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Targeted sequencing using a 47 gene multiple myeloma mutation panel (M³P) in -17p high risk disease

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Summary

We constructed a multiple myeloma (MM)-specific gene panel for targeted sequencing and investigated 72 untreated high-risk (del17p) MM patients. Mutations were identified in 78% of the patients. While the majority of studied genes were mutated at similar frequency to published literature, the prevalence of *TP53* mutation was increased (28%) and no mutations were found in *FAM46C*. This study provides a comprehensive insight into the mutational landscape of del17p high-risk MM. Additionally, our work demonstrates the practical use of a customized sequencing panel, as an easy, cheap and fast approach to characterize the mutational profile of MM.

Keywords

myeloma; cancer genetics; DNA mutation; DRUG resistance; genetic analysis

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Authorship contributions

KMK designed and performed research, analysed data and wrote the paper; EB, RF, HE and AKS designed research, analysed and interpreted data and edited the paper; MDC analysed data; CL, LBu, MK, PL, LR and SK contributed sample and data; LBr, CXS, YXZ, JS, JO, JBE, PJ, JM, SVW and GA performed research and analysed data; All authors revised the paper and approved submission.

Disclosure of conflicts of interest

KMK, CL, EB, JM, LBr, JBE, PL, YXZ, CXS, PJ, JS, JO, LBu, MK, MDC, SVW, GA, LR, SK, HE, AKS and EB have nothing to disclose.

Supporting Information

Additional Supporting Information may be found in the online version of this article:
Fig S1. Survival del17p mutant *versus* wildtype.
Table SI. Variant table.
Table SII. Amplicon details of the M³P gene panel.

In multiple myeloma (MM), risk assessment based on individual tumour cytogenetics, fluorescence *in-situ* hybridization (FISH) and gene expression profiling is already established, however these prognostic indices fail to capitalize on the technological revolution in speed and reduced cost of genomic next generation sequencing (NGS). Two recent large sequencing studies on 270 primary MM samples have essentially defined the mutation landscape for this disease (Bolli *et al*, 2014; Lohr *et al*, 2014). The existence of baseline clonal heterogeneity, linear and branching evolution and therapeutic selection resulting in clonal tides suggest an imminent need for rapid, accurate and comprehensive evaluation of patient genomic profiles to guide precision therapy. Discoveries on the role of *CRBN*, *IKZF1* and *IKZF3* for the efficacy of immunomodulatory drugs (IMiDs), XBP1s and IRE1 in proteasome inhibitor therapy and druggable targets, such as *BRAF* mutations in MM, have had a major impact on the understanding of the disease and emphasize the need for tailored treatment. With this in mind, we generated a 47-gene MM Mutation Panel (M^3P) that requires small amounts of DNA and provides deep coverage results in clinically meaningful timeframes.

Material and methods

Tumour DNA from 72 newly diagnosed MM patients with del17p was collected by the German MM study group (DSMM), including 40 corresponding germline samples. Plasma cells were purified using anti-CD138+ immunomagnetic beads (median purity 95%). DNA was extracted from cell pellets stored at −80°C (AllPrep DNA/RNA Mini Kit, Qiagen, Venlo, The Netherlands). Baseline FISH confirmed del17p in all 72 cases. In a subset, additional abnormalities were screened with 35% (24/68) having gain of 1q21, 81% (58/72) del(13q), 29% (19/66) t(4;14), 40%(16/40) t(11;14) and 3%(1/36) t(14;16). To generate M^3P we selected the top mutated genes (≥3%), (Lohr *et al*, 2014), expressed in public datasets (http://www.broad.mit.edu/mmgp), ending up with a list of 39 genes. Next we added eight genes targeted by commonly used therapies, associated with resistance to IMiDs (*CRBN*, *CUL4A*, *CUL4B*, *DDB1* and *IRF4*), proteasome inhibitors (*PSMG2*, *PSMB5*) and glucocorticoid therapies (*NR3C1*). Overall, 2875 amplicons, covering 96% of the M^3P exons (Tables SI, SII), were analysed per sample, multiplexed in two library preparations, using 20 ng of input DNA. Enriched templates were sequenced using semiconductor technology (PGM™, Life Technologies, Carlsbad, CA, USA) and analysed with Ion Reporter Software v1.6 (Life Technologies). A minimum of 20× depth coverage was required. Mutation calls were considered positive when called by ≥10% variant reads. In already characterized cancer-related mutations (COSMIC database, http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/), the threshold was reduced to 3%. Variants called in samples without corresponding germline available were excluded if listed in the Single Nucleotide Polymorphism Database (dbSNP, http://www.ncbi.nlm.nih.gov/SNP/), as well as single nucleotide variants (SNV) that were identified in multiple samples, unless described in COSMIC (Table I).

Results and discussion

NGS has served as a catalyst in understanding the genomic landscape and subclonal complexity in haematological diseases. Individual genomic profiling prior to therapy and the tracking of clonal evolution during treatment will probably be the basis of individualized treatment decisions and the standard of care in the very near future. We capitalized on the availability of fast turnaround NGS technologies and early access to one of the recent large MM whole exome sequencing efforts to design a comprehensive, MM-specific mutation panel (M^3P), containing the most frequently mutated genes and a selection of clinically relevant genes. A mean sequencing depth coverage of 298× (tumour) and 221× (germline) was achieved. In total, 123 nonsynonymous missense/nonsense SNVs and three indels were detected. 124 mutations were rated by Polyphene-2, SIFT and Provean, 108 were predicted damaging (87%) and 53 (43%) were listed in the COSMIC database (Fig 1). The average mutation prevalence was 1·75 (range:0–8). Mutations were found in 78% (56/72) of the patients and in 66% (31/47) of the genes. More than one mutation in one patient was present in seven genes including *CCND* (Pro18Ser, Cys47Gly), *DIS3* (Tyr246Asp, Gln931*), *FAT1* (Asp3799Gly, Arg549Trp), *FAT4* (Leu784Met, Thr4281Ala), *VCAN* (Gln1009*, Ser2445Phe), *ZFHX4* (Glu3379Asp, Glu3380*) and *TP53* (Lys132Asn, Met237Ile, Val173Met, (6%/4%/5% VR). *TP53* was the most frequently mutated gene (27·8%), followed by *NRAS* (20·8%) and *KRAS* (13·9%). Overall, the MAPK/ERK pathway was mutated in 38%, including three combined *NRAS* and *KRAS* mutations and three *BRAF* mutations, two of which were at the known targetable Val600E position. *NRAS* and *KRAS* is less frequently mutated in high-risk MM, including del17p (Chng *et al*, 2008), and more often in relapsed patients (Lohr *et al*, 2014), both explaining the lower prevalence in our untreated del17p cohort. *TP53* is commonly assumed to be the major target of del17p MM (Munshi & Avet-Loiseau, 2011). Previous work suggested that *TP53* mutations are correlated (Bolli *et al*, 2014) with or even be exclusively present in del17p (Lode *et al*, 2010) and, indeed, it was more prevalent than in published data from unselected MM patients (Bolli *et al*, 2014; Lohr *et al*, 2014). *TP53* mutation has been associated with impaired event-free survival (Bolli *et al*, 2014) and overall survival (OS) (Chng *et al*, 2007) in non-del17p cohorts; however, as survival data was available in only 61 cases no significant difference in progression-free survival ($n = 50$) or OS ($n = 61$) by *TP53* status could be observed (Figure S1), as the observation time was variable (follow up 60–2550 days, median 598 days) and numbers might still be too small to derive meaningful conclusions. *DIS3* was mutated in five patients (Arg418Gly, Ser756Phe, His764Asp, Asp784Asn), including one case with two mutations (Tyr246Asp and Gln931*). *DIS3* mutations have been reported to be exclusively present in t(4;14) and t (11;14) patients (Walker *et al*, 2012) and to be del13q14-dependent. In our del17p cohort all *DIS3*-mutated patients harboured a del13q, however only three of five patients had a t(4;14) or t(11;14). Truncating mutations of *SP140*, involved in the pathogenesis of acute leukaemia and viral infection, have been recently described in MM (Bolli *et al*, 2014), and two (Arg576*, Glu627*) were present in our cohort. *FAT* family genes showed a significant number of mutations, with *FAT1* as the most frequently mutated (9%), followed by *FAT4* (7%) and *FAT3* (4%). Alterations of *FAT* cadherins (Bolli *et al*, 2014; Messina *et al*, 2014) and other large M^3P genes, such as the dynein protein (*DNAH5*; Morgan *et al*, 2012) (3%), the low

density lipoprotein receptorrelated protein 1B (*LRPB1P*; Leich *et al*, 2013) (1%) and the histone modifying enzyme *KMT2C* (*MLL3*; Chapman *et al*, 2011) (6%), have been reported in cancer, including MM. *ZFHX4* (8%) has not yet been associated with MM. As a cautionary note, the frequent occurrence of mutations in implausible genes in sequencing studies has been identified as a confounding issue (Lawrence *et al*, 2013), thus functional investigation of these findings is needed to determine the relevance to MM. *FAM46C* is recurrently mutated in MM, however, understanding of the function of this gene in particular is very limited. An 9% overall mutation prevalence (Boyd *et al*, 2011; Bolli *et al*, 2014; Lohr *et al*, 2014) and impaired survival by abnormal *FAM46C* (Boyd *et al*, 2011) have been reported. Interestingly, no *FAM46C* mutation was seen in our cohort. The correlation of *FAM46C* mutation status and ploidy is controversial: Bolli *et al* (2014) reported a correlation of *FAM46C* mutations to hyperdiploid MM whereas Lohr *et al* (2014) did not find a significant correlation. Ploidy information in our cohort was incomplete, however, del17p is enriched in non-hyperdiploid MM (Van Wier *et al*, 2013), which might partly explain the lack of *FAM46C* mutations in our del17p-restricted cohort. Alternatively, *TP53* and *FAM46C* might activate similar pathways, which would make mutations in both genes redundant. In published data *FAM46C* mutations seem to be extremely uncommon in del17p/mutant *TP53* MM with a mean prevalence of 0·4% (Van Wier *et al*, 2013; Bolli *et al*, 2014; Lohr *et al*, 2014). Almost two-thirds of patients with *FAM46C* mutations were untreated in previous reports (Bolli *et al*, 2014; Lohr *et al*, 2014). We therefore suggest that *FAM46C* mutations might be a marker for lower risk disease rather than of progression, however further investigation is needed. Of note, no mutations in genes related to drug resistance were seen above our chosen cut-off point. However, mutations were identified in minor subclones below cut off threshold in *CUL4B* (Asp426Gly, 9% VR), *DDB1* (Ala971Asp, 4% VR) and *IRF4* (Gly43Ser, 6% VR), affecting the *CRBN* pathway, and in the steroid receptor *NR3C1* (Lys772Asn, 8% VR). The *CUL4B, IRF4* and *NR3C1* mutations were predicted to be damaging by polyphene-2 or SIFT. Furthermore, according to the Universal Protein Resource database (www.uniprot. org) the identified *DDB1* mutation potentially affects interaction with CUL4A, which is part of the ubiquitin ligase complex of CRBN, the *IRF4* mutation is located in the DNA binding and the *NR3C1* mutation in the steroid binding region, thus these findings provide a potential source of later IMiD and steroid drug resistance, respectively.

In summary, we present the first MM-specific gene panel that characterizes the individual mutation status in a fast, accurate and cost-effective manner. Minimal DNA is required to run the assay and sensitivity down to minor clonal frequencies is attainable. We believe that a targeted panel of mutation detection, such as M^3P, will become a diagnostic tool of choice to further improve MM classification, track clonal variability, more precisely predict prognosis and better guide treatment decisions in times of genetic-based risk assessment and personalized medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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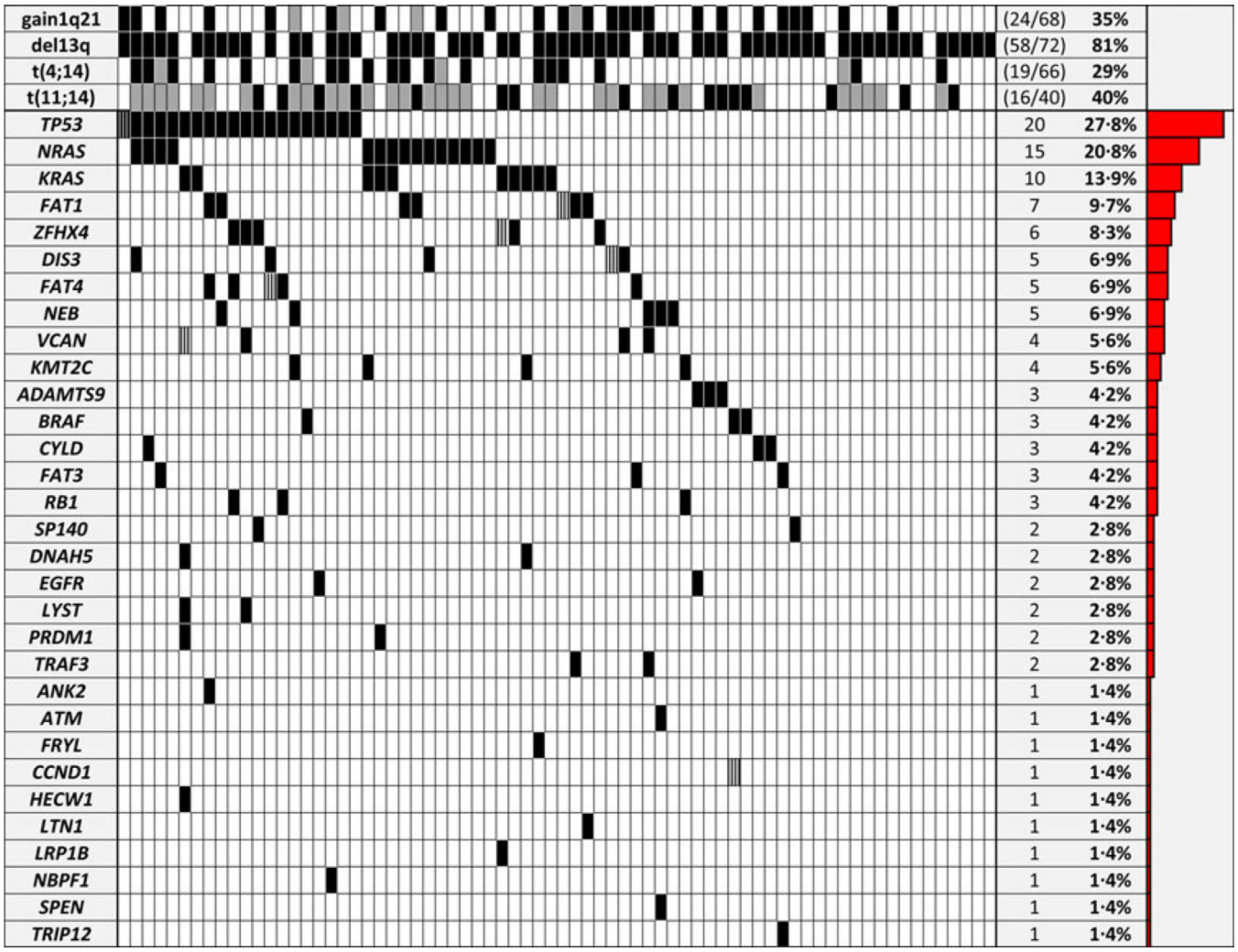

Fig 1.

Mutation prevalence in untreated patients with newly diagnosed and untreated del17p multiple myeloma and fluorescence *in situ* hybridization (FISH) results. All patients had a confirmed del 17p. Grey shading indicates lack of FISH data; black and striped shading indicates, multiple mutations in the same gene and timepoint. No mutation above the chosen threshold was found in *CRBN, CUL4A/B, DDB1, EGR1, FAM46C, IRF4, MECOM, NR3C1, PSMB5, PSMG2, PTPRD, RASA2, TIAM1, XBP1* or *ZFHX3.*

Table I

Genes included in the Multiple Myeloma Mutation Panel (M^3P) v1·0.

M^3P				
ADAMTS9	*DIS3*	*IRF4*	*NRAS*	*TP53*
ANK2	*DNAH5*	*KRAS*	*PRDM1*	*TRAF3*
ATM	*EGFR*	*LRP1B*	*PSMB5*	*TRIP12*
BRAF	*EGR1*	*LTN1*	*PSMG2*	*VCAN*
CCND1	*FAM46C*	*LYST*	*PTPRD*	*XBP1*
CRBN	*FAT1*	*MECOM*	*RASA2*	*ZFHX3*
CUL4A	*FAT3*	*KMT2C*	*RB1*	*ZFHX4*
CUL4B	*FAT4*	*NBPF1*	*SP140*	
CYLD	*FRYL*	*NEB*	*SPEN*	
DDB1	*HECW1*	*NR3C1*	*TIAM1*	