Therapeutic Effects of a Neoantigen Vaccine With an Immune Checkpoint Inhibitor in a Cisplatin-Treated Mouse Model

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Abstract

Background: Cancer immunotherapy is widely used as a treatment for cancer that works by improving the immune system with fewer side effects than conventional methods. Neoantigen vaccines are one form of immunotherapy that use cancer-specific neoantigens that are extracted from cancer patients and are not recognized by normal cells in the immune system.

Methods: In this study, mutant genes of 4T1 mouse breast cancer cells were identified by direct sequence analysis using tumor-specific MHC I (Major Histocompatibility Complex) or MHC II epitopes through *in vivo* experiments.

Results: The neoantigen vaccine with mutant $CD4^+$ or $CD8^+$ T cell-reactive neoantigen peptides was shown to inhibit tumor growth, increase long-term survival, and induce the secretion of IFN-γ (Interferon gamma) in the cisplatin-treated mouse models. In particular, mutant $CD4^+$ T cell neoantigen peptides induced full potential anti-tumor effects, whereas dual treatment with $CD4^+$ (Cluster of differentiation 4) and $CD8^+$ (Cluster of differentiation 8) T cell neoantigen peptides increased the suppression of tumor growth. Moreover, the combination of neoantigen vaccine with mutant $CD4^+$ T cell neoantigen peptide and anti-PD-L1 (Programmed death-ligand 1) as an immune checkpoint inhibitor (ICI) has been shown to have synergistic therapeutic effects in cisplatin-treated mouse models.

Conclusion: This study, therefore, proved that cancer cell-derived neoantigens have great potential to induce immunogenic responses and cancer treatment effects, along with synergistic efficiency when applied to various combinational therapies. Through the methods that were used in our experiments, we could contribute to the development of new adjuvants for evaluating efficacy, discovering unfound neoantigens, and investigating immune checkpoint blockade antibodies for non-clinical studies.

Background

Cancer immunotherapy is a form of therapy that improves our immune system to fight against infection or disease from viruses or bacteria[1]. Although conventional methods (e.g., surgery, chemotherapy, and radiotherapy) have been used for tumor treatment, they often lead to side effects such as damage to normal cells, tumor metastasis, and recurrence[2]. On the other hand, cancer immunotherapy (e.g., Dendritic cell (DC)-based vaccine, DNA or RNA vaccine, CAR-T cell therapy, antibody-based therapy, cytokine-based therapy, etc.) has been appreciated for its safety and increased survival rates in cancer patients[3]. For instance, PROVENGE (Sipuleucel-T), a DC-based vaccine for prostate cancer, has been approved by the FDA (Food and Drug Administration), but it has not been widely used since it is not very efficient[4]. During the anti-tumor immune response, $CD8^+$ T cells or $CD4^+$ T cells recognize antigen epitopes presented on MHC I or MHC II in antigen-presenting cells (APCs), such as dendritic cells (DCs)[5]. This activates T cells in the lymph node or spleen that then infiltrate the tumor microenvironment (TME) to attack malignant cells or to help in the activation of immune cells[6]. Despite the potential of this therapeutics, there are limitations to tumor treatment using immunotherapy such as immune tolerance by

self-antigen, that also causes autoimmune diseases, or immune escape through immunosuppression within the tumor microenvironment (e.g., the secretion of anti-inflammatory cytokines, the expression of immune checkpoints, and especially, deficiency in recognizable tumor antigens)[7]. Thus, tumor-specific neoantigens that can evade immune tolerance and boost the immune response to clear tumors in cancer patients are required for immunotherapy[8].

Neoantigens are newly formed antigens that elicit immune responses as non-self antigens that have not been previously recognized by the immune system[9]. In general, tumor cells have distinct characteristics including a high incidence of mutation in the genome, immortality, increased invasiveness, and an abnormal rapid growth rate compared to normal cells[10]. In particular, a high tumor mutation burden (TMB) may induce an increase in neoantigens in tumors that can be recognized as immunogenic by innate immune cells to initiate adaptive T cell immune responses[11]. Analysis of neoantigens in tumor genomes can be performed by next-generation sequencing, wherein prediction algorithms have been developed to identify which neoantigens will likely elicit immune responses[12]. Among various cancer vaccines (e.g., DNA, mRNA(Messenger RNA), and peptide vaccines), neoantigen vaccines using tumor peptides are widely employed in clinical trials due to their high efficacy, wherein the mutant peptides act as epitopes that can activate $CD8^+$ or $CD4^+$ T cells depending on which class of MHC is presenting the antigens (e.g., epitope on MHC I for $CD8^+$ T cells and epitopes on MHC II for $CD4^+$ T cells)[13]. In a recent study, neoantigen vaccines using $CD4^+$ T cell-activating mutant peptides have shown significant effects in cancer immunotherapy[5]. Therefore, the identification of tumor neoantigens is required to activate not only $CD8^+$ T cells but also $CD4^+$ T cells for tumor treatment[5, 14]. Moreover, the combination of a neoantigen vaccine and chemotherapy that alters the tumor microenvironment to allow permeability of immune cells can induce synergistic effects by increasing the tumor neoantigen-specific T cell immune response[15].

Chemotherapy is a cancer therapy that inhibits the growth of tumors using various anti-cancer drugs (e.g., cisplatin, doxorubicin, vinblastine, paclitaxel, etc.) that induce the release of inflammatory factors from tumor or immune cells to make tumor microenvironment conducive to the production of a tumor-specific adaptive immune response[16–18]. In particular, cisplatin, which is used in our experiment, is a well-known platinum-based chemotherapeutic drug that is widely used to treat a number of cancers (e.g., testicular cancer, ovarian cancer, cervical cancer, breast cancer, bladder cancer, head and neck cancer, etc.) by binding to DNA and inhibiting replication[19]. Thus, the combinational administration of chemotherapy and antigen epitopes could induce tumor-specific T cell immune responses[20, 21]. Nevertheless, side effects have been shown within the tumor microenvironment after chemotherapy, including the expression of immune checkpoints that inhibit activation of effective T cell immune response[22]. For example, PD-L1 expressed in tumors disrupts the anti-tumor immune response through interaction with PD-1(Programmed cell death protein 1) expressed in immune cells[23]. Therefore, together with cisplatin treatment, the administration of immune checkpoint inhibitors (ICIs) can reduce the suppressive reaction in the tumor microenvironment and inhibit tumor growth[6].

In conclusion, a combination therapy using a neoantigen vaccine, chemotherapy, and an immune checkpoint inhibitor is required to compensate for the side effects of cancer immunotherapy and to induce synergistic effects in tumor treatment[8]. This can be done by increasing the exposure of tumor-specific neoantigens, altering the tumor microenvironment to produce a tumor-specific T cell immune response, and suppressing immune escape by the tumor[24, 25]. In our study, the mutant antigen epitopes for $CD8^+$ T cells and, especially, for $CD4^+$ T cells were isolated and synthesized from tumor cells to be used for neoantigen vaccines[5]. Moreover, anti-tumor effects have been shown with administering the neoantigen vaccine and anti-PD-L1 antibody in cisplatin-treated mouse models.

Results

The mouse breast cancer cell line 4T1 has various mutant genes.

It has been studied by another group that mutant MHC class II epitopes drive therapeutic immune responses to cancer[5]. Therefore, we confirmed the presence of mutations in 4T1 genes in this study, where total RNA was isolated from tumor cells, cDNA was synthesized and amplified, followed by identification of mutant genes through direct sequence analysis (Fig. 1a). As a result, 8 genes have shown mutations among different proportions in the base pairs: *Gen1* has 100%; *Gprc5a* has above 50%; *Zfr*, *Cep120*, *Malt1*, and Zzz3 have 50%; and *Polr2a* and *Cenpf* have less than 50%, whereas three genes (*Wdr*, *Kbtbd2*, and *Ilkap*) had no mutation compared to the wild type (Fig. 1b and Table 1). To verify this, tumor cells were colonized following the same procedures wherein the genes were shown to have the same degree of mutation in multiple colonies (e.g., *Gprc5a* has > 50% mutation in a base pair for 8 colonies) (Additional file 1). These results suggest that 4T1 tumor cells have either various mutant gene with different proportions of mutation or genes with no mutation, similar to normal cells.

Table 1. Immunogenic 4T-1 mutation percentage

Gene	Mutated sequence	Substitution (WT, AA#, Mut)	Reactive T cell subtype	Mutation percentage
Gen1	IPHNPRVAVKTT**N**NLVMKNSVCLERDS	K707N	CD4	100%
Polr2a	LAAQSLGEPATQ**I**TLNTFHYAGVSAKN	M1102I	CD4	50%▼
Zfr	AHIRGAKHQKVV**T**LHTKLGKPIPSTEP	K411T	CD4	50%
Cep120	ELAWEIDRKVLHQ**N**RLQRTPIKLQCFA	H68N	CD4	50%
Malt1	FLKDRLLEDKKI**A**VLLDEVAEDMGKCH	T534A	CD4	50%
Wdr11	No mutation	-	CD8	-
Kbtbd2	No mutation	-	CD4	-
Gprc5a	FAICFSCLLAHA**L**NLIKLVRGRKPLSW	F119L	CD8	50%▲
Zzz3	KELLQFKKLKKQ**N**LQQMQAESGFVQHV	K311N	CD8	50%
Ilkap	No mutation	-	CD4	-
Cenpf	RVEKLQLESELN**E**SRTECITATSQMTA	D1327E	CD4	50%▼

(Bold text is mutated sequence site. Symbol ▲; more than, symbol ▼; below)

Neoantigen vaccine using 4T1 mutant CD4$^+$ T cell neoantigen peptides produces potent anti-tumor effects in cisplatin-treated 4T1 tumor-bearing mouse models.

To evaluate the anti-tumor effect of the neoantigen vaccine in cisplatin-treated mouse models, 4T1 murine breast tumor cells (1×10^6 per mouse) were subcutaneously inoculated into BALB/c mice. Next, the tumor-bearing mice were injected intraperitoneally 2 times with cisplatin at intervals of 3 days and were vaccinated intratumorally with mutant neoantigen peptides 5 times at intervals of 3 days (Figure 2a). The combination of cisplatin treatment and neoantigen vaccines strongly controlled 4T1 tumor growth as compared to the control groups with no vaccination or cisplatin treatment or neoantigen vaccine only (Figure 2b). In addition, the mice in groups treated with cisplatin and vaccinated with mutant neoantigen peptides survived for more than 50 days after tumor injection, whereas all the mice treated with cisplatin alone or vaccinated with mutant neoantigen peptides alone died before 40 days (Figure 2c). We next assessed the functional activity of effective T cells by evaluating the levels of IFN-γ through ELISA after isolating splenocytes from the tumor-bearing mice. After re-stimulation with mutant neoantigen peptides for 1 or 2 days, the levels of IFN-γ were significantly increased in groups vaccinated with mutant neoantigen peptides as compared to control groups (Figure 2d). It is concluded that neoantigen vaccines using mutant neoantigen peptides can improve the immunogenicity of tumors for anti-tumor effects in a mouse model undergoing cisplatin treatment.

$CD4^+$ T cell-reactive mutant neoantigen peptide induces an immunogenic response and anti-tumor effect in cisplatin-treated mouse model.

We next confirmed that the use of mutant type neoantigen peptides reactive to $CD4^+$ T cells was more efficient compared to the use of wild type antigens in cisplatin-treated mouse models. First, 4T1 tumor cells (1×10^6 per mouse) were subcutaneously injected into the BALB/c mice which were then treated intraperitoneally with cisplatin, followed by vaccination with $CD4^+$ T cell wild type or mutant type antigen peptide (Figure 3a). The mice treated with cisplatin and vaccinated with mutant $CD4^+$ T cell neoantigen peptide suppressed the growth of 4T1 tumor significantly as compared to control groups with no vaccination or treated with wild type $CD4^+$ T cell antigen peptide (Figure 3b). In addition, the 4T1 tumor-bearing mice treated with cisplatin and vaccinated with mutant $CD4^+$ T cell neoantigen peptide survived for at least 60 days, but all the mice in control groups treated with cisplatin alone or vaccinated with wild-type $CD4^+$ T cell antigen peptide alone died within 45 days (Figure 3c). To evaluate the functional activity of effective T cells in tumor-bearing mice, their splenocytes were isolated and the levels of IFN-γ were measured by ELISA after re-stimulation with wild type or mutant type $CD4^+$ T cell neoantigen peptide. The levels of IFN-γ were potentially increased in the mice treated with cisplatin and vaccinated with mutant $CD4^+$ T cell neoantigen peptide compared to the mice with no vaccination or vaccinated with wild-type $CD4^+$ T cell antigen peptide (Figure 3d). In short, mutant neoantigen peptides reactive to $CD4^+$ T cells have the potential to exert anti-tumor effects and to activate effective T cells in the cisplatin-treated mouse models.

The mutant $CD4^+$ and $CD8^+$ T cell neoantigen peptide has increased anti-tumor effects in cisplatin treated mouse models.

As shown, therapeutic effects and immunogenic responses have been established in the cisplatin-treated mouse models by vaccination with mutant $CD4^+$ T cells or $CD8^+$ T cell neoantigen peptides (Fig. 2)., We next analyzed the increased anti-tumor effects of the neoantigen vaccine with 2 types of T cell mutant neoantigen peptides at the same time. First, 4T1 tumor cells (1×10^6 per mouse) were injected subcutaneously into BALB/c mice, which were then treated with cisplatin twice with an interval of 3 days. Then, mutant $CD4^+$ T cell neoantigen peptide and/or mutant $CD8^+$ T cell neoantigen peptide was used to vaccinate the mice 5 times at intervals of 3 days. The mice were sacrificed 1 week after the last vaccination, and their splenocytes were then isolated to evaluate the levels of IFN-γ (Fig. 4a). Neoantigen vaccination with two types of T cell mutant neoantigen peptides significantly suppressed tumor growth compared to neoantigen vaccination with either mutant $CD4^+$ T cells or $CD8^+$ T cell neoantigen peptides alone in the cisplatin-treated mice (Fig. 4b). In addition, long-term survival was observed in the cisplatin-treated mice vaccinated with both mutant $CD4^+$ and $CD8^+$ T cell neoantigen peptides compared to the cisplatin-treated mice vaccinated with either mutant $CD4^+$ or $CD8^+$ T cell neoantigen peptides alone (Fig. 4c). To evaluate the functional activity of effective T cells in the tumor-bearing mice, their splenocytes were isolated and the levels of IFN-γ were measured by ELISA after re-stimulation with

mutant $CD4^+$ and/or $CD8^+$ T cell neoantigen peptides. The levels of IFN-γ were mostly increased in the cisplatin-treated mice vaccinated with both $CD4^+$ and $CD8^+$ T cell neoantigen peptides compared to the cisplatin-treated mice vaccinated with either mutant $CD4^+$ or $CD8^+$ T cell neoantigen peptide alone (Fig. 4d). These results suggest that the two types of T cell-activating neoantigen peptides synergistically contribute towards significantly increased anti-tumor and immunogenic responses.

Combination of neoantigen vaccination and immune checkpoint inhibitor has synergistic therapeutic effects in cisplatin-treated mouse model.

The therapeutic potential of immune checkpoint blockade is to induce synergistic anti-tumor effects through effective T cell immune responses. To improve the therapeutic effect and immunogenic response, an immune checkpoint inhibitor was used together with the neoantigen vaccine in the cisplatin-treated mouse models. First, 4T1 tumor cells (1×10^6 per mouse) were injected subcutaneously into BALB/c mice, which were then treated with cisplatin twice, with an interval of 3 days in between. Then, the mutant $CD4^+$ T cell neoantigen peptides were injected intratumorally in the mice 5 times at intervals of 3 days, and anti-PD-L1 was injected intraperitoneally in mice 6 times at intervals of 2 days (Fig. 5a). The combination of neoantigen vaccine and anti-PD-L1 synergistically inhibited tumor growth compared to the neoantigen vaccine or anti-PD-L1 alone in the cisplatin-treated mice (Fig. 5b). Moreover, the cisplatin-treated 4T1 tumor-bearing mice in the group vaccinated with neoantigen peptide and immune checkpoint inhibitor survived for more than 70 days; whereas, the mice in the group vaccinated with neoantigen peptide alone died before 60 days (Fig. 5c). The levels of IFN-γ were significantly higher in the cisplatin-treated mice vaccinated with neoantigen and anti-PD-L1 compared to the cisplatin-treated mice vaccinated with neoantigen alone (Fig. 5d). In conclusion, the combination of neoantigen vaccine and immune checkpoint inhibitor contributed to improved cancer immunotherapy with synergistic anti-tumor effects in the cisplatin-treated mouse models.

Discussion

In this study, we identified 11 genes in the 4T1 cancer cell line and selected eight mutant neoantigen peptides to be used in our experiments based on previous paper[5]. We confirmed the anti-cancer effect of the neoantigen vaccine with an immune checkpoint blockade antibody in cisplatin-treated mouse models. After chemotherapy on the 4T1 mouse breast cancer cells in mice, mutant neoantigen peptides that reactivate $CD4^+$ or $CD8^+$ T cells against tumors, and anti-PD-L1 that inhibits the expression of PD-L1 on tumor cells, were used to suppress the growth of tumors and to increase long-term survival. Furthermore, the levels of cytokine IFN-γ secreted from activated and effective T cells were measured after stimulation with mutant neoantigen peptides. In our experiments, we confirmed that mutant $CD4^+$ T cell neoantigen peptides have significant therapeutic effects by helping and improving not only the activation of $CD8^+$ T cells but also the overall immune reaction in the tumor microenvironment through $CD4^+$ T cells.

With the increasing number of cancer patients and cancer-related mortality, various therapeutic methods have been investigated to decrease side effects and improve cancer immunotherapy, including CAR-T cell therapy, neoantigen vaccination, and immune checkpoint blockade[26]. Recently, neoantigen vaccines have been spotlighted because of their efficiency, since they are personalized for each cancer patient by analyzing the genome in their tumor cells[27, 28]. In fact, mass production of tumor neoantigens has been available for tumors that have their genomic information disclosed. In addition, the combination of several tumor neoantigens could induce a more efficient immune response. Moreover, neoantigen vaccines might be effective when co-administrated with immune checkpoint inhibitors that are used in clinical trials to overcome the increase in TMB of proliferating tumor cells.

Various animal models, such as mice or rats, are utilized in experiments to prove the efficiency of neoantigens and cancer cell lines that are distributed worldwide for the development of cancer research. Even though cancer cell lines are derived from the same organ, the proportion of mutations in genes for each cancer cell might be different and should be confirmed before using neoantigen vaccines. As shown in Fig. 1, we identified, through direct sequence analysis, a mutation in the genomic sequence that varied depending on gene copy number variation (CNV) in mouse breast cancer cell line 4T1. For use in our experiments, we made customized neoantigen peptides based on immunogenicity and the occurrence of tumor-specific mutations in varied proportions. Moreover, we confirmed the synergistic tumor treatment effects and long-term survival in cisplatin-treated mouse models treated with the neoantigen vaccine and anti-PD-L1 as an immune checkpoint inhibitor.

Although the combination of multiple cancer immunotherapies has shown increased immunogenic response and anti-cancer therapeutic effects in our experiments, there is a need for advanced research for potentially stronger suppression of tumor growth and better long-term survival. In the future, the methods used in our experiments could be applied to the investigation of noble adjuvants, the discovery of neoantigens, and the development of immune checkpoint blockade antibodies for non-clinical studies. In conclusion, a system needs to be developed to prove the immunogenicity as well as the tumor treatment effect of newly identified neoantigens.

Materials And Methods

Mice and cell

BALB/c mice were purchased from Orient Bio (Seongnam, South Korea) and were kept under pathogen-free conditions. Female mice at 6–8 weeks of age were used in the experiments, according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) of Konkuk University.

A mouse breast cancer cell line, 4T-1, was incubated in RPMI-1640 medium (Biowest, France) supplemented with 10% fetal bovine serum (Biowest, France) and 50 U/mL penicillin streptomycin (Biowest, France) at 37°C and 5% CO_2.

Total RNA isolation and cDNA synthesis

For isolation of RNA from 4T1 mouse breast cancer cells, the cells were collected and centrifuged at 1,600 rpm. Then, pellets were resuspended in 1 mL TRIzol Reagent (Invitrogen, USA) and incubated for 5 min. This was followed by addition of 0.2 mL chloroform (Sigma-Aldrich, Germany) and incubation for 3 min. After centrifugation at 12,000 rpm for 15 min, the top layer of the aqueous phase was collected. Then, RNA was precipitated by adding 0.5 mL isopropanol and incubating for 10 min followed by centrifugation at 12,000 rpm. Pellets were washed with 1 mL 75% ethanol and centrifuged at 12,000 rpm. After evaporating the ethanol, 20 µl RNase-free water (Qiagen) was added to the pellet and heated at 55°C for 5 min. Then, cDNA (Complementary DNA) was synthesized from total RNA using a real-time PCR (Polymerase Chain Reaction) kit (Promega, USA) and amplified by a PCR kit (NanoHelix, South Korea) based on neoantigen gene primers. (The detailed primer sequences are provided in Additional file 2).

Direct sequence analysis

Gel electrophoresis was performed using 1% agarose gel (DYNEBIO INC., South Korea) and a single band at 300–600 bp (base pair) was detected by Core Bio i-MAX™ gel image analysis system (Corebiosystem, South of Korea). DNA was extracted using a gel extraction kit (DYNEBIO INC, South Korea) and the mutation in the sequence was analyzed by Cosmogenetech (Seoul, South Korea). The proportion of mutation in base pairs was evaluated as follows: 100%, a single peak; 50%, double peaks including mutated and non-mutated in equal degrees; and 0%, a peak with no mutation. In addition, >50% indicated double peaks including high proportion of mutated sequences and <50% indicated double peaks including less proportion of mutated than non-mutated sequences.

Neoantigen peptide synthesis

All neoantigen peptides used in our experiments were custom-made by Anygen (Gwangju, South Korea). The genes and their peptide sequences are provided as follows[5]: Mutant *Gen1* (IPHNPRVAVKTTNNLVMKNSVC LERDS), Mutant *Polr2a* (LAAQSLGEPATQITLNTFHYAGVSAKN), Wild type *Zfr* (AHIRGAKHQKVVKLH TKLGKPIPSTEP), Mutant *Zfr* (AHIRGAKHQKVVTHTKLGKPIPSTEP), Mutant *Cep120* (ELAWEIDRKVL HQNRLQRTPIKLQCFA), Mutant *Malt1* (FLKDRLLEDKKIAVLLDEVAEDMGKCH), Mutant *Wdr11* (NDE PDLDPVQELIYDLRSQCDAIRVTKA), Mutant *Kbtbd2* (DAAALQMIIAYAYRGNLAVNDSTVEQL), Mutant *Gprc5a* (FAICFSCLLAHALNLIKLVRGRKPLSW), Mutant *Zzz3* (KELLQFKKLKKQNLQQMQAE SGFVQH V), Mutant *Ilkap* (RKGEREEMQDAHVSLNDITQECNPPSS), Mutant *Cenpf* (RVEKLQLESELNE SRTECITA TSQMTA).

In vivo tumor treatment experiments.

For tumor treatment experiments *in vivo*, 4T-1 cells ($1x10^6$ per mouse) were injected subcutaneously into BALB/c mice (6 per group). Doses of 5 mg/kg cisplatin (Sigma-Aldrich, Germany) were administered via intraperitoneal injection on days 12 and 15. Doses of 20 µg (per mouse) neoantigen peptide were injected intratumorally on days 13, 16, 19, 22, and 25. Doses of 100 µg (per mouse) anti-PD-L1 (clone 10 F.9G2) antibodies (BioXcell, USA) were injected intraperitoneally on days 18, 20, 22, 24, 27, and 29. Tumor sizes were measured twice a week and tumor masses were calculated using the formula (length × $width^2$)/2.

Euthanasia was performed when the size of the tumor exceeded 10% of the weight of the mouse or was 2 cm or more. Euthanasia proceeds with CO2 gas (injection at a rate of slowly filling the euthanasia chamber at a rate of 10-20% per minute).

Enzyme-linked immunosorbent assay (ELISA).

For *in vivo* cytokine analysis, splenocytes were harvested from the tumor-bearing BALB/c mice, one week after the last peptide or antibody injection. The cells were then treated with ACK(Ammonium-Chloride-Potassium) solution (Quality Biological, Gaithersburg, MD, USA) for red blood cell lysis. The splenocytes were then incubated with neoantigen peptide. After incubation for 24–48 h, the supernatants were harvested and assessed for IFN-γ cytokine levels using a mouse IFN gamma ELISA kit (Invitrogen, USA) following the manufacturer's recommendations.

Statistical analysis

The t-tests used represent statistical significance as follows: *P<0.01; **P<0.05; ***P<0.001. All experiments were performed three times independently, and IBM (**I**nternational **B**usiness **M**achines Co.) SPSS (**S**tatistical **P**ackage for the **S**ocial **S**ciences) Statistics Base 22.0 was used as a statistical tool to analyze the differences between the groups in survival experiments.

Declarations

Ethics approval and consent to participate

All animal studies are approved by Konkuk University's Institutional Animal Care Use Committee (IACUC, KU20031)

Consent to publish

In the name of all the authors I give my full consent for publication.

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

SEL, GYJ, HDH, YMP and THK designed the experiments. SEL, GYJ and JWL carried out the experiments. SEL, GYJ and THK analyzed the data and wrote the manuscript. HDH verified the statistical methods. All authors provided critical feedback and contributed to the final manuscript. THK supervised the project.

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Figures

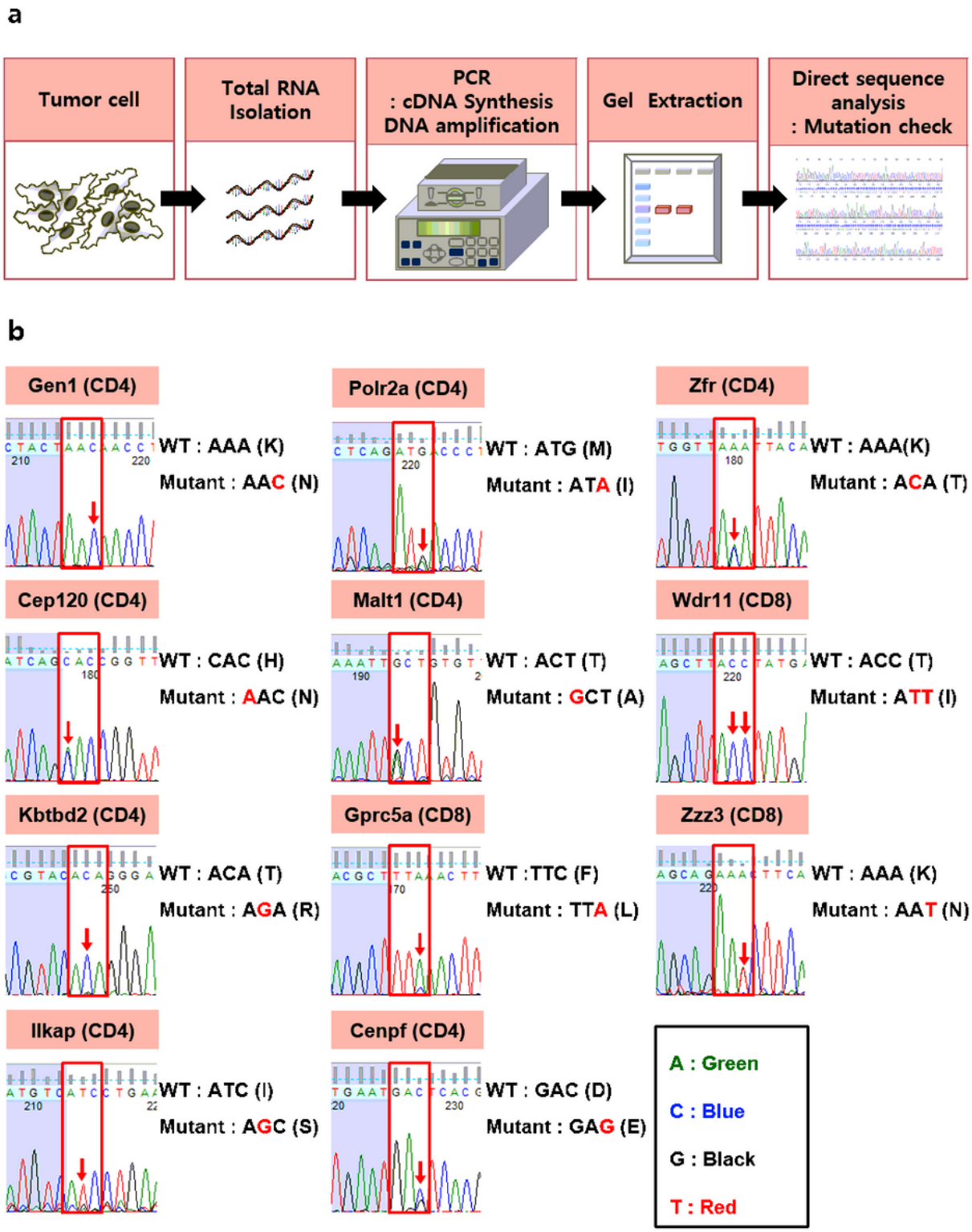

Figure 1

The identification of mutated 4T1 gene sequence

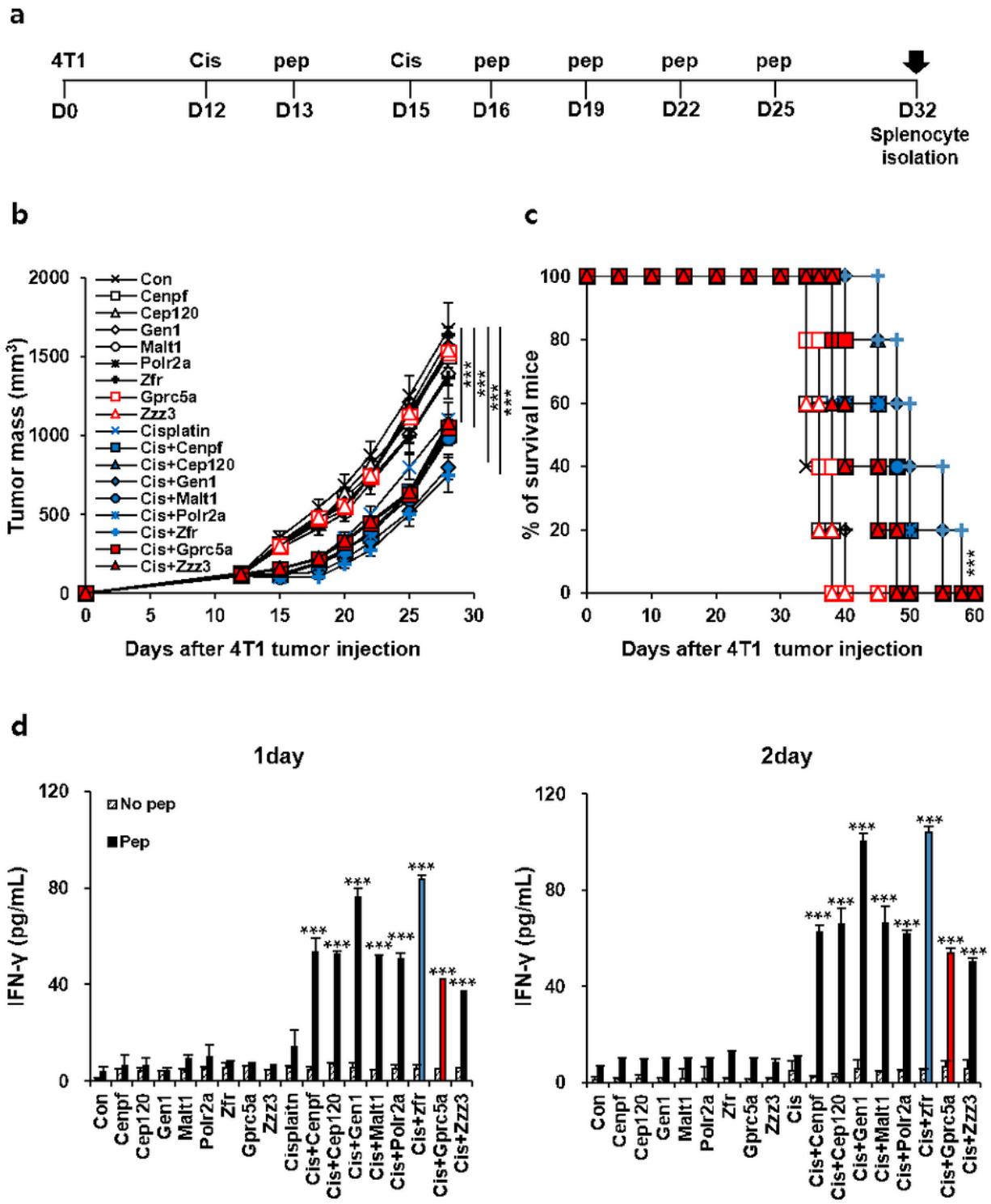

Figure 2

Tumor treatment effects of neoantigen vaccine in cisplatin-treated mouse model

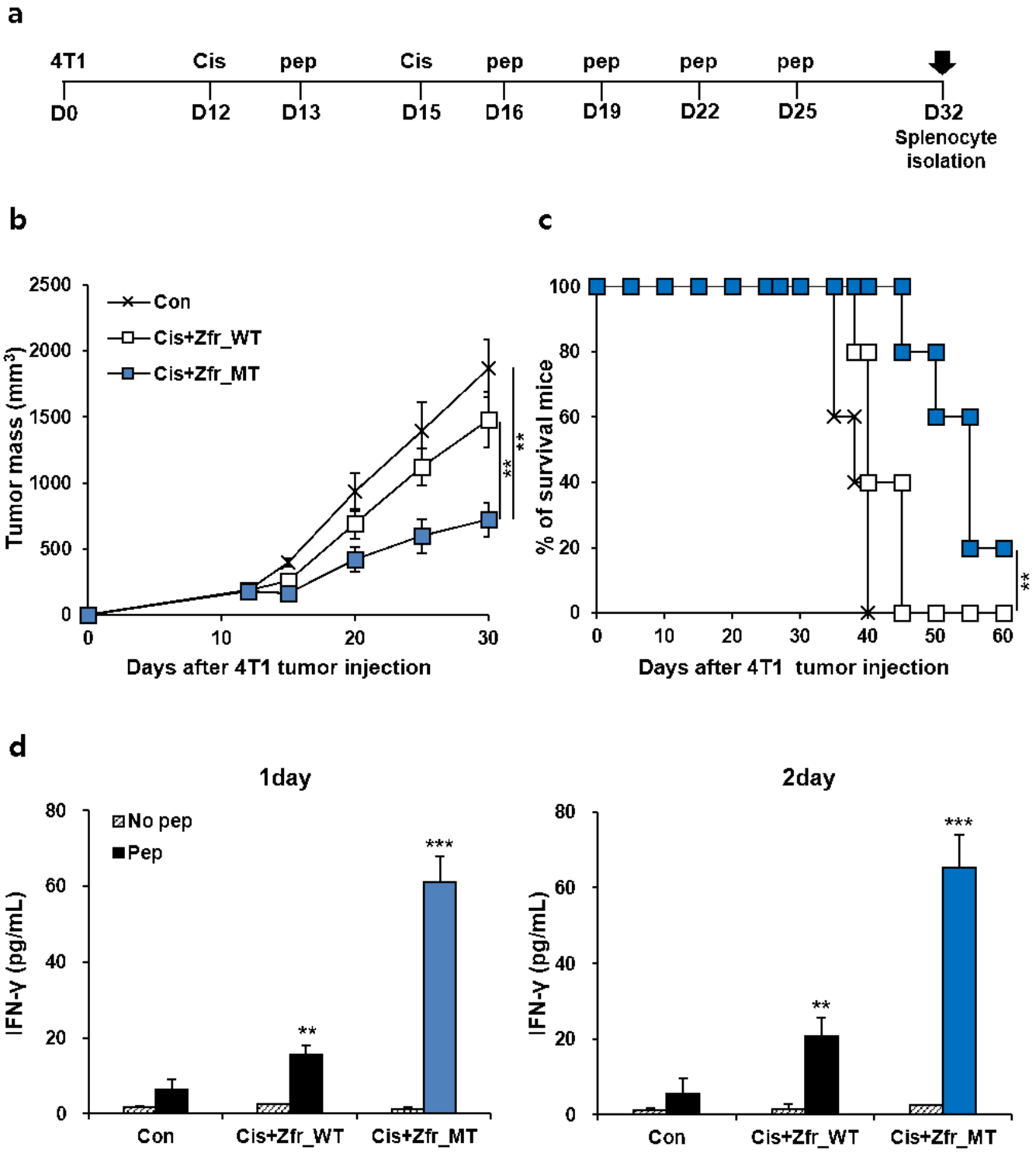

Figure 3

Neoantigen vaccine tumor-treatment effects using mutant CD4+ T cell neoantigen peptide in cisplatin-treated mouse model

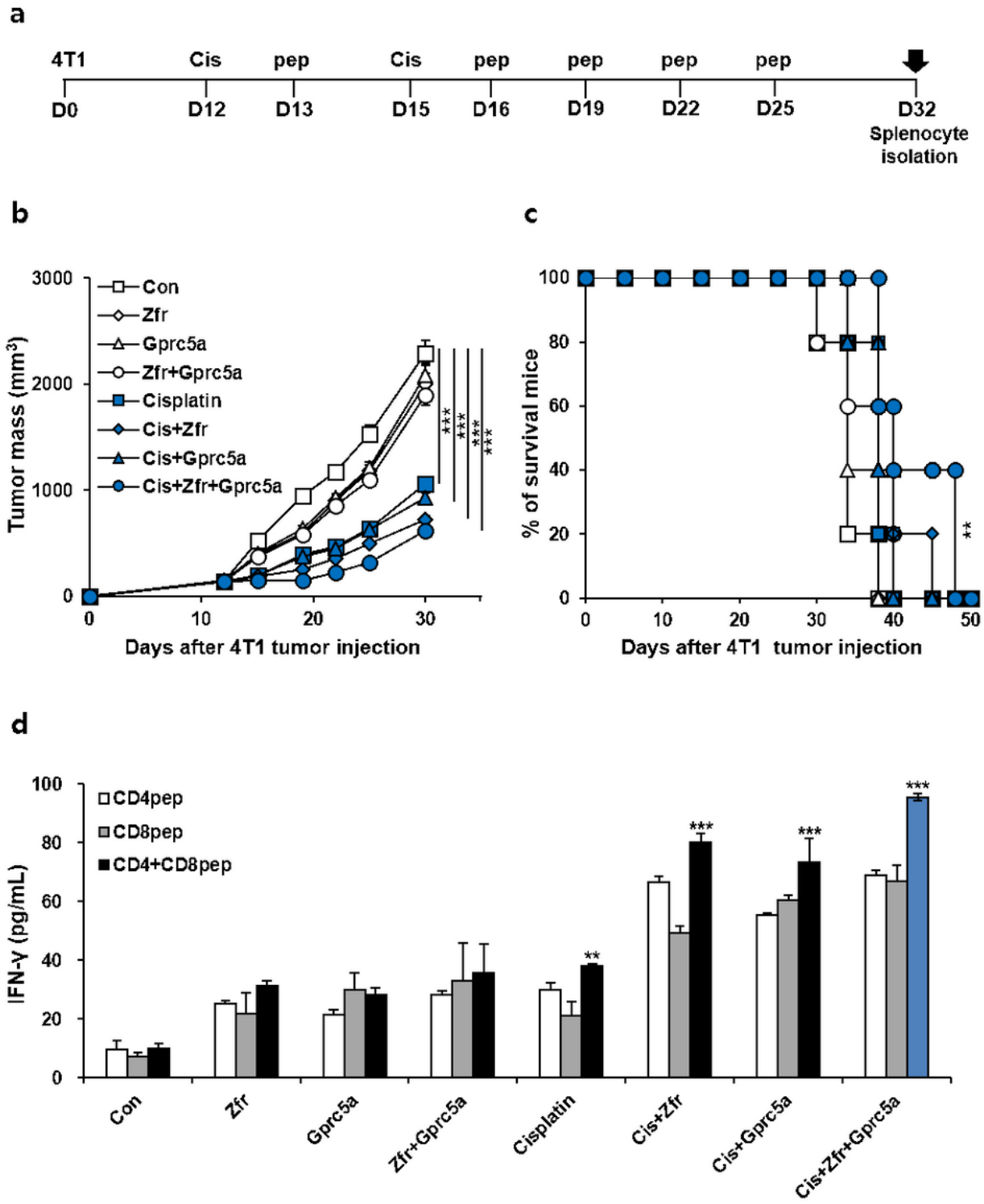

Figure 4

Neoantigen vaccine tumor-treatment effects in mutant CD4+ or CD8+ T cells in cisplatin-treated mouse model

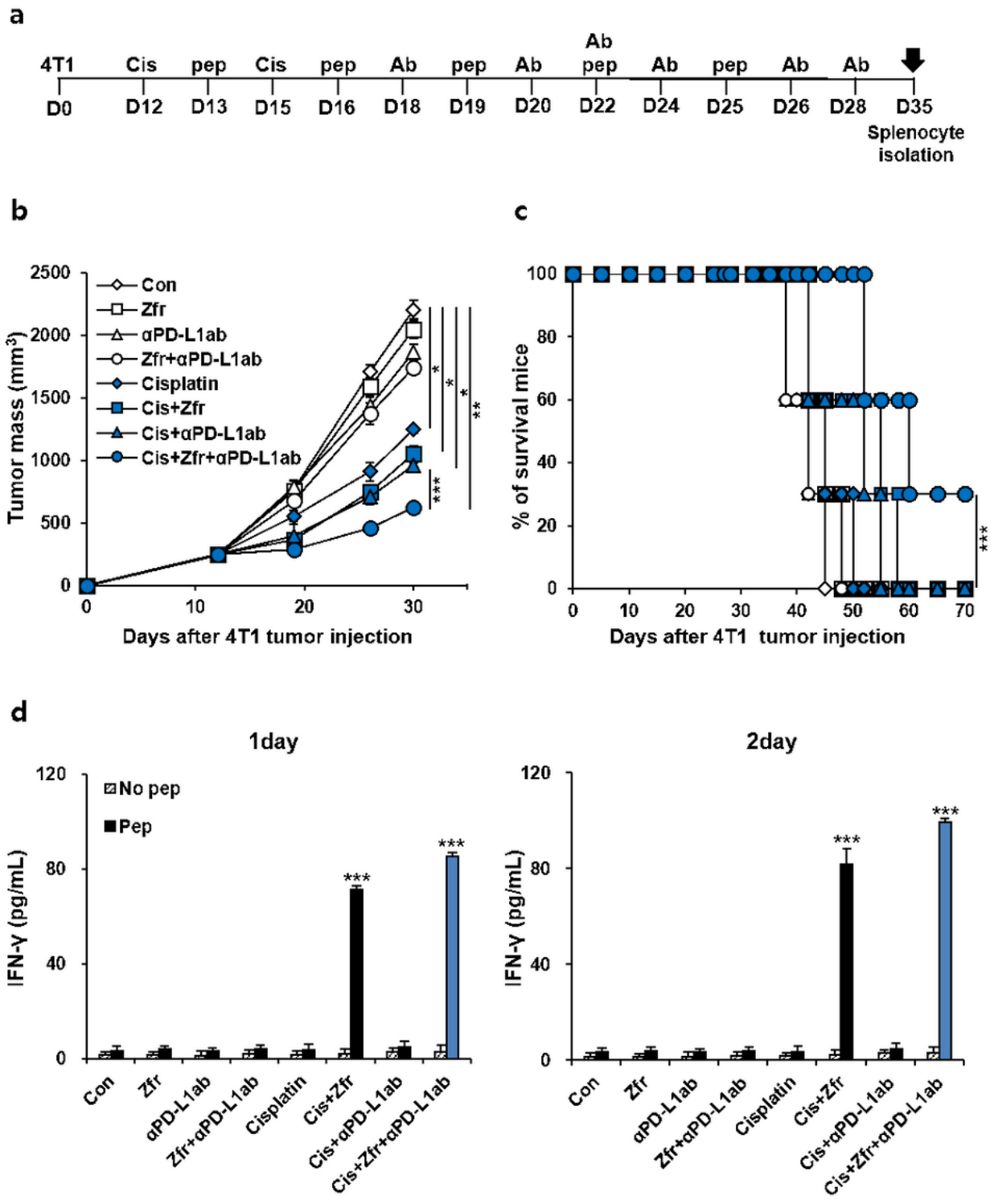

Figure 5

Combinational tumor treatment effects of neoantigen vaccine and immune checkpoint inhibitor in cisplatin-treated mouse model.

Supplementary Files

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- Arriveguidelines.pdf
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