

Relationship Between the Antitumor Activity of *Ispinesib*, a Novel KSP Inhibitor, and Neutropenia in a Human Xenograft Model

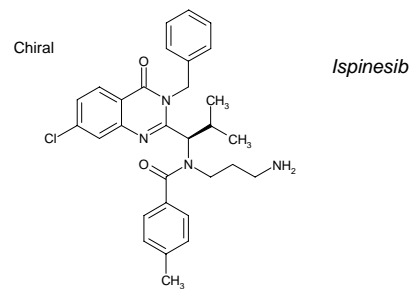
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Abstract

Ispinesib (SB-715992) is a novel kinesin spindle protein inhibitor that is currently in phase II studies. Phase I studies have demonstrated that neutropenia is the dose limiting toxicity (DLT) in patients. Preclinical studies have shown that *ispinesib* has anti-tumor activity in several xenograft models. Studies have now been conducted to determine if *ispinesib* induces neutropenia in mice and if so how does this relate to efficacy. In vitro studies showed that human and murine bone marrow progenitor cells were equally sensitive to *ispinesib* in the presence of either G-CSF or GM-CSF, thus allowing a direct comparison between efficacy and hematopoietic toxicity. *Ispinesib* was administered intraperitoneally to CD-1 nu/nu mice as either a single or multiple intraperitoneal doses. Terminal bleeds were collected and analyzed using an Advia 120 blood analyzer. *Ispinesib* induced dose-dependent pan-leukopenia with no apparent effect on red blood cell or platelet count. Neutrophils were the most affected cell population. The nadir was reached approximately 5 days after a single dose. The neutrophil counts had returned to normal values by day 10. Administration of *ispinesib* on a q4dx3 did not lead to a cumulative effect on neutrophil counts despite dosing in the presence of significant neutropenia. The nadir was delayed until day 9, but the neutrophil counts had returned to normal values by day 14. Thus the recovery period, 5 days, was the same as that following a single dose. The median duration of neutropenia (<500 neutrophils/ul) at 20, 10 and 5mg/kg *ispinesib* (q4dx3) was 8, 7 and 3 days respectively. Severe neutropenia (<100 neutrophils/ul) was noted for 20mg/kg (median duration 5 days) and 10mg/kg (median duration 2 days), but was not seen with 5mg/kg of *ispinesib*. In efficacy studies in nude mice with advanced Colo205 human colon tumors using a q4dx3 schedule, the maximum tolerated dose of *ispinesib* was 20mg/kg. Tumor regression was observed at doses down to 10mg/kg - a dose level that only caused brief severe neutropenia, and tumor growth inhibition was observed at 5 mg/kg. Similar findings were observed using a P388 murine lymphocytic leukemia model in normal B6D2F1 mice where a significant increase in life span and net cell kill were achieved at *ispinesib* doses that did not induce severe neutropenia. In summary, this series of studies confirmed that *ispinesib* did induce neutropenia in mice thus reflecting clinical observations. Use of a more clinically relevant end-point of toxicity in the mouse models may improve their usefulness in preclinical evaluation of novel cytotoxic agents.

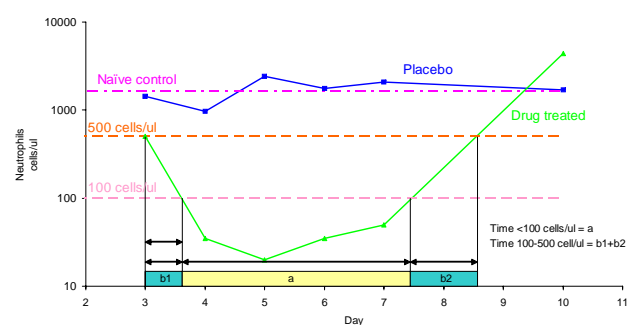
Methods

Female CD-1 nude mice or BDF-1 mice were obtained from Charles River (Wilmington MA and Raleigh, NC respectively). *Ispinesib* was formulated in a vehicle consisting of 2% Cremophor EL (Sigma), 2% N, N-Dimethylacetamide (Sigma), 96% acidified water (pH5) and administered intraperitoneally as either a single dose or three doses each separated by 4 days (q4dx3).



CBC data was generated from 4 mice per group per timepoint. Animals were bled under terminal anesthesia using EDTA-treated needles. Blood samples were collected in Microtainers (EDTA) (Becton Dickinson) and then analyzed using an ADVIA 120 hematology System (Bayer).

Quantification of neutropenia - time below 100cell/ul and time below 100 to 500 cells/ul



Solid tumor studies

Antitumor activity was determined in female CD-1 nu/nu mice with advanced Colo205 xenografts. Tumor volumes were measured at regular intervals for at least 90 days post-implantation. Complete regressions (CR) were defined as tumor volume measurements ≤ 14 cu mm for three consecutive measurements. Partial regressions (PR) were defined as tumor volume measurements $\leq 50\%$ of initial tumor volume for three consecutive measurements. A treatment was defined as toxic if 2 or more animals exhibited signs of toxicity that either prevented further treatment or necessitated euthanasia.

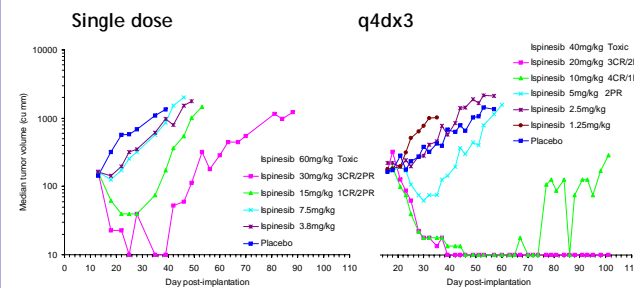
Leukemia studies

Murine P388 lymphocytic leukemia (10^6 cells per mouse) was implanted intravenously on day 0. Study end-points were % increase in median survival (%LS) and net \log_{10} cell kill (NCK) based on a cell titration curve using 10^6 to 10^2 cells per animal.

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

Results

Ispinesib caused complete regression of advanced sc human Colo205 colon carcinoma xenografts

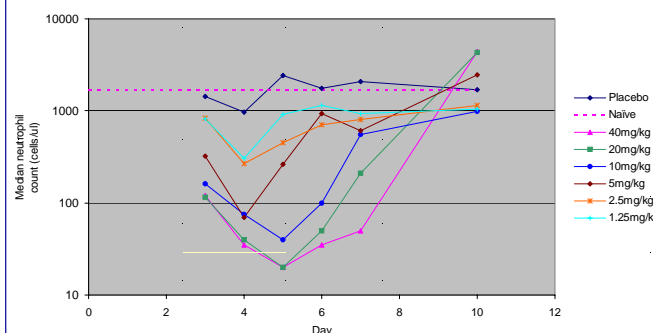


A single dose of *ispinesib* caused pan-leukopenia in both normal and nude mice

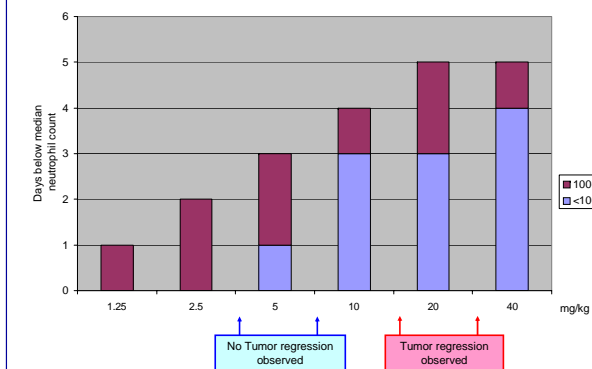
	BDF-1	CD-1 nude
RBC	No effect	No effect
Platelets	↓?	No effect
WBC	↓↓	↓↓
Lymphocytes	↓	↓
Neutrophils	↓↓	↓↓↓
Basophils	No effect	No effect
Eosinophils	↓	↓↓
Monocytes	↓	↓
Atypicals	No effect	No effect

Significant neutropenia is consistent with clinical DLT in patients

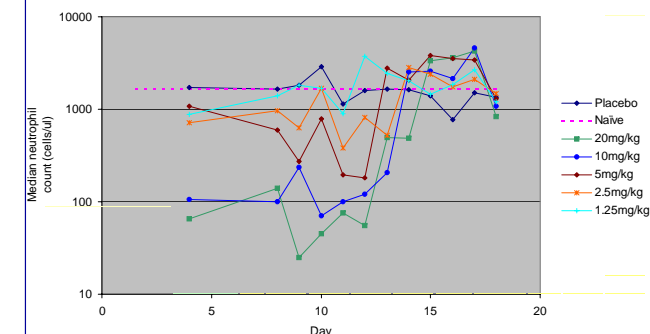
A single dose of *ispinesib* induced neutropenia in nude mice in a dose-dependent manner



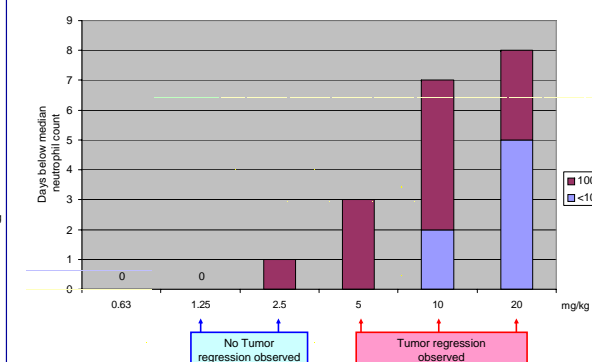
Severe neutropenia was induced in nude mice at therapeutic single doses of *ispinesib*



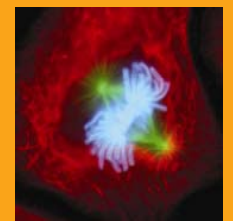
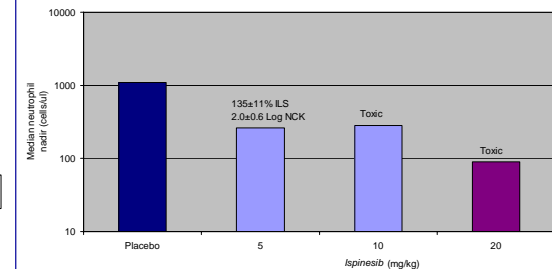
Ispinesib administered q4dx3 induced neutropenia in nude mice in a dose-dependent manner



Administration of multiple doses of *ispinesib* caused tumor regression without inducing severe neutropenia



Administration of multiple doses of *ispinesib* inhibited P388 leukemia without inducing severe neutropenia



Abstract C203

Conclusions

- *Ispinesib* induced dose-dependent neutropenia in both normal and nude mice
- While severe neutropenia (<100 neutrophils/ul) was seen at higher *ispinesib* doses, significant antitumor activity was achieved at *ispinesib* doses that caused mild (100-500 neutrophils/ul) or transient severe neutropenia
- Use of a clinically relevant end-point of toxicity, such as neutropenia, in murine efficacy models may improve their usefulness in preclinical evaluation of novel cytotoxic agents

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. Preclinical studies have shown that *ispinesib* has anti-tumor activity in several xenograft models and P388 murine leukemia at fractions of the drug's maximum tolerated dose. Phase I studies have demonstrated that the dose limiting toxicity (DLT) of *ispinesib* in patients is neutropenia. Based on these observations, studies have now been conducted to determine if *ispinesib* induces neutropenia in mice and if so how does this relate to efficacy in murine efficacy models.