Flavopiridol in patients with relapsed or refractory multiple myeloma: a phase 2 trial with clinical and pharmacodynamic end-points

Flavopiridol downregulates anti-apoptotic regulators including Mcl-1, upregulates p53, globally attenuates transcription through inhibition of P-TEFb, binds to DNA, and inhibits angiogenesis. Eighteen myeloma patients were treated with 1-hour flavopiridol infusions for 3 consecutive days every 21 days. Immunoblotting for Mcl-1, Bcl-2, p53, cyclin D, phosphoRNA polymerase II and phosphoSTAT 3 was conducted on myeloma cells. Ex vivo flavopiridol treatment of cells resulted in cytotoxicity, but only after longer exposure times at higher flavopiridol concentrations than were anticipated to be achieved in vivo. No anti-myeloma activity was observed in vivo. As administered, flavopiridol has disappointing activity as a single agent in advanced myeloma.

Key words: therapy, clinical trial, Mcl-1.

Haematologica 2006; 91:390-393
©2006 Ferrata Storti Foundation

Design and Methods

After providing written informed consent, 18 patients with myeloma, enrolled between November 2002 and November 2003 in a phase II trial approved by the Institutional Review Board, were administered flavopiridol. All patients had disease progression/relapse as documented by standard criteria. Patients were excluded if: (i) creatinine exceeded 3 mg/dL; (ii) aspartate transaminase and/or alkaline phosphatase exceeded 2.5 times the upper limits of normal; (iii) Eastern Cooperative Oncology Group performance score was 3 or higher; or (iv) neutrophils were less than 750/mm³. Flavopiridol was administered at a dose of 50 mg/m² over 1 hour for 3 consecutive days every 3 weeks. Patients were continued on treatment until disease progression or prohibitive toxicities.

Data analysis was conducted in May 2004, at which time all patients had ceased flavopiridol therapy. Patients were evaluated for response prior to each cycle and were continued on study if they had disease response or stabilization according to EBMTR criteria. The primary end-point was objective response (partial response or better). Toxicity was a secondary end-point. Patients were evaluated every three weeks, and adverse events were recorded according to Common Toxicity Criteria Version 2.0 (CTC 2.0) criteria. The initial design was to be a one-stage
design, testing drug efficacy in 32 patients (0.91 power to
detect a hematologic response rate of 20%). However,
because there were no responders in the first 18 patients
accrued, an interim analysis based on a modified Fleming
design (type I error rate=0.07, power=0.9) was undertak-
Under this decision rule, at least one response in the
first 18 patients would have been required to justify fur-
ther accrual, yet no responses were observed. Given the
lack of efficacy of this treatment regimen in this popula-
tion of patients, the trial was permanently closed to
accrual effective on December 31, 2003.

Unilateral iliac crest bone marrow aspirates were
performed prior to and immediately after flavopiridol ther-
apy, and on day 21 in patients treated with flavopiridol as
feasible. Twenty milliliters of marrow aspirate were col-
clected in EDTA tubes, subjected to ammonium chloride
(ACK; BioWhittaker, Walkersville, MD, USA) red cell
lysis and subsequently CD138 positive selection using
MicroBeads and an MS magnetic bead separation column
(Miltenyi Biotec, Auburn, CA, USA).

Ex vivo assessment of flavopiridol

After re-suspension of myeloma cells in MEM supple-
mented with 10% heat-inactivated human serum and 1
µg/mL interleukin-6, aliquots of cells were transferred to
tissue culture plate wells for treatment with flavopiridol
(125, 250, 500 and 1000 nM). Aliquots of cells were with-
drawn at 0, 24, 48 and 72 hours and examined for viabil-
ity (Trypan blue). Sham/diluent-treated myeloma cells
were used as controls.

Immunoblotting

Freshly collected CD138-selected myeloma cells were
washed twice with phosphate-buffered saline and solu-
bilized in alkylation buffer [6 M guanidine hydrochlo-
ride, 250 mM Tris-HCl (pH 8.5 at 21 °C) and 10 mM
EDTA] and supplemented immediately before use with
150 mM β-mercaptoethanol and 1 mM α-phenylmethyl-
sulfonyl fluoride. Resulting lysates were processed for
SDS-polyacrylamide gel electrophoresis and immuno-
blotting as previously described. Transfers were probed
using the following antibodies: Mcl-1 (BD ParrMingen,
San Diego, CA, USA), Bcl-2 (Dako, Glostrup, Denmark),
p53 (Neomarkers, Fremont, CA, USA), cyclin D1
(Calbiochem, San Diego, CA, USA), phosphoRNA poly-
merase II (Covance, Cumberland, VA, USA) phospho-
(Thr)STAT3 (Cell Signaling, Beverly, MA, USA), and
actin (Santa Cruz, Santa Cruz, CA, USA; loading control).

Results and Discussion

Table 1 summarizes the characteristics and disease his-
tory of the 18 patients treated on study. Patients received
a median of three (range, 1-5) treatment regimens prior to
enrollment Sixty-one percent of patients were refractory
to prior therapy and 55% had previously undergone high
dose therapy with hematopoietic stem cell support. As
shown in Table 2, the most frequent adverse effects were
leukopenia and diarrhea, followed by thrombocytopenia,
nausea, and fatigue. One patient with grade 4 thrombo-
cytopenia deemed not related to flavopiridol therapy
died 4 days after starting flavopiridol due to gastrointesti-
nal bleeding. One patient developed renal failure, attrib-
uted to progressive myeloma. No thrombotic episodes
were observed. All patients have ended the active tr eat-
ment phase. The mean number of cycles administered
was 1.6 (range 1-3). Of the 18 treated patients, three dis-
continued therapy because of adverse events, 15 due to
progression, and one due to death. No indication of anti-
myeloma effects of flavopiridol was seen in any study
patient.

Sufficient pre-therapy fresh bone marrow plasma cells
for studying the ex vivo cytotoxicity of flavopiridol were
available from six patients. After 48 hours of continuous

<table>
<thead>
<tr>
<th>Table 1. Patients' characteristics.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number or median (range)*</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Male/Female</td>
</tr>
<tr>
<td>Duration of disease, months</td>
</tr>
<tr>
<td>Prior regimens</td>
</tr>
<tr>
<td>Disease status</td>
</tr>
<tr>
<td>Relapse on therapy</td>
</tr>
<tr>
<td>Relapse off therapy</td>
</tr>
<tr>
<td>Primary refractory</td>
</tr>
<tr>
<td>Plasma cell labeling index ≥1%</td>
</tr>
<tr>
<td>C-reactive protein &gt;0.4</td>
</tr>
<tr>
<td>Lactate dehydrogenase &gt; upper limit of normal</td>
</tr>
<tr>
<td>Bone marrow plasma cells &gt;40%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 2. Adverse events.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 3-4 %</td>
</tr>
<tr>
<td>Hematologic</td>
</tr>
<tr>
<td>Anemia</td>
</tr>
<tr>
<td>Leukopenia</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
</tr>
<tr>
<td>Gastrointestinal*</td>
</tr>
<tr>
<td>Diarrhea</td>
</tr>
<tr>
<td>Nausea</td>
</tr>
<tr>
<td>Vomiting</td>
</tr>
<tr>
<td>Infection</td>
</tr>
<tr>
<td>Cardiovascular Hypotension</td>
</tr>
<tr>
<td>Constitutional Symptoms Fatigue</td>
</tr>
<tr>
<td>Metabolic</td>
</tr>
<tr>
<td>Hypercalcemia</td>
</tr>
<tr>
<td>Hyperglycemia</td>
</tr>
</tbody>
</table>

*For all adverse events (CTC 2.0) which affected at least 10% of patients;
*One patient died secondary to gastrointestinal bleeding in the setting of grade 4 thrombocytopenia.
exposure to flavopiridol (500 nM), there was a variable effect on cell numbers (Figure 1A). In response to this exposure, more than 90% reduction in viability was seen in two patients (patients #10 and 14) and 50-70% reduction in three patients (patients #4, 13 and 15). In patient #18 intermediate cytotoxicity was observed. Because bolus followed by 3- hour flavopiridol infusion has been reported to be quickly cytotoxic to CLL cells in vivo,

in RPMI-8226 cells has been effective in blocking flavopiridol-induced apoptosis, and early reduction in Mcl-1 correlated with the induction of apoptosis.\textsuperscript{13} In our studies of myeloma cell lines, the most consistent flavopiridol-induced changes were decreased Mcl-1 and phosphoRNA polymerase II (Figure 1B). We therefore performed immunoblotting of total cellular proteins from sorted myeloma cells pre-therapy and immediately following the third flavopiridol infusion. Cyclin D1 levels were undetectable in all patients (data not shown), and only minimal levels of phosphoRNA polymerase II were detected. The pattern of alterations of cellular polypeptides observed in vitro was not generally recapitulated in vivo (Figure 1C). In only one of eight assessed patients (patient #15) was decreased Mcl-1 accompanied by an increase in p53 and phospho(Tyr)STAT3 levels. Two other patients (patients #10 and 11) had decreasing levels of Mcl-1 accompanied by unexpectedly lower levels of p53 and p(Tyr)STAT-3. We can only conclude that, although the anticipated effects of flavopiridol were achieved in one patient (#15), the effects were either

Figure 1. \textit{In vivo} and \textit{ex vivo} effects of flavopiridol. A. Flavopiridol inhibits myeloma cell proliferation in both myeloma cell lines and also freshly collected CD138-positive patient myeloma cells exposed to flavopiridol \textit{ex vivo}. All cells were treated with continuous exposure to 500 nM flavopiridol. Cell numbers were obtained by counting trypan blue-excluding cells at the specified time points using a hemocytometer. In \textit{ex vivo} experiments, the numbers of corresponding sham-treated trypan blue-excluding cells at designated time points served as controls. Kas 6/1 (IL-6 dependent) and OCI-MY-5 (IL-6 independent) served as representative cell lines. With the exception of patient #4, all patients were treated with flavopiridol in the trial. Data from cell lines are representative of three independent experiments, with each data point representing an average of triplicate determinations. Data from patients' samples represent single experiments for each patient, as only limited myeloma cells were available from patients. B. Effects of flavopiridol therapy on levels of selected polypeptides in Kas 6/1 and OCI-MY-5 myeloma cell lines. Levels of the indicated polypeptides were assessed by immunoblotting, with 100 µL of total cellular proteins loaded per lane. C. Effects of flavopiridol therapy on levels of selected polypeptides in freshly collected CD138 positive cells from study patients. C indicates results in patient MM cells obtained prior to flavopiridol therapy, while F designates results obtained after the third day of three daily flavopiridol infusions for each indicated patient. Levels of indicated polypeptides were assessed by immunoblotting. SDS-PAGE gels were loaded by cell number (generally 2×10⁶ cells/lane), and actin is shown as a loading control. Representative data from the Kas6/1 and Anbl 6 MM cell lines are shown on the left.
We found that, although reasonably well tolerated, flavopiridol one-hour daily infusions for 3 consecutive days did not provide clinical benefit in patients with heavily pre-treated, refractory MM. Despite a strong rationale and good preclinical data, no cytotoxic or cytostatic activity was observed in our patients. Our in vitro and ex vivo studies, indicating that sustained exposure to micromolar concentrations of drug are required to achieve significant cytotoxicity, suggest that the current treatment schedule was likely inadequate to achieve these required levels. Exposure to flavopiridol for more than 12 hours was required to achieve cytotoxicity in myeloma cells from patients even when concentrations of flavopiridol were as high as 10 µM (data not shown). Currently, loading infusions of flavopiridol followed by maintenance infusions – designed to achieve prolonged micromolar flavopiridol concentrations – are meeting with some success in CLL patients and may be worthy of exploration in MM.

References