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# Pevonedistat (MLN4924), a First-in-Class NEDD8-activating enzyme inhibitor, in patients with acute myeloid leukaemia and myelodysplastic syndromes: a phase 1 study

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## Summary

This trial was conducted to determine the dose-limiting toxicities (DLTs) and maximum tolerated dose (MTD) of the first in class NEDD8-activating enzyme (NAE) inhibitor, pevonedistat, and to investigate pevonedistat pharmacokinetics and pharmacodynamics in patients with acute myeloid leukaemia (AML) and myelodysplastic syndromes (MDS). Pevonedistat was administered via a 60-min intravenous infusion on days 1, 3 and 5 (schedule A, $n = 27$), or days 1, 4, 8 and 11 (schedule B, $n = 26$) every 21-days. Dose escalation proceeded using a standard '3 + 3' design. Responses were assessed according to published guidelines. The MTD for schedules A and B were 59 and 83 mg/m$^2$, respectively. On schedule A, hepatotoxicity was dose limiting. Multi-organ failure (MOF) was dose limiting on schedule B. The overall complete (CR) and partial (PR) response rate in patients treated at or below the MTD was 17% (4/23, 2 CRs, 2 PRs) for schedule A and 10% (2/19, 2 PRs) for schedule B. Pevonedistat plasma concentrations peaked after infusion followed by elimination in a biphasic pattern. Pharmacodynamic studies of biological correlates of NAE inhibition demonstrated target-specific activity of pevonedistat. In conclusion, administration of the first-in-class agent, pevonedistat, was feasible in patients with MDS and AML and modest clinical activity was observed.

**Keywords:** MLN4924, acute myeloid leukaemia, NEDD8, NEDD8-activating enzyme, pevonedistat.

Acute myeloid leukaemia (AML) is predominantly a disease of older patients and fatal for the majority (Burnett *et al*, 2009). Standard chemotherapy has remained essentially unchanged over the last four decades with recent improvements in survival largely attributable to better supportive care and safer stem cell transplantation (SCT) techniques (Burnett *et al*, 2007, 2009, 2010). Outcomes are particularly poor for older patients considered unfit for conventional chemotherapy (Burnett *et al*, 2007). Adverse biological features are more common in these patients, making the disease even more challenging to treat. Consequently, this contributes to rising AML mortality as the aging population continues to increase (Burnett *et al*, 2007, 2009, 2010). New therapies are urgently needed.

The ubiquitin-proteasome system (UPS) is responsible for regulated protein turnover in eukaryotic cells, and plays a key role in a wide variety of cellular processes (Ciechanover, 2005; Hershko, 2005) Protein substrates degraded by the UPS have roles in cell division, signal transduction, growth and differentiation, and apoptosis (Adams, 2004; Herrmann *et al*, 2007). Defects within the UPS have been shown to underlie the pathogenesis of many diseases, including cancer (Ciechanover, 2005; Hershko, 2005). Consequently, the UPS is increasingly being investigated as a potential target for anti-cancer agents (Adams, 2004; Herrmann *et al*, 2007). The clinical activity of the proteasome inhibitor, bortezomib, in multiple myeloma, mantle cell lymphoma and other tumour types has demonstrated the validity of targeting this pathway

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(Reece *et al*, 2011; Kondagunta *et al*, 2004; Richardson *et al*, 2005; Fanucchi *et al*, 2006; Fisher *et al*, 2006; Treon *et al*, 2007; San Miguel *et al*, 2008; Goy *et al*, 2009). Within the UPS, proteins are targeted for degradation by E3 ubiquitin ligases through the addition of ubiquitin chains (polyubiquitination) (Hershko, 2005). The Cullin-RING E3 ubiquitin ligases (CRLs) are the largest family of E3 ligases. CRL activity requires conjugation of the ubiquitin-like protein NEDD8 (neural precursor cell-expressed, developmentally down-regulated 8) to the cullin protein scaffold ('neddylation'), which is regulated by NEDD8-activating enzyme (NAE) (Gong & Yeh, 1999; Read *et al*, 2000; Chiba & Tanaka, 2004; Petroski & Deshaies, 2005). NAE activation of NEDD8 is therefore essential for CRL activity and subsequent proteasomal destruction of CRL substrates (Read *et al*, 2000; Chiba & Tanaka, 2004), which preclinical studies have shown to comprise approximately 20% of UPS-degraded proteins (Soucy *et al*, 2009a).

Pevonedistat is a small molecule inhibitor of NAE (Soucy *et al*, 2009a, 2010; Brownell *et al*, 2010; Lin *et al*, 2010; Milhollen *et al*, 2010, 2011, 2012; Wang *et al*, 2011). In the presence of enzymatically active NAE, pevonedistat covalently binds with NEDD8, forming a pevonedistat-NEDD8 adduct. This adduct remains tightly bound to NAE. Consequently, NAE is unable to process NEDD8 for CRL conjugation, which leads to impaired CRL function and accumulation of CRL dependent substrates (Brownell *et al*, 2010). Many of these substrates have relevance in human cancers (Gstaiger *et al*, 2001; Bloom & Pagano, 2003; Karin, 2006; Chen *et al*, 2010; Crusio *et al*, 2010), thus providing the rationale for investigating pevonedistat as an anti-cancer agent. Preclinical studies indicate that pevonedistat is cytotoxic to a range of solid and haematopoietic tumour cell lines (Soucy *et al*, 2009a; Milhollen *et al*, 2010; Swords *et al*, 2010). Anti-tumour activity has been demonstrated in several mouse xenograft models including of AML (Soucy *et al*, 2009a,b; Milhollen *et al*, 2010; Swords *et al*, 2010; Traore *et al*, 2010).

A variety of CRL substrates of relevance in AML have potential anti-proliferative effects, with roles in cell cycle progression (p27), signal transduction, DNA damage (CDT1), and the stress response (Nrf-2 [NFE2L2]) (Podust *et al*, 2000; Read *et al*, 2000; Petroski & Deshaies, 2005; Soucy *et al*, 2009a; Brownell *et al*, 2010; Chen & Li, 2010; Milhollen *et al*, 2010). Swords *et al* (2010) demonstrated that pevonedistat induced cell death at low nano-molar concentrations in cell lines and primary patient material, independent of FLT3 (Fms-like tyrosine kinase) expression and stromal mediated survival signals. Stabilization of key NAE targets confirmed pathway inhibition and disruption of cellular redox was shown to be a key event in pevonedistat-mediated apoptosis. Treatment of mice bearing AML xenografts led to prolonged disease regression and inhibition of NEDDylated cullins. Consequently, this first-in-human phase 1 study in patients with AML or myelodysplastic syndromes (MDS) was undertaken to investigate the safety, activity, pharmacokinetics, and pharmacodynamics of two schedules of pevonedistat (NCT00677170).

## Materials and methods

### *Patients*

Patients with relapsed and/or refractory AML or MDS for which standard, curative or life-prolonging treatment did not exist or was no longer effective were eligible. Patients ≥60 years old with untreated AML considered unfit for standard induction were also eligible. Other inclusion criteria included: age of at least 18 years; Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; adequate renal function (calculated creatinine clearance >50 ml/min); adequate hepatic function (total bilirubin ≤ normal, transaminases and alkaline phosphatase ≤2·5 × upper limit of normal [ULN] and adequate cardiac function (B-type natriuretic peptide ≤1·5 × ULN, left ventricular ejection fraction ≥45% and pulmonary artery systolic pressure ≤1·5 × ULN). Strict cardiac inclusion criteria were employed because pulmonary hypertension was observed at higher doses of pevonedistat in animal models. Exclusion criteria included: treatment with an investigational anti-leukaemic agent within 14 days prior to entering study; documented history of allergic reactions to compounds of similar chemical or biological composition to pevonedistat; pregnancy or breastfeeding; uncontrolled intercurrent illness and known infection with human immunodeficiency virus and/or viral hepatitis B or C. Institutional review boards at all participating institutions approved the study, which was conducted in accordance with Good Clinical Practice guidelines.

### *Study design*

This open-label, phase 1, dose-escalation study (NCT00911066) was conducted at six sites across the United States. The primary objectives were to determine the dose-limiting toxicities (DLT) and maximum tolerated doses (MTD) of pevonedistat. Secondary objectives included descriptions of pevonedistat pharmacokinetics (PK) in plasma, pharmacodynamic (PD) effects of pevonedistat in leukaemic blasts and whole blood and a preliminary assessment of anti-tumour activity.

Pevonedistat was administered via a 60-min intravenous (IV) infusion on days 1, 3 and 5 (schedule A), or days 1, 4, 8 and 11 (schedule B). Cycles were repeated every 21 days. Dose escalation started at 25 and 147 mg/m$^2$ for schedules A and B, respectively, and MTD determination was based on a standard '3 + 3' design. Dose escalation terminated when 2 (or more) of 6 patients experienced a DLT at the same dose level. The MTD was defined as the highest dose that generated a DLT rate of 0/6 or 1/6 patients during Cycle 1. DLTs were defined in Cycle 1 only as any grade ≥3 toxicity related to pevonedistat (exceptions were: arthralgia/myalgia, fatigue

<1 week, hypophosphataemia and prolonged prothrombin time/activate partial thromboplastin time without clinical bleeding).

### *Safety and efficacy assessments*

Patient demographics and medical history were recorded at baseline. Adverse event assessments, physical examination, vital signs and ECOG performance status were documented at baseline and every week for the duration of the trial. Safety was assessed from informed consent to 30 days after final dose of pevonedistat. Adverse events (AE) were graded according to the National Cancer Institute's Common Terminology Criteria for AEs, version 3.0 (NCI CTEP, 2011). Patients with AML were assessed for efficacy according to published International Working Group criteria (Cheson *et al*, 2003). Responses in MDS were described descriptively.

### *Pharmacokinetic and pharmacodynamic analyses*

Blood samples for PK and PD analyses were collected simultaneously during Cycle 1 at multiple time points, pre- and post-infusion for each schedule. Non-compartmental analyses (using WinNonlin software, Version 6.2, Pharsight Corporation, Cary, NC, USA) were used to estimate the observed maximum concentration ($C_{max}$; theoretically end-of-infusion concentration), the time at which $C_{max}$ occurred ($T_{max}$), the area under the plasma concentration-time curve from time 0 to 24 h post-dose ($AUC_{24\ h}$), the area under the plasma concentration-time curve from time 0 to the end of the dosing interval ($AUC_{0}$-tau) and, data permitting, the terminal disposition phase half-life ($t_{1/2}$).

For PD assessments, the expression levels of 8 mRNA transcripts (*ATF3*, *GCLM*, *GSR*, *AGPAT9*, *NQO1*, *SLC7A11*, *SRXN1*, and *TXNRD1*) were measured in the peripheral blood. These transcripts were previously identified as induced by pevonedistat-mediated NAE inhibition (Walker *et al*, 2011). Percentage change in RT-PCR expression level over baseline was calculated for each gene. In addition, pre- and post-dose bone marrow samples were collected at screening and 3 to 6 h after the second (Day 4 of schedule B) or third (Day 5 of schedule A) dose of pevonedistat. Immunohistochemistry (IHC) was used to assess tissue biomarkers of NAE inhibition in leukaemic blasts [pevonedistat-NEDD8 adduct formation and change in CDT1 levels, as previously described (Shinde *et al*, 2014)].

## Results

### *Patient characteristics*

Fifty-three patients were treated with either schedule A ($n = 27$) or schedule B ($n = 26$). Of these, the most common diagnosis was AML ($n = 50$, 94%). Three patients (6%) were enrolled with MDS. Patients enrolled were heavily pre-treated and included 17 patients (32%) who had prior SCT. Only three patients (6%) were treatment-naive. Pertinent demographics are presented in Table I.

### *Dose escalation and MTD determination*

Patients on schedule A (Days 1,3,5) began escalating doses of pevonedistat at 25 ($n = 3$), 33 ($n = 4$), 44 ($n = 3$), 59 ($n = 3$) and 78 mg/m$^2$ ($n = 4$) mg/m$^2$. At the 78 mg/m$^2$ dose level, one patient experienced grade 3 elevation in transaminases after one dose of pevonedistat, meeting criteria for DLT. This patient was successfully re-challenged with 59 mg/m$^2$ before coming off study during Cycle 4 for disease progression. Another patient treated with 78 mg/m$^2$, received 4 doses of pevonedistat and, on Cycle 2 day 3, presented with sepsis syndrome, elevated transaminases and multi-organ

**Table I.** Demographics and baseline disease characteristics.

| Characteristic | Schedule A $n = 27$ | Schedule B $n = 26$ |
|---|---|---|
| Median age, years (range) | 59·1 (29–84) | 62·7 (19–83) |
| Male, *n* (%) | 17 (63) | 20 (77) |
| Race, *n* (%) | | |
| White | 23 (85) | 25 (96) |
| Asian | 3 (11) | 1 (4) |
| Other | 1 (4) | 0 |
| ECOG performance status, *n* (%) | | |
| 0 | 6 (22) | 5 (19) |
| 1 | 16 (59) | 16 (62) |
| 2 | 5 (19) | 5 (19) |
| Median time from initial diagnosis (months) | 11·4 | 12·7 |
| Primary diagnosis, *n* (%) | | |
| AML | 26 (96) | 24 (92) |
| FAB | | |
| M0 | 2 (7) | 2 (8) |
| M1 | 4 (15) | 2 (8) |
| M2 | 6 (22) | 1 (4) |
| M3 | 0 | 0 |
| M4 | 1 (4) | 2 (8) |
| M5 | 2 (7) | 4 (15) |
| M6 | 0 | 0 |
| M7 | 1 (4) | 0 |
| NOS* | 8 (30) | 13 (50) |
| Unclassifiable | 1 (4) | 0 |
| Missing | 1 (4) | 0 |
| Cytogenetics* | | |
| Favourable | 1 (4) | 0 |
| Intermediate | 12 (44) | 10 (38) |
| Adverse | 9 (33) | 10 (38) |
| Undetermined | 5 (19) | 6 (23) |
| MDS* | 1 (4) | 2 (8) |

*Cytogenetic data were reported as per Cancer and Leukemia Group B criteria (Byrd *et al*, 2002).

ECOG, Eastern Cooperative Oncology Group; AML, acute myeloid leukaemia; FAB, French-American-British classification; NOS, not otherwise specified; MDS, myelodysplastic syndromes.

failure (MOF), which was fatal. This AE, considered related to pevonedistat, happened outside the DLT period. Although the protocol allowed for further dose escalation, severe liver toxicity and MOF had by then been reported in a parallel study of pevonedistat in patients with solid tumours, using a continuous dosing schedule (Kauh *et al*, 2011). Considering this, higher dose levels were not tested on schedule A and the MTD was determined to be 59 mg/m$^2$. An additional 10 patients were enrolled at this dose level and no further DLTs were reported.

Following completion of schedule A, dose escalation for schedule B patients (Days 1,4,8,11) began at 147 mg/m$^2$. The higher starting dose and schedule change was based on then emerging laboratory and clinical experience with pevonedistat. In murine xenograft models, we observed a direct exposure-response relationship, which was schedule-independent. Accordingly, we hypothesized that higher cumulative doses of pevonedistat per cycle, would be more clinically active. In a phase I trial of pevonedistat for patients with lymphoma and myeloma ($n = 16$), pevonedistat was given on Days 1,4,8,11 and dose escalation began at 110 mg/m$^2$ (Shah *et al*, 2009). Only one DLT was reported (grade 3 thrombocytopenia at 110 mg/m$^2$), and pevonedistat plasma exposure did not accumulate between doses. The MTD was 196 mg/m$^2$ on this study and objective responses were reported at this dose level. Grade 3/4 hepatoxicity was not reported. Given the higher cumulative start dose being tested on schedule B and the schedule A MTD, enrollment into the first cohort was staggered. Two patients experienced DLT on this cohort (MOF leading to cardiac failure and death). Both events occurred after a single dose of pevonedistat. Dose de-escalation to 110 mg/m$^2$ proceeded and an additional DLT was reported (orthostatic hypotension; the patient subsequently died of MOF). A fatal fungal pneumonia was also reported in this cohort but did not meet criteria for DLT (unrelated to pevonedistat). Given the severity of both events, dose reduction continued to 83 mg/m$^2$. No further DLT were reported in 19 patents enrolled at this dose level and 83 mg/m$^2$ was declared the MTD for schedule B.

### *Safety*

Patients on schedules A and B were treated for medians of 2 (range 1–19) and 2 (range 1–13) cycles, respectively. All patients were evaluable for toxicity. The majority of AE were mild or moderate (grade 1 or 2); the most common are shown in Table II. Febrile neutropenia, thrombocytopenia and elevated aspartate transaminase (AST) levels were the most frequent grade ≥3 AE reported (occurring in 2 or more patients). Patients with at least 1 treatment-related grade ≥3 AE included 8 (30%) on schedule A and 12 (46%) on schedule B (Table II). Drug-related severe AE (SAE) leading to discontinuation of therapy included one patient (4%) on schedule A (sepsis) and two patients (8%) on schedule B (1 due to MOF, 1 due to heart failure). Deaths related to pevonedistat occurred in three patients with AML, 1 from sepsis on schedule A (78 mg/m$^2$ dose cohort) and 2 from MOF on schedule B (1 each in the 110 mg/m$^2$ and 147 mg/m$^2$ dose cohorts).

**Table II.** Treatment-emergent adverse event (AE) experienced by ≥10% of patients on any schedule.

| AE, *n* (%) | Schedule A *n* = 27 | Schedule B *n* = 26 | Total *n* = 53 |
|---|---|---|---|
| Any AE | 27 (100) | 26 (100) | 53 (100) |
| Pyrexia | 12 (44) | 16 (62) | 28 (53) |
| Diarrhoea | 14 (52) | 9 (35) | 23 (43) |
| Febrile neutropenia | 11 (41) | 8 (31) | 19 (36) |
| Chills | 8 (30) | 11 (42) | 19 (36) |
| Decreased appetite | 11 (41) | 7 (27) | 18 (34) |
| Fatigue | 9 (33) | 9 (35) | 18 (34) |
| Peripheral oedema | 11 (41) | 6 (23) | 17 (32) |
| Nausea | 10 (37) | 7 (27) | 17 (32) |
| Dyspnoea | 6 (22) | 10 (38) | 16 (30) |
| Dizziness | 11 (41) | 4 (15) | 15 (28) |
| Myalgia | 8 (30) | 7 (27) | 15 (28) |
| Vomiting | 8 (30) | 5 (19) | 13 (25) |
| Cough | 7 (26) | 6 (23) | 13 (25) |
| Aspartate transaminase increased | 9 (33) | 3 (12) | 12 (23) |
| Alanine transaminase increased | 8 (30) | 4 (15) | 12 (23) |
| Headache | 5 (19) | 7 (27) | 12 (23) |
| Epistaxis | 6 (22) | 6 (23) | 12 (23) |
| Rales | 9 (33) | 2 (8) | 11 (21) |
| Hypokalaemia | 4 (15) | 6 (23) | 10 (19) |
| Thrombocytopenia | 5 (19) | 5 (19) | 10 (19) |
| Constipation | 6 (22) | 4 (15) | 10 (19) |
| Abdominal pain | 7 (26) | 3 (12) | 10 (19) |
| Confusion | 6 (22) | 4 (15) | 10 (19) |
| Pneumonia | 5 (19) | 4 (15) | 9 (17) |
| Pain | 4 (15) | 5 (19) | 9 (17) |
| Hypocalcaemia | 4 (15) | 4 (15) | 8 (15) |
| Hyperbilirubinaemia | 3 (11) | 5 (19) | 8 (15) |
| Stomatitis | 5 (19) | 3 (12) | 8 (15) |
| Hypoalbuminaemia | 4 (15) | 4 (15) | 8 (15) |
| Hypoxia | 6 (22) | 2 (8) | 8 (15) |
| Any Grade ≥3 AE, n (%)* | | | |
| Thrombocytopenia | 2 (7) | 2 (8) | 4 (8) |
| Febrile neutropenia | 0 | 2 (8) | 2 (4) |
| Increased aspartate transaminase | 2 (7) | 0 | 2 (4) |
| Hypoxia | 1 (4) | 1 (4) | 2 (4) |
| Hypotension | 1 (4) | 1 (4) | 2 (4) |
| Multi-organ failure | 0 | 2 (8) | 2 (4) |
| Fatigue | 2 (7) | 0 | 2 (4) |

*Grade 3 treatment related AEs occurring in >1 patient overall.

### *Pharmacokinetics*

The PK-evaluable population included 19 (70%) and 17 (65%) patients on schedules A and B, respectively. Mean pevonedistat plasma concentration-time profiles obtained

on Day 1 of Cycle 1 are shown in Fig 1. Following IV infusion on Day 1, individual concentration-time profiles declined in a bi-exponential manner, reflecting a rapid distribution of pevonedistat from plasma to peripheral tissues followed by a slower phase, reflecting eventual elimination from the body. Plasma concentrations were generally quantifiable up to 24 h post-infusion at doses of 25 and 33 mg/m$^2$ and up to 48 h post-infusion at doses of 44 mg/m$^2$ or above. Relevant PK parameters of pevonedistat for both schedules are summarized in Table III. Observed increases in mean $C_{max}$ and $AUC_{24\ h}$ were approximately dose proportional between the 25 and 83 mg/m$^2$ doses after single dosing. Inter-individual variability in pevonedistat systemic exposure was modest across the various dosing cohorts (% CV ≤31%).

### *Pharmacodynamics*

Across all dose levels tested, induction of 8 target genes known to be induced by pevonedistat-mediated NAE inhibition (Walker *et al*, 2011) was confirmed. Fig 2A shows expression levels of one of these genes (*NQO1*) across 25 patients treated on schedule A. Increases in gene expression occurred in most patients within 4 to 8 h upon IV infusion of pevonedistat, and returned close to baseline within 24 h, consistent with the observed elimination of pevonedistat from the systemic circulation. Immunohistochemical assays for the presence of pevonedistat-NEDD8 adduct confirmed NAE inhibition in leukaemic blasts in 32 of 35 post-dose samples analysed. In three cases, adduct was detected in the sample labelled 'pre-dose' but not in the post-dose sample,indicating a sample switch, these pairs were therefore excluded from the final analysis (Fig 2B). Additionally, the CRL substrate CDT1, was seen to accumulate in tumour tissue following dosing, providing a downstream indication of NEDD8 pathway inhibition by pevonedistat (Fig 2B and C).

### *Efficacy*

For patients that received doses at or below the MTD ($n$ = 23), two complete responses (CRs, 8%) and two partial responses (PRs, 8%) were documented for a CR/PR rate of 17% on schedule A. On schedule B, there were 2 PRs (10%) documented in those treated at the MTD ($n$ = 19). One patient each in the 44 mg/m$^2$ cohort of Schedule A, the 83 mg/m$^2$ cohort of Schedule B and the 25 mg/m$^2$ cohort of Schedule A, maintained a response for over 12, 10 and 5 months, respectively. All responses documented were in patients with AML. Prolonged stable disease (defined as treatment with ≥5 cycles without evidence of progression or response) was reported in 6 (11%) patients, 4 (15%) on schedule A and 2 (8%) on schedule B. Of the three patients with MDS treated, one patient achieved stable disease beyond 5 cycles of therapy (78 mg/m$^2$ cohort on schedule A). Responses are summarized in Table IV.

## Discussion

In this first-in-human study, two intermittent dosing schedules, selected based on ongoing trials of pevonedistat in other tumour types, were examined. In a phase I study in patients with solid tumours, continuous dosing caused severe liver toxicity and two patients died (due to MOF and disease

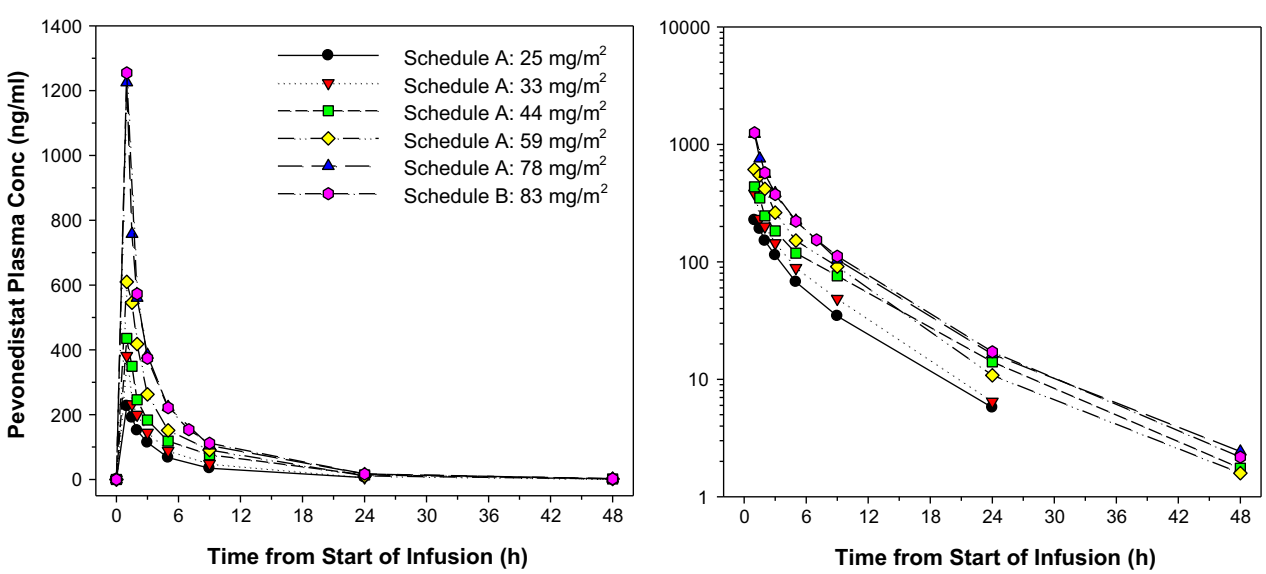


**Fig 1.** Mean pevonedistat plasma concentration-time profiles. Serial samples were obtained on Day 1 of Cycle 1 following a 1-h IV Infusion and presented graphically (linear and semi-log plots, left and right panels respectively). Pevonedistat concentrations (in ng/ml) are plotted on the *y*-axis.

**Table III.** Pevonedistat pharmacokinetic parameters following a 1-h intravenous infusion in patients treated on schedules A and B.

| | Pevonedistat Dose (mg/m$^2$) | | | | | |
|---|---|---|---|---|---|---|
| | Schedule A | | | | | Schedule B |
| Parameter (unit) | 25 $n = 2$ | 33 $n = 4$ | 44 $n = 3$ | 59 (MTD) $n = 6$ | 78 $n = 4$ | 83 (MTD) $n = 17$ |
| $C_{max}$ (ng/ml) | 222 | 374 (22) | 431 (18) | 648 (28) | 1173 (31) | 1212 (25) |
| $T_{max}$ (h)* | 1·07 (1·1,1·1) | 1·08 (1·1,1·2) | 1·03 (1·0,1·1) | 1·08 (0·9,1·6) | 1·04 (1·0,1·2) | 1·05 (0·9,1·3) |
| $AUC_{24\ h}$ (ng.h/ml) | 1034 | 1419 (12) | 1916 (17) | 2506 (28) | 3857 (17) | 3869 (22) |
| $AUC_{0\text{-}t}$ (ng.h/ml) | | | 1829† | 2913 (19)‡ | 3790 (15)§ | |

With the exception of one patient on Schedule B (83 mg/m$^2$), the elimination half-life of pevonedistat could not be accurately estimated due to insufficient sampling in the terminal disposition phase; in this patient, $t_{1/2}$ was estimated to be 12 h in plasma. All other parameters were recorded as shown.

Parameters are presented as geometric means (% coefficient of variance) unless specified otherwise. *Median (range); Schedule A: pevonedistat dosing on Days 1, 3 and 5 followed by a rest period of 16 days; Schedule B: pevonedistat dosing on Days 1, 4, 8 and 11 followed by a rest period of 10 days.

†$n = 2$; ‡$n = 5$; §$n = 3$.

MTD, maximum tolerated dose.

progression). Consequently, further testing was held and intermittent schedules (on days 1, 3 and 5) were explored, permitting recovery of hepatic transaminases between doses. This study formed the basis for the design of schedule A. Doses beyond 78 mg/m$^2$ could have been tested on this schedule, since only one DLT was reported at this dose level. However, as development of pevonedistat continued, schedule B was opened to test the hypothesis that higher cumulative doses per cycle would be more biologically active. In another phase I study for patients with lymphoma and myeloma (Harvey *et al*, 2012), schedule B had already been examined and MTD was declared at 196 mg/m$^2$. In the current trial, dose de-escalation from 147 mg/m$^2$ to 83 mg/m$^2$ (MTD) occurred on this schedule due to MOF in 3 patients with AML. These events led to a series of pre-clinical studies to explore potential mechanism-based toxicity. Rodents treated with combined pevonedistat and TNF-α (but not with single agents alone) exhibited a sepsis-like response, associated with increased circulating cytokines, organ damage (liver, kidney and gastro-intestinal tract) and elevated markers of tissue injury (alanine transaminase [ALT], AST, bilirubin and creatinine) (Wolenski *et al*, 2013). In cell-based assays, pevonedistat was shown to potentiate TNF-α signalling through activation of JNK kinase (Wolenski *et al*, 2013). Collectively, these data suggest that at higher dose levels of pevonedistat, an aberrant cytokine response may have contributed to the MOF observed on this trial.

Although some variability was apparent between cohorts and patients, pevonedistat plasma exposure increased proportionately with dose across the 25–83 mg/m$^2$ dose range. Where it could be assessed, plasma pevonedistat half-life was estimated to be around 12 h (Table III), which is consistent with PK findings from other pevonedistat clinical studies (Shah *et al*, 2009; Bhatia *et al*, 2011; Kauh *et al*, 2011; Harvey *et al*, 2010). To explore pevonedistat pharmacodynamics, we designed a novel immunohistochemical approach to measure pevonedistat-NEDD8 adduct formation. Given that the adduct will only form in the presence of enzymatically active NAE, we demonstrated NAE inhibition directly in leukaemic blasts. Furthermore, when we tested for accumulation of the CRL substrate CDT1 as a consequent effect of NAE inhibition (as previously demonstrated in xenograft studies (Soucy *et al*, 2009a), we confirmed a ≥1·5-fold increase in CDT1 expression in 16 of 29 (55%) evaluable patients, confirming pathway inhibition by pevonedistat at all doses tested. However, the amplitude of CDT1 expression did not predict for response in this study. We looked at expression levels of 8 NAE regulated target genes in whole blood. Induction of all 8 genes was observed, further confirming NAE inhibition by pevonedistat.

Pevonedistat showed clinical activity in relapsed refractory AML patients. Noteworthy responses included a 71-year-old male with primary induction failure who received 44 mg/m$^2$ and achieved a PR before coming off study for disease progression after 19 cycles of therapy. Additionally, two patients with recent relapses after allogeneic SCT for AML responded on study. The first patient was enrolled at the lowest dose level (25 mg/m$^2$) and achieved a complete cytogenetic remission, which was maintained for seven cycles. The second patient achieved a PR and was treated at 25 mg/m$^2$. The possibility that pevonedistat might augment a graft-versus-leukaemia effect is intriguing and currently under investigation. Despite attempts to increase pevonedistat exposure in this and other studies (Kauh *et al*, 2009, 2011; Shah *et al*, 2009; Bhatia *et al*, 2011), most of the responses we observed on this study were observed at lower doses. It is possible that after NAE is fully inhibited, that higher doses of pevonedistat may not confer additional clinical benefit but may cause increased toxicity (at least for patients with AML).

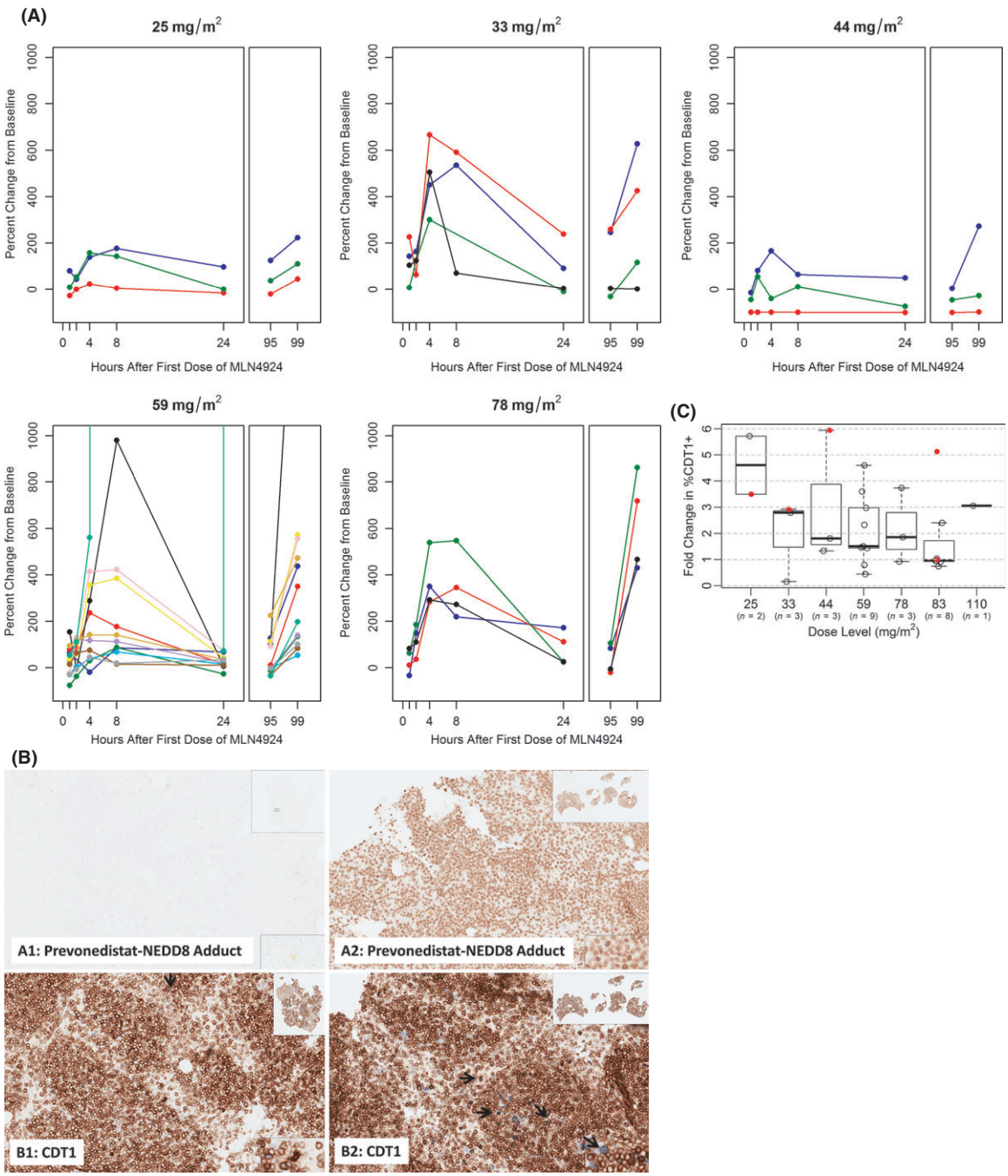


**Fig 2.** (A) Percent change from baseline in *NQO1* expression in blood following pevonedistat dosing at 25–78 mg/m$^2$ on schedule A. Colored lines represent individual patients across the 5 dose levels explored in Schedule A (3 at 25 mg/m$^2$, 4 at 33 mg/m$^2$, 3 at 44 mg/m$^2$, 11 at 59 mg/m$^2$ and 4 at 78 mg/m$^2$). *NQO1* mRNA levels are presented as change from baseline (pre-dose) during the 24 h period following pevonedistat dosing on Day 1 and four hours after dosing on Day 5. The *y*-axis of the 59 mg/m$^2$ plot was capped at change from baseline of 1000% for clarity, the data at 8 h and 99 h off scale were >500 000 and 2000% change respectively. (B) Representative immunohistochemistry images of bone marrow clot preparations stained for pevonedistat-NEDD8-adduct and CDT1. Bone marrow aspirate (from a patient treated on Schedule A at 44 mg/m$^2$) at screening (A1 and B1) and 3–6 h post-third dose of pevonedistat (A2 and B2), stained via immunohistochemistry for pevonedistat-NEDD8-adduct (brown is positive) and CDT1 (brown identifies CD34/CD117 + cells and blue is CDT1). All images contain a 20X image, a zoomed out thumbnail view of the block (top right) and a zoomed-in image (bottom right). Black arrows indicate representative cells that were detected as CD117/34 + and CDT1+. The post-dose increase in percentage of CDT1 positivity reported for this patient was +1·3-fold. (C) Fold change in the percentage of CDT1 positive CD117 + /CD34 + cells by immunohistochemistry in bone marrow before and after pevonedistat dosing. Data are represented as individual patient data (open circles), with median upper and lower quartile (box) and 95% confidence limits (whiskers) plotted per dose group. Dose groups 25–78 mg/m$^2$ were from Schedule A (post-dose sample Cycle 1, Day 5) and dose groups 83–110 mg/m$^2$ were from Schedule B. Red filled circles represent responding patients (complete or partial response).

**Table IV.** Anti-leukaemic activity of pevonedistat for both schedules

| Schedule | Dose (mg/m$^2$) | Disease response | Patients |
|---|---|---|---|
| A* | 25 | CR | 1 |
| A† | 33 | CR | 1 |
| A‡ | 44 | PR | 1 |
| A§ | 59 | PR | 1 |
| B¶** | 83 | PR | 2 |

*29-year-old female with acute myeloid leukaemia (AML) and adverse cytogenetics relapsing post-stem cell transplantation (SCT). Achieved a complete cytogenetic remission that was maintained for 7 cycles of therapy until disease progression.

†82-year-old male with AML and prior azacitidine failure, achieved stable disease for 8 cycles of therapy and then achieved a complete remission (CR) by Cycle 10, which lasted 2·7 months.

‡71-year-old with AML and primary induction failure achieved a partial response (PR) after 2 cycles of therapy and remained on study for 19 cycles.

§51-year-old with AML relapsing post-SCT achieved a PR after one cycle of therapy.

¶78-year-old male with refractory AML (conventional chemotherapy, azacitidine) achieved a PR after Cycle 1 and remained on study for 13 cycles before being withdrawn due to disease progression.

**73-year-old female with azacitidine-refractory AML achieved a PR following Cycle 1 of therapy.

This first-in-human study confirms that treatment with the NAE inhibitor pevonedistat is a feasible new investigational approach for patients with AML. Based on relative ease of administration (3 doses in 1 week followed by 2 weeks off), convenience in terms of future combination therapies and the data reported in this trial, schedule A has been selected for further clinical development. We are confident that this schedule reflects the most clinically and biologically active approach, which will be confirmed in larger studies. The incidence of pevonedistat-associated myelosuppression was low on this study, making it a potentially good candidate for combinations with other established anti-leukaemic agents. Combined therapy with hypomethylating agents (Smith *et al*, 2011) and cytarabine (Nawrocki *et al*, 2010) has already been explored in preclinical models, and a phase Ib investigation of pevonedistat combined with azacitidine is ongoing in patients with AML (NCT01814826). An interim analysis of this study was recently presented (Swords *et al*, 2014). Previously untreated AML patients ≥60 years old, considered unfit or unlikely to benefit from standard induction chemotherapy, were treated with escalating doses of pevonedistat given on days 1, 3 and 5, combined with fixed doses of azacitidine (75 mg/m$^2$ IV/SC on days 1–5 and days 8–9). Cycles were repeated every 28 days. At data cut-off (April 2014), 22 patients had received pevonedistat at 20 mg/m$^2$ ($n$ = 19) and 30 mg/m$^2$ ($n$ = 3). One patient each had grade 3 AST elevation and grade 2 elevated bilirubin at pevonedistat 30 mg/m$^2$ that was dose-limiting. One DLT (grade 4 AST/ALT elevation) occurred in 19 patients treated at the MTD (pevonedistat 20 mg/m$^2$ plus IV/SC azacitidine 75 mg/m$^2$). The most common AEs reported were myelosuppression and constipation. The PK profile of pevonedistat on this study did not change significantly with the addition of azacitidine. In the 15 patients evaluable for response, the CR/PR rate was 53% (4 CRs, 2 PRs). In this study, the addition of pevonedistat to azacitidine was feasible, and justifies the conduct of other rational pevonedistat combination trials in AML.

In the future, treatment emergent mutations may arise as mechanisms of resistance to pevonedistat. In cell lines and xenograft models, heterozygous mutations in the ATP binding pocket and substrate binding cleft of NAE, were identified as primary mechanisms of resistance to pevonedistat (Milhollen *et al*, 2012). An amino acid substitution (A171T) in the adenylate binding site was the most frequent mutation detected. A pan-E1 inhibitor with tighter binding properties was able to potently inhibit mutant enzymes in cells. These data provide a rationale for patient selection and the development of next-generation NAE inhibitors designed to overcome treatment-emergent mutations in the clinic. Larger planned studies of pevonedistat will provide more patients to profile for mechanisms of pevonedistat resistance. In conclusion, the results of the currently reported study indicate that NAE may be a novel therapeutic target and justify further study of pevonedistat, particularly in patients with AML.

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## Author contribution statement

RTS, HPE, DJD, JA, MM, HF, BJD, FJG and BCM were involved in the conception and design of the study. RTS, HPE, DJD, JA, MM, ZH, SB, HF, FS, BJD, FJG and BCM were responsible for the collection and assembly of data. RTS, HPE, DJD, DLB, JA, MM, ZH, SB, HF, FS, BJD, FJG, BCM were involved in data analysis and interpretation. All authors were responsible for writing/reviewing the draft manuscript, and all authors provided approval of the final draft manuscript.

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## References


Adams, J. (2004) The development of proteasome inhibitors as anticancer drugs. *Cancer Cell*, **5**, 417–421.

Bhatia, S., Hamid, O., Pavlick, A.C., Mulligan, G., Smith, P.G., Pickard, M., Shultz, M., Walker, R.M., Dezube, B. & O'Day, S. (2011) MLN4924, an investigational NEDD8-activating enzyme (NAE) inhibitor, in patients (pts) with metastatic melanoma: results of a phase I study. *Journal of Clinical Oncology*, **29**, 8529, (suppl; abstr 8529).

Bloom, J. & Pagano, M. (2003) Deregulated degradation of the cdk inhibitor p27 and malignant transformation. *Seminars in Cancer Biology*, **13**, 41–47.

Brownell, J.E., Sintchak, M.D., Gavin, J.M., Liao, H., Bruzzese, F.J., Bump, N.J., Soucy, T.A., Milhollen, M.A., Yang, X., Burkhardt, A.L., Ma, J., Loke, H.K., Lingaraj, T., Wu, D., Hamman, K.B., Spelman, J.J., Cullis, C.A., Langston, S.P., Vyskocil, S., Sells, T.B., Mallender, W.D., Visiers, I., Li, P., Claiborne, C.F., Rolfe, M., Bolen, J.B. & Dick, L.R. (2010) Substrate-assisted inhibition of ubiquitin-like protein-activating enzymes: the NEDD8 E1 inhibitor MLN4924 forms a NEDD8-AMP mimetic in situ. *Molecular Cell*, **37**, 102–111.

Burnett, A.K., Milligan, D., Prentice, A.G., Goldstone, A.H., McMullin, M.F., Hills, R.K. & Wheatley, K. (2007) A comparison of low-dose cytarabine and hydroxyurea with or without all-trans retinoic acid for acute myeloid leukemia and high-risk myelodysplastic syndrome in patients not considered fit for intensive treatment. *Cancer*, **109**, 1114–1124.

Burnett, A.K., Milligan, D., Goldstone, A., Prentice, A., McMullin, M., Dennis, M., Sellwood, E., Pallis, M., Russell, N., Hills, R.K. & Wheatley, K. (2009) The impact of dose escalation and resistance modulation in older patients with acute myeloid leukaemia and high risk myelodysplastic syndrome: the results of the LRF AML14 trial. *British Journal of Haematology*, **145**, 318–332.

Burnett, A.K., Russell, N.H., Kell, J., Dennis, M., Milligan, D., Paolini, S., Yin, J., Culligan, D., Johnston, P., Murphy, J., McMullin, M.F., Hunter, A., Das-Gupta, E., Clark, R., Carr, R. & Hills, R.K. (2010) European development of clofarabine as treatment for older patients with acute myeloid leukemia considered unsuitable for intensive chemotherapy. *Journal of Clinical Oncology*, **28**, 2389–2395.

Byrd, J.C., Mrózek, K., Dodge, R.K., Carroll, A.J., Edwards, C.G., Arthur, D.C., Pettenati, M.J., Patil, S.R., Rao, K.W., Watson, M.S., Koduru, P.R., Moore, J.O., Stone, R.M., Mayer, R.J., Feldman, E.J., Davey, F.R., Schiffer, C.A., Larson, R.A. & Bloomfield, C.D. (2002) Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood*, **100**, 4325–4336.

Chen, G. & Li, G. (2010) Increased Cul1 expression promotes melanoma cell proliferation through regulating p27 expression. *International Journal of Oncology*, **37**, 1339–1344.

Chen, G., Cheng, Y., Martinka, M. & Li, G. (2010) Cul1 expression is increased in early stages of human melanoma. *Pigment Cell & Melanoma Research*, **23**, 572–574.

Cheson, B.D., Bennett, J.M., Kopecky, K.J., Büchner, T., Willman, C.L., Estey, E.H., Schiffer, C.A., Doehner, H., Tallman, M.S., Lister, T.A., Lo-Coco, F., Willemze, R., Biondi, A., Hiddemann, W., Larson, R.A., Lowenberg, B., Sanz, M.A., Head, D.R., Ohno, R. & Bloomfield, C.D. (2003) Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. *Journal of Clinical Oncology*, **21**, 4642–4649.

Chiba, T. & Tanaka, K. (2004) Cullin-based ubiquitin ligase and its control by NEDD8-conjugating system. *Current Protein and Peptide Science*, **5**, 177–184.

Ciechanover, A. (2005) Intracellular protein degradation: from a vague idea, through the lysosome and the ubiquitin-proteasome system, and onto human diseases and drug targeting (Nobel lecture). *Angewandte Chemie (International ed. in English)*, **44**, 5944–5967.

Crusio, K.M., King, B., Reavie, L.B. & Aifantis, I. (2010) The ubiquitous nature of cancer: the role of the SCF(Fbw7) complex in development and transformation. *Oncogene*, **29**, 4865–4873.

Fanucchi, M.P., Fossella, F.V., Belt, R., Natale, R., Fidias, P., Carbone, D.P., Govindan, R., Raez, L.E., Robert, F., Ribeiro, M., Akerley, W., Kelly, K., Limentani, S.A., Crawford, J., Reimers, H.J., Axelrod, R., Kashala, O., Sheng, S. & Schiller, J.H. (2006) Randomized phase II study of bortezomib alone and bortezomib in combination with docetaxel in previously treated advanced non-small-cell lung cancer. *Journal of Clinical Oncology*, **24**, 5025–5033.

Fisher, R.I., Bernstein, S.H., Kahl, B.S., Djulbegovic, B., M.Robertson, M.J., de Vos, S., Epner, E., Krishnan, A., Leonard, J.P., Lonial, S., Stadtmauer, E.A., O'Connor, O.A., Shi, H., Boral, A.L. & Goy, A. (2006) Multicenter phase II study of bortezomib in patients with relapsed or refractory mantle cell lymphoma. *Journal of Clinical Oncology*, **24**, 4867–4874.

Gong, L. & Yeh, E.T. (1999) Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. *Journal of Biological Chemistry*, **274**, 12036–12042.

Goy, A., Bernstein, S.H., Kahl, B.S., Djulbegovic, B., Robertson, M.J., de Vos, S., Epner, E., Krishnan, A., Leonard, J.P., Lonial, S., Nasta, S., O'Connor, O., Shi, H., Boral, A.L. & Fisher, R.I. (2009) Bortezomib in patients with relapsed or refractory mantle cell lymphoma: updated time-to-event analyses of the multicenter phase 2 PINNACLE study. *Annals of Oncology*, **20**, 520–525.

Gstaiger, M., Lim, M., Jordan, R., Catzavelos, C., Mestan, J., Slingerland, J. & Krek, W. (2001) Skp2 is oncogenic and overexpressed in human cancers. *Proceedings of the National Academy of Sciences*, **98**, 5043–5048.

Harvey, R.D., Lebovic, D., Lonial, S., Jakubowiak, J., Pickard, M., McDonald, A., Mulligan, G., Blakemore, S., Kuan, S., Dezube, B. & O'Connor, O. (2010) MLN4924, an investigational NEDD8-activating (NAE) inhibitor, inpatients with relapsed and/or refractory lymphoma or multiple myeloma (MM): Phase I dose escalation study of twice-weekly dosing. *Haematologica*, **95**[suppl.2]: 160. abs. 0394.

Herrmann, J., Lerman, L.O. & Lerman, A. (2007) Ubiquitin and ubiquitin-like proteins in protein regulation. *Circulation Research*, **100**, 1276–1291.

Hershko, A. (2005) The ubiquitin system for protein degradation and some of its roles in the control of the cell division cycle. *Cell Death and Differentiation*, **12**, 1191–1197.

Karin, M. (2006) Nuclear factor-kappaB in cancer development and progression. *Nature*, **441**, 431–436.

Kauh, J.S., Cohen, R., Clarke, J.W., Harvey, R., Mulligan, G., Venkatakrishnan, K., Berger, A., Smith, P.G., Petruzelli, L. & Sarantopoulos, J. (2009) Pharmacodynamic effects of MLN4924, a novel NAE inhibitor, on blood and skin. *Journal of Clinical Oncology* **27**, 15s (suppl: abstr 2562)

Kauh, J.S., Shapiro, G., Cohen, R.B., Clark, J.W., Harvey, R.D., Cleary, J.M., Mahalingam, D., Kuan, S., McDonald, A., Berger, A., Dezube, B. & Saratopoulos, J. (2011) MLN4924, an investigational NEDD8-activating enzyme (NAE) inhibitor, in patients (pts) with advanced solid tumors: phase I study of multiple treatment schedules. *Journal of Clinical Oncology* **29**, 17s (suppl: abstr 3013).

Kondagunta, G.V., Drucker, B., Schwartz, L., Bacik, J., Marion, S., Russo, P., Mazumdar, M. & Motzer, R.J. (2004) Phase II trial of bortezomib for patients with advanced renal cell carcinoma. *Journal of Clinical Oncology*, **22**, 3720–3725.

Lin, J.J., Milhollen, M.A., Smith, P.G., Narayanan, U. & Dutta, A. (2010) NEDD8-targeting drug MLN4924 elicits DNA rereplication by stabilizing Cdt1 in S phase, triggering checkpoint activation, apoptosis, and senescence in cancer cells. *Cancer Research*, **70**, 10310–10320.

Milhollen, M.A., Traore, T., Adams-Duffy, J., Thomas, M.P., A.Berger, A.J., Dang, L., Dick, L.R., Garnsey, J.J., Koenig, E., Langston, S.P., Manfredi, M., Narayanan, U., Rolfe, M., Staudt, L.M., Soucy, T.A., Yu, J., Zhang, J., Bolen, J.B. & Smith, P.G. (2010) MLN4924, a NEDD8-activating enzyme inhibitor, is active in diffuse large B-cell lymphoma models: rationale for treatment

of NF-{kappa}B-dependent lymphoma. *Blood*, **116**, 1517–1524.

Milhollen, M.A., Narayanan, U., Soucy, T.A., Veiby, P.O., Smith, P.G. & Amidon, B. (2011) Inhibition of NEDD8-activating enzyme induces rereplication and apoptosis in human tumor cells consistent with deregulating CDT1 turnover. *Cancer Research*, **71**, 3042–3051.

Milhollen, M.A., Thomas, M.P., Narayanan, U., Traore, T., Riceberg, J. & Amidon, B.S. (2012) Treatment-emergent mutations in NAEbeta confer resistance to the NEDD8-activating enzyme inhibitor MLN4924. *Cancer Cell*, **21**, 388–401.

National Cancer Institute Cancer Therapy Evaluation Program (NCI CTEP): National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE). Version 3, 09 August 2006, 2011.

Nawrocki, S., Kelly, K., Oberheu, K., Mahalingam, D., Smith, P.G. & Carew, J.S. (2010) Disrupting NEDD8-mediated protein turnover with MLN4924 significantly augments the efficacy of cytarabine. *Blood*, **116**, 1335a, 2010 (suppl; abstr 3255)

Petroski, M.D. & Deshaies, R.J. (2005) Function and regulation of cullin-RING ubiquitin ligases. *Nature Reviews Molecular Cell Biology*, **6**, 9–20.

Podust, V.N., Brownell, J.E., Gladysheva, T.B., Luo, R.S., Wang, C., Coggins, M.B., Pierce, J.W., Lightcap, E.S. & Chau, V. (2000) A Nedd8 conjugation pathway is essential for proteolytic targeting of p27Kip1 by ubiquitination. *Proceedings of the National Academy of Sciences*, **97**, 4579–4584.

Read, M.A., Brownell, J.E., Gladysheva, T.B., Hottelet, M., Parent, L.A., Coggins, M.B., Pierce, J.W., Podust, V.N., Luo, R.S., Chau, V. & Palombella, V.J. (2000) Nedd8 modification of cul-1 activates SCF(beta(TrCP))-dependent ubiquitination of IkappaBalpha. *Molecular and Cellular Biology*, **20**, 2326–2333.

Reece, D.E., U. Hegenbart, U., Sanchorawala, V., Merlini, G., Palladini, G., Bladé, J., Fermand, J.P., Hassoun, H., Heffner, L., Vescio, R.A., Liu, K., Enny, C., Esseltine, D.L., van de Velde, H., Cakana, A. & Comenzo, R.L. (2001) Efficacy and safety of once-weekly and twice-weekly bortezomib in patients with relapsed systemic AL amyloidosis: results of a phase 1/2 study. *Blood*, **118**, 865–873.

Richardson, P.G., Sonneveld, P., Schuster, M.W., Irwin, D., Stadtmauer, E.A., Facon, T., Harousseau, J.L., Ben-Yehuda, D., Lonial, S., Goldschmidt, H., Reece, D., San-Miguel, J.F., Blade, J., Boccadoro, M., Cavenagh, J., Dalton, W.S., Boral, A.L., Esseltine, D.L., Porter, J.B., Schenkein, D. & Anderson, K.C. (2005) Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. *New England Journal of Medicine*, **352**, 2487–2498.

San Miguel, J.F., Schlag, R., Khuageva, N.K., Dimopoulos, M.A., Shpilberg, O., Kropff, M., Spicka, I., Petrucci, M.T., Palumbo, A., Samoilova, O.S., Dmoszynska, A., Abdulkadyrov, K.M., Schots, R., Jiang, B., Mateos, M.V., Anderson, K.C., Esseltine, D.L., Liu, K., Cakana, A., van de Velde, H. & Richardson, P.G. (2008) Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. *New England Journal of Medicine*, **359**, 906–917.

Shah, J., Jakubowiak, A.J., O'Connor, O., Orlowski, R., Patterson, M. & Harvey, D. (2009) Phase 1 dose escalation study of MLN4924, a novel NAE inhibitor, in patients with multiple myeloma and non-hodgkin lymphoma. *American Society of Hematology*, **114**, abs #1854

Shinde, V., Burke, K.E., Chakravarty, A., Fleming, M., McDonald, A.A., Berger, A., Ecsedy, J., Blakemore, S., Tirrell, S.M. & Bowman, D. (2014) Applications of pathology-assisted image analysis of immunohistochemistry-based biomarkers in oncology. *Veterinary Pathology*, **51**, 292–303.

Smith, P.G., Traore, T., Grossman, S., Narayanan, U., Carew, J.S. & Lublinksky, A. (2011) Azacitidine/Decitabine synergism with the NEDD8-activating enzyme inhibitor MLN4924 in preclinical AML Models. *American Society of Hematology Annual Meeting*, **118**, 578. abstr 615.

Soucy, T.A., Smith, P.G., Milhollen, M.A., Berger, A.J., Gavin, J.M., Adhikari, S., Brownell, J.E., Burke, K.E., Cardin, D.P., Critchley, S., Cullis, C.A., Doucette, A., Garnsey, J.J., Gaulin, J.L., Gershman, R.E., Lublinsky, A.R., McDonald, A., Mizutani, H., Narayanan, U., Olhava, E.J., Peluso, S., Rezaei, M., Sintchak, M.D., Talreja, T., Thomas, M.P., Traore, T., Vyskocil, S., Weatherhead, G.S., Yu, J., Zhang, J., Dick, L.R., Claiborne, C.F., Rolfe, M., Bolen, J.B. & Langston, S.P. (2009a) An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. *Nature*, **458**, 732–736.

Soucy, T.A., Smith, P.G. & Rolfe, M. (2009b) Targeting NEDD8-activated cullin-RING ligases for the treatment of cancer. *Clinical Cancer Research*, **15**, 3912–3916.

Soucy, T.A., Dick, L.R., Smith, P.G., Milhollen, M.A. & Brownell, J.E. (2010) The NEDD8 Conjugation Pathway and Its Relevance in Cancer Biology and Therapy. *Genes Cancer*, **1**, 708–716.

Swords, R.T., Kelly, K.R., Smith, P.G., Garnsey, J.J., Mahalingam, D., Medina, E., Oberheu, K., Padmanabhan, S., O'Dwyer, M., Nawrocki, S.T., Giles, F. & Carew, J.S. (2010) Inhibition of NEDD8-activating enzyme: a novel approach for the treatment of acute myeloid leukemia. *Blood*, **115**, 3796–3800.

Swords, R., Savona, M., Maris, M., Erba, H.P., Zhaowei, Z., Faessel, H., Blakemore, S., Sederati, F., Dezube, B. & Medeiros, B. (2014) First-in-class NAE inhibitor pevonedistat (MLN4924), in combination with azacitadine for acute myeloid leukemia (AML) patients consdiered unfit for conventional chemtherapy: results from the C15009 trial. *Haematologica*, **99**[suppl.1]: 223–224. abs # 4024.

Traore, T., Mihollen, M., Garnsey, J., Berger, A., Manfredi, M. & Cosmopolous, K. (2010) Antitumor activity of MLN4924, an investigational inhibitor of NEDD8-activating enzyme (NAE), in preclinical models of melanoma. *Journal of Clinical Oncology* **29**, 16s (suppl; abstr 8594).

Treon, S.P., Hunter, Z.R., Matous, J., Joyce, R.M., Mannion, B., Advani, R., Cook, D., Songer, J., Hill, J., Kaden, B.R., Sharon, D., Steiss, R., Leleu, X., Branagan, A.R. & Badros, A. (2007) Multicenter clinical trial of bortezomib in relapsed/refractory Waldenstrom's macroglobulinemia: results of WMCTG Trial 03-248. *Clinical Cancer Research*, **13**, 3320–3325.

Walker, R.M., Koenig, E., Mulligan, G., Lightcap, E., Pickard, M.D. & Smith, P.G. (2011) Development, validation, and clinical implementation of a peripheral blood RT-PCR pharmacodynamic assay for MLN4924, an investigational small molecule inhibitor of NEDD8-activating enzyme (NAE). Proceedings of the AACR-NCI-EORTC International Conference: Molecular Targets and Cancer Therapeutics; 2011 Nov 12-16; San Francisco, CA. Philadelphia (PA): AACR; Mol Cancer Ther 2011; (suppl; abstr 196).

Wang, M., Medeiros, B.C., Erba, H.P., DeAngelo, D.J., Giles, F.J. & Swords, R.T. (2011) Targeting protein neddylation: a novel therapeutic strategy for the treatment of cancer. *Expert Opinion on Therapeutic Targets*, **15**, 253–264.

Wolenski, F., Fisher, C., Sano, T., Varrone, G., Wyllie, S. & Ciccia, L. (2013) The NAE Inhibitor MLN4924 Synergizes with TNF-a to Induce Death Through Apoptosis and Necroptosis in a Rat Hepatoma Cell Line. August 11-16, Andover, NH, poster presentation.