Aurora kinase A-specific T-cell receptor gene transfer redirects T lymphocytes to display effective antileukemia reactivity

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**Aurora kinase A (AURKA) is overexpressed in leukemias.** Previously, we demonstrated that AURKA-specific CD8+ T cells specifically and selectively lysed leukemia cells, indicating that AURKA is an excellent target for immunotherapy. In this study, we examined the feasibility of adoptive therapy using redirected T cells expressing an HLA-A*0201–restricted AURKA207-215-specific T-cell receptor (TCR). Retrovirally transduced T cells recognized relevant peptide-pulsed but not control target cells. Furthermore, TCR-redirected CD8+ T cells lysed AURKA-overexpressing human leukemic cells in an HLA-A*0201–restricted manner, but did not kill HLA-A*0201+ normal cells, including hematopoietic progenitors. In addition, AURKA207-215-specific TCR-transduced CD4+ T cells displayed target-responsive Th1 cytokine production. Finally, AURKA207-215–specific TCR-transduced CD8+ T cells displayed antileukemia efficacy in a xenograft mouse model. Collectively, these data demonstrate the feasibility of redirected T cell–based AURKA-specific immunotherapy for the treatment of human leukemia. 

**Introduction**

Aurora kinase A (AURKA) is a member of the serine-threonine kinase family that regulates mitotic cell division from G2 through to M phase of the cell cycle.1 The AURKA gene maps to chromosome region 20q13.2. AURKA is expressed at low levels in normal cells, including dividing cells, and overexpression of AURKA has clear oncogenic potential.2,3 Indeed, the AURKA gene is overexpressed in various types of cancer,4 including leukemias.5,6 Furthermore, correlations between the genetic dysregulation of AURKA and susceptibility to cancer, disease status, and prognosis have been described.4 In particular, AURKA gene overexpression correlates with genetic instability and poor differentiation of cancer cells.7,8 As AURKA expression is tightly regulated in normal tissues and overexpression correlates with malignant transformation, small molecular inhibitors have been developed that selectively target this protein in various tumors. A number of such molecules are currently in early phase clinical trials and preliminary data are encouraging.9,12 The overexpression of AURKA in cancer cells, but not in normal tissues, makes it an attractive target for tumor immunotherapy. We have previously shown that testis is the only tissue that expresses detectable levels of AURKA, which suggests that this antigen behaves like cancer/testis antigens.13 Based on these findings, we previously studied the immunotherapeutic potential of AURKA and identified an HLA-A*0201–restricted antigenic nonamer epitope derived from the kinase domain (residues 207-215). The AURKA207-215 epitope (YLILEYAPL) was recognized by CD8+ cytotoxic T lymphocytes (CTLs) generated in vitro.6 Furthermore, leukemic cells endogenously expressing AURKA were killed by these CTLs, indicating that the cognate epitope is naturally processed and presented in the context of HLA-A*0201 at levels sufficient for immunotherapeutic applications. In addition, Kobayashi and colleagues have identified HLA-class II–restricted AURKA-derived pentadecamer epitopes to which they could generate CD4+ helper T cells that expressed antitumor reactivity.14 Immunotherapeutic interventions based on tumor antigen-specific T-cell receptor (TCR) gene transfer to redirect the specificity of other T cells has shown clinical success in patients with advanced melanoma.15 However, this approach is complicated by several potential problems: (1) on-target adverse events directed against normal tissues, especially when affinity-enhanced TCRRs are used16; (2) issues related to chain mispairing between the introduced and endogenous TCR α/β genes; and (3) off-target adverse events because of inherent cross-reactivity of the introduced TCR.17 Although various solutions have been explored to minimize TCR chain mispairing, all current approaches have intrinsic limitations. To this end, we have recently developed a unique vector system that simultaneously delivers siRNAs, which specifically down-regulate endogenous TCR expression, and a siRNA-resistant relevant TCR construct (si-TCR vector).18 Furthermore, the likelihood of adverse events related to expression of the introduced TCR may be minimized by the selection of tumor-specific antigens or cancer/testis antigens, rather than tumor-associated antigens. Indeed, a recent clinical study reported that redirected T-cell therapy using NY-ESO-1–specific TCR gene


*K.N. and T.O. contributed equally to this work.*

An Inside Blood analysis of this article appears at the front of this issue.
transfer displayed antitumor efficacy against metastatic melanoma and metastatic synovial cell sarcoma without obvious toxicities mediated by the transferred T cells.

In this study, we examined the antileukemic efficacy and safety of redirected T cells using HLA-A*0201–restricted AURKA gene transfer both in vitro and in vivo. The data demonstrate the feasibility of this approach for the treatment of human leukemias.

Methods

Cells and cell lines

Approval for this study was obtained from the Institutional Review Board of Ehime University Hospital (Protocol 0909001 and 0909002). Written informed consent was obtained from all patients, healthy volunteers, and parents ofcord blood donors in accordance with the Declaration of Helsinki. B-lymphoblastoid cell lines (B-LCLs) were established by transformation of peripheral blood B-lymphocytes with Epstein-Barr virus. GANMO-1 (HLA-A2*), MEG01 (HLA-A2*), MEG01-A2 (HLA-A*0201 gene-transduced MEG01), OUN-1 (HLA-A2*), and KA2Z (HLA-A2*) leukemia cell lines were cultured in RPMI 1640 with 10% FCS, antibiotics, and L-glutamine. The artificial antigen-presenting cell line C1R-A2 (HLA-A2*) was purchased from Thermo Electron (Greiner Bio-One). leukemia cell lines were cultured in RPMI 1640 with 10% FCS, antibiotics, and L-glutamine. The artificial antigen-presenting cell line C1R-A2 (HLA-A*0201*) was a kind gift from Dr A. John Barrett (National Heart, Lung, and Blood Institute, Bethesda, MD). The Jurkat/MA cell line (kindly provided by Prof Erik Hooijberg, Vrije Universiteit Medisch Centrum, Amsterdam, The Netherlands) is a Jurkat cell subclone that lacks endoge

signaling via newly introduced TCRs. PBMCs and bone marrow mononuclear cells (BMMCs) from leukemia patients and healthy volunteers, and cord blood mononuclear cells (CBMCs) from healthy donors, were isolated by density gradient centrifugation and stored in liquid nitrogen until use. CD34+ cells from CBMCs were isolated using CD34+ cell-isolating immunomagnetic beads (Miltenyi Biotec).

Synthetic peptides and HLA-A*0201/peptide tetrameric complexes

The HLA-A*0201–restricted AURKA gene transfer both in vitro and in vivo. The data demonstrate the feasibility of this approach for the treatment of human leukemias.

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Intracellular expression of Foxp3 and AURKA\textsubscript{207-215}-responsive IFN-γ production by AURKA\textsubscript{207-215}-specific TCR-transduced CD4⁺ T cells were analyzed using anti-Foxp3-PE (e-Bioscience) and anti–IFN-γ–FITC (BD Biosciences). Data were acquired using a FACS Calibur flow cytometer and analyzed with either Cell Quest (BD Biosciences) or FlowJo Version 7.2.2 software (TreeStar Inc).

**CFSE dilution assay**

To measure epitope-responsive proliferation of AURKA\textsubscript{207-215}-specific TCR-transduced CD8⁺ T cells in the presence or absence of similarly redirected CD4⁺ T cells, CD8⁺ T cells were labeled with CFSE (Molecular Probe Inc) as described previously. After 3 days, CFSE dilution within the CD8⁺ T-cell population was assessed by flow cytometry.

**Epitope-responsive luciferase production by AURKA\textsubscript{207-215}-specific TCR-transduced Jurkat/MA cells**

To verify the functionality of the cloned AURKA\textsubscript{207-215}-specific TCR α and β chains, we used the TCR Jurkat/MA cell line, which stably expresses hCD8α and an NFAT-luciferase reporter gene (Jurkat/MA/CD8α/luc), as follows, pMS3-AURKA-TCR was retrovirally transduced into Jurkat/MA/CD8α/luc cells. Cells expressing TCR VβJ2 were isolated for functional analysis. Briefly, HLA-A*0201/B-β-LCL cells were loaded with titrated doses of AURKA\textsubscript{207-215} peptide or the irrelevant SL9 peptide (10 μM; HIV-1 p17 Gag, residues 77-85) and used to stimulate 8 × 10⁶ TCR gene-modified Jurkat/MA/CD8α/luc cells (effectortarget ratio 2:1) for 12 hours. The cells were then lysed and subjected to luciferase assay using the Picogene-Dual-SeaPansy Kit (TOYOInki) according to manufacturer’s instructions. Luciferase activity was measured using a Lumicycle700 (MicrotecNiton).

**IFN-γ secretion assay**

AURKA\textsubscript{207-215}-specific TCR-transduced CD4⁺ or CD8⁺ T cells (5 × 10⁶) were incubated with 10⁶ AURKA\textsubscript{207-215} peptide-pulsed (1 μM) or unpulsed C1R-A2 cells for 24 hours. For the inhibition assay, cells were cultured in the presence of either an anti-HLA class I framework mAb (w6/32; ATCC) or a control anti–HLA-DR mAb (L243; ATCC). Cytokine production patterns were assessed using a bead-based immunoassay kit (Becton Dickinson). IFN-γ in the culture supernatant was measured using an ELISA kit (Pierce) according to the manufacturer’s instructions. Streptavidin-HRP was used for color development, and luminointensity was measured using IMMUNO-MINI (NJ-2300; Microtec).

**Cytotoxicity assay**

Standard ⁵¹Cr release assays were performed as previously described. Briefly, 10⁵ unpulsed or peptide-pulsed target cells were labeled with ⁵¹Cr (Na₂⁵¹CrO₄; MP Bio Japan) and incubated at various ratios with effector cells in 200 μL of medium culture in 96-well round-bottomed plates. To assess HLA class I restriction, target cells were incubated with 10 μg/mL w6/32 mAb or the control L243 mAb for 1 hour, then incubated with effector cells for 5 hours. After incubation, 100 μL supernatant was collected from each well to measure ⁵¹Cr release. The percentage specific lysis was calculated as: (experimental release cpm – spontaneous release cpm)/(maximal release cpm – spontaneous release cpm) × 100 (%).

**Quantitative analysis of AURKA mRNA expression**

Quantitative real-time PCR (qRT-PCR) for AURKA mRNA was performed as described previously. Briefly, total RNA was extracted using an RNAeasy Mini Kit (QIAGEN) and cDNA was synthesized. qRT-PCR for AURKA mRNA (Hs00269212_ml) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA (4326317E) as an internal control was performed using the TaqMan Gene Expression assay (Applied Biosystems) in accordance with the manufacturer’s instructions and an ABI Prism 7700 Sequence Detection System (Applied Biosystems). The expression level of AURKA mRNA was corrected by reference to that of GAPDH mRNA, and the relative amount of AURKA mRNA in each sample was calculated by the comparative ΔΔt method.

**AURKA protein expression analysis by Western blotting**

For the analysis of protein expression, Western blotting was performed as described previously. Briefly, cell lysates were subjected to 10% SDS-PAGE (e-PAGEL, ATTO) and blotted onto PVDF membranes (Bio-Rad Laboratories). The blots were incubated first with anti-AURKA mouse mAb (Abcam), then with HRP-conjugated anti–mouse IgG (GE Healthcare). The probed proteins were visualized using an enhanced chemiluminescence system (GE Healthcare). Subsequently, the blotted membranes were stripped and reprobed with anti–β-actin mouse mAb (Sigma-Aldrich) to confirm equivalent protein loading between samples.

**Antileukemia effect of AURKA\textsubscript{207-215}-specific TCR-transduced T cells in xenograft mouse models**

All in vivo experiments were approved by the Ehime University animal care committee. For the Winn assay, 5 × 10⁶ GANMO-1 cells and 2.5 × 10⁶ AURKA\textsubscript{207-215}-specific TCR gene-transduced or non–gene-modified CD8⁺ T cells were inoculated per mouse (n = 4 per group). The cells were suspended in 300 μL PBS and injected subcutaneously into the left flank of NOG mice (Non-Obese Diabetic/Severe Combined Immunodeficiency/IL-2 receptor γ-chain allelic mutation; NOD/Shi-scid/IL-2R ϶null) aged 5-6 weeks (Central Institute for Experimental Animals). Mice were subsequently injected intravenously with either 5 × 10⁶ AURKA\textsubscript{207-215}-specific TCR gene-modified cells, AUR-2 cells or non-modified CD8⁺ T cells, as per the initial inoculation, on a weekly basis for a total of 5 infusions. Tumor size was measured every 5 days until the mice died or were euthanized because of tumor progression.

For adoptive transfer experiments, NOG mice aged 9 weeks were similarly inoculated with 5 × 10⁶ of GANMO-1 cells. Intravenous administration of either 5 × 10⁶ AURKA\textsubscript{207-215}-specific TCR gene-transduced or non–gene-modified CD8⁺ T cells commenced on the same day (day 0), and was continued on a weekly basis thereafter until the mice died or were euthanized because of tumor progression.

**Statistical analysis**

The paired t test was used to assess differences between groups; a P value < .05 was considered significant.

**Results**

**Generation of a novel HLA-A*0201-restricted AURKA\textsubscript{207-215}-specific CTL clone (AUR-2) and retroviral expression of the full-length TCR α and β genes**

Characteristics of the newly established HLA-A*0201-restricted AURKA\textsubscript{207-215}-specific CTL clone (AUR-2) are shown in Figure 1. AUR-2 was stained uniformly with the HLA-A*0201/Gag 77-85 tetramer, but not with the irrelevant HLA-A*0201/Gag 77-85 tetramer (Figure 1A). In cytotoxicity assays, AUR-2 displayed moderate levels of functional sensitivity in response to cognate peptide (Figure 1B). Epitope-dependent production of IFN-γ was confirmed in ELISPOT assays (Figure 1C). Peptide specificity and HLA restriction were further demonstrated in cytotoxicity assays with different target cells (Figure 1D). In addition, AUR-2 lysed the HLA-A*0201⁺ leukemia cell line GANMO-1, which overexpresses AURKA mRNA, but not the HLA-A*0201⁻ negative cell lines MEG01 and K562, both of which also express AURKA mRNA at high levels (Figure 1E). The rearranged TCR α and β genes of AUR-2 were sequenced and found to comprise the germ line gene segments TRAV3/TRAJ20/TRAC and TRBV10-3/TRBJ1-1/TRBC1, respectively; both full-length genes were cloned into a novel bicistronic retroviral vector (Figure 1F).
were conducted using C1R-A2 target cells loaded with 1M AURKA207-215 peptide and AUR-2 CTL at different input numbers as shown. (D) 51Cr-release assays were conducted using C1R-A2 target cells loaded with 1μM AURKA207-215 Peptide and AUR-2 CTL at different input numbers as shown. (E) IFN-γ ELISPOT assays were conducted using C1R-A2 target cells loaded with 1μM AURKA207-215 Peptide and AUR-2 CTL at different input numbers as shown. (F) Construction of a novel retroviral vector encoding full-length AURKA-specific TCR α and β genes derived from AUR-2. MoMLV indicates Moloney murine leukemia virus; LTR, long terminal repeat; EF1a, elongation factor 1a; PGK, phosphoglycerate kinase promoter; and MSCV, murine stem cell virus. Error bars represent SDs.

**Figure 2.** Functional retroviral expression of the AURKA207-215–specific TCR.

(A) Representative flow cytometry plots showing staining of AUR-2 with the HLA-A*0201/AURKA207-215 tetramer (left) and the irrelevant HLA-A*0201/Gag72-85 tetramer (negative control; right). (B) The cytotoxic activity of AUR-2 was measured in 51Cr-release assays against C1R-A2 or C1R (negative control) cells loaded with a range of AURKA207-215 peptide concentrations as indicated. (C) IFN-γ ELISPOT assays were conducted using C1R-A2 target cells loaded with 1μM AURKA207-215 Peptide and AUR-2 CTL at different input numbers as shown. (D) 51Cr-release assays were conducted using AUR-2 CTL with unpulsed or AURKA207-215 peptide–pulsed (1μM) HLA-A*0201+ autologous or allogeneic B-LCLs, C1R-A2 cells or HLA-A*0201–allogeneic B-LCLs as indicated. E/T indicates effector:target ratio. (E) The cytotoxic activity of AUR-2 CTL against the indicated leukemia cell lines was measured in 51Cr-release assays. (F) Construction of a novel retroviral vector encoding full-length AURKA-specific TCR α and β genes derived from AUR-2. MoMLV indicates Moloney murine leukemia virus; LTR, long terminal repeat; EF1a, elongation factor 1a; PGK, phosphoglycerate kinase promoter; and MSCV, murine stem cell virus. Error bars represent SDs.

**Figure 3.** Characteristics of the AURKA207-215–specific CTL clone AUR-2.

(A) Schematic representation of the luciferase assay using AURKA 207-215–specific TCR-transduced Jurkat/MA cells and the parental CTL clone. (B) The cytotoxic activity of AUR-2 was measured in 51Cr-release assays against H9251 luc cells produced luciferase in response to stimulation with AURKA207-215 peptide–loaded C1R-A2 cells in a dose-dependent manner (Figure 3C). Compared with the parental AUR-2 CTL clone (Figure 1B), the TCR-transduced Jurkat/MA cells displayed low levels of peptide sensitivity. To address this functional discrepancy, we assessed cell-surface expression of TCR α/β, CD3, CD8α, CD11a, and CD28 (supplemental Figure 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article). The TCR-transduced Jurkat/MA cells expressed lower surface levels of TCR α/β, CD3 and CD8α compared with both similarly activated normal CD8+ T cells and the parental AUR-2 CTL clone. Furthermore, CD11a and CD28 were almost absent from the transfectant cells. These findings may explain the observed differences in functional sensitivity between AUR-2 TCR-transduced Jurkat/MA cells and the parental CTL clone.

**Figure 4.** Antileukemia reactivity in vitro

Next, the AURKA207-215–specific TCR was retrovirally introduced into normal CD8+ T cells. Transduction efficiency determined by Vβ12 staining of TCR gene-modified T cells was 50%-70% (data not shown), and 20%-25% of the Vβ12+ cells stained with the HLA-A*0201/AURKA207-215 tetramer (Figure 3A). Isolated Vβ12+ AURKA207-215–specific TCR gene-transduced CD8+ T cells displayed similar antigen sensitivity to the parental AUR-2 CTL clone (Figure 3B-C). Notably, however, the AURKA207-215–specific TCR transductants produced higher quantities of IFN-γ in response to the same peptide-pulsed C1R-A2 targets (Figure 3C). On the basis of these observations, further experiments were carried out using these AURKA207-215–specific TCR gene transfectants.

Transmission Electron Microscopy


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**AURKA207-215–specific TCR gene-transduced CD8+ T cells exert antileukemia reactivity in vitro**

Next, the AURKA207-215–specific TCR was retrovirally introduced into normal CD8+ T cells. Transduction efficiency determined by Vβ12 staining of TCR gene-modified T cells was 50%-70% (data not shown), and 20%-25% of the Vβ12+ cells stained with the HLA-A*0201/AURKA207-215 tetramer (Figure 3A). Isolated Vβ12+ AURKA207-215–specific TCR gene-transduced CD8+ T cells displayed similar antigen sensitivity to the parental AUR-2 CTL clone (Figure 3B-C). Notably, however, the AURKA207-215–specific TCR transductants produced higher quantities of IFN-γ in response to the same peptide-pulsed C1R-A2 targets (Figure 3C). On the basis of these observations, further experiments were carried out using these AURKA207-215–specific TCR gene transfectants.

**AURKA207-215–specific TCR-transduced CD8+ T cells displayed HLA class I–restricted, peptide-dependent IFN-γ production (Figure 3D), and target epitope–specific cytotoxic activity (Figure 3E).** Furthermore, these redirected CD8+ T cells selectively lysed the HLA-A*0201+ leukemia cell line GANMO-1, which overexpresses AURKA, but not the HLA-A*0201–leukemia cell lines, MEG01, KAZZ, and OUN-1, which also overexpress AURKA (Figure 4A, supplemental Figure 2). In contrast,
lymphoblasts and 0.21/H11006 AURKA mRNA expression relative to K562 for each group (mean transduced CD8 indicates effector:target ratio. (C) IFN-γ-transduced CD8 AURKA207-215–specific MEG01-A2 were susceptible to the cytotoxic effects of endogenously processed AURKA207-215 epitope in the context of HLA-DR mAb (Figure 4C-D). To confirm recognition of the diminished by an anti-HLA class-I mAb but not by an anti– AURKA207-215–specific (A) Representative flow cytometry plots showing staining of Figure 4E). Parental MEG01 cells do not express HLA-A*0201, and the cognate antigen-specific proliferative response of AURKA207-215–specific TCR-transduced CD8+ T cells was actually enhanced in the presence of redirected CD4+ T cells but not in the presence of non–gene-modified CD4+ T cells (supplemental Figure 3B). Furthermore, AURKA207-215–specific TCR-transduced CD4+ T cells produced significant amounts of IL-2, TNF-α, and IFN-γ, but not IL-4 or IL-10 (supplemental Figure 4).

These observations suggest that AURKA207-215–specific TCR-transduced CD4+ T cells might be able to function as epitope-specific Th1 helper T cells, and that the interaction between T cell–expressed CD4 and target cell–expressed HLA class II molecules facilitates HLA class I–restricted AURKA207-215–specific IFN-γ production.

AURKA207-215–specific TCR-transduced CD8+ T cells exhibit antileukemia reactivity in vivo

The in vivo antileukemia reactivity of AURKA207-215–specific TCR-transduced CD8+ T cells was assessed using the Winn assay and a therapeutic adoptive transfer model.

In the Winn assay, NOG mice were initially coinjected with GANMO-1 cells (5 × 10⁶) and either 2.5 × 10⁶ AURKA207-215–specific TCR gene-modified or non–gene-modified CD8+ T cells; 5 weekly infusions of the respective CD8+ T-cell populations (5 × 10⁶ cells per infusion) were subsequently administered. Treatment with AURKA207-215–specific TCR-transduced CD8+ T cells completely prohibited the engraftment and growth of inoculated leukemia cells for more than 2 months (Figure 7A), and significantly prolonged survival (Figure 7B). Similar results were obtained with AUR-2 cells in a parallel regimen (supplemental Figure 5). In contrast, non–gene-modified CD8+ T cells did not prohibit leukemia growth. In a therapeutic adoptive transfer model, intravenously injected AURKA207-215–specific TCR-transduced
**CD8⁺ T cells, but not non–gene-modified CD8⁺ T cells, significantly suppressed the growth of inoculated leukemia cells in vivo (P < .02; Figure 7C). Statistically significant tumor suppression was achieved on day 65, after 10 adoptive infusions. Thereafter, all mice (n = 4) treated with non–gene-modified CD8⁺ T cells died by day 85. 2 mice treated with AURKA207-215–specific TCR-transduced CD8⁺ T cells died from other causes (1 on day 45 and 1 on day 70); the other 2 mice in this group survived longer than 90 days and were finally euthanized because of disease progression. Collectively, these observations indicate that AURKA207-215–specific TCR-transduced CD8⁺ T cells exhibit antileukemia reactivity in vivo.**

**Discussion**

In the setting of hematologic malignancies, TCR gene therapy targeting WT1 in leukemia,29 and chimeric antigen receptor (CAR) gene therapy targeting CD33 in myeloid leukemias31 and CD19, CD20, CD22, CD30, and the receptor tyrosine kinase-like orphan receptor 1 (ROR1) in B-cell malignancies,32-38 are currently being investigated in preclinical studies or in early phase clinical trials.

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**Figure 4.** AURKA207-215–specific TCR-transduced CD8⁺ T cells can distinguish leukemia cells from normal cells on the basis of AURKA expression levels. (A) AURKA207-215–specific TCR-transduced CD8⁺ T cells exhibit antileukemia reactivity in an HLA-A*0201-dependent fashion. The HLA-A*0201⁺ leukemia cell line GANMO-1 was lysed by AURKA207-215–specific TCR-transduced CD8⁺ T cells as a function of effector:target (E/T) ratio; no significant lysis was observed with the HLA-A*0201⁻ leukemia cell lines MEG01, KAZZ and OUN-1. All of the tested leukemia cell lines overexpress AURKA mRNA; numbers in parentheses indicate AURKA mRNA expression relative to K562, and correlations with AURKA protein expression are shown in supplemental Figure 2. (B) The same AURKA207-215–specific TCR-transduced CD8⁺ T cells used in panel A at the same E/T ratios were tested in 51Cr-release assays for potentially damaging effects against normal cells. No significant lysis was observed with HLA-A*0201⁺ PBMCs (n = 3). PHA-lymphoblasts representing normal mitotic cells (n = 3) or normal cord blood–derived CD34⁺ cells (CB-CD34⁻) encompassing normal hematopoietic progenitor cells (n = 2). AURKA mRNA expression relative to K562 was 0.02 ± 0.008 for PBMCs, 0.25 ± 0.005 for PHA-lymphoblasts and 0.21 ± 0.09 for CB-CD34⁺ cells (⁎ indicates less than detectable). (C) Effects of HLA class I and class II blockade on the cytotoxic activity of AURKA207-215–specific TCR-transduced CD8⁺ T cells against GANMO-1 leukemia cells. E/T, effector:target ratio. (D) As for panel C, showing the effects of HLA class I and class II blockade on the lysis of autologous B-LCLs loaded with AURKA207-215 peptide (1μM). (E) Flow cytometric confirmation of HLA-A*0201 expression by MEG01-A2 cells. (F) Enhanced lysis of MEG01-A2 cells relative to parental MEG01 cells by AURKA207-215–specific TCR-transduced CD8⁺ T cells confirms recognition of endogenously processed AURKA207-215 peptide presented in the context of HLA-A*0201. E/T indicates effector:target ratio. Error bars represent SDs.

**Figure 5.** AURKA207-215–specific TCR-transduced CD8⁺ T cells kill freshly isolated leukemia cells in vitro. Freshly isolated HLA-A*0201⁺ (n = 3) or HLA-A*0201⁻ (n = 3) acute or chronic myeloid leukemia cells were used as targets in 51Cr-release assays with AURKA207-215–specific TCR-transduced CD8⁺ T cells at the indicated effector:target (E/T) ratios. AML, acute myeloid leukemia; BC, blast crisis; CML, chronic myeloid leukemia. M1, M2, and M4 refer to French-American-British classification subtypes (⁎ indicates the expression of AURKA mRNA relative to the mean expression levels across 5 PBMC samples from healthy donors was determined by qRT-PCR and calculated using the comparative ΔΔCT method). Error bars represent SDs.

**Figure 6.** AURKA207-215–TCR-transduced CD4⁺ T cells display antigen-specific Th1 cytokine production. (A) A representative flow cytometry plot showing surface Vj12 expression by AURKA207-215–specific TCR-transduced CD4⁺ T cells. (B) AURKA207-215–TCR-transduced CD4⁺ T cells produce IFN-γ in response to cognate peptide-loaded (1μM) C1R-A2 cells; unpulsed or irrelevant (HIV p17 Gag SL9) peptide-pulsed C1R-A2 cells were used as negative controls. Cognate antigen-specific IFN-γ production was reduced to background levels in the presence of anti-HLA class I blocking mAb and inhibited in the presence of anti-HLA class II blocking mAb. APC, antigen-presenting cell. Error bars represent SDs.
Although adoptive antileukemia/lymphoma therapy with redirected T cells using tumor antigen-specific TCR or CAR gene transfer remains in its infancy, emerging evidence supports the development of such therapeutic options.

A number of preclinical and clinical studies of tumor antigen-specific TCR gene therapy have underscored the fact that appropriate antigen selection is essential to minimize the likelihood of on-target adverse events mediated by redirected T cell recognition of normal tissues expressing self-derived specificities. This concept is further supported by a recent study of NY-ESO-1–specific TCR gene transfer. In this report, objective clinical responses were observed in 5 of 11 patients with metastatic melanoma and 4 of 6 patients with metastatic synovial cell sarcoma without any toxicity related to engineered T cell activity. Thus, the exploration of novel tumor antigens to identify safe and effective targets for TCR gene therapy is warranted, especially in the context of hematologic malignancies.

Previously, we reported a significant correlation between the overexpression of AURKA mRNA and the aggressiveness of lymphoma cells. Furthermore, we found that AURKA mRNA is overexpressed in a large proportion of freshly isolated human leukemia cells. However, in normal tissues, AURKA mRNA expression is largely limited to the testis. Subsequently, we identified an immunogenic nonamer epitope derived from AURKA that was presented in the context of HLA-A*0201. In the present study, we set out to examine the feasibility of redirected T cell–based adoptive immunotherapy for the treatment of human leukemia using a TCR derived from an HLA-A*0201–restricted AURKA207-215–specific CD8+ T-cell clone (AUR-2). Expression of this TCR in CD8+ T cells conferred antileukemia reactivity both in vitro and in a xenogeneic mouse model of human leukemias. Furthermore, CD4+ T cells could be redirected using this TCR to recognize the same HLA-A*0201–restricted AURKA207-215 epitope. This represents a potentially important advantage, as the same TCR could redirect both helper (CD4+) and cytotoxic (CD8+) functions within the transduced T-cell population, which might sustain the antileukemia response in vivo after adoptive transfer.

Redirected CD8+ T cells expressing the TCR cloned from AUR-2 displayed similar levels of functional sensitivity to the parental CTL clone. In vitro, AURKA207-215–specific TCR–transduced CD8+ T cells were able to lyse HLA-A*0201+ human leukemia line GANMO-1 cells, which overexpress AURKA mRNA, and freshly isolated leukemia cells from HLA-A*0201+ patients. This antileukemia reactivity was implemented through recognition of the endogenously processed AURKA207-215 epitope presented in the context of HLA-A*0201. Importantly, these AURKA207-215–specific TCR–transduced CD8+ T cells did not lyse HLA-A*0201+ normal PBMCs, mitotic PHA-lymphoblasts or cord blood CD34+ cells; these data suggest that on-target adverse effects would be minimal in clinical applications. Furthermore, we demonstrated the efficacy of AURKA207-215–specific TCR–transduced CD8+ T cells in vivo by showing the inhibition of leukemia cell growth in a xenograft mouse model. As many hematopoietic progenitor cells actively proliferate and will therefore have enhanced AURKA expression levels, these cells may become targets for AURKA207-215–specific TCR–transduced CD8+ T cells in vivo, as is the case with selective AURKA inhibitors. However, our observations suggest that redirected CD8+ T cells targeting AURKA may not cause severe bone marrow failure, although further studies are needed to substantiate this point.

Notably, AURKA is overexpressed in the fraction of bone marrow cells that encompasses myeloid leukemia stem cells. Recently, targeting leukemia stem cells has been highlighted as a treatment strategy to prevent disease progression in a durable fashion. Monoclonal antibodies that target leukemia stem cell surface antigens have been proposed for this purpose. Examples of such molecules include CD123 (IL3Rα) and TIM-3. Cellular immunotherapy targeting antigens that are preferentially overexpressed in leukemia stem cells has also been proposed. In this regard, WT1 appears to be a particularly attractive candidate. Indeed, we have cloned an HLA-A*2402–restricted WT1235-243–specific TCR gene into our unique si-TCR vector to address the potential of this approach. With respect to AURKA, we previously described that the CD34+CD38–fraction of bone marrow mononuclear cells from CML patients expressed high levels of AURKA mRNA and that these cells were susceptible to AURKA–specific CTL–mediated lysis. Thus, redirected T cell–based immunotherapy targeting AURKA might be able to suppress leukemia stem cells. Furthermore, such an approach may be synergistic with the administration of selective AURKA inhibitors, for example in the treatment of relapsed leukemia after allogeneic hematopoietic stem cell transplantation.

Strategic options to achieve better clinical responses in the field of TCR gene transfer are much needed. The manipulation of helper CD4+ T cells is one such approach. To date, the adoptive transfer of redirected CD4+ T cells concurrently with CD8+ T cells expressing the same tumor-specific TCR gene has not been described; however, this is an intriguing notion that could enhance the antitumor reactivity of such adoptive transfer approaches in vivo. In recognition of this possibility, we found that AURKA207-215 TCR–transduced CD4+ T cells displayed Th1 cytokine production in response to the HLA-A*0201/AURKA207-215 epitope in vitro. The effects of such activity in vivo, however, remain to be clarified. Another approach to combined immunotherapy employs peptide vaccination. Indeed, vaccination with the relevant peptide has been
shown to enhance the antitumor functionality of infused gene-modified T cells. The feasibility of this combination strategy using AURKA-specific TCR gene transfer as AURKA is also overexpressed in diverse solid tumors, the potential clinical applications of this approach are widespread. Further studies are therefore warranted to investigate the safety and utility of this novel therapy in the clinic.

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Authorship

Contribution: K.N. and T.O. performed the research and wrote the paper; H.F. designed and performed the research, wrote and edited the paper, and provided financial support; J.A., T.S., J.M., H.S., J.J.M., and E.I. discussed and interpreted the experimental results and provided materials; K.K., E.G., and D.A.P. made and supplied the tetramers and edited the paper; and M.Y. discussed and interpreted the experimental results, edited the paper, and provided financial support.

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