
Publishers page: https://doi.org/10.1182/blood.V130.Suppl_1.1771.1771

Please note:
Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher’s version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See http://orca.cf.ac.uk/policies.html for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.
mutations in minor cell populations during the course of CLL evolution.

CONFLICT OF INTEREST
Dr Steven Devereux: Member of Advisory Board of Janssen, Gilead and Merck Sharp and Dohme and recipient of honoraria and travel expenses from Janssen, Gilead and GlaxoSmithKline. Dr Nicholas Chiorazzi Consultant fee from Janssen Pharmaceuticals, and honorarium from Nodality, Inc. The remaining authors declare no conflict of interest.

ACKNOWLEDGEMENTS
This work was supported in part by philanthropic contributions from The Karches Foundation, Marks Foundation, Nash Family Foundation, Jerome Levy Foundation, Leon Levy Foundation, Frank and Mildred Feinberg Foundation, the Mona and Edward Albert Foundation, and the Jean Walton Fund for Leukemia, Lymphoma, and Myeloma Research.

S Marsilio1, H Khiabanian2, G Fabbri3, S Vergani1, C Scuopo3, E Montserrat4, EJ Shpali5, M Hadigol2, P Marin1, KR Rai1, R Rabadán6, S Devereux1, L Pasqualucci3 and N Chiorazzi1
1Karches Center for Oncology Research, The Feinstein Institute for Medical Research, Northwell Health, Manhasset, NY, USA; 2Center for Systems and Computational Biology, Rutgers Cancer Institute of New Jersey, Rutgers University, New Brunswick, NJ, USA; 3Institute for Cancer Genetics, Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY, USA; 4Institute of Hematology and Oncology, Department of Hematology, Hospital Clinic, University of Barcelona, Barcelona, Spain; 5Department of Stem Cell Transplantation and Cell Therapy, University of Texas MD Anderson Cancer Center, Houston, TX, USA; 6Department of Systems Biology, Columbia University College of Physicians and Surgeons, New York, NY, USA and 7Kings College Hospital, NHS Foundation Trust, London, UK
E-mail: NChizzi@Northwell.edu

REFERENCES

Letters to the Editor

Biological and prognostic impact of APOBEC-induced mutations in the spectrum of plasma cell dyscrasias and multiple myeloma cell lines


Tumors are characterized by variable numbers of somatic variants that have accumulated during the life history of the cancer cell as a result of abnormal DNA replication and/or DNA repair processes. The classification of such variants into six types based on the nucleotide change was used in the past to differentiate the crude mutation pattern of different cancers.1 Recently, the S- and 3’-context of each substitution was included in such analyses, expanding the combinations to 96 possible mutation types. This trinucleotide mutational model represents the combined effect of several mutational signatures, and has enough resolution to allow deconvolution of the underlying mutational processes through the non-negative matrix factorization (NNMF) algorithm.2 To date, more than 30 distinct signatures have been identified, opening the field to the investigation of the biological processes responsible for shaping the genome of cancer, and allowing a deeper understanding of their relative contribution in different cancer types.2,3

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


OPEN

Biological and prognostic impact of APOBEC-induced mutations in the spectrum of plasma cell dyscrasias and multiple myeloma cell lines


Tumors are characterized by variable numbers of somatic variants that have accumulated during the life history of the cancer cell as a result of abnormal DNA replication and/or DNA repair processes. The classification of such variants into six types based on the nucleotide change was used in the past to differentiate the crude mutation pattern of different cancers.1 Recently, the S- and 3’-context of each substitution was included in such analyses, expanding the combinations to 96 possible mutation types. This trinucleotide mutational model represents the combined effect of several mutational signatures, and has enough resolution to allow deconvolution of the underlying mutational processes through the non-negative matrix factorization (NNMF) algorithm.2 To date, more than 30 distinct signatures have been identified, opening the field to the investigation of the biological processes responsible for shaping the genome of cancer, and allowing a deeper understanding of their relative contribution in different cancer types.2,3

Accepted article preview online 6 December 2017; advance online publication, 9 January 2018


Leukemia (2018) 1034 – 1051
In multiple myeloma (MM), two independent whole-exome sequencing (WES) studies have revealed four mutational signatures. Two are associated with aberrant activity of APOBEC cytidine deaminases (signatures #2 and #13). The other two reflect processes generating mutations at a steady rate, resulting in a mutation load that is often proportional to the cancer age at the time of sampling: these processes are highlighted by signature #1, arising from spontaneous deamination of methylated cytosines, and by signature #5, a less-understood process that exhibits transcriptional strand bias.\textsuperscript{4-6} Mutational signatures have not been investigated in other primary plasma cell dyscrasias such as monoclonal gammopathy of unknown significance (MGUS) or primary plasma cell leukemia (pPCL). Furthermore, human myeloma cell lines (HMCLs) bear a genomic profile that is only partially recapitulating their primary counterparts,\textsuperscript{3} and mutational signatures have never been studied in that context. Finally, while APOBEC activity has been correlated to increased mutational burden and poor-prognosis \textit{MAF}/\textit{MAFB} translocations in MM at diagnosis\textsuperscript{5}, this has never been confirmed in multivariate analysis in an independent large series.

To answer these questions, we mined two large public MM WES data sets\textsuperscript{4,7} that included six MGUS/Smoldering MM and 255 MM, to which we added 896 MM samples from the IA9 public release of the CoMMpass trial. The CoMMpass data were generated as part of the Multiple Myeloma Research Foundation Personalized Medicine Initiatives (https://research.themff.org and www.themff.org). Furthermore, we included matched WES data from five previously published pPCL patients.\textsuperscript{8} Finally, we used WES mutational catalogs from 18 HMCLs available from the COSMIC cell-line project (v81, http://cancer.sanger.ac.uk/cell_lines; Supplementary Materials and Methods). Interestingly, in cluster B we found an enrichment of \textit{MAF}/\textit{MAFB} translocations and \textit{APOBEC} activity. While some cell lines in this cluster (MC-CAR, IM-9 and ARH-77) are annotated as MM but were found to be compatible with Epstein–Barr virus-transformed lymphoblastoid cells instead (Supplementary Table 1),\textsuperscript{9,10} others are of clear MM or PCL origin, thus underscoring the genomic diversity of HMCLs. Overall, the APOBEC contribution was characterized by a progressive increment from MGUS/MM to MM and pPCL and ‘cluster A’ HMCLs (Figures 1e and f).

We next investigated the prognostic impact of APOBEC signatures at diagnosis using prospective data from the CoMMpass study (median follow-up 435 days (30–1421)). Patients with an absolute APOBEC contribution in the fourth quartile had shorter 2-year progression-free survival (PFS; 47% vs 66%, \textit{P} < 0.0001) and 2-year overall survival (OS; 70% vs 85%, \textit{P} = 0.0033) than patients in the first–third quartiles (Figures 2a and b). As APOBEC contribution correlates with higher mutational burden and \textit{MAF}/\textit{MAFB} translocations, two known poor prognostic factors in MM, we performed a multivariate analysis with Cox regression to assess the independent prognostic value of APOBEC activity against these and other prognostic factors such as the International Staging System (ISS)\textsuperscript{10} and type of treatment (Figure 2c and d, Supplementary Figure 7 and Table 3). In this model, variables such as \textit{IGH} translocations and overall mutational load did not show any independent prognostic significance. Conversely, ISS stage III, as expected, had the highest hazard ratio (HR) and significance as independent prognostic factor for both PFS and OS. Remarkably, fourth quartile APOBEC had an independent adverse prognostic effect of significant magnitude (PFS HR 2.02, \textit{P} = 0.02, OS HR 2.78, \textit{P} = 0.02; Figures 2c and d and Supplementary Table 3). Despite \textit{MAF}/\textit{MAFB}/\textit{MAFA} translocations being associated with high APOBEC activity,\textsuperscript{5} such cases accounted for just 23% of patients included in the fourth APOBEC quartile. The remainder of APOBEC-high patients did not carry \textit{MAF}/\textit{MAFB}/\textit{MAFA} translocations nor overexpression of these genes (Supplementary Figure 8 and Supplementary Table 4). Conversely, they were characterized by a higher APOBEC (particularly APOBEC3B) gene expression compared to other quartiles (Supplementary Figure 9 and Supplementary Table 5).\textsuperscript{7} We went on to combine fourth quartile APOBEC activity with ISS stage III in a two-variable prognostic score, and we found that co-occurrence of these two factors identifies a fraction of high-risk patients with 2-year OS of 53.8% (95% confidence interval (CI) 36.6–79%), while their simultaneous absence identifies long-term survivors with 2-year OS of 93.3% (95% CI 89.6–97.2%; Supplementary Figures 10a and b). This was partially explained by a higher proportion of primary refractory cases among patients carrying both risk factors (Supplementary Figures 10c and d).

In this study, we provided a global overview on the contribution of mutational processes in the largest WES series of plasma cell dyscrasias, from MGUS to MM to pPCL, investigated to date by NNNM. Contrary to what anticipated, we did not identify additional signatures compared to smaller data sets.\textsuperscript{4,5,7} Our data nevertheless suggest that the relative contribution of APOBEC activity may increase during progression through the different phases of MM evolution. Further studies will be necessary to confirm these findings. In primary samples, APOBEC activity showed a continuum of increased contribution that correlated with the overall
Figure 1. APOBEC contribution in plasma cell dyscrasias. (a, b) Barplot of absolute (a) and relative (b) contribution of mutational signatures on three different MM WES series. (c, d) Extraction of mutational signature from 18 HMCLs: (c) unsupervised hierarchical clustering, showing two main clusters A and B characterized by different APOBEC contribution. (d) Barplot representing the absolute APOBEC contribution to the mutational load when NNMF was applied considering clusters A and B as independent series. Asterisks (*) highlight cell lines with ‘canonical’ t(14;16) translocations (IGH/MAF). The template ($) and hash (#) signs mark cell lines carrying alternative MAF/MAFB rearrangements among clusters A and B, respectively. (e, f) Boxplot showing the progressive increase of the APOBEC absolute (e) and relative (f) mutation load from MGUS to Cluster A HMCLs.
mutational burden. In HMCLs instead, we found a clear-cut distinction between a cluster that had a much higher APOBEC contribution as compared to primary samples, and a second cluster where APOBEC activity was minimal or absent. Furthermore, in HMCLs the correlation with mutational burden was apparently lost. This observation is independent from the high number of likely residual germline variants observed in cell lines, as such variants are enriched for age-related signatures, while APOBEC mutations are typically of somatic nature. Furthermore, both in primary MM and HMCLs, the presence of MAF/MAFB/MAFA translocations explained some but not all cases with high APOBEC activity, suggesting other factors may modulate this aberrant

Figure 2. Prognostic role of APOBEC mutations. (a, b) Kaplan–Meier estimated curves of PFS (a) and OS (b) according to APOBEC mutational activity in all patients from the CoMMpass study. (c, d) Forest plot summarizing the results of multivariate analysis for PFS (c) and OS (d).
Supplementary Information accompanies this paper on the Leukemia website (http://www.nature.com/leu)