects as a control group, were enrolled for determination of FAT1 cadherin mRNA by quantitative real time polymerase chain reaction.

Results: FAT1 cadherin mRNA was high (≥ 0.01 copies/ml) in 27/52 (52%) of ALL patients. In the control group, FAT1 expression was low (< 0.01 copies /ml). FAT1 expression was significantly correlated with high total leucocytic count as well as unfavorable cytogenetic analysis. Regarding the clinical outcome of ALL patients, out of 25 patients with low FAT1 expression, 20 (80%) showed continuous complete remission (CCR), while 5 (20%) failed to achieve CCR (relapsed or died). On the other hand, among 27 patients with high FAT1 expression, 11 (41%) showed CCR, while 16(59%) failed to achieve CCR (relapsed or died), revealing a highly significant relationship between high FAT1 expression and poor clinical outcome ($P < 0.001$).

Conclusion: FAT1 is expressed on leukemic cells in adult patients with ALL, but not on normal cells, its expression is associated with poor clinical outcome. These data make FAT1 a promising candidate for disease monitoring, risk stratification as well as development of targeted therapies.

INTRODUCTION

Advances in leukemia therapy have resulted in a 5-year event-free survival rates of nearly 90% of patients with acute lymphoblastic leukemia (ALL). However a considerable proportion of these patients suffer relapse due to the persistence of leukemic cells within the peripheral blood (PB) or bone marrow which are often proportional to risk of relapse (1,2). The detection of reliable prognostic markers provide valuable information for adjusting the future management treatment strategies (3,4).

FAT1 is a type 1 transmembrane protein belonging to the cadherin superfamily, overexpressed in about 60% of patients with ALL, while having no significant expression across all normal hematopoietic cells (5).

The cadherin gene FAT1, is located on chromosome 4q34-35(6) within a region frequently deleted in human cancers (7), encodes a large protein with 34 extracellular cadherin repeats (8). In solid tumors, aberrant expression of FAT1 was found to be associated with disease progression (9).

FAT1 has a potential role in leukemogenesis, as well as its role in the interaction of leukemic cells with the microenvironment, FAT1 is associated with cell migration, polarity, cell-cell adhesion and direct interaction with β-Catenin (7,9,10). FAT1 might have a role in the stabilization of the interaction of leukemic cells with the bone marrow niche and/or thymic homing. Inactivating mutations of FAT1 in different human cancers have been linked to the inability to bind β-catenin and deregulated activation of the WNT pathway (7). These mechanisms might hae a role in solid cancer leading to higher treatment sensitivity and evasion of tumor metastasis (7,11). Although FAT1 is overexpressed in ALL, there have been few follow-up studies that systematically analyzed this molecular marker in ALL. Therefore this study aimed to investigate FAT1 expression in ALL and its relation to clinicopathologic characteristics of patients at presentation and to evaluate its prognostic value and impact on disease outcome.

Subjects and methods

This study was conducted on 52 newly diagnosed ALL patients recruited from the department of internal medicine Hematology oncology unit, Ain shams University hospital in the period from July 2012 to January 2013. They were investigated at presentation and followed up for 18 months to assess the fate of the disease. Thirty two of them were males and twenty were females with male to female ratio of 1.6:1. Their ages ranged from 18-62 with a median of 38 years. Ten healthy, hematologically free age and sex matched individuals were included in the study as a control group. Patients were subjected to the following: 1) thorough clinical history and examination. 2) Complete blood count (CBC) using coulter counter T-450 with examination of Leishman stained peripheral blood smears. 3) Bone marrow aspiration and examination. 4) immunophenotyping (IPT) using coulter EPICS-X flow cytometer utilizing a standard panel of monoclonal antibodies. 5) Conventional cytogenetic analysis (CCA) and molecular cytogenetics using fluorescence in situ hybridization (FISH). 6) Real time quantitative polymerase chain reaction to quantify the expression of FAT1 mRNA.

Sampling

Peripheral blood (PB) and bone marrow (BM) samples were collected on ethylene diaminetetraacetic acid (EDTA) (1.5 mg/ml) for CBC, IPT. Samples on lithium heparin for cytogenetic analyses. BM samples were obtained for determination of mRNA FAT1 by quantitative RT-PCR.

Determination of fat by quantitative RT-PCR

RNA is extracted after sample collection using (Illustra RNAspin Mini Kit, GE healthcare, Rydalmere, NSW, Australia). Total RNA was reverse transcribed to cDNA using the transcriptor High Fidelity cDNA synthesis kit with random hexamer primers (Roche Diagnostics, Castle Hill, NSW, Australia). PCR primer sequences are shown in table (1). PCR reactions performed using the SensiMixTM sYBR green master mix (30ng cDNA diluted in final volume of 12.5 µl; Bioline, eveleigh, NSW, Australia). Cycling conditions were 10 minutes at 95ºC followed by 45 cycles at 95ºC/15 seconds and 60ºC/60 seconds with subsequent melt curve analysis (95ºC 15 seconds/ 60ºC 60 seconds /95ºC 15 seconds) using an ABI-applied Biosystems 7500 thermal cycler (Life Technologies, Mulgrave, VIC, Australia). Results were normalized againt two housekeeping genes Gus B and ABL1 using relative comparative quantification algorithms (ΔC_t method) (12).

After the PCR was done, cases were divided into 2 groups according to a cut-off value established by ROC curve, high expression (≥ 0.01 copies/ml) and low expression (< 0.01 copies/ml).

Statistical analysis

Analysis of data was done by IBM computer using statistical program for social science version 15 (SPSS Inc., Chicago, IL, USA). Quantitative variables were described in the form of median, mean ±Standard deviation and range and qualitative variables were described as number and percent. Comparison of nonparameteric quantitative variables between two groups was done using Mann-Whitney test, while Kruskal-Wallis was used for comparison of more than two groups. Qualitative variables were compared using Chi-Sqaure (X^2) and Fisher's exact test. A receiver operating characteristic (ROC) to determine the best cut-off value for prognostic marker under evaluation.

Results:

Table (1): PCR primer sequences.

Primer	Sequence(5'3')	GeneBank ID
FAT1 F	GTGTGATTCGGGTTTTAGGG	NM_005245
FAT1 R	CTGTACTCGTGGCTGCAGTT	
GusB F	GCCAATGAAACCAGGTATCCC	NM_000181
GusB R	GCTCAAGTAAACAGGCTGTTTTCC	
ABL F	TGGAGATAACACTCTAAGCATAACTAAAGGT	NM_005157
ABL R	GATGTAGTTGCTTGGGACCCA	

The clinical and laboratory data of ALL patients are summarized in table 2.

FAT1 expression among ALL patients and control groups

A cut-off level for FAT1 was established according to ROC curve subdividing patients into two groups. Patients with FAT1 expression below 0.01 copies/ml were designated as having low FAT1 expression and those with levels above this cut-off had high expression.

Molecular analysis using quantitative realtime PCR revealed that 27 out of 52 patients (52%) showed high FAT1 expression. On the other hand, the entire control group showed low expression of FAT1.

Association between FAT1 expression and clinical and hematological features:

Table 3 compares the standard clinical and hematological features in ALL patients with high and low expression of FAT1. No statistically significant difference was found between cases with high FAT1 expression and those with low expression as regarding gender, leukemic burden (hepatosplenomgaly and/or lymphadenopathy), Hb level, platelet count as well as FAB subtypes ($P > 0.05$).

However, a statistically significant association was detected between high FAT1 expression and each of increased WBCs count and unforvorable cytogenetic analysis ($P< 0.001$, 0.041 respectively).

Association between FAT1 expression and disease outcome

Table 4 and figure 1 correlate the expression of FAT1 with the clinical outcome of the ALL patients revealing a highly significant correlation ($P<0.001$).

Continuous complete remission (CCR) was achieved in 20/25 (80%) of patients with low FAT1, while it was achieved in 11/27 (41%) of patients with high FAT1 expression. Poor outcome (relapse/death) was also

significantly associated with high FAT1 expression as it occurred in 16/27 (59%) of patients with high expression compared to 5/25 (20%) of patients with low FAT1 expression.

Cox proportional hazard showed that there was a statistical significant association between high FAT1 expression and poor disease outcome revealing that FAT1 is an independent prognostic marker (Hazard risk 9.640, 95% CI 1.4038-66.19, p = 0.02).

Table (2): Clinical and laboratory features of ALL patients.

Clinical	Hematological
Patient no: 52 - Age (year) $\bar{x}$±SD 41±12 , Range 18-62 - Sex (M/F), 1.6:1 - Lymphadenopathy n (%): 21 (40%) - Hepatosplenomegaly n (%): 25 (48%)	- FAB subtypes B – ALL 24 (46%) T – ALL 28 (54%) - WBCS ($x10^9$/L) $\bar{x}$±SD (29.3±10.3) - HB (g / dl) $\bar{x}$±SD (7.75±1.4) - Platelets ($x10^9$/ L) $\bar{x}$±SD (152.5±29.1) - Cytogenetic analysis Favorable 28 (54%) Intermediate 17 (33%) Unfavorable 7 (13%)

Table (3): Relation between FAT1 expression and standard Clinical and Laboratory prognostic features in ALL patients.

Parameters	Low FAT1 (n=25) N (%)	High FAT1 (n=27) N (%)	P-value
• Gender			
Male	18 (72)	14(52)	
Female	8 (28)	12(48)	0.254
• Lymphadenopathy			
-ve	16(64)	15(56)	
+ve	9(36)	12(44)	0.535
• Hepatosplenomegaly			
-ve	14(56)	13(48)	0.571
+ve	11(44)	14(52)	
• WBCS ($x10^9$/L)	15.4 ± 8.1	43.2 ± 12.5	<0.001
• Plat ($x10^9$/L)	160 ± 30.4	145 ± 27.8	0.072
• HB (g / dl)	8.1 ± 3.5	7.4 ± 1.6	0.084
• FAB subtypes			
B – ALL	15 (60)	9 (33)	0.054
T – ALL	10 (40)	18 (67)	
Cytogenetic analysis			
Favorable	15 (60)	13 (48)	
Intermediate	9 (36)	8 (30)	0.041
Unfavorable	1 (4)	6 (22)	

Table (4): Relationship between FAT1 expression and the clinical outcome of ALL patients.

Clinical outcome	Low FAT1 (n=25) N (%)	High FAT1 (n=27) N (%)	P-value

- CCR	20 (80)	11 (41)	< **0.001**
- Relapse / death	5 (20)	16 (59)	

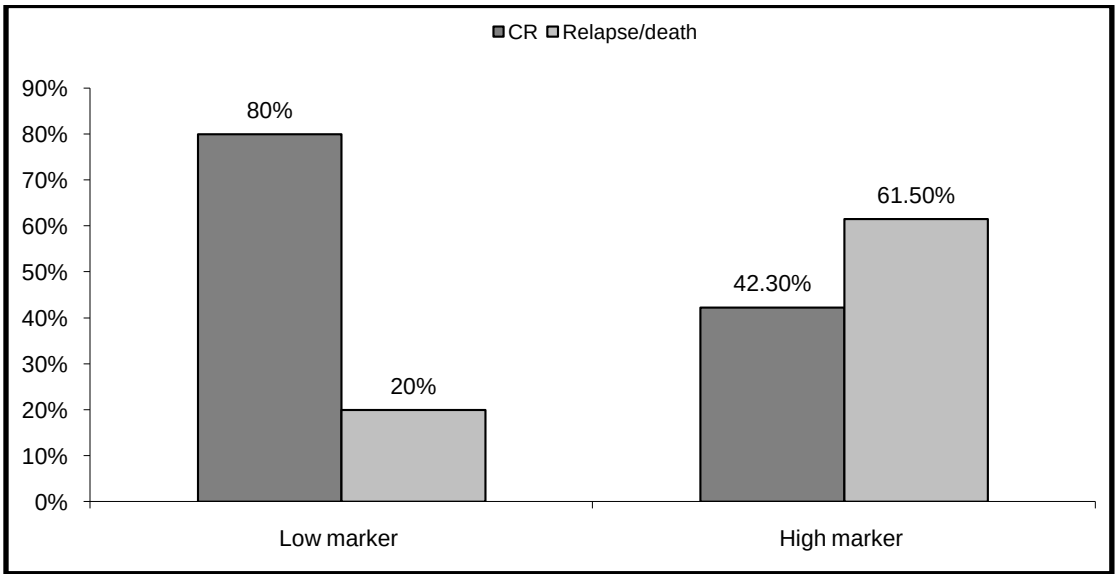

Figure (1): Relation between FAT1 expression and disease outcome.

Discussion

In this study, we aimed to evaluate the role of FAT1 expression in adult patients with ALL regarding its impact on prognosis and disease outcome. We investigated the expression of FAT1 using quantitative real-time PCR in 52 newly diagnosed adult ALL patients. FAT1 expression was elevated in bone marrow leukemia cells of ALL patients compared with normal controls. A cut-off level was established according to ROC curve subdividing patients into high and low FAT1 expression groups. FAT1 was highly expressed in 27 out of 52 patients (52%). On the other hand it showed a low (negative) expression in the entire control group and this comes in accordance with the study done by **Neumann et al. (13)** who stated that normal hematopoietic cells lacked FAT1 expression. **Coustan et al. (14)** stated that the first major criteria for a candidate MRD marker is its differential expression between leukemic and normal cells. Being high in leukemic and low or negative in normal counter parts, since MRD is the measurement of rare cells, ideally other normal blood cells do not express the MRD marker (15-17), FAT1 meets with this criterion suggesting it to be of a beneficial role in the detection of MRD in ALL. In this study, comparative analysis done between patients with high versus low FAT1 expression, revealed that high FAT1 expression was significantly associated with high WBCs count as well as unfavorable cytogenetic analysis. This comes in agreement with **Neumann et al. (13)** who found in his study that patients with high FAT1 expression showed higher WBCs count compared to those with low FAT1 expression, indicating the possibility of considering FAT1 as an adverse prognostic marker. FAT1 expression was identified as a contributor to leukemogenesis. One potential mechanism may involve disturbances in cell polarity and asymmetric cell division, since these are important for the self-renewal of somatic stem cells and proposed to be awry in leukemia (18). Hence, FAT1 expression may disturb cell polarity and asymmetric cell division aiding leukemogenesis.

Functional studies have shown a role of FAT1 in Wnt signaling pathway (19,20) as well as the ability to alter actin filament organization, cell-cell adhesion and cell migration (21). The Wnt/β-catenin pathways have been shown to be essential in the development of all hematopoietic cells, and mutations in this pathway lead to leukemia (22). In addition, the t(1;19)(E2A—PBX1) translocation has been shown to induce high expression of Wnt 16 and to regulate leukemia-stroma interactions, with β-catenin expression highly expressed and localized at the cell membrane (23). Previous studies showed FAT1 transcript is enriched in those cases harboring the t(1;19) E2A-PBX1) translocation (24-27). Although this translocation is no longer associated with poor prognosis in pediatric

patients due to new and intensified chemotherapy (28-29), they do suffer from increased risk of central nervous system relapse (30-31).

Previous reports have established high FAT1 expression within the central nervous system and its importance for central nervous system development (32-34). So it is speculated that high Fat1 expression associated with t(1;19)(E2A-PBX1) translocation, together with the ability of the cytoplasmic tail of FAT1 to directly bind β-catenin (35), may provide another potential mechanism whereby FAT1 expression can either aid central nervous system infiltration or stabilize leukemia-stroma interactions with its unknown extracellular ligand. FAT1 protein is expressed as both an unprocessed full-length protein and furin-processed heterodimer on the surface of melanoma cells (36). These dual forms of FAT1 could perform different signaling functions. This observation highlights that changes in both transcriptional and/or post-translational mechanisms may contribute to any functional role for FAT1 in leukemia onset, progression or relapse.

In this study, high FAT1 expression was significantly associated with poor outcome as (59%) of patients with high FAT1 expression had poor clinical outcome (relapse/death) compared to those with low FAT1 expression (20%).

To date, there remains a great need to identify new therapeutic targets for ALL. Currently the use of mAbs, whether simply humanized or further modified by coupling to toxins or radioactive isotopes are emerging as an important therapeutic tool in leukemia. FAT1 would provide an ideal target for the generation of a novel antibody based therapy. Recently new anti-FAT1 are to be generated which are raised against a natively folded FAT1 protein antigen, paving the way for a potential novel targeted therapeutic agent (37-40).

In conclusion, FAT1 expression was detected on leukemic cells of adult ALL patients but not on normal cells, suggesting it to be a useful prognostic marker which has an adverse impact on disease outcome and survival as well as a beneficial treatment target, supporting the possibility of using anti-FAT1 as a novel therapeutic strategy for this disease.

References

1. **Campana D (2004):** Minimal residual disease studies in acute leukemia. Am J Clin Pathol.;122(Suppl):S47–57.

2. **Stow P, Key L, Chen X, Pan Q, Neale GA, Coustan-Smith E, et al. (2010):** Clinical significance of low levels of minimal residual disease at the end of remission induction therapy in childhood acute lymphoblastic leukemia. Blood. 2010;115:4657–63.

3. **Campana D (2010):** Minimal residual disease in acute lymphoblastic leukemia. Hematology/the Education Program of the American Society of Hematology. American Society of Hematology. Educ Program.; 2010:7–12.

4. **Campana D (2009):** Role of minimal residual disease monitoring in adult and pediatric acute lymphoblastic leukemia. Hematol/Oncol Clin North Am.; 23:1083–98, vii.

5. **de Bock CE, Ardjmand A, Molloy TJ, Bone SM, Johnstone D, Campbell DM, et al. (2012):** The FAT1 cadherin is overexpressed and an independent prognostic factor for survival in paired diagnosis-relapse samples of precursor B-cell acute lymphoblastic leukemia. Leukemia. 2012;26:918–26.

6. **Dunne J, Hanby AM, Poulsom R, Jones TA, Sheer D, Chin WG, et al. (1995):** Molecular cloning and tissue expression of FAT, the human homologue of the Drosophila fat gene that is located on chromosome 4q34-q35 and encodes a putative adhesion molecule. Genomics.; 30(2):207-23.

7. **Morris LG, Kaufman AM, Gong Y, Ramaswami D, Walsh LA, Turcan S, et al. (2013):** Recurrent somatic mutation of FAT1 in multiple human cancers leads to aberrant Wnt activation. Nat Genet.; 45(3):253-61.

8. **Mahoney PA, Weber U, Onofrechuk P, Biessmann H, Bryant PJ, Goodman CS (1991):** The fat tumor suppressor gene in Drosophila encodes a novel member of the cadherin gene superfamily. Cell.; 67(5):853-68.

9. **Sadeqzadeh E, de Bock CE, Thorne RF (2014):** Sleeping giants: emerging roles for the fat cadherins in health and disease. Med Res Rev; 34(1):190-221.

10. **Hou R, Liu L, Anees S, Hiroyasu S, Sibinga NE (2006):** The FAT1 cadherin integrates vascular smooth muscle cell growth and migration signals. J Cell Biol; 173(3):417-29.

11. **India Project team of the international cancer genome consortium (2013):** Mutational landscape of gingivo-buccal oral squamous cell carcinoma reveals new recurrently-mutated genes and molecular subgroups. Nat Commun; 4: 2873.

12. **Pfaffl MW (2004):** Quantification strategies in real time PCR. In: Bustin SA, (ed.) A-Z of quantitative PCR. La Jolla, CA: International University Line; pp. 87-112.

13. **Neumann M, Seehawer M, Schlee C, Vosberg S, Heesch S, von der Heide EK, et al. (2014):** FAT1 expression and mutations in adult acute lymphoblastic leukemia. Blood Cancer J; 4:e224.

14. **Coustan-Smith E, Song G, Clark C, Key L, Liu P, Mehrpooya M, et al. (2011):** New markers for minimal residual disease detection in acute lymphoblastic leukemia. Blood. ; 117:6267–76.

15. **Chen JS, Coustan-Smith E, Suzuki T, Neale GA, Mihara K, Pui CH, et al. (2001):** Identification of novel markers for monitoring minimal residual disease in acute lymphoblastic leukemia. Blood.; 97:2115–20.

16. **Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, et al. (2004):** Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell. ; 118:149–61.

17. **Tohmiya Y, Koide Y, Fujimaki S, Harigae H, Funato T, Kaku M, et al. (2004):** Stanniocalcin-1 as a novel marker to detect minimal residual disease of human leukemia. Tohoku J Exp Med.; 204:125–33.

18. **Hawkins ED, Russell SM (2008):** Upsides and downsides to polarity and asymmetric cell division in leukemia. Oncogene.; 27(55):7003-17.

19. **Grusche FA, Richardson HE, Harvey KF (2010):** Upstream regulation of the hippo size control pathway. Curr Biol.; 20(13):R574-82.

20. **Varelas X, Miller BW, Sopko R, Song S, Gregorieff A, Fellouse FA, et al. (2010):** The Hippo pathway regulates Wnt/beta-catenin signaling. Dev Cell.; 18(4):579-91.

21. **Tanoue T, Takeichi M (2004):** Mammalian FAT1 cadherin regulates actin dynamics and cell-cell contact. J Cell Biol.; 165(4):517-28.

22. **Ge X, Wang X (2010):** Role of Wnt canonical pathway in hematological malignancies. J Hematol Oncol; 3:33.

23. **Nygren MK, Dosen-Dahl G, Stubberud H, Walchli S, Munthe E, Rian E (2009):** Beta-catenin is involved in N-cadherin-dependent adhesion, but not in canonical Wnt signaling in E2A-PBX1-positive B acute lymphoblastic leukemia cells. Exp Hematol.; 37(2):225-33.

24. **Den Boer ML, van Slegtenhorst M, De Menezes RX, Cheok MH, Buijs-Gladdines JG, Peters ST, et al. (2009):** A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. Lancet Oncol; 10(2):125-34.

25. **Li Z, Zhang W, Wu M, Zhu S, Gao C, Sun L, et al. (2009):** Gene expression-based classification and regulatory networks of pediatric acute lymphoblastic leukemia. Blood.; 114(20):4486-93.

26. **Ross ME, Zhou X, Song G, Shurtleff SA, Girtman K, Williams WK, et al. (2003):** Classification of pediatric acute lymphoblastic leukemia by gene expression profiling. Blood.; 102(8):2951-9.

27. **Yeoh EJ, Ross ME, Shurtleff SA, Williams WK, Patel D, Mahfouz R, et al. (2002):** Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell.; 1(2):133-43.

28. **Raimondi SC, Behm FG, Roberson PK, Williams DL, Pui CH, Crist WM, et al. (1990):** Cytogenetics of pre-B-cell acute lymphoblastic leukemia with emphasis on prognostic implications of the t(1;19). J Clin Oncol.; 8(8):1380-8.

29. **Vey N, Thomas X, Picard C, Kovascovicz T, Charin C, Cayuela JM, et al. (2006):** Allogeneic stem cell transplantation improves the outcome of adults with t(1;19)/E2A-PBX1 and t(4;11)/MLL-AF4 positive B-cell acute lymphoblastic leukemia: results of the prospective multicenter LALA-94 study. Leukemia.; 20(12):2155-61.

30. **Jeha S, Pei D, Raimondi SC, Onciu M, Campana D, Cheng C, et al. (2009):** Increased risk for CNS relapse in pre-B cell leukemia with the t(1;19)/TCF3-PBX1. Leukemia; 23(8):1406-9.

31. **Pui CH, Campana D, Pei D, Bowman WP, Sandlund JT, Kaste SC, et al. (2009):** Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med.; 360(26):2730-41.

32. **Ponassi M, Jacques TS, Ciani L, ffrench Constant C (1999):** Expression of the rat homologue of the Drosophila fat tumour suppressor gene. Mech Dev; 80(2):207-12.

33. **Ciani L, Patel A, Allen ND, ffrench-Constant C (2003):** Mice lacking the giant protocadherin mFAT1 exhibit renal slit junction abnormalities and a partially penetrant cyclopia and anophthalmia phenotype. Mol Cell Biol; 23(10):3575-82.

34. **Smith TG, Van Hateren N, Tickle C, Wilson SA (2007):** The expression of Fat-1 cadherin during chick limb development. Int J Dev Biol; 51(2):173-6.

35. **Hou R, Liu L, Anees S, Hiroyasu S, Sibinga NE (2006):** The FAT1 cadherin integrates vascular smooth muscle cell growth and migration signals. J Cell Biol; 173(3):417-29.

36. **Sadeqzadeh E, de Bock CE, Zhang XD, Shipman KL, Scott NM, Song C, et al. (2011):** Dual processing of FAT1 cadherin protein by human melanoma cells generates distinct protein products. J Biol Chem.; 286(32):28181-91.

37. **Kawamata N, Ogawa S, Zimmermann M, Kato M, Sanada M, Hemminki K, et al. (2008):** Molecular allelokaryotyping of pediatric acute lymphoblastic leukemias by high-resolution single nucleotide polymorphism oligonucleotide genomic microarray. Blood.; 111(2):776-84.

38. **Mullighan CG, Goorha S, Radtke I, Miller CB, Coustan-Smith E, Dalton JD, et al. (2007):** Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. Nature.; 446(7137):758-64.

39. **Iacobucci I, Storlazzi CT, Cilloni D, Lonetti A, Ottaviani E, Soverini S, et al. (2009):** Identification and molecular characterization of recurrent genomic deletions on 7p12 in the IKZF1 gene in a large cohort of BCR-ABL1-positive acute lymphoblastic leukemia patients: on behalf of Gruppo Italiano Malattie Ematologiche dell'Adulto Acute Leukemia Working Party (GIMEMA AL WP). Blood.; 114(10):2159-67.

40. **Mullighan CG, Phillips LA, Su X, Ma J, Miller CB, Shurtleff SA, et al. (2008):** Genomic analysis of the clonal origins of relapsed acute lymphoblastic leukemia. Science; 322(5906):1377-80.