X4P-001, an Orally Bioavailable CXCR4 Antagonist, Increases T Cell Infiltration in Human Metastatic Melanoma

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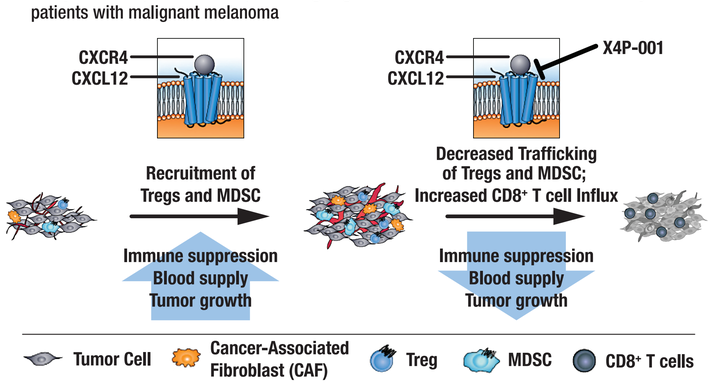
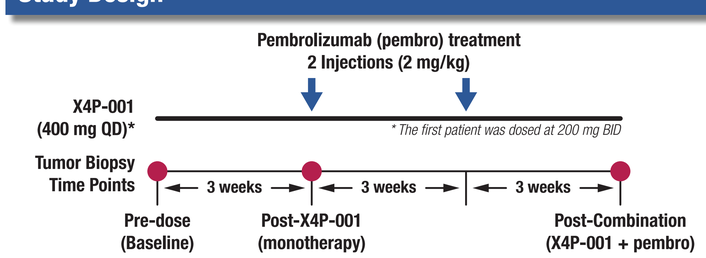
Background

- The CXCR4/CXCL12 axis plays a central role in the trafficking of key immune cells in the tumor microenvironment (TME)[1]
- Enhanced survival is reported in multiple syngeneic mouse models when a CXCR4 antagonist is combined with a checkpoint inhibitor[2,3]
- X4P-001 is an oral, selective, allosteric inhibitor of CXCR4. CXCR4 antagonist treatment alone demonstrated robust inhibition of murine B16-OVA melanoma growth[4]
- It is hypothesized that disruption of CXCR4/CXCL12 signaling by X4P-001 will modulate the immune cell profile within the TME and increase CD8+ T cell infiltration, which will favor an improved response to checkpoint inhibitors
- Study X4P-001 (NCT02823405) is an ongoing biomarker-driven Phase 1b clinical study in patients with malignant melanoma



Study Design



Key Eligibility Criteria

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

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

Immunohistochemistry and NanoString Analysis

- As of August 2nd 2017, 13 patients have been enrolled, and biopsies from 11 patients have been analyzed:
  - Five had both pre-dose and post-X4P-001 treatment-evaluable biopsies, one of whom had an additional biopsy at the end of treatment with combination therapy
  - One had pre-dose and post-combination treatment-evaluable biopsies
- Multiplex immunohistochemistry (IHC) panel included CD4, PD-1, PD-L1, macrophage cocktail (CD68 + CD163), and FoxP3 with DAPI as a nuclear counterstain
- Single-marker IHC (CD8 and granzyme B) and multiplex IHC staining were analyzed by HALO™ (Indica Labs), and the entire tumor area of each specimen was scored
- NanoString nCounter analysis was conducted with the PanCancer Immune probe set using RNA extracted from FFPE slides. Raw counts were normalized using the geometric mean of housekeeping genes

Demographics and Baseline Characteristics

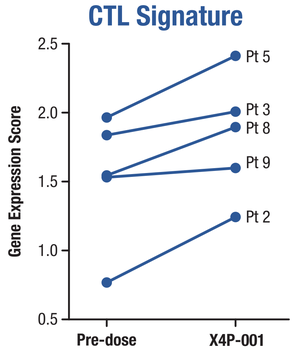
- Mean patient age was 73.8 (± 10.4 years); the median age was 73 (range 53–90 years)
- Of the 13 patients enrolled, 8 (62%) were male and 5 (39%) were female
- 12 patients (92%) were White and 1 (8%) was Asian
- 7 patients (54%) had a screening ECOG status score of 0 and 6 (46%) had a score of 1

Safety

- X4P-001 was generally well-tolerated
- Adverse Events (AEs) assessed as related to X4P-001 during monotherapy (> 10%) were diarrhea (31%) and chills (15%)
- AEs assessed as related to either X4P-001 or pembrolizumab (> 10%) at any time were diarrhea (39%), maculo-papular rash and fatigue (31% each), chills, and acute kidney injury (15% each)
- Grade 3 AEs assessed as related to either X4P-001 or pembrolizumab at any time were maculo-papular rash (15%), diarrhea, acute kidney injury, alanine aminotransferase increased, aspartate aminotransferase increased, blood bilirubin increased, hypertension, and stomatitis (8% each)
- There were no Grade 4 or Grade 5 AEs at any time during the study

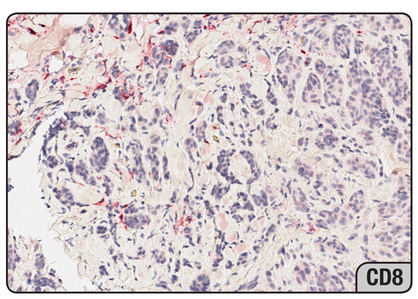
Increased Cytotoxic CD8+ T Cells Post-X4P-001 Treatment

CTL Signature

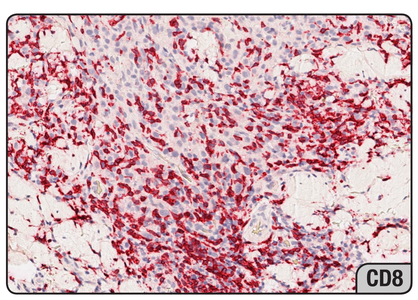


RNA was extracted from FFPE slides using Qiagen's AllPrep kit and analyzed using the NanoString nCounter platform with the PanCancer Immune probe set. Raw counts were normalized using the geometric mean of housekeeping genes. The cytotoxic T lymphocyte (CTL) gene signature was calculated for each patient sample from the geometric mean of normalized counts for *CD8A*, *CD8B*, *FLTLG*, *GZMM*, and *PRF1*. The mean was Log10-transformed to generate the Gene Expression score.

Patient #5 Pre-dose

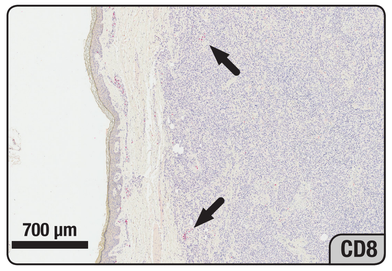


Patient #5 Post-X4P-001

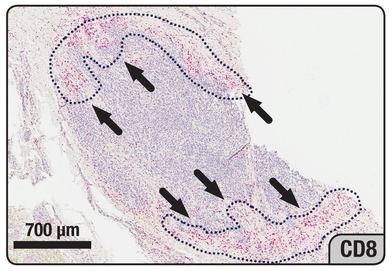


Increased CD8+ T Cells in Tumor Margin Post-Combination Treatment

Patient #4 Pre-dose

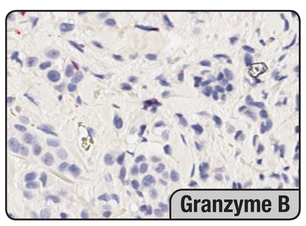


Patient #4 Post-Combination

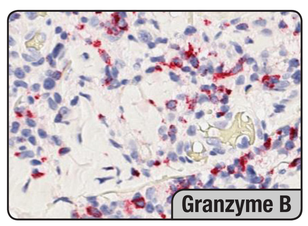


Increased Granzyme B Signal in TME Post-X4P-001 Treatment

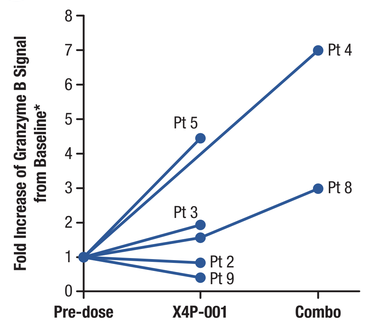
A Patient #5 Pre-dose



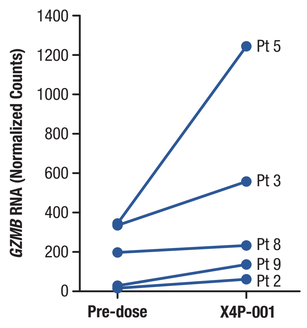
B Patient #5 Post-X4P-001



C Granzyme B/IHC



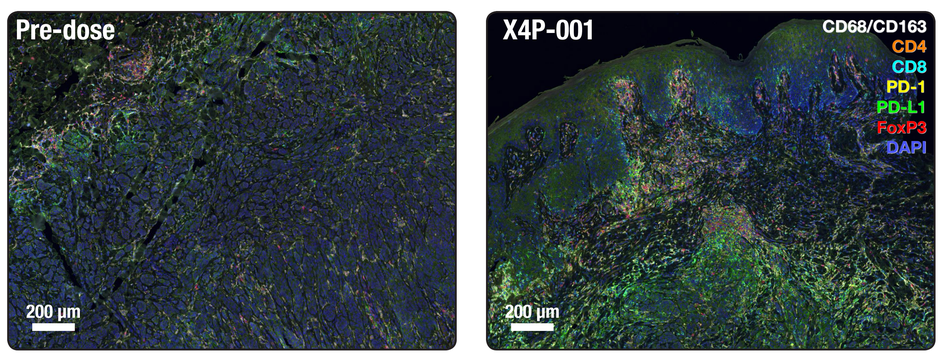
D Granzyme B/NanoString

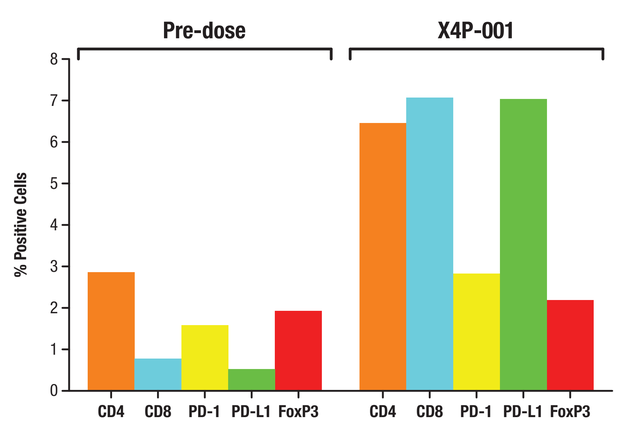


Representative granzyme B IHC staining is shown at baseline (panel A) and following 21 days of X4P-001 treatment (panel B). Panel C shows the fold change of granzyme B positivity post-treatment for all evaluable samples. Quantification was performed using HALO™ software and the entire tumor area was scored. Panel D shows the granzyme B RNA expression level for 5 patients with both pre- and post-X4P-001 single-agent treatment-evaluable biopsies.

References: 1) Duda DG, Kozin SV, Kirkpatrick ND, et al. CXCL12 (SDF1a)-CXCR4/CXCR7 Pathway Inhibition: An Emerging Sensitizer for Anticancer Therapies? *Clin Cancer Res.* 2011;17(8):2074-2080. **2)** Feig C, Jones JO, Kramana, et al. Targeting CXCL12 from FAP-expressing carcinoma associated fibroblasts synergizes with anti–PD-L1 immunotherapy in pancreatic cancer. *PNAS.* 2013;110(50):20212-20217. **3)** Righi E, Kashiwagi S, Yuan J, et al. CXCL12/CXCR4 Blockade Induces Multimodal Antitumor Effects That Prolong Survival in an Immunocompetent Mouse Model of Ovarian Cancer. *Cancer Res.* 2011; 71(16):5522-5534. **4)** Saxena, Wang and Mier. Efficacy and mechanism of action of CXCR4 inhibition in B16 OVA melanoma model. SITC, Nov 2017. **5)** Ayers, Lunceford, Nebozhyn et al. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. *Clin Invest.* 2017;127(8):2930–2940.

Increased CD8 : FoxP3 Ratio and PD-L1 in TME Post-X4P-001 Treatment



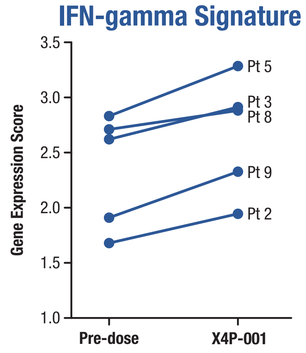


Based on analysis of multiplex IHC staining of tumor samples from patient #5 using HALO™ software:
- X4P-001 increased the percentage of CD4, CD8, PD-1, and PDL-1 positive cells in the TME
- The percentages of Treg (FoxP3 positive) cells and macrophages (CD68/CD163positive; 24.1% vs. 25.4%; not shown) were not altered

Formalin-fixed paraffin-embedded melanoma samples were stained sequentially with a 6-component immunophenotyping antibody panel, including CD4, CD8, PD-1, PD-L1, macrophage cocktail (CD68 + CD163), and FoxP3. DAPI was used as a nuclear counterstain. Antibodies were detected using HRP-catalyzed deposition of fluorescent tyramide substrates (Opal, Perkin-Elmer). Images were obtained using spectral imaging, autofluorescence subtraction and unmixing (Vectra 3.0, Perkin-Elmer), and analyzed using HALO™ image analysis software.

X4P-001 Increased the IFN-gamma Signature in the TME

IFN-gamma Signature



The Interferon-gamma gene signature, based on Ayers et al[5], was calculated from RNA samples extracted from patient FFPE slides, as previously described. The geometric mean was determined from the normalized counts of six genes (*IFN-gamma*, *CXCL9*, *CXCL10*, *HLA-DRA*, *IDO1*, *STAT1*) and then Log10-transformed to generate the Gene Expression score.

Conclusions

- X4P-001 as a single agent and in combination with pembrolizumab is generally safe and well-tolerated
- Preliminary evidence of enhanced immune cell infiltration and activation is observed in the tumor microenvironment with X4P-001 alone:
  - Increased CD8+ T cells
  - Increased cytotoxic T lymphocyte (CTL) gene expression signature score
  - Increased granzyme B signal
  - Increased IFN-gamma gene expression signature score
  - No change in FoxP3-expressing immune-suppressive cells
- Increased IFN-gamma gene expression signature scores and PD-L1 levels after single-agent X4P-001 treatment support the use of X4P-001 in combination with anti-PD-1 therapy
- Enrollment is ongoing; further biomarker analysis is in progress

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