

# 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<sup>+</sup> 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 AURKA<sub>207-215</sub>-specific**

**T-cell receptor (TCR). Retrovirally transduced T cells recognized relevant peptide-pulsed but not control target cells. Furthermore, TCR-redirection CD8<sup>+</sup> T cells lysed AURKA-overexpressing human leukemic cells in an HLA-A\*0201-restricted manner, but did not kill HLA-A\*0201<sup>+</sup> normal cells, including hematopoietic progenitors. In addition, AURKA<sub>207-215</sub>-specific TCR-transduced CD4<sup>+</sup>**

**T cells displayed target-responsive Th1 cytokine production. Finally, AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> 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. (*Blood*. 2012;119(2):368-376)**

## Introduction

Aurora kinase A (AURKA) is a member of the serine-threonine kinase family that regulates mitotic cell division from G<sub>2</sub> through to M phase of the cell cycle.<sup>1</sup> 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.<sup>2,3</sup> Indeed, the *AURKA* gene is overexpressed in various types of cancer,<sup>4</sup> including leukemias.<sup>5,6</sup> Furthermore, correlations between the genetic dysregulation of *AURKA* and susceptibility to cancer, disease status, and prognosis have been described.<sup>4</sup> In particular, *AURKA* gene overexpression correlates with genetic instability and poor differentiation of cancer cells.<sup>7,8</sup> 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.<sup>9-12</sup>

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.<sup>13</sup> 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 *AURKA*<sub>207-215</sub> epitope (YLILEYAPL) was recognized by CD8<sup>+</sup> cytotoxic T lymphocytes (CTLs) generated in vitro.<sup>6</sup> 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<sup>+</sup> helper T cells that expressed antitumor reactivity.<sup>14</sup>

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.<sup>15</sup> However, this approach is complicated by several potential problems: (1) on-target adverse events directed against normal tissues, especially when affinity-enhanced TCRs are used<sup>16</sup>; (2) issues related to chain mispairing between the introduced and endogenous *TCR*  $\alpha/\beta$  genes; and (3) off-target adverse events because of inherent cross-reactivity of the introduced TCR.<sup>17</sup> 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).<sup>18</sup> 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

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transfer displayed antitumor efficacy against metastatic melanoma and metastatic synovial cell sarcoma without obvious toxicities mediated by the transferred T cells.<sup>19</sup>

In this study, we examined the antileukemic efficacy and safety of redirected T cells using HLA-A\*0201–restricted AURKA<sub>207-215</sub>–specific *TCR* 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 Ehome University Hospital (Protocol 0909001 and 0909002). Written informed consent was obtained from all patients, healthy volunteers, and parents of cord 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<sup>+</sup>), MEG01 (HLA-A2<sup>-</sup>), MEG01-A2 (HLA-A\*0201 gene-transduced MEG01), OUN-1 (HLA-A2<sup>-</sup>), and KAZZ (HLA-A2<sup>-</sup>) 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<sup>+</sup>) 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 endogenous TCR expression and stably expresses both the human *CD8α* gene (*hCD8α*) and an *NFAT-luciferase* gene construct for the detection of signaling via newly introduced TCRs.<sup>20</sup> 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<sup>+</sup> cells from CBMCs were isolated using CD34<sup>+</sup> cell-isolating immunomagnetic beads (Miltenyi Biotec).

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

The HLA-A\*0201–restricted AURKA<sub>207-215</sub> nonameric peptide (YLI-LEYAPL) was purchased from Thermo Electron (Greiner Bio-One). Biotin-tagged soluble HLA-A\*0201/AURKA monomers were produced as previously described.<sup>21</sup> Fluorochrome-labeled tetrameric complexes were generated by conjugation to streptavidin-PE (Prozyme) at a molar ratio of 4:1.<sup>22</sup> HLA-A\*0201 tetramers were also produced with the HIV-1 p17 Gag-derived peptide epitope SL9 (SLYNTVATL, residues 77-85) for control purposes.

### Generation of an AURKA<sub>207-215</sub>–specific CTL clone

A novel AURKA<sub>207-215</sub>–specific CTL clone designated AUR-2 was generated as previously described.<sup>23</sup> Briefly, monocyte-derived dendritic cells (Mo-DCs) were generated from CD14<sup>+</sup> PBMCs using 10 ng/mL recombinant human IL-4 and 75 ng/mL recombinant human GM-CSF (R&D systems), then matured with 100 U/mL recombinant human TNF-α (Dainippon Pharmaceutical). CD8<sup>+</sup> T cells (1 × 10<sup>5</sup>) were stimulated with 10<sup>4</sup> autologous mature AURKA<sub>207-215</sub> peptide-loaded (10 μM) Mo-DCs in a 96-well round-bottomed plate. One week later, the CD8<sup>+</sup> T cells were restimulated similarly, and 10 U/mL recombinant human IL-2 (Roche) was added after a further 4 days. Thereafter, CD8<sup>+</sup> T cells were restimulated weekly with 10<sup>5</sup> autologous AURKA<sub>207-215</sub> peptide-pulsed (10 μM) PBMCs treated with mitomycin-C (MMC; Kyowa Hakko). Epitope-dependent target cell cytotoxicity was examined using standard<sup>51</sup>chromium (<sup>51</sup>Cr)–release assays.

### ELISPOT

ELISPOT assays were conducted as previously described.<sup>22</sup> Briefly, 96-well flat-bottomed MultiScreen-HA plates with a nitrocellulose base

(Millipore) were coated overnight at 4°C with 10 μg/mL anti-IFN-γ monoclonal antibody (mAb; R&D Systems). After washing with PBS, cultured CD8<sup>+</sup> T cells were stimulated with 5 × 10<sup>4</sup> AURKA<sub>207-215</sub> peptide-pulsed (1 μM) or unpulsed C1R-A2 cells/well for 20 hours at 37°C in a 5% CO<sub>2</sub> atmosphere. Subsequently, the wells were vigorously washed with PBS/0.05% tween 20 and incubated with polyclonal rabbit anti-IFN-γ Ab (Endogen) for 90 minutes at room temperature. The wells were then washed again and incubated for 90 minutes with peroxidase-conjugated goat anti-rabbit IgG Ab (Zymed). Spots were visualized by the addition of 100 μL substrate, comprising 0.1M sodium acetate buffer (pH 5.0) containing 3-amino-9-ethylcarbazole (Sigma-Aldrich) and 0.015% H<sub>2</sub>O<sub>2</sub>, for 40 minutes at room temperature and counted under a light microscope.

### Cloning of full-length *TCR α* and *β* chain genes from the AUR-2 CTL clone and retroviral vector construction

Total RNA was extracted from the HLA-A\*0201–restricted AURKA<sub>207-215</sub>–specific CTL clone AUR-2 using the FastPure RNA Kit (Takara Bio) according to the manufacturer's instructions. Full-length *TCR α* and *β* genes were cloned as previously described.<sup>24</sup> Briefly, cDNA was amplified using a 5'-RACE primer and 3'-constant region primers as follows: (1) 5'-TCAGCTGGACCACAGCCGACGCT-3' for *TCR Cα*; (2) 5'-TCAGAAATCCTTTCTCTTGAC-3' for *TCR Cβ1*; and (3) 5'-CTAGCCTCTGGAATCCTTTCTCTT-3' for *TCR Cβ2*. The conditions for PCR were: one cycle at 94°C for 3 minutes, followed by 30 cycles at 94°C for 40 seconds, 58°C for 40 seconds and 72°C for 1 minute, with a final extension phase at 72°C for 5 minutes. Each *TCR α* and *β* chain amplicon was cloned into the pMD20 TA cloning vector (Takara Bio), and sequenced using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and an ABI 3730xl sequencer (Applied Biosystems). Full-length *TCR α* and *β* genes were then cloned into the bicistronic pMS3 retroviral vector, which is a pME1-5 derivative that contains the murine stem cell virus (MSCV) LTR (Takara Bio) in place of the 3'LTR (pMS3-AURKA-TCR, Figure 1). Ecotropic retrovirus particles were obtained by transient transfection of HEK293 T cells with the TCR construct and helper plasmids (pGP vector and pE-eco vector; Takara Bio). GaLV-pseudotyped retrovirus particles were obtained by consecutive transfection into PG13 cells. The pMS3-AURKA-TCR GaLV-pseudotyped retroviruses were used for AURKA-specific *TCR α* and *β* gene transduction.

### Transduction of AURKA<sub>207-215</sub>–specific *TCR genes*

Jurkat/MA cells and healthy donor T cells were genetically modified to express the AURKA-specific TCR using RetroNectin (Takara Bio) as previously described.<sup>18</sup> Briefly, 1 × 10<sup>6</sup> healthy donor T cells per well in GT-T503 (Takara Bio) with 5% human serum, 0.2% human albumin, 50 U/mL recombinant human IL-2 (R&D Systems), 5 ng/mL recombinant human IL-7 (R&D Systems), 10 ng/mL recombinant human IL-15 (Pepro-Tech Inc), and 100 ng/mL recombinant human IL-21 (Shenandoah Biotechnology Inc) were added on day 1 to a 24-well culture plate pretreated with anti-human CD3 mAb (BioLegend). Jurkat/MA cells were cultured in IMDM with 8% FCS and 50 μg/mL hygromycin B (Invitrogen). On day 3, cultured T cells or Jurkat/MA cells were transferred on to a retrovirus-preloaded RetroNectin-coated 24-well plate, centrifuged at 2000g for 2 hours and rinsed with PBS. Cells were then applied to the retrovirus-preloaded RetroNectin-coated 24-well plate again for the second transduction. AURKA-specific *TCR*-transduced T cells were stimulated weekly with MMC-treated C1R-A2 cells loaded with AURKA<sub>207-215</sub> peptide (1 μM) for further functional experiments.

### Flow cytometric analysis

The AUR-2 CTL clone expresses the *TRBV10-3* gene, denoted in IMGT nomenclature.<sup>25</sup> This corresponds to *TCR Vβ12* in the Arden nomenclature.<sup>26</sup> Accordingly, anti-TCR Vβ12 mAb was used to detect AURKA<sub>207-215</sub>–specific *TCR*-transduced cells. After 4 to 6 days, transduced cells were analyzed by flow cytometry using anti-TCR Vβ12-FITC (Beckman Coulter), anti-CD8-FITC (BD Biosciences) or anti-CD8-PE (BioLegend), and HLA-A\*0201/AURKA<sub>207-215</sub> tetramer-PE (only with anti-CD8-FITC).

Intracellular expression of Foxp3 and AURKA<sub>207-215</sub>-responsive IFN- $\gamma$  production by AURKA<sub>207-215</sub>-specific TCR-transduced CD4<sup>+</sup> T cells were analyzed using anti-Foxp3-PE (e-Bioscience) and anti-IFN- $\gamma$ -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<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells in the presence or absence of similarly redirected CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells were labeled with CFSE (Molecular Probe Inc) as described previously.<sup>27</sup> After 3 days, CFSE dilution within the CD8<sup>+</sup> T-cell population was assessed by flow cytometry.

### Epitope-responsive luciferase production by AURKA<sub>207-215</sub>-specific TCR-transduced Jurkat/MA cells

To verify the functionality of the cloned AURKA<sub>207-215</sub>-specific TCR  $\alpha$  and  $\beta$  chains, we used the TCR<sup>-</sup> Jurkat/MA cell line, which stably expresses *hCD8 $\alpha$*  and an *NEAT-luciferase* reporter gene (Jurkat/MA/CD8 $\alpha$ /luc), as follows. pMS3-AURKA-TCR was retrovirally transduced into Jurkat/MA/CD8 $\alpha$ /luc cells. Cells expressing TCR V $\beta$ 12 were isolated for functional analysis. Briefly, HLA-A\*0201<sup>+</sup> B-LCL cells were loaded with titrated doses of AURKA<sub>207-215</sub> peptide or the irrelevant SL9 peptide (10  $\mu$ M; HIV-1 p17 Gag, residues 77-85) and used to stimulate  $8 \times 10^5$  TCR gene-modified Jurkat/MA/CD8 $\alpha$ /luc cells (effector:target ratio 2:1) for 12 hours. The cells were then lysed and subjected to luciferase assay using the PicaGene-Dual-SeaPansy Kit (TOYOINKI) according to manufacturer's instructions. Luciferase activity was measured using a Lumiscouter700 (MicrotecNition).

### IFN- $\gamma$ secretion assay

AURKA<sub>207-215</sub>-specific TCR-transduced CD4<sup>+</sup> or CD8<sup>+</sup> T cells ( $5 \times 10^5$ ) were incubated with  $10^5$  AURKA<sub>207-215</sub> peptide-pulsed (1  $\mu$ M) 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- $\gamma$  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 <sup>51</sup>Cr release assays were performed as previously described.<sup>28</sup> Briefly,  $10^4$  unpulsed or peptide-pulsed target cells were labeled with <sup>51</sup>Cr (Na<sub>2</sub><sup>51</sup>CrO<sub>4</sub>; MP Bio Japan) and incubated at various ratios with effector cells in 200  $\mu$ L of culture medium in 96-well round-bottomed plates. To assess HLA class I restriction, target cells were incubated with 10  $\mu$ g/mL w6/32 mAb or the control L243 mAb for 1 hour, then incubated with effector cells for 5 hours. After incubation, 100  $\mu$ L supernatant was collected from each well to measure <sup>51</sup>Cr release. The percentage specific lysis was calculated as: (experimental release cpm - spontaneous release cpm)/(maximal release cpm - spontaneous release cpm)  $\times$  100 (%).

### Quantitative analysis of AURKA mRNA expression

Quantitative real-time PCR (qRT-PCR) for AURKA mRNA was performed as described previously.<sup>6</sup> Briefly, total RNA was extracted using an RNeasy Mini Kit (QIAGEN) and cDNA was synthesized. qRT-PCRs for AURKA mRNA (Hs00269212\_ml) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) mRNA (4326317E) as an internal control were 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  $\Delta$ Ct method.

### AURKA protein expression analysis by Western blotting

For the analysis of protein expression, Western blotting was performed as described previously.<sup>6</sup> Briefly, cell lysates were subjected to 10% SDS-PAGE (e-PAGE, 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- $\beta$ -actin mouse mAb (Sigma-Aldrich) to confirm equivalent protein loading between samples.

### Antileukemia effect of AURKA<sub>207-215</sub>-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,  $5 \times 10^6$  GANMO-1 cells and  $2.5 \times 10^7$  AURKA<sub>207-215</sub>-specific TCR gene-transduced or non-gene-modified CD8<sup>+</sup> T cells were inoculated per mouse (n = 4 per group). The cells were suspended in 300  $\mu$ L PBS and injected subcutaneously into the left flank of NOG mice (Non-Obese Diabetic/Severe Combined Immunodeficiency/IL-2 receptor  $\gamma$ -chain allelic mutation; *NOD/Shi-scid/IL-2R $\gamma$ <sup>null</sup>*)<sup>29</sup> aged 5-6 weeks (Central Institute for Experimental Animals). Mice were subsequently injected intravenously with either  $5 \times 10^6$  AURKA<sub>207-215</sub>-specific TCR gene-modified cells, AUR-2 cells or unmodified CD8<sup>+</sup> 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  $5 \times 10^6$  of GANMO-1 cells. Intravenous administration of either  $5 \times 10^6$  AURKA<sub>207-215</sub>-specific TCR gene-transduced or non-gene-modified CD8<sup>+</sup> 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.

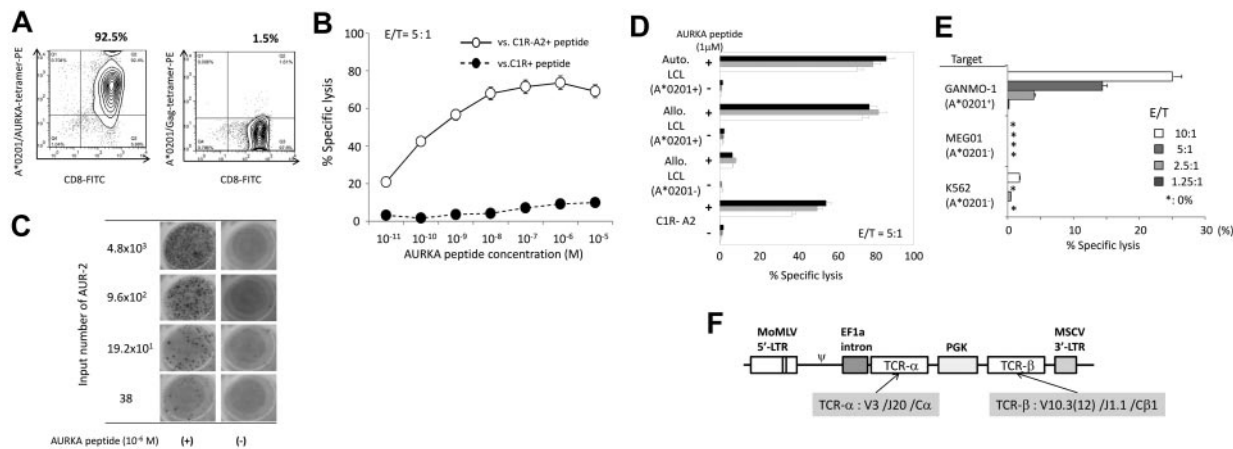
### Statistical analysis

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<sub>207-215</sub>-specific CTL clone (AUR-2) and retroviral expression of the full-length TCR $\alpha$ and $\beta$ genes

Characteristics of the newly established HLA-A\*0201-restricted AURKA<sub>207-215</sub>-specific CTL clone (AUR-2) are shown in Figure 1. AUR-2 was stained uniformly with the HLA-A\*0201/AURKA<sub>207-215</sub> tetramer, but not with the irrelevant HLA-A\*0201/Gag<sub>77-85</sub> 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- $\gamma$  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<sup>+</sup> leukemia cell line GANMO-1, which overexpresses AURKA mRNA, but not the HLA-A\*0201<sup>-</sup> negative cell lines MEG01 and K562, both of which also express AURKA mRNA at high levels (Figure 1E). The rearranged TCR  $\alpha$  and  $\beta$  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).

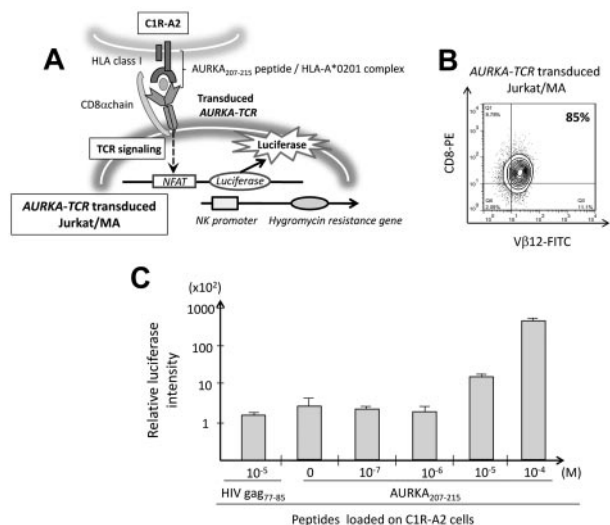


**Figure 1. Characteristics of the AURKA<sub>207-215</sub>-specific CTL clone AUR-2.** (A) Representative flow cytometry plots showing staining of AUR-2 with the HLA-A\*0201/AURKA<sub>207-215</sub> tetramer (left) and the irrelevant HLA-A\*0201/Gag<sub>77-85</sub> tetramer (negative control; right). (B) The cytotoxic activity of AUR-2 was measured in <sup>51</sup>Cr-release assays against C1R-A2 or C1R (negative control) cells loaded with a range of AURKA<sub>207-215</sub> peptide concentrations as indicated. E/T indicates effector:target ratio. (C) IFN- $\gamma$  ELISPOT assays were conducted using C1R-A2 target cells loaded with 1  $\mu$ M AURKA<sub>207-215</sub> peptide and AUR-2 CTL at different input numbers as shown. (D) <sup>51</sup>Cr-release assays were conducted using AUR-2 CTL with unpulsed or AURKA<sub>207-215</sub> peptide-pulsed (1  $\mu$ M) HLA-A\*0201<sup>+</sup> autologous or allogeneic B-LCLs, C1R-A2 cells or HLA-A\*0201<sup>-</sup> 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 <sup>51</sup>Cr-release assays. GANMO-1, HLA-A\*0201<sup>+</sup>; MEG01 and K562, HLA-A\*0201<sup>-</sup>. Expression of AURKA mRNA and AURKA protein in these leukemia cell lines is shown in supplemental Figure 2. E/T indicates effector:target ratio. (F) Construction of a novel retroviral vector encoding full-length AURKA-specific TCR  $\alpha$  and  $\beta$  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.

**Functional reconstitution of the AURKA<sub>207-215</sub>-specific TCR heterodimer in Jurkat/MA cells**

To validate the functionality of the cloned TCR genes, both chains were expressed in the TCR<sup>-</sup> cell line Jurkat/MA/CD8 $\alpha$ /luc, which contains a luciferase reporter gene to monitor TCR signaling (Figure 2A). AUR-2TCR-transduced, V $\beta$ 12-selected Jurkat/MA/CD8 $\alpha$ /luc cells (Figure 2B) were incubated with C1R-A2 cells pulsed with a range of AURKA<sub>207-215</sub> peptide concentrations, then assayed for luciferase activity. The TCR gene-modified Jurkat/MA/CD8 $\alpha$ /luc cells produced luciferase in response to stimulation with AURKA<sub>207-215</sub> peptide-loaded C1R-A2 cells in a dose-dependent

manner (Figure 2C). 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  $\alpha/\beta$ , CD3, CD8 $\alpha$ , 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  $\alpha/\beta$ , CD3 and CD8 $\alpha$  compared with both similarly activated normal CD8<sup>+</sup> 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.

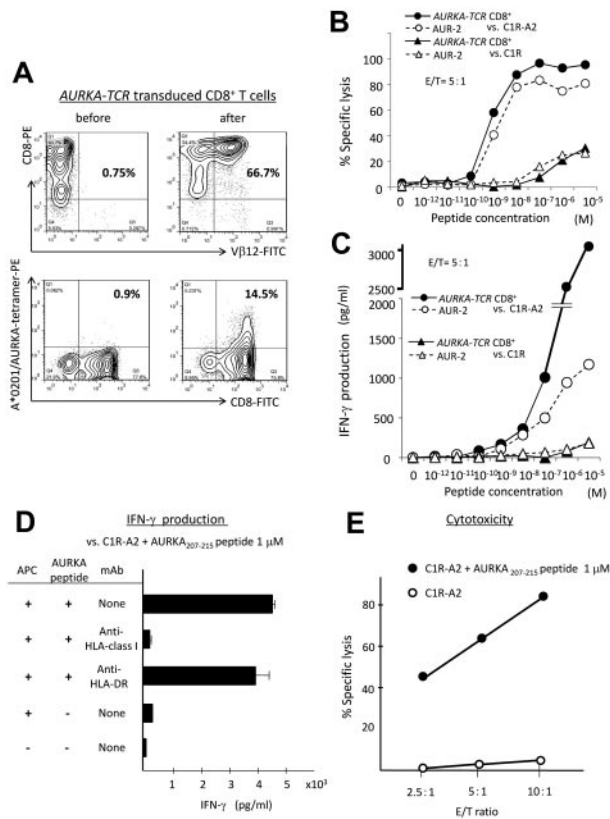


**Figure 2. Functional retroviral expression of the AURKA<sub>207-215</sub>-specific TCR.** (A) Schematic representation of the luciferase assay using AURKA<sub>207-215</sub>-specific TCR-transduced Jurkat/MA cells. NFAT indicates nuclear factor activated T cells; and NK, natural killer. (B) AURKA<sub>207-215</sub>-specific TCR-transduced Jurkat/MA cells express V $\beta$ 12 but label poorly with cognate tetramer (data not shown), probably because of the low levels of surface CD8 $\alpha$  expression. (C) AURKA<sub>207-215</sub>-specific TCR-transduced Jurkat/MA cells were stimulated with peptide-pulsed C1R-A2 cells as shown and subjected to luciferase assay. Error bars represent SDs.

**AURKA<sub>207-215</sub>-specific TCR gene-transduced CD8<sup>+</sup> T cells exert antileukemia reactivity in vitro**

Next, the AURKA<sub>207-215</sub>-specific TCR was retrovirally introduced into normal CD8<sup>+</sup> T cells. Transduction efficiency determined by V $\beta$ 12 staining of TCR gene-modified T cells was 50%-70% (data not shown), and 20%-25% of the V $\beta$ 12<sup>+</sup> cells stained with the HLA-A\*0201/AURKA<sub>207-215</sub> tetramer (Figure 3A). Isolated V $\beta$ 12<sup>+</sup> AURKA<sub>207-215</sub>-specific TCR gene-transduced CD8<sup>+</sup> T cells displayed similar antigen sensitivity to the parental AUR-2 CTL clone (Figure 3B-C). Notably, however, the AURKA<sub>207-215</sub>-specific TCR transductants produced higher quantities of IFN- $\gamma$  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 AURKA<sub>207-215</sub>-specific TCR gene transfectants.

AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells displayed HLA class I-restricted, peptide-dependent IFN- $\gamma$  production (Figure 3D), and target epitope-specific cytotoxic activity (Figure 3E). Furthermore, these redirected CD8<sup>+</sup> T cells selectively lysed the HLA-A\*0201<sup>+</sup> leukemia cell line GANMO-1, which overexpresses AURKA, but not the HLA-A\*0201<sup>-</sup> leukemia cell lines, MEG01, KAZZ, and OUN-1, which also overexpress AURKA (Figure 4A, supplemental Figure 2). In contrast,



**Figure 3. AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells display epitope-specific functionality.** (A) Representative flow cytometry plots showing staining of AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells with anti-TCR Vβ12 mAb and HLA-A\*0201/AURKA<sub>207-215</sub> tetramer. (B) The same AURKA<sub>207-215</sub> TCR-transduced CD8<sup>+</sup> T cells shown in panel A were tested in <sup>51</sup>Cr-release assays against C1R (negative control) and C1R-A2 cells pulsed with the indicated concentrations of AURKA<sub>207-215</sub> peptide. The parental AUR-2 CTL clone was tested in parallel. E/T indicates effector:target ratio. (C) IFN-γ production by AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells was measured in a similar format to that described for panel B. (D) Effects of HLA class I and class II blockade on the production of IFN-γ by AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells stimulated with cognate peptide-loaded (1 μM) C1R-A2 cells. (E) Cytotoxic activity of AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells against unpulsed or cognate peptide-loaded (1 μM) C1R-A2 cells as a function of effector:target (E/T) ratio. Error bars represent SDs.

HLA-A\*0201<sup>+</sup> PBMCs (n = 3), PHA-stimulated lymphoblasts representing highly mitotic normal cells (n = 3), and normal cord blood CD34<sup>+</sup> cells (CB-CD34<sup>+</sup> cells; n = 2) were not lysed by these AURKA<sub>207-215</sub>-specific TCR transductants (Figure 4B). AURKA mRNA expression relative to K562 for each group (mean ± SD) was 0.02 ± 0.008 for PBMCs, 0.25 ± 0.005 for PHA-lymphoblasts and 0.21 ± 0.09 for CB-CD34<sup>+</sup> cells, which indicated relatively low expression levels of AURKA mRNA among these cells compared with K562. The cytotoxic activity of AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells against GANMO-1 and cognate peptide-loaded B-LCLs was significantly diminished by an anti-HLA class-I mAb but not by an anti-HLA-DR mAb (Figure 4C-D). To confirm recognition of the endogenously processed AURKA<sub>207-215</sub> epitope in the context of HLA-A\*0201 expressed by leukemia cells, we retrovirally transduced the HLA-A\*0201 gene into MEG01 cells (MEG01-A2; Figure 4E). Parental MEG01 cells do not express HLA-A\*0201, but abundantly overexpress both AURKA mRNA and AURKA protein (supplemental Figure 2). Compared with MEG01, MEG01-A2 were susceptible to the cytotoxic effects of AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells (Figure 4F). Collectively, these data indicate that the antileukemia reactivity

mediated by AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells occurs through the recognition of endogenously processed and presented AURKA<sub>207-215</sub> peptide in the context of HLA-A\*0201 on the surface of leukemia cells.

Next, the antileukemia reactivity mediated by these redirected AURKA<sub>207-215</sub>-specific CD8<sup>+</sup> T cells was tested against freshly isolated leukemia cells in vitro (Figure 5). AURKA mRNA was overexpressed in all 6 leukemia samples as determined by qRT-PCR. Leukemia cells isolated from HLA-A\*0201<sup>+</sup> patients (1-3), but not HLA-A\*0201<sup>-</sup> patients (4-6) were lysed by the AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells. Thus, our data show that the AUR-2 TCR confers AURKA<sub>207-215</sub> specificity to donor CD8<sup>+</sup> T cells transduced with both TCR chains, and that normal cells, including actively cycling cells and hematopoietic progenitor cells, are not lysed by these redirected T cells.

### AURKA<sub>207-215</sub>-specific TCR-transduced CD4<sup>+</sup> T cells respond to cognate antigen

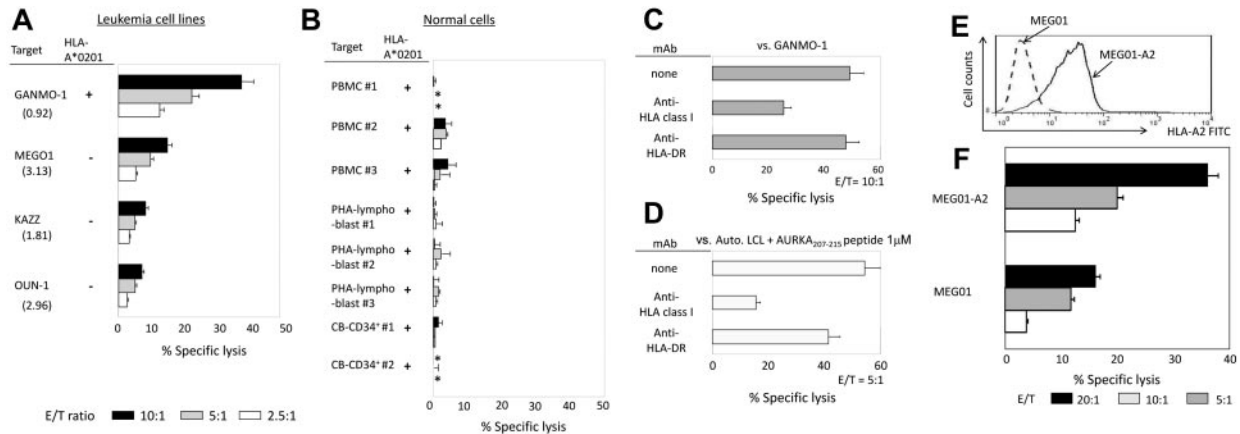
Next, we examined antigen reactivity in isolated populations of Vβ12<sup>+</sup> AURKA<sub>207-215</sub>-specific TCR-transduced CD4<sup>+</sup> T cells (Figure 6A). AURKA<sub>207-215</sub>-specific TCR-transduced CD4<sup>+</sup> T cells successfully produced IFN-γ in response to stimulation with AURKA<sub>207-215</sub> peptide-loaded C1R-A2 cells; this response was substantially reduced by HLA class I blockade, and a partial response reduction was also observed with HLA class II blockade (Figure 6B). These redirected CD4<sup>+</sup> T cells did not express Foxp3, which is a key molecular signature of regulatory T cells (supplemental Figure 3A), and the cognate antigen-specific proliferative response of AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells was actually enhanced in the presence of redirected CD4<sup>+</sup> T cells but not in the presence of non-gene-modified CD4<sup>+</sup> T cells (supplemental Figure 3B). Furthermore, AURKA<sub>207-215</sub>-specific TCR-transduced CD4<sup>+</sup> 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 AURKA<sub>207-215</sub>-specific TCR-transduced CD4<sup>+</sup> 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 AURKA<sub>207-215</sub>-specific IFN-γ production.

### AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells exhibit antileukemia reactivity in vivo

The in vivo antileukemia reactivity of AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> 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 × 10<sup>6</sup>) and either 2.5 × 10<sup>7</sup> AURKA<sub>207-215</sub>-specific TCR gene-modified or non-gene-modified CD8<sup>+</sup> T cells; 5 weekly infusions of the respective CD8<sup>+</sup> T-cell populations (5 × 10<sup>6</sup> cells per infusion) were subsequently administered. Treatment with AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> 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<sup>+</sup> T cells did not prohibit leukemia growth. In a therapeutic adoptive transfer model, intravenously injected AURKA<sub>207-215</sub>-specific TCR-transduced



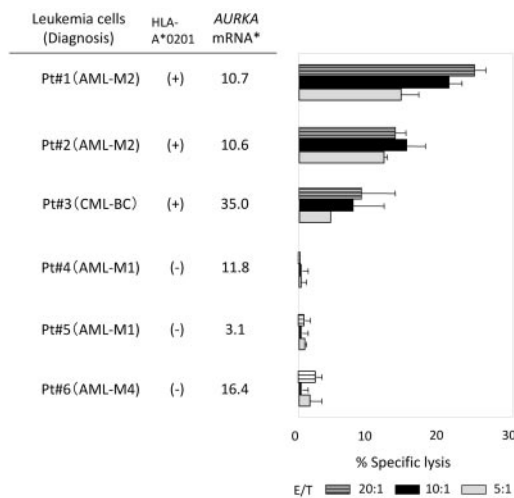
**Figure 4.** *AURKA*<sub>207-215</sub>-specific *TCR*-transduced CD8<sup>+</sup> T cells can distinguish leukemia cells from normal cells on the basis of *AURKA* expression levels. (A) *AURKA*<sub>207-215</sub>-specific *TCR*-transduced CD8<sup>+</sup> T cells exhibit antileukemia reactivity in an HLA-A\*0201-dependent fashion. The HLA-A\*0201<sup>+</sup> leukemia cell line GANMO-1 was lysed by *AURKA*<sub>207-215</sub>-specific *TCR*-transduced CD8<sup>+</sup> T cells as a function of effector:target (E/T) ratio; no significant lysis was observed with the HLA-A\*0201<sup>-</sup> 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 *AURKA*<sub>207-215</sub>-specific *TCR*-transduced CD8<sup>+</sup> T cells used in panel A at the same E/T ratios were tested in <sup>51</sup>Cr-release assays for potentially damaging effects against normal cells. No significant lysis was observed with HLA-A\*0201<sup>+</sup> PBMCs (n = 3), PHA-lymphoblasts representing normal mitotic cells (n = 3) or normal cord blood–derived CD34<sup>+</sup> cells (CB-CD34<sup>+</sup>) 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<sup>+</sup> cells (\* indicates less than detectable). (C) Effects of HLA class I and class II blockade on the cytotoxic activity of *AURKA*<sub>207-215</sub>-specific *TCR*-transduced CD8<sup>+</sup> 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 *AURKA*<sub>207-215</sub> peptide (1 μM). (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 *AURKA*<sub>207-215</sub>-specific *TCR*-transduced CD8<sup>+</sup> T cells confirms recognition of endogenously processed *AURKA*<sub>207-215</sub> peptide presented in the context of HLA-A\*0201. E/T indicates effector:target ratio. Error bars represent SDs.

CD8<sup>+</sup> T cells, but not non–gene-modified CD8<sup>+</sup> 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<sup>+</sup> T cells died by day 85. 2 mice treated with *AURKA*<sub>207-215</sub>-specific *TCR*-transduced CD8<sup>+</sup> 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 progres-

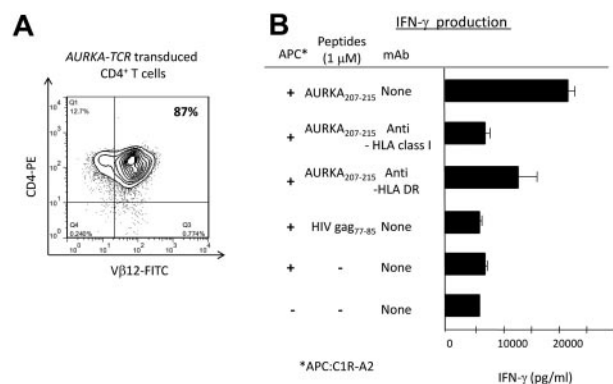
sion. Collectively, these observations indicate that *AURKA*<sub>207-215</sub>-specific *TCR*-transduced CD8<sup>+</sup> T cells exhibit antileukemia reactivity in vivo.

### Discussion

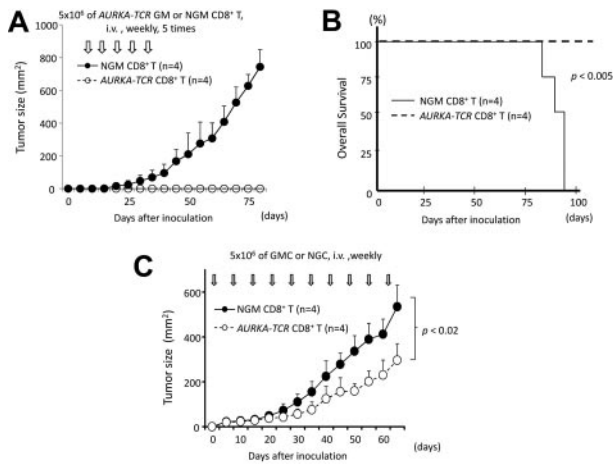
In the setting of hematologic malignancies, *TCR* gene therapy targeting WT1 in leukemia,<sup>30</sup> and *chimeric antigen receptor (CAR)* gene therapy targeting CD33 in myeloid leukemias<sup>31</sup> and CD19, CD20, CD22, CD30, and the receptor tyrosine kinase-like orphan receptor 1 (ROR1) in B-cell malignancies,<sup>32-38</sup> are currently being investigated in preclinical studies or in early phase clinical trials.



**Figure 5.** *AURKA*<sub>207-215</sub>-specific *TCR*-transduced CD8<sup>+</sup> T cells kill freshly isolated leukemia cells in vitro. Freshly isolated HLA-A\*0201<sup>+</sup> (n = 3) or HLA-A\*0201<sup>-</sup> (n = 3) acute or chronic myeloid leukemia cells overexpressing *AURKA* mRNA were used as targets in <sup>51</sup>Cr-release assays with *AURKA*<sub>207-215</sub>-specific *TCR*-transduced CD8<sup>+</sup> 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 ΔCt method). Error bars represent SDs.



**Figure 6.** *AURKA*<sub>207-215</sub>-*TCR* transduced CD4<sup>+</sup> T cells display antigen-specific Th1 cytokine production. (A) A representative flow cytometry plot showing surface Vβ12 expression by *AURKA*<sub>207-215</sub>-specific *TCR*-transduced CD4<sup>+</sup> T cells. (B) *AURKA*<sub>207-215</sub>-*TCR* transduced CD4<sup>+</sup> T cells produce IFN-γ in response to cognate peptide-loaded (1 μM) 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.



**Figure 7.** AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells mediate antileukemia reactivity in vivo. (A) Winn assay: tumor suppression curve. NOG mice were coinjected with GANMO-1 cells ( $5 \times 10^6$ ) and either  $2.5 \times 10^7$  AURKA<sub>207-215</sub>-specific TCR gene-modified (AURKA-TCR) or non-gene-modified (NGM) CD8<sup>+</sup> T cells ( $n = 4$  group). Subsequently, 5 weekly infusions of the respective CD8<sup>+</sup> T-cell populations ( $5 \times 10^6$  cells per infusion) were administered intravenously. Tumor growth was monitored every 5 days. (B) Winn assay: survival curve. Treatment with AURKA<sub>207-215</sub>-specific TCR gene-modified (AURKA-TCR) CD8<sup>+</sup> T cells significantly prolonged survival ( $P < .005$ ). (C) Therapeutic adoptive transfer model. NOG mice ( $n = 4$  per group) were inoculated with  $5 \times 10^6$  of GANMO-1 cells. Intravenous administration of either  $5 \times 10^6$  AURKA<sub>207-215</sub>-specific TCR gene-modified (AURKA-TCR) or non-gene-modified (NGM) CD8<sup>+</sup> T cells commenced on the same day and was continued weekly thereafter. Therapeutic infusions of AURKA<sub>207-215</sub>-specific TCR gene-modified CD8<sup>+</sup> T cells significantly suppressed tumor growth ( $P < .02$ ). 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.<sup>39</sup> This concept is further supported by a recent study of NY-ESO-1-specific TCR gene transfer.<sup>19</sup> 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.<sup>19</sup> 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.<sup>13</sup> Furthermore, we found that AURKA mRNA is overexpressed in a large proportion of freshly isolated human leukemia cells.<sup>6</sup> However, in normal tissues, AURKA mRNA expression is largely limited to the testis.<sup>13</sup> Subsequently, we identified an immunogenic nonamer epitope derived from AURKA that was presented in the context of HLA-A\*0201.<sup>6</sup> 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 AURKA<sub>207-215</sub>-specific CD8<sup>+</sup> T-cell clone (AUR-2). Expression of this TCR in CD8<sup>+</sup> T cells conferred antileukemia reactivity both in vitro and in a xenogeneic mouse model of human leukemias. Furthermore, CD4<sup>+</sup> T cells could be redirected using this TCR to recognize the same HLA-A\*0201-restricted AURKA<sub>207-215</sub> epitope.

This represents a potentially important advantage, as the same TCR could redirect both helper (CD4<sup>+</sup>) and cytotoxic (CD8<sup>+</sup>) functions within the transduced T-cell population, which might sustain the antileukemia response in vivo after adoptive transfer.

Redirected CD8<sup>+</sup> T cells expressing the TCR cloned from AUR-2 displayed similar levels of functional sensitivity to the parental CTL clone. In vitro, AURKA<sub>207-215</sub>-specific TCR gene-transduced CD8<sup>+</sup> T cells were able to lyse HLA-A\*0201<sup>+</sup> human leukemia line GANMO-1 cells, which overexpress AURKA mRNA, and freshly isolated leukemia cells from HLA-A\*0201<sup>+</sup> patients. This antileukemia reactivity was implemented through recognition of the endogenously processed AURKA<sub>207-215</sub> epitope presented in the context of HLA-A\*0201. Importantly, these AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells did not lyse HLA-A\*0201<sup>+</sup> normal PBMCs, mitotic PHA-lymphoblasts or cord blood CD34<sup>+</sup> cells; these data suggest that on-target adverse effects would be minimal in clinical applications. Furthermore, we demonstrated the efficacy of AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> 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 AURKA<sub>207-215</sub>-specific TCR-transduced CD8<sup>+</sup> T cells in vivo, as is the case with selective AURKA inhibitors.<sup>40</sup> However, our observations suggest that redirected CD8<sup>+</sup> 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.<sup>6,41</sup> Recently, targeting leukemia stem cells has been highlighted as a treatment strategy to prevent disease progression in a durable fashion.<sup>42</sup> Monoclonal antibodies that target leukemia stem cell surface antigens have been proposed for this purpose. Examples of such molecules include CD123 (IL3R $\alpha$ )<sup>43</sup> and TIM-3.<sup>44</sup> 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.<sup>45</sup> Indeed, we have cloned an HLA-A\*2402-restricted WT1<sub>235-243</sub>-specific TCR gene into our unique si-TCR vector to address the potential of this approach.<sup>46</sup> With respect to AURKA, we previously described that the CD34<sup>+</sup>CD38<sup>-</sup> 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.<sup>6</sup> 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<sup>+</sup> T cells is one such approach.<sup>24,47,48</sup> To date, the adoptive transfer of redirected CD4<sup>+</sup> T cells concurrently with CD8<sup>+</sup> 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 AURKA<sub>207-215</sub> TCR-transduced CD4<sup>+</sup> T cells displayed Th1 cytokine production in response to the HLA-A\*0201/AURKA<sub>207-215</sub> 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.<sup>49</sup> The feasibility of this combination strategy using AURKA<sub>207-215</sub> peptide vaccination is currently under investigation.

In summary, we have demonstrated the feasibility of antileukemia adoptive therapy using AURKA-specific TCR gene transfer. As AURKA is also overexpressed in diverse solid tumors,<sup>4</sup> 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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## References

- Marumoto T, Zhang D, Saya H. Aurora-A—a guardian of poles. *Nat Rev Cancer*. 2005;5(1):42-50.
- Carmena M, Earnshaw WC. The cellular geography of aurora kinases. *Nat Rev Mol Cell Biol*. 2003;4(11):842-845.
- Bischoff JR, Anderson L, Zhu Y, et al. A homologue of *Drosophila* aurora kinase is oncogenic and amplified in human colorectal cancers. *EMBO J*. 1998;17(11):3052-3065.
- Gautschi O, Heighway J, Mack PC, Pumell PR, Lara PN Jr, Gandara DR. Aurora kinases as an anticancer targets. *Clin Cancer Res*. 2008;14(6):1639-1648.
- Ikezoe T, Yang J, Nishioka C, et al. A novel treatment strategy targeting Aurora kinases in acute myelogenous leukemia. *Mol Cancer Ther*. 2007;6(6):1851-1857.
- Ochi T, Fujiwara H, Suemori K, et al. Aurora-A kinase: a novel target of cellular immunotherapy for leukemia. *Blood*. 2009;113(1):66-74.
- Ewart-Toland A, Briassoulis P, de Koning JP, et al. Identification of *Stk6/STK15* as a candidate low-penetrance tumor-susceptibility gene in mouse and human. *Nat Genet*. 2003;34(4):403-412.
- Xu HT, Ma L, Qi FJ, et al. Expression of serine threonine kinase 15 is associated with poor differentiation in lung squamous cell carcinoma and adenocarcinoma. *Pathol Int*. 2006;56(7):375-380.
- Boss DS, Beijnen JH, Schellens JH. Clinical experience with aurora kinase inhibitors: a review. *Oncologist*. 2009;14(8):780-793.
- Dees EC, Infante JR, Cohen RB, et al. Phase 1 study of MLN8054, a selective inhibitor of Aurora A kinase in patients with advanced solid tumors. *Cancer Chemother Pharmacol*. 2011;67(4):945-954.
- Steehns N, Eskens FA, Gelderblom H, et al. Phase I pharmacokinetic and pharmacodynamic study of the aurora kinase inhibitor danusertib in patients with advanced or metastatic solid tumors. *J Clin Oncol*. 2009;27(30):5094-5101.
- Traynor AM, Hewitt M, Liu G, et al. Phase I dose escalation study of MK-0457, a novel Aurora kinase inhibitor, in adult patients with advanced solid tumors. *Cancer Chemother Pharmacol*. 2011;67(2):305-314.
- Hamada M, Yakushiji Y, Ohtsuka M, Kakimoto M, Yasukawa M, Fujita S. Aurora2/BTAK/STK15 is involved in cell cycle checkpoint and cell survival of aggressive non-Hodgkin's lymphoma. *Br J Haematol*. 2003;121(3):439-447.
- Kobayashi H, Azumi M, Hayashi S, et al. Characterization of human CD4 helper T cell responses against Aurora kinase A. *Cancer Immunol Immunother*. 2010;59(7):1029-1039.
- Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science*. 2006;314(5796):126-129.
- Johnson LA, Morgan RA, Dudley ME, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. *Blood*. 2009;114(3):535-546.
- Bendle GM, Linnemann C, Hooijkaas AI, et al. Lethal graft-versus-host disease in mouse models of T cell receptor gene therapy. *Nat Med*. 2010;16(5):565-570.
- Okamoto S, Mineno J, Ikeda H, et al. Improved expression and reactivity of transduced tumor-specific TCRs in human lymphocytes by specific silencing of endogenous TCR. *Cancer Res*. 2009;69(23):9003-9011.
- Robbins PF, Morgan RA, Feldman SA, et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J Clin Oncol*. 2011;29(7):917-924.
- Calogero A, Hospers GA, Kruse KM, et al. Retargeting of a T cell line by anti MAGE-3/HLA-A2 alpha beta TCR gene transfer. *Anticancer Res*. 2000;20(3A):1793-1799.
- Price DA, Brenchly JM, Ruff LE, et al. Avidity for antigen shapes clonal dominance in CD8+ T cell populations specific for persistent DNA viruses. *J Exp Med*. 2005;202(10):1349-1361.
- Kuzushima K, Hayashi N, Kimura H, Tsurumi T. Efficient identification of HLA-A\*2402-restricted cytomegalovirus-specific CD8+ T-cell epitopes by a computer algorithm and an enzyme-linked immunospot assay. *Blood*. 2001;98(6):1872-1881.
- Ohnami H, Yasukawa M, Fujita S. HLA class I-restricted lysis of leukemia cells by a CD8+ cytotoxic T-lymphocyte clone specific for WT1 peptide. *Blood*. 2000;95(1):286-293.
- Tsuji T, Yasukawa M, Matsuzaki J, et al. Generation of tumor-specific, HLA class I-restricted human Th1 and Tc1 cells by cell engineering with tumor peptide-specific T-cell receptor genes. *Blood*. 2005;106(2):470-476.
- Folch G, Scaviner D, Contet V, Lefranc MP. Protein displays of the human T cell receptor alpha, beta, gamma and delta variable and joining regions. *Exp Clin Immunogenet*. 2000;17(4):205-215.
- Arden B, Clark SP, Kabelitz D, Mak TW. Human T-cell receptor variable gene segment families. *Immunogenetics*. 1995;42(6):455-500.
- Jedema I, van der Werff NM, Barge RM, Willemze R, Falkenburg JH. New CFSE-based assay to determine susceptibility to lysis by cytotoxic T cells of leukemic precursor cells within a heterogeneous target cell population. *Blood*. 2004;103(7):2677-2682.
- Yasukawa M, Ohnami H, Arai J, Kasahara Y, Ishida Y, Fujita S. Granule exocytosis, and not the fas/fas ligand system, is the main pathway of cytotoxicity mediated by alloantigen-specific CD4+ as well as CD8+ cytotoxic T lymphocytes in humans. *Blood*. 2000;95(7):2352-2355.
- Ito M, Hiramatsu H, Kobayashi K, et al. NOD/SCID/gamma(c) (null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood*. 2002;100(9):3175-3182.
- Xue SA, Gao L, Hart D, et al. Elimination of human leukemia cells in NOD/SCID mice by WT1-TCR gene-transduced human T cells. *Blood*. 2005;106(9):3062-3067.
- Finney HM, Akbar AN, Lawson AD. Activation of



- resting human primary T cells with chimeric receptors: costimulation from CD28, inducible costimulator, CD134, and CD137 in series with signals from the TCR zeta chain. *J Immunol*. 2004; 172(1):104-113.
32. Brentjens RJ, Santos E, Nikhamin Y, et al. Genetically targeted T cells eradicate systemic acute lymphoblastic leukemia xenografts. *Clin Cancer Res*. 2007;13(18 Pt 1):5426-5435.
  33. Hollyman D, Stefanski J, Przybylowski M, et al. Manufacturing validation of biologically functional T cells targeted to CD19 antigen for autologous adoptive cell therapy. *J Immunother*. 2009;32(2):169-180.
  34. Wang J, Press OW, Lindgren CG, et al. Cellular immunotherapy for follicular lymphoma using genetically modified CD20-specific CD8<sup>+</sup> cytotoxic T lymphocytes. *Mol Ther*. 2004;9(4):577-586.
  35. James SE, Greenberg PD, Jensen MC, et al. Antigen sensitivity of CD22-specific chimeric TCR is modulated by target epitope distance from the cell membrane. *J Immunol*. 2008;180(10):7028-7038.
  36. Hombach A, Mucic JM, Gerken M, et al. T cells engrafted with a recombinant anti-CD30 receptor target autologous CD30<sup>+</sup> cutaneous lymphoma cells. *Gene Ther*. 2001;8(11):891-895.
  37. Hudecek M, Schmitt TM, Baskar S, et al. The B-cell tumor-associated antigen ROR1 can be targeted with T cells modified to express a ROR1-specific chimeric antigen receptor. *Blood*. 2010; 116(22):4532-4541.
  38. Till BG, Jensen MC, Wang J, et al. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. *Blood*. 2008;112(6):2261-2271.
  39. Offringa R. Antigen choice in adoptive T-cell therapy of cancer. *Curr Opin Immunol*. 2009; 21(2):190-199.
  40. Moore AS, Blagg J, Linardopoulos S, Pearson ADJ. Aurora kinase inhibitors: novel small molecules with promising activity in acute and Philadelphia-positive leukemias. *Leukemia*. 2010;24: 671-678.
  41. Ye D, Garcia-Manero G, Kantarjian HM, et al. Analysis of Aurora kinase A expression in CD34<sup>+</sup> blast cells isolated from patients with myelodysplastic syndromes and acute myeloid leukemia. *J Hematop*. 2009;2(1):2-8.
  42. Misaghian N, Ligresti G, Steelman LS, et al. Targeting the leukemic stem cell: the Holy Grail of leukemia therapy. *Leukemia*. 2009;23(1):25-42.
  43. Jin L, Lee EM, Ramshaw HS, et al. Monoclonal antibody-mediated targeting of CD123, IL-3 receptor alpha chain, eliminates human acute myeloid leukemic stem cells. *Cell Stem Cell*. 2009; 5(1):31-42.
  44. Kikushige Y, Shima T, Takayanagi S, et al. TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells. *Cell Stem Cell*. 2010; 7(6):708-717.
  45. Saito Y, Kitamura H, Hijikata A, et al. Identification of therapeutic targets for quiescent, chemotherapy-resistant human leukemia stem cells. *Sci Transl Med*. 2010;2(17):17ra9.
  46. Ochi T, Fujiwara H, Okamoto S, et al. Novel adoptive T-cell immunotherapy using a WT1-specific TCR vector encoding silencers for endogenous TCRs show marked antileukemia reactivity and safety. *Blood*. 2011;118(6):1495-1503.
  47. Morris EC, Tsallios A, Bendle GM, Xue SA, Stauss HJ. A critical role of T cell antigen receptor-transduced MHC class I-restricted helper T cells in tumor protection. *Proc Natl Acad Sci U S A*. 2005;102(22):7934-7939.
  48. Ray S, Chhabra A, Chakraborty NG, et al. MHC-I-restricted melanoma antigen specific TCR-engineered human CD4<sup>+</sup> T cells exhibit multifunctional effector and helper responses, in vitro. *Clin Immunol*. 2010;136(3):338-347.
  49. Ly LV, Sluijter M, Versluis M, et al. Peptide vaccination after T-cell transfer causes massive clonal expansion, tumor eradication, and manageable cytokine storm. *Cancer Res*. 2010;70(21):8339-8346.