

KPC34: A Co-Drug that Combines a DNA Damaging Agent with a Targeted Therapy for the Treatment of AML

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Abstract #4059

Introduction

AML occurs in the elderly with ~18,000 new US cases in 2014. Mortality is an estimated 10,050 US deaths in 2014. Elderly patient prognosis is a dismal 6.6%, 5 year survival rate. AML treatment involves induction chemotherapy with Ara-C followed by consolidation therapy or stem cell transplant. The major obstacle to long-term disease-free survival is relapse after treatment due to chemoresistance. Chemoresistance occurs by several mechanisms including reduced activation of Ara-C via down-regulated deoxycytidine kinase (dCK) levels or decreased uptake via lowered Equilibrative Nucleoside Transporter 1 (ENT-1) levels. The low expression of both dCK and ENT-1 correlates with a poor prognosis in patients with acute leukemias. Recurrent disease is more difficult to treat successfully. Elderly patients often have trouble tolerating repeated chemotherapy treatments. Activation of NF- κ B by protein kinase C (PKC) promotes proliferation and inhibits apoptosis in AML. Increased expression of PKC isoforms has been detected in AML. The activity of these isoforms promotes leukemic proliferation and survival by activation of ERK and BCL-2. KPC34 is a novel synthetic phospholipid/deoxycytidine analog conjugate. KPC34's physiochemical properties result in improved pharmacokinetics. It overcomes chemoresistance to Ara-C by bypassing ENT-1 uptake and dCK activation. KPC34 is administered orally. It crosses the blood-brain barrier, targeting CNS-infiltrating leukemias. Finally, KPC34's lipid moiety inhibits PKC activity. KPC34 is highly cytotoxic to a variety of leukemic cell lines *in vitro*, with IC₅₀s in the nM range. The mean IC₅₀ was 45.92 nM (range from 7.39-175.6 nM). **The study's hypothesis is that KPC34 is active against AML *in vivo*.**

Methods

To assess the activity of KPC34 *in vivo*, we injected NSG mice with OCI-AML-3 cells expressing luciferase. Mice were imaged using an IVIS100 system. Mice were injected with 150 mg/kg D-luciferin, and imaged for 2 min. Chemotherapy was initiated upon detection of signal. Mice were randomly assigned to treatment groups and treated as

described. Following engraftment, mice were treated with KPC34, Ara-C, or water on days 1-4. For combination studies, mice were treated with KPC34 or Ara-C once a day for four days and doxorubicin at 3 mg/kg once a day for three days.

Results

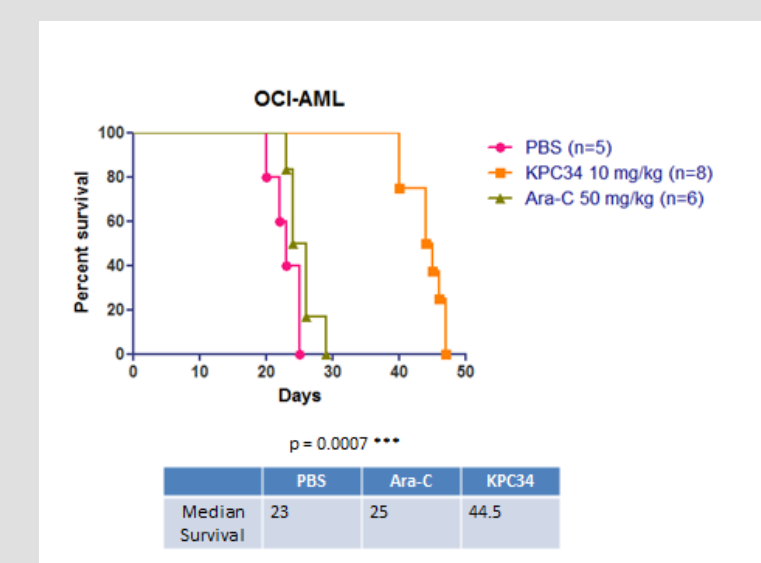


Figure 1. KPC34 Is Active *In Vivo* Against the OCI-AML Murine Model of AML. A significant prolongation of survival was observed in the KPC34-treated mice (median survival 44.5 days vs. 25 days for Ara-C-treated mice, p=0.0033, by log-rank test).

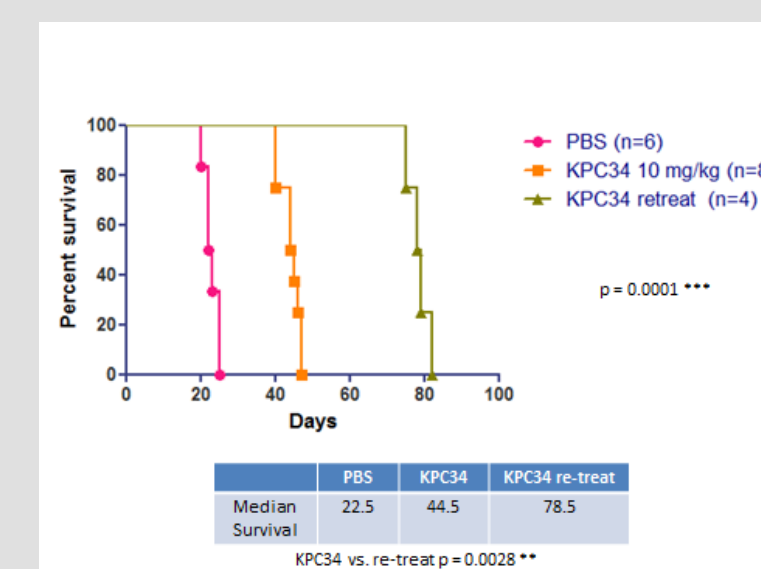


Figure 2. Retreatment of OCI-AML Mice with KPC34 after Relapse. Retreatment of the mice with additional KPC34 resulted in a median survival time of 78.5 days (p=0.0028, single KPC34 course vs. 2 doses/week indefinitely, p=0.0001, PBS control vs. repeated doses).

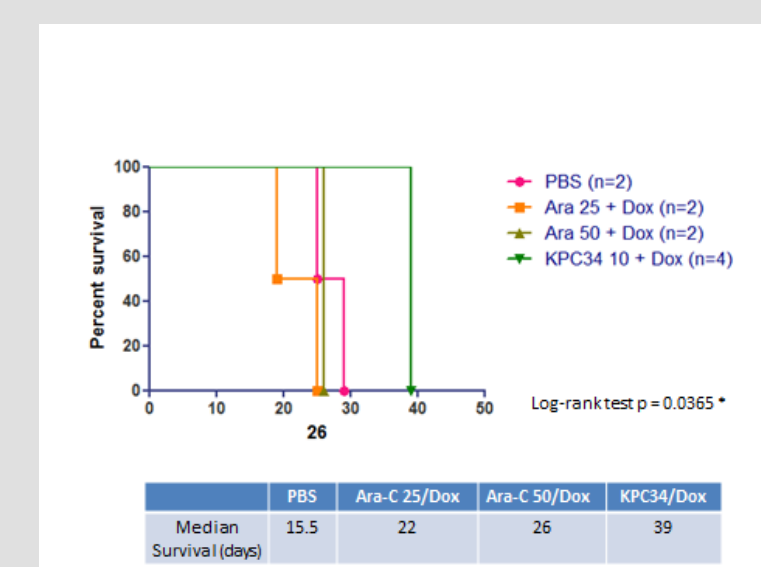


Figure 3. KPC34 + Dox Is More Effective Than Ara-C + Dox in Treatment of Naïve AML *In Vivo*. KPC34 (10 mg/kg) + doxorubicin is superior to the treatment of mice with Ara-C + doxorubicin (39 days vs. 22 and 26 days following 25 and 50 mg/kg Ara-C + doxorubicin, p=0.0365).

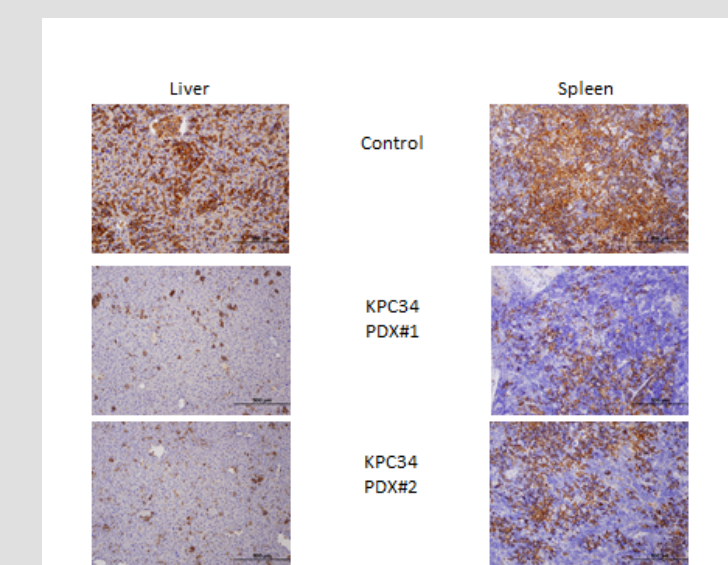


Figure 4. KPC34 Reduces Tumor Burden in a PDX Model. KPC34 (10 mg/kg) showed a reduction in leukemic burden in the liver and spleen using a human CD33 stain. The AML cells were from a 53 y.o. male with normal cytogenetics, FLT3-IDT+, FAB M5.

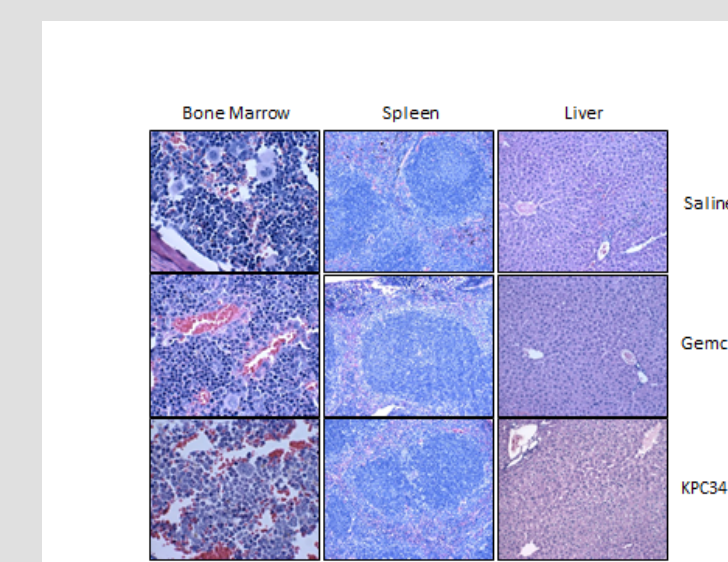


Figure 5. KPC34 Is Well Tolerated *In Vivo*. C57/Bl6 mice were treated with saline, gemcitabine (7.5 mg/kg), or KPC34 (17 mg/kg). The mice were sacrificed and evaluated for toxicity in the bone marrow, spleen, and liver.

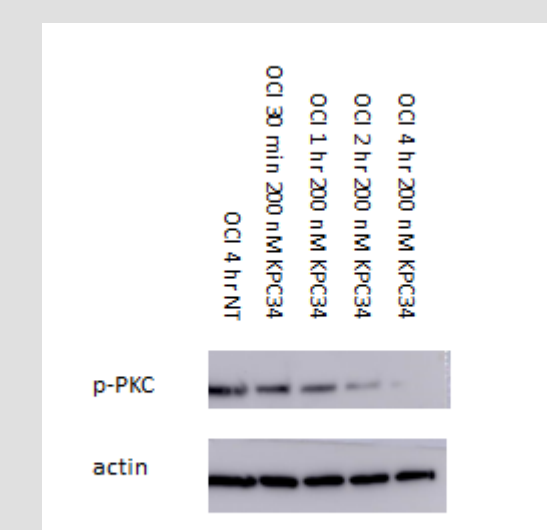


Figure 6. KPC34 Inhibits PKC Signaling in OCI-AML Cells *In Vitro*. OCI-AML cells were treated with 200 nM KPC34 for up to 4 hours. The cells were harvested, lysed, and the proteins separated by gel electrophoresis. The proteins were probed with a phospho-PKC specific antibody.

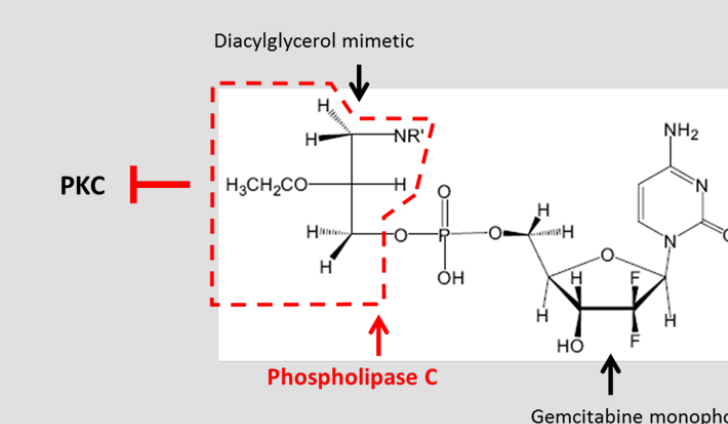
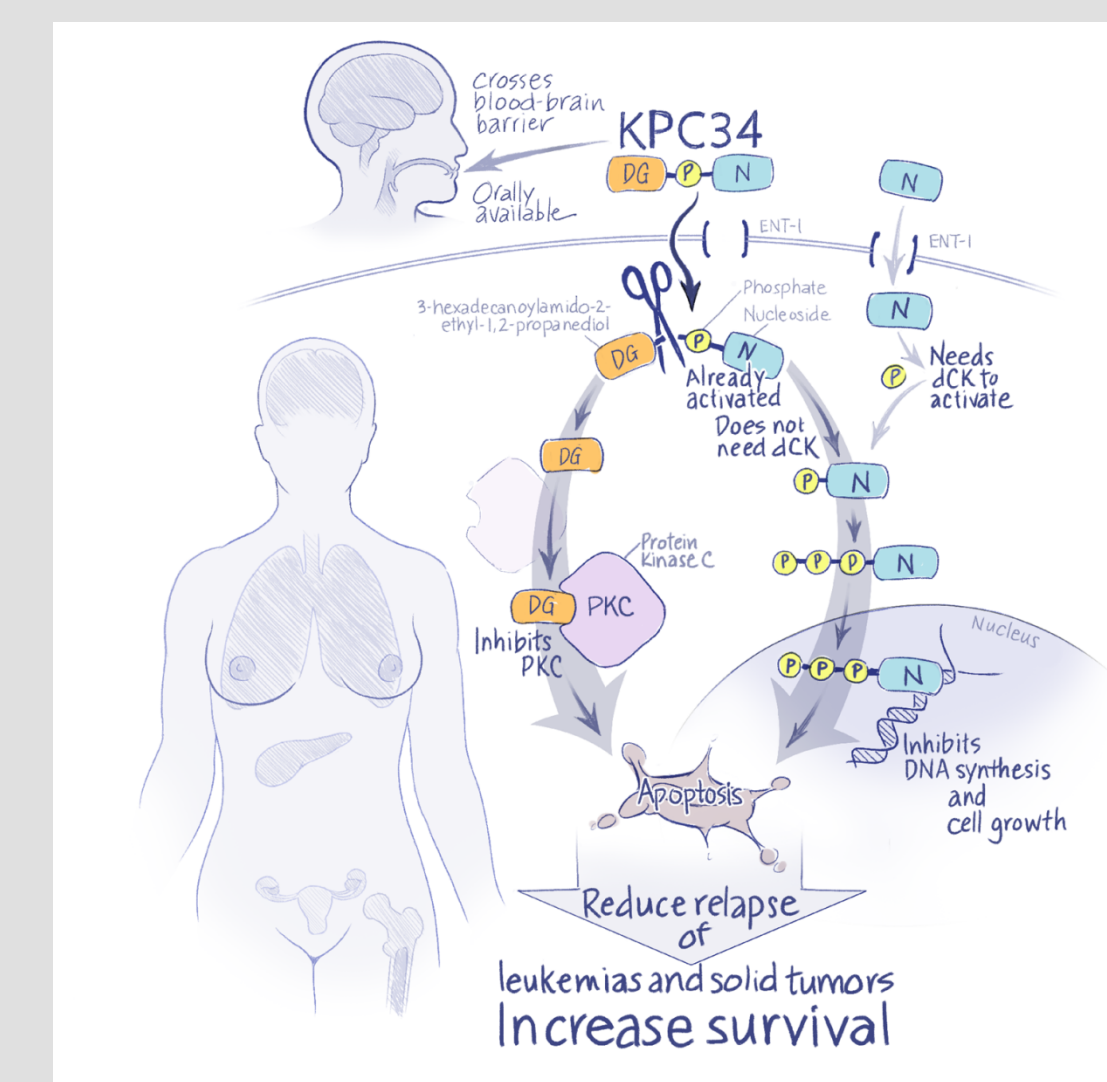


Figure 7. KPC34 Structure and Proposed Mechanism.



Conclusions

1. KPC34 is superior to Ara-C against AML *in vivo*
2. Additional KPC34 treatments increase survival time
3. It is more effective than Ara-C combined with doxorubicin
4. The co-drug reduces tumor burden in a PDX model of AML
5. It is well tolerated by mice
6. There is a benefit with p.o. vs. i.p. KPC34 delivery (data submitted for publication)

KPC34 is a novel drug effective against multiple models of naïve and chemoresistant leukemia. It has two distinct mechanisms of action: 1) It can inhibit PKC, and 2) It causes DNA termination. This co-drug has the potential to replace Ara-C-based leukemia treatment regimens as either a single agent or as a combination in naïve acute leukemia. It also could be used as a single agent in relapsed/refractory disease, and it could serve as a CNS prophylactic agent.