

S-1-1

Gene Expression Profiling To Model Prognosis of Myeloma Treated with Tandem Auto Transplants

John Shaughnessy, Fenghuang Zhan, Erik Rasmussen, and Bart Barlogie

Donna D. and Donald M. Lambert Laboratory of Myeloma Genetics, Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, Little Rock

Multiple Myeloma: The Disease, Its Therapy and Prognosis

Myeloma is a plasma cell dyscrasia that causes a constellation of disease manifestations including osteolytic lesions due to osteoblast inactivation and osteoclast-activation, anemia and immunosuppression due to loss of normal hematopoietic stem cell function, and end organ damage due to monoclonal immunoglobulin secretion.¹ The presence of somatic hypermutations of the immunoglobulin variable region genes in myeloma plasma cells suggests that malignant transformation occurs in a B cell that has traversed the germinal centers of lymph nodes. However, the hypoproliferative nature of myeloma has led to hypothesis that the bulk of the tumor arises from a transformed B-cell with the capacity for both self-renewal and production of terminally differentiated progeny.²⁻⁴

The clinical course of patients requiring therapy for myeloma varies markedly. Even with tandem autotransplants yielding CR rates in excess of 60%, survival ranges from a few months to greater than 15 years. The extended time of almost 2 years required for all eventual candidate patients to achieve clinical CR and the even longer time to achieve MRI-CR strongly suggests enormous tumor cell population heterogeneity in terms of drug responsiveness/resistance.

Traditional prognostic factors such as β 2microglobulin (β 2M), albumin, and C-reactive protein (CRP) account for only 15–20% of outcome heterogeneity. Abnormal metaphase karyotypes, present in one third of newly diagnosed patients and reflecting stroma independence, have been consistently

associated with a rapidly fatal outcome so that fewer than 10% of patients with these abnormalities survive >5 years.

Recent advances in molecular cytogenetics have identified primary translocations involving the immunoglobulin heavy chain locus at 14q32 in 40% of patients.⁵ According to a consensus report of a recent Paris workshop on myeloma genetics, hyperdiploid and t(11;14)(q13,q32)-positive myeloma are associated with a good prognosis whereas non-hyperdiploidy, often associated with translocations other than t(11;14) and chromosome 13 deletion, imparts a strikingly dismal prognosis.⁶

Gene Expression Profiling to Model the Effectiveness of Tandem Autotransplantation for The Treatment of Myeloma

Gene expression profiling (GEP) has emerged as powerful means of developing risk-adapted prognostic models for leukemias and lymphomas.⁷⁻¹³ The highly variable outcome in patients with multiple myeloma, with very little of this variability being accounted for by current laboratory tests, prompted us to investigate if GEP could better model event-free and overall survival in this disease as well. We initiated GEP studies on CD138-enriched (> 90% CD38+/CD45-) plasma cells from bone marrow aspirates taken from newly diagnosed myeloma patients entering the Total Therapy 2 protocol nearly 5 years ago. In addition to tandem transplants with melphalan (200 mg/m²), Total Therapy 2 also randomized patients upfront to thalidomide or no thalidomide, provided 1 year of intensive consolidation therapy,

and an additional year of dexamethasone maintenance. Since beginning these studies in early in 2000 we have performed GEP on plasma cells from 350 consecutive patients entering the trial using Affymetrix® high-density oligonucleotide microarrays.

Unsupervised Hierarchical Clustering of Global Gene Expression Patterns of Pretreatment Myeloma Plasma Cells Defines Biological and Clinical Subgroups

Unsupervised hierarchical cluster analysis based on a set of 4,580 highly variable genes from ~10,000 tested using the U95Av2 microarray, segregated 221 myelomas into four discrete subgroups. These groups were characterized by significant differences in genetic features such as chromosome ploidy ($P < 0.01$), trisomy 11 ($P < 0.001$) and 14q32 *IGH* translocations ($P < 0.01$), as well as and clinical parameters such as IgA isotype, albumin (< 3.5 g/dL), β 2M (> 4 mg/L), creatinine (> 2 mg/d), MRI lesions ($P < 0.05$) and event-free ($P = 0.002$) and overall survival ($P = 0.06$). In the current unsupervised hierarchical cluster analysis we noted a nonrandom distribution of spikes of common translocation partners within the cluster-defined subgroups. The data revealed that 26 of 30 *CCND1* spikes were clustered in one subgroup whereas 28 of 32 *MMSET/FGFR3*, 4 of 5 *MAF* and 3 of 4 *MAFB* spikes were all located in the same cluster branch. *CCND1* expression, either low level expression and associated with hyperdiploid karyotypes or spiked expression linked to the t(11;14)(p13;q32) translocation and normal karyotypes, defined two distinct subgroups with relatively low risk for relapse. Elevated expression of proliferation-associated genes or *MMSET/MAF/MAFB* defined two poor prognostic subgroups. The *MMSET/MAF/MAFB* groups was more frequently associated with hypodiploid karyotypes, an IgA isotype, increased incidence of 14q32 translocations by FISH, and reduced incidence of MRI lesions whereas the *CCND1* spike group was significantly associated with normal cytogenetics. We are currently validating these findings using the U133Plus2.0 data on 350 cases and will be submitted as a full-length manuscript next

year. Taken together, our results highlight the relationship between the transcriptome, cytogenetics, and the biological and clinical features of myeloma. These findings extend and refine our previous gene expression classification system and provide further evidence that distinct molecular entities of myeloma exist.

Cox Regression Modeling of Gene Expression Patterns in Pretreatment Plasma Cells Identifies Three Genes Associated with Rapid Relapse

Because microarrays are not likely to become routine clinical tests, comprehensive global GEP studies will probably be used to identify a small subset of genes whose expression can be applied in the development of robust risk-adapted patient stratification, as has been recently done in lymphoma.¹¹ We used Cox regression modeling of gene expression on EFS in our cohort of patients. We used data from U95Av2 microarray (~10,000) genes on 212 newly diagnosed myeloma patients. The median follow-up time at the time of this analysis was 20 months. There were 34 events representing either disease-specific death or progression/relapse. EFS was modeled using standard prognostic values as well as GEP using the Affymetrix signal. A generalized estimate of R^2 was obtained using the approach suggested by Cox and Snell.¹⁴ Gene expression values considered were based on significance of univariate association with EFS. The median signal call was used as a cut point prior to modeling inclusion. The 100 genes most significantly associated with EFS based on the score test were potential variables in multivariate modeling. Using standard prognostic variables only, the model that best fit disease-specific EFS was FISH13 (chromosome 13 deletion detected by fluorescence *in situ* hybridization [FISH]) and the presence of chromosomal abnormalities (CA).

Adjusting for CA and FISH13, three genes: *RAN*, *ZHX-2*, and *CHC1L*, were simultaneously significant, each at the 0.005 level. After adjustment for other model variables, patients with high *RAN* expression had increased risk of event, while patients with high *ZHX-2* or high *CHC1L* had decreased

risk of event. After adjusting for these three genes and each other, FISH13 patients had an increased risk of event but those with CA did not. R^2 for the FISH13 and CA model was 30%. The R^2 value for the *RAN*, *ZHX-2*, and *CHC1L* model was 66%. It is important to note that each of these genes has independent prognostic influence so that the combined overexpression of *RAN*, and loss of expression of *ZHX-2* and *CHC1L* represents a much more powerful model than any other combination.

We next compared the EFS between a high-risk entity (patients whose plasma cells express *RAN* above median and *ZHX-2* and *CHC1L* below the median; n=45) and a low risk entity (all remaining cases; n = 165). In this population, there were 30 events in the 45 high-risk patients (66%) and 30 events in the 165 low risk patients (18%) ($P < .0001$). We next calculated the EFS based on the combination of GEP risk groups and CA. EFS rates were not significantly different between high-risk and low-risk disease with and without CA. However, high-risk and low-risk disease groups were significantly different irrespective of the presence of CA ($P < .0001$).

1. Barlogie B, Shaughnessy J, Munshi N, Epstein J. Plasma Cell Myeloma. In: Beutler E, Lichtman M, Coller B, Kipps T, Seligsohn U, eds. Williams Hematology (ed 6). New York: McGraw-Hill; 2001:1279-1304
2. Billadeau D, Ahmann G, Greipp P, Van Ness B. The bone marrow of multiple myeloma patients contains B cell populations at different stages of differentiation that are clonally related to the malignant plasma cell. *J Exp Med.* 1993;178:1023-1031
3. Corradini P, Boccadoro M, Voena C, Pileri A. Evidence for a bone marrow B cell transcribing malignant plasma cell VDJ joined to C mu sequence in immunoglobulin (IgG)- and IgA-secreting multiple myelomas. *J Exp Med.* 1993;178:1091-1096
4. Szczepek AJ, Seeberger K, Wizniak J, Mant MJ, Belch AR, Pilarski LM. A high frequency of circulating B cells share clonotypic Ig heavy-chain VDJ rearrangements with autologous bone marrow plasma cells in multiple myeloma, as measured by single-cell and in situ reverse transcriptase-polymerase chain reaction. *Blood.* 1998;92:2844-2855
5. Kuehl WM, Bergsagel PL. Multiple myeloma: evolving genetic events and host interactions. *Nat Rev Cancer.* 2002;2:175-187
6. Fonseca R, Barlogie B, Bataille R, Bastard C, Bergsagel PL, Chesi M, Davies FE, Drach J, Greipp PR, Kirsch IR, Kuehl WM, Hernandez JM, Minvielle S, Pilarski LM, Shaughnessy JD, Jr., Stewart AK, Avet-Loiseau H. Genetics and cytogenetics of multiple myeloma: a workshop report. *Cancer Res.* 2004;64:1546-1558
7. Yeoh EJ, Ross ME, Shurtleff SA, Williams WK, Patel D, Mahfouz R, Behm FG, Raimondi SC, Relling MV, Patel A, Cheng C, Campana D, Wilkins D, Zhou X, Li J, Liu H, Pui CH, Evans WE, Naeve C, Wong L, Downing JR. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. *Cancer Cell.* 2002;1:133-143
8. Ross ME, Zhou X, Song G, Shurtleff SA, Girtman K, Williams WK, Liu HC, Mahfouz R, Raimondi SC, Lenny N, Patel A, Downing JR. Classification of pediatric acute lymphoblastic leukemia by gene expression profiling. *Blood.* 2003;102:2951-2959
9. Bullinger L, Dohner K, Bair E, Frohling S, Schlenk RF, Tibshirani R, Dohner H, Pollack JR. Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. *N Engl J Med.* 2004;350:1605-1616
10. Valk PJ, Verhaak RG, Beijen MA, Erpelinck CA, Barjesteh van Waalwijk van Doorn-Khosrovani S, Boer JM, Beverloo HB, Moorhouse MJ, van der Spek PJ, Lowenberg B, Delwel R. Prognostically useful gene-expression profiles in acute myeloid leukemia. *N Engl J Med.* 2004;350:1617-1628
11. Lossos IS, Czerwinski DK, Alizadeh AA,

- Wechsler MA, Tibshirani R, Botstein D, Levy R. Prediction of survival in diffuse large-B-cell lymphoma based on the expression of six genes. *N Engl J Med.* 2004;350:1828-1837
12. Shipp MA, Ross KN, Tamayo P, Weng AP, Kutok JL, Aguiar RC, Gaasenbeek M, Angelo M, Reich M, Pinkus GS, Ray TS, Koval MA, Last KW, Norton A, Lister TA, Mesirov J, Neuberger DS, Lander ES, Aster JC, Golub TR. Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. *Nat Med.* 2002;8:68-74
 13. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, Gascoyne RD, Muller-Hermelink HK, Smeland EB, Giltnane JM, Hurt EM, Zhao H, Averett L, Yang L, Wilson WH, Jaffe ES, Simon R, Klausner RD, Powell J, Duffey PL, Longo DL, Greiner TC, Weisenburger DD, Sanger WG, Dave BJ, Lynch JC, Vose J, Armitage JO, Montserrat E, Lopez-Guillermo A, Grogan TM, Miller TP, LeBlanc M, Ott G, Kvaloy S, Delabie J, Holte H, Krajci P, Stokke T, Staudt LM. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med.* 2002;346:1937-1947
 14. Cox DR, Snell EJ. *The analysis of binary data* (ed Second). London: Chapman & Hall; 1989