C007

ARC-8

Quemliclustat (CD73 Inhibitor) Reduces Adenosine-Regulated NR4A Gene Expression and Increases mPDAC Inflammation in Patients From the ARC-8 Trial

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Objectives

- To investigate the mechanism of action of quemliclustat (quemli) and treatment-related changes in adenosine-regulated genes using transcriptomic analysis of matched pairs of pre- and post-treatment tumor samples from patients enrolled in the ARC-8 clinical trial
- To assess the relationship between clinical benefit with quemli-containing regimens and tumor adenosine biology at baseline and post-treatment

Conclusions

- Treatment with quemli-containing regimens with or without zimberelimab led to a reduction in the expression of adenosine-regulated NR4A genes and an increase in the levels of tumor inflammation among patients with mPDAC who were enrolled in ARC-8
  - Paired tumor samples from ARC-8 showed a reduction in NR4A gene expression following treatment with quemli
  - Decreased tumor NR4A gene expression on treatment was accompanied by an increase in T-cell activation signature
- Quemli-derived clinical benefit may be linked to adenosine biology in ARC-8
- Modulation of NR4A gene expression in tumors is associated with PFS/OS in ARC-8 tumor PD cohort
- Clinical benefit of a quemli-containing regimen may be most pronounced in cases with high baseline tumor expression levels of NR4A
- A phase 3 study (PRISM-1) is planned to assess the clinical benefit of quemli in combination with chemotherapy in treatment-naive patients with mPDAC

Introduction

- In pancreatic ductal adenocarcinoma (PDAC), extracellular adenosine suppresses anti-tumor immune responses in the tumor microenvironment
- Quemliclustat (quemli) is a potent and selective small-molecule inhibitor of soluble and cell-bound CD73,[1,2] a key enzyme involved in the production of extracellular adenosine within the tumor microenvironment
- In the ARC-8 clinical trial, quemli, in combination with gemcitabine/nab-paclitaxel (G/nP) with or without the anti-PD-1 antibody zimberelimab, demonstrated encouraging clinical activity in first-line metastatic PDAC (mPDAC) with a median overall survival (OS) ranging from 14.6 to 19.4 months[3]
- When activated, Gs-coupled adenosine receptors A_{2a} and A_{2b} increase levels of cyclic adenosine monophosphate (cAMP) and phosphorylation of cAMP response element-binding protein (CREB). The nuclear receptor subfamily 4A (NR4A) of orphan nuclear receptors is upregulated by CREB activation, suggesting that NR4A expression may be indicative of extracellular adenosine levels[4]
- Measuring adenosine levels in tumor samples directly is not technically feasible in a clinical setting. Using a potential surrogate for adenosine levels (ie, NR4A gene expression), we investigated the effect of quemli-containing regimens on adenosine levels

Methods

In Vitro NR4A1 Analysis

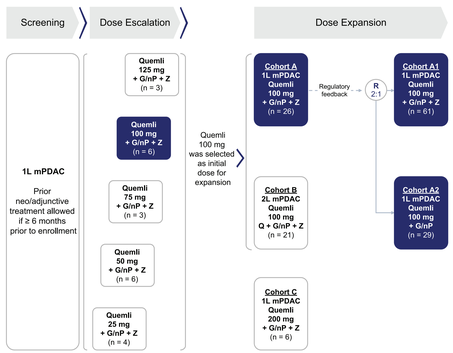
- Isolated human primary or cancer cell lines were stimulated with the adenosine receptor agonist 5'-(N-ethylcarboxamido) adenosine (NECA) or provided AMP that was converted to adenosine by CD73 on the cell surface
- Cancer cell lines NCI-H441 and NCI-H650 (lung) were examined for NECA (adenosine analog)-stimulated gene expression using RNA-sequencing (RNA-seq)
- PANC-1 (pancreatic) and pancreatic tumor fibroblasts were evaluated for quemli treatment effect on NR4A gene expression by RT-PCR

Biomarker Analysis

- ARC-8 (NCT04104672) was a phase 1b, dose-escalation, dose-expansion study in patients with mPDAC who were treatment-naive **(Figure 1)**
- Tumors were microdissected to enrich to 70% or greater 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 voom-transformed to obtain gene expression values
  - Single sample Gene Set Enrichment Analysis (ssGSEA) was used to calculate pathway enrichment scores[5]
- ssGSEA scores of the NR4A family of genes (NR4A1, 2, and 3) were calculated from RNA-seq data from 37 patients who received quemli 100 mg with or without zimberelimab and had paired pre- and post-treatment sequencing results
- Cox regression, Kaplan-Meier curves, and log-rank tests were used for survival analysis
- Paired Wilcoxon rank sum and signed rank tests were used for calculating statistical significance for pharmacodynamic (PD) changes

Study Design and Treatment

Figure 1. ARC-8 Study Design



mPDAC, metastatic pancreatic ductal adenocarcinoma; Q, quemliclustat; quemli, quemliclustat; R, randomization; G/nP, gemcitabine/nab paclitaxel; Z, zimberelimab.

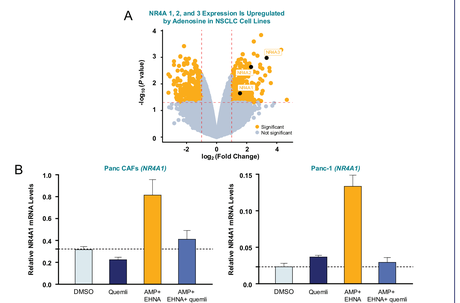
- Quemli PD analysis population includes all patients receiving quemli 100 mg with matched pre- and post-treatment tumor RNA-seq analysis

Results

In Vitro NR4A Analysis

- Adenosine receptor agonism (ie, NECA) significantly upregulated all 3 NR4A genes in NSCLC cell lines **(Figure 2A)**
- Adenosine derived from CD73-mediated AMP hydrolysis stimulated NR4A expression, which was inhibited by the addition of quemli **(Figure 2B)**

Figure 2. Adenosine Signaling Increased NR4A Gene Expression In Vitro, Which Is Reversed by a Quemli-Containing Regimen



In panel A, three NR4A family genes are labeled for emphasis.
AMP, adenosine monophosphate; CAFs, cancer-associated fibroblasts; DMSO, dimethylsulfoxide; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine; mNRA, messenger RNA; NECA, 5'-(N-ethylcarboxamido) adenosine; NR4A, nuclear receptor subfamily 4A; NSCLC, non-small cell lung cancer; PANC-1, human pancreatic cancer cell line; quemli, quemliclustat with or without zimberelimab.

Biomarker Analysis Population

- As of the June 19, 2023 cutoff date, 122 patients with mPDAC who were treatment-naive received quemli-based regimens
- For patients with available tumor sequencing data (baseline, n = 80; paired pre- and post treatment, n = 37), baseline characteristics and outcomes were similar to the intention-to-treat population **(Table)**

Table. Baseline Demographic and Clinical Characteristics

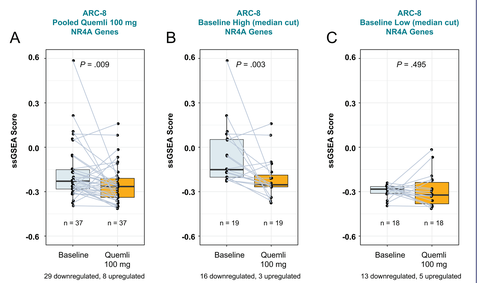
Characteristic	Total Quemli Treated (n = 122)	RNA-Seq Evaluable Baseline (n = 80)	RNA-Seq Evaluable All Paired Biopsies (n = 37)
Age, years, median (range)	65.5 (41, 81)	67.5 (42, 81)	66 (44, 81)
Sex, n (%) Female Male	58 (47.5) 64 (52.5)	37 (46.3) 43 (53.8)	20 (54.1) 17 (45.9)
ECOG PS, 0/1, (%)	34.4/65.6	36.3/63.7	48.6/51.4
Liver metastases present at baseline, n (%)	79 (64.8)	56 (70.0)	26 (70.3)
Median overall survival, months (95% CI)	15.7 (12.4, 19.5)	13.9 (10.6, 21.5)	16.53 (12.5, NR)

ECOG PS, Eastern Cooperative Oncology Group Performance Status; NR, not reached; quemli, quemliclustat with or without zimberelimab.

ARC-8 PD Analysis

- Expression of NR4A genes was significantly downregulated in samples post-treatment versus baseline (P = .009; **Figure 3A**)
- Tumor samples with higher baseline levels of NR4A had a larger decrease in NR4A gene expression **(Figure 3B)**

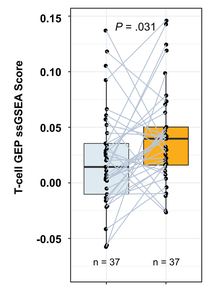
Figure 3. NR4A Gene Tumor Expression Is Downregulated by a Quemli-Containing Regimen



Boxplots represent 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.
NR4A, nuclear receptor subfamily 4A; quemli, quemliclustat with or without zimberelimab; ssGSEA, single sample Gene Set Enrichment Analysis.

- Treatment with quemli-containing regimens led to an increase in tumor inflammation, as measured by a T-cell activation signature (T-cell–inflamed GEP[6]; **Figure 4**)

Figure 4. Treatment With a Quemli-Containing Regimen Led to an Increase in Tumor Inflammation

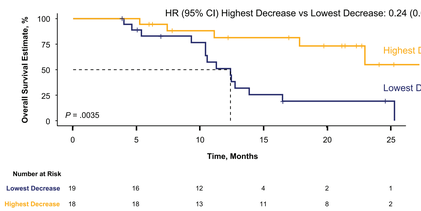


GEP, gene-expression profile; ssGSEA, single sample Gene Set Enrichment Analysis; quemli, quemliclustat with or without zimberelimab.

Quemli Treatment-Benefit May Be Linked to Adenosine Biology

- Within the ARC-8 tumor PD biomarker evaluable population (n = 37), the most pronounced decrease in tumor NR4A gene expression after treatment with quemli-containing regimens was associated with OS benefit compared with cases with the lowest decrease in NR4A family gene expression after treatment (HR [95% CI]: 0.24 [0.08, 0.67]; **Figure 5**)

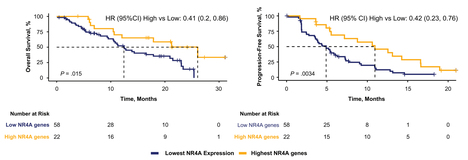
Figure 5. Patients With the Highest Decreases in Tumor NR4A Gene Expression Experience an OS Benefit in ARC-8



Kaplan-Meier estimate of OS according to the magnitude of treatment-dependent changes in NR4A gene expression in tumors by median cut. Ticks in Kaplan-Meier curves denote censoring events.
HR, hazard ratio; NR4A, nuclear receptor subfamily 4A; OS, overall survival.

- Kaplan-Meier estimate of OS and progression-free survival, according to baseline levels of tumor NR4A gene expression, showed that patients with higher levels of NR4A gene expression experienced a greater benefit with quemli treatment **(Figure 6)**

Figure 6. Patients With the Highest Baseline NR4A Gene Expression Derived Greater Benefit From a Quemli-Containing Regimen



Kaplan-Meier estimate of OS and PFS according to the baseline NR4A gene expression in tumors using best cut. Ticks in Kaplan-Meier curves denote censoring events.
HR, hazard ratio; NR4A, nuclear receptor subfamily 4A; OS, overall survival; PFS, progression-free survival; quemli, quemliclustat with or without zimberelimab.

References | Disclosures | Acknowledgments

References: 1. Lawson KV, *et al. J Med Chem.* 2020;63(20):11448–68. 2. Bowman CE, *et al. Biochemistry.* 2019;58(31):3331–34. 3. Wainberg Z, et al. *J Clin Oncol.* 2024;42(3 suppl):Abstract 665. 4. Hawk J and Abel T. *Brain Res Bull.* 2011;85(1-2):21–9. 5. Barbie DA, *et al. Nature.* 2009;462(7269):108–12. 6. Cristescu R, et al. *Science.* 2018;362(6411):eaar3593.

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