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A novel rapid-onset high-penetrance plasmacytoma mouse model driven by deregulation of cMYC cooperating with KRAS12V in BALB/c mice

Y Hu¹, M Zheng², R Gali³, Z Tian¹, G Topal Görgün¹, NC Munshi¹, CS Mitsiades¹ and KC Anderson¹

INTRODUCTION

Multiple myeloma (MM) is a B-cell neoplasm characterized by accumulation of monoclonal plasma cells.¹ Neoplastic transformation in MM is associated with genomic and epigenetic dysregulation.² Previous studies have revealed that 40% of MM harbor chromosome translocations, including CCND1, CCND2, MAFB and FGF3/WHSC1, with immunoglobulin heavy chain (IgH).³ Deletions of chromosome 13 are frequently detected in early and late stage MM.⁴ During disease progression, genetic lesions accumulate, including mutations of NRAS and KRAS, overexpression of cMYC and downregulation of P53.⁵ Using whole-genome sequencing and mutations of NRAS and KRAS, overexpression of cMYC and downregulation of P53, more genetic lesions have been identified. Thus, it is critical to develop a short latency in vivo model to functionally evaluate the roles of these dysregulated genes in MM pathogenesis.

Mouse models both facilitate evaluation of the roles of genetic lesions detected in MM and provide for assessing therapeutic agents. The earliest mouse model for MM was induced by intraperitoneal injection of mineral oil, adjuvant and alkanes in BALB/c mice.¹¹ Several transgenic mouse models have been widely used to study acute myeloid leukemia,¹⁷ chronic myeloid leukemia,¹⁸ B-cell acute lymphoid leukemia¹⁹ and the majority of myeloproliferative neoplasms.²⁰ Based on previous studies,¹² we hypothesized that retroviral delivery of cMYC into a later stage B-cell subset might induce plasmacytomas in mice. MYC requires the cooperation or complementation with other oncogenes, such as v-H-ras or v-raf, for transformation.²¹ We here therefore introduced KRAS (KRAS12V) mutation to complement the function of cMYC with KRAS12V in T2 B cells rapidly generates a plasma cell disease in mice, suggesting utility of this model both to elucidate molecular pathogenesis and to validate novel targeted therapies.

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Keywords: multiple myeloma (MM); cMYC; KRAS12V; retroviral transduction and transplantation; BALB/c mouse; plasmacytoma

Our goal is to develop a rapid and scalable system for functionally evaluating deregulated genes in multiple myeloma (MM). Here, we forcibly expressed human cMYC and KRAS12V in mouse T2 B cells (IgM⁺ B220⁺ CD38⁺ CD19⁻) using retroviral transduction and transplanted these cells into lethally irradiated recipient mice. Recipients developed plasmacytomas with short onset (70 days) and high penetrance, whereas neither cMYC nor KRAS12V alone induced disease in recipient mice. Tumor cell morphology and cell surface biomarkers (CD138⁺, CD19⁻, IgM⁺, GFP⁺) indicate a plasma cell neoplasm. Gene set enrichment analysis further confirms that the tumor cells have a plasma cell gene expression signature. Plasmacytoma cells infiltrated multiple loci in the bone marrow, spleen and liver; secreted immunoglobulins; and caused glomerular damage. Our findings therefore demonstrate that deregulated expression of cMYC with KRAS12V in T2 B cells rapidly generates a plasma cell disease in mice, suggesting utility of this model both to elucidate molecular pathogenesis and to validate novel targeted therapies.

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cMYC in tumorigenesis. With this strategy, we generated a rapid-onset high-penetrance plasmacytoma mouse model by enforced expression of cMYC and KRAS12V in later transition (T2) B-cell subset. This model provides a rapid tool to functionally evaluate genes in MM pathogenesis, as well as evaluate novel targeted therapies.

**MATERIALS AND METHODS**

**DNA constructs**

All PCR products were cloned into T-easy vector (Promega BioSystems, Sunnyvale, CA, USA) and completely sequenced to verify the correct reading frame.

To make a subclone vector MSCVm, MSCV-IRES-eGFP (MIG) was digested with BglII and CalI to remove IRES and eGFP, and ligased with an oligonucleotide (5'-GATCGGATCCGGATCCGGCCCAAGCTTCAATTGGCGGCCGAGTCGAGAT-3') containing multiple clone sites, EcoRI, Hpal, BamHI, Apal, HindIII, Mfel, NotI, BglII, XhoI and Clal.

Vector MIGm was constructed by introducing IRES-eGFP generated by PCR with a 5' primer containing an EcoRI and a 3' primer containing a Mfel site into the Mfel site of MSCVm vector. MIGm-cMyc was generated by introducing cMYC released from MSCV-hcMYC-IRES-GFP vector (Addgene, Cambridge, MA, USA) with EcoRI into EcoRI site of MIGm vector. MIGm-KRAS12V was made by inserting KRAS12V obtained from T vector with NotI (blunt) and MfeI into MIGm vector digested with EcoRI and Hpal.

**Figure 1.** cMYC/KRAS12V can transform BaF3 cells independent of IL3 and transduce T2 B cells in vitro. (a) Workflow for generation of the adoptive plasmacytoma mouse model. (b) Schematic diagram of MSCV-based retroviral vectors: MIG, cMYC, KRAS12V and cMYC/KRAS12V. (c) Western blot analysis showed expression of MIG, cMYC, KRAS12V and cMYC/KRAS12V in transfected 293T cells. Total protein was analyzed against human MYC (upper) and RAS (middle); GAPDH (lower) served as a loading control. (d) KRAS12V and cMYC/KRAS12V drove BaF3 cells to grow independent of IL3 in vitro. Representative cells from two independent experiments are shown. (e) The purification of mouse IgM⁺ spleen B cells after micro-bead isolation is shown (left panel). The cell population transited to T2 B-cell subset (IgM⁺ B220⁺ CD38⁺ IgD⁺) after stimulation with LPS and mIL4 for 48 h (right three panels). Assays were independently and repeatedly performed. (f) Colonies in methylcellulose assays were observed only in cells transduced by cMYC/KRAS12V but not other groups. Three independent experiments were performed.

**Figure 2.** cMYC/KRAS12V-induced plasmacytoma in BALB/c mice. (a) Recipients receiving MIG- or cMYC- or KRAS12V-transfected donor cells remained tumor free, whereas all recipients of cMYC/KRAS12V cells died with plasmacytomas. Secondary transplantation recipients of cMYC/KRAS12V-transduced tumor cells died with similar syndromes (2nd, secondary transplantation). Primary transplantation was repeated five times, and secondary transplantation was independently and repeatedly performed. Group sizes and survival times are indicated. (b) Tumor in peritoneal cavity and splenomegaly were observed in the cMYC/KRAS12V group animals (n = 20, from five independent experiments). (c) H&E staining showing tumor cell morphology (n = 6). (d) Tumor cells from peritoneal cavity (P.C.) and ascites (A.C.) (n = 10) were characterized as CD138⁺ B220⁺ IgM⁺ GFP⁺ by flow cytometry.
MSCV-m-cMYC-2A-eGFP-ires-KRAS12V (MIKMGG) was made by the following process: first, MSCVm was inserted a 2A sequence (5′-GATCCCGAGCTGTTGAATTTTGACCTTCTTAAGCTTGCGGGAGACGTCGAGTCCAACCCCGGGCC-3′), which contains a BamHI and an Apal competent site at 5′ and 3′ end, respectively; then, an eGFP coding sequence was inserted into MSCVm-2A vector, which was generated by PCR with a 5′ primer.
containing a Apal site without its start code sequence and 3’ primer containing an MfeI site; next, IRES element was cloned into MSCVm-2A-eGFP vector at MfeI site, which was generated by PCR with a 5’ primer containing an EcoR1 site and 3’ primer containing an MfeI site; the open reading frame encoding cMYC generated by PCR from MIG-hcMYC vector (Addgene, 18119), with a 5’ primer containing an EcoR1 site and a
3' primer containing a BamH1 site without stop coding sequence, was inserted into MSCvM-2a-eGFP-IRES with EcoRI and BamHI. KRAS12V open reading frame generated by PCR from pBabe-KRAS12V (Addgene, 12544) was cloned into MSCvM-cMYC-2a-eGFP-IRES with MfeI and NtIiBott behind the IRES element.

Viral stock
All DNAs were purified in CsCl gradients. Lenti-X 293T cells (Clontech Laboratories, Mountain View, CA, USA) were co-transfected with 10 µg retroviral vector DNA and 5 µg pCL-EcoRipac packaging vector DNA using CaCl2 method. Medium was changed at 24 h post transfection, and the supernatant was harvested at 48 h after transfection. Supernatant was filtered with 0.45-µm filters, aliquoted and frozen in a freezer at –80°C. The virus titer was determined by transduction of NIH 3T3 cells, and the percentage of GFP+ cells was screened by flow cytometry. After calculation, all viruses had titters > 4.8 x 10⁶ GFP+/ml.

Mouse IgM+ B-cell isolation
All animal experiments were approved by and conformed to the standards of the Institutional Animal Care and Use Committee at the DFCI. BALB/c or C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME, USA; 000651) aged 6–12 weeks were used in all experiments. Mice were killed by CO2 asphyxiation. Mouse IgM+ splenic B cells were isolated using magnetic microbeads and LS MACS separation columns (Miltenyi Biotec, Cambridge, MA, USA).

Retroviral transfection and transplantation
IgM+ cells were counted and plated at 2 x 10⁶ cells per 10-cm plate in pre-stimulation medium of RPMI1640 containing 5% (vol/vol) fetal calf serum, 1% (vol/vol) penicillin/streptomycin, 100 µg/ml ciprofloxacin, 200 µM L-glutamine, 10 ng/ml recombinant murine interleukin 4 (ml4); Sigma, St Louis, MO, USA), and 50 µg/ml lipopolysaccharide (LPS; Sigma). After pre-stimulation for 24 h, viable cells were counted and transduced with retroviral stocks in the same medium containing 50% retroviral supernatant, 10 µl Hepes, pH 7.4 and 2 µg/ml polybrene. To increase transduction efficiency, virus and cells were cosedimented at 1000 g for 90 min in a Sorvall RT-5.3 centrifuge. Medium was changed after a 3-h adsorption period. On the next day, a second round of transduction and cosedimentation was performed using the same conditions. After another 3 h, cells were harvested and 5 x 10⁶ cells were injected via the lateral tail vein with 5 x 10⁶ BM into lethally irradiated recipients.

Flow cytometry
Red blood cells were removed from single-cell suspensions of peripheral blood, BM, peritoneal fluid and spleen by RBC lysis buffer. Cells were then washed once with PBS and stained for 15 min at room temperature with the combination of the following antibodies: IgM-PE, B220-PECy7, CD138-APC, CD38-PE and Igd-PE washing once with PBS, and then cells were analyzed on a FACSCalibur machine (Becton Dickinson, Franklin Lakes, NJ, USA) using Cell Quest software (Becton Dickinson).

Western blots
Whole-cell extracts from target cells were prepared, electrophoresed onto NC membranes (Amer sham, Wilson, OK, USA) and probed with primary antibodies according to the standard procedures. The anti-cMYC, RAS and eGFP antibodies were obtained from Cell Signaling. Following incubation with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse secondary antibody (Cell Signaling, Danvers, MA, USA), bound Igs were detected using ECL detection solutions (Pierce, Rockford, IL, USA). Anti-GAPDH served as a loading control.

Southern blot analysis of the IgH gene
Genomic DNA was prepared from IgM+ B cells or cMYC/KRAS12V-induced plasmacytoma cells with Qiagen DNeasy 96 Blood and Tissue Kit (Qiagen, Valencia, CA, USA). DNA was digested with EcoRI. Blots were hybridized with a JH4 probe of the mouse IgH locus. The JH4 probe was generated by PCR using a plasmid containing a 1.9 BamHI-EcoRi genomic fragment of mouse heavy-chain locus as template and primers JH4f (5′-TATTGCG-TATGGACTACTGG-3′) and JH4r (5′-CCTCGAGGTCCGCTGTAAC-3′). Southern blot hybridization was performed as described.22

Gene expression profiling
RNA from purified IgM+ B cells and plasmacytoma cells was extracted with RNA mini kit (Grand Island, NY, USA), and gene expression profiling was performed using the Affymetrix mouse 430A2.0 gene chip (Affymetrix, Cleveland, OH, USA). Microarray data were analyzed with OneChannelGUI package in R workplace. Differential expression was determined using the LIMMA model. Gene signatures were analyzed with gene set enrichment analysis (GSEA) in Molecular Signatures Database (MsigDB).

RESULTS
Design of multiple gene expression MSCV-based vectors
To develop an adoptive mouse plasmacytoma model, we purified splenic IgM+ B cells, which were cultured with LPS and mL4 for 24 h. These target cells were transfected twice within 24 h and then injected via tail vein (intravenous) into lethally irradiated syngeneic recipients (Figure 1a). To develop oncogene expression vectors, we modified the retroviral vector MIG (MSCV-ires-eGFP) by inserting multiple cloning sites. We constructed three oncogene expression vectors: cMYC (5′-LTR-cMYC-ires-eGFP-3′LTR), KRAS12V (5′-LTR-KRAS12V-ires-eGFP-3′LTR) and cMYC/KRAS12V (5′-LTR-cMYC-2a-eGFP-ires-KRAS12V-3′LTR). All oncogenes were driven by the cytomegalovirus type I enhancer and the mouse sarcoma virus promoter within the 5′ LTR (long terminal repeat; Figure 1b). Target genes were efficiently expressed by the vectors in transiently transfected 293T cells (Figure 1c).

To investigate the biological and functional sequelae of these oncogenes, we transfected BaF3 cells with MIG, cMYC, KRAS12V or cMYC/KRAS12V. Two days after transfection, GFP+ cells were sorted and cultured without IL3. After a week, both KRAS12V and cMYC/KRAS12V, but not cMYC or MIG alone, drove BaF3 cells growth in vitro independent of IL3 (Figure 1d). These data suggest...
that KRAS12V has the capacity to suppress cMYC-induced apoptosis in BaF3 cells.

Previous studies have shown that overexpression of oncogenes cMYC, cMAF, and XBP-1 driven by $\text{E}_{\text{mu}}$ promoter in immature B cells induces plasma cell neoplasms in mice. Here we used T2 B-cell subset (IgM$^{+}$ B220$^{+}$ CD38$^{+}$ IgD$^{+}$) as target cells, which are generated from stimulating purified IgM$^{+}$ B cells with LPS and mIL4 for 48 h (Figure 1e). To determine whether cMYC-, KRAS12V- or cMYC/KRAS12V-transfected cells could grow in vitro, we performed soft agar colony assays. T2 B cells transfected with MIG, cMYC, KRAS12V or cMYC/KRAS12V were seeded in soft agar culture media, and after 2 weeks, only cMYC/KRAS12V-transduced cells formed colonies in vitro (Figure 1f). These results suggested that cMYC/KRAS12V had the capacity to promote independent T2 B cells growth in vitro.

**cMYC/KRAS12V induced plasmacytomas in BALB/c mice**

To determine whether cMYC/KRAS12V can transform T2 B cells and induce plasmacytomas in vivo, we transplanted T2 B cells transfected with MIG, cMYC, KRAS12V or cMYC/KRAS12V into lethally irradiated syngeneic recipient mice. Only mice that received cMYC/KRAS12V-transfected T2 B cells developed fatal tumors within 10 weeks post transplantation (Figure 2a). In diseased mice, peritoneal tumor, splenomegaly, and ascites were noted (Figure 2b). Tumor cells had dispersed nuclear chromatin, a low nuclear-to-cytoplasmic ratio and amphophilic cytoplasm with paranuclear hof. Binucleate cells and mitoses were rarely observed (Figure 2c). The analyses by flow cytometry showed tumor cell surface positivity for GFP and CD138 (Figure 1e). The analyses of disease manifestations in MM mice include IgG1, IgM and IgA heavy chains and both of $\kappa$ and $\lambda$ light chains (Figure 4b). Southern blot analysis of IgH gene rearrangement in plasmacytoma cells indicated that cMYC/KRAS12V-induced plasmacytomas were clonal (Figure 4c).

In human MM, development of Ig heavy and light chain, as well as infiltration of tumor cells, leads to both renal tubular obstruction and glomerular damage. We observed glomerular shrinkage in cMYC/KRAS12V mice but not in other groups (Figure 5a). These renal lesions were similar to pathological manifestations in MM. To assess Ig deposition, we performed IHC assays with antibodies specific for mouse Igs IgG, IgM, $\kappa$ and $\lambda$. Ig chains were deposited in both the tubules and glomeruli (Figure 5b).

Another hallmark of human MM is osteolytic bone lesions. To determine whether the bone lesions developed in diseased mice, we examined bone structure changes with micro-computed tomography (micro-CT). No bone oseolysis was detected, even in the cMYC/KRAS12V group mice (Figure 5c).

**Gene expression profiling in cMYC/KRAS12V-induced plasmacytoma cells**

To gain further insights into the roles of cMYC/KRAS12V in tumorigenesis and identify the tumor type, we analyzed the gene expression signatures of cMYC/KRAS12V-induced tumor cells by using global transcriptional profiling analysis and comparing tumor cells to syngeneic murine IgM$^{+}$ B cells. Genes were considered significantly altered based on more than twofold change in mean expression ($P<0.05$). Using these criteria, 4466...
genes were significantly altered in tumor cells: 2333 were upregulated and 2133 were downregulated. Transcription factors required for plasma cell differentiation and survival were significantly increased: PRDM1 (Blimp-1), IRF4 and XBP-1 were increased 5.9-, 4.8- and 3.6-fold, respectively. Conversely, B-cell transcription factors absent or downregulated in plasma cells, including BCL-6, CIITA, MTA3, EBF1, STAT3, PAX5 and SPIB, were also significantly downregulated in plasmacytoma cells (Supplementary Data, Supplementary Table S1). Expression of several hallmark plasma cell differentiation and survival genes were confirmed using quantitative real-time PCR (Figure 6a).

GSEA also revealed significant enrichments for upregulation of genes related to plasma cell differentiation and survival genes were confirmed using quantitative real-time PCR (Figure 6a). GSEA also revealed significant enrichments for upregulation of genes related to plasma cell differentiation and survival (Figure 6b, Supplementary Figure S1A), whereas gene sets related to B-cell receptor signaling were downregulated (Figure 6d). As expected, genes regulated by IRF4 and XBP1 were significantly upregulated (Figure 6e and Supplementary Figures S1B and C). Finally, several components of the nuclear factor-κB (NF-κB) pathways, including NF-κB1 and NF-κB2, and Rel, RelA and RelB were also downregulated in tumor cells (Figure 6f and Supplementary Figure S1D). These data are consistent with previous comparison of plasma cells versus IgM⁺ B cells.29–32

To confirm the functional roles of cMYC and KRAS12V in plasmacytoma genesis, we performed GSEA with four well-established canonical transcriptional signatures of MYC-dependent genes.36,33–35 Most genes in these gene sets were significantly upregulated in plasmacytoma cells (Figure 7a and Supplementary Figure S2A). Previous studies have demonstrated that RAS suppressed cMYC-induced apoptosis via activating PI3K/AKT pathway.36 Activated AKT in turn phosphorylates and activates mammalian target of rapamycin (mTOR), an essential component of mTORC1 that promotes tumor cell proliferation and survival.37 In cMYC/KRAS12V-transduced plasmacytoma cells, genes in mTOR target gene set38 were significantly upregulated (Figure 7b and Supplementary Figure S2B). These results further confirm the formation of plasmacytoma due to cooperation of cMYC and KRAS12V. As in other tumors, genes regulating cell cycle,39 as well E2F40 target genes, and genes suppressed by RB141 were also significantly upregulated (Supplementary Figure S2C). Apoptotic signaling was inhibited by cMYC/KRAS12V: specifically, genes suppressed by P53 and P73 during cell growth arrest and apoptosis were enriched and upregulated in plasmacytoma cells (Figure 7c and Supplementary Figure S2C). Several other gene sets were also enriched and upregulated plasmacytoma cells, including...
EZH2,42 NRF243 and YAP44 (Figure 7d). These results provide further insight into the role of cMYC/KRAS12V in plasmacytoma pathogenesis and identify potential therapeutic targets.

Identifying provirus integration sites and altered genes

Single oncogene transgenic MM mouse models may have long disease latency until the development of disease, as additional genetic lesions and/or epigenetic dysregulations in tumor-initiating cells may be required for tumor development.12,13 In an adoptive model, provirus integration into host genome can alter host-cell gene expression.15 To determine whether provirus integrations are involved in the pathogenesis in our model, we identified MSCV integration sites using ligation-mediated PCR method. In total, we identified 11 retroviral integration sites in three diseased mice (Figure 8a). The integration sites are not recurrent, and

**Figure 6.** Plasma cell transcription signatures in cMYC/KRAS12V-transduced plasmacytoma cells. (a) Expression of selected genes from microarray results were confirmed using quantitative real-time PCR. All data represent mean of triplicate experiments. (b) GSEA showed a plasma cell gene expression signature of plasmacytoma cells. (c) GSEA showed genes associated with B-cell receptor signaling to be significantly downregulated in plasmacytoma cells. (d) GSEA showed genes in IRF4 target gene set were upregulated in plasmacytoma cells. (e) Table of the gene sets related to plasma cell differentiation and survival.
there is no evidence indicating an association with MM (Figure 8b). These data indicate that the plasmacytomas derived by MSCV-cMYC/KRAS12V did not require additional genetic lesions.

DISCUSSION

Here we report a rapid-onset high-penetrance mouse model of plasmacytoma based on enforced expression of both cMYC and KRAS12V in T2 B subset population cells via retroviral transduction, followed by transplantation into lethally irradiated mice. The disease latency was about 7 weeks, all recipients developed disease, and most recipients died within 10 weeks. Compared with transgenic models, this adoptive model significantly reduces both time and cost and provides a highly efficient system to evaluate oncogenes. Moreover, it is feasible to combine oncogenes to study their cooperation effects. Conversely, we can introduce RNA-interfering techniques to suppress the expression of specific targets associated with oncogenes. This rapid model therefore provides a faithful in vivo system to functionally evaluate the genetic lesions in cancer, both elucidating pathogenesis and evaluating therapeutic targets.

We selected cMYC and KRAS12V as examples to examine the possibility that enforced expression of oncogenes in a specific B-cell population could model plasmacytoma in mice. Deregulated activity of cMYC is highly associated with MM, and previous transgenic mouse models and chemically induced plasmacytomas in BALB/c mice have demonstrated the roles of cMYC in the development of plasmacytomas. KRAS as a secondary oncogene was selected as KRAS mutations can suppress cMYC-induced apoptosis in rat fibroblasts via PI3K/AKT signaling, and KRAS mutations are frequently detected in MM. Moreover, previous studies showed that KRAS12V could enhance cMYC-induced apoptosis via constitutively activating Raf pathway in rat fibroblasts. Of note, v-myc and v-raf had synergistic roles in plasmacytomas in BALB/c mice induced by pristine priming, suggesting that the role of Raf signaling might vary with cell types or genetic backgrounds. As expected, KRAS12V efficiently suppressed cMYC-induced apoptosis in a pre-B-cell line (murine BaF3 cells) in vitro and successfully induced plasmacytomas in vivo by cooperating with cMYC. Finally, in our adoptive mouse model, neither cMYC nor KRAS12V alone was sufficient to induce plasmacytomas in BALB/c mice. Previous transgenic mouse models already indicate that secondary mutations are required for the development of plasmacytomas contributing to their long latency time. Here, we provide KRAS12V as a required secondary gene lesion, which significantly reduces disease latency time.
micro-CT may be associated with lethal irradiation\textsuperscript{48} or could be manifested at later points of time. Other MM features, such as hyperglobulinemia and renal injury, were mimicked in this model. After secondary transplantation of cMYC/KRAS12V-induced plasmacytomas, all recipients developed disease at short latency, 21 days post transplantation compared with 70 days primary transplantation recipients. The major disease features of secondary transplant recipients resemble those in primary transplant recipients. Thus, secondary transplant recipients may represent an even more suitable system for preclinical drug evaluations.

Previous studies have demonstrated that chemically induced plasmacytoma are mouse strain specific.\textsuperscript{49} Consistent with these findings, we find that BALB/c, but not C57BL/6, mice are susceptible to cMYC/KRAS12V-induced plasmacytoma. This provides us a way to investigate the role of tumor-suppressor genes in MM pathogenesis, such as P53 and RB1. For example, in both human MM and murine plasmacytoma, loss of function of P53 or RB1 is commonly observed. Thus, our model could be used to determine whether P53 or RB1 has a critical role in preventing the development of cMYC/KRAS12V-induced plasmacytoma.

In summary, this adoptive plasmacytoma mouse model faithfully reflects most of the major characteristics of MM in patients. It affords a system to evaluate the oncogenic events in MM, elucidate mechanism of these events and identify potential targets for future therapy. It will also be useful to study microenvironmental and epigenetic factors in MM, as well as assess potential targeted therapies.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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