A Pan-cancer Clinical Study of Personalized Neoantigen Vaccine Monotherapy in Treating Patients with Various Types of Advanced Solid Tumors

Yong Fang1, Fan Mo2,3,4,5, Jiawei Shou1, Huimin Wang2, Kai Luo2, Shanshan Zhang2,6, Ning Han2, Hongsen Li1, Shengli Ye7, Zhan Zhou3, Rongchang Chen2, Lin Chen2, Liang Liu2, Huina Wang8, Hongming Pan1, and Shuqing Chen2,3,6

ABSTRACT

Purpose: Because of their high tumor specificity and immunogenicity, neoantigens have been considered as ultimate targets for cancer immunotherapy. Neoantigen-based vaccines have demonstrated promising efficacy for several cancer types. To further investigate the antitumor potentials for other types of solid tumors, we designed a peptide-based neoantigen vaccine, iNeo-Vac-P01, and conducted a single-arm, open-labeled, investigator-initiated clinical trial (NCT03662815).

Patients and Methods: Personalized neoantigen vaccines were designed and manufactured according to our bioinformatics analysis results from the whole-exome sequencing of tumor and peripheral blood cell DNAs. Patients were scheduled to be vaccinated subcutaneously with adjuvant on days 1, 4, 8, 15, and 22 (prime phase), and days 78 and 162 (boost phase). Additional immunizations were administered every 2–3 months as per patient’s potential benefit. The safety and efficacy were assessed through adverse events (AE), progression-free survival (PFS), overall survival (OS), and other parameters.

Results: Of the 22 patients enrolled with advanced malignancies, 20 had no or mild AEs, while 2 had grade 3 or 4 acute allergic reactions only after their sixth boost vaccination. The disease control rate was 71.4%. The median PFS was 4.6 months, whereas the median OS was not reached (12-month OS = 55.1%). Around 80% of individual peptides or peptide pools elicited measurable specific immune response. In addition, our findings revealed several potential biomarkers for the prediction of better response.

Conclusions: iNeo-Vac-P01 as monotherapy is feasible and safe for patients with advanced solid tumors. It could elicit T-cell-mediated immune response targeting tumor neoantigens, and might have promising antitumor efficacy.

See related commentary by Filderman and Storkus, p. 4429

Introduction

In the past decade, immunotherapy has attracted intensive attention as an effective alternative cancer therapy. In particular, immune checkpoint blockade (ICB) has shown remarkable clinical responses with low toxicity and few side-effects in several cancer types, including advanced non–small cell lung cancer, melanoma, bladder cancer, gastric cancer, hepatocellular carcinoma, and colorectal cancer with DNA mismatch repair deficiency (1–5). Further intensive analysis of those patients with tumor regression after ICB treatment showed that high tumor mutation burden (TMB) could be related to better prognosis. Therefore, neoantigens, derived from tumor somatic mutations, are considered as critical and optimal targets for immunologic recognition of cancer cells.

It has been widely accepted that ICBs show better clinical response in tumors with high levels of infiltrating T cells and more antigens (“hot” tumor) rather than those lacking tumor-reactive infiltrating T cells (“cold” tumor). Neoantigen vaccines are designed to present tumor-specific neoantigens and activate cytotoxic T cells to recognize and infiltrate into tumor cells, turning “cold” tumor into “hot” tumor. Also, the process involves the training of immune system to target and kill tumor cells. As generated mostly by nonsynonymous mutations in cancer cells (6–8), neoantigens are exempted from central tolerance. Therefore, neoantigen vaccines are more likely to generate robust immune responses (9, 10), functioning as bona fide antigens to facilitate tumor regression (11).

Therapeutic neoantigen cancer vaccines are safe, tolerable, and capable of eliciting robust T-cell responses to kill tumor cells (12, 13). Plenty of related clinical trials have been launched in the past 5 years. Two studies on melanoma demonstrated that neoantigen peptide or RNA vaccines could not only benefit for the regression of advanced melanoma, but also provide long-term protection against tumor relapse and metastasis (12, 13). In subsequent investigation, neoantigen vaccine along with adjuvant could induce predominantly T-cell responses against predicted neoepitopes in patients with newly diagnosed glioblastoma (14, 15), and increase the number of tumor-infiltrating T cells (15). In addition, adoptive transfer of mutant protein–specific tumor-infiltrating lymphocytes have been applied to mediate complete durable regression of metastatic breast cancer. These personalized neoantigen vaccines could elicit sustained responses of T cells and display great potentials for further development (14, 15).
were revealed.

Several biomarkers potentially predictive for patient’s better response to the iNeo-Vac-P01 monotherapy were identified. Preliminary results indicated the antitumor potential of neoantigen vaccine has not been proven in patients’ response. Despite the relatively promising clinical results, a few patients (no more than 17) of a single cancer type, and tumor tissues and normal tissues.

As mentioned above, all of these exploratory studies were done with a few patients (no more than 17) of a single cancer type, and combination therapies were applied in these studies to improve patients’ response. Despite the relatively promising clinical results, the antitumor potential of neoantigen vaccine has not been proven in broader cancer types. In principle, the antitumor potential of neoantigen vaccine was not limited to certain tumor type as long as appropriate tumor neoantigens were identified. Therefore, a pan-cancer clinical study focused on neoantigen vaccine monotherapy instead of combination therapy was conducted to demonstrate the antitumor efficacy of neoantigen vaccine in various types of advanced solid tumors. Preliminary results indicated the iNeo-Vac-P01 monotherapy could elicit specific T-cell activation and induce broad spectrum of antitumor effects, without limitation to tumor type. In addition, several biomarkers potentially predictive for patient’s better response were revealed.

Patients and Methods

Patients

Eligible patients were at least 18 years old with advanced malignant tumors confirmed histologically or cytologically. Patients had disease progression after two or more lines of standard treatment; at least one measurable lesion as per investigator-assessed RECIST version 1.1; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; good functioning main organs, such as heart, liver, and kidney; and could provide sufficient tumor tissue and blood samples for DNA-sequencing assay or qualified genome/exome-sequencing data of tumor tissues and normal tissues.

Key exclusion criteria included: having other malignant tumor, except for cured basal cell carcinoma, thyroid carcinoma, or cervical dysplasia; lack of identified neoantigen in the sequencing data; received bone marrow or stem cell transplants; and allergic to any drug, polypeptide, or other potential immunotherapies.

Trial design and treatment

This was a single-arm, open-label, investigator-initiated clinical study at Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University (Hangzhou, Zhejiang, China). The final study protocol was approved by institutional review board and independent ethics committee, and conducted in accordance with Declaration of Helsinki and the International Conference on Harmonisation Guidelines for Good Clinical Practice. All patients had signed informed consent forms before treatment. The primary endpoints of the study were safety and feasibility, and the secondary endpoints were efficacy based on progression-free survival (PFS), overall survival (OS), and neoantigen-specific immune responses. The safety of the study was assessed on the basis of occurrence of adverse events (AE). The feasibility of this trial was assessed by whether neoantigen could be identified and the vaccines could be synthesized for clinical use.

iNeo-Vac-P01 comprises 5–20 peptides at lengths of 15–35 amino acids. The peptides were grouped into 2–4 pools (based on HLA typing, affinity, and allele frequency) and then injected subcutaneously at a quantity of 0.1 or 0.3 mg per peptide in upper arms and para-umbilical area, respectively. Patients were scheduled to receive iNeo-Vac-P01 with GM-CSF as adjuvant on days 1, 4, 8, 15, and 22 (i.e., prime phase), as well as on days 78 and 162 (i.e., boost phase; refs. 12, 15–18). The adjuvant GM-CSF was injected subcutaneously 30 minutes before the administration of iNeo-Vac-P01 at a quantity of 40 μg per injection nearby the injection site of iNeo-Vac-P01. Additional boost vaccines might be administered depending on ethics and patients’ potential benefit according to the clinical research protocol.

Clinical assessment, monitoring, and follow-up in this research were conducted, including physical examination, ECOG performance, vital sign, blood test, and urinalysis to assure the safety of each immunization; imaging examination at baseline and approximately every 8 weeks postvaccination to assess clinical efficacy; and enzyme-linked immunospot (ELISpot), T-cell receptor (TCR) sequence, and flow cytometry (T-cell subsets and cytokines) conducted pretreatment and every 8–12 weeks after treatment for the detection of specific immune response.

Tumors were assessed by investigators according to RECIST v1.1 criterion at baseline and approximately every 8 weeks thereafter. Patients’ conditions were monitored while receiving neoantigen vaccine treatment and every 3 months after treatment discontinuation. The related AEs were recorded and graded for safety evaluation according to the NCI Common Terminology Criteria for Adverse Events (CTCAE version 4.0) throughout whole treatment period.

Generation of personalized neoantigen vaccines

To identify mutation-derived neoantigens, some tumor tissues and blood samples were obtained directly by surgery, while others were obtained by biopsy or intravenous blood sampling. Whole-exome sequencing (WES) with coverage depths of 500–× for tumor and 100–× for blood cells was conducted on these samples using HiSeq 4000 NGS platforms (Illumina; AcornMed Biotechnology Co., Ltd.); the raw sequence data has been deposited in Genome Sequence Archive under accession number HRA000171 at http://bigd.big.ac.cn/gsa-human; refs. 19–23). In case of unavailability of fresh tumor samples, formalin-fixed, paraffin-embedded (FFPE) samples were used instead.

The bioinformatics analysis, which consists modules of sequencing read filtering, genome alignment, mutation calling, HLA typing, MHC affinity prediction, gene expression profiling, vaccine peptide sequence design, and mutation-centered prioritization based on therapeutic potency, was performed by our in-house pipeline iNeo-Suite (Supplementary Materials and Methods; Supplementary Fig. S1).
To generate personalized neoantigen vaccines, the customized clinical grade long peptides were manufactured through chemical synthesis at GMP-like standard (bacteria-free, >95.0% purity, and quantities of bacterial endotoxin less than 10 EU/mg). The water solubility of peptides was tested after synthesis, followed by the removal of insoluble peptides from iNeo-Vac-P01.

IFN\(_\gamma\) ELISpot assay

To confirm the immunogenicity of iNeo-Vac-P01, ELISpot assays were performed for each patient at a series of time points pre- and postvaccination. Peripheral blood mononuclear cells (PBMC) were isolated from the peripheral blood (10–30 mL) collected from each patient and coincubated (2 × 10\(^5\) cells per well) with peptides for 16–24 hours using Human IFN\(_\gamma\) precoated ELISpot kit according to the standard protocol. An automatic plate reader with appropriate parameters was used to count the spots in ELISpot plates (Supplementary Materials and Methods).

TCR sequence

To observe the change of T-cell population after vaccination, TCR \(\beta\) chain was sequenced for each patient before and after vaccination. Peripheral blood (10 mL) was collected from each patient for the extraction of RNA from PBMCs. Samples were analyzed by high-throughput sequencing of TCR using ImmuHub TCR Profiling System at a deep level (ImmunoQuand Biotech; Supplementary Materials and Methods).

Multiplexed immunofluorescence

To examine tumor infiltrated T cells, multiplexed immunofluorescence (IF) was performed by staining 4-μm thick FFPE whole-tissue sections with standard primary antibodies sequentially, and pairing with a unique fluorochrome before DAPI staining. Slides were air dried, mounted with Prolong Diamond Antifade Mounting Medium (*P36965, Thermo Fisher Scientific*), and observed with Aperio Versa 8 Tissue Imaging System (Leica). Sample images were analyzed using Indica Halo Software (Version 2.3.2089.52; refs. 24, 25; Supplementary Materials and Methods).

Cytometric analysis of T lymphocyte and cytometric bead array analysis of cytokines

To quantify the activation of T cells after vaccinations, patients’ peripheral T cells were extracted and labeled with several antibodies for cytometry analysis of the proportions of various types of T cells. To examine the cytokines secreted from the T cells activated by iNeo-Vac-P01, the concentrations of cytokines in patients’ peripheral blood were measured by cytometric bead array according to the manufacturer’s protocol (Supplementary Materials and Methods).

Statistical analysis

Data from the patients who received at least one dose of iNeo-Vac-P01 were included in the safety and clinical effects analysis. Descriptive statistics were used to determine the characteristics of baseline and patients’ peripheral blood cells as normal control (Supplementary Table S2). Neoantigens were predicted and prioritized using our in-house pipeline iNeo-Suite (Supplementary Materials and Methods), which leveraged information including the allelic frequency of mutation, gene expression, the affinity between the mutated peptide and HLA complex class I and II.

### Table 1. Demographic and disease characteristics at baseline.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (N = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
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</tr>
<tr>
<td>Mean</td>
<td>59 ± 10</td>
</tr>
<tr>
<td>Range</td>
<td>28–79</td>
</tr>
<tr>
<td>Age category, no. (%)</td>
<td></td>
</tr>
<tr>
<td>&lt;65 years</td>
<td>16 (72.73)</td>
</tr>
<tr>
<td>≥65 years</td>
<td>6 (27.27)</td>
</tr>
<tr>
<td>Sex, no. (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>12 (54.55)</td>
</tr>
<tr>
<td>Female</td>
<td>10 (45.45)</td>
</tr>
<tr>
<td>Metastatic sites, no. (%)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>5 (22.72%)</td>
</tr>
<tr>
<td>Lung</td>
<td>4 (18.18%)</td>
</tr>
<tr>
<td>Both liver and lung</td>
<td>6 (27.27%)</td>
</tr>
<tr>
<td>Bone</td>
<td>4 (18.18%)</td>
</tr>
<tr>
<td>ECOG performance status score, no. (%)(^{a})</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>7 (31.82)</td>
</tr>
<tr>
<td>1</td>
<td>15 (68.18)</td>
</tr>
<tr>
<td>Radiotherapy, no. (%)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11 (50.00)</td>
</tr>
<tr>
<td>No</td>
<td>11 (50.00)</td>
</tr>
<tr>
<td>Lines of prior systematic therapy, no. (%)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7 (31.82)</td>
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<tr>
<td>≥3</td>
<td>15 (68.18)</td>
</tr>
<tr>
<td>Tumor type, no. (%)</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td>4 (18.18)</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>4 (18.18)</td>
</tr>
<tr>
<td>NSCLC</td>
<td>5 (22.73)</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>2 (9.09)</td>
</tr>
<tr>
<td>Biliary tract cancer</td>
<td>2 (9.09)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>2 (9.09)</td>
</tr>
<tr>
<td>Others</td>
<td>5 (22.73)</td>
</tr>
</tbody>
</table>

\(^{a}\)ECOG performance status scores range from 0 to 5, with 0 indicating no symptoms and higher scores indicating increasing disability.

### Results

**Patients and demographics**

A total of 22 patients with cancer with advanced stage of various tumor types, including non–small cell lung cancer (NSCLC), colorectal cancer, melanoma, pancreatic cancer, biliary tract cancer, ovarian cancer, small-cell lung cancer, adrenal sebaceous adenocarcinoma, breast cancer, parotid carcinoma, and gastric carcinoma, were enrolled in the trial from February 7, 2018. All these patients failed to respond or were unable to tolerate the standard treatment. The patient demographics, baseline disease characteristics, and previous treatments are presented in Table 1, indicating that 5 (22.72%) patients had liver metastases, 4 (18.18%) patients had lung metastases, 6 (27.27%) patients had both, and 4 (18.18%) patients had bone metastases (Supplementary Table S1). All the data in this article were collected and summarized before May 31, 2019.

**Feasibility of iNeo-Vac-P01 manufacturing for pan-cancer patients**

WES was conducted on patients’ tumor fresh tissue and peripheral blood cells as normal control (Supplementary Table S2). Neoantigens were predicted and prioritized using our in-house pipeline iNeo-Suite (Supplementary Materials and Methods), which leveraged information including the allelic frequency of mutation, gene expression, the affinity between the mutated peptide and HLA complex class I and II.
as well as the feasibility of peptide synthesis. Clinical grade long peptides were synthesized at lengths of 15–35 amino acids incorpo-
rating multiple neoepitopes of both HLA class I and II (Supplementary
Tables S3–S6). The turn-around time of the whole process was mostly
between 1.5 and 3 months, and no preference in tumor type was
witnessed. In total, 91.7% (22/24) patients received iNeo-Vac-01
immunization, while 2 other patients dropped out because of rapid
disease progression.

Although patients enrolled were bearing various types of tumors
with the purity estimated by WES data ranging from 12.4% to 83.7%,
sufficient neoantigens were predicted for the successful manufactur-
ing of long peptide vaccines for each patient. To be specific, there was a
median of 14 long peptides immunized, which comprised a median of
10 class I neoepitopes and 26 class II neoepitopes per peptide. Most
patients (17/22) received vaccines containing more than 10 peptides
(Supplementary Tables S5 and S7).

Study treatment

Each patient’s peptides were pooled into 2–4 groups with maximum
five peptides per pool. The vaccine was administrated subcutaneously
in two flanking sites of navel and tail-end of arms following the
administration of GM-CSF as adjuvant. Peripheral cytokine and
T-cell subtypes monitoring, TCR sequencing, and IFNγ ELISpot assay
in vitro were detected at a series of time points before and after
vaccination according to the study protocol and recorded in a case
report form.

Patients were scheduled to receive iNeo-Vac-P01 with GM-CSF as
adjuvant on days 1, 4, 8, 15, and 22 (i.e., prime phase), as well as on days
78 and 162 (i.e., boost phase). Additional boost vaccines might be
administered depending on ethics and patients’ potential benefit
(Fig. 1A). The median duration of follow-up was 9.8 months, ranging
from 0.9 to 14.5 months before the deadline, May 31, 2019. Twenty-
one patients completed prime phase vaccinations except for P014
because of the withdrawal of informed consent form. Seventeen of
22 patients received additional boost vaccines (Fig. 1A). As shown, the
median duration of treatment was 4.15 months (range, 16 days to
12.1 months). Patients P005 and P015 had SD ever since enrolled,
while P005 dropped out because of grade 3 acute allergic reactions. No
progress for 2 patients (P001 and P011) was recorded until death. Till
May 31, 2019, 20 patients had ceased iNeo-Vac-P01, while 2 patients
(P015 and P019) were still receiving the assigned treatment.

Safety and tolerability

Defined by NCI CTCAE 4.03, treatment-related AEIs occurred in
54.55% of the patients (Table 2). Most witnessed AEIs were in grade 1–
2, mainly including fatigue (36.36%), chill (18.18%), and fever
(13.64%). However, grade 3–4 treatment-related acute allergic reac-
tions were only observed in 2 patients (9.09%) after their sixth round of
boost vaccinations, leading to their drop out from the study. The rest of
the cases of AE observed throughout the treatment were reversible
without particular nursing or treatment. No apparent association was
found between the presence or the type of AEIs and the tumor type. No
treatment-related serious AE (SAE) and death was witnessed.

Immune response

By performing IFNγ ELISpot assay in vitro using autologous PBMCs
after vaccination, T-cell activation induced by iNeo-Vac-P01 was
confirmed in 19 of 21 patients. Overall, 99 of 125 (79.2%) individual
long peptides and 43 of 51 (84.3%) long peptide pools elicited mea-
surably increased abundance detected posttreatment in 17 (77.3%)
and 12 (54.5%) patients (Fig. 1B; Supplementary Table S9).

To detect the activation of both CD8+ and CD4+ T cells in tumor
microenvironment during treatment, multiplexed IF on FFPE samples
with antibodies of CD4, CD8, and Granzyme B (Supplementary
Fig. S3) were applied to analyze the tumor regions of pre- and
postvaccination FFPE samples for 3 patients (P008, P013, and
P015). To locate the tumor regions for the analysis, hematoxylin and
eosin (H&E) staining was performed to analyze the adjacent slices of all
multiplexed IF samples, except the postvaccination sample of P008
(H&E staining images obtained from Department of Pathology at Sir
Run Run Shaw Hospital, Hangzhou, Zhejiang, China) was used instead
(Supplementary Fig. S4). As shown in Fig. 1C, the proportion of both
activated CD8+ T cells (Granzyme B positive) and activated CD4+
T cells (Granzyme B positive) were found to be increased after
vaccination in 3 patients (5.5 months postvaccination for P008,
7 months postvaccination for P013, and 8 months postvaccinations
for P015), as well as the densities of these two types of T cells. These
findings suggested that iNeo-Vac-P01 had the potential to activate
tumor-specific CD8+ T cells and CD4+ T cells, which could subse-
quently infiltrate into tumor tissue and generate antitumor effects.

Clinical response

Except for patient P005, standard RECIST 1.1 criteria were applied to
all other patients during assessment. For P005, iRECIST guideline was
applied as a result of his pseudoprogression. For 21 of 22 patients, who
completed five prime immunizations, at least one posttreatment target
lesion was evaluated. Tumor reduction was observed in the assessment
of 8 of 21 patients (38.1%), with a 16.7% maximum reduction of target
lesions compared with baseline (Fig. 2A and B). The calculated DCR
was 71.4% (15/21). In Fig. 2B, 2 patients were excluded because of
new lesion appearance (P002) and the progression of nontargeted lesion
occurrence (P006) at first posttreatment assessment, respectively. Fif-
teen of 21 patients (71.4%) displayed SD (8 with tumor size reduction
ranging from 1.9% to 18.2%, 7 with tumor size growth ranging from
1.1% to 9.0%) with a median duration of 4.4 months (ranging from 1.7
to 14.5+ months, “+” indicates some patients maintained SD at cut-off
date). The median PFS of 21 treated patients was 4.6 months [95%
confidence interval (CI), 2.5–5.2], and the estimated percentage of
patients without disease progression at 6th month was 27.3% (95% CI,
10.3%–46.8%; Fig. 2C). Seven of all treated patients (N = 22) had died.
As shown in Fig. 2D, the estimated OS at 12th month was 55.1% (95%
CI, 25.9%–76.9%), while the median OS had not been reached yet (95%
CI, 9.4–not reached). In particular, no evident differences were observed
between various types of cancer, which indicated that iNeo-Vac-P01 has
broad spectrum antitumor effects.

Potential biomarkers for clinical response

Patients with SD lasting more than 3 months were considered
to have better response than the others. Several mutant genes
and copy-number variants detected by WES were found to be
predictive for better response (Supplementary Fig. S5). The muta-
tions of genes MUC (mucins), ZFHX3 (Zinc Finger Homeobox 3),

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Figure 1.
Ineo-Vac-P01 induced specific T-cell response and antitumor activation. A, Swimmer plot showed the follow-up information of enrolled patients (N = 22) in a time range of 16 months. Light gray lines and yellow dots indicated the period of prime and the time points of boost vaccinations, respectively. The depiction of disease condition and patient status were indicated by lines and dots with various colors. Patient numbers are shown as the values of y-axis. B, For each patient marked in x-axis, green triangle and red diamond represented the relative response rates, which are equal to the ratios of peptides (or peptide pools) with positive ELSpot results to all immunized peptides (or peptide pools) before and after vaccination, respectively. The bar chart with secondary y-axis represents the IFNγ spots per 10^5 PBMCs of the peptide or peptide pool with best response for each patient. C, The multiplexed IF images of FFPE samples obtained from patients P008, P013, and P015 pre- (prevax) and postvaccination (postvax). CD8 (red in P008 and P013, and pink in P015) and Granzyme B (GZB, yellow) double positive T cells, CD4 (green) and Granzyme B (yellow) double positive T cells, and the merged signals are shown on the left. The proportion (%) and density (counts per mm^2) of the CD8 and Granzyme B double positive T cells and CD4 and Granzyme B double positive T cells are shown on the right.

and ABL1 (ABL Proto-Oncogene 1) seemed to be associated with faster disease progression and poor response, while copy-number variations of genes TNFRSF (tumor necrosis factor receptor superfamily), SOX3 (Sex determining region Y-box transcription factor 3), and MAGE family members (Melanoma-associated antigens) were relatively associated with better response (Supplementary Fig. S5; Supplementary Results).

The changes of peripheral T cells during treatment might provide potential predictive biomarkers for clinical response because T cells play a major role in antitumor response. After treatment, the proportions of effector CD8^+ T cells to total T cells in patients’ peripheral blood samples all increased to certain extend. Although insignificantly at most time points after vaccination, the effector CD8^+ T cells of patients with good response had a relatively higher boost compared with those of patients with poor response (Supplementary Fig. S6A). Similarly, the level of IFNγ in the patients’ peripheral blood samples all increased at prime phase. Notably, different from patients with poor response, patients with better response maintained a relatively higher boost compared with those of patients with poor response (Supplementary Fig. S6B). On the contrary, a continuous increase of IL6 during treatment was observed in the peripheral blood samples of patients with poor response (Supplementary Fig. S6C). These findings implied that the clinical
eFicacy might be predicted by the peripheral CTLs’ proliferation and activation (Supplementary Results).

Case report of a patient with advanced hepatic biliary tract cancer

In the case of patient P005, a 63-year-old male, initially diagnosed with intrahepatic biliary tract cancer in 2013, was treated by surgical excision in June 2013 followed by postoperative chemotherapy till April 2014. Tumor recurrence and metastases were confirmed by CT scan and pathologic tissue biopsy in April 2017. Then he was treated with apatinib for 6 months. He enrolled in a clinical trial of a PD-1 antibody (IBI308) for six cycles after failing to respond to chemotherapy again, and dropped out because of progressive disease (in November 2017, on a clinical trial in The First Affiliated Hospital, Zhejiang University in China, Hangzhou, China).

On March 22, 2018, he started to receive iNeo-Vac-P01. The treatment scheme is displayed in Fig. 3A, including five prime vaccinations and six subsequent boost vaccinations. Compared with standard treatment scheme with only two boost vaccinations, four more boost vaccinations were added under patient’s consent, based on the facts that his symptoms were greatly relieved and the level of a tumor marker (Carbohydrate Antigen 72–4) was significantly reduced.

After the last boost vaccination, a grade 3–4 acute allergic reaction occurred along with clinical manifestations: nausea, vomiting, and rash. Radiographic imaging was performed at 2nd, 5th, 8th, 10th, and 12th months after the first vaccination. The CT scans demonstrated an

Table 2. Treatment-related AEs in all treated patientsa.

<table>
<thead>
<tr>
<th>Patients (N = 22)</th>
<th>Any grade</th>
<th>Grades 3–4</th>
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<tbody>
<tr>
<td>Any AE</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Fatigue</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Chill</td>
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<td>0</td>
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<tr>
<td>Fever</td>
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<td>0</td>
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<td>Emesis</td>
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<td>0</td>
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<tr>
<td>Muscle soreness</td>
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<td>0</td>
</tr>
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<td>Injection site reaction</td>
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<td>0</td>
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<td>Dizzy</td>
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<td>Nausea</td>
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<td>0</td>
</tr>
<tr>
<td>Upper gastrointestinal hemorrhage</td>
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<td>0</td>
</tr>
<tr>
<td>Lose weight</td>
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</tr>
<tr>
<td>Acute allergic reaction</td>
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<td>0</td>
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</table>

*aIncluding all patients who received at least one dose of a trial treatment. Events were attributed to treatment by the investigator and are listed as indicated by the investigator on the case report form.

Figure 2.

Clinical response induced by iNeo-Vac-P01. A, Percentage changes of tumor lesion size from baseline were recorded over a period of 55 weeks. B, The waterfall plots recorded the best clinical response of patients enrolled. Dashed lines above and below indicate 20% increase or 30% reduction of the sum of the longest diameter of the tumor, respectively, which are in accordance with the cut-off value for progressive disease and PR by RECIST 1.1. Patient P005 was diagnosed as pseudoprogression according to the change of target tumor lesion. The Kaplan–Meier survival curves with PFS (C) and OS (D). Tick marks represent data censored at the last time that patient was known to be SD or unknown (C) and alive (D).
evident increase of tumor size (maximum diameter of target lesions was 122.9 mm) at 5th month compared with baseline (maximum diameter of target lesions was 89.1 mm), and a significant shrinkage of tumor size (maximum diameter of target lesions was 75.4 mm) at 8th month, indicating a pseudoprogression previously occurred (Fig. 3B).

His disease continually maintained stable at cut-off date (over 14.5 months). PBMCs were tested for the reactivity against the immunizing peptides. Robust de novo immune response against predicted neoantigen (mutant PIGK) was generated after 8-week vaccination, analyzed using ex vivo IFNγ ELISpot (Fig. 3C and D). CEF, DMSO was used as negative control and mixed peptides from CEF (including peptides of cytomegalovirus, Epstein–Barr virus, and influenza virus) were used as positive control. E, Increased abundance of peripheral T-cell clones after vaccination was detected by TCR sequencing.

These data suggest that a subset of T cells with specificities induced by iNeo-Vac-P01 can be successfully activated and kill tumor cells.

**Case report of a patient with pancreatic cancer**

In the case of patient P008, a 65-year-old female, diagnosed with pancreatic cancer in July 2016, and later subjected to surgery, pathology showed low differentiated adenocarcinoma in the tail of her pancreas. A total of six cycles of postsurgery chemotherapy were conducted based on a gemcitabine regimen. In June 2017, upper abdomen MRI enhancement scan showed relapse with hepatic metastases. Soon afterwards, she received chemotherapy and radiofrequency ablation (RFA) for liver metastases.

On April 6, 2018, she started to receive iNeo-Vac-P01, and underwent a total of eight doses of iNeo-Vac-P01, including five prime and...
three boost vaccinations. During treatment, the changes of target lesions were monitored every 2 months. MRI showed liver lesion regression at 2nd and 4th month after vaccination compared with baseline level (Fig. 4A). Grade 1 and 2 side effects, including chill, fatigue, and muscle soreness were observed and recorded during regimen. No SAE was noted through the whole treatment period. Ex vivo IFNγ ELISpot of PBMCs showed markedly stronger response (except week 18 due to the poor condition of PBMCs) to all peptide pools at week 3 (average 132.8 spots), week 9 (average 213.9 spots), and week 32 (average 87.1 spots) compared with that of baseline (week 0, average 6.1 spots; Fig. 4B and C). Notably, pretreatment PBMCs also showed measurable response to all five peptide pools, suggesting that previous RFA might lead to release of tumor neoantigens which further activated specific T cells.

**Discussion**

Patients with various types of advanced solid tumors were enrolled in this study. Despite the significant differences in their TMBs, for example, those between P009 (pancreatic cancer) and P011 (ovarian cancer) in particular, effective vaccines were designed and prepared for all patients, following the successful identification of patients’
neoadjuvant by iNeo-Suite. Here, several different vaccine immunization schemes were applied for the patients’ maximum profit and safety, exploring the impact of peptide dosage (100 μg or 300 μg peptide) and boost immune cycles (1-month or 2-month boost intervals as well as 2–6 boost times) on vaccines’ safety and efficacy (Supplementary Table S1). As a result, two cases of acute allergic reaction with similar clinical manifestations occurred in the study, which might result from the increase of boost times, owing to peptide-specific antibody accumulation. This hypothesis needs experimental validation by ELISA. No significant differences in terms of immunologic responses, clinical activities, and AEs were observed in this study while applying 100 and 300 μg prime doses. However, it was noticed that patients with more AEs usually had better response, indicating a potential relationship between clinical response and AEs (Supplementary Table S1), which requires further validation.

Although none of the enrolled patients was observed with CR or PR, their DCR was relatively high (71.4%, 15/21), with PFS and OS similar to the reported data of Neon Therapeutics Inc. In addition, the values of these indexes could be higher if iRECIST were used instead of standard RECIST v1.1 for our data analysis. So far, both advanced pancreatic cancer and biliary tract cancer are known for their poor prognosis and short survival times. For example, the first-line standard therapy for metastatic pancreatic cancer is nanoliposomal irinotecan plus fluorouracil and folinic acid with median OS of 6.1 months and PFS of 3.1 months (26). However, as of data cutoff, the PFS of our enrolled 2 patients with pancreatic cancer was 4.2 months and 6.3 months, respectively, while their OS was 14.0+ months and 13.3+ months, respectively. In addition, the enrolled 2 patients with biliary tract cancer were under deduced SD for 10.8+ months and 14.5+ months, respectively, potentially longer than the 11.7-month median OS of cisplatin/gemcitabine as the first-line standard treatment in advanced biliary cancer (27), and these enrolled patients with advanced pancreatic and biliary tract cancer failed to routine line of chemotherapy, immunotherapy, or small-molecule target therapy. Although these cases cannot stand for a large cohort, the above data suggested that the patients with pancreatic cancer and biliary tract cancer in this study might have benefited from our personalized peptide vaccine.

In this study, both the median PFS (4.6 months, 95% CI, 2.5–5.2) and OS with a 55.1% estimated percentage at the 12th month (95% CI, 25.9%–76.9%). Take some typical clinical trials of cancers with high incidence rates for example. In a phase III clinical trial (CheckMate 078) for NSCLC progressed during/after platinum-based doublet chemotherapy, the median OS and PFS of nivolumab (anti-PD-1 antibody) treatment were 12.0 months (95% CI, 10.4–14.0) and 2.76 months (95% CI, 2.37–3.35), respectively (28). In ATTRAC-TION-2, a phase III trial for patients with advanced gastric or gastroesophageal junction cancer failed after at least two previous chemotherapy regimens, the median OS and PFS of nivolumab treatment were 5.26 months (95% CI, 4.60–6.37) and 1.61 months (95% CI, 1.54–2.30), respectively (29). In addition, a phase II study of anti-CTLA-4 mAb (tremelimumab) for patients with refractory metastatic colorectal cancer demonstrated a median PFS of 2.3 months (95% CI, 2.1–2.6) and a median OS of 4.8 months (95% CI, 4.1–7.7; refs. 30). Although the sample size was small, the personalized neoadjuvant vaccine iNeo-Vac-P01 had shown promising antitumor efficacy, which needs further study with a larger sample size.

Interestingly, besides patient P008 (Fig. 4B and C), 3 other patients (P004, P014, and P019) of the 9 post-RFA patients (44%) also displayed evident neoadjuvant-specific T-cell response before vaccination, while there was only 1 of 13 patients (8%) in non-RFA group found having prevaccination response (Fig. 1B; Supplementary Table S10). It is probably due to the fact that, different from complete surgical resection, RFA treatment can result in tumor necrosis, which will become an immunogenic source, providing proinflammatory signals (31, 32). After RFA, it may activate and/or generate a large amount of IFN-γ and/or neoantigen, while improving the expression of other costimulating factors and the presenting of tumor antigen to T cells. In addition, the prevaccination responses seemed unrelated with tumor type, position of RFA, or time interval between last RFA and vaccination (Supplementary Table S10). The iNeo-Vac-P01 vaccination post-RFA could form an effective antitumor T-cell response, which is worth studying further.

Furthermore, compared with non-RFA group, RFA group seemed to have a longer survival (unpublished data). Similarly, in the case study of patient P005 who received iNeo-Vac-P01 after PD-1 antibody treatment, we noticed that the combinatorial therapies of PD-1/L1 antibodies and personalized neoantigen vaccine may be able to provide better anticancer therapeutic effects. More work needs to be done to evaluate the potential benefits from the combinatorial therapies of iNeo-Vac-P01 with RFA or PD1/L1 antibodies.

In addition, we also identified several potential biomarkers for clinical response, which required further validation. For example, the mutations of genes MUC, ZFHX3, and ABL1 seemed to predict poor response, whereas the copy-number variations of genes TNFRSF, SOX3, and MAGE were relatively associated with better response. Meanwhile, the increase of peripheral T cells during treatment implied patients’ better response. More patients would be enrolled in future study to validate our hypothesis.

In general, the preliminary results demonstrated the feasibility, safety, and efficacy of iNeo-Vac-P01 on patients with various types of advanced solid tumors. iNeo-Vac-P01 monotherapy could elicit robust neoantigen-specific T-cell response, significantly increase T-cell infiltration into tumors, and remodel tumor immune microenvironment alone, suggesting its great potential as cancer immunotherapy. Until now, 22 patients have participated in this clinical trial, more than all of the previous reported patient numbers that we found in the similar kind of clinical trials. In future, we plan to enroll more patients with early- and intermediate-stage cancers, including those who require recurrence prevention after surgery, making further efforts to explore the antitumor and antirecurrence efficacy of iNeo-Vac-P01. The combination of iNeo-Vac-P01 with RFA and/or anti-PD-1/L1 antibody is worth further exploring in the future for patients with advanced cancer, especially oligometastatic patients.

**Disclosure of Potential Conflicts of Interest**

F. Mo is an employee for Hangzhou Neoantigen Therapeutics Co., Ltd. H. Wang is an employee for Hangzhou Neoantigen Therapeutics Co., Ltd. N. Han is an employee for Hangzhou Neoantigen Therapeutics Co., Ltd. H. Li is an employee of Sir Run Run Shaw Hospital, Zhejiang University School of Medicine. R. Chen is an employee for Hangzhou Neoantigen Therapeutics Co., Ltd. L. Chen is an employee for Hangzhou Neoantigen Therapeutics Co., Ltd. L. Liu is an employee for Hangzhou Neoantigen Therapeutics Co., Ltd. S. Chen is an employee for Hangzhou Neoantigen Therapeutics Co., Ltd. No potential conflicts of interest were disclosed by the other authors.

**Authors’ Contributions**

Conception and design: Y. Fang, F. Mo, J. Shou, L. Liu, H. Pan, S. Chen Development of methodology: Y. Fang, F. Mo, H. Wang, K. Luo, S. Zhang, Z. Zhou, L. Liu, H. Wang, H. Pan, S. Chen Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Fang, J. Shou, H. Wang, H. Li, L. Liu, H. Pan

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A Pan-cancer Clinical Study of Personalized Neoantigen Vaccine Monotherapy in Treating Patients with Various Types of Advanced Solid Tumors

Yong Fang, Fan Mo, Jiawei Shou, et al.


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