Dynamic transcriptional reprogramming leads to immunotherapeutic vulnerabilities in myeloma

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While there is extensive evidence for genetic variation as a basis for treatment resistance, other sources of variation result from cellular plasticity. Using multiple myeloma as an example of an incurable lymphoid malignancy, we show how cancer cells modulate lineage restriction, adapt their enhancer usage and employ cell-intrinsic diversity for survival and treatment escape. By using single-cell transcriptome and chromatin accessibility profiling, we show that distinct transcriptional states co-exist in individual cancer cells and that differential transcriptional regulon usage and enhancer rewiring underlie these alternative transcriptional states. We demonstrate that exposure to standard treatment further promotes transcriptional reprogramming and differential enhancer recruitment while simultaneously reducing developmental potential. Importantly, treatment generates a distinct complement of actionable immunotherapy targets, such as CXCR4, which can be exploited to overcome treatment resistance. Our studies therefore delineate how to transform the cellular plasticity that underlies drug resistance into immuno-oncologic therapeutic opportunities.

Although most patients with cancer respond to initial treatment, responses are often short-lived as drug resistance frequently develops. Non-genetic cellular plasticity and adaptive state changes have recently emerged as a basis for therapeutic resistance in cancer1–3, and a better understanding of how cell-state transitions are regulated is critical to develop therapeutic approaches that can overcome drug resistance. These transitions are mediated by dynamic transcriptional changes and can involve epigenetic remodelling of the open chromatin landscape or changes in the activity of transcriptional regulators4–7.

To investigate lineage infidelity and drug resistance at the single-cell level, we studied multiple myeloma (MM), a malignancy of antibody-producing plasma cells in the bone marrow. The notion that plasma cells are terminally differentiated cells has long prevented intensive research into phenotypic plasticity and differentiation in MM; however, it has recently been demonstrated that normal and malignant plasma cells differ in their differentiation state8–9. Furthermore, it has been suggested that more immature states may correlate with drug resistance8. Yet, it remains unclear how heterogeneous differentiation states are regulated at the transcriptional and epigenetic level and whether they change with treatment.

Here, we use single-cell transcriptome and chromatin accessibility profiles to define the transcriptional and cis-regulatory alterations that underlie cell-state transitions in malignant plasma cells. We infer gene regulatory network (GRN) configurations during the establishment of distinct cell states and highlight regulatory factors driving phenotypic changes. These phenotypic changes in turn provide the rationale for targeted therapeutic strategies to overcome drug resistance.

Results

Coexisting transcriptional programmes in single myeloma cells. To define transcriptional states in myeloma at single-cell resolution, we used SmartSeq2 (ref. 10) to perform full-length single-cell RNA sequencing (scRNA-seq) of myeloma cells and CD45+ immune cells from the bone marrow or blood of eight patients with relapsed/refractory MM (RRMM) and of two healthy donors (Fig. 1a and Extended Data Fig. 1). Patients were treated with elotuzumab (Elo), pomalidomide (P), bortezomib (V) and dexamethasone (D) in a clinical trial (Elo-PVD; ClinicalTrials.gov identifier NCT02718833; Supplementary Table 1), and we obtained bone marrow samples at screening and following treatment. A total of 6,955 cells were analysed and underwent quality control (QC) filtering (Extended Data Fig. 2 and Supplementary Table 2). As an initial examination of transcriptional heterogeneity, we performed PAGODA2 clustering11 (Fig. 1b,c). Reference-based annotation of the single-cell profiles using a dataset of 21 immune cell populations12 showed that clusters 20, 1, 5, 7, 14, 12, 3 and 13 corresponded to CD4+ and CD8+ immune cells (Fig. 1d).

We next set out to distinguish malignant from non-malignant cells. We estimated copy number variations (CNVs), which occur in virtually all patients with MM. CNVs were highly enriched in...
presumed malignant clusters, which was highly concordant with results from clinical cytogenetics (Extended Data Fig. 3a and Supplementary Table 3), and were distinct between individual clusters (Fig. 1c and Extended Data Fig. 3b). As the immunoglobulin sequences are typically monoclonal or rarely oligoclonal in myeloma cells, we reconstructed the CDR3 region for each cell. Indeed, presumed malignant cells from individual patients displayed a high degree of clonality, with the most common CDR3 sequence found in the majority of cells (Fig. 1e). As MM cells frequently harbour translocations involving the immunoglobulin heavy chain locus, we next assessed the expression of translocation target genes CCND1 and MAF and detected overexpression in clusters 6, 17 and 2, and in cluster 8, respectively, which was concordant with the fluorescence in situ hybridization results (Fig. 1e and Extended Data Fig. 3a).

We then employed a random forest (RF) model to determine genes that best discriminate presumed malignant cells and normal plasma cells (Fig. 1e and Extended Data Fig. 3c–e). The RF model identified several genes that have previously been described as drivers in MM, such as CCND1 and the modulator of Wnt signalling FRZB. Myeloma marker genes, which thus far have not been described in MM, include the cytoskeletal linker protein dystonin (DST). Using an aggregate approach combining myeloma-specific gene expression with copy number and clonotype analysis, we were able to robustly distinguish myeloma cells from normal plasma cells, even within patients, including cases for which PAGODA2 clustering alone was ambiguous (Fig. 1f and Extended Data Fig. 3f,g).

Characterization of malignant clusters through marker gene analysis identified known myeloma driver genes, such as MAF consistent with a t(14;16) translocation in patient MM7 (Extended Data Fig. 3h and Supplementary Table 4). Interestingly, some driver genes were highly expressed in more than one MM cluster, for example, CCND1 in 3 out of 8 patients (Fig. 1e). We therefore asked whether the distinct clustering of myeloma cells from patients with the same established genomic classifier (11;14), which results in the overexpression of CCND1 (Extended Data Fig. 3a,d,h), could be due to the presence of multiple gene expression signatures in the same cells. We indeed observed that individuals with CCND1 translocation expressed the corresponding CD1 signature, but further manifested distinct expression of other signatures, such as the NFKB signature (patient MM1) or the proliferation signature PR (patient MM8) (Fig. 1g). We detected the co-expression of unrelated signatures in the same single cells in each individual, which argues that there are widespread transcriptional differences not captured by established gene expression classifiers.

To investigate transcriptional heterogeneity in greater detail, we first assessed the effect of the cell cycle as a potential source of heterogeneity. We did not observe cell-cycle-driven effects on PAGODA2 clustering, with the exception of cluster 10, which consisted of cycling cells derived from multiple patients (Fig. 2a,b). We next scored the expression of 11 recurrent heterogeneity programmes identified across 198 cell lines reflecting 22 cancer types (Fig. 2c). These programmes representing diverse biological processes, including the cell cycle, the stress response and protein maturation, revealed widespread inter- and intra-patient heterogeneity.

To identify other cellular states displaying variability in myeloma cells, we employed non-negative matrix factorization to identify expression programmes in our dataset (Fig. 2d). Using this approach, we detected six robust expression programmes across patients. Apart from the cell cycle, these reflected cell signalling states, such as KRAS–MAPK signalling, IL-2–STAT5 signalling, the interferon (IFN) response and IL-6–STAT3 signalling, a known survival pathway in MM (Fig. 2d–f). The sixth programme consisted of genes related to the endoplasmic reticulum, a cellular component involved in protein maturation and secretion, processes that are of paramount importance for myeloma cells. Scoring of these expression signatures revealed widespread transcriptional variability across the identified expression programmes between and within patients (Fig. 2g).

Lineage infidelity and plasticity in myeloma cells. We next sought to define the nature of the transcriptional profiles that are co-expressed in individual cancer cells. We observed the expression of genes not normally detectable in plasma cells, such as MS4A1 (which encodes CD20), BLK (which encodes the B lymphoid tyrosine kinase) and CTSC (which encodes serine protease C) (Extended Data Fig. 4a). To investigate whether myeloma cells acquire transcriptional states from less mature or entirely different haematopoietic lineages, we defined expression signatures derived from the BLUEPRINT dataset. We found that the transcriptional states of myeloma cells indeed diverged from normal plasma cells towards more immature progenitor populations of the lymphoid or entirely different haematopoietic precursors (Fig. 3a,b). Notably, these transcriptional signatures were co-expressed in the same single cells. We validated our observations using signatures from the Human Cell Atlas (Extended Data Fig. 4b,c).

To capture the differentiation trajectories between different developmental stages, we performed cluster analysis based on the expression of progenitor signatures (Supplementary Table 5) and performed RNA velocity analysis. We identified a differentiation trajectory from less to more differentiated cells (Fig. 3c,d). The plasma cells from healthy donors clustered towards the endpoint of the trajectory, which is consistent with their state of terminal differentiation (Extended Data Fig. 4d,e). Cycling cells were enriched towards the starting point of the trajectory (Extended Data Fig. 4f). As transcriptional diversity is a hallmark of developmental potential, which is progressively restricted as cells differentiate, we assessed the number of genes expressed per cell and transcriptional entropy (Extended Data Fig. 4g–j) and detected increases in both measures in MM (P < 2.2 × 10−16 by two-sided Wilcoxon test). Using CytoTRACE, we predicted a higher developmental potential for myeloma cells than for normal plasma cells (Fig. 3e and Extended Data Fig. 4k). The cells with the highest CytoTRACE score (that is, the most immature cells) mapped to the starting point of the trajectory by RNA velocity.

To further define the two divergent differentiation endpoints, we determined the top ranked genes correlated with developmental potential (Supplementary Table 6), which included LAMP5, a recently identified marker of MM upregulated through epigenetic activation (Fig. 3f). Gene sets associated with immaturity included...
metabolic processes, Myc activation and a plasmablast-like signature (Fig. 3g). Interestingly, we observed that the starting and endpoints of the differentiation trajectory were associated with divergent signalling states characterized by mutually exclusive activation of either the MAPK pathway or the PI3K pathway (Fig. 3h,i). While MAPK activity was detected in a subset of cycling and
immature cells, the more mature cells showed activation of PI3K, which is consistent with previous reports showing that PI3K activity induces PRDM1 and terminal differentiation of B cells\(^2\). These findings suggest that MM cells arrange along a spectrum of two divergent states, which are characterized by heterogeneity in differentiation state and signalling activity.

**Fig. 2** | Transcriptional heterogeneity in single myeloma cells. a, tSNE plot coloured by cell-cycle phase predicted by SEURAT. b, Stacked bar plot showing the relative proportion of cells in each cell-cycle phase per cluster. c, Heatmap showing the expression scores for recurrent heterogeneity programmes in patients with MM (scores identified across 198 cell lines reflecting 22 cancer types\(^1\)). d, Consensus matrix depicting pairwise similarities between NMF programmes ordered by hierarchical clustering. Six clusters corresponding to the six identified programmes and assignment of patients are indicated on the top. Functional annotation and selected marker genes are shown below. e, Heatmap showing the top 50 genes based on the highest NMF scores selected as signature genes for each programme, with selected genes labelled. f, Functional enrichment (\(-\log_{10}(\text{FDR})\)) of heterogeneity programmes with six annotated gene sets. g, Heatmap showing expression scores for the six heterogeneity programmes identified in d. Enlarged heatmaps in c and g are provided in the Supplementary Information.
To distinguish whether the observed results reflect defective differentiation or a loss of lineage restriction, we assessed the expression of transcriptional regulators of plasma cell lineage, including XBP1, PRDM1 and IRF4. Expression of these regulators was preserved in MM (Extended Data Fig. 4l), which argues that MM cells retain plasma cell identity and manifest a loss of lineage restriction rather than a block in differentiation.

Regulatory network usage and chromatin accessibility in MM. As GRNs play a key role in the regulation of cell fates, we set out to define the underlying GRN in MM. We therefore defined transcription factor (TF; regulon) activity using SCENIC. We identified transcriptional modules present in normal haematopoiesis in the BLUEPRINT dataset and determined the dominating module containing plasma cell transcriptional regulators (Fig. 4a and Extended Data Fig. 4l). In our healthy donor plasma cells, we detected activity predominantly in the plasma cell module (Fig. 4b). By contrast, GRN activity of myeloma cells was no longer confined to the plasma cell module but was observed across other modules assigned to haematopoietic cells, including haemopoietic stem cell and macrophage modules (Fig. 4b and Extended Data Fig. 4c–d).

To identify state-specific GRNs in MM, we next constructed a network based on MM cell regulon activity, as well as a network based on normal haematopoietic cells (Extended Data Fig. 4c), and created an overlay (Fig. 4c). Interestingly, while the majority of regulons have activity in healthy haematopoietic cells and in myeloma...
cells, we identified many regulons that were largely inactive or entirely absent in normal haematopoiesis (Fig. 4c,d, Extended Data Fig. 5e and Supplementary Tables 7 and 8). ELF3 and TEAD4 are two examples of regulons that gain activity in MM cells (Extended Data Fig. 5f). ELF3 is a member of the epithelium-specific ETS TFs expressed predominantly in epithelial tissues\(^23\), while TEAD4 acts as a downstream regulator of the Hippo pathway and binds to the M-CAT motif found primarily in muscle-specific genes\(^24\). Among their target genes are those not normally expressed in plasma cells, including CD3E, CD5 and CD300A (Supplementary Table 9). These data illustrate an aberrant and promiscuous activation of transcriptional regulators in MM that are not active in normal haematopoietic cells, which is in line with a loss of lineage restriction.

Importantly, we also identified a shared set of regulons between malignant and normal plasma cells, including XBP1, IRF4 and PRDM1, which indicates that myeloma cells retain lineage-specific regulons (Extended Data Fig. 5g). This is consistent with our previous finding that MM cells retain plasma cell identity. Interestingly, even regulators that define plasma cell lineage, such as XBP1, gained network connections in myeloma cells, which suggests that while myeloma cells maintain activity of canonical plasma cell regulators, they acquire additional target genes outside the plasma cell module (Fig. 4d).

We therefore determined the TFs with the highest rewiring score for which connections between transcriptional regulators and target genes are altered\(^2\) (Fig. 4e and Supplementary Table 10). We identified ELF3 and TEAD4 as the top-most rewired TFs, followed by XBP1 (Fig. 4e and Supplementary Table 10), which indicates that rewired TFs include lineage-defining and non-lineage TFs. Other rewired TFs included E2F1, which has been described as a master regulator of proliferation\(^2\). E2F1 has been shown to be essential in myeloma cells and has been identified as a potential therapeutic target\(^5\), which suggests that other transcriptional nodes, in particular TFs with a high rewiring score, could represent attractive therapeutic targets.

We next identified the top rewired cell surface markers that may represent potential therapeutic targets. Deregulated surface molecules included the growth factor receptors PDGFR receptor A and B (encoded by PDGFR\(A\) and PDGFR\(B\), respectively), as well as the B cell maturation antigen BCMA (encoded by TNFRSF17) (Fig. 4f). These data illustrate that substantial transcriptional rewiring occurs in myeloma cells even though the expression of TFs that define plasma cell lineage is preserved, and misexpression of surface proteins appears to be an important consequence of this rewiring.

To assess whether the identified rewired TFs represent therapeutic vulnerabilities, we investigated CRISPR-based screening data from the Depmap portal (https://depmap.org/portal/). We found that some rewired TFs, such as XBP1, are essential in MM (Fig. 4g,h). Essential TFs were strongly enriched in B cell/plasma cell TFs (Supplementary Table 11), including XBP1, IRF4 and IKZF1 (Fig. 4i,j), thereby reinforcing the notion that MM cells do not lose their plasma cell identity and that plasma-cell-defining TFs are essential for cell survival. However, other rewired TFs that do not define the plasma or B cell lineages, such as TAL1, do not generate lineage dependencies in myeloma, which is probably due to plasticity and redundancy in the transcriptional network (Fig. 4g,k).

To assess whether increased transcriptional diversity could be attributed to greater chromatin accessibility, we performed single-cell ATAC sequencing (scATAC-seq) on myeloma cells from a subset of patients as well as healthy donor plasma cells (Fig. 5a). A total of 1,483 cells were analysed and underwent QC filtering (Methods, Extended Data Fig. 6a–d and Supplementary Table 2). As an initial assessment of heterogeneity, we performed clustering and dimensionality reduction (Extended Data Fig. 6e–h). Annotation of the scATAC profiles through integration with our scRNA-seq data revealed that clusters 1–6 corresponded to plasma cells, while clusters 7, 8 and 9 corresponded to monocytes, B cells and NK cells, respectively (Extended Data Fig. 6i–k). Visual inspection of tracks with aggregated ATAC profiles and gene scores for the marker genes SDC1, LYZ, MSAA1 and FCGR3A confirmed these cell-type annotations (Extended Data Fig. 6l–s). For further characterization of the single-cell accessibility profiles of myeloma cells, we retained clusters annotated as plasma cells only. Similar to our findings from transcriptional profiling, we observed patient-specific clustering, thereby arguing that individual patients have distinct chromatin profiles (Fig. 5b,c).

We next performed peak calling (Extended Data Fig. 7a–c) and found that the majority of accessible regions in normal plasma cells are shared with MM cells, with only 7,944 out of 46,935 peaks unique to the normal plasma cells (Extended Data Fig. 7b). However, we identified a substantial number of peaks unique to MM (29,761; Extended Data Fig. 7b), which suggests that MM cells retain accessible regions of normal plasma cells but gain additional peaks. Consistent with this finding, using DESeq2, we observed a higher number of differentially accessible peaks gained in MM (3,421 peaks gained versus 887 lost in MM compared to healthy donor plasma cells; false discovery rate (FDR)\(≤0.1\)) (Extended Data Fig. 7c,d).

To characterize genomic regions with peak enrichment, we annotated chromatin states using ChromHMM annotations in GM12878, a widely used model for normal B cells, as reference. We observed that a greater fraction of accessible regions fell into heterochromatin in MM (\(P = 0.0029\) by \(t\)-test; Extended Data Fig. 7e,f). Furthermore, a greater fraction of peaks was localized in intronic and intergenic regions (Extended Data Fig. 7g), which is in line with a gain of enhancers. Accessible regions in myeloma cells were also located at a greater distance from the transcription start site (TSS) (\(P < 2.2 \times 10\(^{-16}\) by Wilcoxon test; Extended Data Fig. 7h), which is consistent with reports describing increased H3K27 acetylation levels in myeloma\(^8\).

We next used GREAT to assign genes to enhancers\(^9\) and noted that many genes in MM were associated with ten or more

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**Fig. 4** Differential regulon activity and transcriptional rewiring in MM underlie alternative differentiation states. a, A GRN constructed based on transcriptional modules present in normal haematopoiesis. Highlighted are the plasma cell module and its dominating TFs. b, Gene expression in healthy donor plasma cells (top) and myeloma cells (bottom) projected onto a normal haematopoiesis network. The plasma cell module is highlighted. c, A GRN constructed exclusively from myeloma cells (red) overlayed with a GRN from normal hematopoietic cells (green). d, Regulons, that is, TFs with their target genes, as part of network layouts in normal haematopoiesis (BLUEPRINT, top) and myeloma (bottom). Regulons were chosen on the basis of their rewiring score (e). e, TFs (e) or CD markers (f) ordered by rewiring score determined using the DyNet algorithm. g, k, Dependency data were downloaded from the Cancer Dependency Map (https://depmap.org/portal/). g, Lineage-dependency score in MMCLs for the toprewired TFs in plasma cells. Data are presented as the mean ± standard error. n = 20 cell lines. h, Scaled dependency scores for 675 cell lines from 21 different lineages ordered by dependency for XBP1. Myeloma cell lines (plasma cell lineage) are highlighted in pink. i, Venn diagram showing the overlap between lineage-dependency genes and rewired TFs. Shown are TFs that overlap, j, TFs that overlap between lineage dependencies and rewired TFs ordered by lineage-dependency score in MMCLs. Data are presented as the mean ± standard error. n = 20 cell lines. For i and j, lineage TFs are highlighted in pink. k, Scaled dependency scores for 675 cell lines from 21 different lineages ordered by dependency for TAL1. Myeloma cells (plasma cell lineage) are highlighted in pink.
When comparing the accessibility of enhancers associated with the top multi-enhancer genes, we found that a subset was differentially accessible in MM (Extended Data Fig. 7i). Gene set enrichment analysis showed that the top multi-enhancer genes are involved in cellular processes such as differentiation, cell death and signalling, which indicates that changes in chromatin accessibility modulate these critical processes. As these results depend on accurate enhancer–gene maps, we validated our find-
Fig. 5 | Transcriptional reprogramming in MM and associated changes in chromatin accessibility and enhancer rewiring. a, Schematic illustrating the experimental set-up. b, tSNE plot showing clustering of single myeloma cells coloured by clusters based on scATAC. c, tSNE plot showing clustering of single myeloma cells coloured by patient based on scATAC. d, The number of associated enhancers per gene for genes associated with ≥10 enhancers in healthy donor plasma cells or patient-derived myeloma cells based on association using GREAT. Boxplots show the median and the interquartile range, and whiskers extend to 1.5 × the interquartile range. n = 1,020 and 1,159 genes, respectively. ****P = 5.5 × 10^-8 by two-sided Wilcoxon test. e, Pseudotime trajectory. f, Heatmap showing the correlation of gene scores with pseudotime. Pseudotime increases from left to right. g, Heatmap showing the correlation of motif accessibility with pseudotime. Pseudotime increases from left to right. h, tSNE plots of scATAC-seq coloured on the basis of bias-corrected z-scores of selected rewired TFs that bind differentially accessible motifs. Shown are TEAD4, MEF2A, IRF8 and FOXO3. i, j, Peak-to-gene linkage determined on the basis of integrated scATAC for the TEAD4 locus (i) and the ELF3 locus (j). k, Model of developmental potential and transcriptional diversity in MM.
ings using the activity-by-contact (ABC) model to link regulatory regions to target genes\(^\text{2}^\text{(Extended Data Fig. 7j–l).}\)

To assess variability in the scATAC dataset, we next performed pseudotime ordering of the scATAC profiles (Fig. 5e). We found that genes known to play a role in MM, such as \textit{CCND1} and \textit{ELF3}, displayed higher accessibility towards the start of the trajectory (Fig. 5f). Analysing motif accessibility across pseudotime (Fig. 5g), we further observed that the NF-xB family members \textit{REL}, \textit{RELA} and \textit{NFKB1} scored higher towards the end of the trajectory in the normal donor plasma cells. We therefore performed differential motif enrichment analysis using chromVAR\(^\text{3}\) and detected differential enrichment of a total of 276 TFs (FDR \(p<0.01\); Extended Data Fig. 7n), including \textit{TEAD4}, \textit{ELF3} and other TFs such as \textit{IRF8}, \textit{FOXO3} and \textit{MEF2A} (Fig. 5h). This finding indicates that a gain in accessibility of TF sites contributes to rewiring and transcriptional reprogramming.

The single-cell chromatin accessibility data further allowed us to predict cis-regulatory interactions by defining peak-to-gene linkage. The loci of the TFs \textit{ELF3} and \textit{TEAD4} manifested further evidence for enhancer activation in MM (Fig. 5i,j), which demonstrates that there are widespread alterations in cis-regulatory regions in MM.

Taken together, these data suggest that derepression of heterochromatin in MM allows for binding of TFs and expression of genes not normally expressed in this lineage, ultimately leading to the promiscuous acquisition of alternative cell states (Fig. 5k).

**Treatment reduces developmental potential.** To assess how treatment modulates the differentiation state of single myeloma cells, we investigated myeloma cells from patients treated with Elio-PVD at cycle 5 day 1 (C5D1), that is, persisting after 4 months of treatment. Alternative splicing resulting in multiple transcript variants is known to regulate key developmental decisions, including maintenance of pluripotency and differentiation\(^\text{3}\). Differential isoform expression analysis showed differential expression of 312 transcripts with treatment (Extended Data Fig. 8a). Gene set enrichment analysis revealed that transcripts involved in processes such as RNA binding, cell activation and regulation of gene expression were differentially spliced between timepoints (Extended Data Fig. 8b).

To further investigate differential splicing probabilities in single cells, we performed Louvain clustering based on the splicing probability (measured as the percent of spliced in (PSI)) and identified six clusters, two of which were enriched in treated cells, which suggests that treatment is the cause of cluster formation (Extended Data Fig. 8c–e). Indeed, we observed that transcripts with a high splicing probability showed exon inclusion less frequently after treatment, that is, had a higher probability of exon skipping (Fig. 6a). \textit{PDCD4} and \textit{TSC1}, a negative regulator of mTORC1 signalling, were among the transcripts with reduced splicing probabilities at C5D1, while \textit{CALU}, which is involved in protein folding\(^\text{31}\), showed higher splicing probabilities (Extended Data Fig. 8f).

We also generated mixture of isoform (MISO) plots to visualize potential exon-skipping events or alternative splice site usage in individual transcripts following treatment (Extended Data Fig. 8g–i). We hypothesized that \textit{SLAMF7}, the target of elotuzumab treatment, might be alternatively spliced following treatment. We identified various splice isoforms resulting in skipping of exon 3, which encodes the antibody binding site\(^\text{41}\) (Extended Data Fig. 8j). However, we did not observe differential splicing following treatment in this transcript (Extended Data Fig. 8f(i)), which argues that alternative splicing of \textit{SLAMF7} does not significantly contribute to treatment evasion in this cohort.

We next investigated whether developmental potential changed with treatment. Notably, we observed a decrease in the number of genes expressed (5,700 at screening versus 4,473 at C5D1, \(P<2.2\times10^{-16}\) by \(t\)-test) (Extended Data Fig. 9a). CytoTRACE values were decreased following treatment (\(P<2.2\times10^{-16}\) by \(t\)-test; Fig. 6b), which suggests that developmental potential and transcriptional diversity are reduced by treatment, consistent with persisting cells assuming a more quiescent state. We further observed a minor reduction in cycling cells following treatment (Extended Data Fig. 9b).

We therefore hypothesized that treatment might modulate TF activity. Notably, we observed that overall regulon activity was increased following treatment, with 160 out of 365 decreased and 205 out of 365 regulons upregulated (Fig. 6c). Regulons that increased in activity included the B cell TF FOXO3 and the regulators of plasma cell fate IRF4 and PRDM1, while downregulated regulons included the rewired non-lineage TFs \textit{ELF3} and \textit{TEAD4} (Fig. 6d). Among the lineage TFs, we observed an increase in regulon activity in 17 out of 38 (including the IRF family members IRF4, IRF2 and IRF8) (Extended Data Fig. 9c,d).

To investigate whether cells with increased regulon activity were selected for by treatment or whether treatment directly induced these transcriptional changes, we modelled short-term drug treatment in vitro. Treatment resulted in a reduction in developmental potential, which indicates that this change is a direct effect of treatment rather than an effect of selection (\(P<2.2\times10^{-16}\) by \(t\)-test; Fig. 6e). Short-term treatment further resulted in changes in regulon activity consistent with those observed in patients with MM (245 out of 434 upregulated and 189 out of 434 downregulated), including upregulation of IRF factors and B cell lineage TFs such as FOXO3 (Fig. 6f,g).

We hypothesized that changes in regulon activity could be attributed to differential chromatin accessibility, so we performed ATAC-seq of the MM cell line (MMCL) MOLP2 after 72 h of treatment with PVD (Fig. 6h,i). Differential accessibility analysis by DESeq2 revealed 5,268 peaks gained and 3,154 peaks lost with treatment (Fig. 6j), which indicates that treatment induced significant changes in chromatin accessibility. Gene Ontology (GO) term analysis of the differential peaks revealed enrichment of several terms related to differentiation (lymphocyte differentiation and cell fate commitment) in peaks gained following treatment, while peaks lost after treatment were enriched in lymphocyte proliferation (Extended Data Fig. 9e,f). As we detected fewer genes expressed in the treated cells without a concomitant decrease in accessible regions, we hypothesized that more enhancers were connected to individual genes. Using GREAT to assign genes to enhancers, we noted that following treatment, the average number of enhancers per gene was increased (11 in the PVD-treated group versus 10 in the dimethylsulfoxide (DMSO)-treated group, \(P=0.00012\) by \(t\)-test), with a greater number of genes connected to ten or more enhancers (Fig. 6k).

Gene set enrichment analysis of the top genes gaining enhancers revealed that these are involved in the regulation of cellular processes, such as response to cytokines and cell motility (Extended Data Fig. 9g), which suggests that enhancer rewiring supports the expression of a subset of genes needed for persistence under drug pressure. We validated these results using the ABC model for gene region annotation (Extended Data Fig. 9h,i).

Motif enrichment analysis in accessible regions showed enrichment of several IRF factors after treatment, which is consistent with increases in regulon activities (Fig. 6l). Rewired TFs with differentially accessible motifs also included the IRF factors IRF2, IRF9 and IRF1 (Extended Data Fig. 9j). Collectively, these results suggest that treatment directly modulates the transcriptional landscape.

**Immunotherapeutic targets in reprogrammed cancer cells.** Having identified deregulated expression of surface markers in MM, we hypothesized that these might represent attractive therapeutic targets as surface markers are easily accessible and can be targeted by immunotherapy. Therefore, we assessed the expression of the surface markers CD33, CD4, CD5 and CD20 (Fig. 7a) in MM cells

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Fig. 6 | Treatment reduces developmental potential while increasing regulon activity. 

**a-d.** Comparison of primary MM cells on treatment (CSD1) compared to the screening timepoint. 

**a.** The exon inclusion/exclusion ratio (PSI) quantified at screening versus CSD1 for transcripts with low, medium or high splicing probability (**standard error of the mean (SEM)**). 

**b.** CytoTRACE values in MM cells before and after treatment (PSI < 2.2 × 10^{-16} by two-sided t-test). 

**c.** The number of regulons downregulated (160 out of 365) and upregulated (205 out of 365) following treatment. 

**d.** The change in regulon activity after treatment. Selected lineage and non-lineage TFs are highlighted. 

**e-g.** The MMCL MOLP2 was treated with PVD for 72 h before scRNA-data. 

**e.** CytoTRACE values for cells treated with PVD or DMSO (PSI < 2.2 × 10^{-16} by two-sided t-test). 

**f.** Regulons downregulated and upregulated in cells treated with PVD compared to DMSO. 

**g.** The change in regulon activity in cells treated with PVD compared to DMSO. Selected lineage TF regulons are highlighted. 

**h.** ATAC-seq was performed on MOLP2 cells after 72 h of treatment with PVD or DMSO. 

**i.** Venn diagram illustrating the overlap of peaks in cells treated with DMSO or PVD. 

**j.** The number of genes associated with ≥10 enhancers in cells treated with DMSO or PVD using GREAT. 

**k.** Motif enrichment in MOLP2 cells after 72 h of treatment with PVD versus DMSO. Enriched TF motifs are highlighted in red. For a, b and e, boxplots show the median and the interquartile range, and whiskers extend to 1.5× the interquartile range. n = 1,374 (a, b) or 192 (e) cells.
Fig. 7 | Inducible and stable expression of putative immunotherapy targets on myeloma cells. **a**. Expression of selected surface markers not found in normal plasma cells in single myeloma (MM) cells or healthy donor plasma cells (ND). **b**. Deconvolution of surface marker expression in healthy donor plasma cells, B cells and myeloma cells from patient MM1 at screening and at C5D1 based on the enrichment of surface marker signatures derived from the BLUEPRINT dataset. **c, d**. CXCR4 mRNA (**c**) and surface protein (**d**) expression in single myeloma cells from patient MM1 before treatment and at C5D1. **e**. Co-accessibility determined on the basis of scATAC data showing the CXCR4 locus. Aggregated scATAC tracks show chromatin accessibility upstream of CXCR4 at screening and at C5D1 with differential peaks highlighted in grey. IRF4 motifs in peaks and IRF4 ChIP peaks from KMS12BM are displayed. **f**. Inducible and stable expression of putative immunotherapy targets on myeloma cells. **g**. Expression of selected surface markers not found in normal plasma cells in single myeloma (MM) cells or healthy donor plasma cells (ND). **h**. Inducible and stable expression of putative immunotherapy targets on myeloma cells. **i**. Treatment of MOLP2 myeloma cells with dexamethasone (D) or a combination of PVD resulting in the upregulation of CXCR4 at the cell surface. **j**. Quantitative analysis of CXCR4 surface protein expression changes on OPM2 myeloma cells with drug exposure by flow cytometry (MFI, mean fluorescence intensity). Data are presented as the mean ± s.d., n = 3 independent experiments. **k**. Drug removal resulted in the downregulation of CXCR4 surface expression in MOLP2 cells. **l**. Cell viability following treatment with the CXCR4 inhibitors BKT140 (k) and plerixafor (l) following pretreatment with PVD in the MMCL OPM2. Data are presented as the mean ± s.d., n = 3 independent experiments. **P** ≤ 0.01 and **P** ≤ 0.001 by unpaired two-sided t-test. BFU-E, burst forming unit-erythroid; CDC, conventional dendritic cell; EP, erythroid progenitor; GCB, germinal center B cell; NS, non-switched.
at screening as examples of CD markers not normally expressed in this lineage but upregulation was detected in individual patients.

To further assess whether altered differentiation states are reflected in distinct surface marker profiles, we performed deconvolution of cell types based on mRNA expression of cell surface markers. While normal plasma cells manifested a surface marker profile closest to plasma cells and other B cell subtypes, in MM, we identified signatures of different lineages such as endothelial progenitors and common myeloid progenitors (Fig. 7b). This finding suggests that the lineage infidelity that we identified in MM cells is associated with widespread changes in expression of surface markers.

Notably, we observed a further shift in surface marker expression with treatment, which argues that treatment itself may induce the expression of different surface molecules (Fig. 7b). Interestingly, we identified the surface marker CXCR4 among the top upregulated genes following treatment (Extended Data Fig. 10a). CXCR4 has been implicated in MM for playing a role in disease progression and inducing an epithelial–mesenchymal transition (EMT)-like phenotype. CXCR4 therefore represents an attractive therapeutic target, particularly as CXCR4 antagonists are already being tested in applications. We validated the surface expression of CXCR4 following treatment by flow cytometry (Fig. 7c,d).

To assess its regulation after treatment, we investigated chromatin accessibility in the scATAC data and detected numerous intergenic enhancers linked to CXCR4 (Fig. 7e). We observed significantly greater accessibility in an enhancer peak associated with CXCR4 following treatment, which suggests that there is differential epigenetic activation of CXCR4. As it has been established that IRF4 regulates CXCR4 expression in pre-B cells, we compared IRF4 peaks from chromatin immunoprecipitation assays with sequencing (ChIP-seq) with our ATAC-seq profiles. We observed IRF4 binding at the promoter and enhancer regions of CXCR4, which argues that IRF4 regulates CXCR4 expression in MM. We therefore investigated chromatin accessibility in the MMCL MOLP2 after treatment with PVD and found that the IRF4 motif is enriched in ATAC peaks following treatment (Fig. 7f and Extended Data Fig. 10b). Consistent with this observation, we detected a significant increase in IRF4 regulon activity following treatment ($P = 1.3 \times 10^{-3}$ by t-test; Fig. 7g). These data argue that IRF4 regulates CXCR4 and is activated following treatment, thus resulting in increased CXCR4 expression.

To determine whether CXCR4 expression can be directly induced by treatment, we treated MMCLs in vitro with PVD. We observed upregulation of CXCR4 in multiple MMCLs (Fig. 7h,i), but only minimal cell death (Extended Data Fig. 10c–e) following treatment. Quantitative analysis of CXCR4 surface protein expression revealed dexamethasone as the drug that had the greatest effect on this phenotype (Fig. 7i and Extended Data Fig. 10f,g). Drug removal resulted in downregulation back to starting levels (Fig. 7i), which suggests that this phenotype is indeed induced by drug treatment and is reversible. To investigate whether we could exploit this finding for therapeutic targeting, we treated MMCLs with the CXCR4 inhibitors BKT140 and plerixafor (Fig. 7k,l and Extended Data Fig. 10h,i). We observed greater cell death when adding BKT140 and plerixafor following pretreatment with PVD, which argues that we can induce phenotypic changes that provide the rationale for targeted therapeutic strategies. Collectively, these results demonstrate that drug treatment can indeed modulate surface marker expression and can induce the expression of potential therapeutic targets.

We detected widespread transcriptional reprogramming in myeloma cells, with expression of genes not normally detected in this cell type. Our analyses demonstrated greater developmental potential and expression of markers associated with immaturity. This is of particular interest as plasma cells, the cell of origin for MM, are terminally differentiated and this process is generally considered irreversible. Our data suggest that while MM cells do not lose their plasma cell identity, they acquire expression of gene signatures from earlier developmental stages or entirely different lineages. We showed that differential regulon usage and transcriptional rewiring underlie these alternative differentiation states and identified differential TF motif accessibility as a possible mechanism.

We detected widespread changes in chromatin accessibility, with derepression of heterochromatin regions and increased accessibility in enhancer regions. These findings are consistent with previous reports demonstrating that enhancer activation is a key feature of myeloma. We also found that a subset of genes enriched for regulation of differentiation and cell death is associated with many more enhancer regions in MM, which is reminiscent of previous suggestions that establishment of superenhancers is a key feature of MM biology.

We argue that permissive chromatin states in myeloma lower epigenetic barriers, allowing for promiscuous sampling of alternative cell states. This plasticity allows MM cells to aberrantly activate alternative gene regulatory programmes, resulting in widespread transcriptional reprogramming and dedifferentiation in this malignancy derived from terminally differentiated plasma cells. Importantly, we showed that this plasticity can lead to therapeutic opportunities, as it results in the expression of surface markers not normally expressed in this lineage. Using CXCR4 as a proof of principle, we further showed that surface marker expression can be modulated by treatment and exploited for immunotherapeutic targeting, and we identified increased IRF4 activity as a possible mechanism driving CXCR4 upregulation. Interestingly, we observed dedifferentiation independent of genotype, thereby arguing that there are convergent cell states dictated by derepression of chromatin that supersede genetic background and heterogeneity. Targeting MM cell states, rather than distinct genotypes, may therefore represent a way to overcome the extensive genetic heterogeneity of this disease. We propose that identifying GRNs and the associated complement of surface proteins in cancer can reveal actionable targets for immunotherapies.

Online content
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Methods

**Patient samples.** Banked blood or bone marrow samples from patients with RMM (Supplementary Table 1) who had signed consent and were enrolled into a Phase II clinical trial investigating Eleo-PVD (NCT02721883) were used in this study. The research was determined to be not human participant research by DF/HCC IRB (19-511). Samples from 8 patients (5 males, 3 females) were analysed, and the median age at registration was 68.5 years (range 45–80 years). Myeloma cells were enriched using an EasySep Human CD138-positive selection kit (Stemcell Technologies). Bone marrow from healthy donors was obtained from ABCCells.

**Single-cell sorting.** Bone marrow cells were stained with antibodies against CD38 FITC (multi-epitope, Cytognos; 1:200), CD138 PE (44F9, Miltenyi; 1:100), CD19 PE (HIB19, BioLegend; 1:100) or with antibodies against CD45 FITC (HI30, eBioscience; 1:100), CD38 FITC (multi-epitope, Cytognos; 1:200), CD138 PE (44F9, Miltenyi; 1:100), SLAMF7 APC (162.1, BioLegend; 1:100) or with antibodies against CD45 FITC (HI30, eBioscience; 1:100), CD3 PerCP-Cy5.5 (OKT3, eBioscience; 1:100), CD14 APC-Cy7 (MoP9, BD Biosciences; 1:100) and CD19 PE (HIB19, BioLegend; 1:100). Cells were stained with 4,6-diamidino-2-phenylindole (DAPI; 1 μg/ml, Sigma–Aldrich) to exclude dead cells. Single cells were sorted into 96-well plates using a Sony SH800 sorter (Extended Data Fig. 1). Immediately after sorting, plates were spun down and stored at −80°C until further processing.

**scRNA-seq library preparation.** Full-length scRNA-seq libraries were prepared using the SmartSeq2 protocol. Briefly, single cells were sorted into 96-well plates with each well containing 10 μl of TCM buffer (Qagen) and 1% 2-mercaptoethanol. RNA purification was performed using RNA Clean XP beads (Beckman Coulter) before complementary DNA amplification. cDNA was subjected to tagmentation using a Nextera XT kit (Illumina) and cDNA was amplified with Nextera indexes. Pooled libraries were paired-end sequenced using a 75 cycle kit on a NextSeq 500 sequencer (Illumina) with an average sequencing depth of 3 × 10^6 reads per cell.

**Cytogenetics.** Human MMCLs were obtained from the American Type Culture Collection (ATCC; MM1.S, CRL-2974) or DSMZ (MOLP2, ACC 607; OPM2, ACC 50). The cell lines MOLP2, OPM2 and MM1.S were cultured in RPMI-1640 (Gibco) medium with 10% (MM1.S and OPM2) or 20% (MOLP2) heat-inactivated fetal bovine serum, 100 μM penicillin–streptomycin (Invitrogen), 2 mM l-glutamine (Sigma), 1 mM sodium pyruvate (Sigma), 1× NEAA (Sigma), 20 mM HEPES buffer (Sigma) and 0.5 mM 2-mercaptoethanol. Cell identity was confirmed by short tandem repeat profiling and cultures were tested for mycoplasma every 3 months.

**In vitro inhibitor treatment.** MMCLs were treated for 72 h with pomalidomide (Selleck), bortezomib (Selleck) and dexamethasone (MilliporeSigma) individually or in combination (half IC50 concentration after 72 h) and MMCLs were cultured in RPMI-1640 (Gibco) medium with 10% (MM1.S and OPM2) or 20% (MOLP2) heat-inactivated fetal bovine serum, 100 μM penicillin–streptomycin (Invitrogen), 2 mM l-glutamine (Sigma), 1 mM sodium pyruvate (Sigma), 1× NEAA (Sigma), 20 mM HEPES buffer (Sigma) and 0.5 mM 2-mercaptoethanol. Cell identity was confirmed by short tandem repeat profiling and cultures were tested for mycoplasma every 3 months.

**scATAC-seq library preparation.** A total of 2,000–5,000 plasma cells were bulk sorted as described above, and scATAC-seq libraries were prepared as described by Chen et al. Libraries were paired-end sequenced using a 75 cycle kit on a NextSeq 500 sequencer (Illumina) with an average sequencing depth of 0.5–1 million reads per cell.

**MMCLs.** Human MMCLs were obtained from the American Type Culture Collection (ATCC; MM1.S, CRL-2974) or DSMZ (MOLP2, ACC 607; OPM2, ACC 50). The cell lines MOLP2, OPM2 and MM1.S were cultured in RPMI-1640 (Gibco) medium with 10% (MM1.S and OPM2) or 20% (MOLP2) heat-inactivated fetal bovine serum, 100 μM penicillin–streptomycin (Invitrogen), 2 mM l-glutamine (Sigma), 1 mM sodium pyruvate (Sigma), 1× NEAA (Sigma), 20 mM HEPES buffer (Sigma) and 0.5 mM 2-mercaptoethanol. Cell identity was confirmed by short tandem repeat profiling and cultures were tested for mycoplasma every 3 months.

**In vitro inhibitor treatment.** MMCLs were treated for 72 h with pomalidomide (Selleck), bortezomib (Selleck) and dexamethasone (MilliporeSigma) individually or in combination (half IC50 concentration for bortezomib and half IC10 for dexamethasone) (Selleck), bortezomib (Selleck) and dexamethasone (MilliporeSigma) individually or in combination (half IC10 concentration for bortezomib and half IC10 for dexamethasone) (Selleck), bortezomib (Selleck) and dexamethasone (MilliporeSigma) individually or in combination (half IC10 concentration for bortezomib and half IC10 for dexamethasone) (Selleck), bortezomib (Selleck) and dexamethasone (MilliporeSigma) individually or in combination (half IC10 concentration for bortezomib and half IC10 for dexamethasone) (Selleck). A composite malignancy score was defined by combining the relative expression of the positive marker genes and subtracting the average relative expression of the negative markers.

**Classification of malignant cells.** MMCLs were classified into homogeneous and heterogeneous groups. The classification was evaluated using varImpPlot function.

A composite malignancy score was calculated by scoring the relative expression of positive marker genes and adding a value of +1 if (1) the positive marker genes were upregulated and (2) the dominant CDR3 region was detected. A network classifier was built in R defining patient-specific clusters showing separation for copy-number aberrations as malignant. We randomly split the dataset into non-overlapping training and test sets, using 30% of cells for testing. The optimum parameters—mt (62) and ntrees (500) for randomforest were determined using tuneRF function.

Defining heterogeneity programmes. MMCLs at screening were classified into homogeneous and heterogeneous groups. The classification was evaluated using varImpPlot function.

Comparison to published RNA-seq datasets. Gene expression data from bulk-sorted populations of immune cells from the BLUEPRINT database were used to assess the expression of marker genes in progenitor signatures (common lymphoid progenitor (CLP), common myeloid progenitor (CMP), multipotent progenitor (MPP), megakaryocyte–erythroid progenitor (MEP)) along with B cell and plasma cell signatures from the Human Cell Atlas.
potential. The top 100 genes correlated or anti-correlated with CytoTRACE values (Supplementary Table 6) were chosen as markers for immaturity and differentiation, respectively. Gene set enrichment analysis was performed using MSigDB (https://www.gSEA-msigdb.org/gsea/msigdb/index.jsp). Signaling activity was predicted based on perturbation experiments using PROGENy64. Entropy was calculated using the R package LandSCENT65 with a previously defined protein–protein interaction network provided with the LandSCENT package available on github under the filename “net13Jun12.m.RData.” For validation, we used a subsampled version of the protein interaction network also provided on github under the filename “net1Jan2016.m.RData,” which contains a greater number of protein–protein interactions (11,751 versus 8,434 columns; Extended Data Fig. 4j).

**Construction of the regulon network.** The bulk RNA-seq data from the BLUEPRINT consortium12 was used to construct a reference network. Only the high-confidence regulon targets with Genie3Weight ≥0.01 were filtered from the file “2.5_regulonTargetsInfo.Rds” (Supplementary Table 9) generated by SCENIC6. The modules in the constructed network were detected using the ModulLand plugin in cytoscape66. Similarly, the network was constructed for the disease state by using the scRNA-seq data generated from our study from malignant and normal plasma cells. We compared the regulon network layout of the plasma cells in BLUEPRINT with that of the healthy donor plasma cells and myeloma cells at screening.

While for the BLUEPRINT plasma cell type we averaged the area under the curve (AUC) values for each regulon for the scRNA-seq data generated from our study, we averaged the AUC values for each regulon for MM or healthy donor plasma cells. Finally, for each cell type/group, we centered the averaged AUC values by the mean over regulons and scaled by the standard deviation (Supplementary Tables 7 and 8). Whenever a regulon TF produced another (extended) regulon with a different number of target genes, we retained only the one having high-confidence target genes, which was identified by having the same or close number of target genes as shown in the high-confidence regulon targets data. To project the scaled regulon activity onto the BLUEPRINT and ELO networks, we integrated the scaled regulon activity data to their corresponding regulon target data.

Both the normal haematopoietic and disease networks were compared to analyze the rewiring of regulon networks using the DyNet algorithm (Supplementary Table 10). Lineage TFs were identified as TFs present in the B cell or plasma cell modules with selected TFs added from the literature (Supplementary Table 11).

**Expression of surface markers.** Gene expression data from bulk-sorted populations of immune cells from the BLUEPRINT dataset13 were used to score enrichment of surface markers from other cell types in MM cells. Only CD markers were selected, and their enrichment was scored using the R package TissueEnrich.

**Detection of alternative splicing.** We quantified the exon inclusion/exclusion ratio (PSI) using BRIE2 (Bayesian regression for isoform estimate, v.2.0) python package developed under the Python2 environment7. A total of 7,516 predefined annotations for exon-skipping splicing of human data were downloaded from https://sourceforge.net/projects/brie-rna/files/annotation/. The produced PSI values were used for projection of selected events and downstream analysis by creating a PSI-based uniform manifold approximation and projection (UMAP). Poor-quality genes were filtered out using the defined arguments in brie-quant (minCount, minUniqCount, minCell and minMIF), choosing a minimum of 50 counts, a minimum of 10 unique counts across all cells, a minimum of 30 cells with unique counts and a minimum of 0.001 minor isoform frequency in unique counts, respectively.

Single-cell samples in each experimental condition were selected to generate Sashimi plots using MISO (v0.5.4)7. Skipped exon events (diff-annotation files) were downloaded from https://miso.readthedocs.io/en/fastmiso/index.html#event-annotation.

**Processing and analysis of the scATAC-seq data.** Single-cell chromatin accessibility profiles were processed with ArchR8. QC assessment was performed using the QCQC function implemented in Seurat. QC parameters and their enrichment score greater than 4 and greater than 1,000 unique nuclear fragments were retained. Doublet filtering resulted in a further removal of 11 cells (1% of cells). QC measures and sample statistics are shown in Extended Data Fig. 6a–d and Supplementary Table 2.

We next performed dimensionality reduction using iterative latent semantic indexing and clustering using a graph-based approach implemented in Seurat with the FindClusters function. We performed batch effect correction using Harmony before clustering again (Extended Data Fig. 6g,h). To define cluster identities, we performed integration with our scRNA-Seq dataset using the FindTransferAnchors function integrated in Seurat.

For pseudotime ordering of scATAC profiles, we defined the patient with the highest CytoTRACE score (MMCL MOLP2) as the starting point of the healthy donor plasma cells as the endpoint of the trajectory (Fig. 5e). To predict cis-regulatory interactions, we defined peak-to-gene linkage in ArchR. A correlation cutoff of 0.45 and a resolution of 1 were selected.

**Differential motif accessibility was investigated using the R package ChIPQuest.** We identified a set of background peaks that were matched in GC content and accessibility and calculated bias-corrected deviations and z-scores. Motifs were imported from the JASPAR 2018 database, and differential motif accessibility was determined specifying MM and healthy donor plasma cells as groups for comparison based on the z-scores.

For peak annotation in aggregated scATAC data, samples from individual patients for greater than 50 cells were retained, downsampled to obtain comparable numbers of reads and analysed using the ncfc/ataseq pipeline v1.0.0 with the --narrowPeak parameter9 (Extended Data Fig. 7a–c). To annotate chromatin states, we obtained ChromHMMP annotations for GM12878 cells from ENCODE. GM12878 ChromHMMP states were intersected with the ATAC-seq peaks using bedtools intersect.

For gene–enhancer relationships, we used annotations from GREAT with default settings10, whereby GREAT assigns each gene a regulatory domain consisting of a basal domain that extends 5-kb upstream and 1-kb downstream from its TSS, and an extension upstream and downstream up to the basal regulatory domain of the nearest gene within a 5-Mb. GREAT further refines the assignment using curated experimentally determined regulatory domains.

We further implemented the ABC model to predict enhancer–gene interactions. To this end, publicly available HXK27 acetylation ChIP-seq data were downloaded from the European Nucleotide Archive (accession number PRJEB25605), which was used for visualized for comparison. For enrichment, the genome-wide GREAT tracks were imported from UCSC, and their enrichment was scored using the R package TissueEnrich.

For gene–enhancer relationships, we used annotations from GREAT with default settings10, whereby GREAT assigns each gene a regulatory domain consisting of a basal domain that extends 5-kb upstream and 1-kb downstream from its TSS, and an extension upstream and downstream up to the basal regulatory domain of the nearest gene within a 5-Mb. GREAT further refines the assignment using curated experimentally determined regulatory domains.

The R package Cicero was used to define co-accessibility of genomic regions based on the scATAC data11. A co-accessibility cut-off of 0.25 was chosen, and only connections with the respective TSS were selected using the viewpoint feature. The R package GVIZ was used for visualization. For comparison, double elite GeneHancer tracks were imported from UCSC, and their enrichment was scored using the Cicero/Chipseq pipeline v1.0.0 (https://github.com/nfc/core/cipseq) with default settings. CUT&TAG data for HXK27 acetylation were generated for MOLP2 as previously described7. Expression data were provided as average log counts from the scRNA-seq data. The ABC score was calculated using average HiC data provided by the package and default options. Elements with an ABC score of below 0.02 were discarded.

The R package Cicero was used to define co-accessibility of genomic regions based on the scATAC data11. A co-accessibility cut-off of 0.25 was chosen, and only connections with the respective TSS were selected using the viewpoint feature. The R package GVIZ was used for visualization. For comparison, double elite GeneHancer tracks were imported from UCSC, and their enrichment was scored using the Cicero/Chipseq pipeline v1.0.0 (https://github.com/nfc/core/cipseq) with default settings. CUT&TAG data for HXK27 acetylation were generated for MOLP2 as previously described7. Expression data were provided as average log counts from the scRNA-seq data. The ABC score was calculated using average HiC data provided by the package and default options. Elements with an ABC score of below 0.02 were discarded.

**Dependency scores.** For peak annotation in aggregated scATAC data, samples from individual patients for greater than 50 cells were retained, downsampled to obtain comparable numbers of reads and analysed using the ncfc/ataseq pipeline v1.0.0 with the --narrowPeak parameter9 (Extended Data Fig. 7a–c). To annotate chromatin states, we obtained ChromHMMP annotations for GM12878 cells from ENCODE. GM12878 ChromHMMP states were intersected with the ATAC-seq peaks using bedtools intersect.

For peak annotation in aggregated scATAC data, samples from individual patients for greater than 50 cells were retained, downsampled to obtain comparable numbers of reads and analysed using the ncfc/ataseq pipeline v1.0.0 with the --narrowPeak parameter9 (Extended Data Fig. 7a–c). To annotate chromatin states, we obtained ChromHMMP annotations for GM12878 cells from ENCODE. GM12878 ChromHMMP states were intersected with the ATAC-seq peaks using bedtools intersect.

**Statistics and reproducibility.** Results were plotted and quantified using R-based packages or GraphPad Prism 8. Significance was calculated using the indicated statistical tests. No statistical method was used to predetermine sample sizes. No data were excluded from the analyses. For the scRNA and scATAC experiments, low-quality cells were filtered and excluded from downstream analyses as described. No attempts of replication failed. The experiments were not randomized. The investigators were not formally blinded to allocation during experiments and outcome assessment.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability.** The data collected in this paper included next-generation sequencing (NGS) data. The data generated in this study include (1) scRNA and datasets from primary myeloma and CD45+ immune cells and normal donor bone marrow plasma cells and CD45+ immune cells; (2) scRNA-seq datasets from the MMCL MOLP2 treated with PVD or DMSO; (3) scATAC-seq datasets from primary myeloma and normal donor plasma cells; (4) bulk ATAC-seq datasets from the MMCL MOLP2 treated with PVD or DMSO. FASTQ files were generated following Illumina sequencing and further analysed as described below in and the Methods. The scRNA-seq and ATAC-seq datasets and CUT&Tag data generated in this
study have been deposited in the Gene Expression Omnibus under the accession number GSE162337. The cytosome files for the final GRNs are available for download at the Broad Institute Single Cell Portal (https://singlecell.broadinstitute.org/singlecell/). SCENIC (v.2.1.1), PAGODA2 (v.0.1.7), GREAT (http://great.stanford.edu/public/html/) and GSEA (v.3.32.0), NMF (v.0.23.0), BRIE2 (v.2) and MISO (v.0.5.4); also FlowJo (v.10), CytoTRACE (v.0.1.0), PROGENy (1.6.0), LandSCENT (0.99.3), SCENIC (1.1.2-2), PAGODA2 (v.0.1.1), GENIE3 (v.2.1.1), SingleR (v.1.0.1), scran (1.12.1), InferCNV (1.4.0), CONICSmant (v.0.0.0.1), MIXCR, randomFOREST (v.4.6-14), SCAPNY (v.1.4.6), CytoTRACE (v.1.0.1), PROGENY (1.6.0), LandsCEN'T (0.99.3), SCENITIC (v.1.2.2), Sortat (v.3.2.1), velocyto, scvelo (v.0.2.0), TissueEarshear (v.1.4.1), Cicero (v.1.2.0), chromVAR (v.1.6.0), GVIZ (v.1.28.3), deepTools (v.3.0.2), ArchR (v.0.9.5), edgeR (v.3.2.0), NMF (v.2.23.0), BRIE2 (v.2) and MISO (v.5.8.4); also FlowJo (v.10), CytoTRACE (v.3.8.0), GREAT (http://great.stanford.edu/public/html/) and GSEA (https://www.gsea-msigdb.org/gsea/index.jsp).

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Author contributions

J.F. designed and performed experiments, analysed data and wrote the manuscript. P.A., R.A.P, N.S. and H.S. performed data analyses. M.S.N. performed experiments and data analyses. T.V., J.M.W., S.P., J.A.K., A.K. and V.D. performed sample processing, sequencing or provided scientific input. N.S.R. and A.J.Y. provided project leadership, coordinated sample acquisition and clinical data integration. M.M., K.C.A., P.G.R. and J.P.L. oversaw clinical data analyses and integration. B.K. and J.G.L. designed experiments, provided project leadership, supervised the analysis and wrote the manuscript. All authors discussed the results and reviewed the manuscript.

Competing interests

A.J.Y. has a consulting role for Adaptive Biotechnologies, Amgen, BMS, Celgene, GSK, Janssen, Karyopharm, Sanofi and Takeda. The remaining authors declare no competing interests.

Additional information

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Peer review statement Nature Cell Biology thanks the anonymous reviewers for their contribution to the peer review of this work.
Extended Data Fig. 1 | Sorting strategy. a) Sorting strategy for myeloma cells and normal donor plasma cells with representative flow cytometry plots. CD38+CD138+ cells were sorted after EasySep enrichment of CD138+ cells from bone marrow or peripheral blood. b) Sorting strategy for immune cell subsets. CD3+, CD19+, CD14+ and CD45+Lin- cells were sorted following exclusion of dead cells and doublets. c) Sorting strategy for NK cells. CD56+CD16+ cells (Q1-3) were sorted after exclusion of dead cells, doublets, CD3+ cells and CD138+ cells.
Extended Data Fig. 2 | Quality assessment and filtering of single cell RNA data. a) Distribution of library size (i), number of detected genes (ii), percentage of counts mapping to mitochondrial genes (iii), and percentage of counts mapping to house-keeping genes (HKGs) (iv) per cell. b) Scatter plot depicting principal component analysis using the top two dimensions. The PCA was performed on the four features depicted in a) for all cells in the unfiltered dataset. The outliers, highlighted in orange, were identified using the mvoutlier package. c) The distribution of features shown in a) after filtering out cells identified as outliers. d) Scatter plot depicting expression frequency vs mean read counts per gene in the filtered dataset. e) Density plot showing the contribution of various technical factors (timepoint, run date, individual, total features, total counts, percentage of counts mapping to mitochondrial genes, and percentage of counts mapping to house-keeping genes (HKGs)) to the total variation observed in the dataset.
Extended Data Fig. 3 | Characterization of scRNA-Seq dataset of primary myeloma cells. a) Patient characteristics: Genetic aberrations detected by FISH. b) Copy number profiles of myeloma patients were inferred from scRNA expression data using InferCNV. Normal donor plasma cells served as a control. See the enlarged version in Supplementary Information. c-e) Random forest model identifying genes that best discriminate myeloma from normal plasma cells. c) Plot depicting the error vs number of trees used by random forest model on malignant (green), non-malignant (red) and combined (black) cells. d) Relative importance of each gene in the model (mean decrease in Gini coefficient). e) Confusion matrix showing classification and error rates during training of the model, for prediction on the training set (predict_train) and the test set (predict_test). f) Detailed heatmap showing classification of malignant and normal plasma cells based on CNVs, CDR3 sequence, expression of translocation targets, and genes identified by random forest (RF) model that best discriminate between normal and malignant cells, and composite malignancy score for patient MM5. g) tSNE plots showing PAGODA2 clustering highlighting cells from patient MM5 with malignancy scores ≥ 1 or < 1. h) Heatmap with relative expression of marker genes for individual patients in single myeloma and normal plasma cells.
Extended Data Fig. 4 | See next page for caption.
**Extended Data Fig. 4 | Transcriptional diversity is increased in MM.**

**a)** tSNE plots showing expression of selected genes not normally expressed in plasma cells (log2-transformed counts). Depicted are CD20 (MS4A1), the B Lymphoid Tyrosine Kinase BLK, usually expressed in earlier stages of B cell development and the myeloid restricted serine protease C (CTSC).

**b)** Heatmap with lineage scores from single cell RNA-Seq derived datasets from the Human Cell Atlas in single myeloma cells from patients, single plasma cells from normal donors or single B cells. HSC, hematopoietic stem cell; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; MKP, MK progenitor; ERP, ER progenitor; CD34 Gran, CD34 + Granulocyte progenitor, MixLin, mixed lineage progenitor; PreB, Pre-B cell; ProB, Pro-B cell; PC, plasma cell. **c)** Heatmap showing relative expression of individual genes from different lineages from the Human Cell Atlas dataset in single myeloma cells, single normal donor plasma cells or single B cells. The enlarged heatmaps in b-c are provided in Supplementary Information.

**d)** UMAP colored by patient. **e)** RNA velocity estimates of single myeloma cells and normal donor plasma cells projected onto two-dimensional UMAP. Normal donor plasma cells are indicated in red.

**f)** Cells colored based on cell cycle phase. **g)** Number of genes detected per cell in single myeloma vs normal donor plasma cells. Boxplots show the median and interquartile range, whiskers extend to 1.5x the interquartile range. n = 1,162 cells. p < 2.2e-16 by two-sided Wilcoxon-test.

**h, i)** Entropy is increased in myeloma vs normal donor plasma cells. Boxplots show the median and interquartile range, whiskers extend to 1.5x the interquartile range. n = 1,162 cells. p < 2.2e-16 by two-sided Wilcoxon-test.

**j)** Validation of entropy using an alternative protein-protein interaction network. Boxplots show the median and interquartile range, whiskers extend to 1.5x the interquartile range. n = 1,162 cells. p < 2.2e-16 by two-sided Wilcoxon-test.

**k)** Predicted ordering by CytoTRACE, which orders MM cells based on their developmental potential from most mature (lowest values) to most immature (highest values). Boxplots show the median and interquartile range, whiskers extend from min to max.

**l)** tSNE plots showing expression of plasma cell lineage transcription factors XBP1, IRF4, PRDM1, FOS, POU2AF1 and ZBTB20 (log2-transformed counts) in single myeloma cells, normal donor plasma cells and B cells.
Difference in regulon activity

Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Gene regulatory network activity in different cell types. a–d) Gene regulatory network activity for different cell types was determined from the BLUEPRINT dataset. a) Gene expression in normal plasma cells. b) Gene expression in hematopoietic stem cells (HSCs). c) Gene expression in CLP (common lymphoid progenitor) population. d) Gene expression in macrophages. e) Network layouts for normal hematopoiesis based on the BLUEPRINT dataset (top) and based on our single-cell RNA data (bottom) illustrating extensive rewiring, gain of new connections and changes in relative activity. Edges are colored based on regulon activity where high activity is indicated in red, low activity in blue. Target genes are depicted in white, transcription factors which are not among regulons are shown in green. f) Changes in regulon activity in myeloma compared to normal donor plasma cells are projected onto the network. Transcription factors with the largest difference in regulon activity in myeloma compared to normal donor plasma cells are highlighted in insets. g) tSNE plots showing regulon activity (area under the curve, AUC) of plasma cell lineage transcription factors XBP1, IRF4, PRDM1, and FOS in single myeloma and normal plasma cells.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Quality assessment and filtering of single cell ATAC data. **a**) Filtering of single cell ATAC profiles based on TSS enrichment and number of unique nuclear fragments. **b**) Fragment length distribution of filtered scATAC profiles showing characteristic distribution with nucleosome-free region and mononucleosome peaks. **c**) TSS enrichment after filtering per sample. **d**) Number of unique fragments per sample. **e,f**) tSNE plot colored by clusters (e) or individual (f). **g,h**) tSNE plots following batch effect removal by Harmony colored by clusters (g) or individual (h). **i-k**) Defining cluster identities following integration with scRNA-seq data. **i**) tSNE plot colored by predicted cell type identities. **j**) tSNE plot showing cell type identities by cluster. **k**) Heatmap showing confusion matrix for predicted cell type identities by cluster. **l-o**) Tracks with aggregated ATAC profiles for each cluster for marker genes SDC1, LYZ, MS4A1 and FCGR3A, respectively. **p-s**) tSNE plots colored by gene scores for marker genes SDC1, LYZ, MS4A1 and FCGR3A, respectively.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Annotation of peaks from aggregated scATAC data. a) Processing of scATAC profiles. b) Intersection of peaks in aggregate scATAC samples showing the overlap of peaks between MM and normal donor (ND) samples. c) Number of peaks significantly different by DESeq2 enriched in ND or MM (FDR ≤ 0.1, absolute log2FC ≥ 1). d) Heatmap showing differentially accessible peaks from c) in ND and MM, sorted by the ND sample. e) ChromHMM state annotation in aggregate scATAC samples. Depicted is the fraction of peaks in each of the indicated states. f) Number of peaks in ChromHMM state 13 corresponding to heterochromatin. **p = 0.0029 by two-sided t-test. g) Fraction of peaks in indicated genomic regions. h) Boxplot comparing distance to TSS in ND vs MM. ****p < 2.2e-16 by two-sided Wilcoxon test. Boxplots show the median and interquartile range, whiskers extend to 1.5x the interquartile range. n = 46,935 peaks and 68,752 peaks, respectively. i) Heatmap showing accessibility of enhancers (n = 15,748) associated with the top multi-enhancer genes, sorted by the ND sample. j) Barplot showing genes with ≥ 2 enhancer interactions by ABC model. k) Heatmap showing multi-enhancer genes by ABC model (n = 16,635), sorted by the ND sample. l) Barplot showing gene set enrichment analysis for multi-enhancer genes in MM by ABC model. m) Barplot showing differential motif enrichment analysis of the single cell ATAC-Seq dataset comparing myeloma cells with normal donor plasma cells. Shown are the top 40 differentially enriched transcription factor motifs ordered by FDR. n) Venn diagram showing overlap of differentially enriched motifs and rewired transcription factors determined by DyNet algorithm.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Alternative splicing following treatment in MM. a) Quantification of exon inclusion/exclusion ratio (percent of spliced in, psi). Volcano plot showing differential splicing at C5D1 vs screening timepoints with FDR < 0.05. b) Barplot showing gene set enrichment analysis of differentially spliced transcripts. c) UMAP colored by Louvain clusters based on calculated psi (percent of spliced in) values. d) psi-based UMAP colored by individual. e) psi-based UMAP colored by timepoint. f) Violin plots showing the single cell distribution of logit (percent spliced in) values at screening and C5D1 for selected differentially spliced transcripts. Boxplots show the median and interquartile range, whiskers extend to 1.5x the interquartile range. n = 1,374 cells. **** p ≤ 0.0001 and * p ≤ 0.05 by two-sided Wilcoxon. g-j) Miso plots visualizing splice junctions and potential exon skipping events in differentially spliced transcripts for TSC1 (g), CD200 (h) and CALU (I) as well as SLAMF7 (j). Splice probabilities are shown on the right.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Transcriptional diversity following treatment. 

a) Number of genes expressed in primary MM cells before and after treatment (p < 2.2e-16 by two-sided t-test). Boxplots show the median and interquartile range, whiskers extend to 1.5x the interquartile range. n = 1,374 cells. 

b) Bar graph showing relative proportion of cells in each cell cycle phase at screening and CSD1. 

c) Shown are the lineage TF regulons downregulated and upregulated upon treatment. 

d) Change in regulon activity of lineage TFs upon treatment. 

e,f) GO-term enrichment of ATAC-Seq peaks gained (e) and lost (f) in MOLP2 cells following 72 h treatment with PVD. 

g) Gene set enrichment analysis of the top 500 genes gaining enhancers following treatment with PVD. 

h) Genes with ≥ 5 enhancer interactions by ABC model. 

i) Gene set enrichment analysis of genes gaining enhancer interactions following treatment with PVD by ABC model. 

j) Top rewired TFs with differentially accessible motifs upon treatment.
Extended Data Fig. 10 | Surface marker expression in MM. a) Genes upregulated upon treatment. Shown is a barplot with log2 fold change values compared to screening timepoint. b) Motif enrichment in differential ATAC-Seq peaks in MMCL MOLP2 after PVD treatment (right), comparing to untreated (left). p values are calculated using binomial test. Significance was assessed using a two-sided t-test with Welch’s correction, with * p ≤ 0.05 and **** p ≤ 0.0001. Data are presented as mean ± SD, n = 3 independent experimental replicates. c-f) Live cell counts following 72 h of treatment with pomalidomide (Pom), bortezomib (Bor), dexamethasone (Dex) and combination of all three drugs (PVD) in myeloma cell lines MOLP2 (c), MM1.S (d) and OPM2 (e) as a percentage of total cell numbers. g-i) Quantification of CXCR4 surface levels by flow cytometry following treatment with pomalidomide (Pom), bortezomib (Bor), dexamethasone (Dex) and combination of all three drugs (PVD) in myeloma cell lines MOLP2 (f) and MM1.S (g). Significance was assessed using a two-sided t-test with Welch’s correction, with * p ≤ 0.05 and **** p ≤ 0.0001. Data are presented as mean ± SD, n = 3 independent experimental replicates. h-i) Cell viability following treatment with CXCR4 inhibitors BKT140 (h) and Plerixafor (i) following pre-treatment with PVD in MMCL MOLP2. Data are presented as mean ± SD, n = 3 independent experiments. n.s. p > 0.05, * p ≤ 0.05, *** p ≤ 0.001 and **** p ≤ 0.0001 by two-sided t-test.
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Software and code

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| Data collection | FASTQ files were generated following Illumina sequencing and further analyzed as described below and in the Methods section. Flow cytometry data was collected using the SONY SH800 sorter or the BD Accuri C6 Flow Cytometer and was analysed using FlowJo software (v10). |

Data analysis

For processing of sequencing data, we used trimmomatic, STAR aligner, HTSeq, RSEM and the nfCore/rnaseq pipeline v1.0.0, and for downstream analysis R (v3.6.1 or 4.0), python (v2.7.2 or 3.6.0) and the following packages: mvoutlier (v2.1.1), PAGODA2 (v0.1.1), SingleR (v1.0.1), scran (1.12.1), InferCNV (1.4.0), CONICSmat (v0.0.0.1), MIXCR, randomForest (v4.6-14), ScanPy (v1.4.6), Cytotrache (v0.1.0), PROGENY (v1.6.0), LendSCENT (0.99.3), SCENIC (v1.1.2-2), Seurat (v3.2.1), velocyto, scvelo (v0.2.0), TissueEnrich (v1.4.1), Cicero (v1.2.0), chromVAR (v1.6.0), GVI2 (v1.28.3), deepTools (v3.0.2), ArchR (v0.9.5), edgeR (v3.37.0), NMF (v0.23.0), BRIF2 (v2) and MISO (v0.5.4); also FlowJo (v10), cytoscape (v3.8.0), DyNet (v1.0.0), GREAT (http://great.stanford.edu/public.html), GSEA (https://www.gsea-msigdb.org/gsea/index.jsp). |

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The data collected in this paper include next-generation sequencing data. The NGS data consist of 1) single cell RNA sequencing datasets from primary myeloma and CD45+ immune cells and normal donor bone marrow plasma cells and CD45+ immune cells, and 2) single cell RNA sequencing datasets from multiple myeloma cell
Line MOLP2 treated with PVD or DMSO; 3) single-cell ATAC sequencing datasets were generated from primary myeloma and normal donor plasma cells; 4) bulk ATAC-sequencing datasets from myeloma cell line MOLP2 treated with PVD or DMSO. FASTQ files were generated following illumina sequencing and further analyzed as described below and in the Methods section. The scRNA and ATAC-sequencing data and CUT&Tag data generated in this study have been deposited in GEO under the accession number GSE162337. The cytoscape files for the final gene regulatory networks are available for download at the Broad Institute Single Cell Portal (https://singlecell.broadinstitute.org/single_cell/study/SCP1511/dynamic-transcriptional-reprogramming-leads-to-novel-immunotherapeutic-vulnerabilities-in-myeloma).

Previously published IRF4 Chip-Seq data that were re-analysed here are available under accession code PRJEB25605 from the European Nucleotide Archive. Previously published H3K27ac Chip-Seq data that were re-analysed here are available from the European Nucleotide Archive under accession codes PRJEB2560542 (for patient samples), and PRJNA60882103 (for normal plasma cells). Additionally, we used the following publicly available datasets: the dependency scores available through the deepmap download portal (https://deepmap.org/portal/download/); the BLUEPRINT dataset (Fernandez et al., 2016), JASPAR motif database. Source data are provided with this study. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

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Sample size

As this was an exploratory study, we did not determine sample size prior to the analysis. We based our sample size on being able to capture a patient population that reflects the heterogeneity of the disease, with 3/8 patients with confirmed t11;14 translocation, 1 patient with t11;16 translocation and the remaining patients showing other genetic aberrations. While sample size was not predetermined with statistical methods, sample size was determined based on previous experience and current standards in the field.

Data exclusions

Single cell RNA: No biological samples were excluded from the analyses. Low-quality cells (based on distribution of library size (I), number of detected genes (II), percentage of counts mapping to mitochondrial genes (III), and percentage of counts mapping to house-keeping genes (IHKGs) [IV] per cell) were filtered and excluded from downstream analyses, as described in the Methods section of the manuscript. Single cell ATAC: No biological samples were excluded from the analysis. Low quality cells (based on number of unique nuclear fragments, TSS enrichment score) were filtered as detailed in the manuscript. For further analysis only myeloma cells were retained. For the analysis of collapsed scATAC-seq, samples with fewer than 50 cells following filtering were excluded due to lack of complexity, resulting in exclusion of sample MM1. For bulk ATAC sequencing and all in vitro experiments no data were excluded.

Replication

No attempt of replication failed. Reproducibility of experimental findings was ensured using various approaches. We are showing all data for single cell sequencing experiments, and validated results with other techniques, such as in vitro treatment of myeloma cells lines. Those experiments were replicated at least three times with similar results. The sample size and number of replicates for each experiment is described in the figure legends.

Randomization

Randomization was not performed as part of this study. While we are investigating samples from a clinical trial, we are comparing between individual myeloma patients and with normal donors, and thus are not drawing conclusions as to the efficacy of the treatment.

Blinding

Blinding of investigators was not performed in this study. However, unbiased analyses were performed, such that the investigators were unaware of outcomes.

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Antibodies

**Antibodies used**

CD38 FITC (multi-epitope, Cytognos, cat. #CYT-38F2), CD138 PE (Milenyi, cat. #130-119-840), SLAMF7 APE (162.1, Biologend, cat. #331806), BCMA PE-Cy7 (19F2, Biologend, cat. #357508), CD45 FITC (H130, eBioscience, cat. #11-0459-42), CD3 PerCP-Cy5.5 (OKT3, eBioscience, cat. #45-0037-42), CD14 APC-Cy7 (MoP9, BD Biosciences, cat. #557831), CD19 PE (HIB19, Biologend, cat. #302208) and PE/Cy7 anti-human CXC CR4 (12G5, Biologend, cat. #306514), H3K27ac (abcam, cat. #ab4729).

**Validation**

CD38 FITC (multi-epitope, Cytognos, cat. #CYT-38F2): CYT-38F2 is designed for Flow Cytometry (FC) use for the identification and enumeration of human CD38 antigen-expressing cells even when cells are hampered by other monoclonal antibody.

After an extensive selection of clones against different parts of the CD38 molecule, Cytognos has developed a multi-epitope reagent that allows the identification by FC of CD38 in plasma cells even if the patient is under treatment with Anti-CD38 (source: manufacturer website).

CD138 PE (Milenyi, cat. #130-119-840): FC Quality tested. References:
1. Couchman, J. R. (1996) Syndecans, signaling, and cell adhesion. J. Cell. Biochem. 61 (): 578 -584
2. Bernard, M. (1992) Biology of the syndecans: a family of transmembrane heparan sulfate proteoglycans. Annu. Rev. Cell Biol. 8 (): 365 -393

SLAMF7 APC (162.1, Biologend, cat. #331806): FC Quality tested. References:
1. Beyer M, et al. 2012. PLoS One. 7:45466.
2. Tartif V, et al. 2019. Nat Commun. 10:823.
3. Jenks SA et al. 2018. Immunity. 49(4):725-739.

BCMA PE-Cy7 (19F2, Biologend, cat. #357508): FC Quality tested. Reference:
Alhjaj Hussen K, et al. 2017. Immunity. 47:680.

CD45 FITC (H130, eBioscience, cat. #11-0459-42): Selected references:
1. Khaja S, et al. 2017. Front Immunol. 8:519. doi: 10.3389/fimmu.2017.00519
2. Liu Z, et al. 2016. Sci Rep. 6:39808. doi: 10.1038/srep39808

CD3 PerCP-Cy5.5 (OKT3, eBioscience, cat. #45-0037-42): Selected reference:
Rotoalo A, et al. 2018. Cancer Cell. 34(4):396-610.e11. doi: 10.1016/j.ccell.2018.08.017

CD14 APC-Cy7 (MoP9, BD Biosciences, cat. #557831): FC Quality tested. Reference:
Dimitriru-Bona A, et al. 1983. J Immunol. 130(1):145-152.

CD19 PE (HIB19, Biologend, cat. #302208): FC Quality tested. Selected references:
1. Boier C, et al. 2018. Dev Cell. 44:362.
2. Palazzo A, et al. 2018. J Immunol. 200:2304.
3. Kagoya Y, et al. 2018. Nat Commun. 9:2915.

PE/Cy7 anti-human CXC CR4 (12G5, Biologend, cat. #306514): FC Quality tested. Selected references:
Raposo R, et al. 2013. J Leukoc Biol. 94:1051.
Martínez-Cingolani C, et al. 2014. Blood. 124:3411.
Lourenço S, et al. 2015. J Immunol. 194:3463.

H3K27ac (abcam, cat. #ab4729): CHIP Grade. References:
1. Chen Z, et al. Imaging assisted evaluation of antitumor efficacy of a new histone deacetylase inhibitor in the castration-resistant prostate cancer. Eur J Nucl Med Mol Imaging 48:53-66 (2021).
2. Wei J, et al. Genome-wide CRISPR Screens Reveal Host Factors Critical for SARS-CoV-2 Infection. Cell 184:76-91.e13 (2021).
3. Zhao Y, et al. Ubiquitin-Specific Processing Protease 7 Regulates Female Germ Cell Self-Renewal Through DNA Methylation. Stem Cell Rev Rep N/A:NA/NA (2020).

Eukaryotic cell lines

**Policy information about** cell lines

**Cell line source(s)**

Human multiple myeloma cell lines (MMCL) were obtained from ATCC [MM1.5, CRL-2974] or DSMZ [MOLP-2, ACC 607, OPM-2 ACC 50].

**Authentication**

Cell lines were authenticated by STR profiling.

**Mycoplasma contamination**

All cell lines tested negative for mycoplasma contamination.

**Commonly misidentified lines**

See cell line register.

**This study did not include any commonly misidentified cell lines.**

Human research participants

**Policy information about** studies involving human research participants

**Population characteristics**

Banked blood or bone marrow samples from patients with relapsed/refractory multiple myeloma (Supplementary Table 1), who had signed consent and were enrolled on a phase II clinical trial investigating with elotuzumab, pomalidomide, bortezomib and dexamethasone on a clinical trial (E10-PVD; NCT02718833) were used in this study. The research was determined not human subject research by DF/HCC IRB (19-511).

**Recruitment**

NA

**Ethics oversight**

DF/HCC-IRB
Flow Cytometry

Plots

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
Fresh bone marrow aspirates/peripheral blood samples were obtained from all patients. RBC lysis was performed prior to further processing. Myeloma cells were enriched using the EasySep Human CD138 positive selection kit (STEMCELL Technologies). Bone marrow from 2 normal donors was obtained from AllCells.

Instrument
Primary patient samples were sorted using a SONY SH800 sorter. For in vitro experiments, cells were analyzed using the BD Accuri C6 Flow Cytometer.

Software
Analysis was performed using FlowJo software v10.

Cell population abundance
Single cell sorting was performed directly into plates with lysis buffer.

Gating strategy
For primary patients samples, dead cells were excluded based on DAPI staining, size was used to exclude debris, and doublets were excluded prior to further gating. For myeloma cells, CD38+CD138+ cells were sorted. Immune cell populations were gated off CD45+ cells. Sorting strategies for individual populations are shown in Extended Data Figure 1. For in vitro experiments, dead cells were excluded based on Annexin V staining.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.