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INTRODUCTION

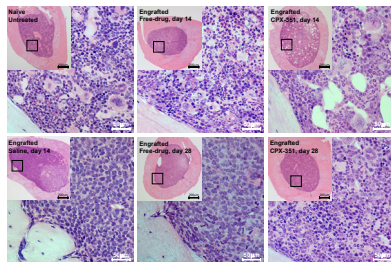
The combination of cytarabine (Cyt) and daunorubicin (Daun) is a widely used chemotherapy regimen against acute myeloid leukemia (AML). These agents exhibit drug ratio-dependent cytotoxicity *in vitro* and a liposomal co-formulation of Cyt:Daun (CPX-351) was developed to maintain the synergistic 5:1 molar drug ratio *in vivo*. This formulation demonstrated superior therapeutic activity relative to either the saline-based drug cocktail or the individual liposomal agents, leading to enhanced efficacy in xenograft and murine leukemia models (P. Tardi *et al.*, Leukemia Research 33:129-139, 2009). In Phase I clinical studies, CPX-351 also exhibited promising anti-leukemic activity in patients with advanced disease (E. Feldman *et al.*, Blood ASH Annual Meeting Abstracts 2008; 112: 2984), including patients with refractory and multiple relapsed AML. Currently two randomized Phase II clinical studies are comparing CPX-351 with standard of care in newly diagnosed elderly AML patients and 1st-relapse AML patients. In these trials, CPX-351 is administered on a day(1,3,5) schedule for induction treatment. In consolidation treatments, the schedule is typically truncated to day(1,3) based on the conventions of using free Cyt and Daun combinations. Given the extremely prolonged circulation half-life of CPX-351 compared to free Cyt and Daun, we investigated the relationship between dosing schedule and anti-leukemic activity as well as toxicity to normal hematopoietic cells for consolidation therapy in a human leukemia xenograft model.

KEY OBSERVATIONS

A leukemia xenograft model was generated in Rag2-M mice with CCRF-CEM leukemia cells. Tumor engraftment in the bone marrow and superior anti-leukemic activity of CPX-351 relative to the free drug cocktail were demonstrated. The addition of consolidation treatments to CPX-351 therapy further amplified its therapeutic efficacy, attaining the only long-term cures among all tested treatments. With the consolidation schedules evaluated here the most favorable efficacy observed was obtained using intermediate day(1,3) and day(1,5) dosing frequencies. Improved efficacy with the latter dosing schedules correlated to increased bone marrow drug levels. Furthermore, recovery time of normal bone marrow cells following these consolidation schedules was similar to that following a lower dosing frequency.

RESULTS

1 Superior anti-leukemic activity of CPX-351 treatment in the CCRF-CEM human leukemia xenograft model.

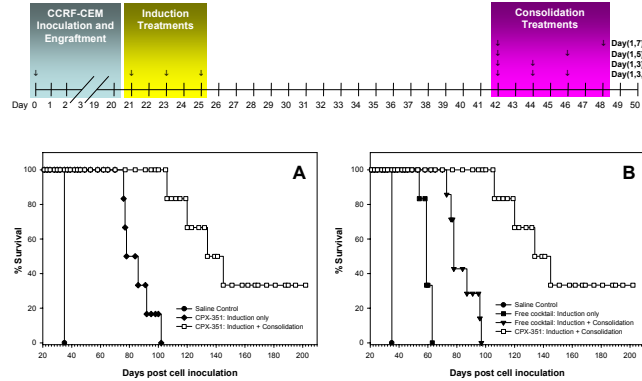


Rag2-M mice were implanted with human acute T-cell lymphoblastic leukemia CCRF-CEM cells. Twenty-one days after implantation, engrafted mice were treated with either saline, a cocktail of free Cyt and Daun, or CPX-351. On days 14 and 28 after treatments (35 and 49 days post CCRF-CEM implantation), histology cross sections of femurs were assessed for growth of leukemic and normal bone marrow cells. Similar to clinical acute leukemia progression, saline-treated tumor supplanted normal bone marrow cells on day 14, whereas tumors were ablated and normal bone marrow cells repopulated with free cocktail and CPX-351 treatments. On day 28, free cocktail-treated mice relapsed while CPX-351-treated mice remained tumor-free.

Methods: 1x10⁷ CCRF-CEM human acute T-cell lymphoblastic leukemia cells were inoculated IV into the lateral tail vein of female Rag2-M mice. On days 21, 23, and 25 after cell inoculation, saline, free cocktail at MTD (Cyt:Daun 300:4.5 mg/kg), or CPX-351 at MTD (Cyt:Daun 10:4.4 mg/kg) were administered by IV injection. At indicated times after treatments, femurs were resin-embedded, cross-sectioned and H&E-stained for tumor assessment.

2 Consolidation therapy with CPX-351 markedly improves anti-leukemic activity.

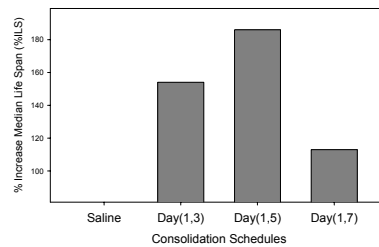
Timeline of CCRF-CEM xenograft model and treatment schedules



(A) CCRF-CEM tumor-engrafted mice were treated with either saline, a CPX-351 induction course alone, or a CPX-351 induction course followed by a CPX-351 consolidation course. Induction and consolidation courses were administered using a day(1,3,5) dosing schedule. While improved survival was observed with CPX-351 induction treatment, long-term cure was achieved when CPX-351 consolidation therapy followed. (B) CCRF-CEM tumor-engrafted mice were treated with either saline, a free cocktail induction course alone, or a free cocktail induction course followed by a free cocktail consolidation course. A free cocktail consolidation subsequent to a free cocktail induction improved survival compared to free cocktail induction alone. However, long-term cure achieved by CPX-351 consolidation was not observed in free cocktail treatments. Each symbol represents an observation day in the study. N=6 to 8 mice per treatment group.

Methods: 1x10⁷ CCRF-CEM cells were inoculated IV into the lateral tail vein of female Rag2-M mice. For induction treatment, mice were administered IV with saline, free cocktail (Cyt:Daun 300.3 mg/kg), or CPX-351 (Cyt:Daun 10:4.4 mg/kg) on days 21, 23, and 25 after CCRF-CEM inoculation. For consolidation treatment, an additional course of drugs at the same doses were administered on days 42, 44, and 46 (a day(1,3,5) schedule). Mice were monitored daily for their general well being and indications of excessive tumor burden. Moribund animals were humanely euthanized and necropsied.

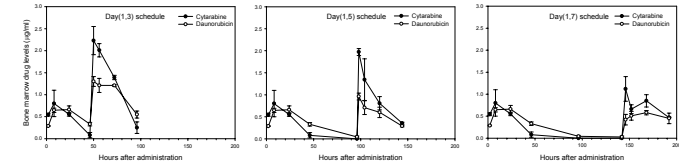
3 Intermediate-frequency consolidation schedules improved efficacy of CPX-351.



All CCRF-CEM-engrafted mice received a CPX-351 induction at MTD or saline treatment on days 21, 23 and 25 post cell inoculation. The following consolidation treatments with CPX-351 at MTD were administered on a day(1,3), (1,5), or (1,7) schedule starting 42 days post CCRF-CEM implant. Percent increase of lifespan (%ILS) was calculated by comparing median lifespan of CPX-351-treated mice to that of saline-treated mice. N=6 mice per treatment group.

Methods: 1x10⁷ CCRF-CEM cells were inoculated IV into the lateral tail vein of female Rag2-M mice. Induction and consolidation dose schematic for CPX-351 were as detailed in Figure 2. Mice were monitored daily for their general well being and indications of excessive tumor burden. Moribund animals were humanely euthanized and necropsies performed.

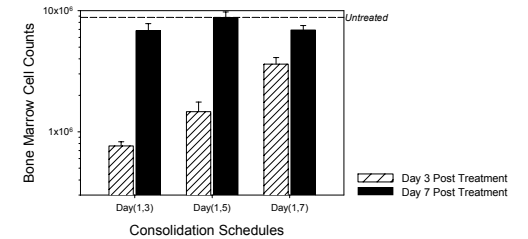
4 Bone marrow drug levels increase following day(1,3) and day(1,5) but not day(1,7) consolidation treatment schedules.



CCRF-CEM-engrafted mice were treated at the MTD of CPX-351 via an induction schedule starting on day 21 followed by either a day(1,3), (1,5) or (1,7) consolidation treatment schedule. Bone marrow levels of Cyt and Daun were determined at indicated times throughout the dosing schedule starting on day 42. The second doses of day(1,3) and day(1,5), but not the day(1,7), consolidation schedules increased bone marrow drug levels. Note that the drug elimination kinetics for mice are roughly 2-fold faster than in humans so that the day(1,5) schedule most closely resembles a day(1,3) schedule in humans.

Methods: Freshly harvested bone marrow samples were extracted with methanol or acidified methanol; gemcitabine and idarubicin were used as internal standards. Cyt was quantified using ion-exchange HPLC on a Phenomenex SCX column. Ammonium formate (10 mM, pH 3.0) was used as the mobile phase running; 1.5 mL/min and UV detection at 278 nm. Daunorubicin was quantified using reverse phase HPLC on a Phenomenex Luna C18(2) column, with 67.5:32.5 (v/v) 25 mM ammonium acetate (pH 4.8) : acetonitrile as mobile phase; 1 mL/min and fluorescence detection (Ex = 480 nm, Em = 560 nm). N=3 mice per time point. For each mouse the marrow from both femurs were pooled prior to extraction. On average each femur had a bone marrow volume of 8.9 µL/mur. Femur volumes were estimated from microscopic measurements of internal femur dimensions from decalcified and H&E cross sections. The cross sectional area was determined using the formula for an ellipse where A = 7 (a) (b) in which a = 1/2 major dimension and b = 1/2 minor dimension.

5 Bone marrow cellularity recovers at similar times following either a day(1,3), (1,5), or (1,7) consolidation treatment in BDF-1 mice.



Tumor-free BDF-1 mice were administered an induction course of CPX-351 at MTD on days 1, 3, and 5 of the experiment. Starting on day 22, consolidation CPX-351 treatments at MTD were administered at various schedules. Bone marrow cellularity was analyzed by flow cytometry on days 3 and 7 following the last consolidation treatment. Day 3 was the nadir of bone marrow cellularity in most treatments. On day 7, bone marrow recovery approached cellularity of untreated bone marrow regardless of consolidation schedules. N=3 mice per time point per group.

Methods: Femoral bone marrow from BDF-1 mice were isolated at the indicated days following the last dose of the consolidation treatments. Nucleated cells isolated from the femoral bone marrows were quantified by flow cytometry by side scatter and electronic volume analyses.

CONCLUSIONS

- CPX-351 is superior to Cyt:Daun free drug cocktail in ablating leukemic bone marrow cells in the CCRF-CEM xenograft model.
- Median survival was improved by consolidation treatments in both CPX-351 and free drug cocktail therapies but long-term cure was achieved *only* in CPX-351 induction + consolidation therapy.
- Day(1,3) and day (1,5) consolidation schedules are superior to day(1,7) in survival improvement and bone marrow drug accumulation.
- Recovery times of normal bone marrow cellularity following consolidation treatments were comparable among different schedules.
- Intermediate dosing frequencies improved the therapeutic index in a consolidation treatment setting.