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Brief Communications

Bacterial Lipopolysaccharide as a Negative Predictor of Adjuvant Gemcitabine Efficacy in Pancreatic Cancer

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Abstract

Adjuvant gemcitabine (aGC) is one standard of care after pancreatic ductal adenocarcinoma (PDAC) resection. No biomarker for its efficacy is established. As bacteria mediate gemcitabine resistance, we analyzed whether lipopolysaccharide (LPS) as surrogate for bacterial colonization is prognostic in PDAC patients treated with aGC or without aGC adjuvant gemcitabine. We detected LPS in 86 tumors from 376 patients, which defined a specific microbiome as revealed by 16 s-rRNA-sequencing. In the 230 aGC patients, LPS conferred worse disease-free survival (8.3 vs 13.7 months; hazard ratio = 1.75, 95% confidence interval = 1.22 to 2.49; log-rank $P = .002$) and overall survival (21.7 vs 28.5 months; hazard ratio = 1.80, 95% confidence interval = 1.23 to 2.57; log-rank $P = .001$) but not in the 146 naGC patients, which was confirmed in an independent validation cohort (n = 178). LPS may serve as a negative predictor for aGC efficacy in PDAC, which suggests a role for microbiome modification to overcome bacteria-mediated chemotherapy resistance.

The dismal prognosis of pancreatic ductal adenocarcinoma (PDAC) is improved by adjuvant chemotherapy, for which gemcitabine remains a therapeutic mainstay in the clinically unfit patient (1); however, no biomarker for efficacy prediction is established. The tumor microbiome in PDAC affects patient prognosis (2) as well as response to gemcitabine-based chemotherapy in preclinical models (3) and advanced disease stages (4) in which single-agent gemcitabine is replaced by more efficient regimens (1). However, the effect of the tumor microbiome on adjuvant gemcitabine (aGC) efficacy has not been examined to date.

We retrieved formalin-fixed, paraffin-embedded primary tumor tissue from PDAC resections from the archives of the Institute of Pathology of Ludwig-Maximilians-University (LMU). Clinicopathological, outcome, and treatment data were derived from the databases of the Institute of Pathology, the Munich Cancer Registry, and the LMU University Hospital. We updated each cases' TNM classification to the current Union Internationale Contre le Cancer (UICC) staging system (5). The ethics committee at the LMU medical faculty approved the study (20-081). Tissue microarray construction, lipopolysaccharide (LPS) immunohistochemistry, and staining were described previously (4). To evaluate the association of LPS detection with disease-free survival (DFS) and overall survival (OS) independent of other clinicopathologic factors, we employed log-rank statistics and univariate and multivariate Cox regression models. DFS was calculated from adjuvant therapy initiation or surgery (in the patients without adjuvant therapy) to clinically apparent disease relapse. OS was calculated from surgery to death by disease excluding patients deceased within 30 days postsurgery. Statistical significance was indicated by a P value less than .05. All statistical tests were 2-sided where appropriate. We examined the intratumoral microbiome by sequencing the bacterial 16 s rRNA locus (16 s rRNA-seq) using tumor DNA extracted from formalin-fixed, paraffin-embedded tissue (6) (Supplementary Methods, available online). Propensity score matching was conducted using pymatch (https://github.com/benmiroglio/pymatch) for Python (Anaconda

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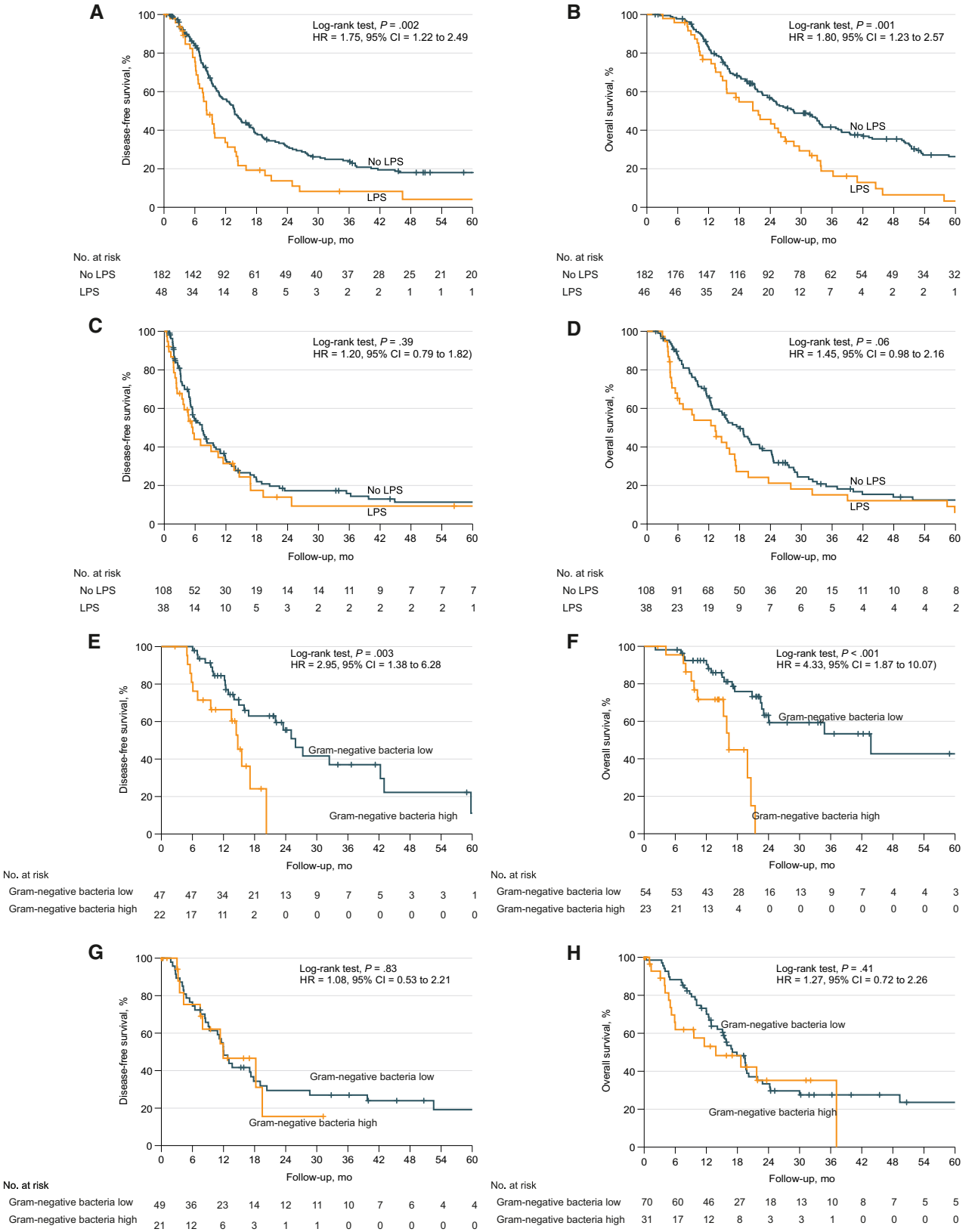

Figure 1. Intratumoral LPS detection and the abundance of gram-negative bacteria are associated with poor disease-free survival and overall survival in pancreatic cancer patients treated with adjuvant gemcitabine (aGC). Intratumoral LPS detection and the abundance of gram-negative bacteria are negatively associated to disease-free survival and overall survival in **(A, B)** the aGC study cohort (n = 230) and **(C, D)** the naGC study cohort (n = 146) as well as in **(E, F)** the aGC validation cohort (n = 101) and **(G, H)** the naGC validation cohort (n = 77). All statistical tests were 2-sided. CI = confidence interval; HR = hazard ratio; LPS = lipopolysaccharide.

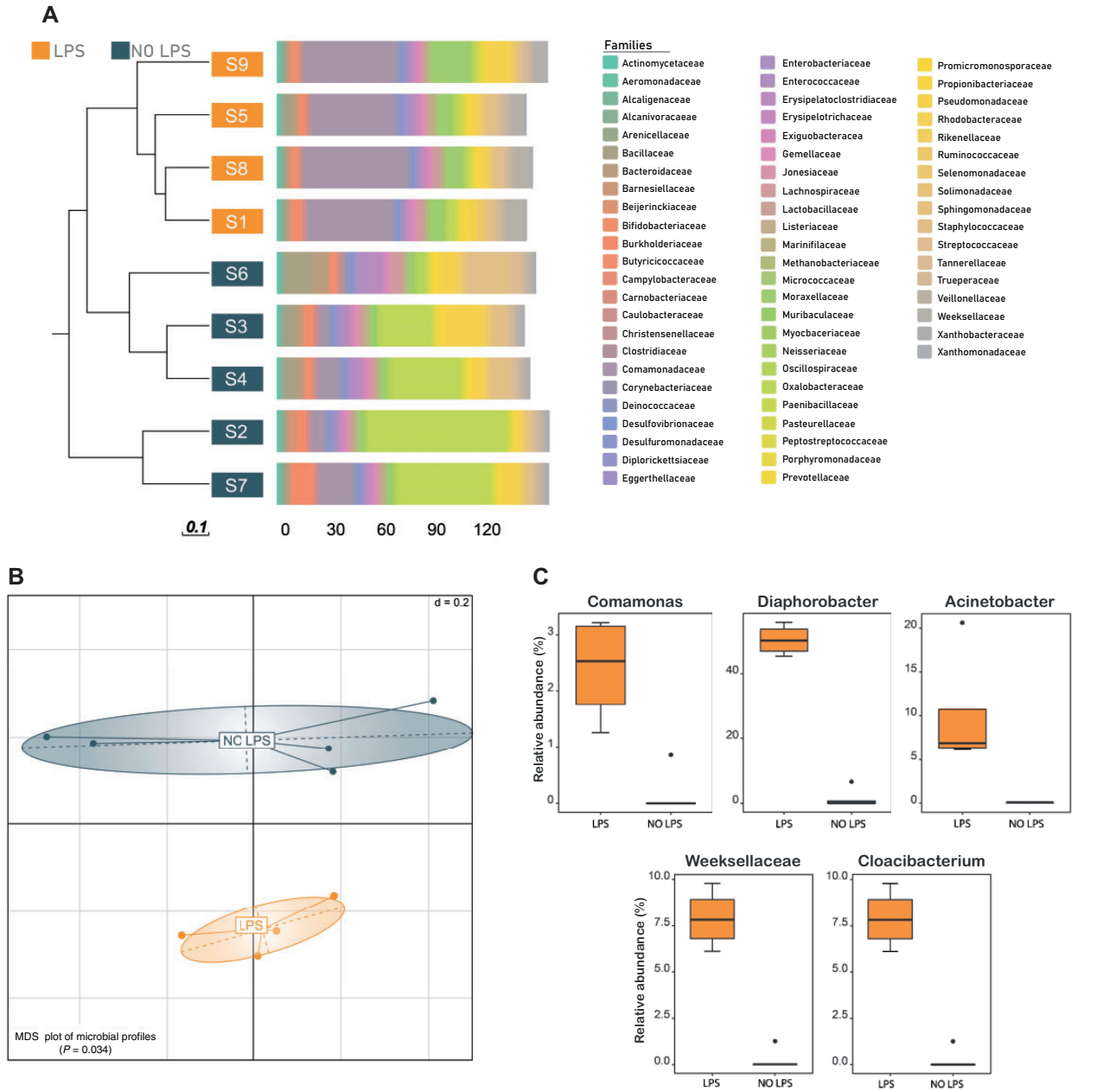

Figure 2. Lipopolysaccharide (LPS) positivity defines a specific tumor microbiome as determined by 16 s rRNA sequencing. **A)** Phylogenetic distance tree calculated by generalized unique fraction metric (UniFrac) distances, grouped by hierarchical clustering and taxonomic composition on family level based on the relative sequence abundances (**colored bar plots**). **B)** Multidimensional scaling shows a significant clustering according to LPS positivity and a high level of dissimilarity between LPS-positive and LPS-negative samples (beta-diversity) based on generalized UniFrac distances. **C)** Relative abundances of the 5 main differentially detected species between LPS-positive and LPS-negative samples by systematic testing of all available operational taxonomic units using the nonparametric Kruskal-Wallis rank sum test correcting the calculated pairwise test significance values for multiple testing using the Benjamini-Hochberg method. All statistical tests were 2-sided. MDS = multidimensional scaling.

Inc, Austin, TX, USA). Normalized abundance microbial data were downloaded from the data repository of The Cancer Genome Atlas as described (7). Corresponding clinical patient information was downloaded from Broad Genome Data Analysis Center (GDAC) Firehose and National Cancer Institute Genomic Data Commons (GDC Data Release v29.0; Supplementary Methods, available online).

The study cohort comprised 197 men and 179 women (median age = 66 years, range = 41-83 years) of which 230 (61.2%) received adjuvant gemcitabine (aGC) and 146 (38.8%) received either nongemcitabine based (n = 29) or no adjuvant (n = 117) (naGC) treatment (Supplementary Table 1, available online). The median follow-up was 88.02 (95% confidence interval [CI] = 72.2 to 103.8) months. The aGC therapy conferred superior DFS and OS over naGC treatment (DFS 12.7 vs 6.9 months, hazard ratio [HR] = 0.65, 95% CI = 0.52 to 0.83; log-rank $P < .001$; OS 25.8 vs 15.6 months, HR = 0.59, 95% CI = 0.46 to 0.74; log-rank $P < .001$). We detected intratumoral LPS at similar rates in both cohorts (aGC cohort = 20.9 %, naGC cohort = 26.0 %; Pearson χ^2 $P = .25$; Supplementary Table 1, available online). LPS positivity conferred reduced DFS (8.3 vs 13.7 months, HR = 1.75, 95% CI = 1.22 to 2.49; log-rank $P = .002$; Figure 1, A) and OS (21.7 vs 28.5 months, HR = 1.80, 95% CI = 1.23 to 2.57; log-rank $P = .001$;

Figure 1, B) in the aGC cohort but not in the naGC cohort (DFS 5.6 vs 7.4 months, HR =1.20, 95% CI = 0.79 to 1.82; log-rank $P = .39$; OS 13.3 vs 18.7 months, HR = 1.45, 95% CI = 0.98 to 2.16; log-rank $P = .06$; Figure 1, C and D). LPS positivity also reflected on 5-year survival rates of 21.1% vs 2.4% (LPS negative vs LPS positive tumors; Pearson χ^2 $P = .004$) in patients of the aGC cohort, whereas no differences in the naGC cohort were detected (8.7% vs 5.7%; Pearson χ^2 $P = .58$). LPS did not correlate to preoperative antibiotic treatment, bile duct intervention, or diabetes (all Pearson χ^2 $P > .2$). Multivariate analyses confirmed LPS as a negative predictor for DFS (HR = 1.83, 95% CI = 1.26 to 2.65; Cox $P = .001$) and OS (HR = 1.82, 95% CI = 1.26 to 2.62; Cox $P = .001$) in the aGC cohort. Propensity score matching compensated imbalances between the cohorts (Supplementary Table 1, available online), resulted in balanced subgroups (n = 100 each), and confirmed the findings evidently (DFS in the aGC cohort 9.4 vs 15.1 months, HR = 2.3, 95% CI = 1.37 to 3.88; log-rank $P = .001$; in the naGC cohort 5.8 vs 7.4 months, HR = 0.94, 95% CI = 0.57 to 1.68; log-rank $P = .94$). For validation, we determined whether the abundance of gram-negative bacteria affect outcome dependent on adjuvant chemotherapy in The Cancer Genome Atlas dataset (n = 178; Supplementary Table 2, available online). Abundant intratumoral gram-negative bacteria conferred inferior DFS and OS in aGC patients (n = 77), whereas in naGC patients (n = 101), we observed no effect on outcome (Figure 1, E-H). The 16 s rRNA-seq from 9 advanced PDAC tumors (4,8) revealed that LPS positivity defined a specific tumor microbiome correlating with the relative abundance of the genera Comamonas, Diaphorobacter, and Acinetobacter within the phylum of Proteobacteria as well as Weeksellaceae and Cloacibacterium within the phylum of Bacteriodetes. The phylum proteobacteria, to which the vast majority of bacteria belong that express the long isoform of cytidine deaminase, has been shown to cause gemcitabine resistance in vitro and in vivo (3) (Figure 2, A-C).

Here we show that intratumoral LPS is associated with inferior DFS and OS in PDAC patients treated with gemcitabine-based adjuvant chemotherapy and that it defines a specific tumor microbiome. In patients receiving either no or nongemcitabine-based adjuvant chemotherapy, LPS had no prognostic impact. This correlation was even more pronounced after propensity score matching, and we confirmed this association in a validation dataset. Thus, in line with previously published data on gemcitabine resistance mediated by long isoform of cytidine deaminase to expressing bacteria (3), we reason that an LPS-positive tumor microbiome serves as a negative predictor of aGC efficacy. Our observations are limited by the retrospective nature of this single-center study and lacking information on the tumor microbiome during disease progression, as we examined primary tumor tissue only. However, as most patients relapse eventually, PDAC is considered a systemic disease on diagnosis (9), which explains the negative predictive effect of LPS in the primary tumor. The nonsignificant trend toward decreased OS in LPS-positive naGC cases may be due to the negative predictive effect of LPS on palliative gemcitabine-based therapy (4), which many patients received after relapse. aGC is partly replaced by more efficient regimens in selected patients (10). However, it is still widely used and recommended for patients with Eastern Cooperative Oncology Group performance status scale (ECOG) > 1 by National Comprehensive Cancer Network guidelines (11), as many cannot receive more toxic adjuvant therapies because of their limited condition. Further studies on the tumor microbiome impact on outcome in adjuvant treatment randomized controlled trials are required to verify our findings and to clarify whether they are limited to gemcitabine-based therapies. Our results offer a potential predictive biomarker for clinical decisions on adjuvant treatment. Additionally, they provide a rationale to address the tumor microbiome as a therapeutic target in PDAC as it may be modified by antibiotics or microbiome transplantation, which has already been established for gastrointestinal diseases (12) and in the context of immune therapy (13).

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Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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