Brief report

Costimulated tumor-infiltrating lymphocytes are a feasible and safe alternative donor cell therapy for relapse after allogeneic stem cell transplantation

Nancy M. Hardy,[1] Vicki Fellowes,[1] Jeremy J. Rose,[1] Jeanne Odom,[1] Stefania Pittaluga,[2] Seth M. Steinberg,[3] Bazetta Blacklock-Schuver,[1] Daniele N. Avila,[1] Sarfraz Memon,[1] Roger J. Kurlander,[4] Hahn M. Khuu,[5] Maryalice Stetler-Stevenson,[2] Esther Mena,[6] Andrew J. Dwyer,[7] Bruce L. Levine,[8] Carl H. June,[8] Ran Reshef,[8] Robert H. Vonderheide,[8] Ronald E. Gress,[1] Daniel H. Fowler,[1] Frances T. Hakim,[1] and Michael R. Bishop[1,9]

[1]Experimental Transplantation and Immunology Branch, [2]Laboratory of Pathology, and [3]Biostatistics and Data Management Section, Center for Cancer Research (CCR), National Cancer Institute (NCI), [4]Department of Laboratory Medicine, Clinical Center (CC), [5]Department of Transfusion Medicine, CC, [6]Molecular Imaging Program, CCR, NCI, and [7]Department of Radiology and Imagine Sciences, CC, National Institutes of Health (NIH), Bethesda, MD; [8]Abramson Cancer Center, Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; and [9]Division of Hematology and Oncology, Clinical Cancer Center, Medical College of Wisconsin, Milwaukee, WI

Donor lymphocyte infusion (DLI), a standard relapse treatment after allogeneic stem cell transplantation (AlloSCT), has limited efficacy and often triggers GVHD. We hypothesized that after AlloSCT tumor-infiltrating donor lymphocytes could be costimulated ex vivo to preferentially activate/expand antitumor effectors. We tested the feasibility and safety of co-stimulated, tumor-derived donor lymphocyte (TDL) infusion in a phase 1 trial. Tumor was resected from 8 patients with B-cell malignancy progression post-AlloSCT; tumor cell suspensions were costimulated with anti-CD3/anti-CD28 Ab-coated magnetic beads and cultured to generate TDL products for each patient. Costimulation yielded increased proportions of T-bet$^+$FoxP3$^-$ type 1 effector donor T cells. A median of 2.04×10^7 TDL/kg was infused; TDLs were well tolerated, notably without GVHD. Two transient positron emission tomography (PET) responses and 2 mixed responses were observed in these refractory tumors. TDL are a feasible, tolerable, and novel donor cell therapy alternative for relapse after AlloSCT. This trial is registered at clinical-trials.gov as no. NCT00445666. (*Blood*. 2012;119(12):2956-2959)

Introduction

Donor lymphocyte infusion (DLI) is standard relapse treatment after allogeneic hematopoietic stem cell transplantation (AlloSCT), with durable responses reported in relapse of chronic hematologic malignancies, but is not available for cord blood or many unrelated donor recipients. DLI response rates vary but, except for early relapse of chronic myelogenous leukemia, are generally $< 50\%$.[1-3] Responses are strongly associated with GVHD, affecting up to 60%, with up to 11% treatment-related mortality.[4] We hypothesized that relapsed tumor post-AlloSCT would be infiltrated with tumor-specific donor lymphocytes that might be primed but inadequately costimulated for tumor cell killing. Their costimulation and expansion ex vivo might yield a donor lymphocyte product with enhanced tumor specificity and graft-versus-tumor (GVT) potency. Safety of ex vivo CD3/CD28-costimulated DLI has been demonstrated,[5-8] as has feasibility of CD3/CD28-expanded marrow lymphocytes from myeloma patients.[9] Preclinical feasibility studies with tumor-infiltrating donor lymphocytes[10] provided the basis for a phase 1 clinical trial of tumor-derived donor lymphocyte (TDL; BB-IND13226, MRB) infusion for relapsed B-lymphoid malignancy.

Methods

Eligible adults had: progression of B-cell malignancies despite full-donor T-cell engraftment after AlloSCT; trial of immune suppression withdrawal;

DLI (if available); minimal active GVHD; and resectable tumor. The National Cancer Institute Institutional Review Board approved this study, and all subjects provided informed, written consent in accordance with the Declaration of Helsinki. Primary endpoints were feasibility (at least 80% success in generating the TDL product) and safety ($\leq 60\%$ grade 2-4 acute/late-acute GVHD within 28 days). Tumor response was a secondary end point. Accrual stopped subsequent to a planned interim analysis of the first 8 of 15 subjects, when the investigator-held investigational new drug application changed institutions.

After surgical tumor harvest, single-cell suspensions were prepared and costimulated by incubating with anti-CD3/anti-CD28 Ab-coated magnetic beads (BB-IND6675, CHJ) at a 3:1 bead/T-cell ratio and expanded in TDL media (supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Cultures were harvested when yields reached infusion targets, with $\geq 50\%$ CD3$^+$ T cells and $\leq 5\%$ CD19$^+$ tumor cells by flow cytometry. Expanded TDL products were cryopreserved and tested for release criteria (sterility, donor chimerism $> 90\%$, and purity: residual tumor $< 5\%$ by clinical pathology and microscopic bead count < 100 per 3×10^6 cells). TDL dose was determined by culture yield, with $1\text{-}100 \times 10^6$ TDL/kg infused without additional immunoprophylaxis or exogenous cytokines. Subjects were monitored for toxicity[11] for 24 hours in-hospital and at 1, 2, 4, 8, and 12 weeks; response was assessed at 4, 8, and 12 weeks (supplemental Figure 1).[12,13]

Fresh tumor-infiltrating lymphocytes and the expanded TDL products were phenotyped with multicolor flow cytometry for T-cell and effector subsets; TDL clonality was assessed with PCR for TCR-γ-chain (*TRG* locus) gene rearrangements and Vβ spectratyping; and tumor-associated

Table 1. Patient, tumor, and TDL product characteristics and clinical outcomes.

						Maximum prior GVHD				Tumor		TDL					Clinical Outcomes		
Patient no.	Primary diagnosis	Donor	Conditioning (TCD)	TTP post-AlloSCT, mo	Relapse duration, mo	Acute skin/liver/GI	Chronic grade	Post-Allo ChemoRx	Post-Allo donor cells	Tumor harvest	T cell, %	Culture, d	T cell, %	Expansion: T, fold	Donor, %	Cell dose, 10^6/kg	Toxicity	Response (duration)	Survival, mo*
1	DLBCL/t-FL	MRD	RIC (none)	0	10	2/0/2	None	3	1	Flank	5	28	98	54	100	5.8		PD	1.1
2	Lymphoblastic	UCB (2)	MA (none)	10	8	2/0/0	Mild	2†	0	Neck	0.5	36‡	82	60	92	14.5		PD	**1.4**
3	HL (1° Ref)	MUD	MA (R-ATG)	6	16	0/0/0	None	1	1	Axilla	56	12	91	65	100	10.6		NE	**2.1**
4	HL (Rel)	MRD	RIC (none)	15	54	0/0/0	Moderate	5	2	Axilla	63	9	88	22	100	9.3		SD (5 mo)	33.9
5	HL (1° Ref)	MUD	RIC (alemtuzumab)	3	16	0/0/0	None	1	2	Groin	71	7	93	39	100	100	Grade 4 pneumonitis‖	NE/SD (1 mo)	*10.7*
6	HL (Rel)	MRD	MA (post-SCT Cy)	3	26	0/0/0	Mild	1	0	Axilla§	44	12	95	91	100	26.3	Grade 2 pain	SD (3M) *PET response*	6.4
7	P53⁺ CLL	MUD	RIC (alemtuzumab)	13	13	0/0/0	Mild	2	3	Axilla	8	8	97	144	99	98.6		SD (2 mo)	16.4
8	HL (Rel)	MRD	RIC (none)	7	64	2/0/0	Mild	2	1	Axilla	54	12	96	30	100	91.2	Grade 2 cytokine release	SD (4 mo) *PET response*	9.5
Median				6	16						49	12	94	57	100	20.4			8.0

Acute indicates acute GVHD stage per Keystone Consensus Criteria; chronic, chronic GVHD grade per IBMTR Criteria (then in we); CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; GI, gastrointestinal; HL, Hodgkin lymphoma (1° Ref, primary refractory; Rel, relapsed); MA, myeloablative; MRD, matched, related (sibling) donor; MUD, matched unrelated donor; NE, not evaluated; PD, progressive disease; PET response, partial response by positron emission tomography; PR, partial response; RIC, reduced-intensity conditioning; SD, stable disease; t-FL, transformed follicular lymphoma; TDL, tumor-derived donor lymphocyte; TTP, time to progression; and UCB, unrelated cord blood.

Post-Allo ChemoRx indicates number of relapse regimens received since AlloSCT; post-Allo donor cells, number of prior donor cell product infusions since AlloSCT; R-ATG post-SCT Cy, high-dose cyclophosphamide on days +3 and +4; and TCD, in vivo T-cell depletion for GVHD prophylaxis.

Acute GVHD staged per Keystone Consensus Criteria; Chronic GVHD Grade according in IBMTR Criteria, then in use.

*Survival censored July 21, 2011. Bold values indicate deceased; italicized values indicate last contact.

†PN2 received one cycle of cyclophosphamide, vincristine, doxorubicin and dexamethasone (Hyper-CVAD) after harvest, TDL infusion on day 19.

‡PN2 TDL product required extension of culture beyond the expansion target in order to meet criteria for residual tumor contamination.

‖PN6 required a second tumor harvest; the initial excision yielded a wholly sclerotic specimen.

§PN5 presented to his local emergency room 22 days after TDL infusion with fever and shortness of breath. Computerized tomography (CT, pulmonary embolism-protocol) demonstrated stable tumor with new perinodular infiltrates. He refused bronchoscopy and was treated with broad-spectrum antimicrobials without improvement. He consented and underwent bronchoscopy with bronchoalveolar lavage 1 week later, and required intubation for the procedure. Microbiologic stains and cultures and cytopathologic examination were nondiagnostic. He was treated with steroids with prompt resolution of hypoxia and infiltrates, was extubated on the third day after the procedure, and was discharged 4 days later. Hypoxia and infiltrates recurred during rapid steroid taper and again resolved with increased oral steroid dose. Symptoms flared again with taper; he was treated with gemcitabine, vinorelbine, and liposomal doxorubicin with resolution of infiltrates and complete remission of HL.
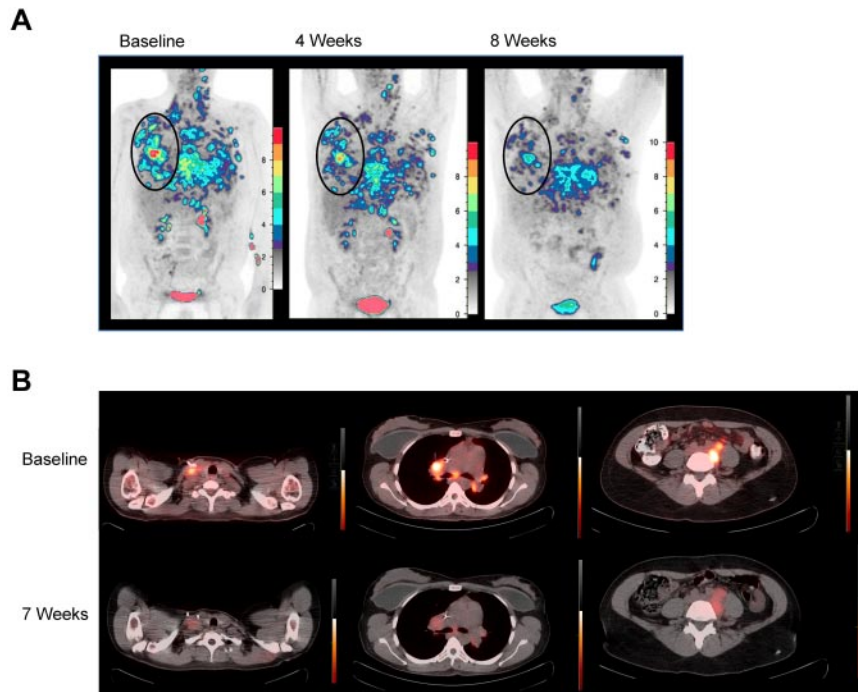
Figure 1. ^{18}FDG-PET responses. (A) ^{18}FDG-PET before and at 4 and 8 weeks after TDL. PN6, a 36-year-old with primary-refractory HL with unabated disease progression following myeloablative conditioning and a 6 of 6 HLA-matched sibling-donor AlloSCT with posttransplantation in vivo T-cell depletion with cyclophosphamide. Transient responses followed radiation and gemcitabine, vinorelbine, and liposomal doxorubicin therapies. Twenty-eight months post-AlloSCT, TDL were generated from a right axillary tumor harvest and 26.3 × 10^6 TDL/kg were infused. Moderate pain and tenderness at sites of chest wall tumor and worsening of pleural effusions developed 1 week after infusion. At restaging, ^{18}FDG-PET-CT demonstrated decreasing standardize uptake values (SUV) in all lesions over 3 consecutive months. By 4 months, however, PET showed increasing SUV in prior sites of disease, which correlated with clinical progression. Shown are coronal maximum intensity projection (MIP) images with a cutoff intensity of 2.5 SUV. (B) Transverse ^{18}FDG-PET-CT fusion images demonstrate decreased SUV in PN8, a 35-year-old with relapsed HL, whose disease progressed from best partial response 6 months after reduced-intensity conditioning and a T-cell replete, 6 of 6 HLA-matched sibling-donor AlloSCT. The patient was treated with vinblastine and subsequently with 3 cycles of dose-adjusted EPOCH (etoposide, vincristine, adriamycin, prednisone, and cyclophosphamide) plus DLI. Seventy months from AlloSCT, TDL were generated from a right axillary tumor harvest, and 91.2 × 10^6 TDL/kg were infused. Grade 2 cytokine release syndrome (culture-negative fevers to 39.5°C, mild hypotension, and tachycardia) was observed from 4 to 24 hours after infusion. ^{18}FDG-PET-CT was stable at 4 weeks, then demonstrated decrease in SUV in multiple sites of disease at 2 months; tumor size was stable by CT. At 4 months, although PET-CT did not show clear progression, the patient developed severe radicular pain associated with tumor-involved cervical lymph nodes requiring additional therapy.

Ag–specific CD8$^+$ donor T cells were sought with an MHC/peptide tetramer library.[14] Proliferative alloresponses and antitumor cytotoxicity were assessed in mixed lymphocyte culture, with CFSE and caspase-8 cleavage, respectively (supplemental Methods).

Results and discussion

Primary feasibility and safety aims were met with the initial 8 patients (Table 1). All surgically harvested tumors demonstrated donor lymphocyte infiltration; T cells comprised 0.5% to 71% of tumor cell suspensions, with donor origin confirmed by short tandem repeat chimerism analysis. CD3/CD28 costimulation and culture yielded clinical TDL products for 8 of 8 patients (95% CI for feasibility: 63.1%-100%), generating a median of 2.04 × 10^9 cells in a median of 12 days. A median 95% of TDL were CD3$^+$ with variable expansion of both CD4$^+$ and CD8$^+$ T cells yielding a median CD4/CD8 ratio of 3.7.

T cells with a type 1 effector phenotype predominated in all TDL products (supplemental Figure 2), with the majority expressing the type 1 transcription factor T-bet (86% of CD4$^+$ and 90% of CD8$^+$ T cells), while the proportion of CD4$^+$CD25$^+$FoxP3$^+$ Tregs decreased in all cultures (from median 19% to 3%). Likewise, the proportion of T cells expressing cell-surface markers of effector function increased with costimulation: CD4$^+$ T cells expressing CD40L uniformly increased (from median 1% to 19%); CD8$^+$ T-cell expression of NKG2D generally increased as well, although expression was more heterogeneous at baseline and increased less consistently from tumors with a high initial proportion of CD8$^+$NKG2D$^+$ T cells (from median 13% to 61%). CD27 expression, lost with terminal differentiation of effector T cells, is a proposed marker of T-cell product potential for in vivo persistence and expansion.[15,16] Predictably, the frequency of CD27$^+$ T cells decreased with costimulation/expansion, yet a significant proportion of TDL retained CD27 (median 36% of CD4$^+$ and 39% of CD8$^+$ T cells).

As expected, CD3/CD28 costimulation yielded polyclonal TDL products, without evidence of a dominant *TRG* gene rearrangement or skewing of TCR repertoire by Vβ spectratyping. PN6 and PN8 lymphocytes could be evaluated with HLA-A2/TAA-peptide tetramers for Ag-specific CD8$^+$ T cells; in both, modest TAA-tetramer$^+$ populations could be found in TDL and in recipient peripheral blood. Although no GVHD was observed, alloreactivity could be detected in TDL products with in vitro proliferative responses against third-party targets. Furthermore, coincubation of PN7's TDL with recipient chronic lymphocytic leukemia (CLL) targets also demonstrated a proliferative response, as well as a cytotoxic effect (TDL vs costimulated donor PBL, 28.7% vs 14.8%, respectively), consistent with a small CLL-reactive population and with the minimal clinical response observed in this patient (supplemental Figure 3).

All patients received TDL infusion. Acute toxicity was minimal, with grade 2 cytokine release syndrome in one patient (PN8). None developed GVHD (95% CI for safety: 0%-36.9%). One patient (PN5) developed grade 4 pneumonitis 22 days after infusion, with acute onset of fever, hypoxia, and pulmonary infiltrates surrounding parenchymal tumor (95% CI for incidence of severe adverse events: 0.3%-52.7%). Extensive evaluation was nondiagnostic and rapid resolution followed steroid treatment (Table 1).

Five patients were evaluable for response. PN1, PN2, and PN3 experienced unabated, rapid progression, and clinical deterioration requiring additional treatment and/or initiation of hospice before 4-week restaging. Four of the 5 subsequent patients maintained stable disease through at least 2 evaluations and each manifested some evidence of antitumor activity. PN6 and PN8 demonstrated transient responses by positron emission tomography (PET), with decreased [18]FDG uptake in all measurable lesions (Figure 1), and PN4 and PN7 demonstrated mixed responses. PN5 may also have had an antitumor effect from TDL, if pneumonitis reflected a tumor-directed immune response. Interestingly, both PET responses were in patients with Hodgkin lymphoma (HL) whose tumors were enriched with donor-derived reactive cells. Plausibly, in HL, gain of type 1 effector function and retained tumor homing permitted infused TDL to transiently disrupt the HL microenvironment.[17,18]

Ex vivo expansion of TDL by CD3/CD28 costimulation is feasible, generating large numbers of type 1 effector donor T cells. TDL appear relatively safe; remarkably, no GVHD was observed after infusing these type 1 polarized TDL. Although significant GVHD did exclude enrollment, 4 of 8 subjects had received in vivo T cell–depleted allografts so likely were at significant risk for DLI-associated GVHD. TDL may be a therapeutic option for cord blood recipients and others without access to DLI. Suggestive tumor-directed biologic effects and potential GVT activity in these few, heavily treated patients warrants further investigation of this therapeutic approach. Planned evaluation of pre-TDL immune depletion to support TDL expansion and effector function in vivo may improve magnitude and durability of responses.

Authorship

Contribution: N.M.H., S.M.S., R.E.G., D.H.F., and M.R.B. contributed to study conception and design; N.M.H., S.P., B.B.-S., D.N.A., R.J.K., H.M.K., M.S.-S., E.M., A.J.D., B.L.L., C.H.J., F.T.H., and M.R.B. provided study materials and/or patients; N.M.H., V.F., J.J.R., J.O., S.P., S.M., R.R., R.H.V., F.T.H., and M.R.B. collected and assembled data; N.M.H., V.F., J.J.R., S.M.S., S.M., R.R., R.H.V., D.H.F., F.T.H., and M.R.B. analyzed and interpreted data; N.M.H. wrote the manuscript; and all authors reviewed the final manuscript.

Conflict-of-interest disclosure: C.H.J. has received royalties from United States government–owned patents and patent applications in the field of adoptive immunotherapy; this arrangement is under compliance with the policies of the University of Pennsylvania. The remaining authors declare no competing financial interests.

Correspondence: Nancy M. Hardy, MD, Experimental Transplantation and Immunology Branch, CCR, NCI, 10 Center Dr, Hatfield Clinical Research Center, Room 3E-3330, Bethesda, MD 20892-1203; e-mail: hardyn@mail.nih.gov.

References

1. Collins RH Jr, Shpilberg O, Drobyski WR, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. *J Clin Oncol.* 1997;15(2):433-444.

2. Lokhorst HM, Schattenberg A, Cornelissen JJ, et al. Donor lymphocyte infusions for relapsed multiple myeloma after allogeneic stem-cell transplantation: predictive factors for response and long-term outcome. *J Clin Oncol.* 2000;18(16):3031-3037.

3. Russell NH, Byrne JL, Faulkner RD, Gilyead M, Das-Gupta EP, Haynes AP. Donor lymphocyte infusions can result in sustained remissions in patients with residual or relapsed lymphoid malignancy following allogeneic haemopoietic stem cell transplantation. *Bone Marrow Transplant.* 2005;36(5):437-441.

4. Frey NV, Porter DL. Graft-versus-host disease after donor leukocyte infusions: presentation and management. *Best Pract Res Clin Haematol.* 2008;21(2):205-222.

5. Fowler D, Hou J, Foley J, et al. Phase I clinical trial of donor T-helper type-2 cells after immunoablative, reduced intensity allogeneic PBSC transplant. *Cytotherapy.* 2002;4(5):429-430.

6. Fowler DH, Odom D, Castro K, et al. Co-stimulated Th2 cells for modulation of acute GVHD: phase I clinical trial results. *Blood.* 2002;100(11):804.

7. Fowler DH, Odom J, Steinberg SM, et al. Phase I clinical trial of costimulated, IL-4 polarized donor CD4+ T cells as augmentation of allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant.* 2006;12(11):1150-1160.

8. Porter DL, Levine BL, Bunin N, et al. A phase 1 trial of donor lymphocyte infusions expanded and activated ex vivo via CD3/CD28 costimulation. *Blood.* 2006;107(4):1325-1331.

9. Noonan K, Matsui W, Serafini P, et al. Activated marrow-infiltrating lymphocytes effectively target plasma cells and their clonogenic precursors. *Cancer Res.* 2005;65(5):2026-2034.

10. Fellowes VS, Rose JJ, Hakim FT, Hardy NM, Bishop MR. Pre-clinical development of allogeneic tumor-derived donor lymphocyte immunotherapy product. Annual Meeting of the International Society for Cellular Therapy, May 23-26, 2010. Philadelphia, PA.

11. CTEP. Common Terminology Criteria for Adverse Events (CTCAE v3.0). Rockville, MD: National Cancer Institute; 2006.

12. Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. *J Clin Oncol.* 1999;17(4):1244.

13. Cheson BD, Bennett JM, Grever M, et al. National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood.* 1996;87(12):4990-4997.

14. Beatty GL, Smith JS, Reshef R, et al. Functional unresponsiveness and replicative senescence of myeloid leukemia antigen-specific CD8+ T cells after allogeneic stem cell transplantation. *Clin Cancer Res.* 2009;15(15):4944-4953.

15. Huang J, Khong HT, Dudley ME, et al. Survival, persistence, and progressive differentiation of adoptively transferred tumor-reactive T cells associated with tumor regression. *J Immunother.* 2005;28(3):258-267.

16. Zhou J, Shen X, Huang J, Hodes RJ, Rosenberg SA, Robbins PF. Telomere length of transferred lymphocytes correlates with in vivo persistence and tumor regression in melanoma patients receiving cell transfer therapy. *J Immunol.* 2005;175(10):7046-7052.

17. Steidl C, Connors JM, Gascoyne RD. Molecular pathogenesis of Hodgkin's lymphoma: increasing evidence of the importance of the microenvironment. *J Clin Oncol.* 2011;29(14):1812-1826.

18. Fozza C, Longinotti M. T-cell traffic jam in Hodgkin's Lymphoma: pathogenetic and therapeutic implications. *Adv Hematol.* 2011;2011:501659.