The genetic heterogeneity and drug resistance mechanisms of relapsed refractory multiple myeloma

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Multiple myeloma is the second most common hematological malignancy. Despite significant advances in treatment, relapse is common and carries a poor prognosis. Thus, it is critical to elucidate the genetic factors contributing to disease progression and drug resistance. Here, we carry out integrative clinical sequencing of 511 relapsed, refractory multiple myeloma (RRMM) patients to define the disease’s molecular alterations landscape. The NF-κB and RAS/MAPK pathways are more commonly altered than previously reported, with a prevalence of 45–65% each. In the RAS/MAPK pathway, there is a long tail of variants associated with the RASopathies. By comparing our RRMM cases with untreated patients, we identify a diverse set of alterations conferring resistance to three main classes of targeted therapy in 22% of our cohort. Activating mutations in IL6ST are also enriched in RRMM. Taken together, our study serves as a resource for future investigations of RRMM biology and potentially informs clinical management.
Multiple myeloma (MM) is a malignancy of plasma cells, mature B lymphocytes dedicated to producing immunoglobulins. The median age of diagnosis of MM is 70 years, and MM affects individuals of African descent two- to three-fold more than those of European descent, accounting for significant health disparities.\(^2,3\) Over the last two decades, many novel treatments have been developed for MM, including proteasome inhibitors, immunomodulatory drugs, monoclonal antibodies, and CAR-T-cell therapies.\(^4\) The 5-year relative survival rate across all stages combined is 54%, which has improved based on the advent of these new treatments.\(^5\) Despite this, the majority of MM patients suffer a relapse, and each subsequent relapse limits treatment options and reduces the ability to control disease progression.\(^1,2\) Thus, understanding the genetic heterogeneity of relapsed, refractory RRMM (RRMM) will shed light on MM disease progression as well as elucidate therapeutic resistance mechanisms. Newly diagnosed MM (NDMM), like other primary cancers, has been genomically dissected by a number of groups, including the Multiple Myeloma Research Foundation’s (MMRF) CoMMpass Study (NCT01454297).\(^6\) Less is known about the genomic heterogeneity and resistance mechanisms of RRMM.

In this work, as part of the MMRF’s molecular profiling initiative (NCT02884102) which included 22 academic medical centers, we carry out clinical-grade targeted sequencing (tumor/normal) and whole transcriptome sequencing of 511 RRMM patients and reanalyzed equivalent data from 965 patients enrolled in the CoMMpass Study to systematically compare alterations in RRMM with NDMM. Our uniform integrative analyses uncover a wide range of genetic alterations, implicate known oncogenic MM pathways often at a much higher prevalence than previously known, and provide a comprehensive genetic basis for drug resistance mechanisms in RRMM.

**Results**

The landscape of genomic alterations in relapsed refractory multiple myeloma. As part of the MMRF molecular profiling initiative, a consecutive series of 762 RRMM patients were enrolled and provided CLIA genomic sequencing from May 2017 to June 2020. As part of this precision oncology study, molecular reports were provided to the participating physicians within a 10-day turnaround period. After the exclusion of patients that either had allologeneic stem cell transplants (allo-SCT), or had low tumor purity (<20%) following CD138 + selection, we compiled a cohort of 511 RRMM patients with comprehensive 1700-gene tumor/normal DNA sequencing and whole transcriptome sequencing\(^7–10\) for integrative analyses (Supplementary Fig. 1a and Supplementary Data 1). Overall, RRMM samples were contributed by 22 academic medical centers that participate in the Multiple Myeloma Research Consortium (MMRC), with the University of Michigan Rogel Cancer Center, Mt. Sinai Medical Center, and Hackensack Medical Center as the top three enrolling centers (Supplementary Fig. 1b). The cohort was comprised of 53.3% males and 46.7% females. Light chain type statistics included 52.4% kappa, 31.5% lambda, and 4.0% biclonal; 15.7% did not have available information. After CD138 + selection, the average tumor content of our RRMM cohort was 62%. For each patient, we performed targeted sequencing (Onco1700 panel)\(^10\) on DNA from tumor and matched normal samples to call somatic and germline genetic aberrations (Methods, Supplementary Data 2). Mean target coverages for tumor and normal libraries were 655X and 483X, respectively, which is a depth conducive for subclonal assessments. RNA-sequencing for all but one tumor sample was available and performed by capture transcriptome sequencing with a cohort average of 44.5M uniquely mapped reads. Our computational pipeline\(^9\) also called copy-number alterations (CNAs) (Supplementary Data 3), gene fusions (Supplementary Data 4), and provided gene expression levels on a per-sample basis (Supplementary Fig. 1c). FASTQ files of 1108 NDMM samples from the CoMMpass Study were downloaded from dbGaP as of August 2020 and analyzed with the same bioinformatics pipeline to facilitate comparisons with RRMM. In total, 965 complete CoMMpass cases were available for downstream integrative analyses (Methods and Supplementary Fig. 1a, c).

Employing an ensemble approach of statistical tools\(^11\) to discover cancer drivers based on single nucleotide variants (SNV) and indels, we identified a set of 43 high-confident, significantly mutated genes (Fig. 1a and Supplementary Data 2). Their biological functions can be classified into the following categories: (1) RAS-MAPK pathway (KRAS, NRAS, BRAF, PTPN11, NF1, IL6ST), (2) NF-xB pathway (CYLD, TRAF3, TRAF2, NFKBIA, IRAK1), (3) MYC pathway (MYC, MAX, EP300, CREBBP, SP3), (4) cell cycle and DNA damage checkpoints (TP53, RB1, CDKN2C, CDKN1B, ATM, FGFR3, LATS2), (5) RNA processing machinery (DDX3, FAM46C, DDX3X, DDX5), (6) epigenetic modifiers and transcriptional co-activators/co-repressors (KDM3B, SETD2, ARID1A, ARID2, MB1, IDH1, BCR/ABL1, ATAD2B), (7) B lymphocyte development (PRDM1, SP140, UBR5, IRF4), and (8) genes that likely acquired mutations due to MM treatment (CRBN, CUL4B, NR3C1) (Supplementary Fig. 2a, b)\(^12\). The average mutation rate of RRMM was 3.43 point mutations/megabase. Hypermutation was observed in 9.8% of cases due to the APOBEC mutational process and in another 2.7% due to undefined mechanisms (Supplementary Fig. 2c). Mutations in cancer driver genes were also detected in matched-normal sequencing libraries with a wide range of variant allele fraction (VAF) due to the presence of circulating tumor cells (CTCs) in advanced patients (Supplementary Fig. 2d).

Global copy-number analyses identified recurrent arm-level and chromosome-level gain of 1q, 2, 3, 5, 7, 9, 11, 15, 17q, 19, and 21 (e.g., “hyperdiploid”) and loss of 6q, 8p, 13,16, 22q and X (Fig. 1b, Supplementary Fig. 3a, and Supplementary Data 3). Frequent focal losses tended to center at or near known tumor suppressors in MM, such as GFI1, FAM46C, CDKN2C, ARI1B, NKK3-1, CDKN2A/B, BIRC2/3, CDKN1B, RB1, TRAF3, and CYLD, while focal gains were at or near oncogenes including MYC, CCND1, and NR3C1 (Supplementary Fig. 1b)\(^13\). Interestingly, there were recurrent homozygous deletions of diaphanous-related formin 2 (DIAPH2) on the X chromosome. These deletions were focal, affecting single exons or a group of exons, and affected both males and females in NDMM and RRMM (Supplementary Fig. 3b, c).

Integrative analysis of trinucleotide mutational signatures, gene expression, and copy-number identified distinct transcriptional signatures associated with high expression (presumably due to translocation) of WHSCI, CCND1, and MAF family genes (MAF, MAFa, and MAFb) (Supplementary Fig. 4a, b). As previously reported, samples with high expression of MAF family genes tended to associate with the APOBEC-enriched trinucleotide mutational signature (Supplementary Fig. 4a)\(^14\) and had elevated expression of APOBEC3G (Supplementary Fig. 4c). A small subset (2.9%) of patients with high expression of CCND1 also exhibited high expression of pre-B cell markers, such as FCER2 (CD23), VPREB3, PAX5, and TNFRSF13C (Supplementary Fig. 4a, d), a finding which was also observed in NDMM\(^15\).

Highly prevalent, diverse mechanisms of NF-xB pathway activation. The NF-xB pathway functions as an anti-apoptotic signal in myeloma cells and thus, mutations that lead to constitutive activation of NF-xB are selected for.\(^16–18\) Our findings in
RRMM were in line with previous studies in NDMM in that genes involved in alternative (non-canonical) NF-κB signaling via the cell surface TNF family receptors, including CD40, LTBR, TNFRSF17 (BCMA), and TNFRSF13B (TACI), were the most frequently affected by alterations (Fig. 2a, b). These alterations also induced robust activation of a previously defined NF-κB transcriptomic signature (Fig. 2a, top). Our integrative cross-cohort analysis further identified in-frame insertions and deletions in the transmembrane domains of TNFRSF17 (NDMM \( n = 7 \), RRMM \( n = 4 \)) and CD40 (NDMM \( n = 3 \)) (Fig. 2c, d). Cloning of these mutations confirmed their activating potential in a cell-based, NF-κB reporter assay (Supplementary Fig. 5a, b). Thus, we hypothesize that in-frame indels involving amino acids in the transmembrane domain of these cell surface receptors induce local conformational alterations that facilitate ligand-independent oligomerization, leading to constitutive activation of downstream signaling. In addition to alterations in the alternative NF-κB pathway, our integrative analysis revealed recurrent alterations in genes of the classical (canonical) NF-κB signaling pathway, including Toll-like receptors (TLR), B cell receptor (BCR), and TNF-α (Fig. 2a, b right).

MAP3K14 (NIK), the central kinase of the alternative NF-κB pathway, was also frequently truncated at the N-terminus by rearrangements that form in-frame fusion transcripts (Supplementary Fig. 5c)\(^\text{19}\). These chimeric products have an intact kinase domain but lack the TRAF3 binding domain, and thus escape cIAP-TRAF2-TRAF3-mediated proteasomal degradation and remain stabilized in MM cells\(^\text{20}\). We also observed N-terminal intragenic deletions of MAPK314 in both cohorts (Fig. 2e) which result in in-frame transcripts with a translation start site (methionine) located before the kinase domain. More complex rearrangements were out of frame or apparently lacked a methionine before the kinase domain. These co-occurred with a secondary event to restore the translation frame or introduce a de novo methionine. Examples of such a “second hit” were a frameshift mutation (P254fs) or intron retention/de novo splice site (Fig. 2e and Supplementary Fig. 5d). Interestingly, one case harbored a start-loss (M1I) mutation of MAP3K14 while maintaining a robust NF-κB transcriptomic signature (Fig. 2e, last row). MAP3K14 was recently reported to have an N-terminal binding site for BIRC2 (c-IAP)\(^\text{21}\). The start-loss mutation at M1 may force an alternative translation site (M4), thus disrupting the binding of BIRC2 and evading proteolytic degradation by the cIAP-TRAF2-TRAF3 complex. In-frame C-terminal fusions and deletions were also observed in NFKB2 (p100) and NFKB1 (p105) (Fig. 2f and Supplementary Fig. 6a). These rearrangements had breakpoints located in the ankyrin repeats, the domains on the precursor forms (p100 and p105) that bind the preformed NF-κB dimers (e.g., p50:RelA and p52:RelB), and thus disrupt the inhibitory activities of the precursors\(^\text{22}\).
While inspecting CD40 across the MM cohorts, we observed cases with outlier gene expression levels coincident with robust NF-κB transcriptional signatures. This finding prompted us to perform a systematic screening for patients with rare outlier expression (Supplementary Fig. 6b) and to search for genomic rearrangements to explain these events. Our analyses revealed that cell surface receptors (CD40, LTBR) and the kinase MAP3K14 exhibited outlier expression levels in select cases (Supplementary Fig. 6c). Overexpression of the receptors could potentially lead to oligomerization, activating downstream signaling. Whole-genome sequencing of representative cases across the MM cohorts, we observed more independent of the bone marrow environment in an autocrine manner. This exemplary case serves as evidence of positive selection for tumor cells that can remodel the micro-environment to facilitate their survival.

While plasma cells represent the end-stage of differentiation in B lymphocytes, a subset of MM patients shared common features with earlier stage B cell malignancies and had TLR and BCR-mediated NF-κB signaling activated by mutation (Fig. 2a, b) and missense mutations in the coiled-coil domain of CARD11 (Fig. 2h). An in-frame deletion in the transmembrane domain of CD79B was observed in one RRMM patient which would potentially activate NF-κB in the same manner as the aforementioned in-frame indels in TNFRSF17 and CD40.

Integrative heatmap for alterations in the NF-κB pathway activated by mutation (Fig. 2a, b) and missense mutations in the coiled-coil domain of CARD11 (Fig. 2h). An in-frame deletion in the transmembrane domain of CD79B was observed in one RRMM patient which would potentially activate NF-κB in the same manner as the aforementioned in-frame indels in TNFRSF17 and CD40.

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Fig. 6d). Substitutions at these key residues would render the protein kinase-dead, and interestingly, have not been reported in any other hematological tumor. They also provide genetic evidence of IRAK1 functioning as a context-specific negative regulator of survival or proliferation in addition to its role in the formation of the Myddosome28.

In summation, previous studies profiling NDMM have implicated alternative NF-κB alterations at a prevalence of 20%17. Our comprehensive integrative approach included all characterized NF-κB pathways and encompassed diverse mechanisms, indicating that over 60% of MM harbor alterations in this key cell survival pathway.

Heterogeneity of alterations in the RAS-MAPK pathway including Rasopathy-related genes. Similar to NDMM, alterations in the RAS-MAPK pathway play a major role in RRMM (Fig. 3a, b). KRAS was the most frequently mutated gene in RRMM with a frequency of 26%, followed by NRAS at 25% and BRAF at 15% (Fig. 1a). The distribution of mutated KRAS codons (Fig. 3c, left) was distinct from that observed in a pan-cancer assessment29. Although codon G12 was still the most common (34%) in MM, there was strong enrichment for Q61 (28.1%) and G13 (12.6%)29. Across all cancers, non-G12/G13/Q61 codons constituted around 2% of total KRAS mutations. By contrast, the incidence for recurrent atypical codons was higher in RRMM: 8.1% for A146, 3% for K117, and 3% for Q22 (Fig. 3c, left). NRAS codon distribution more closely resembled the pan-cancer assessment, with Q61 as the dominant codon observed in 73% of cases, and non-G12/G13/Q61 codons in 7% (Fig. 3c, middle). Across cancers, NRAS G13 was twice as frequent as G1229, while NRAS G12 and G13 were both observed at 10% in RRMM.

BRAF mutations in MM can be stratified into three classes based on their kinase activities30,31. All three classes of BRAF mutations were well-represented in our MM cohorts (Fig. 3c, right). Class 1 mutations (V600E monomer) were the most common at 32%, followed by Class 3 mutations (kinase-impaired or dead) at 28%, and Class 2 alterations (constitutive dimers, including N-terminal BRAF fusions) at 17%. Among the BRAF alterations whose kinase activities have not yet been classified include the in-frame deletion involving codons 485 to 490 (Fig. 3c, right). These rare in-frame indels have been demonstrated to form BRAF homodimers and are potentially
located within the PEST sequence, a domain required for efficient proteolysis\textsuperscript{45}, and this alteration may disrupt post-translational modifications and enhance MYC stability\textsuperscript{45}. MYCL and MYCN were also affected by structural rearrangements that led to overexpression (Supplementary Fig. 7c, d).

**Alterations related to disease progression and drug resistance.** We next systematically compared the mutational landscape of RRMM with NDMM from the CoMMpass cohort. We observed a significantly higher incidence of mutations and copy-number loss in tumor suppressors such as *TP53*, *RB1*, *CDKN2A/B*, *BIRC2/3*, and *CDKN2C* (Fig. 4a, b and Supplementary Fig. 8a–c), likely due to progression. Furthermore, in RRMM relative to NDMM, we uncovered a diverse range of mutations in genes that confer resistance to three classes of MM therapies - immunomodulatory imide drugs (iMiDs), synthetic glucocorticoids, and monoclonal antibodies (Fig. 4c–i). Immunomodulatory imide drugs (such as thalidomide, lenalidomide, and pomalidomide) function by binding to a tri-tryptophan pocket in CRBN, the substrate receptor of the CUL4–ROC1–DDB1–CRBN (CRL4\textsuperscript{CRBN}) E3 ubiquitin ligase\textsuperscript{46,47}. The binding of iMiDs induces a conformational change and shifts the target of degradation from endogenous substrates to IKZF1 and IKZF3, two essential transcription factors in MM\textsuperscript{46,47}. Various types of alterations frequently targeted two genes of the CRL4\textsuperscript{CRBN} complex in RRMM, CRBN, and *CUL4B*, at a cohort frequency of 11 and 5%, respectively (Fig. 4c, e, f). Missense substitutions were observed throughout the gene body of CRBN (Fig. 4e), including those at or near the binding pocket (E377K, H353N, G354E) (Fig. 4e) and Supplementary Fig. 8d, e). CRBN was also frequently affected by truncating mutations (frameshift, stop-gain, and splice site) and hemizygous deletion involving cytoband 3p26 (Fig. 4b and Supplementary Fig. 8a, b). *CUL4B* harbored truncating mutations and missense mutations clustered in the cullin domain, which could significantly disrupt the scaffold’s structure (Fig. 4f).

At high doses, glucocorticoids such as dexamethasone and prednisone affect MM cells potentially via an anti-NF-κB transcriptional program mediated by the glucocorticoid receptor (*NR3C1*)\textsuperscript{49}. In addition to truncating mutations, missense mutations were observed in the ligand-binding domain and N-terminal domain of *NR3C1* in the RRMM cohort (Fig. 4c, g). One case had an in-frame intragenic deletion that removed the DNA-binding domain of *NR3C1* (Supplementary Fig. 9a). This deletion may disrupt *NR3C1*’s transcriptional activity while retaining protein interactions, such as binding to co-activators. Interestingly, retinoic acid receptor alpha (*RARα*), another nuclear receptor, harbored mutations enriched in the RRMM cohort compared to the NDMM cohort (Fig. 4a). *RARα* mutations clustered in the ligand-binding domain (Fig. 4h), a pattern similar to those found in fibroepithelial breast tumors\textsuperscript{50}. It has been demonstrated that these mutations may not disrupt RARA’s ligand-binding ability but enhance its interaction with other co-activators\textsuperscript{50}.

Monoclonal antibodies targeting CD38 may induce cell death via antibody-dependent cellular phagocytosis (ADCP) or antibody-dependent cellular cytotoxicity (ADCC)\textsuperscript{51}. In RRMM relative to NDMM, we screened for alterations in *CD38* and identified several loss-of-function events, such as homozygous deletions, truncating mutations, and fusions in which the

**Alterations in the MYC pathway.** Dysregulation of MYC pathways is common in MM\textsuperscript{44}. In our analyses, focal amplification and rearrangements involving the MYC/PVT1 locus producing chimeric transcripts were detected in at least 10% of samples in the cohort (Fig. 1b and Supplementary Fig. 7a). Recurrent mutations in MYC were detected in the combined NDMM and RRMM cohorts, including S161L (*n* = 2 RRMM, *n* = 3 NDMM) and an uncharacterized in-frame deletion at Val 280 (*n* = 3 RRMM, *n* = 6 NDMM) (Supplementary Fig. 7b). Val 280 is
extracellular domain was lost (Fig. 4c, i, top, Supplementary Fig. 9b, c). Systematic analysis of de novo splice junctions identified two cases with exon 6 skipping (Fig. 4i, bottom). One case exhibited a splice donor mutation, while another had a point substitution of two nucleotides upstream of the splice site. This point mutation could function as a stop-gain (R251*), but instead unexpectedly induced in-frame exon skipping and removed most of the epitopes on CD38 that interact with daratumumab. These events may facilitate the evasion of MM cells from binding by daratumumab, while still retaining a major portion of the extracellular domain (Fig. 4i, j). Overall, CD38 mutations were associated with lower CD38 expression than the rest of the cohort.
Fig. 4 Alters enriched in relapsed refractory multiple myeloma associated with drug resistance or disease progression. a Scatter plot of mutation frequency in RRMM vs. NDMM. The size of the circles correlates with log2 of \( q \) value two-sided Fisher exact test. Genes with top coefficients from the regression model (Methods) are highlighted in red. b Comparison of focal mutations (less than 20MB) in NDMM vs. RRMM. * ** *** **** indicate \( P < 0.05, 0.01, 0.001, \) respectively for the two-sided Fisher exact test. \( P \) values obtained: \( 7.1 \times 10^{-6} \) (TP53), \( 1.4 \times 10^{-5} \) (CRBN), \( 1.3 \times 10^{-4} \) (CDKN2A/B), \( 2.2 \times 10^{-3} \) (RB1), 0.025 (BIRC2/3), and 0.035 (CDKN2C). c Heatmap of drug resistance-related genes identified in RRMM. The types of alterations are indicated in the legend. There were cases with alterations observed in more than two genes or different types of alterations per gene, reflecting the complex history of tumor evolution through several treatments. d Overview of therapies in MM with observed resistance mechanisms. Genes highlighted in blue are resistance mutations found in RRMM. ADCP antibody-dependent cellular phagocytosis, ADCC antibody-dependent cellular cytotoxicity, mABs monoclonal antibodies, GCs glucocorticoids, iMiDs immunomodulatory imide drugs. e-h Lollipop plots for CRBN, CUL4B, NRC31, and RARA. i Top panel, lollipop plot for CD38. Frameshift mutations would completely abolish CD38, and fusions that truncate the extracellular domain would disrupt binding events. Bottom panel, Sashimi plot for two cases with exon 6 skipping. MM_5034 had a splice donor mutation. MM_S1B3 had a missense mutation that was two nucleotides upstream of the splice site, which would naturally introduce a new stop codon (R251*). Unexpectedly, this mutation functioned as a splice donor mutation instead, which also induced the skipping of exon 6. MM_S191 is included as a control. j The skipping of CD38 exon 6 in MM_5034 and MM_S1B3 was in-frame and would delete 29 amino acids (highlighted in red), including the epitope of daratumumab (PDB structure 7DUO)52.

Discussion

RRMM is difficult to manage malignancy with an aggressive disease course34. Our study represents comprehensive integrative analyses of RRMM genetics and, further, systematically compares the genetic landscape of RRMM with that of NDMM. By covering somatic mutations, indels, copy-number alterations, gene fusions, gene expression, and outlier expression, we demonstrate that a majority of MM patients harbor alterations in the NF-κB and RAS-MAPK pathways; this is a level of prevalence that has not been previously reported and includes many alterations with diverse mechanisms of action.

It was surprising to observe that at least 45% of MM harbors alterations in the NF-κB pathway. Several activating mechanisms were identified, including: (1) in-frame insertions and deletions in the transmembrane domain of TNFRSF17 and CD40, (2) N-terminal intragenic deletions of MAP3K14, (3) in-frame C-terminal deletions and deletions in NFKB2 (p100) and NFKB1 (p105), (4) genomic rearrangements or translocations of CD40, LTBR, and TNFSF13, and (5) diverse alterations in A20, CARD11, CD79B, and IRAK1. Notably, mid-size to larger indels in CD40, TNFRSF17, and MAP3K14 already occurred in the published CoMMpass dataset but have not been reported by any analyses. These results highlighted the importance of thorough bioinformatics analyses in variant calling. It is fascinating to note that recurrent mutations in IRAK1 were kinase-dead mutants in MM. Our finding agrees with a previous study that showed IRAK1 is an important upstream adapter for NF-κB signaling via TLRs, but its kinase domain is dispensable for signaling activity53. It is also possible, as is the case for another receptor-associated kinase of the NF-κB pathway, RIPK1, that the kinase domain of IRAK1 plays a role in inducing apoptosis54.

Alterations in the RAS-MAPK pathway in MM are even more prevalent than alterations in the NF-κB pathway. In addition to the well-characterized NRAS, Kras, and BRAF genes, our study revealed that the germline Rasopathy genes represent a long tail of somatic alterations linking MM to these rare, congenital RAS-pathway-related diseases. If the designation of Rasopathy is extended to include mosaic conditions, such as keratinocytic epidermal nevus syndrome55, alterations in FGFR3 can also be integrated into this long tail, making alterations in the RAS-MAPK pathway even more prevalent in RRMM. It is generally observed that the spectrum of mutations in the Rasopathies and in cancer minimally overlap, as exemplified by PTEN11 and BRAF56,57. One possible explanation is that cancer-associated RAS-MAPK mutations would be lethal for embryonic development, while the Rasopathy-associated RAS-MAPK mutations are too mild to evade apoptosis in malignant transformation. Interestingly, RAS-MAPK aberrations in RRMM are a conglomeration of both, making RRMM an ideal model to study strong and weak RAS-activating events. Future studies should investigate the correlation between strong and weak RAS activators with the clinical history and outcome of RRMM patients.

We observed that IL6ST aberrations are enriched in RRMM, suggesting that this gene is associated with MM progression. IL6ST engages in both RAS-MAPK signaling via PTPN11 and JAK/STAT3 signaling via JAK40,41. As in inflammatory hepatocellular carcinoma, point mutations and in-frame indels of IL6ST occurred within or nearby the dimerization interface42,43 and could activate STAT3. Follow-up studies could investigate the use of approved JAK inhibitors, like ruxolitinib and tofacitinib, as potential therapeutic strategies for a subset of relapsed refractory multiple myeloma patients.

A unique opportunity afforded by this study was the ability to systematically compare the MMRF’s multi-institutional cohorts of NDMM patients with RRMM patients sequenced at our center. In addition to IL6ST mentioned above, mutations and copy-number loss in tumor suppressors such as TP53, RB1, CDKN2A/B, BIRC2/3, and CDKN2C were enriched in RRMM, suggesting that these are also likely events associated with disease progression. Our analyses also identified resistance alterations that develop in the context of commonly used MM therapies, including iMiDs (CRBN and CUL4B), synthetic glucocorticoids (NR3C1), and monoclonal antibodies (CD38), which expands upon previous studies58,59. The most common resistance alterations were associated with iMiDs, and these alterations have significant implications for clinical research and drug development beyond MM. For example, given that CRBN-based PROTACs are emerging as a promising approach for targeted
therapy\(^6^{0}\), developments in this drug class must anticipate possible resistance via acquired alterations in CRBN and CUL4B. Indeed, in vitro CRISPR-Cas9-mediated loss-of-function screening in prostate cancer cells treated with a CRBN-based BRD4 degrader also predicted CRBN as the most likely candidate for drug resistance\(^6^{0}\). Furthermore, as mutations in CRBN, CUL4B, and NR3C1 were detected in peripheral blood in a subset of RRMM patients, these genes should be included in CTC screening panels employing next-generation sequencing. Interestingly, despite our unbiased effort to search for the enrichment of mutations and copy-number alterations in RRMM compared to NDMM, a genetic basis for resistance against proteasome inhibitor drug class remains elusive. We suggest that genome-wide gain-of-function or loss-of-function CRISPR screening could help to narrow the candidate gene list.

Another clinically relevant aspect of our study concerns the monoclonal antibodies targeting CD38. Although potent as MM therapies, they were subject to resistance mutations and copy losses, according to our analyses. Two of our patients harbored distinct mutations that converged into the in-frame exon skipped affected codons 221 to 250, presumably only by disrupting the epitope of daratumumab while retaining a major portion of the extracellular domain. In theory, such patients could still benefit from isatuximab, another monoclonal therapy targeting CD38. As ref.\(^5^{2}\) pointed out, that the epitope of isatuximab is composed of residues from codons 34 to 189, thus completely unaffected by this exon skipping the event. Future structural and clinical studies should explore this direction to widen the therapeutic options for patients who relapse on daratumumab. Together, this study defines the genetic landscape and pathways of progression to RRMM while also identifying targeted therapy resistance mechanisms likely to impact the clinical management of RRMM.

**Methods**

**Patient samples.** All samples were acquired after patients provided written informed consent in compliance with the Multiple Myeloma Research Foundation Institutional Review Board (IRB) (Protocol MMRF-002; IRB Tracking Number 20151186) and the University of Michigan IRB. Germline assessment was carried out using blood specimens. CD138+ cells from fresh bone marrow mononuclear cells were isolated by immunomagnetic selection. In brief, mononuclear cells were separated from bone marrow by density centrifugation by Ficoll-Paque (GE Healthcare). Mononuclear cells were then incubated with CD138 antibody-conjugated microbeads and loaded onto a MACS column according to the manufacturer’s instructions (Miltenyi Biotec). After washing with 10 volumes of PBS buffer, the column was removed from the magnetic stand, and CD138+ tumor cells were eluted. Smears of the selected cells were prepared by cytospin centrifuge and stained by HEMA-DIFF (+) tumor cells and matched-normal blood mononuclear cells were resuspended in RLT lysis buffer (Qiagen) and disrupted by 5 mm beads on a Tissuelyser II (Qiagen). Genomic DNA and total RNA were purified from the same sample using the AllPrep DNA/RNA/miRNA kit (Qiagen). Matched-normal genomic DNA from blood, buccal swab, or saliva was isolated using a DNeasy Blood & Tissue Kit (Qiagen). RNA integrity was measured on an Agilent 2100 Bioanalyzer using RNA Nano reagents (Agilent Technologies). RNA-sequencing was performed by the exome-capture transcription platform developed in our lab and as described previously\(^6^{0}\). In brief, capture transcriptome libraries were prepared using 1–2 μg of total RNA. Following the steps of cDNA synthesis, end-repair, A-base addition, and ligation of adapters, pre-capture libraries were size-selected by the PippenHT system (Sage Science). Recovered fragments were enriched by PCR using Phusion DNA polymerase (New England Biolabs) and index primers and purified by AMPure XP beads (Beckman Coulter). Coding exons were then captured by Agilent SureSelect Human All Exon v4 probes using the manufacturer’s protocol. Final sequencing libraries were analyzed by Agilent 2100 Bioanalyzer for product size and concentration. Libraries were sequenced by the Illumina HiSeq 2500 (2 × 126-nucleotide read length), with a sequencing coverage of 40–60 million paired reads. Reads that passed the chastity filter of Illumina BaseCall software were used for subsequent analysis.

Exome libraries of matched pairs of tumor/normal DNA were prepared as previously described\(^6^{0}\). In brief, 1 μg of genomic DNA was sheared using a Covaris S2 (Covaris) to a peak target size of 250 bp. Fragmented DNA was purified using AMPure beads, followed by end-repair, A-base addition, and ligation of adapters using the Kapa HyperPrep kit and protocols (Roche/Kapa Biosystems). DNA molecules were size-selected by the PippenHT system (Sage Science). Fragments between 300–350 bp were recovered, amplified by KAPA HiFi HotStart Mix and index primers, and purified by AMPure beads. One microgram of the pre-capture library was hybridized to an in-house developed Oncoseq targeted gene panel containing 1711 genes with suggestive links to cancer (probes synthesized by Roche). The targeted exon fragments were captured and enriched following the manufacturer’s protocol (Roche). Final sequencing libraries were analyzed by Agilent 2100 Bioanalyzer and sequenced by Illumina HiSeq 2500 (Illumina; 2 × 126-nucleotide read length).

**Alignment, mutation calling, and filtering.** The FASTQ files were aligned to the reference genome build hg19/GRCh37 using Novoalign\(^6^{0}\) Multithreaded (version 3.3.02.08) (Novocraft) and converted into BAM files using Samtools\(^6^{0}\) (version 0.1.19) (Broad Institute). BAM files were sorted and marked for duplication (version 1.03.02). Single nucleotide variants (SNVs) and small indels were called by freebayes\(^6^{5}\) (version 1.0.1). Larger indels and exon-level structural arrangements were called with pindel\(^6^{6}\) (version 0.2.359). Freebayes and pindel calls were then compiled and annotated to RefSeq and COSMIC\(^6^{0}\) v90, dbSNP\(^6^{7}\) v146, ExAC\(^6^{8}\) v0.3, and 1000 Genomes phase 3\(^9\) databases using snpEff\(^6^{5}\) (version 4.3t) and snpSift\(^6^{7}\) (version 4.3t).

We employed a two-step filtering strategy to detect somatic mutations when there was contamination of CTCs in peripheral blood controls. First, mutation calling was performed on normal libraries to estimate the level of contamination. This information would be used to adjust the somatic filtering threshold accordingly. To be more specific:

- In the normal-only variant filtering step, a normal-contaminated mutation was identified as the call that: (1) was supported by at least five reads, (2) had the population minor allele frequency (MAF) <0.05%, (3) passed the filtering against an in-house database of recurrent sequencing artifacts (e.g., pool-filtering) constructed from 2000 genomic sequencing libraries. Known hotspot mutations (such as those from TP53, NRAS, KRAS, BRAF, PIK3CA, etc.) were inferred from COSMIC v90 and 2000 genomic libraries of the same sequencing platform at our center, then “white-listed” out from the pool-normal. This is because variants at these locations are more likely to be real mutations than artifacts. To enhance the specificity, variants with ultra-low Variation Allelic Fraction VAF (<5%) were (4) further filtered for K introartifacts\(^5^{3}\), (5) passed stringent evidence supported by reads in both forward- and reverse- strands. Strand-specific reads at a given position were counted by bam-readcount\(^7^{4}\) (version 0.8.0) for SNV and small indel up to 5 bp. The maximum VAF of all contaminated mutations in each normal library was then used as a threshold to filter somatic mutations in the next step. This threshold could be more than 30% for some samples in our cohort (Supplementary Fig. 2d). This approach allowed us to discover CTC-associated mutations that may not even be present at the biopsy site (Supplementary Fig. 9g).

- In the tumor-normal variant filtering step, in addition to satisfying criteria (1–5) above, a somatic mutation should tolerate a matched-normal VAF up to the contamination threshold (if there is no normal contamination, an arbitrary threshold of 2% was applied, Supplementary Fig. 2d, horizontal dashed line). To further distinguish recurrent indel artifacts in homopolymer regions from true somatic variants, especially in cases where instability matches the proportion of the process model using PCR duplication rate as a covariate to model variant and total read counts were applied to improve the read cut-off for indels, as detailed previously\(^7^{5}\).

Variant calling for SNVs and small indels from RNA-seq (tumor-only) was performed using sentence\(^\ast\) (version 202010.02) (Sentience, Inc.). Calls were annotated to RefSeq and COSMIC\(^6^{0}\) v90, dbSNP\(^8\) v146, ExAC\(^6^{9}\) v0.3, and 1000 Genomes phase 3. We adopted a similar variant filtering strategy to the normal-only variant filtering step detailed above, and further filtered calls against RADAR\(^7\), a database for A-to-T RNA editing. Potential variants in CD38 were validated by whole-exome sequencing using Agilent Human All Exon v4 reagents.

**Copy-number analysis.** Targeted sequencing and whole-exome sequencing data were analyzed for copy-number using an in-house pipeline (cnaTools), as previously described\(^7\). Circular dichromatic segmentation (CDS) algorithm (as implemented in DNAcopy\(^6^{0}\) version 1.48.0) was used to jointly segment B-allele frequencies and log2-transformed tumor/normal coverage ratios across targeted
regions. The expectation-maximization (EM) algorithm was used to jointly estimate tumor purity and classify regions by copy-number status, namely gain, loss, and loss of heterozygosity. CNVkit (version 0.6.16) with CBB and haaf segmentation method were also run in all samples. Results from cnatools and CNVkit were compiled by a customized script to inspect discrepancies in segmentation, especially for highly heterogeneous samples with subclonal CNAs. For each result from cnatools, different ploidy models were manually reviewed by D.R., Y.-M.W., and I.N.V. to account for the possibility of whole-genome duplication (tetraploidy).

GISTIC2.0 was used to locate arm-level and focal peaks of recurrent copy-number gains and losses. Germline CNAs, IgH, Igk, TCR loci, and recurrent noisy segments were removed before running GISTIC2.0. Significant peaks were defined as those with a p-value < 0.05.

**Trinucleotide mutational analysis.** The limited number of mutations detected from targeted sequencing in our study did not allow us to discover de novo trinucleotide mutational signatures. However, APOBEC-enriched patients can be assessed as in Roberts et al.2. For each case, within a fraction of the captured genome, the number of C-to-T or C-to-G, and G-to-A or G-to-C substitutions that occurred in and out of the APOBEC motif (TCW or WGA), as well as the total number of C or G occurred in and out of APOBEC motif, was tabulated. One-sided Fisher’s exact tests were performed, and P values were corrected using the Benjamini–Hochberg method. Samples with FDR < 0.05 were marked as APOBEC-enriched.

**Gene expression and fusion analysis.** Strand-specific RNA-seq FASTQ files were aligned to reference genome build hg19/GRCh37 in chimeric alignment mode by STAR aligner (version 2.7.4a). After alignment, libraries with ribosomal content ≥60% mapped reads (i.e., failed ribosomal removal) and libraries with a low number of splice junctions (<25th percentile of all in-house libraries) were excluded from the final cohort. Gene expression was quantified with featureCounts (version 2.0.0), and gene fusions were called using an in-house pipeline as previously described. Highly recurrent RNA chimeric transcripts (e.g., “trans-splicing”) were filtered out from the reported fusions (Supplementary Data 4). Differential gene expression analysis was performed with edger (version 3.34.0).

**CoMMpass data re-analysis.** Using CoMMpass consortium data, we reanalyzed raw (FASTQ) NGS data using our integrative pipeline so that direct comparisons could be made to RMM samples sequenced in this study. FASTQ files were downloaded from dbGaP with the accession number phs000748 and analyzed for mutation and copy-number, as outlined above. We adjusted critical parameters and reference files as CoMMpass used a different probe set for whole-exome sequencing (Agilent Capture V5 + 5’UTR). After excluding duplicate entries, and samples without available matched peripheral blood normal or CD3 + selection normal, the remaining samples were included for mutation and copy-number analyses. A small number of CoMMpass samples were subjected to whole-exome sequencing more than once. To fairly compare mutation and CNA incidence, we only included the cases with the highest number of mutations. Finally, we excluded samples that did not pass quality control assessed from Picard’s AT-dropout metrics, which resulted in noisy copy-number results.

**Nomination of cancer driver genes panel.** Integration of results from various statistical tools that predict significantly mutated genes would result in a more comprehensive list of key cancer driver genes since these tools are complementary. We, therefore, ran oncodriveCLUSTR (version 1.1.3), MutsigCV88, MutsigCV89, and 20+20+90 (version 1.0.1) on RMM SNV and small indels. A relaxed threshold FDR <0.2 was applied in each tool to call significantly mutated genes. Finally, we only included genes that were predicted to be significantly mutated in at least two different statistical tools (Supplementary Fig. 2a).

**Outlier expression analysis.** To search for rare outlier expression, we used the statistical approach employed by the R package OUTRIDER (version 1.7.1), which models RNA-seq read counts with a negative binomial distribution and corrects for variations in sequencing depth and co-variations across samples. Before parameter fitting, OUTRIDER requires the control of confounders, which was performed by PEER implementation on 100 factors. Finally, genes with outliers were prioritized by the minimum p-value (or maximum log p-value) among all detected samples.

**Clonality analysis.** The cancer cell fraction (CCF) of a variant (including point mutations or small insertions) was defined as in ref 95. Briefly, the relationship between mutation multiplicity $m_i$ of a variant and its cancer cell fraction CCF is considered as the following:

$$ u_i = \text{CCF}_i m_i $$

**Comparing point mutation frequencies between cohorts.** The number of mutations affected by point mutations in the RRMM and NDMM cohorts was tabulated for each gene in the Onco1700 panels. Functionally, point mutations can be synonymous, missense, stop-gain, start-loss, and splice site. Two-sided Fisher exact tests were performed, and FDR was calculated using the Benjamini–Hochberg method. Samples with FDR < 0.05 were marked as APOBEC-enriched.  

**Structural rearrangements analyses for whole-genome sequencing.** Whole-genome sequencing FASTQ files were aligned to the reference genome build hg19/GRCh37 with bwa-mem95 (version 0.7.17). BAM files were sorted, indexed with Samtools, and marked duplicates with samblaster96 (version 0.1.25). Structural rearrangements such as chromosomal translocations and deletions were called with LUMPY97 (version 0.3.1). Split reads supporting the breakpoints were further confirmed by Blast98.

**Data visualization.** Molecular graphics were generated with Pymol100 (version 1.8.2) and some illustrations were created using BioRender.com.

**NF-kB reporter assay.** The impact of mutations in CD40 and TNFRSF17 on the NF-kB signaling pathway was assessed by NF-kB reporter assays. HEK-293FT cells (purchased from the ThermoFisher/Invitrogen) were plated in 24-well plates at a density of 10^5 cells per well in Dulbecco’s Modified Eagle medium (DMEM) containing 10% fetal bovine serum and antibiotics. The next day, cells were transiently co-transfected with CD40 or TNFRSF17 wt and mutant expression plasmids at 250 ng/well, the pGL4.32[luc2P-NF-kB-RE/Hygro] reporter plasmid at 250 ng/well (Promega), and the pRL-TK internal control plasmid at 25 ng/well (Promega) using the FuGene-HD transfection protocol (Promega). The pGL4.32[luc2P-NF-kB-RE/Hygro] plasmid contains five copies of an NF-kB response element that produces luciferase reporter protein. Cells were harvested 48 hours post-transfection, and luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega).

**Functional analysis of IL6ST variants.** The full-length open reading frame of the wild type IL6ST was generated by PCR. Patient-derived IL6ST mutations were subsequently generated by site-directed mutagenesis (QuikChange, Agilent). IL6ST variants were then cloned in the lentivector pDH510 for mammalian expression. Expression constructs were transfected into HEK-293FT cells using FuGene-HD transfection reagent (Promega). Transfected cells were cultured in DMEM medium supplemented with 10% FBS for 48 and harvested for Western blot analysis. HEK-293FT was purchased from the ThermoFisher/Invitrogen and validated by genotyping. Antibodies and their commercial sources are as follows: anti-IL6ST/Epitope130 (Abcam, ab283685, 1:1,000 dilution), anti-epsilon-tubulin (Abcam, ab184577, 1:3,000 dilution), anti-phospho-STAT3+Y705 (Cell
can be found on dbGaP website. All structures used in the analysis (7DUO52 and 4CI246) datasets must be renewed annually. Additional information about the application process for phenotype information. Principal investigators wishing to access these data must submit an application to dbGaP. The remaining data are available within the Article, Supplementary Information, or Source Data file. Source data are provided with this paper.

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References
1. van de Donk, N. W. C. J., Pawlyn, C. & Yong, K. L. Multiple myeloma. Lancet 397, 410–427 (2021).
2. Greenberg, A. J., Vachon, C. M. & Rajkumar, S. V. Disparities in the treatment of multiple myeloma. Blood 129, 653–667 (2012).
3. Bazarbachi, A. H., Al Hamed, R., Malard, F., Harousseau, J.-L. & Mohty, M. Treatment of relapsed and refractory multiple myeloma: recommendations from the International Myeloma Working Group. Lancet Oncol. 22, e105–e118 (2021).
4. Keats, J. et al. Interim analysis of the MMRF CoMMpass study: comprehensive characterization of multiple myeloma patients at diagnosis reveals distinct molecular subtypes and clinical outcomes. Clin. Lymphoma Myeloma Leuk. 15, e44–e45 (2015).
5. Roychowdhury, S. et al. Personalized oncology through integrative high-dimensional data analysis. Nat. Rev. Clin. Oncol. 16, 39–53 (2019).
6. Robinson, D. R. et al. Integrative clinical genomics of metastatic cancer. Nature 548, 297–303 (2017).
7. Cobain, E. F. et al. Assessment of clinical benefit of integrative genomic profiling in advanced solid tumors. JAMA Oncol. 7, 525–533 (2021).
8. Bailey, M. H. et al. Comprehensive characterization of cancer driver genes and mutations. Cell 174, 1031–1045 (2018).
9. Morgan, G. J., Walker, B. A. & Davies, F. E. The genetic architecture of multiple myeloma. Nat. Rev. Cancer 12, 335–348 (2012).
10. Yang, Y. et al. Identification of novel mutation drivers reveals oncogenic dependencies in multiple myeloma. Blood 132, 587–597 (2018).
11. Walker, B. A. et al. APOBEC family mutational signatures are associated with poor prognosis translocations in multiple myeloma. Nat. Commun. 6, 6979 (2015).
12. Zhou, Y., Barlogie, B. & Shaughnessy, J. D. Jr. The molecular characterization and clinical management of multiple myeloma in the post-genome era. Leukemia 23, 1941–1956 (2009).
13. Demchenko, Y. N. et al. Classical and/or alternative NF-kB pathway activation in multiple myeloma. Blood, J. Am. Soc. Hematol. 115, 3541–3552 (2010).
14. Keats, J. et al. Promiscuous mutations activate the non-canonical NF-κB pathway in multiple myeloma. Cancer Cell 12, 134–144 (2007).
15. Demchenko, Y. N. & Kuehl, W. M. A critical role for the NFκB pathway in multiple myeloma. Oncotarget 1, 59–68 (2010).
16. Morgan, G. J. et al. Kinase domain activation through gene rearrangement in multiple myeloma. Leukemia 32, 2435–2444 (2018).
17. Zarnegar, B. J. et al. Noncanonical NF-κB activation requires coordinated assembly of a regulatory complex of the adaptors cIAP1, cIAP2, TRAF2 and the kinase NIK. Nat. Immunol. 9, 1317–1327 (2008).
18. Lee, S., Chall-a-Malladi, M., Bradton, S. B. & Wright, C. W. Nuclear factor-κB-inducing kinase (NIK) contains an amino-terminal inhibitor of apoptosis (IAP) binding motif (IBM) that potentiates NIK degradation by cellular IAP1 (c-IAP1). J. Biol. Chem. 289, 30680–30689 (2014).
19. Hayden, M. S. & Ghosh, S. Signaling to NF-kappaB. Genes Dev. 18, 2195–2224 (2004).
20. Brennan, P. J., Kumagai, T., Berezov, A., Murali, R. & Greene, M. I. HER2/new: mechanisms of dimerization/oimerization. Oncogene 19, 6093–6101 (2000).
21. MacLennan, I. C. M. & Vinuesa, C. G. Dendritic cells, BAFF, and APRIL define a new cytokine family. J. Biol. Chem. 273, 235–238 (2002).
22. Yan, Q. et al. BCR and TLR signaling pathways are recurrently targeted by genetic changes in splenic marginal zone lymphomas. Haematologica 97, 595–598 (2012).
23. Lenz, G. et al. Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. Science 319, 1676–1679 (2008).
24. Pasqualucci, L. et al. Genetics of follicular lymphoma transformation. Cell Rep. 6, 130–140 (2014).
25. De Nardo, D. et al. Interleukin-1 receptor–associated kinase 4 (IRAK4) plays a dual role in myeloid formation and Toll-like receptor signaling. J. Biol. Chem. 293, 15195–15207 (2018).
26. Prieto, I. A., Hood, F. E. & Hartley, J. L. The frequency of Ras mutations in cancer. Cancer Res. 80, 2969–2974 (2020).
27. Yao, Z. et al. BRAF mutants evade ERK-dependent feedback by different mechanisms that determine their sensitivity to pharmacologic inhibition. Cancer Cell 28, 370–383 (2015).
28. Yao, Z. et al. Tumours with class 3 BRAF mutants are sensitive to the BRAFI inhibitor vemurafenib. Nature 548, 234–238 (2017).
29. Chen, S.-H. et al. Oncogenic BRAF deletions that function as homodimers and are sensitive to inhibition by RAF dimer inhibitor LY3009120. Cancer Discov. 6, 300–315 (2016).
30. Rivas, J. et al. Promiscuous mutations activate the non-canonical NF-kappaB pathway: stabilization of c-Myc in Burkitt’s lymphoma cells. Mol. Cell. Biol. 30, a044244 (2020).
31. Rauen, K. A. The RASopathies. Annu. Rev. Genomics Hum. Genet. 14, 355–369 (2013).
32. Aoki, Y., Nihori, T., Inoue, S.-I. & Matsubara, Y. Recent advances in the RASopathies. J. Hum. Genet. 61, 33–39 (2016).
33. Tidyman, W. E. & Rauen, K. A. Expansion of the RASopathies. Curr. Genet. Med. Rep. 4, 57–64 (2016).
34. Nihori, T. et al. Germline KRAS and BRAF mutations in cardio-facio-cutaneous syndrome. Nat. Genet. 38, 294–296 (2006).
35. Rassuque, M. et al. Germline gain-of-function mutations in RAF1 cause Noonan syndrome. Nat. Genet. 43, 1013–1017 (2011).
36. Bigenzahn, J. W. et al. LZTR1 is a regulator of Ras ubiquitination and signaling. Science 362, 1171–1177 (2018).
37. Ohtani, T. et al. Dissection of signaling cascades through gp130 in vivo: reciprocal roles for STAT3- and SHP2-mediated signals in immune responses. Immunity 15, 95–103 (2000).
38. Fukushima, T. et al. Two signals are necessary for cell proliferation induced by a cytokine receptor gp130: involvement of STAT3 in anti-apoptosis. Immunity 5, 449–460 (1996).
39. Reburn, S. et al. Frequent in-frame somatic deletions activate gp130 in inflammatory hematopoietic tumours. Nature 547, 200–204 (2019).
40. Poussin, K. et al. Biochemical and functional analyses of gp130 mutants unveil Jak1 as a novel therapeutic target in human inflammatory hematopoietic adenocarcinoma. Oncoimmunology 2, e270990 (2013).
41. Misund, K. et al. MYC dysregulation in the progression of multiple myeloma. Leukemia 34, 322–326 (2020).
42. Gregory, M. A. & Hann, S. R. c-Myc proto-oncogene pathway: stabilization of c-Myc in Burkitt’s lymphoma cells. Mol. Cell. Biol. 20, 2432–2433 (2000).
43. Fischer, E. S. et al. Structure of the DDB1-CRB1 E3 ubiquitin ligase in complex with thalidomide. Nature 512, 49–53 (2014).
44. Kronke, J. et al. Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. Science 343, 301–305 (2014).
45. Kregel, S. et al. Functional and mechanistic interrogation of BET bromodomain degraders for the treatment of metastatic castration-resistant prostate cancer. Clin. Cancer Res. 25, 4038–4048 (2019).
46. Alexanian, R., Barlogie, B. & Dixon, D. High-dose glucocorticoid treatment of resistant myeloma. Ann. Intern. Med. 105, 8–11 (1986).
47. Tan, J. et al. Genomic landscapes of breast fibroepithelial tumours. Nat. Genet. 47, 1341–1345 (2015).
Multiple Myeloma Research Consortium

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