

TO THE EDITOR:

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

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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 study⁸ 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

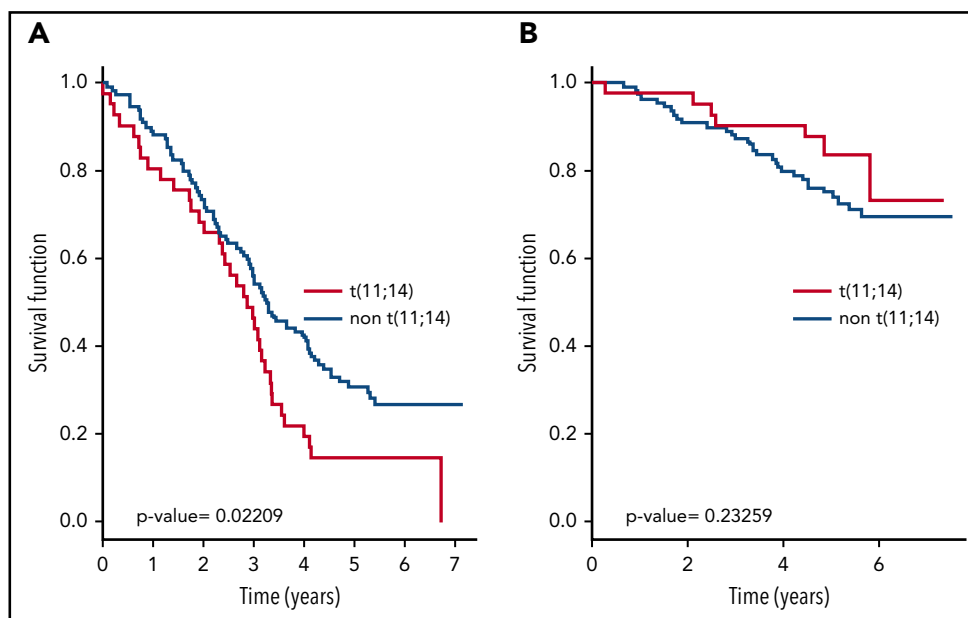


Figure 1. Survival of t(11;14) myeloma patients. Analysis was done with 40 t(11;14) patients and 110 non-t(11;14) patients. The median follow-up was 5 years. (A) Event-free survival. (B) Overall survival.

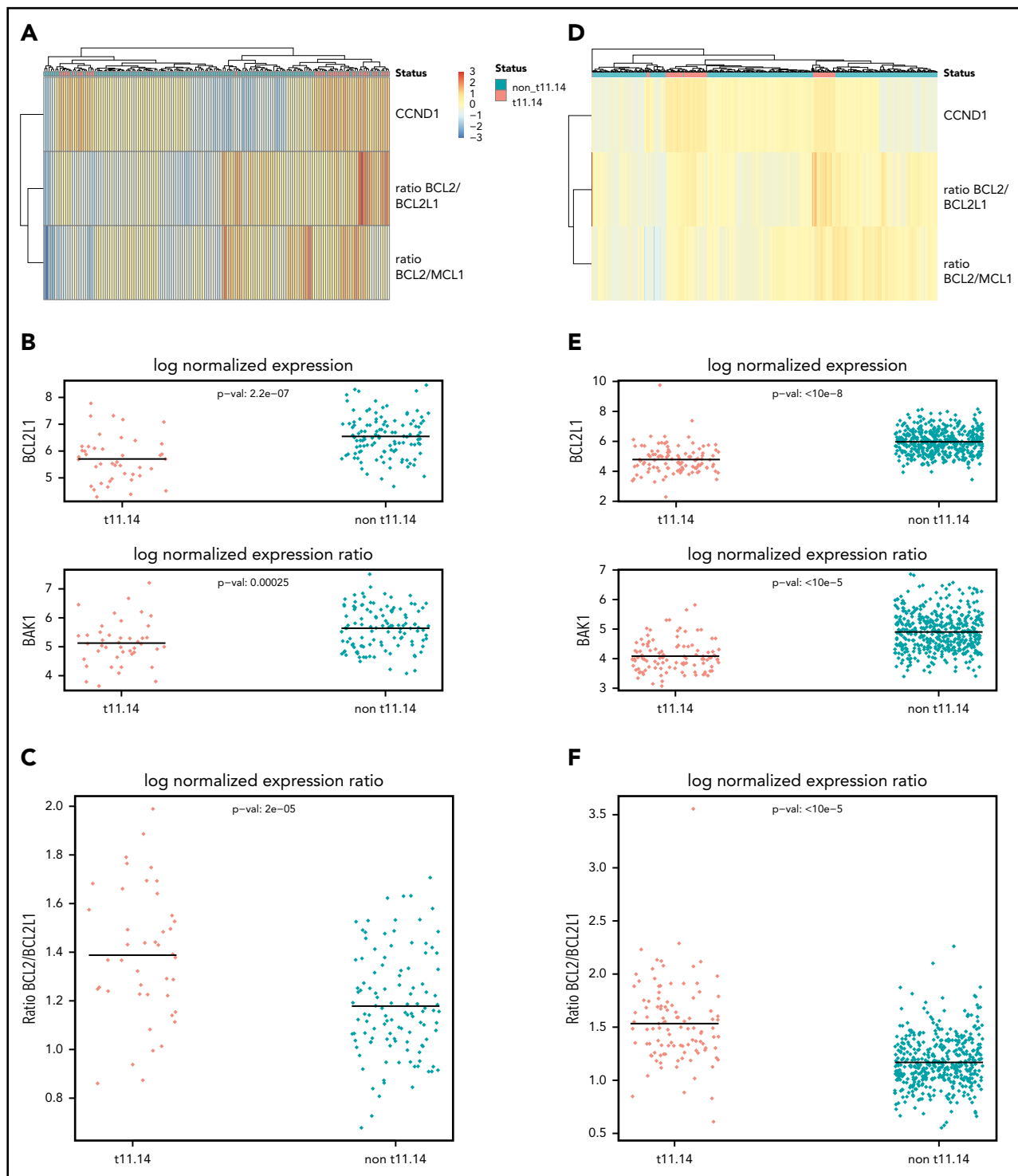


Figure 2. Transcriptomic comparison of t(11;14) and other MM patients. (A-C) The Randomized Study Comparing Conventional Dose Treatment Using a Combination of Lenalidomide, Bortezomib and Dexamethasone to High-Dose Treatment With ASCT in the Initial Management of Myeloma in Patients up to 65 Years of 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 non-responder 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.

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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.

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