TCA Cycle Inhibition by CPI-613 Increases Sensitivity to Chemotherapy in Older and Poor Risk Acute Myeloid Leukemia (AML)

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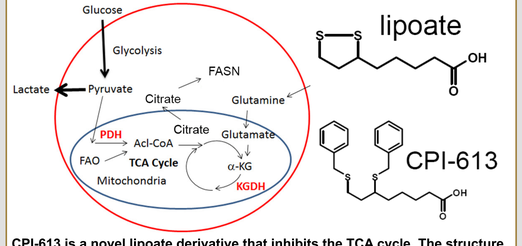
Introduction

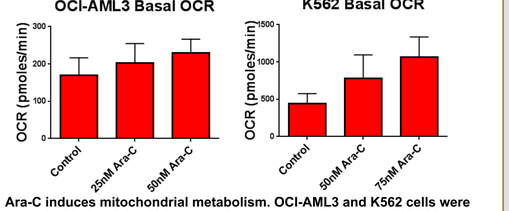
Acute myeloid leukemia (AML) is an aggressive malignancy. Outcomes in relapsed disease are dismal especially in older patients and those with poor risk cytogenetics. New therapies are desperately needed. Mitochondrial metabolism is aberrant in AML and is associated with resistance. Pyruvate dehydrogenase complex (PDH) and α-Ketoglutarate dehydrogenase complex (KGDH) are 2 key mitochondrial TCA cycle enzymes. CPI-613 is a non-redox active lipoate derivative developed by Cornerstone Pharmaceuticals that inhibits these enzymes.

CPI-613 is a Novel Lipoate Derivative



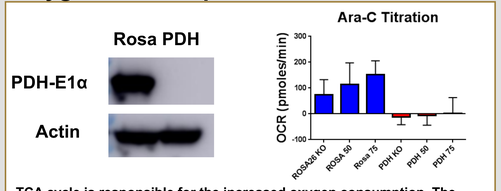
CPI-613 is a novel lipoate derivative that inhibits the TCA cycle. The structure of lipoate and CPI-613 are shown on the right. On the left is a simplified schematic of carbon metabolism with the CPI-613 targets shown in red.

Chemotherapy Induces Mitochondrial Metabolism in AML Cells



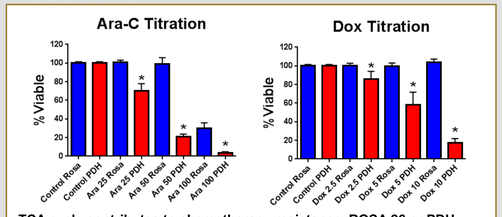
Ara-C induces mitochondrial metabolism. OCI-AML3 and K562 cells were treated with Ara-C for 16 hours and oxygen consumption rate (OCR) was assessed. Shown is the average OCR from 3 independent experiments each done in triplicate. P value for OCI-AML3 is 0.0387 and for K562 is <0.0001.

TCA Cycle is Responsible for Increased Oxygen Consumption



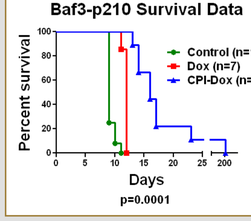
TCA cycle is responsible for the increased oxygen consumption. The murine AML cell line MFL2 was infected with a constitutive Cas9 expressing vector and then sgRNAs against ROSA 26 or PDH E1a were introduced. Western Blot confirmed knock out (left panel). Cells were incubated with the indicated concentration of Ara-C for 16 hours and oxygen consumption rate (OCR) was assessed.

TCA Cycle Contributes to Chemotherapy Resistance



TCA cycle contributes to chemotherapy resistance. ROSA 26 or PDH deleted AML cells from above were incubated with Ara-C (Ara) or doxorubicin (Dox) at the indicated nM concentration for 72 hours and viability determined. * denotes a significant difference when compared by Sidak's Multiple Comparisons test.

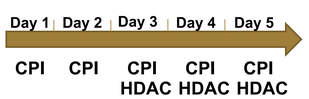
CPI-613 is Synergistic with Chemo *In Vivo*



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. P value shown is for Dox vs CPI-Dox.

Phase I Clinical Trial Schema

Schema:



HDAC= 3gm/m² Q12hr for 5 doses (1.5gm if age ≥60)
Mito = 6mg/m² QD for 3 doses

Nadir marrow done on day 14 and if residual leukemia present a second cycle was allowed

Starting dose =500 mg/m², 1-3-6 Escalation Scheme

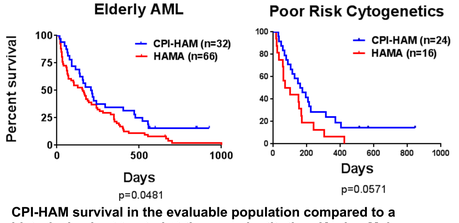
Expanded 1500, 2000 and 2500 dosing cohorts

CPI-613 Clinical Activity Summary

Cohort	HAMA (94)		CPI-HAM (n=62)	
Response	CR	CR + CRi	CR	CR + CRi
Overall	34%	41%	42%	50%
≥60 y.o.	27%	33%	38%	47%
Poor Karyotype	6%	19%	36%	46%
FLT3 Mutated	NA	NA	54%	69%

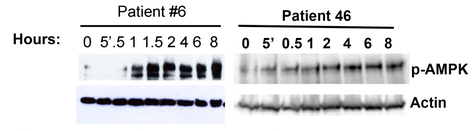
CR= complete remission, CRi= complete remission with incomplete count recovery. Four patients were not evaluable for a response.

Survival Compared to Historical Cohort

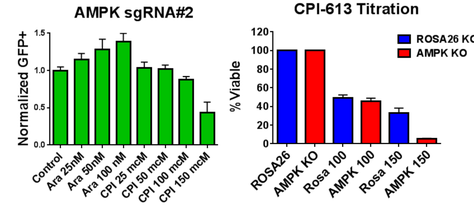


CPI-HAM survival in the evaluable population compared to a historical cohort treated at the same institution. Kaplan-Meier estimates were compared by log-rank test.

AMPK Contributes to Resistance to CPI-613

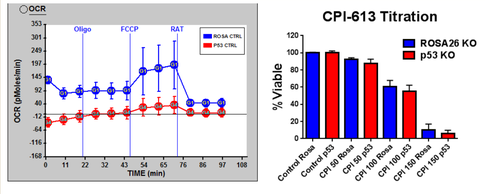


CPI-613 induces AMPK phosphorylation. Blood samples were taken on day one of treatment at the indicated time points following infusion of CPI-613. Red blood cells were lysed, and mononuclear cells were isolated. Extracts were prepared and blotted for phosphorylated AMPK. Both patients had circulating blasts at the time of sampling. Neither patient achieved a remission. Actin was used as a loading control.



AMPK promotes resistance to CPI-613. Left: Competition assay. Cas9 expressing MFL2 cells were partially infected with an sgRNA vector targeting AMPK tagged with GFP. Cells were incubated with the indicated drug for 72 hours and the %GFP+ determined in the viable population. Right: Viability assay. Clones of Cas9 expressing MFL2 cells with sgRNAs targeting AMPK or ROSA26 were incubated with the indicated drug for 72 hours and viability determined.

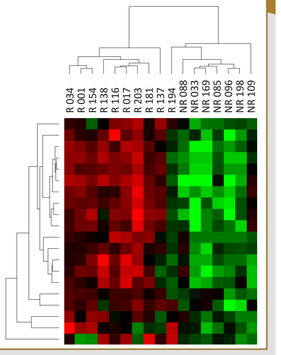
Mitochondrial Oxygen Consumption is Impaired in p53 KO Cells



Loss of p53 impairs mitochondrial oxygen consumption. MFL2 cells expressing Cas9 were infected with sgRNAs against ROSA26 or p53 and oxygen consumption measured (Left panel). The same cells were treated with the CPI-613 for 72 hours. Following treatment viability was assessed (Right Panel).

An Immune Expression Signature is Overexpressed in Responders

RNA seq analysis of baseline samples marrow samples. The transcriptomes of mononuclear cells from 17 baseline patient bone marrow samples were sequenced. These samples corresponded to 10 responders and 7 non-responders. Analysis for enrichment of gene ontologies revealed several significant biological categories for the genes overexpressed in responders, while none were identified for the non-responders. The responders showed significant enrichment for gene categories related to immune involvement. Heat map on the right shows the top 20 differentially expressed genes between responders (R) and non-responders (N).



Conclusions

- Chemotherapy induces mitochondrial oxygen consumption via the TCA cycle.
- The TCA cycle is a source of resistance to standard therapy in AML.
- TCA cycle inhibition with the novel agent CPI-613 sensitizes leukemia cells to chemotherapy in preclinical models.
- CPI-613 in combination with high dose cytarabine and mitoxantrone (CPI-HAM) was well tolerated even in older adults.
- Median overall survival for the evaluable cohort and responders was 6.7 and 13.2 months respectively.
- The response rate was 50% (26CR+5CRi) out of 62 evaluable patients.
- In patients ≥60 years old the CR/CRi rate was 47% (12CR+3CRi out of 32 evaluable patients).
- In patients with poor risk cytogenetics the CR/CRi rate was 46% (9CR+2CRi out of 24 evaluable patients) with a median survival of 5.5 months.
- AMPK activation contributes to resistance to TCA cycle inhibition.
- Loss of p53 reduces mitochondrial oxygen consumption and does not promote resistance to CPI-613.
- The presence of an immune cell signature may be predictive of those patients most likely to respond to this approach.
- CPI-613 in combination with HDAC and mitoxantrone is a promising salvage regimen, especially in older patients and those with high risk cytogenetics.

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