Combination of mTOR inhibitor ridaforolimus and HDAC inhibitor vorinostat exhibits *in vitro* synergism in synovial sarcoma, osteosarcoma, and a range of other tumor subtypes

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Abstract

Background: Curative treatments for patients with metastatic synovial sarcoma (SS) do not exist, and such patients have a poor prognosis. We explored combinations of molecularly-targeted and cytotoxic agents to identify synergistic treatment combinations in SS cells.

Methods: Two SS cell lines (HS-SY-II and SYO-I) were treated with single agents or combinations of molecularly targeted therapies (HDAC inhibitor, vorinostat; mTOR inhibitor, ridaforolimus) and cytotoxic agents. After 72 hours, cell viability was measured using the cell proliferation assay (MTS). Combination Indices (CI) were calculated to determine whether each combination was synergistic, additive, or antagonistic. The most active combination was selected for further confirmation in other tumor subtypes.

Results: Ridaforolimus IC_{50} was 10.9 nM in HS-SY-II and 23.1 nM in SYO-I; vorinostat IC_{50} was 440 nM in HS-SY-II and 561 nM in SYO-I; doxorubicin IC_{50} was 9.4 nM in HS-SY-II and 7.4 nM in SYO-I; and melphalan IC_{50} was 687 nM in HS-SY-II and 859 nM in SYO-I. Synergism was observed in cells treated with ridaforolimus/vorinostat: CI was 0.28 and 0.63 in HS-SY-II and SYO-I, respectively. Both ridaforolimus/doxorubicin and ridaforolimus/melphalan exhibited synergism: CI ranged from 0.50 to 0.59 in HS-SY-II and SYO-I. Additive effects were observed when vorinostat was combined with doxorubicin or melphalan. Given its strong synergism, the ridaforolimus/vorinostat combination was assessed in osteosarcoma (U2OS, M189, and P16T), metastatic melanoma (Stew1 and Stew2), pancreatic cancer (Panc1 and BxPC3), and non-small cell lung cancer (A549) cell lines. The combination was synergistic in all cell lines: CI ranged from 0.37 to 0.77, except in Panc1, where it was additive (CI was 0.92).

Conclusions: The combination of ridaforolimus and vorinostat is synergistic *in vitro* in SS as well as in a variety of tumor types, including osteosarcoma, melanoma, pancreatic cancer, and non-small cell lung cancer. In anticipation of human studies, further *in vitro* studies are planned to assess the activity of this combination in other sarcomas subtypes and *in vivo*. Studies to assess the molecular basis for this synergism are also planned.

Background

- STS is responsible for approximately 4,000 deaths in US in 2010.
- At least 50 distinct histological STS subtypes exist.
- Unresectable or metastatic disease occurs in approximately 40-60% of patients, which portends poor prognosis.
- First line treatment is doxorubicin, with typical response of 26%.
- Curative systemic treatment options for patients with unresectable or metastatic STS are currently not available.
- Effective systemic therapies are desperately needed to treat unresectable and metastatic STS.

Project Goal

Evaluate the *in vitro* sensitivity of synovial sarcoma cells to vorinostat and ridaforolimus alone and in combination with standard chemotherapeutic agents.

Results

Table 1. The average calculated (and range of) IC_{50} for each agent alone in HS-SY-II and SYO-I

Cell Proliferation Assay:

Cells were seeded in quadruplicate in 96-well plates at a density of 4.0 x 10^3 cells per well for 24 hours followed by incubation with vehicle or drug for 72 hours. Cell viability was measured using the MTS CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (Promega).

Treatments	HS-SY-II	SYO-I
Ridaforolimus	**10.9 nM** (2.1 to 23 nM)	**23.1 nM** (3.4 to 33.2 nM)
Vorinostat	**440 nM** (216 to 612 nM)	**561 nM** (341 to 702 nM)
Dalotuzumab	Indeterminate*	Indeterminate*
Doxorubicin	**9.4 nM** (5.98 to 14.8 nM)	**7.4 nM** (3.1 to 16.2 nM)
Melphalan	**687 nM** (432 to 1227 nM)	**859 nM** (401 to 1472 nM)
Gemcitabine	**2.6 nM** (2.1 to 2.9 nM)	**2.4 nM** (0.9 to 3.43 nM)

Table 2. Summary of the combination index (CI) values for each treatment combination

HS-SY-II			SYO-I		
	Ridaforolimus	Vorinostat		Ridaforolimus	Vorinostat
Vorinostat	0.28	n/a	Vorinostat	0.63	n/a
Doxorubicin	0.56	1.1	Doxorubicin	0.50	0.98
Melphalan	0.51	0.91	Melphalan	0.59	0.90
Gemcitabine	3.1	1.54	Gemcitabine	2.6	2.25

CI < 0.90 Synergistic | CI = 0.9–1.1 Additive | CI > 1.10 Antagonistic

Calculation of Combination Index (CI) values:

CI was calculated using the median-effect analysis method of Chou and Talalay.

$$CI = \frac{D_1}{(D_x)_1} + \frac{D_2}{(D_x)_2}$$

where D_1 and D_2 are doses of drugs 1 and 2 that have x% effect when used in combination, and $(D_x)_1$ and $(D_x)_2$ are doses of drugs 1 and 2 that have the same x% when used alone as single agents.

Table 3. Combination of ridaforolimus and vorinostat in a panel of cell lines.

Cell Proliferation Assay was used to determine the effects of the combination of ridaforolimus and vorinostat in a variety of cell lines, including non-small cell lung cancer, pancreatic cancer, osteosarcoma, and metastatic melanoma. CI values for the ridaforolimus/vorinostat combination in these cell lines are presented here.

CI < 0.90 Synergistic | CI = 0.9–1.1 Additive | CI > 1.10 Antagonistic

Cell Line	Disease	CI
A549	Non-Small Cell Lung Cancer	0.54
Panc1	Pancreatic Cancer	0.92
BxPC3	Pancreatic Cancer	0.50
U2-OS	Osteosarcoma	0.56
M189	Osteosarcoma	0.59
P16T	Osteosarcoma	0.77
Stew1	Metastatic Melanoma	0.46
Stew2	Metastatic Melanoma	0.37

Conclusions

- The combination of ridaforolimus and vorinostat is synergistic in synovial sarcoma cell lines (HS-SY-II and SYO-I) as well as other cell lines (non-small cell lung cancer, pancreatic cancer, osteosarcoma, and metastatic melanoma).
- Ridaforolimus in combination with doxorubicin or melphalan is synergistic in both synovial sarcoma cell lines.
- Vorinostat in combination with doxorubicin and melphalan is additive in both synovial sarcoma cell lines.
- Gemcitabine antagonizes the effects of ridaforolimus and vorinostat in synovial sarcoma cell lines (HS-SY-II and SYO-I).

Discussion

- Synergy between ridaforolimus and vorinostat *in vitro* appears very promising.
- Synergistic and additive effects of ridaforolimus or vorinostat with cytotoxic chemotherapies (e.g. doxorubicin) may have clinical relevance.
  i. Ridaforolimus or vorinostat may serve as a chemotherapy-sparing agent, reducing the dose-limiting toxicities associated with a particular agent.
  ii. This may be especially important with doxorubicin, a backbone therapy for STS, but hampered by dose-limiting cardiotoxicity.

Future Directions

1. Determine whether the combination of ridaforolimus/vorinostat is synergistic in other STS subtypes.
2. Determine the effects of ridaforolimus/vorinostat combination, compared to single agents, in an *in vivo* STS mouse model.
3. Characterize the molecular mechanisms that mediate the synergistic effects between ridaforolimus and vorinostat.
4. Determine the effects of this combination in STS patients.

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