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Abstract:

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Mutation landscape of multiple myeloma measurable residual disease: identification of targets for precision medicine

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Multiple myeloma (MM) measurable residual disease (MRD) persisting after treatment is an adverse prognostic factor for progression free survival (PFS) and overall survival.[1] Genomic mutations occurred in the remaining clonal aberrant plasma cells (A-PCs) are linked to the development of drug resistance and disease relapse.[2] Thus, personalised treatment based on the genomic profile of MRD could be highly beneficial and ultimately increase patients' survival. However, while large-scale sequencing studies have

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characterised the genome of many malignancies including MM,[3–8] the genomic mutations present in MM MRD are at the beginning of investigation.[9] Here, we set up an exome sequencing analysis to identify genomic mutations characteristic for MM MRD and explore if they could mediate drug response, resistance or disease progression.

Samples of peripheral blood and sorted clonal bone marrow A-PCs were collected from 22 patients after bortezomib-based treatment (Table S1, Figure S1), upon signing the informed consent form. The study was approved by the institutional ethics board of the University Hospital Ostrava (reference number 500/2016) was conducted in accordance with the Declaration of Helsinki. All methodological details used in this study are provided in supplementary methods; importantly, clonal A-PCs were sorted according to pathological immunophenotype using CD38, CD45, CD19, CD56 (CD117 when necessary)[10] (Figure S2) with the purity exceeding 95%, which led to a median of 2000 cells per patient. DNA from those cells was amplified and its exome was further analyzed. Here we report only non-synonymous somatic variants with frequency in human population below 1% (Table S2, Figure S3, Supp methods). In total, we identified 278 variants, with a median of 12.5 mutations per patient and a median coverage 71. These variants were located in exons of 263 genes, which account for a median of 12.5 mutated genes per patient (Figure 1A). In the results (Figure 1, 2), we focused only on genes expressed in our independent cohort of 10 MM patients' A-PCs (unpublished) and thus potentially playing a role in the MRD cells biology. From all analysed MM MRD exomes, 8 genes were mutated in at least 2 patients (Figure 1A), which is consistent with high MM heterogeneity.[3–7] Recurrently mutated genes included KRAS, DIS3, TRAF3, OGT, FRG1, UNC13C, FRMPD3 and TRAPPC8. Genes KRAS, DIS3 and TRAF3 are known MM drivers[6], OGT encodes a glycosyltransferase and O-GlcNAcylation catalyzed by OGT is essential for stabilization of NRF1, a transcription factor of proteasome subunit genes, potentially linked to proteasome inhibitor resistance.[11] FRG1 participates on mRNA processing and its decreased expression promotes cancer progression, cell migration, invasion and angiogenesis.[12,13] UNC13C play a role in vesicle maturation during exocytosis and acts as a tumor suppressor in solid cancers.[14] TRAPPC8 in involved in endoplasmic reticulum to Golgi apparatus trafficking[15] and was often mutated in solid cancers.[16]

Comprehensive analysis of driver genes is not feasible in such small MRD cohort, thus we compared our results with a list of known drivers and other MM associated genes to better understand MRD pathogenesis.[4–7] Our dataset contained 9 MM genes from 8 patients (Table S3), including KRAS, NRAS, DIS3, TRAF3, SF3B1, NKFBIA, MYC, IKZF3, BTG1. Interestingly, NRAS mutations were undetectable in a recently published MRD cohort.[9] In 12 patients (55%) we did not identify any mutations in above mentioned genes, nor they share some other common mutations, however several of those patients relapsed, thus the malignant characteristics of plasma cells is likely underlied by different mechanisms.

To uncover possible common patterns underlying the heterogenous mutation profile in MRD cohort, we ran pathway analysis using 7 gene set collections, together including 7 627 gene sets for each patient (Table S4). The results showed no pathways significantly enriched and simultaneously commonly mutated among patients (Table S5). Simple overlap with pathways typical for MM[17] revealed mutations in MAPK[18] pathway (7 patients, 32 %), NFκB[18] pathway (3 patients, 14 %), P53 pathway[18] (0 patients), proteasome subunits[19] (1 patient, 5 %), cereblon[20] (2 patients, 9 %) (Table S6). The most commonly shared pathways with at least 1 affected gene are shown in Figures S4 – S11.

To depict novel genes or pathways important for our MM MRD cohort, we performed survival analysis for genes and pathways (Tables S7-8, Figures S12-S18). Only mutations in FRMPD3 present in two patients were associated with shorter PFS (Figure 2B, C). This gene is involved in signal transduction and it was not found frequently mutated in previous MM studies.[3,16] Interestingly, two RAS related pathways, „KRAS.600_UP.V1_UP" including synthetic lethal partners of oncogenic KRAS (FDR 0.015) and "GO_RAS_PROTEIN_SIGNAL_TRANSDUCTION" (FDR 0.049), including 4 and 6 samples showed significant difference in PFS (Figure 1B, C). This finding is consistent with known frequent impairment of RAS signalling in MM and offers new genes with potential applicability of RAS inhibitors.[21]

Our ultimate goal was to identify novel genes that could be targeted by available drugs. Therefore, we annotated the geneset with known pharmacological information. We used The Drug-Gene Interaction Database[22] covering broad spectrum of drugs and diseases and Precision Oncology Knowledge Base[23] (OncoKB) summarizing druggable mutations and the literature search to retrieve genes important for myeloma drug resistance. Overall, we have generated a set of mutations in 8 drug-interacting genes with evidence of expression in plasma cells that were mutated in 7 patients (Figure 2A, Table S9-S11). The most interesting hit was a mutation in PSMC6 gene (R256Q), coding subunit of 19S proteasome complex, present in a patient treated with bortezomib, who reached VGPR and MRD depth $10e^{-3}$ and had one of the shortest PFS (18 months). Mutations in this gene were previously found only in 4 patients in the CoMMpass study[16] but the gene was already shown to be important in Bortezomib resistance.[24] The effect of the specific substitution R256Q was confirmed by in-vitro functional tests (manuscript in prep). Of note, mutation of BCMA gene, frequent target of CAR-T cell immunotherapy, was detected in one case. Mutations in this gene could be potentially important for the binding between the BCMA epitope and the antibody. KRAS was the only druggable gene mutated in more than one patient (Figure 1A) and also possessed affected amino acids exactly fitting with the positions for selective treatment (G13D, Q61H).

In summary, we performed whole exome analysis of somatic variants in pure population of sorted MM MRD samples with low A-PC infiltration to describe its mutation pattern and to reveal its further utilization in clinics. Limited number of aberrant present at MRD stage and application of whole genome amplification did not allow to analyze larger genomic changes than SNVs and short indels. Copy number variant analysis revealed ambiguous results without a clear pattern (Figure S19). In the heterogeneous spectrum of mutated genes we did not reveal any unifying feature of MRD clones. In context of that, there is very interesting exposure of the mutation in proteasome subunit PSMC6 that despite being scarcely mutated in myeloma population, it was confirmed in cell lines as a bortezomib-resistance causing mutation and thus it may still be useful for patient's treatment design. The survival analysis revealed mutations in two RAS associated pathways that were linked to shorter PFS and thus can be important for disease progression. Discovery of new genetic aberrations with yet unknown role in MM opens new avenues for further investigation in preclinical studies and can provide new targets for treatment upon validation in laboratory and clinics.

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Data availability: All sequencing data (BAM files) are deposited in European Genome-phenome Archive (EGA) with accession number EGAS00001004855.
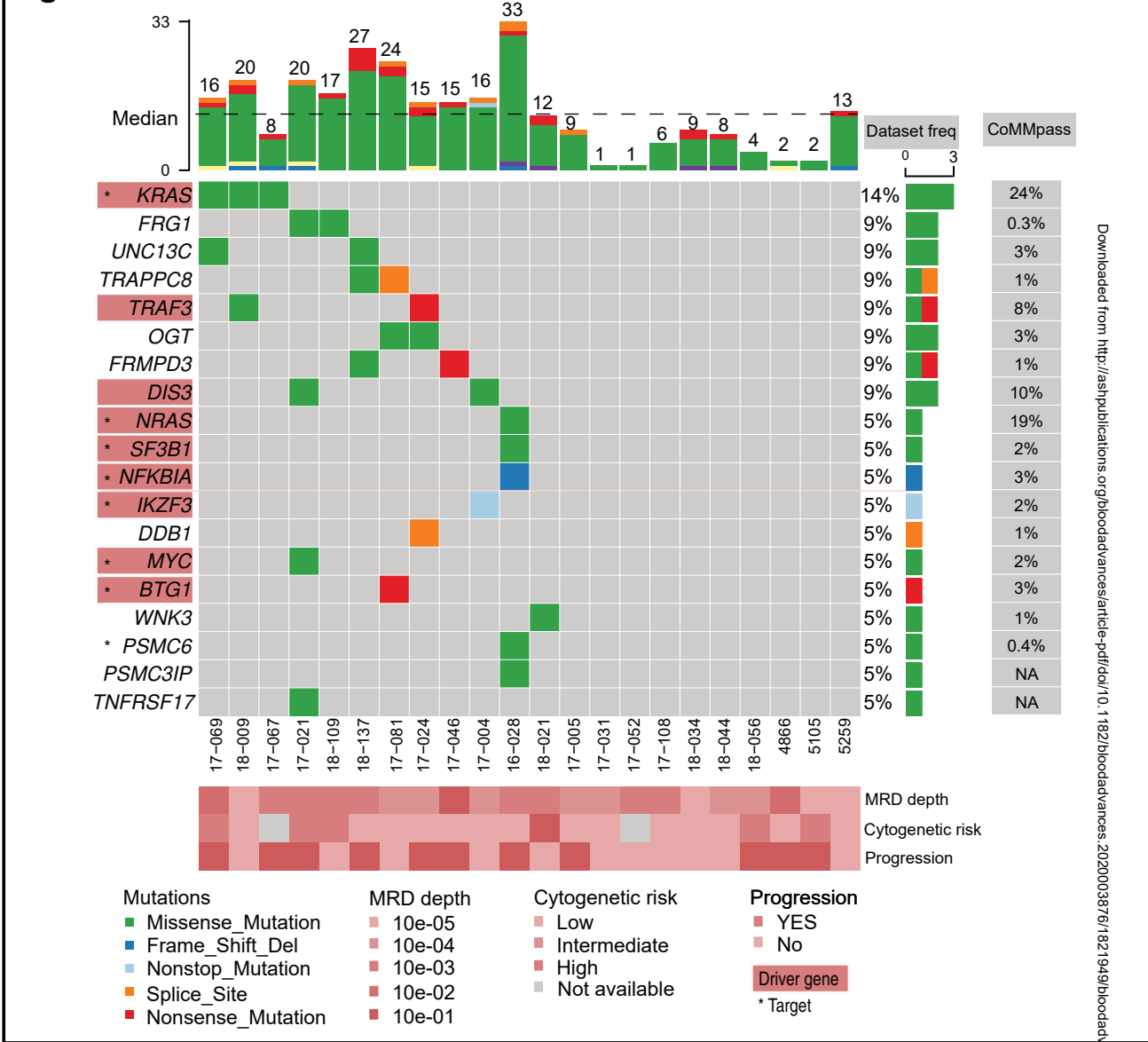
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Figure 1: Mutation profile of the MRD cohort. A) Recurrently mutated genes and functionally important hits. Patients are depicted as columns, genes as rows. Previously identified MM associated genes (Table S3) are highlighted in red rectangles; star symbols indicate potentially actionable targets. Total number of SNVs in particular patients is given on the top. Driver frequencies from other studies were obtained from 5 papers[3–7] B,C) Kaplan Meier curves showing association of PFS with RAS related pathways. Pathways KRAS.600_UP.V1_UP included synthetic lethal partners of oncogenic KRAS. Ras protein signal transduction pathway is a series of molecular signals within the cell that are mediated by a member of the Ras superfamily of proteins switching to a GTP-bound active state. List of genes included in respective pathways is provided below each graph.

Figure 2: SNV overview of important MM MRD genes. Functional domains are shown for each gene, mutated positions are represented by coloured lollipop marks (red – single nucleotide change, blue – splice site/nonsense mutation). A) Mutations in genes potentially useful in clinics, suggested for preclinical studies. Interacting drugs are given on the right. B) Genes identified as drivers without assigned treatment and gene FRMPD3 are schematically shown. C) Kaplan Meier curve showing gene FRMPD3 that is the only shared gene with significant PFS association.

Figure 1

Figure 1A

B — MSigDB - Oncogenic signature
KRAS.600_UP.V1_UP

FDR: 0.0116

Genes: KIF5C, EFCAB6, SCN2A, SORL1

C — Gene Ontology - Biological process
RAS protein signal tranduction

FDR: 0.04780

Genes: KRAS, NRAS, RASGRP3, GRAP2, PLCE1

Figure 2

Figure 2A — Druggable genes

KRAS — Ras family — Trametinib and more
(G13D, Q61H; 0, 13, 61, 165, 189)

NRAS — Ras family — Trametinib and more
(G12C; 0, 12, 165, 189)

SF3B1 — SF3b1 — H3B-8800
(G895R, S702C; 0, 329, 452, 895, 1304)

MYC — Myc amino-terminal region — HLH — Myc-LZ — Prednisone and more
(A59T; 0, 345, 407, 439)

DDB1 — MMS1_N — CPSF_A — Imid resistance
(X409_splice; 57, 0, 75, 409, 543, 788, 1140)

IKZF3 — zf-C2H2, zf-C2H2 — Imid resistance
(*510R; 0, 146, 202, 509)

PSMC6 — Prot_ATP_iD_OB — AAA — AAA_lid_3 — PI resistance
(R256Q; 0, 57, 170, 303, 389)

NFKBIA — Ank Ank Ank Ank — Aspirin
(R53Afs*37; 0, 53, 111, 142, 176, 213, 248, 317)

B Drivers + FRMPD3

TRAF3 — zf-TRAF zf-TRAF
(R163*, G466R; 0, 136, 251, 568)

DIS3 — PIN domain — Rrp44_CSD1 — OB_Dis3 — RNB domain
(D479H, R780K; 0, 66, 225, 438, 846, 958)

BTG1 — BTG family
(F40*; 0, 11, 124, 171)

FRMPD3 — PDZ — FERM_M
(D427H, R1393*; 0, 54, 270, 1810)

C FRMPD3

Progression probability — months

FDR: 0.0146