A combined mregDC and T_{reg} signature associates with antitumor efficacy of CCR4 antagonist tivumecirnon (FLX475)

Adam Grant*[1], Juraj Adamik*[1], Jingtao Qiu*[1], Jacob Wert[1], Molly Grandcolas[1], Scott Jacobson[1], Rakesh K. Goyal[1], William Ho[1], Shoji Ikeda[1], Marvin Au[1], Damian Trujillo[1], Michael Chisamore[2], Qingfeng Zhu[3], Robert A. Anders[3], Denise de Almeida Nagata[1], Mohsen Sabouri Ghomi[1], Dirk G. Brockstedt[1], Paul D. Kassner[1], George E. Katibah[1]

[1]RAPT Therapeutics, Inc., South San Francisco CA, USA, [2]Merck & Co., Inc, Rahway NJ, USA, [3]Johns Hopkins University School of Medicine, Baltimore MD, USA

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Abstract

Cancers evade immune surveillance through multiple mechanisms including the recruitment of immunosuppressive regulatory T cells (T_{reg}). Tivumecirnon (formerly known as FLX475), a small molecule oral CCR4 antagonist under clinical evaluation, blocks the binding of CCR4 to its ligands CCL17 and CCL22 and thereby reduces T_{reg} infiltration into the tumor microenvironment (TME). This relieves T_{reg}-mediated immune suppression, resulting in increased antitumor immunity. Our clinical study (NCT03674567) demonstrated that tivumecirnon can block the local accumulation of T_{reg} in the TME and has clinical activity as monotherapy as well as in combination with pembrolizumab. In both mouse and human tumors, we identified a subset of tumor infiltrating dendritic cells (DC) producing high levels of CCL22, previously identified as mregDC, suggesting a potential role in the CCR4-dependent recruitment of T_{reg}.

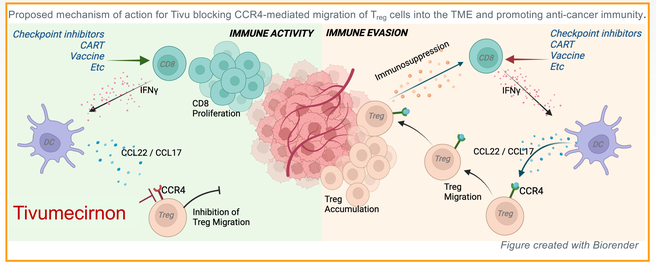
To study the role of mregDC in the recruitment of T_{reg}, non-clinical studies were performed. CD45+ immune cells from the TME of MB49 tumor bearing mice treated with tivumecirnon or vehicle and were analyzed by flow cytometry, scRNA-seq and CITE-seq. We confirmed that mregDC cells are the primary producer of CCL22 in the TME and that tivumecirnon treatment reduced the number of T_{reg} cells in the TME and increased the percentage of IFN gamma and granzyme B expressing CD4+ T cells.

Based on these findings we interrogated tumor biopsies from patients (n = 27) from our clinical study prior to treatment with tivumecirnon and pembrolizumab. We found that higher mregDC + T_{reg} signature levels at baseline trend towards response to tivumecirnon and pembrolizumab therapy. Analysis of TCGA gene expression data demonstrated a positive correlation with mregDC and T_{reg} cells across human tumor types, indicating a previously unknown relationship between these cell types.

Overall, this study demonstrated that inhibition of the CCR4 pathway using tivumecirnon can disrupt the immunosuppressive mregDC – T_{reg} axis, leading to enhanced antitumor activity. Furthermore, the levels of mregDC and T_{reg} may be used as a biomarker for selection of patients more likely to respond to tivumecirnon in combination with anti-PD-1. Additional studies are required to confirm these results.

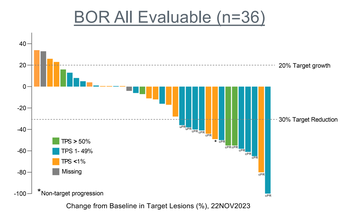
Background

- Immune cells follow chemokine gradients to migrate into target tissues and position immune cells in the local environment.
- CCR4 is the primary chemokine receptor expressed on human T_{reg}.
- Cells in the tumor microenvironment express chemokines CCL17 and CCL22 (ligands for CCR4), inducing the migration of T_{reg} into tumors leading to T_{reg} suppression of antitumor activity of effector T cells.
- Tivumecirnon is a potent, orally-available, selective, small molecule antagonist of CCR4 designed to specifically block the recruitment of T_{reg} into tumors, leading to a shift in the T_{eff}/T_{reg} balance in favor of tumor elimination.
- Tivumecirnon does not result in depletion of CCR4-expressing cells and, hence, is unlikely to cause autoimmunity due to systemic depletion of T_{reg}.

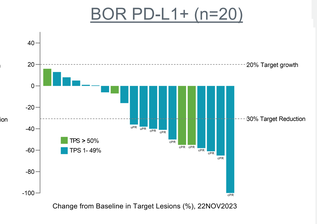


Clinical activity of tivumecirnon + pembrolizumab observed in CPI-naive NSCLC cohort

Best Change From Baseline in Target Lesions
All Evaluable CPI-naïve Subjects (n=36)

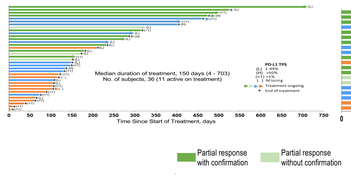


Best Change From Baseline in Target Lesions
PD-L1+ (TPS ≥1%) CPI-naïve Subjects (n=20)

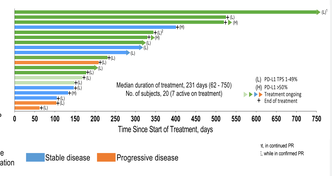


- Overall confirmed ORR: 31% (11/36), regardless of PD-L1 status
- 22C3 pharmDx assay used in 32/36; unknown assay in 2/36; missing data in 2/36
- PD-L1+ confirmed ORR = 45% (9/20)
- PD-L1+ median PFS = 6.3 mo

Response Duration
All Evaluable CPI-naïve Subjects (n=36)



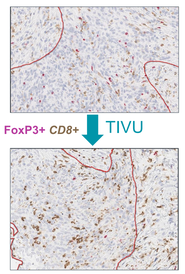
Response Duration
PD-L1+ (TPS ≥1%) CPI-naïve Subjects (n=20)



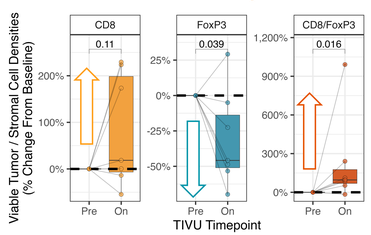
Tivumecirnon treatment alters spatial organization and immunological phenotype in tumors

We investigated the spatial relationship between CD8+ cells and Foxp3+ cells in tivumecirnon monotherapy-treated patients.

Tivumecirnon impacts CD8/T_{reg} ratio and sub-localization of immune cells. Reduction of Treg cell proportions in viable tumor regions

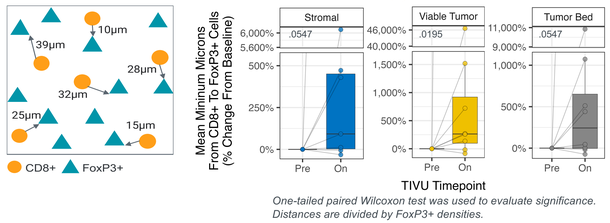


Representative staining derived from a nasopharyngeal cancer patient.



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

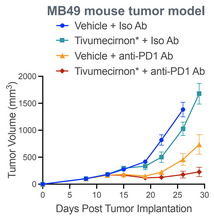
Spatial distance between CD8+ and T_{reg} in tumors is increased after tivumecirnon monotherapy



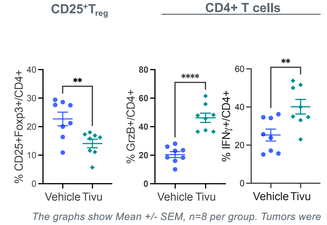
One-tailed paired Wilcoxon test was used to evaluate significance. Distances are divided by FoxP3+ densities.

Tivumecirnon remodels the tumor microenvironment in preclinical tumor studies

Tivumecirnon promotes antitumor activity by reducing T_{reg} frequency

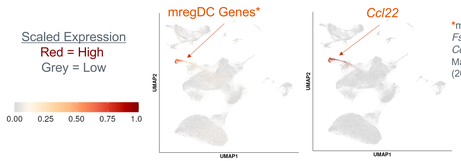


*The graph shows the mean +/- SEM, n=10 per group. *Tivumecirnon (40 mpk, PO, QD)*



*The graphs show Mean +/- SEM, n=8 per group. Tumors were collected on day 7 post-tx. Unpaired T test; **p<0.01, ****p<0.0001*

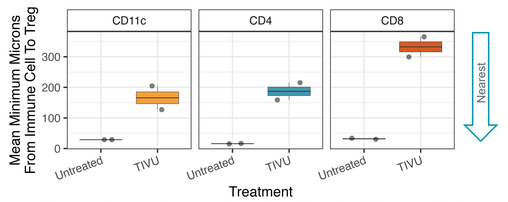
A subset of activated dendritic cells produce high levels of the CCR4 ligand CCL22 in the TME



*mregDC signature: *Fscn1, Ccr7, Pdcd1lg2, Cd200, Cd40, Il12b* Maier et al., *Nature* 580.7802 (2020): 257-262.

CD45+ cells were isolated from tumors for scRNA and libraries prepared on a 10x Chromium Device. Data is representative of one experiment.

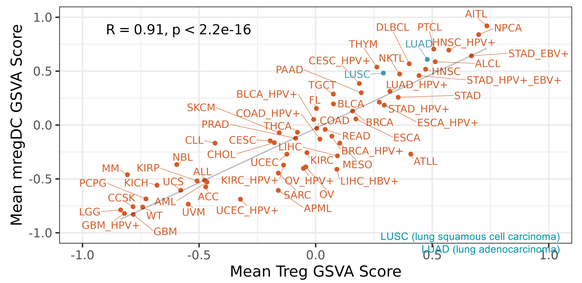
Tivumecirnon reduces the spatial proximity of T_{reg} to immune cells in the TME



Spatial profiling was performed (n=2, tumors) with a multicolor IF panel on FFPE tumors. Cell distances were calculated with QuPath. Distances are divided by T_{reg} percentages. Data is representative of one experiment.

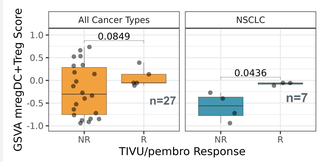
mregDC and T_{reg} signatures have a strong positive correlation across diverse human cancer types

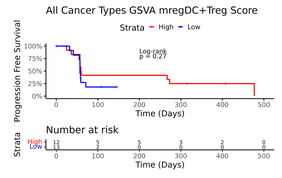
Gene sets for mregDC and T_{reg} cells were developed and tested across diverse human tumor types



Gene set variation analysis (GSVA) was performed on publicly available bulk RNA-seq data to quantify the mregDC signature (CCL22, LAMP3, CCR7, CCL19) and the T_{reg} signature (CCR4, FOXP3, CCR8, ICOS, IL1R1, TNFRSF9). A Pearson correlation was used for statistics.

mregDC + T_{reg} gene signature tend to be associated with response to tivumecirnon + pembrolizumab and a better PFS in CPI-naïve patients

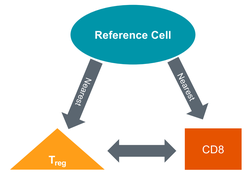




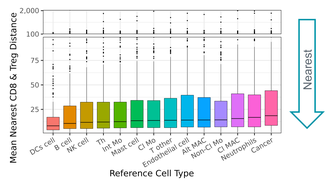
Bar graph: A two-tailed t-test was used for statistics
PFS: Median values were used to stratify patients into high and low groups

External single cell database suggests that dendritic cells are positioned closely with T_{reg} and CD8+ T cells in human lung tumors

Dendritic cells were found to be the cell type in closest proximity to T_{reg} and CD8 cells

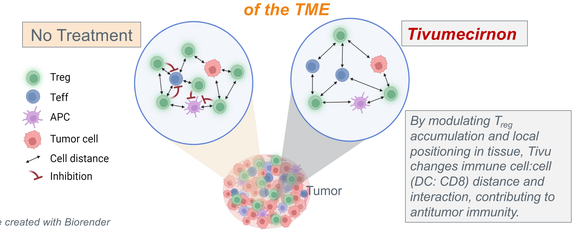


*Distances are divided by Treg and CD8 percentages



Data from Sorin, M. et al. Nature 614, 548–554 (2023)

Proposed model for tivumecirnon activity in the spatial landscape of the TME



By modulating T_{reg} accumulation and local positioning in tissue, Tivu changes immune cell:cell (DC: CD8) distance and interaction, contributing to antitumor immunity.

Figure created with Biorender

Conclusions

- The clinical activity of tivumecirnon in combination with pembrolizumab in CPI-naïve NSCLC is an important POC of how modulating T_{reg} can enhance the antitumor activity of anti-PD1.
- In patients, tivumecirnon as monotherapy remodels the tumor microenvironment by increasing CD8:T_{reg} ratio.
- Similarly, in the preclinical mouse tumor model, tivumecirnon decreases the frequency of T_{reg}, promoting cytotoxicity (increased granzyme B and IFNg), altering the spatial relation of immune cells in the tumor microenvironment, and inhibiting tumor growth.
- In this same preclinical mouse tumor model, single-cell RNA seq analysis revealed that the CCR4-ligand CCL22 is expressed at high levels by a single cluster of activated mregDCs.
- mregDCs are a significant source of CCR4 ligand (CCL22) and are reported to represent an activated dendritic cell state with high levels of antigen capture alongside expression of potent T cell activation molecules.
- We hypothesize that tivumecirnon has the potential to reduce T_{reg} trafficking and alter the spatial distribution of local T_{reg} in areas of mregDC-mediated T cell activation, thereby enhancing antitumor immunity.
- Using publicly available data, we observed a correlation in gene signatures for T_{reg} and mregDCs across tumor types, with these cells in close proximity to CD8 T cells in lung cancer.
- We find a higher baseline mregDC + T_{reg} gene expression score appears to be associated with response to tivumecirnon + pembrolizumab in CPI naïve NSCLC patients and trends with response and PFS in other tumor types.

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