MLN0905, a Small-Molecule PLK1 Inhibitor, Induces Antitumor Responses in Human Models of Diffuse Large B-cell Lymphoma

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Abstract

Diffuse large B-cell lymphoma (DLBCL) is the most common of the non–Hodgkin lymphomas, accounting for up to 30% of all newly diagnosed lymphoma cases. Current treatment options for this disease are effective, but not always curative; therefore, experimental therapies continue to be investigated. We have discovered an experimental, potent, and selective small-molecule inhibitor of PLK1, MLN0905, which inhibits cell proliferation in a broad range of human tumor cells including DLBCL cell lines. In our report, we explored the pharmacokinetic, pharmacodynamic, and antitumor properties of MLN0905 in DLBCL xenograft models grown in mice. These studies indicate that MLN0905 modulates the pharmacodynamic biomarker phosphorylated histone H3 (pHisH3) in tumor tissue. The antitumor activity of MLN0905 was evaluated in three human subcutaneous DLBCL xenograft models, OCI LY-10, OCI LY-19, and PHTX-22L (primary lymphoma). In each model, MLN0905 yielded significant antitumor activity on both a continuous (daily) and intermittent dosing schedule, underscoring dosing flexibility. The antitumor activity of MLN0905 was also evaluated in a disseminated xenograft (OCI LY-19) model to better mimic human DLBCL disease. In the disseminated model, MLN0905 induced a highly significant survival advantage. Finally, MLN0905 was combined with a standard-of-care agent, rituximab, in the disseminated OCI LY-19 xenograft model. Combining rituximab and MLN0905 provided both a synergistic antitumor effect and a synergistic survival advantage. Our findings indicate that PLK1 inhibition leads to pharmacodynamic pHisH3 modulation and significant antitumor activity in multiple DLBCL models. These data strongly suggest evaluating PLK1 inhibitors as DLBCL anticancer agents in the clinic.

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non–Hodgkin lymphoma (1), accounting for up to 30% of all newly diagnosed non–Hodgkin cases (2). DLBCL is an aggressive fast-growing cancer, which arises in either lymph nodes or outside the lymphatic system, in gastrointestinal tract, testes, thyroid, skin, breast, bone, or brain (2). This disease is more common in men (2) and the median age of diagnosis in both men and women is mid-60’s (3). Unfortunately, 40% to 50% of the patients diagnosed with DLBCL have extranodal disease at the time of diagnosis (4). Current treatment options for DLBCL include cyclophosphamide–adriamycin–vincristine–prednisone (CHOP) ± Rituxan, radiation, and bone marrow or stem cell transplantation (5). These treatment options can be effective but are not always curative (6); therefore, targeted investigational treatment options are being evaluated to improve clinical outcome.

Polo-like kinase 1 (PLK1) is a serine/threonine protein kinase that plays a key role in cell-cycle control (7). This mitotic kinase controls entry into and progression through mitosis at multiple stages by regulation of centrosome maturation, activation of initiating factors, degradation of inhibitory components, chromosome condensation, and exit from mitosis (reviewed in ref. 8). PLK1 is reported to be overexpressed in numerous cancers including melanoma, prostate, ovarian, colorectal, pancreatic, non–small cell lung, esophageal, endometrial, glioma, squamous cell carcinoma of the head and neck, and non–Hodgkins lymphoma (reviewed in ref. 8). Moreover, overexpression of PLK1 in DLBCL is linked to poor patient prognosis (9).
We have discovered a highly potent small-molecule inhibitor of PLK1 (MLN0905) that inhibits PLK1 enzyme activity in vitro using a final concentration of 5 nmol/L (IC_{50} value; ref. 10). In a cell-based assay measuring phosphorylation of a direct PLK1 substrate, CDc25C-T96, MLN0905 was shown to be a highly potent PLK1 inhibitor (EC_{50} = 29 nmol/L; ref. 10). In addition, tissue culture experiments show that cells treated with MLN0905 undergo a strong mitotic arrest followed by subsequent apoptosis (10), phenotypes consistently showed to be associated with PLK1 inhibition (using both small-molecule and genetic knockdown approaches; refs. 11–14). Tissue culture cell viability experiments show that MLN0905 yields LD_{50} values in the double-digit nanomolar range for solid tumor cell lines (10). In vivo, MLN0905 shows significant antitumor activity in an HT-29 solid tumor model of human cancer (10). In the current report, we evaluated the pharmacokinetic, pharmacodynamic, and antitumor properties of MLN0905 in mouse models harboring human DLBCL disease. Our findings indicate that MLN0905 displays drug-like pharmacokinetic properties, modulates an in vivo pharmacodynamic marker, and yields significant antitumor activity in multiple models of DLBCL. To our knowledge, this is the first time a small-molecule PLK1 inhibitor has been evaluated in preclinical models of DLBCL.

Materials and Methods

Cell culture and reagents

HT-29, Daudi, Pfeiffer, and Ramos cells were obtained from American Type Culture Collection. All cell lines used were passaged for less than 6 months after receipt or resuscitation (no further authentication was carried out). HT-29 cells were cultured in McCoy’s 5A media supplemented with 10% FBS. Daudi, Pfeiffer, and Ramos cells were cultured in RPMI-1640 supplemented with 5% FBS. WSU cells were obtained from Wayne State and cultured in RPMI-1640 with 10% FBS and 1% L-glutamine. The OCI LY-19 and OCI LY-10 cells were a kind gift from Lou Staudt (National Cancer Institute, Bethesda, MD). OCI LY-10 cells were cultured in Iscove’s Modified Dulbecco’s Medium supplemented with 10% FBS, 1% L-glutamine, and 55 μmol/L β-mercaptoethanol. Generation of the OCI LY-19-Luc cells was previously described (15); and cells were cultured in the presence of 1 mg/mL G418.

RNAi experiments

Cells (4 x 10^5) were transfected as previously described (16). The PLK1 sequence used was as follows: sense, 5’CCGAGUUAAUCAUCGAGACG3’. Immunofluorescent staining in tissue culture

Methodology was as previously described (16). Antibodies used were anti-pH3 (Cell Signaling), anti-tubulin (Sigma), and secondary antibodies included the Alexa-Fluor series (Invitrogen). Cells were counterstained with Hoechst # 33342 (Invitrogen). Western blotting

Floating and adherent cells were harvested as previously described (17). Antibodies used included: anti-β-actin (Abcam Inc.), anti-PLK1 (Invitrogen), and goat anti-mouse (IgG)-horseradish peroxidase (Santa Cruz).

ATPit assay

As previously described (15).

In vivo efficacy studies

The OCI LY-10 and OCI LYC-19 Luc models were previously described (15). The PHTX-22L primary lymphoma tumor was obtained from the Cooperative Human Tissue Network, University of Pennsylvania Medical Center (Philadelphia, PA). PHTX-22L tumor fragments were implanted into the dorsal flank of female nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice (Taconic Farms; ref. 15).

In vivo efficacy statistical analysis with MLN0905 used as a single agent

Statistical methodology used to compare MLN0905 treatment to control has been previously described (10). Efficacy study P values shown refer to dAUC comparisons.

In vivo efficacy statistical analysis when combining MLN0905 with rituximab

Analysis during the treatment phase. Photons flux area under the curve (AUC) from each animal was calculated from days 0 to 22. Let AUC_i be the transformed photon flux level for the ith mouse, i = 1, 2, 3, ..., 40. Then the mean of AUC_i is modeled as

\[ \text{AUC}_i = \beta_0 + \beta_1 D_1 + \beta_2 D_2 + \beta_3 D_1 D_2 \]

and the AUC_i is assumed to be normally distributed and independent across mice. The following hypothesis was tested H_0: β_3 ≥ 0 versus H_1: β_3 < 0. Rejecting the null hypothesis H_0 indicates that the effects of rituximab and MLN0905 are synergistic.

Survival analysis. The survival rates of the animals in each treatment group were plotted using Kaplan–Meier curves and a log-rank test was used to compare the survival rates among treatment group to the vehicle control group. For the synergy analysis the median (50%) survival time (MST) was used as benchmark. The following equation was used to determine synergy: β = MST (combination of drugs A and B) − MST (vehicle) − MST (drug A) − MST (vehicle) + MST (drug B) − MST (vehicle). If β is significantly greater than 0, the effects of drug A and B are synergistic. The P value (for one-sided null hypothesis) is calculated on the basis of bootstrap resampling. It is correct only if we assume that the distribution for the estimator of β under alternative hypothesis is a location shift of the distribution for the estimator of β under null hypothesis. Ninety-five percent confidence lower bounds for β are calculated on the basis of B = 10,000.
bootstrap resampling of time to event data within each group.

**In vivo immunohistochemistry**

OCI LY-19-Luc subcutaneous tumors were processed, stained, and quantified as previously described (17). One tissue section per animal (*n* = 3 animals/group) was used for the analysis.

MLN0905 pharmacokinetic analysis

MLN0905 was formulated and analyzed as previously described (10).

**Results**

In cells, MLN0905 phenocopies the effects of PLK1 knockdown using RNAi

Previous studies indicate that PLK1 inhibition, using both small-molecule and genetic RNAi knockdown approaches, lead to mitotic arrest, monopolar spindle formation, and subsequent cell death (11, 18). Here, we treated HT-29 cells with a small-molecule PLK1 inhibitor, MLN0905 (structure shown in Fig. 1A), or PLK1 RNAi, and evaluated cells for mitotic arrest, that is, pHisH3, and monopolar spindle formation. Targeting PLK1 with RNAi efficiently reduced PLK1 protein levels in HT-29 cells (Fig. 1B). Both RNAi and MLN0905 treatment yielded strong mitotic arrest characterized by robust pHisH3 staining (Fig. 1C). These data are consistent with a previous report showing a robust dose-dependent pHisH3 increase in HT-29 cells treated with MLN0905 (10). In addition, both RNAi and MLN0905 treatments yielded monopolar spindle formation. Monopolar spindle formation and mitotic arrest are phenotypes consistently shown to be associated with PLK1 inhibition (11, 18).

**Evaluating cell viability in DLBCL cells treated with MLN0905**

MLN0905 has been shown to reduce cell viability in solid tumor cells with *LD*$_{50}$ values in the double-digit nanomolar range (10). In the current report, we treated a panel of human lymphoma cells with MLN0905 and evaluated cell viability at 72 hours using the ATPlite assay. Supplementary Table S1 indicates that lymphoma cell lines are highly sensitive to MLN0905 treatment with *IC*$_{50}$ values between 3 and 24 nmol/L.

**Evaluating plasma and tumor pharmacokinetics following oral administration of MLN0905 into CB17-SCID mice bearing OCI LY-19 Luc xenograft tumors**

To determine the pharmacokinetic parameters of MLN0905 *in vivo*, CB17-SCID mice bearing OCI LY-19 Luc xenograft tumors were treated orally with increasing doses of MLN0905; and both plasma and tumor tissues were harvested and analyzed for MLN0905 exposure. After a single 1.6, 3.12, and 6.25 mg/kg dose, MLN0905 had plasma and tumor exposures (Supplementary Fig. S1 and Supplementary Table S2) that were greater than dose proportional, especially at the 6.25 mg/kg dose level. MLN0905 was rapidly distributed to tumor with a tumor-to-plasma AUC$_{0–24}$ of 2.1 to 2.9 across all dose levels. The compound showed reasonable retention in tumor with tumor half-life ranging from 4.5 to 6.9 hours.

**Pharmacodynamic response of phosphorylated histone H3 in OCI LY-19-Luc xenograft tumors following oral administration of MLN0905**

Phosphorylated histone H3 [pHisH3 (ser 10)] is a biomarker routinely used to identify cells arrested in mitosis. Here, pHisH3 was used as a downstream pathway biomarker to identify the percentage of cells arrested in mitosis following PLK1 inhibition. First, we treated OCI LY-19-Luc (and OCI LY-10) cells with MLN0905 in tissue culture and then evaluated pHisH3 levels using Western blotting. Supplementary Fig. S2 shows that MLN0905 leads to an increase of pHisH3 protein levels in the tissue culture setting. Next, we wanted to evaluate the pHisH3 levels in HT-29 cells treated with MLN0905.
levels in vivo using animals bearing subcutaneous OCI LY-19 Luc xenograft tumors. Animals were given a single oral dose of MLN0905 (1.6, 3.12, and 6.25 mg/kg), and tumors were harvested for pHisH3 analysis at 0, 1, 2, 4, 8, 24, 48, and 72 hours. Immunofluorescent staining was used to determine the percentage of tumor area staining positive for pHisH3 (Fig. 2). A significant increase in pHisH3 staining (over control) was observed (Supplementary Fig. S3) in all MLN0905-treated groups as follows: 3.12 mg/kg daily (T/C = 0.57, dAUC P < 0.001), 6.25 mg/kg daily (T/C = 0.15, dAUC P < 0.001), 10 mg/kg QD x 3/wk (T/C = 0.34, dAUC P < 0.001), and 14.5 mg/kg QD x 3/wk (T/C = 0.26, dAUC P < 0.001). An antitumor dose response was observed for the daily schedule but not for the QD x 3/wk schedule, likely due to efficacy saturation in the latter. All doses and schedules were well-tolerated with mean body weight loss less than 5% (data not shown).

Within this efficacy study the antitumor effect associated with dosing schedule (intermittent vs. continuous) was compared. Comparing the antitumor effect of daily dosing to the 3-on/4-off (QD x 3/wk) schedule was possible, because the same amount of drug was delivered (44 mg/kg) per week using the 6.25 mg/kg daily and 14.5 mg/kg QD x 3/wk groups. No significant antitumor difference was observed when comparing these 2 groups, using the dAUC analysis, suggesting that the antitumor effect was independent of schedule and driven by the total dose administered.

Evaluating the antitumor activity of MLN0905 in a disseminated OCI LY-19-Luc lymphoma xenograft model

To better mimic lymphoma disease in humans, a disseminated lymphoma model was used to evaluate the antitumor effect associated with MLN0905 treatment. To generate this model, OCI LY-19-Luc cells were inoculated into the tail vein of female SCID mice. The OCI LY-19-Luc cells constitutively express a luciferase reporter, allowing photon flux detection (following luciferin injection) with Xenogen imaging. Throughout the study, animals were imaged once/wk to determine tumor burden. Cancer hotspots were detected in femur, spine, and thymus (Fig. 3B, see vehicle-treated), thereby mimicking lymphoma disease in humans. Once tumor burden reached an average photon flux of 1.8 x 10^8 units (10 days postinoculation), animals were treated daily for 21 days with MLN0905 (1.6, 3.12, and 6.25 mg/kg). Antitumor activity was assessed by calculating T/C values on day 20 and by calculating tumor growth curve AUC values during treatment. Significant (P < 0.001, a log-rank Mantel–Cox test) antitumor activity was observed using the medium and high dose levels of MLN0905 (Fig. 3A; 3.12 mg/kg, T/C = 0.31, dAUC = 32.9; 6.25 mg/kg, T/C = 0.02, dAUC = 65.9). In the 6.25 mg/kg group, photon flux was almost undetectable on day 20 (Fig. 3B). When comparing the antitumor effect of MLN0905 in the subcutaneous (Supplementary Fig. S5) versus disseminated LY19-Luc model, MLN0905 yielded a better antitumor activity in the disseminated model. All treatments in this study were well-tolerated with no body weight loss.

To determine whether MLN0905 treatment provided a survival advantage, animals were monitored for advanced disease (paralysis), a predefined humane endpoint, for 110 days after treatment initiation. A Kaplan–Meier graph (Fig. 3C) summarizes mouse survival throughout the study. All vehicle-treated mice reached paralysis and were euthanized between days 26 and 37

Figure 2. pHisH3 was used as a biomarker to determine the level of mitotic arrest in OCI LY-19-Luc xenograft tumors. Tumor-bearing mice were treated with a single oral dose of MLN0905 (vehicle was used as a control (c)). At the indicated times, tumor tissue was harvested and assayed for pHisH3 using immunohistochemistry. A 2-tailed t test for unequal variances was used to determine statistical significance (*, P < 0.05, n = 3/time point).

Evaluating the antitumor activity of MLN0905 in a subcutaneous OCI LY-19-Luc lymphoma xenograft model

To determine the antitumor activity of MLN0905 in a model of human lymphoma, OCI LY19-Luc cells were grown as a s.c. xenograft tumor in SCID mice. Tolerability experiments in SCID mice determined the maximum-tolerated dose (MTD) of MLN0905 on the QD (daily) schedule to be 6.25 mg/kg and on the QD x 3/wk (3-days on/4-days off) schedule to be 14.5 mg/kg. Female SCID mice bearing s.c. OCI LY-19-Luc tumors were treated orally with MLN0905 on a QD x 3/wk schedule using 10 and 14.5 mg/kg or on a daily schedule using 3.12 and 6.25 mg/kg. Animals were dosed for 21 days and the antitumor effect was calculated using the following 2 analyses: (i) a T/C analysis, which calculates the mean tumor volume of the treatment group divided by the mean tumor volume of the control group, and (ii) an area under the tumor growth curve analysis (dAUC), which accounts for tumor volume over all treatment days. Using these 2 analyses, a significant antitumor effect was observed (Supplementary Fig. S3) in all MLN0905-treated groups as follows: 3.12 mg/kg daily (T/C = 0.57, dAUC P < 0.001), 6.25 mg/kg daily (T/C = 0.15, dAUC P < 0.001), 10 mg/kg QD x 3/wk (T/C = 0.34, dAUC P < 0.001), and 14.5 mg/kg QD x 3/wk (T/C = 0.26, dAUC P < 0.001). An antitumor dose response was observed for the daily schedule but not for the QD x 3/wk schedule, likely due to efficacy saturation in the latter. All doses and schedules were well-tolerated with mean body weight loss less than 5% (data not shown).

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Evaluating the antitumor activity of MLN0905 in a disseminated OCI LY-19-Luc lymphoma xenograft model

To better mimic lymphoma disease in humans, a disseminated lymphoma model was used to evaluate the antitumor effect associated with MLN0905 treatment. To generate this model, OCI LY-19-Luc cells were inoculated into the tail vein of female SCID mice. The OCI LY-19-Luc cells constitutively express a luciferase reporter, allowing photon flux detection (following luciferin injection) with Xenogen imaging. Throughout the study, animals were imaged once/wk to determine tumor burden. Cancer hotspots were detected in femur, spine, and thymus (Fig. 3B, see vehicle-treated), thereby mimicking lymphoma disease in humans. Once tumor burden reached an average photon flux of 1.8 x 10^8 units (10 days postinoculation), animals were treated daily for 21 days with MLN0905 (1.6, 3.12, and 6.25 mg/kg). Antitumor activity was assessed by calculating T/C values on day 20 and by calculating tumor growth curve AUC values during treatment. Significant (P < 0.001, a log-rank Mantel–Cox test) antitumor activity was observed using the medium and high dose levels of MLN0905 (Fig. 3A; 3.12 mg/kg, T/C = 0.31, dAUC = 32.9; 6.25 mg/kg, T/C = 0.02, dAUC = 65.9). In the 6.25 mg/kg group, photon flux was almost undetectable on day 20 (Fig. 3B). When comparing the antitumor effect of MLN0905 in the subcutaneous (Supplementary Fig. S5) versus disseminated LY19-Luc model, MLN0905 yielded a better antitumor activity in the disseminated model. All treatments in this study were well-tolerated with no body weight loss.

To determine whether MLN0905 treatment provided a survival advantage, animals were monitored for advanced disease (paralysis), a predefined humane endpoint, for 110 days after treatment initiation. A Kaplan–Meier graph (Fig. 3C) summarizes mouse survival throughout the study. All vehicle-treated mice reached paralysis and were euthanized between days 26 and 37
Similarly, mice receiving 1.6 mg/kg of MLN0905 reached the predefined endpoint between days 26 and 35 (median = 33 days). Medium survival for animals treated with either 3.12 (42 days) or 6.25 mg/kg (60.5 days) were significantly longer (P < 0.0001) than that in the vehicle group (26.5 days). The MLN0905 survival advantage correlated nicely with pharmacodynamic response (Fig. 2), suggesting the emergence of pharmacodynamic/efficacy relationship.

**Evaluating the antitumor activity of MLN0905 in a subcutaneous OCI LY-10 lymphoma xenograft model**

To evaluate the antitumor effect of MLN0905 in a second model of human lymphoma the OCI LY-10 subcutaneous lymphoma model was used. Female CB-17 SCID mice bearing OCI LY-10 tumors were treated orally with MLN0905 on a daily schedule using 1.6, 3.12, and 6.25 mg/kg for 21 days (treatment initiated on day 0). A, tumor cell photon flux was measured using photon flux imaging and the tumor growth curves are shown using a linear scale. B, photon flux signal from 5 representative animals are shown for both the vehicle and the 6.25 mg/kg MLN0905 groups on day 20. Heat map key: bright red, highest photon emission; cool blue, lowest photon emission. C, Kaplan–Meier survival curves show animal survival during and after the MLN0905 treatment. *P < 0.0001* from the vehicle. QD, daily.
MLN0905 synergizes with rituximab in a disseminated OCI LY-19-Luc lymphoma model

Rituximab is a therapeutic monoclonal antibody directed against the B-cell lymphoma phosphoprotein CD20 (19). Rituximab is routinely used in the clinic as a standard-of-care agent in combination with CHOP. Using flow cytometry, the OCI LY-19-Luc cell line was confirmed to express cell surface CD20 (data not shown).
The main goal of this OCI LY-19-Luc efficacy study was to determine whether rituximab would synergize with the PLK1 inhibitor MLN0905 in a disseminated model of lymphoma. Before the efficacy study, it was determined that rituximab did not alter the pharmacokinetic profile of MLN0905 in either mouse plasma or OCI LY-19-Luc tumor tissue (data not shown). For the efficacy study, animals with established OCI LY-19-Luc disease were treated for 3 weeks with either rituximab (10 mg/kg) i.v. 1 x/wk), MLN0905 (3.64 and 7.28 mg/kg, QD x 3/wk), or simultaneous combination. Figure 6A shows the average tumor Xenogen signal plotted as a function of time during the treatment period. The antitumor effect was evaluated using T/C values on day 22 and using an area under the tumor growth curve analysis during the treatment period (days 0–22). Using a χ2 evaluation, significant antitumor activity was observed with single-agent rituximab alone (T/C = 0.04, dAUC = 51.5, P < 0.001) and using the high-dose (7.28 mg/kg) MLN0905 (T/C = 0.22, dAUC = 17.0, P < 0.001). Combining rituximab with MLN0905 significantly enhanced the antitumor activity, and more importantly, yielded a synergistic antitumor response (Fig. 6A). All doses in this study were well-tolerated with no observed weight loss (data not shown).

To determine whether MLN0905 + rituximab treatment provided a survival advantage, animals were monitored for paralysis out to day 130 after treatment initiation. All vehicle-treated mice reached the predefined endpoint of paralysis, and were euthanized between days 16 and 35 (median = 29 days, Fig. 6B). Similarly, mice receiving single-agent MLN0905 (3.64 mg/kg) reached the predefined endpoint between days 24 and 36 (median = 28), neither significantly different from the vehicle group. In contrast, the medium survival time for animals treated with the high dose (7.28 mg/kg) MLN0905 (36 days, P < 0.0134) or with 10 mg/kg rituximab alone (57 days, P < 0.0001) was statistically longer than that in the vehicle group (29 days).

To determine whether combining rituximab with MLN0905 yielded synergistic survival advantage, the 50% survival mark was used as a benchmark (see Materials and Methods). Low-dose MLN0905 combined with rituximab did not yield a synergistic response, likely due to a suboptimal MLN0905 dosing. In contrast, combining rituximab with the high-dose (7.28 mg/kg) MLN0905 yielded a synergistic enhancement of life span (P = 0.0008).

Discussion

Small-molecule PLK1 inhibitors have entered phase I/II clinical trials both in solid and hematologic settings (20). Despite this advancement, little clinical data are available for evaluating PLK1 inhibition in patients diagnosed with DLBCL, and to our knowledge no preclinical data exists evaluating a PLK1 inhibitor in xenograft models of lymphoma. In 2008, phase I clinical trial data were presented evaluating PLK1 inhibition (BI-2536) in 41 patients with non–Hodgkin lymphoma (21). The number of patients with DLBCL treated in this trial was not disclosed, however the authors stated that one patient with DLBCL had a complete disease response. The observation that PLK1 inhibition led to a complete response in this patient is particularly encouraging.

In our current report, our efforts were focused on evaluating PLK1 as an oncology drug target in preclinical models of DLBCL. We have discovered a potent small-molecule inhibitor of PLK1, MLN0905 (10). MLN0905 was screened against a panel of 359 known kinases (Ambit; ref. 10) and found to have partial inhibitory activity against anaplastic lymphoma kinase (ALK). ALK translocations are rarely found in DLBCLs (22, 23) and all of the cell lines used in our studies were negative for ALK phosphorylation of Y1604 (data not shown), a marker indicative of ALK translocation activity (24). The absence of this marker provides evidence that the ALK translocation is unlikely relevant in the model systems used in our study. Moreover, the observed cellular phenotype (G2–M mitotic arrest) is consistent with that of PLK1 inhibition not an ALK inhibitor.

In our current report, our efforts were focused on evaluation of PLK1 as an oncology drug target in...
preclinical models of DLBCL. Using MLN0905 as a small-molecule PLK1 inhibitor, we show that pHisH3 can be used as a biomarker to track PLK1 pathway modulation in in vivo models of DLBCL. Moreover, the antitumor activity associated with PLK1 inhibition was evaluated in 3 human xenograft DLBCL models. In the OCI LY-10 model, MLN0905 treatment induced significant antitumor activity when administered using a variety of different doses and schedules. The highlight of this model was that 20% of the animals treated with the daily MTD responded with complete responses. In the OCI LY-19 model, the antitumor activity of MLN0905 was evaluated in animals harboring both subcutaneous and disseminated lymphoma disease. Significant antitumor activity was observed in both settings, and most importantly, MLN0905 treatment yielded a significant survival advantage (over control) in mice harboring the disseminated disease, the most fatal setting in humans. Also in the OCI LY-19 model, combining MLN0905 with the standard-of-care agent rituximab, yielded both synergistic antitumor and synergistic survival activity. These synergistic activities suggest that, in the clinical setting, a PLK1 inhibitor could be successfully combined with a standard-of-care agent to realize enhanced antitumor responses. The antitumor activity of MLN0905 was also evaluated in a primary lymphoma model that was recently harvested from a patient with DLBCL and represents, in many ways, the closest experimental system for mimicking dosing MLN0905 in a human patient. MLN0905 yielded significant antitumor activity in this primary model. When taken together, the above preclinical data indicate that PLK1 is an exciting oncology drug target. Combining our
Antimotic Activity of MLN0905 in Xenograft Models of DLBCL

preclinical data package with the encouraging clinical data from the BI-2536 phase I trial suggests PLK1 inhibitors should be evaluated in the clinical DLBCL setting. MLN0905 was also used to compare the effect scheduling imparts on antimitotic activity when dosing a PLK1 inhibitor on either a continuous or intermittent dosing schedule. In 3 different models (OCI LY-19, OCI LY-10, and PHTX-22L) the antimitotic effect was similar when comparing the continuous to the intermittent dosing schedules. These data suggest that, when comparing these 2 schedules, the primary driver of efficacy is the total amount of drug given, highlighting dosing flexibility.

In conclusion, PLK1 inhibition leads to robust antimitotic activity in multiple DLBCL xenograft models using both a subcutaneous and disseminated setting. These preclinical data validate PLK1 as an oncology drug target in the indication of DLBCL. With dosing flexibility as an option, PLK1 inhibitors should be further evaluated in patients suffering from DLBCL.

Disclosure of Potential Conflicts of Interest
At the time work was carried out, all authors were full-time Millennium employees.

References

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