**γδ T cells for cancer immunotherapy**

**A systematic review of clinical trials**

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**Keywords:** adoptive cell transfer, aminobisphosphonate, cancer, immunotherapy, clinical trials, γδ T cell, systematic review

**Abbreviations:** BrHPP, bromohydrin pyrophosphate

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γδ T cells contribute to the front line of lymphoid antitumor surveillance and bridge the gap between innate and adaptive immunity. They can be readily expanded to high numbers in vivo and in vitro, starting from the blood of cancer patients, and a number of Phase I trials have demonstrated that these cells can be employed in cancer immunotherapy. Sufficient patients have received γδ T cell-based immunotherapies in the context of clinical trials to evaluate their utility, and to inform the direction of new trials. A systematic approach was used to identify Phase I, Phase II, and feasibility studies testing γδ T cell-based immunotherapy in cancer patients. Studies were excluded from further analysis if they did not provide patient-specific data. Data were compiled to evaluate efficacy, with stratification by treatment approach. When possible, comparisons were made with the efficacy of second-line conventional therapeutic approaches for the same malignancy. Twelve eligible studies were identified, providing information on 157 patients who had received γδ T cell-based immunotherapy. The comparison of objective response data suggests that γδ T cell-based immunotherapy is superior to current second-line therapies for advanced renal cell carcinoma and prostate cancer, but not for non-small cell lung carcinoma. An evaluation of pooled data from 132 published in vitro experiments shows a consistent improvement in the cytotoxicity of γδ T cells in the presence of antitumor antibodies. Immunotherapy using γδ T cells alone shows promising clinical activity, but there is a strong preclinical rationale for combining this treatment modality with cancer-targeting antibodies to augment its efficacy.

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**Introduction**

γδ T cells recognize pathogens and transformed cells in a HLA-unrestricted manner. These lymphocytes respond to markers of cellular stress including phosphoantigens, which are released by transformed cells as by-products of the mevalonate biosynthetic pathway.¹ Furthermore, γδ T cells share characteristics of both the innate and adaptive immune system, displaying both innate cytotoxic functions and antigen-presenting capability,²,³ particularly in the presence of antibody-opsonized target cells.⁴ This dual capacity makes them an exciting candidate for cancer immunotherapy.

The most abundant subset of circulating γδ T cells, Vγ9Vδ2 cells, can be activated and expanded in vitro following a single treatment with the phosphoantigen isopentenyl pyrophosphate (IPP), with an EC₅₀ of 3 μM.⁵ Naturally occurring or synthetic non-peptide prenyl pyrophosphate analogs of IPP can serve as ligands of the Vγ9Vδ2 T-cell receptor (TCR), including the synthetic analog bromohydrin pyrophosphate (BrHPP, EC₅₀ 0.15 μM, from Innate Pharma, France), which has been evaluated in Phase I and II clinical trials.⁶,⁷ Inhibitors of farnesyl pyrophosphate synthase (FPPS) such as the 3rd generation aminobisphosphonates zoledronate and pamidronate lead to IPP accumulation. Originally intended as inhibitors of osteoclast-mediated bone resorption for the treatment of osteoporosis and hypercalcemia, aminobisphosphonates potentially provide secondary benefit as part of γδ T cell-based immunotherapy and have been shown to be very well tolerated in combination with chemotherapy by cancer patients of all age ranges.

Similar to natural killer (NK) cells, the activation of γδ T cells is regulated by a balance between stimulatory and inhibitory signals. They can be activated by γδ TCR ligands such as phosphoantigens, or by MHC-associated ligands of the activatory receptor killer cell lectin-like receptor subfamily K, member 1 (KLRK1, best known as NKG2D, such as MHC class I polypeptide-related sequence A (MICA), MICB, and various members of the UL16-binding protein (ULBP) family. γδ T cells also express killer-cell immunoglobulin-like receptors (KIRs), which can be either activatory or inhibitory, including killer cell immunoglobulin-like receptor, 2 domains, long cytoplasmic tail, 1 (KIR2DL1)⁸ and killer cell immunoglobulin-like receptor, 3 domains, long cytoplasmic tail, 1 (KIR3DL1).⁹ Tumors possess the ability to manipulate this balance to stimulate tolerance by inhibitory signals, including soluble NKG2D ligands, transforming growth factor β1 (TGFβ1), galectin 3 and prostaglandin E₂ (PGE₂)¹⁰,¹¹,¹²,¹³ Elevated circulating levels of sMICA, sMICB, and sULBP1 might be particularly active against effector γδ T cells, as the latter express high amounts of NKG2D. Of interest, the NKG2D ligand ULBP4 may bind to the TCR of some γδ T-cell subsets, and this may exacerbate their inhibition by neuroblastoma cells.¹⁴
The balance between inhibitory and activatory signals can be tilted toward tumor control by boosting tumor-specific cytotoxic functions. With γδ T cells, this is achieved upon activation by phosphoantigens such as IPP, an effect that is exacerbated if target cells are opsonized by an appropriate antibody, making the combination of antitumor antibodies with γδ T cell-based immunotherapy an attractive therapeutic prospect.

**Treatment Approaches—In Vivo Expansion Vs. Adoptive Transfer**

Following the recognition that γδ T cells can be expanded to form potent antitumor effectors in vitro and in vivo, numerous clinical trials have attempted to capitalize on these properties for cancer immunotherapy. Adoptive transfer—a process that requires the expansion (and activation) of autologous T cells ex vivo and their reinjection into patients, is becoming a popular paradigm of cellular immunotherapy. The potential to expand γδ T cells in vivo using combinations of aminobisphosphonates and cytokine offers a comparatively cheaper and more straightforward delivery alternative.

The expansion of γδ T cells ex vivo allows for the optimization and control of the effector cell population. Strategies that are currently under investigation in this sense include the use of naturally occurring and genetically-modified tumor-specific effectors. The benefits of controlling the effector cell population for adoptive transfer are significant, but must be balanced against the cost of preparing and administering the treatment. γδ T cells from cancer patients can be reproducibly expanded ex vivo to large numbers using phosphoantigens, aminobisphosphonates, or immobilized anti-γδ TCR antibodies.

Treating patients with aminobisphosphonates or synthetic phosphoantigens leads to an increase in circulating Vγ9Vδ2 T cells that are able to kill autologous tumor cells in vitro. The 3rd generation aminobisphosphonate zoledronate has been the most commonly used agent for the activation/expansion of γδ T cells in clinical trials, as it has been administered to 61/80 (72%) of patients. The EC50 of zoledronate for human γδ T-cell activation is favorable (0.003 μM) and is well within the concentrations achievable with a standard dose of 4 mg. Zoledronate has been shown to improve the survival of multiple myeloma patients and to reduce the progression of skeletal-related events.

Zoledronate inhibits FPPS, resulting in the compensatory upregulation of non-prenylated small GTPases such as RAP1A and the accumulation of IPP. These effects not only activate Vγ9Vδ2 T cells, they also inhibit the growth of cancer cells by suppressing protein prenylation. Zoledronate is rapidly cleared from the plasma following intravenous infusion, most likely due to sequestration into the bone. Following the administration of 4 mg zoledronate in cancer patients with normal renal function, mean peak plasma concentration was 1.13 μM. A pharmacokinetic study of zoledronate infusions in patients with renal impairment showed that plasma concentrations 24 h upon infusion were < 1% of peak concentrations, but were still sufficient to elicit consistent γδ T-cell activation in vitro. The pharmacokinetics of zoledronate in children aged 3–17 are similar to those in adults when a comparable dose (mg/kg) is used.

**Search Methods**

The NCBI PubMed database was queried using the MeSH terms outlined in Table S1. In addition, the bibliographies of review articles listing γδ T lymphocytes in the title and published in the last year were searched for references to clinical studies.

Articles were included if the study pertained to cancer immunotherapy in humans, measured clinical outcomes and contained a clear treatment protocol that could be linked to each patient included in the study. Clinical outcome data either in the form of Response Evaluation Criteria In Solid Tumors (RECIST) assessment, progression-free survival or overall survival for each patient were also required. Full texts only that provided summarized statistics of patient data or no patient-specific information regarding cancer type or response were excluded. In these cases, the corresponding authors were contacted and asked if unpublished data were available on the patient characteristics, treatment received and clinical outcome.

**Results**

**Patient demographics and diagnoses**

Fifty-five studies were identified from the initial literature review, of which 15 were found to be suitable for screening. Three studies of 15 were excluded because of insufficient clinical data. Data were therefore available for 12 clinical studies, involving a total of 157 patients. Seventy-seven of these 157 patients had received infusions of γδ T cell-enriched populations, and 80 had received drugs to expand and activate endogenous γδ T cells. Of these, 68/77 patients subjected to adoptive γδ T cell transfer and 62/80 patients receiving γδ T cell-expanding drugs had RECIST data available. A PRISMA flow sheet of the screening process is shown in Figure S1. Patients with solid tumors were most often treated with adoptive T-cell transfer (71) as compared with γδ T cell-expanding drugs (47), whereas patients affected by hematological malignancies were...
more often treated with drugs (33) than with γδ T cells expanded ex vivo (6) (Fig. 1). The mean age of patients enrolled in adoptive T-cell transfer trials was 60 y (range 18–85 y, n = 67, 10 missing values), as opposed to 63 y (range 29–83 y, n = 58, 22 missing values) for trials testing γδ T cell-expanding drugs. There was no significant difference in the age of patients in each group.

Prior therapies
As the studies reviewed were either Phase I, Phase II, or feasibility studies, participants had already received extensive treatment for their primary disease. 83.8% of patients enrolled into trials testing in vivo γδ T-cell expansion had previously received myelosuppressive chemotherapy, as compared with only 41.6% of patients allocated to γδ T-cell transfer. Conversely, 41.6% of the participants in adoptive γδ T-cell transfer trial had previously received some form of immunotherapy, as compared with only 16.3% of patients receiving γδ T cell-expanding drugs (Fig. 1). Prior treatments reflect the predominant diseases in each trial type. For example, a high proportion of patients previously treated with immunotherapy had melanoma or RCC.

Trials testing γδ T cell-stimulatory drugs
These trials used aminobisphosphonates +/- IL-2 to stimulate γδ T cells in vivo. Two studies involved standard Phase I dose-escalation protocols and 3 studies were Phase II clinical trials. In all but one case, the dose of IL-2 administered to participants within each trial was kept consistent, though there were variations between studies, as shown in Table 1.

In an intra-patient dose-escalation study by Wilhelm and coworkers,38 gradually increasing doses of IL-2 were administered to 19 patients with hematological malignancies to determine the effects of IL-2 dose and administration route. Following

Figure 1. Diagnosis and previous treatments of patients enrolled in clinical trials testing γδ T cell-based immunotherapy. AML, acute myeloid leukemia; CA, carcinoma; CLL, chronic lymphocytic leukemia; NSCLC, non-small cell lung carcinoma; PBMC, peripheral blood mononuclear cell; PBSCT, peripheral blood stem cell transplantation; RCC, renal cell carcinoma.
very high concentration of IL-2 (1000 U/mL). The expansion protocols varied substantially, and in some studies they involved a culture with pamidronate and IL-2 (Table S1).

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### Table 1. Treatment protocols aimed at expanding γδ T cells in vivo using zoledronate and IL-2

| Paper       | n  | Disease (n)                        | Patients screened for γδ T cell expansion? | Sub groups within trial (n) | ZOL dose (mg) | IL-2 dose/m² (MU) | IL2 dose if not by BSA (MU) | Days of IL-2 per cycle | Cycle length (d) | Mean cycles | Lower 95% CI | Upper 95% CI |
|-------------|----|-----------------------------------|-------------------------------------------|----------------------------|---------------|------------------|-----------------------------|------------------------|----------------|-------------|-------------|-------------|
| Kunzmann 2012⁵⁷ | 21 | Advanced renal cell carcinoma (7) Multiple myeloma (6) AML (8) | Yes (21)                                  | 21                         | 4             | -                | 2                           | 6                      | 28             | 2.8         | 2.0         | 3.5         |
| Lang 2011⁵⁹   | 12 | Advanced renal cell carcinoma (12) | No (12)                                   |                            | 6             | 4                | -                           | 15                     | 28             | 3.7         | 0.6         | 6.8         |
|              |    |                                   |                                           |                            | 2             | 4                | -                           | 15                     | 28             | 17.0        | -           | -           |
|              |    |                                   |                                           |                            | 1             | 4                | 1–2                         | 15                     | 28             | 3.0         | -           | -           |
|              |    |                                   |                                           |                            | 2             | 3                | 1                           | 15                     | 28             | 11.5        | -           | -           |
|              |    |                                   |                                           |                            | 1             | 1.5              | 1                           | 15                     | 28             | 4.0         | -           | -           |
| Dieli 2007⁴⁰  | 18 | Advanced prostate cancer (18)      | No (18)                                   | 9                          | 4             | 0                | 0                           | 0                      | 21             | 9.2         | 5.3         | 13.1        |
|              |    |                                   |                                           |                            | 9             | 4                | 0.6                         | 1                      | 21             | 14.4        | 12.3        | 16.5        |
| Meraviglia 2010⁴¹ | 10 | Advanced breast cancer (10)        | No (10)                                   | 10                         | 4             | -                | 1                           | 1                      | 21             | Not specified |

Abbreviations: CI, confidence interval; IL-2, interleukin-2; MU, mega unit; ZOL, zoledronate.

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Disappointing results in patients receiving continuous subcutaneous infusions on day (D)3–8 of each treatment cycle, the protocol was altered to 6 h intravenous bolus infusions on D1–6. It is not possible to compare the overall efficacy of these IL-2 delivery techniques from this study as patients were enrolled in the cohort treated under the altered protocol only if they had a significant expansion of γδ T cells in vitro (> 20% proliferation at D8 of culture with pamidronate and IL-2) (Table S1).

**Adoptive transfer of enriched γδ T-cell populations**

Clinical trials testing the adoptive transfer of γδ T cells were more homogenous in their protocol design than those investigating γδ T cell-stimulatory drugs. Indeed, the former mainly varied relative to the stimulus used to expand γδ T cells ex vivo, and the number and timing of the γδ T-cell infusions. Adoptive transfer protocols involve obtaining lymphocytes from the patient and then culturing them in conditions that selectively promote γδ T-cell proliferation. After a period of proliferation (usually 14 d), γδ T cells are re-infused into the patient, along with further immunostimulatory agents in some cases. Ex vivo expansion requires specialized laboratories and expertise in handling cellular therapy products.

The majority (78%) of patients enrolled in adoptive transfer studies had solid tumors. The major variable across protocols lies in ex vivo expansion methods. Additional variables include the cell source (leukopheresis, n = 2, or the peripheral blood, n = 5), and the length of time between subsequent γδ T-cell infusions. In addition, 2 studies administered IL-2, 1 zoledronate and 1 both agents alongside adoptively transferred cells⁶⁻⁴⁴ (Table 2). Expansion protocols varied substantially, and in some studies they involved a very high concentration of IL-2 (1000 U/mL).⁵⁵⁻⁵⁷ The expansion of γδ T cells from the peripheral blood is clearly feasible, while studies involving leukopheresis did not achieve significantly higher numbers of γδ T cells than those based on γδ T-cell expansion from the whole blood (leukopheresis, mean 11.3 x 10⁹ cells, 95% CI 5.8–16.9 x 10⁹ cells: whole blood, mean 16.2 x 10⁹ cells; 95% CI 12.5–19.9 x 10⁹ cells). This suggests that leukopheresis is not required to generate satisfactory γδ T-cell products.

**Clinical responses to γδ T-cell immunotherapy as compared with conventional second-line treatments**

To compare the clinical response to γδ T-cell immunotherapy with standard-of-care second-line treatment approaches, we selected 3 cancer types for which national guidelines for second-line treatment exist in the UK (from the National Institute for Clinical Excellence, NICE) or US (from the National Comprehensive Cancer Network, NCCN), namely, renal cell carcinoma (RCC), non-small cell lung carcinoma (NSCLC), and prostate cancer. Outcomes in terms of clinical responses were compared. These 3 cancers also represented the commonest types of tumor patients enrolled in γδ T-cell immunotherapy trials.

The only second-line regimen currently recommended by the NICE for the treatment of refractory/relapsed advanced prostate cancer is the combination of docetaxel and prednisolone.⁴⁸ Disease outcome data regarding this combination is available from numerous sources.⁴⁹⁻⁵¹ There is currently no NICE recommended second-line treatment for advanced/metastatic RCC, but the NCCN recommends everolimus, much of the evidence in support of this option coming from the RECORD-1 trial.⁵² Docetaxel or erlotinib are recommended for second-line chemotherapy in patients with Stage III-IV NSCLC.⁵³ A recent comparator study⁵⁴ demonstrated a slight superiority for erlotinib over docetaxel for the second-line treatment of advanced NSCLC. Both these therapeutic options are permissible under current NICE guidelines.

Data on disease outcome from large studies that were used in the formulation of the treatment guidelines for these 3 tumor types are shown in Table 3, alongside comparisons with disease outcome from corresponding γδ T-cell immunotherapy trials. A more extensive breakdown of the results from γδ T-cell immunotherapy trials is shown in Table S2. Although direct statistical comparisons are not possible, the proportion of objective responses among patients enrolled in clinical trials testing γδ T cell-based immunotherapy...
is superior to that achieved with established second-line therapy in patients with advanced prostate cancer (33.3% with γδ T cells vs. 25.2% with prednisolone + docetaxel) and advanced RCC (4.8% with γδ T cells vs. 1.8% with everolimus), but not advanced NSCLC patients (7.6% with erlotinib, 12.2% with docetaxel, 0% with γδ T cells). While this could be explained through patient selection, all individuals analyzed had relapsed or recurrent disease and so are broadly comparable in terms of prognosis.

**Variation in γδ T-cell expansion capacity between patients**

Tumor immune evasion can be facilitated by host cells. Regulatory T cells (Tregs) are an important immunosuppressive cell population that prevent autoimmune responses and excessive reactions against self entities. Tumors that recruit high levels of CD4+CD25+FOXP3+ Tregs among tumor-infiltrating lymphocytes (TILs) are associated with invasive disease.55-57 γδ T cells from healthy individuals and cancer patients can be expanded to clinically useful numbers, even if patients have previously received chemotherapy,19,58 but there is a high degree of inter-individual variation in expansion capacity. In one study, γδ T cells from only 49% (20/41) healthy donors expanded in vitro in response to γδ T-cell-stimulating drugs were stratified based on in vitro γδ T-cell expansion rate in response to the same stimulus used in the trial. While the overall response of these “positive responders” is better than that of unscreened patients receiving γδ T-cell-stimulating drugs (overall response rate 16.2% vs. 8.1%) these populations are too small and heterogeneous for firm conclusions (Table S3).

**Antibody-dependent γδ T cell-mediated cytotoxicity**

The initial evaluation of γδ T-cell-based immunotherapy shows some promise but there is large room for improvement. Overall, conventional response rates are poor, with only 14/130 (10.8%) objective responses documented across all of the trials assessed. However, 51 (39.2%) patients achieved disease stabilization, a successful outcome of immunotherapy, indicating that clinical benefits can be achieved by a high proportion of patients subject to this treatment.

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**Table 2. Comparison of clinical trials using adoptively transferred γδ T cells**

| Paper | n | Disease (n) | Cell source | Expansion conditions | Cycles | Cumulative cell dose (x10⁶) | Additional treatments |
|-------|---|------------|-------------|---------------------|--------|---------------------------|---------------------|
| Bennouna et al. 2003³⁵ | 10 | RCC (10) | L | IL-2 (U/mL) | [aBP or PAg] | Cycle length (d) | Mean no. of cycles | Lower 95% CI | Upper 95% CI | ZOL (mg) | IL-2 (MU/m²) | d/cycle |
| Kobayashi et al. 2007⁴³ | 7 | RCC (7) | PB | [3M3B1-PP (100 μM)] | 7 | (n = 4), 14(n = 3) | 9.6 | 7.5 | 11.7 | 14.2 | 4.4 | 24.0 | - | 0.7 [1d] |
| Nakajima et al. 2010⁵⁵ | 10 | NSCLC (10) | PB | IL-2 (U/mL) | 100 | ZOL (5 μM) | 14 | 6.5 | 4.7 | 8.3 | 14.5 | 8.6 | 20.3 | - | - |
| Abe et al. 2009⁶⁶ | 6 | MM (6) | PB | IL-2 (U/mL) | L 600 | BrHPP (3 μM) | 9.6 | 7.5 | 11.7 | 14.2 | 4.4 | 24.0 | - | - |
| Kobayashi et al. 2011⁵⁵ | 11 | RCC (11) | PB | IL-2 (U/mL) | L 600 | ZOL (5 μM) | 14 | 6.8 | 5.7 | 8.0 | 9.3 | 4.9 | 13.7 | - | - |
| Sakamoto et al. 2011⁵⁷ | 15 | NSCLC (15) | PB | IL-2 (U/mL) | L 600 | ZOL (5 μM) | 14 | 6.5 | 5.2 | 7.7 | 18.4 | 12.2 | 24.7 | - | - |
| Nicol et al. 2011⁶⁸ | 18 | MML (7) OC (2) CAC (3) BC (2) CC (1) CVC (1) DC (1) AC (1) | L | IL-2 (U/mL) | L 600 | ZOL (5 μM) | 14 | 6.5 | 5.2 | 7.7 | 18.4 | 12.2 | 24.7 | - | - |

**Abbreviations:** aBP, aminobisphosphonate; AC, adenocarcinoma; BC, breast carcinoma; CC, colon cancer; CI, confidence interval; CVC, cervical carcinoma; DC, duodenal carcinoma; IL-2, interleukin-2; L, leukopheresis; MM, multiple myeloma; MML, metastatic melanoma; MU, mega unit; NSCLC, non-small cell lung carcinoma; OC, ovarian carcinoma; PAg, phoshhoantigen; PB, peripheral blood; RCC, renal cell carcinoma; U, unit; ZOL, zoledronate.
Table 3. Clinical outcomes of commonly used second-line anticancer agents as compared with γδ T-cell immunotherapy.*

| Disease                        | Second-line treatments                      | CR  | 95% CI | PR  | 95% CI | SD  | 95% CI | PD  | 95% CI |
|--------------------------------|--------------------------------------------|-----|--------|-----|--------|-----|--------|-----|--------|
| Advanced prostate cancer      | Prednisolone + docetaxel (n = 101, 3 randomized controlled trials) | 0   | 0      | 25.2| 8.4–41.8| 44.3| 32–56.8| 30.4| 14.5–46.2|
|                               | In-vivo expansion of γδT cells (n = 12, 6 missing) | 0   | 33.3   | 41.6| 25.0   |
| Advanced renal cell carcinoma | Everolimus (n = 277, 1 randomized phase 3 study) | 0   | 0      | 1.8 | -      | 66.5| -      | 31.7| -      |
|                               | Adoptive transfer of γδT cells (n = 21, 7 missing) | 4.8 | 0      | 42.9| 52.4   |
|                               | In-vivo expansion of γδT cells (n = 15, 4 missing) | 0   | 0      | 66.7| 33.3   |
| Advanced NSCLC                | Erlotinib (n = 3324, 2 randomized controlled trials) | 0.4 | 0.17–0.73| 7.2 | 1.88–10.89| 33.9| 4.9–43.4| 58.5| 6.9–72.1|
|                               | Docetaxel (n = 385, 2 randomized controlled trials) | 2.6 | 0.3–4.9| 9.6 | 8.9–10.2| 37.7| 30.0–45.3| 50.2| 45.5–55.0|
|                               | Adoptive transfer of γδT cells (n = 24, 1 missing) | 0   | 0      | 54.2| 45.8   |

*Data are pooled from clinical trials and standard of care treatments were selected based upon current UK or US guidelines for treatment of the tumors in question. A more detailed breakdown of γδT cell immunotherapy results is included in Table S2. CI, confidence interval; CR, complete response; NSCLC, non-small cell lung carcinoma; PD, progressive disease; PR, partial response; SD, stable disease.

γδ T cells are a potential alternative to αβ T cells for cellular immunotherapy. They have a number of advantages that could be exploited, not least the fact that they can be easily expanded in vivo upon the administration drugs with well established safety records in adults and children. The sequential nature of the lymphoid immune response is governed by the time required to expand sufficient effector numbers to generate antimicrobial or antitumor reactivity.10 The activation of γδ T cells in response to a range of stress signals such as NKG2D ligands, endogenous phosphoantigens, or TLR agonists is independent of HLA molecules. The kinetics of the γδ T-cell response in vivo is faster than that of the αβ T-cell response, as the former requires neither priming by dendritic cells in lymph nodes, nor clonal expansion. In an immunodeficient mouse model, adoptively transferred human Vγ9Vδ2 cells mounted almost immediate anti-bacterial responses following administration.61 γδ T cells also acquire professional antigen-presenting function upon activation, implying that they may have a value as cellular vaccines that goes beyond their ability to exert cytotoxic functions.2,4,62

Adoptive transfer of T cells

The possibility to enhance antitumor immune responses using tumor-specific αβ T cells expanded ex vivo was first demonstrated in melanoma63 and RCC patients,64 from whom tumor-infiltrating lymphocytes (TILs) can be readily obtained. The isolation of TILs has indeed proved problematic in patients affected by most other tumor types, and no data are available on tumor-infiltrating γδ T cells. Without prior lymphodepletion, adoptively transferred TILs are short-lived and clinical benefits are transient. Lymphodepletion significantly improves the clinical benefit of this immunotherapeutic regimen. In a cohort of melanoma patients, lymphodepletion followed by adoptive T-cell transfer resulted in a response rate of 56% and many patients still remain disease-free at follow up (4–10 y).65 Interestingly, autologous γδ T cells expanded ex vivo...
have been shown to persist in the circulation of cancer patients receiving IL-2 but no prior lymphodepletion for over 12 wk.\textsuperscript{45,66}

TILs are unavailable for a majority of patients affected tumors other than melanoma and RCC, implying that T cells must be expanded or engineered ex vivo to generate a bulk population of tumor-reactive cells for adoptive transfer. The efficacy of adoptively transferred tumor-reactive αβ T cells can decrease upon the loss of antigen expression by malignant cells, which occurs frequently in response to the selective pressure of therapy itself. γδ T cells, which recognize a broad range of stress signals emitted by malignant cells, are not subjected to this limitation. Moreover, as the cytotoxic potential of γδ T cells is independent of HLA molecules, limits the need for engineering in this sense. Nonetheless, the adoptive transfer of γδ T cells could be combined with T-cell engineering to enhance functions other than cytotoxicity.

**γδ T cells as vaccines**

A further advantage of γδ T cells over αβ T cells is that the former acquire professional antigen-presenting capacity upon stimulation, expressing increased levels of co-stimulatory molecules such as CD80 and CD86, as well as of molecules associated with the homing to lymph nodes.\textsuperscript{2,4,62,67} γδ T cells also share some properties with NK cells and cytokine-induced killer (CIK) cells, such as the innate cytotoxic potential and the ability to mediate antibody-dependent cell-mediated cytotoxicity. The antigen-presenting capacity of dendritic cells has already been harnessed in clinical trials that have been running for over ten years.\textsuperscript{68} Adoptively transferred NK cells showed efficacy in metastatic RCC,\textsuperscript{69} breast cancer,\textsuperscript{70} and malignant glioma patients.\textsuperscript{71} The combination of innate cytotoxic and antigen-presenting functions raises the intriguing possibility that γδ T cells could be used as a cellular vaccine that would kill malignant cells in situ and cross-present tumor-associated antigens to αβ T cells, hence generating a potent and long-lasting immune response. γδ T cells can be expanded in vitro and in vivo and their role as antigen-presenting cells, alone or combined with antibodies that enhance their effector functions, can be evaluated in clinical trials.

**Overcoming inhibitory signals**

The immunosuppressive nature of the tumor microenvironment is one of the biggest obstacles against successful immunotherapy. Strategies for unpicking these barriers are continuously progressing, the discovery that blocking the PD-1/PD-L1 interaction significantly reduces immune evasion and provides objective clinical benefits perhaps being the most recent example.\textsuperscript{72} Inhibiting the immunosuppressive activity of CTLA4 with the anti-CTLA4 antibody ipilimumab is also highly effective in metastatic melanoma patients,\textsuperscript{73} and is likely to provide clinical benefits to patients affected by other malignancies, such as prostate cancer.\textsuperscript{74} In line with their central role in innate immunity, the activation of γδ T cells is controlled by a balance of activatory and inhibitory signals.\textsuperscript{10} Tumors are known to produce several mediators that inhibit γδ T and NK-cell functions including soluble NKG2D ligands as well as TGFβ1 and PGE\textsubscript{2}.\textsuperscript{10,13,75} However, compelling in vitro and in vivo evidence indicate that γδ T cells and antitumor antibodies can be successfully combined for the treatment of both hematological and solid malignancies,\textsuperscript{3,4,35-38} indicating that tumor-elicited immunosuppression can be overcome. The combination of γδ T cell-expanding agents and tumor-targeting antibodies could tip a failing immune response dominated by inhibitory cells such as T\textsubscript{reg},\textsuperscript{76} myeloid-derived suppressor cells, and inhibitory/tolerizing dendritic cells\textsuperscript{76} and the activation of immune checkpoints mediated by CTLA4 and the B7 protein family,\textsuperscript{77} toward a robust cytotoxic T-cell response. Although malignant cells accumulate higher amounts of phosphoantigens than healthy cells,\textsuperscript{7} an effect that is magnified by the administration of aminobisphosphonates,\textsuperscript{78} this appears to be insufficient to fully overcome tumor-elicited immunosuppression. While TIL-derived γδ T cells derived will selectively kill transformed cells and spare healthy bystanders,\textsuperscript{79} tumor-targeting antibodies may be required for achieving optimal cytotoxic γδ T-cell responses. Thus, combining γδ T cell-based immunotherapy with tumor-specific antibodies might spare healthy cells expressing tumor-associated antigens if the engagement of the γδ TCR is also required for optimal effector functions. Moreover, this approach might result in full-blown activation of professional antigen-presenting cells at the tumor site. The combination of γδ T cells with immunomodulatory and/or cytolytic antibodies is an attractive prospect for future clinical trials.

**Conclusions**

Successful immunotherapy relies on the control of the balance between antitumor cytotoxicity and immunological tolerance. In this context, adoptively transferred αβ T-cell populations potentially attack specific targets but are limited by their specificity. dendritic cells lack cytotoxic functions of their own and NK cells have given inconsistent results in clinical trials.\textsuperscript{69,71} γδ T cells in combination with tumor-targeting antibodies might provide a direct but not antigen-exclusive response, potentially mediating not only antitumor cytotoxic effects but also long-lasting protection upon antigen presentation. Results from Phase I and II clinical trials indicate that the efficacy of γδ T-cell-based immunotherapy is comparable to that of conventional second-line therapies. Combining agents that promote γδ T-cell expansion and activation with cytolytic tumor-specific antibodies is a feasible and logical approach with an (expectedly) favorable toxicity profile.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

The authors would like to acknowledge Dr Darren Hargrave for his input regarding comparison of phase I and II trials. Dr Fisher is funded by the Wellcome Trust, Sparks and the Dubois Child Cancer Fund. John Anderson holds a Great Ormond Street Hospital Charity Leadership award. MY is funded by a project grant from Leukaemia Lymphoma Research (UK).

**Supplemental Materials**

Supplemental materials may be found here: http://www.landesbioscience.com/journals/oncoimmunology/article/27572/
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