Clinical And Biological Activity Of FLX475, An Oral CCR4 Antagonist, In Combination With Pembrolizumab In Advanced Cancer

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Introduction

FLX475 is a potent and selective CCR4 antagonist, designed to block the recruitment of immunosuppressive regulatory T cells (Treg) into tumors without affecting healthy tissues. Blocking migration of Treg into the tumor microenvironment (TME) has the potential to restore antitumor immunity and provide an additive effect with a variety of conventional and immunotherapy-based approaches to overcome immune resistance and broaden clinical efficacy. In a recent interim clinical update from the ongoing FLX475-02 Phase 1/2 trial (NCT03674567), evidence of monotherapy and combination activity were reported. FLX475 monotherapy induced complete responses in two of the six evaluable subjects enrolled with EBV+ NK/T cell lymphoma. In checkpoint inhibitor naïve non-small-cell lung cancer (NSCLC), 4/13 subjects (31%) had confirmed partial responses (PRs) following treatment with the combination of FLX475 and pembrolizumab. In this analysis we present biomarker data from patients with a broad range of tumor types treated with the combination of FLX475 and pembrolizumab.

Methods

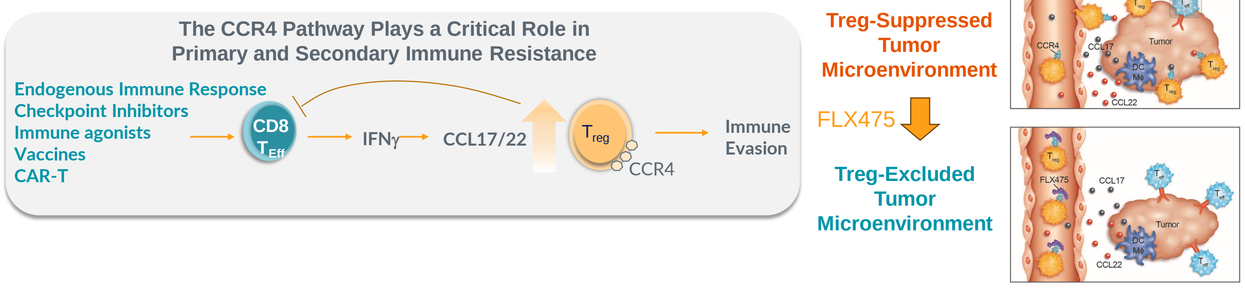
Circulating Treg (CD25+CD127-/low CD4+) were analyzed by flow cytometry. CD8 and FOXP3 positive cells in tumor biopsies were quantified by immunohistochemistry (IHC). RNAseq data derived from tumor biopsies prior to, and after approximately 6 weeks of treatment with FLX475/pembrolizumab were compared to published biopsy data from anti-PD-1 treated patients[1-4]. Gene set variance analysis, immune deconvolution and machine learning approaches were used to interrogate these datasets to identify differences conferred by FLX475.

Results

FLX475/pembrolizumab treatment results in a small but significant increase in proportion of circulating Treg by day 21 of treatment. Transcriptomic analysis of paired biopsies from both anti-PD-1 (publicly available datasets) and FLX475/pembrolizumab regimens significantly increased T cell infiltration immune signatures (CD8A, GZMB, IFNG, PRF1) and the published “expanded immune gene signature” (associated with response to pembrolizumab) indicating potentially turning cold tumors hotter. However, FLX475/pembrolizumab prevented coordinated increase of Foxp3+ Treg in the TME. This is consistent with the finding that significant increase in expression of CCR4 and its ligands CCL17 and CCL22 were only observed in biopsies of patients receiving anti-PD-1 treatment compared to FLX475/pembrolizumab. Supervised classification between responders and non-responders identified that baseline gene expression profiles of FLX475/pembrolizumab patients are distinct and better predictors of response than profiles associated with anti-PD-1 response. Positive predictive features of FLX475/pembrolizumab response were statistically enriched in Treg related gene sets in contrast to anti-PD-1 predictive features consistent with our therapeutic hypothesis.

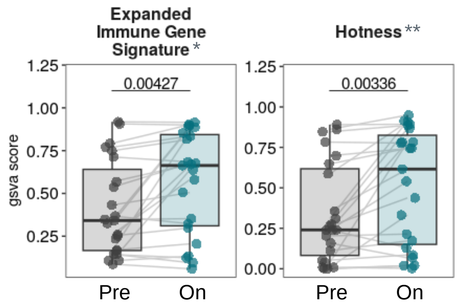
Conclusions

FLX475/pembrolizumab therapy results in beneficial changes in the TME consistent with its proposed mechanism of action. Baseline markers associated with favorable response are different for the combination treatment compared to anti-PD-1 monotherapies suggesting that new populations of patients might benefit from the FLX475/pembrolizumab combination.



FLX475 Limits Treg Accumulation In The TME Associated With Anti-PD-1 Treatment In CPI-naïve Patients

FLX475/pembrolizumab Increased Immune Signatures in TME

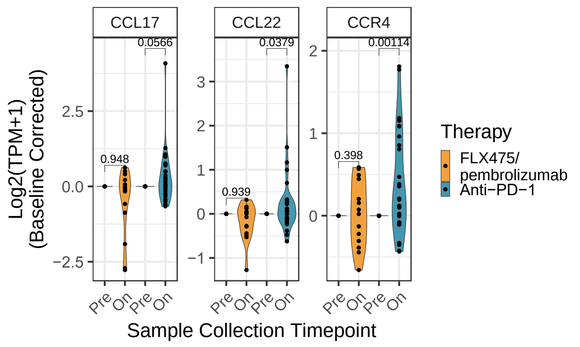


- Gene Set Variance Analysis (GSVA) was used to evaluate immune infiltration gene signature changes
- Two-sided paired t-test was used for significance

*Ayers et.al. JCI (2017) PMID: 28650338

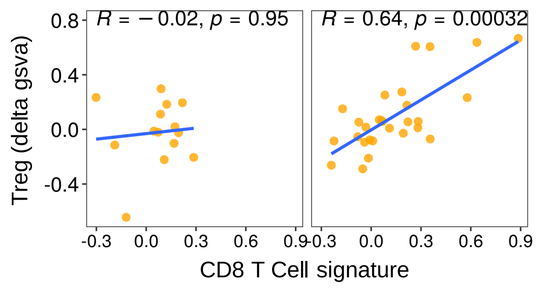
**Hotness includes CD8A, INFG, GZMB, PRF1

Treg-recruitment Genes CCL17, CCL22 and CCR4 are Elevated In Anti-PD-1 Monotherapy Contrary To FLX475/pembrolizumab



- One-sided paired t-test was used to test significance
- Anti-PD-1 datasets obtained from references 2, 4

FLX475 /pembrolizumab Results in a CD8 Increase Without Accumulation of Treg Observed on Anti-PD1 Alone



- Gene Set Variance Analysis (GSVA) was used to evaluate immune infiltration gene signature changes
- Two-sided paired t-test was used for significance
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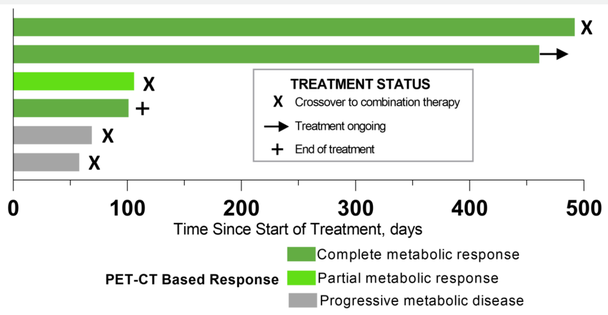
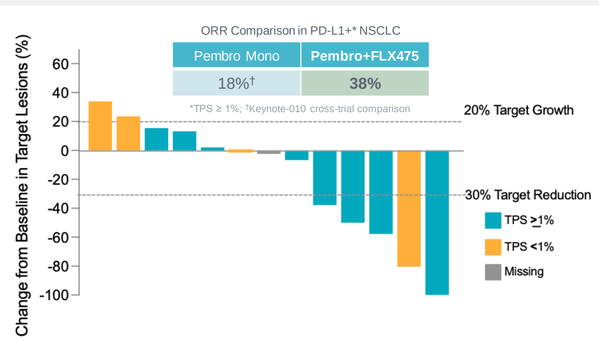
Complete and Durable Responses of NK/T Cell Lymphoma on FLX475 Monotherapy



- Subjects with NK/T Cell lymphoma treated with FLX475 monotherapy. (NCT03674567)
- Response duration based on investigator assessment per Lugano criteria is plotted and color coded by metabolic response based on PET-CT.

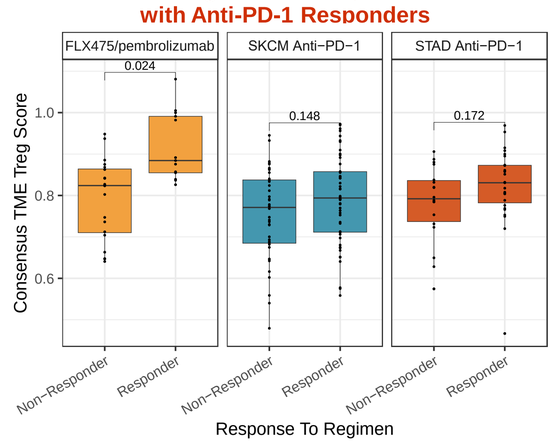
Encouraging Responses in CPI-naïve NSCLC on FLX475/pembrolizumab Combo



- Subjects with checkpoint inhibitor naïve NSCLC treated with FLX475/pembrolizumab combination. (NCT03674567)
- Percent change from baseline measurement of target lesions is plotted and color coded by PD-L1 tumor proportion score.

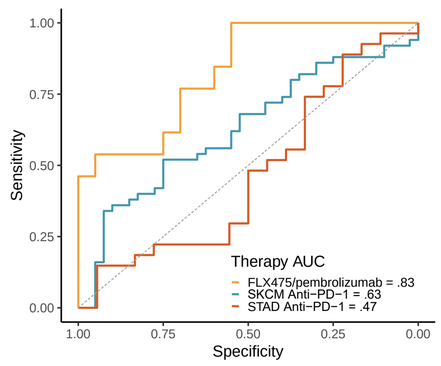
High Intratumoral Treg Signature is a Potential Patient Selection Marker for FLX475/pembrolizumab Combination Therapy, but not Anti-PD-1 Monotherapy

FLX475/pembrolizumab Responders Have Increased Treg Populations, Not Observed with Anti-PD-1 Responders



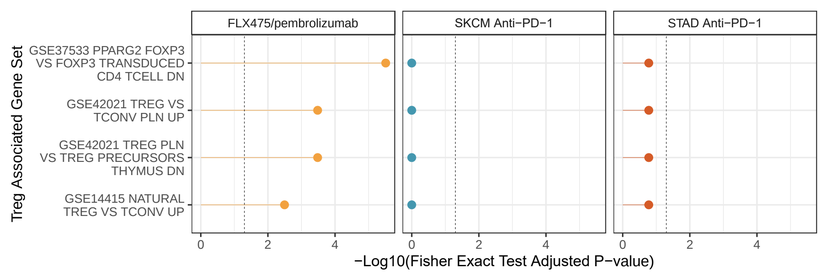
- Responders include complete or partial remission and stable disease >6 months
- P-values were adjusted for cancer type and study via logistic regression
- Similar results were observed using Quantiseq and xCell immune deconvolution methods
- SKCM data from references 1,2 and 4; STAD data from reference 3

Gene Expression Profiles From FLX475/pembrolizumab Patients Are More Predictive Of Response Than Those from Anti-PD-1 Monotherapy Patients



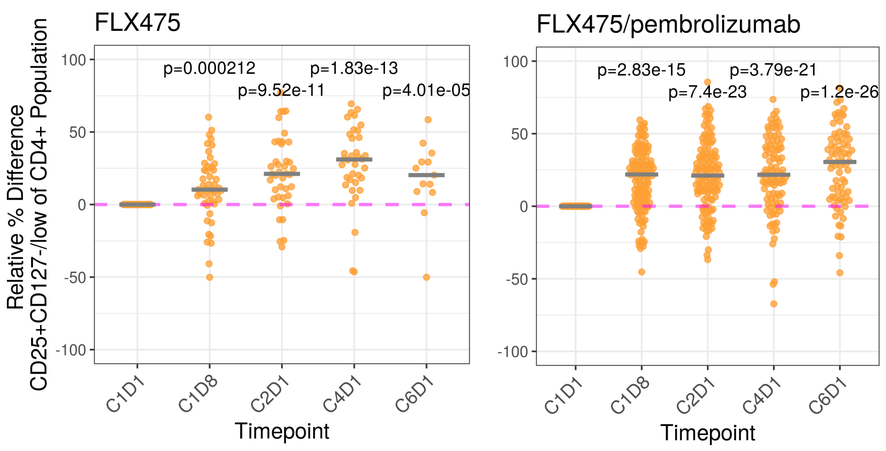
- Predictivity was measured using logistic regression classification with a 5-fold cross validation
- Each model was independently trained and tested using baseline gene expression
- Cancer type did not influence the predictive power of FLX475/pembrolizumab
- Results suggest that FLX475/pembrolizumab overcomes some anti-PD-1 mechanisms of resistance

FLX475/pembrolizumab Predictive Features Were Enriched for Treg Signatures



- A hypergeometric test was performed on predictive features that are upregulated in responders
- Points on the right of the dotted line have an adjusted p-value less than 0.05
- Treg gene sets were selected from the MSigDB C7 immunologic signature data set

FLX475 Monotherapy and FLX475/pembrolizumab Significantly Increase Peripheral Tregs Throughout FLX475 Treatment



- Cross bars represent median of the points across visits
- 1 outlier at C1D8 below -100 in the FLX475/pembrolizumab group is outside of the vertical boundary of the graph
- Linear mixed effect model was used to test statistical significance

External Anti-PD-1 RNA-seq Datasets Used For Comparative Analyses with FLX475/pembrolizumab

Study	Drug	Cancer Type	Year	PMID
[1]Hugo et al.	Pembrolizumab	SKCM (Skin Cutaneous Melanoma)	2016	26997480
[2]Riaz et al.	Nivolumab	SKCM (Skin Cutaneous Melanoma)	2017	29033130
[3]Kim et al.	Pembrolizumab	STAD (Stomach Adenocarcinoma)	2018	30013197
[4]Gide et al.	Pembrolizumab, Nivolumab	SKCM (Skin Cutaneous Melanoma)	2019	30753825

- All data sets were processed using the same RNA-seq pipeline to reduce batch effects

Conclusions

- **FLX475 monotherapy and in combination with pembrolizumab induced a small, but significant and durable increase in peripheral Treg consistent with its mechanism of action**
- **FLX475/pembrolizumab therapy results in beneficial changes in the TME consistent with our proposed mechanism of action**
- **FLX475/pembrolizumab increased immune signatures but prevented coordinate upregulation of Treg signatures and reduced expression of Treg-associated genes CCL17, CCL22 and CCR4 in the TME in contrast to anti-PD-1 monotherapies (based on publicly available reference data)**
- **TME gene expression profiles from FLX475/pembrolizumab patients were enriched for Treg signatures and more predictive of response than gene expression profiles from anti-PD-1 monotherapy patients**
- **Baseline markers associated with favorable response are different for the combination treatment compared to anti-PD-1 monotherapies suggesting that different patient populations might benefit from FLX475/pembrolizumab combination**
- **Comparisons to anti-PD-1 monotherapy are from published data from separate trials in different tumor types and therefore should be seen as preliminary and in need of confirmation in properly controlled trials**

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