

# Cutaneous T-cell lymphoma: molecular pathogenesis and clinical behaviour.

Doorn, R. van

### Citation

Doorn, R. van. (2005, October 26). *Cutaneous T-cell lymphoma: molecular pathogenesis and clinical behaviour*. Retrieved from https://hdl.handle.net/1887/3630

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/3630

**Note:** To cite this publication please use the final published version (if applicable).

## **Summary and Discussion**

Studies presented in this thesis focused on clinicopathological determinants of disease behaviour in patients with CTCL, and molecular studies aimed at identifying (epi)genetic features of malignant T cells relevant in the development and progression of these T-cell malignancies.

#### **Disease progression in MF**

In chapter 2 we determined the disease course of 309 Dutch patients with MF stratified per clinical stage and defined clinical factors related to disease progression and survival. Consistent with previous reports, the disease-specific 5-year survival rate of patients with MF was approximately 89% and even higher for patients with localized or generalized patches or plaques.<sup>1-4</sup> As an indicator of disease evolution in addition to survival rate, we studied disease progression, defined as the development of skin tumors or extracutaneous dissemination, in this patient cohort. The risk of disease progression for the entire group of patients with MF within the first 5 years of diagnosis was 24%. These results confirm clinical experience that patients with MF usually have stable disease for years and that the development of skin tumors or potentially lethal extracutaneous dissemination takes place in a minority of cases. The estimated disease-specific 5-year survival was 89%. In addition this study includes disease progression and survival data stratified per clinical stage. Multivariate analysis revealed that patients presenting with extracutaneous disease or extensive skin involvement, patients who had an incomplete response to initial treatment or patients who had folliculotropic MF had a higher risk of disease progression and a lower disease-specific survival rate. The detailed prognostic data obtained from this long-term follow-up study of a large representative patient cohort represent useful information for clinicians involved in the management of patients with MF.

#### **Folliculotropic MF: a distinct variant of MF**

The results of multivariate analysis presented in chapter 2, suggesting that patients with folliculotropic MF have a worse prognosis independently of other prognostic parameters, prompted us to study a larger group of patients with this type of CTCL. In chapter 3 the results of this study on the clinicopathologic and prognostic features of 51 Dutch patients diagnosed with folliculotropic MF are outlined. Our observations confirm that the morphology and distribution of the skin lesions are very different from those of patients with the classic epidermotropic type of MF. Moreover, folliculotropic MF was more refractory to treatment and was associated with a significantly higher risk of disease progression and disease-related mortality. Based on these findings we advocate radiotherapy in the treatment of folliculotropic MF.

Our conclusions concerning the aggressive behaviour of folliculotropic MF have been supported by subsequent reports from other research groups.<sup>5,6</sup> However, in a subsequent study by Cerroni and colleagues it was argued that also idiopathic follicular mucinosis, with no pathologic evidence of MF, may be considered a variant of MF.<sup>7</sup> This was concluded from the fact that there was a considerable overlap in the histological features, molecular analyses and clinical presentation between patients with idiopathic and MF-associated variants of follicular mucinosis. When considering the compound group of patients with idiopathic and MF-associated follicular mucinosis

they found the disease course to be relatively favorable. The design of this study and the implication of the conclusion, namely that one could diagnose a malignant lymphoma on the basis of the presence of reactive mucinous degeneration of follicular epithelium without the demonstration of malignant cells, have been criticized and dismissed by others.<sup>8</sup> The results of our study furthermore support the contention that folliculotropism of the neoplastic T-cell infiltrate and not the presence of follicular mucinosis is the essential feature of this disease: the clinical presentation and disease course of patients with the characteristic features of folliculotropic MF but without follicular mucinosis. This view has been reflected in the recently published WHO-EORTC classification, that makes no distinction between cases with and without follicular mucinosis.<sup>9</sup>

#### <u>CD8<sup>±</sup> T cells in CTCL skin lesions</u>

Several lines of evidence indicate that an anti-tumor immune response, primarily mounted by cytotoxic CD8<sup>+</sup> T cells, exists in CTCL and partly determines the clinical behaviour of this malignancy. Bagot and colleagues demonstrated that CD8<sup>+</sup> T cells obtained from an MF skin lesion have cytolytic activity to autologous neoplastic T cells.<sup>10</sup> However in two previous studies, correlation between the relative numbers of CD8<sup>+</sup> cytotoxic T cells in MF skin lesions and clinical behaviour had led to contradicting results.<sup>11,12</sup> To further characterize the role of cytotoxic CD8<sup>+</sup> T cells in CTCL, we investigated the expression of cytotoxic markers by these cells and determined the proportion of CD8<sup>+</sup> to neoplastic T cells in skin lesions of patients with MF and CD30<sup>-</sup> LTCL in chapter 4. We found that CD8<sup>+</sup> T-cells in MF and CD30<sup>-</sup> LTCL skin lesions express TIA-1 and Fas ligand indicative of cytolytic capacity. The proportion of cytotoxic CD8<sup>+</sup> T cells to neoplastic CD4<sup>+</sup> T cells was significantly higher in plaque-stage MF than in advanced tumor-stage MF and CD30<sup>-</sup> LTCL. Moreover, within the group of patients with tumor-stage MF a low proportion of CD8<sup>+</sup> T cells was associated with poor prognosis. This study shows that the balance between cytotoxic CD8<sup>+</sup> T cells and neoplastic T cells shifts during progression of disease and supports the notion that cytotoxic activity of CD8<sup>+</sup> T cells limits this progression. Furthermore the fact that higher proportions of CD8<sup>+</sup> T cells in skin lesions predict better prognosis may be applied in a clinical setting. Correspondingly, Abeni and colleagues found that a low number of CD8<sup>+</sup> T cells in peripheral blood of patients with MF predicted a poor survival.<sup>13</sup>

#### Alternative splicing and point mutations of the Fas gene in CTCL

Defective apoptosis signaling in malignant T cells of patients with CTCL is suggested by the fact that these malignant cells have a low mitosis index and are resistant to anti-tumor immune responses and treatment with genotoxic agents.<sup>14</sup> In addition, mechanisms governing T cell homeostasis, presumed to be disrupted in CTCL, are critically dependent on apoptosis as a means of eliminating clonally expanding cells. A key regulator of apoptosis in mature T cells is the Fas (CD95) death receptor, that activates a signaling cascade eventuating in apoptosis upon cross-linking to Fas ligand. A previous study by our research group demonstrated that expression of Fas protein was diminished in aggressive subtypes and advanced stages of CTCL.<sup>15</sup> The function and expression of the Fas receptor may be affected by deleterious point mutations as well as by alternative splicing affecting its death and transmembrane domain.<sup>16-19</sup>

In chapter 5 we demonstrate the occurrence of a specific splice variant of the *Fas* gene that displays retention of intron 5 in malignant T cells of 13 of 22 patients (59%) with CTCL. Translation of this aberrantly spliced variant results in a protein that terminates prematurely and does not contain a so-called death domain, essential for propagating the pro-apoptotic signal. Since the truncated protein also lacks a functional transmembrane anchor domain, splicing of the *Fas* gene may also be related to diminished expression of the Fas receptor protein. In addition several point mutations in the *Fas* gene were detected in malignant T cells. These findings shed light on the basis for resistance to apoptosis of malignant T cells in CTCL, postulated as central in the pathogenesis of this group of malignancies, and point to alternative splicing as an important underlying mechanism regulating the function of the *Fas* unor suppressor gene. Providing a possible explanation, in chapter 6 we show that a number of genes encoding proteins involved in pre-mRNA splicing were downregulated in malignant T cells of patients with Sz when compared with benign T cells.

To evaluate whether the intron 5-retaining Fas transcript was exclusively expressed in malignant T cells of CTCL patients and its detection might be used in early diagnosis of CTCL, we recently examined a large panel of skin biopsies from inflammatory dermatoses. We found that the isoform was also expressed in a small proportion of auto-immune skin diseases, indicating that in benign T cells the function of Fas may under certain circumstances be regulated at the level of pre-mRNA splicing (unpublished results). In subsequent studies by other research groups, alternative splicing of the *Fas* gene associated with deletion of the death domain and functional inactivation has been shown in several hematopoietic malignancies including adult T-cell leukaemia, acute lymphoblastic leukaemia and nasal-type natural killer/T-cell lymphoma.<sup>20-22</sup> Further functional studies into the underlying mechanism and consequences of alternative splicing of Fas transcript are warranted.

#### **Expression profiling of Sézary syndrome**

As noted in chapter 1, the use of microarray technology to investigate gene expression patterns has meant a great advance for the study of pathogenetic mechanisms in cancer. In chapter 6 we applied oligonucleotide microarray analysis to study expression patterns of malignant T cells from peripheral blood of patients with Sz, a leukemic variant of CTCL. In order to identify gene expression alterations that are related to the malignant phenotype, we compared expression patterns of malignant T cells of patients with Sz to those of benign T cells from healthy subjects and from patients with benign forms of erythroderma. From the great amount of data obtained in this study we focused on altered expression of oncogenes and tumor suppressor genes. Collectively our results point to dysregulation of multiple growth-regulatory pathways in Sz. Among hematopoietic malignancy-linked tumor suppressor genes downregulated in Sz were *TGF-beta receptor II*, *Forkhead Box O1A*, *CREB-binding protein* and *Mxi1*. Downregulation of the tumor suppressor genes *BCL11a* and *BCL2-like 11* (also known as *BCL2-interacting protein*, *Bim*) in Sz may be specifically implicated in resistance to apoptosis.

As discussed in chapter 6, high expression of the potential oncogenes *Twist* and *EphA4* was detected in malignant T cells of patients with Sz. The occurrence and the role of Twist overexpression in malignant tumors has received much attention

recently. The pro-oncogenic effects of Twist overexpression are now thought to be primarily related to its inhibition of the ARF/p53 pathway.<sup>23</sup> Inhibition of this pathway protects cells from the proapoptotic effects of c-myc, the activity of which may be increased in Sz through altered expression of the regulators of its activity Mxi1, Mnt and MycBP.

The EphA4 transmembrane tyrosine kinase, overexpressed by malignant T cells in patients with Sz and MF, may serve as a target for directed therapeutic intervention using monoclonal antibodies. The EphA4 receptor belongs to a family of growth-regulatory Eph receptors, of which several members are overexpressed in cancer.<sup>24,25</sup> We are currently conducting further studies on the functional significance of *EphA4* expression.

Systemic immunosuppression is the major cause of death in patients with Sz. T-cell depletion and reduction in the T-cell repertoire complexity concomitant with expansion of the malignant T-cell clone has been held responsible, but the underlying mechanisms are still unclear.<sup>26</sup> We have found markedly increased expression of the TNF receptor family ligands *CD70* and *RANKL* in malignant T cells of patients with Sz. Since CD70-transgenic mice similarly show depletion of naive T cells, we propose that the induction of inappropriate immune activation by these costimulatory membrane molecules may be responsible for depletion of the naïve T-cell pool and immunodeficiency in patients with Sz.<sup>27</sup>

Due to the multiplicity of potentially oncogenic alterations observed in neoplastic T cells from patients with Sz in this study and the fact that many of the alterations have not yet been confirmed on the protein level, it would be premature to propose a model of essential pathogenetic alterations and aberrant signaling in Sz. Functional studies will be necessary to discern from the multiplicity of potentially oncogenic alterations observed, the essential (epi)genetic events in the pathogenesis of Sz and related CTCLs.

#### **Promoter hypermethylation in CTCL**

In chapter 7 we demonstrated that malignant T cells of patients with CTCL display widespread promoter hypermethylation associated with inactivation of several tumor suppressor genes.

In recent years it has been recognized that the epigenetic mechanism of promoter hypermethylation is at least as common as genetic mechanisms such as mutation and chromosomal loss in inactivating classic tumor suppressor genes in cancer cells. In contrast to genetic lesions, promoter hypermethylation is potentially reversible by treatment with chemical demethylating agents.

Using a microarray-based epigenomic screening method we identified 35 CpG islands frequently methylated in CTCL, several of which are located in the promoters of tumor suppressor genes.

Strikingly, one of the most differentially methylated CpG islands was located in the first exon of *BCL7a*, a gene that had previously been shown to be downregulated in MF and peripheral T-cell lymphoma using gene expression profiling.<sup>28,29</sup> We demonstrated for *BCL7a* and the tumor suppressor gene *protein tyrosine phosphatase receptor gamma* that promoter hypermethylation was associated with transcriptional downregulation. Disruption of the *BCL7a* gene directly contributes to the malignant phenotype in B-cell lymphoma and predicts unfavorable prognosis in these malignancies.<sup>30,31</sup> Epigenetic silencing of the BCL7a tumor suppressor gene, that

occurred more often in CTCL entities with an unfavourable prognosis, therefore may well be a functionally significant event in CTCL.

In addition, we detected frequent promoter hypermethylation of selected tumor suppressor genes in CTCL, inactivation of which may lead to cell cycle dysregulation (*CDKN2A* encoding p16, *CDKN2B*, p73), defective DNA repair (*MGMT*), disruption of apoptosis signaling (*TMS1*, p73) and chromosomal instability (*CHFR*). Evidently epigenetic instability, as displayed by CTCL cells, results in oncogenic signaling alterations in CTCL. Especially promoter hypermethylation of p73 may be a relevant finding in CTCL, as inactivation of this p53-homologue interferes with activation-induced cell death, the propriocidal mechanism ensuring elimination of certain tumor suppressor genes in CTCL not only gives insight into the molecular pathogenesis of these malignancies, but in addition provides a rationale for treating CTCL patients with demethylating agents.

#### **Conclusion and future perspectives**

In the first part of this thesis it was demonstrated that the presence of extracutaneous involvement, extensive skin disease at time of diagnosis, response to therapy (clinical), folliculotropism of malignant T cells and low proportions of cytotoxic CD8<sup>+</sup> T cells in CTCL skin lesions (pathological) are determinants of the disease course of CTCL.

In the second part of this thesis we found that malignant T cells of CTCL patients express an alternatively spliced variant of the *Fas* gene encoding a truncated protein that dominantly interferes with the function of the Fas apoptosis receptor, known to have a crucial role in the elimination of inappropriately proliferating T cells and in the susceptibility to cellular cytotoxicity. Furthermore it was shown that the gene expression patterns of malignant T cells in Sz are characterized by selective high expression of *Twist* and *EphA4* as well as by diminished expression of several genes with tumor suppressive properties in hematopoietic malignancies, such as *Mxi1* and *Forkhead Box O1A*. We observed aberrant expression of the TNF receptor ligands *CD70* and *RANKL* in malignant T cells of patients with Sz and suggested that this may contribute to chronic immunosuppression often observed in patients with this CTCL. In addition we identified promoter hypermethylation of new methylation targets as well as of several established tumor suppressor genes, including *BCL7a*, *PTPRG* and *p73*, in malignant T cells of CTCL, inactivation of which will interfere with growth and apoptosis regulation.

The greatest advances in our understanding of the pathobiology of CTCL may be expected to come from integration of data obtained from analysis of expression patterns, DNA methylation patterns and genomic imbalances in early and advanced CTCL skin lesions. To this aim we are currently conducting further gene expression profiling and array-based comparative genomic hybridization studies on DNA and RNA isolated from CTCL skin lesions.

A great number of genes have been and will be shown to be affected by deletion, amplification, mutation or promoter hypermethylation in CTCL. Subsequent studies should be aimed at delineation of these (epi)genetic lesions that are essential for the development and progression of CTCL. However, investigating the functional significance of particular genetic defects will be hampered by the fact that representative model systems for CTCL are currently lacking.

The identification of membrane-associated proteins that are exclusively or predominantly expressed by the malignant T-cell population may prove to be an easier

attainable and clinically useful goal. Such proteins would constitute useful markers for the early molecular diagnosis of CTCL and possibly targets for directed therapeutic intervention using monoclonal antibodies. Furthermore it may be expected that future studies of gene and protein expression profiles and chromosomal alterations may contribute to a more accurate classification of cutaneous lymphomas.

It appears reasonable to predict that the expansion of our understanding of the molecular genetics of CTCL will in the near future lead to improved therapies for patients with this group of malignancies.

#### **References**

- 1. Weinstock MA, Horm JW. Mycosis fungoides in the United States. Increasing incidence and descriptive epidemiology. JAMA. 1988;260:42-6.
- 2. Kim YH, Liu HL, Mraz-Gernhard S, et al. Long-term outcome of 525 patients with mycosis fungoides and Sezary syndrome: clinical prognostic factors and risk for disease progression. Arch Dermatol. 2003;139:857-66.
- 3. Kim YH, Chow S, Varghese A, et al. Clinical characteristics and long-term outcome of patients with generalized patch and/or plaque (T2) mycosis fungoides. Arch Dermatol. 1999;135:26-32.
- 4. Kim YH, Jensen RA, Watanabe GL, et al. Clinical stage IA (limited patch and plaque) mycosis fungoides. A long-term outcome analysis. Arch Dermatol. 1996;132:1309-13.
- 5. Bonta MD, Tannous ZS, Demierre MF, et al. Rapidly progressing mycosis fungoides presenting as follicular mucinosis. J Am Acad Dermatol. 2000;43:635-40.
- 6. Apisarnthanarax N, Ha CS, Duvic M. Mycosis fungoides with follicular mucinosis displaying aggressive tumor-stage transformation: successful treatment using radiation therapy plus oral bexarotene combination therapy. Am J Clin Dermatol. 2003;4:429-33.
- Cerroni L, Fink-Puches R, Back B, et al. Follicular mucinosis: a critical reappraisal of clinicopathologic features and association with mycosis fungoides and Sezary syndrome. Arch Dermatol. 2002;138:182-9.
- 8. Vollmer RT. Mycosis fungoides and follicular mucinosis. Arch Dermatol. 2002;138:1613-4.
- 9. Willemze R, Kerl H, Sterry W, et al. EORTC classification for primary cutaneous lymphomas. A Proposal from the Cutaneous Lymphoma Study Group of the European Organization for Research and Treatment of Cancer (EORTC). Blood. 1997; 90:354-371.
- Bagot M, Echchakir H, Mami-Chouaib F, et al. Isolation of tumor-specific cytotoxic CD4+ and CD4+CD8dim+ T-cell clones infiltrating a cutaneous T-cell lymphoma. Blood. 1998;91:4331-41.
- 11. Vonderheid EC, Tan E, Sobel EL, et al. Clinical implications of immunologic phenotyping in cutaneous T cell lymphoma. J Am Acad Dermatol. 1987;17:40-52.
- 12. Hoppe RT, Medeiros LJ, Warnke RA, et al. CD8-positive tumor-infiltrating lymphocytes influence the long-term survival of patients with mycosis fungoides. J Am Acad Dermatol. 1995;32:448-53.
- Abeni D, Frontani M, Sampogna F, et al. Circulating CD8+ lymphocytes, white blood cells, and survival in patients with mycosis fungoides. Br J Dermatol. 2005;153:324-30.
- 14. Kikuchi A, Nishikawa T. Apoptotic and proliferating cells in cutaneous lymphoproliferative diseases. Arch Dermatol. 1997;133:829-33.
- 15. Zoi-Toli O, Vermeer MH, De Vries E, et al. Expression of Fas and Fas-ligand in primary cutaneous T-cell lymphoma (CTCL): association between lack of Fas expression and aggressive types of CTCL. Br J Dermatol. 2000;143:313-9.
- 16. Muschen M, Warskulat U, Beckmann MW. Defining CD95 as a tumor suppressor gene. J Mol Med. 2000;78:312-25.
- 17. Gronbaek K, Straten PT, Ralfkiaer E, et al. Somatic Fas mutations in non-Hodgkin's lymphoma: association with extranodal disease and autoimmunity. Blood. 1998;92:3018-24.
- 18. Otsuki T, Sakaguchi H, Tomokuni A, et al. Detection of alternatively spliced variant messages of Fas gene and mutational screening of Fas and Fas ligand coding regions in peripheral blood mononuclear cells derived from silicosis patients. Immunol Lett. 2000;72:137-43.

- Cascino I, Papoff G, De Maria R, et al. Fas/Apo-1 (CD95) receptor lacking the intracytoplasmic signaling domain protects tumor cells from Fas-mediated apoptosis. J Immunol. 1996;156:13-7.
- 20. Shen L, Liang AC, Lu L, et al. Frequent deletion of Fas gene sequences encoding death and transmembrane domains in nasal natural killer/T-cell lymphoma. Am J Pathol. 2002;161:2123-31.
- 21. Wood CM, Goodman PA, Vassilev AO, et al. CD95 (APO-1/FAS) deficiency in infant acute lymphoblastic leukemia: detection of novel soluble Fas splice variants. Eur J Haematol. 2003;70:156-71.
- 22. Tawara M, Maeda T, Yamada Y, et al. Aberrant processing of Fas transcripts in adult T-cell leukemia: a possible role in tumor cell survival. Cancer Lett. 2003;193:235-42.
- 23. Valsesia-Wittmann S, Magdeleine M, Dupasquier S, et al. Oncogenic cooperation between H-Twist and N-Myc overrides failsafe programs in cancer cells. Cancer Cell. 2004;6:625-30.
- 24. Surawska H, Ma PC, Salgia R. The role of ephrins and Eph receptors in cancer. Cytokine Growth Factor Rev. 2004;15:419-33.
- 25. Walker-Daniels J, Hess AR, Hendrix MJ, et al. Differential regulation of EphA2 in normal and malignant cells. Am J Pathol. 2003;162:1037-42.
- 26. Yawalkar N, Ferenczi K, Jones DA, et al. Profound loss of T-cell receptor repertoire complexity in cutaneous T-cell lymphoma. Blood. 2003;102:4059-66.
- 27. Tesselaar K, Arens R, van Schijndel GM, et al. Lethal T cell immunodeficiency induced by chronic costimulation via CD27-CD70 interactions. Nat Immunol. 2003;4:49-54.
- 28. Tracey L, Villuendas R, Dotor AM, et al. Mycosis fungoides shows concurrent deregulation of multiple genes involved in the TNF signaling pathway: an expression profile study. Blood. 2003;102:1042-50.
- 29. Martinez-Delgado B, Melendez B, Cuadros M, et al. Expression profiling of T-cell lymphomas differentiates peripheral and lymphoblastic lymphomas and defines survival related genes. Clin Cancer Res. 2004;10:4971-82.
- 30. Zani VJ, Asou N, Jadayel D, et al. Molecular cloning of complex chromosomal translocation t(8;14;12)(q24.1;q32.3;q24.1) in a Burkitt lymphoma cell line defines a new gene(BCL7A) with homology to caldesmon. Blood. 1996;87:3124-34.
- 31. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000;403:503-11.
- 32. Lissy NA, Davis PK, Irwin M, et al. A common E2F-1 and p73 pathway mediates cell death induced by TCR activation. Nature. 2000;407:642-5.