

Clinical benefit from treatment of advanced MSS mCRC with the A_{2a}R/A_{2b}R antagonist etrumadenant is associated with adenosine-mediated T-cell exclusion and enhancement of the effectiveness of immunotherapy

Ji Yun Kim^{1,*}, Emily J Brown^{1,*}, Shravani Shitole^{1,*}, Madhura Joglekar¹, Haben Ghermazien¹, Jose Aquino¹, Tingting Zhao¹, Jennifer Scott¹, Soonweng Cho¹, Angelo Kaplan¹, Omar Kabbarah¹

1. Arcus Biosciences, Hayward CA.
*Authors contributed equally.

Background

- Advanced microsatellite stable (MSS) metastatic colorectal cancers (mCRCs) are refractory to checkpoint inhibitors and represent a significant unmet clinical need.¹
- mCRCs express elevated levels of adenosine-generating enzyme CD73, which leads to reduced effector T cell-mediated antitumor immunity.²
- Etrumadenant (etruma) is an orally bioavailable, selective adenosine 2a/2b receptor antagonist designed to reverse adenosine-mediated immunosuppression.
- Etruma, in combination with zimberelimab (Z) (an anti-PD-1 antibody) and FOLFOX/bevacizumab (bev) (EZFB regimen) demonstrated a significant progression free survival (PFS) and overall survival (OS) benefit over regorafenib (rego) in third-line, MSS, chemo-resistant mCRC in Cohort B of the ARC-9 Phase 2 study (NCT04660812).³
- We examined the relationship between tumor expression of the adenosine producing enzyme CD73, T cell localization and activation, CD274 (PD-L1) expression, and clinical benefit on EZFB.

Methods

Study Design

- Cohort B in ARC-9 (NCT04660812) is a phase 1b/2, randomized, open-label study investigated the efficacy and safety of the EZFB regimen against rego in patients with 3L mCRC (Figure 1).

Sequencing Analysis

- Tumors were macro-dissected to enrich >70% where possible.
- RNA-seq was performed using the Illumina TruSeq™ RNA Exome Kit.
- Whole Exome Sequencing was performed using the IDT xGen Exome panel.

CD73 Immunohistochemistry (IHC):

- IHC staining for CD73 detection was performed using an IHC assay, which was designed and validated as a laboratory developed test (LDT), and scored visually by a board-certified pathologist.

Multiplex IF (mIF) Analysis

- A custom multiplex immunofluorescence (mIF) panel was developed to quantify pan cytokeratin (panCK), CD73, CD3, CD8, granzyme B (GZMB), and fibroblast activation protein (FAP) proteins.
- Digital pathology was employed to assess the number and spatial distribution of cells expressing these six markers in tumor tissues.

Figure 1: ARC-9 Study Design and Biomarker Evaluable Population (BEP)

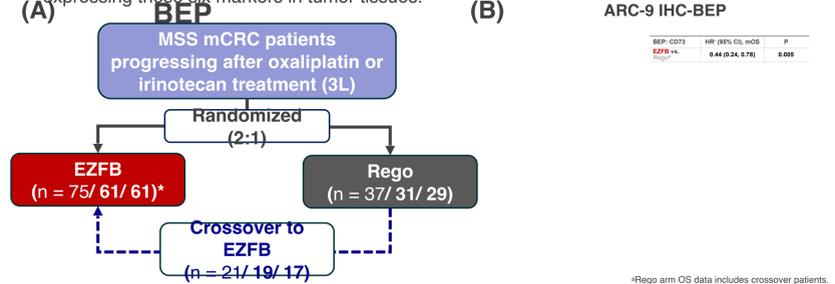


Figure 1: ARC-9 Study Design and Biomarker Evaluable Population (BEP). (A) ARC-9 cohort B study design. *Numbers correspond to ITT / IHC-BEP / BEP-RNA-seq. BEP: 3L, third line; EZFB, etrumadenant and zimberelimab with modified FOLFOX-6 and bevacizumab; mCRC, metastatic colorectal cancer; R, randomization; rego, regorafenib. (B) Kaplan-Meier curves for ARC-9 BEP-IHC comparing overall survival (OS) across arms. Cox regression HRs stratified by region shown in inset table. Data cut off: 2023-11-13.

Results

Subjects with CD73 positive tumors derive increased benefit with EZFB treatment compared to CD73 negative cases

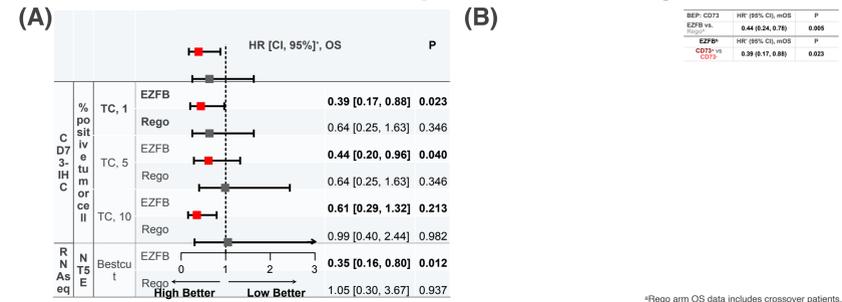


Figure 2: CD73 positivity is associated with better response to EZFB treatment but not rego.

(A) Forest plot of hazard ratios (HR) and 95% confidence intervals (CIs) of Cox model for overall survival (OS) stratified for region comparing CD73+ vs CD73- tumors by IHC at 1%, 5%, and 10% tumor positivity (top), and high vs low NT5E (CD73) RNA expression. NT5E best cut ≥ 4.38. CD73 ≥ 1% is used as the IHC cut-off for further analyses. (B) KM analysis of EZFB-all, EZFB-CD73+, EZFB-CD73-, and rego-all arms. Cox regression HRs stratified by HR shown in inset table. Data cut off: 2023-11-13. CD73+ tumors ≥ 1% tumor positivity.

CD73 positive tumors have elevated CD8 effector T cells and expression of T cell effector pathways

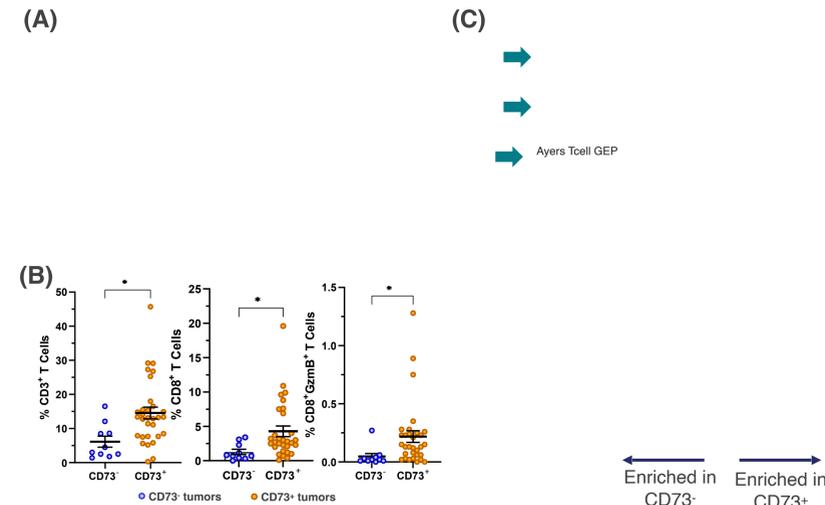


Figure 3: CD73 positivity is independent of other clinically relevant factors and shows increased T cell infiltration, as well as enrichment of IFN and T effector signaling gene sets. (A) Pie charts of proportions for sidedness, PD-L1 status ≥ median CD274 RNA, RAS, and CMS status by CD73 status^a. Fisher's exact test shows no statistically significant difference between CD73 groups. (B) CD3+, CD8+, and CD8+GzmB+ T cells from mIF as a percent of total cells in CD73+ and CD73- tumors^b (n=40). (C) Ridgeplot of GSEA leading-edge genes comparing CD73+ vs CD73- tumors^a. Pathways with significant enrichment are shown. Arrows denote IFN and T cell gene expression (GEP) pathways.

^aCD73+ defined as ≥ 1% tumor positive by IHC in (a) and (c); ^bCD73+ defined as % CD73 positive tumor cells above the 25th percentile by mIF in (b).

Results

CD73 positive tumors show a propensity toward immune exclusion in the TME away from CD73+ cancer cells

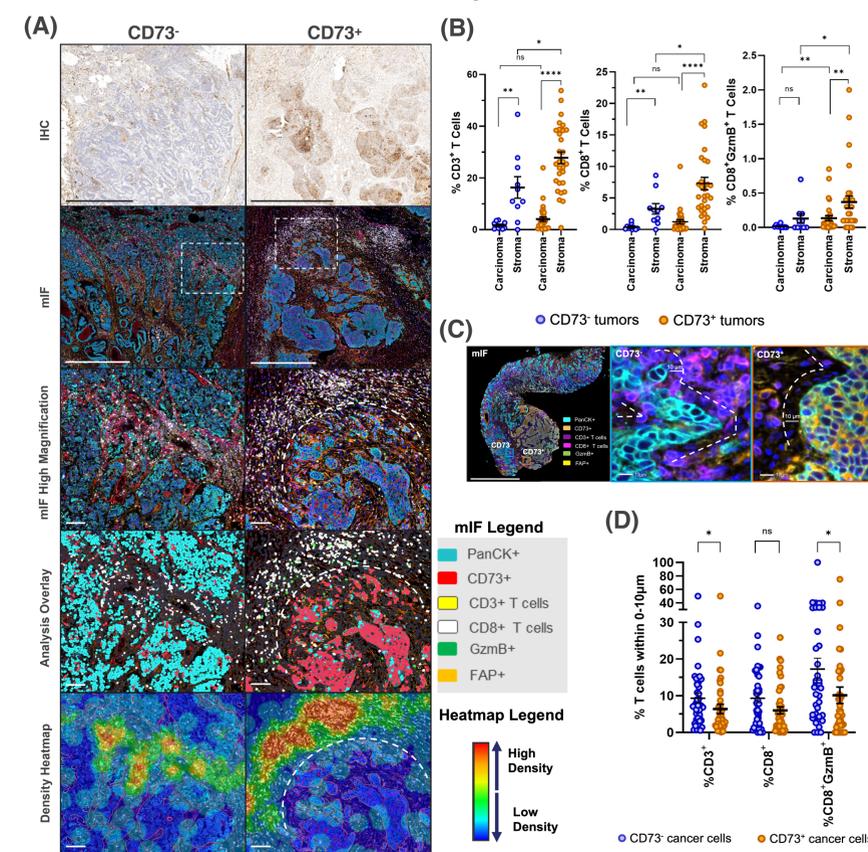


Figure 4: TME immunophenotyping shows preferential localization of T cells to the stroma and distal to CD73+ cancer cells. (A) mIF CD73 staining is concordant with the chromogenic IHC assay (top row) in representative CD73- and CD73+ cases (left and right columns, respectively). At low magnification (second row), increased T cells (yellow), including CD8+ T cells (white), are observed in the stroma surrounding CD73+ cancer nests, while more evenly distributed in the CD73- tumor. Localization difference is more clearly shown at higher magnification (third and fourth rows), with a dotted line representing the boundary between the carcinoma nests and adjacent stroma. The bottom row shows the CD8+ T cell density represented by a heatmap, where high density is observed in the stroma of the CD73+ tumor (right) and a more even distribution throughout the cancer nests (red outline) and stroma can be seen in the CD73- tumor (left). (B) Quantification of CD3+ T cells, CD8+ T cells, and activated CD8+GzmB+ effector T cells according to their frequency out of total cells in carcinoma (intraepithelial) vs stromal compartments. (C) Representative mIF image showing CD73+ and CD73- cancer cells and the corresponding relationship to T cell populations which are preferentially localized >10 μm away from CD73+ cancer cells. (D) Proximity analysis shows significantly fewer T cells and activated cytotoxic T cells within 10 μm from CD73+ cancer cells, as a percentage of total T cells in the entire tissue, compared to CD73- cancer cells. Scale bars: low mag = 1 mm, high mag = 100 μm. CD73+ ≥ 25th percentile cancer cell expression.

Results

Stratification by CD73 and PD-L1 defines tumors with exceptional response to EZFB treatment but poor performance with rego

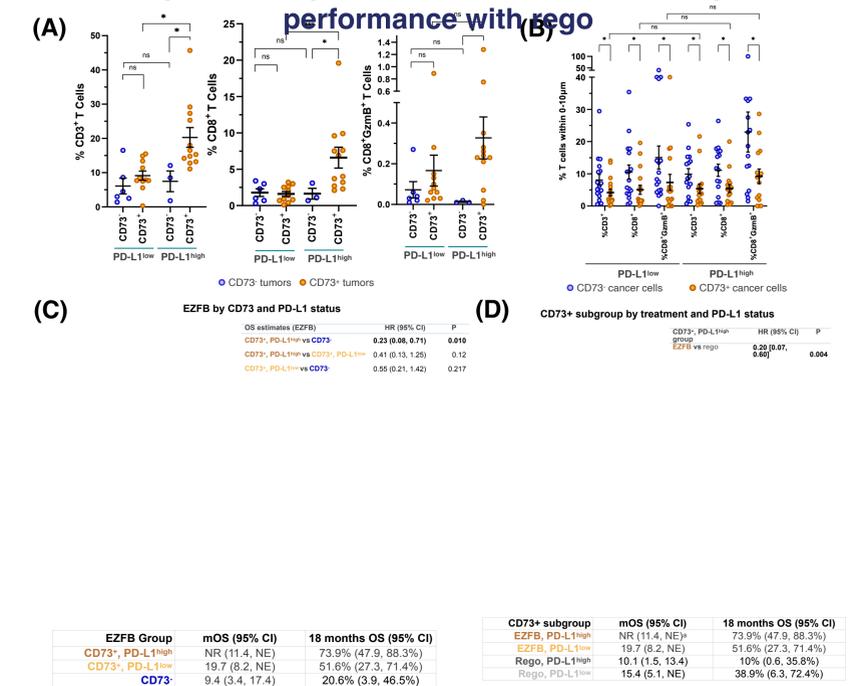


Figure 5: CD73+, PD-L1^{high} tumors have elevated T cell infiltration but greater distance to CD73+ cancer cells and exhibit exceptional survival on the EZFB regimen but poorer performance with rego. (A) CD3+, CD8+, and CD8+GzmB+ T cells quantification by PD-L1 and CD73 status. (B) Distal localization of T cells (≥10 μm) from CD73+ cancer cells is not influenced by PD-L1 status. (C) KM analysis of OS in EZFB cohort by CD73 and PD-L1 status. (D) KM analysis of OS in CD73+ tumors by the treatment and PD-L1 status. CD73+ tumor status defined by ≥1% tumor positivity. PD-L1 status defined by median CD274 RNA expression. (C), (D), inset tables show Cox model for OS estimates, stratified by region. *At 2023-11-13 DCO with 20.4 months follow-up. CD73+, PD-L1^{high} group treated with EZFB did not reach mOS, so the landmark OS analyses were performed at 18 months. NR: not reached, NE: not estimable.

Conclusions

- ARC-9 patients with 3L MSS mCRC derived benefit from EZFB regimen compared to regorafenib.
- CD73 positivity is associated with improved OS in EZFB arm independent of clinically relevant covariates, linking clinical benefit with EZFB to adenosine biology.
- While CD73 positive tumors show increased T cell infiltration and signaling, CD8+GzmB+ T cells localize in the stromal compartments and distally to CD73+ cancer cells. Distal localization is true regardless of PD-L1 status, linking adenosine biology to a suppressive immune phenotype.
- CD73+, PD-L1^{high} tumors show exceptional OS with EZFB treatment.