The Adenosine Receptor Antagonist Etrumadenant Reduces Tumor Adenosine-Regulated NR4A Gene Expression and Increases mCRC Inflammation in Patients From the ARC-9 Trial

Poster 52
ARC-9

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Objectives

- To investigate the mechanism of action of etrumadenant and treatment-related changes in adenosine levels by assessing the transcriptomic profiles of matched pairs of pre- and post-treatment tumor samples from patients with metastatic colorectal cancer (mCRC) enrolled in the ARC-9 clinical trial
- To investigate CD73 as a potential predictive biomarker in the treatment of mCRC with EZFB (etrumadenant and zimberelimab with modified FOLFOX-6 and bevacizumab)

Conclusions

- Treatment of mCRC with EZFB was associated with a reduction in the expression of adenosine-regulated nuclear receptor subfamily 4A (NR4A) genes and an increase in tumor inflammation, presumably reflecting a reversal of tumor immunosuppression
- Consistent with an adenosine-targeting mechanism of action, patients with CD73+ tumors derived additional benefit with EZFB treatment compared with regorafenib
- Findings from this analysis highlight a potential mechanistic link between the efficacy of EZFB in third-line mCRC and adenosine-related biology and warrant further investigation

Methods

In Vitro NR4A Analysis

- Isolated human primary or cancer cell lines were stimulated with the adenosine receptor agonist 5′-(N-ethylcarboxamido) adenosine (NECA)
- Non-small cell lung cancer cell lines (NCI-H441 and NCI-H650) were examined for NECA (adenosine analog)-stimulated gene expression using RNA-sequencing (RNA-seq)
- HCT116 (colorectal cancer cell line), PANC-1 (pancreatic cancer cell line), pancreatic cancer-associated fibroblasts (CAFs), and M0 and M2-like macrophages were evaluated for induction of NR4A gene expression by adenosine signaling and reversal by etruma using reverse transcription-polymerase chain reaction
- M0 and M2 human monocyte-derived macrophages were derived from CD14+ monocytes isolated from healthy donors and cultured in complete growth medium
- M0 macrophages were differentiated by culturing for 7 days in the presence of macrophage colony-stimulating factor, while M2 polarization was achieved by culturing for the final 24 hours in interleukin-4

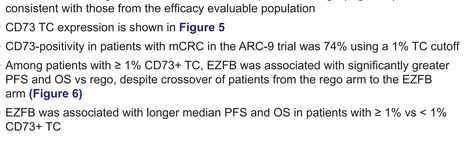
Biomarker Analysis

- This analysis included patients from cohort B of ARC-9 (third-line mCRC; randomization to treatment was stratified by geographic region [US or the rest of the world])
- Tumors were microdissected to enrich to ≥ 70% tumor content, where possible
- RNA and DNA were extracted using the MagMax™ Total Nucleic Isolation Kit (ThermoFisher); RNA-seq was performed using the Illumina TruSeq™ RNA Exome Kit
  - RNA-seq data were processed using a custom pipeline utilizing STAR and salmon to obtain expected gene counts, which were then converted to counts per million, normalized using trimmed mean of M-values, and voom-transformed to obtain gene expression values
  - Single sample Gene Set Enrichment Analysis (ssGSEA) was used to calculate pathway enrichment scores[6]
- ssGSEA scores of the NR4A family of genes (NR4A1, 2, and 3) were calculated from RNA-seq data from 10 patients who received EZFB and had paired pre- and post-treatment sequencing results
- Paired Wilcoxon rank sum and signed-rank tests were used to calculate the statistical significance of pharmacodynamic changes
- CD73 protein expression level was assessed in FFPE slides from pre-treatment biopsy tissue using immunohistochemistry (IHC)
  - Staining was performed on the automated Leica Bond Rx platform using a standard IHC protocol and chromogenic detection
  - A board-certified pathologist scored immunostainings to quantify the tumor cell (TC) percent positive, fractional intensities (0–3+), H-scores, and percent and intensity scores for stroma and tumor-infiltrating immune cells
- Survival outcomes (based on Cox regression, Kaplan-Meier curves, and log-rank tests) were assessed in the CD73-IHC population (patients with CD73 protein expression data available at baseline); impact of CD73 protein expression on survival was assessed by the level of CD73 expression (≥ 1% CD73+ TC vs < 1% CD73+ TC)

Introduction

- Human colorectal cancer tissue expresses high levels of CD73, a key enzyme involved in production of extracellular adenosine[1]
- Adenosine-mediated signaling via A_{2a} and A_{2b} receptors impairs activation, proliferation, and cytotoxic activity of effector T cells, resulting in reduced anti-tumor immunity[2]
- Etrumadenant (etruma) is a small molecule, selective dual antagonist of A_{2a} and A_{2b} receptors that prevents adenosine-mediated immunosuppression
- ARC-9 (NCT04660812) is a phase 1b/2, randomized, open-label study in patients with metastatic colorectal cancer (mCRC) that investigated the efficacy and safety of etruma in combination with zimberelimab (anti–PD-1 antibody), modified FOLFOX-6, and bevacizumab (EZFB regimen; **Figure 1**)
- In the primary analysis of the ARC-9 study, treatment with EZFB was associated with longer median progression-free survival (PFS; 6.2 vs 2.1 months; hazard ratio, 0.27 [95% CI: 0.17, 0.43]) and overall survival (OS; 19.7 vs 9.5 months; hazard ratio, 0.37 [95% CI: 0.22, 0.63]) compared with regorafenib (rego) in third-line mCRC[3]
  - 21/37 (56.8%) patients crossed over from the rego arm to the EZFB arm
  - OS benefit was achieved with EZFB despite crossover from rego
- Activation of Gs-coupled adenosine receptors A_{2a} and A_{2b} leads to increased phosphorylation of cyclic adenosine monophosphate response element-binding protein (CREB).[4] Activation of CREB upregulates expression of nuclear receptor subfamily 4A (NR4A),[5] suggesting that NR4A expression may be reflective of extracellular adenosine levels
- In this analysis, we investigated the impact of EZFB on adenosine signaling by using NR4A gene expression as a surrogate for adenosine and explored whether the adenosine-generating enzyme CD73 is a predictive biomarker in the treatment of mCRC with EZFB

Figure 1. ARC-9 Study Design



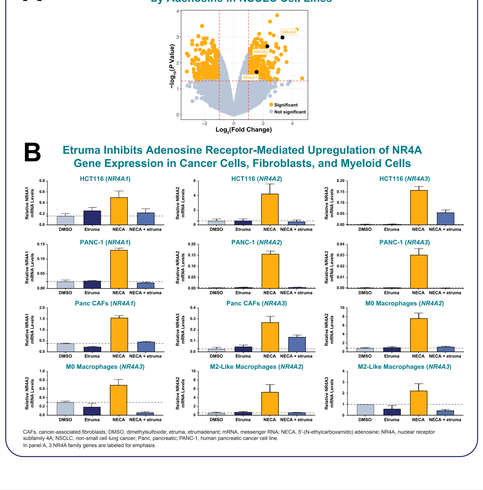
3L, third line; EZFB, etrumadenant and zimberelimab with modified FOLFOX-6 and bevacizumab; mCRC, metastatic colorectal cancer; R, randomization; rego, regorafenib.
[a]Stratified by geographic region (US or the rest of the world).
[b]Oral etrumadenant 150 mg once daily and intravenous zimberelimab 480 mg once every 4 weeks combined with intravenous modified FOLFOX-6 plus intravenous bevacizumab 5 mg/kg once every 2 weeks; bevacizumab was included for all patients unless contraindicated.
[c]Oral rego 160 mg once daily (days 1–21 every 4 weeks).

Results

In Vitro NR4A Analysis

- In non-small cell lung cancer cell lines, adenosine receptor agonism with NECA significantly upregulated NR4A gene expression **(Figure 2A)**
- Adenosine receptor agonism upregulated NR4A genes in colorectal cancer cells, pancreatic cancer cells, pancreatic CAFs, and macrophages, which was inhibited by the addition of etruma **(Figure 2B)**

Figure 2. Adenosine Signaling Increased NR4A Gene Expression in Vitro, Which Is Reversed by Etruma



CAFs, cancer-associated fibroblasts; DMSO, dimethylsulfoxide; etruma, etrumadenant; mRNA, messenger RNA; NECA, 5′-(N-ethylcarboxamido) adenosine; NR4A, nuclear receptor subfamily 4A; NSCLC, non-small cell lung cancer; Panc, pancreatic; PANC-1, human pancreatic cancer cell line.
In panel A, 3 NR4A family genes are labeled for emphasis.

Biomarker Analysis Populations

- As of November 13, 2023, 112 patients with mCRC (EZFB, n = 75; rego, n = 37) were randomized in the ARC-9 study
- Baseline characteristics and outcomes were similar between the biomarker evaluable (RNA-seq and CD73-IHC) and efficacy evaluable populations **(Table)**

Table. Baseline Characteristics and Overall Survival

Characteristic, n (%)	EEP (n = 112)	CD73-IHC (n = 92)	RNA-Seq (n = 91)
Age, < 65 years	77 (68.8)	64 (69.6)	63 (69.2)
Sex, female	37 (33.0)	26 (28.3)	25 (27.5)
ECOG PS, 1	64 (57.1)	50 (54.3)	49 (53.8)
Primary diagnosis, colon	83 (74.1)	65 (70.7)	66 (72.5)
Liver metastases present at baseline	82 (73.2)	69 (75.0)	64 (70.3)

Median Overall Survival, months (95% CI)	EEP (n = 112)	CD73-IHC (n = 92)	RNA-Seq, Paired Biopsies (n = 8)
EZFB	n = 75 19.7 (14.7, 20.6)	n = 61 19.7 (10.5, NE)	n = 7 15.7 (11.4, 19.7)
Rego	n = 37 9.5 (8.0, 12.5)	n = 31 10.3 (6.2, 13.4)	n = 1 NA

ECOG PS, Eastern Cooperative Oncology Group Performance Status; EEP, efficacy evaluable population; EZFB, etrumadenant and zimberelimab with modified FOLFOX-6 and bevacizumab; IHC, immunohistochemistry; NA, not applicable; NE, not evaluable; rego, regorafenib; RNA-seq, RNA-sequencing.

ARC-9 Tumor Pharmacodynamic Analysis

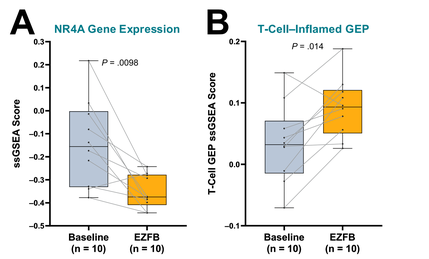
- Treatment with EZFB significantly downregulated expression of NR4A genes (P = .0098 vs baseline; **Figure 3A**) and significantly increased tumor inflammation (P = .014 vs baseline), as measured by a T-cell activation signature (T-cell–inflamed GEP[7]; **Figure 3B**)

Survival Benefit With EZFB May Be Linked to Adenosine Biology

- Within the ARC-9 biomarker evaluable population (CD73-IHC, n = 92), EZFB was associated with longer median PFS and OS compared with rego **(Figure 4)**; results were consistent with those from the efficacy evaluable population
- CD73 TC expression is shown in **Figure 5**
- CD73-positivity in patients with mCRC in the ARC-9 trial was 74% using a 1% TC cutoff
- Among patients with ≥ 1% CD73+ TC, EZFB was associated with significantly greater PFS and OS vs rego, despite crossover of patients from the rego arm to the EZFB arm **(Figure 6)**
- EZFB was associated with longer median PFS and OS in patients with ≥ 1% vs < 1% CD73+ TC

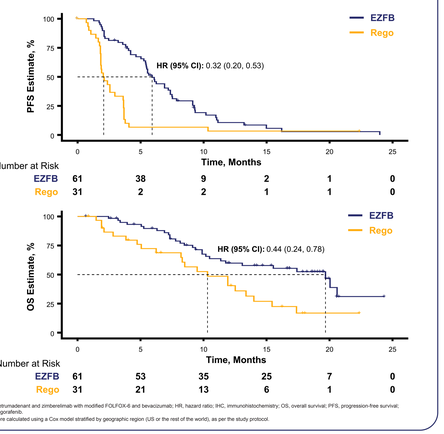
Results *cont'd.*

Figure 3. EZFB Downregulated NR4A Gene Tumor Expression and Increased Tumor Inflammation



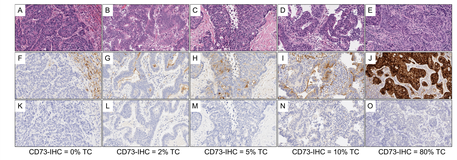
GEP, gene-expression profile; EZFB, etrumadenant and zimberelimab with modified FOLFOX-6 and bevacizumab; NR4A, nuclear receptor subfamily 4A; ssGSEA, single sample Gene Set Enrichment Analysis.
The left boxplot represents the ssGSEA score distribution of the pre- and post-treatment samples, and each point represents a patient sample in the study. The grey lines connect the pre- and post-treatment samples from the same patient.

Figure 4. Survival Outcomes in the CD73-IHC Population Were Comparable With the Efficacy Evaluable Population



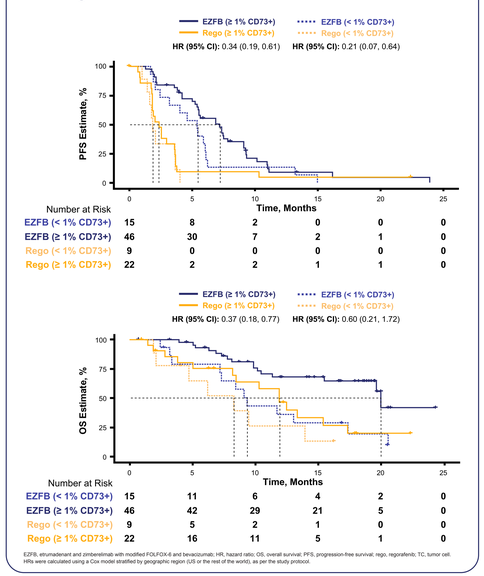
EZFB, etrumadenant and zimberelimab with modified FOLFOX-6 and bevacizumab; HR, hazard ratio; IHC, immunohistochemistry; OS, overall survival; PFS, progression-free survival; rego, regorafenib.
HRs were calculated using a Cox model stratified by geographic region (US or the rest of the world), as per the study protocol.

Figure 5. CD73 Protein Assessed by IHC Showed a Range of TC Expression Across ARC-9 Biopsy Samples



IHC, immunohistochemistry; TC, tumor cell.
CD73-IHC was visually scored for tumor expression with examples in panels F–J ranging from low to high. Corresponding images of hematoxylin and eosin (H&E) staining and isotype controls are shown in panels A–E and K–O, respectively. CD73+ normal adjacent liver is shown in the top right corner of panel F.

Figure 6. EZFB Significantly Improved Survival Compared With Rego in Patients With ≥ 1% CD73+ TC



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HRs were calculated using a Cox model stratified by geographic region (US or the rest of the world), as per the study protocol.

References | Disclosures | Acknowledgments

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