blood®
Brief Report

LYMPHOID NEOPLASIA

Subclonal *TP53* copy number is associated with prognosis in multiple myeloma

Vallari Shah,[1] David C. Johnson,[1] Amy L. Sherborne,[1] Sidra Ellis,[1] Frances M. Aldridge,[2] Julie Howard-Reeves,[2] Farzana Begum,[1] Amy Price,[1] Jack Kendall,[1] Laura Chiecchio,[3] Suvi Savola,[4] Matthew W. Jenner,[5] Mark T. Drayson,[6] Roger G. Owen,[7] Walter M. Gregory,[8] Gareth J. Morgan,[9] Faith E. Davies,[9] Richard S. Houlston,[1] Gordon Cook,[10] David A. Cairns,[8] Graham Jackson,[11] and Martin F. Kaiser,[1] on behalf of the National Cancer Research Institute Haematology Clinical Studies Group

[1]Division of Molecular Pathology, Institute of Cancer Research, London, United Kingdom; [2]Centre for Molecular Pathology, The Royal Marsden Hospital, London, United Kingdom; [3]Wessex Regional Genetics Laboratory, Salisbury NHS Foundation Trust, Salisbury, United Kingdom; [4]MRC-Holland, Amsterdam, Netherlands; [5]Department of Haematology, University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom; [6]Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, United Kingdom; [7]Haematological Malignancy Diagnostic Service, St. James's University Hospital, Leeds, United Kingdom; [8]Clinical Trials Research Unit, Leeds Institute of Clinical Trials Research, Leeds, United Kingdom; [9]Myeloma Institute, University of Arkansas for Medical Sciences, Little Rock, AR; [10]Leeds Institute of Cancer and Pathology, University of Leeds, Leeds, United Kingdom; and [11]Department of Haematology, University of Newcastle, Newcastle upon Tyne, United Kingdom

KEY POINTS

- ***TP53* deletion of minor tumor subclones is independently prognostic in newly diagnosed multiple myeloma.**
- **Assessment of subclonal *TP53* deletions by MLPA is readily applicable in standard diagnostics, enabling stratified patient management.**

Multiple myeloma (MM) is a genetically heterogeneous cancer of bone marrow plasma cells with variable outcome. To assess the prognostic relevance of clonal heterogeneity of *TP53* copy number, we profiled tumors from 1777 newly diagnosed Myeloma XI trial patients with multiplex ligation-dependent probe amplification (MLPA). Subclonal *TP53* deletions were independently associated with shorter overall survival, with a hazard ratio of 1.8 (95% confidence interval, 1.2-2.8; $P = .01$). Clonal, but not subclonal, *TP53* deletions were associated with clinical markers of advanced disease, specifically lower platelet counts ($P < .001$) and increased lactate dehydrogenase ($P < .001$), as well as a higher frequency of features indicative of genomic instability, del(13q) ($P = .002$) or del(1p) ($P = .006$). Biallelic *TP53* loss-of-function by mutation and deletion was rare (2.4%) and associated with advanced disease. We present a framework for identifying subclonal *TP53* deletions by MLPA, to improve patient stratification in MM and tailor therapy, enabling management strategies. (*Blood*. 2018;132(23):2465-2469)

Introduction

Despite recent improvements in survival, patient outcomes remain variable in multiple myeloma (MM). It is increasingly recognized that tumor heterogeneity is a determinant of patient outcome for many cancers, and the identification of subclonal driver events is central to better patient stratification.[1,2] Aberrations in *TP53* are recognized to be one of most important markers of poor prognosis in MM.[3] These are secondary driver events with variable subclonal distribution, with *TP53* typically being deleted and point mutations being relatively rare.[4] However, defining the prognostic association of subclonal deletion of *TP53* in MM at diagnosis with a cutoff for diagnostic purposes has been problematic as a result of the technical challenges of using interphase fluorescence in situ hybridization (iFISH) in MM to quantify subclonal populations.[5] To assess the prognostic relevance of subclonal *TP53* deletion at diagnosis, we profiled 1777 MM trial patients using multiplex ligation-dependent probe amplification (MLPA), which is readily applicable in diagnostic settings.

Methods

Myeloma IX and XI trial patients

We studied 1777 patients with MM enrolled in the UK NCRI Myeloma XI trial, and a subset from MRC Myeloma IX and Myeloma XI underwent comparison of MLPA and iFISH (supplemental Methods, available on the *Blood* Web site).

Copy number, translocation calling, and mutation detection

Bone marrow aspirates were processed as detailed in supplemental Methods. Details about iFISH profiling of Myeloma IX and Myeloma XI have been published previously and are described in supplemental Methods.[6] Myeloma XI cases were profiled for copy number by MLPA and translocations determined by quantitative PCR, as previously reported.[7]

The MLPA P425 probemix (MRC-Holland) interrogates *TP53* exons 4, 7, and 10. *TP53* was considered deleted when

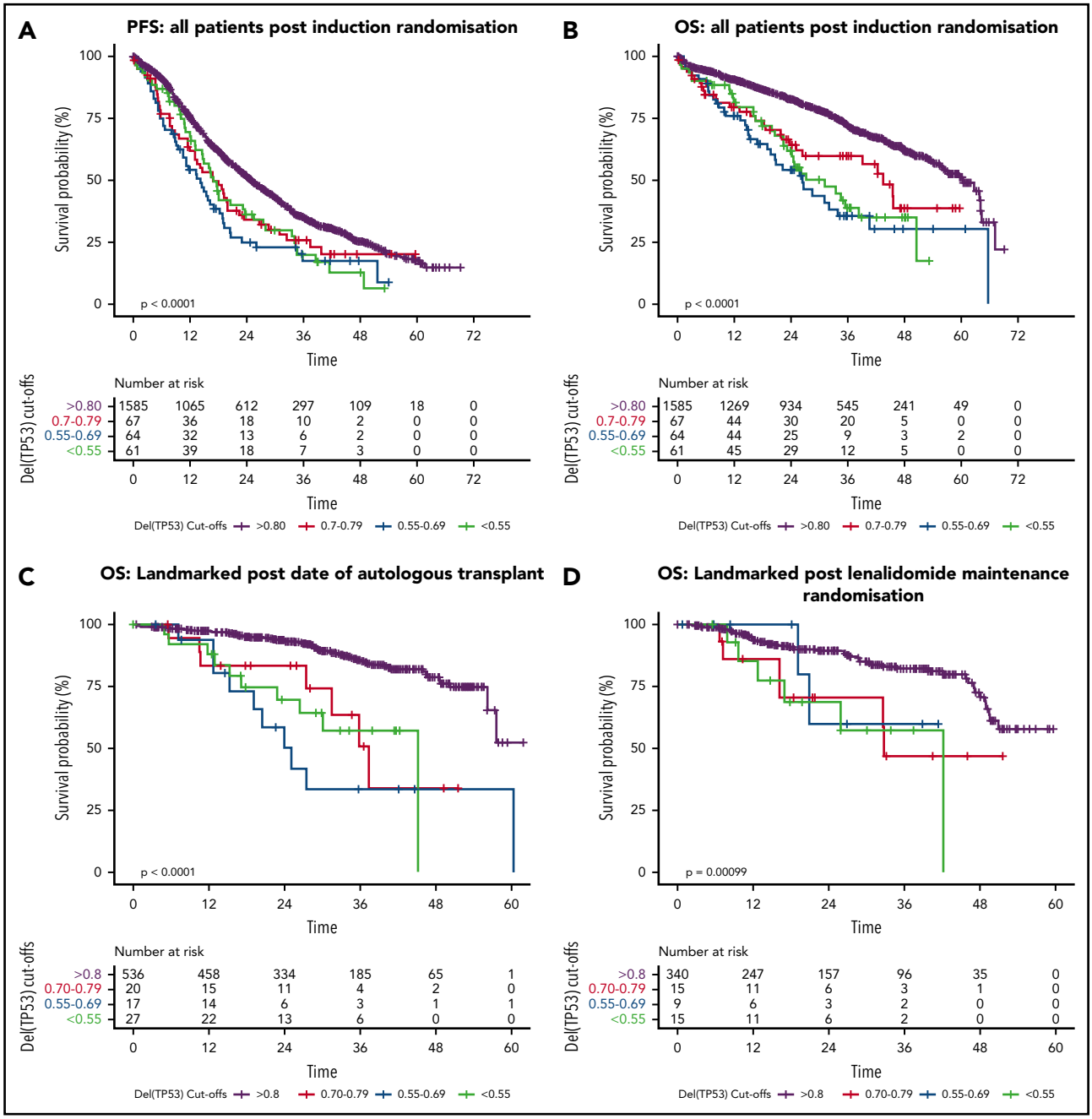

Figure 1. Association between subclonal and clonal *TP53* deletion and survival in newly diagnosed myeloma. Kaplan-Meier survival curves showing progression-free survival (A) and OS (B) of 3 approximately equal-sized *TP53*-deleted clonal subgroups vs no *TP53* deletion in 1777 patients in the Myeloma XI trial. OS evaluation of the above subgroups in landmarked analysis from the time of high-dose melphalan and autologous stem cell transplant (C) and from the time of maintenance randomization (D).

normalized copy number values of 2 of 3 MLPA probes were below the defined cutoff. A total of 1357 patient tumors was further analyzed with probemix X073, covering all exons of *TP53*. Previously published exome sequencing was available for 463 patients.[4]

Statistical analysis

Statistical analyses were performed in R (version 3.4.1) using subroutines survival, survC1, and survivalROC. Progression-free survival was defined as time from randomization to progression or death, and overall survival (OS) was defined as time from randomization to death. To define the optimal prognostic normalized MLPA cutoff value for *TP53* deletion calling, we analyzed subgroups defined by descending (0.05 steps from 1.0 [equivalent to normal diploid copy number]) normalized MLPA value using time-dependent receiver operator curve AUCi (integrated area under the curve) estimates for OS for each cutoff.[8]

Cox proportional hazards regression was used to estimate univariate and multivariable hazard ratios (HRs) and 95% confidence intervals (CIs). Kaplan-Meier survival curves were generated, and homogeneity between groups was assessed using the log-rank test. The association between categorical variables was examined using the Fisher exact test, and the association

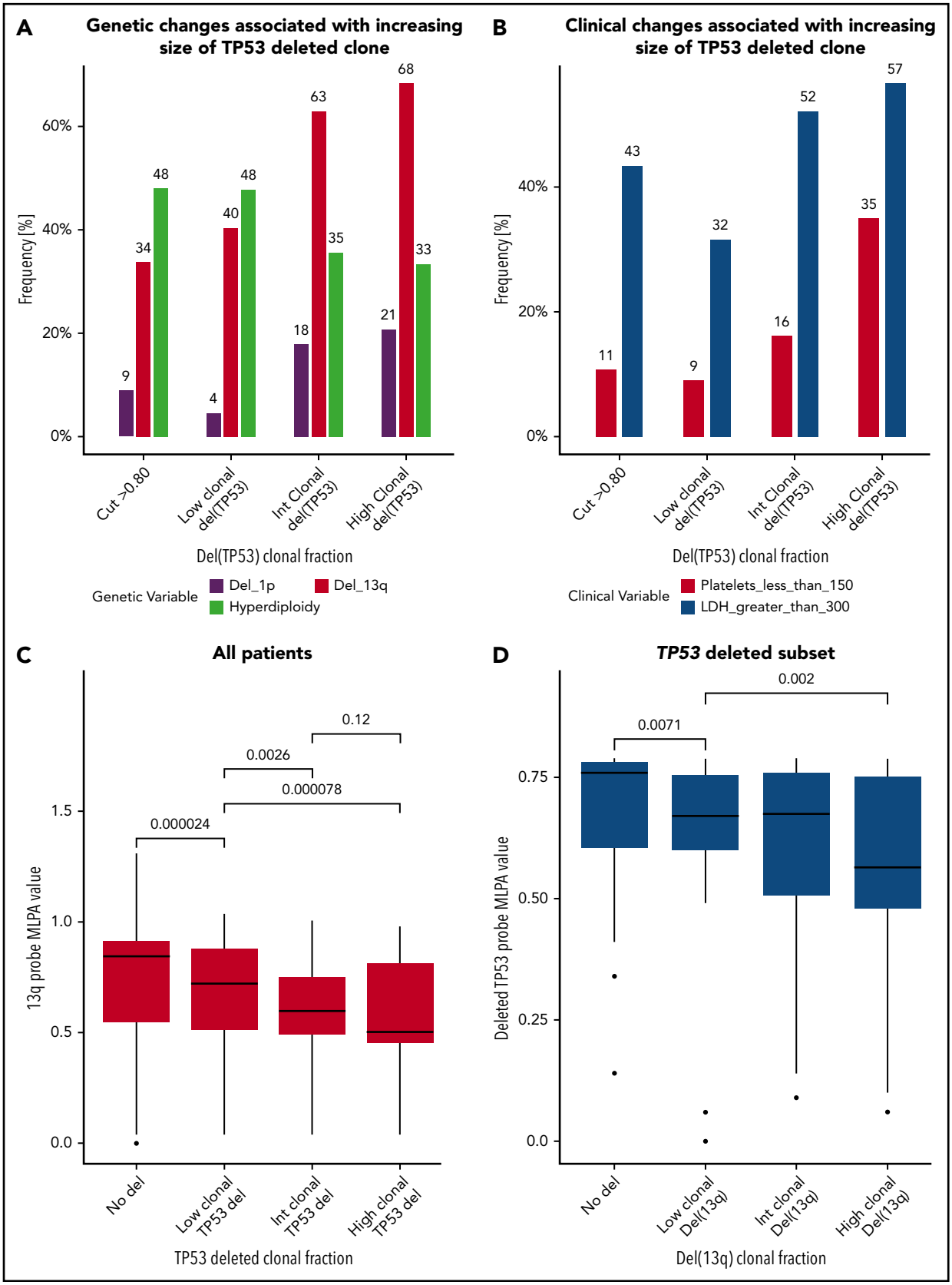

Figure 2. Relationship between subclonal and clonal *TP53* deletion and clinical and genetic characteristics of myeloma. Percentage frequency of genetic changes (A) and clinical changes (B) associated with low, intermediate, and high deletion of *TP53* clone. (C) MLPA values normalized for 13q probes in the same patients with low, intermediate, and high deletion of *TP53* clone. (D) MLPA values across the subset of patients with del(*TP53*) and increasing size of del(13q) clone. Lower MLPA values represent increasing size of deleted clone.

between continuous variables was determined using the Wilcoxon signed-rank test. A 2-sided P value $<.05$ was considered significant.

Results and discussion

To identify the clinically relevant threshold for subclonal *TP53* deletions, we interrogated step-wise increasing fractions of *TP53* deletion by MLPA using the time-dependent receiver operating characteristic curve analysis method (AUCi) for OS.[9] We identified a normalized *TP53* MLPA value <0.8 as the cutoff providing optimal prognostic power, identifying 192 of 1777 (10.8%) tumors as *TP53* deleted (supplemental Figure 1). These results were consistent in intensively (transplant eligible) and nonintensively treated patients (nontransplant eligible) (supplemental Table 1). The optimized <0.8 MLPA cutoff is equivalent to 10% to 20% subclonal 17p deletion, MLPA levels <0.6 were equivalent to clonal deletions with $\geq$50% tumor fraction, and MLPA values <0.5 were equivalent to fully clonal (95% to 100%) del(17p) compared with iFISH in a matched data set from the Myeloma IX[6] and Myeloma XI trials (supplemental Figure 2B). The distribution of MLPA-normalized values for *TP53* probes across 1777 Myeloma XI tumors is shown in supplemental Figure 2. Inclusion of subclonal deletions using an MLPA cutoff <0.8 was confirmed as prognostically most informative by univariate Kaplan-Meier log-rank testing ($P = 6.7 \times 10^{-15}$), Cox regression (Wald $P = 4.1 \times 10^{-14}$), and C-statistic by Uno et al[10] (supplemental Table 2). A limitation of the study is the lack of a validation trial data set.

Treatment allocation, key demographics, and induction response were comparable between patients with *TP53*-deleted and nondeleted tumors, as defined by MLPA <0.8. However, patients with *TP53* deletion showed features of advanced disease and associated morbidity, specifically reduced platelet counts $<150 \times 10^9$/L ($P = 5.1 \times 10^{-4}$) and poorer performance status (World Health Organization [WHO] performance status ≥ 2) ($P = .0012$) (supplemental Table 3). Although WHO was independently associated with shorter survival, the association between WHO and *TP53* deletion suggests an interrelationship with genetic and clinical features that are normally thought of as patient related rather than disease related.

To characterize the features of subclonal vs clonal deletion, *TP53*-deleted tumors were divided into 3 equal-sized subgroups based on MLPA values: subclonally deleted tumors (n = 67; MLPA cutoff $\geq 0.7 < 0.8$), intermediate clonal tumors (n = 64; MLPA $\geq 0.55 < 0.7$), and clonally *TP53* deleted tumors (n = 61; MLPA <0.55). All 3 groups were independently associated with OS, with a subclonally deleted HR of 1.8 (95% CI, 1.2-2.8; $P = .01$), an intermediate-deleted HR of 2.9 (95% CI, 1.9-4.4; $P = 5.6 \times 10^{-7}$), and a clonally deleted HR of 2.2 (95% CI, 1.4-3.2; $P = .0002$) (Figure 1A-B; supplemental Table 4). Landmarked analyses from autologous stem cell transplant and lenalidomide maintenance randomization show consistent results for all 3 groups (Figure 1C-D; supplemental Table 4).

Correlating clinical characteristics, patients with clonal, rather than subclonal, *TP53* deletion had markers of high disease burden, specifically reduced platelet counts ($<150 \times 10^9$/L, 35% vs 9%; $P = .00047$) and high LDH levels (>300 U/L, 57% vs 32%; $P = .012$) (Figure 2B). Clonal vs subclonal deletion of *TP53* was associated with higher rates of del(13q) (68% vs 40%; $P = .002$) and/or del(1p) (21% vs 4%; $P = .006$) (Figure 2A). The rate of *TP53* mutations was increased in clonal deletions (3/18) vs subclonal deletions (1/21). Although MLPA cannot comprehensively assess clonal architecture, an association between *TP53* deletion clonality and an increasing size of del(13q) clone ($P = .002$) (Figure 2C-D; supplemental Figure 5) raises the possibility of coevolution of these lesions. Deletion of *TP53* and *RB1* on chromosome 13q have been shown to be important in cell cycle[11] and senescence,[12] suggesting possible mechanisms for how their codeletion may confer a competitive advantage.

Clonal homozygous *TP53* deletions defined by MLPA values <0.25 were present in 9 of 1777 tumors (0.5%) analyzed with the P425 MLPA probemix for exons 4, 7, and 10 of *TP53*. To identify patterns of focal homozygous deletions, all 11 *TP53* exons were analyzed using a specifically designed X073 MLPA probemix in 1357 patients. Homozygous deletion frequency was low (0.6%), and deletions were focal and not restricted to the DNA-binding domain of *TP53*. Homozygous *TP53* deletion was associated with a very short median OS of 22.4 months and an HR for OS of 3.7 (95% CI, 1.5-8.9; $P = .004$) (supplemental Figure 3; supplemental Table 5). Most patients with homozygous *TP53* deletions had markers of clinically and molecularly advanced disease, with elevated LDH (>300 U/L) in 67%, reduced platelet counts ($<150 \times 10^9$/L) in 67%, and del(13q) in 88%. Exome-sequencing data were available for 422 of the tumors profiled using MLPA. Of these, 10 tumors (2.4%) had biallelic (mutation+deletion) *TP53* loss of function, and 47 tumors (11.1%) had monoallelic loss. Biallelic and monoallelic *TP53* loss was independently associated with inferior survival (supplemental Figure 3, supplemental Table 5).

In summary, we demonstrate an independent association between subclonal *TP53* deletions and MM outcome. Detection of subclonal *TP53* deletion by MLPA is readily applicable within diagnostic settings and could enable stratified treatment approaches aimed at preventing subsequent rapid disease evolution.

Acknowledgments

The authors thank the NCRI Haemato-oncology subgroup and all investigators for recruiting patients to Myeloma XI. The principal investigators at the 4 top recruiting centers were Jindriska Lindsay (Kent and Canterbury Hospital), Nigel Russell (Nottingham University Hospital), Mamta Garg (Leicester Royal Infirmary), and Salim Shafeek (Worcestershire Royal Hospital). The support of the Clinical Trials Research Unit at The University of Leeds was essential to the successful running of the Myeloma IX and Myeloma XI studies, and we thank all staff, past and present, who contributed. We acknowledge the work of Bektas Aygun (MRC-Holland) in the development and optimization of the novel X073 MLPA assay. Finally, we are grateful to all patients whose participation made this study possible.

This work was supported by Myeloma UK, Cancer Research UK Clinical Trials Awards and Advisory Committee sample collection grants (C2470/A12136 and C2470/A17761), and a Cancer Research UK Biomarkers and Imaging Discovery and Development grant (C2470/A14261). M.K. was supported by a Jacquelin Forbes-Nixon Fellowship. The authors also acknowledge support from the National Institutes of Health Biomedical Research Centre at the Royal Marsden Hospital.

Authorship

Contribution: V.S., D.C.J., R.S.H., and M.F.K. conceived and designed the study; all authors acquired data; V.S., D.A.C., R.S.H., and M.F.K. analyzed data; and V.S., R.S.H., and M.F.K. wrote the manuscript.

Conflict-of-interest disclosure: V.S. received travel support from Sanofi and Janssen. S.S. is an employee of MRC-Holland. M.W.J. acted as a consultant for Janssen, Takeda, Amgen, Celgene Corporation, and Novartis; received travel support and honoraria from Janssen, Takeda, and Amgen; received honoraria from Celgene Corporation and Novartis; and received research funding from Janssen and Celgene Corporation. M.T.D. has equity ownership in and serves on the board of directors of Abingdon Health. R.G.O. received honoraria from Takeda and Celgene Corporation; received travel support from Takeda and Janssen; acted as a consultant for Janssen and Celgene Corporation; and received research funding from Celgene Corporation. G.J.M. received research funding from Janssen and Celgene Corporation and acted as a consultant for and received research funding from Bristol-Myers Squibb, Takeda, and Celgene Corporation. W.M.G. received research funding from Celgene Corporation, Amgen, Merck Sharp and Dohme; acted as a consultant for Celgene Corporation; received honoraria from Janssen and Abbvie. F.E.D. acted as a consultant for and received honoraria from Amgen, AbbVie, Takeda, Janssen, and Celgene Corporation. G.C. acted as a consultant for and received honoraria from Takeda, Glycomimetics, Sanofi, Celgene Corporation, Janssen, Bristol-Myers Squibb, and Amgen; received research funding from Takeda, Celgene Corporation, Janssen, and Amgen; and is a member of the speakers bureau for Sanofi, Celgene Corporation, Janssen, and Amgen. D.A.C. received research funding from Celgene Corporation, Amgen, and Merck Sharp and Dohme. G.J. acted as a consultant for and received honoraria from Roche, Amgen, Janssen, Merck Sharp and Dohme, Celgene Corporation, and Takeda; received travel support from Celgene Corporation and Takeda; received research funding from Takeda; and is a member of the speakers bureau for Roche, Amgen, Janssen, Merck Sharp and Dohme, Celgene Corporation, and Takeda. M.F.K. acted as a consultant for Bristol-Myers Squibb, Chugai, Janssen, Amgen, Takeda, and Celgene Corporation; received travel support from Bristol-Myers Squibb and Takeda; received honoraria from Janssen, Amgen, and Celgene Corporation; and received research funding from Celgene Corporation. The remaining authors declare no competing financial interests.

A complete list of the members of the National Cancer Research Institute Haematology Clinical Studies Group appears in "Appendix."

ORCID profiles: L.C., 0000-0002-6221-8617; R.S.H., 0000-0002-5268-0242; M.F.K., 0000-0002-3677-4804.

Correspondence: Martin F. Kaiser, The Institute of Cancer Research, 123 Old Brompton Rd, Kensington, London SW7 3RP, United Kingdom; e-mail: martin.kaiser@icr.ac.uk.

Footnotes

Submitted 12 June 2018; accepted 3 October 2018. Prepublished online as *Blood* First Edition paper, 29 October 2018; DOI 10.1182/blood-2018-06-857250.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Appendix: study group members

The members of the National Cancer Research Institute Haematology Clinical Studies Group are: Peter Hillmen, Alan Chant, Gordon Cook, Mhairi Copland, Charles Craddock, Lavinia Davey, Walter Gregory, Sally Killick, Amy Kirkwood, Judith Marsh, Adam Mead, Gillian Murphy, Kikkeri Naresh, Stephen O'Brien, Andy Peniket, Alasdair Rankin, Clare Rowntree, Anna Schuh, Shamyla Siddique, Simon Stanworth, Simon Watt, Kwee Yong.

REFERENCES

1. McGranahan N, Swanton C. Clonal heterogeneity and tumor evolution: past, present, and the future. *Cell*. 2017;168(4):613-628.
2. Nadeu F, Delgado J, Royo C, et al. Clinical impact of clonal and subclonal TP53, SF3B1, BIRC3, NOTCH1, and ATM mutations in chronic lymphocytic leukemia. *Blood*. 2016;127(17):2122-2130.
3. Manier S, Salem KZ, Park J, Landau DA, Getz G, Ghobrial IM. Genomic complexity of multiple myeloma and its clinical implications. *Nat Rev Clin Oncol*. 2017;14(2):100-113.
4. Walker BA, Boyle EM, Wardell CP, et al. Mutational spectrum, copy number changes, and outcome: results of a sequencing study of patients with newly diagnosed myeloma. *J Clin Oncol*. 2015;33(33):3911-3920.
5. Ross FM, Avet-Loiseau H, Ameye G, et al; European Myeloma Network. Report from the European Myeloma Network on interphase FISH in multiple myeloma and related disorders. *Haematologica*. 2012;97(8):1272-1277.
6. Boyle EM, Proszek PZ, Kaiser MF, et al. A molecular diagnostic approach able to detect the recurrent genetic prognostic factors typical of presenting myeloma. *Genes Chromosomes Cancer*. 2015;54(2):91-98.
7. Kaiser MF, Walker BA, Hockley SL, et al. A TC classification-based predictor for multiple myeloma using multiplexed real-time quantitative PCR. *Leukemia*. 2013;27(8):1754-1757.
8. Guinney J, Wang T, Laajala TD, et al; Prostate Cancer Challenge DREAM Community. Prediction of overall survival for patients with metastatic castration-resistant prostate cancer: development of a prognostic model through a crowdsourced challenge with open clinical trial data. *Lancet Oncol*. 2017;18(1):132-142.
9. Hung H, Chin-Tsang C. Estimation methods for time-dependent AUC models with survival data. *Can J Stat*. 2010;38(1):8-26.
10. Uno H, Cai T, Pencina MJ, D'Agostino RB, Wei LJ. On the C-statistics for evaluating overall adequacy of risk prediction procedures with censored survival data. *Stat Med*. 2011;30(10):1105-1117.
11. Otto T, Sicinski P. Cell cycle proteins as promising targets in cancer therapy. *Nat Rev Cancer*. 2017;17(2):93-115.
12. Huun J, Lønning PE, Knappskog S. Effects of concomitant inactivation of p53 and pRb on response to doxorubicin treatment in breast cancer cell lines. *Cell Death Discov*. 2017;3:17026.