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X4P-001, an Orally Bioavailable CXCR4 Antagonist, Enhances Immune Cell Infiltration and Activation in the Tumor Microenvironment of Melanoma

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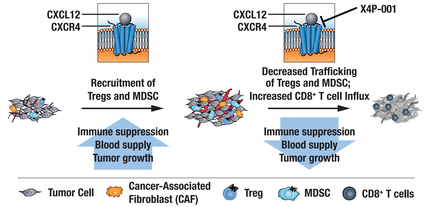
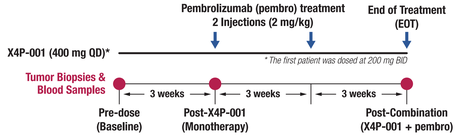
Background

- The CXCR4/CXCL12 pathway plays a central role in the trafficking of key immune cells in the tumor microenvironment (TME).[1]
- 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 CXCR4/CXCL12 signaling by X4P-001 will favor an improved response to checkpoint inhibitors by modulating the immune cell profile within the TME and increasing CD8⁺ T cell infiltration.
- A biomarker-driven Phase 1b clinical study is being conducted in melanoma patients to test this hypothesis (NCT02823405).



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

Methods

Sample Assessments: Sixteen patients have been enrolled. Clinical data for the first 13 patients is presented in this poster. Biopsies and blood samples from 11 patients have been analyzed at the time points depicted in the Study Design diagram. Five had both pre-dose and post-X4P-001 treatment-evaluable biopsies (one of whom had an additional biopsy at the end of combination therapy). A sixth patient had pre-dose and post-combination treatment-evaluable biopsies. Sera was prepared from the collected blood samples, and the concentrations of specific chemokines, cytokines, and growth factors were measured using the Multi-Analyte Profile platform (Myriad RBM).

Multiplex immunofluorescence (mIF): Formalin-fixed paraffin-embedded (FFPE) tumor samples were obtained from melanoma patients at the treatment time points shown in the Study Design diagram. Slides were sequentially stained with antibody panels after rounds of heat-induced epitope retrieval and detected by antibody-binding HRP-containing polymers in conjunction with fluorescent tyramide substrates (Opal, Perkin-Elmer). DAPI was used as a nuclear counterstain. Fluorochromes with spectral overlap were imaged using spectral deconvolution and autofluorescence-subtraction (Vectra 3.0, Perkin-Elmer). Whole-slide scans were imaged using the Aperio-FL system (Leica Biosystems) and transferred into HALO (Indica Labs) for quantitative digital image analysis.

Nanostring: RNA was extracted from FFPE slides for Nanostring analysis and analyzed using the PanCancer Immune Profiling and PanCancer Progression Panels supplemented with 30 user-defined genes (Nanostring Technologies). Raw counts were normalized using the geometric mean of housekeeping genes, and the normalized data from both panels were merged and scaled based on the expression of 134 overlapping probes using nSolver software (Version 4.0). The tumor inflammatory signature (TIS) was calculated from 18 genes by taking the Log10 of the geometric mean of the normalized counts across each gene set to generate a "Gene signature score".[3]

Demographic and Baseline Characteristics

X4P-001 + Pembro (N = 13)		
Age (Years)	Mean (±SD)	73.8 (± 10.4)
	Median (min, max)	73 (53, 90)
Gender	Male	8 (62%)
	Female	5 (39%)
Ethnicity	Not Hispanic or Latino	13 (100%)
Race	White	12 (92%)
	Asian	1 (8%)
Screening ECOG Status	0	7 (54%)
	1	6 (46%)
Disease Characteristics (N = 13)		
Resectable Disease	Yes	9 (69%)
	No	4 (31%)
Stage of Disease at Enrollment	IIIB	4 (31%)
	IIIC	7 (54%)
	IV M1A	2 (15%)
# of Prior SystemicTherapies	0	12 (92%)
	1	1 (8%)

Safety

Adverse Events (≥10%) Related to X4P-001 Monotherapy During Initial 3-Week Treatment (N = 13)			
AE Preferred Term	All N (%)	Grade 3 N (%)	≥ Grade 4 N (%)
All	9 (69)	0	0
Diarrhea	4 (31)	1 (8)	0
Chills	2 (15)	0	0

Adverse Events (≥10%) Related to X4P-001 or Pembrolizumab During 9-Week Treatment (N = 13)			
AE Preferred Term	All N (%)	Grade 3 N (%)	≥ Grade 4 N (%)
All	10 (77)	4 (31)	0
Diarrhea	6 (46)	1 (8)	0
Rash Maculopapular	4 (31)	2 (15)	0
Fatigue	4 (31)	0	0
Acute Kidney Injury	2 (15)	1 (8)	0
Chills	2 (15)	0	0
Oral Candidiasis	2 (15)	0	0

- X4P-001 was well-tolerated as monotherapy or in combination with pembrolizumab.
- Adverse events (AEs, regardless of relationship; > 20%) were diarrhea (54%), fatigue (46%), rash maculopapular (31%), and constipation (23%) for monotherapy and combination treatment.
- There were no Grade 4 or Grade 5 AEs at any time during the study.
- Of the 13 pts, 9 completed the study and 4 discontinued treatment due to AEs:
  - One patient discontinued during X4P-001 monotherapy due to acute diarrhea.
  - Three patients discontinued during combination treatment:
    - » Grade 3 immune-mediated drug reaction (1 pt)
    - » Grade 3 rash maculopapular (1 pt)
    - » Grade 3 mucositis and Grade 2 palmar-plantar erythrodysesthesia (1 pt)

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References: 1) Duda DG, Kozin SV, Kirkpatrick ND, et al. CXCL12 (SDF1α)-CXCR4/CXCR7 Pathway Inhibition: An Emerging Sensitizer for Anticancer Therapies? *Clin Cancer Res.* 2011;17(8):2074-2080. **2)** Saxena, Wang and Mier SITC, Nov 2017. **3)** Ayers, Lunceford, Nebozhyn et al. IFN-γ-related mRNA profile predicts clinical response to PD-1 blockade. *Clin Invest.* 2017;127(8):2930–2940. **4)** Yamazaki N, Kiyohara Y, Uhara H, et al. Cytokine biomarkers to predict antitumor responses to nivolumab suggested in a phase 2 study for advanced melanoma. *Cancer Sci.* 2017; 108:1022-1031.

Increased CD8:FoxP3 Ratio in TME Post-X4P-001 Monotherapy

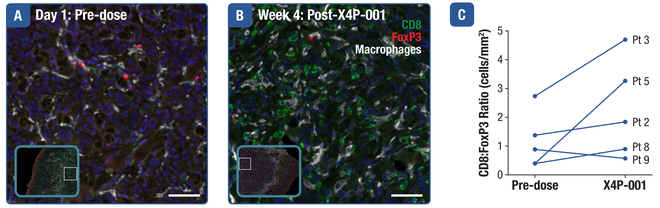
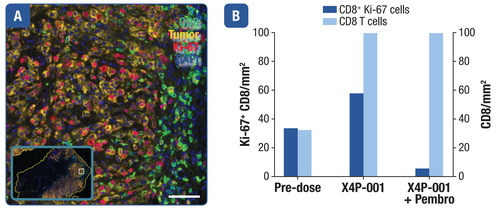


Figure 1: X4P-001 induces proinflammatory changes in the TME. Matched FFPE tumor samples from melanoma patients were stained sequentially by mIF for CD8 (cytotoxic T cells), FOXP3, and a CD68/CD163 cocktail (macrophages) and imaged using spectral deconvolution and auto fluorescence-subtraction, as described in Methods. **(A)** High-power scan of stained pre-treatment tumor tissue; inset shows a low-power biopsy scan with a white box outlining the area imaged in the large panel. **(B)** High-power scan of stained tumor tissue after 3 weeks of X4P-001 monotherapy (scale bar = 100 µm); inset shows a low-power biopsy scan with a white box outlining the area imaged in the large panel. **(C)** Proinflammatory changes in CD8/FOXP3 ratio after X4P-001 monotherapy calculated using HALO-based quantitation for all 5 patients with evaluable matched biopsy samples.

Increased CD8 Density and Density of Proliferating CD8 T cells Post-X4P-001 Monotherapy

Figure 2: X4P-001 increases the density of proliferating CD8⁺ T cells. Multiplex IHC was performed as described in Figure 1 for CD8 (cytotoxic T cells), Novus melanoma marker cocktail (melanoma cells), and Ki-67 (proliferating cells). Whole-slide scans were imaged using HALO without spectral deconvolution. **(A)** High-power image (scale bar = 100 µm) showing the invasive front, where CD8⁺ T cells (green) abut and infiltrate the periphery of the tumor mass (yellow). Ki-67 (red) labels proliferating melanoma cells and CD8⁺ T cells. The inset shows a low-power image; a white box outlines the area imaged in the large panel. **(B)** HALO-based analysis of total CD8⁺ T cell density (CD8⁺/mm²) and the density of proliferating CD8⁺ T cells (Ki-67+ CD8/mm²), across the entire tissue sample. X4P-001 monotherapy increased the density of CD8⁺ T cells and had an even stronger effect on the density of proliferating CD8⁺ T cells. At EOT, no residual tumor mass remains, and proliferation of residual CD8⁺ T cells is sharply reduced, consistent with a clearance of tumor antigen from the microenvironment.



X4P-001 Monotherapy Increased CD8⁺ T Cell Infiltration

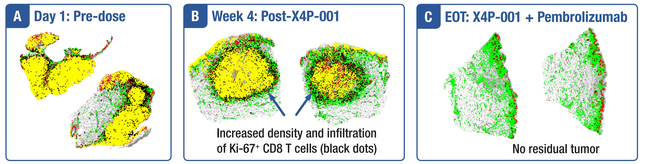
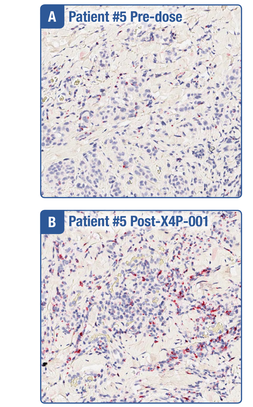
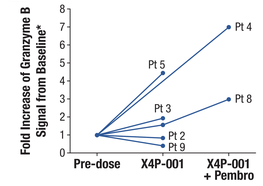


Figure 3: CXCR4 inhibition with X4P-001 increases the density of proliferating CD8⁺ T cells at the tumor margin and infiltration into the tumor mass. FFPE biopsies of melanoma lesions stained by mIF for CD8 (cytotoxic T cells), Ki-67 (proliferating cells), and a cocktail of melanoma-specific antibodies to label tumor as described above. The images represent the graphical output from the nearest neighbor analysis module, with unlabeled cells rendered as gray. After X4P-001 monotherapy, proliferative CD8⁺ T cells surround and infiltrate the tumor lesion. The average distance between CD8⁺ cells and the nearest tumor cell decreases from 95 microns at baseline to 43 microns after X4P-001 monotherapy, and the number of unique neighbors increases, indicating enhanced infiltration.

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



C Granzyme B/IHC



D Granzyme B/Nanostring

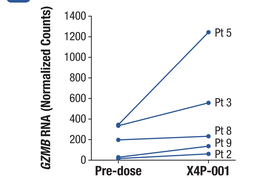
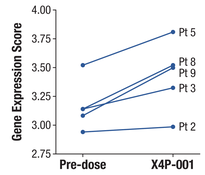


Figure 4: Representative granzyme B IHC staining is shown at baseline **(A)** and following 21 days of X4P-001 monotherapy **(B)**. The fold change of granzyme B positivity post-treatment for all evaluable samples is shown in **(C)**. Quantification was performed using HALO software and the entire tumor area was scored. **(D)** shows the granzyme B RNA expression level for 5 patients with both pre- and post-X4P-001 monotherapy treatment-evaluable biopsies.

Increased Antigen Presentation/Processing Genes Post-X4P-001 Monotherapy

Nanostring Analysis

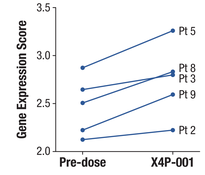
Genes		
B2M	*CD74*	*CTSL*
CTSS	*HLA-DMA*	*HLA-DMB*
HLA-DOB	*HLA-DPA1*	*HLA-DPB1*
HLA-DQA1	*HLA-DQB1*	*HLA-DRA*
HLA-DRB1	*HLA-DRB3*	*PSMB8*
PSMB9	*TAP1*	*TAP2*



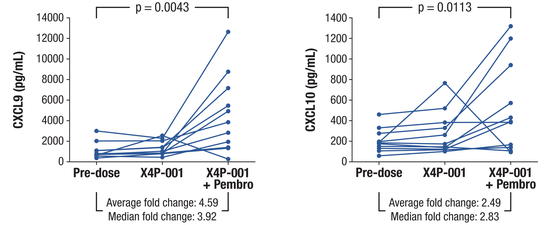
X4P-001 Monotherapy Increased the Tumor Inflammation Signature (TIS) in the TME

Nanostring Analysis

Genes		
CCL5	*CD27*	*CD274*
CD276	*CD8A*	*CMKLR1*
CXCL9	*CXCR6*	*HLA-DQA1*
HLA-DRB1	*HLA-E*	*IDO1*
LAG3	*NKG7*	*PDCD1LG2*
PSMB10	*STAT1*	*TIGIT*



Combination Treatment Robustly Increases Serum Concentrations of CXCL9 & CXCL10



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 CD8/FoxP3 ratio
  - Increased proliferating CD8⁺ cells
  - Decreased distance between CD8⁺ T cells and the nearest tumor cells
  - Increased granzyme B signal
  - Increased antigen presentation/processing genes
  - Increased Tumor Inflammation Signature (TIS)
  - No change in FoxP3-expressing immune-suppressive cells
- Serum concentrations CXCL9 and CXCL10 are increased after combination treatment by 4.5- and 2.5-fold respectively, consistent with immune stimulation[4]
- Enrollment is near completion; biomarker analysis of all evaluable patients will be presented at future scientific conferences

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