deaths occurred secondary to treatment toxicity.

These results provide hope for treating patients with T-cell lymphoma with CAR T cells and the ability to overcome some of the challenges and concerns related to manufacturing and infectious complications. However, follow-up is still short, and only 2 of the 9 patients were still alive at time of publication: 1 after alloSCT and 1 after progression; and in this small cohort, durable remissions without transplant were not seen. Future studies will show whether CD5.CAR T therapy can be curative on its own or whether it will serve only as a bridge to alloSCT. In addition, these results underscore the challenges of treating patients with T-cell lymphoma with autologous CAR T cell products given how aggressive their disease is. Almost half (8/17) of enrolled patients were unable to be treated, and in half (n = 4) of these patients, it was due to death and/or progressive lymphoma. These patients often have rapid progression that cannot be effectively controlled with bridging therapy while awaiting CAR T manufacturing. The median time from consent to infusion was 8 weeks; if this could be shortened, more patients might be able to be treated. Finally, allogeneic CAR T cells may be an ideal approach for being able to successfully treat patients as this might be the fastest way to get CAR T cells to patients. We eagerly await more studies that tackle this challenging disease.

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LYMPHOID NEOPLASIA

Comment on Durand et al, page 1242

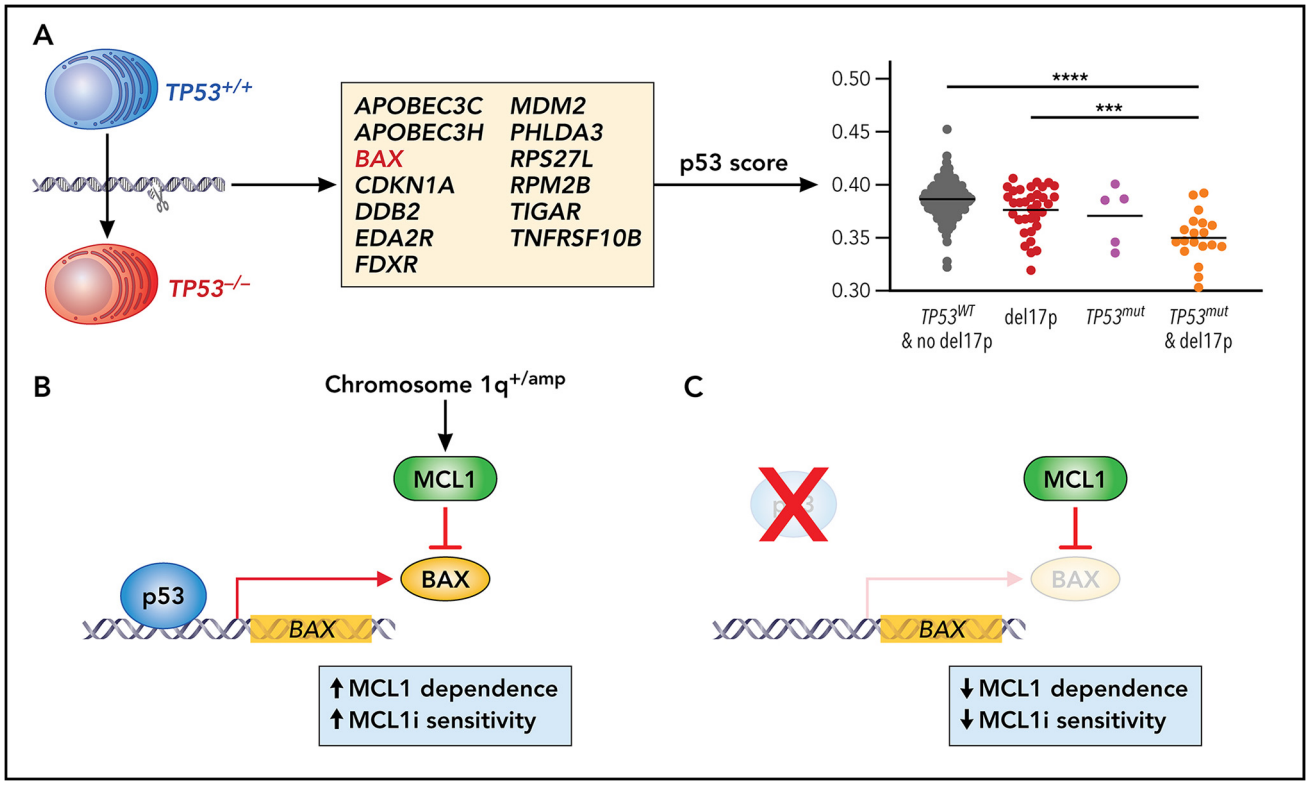
TP53 function over forms in multiple myeloma

Paola Neri[1] **and Lawrence H. Boise**[2] | [1]University of Calgary and [2]Emory University

In this issue of *Blood*, Durand et al describe a functional score that identifies cells with loss of *TP53* and demonstrate a role for *TP53* function in the response to BH3 mimetics in multiple myeloma (MM).[1]

MM is a disease that is defined in part by structural variations that occur during the germinal center reaction, consistent with dysfunctional class switch recombination. Approximately 50% of patients with MM harbor translocations that result in the juxtaposition of oncogenes to the strong enhancers of the *IGH* locus.[2] However, these truncal events are soon followed by additional alterations that help drive disease progression and can influence disease outcome. These secondary events include copy number variations, non-*IGH* translocations, and single-nucleotide variations (SNVs). Independently, few of these have a significant impact on outcome, with the notable exceptions of gains/amplifications of chromosome 1q ($1q^+$ or amp) and loss of chromosome 17p (del17p).[3] The latter is where the tumor suppressor *TP53* is found and is the only gene where SNVs have been shown to be a predictor of outcome,[4] likely due to the fact that most *TP53* SNVs occur in patients who harbor del17p and reflect biallelic loss of function.[5,6] Therefore, identifying patients with biallelic loss of *TP53* to be able to consider alternative treatment options, given their poor response to standard therapies, is necessary.

To address these issues, Durand et al aimed to identify a gene expression signature that could be used to functionally identify biallelic loss of *TP53*. Previous work on *RAS*/*RAF* mutations in MM has demonstrated that a functional output, in that case gene expression associated with mitogen-activated protein kinase pathway activation, can be more accurate in predicting outcome than just determining if there are mutations in *NRAS*, *KRAS*, or *BRAF*.[7] For the current studies, a *TP53* gene signature was derived by comparing expression of cells where *TP53* expression was inhibited by gene editing in 2 human myeloma cell lines with wild-type *TP53*. The authors found downregulation of 16 genes (including *TP53*). To derive a functional readout of p53 activity, they focused on 13 genes that have been previously shown to be early targets in the pathway (see figure panel A). More important, when similar editing was performed in cells that lacked a functional p53 due to mutations and deletions, no change in the expression of these genes was observed. In addition, MDM2 inhibition with nutlin3a only increased the p53 score in cell lines that had wild-type *TP53*, and a low score also accurately predicted biallelic loss of



(A) Using gene editing and transcriptional profiling in human MM cell lines, Durand et al defined a 13 gene signature to generate a measure of p53 activity. This p53 score could discriminate patient samples that had biallelic loss of TP53. (B-C) The authors then focused on the function of 1 gene in the p53 score, *BAX*, and demonstrated that *BAX* expression resulted in increased dependence of cells on *MCL1* and MCL1 inhibitor (MCL1i) sensitivity. Loss of *TP53* and subsequent lower *BAX* expression render cells less dependent on *MCL1*; therefore, they are less sensitive to MCL1i. Professional illustration by Patrick Lane, ScEYEnce Studios.

TP53 in cell lines from other tumor types.

Once validated in cell lines, the authors then determined if their p53 score had prognostic value for determining outcomes in patients with MM. They applied their score to 2 clinical studies with newly diagnosed patients (CASSIOPEA and MMRF-CoMMpass) and in both cases found that the cohort of patients with the lowest p53 score were more likely to have biallelic loss of *TP53* and experienced significantly worse outcomes. More important, in both studies, a significant proportion of patients with del17p and no second hit were in all groups defined by the p53 score, despite likely being defined as high risk using current guidelines.[3] In addition, there was also a small proportion of patients with both *TP53* SNVs and del17p who were classified in the higher p53 score cohorts, consistent with findings demonstrating that not all TP53 SNVs result in complete loss of p53 function.[8]

The authors then turn their attention to the potential role of the genes in the p53 score in disease biology and how this may be targeted in MM. They focused on *BAX* because of its central role in apoptosis and potential impact in the response to a wide variety of therapies. To determine the consequence of decreased *BAX* expression in cells that lack *TP53*, they used specific inhibitors of anti-apoptotic BCL2 proteins (aka, BH3 mimetics) as well as BH3 profiling and found that loss of *TP53* selectively effects *MCL1* priming, resulting in decreased sensitivity to the MCL1 inhibitor S63845 (see figure panels B-C). At first glance this is somewhat surprising as BAX can also bind to BCL2 and BCL-X_L and could be activated by BH3-only proteins, such as BIM, that could be released from any of these prosurvival proteins. However, all the cell lines used in this study have t(4;14) and are already resistant to venetoclax due to a lack of BCL2 priming; therefore, it is impossible to change their resistance status. In addition, t(4;14) MM is associated with $1q^+$ and 1q amp and *MCL1* is found at 1q21.[4,9] Although correlative data presented here in a larger set of cell lines support the hypothesis that loss of p53 function is more likely to influence MCL1 dependence, future testing in cells with other backgrounds, such as t(14;16) and t(11;14), is warranted to determine the effects of p53 and *BAX* in BCL2- or BCL-X_L–dependent cells.

Finally, taking into consideration the heterogeneity of MM cells in patients,[10] the authors investigated if the p53 score was altered by BH3 mimetic treatment. Using single-cell RNA sequencing on 24 samples, they were able to determine that following treatment, the p53 score was lower in approximately one-third of the clusters, and this was more common in clusters that did not have del17p. This suggests that treatment with BH3 mimetics could select for loss of p53 function, having implications for how and when to use them in the MM therapy.

There are pros and cons to both the genomic and functional approaches for determining the consequences of *TP53* loss. The functional approach can identify samples that are functionally equivalent to biallelic loss of *TP53* without having the genetic lesions and determine if the mutations actually result in a functional loss. However, unlike the genomic

approach, detection of a small subclonal fraction of cells is difficult unless one incorporates single-cell analysis, which may not be practical for routine use. Thus, the p53 score will likely be best used in concert with current approaches to assess the prognostic impact and aid most accurately in treatment decisions.

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LYMPHOID NEOPLASIA

Comment on Bomsztyk et al, page 1259

AL amyloidosis response: a move in the "light" direction

Angela Dispenzieri | Mayo Clinic

As redemonstrated by Bomsztyk et al[1] in this issue of *Blood*, top-down mass spectrometry (MS) of blood has been transformative for diagnosing and monitoring plasma cell disorders,[2-7] and immunoglobulin light chain (AL) amyloidosis is no exception.[3,8,9]

To date, every comparator study using a 5-bead MS-based assessment of hematologic response compared with standard immunofixation in either multiple myeloma or AL amyloidosis has favored the MS approach because of both improved sensitivity and specificity of the MS assays.[5-9] Bomsztyk et al take this a step further in AL amyloidosis, which is a disease primarily of elevated free light chains (FLCs), by using an MS assay that detects FLCs (FLC-MS) rather than total light chains (those light chains bound to intact immunoglobulins plus FLCs [see figure panel A]).[1] The authors studied 487 patients who were newly diagnosed with AL amyloidosis who were treated with bortezomib-based regimens with serial measures. All but 4 had their FLC peak identifiable by FLC-MS. Using FLC-MS, they demonstrated that fewer patients had as deep a response as estimated by standard hematologic measures (ie, the International Society of Amyloidosis amyloid response criteria, which are dependent on immunofixation and the nephelometric FLC assay) (see figure panel B, panel 1). Overall, only 21% of patients were FLC-MS negative at 12 months, and the likelihood of being FLC-MS negative increased with superior hematologic response status (see figure panel B, panel 2). Notably, 12 months after diagnosis, 32% of their patients had been classified as amyloid complete hematologic response, but only 39% of these had a negative FLC-MS, making only 13% of the entire cohort both amyloid complete response and FLC-MS negative.

FLC-MS negativity translated into a higher likelihood of organ response at 12 months, with 70% and 38% of organ response–eligible patients having a cardiac and renal response, respectively. Overall survival was also better among patients who had a negative (compared with a positive) FLC-MS in each of the following patient populations: (1) all, regardless of standard hematologic response; (2) complete hematologic responders; and (3) very good partial responders (see figure panel C). Patients achieving complete hematologic response but who remained FLC-MS positive had similar overall survival compared with patients in very good partial response.

These results are exciting, but there are several limitations to this study. First, although there are 2 commercially available top-down MS assays (Mass-Fix and EXENT), the FLC-MS, the assay used in this study, is not currently commercially available (see figure panel A), making this article academically interesting, but not yet applicable for general practice. It would have been useful had they contextualized the FLC-MS results relative to the EXENT assay. How do the existing MS assays perform relative to the FLC-MS assay? Investigators from the Mayo Clinic compared the FLC-MS with the Mass-Fix assay in the sera of 167 patients (see figure panel D).[10] Concordance between Mass-Fix and FLC-MS assays was 74%, with the vast majority of the discrepancy due to the greater sensitivity of the FLC-MS. When the results of the FLC ratio derived from the FLC nephelometric assay were combined with the Mass-Fix result, concordance between these 2 commercially available assays and the FLC-MS assay was 88%, with the discordance relating predominantly to abnormal FLC ratios (typically favoring κ) in the setting of a negative FLC-MS. The ability of this combination rivaled the FLC-MS for detecting λ clones. Extrapolation to the