FULL-LENGTH ARTICLE

Immunotherapy

C-Met is a chimeric antigen receptor T-cell target for treating recurrent nasopharyngeal carcinoma

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ABSTRACT

Background aims: Radiation therapy is the standard treatment for patients with nasopharyngeal carcinoma (NPC), but relapse occurs in 10% to 20% of patients. The treatment of recurrent nasopharyngeal carcinoma (rNPC) remains challenging. Chimeric antigen receptors (CAR)-T-cell therapy has achieved good outcomes in the treatment of leukemia and seems to be a promising therapeutic strategy for solid tumors. c-Met has been found to be highly expressed in multiple cancer types, and the activation of c-Met leads to the proliferation and metastasis of cancer cells. However, the expression of c-Met in NPC tissues and whether it can be used as a target for CAR-T therapy in rNPC remain to be investigated.

Methods: We detected the expression of c-Met in 24 primary human NPC tissues and three NPC cell lines and constructed two different antibody-derived anti-c-Met CARs, namely, Ab928z and Ab1028z. To estimate the function of these two different c-Met-targeted CAR-T cells, CD69 expression, cytotoxicity, and cytokine secretion of CAR-T cells were assessed after coculture with target cells. A cell line-derived xenograft mouse model was also used to evaluate these two anti-c-Met CAR-T cells. Furthermore, we determined whether combination with an anti-EGFR antibody could promote the antitumor effect of CAR-T cells in a patient-derived xenograft mouse model.

Results: High c-Met expression was detected in 23 of 24 primary human NPC tissues by immunohistochemical staining and in three NPC cell lines by flow cytometry. Ab928z-T cells and Ab1028z-T cells showed significantly upregulated expression of CD69 after coculture with targeted cells. However, Ab1028z-T cells showed superior cytokine secretion and antitumor activity. Furthermore, Ab1028z-T cells effectively suppressed tumor growth compared with control CAR-T cells, and the combination with nimotuzumab further enhanced the tumor-clearing ability of Ab1028z-T cells.

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Background

Nasopharyngeal carcinoma (NPC) is a malignant epithelial tumor of the nasopharynx that demonstrates a unique geographic distribution, with the greatest incidence in Southern China [1,2]. As NPC is highly sensitive to radiotherapy, intensity-modulated radiotherapy is the preferred treatment; however, 10–20% of patients experience recurrence at the primary or regional site after radiotherapy [3,4]. Radiotherapy [5], chemotherapy [6], surgery [7], targeted therapy [8] and other methods have been used to treat recurrent nasopharyngeal carcinoma (rNPC). However, each therapy has drawbacks, including radio-resistance [9,10], severe side effects of chemotherapy [11], narrow surgical indications [12], and unstable targeted therapy effects [13]. Chimeric antigen receptor (CAR)-T-cell immunotherapy appears to circumvent these disadvantages, eliminating cancer cells by directly targeting cancer-cell membrane antigens.

CARs are synthetic receptors consisting of single-chain variable fragments (scFvs) that recognize antibodies to tumor-associated antigens, a transmembrane structural domain and intracellular signals [14–17]. CAR-T cells lyse cancer cells with high specificity in major histocompatibility complex–independent mechanisms [18]. CAR-T-cell immunotherapy is a promising approach for the treatment of cancer and has achieved striking results in the treatment of both hematologic and solid tumors [19,20]. However, most potential targets for solid tumors are nonspecific, and selecting appropriate targets for solid tumors remains challenging [21,22]. In particular, for rNPC, few targets have been reported. Therefore, it is extremely urgent to explore specific tumor-associated antigens for the treatment of NPc. c-Met is a receptor tyrosine kinase and is also known as hepatocyte growth factor receptor [23]. c-Met plays a significant role in promoting tumor invasion, angiogenesis and metastasis in a variety of human cancers, including breast cancer, colorectal cancer, lung cancer, gastric cancer and hepatocellular carcinoma [24–29]. The elevated expression of c-Met also was demonstrated in NPC and correlated with increased cancer-cell proliferation and metastasis [30,31]. c-Met has been proposed as a target for CAR-T therapy in hepatocellular carcinoma, breast cancer and renal cancer [32–34]. Nevertheless, the expression profile of c-Met in rNPC and the feasibility of CAR-T cells targeting c-Met to treat rNPC need further study.

Materials and Methods

Ethics approval and consent to participate

Umbilical cord blood was obtained from healthy pregnant women at the Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou, China. rNPC tumor tissues were provided by the Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou, China. All donors who provided umbilical cord blood or primary specimens gave informed consent for the use of their samples for research purposes, and all procedures were approved by the Ethics Committee of the Fifth Affiliated Hospital of Guangzhou Medical University (approval number: KY01-2021-11-11).

All animal experiments were performed at the Laboratory Animal Center of the Guangzhou Institutes of Biomedicine and Health (GIBH), and all procedures were approved by the Animal Welfare Committee of GIBH. NOD-SCID-IL2Rgf−/− (NSI) mice were derived at GIBH. All mice were maintained in specific pathogen-free cages and provided autoclaved food and water. Animal protocols were approved by the Institutional Animal Care and Use Committee.

Construction of the CAR vector

To generate c-Met–targeted CARs, we synthesized two antibody-derived anti-c-Met scFv sequences, namely, Ab9 and Ab10. The basic skeleton of the CAR vector was composed of the CSF2RA signal peptide, scFv, CD28 transmembrane domain, CD28 and CD3ζ intracellular domains. These CARs were cloned into the lentiviral vector pWPXLd-2A-enhanced green fluorescent protein (GFP).

Production of c-Met CAR lentivirus

The vector was preliminarily identified by restriction endonuclease sites SaI and SpeI enzyme digestion, and the extracted plasmids were used for lentiviruses package after sequencing. The pWPXLd-based CAR vector plasmid and the two packaged plasmids, psPAX2 and pMD.2G, were cotransfected into HEK-293T cells with polyethylenimine (Sigma–Aldrich, St. Louis, MO, USA) at a ratio of 3:1:4, totaling 24 μg. Each plate of 293T cells was starved with 7 mL of lentiviral medium for 1–2 h before adding the constructed plasmid. Lentiviral supernatants were collected at 24 h, 48 h and 72 h posttransfection and filtered through a 0.45-μm filter.

Isolation and activation of primary human T cells

Peripheral blood mononuclear cells were isolated from the umbilical cord blood of healthy pregnant women aged 20 years or older recruited by the Fifth Affiliated Hospital of Guangzhou Medical University, and all procedures were approved by the Research Ethics Committee. Lymphoprep (Fresenius Kabi Norge, AS, Berg i Østfold, Norway) was used to separate peripheral blood mononuclear cells from cord blood by density gradient centrifugation. T cells were selected from negative peripheral blood mononuclear cells using the EasySep Human T-Cell Isolation Kit (STEMCELL, Vancouver, British Columbia, Canada) and activated in T551 for 48 h using microspheres coated with anti-human CD3 and anti-human CD28 antibodies (Milenyi Biotec, Bergisch Gladbach). In this study, 5% heat-inactivated fetal bovine serum (MG), 1000 IU/mL IL-2 and 1% penicillin/streptomycin were added.

Lentivirus transduction

Five to 10 mL of CAR lentivirus supernatant and 1 mL of T551 containing 5% fetal bovine serum (FBS; Gibco/Life Technologies, Carlsbad, CA, USA) were added per 1 × 10⁶ T cells, and the cells were incubated for 12 h. After transduction, T cells were cultured in fresh T551 medium containing interleukin (IL-2; 1000 IU/mL). Subsequently, fresh medium was added every 2–3 days to maintain the cell density in the range of 0.5–1 × 10⁶ cells/mL. The percentage of CAR-positive T cells was measured by flow cytometry after 2 days of transduction.

Cells and culture conditions

HEK-293T cells were maintained in Dulbecco’s modified Eagle’s medium (Gibco, Grand Island, NY, USA). NCI-H522 (human non–small cell lung cancer cell line) was obtained from CTCC (Meisen CTCC, Zhejiang, China) and maintained in RPMI-1640 medium.
**Flow cytometry**

All samples were analyzed using a NovoCyteTM (ACEA Biosciences, San Diego, CA), LSR Fortessa or C6 flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA), and data were analyzed using FlowJo software (FlowJo, LLC, Ashland, OR, USA). The antibodies used included anti-c-Met-biotin (clone eBioclone 97), APC Streptavidin (CAT405243), Rat IgG1 kappa Isotype Control-APC (eBRG1), APC/Cyanine7 anti-human CD3 Antibody (clone OKT3), APC anti-human CD4 Antibody (clone OKT4), APC/Cyanine7 anti-human CD4 Antibody (clone OKT4), PE anti-human CD8a Antibody (clone HIT8a), phycoerythrin/Cyanine7 anti-human CD25 Antibody (clone M-A251) and APC anti-human CD69 Antibody (clone FN50). All fluorescence-activated cell sorting–related staining procedures were performed on ice for 30 minutes, and the cells were then washed with phosphate-buffered saline (PBS) containing 1% FBS before cytometry analysis.

**In vitro tumor killing assays**

Target SUNE1-GL, CNE1-GL and NP69-GL cells and negative control NCI-H522-GL cells were incubated with 1928z-T cells, Ab28z-T cells and Ab1028z-T cells at a 1:1, 1:2 and 1:4 ratio in triplicate wells of white 96-well plates. Target cell viability was monitored 24 hours later by adding 100 μl/well D-luciferin (potassium salt) (Yeasen, Shanghai, China) at 100 μg/mL. Background luminescence was negligible (1% of the signal from wells containing only target cells). The percent viability (%) was calculated as experimental signal/maximal signal × 100, and the percent lysis was equal to 100 percent viability.

**Cytokine release assays**

Enzyme-linked immunosorbent assay (ELISA) kits for IL-2, interferon (IFN)-γ, tumor necrosis factor (TNF)-α and granzyme B were purchased from DAKEWEI (Beijing, China), and all ELISAs were performed according to the manufacturer’s protocols. T cells were cocultured with target cells at an E:T ratio of 1:2 for 24 hours. The culture supernatants were analyzed for the secretion of IL-2, IFN-γ, TNF-α and granzyme B using ELISA kits.

**Cell line–derived xenograft and patient-derived xenograft (PDX) models for CAR-T-cell treatment**

Animal experiments were performed in the Laboratory Animal Center of GIBH, and all animal procedures were approved by the Animal Welfare Committee of GIBH. All protocols were approved by the relevant Institutional Animal Care and Use Committee. We used NSI mice aged 6–8 weeks, which were maintained in specific pathogen-free (SPF)-grade cages and provided autoclaved food and water.

For the subcutaneous cell line–derived xenograft models, 1 × 10^6 SUNE1 cells in 100 μl of PBS were injected subcutaneously into the right flanks of NSI mice. When tumor nodules were palpable, mice were divided into three groups (1928z-T, Ab928z-T and Ab1028z-T) and received a peritumoral injection of 5 × 10^6 CAR-T cells in 100 μl of PBS. Tumor volume was measured every 3 days with a vernier caliper and calculated by the following equation: tumor volume = (length × width^2)/2.

For the subcutaneous PDX models, rNPC tissues were obtained from The Fifth Affiliated Hospital of Guangzhou Medical University (Guangzhou, China) with informed consent from the patients. Tumors were dissected into ~25-mm³ pieces and subcutaneously transplanted into NSI mice within a 1-h period. When the tumor reached 1000 mm³, it was excised and transplanted into new NSI mice to produce the next generation. On day –9, the mice were subcutaneously transplanted after anesthetization. On day 0, the mice were divided into four groups, which received 1928z-T cells, 1928z-T cells + nimotuzumab, Ab1028z-T cells and Ab1028z-T cells + nimotuzumab. Each mouse was intravenously injected with 5 × 10^6 CAR-T cells. Nimotuzumab was intravenously injected within 200 μL (1 mg/mouse) every 3 days four times consecutively. The 1928z-T-cell and Ab1028z-T-cell groups were injected with an equal volume of PBS. Tumors were measured every 3 days with a vernier caliper to determine the subcutaneous growth rate. The tumor volume was calculated using the following equation: (length × width^2)/2.

**Immunohistochemistry (IHC)**

Tumor tissue sections were fixed with 10% paraformaldehyde, embedded in paraffin, sectioned at a thickness of 4 μm and stained using a standard hematoxylin and eosin technique. Paraffin sections were also immunostained with antibodies specific for c-Met and EGFR (Abcam, Cambridge, UK) overnight at 4°C, followed by secondary staining with goat anti-rabbit IgG (D110073-0005) (Sangon, Shanghai, China). Images of all sections were obtained with a digital slide scanner (PANNORAMIC Midi; CaseViewer, Budapest, Hungary).

**Statistics**

Data are presented as the means ± standard error of the means. Student’s t-test was used to determine the statistical significance of differences between samples, and a P value <0.05 indicated a significant difference. All statistical analyses were performed using Prism software, version 8.4.0 (GraphPad, Inc., San Diego, CA, USA).

**Results**

**Expression profile of c-Met in primary rNPC tissues**

Cancer cells recognized by CAR-T cells require the expression of certain tumor-associated antigens on the cancer cell surface. To determine whether c-Met was expressed in rNPC tissues, we performed an IHC staining assay in 24 patient-derived rNPC tissues. The results showed that 23 of 24 rNPC tissues expressed c-Met (Figure 1A, supplementary Figure 1A). The clinical information of the 24 patients with rNPC is shown in Table 1. Moreover, we transplanted rNPC tumor tissues from 12 patients into NSI mice, and seven tumors did not grow in the first generation. Five rNPC PDXs were successfully implanted into NSI mice and could be stably transmitted to the next generation. The expression of the xenograft transplant histology demonstrated that transplanted tumors retained the characteristics of the primary rNPC tumor. Subsequently, we performed IHC staining of successfully passed five rNPC PDX tissues, which also expressed the c-Met (Figure 1A). Moreover, we attempted to detect the expression of c-Met in normal nasopharyngeal tissues, but it was difficult to collect normal nasopharyngeal tissues in the clinic. We then evaluated the expression of c-Met in 4 nasopharyngitis tissues and found very limited expression of c-Met in 2 nasopharyngitis tissues (Figure 1B). Overall, we proved that c-Met is highly expressed in rNPC tissues and would be a promising CAR target in rNPC.
Construction and manufacture of c-Met--targeted CAR-T cells

To enable T cells to accurately recognize c-Met antigens on the surface of rNPC tumors, we generated two different scFv sequences, Ab9 and Ab10, targeting c-Met. We cloned the Ab9 and Ab10 scFv sequences into lentiviral vectors containing the CD28 transmembrane domain, CD28 and CD3ζ T-cell activation domains, 2A peptide and enhanced GFP sequence, respectively (Figure 2A). T cells were

Figure 1. Expression profile of c-Met in primary rNPC tissues and nasopharyngitis tissues. (A) Immunohistochemical staining for c-Met in five successfully passaged primary samples and their related PDX samples; scale bar = 50 μm. (B) Immunohistochemical staining for c-Met in four nasopharyngitis tissues; scale bar = 50 μm. H&E, hematoxylin and eosin.
transduced with lentivirus supernatants after activation. T-cell transduction efficiency was measured by flow cytometry (Figure 2B-C). T cells were cultured in T551 medium supplemented with 1000 IU/mL exogenous IL-2, and a 140-fold expansion was observed on day 10. There was no significant difference in the growth of 1928z-T, Ab928z-T and Ab1028z-T cells (Figure 2D).

Both Ab928z-T and Ab1028z-T cells could specifically target c-Met–positive NPC cells in vitro

We tested the expression of c-Met in 3 NPC cell lines by flow cytometry, and this antigen was expressed by all three NPC cell lines. The NCI-H522 cell line without c-Met expression was used as a negative control [33] (Figure 3A). To verify whether Ab928z-T and Ab1028z-T cells could be activated by c-Met–positive target cells, we chose the c-Met+ cell line SUNE1 to coculture with 1928z-T, Ab928z-T and Ab1028z-T cells at a ratio of 1:2. CD69 expression in CD4 and CD8 cells was detected by flow cytometry. The NCI-H522 cell line without c-Met expression was used as a negative control [33] (Figure 3A). Mice were randomly divided into three groups (n = 5 per group): (i) 1928z-T cells, (ii) Ab928z-T cells and (iii) Ab1028z-T cells.

We performed IHC staining of EGFR in 24 primary rNPC specimens, and 23 specimens were EGFR staining positive (supplementary Figure 2A). The combination of nimotuzumab can enhance the antitumor activity of CAR-T-cell therapy in the rNPC PDX model.

Studies have shown that EGFR is highly expressed in more than 80% of patients with NPC [35] and is associated with poor outcomes [36]. Nimotuzumab is a humanized monoclonal antibody targeting epithelial growth factor receptor (EGFR) [37], which inhibits EGF binding by binding to the extracellular domain of EGFR [38]. In patients with NPC, combination therapy with nimotuzumab is more effective than monotherapy. Therefore, we explored whether the combination with nimotuzumab would further improve the efficacy of CAR-T-cell therapy in the rNPC PDX model.

We performed IHC staining of EGFR in 24 primary rNPC specimens, and 23 specimens were EGFR staining positive (supplementary Figure 2A). PDX sample expression of c-Met and EGFR from patient 4 (P4) was chosen to develop the rNPC PDX model (Figure 5A-B). Ten days after tumor tissue transplantation, the mice were randomly divided into four groups (1928z-T, 1928z-T + nimotuzumab, Ab1028z-T and Ab1028z-T + nimotuzumab) and treated respectively (Figure 5B). CAR-T-cell persistence in the peripheral blood of mice was detected on day 26. Moderately greater CAR-T-cell percentage was detected in Ab1028z-T + nimotuzumab group (Figure 5C). Compared with 1928z-T group, tumor growth was significantly suppressed in 1928z-T + nimotuzumab group, Ab1028z-T group and Ab1028z-T + nimotuzumab group. Notably, Ab1028z-T + nimotuzumab group showed strongest efficacy in inhibiting tumor growth (Figure 5D). The tumor weights were analyzed after the mice were euthanized and the results showed that the Ab1028z-T + nimotuzumab group had the lowest tumor burden among the four groups (Figure 5E-F). However, there was a lot of serous fluid in the tumors, and it flowed out during tumor dissection, resulting in weight loss of tumors, especially in the 1928z-T + nimotuzumab group. Further analysis demonstrated that persistence of CAR-T cell in tumors in the Ab1028z-T + nimotuzumab group and the Ab1028z-T group was significantly greater than that in the 1928z-T group and the 1928z-T + nimotuzumab group (Figure 5G), indicating the strong infiltrating ability and viability of Ab1028z-T cells. In conclusion, the combination of nimotuzumab can enhance the antitumor activity of c-Met CAR-T cells.

**Discussion**

Remarkable achievements in the treatment of hematological tumors have been made with CAR-T therapy. In recent years, the
application of CAR-T cells for solid tumors has been extensive. People with rNPC, especially in the advanced stage, undergo multiple treatments with radiotherapy, chemotherapy and even secondary radiotherapy and surgery, which leads to severe complications and low 5-year survival rates. The current first-line treatment for advanced rNPC is platinum-based chemotherapy [39]; however, the long-term efficacy is limited [11]. Epstein–Barr virus–specific T lymphocytes or cancer vaccines also have been introduced for immunotherapy in recent years, but the in vivo immune response elicited in patients with NPC is generally low and transient [40,41]. CAR-T-cell immunotherapy is an antigen-targeted therapy that seems to avoid the drawbacks associated with the aforementioned treatments, providing a
Figure 3. Both Ab928z-T and Ab1028z-T cells specifically target c-Met–positive NPC cells in vitro. (A) Expression profile of c-Met in three NPC cell lines and NCI-H522. The NCI-H522 cell line was used as the negative control. (B) CD69 expression was measured before and after 1928z-T, Ab928z-T or Ab1028z-T cells cocultured with c-Met–positive SUNE1 cells at a ratio of 1:2. Data are representative of three biological repetitions and are presented as means ± standard error of the mean (SEM). The results were compared with two-way analysis of variance (ANOVA). *P < 0.05, **P < 0.01, ***P < 0.001. (C) 1928z-T, Ab928z-T and Ab1028z-T cells were subjected to a 24-h in vitro killing assay against three NPC cell lines (CNE1-GL, SUNE1-GL, NP69-GL) and NCI-H522-GL cells at each E:T ratio. Data are representative of three biological repetitions and are presented as means ± SEM. (D) The concentrations of IL-2, IFN-γ, TNF-α and granzyme B released by 1928z-T, Ab928z-T and Ab1028z-T cells after coculture with SUNE1 cells for 24 h at an E:T ratio of 1:1 were shown. Data are representative of three biological repetitions and are presented as means ± SEM. The results were compared with one-way ANOVA. *P < 0.05; **P < 0.01; ***P < 0.001. E:T, effector to target.
new treatment option for patients with rNPC. The crucial factor in CAR-T-cell therapy for rNPC is the selection of specific target. c-Met protein plays an essential role in the development and progression of NPC [42]; a high c-Met protein expression level correlates with poorer survival in the late stage NPC [43]. In our study, c-Met was found to be highly expressed in both primary rNPC specimens and NPC cell lines. The reactivity and efficacy of c-Met–targeted CAR-T cells against tumors have been demonstrated in vitro and in vivo. c-Met–targeted CAR-T cells with different scFv sequences, Ab928z and Ab1028z, can specifically kill NPC cells, and the latter has relatively stronger antitumor activity. Notably, this result is in line with the in vivo results. In addition, we noted that CD69 expression in CD4 of Ab928z-T cells was greater than that in 1928z-T and Ab1028z-T cells before co-culture with target cells. This might be due to Ab928z-CAR–induced T-cell self-activation and could lead to T-cell exhaustion, which is similar to anti-GD2 (14g2a-E101K) CAR-T cells with high affinity [44]. But this needs further phenotyping with exhaustion markers. In general, we demonstrated that Ab1028z-T cells showed superior antitumor ability than Ab928z-T cells, suggesting the potent antitumor activity of Ab1028z-T cells against c-Met–expressing NPC cells. Tchou et al. [33] conducted clinical trials related to c-Met CAR-T cells and found that mRNA c-Met CAR-T cells were well tolerated when injected intratumorally into patients with metastatic breast cancer expressing c-Met. This study preliminarily
Figure 5. c-Met–specific CAR-T cells suppressed the growth of NPC patient-derived xenograft tumors in vivo. (A) Hematoxylin and eosin- and IHC-stained tissues of P4 third-generation PDXs. The IHC staining included c-Met and EGFR. (B) Schematic representation of the experiments (five mice in each group). (C) Percentage of CAR-T cells in the PB of the patient-derived xenograft models. (D) Growth curve of NPC patient-derived xenograft tumors. Tumor volume was calculated according to the following formula: length × width × width/2. Error bars denote the standard error of the mean (SEM), and the results were compared with two-way analysis of variance (ANOVA). *P < 0.05, **P < 0.01, ***P < 0.001. (E) The figure shows tumors at day 27 in mice treated with 1928z-T, 1928z-T + nimotuzumab, Ab1028z-T or Ab1028z-T + nimotuzumab cells. (F) Tumor weights of mice in the four groups are shown. Error bars denote the SEM, and the results were compared with one-way ANOVA. *P < 0.05; **P < 0.01; ***P < 0.001. (G) Percentage of CAR-T cells in the tumors of the patient-derived xenograft models. Error bars denote the SEM, and the results were compared with two-way ANOVA. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.
demonstrated the safety of c-Met as a CAR-T target. Further clinical trials are needed to demonstrate the safety and efficacy of lentivirus-transduced c-Met CAR-T cells for treating rNPC.

In this study, nimotuzumab was used as a treatment regimen in combination with c-Met CAR-T cells in a rNPC PDX model. We developed a PDX mouse model and measured the expression of c-Met and EGFR in transplanted mouse tumors. We demonstrated that c-Met CAR-T cells combined with nimotuzumab was more effective than CAR-T cells alone. Recent studies have found that overactivation of EGFR in cancer cells upregulates programmed death-ligand 1, while nimotuzumab downregulates programmed death-ligand 1 expression in cancer cells by blocking EGFR activation and further reducing the inhibitory effect on T cells inside the tumor [45], which might be beneficial for CAR-T cell therapy. In addition, some researchers have found that nimotuzumab stimulates the maturation of dendritic cells, which is more conducive to the elimination of cancer cells by T cells [46]. However, whether nimotuzumab has a direct effect on CAR-T cells needs our further study.

To date, this is the first novel immunotherapy for rNPC to rigorously screen c-Met CAR-T cells in vitro and in vivo, and the combination of CAR-T cells with nimotuzumab provides a rational basis for promoting clinical translation.

Conclusions

c-Met was shown to be highly expressed in rNPC and might be a potential target for CAR-T therapy. We designed two CARs to target c-Met and demonstrated that Ab1028z-T cells had superior antitumor activity. The combination with anti-EGFR antibody further improved the antitumor activity of Ab1028z-T cells. Our study provides a promising strategy for the treatment of rNPC.

Declaration of Competing Interest

The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

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Author Contributions

Conception and design of the study: Q.H., J.L. and J.Z. and G.C. Acquisition of data: Q.H., J.Z., J.L., H.H., H.Y., Z.X., Y.W., Y.W., Y.Q., Y.Y., A.H., Y.C., L.Q. and G.C. Analysis and interpretation of data: Q.H., and J.L. Drafting or revising the manuscript: Q.H., and J.L. All authors have approved the final article.

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Data Availability

All data generated or analyzed during this study are included in this article.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jcyt.2023.06.004.

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