

# Cisplatin Enhances the Activity of Ispinesib, a Novel KSP Inhibitor, Against Murine P388 Lymphocytic Leukemia

David Sutton<sup>1</sup>, Melody Diamond<sup>1</sup>, James Onori<sup>1</sup>, ShuYun Zhang<sup>1</sup>, Michele Giardiniere<sup>1</sup>, Leo Faucette<sup>1</sup>, Lisa Belmont<sup>2</sup>, Kenneth W. Wood<sup>2</sup>, Jeffrey R Jackson<sup>1</sup>, Pearl Huang<sup>1</sup>. 1. Department of Molecular and Cellular Oncology, GlaxoSmithKline, Collegeville, PA. 2. Cytokinetics Inc., South San Francisco, CA

## Abstract

*Ispinesib* (SB-715992) is a novel kinesin spindle protein inhibitor that is currently in phase II studies. This compound has anti-tumor activity in several xenograft models and causes mitotic arrest with monopolar spindles in cultured cells and in dividing cells *in vivo*. Combination therapy is a cornerstone of cancer treatment and often involves combining drugs with diverse mechanisms of action. This can sometimes present a challenge for agents that have different cell cycle-specific actions. In these studies, we have tested how a widely-used DNA damaging agent (cisplatin) combines with the anti-mitotic drug *ispinesib*, and whether the order of treatment has any effect on outcome. The P388 murine lymphocytic leukemia was used to explore the effect of dose scheduling on efficacy. Female B6D2F1 mice were implanted intravenously with 10<sup>6</sup> P388 cells. Study endpoints were % increase in lifespan (%ILS) based on median survival time and log net cell kill (NCK). A preliminary study in mice demonstrated that intraperitoneal administration of *ispinesib* and cisplatin 1h apart on a q4dx3 schedule resulted in greater than predicted activity. Interestingly, combination of single-agent maximum tolerated doses (MTD) of *ispinesib* and cisplatin were found to be well tolerated and led to a significant improvement in %ILS. Additional studies explored the effect of dose scheduling on efficacy. The two drugs were administered in a dose matrix on one of three schedules; 1. *ispinesib* (days 2, 6 and 10) plus cisplatin (days 3, 7 and 11); 2. cisplatin (days 2, 6 and 10) plus *ispinesib* (days 3, 7 and 11); *ispinesib* and cisplatin (days 2, 6 and 10). At its MTD, *ispinesib* (5-10mg/kg) led to 160 to 184% ILS corresponding to approximately 4 log NCK. In contrast, cisplatin at its respective MTD (4mg/kg) led to maximum of 140% ILS corresponding to approximately 2 log NCK. Combinations of the two drugs, each at doses < MTD, resulted in better efficacy than either drug alone at its MTD. For example, *ispinesib* (5mg/kg; half MTD) and cisplatin (1mg/kg; half MTD), led to a 230% ILS (with 1 long term survivor) and a 7.7 log NCK. The order of drug administration influenced the effectiveness of the combination especially at the lower doses of each agent. Administration of cisplatin 24h before *ispinesib* was the most effective schedule while co-administration was the least. These data suggest that inducing DNA damage *in vivo* makes tumor cells more sensitive to the effects of *ispinesib*.

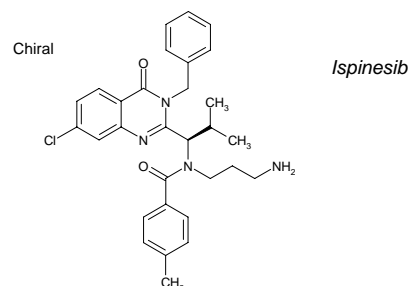
## Introduction

*Ispinesib* (SB-715992) is an inhibitor of the mitotic kinesin KSP that blocks assembly of a functional mitotic spindle, thereby causing cell cycle arrest in mitosis and subsequent cell death. The mitotic spindle has long been an important functional target in cancer chemotherapy as demonstrated by the anti-tubulin agents vincristine, vinblastine and vinorelbine (Vinca alkaloids), and the taxanes paclitaxel and docetaxel. *Ispinesib* acts via a novel mechanism: inhibition of a mitotic kinesin motor protein, KSP. The expression profiles of KSP mRNA in normal human tissues are consistent with expression of KSP only in proliferating cells, as is overexpression in tumor tissue relative to normal adjacent tissue. Moreover, a correlation of KSP protein and mRNA levels has been observed in human cell lines cultured *in vitro*, including absence of expression in terminally differentiated post-mitotic neurons. In total, KSP appears to function exclusively in mitosis, and is required for centrosome separation and formation of a bipolar mitotic spindle. No role for KSP outside of mitosis has been demonstrated. As KSP is not involved in non-mitotic processes such as neuronal transport, it is expected that *ispinesib* would not cause the neuropathy often associated with the tubulin agents.

Combination therapy is a cornerstone of cancer treatment and often involves combining drugs with diverse mechanisms of action. This can sometimes present a challenge for agents that have different cell cycle-specific actions. In these studies, we have tested how cisplatin, a widely-used DNA damaging agent, combines with *ispinesib*, and whether the order of treatment has any effect on outcome. The murine P388 lymphocytic leukemia was selected for this series of studies as preliminary experiments had shown that P388 was sensitive to both agents and the nature of the model allowed multiple dose levels and schedules to rapidly explored.

## Methods

Female BDF-1 mice were obtained from Charles River (Raleigh, NC). *Ispinesib* was formulated in a vehicle consisting of 2% Cremophor EL (Sigma), 2% N, N-Dimethylacetamide (Sigma), 96% acidified water (pH5) and Cisplatin (Bedford Laboratory) was diluted to the required concentration in normal saline. Both agents were administered intraperitoneally as three doses each separated by 4 days (q4dx3).



Murine P388 lymphocytic leukemia (10<sup>6</sup> cells per mouse) was implanted intravenously on day 0. Study end-points were % increase in median survival (%ILS) and net log<sub>10</sub> cell kill (NCK) based on a cell titration curve using 10<sup>6</sup> to 10<sup>2</sup> cells per animal. The group size was 5.

Drug treatment began on Day 2. Three separate studies were conducted: both compounds administered approximately 1 hour apart (co-administration), Cisplatin dosed 24 hours before *ispinesib* and cisplatin dosed 24 hours after *ispinesib*.

All *in vivo* procedures were carried out in accordance with protocols approved by the GSK Institutional Animal Care and Use Committee.

### Key to tables

%ILS - percent increase in lifespan compared to placebo

NCK - log<sub>10</sub> net cell kill

LTS - long term survivor

Enhanced activity - Net cell kill greater than either agent alone

Reduced activity - Net cell kill less than either agent alone

## Results

### *Ispinesib* and cisplatin have enhanced activity against P388 lymphocytic leukemia in BDF1 mice

#### *Ispinesib* administered prior to cisplatin

%ILS (NCK) following treatment with cisplatin (mg/kg) q4dx3 from day 3	%ILS (NCK) following treatment with <i>ispinesib</i> (mg/kg) q4dx3 from day 2				
	0	1.25	2.5	5	10
0	0 (0)	10 (0)	50 (0)	110 (0.9)	160 (3.7)
0.5	20 (0)	50 (0)	90 (0)	130 (2.0)	220 (>7.0)
1	50 (0)	60 (0)	80 (0)	190 (5.4)	260 (>7.0)
2	60 (0)	90 (0)	130 (2.0)	230 (>7.0) 1 LTS	390 (>7.0) 2 LTS
4	100 (0.3)	140 (2.5)	170 (4.2)	880 (>7.0) 4 LTS	20 (0) 3 LTS

Combinations of *ispinesib* and cisplatin had enhanced activity  
10mg/kg *ispinesib* + 0.5-2mg/kg cisplatin  
5mg/kg *ispinesib* + 0.5-4mg/kg cisplatin  
2.5mg/kg *ispinesib* + 2-4mg/kg cisplatin  
1.25mg/kg *ispinesib* + 4mg/kg cisplatin

Combination of sub-MTD doses of *ispinesib* and cisplatin were superior to either agent alone at their MTD's

Combining *ispinesib* and cisplatin at their respective MTD's led to toxicity  
However, long term survival was observed in mice that could tolerate the combination

#### Cisplatin administered prior to *ispinesib*

%ILS (NCK) following treatment with cisplatin (mg/kg) q4dx3 from day 2	%ILS (NCK) following treatment with <i>ispinesib</i> (mg/kg) q4dx3 from day 3				
	0	1.25	2.5	5	10
0	0 (0)	11 (0)	88 (0)	111 (0.5)	166 (3.0)
0.5	33 (0)	77 (0)	111 (0.5)	156 (2.5)	322 (>7.0)
1	33 (0)	88 (0)	133 (1.5)	189 (4.0)	356 (>7.0) 2 LTS
2	66 (0)	115 (0.5)	155 (2.5)	355 (>7.0)	467 (>7.0) 4 LTS
4	133 (1.5)	178 (3.5)	277 (>7.0)	478 (>7.0) 1 LTS	77 (0)

Combinations of *ispinesib* and cisplatin had enhanced activity  
10mg/kg *ispinesib* + 0.5-2mg/kg cisplatin  
5mg/kg *ispinesib* + 0.5-4mg/kg cisplatin  
2.5mg/kg *ispinesib* + 0.5-4mg/kg cisplatin  
1.25mg/kg *ispinesib* + 2-4mg/kg cisplatin

Combination of sub-MTD doses of *ispinesib* and cisplatin were more active than either agent alone at their MTD's

Combining *ispinesib* and cisplatin at their respective MTD's was less effective than either agent alone probably due to toxicity

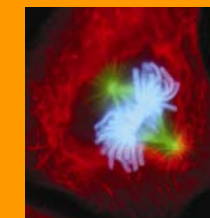
#### Co-administration of *ispinesib* and cisplatin

%ILS (NCK) following treatment with cisplatin (mg/kg) q4dx3 from day 2	%ILS (NCK) following treatment with <i>ispinesib</i> (mg/kg) q4dx3 from day 2				
	0	1.25	2.5	5	10
0	0 (0)	47 (0)	89 (0)	184 (4.1)	173 (3.6)
0.5	47 (0)	68 (0)	89 (0)	142 (2.1)	121 (1.1)
1	68 (0)	89 (0)	121 (1.1)	184 (4.1)	331 (>7.0)
2	110 (0.6)	100 (0.1)	142 (2.1)	226 (5.9)	47 (0)
4	142 (2.1)	184 (4.1)	184 (4.1)	395 (>7.0) 3 LTS	5 (0) 3 LTS

Combinations of *ispinesib* and cisplatin had enhanced activity  
5mg/kg *ispinesib* + 2-4mg/kg cisplatin  
2.5mg/kg *ispinesib* + 1-4mg/kg cisplatin  
1.25mg/kg *ispinesib* + 4mg/kg cisplatin

Combination of sub-MTD doses of *ispinesib* and cisplatin were more active than either agent alone at their MTD's

Combining *ispinesib* and cisplatin at their respective MTD's led to toxicity but long term survival in mice that could tolerate the combination



Abstract C204

## Conclusions

- Cisplatin enhanced the activity of *ispinesib* against P388 lymphocytic leukemia
- Combining doses that were suboptimal alone resulted in activity superior to that observed with either *ispinesib* or cisplatin at their respective maximum tolerated doses
- The order of administration influenced the effectiveness of the combinations
- Administration of cisplatin 24h before *ispinesib* was the most effective combination
- Co-administration was the least effective combination
- The data suggest that inducing DNA damage *in vivo* makes tumor cells more sensitive to the effects of *ispinesib*.