TO THE EDITOR:

Variable BCL2/BCL2L1 ratio in multiple myeloma with t(11;14)

Alice Cleynen,[1] Mehmet Samur,[2,3] Aurore Perrot,[4] Laure Buisson,[5,6] Sabrina Maheo,[5,6] Mariateresa Fulciniti,[2] Michel Attal,[5,6] Nikhil Munshi,[2,7] Hervé Avet-Loiseau,[5,6] and Jill Corre[5,6]

[1]Institut Montpellierain Alexander Grothendieck, CNRS, University of Montpellier, Montpellier, France; [2]Medical Oncology and [3]Department of Biostatistics and Computational Biology, Dana-Farber Cancer, Institute Harvard Medical School, Boston, MA; [4]Hematology Department, University Hospital, Nancy, France; [5]Unit for Genomics in Myeloma, Institut Universitaire du Cancer-Oncopole, Toulouse, France; [6]Centre de Reserches en Cancérologie de Toulouse, INSERM U1037, Toulouse, France; and [7]VA Boston Healthcare System, West Roxbury, MA

Multiple myeloma (MM) is characterized by a large diversity of genetic abnormalities.[1-3] They can be classified in 3 categories: copy number changes, mutations, and translocations involving mainly the *IGH* gene at 14q32. Translocations are usually balanced translocations with various partner genes: *CCND1* at 11q13 (15%-20% of the patients), *MMSET/FGFR3* at 4p16 (12%-15%), *MYC* at 8q24 (15%), *MAF* at 16q23 (3%), *MAFB* at 20q11 (1%), and *CCND3* at 6p21 (1%). Several papers characterized the t(4;14) and t(14;16), but few analyzed the most frequent 1, t(11;14),[4,5] mainly because, in contrast to the other 2, which are associated with a poor prognosis,[6] the t(11;14) was not associated with a specific outcome until recently. However, recent reports suggest that outcome of patients displaying t(11;14) is inferior to other standard risk patients.[7,8] The development of novel strategies and drugs in the past decades dramatically improved the survival of MM patients.[9-12] The next step would be to define the right treatment of the right patient. In line with this goal, translocation t(11;14) is becoming more interesting for physicians with the demonstration of a specific good response (40% of overall response rate) with venetoclax, a BCL-2 inhibitor.[13,14] Even if direct comparison cannot be done because recent clinical trials did not report specific t(11;14) response rate, these results obtained in heavily pretreated relapsed/refractory with an oral single agent are better than expected.

We thus decided to characterize the transcriptomic profile of a large number of patients with t(11;14). All patients provided signed consent for these genetic analyses in accordance with the Declaration of Helsinki. This study was approved by Toulouse Ethic Committee. RNA sequencing experiments (with tumor purity >90%) were conducted on 157 patients at diagnosis within the Randomized Study Comparing Conventional Dose Treatment Using a Combination of Lenalidomide, Bortezomib and Dexamethasone to High-Dose Treatment With ASCT in the Initial Management of Myeloma in Patients up to 65 Years of Age 2009 trial for which fluorescence in situ hybridization was performed, allowing the identification of 43 (27%) patients with a t(11;14). All patients received pretransplant bortezomib-lenalidomide-dexamethasone induction. In accordance to previous reports[4,5] supporting a standard risk profile of t(11;14), a 5-year median follow-up of our patients could not associate the presence of this translocation with a specific outcome (Figure 1). By contrast, a recent study[8] reported a subgroup t(11;14) displaying a significantly shorter survival than a non-14q32 translocation group. The very nature of this comparison, added to the demographical and treatment features (only 60% were transplant eligible, and most patients received 1 novel agent-based induction [proteasome inhibitor or Immunomodulatory imide drugs]), may explain this different interpretation. Of note, in our study, the incidence of 17p deletion, 1q gain, and 1p32 deletion were comparable between t(11;14) and non-t(11;14) subgroups. We then identified a specific transcriptomic signature with 2345 differentially

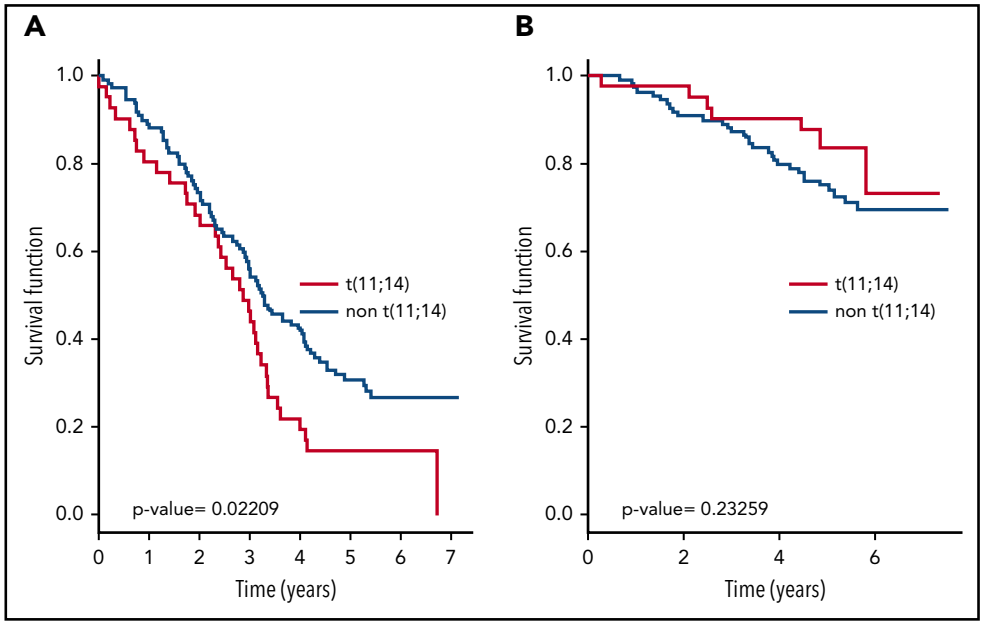

Figure 1. Survival of t(11;14) myeloma patients. Analysis was done with 40 t(11;14) patients and 110 non-t(11;14) patients. The median follow-up was 5 years. (A) Event-free survival. (B) Overall survival.

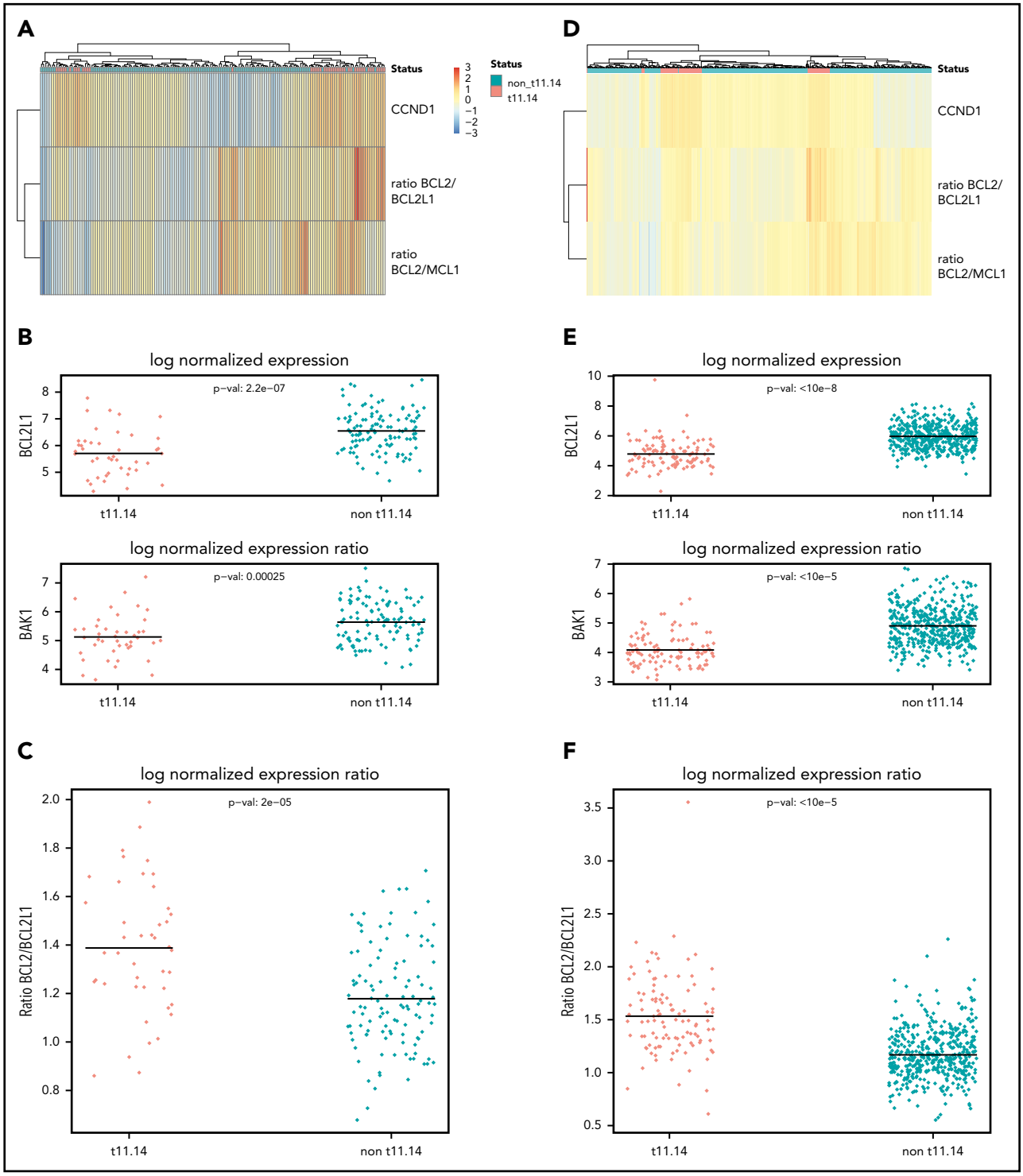

Figure 2. Transcriptomic comparison of t(11:14) and other MM patients. (A-C) The Randomized Study Comparing Conventional Dose Treatment Using a Combination of Lenalidomide, Bortezomib and Dexamethasone to High-Dose Treatment With ASCT in the Initial Management of Myeloma in Patients up to 65 Years of Age 2009 cohort and (D-F) the independent Multiple Myeloma Research Foundation cohort. (A,D) Heat map of the normalized expression of CCND1 and *BCL2/BCL2L1* and BCL2/MCL1 expression ratio in the cohorts. The t(11;14) patients (indicated in pink on the first line) cluster in 2 distinct groups based on their *BCL2/BCL2L1* ratio. (B,E) Normalized expression of differentially expressed genes from the apoptosis family between t(11;14) and non-t(11;14) patients. (C,F) Normalized expression ratio of *BCL2/BCL2L1* between t(11;14) and non-t(11;14) patients.

expressed genes in patients displaying t(11;14) compared with the rest of the patients. As expected, the most discriminant gene was *CCND1* (Figure 2A), which was highly expressed in patients with t(11;14). However, 11 genes from the apoptosis family were differentially expressed. These included 2 members of the *BCL2* family, *BCL2L1* and *BAK1*, that were underexpressed by t(11;14) patients (Figure 2B), 7 members of the tumor necrosis factor (TNF) receptor superfamily (*TNFRSF18*, *TNFRSF4*, *FAS*, *TNFRSF10B*, *TNFRSF10D*, and *TNFRSF13C* were overexpressed, and *TNFRSF17* was underexpressed) and 2 members of the BH3-only family,

namely *PMAIP1* [*NOXA*] which was overexpressed, and *BIK* which was underexpressed in the patients with t(11;14). Interestingly, no member of the caspase family or other apoptotic genes was differentially expressed.

Based on the recent encouraging clinical activity of the BCL-2 inhibitor venetoclax in t(11;14) MM, with a good correlation with high *BCL2*/*BCL2L1* and/or *BCL2*/*MCL1* messenger RNA ratio, we analyzed these ratios in this population. Although the *BCL2*/*MCL1* did not discriminate the 2 populations, overall the *BCL2*/*BCL2L1* ratio was generally significantly higher in t(11;14) patients (Student 2-sided $P = 2 \times 10^{-5}$; Figure 2A,C). The *BCL2*/*BCL2L1* ratio separates the t(11;14) patients in 2 groups, with only two-thirds of the patients (30/43) displaying a high ratio despite all patients having high *CCND1* expression. This separation does not correlate with the CD-1/CD-2 molecular classification of the *CCND1*/t(11;14) myeloma patients,[15,16] and *BCL2L1* was the only gene whose expression differentiates both groups. Interestingly, high *BCL2*/*BCL2L1* ratio was frequently identified in a subgroup of non-t(11;14) patients as well (Figure 2A), with a transcriptomic signature of >3000 genes characterizing those high-ratio patients. Although *BCL2L1* and *BCL2* were the most differentially expressed genes, it is likely that this signature is a result of a large heterogeneity of patients in the groups. These results were confirmed by the independent study of the Multiple Myeloma Research Foundation RNA-sequencing cohort (performed on 564 patients, including 104 patients with t(11;14); , the *BCL2*/*BCL2L1* ratio in this cohort being significantly higher [$P = 2 \times 10^{-16}$]; Figure 2D-F). *BCL2L1* encodes the anti-apoptotic Bcl-X_L protein, whose expression has been correlated to resistance to venetoclax in a preclinical MM model.[17]

So, which marker best predicts response to venetoclax? Because our patients were not treated with venetoclax, we cannot answer this important question. Our data may explain the better responses in t(11;14) patients, but not in all of them. In contrast, some patients lacking the t(11;14) also display high ratios and low *BCL2L1* or *BAK* expression. These patients might be good candidates for venetoclax treatment. We believe that these issues should be addressed specifically in the ongoing and upcoming venetoclax trials by comparing transcriptome from plasma cells obtained in responder vs nonresponder patients. If 1 predictive marker is actually identified, a more accessible method will have to be developed for clinical use, such as flow cytometry or reverse transcription polymerase chain reaction, making the opportunity to practice targeted-based precision medicine in multiple myeloma.

In summary, we confirm that t(11;14) is not associated with a specific outcome, but this may change if clinical efficacy of venetoclax is confirmed in this subgroup. A high ratio *BCL2*/*BCL2L1* or low expression of *BCL2L1* or *BAK* might select patients with a high probability of response. Further studies are needed to establish a potential link with response to venetoclax.

Acknowledgments

The authors thank the Intergroupe Francophone du Myélome for providing patient samples and clinical data.

This work was supported by National Institutes of Health, National Cancer Institute grants PO1-155258 and P50-100707 (H.A.L.) and the Cancer Pharmacology of Toulouse and Region (CAPTOR) program. The Centre de Recherches en Cancérologie de Toulouse Team 13 is supported by la Fondation ARC (Association pour la Recherche sur la Cancer, grant PGA1*20160203788).

Authorship

Contribution: A.C., N.M., H.A.-L., and J.C. designed research, analyzed data, and wrote the manuscript, which was reviewed and edited by the other coauthors; L.B. and S.M. performed laboratory work; A.C. and M.S. analyzed the data; and A.P., M.F., and M.A. provided samples and clinical data.

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

ORCID profiles: A.P., 0000-0003-0131-8689; J.C., 0000-0003-1580-6106.

Correspondence: Jill Corre, Unit for Genomics in Myeloma, Institut Universitaire du Cancer-Oncopole, 1 Avenue Irène Joliot-Curie, 31100 Toulouse, France; e-mail: corre.jill@iuct-oncopole.fr.

REFERENCES

1. Corre J, Munshi N, Avet-Loiseau H. Genetics of multiple myeloma: another heterogeneity level? *Blood*. 2015;125(12):1870-1876.
2. Robiou du Pont S, Cleynen A, Fontan C, et al. Genomics of multiple myeloma. *J Clin Oncol*. 2017;35(9):963-967.
3. Manier S, Salem KZ, Park J, Landau DA, Getz G, Ghobrial IM. Genomic complexity of multiple myeloma and its clinical implications. *Nat Rev Clin Oncol*. 2017;14(2):100-113.
4. Fonseca R, Blood EA, Oken MM, et al. Myeloma and the t(11;14)(q13;q32); evidence for a biologically defined unique subset of patients. *Blood*. 2002; 99(10):3735-3741.
5. An G, Xu Y, Shi L, et al. t(11;14) multiple myeloma: a subtype associated with distinct immunological features, immunophenotypic characteristics but divergent outcome. *Leuk Res*. 2013;37(10):1251-1257.
6. Rajkumar SV. Multiple myeloma: 2016 update on diagnosis, risk-stratification, and management. *Am J Hematol*. 2016;91(7):719-734.
7. Kaufman GP, Gertz MA, Dispenzieri A, et al. Impact of cytogenetic classification on outcomes following early high-dose therapy in multiple myeloma. *Leukemia*. 2016;30(3):633-639.
8. Lakshman A, Alhaj Moustafa M, Rajkumar SV, et al. Natural history of t(11;14) multiple myeloma. *Leukemia*. 2018;32(1):131-138.
9. Richardson PG, Weller E, Lonial S, et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. *Blood*. 2010;116(5):679-686.
10. Moreau P, Masszi T, Grzasko N, et al; TOURMALINE-MM1 Study Group. Oral ixazomib, lenalidomide, and dexamethasone for multiple myeloma. *N Engl J Med*. 2016;374(17):1621-1634.
11. Dimopoulos MA, Oriol A, Nahi H, et al; POLLUX Investigators. Daratumumab, lenalidomide, and dexamethasone for multiple myeloma. *N Engl J Med*. 2016;375(14):1319-1331.
12. Stewart AK, Rajkumar SV, Dimopoulos MA, et al; ASPIRE Investigators. Carfilzomib, lenalidomide, and dexamethasone for relapsed multiple myeloma. *N Engl J Med*. 2015;372(2):142-152.
13. Kumar S, Kaufman JL, Gasparetto C, et al. Efficacy of venetoclax as targeted therapy for relapsed/refractory t(11;14) multiple myeloma. *Blood*. 2017;130(22):2401-2409.
14. Touzeau C, Maciag P, Amiot M, Moreau P. Targeting Bcl-2 for the treatment of multiple myeloma. *Leukemia*. 2018;32(9):1899-1907.
15. Zhan F, Huang Y, Colla S, et al. The molecular classification of multiple myeloma. *Blood*. 2006;108(6):2020-2028.
16. Weinhold N, Heuck CJ, Rosenthal A, et al. Clinical value of molecular subtyping multiple myeloma using gene expression profiling. *Leukemia*. 2016;30(2):423-430.
17. Punnoose EA, Leverson JD, Peale F, et al. Expression profile of BCL-2, BCL-XL, and MCL-1 predicts pharmacological response to the BCL-2 selective antagonist venetoclax in multiple myeloma models. *Mol Cancer Ther*. 2016;15(5):1132-1144.

DOI 10.1182/blood-2018-09-876433