Clinical and Biological Activity of FLX475, an Oral CCR4 Antagonist, in Advanced Cancer

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Abstract: 421932

ABSTRACT

Introduction

FLX475 is a potent and selective CCR4 antagonist, designed to block the recruitment of immunosuppressive regulatory T cells (T_{reg}) into tumors without affecting healthy tissues. Blocking migration of T_{reg} into the tumor microenvironment (TME) has the potential to restore antitumor immunity and synergize 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, 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 broad range of tumor types treated with FLX475 monotherapy. We provide evidence to substantiate the mechanism of action and support the combination of FLX475 with pembrolizumab. Flow cytometry was used to measure relative proportions of T_{reg} in the periphery. CD8 and FOXP3 positive cells in tumor biopsies were quantified by immunohistochemistry (IHC). Gene set enrichment analysis and immune deconvolution methods were used to interrogate RNAseq data derived from tumor biopsies.

Results

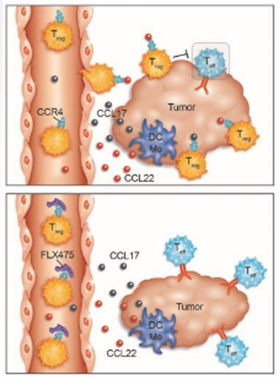
FLX475 monotherapy results in a small but significant increase in proportion of circulating CD25+CD127-/low CD4+ Treg by day 8 of treatment. IHC analysis revealed a trend towards increased CD8:FOXP3 ratio in the TME of patient biopsies. Transcriptomic profiles from tumor biopsies of FLX475 monotherapy treated patients exhibited significant changes in immune pathways likely to benefit the antitumor response. Using global gene expression analyses we identified that FLX475 monotherapy altered the transcriptome of tumors to resemble those from patients with favorable clinical outcome to anti-PD(L)-1 treatment. Consistent with the proposed mechanism of action, immune deconvolution identified that FLX475-treated patients experienced a decrease in Treg cell populations.

Conclusions

FLX475 monotherapy results in beneficial changes in the TME consistent with our proposed mechanism of action. FLX475 monotherapy is modifying the tumor microenvironment toward a phenotype associated with response to anti-PD-1/anti-PD-L1.

BACKGROUND

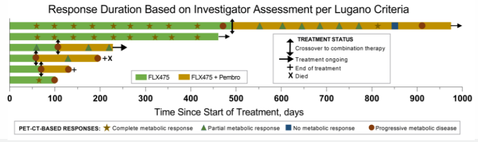
FLX475: Designed to Enhance Antitumor Immune Response



- Immune cells follow chemokines to migrate into target tissues
- CCR4 is the primary chemokine receptor expressed on human T_{reg}
- In response to inflammation, tumor cells and other cells in the TME highly express the chemokines CCL17 and CCL22 (ligands for CCR4), inducing the migration of T_{reg} into tumors
- T_{reg} can suppress the antitumor activity of effector T cells
- FLX475 is a potent, orally-available, small molecule antagonist of CCR4 designed to specifically block the recruitment of T_{reg} into tumors
  - Shifting the T_{eff}/T_{reg} balance in favor of tumor elimination
  - Unlike a CCR4-depleting antibody, FLX475 should neither cause autoimmunity due to non-specific T_{reg} depletion, nor should it cause immunosuppression by depleting CCR4$^+$ effector cells

Clinical Data*

FLX475 Monotherapy Activity in EBV+ NK/T Cell Lymphoma



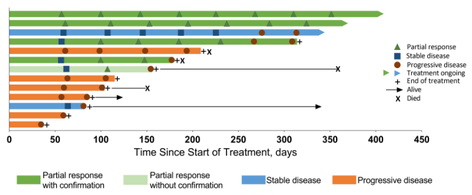
- 4 of 6 subjects with EBV+ NK/T cell lymphoma enrolled to date have responses to FLX475 monotherapy
  - 2 durable complete metabolic responses to FLX475 monotherapy
  - 1 unconfirmed CMR and 1 unconfirmed PMR as best overall response to FLX475 monotherapy
  - 4 have crossed over to combination therapy, with responses as noted above
- Response assessments by Lugano criteria
- Data cutoff 21Nov2022 ; NCT03674567

FLX475 + Pembrolizumab Combination CPI-Naïve NSCLC Stage 1 Cohort

Best Change From Baseline in Target

- 5 of 13 subjects with CPI-naïve NSCLC had responses to FLX475 + pembrolizumab combination therapy
- 4 confirmed PRs, 1 unconfirmed PR as best overall response

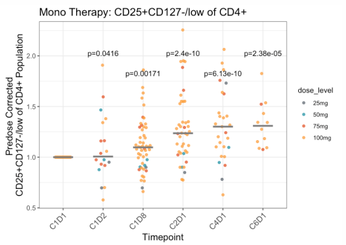
Response Duration



- Based on investigator assessment per RECIST v1.1
- Data cutoff 21Nov2022; clinicaltrials.gov NCT03674567
- *** previously presented: Lin C.-C. et al ESMO-IO 2022 Dec; Presentation #187P**

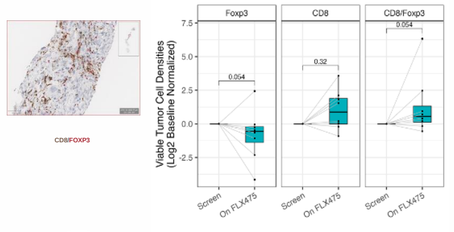
Peripheral and Tumor Biopsy IHC Biomarkers

Increased Proportions of T_{reg} in the Periphery Following Monotherapy



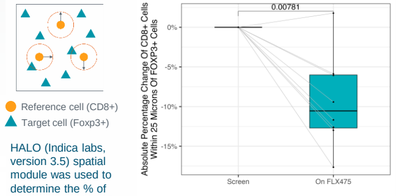
CnDn indicates Cycle number and Day of that cycle samples are collected with a cycle being 21 days

Trend Toward Reduced T_{reg} and Increase in the CD8:FOXP3 Ratio Observed in Paired Tumor Biopsies From Patients Treated with FLX475



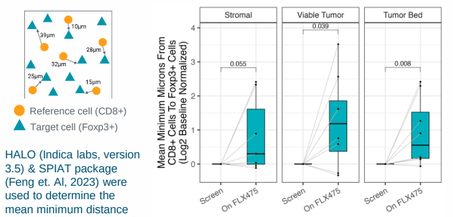
- Density of CD8 and FOXP3 determined by IHC of core tumor biopsies taken prior to treatment and after 2 cycles (6 weeks) of treatment
- Only viable tumor region represented in this figure (stromal regions not included)
- Ratio of CD8/FOXP3 expressed as Log2 values normalized to screening biopsy

FLX475 Monotherapy Decreases the Percentage of CD8+ Cells Within 25 Microns Of Foxp3+ Cells



HALO (Indica labs, version 3.5) spatial module was used to determine the % of CD8+ cells within 25µm of Foxp3+ cells in the TME

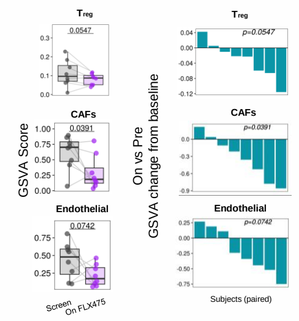
The Mean Minimum Distance From CD8+ Cells To Foxp3+ Cells Is Significantly Higher After FLX475 Monotherapy



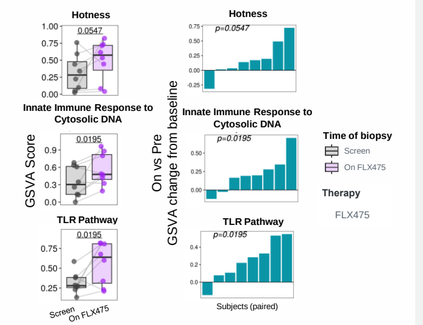
HALO (Indica labs, version 3.5) & SPIAT package (Feng et. Al, 2023) were used to determine the mean minimum distance between CD8+ and Foxp3+ cells in TME

TME Gene Expression Biomarkers

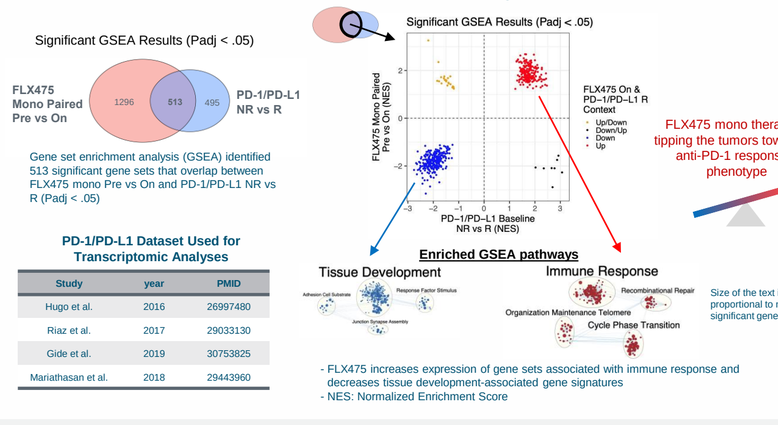
FLX475 Monotherapy Results in a Decrease in T_{reg}, Cancer Associated Fibroblasts (CAFs) & Endothelial Cell Gene Signatures



FLX475 Monotherapy Results in an Increase Towards Hotness, Innate Immune Response to Cytosolic DNA & TLR Pathway and Signatures On-treatment in TME



FLX475 Monotherapy Enhances Expression of Gene Sets That Are Important for Favorable Anti-PD-1/PD-L1 Response



Gene set enrichment analysis (GSEA) identified 513 significant gene sets that overlap between FLX475 mono Pre vs On and PD-1/PD-L1 NR vs R (Padj < .05)

FLX475 mono therapy is tipping the tumors toward an anti-PD-1 responsive phenotype

Size of the text is proportional to number of significant gene sets

- FLX475 increases expression of gene sets associated with immune response and decreases tissue development-associated gene signatures
- NES: Normalized Enrichment Score

PD-1/PD-L1 Dataset Used for Transcriptomic Analyses

Study	year	PMID
Hugo et al.	2016	26997480
Riaz et al.	2017	29033130
Gide et al.	2019	30753825
Mariathasan et al.	2018	29443960

CONCLUSIONS

Consistent with the clinical activity observed to date with FLX475 monotherapy in EBV+ NK/T cell lymphoma and with FLX475 + pembrolizumab in CPI-naïve NSCLC, biomarker studies have demonstrated several lines of evidence supporting the proposed MOA of CCR4 antagonism:

Peripheral biomarkers

- FLX475 treatment results in a small increase in proportion of circulating T_{reg}

Tumor microenvironment

- FLX475 treatment increased the CD8/FOXP3 ratio
- FLX475 increased the proximity between CD8+ to Foxp3+ cells in the TME
- FLX475 treatment results in decreased T_{reg}, endothelial cells and CAFs along with increased hotness and innate immune activation pathways
- FLX475 induced gene expression changes which resemble TME of patients that responded to anti-PD-1 monotherapy
- FLX475 conditions TME for Anti-PD-1/PD-L1 response by increasing expression of gene sets associated with immune response

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