

## **Gamma delta T-cell phenotype is associated with significantly decreased survival in cutaneous T-cell lymphoma**

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## ABSTRACT

**BACKGROUND:** The importance of  $\alpha\beta$  vs.  $\gamma\delta$  T-cell subset antigen expression in the classification of peripheral T-cell lymphomas is still unclear. The objective of this study was to investigate the prognostic value of TCR $\delta$ 1 expression in primary cutaneous T-cell lymphomas.

**METHODS:** TCR $\delta$ 1 cellular expression was assessed in skin biopsy specimens of 104 individuals with cutaneous T-cell lymphoma by immunohistochemistry. Both univariate (Kaplan-Meier) and multivariate (Cox regression) analyses were conducted to determine which variables (T-cell subtype, hemophagocytosis, histologic profile, age, sex, and adenopathy) were significantly associated with survival.

**RESULTS:** Univariate analysis indicated that there was a statistically significant difference in survival between the patients with  $\alpha\beta$  cutaneous T-cell lymphoma and patients with  $\gamma\delta$  cutaneous T-cell lymphoma ( $p < 0.0001$ ). There was also a statistically significant decrease in survival among patients who had subcutaneous involvement compared with patients who had epidermotropic and/or dermal involvement ( $p < 0.0001$ ). Cox model analysis indicated that TCR $\delta$ 1 expression was the factor that was most closely associated with decreased survival ( $P < 0.0001$ ). Among those patients with cutaneous  $\gamma\delta$  T-cell lymphoma ( $n = 33$ ), there was a trend for decreased survival for patients who had histological evidence of subcutaneous fat involvement in comparison to patients who had epidermotropic or dermal patterns of infiltration ( $P = 0.067$ ). No other prognostic factors were identified as having a notable association with outcome in this subgroup.

**CONCLUSION:** TCR $\delta$ 1 expression in primary cutaneous lymphomas is an independent prognostic factor associated with decreased survival.

## INTRODUCTION

Peripheral T-cell lymphomas overall represent 10% to 15% of Non-Hodgkin lymphoma (NHL) and are a diverse group of lymphoid neoplasms manifesting heterogeneous clinical, histologic, immunophenotypic, cytogenetic and molecular features.<sup>1,2</sup> The subclassification of primary cutaneous T-cell lymphomas in the Revised European American Lymphoma (REAL) classification and the World Health Organization (WHO) includes: mycosis fungoides/ Sezary syndrome (MF/SS), CD30+ T-cell lymphoproliferative disease, and subcutaneous panniculitis-like T-cell lymphoma (SPTCL).<sup>3,4</sup> Cutaneous gamma delta T-cell lymphomas (CGD-TCL) are not defined as a specific entity in the WHO or REAL classification, nor are they delineated in the classification of primary cutaneous lymphomas proposed by the European Organization for Research and Treatment of Cancer (EORTC).<sup>5</sup> However, these tumors appear to have distinctive features.<sup>6</sup> Conventional cutaneous T-cell lymphoma (CTCL) typically represents MF/SS and expresses CD4 surface markers. It is a distinct disease from CGD-TCL that by definition lacks CD4 surface marker expression despite occasional cases of CGD-TCL showing epidermotropism.<sup>7</sup>

The T-cell receptor consists of either a gamma-delta ( $\gamma\delta$ ) or alpha beta ( $\alpha\beta$ ) heterodimer expressed in association with the CD3 complex of proteins on the cell surface.<sup>8</sup> The majority of mature T cells express the  $\alpha\beta$  T-cell receptor. However, 5% of normal T-cells express the  $\gamma\delta$  T-cell receptor.<sup>8</sup> Gamma delta T-cells have cytotoxic capabilities and can respond to stimuli with lymphokine production and proliferation. Most  $\gamma\delta$  T-cells lack CD4 and CD8 surface markers. However, some  $\gamma\delta$  T-cells in human peripheral blood are CD8+. The exact function of  $\gamma\delta$  T-cells remains unknown.<sup>9</sup> Gamma delta T-cell malignancies are rare and have been found among cases of T-cell lymphoblastic lymphoma/leukemia,<sup>10,11</sup> hepatosplenic T-cell lymphoma (HS TCL),<sup>12,13</sup> nasal and extranodal NK/T-cell lymphoma<sup>14</sup> and cutaneous-mucosa associated T-cell lymphomas including SPTCL.<sup>6,14-16</sup> HS-TCLs are nearly always of  $\gamma\delta$  T-cell origin, as opposed to cutaneous T-cell lymphomas, in which  $\alpha\beta$  T-cells usually predominate.<sup>12</sup> Furthermore,  $\gamma\delta$  HS-TCL and  $\gamma\delta$  SPTCL express different V $\delta$  subsets of  $\gamma\delta$  T-cell lymphocytes. Whereas  $\gamma\delta$  HS-TCL belongs usually to the V $\delta$ 1 subset,  $\gamma\delta$  SPTCL represent the V $\delta$ 2

subset.<sup>17</sup> While HS-TCLs are comprised of functionally immature cells lacking granzyme B and perforin,<sup>12</sup> most other gamma-delta T-cell lymphomas are derived from activated cytotoxic T-cells and present preferentially in cutaneous or mucosal sites.<sup>18</sup> This clinical distribution corresponds to the distribution of normal gamma-delta T-cells.<sup>19</sup>

Cutaneous gamma-delta T-cell lymphomas (CGD-TCL) are not common. In a recent report of 62 cases of cutaneous T-cell lymphoma, only two cases were gamma delta positive.<sup>20</sup> To our knowledge, approximately 40 cases of gamma delta cutaneous T-cell lymphoma have been reported.<sup>6,7,14,16,17,20-30</sup> We recently described the clinical, histologic and immunohistochemical features of primary cutaneous  $\gamma\delta$  T-cell lymphoma.<sup>6</sup> CGD-TCL shows preferential involvement of the extremities with plaques, tumors and subcutaneous nodules, some of which ulcerate. CGD-TCL can exhibit diverse histological patterns, often in the same patient, including epidermotropism and dermal or subcutaneous involvement. CGD-TCLs are EBV-negative clonal T-cell lymphomas that express a mature cytotoxic phenotype with frequent apoptosis.<sup>6</sup> Although our previous study and individual case reports suggested that CGD-TCLs have an aggressive clinical course, the significance of TCR $\delta$ 1 expression on the outcome of patients with cutaneous lymphoma has not been studied. In the present study, we evaluated the clinical relevance of TCR $\delta$ 1 expression in predicting overall survival in individuals affected with primary cutaneous T cell lymphoma.

## **PATIENTS AND METHODS**

### **Patient evaluation**

Cases with the diagnosis of cutaneous T-cell lymphoma that had frozen skin biopsy specimens available during the period of July 1976 to July 2001 were included in the study, since staining of TCR $\delta$ 1 is only possible in frozen tissue sections. Clinical evaluation documented primary cutaneous disease without evidence of systemic spread within one year of diagnosis. Patients seen and treated at the NIH were enrolled in an IRB approved protocol. The remaining cases were submitted in consultation for diagnostic evaluation. Because the NIH is a tertiary hospital, the patients enrolled in our study do not represent a truly unselected population. Cases were classified according to the WHO classification.<sup>4</sup> T-cell lymphoma subtypes eligible for inclusion in the study

were: mycosis fungoides, subcutaneous panniculitis-like T-cell lymphoma, and peripheral T-cell lymphoma (PTCL), unspecified. PTCL, unspecified is a heterogeneous category in the WHO classification, and includes all mature T-cell lymphomas that cannot be assigned to a specific entity. Cases of primary cutaneous CD30+ T-cell lymphoproliferative<sup>31</sup> disease including, primary cutaneous anaplastic large cell lymphoma, were excluded as expression of TCR $\delta$ 1 was not observed in this subset of cases. In addition, primary cutaneous CD30+ T-cell lymphoproliferative disease is a distinct clinicopathological entity with a known favorable prognosis, and distinctive clinical, pathological, and immunophenotypic features.<sup>32</sup> Each patient had a detailed medical history and clinical examination of the skin and lymph nodes. As only 23/104 patients had a diagnosis of mycosis fungoides, we did not employ the staging system traditionally used for cutaneous T-cell lymphomas.<sup>33</sup> However, we did evaluate the major risk factors identified in this staging scheme in all patients. Thirty-six patients had lymph node biopsies and 68 had examination of the bone marrow. Routine morphological studies were done on 4-mm hematoxylin-eosin (H&E)–stained sections. We classified skin biopsies by the predominant pattern of lymphomatous involvement as epidermotropic, dermal or subcutaneous.

The immunophenotypic panel included monoclonal antibodies directed against the lymphocyte-associated antigens: CD3, CD4, CD 8, CD5, CD7, CD20,  $\beta$  F1, granzyme B, TIA-1 and perforin. Immunohistochemistry and antigen retrieval were performed as previously described.<sup>34</sup> Staining for TCR1 $\delta$  (T Cell Diagnostics, Inc. Woburn, MA) was performed in frozen sections. TCR  $\delta$ 1 staining was considered positive when more than 80% of tumor cells showed membrane positivity.

National Cancer Institute (NCI)-treated patients with cutaneous T-cell lymphoma were enrolled into successive NCI treatment protocols in which combined modality therapy (electron beam therapy, chemotherapy and topical treatment) was evaluated.<sup>35,36</sup> Patients with CGD-TCL did not achieve durable complete remissions with any of the available treatment protocols utilized at the NCI. These included conventional CTCL therapies such as topical steroids (1 individual), PUVA (7 individuals), IFN- $\alpha$  (4

individuals), IFN- $\gamma$  (1 individual), retinoids, and more aggressive treatments including radiation therapy (8 individuals), CHOP (cyclophosphamide, doxorubicin hydrochloride, vincristine sulfate, and prednisone)(6 individuals), polychemotherapy (8 individuals), bone marrow transplant (1 individual) or investigational therapies including anti-Tac (1 individual) or UCN-01( 7-hydroxy analogue of staurosporine, a tyrosine kinase inhibitor (1 individual). Therefore, the survival of patients in this series represents the natural history of disease altered by the best treatment available during the study period.

### **Statistical analysis**

Survival time was measured in months from time of diagnosis until date of death or last follow-up. Initially, univariate analyses were performed in order to screen for parameters to evaluate in a more definitive model. The Kaplan-Meier method was used to construct survival curves, and the probabilities of survival between pairs or sets of curves were compared with a log-rank test.<sup>37</sup> The following variables were considered for evaluation in the univariate analysis (levels compared are in parentheses): T-cell type (TCR $\delta$ + vs. TCR $\delta$ -), histologic profile (epidermotropic and/or dermal vs. subcutaneous), age (divided into four groups based on the quartiles: 41, 59, 69), sex (male vs. female) and adenopathy (yes vs. no). Additionally, actuarial analyses were performed using hemophagocytosis (yes vs. no), histologic profile (epidermotropic or dermal vs. subcutaneous), age (quartiles: 41, 49, 65), and sex among those patients classified as  $\gamma\delta$  (n=33). The Cox proportional hazards model method was then used to identify the significance of those parameters found to be potentially useful in the univariate analyses, when considered jointly.<sup>38</sup> In addition, a likelihood ratio test was performed to determine whether T-cell type was significantly associated with survival after adjustment for other common parameters evaluated.

## **RESULTS**

### *Clinicopathological characteristics*

The general characteristics of the patients studied are listed in **Table 1**. Our cohort included 66 men and 38 women with a median age of 59 years. The age range was 13-

84, with only two patients under the age of 21. Thirty-three of 104 cases expressed TCR $\delta$ 1 and were  $\beta$ F1 negative. Therefore, they were designated as cutaneous  $\gamma\delta$  T-cell lymphoma (CGD-TCL). All such cases were either CD4-/CD8- or CD8+ and expressed cytotoxic molecules in all cases studied (data not shown). Patients with CGD-TCL had a distinctive clinical presentation with a predominant involvement of the extremities with plaques, tumors and subcutaneous nodules, some of which ulcerated (**Figure 1**). Fifty-seven per cent (41/71) of patients with primary cutaneous  $\alpha\beta$  T-cell lymphomas presented with tumors, as compared to 73% (24/33) of patients with CGD-TCL. Histological patterns of involvement were evaluated in all 104 patients. Dominant epidermotropic or dermal involvement was present in 71 cases. Of this group 23 were classified within the spectrum of mycosis fungoides and 48 were classified as PTCL, unspecified. In addition, 33 cases had subcutaneous involvement. Of these cases, 10 cases were PTCL, unspecified and 23 cases were classified as SPTCL: 9 were  $\alpha\beta$  and 14 were  $\gamma\delta$ . The designation of SPTCL was based on a predominant pattern of subcutaneous infiltration, rimming of fat spaces by cytotoxic neoplastic T-cell lymphocytes, and prominent apoptosis as previously described.<sup>15</sup>

#### *Univariate Analysis*

The results of tests for significance from the univariate analysis are summarized in **Table 2**. There was a very large and statistically significant difference in survival between the patients with cutaneous  $\alpha\beta$  T-cell lymphoma and individuals affected with cutaneous  $\gamma\delta$  T-cell lymphoma ( $p < 0.0001$ ) (**Figure 2A**). In addition, there was a statistically significant decrease in survival in individuals who had subcutaneous involvement in comparison with patients who had epidermotropic and/or dermal involvement ( $p < 0.0001$ ) (**Figure 2B**). However, this was not consistent in magnitude between the patients with cutaneous  $\alpha\beta$  T-cell lymphoma and patients with cutaneous  $\gamma\delta$  T-cell lymphoma ( $p = 0.73$  within  $\alpha\beta$ , and  $p = 0.067$  within  $\gamma\delta$ ). Thus, an interaction between these two parameters was worthy of investigation in the Cox models (see below).

There was a marginally statistically significant difference in survival between individuals with and without bone marrow involvement, although data were only available to evaluate on 65% of all patients ( $p = 0.05$ ). There was no statistical difference in survival

between individuals with and without adenopathy ( $p=0.66$ ). There was a marginally statistically significant difference in gender when data from all patients were analyzed ( $p=0.047$ ). However, age was not associated with decreased survival ( $p=0.93$ ). To determine if the poorer prognosis of CGD TCL was related to the higher incidence of either clinical tumors or subcutaneous involvement in these patients, we examined the prognostic significance of immunophenotype ( $\alpha\beta$  vs.  $\gamma\delta$ ) within these subsets. In the subset of patients presenting with clinical tumors,  $\gamma\delta$  immunophenotype still predicted for poor prognosis ( $p < 0.0001$ ) (**Figure 2D**). Moreover, individuals with subcutaneous involvement and a  $\gamma\delta$  immunophenotype had a poorer survival than individuals with subcutaneous involvement and an  $\alpha\beta$  immunophenotype ( $p=0.0003$ ).

We also examined the statistical significance of a variety of parameters within the subset of patients with cutaneous  $\gamma\delta$  T-cell lymphoma. As shown in **Table 2**, subcutaneous involvement ( $p=0.067$ ) was the only parameter, which showed nearly statistical significance within this subset of patients (**Figure 2C**). The development of a hemophagocytic syndrome did not reach statistical significance ( $p = 0.21$ ).

#### *Cox Proportional Hazards Model Analysis*

The results from the Cox regression analysis are summarized in **Table 3**. Based on the results from the univariate analyses, the following variables were considered for inclusion in the Cox regression analysis: T-cell type ( $\alpha\beta$  vs.  $\gamma\delta$ ), histologic profile (epidermotropism and/or dermal vs. subcutaneous involvement), and sex (male vs. female). No other parameters were included since all other univariate  $p$ -values were  $>0.15$ . Since multiple factors did not emerge with respect to being potentially statistically significant in the patients with a  $\gamma\delta$  phenotype, no Cox model was created for that subset.

A Cox regression analysis, initially excluding T-cell type in order to establish the joint importance of all other factors under consideration, showed that histologic profile (epidermotropic and/or dermal vs. subcutaneous involvement) was significantly

associated with survival (model 1). Once T-cell subtype was added to the model (model 2), a likelihood ratio test showed that T-cell subtype was significantly associated with survival ( $P < 0.0001$ ) after adjusting for histologic profile. However, histologic profile was no longer statistically significantly associated with survival, which was also confirmed by a likelihood ratio test ( $p = 0.11$ ). When the histologic profile was removed from the two-factor model, model 2, leaving only T cell type (model 3), T-cell type showed a strong association with survival.

T-cell type and histologic profile interactions were also tested, with both T-cell type and histologic profile effects in the model, since there appeared to be a differential effect in the univariate analyses. However, there was no statistically significant interaction between T-cell type and histologic profile (likelihood ratio  $P = 0.22$ ).

A likelihood ratio test was also performed to test whether gender was associated with survival after adjusting for T-cell type. In addition, T-cell type by gender interaction was evaluated after inclusion of both T-cell type and gender effects in the model. However, neither gender (likelihood ratio  $P = 0.26$ ) nor T-cell type by gender interaction (likelihood ratio  $P = 0.13$ ) was significantly associated with survival.

## **DISCUSSION**

While the emphasis of the WHO classification is on the definition of disease entities, peripheral or mature T-cell lymphomas remain poorly understood. Lineage is the starting point in the subclassification of most lymphoid malignancies, yet the literature on primary cutaneous  $\gamma\delta$  T-cell lymphoma has been limited, because of the rarity of this lymphoma and the inability to study large numbers of patients. Most small series and case reports have indicated an aggressive clinical course. However, no other study has evaluated the impact of a  $\gamma\delta$  immunophenotype on the clinical outcome of primary cutaneous T-cell lymphoma in patients with diverse patterns of involvement. This study was designed to evaluate if TCR $\delta$  expression was of prognostic significance in primary cutaneous T-cell lymphomas. Immunohistochemical studies for TCR $\delta$ 1 were utilized to

provide direct evidence of  $\gamma\delta$  T-cell derivation. In addition, all TCR $\delta$ 1 positive cases tested were  $\beta$ F1 negative consistent with a  $\gamma\delta$  T-cell derivation (data not shown). We found that there was a statistically significant decrease in survival among individuals affected with  $\gamma\delta$  cutaneous T-cell lymphoma in comparison with individuals with  $\alpha\beta$  cutaneous T-cell lymphoma (**Figure 2A**). The median survival for individuals with  $\gamma\delta$  T-cell lymphoma was 15 months, while the median survival of individuals with  $\alpha\beta$  T-cell lymphoma was 166 months. Cox proportional hazard model analysis confirmed that TCR $\delta$ 1 expression was strongly associated with decreased survival.

We then investigated whether there was a difference in survival related to the depth of cutaneous involvement for all patients. We found a statistically significant decrease in survival in individuals who had subcutaneous involvement in comparison with individuals who had epidermotropic or dermal involvement (**Figure 2B**). We further examined the significance of these histological parameters within only the  $\gamma\delta$  T-cell group. Subcutaneous involvement in CGD-TCL was associated with a trend for decreased survival in comparison to individuals who had epidermotropic or dermal involvement (**Figure 2C**). Those with subcutaneous involvement had a median survival of 13 months as compared with those with epidermotropic or dermal involvement who had a more favorable median survival of 29 months. We also sought to identify other clinical and pathologic features of prognostic significance in patients with CGD TCL. However, age, gender, adenopathy and hemophagocytic syndrome did not have a significant association with survival.

Clinical tumors and subcutaneous involvement were more commonly seen in patients with CGD-TCL than in patients with  $\alpha\beta$  T-cell tumors (Table 1). However, these factors alone cannot account for the poor survival of patients with  $\gamma\delta$  T-cell disease. In the subset of patients presenting with clinical tumors,  $\gamma\delta$  immunophenotype still predicted for poor prognosis ( $p < 0.0001$ ) (**Figure 2D**). Moreover, individuals with subcutaneous involvement and a  $\gamma\delta$  immunophenotype had a poorer survival than individuals with subcutaneous involvement and an  $\alpha\beta$  immunophenotype ( $p=0.0003$ ).

In the present study we have demonstrated that TCR $\delta$ 1 expression can be useful to predict the clinical outcome of patients with primary cutaneous T-cell lymphoma. Clinically, patients had aggressive disease and were resistant to multiagent chemotherapy and /or radiation. Sixty-six percent (22/33) patients were dead of their disease within 5 years of diagnosis and seven patients had progressive disease. It is of interest that none of the patients with CGD-TCL had bone marrow involvement, only one had lymph node involvement and only 4 had adenopathy. However, biopsy evaluation of these sites was limited; only 36 patients had lymph node biopsies, and 68 had bone marrow biopsies. Similar to MF/SS, CGD-TCL may spare the bone marrow even in advanced and leukemic stages. This pattern of dissemination is consistent with the tendency of CGD-TCL to preferentially involve mucocutaneous sites, and also may be a reflection of the migratory features of “cutaneous T-cells”. However, because of the poor clinical outcome, early diagnosis is indicated in patients with CGD-TCL.

Prior studies have indicated that mucosal /cutaneous  $\gamma\delta$  T-cell lymphoma represents a proliferation of functionally mature T-cell lymphocytes that express TIA-1 and release the cytotoxic proteins granzyme B and perforin, capable of inducing cellular apoptosis.<sup>6,14</sup> This distribution of disease reflects the localization of normal  $\gamma\delta$  T-cells, which are believed to play a role in host mucosal and epithelial immune responses.<sup>8,19</sup> Mucosal and cutaneous  $\gamma\delta$  T-cell lymphomas differ from hepatosplenic  $\gamma\delta$  T-cell lymphomas, which are derived from functionally immature  $\gamma\delta$  T-cells, positive for TIA-1, but negative for granzyme B and perforin.<sup>12,18,39</sup> It is possible that the activated cytotoxic phenotype contributes to the aggressive clinical behavior as suggested in prior studies.<sup>7</sup> For example, cutaneous epidermotropic CD-8+ cytotoxic T-cell lymphoma is characterized by generalized patches, plaques, papulonodules, and tumors, spread to unusual sites but not to the lymph nodes, and an aggressive course (median survival, 32 months). Histologically, it shows a band-like infiltrate of epidermotropic T-cells and necrosis. The neoplastic cells express CD3, CD8, CD7, CD45RA,  $\beta$ F1, and TIA-1 markers, whereas CD2 and CD5 were frequently absent.<sup>7</sup> However, not all cutaneous

lymphomas of cytotoxic phenotype are aggressive. For example, cutaneous ALCL has an indolent clinical course but expresses a cytotoxic phenotype.<sup>34</sup>

All patients in this study presented with disease initially confined to the skin. A characteristic clinical feature of patients with CGD-TCL was the presence of necrotic tumors or nodules, primarily affecting the extremities. This clinical feature should raise a suspicion for the diagnosis of CGD-TCL. We had found this characteristic quite helpful in identifying cases during physical examination. We identified three histological patterns of involvement in the skin: epidermotropic, dermal and subcutaneous involvement. However, usually more than one histological pattern was present in the same patient in different biopsy specimens or even in some cases within a single biopsy specimen (**Figure 1**). Epidermal infiltration from mild epidermotropism to pagetoid reticulosis-like has been reported previously in some cases of CGD-TCL.<sup>6,7</sup> We only observed mild to moderate epidermotropism in our cases.

Subcutaneous involvement has been reported in  $\gamma\delta$  T-cell lymphoma.<sup>24</sup> Moreover, in several recent studies approximately 25% of cases of SPTCL were identified as being of  $\gamma\delta$  T-cell derivation based on expression of TCR $\delta$ 1 or inferential evidence based on absent staining for  $\beta$ F-1 in conjunction with a double negative cytotoxic T-cell phenotype.<sup>15,16,40</sup> CGD-TCL with subcutaneous involvement shares many clinical and histological features with SPTCL of  $\alpha\beta$  derivation including expression of cytotoxic molecules such as TIA-1 and perforin, and the presence of an atypical lymphocytic infiltrate with rimming of fat spaces within the subcutaneous tissue. However in the published cases, certain differences with classical SPTCL of  $\alpha\beta$  origin have been noted. For example, the cases of  $\gamma\delta$  SPTCL have been noted to exhibit dermal involvement, in addition to the classic subcutaneous panniculitis-like infiltrate, as well as aggressive clinical behavior.<sup>15,16,40</sup> Similar to previous reports, we found that cases of CGD-TCL with panniculitic features often manifested dermal as well as subcutaneous infiltrates, and variations in the histologic pattern were seen. However, not all cases with subcutaneous involvement in our series fulfilled the criteria for SPTCL. Of 33 cases with subcutaneous involvement, only 23 were diagnosed as SPTCL, 14 of  $\gamma\delta$  phenotype and 9 of  $\alpha\beta$  phenotype.

Arnulf et al. made the observation that mucocutaneous  $\gamma\delta$  T-cell lymphomas could resemble SPTCL, nasal NK/T-cell lymphoma or even enteropathy-associated T-cell lymphoma.<sup>14</sup> Our study expands the diversity of histological patterns described in the skin in  $\gamma\delta$  T-cell lymphomas, and more importantly shows that irrespective of histological pattern,  $\gamma\delta$  immunophenotype has important prognostic implications.

The distinctive clinical presentation in conjunction with a spectrum of histologic patterns and the fact that TCR $\delta$ 1 expression is an independent prognostic factor associated with decreased survival suggests that CGD-TCL has distinctive features. Further studies are needed to determine if mucocutaneous  $\gamma\delta$  T-cell lymphoma should be designated as a separate disease entity in future classification schemes.<sup>39</sup> Analysis of T-cell subtype expression ( $\alpha\beta$  vs.  $\gamma\delta$ ) may be clinically indicated in the evaluation of patients with primary cutaneous T-cell lymphomas.

## References

1. Jaffe ES, Krenacs L, Raffeld M. Classification of T-cell and NK-cell neoplasms based on the REAL classification. *Ann. Oncol.* 1997;8 (suppl. 2):S17-S24
2. Kluijn PM, Feller A, Gaulard P, Jaffe ES, Meijer CJ, Muller-Hermelink HK, Pileri S. Peripheral T/NK-cell lymphoma: a report of the IXth Workshop of the European Association for Haematopathology Conference report. *Histopathology.* 2001;38:250-270.
3. Harris NL, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, Delsol G, De Wolf Peeters C, Falini B, Gatter KC, Grogan TM, Isaacson PG, Knowles DM, Mason DY, Muller-Hermelink H-K, Pileri S, Piris MA, Ralfkiaer E, Warnke RA. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood.* 1994;84:1361-1392
4. Jaffe ES, Harris NL, Stein H, Vardiman J. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. World Health Organization Classification of Tumours. Lyon, France: IARC Press; 2001
5. Willemze R, Kerl H, Sterry W, Berti E, Cerroni L, Chimenti S, Diaz-Perez JL, Geerts ML, Goos M, Knobler R, Ralfkiaer E, Santucci M, Smith N, Wechsler J, van Vloten WA, Meijer CJ. EORTC classification for primary cutaneous lymphomas: a proposal from the Cutaneous Lymphoma Study Group of the European Organization for Research and Treatment of Cancer. *Blood.* 1997;90:354-371
6. Toro JR, Beaty M, Sorbara L, Turner ML, White J, Kingma DW, Raffeld M, Jaffe ES. Gamma delta T-cell lymphoma of the skin: a clinical, microscopic, and molecular study. *Archives of Dermatology.* 2000;136:1024-1032
7. Berti E, Cerri A, Cavicchini S, Delia D, Soligo D, Alessi E, Caputo R. Primary cutaneous gamma/delta T-cell lymphoma presenting as disseminated pagetoid reticulosis. *J. Invest. Dermatol.* 1991;96:718-723
8. Bluestone JA, Khattry R, Sciammas R, Sperling AI. TCR gamma delta cells: a specialized T-cell subset in the immune system. *Annu Rev Cell Dev Biol.* 1995;11:307-353.
9. Janis EM, Kaufmann SH, Schwartz RH, Pardoll DM. Activation of gamma delta T cells in the primary immune response to *Mycobacterium tuberculosis*. *Science.* 1989;244:713-716.
10. Biondi A, Champagne E, Rossi V, Giudici G, Cantu-Rajoldi A, Masera G, Mantovani A, Mak TW, Minden MD. T-cell receptor delta gene rearrangement in childhood T-cell acute lymphoblastic leukemia. *Blood.* 1989;73:2133-2138
11. Gouttefangeas C, A. B, Boumsell L. Utilization of two different T-cell receptors by T-cell acute lymphoblastic lymphoma and leukemia. *Nouv. Rev. Fr. Hematol.* 1990;32:337-340
12. Cooke CB, Krenacs M, Stetler-Stevenson M, Greiner TC, Raffeld M, Kingma DW, Abruzzo L, Frantz C, Kaviani M, Jaffe ES. Hepatosplenic gamma/delta T-cell lymphoma: A distinct clinicopathologic entity of cytotoxic gamma/delta T-cell origin. *Blood.* 1996;88:4265-4274

13. Gaulard P, Zafrani ES, Mavier P, Rocha FD, Farcet J-P, Divine M, Haioun C, Pinaudeau Y. Peripheral T-cell lymphoma presenting as predominant liver disease: a report of three cases. *Hepatology*. 1986;6:864-868
14. Arnulf B, Copie-Bergman C, Delfau-Larue MH, Lavergne-Slove A, Bosq J, Wechsler J, Wassef M, Matuchansky C, Epardeau B, Stern M, Bagot M, Reyes F, Gaulard P. Nonhepatosplenic gamma-delta T-cell lymphoma: a subset of cytotoxic lymphomas with mucosal or skin localization. *Blood*. 1998;91:1723-1731
15. Kumar S, Krenacs L, Elenitoba-Johnson K, Greiner T, Sorbara L, Kingma D, Raffeld M, Jaffe E. Subcutaneous panniculitic T-cell lymphoma is a tumor of cytotoxic T-lymphocytes. *Hum Pathol*. 1998;29:397-403
16. Salhany KE, Macon WR, Choi JK, Elenitsas R, Lessin SR, Felgar RE, Wilson DM, Przybiski GK, Lister J, Wasik MA, Swerdlow SH. Subcutaneous panniculitis-like T-cell lymphoma: clinicopathologic, immunophenotypic, and genotypic analysis of alpha/beta and gamma/delta subtypes. *Am J Surg Pathol*. 1998;22:881-893
17. Przybyski GK, Wu H, Macon WR, Finan J, Leonard DG, Felgar RE, DiGiuseppe JA, Nowell PC, Swerdlow SH, Kadin ME, Wasik MA, Salhany KE. Hepatosplenic and subcutaneous panniculitis-like gamma/delta T cell lymphomas are derived from different Vdelta subsets of gamma/delta T lymphocytes. *J Mol Diagn*. 2000;2:11-19.
18. Boulland ML, Kanavaros P, Wechsler J, Casiraghi O, Gaulard P. Cytotoxic protein expression in natural killer cell lymphomas and in alpha beta and gamma delta peripheral T-cell lymphomas. *J Pathol*. 1997;183:432-439.
19. Williams N. T cells on the mucosal frontline. *Science*. 1998;280:198-200.
20. Ralfkiaer E, Wolff-Sneedorff A, Thomsen K, Geisler C, Vejlsgaard GL. T-cell receptor gamma delta-positive peripheral T-cell lymphomas presenting in the skin: a clinical, histological and immunophenotypic study. *Exp Dermatol*. 1992;1:31-36
21. Alaibac M, Chu A. Pagetoid reticulosis; a gamma delta T-cell lymphoma? *Eur J Dermatol*. 1992;2:109-111
22. Burg G, Dummer R, Wilhelm M, Nestle F, Ott MM, Feller A, Hefner H, Lanz U, Schwinn A, Wiede J. A subcutaneous delta-positive T-cell lymphoma that produces interferon gamma. *N Engl J Med*. 1991;325:1078-1081
23. Fujita M, Miyachi Y, Furukawa F, Toichi E, Furukawa I, Nakajima N, Imamura S. A case of cutaneous T-cell lymphoma expressing gamma delta T-cell receptors. *J Am Acad Dermatol*. 1993;28:355-360
24. Avinoach I, Halevy S, Argov S, Sacks M. Gamma/delta T-cell lymphoma involving the subcutaneous tissue and associated with a hemophagocytic syndrome. *Am J Dermatopathol*. 1994;16:426-433

25. Munn SE, McGregor JM, Jones A, Amlot P, Rustin MH, Russell Jones R, Whittaker S. Clinical and pathological heterogeneity in cutaneous gamma-delta T-cell lymphoma: a report of three cases and a review of the literature. *Br J Dermatol.* 1996;135:976-981.
26. Barzilai A, Goldberg I, Shibi R, Kopolovic J, Trau H. Mycosis fungoides expressing gamma/delta T-cell receptors. *J Am Acad Dermatol.* 1996;34:301-302.
27. Aractingi S, Marolleau JP, D'Agay MF, Flageul B, Gisselbrecht C, Dubertret L. [Epidermotropic T-cell gamma/delta lymphoma]. *Ann Dermatol Venereol.* 1993;120:794-796.
28. Amoric JC, Bodemer C, Donadieu J, Brousse N, Agache P, Ranfraing E, Griscelli C, De Prost Y. [Cutaneous manifestations disclosing T-cell gamma/delta lymphoma in a 13-year-old-girl]. *Ann Dermatol Venereol.* 1993;120:792-793.
29. Marzano AV, Berti E, Paulli M, Caputo R. Cytophagic histiocytic panniculitis and subcutaneous panniculitis-like T-cell lymphoma: report of 7 cases. *Arch Dermatol.* 2000;136:889-896.
30. Jones D, Vega F, Sarris AH, Medeiros LJ. CD4-CD8-"Double-negative" cutaneous T-cell lymphomas share common histologic features and an aggressive clinical course. *Am J Surg Pathol.* 2002;26:225-231.
31. Beljaards RC, Kaudewitz P, Berti E, Gianotti R, Neumann C, Rosso R, Paulli M, Meijer CJ, Willemze R. Primary cutaneous CD30-positive large cell lymphoma: definition of a new type of cutaneous lymphoma with a favorable prognosis. A European Multicenter Study of 47 patients. *Cancer.* 1993;71:2097-2104
32. Willemze R, Beljaards RC. Spectrum of primary cutaneous CD30 (Ki-1)-positive lymphoproliferative disorders. A proposal for classification and guidelines for management and treatment. *J Am Acad Dermatol.* 1993;28:973-980
33. Bunn PA, Jr., Lamberg SI. Report of the Committee on Staging and Classification of Cutaneous T-Cell Lymphomas. *Cancer Treat Rep.* 1979;63:725-728.
34. Krenacs L, Wellmann A, Sorbara L, Himmelmann AW, Bagdi E, Jaffe ES, Raffeld M. Cytotoxic cell antigen expression in anaplastic large cell lymphomas of T- and null-cell type and Hodgkin's disease: evidence for distinct cellular origin. *Blood.* 1997;89:980-989
35. Winkler CF, Sausville EA, Ihde DC, Fischmann AB, Schechter GP, Kumar PP, Nibhanupdi JR, Minna JD, Makuch RW, Eddy JL, et al. Combined modality treatment of cutaneous T cell lymphoma: results of a 6-year follow-up. *J Clin Oncol.* 1986;4:1094-1100.
36. Kaye FJ, Bunn PA, Jr., Steinberg SM, Stocker JL, Ihde DC, Fischmann AB, Glatstein EJ, Schechter GP, Phelps RM, Foss FM, et al. A randomized trial comparing combination electron-beam radiation and chemotherapy with topical therapy in the initial treatment of mycosis fungoides. *N Engl J Med.* 1989;321:1784-1790.
37. Kaplan E, Meier P. Non-parametric estimation from incomplete observations. *J Am Stat Assoc.* 1958;53:457-481
38. Cox DR. Regression models and life tables. *J Royal Stat Society.* 1972;34:187-202

39. de Wolf-Peeters C, Achten R. Gamma delta T-cell lymphomas: a homogeneous entity? Histopathology. 2000;36:294-305.

40. Massi D, Pimpinelli N, Berti E, Cerroni L, Kadin ME, Meijier CJLM, Muller-Hermelink HK, Pauli M, Wechsler J, Willemze R, Santucci M. Subcutaneous panniculitis-like T-cell lymphoma. J.Clin.Pathol. 2002;55 (Suppl. I):38A

**Table 1. Clinical characteristics of patients with primary cutaneous T-cell lymphomas**

		<b><math>\alpha\beta</math> (n=71)</b>	<b><math>\gamma\delta</math> (N=33)</b>
<b>Age</b>			
Median	59	62	49
Range	(13-84)	(17-84)	(13-82)
<b>Gender</b>			
Male	66	47	19
Female	38	24	14
<b>Adenopathy ( 6 not done)</b>			
Positive	26	22	4
Negative	72	44	28
<b>Histologic profile</b>			
Epidermotropic or Dermal	71	59	12
Subcutaneous	33	12	21
<b>Provisional subtype in WHO classification</b>			
Mycosis fungoides	23	19	4
SPTCL	23	9	14
PTL, unspecified	58	43	15
<b>Bone Marrow (36 not done)</b>			
Positive	5	5	0
Negative	63	45	18
<b>Node Biopsy (68 not done)</b>			
Positive	5	4	1
Negative	31	21	10

## Table 2. Univariate Analysis

### A. Univariate analysis of primary cutaneous T-cell lymphomas

Variable	P-value (Log-rank, two-tailed)
T-cell type ( $\alpha\beta$ vs. $\gamma\delta$ )	<0.0001
Histologic profile (epidermotropic or dermal vs. subcutaneous)	<0.0001
Age (among 4 groups)	0.93
Sex	0.047
Adenopathy (normal vs. positive)	0.66
Bone marrow (normal vs. positive)	0.05

### B. Univariate analysis of cutaneous $\gamma\delta$ T-cell lymphomas

Variable	P-value (Log-rank, two-tailed)
Histologic profile (epidermotropic or dermal vs subcutaneous)	0.067
Age (among 4 groups)	0.14
Sex	0.77
Adenopathy (normal vs. positive)	0.17
Hemophagocytic syndrome (negative vs. positive)	0.21

### Table 3. Multivariate Analysis

#### A. Multivariate analysis of primary cutaneous T-cell lymphomas

Model	Variables in model	P*	Hazard ratio (95% confidence interval)
1	Histologic profile	<0.0001	0.25 (0.13-0.48)
2	Histologic profile	0.11	0.55 (0.26-1.14)
	T-cell type	<0.0001	0.16 (0.08-0.35)
3	T-cell type	<0.0001	0.13 (0.07-0.25)

\*Chi-square p-value



**Figure. 1** Dermatological and histologic features of cutaneous gamma delta T-cell lymphoma.

**A.** Characteristic ulcerated tumors covered with hemorrhagic crust.

**B.** Multiple pink to plum-colored tumors in an upper extremity.

**C.** Low magnification shows an example of the coexistent patterns epidermotropic, dermal and subcutaneous patterns of involvement in a single biopsy. There is psoriasiform epidermal hyperplasia and a band-like lymphocytic infiltrate in the papillary dermis with epidermotropism. The infiltrate extends into the reticular dermis with a perivascular pattern. In addition, there is infiltration of subcutaneous tissue reminiscent of lobular panniculitis.

**D.** The dermal infiltrate is positive for TCR $\delta$ 1 by immunohistochemistry (ABC immunoperoxidase; methyl green counter stain).

Note: Some components of this figure were previously published in Toro et al.(6), and reproduced with the permission of the journal, in part, in (4).

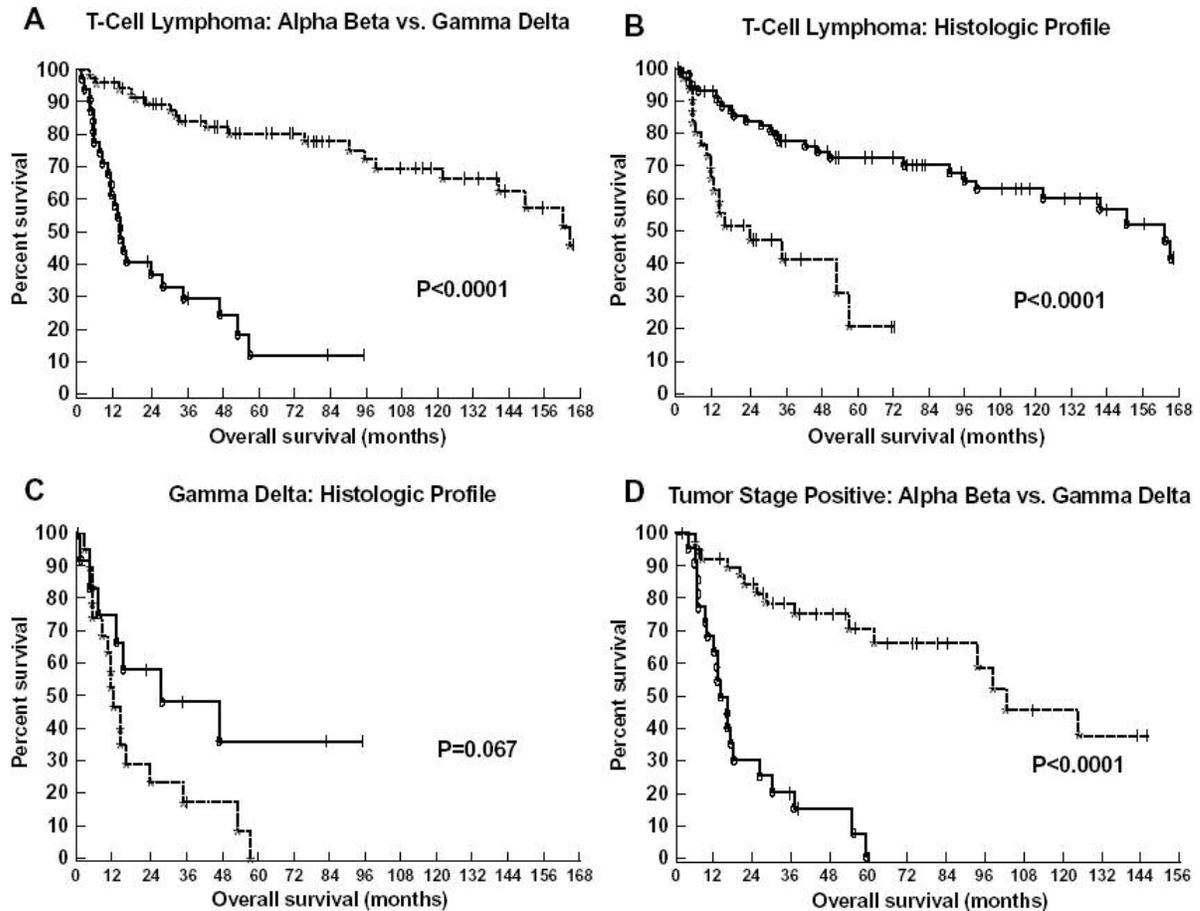


Figure 2

**Figure 2.** Kaplan and Meier plots of patients with cutaneous T-cell lymphoma.

**A.** Survival of individuals with cutaneous T-cell lymphoma according to T-cell receptor immunophenotype. A comparison of was made between patients with alpha beta (dotted line) and gamma delta (solid line) cutaneous T-cell lymphomas. Significance was determined by the log rank test.

**B.** Survival of individuals with cutaneous T-cell lymphoma according to histologic profile. A comparison of survival was made between patients with subcutaneous involvement (dotted line), and epidermotropic and/or dermal involvement (solid line). Significance was determined by the log rank test.

**C.** Survival of individuals with cutaneous gamma delta T-cell lymphoma according to histologic profile. A comparison of survival was made between patients with subcutaneous involvement (dotted line), and epidermotropic and/or dermal involvement (solid line). Significance was determined by the log rank test.

Survival of patients presenting with cutaneous tumors according to T-cell receptor immunophenotype. A comparison of was made between patients with alpha beta (dotted line) and gamma delta (solid line) cutaneous T-cell lymphomas. Significance was determined by the log rank test.