ISR Failure for 5-Fluorouracil Analysis from Patients Dosed with Capecitabine

Janine McKnight1, Chris Schmidt1, Kevin Lam1, Kristy Strooisma1, Sara Andersen1, Brian Dean2 and Xiao Ding2
1Covance Laboratories Inc., Madison, WI; 2Genentech, A Member of the Roche Group, South San Francisco, CA

Introduction

Here we describe the investigation of an Incurred Sample Reanalysis (ISR) failure for the measurement of 5-fluorouracil (5-FU) in human subjects dosed with capecitabine.

Materials and Methods

5-fluorouracil on samples from patients that were dosed orally with capecitabine and from patients dosed by infusion with 5-FU. ISR analysis for 5-fluorouracil on samples from patients that were dosed orally with capecitabine failed this evaluation while 5-FU IV dosed patients in the same study had acceptable ISR results, shown at right.

Background

Capacitabine (Xeloda, Roche) is an orally-administered chemotherapy agent for treating breast and colorectal cancers. Capacitabine is a prodrug which is enzymatically converted to the active drug 5-fluorouracil (5-FU) within the tumor. 5-Fluorouracil (Adrucil, Teva Generics) is administered by infusion and inhibits action of the enzyme, thymidylate synthase (required for DNA replication) which causes cancerous cells to undergo cell death. It is used to treat breast, colorectal, stomach, pancreatic and skin cancers. A clinical study involved the measurement of 5-FU from patients who were dosed orally with capacitabine and from patients dosed with infusion of 5-FU. ISR analysis for 5-fluorouracil on samples from patients that were dosed orally with capacitabine failed this evaluation while 5-FU IV dosed patients in the same study had acceptable ISR results, shown at right.

Extraction Method

1) Aliquot 100 μL plasma
2) Add 25 μL internal standard solution
3) Add 100 μL, 10% acetic acid in water
4) Vortex-mix, load sample to SLE 200+ plate (Isolute)
5) Elute with 1000 μL ethyl acetate
6) Dry eluent under nitrogen
7) Reconstitute in 100 μL 5% methanol in water

LC-MS/MS Method

LC system: LC-20AD, Shimadzu
HPLC column: Waters Atlantis dC18, 150 x 2.1 mm, 5 μm particle size
Mobile phases: 10 mM ammonium formate, methanol
Flow Rate: 0.400 mL/min
Typical injection volume: 5 μL
Mass spectrometer: API 5000 (AB Sciex)
Detection: ESI negative mode
Mass spectrometer: API 5000 (AB Sciex)
Typical injection volume: 5 μL
Flow Rate: 0.400 mL/min
Typical injection volume: 5 μL
Mass spectrometer: API 5000 (AB Sciex)
Detection: ESI negative mode
Mass spectrometer: API 5000 (AB Sciex)

Investigation Part 1: Which metabolite is converting to 5-FU?

Blank plasma was spiked individually with capacitabine, 5'-DFCR and 5'-DFUR at potential C50 concentrations.

These individually fortified test pools were left at room temperature for 2 hours and then analyzed to determine 5-FU concentrations.

Remaining individual test pools were stored at -70°C for at least 24 hours, thawed and then analyzed to determine 5-FU concentrations.

Results

5-FU ISR Results for Clinical Study

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Test condition</th>
<th>Concentration</th>
<th>Original Conc. (nM)</th>
<th>Mass Spec Conc. (nM)</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>No inhibitor</td>
<td>24 h on wet ice</td>
<td>1.0</td>
<td>1.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>No inhibitor</td>
<td>6 h on wet ice</td>
<td>1.0</td>
<td>1.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>No inhibitor</td>
<td>24 h on room temp</td>
<td>1.0</td>
<td>1.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>5-fluoro-5'-deoxyuridine (5'-DFUR)</td>
<td>24 h on wet ice</td>
<td>1.0</td>
<td>1.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>5-fluoro-5'-deoxyuridine (5'-DFUR)</td>
<td>6 h on wet ice</td>
<td>1.0</td>
<td>1.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>5-fluoro-5'-deoxyuridine (5'-DFUR)</td>
<td>24 h on room temp</td>
<td>1.0</td>
<td>1.1</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

Impact of Acids on Conversion of 5'-DFUR to 5-FU in Plasma

<table>
<thead>
<tr>
<th>Acid</th>
<th>Test condition</th>
<th>Concentration</th>
<th>Original Conc. (nM)</th>
<th>Mass Spec Conc. (nM)</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% formic acid</td>
<td>24 h on wet ice</td>
<td>1.0</td>
<td>1.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>0.1% acetic acid</td>
<td>24 h on wet ice</td>
<td>1.0</td>
<td>1.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>0.1 mg/mL THU</td>
<td>24 h on wet ice</td>
<td>1.0</td>
<td>1.1</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion

After testing capacitabine, 5'-DFCR and 5'-DFUR, it was determined that 5'-DFUR was the most effective inhibitor. This new approach has resulted in a clinical study with acceptable ISR results for 5-FU from patients dosed with capacitabine utilizing the above approach sample collection procedure.

Acknowledgements

The authors gratefully acknowledge Fengxia Li, Yifan Shi, Harumi Shimizu, and Abigail Trenchak for their expertise.

References


5-FU ISR Results for New Clinical Study for Patients Dosed with Capacitabine

References