Abstract #3565

Birinapant (TL32711), a Small Molecule Smac Mimetic, Induces Regressions in Childhood Acute Lymphoblastic Leukemia (ALL) Xenografts that Express TNFα and Synergizes with TNFα *in Vitro* – A Report from the Pediatric Preclinical Testing Program (PPTP)

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BIRINAPANT (TL32711)

- Birinapant is a small molecule mimetic of Smac that potently and specifically antagonizes multiple inhibitors of apoptosis proteins (IAPs).
- Birinapant rapidly degrades cIAPs and enables cytokines (TNFα, TRAIL) to activate the extrinsic apoptosis pathway, while it rapidly turns off the canonical NF-κB survival pathway, causing cancer cell death.
- Preclinical studies using adult cancer models have shown that birinapant causes tumor regressions as a single agent in selected models and that it has potent antitumor activity when combined with chemotherapies and death receptor ligands.

BIRINAPANT *IN VITRO* METHODS

- Birinapant was evaluated against the 23 cell lines of the PPTP *in vitro* panel (including 1 AML and 5 ALL lines) using 96 hour exposure at concentrations from 1.0 nM to 3.0 μM, both as a single agent and in combination with TNFα (10 ng/mL) or TRAIL (10 ng/mL).
- Relative IC_{50} (rIC_{50}) is the concentration of agent that gives a half-maximal response.
- Relative In/Out (I/O)% values represent the percentage difference between the Ymin value and the estimated starting cell number and either the control cell number (for agents with Ymin > starting cell number) or 0 (for agents with Ymin < estimated starting cell number).
- Relative I/O% values range between 100% (no treatment effect) to -100% (complete cytotoxic effect), with a Relative I/O% value of 0 being observed for a completely effective cytostatic agent.

BIRINAPANT *IN VITRO* ACTIVITY

- Only 4 of 23 PPTP cell lines showed rIC_{50} values < 3 μM, including 1 of 4 rhabdomyosarcoma cell lines, 1 of 5 ALL cell lines (CCRF-CEM), the AML cell line Kasumi-1, and the anaplastic large cell lymphoma cell line Karpas-299.
- Only the AML cell line Kasumi-1 showed a Relative In/Out% (Rel I/O%) value < 0%. It had an rIC_{50} of 37 nM.

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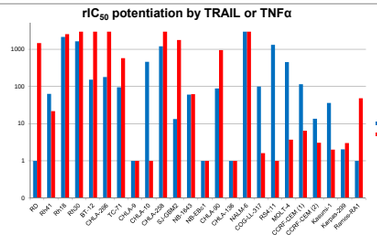
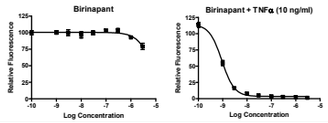
BIRINAPANT *IN VITRO* ACTIVITY IS POTENTIATED BY TNFα OR TRAIL

- Marked potentiation of birinapant was observed for a subset of cell lines with the addition of TNFα or TRAIL.
- The 5 ALL cell lines showed a median rIC_{50} value of 3.6 nM for birinapant in combination with TNFα, representing a potentiation factor of 10- to 1000-fold (figure below, top).
- Birinapant plus TNFα produced relative I/O% values between -50% and -100% (indicative of a cytotoxic effect) for each of the ALL cell lines (figure below, bottom).
- Four of 5 ALL cell lines showed little or no potentiation of birinapant activity with the addition of TRAIL (figure below, top).
- Among solid tumor cell lines, potentiation of birinapant activity was observed for selected rhabdomyosarcoma, rhabdoid tumor, Ewing sarcoma, and neuroblastoma cell lines with the addition of either TNFα or TRAIL.



Example of potentiation of birinapant *in vitro* activity by TNFα [birinapant alone (left) and with TNFα (right)] for NALM-6 cells.



IN VIVO METHODS

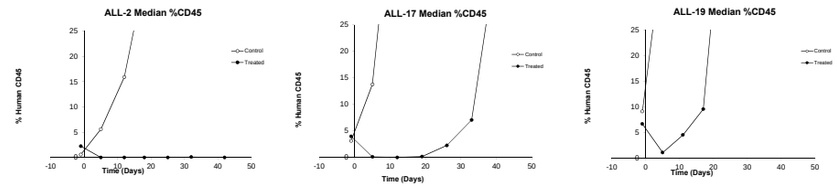
- Birinapant was tested against the PPTP solid tumor xenografts (using SCID mice) at a dose of 30 mg/kg administered by the intraperitoneal route Q3day x 5.
- For the ALL panel (using NOD-SCID mice), the maximum tolerated dose was also 30 mg/kg, and this dose was used for efficacy testing.
- The total planned treatment and observation period was 6 weeks.
- The primary readout for activity was the "objective response" metric that for the ALL panel used definitions described below:
  - An event is defined as hCD45 cells above 25% in the peripheral blood.
  - Individual mice were classified as SD if their percentage of hCD45 cells never dropped below 1% and no event occurred before the end of the study.
  - PR was assigned if the percentage of cells dropped below 1% for any one time point regardless of whether the percentage eventually reached 25%.
  - A CR was assigned if the percentage of hCD45 cells dropped below 1% for 2 consecutive weeks of the study and regardless of whether the percentage reached 25% or not.
  - A CR was considered maintained (i.e., MCR) if the percentage of hCD45 was less than 1% for the last three measurements of the study.
  - The objective response for each model represents the median objective response score for all treated animals for that model.

IN VIVO RESULTS

- Birinapant was well tolerated *in vivo*.
- Birinapant induced significant differences in event-free survival (EFS) distribution compared to control in 3 of 3 (100%) of the B-precursor ALL xenografts, but in none of the solid tumor or ALCL xenografts.
- Objective responses were not observed for the solid tumor and ALCL xenografts.
- For the ALL panel one xenograft (ALL-17) achieved a complete response (CR) and another (ALL-2) achieved a maintained CR, with treated animals remaining in remission at day 42, approximately 30 days after their last treatment with birinapant.

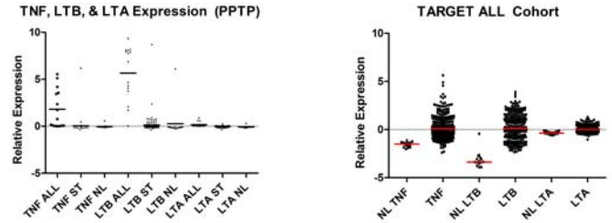
Xenograft Line	Histology	Median Time to Event	P-value	EFS T/C	Median Final RTV or huCD45%	Objective Response
Karpas-299	Anaplastic Large Cell Lymphoma	7.1	0.144	1.0	>4	PD1
CHLA258	Ewing	12.2	0.719	1.3	>4	PD1
Rh30	ALV Rhabdomyosarcoma	11.3	0.414	1.0	>4	PD1
ALL-2	ALL B-precursor	> EP	<0.001	> 2.8	0.0	MCR
ALL-17	ALL B-precursor	39.0	<0.001	4.9	>25	CR
ALL-19	ALL B-precursor	20.6	0.001	4.1	>25	SD

EXAMPLES OF BIRINAPANT *IN VIVO* ACTIVITY (ALL XENOGRAFTS)



TNF FAMILY EXPRESSION IN ALL

- Given the mechanism of action of Smac mimetics, TNFα expression was examined for the PPTP xenografts using Affymetrix U133 Plus 2 expression data. TNFα expression was significantly higher for the PPTP ALL xenografts compared to the PPTP solid tumor xenografts (ST) and to 15 normal tissues (NL, figure below, left).
- TNFα expression in ALL clinical specimens was examined using the TARGET ALL gene expression data (Affymetrix U133 Plus 2), with the observation that its expression was significantly higher for high-risk B-precursor ALL compared to a set of normal tissues (NL), but with a wide range of TNFα expression among ALL cases (figure below, right).
- Lymphotoxin B and Lymphotoxin A also show significantly elevated expression in ALL xenografts and clinical specimens compared to normal tissues.
- Among the ALL xenografts tested with birinapant, the best responding xenograft (ALL-2) showed the highest TNFα expression. Karpas-299, which did not respond in vivo to TL32711, also showed high TNFα expression, but the two solid tumor xenografts tested in vivo did not.



CONCLUSIONS

- Birinapant showed little single agent *in vitro* activity against ALL cell lines, though its activity was markedly potentiated by the addition of exogenous TNFα for these cell lines.
- *In vivo*, birinapant showed remission-inducing activity against 2 of 3 ALL xenografts, with one of these showing a maintained CR.
- TNFα is mechanistically associated with the activity of Smac mimetics, and the initial PPTP *in vivo* data for ALL xenografts are consistent with a relationship between TNFα expression and responsiveness to birinapant.
- The PPTP results suggest that birinapant may show high level activity against a subset of childhood ALL, and additional *in vivo* testing is ongoing to better identify predictive markers that can reliably select responsive cases.