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# Toll-like receptor 2 costimulation potentiates the antitumor efficacy of CAR T Cells. Postprint

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## Abstract

Chimeric antigen receptor (CAR) T-cell immunotherapies have shown unprecedented success in treating leukemia but limited clinical efficacy in solid tumors. Here, we generated 1928zT2 and m28zT2, targeting CD19 and mesothelin, respectively, by introducing the Toll/interleukin-1 receptor domain of Toll-like receptor 2 (TLR2) to 1928z and m28z. T cells expressing 1928zT2 or m28zT2 showed improved expansion, persistency and effector function against CD19+ leukemia or mesothelin+ solid tumors respectively in vitro and in vivo. In a patient with relapsed B-cell acute lymphoblastic leukemia, a single dose of 5 * 104/kg 1928zT2 T cells resulted in robust expansion and leukemia eradication and led to complete remission. Hence, our results demonstrate that TLR2 signaling can contribute to the efficacy of CAR T cells. Further clinical trials are warranted to establish the safety and efficacy of this approach.Leukemia advance online publication, 25 August 2017; doi:10.1038/leu.2017.249.

## Full Text

## Preamble

### Toll-like Receptor 2 Costimulation Potentiates the Antitumor Efficacy of CAR T Cells

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**One Sentence Summary:** The innate immune receptor TLR2, incorporated into CARs, enhanced the efficacy of CAR T cells in eliminating leukemia and solid tumors.

## Abstract

Chimeric antigen receptor (CAR) T cell immunotherapies have shown unprecedented success in treating leukemia but limited clinical efficacy in solid tumors. Here, we generated 1928zT2 and m28zT2, targeting CD19 and mesothelin, respectively, by introducing the Toll/interleukin-1 receptor (TIR) domain of Toll-like receptor 2 (TLR2) to 1928z and m28z. T cells expressing 1928zT2 or m28zT2 showed improved expansion, persistency and effector function against CD19+ leukemia or mesothelin+ solid tumors respectively in vitro and in vivo. In a patient with relapsed B cell acute lymphoblastic leukemia, a single dose of 5×10 /kg 1928zT2 T cells resulted in robust expansion and leukemia eradication and led to complete remission. Hence, our results demonstrate that TLR2 signaling can contribute to the efficacy of CAR T cells. Further clinical trials are warranted to establish the safety and efficacy of this approach.

## Introduction

The success of the adoptive transfer of CD19-specific chimeric antigen receptor (CAR) T cells represents a major step in cancer immunotherapy. Both CD3 and costimulatory domains are needed to generate effective CARs that provide the signals necessary for full activation, expansion and survival of CAR T cells. The incorporation of costimulatory molecules CD28, 4-1BB, OX-40, CD27 and ICOS significantly improves the expansion, cytokine production, in vivo persis-

tence and antitumor efficacy of CAR T cells. Moreover, the efficacy of CAR T cells for solid tumors is substantially poorer than that for leukemia. Given the few costimulatory molecules that have been used in the design of CARs, novel intracellular signals are needed to improve their efficacy so as to effectively treat not only leukemia but also solid tumors.

The intracellular signaling domains of CARs mainly derive from costimulatory receptors expressed on T cells that are critical for their adaptive immune responses. Nevertheless, the innate immune receptor Toll-like receptor 2 (TLR2) is highly expressed on activated and memory T cells, and TLR2 signaling increases T cell expansion and cytokine production, lowers the activation threshold for costimulatory signals delivered by antigen presenting cells (APCs), facilitates the generation of memory CD8 T cells, and directly triggers Th1 effector functions. In addition, TLR2-mediated signaling can abolish the suppressive capacities of regulatory T (Treg) cells. Furthermore, T cell hyporesponsiveness with impaired IFN- and IL-2 production can partially be accounted for by diminished expression of TLR2 on T cells. Strikingly, TLR2 ligands induce the regression of established leukemia and solid tumors through compromising the suppressive function of Foxp3+ Treg cells and enhancing the cytotoxicity of tumor-specific T cells. TLR2 can form heterodimers with TLR1 or TLR6 to initiate a proinflammatory response. However, TLR2 may also form weak homodimers, which allow it to directly bind to MyD88. Therefore, we proposed that TLR2 signaling could potentiate the antitumor activity of CAR T cells.

To test our hypothesis, we generated CARs targeting CD19 (1928z) and mesothelin (m28z) by linking scFv with CD28 and CD3 signaling domains and then generated 1928zT2 or m28zT2 by adding the TIR domain of TLR2 to the 3' end of CD3 . We compared the functions of CAR T cells with or without the TLR2 signaling domain and found that the addition of a TLR2 domain led to enhanced antitumor responses for both leukemia and solid tumors. To test the efficacy and safety of TLR2 construct in humans, we conducted a pilot trial in a patient with relapsed B cell acute lymphoblastic leukemia (B-ALL) with 1928zT2 T cells and found that a single dose (5×10 /kg) of 1928zT2 T cells led to complete remission. Our data revealed that the innate immune receptor TLR2, in addition to canonical costimulatory receptors, can improve the antitumor efficacy of CARs for both leukemia and solid tumors.

## Results

### TLR2 Enhanced the Effector Functions of CD19-Specific CAR T Cells In Vitro

The CD19-specific second-generation CARs have generated exciting results in clinical trials. We used 1928z, a CAR-linking FMC63-scFv, CD28 transmembrane and endodomain, and the CD3 signaling domain as benchmarks to evaluate TLR2 function in CARs. The TIR domain (amino acid 639-784) of TLR2 was appended to the CD3 signaling domain. Lentiviral vectors that contained

GFP (negative control), 1928z and 1928zT2 (Supplementary Figure 1a [Figure 1: see original paper]) were transfected into T cells to generate GFP T, 1928z T and 1928zT2 T cells, respectively. The transduction efficiencies were measured by the percentages of GFP+ cells (Supplementary Figure 1b). To confirm the role of GFP in representing CAR expression, we detected the coexpression of GFP and anti-CD19 CAR (Supplementary Figure 1c). K562, K562-CD19, NALM6 and REH cells were tagged with both GFP and luciferase (GL). K562-CD19-GL, NALM6-GL, and REH-GL cells, but not K562-GL cells, were specifically killed by 1928z T and 1928zT2 T cells, and notably, the killing percentages of 1928zT2 T cells were higher than those of 1928z T cells at low effector to target (E/T) ratios (Figure 1a). Expression of GZMB, PRF1 and IFNG were higher in 1928zT2 T cells compared to 1928z T cells upon stimulation by NALM6 cells (Supplementary Figure 2 [Figure 2: see original paper]). Consistently, 1928zT2 T cells secreted significantly higher amounts of IL-2, IFN- and GM-CSF, but a lower level of TNF- (Figure 1b). Interestingly, suppression of TNF- secretion from macrophages by TLR2 agonist has been reported. In addition, the capacities of 1928zT2 T cells to expand upon serial stimulations and to continuously eliminate target cells were higher than 1928z T cells (Figure 1c). Moreover, the killing capacity of 1928zT2 T cells was enhanced compared with 1928BBz T cells (Supplementary Figure 3 [Figure 3: see original paper]). Taken together, the incorporation of the TLR2 signaling domain into 1928z led to enhanced anti-tumor effector functions in vitro.

**1928zT2 T Cells Exhibited Enhanced Antitumor Efficacy In Vivo**

Next, the killing capacities of 1928z T and 1928zT2 T cells were compared in cell line-derived xenografts. Immunodeficient NSI mice were intravenously injected with 1×10 NALM6-GL cells followed by two infusions of 5×10 GFP T, 1928z T or 1928zT2 T cells. Bioluminescence imaging (BLI) showed that a reduced tumor burden was present in mice infused with 1928zT2 T cells compared with 1928z T cells (Figure 2a). Consistently, the percentages of NALM6-GL cells in the peripheral blood mononuclear cells (PBMC) were significantly lower in mice treated with 1928zT2 T cells compared with 1928z T cells (Figure 2b). In addition, the mice treated with 1928zT2 T cells exhibited delayed weight loss (Supplementary Figure S4a). Furthermore, mice treated with 1928zT2 T cells showed enhanced secretion of IL-2, IFN- and GM-CSF, but not TNF- , in serum (Supplementary Figure 4b [Figure 4: see original paper]). To confirm the improved efficacy of the TLR2 construct in terms of survival, 2×10 NALM6 cells, and two days later, 2×10 GFP T, 1928z T, 1928zT2 T or no T cells, were intravenously injected into each NSI mice (n=10). 1928zT2 T cells improved the survival of NALM6 burdened mice better than 1928z T cells (Figure 2c). The percentages of NALM6 cells and T cells in BM when mice died (Supplementary Figure 4c) indicated that the major cause of death was high NALM6 burden for mice treated with no T, GFP T and 1928z T cells, but was graft-versus-host disease (GVHD) for mice treated with 1928zT2 T cells as severe symptoms such as loss of hair and weight were observed. Thus, 1928zT2 T cells were more

potent than 1928z T cells at killing NALM6-GL cells in vivo.

Furthermore, the efficacies of 1928z T and 1928zT2 T cells were compared in patient-derived xenograft (PDX) models. B-ALL cells were eliminated by 1928zT2 T cells, but not by 1928z T cells, and the percentages of total human T cells and CAR T cells were higher in 1928zT2 group compared with 1928z group (Supplementary Figure 5a [Figure 5: see original paper]). Due to the less aggressiveness of primary B-ALL cells, all the B-ALL burdened mice including the GFP T group survived until when the mice were culled for analysis on day 78, and splenomegaly was found only in the GFP and 1928z groups, but not in the 1928zT2 group (Supplementary Figure 5b). Collectively, these results demonstrated that 1928zT2 T cells had significantly higher anti-leukemia efficacy than 1928z T cells in vivo.

### TLR2 Costimulation Upregulated Genes Associated with Cell Adhesion, Synaptic Transmission, T Cell Migration and mTOR Signaling

To decipher the molecular mechanisms of TLR2 costimulation, we performed RNA sequencing to compare the transcriptomes of 1928z T and 1928zT2 T cells with or without stimulation by NALM6 cells. Approximately 11013 of the 33382 RefSeq genes were detectably expressed in all four populations. We identified the genes specifically upregulated in stimulated 1928zT2 T cells and found that most of these genes were related to cell adhesion and synaptic transmission (Figure 3a). Quantitative real-time PCR confirmed the upregulation of genes associated with cell adhesion and synaptic transmission, along with two genes IRF3 and IRF7 downstream of the TLRs pathway (Figure 3b). TLR2 signaling has been reported to enhance mTOR signaling and expression of T-bet. Here, we also detected elevated transcription of T-bet, mTOR, 4E-BP1 and P70S6K (Figure 3b). Matrix metallopeptidases (MMPs) have been shown to play critical roles in T cell activation and migration into tissues. TLR2 incorporation also induced the upregulation of MMP2 and MMP24 expression in 1928zT2 T cells (Figure 3a and 3b). Therefore, the migration capacities of GFP T, 1928z T and 1928zT2 T cells were compared and results indicated that 1928zT2 T cells transmigrated the matrigel more efficiently than 1928z T cells (Figure 3c). These results suggest that TLR2 incorporation enhances the antitumor efficacy of CAR T cells partly through improved adhesion, synaptic transmission and migration capacities, and enhanced T-bet expression through mTOR pathway.

### TLR2 Costimulation Enhanced Antitumor Efficacy of Mesothelin-Specific CAR T Cells

Mesothelin is highly expressed in various solid tumors including adenocarcinoma cells, and clinical trials of mesothelin-targeting CAR T cells are ongoing (such as NCT02414269 and NCT02930993). Thus, to assess the effect of TLR2 costimulation on CAR T cells targeting solid tumors, we incorporated TLR2 into a CAR specific to mesothelin and generated GFP T, m28z T and m28zT2 T cells through lentiviral transfection (Supplementary Figure 6a [Figure 6: see origi-

nal paper] and 6b). m28zT2 T cells showed enhanced killing capacity against mesothelin+ A549-GL cells compared with m28z T cells (Figure 4a). m28zT2 T cells secreted more IL-2, IFN- and GM-CSF, but reduced TNF- compared with m28z T cells upon stimulation by A549 cells (Figure 4b). The expansion of m28zT2 T cells during repetitive stimulation was improved compared with m28z T cells, and the potency of m28zT2 T cells at killing A549 cells were significantly higher than m28z T cells (Supplementary Figure 7a [Figure 7: see original paper]). Meanwhile the exhaustion markers on T cells after repetitive coculture were compared, and the expression of TIM-3, but not PD-1 or LAG-3, was lower on m28zT2 T cells relative to m28z T cells (Supplementary Figure 7b). These results indicated that TLR2 incorporation in mesothelin-specific CAR also improved its effector functions in vitro.

We further compared the antitumor efficacy of m28zT2 T, m28z T and GFP T cells in subcutaneous A549-GL xenografted mice. Tumors from m28zT2 group were smaller than those from the blank, GFP and m28z groups (Figure 4c). Moreover, mice treated with m28zT2 T cells produced more cytokines IL-2, IFN- and GM-CSF, but not TNF- , than those treated with m28z T cells (Supplemental Figure 8 [Figure 8: see original paper]). Since metastasis is a major hurdle in the treatment of solid tumors, we compared the efficacies of m28z T and m28zT2 T cells against metastasized tumors through the blood in intravenous A549-GL xenografted mice. The BLI results showed that m28zT2 T cells efficiently inhibited the growth of A549-GL cells in NSI mice; however, surprisingly, the mice grew a greater burden of tumors when treated with m28z T cells than the mice that were injected with GFP T cells (Supplementary Figure 9a [Figure 9: see original paper]). The histologic analysis confirmed that the tumor cells in the uterus of the mice from the m28z group were HLA+ A549-GL cells (Supplementary Figure 9b). These results indicated that TLR2 incorporation could enhance the efficacy of mesothelin-specific CAR T cells against solid tumors.

### Clinical Trial of 1928zT2 T Cells in a Patient with Relapsed and Refractory B-ALL

We conducted a pilot clinical trial in a patient with relapsed and refractory B-ALL (NCT02822326). 1928zT2 T cells were produced and well characterized phenotypically and functionally (Supplementary Figure 10a [Figure 10: see original paper]-f). The patient received two days of treatment with Fludarabine (30 mg/$m^2$/day) and cyclophosphamide (300 mg/$m^2$/day) for lymphocyte depletion, followed by one dose of 1928zT2 T cells at 5×10  cells/kg (Supplementary Figure 11a [Figure 11: see original paper]). After infusion of 1928zT2 T cells, the patient experienced side effects including fever, coughing, skin rashes and hypotension, but not neurological defects. During this trial, the symptoms of the patients did not necessitate ICU stay, vasopressor support or any steroids treatment. A marked elevation of serum IL-6 was detected on day 2 and tocilizumab, an anti-IL-6R monoclonal antibody, was administered at 4 mg/kg,

which resulted in rapid defervescence and complete reversal of symptoms over a period of 4 days; however, the patient' s concentration of C-reactive protein (CRP) fluctuated in another pattern, and the highest peak on day 45 was due to urological infection (Supplementary Figure 11b). In addition, we monitored the dynamic changes of other cytokines (Supplementary Figure 11c). B-ALL cells comprised approximately 15% of the PBMC and more than 70% of the BM before treatment (Figure 5a), but decreased to an undetectable level on day 22 (Figure 5b). Complete remission (CR) was formally diagnosed on day 51 (Figure 5c). Percentage changes of 1928zT2 T cells and CD19+ B-ALL cells in PBMC (Figure 5d) and BM (Figure 5e) were depicted. On day 22, the percentages of CD4+CD8-, CD62L+CD25- and CD45RO+CD45RA- populations in 1928zT2 T cells were higher than those in wild-type T cells in both PBMC and BM (Supplementary Figure 12a [Figure 12: see original paper]). Interestingly, the elevation of CD4+ 1928zT2 T cell percentage was concomitant with the increase of the percentage of 1928zT2 T cells in both PBMC and BM (Supplementary Figure 12b and 12c). In addition, the percentages of CD25+ 1928zT2 T cells were negatively correlated with the percentages of 1928zT2 T cells in PBMC (Supplementary Figure 12d), while CD62L expression on 1928zT2 T cells in PBMC remained high until the B-ALL cells were eradicated (Supplementary Figure 12e). 1928zT2 T cells were detected cerebrospinal fluid, indicating the potential role of 1928zT2 T cells in eradicating leukemia in the central nervous system (Supplementary Figure 12f). The clinical results here indicated that small dosages of 1928zT2 T cells could kill leukemic cells with potent efficacy.

## Discussion

Although CD19-specific CAR T cell immunotherapy has achieved promising results in clinical trials, efficacy of other CAR T cells needs improvement, especially those for solid tumors. The development of CARs from the first generation (with only the CD3 signaling domain) to the second generation (with CD3 and a costimulatory domain) has revealed a critical role of costimulatory molecules in CARs. Incorporation of novel costimulatory molecules into CARs should be a feasible strategy to improve their efficacy.

Interestingly, the innate immune receptor TLR2 is expressed on activated T cells as a costimulatory receptor. Innate immunity serves as an immediate defense mechanism against pathogenic organisms. Adaptive immunity plays a delayed but more delicate role in controlling diseases and forming memories. The two types of immunity are not independent of each other and the interaction between them is critical to the outcome of overall immune response. CARs with combinatorial moieties of both adaptive and innate immunity may exert dual functions of mobilizing innate responses as well as promoting T cell expansion, antigen-specific reaction, T cell contraction and memory formation. We demonstrated that 1928zT2 and m28zT2 T cells were more potent at killing CD19+ leukemia cells and mesothelin+ lung cancer cells in vitro. TLR2 signaling in CD8 T cells can augment T-bet protein levels and IFN- , perforin and granzyme

B expression through the mTOR pathway, and contribute to the efficient clearance of intracellular pathogens, reminiscent of the results we provide here. To decipher other mechanisms of TLR2-enhanced antitumor efficacy, we found the genes upregulated by TLR2 costimulation, most of which were associated with cell adhesion, synaptic transmission and migration. Therefore, TLR2 costimulation can serve as an innate immune moiety to enhance the effector functions and promote adhesion and immune synaptic signaling between CAR T cells and target cells.

In our pilot study, only 5×10 /kg of 1928zT2 T cells expanded to a population that constituted approximately 40% of lymphocytes in patient PBMC, indicating the remarkable expansion capacity of 1928zT2 T cells. Since correlation between infused CAR T cell dose and incidence and severity of cytokine release syndrome (CRS) has been suggested, it should be beneficial to reduce the CAR T cell dose. In addition, while large numbers of CAR T cells can be generated, a reduction in CAR-T cell dose may reduce the time for producing enough CAR T cells for therapy and manufacturing cost, and provide capacity advantages, which will be important for widespread adoption of CAR-T cell therapies. Nevertheless, other patients in our clinical trial showed less severe side effects and none needed tocilizumab treatment (data unpublished). Therefore, our TLR2 construct should be potent but without increased incidence and severity of CRS. We provided an unprecedentedly detailed dynamic changes of CAR T cells and B-ALL cells in the patient, a pattern which could be reference for other patients to predict CR. However, results of our ongoing clinical trial will be needed to further determine the safety and efficacy of TLR2 co-stimulation in larger patient cohorts.

CAR T cells for solid tumors face five major challenges –trafficking, tumor recognition and killing, proliferation and persistence, counteract of microenvironment and control of CAR T cells, and the former four of which can be tackled by optimization of costimulations in CARs. In our A549-GL metastatic xenograft mouse models (Supplementary Figure 9a), the tumors in the m28z group were larger than that not only in the m28zT2 group but also in the GFP group. Conversely, we demonstrated that m28z T cells were able to kill A549-GL cells in vitro (Figure 4a) and suppress the growth of A549-GL in subcutaneous xenograft models (Figure 4c). The differences of the results from these experiments show the importance of validating the efficacies of cellular immunotherapies in multiple models. The size of tumors is determined by the combination effects of tumor growth and tumor killing in vivo. It is possible that inflammatory factors secreted by m28z T cells or tumor cells, and cell debris during killing stimulated and recruited mouse tumor-supportive cells, such as MDSCs that easily access to and accumulate in uterus. Then the tumor-supportive cells promoted the expansion of A549-GL cells and overcame the anti-tumor effects of m28z T cells. However, m28zT2 T cells eliminated A549-GL cells efficiently enough so that tumors hardly formed and grew in NSI mice. Of course, further studies were required to test this hypothesis in the future.

To summarize, our data demonstrate that TLR2 incorporation can generate more powerful CARs, which have superior anti-tumor efficacies in vitro and in vivo and can be used in patients. These findings reveal an important new strategy for CAR design which bridges the conventional costimulation of adaptive immunity with unconventional innate immune signaling.

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## Conflict of Interest Statement

The authors declare no competing financial interests.

## Figure Legends

**Figure 1.** 1928zT2 T cells showed enhanced effector functions compared with 1928z T cells in vitro. (a) Killing percentages of K562-GL, K562-CD19-GL, NALM6-GL and REH-GL cells by GFP T, 1928z T and 1928zT2 T cells after 18h coculture in vitro. E:T ratios mean the ratios of absolute number of CAR T cells to target cells. The GFP percentages of CAR T cells were equalized by non-transduced T cells from the same donor. Results are representative of at least three independent experiments with T cells from different healthy donors. (b) IL-2, IFN- , GM-CSF and TNF concentration in the supernatant of 24h coculture of the indicated T cells with K562-GL or K562-CD19-GL cells (E:T=1, 2×10 cells each). (c) Enhanced expansion and serial killing potency of 1928zT2 T cells compared with 1928z T cells. 1928z T and 1928zT2 T cells were serially co-cultured with NALM6 cells at 1:4 E/T ratio, the numbers of 1928z T cells and 1928zT2 T cells were counted (left), and percentages of CD19+ NALM6 cells (right) were detected by flow cytometry, every 24h for three times.

Error bars denote s.e.m and results were compared through two-sided unpaired t-test. *P<0.05,* ***P<0.01,*** P<0.001.

**Figure 2.** 1928zT2 T cells showed superior anti-leukemia efficacy in vivo. (a) Bioluminescence imaging of NALM6-GL-burdened mice treated with GFP T, 1928z T or 1928zT2 T cells. 1×10 NALM6-GL cells were injected intravenously into NSI mice, and on day 2 and 7, 5×10 GFP T, 1928z T or 1928zT2 T cells were injected intravenously into each NALM6-GL-NSI mice (n=5). On day 8, 14 and 19, bioluminescence imaging was conducted. (b) The percentages of CD19+ (NALM6-GL) cells in the PBMC of mice on day 14. (c) Survival analysis of mice treated with GFP T, 1928z T or 1928zT2 T cells (n=10). 2×10 NALM6 cells were intravenously injected into NSI mice, and two days later, 2×10 T cells were injected (GFP% normalized to 10%), and survival of mice was observed. Error bars denote s.e.m and groups were compared through two-sided unpaired t-test. *P<0.05,* ***P<0.01,*** P<0.001.

**Figure 3.** TLR2 costimulation up-regulated genes associated with cell adhesion, synaptic transmission, infiltration and mTOR pathway. (a) Heatmap of RNA sequencing results showing genes specifically up-regulated in 1928zT2 T cells after stimulation (+sti) (left). These genes were associated with cell adhesion, synapse components and transmission and cell invasion of T cells (right). (b) Relative expression of genes associated with cell adhesion (CD24, CDH2, TYRO3, CXADR), T cell infiltration (MMP2), synaptic transmission (CADPS2), TLRs pathway (IRF3 and IRF7) and mTOR pathway (T-bet, mTOR, 4E-BP1 and p70S6K). (c) Transwell cell migration assay indicated an improved capacity of 1928zT2 T cells to degrade and transmigrate Matrigel. T cell culture medium was used as a chemoattractant in the lower chamber. T cells were cultured in the insert with or without coated Matrigel for 24h, and the cells that transmigrated to the lower chamber were counted. The percentage of invasion was calculated as follows: (mean of cells migrating through the Matrigel chamber membrane/mean of cells migrating through the control insert membrane) ×100. Error bars denote s.e.m and groups were compared through two-sided unpaired t-test. *P<0.05,* ***P<0.01,*** P<0.001.

**Figure 4.** m28zT2 T cells showed enhanced antitumor efficacy compared with m28z T cells. (a) Killing percentages of mesothelin+ A549-GL and mesothelin- H23-GL cells after 18h coculture with GFP T, m28z T or m28zT2 T cells at the indicated ratios. (b) IL-2, IFN- , GM-CSF and TNF- concentration in the supernatant of 24h coculture of GFP T, m28z T or m28zT2 T cells with A549-GL cells. E:T=1:1, 2×10 cells each. (c) Weight of subcutaneous A549-GL tumors from NSI mice treated with no T cells, GFP T, m28z T or m28zT2 T cells. On day 0, 2×10 A549-GL cells were injected subcutaneously into NSI mice, and on day 7 and 9, no T, 5×10 GFP T, m28z T or m28zT2 T cells were injected through the tail vein into each mouse (n=6). On day 43, mice were sacrificed and tumors were analyzed. Error bars denote s.e.m and groups were compared through two-sided unpaired t-test. *P<0.05,* ***P<0.01,*** P<0.001.

**Figure 5.** 1928zT2 T cells showed potent efficacy in a patient with relapsed

B-ALL. (a) FACS analysis of B-ALL cells in PBMC and BM from the patient on the day before infusion of 1928zT2 T cells. (b) B-ALL cells were eradicated from PBMC and BM of the patient on day 19 and 22 respectively. (c) Wright-Giemsa staining of BM biopsies of the patient on day 18 before infusion (left) and on day 51 post-infusion (right) of 1928zT2 T cells. Scale bar = 10 m. (d) Dynamic changes of the percentages of CD19+ cells and 1928zT2 T cells in PBMC of the patient. (e) Dynamic changes of the percentages of CD19+ cells and 1928zT2 T cells in BM of the patient.

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