Immune Combination Therapy With NK Cell and Pembrolizumab Showed Therapeutic Efficacy in Treating Advanced Solid Tumors

Jiankun Jia
Southwest Hospital, Third Military Medical University (Army Medical University)
Gang Heng
Southwest Hospital, Third Military Medical University (Army Medical University)
Meiling Wang
Southwest Hospital, Third Military Medical University (Army Medical University)
Linling Wang
Southwest Hospital, Third Military Medical University (Army Medical University)
Yunyan Li
Southwest Hospital, Third Military Medical University (Army Medical University)
Yingzi Zhang
Southwest Hospital, Third Military Medical University (Army Medical University)
Chengcheng Zhang
Southwest Hospital, Third Military Medical University (Army Medical University)
Zhi Yang
Southwest Hospital, Third Military Medical University (Army Medical University)
Zhihua Ruan
Southwest Hospital, Third Military Medical University (Army Medical University
Cheng Qian (qianc202111@163.com)
Southwest Hospital, Third Military Medical University (Army Medical University)

Research Article

Keywords: Immune combination therapy, NK, Pembrolizumab

Posted Date: December 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1115691/v1

Abstract

Background: Natural killer cells are innate cytotoxic lymphocytes that play an important role in the anti-tumor immune response. However, in the microenvironment of solid tumors, the effector functions of NK cells are often impaired by the induction of immune checkpoint inhibitors, including PD-1.

Methods: In this study, we conducted a two-phase study treating advanced solid patients with NK cell therapy (phase 1) or NK and anti-PD-1 inhibitor, pembrolizumab (phase 2).

Results: After treatment, only 3 of 9 patients achieved stable disease after accepting NK cell therapy in the phase 1 study. While in the phase 2 study, 4 patients achieved stable diseases and 1 patient achieved partial response. Remarkably, no severe adverse event was observed in patients treated by NK cell and pembrolizumab combination therapy.

Conclusion: The results in our study indicated that immune combination therapy with NK cell and pembrolizumab might be a promising and safe approaches to treating advanced solid tumors.

Background

Nowadays, tumor immunotherapy has shown great promise in treating solid and hematological malignancies (1). Among those modalities, immune checkpoint blockade therapies and chimeric antigen receptor T cell (CAR-T) therapy have demonstrated unprecedented clinical success (2). However, as an indispensable part of tumor immunotherapy, natural killer (NK) cell therapy has not yet achieved the same degree of clinical success.

NK cells play a significant role in the innate immune surveillance because of their ability to kill infected cells and tumor cells without the need of MHC molecules (main histocompatibility complex) (3). As found in T cells, multiple activating receptors and inhibitory receptors are also expressed on the surfaces of NK cells. The activation and function of NK cells rely on a balance between signaling from those inhibitory and activating receptors (4, 5). However, there are increasing data indicating that chronic infection or the inhibitory tumor micro environment (TME) can up-regulate inhibitory receptors on the surfaces of NK cells, such as NKG2A, PD-1, TIM-3 and TIGIT (6, 7). Signals from these inhibitory receptor, severely depress the activation and effector function of NK cells, eventually result in the exhaustion of NK cells (5, 8). This might be the major reason of the limited efficacy of NK cell therapy in treating malignant tumors.

Although it was initially thought that blocking PD-1/PD-L1 axis with PD-1 or PD-L1 antibodies would only rescue the T cell response, it is now becoming clear that NK cell responses may also be potentiated through this strategy (9). Besides, another research carried out by Barry KC found that NK cells could increase the responsiveness of patients to anti-PD-1 immunotherapy, through the production of Fms-related tyrosine kinase 3 ligand (FLT3LG) in tumor (10). Therefore, it is important and necessary to investigate the anti-tumor efficacy of the combined therapy of the checkpoint blockade and NK cells in treating patients with malignant tumor.

Methods

Study design

This clinical trial was an investigator-initiated clinical study approved by the institutional review board (IRB) of the Southwest Hospital of Third Military Medical University (Chongqing, China) and all patients signed informed consents. The study was performed according to the principles of the Declaration of Helsinki. All patients or their guardians provided written informed consent before they were recruited into this study. One course of infusion was defined as a round of NK and/or Keytruda administration. Follow-up tests including tumor imaging and blood tests were conducted every month during the first 3 months, every 2 months during the next 6 months, and every 3 months afterwards. Patients who were response-evaluable must accept at least two courses of infusion, and did not accept other anti-tumor therapy during the study period. In this study, there was a general procedure for the treatment of each patient (Figure 1A and B). The first group patients received traditional NK cell therapy; the second group patients received NK cell and Pembrolizumab (Keytruda) therapy. Basic laboratory and radiological tests were administrated to judge whether the patient fit the enrollment criteria and gave the baseline disease characteristics of each patient.

Patient eligibility

Eligibility criteria included patients with advanced cancer who were resistant to standard therapy, had a Karnofsky performance status (KPS)>60 and adequate organ function (Aspartate transaminase/Alanine transaminase <5 times upper limits of the normal level, creatinine <2 mg/dL or calculated creatinine clearance >50 ml/min platelet >80×10^9/L, hemoglobin >100 g/L and an absolute neutrophil count >1×10^9/L). Corticosteroids and immunosuppressive medications were not administrated for at least 3 weeks prior to this study entry. All patients in this study had failed at prior therapies and were in the advanced stage (Ⅲa to Ⅳ) for current tumor disease.

The generation and expansion of NK cells

NK cells were generated and expanded on the basis of pre-established procedures. Briefly, human PBMCs were cultured in a medium which was coated by NK cell activating cytokines. IL-2 (Satellite Pharmacy, Inc) and self-serum were added into this medium. About 14-18 days were needed to obtain enough NK cells for clinical infusion. Before infusion, the phenotypes of NK cells, including the percentage of CD3+ and CD56+ cells, were calculated by flow cytometry to assess the quality of these cells.

Laboratory and radiological tests

Laboratory tests, including routing blood analysis, liver function examination, renal function examination, C-reactive protein (CRP) and tumor markers, were administrated during the treatment and follow-up periods.

CT, MRI or PET-CT was conducted for direct assessment of the tumor numbers and volumes before and after this treatment. The specific radiological method was chosen according to the disease characteristics and economy status of patients.

Results

Patient characteristics

From September 2016 to June 2019, 14 patients with advanced solid tumors (5 breast cancer, 3 NSCLC, 2 colorectal cancer, 1 ovarian cancer, 1 esophageal cancer, 1 soft tissue sarcoma, 1 pancreatic cancer) were included in this study, the median age of these patients was 55 (ranging from 45 to 82) years old. Previously, all these patients had received several cycles of standard therapies, including surgical resection, traditional chemotherapy, radiotherapy and immune therapy.

In the first phase of this study, 9 patients received NK cell therapy, and all of them completed at least 2 courses of infusion (4 completed a full cycle of infusion, 2 received multi-cycle infusions, 3 patients received 2 or 3 courses of infusion). The median dose of NK cells was 10.6×10^9 cells.

In the second phase, 5 patients received NK cell and Keytruda therapy. Among them, 3 patients (P10, P11 and P12) received 2 cycles of infusions and 2 patient (P13 and P14) received 2 courses of infusion. The median dose of NK cells was 10.9×10^9 cells. The Keytruda was administrated at 200 mg each course for adult patients. The specific details were provided in the Table 1.

As shown in Figure 1C, the major phonotype of NK cells was CD3-CD56+, which was significant higher than the percentage of CD3+CD56- and CD3+CD56+ cells (median, 72.6% vs. 17.5% and 9.4%, P< 0.0001).

Clinical outcomes

In the first phase, 3 of 9 patients achieved a stable disease (SD) 4 weeks after NK cell therapy; 5 patients experienced progressive disease (PD) after treatment; 1 patient (P4) was response non-evaluable (NE) as he refused to accept the second course of infusion and withdraw from the study. Among the 3 patients who achieved SD after treatment, P2 was a 64 years old man with a diagnosis of esophageal cancer (stage □b) accompanying lymph nodes and left pleura metastasis. After infusion of 2 courses of NK cells, the tumor remained the primary size for 4 weeks detected by CT and ultrasound test. Besides, the tumor makers (CA-199 and CA-242) had a significant decrease after treatment. Especially, the CA-242 evidently decreased from over 200 KU/L to 143 KU/L 4 weeks after infusion (Figure 2A and B). For patient 7 who was diagnosed as soft tissue sarcoma with lung and colon metastasis, a stable disease was achieved after treatment. As shown in Figure 3A and B, the lung metastasis remained the primary size 4 weeks after NK cells infusion. For 2 patients (P8 and P9) with advanced breast cancer, 3 and 2 cycles of infusion were conducted in them respectively. For patient 8, she had a stable disease after each cycle of infusion, and this SD remained for about 8 weeks after treatment. However, for patient 9, the disease had a significant progression after 2 cycles of infusion, and the patient finally died of it.

In the second stage of this study, 5 patients received NK cell and Keytruda therapy, and 1 of them (P12) achieved partial remission (PR), 4 patients (P10, P11, P13 and P14) achieved SD after treatment. For patient 10, she had received anti-CEA chimeric antigen receptor (CAR)-T cell therapy for refractory/relapsed rectal cancer 4 months ago, and a stable disease was achieved after CAR-T. However, the disease had a progression 2 months after CAR-T therapy and this patients turned to this study accepting NK and Keytruda treatment. After 2 cycles of treatment, her tumor remained stable, the metastasis in the lung was no longer progressed (Figure 3C and D). Patient 11 was diagnosed as stage □ NSCLC with lymph nodes and spine metastasis, receiving 2 cycles of NK and Keytruda treatment. As shown in Figure 2C-F, although the tumor markers had some slight fluctuations after treatment, they still remained at the normal range. The metastasis in the spine was no longer progressed and remained stable for 4 months after treatment (Figure 3E-H).

For patient 12, he was diagnosed as NSCLC (stage □) with metastases in the lymph nodes, bone and brain, and accepted anti-CEA CAR-T cell therapy 5 months before this study. After CAR-T, his disease remained stable for about 3 months but rapidly progressed afterwards. In this study, he accepted 2 cycles of NK and Keytruda treatment. Surprisingly, levels of the tumor markers including CA-125, CA-199, CA-242 and CEA had an evident decrease after 2 cycles of infusion (Figure 2G-J). Besides, the primary lesions in the lungs showed apparent attenuation and almost disappeared after treatment (Figure 4).

Toxicities

In the NK cell group, all fusions of NK cells were well tolerated by all patients. The mild fever was the most common adverse event observed in this group, and only one patient (P8) experienced high fever (>39℃). All symptoms were quickly relieved after conventional treatments.

In the NK cell and Keytruda group, the degree of adverse events was relatively more serious than that in NK cell group. 4 patients (P10, P11, P12 and P14) experienced mild to moderate fever and chills after infusion, especially after Keytruda administration. Besides, mild local erythroderma (grade 1) was observed after Keytruda administration in P11 and P12, which was relieved after symptomatic treatments and did not have a negative effect on the overall treatment schedule in this study. No obvious adverse event was observed in P13.

Discussion

NK cells were the first subtype of innate lymphoid cells to be identified and can respond to virally infected and cancerous cells without the restriction of MHC molecules. However, NK cell function is often impaired during the process of tumor progression and development. Numbers of NK cell infiltrating into tumor sites are usually decreased and the cell function and activation are severely inhibited (5). Decreased NK cell cytolytic activity and cytokine secretion promote the progression and metastasis of tumors. In various cancers, the immunosuppressive tumor microenvironment alters the balance between activating receptors and inhibitory receptors on NK cells, through downregulating activating receptors and upregulating inhibitory receptors, including PD-1, TIM-3, LAG-3 and TIGIT (11). The interaction between checkpoint receptors and their respective ligands plays the major role in contributing to the NK cell dysfunction (12). Therefore, it is necessary and promising to combine NK cell therapy with anti-PD-1 blockade therapy in treating patients with advanced tumors.

Although single immunotherapy, particularly immune checkpoint inhibitors or immune cells therapy, has demonstrated marked success in improving the survival of patients with advanced malignancy, the response rates are still limited until now (13, 14). Many patients do not respond to or even develop resistance to these therapeutic approaches. It is likely that interrupting a single checkpoint or infusing a single type of immune cells is not enough to recover the function of immune systems, and some cancers may develop primary or acquired resistance to these therapies in a short period (15, 16). Combined immunotherapies might be an effective approaches to overcome this resistance, enhance the anti-tumor efficacy and increase the response rates. A prime example of combination immune therapy is the use of combined therapy with blocking antibodies against CTLA-4 and PD-1, which results in significantly higher response rates and improved survival in patients with metastatic melanoma (17, 18). Other strategies combining immune modulation of the tumor microenvironment with immune checkpoint inhibitor therapy are currently being tested in clinical trials (19). Besides, vaccine strategies against identified neoantigen epitopes are also being combined with immunotherapeutic approaches, though relative data are not available now. In this study, we found that NK cell and anti-PD-1 combination therapy showed superior anti-tumor activities than single NK cell or anti-PD-1 therapy in treating advanced solid tumors. Among 5 patients accepting NK cell and anti-PD-1 combination therapy, all of them had previously accepted several doses of Keytruda, a PD-1 inhibitor, while no obvious efficacy was achieved after Keytruda treatment.

However, it is believed that combined immune therapy is associated with an increased risk of some immune related adverse events (20). For example, patients who accepted anti-PD-1 and anti-CTLA-4 combination therapy developed more severe adverse events than single therapy (21). In this study, we found that NK cell and anti-PD-1 combination therapy did not increase organ toxicities, compared to traditional NK cell therapy.

Conclusion

We primarily showed that NK cell and anti-PD-1 combination therapy could be a promising and safe approach to treating patients with advanced solid tumors, and it is worth expanding the sample sizes to further verify the efficacy and safety of this therapy.

Abbreviations

NK: natural killer; CAR-T: chimeric antigen receptor T; DC: dendritic cells;

TME: tumor micro environment; KIRs: killer immunoglobulin-like receptors; PD-1: Programmed Death 1; PD-L1: Programmed Death Ligand 1; FLT3LG: Fms-related tyrosine kinase 3 ligand; IRB: institutional review board; CRP: C-reactive protein; NSCLC: non-small cell lung cancer; SD: stable disease; PD: progressive disease.

Declarations

Ethics approval and consent to participate

This clinical trial was an investigator-initiated clinical study approved by the institutional review board (IRB) of the Southwest Hospital of Third Military Medical University (Chongqing, China). In this study, all methods were carried out in accordance with relevant guidelines and regulations and informed consent was obtained from all subjects or their legal guardian(s).

Consent for publication

All patients signed informed consents.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by National Key Research and Development Program (2016YFC1303405), National Natural Science Foundation of China (81520108025), Chongqing Precision Biotech Co., Ltd (JSXM-1).

Authors' Contributions

Cheng Qian, Zhihua Ruan and Zhi Yang designed the research study. Jiankun Jia and Gang Heng performed the research. Meiling Wang conducted the flow cytometry tests. Yunyan Li and Linling Wang cultivated NK cells. Zhihua Ruan Yingzi Zhang and Chengcheng Zhang managed the adverse events. Jiankun Jia and Gang Heng wrote the paper.

Acknowledgments

Not applicable

References

1. Yang Y. Cancer immunotherapy: harnessing the immune system to battle cancer. The Journal of clinical investigation. 2015;125(9):3335–7.
2. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Delbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. The Lancet. 2015;385(9967):517–28.
3. Herberman RB, Nunn ME, Holden HT, Lavrin DH. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. International journal of cancer. 1975;16(2):230–9.
4. Bryceson YT, March ME, Ljunggren HG, Long EO. Activation, coactivation, and costimulation of resting human natural killer cells. Immunological reviews. 2006;214:73–91.
5. Long EO. Negative signaling by inhibitory receptors: the NK cell paradigm. Immunological reviews. 2008;224:70–84.
6. Barrow AD, Martin CJ, Colonna M. The Natural Cytotoxicity Receptors in Health and Disease. Front Immunol. 2019;10:909.
7. Zingoni A, Molfetta R, Fionda C, Soriani A, Paolini R, Cippitelli M, et al. NKG2D and Its Ligands: "One for All, All for One". Front Immunol. 2018;9:476.
8. Parham P, Norman PJ, Abi-Rached L, Guethlein LA. Human-specific evolution of killer cell immunoglobulin-like receptor recognition of major histocompatibility complex class I molecules. Philosophical transactions of the Royal Society of London Series B, Biological sciences. 2012;367(1590):800–11.
9. Hsu J, Hodgins JJ, Marathe M, Nicolai CJ, Bourgeois-Daigneault MC, Trevino TN, et al. Contribution of NK cells to immunotherapy mediated by PD-1/PD-L1 blockade. The Journal of clinical investigation. 2018;128(10):4654–68.
10. Barry KC, Hsu J, Broz ML, Cueto FJ, Binnewies M, Combes AJ, et al. A natural killer-dendritic cell axis defines checkpoint therapy-responsive tumor microenvironments. Nature medicine. 2018;24(8):1178–91.
11. Vitale M, Cantoni C, Pietra G, Mingari MC, Moretta L. Effect of tumor cells and tumor microenvironment on NK-cell function. European journal of immunology. 2014;44(6):1582–92.
12. Myers JA, Miller JS. Exploring the NK cell platform for cancer immunotherapy. Nature reviews Clinical oncology. 2021;18(2):85–100.
13. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for First-Line Treatment of Metastatic Nonsquamous NSCLC. The New England journal of medicine. 2018;378(24):2288–301.
14. Peters S, Kerr KM, Stahel R. PD-1 blockade in advanced NSCLC: A focus on pembrolizumab. Cancer treatment reviews. 2018;62:39–49.
15. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. Cell. 2017;168(4):707–23.
16. Barrueto L, Caminero F, Cash L, Makris C, Lamichhane P, Deshmukh RR. Resistance to Checkpoint Inhibition in Cancer Immunotherapy. Translational oncology. 2020;13(3):100738.
17. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. The New England journal of medicine. 2015;372(21):2006–17.
18. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. The New England journal of medicine. 2015;373(1):23–34.
19. Wrangle JM, Velcheti V, Patel MR, Garrett-Mayer E, Hill EG, Ravenel JG, et al. ALT-803, an IL-15 superagonist, in combination with nivolumab in patients with metastatic non-small cell lung cancer: a non-randomised, open-label, phase 1b trial. The Lancet Oncology. 2018;19(5):694–704.
20. Zhang B, Wu Q, Zhou YL, Guo X, Ge J, Fu J. Immune-related adverse events from combination immunotherapy in cancer patients: A comprehensive meta-analysis of randomized controlled trials. International immunopharmacology. 2018;63:292–8.
21. Pollack MH, Betof A, Dearden H, Rapazzo K, Valentine I, Brohl AS, et al. Safety of resuming anti-PD-1 in patients with immune-related adverse events (irAEs) during combined anti-CTLA-4 and anti-PD1 in metastatic melanoma. Annals of oncology: official journal of the European Society for Medical Oncology. 2018;29(1):250–5.

Tables

Table 1: Basic characteristics and clinical outcomes of patients

Patient	Gender	Age	Diagnosis	Stage	Metastasis	Prior therapy	Current therapy	Keytruda dose (mg)	Total NK cells (10^9)	Outcome
P1	F	45	Ovarian cancer		LNs; Liver	Radical resection of ovarian cancer; Cis-platinum+paclitaxel; Carboplatin+ cyclophosphamide+pharmorubicin +bevacizumab	NK	/	7.8	PD
P2	M	64	Esophageal cancer	b	LNs; Left pleura	Radical resection of esophageal cancer; Oxaliplatin+Tegafur,Gimeracil and Oteracil Porassium	NK	/	3.7	SD
P3	F	60	Breast cancer		LNs; Left wall of the chest; Left arm	TE; Modified mastectomy of left breast; Letrozole; Gemcitabine +xeloda; Paclitaxel+carboplatin	NK	/	10.3	PD
P4	F	47	Breast cancer		LNs; Bone	Docetaxel+Epirubicin; Modified mastectomy of right breast; Docetaxel+Capecitabine; DC-CIK;	NK	/	4	NE
P5	M	69	NSCLC	a	LNs; Left back	Left pneumonectomy; Paclitaxel+Nedaplatin	NK	/	9.1	PD
P6	F	65	Breast cancer		LNs; Bone	Modified mastectomy of left breast; Letrozole; Gemcitabine +xeloda;	NK	/	15.5	PD
P7	F	82	Soft tissue sarcoma		Lung; Colon	Mass resection (right leg+colon); I-125 implantation	NK	/	10.9	SD
P8-1	F	51	Breast cancer		LNs; Right wall of the chest; Left breast	Docetaxel+Epirubicin; Modified mastectomy of right breast; Herceptin; Gemcitabine+Capecitabine	NK	/	11.5	SD
P8-2							NK	/	20.2	SD
P8-3							NK	/	21	SD
P9-1	F	47	Breast cancer		LNs; Right wall of the chest;	Modified mastectomy of right breast; AC-T; Tamoxifen; Letrozole; Paclitaxel+carboplatin	NK	/	9.4	PD
P10-1	F	53	Rectal cancer		LNs; Lung	Radical resection; FOLFOX; Endostar; FOLFIRI; RFA; Keytruda; CAR-T	NK+Keytruda	600	10.4	SD
P10-2								600	9.9	SD
P11-1	M	51	NSCLC		LNs; Bone	pulmonary lobectomy; TP,Tarceva; Iodine seeds implantation; Keytruda	NK+Keytruda	600	10.5	SD
P11-2								600	11.3	SD
P12-1	M	46	NSCLC		LNs; Bone; Brain	pulmonary lobectomy; GL,DC; Bevacizumab; Temozolomide; Iodine seeds implantation; Keytruda; CAR-T	NK+Keytruda	600	14.4	PR
P12-2								600	12.1	PR
P13	F	71	Pancreatic cancer		LNs; Liver	Tegafur,Gimeracil and Oteracil Porassium; Keytruda	NK+Keytruda	400	6.9	SD
P14	M	57	Colon cancer		LNs; Liver; Lung	XE-LOX; XE-LOX+Bevacizumab Capecitabine+ Bevacizumab; Keytruda; Stivarga+ Irinotecan	NK+Keytruda	400	13.2	SD

Figures

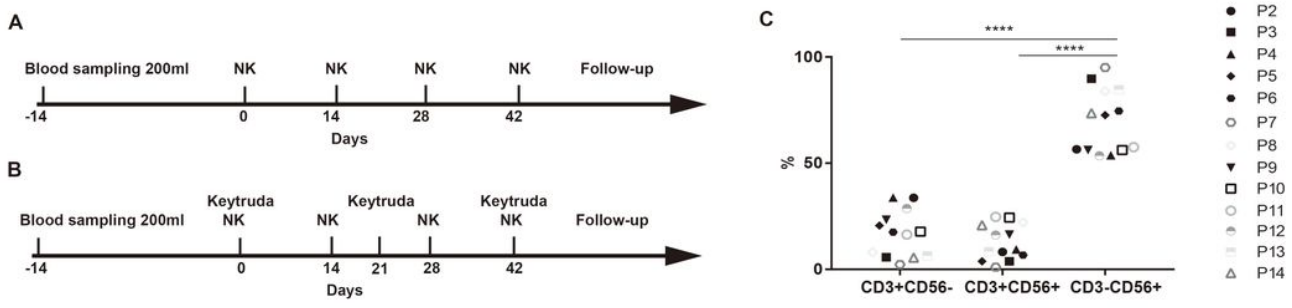

Figure 1

The schema diagram of this study and the phenotypes of infused NK cells. (A) The infusion schema of NK cells in the phase 1 study. (B) The infusion schema of NK cells and Keytruda in the phase 2 study. (C) The phenotypes of NK cells used in the study.

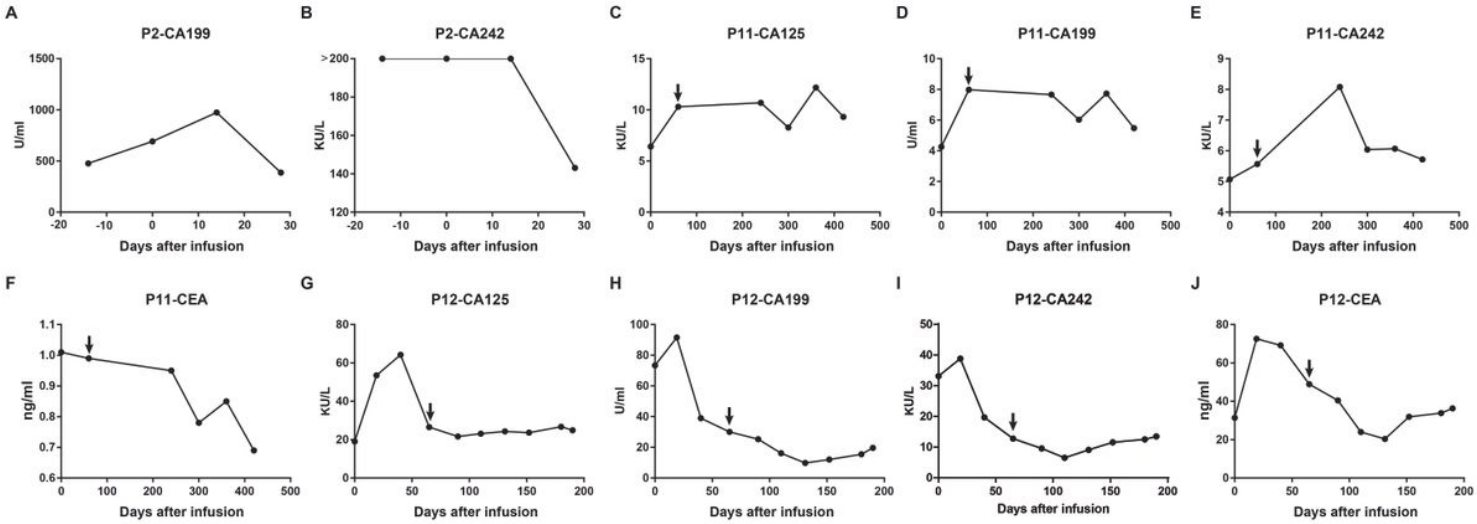

Figure 2

The dynamics of tumor bio-markers in 3 patients after treatment. (A-B) The dynamics of CA199 and CA242 in patient 2 after accepting NK cell therapy. (C-E) The dynamics of CA125, CA199, CA242 and CEA in patient 11 after accepting NK and Keytruda therapy. (G-J) The dynamics of CA125, CA199, CA242 and CEA in patient 11 after accepting NK and Keytruda therapy.

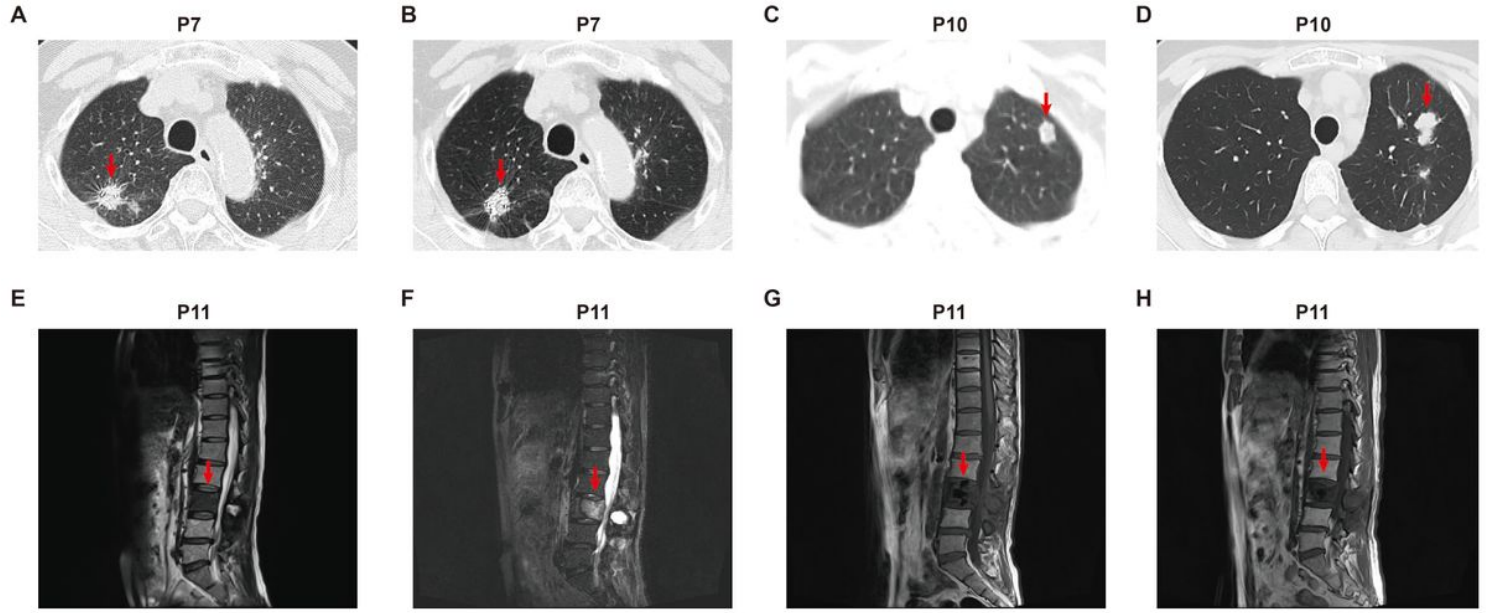

Figure 3

The imaging features of tumors in 3 patients who achieved SD after therapy. (A-B) The dynamics of tumor size in the lung before and after therapy in patient 7. (C-D) The dynamics of tumor size in the lung before and after therapy in patient 10. (E-H) The dynamics of tumor size in the spine before and after therapy (1,2 and 4 months) in patient 11.

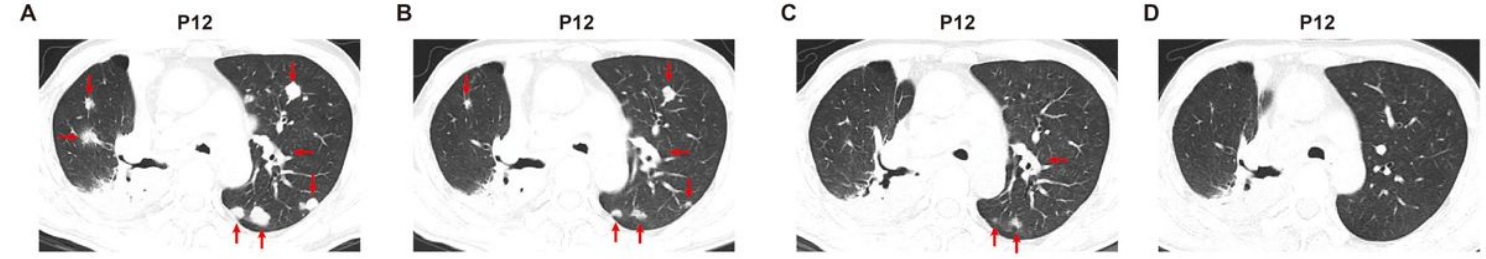

Figure 4

The imaging features of tumors in patient 12 who achieved PR after NK and Keytruda therapy. (A-D) The dynamics of tumor size in the lung before and after therapy (1,2 and 4 months) in patient 12.