Published anti-PD-(L)1 RNA-seq datasets used for comparative analyses with FLX475 +/- pembrolizumab

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cancer Type</th>
<th>Year</th>
<th>PMID</th>
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<tbody>
<tr>
<td>Nivolumab, Pembrolizum</td>
<td>SKCM (Skin Cutaneous Melanoma)</td>
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<td>Fluorinated (2)</td>
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<td>Pembrolizum</td>
<td>NSCLC (Non-Small Cell Lung Cancer)</td>
<td>2019</td>
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*All data sets were processed using the same RNA-seq pipeline to reduce batch effects.

FLX475 is a potent and selective CCR4 antagonist, designed to block immune-suppressive regulatory T cell (Treg) migration into the tumor microenvironment (TME), which has the potential to overcome immune resistance and broaden efficacy to a variety of conventional and immunotherapy-based approaches. In a recent interim clinical update from the ongoing FLX475-ID Phase 1/2 trial (NCT02357377), evidence of monotherapy and combination activity were reported. Here, we present biomarker data from patients with metastatic skin tumor types treated with FLX475 as monotherapy and in combination with pembrolizumab. These data support the beneficial effects of FLX475 in the modification of the TME and promotion of anti-cancer immunity.

As determined by flow cytometry, a small but significant increase in proportion of circulating Treg (CD4+CD25+CD127low) was observed in patients by day 8 of treatment (immunohistochemistry (IHC) revealed that FLX475 monotherapy increased CCR4/Foxp3 density ratio; increased distance between CD8+ and Foxp3+ cells, and reduced migration of Foxp3+ cells from stromal to viable tumor regions). RNA-seq data derived from tumor biopsies prior to (n = 20) and after pembrolizumab (n = 22, paired samples) with FLX475+pembrolizumab were compared to published bio-data from anti-PD-1 (treated patients). Transcriptional changes from tumor biopsies of FLX475 monotherapy treated patients exhibited significant changes in immune pathways to resemble profiles from patients with favorable clinical outcome in anti-PD-1/L1 treatment. Analysis of panel biopsies from both FLX475/pembrolizumab and anti-PD-1 regimen showed significantly increased 1 cell signatures; however, the FLX475+Pembrolizumab group had a significant increase in cell signature compared to P-1 alone. Consistent with these findings, increased expression of CCR4 and its ligands CCL17 and CCL22 were observed in biopsies of patients receiving PD-1 treatment but not FLX475 monotherapy. To identify patients more likely to benefit from FLX475/pembrolizumab therapy, baseline transcriptional profiles were analyzed. Patients with clinical benefit (CR, PR, and stable disease ≥6 months) were found to have elevated Treg/patient populations compared to those without clinical benefit (PD and SD <6 months). This phenomenon was not observed in external anti-PD-1 datasets.

FLX475 monotherapy and in combination with pembrolizumab result in beneficial changes in the TME. FLX475 monotherapy appears to modify the TME toward a phenotype associated with response to anti-PD-1/L1. Baseline parameters associated with favorable outcome are different for the combination treatment compared to anti-PD-1/L1 monotherapy suggesting that patients with enriched intratumoral Treg may benefit from FLX475/pembrolizumab combination rather than pembrolizumab monotherapy.

Gene Set Variance Analysis (GSVA) was used to evaluate immune landscape gene signature changes. Significant GSEA results are shown in Table 1. Significant GSEA results in CCR4 increase in Tregs with the accumulation of Treg observed with anti-PD-1 monotherapy.

One unlinked monotherapy (FLX475) was used to evaluate significance

Baseline levels of intratumoral Treg has the potential to predict clinical benefit to FLX475/pembrolizumab and be used for patient stratification.

FLX475+pembrolizumab predictive features in tumors are enriched for Treg signatures, which is not seen in anti-PD-1 monotherapy

Flux475/pembrolizumab responders have higher intra-tumoral Treg at baseline, not seen in anti-PD-1 monotherapy responders

Conclusions

- FLX475 monotherapy shows biological activity in patients, demonstrated by alteration in intratumoral immunophenotypic, spatial balancing of immune cells and upregulation of gene signatures associated with anti-tumor immunity and anti-PD-1/L1 response.
- FLX475 monotherapy or in combination with pembrolizumab leads to a significant increase in frequency of Treg cells in the blood, acting as a pharmacodynamic biomarker for drug activity.
- Comparative analyses with published anti-PD-1 RNAseq datasets revealed that intratumoral CCR4 and its ligands (CCL17/CCL22) are upregulated in CP-mediated patients upon anti-PD-1 treatment, suggesting increased Treg cell trafficking. On the contrary, FLX475 limited CCR4-associated accumulation as TME in patients receiving FLX475/pembrolizumub combination.
- In contrast to anti-PD-1 monotherapy, FLX475/pembrolizumab responders significantly higher intratumoral Tregs relative to nonresponders at baseline. This suggests that patient populations with enriched intratumoral Tregs may benefit from FLX475/pembrolizumab as compared to anti-PD-1 alone.
- In peripheral blood, lower levels of Tregs at baseline correlate with favorable response and better PFS for patients treated with FLX475/pembrolizumab.