Identification and Characterization of Small Molecules That Inhibit Nonsense-Mediated RNA Decay and Suppress Nonsense p53 Mutations

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Abstract

Many of the gene mutations found in genetic disorders, including cancer, result in premature termination codons (PTC) and the rapid degradation of their mRNAs by nonsense-mediated RNA decay (NMD). We used virtual library screening, targeting a pocket in the SMG7 protein, a key component of the NMD mechanism, to identify compounds that disrupt the SMG7–UPF1 complex and inhibit NMD. Several of these compounds upregulated NMD-targeted mRNAs at nanomolar concentrations, with minimal toxicity in cell-based assays. As expected, pharmacologic NMD inhibition disrupted SMG7–UPF1 interactions. When used in cells with PTC-mutated p53, pharmacologic NMD inhibition combined with a PTC "read-through" drug led to restoration of full-length p53 protein, upregulation of p53 downstream transcripts, and cell death. These studies serve as proof-of-concept that pharmacologic NMD inhibitors can restore mRNA integrity in the presence of PTC and can be used as part of a strategy to restore full-length protein in a variety of genetic diseases. Cancer Res; 74(11) June 1, 2014; DOI: 10.1158/0008-5472.CAN-13-2235.

Introduction

Human genetic disorders are caused by diverse types of mutations. Many of these mutations, including nonsense mutations, frameshift mutations, and mutations that cause alternative splicing events, result in premature termination codons (PTC). For example, approximately 15% of the mutations leading to Duchenne muscular dystrophy, 10% of the mutations responsible for cystic fibrosis, and common β-globin mutations responsible for thalassemia are PTC mutations (1–3). It has been estimated that up to 30% of all mutations resulting in human genetic disorders result in PTCs (4). In addition, many acquired mutations in cancer, including those that disable p53 and BRCA1, ATM, VHL, and NF1 and NF2, result in PTCs (5).

Many transcripts carrying a PTC are targeted for rapid degradation before they can be translated into protein through a multistep process termed nonsense-mediated RNA decay (NMD). The molecular mechanism of NMD has not been fully delineated, though working models have been proposed. During the processing of mammalian pre-mRNA, introns are excised and marked by a multiprotein exon junction complex (EJC; refs. 6–8). When the translation complex pauses at a PTC that is upstream of an EJC, eukaryotic release factors recruit the RNA helicase UPF1, a vital component of the NMD mechanism (9). UPF1 then associates with UPF2, a component of the EJC, and is phosphorylated at several sites by SMG1 (10). Phosphorylated UPF1 recruits SMG7 and SMG5, two 14-3-3 proteins that each bind to UPF1 and form a heterodimer, and results in the dephosphorylation of UPF-1 by PP2A (11, 12). The phosphorylation and dephosphorylation of UPF1 are necessary steps before the degradation of the transcript by exonucleases (12, 13).

One approach to treat genetic disorders and to restore tumor-suppressor genes with PTC mutations is to pharmacologically promote ribosomes to read-through a PTC and produce a full-length protein (14). For example, treating cells with the antibiotic gentamicin increases the full-length expression of both PTC-mutated β-globin and PTC-mutated p53 (15, 16). Gentamicin treatment of cells with PTC mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) can increase cellular chloride transport (17, 18) and patients with PTC mutations treated with intranasal gentamicin increase CFTR protein expression and improve the potential difference of nasal epithelial cells (19). Although application of gentamicin or the closely related geneticin (G418) is not practical clinically, a recent high-throughput screen identified a small molecule, Ataluren, which has similar properties but seems to be more potent and less toxic (20), and the use of antisense oligonucleotides to promote exon skipping are also being tested in muscular dystrophy (reviewed in ref. 21). The clinical activities of these approaches are likely to be enhanced with higher cellular concentrations of PTC-mutated mRNA. Indeed, in vitro experiments have demonstrated that the expression of PTC-mutated CFTR, as well as CFTR-mediated chloride transport, is improved in gentamicin-treated cells when UPF1 or UPF2 are also depleted (22).
Because translation is necessary for NMD (23), inhibitors of translation are effective inhibitors of NMD (24). Unfortunately these drugs are not candidates for clinical therapies because of general toxicity and, as translation is inhibited, the ultimate goal of restoring protein expression is not achieved. We have recently demonstrated that NMD inhibition can be achieved via other mechanisms. Specifically, we have shown that phosphorylation of the translation initiation factor eIF2α by a variety of cellular stresses inhibits NMD (5, 25, 26). We have also determined that modest (80%) depletion of UPF1 or UPF2 can suppress NMD activity without diminishing the proliferation or survival of cells (26). These observations suggest that the pharmacologic inhibition of NMD can be achieved with limited toxicity or adverse effects. We hypothesized that the combination of a drug that can bypass a PTC and/or promote exon skipping in combination with a drug that inhibits NMD would be a synergistic combination and serve as an effective platform to treat genetic diseases. To pursue this approach, we performed a virtual screen to identify commercially available compounds predicted to dock within a SMG7 pocket previously shown to be vital in the binding of UPF1. We then tested whether these compounds interfere with NMD, and using PTC-mutated p53 as a proof-of-principle model, whether the pharmacologic inhibition of NMD would make ribosomal read-through strategies more effective in producing full-length proteins.

Materials and Methods

Virtual library screening

The crystallographic structure of SMG7 (PDB ID, 1ya0) was analyzed using the PocketFinder algorithm available in the Internal Coordinate Mechanics (ICM) software program (Molsoft, LLC). An appropriately sized pocket (based on criteria previously reported in ref. 27) was selected as the target site for virtual library screening (VLS) using ICM-VLS software (Molsoft, LLC). ICM-VLS uses global optimization with a biased probability Monte Carlo conformational search to rapidly dock fully flexible, full-atom models of the ligands to a set of grid potential maps calculated from the coordinates of the atoms in the protein receptor. Each ligand-docking conformation is then evaluated with a scoring function. The ICM-scoring function integrates van der Waals energy, electrostatics, hydrogen bonding, conformational entropy loss, and solvation electrostatic energy change (28). ICM-VLS was used to dock the entire set of 436,115 compounds from the ChemBridge Express Library using default ICM-docking parameters on three 3.0-GHz Intel Xeon processors.

Cell lines and reagents

U2OS, Hela, 293, BJ-hTERT (BJ-human telomerase reverse transcriptase), PBMC (peripheral blood mononuclear cell), MDA231, DU145, T98G, and HCT116 cells were grown as previously described (29, 30). N417 cells were grown in RPMI containing 10% FBS. Calu-6 (gift of E. Welch) and HDQ1 cell lines (DSMZ German Collection of Microorganisms and Cell Cultures) were grown in Dulbecco’s Modified Eagle Medium with 10% FBS. Pools of β-globin mRNA-expressing cells were generated as previously described (25). Compounds were obtained from Chembridge. UPF1 shRNA (short hairpin RNA; ref. 26) and SMG7 shRNA (TRCN0000127998) were obtained from Sigma. Primer sequences for β-globin, endogenous NMD targets, p53, and p53 downstream targets are available upon request.

Immunoblots and RNA assessment

Immunoblots were prepared as previously described (29) and membranes were probed with antibodies against p53 (Abcam Inc.; ab26), SMG1 (Santa Cruz Biotechnology, Inc.; 6-RE13), UPF1/Rent1 (SC H-300), SMG7 (A302-170A; Bethyl Laboratories, Inc.). Immunoprecipitations were performed as described previously (10). Briefly, cells were lysed and incubated 30 minutes at 4°C with buffer containing 50 mmol/L Tris-HCL at pH 7.4, 50 mmol/L NaCl, 0.05% Tween-20, 10 mmol/L tetrasodium pyrophosphate, 100 mmol/L NaF, 17.5 mmol/L β-glycerophosphate, 1 mmol/L phenylmethylsulfonyl-fluoride, and protease inhibitor (Roche). Lysates were clarified and incubated with 200 μg/mL RNaseA (Qiagen) at room temperature for 15 minutes with gentle rotation before 4°C overnight immunoprecipitation with the appropriate antibodies. Isolation of RNA for cDNA generation, real-time PCR (RT-PCR), DRB (5′,6-dichloro-1-β-D-ribofuranosylbenzimidazole) treatment, RNA stability experiment, expression profiling, and expression array data analysis were as described previously (25, 26). For unbiased gene expression experiments, cells were treated with 50 μmol/L NMDI14 for 6 hours, 100 μg/mL emetine for 3 hours, or depleted of UPF1 and RNA was harvested, prepared, hybridized to Affymetrix HG-U133 Plus 2.0 GeneChips and analyzed as previously described (26).

Cell viability assay and proliferation assay

To assess viability, cells were cultured in 6-well dishes and incubated with dimethyl sulfoxide (DMSO), G418, NMDI (NMD inhibitor) alone, or G418 with NMDI together for the indicated hours. After incubations, cells and media were collected and cell viability was measured as described (31). To assess cell proliferation U2OS, Hela, and BJ-hTERT cells were cultured in 6-well plates and, after 24 hours, treated with NMDI14 for 0, 24, 48, and 72 hours. The cells were collected and viable cells were counted by using the Countess Automated Cell Counter (Invitrogen).

Protein synthesis ([35S] incorporation assay)

U2OS, Hela, and BJ-hTERT cells were incubated with DMSO, NMDI14, or emetine as indicated. The cells were labeled with 100 μCi/mL [35S] Methionine for 30 minutes and the protein was precipitated using the standard trichloroacetic acid precipitation method as described previously (26). The precipitated protein was collected in glass fiber filters and [35S]Methionine incorporation was measured by using liquid scintillation counter (PerkinElmer, Inc.).

Results

Identification of potential NMD inhibitors

Prior studies have demonstrated that NMD activity is dependent on the formation of a UPF1–SMG7 complex (Fig. 1A), which has been crystallographically resolved (11).
Though UPF1 and SMG7 may have additional non-NMD roles, the interaction between the two seems to be unique for NMD. Thus, the disruption of this complex is an ideal target for NMD inhibition while minimizing the toxicity that might be seen with other known mechanisms of NMDIs, including translation inhibitors and inducers of eIF2α phosphorylation (24, 25).

We elected to take a three-dimensional (3D) structure activity approach to NMDI discovery by targeting this protein interface. Only one pocket on the 3D structure of SMG7 was both of suitable size and volume (between 150 and 550 Å³) and was lined with functionally sensitive amino acid side chains that mediate the interaction with UPF1 (Fig. 1B; ref. 11). This pocket was selected as the target site for VLS of a collection of 436,115 compounds from the ChemBridge Express Library. The resulting compounds achieving a docking score of $< -32$ (see Materials and Methods) were further filtered by selecting those with extensive hydrogen bonds and van der Waals contacts, followed by hierarchical clustering for a diversity of scaffolds, resulting in the identification of 31 diverse compounds highly structurally and chemically compatible with the target SMG7 pocket (Fig. 1C and D).

**Identified compounds inhibit NMD in a cell-based assay**

To test these putative NMDIs, we used a common and straightforward assay for NMD. We first generated fibroblasts stably expressing either a wild-type β-globin construct or a PTC 39-mutated β-globin gene construct well established to be degraded by NMD (32). In our initial screen, we treated cells expressing these genomic constructs with 50 μmol/L of the 31 identified compounds for 6 hours, a condition that led to no
gross toxicity in these cells. RNA was then harvested and expression of PTC β-globin and wild-type β-globin mRNA was determined using RT-PCR.

We identified 10 compounds that selectively increased the expression level of PTC 39 β-globin greater than two-fold, without affecting wild-type β-globin expression, with a P value of <0.05 (Fig. 2A). Because of rapid degradation by NMD, the steady-state mRNA expression of the PTC39 β-globin was approximately 3% of the wild-type β-globin expression. Treating cells with NMDI14 for 6 hours, for example, led to an increase of PTC 39 β-globin to 12%, a relative four-fold increase that, if resulting in biologically active hemoglobin, would be sufficient to ameliorate the clinical symptoms of thalassemia (Fig. 2B). When these 10 compounds were tested at decreasing concentrations, we identified several NMDIs active at nanomolar concentrations, with some active over a wide range of concentrations and others exhibiting more narrow effective concentration, which peaked at micromolar concentrations and displayed even a biphasic effect (Fig. 2C and data not shown). These compounds, previously not reported to affect NMD, represent a diverse group of chemical scaffolds (Fig. 2D and data not shown). We chose to focus the majority of our further studies on one of these compounds, ethyl 2-[(6,7-dimethyl-3-oxo-1,2,3,4-tetrahydro-2-quinoxalinyl)acetyl]amino]-4,5-dimethyl-3-thiophenecarboxylate (NMDI14), which demonstrated dose-dependent activity, with significant activity at low concentrations.

Although the activity of our identified NMDIs does not depend on the mechanism of action, because these drugs were screened in silico to fit into the SMG7 pocket necessary to bind...
UPF1, we expected that NMDI14 would interfere with the interaction of UPF1 and SMG7. We immunoprecipitated SMG7, in the presence of RNase and confirmed that both overexpressed (Fig. 2E, left) and endogenous (Fig. 2E, right) SMG7 interacted with overexpressed and endogenous UPF1, respectively, and observed that this interaction was disrupted when cells were treated with NMDI14.

NMDI can be achieved with minimal cellular toxicity in multiple cell lines

NMD inhibition can result in the upregulation of nonmutated, mutated, and alternatively spliced mRNAs (5). Thus, NMD inhibition might be predicted to result in widespread biologic effects. In fact, we have recently reported that the genetic inhibition of NMD subtly but significantly increases autophagy, presumably in an attempt to rid the cell of mutated and misfolded proteins that accumulate with NMD suppression (31). To determine the cellular toxicity of select NMDIs, we assessed viability in treated Hela, U2OS, and Calu-6 cell lines, and in immortalized/nontransformed human fibroblasts. These cells were treated with 50 μmol/L of NMDI14 and two other effective NMDIs, as an assessment of any class toxicity effects, for 48 hours, and viability was assessed. We noted almost no toxicity with NMDI14 and NMDI25, and minimal toxicity with NMDI9, suggesting the pharmacologic inhibition of NMD is not unduly toxic in contrast with 24 hours of treatment with emetine, which blocks NMD by inhibiting translation (Fig. 3A).

To determine whether the effective pharmacologic inhibition of NMD exerted a sublethal effect on proliferation, we assessed proliferation in NMDI14-treated transformed and nontransformed cell lines (Fig. 3B). Three days of treatment resulted in no decrease in cell counts, demonstrating that the pharmacologic inhibition of NMD can be achieved without subtle changes in proliferation.

Because suppression of translation is a potent mechanism to inhibit NMD, previously described inhibitors of NMD also inhibit translation. Although we did not predict this mechanism of action for our compounds, we tested protein synthesis after 6 to 24 hours of NMDI14 treatment. During the last hour of treatment cells were pulsed with S35 methionine, and protein was then precipitated and the incorporated S35 assessed. NMDI14 had no effect on S35 incorporation (Fig. 3C). Together, these data suggest that the pharmacologic inhibition of NMD can be achieved without cellular toxicity.

NMDI14 increases the expression of endogenous NMD targets

The effectiveness of NMDI14 in inhibiting NMD was originally determined by assessing the expression of an NMD-degraded reporter transcript that harbors a premature stop codon (Fig. 2). Although NMD has been primarily appreciated as a mechanism to rapidly degrade mutated transcripts, we and others have demonstrated that a wide variety of nonmutated transcripts are also degraded by NMD, which have several distinct motifs, as well as unknown features, which render them sensitive to NMD (25, 26, 33). We noted that the steady-state mRNA expression, reflective of both the rate of synthesis and the rate of decay, of seven nonmutated NMD targets that we have validated (26) increased in U2OS and/or Hela cells after treatment with NMDI14, whereas no such increase was seen in non–NMD-targeted mRNAs (Fig. 4A).

Figure 3. NMDIs are not unduly toxic. A, U2OS, Hela, Calu-6, and BJ-hTERT cells were treated with three NMDIs at 50 μmol/L for 48 hours, and viability was assessed. Toxicity with translation/NMDI emetine is shown as control. Average of three readings shown. B, U2OS, Hela, and BJ-hTERT cells were treated with NMDI14 at 5 μmol/L. The cell numbers were determined at 0, 24, 48, and 72 hours. C, U2OS, Hela, and BJ-hTERT cells were treated with 5 μmol/L of NMDI14 for 6 and 24 hours. The cells were pulsed with S35 methionine (100 μCi/mL) for last 1 hour and protein synthesis was measured by S35 incorporation. The protein synthesis with translation/NMDI emetine is shown as control.
To assess potential off-target effects of NMDI14, we used expression arrays to globally assess gene expression in U2OS cells treated with NMDI14, emetine, or with UPF1 depletion and compared this with gene expression in control U2OS cells. Of note, 941 genes were increased >1.5-fold with NMDI14 (Fig. 4B). There was a significant overlap (22%, \( P = 1.2 \times 10^{-6} \), \( \chi^2 \) test) of genes upregulated by NMDI with genes upregulated by emetine treatment (which inhibits NMD potently but clearly has other effects), and with UPF1 depletion (11%). In comparison, 20% of the 5,290 genes upregulated by emetine were also upregulated in UPF1-depleted cells. The overlap of genes regulated by NMDI14 and those regulated by either emetine or UPF1 depletion is significantly higher than what would be expected by chance (\( \chi^2 \) test). Genes upregulated by NMDI14 but not regulated by UPF1 or emetine, likely representative of off-target effects, were not particularly enriched in any specific functional group, and only demonstrated a greater than two-fold enrichment in ossification, actin binding, and cytoskeletal binding, suggesting that NMDI14 may not have specific toxicities.

We next determined whether our NMDIs are effective in upregulating mutated endogenously expressed mRNAs. The small-cell lung cancer cell line N417 has a p53 tumor-suppressor gene that is deleted on one allele and on the other allele carries a PTC mutation in the 298th codon that renders it a target for NMD (34). When treated with an NMDI, we noted increased expression of the mutated p53 mRNA (Fig. 4C). Similar effects were seen in the breast cancer HDQP-I cell line, which carries a PTC mutation in the 213th codon of the p53 gene (35), and the lung adenocarcinoma cell line Calu-6, which carries a codon 196 PTC-mutated p53 (36). The treatment of N417 cells with NMDI14 for 6 hours led to a steady-state expression of p53 similar to that seen in U2OS cells (Fig. 4D). No such effect was seen in cells expressing wild-type p53 in two other cell lines (Fig. 4C).

NMDI, along with a PTC "read-through" drug, increases the protein expression of endogenous NMD targets

Although NMDI14 increases the stability and expression of NMD-targeted mRNAs, a clinically effective strategy demands the expression of a full-length, biologically active protein. As noted previously, there are several drugs that can promote read-through of PTCs. Although G418 has a modest effect of suppressing NMD, the most effective agent at nontoxic concentrations, Ataluren, does not protect mRNAs from NMD (20). We reasoned that if there were more mutated mRNA present, PTC read-through drugs might be more effective.
We noted minimal expression of full-length p53 in N417 cells treated with G418, but significantly more in cells treated with both G418 and NMDI14 (Fig. 5A). This increase was seen over a variety of dose concentrations of G418, demonstrating that in the presence of NMDI14 higher full-length p53 expression can be achieved at lower and potentially less toxic G418 concentrations (Fig. 5B). Similarly, the HDQP-1 and Calu-6 cell lines, which carry p53 PTC mutations, showed significant increased full-length p53 expression with the combination of G418 and NMDI14 (Fig. 5A). The expression of wild-type p53 in HCT116 cells was unaltered by these treatments (Fig. 5A). The effectiveness of the two drugs varied among cell lines, which could either reflect variability in ribosomal read-through (which depends on the termination codon sequence; ref. 20), or cell-type–dependent NMD14 metabolism. Consistent with an increase in full-length/active p53 restoration, the combination of both drugs led to a synergistic increase in multiple p53 target genes mRNAs, including p21, BAX, and PUMA in N417 and cells (Fig. 5C). This led to an increase in the mRNA levels of the proapoptotic BAX and PUMA similar to those seen in U2OS cells (Fig. 5D). As expected by the lack of p53 mRNA induction in nonmutated p53 cells with NMDI14 (Fig. 4C), in p53 wild-type cell lines HCT116 and U2OS NMDIs had no effect on p53 protein expression or p53 target gene expression over a wide range of concentrations (Fig. 5A, C and data not shown). Together, these data indicate that NMDIs can increase endogenous mutated mRNAs and, in combination with a PTC bypass drug, can result in full-length protein production.

The restoration of full-length p53 with NMD inhibition and a "read-through" drug leads to cell death in cells with a PTC-mutated p53

The frequently mutated p53 gene is a tumor suppressor that can induce apoptosis. The reestablishment of p53 in cancer cells, with or without chemo/radiation, has been a therapeutic strategy pursued in a wide variety of cancer for almost two decades, but this approach has been hampered by problems in delivery of wild-type p53 (37, 38). Although NMDIs are relatively nontoxic, we hypothesized that the combination of NMDI with a 'read-through' drug would restore full-length p53 and result in a p53-mediated cell death. We treated N417 and HDQP-1 cells with a combination of read-through drug and a dose of NMDI14 that results in full-length p53 expression. We noted a synergistic cell death only with the combination of drugs that led to full-length p53 expression (Fig. 5E). No such death was seen when G418 and NMDI14 was used on cells with wild-type p53, including U2OS and primary PBMCs (Fig. 5E).

Although the combination of G418 and NMDI14 was not toxic in p53 wild-type cells, we examined a number of additional cell lines as controls (Fig. 5F). These included HCT116 p53 wild-type and isogenic HCT116 p53 null cells, to determine whether the combination of NMDI14 and G418 is toxic only to cells without a functional p53. We also tested the breast cancer cell line MDA-231, the prostate cancer cell line DU145, and the glioblastoma cell line T98G, all of which carry single-nucleotide p53 mutations that do not result in PTCs and are, thus, not NMD targets. As with other cell lines we examined, 48 hours treatment with NMDI14 alone was minimally toxic (<5% cell death) in these cell lines. G418 alone showed variable toxicity. However, similar to the p53 wild-type cells and in contrast with those cell lines with NMD-provoking p53 mutations. In no case did the addition of NMDI14 and G418 lead to a synergistic cell death.

Discussion

We have used a virtual screening strategy to identify small compounds that inhibit NMD. The inhibition of NMD augments the expression of PTC-mutated mRNAs and nonmutated mRNAs normally targeted by NMD. Several of these identified NMDIs, representing diverse chemical backbones, are active at the nanomolar level even without further chemical modification to optimize their solubilities or pharmacokinetic properties. The few pharmacologic inhibitors previously described have been useful in delineating the mechanism of NMD, but either inhibit translation, interfere with mRNA splicing, have not been demonstrated to upregulate endogenous NMD targets, have weak activity, do not synergize with read-through drugs, were identified through functional screening assays, and/or have unknown mechanisms of action (24, 39–41). The rational design of our screening makes it likely that our identified compounds are more specific with less off-target effects than these. Although several of our NMDIs do not seem excessively toxic in a variety of cell lines and have no effect on the expression of mRNAs that are not targeted by NMD, further work is needed to document and potentially improve the specificity, in vivo effectiveness, and off-target toxicity of these lead compounds.

Because of the important role NMD plays in degrading many mutated, alternatively spliced, and physiologically expressed mRNAs, even specific inhibition of NMD without any off-target effects might be theorized to be toxic. Indeed, the complete depletion of UPF2 in mice results in nonviability (42). However, we and others have demonstrated that the more modest depletion of UPF1 and UPF2, through shRNA, does not adversely affect cell proliferation or survival (26). In addition,
inhibition of NMD by UPF2 deletion in murine hematopoietic cells results only in diminished hematopoietic progenitor cells with almost no effect on differentiated hematopoietic cells (42), and widespread constitutive expression of a dominant negative UPF1 only modestly affects the differentiating mouse thymus and spleen (43). Although the inhibition of NMD by these stresses normally occurs in growing tumors and in fact is necessary for the 3D growth of cells in soft agar and as explants, the depletion of UPF1 from cells with shRNA does not increase their 3D growth, and, thus, we would not expect inhibition of NMD to necessarily augment tumorigenesis (26). The future clinical use of these or other NMDIs, however, will obviously require further studies to determine the short- and long-term effects of potent NMD inhibition.

Although NMD requires the pausing of a ribosome at a PTC, the use of the PTC read-through drug Ataluren does not inhibit NMD, potentially because only a small percentage of mutated mRNAs are read-through by Ataluren. Thus, we have predicted that Ataluren, as well as other strategies, would be more effective in the presence of more substrate mRNA. We have validated this approach by demonstrating that the combination of a read-through drug and NMDI results in the increased protein expression of a PTC-mutated p53, resulting in improved p53 function. Many genetic disorders have mutations that either directly (nonsense) or indirectly (e.g., splicing mutations, additions or deletions that alter reading frame, alternations in poly-A processing) can render an mRNA sensitive to NMD. These include cancer (e.g., p53 and BRCA1). Because truncated proteins may be able to replicate the function of the full-length protein, may be completely nonfunctional, or may act as dominant negatives against the full-normal allele, the effects of inhibiting NMD will have to be carefully considered, particularly without a simultaneous consideration of restoring a full-length protein with ribosome read-through. However, based on our data the concomitant pharmacologic inhibition of NMD remains an attractive therapeutic strategy for many genetic diseases, including cancer.

Disclosure of Potential Conflicts of Interest
L.B. Gardner, L. Martin, and T. Cardozo have ownership interests in a patent. No potential conflicts of interest were disclosed by the other authors.

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Grant Support
This work was supported by R01DK081641 (L.B. Gardner), IDP20D004631 (T. Cardozo), UL1 TR000038 from the National Center for the Advancement of Translational Science (NCATS), NIH (L.B. Gardner and T. Cardozo), and R01HL102449 and FP7-HEALTH-2012-INNOVATION from the European Community and the Children’s Cancer and Blood Foundation (S. Rivella). L.B. Gardner is the Saul J. Farber Associate Professor of Medicine and a Saul Farber Scholar. The Gardner lab is supported by generous gifts from Dr. Howard Furst and Ms. D. Lederman.

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Received August 6, 2013; revised January 8, 2014; accepted March 4, 2014; published OnlineFirst March 24, 2014.

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