Translational Exposure–Efficacy Modeling to Optimize the Dose and Schedule of Taxanes Combined with the Investigational Aurora A Kinase Inhibitor MLN8237 (Alisertib)

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Abstract

Aurora A kinase orchestrates multiple key activities, allowing cells to transit successfully into and through mitosis. MLN8237 (alisertib) is a selective Aurora A inhibitor that is being evaluated as an anticancer agent in multiple solid tumors and heme-lymphatic malignancies. The antitumor activity of MLN8237 when combined with docetaxel or paclitaxel was evaluated in in vivo models of triple-negative breast cancer grown in immunocompromised mice. Additive and synergistic antitumor activity occurred at multiple doses of MLN8237 and taxanes. Moreover, significant tumor growth delay relative to the single agents was achieved after discontinuing treatment; notably, durable complete responses were observed in some mice. The tumor growth inhibition data generated with multiple dose levels of MLN8237 and paclitaxel were used to generate an exposure–efficacy model. Exposures of MLN8237 and paclitaxel achieved in patients were mapped onto the model after correcting for mouse-to-human variation in plasma protein binding and maximum tolerated exposures. This allowed rank ordering of various combination doses of MLN8237 and paclitaxel to predict which pair would lead to the greatest antitumor activity in clinical studies. The model predicted that 60 and 80 mg/m² of paclitaxel (every week) in patients lead to similar levels of efficacy, consistent with clinical observations in some cancer indications. The model also supported using the highest dose of MLN8237 that can be achieved, regardless of whether it is combined with 60 or 80 mg/m² of paclitaxel. The modeling approaches applied in these studies can be used to guide dose-schedule optimization for combination therapies using other therapeutic agents. Mol Cancer Ther; 13(9); 2170–83. © 2014 AACR.

Introduction

Antimitotics are among the most successful classes of chemotherapy used in cancer care. This class of agents, including the taxanes, vinca alkaloids, and epothilones, is used to treat diverse solid and hematologic malignancies as single agents or as part of combination regimens. Paclitaxel (brand name Taxol), a taxane, identified in the 1960s and was first approved for use in patients with metastatic ovarian cancer in 1992 and in 1994 in patients with metastatic breast cancer. Paclitaxel binds to β-tubulin and prevents the disintegration of spindle microtubules during mitosis (1), thereby preventing the normal assembly/disassembly dynamics necessary for spindle microtubules to appropriately attach chromosomes and subsequently segregate the sister chromatids to the daughter cells. As a result, cells treated with paclitaxel arrest in mitosis via the activation of the spindle-assembly checkpoint and either undergo apoptosis directly out of mitosis or exit mitosis without completion of cytokinesis, a process known as mitotic slippage (2, 3). In the latter case, these cells can die by apoptosis, arrest by senescence or reenter the cell cycle by endoreduplication.

Docetaxel (brand name Taxotere) is a more soluble and potent synthetic derivative of paclitaxel and was approved for use in breast cancer in 1998. At the cellular level, docetaxel and paclitaxel share a similar mechanism of action. In addition to agents that directly perturb microtubule dynamics, anticancer therapies are being developed to directly inhibit enzymes that drive normal mitotic progression (4). Among these targets are the Aurora kinases, a...
family of serine/threonine kinases that comprises 3 iso-
forms, Aurora A, Aurora B, and Aurora C. As Aurora C
to expression is predominantly limited to germ cells, most
attempts at targeting the Aurora kinases have focused on
developing selective inhibitors of Aurora A, Aurora B, or
both (5, 6).

Aurora A mediates multiple steps throughout mitosis,
including centrosome maturation and separation, mitotic
tentry, formation of mitotic spindle poles and spindles,
alignment of chromosomes during metaphase, and their
subsequent separation during anaphase (7–11). The out-
comes associated with targeted inhibition of Aurora A
kinase have been studied using multiple experimental
modalities, including RNA interference, antibody micro-
injection, targeted knockout in mice, and with use of
small-molecule inhibitors (12–17). In mitosis, Aurora A
inhibition causes abnormal formation of the mitotic spin-
dles, resulting in mitotic arrest that is mediated by ac-
tivation of the spindle-assembly checkpoint. The fate of
these arrested cells can vary, and includes apoptosis
directly out of mitosis, exit from mitosis without under-
going cytokinesis resulting in G1 tetraploidy, or complet-
ed cytokinesis albeit with severe chromosome segregation
defects. In the latter 2 outcomes, the abnormal mitotic
divisions can lead to deleterious aneuploidy resulting in
cell death or senescence (13, 18).

MLN8237 (alisertib) is a selective ATP competitive
inhibitor of Aurora A kinase (19) studied in a number of
phase I and II clinical trials as a single agent and in
combination with other therapeutics, including paclitaxel
in recurrent ovarian cancer (NCT01091428) and with
docetaxel in prostate and other advanced solid cancers
(NCT01094288). Multiple preclinical studies demonstrat-
ed beneficial antitumor activity in cultured tumor cells
and in efficacy studies in vivo when combining Aurora
kinase inhibitors or Aurora kinase–targeted RNA inter-
ference in a variety of solid and heme-lymphatic cancer
models with paclitaxel and docetaxel (20–30). Aurora A
inhibition using the selective Aurora A kinase inhibitor
MLN8054 or RNA interference was shown to abrogate the
spindle-assembly checkpoint mediated mitotic delay
induced by paclitaxel (31). These cells rapidly exited
mitosis without completing cytokinesis via mitotic slip-
page and enter the G1 portion of the cell cycle with a
tetraploid DNA content. Interestingly, Aurora A over-
expression also abrogated the spindle-assembly check-
point in the presence of microtubule-perturbing agents
(32, 33).

Here, we demonstrate in preclinical models that the
Aurora A kinase inhibitor MLN8237 significantly
enhances the preclinical antitumor activity of docetaxel
and paclitaxel in triple-negative breast cancer models.
Triple-negative breast cancers are characterized as not
expressing estrogen receptor, progesterone receptor, or
HER-2; therefore, these tumors are not susceptible to
hormone- or HER-2–targeted therapies. Treatment stra-
egies for triple-negative breast cancer include multiple
chemotherapeutic agents, including taxanes (34).

Although these agents do provide some benefit to
patients, there remains a significant unmet need in this
population; therefore, alternative options need to be test-
ed, including combination therapy. Here, we build a
quantitative translational exposure–efficacy model using
both preclinical and clinical data for MLN8237 and pac-
litaxel to guide combination dose and schedule strategies
for these agents in patients in order to optimize the
potential antitumor activity.

Materials and Methods

Tumor cell culture and primary human tumors

MDA-MB-231 cells were obtained from the ATCC
and cultured in DMEM supplemented with heat inacti-
vated 10% FBS and 1% l-glutamine (Life Technologies).
MDA-MB-231 cells were purchased in 2002 and in-
house testing showed them to be free of mycoplasma
and murine pathogens. All experiments were con-
ducted with low-passage cells from recently resuscit-
ated frozen stocks. MDA-MB-231 cells (2 × 10^6) were
injected orthotopically into the mammary fat pad of
NCr nude mice. The primary human tumor xenografts
PHTX-02B and PHTX-14B were developed at Takeda
Pharmaceuticals International Co. from tumors that
were originally obtained from patients with breast can-
cer through the Cooperative Human Tissue Network
and were passed by trocar subcutaneously into the flank
of NOD SCID mice.

In vivo efficacy studies

MDA-MB-231, PHTX-02B, or PHTX-14B tumor-bearing
mice (n = 10 animals per group) were dosed orally (p.o.)
with vehicle (10% HPbCD + 3.5% NaHCO₃) or MLN8237
(3, 10, or 20 mg/kg) for 21 days using a once daily schedule
(every day) or for 3 days on/4 days off over 3 consecutive
weeks. Docetaxel (5 and 10 mg/kg) and paclitaxel (5,
10, 15, 20, and 30 mg/kg) were administered intravenously
(i.v.) on a once weekly schedule (every week) for a total
of 3 doses. Tumor growth was measured using vernier
calipers. Tumor growth inhibition (TGI) was determined
as the average change in vehicle treated tumors (ΔVehicle)
minus the average change in test agents treated tumors
(ΔTreated) divided by ΔVehicle and expressed as a per-
centage. Tumor growth delay (TGD) was the difference
in the number of days required for each test agent treatment
group to reach an average tumor volume of 1,000 mm³
relative to the vehicle-treated group. Drug combinations
were assessed for synergy using observed area under the
curve (AUC) values from the efficacy studies over the 21
days of dosing. The change in AUC relative to the control
was calculated for both single-agent treatment groups as
well as the combination group. The interaction between
the 2 compounds was then assessed by comparing the
change in AUC observed in the combination group to
the sum of the changes observed in both single agents.
The synergy score for the combination of MLN8237 (M) and
either taxane (T) was defined as 100 × [mean(AUC_MT) –
mean(AUC_M) – mean(AUC_T) + mean(AUC_C) + mean(AUC_v)]/
mean(AUC_{vehicle}). A 2-sided t test was used to determine if the synergy score was significantly different from zero. If the synergy score was less than zero and the P-value was below 0.05, then the combination was considered to be synergistic. If the synergy score was above zero and the P-value was below 0.05, then the combination was considered to be subadditive or antagonistic. Otherwise, the combination was considered to be additive.

**Immunohistochemistry**

MDA-MB-231 or PHTX-148 tumor-bearing NCr female nude or NOD SCID mice were dosed with MLN8237 at 10 mg/kg or docetaxel at 5 mg/kg or the 2 agents combined. Tumor tissue was harvested after multiple days of treatment and fixed in 10% neutral buffered formalin. Tumor sections were stained for phosphorylation of Histone H3 on serine 10 (pHistH3; Millipore) and MPM2 (Millipore) as described previously (35). The number of cells positive for pHistH3 were counted and averaged in 5 fields of view and DAPI nuclear staining was used to estimate the total number of cells in the fields. Apoptotic cells in tumor xenograft sections were evaluated by immunohisto staining using cleaved caspase-3 (Asp 175) antibody (Cell Signaling) and were quantified using Aperio Image analysis software (Leica Microsystems Vista). For histopathologic evaluation, 5-μm sections of formalin fixed, paraffin-embedded tumor samples were stained with hematoxylin and eosin (H&E) using a Leica Autostainer XL (Leica Biosystems). Regions of interest were manually drawn on H&E images using Aperio software to exclude areas of artifacts. Definiens Tissue Studio software (Definiens) was then used to identify tumor versus nontumor regions.

**Pharmacokinetics**

Female Balb/c nude mice bearing the MDA-MB-231 tumor (approximately 500 mm³) received a single dose of vehicle (10% HPβCD) p.o., MLN8237 p.o., docetaxel, or paclitaxel i.v., or a combination of MLN8237 p.o. with docetaxel or paclitaxel i.v. Whole blood and tumor samples were collected at specified time points. Blood samples were collected into tubes containing EDTA and placed on ice then centrifuged for 5 minutes at 10,000 rpm. Plasma was removed into fresh tubes and stored at −80°C. Tumors were dissected from the mice, weighed, and immediately frozen on dry ice. Homogenates were prepared in diH2O from frozen tumors using a FastPrep24 tissue homogenizer. Plasma and tumor homogenates were thawed at room temperature and the concentration of MLN8237 and paclitaxel or docetaxel in mouse plasma and tumor samples was determined by HPLC with MS-MS detection.

Pharmacokinetic (PK) analysis was performed in NONMEM (Icon plc). Plasma concentrations after a single dose of MLN8237 were fitted to a 2-compartment model with absorption, and plasma concentrations after a single dose of paclitaxel were fitted using a 2-compartment model with saturable clearance.

**Exposure-efficacy modeling**

Total MLN8237 exposures (AUC_{0-21d}) on each dosing regimen (every day and 3 days on/4 days off), and total paclitaxel exposures (AUC_{0-21d}) from every week dosing, were calculated from simulations of plasma concentration based on the fitted PK models. The simulated plasma concentration time courses for paclitaxel after i.v. dosing and for MLN8237 after p.o. dosing are shown in Supplementary Fig. S1A and S1B respectively, and the PK parameters used are shown in Supplementary Fig. S1C. Free fractions in female Balb/c Nude mice of 3.4% and 4.2% for paclitaxel and MLN8237 respectively, were determined using rapid equilibrium dialysis. Kinetic tumor xenograft volume data were converted to TGI using Eq. (1). The TGI values were fit using Pharsight Phoenix (Certara) software with a combination \( E_{\text{max}} \) model defined by Eq. (2), where \( \text{AUC}_{\text{MLN}} \) and \( \text{AUC}_{\text{Tax}} \) represent the total cycle-free exposures of MLN8237 and paclitaxel, respectively. Supplementary Table S1 contains a list of the best-fit parameters. The resulting best fits are shown in Supplementary Fig. S2.

%TGI = \frac{V_{\text{treated, 21d}} - V_{\text{control, 21d}}}{V_{\text{control, 21d}}} \times 100% \quad (1)

%TGI(\text{AUC}_{\text{MLN}}, \text{AUC}_{\text{Tax}}) = \frac{E_{\text{max, MLN}} \text{AUC}_{\text{MLN}}}{\text{AUC}_{\text{MLN}} + E_{\text{50, MLN}}} + \frac{E_{\text{max, Tax}} \text{AUC}_{\text{Tax}}}{\text{AUC}_{\text{Tax}} + E_{\text{50, Tax}}} \quad (2)

Clinical exposures were estimated for MLN8237 and paclitaxel as follows. The total cycle AUC of MLN8237 for doses of 10 to 50 mg twice a day administered on days 1 to 3, 8 to 10, 15 to 17 of 28-day cycles was calculated based on a previously reported geometric mean apparent oral clearance of 4.45 L/h in patients with advanced hematologic malignancies (36). The corresponding unbound plasma exposures were calculated based on a free fraction of 2.5% in human plasma using rapid equilibrium dialysis. The total cycle AUC of paclitaxel for doses of 60 to 90 mg/m² administered on days 1, 8, and 15 of 28-day cycles was calculated based on a previously reported clearance of 5.5 mL/min/kg (~223 mL/min/m²; ref. 37). The corresponding unbound plasma exposures were calculated based on a free fraction of 4.4% in human plasma using rapid equilibrium dialysis. For comparison with unbound preclinical exposures, a scaling was applied to the unbound clinical exposures factor based on the unbound exposure ratio between mouse and human as well as on the maximum tolerated dose between mouse and humans for MLN8237 (20 mg/kg twice a day every day for 21 days and 50 mg twice a day for 7 days on a 21-day schedule, respectively) and paclitaxel (30 mg/kg every week for 3 weeks and 80 mg/m² every week for 3 weeks on a 28-day schedule, respectively).

**Results**

The antitumor activity of MLN8237 combined with docetaxel was tested in xenograft and primary human
Figure 1. MLN8237 combined with docetaxel results in antitumor activity in three models of triple-negative breast cancer, including the cell line xenograft MDA-MB-231 (A), the primary human breast tumor xenograft PHTX-02B (B), and the primary human breast tumor xenograft PHTX-14B (C). Mice were treated for 21 days with MLN8237 (p.o., every day), docetaxel (i.v., every week × 3), or the combination of both at the indicated doses. Tumors were measured twice weekly with vernier calipers; error bars, SEM. The dotted line box indicates the 21-day treatment period.
tumor-derived triple-negative breast tumor models in immunocompromised mice. In most mouse strains, the maximum tolerated dose for MLN8237 is 30 mg/kg dosed every day or 20 mg/kg dosed twice a day with an 8-hour break between doses. The maximum tolerated dose for docetaxel was determined to be 15 mg/kg dosed once weekly (every week) as doses above this led to body weight loss exceeding 10%. In the MDA-MB-231 xenograft, 3 and 10 mg/kg MLN8237 (every day × 21 days) combined with 5 and 10 mg/kg docetaxel (every week, days 1, 8, and 15) led to synergistic antitumor activity (Fig. 1A and Table 1). Importantly, MLN8237 at 3 and 10 mg/kg combined with 10 mg/kg docetaxel led to regressions and prolonged TGD (difference in days between the control and treated groups to reach 1,000 mm³), and in some mice tumors never reformed even after discontinuing treatment. In comparison, MLN8237 dosed at the maximum tolerated dose in mice bearing the MDA-MB-231 xenograft did not lead to regressions (19). In the primary human tumor xenograft PHTX-02B, 10 and 20 mg/kg MLN8237 (every day × 21 days) combined with 5 mg/kg docetaxel (every week days 1, 8, and 15) led to additive or synergistic antitumor activity (Fig. 1B and Table 1). Additive or synergistic antitumor activity also occurred in the PHTX-14B xenograft with 10 and 20 mg/kg (every day) MLN8237 and 5 mg/kg docetaxel, or the combination of both (Fig. 2A). In plasma and tumor tissue, the exposures of both MLN8237 and docetaxel were similar whether dosed alone or in combination with the other agent.

MLN8237 and docetaxel lead to a transient accumulation of cells in mitosis. Therefore, the effect of a single dose of MLN8237 and docetaxel alone or combined on the tumor mitotic index was evaluated in mice bearing the MDA-MB-231 xenograft. Tumor mitotic index was

<table>
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<th>Model</th>
<th>MLN8237 dose (every day or 3 on/4 off)</th>
<th>Docetaxel dose (every 7 days × 3)</th>
<th>TGI (%)</th>
<th>TGD (days)</th>
<th>Outcome (AUC)</th>
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<td>MDA-MB-231</td>
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<td>10 mg/kg</td>
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<tr>
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<td>5 mg/kg</td>
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<td>49</td>
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<tr>
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*Orthotopic MDA-MB-231 xenografts were grown in the fat pad of nude mice and treated with MLN8237 administered orally for 21 days with docetaxel dosed i.v. once per week.

*Primary breast cancer models were grown subcutaneously in SCID (PHTX-14B) or NOD (PHTX-02B) mice and treated with MLN8237 administered orally for 21 days with docetaxel dosed i.v. once per week.

†TGI = (Δ treated/Δ control) × 100/Δ control, was calculated on the last day of treatment.

‡TGD, the difference in days between the control and the treated groups to reach 1,000 mm³. > denotes that the treatment group was terminated before reaching 1,000 mm³.

§Synergy analysis based on the AUC values days 0 to 20.
evaluated using 2 independent markers for mitotic cells, pHistH3, and a mitotic-specific antigen MPM2. In all cases, the mitotic index increased within 2 hours after dosing; however, there were no notable differences in the mitotic index in the combination relative to the single agents (Fig. 2B).

As no marked change in the mitotic index was observed with combined MLN8237 and docetaxel relative to the single agents in MDA-MB-231 model, the effect of the MLN8237 and docetaxel combination on tumor morphology was evaluated after treating PHTX-14B and MDA-MB-231 tumor-bearing mice with 10 mg/kg MLN8237 (every day \( \times \) 10 days), 5 mg/kg docetaxel (every week days 1 and 8), or the combination of MLN8237 and docetaxel (Fig. 3). In the PHTX-14B tumors, the combination of MLN8237 and docetaxel led to marked changes in tumor morphology, with increased mitotic and multinucleated cells and significant fibrosis (Fig. 3A).

In regions of the tumors where viable cells remained, there was a significant increase in nontumor tissue that comprised necrotic and fibrotic regions along with stromal infiltrate (Fig. 3B). Only a modest increase in apoptotic cells as determined by cleaved caspase-3 staining was observed with single-agent docetaxel and the combination (Supplementary Fig. S3), potentially because of the transient nature of this marker during the apoptotic cascade. The morphologic effects of the combination were less evident in the MDA-MB-231 model, however mitotic and multinucleated cells were observed (Fig. 3C and D).
One of the primary dose limiting toxicities of MLN8237 in patients with cancer is myelosuppression (38, 39). Therefore, there is risk for overlapping toxicity when combining MLN8237 with docetaxel or paclitaxel as myelosuppression is a common dose-limiting toxicity for taxanes as well. One path toward reducing the risk of overlapping toxicity is to decrease the dosing frequency for MLN8237. Therefore, the antitumor activity of MLN8237 dosed intermittently (3 days on/4 days off) with docetaxel once every week for 3 consecutive weeks was evaluated. In the PHTX-02B model, 20 mg/kg MLN8237 dosed 3 days on/4 days off with 5 mg/kg docetaxel (every week days 1, 8, and 21) resulted in significant TGI and TGD relative to the single agents (Fig. 4A and Table 1). MLN8237 dosed 3 days on/4 days off with docetaxel dosed weekly resulted in synergistic antitumor activity in the PHTX-14B model as well (Fig. 4B and Table 1). In fact, the extent of antitumor activity (TGI and TGD) achieved with the combination in both models with the 3 days on/4 days off schedule equaled that observed when MLN8237 was dosed consecutively for 21 days at an identical total daily dose. These data support dosing MLN8237 on an intermittent schedule as a potential means to minimize overlapping dose-limiting toxicities while maintaining antitumor activity when combined with weekly taxanes.

The in vivo antitumor activity of MLN8237 was also tested in combination with paclitaxel in the MDA-MB-231 and PHTX-14B xenografts (Fig. 5 and Table 2). The maximum tolerated dose for paclitaxel was determined to be 30 mg/kg dosed once every week as doses above this led to body weight loss exceeding 10%. In the MDA-MB-231 xenograft, 3, 10, and 20 mg/kg MLN8237 (every day) combined with 5, 10, 15, 20, or 30 mg/kg paclitaxel (every week days 1, 8, and 15) led to additive or synergistic antitumor activity (Fig. 5A and Table 2). With 10 and 20 mg/kg MLN8237, paclitaxel at 20 and 30 mg/kg led to substantial TGD (Table 2). In the primary human tumor xenograft PHTX-14B, 20 mg/kg MLN8237 (every day) combined with 10 and 20 mg/kg paclitaxel (every week) led to synergistic antitumor activity (Fig. 5B and Table 2) with TGD extending beyond the observation period. The
antitumor activity of MLN8237 dosed intermittently (3 days on/4 days off) with paclitaxel once every week for 3 consecutive weeks was also evaluated. In the MDA-MB-231 model, 20 mg/kg MLN8237 dosed 3 days on/4 days off with 20 mg/kg paclitaxel (every week days 1, 8, and 21) resulted in additive TGI relative to the single agents (Supplementary Fig. S4). Total body weight loss in mice for all MLN8237 and paclitaxel treatment regimens never exceeded 10%. In addition, no PK drug–drug interaction between MLN8237 and paclitaxel was observed in mice as the PK profiles for each agent in plasma and MDA-MB-231 tumor tissue were similar whether dosed alone or in combination with the other agent (Supplementary Fig. S5).

The durable antitumor activity of MLN8237 combined with both docetaxel and paclitaxel in preclinical tumor models presented provided part of the rationale for evaluating the safety and antitumor activity of MLN8237 combined with paclitaxel in patients with recurrent ovarian cancer (NCT01091428). In this clinical study, MLN8237 was dosed twice a day 3 days on/4 days off concomitantly with paclitaxel dosed weekly (every week days 1, 8, and 21) at 60 or 80 mg/m² on a 28-day schedule (40). To guide dose-schedule selection for combined MLN8237 and paclitaxel in patients with cancer, an exposure–efficacy model based on nonclinical and clinical data were developed to predict which MLN8237/paclitaxel combinations results in the greatest antitumor efficacy. An exposure–efficacy surface plot (Fig. 6A) and isobologram (Fig. 6B) relating MLN8237 and paclitaxel exposures to TGI was generated from in vivo efficacy studies in tumor-bearing mice (Table 2). The free-fraction corrected clinical exposures of MLN8237 dosed twice a day based on previously published PK data (36, 39) and the clinical exposures of paclitaxel at 60 or 80 mg/m² doses of paclitaxel determined from its human plasma clearance (37, 41) were mapped onto the isobologram by correcting for mice–human variation in plasma protein binding and maximum tolerated exposures for both agents. A combination $E_{\text{max}}$ exposure–efficacy model provided a reasonable fit to the data, as is shown in the diagnostic plots (Supplementary Fig. S5).

Figure 4. MLN8237 administered on an intermittent 3 days on/4 days off schedule combined with docetaxel results in significant antitumor activity in two primary breast tumor models, PHTX-02B (A) and PHTX-14B (B). Tumor-bearing mice were treated with MLN8237 administered once daily for 3 days for 3 weeks (3on/4off), docetaxel administered once weekly for 3 weeks, or the combination of both. Tumors were measured twice weekly with vernier calipers; error bars, SEM. The dotted line box indicates the 21-day treatment period.
This translational approach demonstrated allowed placing the clinically achieved exposures of the combination in the context of the preclinically observed antitumor efficacy. Placed in this context, the model predicted that 80 and 60 mg/m² of paclitaxel lead to similar levels of efficacy (Fig. 6B and C), consistent with clinical observations in some cancer indications (42, 43). In contrast, placing the MLN8237 exposures in the context of the preclinical antitumor efficacy suggests that increasing the dose of MLN8237 from 10 to 50 mg twice a day would result in increasing antitumor activity (Fig. 6B and C). This approach allows for rank ordering various combination doses and schedules of MLN8237 and paclitaxel to predict which pair leads to the greatest antitumor activity. For example, overlapping toxicities could prevent escalation of MLN8237 to a biologically active exposure range when combined with 80 mg/m² paclitaxel. If reducing the dose of paclitaxel to 60 mg/m² can mitigate overlapping toxicities allowing for higher MLN8237 doses, the translational approach demonstrated here suggests that this would also result in increased antitumor activity relative to 80 mg/m² single-agent paclitaxel.

Discussion

Antimitotic therapies are a mainstay for cancer care, as they are used broadly in both solid and hematologic cancers. Traditionally, these therapies have comprised agents that directly target microtubules, and include the taxanes, vinca alkaloids, and epothilones. Recently, encouraging activity has been observed with microtubule-perturbing agents such as the microtubule destabilizer mono-methyl aurastatin E conjugated to antibodies, including brentuximab vedotin and trastuzumab-DM1 for treating CD30⁺ lymphomas and Her2⁺ breast cancer respectively (44, 45). Despite the success of antimitotic therapies across many indications, strategies to improve response rates and extend responses in patients are needed. Here, we demonstrated improved antitumor activity and extended duration of response in multiple triple-negative breast cancer models by combining 2 classes of antimitotic agents, taxanes, and the Aurora A kinase inhibitor MLN8237.

Mice bearing 3 xenograft models of triple-negative breast cancer, including 2 primary models, were treated with various doses of MLN8237 combined with docetaxel or paclitaxel. In each tumor model, the combination led to greater TGI relative to the single agents, additive or synergistic antitumor activity while dosing, and prolonged TGD after discontinuing treatment. Notably, in several cases, the combination of MLN8237 and either taxane led to tumor regressions and in some mice the tumors never reformed after discontinuing treatment; outcomes that did not occur with the single agents when dosed at the individual maximum tolerated dose.

In MDA-MB-231 tumor xenografts treated with combined MLN8237 and docetaxel, there was no notable difference in the mitotic index, necrotic and fibrotic content, or stromal infiltrate compared with tumors treated with the single agents after 10 days of dosing. It is possible that the timing of this analysis in this tumor model was not optimal to capture the events underlying the antitumor activity observed after 21 days of dosing. However, the impact of this combination on tumor morphology was...
more evident in the PHTX-14B tumor xenograft as a histopathologic assessment revealed distinct tissue morphology changes in combination treated tumors relative to the single-agent treated tumors, including increased nontumor tissue (necrotic cells, fibrosis, stromal infiltrate) and multinucleated tumor cells. The multinucleated phenotype is consistent with previous observations in cultured tumor cells, demonstrating that concurrent Aurora A kinase inhibition using the selective small-molecule inhibitor MLN8054 or siRNA with microtubule-perturbing agents, including taxanes, caused cells to exit mitosis via mitotic slippage (31). Cells that exit mitosis by mitotic slippage enter the G1 stage of the cell cycle with a tetraploid DNA content often accompanied by multiple nuclei because of hyper-karyokinesis that can occur when the nuclear membrane reforms (2, 4). Depending on several underlying genetic factors, these cells can reenter the cell cycle and undergo another round of DNA replication through a process known as endoreduplication where they subsequently are characterized as polyploid (>4N). Therefore, the multinucleated phenotype observed in the tumor tissue with combined MLN8237 and docetaxel suggests that the mechanism elucidated in cell culture with Aurora A inhibition combined with taxanes occurs in \textit{in vivo} tumor models as well.

MLN8237 has been evaluated in multiple PI and PII clinical studies (36, 39, 46, 47). In the first-in-human PI study, a partial response was achieved in one patient with platinum and radiation refractory ovarian cancer that lasted for greater than 1 year (38). Single-agent MLN8237 was subsequently investigated in a phase II study in patients with platinum-resistant or platinum-refractory ovarian, primary peritoneal, and fallopian tube cancers (48). In this study, objective responses occurred in 10% of patients (\(n=3\) of 31) as determined by Response Evaluation Criteria in Solid Tumors (RECIST) and/or reduction in plasma CA-125, warranting further MLN8237 studies in this indication in combination with other therapeutics, including with taxanes.

MLN8237 was tested in combination with weekly paclitaxel in patients with recurrent ovarian cancer (NCT01091428; ref. 40). Given that myelosuppression is a common adverse event for both MLN8237 and paclitaxel, weekly paclitaxel (every week/3 28-day cycle) was selected for this study as it is known to have a decreased incidence of myelosuppression relative to paclitaxel dosed once every 3 weeks (49, 50). For MLN8237, an intermittent schedule of 3 days on (twice a day)/4 days off was selected for combining with

| Table 2. Antitumor activity summary of MLN8237 combined with paclitaxel |
|---------------------------|------------------|----------------|-----------------|----------------|
| Model                    | MLN8237 dose (every day) | Paclitaxel dose (every 7 days \(\times 3\)) | TGI\(^a\) (%) | TGD (days)\(^d\) | Outcome (AUC)\(^e\) |
| MDA-MB-231\(^a\)         | 20 mg/kg          | 30 mg/kg          | 101.4         | 35             | Synergistic      |
|                          | 20 mg/kg          | 20 mg/kg          | 94.3          | 26             | Synergistic      |
|                          | 20 mg/kg          | 20 mg/kg          | 96.3          | 24             | Synergistic      |
|                          | 20 mg/kg          | 15 mg/kg          | 85.7          | 16             | Additive         |
|                          | 20 mg/kg          | 10 mg/kg          | 45.87         | 4              | Additive         |
|                          | 20 mg/kg          | 5 mg/kg           | 43.6          | 4              | Additive         |
|                          | 10 mg/kg          | 30 mg/kg          | 102.4         | 31             | Synergistic      |
|                          | 10 mg/kg          | 20 mg/kg          | 81.9          | 13             | Additive         |
|                          | 10 mg/kg          | 15 mg/kg          | 85.6          | 14             | Additive         |
|                          | 10 mg/kg          | 10 mg/kg          | 42.3          | 4              | Additive         |
|                          | 3 mg/kg           | 20 mg/kg          | 69.2          | 10             | Additive         |
|                          | 3 mg/kg           | 20 mg/kg          | 60.5          | 7              | Additive         |
|                          | 3 mg/kg           | 10 mg/kg          | 21.7          | 2              | Additive         |
|                          | 3 mg/kg           | 5 mg/kg           | 20.8          | 2              | Additive         |
| PHTX-14B\(^b\)           | 20 mg/kg          | 20 mg/kg          | 103           | >14            | Synergistic      |
|                          | 20 mg/kg          | 10 mg/kg          | 84            | >14            | Synergistic      |
|                          | 3 mg/kg           | 20 mg/kg          | 72            | >14            | No data         |

\(^a\)Orthotopic MDA-MB-231 xenografts were grown in the fat pad of nude mice and treated with MLN8237 administered orally for 21 days with paclitaxel dosed i.v. once per week.

\(^b\)Primary breast cancer models were grown in SCID mice and treated with MLN8237 administered orally for 21 days with paclitaxel dosed i.v. once per week.

\(^c\)TGI = \((A \text{ treated}/A \text{ control}) \times 100/A \text{ control}, was calculated on the last day of the treatment.

\(^d\)TGD, the difference in days between the control and the treated groups to reach 1,000 mm\(^3\). > denotes that the treatment group was terminated before reaching 1,000 mm\(^3\).

\(^e\)Synergy analysis based on the AUC values days 0 to 20.
Figure 6. A surface response plot relating MLN8237 and paclitaxel exposures to TGI (% TGI) generated from multiple in vivo efficacy studies in mice bearing the MDA-MB-231 xenograft (blue dots; A). An isobologram derived from the surface response plot (B). Clinically achieved exposures of MLN8237 (10 or 40 mg twice a day) and paclitaxel (60 or 80 mg/m²) from the NCT01091428 study represented by the red stars were mapped onto the isobologram by correcting for mouse-to-human variation in plasma protein binding and maximum tolerated exposures for both agents [AUCu/CF (correction factor)]. Predicted TGI derived from the exposure–efficacy surface response plot with increasing doses of MLN8237 administered twice a day with or without paclitaxel (G).
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Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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weekly paclitaxel, rather than using the single-agent MLN8237 recommended schedule of days 1 to 7 on a 21-day cycle (38, 39). This intermittent schedule allows for concurrent administration of MLN8237 with weekly paclitaxel, which may be necessary for amplifying the mitotic defects caused by this combination. Importantly, this intermittent MLN8237 schedule may also further reduce the risk for overlapping toxicities such as myelosuppression. Previous studies modeling hematologic toxicity by assessing the PK–absolute neutrophil count (ANC) relationship in rats using methods similar to those described by Friberg and colleagues (51) predicted MLN8237 dosed on an intermittent schedule of 3 days on (twice a day)/4 days off concomitantly with the weekly taxanes will decrease the incidence of dose limiting neutropenia compared with a 7-day continuous schedule (52). Moreover, the approximate 23-hour mean steady-state half life of the drug in patients allows for near complete MLN8237 clearance during the 4-day break, which should allow for reversion of myelosuppressive effects caused by MLN8237 (38). In our in vivo efficacy experiments we showed that MLN8237 dosed 3 days on/4 days off was synergistic when combined with weekly docetaxel. Of note, the extent of the antitumor activity for docetaxel combined with MLN8237 dosed 3 days on/4 days off was nearly identical to that of MLN8237 dosed for 21 consecutive days. Importantly the intermittent dosing schedule enabled a significant decrease in the total dose of MLN8237 by 57%. In the MDA-MB-231 model, continuous dosing of MLN8237 led to slightly greater antitumor activity relative to dosing 3 days on/4 days off when combined with paclitaxel, however, both MLN8237 schedules led to additive antitumor activity.

We developed an exposure–efficacy model to relate MLN8237 and paclitaxel exposures to antitumor activity using TGI from the efficacy studies performed with the MDA-MB-231 xenograft. This model was translationally applied in context of clinical exposures of paclitaxel and MLN8237 after interspecies corrections for plasma protein binding and maximum tolerated exposures of the 2 agents. An isobolographic representation of the response surface was used to rank order pairs of doses of the 2 agents in the combination setting. This translatable PK–efficacy prediction that the combination of MLN8237 and paclitaxel at the doses explored in the clinic will have greater antitumor activity than the single agent standard dose for paclitaxel (80 mg/m²) and MLN8237 (50 mg twice a day; Fig. 6C). In addition, the modeling predicted that 80 and 60 mg/m² paclitaxel lead to similar levels of efficacy alone or in combination with MLN8237. Several observations have been reported that paclitaxel as a single agent or in combination with other therapeutics dosed weekly at 60 mg/m² provides similar efficacy to paclitaxel at 80 mg/m², however 60 mg/m² is better tolerated (42, 43). The model also predicted that higher doses of MLN8237 with either dose of paclitaxel will lead to increased antitumor activity. Therefore, in patients, if higher doses of MLN8237 can be achieved with 60 mg/m² rather than 80 mg/m² of paclitaxel, the model predicts increased antitumor activity. In addition, an exposure related pharmacodynamic effect in tumors was demonstrated during phase 1 testing of MLN8237 (39), suggesting that the doses of MLN8237 between 30 and 50 mg twice a day are likely to result in biologically active exposures in regard to Aurora A kinase inhibition in tumors. Therefore, both the exposure–efficacy model and the phase 1 pharmacodynamic results support using higher doses of MLN8237 when combined with paclitaxel.

Viewed from a broader perspective, more generalized applications of the quantitative model-based translational pharmacology approach applied in this analysis of the MLN8237–paclitaxel combination are readily apparent. As dose-escalation studies in patient populations with advanced cancers can be resource and time-consuming, and not all dose pairs can be clinically evaluated, it is envisioned that systematic analysis of the exposure–efficacy surface for antitumor activity in preclinical xenograft models may represent a key enabler for clinical development of oncology drug combinations. These results, when coupled with clinical PK, pharmacodynamics, and safety data analyses, offer potential to objectively guide prioritization and optimization of dose finding in combination phase I trials to support qualification of the therapeutic window for optimal benefit-risk balance in anticancer drug development.

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No potential conflicts of interest were disclosed.

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Dose Schedule Optimization with Combined MLN8237 and Taxanes


Molecular Cancer Therapeutics

Translational Exposure–Efficacy Modeling to Optimize the Dose and Schedule of Taxanes Combined with the Investigational Aurora A Kinase Inhibitor MLN8237 (Alisertib)

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