Expression Of FGFR2b In Gastric Cancer As Measured By Immunohistochemistry With A Highly Specific Monoclonal Antibody

Amit M. Deshpande,1 Servando Palencia,1 David I. Bellovin,1 Abigail T. Gemo1, Tina Giese1, Bradley Stohr2, Kristen L. Pierce1, Gerrit Los1
1Five Prime Therapeutics, Inc., South San Francisco, CA; 2University of California San Francisco, San Francisco, CA

#2845

**MATERIALS AND METHODS**

Immunohistochemistry (IHC) and Fluorescence In-Situ Hybridization (FISH)

FGFR2b-D is a mouse monoclonal antibody generated at Five Prime. IHC protocols were performed using the ready-to-use VECTASTAIN Elite ABC kit (Vector labs, Burlingame, CA). All staining was performed using the Dako Autostainer.

Fish analyses were performed using standard protocols with FGFR2 probes obtained from Empire Genomics (Buffalo, NY).

Specificity and Sensitivity studies

23T cells transfected with various FGFRs, including FGFR1, FGFR2b, FGFR2c, FGFR3, and FGFR4 were used to define specificity of FGFR2b. 7 commercially available anti-FGFR2 antibodies were used for comparison. 90K cells stably expressing a dioxycycline-inducible FGFR2b construct were used to define sensitivity of FPR2-D. FGFR2b expression was induced by culturing the cells with dioxycycline. Cells were collected on day 3 for processing into FFPE blocks.

FGFR2b amplification and Expression Analysis in Clinical Samples

FGFR2 copy number and protein expression were evaluated in archival samples from 186 gastric cancer samples by FISH and IHC analyses respectively. Amplification of FGFR2 was defined as a ratio of FGFR2/CEN10 >3. IHC expression was reported using a qualitative 0-3+ scale.

**RESULTS**

Antibody FPR2-D specifically detects FGFR2b:

FPR2-D was able to detect low levels of FGFR2b expressed in 90K/3 cells following induction with dioxycycline (panel A). Western blot analysis shows relative levels of FGFR2b following induction (panel B).

FPR2-D was shown to detect FGFR2b in 20% of gastric cancer cells with FGFR2 amplification. In contrast, 7 commercially available anti-FGFR2 antibodies cross-reacted with various FGFRs. Staining using anti-FGFR antibody ab58201 (Abcam, Cambridge, MA) which has been used extensively in literature as shown in an example. In addition, FPR2-D also showed significantly higher sensitivity compared to the Abcam antibody as evident in panels for FGFR2 expressing cells.

**Table 1: Summary of FISH and IHC analyses for FGFR2 copy number and expression.**

<table>
<thead>
<tr>
<th></th>
<th>IHC</th>
<th>FISH Positive</th>
<th>FISH (%)</th>
<th>Total (%)</th>
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<tbody>
<tr>
<td>3+</td>
<td>11</td>
<td>(5.9%)</td>
<td>20 (10.8%)</td>
<td>31 (16.7%)</td>
</tr>
<tr>
<td>2+</td>
<td>0</td>
<td>30 (16.1%)</td>
<td>30 (16.1%)</td>
<td></td>
</tr>
<tr>
<td>0/1+</td>
<td>0</td>
<td>125 (67.2%)</td>
<td>125 (67.2%)</td>
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**SUMMARY**

1. Five Prime Therapeutics has developed a sensitive and specific monoclonal antibody, FPR2-D, for detecting expression of the b-isoform of FGFR2, a known driver of tumorigenesis in gastric and potentially other cancers.
2. Analysis of archival tissue from 186 gastric cancer cases identified ~6% cases with amplification of the FGFR2 gene and an additional 10.8% cases with strong IHC reactivity for FGFR2b.
3. These results suggest that a potentially larger percentage of patients with gastric cancer may be appropriately identified as candidates for FGFR2b-specific therapies. We intend to explore the use of this antibody as a potential companion diagnostic for our FPA144 program.