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γδ T cells for cancer immunotherapy
A systematic review of clinical trials

Jonathan PH Fisher[1], Jennifer Heuijerjans[1], Mengyong Yan[1], Kenth Gustafsson[2], and John Anderson[1,*]

[1]UCL Institute of Child Health; London, UK; [2]UCL Institute of Child Health; Molecular Immunology Unit; London, UK

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Abbreviations: BrHPP, bromohydrin pyrophosphate

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.

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 EC_{50} 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, EC_{50} 0.15 μM, from Innate Pharma, France),[5] 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 E_2 (PGE_2)[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]

*Correspondence to: John Anserson; Email: j.anderson@ucl.ac.uk
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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,[3,4,15-18] 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 reinfusion 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. γδT cells from cancer patients can be reproducibly expanded ex vivo to large numbers using phosphoantigens,[19] aminobisphosphonates,[20] or immobilized anti-γδ TCR antibodies.[21]

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.[22] 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 EC_{50} of zoledronate for human γδ T-cell activation is favorable (0.003 μM)[5] 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.[23] Zoledronate inhibits FPPS, resulting in the compensatory upregulation of non-prenylated small GTPases such as RAP1A[24] 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.[24,25] 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.[26] 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 (source: European Medicines Agency data, EMEA/H/C/000336 -A20/0026). Hence, at well-tolerated doses, zoledronate achieves plasma levels that are capable of activating Vγ9Vδ2 T cells.

Interleukin (IL)-2 is required to expand γδ T cells in vitro, has modest clinical activity as a standalone therapeutic agent in renal cell carcinoma (RCC) and melanoma patients,[27-29] and has been shown to reduce the incidence of relapse among patients with hematological malignancies who underwent bone marrow transplantation.[30] The antineoplastic effects of IL-2 are indirect, following the activation and expansion of immune effectors. The toxicity of IL-2 at high doses is problematic, leading to hyperpyrexia, capillary leak, and hypotension. Low doses of IL-2 are well tolerated but might have undesirable effects for cancer immunotherapy. For example, while low-dose IL-2 drives T cells toward the effector memory (T_{EM}) phenotype upon TCR stimulation, it also increases the number of circulating regulatory T cells (T_{reg}), resulting in a robust immunosuppressive effect.[31] For this reason, the precise dose and schedule for IL-2 administration appears to be critical for the elicitation of optimal antitumor responses.

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[32-36] 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 that only 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

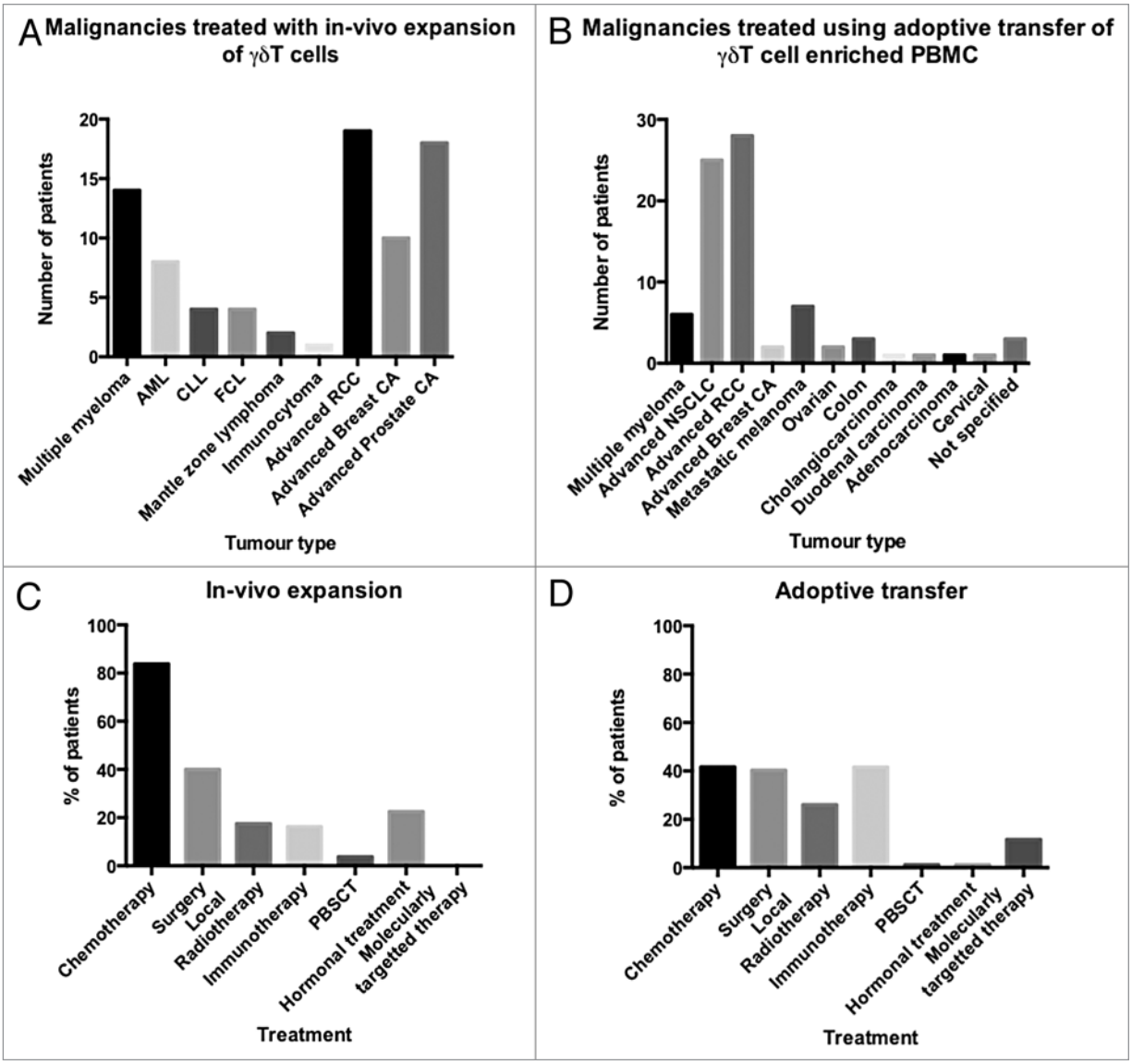

Figure 1. Diagnosis and previous treatments of patients enrolled in clinical trials testing γδ T cell-based immunotherapy. AML, acute myeloid leukemia; CA, carcinoma; CLL, chronic lymphocytic leukemia; NSCLC, non-small cell lung carcinoma; PBMC, peripheral blood mononuclear cell; PBSCT, peripheral blood stem cell transplantation; RCC, renal cell carcinoma.

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.[37-41] 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

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 2012[37]	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 2011[39]	12	Advanced renal cell carcinoma (12)	No (12)	6	4	7	-	15	28	3.7	0.6	6.8
				2	4	1	-	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 2007[40]	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 2010[41]	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.

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 cells[6,42-44] (**Table 2**). Expansion protocols varied substantially, and in some studies they involved a very high concentration of IL-2 (1000 U/mL).[45-47] 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 10^9 cells, 95% CI 5.8–16.9 x 10^9 cells: whole blood, mean 16.2 × 10^9 cells; 95% CI 12.5–19.9 × 10^9 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 in 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 study[54] 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

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

Paper	n	Disease (n)	Cell source	Expansion conditions		Cycles				Cumulative cell dose (x10^9)			Additional treatments	
				IL-2 (U/mL)	[aBP or PAg]	Cycle length (d)	Mean no. of cycles	Lower 95% CI	Upper 95% CI	Mean	Lower 95% CI	Upper 95% CI	ZOL (mg)	IL-2 (MU/m^2) [d/cycle]
Bennouna et al. 2003[6]	10	RCC (10)	L	600	BrHPP (3 μM)	21	3	3	3	26.7	17.0	36.4	-	2 [7d]
Kobayashi et al. 2007[43]	7	RCC (7)	PB	100	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. 2010[45]	10	NSCLC (10)	PB	1000	ZOL (5 μM)	14	6.5	4.7	8.3	14.5	8.6	20.3	-	-
Abe et al. 2009[46]	6	MM (6)	PB	1000	ZOL (5 μM)	14	6.8	5.7	8.0	9.3	4.9	13.7	-	-
Kobayashi et al. 2011[42]	11	RCC (11)	PB	100	3M3B1-PP (100 μM)	28	4.2	3.0	5.4	20.5	9.5	31.5	4	1.4 [5d]
Sakamoto et al. 2011[47]	15	NSCLC (15)	PB	1000	ZOL (5 μM)	14	6.5	5.2	7.7	18.4	12.2	24.7	-	-
Nicol et al. 2011[44]	18	MML (7) OC (2) CAC (3) BC (2) CC (1) CVC (1) DC (1) AC (1)	L	700	ZOL (1 μM)	NS	7.6	7.3	7.93	2.8	1.9	3.6	2	-

Abbreviations: aBP, aminobisphosphonate; AC, adenocarcinoma; BC, breast carcinoma; AC, colonic adenocarcinoma; CC, cholangiocarcinoma; 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.

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 $CD4^+CD25^+FOXP3^+$ T_{regs} 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 88% (14/16) healthy donors expanded in vitro in response to IL-2 + pamidronate, whereas γδ T cells from only 49% (20/41) cancer patients (including multiple myeloma, MM, non-Hodgkin's lymphoma, NHL and, B-cell chronic lymphocytic leukemia (B-CLL) expanded following the same stimuli.[38] There is an inverse correlation between the frequency of circulating T_{regs} and the ability of γδ T cells from cancer patients to proliferate upon phosphoantigen stimulation in vitro. However, T_{regs} do not directly suppress the cytotoxic activity of γδ T cells or their ability to produce cytokines,[59] a factor that is in favor of the adoptive transfer of γδ T cells over the use of γδ T cell-stimulatory agents, as the cells can be expanded in optimized conditions.

The variability in γδ T-cell proliferation was documented in a number of trials in which patients were screened before enrolment to determine whether their γδ T cells would expand in vitro. Thirty-five out of 77 patients treated with adoptively transferred γδ T cells and 31/80 patients treated with 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 subjected

Table 3. Clinical outcomes of commonly used second-line anticancer agents as compared with γδ T-cell immunotherapy.*

Disease	Second-line treatments	CR		PR		SD		PD	
		%	95% CI	%	95% CI	%	95% CI	%	95% CI
Advanced prostate cancer[48]	Prednisolone + docetaxel (n = 101, 3 randomized controlled trials)	0	0	**25.2**	8.4–41.8	**44.43**	32–56.8	**30.40**	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[52]	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**	0	**42.9**		**52.4**	
	In-vivo expansion of γδT cells (n = 15, 4 missing)	0	0	**0**	0	**66.7**		**33.3**	
Advanced NSCLC[53,54]	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.

to γδ T cell-based immunotherapy. The capacity of Vγ9Vδ2 γδ T cells to kill malignant cells in vitro is well documented, and means of augmenting the cytotoxic activity of γδ T cells should be investigated. A number of studies have demonstrated the potential benefit of combining γδ T cells with therapeutic tumor-targeting antibodies.[32,34] To evaluate the evidence in support of this notion we reviewed papers that reported the cytotoxicity of γδ T cells in vitro and in vivo, in the presence or in the absence of tumor-targeting antibodies. The addition of an appropriate tumor-targeting antibody improved the cytotoxic activity of γδ T cells in 132 separate experiments, reported in 6 different publications, based on 12 different cell lines or 5 primary tumor tissues, providing a statistically significant improvement in 94/132 (71.2%) experimental settings.[3,4,15-18] These effect was observed in both hematological and solid tumor models, including $CD20^+$ hematological malignancies, $CD52^+$ lymphomas and *v-erb-b2* avian erythroblastic leukemia viral oncogene homolog 2 $(ERBB2)^+$ breast cancers. The addition of the anti-CD20 antibody rituximab significantly prolonged the clearance of malignant B cells from the circulation of cynomolgus macaques when combined with the γδ T cell-stimulator BrHPP.[3] In a mouse model of ERBB2+ breast carcinoma, the addition of the anti-ERBB2 antibody trastuzumab (Herceptin®) significantly increased tumor infiltration by $Vγ9^+$ γδ T cells. In this study, mice treated with adoptively transferred γδ T cells and trastuzumab achieved a superior control of tumor growth, as compared with animals receiving γδ T cells alone, trastuzumab alone or vehicle.[17]

Discussion

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.[60] 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 melanoma[63] 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.[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.[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.[68] Adoptively transferred NK cells showed efficacy in metastatic RCC,[69] breast cancer,[70] and malignant glioma patients.[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.[72] Inhibiting the immunosuppressive activity of CTLA4 with the anti-CTLA4 antibody ipilumumab is also highly effective in metastatic melanoma patients,[73] and is likely to provide clinical benefits to patients affected by other malignancies, such as prostate cancer.[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.[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_2.[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,[3,4,15-18] 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_{regs}, myeloid-derived suppressor cells, and inhibitory/tolerizing dendritic cells[76] and the activation of immune checkpoints mediated by CTLA4 and the B7 protein family,[77] toward a robust cytotoxic T-cell response. Although malignant cells accumulate higher amounts of phosphoantigens than healthy cells,[1] an effect that is magnified by the administration of aminobisphosphonates,[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,[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 potently 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.[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.

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Supplemental Materials

Supplemental materials may be found here:

http://www.landesbioscience.com/journals/oncoimmunology/article/27572/

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