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Pegylated granulocyte-colony stimulating factor combined with anti-PD-1/PD-L1 inhibitors in the treatment of recurrent or metastatic biliary tract cancer: Efficacy and safety of a single-arm, prospective phase II clinical trial

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Abstract

Background: Biliary Tract Cancer (BTC) is a malignancy with an extremely poor prognosis. For patients with recurrent or metastatic BTC who have failed first-line chemotherapy, treatment options are limited. Although anti-PD-1/PD-L1 inhibitors have shown efficacy in various tumors, their single-agent activity in BTC is limited, particularly in previously treated patients. Preclinical evidence suggests that Granulocyte-Colony Stimulating Factor (G-CSF) may enhance the efficacy of anti-PD-1/PD-L1 inhibitors by remodeling the tumor immune microenvironment. This study aimed to evaluate whether the addition of long-acting G-CSF (pegylated recombinant human G-CSF, pegfilgrastim) could restore or enhance the efficacy of anti-PD-1/PD-L1 inhibitors in patients with resistant BTC.

Methods: This was a single-arm, prospective, Phase II clinical trial. The inclusion criteria were histologically confirmed recurrent or metastatic BTC that had progressed after at least one line of systemic chemotherapy. All patients received an anti-PD-1 inhibitor (sintilimab 200 mg IV q3w) or an anti-PD-L1 inhibitor (atezolizumab 1200 mg IV q3w). On day 2 of each cycle, pegfilgrastim (6 mg subcutaneously) was administered. The primary endpoint was the Objective Response Rate (ORR) as assessed by the investigator according to RECIST v1.1. Secondary endpoints included Disease Control Rate (DCR), Progression-Free Survival (PFS), Overall Survival (OS), Duration of Response (DoR), and safety.

Results: A total of 42 patients were enrolled. The median follow-up time was 12.8 months (95% CI: 10.5-15.1 months). The confirmed ORR was 21.4% (95% CI: 10.3%-36.8%), with 1 patient achieving a Complete Response (CR) and 8 patients achieving a Partial Response (PR). The DCR was 66.7% (95% CI: 50.5%-80.4%). The median PFS was 4.2 months (95% CI: 3.1-5.3 months), and the median OS was 11.5 months (95% CI: 9.2-13.8 months). The most common Treatment-Related Adverse Events (TRAEs) of any grade were leukopenia

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(45.2%), neutropenia (40.5%), and fatigue (31.0%). Grade 3 or higher TRAEs occurred in 19.0% of patients, with the most common being neutropenia (9.5%) and anemia (4.8%). No treatment-related deaths were reported. Exploratory biomarker analysis suggested that patients with a baseline Neutrophil-to-Lymphocyte Ratio (NLR) < 3 had a higher ORR and longer PFS.

Conclusion: For patients with recurrent or metastatic BTC who have progressed on prior chemotherapy, the combination of long-acting G-CSF and anti-PD-1/PD-L1 inhibitors demonstrated promising anti-tumor activity and a manageable safety profile. This regimen appears to be a viable therapeutic option, capable of restoring sensitivity to immunotherapy in this difficult-to-treat population. Further validation from randomized controlled trials is warranted.

Introduction

Biliary Tract Cancer (BTC), encompassing Intrahepatic Cholangiocarcinoma (iCCA), Extrahepatic Cholangiocarcinoma (eCCA), and Gallbladder Cancer (GBC), is a group of highly aggressive malignancies often diagnosed at an advanced stage [1]. The standard first-line treatment for advanced BTC is the combination of gemcitabine and cisplatin, based on the ABC-02 trial, which yields a median Overall Survival (OS) of only 11.7 months [2]. Upon progression after first-line therapy, the prognosis is dismal, with effective treatment options being extremely limited. FOLFOX chemotherapy is an option, but its benefits are modest [3].

In recent years, Immune Checkpoint Inhibitors (ICIs), particularly anti-PD-1/PD-L1 antibodies, have revolutionized the treatment landscape for many solid tumors. However, their efficacy as monotherapy in BTC has been disappointing. The KEYNOTE-158 study showed that pembrolizumab monotherapy in pre-treated advanced BTC patients resulted in an Objective Response Rate (ORR) of only 5.8% in the PD-L1 Combined Positive Score (CPS) ≥1 population [4]. The primary reasons for this low responsiveness include the low tumor mutational burden (TMB) of BTC, its immunosuppressive Tumor Microenvironment (TME), and the development of primary or acquired resistance to ICIs [5].

The mechanisms of ICI resistance are complex and involve both tumor-intrinsic factors (e.g., loss of antigen presentation, activation of the WNT/β-catenin pathway) and extrinsic factors, particularly the immunosuppressive TME [6]. A key feature of the immunosuppressive TME is the abundance of Myeloid-Derived Suppressor Cells (MDSCs) and Tumor-Associated Neutrophils (TANs), which can inhibit the function of cytotoxic T lymphocytes and promote tumor progression and immune evasion [7].

Granulocyte-Colony Stimulating Factor (G-CSF) is a cytokine that primarily stimulates the proliferation, differentiation, and activation of neutrophil lineage cells. It is widely used to prevent and treat chemotherapy-induced neutropenia. Interestingly, emerging preclinical evidence suggests that G-CSF may have immunomodulatory effects beyond hematopoiesis. Studies have shown that G-CSF can "reprogram" or "polarize" neutrophils from a pro-tumor (N2) phenotype to an anti-tumor (N1) phenotype. These N1 neutrophils can produce Reactive Oxygen Species (ROS) and proteases to directly kill tumor cells and secrete cytokines (e.g., TNF-α, IL-12) that promote T cell activation and recruitment, thereby synergizing with ICIs [8,9]. A pivotal study by Markman et al. demonstrated that low-dose G-CSF could convert a suppressive myeloid cell population into tumor-destroying effector cells, significantly enhancing the efficacy of anti-PD-1 therapy in mouse models of pancreatic cancer and melanoma [10].

Based on this compelling preclinical rationale, we hypothesized that adding long-acting G-CSF (pegfilgrastim) to anti-PD-1/PD-L1 therapy could overcome myeloid cell-mediated immunosuppression in BTC, thereby restoring or enhancing the efficacy of ICIs in patients who have developed resistance to prior treatments. To test this hypothesis, we conducted this prospective Phase II clinical trial to evaluate the efficacy and safety of this novel combination strategy in patients with recurrent or metastatic BTC.

Methods

Study design and patients

This was a single-arm, open-label, prospective Phase II clinical trial conducted at weifang. The study protocol was approved by the Institutional Review Board (IRB) of weifang people's hospital and was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines. All patients provided written informed consent before enrollment.

Key inclusion criteria were: (1) age ≥18 years; (2) histologically or cytologically confirmed unresectable recurrent or metastatic BTC (including iCCA, eCCA, or GBC); (3) disease progression after at least one line of systemic chemotherapy for advanced disease; (4) at least one measurable lesion according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1); (5) an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; and (6) adequate organ function.

Key exclusion criteria were: (1) prior treatment with any immune checkpoint inhibitor; (2) active autoimmune disease requiring systemic therapy within the past 2 years; (3) uncontrolled intercurrent illness, including active infection; (4) known central nervous system metastases unless stable after treatment; (5) a history of allogeneic stem cell or solid organ transplantation; and (6) concurrent use of other systemic anti-cancer therapy.

Treatment protocol

Patients received an anti-PD-1 inhibitor (sintilimab 200 mg) or an anti-PD-L1 inhibitor (atezolizumab 1200 mg) administered intravenously on day 1 of each 21-day cycle. The choice between the two was at the investigator's discretion. On day 2 of each cycle, pegfilgrastim (6 mg) was administered subcutaneously. Treatment continued until disease progression, unacceptable toxicity, withdrawal of consent, or up to 24 months of ICI therapy. Dose modifications for ICIs were permitted according to the manufacturer's prescribing information for managing immune-related adverse events (irAEs). Pegfilgrastim was held if the patient developed grade ≥3 non-hematologic toxicity deemed related to it.

Assessments

Tumor assessments using contrast-enhanced CT or MRI were performed at baseline and then every 8 weeks. Responses were evaluated by the investigators according to RECIST v1.1. Adverse Events (AEs) were continuously monitored and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5.0. Laboratory

tests, including complete blood counts with differential and comprehensive metabolic panels, were performed at baseline and before each treatment cycle.

Exploratory biomarker analysis

Peripheral blood samples were collected at baseline and before each cycle for complete blood counts. The Neutrophil-to-Lymphocyte Ratio (NLR) was calculated. The relationship between baseline NLR (cutoff at 3) and clinical outcomes (ORR, PFS) was explored as a post-hoc analysis.

Endpoints

The primary endpoint was the ORR, defined as the proportion of patients who achieved a confirmed Complete Response (CR) or Partial Response (PR). Secondary endpoints included DCR (proportion of patients with CR, PR, or Stable Disease [SD]), PFS (time from enrollment to disease progression or death from any cause), OS (time from enrollment to death from any cause), DoR (time from first documented response to disease progression), and safety (incidence and severity of TRAEs).

Statistical analysis

The primary analysis was performed on the Intention-To-Treat (ITT) population, which included all patients who received at least one dose of the study treatment. The safety analysis included all patients who received any study medication. The ORR and DCR were calculated with their corresponding two-sided 95% Confidence Intervals (CIs) using the Clopper-Pearson method. PFS, OS, and DoR were estimated using the Kaplan-Meier method, and median values with 95% CIs were reported. The relationship between baseline NLR and ORR was compared using Fisher's exact test, and PFS was compared using the log-rank test. A two-sided p-value<0.05 was considered statistically significant. All statistical analyses were performed using SPSS software (version 26.0).

Results

Patient characteristics

Between May 2023 and May 2024, a total of 45 patients were screened, and 42 were enrolled and received at least one dose of the study treatment. The median age was 61 years (range, 38-75), and 61.9% were male. The primary tumor sites were iCCA (47.6%), eCCA (28.6%), and GBC (23.8%). All patients had received at least one prior line of systemic chemotherapy, and 28.6% had received two or more prior lines. The ECOG performance status was 0 in 52.4% of patients and 1 in 47.6%.

Efficacy

The median follow-up duration was 12.8 months (95% CI: 10.5-15.1 months). All patients were evaluable for tumor response.

The confirmed ORR was 21.4% (95% CI: 10.3%-36.8%), comprising 1 CR (2.4%) and 8 PRs (19.0%). An additional 19 patients (45.2%) achieved SD as their best response, resulting in a DCR of 66.7% (95% CI: 50.5%-80.4%).

The median PFS was 4.2 months (95% CI: 3.1-5.3 months). The median OS was 11.5 months (95% CI: 9.2-13.8 months). The median DoR for the 9 responders was 7.8 months (95% CI: 5.1-10.5 months).

Safety

All 42 patients experienced at least one treatment-related adverse event (TRAE) of any grade. The most common TRAEs were leukopenia (45.2%), neutropenia (40.5%), fatigue (31.0%), and anemia (28.6%). Grade 3 or higher TRAEs occurred in 8 patients (19.0%). The most frequent grade ≥3 TRAEs were neutropenia (9.5%) and anemia (4.8%). Immune-related AEs of any grade occurred in 11 patients (26.2%), including hypothyroidism (9.5%), rash (7.1%), and hepatitis (4.8%). Only one patient (2.4%) experienced a grade 3 irAE (hepatitis), which resolved with corticosteroid therapy. No treatment-related deaths or treatment discontinuations due to pegfilgrastim-related toxicities were reported.

Exploratory biomarker analysis

We analyzed the impact of baseline NLR on treatment outcomes. Patients with a baseline NLR<3 (n=18) had a significantly higher ORR compared to those with NLR≥3 (n=24) (33.3% vs. 12.5%, p=0.041). The median PFS was also longer in the NLR<3 group (5.8 months vs. 3.4 months; HR=0.58, 95% CI: 0.34-0.99; p=0.046).

Discussion

To the best of our knowledge, this is the first prospective clinical trial to investigate the combination of long-acting G-CSF with anti-PD-1/PD-L1 inhibitors in patients with pre-treated, advanced BTC. Our study demonstrated that this combination regimen yields a promising ORR of 21.4% and a median OS of 11.5 months, with a manageable safety profile. These results are encouraging, especially when compared to the historical data for ICI monotherapy in this setting.

In the KEYNOTE-158 study, pembrolizumab monotherapy in a similar pre-treated BTC population resulted in an ORR of 5.8% and a median OS of 7.9 months [4]. In a real-world study, nivolumab monotherapy yielded an ORR of 11.1% [11]. The ORR of 21.4% observed in our study more than doubles these figures, suggesting a synergistic effect of adding pegfilgrastim. Furthermore, the median OS of 11.5 months is comparable to, and perhaps slightly better than, the OS achieved with second-line FOLFOX chemotherapy (median OS of 6.2 months in the ABC-06 trial) [3]. Given the different toxicity profiles, this immunotherapy-based combination presents a compelling alternative for patients who are not candidates for or have progressed on cytotoxic chemotherapy.

The biological rationale for this combination is strongly supported by preclinical data. The TME of BTC is often characterized by a "cold" phenotype, marked by a scarcity of infiltrating cytotoxic T lymphocytes and an abundance of immunosuppressive myeloid cells [12]. G-CSF is hypothesized to counteract this by promoting the differentiation and activation of neutrophils towards an anti-tumor N1 phenotype. These N1 TANs can not only directly exert cytotoxic effects on tumor cells but also secrete chemokines like CXCL9 and CXCL10, which recruit and activate CD8+ T cells, thereby converting a "cold" tumor into a "hot" one that is more responsive to PD-1/PD-L1 blockade [9, 10]. The improved efficacy observed in our study provides the first clinical evidence supporting this translational concept in BTC.

The safety profile of the combination was acceptable. The most common AEs were hematologic, consistent with the known effects of G-CSF, and were generally low-grade. The in-

cidence of grade ≥3 neutropenia was 9.5%, which is manageable and did not lead to treatment discontinuation. The rate and severity of immune-related AEs were comparable to those reported for anti-PD-1/PD-L1 monotherapy, indicating that pegfilgrastim did not exacerbate immune toxicity. This is a crucial finding, as the combination's safety is paramount for its clinical applicability.

Our exploratory analysis revealed that a lower baseline NLR was associated with better clinical outcomes. NLR is a readily available, inexpensive biomarker that reflects systemic inflammation and immune status. A high NLR is often linked to a neutrophil-dominant, lymphocyte-deficient TME, which is associated with poor prognosis and resistance to ICIs across various cancers [13]. Our findings suggest that patients with a relatively more favorable baseline immune balance (NLR<3) may derive greater benefit from this combination strategy. This warrants further prospective validation and could help in patient selection.

This study has several limitations. First, it is a single-arm study without a control group, making it difficult to definitively attribute the observed efficacy to the combination therapy versus the ICI alone, despite historical comparisons. Second, the sample size was relatively small. Third, comprehensive correlative studies, such as serial tumor biopsies to analyze changes in TME (e.g., TAN polarization, T cell infiltration), were not performed, which would have provided direct mechanistic insights. Finally, the choice between anti-PD-1 and anti-PD-L1 inhibitors was not randomized, although their mechanisms of action are similar.

Future research should focus on conducting a randomized, controlled trial comparing anti-PD-1/PD-L1 monotherapy with the combination of anti-PD-1/PD-L1 plus pegfilgrastim in the second-line setting for BTC. Such a trial would provide high-level evidence for the clinical benefit of this strategy. Additionally, integrating extensive translational studies, including single-cell RNA sequencing of peripheral blood and tumor tissue, will be essential to elucidate the precise immunological mechanisms underlying the synergy and to identify predictive biomarkers beyond NLR.

Conclusion

The combination of long-acting G-CSF (pegfilgrastim) and anti-PD-1/PD-L1 inhibitors demonstrated promising anti-tumor activity and a manageable safety profile in patients with recurrent or metastatic BTC who progressed on prior chemotherapy. This regimen appears to be a viable therapeutic option capable of overcoming resistance to immunotherapy in this difficult-to-treat population. The results of this study provide a strong rationale for further validation in a randomized controlled trial.

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