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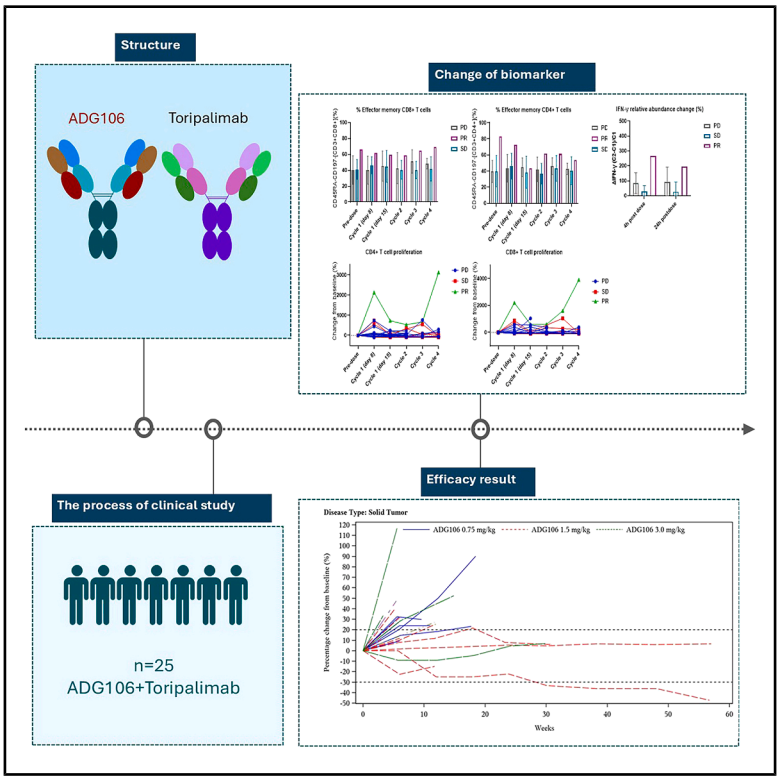
Phase 1b/2 study of ADG106, a 4-1BB/CD137 agonist, in combination with toripalimab in patients with advanced solid tumors

Graphical abstract



Highlights

- ADG106 is an agonistic antibody that blocks ligands, specifically targeting CD137
- ADG106 in combination with toripalimab demonstrated a manageable safety profile
- Balancing the efficacy and safety of ADG106 combined with toripalimab remains challenging

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In brief

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Phase 1b/2 study of ADG106, a 4-1BB/CD137 agonist, in combination with toripalimab in patients with advanced solid tumors

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SUMMARY

This phase 1b/2 clinical trial (NCT04775680) evaluated the safety, efficacy, pharmacokinetics and pharmacodynamics of ADG106, a ligand-blocking agonistic antibody targeting CD137 (4-1BB), combined with toripalimab in patients with advanced malignancies. ADG106 0.75–3 mg/kg plus toripalimab 240 mg were administered every 3 weeks. One dose-limiting toxicity occurred in 1 subject at 1.5 mg/kg and 2 in another subject at 3 mg/kg. Grade ≥ 3 treatment related adverse events occurred in 4/25 patients (16%). The overall disease control rate was 29.2% (7/24), including 1 partial response (PR) patient with a duration of response and a progression-free survival of 17.6 and 24.5 months. Circulating biomarkers suggested increased soluble CD137, $CD3^{-}CD16^{+}CD56^{+}$ natural killer (NK) cells, interferon γ (IFN-γ), TNFα, and IL-6 after therapy. Elevated baseline memory T cells and PD-L1, activation of immune-related pathways, along with enhanced T cell proliferation and increased IFN-γ following treatment were observed in the PR patient. ADG106 in combination with toripalimab demonstrated a manageable safety profile but no efficacy conclusions could be drawn.

INTRODUCTION

Current research has firmly confirmed that the T cell immune response plays a crucial role in tumor control. As a key component of the immune system, T cells exert their core function by identifying and attacking abnormal cells, including tumor cells. Therefore, the emergence of T cell coinhibitory receptor antagonists represents a breakthrough in cancer treatment, particularly the antibodies targeting programmed cell death protein 1 (PD-1)/programmed cell death 1 ligand 1 (PD-L1). Currently, anti-PD-1/PD-L1 monoclonal antibody (mAb) treatment changed the landscape of cancer treatment.[1,2] However, the response rate of anti-PD-1/PD-L1 monotherapy is still modest with only a few patients driving clinical benefits. Therefore, exploring of novel agonists targeting costimulatory molecules or coinhibitory checkpoints in combination with anti-PD-1/PD-L1 mAb is still an urgent need to improve clinical outcomes for patients with advanced tumor malignancies.[3,4]

CD137 (4-1BB) is a member of the tumor necrosis factor receptor superfamily (TNFRSF) and serves as a costimulatory molecule. It is expressed on various activated immune cells, including natural killer (NK) cells, T cells, and dendritic cells (DCs). Upon activation, CD137 transduces intracellular signals through the nuclear factor κB (NF-κB) and mitogen-activated protein kinases (MAPKs) pathways, enhancing cytokine production, cell proliferation, survival, and cytotoxic T cell activity. Furthermore, compelling evidence suggests that introducing an agonist antibody or CD137 ligand into tumor cells can effectively eliminate tumors, highlighting CD137 as a promising therapeutic target.[5–7]

ADG106 stands out as a fully human IgG4 antibody, precisely targeting a unique and cross-reactive epitope of CD137 that is conserved across various species, including humans, monkeys, and mice. This antibody not only effectively activates CD137 through FcγRIIB-mediated crosslinking but also serves to antagonize CD137 ligands. *In vitro* study demonstrates that

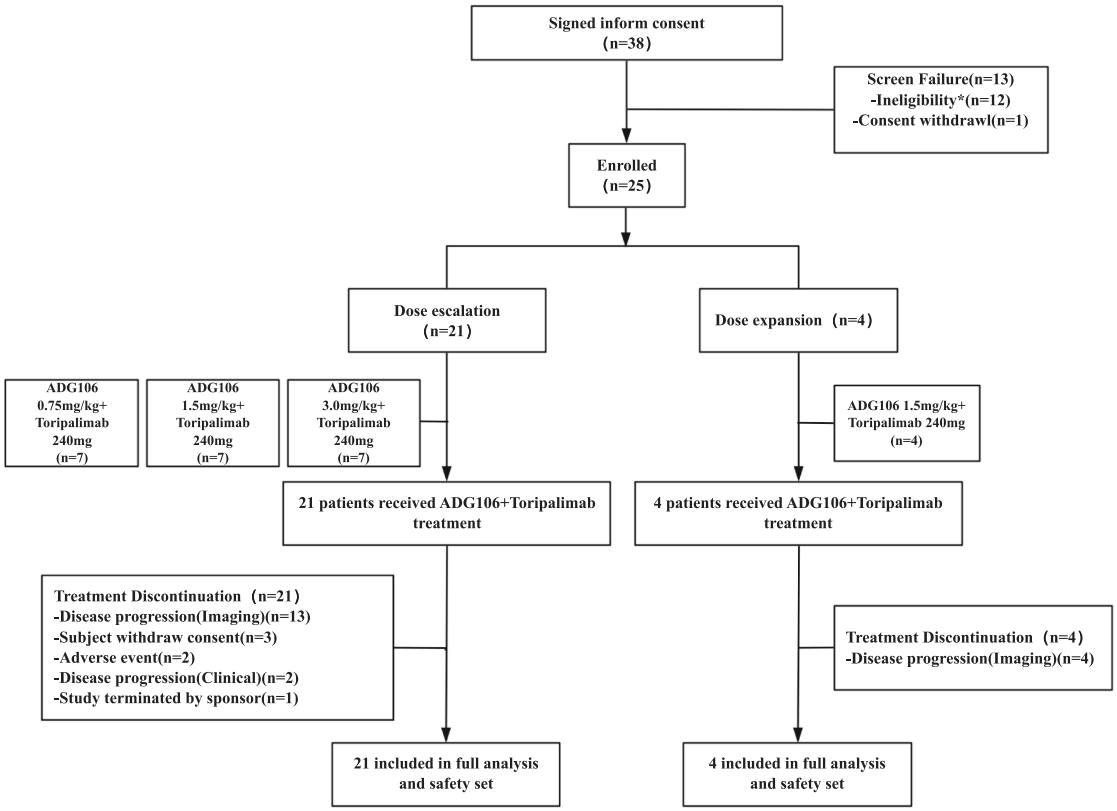

Figure 1. Study subjects included in the clinical study process

ADG106 is a strong agonist under crosslinking conditions manifested by NF-κB dependent signaling activation in a reporter assay, and functions to costimulate T cell activation in a $CD8^+$ T cell proliferation and interferon γ (IFN-γ) release assay when $CD8^+$ T cells are primed by sub-optical concentrations of anti-CD3 antibody. In preclinical animal studies, ADG106 exhibits robust antitumor response in multiple syngeneic tumor models, with no significant toxicity in mouse, rats and monkeys. In the first-in-human phase 1 trial designated as ADG106-1002, ADG106 displayed notable safety benefits, particularly its minimal hepatic toxicity, while also showing encouraging preliminary anti-tumor efficacy.[8]

Previous reports on solid tumor models have demonstrated that the combination of 4-1BB/CD137 agonists with PD-1 inhibitor may yield additive or synergistic antitumor effects, substantiated by the coexpression and functional interplay of PD-1 and CD137 noted in human tumor-infiltrating lymphocytes and mouse models.[9–11] The expression of 4-1BB and PD-1 proteins was augmented on the surface of CD8(+) T cells due to the anti-4-1BB/anti-PD-1 cotreatment.[10] Moreover, the anti-4-1BB/anti-PD-1 combination therapy is adequate to provoke a vigorous antitumor effector/memory T cell response in a highly aggressive tumor model and thus emerges as a promising candidate for combination trials in patients.[12]

In current investigation, we delved deeper into exploring the clinical safety profile, early anti-tumor activity, as well as the pharmacokinetics and pharmacodynamics of ADG106 when administered with anti-PD-1 mAb toripalimab in patients diagnosed with advanced solid tumors.

RESULTS

Patients

A total of 38 patients underwent screen in this study, 25 patients from whom were enrolled (Figure 1). The patients were assigned to ADG106 0.75 mg/kg (n = 7), 1.5 mg/kg (n = 11), and 3 mg/kg (n = 7), in combination with a fixed-dose (240 mg) of toripalimab based on the time of enrollment. Out of the 25, 21 patients were enrolled in the dose-escalation phase, including 7 patients in at 0.75 mg/kg, 7 patients at 1.5 mg/kg, and 7 cases at 3.0 mg/kg. The other 4 patients were enrolled in the dose expansion phase (ADG106 1.5 mg/kg). The baseline characteristics were displayed in Table 1. Out of the 25, 11 (44.0%) were diagnosed with nasopharyngeal carcinoma (NPC). The mean age (± standard deviation [SD]) of all patients was 48.1 years (±11.7 years), and the majority of patients (23/25, 92.0%) were 65 years or older. The ECOG score was 0 (12/25, 48.0%) or 1 (13/25, 52.0%). The median prior lines of therapy was 3.

Safety, DLT, and MTD

As detailed in Table 2, 8/25 patients (32.0%) had CTCAE grade ≥ 3 treatment-emergent adverse events (TEAEs), including

Table 1. Baseline characteristics of study population

Toripalimab 240 mg + ADG106				
ADG106	0.75 mg/kg *N* = 7	1.5 mg/kg *N* = 11	3 mg/kg *N* = 7	Total *N* = 25
Mean age, range				
Age, ≥ 65 years, n (%)	7 (100)	10 (90.9)	6 (85.7)	23 (92.0)
Age, < 65 years, n (%)	0	1 (9.1)	1 (14.3)	2 (8.0)
Gender, n (%)				
Male	3 (42.9)	7 (63.6)	5 (71.4)	15 (60.0)
Female	4 (57.1)	4 (36.4)	2 (28.6)	10 (40.0)
Race, n (%)				
Asian	7 (100)	11 (100)	7 (100)	25 (100)
Other	0	0	0	0
ECOG, n (%)				
0	3 (42.9)	7 (63.6)	2 (28.6)	12 (48.0)
1	4 (57.1)	4 (36.4)	5 (71.4)	13 (52.0)
Primary cancer, n (%)				
Nasopharyngeal carcinoma	2 (28.6)	6 (54.5)	3 (42.9)	11 (44.0)
Melanoma	1 (14.3)	2 (18.2)	0	3 (12.0)
Lung cancer	4 (57.1)	1 (9.1)	1 (14.3)	6 (24.0)
Liver cancer	0	0	2 (28.6)	2 (8.0)
TNBC	0	1 (9.1)	0	1 (4.0)
Thymoma	0	0	1 (14.3)	1 (4.0)
Mesothelioma	0	1 (9.1)	0	1 (4.0)
Prior systemic anticancer therapy, n (%)				
Chemotherapy	4 (57.1)	6 (54.5)	6 (85.7)	16 (64.0)
Immunotherapy	5 (71.4)	6 (54.5)	2 (28.6)	13 (52.0)
Targeted therapy	4 (57.1)	3 (27.3)	3 (42.9)	10 (40.0)
Other	4 (57.1)	9 (81.8)	3 (42.9)	16 (64.0)
Prior radiotherapy, n (%)	3 (42.9)	8 (72.7)	3 (42.9)	14 (56.0)
Prior anticancer surgery, n (%)	1 (14.3)	4 (36.4)	2 (28.6)	7 (28.0)
Prior lines of therapy				
1st-line	2 (28.6)	1 (9.1)	1 (14.3)	4 (16.0)
2nd-line	1 (14.3)	3 (27.3)	2 (28.6)	6 (24.0)
3rd-line	2 (28.6)	5 (45.5)	3 (42.9)	10 (40.0)
4th-line or above	2 (28.6)	2 (18.2)	1 (14.3)	5 (20.0)

4/7 cases (57.1%) in 0.75 mg/kg group, 2/11 cases (18.2%) in 1.5 mg/kg group, and 2/7 cases (28.6%) in 3.0 mg/kg group. The most common (≥ 15%) treatment-related TEAEs (TRAEs) included fatigue (48.0%), anemia (24.0%), lymphopenia (16.0%), and leukopenia (16.0%), nausea (16.0%), vomiting (16.0%), and rash (16.0%). A total of 7/25 (28.0%) patients had TEAEs that resulted in simultaneous discontinuation of ADG106 and toripalimab, and 2 were assessed as possibly related to ADG106 and probably related to toripalimab, including immune-mediated myocarditis and leukopenia. Three drug related serious adverse events (SAEs) occurred in 2 patients.

Dose limited toxicity (DLT) analysis set included 16 subjects (3 at 0.75 mg/kg, 6 at 1.5 mg/kg, and 7 at 3.0 mg/kg). One subject (16.7%) in the 1.5 mg/kg group exhibited a DLT of grade 4 neutropenia, and one subject (14.3%) in the 3 mg/kg group experienced 2 DLTs of grade 4 immune-mediated myocarditis and grade 5 respiratory failure, leading to the discontinuation of the study drug. The maximum tolerated dosage (MTD) of ADG106 in combination with toripalimab has not been achieved. The subject encountered immune myocarditis subsequent to the initial administration of ADG106 at a dosage of 3 mg/kg alongside toripalimab. Upon confirmation of the immune myocarditis, immediate measures, including the administration of corticosteroids, supportive care, and other medical interventions, were undertaken. Regrettably, the disease progressed swiftly, resulting in the subject's death due to type II respiratory failure.

Pharmacokinetic and immunogenicity results

ADG106 drug concentration-time curve following different dosages in combination with toripalimab was presented in Figure S1. Mean maximum drug concentration (Cmax) in the first treatment cycle at 0.75 mg/kg, 1.5 mg/kg, and 3 mg/kg were 11.4 μg/mL, 27.7 μg/mL, and 48.9 μg/mL, respectively. The area under the predicted infinite drug exposure (AUC_{inf}) values at 0.75 mg/kg, 1.5 mg/kg, and 3 mg/kg were 84.3 days* μg/mL, 220.4 days* μg/mL, and 348.2 days* μg/mL, respectively. A nearly linear PK profile was observed within the investigated clinical dosage spectrum. The concentrations of ADG106 with repeated administration every three weeks were found to be largely non-cumulative or less than twice the cumulative levels in a limited number of patients, in comparison to the mean (±SD) of the initial dosing cycle as per the non-compartmental model analysis. The half-life of ADG106 was 6.9 ± 1.3 days.

The exposure of ADG106 co-administrated with toripalimab was detailed in Supplementary materials and displayed in Tables S1 and S2. As detailed in Tables S1 and S2, the mean exposure time of ADG106 and toripalimab in ADG106 0.75 mg/kg, ADG106 1.5 mg/kg, and ADG106 3.0 mg/kg group were 12.16 weeks, 19.94 weeks, and 8.97 weeks, respectively. The average total exposure time was 14.69 weeks. In the group receiving ADG106 at a dose of 0.75 mg/kg, 2/7 (28.6%), 3/7 (42.9%), and 2/7 (28.6%) completed 2, 4, and 6 cycles of ADG106 plus toripalimab. On the other hand, in the group receiving ADG106 at a dose of 1.5 mg/kg, 1/11 (9.1%), 4/11 (36.4%), 3/11 (27.3%), and 3/11 (27.3%) patients completed 1 cycle, 2, 4, and more than 6 cycles of combination therapy, respectively. In the ADG106 3.0 mg/kg dose group, 3/7 (42.9%), 2/7 (28.6%), 1/7 (14.3%), and 1/7 (14.3%) patients completed 1 cycle, 2, 4, and more than 6 cycles of combination therapy, respectively. A total of 4/25 (16.0%) patients received more than 6 cycles of treatment.

The average actual cumulative doses of ADG106 in the three dose groups were 160.961 mg, 593.866 mg, and 426.750 mg, respectively, and the total average actual cumulative dose was 425.860 mg. The average cumulative doses of toripalimab were 925.7 mg, 1592.7 mg, and 720.0 mg, respectively, and the total mean cumulative dose was 1161.6 mg.

The validated ADA method was used to assess the immunogenicity of ADG106 at baseline and post treatment. Of the

Table 2. Summary of TRAE and TEAE by system organ classification

Toripalimab 240 mg + ADG106				
System Organ Classification	ADG106	ADG106	ADG106	All Subjects
Preferred Term	0.75 mg/kg	1.5 mg/kg	3.0 mg/kg	
	N = 7	*N* = 11	*N* = 7	*N* = 25
	n (%)	n (%)	n (%)	n (%)
≥3 grade TRAE				
At least one TRAEs(≥ 3 grade)	2 (28.6)	1 (9.1)	1 (14.3)	4 (16.0)
Leukopenia	1 (14.3)	1 (9.1)	1 (14.3)	3 (12.0)
Lymphopenia	0 (0)	1 (9.1)	1 (14.3)	2 (8.0)
Neutropenia	1 (14.3)	1 (9.1)	1 (14.3)	3 (12.0)
Anemia	0 (0)	0 (0)	1 (14.3)	1 (4.0)
Febrile neutropenia	0 (0)	1 (9.1)	0 (0)	1 (4.0)
Lymphopenia	1 (14.3)	0 (0)	0 (0)	1 (4.0)
Hyponatraemia	0 (0)	0 (0)	1 (14.3)	1 (4.0)
Respiratory failure	0 (0)	0 (0)	1 (14.3)	1 (4.0)
Immune-mediated myocarditis	0 (0)	0 (0)	1 (14.3)	1 (4.0)
Immune-mediated hepatic disorder	0 (0)	0 (0)	1 (14.3)	1 (4.0)
Drug related SAEs				
Immune-mediated myocarditis	0 (0)	0 (0)	1 (14.3)	1 (4.0)
Neutropenia	0 (0)	1 (9.1)	0 (0)	1 (4.0)
Respiratory failure	0 (0)	0 (0)	1 (14.3)	1 (4.0)
All TEAEs				
Any AEs	7 (100)	11 (100)	7 (100)	25 (100)
Any TEAEs	7 (100)	11 (100)	7 (100)	25 (100)
≥ grade 3 TEAEs	4 (57.1)	2 (18.2)	2 (28.6)	8 (32.0)
Any treatment related TEAEs	7 (100)	11 (100)	4 (57.1)	22 (88.0)
≥ grade 3 treatment related TEAEs	2 (28.6)	1 (9.1)	1 (14.3)	4 (16.0)
Any ADG106-related TEAEs	7 (100)	11 (100)	4 (57.1)	22 (88.0)
≥ grade 3 ADG106-related TEAEs	2 (28.6)	1 (9.1)	1 (14.3)	4 (16.0)
Any toripalimab-related TEAEs	7 (100)	11 (100)	4 (57.1)	22 (88.0)
≥ grade 3 toripalimab-related TEAEs	2 (28.6)	1 (9.1)	1 (14.3)	4 (16.0)
Any TEAEs leading to study treatment interruption	2 (28.6)	2 (18.2)	3 (42.9)	7 (28.0)
Any TEAEs leading to ADG106 interruption	2 (28.6)	2 (18.2)	3 (42.9)	7 (28.0)
Any TEAEs leading to toripalimab interruption	2 (28.6)	2 (18.2)	3 (42.9)	7 (28.0)

74 ADA samples tested, 34 samples (34/74, 45.9%) from 11 patients (11/25, 44.0%) were tested positive at ≥ 1 time point regardless of baseline ADA status. The presence of positive ADA at baseline (2/11) was likely due to preexisting host antibodies that were cross reactive with ADG106. Nine patients (9/11, 81.8%) exhibited treatment induced ADA, and none of the patients had treatment boosted ADA. The median onset of ADA was 42 days. A total of 16 patients were assessed for ADA levels at end of the treatment (EOT), and 7 patients (43.8%) had positive titer results. ADG106 drug exposure was apparently affected in two ADA positive patients. There was no evidence that the efficacy was affected by ADA incidence.

Pharmacodynamics

Increasing levels of sCD137 were observed in patient's serum after ADG106 and toripalimab combo therapy across different ADG106 dosing levels (Figure S2). And there was no relationship between doses of ADG106 and the magnitude of sCD137 release.

Lymphocyte subpopulation

Activated T lymphocytes, predominantly in the $CD8^+$ subset, as well as NK cells, have the capacity to express sCD137. Consequently, various lymphocyte subsets in the peripheral blood, such as $CD3^-CD16^+CD56^+$ NK cells, $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, and $CD19^+$ B cells, were monitored during the administration of ADG106 and toripalimab (Figure S3A).The findings revealed a general elevation in $CD3^-CD16^+$ $CD56^+$ NK cells following treatment with increasing doses of ADG106 (ranging from 0.75 mg/kg to 3 mg/kg) and toripalimab. Conversely, $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, and $CD19^+$ B cells exhibited no discernible alterations from baseline levels post-administration.

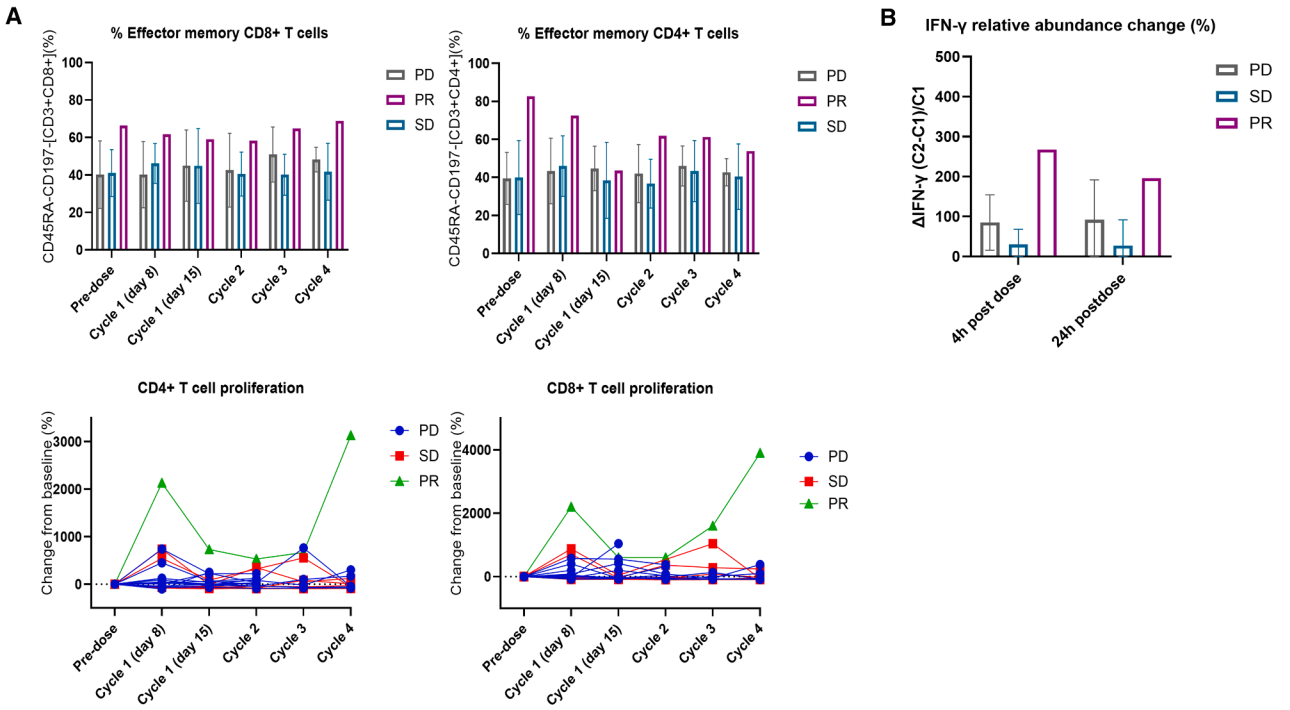

Figure 2. T cell proliferation, memory T cell subpopulation and cytokine profile of ADG106 in combination with toripalimab

(A) Changes in memory CD8$^+$T cells, memory CD4$^+$T cells and proliferation of CD4$^+$ and CD8$^+$T cells detected from baseline in individual patients by efficacy groups.

(B) Changes in IFN-γ levels detected from baseline in individual patients by efficacy group. Data are represented as mean ± SEM.

See also Figure S3.

In the partial response (PR) patient (Figure 2A), a trend of high baseline memory T cells including memory CD8$^+$T cells (CD45RA-CD197-/[CD3$^+$/CD8$^+$T]) and memory CD4$^+$T cells (CD45RA-CD197-/[CD3$^+$/CD4$^+$T]) were observed. After ADG106 and toripalimab combination therapy, proliferations of CD4$^+$ and CD8$^+$T cells increased, which were indicated by increasing in percentage of Ki67 positive cells in both CD4$^+$ and CD8$^+$ T cells.

Cytokine

As an immune checkpoint agonist, ADG106 serves to engage T cells, augment T cell proliferation, and stimulate the secretion of T cell-derived cytokines, including IL-2, IL-4, IL-6, IL-10, TNFα, and IFN-γ. Serum cytokine analyses after one cycle of treatment were conducted to explore potential correlations between cytokine activation and adverse reactions or therapeutic response. In this study, patients exhibited a tendency toward heightened levels of IFN-γ, TNFα, and IL-6 after treatment across all dosage cohorts, and there was no apparent relationship between dosing groups (Figure S3B). As presented in Figure 2B, patient with PR showed a trend toward higher levels of IFN-γ after treatment versus nonresponders. Notably, IL-2, IL-10, and IL-4 were not tested in most of the samples (data not shown).

RP2D

RP2D for dose expansion was determined as 1.5 mg/kg after a comprehensive evaluation of safety, efficacy, and PK/PD data, based on the following rationales: (1) The 1.5 mg/kg dose group exhibited superior safety profiles than 3 mg/kg. (2) Although the overall levels of sCD137 were higher in the group receiving a dosage of 3 mg/kg after the first cycle (in contrast to the group receiving 1.5 mg/kg), consistent alterations in IFN-γ levels (as per the mean statistical evaluation) and comparable total sCD137 levels prior to successive treatment cycles among all dosage cohorts suggested the possibility of similar immune activation effects. (3) Based on the receptor occupancy (RO) of CD137, derived from the amalgamation of individual PK data from ADG106-1008 and relevant theoretical antibody affinity calculations, assuming a tumor tissue distribution of 10% (tumor drug concentration divided by blood concentration), the mean RO levels within the tumor tissue at dosages of 1.5 mg/kg and 3.0 mg/kg were approximately 30%–80% and 60%–90%, respectively. Previous *in vitro* studies have shown that ADG106 concentrations of ∼100 ng/mL (with a theoretical RO of 15%) can activate the CD137 signaling pathway, while concentrations of 1 μg/mL (with a theoretical RO of 65%) exhibit robust *in vitro* activity. Therefore, these data suggested that we did not require 100% RO to drive the pharmacological effects of ADG106. In conclusion, 1.5 mg/kg was selected as the RP2D for this study.

Efficacy results

The details on patient response and outcome data were displayed in Figure 3. The analysis of the optimal efficacy evaluation

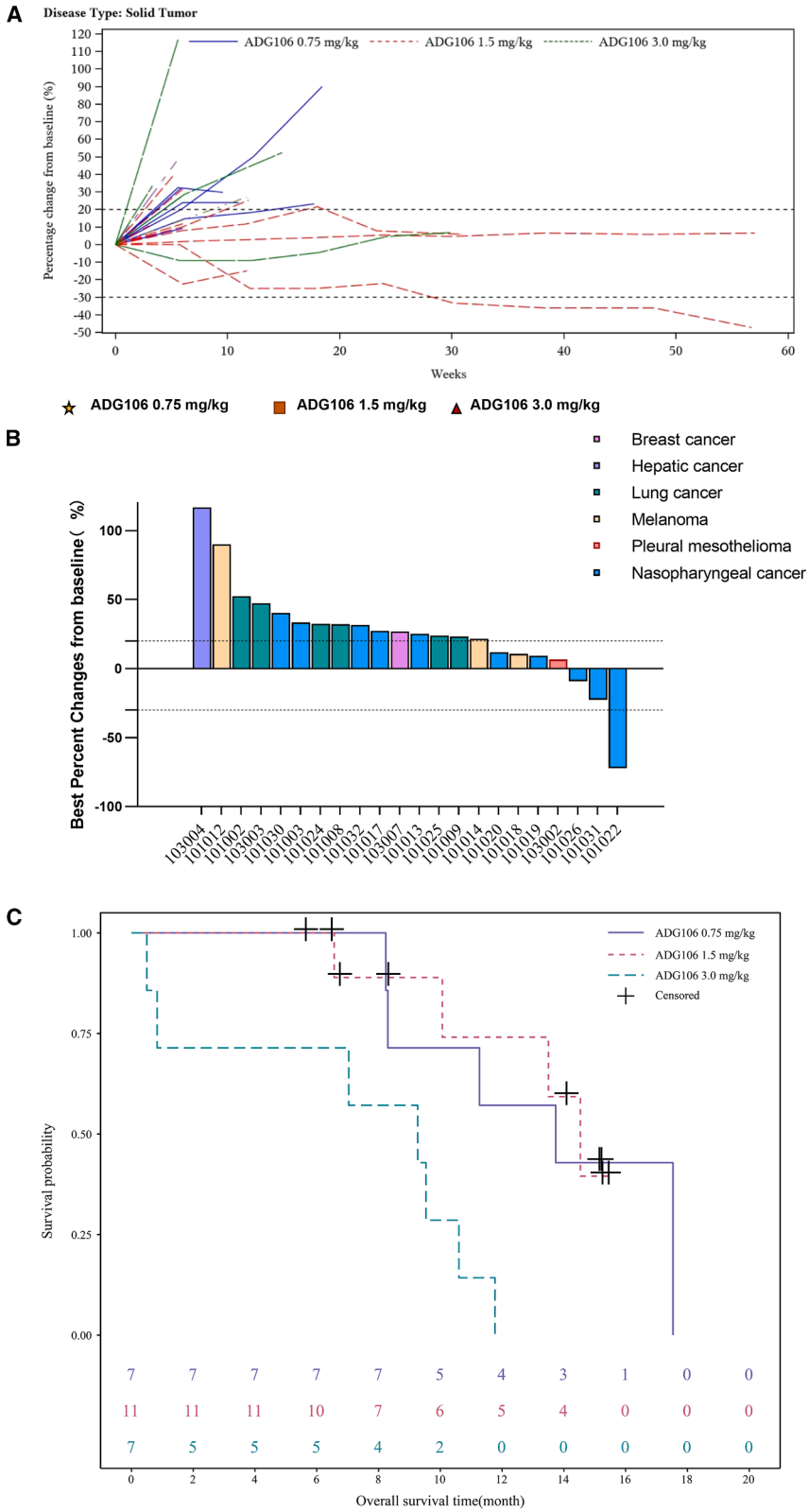

Figure 3. Details on patient response and outcome data

(A) Spider plot of percentage changes from baseline in tumor measurement of target lesion.

(B) Waterfall plot of maximal percentage change from baseline in tumor measurement of target lesion.

(C) The Kaplan-Meier curve for overall survival.

See also Figure S4, Tables S3 and S4.

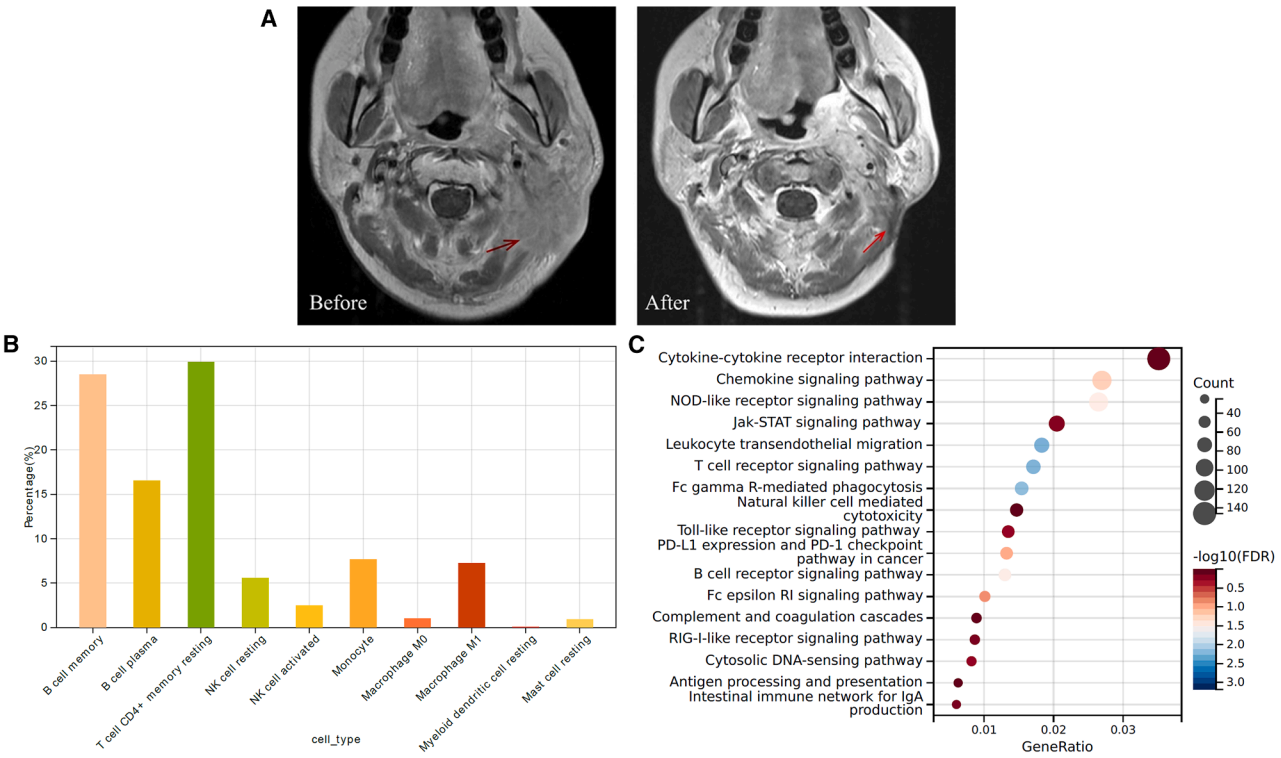

Figure 4. Antitumor efficacy and RNA-seq analyses of the PR patient

(A) Baseline and post-treatment scan images.

(B) Boxplots illustrate the immune cell proportions.

(C) The KEGG analysis of immune-related pathways.

of 24 patients in the response-evaluable analysis set (RES) showed that the efficacy evaluation of 1 subject with NPC in the ADG106 1.5 mg/kg group was PR after 10 cycles of treatment, and the objective response was confirmed, with a duration of response (DOR) of 76.6 weeks. The overall response rate (ORR) was 4.1% (1/24). The overall DCR was 29.2% (7/24, 95% CI: 12.62%–51.09%), with 1 PR and 6 SD. The DCR were 28.6% (2/7 SD) in 0.75 mg/kg group, 36.4% (1/11 PR, 3/11 SD) in 1.5 mg/kg group, and 16.7% (1/6 SD) in 3.0 mg/kg group. The overall DCR by irRECIST were the same as RECIST v1.1. The progression-free survival (PFS) results were detailed in Supplementary materials. The median overall survival (OS) was 13.6 months (95% CI: 8.2-NA) for ADG106 0.75 mg/kg, 14.3 months (95% CI: 9.9-NA) for ADG106 1.5 mg/kg, and 9.1 months for ADG106 3.0 mg/kg group (95% CI: 0.8–10.5). The median OS for all treatment groups combined was 11.1 months (95% CI: 9.1–14.3).

Median PFS assessed by RESIST 1.1 criteria (Table S3 and Figure S4A) were 6.0 weeks in the ADG106 0.75 mg/kg group (95% CI: 5.9-NA), 6.0 weeks (95% CI: 5.4–18.0) in the ADG106 1.5 mg/kg group and 5.9 weeks (95% CI: 3.3-NA) in the ADG106 3.0 mg/kg group. The median PFS for all treatment groups combined was 6.0 weeks (95% CI: 5.9–12.3). The 3-month, 6-month, and 9-month PFS rates in all response evaluation population were 27.7%, 16.6%, and 16.6%, respectively. Median PFS assessed by iRESIST 1.1 criteria (Figure S4B) were 6.0 weeks (95% CI: 5.9-NA) in the ADG106 0.75 mg/kg group and 6.0 weeks (95% CI: 5.4-NA) in the 1.5 mg/kg group. The survival rate in the ADG106 3.0 mg/kg group was not less than 50% at the end of the study, so the median PFS was NA (95% CI: 3.3-NA).

Among the 24 patients with evaluable efficacy, 11 were immunotherapy-naive, and 13 were immunotherapy-treated. The NPC subject achieved PR was immunotherapy-naive. The overall efficacy of immunotherapy-naive and immunotherapy-treated patients was shown in Table S4.

The NPC patient with PR was a 39-year-old female who had previously received docetaxel, cisplatin, 5-fluorouracil, and nimotuzumab as induction chemotherapy followed by concurrent chemoradiotherapy plus cisplatin and nimotuzumab. This patient experienced a recurrence after 15 months of disease-free survival and received gemcitabine plus endostar as the first-line therapy (BOR of PD) and capecitabine as the second-line therapy (BOR of PR). She experienced a DOR and a PFS of 17.6 and 24.5 months and discontinued treatment after reaching the maximum 2-year treatment. The OS was not reached. CT scans before and after treatment was shown in Figure 4A. A dramatic reduction of EBV DNA load was observed along with tumor response, from an initial 7600 copies/mL to 87 copies/mL upon PR. The baseline formalin fixed paraffin embedded tumor specimen for RNA-sequencing (RNA-seq) analysis was procured from the PR patient. The 22 immune cell proportions and the

immune-related pathways were subsequently examined. The proportions of infiltrating immune cells of the 22 types were deduced from standardized gene expression data utilizing the CIBERSORT algorithm with 1000 permutations.[13] The analysis showed that the PR patient had high proportions of B cell memory, B cell plasma, and T cell $CD4^+$ memory resting (Figure 4B). The PD-L1 expression was 56 (fragments per kilobase of transcripts per million mapped reads, FPKM). The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis unveiled that the differentially expressed genes were predominantly enriched in immune-related categories, such as NOD-like receptor signaling pathway, leukocyte transendothelial migration, T cell receptor signaling pathway, and Fc gamma R-mediated phagocytosis (Figure 4C).

DISCUSSION

The current study reported the results of a mini dose escalation phase 1b and single-arm phase 2 trial of a 4-1BB/CD137 agonist (ADG106) and anti-PD-1 antibody (tolipalimab) combination in advanced solid tumor patients. The primary endpoints were successfully accomplished with the RP2D determined, and the MTD not reached. Also, to our knowledge, this's the first report of a complete PK, PD profile of CD137 and anti-PD-1 combination. Herein, we explored the systemic PK, cytokine, RO, and soluble CD137 of this combination. A tolerable safety profile without severe hepatic toxicity was observed; however, the relatively weak anti-tumor activity led to the early discontinuation of phase 2 or even further trials of this combination therapy.

ADG106 showed a manageable safety profile in combination with toripalimab in heavily pretreated patients with advanced solid tumors. Urelumab, a potent CD137 agonist, is a monoclonal human IgG4 antibody administered as a monotherapy for non-Hodgkin's lymphoma (NHL) and melanoma. However, doses exceeding 0.3 mg/kg have been associated with severe hepatic toxicity, leading to the discontinuation of its research and development.[14] On the other hand, utomilumab, another CD137 antibody, has shown no treatment-related grade 3 to 4 liver toxicity even combined with pembrolizumab.[15] GEN1046, a bispecific antibody targeting PD-L1 and 4-1BB, resulted in treatment-related grade 3 transaminase elevations in 9.8% of patients.[16] Comparing favorably to urelumab and GEN1046, no treatment-related grade 3 to 4 liver toxicity was observed at the RP2D of ADG106 when combined with toripalimab. Meanwhile, the occurrence of grade 3 and previous treatment-related adverse events appeared comparable between the combination of ADG106 with toripalimab (16.0%), ADG106 alone (21.0%),[8] and toripalimab monotherapy (18.2%).[17]

Effectively targeting CD137 while circumventing the key safety limitation of hepatotoxicity seems fraught with challenges. The previous reported CD137 antibodies demonstrated limited antitumor activity, whether administered as monotherapy or in combination with PD-1/PD-L1 inhibitors.[15,16,18] While 6 patients (26.1%) exhibited confirmed complete or partial responses when co-administered with utomilumab and pembrolizumab,[15] the specific contribution of utomilumab to the observed antitumor activity remains indeterminate, as all responders were immunotherapy-naive and pembrolizumab demonstrates efficacy as a monotherapy across various malignancies.[19]

A bispecific antibody targeting PD-L1 and 4-1BB, GEN1046, is a new wave of CD137 agonists targeting tumors.[20] Though with an acceptable safety profile, GEN1046 offered limited signs of clinical activity, with confirmed PRs observed only in two patients who had not previously undergone immunotherapy.[16] Within our study, a confirmed response was shown in an immunotherapy-naive NPC patient, demonstrating a sustained DOR and PFS of 17.6 and 24.5 months, while OS had not been reached by the data cut-off. The reported median PFS for PD-1/PD-L1 monotherapy in patients with NPC ranged from 1.9 to 6.5 months in the second-line or latter setting.[21] Given the low response rate and the exploratory nature of this study, it is challenging to ascertain the efficacy in this dose-finding combination study. Whether dual targeting of 4-1BB and PD-L1 may improve antitumor activity in a human setting awaits further confirmation. Novel waves of CD137 agonists are currently under development, aiming to expand the therapeutic window of 4-1BB-targeting agents while achieving a more harmonious balance between efficacy and toxicity.[22–24] FS222, a conditionally activated CD137 agonist designed to target CD137 activation to regions expressing PD-L1, demonstrated no signs of hepatotoxicity at doses up to 30 mg/kg in cynomolgus monkeys, presenting itself as a promising compound advancing through clinical development.[22] Taken together, the CD137 agonist and PD-1/L1 inhibition combination aimed to overcome the resistance of immune checkpoint inhibitor. However, current reports (including this study) show poor efficacy in those immune therapy resistance patients, further exploration in optimizing CD137 agonist or discovering advantage population need to be done.

In conjunction within this study, complete pharmacokinetics, immunogenicity, pharmacodynamics, and cytokine were evaluated. A nearly linear PK profile was observed within the dose range that investigated. The exposure of ADG106 co-administrated with toripalimab was comparable with that observed in monotherapy clinical trials.[8] The frequency of ADA observed in this study was 44%, and there was no evidence that the efficacy was affected by ADA incidence. Pharmacodynamic revealed heightened levels of sCD137 after the combination therapy of ADG106 and toripalimab, aligning with the results observed with ADG106 monotherapy.[8] The elevation of sCD137 in serum could be influenced by the entrapment of this soluble form by therapeutic antibodies, which may prolong its half-life in circulation. Soluble forms of 4-1BB have been observed in sera of patients with autoimmune diseases and some cancers, suggesting an association with immune activation.[25] It is reported that soluble CD137 increased after anti-CD137 therapy in preclinical studies[26] and in clinical trials after utomilumab treatment.[18] Cytokine analysis showed a tendency toward increased IFN-γ, TNFα, and IL-6 following the co-administration of ADG106 and toripalimab across various doses, effects not observed with the concurrent use of utomilumab and pembrolizumab,[15] which warrants further studies specifically designed to formally assess these trends.

Lymphocyte subpopulation in the peripheral blood of the PR patient indicates a high baseline level of memory T cells including memory $CD8^+$T cells and memory $CD4^+$T cells, and this is

consistent with RNA-seq examination data of immune cell proportions and the immune-related pathways. Compared with nonresponders, the PR patient exhibited an increased proliferation of $CD4^+$ and $CD8^+$T cells after ADG106 and toripalimab combination therapy. This aligned with the observation that an increased $CD8^+$/regulatory T cell ratio and heightened activity of tumor-specific cytotoxic T lymphocytes were associated with the antitumor activity of the combined 4-1BB agonist and PD-1 antagonist therapy in tumor model.[10] Within the tumor microenvironment, 4-1BB is exhibited by a subset of tumor-infiltrating $CD8^+$ T cells, distinguished by the coexpression of numerous TCR-inducible molecules, including elevated levels of PD-1.[27,28] The PR patient provided an exemplary case illustrating that individuals exhibiting elevated PD-L1 levels, coupled with an abundance of $CD8^+$ T cells and the activation of immune-related pathways, such as the T cell receptor signaling pathway, might derive significant benefit from the combination therapy of ADG106 and toripalimab. This observation necessitates further investigation and validation due to the current paucity of evidence.

ADG106 combined with toripalimab displayed a manageable safety profile, notably overcoming the principal safety concern of liver toxicity typically linked with 4-1BB agonists. However, due to the limited clinical activity and exploratory nature of this study, we cannot draw an efficacy conclusion for ADG106 and toripalimab combination therapy for cancer patients. Nevertheless, the biomarker analysis of the PR patient provided an indication that patients with higher baseline levels of memory T cells and PD-L1, alongside the activation of immune-related pathways and elevated proliferations of $CD4^+$ and $CD8^+$T cells after therapy might be the dominant population responsive to ADG106 and toripalimab combination treatment. Our study provided enlightenment for subsequent studies to further explore the correlation with these biomarkers to the clinical advantages of the CD137/4-1BB antibody when used in conjunction with PD-1 pathway inhibitors. The development of novel CD137-based constructs is essential for further exploration in tumors where a considerable unmet medical need persists in the field of immunotherapy.

Limitations of the study

This study is not without its limitations. While the combination of ADG106 and toripalimab exhibited a tolerable safety profile, the clinical efficacy was constrained, resulting in the premature cessation of phase 2 and potentially subsequent trials of this combination therapy. Furthermore, the biomarker analysis conducted on the PR patient offered merely preliminary insights and necessitated further investigation. There remains a pressing need for the development of innovative CD137-based constructs and combination therapies.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to corresponding author, Hongyun Zhao (zhaohy@sysucc.org.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Adagene, Inc. will provide access to individual de-identified participant data, protocols, and statistical analysis plans, contingent upon specific criteria, conditions, and exceptions. Data requests may be directed to info@adagene.com. This paper does not report any original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request

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AUTHOR CONTRIBUTIONS

H.Z., L.Z., and P.L.: conceptualization, formal analysis, supervision, validation, investigation, visualization, methodology, writing original draft, project administration, and writing—review and editing. S.L., Y.M., and Y.W.: conceptualization, formal analysis, validation, investigation, visualization, methodology, writing original draft, and writing—review and editing. Y.Y., J.X., Y.H., Y. Zhao, W.F., S.H., Y. Zhang, and Q.L.: investigation, methodology, writing—review and editing. G.L., X.S., J.Z., S.Z., and Y.L.: conceptualization, formal analysis, validation, methodology, and writing—review and editing. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
BV421 Mouse Anti-Human CD45	BD Horizon™	Cat#563879; RRID:AB_2744402
FITC Mouse Anti-Human CD3	BD Pharmingen™	Cat#555332, 555339; RRID:AB_395739
BB700 Mouse Anti-Human CD4	BD Horizon™	Cat#566392; RRID:AB_2744421
APC-H7 Mouse Anti-Human CD8	BD Pharmingen™	Cat#560179; RRID:AB_1645481
PE Mouse Anti-Human CD19	BD Pharmingen™	Cat#555413; RRID:AB_395813
APC Mouse Anti-Human CD16	BD Pharmingen™	Cat#561304; RRID:AB_10714780
APC Mouse Anti-Human CD56 (NCAM-1)	BD Pharmingen™	Cat#555518; RRID: AB_398601
BV421 Anti-Human CD45RA	Biolegend	Cat#304130; RRID: AB_10900421
PerCP Anti-Human CD45	Biolegend	Cat#304026; RRID:AB_893341
FITC Anti-Human CD3	Biolegend	Cat#317306; RRID: AB_571906
BV785 Anti-Human CD4	Biolegend	Cat#317442; RRID: AB_2563242
BV510 Anti-Human CD8a	Biolegend	Cat#301048; RRID: AB_2561942
PE Anti-Human CD197 (CCR7)	Biolegend	Cat#353204; RRID: AB_10913813
BV510 Mouse Anti-Human CD8	BD Horizon™	Cat#563256; RRID:AB_2738101
BV650 Mouse Anti-Ki-67	BD Horizon™	Cat#563757; RRID: AB_2688008
Biological samples		
Blood	Patients in this study	N/A
Chemicals, peptides, and recombinant proteins		
ADG106	Adagene Inc.	N/A
Toripalimab	TopAlliance	N/A
Critical commercial assays		
V-PLEX Custom Human Cytokine Kits	MSD	Cat#K151A0H-2, K151A9H-2
R-PLEX Human 4-1BB/TNFRSF9 Antibody Set	MSD	Cat#F218U-3
Deposited data		
The data of patients	This manuscript	
Software and algorithms		
BD DIVA	BD	BD FACS Diva Software V8.0.1.1
GraphPad Prism	GraphPad Software	10.2.3
Phoenix WinNonlin	Certara	8,4
SAS	SAS	9.4

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Samples and ethical statement

The human subjects were obtained from an open-label, single-arm, phase Ib/II study (NCT04775680) of ADG106, a 4-1BB/CD137 agonist, in combination with toripalimab in patients with advanced solid tumors. A total of 25 patients were enrolled in this single-arm trial and received ADG106 combined with toripalimab. The study protocol received approval from the ethics board at Sun Yat-sen University Cancer Center (ID: A2020-017). Written informed consent was acquired from all participants. The research was conducted in alignment with the Declaration of Helsinki and the principles of Good Clinical Practice Guidelines.

METHOD DETAILS

Patients

Patients were included in the study if they had a histologic or cytologic diagnosis of advanced solid tumor that had progressed after standard therapy or for which no standard therapy was available. Patients were excluded if they had a primary central nervous system

malignancy, active epileptic seizure, spinal cord compression or carcinomatous meningitis; Prior treatment of anti-4-1BB antibody therapy was not allowed. Patients were also excluded if they had irAE $\geq$ Grade 3 or treatment discontinuation after immune therapy treatment; had an active interstitial lung disease (ILD) or pneumonia or a documented history of ILD and pneumonia that required steroid or immunosuppressive agents.

The study was approved by Center for Drug Evaluation (CDE) and independent ethics committees of the participating centers and followed the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice guidelines. All patients provided informed consent to participate in the study. The study was sponsored by Adagene. Inc and registered at ClinicalTrials.gov (NCT04775680).

Study design, objectives, and treatment

The primary objective of this phase Ib/II, open-label, dose-escalation study was to estimate the maximum tolerated dosage (MTD), determine the recommended phase II dose (RP2D) (Phase Ib) and evaluate the preliminary anti-tumor activity of ADG106 in combination with toripalimab in advanced solid tumors (Phase II). The primary endpoints were dose limited toxicity (DLT) occurring in the first treatment cycles (3 weeks) of the dose escalation stage (Phase Ib) and the overall response rate (ORR) in the study patients (Phase II). The secondary endpoints included the overall safety profile, pharmacokinetic (PK) parameters, antidrug antibody (ADA) levels, duration of response (DOR), time to response (TTR), progression-free survival (PFS) and overall survival (OS). Solid tumor response was evaluated using the Response Evaluation Criteria in Solid Tumor (RECIST v1.1 and iRECIST criteria). Furthermore, exploratory endpoints included cytokine levels (IFN-γ, IL-2, IL-6, IL-10, TNF-α) in serum, soluble CD137/4-1BB and immune subset (T, B, NK, Treg, Memory T cells, etc.) changes in peripheral blood.

Patients received ADG106 in combination with flat dose of toripalimab (240mg) on the first day of each 21-day cycle. The study used a traditional 3+3 dosage escalation design. Three doses of ADG106, 0.75 mg/kg, 1.5 mg/kg and 3 mg/kg, were evaluated in this study. In the actual enrollment process, 3 patents were first enrolled in dose cohort of 3 mg/kg in combination with toripalimab following the clinical protocol (ADG106-1008 V2.1), resulting in 1 DLT (1/3). Considering toxicity synergetic or additive effect of ADG106 and toripalimab, the dose of ADG106 was decreased to 0.75 mg/kg for safety evaluation after discussion with investigators and sponsor. Then doses of ADG106 were escalated to 1.5 mg/kg and 3 mg/kg for further safety evaluation. A maximum of 2 years of treatment was planned for the study. Treatment was to be continued until confirmed progression of disease (PD), unacceptable toxicity, death, withdraw of consent, loss to follow-up or study termination by sponsor or the study endpoint, whichever occurred first.

Assessments

AEs were graded using NCI CTCAE V5.0. DLTs related to ADG106/toripalimab during the first cycle were defined based on hematologic/non-hematologic criteria, with MTD set before $\geq$2/6 DLTs. Tumor response was assessed by CT/MRI at baseline, every 2 cycles (6 weeks ± 7 days) for the first 10 cycles, then every 3 cycles (9 weeks). ORR was the proportion of CR/PR patients. DCR was CR/PR/SD patients. DOR was time from first response to PD/death. Durations could be converted to months/weeks. TTR was time from first dose to response. PFS was from first dose to progression/death per RECIST/iRECIST. OS was defined as time from the first dose of administration to the date of death due to any cause.

Pharmacokinetics and immunogenicity

Blood samples were collected for pharmacokinetic analyses of ADG106 on day 1 (predose, 30 minutes, 4 hours after the end of toripalimab infusion), day 2, day 7 and day 14 of cycles 1; day 1 (predose and 30 min after the end of toripalimab infusion) of cycle 2 to cycle 5, then every 2 cycles up to cycle 11, every 4 cycles thereafter, and at the end of the treatment (EOT). Samples were analyzed for ADG106 using validated enzyme-linked immunosorbent assay (ELISA) methods. Standard serum pharmacokinetic parameters, including maximum observed serum concentration (C_{max}), serum trough concentration (C_{trough}), time to maximum serum concentration (t_{max}), area under the concentration–time curve up to the last measurable concentration ($AUC_{0\text{-}last}$), area under the concentration–time curve up to infinity ($AUC_{0\text{-}\infty}$), the clearance rate (CL) and apparent volume of distribution were estimated for ADG106 using noncompartmental analysis by WinNonlin software. Blood samples for ADG106 immunogenicity testing were collected at predose every 2 cycles up to cycle 11, at predose of every 4 cycles thereafter and at end of treatment (EOT). Blood samples were tested for anti-drug antibody (ADA) against using validated electro chemiluminescence (ECL) method.

Pharmacodynamics and biomarker analysis

Patient serum samples were analyzed for the cytokines IFN-γ, IL2, IL6, IL10, and TNFα, at predose, 4 hours and 24 hours on day 1 of cycles 1. All cytokines were assayed using the V-Plex Custom Human Cytokine Kits (MSD) by Electrochemiluminescence method. Soluble CD137/4-1BB in serum were tested at predose, 168 hours and 336 hours after the first cycle, and predose of cycle 2 to cycle 4, using R-PLEX Human 4-1BB/TNFRSF9 Antibody Set assay kit (MSD) by Electrochemiluminescence method. Lymphocyte subpopulations (T, B, NK, Treg, Memory T cells, etc.) were analyzed by flow cytometry of peripheral blood samples collected on day 1 of cycle 1 (predose) and at 168, and 336 hours after end of infusion, and at predose of cycle 2 to cycle 4. The analyses were performed

on a FACS Celesta flow cytometer (BD Biosciences) using three antibody panels: (i) CD45, CD3, CD4, CD8, CD19, CD16, CD56; (ii) CD45, CD3, CD4, CD8, CD25, CD45RA, CD197, CD137; (iii) CD3, CD4, CD8, CD25, FoxP3, PD-1, CTLA-4, Ki67. Reagents were procured from BD Biosciences and BioLegend.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses and generation of tables, figures, subject data listings and statistical outputs were carried out using SAS Version 9.4 or higher. ORR was reported in descriptive statistics. The corresponding two-sided 95% confidence intervals (CIs) were derived using the Clopper-Pearson method. PFS and OS were estimated using the Kaplan-Meier (KM) product-limit method. The corresponding two-sided 95% CIs of median event time were computed using the Brookmeyer-Crowley approach. Survival rates at fixed time point were derived from the KM estimator and the corresponding two-sided 95% CIs were derived based on the Greenwood's formula. Pharmacokinetic parameters were analyzed with non-compartmental model using Phonix WinNonlin V8.3 and summarized with descriptive statistics. Pharmacodynamic data was determined at baseline and after treatment, with calculation of the percentage change from baseline. The graphs were analyzed using Graphpad prism V10.

ADDITIONAL RESOURCES

This study has been registered on clinicaltrials.gov (NCT04775680).