Preclinical Pharmacologic Evaluation of Pralatrexate and Romidepsin Confirms Potent Synergy of the Combination in a Murine Model of Human T-cell Lymphoma

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Abstract

Purpose: T-cell lymphomas (TCL) are aggressive diseases, which carry a poor prognosis. The emergence of new drugs for TCL has created a need to survey these agents in a rapid and reproducible fashion, to prioritize combinations which should be prioritized for clinical study. Mouse models of TCL that can be used for screening novel agents and their combinations are lacking. Developments in noninvasive imaging modalities, such as surface bioluminescence (SBL) and three-dimensional ultrasound (3D-US), are challenging conventional approaches in xenograft modeling relying on caliper measurements. The recent approval of pralatrexate and romidepsin creates an obvious combination that could produce meaningful activity in TCL, which is yet to be studied in combination.

Experimental Design: High-throughput screening and multimodality imaging approach of SBL and 3D-US in a xenograft NOG mouse model of TCL were used to explore the in vitro and in vivo activity of pralatrexate and romidepsin in combination. Corresponding mass spectrometry–based pharmacokinetic and immunohistochemistry-based pharmacodynamic analyses of xenograft tumors were performed to better understand a mechanistic basis for the drug-drug interaction.

Results: In vitro, pralatrexate and romidepsin exhibited concentration-dependent synergism in combination against a panel of TCL cell lines. In a NOG murine model of TCL, the combination of pralatrexate and romidepsin exhibited enhanced efficacy compared with either drug alone across a spectrum of tumors using complementary imaging modalities, such as SBL and 3D-US.

Conclusions: Collectively, these data strongly suggest that the combination of pralatrexate and romidepsin merits clinical study in patients with TCLs. Clin Cancer Res, 21(9): 2096–106. ©2015 AACR.

Introduction

The peripheral T-cell lymphomas (PTCL) represent a heterogeneous group of lymphoma’s with a poor prognosis (1). Only 10% to 15% of patients will experience long-term survival with standard cyclophosphamide–doxorubicin–vincristine–predni-}

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).
Combination cHTS procedure

H9 and H1 cell lines were plated at optimal density into 384-well tissue culture plates (Greiner 781080) at 50 μL per well, incubated for 24 hours before drug addition (18). A total of 10 concentrations per drug in the combination were added in three plate replicates with DMSO (0.2%). Drugs were added using HP D300 Digital Dispenser. Twenty-five microliter of Cell Titer Glo (Promega) was added, and viability was measured at 24, 48, and 72 hours. The standard reference model of Bliss independence was employed. Bliss predicts the combined response C for two single compounds with effects A and B as C = A + B – A × B, where each effect is expressed as fractional inhibition between 0 and 19. The difference between Bliss expectation and observed growth inhibition induced by the combination of agents A and B at the same dose is the “Bliss excess” (18, 19).

RNA analyses

Total RNA was isolated with Trizol reagent (Invitrogen). RNA (1 μg) was reverse transcribed using a commercially available cDNA synthesis kit (Bioline). Quantitative PCR was performed on RNA that was reverse transcribed with random primers (Invitrogen) and amplified in a LightCyclerThermocycler using probes from the Roche Universal ProbeLibrary (Roche Diagnostics). Primers and probe sets are shown in Supplementary Table S1. Each experiment was repeated 3 to 4 times. Induction of each mRNA was expressed relative to the untreated control, after normalization to rRNA.

Transfection of cell lines

The H9 and HuT-78 cell lines were transfected with the previously described pGLCherryluciferase plasmid using the transfection reagent, Effectene (12).

Analysis and sorting of transfected cells by flow cytometry

Cells were harvested 48 hours after transfection, washed, and resuspended in PBS. The cells were analyzed by mCherry expression and sorted using the MoFlo Legacy cell sorter (Beckman Coulter). The sorted cells were propagated and followed by repeated sorting until a stably transfected cell line wherein >80% H9 and HuT-78 cells demonstrating fluorescent activity in vitro was achieved.

Mouse xenograft models and in vivo bioluminescence imaging

In vivo experiments were performed as follows: 5- to 7-week-old female NOD/Shi-scid/IL-2Rγnull (NOG) mice (Taconic Farms) were injected with up to 2 million mCherry luciferase-expressing H9 cells subcutaneously in the flank. In vivo BLI analysis was conducted on a cryogenically cooled IVIS system (Xenogen Corp) as previously described (12). Initial experiments explored complementary antitumor effects and toxicities of various doses and schedules of romidepsin and pralatrexate in these mice. Drugs were administered by i.p. injection as follows: romidepsin 1.2 mg/kg and 2 mg/kg on days 1, 4, 8, and 11 and 3 mg/kg weekly for 3 of 4 weeks; and pralatrexate 15 mg/kg and 30 mg/kg on days 1, 4, 8, and 11. Based on these results, a subsequent experiment was performed, where mice were inoculated in the flank with 2 million H9-mCherry luciferase cells. Animals were randomized into 4 groups of 6 animals each as follows: (i) control group treated with normal saline alone; (ii) romidepsin group in which the drug was administered at 2 mg/kg on days 1, 8, and 14; (iii) pralatrexate group in which the drug was administered at 15 mg/kg on days 1,
and schedule of romidepsin and pralatrexate was performed with the mCherry-luciferase-expressing HuT-78 cells.

Mouse xenograft model and US imaging

In a second parallel in vivo experiment, 5- to 7-week-old female NOG mice were injected with 2 million H9 cells subcutaneously in the flank. 3D-US imaging data sets were collected for each xenograft using a Vevo2100 US microimaging system (VisualSonics Inc.) designed for small animal imaging. Three-dimensional B-mode data were acquired by automated translation of the 30 MHz US transducer along the entire length of the xenograft. The resulting data sets had a 17 mm × 17 mm field of view with an in-plane pixel resolution of 33.2 × 33.2 μm and an interslice spacing of 101.6 μm, resulting in 33.2 × 33.2 × 101.6 μm voxels. For analysis of US data, images were imported into Amira 5.2 (Visage Imaging) for volumetric analysis.

Morphology and immunohistochemistry for cell proliferation and apoptosis

Twenty-one days after drug administration in the BLI intensity H9 xenograft experiment, mice were euthanized, tumors were excised, and weighed. One part was fixed in 10% neutral-buffered formalin overnight before processing, embedded in paraffin, and sections (4 μm) were stained with hematoxylin and eosin to determine the presence of tumor for immunohistochemistry. The remainder was stored at −80°C. One 5 μm section of tissue was examined by hematoxylin–eosin staining to verify that adequate tumor tissue was present, and blocks were then randomly arrayed in a 4-mm tissue array (three tumors per group in and two sections per marker). Immunohistochemical staining was performed after 5 μm sections from these arrays were used. After deparaffinization of tissue sections, endogenous peroxidase was blocked by 3% H2O2, and endogenous avidin and biotin were blocked according to the supplied protocol. The formalin-fixed, paraffin-embedded 5 μm thick sections of all tumor samples were used to identify apoptotic cells by terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) staining using tumor TACS in situ apoptosis detection kit (R & D Systems, Inc.) as detailed recently (20). Tumors were scored by the

Quantification of romidepsin and pralatrexate in mouse plasma and tumor tissue

To define and compare the pharmacokinetic profile of the two agents and their combination in blood and tumor tissue, serial blood collections were performed at 6, 18, and 24 hours after the first dose administration in 3 mice from each group in the BLI experiment. After the 24-hour blood collection, mice were euthanized and tumor tissue was harvested. Plasma was prepared by centrifugation at room temperature. Plasma and tumor tissue were stored at −80°C before analysis. Romidepsin and pralatrexate were quantified using 25 μL of plasma or approximately 20 mg of tumor tissue. Tissue was homogenized in 100 μL of saline using a 1 mL dounce homogenizer. The homogenate was rinsed with 100 μL of saline. Proteins from plasma and tumor homogenates were precipitated with 1 mL of acetonitrile/methanol (4:1). After vortexing for 60 seconds, the samples were centrifuged (14,000 × g for 10 minutes). Supernatant was evaporated with nitrogen and resolubilized with 75 μL of 10% methanol. Five microliters of each sample was injected onto a Poroshell 120 EC-C18 2.1 × 50 mm 2.7-μm column (40°C; Agilent Technologies) using an Agilent 1290 Infinity UHPLC (Agilent Technologies) with the initial conditions of 100% of 0.1% formic acid in water (0.5 mL/min) and ramped linearly to 40% of 0.1% formic acid in acetonitrile over 3 minutes. The column was cleaned with 95% of 0.1% formic acid in acetonitrile for 1 minute and then re-equilibrated to the initial conditions for 1.8 minutes (total run time: 6.3 minutes). Romidepsin and pralatrexate were detected with an Agilent6410 tandem mass spectrometer with positive electrospray ionization. Drugs were quantified using multiple reactions monitoring of the +H ion with the transition 541.2 to 424.1 (collision energy = 13 V; Fragmentor = 135 V) and 478.2 to 175.1 (collision energy = 29 V; Fragmentor = 175 V) for romidepsin and pralatrexate, respectively. Spiked plasma was used to create a standard curve, which was linear from 1 ng/mL to 2,500 ng/mL, with an limit of quantification (LOQ) and limit of detection (LOD) of 1.0 ng/mL and 0.5 ng/mL, respectively. Quantification of romidepsin and pralatrexate in both plasma and tumor tissue was calculated relative to the spiked plasma standard curve. The mass spectrometry (MS) conditions were as follows: gas temperature = 300°C; gas flow = 13 L/minute; nebulizer = 45 psi; capillary = 4500 V; desolvation gas flow = 500 L/h; cone gas flow = 50 L/h; collision energy = 30 V.

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percentage of cells positive for BrdUrd (as a measure of cell proliferation), necrosis, and for TUNEL (as a measure of apoptosis) using an Olympus BX41 microscope, with total magnification of ×400 (Olympus America Inc.).

Statistical analysis

Log-linear mixed models were used to model the tumor intensity in the BLI experiment groups or 3D tumor volume in the US group (21). The model assumed that the logarithm of the tumor intensity (or volume) is linear in time, and allowed difference intercepts and slopes for different treatment groups. A random effect of individual mice to account for the within-mouse correlation was also included in the model. Because of the small sample sizes, permutation tests were performed to determine whether the single-drug experimental groups and the control group were significantly different from the combination group on each of the days. The Kaplan–Meier survival functions were calculated with any of the single-drug treatments. C, Heatmap showing quantitative PCR analysis of RNA expression following romidepsin and pralatrexate. Response of genes with potential impact on pralatrexate sensitivity or resistance is shown. Positive controls, B1 [ABCB1 (ATP-binding cassette, sub-family G, member 2), a potential mechanism of resistance for pralatrexate (22–24), although positive controls for romidepsin gene induction were confirmed, no alterations were observed in combination with pralatrexate in the RFC, DHFR (dihydrofolate reductase), GGH (Gamma-glutamyl hydrolase), FPGS, or ABCG2 genes in any of the 3 cell lines (Fig. 1C, 24-hour

RNA analyses of folate pathway gene expression

A potential synergistic mechanism would be to increase levels of RFC (reduced folate carrier) or FPGS (folylpolyglutamate synthase), which would promote intracellular retention of pralatrexate. We examined gene expression following the combination of romidepsin and pralatrexate, and the drugs alone at 12, 24, and 48 hours. In addition, romidepsin has been shown to induce expression of drug resistance genes, including ABCG2 (ATP-binding cassette, sub-family G, member 2), a potential mechanism of resistance for pralatrexate (22–24). Although positive controls for romidepsin gene induction were confirmed, no alterations were observed in combination with pralatrexate in the RFC, DHFR (dihydrofolate reductase), GGH (Gamma-glutamyl hydrolase), FPGS, or ABCG2 genes in any of the 3 cell lines (Fig. 1C, 24-hour

**Results**

Romidepsin and pralatrexate is synergistic in TCL lines

A HTS protocol and Bliss independence were used to quantify synergy (18, 19). Two cell lines, H9 and HH, were treated with romidepsin at the IC10 or IC20 [corresponding to 2 nmol/L either alone or in combination with pralatrexate at concentrations ranging from 20 nmol/L to 1 μmol/L (IC10 to IC20)], and evaluated following 24, 48, and 72 hours of exposure. Excess over Bliss (EOB) scores are presented (Fig. 1, Tables A and B). Synergy was observed over a range of concentrations in both cell lines. EOB scores of >10 represent synergy. The full matrix of the CHTS results, highlighting synergy in H9 and HH cell lines at 48 and 72 hours, respectively, is included in Supplementary Fig. S1. There was no observed difference of the combination at 24 hours compared with any of the single-drug treatments.

**Figure 1.**

Table A and B, in vitro synergy of romidepsin and pralatrexate (PDX) in TCL cell lines. HTS procedure was used to explore the combination of romidepsin and pralatrexate in vitro in TCL cell lines H9 and HH. Romidepsin was dosed at 0.002 μmol/L and pralatrexate was dosed in a range of 0.02 to 1 μmol/L after testing 10 different concentrations of each drug. Based on the excess over bliss model, values >10 were suggestive of synergistic effect of combination versus additive. Each value reported is the average of at least 3 independent experiments run in triplicate. H9 cell line demonstrated synergy at 48 hours, whereas HH exhibited synergistic effect at 72 hours as exhibited in Tables A and B, respectively. There was no observed difference of the combination at 24 hours compared with any of the single-drug treatments. C, Heatmap showing quantitative PCR analysis of RNA expression following romidepsin and pralatrexate. Response of genes with potential impact on pralatrexate sensitivity or resistance is shown. Positive controls, B1 [ABCB1 (ATP-binding cassette, sub-family G, member 1)] or MDRI (multi-drug resistance gene), FOS (Proto-oncogene c-FOS), and p21, often found upregulated in response to romidepsin (Romi), are shown for each cell line. Genes with potential impact on pralatrexate intracellular retention or resistance: ABCG2 (ATP-binding cassette, sub-family G, member 2), GGH, FPGS, DHFR, and RFC. Scale is shown next to the figure ranging from X to X-fold induction.
Although RNA induction did not explain the synergistic effects with the histone deacetylase (HDAC) inhibitor, it also would not interfere with cell death induced independently by the two agents.

Development of a bioluminescent xenograft mouse model of human TCL

Stable cell lines of H9 and HuT-78 expressing mCherry and luciferase were generated (Supplementary Fig. S2). Various routes (subcutaneous, intravenous) and concentrations (up to 20 million cells) of H9mCherryluciferase cells were injected into 5- to 7-week-old SCID/Beige mice. No evidence of tumor engraftment was noted. We then investigated the engraftment of the mCherryluciferase-expressing H9 and HuT-78 cells in NOD/Shi-scid/IL-2Rγnull (NOG) mice. Two million cells were injected subcutaneously in the flank of 5- to 7-week-old female NOG mice. Bioluminescent imaging was initiated 48 hours after inoculation of cells and demonstrated uniform linear increase in light intensity over time at the site of tumor cell injection, suggesting engraftment in all mice. Exploratory toxicity experiments were performed (Fig. 2). When 1.2 mg/kg of romidepsin was administered on days 1, 4, 8, and 11, there was minimal effect on tumor reduction but was well tolerated (A), pralatrexate (30 mg/kg dose), demonstrated marked antitumor activity but associated with high toxicity (B), romidepsin (2 mg/kg dose), exhibited modest activity but associated with toxicity (C), pralatrexate (15 mg/kg dose), revealed minimal antitumor activity but was well tolerated (D). All drugs were administered on days 1, 4, 8, and 11.

Figure 2.

Determination of MTD for romidepsin and pralatrexate and associated antitumor activity. In vivo surface bioluminescence acquired images of H9 human TCL xenograft tumors in mice with corresponding mean BLI response to i.p. treatment with: romidepsin (1.2 mg/kg dose), showed minimal effect on tumor reduction but was well tolerated (A), pralatrexate (30 mg/kg dose), demonstrated marked antitumor activity but associated with high toxicity (B), romidepsin (2 mg/kg dose), exhibited modest activity but associated with toxicity (C), pralatrexate (15 mg/kg dose), revealed minimal antitumor activity but was well tolerated (D). All drugs were administered on days 1, 4, 8, and 11.
Figure 3.
Analysis of bioluminescent intensity (BLI) of H9 xenograft tumors to romidepsin and pralatrexate. In vivo surface BLI images were acquired \((n = 6\) in each group\) to determine tumor response of H9 xenograft to i.p. treatment with romidepsin and pralatrexate. A control group was administered normal saline on days 1, 4, 8, 11, and 14. Two single-agent cohorts received treatment with romidepsin (2 mg/kg dose) on days 1, 8, and 14, and pralatrexate (15 mg/kg dose) on days 1, 4, 8, and 11. A combination cohort received simultaneous treatment with romidepsin and pralatrexate at the same dose and schedule as single-agent groups. All cohorts were imaged on days 1, 4, 8, 11, 14, 18, and 21 using IVIS Spectrum Imaging System before administration of drugs. Tumor location is highlighted by BLI (A).

In vivo surface BLI images were acquired \((n = 6\) in each group) 7 days after completion of one cycle of treatment (control, romidepsin, pralatrexate, and romidepsin plus pralatrexate combination) was analyzed for necrosis, apoptosis, and cell proliferation. Apoptotic cells were identified by TUNEL staining. Corresponding photomicrographs of apoptosis and necrosis at different magnifications across the treatment groups are displayed in C, and histogram demonstrating greater apoptosis in the combination group in contrast with the other treatment groups is displayed in D. Cell proliferation was measured through BrdUrd staining. The level of proliferation inhibited with romidepsin plus pralatrexate combination was higher in contrast with the other treatment groups (D).

4, 8, and 11; and (iv) a combination group of both drugs administered at the same dose and schedule as above. After 21 days from the start of treatment, the combination group treated demonstrated a statistically significant reduction in the BLI intensity compared with the romidepsin alone \((P < 0.05)\), pralatrexate alone \((P < 0.05)\), and control \((P < 0.05)\) groups (Fig. 3A and B). Complete remissions (CR) were observed by day 18 only in the combination cohort where all 6 mice experienced CR. Neither significant weight loss nor death was observed in any of the cohorts (day 21) when all mice were euthanized to harvest tumor.

Pharmacodynamic analysis of romidepsin and pralatrexate in xenograft tumors by immunohistochemistry
Given the statistically significant tumor growth inhibition by the combination treatment (Fig. 3), we analyzed its effect on necrosis and apoptosis by TUNEL staining. Quantification of necrosis and TUNEL-positive apoptotic cells was increased in the combination mouse (80% and 75%, respectively) compared with control (40% and 15%, respectively), romidepsin alone (60% and 25%, respectively), and pralatrexate alone (60% and 38%, respectively) shown in Fig. 3C and D. We investigated cell proliferation by assessing the uptake of BrdUrd by tumor cells. A representative
The histogram of BrdUrd staining from a mouse xenograft tumor from each treatment group is depicted in Fig. 3D. The fraction of actively proliferating cells was lower in the combination mouse (20%) compared with control (60%), romidepsin alone (40%), and pralatrexate alone (40%) treated mice in agreement with the gross decrease in tumor burden noted in the combination group compared with control and other treatment groups. These effects were confirmed in the HuT-78 xenograft tumors (Fig. 4). These data validate the utility of the BLI xenograft murine model of human TCL and support our original hypothesis.

Therapeutic effects in high volume tumors by 3D-US

The in vivo efficacy of the combination observed in the BLI experiment was investigated in another xenograft experiment, using higher tumor starting volumes using 3D-US. Two million mCherryLuciferase-expressing H9 cells were injected subcutaneously in the flank of 5- to 7-week-old female NOG mice. 3D-US imaging datasets were collected for each xenograft starting 4 days after inoculation of tumor cells twice a week. Once the diameter of the xenograft tumors reached 3 to 5 mm corresponding to 70 to 90 mm³ tumor volume on US imaging, mice were randomized to one of the 4 treatment groups (n = 9 in each cohort), including (i) a control group treated with normal saline alone; (ii) a romidepsin group administered 2 mg/kg dose on days 1, 8, and 14; (iii) a pralatrexate group in which administered 15 mg/kg dose on days 1, 4, 8, and 11; and the (iv) combination group received drugs at the same dose and schedule as single agents. All cohorts were imaged on days 1, 4, 8, 11, and 14 using IVIS Spectrum Imaging System, and any respective drug dose was given after completing the imaging process. Tumor location is highlighted by the BLI (A). With the images collected, we generated a mean BLI curve of human TCL xenografts. The combination arm demonstrated a greater decline in their BLI from day 4 onwards after the start of treatment in comparison with the other cohorts (B). P value was calculated to show the results that were statistically significant at several time points in comparison with the other treatment groups (C).

Survival analysis of mice in the US group

Treatment was terminated after 1 cycle of therapy (3 weeks) across all treatment groups. Mice were subsequently followed for survival analysis until they had to be euthanized for disease progression. Kaplan–Meier survival functions were calculated for each group and demonstrated that survival distributions for the
four treatment groups were significantly different in general (Fig. 5C). The log-rank test for comparison of median survival time among the four treatment groups (control, romidepsin alone, pralatrexate alone, and romidepsin plus pralatrexate) exhibited marked increase in survival for the combination group versus all other cohorts (Fig. 5D).

Preclinical pharmacokinetic analysis of romidepsin and pralatrexate in mouse plasma and tissues by high affinity liquid chromatography and tandem mass spectrometry

A high affinity liquid chromatography and tandem mass spectrometry (HPLC-MS/MS) method was developed for the simultaneous quantification of romidepsin and pralatrexate, in mice plasma and tissues. The method is selective and highly sensitive with a detection limit of 0.5 ng/mL in plasma. The concentration of romidepsin and pralatrexate in mice plasma samples at 6, 18, and 24 hours after the first i.p. dose administration in 3 mice from each group in the BLI H9 xenograft experiment was determined by this method. Intratumoral drug concentrations were quantified by this method 24 hours after the first dose. The mean plasma concentration of romidepsin versus time curves and pralatrexate versus time curves is presented in Fig. 6A and B respectively. Relatively high concentrations were achieved at 6 hours after intraperitoneal injection for both drugs. Plasma levels then declined and were undetectable in most samples by 24 hours. Mean concentrations of romidepsin and pralatrexate in tissue

Figure 5.

In vivo US images of H9 human TCL xenograft tumors in mice and their response to romidepsin and pralatrexate. In vivo US images were acquired (n = 9 in each group) to determine tumor response of H9 xenografts to i.p. treatment with romidepsin and pralatrexate. On days 1, 8, and 14, mice were treated i.p. with normal saline control (A–D), and single-agent romidepsin (2 mg/kg; E–H). Single-agent pralatrexate (15 mg/kg) was administered on days 1, 4, 8, and 11 (I–L). Combination of romidepsin and pralatrexate was administered at the same dose and schedule as single agents (M–P). All cohorts were imaged on days 1, 8, 14, and 21 using VisualSonics Vevo 2100 Imaging System, and any respective drug dose was given after completing the imaging process. Tumor location is outlined with blue lines (A). The US system acquired sequential images for each tumor, resulting in a 3D representation of the tumors. The 3D images were used to calculate the estimated tumor volume and generate a tumor response curve. As shown in the curve, the combination cohort demonstrated a greater decline in the estimated tumor volumes after day 4 of treatment in comparison with the other treatment groups. We followed the mice for survival, and generated Kaplan–Meier survival plot curve of the 4 cohorts in the experiment. Mice receiving the combination of romidepsin plus pralatrexate showed longer survival compared with other treatment groups (C). P value and 95% confidence interval analysis were performed to determine that the survival curve was statistically significant in contrast with other treatment groups through (D).
The TCLs represent a heterogeneous group of diseases with few well-described in vivo models of TCL exist (25, 26). One objective of these studies was to develop a noninvasive in vivo model and generate a “proof-of-concept” study demonstrating both efficacy of the combination in vivo, and to define pharmacologic features. We began with pralatrexate and romidepsin as these were the first drugs approved for relapsed/refractory PTCL. Most of the available published models have not been employed for evaluating experimental drugs and their combinations, or for determining their pharmacokinetic and pharmacodynamic profile for translation to early phase clinical trials. Few of the current in vivo models use noninvasive imaging techniques and are not optimized to measure tumor-specific variables or plasma: tumor drug ratios. Romidepsin and pralatrexate are the first two agents approved for the treatment of relapsed/refractory PTCL. These agents, in addition to a host of others now emerging, raise the prospect that new small molecules with lineage-specific activity could be combined to develop novel treatment platforms for TCL. Making an assumption that every doublet will be complementary in combination is flawed. To prioritize the potential clinical study of these combinations, we need better preclinical tools to better explore the merits of novel combinations.

Initial in vitro studies based on HTS and a Bliss independence model demonstrated synergy of romidepsin and pralatrexate
across TCL lines. Initial attempts to engraft the H9 TCL cell line in SCID/Beige mice were unsuccessful. Subsequently, NOG mice permitted 100% engraftment of both H9 and HuT-78, suggesting that it may be a superior xenotransplantation recipient. This strain of mouse is being increasingly used as a xenotransplantation tool in a variety of hematologic and solid tumor malignancies (27, 28). One of the limitations of this murine model is the inability to assess the influence of the tumor microenvironment on tumor progression and growth due to the immunological deficits of the mice. Another drawback may include the inability to examine the influence of specific genes in tumor progression and response to therapy. Subsequent experiments concentrating on identifying the dose and schedule of romidepsin and pralatrexate for combination studies demonstrated that the MTD of these agents varied with the strain of mice being used. Previous work from our laboratory demonstrated that in SCID/Beige mice the MTD of pralatrexate was 60 mg/kg on days 1, 4, 8, and 11, whereas in this NOG mouse model, even the 30 mg/kg dose twice weekly proved toxic albeit effective (16). At the doses used for combination studies, neither of the drugs exhibited significant antitumor activity as single agents. However, when they were used in combination, a statistically significant marked reduction in tumor burden leading to increased survival was observed. These findings underscore the synergistic activity of the combination regimen in vivo. The intensity of the BLI signal in murine models using luciferase-transfected cells can be reliably used as a surrogate for tumor volume in tracking growth and regression in response to drugs and their combinations (12). One criticism of these strategies is that these models may recapitulate disease states with a very low tumor burden, which might select for better therapeutically active than actually exists under clinical scenarios. To address this consideration, we adopted a strategy in which we investigated xenograft tumor responses across a greater spectrum of volumes in a parallel experiment with an additional imaging modality like 3D-US. We observed that despite the differences in techniques and the tumor burden at the start of treatment, the results were strikingly similar. Even in these more challenging treatment scenarios, the romidepsin and pralatrexate combination emerged as superior in effecting tumor reduction compared with control and single agents. Our data suggest that surface BLI imaging may be useful in tracking early tumor engraftment and response in drug intervention studies. A complementary imaging modality like US may be more beneficial in monitoring tumor burden in advanced stages of disease. Hence, the integration of these two monitoring approaches could provide a more comprehensive evaluation of tumor response in vivo models.

Clear explanations for the effectiveness of good combinations in clinical oncology are often elusive. In the case of romidepsin and pralatrexate, several possibilities exist. Pralatrexate preferentially enters cancer cells via the RFC, has increased intracellular retention due to polyglutamylation, and impairs DNA synthesis via inhibition of DHFR (29). We investigated whether romidepsin would increase the levels of proteins that promote intracellular retention (or conversely, reduce the levels), but saw no impact at the RNA level. However, HDAC inhibitors, including romidepsin, have been shown to induce DNA damage through both acetylation-mediated events and inhibition of DNA repair. Thus, romidepsin might block recovery of DNA synthesis following its inhibition by pralatrexate. Confirmation of the combination activity in the clinic will provide the impetus to understanding the mechanism in detail. Pharmacokinetic and pharmacodynamic studies were performed on both the individual mouse, tumor, and tumor cells. HPLC-MS/MS-based quantification of drug concentrations in mouse plasma and tumor tissue highlighted that the kinetics of the two agents were not antagonistic and did not adversely affect the activity of the other drug. Both agents were retained in the tumor tissue at higher levels even when their corresponding plasma levels were undetectable, which provides a robust rationale for the rapid activity seen by this combination, and the selective accumulation of these drugs in the target tumor. Immunohistochemical studies of xenograft tumor tissue confirmed a greater inhibition of cell proliferation and apoptosis with the combination in comparison with single agents and control groups.

In summary, we have developed a BLI mouse model of human TCL to rapidly screen promising agents and their combinations in a noninvasive longitudinal fashion. Our preclinical murine data demonstrate that the combination of romidepsin and pralatrexate was an effective strategy in tumor reduction in comparison with the single agents. The multimodality approach we adopted to evaluate this strategy was able to screen for the activity of this regimen against a wide spectrum of tumor volumes and may offer researchers greater confidence in preclinical drug development. These results were supported by the correlative pharmacokinetic and pharmacodynamic data. An early phase I/IIA clinical trial to investigate this combination started.

Disclosure of Potential Conflicts of Interest
S.E. Bates reports receiving commercial research grants from Celgene. O.A. O’Connor reports receiving commercial research grants from Celgene and Spectrum. No potential conflicts of interest were disclosed by the other authors.

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References


Preclinical Pharmacologic Evaluation of Pralatrexate and Romidepsin Confirms Potent Synergy of the Combination in a Murine Model of Human T-cell Lymphoma


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