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

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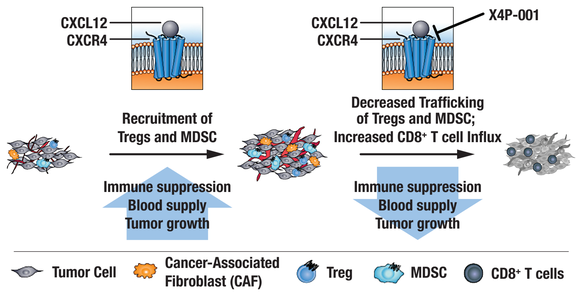
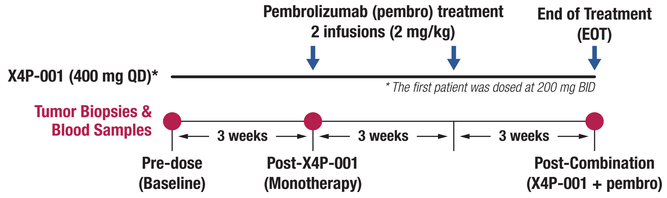
Background

- The CXCR4/CXCL12 pathway plays a central role in the trafficking of key immune cells in the tumor microenvironment (TME).[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 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).



Study Design



Key Eligibility 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 HIV, hepatitis C virus, 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. The biopsy analyses focused primarily on post-X4P-001 monotherapy comparisons to baseline due to limited sample availability post-combination treatment.
- Multiplex immunohistochemistry (IHC) images were acquired on a Perkin Elmer Vectra 3.0 (6-plex images) or an Aperio FL (3-plex images). All images were analyzed using Indica Labs HALO™ analysis software (High-plex and Cytonuclear FL modules).
- Sera was prepared from collected blood samples, and the concentrations of panels of chemokines, cytokines, and growth factors were measured using the Multi-Analyte Profile platform (Myriad RBM). The proteins that were examined include the ones in HCANCER2, HMP8, HMPC19, HMPC42 MAPs and IL15, IFN-gamma, and IL2 by Simoa.
- RNA was extracted from formalin-fixed paraffin-embedded (FFPE) slides 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 analyzed with nSolver software (Version 4.0). The tumor inflammatory signature (TIS) was calculated from 18 genes by taking the $\log_{10}$ of the geometric mean of the normalized counts across each gene set to generate a "gene signature score".[3] The interferon-gamma (IFN-gamma) gene signature was determined from the normalized counts of six genes (*IFN-gamma*, *CXCL9*, *CXCL10*, *HLA-DRA*, *IDO1*, and *STAT1*), as described by Ayers et al.[4]

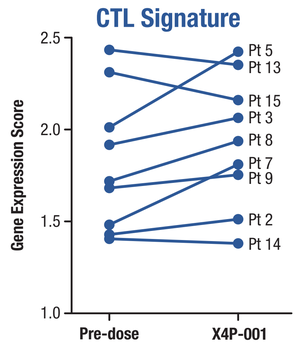
Demographics and Baseline Characteristics

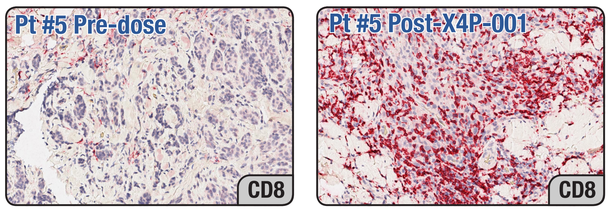
- Mean patient age was 74.6 (± 9.6 years); the median age was 74.5 (range 53-91 years)
- Of the 16 patients enrolled, 10 (62.5%) were male and 6 (37.5%) were female
- 15 patients (94%) were White and 1 (6%) was Asian
- 9 patients (56%) had a screening ECOG status score of 0 and 7 (44%) had a score of 1
- At study entry, 4 patients (25%) were stage IIIB, 10 patients (62.5%) were stage IIIC, and 2 patients (12.5%) were stage IV M1A

Safety

- X4P-001 was well-tolerated as monotherapy and in combination with pembrolizumab
- Adverse Events (AEs) assessed as related to X4P-001 during monotherapy (> 15%) was diarrhea (31%)
- AEs assessed as related to either X4P-001 or pembrolizumab (> 15%) at any time were diarrhea (44%), fatigue (38%), rash macro-papular (25%), and dry eye (19%)
- No X4P-001 related Grade 3 AEs were observed during the monotherapy period
- Grade 3 AEs assessed as related to either X4P-001 or pembrolizumab at any time were rash maculo-papular (13%), hypertension, acute kidney injury, ALT increased, AST increased, blood bilirubin increased, diarrhea, immune-mediated adverse reaction, and stomatitis (6% each)
- There were no Grade 4 or Grade 5 AEs at any time during the study

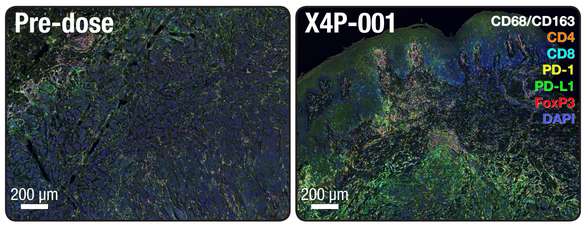
Increased Cytotoxic CD8+ T Cells Post-X4P-001 Treatment



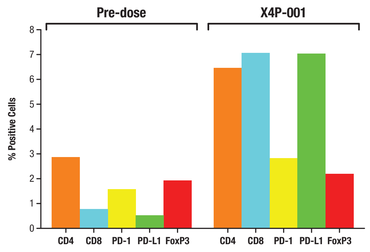


NanoString analysis and mIF staining were conducted as described in Methods. Raw counts were normalized using the geometric mean of housekeeping genes. The cytotoxic T lymphocyte (CTL) gene signature was calculated for each patient sample from the geometric mean of normalized counts for *CD8A*, *CD8B*, *FLTLG*, *GZMM*, and *PRF1*. The mean was $\log_{10}$-transformed to generate the gene expression score.

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

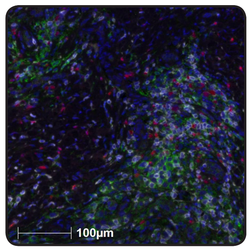


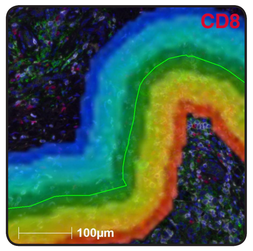
Formalin-fixed paraffin-embedded melanoma samples were stained sequentially with a 6-component immunophenotyping antibody panel, including CD4, CD8, PD-1, PD-L1, macrophage cocktail (CD68 + CD163), and FoxP3. DAPI was used as a nuclear counterstain. Antibodies were detected using HRP-catalyzed deposition 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.

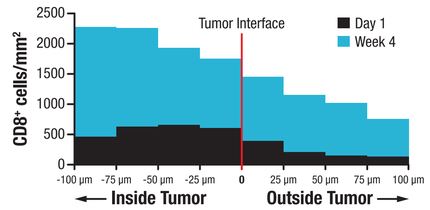


Based on analysis of multiplex IHC staining of tumor samples from patient #5 using HALO software:
- X4P-001 increased the percentage of CD4, CD8, PD-1, and PDL-1 positive cells in the TME
- The percentages of Treg (FoxP3 positive) cells and macrophages (CD68/CD163positive; 24.1% vs. 25.4%; not shown)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

X4P-001 Monotherapy Increases Intratumor CD8+ Cell Density



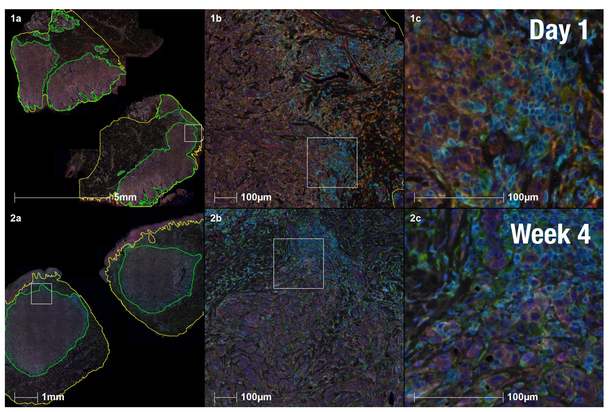




Timepoint	CD8 Count	CD8 Within Interface Area	CD8 Average Distance to Interface (µm)	Total Interface Area (mm²)	CD8 Average Density (cells/mm²)
Day 1	8924	5233	-21.21	13.7694	380.0449
Week 4	25894	7557	-18.36	4.8738	1550.5403

The number of CD8+ T cells at the melanoma tumor interface with normal tissue was quantified using multiplex IHC and HALO image analysis. CD8-labeled cells within 100 µm of the inside or outside of the tumor boundary with normal tissue were counted. The number of CD8+ cells/mm² was plotted against distance from the boundary in 25 µm bands. After 3 weeks of X4P-001 monotherapy, the total density of CD8+ cells within the boundary area was increased four-fold compared with baseline.

Increased CD3 and Decreased VISTA Post Single Agent Treatment of X4P-001

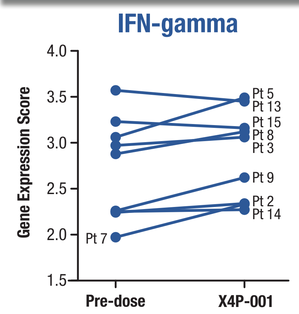


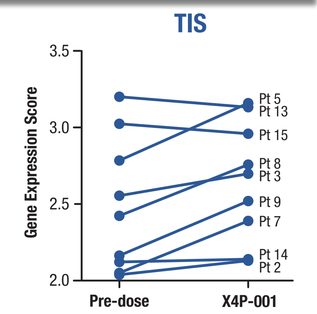
Biopsy samples were obtained at baseline (top row) and at the end of X4P-001 monotherapy (bottom row). The left column shows biopsy samples with outlines of normal tissue (yellow) and the tumor border (green). The center column shows the enlarged boxed regions from the left column stained with the markers listed in the figure key. The right column contains higher magnification views of the boxed regions in the center panel. X4P-001 leads to increased numbers of CD3+ cells within tumor borders and decreased expression of VISTA, a check point molecule that inhibits T cell activation and proliferation.

CD163 CD206 VISTA COX-2 CD3 B7H3 DAPI

Timepoint	ROI	Cells/mm²	CD3/mm²	COX-2/mm²	CD206/mm²	VISTA/mm²	B7H3/mm²	CD163/mm²
Day 1	Whole Tissue	4149.31	308.53	2.87	259.38	1673.17	2998.14	464.86
	Tumor	5758.23	239.91	3.40	264.79	2640.08	4826.36	426.51
	Non-tumor	2057.63	400.31	2.07	252.20	410.11	615.63	514.86
Week 4	Whole Tissue	3572.93	738.82	1.97	182.26	457.53	2288.84	552.27
	Tumor	5297.64	1050.68	2.98	161.25	852.86	3952.27	661.21
	Non-tumor	2217.73	494.97	1.22	199.30	144.72	980.49	467.25

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

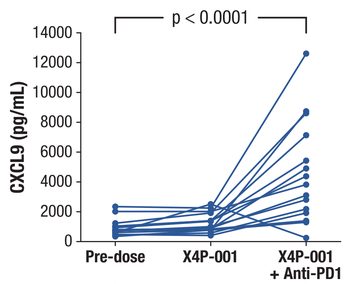


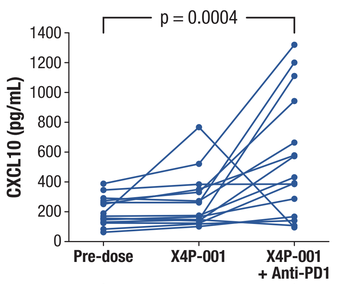


Significant Serum Cytokine and Chemokine Changes at Week 4 of X4P-001 Monotherapy Compared to Baseline

Biomarker	Signed Rank	Student's t
Increase		
TNF-Related Apoptosis-Inducing Ligand Receptor 3 (TRAIL-R3)	< 0.0001	< 0.0001
Interleukin-6 receptor (IL-6r)	0.0002	0.0007
Myeloid Progenitor Inhibitory Factor 1 (MPIF-1)	0.0002	< 0.0001
Tumor necrosis factor receptor 2 (TNFR2)	0.0004	0.0005
Interleukin-2 Simoa (IL-2 Simoa)	0.0006	0.0063
Monokine Induced by IFN-gamma (MIG; CXCL9)	0.0012	0.0194
EN-RAGE	0.0020	0.0041
Tumor Necrosis Factor Receptor I (TNF RI)	0.0021	0.0032
Eotaxin-2	0.0026	0.1533
Chemokine CC-4 (HCC-4)	0.0034	0.0006
Urokinase-type plasminogen activator receptor (uPAR)	0.0034	0.0029
Interleukin-2 receptor alpha (IL-2 receptor alpha)	0.0103	0.0073
Macrophage Inflammatory Protein-1 beta (MIP-1 beta)	0.0103	0.0264
IFN-gamma Induced Protein 10 (IP-10; CXCL10)	0.0157	0.1099
6Ckine	0.0210	0.1296
Macrophage inflammatory protein 3 beta (MIP-3 beta)	0.0353	0.0807
Macrophage-Derived Chemokine (MDC)	0.0353	0.0680
AXL Receptor Tyrosine Kinase (AXL)	0.0463	0.0555
Tissue Inhibitor of Metalloproteinases 1 (TIMP-1)	0.0616	0.0278
Decrease		
Plasminogen Activator Inhibitor 1 (PAI-1)	0.0007	0.0007
Brain-Derived Neurotrophic Factor (BDNF)	0.0081	0.0040
Epidermal Growth Factor (EGF)	0.0237	0.0302
E-Selectin	0.0327	0.3136
Monocyte Chemotactic Protein 2 (MCP-2)	0.0377	0.0289

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 IFN-gamma gene expression signature score
  - Increased Tumor Inflammation Signature (TIS)
  - Increased tumor-infiltrating CD8+ T cells and CD8/FoxP3 ratio
  - Decreased expression of the check point inhibitor VISTA in tumor biopsies
- Increases in multiple chemoattractant factors in serum were observed after single agent treatment with X4P-001. This observation is consistent with increased trafficking of immune cells post CXCR4 inhibition.
- Mean serum concentration for CXCL9 and CXCL10 are increased after combination treatment by 5.2 and 2.8-fold respectively, consistent with immune stimulation[5]
- 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 check point inhibitors

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