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Clinical Cancer Research

# Phase I Trial of Inducible Caspase 9 T Cells in Adult Stem Cell Transplant Demonstrates Massive Clonotypic Proliferative Potential and Long-term Persistence of Transgenic T Cells

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## Abstract

**Purpose:** Inducible caspase 9 (*iCasp9*) is a cellular safety switch that can make T-cell therapy safer. The purpose of this phase I trial was to investigate the use of *iCasp9*-transduced T-cell addback in adult patients undergoing haploidentical stem cell transplantation for high-risk hematologic malignancies.

**Patients and Methods:** Patients undergoing myeloablative, CD34-selected haploidentical stem cell transplantation were treated with $0.5-1.0 \times 10^6$/kg donor-derived *iCasp9*-transduced T cells on day +25 or 26 post-transplant, with additional doses allowed for disease relapse, infection, or mixed chimerism.

**Results:** Three patients were enrolled. *iCasp9*-transduced T cells were readily detectable by 4 weeks post-infusion in all patients and remained at high level (114 cells/μL, 11% of T cells) in 1 patient alive at 3.6 years. One patient developed donor-derived Epstein–Barr virus-associated post-transplant lymphoproliferative disease (EBV-PTLD), which was followed by a marked expansion of *iCasp9* T cells and cytokine release syndrome (CRS). These *iCasp9*-transduced T cells infiltrated the affected lymph nodes and secreted IFNγ and IL-10. They peaked at 1,848 cells/μL and were found to be monoclonal by T-cell receptor (TCR) clonotype and oligoclonal by viral integrant analysis, representing a 6-log *in vivo* expansion of the dominant T-cell clone. These T cells were not autonomous and contracted with the resolution of EBV-PTLD, which did not recur.

**Conclusions:** *iCasp9*-transduced T cells could persist long-term. They retained very high *in vivo* clonotypic proliferative capacity and function, and could cause CRS in response to *de novo* lymphoma development.

## Introduction

Inducible caspase 9 (*iCasp9*) is a cellular safety switch that enables the conditional elimination of gene-modified cells in the setting of adverse events (1). It consists of a human caspase 9 domain that is linked to a drug-binding domain which is derived from FK506-binding protein (1). A nontoxic small-molecule drug, AP1903, induces dimerization of the caspase 9 domains, leading to activation of the intrinsic apoptotic pathway and rapid cell-cycle–independent cell death (1, 2). Proof of concept of *iCasp9* effectiveness as a safety switch was first demonstrated in the setting of T-cell–deplete allogeneic stem cell transplantation in which addback *iCasp9*-transduced donor T cells could be rapidly eliminated in the setting of acute graft-versus-host disease (GVHD), with abrogation of clinical GVHD within 24–48 hours (3–5). Following these promising early findings, *iCasp9* has been incorporated as a safety switch in other forms of T-cell cancer therapy, including chimeric antigen receptor (CAR) T cells (6) and other gene-modified antigen-specific T-cell therapy (7). However, the use of *iCasp9* as a safety strategy is tempered by a theoretical concern that there may be a degree of basal, dimerizer-independent, dimerization of the engineered caspase 9 domains, which can in theory trigger unintended cell death and thus reduce the persistence and proliferative potential of *iCasp9*-transduced cells (8).

We conducted a phase I clinical trial to investigate the use of *iCasp9*-transduced T-cell addback in patients undergoing T-cell–deplete haploidentical stem cell transplantation for high-risk hematologic malignancies. The primary aim was to study the safety of this approach in adult patients receiving uniform myeloablative conditioning because the two phase I clinical trials conducted to date had enrolled predominantly pediatric patients (3–5). Our study was terminated early after the

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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## Translational Relevance

Inducible caspase 9 (*iCasp9*) is increasingly used as a safety switch in clinical T-cell therapy, including chimeric antigen receptor (CAR) and other targeted T-cell therapy. This safety switch, which is activated by a small-molecule dimerizer, has been shown to be highly effective in small early-phase clinical trials, but its widespread use is tempered by a theoretical concern that basal dimerizer–independent dimerization of caspase 9 domains may occur and impair the *in vivo* proliferative capacity and persistence of *iCasp9*-transduced T cells. This phase I trial demonstrates that a single clone of *iCasp9*-transduced T cell can rapidly expand by 6-log fold *in vivo* in response to *de novo* virus-associated lymphoma development, and *iCasp9* T cells can persist in high levels long-term. Our findings provide added reassurance that the *iCasp9* safety switch does not have any deleterious effect on the proliferative capacity or long-term persistence of transduced T cells.

enrollment of 3 patients because of slow recruitment and our transplant unit's transition to a T-cell–replete haploidentical transplant approach using post-transplant cyclophosphamide (9). However, our detailed immunologic analysis has provided critical insights into the long-term persistence and proliferative capacity of *iCasp9*-transduced T cells. We demonstrate that despite prior *in vitro* expansion, *iCasp9*-transduced T cells could nonetheless undergo massive clonotypic proliferation and activation when driven by *de novo* Epstein–Barr virus-associated post-transplant lymphoproliferative disease (EBV-PTLD), resulting in delayed cytokine release syndrome (CRS).

## Patients and Methods

### Study protocol

We conducted a phase I study using dose-escalated addback of *iCasp9*-transduced donor T cells in patients undergoing myeloablative T-cell–deplete haploidentical stem cell transplantation. Patients ages 18–59 years old who lacked a fully HLA-matched donor and had poor-risk hematologic malignancies were eligible. Conditioning consisted of 8 Gy total body irradiation, thiotepa (5 mg/kg/day × 2), fludarabine (40 mg/m$^2$/day × 5), and rabbit antithymocyte globulin (ATG, Thymoglobulin, Sanofi Genzyme; 1.5 mg/kg/day × 4; ref. 10). Rituximab was not used in conditioning. Donor G-CSF–mobilized peripheral blood stem cells were CD34$^+$-selected (CliniMACS Plus, Miltenyi Biotec) and infused on day 0 without post-transplant immunosuppression. On day +21 or later, the patients were infused with a dose of *iCasp9*-transduced donor T cells, with planned dose increment in cohort size of two: $5 \times 10^5$/kg, $1 \times 10^6$/kg, $5 \times 10^6$/kg, and $1 \times 10^7$/kg. Plasmapheresis was performed prior to T-cell addback to remove residual ATG in circulation. Additional *iCasp9*-transduced T-cell addback was allowed for standard indications, including disease relapse, severe infection, and mixed chimerism. Patients who developed acute GVHD grade ≥II were given an infusion of AP1903 at 0.4 mg/kg (Rimiducid, Bellicum Pharmaceuticals; ref. 11). Additional protocol details are provided in Supplementary Materials and Methods. This trial was prospectively approved by the Human Research Ethics Committee (Institutional Review Board) of Royal Brisbane and Women's Hospital. It was conducted in accordance with the Declaration of Helsinki and the Australian National Statement on Ethical Conduct in Human Research. Written informed consent was obtained from both donors and recipients. The trial was prospectively registered at www.anzctr.org.au as ACTRN12614000290695. It opened in April 2014 and was closed to recruitment in September 2017.

### Cell manufacture

Alloreplete *iCasp9*-transduced T cells were generated at Q-Gen Cell Therapeutics, which is the Good Manufacturing Practice facility at QIMR Berghofer Medical Research Institute (Queensland, Australia), using methods described previously (2–5). In brief, peripheral blood mononuclear cells (PBMC) were activated with immobilized CD3 antibody (clone OKT3, Miltenyi Biotec) and transduced with SFG.iCasp9.2A.dCD19 retroviral vector (Baylor College of Medicine, Houston, TX). The cells were enriched for CD19 by immunomagnetic selection (CliniMACS Plus, Miltenyi Biotec), further expanded, and cryopreserved. Replication-competent retrovirus screening was performed by qRT-PCR for the gibbon ape leukemia virus (GALV) envelope gene (12) and the inGluc-MLV-DERSE assay (13).

### Flow cytometry

Monoclonal antibodies (mAbs) were obtained from BioLegend and are listed in Supplementary Materials and Methods. For intracellular cytokine staining, cells were stained with Live/Dead Fixable Staining Kit (Molecular Probes) and surface antibodies, then fixed and permeabilized (Transcription Factor Fixation/Permeabilization Kit, eBiosciences), and stained for intracellular cytokines. Flow cytometry was performed on LSR Fortessa (BD Biosciences) and analyzed with FlowJo software v9.9 (FlowJo LLC). Plasma cytokine analysis was performed using Cytometric Bead Array (BD Biosciences).

### Molecular analyses

Viral integration site analysis was performed by inverse PCR followed by sequencing on MinION (Oxford Nanopore Technologies). Detailed methods are provided in Supplementary Materials and Methods. T-cell receptor (TCR) beta clonotype analysis was performed by Adaptive Biotechnologies by deep sequencing of genomic DNA. *iCasp9* copy number was determined by qPCR as described previously (3).

## Results

### Generation of iCasp9 T cells

Cell products were successfully generated for all patients. Median net cell expansion was 25-fold (13–33) within 9 days, which represented 111-fold (65–135) cell proliferation after taking into account cell loss from transduction and CD19 immunomagnetic selection (Supplementary Fig. S1). The final cell products consisted of >95% T cells, of which >90% were CD3$^+$CD19$^+$ *iCasp9* transgenic T cells. Composition of the cell products is detailed in Supplementary Table S1.

### Patient characteristics and outcome

The patient characteristics are detailed in Table 1. The patients received an infusion of *iCasp9* T cells on day +25 or +26 per study protocol: $0.5 \times 10^6$/kg in the first 2 patients and $1.0 \times 10^6$/kg in the third patient. Despite evidence of *iCasp9* T-cell engraftment, all 3 patients received additional doses of *iCasp9* T cells for the

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**Table 1.** Patient characteristics, cell dose, and outcome

| Pt | Age (years), gender | Diagnosis | Disease status | Donor and HLA matching | Graft infused on day 0 | | *iCasp9* T cells - cells/kg (day post-transplant) | Outcome | Current status |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | $CD34^+$/kg | $CD3^+$/kg | | | |
| 1 | 53, M | Secondary AML (transformed MDS) | CR1 | Brother; 5/10 | $6.5 \times 10^6$ | $8.0 \times 10^3$ | $0.5 \times 10^6$ (day +26)<br>$1.0 \times 10^6$ (day +98) | Ongoing remission | Alive in remission (day +1332 ongoing) |
| 2 | 51, F | AML with myelodysplasia-related changes | CR2 | Daughter; 5/10 | $9.8 \times 10^6$ | $3.9 \times 10^4$ | $0.5 \times 10^6$ (day +25)<br>$1.0 \times 10^6$ (day +162)<br>$3.5 \times 10^6$ (day +190) | Relapsed AML (day +161) | Died from relapse (day +303) |
| 3 | 30, F | Philadelphia-positive B-ALL | CR1 | Brother; 6/10 | $12.7 \times 10^6$ | $3.3 \times 10^3$ | $1.0 \times 10^6$ (day +26)<br>$1.0 \times 10^6$ (day +279) | EBV-PTLD (day +61)<br>Wasting syndrome, gut infection (norovirus and microsporidium), probable gut GVHD | Died in remission (day +368) |

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CR, complete remission; MDS, myelodysplastic syndrome.

following indications: mixed chimerism (patient #1), leukemia relapse (patient #2), and recurrent infection (patient #3).

Patients #1 and #2 had uncomplicated cytomegalovirus (CMV) reactivation at 5 weeks post-transplant. In patient #1, a significant proportion of the persisting recipient T cells, identifiable by disparate HLA-A2 expression, were CMV-specific: 66% of CD8 and 10% of CD4 T cells were specific for CMV pp65, IE-1 or glycoprotein B; whereas no CMV-reactivity was detectable within the seronegative donor T-cell compartment (Supplementary Fig. S2A). This provided direct evidence that recipient CMV-specific T cells contribute to mixed chimerism in CMV-disparate transplantation (14). In patient #2, CMV pp65-specific CD8 T cells were present within both $CD19^+$ and $CD19^-$ compartments in similar proportions (Supplementary Fig. S2B), consistent with the retention of anti-viral reactivity in *iCasp9* T cells. This patient died from relapsed leukemia on a background of very high-risk relapsed disease with a first remission duration of only 3 months. Patient #3 developed EBV-PTLD, which will be further discussed below.

### Engraftment and persistence of iCasp9 T cells

Transgenic $CD3^+CD19^+$ T cells could be detected by flow cytometry in all patients within 4 weeks of the first dose of *iCasp9* T-cell infusion and remained detectable throughout the follow-up

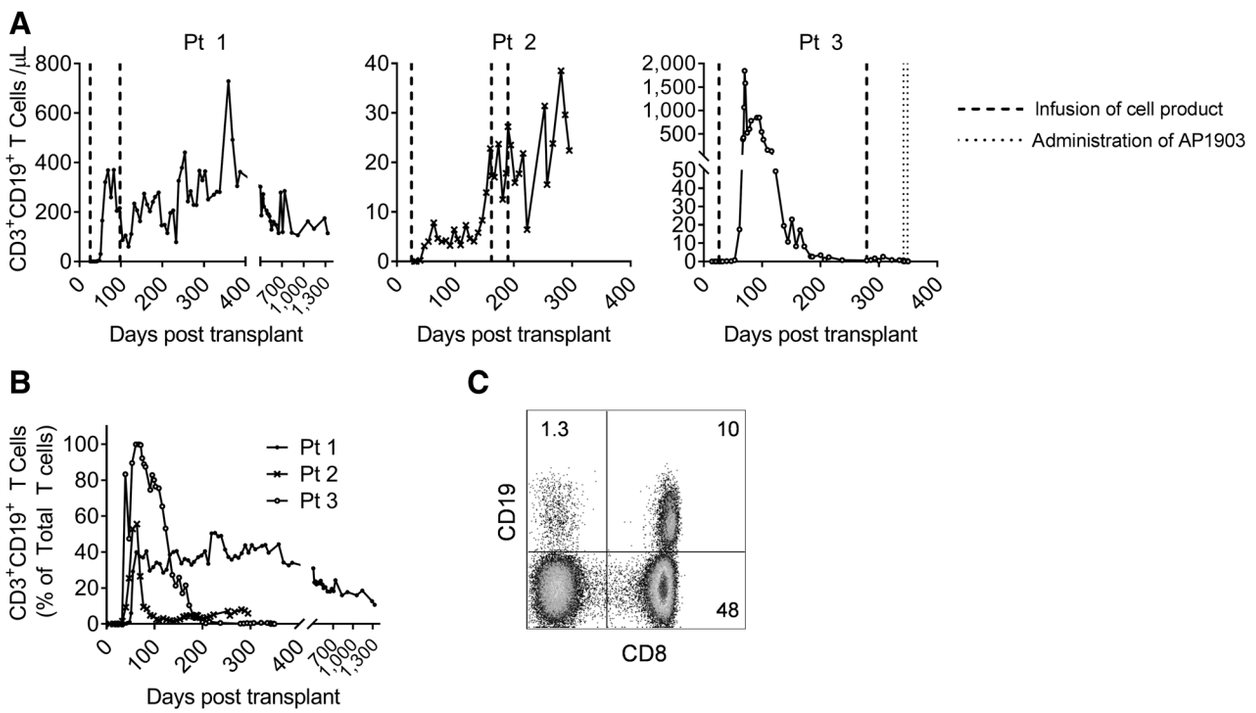


**Figure 1.**

Engraftment of $CD3^+CD19^+$ *iCasp9* transgenic T cells. **A,** Absolute number of $CD3^+CD19^+$ T cells. Dashed lines indicate *iCasp9* T-cell infusion and dotted lines indicate AP1903 administration. **B,** $CD3^+CD19^+$ T cells as a percentage of total T cells. **C,** Flow cytometry plot from patient #1 at day +1332 post-transplant, gated on donor T cells.

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period (Fig. 1A and B). This was corroborated by qPCR for *iCasp9* transgene (Supplementary Fig. S3A). Patient #1 is in ongoing remission and the *iCasp9* T cells have persisted at high level (114 cells/μL, 11% of total T cells) at 3.6 years after transplant (Fig. 1C).

### Massive clonotypic expansion of iCasp9 T cells and CRS in response to EBV-PTLD

Patient #3 developed monomorphic B-cell EBV-PTLD on day +61, which involved lymph nodes, liver, spleen, and bones (Fig. 2A and B). This was confirmed to be donor-derived by XY FISH analysis. The affected lymph nodes demonstrated extensive T-cell infiltration, almost all of which were $CD3^+CD19^+$ *iCasp9* T cells (Fig. 2B and C). There was also marked peripheral blood B-cell ($CD3^-CD19^+$) lymphocytosis, which was closely followed by a sharp rise in $CD8^+CD19^+$ T-cell numbers to a peak of 1,848/μL (Fig. 2C and D). This was accompanied by fevers to 40.5°C, tachycardia, thrombocytopenia, and hepatic transaminitis, which together were consistent with moderate CRS (15). This was corroborated by very high levels of IL-6 (647 pg/mL), IL-10 (460 pg/mL), and IFNγ (10,869 pg/mL; Fig. 2E). The *iCasp9*

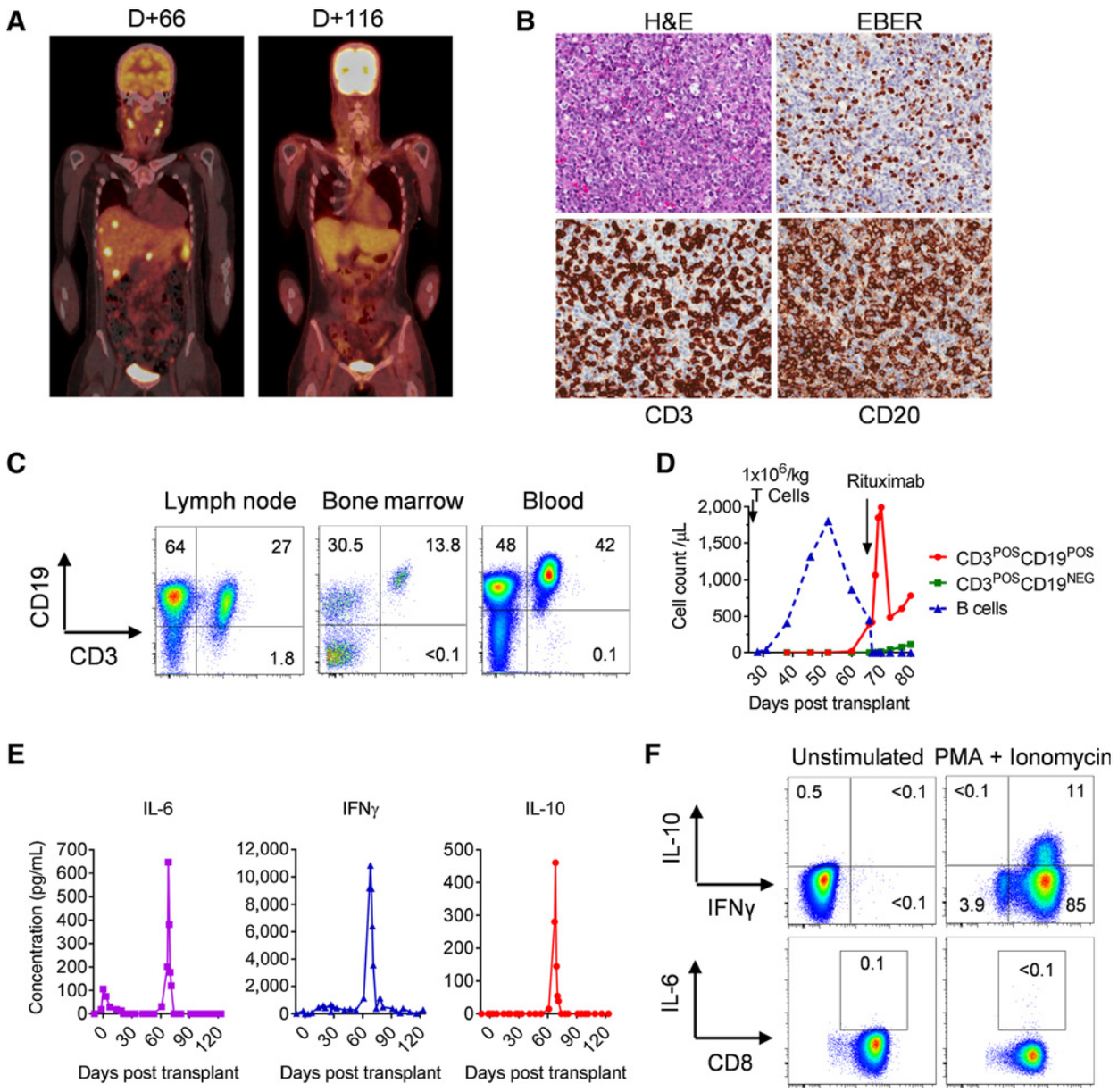


**Figure 2.**

*iCasp9* T-cell expansion and function in response to EBV-PTLD. **A,** PET scan from patient #3 at PTLD diagnosis (day +66 post-transplant) and after disease resolution (day +116 post-transplant). **B,** Lymph node biopsy showing involvement with EBV-PTLD and infiltration by T cells. EBER, EBV-encoded RNA *in situ* hybridization. **C,** Flow cytometry plots showing infiltration of lymph nodes and bone marrow by $CD3^+CD19^+$ T cells, and circulating $CD3^+CD19^+$ T cells. **D,** Peripheral blood lymphocyte counts during course of EBV-PTLD. **E,** Plasma cytokine profile. **F,** Cytokine secretion by $CD3^+CD8^+CD19^+$ T cells during EBV-PTLD at day +70 post-transplant after 4-hour incubation with phorbol 12-myristate 13-acetate (PMA), ionomycin, and brefeldin A.

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T cells secreted IL-10 and IFNγ, but not IL-6 (Fig. 2F). The patient was treated with rituximab and the transgenic T cells contracted with the resolution of PTLD, which did not recur.

Remarkably, the expanded T cells were dominated by a single TCR Vbeta clonotype, which accounted for >97% of TCR clonotype in the lymph node and peripheral blood at peak T-cell expansion, and 72% of TCR clonotype after contraction (Fig. 3A and B). Sequencing of 33,000 FACS-sorted $CD8^+CD19^+$ T cells from the infused cell product yielded 25,879 productive TCR clonotypes, which did not include the expanded T-cell clonotype. Viral integrant analysis showed the insertion sites to be polyclonal in the cell product but oligoclonal in samples taken at peak expansion and post-PTLD contraction, with dominance of one viral integrant site (Fig. 3A and C). On the basis of the infusion of $1 \times 10^6$ T cells/kg and circulating blood volume of around 80 mL/kg, the estimated *in vivo* expansion of the dominant clone was at least $10^6$-fold. The actual degree of expansion was likely much greater when T cells within the lymphoid organs and bone marrow were considered. There was no suggestion of autonomous proliferation and no late resurgence of *iCasp9* T cells in the absence of cognate antigen. We were unable to determine the EBV specificity of the T-cell clone because of insufficient residual samples.

### Elimination of iCasp9 T cells

Patient #3 had very delayed immune reconstitution and received a second infusion of $1 \times 10^6$/kg *iCasp9* T cells on day +279 because of refractory norovirus and microsporidium gut infection. Her abdominal pain and diarrhea worsened 8 weeks later and gut biopsy showed increased apoptosis, raising the possibility of GVHD. AP1903 was administered on day +342 at a time when the transgenic T cells represented <1% of total circulating T cells. The $CD3^+CD19^+$ T-cell numbers measured by flow cytometry fell from 0.7 cells/μL to 0.1 cells/μL 30 minutes after AP1903, which was corroborated by *iCasp9* qPCR, with transgene level falling from 4.5 to 0.3 copies/μL whole blood (Supplementary Fig. S3B and S3C). However, the patient's

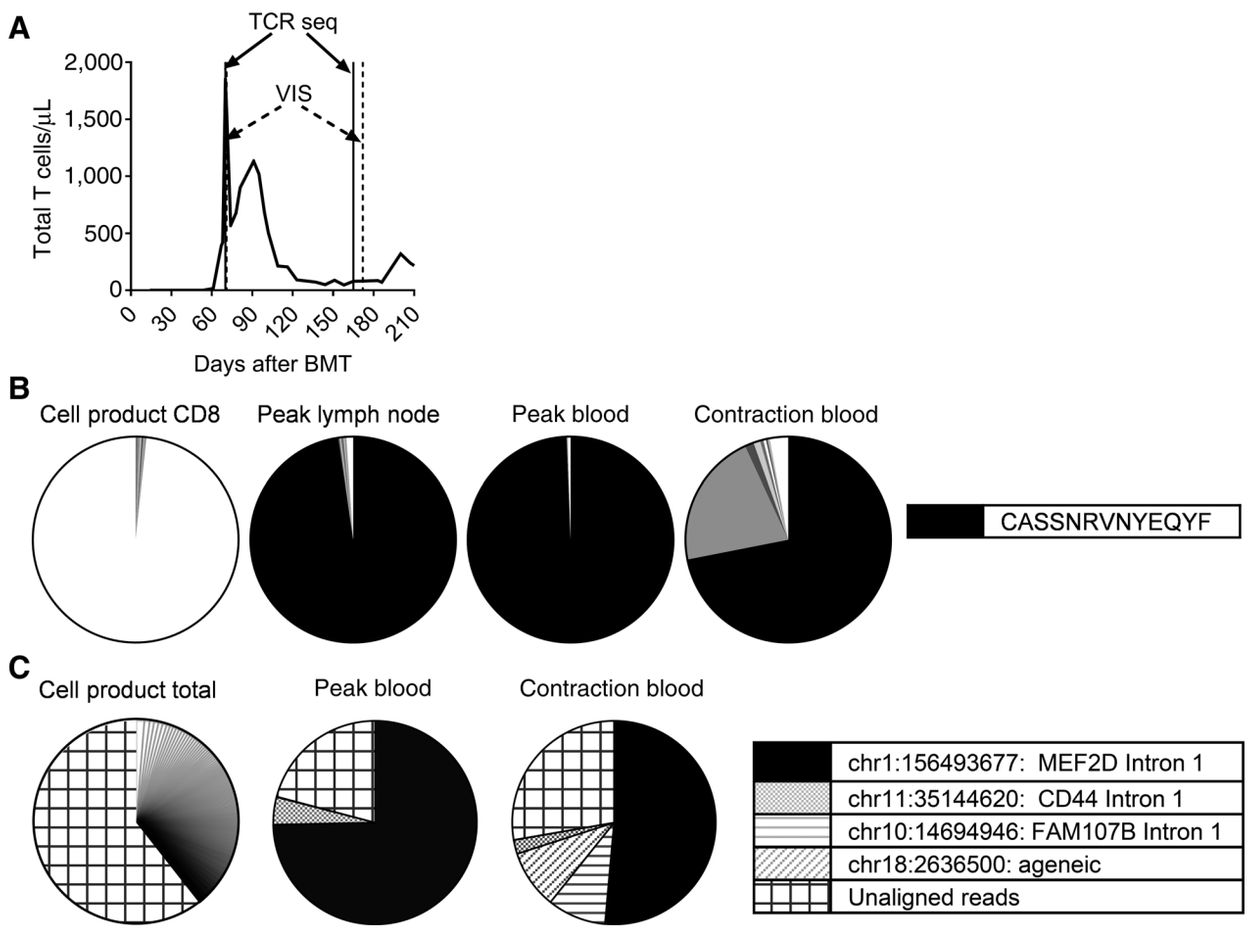


**Figure 3.**

Clonal analysis of *iCasp9* T cells in patient #3. **A,** Time points of sample collection for TCR sequencing and viral integrant analysis (VIS). **B,** TCR beta clonotypes in patient #3: *iCasp9* T-cell product after FACS-sorting for $CD8^+CD19^+$ T cells, lymph node biopsy, and peripheral blood samples at day +70 and +165. The dominant clone found in the patient is shown in black. This clone was not present within the sampled cell product. The top 10 clones in each sample are individually shaded, with the remaining clones shown in white. **C,** Viral integrant analysis of samples from patient #3: cell product, PBMC at peak *iCasp9* T-cell expansion on day +71 post-transplant, and PBMC at contraction of *iCasp9* T cells on day +172 post-transplant. Shown are all reads that spanned both flanking sequences. The dominant clone is shown in black. A proportion of reads could not be aligned to the human genome because of short read-lengths between the flanking sequences and are shown in large squares.

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presumptive GVHD did not respond clinically to this or a second infusion of AP1903.

## Discussion

We conducted a phase I clinical trial to study the use of *iCasp9* T cells in adult patients undergoing a uniform T-cell–deplete myeloablative haploidentical transplant protocol based on high-dose total body irradiation and ATG lymphodepletion (10). This trial was important because published studies had enrolled mainly pediatric patients, with only 3 of 22 subjects older than 18 years old, and only one of whom received myeloablative conditioning (3–5). Our clinical trial was stopped early in part driven by our unit's transition to a T-cell–replete haploidentical transplant strategy with post-transplant cyclophosphamide (9). This T-cell–replete strategy is increasingly used worldwide because of its relative ease and lower cost, but relapse remains a concern and there is at least one planned clinical trial in the United States for using *iCasp9* T-cell addback in this setting.

Our study provided new data on the clinical fate of *iCasp9* T cells. We showed that *iCasp9* T cells could persist long-term and retained massive single-cell proliferative capacity *in vivo* despite prior 2-log *in vitro* expansion. Although *iCasp9* T cells did not prevent the development of a first episode of viral reactivation (4, 5), consistent with the need for antigen exposure to generate sufficient T-cell numbers, they were poised to respond and could expand massively and rapidly. We were unable to attribute clearance of EBV-PTLD wholly to *iCasp9* T cells, but the reduction in circulating B-cell numbers before the administration of rituximab and coincident with *iCasp9* T-cell expansion, and the maintenance of remission until death from other causes, would suggest that the *iCasp9* T cells had some clinical efficacy.

CRS is a well-recognized complication of T-cell transfer and has been described following the infusion of CAR T cells (15, 16), virus-specific T cells (15), and also in T-cell–replete haploidentical transplantation (17). It typically occurs within the first 2 weeks of T-cell infusion and is more common in the setting of large T-cell doses and high tumor burden or antigen load (16, 18), which result in the proliferation and activation of a large number of T cells. Our patient had an atypical pattern of delayed CRS that was not a result of preexisting antigens or large T-cell dose; rather, a very small number of T-cell clones, all bearing identical TCR, had responded vigorously to a rapidly developing *de novo* virus-associated lymphoma. These T cells were highly functional and produced large amounts of IFNγ and IL-10. However, they were not the direct source of IL-6, which was consistent with a recent report suggesting that the latter was largely derived from vascular endothelial cells and other non-T cells (19).

The lack of clinical response in patient #3 despite effective elimination of *iCasp9* T cells raised a number of considerations. The circulating T cells were >99% non-transgenic and the pathogenic gut-infiltrating T cells were likely similarly non-transgenic—confirmation with CD19 immunostaining would have been ideal, but unfortunately all available immunohistochemistry antibodies were directed at the intracellular domain, which was absent in our truncated dCD19. Although AP1903 has been shown to be effective in early visceral GVHD (3, 5), extensive tissue damage could have precluded a clinical response. In order to ensure optimum tissue concentration, we administered a second dose of AP1903, which was also clinically ineffective. Finally, some GVHD are primarily mediated by cytokines rather than cognate T-cell interaction, and our patient also had concurrent refractory gut infection, both of which would not have responded to *iCasp9* T-cell deletion.

The current era of CAR T cells and other antigen-specific T-cell cancer therapy has seen a large number of preclinical research being translated into early-phase clinical trials. These exciting new therapies can cause unexpected adverse events and the use of safety switches to mitigate risks is an important consideration (20). The *iCasp9* safety switch has a number of significant advantages over other safety switches: it is highly effective and acts rapidly, with >90% cell kill within 30 minutes and a further 0.5–1 log cell kill within the next 24 hours (3); it is largely human-derived and hence unlikely to be immunogenic (1, 2); and it does not interfere with the administration of other drugs (20). One of the drawbacks of iCasp9 is a theoretical concern that high level expression of the engineered caspase 9 domain can lead to spontaneous, nondimerizer–induced dimerization, resulting in unintended cell death and therefore a reduced capacity for *in vivo* proliferation or long-term persistence (8). Our data showed that this is unlikely to be a significant clinical concern as *iCasp9* gene modification is compatible with durable and functional engraftment of T cells.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Authors' Contributions

**Conception and design:** G.A. Kennedy, G.R. Hill, S.-K. Tey
**Development of methodology:** P. Zhang, M.P. Brown, L.J. Coin, C.J. Hutchins, G.A. Kennedy, G.R. Hill, S.-K. Tey
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**Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):** P. Zhang, J. Raju, M.A. Ullah, A. Varelias, L.J. Coin, D. Ganesamoorthy, A.J. Morton, S.-K. Tey
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**Study supervision:** P. Zhang, G.A. Kennedy, S.-K. Tey
**Other (recruitment of patients):** A.J. Morton
**Other (clinical care of patients in this study):** C.I. Curley, G.A. Kennedy, G.R. Hill, A.J. Morton, S.-K. Tey

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Clinical Cancer Research

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