NOVEL NON-CODING CANDIDATE DRIVERS OF MULTIPLE MYELOMA LINKED TO PLASMA CELL TUMOR BIOLOGY AND B CELL DEVELOPMENT

JB Alberge[1,2,3], AK Dutta[1,2,3], A Poletti[1,4,5], O Priebe[3], THH Coorens[3], ED Lightbody[1,2,3], R Toenges[1,2,3], X Loinaz[3], S Wallin[1], AJ Dunford[3], CJ Boehner[1], EM Horowitz[1], NK Su[1], H Barr[1], L Hevenor[1], K Towle[1], R Beesam[1], JD Beckwith[1], J Perry[1], DM Cordas dos Santos[1,2,3], L Bertamini[1,2,3], K Kübler[3,6], C Terragna[4], E Zamagni[4,5], J Hess[3], R Sklavenitis-Pistofidis[1,2,3], C Stewart[3], G Getz[2,3,7,8], and IM Ghobrial[1,2,3]

1. Dana-Farber Cancer Institute, Boston, MA; 2. Harvard Medical School, Boston, MA; 3. Broad Institute of MIT and Harvard, Cambridge, MA; 4. IRCCS Azienda Ospedaliero-Universitaria di Bologna, Istituto di Ematologia "Seràgnoli", Bologna, Italy; 5. Department of Medical and Surgical Sciences - University of Bologna, Bologna, Italy; 6. Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Germany; 7. Department of Pathology, Massachusetts General Hospital, Boston, MA; 8. Krantz Family Center for Cancer Research and Dept. of Pathology, Massachusetts General Hospital, Boston, MA

INTRODUCTION

Few mutations in non-coding elements, such as the *TERT* and *FOXA1* promoter regions, have been shown to drive solid cancers[1-4].

In multiple myeloma (MM), the significance and function of non-coding mutation hotspots remain unknown.

AIMS

- To conduct discovery analysis for non-coding driver elements in multiple myeloma and its precursor conditions
- To link candidate drivers to the biological processes of precancerous and cancerous stages of MM
- To characterize significant mutations in the promoter of *ILF2* and in super-enhancer regions of B cells.

METHOD

Whole-genome sequencing (WGS) data from 969 individuals with MM (n=812) and its precursor conditions (monoclonal gammopathy of undetermined significance [MGUS], n=39, smoldering multiple myeloma [SMM], n=120) was analyzed homogeneously with the Cancer Genome Analysis pipeline from the Cancer Program of the Broad Institute of MIT and Harvard.

Non-coding hotspots were discovered with the DIG method[5].

Candidate drivers were annotated for associated putative gene targets and mutational processes, and their presence was assessed in WGS data from publicly available normal naïve and memory B cells[6].

RESULTS

- Among 969 individuals, we identified **66 non-coding** elements with significantly recurrent mutations across MGUS/SMM and MM (q<0.05) [*Fig. A* and *Table 1*].

Table 1 — Significant non-coding elements mutated across MGUS, SMM, and MM (q<0.05)

Element	N signif.	Genes or likely target genes
3' UTR	4	*BMP6*, *TXNDC5*, *BTG2*, *SYBU*
5' UTR	11	*EGR1*, *BACH2*, *DNMT1*, *BCL7A*, *RFTN1*, *LPP*, *DTX1*, *SERPINA9*, *LRMP*, *BCL6*, *CCND1*
Promoter	13	*ILF2*, *ACTB*, *STRIP1*, *IKZF3*, *BACH2*, *PAX5*, *DTX1*, *LPP*, *RFTN1*, *LRMP*, *BCL6*, *SERPINA9*, *CCND1*
Enhancer	1	*TXNDC5*
miRNA and lncRNA	18	*MALAT1*, *hsa-mir-4537*, etc.
Immunoglobulin loci	12	IGH, IG Kappa, IG Lambda
Likely artifacts	7	-

- Mutations in B cell-related genes are consistent with off-target AID activity.
- Of the remaining 11 candidates, 6 were found in known MM drivers or key genes.
- ***ILF2* promoter** (1q21.2) was exclusively mutated in the MAF subgroup of patients, enriched with the APOBEC signature, and mostly clonal [*Fig. B*].

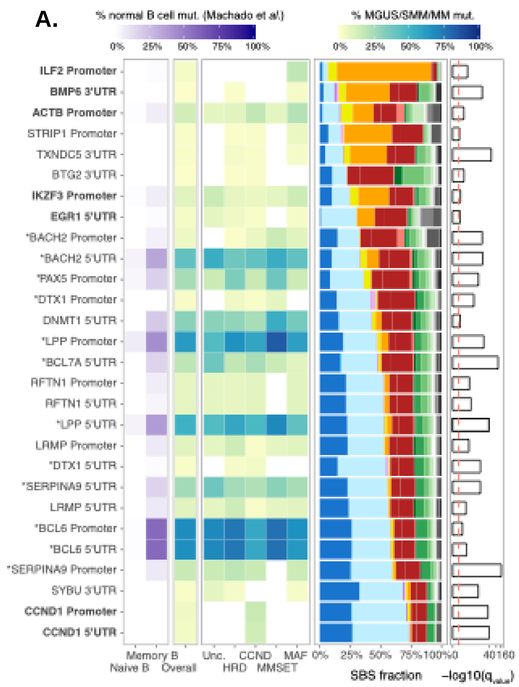

Figure A. Noncoding mutation hotspots across multiple myeloma stages reflect the history of the disease and novel candidate driver genes. Gene and regulatory elements detected with the DIG algorithm on MGUS/SMM/MM participants (Filtered heatmap representing significantly hypermutated non-coding WG subgroup in yellow-green-blue shades). Left: Fraction of normal B cell expansions with mutations in the same elements[6]. Middle-right panel: mutational signature weights in each element. Right panel: q score for each element hypermutation in MGUS/SMM/MM. Bold: non-coding elements associated with known drivers of myeloma, asterisk: non-coding elements that are hypermutated in normal B cells.

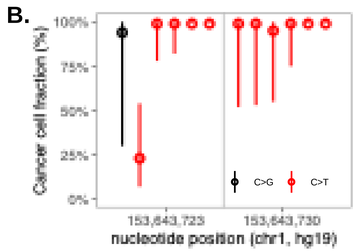

Figure B. For the *ILF2* promoter element, genomic coordinates and cancer cell fraction (CCF) estimates from ABSOLUTE for each mutant allele with deep WGS of this study

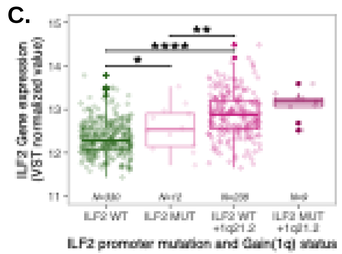

Figure C. *ILF2* gene expression levels by mutation status of the promoter and of gain(1q) from the CoMMpass study

- In CoMMpass WGS, *ILF2* promoter hotspot mutations were found in 26 patients enriched in the MAF subgroup (13/26; Fisher's Exact p=4.7x10-11) and correlated with higher *ILF2* expression levels.

CONCLUSIONS

Overall, we present:

- The first comprehensive list of significantly mutated **non-coding elements in Multiple Myeloma**, particularly in its precursor conditions.
- Newly identified hotspots associated with known MM driver genes, in particular in ***ILF2* promoter**.
- A catalog of **B-cell-specific mutations** that likely reflect the differentiation from B cells to plasma cells

REFERENCES

1. **Horn S**, et al. TERT promoter mutations in familial and sporadic melanoma. *Science*. 2013;339(6122):959-61.
2. **Huang FW**, et al. Highly recurrent TERT promoter mutations in human melanoma. *Science*. 2013;339(6122):957-9.
3. **Rheinbay E**, et al. Recurrent and functional regulatory mutations in breast cancer. *Nature*. 2017;547(7661):55-60.
4. **Rheinbay E**, et al. Analyses of non-coding somatic drivers in 2,658 cancer whole genomes. *Nature*. 2020;578(7793):102-11.
5. **Sherman MA**, et al. Genome-wide mapping of somatic mutation rates uncovers drivers of cancer. *Nat Biotechnol*. 2022;40(11):1634-43.
6. **Machado HE**, et al. Diverse mutational landscapes in human lymphocytes. *Nature*. 2022;608(7924):724-32.

ACKNOWLEDGEMENTS

We acknowledge funding support from the Multiple Myeloma Research Foundation (MMRF), Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (AMRF), International Myeloma Society (IMS; J-B.A), Krouse Family Foundation (I.M.G.), NIH R35CA263817 (I.M.G), Stand Up To Cancer Dream Team Research Grant (grant number: SU2C-AACR-DT-28-18).

CONTACT INFORMATION

Correspondence to jalberge@broadinstitute.org (J-B.A.), gadgetz@broadinstitute.org (G.G.) and Irene_Ghobrial@dfci.harvard.edu (I.M.G.)