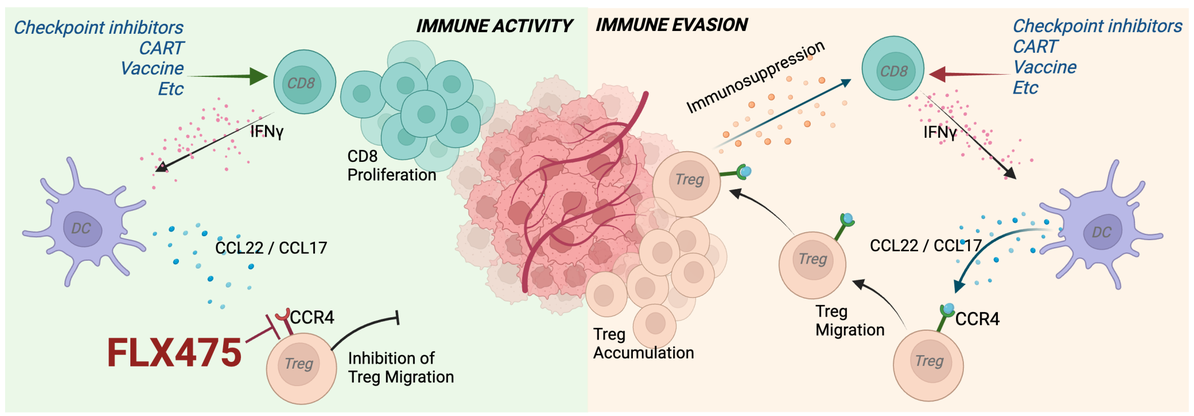
Biological activity of FLX475, an oral CCR4 antagonist, as monotherapy and in combination with pembrolizumab in advanced cancer

Juraj Adamik*, Adam Grant*, Damian Trujillo, Rakesh Kumar Goyal, William Ho, Michael Chisamore, Shoji Ikeda, Marvin Au, Denise de Almeida Nagata, Eugene Lurie, Qingfeng Zhu, Robert A. Anders, Dirk G. Brockstedt, Mohsen Sabouri Ghomi, Paul D. Kassner; Merck & Co., Inc., Rahway NJ, USA; Johns Hopkins University School of Medicine, Baltimore MD, USA; RAPT Therapeutics, Inc., South San Francisco CA, USA

FLX475 is a potent and selective CCR4 antagonist, designed to block immunosuppressive regulatory T cell (Treg) migration into the tumor microenvironment (TME), which has the potential to overcome immune resistance and broaden clinical efficacy to a variety of conventional and immunotherapy-based approaches. In a recent interim clinical update from the ongoing FLX475-02 Phase 1/2 trial (NCT03674567), evidence of monotherapy and combination activity were reported. Here, we present biomarker data from patients with multiple tumor types treated with FLX475 as monotherapy and in combination with pembrolizumab. These data support the beneficial effects of FLX475 in modification of the TME and promotion of anti-cancer immunity.

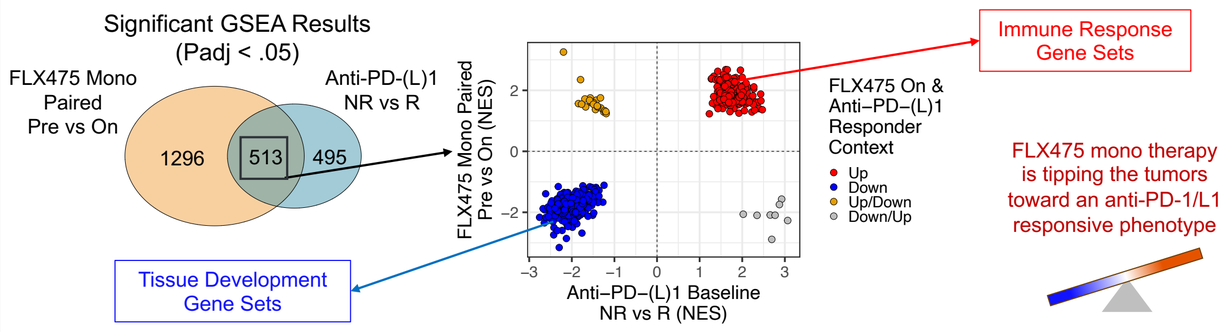
As determined by flow cytometry, a small but significant increase in proportion of circulating Treg (CD4+CD25+CD127-/low) was observed in patients by day 8 of treatment. Immunohistochemistry (IHC) revealed that FLX475 monotherapy increased CD8/Foxp3 density ratio, increased distance between CD8+ and Foxp3+ cells, and reduced migration of Foxp3+ cells from stroma to viable tumor regions. RNA-seq data derived from tumor biopsies prior to (n = 33), and after approximately 6 weeks of treatment (n = 22, paired samples) with FLX475+/-pembrolizumab were compared to published biopsy data from anti-PD-(L)1 treated patients. Transcriptomic profiles from tumor biopsies of FLX475 monotherapy treated patients exhibited significant changes in immune pathways to resemble profiles from patients with favorable clinical outcome to anti-PD-(L)1 treatment. Analysis of paired biopsies from both FLX475/pembrolizumab and anti-PD-1 regimens showed significantly increased T cell signatures. However, FLX475/pembrolizumab prevented coordinated increase of Treg cell signatures observed in the TME of patients treated with anti-PD-1 alone. Consistent with this finding, increased expression of *CCR4* and its ligands *CCL17* and *CCL22* were observed in biopsies of patients receiving anti-PD-1 treatment but not FLX475/pembrolizumab. To identify patients more likely to benefit from FLX475/pembrolizumab therapy, baseline transcriptomic profiles were analyzed. Patients with clinical benefit (CR, PR, and stable disease ≥6 months) were found to have elevated T_{reg} 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-(L)1. Baseline markers associated with favorable outcome are different for the combination treatment compared to anti-PD-1 monotherapy suggesting that patients with enriched intratumoral Tregs might benefit from FLX475/pembrolizumab combination than pembrolizumab alone.



- *Proposed mechanism of action for FLX475 blocking CCR4-mediated migration of Tregs into the TME and promoting anti-cancer immunity.*

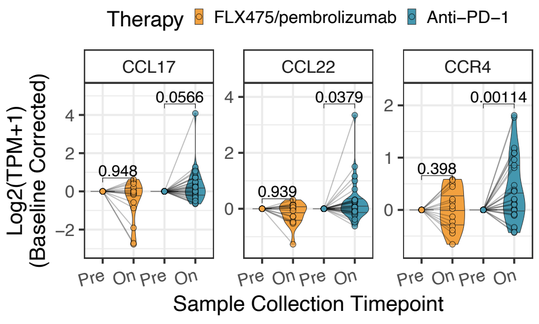
FLX475 monotherapy alters TME expression to resemble anti-PD-(L)1 responders



- *GSEA: gene set enrichment analysis, NR: non-responder, R: responder, NES: normalized enrichment score from GSEA*
- *FLX475 increases expression of gene sets associated with immune response and decreases tissue development-associated gene signatures*
- *Anti-PD-1/PD-L1 Datasets obtained from references 1, 2, 4, 5*

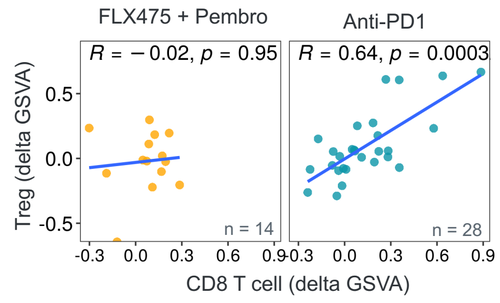
FLX475 limits CCR4-associated Treg accumulation in the TME observed with anti-PD-1 treatment

Contrary to FLX475/pembrolizumab, anti-PD-1 monotherapy upregulates CCR4 and CCR4 ligands favoring Treg cell recruitment into the tumor



- *CPI-naïve patient samples analyzed by RNAseq of turmor biopsy*
- *One-sided paired t-test was used to test significance*
- *Anti-PD-1 datasets obtained from references 2, 5*

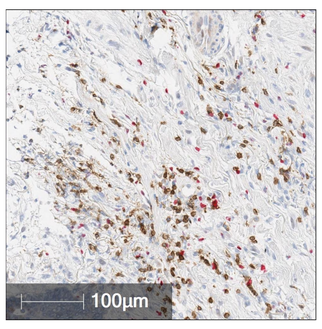
FLX475/pembrolizumab results in CD8 increase without the accumulation of Tregs observed with anti-PD-1 monotherapy



- *Gene Set Variance Analysis (GSVA) was used to evaluate immune infiltration gene signature changes in CPI-naïve patient samples*
- *Spearman test was used for significance*
- *Anti-PD-1 datasets obtained from references 2, 5*

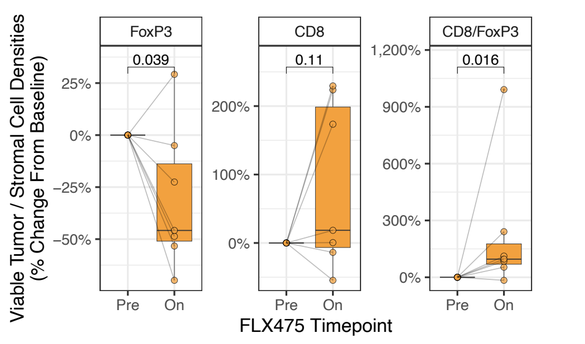
FLX475 monotherapy alters spatial organization and immunological phenotype in tumors

FLX475 monotherapy impacts CD8/Treg ratio and sub-localization of immune cells Reduction of Treg cell migration from stroma to viable tumor regions



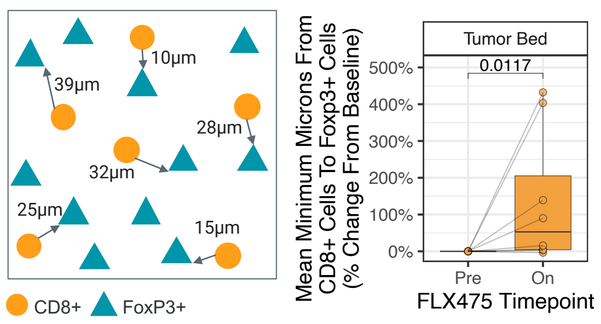
FOXP3 CD8

- *Representative staining derived from esophageal cancer patient*



- *One-tailed paired Wilcoxon test was used to evaluate significance*
- *HALO (Indica Labs, version 3.5) was used to quantify cell densities and proximities*

Spatial proximity between CD8+ and Treg are increased after FLX475 monotherapy



- *One-tailed paired Wilcoxon test was used to evaluate significance*

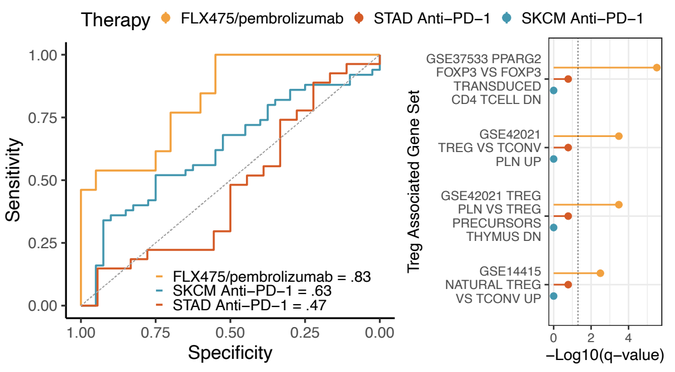
FLX475 decreased Treg and increases pro-immunity associated gene signatures in the TME

- *Gene Set Variance Analysis (GSVA) was used to evaluate immune infiltration gene signature changes*
- *One-tailed paired Wilcoxon test was used for significance*

***Hotness includes CD8A, INFG, GZMB, PRF1*
***Treg includes FOXP3, CCL17, CCL22*

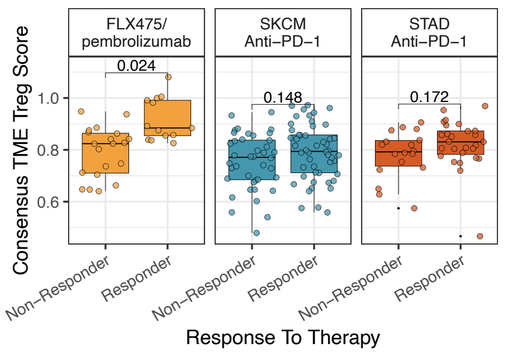
Baseline levels of intra-tumoral Treg has the potential to predict clinical benefit to FLX475/pembrolizumab and be used for patient stratification

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



- *An unbiased machine learning method was applied to identify gene expression features up-regulated in responders vs non-responders (hypergeometric test performed separately on each dataset)*
- *Points to the right of the dotted line have an adjusted p-value < 0.05*
- *Treg gene sets were selected from the MSigDB C7 immunologic signatures*

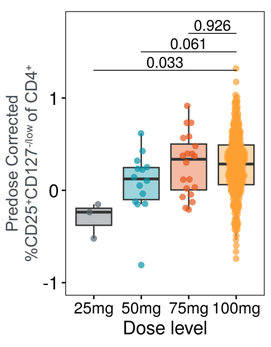
FLX475/pembrolizumab responders have higher intra-tumoral Tregs at baseline, not seen in anti-PD-1 monotherapy responders



- *P-values were adjusted for cancer type and study*
- *Similar results were observed using Quantiseq and xCell immune deconvolution methods*
- *SKCM data from references 1,2 and 5; STAD data from reference 3*

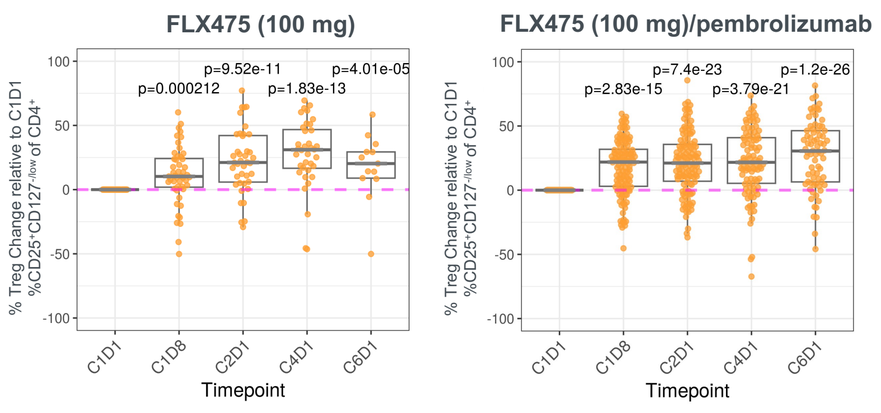
Peripheral Treg cell accumulation upon FLX475 +/- pembrolizumab is a biomarker for biological activity of the drug

FLX475 +/- pembrolizumab increases Treg in a dose-dependent manner



- *Change in Treg at C2D1 is shown*
- *Stats: two-sided t-test was used for pair-wise comparisons*

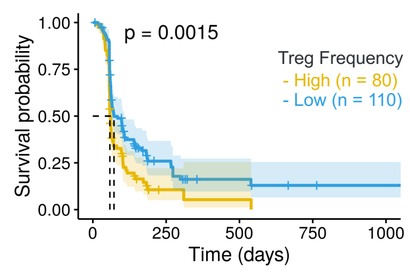
Peripheral Treg increase over time upon FLX475 +/- pembrolizumab



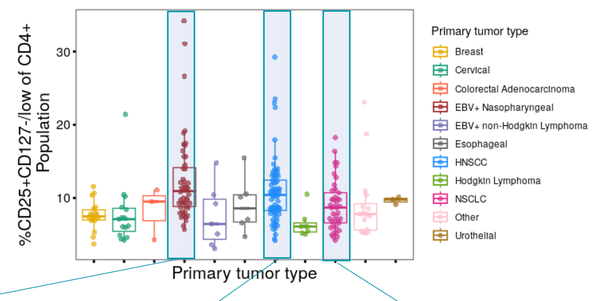
- *Cross bars represent median of the points across visits*
- *1 outlier at C1D8 below -100 in the FLX475/pembrolizumab group is outside of the vertical boundary of the graph*
- *Linear mixed effect model was used to test statistical significance*
- *Purple dashed line marks 25% change for visualization purposes*

Lower levels of peripheral Treg cells at baseline is associated with favorable response to FLX475/pembrolizumab

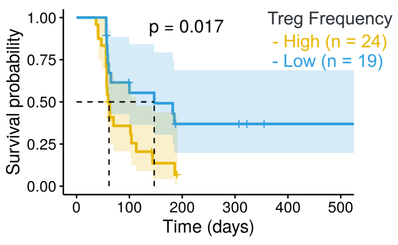
Progression free survival All indications



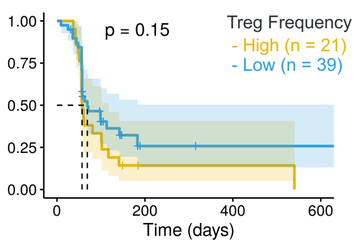
Baseline peripheral levels of Treg cells by indication Cohorts with higher number of patients are highlighted



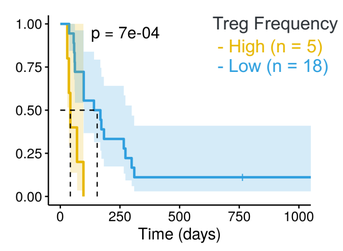
NPC EBV+



HNSCC



NSCLC *CPI-experienced*



- *Peripheral Treg populations were assessed by flow cytometry ($CD25^+CD127^{-/low}$ of CD4)*
- *Minimum p-value approach was used for high vs low Treg frequency stratification for Progression Fee Survival (PFS) analysis*
- *NSCLC includes CPI-experienced patients*
- *HNSCC includes both CPI-experienced and CPI-naïve patients*

Conclusions

- FLX475 monotherapy shows biological activity in patients, demonstrated by alteration in intra-tumoral immunophenotype, spatial distancing 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 CPI-naïve patients upon anti-PD-1 treatment, suggesting increased Treg cell trafficking. On the contrary, FLX475 limited CCR4-associated Treg accumulation in the TME of patients receiving FLX475/pembrolizumab combination.
- In contrast to anti-PD-1 monotherapy, FLX475/pembrolizumab responders have significantly higher intra-tumoral Tregs relative to non-responders 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 Treg cells at baseline correlate with favorable response and better PFS for patients treated with FLX475/pembrolizumab.

Published anti-PD-(L)-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]Mariathasan et al.	Atezolizumab	BLCA (Bladder Urothelial Carcinoma)	2018	29443960
[5]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

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