
**UNIVERSITI PUTRA MALAYSIA**

***EVALUATING A RAPID METHOD TO QUANTIFY MULTIPLE GENE EXPRESSION IN ACUTE MYELOID LEUKAEMIA***

**LEE CIN DEE**

**IB 2013 46**

# EVALUATING A RAPID METHOD TO QUANTIFY MULTIPLE GENE EXPRESSION IN ACUTE MYELOID LEUKAEMIA

By

LEE CIN DEE

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

July 2013

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

**EVALUATING A RAPID METHOD TO QUANTIFY MULTIPLE GENE EXPRESSION IN ACUTE MYELOID LEUKAEMIA**

By

**LEE CIN DEE**

**July 2013**

**Chairman : Maha Abdullah, PhD**
**Faculty : Institute of Bioscience**

Acute myeloid leukaemia (AML) is a clonal disease characterized by the proliferation and accumulation of myeloid progenitor cells in the bone marrow and peripheral blood. Prognostic markers are needed to predict patient's response to therapy and reduce under- or over-treatment. Age, white blood counts and cytogenetics are currently routine prognostic markers but are limited by the test and the number of patients who can benefit from it. Large scale gene profiling methods have further identified genetic aberrations such as microdeletions and mutations associated with pathogenesis of disease and outcome. Thus, further screening and confirmation using reliable and accurate high-throughput methods are required. A pool of genes with potential markers was isolated from good and poor prognosis patients in the previous study, where subtractive hybridisation was applied on randomly selected patient based on their prognosis. The expressions of these markers were then evaluated on leukaemia cell lines. This study, aims to further examine the expression of these markers on AML samples and confirm its prognostic properties. Thirty newly diagnosed acute myeloid leukaemia (AML) cases were included in the study. The diagnosis and treatment outcome of these cases were retrieved from haematological reports and clinical data from the hospital involved. Nineteen potential genes identified from earlier study and four housekeeping genes were selected and the level of expression was determined using the GeXP method and confirmed using the real-time PCR method. Results revealed that several genes including CALM2, CSTB and TMSB4X may be related to good prognosis while SON, PGK1 and SF3B1 may be related to poor prognostic in acute myeloid leukaemias. The GeXP method has several advantages over real-time PCR including the requirement for less samples, simultaneous optimization and examination of all target and reference genes in a reaction and no requirement for repeated standard curves which are all important considerations to the study of clinical samples. This study has provided much insight into reliable techniques for selection of biomarkers and identified potential prognostic markers for acute myeloid leukaemias. This will in turn lead towards more-directed therapy for patients.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

**EVALUASI KAEDAH PANTAS UNTUK MENGUKUR ESPRESI GEN DALAM LEUKEMIA MYELOID AKUT (AML)**

Oleh

**LEE CIN DEE**

**Julai 2013**

**Pengerusi : Maha Abdullah, PhD**
**Fakulti : Institut Biosains**

Leukemia myeloid akut (AML) adalah penyakit klonal yang dicirikan oleh proliferasi dan pengumpulan sel anak myeloid di dalam sum-sum tulang dan darah periferi. Penanda ramalan perlu untuk meramal respons pesakit terhadap terapi serta mengawal terhadap pemberian ubatan kekurangan atau melampau. Umur, bacaan sel darah putih dan sitogenetik adalah penanda ramalan rutin tetapi terhad kepada kemampuan ujian tersebut disamping hanya memanfaatkan sebilangan pesakit sahaja. Kaedah profil gen secara besar-besaran telah mengenal pasti aberasi genetik seperti *microdeletions* dan mutasi yang berkenaan dengan patogenesis penyakit dan kesannya. Oleh itu, saringan lanjut dan pengesahan dengan bilangan sampel yang tinggi dengan kaedah ujian yang dipercayai serta tepat adalah perlu. Sekumpulan gen dengan penanda berpotensi telah diasingkan daripada sampel pesakit prognosis baik dan buruk dalam kajian sebelum ini. Ekspresi penanda-penanda ini kemudiannya telah dinilai pada *cell line*. Kajian ini bertujuan untuk memeriksa ekspresi penanda-penanda tersebut pada sampel AML dan seterusnya mengesahkan ciri-ciri prognostiknya. Tiga puluh kes AML baru disertakan ke dalam kajian ini. Diagnosis dan kesan rawatan diperoleh daripada laporan hematologi dan data klinikal dari hospital yang terlibat. Sembilan belas gen berpotensi yang dikenalpasti daripada kajian sebelum ini, yang mana kaedah "subtractive hybridization" telah digunakan terhadap pesakit yang dipilih secara rambang berdasarkan prognosis mereka. Empat gen rujukan juga telah dipilih dan tahap ekspresi gen-gen tersebut ditentukan menggunakan kaedah GeXP dan disahkan melalui kaedah *real-time PCR*. Keputusan mendedahkan bahawa beberapa gen termasuk CALM2, CSTB and TMSB4X mungkin berkaitan dengan prognosis baik manakala SON, PGK1 and SF3B1 mungkin berkaitan dengan prognosis buruk dalam AML. Kaedah GeXP mempunyai beberapa kelebihan berbanding dengan *real-time PCR* iaitu keperluan sampel yang sedikit, pengoptimaan serentak dan pemeriksaan semua gen sasaran dan rujukan dalam satu reaksi serta tidak perlu pengulangan lengkung standard dalam setiap esei. Semua ini adalah pertimbangan penting dalam kajian berkaitan sampel klinikal. Kajian ini telah memberi pemahaman kepada teknik yang dapat dipercayai bagi pemilihan penandabio dan mengenalpasti penanda prognostik berpotensi bagi

AML. Dengan ini, ia akan dapat membawa kepada terapi yang lebih sesuai dan berkesan kepada pesakit.

**ACKNOWLEDGEMENTS**

I fondly acknowledge the influence of my parents who instilled in me the importance of learning and helped me appreciate the indispensable value of intellectual honesty and boundless curiosity.

Also, I owe special thanks to my supervisor Assoc. Prof. Dr. Maha Abdullah for all the constructive feedback and constant guidance as well as suggestions throughout the process of producing this dissertation. A special note of thanks to Assoc. Prof. Dr. Chong Pei Pei and Dato' Dr. Chang Kian Meng as my co-supervisors for their invaluable advice.

In addition, I am thankful for the financial support from UPM Graduate Research Fellowship Scheme. Lastly, I would like to express my sincere gratitude and appreciation to all who have directly and indirectly assisted me especially my fellow labmates. More importantly, I thank God for His blessings and seeing me through this challenging journey.

Thank you.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science**.** The members of the Supervisory Committee were as follows:

**Maha Abdullah, PhD**
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

**Chong Pei Pei, PhD**
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

**Chang Kian Meng, MBBS**
Consultant Haematologist
Department of Haematology
Hospital Ampang
(Member)



**ROBIAH BINTI YUNUS, PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

**Declaration by graduate student**

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: Lee Cin Dee (GS24326)

**Declaration by Members of Supervisory Committee**

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Maha Abdullah, PhD

Signature: _____
Name of Member of Supervisory Committee: Chong Pei Pei, PhD

Signature: _____
Name of Member of Supervisory Committee: Chang Kian Meng, MBBS

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AAI | Anti-apoptosis index |
| ALL | Acute Lymphoblastic Leukaemia |
| AML | Acute myeloid leukaemia |
| ANAE | Alpha naphthyl acetate esterase |
| ANBE | Alpha naphthyl butyrate esterase |
| AP | Acid phosphatase |
| APML | Acute promyelocytic leukaemia |
| ASO | Allele-specific oligonucleotide |
| BAALC | Brain and Acute Leukemia, Cytoplasmic |
| BLAST | Basic local alignment search tool |
| BMA | Bone marrow aspirate |
| BMT | Bone marrow transplantation |
| bp | Base pair |
| CAE | Chloroacetate esterase |
| CBF | Core-binding factor |
| cDNA | Complementary DNA |
| CEBPA | CCAAT enhancer-binding protein-α |
| CLL | Chronic Lymphoid Leukaemia |
| CML | Chronic Myeloid Leukaemia |
| cMPO | Cytoplasmic myeloperoxidase |
| CN-AML | Cytogenetically normal acute myeloid leukaemia |
| CR | Complete remission |
| CR1 | First complete remission |
| $C_T$ | Threshold cycle |
| DEPC | Diethylpyrocarbonate |
| DFS | Disease-free survival |

| | |
|---|---|
| DI | Deformability index |
| DMSO | Dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| EDTA | Ethylenediaminetetraacetic acid |
| ERG | ETS-related gene |
| EtBr | Ethidium bromide |
| FAB | French-American-British |
| FISH | Fluorescence in-situ hybridization |
| FLT3 | Fms-like tyrosine kinase 3 |
| GEP | Gene expression profiling |
| GeXP | GenomeLab GeXP Genetic Analysis System (Beckman Coulter) |
| GVL | Graft-versus-leukaemia |
| HBSS | Hank's Balanced Salt Solution |
| HCT | Haematopoietic cell transplantation |
| HIDAC | High-dose Ara-C |
| HSC | Hematopoietic stem cell |
| HSCT | Hematopoietic stem cell transplantation |
| ITD | Internal tandem duplication |
| LSCs | Leukemic stem cells |
| MDR | Multidrug resistance |
| MDS | Myelodysplastic syndromes |
| MGG | May-Grünwald/Giemsa |
| MIC-M | Morphologic, Immunologic, Cytogenetic and Molecular Genetics |
| MK | Monosomal karyotype |
| M-MLV | Moloney murine leukaemia virus |
| MoAb | Monoclonal antibodies |

| | |
|---|---|
| MPO | Myeloperoxidase |
| MRD | Minimal residual disease |
| mRNA | Messenger RNA |
| NCBI | National Center for Biotechnology Information |
| NGS | Next generation sequencing |
| NPM1 | Nucleophosmin 1 |
| NSE | Non-specific esterase |
| OS | Overall survival |
| PAS | Periodic acid–Schiff |
| PBF | Peripheral blood film |
| PBMC | Peripheral blood mononuclear cells |
| PBS | Phosphate buffer saline |
| PCR | Polymerase chain reaction |
| PTD | Partial tandem duplication |
| qPCR | Real-time PCR |
| RFU | Relative fluorescence unit |
| RNA | Ribonucleic acid |
| RPMI | Roswell Park Memorial Institute |
| RT | Reverse transcription |
| RTK | Receptor tyrosine kinase |
| SBB | Sudan black B |
| SD | Standard deviation |
| SEM | Standard error of mean |
| SNP | Single nucleotide polymorphism |
| SSCP | Single-strand conformation polymorphism |
| WBC | White blood cells |
| WHO | World Health Organization |

# CHAPTER 1

# INTRODUCTION

Acute myeloid leukaemia (AML) is a clonal disease characterized by the proliferation and accumulation of myeloid progenitor cells in the bone marrow, which ultimately leads to hematopoietic failure (Robak and Wierzbowska, 2009). More than one quarter of a million adults throughout the world are diagnosed annually with acute myeloid leukaemia (AML) (Rowe and Tallman, 2010). Compared to younger adults, older AML patients are more likely to have AML with poor-risk cytogenetics (Sekeres, 2008). The duration of survival also varies in these patients. Age, white blood and cytogenetics are current risk factors and are essential to provide risk-adapted therapy to reduce over-treatment or under-treatment of patients. However, these are useful for only a fraction of patients as only 40 percent of patients have normal karyotype. Older adults with leukaemias typified by core-binding factor (CBF) abnormality (Sangle and Perkins, 2011) experiencing five-year overall survival (OS) rates is only 20 percent and even worse, zero percent for those with poor-risk features (Farag et al., 2006). Younger adults with AML who receive standard remission induction therapy experience complete remission (CR) rates of 65 to 85 percent, a full 25 percent higher than all older adults (Sekeres, 2008). To date, gene expression profiling of AML has largely confirmed the presence of well-established recurring cytogenetic abnormalities, such as translocations, deletions, point mutations, as well as microaberrations in apparently normal karyotypes. This has led to the identification of sets of genes reflective of these abnormalities (Willman, 2008) and identified new prospective prognosis categories. Genome wide search for suitable markers has identified multitudes of genes and uncharacterized transcripts but has not led to the identification of many novel therapeutic targets in AML. Selection of potential markers requires further screening, quantification and confirmation of genes expressed in a sufficiently large number of acute myeloid leukaemia samples. Current gold standard using real-time polymerase chain reaction (PCR) method is laborious and time consuming. The GenomeLab GeXP system provides an alternative method for rapid, simultaneous quantification of gene expressions in multiple samples. We hypothesized that GenomeLab GeXP system would be able to quantify multiple gene expressions more rapidly as compared to conventional PCR and there would be a significant correlation between treatment outcomes of the patients with their level of gene expressions.

## 1.1 Aim

To screen and confirm a panel of genes as well as to identify prognostic markers for acute myeloid leukaemias (AML).

## 1.2 Objectives

The objectives of the study are:-

a) To quantify expression of genes in acute myeloid leukaemia samples using the GenomeLab GeXP Genetic Analysis System.
b) To compare and confirm expression levels of selected genes in acute myeloid leukaemia samples from GenomeLab GeXP Genetic Analysis System with real-time PCR method.
c) To correlate treatment outcome of acute leukaemia patients with level of gene expression in samples.

## REFERENCES


(1988). Survival in acute myeloblastic leukemia is not prolonged by remission maintenance or early reinduction chemotherapy. The Toronto Leukemia Study Group. Leuk Res *12*, 195-200.

Aad, G., Abajyan, T., Abbott, B., Abdallah, J., Abdel Khalek, S., Abdelalim, A.A., Abdinov, O., Aben, R., Abi, B., Abolins, M.*, et al.* (2013). Search for Dark Matter Candidates and Large Extra Dimensions in Events with a Photon and Missing Transverse Momentum in pp Collision Data at sqrt[s]=7 TeV with the ATLAS Detector. Phys Rev Lett *110*, 011802.

Abkowitz, J.L., Catlin, S.N., McCallie, M.T., and Guttorp, P. (2002). Evidence that the number of hematopoietic stem cells per animal is conserved in mammals. Blood *100*, 2665-2667.

Adamo, A., Sharei, A., Adamo, L., Lee, B., Mao, S., and Jensen, K.F. (2012). Microfluidics-based assessment of cell deformability. Anal Chem *84*, 6438-6443.

Ahn, E.Y., DeKelver, R.C., Lo, M.C., Nguyen, T.A., Matsuura, S., Boyapati, A., Pandit, S., Fu, X.D., and Zhang, D.E. (2011). SON controls cell-cycle progression by coordinated regulation of RNA splicing. Mol Cell *42*, 185-198.

Ahn, E.Y., Yan, M., Malakhova, O.A., Lo, M.C., Boyapati, A., Ommen, H.B., Hines, R., Hokland, P., and Zhang, D.E. (2008). Disruption of the NHR4 domain structure in AML1-ETO abrogates SON binding and promotes leukemogenesis. Proc Natl Acad Sci U S A *105*, 17103-17108.

Akane, A., Matsubara, K., Nakamura, H., Takahashi, S., and Kimura, K. (1994). Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction (PCR) amplification. J Forensic Sci *39*, 362-372.

Al-Soud, W.A., Jonsson, L.J., and Radstrom, P. (2000). Identification and characterization of immunoglobulin G in blood as a major inhibitor of diagnostic PCR. J Clin Microbiol *38*, 345-350.

Al-Soud, W.A., and Radstrom, P. (2001). Purification and characterization of PCR-inhibitory components in blood cells. J Clin Microbiol *39*, 485-493.

Ali, M., Markham, A.F., and Isaacs, J.D. (2001). Application of differential display to immunological research. J Immunol Methods *250*, 29-43.

Aliev, G., Smith, M.A., de la Torre, J.C., and Perry, G. (2004). Mitochondria as a primary target for vascular hypoperfusion and oxidative stress in Alzheimer's disease. Mitochondrion *4*, 649-663.

Alison, M.R., Islam, S., and Wright, N.A. (2010). Stem cells in cancer: instigators and propagators? J Cell Sci *123*, 2357-2368.

Alvarez, R.H., Kantarjian, H.M., and Cortes, J.E. (2006). The role of Src in solid and hematologic malignancies: development of new-generation Src inhibitors. Cancer *107*, 1918-1929.

Andersen, J.S., Lyon, C.E., Fox, A.H., Leung, A.K., Lam, Y.W., Steen, H., Mann, M., and Lamond, A.I. (2002). Directed proteomic analysis of the human nucleolus. Curr Biol *12*, 1-11.

Appelbaum, F.R., Rosenblum, D., Arceci, R.J., Carroll, W.L., Breitfeld, P.P., Forman, S.J., Larson, R.A., Lee, S.J., Murphy, S.B., O'Brien, S.*, et al.* (2007). End points to establish the efficacy of new agents in the treatment of acute leukemia. Blood *109*, 1810-1816.

Aref, S., Salama, O., Al-Tonbary, Y., Fouda, M., Menessy, A., and El-Sherbiny, M. (2002). L and E selectins in acute myeloid leukemia: expression, clinical relevance and relation to patient outcome. Hematology *7*, 83-87.

Bacher, U., Kern, W., Alpermann, T., Schnittger, S., Kohlmann, A., Klein, H.U., Dugas, M., Haferlach, C., and Haferlach, T. (2011). Prognosis in patients with MDS or AML and bone marrow blasts between 10% and 30% is not associated with blast counts but depends on cytogenetic and molecular genetic characteristics. Leukemia *25*, 1361-1364.

Bacher, U., Kohlmann, A., Haferlach, C., and Haferlach, T. (2009). Gene expression profiling in acute myeloid leukaemia (AML). Best Pract Res Clin Haematol *22*, 169-180.

Bacher, U., Kohlmann, A., and Haferlach, T. (2010). Gene expression profiling for diagnosis and therapy in acute leukaemia and other haematologic malignancies. Cancer Treat Rev *36*, 637-646.

Bain, B.J. (2001). Overview. Cytogenetic analysis in haematology. Best Pract Res Clin Haematol *14*, 463-477.

Bakker, A.B., van den Oudenrijn, S., Bakker, A.Q., Feller, N., van Meijer, M., Bia, J.A., Jongeneelen, M.A., Visser, T.J., Bijl, N., Geuijen, C.A.*, et al.* (2004). C-type lectin-like molecule-1: a novel myeloid cell surface marker associated with acute myeloid leukemia. Cancer Res *64*, 8443-8450.

Balkhi, M.Y., Trivedi, A.K., Geletu, M., Christopeit, M., Bohlander, S.K., Behre, H.M., and Behre, G. (2006). Proteomics of acute myeloid leukaemia: Cytogenetic risk groups differ specifically in their proteome, interactome and post-translational protein modifications. Oncogene *25*, 7041-7058.

Barjesteh van Waalwijk van Doorn-Khosrovani, S., Erpelinck, C., van Putten, W.L., Valk, P.J., van der Poel-van de Luytgaarde, S., Hack, R., Slater, R., Smit, E.M., Beverloo, H.B., Verhoef, G.*, et al.* (2003). High EVI1 expression predicts poor survival in acute myeloid leukemia: a study of 319 de novo AML patients. Blood *101*, 837-845.

Basara, N., Schulze, A., Wedding, U., Mohren, M., Gerhardt, A., Junghanss, C., Peter, N., Dolken, G., Becker, C., Heyn, S.*, et al.* (2009). Early related or unrelated haematopoietic cell transplantation results in higher overall survival and leukaemia-free survival compared with conventional chemotherapy in high-risk acute myeloid leukaemia patients in first complete remission. Leukemia *23*, 635-640.

Bennett, J.M., Catovsky, D., Daniel, M.T., Flandrin, G., Galton, D.A., Gralnick, H.R., and Sultan, C. (1976). Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol *33*, 451-458.

Bennett, J.M., Catovsky, D., Daniel, M.T., Flandrin, G., Galton, D.A., Gralnick, H.R., and Sultan, C. (1985). Proposed revised criteria for the classification of acute myeloid

leukemia. A report of the French-American-British Cooperative Group. Ann Intern Med *103*, 620-625.

Bernier M Fau - Massy, M., Massy M Fau - Deleeuw, N., Deleeuw N Fau - Bron, D., Bron D Fau - Debusscher, L., Debusscher L Fau - Stryckmans, P., and Stryckmans, P. Immunological definition of acute minimally differentiated myeloid leukemia (MO) and acute undifferentiated leukemia (AUL).

Bhatia, S., Robison, L.L., Francisco, L., Carter, A., Liu, Y., Grant, M., Baker, K.S., Fung, H., Gurney, J.G., McGlave, P.B.*, et al.* (2005). Late mortality in survivors of autologous hematopoietic-cell transplantation: report from the Bone Marrow Transplant Survivor Study. Blood *105*, 4215-4222.

Bolufer, P., Barragan, E., Collado, M., Cervera, J., Lopez, J.A., and Sanz, M.A. (2006). Influence of genetic polymorphisms on the risk of developing leukemia and on disease progression. Leuk Res *30*, 1471-1491.

Bonnet, D., and Dick, J.E. (1997). Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med *3*, 730-737.

Booken, N., Gratchev, A., Utikal, J., Weiss, C., Yu, X., Qadoumi, M., Schmuth, M., Sepp, N., Nashan, D., Rass, K.*, et al.* (2008). Sezary syndrome is a unique cutaneous T-cell lymphoma as identified by an expanded gene signature including diagnostic marker molecules CDO1 and DNM3. Leukemia *22*, 393-399.

Bradstock, K., Matthews, J., Benson, E., Page, F., and Bishop, J. (1994). Prognostic value of immunophenotyping in acute myeloid leukemia. Australian Leukaemia Study Group. Blood *84*, 1220-1225.

Breems, D.A., and Lowenberg, B. (2007). Acute myeloid leukemia and the position of autologous stem cell transplantation. Semin Hematol *44*, 259-266.

Breems, D.A., Van Putten, W.L., De Greef, G.E., Van Zelderen-Bhola, S.L., Gerssen-Schoorl, K.B., Mellink, C.H., Nieuwint, A., Jotterand, M., Hagemeijer, A., Beverloo, H.B.*, et al.* (2008). Monosomal karyotype in acute myeloid leukemia: a better indicator of poor prognosis than a complex karyotype. J Clin Oncol *26*, 4791-4797.

Breems, D.A., Van Putten, W.L., Huijgens, P.C., Ossenkoppele, G.J., Verhoef, G.E., Verdonck, L.F., Vellenga, E., De Greef, G.E., Jacky, E., Van der Lelie, J.*, et al.* (2005). Prognostic index for adult patients with acute myeloid leukemia in first relapse. J Clin Oncol *23*, 1969-1978.

Bruisten, S.M., Oudshoorn, P., van Swieten, P., Boeser-Nunnink, B., van Aarle, P., Tondreau, S.P., and Cuypers, H.T. (1997). Stability of HIV-1 RNA in blood during specimen handling and storage prior to amplification by NASBA-QT. J Virol Methods *67*, 199-207.

Budihna, M., Strojan, P., Smid, L., Skrk, J., Vrhovec, I., Zupevc, A., Rudolf, Z., Zargi, M., Krasovec, M., Svetic, B.*, et al.* (1996). Prognostic value of cathepsins B, H, L, D and their endogenous inhibitors stefins A and B in head and neck carcinoma. Biol Chem Hoppe Seyler *377*, 385-390.

Buss, E.C., and Ho, A.D. (2011). Leukemia stem cells. Int J Cancer *129*, 2328-2336.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L.*, et al.* (2009). The MIQE guidelines: minimum

information for publication of quantitative real-time PCR experiments. Clin Chem *55*, 611-622.

Bustin, S.A., Benes, V., Nolan, T., and Pfaffl, M.W. (2005). Quantitative real-time RT-PCR--a perspective. J Mol Endocrinol *34*, 597-601.

Byrne, J.L., Dasgupta, E., Pallis, M., Turzanski, J., Forman, K., Mitchell, D., Haynes, A.P., and Russell, N.H. (1999). Early allogeneic transplantation for refractory or relapsed acute leukaemia following remission induction with FLAG. Leukemia *13*, 786-791.

Care, R.S., Valk, P.J., Goodeve, A.C., Abu-Duhier, F.M., Geertsma-Kleinekoort, W.M., Wilson, G.A., Gari, M.A., Peake, I.R., Lowenberg, B., and Reilly, J.T. (2003). Incidence and prognosis of c-KIT and FLT3 mutations in core binding factor (CBF) acute myeloid leukaemias. Br J Haematol *121*, 775-777.

Cason, J.D., Trujillo, J.M., Estey, E.H., Huh, Y.O., Freireich, E.J., and Stass, S.A. (1989). Peripheral acute leukemia: high peripheral but low-marrow blast count. Blood *74*, 1758-1761.

Cassileth, P.A., Harrington, D.P., Appelbaum, F.R., Lazarus, H.M., Rowe, J.M., Paietta, E., Willman, C., Hurd, D.D., Bennett, J.M., Blume, K.G., *et al.* (1998). Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. N Engl J Med *339*, 1649-1656.

Cassileth, P.A., Harrington, D.P., Hines, J.D., Oken, M.M., Mazza, J.J., McGlave, P., Bennett, J.M., and O'Connell, M.J. (1988). Maintenance chemotherapy prolongs remission duration in adult acute nonlymphocytic leukemia. J Clin Oncol *6*, 583-587.

Cassileth, P.A., Lynch, E., Hines, J.D., Oken, M.M., Mazza, J.J., Bennett, J.M., McGlave, P.B., Edelstein, M., Harrington, D.P., and O'Connell, M.J. (1992). Varying intensity of postremission therapy in acute myeloid leukemia. Blood *79*, 1924-1930.

Chapman, M., Warren, E.H., 3rd, and Wu, C.J. (2012). Applications of next-generation sequencing to blood and marrow transplantation. Biol Blood Marrow Transplant *18*, S151-160.

Chen, B., Borinstein, S.C., Gillis, J., Sykes, V.W., and Bogler, O. (2000). The glioma-associated protein SETA interacts with AIP1/Alix and ALG-2 and modulates apoptosis in astrocytes. J Biol Chem *275*, 19275-19281.

Chen, J., Kobayashi, M., Darmanin, S., Qiao, Y., Gully, C., Zhao, R., Yeung, S.C., and Lee, M.H. (2009). Pim-1 plays a pivotal role in hypoxia-induced chemoresistance. Oncogene *28*, 2581-2592.

Chen, Q.R., Vansant, G., Oades, K., Pickering, M., Wei, J.S., Song, Y.K., Monforte, J., and Khan, J. (2007). Diagnosis of the small round blue cell tumors using multiplex polymerase chain reaction. J Mol Diagn *9*, 80-88.

Chen, Y., Garrison, S., Weis, J.J., and Weis, J.H. (1998). Identification of pactolus, an integrin beta subunit-like cell-surface protein preferentially expressed by cells of the bone marrow. J Biol Chem *273*, 8711-8718.

Cheson, B.D., Bennett, J.M., Kopecky, K.J., Buchner, T., Willman, C.L., Estey, E.H., Schiffer, C.A., Doehner, H., Tallman, M.S., Lister, T.A., *et al.* (2003). Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria,

Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol *21*, 4642-4649.

Cheson, B.D., Cassileth, P.A., Head, D.R., Schiffer, C.A., Bennett, J.M., Bloomfield, C.D., Brunning, R., Gale, R.P., Grever, M.R., Keating, M.J.*, et al.* (1990). Report of the National Cancer Institute-sponsored workshop on definitions of diagnosis and response in acute myeloid leukemia. J Clin Oncol *8*, 813-819.

Cheung, W.Y. (1980). Calmodulin plays a pivotal role in cellular regulation. Science *207*, 19-27.

Chien, J.H., Tang, J.L., Chen, R.L., Li, C.C., and Lee, C.P. (2008). Detection of BCR-ABL gene mutations in Philadelphia chromosome positive leukemia patients resistant to STI-571 cancer therapy. Leuk Res *32*, 1724-1734.

Chisaka, O., and Kameda, Y. (2005). Hoxa3 regulates the proliferation and differentiation of the third pharyngeal arch mesenchyme in mice. Cell Tissue Res *320*, 77-89.

Clevers, H. (2006). Wnt/beta-catenin signaling in development and disease. Cell *127*, 469-480.

Court, E.L., Smith, M.A., Avent, N.D., Hancock, J.T., Morgan, L.M., Gray, A.G., and Smith, J.G. (2004). DNA microarray screening of differential gene expression in bone marrow samples from AML, non-AML patients and AML cell lines. Leuk Res *28*, 743-753.

Craig, C.M., and Schiller, G.J. (2008). Acute myeloid leukemia in the elderly: conventional and novel treatment approaches. Blood Rev *22*, 221-234.

D'Alo, F., Di Ruscio, A., Guidi, F., Fabiani, E., Greco, M., Rumi, C., Hohaus, S., Voso, M.T., and Leone, G. (2008). PU.1 and CEBPA expression in acute myeloid leukemia. Leuk Res *32*, 1448-1453.

Dang, H., Jiang, A., Kamel-Reid, S., Brandwein, J., and Chang, H. (2013). Prognostic value of immunophenotyping and gene mutations in elderly patients with acute myeloid leukemia with normal karyotype. Hum Pathol *44*, 55-61.

Das, A., Beckham, T.R., and McIntosh, M.T. (2011). Comparison of methods for improved RNA extraction from blood for early detection of Classical swine fever virus by real-time reverse transcription polymerase chain reaction. J Vet Diagn Invest *23*, 727-735.

Davis, R.J. (2000). Signal transduction by the JNK group of MAP kinases. Cell *103*, 239-252.

de Franchis, R., Cross, N.C., Foulkes, N.S., and Cox, T.M. (1988). A potent inhibitor of Taq polymerase copurifies with human genomic DNA. Nucleic Acids Res *16*, 10355.

de Greef, G.E., van Putten, W.L., Boogaerts, M., Huijgens, P.C., Verdonck, L.F., Vellenga, E., Theobald, M., Jacky, E., and Lowenberg, B. (2005). Criteria for defining a complete remission in acute myeloid leukaemia revisited. An analysis of patients treated in HOVON-SAKK co-operative group studies. Br J Haematol *128*, 184-191.

de Nully Brown, P., Jurlander, J., Pedersen-Bjergaard, J., Victor, M.A., and Geisler, C.H. (1997). The prognostic significance of chromosomal analysis and immunophenotyping in 117 patients with de novo acute myeloid leukemia. Leuk Res *21*, 985-995.

Deligezer, U., Akisik, E., and Dalay, N. (2003). Genotyping of the MTHFR gene polymorphism, C677T in patients with leukemia by melting curve analysis. Mol Diagn *7*, 181-185.

Dieffenbach, C.W., Lowe, T.M., and Dveksler, G.S. (1993). General concepts for PCR primer design. PCR Methods Appl *3*, S30-37.

Dohner, H., Estey, E.H., Amadori, S., Appelbaum, F.R., Buchner, T., Burnett, A.K., Dombret, H., Fenaux, P., Grimwade, D., Larson, R.A.*, et al.* (2010). Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood *115*, 453-474.

Dohner, H., and Gaidzik, V.I. (2011). Impact of genetic features on treatment decisions in AML. Hematology Am Soc Hematol Educ Program *2011*, 36-42.

Dohner, K., Schlenk, R.F., Habdank, M., Scholl, C., Rucker, F.G., Corbacioglu, A., Bullinger, L., Frohling, S., and Dohner, H. (2005). Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood *106*, 3740-3746.

Dohner, K., Tobis, K., Ulrich, R., Frohling, S., Benner, A., Schlenk, R.F., and Dohner, H. (2002). Prognostic significance of partial tandem duplications of the MLL gene in adult patients 16 to 60 years old with acute myeloid leukemia and normal cytogenetics: a study of the Acute Myeloid Leukemia Study Group Ulm. J Clin Oncol *20*, 3254-3261.

Douer, D. (2003). The epidemiology of acute promyelocytic leukaemia. Best Pract Res Clin Haematol *16*, 357-367.

Drabkin, H.A., Diaz, M., Bradley, C.M., Le Beau, M.M., Rowley, J.D., and Patterson, D. (1985). Isolation and analysis of the 21q+ chromosome in the acute myelogenous leukemia 8;21 translocation: evidence that c-mos is not translocated. Proc Natl Acad Sci U S A *82*, 464-468.

Drabkin, H.A., Parsy, C., Ferguson, K., Guilhot, F., Lacotte, L., Roy, L., Zeng, C., Baron, A., Hunger, S.P., Varella-Garcia, M.*, et al.* (2002). Quantitative HOX expression in chromosomally defined subsets of acute myelogenous leukemia. Leukemia *16*, 186-195.

Drew, J.E., Mayer, C.D., Farquharson, A.J., Young, P., and Barrera, L.N. (2011). Custom design of a GeXP multiplexed assay used to assess expression profiles of inflammatory gene targets in normal colon, polyp, and tumor tissue. J Mol Diagn *13*, 233-242.

Duan, Z., Lamendola, D.E., Yusuf, R.Z., Penson, R.T., Preffer, F.I., and Seiden, M.V. (2002). Overexpression of human phosphoglycerate kinase 1 (PGK1) induces a multidrug resistance phenotype. Anticancer Res *22*, 1933-1941.

Dunican, D.S., McWilliam, P., Tighe, O., Parle-McDermott, A., and Croke, D.T. (2002). Gene expression differences between the microsatellite instability (MIN) and chromosomal instability (CIN) phenotypes in colorectal cancer revealed by high-density cDNA array hybridization. Oncogene *21*, 3253-3257.

Dunphy, C.H. (2009). Bone marrow evaluation of monocytosis. Diagnostic Histopathology *15*, 116-124.

Duvigneau, J.C., Sipos, W., Hartl, R.T., Bayer, M., Moldzio, R., Stevenson, L., Adair, B., and Gemeiner, M. (2007). Heparin and EDTA as anticoagulant differentially affect cytokine

mRNA level of cultured porcine blood cells. Journal of Immunological Methods *324*, 38-47.

Ebert, E., Werle, B., Julke, B., Kopitar-Jerala, N., Kos, J., Lah, T., Abrahamson, M., Spiess, E., and Ebert, W. (1997). Expression of cysteine protease inhibitors stefin A, stefin B, and cystatin C in human lung tumor tissue. Adv Exp Med Biol *421*, 259-265.

Estey, E., and Dohner, H. (2006). Acute myeloid leukaemia. Lancet *368*, 1894-1907.

Estey, E., Thall, P., Beran, M., Kantarjian, H., Pierce, S., and Keating, M. (1997). Effect of diagnosis (refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, or acute myeloid leukemia [AML]) on outcome of AML-type chemotherapy. Blood *90*, 2969-2977.

Estey, E.H. (2000). Treatment of relapsed and refractory acute myelogenous leukemia. Leukemia *14*, 476-479.

Farag, S.S., Archer, K.J., Mrozek, K., Ruppert, A.S., Carroll, A.J., Vardiman, J.W., Pettenati, M.J., Baer, M.R., Qumsiyeh, M.B., Koduru, P.R.*, et al.* (2006). Pretreatment cytogenetics add to other prognostic factors predicting complete remission and long-term outcome in patients 60 years of age or older with acute myeloid leukemia: results from Cancer and Leukemia Group B 8461. Blood *108*, 63-73.

Foran, J.M. (2010). New prognostic markers in acute myeloid leukemia: perspective from the clinic. Hematology Am Soc Hematol Educ Program *2010*, 47-55.

Foss, B., Tronstad, K.J., and Bruserud, O. (2010). Connexin-based signaling in acute myelogenous leukemia (AML). Biochim Biophys Acta *1798*, 1-8.

Frank, O., Brors, B., Fabarius, A., Li, L., Haak, M., Merk, S., Schwindel, U., Zheng, C., Muller, M.C., Gretz, N.*, et al.* (2006). Gene expression signature of primary imatinib-resistant chronic myeloid leukemia patients. Leukemia *20*, 1400-1407.

Galimberti, S., Guerrini, F., Palumbo, G.A., Consoli, U., Fazzi, R., Morabito, F., Santini, V., and Petrini, M. (2004). Evaluation of BCRP and MDR-1 co-expression by quantitative molecular assessment in AML patients. Leuk Res *28*, 367-372.

Ganzel, C., and Rowe, J.M. (2011). Prognostic factors in adult acute leukemia. Hematol Oncol Clin North Am *25*, 1163-1187.

Ghadessy, F.J., Ong, J.L., and Holliger, P. (2001). Directed evolution of polymerase function by compartmentalized self-replication. Proc Natl Acad Sci U S A *98*, 4552-4557.

Ghossein, R.A., and Bhattacharya, S. (2000). Molecular detection and characterisation of circulating tumour cells and micrometastases in solid tumours. Eur J Cancer *36*, 1681-1694.

Ghossein, R.A., Bhattacharya, S., and Coit, D.G. (2001). Reverse transcriptase polymerase chain reaction (RT-PCR) detection of melanoma-related transcripts in the peripheral blood and bone marrow of patients with malignant melanoma. What have we learned? Recent Results Cancer Res *158*, 63-77.

Goardon, N., Marchi, E., Atzberger, A., Quek, L., Schuh, A., Soneji, S., Woll, P., Mead, A., Alford, K.A., Rout, R.*, et al.* (2011). Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. Cancer Cell *19*, 138-152.

Godley, L.A., Cunningham, J., Dolan, M.E., Huang, R.S., Gurbuxani, S., McNerney, M.E., Larson, R.A., Leong, H., Lussier, Y., Onel, K., *et al.* (2011). An integrated genomic approach to the assessment and treatment of acute myeloid leukemia. Semin Oncol *38*, 215-224.

Goh, S.H., Hong, S.H., Lee, B.C., Ju, M.H., Jeong, J.S., Cho, Y.R., Kim, I.H., and Lee, Y.S. (2011). eIF3m expression influences the regulation of tumorigenesis-related genes in human colon cancer. Oncogene *30*, 398-409.

Grandage, V.L., Gale, R.E., Linch, D.C., and Khwaja, A. (2005). PI3-kinase/Akt is constitutively active in primary acute myeloid leukaemia cells and regulates survival and chemoresistance via NF-kappaB, Mapkinase and p53 pathways. Leukemia *19*, 586-594.

Gratwohl, A., Baldomero, H., Aljurf, M., Pasquini, M.C., Bouzas, L.F., Yoshimi, A., Szer, J., Lipton, J., Schwendener, A., Gratwohl, M., *et al.* (2010). Hematopoietic stem cell transplantation: a global perspective. JAMA *303*, 1617-1624.

Greenhalf, W., Lee, J., and Chaudhuri, B. (1999). A selection system for human apoptosis inhibitors using yeast. Yeast *15*, 1307-1321.

Gregory, T.K., Wald, D., Chen, Y., Vermaat, J.M., Xiong, Y., and Tse, W. (2009). Molecular prognostic markers for adult acute myeloid leukemia with normal cytogenetics. J Hematol Oncol *2*, 23.

Grimwade, D., Walker, H., Harrison, G., Oliver, F., Chatters, S., Harrison, C.J., Wheatley, K., Burnett, A.K., and Goldstone, A.H. (2001). The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. Blood *98*, 1312-1320.

Grimwade, D., Walker, H., Oliver, F., Wheatley, K., Harrison, C., Harrison, G., Rees, J., Hann, I., Stevens, R., Burnett, A., *et al.* (1998). The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood *92*, 2322-2333.

Grzybowska-Izydorczyk, O., Cebula, B., Robak, T., and Smolewski, P. (2010). Expression and prognostic significance of the inhibitor of apoptosis protein (IAP) family and its antagonists in chronic lymphocytic leukaemia. Eur J Cancer *46*, 800-810.

Gu, J., Zhang, Q.H., Huang, Q.H., Ren, S.X., Wu, X.Y., Ye, M., Huang, C.H., Fu, G., Zhou, J., Niu, C., *et al.* (2000). Gene expression in CD34(+) cells from normal bone marrow and leukemic origins. Hematol J *1*, 206-217.

Guescini, M., Sisti, D., Rocchi, M.B., Stocchi, L., and Stocchi, V. (2008). A new real-time PCR method to overcome significant quantitative inaccuracy due to slight amplification inhibition. BMC Bioinformatics *9*, 326.

Guzman, M.L., Neering, S.J., Upchurch, D., Grimes, B., Howard, D.S., Rizzieri, D.A., Luger, S.M., and Jordan, C.T. (2001). Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood *98*, 2301-2307.

Hampson, P., Chahal, H., Khanim, F., Hayden, R., Mulder, A., Assi, L.K., Bunce, C.M., and Lord, J.M. (2005). PEP005, a selective small-molecule activator of protein kinase C, has

potent antileukemic activity mediated via the delta isoform of PKC. Blood *106*, 1362-1368.

Haouas, H., Haouas, S., Uzan, G., and Hafsia, A. (2010). Identification of new markers discriminating between myeloid and lymphoid acute leukemia. Hematology *15*, 193-203.

Harousseau, J.L., Cahn, J.Y., Pignon, B., Witz, F., Milpied, N., Delain, M., Lioure, B., Lamy, T., Desablens, B., Guilhot, F.*, et al.* (1997). Comparison of autologous bone marrow transplantation and intensive chemotherapy as postremission therapy in adult acute myeloid leukemia. The Groupe Ouest Est Leucemies Aigues Myeloblastiques (GOELAM). Blood *90*, 2978-2986.

Harris, N.L., Jaffe, E.S., Diebold, J., Flandrin, G., Muller-Hermelink, H.K., Vardiman, J., Lister, T.A., and Bloomfield, C.D. (1999). World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. J Clin Oncol *17*, 3835-3849.

Hatch, C.L., and Bonner, W.M. (1995). Characterization of the proximal promoter of the human histone H2A.Z gene. DNA Cell Biol *14*, 257-266.

Hayashi, E., Aoyama, N., and Still, D.W. (2008). Quantitative trait loci associated with lettuce seed germination under different temperature and light environments. Genome *51*, 928-947.

Heckmann, L.H., Connon, R., Hutchinson, T.H., Maund, S.J., Sibly, R.M., and Callaghan, A. (2006). Expression of target and reference genes in Daphnia magna exposed to ibuprofen. BMC Genomics *7*, 175.

Heid, C.A., Stevens, J., Livak, K.J., and Williams, P.M. (1996). Real time quantitative PCR. Genome Res *6*, 986-994.

Hembruff, S.L., Villeneuve, D.J., and Parissenti, A.M. (2005). The optimization of quantitative reverse transcription PCR for verification of cDNA microarray data. Analytical Biochemistry *345*, 237-249.

Hess, C.J., Berkhof, J., Denkers, F., Ossenkoppele, G.J., Schouten, J.P., Oudejans, J.J., Waisfisz, Q., and Schuurhuis, G.J. (2007). Activated intrinsic apoptosis pathway is a key related prognostic parameter in acute myeloid leukemia. J Clin Oncol *25*, 1209-1215.

Heuser, M., Wingen, L.U., Steinemann, D., Cario, G., von Neuhoff, N., Tauscher, M., Bullinger, L., Krauter, J., Heil, G., Dohner, H.*, et al.* (2005). Gene-expression profiles and their association with drug resistance in adult acute myeloid leukemia. Haematologica *90*, 1484-1492.

Himori, T., Tanaka, T., and Ohnuma, T. (1985). Ultrastructural peroxidase cytochemistry of three established human myelogenous leukemia cell lines, HL-60, KG-1 and ML-2. Leuk Res *9*, 913-919.

Holland, N.T., Smith, M.T., Eskenazi, B., and Bastaki, M. (2003). Biological sample collection and processing for molecular epidemiological studies. Mutat Res *543*, 217-234.

Horowitz, M.M., Gale, R.P., Sondel, P.M., Goldman, J.M., Kersey, J., Kolb, H.J., Rimm, A.A., Ringden, O., Rozman, C., Speck, B.*, et al.* (1990). Graft-versus-leukemia reactions after bone marrow transplantation. Blood *75*, 555-562.

Hosen, N., Park, C.Y., Tatsumi, N., Oji, Y., Sugiyama, H., Gramatzki, M., Krensky, A.M., and Weissman, I.L. (2007). CD96 is a leukemic stem cell-specific marker in human acute myeloid leukemia. Proc Natl Acad Sci U S A *104*, 11008-11013.

Huang, Y.H., Hu, X.X., Xu, W.Z., Gao, Y., Feng, J.D., Sun, H., and Li, N. (2003). [The factors affecting the efficiency of mutiplex PCR]. Yi Chuan *25*, 65-68.

Huh, Y.O., Jilani, I., Estey, E., Giles, F., Kantarjian, H., Freireich, E., and Albitar, M. (2002). More cell death in refractory anemia with excess blasts in transformation than in acute myeloid leukemia. Leukemia *16*, 2249-2252.

Jain, M., Bakhshi, S., Shukla, A.A., and Chauhan, S.S. (2010). Cathepsins B and L in peripheral blood mononuclear cells of pediatric acute myeloid leukemia: potential poor prognostic markers. Ann Hematol *89*, 1223-1232.

Janssens, N., Janicot, M., Perera, T., and Bakker, A. (2004). Housekeeping genes as internal standards in cancer research. Mol Diagn *8*, 107-113.

Jedema, I., Barge, R.M., Frankel, A.E., Willemze, R., and Falkenburg, J.H. (2004). Acute myeloid leukemia cells in G0 phase of the cell cycle that are unresponsive to conventional chemotherapy are sensitive to treatment with granulocyte-macrophage colony-stimulating factor/diphtheria toxin fusion proteins. Exp Hematol *32*, 188-194.

Ji, P., Diederichs, S., Wang, W., Boing, S., Metzger, R., Schneider, P.M., Tidow, N., Brandt, B., Buerger, H., Bulk, E.*, et al.* (2003). MALAT-1, a novel noncoding RNA, and thymosin beta4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene *22*, 8031-8041.

Jin, L., Lee, E.M., Ramshaw, H.S., Busfield, S.J., Peoppl, A.G., Wilkinson, L., Guthridge, M.A., Thomas, D., Barry, E.F., Boyd, A.*, et al.* (2009). Monoclonal antibody-mediated targeting of CD123, IL-3 receptor alpha chain, eliminates human acute myeloid leukemic stem cells. Cell Stem Cell *5*, 31-42.

Kahl, C.R., and Means, A.R. (2003). Regulation of cell cycle progression by calcium/calmodulin-dependent pathways. Endocr Rev *24*, 719-736.

Kaida, D., Motoyoshi, H., Tashiro, E., Nojima, T., Hagiwara, M., Ishigami, K., Watanabe, H., Kitahara, T., Yoshida, T., Nakajima, H.*, et al.* (2007). Spliceostatin A targets SF3b and inhibits both splicing and nuclear retention of pre-mRNA. Nat Chem Biol *3*, 576-583.

Kang, J.-E., Hwang, S.-H., Lee, J.H., Park, D.Y., and Kim, H.-H. (2011). Effects of RBC removal and TRIzol of peripheral blood samples on RNA stability. Clinica Chimica Acta *412*, 1883-1885.

Kantarjian, H.M., Keating, M.J., Walters, R.S., McCredie, K.B., and Freireich, E.J. (1988). The characteristics and outcome of patients with late relapse acute myelogenous leukemia. J Clin Oncol *6*, 232-238.

Karlen, Y., McNair, A., Perseguers, S., Mazza, C., and Mermod, N. (2007). Statistical significance of quantitative PCR. BMC Bioinformatics *8*, 131.

Kaufmann, W.K., Nevis, K.R., Qu, P., Ibrahim, J.G., Zhou, T., Zhou, Y., Simpson, D.A., Helms-Deaton, J., Cordeiro-Stone, M., Moore, D.T.*, et al.* (2008). Defective cell cycle checkpoint functions in melanoma are associated with altered patterns of gene expression. J Invest Dermatol *128*, 175-187.

Keilholz, U., Goldin-Lang, P., Bechrakis, N.E., Max, N., Letsch, A., Schmittel, A., Scheibenbogen, C., Heufelder, K., Eggermont, A., and Thiel, E. (2004). Quantitative detection of circulating tumor cells in cutaneous and ocular melanoma and quality assessment by real-time reverse transcriptase-polymerase chain reaction. Clin Cancer Res *10*, 1605-1612.

Keppler, D. (2006). Towards novel anti-cancer strategies based on cystatin function. Cancer Lett *235*, 159-176.

Klobusicka, M., Kusenda, J., and Babusikova, O. (2005). Myeloid enzymes profile related to the immunophenotypic characteristics of blast cells from patients with acute myeloid leukemia (AML) at diagnosis. Neoplasma *52*, 211-218.

Konoplev, S., and Bueso-Ramos, C.E. (2006). Advances in the pathologic diagnosis and biology of acute myeloid leukemia. Ann Diagn Pathol *10*, 39-65.

Krauter, J., Wagner, K., Schafer, I., Marschalek, R., Meyer, C., Heil, G., Schaich, M., Ehninger, G., Niederwieser, D., Krahl, R.*, et al.* (2009). Prognostic factors in adult patients up to 60 years old with acute myeloid leukemia and translocations of chromosome band 11q23: individual patient data-based meta-analysis of the German Acute Myeloid Leukemia Intergroup. J Clin Oncol *27*, 3000-3006.

Kurinna, S., Konopleva, M., Palla, S.L., Chen, W., Kornblau, S., Contractor, R., Deng, X., May, W.S., Andreeff, M., and Ruvolo, P.P. (2006). Bcl2 phosphorylation and active PKC alpha are associated with poor survival in AML. Leukemia *20*, 1316-1319.

Langer, C., Radmacher, M.D., Ruppert, A.S., Whitman, S.P., Paschka, P., Mrozek, K., Baldus, C.D., Vukosavljevic, T., Liu, C.G., Ross, M.E.*, et al.* (2008). High BAALC expression associates with other molecular prognostic markers, poor outcome, and a distinct gene-expression signature in cytogenetically normal patients younger than 60 years with acute myeloid leukemia: a Cancer and Leukemia Group B (CALGB) study. Blood *111*, 5371-5379.

Lay, A.J., Jiang, X.M., Kisker, O., Flynn, E., Underwood, A., Condron, R., and Hogg, P.J. (2000). Phosphoglycerate kinase acts in tumour angiogenesis as a disulphide reductase. Nature *408*, 869-873.

Lee, E.J., George, S.L., Amrein, P.C., Paciucci, P.A., Allen, S.L., and Schiffer, C.A. (1998). An evaluation of combinations of diaziquone, etoposide and mitoxantrone in the treatment of adults with relapsed or refractory acute myeloid leukemia: results of 8722, a randomized phase II study conducted by Cancer and Leukemia Group B. Leukemia *12*, 139-143.

Li, J., Mao, N.Y., Zhang, C., Yang, M.J., Wang, M., Xu, W.B., and Ma, X.J. (2012). The development of a GeXP-based multiplex reverse transcription-PCR assay for simultaneous detection of sixteen human respiratory virus types/subtypes. BMC Infect Dis *12*, 189.

Li, K., Yang, M., Sablok, G., Fan, J., and Zhou, F. (2013). Screening features to improve the class prediction of acute myeloid leukemia and myelodysplastic syndrome. Gene *512*, 348-354.

Li, X., Zimmerman, A., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., and Yin, H.L. (1996). The mouse thymosin beta 4 gene: structure, promoter identification, and chromosome localization. Genomics *32*, 388-394.

Liang, P., and Pardee, A.B. (1992). Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science *257*, 967-971.

Link, D.C. (2012). Molecular genetics of AML. Best Pract Res Clin Haematol *25*, 409-414.

Lowenberg, B., Downing, J.R., and Burnett, A. (1999). Acute myeloid leukemia. N Engl J Med *341*, 1051-1062.

Lowenberg, B., Griffin, J.D., and Tallman, M.S. (2003). Acute myeloid leukemia and acute promyelocytic leukemia. Hematology Am Soc Hematol Educ Program, 82-101.

Luo, J., Manning, B.D., and Cantley, L.C. (2003). Targeting the PI3K-Akt pathway in human cancer: rationale and promise. Cancer Cell *4*, 257-262.

Lutterbach, B., and Hiebert, S.W. (2000). Role of the transcription factor AML-1 in acute leukemia and hematopoietic differentiation. Gene *245*, 223-235.

Ma, W., Wang, M., Wang, Z.Q., Sun, L., Graber, D., Matthews, J., Champlin, R., Yi, Q., Orlowski, R.Z., Kwak, L.W.*, et al.* (2010). Effect of long-term storage in TRIzol on microarray-based gene expression profiling. Cancer Epidemiol Biomarkers Prev *19*, 2445-2452.

Majhail, N.S., and Lichtin, A.E. (2004). Acute leukemia with a very high leukocyte count: confronting a medical emergency. Cleve Clin J Med *71*, 633-637.

Marchesi, F., Annibali, O., Cerchiara, E., Tirindelli, M.C., and Avvisati, G. (2011). Cytogenetic abnormalities in adult non-promyelocytic acute myeloid leukemia: a concise review. Crit Rev Oncol Hematol *80*, 331-346.

Marcucci, G., Baldus, C.D., Ruppert, A.S., Radmacher, M.D., Mrozek, K., Whitman, S.P., Kolitz, J.E., Edwards, C.G., Vardiman, J.W., Powell, B.L.*, et al.* (2005). Overexpression of the ETS-related gene, ERG, predicts a worse outcome in acute myeloid leukemia with normal karyotype: a Cancer and Leukemia Group B study. J Clin Oncol *23*, 9234-9242.

Mayer, R.J., Davis, R.B., Schiffer, C.A., Berg, D.T., Powell, B.L., Schulman, P., Omura, G.A., Moore, J.O., McIntyre, O.R., and Frei, E., 3rd (1994). Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. N Engl J Med *331*, 896-903.

Mayur, Y.C., Jagadeesh, S., and Thimmaiah, K.N. (2006). Targeting calmodulin in reversing multi drug resistance in cancer cells. Mini Rev Med Chem *6*, 1383-1389.

McCulloch, E.A., Howatson, A.F., Buick, R.N., Minden, M.D., and Izaguirre, C.A. (1979). Acute myeloblastic leukemia considered as a clonal hemopathy. Blood Cells *5*, 261-282.

McHale, R.H., Stapleton, P.M., and Bergquist, P.L. (1991). Rapid preparation of blood and tissue samples for polymerase chain reaction. Biotechniques *10*, 20, 22-23.

Milech, N., Kees, U.R., and Watt, P.M. (2001). Novel alternative PBX3 isoforms in leukemia cells with distinct interaction specificities. Genes Chromosomes Cancer *32*, 275-280.

Milella, M., Kornblau, S.M., Estrov, Z., Carter, B.Z., Lapillonne, H., Harris, D., Konopleva, M., Zhao, S., Estey, E., and Andreeff, M. (2001). Therapeutic targeting of the MEK/MAPK signal transduction module in acute myeloid leukemia. J Clin Invest *108*, 851-859.

Miller, M.B., and Tang, Y.W. (2009). Basic concepts of microarrays and potential applications in clinical microbiology. Clin Microbiol Rev *22*, 611-633.

Miranda, M.B., Redner, R.L., and Johnson, D.E. (2007). Inhibition of Src family kinases enhances retinoic acid induced gene expression and myeloid differentiation. Mol Cancer Ther *6*, 3081-3090.

Moon, J.H., Sohn, S.K., Lee, M.H., Jang, J.H., Kim, K., Jung, C.W., and Kim, D.H. (2010). BCL2 gene polymorphism could predict the treatment outcomes in acute myeloid leukemia patients. Leuk Res *34*, 166-172.

Morrison, S.J., Uchida, N., and Weissman, I.L. (1995). The biology of hematopoietic stem cells. Annu Rev Cell Dev Biol *11*, 35-71.

Morrissette, J.J., and Bagg, A. (2011). Acute myeloid leukemia: conventional cytogenetics, FISH, and moleculocentric methodologies. Clin Lab Med *31*, 659-686, x.

Mrozek, K., Heerema, N.A., and Bloomfield, C.D. (2004). Cytogenetics in acute leukemia. Blood Rev *18*, 115-136.

Murphy, J., and Bustin, S.A. (2009). Reliability of real-time reverse-transcription PCR in clinical diagnostics: gold standard or substandard? Expert Rev Mol Diagn *9*, 187-197.

Nasedkina, T.V., Zharinov, V.S., Isaeva, E.A., Mityaeva, O.N., Yurasov, R.N., Surzhikov, S.A., Turigin, A.Y., Rubina, A.Y., Karachunskii, A.I., Gartenhaus, R.B.*, et al.* (2003). Clinical screening of gene rearrangements in childhood leukemia by using a multiplex polymerase chain reaction-microarray approach. Clin Cancer Res *9*, 5620-5629.

Neame, P.B., Soamboonsrup, P., Browman, G.P., Meyer, R.M., Benger, A., Wilson, W.E., Walker, I.R., Saeed, N., and McBride, J.A. (1986). Classifying acute leukemia by immunophenotyping: a combined FAB-immunologic classification of AML. Blood *68*, 1355-1362.

Nieborowska-Skorska, M., Hoser, G., Kossev, P., Wasik, M.A., and Skorski, T. (2002). Complementary functions of the antiapoptotic protein A1 and serine/threonine kinase pim-1 in the BCR/ABL-mediated leukemogenesis. Blood *99*, 4531-4539.

Nygard, A.B., Jorgensen, C.B., Cirera, S., and Fredholm, M. (2007). Selection of reference genes for gene expression studies in pig tissues using SYBR green qPCR. BMC Mol Biol *8*, 67.

Ogawa, M. (1993). Differentiation and proliferation of hematopoietic stem cells. Blood *81*, 2844-2853.

Oscier, D.G., Rose-Zerilli, M.J., Winkelmann, N., Gonzalez de Castro, D., Gomez, B., Forster, J., Parker, H., Parker, A., Gardiner, A., Collins, A.*, et al.* (2013). The clinical significance of NOTCH1 and SF3B1 mutations in the UK LRF CLL4 trial. Blood *121*, 468-475.

Ottone, T., Ammatuna, E., Lavorgna, S., Noguera, N.I., Buccisano, F., Venditti, A., Gianni, L., Postorino, M., Federici, G., Amadori, S.*, et al.* (2008). An allele-specific rt-PCR assay to detect type A mutation of the nucleophosmin-1 gene in acute myeloid leukemia. J Mol Diagn *10*, 212-216.

Park, J.Y., Cho, M.O., Leonard, S., Calder, B., Mian, I.S., Kim, W.H., Wijnhoven, S., van Steeg, H., Mitchell, J., van der Horst, G.T.*, et al.* (2008). Homeostatic imbalance between apoptosis and cell renewal in the liver of premature aging Xpd mice. PLoS One *3*, e2346.

Passegue, E., Jamieson, C.H., Ailles, L.E., and Weissman, I.L. (2003). Normal and leukemic hematopoiesis: are leukemias a stem cell disorder or a reacquisition of stem cell characteristics? Proc Natl Acad Sci U S A *100 Suppl 1*, 11842-11849.

Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res *29*, e45.

Pfaffl, M.W., Horgan, G.W., and Dempfle, L. (2002). Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res *30*, e36.

Plesa, C., Chelghoum, Y., Plesa, A., Elhamri, M., Tigaud, I., Michallet, M., Dumontet, C., and Thomas, X. (2008). Prognostic value of immunophenotyping in elderly patients with acute myeloid leukemia: a single-institution experience. Cancer *112*, 572-580.

Plotkin, L.I., and Bellido, T. (2001). Bisphosphonate-induced, hemichannel-mediated, anti-apoptosis through the Src/ERK pathway: a gap junction-independent action of connexin43. Cell Commun Adhes *8*, 377-382.

Pomp, D., and Medrano, J.F. (1991). Organic solvents as facilitators of polymerase chain reaction. Biotechniques *10*, 58-59.

Preudhomme, C., Sagot, C., Boissel, N., Cayuela, J.M., Tigaud, I., de Botton, S., Thomas, X., Raffoux, E., Lamandin, C., Castaigne, S., *et al.* (2002). Favorable prognostic significance of CEBPA mutations in patients with de novo acute myeloid leukemia: a study from the Acute Leukemia French Association (ALFA). Blood *100*, 2717-2723.

Radonic A Fau - Thulke, S., Thulke S Fau - Mackay, I.M., Mackay Im Fau - Landt, O., Landt O Fau - Siegert, W., Siegert W Fau - Nitsche, A., and Nitsche, A. Guideline to reference gene selection for quantitative real-time PCR.

Radstrom, P., Knutsson, R., Wolffs, P., Lovenklev, M., and Lofstrom, C. (2004). Pre-PCR processing: strategies to generate PCR-compatible samples. Mol Biotechnol *26*, 133-146.

Rai, A.J., Kamath, R.M., Gerald, W., and Fleisher, M. (2009). Analytical validation of the GeXP analyzer and design of a workflow for cancer-biomarker discovery using multiplexed gene-expression profiling. Anal Bioanal Chem *393*, 1505-1511.

Ravandi, F. (2011). New treatments and strategies in acute myeloid leukemia. Clin Lymphoma Myeloma Leuk *11 Suppl 1*, S60-64.

Rebrikov, D.V. (2008). Identification of differential genes by suppression subtractive hybridization: I. Preparation of subtracted cDNA or genomic DNA library. CSH Protoc *2008*, pdb prot4855.

Rho, H.W., Lee, B.C., Choi, E.S., Choi, I.J., Lee, Y.S., and Goh, S.H. (2010). Identification of valid reference genes for gene expression studies of human stomach cancer by reverse transcription-qPCR. BMC Cancer *10*, 240.

Robak, T., and Wierzbowska, A. (2009). Current and emerging therapies for acute myeloid leukemia. Clin Ther *31 Pt 2*, 2349-2370.

Rowe, J.M., Kim, H.T., Cassileth, P.A., Lazarus, H.M., Litzow, M.R., Wiernik, P.H., and Tallman, M.S. (2010). Adult patients with acute myeloid leukemia who achieve complete remission after 1 or 2 cycles of induction have a similar prognosis: a report on 1980

patients registered to 6 studies conducted by the Eastern Cooperative Oncology Group. Cancer *116*, 5012-5021.

Rowe, J.M., and Tallman, M.S. (1997). Intensifying induction therapy in acute myeloid leukemia: has a new standard of care emerged? Blood *90*, 2121-2126.

Rowe, J.M., and Tallman, M.S. (2010). How I treat acute myeloid leukemia. Blood *116*, 3147-3156.

Rubie, C., Kempf, K., Hans, J., Su, T., Tilton, B., Georg, T., Brittner, B., Ludwig, B., and Schilling, M. (2005). Housekeeping gene variability in normal and cancerous colorectal, pancreatic, esophageal, gastric and hepatic tissues. Mol Cell Probes *19*, 101-109.

Ryan, U.S., and Worthington, R.E. (1992). Cell-cell contact mechanisms. Curr Opin Immunol *4*, 33-37.

Sahu, R.K., Zelig, U., Huleihel, M., Brosh, N., Talyshinsky, M., Ben-Harosh, M., Mordechai, S., and Kapelushnik, J. (2006). Continuous monitoring of WBC (biochemistry) in an adult leukemia patient using advanced FTIR-spectroscopy. Leuk Res *30*, 687-693.

Sangle, N.A., and Perkins, S.L. (2011). Core-binding factor acute myeloid leukemia. Arch Pathol Lab Med *135*, 1504-1509.

Schlenk, R.F., Benner, A., Hartmann, F., del Valle, F., Weber, C., Pralle, H., Fischer, J.T., Gunzer, U., Pezzutto, A., Weber, W.*, et al.* (2003). Risk-adapted postremission therapy in acute myeloid leukemia: results of the German multicenter AML HD93 treatment trial. Leukemia *17*, 1521-1528.

Schlenk, R.F., Dohner, K., Krauter, J., Frohling, S., Corbacioglu, A., Bullinger, L., Habdank, M., Spath, D., Morgan, M., Benner, A.*, et al.* (2008). Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med *358*, 1909-1918.

Sekeres, M.A. (2008). Treatment of older adults with acute myeloid leukemia: state of the art and current perspectives. Haematologica *93*, 1769-1772.

Sekeres, M.A., Elson, P., Kalaycio, M.E., Advani, A.S., Copelan, E.A., Faderl, S., Kantarjian, H.M., and Estey, E. (2009). Time from diagnosis to treatment initiation predicts survival in younger, but not older, acute myeloid leukemia patients. Blood *113*, 28-36.

Shaffer, B.C., Gillet, J.P., Patel, C., Baer, M.R., Bates, S.E., and Gottesman, M.M. (2012). Drug resistance: still a daunting challenge to the successful treatment of AML. Drug Resist Updat *15*, 62-69.

Shahbazian, D., Parsyan, A., Petroulakis, E., Hershey, J., and Sonenberg, N. (2010). eIF4B controls survival and proliferation and is regulated by proto-oncogenic signaling pathways. Cell Cycle *9*, 4106-4109.

Sharma, K. (1993). Cellular deformability studies in leukemia. Physiol Chem Phys Med NMR *25*, 293-297.

Shim, J.S., Lee, J., Kim, K.N., and Kwon, H.J. (2007). Development of a new Ca2+/calmodulin antagonist and its anti-proliferative activity against colorectal cancer cells. Biochem Biophys Res Commun *359*, 747-751.

Shiraishi, T., Mori, M., Tanaka, S., Sugimachi, K., and Akiyoshi, T. (1998). Identification of cystatin B in human esophageal carcinoma, using differential displays in which the gene expression is related to lymph-node metastasis. Int J Cancer *79*, 175-178.

Sidhu, R.S., Clough, R.R., and Bhullar, R.P. (2003). Ca2+/calmodulin binds and dissociates K-RasB from membrane. Biochem Biophys Res Commun *304*, 655-660.

Sikorsky, J.A., Primerano, D.A., Fenger, T.W., and Denvir, J. (2004). Effect of DNA damage on PCR amplification efficiency with the relative threshold cycle method. Biochem Biophys Res Commun *323*, 823-830.

Simon, M., Grandage, V.L., Linch, D.C., and Khwaja, A. (2005). Constitutive activation of the Wnt/beta-catenin signalling pathway in acute myeloid leukaemia. Oncogene *24*, 2410-2420.

Slovak, M.L., Kopecky, K.J., Cassileth, P.A., Harrington, D.H., Theil, K.S., Mohamed, A., Paietta, E., Willman, C.L., Head, D.R., Rowe, J.M.*, et al.* (2000). Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. Blood *96*, 4075-4083.

Smith, M., Barnett, M., Bassan, R., Gatta, G., Tondini, C., and Kern, W. (2004). Adult acute myeloid leukaemia. Crit Rev Oncol Hematol *50*, 197-222.

Sperr, W.R., Jordan, J.H., Baghestanian, M., Kiener, H.P., Samorapoompichit, P., Semper, H., Hauswirth, A., Schernthaner, G.H., Chott, A., Natter, S.*, et al.* (2001). Expression of mast cell tryptase by myeloblasts in a group of patients with acute myeloid leukemia. Blood *98*, 2200-2209.

Spertini, O., Callegari, P., Cordey, A.S., Hauert, J., Joggi, J., von Fliedner, V., and Schapira, M. (1994). High levels of the shed form of L-selectin are present in patients with acute leukemia and inhibit blast cell adhesion to activated endothelium. Blood *84*, 1249-1256.

Stahlberg, A., Aman, P., Ridell, B., Mostad, P., and Kubista, M. (2003). Quantitative real-time PCR method for detection of B-lymphocyte monoclonality by comparison of kappa and lambda immunoglobulin light chain expression. Clin Chem *49*, 51-59.

Stasi, R., Venditti, A., Del Poeta, G., Aronica, G., Abruzzese, E., Pisani, F., Cecconi, M., Masi, M., and Amadori, S. (1996). High-dose chemotherapy in adult acute myeloid leukemia: rationale and results. Leuk Res *20*, 535-549.

Steeber, D.A., Green, N.E., Sato, S., and Tedder, T.F. (1996). Lyphocyte migration in L-selectin-deficient mice. Altered subset migration and aging of the immune system. J Immunol *157*, 1096-1106.

Stier, S., Ko, Y., Forkert, R., Lutz, C., Neuhaus, T., Grunewald, E., Cheng, T., Dombkowski, D., Calvi, L.M., Rittling, S.R.*, et al.* (2005). Osteopontin is a hematopoietic stem cell niche component that negatively regulates stem cell pool size. J Exp Med *201*, 1781-1791.

Stoiser, B., Knobl, P., Fonatsch, C., Haas, O.A., Mitterbauer, G., Weltermann, A., Geissler, K., Valent, P., Sperr, W., Pabinger, I.*, et al.* (2000). Prognosis of patients with a second relapse of acute myeloid leukemia. Leukemia *14*, 2059-2063.

Stone, R.M. (2002). The difficult problem of acute myeloid leukemia in the older adult. CA Cancer J Clin *52*, 363-371.

Strojan, P., Budihna, M., Smid, L., Svetic, B., Vrhovec, I., Kos, J., and Skrk, J. (2000). Prognostic significance of cysteine proteinases cathepsins B and L and their endogenous inhibitors stefins A and B in patients with squamous cell carcinoma of the head and neck. Clin Cancer Res *6*, 1052-1062.

Suciu, S., Mandelli, F., de Witte, T., Zittoun, R., Gallo, E., Labar, B., De Rosa, G., Belhabri, A., Giustolisi, R., Delarue, R*., et al.* (2003). Allogeneic compared with autologous stem cell transplantation in the treatment of patients younger than 46 years with acute myeloid leukemia (AML) in first complete remission (CR1): an intention-to-treat analysis of the EORTC/GIMEMAAML-10 trial. Blood *102*, 1232-1240.

Sun, Y., and Cook, J.R. (2010). Fluorescence in situ hybridization for del(5q) in myelodysplasia/acute myeloid leukemia: comparison of EGR1 vs. CSF1R probes and diagnostic yield over metaphase cytogenetics alone. Leuk Res *34*, 340-343.

Tam, W.F., and Gary Gilliland, D. (2008). Can FLT3 inhibitors overcome resistance in AML? Best Pract Res Clin Haematol *21*, 13-20.

Terry, P.D., Shore, D.L., Rauscher, G.H., and Sandler, D.P. (2005). Occupation, hobbies, and acute leukemia in adults. Leuk Res *29*, 1117-1130.

Toutenhoofd, S.L., Foletti, D., Wicki, R., Rhyner, J.A., Garcia, F., Tolon, R., and Strehler, E.E. (1998). Characterization of the human CALM2 calmodulin gene and comparison of the transcriptional activity of CALM1, CALM2 and CALM3. Cell Calcium *23*, 323-338.

Trinkaus, M., Vranic, A., Dolenc, V.V., and Lah, T.T. (2005). Cathepsins B and L and their inhibitors stefin B and cystatin C as markers for malignant progression of benign meningiomas. Int J Biol Markers *20*, 50-59.

van Beijnum, J.R., Dings, R.P., van der Linden, E., Zwaans, B.M., Ramaekers, F.C., Mayo, K.H., and Griffioen, A.W. (2006). Gene expression of tumor angiogenesis dissected: specific targeting of colon cancer angiogenic vasculature. Blood *108*, 2339-2348.

Van Limbergen, H., Poppe, B., Michaux, L., Herens, C., Brown, J., Noens, L., Berneman, Z., De Bock, R., De Paepe, A., and Speleman, F. (2002). Identification of cytogenetic subclasses and recurring chromosomal aberrations in AML and MDS with complex karyotypes using M-FISH. Genes Chromosomes Cancer *33*, 60-72.

van Rhenen, A., Feller, N., Kelder, A., Westra, A.H., Rombouts, E., Zweegman, S., van der Pol, M.A., Waisfisz, Q., Ossenkoppele, G.J., and Schuurhuis, G.J. (2005). High stem cell frequency in acute myeloid leukemia at diagnosis predicts high minimal residual disease and poor survival. Clin Cancer Res *11*, 6520-6527.

van Stijn, A., Feller, N., Kok, A., van der Pol, M.A., Ossenkoppele, G.J., and Schuurhuis, G.J. (2005). Minimal residual disease in acute myeloid leukemia is predicted by an apoptosis-resistant protein profile at diagnosis. Clin Cancer Res *11*, 2540-2546.

Vanderhoek, M., Juckett, M.B., Perlman, S.B., Nickles, R.J., and Jeraj, R. (2011). Early assessment of treatment response in patients with AML using [(18)F]FLT PET imaging. Leuk Res *35*, 310-316.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., and Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol *3*, RESEARCH0034.

Vansant, G., Pezzoli, P., Saiz, R., Birch, A., Duffy, C., Ferre, F., and Monforte, J. (2006). Gene expression analysis of troglitazone reveals its impact on multiple pathways in cell culture: a case for in vitro platforms combined with gene expression analysis for early (idiosyncratic) toxicity screening. Int J Toxicol *25*, 85-94.

Vardiman, J.W., Harris, N.L., and Brunning, R.D. (2002). The World Health Organization (WHO) classification of the myeloid neoplasms. Blood *100*, 2292-2302.

Vendittelli, F., Santonocito, C., Paradisi, A., Romitelli, F., Concolino, P., Silveri, S.L., Sisto, T., Capizzi, R., Catricala, C., Mule, A.*, et al.* (2009). A new standardized absolute quantitative RT-PCR method for detection of tyrosinase mRNAs in melanoma patients: technical and operative instructions. Clin Chim Acta *409*, 100-105.

Vermeulen, K., Van Bockstaele, D.R., and Berneman, Z.N. (2003). The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer. Cell Prolif *36*, 131-149.

Vito, P., Lacana, E., and D'Adamio, L. (1996). Interfering with apoptosis: Ca(2+)-binding protein ALG-2 and Alzheimer's disease gene ALG-3. Science *271*, 521-525.

Wang, Z., Bhattacharya, N., Weaver, M., Petersen, K., Meyer, M., Gapter, L., and Magnuson, N.S. (2001). Pim-1: a serine/threonine kinase with a role in cell survival, proliferation, differentiation and tumorigenesis. J Vet Sci *2*, 167-179.

Weiler, S.R., Gooya, J.M., Ortiz, M., Tsai, S., Collins, S.J., and Keller, J.R. (1999). D3: a gene induced during myeloid cell differentiation of Linlo c-Kit+ Sca-1(+) progenitor cells. Blood *93*, 527-536.

Wen, W.H., Bernstein, L., Lescallett, J., Beazer-Barclay, Y., Sullivan-Halley, J., White, M., and Press, M.F. (2000). Comparison of TP53 mutations identified by oligonucleotide microarray and conventional DNA sequence analysis. Cancer Res *60*, 2716-2722.

Werle, B., Lotterle, H., Schanzenbacher, U., Lah, T.T., Kalman, E., Kayser, K., Bulzebruck, H., Schirren, J., Krasovec, M., Kos, J.*, et al.* (1999). Immunochemical analysis of cathepsin B in lung tumours: an independent prognostic factor for squamous cell carcinoma patients. Br J Cancer *81*, 510-519.

Whitman, S.P., Archer, K.J., Feng, L., Baldus, C., Becknell, B., Carlson, B.D., Carroll, A.J., Mrozek, K., Vardiman, J.W., George, S.L.*, et al.* (2001). Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: a cancer and leukemia group B study. Cancer Res *61*, 7233-7239.

Willemze, R., Suciu, S., Mandelli, F., de Witte, T., Amadori, S., Hayat, M., Petti, M.C., Solbu, G., and Zittoun, R. (1997). New trends in the treatment of adult acute myeloid leukaemia. European Journal of Cancer *33, Supplement 4*, S7-S14.

Willman, C.L. (2008). Has gene expression profiling improved diagnosis, classification, and outcome prediction in AML? Best Pract Res Clin Haematol *21*, 21-28.

Wilson, I.G. (1997). Inhibition and facilitation of nucleic acid amplification. Appl Environ Microbiol *63*, 3741-3751.

Xia, Q., Wang, H.X., Wang, J., Zhang, J.Y., Liu, B.Y., Li, A.L., Lv, M., Hu, M.R., Yu, M., Feng, J.N.*, et al.* (2005). Proteomic analysis of interleukin 6-induced differentiation in mouse myeloid leukemia cells. Int J Biochem Cell Biol *37*, 1197-1207.

Xiang-Hong, J., Yan-Hong, Y., Han-Jin, X., Li-Long, A., Ying-Mei, X., Pei-Rong, J., and Ming, L. (2011). Selection of reference genes for gene expression studies in PBMC from Bama miniature pig under heat stress. Vet Immunol Immunopathol *144*, 160-166.

Xu, Q., Simpson, S.E., Scialla, T.J., Bagg, A., and Carroll, M. (2003). Survival of acute myeloid leukemia cells requires PI3 kinase activation. Blood *102*, 972-980.

Yamamoto, J.F., and Goodman, M.T. (2008). Patterns of leukemia incidence in the United States by subtype and demographic characteristics, 1997-2002. Cancer Causes Control *19*, 379-390.

Yang, M.J., Luo, L., Nie, K., Wang, M., Zhang, C., Li, J., and Ma, X.J. (2012). Genotyping of 11 human papillomaviruses by multiplex PCR with a GeXP analyzer. J Med Virol *84*, 957-963.

Yong Wu, M.R.G., Kathryn E. Sciabica, Jane Luo. Quantitative Multiplexed Gene Expression: An Analysis of Sensitivity, Accuracy and Precision (Beckman Coulter, Inc.).

Zajc, I., Sever, N., Bervar, A., and Lah, T.T. (2002). Expression of cysteine peptidase cathepsin L and its inhibitors stefins A and B in relation to tumorigenicity of breast cancer cell lines. Cancer Lett *187*, 185-190.

Zenz, T., Krober, A., Scherer, K., Habe, S., Buhler, A., Benner, A., Denzel, T., Winkler, D., Edelmann, J., Schwanen, C.*, et al.* (2008). Monoallelic TP53 inactivation is associated with poor prognosis in chronic lymphocytic leukemia: results from a detailed genetic characterization with long-term follow-up. Blood *112*, 3322-3329.

Zhang, Q.Y., Garner, K., and Viswanatha, D.S. (2002). Rapid detection of leukemia-associated translocation fusion genes using a novel combined RT-PCR and flow cytometric method. Leukemia *16*, 144-149.

Zhang, Z., and Gerstein, M. (2003). Identification and characterization of over 100 mitochondrial ribosomal protein pseudogenes in the human genome. Genomics *81*, 468-480.

Zhang, Z., Kermekchiev, M.B., and Barnes, W.M. (2010). Direct DNA amplification from crude clinical samples using a PCR enhancer cocktail and novel mutants of Taq. J Mol Diagn *12*, 152-161.

Zhao, S., Konopleva, M., Cabreira-Hansen, M., Xie, Z., Hu, W., Milella, M., Estrov, Z., Mills, G.B., and Andreeff, M. (2004). Inhibition of phosphatidylinositol 3-kinase dephosphorylates BAD and promotes apoptosis in myeloid leukemias. Leukemia *18*, 267-275.

Zheng, G.H., Fu, J.R., Xu, Y.H., Jin, X.Q., Liu, W.L., and Zhou, J.F. (2009). Screening and cloning of multi-drug resistant genes in HL-60/MDR cells. Leuk Res *33*, 1120-1123.

Zheng, J., Wang, X., Hu, Y., Yang, J., Liu, J., He, Y., Gong, Q., Yao, J., Li, X., Du, W.*, et al.* (2008). A correlation study of immunophenotypic, cytogenetic, and clinical features of 180 AML patients in China. Cytometry B Clin Cytom *74*, 25-29.

Zhu X Fau - Yeadon, J.E., Yeadon Je Fau - Burden, S.J., and Burden, S.J. AML1 is expressed in skeletal muscle and is regulated by innervation.

Zolota, V., Sirinian, C., Melachrinou, M., Symeonidis, A., and Bonikos, D.S. (2007). Expression of the regulatory cell cycle proteins p21, p27, p14, p16, p53, mdm2, and

cyclin E in bone marrow biopsies with acute myeloid leukemia. Correlation with patients' survival. Pathol Res Pract *203*, 199-207.

Zuo, Z., Chandra, P., Wen, Y.H., and Koeppen, H. (2009). Molecular diagnostics of acute myeloid leukaemia. Diagnostic Histopathology *15*, 531-539.