Altered Lipid and Mitochondrial Metabolism are Viable Targets in Acute Leukemia

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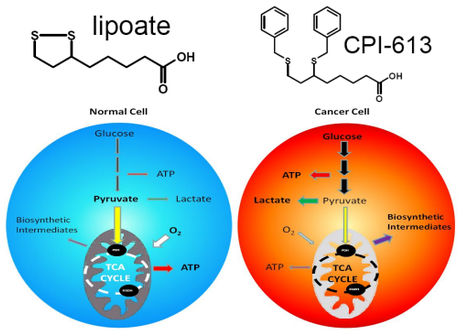
Introduction

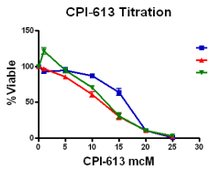
Acute Myeloid Leukemia (AML) is an aggressive myeloid malignancy that leads to marrow failure and death. This disease affects approximately 12,000 people per year in the United States, causing 9,000 deaths. Despite decades of research, therapy remains essentially unchanged and outcomes are poor. The prognosis for elderly patients, often defined as age>60, is poor with fewer than 10% of patients alive at 5 years. This high mortality is driven by the combination of biological and clinical differences in older patients. These differences include an increased incidence of co-morbidities and increased proportion of patients with multidrug resistant disease. This is compounded by the fact that AML is a disease of the elderly with the median age of onset of 72 years old. There is clearly a desperate need for additional active therapies for AML with acceptable toxicity profiles. Nearly all tumor cells will preferentially utilize anaerobic glycolysis, a phenomenon known as the Warburg effect. This altered mitochondrial metabolism represents a possible therapeutic target. CPI-613, developed by Cornerstone Pharmaceuticals, is a first in class agent that targets at least one mitochondrial enzyme involved in this aberrant metabolism, including the pyruvate dehydrogenase complex. Its activity against AML and other hematologic malignancies is being investigated in the laboratory and in a phase I clinical trial.

CPI-613 is a Novel Lipoate Derivative



Lipoate is a necessary co-factor of both pyruvate dehydrogenase (PDH) and alpha-ketoglutarate dehydrogenase (KGDH) complexes. These 2 enzymes play key roles in mitochondrial carbon metabolism as their activity is required for the interconversion of both pyruvate and alpha-ketoglutarate to key biosynthetic intermediates. CPI-613 is a non-redox active lipoate derivative that inhibits mitochondrial energy metabolism.

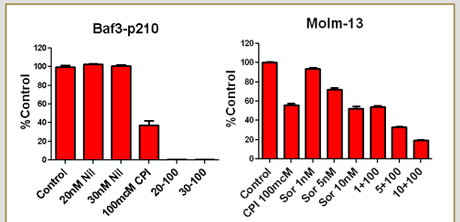
CPI-613 is Active Against Multiple Leukemia Cell Lines



CPI-613 has activity against acute leukemia. Cells were subjected to 72 hour exposures to CPI-613 in RPMI with 0.5% FBS prior to viability assessment. Graph represents combined results of 3 separate experiments carried out in triplicate.

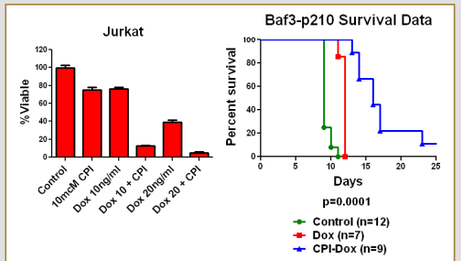
Cell Line:	IC_{50}
HL60	16.36 µM (15.58 to 17.17)
Jurkat	13.40 µM (10.98 to 16.36)
K562	12.19 µM (10.81 to 13.76)
95% confidence intervals are shown in the parenthesis.	

CPI-613 is Synergistic with Tyrosine Kinase inhibitors



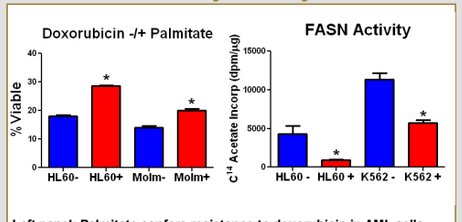
CPI-613 is synergistic with nilotinib against BCR-ABL expressing cells and with sorafenib against Flt3 ITD expressing cells. Cells were incubated with the indicated drugs in 10% FBS media for 72 hours prior to viability assessment. Combinatorial index (CI) values for Baf3-p210 (Nilotinib 30 nM + CPI) was 0.059 (+/-0.002) and for Molm-13 (Sorafenib 10nM + CPI) was 0.581 (+/-0.052).

CPI-613 is Synergistic with Doxorubicin *In Vitro* and *In Vivo*



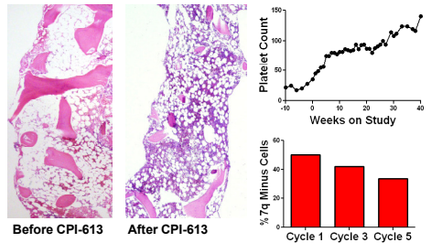
Left panel: CPI-613 is synergistic with doxorubicin *in vitro*. Cells were subjected to the indicated treatment for 72 hours prior to viability assessment. CI value for Jurkat (Dox 20 + CPI) was 0.657. Right Panel: CPI-613 is synergistic with doxorubicin *in vivo*. Balb/c mice were injected with Baf3-p210 cells and on day 3 treated with saline (control), Doxorubicin at 3mg/kg or CPI at 250mg/kg plus doxorubicin. Dox= Doxorubicin, CPI=CPI-613.

Fatty Acids Confer Resistance to AML Cells, CPI-613 Inhibits Fatty Acid Synthesis



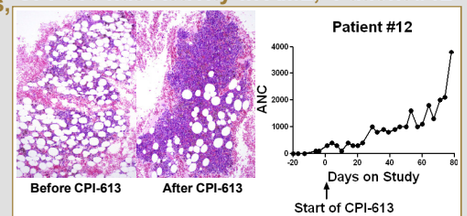
Left panel: Palmitate confers resistance to doxorubicin in AML cells. Cells were incubated with doxorubicin 40ng/ml ± 100 µM palmitate for 72 hours. Viability was determined following exposure and normalized to control samples without doxorubicin. Right panel: CPI-613 inhibits Fatty Acid Synthase (FASN). Cells were incubated with 50 µM CPI-613 for 4 hours and assessed for FASN activity by incorporation of C^{14} labeled acetate into lipids. *= p value <0.05.

CPI-613 has Activity in AML, Patient #4



Patient #4, a 49 y/o male diagnosed with AML with normal Karyotype in 2004. After standard therapy achieved CR1. Relapsed in 2008, received a HDAC based regimen and achieved CR2. Underwent autologous BMT in 2010 while in CR2. Delayed count recovery, red cell and platelet transfusion dependent. Three months post transplant found to have 8.5% cells with 7q-. Marrow 6 months post transplant showing 41% cells with 7q-, remained transfusion dependent. Patient has been transfusion independent since starting CPI-613 and now is in CR3.

CPI-613 has Activity in AML, Patient #12



Patient #12, 66 y/o female with refractory AML. She received induction therapy with 7+3, 1st salvage attempt with cytoxan-etoposide, next salvage with high dose cytarabine-mitoxantrone-L-spar, then decitabine and finally azacytidine before starting on CPI-613. Pre-trial bone marrow biopsy showed hypocellular marrow with 9% blasts and 22% immature monos. After 2 cycles repeat biopsy shows hypercellular marrow with no evidence of disease. She has now be removed from study to undergo reduced intensity conditioned allogeneic stem cell transplant. Shown top left marrow clot section before and after 2 cycles CPI613, top right absolute neutrophil count (ANC).

CPI-613 Clinical Activity Summary

Patient #	Diagnosis	Dose mg/m²	Best response	More than 1 cycle?
1	NHL	420	NA	No
2	AML	420	PD	No
3	Myeloma	840	PD	No
4	AML	840 ->2100	CR	Yes (12 cycles)
5	Hodgkin's	840	PD	No
6	AML	1386	PD	No
7	Myeloma	1386	SD	Yes (3 cycles)
8	Myeloma	1386	SD	Yes (4 cycles)
9	AML	1386	PD	No
10	MDS (REAB-2)	2100	SD	Yes (5 cycles)
11	MDS (RA)	2100	SD	Yes (4 cycles)
12	AML	2100	MLFS	Yes (2 cycles)
13	AML	2940	NA	No
14	Burkitt's	2940	SD	Yes (2 cycles)
15	AML	2940	PD	No

NA=not assessable, SD=Stable Disease, CR=Complete Remission, MLFS= Morphologically Leukemia Free State, PD=Progressive Disease

Conclusions

CPI-613 is a first in class non-redox active lipoate derivative currently under study in the laboratory and in phase I clinical trial for patients with relapsed or refractory hematological malignancies. To date we have shown:

- **CPI-613 is active in vitro against several human leukemia cell lines with IC50 values in the low µM range.**
- **CPI-613 displays strong synergy with tyrosine kinase inhibitors in ALL and AML cell lines.**
- **CPI-613 displays synergy with the anthracycline doxorubicin *in vitro* and *in vivo*.**
- **CPI-613 inhibits fatty acid synthesis**
- **No DLT identified even at a dose of 2940 mg/m²**
- **CPI-613 has activity in multiple hematologic malignancies. Of the eight evaluable patients with a diagnosis of AML or MDS one achieved a CR, one a morphologic leukemia free state and two had stable disease for an overall response rate of 50%.**

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