ABSTRACT

PURPOSE: TRC102 (methoxyamine) reverses resistance to alkylating agents by inhibiting base excision repair (BER; a mechanism of DNA repair), thereby increasing TRC102 Bound AP Sites are Refractory to the Repair TRC102 Bound AP Sites are Refractory to the Repair

AP Sites Detected in H460 Cells

TRC102 Reduced the Number of Available AP Sites in Pemetrexed-Treated Cells (by 60-80%), Indicating Successful Inhibition of BER. TRC102 Treatment Increased DNA Strand Breaks (2-fold Increase versus Treatment with Pemetrexed Alone) and Apoptosis. TRC102 Increased the Activity of Pemetrexed in Vivo (Tumor Growth Delay of 2 Days in Mice Bearing H460 or A549 Lung Cancer Xenografts Treated with 150 mg/kg Pemetrexed Alone versus 9 Days in Mice Treated with 150 mg/kg Pemetrexed + 4 mg/kg TRC102, p < 0.05). In Vivo Systemic Toxicity Was Not Increased and TRC102 Alone Had No Effect in Vivo or In Vivo. TRC102 Also Increased Pemetrexed Activity on HCT116 Colorectal and MDA-MB-468 Breast Cancer Xenografts. Moreover, the Combination Selectively up-regulated the BER proteins, uracil DNA glycosylase and polymerase β, which Provides Strong Evidence That DNA Damage Induced by the Drug Combination Induces BER. CONCLUSION: TRC102 Effectively Inhibits BER in Cancer Cells Treated with Pemetrexed. Inhibition of BER by TRC102 Results in an Increase in DNA Strand Breaks and Apoptosis, and Improved Anti-Tumor Activity Versus Treatment with Pemetrexed Alone. Given Its Preclinical Safety and Efficacy Profile, Clinical Study of TRC102 Combined with Pemetrexed is Warranted.

RESULTS

TRC102 Enhanced Antitumor Effect of Pemetrexed in Nude Mice Carrying Human Tumors

<table>
<thead>
<tr>
<th>Tumor Volume (mm³)</th>
<th>Control</th>
<th>Pemetrexed</th>
<th>P+T</th>
<th>TRC102</th>
<th>Pem+TRC102</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemetrexed alone</td>
<td>2000</td>
<td>1500</td>
<td>1200</td>
<td>1000</td>
<td>800</td>
</tr>
<tr>
<td>4 mg/kg pemetrexed + 4 mg/kg TRC102</td>
<td>1000</td>
<td>800</td>
<td>600</td>
<td>500</td>
<td>400</td>
</tr>
</tbody>
</table>

Hypotheses:

1) Pemetrexed Inhibits Several Key Enzymes in the De Novo Pathways of Pyrimidine and Purine Biosynthesis, Leading to Nucleotide Pool Imbalances, Which Favor the Incorporation of Mismatched Bases to Initiate Base Excision Repair (BER).

2) TRC102 Blocks BER and Enhances Cytotoxicity of Pemetrexed.

SUMMARY

1) Pemetrexed Induces the Incorporation of Abnormal Bases That Are Removed by DNA Glycosylases as Part of Base Excision Repair (BER), Thereby Producing AP Sites.

2) TRC102 Blocks BER and Enhances AP Sites.

3) Inhibition of BER by TRC102 Enhances Pemetrexed Antitumor Activity Against Several Human Solid Tumors, Including Lung, Breast and Colorectal Cancers.