Phase I trial of KN046, a novel bispecific antibody targeting PD-L1 and CTLA-4 in patients with advanced solid tumors

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ABSTRACT

Background KN046 is a novel bispecific antibody targeting programmed death ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4). This multicenter phase I trial investigated the safety, tolerability, pharmacokinetics (PK), and efficacy of KN046 in patients with advanced solid tumors.

Methods Patients who failed standard treatment were included. KN046 was administered at doses of 1, 3, and 5 mg/kg every 2 weeks (Q2W), 5 mg/kg every 3 weeks (Q3W), and 300 mg Q3W based on the modified toxicity probability interval method in the dose-escalation phase; the recommended dose was used in the expansion phase. Primary objectives were maximum tolerated dose (MTD) and recommended phase II dose (RP2D) in escalation and preliminary efficacy in expansion. Secondary objectives included PK, pharmacodynamics, safety, and tolerability of KN046. We also explored biomarkers based on PD-L1 expression, multiplex immunofluorescence (mIF) staining, and RNAseq-derived nCounter platform.

Results Totally, 100 eligible patients were enrolled, including 59 with nasopharyngeal carcinoma (NPC), 36 with epidermal growth factor receptor (EGFR)-mutated non-small-cell lung cancer (NSCLC), and those with other advanced solid tumors. The most common treatment-related adverse events (TRAEs) were rash (33.0%), pruritus (31.0%), and fatigue (20.0%). Grade ≥3 TRAEs were observed in 14.0% of participants. No dose-limiting toxicity occurred in the dose-escalation phase, and the MTD was not reached. The RP2D was determined as 5 mg/kg Q2W according to the pharmacokinetic–pharmacodynamic model, the preliminary exposure–response analysis, and the overall safety profile. Among 88 efficacy-evaluable participants, the objective response rate (ORR) was 12.5%, and the median duration of response was 16.6 months. In the NPC subgroup, the ORR was 15.4%, and the median overall survival (OS) was 24.7 (95% CI 16.3 to not estimable) months. In the EGFR-mutant NSCLC subgroup, the ORR was 6.3%. mIF analysis results showed patients with high CD8 expression showed longer median OS (27.1 vs 9.2 months, p=0.02); better prognosis was observed in patients with high CD8 and PD-L1 expression.

Conclusions KN046 was well tolerated and showed promising antitumor efficacy in advanced solid tumors, especially in patients with NPC. The combination of both CD8 and PD-L1 expression improved the prediction of KN046 response.

WHAT IS ALREADY KNOWN ON THIS TOPIC

- A combination of programmed death 1/programmed death ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4) blockade showed favorable outcomes in advanced solid tumors, while such combinations are associated with more severe adverse events and a lack of putative biomarkers.

WHAT THIS STUDY ADDS

- KN046 is a novel bispecific antibody targeting PD-L1 and CTLA-4. This is the first reported KN046 study that showed a relatively lower grade of ≥3 treatment-related adverse events rate and promising efficacy in advanced solid tumors, especially for patients with nasopharyngeal carcinoma (NPC). Biomarker analysis demonstrated that patients with high CD8 expression and high PD-L1 expression had longer median overall survival.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

- This study provided evidence for a further clinical trial of KN046 in patients with NPC and may lay the foundation of biomarker discovery related to advanced population from bispecific antibody.

Trial registration numbers NCT03733951.

BACKGROUND

In recent years, the advent of immunotherapy has changed the treatment strategy for solid tumors.1 Several anti-programmed death 1 (PD-1)/programmed death ligand 1 (PD-L1) and anti-cytotoxic T lymphocyte-associated protein 4 (CTLA-4) immune checkpoint inhibitors (ICIs) have been developed, with proven efficacy and safety in many tumor types.2,3 One of the most promising strategies for improving the outcome of immunotherapy is the combination of two ICIs or the use of bispecific antibodies with dual checkpoint blockade.4,5 Recent studies support that inhibiting PD-1/PD-L1 and CTLA-4 simultaneously achieves favorable...
outcomes in urothelial carcinoma, non-small-cell lung cancer (NSCLC), and melanoma. However, such combinations are expensive and associated with a higher rate of adverse events compared with single-agent immunotherapy. The development of bispecific antibodies by binding two distinct epitopes on the same or different antigens provides an option for enhancing the immune response and potentially minimizing toxicity. Several bispecific antibodies have been approved for global marketing, including blinatumomab (CD3×CD19), tisotumumab vedotin (CD3×T iso), and avolumizomab (estimated glomerular filtration rate (eGFR)×cellular-mesenchymal epithelial transition factor (c-MET)), and numerous advances have been observed for bispecific antibody drugs. However, bispecific antibodies targeting PD-L1 and CTLA-4 in solid tumors have not been applied clinically.

KN046, produced from Chinese hamster ovary cells, is a novel bispecific domain antibody fused with human IgG1 Fc, which blocks PD-L1 interaction with PD-1 and CTLA-4 interaction with CD80/CD86. PD-1-mediated immune suppression occurs in the antigen-elimination phase in the tumor, while CTLA-4-mediated suppression of T-cell activation occurred in the antigen-presentation phase; therefore, blockade of both PD-L1 and CTLA-4 might result in synergistic antitumor effects. The wild-type IgG1 Fc portion of KN046 preserves the intact effector functions, including antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity. A preclinical study indicated KN046 targets PD-L1 and CTLA-4, showed higher affinity for PD-L1, and mediated the depletion of regulatory T cells in the tumor microenvironment, which could enhance the antitumor immune response and reduce immunosuppressive effects. Compared with dual ICI therapy, KN046 might also reduce side effects and treatment costs.

Here, we conducted this multicenter phase I trial to assess the safety, maximum tolerated dose (MTD), recommended phase II dose (RP2D), and preliminary efficacy of KN046 in patients with advanced solid tumors who failed standard treatment. We also explored potential biomarkers for efficacy including PD-L1 expression, multiplex immunofluorescence (mIF) staining, and the nCounter PanCancer IO 360 panel.

**Patients and Methods**

**Study design**

This study included a dose-escalation phase and a schedule-expansion phase. The dose-escalation phase followed the modified toxicity probability interval (mTPI) method. A scientific monitoring committee discussed and adjusted the schedule-expansion phase. KN046 was administered at doses of 1, 3, and 5 mg/kg every 2 weeks (Q2W), 5 mg/kg every 3 weeks (Q3W) and 300 mg Q5W based on the mTPI method in the dose-escalation phase and the recommended dose was used in the expansion phase.

KN046 was administered intravenously until image-proven disease progression, significant clinical deterioration, unacceptable toxicity, and loss to follow-up, or administered for 2 years. In case of dose-limiting toxicity (DLT) or grade of ≥3 toxicity related to KN046, KN046 administration was discontinued until the treatment-related toxicity returned to a grade of ≤1. The specific adjustment scheme is presented in the online supplemental methods.

For the dose-escalation phase, the primary objectives were safety, MTD, and/or the RP2D. For the dose-expansion phase, the primary objective was preliminary efficacy. MTD is defined as the dose at which the DLT occurred in no more than 33% of the patients during 28 days in the Q2W dosing group or 21 days in the Q3W dosing group. DLT was defined as a severe adverse event related to KN046 treatment, including severe hematological or non-hematological toxicities, detailed in the online supplemental methods. The RP2D was determined as the MTD if the criteria was met. Otherwise, the RP2D was determined according to the pharmacokinetic–pharmacodynamic model.

Secondary objectives included pharmacokinetics (PK), pharmacodynamics (PD), safety, and tolerability of KN046. The exploratory endpoint was the associations of biomarkers (including PD-L1 expression, mIF, and RNAseq-derived nCounter platform) with efficacy.

**Patients**

This multicenter phase I trial included patients with advanced solid tumors who failed standard treatment. The inclusion and exclusion criteria are detailed in the online supplemental methods. The key inclusion criteria were (1) ≥18 years of age; (2) histologically or cytologically confirmed advanced, unresectable, or metastatic solid tumors, progression after standard treatment, no standard treatment option available, or could not tolerate the standard treatment; (3) measurable lesions at baseline; (4) an Eastern Cooperative Oncology Group performance status score of 0–1. The key exclusion criteria were (1) untreated active brain metastasis or (2) received radical radiotherapy within 3 months before enrollment.

**Assessments**

Treatment-emergent adverse events (TEAEs) graded according to National Cancer Institute Common Terminology Criteria for Adverse Events V.5.0, treatment-related adverse events (TRAEs) were judged by investigators. Objective response rate (ORR), duration of response (DOR), disease control rate (DCR), complete response (CR), partial response (PR) or stable disease (SD) for ≥6 weeks, clinical benefit rate (CBR, CR, PR, or SD for ≥12 weeks), 12-month progression-free survival (PFS), 12-month overall survival (OS), and pharmacokinetic parameters. Disease progression was evaluated according to Response Evaluation Criteria in Solid Tumors V.1.1.

CT or MRI examination was performed every 6 weeks in the first year and every 12 weeks thereafter. The safety was followed up to 90 days after the end of the treatment or the start of new antitumor treatment, whichever occurred first.
Pharmacokinetic–pharmacodynamic model development

Blood samples for pharmacokinetic analysis were collected predose, post dose (15 min after infusion), and 0.5, 1.0, 4.0, 24.0, 48.0, 72.0, 168.0, 336.0, and 504.0 hours after the administration of the first dose. For robustness, the pharmacokinetic data of KN046 in another phase I trial in Australia (NCT03529526) were included. The pharmacodynamic model was developed based on ex vivo interleukin-2 (IL-2) data. As an indirect measure of target KN046 occupancy on T cells, the ex vivo IL-2 stimulation ratio was measured predose and post dose. Blood samples were drawn before dosing, and 1, 7, 14, and 21 days after the administration of the first dose. Different concentrations of KN046 and 800ng/mL of staphylococcal enterotoxin-B were added into the peripheral blood mononuclear cell solutions to evaluate endogenous IL-2 levels by the ELISA method.

A two-compartmental first-order elimination model was fitted to multiple dose pharmacokinetic data in humans to build a population pharmacokinetic model (online supplemental methods). The relationship between KN046 concentration and the reduction of IL-2 stimulation ratio was described using a Sigmoid Imax model (online supplemental methods). Target trough concentration (C\text{\tiny{trough_target}}) was defined as the KN046 concentration reaching 95% inhibition of ex vivo IL-2 release (IC95). Simulations were performed to predict KN046 concentrations over time under different dose levels based on the population pharmacokinetic model. Patients in different dose groups predicted to attain the C\text{\tiny{trough_target}} were analyzed, and the percentage (preset ≥80% for RP2D) of individuals whose trough concentration exceeded the target concentration was calculated based on the simulation results.

Biomarker analyses

Archived or fresh tumor samples were retrieved for immunohistochemical staining of PD-L1 expression in tumor cells with SP263 antibody (Abcam) in a central laboratory. mIF staining of immune-related biomarkers including CD86, CD200, CD11B, CD4, CD8, FOXP3, GZMB, and PANCK was also conducted in a central laboratory. The expression of immune-related biomarkers was digitally measured and quantified as percentages of positive expression. We used the median percentage as a cut-off.

Gene expression analysis was performed on all available baseline formalin-fixed paraffin-embedded samples by the nCounter PanCancer IO 360 panel. Immune cell abundance was evaluated according to the algorithm of the average log-transformed expression values of marker genes. The details of mIF and the nCounter PanCancer IO 360 panel are shown in the online supplemental methods.

Statistical analysis

The safety analysis set included all participants who received KN046 at least once. The efficacy analysis set included all participants who had at least one efficacy evaluation. The DLT analysis set included participants who received ≥80% of the planned KN046 dosing in the dose-escalation phase. The pharmacokinetic analysis set included participants who had at least one pharmacokinetic sample available. Pharmacokinetic parameters were calculated using Phoenix WinNonlin V.8.3 (Certara, Princeton, New Jersey, USA).

For ORR, DCR, and CBR, the 95% CI calculated by the Clopper-Pearson method was reported in the total population and different doses and/or tumor species. For DOR, PFS, and OS, the median and 95% CI were calculated using the Kaplan-Meier method. Univariable Cox regression analysis was applied to explore the associations of immune-related biomarkers with OS. The correlation was examined with the Spearman rank correlation coefficients. All statistical analyses were performed using SAS V.9.4 or R V.4.0.3.

RESULTS

Patients

From November 2018 to April 2020, a total of 100 patients were enrolled (figure 1), including 59 with nasopharyngeal carcinoma (NPC), 36 with EGFR-mutated NSCLC, 2 with gastric cancer, 1 with small-cell lung cancer (SCLC), 1 with melanoma, and 1 with external auditory canal carcinoma. There were 24 (40.7%) participants with NPC and 9 (25%) participants with NSCLC who failed prior immunotherapy. The data cut-off date was August 31, 2021. The median follow-up was 22.6 (range 19.9–25.3) months. Patient characteristics are listed in table 1.

Safety

A total of 100 patients were included in the safety analysis. Of these, 12 cases were included in the DLT analysis set. No DLT occurred in the dose-escalation phase. TRAEs were observed in 83.0% (83/100) of all participants, including 14 (14.0%) participants with grade ≥3 TRAEs, and 9 (9.0%) with serious TRAEs. The most common TRAEs were rash (33.0%), pruritus (31.0%), and fatigue (20.0%) (table 2). The most common TEAEs are listed in online supplemental table S1. Immune-related adverse events (irAEs) and grade≥3 irAEs were observed in 47.0% and 4.0% of participants, respectively (table 2). The median onset time of irAEs was 2 weeks (range 0.1–74.3). The incidence rate of irAEs in 0–6 weeks was 35.0%. Among all participants, 14 (14.0%) had TRAEs leading to treatment suspension; 7 (7.0%) had TRAEs leading to treatment termination; and 1 (1.0%) had TRAE leading to death (table 2). One participant in the 300 mg Q3W dose group died of unknown reasons related to KN046.

Pharmacokinetics

A total of 62 subjects were included in the systemic pharmacokinetic analysis. Following KN046 administration at 1 mg/kg Q2W, 3 mg/kg Q2W, 5 mg/kg Q2W, 5 mg/kg Q3W, and 300 mg Q3W, no significant changes were observed in half-life (t\text{\text{1/2}}, 111.0–137.4 hours), the volume of distribution (4400.0–6138.8 mL), and clearance.

(27.4–34.65 mL/hour) in the different dose cohorts (n=62). Maximum concentration and area under the time–concentration curve0–t increased in an approximately dose-proportional manner (online supplemental table S2 and figure 2). In popPK analysis, two-compartmental first-order elimination combined with a proportional and additive residual error model well described the observed human pharmacokinetic data (online supplemental figure S1 and table S3).

**RP2D decision**

As no DLT was observed, the MTD was not determined. RP2D determination was based on the pharmacokinetic–pharmacodynamic model and the preliminary exposure–response analysis from the dose-escalation phase and the Australian cohort.

Data obtained from blood samples in 16 patients were included in the pharmacokinetic–pharmacodynamic model. The KN046 concentration required to cause IC₉₅ was estimated to be 2629 ng/mL, which was set as a Cₘₜₜₜₜ_target to reach the maximum target engagement. Due to the relatively small sample size, to provide uncertainty in estimates of the target engagement, the upper bound of 95% CI estimation of the corresponding calculated IC₉₅ (8683 ng/mL) was considered as a worst-case scenario (online supplemental table S4). To improve the robustness of the estimation for the target concentration, the association of plasma concentration with objective response was analyzed. Preliminary exposure–response for efficacy showed a median Cₘₜₜₜₜ of 8703 ng/mL (lower quartile, 7150 ng/mL) in patients who achieved disease control (CR, PR, or SD), which was higher than the median Cₘₜₜₜₜ (4048 ng/mL) in patients with progressive disease. The results indicated that efficacy might be correlated with Cₘₜₜₜₜ.

To increase the DCR, the selected RP2D should have a Cₘₜₜₜₜ over the preset Cₘₜₜₜₜ-target. Using the popPK simulation model, we applied the estimated Cₘₜₜₜₜ-target in the simulated concentration–time curves of 3 mg/kg Q2W, 5 mg/kg Q2W, and 5 mg/kg Q3W (online supplemental figure S2). The percentage of patients predicted to reach or exceed the set Cₘₜₜₜₜ-target was simulated. There were 73.5%, 85.1%, and 62.3% probability of Cₘₜₜₜₜ over Cₘₜₜₜₜ-target (2629 ng/mL) in doses of 3 mg/kg Q2W, 5 mg/kg Q2W, and 5 mg/kg Q3W, respectively (online supplemental table S5). When applying the upper limit of the 95% CI of the Cₘₜₜₜₜ-target (8683 ng/mL), the highest probability (51.4%) of Cₘₜₜₜₜ over Cₘₜₜₜₜ-target was observed at 5 mg/kg Q2W (online supplemental table S5). Considering the highest probability of Cₘₜₜₜₜ over Cₘₜₜₜₜ-target, along with the overall safety and tolerability of KN046, we determined R2PD to be 5 mg/kg Q2W.

**Efficacy**

Eighty-eight participants were evaluable for treatment response, including 11 who achieved a PR with an ORR
<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline characteristics of the participants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (N=100)</td>
</tr>
<tr>
<td>Age (years), median (range)</td>
<td>51.5 (25–73)</td>
</tr>
<tr>
<td>Age ≥60 years, n (%)</td>
<td>30 (30.0)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>82 (82.0)</td>
</tr>
<tr>
<td>BMI, mean±SD</td>
<td>21.8±3.2</td>
</tr>
<tr>
<td>ECOG score of 1, n (%)</td>
<td>47 (47.0)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td></td>
</tr>
<tr>
<td>Former smoker</td>
<td>32 (32.0)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>10 (10.0)</td>
</tr>
<tr>
<td>Diagnosis, n (%)</td>
<td></td>
</tr>
<tr>
<td>Nasopharyngeal carcinoma</td>
<td>59 (59.0)</td>
</tr>
<tr>
<td>NSCLC (all EGFR-mutated)</td>
<td>36 (36.0)</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>Right external auditory canal carcinoma</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Small cell lung cancer</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>1 (1.0)</td>
</tr>
<tr>
<td>Pathological classification, n (%)</td>
<td></td>
</tr>
<tr>
<td>Non-keratinizing carcinoma</td>
<td>47 (79.7)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>5 (8.5)</td>
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<tr>
<td>Unknown</td>
<td>3 (5.1)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (6.8)</td>
</tr>
<tr>
<td>Brain metastasis</td>
<td>14 (14.0)</td>
</tr>
<tr>
<td>Baseline PD-L1 expression, n (%)</td>
<td></td>
</tr>
<tr>
<td>&lt;1%</td>
<td>19 (19.0)</td>
</tr>
<tr>
<td>1%–49%</td>
<td>42 (42.0)</td>
</tr>
<tr>
<td>≥50%</td>
<td>32 (32.0)</td>
</tr>
<tr>
<td>Lost</td>
<td>7 (7.0)</td>
</tr>
<tr>
<td>Time from diagnosis to enrollment (months), median (range)</td>
<td>28.6 (3.7–137.7)</td>
</tr>
<tr>
<td>Prior surgery, n (%)</td>
<td>19 (19.0)</td>
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<tr>
<td>Prior radiotherapy, n (%)</td>
<td>64 (64.0)</td>
</tr>
<tr>
<td>Prior immunotherapy, n (%)</td>
<td></td>
</tr>
<tr>
<td>Anti-CTLA-4</td>
<td>1 (1.0)</td>
</tr>
</tbody>
</table>

Continued
of 12.5% (95% CI 6.4% to 21.3%) (online supplemental table S6 and figure S). With a median follow-up of 23.2 (range 20.5–25.9) months, the median DOR was 16.6 (95% CI 4.2 to not estimable (NE)) months, the median PFS was 1.7 (95% CI 1.3 to 2.7) months, and the median OS was 16.6 (95% CI 10.6 to 20.2) months. The efficacy for immunotherapy-naïve and treated patients with NPC and NSCLC is described in online supplemental table S7. In the NPC subgroup, the ORR was 15.4% (95% CI 6.9% to 28.1%); the median DOR was 8.31 (95% CI 2.83 to NE) months with a 1-year DOR rate of 46.9% (95% CI 12.0% to 76.3%). The median PFS was 1.5 (95% CI 1.3 to 2.7) months, and the median OS was 24.7 (95% CI 16.3 to NE) months with a 1-year OS rate of 66.1% (95% CI 52.1% to 76.9%). In the NSCLC subgroup, all patients were with EGFR mutation. the ORR was 6.3% (95% CI 0.8% to 20.8%), and the median DOR was 17.3 (95% CI 16.6 to NE) months with a 1-year DOR rate of 100.0%. The median PFS was 2.5 (95% CI 1.3 to 2.8) months; the median OS was 7.8 (95% CI 5.3 to 14.8) months with a 1-year OS rate of 41.7% (95% CI 25.6% to 57.0%) (online supplemental figure S3).

Biomarkers

Tumor PD-L1 expression was assessed in 93 patients (NPC, n=57; NSCLC, n=32; SCLC, n=1; gastric cancer, n=2; and melanoma, n=1) (online supplemental table S8). Among them, 81 participants were available for efficacy evaluation; 15 were PD-L1− (PD-L1+ staining on <1% of tumor cells), and 66 were PD-L1+ (online supplemental figure S4). The ORRs were 0% and 13.6% (95% CI 6.4 to 24.3) in patients with PD-L1 expressions of <1% and ≥1%, respectively (figure 4A). Prolonged median PFS was observed in patients with PD-L1+ (mPFS 2.5 months, 95% CI 2.0 to 3.1) than those with PD-L1− (mPFS 1.3 months, 95% CI 1.3 to 1.4, p=0.0098; online supplemental table S9). In addition, longer median OS was observed in patients with PD-L1+ (19.9 months, 95% CI 12.6 to 27.2) than those with PD-L1− (5.4 months, 95% CI 0.0 to 23.9, p=0.014; figure 4B).

mIF staining was carried out in 25 patients with PD-L1 tested (NPC, n=13; NSCLC, n=12) (online supplemental figure S4), and the relative expression of immune-related biomarkers was analyzed (online supplemental figure S5). Univariate Cox regression analysis identified CD8 expression was associated with better OS (online supplemental figure S6; HR=0.22, 95% CI 0.051 to 0.94, p=0.04). No significant associations were observed between OS and other immune-related biomarkers (online supplemental figure S6). Patients with high CD8 expression (>median, n=12) showed longer OS, with a median OS of 27.1 vs 9.2 months in the low-CD8 expression group (n=13, p=0.029; figure 4C). The ORRs were 8.3% and 7.7% in patients with high and low CD8 expressions, respectively; also, no significant difference in median PFS (p=0.33) was observed (online supplemental table S9). We then investigated the potential predictive value of combined biomarkers. Patients with both PD-L1+ and...
Table 2  TRAE grouped by dose level

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>1mg/kg Q2W (n=1)</th>
<th>3mg/kg Q2W (n=30)</th>
<th>5mg/kg Q2W (n=57)</th>
<th>5mg/kg Q3W (n=6)</th>
<th>300mg Q3W (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any TRAE</td>
<td>83 (83.0)</td>
<td>0</td>
<td>27 (90.0)</td>
<td>45 (78.9)</td>
<td>6 (100)</td>
<td>4 (66.7)</td>
</tr>
<tr>
<td>Grade ≥3 TRAE</td>
<td>14 (14.0)</td>
<td>0</td>
<td>6 (20.0)</td>
<td>5 (8.8)</td>
<td>2 (33.3)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Serious</td>
<td>9 (9.0)</td>
<td>0</td>
<td>5 (16.7)</td>
<td>2 (3.5)</td>
<td>1 (16.7)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Lead to suspension</td>
<td>14 (14.0)</td>
<td>0</td>
<td>8 (26.7)</td>
<td>5 (8.8)</td>
<td>1 (16.7)</td>
<td>0</td>
</tr>
<tr>
<td>Lead to termination</td>
<td>7 (7.0)</td>
<td>0</td>
<td>3 (10.0)</td>
<td>4 (7.0)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lead to death</td>
<td>1 (1.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>irAE</td>
<td>47 (47.0)</td>
<td>0</td>
<td>21 (70.0)</td>
<td>20 (35.1)</td>
<td>5 (83.3)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td>Grade ≥3 irAE</td>
<td>4 (4.0)</td>
<td>0</td>
<td>3 (10.0)</td>
<td>1 (1.8)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Infusion-related reaction</td>
<td>20 (20.0)</td>
<td>0</td>
<td>9 (30.0)</td>
<td>9 (15.8)</td>
<td>2 (33.3)</td>
<td>0</td>
</tr>
<tr>
<td>Grade ≥3 infusion-related reaction</td>
<td>5 (5.0)</td>
<td>0</td>
<td>3 (10.0)</td>
<td>2 (3.5)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Most common TRAE (≥5% of the total population)
- Rash: 33 (33.0) 0 14 (46.7) 15 (26.3) 3 (50.0) 1 (16.7)
- Pruritus: 31 (31.0) 0 11 (36.7) 15 (26.3) 4 (66.7) 1 (16.7)
- Fatigue: 20 (20.0) 0 8 (26.7) 8 (14.0) 3 (50.0) 1 (16.7)
- Increased AST: 19 (19.0) 0 9 (30.0) 8 (14.0) 1 (1.8) 0 |
- Increased ALT: 15 (15.0) 0 6 (20.0) 6 (10.5) 2 (33.3) 1 (16.7)
- Hypothyroidism: 15 (15.0) 0 4 (13.3) 9 (15.8) 2 (33.3) 0 |
- Fever: 11 (11.0) 0 4 (13.3) 7 (12.3) 0 | 0 |
- Joint pain: 9 (9.0) 0 5 (16.7) 4 (7.0) 0 | 0 |
- Increased TSH: 5 (5.0) 0 1 (3.3) 2 (3.5) 1 (16.7) 1 (16.7)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; irAE, immune-related adverse event; Q2W, every 2 weeks; Q3W, every 3 weeks; TRAE, treatment-related adverse event; TSH, thyroid stimulating hormone.

Figure 2  KN046 plasma time–concentration curve. Mean KN046 plasma time–concentration curve (ng/mL) by dose level after administration of a single dose. The ordinate of the left graph is a linear scale, and the ordinate of the right graph is a log-linear scale. Q2W, every 2 weeks; Q3W, every 3 weeks.
high CD8 expression (n=10) had better OS compared with the remaining patients (27.1 vs 9.2 months, p=0.027; figure 4D). Prior applicability test indicated no correlation between PD-L1 and CD8 expression (Spearman’s p=0.166). We also tested samples from 4 patients (among 25 with mIF) using the nCounter PanCancer IO360 panel for RNAseq-derived analysis (2 NPC and 2 NSCLC, all immunotherapy-treated). Consistent with the mIF results, higher CD8 T-cell infiltration was observed in patients with OS exceeding 24 months (online supplemental table S10 and figure S7). One patient with high PD-L1 expression but low CD8 expression had a relatively poor OS (patient number 1101062, OS=7.52 months; online supplemental table S10).

**DISCUSSION**

To our knowledge, this is the first large phase I trial of a bispecific antibody with dual blockade of PD-L1 and CTLA-4 in advanced solid tumors. Our results indicate...
that KN046 monotherapy was well tolerated and had a satisfactory safety profile with a low rate of grade 3 TRAEs. Also, KN046 showed potential survival benefits in different tumor types (median DOR of 16.6 months and prolonged OS). The RP2D of KN046 monotherapy was determined as 5 mg/kg Q2W using a comprehensive strategy based on pharmacokinetic–pharmacodynamic model, preliminary exposure–response analysis, and overall safety profile. Biomarker analysis demonstrated that high PD-L1 expression combined with high CD8 expression was associated with a good prognosis after KN046 treatment.

Previous studies have reported promising clinical effects for the combination of two ICIs, while high toxicity and treatment interruption were also observed.\textsuperscript{7,9,15,22,23} The incidence rate of grade $\geq$ 3 TRAEs was numerically low for the KN046 treatment (14.0%), with 27%–53% for two ICI combination treatments in advanced solid tumors.\textsuperscript{7,9,15,23} Furthermore, we also found that the incidence of TRAEs leading to treatment termination was relatively low (7.0%) after KN046 treatment, with 7.1%–22.5% reported in anti-PD-1/PD-L1 and anti-CTLA-4 antibody combinations.\textsuperscript{7,22} The $t_{1/2}$ of KN046 is about 5 days, much shorter compared with 11–15 days reported for ipilimumab,\textsuperscript{24} and intermittent continuous exposure to anti-CTLA-4 may be attributed to relatively lower toxicity. Based on the aforementioned data, the bispecific antibody KN046 showed the advantage of good safety compared with the combination of the two ICIs. Considering the lower toxicity and improved tolerability, long period treatment of KN046 may translate into long-term disease control and sustained survival benefits.

Traditional RP2D determination based on DLT observation is commonly not optimal for PD-L1/PD-L1 antibodies or bispecific antibodies due to DLT seldom occurred during the first treatment cycle.\textsuperscript{25,26} A previous study integrated clinical PK and IL-2 stimulation data from the KEYNOTE-001 trial to establish a model to reveal the relationship between PK and PD of monoclonal antibody therapy.\textsuperscript{19} In this study, as an indirect measure of target KN046 occupancy on T cells, which are the major participant of immune reactions in the tumor microenvironment, the ex vivo IL-2 stimulation ratio was measured, and the simulated pharmacokinetic–pharmacodynamic model was used. This is also the first time an IL2-based
pharmacokinetic–pharmacodynamic model was used in the analysis of a bispecific antibody. As no DLT was observed in this study, the R2PD of KN046 was recommended as 5mg/kg Q2W based on the consideration of overall safety, tolerability, and preliminary efficacy, alongside the results of the pharmacokinetic–pharmacodynamic model. Similarly, the RP2D of bintrafusp alfa, a bifunctional fusion protein targeting transforming growth factor beta and PD-L1 was determined based on PK–PD, the population pharmacokinetic model, and exposure–response for efficacy and safety. This novel and constructive pharmacokinetic–pharmacodynamic model and RP2D determination method may provide a reference for the application of a model-based design in the dose selection of bispecific antibody in the future.

The efficacy of KN046 was promising, especially for patients with NPC. Although the ORR and PFS were relatively low in patients with NPC, the median DOR (8.3 months) and the 1-year DOR rate (46.9%) were prolonged, suggesting that antitumor effects in responders might be sustained over time. Besides, the median OS of pretreated patients with NPC was 21.1 months (online supplemental table S7), suggesting potential survival benefit from KN046 treatment. As a reference, the median OS reported were 17.2 and 17.4 months for toripalimab and camrelizumab, respectively. To our knowledge, this is the first report on the efficacy of bispecific antibodies in patients with NPC. Sustained efficacy observed in responsive patients suggested KN046 may provide a new treatment option for patients with metastatic or recurrent NPC. It also needs to be noted that the median PFS was only 1.5 months; further biomarker studies are wanted to find responsive patients. Objective response was observed in patients with EGFR-mutated NSCLC who failed standard therapies. For patients with EGFR-mutated NSCLC who progressed after treatment with tyrosine kinase inhibitors, further clinical trials of KN046 combined with chemotherapy or angiogenesis inhibitor should be considered.

Currently, studies identifying populations with potential benefits from bispecific antibodies targeting PD-L1 and CTLA-4 are limited. Recently, Song et al provided a signature consisting of 18 protein candidates associated with treatment response based on spatial multomics analysis in patients treated with KN046 (n=18). In our study, we found that PD-L1 positivity was associated with better ORR, PFS, and OS after KN046 treatment, suggesting that PD-L1 expression is a predictor of the bispecific antibody. This part of our results was consistent with the research of Song et al, who also found that the high expression of PD-L1 is related to the treatment efficacy. In our mIF analysis, we selected immune-related biomarkers, including CD86, CD206, CD11B, CD4, CD8, FOXP3, and GZMB. Regardless of PD-L1 expression, CD8 was independently associated with prognosis. Studies have found that the combination of mIF-based markers and PD-L1 could help more accurately identify people who may benefit from immunotherapy. Similarly, we also found that using CD8 expression jointly with PD-L1 expression may help precisely identify patients who could potentially respond to KN046 treatment. This provides insights for the identification of patients with optimal eligibility for KN046 treatment. It should be noted that the biomarker analysis was a preliminary exploration which needs to be validated in further clinical trials.

This study had some limitations. Major limitations included this study being a preliminary safety and efficacy evaluation of a phase I study, with a lack of randomization; the trial included patients with multiple tumor types; and the generalizability of efficacy data is limited. The post hoc biomarker analysis was conducted in a quite small cohort, which may confer bias.

In conclusion, in this phase I trial, KN046, a novel bispecific antibody targeting PD-L1 and CTLA-4, was well tolerated and showed promising antitumor efficacy in advanced solid tumors. Potential OS benefit may be favored through KN046 treatment, especially in patients with pretreated advanced NPC. Besides, patients with high PD-L1 and CD8 expression might benefit from KN046 treatment. Investigations of KN046-based regimens with larger sample sizes in various solid tumors are ongoing.

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Hospital (ethics approval number A2018-055-01). This trial was registered at ClinicalTrials.gov. Written informed consent was obtained from all patients before any study procedure. This trial was conducted according to the tenets of the Declaration of Helsinki and Good Clinical Practices.

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