ACKNOWLEDGEMENTS

We thank P Jouany, S Katsahian and V Millul for study management, C Pautas and Y Hicheri for clinical care and E Atti and T Berthy for technical assistance (all in Assistance Publique-Hôpitaux de Paris, France). The study was sponsored and monitored by the Regional Clinical Research Office, Paris and supported by a grant from the Programme Hospitalier de Recherche Clinique (PHRC ID, P 010506) and recurrent funding from the Centers for Clinical Investigation in Biotherapy from the Henri Mondor and Pitié-Salpêtrière hospitals. The sponsors had no role in study design, data analysis, data interpretation or writing of the report.

S Maury^{1,2,3}, M Rosenzweig^{4,5,6,7,8}, R Redjoul¹, A Marçais⁹,
A Xhaard¹⁰, M Cherai^{4,5}, L Cabanne¹, G Churlaud⁴, F Suarez⁹,
G Socié¹⁰, L Gregoire¹¹, K Debbache¹, C Bernard^{4,5,6},
J-L Beaumont¹², N Azar⁴, O Boyer¹³, F Roudot-Thoraval^{2,11},
JL Cohen^{2,3}, C Cordonnier^{1,2}, FM Lemoine^{4,5,13} and
D Klatzmann^{4,5,6,7,8}

¹AP-HP, Hôpital Henri Mondor, Department of Hematology,
DHU Virus-Immunity-Cancer, Créteil, France;

²IMRB, University Paris Est Créteil (UPEC), INSERM U955 team 21,
Créteil, France;

³Center for Clinical Investigation in Biotherapy, Créteil, France;

⁴AP-HP, Hôpital Pitié-Salpêtrière, Biotherapy Department,
Paris, France;

⁵AP-HP, Hôpital Pitié-Salpêtrière, Clinical Investigation Center in
Biotherapy (CIC-BTi), Paris, France;

⁶AP-HP, Hôpital Pitié-Salpêtrière, Inflammation-Immunopathology-
Biotherapy Department (i2B), Paris, France;

⁷Sorbonne Université, UPMC Univ Paris 06, UMR S 959,

Immunology-Immunopathology-Immunotherapy (I3), Paris, France;

⁸INSERM, UMR S 959, Immunology-Immunopathology-
Immunotherapy (I3), Paris, France;

⁹Department of Hematology, Hôpital Necker, Paris, France;

¹⁰Department of Hematology-Transplantation,
Hôpital St Louis, Paris, France;

¹¹Department of Public Health, Créteil, France;

¹²Department of Hemapheresis, Etablissement Français du Sang,
Créteil, France and

¹³Sorbonne Universités, UPMC Univ Paris 06, UMR-S INSERM 1135,
CIMI-Paris, Paris, France

E-mail: sebastien.maury@hmn.aphp.fr or david.klatzmann@upmc.fr

REFERENCES

- Schmid C, Labopin M, Nagler A, Bornhauser M, Finke J, Fassas A *et al*. Donor lymphocyte infusion in the treatment of first hematological relapse after allogeneic stem-cell transplantation in adults with acute myeloid leukemia: a retrospective risk factors analysis and comparison with other strategies by the EBMT Acute Leukemia Working Party. *J Clin Oncol* 2007; **25**: 4938–4945.
- Porter DL, Alyea EP, Antin JH, Delima M, Estey E, Falkenburg JH *et al*. NCI First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation: Report from the Committee on Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation. *Biol Blood Marrow Transplant* 2010; **16**: 1467–1503.
- Miller JS, Weisdorf DJ, Burns LJ, Slungaard A, Wagner JE, Verneris MR *et al*. Lymphodepletion followed by donor lymphocyte infusion (DLI) causes significantly more acute graft-versus-host disease than DLI alone. *Blood* 2007; **110**: 2761–2763.
- Lemoine FM, Mesel-Lemoine M, Cherai M, Gallot G, Vie H, Leclercq V *et al*. Efficient transduction and selection of human T-lymphocytes with bicistronic Thy1/HSV1-TK retroviral vector produced by a human packaging cell line. *J Gene Med* 2004; **6**: 374–386.
- Blair A, Hogge DE, Ailles LE, Lansdorp PM, Sutherland HJ. Lack of expression of Thy-1 (CD90) on acute myeloid leukemia cells with long-term proliferative ability in vitro and in vivo. *Blood* 1997; **89**: 3104–3112.
- Tiberghien P, Ferrand C, Lioure B, Milpied N, Angonin R, Deconinck E *et al*. Administration of herpes simplex-thymidine kinase-expressing donor T cells with a T-cell-depleted allogeneic marrow graft. *Blood* 2001; **97**: 63–72.
- Burt RK, Drobyski WR, Seregina T, Traynor A, Oyama Y, Keever-Taylor C *et al*. Herpes simplex thymidine kinase gene-transduced donor lymphocyte infusions. *Exp Hematol* 2003; **31**: 903–910.
- Ciceri F, Bonini C, Stanghellini MT, Bondanza A, Traversari C, Salomoni M *et al*. Infusion of suicide-gene-engineered donor lymphocytes after family haploidentical haemopoietic stem-cell transplantation for leukaemia (the TK007 trial): a non-randomised phase I-II study. *Lancet Oncol* 2009; **10**: 489–500.
- Borchers S, Provasi E, Silvani A, Radizzani M, Benati C, Dammann E *et al*. Genetically modified donor leukocyte transfusion and graft-versus-leukemia effect after allogeneic stem cell transplantation. *Hum Gene Ther* 2011; **22**: 829–841.
- Cieri N, Mastaglio S, Oliveira G, Casucci M, Bondanza A, Bonini C. Adoptive immunotherapy with genetically modified lymphocytes in allogeneic stem cell transplantation. *Immunol Rev* 2014; **257**: 165–180.
- Hoyos V, Savoldo B, Quintarelli C, Mahendravada A, Zhang M, Vera J *et al*. Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to enhance their anti-lymphoma/leukemia effects and safety. *Leukemia* 2010; **24**: 1160–1170.
- Maury S, Lemoine FM, Hicheri Y, Rosenzweig M, Badoual C, Cherai M *et al*. CD4 +CD25+ regulatory T cell depletion improves the graft-versus-tumor effect of donor lymphocytes after allogeneic hematopoietic stem cell transplantation. *Sci Transl Med* 2010; **2**: 41ra52.
- Shimoni A, Kroger N, Zander AR, Rowe JM, Hardan I, Avigdor A *et al*. Imatinib mesylate (STI571) in preparation for allogeneic hematopoietic stem cell transplantation and donor lymphocyte infusions in patients with Philadelphia-positive acute leukemias. *Leukemia* 2003; **17**: 290–297.
- Kochenderfer JN, Dudley ME, Carpenter RO, Kassim SH, Rose JJ, Telford WG *et al*. Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. *Blood* 2013; **122**: 4129–4139.
- Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR *et al*. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med* 2013; **368**: 1509–1518.

Supplementary Information accompanies this paper on the Leukemia website (<http://www.nature.com/leu>)

OPEN

Five gene probes carry most of the discriminatory power of the 70-gene risk model in multiple myeloma

Leukemia (2014) **28**, 2410–2413; doi:10.1038/leu.2014.232

The prognostic value of gene expression profiling (GEP) in multiple myeloma (MM) has been reported by several groups.^{1–4} We have previously published a 70-gene classifier (GEP70) that identifies patients with high risk for short progression-free

survival (PFS) and overall survival (OS).¹ The GEP70 model was developed from data on patients enrolled in Total Therapy 2 (TT2).¹ Its discriminatory power has been validated in several published data sets in the transplant, non-transplant and relapse settings (reviewed in Johnson *et al*.⁵). We applied the GEP70 model to 56 previously treated patients with available baseline GEP information who were enrolled in Total Therapy 6 (TT6), a

Accepted article preview online 31 July 2014; advance online publication, 5 September 2014

tandem transplant trial the details of which are provided in Supplementary Methods. The gene expression profiles have been deposited at the NCBI GEO data repository (<http://www.ncbi.nlm.nih.gov/geo/>) under GEO accession number GSE57317. Sample procurement and processing for GEP, as well as calculations of the GEP70 risk score, have been reported previously.¹ The estimated 1-year survival was 62% for the high-risk group and 97% for the low-risk group by GEP70 (Supplementary Figure S1A, $P < 0.0001$). To investigate whether this striking difference in outcomes was driven by a few genes, all 70 probe sets of the GEP70 risk model were ranked by their P -values, based on univariate Cox regression analysis for OS in TT6 (Supplementary Table S1). The five probe sets with the smallest P -values (*ENO1*, *FABP5*, *TRIP13*, *TAGLN2* and *RFC4*) were combined to create a continuous score, using methodology similar to that used to develop the GEP70 model.¹ Because each of the five probe sets had a positive association with short OS in TT6, the GEP5 score was simply the mean of log2 transformed expression levels of the five probe sets. An optimal cutoff for the new risk score (hereafter referred to as GEP5) was then established with the running log-rank test, so that patients with scores higher than the cutoff were deemed to have high-risk MM and others to have low-risk (Figure 1a), with an estimated OS at 1 year of 60% and 95%, respectively (1-year PFS 50% and 91%, respectively).

All five genes identified in this study were previously reported to be involved in cell proliferation and have been associated with development and survival in different cancers. *ENO1* encodes alpha-enolase. Initiation of translation at an alternative translation start site results in a shorter isoform that produces MYC binding protein 1, which acts as a transcriptional repressor and possibly as a tumor suppressor.⁶ Overexpression of *FABP5*, a member of the family of fatty acid-binding proteins, was associated with poor survival in triple-negative breast cancer and with resistance to all-*trans* retinoic acid in a preclinical model of pancreatic ductal adenocarcinoma.^{7,8} *TRIP13* encodes a hormone-dependent transcription factor that interacts with the ligand-binding domain of thyroid hormone receptors and may play a role in early-stage non-small-cell lung cancer.⁹ Association of *TAGLN2* overexpression and short survival, metastasis and disease progression has been shown for several cancers.^{10,11} *RFC4* encodes the 37-kDa subunit of the replication factor C protein complex, which, together with the proliferating cell nuclear antigen, is required for DNA elongation.¹²

Because the number of patients treated on TT6 was relatively small and follow-up short (median follow-up 26.5 months), a larger data set of 275 uniformly treated patients on TT3a with a longer follow-up was then used to investigate the new GEP5 score's applicability to previously untreated myeloma. We validated the new GEP5 cutoff for patients enrolled in TT3b ($n=166$).¹³ Gene expression data for TT3a and TT3b have previously been published and are deposited in the ArrayExpress archive (<http://www.ebi.ac.uk/arrayexpress>) under the accession number E-TABM-1138. A new optimal cutoff for the GEP5 model of 10.68 was identified from TT3a using the running log-rank statistics, which identified significant differences in OS and PFS for the groups with high- and low-risk disease. Importantly, these differences are comparable to those obtained by the GEP70 risk model with its established cutoff¹ (Figure 1b and Supplementary Figure S1B). In the validation cohort (TT3b), risk distinction using GEP5 was very similar to GEP70 (Figure 1c and Supplementary Figure S1C) and both were comparable to results in the TT3a training set. We also applied GEP5 to a publicly available external data set of previously untreated patients (HOVON65/GMMG-HD4, $n=288$)⁴ as a second validation set, where GEP5 also differentiated between a high-risk and

a low-risk population with significantly different survival (Figure 1d).

In order to address the question whether the five probe sets in the GEP5 were truly the best choice, we randomly selected 10 000 quintuplets from all the probe sets within the 70 gene model to create 10 000 continuous scores using the same methodology as for the GEP5 score. Among the 10 000 random scores tested, only 40 performed better in TT6. Of these 40 only 1 performed better in the TT3 test set and none was superior to GEP5 in the TT3b validation set (Supplementary Figure S2 and Supplementary Table S2). We also examined randomly selected continuous scores in TT6 with probe sets ranging between 1 and 10. Of a total of 42 485 models considered, only 1236 had a smaller P -value than GEP5 in TT6. Among those 1236 scores, 68 had a smaller P -value when tested in the TT3a test set and none performed better than GEP5 in the TT3b validation set (Supplementary Figure S3 and Supplementary Table S3). Although some of these random scores showed a better correlation with survival in single data sets, none were consistently better than the GEP5 score across different data sets. The GEP5 always ranked among the top 2% of all scores in all data sets analyzed (data not shown).

On multivariate stepwise analysis, the GEP5-defined high-risk designation was selected as the most adverse variable linked to inferior PFS, with an estimated hazard ratio of 3.44 (95% CI: 2.02–5.86), whereas the GEP70 model was selected for OS (Supplementary Table S4). Table 1 summarizes the univariate survival analysis of the GEP5 and GEP70 models. Cross-tabulation of GEP70 and GEP5 risk (low vs high) for TT3A, and TT3B showed an agreement rate between the two models of 0.89, and 0.87, respectively (Supplementary Table S5).

GEP70 and GEP5 currently require the use of microarray technology that interrogates the expression levels of more than 47 000 transcripts and variants simultaneously. To assess whether a more targeted approach, only measuring the expression of a small number of genes, could reliably predict risk in MM, we analyzed 48 RNA samples of previously untreated patients on TT3a and TT3b with available GEP data using the nanoString nCounter, with a code set consisting of all five genes (*ENO1*, *FABP5*, *TAGLN3*, *TRIP13* and *RFC4*) of the GEP5 signature and the housekeeping genes *RPL27*, *RPL30*, *RPS13*, *RPS29* and *SRP14* (code set sequences are provided in Supplementary Table S6). Technical and biological normalization were performed using the nSolver software provided by nanoString. The correlation between microarray and nanoString-based gene expression for all five genes was between $r=0.64$ and $r=0.87$. Using the normalized nanoString data, we computed a nanoString-based GEP5 score (nsGEP5) applying the same methodology as for the microarray-based GEP5. nsGEP5 and GEP5 correlated very well with $r=0.852$ (Supplementary Figure S4A). The receiver operator curve revealed an area under the curve of 0.897, suggesting that GEP5 high/low risk can be predicted using nsGEP5 (Supplementary Figure S4B).

In summary, high-risk myeloma remains one of the greatest therapeutic challenges. The striking difference in survival of previously treated patients among GEP70 low- and high-risk groups motivated our search for fewer responsible genes. We indeed identified a set of five genes that are highly predictive of survival in multiple independent data sets. The nsGEP5 based on targeted evaluation of the expression levels of these five genes using the nanoString technology showed a very good correlation with GEP5 (based on microarray data). This new technology could reduce cost and sample requirements and has the great potential of making gene expression-driven risk assessment available to a broader patient population. However, the nsGEP5 will have to be evaluated in an independent homogeneous set of clinical samples before it can be utilized in the routine clinical setting. Recently a large-scale proteomics experiment involving 85 patients with MM identified *ENO1*,

Figure 1. GEP5 distinguishes a high- and a low-risk group with significantly different OS and PFS in the TT6 discovery set, the TT3A training set, and the TT3B and HOVON65/GMMG-HD4 validation sets. Left panels show overall survival, right panels progression-free survival. (a) TT6 discovery set; (b) TT3A training set; (c) TT3B validation set; (d) HOVON65/GMMG-HD4 validation set ($P < 0.0001$, all panels).

Table 1. Summary of the GEP70 and GEP5 models by *P* values from univariate analysis when GEP5 and GEP70 were considered as both binary and continuous variables

Protocol	Outcome variable	Gene predictor	<i>P</i> in continuous Cox analysis	<i>P</i> in binary log-rank analysis
TT3a	OS	GEP5	1.76E–10	3.87E–05
		GEP70	1.65E–11	2.98E–10
	PFS	GEP5	1.14E–05	0.001044
		GEP70	7.75E–10	2.75E–08
TT3b	OS	GEP5	4.17E–10	5.67E–06
		GEP70	2.11E–08	1.09E–06
	PFS	GEP5	1.72E–10	3.35E–07
		GEP70	3.50E–08	1.85E–05

FABP5 and *TAGLN2* among a set of 24 proteins that are associated with short OS.¹⁴ This set of 85 patients included 47 who were enrolled in TT3b. The correlation of expression at both mRNA (via our GEP analyses) and protein levels supports the biological relevance of the genes included in the GEP5 model. Work is in progress to identify agents that can effectively target these prognostic genes.

CONFLICT OF INTEREST

BB has received research funding from Celgene and Millennium, is a consultant to Celgene and Millennium, and is a co-inventor on patents and patent applications related to use of GEP in cancer medicine that have been licensed to Myeloma Health, LLC. SZU is a consultant to Celgene, Millennium and Onyx. He has received research funding from Onyx and Celgene, and speaking honoraria from Celgene. The remaining authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Peggy Brenner, a science editor supported by the University of Arkansas for Medical Sciences, provided editorial assistance to the authors during preparation of this manuscript. This manuscript was supported by a grant from the National Institutes of Health (P01CA055819).

AUTHOR CONTRIBUTIONS

BB, JE, JC and CJH conceived and planned the project; PQ, QZ, AH and JC conducted statistical analyses; patients were accrued by BB, FvR, SW and SZU; all authors wrote and approved the manuscript.

CJ Heuck¹, P Qu², F van Rhee¹, S Waheed¹, SZ Usmani¹, J Epstein¹, Q Zhang¹, R Edmondson¹, A Hoering², J Crowley² and B Barlogie¹
¹Myeloma Institute for Research and Therapy, Little Rock, AR, USA and
²Cancer Research and Biostatistics, Seattle, WA, USA
 E-mail: cjheuck@uams.edu

REFERENCES

- Shaughnessy JD Jr, Zhan F, Burington BE, Huang Y, Colla S, Hanamura I *et al.* A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. *Blood* 2007; **109**: 2276–2284.
- Decaux O, Lode L, Magrangeas F, Charbonnel C, Gouraud W, Jezequel P *et al.* Prediction of survival in multiple myeloma based on gene expression profiles reveals cell cycle and chromosomal instability signatures in high-risk patients and hyperdiploid signatures in low-risk patients: a study of the Intergroupe Franco-phone du Myelome. *J Clin Oncol* 2008; **26**: 4798–4805.
- Wu P, Walker BA, Brewer D, Gregory WM, Ashcroft J, Ross FM *et al.* A gene expression-based predictor for myeloma patients at high risk of developing bone disease on bisphosphonate treatment. *Clin Cancer Res* 2011; **17**: 6347–6355.
- Kuiper R, Broyl A, de Knecht Y, van Vliet MH, van Beers EH, van der Holt B *et al.* A gene expression signature for high-risk multiple myeloma. *Leukemia* 2012; **26**: 2406–2413.
- Johnson SK, Heuck CJ, Albino AP, Qu P, Zhang Q, Barlogie B *et al.* The use of molecular-based risk stratification and pharmacogenomics for outcome prediction and personalized therapeutic management of multiple myeloma. *Int J Hematol* 2011; **94**: 321–333.
- Subramanian A, Miller DM. Structural analysis of alpha-enolase. Mapping the functional domains involved in down-regulation of the c-myc protooncogene. *J Biol Chem* 2000; **275**: 5958–5965.
- Liu RZ, Graham K, Glubrecht DD, Germain DR, Mackey JR, Godbout R. Association of *FABP5* expression with poor survival in triple-negative breast cancer: implication for retinoic acid therapy. *Am J Pathol* 2011; **178**: 997–1008.
- Gupta S, Pramanik D, Mukherjee R, Campbell NR, Elumalai S, de Wilde RF *et al.* Molecular determinants of retinoic acid sensitivity in pancreatic cancer. *Clin Cancer Res* 2012; **18**: 280–289.
- Kang JU, Koo SH, Kwon KC, Park JW, Kim JM. Gain at chromosomal region 5p15.33, containing TERT, is the most frequent genetic event in early stages of non-small cell lung cancer. *Cancer Genet Cytogenet* 2008; **182**: 1–11.
- Zhang Y, Ye Y, Shen D, Jiang K, Zhang H, Sun W *et al.* Identification of transgelin-2 as a biomarker of colorectal cancer by laser capture microdissection and quantitative proteome analysis. *Cancer Sci* 2010; **101**: 523–529.
- Nohata N, Sone Y, Hanazawa T, Fuse M, Kikkawa N, Yoshino H *et al.* miR-1 as a tumor suppressive microRNA targeting *TAGLN2* in head and neck squamous cell carcinoma. *Oncotarget* 2011; **2**: 29–42.
- Loor G, Zhang SJ, Zhang P, Toomey NL, Lee MY. Identification of DNA replication and cell cycle proteins that interact with PCNA. *Nucleic Acids Res* 1997; **25**: 5041–5046.
- Nair B, van Rhee F, Shaughnessy JD Jr, Anaissie E, Szymonifka J, Hoering A *et al.* Superior results of Total Therapy 3 (2003-33) in gene expression profiling-defined low-risk multiple myeloma confirmed in subsequent trial 2006-66 with VRD maintenance. *Blood* 2010; **115**: 4168–4173.
- Edmondson R, Chavan S, Heuck C, Epstein J, Barlogie B. Combining proteomics and gene expression profiling identifies proteins/genes associated with short overall survival in multiple myeloma. *Blood (ASH Annu Meet Abstr)* 2012; **120**: Abstract 197.

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-sa/4.0/>

Supplementary Information accompanies this paper on the Leukemia website (<http://www.nature.com/leu>)

Phase II study of pomalidomide in high-risk relapsed and refractory multiple myeloma

Leukemia (2014) **28**, 2413–2415; doi:10.1038/leu.2014.248

Pomalidomide (Pom) is an IMiD immunomodulatory agent that is now FDA approved for treatment of patients who have

received ≥ 2 prior therapies, including lenalidomide (Len) and bortezomib (Bor), and have demonstrated disease progression on or within 60 days of completion of the last line of therapy.¹ There are limited data evaluating efficacy of Pom in high-risk RRMM with prior exposure/refractoriness to Len and how best to

Accepted article preview online 25 August 2014; advance online publication, 16 September 2014