X4P-001, an Orally Bioavailable CXCR4 Antagonist Increases Immune Cell Infiltration and Tumor Inflammatory Status in the Microenvironment of Melanoma

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Background

- The CXCR4/CXCL12 pathway plays a central role in the trafficking of key immune cells in the tumor microenvironment (TME).
- X4P-001 is an oral, selective, allosteric CXCR4 inhibitor. CXCR4 antagonist treatment alone demonstrates robust inhibition of murine B16-OVA melanoma growth.²
- We hypothesize that the disruption of the CXCR4/CXCL12 signaling by X4P-001 will favorably modulate the immune cell profile in the TME and increase CD8⁺ T cell infiltration, improving responses to checkpoint inhibitors and other backbone therapies.
- A biomarker-driven Phase 1b clinical study was conducted in melanoma patients to test this hypothesis (NCT02823405).

Key Efficacy Criteria

Inclusion:
- ≥ 18 years of age
- Histologically confirmed malignant melanoma
- ≤ 2 separate cutaneous or subcutaneous lesions suitable for biopsies (≥ 3 mm)

Exclusion:
- ECOG Performance Status ≥ 2
- Prior checkpoint inhibitor therapies (anti-CTLA-4, PD-1, PD-L1) or oncolytic virus therapy
- Ongoing IV, INTRAVENOUS C, or uncontrolled infection
- Occurrences of myocardial infarction, ≥ Grade 3 hemorrhage, chronic liver disease, or other active malignancies in the past 6 months

Methods

As of October 3, 2018 16 patients have been enrolled, and biopsies from 13 patients have been analyzed.

- Nine had both pre-dose and post-X4P-001 treatment-evaluable biopsies.
- Biopsies were analyzed. Nine had both pre-dose and post-X4P-001 treatment-evaluable biopsies.
- The biopsy sample from patient #5 using HALO software:

Conclusions

- Treatment with X4P-001 as a single agent and in combination with pembrolizumab is well-tolerated.
- X4P-001 monotherapy enhances immune cell infiltration and activation in the TME, as evidenced by:
  - Increased IFN-γ gene expression signature score
  - Increased tumor inflammation signature (TIS)
  - Increased tumor-infiltrating CD8⁺ T cells and CD8⁺/PD-L1⁺ cells

Increased CD8⁺ T Cells Post-X4P-001 Treatment

Increased CD8⁺ T cells in the melanoma tumor interface with normal tissue was quantified using multiplex IHC and HALO image analysis software. CD8⁺-labeled cells within 100 µm of the inside or outside of the tumor boundary with normal tissue were counted.

Increased CD8⁺ T Cells Post-X4P-001 Treatment

CD8 Average Density (cells/mm²)

<table>
<thead>
<tr>
<th>Group</th>
<th>Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dose</td>
<td>2057.63</td>
<td>0.0026</td>
</tr>
<tr>
<td>X4P-001</td>
<td>2640.08</td>
<td>&lt;0.0001</td>
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</tbody>
</table>

Increased Tumor Inflammation Signature (TIS) in the TME

- Based on analysis of multiple IHC staining of tumor samples from patient #5 using HALO software:
  - X4P-001 increased the percentage of CD4, CD8, PD-1, and PD-L1-positive cells in the TME
  - The percentages of Treg (FoxP3⁺) positive cells and macrophages (CD68/CD163/CD16++) in 24% vs. 25.4%, not shown were not altered

Increased Tumor Inflammation Signature (TIS) in the TME

<table>
<thead>
<tr>
<th>Group</th>
<th>Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dose</td>
<td>199.30</td>
<td>0.0012</td>
</tr>
<tr>
<td>X4P-001</td>
<td>2640.08</td>
<td>&lt;0.0001</td>
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Combination Treatment Robustly Increases Serum Concentrations of CCL9 & CCL10

- Increases in multiple chemokinetic factors in serum were observed after single agent treatment with X4P-001. This observation is consistent with increased trafficking of immune cells post CCL9/CCL10 inhibition.
- Mean serum concentration for CXCL9 and CXCL10 are increased after combination treatment.

References:

1. Reference 1.2.3.4.5.6.7.8.
2. Reference 2.3.4.5.6.7.8.
3. Reference 3.4.5.6.7.8.
4. Reference 4.5.6.7.8.
5. Reference 5.6.7.8.
7. Reference 7.8.

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