Abstract

Cytoreg® is a novel anticancer drug candidate which uses Hydrofluoric and inorganic acids as active compounds. The drug has cytotoxic, selective apoptotic, antiviral and antineoplastic properties. It does not change concentration with time and is not photostable. Cytoreg is toxic in blood, and can be administered orally and by IV. Cytoreg has demonstrated potent activity in vitro and in vivo. These studies are being expanded into in vivo animal models to validate drug biomarkers and mechanism of action.

Study Objective

The specific aim of this small pilot study was to determine the effect of Cytoreg on expression of select biomarkers in B16-F10 murine melanoma lung metastases. The aim of this pilot study was to evaluate the effects of the drug on biomarkers using in vivo study. Quantitative analysis of biomarkers (Bcl-2, Bax, Survivin, Smac/Diablo, Raf-1 and its activated form) was performed.

Materials And Methods

Animal Tissue

Murine lung tissue with B16-F10 melanoma metastases was harvested by the Preclinical Research Laboratory at the University of Texas Health Science Center at San Antonio. The lung tissue was harvested from five different mice. The tissue was then deparaffinized and stained with anti-murine antibodies as follows: Bcl-2 (NeoMarkers, Fremont, CA), Bax (Cell Signaling, Beverly, MA), Smac/Diablo (Santa Cruz Biotechnology, Santa Cruz, CA), Survivin (Novus, Littleton, CO), Raf-1 (Santa Cruz Biotechnology, Santa Cruz, CA) (P) Raf-1 (serine 259) (Cell Signaling, Beverly, MA). Quantitative analysis of biomarkers was performed in thin sections from three representative tumor metastases selected from the drug treatment group (CytoReg at 0.654 mg/mouse p.o. qd x 5 to end) and the corresponding control group (vehicle p.o. qd x 5 to end) - Table 1 - by immunostaining with murine-specific antibodies.

Biomarker Analysis

The tissue was dehydrated through graded ethanols and embedded in paraffin. Thin sections were then prepared from paraffin blocks. Slides were deparaffinized and stained with anti-murine antibodies as follows: Bcl-2 (NeoMarkers, Fremont, CA), Bax (Cell Signaling, Beverly, MA), Smac/Diablo (Santa Cruz Biotechnology, Santa Cruz, CA), Survivin (Novus, Littleton, CO), Raf-1 (Santa Cruz Biotechnology, Santa Cruz, CA) (P) Raf-1 (serine 259) (Cell Signaling, Beverly, MA). Quantitative analysis of biomarkers was performed in thin sections from three representative tumor metastases selected from the drug treatment group (CytoReg at 0.654 mg/mouse p.o. qd x 5 to end) and the corresponding control group (vehicle p.o. qd x 5 to end) - Table 1 - by immunostaining with murine-specific antibodies.

Bax (CytoReg) and Bcl-2 (Control) images of Survivin in lung metastases and the control tissue are shown in picture. The SURVIVIN Score decreased from a total of 7 in the vehicle control to a score of 5 in the Cytoreg treated group.

Results And Discussion

In vivo experiments conducted by the Preclinical Research Laboratory at the CTRC Institute for Drug Development have shown that Cytoreg reduced the number of lung metastases in the B16-F10 murine melanoma model. The goal of this pilot study was to evaluate the effects of the drug on biomarkers using diverse pathways such as apoptosis (Bcl-2, Bax, Smac/Diablo), signal transduction (Raf-1, P-Raf-1), and survival (Survivin). The in vivo results (Table 2), representative tumor metastases were selected in the treatment groups corresponding to the control group (vehicle p.o. qd x 5 to end) and the lowest dose of Cytoreg group (group 5; CytoReg at 0.654 mg/mouse p.o. qd x 5 to end). Notably, this dose of Cytoreg reduced the number of lung metastases by 75%. For a preliminary assessment of drug effects on biomarker expression, we have selected three representative specimens in the control and drug treatment group, based on the numbers of metastases closest to the means in each group.

The expression of Bcl-2, Bax, Survivin, Smac/Diablo and Raf-1 was determined using in vivo study. A summary of the immunohistochemical data is shown in Table 2, along with the total scores for the examined biomarkers.

Table 1 - Cytoreg® vs. B16-F10 Murine Metastatic Melanoma Model

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bcl-2 Score</th>
<th>Bax Score</th>
<th>Survivin Score</th>
<th>Raf-1 Score</th>
<th>P-Raf-1 Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle p.o.</td>
<td>7</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>CytoReg 0.654 mg/mouse p.o. qd x 5</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Summary and Conclusions

We have examined the expression of six tumor biomarkers in B16-F10 lung metastases from 3 vehicle controls and 3 Cytoreg-treated animals. Surprisingly, some biomarkers (Bcl-2, Smac/Diablo) were undetected in the metastases, although the expression of Raf-1 was apparently lower in the Cytoreg group. Taken together with other evidence, this suggests that Cytoreg® may have an effect on Raf-1 activation and regulation of the Raf-1 signaling pathway.