haematologica
Journal of Hematology

ISSN 1592-8721
educational edition

Volume 88
Supplement no. 5
April 2003

Published by the
Ferrata-Storti
Foundation,
Pavia, Italy

S5

Cutaneous Lymphomas

Are they separated entities?
Which is the best treatment?

Bologna, Italy, April 8th-9th, 2003

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Direttore responsabile: Prof. Edoardo Ascari; Autorizzazione del Tribunale di Pavia n. 63 del 5 marzo 1955.
Editing: Mikimos - Medical Editions via gen. C.A. Dalla Chiesa 22, Voghera, Italy
Printing: Tipografia PI-ME via Vigentina 136, Pavia, Italy

Printed in April 2003

Haematologica is sponsored by educational grants from the following institutions and companies

IRCCS Policlinico S. Matteo, Pavia, Italy

University of Pavia, Italy

José Carreras International Leukemia Foundation

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International Conference on
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2003; Volume 88; supplement No. 5 - April 2003
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Epidemiology of primary cutaneous lymphomas

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Non-Hodgkin's lymphomas (NHL) represent a heterogeneous group of histologically and possibly biologically distinct malignancies that arise from the lymphopoietic system. The incidence rate of all NHL varies from less than 1.0 case per 100,000 inhabitants per year in some areas of Asia and Africa, to approximately 10.0 case per 100,000 inhabitants per year in the Unites States, Canada, Australia and some European countries.[1]

Data from the Surveillance, Epidemiology and End Results (SEER) program of the National Cancer Institute indicate that the incidence of NHL has been rapidly increasing from the 1970s and that this increase has been higher than for all other cancer sites, except lung cancer in women and melanoma in both sexes. Some recent data suggest that this increase leveled off in the 1990s, particularly in men.[2] The trend of increasing incidence of NHL has also been reported in Europe.[3,4] It is widely accepted that changes in classifications and diagnostic procedures can only explain this increase in part. An analysis of temporal trends considering different subgroups separately suggests that the increase in incidence rates in USA is more pronounced for high grade lymphomas and extranodal disease.[5] Primary cutaneous lymphomas (CL) are, together with gastric lymphomas, one of the most common extra-nodal NHL. In population-based series they have been estimated to represent approximately twenty percent of all extranodal NHL.[6,7]

A reliable estimate of the incidence rates and an evaluation of geographical differences and temporal trends for CL is quite difficult. Population-based data are scarce and data based on clinical series (that is, patients treated in one or more hospitals) are difficult to compare and to transfer to the general population; in addition, changes in diagnostic procedures and classification criteria over the past several years could be partially responsible for the observed variation in incidence.

Population-based Cancer Registries do not routinely provide data on extranodal lymphomas (except for mycosis fungoides).

Table 1 presents the world population age-standardized incidence rates per 100,000 persons per year for the period 1988-1992 for selected Registries. The incidence of mycosis fungoides (MF) varies from 0.90 per 100,000 to 0.10 per 100,000 among males and from 0.50 to rates lower than 0.01 among females, with rates generally higher in western countries.[8]

In a collaborative study based on data from Cancer Registries, the world standardized rates of CL ranged from 0.003 in Philippines to 0.42 in Black Americans.[9] In a recent study using a population-based specialist Registry on hematologic malignancies, covering a population of 11 million people in England and Wales, in the eight-year period 1986-1993 the annual world population age-standardized incidence rates were 0.42 per 100,000 for CL and 0.14 for MF. An increasing trend in CL was reported, while this increase was not evident when MF alone was considered. This finding is apparently in contrast with a previous study[10] based on SEER program data on incident cases of MF from 1973 to 1984 in which a dramatic increase in incidence of MF was found; however in an update including incident cases up to 1992, the incidence rates of MF appeared relatively stable.[11]

In an ad hoc study carried out in Israel, the incidence of MF and non-MF CL were estimated in the period 1985-1993 using data from a Cancer Register supplemented by a field survey carried out to achieve a complete and accurate identification of cases The world population age–standardized incidence rates were high among Israeli-Jews compared with those reported by other population-based studies for all CL (1.18 per 100,000 in males and 0.63 in females) and MF was more frequent than other CL.[12]

A population-based study on the incidence of CL in the period 1986-1995 has been carried out in the Provinces of Florence and Prato (1,100,000 inhabitants). All incident cases with a histologically confirmed diagnosis of CL occurring in the period 1985-1996 among residents in the area were identified through a survey involving all dermatology, hematology, pathology and outpatients departments of the area. The completeness of the series was verified through a linkage with the Tuscany Cancer Registry that has been active in the area from 1985. We identified 132 incident cases of CL (76 in males); 88 were B cell CL and 37 T cell CL. The world population age-standardized incidence rates were 0.94 per 100,000 in males and 0.54 in females for all CL. The incidences of B cell CL and T cell CL were 0.50 per 100,000 and 0.20 per 100,000, respectively.[13] The higher proportion of B-cell CL in our population-based series is in agreement with data from the England and Wales Registry of hematologic malignancies in which MF accounts for 25% of all CL. These data and the observation of an increasing incidence of non-MF CL, possibly, as suggested in our study, for B-cell lym-

Table 1. Mycosis fungoides: world age standardized incident rates per 100,000 per year from selected Cancer Registries, by gender. Period 1988-1992.

	ASR (World)	
	Males	*Females*
USA, SEER: Whites	0.5	0.2
USA, SEER: Blacks	0.6	0.5
Canada, Ontario	0.5	0.3
USA, Puerto Rico	0.2	0.1
Japan, Nagasaki	0.1	0.0
UK, Yorkshire	0.5	0.2
Denmark	0.2	0.1
Switzerland, Vaud	0.3	0.1
Italy, Turin	0.3	0.1
Italia, Varese	0.4	0.2
Italia, Florence	0.2	0.0
France, Bas-Rhin	0.9	0.5
France, Calvados	0.2	0.3
Spain, Murcia	0.1	0.2
Israel (Jews)	0.5	0.3
Kuwait (Natives)	0.5	-
Australia, Victoria	0.2	0.2

Modified from: D.M. Parkin, S.L. Whelan, J. Ferlay, L. Raymond and J. Young, eds. Cancer Incidence in Five Continents, Vol. VII.IARC Scientific Publications No. 143, 1997.

phomas deserve further investigations.

The current knowledge on the etiology of NHL is poor. The well-known risk factors (such as acquired and congenital immune deficiency, autoimmune disorders, HIV infection) account for a relatively small fraction of NHL and are unlikely to explain the increasing temporal trends. The role of other, more diffuse exposures in the etiology of NHL is not completely understood and requires further investigation. It is possible that epidemiological studies focused on specific subtypes of NHL or on specific extranodal sites could be more informative.

Data on risk factors for CL are scarce, being mostly based on case series and limited to MF.

Some reports on CL in subjects occupationally exposed to chlorophenols and phenoxy acids, chemicals associated with NHL in epidemiological studies, were published at the beginning of 1980s.[14,15] Exposure to chemicals has been related to MF in some case series.[16,17] In a hospital-based case-control study, patients with an industrial-related occupation were found to have an increased risk: a machine operator and machinist were the occupations most frequently reported.[18] Nevertheless, no evidence of an association with any occupation or chemical exposure was evident in a subsequent larger case-control study based on 174 MF patients and 294 population controls.[19] A record linkage study carried out in Sweden found an increased risk of MF in the cotton industry, trolley and bus transportation, streetcars drivers and circular saw operators.[20]

The association of CL with sunlight, a recently suggested risk factor for NHL, was investigated in an ecological study but no correlation was found between ambient levels of solar ultraviolet radiation and incidence of CL in 10 areas in USA.[9]

Associations between MF and smoking[19] and high consumption of alcohol[21] were also suggested.

An association with previous skin diseases, particularly those related to chronic antigenic stimulation was reported but was not confirmed in case-control studies.[19,22] A familial predisposition to hematolymphopoietic malignancies has been suggested.[18]

Various infection agents, both viral and bacterial, have been proposed to play a role in the development of CL, but molecular studies on case series produced controversial results.[23,24]

In the frame of the multicenter Italian case-control study on hematolymphopoietic malignancies, involving 12 centers in the period 1991-1993 and focused mainly on occupational exposures,[25,26] in Florence we carried out a case-control study on CL.[27] All incident cases of histologically verified primary CL in the period 1986-1995, diagnosed in residents of both sexes, aged 20-74 years, in the provinces of Florence and Prato, were identified and confirmed in close collaboration with the dermatology and hematology departments of the area. A frequency matched group of population controls was also identified. Overall 105 cases and 470 controls were identified. Subjects who agreed to participate in the study (87.6% of the cases and 80.9% of the controls) filled in a questionnaire focused on smoking habits, occupational history, personal and family history of diseases. Preliminary results indicate that some occupational exposures, smoking and a family history of malignancies are risk factors for CL.

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Classification of cutaneous lymphomas

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Primary cutaneous lymphomas (PCLs) are defined as lymphomas that present in and are limited to the skin without evidence of extracutaneous disease. The skin is the second most common site of extranodal lymphomatous involvement: mycosis fungoides (MF) represents the most frequent type (about 50% of the cases), the remaining 50% being aproximately shared by non-MF T-cell lymphomas (TCL) and peripheral B-cell lymphomas. PCLs are cause of a long-lasting debate among pathologists and dermatologists because of their relatively more indolent clinical behavior than that of corresponding histotypes primarily arising in the lymph node. Consistently, PCLs represent a field in hematopathology in which careful correlations between immunohistology, clinical onset and course are needed for optimal management of the patients.

Thanks to the increasing knowledge on lymphocyte biology, immunohistochemical and molecular markers acquired in the last decade, the diagnosis and approach to lymphomas have been greatly and positively improved, as demonstrated by the worldwide consensus reached first by the REAL classification[1] and more recently by the WHO classification of lymphoid neoplasms (Table 1),[2] which largely adopted the former as far as structure and diseases are concerned. The diagnostic approach based on the amalgamation of several parameters (from pure morphology to genetics passing through phenotype) addressed in the REAL and WHO classifications has obviously been of aid also for PCLs as demonstrated by the fact that, apart from exceptional cases, no reliable diagnosis can nowadays be made without the aid of ancillary techniques. Nonetheless, the histological classification of PCLs is still matter of debate and at present pathologists can choose to assign their diagnosis following either the WHO/REAL classification or the EORTC scheme;[3] both have shortcomings.

The REAL/WHO[1,2] classification represents the first attempt to widen the concept of diagnosing lymphoma in that it is based on a multi-step approach that goes from morphology to genotype, with the necessary contribution of clinical data: on the whole it is a biologically oriented classification scheme. On the other hand, although the EORTC classification[3] also recommends the evaluation of different immunomorphologic and molecular parameters, it basically appears to be strongly clinically-oriented. The REAL/WHO approach had the aim to unify different classifications and makes studies carried out in different countries comparable in order to gather as much information as possible to understand, or at least to try to, the biology of all lymphomas. Without underscoring the clinical peculiarity of PCLs, the WHO proponents think that efforts should be made in the direction of expanding and improving the WHO scheme instead of building up parallel classifications. Organ-specific classifications and the use of ambiguous terminology do not allow the understanding of diseases with similar features although occurring at different anatomic sites. What should be emphasized when using the WHO scheme in diagnosing a PCL is the primary site of onset, clearly addressing the clinical knowledge that lymphomas with similar immunomorphologic features can behave differently at different sites.

It is likely that with the EORTC contribution, the REAL/WHO classification could be enlarged to include variants of the main lymphoma categories.

For example, the definition of follicle center cell lymphoma (FCCL) of the skin is certainly one of the most debated issues in the literature.[4,5] Several groups, however, have recently reported data that convincingly show their germinal center cell derivation.[6–12] In the past, the frequent negativity for the BCL-2 protein had, in fact, led to the speculation that cutaneous FCCLs were unrelated to nodal ones. As a matter of fact, a significant proportion of cases histologically labeled as FCCL of the skin do express the BCL-2 protein, as well as the other *follicular markers* CD10 and BCL-6.[6–12] Additionally, in a percentage of cases varying from 20%[7] to 34% of cases,[10] a t(14;18) can be detected by PCR, more often in the BCL-2 positive cases. Mirza *et al.*[10] could not find any significant difference in the clinical outcome between the BCL-2$^+$ and/or t(14;18)$^+$ cases on the one hand and the t(14;18)- ones on the other. Nonetheless, some authors[11] suggest careful investigation for systemic disease in t(14;18)$^+$ FCCL presenting in the skin, as this aberration might indicate secondary cutaneous involvement by a still clinically occult process. In addition, molecular alterations suggestive of germinal center cell origin have also been detected in cases of so-called large B-cell lymphoma of the leg.[12] These data together show that time and research are clarifying issues that have for long been object of debate, in this case confirming that primary cutaneous FCCL does apparently belong to the wider category of follicular lymphomas: once again, until we actually demonstrate that cutaneous FCCL has a different biology and/or origin from nodal/extracutaneous FCCL, is does not seem necessary to separate skin lesions from the nodal/non-skin ones, while it is mandatory to warn clinicians of the relatively indolent clinical course of the former process.

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Table 1. Classification of lymphoid tumors according to WHO (excluding Hodgkin's lymphoma).

B-cell lymphomas

Lymphomas from precursors of B lymphocytes
- Precursor B-lymphoblastic leukemia/lymphoma

Mature B-cell neoplasms
- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- B-cell prolymphocytic lymphoma
- Lymphoplasmacytic lymphoma
- Splenic marginal zone lymphoma
- Hairy cell leukemia
- Plasma cell neoplasms (plasma cell myeloma, plasmacytoma, monoclonal immunoglobulin deposition disease, heavy chain disease)
- Extranodal marginal zone B-cell lymphoma (MALT lymphoma)
- Nodal marginal zone B-cell lymphoma
- Follicular lymphoma
- Mantle cell lymphoma
- Diffuse large B-cell lymphoma
- Mediastinal (thymic) large B-cell lymphoma
- Intravascular large B-cell lymphoma
- Primary effusion lymphoma
- Burkitt's lymphoma

T/NK-cell neoplasms

Lymphomas from precursors of T/Nk lymphocytes
- Lymphoblastic T-cell leukemia /Nk/lymphoma
- Blastic Nk cell lymphoma (*natural killer*)

Mature T-Cell And Nk-Cell Neoplasms
- T- cell prolymphocytic leukemia
- T- cell large granular lymphocytic leukemia
- Aggressive NK-cell leukemia
- Adult T-cell leukemia/lymphoma ($HTLV^{-}1^{+}$)
- Extranodal NK/T-cell lymphoma, nasal type
- Enteropathy-type T-cell lymphoma
- Hepatosplenic T-cell lymphoma
- Subcutaneous panniculitis-like T-cell lymphoma
- Mycosis fungoides/ Sézary syndrome
- Primary cutaneous CD30 positive T-cell lymphoproliferative disorders (primary cutaneous anaplastic large cell lymphoma, lymphomatoid papulosis, border-line lesions)
- Angioimmunoblastic T- cell lymphoma
- Peripheral T-cell lymphoma , unspecified
- Anaplastic large cell lymphoma

Similar speculations can be made concerning primary cutaneous marginal zone lymphomas and immunocytomas: there are no data in the literature that allow us to consider these tumors as biologically different from extra-cutaneous MALT lymphomas and nodal immunocytomas.[13]

Like the EORTC,[3] the WHO[2] introduced the group of CD30 lymphoproliferative disorders of the skin, clearly stating that lymphomatoid papulosis is a benign spontaneously regressing lesion. Agreement exists on the absolute need for careful clinico-pathological correlations[14,15] in such cases and above all in those that the WHO calls border-line cases, in which there are discrepancies between clinical course and histology. Unfortunately, the reports of border-line lesions are few and although the prognosis seems favorable, the collection of larger series is mandatory.

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Classification of primary cutaneous lymphomas

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Primary versus secondary cutaneous lymphomas

Cutaneous lymphomas represent clonal proliferations of neoplastic T- or B-lymphocytes, which can involve the skin primarily or secondarily. The term *primary cutaneous lymphoma* refers to cutaneous T-cell lymphomas (CTCL) and cutaneous B-cell lymphomas (CBCL), which present in the skin with no evidence of extracutaneous disease at the time of diagnosis. After the group of gastro-intestinal lymphomas, the cutaneous lymphomas form is the second most common group of extranodal lymphomas, with an estimated annual incidence of 0.5-1.0 cases per 100,000 population. Primary cutaneous lymphomas often have a completely different clinical behavior and prognosis compared to their nodal counterparts with or without secondary cutaneous involvement, and therefore require a different type of treatment. In addition, differences in the presence of specific translocations and in the expression of oncogenes, viral sequences or antigens, and adhesion receptors involved in tissue-related lymphocyte homing have been reported. Such differences underscore that these primary cutaneous lymphomas represent a distinct group of lymphomas, and may explain, at least in part, their different clinical behaviour. Furthermore, they may be used as additional diagnostic criteria to differentiate between primary and secondary cutaneous lymphomas. Perhaps the most unique features of these primary cutaneous lymphomas are that they can be seen and biopsied easily, allowing an optimal correlation between clinical appearance and clinical behavior on the one hand, and histologic, phenotypic and genetic aspects on the other. In the last decade these features resulted in the delineation of distinct types of CTCL and CBCL, which formed the basis of new classifications for the group of primary cutaneous lymphomas.[1]

Classification of primary cutaneous lymphomas: EORTC and WHO classification

Until recently, the classification systems for non-Hodgkin's lymphomas, such as the Kiel classification and the Working Formulation, were based on histologic criteria, and within the different categories distinction by site was not made. Since these classification systems did not recognize the special character of primary cutaneous lymphomas, and did not convey to the clinician that these lymphomas have other clinical behaviors and require different therapeutic approaches, from those used for their nodal counterparts, primary cutaneous lymphomas were not uncommonly diagnosed incorrectly, and/or treated inappropriately with unnecessarily aggressive regimens. This was the main reason why the European Organization for Research and Treament of Cancer (EORTC) Cutaneous Lymphoma Group drew up a separate classification for the group of primary cutaneous lymphomas (Table 1). The EORTC classification is the only classification, that is designed specifically for the group of primary cutaneous lymphomas. It contains a limited number of well-defined types of CTCL and CBCL, which together comprise more than 95% of all primary cutaneous lymphomas. In addition, it contains a number of provisional entities, which mostly display characteristic histologic features, but for which distinctive clinical presentations and/or clinical outcomes have not yet been defined. Distinction is made between primary cutaneous lymphomas with an indolent, intermediate or aggressive clinical behavior. The clinical validity of this new classification has now been substantiated by three large studies including follow-up data of more than 1,000 patients with primary cutaneous lymphoma.[1-3]

It is fortunate that in recent years there has been growing awareness among hematopathologists that also classification systems for non-Hodgkin's lymphomas should no longer be based on purely histological criteria, but should take into account immunohistochemical, genetic and clinical features, such as site, and thus should attempt to include only well-defined disease entities.[4,5] In the WHO classification this principle has been applied particularly to the group of mature T/NK-cell neoplasms, a group of malignant lymphomas that thus far defied a reproducible classification because of their enormous cytological and immunophenotypic heterogeneity. In the WHO system the classification of these lymphomas relies heavily on clinical features and site of presentation rather than on cytology and phenotype, as illustrated by the terminology used. It is, therefore, not surprising that the different types of CTCL of the EORTC classification, with its greater emphasis on clinical aspects, could so easily be incorporated into the WHO classification (Table 1). With respect to the mature B-cell neoplasms there was much less need to incorporate clinical features. Although the terminology has changed, and some extranodal groups

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Table 1. EORTC Classification for primary cutaneous lymphomas and corresponding categories in the proposed WHO clasification [5]

EORTC CLASSIFICATION [1]	*Frequency*[#]	*5-year-survival*[#]	*WHO CLASSIFICATION* [5]
Cutaneous T-cell lymphoma			
Indolent clinical behaviour			
Mycosis fungoides	40%	89%	Mycosis fungoides
Mycosis fungoides variants			*Mycosis fungoides variants*
Follicular MF	4%	75%	*Follicular MF*
Pagetoid reticulosis	< 1%	100%	*Pagetoid reticulosis*
CTCL, large cell, CD30-positive	10%	93%	Primary cutaneous CD30+ ALCL
Lymphomatoid papulosis	14%	100%	[*CD30+ lymphoproliferative disease, including lymphomatoid papulosis*]
Aggressive clinical behaviour			
Sezary syndrome	3%	11%	Sezary syndrome
CTCL, large cell, CD30-negative	8%	15%	peripheral T-cell lymphoma, unspecified (most) extranodal NK/T-cell lymphoma, nasal type (some) [blastic NK cell lymphoma]
Provisional entities			
CTCL, pleomorphic, small/ medium-sized	2%	62%	
Subcutaneous panniculitis-like T-celllymphoma	< 1%	NDA	Subcutaneous panniculitis-like T-cell lymphoma
Cutaneous B-cell lymphoma			
Indolent clinical behaviour			
Primary cutaneous immunocytoma/ marginal zone B-cell lymphoma	2%	100%	extranodal marginal zone B-cell lymphoma of MALT type
Primary cutaneous follicle center cell lymphoma	13%	97%	extranodal marginal zone B-cell lymphoma(1) follicular lymphoma (2) diffuse large B-cell lymphoma (3)
Intermediate clinical behaviour			
Primary cutaneous large B-cell lymphoma of the leg	2%	55%	diffuse large B-cell lymphoma
Provisional entities			
Primary cutaneous plasmacytoma	< 1%	100%	Plasmacytoma
Intravascular large B-cell lymphoma	< 1%	50%	*Diffuse large B-cell lymphoma (intravascular)*

[#] based on 960 primary cutaneous lymphomas included in the Dutch registry between 1985 and 1998
(1) for cases with a predominance of small neoplastic B-cells; (2) for rare cases showing a follicular growth pattern
(3) for cases showing a predominance of large neoplastic B-cells (most cases); NDA: no data available

of B-cell lymphomas have been included, the WHO classification for B-cell neoplasms is still essentially a histologic classification. This also explains why there is still considerable confusion and disagreement between the EORTC and WHO classifications regarding the terminology and classification of the group of primary CBCL.

Classification of CTCL

Comparison between the EORTC and WHO classification schemes shows that there is consensus on the categorization of almost 90% of CTCL, including MF, MF variants, Sézary syndrome, the group of primary cutaneous CD30-positive lymphoproliferative disorders [primary cutaneous CD30-positive (anaplastic) large T-cell lymphoma and lymphomatoid papulosis] and subcutaneous panniculitis-like T-cell lymphoma. There is, however, controversy regarding the classification of CD30-positive large T-cell lymphomas with a non-anaplastic morphology. Since the clinical presentation and clinical behavior of primary cutaneous CD30-positive lymphomas with anaplastic morphology and those with non-anaplastic morphology do not differ,[6] no distinction is made between anaplastic and non-anaplastic cases in the EORTC classification, and both groups are included in a category CD30-positive large T-cell lymphoma. In contrast, according to WHO classification, primary cutaneous CD30-positive lymphomas with a non-anaplastic morphology will be categorized as peripheral T-cell lymphoma, unspecified, with the unfortunate consequence that these indolent lymphomas will be treated with unnecessarily aggressive systemic chemotherapy.

Consensus is also still lacking for the remaining 10% of CTCL. In the EORTC classification most of these lymphomas are classified as primary cutaneous CD30-negative large T-cell lymphoma or primary cutaneous CD30-negative small/medium-sized pleomorphic T-cell lymphoma. These groups include not only $CD4^+$ but also $CD8^+$ CTCL, such as the recently defined group of epidermotropic CD8-positive cytotoxic CTCL.[7] Following the criteria of the WHO classification over 90% of these cases will be classified as peripheral T-cell lymphoma, unspecified. Only exceptional cases will be classified as nasal type NK/T-cell lymphoma, whereas some cases are now recognized as belonging to the group of blastic NK cell lymphomas. These $CD56^+$ blastic NK cell lymphomas represent a distinct entity, which often present in the skin with or without concurrent systemic disease.[5] However, they do not belong to the spectrum of CTCL; possibly they are derived from precursor plasmacytoid dendritic cells and they are closely related to acute myeloid leukemia. It should be kept in mind that, apart from a small group of primary cutaneous CD30-negative small/medium-sized pleomorphic T-cell lymphomas with a $CD3^+$, $CD4^+$, $CD8^-$ phenotype, all lymphomas belonging to this remaining 10% of CTCL have an aggressive clinical course, and should be treated accordingly.[8]

Classification of CBCL

Three main groups of CBCL are recognized in the EORTC classification: the group of primary cutaneous follicle center cell lymphomas (PCFCCL), primary cutaneous marginal zone B-cell lymphomas, and the group of primary cutaneous large B-cell lymphomas of the leg (PCLBCL-leg). The group of PCFCCL with its characteristic presentation on the head or trunk has been recognized as a distinct disease entity for more than 40 years, and was originally described as reticulohistiocytoma dorsi. In the WHO classification cutaneous follicular lymphoma is included as a variant of nodal follicular lymphoma, but the relationship between both entities is questioned and exact criteria for the diagnosis of cutaneous follicular lymphoma are lacking. In daily practise most cases PCFCCL will be classified as diffuse large B-cell lymphoma when the WHO classification is used. In very early lesions with a minor population of blast cells the diagnosis of extranodal marginal zone B-cell lymphoma will be made. Thus following the (histologic) criteria of the WHO classification this well-defined disease entity will be classified either as a low grade malignant lymphoma or as a high grade malignant lymphoma resulting in inappropriate treatment with systemic chemotherapy.

There is also controversy over whether or not PCLBCL-leg should be considered as a separate group of CBCL. The neoplastic B-cells in these lymphomas also have the morphologic and phenotypic (e.g. bcl-6 expression) features of follicle center cells, and originally these lymphomas were included in the group of PCFCCL. The reason that the EORTC group considers this group separately was the clinical experience that CBCL with the histology of a diffuse large B-cell lymphoma presenting on the legs had a much poorer prognosis than diffuse large B-cell lymphomas presenting on the head or trunk. Whereas these PCLBCL-leg have a clinical behavior and prognosis comparable with those of diffuse large B-cell lymphomas in general, PCFCCL with the histology of a diffuse large B-cell lymphoma behave like a low grade malignant lymphoma, and should be treated accordingly. In addition, whereas most PCLBCL-leg are composed of large, non-cleaved follicle center cells and express the bcl-2 protein, PCFCCL generally show a predominance of large cleaved cells and do not express bcl-2 protein.[9] One of the main reasons for the current controversy is that this poorer prognosis of PCLBCL-leg is not found by all cutaneous lymphoma groups. Nevertheless, recent CGH and gene profiling studies support the view that the

two groups of *primary cutaneous large B-cell lymphoma* are different clinical and biologic entities.

Conclusions

The paramount rationale for designing the WHO classification was to allow pathologists and clinicians to use the same classification, i.e. terminology, facilitating optimal communication between both groups. In comparison, the basic philosophy of the EORTC classification, which was based on detailed clinicopathologic studies performed by collaborative groups of dermatologists and pathologists, has always been that a classification should be, above all, clinically relevant, and that the terms used in such a classification should provide the clinician all information necessary for adequate management and treatment. It is fortunate that the EORTC definitions of most types of CTCL have been incorporated in the WHO classification. For the group of CBCL the EORTC classification is still the best guide to optimal treatment and management, since it includes more completely defined and recognizable disease entities. Applying the WHO classification to the group of CBCL may still result in unnecessarily aggressive treatment of some cases.

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Mycosis fungoides

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Mycosis fungoides (MF) is the most common type of cutaneous T-cell lymphoma.[1,2] Traditionally it is divided into three clinical phases: patch-, plaque-, and tumor-stage. The clinical course can be protracted over years or decades. The term *mycosis fungoides* should be restricted to the classical so-called *Alibert-Bazin* type of the disease, characterized by the typical slow evolution and protracted course. More aggressive entities (e.g., MF *a tumeur d'emblée*), characterized by onset with plaques and tumors, aggressive course, and bad prognosis, are better classified among the recently described group of so-called cutaneous cytotoxic T-cell lymphomas.

In the past MF was considered as an *incurable,* albeit slowly progressive disease, which inevitably ended with the death of the patients. Recently, identification and definition of criteria for clinicopathologic diagnosis of small patches of the disease allowed identification of patients with early MF,[3-5] who often have a relatively mild course with prolonged survival, without progression into plaque- and tumor-stages. The inevitability of disease progression and death due to the lymphoma, has, therefore, been questioned.

A staging classification system for MF was proposed in 1978 (TNMB staging) (Table 1). This system takes into account the percentage of body area covered by lesions, and the presence of lymph node or visceral involvement. Although the presence of malignant circulating cells in the blood should be recorded for each patient, these data are not used for staging. More recently, a new staging classification for MF has been proposed in the new World Health Organization (WHO) classification of hematopoietic neoplasms (Table 2).[6]

Some centers specialized in the study and management of skin lymphomas, however, do not utilize either the TNM or WHO staging scheme, but rather classify MF according to the *type* of skin lesions (patches, plaques and tumors) and the presence or absence of large cell transformation and/or extracutaneous involvement.

Clinical features

Patch-stage

Patches of MF are characterized by variably large, erythematous, finely scaling lesions with a predilection for the buttocks and other sun-protected areas. Itching is often a prominent symptom. In early phases, a *digitate* pattern can be observed (alone or in combination with larger patches). In the past, cases characterized by small lesions alone were variously diagnosed as *digitate* dermatosis, chronic superficial scaly dermatitis, or small-plaque parapsoriasis. Molecular genetic techniques revealed that some of these lesions contain a monoclonal population of T-lymphocytes. Moreover, similar lesions can be observed in patient with *classic* MF, thus underlying the close relationship between small-plaque parapsoriasis and MF.

Plaque-stage

Plaques of MF are characterized by infiltrated, scaling, reddish-brown lesions. Typical patches are usually observed contiguous to plaques or at other sites on the body.

Tumor-stage

In tumor-stage MF a combination of patches, plaques and tumors is found. Tumors may be solitary or, more often, localized or generalized. Ulceration is common. The onset of tumors considerably worsens the prognosis.

Histopathology, Immunohistology and Molecular Biology

Histopathology

Early lesions of MF have a patchy lichenoid or band-like infiltrate in an expanded papillary dermis. A psoriasiform hyperplasia of the epidermis may frequently be seen. Small lymphocytes predominate; and atypical cells are uncommon or completely absent. Epidermotropism of solitary lymphocytes is usually observed, but so-called Pautrier's microabscesses are rare. Useful diagnostic clues are the presence of epidermotropic lymphocytes with nuclei larger than those of lymphocytes within the upper dermis, and/or the presence of lymphocytes aligned along the basal layer of the epidermis. In this context, it should be emphasized that in some cases epidermotropism may be missing. The papillary dermis shows a moderate to marked fibrosis with coarse bundles of collagen.

The histopathologic diagnosis of early MF may be extremely difficult. In some instances differentiation from inflammatory skin conditions (e.g., psoriasis, chronic contact dermatitis) may be impossible on histopatho-

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Table 1. TNMB STAGING OF MYCOSIS FUNGOIDES

Skin			
T_1	Patches, papules or plaques covering <10% of the skin surface		
T_2	Patches, papules or plaques covering >10% of the skin surface		
T_3	Tumours		
T_4	Generalised erythroderma		
Lymph Nodes			
N_0	No clinically abnormal lymph nodes; histology negative		
N_1	Clinically abnormal peripheral lymph nodes		
N_{1o}	histology not performed		
N_{1n}	histology negative		
N_{1r}	histology reactive		
N_{1d}	dermopathic lymphadenitis		
N_2	No clinically abnormal peripheral lymph nodes; histology positive		
N_3	Clinically abnormal peripheral lymph nodes; histology positive		
Visceral Organs			
M_0	No visceral involvement		
M_1	Visceral involvement		
Blood			
B_0	<5% of atypical circulating cells		
B_1	>5% of atypical circulating cells		
Stage			
Ia	$T_1N_0M_0$	**Ib**	$T_2N_0M_0$
IIa	$T_{1-2}N_1M_0$	**IIb**	$T_3N_{0-1}M_0$
III	$T_4N_{0-1}M_0$		
IVa	$T_{1-4}N_{2-3}M_0$	**IVb**	$T_{1-4}N_{0-3}M_1$

logic grounds alone. In these cases correlation with the clinical findings is crucial in order to make a definitive diagnosis. Plaques of MF are characterized by a dense, band-like infiltrate within the upper dermis. Intraepidermal lymphocytes arranged in so-called Pautrier's collections are a common finding at this stage. Cytomorphologically, small pleomorphic (cerebriform) cells predominate.

In tumors of MF a dense, nodular or diffuse infiltrate is found within the entire dermis involving the subcutaneous fat. Epidermotropism may be lost.

Large cell transformation

In later stages, patients with MF usually develop lesions with many large cells (immunoblasts, large pleomorphic cells, or large anaplastic cells). Large cell transformation in MF is defined as large cells exceeding 25% of the infiltrate, or of large cells forming microscopic nodules.[7] Large cell transformation has been detected in more than 50% of patients with tumor stage MF.[7] Clusters of large cells may be observed sometimes also in plaques of MF (usually in patients with tumors at other sites of the body). Tumors with a large cell morphology may or may not express CD30. Expression of the antigen does not have any prognostic significance in these patients. Large cell transformation of MF bears a poor prognosis, and usually heralds the terminal stage of the disease.

Immunohistology

MF is characterized by an infiltrate of T-helper lymphocytes. A few cases with predominance of T-suppressor lymphocytes have, however, been reported. In late stages there may be a (partial) loss of pan-T-cell antigen expression. In plaque and tumor lesions some neoplastic T-cells may express the CD30 antigen. In contrast to primary cutaneous $CD30^+$ lymphoproliferative disorders, expression of this antigen in MF does not have any prognostic significance.

Molecular genetics

There are no specific abnormalities reported for MF. Rearrangement of the T-cell receptor gene is commonly found in plaques and tumors, but may be absent in early (patch) lesions.[8,9]

Clinical and histopathological variants

Several clinical and/or histopathologic variants of MF have been described (Table 3).[10,11] Patients with these variants of the disease often also show features of *classical* MF at other sites of the body.

Many of the clinicopathologic variants listed in Table 3 are quite rare. Some of them in the past were separated from MF and considered as distinct entities. In the following we will summarize the main characteristics of some of these forms.

Table 2. STAGING OF MYCOSIS FUNGOIDES ACCORDING TO THE WHO.

Stage	
I	disease confined to the skin
Ia	limited patches / plaques
Ib	disseminated patches / plaques
Ic	tumors
II	enlarged lymph nodes (histology negative)
III	lymph node involvement (histology positive)
IV	visceral involvement

Table 3. CLINICOPATHOLOGIC VARIANTS OF MYCOSIS FUNGOIDES.

Acanthosis nigricans-like mycosis fungoides
Angiocentric mycosis fungoides
Bullous mycosis fungoides
Dyshidrotic mycosis fungoides
Erythrodermic mycosis fungoides
Granulomatous mycosis fungoides / Granulomatous slack skin
Hyperpigmented mycosis fungoides
Hypopigmented mycosis fungoides
Ichtyosis-like mycosis fungoides
Mycosis fungoides with eruptive cysts
Mycosis fungoides with follicular mucinosis
large cell transformation
Pagetoid reticulosis
Perioral dermatitis-like mycosis fungoides
Pigmented purpura-like mycosis fungoides
Pilotropic mycosis fungoides
Poikilodermatous mycosis fungoides
Pustular mycosis fungoides
Verrucous mycosis fungoides
Zosteriform mycosis fungoides

Mycosis fungoides with follicular mucinosis

Some patients with MF present with follicular papules and plaques characterized histopathologically by abundant deposits of mucin within follicular structures that are surrounded by a more or less dense infiltrates of T-lymphocytes. The follicles are infiltrated by the lymphocytes (*pilotropism*). It has been suggested that so-called *idiopathic generalized follicular mucinosis* does, in fact, represent a variant of MF with marked deposition of mucin within hair follicles. Cases of progression to tumor-stage MF and death of the patients have been well documented.[12]

Some authors reported that patients with MF associated with follicular mucinosis have a worse prognosis than patients with *common* MF.[13]

Localized pagetoid reticulosis (Woringer-Kolopp)

Localized pagetoid reticulosis is a variant of MF presenting with solitary, psoriasiform, scaly erythematous patches or plaques, usually located on the extremities. The histologic picture shows an hyperplastic epidermis with marked epidermotropism of T-lymphocytes. Intraepidermal lymphocytes are characterized usually by medium-sized pleomorphic nuclei. Both T-helper and T-suppressor phenotypes have been described.

The term pagetoid reticulosis should be restricted to solitary lesions. Patients with the *generalized* form of pagetoid reticulosis probably have either classical MF, or, more frequently, one of the recently described primary cutaneous cytotoxic lymphomas (aggressive epidermotropic $CD8^+$ T-cell lymhoma, γ/δ cutaneous T-cell lymphoma).

The prognosis of patients with solitary pagetoid reticulosis is excellent, and involvement of internal organs has never been observed. The treatment of choice is surgical excision or local radiotherapy.

Hypopigmented mycosis fungoides

Patients with MF may develop hypopigmented patches and plaques. These lesions may be misinterpreted clinically as those of pityriasis versicolor or vitiligo. Histology reveals features typical of MF. Hypopigmented MF is observed more frequently in dark-skinned individuals, and is one of the most

frequent variants seen in children.[14,15] Repigmentation usually takes place after successful treatment of the lesions.

Treatment

The standard treatment[16,17] of early lesions of MF includes psoralens in association with UV-A irradiation (PUVA), interferon α2a, retinoids, or a combination of these three modalities. Several other treatments have been used in the past (and are still in use at present), including topical steroids, UV-B irradiation (311nm), and topical application of chemotherapeutic agents. The administration of total body irradiation, proposed by some authors as a first-line treatment, should probably be restricted to patients with MF in later stages. In the last years, many new protocols have been introduced, including photodynamic therapy with 5-aminolaevulinic acid, new retinoids such as bexarotene (administered orally or topically), and immune response modifiers such as imiquimod. At present there are not enough data to evaluate the efficacy of these new modalities in terms of remission and recurrence rates.

In late stages, in addition to PUVA, retinoids and interferon α2a, conventional systemic chemotherapy, extracorporeal photopheresis and radiotherapy have all been applied. Allogeneic stem cell transplantation has been performed in a few patients. New chemotherapeutic or immunologic agents, including gemcitabine, fludarabine, pegylated doxorubicin, pentostatin, interleukin-12, DAB_{389}-IL-2 fusion protein, and trimetrexate, have also been used in a few patients. Treatment of advanced (tumor stage) MF is unsatisfactory, and the disease usually progresses in spite of aggressive therapy.

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Non-mycosis fungoides cutaneous T-cell lymphomas

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For a long time, primary cutaneous T-cell lymphomas were considered to consist almost exclusively of mycosis fungoides and its variants. Probably as a consequence of this belief, about 30 years ago, Edelson proposed the unifying concept of cutaneous T-cell lymphomas (CTCLs), to define all T-cell lymphomas which primarily developed in skin. Although attractive, this idea was too simple because CTCLs have many faces and comprise a group of lymphomas with very heterogeneous clinical presentation, histomorphology and outcome. In 1988 the updated Kiel classification was presented, but this was dedicated to nodal lymphomas with the exception of mycosis fungoides and Sézary's syndrome, both classified among the cytologically low-grade T-cell lymphomas. Thus a group of dermatologists and pathologists of the European Cutaneous Lymphoma Project Group of the EORTC (*European Organization for Cancer Research and Treatment*) proposed a scheme for the histopathologic classification of the CTCLs in relation to their cytologic spectrum; this scheme represented a *bona-fide* modified Kiel classification, which was applied to the specialized field of extranodal (cutaneous) lymphomas. Subsequently, various new techniques (e.g. immunohistochemistry and molecular biology) have become available which have allowed better definition of the morphofunctional and biological fetaures of lymphoma cells. Furthermore, evidence has accumulated indicating that clinical and biological differences exist between primary nodal and primary cutaneous lymphomas, suggesting the need for a special sub-classification scheme for extranodal lymphomas. On these bases, the most recent lymphoma classifications, in particular the WHO 97 lymphoma classification, have paid special attention to lymphomas, including T and NK-cell subtypes (Table 1), which most frequently affect the skin, primarily or secondarily (Table 1). Another, but very similar, classification scheme (Table 2) for cutaneous T-cell lymphomas is also contained in the last EORTC cutaneous lymphoma classification (Table 2). We shall now focus briefly on the most relevant primary cutaneous T-cell lymphoma subtypes other than mycosis fungoides.

Primary cutaneous CD30$^+$ lymphoproliferative disorders

A spectrum of CD30$^+$ primary cutaneous lymphoproliferative disorders (PCLD) with a relatively favorable prognosis has recently been identified. This group consists of lymphomatoid papulosis (LyP), anaplastic large cell lymphoma (ALCL) and a group of lesions which display histomorphologic and often clinical features at the border between LyP and ALCL. Cases previously diagnosed as *regressing atypical histiocytosis (RAH)* are now also considered ALCL. All of these disorders have in common large atypical CD30$^+$ cells, some of which morphologically resemble Hodgkin and Sternberg-Reed (SR) cells.

Lymphatoid papulosis (LyP)

This is a self-healing rhythmic, paradoxical, often generalized eruption histologically mimicking a malignant lymphoma, but clinically benign. LyP presents with recurrent crops of reddish papulo-nodular lesions which regress spontaneously (within a few weeks) leaving only a small scar or area of altered pigmentation. It usually affects adults but less frequently also elederly patients and children. Morphologically, the classic LyP lesions show a typical *wedge-shaped* pattern of dermal involvement with superficial and deep perivascular lymphoid infiltrates. LyP cellular infiltrates consist of CD30$^+$ atypical cells, scattered or in small clusters, and admixed with an inflammatory reactive cellular background of neutrophils, eosinophils and histiocytes. Atypical LyP cells usually exhibit a CD4 T-helper phenotype (CD3$^+$ CD4$^+$CD8$^-$) but aberrant phenotypes with loss of one or the other T-cell antigen may occur. In addition the CD30$^+$ cells express the proteins associated with cytotoxic granules (TIA-1, perforin and granzyme B). The expression of CD15 has been sporadically reported whereas the reaction for the ALK protein is uniformly negative. Clonal rearrangement of TCR genes can be detected in about 50% of cases whereas searches for EBV viral RNA or EBV gene products are negative. LyP must be differentiated from others CD30$^+$ PCLDs and rare cases of primary or secondary cutaneous Hodgkin's lymphoma. In addition LyP must be differentiated from CD30$^+$ skin lymphoid infiltrates including follicular lymphoid hyperplasia with a high content of activated CD30$^+$ blast cells, cutaneous infection by parapoxvirus (i.e. milker's nodule) and drug reactions. Although the possible association of LyP with malignant lymphomas has often been underlined, the vast majority of LyP patients have an excellent prognosis, thus making aggressive treatments unnecessary.

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Table 1. T-cell and NK- lymphomas (according to the WHO 97 lymphoma classification) with frequent or constant skin involvement.

Primary Cutaneous T-cell Lymphomas
-Mycosis Fungoides
-Sezary syndrome
-Primary Cutaneous CD30+ Lymphoproliferative disorders
-Subcutaneous panniculitis-like T-cell lymphoma

Other T-or NK-Cell Lymphomas with primary or secondary skin involvement.
- Extranodal NK/T-cell lymphoma, nasal type
-Blastic NK-cell lymphoma/leukemia
-Precursor T-lymphoblastic lymphoma/leukemia

Table 2. Primary Cutaneous T-cell Lymphoma, other than MF, listed in the EORTC Classification.

Indolent
-Large cell CD30+ CTCL
Anaplastic
Immunoblastic
Pleomorphic
-Lymphomatoid papulosis

Aggressive
-Large cell CD30 negative CTCL
Immunoblastic
Pleomorphic
-CTCL, pleomoprhic small/medium-sized*
-Subcutaneous Panniculitis-like T-cell lymphoma*

*= Provisional entities

Anaplastic large cell lymphoma (ALCL)

ALCL usually presents as a single or multiple medium-sized to large nodules or tumors that may show temporary regression initially but often persist; regional lymph node involvement may occur but visceral spread is rare. Cases of RAH are now included among the ALCLs. $CD30^+$ cutaneous ALCL usually affect adults and elderly patients but less frequently also children and adolescents. Morphologically the ALCL lesions show a grossly nodular and/or diffuse pattern of dermal involvement with frequent extension subcutaneously. In some cases the overlying epidermis may show a variable degree of pesudo-epitheliomatous hyperplasia. The lymphoma population consists of large anaplastic cells grouped in large and confluent cohesive sheets. The anaplastic lymphoma cells uniformly express CD30 and exhibit, in most cases, a $CD4^+$ T-helper phenotype; cases with a *null* (non-B, non-T) phenotype are rare. The CD30 anaplastic cells also express other activation antigens (often including the EMA) as well as the cytotoxic granule-associated proteins, TIA-1, granzyme B and perforfin. Immunostainings for the CD15 and ALK protein are negative. Expression of CD56 antigens has been sporadically reported in ALCL but the possible biological relevance of such findings is still largely unknown. Most of primary cutaneous ALCL, including some of the rare cases with a *null* phenotype, clonally rearrange T-cell receptor (TCR) genes. At present widely accepted that the t(2;5) is not associated with cutaneous ALCL. $CD30^+$ cutaneous ALCL

must be differentiated from other CD30+ PCLDs and from large cell pleomorphic/anaplastic but CD30-negative primary cutaneous T-cell lymphoma. It is also clinically relevant to differentiate between primary cutaneous CD30+ ALCL and CD30+ ALCL that has evolved from other primary cutaneous lymphomas (most frequently, mycosis fungoides), the latter usually characterized by a very aggressive clinical course and poor outcome. Although loco-regional involvement may occur in primary cutaneous CD30+ ALCL, it does not seem to affect prognosis, which remains favorable in most cases. A partial or less frequently complete spontaneous regression of this lymphoma has been reported in up to 25% of cases in certain series. Risk factors predicting possible lymphoma dissemination (however occurring in less than 10% of cases) are currently unknown.

Borderline lesions

Within the spectrum of CD30+ PCLDs, this term identifies a group of peculiar cases characterized by divergences between clinical features and histologic apperance, which prevent them from being readily distinguished from LyP or CD30+ ALCL. Morphologically these cases exhibit features intermediate between LyP and ALCL. They usually have a nodular pattern of dermal involvement, sometimes with subcutaneous extension. Cellular infiltrates consist of atypical CD30+ anaplastic cells grouped in clusters and admixed with a variable number of inflammatory cells. In some areas, mostly in the deepest part of the lesions, the CD30+ cells may form large confluent sheets. Apoptotic features may be numerous. Associated dermal and/or epidermal modifications include: spongiosis, parakeratosis, epidermal pseudoepitheliomatous hyperplasia and, often, ulceration. Borderline lesions are rare; however, the (limited) available data corroborate their close relationships with the atypical cells of LyP and CD30+ ALCL. Borderline lesions must be differentiated from the other CD30+ PCLDs, but no definitive clinicopathologic criteria are currently available which allow a clear-cut distinction to be made. Prognostically, although it has been suggested that patients with a border lesion have a greater risk of developing CD30+ ALCL than do patients with LyP, most borderline lesions seem to have a very favourable outcome similar to that of LyP.

Cutaneous lymphomas expressing a T-cytotoxic or natural killer cell phenotype

A recent study (in press) which details the result of a previous Histopathologic Workshop of the E.O.R.T.C. Cutaneous Lymphoma Task Force, has confirmed that «*cutaneous T-cell lymphomas expressing a cytotoxic or natural killer (NK) cell phenotype represent a group of lymphoproliferative disorders for which there is currently much confusion and little consensus regarding the best nomenclature and classification*». Furhermore, it is still matter of debate whether at least some of these special lymphoid malignancies really represent primary cutaneous lymphomas or alternatively they must only be considered as the first (cutaneous) site of presentaion of a systemic disease. We shall focus briefly on the major subtypes of T/NK lymphomas which have been listed in the WHO lymphoma classification: panniculitis-like T-cell lymphoma, extranodal NK/T-cell lymphoma, nasal type, and blastic NK-cell lymphoma.

Subcutaneous panniculitis-like T-cell lymphoma (SPTCL)

This is a T-cell lymphoma with a cytotoxic phenotype, which preferentially infiltrates subcutaneous tissue. In the past similar cases have been diagnosed as cytophagic histiocytic panniculitis (CHP) but now evidence has accumulated that CHP and SPCTL may span a clinicopathologic spectrum in which there is a natural disease progression from CHP to SPCTL. SPTCL is rare (about 1% of all NHL) and affects a broad age range including children and young adults. The most common sites of lymphoma involvement are the trunk and the extremities, in the form of multiple subcutaneous nodules. Some patients may present with hemophagocytic syndrome whereas lymphoadenopathy is usually absent. Clinical manifestations of systemic involvement are fever, hepatosplenomegaly, mucosal ulcers and sometimes serosal effusions. Morphologically, lymphoma infiltrates the subcutaneous tissue, usually in the absence of sparing septae. The overlying epidermis and dermis are usually uninvolved, at least during the initial phase of the disease. Lymphoma cells are small to medium in size; the nuclei may be round to irregular to indented/pleomorphic and are often hyperchromatic with a moderate amount of pale-cytoplasm. Often the lymphoma cells tend to surround individual fat cells resulting in a typical rimmed appearance. A variable number of reactive non-neoplastic CD68+ histiocytes are admixed and more abundant in areas of necrosis. The lymphoma cells have a peripheral CD8+; CD3+ T-cell phenotype and express cytotoxic molecules including TIA-1, granzyme B and perforin; CD56+ is variably expressed. Rearrangement of the TCR occurs in most cases, EBV markers are negative. About 25% of PLTCL have a γ/δ origin and exhibit a CD56+ but CD4– and CD8– phenotype. The clinical behavior and outcome of PLTCL is variable. Although nodal and/or visceral spread may occur late in the disease course and good results have been obtained by combined chemotherapy, some cases have a very aggressive clinical course and are often complicated by severe hemophagocytic syndrome.

Extranodal NK/T-cell lymphoma, nasal type

NK/T-cell lymphomas, nasal and nasal type, are prevalent in Asia, Mexico, Central and South America but rarely occur in European countries. This lymphoma has a predilection for extranodal sites, particularly the nasal cavity and nasopharynx but skin and/or soft-tissues may also be involved. In the past lesions located on the nose were called *lethal midline granuloma*. This lymphoma presents in the form of frequently ulcerated skin nodules; areas involved by lesions include the face, trunk and the extremities. However, the patients commonly have high stage disease at presentation with simultaneous involvement of multiple extranodal sites and often also peripheral blood involvement. The lymphoma has a diffuse growth pattern involving the dermis and subcutaneous layer. Angiogentric and angiodestructive features are frequent and associated with fibrinoid changes, coagulative necrosis and apoptotic bodies. Lymphoma cells show a broad spectrum of cytological appearances, but are mostly medium to large in size. Nuclei may be irregular, elongated or vescicular; the cytoplasm is usually pale to clear and moderate in amount. The lymphoma cells express CD2, CD56, and CD3c but they lack surface CD3. Other T-and NK cell markers (CD4, CD5, CD8, CD16 and CD57) are usually negative. Cytotoxic granule-associated proteins are positive in most cases. CD30 and/or CD7 may be sporadically expressed. TCR and immunoglobulin genes are in germline configurations in most cases whereas EBV can be demonstrated in the vast majority of these cases. The prognosis of these lymphomas is variable but usually poor for those cases of nasal-type NK/T-cell lymphomas occurring outside the nasal cavities.

Blastic NK-cell lymphoma/leukemia

This malignancy often presents in the skin and bone marrow and usually has an aggressive clinical course. Other possible sites of lymphoma involvement include lymph nodes, soft-tissues and peripheral blood. The skin lesions consist of a densely packed proliferation of $CD56^+$ blasts (resembling lymphoblasts or myeloblasts) which often express CD4 but less frequently CD2 and CD7. The terminal nucleotidyl transferase (TdT) may be positive whereas markers for mature cytotoxic and NK-cells are negative. Because of the morphologic and , in part, antigenic overlap between lymphoblastic and myeloblastic neoplasms the diagnosis of blastic NK lymphoma requires caution and should only be made in the absence of commitment to the T-cell or myeloid lineages (negativity for CD3s, MPO and CD33). Prognosis is unfavorable in most cases, there being a poor response to regimens used for NHLs.

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Cutaneous B-cell lymphoma

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Primary extranodal non-Hodgkin's lymphomas must be distinguished from primary nodal lymphomas, even if their histologic and immunophenotypic features are similar. This is especially true for primary cutaneous B-cell lymphomas (PCBCLs), which as a rule are associated with a much better prognosis than their nodal counterparts. The established lymphoma classification systems, such as the updated Kiel classification or the Working formulation, were originally designed for malignant lymphomas arising in lymph nodes. Their direct transfer to primary cutaneous lymphomas often created an overestimation of malignancy from the clinical point of view. Even the unifying and partially extended conception of the REAL classification[1] was not able to solve this problem.

Since primary cutaneous lymphomas are not recognized as a distinct group, referral centers for these diseases are frequently confronted with patients with an indolent type of lymphoma, who have been treated with unnecessarily aggressive therapy. It was, therefore, felt necessary to produce a clinically relevant classification, which should contribute to proper diagnosis and treatment of patients with a primary cutaneous lymphoma. Since primary cutaneous lymphomas cannot be adequately defined by histologic criteria alone, and modification of existing histologic classifications was not considered as a realistic option, a separate classification of primary cutaneous lymphoma was proposed by the *Cutaneous Lymphoma Study Group of the European Organization for Research and Treatment of Cancer* (EORTC).[2] Besides histologic and immunophenotypic criteria, this classification also includes clinical criteria, and thus contains disease entities rather than histologic subgroups.

The majority of PCBCLs have been classified as follicle center cell lymphoma (FCCL) and only a minority as immunocytoma/marginal zone B-cell lymphoma (MZL) or large B-cell lymphoma of the leg. Within the group of PCBCLs there are some differences when comparing the EORTC scheme with the REAL classification. The main difference lies in the entity of primary cutaneous FCCL. According to the REAL classification, follicular center lymphoma presents with a follicular pattern of growth in the great majority of cases, and almost all cases with a diffuse pattern of growth are classified among diffuse large B-cell lymphomas. Conversely, FCCL according to the EORTC is a broader term encompassing all lymphomas composed of cells resembling centroblasts and centrocytes, including the very rare primary cutaneous follicular lymphomas and the more common diffuse large B-cell lymphoma at a minimum. In fact, there is evidence that cases classified as FCCL by the EORTC approach show MALT-type features, when assessed using the criteria of the REAL classification, and are therefore better classified as MALT-type lymphoma/MZL.[3]

A second difference concerns the category of large B-cell lymphomas. In the EORTC scheme, a distinct entity (termed large B-cell lymphoma of the leg) is recognized only when such lymphomas, predominantly composed of large cells, occur on the leg(s). Histologically similar processes occurring on other sites are conversely termed FCCL.

The recent WHO classification of hematologic malignancies[4] has partially absorbed the issues of the EORTC classification, incorporating, as variants of follicular lymphoma, two entities: the cutaneous follicle center lymphoma (composed of cells that resemble centrocytes, often large, and centroblasts, with a partial follicular pattern) and the diffuse follicle center lymphoma (cellular composition similar to follicular lymphoma, but entirely diffuse pattern). However, there is no recognition of the biological peculiarities of large B-cell lymphoma of the leg in comparison to similar tumors occurring at other sites in the skin.

A recent study compared the applicability of the EORTC and WHO classification for primary cutaneous lymphomas and concluded that the EORTC scheme allows a more precise categorization and better management of the patients, especially those with PCBCLs.[5]

Primary cutaneous follicle center cell lymphoma presents with non-scaling, solitary or grouped papules, plaques, and/or tumors, which may be surrounded by (annular) erythematous lesions. In most cases the skin lesions are confined to a circumscribed area in the head and neck region or on the trunk. If untreated, skin lesions gradually increase in size over years, but dissemination to extracutaneous sites is uncommon. From the histopathologic point of view, FCCL shows nodular or diffuse infiltrates, with almost constant sparing of the epidermis. The histologic picture is variable, and primarily related to the age and growth rate of the skin lesions.[6] Small and early lesions contain a mixture of small cleaved cells, relatively few large cleaved and non-cleaved cells, and many reactive T-cells, and are classified as centroblastic/centrocytic lymphoma in the Kiel classification, as follicular center lymphoma in the REAL classification, and, at least in part, as cutaneous follicle centre lymphoma in the WHO classification. Reactive follicle centers and/or their remnants may be observed. Neoplastic follicles are extremely rare. With further progression to tumorous lesions, both the number and size of the neoplastic B cells increase, whereas the number of tumor-

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infiltrating T-cells decreases. Rapidly growing tumors often show a monotonous infiltrate of large cells. These lesions are classified as one of the subtypes of centroblastic lymphoma or immunoblastic lymphoma in the updated Kiel classification, and as a diffuse large B-cell lymphoma in the REAL and WHO classifications. Such lesions are usually not associated with a more unfavorable prognosis, which is probably related to the fact that dissemination to extracutaneous sites is extremely rare in these lymphomas.[6] Neoplastic B-cells express B-cell-associated antigens (CD19, CD20, CD22, CD79a) and show monotypic sIg$^+$ (the lack of detectable sIg is common in tumorous lesions with large cell histology). Unlike nodal follicular lymphoma, FCCLs do not generally express CD10, are not associated with the t(14;18) translocation,[7] and very rarely express the bcl-2 protein. The estimated 5-year survival is over 97%. Radiotherapy is the treatment of choice. In case of generalized skin lesions and/or extracutaneous dissemination multiagent chemotherapy is recommended.

Primary cutaneous *immunocytoma/marginal zone B-cell lymphoma* presents with solitary or multiple (sub)cutaneous tumors, preferentially involving the extremities. Typical findings include nodular or diffuse infiltrates composed of small lymphocytes, lymphoplasmacytoid, centrocyte-like cells, and plasma cells admixed with variable numbers of centroblasts, and immunoblast-like cells. The monotypic lymphoplasmacytoid and/or plasma cells are usually located at the periphery of the infiltrates. The central areas of the infiltrates may contain variable numbers of reactive T-cells, small CD20$^+$ B-cells, and in many cases well-evident reactive follicles may be observed. PAS-positive intranuclear or intracellular inclusions are frequently seen. Tumor cells are monotypic cIg$^+$, CD79a$^+$, CD5$^-$. Clonal rearrangement of Ig genes can be demonstrated. There are no known specific translocations associated with this type of PCBCL. In the updated Kiel classification these lymphomas are termed immunocytomas, while in the REAL and WHO classifications they are classified as extranodal marginal zone B-cell lymphomas or MALT-type lymphomas. They have an excellent prognosis; the estimated 5-year survival is over 95%. Radiotherapy is the treatment of choice. *Borrelia burgdorferi* plays a role in the pathogenesis of some of these lymphomas.

There is at present little consensus regarding the definition of the term MZL. By emphasizing histologic heterogeneity as a characteristic feature of these lymphomas, the end of this spectrum of MZL is open to debate, and MZL is likely to become an encompassing term for all or most PCBCLs, including over 90% of primary cutaneous FCCLs.[8] This suggestion was based on the observation that most PCBCLs, as the MALT lymphomas, but unlike follicular lymphomas in lymph nodes, do not express CD5 and CD10 antigens, and are not associated with the interchromosomal t(14;18) translocation.

According to recent studies,[9] immunohistochemistry can furnish valuable help in distinguishing follicular lymphoma from MZL in the skin. In fact, neoplastic cells of follicular lymphoma were constantly found to express bcl-6 and frequently to show CD10 positivity, especially when lesions had a nodular or mixed follicular and diffuse pattern of growth, while tumor cells of MZL were constantly bcl-6$^-$ and CD10$^-$.

According to the definition of the E.O.R.T.C. classification, primary cutaneous *large B-cell lymphoma of the leg* is a lymphoma with a predominance of large B cells presenting on and confined to the leg(s). These lymphomas predominantly affect elderly patients. The patients present with red or bluish nodules or tumors on one or sometimes both legs. Patients present with diffuse nonepidermotropic infiltrates predominantly composed of large B-cells with variable proportions of centroblast- and immunoblast-like cells. There are relatively few small cleaved cells and admixed inflammatory cells. In most cases the majority of the neoplastic B-cells have the morphology of large follicle center cells, and are classified as one of the subtypes of centroblastic lymphoma in the updated Kiel classification. Some cases show an almost pure population of immunoblasts (B-immunoblastic lymphoma). However, since distinction between these two groups is sometimes arbitrary, and not relevant from a clinical point of view, the unifying term large B-cell lymphoma of the leg is preferred. In the REAL and WHO classifications these lymphomas are also classified as diffuse large B-cell lymphomas. The tumor cells express monotypic sIg and/or cIg as well as CD19, CD20, CD22, and CD79a. In contrast to FCCLs on the head and trunk, these lymphomas generally express bcl-2 protein. Expression of bcl-2 protein is not associated with the t(14;18) translocation. Clonal rearrangement of Ig genes can be demonstrated. In case of solitary or localized skin lesions radiotherapy is preferred; in all other cases, multiagent chemotherapy is recommended.

In the experience of the *Dutch Cutaneous Lymphoma Working Group* (DCLWG), the prognosis of these lymphomas is less favorable than that of morphologically similar large cell lymphomas on the head and trunk.[10] The estimated 5-year survival of 18 cases included in the DCLWG is 58%. However, this more aggressive behavior is still a matter of debate (*personal communication*).[11,12]

The results of a European multicenter study on primary cutaneous large B-cell lymphomas[13] seem to suggest that round-cell morphology (RR: 8.5; 95% CI: 2.7-27), location on the leg (RR: 4.2; 95% CI: 1.5-11.5), and multiple skin lesions (RR: 4.2; 95% CI: 1.2-15) are significantly associated with a worse prognosis, and these elements should be taken into

account for a more accurate management and treatment of these patients.

A recent study[14] on primary cutaneous large B-cell lymphoma of the leg documented that all cases investigated stained for the L-26/CD20cy and CD79a antigens and expressed the *bcl-2*, *bcl-6*, and MUM-1/IRF4 proteins but were negative for both the CD10/CALLA and CD138/syndecan-1 antigens. With respect to molecular analysis, the lymphoma population of all these cases carried hypermutation of Ig genes, and all but 1 case also harbored mutations of the *bcl-6* gene. These results indicated that primary cutaneous large B-cell lymphoma of the leg has similar morphofunctional and molecular profiles to those of most diffuse large B-cell lymphomas of other sites. Thus, caution seems justified before definitely considering primary cutaneous large B-cell lymphoma of the leg as a distinct entity. In addition two provisional entities have been included in the EORTC scheme. The term provisional entity refers to PCBCLs which display typical histologic and immunophenotypic features but lack a distinctive clinical presentation or a defined clinical outcome.

According to the definition of the EORTC scheme, *intravascular large B-cell lymphoma*, formerly considered as a vascular proliferation and termed malignant angioendotheliomatosis, is characterized by an accumulation of large neoplastic B-cells within blood vessels. These lymphomas clinically present with indurated violaceous patches and plaques sometimes reminiscent of panniculitis, generally on the legs or the trunk. Histopathologically, dilated blood vessels in the dermis and subcutis are filled and often dilated by a proliferation of large neoplastic lymphoid cells. These cells may cause vascular occlusion. In a limited proportion of cases a slight accumulation of atypical cells can be observed in perivascular areas. Neoplastic cells are CDl9$^+$, CD20$^+$, CD22$^+$, CD79a$^+$, and monotypic sIg$^+$. Clonal rearrangement of Ig genes can be demonstrated. The estimated 5-year survival in the DCLWG series is 50%. Multiagent chemotherapy is the preferred treatment. Intravascular lymphomas with only skin lesions may occur, and generally have a more favorable clinical behavior than those affecting both the central nervous system and the skin.

According to the definition of the EORTC scheme, *plasmacytoma* is a extremely rare type of PCBCL characterized by a clonal proliferation of plasma cells developing primarily in the skin (extramedullary plasmacytoma of the skin) without underlying multiple myeloma. Many cases reported in the literature more likely represent reactive plasma cell proliferations or immunocytomas rather than true plasmacytomas. A workshop organized by the *EORTC Cutaneous Lymphoma Task Force* on plasma cell-rich cutaneous infiltrates (Bilbao, Spain, November 30th-December 1st, 2001) concluded that probably most if not all cases of primary plasma cell-rich infiltrate of the skin can be included in the spectrum of MZL or virus/bacteria-linked lymphomas.

Finally, mantle cell lymphoma is a small cell B-cell lymphoma characterized by expression of CD5 antigen and cyclin D1 protein, which almost without exception involves the skin secondarily, and for that reason has not been included in the EORTC classification. In fact, only one report on a primary cutaneous mantle cell lymphoma has been published so far.[14]

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Cutaneous lymphomas: rare subtypes

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This group include several forms originating from cytotoxic T-lymphocytes or natural killer (NK) cells. Most of these forms show an aggressive clinical course, and it may sometimes be very difficult to be sure of the primary cutaneous origin, particularly considering the short time between skin presentation and systemic progression. The clinicopathologic, immunophenotypic and molecular data in most cases suggest a clinical entity, however the number of well documented cases reported is too low to define the clinical entities.

Pleomorphic small/medium T-cell lymphoma

This form surely exists, although it is very difficult to define because of the absence of specific criteria. In fact, we observed two types of presentations: 1) with a single nodule or localized plaques, similar to pseudo-T-cell lymphomas, with an excellent prognosis; 2) with multiple plaques and deep nodules, without any evidence of precursor mycosis fungoides lesions, showing an intermediate/ aggressive clinical course. Histologically the infiltrate formed by pleomorphic small/medium lymphocytes involves the dermis, and is frequently periadnexal and perivascular showing minimal epidermotropism. The immunophenotype is variable, most of the cases are peripheral T-cell $CD4^+$, but cytotoxic $CD8^+$ cases are not rare.

CD30-large T-cell lymphoma

Clinical presentation, with nodules, large ulcerated tumors or plaques, is similar to that of tumoral stage mycosis fungoides or large $CD30^+$ lymphomas. However, these patients frequently present disseminated cutaneous lesions and systemic symptoms, as in secondary skin lymphomas. Histologically, the infiltrate invades the entire dermis and the hypodermis. It is formed by pleomorphic or immunoblastic T-cells, with a high mitotic index. Most of the cases are $CD4^+$ and show a highly defective T-cell immunophenotype with loss of expression of CD2, CD5, CD7 and CD3. Subcutaneous panniculitis-like T-cell lymphoma This is a rare lymphoproliferative disorder with an aggressive course (around 22 months), previously reported also under the name of cytophagic malignant histiocytosis. This peculiar type of lymphoma shows specific involvement of the subcutaneous fat, presenting with multiple deep soft nodules, showing an inflammatory panniculitis-like evolution. Initially, most patients present with symptoms and laboratory abnormalities suggesting systemic involvement or a hemophagocytic syndrome. Histologically the infiltrate is formed by small/medium size pleomorphic cells, suggesting lobular panniculitis, with necrosis of the adipose tissue, cytophagia and leucoerythrophagocytosis. Neoplastic cells show the immunophenotype of cytotoxic (TIA-1^+, Granzyme-B^+) α/β ($CD8^+$, $CD45RO^+$) or γ/δ ($CD8^{+/-}$, $CD45RO^+$, $CD56^+$) T-cells. Rare cases showing $CD4^+$ T-cell or NK immunophenotype have also been reported. However cases characterized by localized lesions and lobular lymphocytic infiltrate, simulating *chronic* granulomatous or lupus panniculitis, showing an *indolent* more benign course, can also be observed. *Aggressive* epidermotropic $CD8^+$ cytotoxic lymphoma. This is a very aggressive lymphoproliferative disorder characterized by rapid, systemic progression (median survival less than two years), resembling secondary cutaneous lymphomas. Clinically it may present with disseminated hyperkeratotic nodules and plaques, as in *disseminated pagetoid reticulosis* or with large ulcerated hemorrhagic/purpuric tumors or plaques. The progression of the disease typically involves vital organs, such as lungs, heart, liver, kidney, brain, and gut, but sparing, in most cases, the lymph nodes. The infiltrate is nodular and diffuse, perivascular, and formed by medium size pleomorphic T-cell showing a peculiar epidermotropism or adnexotropism. The immunophenotype is characteristic and highly defective: $CD3^+$, $CD8^+$, $CD45RA^+$, TIA-1^+, Granzyme-B^+ and $CD2^-$, $CD5^-$, $CD7^-$, $CD45RO^-$. To avoid overdiagnosis of this rare disorder, a differential diagnosis may be made from cases of mycosis fungoides, localized pagetoid reticulosis, $CD30^+$ disorders, or subcutaneous panniculitislike T-cell lymphoma showing the $CD8^+$ phenotype. It is really important to consider the clinical and histologic data in the context of the immunophenotype of these neoplastic cells. Finally we and others reported cases of cutaneous epidermotropic γ/δ T-cell lymphoma showing a similar clinico-pathologic presentation and evolution. *$CD56^+$ nasal or nasal type NK/T cell lymphoma*. This is a rare lymphoproliferative disorder, with an aggressive clinical course. Ulcerated cutaneous lesions can be observed in the nasal area (midline malignant granuloma), but in other cases the disease presents with fast growing, hemorrhagic and ulcerated nodular and tumoral soft lesions in nasal or extranasal areas. In most cases systemic evolution can be observed in a short time. The infiltrate is angiocentric and angiodestructive, and

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is formed by pleomorphic medium size lymphoid cells showing the $CD2^+$, CD3 epsilon$^+$, $CD45RO^+$, $CD56^+$, $CD94^+$, TIA-1^+ and $NKp46^+$ immunophenotype characteristic of mature, differentiated NK/T-cells. In most cases EBV virus is found integrated in the genome of the neoplastic cells.

$CD4^+$, $CD56^+$ blastoid or precursor NK-lymphoma. Recently there have been several reports of cases with an apparently primary cutaneous presentation and an immature NK-like/myeloid immunophenotype $CD4^+$, $CD56^+$, $CD45RA^+$, $CD123^+$, $CD68^{+/-}$, $CD3\varepsilon^{-/+}$, TIA-$1^{-/+}$, $CD2^{-/+}$, EBV^-, $CD94^-$, $NKp46^-$. A similar phenotype can be expressed by plasmacytoid monocytoid dendritic cells, suggesting a common precursor. Clinical presentations, with multiple cutaneous lesions in most cases and systemic progressin, sometimes with leukemic involvement in a few years, are suggestive of secondary lymphomas. In some cases, involvement of the nasal mucosa was also observed as in nasal lymphoma. Histologically, an angiocentric pleomorphic medium size lymphoid infiltrate was detected in most cases. The differential diagnosis must consider other aggressive lymphomas, but also myelomonocytic leukemia.

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Pitfalls in the diagnosis of cutaneous lymphoma

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Pitfalls in the diagnosis of cutaneous lymphoid proliferations are innumerable and involve a variety of themes.

In our opinion, an important topic and pitfall of prime interest is the differential diagnosis between *pseudolymphoma* and authentic malignant lymphoma in the skin. This distinction is one of the most vexing problems in dermatology.

Cutaneous pseudolymphomas are inflammatory diseases of the skin that simulate malignant lymphomas either clinically, histopathologically, or both. They are traditionally divided into T-cell predominant types and B-cell predominant pseudolymphomas according to their histopathological and immunophenotypic patterns.

Tables 1 and 2 show a proposal for a modern clinicopathological classification of conditions that are currently viewed as cutaneous pseudolymphomas.

In this article, we would like to focus on criteria for the differential diagnosis of cutaneous B-cell lymphomas from B-cell pseudolymphomas.

Certain clinical features may be helpful. Pseudolymphomas often present as a single papule or nodule with a smooth surface in a special location (e.g., earlobe), whereas lymphomas tend to be associated with many tumors and can show ulceration. Unlike lymphomas, pseudolymphomas occur frequently in children. In pseudolymphomas there may be a history of a causative event (e.g., *Borrelia* infection), whereas in lymphomas the cause is usually obsure.

By conventional microscopy, as a rule, infiltrates in pseudolymphomas are less dense and less deep than those of lymphomas. They are usually relatively symmetrical, rather well circumscribed, and denser in the upper part of the dermis than in the lower part. Often the infiltrates assume a wedge-shaped pattern. In contrast, the infiltrates of lymphomas tend to be more massive, asymmetrical, and poorly circumscribed. In cutaneous infiltrates with a follicular growth pattern, confluence of follicles and lack of stainable body macrophages in germinal centers favor the diagnosis of malignancy.

Immunohistologic features can be studied on routinely fixed, paraffin-embedded biopsy specimens. Malignant cell populations of B-lymphocytes usually show a monoclonal restriction to either κ or γ light chain, whereas benign infiltrates exhibit a polyclonal pattern with expression of both light chains. Unfortunately, however, there are several cases of B-cell lymphoproliferative disorders, both benign and malignant, in which the cells do not express immunoglobulins. A useful clue for the diagnosis of follicle center-cell lymphomas with a follicular growth pattern is the diminished proliferative activity of malignant germinal centers, as revealed by the Ki67/MIP-1 antibody. Reactive germinal centers show high proliferation, whereas malignant ones are often characterized by a much lesser degree of positivity.

About 80% of follicular lymphomas and 15% to 30% of high-grade malignant non-Hodgkin`s lymphomas in the lymph nodes are characterized by the t(14;18), which is associated with an overexpression of the bcl-2 oncogene. By contrast, investigation of cutaneous cases showed that t(14;18) expression is rare in primary cutaneous B-cell lymphomas. Bcl-2 positivity is found in a small minority of cutaneous follicular center cell lymphomas.

It has been recently demostrated that CD10 and bcl-6 expression, which support a germinal center-cell origin, may be useful in the differential diagnosis of follicle center-cell lymphomas from pseudolymphomas. The presence of small clusters of CD10- and bcl-6-positive cells outside the follicles and – if present – positivity of bcl-2 within follicular center cells are criteria suggestive of malignancy.

Low-grade malignant lymphomas of B-cell lineage may show aberrant expression of some T-cell-associated markers. CD5 is a pan-T-cell marker that reacts with the cells of most cases of B-cell chronic lymphocytic leukemia. Normal B-lymphocytes are CD43 negative, but several low-grade B-cell lymphomas are CD43 positive. The detection of an aberrant phenotype of the B-lymphocytes ($CD20^+$, $CD5^+$, $CD43^+$) is considered a sign of malignancy.

High-grade malignant cutaneous B-cell lymphomas often show partial loss of one or more B-cell-associated antigens (CD20).

Concerning the molecular analysis of cutaneous B-cell lymphoid proliferations, the detection of clonal IgH-gene rearrangements using polymerase chain reaction

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Table 1. Cutaneous Pseudolymphomas: T-cell pattern/predominance.

Chronic actinic dermatitis
Lymphomatoid contact dermatitis
Lymphomatoid drug reaction (T-cell type)
Solitary T-cell pseudolymphoma
Lichenoid purpura
Lymphomatoid keratosis
Atypical lymphoid infiltrates associated with: molluscum contagiosum, herpes, ORF, milker`s nodule
Lichen sclerosus et atrophicus
Arthropod reactions
Lymphocytic infiltration

Table 2. Cutaneous Pseudolymphomas: B-cell pattern/predominance.

Lymphocytoma
Lymphomatoid drug reaction (B-cell type)
Arthropod reactions
Perniosis
Lupus panniculitis / Lupus profundus
Morphea, inflammatory stage
Syphilis II
Inflammatory pseudotumor
Acral pseudolympomatosus angiokeratoma of children
Pseudolymphomas associated with: tattoos, vaccination, hirudo medicinalis
Unclassifiable

(PCR) is a very valuable method for assessing clonality. Highly sensitive and specific PCR methods are, however, required.

Looking towards the future, a diagnosis of pseudolymphoma will soon no longer be made. It will be possible to characterize many of the *lymphoma simulators* by cause and molecular mechanisms.

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Immunobiology of cutaneous T-cell lymphomas

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Cutaneous T-cell lymphomas are a heterogeneous group of diseases that are characterized by a clonal accumulation of T-lymphocytes in the skin.

Cutaneous T-cell lymphomas (CTCL) represent the largest group (65%) of cutaneous lymphomas (CL) comprising clinical variants such as mycosis fungoides (MF), erythrodermic Sézary syndrome (SS), and pagetoid reticulosis (circumscribed or disseminated).

Cytomorphologically, these forms are composed of small cerebriform or pleomorphic cells. They usually have a good prognosis, unlike the disseminated large T-cell lymphomas (pleomorphic, anablastic) which usually exhibit a rapid and aggressive course.

From a view point integrating molecular biology, histology and clinical features, CTCL include a spectrum of clonal T-cell accumulations in the skin having clinically benign, possible prelymphomatous diseases at one end and definite aggressive lymphomas at the other end.

Clonal dermatitis[1] is one possible precursor of low-grade CTCL. This term includes chronic inflammatory eczematous skin lesions that do not fulfill the criteria for the diagnosis of CTCL. Some patients with this disorder may eventually develop CTCL. The proportion of such cases is rather low. Another inflammatory skin disease that typically shows an exanthematic distribution is small plaque parapsoriasis (*an abortive lymphoma*).[2] Although this disease has a benign clinical cause, clonal T-cell populations have been detected by polylmerase chain reaction (PCR) investigations in some cases. Only a minority of patients will develop CTCL.

Recently, a large group of patients suffering from *idiopathic hypereosinophilic syndrome* has been intensively studied. A substantial proportion of patients presented atypical T-cell populations in the peripheral blood.[3] Since some of these patients developed CTCL in the following years, this condition must be considered as a possible pre-lymphoma disease. Lymphomatoid papulosis (*latent lymphoma*) is another lymphoproliferative disorder with characteristic clinical and histologic features that may develop into aggressive CTCL or Hodgkin's disease in a minority of patients.

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Genetic factors including chromosomal abnormalities

Genomic abnormalities are frequently found in CTCL, but they seem to be non-directed and of heterogeneous origin. A limited genetic diathesis is suggested by the enhanced risk of CTCL in first-degree relatives of CTCL patients. In addition, an association of certain histocompatibility antigens with the susceptibility to develop CTCL has been described. For example, HLA-DR5 is frequently found in CTCL patients (31.5% versus 11% in healthy controls). Similar associations have been reported for HLA-DQB1*03 (72% versus 49% in healthy controls).[4] However, it is more likely that chromosomal aberrations are involved in the onset of CTCL. Chromosomal instability and karyotype variations are typical features of CTCL. Most patients display multiple chromosomal abnormalities.[5]

Statistically increased aberrations have been found in chromosomes #3, #6, #8, #9, #11, #13 and #17, all of which carry oncogenes and/or tumor suppressor genes/regions.[6] Frequently found chromosomal defects are structural abnormalities in chromosome #1[5,7] with translocations, insertions and deletions.[8] Chromosome #9 aberrations and deletions of tumor suppressive regions in 6q are known to be associated with subsets of non-Hodgkin's lymphoma.[9] NF-κB2 was found to be rearranged in certain types of lymphoma and more commonly in CL.[10] The gene codes for a protein of the NF-κB family, which is a transcription factor, involved in immune response and cell growth, and a candidate for a proto-oncogene.[8] NF-κB2 rearrangements occurred within exon 18 and 20 of the gene and involved recombinations with distinct regions at 10q24. The translocation led to specific carboxy-terminal truncations of NF-kB2 generating abnormal constitutively expressed nuclear proteins. The t(2;5) (p23;q35) translocation is frequently found (40%) in CD30$^+$ anaplastic large cell nodal T-cell lymphoma.[11] In contrast, large cell CD30+ CTCL was not found to be associated with t(2;5) (p23;q35) chromosomal translocation, in spite of morphologic and immunophenotyic similarities with CD30$^+$ anaplastic large cell lymphoma.[12] In addition to structural abnormalities, numerical abnormalities such as trisomies have been found but less frequently. Taken together, the genomic defects in CTCL are heterogeneous and follow no clear rules.

Environmental factors including infectious factors

A few case control studies have investigated a possible association of environmental factors with the etiology of MF. The first recorded a high incidence of allergies, of fungal and viral infections and found a higher than expected proportion of patients in the petrochemical, textile, metal and machine industries in the USA.[13] A second study from Scotland failed to confirm these observations, but recorded a higher incidence than expected of atopic diathesis in MF patients.[14] The third study from the USA failed to confirm any difference due to occupational environmental exposure but noticed an increased rate of other malignancies, including skin cancers.[15] Additional investigations clarified that the malignancies do not precede CTCL but are indeed second malignancies.[16] This increased risk of second malignancies found in another study is probably due to the disease-induced systemic immunosuppression.[17,18]

Numerous studies have illuminated the impact of human T-cell lymphoma virus I (HTLV-I) on the pathogenesis of CTCL. However, recent molecular studies using advanced PCR technique showed that this virus plays only a minor role in a small subgroup of CTCL. This is especially true in Europe.[19] One epidemiologic study did not detect any hints to a retroviral factor in the pathogenesis of CTCL.[16]

HTLV-1 is a retrovirus which infects T lymphocytes ($CD4^+$). It is causally associated with a spectrum of human diseases including leukemia/lymphoma. The three major routes of infection are from mother to baby through breast-feeding, blood transfusion and sexual intercourse. There is a long latency period of up to 40 years before the development of adult T-cell leukemia/lymphoma (ATL). The diagnosis is established by the detection of monoclonal integration of HTLV-I proviral DNA in lymphocytes.[20] ATL has been reported worldwide. Areas of high incidence include Japan (particularly the south-west), Central and South America, northern Iran, West and Central Africa.

For two decades now it has been suspected that the cutaneous T-cell lymphoma, mycosis fungoides, and its leukemic variant, the Sézary syndrome, may also be causally related to a retroviral infection.

A number of studies in CTCL have evaluated serum antibodies to retroviral proteins, electron microscopy to identify virus-like particles, and Southern blot analysis and PCR amplification to detect proviral DNA. The reported data are, however, very inconsistent and contradictory, emphasizing the need for critical evaluation of the experimental methods applied. Several interesting observations have included: 1) serologic evidence of HTLV-I infection in a small subset of CTCL patients; 2) cloning of a deleted HTLV-I proviral genome from a B-cell line established from the peripheral blood of a CTCL patient; 3) detection of retrovirus in Langerhans' cells and B-cells, and 4) molecular evidence for the presence of an HTLV-I-like retrovirus.[21]

Arguments against the concept of the involvement of HTLV-I in MF or Sézary syndrome (SS) include the finding that only a small number of MF patients have antibodies to HTLV-I, and that attempts to detect proviral sequences by Southern hybridization of extracted DNA usually meet with failure.[22]

Thus, it seems unlikely that HTLV-I is involved in the pathogenesis of CTCL.

Immunologic factors

The clinical course of CTCL is accompanied by a dysregulated synthesis of cytokines. As recently reviewedn[23] cytokines can influence tumor cells in an autocrine, paracrine or endocrine fashion. There is some controversy relating to the immune biology of MF and SS with respect to the T helper1/2 system.[24] Two major subdivisions of the T helper system can be defined according to the preferentially produced cytokines: T helper 1 (TH1) clones secrete mainly interleukin (IL)-2 and interferon (IFN)-γ, whereas T helper 2 (TH2) clones produce IL-4, IL-5, IL-6 and IL-10.[25] TH1 cells are involved in cell-mediated inflammatory functions, e.g. the induction of delayed-type hypersensitivity. TH2 cells encourage antibody production, in particular IgE responses and enhance eosinophil proliferation and function. Since IL-5 and IL-10 are predominantly found in SS-derived tumor cells, the malignant clone is most likely of TH2 subtype. Asadullah and co-workers used a quantitative reverse transcriptase-PCR to evaluate the IL-10 mRNA in lesional skin of MF patients and found that the IL-10 mRNA levels correlated with the stage of the disease.[26]

Using antibodies against Vα/β of the T-cell receptor we identified malignant T-cell clones in six SS patients. Their phenotype was $CD3^+$, $CD4^+$, $CD5^+$, $CD45RO^+$,[27,28] compatible with peripheral T-memory cells. We expected that all clonal T-cells lack CD7 expression. However, two out of six clonal T-cells were $CD7^+$. All cells produced IL-10 and IL-5. Furthermore, we have shown that IL-10 protein co-localizes with expression of the non-classical HLA molecule HLA-G.[29] HLA-G is able to inhibit cytotoxic cells such as cytotoxic $CD8^+$ cells or NK-cells. Furthermore, we have recently shown, by gene array analysis, that cutaneous T-cell lymphoma cells *in vitro* and *in vivo* express the interferon-γ inhibiting cytokine, IK, in addition to IL-10.[30]

IL-10 indirectly prevents antigen-specific T-cell activation, which is in turn associated with down-regulation of antigen presentation and accessory

cell functions of monocytes, macrophages, Langerhans' cells and dendritic cells. In addition, IL-10 limits T-cell expansion by directly inhibiting IL-2 production by these cells.[31,32]

Since TH1 cells are the principle effectors for cell-mediated immunity against tumor cells and delayed-type hypersensitivity reactions, it seems to be an advantage for the malignant cells to switch the immune response of the host to a TH2 type.[33] This switch is suspected because of the IL-10 transcription and secretion of reactive non-clonal T-cells and the lack of IL-2 and IFN-γ transcription in non-sorted PBMC of SS patients.[34] The dominance of the TH2 cells probably explains the well-known clinical phenomena seen in SS patients and other CTCL patients, such as reduced cutaneous delayed-type hypersensitivity reactions, hypereosinophilia, alterations in serum immunoglobulin levels (IgE, IgA), an increased risk of second malignancies and immunologic abnormalities of PBMC such as reduced natural killer cell activity and decreased mitogen-induced proliferation.[35]

The interferon-inducible protein 10 (IP-10), secreted by keratinocytes, is a chemotactic for normal $CD4^+$ lymphocytes and inhibits proliferation of early subsets of normal and of leukemic hematopoetic progenitors.[36] IP-10 is overexpressed in lesional keratinocytes of CTCL patients and may influence growth control in CTCL.[37] It has been discussed that TNF-α and IFN-γ may cause epidermotropism by inducing IP-10.[38] thus contributing to the clinical manifestation of the disease.

IL-7 mRNA, produced by keratinocytes, is not overexpressed in MF, and is not an autocrine growth factor in CTCL,[39] but has been shown to act as a growth factor for SS cells.[40]

During the last years IL-15 has been intensively studied by our group.[41] IL-15 is produced by keratinocytes and other cells located in the skin. Additionally, it is found frequently in CTCL lesions. Therefore, IL-15 is a candidate responsible for accumulation of malignant T-cells in the skin. In this context, it is important to evaluate whether IL-15 can serve as a proliferation or a viability factor. Immunostainings for Ki-67 can be used as a marker for proliferation and staining for BCL-2 can be used as a marker for viability. We showed that BCL-2 is present in all stages of the disease in several MF-patients. In contrast, Ki-67 was only detectable in advanced stages of the tumor.[42] *In vitro* experiments with SS-derived cells showed that IL-15 could prolong the lifespan of malignant T-cells, but was not able to induce proliferation of the tumor cells.[41] In this context it is important to look at the signal transduction of IL-15 in tumor cells. The IL-15 receptor is composed of three subunits, the α-, β- and γ-chain. During signal transduction, signal transducer and activator of transcription (STAT) factors are recruited and promote transcription of relevant genes, such as BCL-2. The analysis of intracellular BCL-2 levels in malignant and non-malignant T-cells revealed significantly higher amounts of BCL-2 in malignant T-cells. These findings are in perfect correlation with the observed reduced sensitivity to radiation in malignant T cells followed by a lower apoptosis rate.

The basal activation of the JAK3/STAT5 pathway involved in IL-2R signal transduction in SS cells may play a role in the pathgenesis of CTCL as well, since *in vitro* stimulation of SS-cells with IL-2 maintained the level of JAK3 and STAT5 phosphorylation, thus leading to transcription of proliferation genes.[43] On the other hand, Sun and co-workers reported an IFN-α-resistant CTCL cell line that lacked STAT1 expression.[44] TH2 cells in SS-patients showed severely reduced levels of STAT4, which is involved in IL-12 signal transduction.[45] A defect in IL-12 production led to a depression of cell-mediated immunity.[46]

Pernis and co-workers[47] have suggested a concept for the molecular basis for the T-helper 1/2 dichotomy of cytokine responsiveness in murine lymphocytes. Their results indicate that IFN-γ cannot down-regulate TH1 lymphocytes because their IFN-γ signaling pathway lacks AF-1 (interferon γ receptor chain two, IFNGR2). We have demonstrated the presence of mRNA encoding the AF-1 molecule in all SS-derived malignant clonal T-helper lymphocytes investigated. Serum of rabbits immunized with the extracellular domain of a recombinant human AF-1 protein was used to show membrane-bound immunoreactivity for AF-1 in four SS clones, but not in the PBMC of healthy donors. We did not detect AF-1 mRNA in non-purified mRNA from five SS patients and in mRNA from non-clonal $CD4^+$ cells of one patient.[28] These findings suggest that AF-1 was strongly overexpressed in the SS clones investigated. However, purified clonal T-cells from patients with Sézary syndrome did not respond adequately to stimulation with interferon-α or interferon-γ. This interferon resistance can be targeted by replicating viruses.[48]

Summarizing the different data published in recent years, there is no single event responsible for the onset of CTCL, nor a certain rule for the pathogenesis of the disease. It is our understanding that various interventions at different levels of control of the dynamic lymphocyte balance can shift a T-cell population into malignancy.

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Management and prognosis of cutaneous T-cell lymphomas: indolent subtypes

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According to the EORTC classification, indolent subtypes of cutaneous T-cell lymphoma are: mycosis fungoides and its variants, pagetoid reticulosis, large T-cell lymphoma CD30+ (LCL) and lymphomatoid papulosis (LP). Mycosis fungoides (MF), which is by far the most frequent type, is clinically characterized by patches followed by plaques and eventually nodules/tumors. In contrast, the typical manifestations of LP and LCL CD30+ are papules or nodules. Despite the different clinical and histopathologic aspects, indolent cutaneous T-cell lymphomas are characterized by slow progression over years or decades and a good or excellent prognosis. Transformation to a high grade lymphoma may occur in some cases of MF and therefore careful monitoring of the patient is recommended. The therapeutic approach of MF may vary according to the stage of the disease. At least in the early phase, skin lesions may be treated with topical corticosteroids, carmustine, mecloretamine or phototherapy. Recently, excimer laser as well as topical retinoids have been proposed as additional treatments, and used with a certain success. Advanced stages can be treated with interferon + retinoids, interferon + psoralen ultraviolet light A (PUVA), total electron beam radiation or photopheresis. Gemcitabine and oral retinoids (tagretin) are, at the moment, undergoing evaluation in clinical trials.

Radiotherapy and surgical excision are the treatment of choice for LCL CD30+ with single or localized skin lesions, whereas chemotherapy should be performed for disseminated lesions. Treatment of LP may vary according to the number of lesions as well as time of spontaneous clinical remission. In some cases no therapy is necessary (*watchful waiting*) or topical corticosterois may be used. In cases of disseminated, painful or persistent lesions, methotrexate, PUVA therapy or systemic corticosteroids may control the disease.

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Aggressive subtypes of cutaneous T-cell lymphoma: management and prognosis

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Aggressive primary cutaneous T-cell lymphoma (CTCL) subtypes encompass a wide variety of clinico-pathological entities characterized by a constant cutaneous involvement, high risk of extracutaneous spreading and an unfavorable clinical course. Two major well-defined subtypes are identifiable according to the recently proposed EORTC classification:[1] the Sézary syndrome (SS)[2] and CD30$^-$ large-cell CTCL.[3] However, other poorly recognized entities, such as the extranodal nasal- and nasal-type NK cell lymphoma (angiocentric lymphoma),[4] subcutaneous panniculitis-like T-cell lymphoma, and primary cutaneous CD8-positive epidermotropic cytotoxic T-cell lymphomas,[5] which are so far included in the provisional group of the EORTC classification, share an aggressive clinical course. Moreover, even if mycosis fungoides (MF) is classified as an indolent CTCL in the EORTC classification, the advanced-stage disease (stage IIb to IV according to the TNMB classification, i.e. disseminated cutaneous tumoral lesions or erythroderma with or without extracutaneous spread) is usually characterized by an unfavorable prognosis and should therefore be considered in the group of aggressive CTCL subtypes.[6]

The aim of the present study was to analyze the clinico-pathologic findings and disease course of a large cohort of patients with aggressive CTCL, diagnosed, treated and prospectively followed-up at our institutions. The review of the clinical data will be predominantly focused on the treatment modalities,response rates (RT), remission durations and overall survival.

A total of 439 primary CTCL patients were diagnosed, treated and followed up at our institutions, from January 1975 to December 2002. The diseases were classified on the basis of the EORTC classification system:[1] 332 were diagnosed as MF, 75 as SS and 46 as non MF/SS CTCL (13 with CD30$^+$ large cell and 33 with CD30$^-$ large cell CTCL). One hundred and ninety-four patients were identified as having an aggressive CTCL subtype i.e. the cohort included the 75 patients with SS, the 33 with CD30$^-$ large cell CTCL and 86 patients with stage IIb to IV MF.

The diagnosis was made on the basis of clinical, immuno-pathologic and molecular data.

The diagnostic criteria for SS were:[2,7] a) erythroderma and peripheral lymphadenopathies; b) peripheral blood involvement by circulating Sézary cells (SC); c) cutaneous biopsy-proven CTCL, confirmed by the finding of a clonal T-cell receptor (TCR)-γ gene rearrangement. Peripheral blood involvement was defined, according to the criteria recently proposed by the International Society for Cutaneous Lymphoma, only in the presence of two major criteria: absolute circulating SC count > 1,000 mm^3; polymerase chain reaction detection of a dominant TCR-γ gene rearrangement in the peripheral blood. Additional criteria (not necessarily needed for the diagnosis of peripheral blood involvement) were: CD4/CD8 ratio> 10; circulating CD4$^+$CD7$^-$ cells ≥ 40%; aberrant expression of T-cell markers; and chromosomally-abnormal T-cell clone.[7] Moreover, according to our studies suggesting that the lack of CD26 expression is a characteristic phenotypic feature of circulating SC in both SS and MF patients with peripheral blood involvement, a CD4$^+$CD26$^-$ percentage value of more than 30% of peripheral blood lymphocytes was used as a useful threshold value for diagnosis and monitoring of peripheral blood involvement.[8] Circulating SC were morphologically identified, on peripheral blood smears stained with May-Grünwald-Giemsa, on the basis of their characteristic cerebriform nuclei, as previously reported.[2]

The diagnosis of MF was based on standard clinical, histologic and immunohistochemical criteria, according to the EORTC classification.[1,6] All the patients with tumor stage had a prior history of and/or concurrent histologically proven MF patch/plaque lesions. MF patients were staged according to the TNM classification system. Lymph node histopathology was classified according to the NCI classification system, as LN-0, -1, -2, -3, or -4.[9]

For patients with MF/SS, evaluation was based on detailed medical history, physical examination, complete blood cell count (including SC count on May-Grünwald stained smears) and routine laboratory tests. Staging procedures included skin biopsy and computed tomograhpy of the abdomen, pelvis and lungs; lymph node biopsy was performed in the presence of palpable lymphadenopathies. MF patients were staged according to the TNMB classification system.

The diagnosis of primary cutaneous lymphoma was made on the basis of the absence of clinically evident extracutaneous involvement both at diagnosis and within 6 months after, for patients with non MF/SS CTCL. The patients' evaluation was based on physical examination, routine laboratory tests, including lactate dehydrogenase serum levels and complete blood cell

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count with quantification of circulating atypical cells, bone marrow biopsy, computed tomography of the head, neck, chest and abdomen and endoscopy of the upper digestive tract and nasopharynx endoscopies. Lymph node biopsy was performed in the presence of clinically detectable adenopathies.

Systemic polychemotherapy has been shown to play only a palliative role in the treatment of advanced stage MF/SS patients with progressive cutaneous disease refractory to standard therapies or with extracutaneous spread.[10] In fact, even though the response rate (complete plus partial responses; RR) is initially high, remission duration is short-lived and no patient has been cured by this approach. Conversely, systemic polychemotherapy can be considered the first-line treatment in patients with non-MF/SS CD30$^-$ CTCL. In our experience, first generation regimens (COP or CHOP like) give a 40% RR and a median response duration of 5.9 months.[11] Third generation sequential protocols are associated with a higher RR (84%), even if the response duration does not exceed 9 months.[12] Forty patients (18 MF, 13 SS, 4 pleomorphic lymphoma and 5 immunoblastic ymphoma) were treated with a COP or CHOP regimen, whereas 32 patients (23 MF, 2 SS and 7 T pleomorphic lymphoma) were given a 12-week combination of etoposide, idarubicin, cyclophosphamide, vincristine, prednisone and bleomycin (VICOP-B). The overall objective RR was 35%, with a complete response (CR) rate of 20% in the COP/CHOP treated group.[11] The median response duration was 5.4 months. The RR among the MF/SS patients was 29%, with a median response duration of 5.9 months and a median survival of 22 months. MF patients showed a higher RR than did SS patients (44% vs 8%). All the patients with pleomorphic/immunoblastic CTCL achieved a short-lived CR (5-year survival: 28%). The overall RR was 78%, with a 39% CR rate in the VICOP-B group (12). MF patients had an 83% RR, with a median response duration of 8.4 months; no responses were seen in the 2 SS patients. All patients with pleomorphic CTCL achieved an objective response, with a median duration of 3 months and a 30% 5-year survival. Dose-limiting toxicity was hematologic (40% of patients with grade IV). Peripheral neuropathies were observed in 30% of cases. The VICOP-B regimen is effective and feasible as first-line chemotherapy in advanced MF patients, while COP/CHOP results are unsatisfactory. Conversely, there are no indications for the use of both regimens as first-line treatment in SS patients. Despite the high response rate, no survival improvement was achieved in non-MF/SS CD30$^-$ CTCL patients because of the high rate of early relapses.

Although a series of therapeutic approaches have been described in SS patients, their efficacy is still controversial and none of them has been widely accepted as first-line therapy. In a recent paper, we showed that the presence of PAS-positive inclusions in the cytoplasm of circulating Sézary cells, a CD7- circulating SC phenotype and the finding of circulating large SC may be independent adverse prognostic factors in SS. Therefore, on the basis of these data, two low/high risk groups were singled out: one with a slow course and relatively favorable prognosis (5-yr survival: 58%), the other with an aggressive course (5-yr survival: 5%).[2] Of the 75 SS patients included in this study, 25 were females and 50 males, with a median age of 69 years (range, 35 to 90 years). According to the proposed ISCL criteria, a B1 blood rating was observed in 10 patients (13%), with an absolute Sézary cell count lower than 1,000 per mm^3 at diagnosis. Immunophenotyping showed a loss of T-cell lineage markers in 15 of 75 (20%) of the patients. The presence of lymphocytes expressing an aberrant immunophenotype was significantly associated with a poorer prognosis (median survival: 20 versus 46 months; 5-year survival: 8 % versus 36%) with a hazard ratio of 2.39 (95% CI 1.51 to 7.76; p= 0.003). A CD7 positive phenotype was found at diagnosis and during follow-up in 31/75 patients (41%). It is noteworthy that all the cells with aberrant phenotypes were found within the CD4$^+$CD7$^-$ population. Patients with CD7$^+$ Sézary cells had a significantly longer overall survival than that observed in CD7$^-$ patients (median, 57 versus 21 months; 5-year survival, 47% versus 18%), with a hazard ratio of 2.53 (95% CI 1.48 to 4.45; p=0.0007). Initial preliminary results suggested that the lack of DPP-IV/CD26 expression was a constant feature of circulating neoplastic T-cells in SS and that the levels of the CD26$^-$ subpopulation correlated to the extent of peripheral blood involvement. We confirmed these results in 1998 on a cohort of 53 patients with SS.[2] In 2001, we demonstrated a clear increase in the CD4$^+$CD26$^-$ subpopulation in 21 patients with SS and in 14 cases of MF with leukemic involvement. A cut-off value of 30% had a sensitivity of 97%, a specificity of 100% and a positive predictive value of 100% in the diagnosis of peripheral blood involvement.[8]

The 75 patients were treated with the best treatment available at that time. A significantly lower RR was found in patients with atypical phenotypes (33% vs 60%) as well as those with a CD7$^-$ phenotype (29% vs 64%). The results of polychemotherapy were unsatisfactory: out of 44 patients, only 6 responses were observed (13.6% RR), with a response duration of no more than 6 months; moreover, the disease-related immunosuppression was worsened by the therapy-induced leukopenia, giving rise to a high percentage of disseminated viral and/or bacterial infections. When the classic combination of chlorambucil plus prednisone was used it was not able to modify the clinical course of the

disease, achieving a RR of only 22%. The therapeutic use of the pentapeptide thymopentin (TP5), which is the active sequence of the thymic hormone thymopoietin, was associated with a high RR but an increased overall survival was observed only in the patients belonging to the low-risk group.[13] More recently, two different therapeutic strategies have been introduced for the treatment of SS patients: i.e. monochemotherapy with the purine analogs, fludarabine monophosphate (FAMP), deoxycoformycin (pentostatin), and 2-chlorodeoxyadenosine (2-CdA), and extracorporeal photochemotherapy (ECP).

Von Hoff *et al.* treated 31 CTCL patients with FAMP, obtaining a 19% RR but only a 3% complete response rate.[14] Although the addition of continuous low-dose interferon seemed to improve the clinical efficacy (51% RR),[15] it was associated with a significant increase in systemic toxicities. Recently, Scarisbrick *et al.* reported the use of FAMP associated with cyclophosphamide in 12 advanced CTCL patients, and showed a response in 5 SS patients without a significant survival improvement coupled with a severe bone marrow toxicity.[16] A large number of phase II studies have shown the clinical activity of ECP in the management of CTCL patients, with higher RR in erythrodermic patients than in plaque or tumor stage patients.[17-19] Nevertheless, a recent non-randomized trial failed to demonstrate any survival improvement in ECP-treated SS patients.[20]

Forty-four patients with stage IIb to IV MF or SS were treated with FAMP. The overall objective RR was 29.5% (13/44), with a 9.1% CR rate (4/44). The median induction response time was 3.5 months (range: 2-4.5 months). A clear-cut difference in the RR was observed between MF and SS patients. MF patients had a RR of 25.9% (7/27) with 1 CR. The CR and 1 PR were observed in the stage III patients; the other PRs were obtained in 4 stage IV patients (2 T2, 1 T3, 1 T4) and in the patient with stage 1B and peripheral blood involvement. The SS patients achieved 3 CR and 3 PR (RR: 35.3%). The three CR were confirmed by the PCR finding of a germ-line configuration of the TCR g-chain gene in the blood. Two other patients showed stable cutaneous disease, with more than a 50% reduction in the number of circulating Sézary cells.

ECP was performed in 40 CTCL patients between August 1992 and December 2001. Fifteen had MF stage IIB to IV, 21 SS and 4 LyP (2 of them in association with MF). Treatment was usually given on 2 consecutive days once a month according to standard procedures.[21] From 1992 to 1996, the photoactive drug 8-methoxypsoralen (8-MOP) was given orally at a dose of 0.6 mg/kg one hour before ECP. As from 1997, a new liquid form of 8-MOP was added directly to the buffy coat, resulting in reliable and sufficient drug levels in the cell suspension during the irradiation period. The results of ECP seem to be encouraging, with a 42% overall RR. In particular, we have recently evaluated the potential synergistic activity of the sequential association of FAMP and ECP, which produced a RR of 63.2% (3 CR and 9 PR) and stable disease in 26.3%. Overall, the RR of the FAMP-ECP group seemed to be significantly higher than that of the FAMP monotherapy group. The time to progression of the patients treated with the FAMP-ECP combination therapy was longer (median: 13 months; range: 3-91) than that of patients treated with FAMP alone (median: 7 months; range: 2-83), even if this difference was not statistically significant. No difference in survival was found between FAMP-treated (median: 25.5 months; range: 2-84) and FAMP-ECP-treated patients (median: 20.1 months; range: 8.5-91). To the best of our knowledge, there are currently no reports in literature on the sequential association of ECP and FAMP. The reduction in the peripheral $CD4^+$ counts induced by FAMP, together with the $FAMP^-$ and ECP-induced apoptosis, could result in higher CTCL suppression.[21-22] Although it has been shown that immunosuppressive chemotherapy, such as FAMP is related to a significant decrease in the levels of the cytotoxic T-cells and, therefore, partially impairs the immune system stimulation induced by ECP, no modification in either the $CD3^+CD8^+$ or $CD3^-CD16^+/CD56^+$ circulating subpopulations was found in our patients during FAMP treatment. Moreover, the reduction in the $CD3^+CD4^+$ levels of the circulating subpopulation was correlated to a decrease in the percentage of atypical lymphoma-related $CD4^+CD26^-$ cells, whereas the *normal* $CD4^+CD26^+$ circulating lymphocytes showed no modification.

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Cutaneous B-cell lymphomas: management and prognosis

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Definition, classification and prognosis

Primary cutaneous B-cell lymphomas (CBCL) are a definitely established entity, and must be clearly separated from secondary CBCL. In fact, the former are defined by their primary cutaneous presentation in the skin without any documentable sign of extracutaneous localization,[1,2] unlike the latter which are the expression of dissemination from a primarily extracutaneous site. It remains to be clarified whether concurrent CBCL, i.e., those simultaneously presenting in the skin plus lymphoid organs and/or other extranodal sites (e.g., MALT, mucosa-associated lymphoid tissue), behave differently from secondary CBCL. In our experience, the comparison of primary, concurrent, and secondary CBCL, although biased by the different sizes of the studied groups of patients, indicates that concurrent CBCL do have clinico-pathologic, immunologic, and prognostic features much closer to those of primary CBCL.[3]

According to the EORTC classification of primary cutaneous lymphomas,[2] primary CBCL are subgrouped into three categories. Two of them, the so-called follicle center cell lymphomas (FCCL) and immunocytoma/marginal zone lymphomas (MZL), account for nearly 90% of all patients with a definite diagnosis of primary CBCL (89.4% in our series of 274 patients). These lymphomas have a mostly regional extension (trunk, head and neck more frequently than limbs), variable grade histology (low grade in early lesions, high grade in late ones), and a indolent clinical behavior, with a lack of correlation between histologic progression to diffuse large cell infiltrate and either the clinical course or the prognosis of the disease. The prognosis, despite relatively frequent relapses (24.7% in our series, with a median disease-free interval of 42 months) remains invariably excellent (98.5% 5-year survival in our series), unlike that of primary nodal and even other types of extranodal B-cell lymphomas, e.g., MALT lymphoma).[1,2,4-7] Therefore, as regards management and prognosis, they can be readily considered as a single, clinically low-grade entity.[1,2] A much smaller subgroup with intermediate prognosis (the so-called large B-cell lymphoma of the leg)[8] is identified in the EORTC classification.[2,4] This type of lymphoma is characterized by rapid growth of skin lesions (plaques, nodules and/or tumors), *de novo* diffuse large cell infiltrate histology (diffuse large B-cell lymphoma, DLBCL), high proliferation rate, strong bcl-2 protein expression, predilection for the elderly (>70 years of age) and selected skin sites (lower legs), and a much less favorable prognosis (58% 5-year survival). The results of a recent European multicenter study on a large group of patients[9] indicate that large *round* cell (i.e., centroblast- and immunoblast-like) histology and old age (>70 years) are the most potent factors significantly associated with a more aggressive behavior and worse prognosis as compared to low grade, indolent CBCL. Our experience in 29 patients with DLBCL (10.6% in our series of 274 patients) is in line with all the above features (old age, median 79 years, at presentation; higher relapse rate, 41.3% vs. 24.7% in the indolent group; shorter disease-free interval, 15 vs. 42 months; relative predilection for the lower limbs, 17/29 patients), except for prognosis (95.1% vs. 98.5%). This finding was also recently reported by other groups in the UK.[10]

Staging, treatment and follow-up

Once biopsy-confirmed at presentation, the diagnosis of CBCL must be supported by careful staging procedures. Among these, complete physical examination, basic blood investigations (hemogram, lactate dehydrogenase and β-2-microglobulin serum levels, and quantification of immunoglobulins), and neck/thorax/abdomen computed tomography scans are mandatory. Based on the major literature data,[1,2] the case for bone marrow biopsy differs, in that this should be considered mandatory at presentation in DLBCL only. *Vice versa*, it can be restricted to cases with other documentable signs of progression (rising lactate dehydrogenase) and/or frank extracutaneous spread in low-grade, indolent forms.

CBCL are highly responsive to radiotherapy (RT). Therefore, notwithstanding the lack of controlled, randomized trials, most leading groups currently agree that RT should be considered the elective treatment for most CBCL.[1,2,11-13] Concerning the most suitable techniques and the recommended doses, local orthovolt RT (LoRT) showed very good results,[1,11,12] although electron beam and photon beam have also been successfully used.[13] We have been using LoRT (half-deep X-rays, contact X-rays, soft X-rays) for the last two decades, with excellent results, according to the following protocol: potency range 50-120 kV; single dose per field 2.5-5 Gy, total dose of 15-25 Gy delivered in 2-3 weeks in 4-8 fractions

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(according to the thickness of the lesion and the site of irradiation); irradiation fields (up to 20 cm in diameter) almost invariably including 1-2 cm of healthy skin around the lesions. In particular, the slightly infiltrated erythematous patches and plaques surrounding the larger nodules or tumors should be included in the irradiation field. A complete remission was observed in 99.2% of 164 patients treated with LoRT at presentation in our institution. Given that the large majority of relapses are limited to the skin, very often outside the previously irradiated area, they can be successfully and safely treated again with LoRT. Small, isolated lesions can be surgically excised, with or without additional RT. The treatment with multiagent chemotherapy (COP- and CHOP-like regimens), according to the recommendations of the *Italian Group for Cutaneous Lymphomas* (GILC, *Gruppo Italiano Linfomi Cutanei*) prepared on the basis of extensive review of the literature, should be restricted at presentation to the occasional patients with disseminated lesions. Of course, chemotherapy is the rule in patients refractory to RT and those who have repeated relapses after RT, and — of course — in those experiencing extracutaneous spread of disease.[1,2,11-13] Other treatment modalities – e.g., anti-CD20 immunotoxin administered systemically[14-16,18,19] or intralesionally[14,17,18] — should currently be considered as experimental, although very promising and especially useful in *difficult* cases.

The follow-up recommendations, proposed by the GILC, prepared on the basis of extensive review of the literature, are as follows: clinical examination twice a year (and on relapse); restaging once a year (and in case of significant relapse).

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Topical therapy of cutaneous T cell lymphoma

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Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of T-cell lymphomas with initial clinical manifestations localized to the skin.[1,2] The CTCL spectrum is composed of mycosis fungoides (MF), which represents about 80% of cases, erythrodermic expressions of CTCL including Sézary syndrome, which represent 10-15% of cases, and a variety of other peripheral T-cell lymphomas that arise in the skin. In the new World Health Organization (WHO) classification of lymphomas, classic MF and its variant with follicular mucinosis and Sézary syndrome are retained as distinct entities.[3]

One of the salient histopathologic features of MF and Sézary syndrome is the predilection for the neoplastic T-cells to infiltrate into the epidermis, particularly in early phases of the disease (epidermotropism). The neoplastic cells tend to localize along the basal layer of the epidermis or form clusters around Langerhans' cells in more superficial layers (Pautrier microabscesses). When present these features are useful for histologic diagnosis.[4-6] Recent studies have implicated interferon gamma-inducible chemokines (IP-10) and thymus and activation-regulated chemokine (TARC/CCL17) as soluble factors released by keratinocytes that probably mediate the epidermotropism of neoplastic T-cells.[7-9] Possibly as a consequence of the loss of interferon-γ activity that occurs with the switch from a TH1 to TH2 cytokine profile with disease progression,[10,11] epidermotropism is often lost in advanced phases of MF. Other studies have shown that in early lesions of MF (patch and thin plaques), the neoplastic cells are restricted to the epidermis and adjacent papillary dermis.[12-16] In addition, the neoplastic T cells in the epidermis undergo proliferation, presumably in response to an as yet unknown antigenic stimulus.[17,18] These observations indicate that a variety of externally applied therapies delivered to the skin (herein called topical therapies) would be particularly effective in early MF when neoplastic cells preferentially home into the epidermis and are dependent on epidermal factors for tumor cell proliferation and survival.

Traditional topical chemotherapy

Two drugs widely utilized for the topical chemotherapy of MF are mechlorethamine hydrochloride (abbreviated as HN2) and carmustine (BCNU). Both HN2 and BCNU may be dissolved in water and administered to the entire skin surface if desired or prepared in an ointment vehicle for local application to individual lesions.

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Mechlorethamine

HN2 [methyl-bis(2-chloroethyl)amine HCL] has been utilized for the topical chemotherapy of MF for almost 50 years. First used by Sipos in 1956 to treat MF,[19] topical HN2 was subsequently tested for its therapeutic efficacy for psoriasis and other dermatoses in the 1960s.[20] The drug is a highly reactive alkylating agent that can bind to many electron-donating molecules, so it has been a mystery how the drug could penetrate the epidermal barrier and eliminate neoplastic (or normal) lymphocytes.[21] The beneficial effect of HN2 for MF has been attributed to the direct cytotoxic effect of the drug on neoplastic T-cells via cross linking (alkylation) and inhibition of DNA synthesis; however, this has never been demonstrated *in vivo*. One might speculate that the topically applied HN2 might also suppress the production of keratinocyte-derived cytokines that are needed to support neoplastic T-cell growth or inhibit Langerhans' cell-neoplastic T-cell interactions as additional mechanisms that inhibit neoplastic T-cells. The drug is also a weak allergen by binding as a haptene to epidermal proteins. Therefore, some of the beneficial effects may be related to its immunostimulatory properties, and some investigators have administered HN2 topically for the immunotherapy of MF.[22, 23]

The procedures used to apply topical HN2 in aqueous solutions vary widely.[22,24-29] The author's method involves having the patient (or health care provider) dissolve 10 mg of the drug in 50 to 60 mL of water immediately before use, and then the solution is self applied by the patient to the entire skin surface proceeding from top to bottom except the genital skin unless this is involved by the disease.[21,22] If there is concern about potential irritation, as might occur in elderly or heavily pretreated patients or patients with considerable sun damage, the starting dose of aqueous HN2 may be decreased to 5 mg in 50 mL, or a topical corticosteroid such as triamcinolone cream 0.1% used as a counterirritant. A teaspoon of emulsified bath oil may be added to the HN2 mixture for patients with dry skin. Treatments are usually given after bathing and are best given with the patient standing in a bath tub or in an area that is well ventilated. The hands are protected with plastic gloves to avoid irritation between the fingers, and then treated after the drug has been applied elsewhere. The face, including the eyelids, is treated by passing from the forehead to the cheeks with the eyes closed; irritation of the eyes or nasal passages has rarely been encountered. The scalp is treated last by diluting the remaining solution with extra water if needed to provide a sufficient amount. If an assistant

helps the patient, e.g., by applying the drug to hard to reach areas such as the mid upper back, then the assistant must wear a heavier protective glove to avoid exposure to the drug.

The main adverse effect of topical HN2 is contact dermatitis which occurs in about 50-60% of patients treated with aqueous solutions.[27-29] Approximately one third of these cases are due to a delayed hypersensitivity reaction to HN2 (allergic contact dermatitis) confirmed by patch testing.[28,29] The clinical differentiation between irritant and allergic contact dermatitis may be difficult, but irritation is more likely when the reaction is localized to MF lesions, chronic photo-damaged skin or intertriginous zones and the eruption is less pruritic. Indeed, the irritant effects of topical HN2 at lesion sites often makes clinically unapparent skin involvement more evident, much like the effect that 5-fluoruracil has on actinic keratoses.[21] Conversely, allergic contact dermatitis tends to occur more diffusely on the skin, is quite pruritic and typically begins between 1 to 5 weeks after starting to use the drug.[22] Irritant contact dermatitis may be managed by temporarily reducing the concentration of the solution, avoiding the irritated areas and/or applying a topical corticosteroid; allergic contact dermatitis requires discontinuation of the drug and patch testing after the reaction subsides to determine whether topical desensitization is feasible.

When allergic contact dermatitis is suspected, patch testing can be performed by applying a small amount of the drug that has been prepared in the usual way for topical administration, i.e., 10 mg in 50 mL of water, to a small area of normal skin after the contact dermatitis has resolved. More severe reactions at patch test sites, i.e., intense erythema with edema or vesicular reactions, or histologic evidence of spongiotic dermatitis signify that successful topical desensitization to achieve full therapeutic doses is unlikely.[29] However, if patch testing to HN2 reveals no reaction or only erythema without edema or blistering (+1 reaction), then the patient may be restarted on topical HN2 using diluted solutions with gradual escalations in the concentrations over time in an effort to *harden* the skin if irritation is the problem (negative patch test) or desensitize the skin if allergy is involved (positive patch test).[21,30,31] The author typically advises patients to resume daily applications of topical HN2 with concentrations of 10 mg dissolved in a half gallon (1.89 liter) of water and if no dermatitis recurs after 2 weeks, then the concentration is doubled to 10 mg in a quart (0.95 liter) of water for 2 additional weeks; if no reaction occurs, then the concentration is again doubled to 10 mg in a pint (0.47 liter) of water and applied for 2 additional weeks and so on, until the intended therapeutic dose of 10 mg in 50 mL of water is achieved. If recurrent contact dermatitis develops along the way, then longer intervals of time between dose changes or smaller incremental increases are used. The success rate of the topical desensitization procedure varies according to the willingness of the patient and physician to persist with treatment and the proportion and degree of true allergic sensitization among patients. Ramsay reported complete desensitization over many months to years in almost all patients.[30] The author estimates that about one third to one half of patients who have mildly positive patch tests to HN2 can resume full therapeutic doses using the desensitization protocol described above.

The frequency of allergic contact dermatitis (confirmed by patch test) to topical HN2 in aqueous solutions decreases in patients with advanced MF, a reflection of the immunosuppression that accompanies advanced disease.[22,32] The frequency of developing allergic contact dermatitis to HN2 is lower when the drug is administered in a water-free ointment vehicle such as Aquaphor or polyethylene glycol than when it is administered in an aqueous solution; however, in the author's experience the complete clinical response (CCR) rate with use of HN2 ointment for patients with patch/plaque phase MF is about 15% less than that with the aqueous preparation (unpublished observations). This observation differs from the experience at Stanford University in which the CCR rates were 52% and 54% for comparable patch/plaque MF patients treated with ointment versus aqueous preparations, respectively.[33] The reason for the difference between the Stanford experience with aqueous topical HN2 (54% CCR rate) and that of the author (72% CCR rate) is unclear, but may relate to the philosophy of the author to continue to use topical HN2 at reduced concentrations in patients who develop contact dermatitis to the drug with the aim of achieving desensitization. Similar response rates (70% CCR) were reported for patients treated by Ramsay utilizing a similar approach (Table 1).

Other potential adverse effects of topical HN2 include urticaria, xerosis, hyperpigmentation, and induction of other skin cancers. Urticaria is reported to occur in about 5% of patients, and if this occurs, topical HN2 is usually discontinued because of the potential for systemic anaphylaxis.[34,35] Localized bullous reactions or Stevens-Johnson syndrome have also been reported but are rare.[36,37] Concern has been expressed about the possibility that HN2 could irritate the respiratory tract or contaminate the environment with risk to others,[24,38–40] but this has not been a problem in the author's experience. In addition, chest X-rays of patients treated with topical HN2 have not revealed evidence of pulmonary fibrosis.[41] Atypical histologic changes have been observed in otherwise normal skin of patients treated for long periods.[42] Long-term administration of topical HN2 was found by the author to be associated with an increased risk of developing basal

and squamous cell carcinoma,[43] but this finding has not been confirmed in other large series.[30,33] The reason for this divergence is unclear, but may relate to previous treatments and/or the more intensive use of topical HN2 for long-term maintenance of CCR achieved in MF by the author prior to 1989.[43] In addition to shortening the maintenance intervals from 3 years to 6 months in patients with patch/plaque MF who achieve CCRs, the author now minimizes the use of topical HN2 on areas prone to develop skin cancers, i.e., sun damaged or genital skin, and emphasizes the need for protection from excessive sun exposure. Malignant melanomas may occur in patients with MF who are treated with topical HN2, but the risk appears to be no greater than that expected for the general population. Indeed, it is conceivable that topical HN2 may actually enhance the early detection of melanomas through stimulation of hyperpigmentation.[44,45] Because of its high chemical reactivity, topical HN2 is not absorbed through the skin and thus does not cause systemic effects such as bone marrow suppression, increase in internal malignancies, or increased incidence of birth defects. Studstrup reported no increase in sister chromatid exchanges in peripheral blood lymphocytes in patients treated with topical HN2.[46] Nevertheless, the author advises periodic monitoring of peripheral blood counts in patients with large areas of denuded skin (loss of barrier function) and avoidance of this treatment in women who are planning to become pregnant.

Table 1. Topical mechlorethamine (HN2) in patch/plaque mycosis fungoides (10 or more cases in the series).*

Series	*No. Pts.*	*OR (%)*	*CCR (%)*
Molin, 1979[a]	21	19 (90)	10 (62)
Hamminga, 1982	17	17 (100)	14 (82)
Zachariae, 1985	33	26 (79)	14 (42)
Vonderheid, 1989[b]	201		144 (72)
Ia	89		71 (80)
Ib	66		45 (68)
IIa	46		28 (61)
Ramsay, 1997[c]	301		210 (70)
Foulc, 2002[d]	22	18 (82)	13 (59)
Kim, 2003[e]	195	163 (84)	100 (51)
T1	107	100 (93)	70 (65)
T2	88	63 (72)	30 (34)

* Review limited to most recent update by group. Abbreviations: OR = objective response rate, i.e., partial plus complete responses; CCR = clinical complete response rate. [a]Series includes 2 patients with erythroderma. [b]Series includes some patients treated with additional therapies. [c]Data provided by David Ramsay, M.D., personal communication. [d]Series limited to patch/plaque MF without lymphadenopathy (stage Ia-Ib); HN2 solution washed off after 1 hour. [e]Series includes patients treated mostly with topical HN2 in an ointment preparation.

Carmustine

BCNU [bis-chlorethylnitrosourea] was introduced for the topical therapy of MF by Zackheim in 1972, and so far his is the only published experience with this agent.[30,47-50] Like HN2, the drug presumably works by interfering with DNA synthesis of the neoplastic T-cells through alkylation, although other mechanisms might also be playing a role as discussed above. BCNU is more lipid soluble than HN2 and will, therefore, penetrate the epidermal barrier to a greater degree.[51] The positive effect of this is that the agent may be more effective than HN2 in body regions that have a thickened stratum corneum (palms and soles) and perhaps for lesions that have marked hyperkeratosis. The drug might also be more likely to clear lesions with infiltrates that extend into the mid dermis (thicker plaques, follicular mucinosis) although this impression of the author has never been formally evaluated in clinical studies. The negative aspect of the better penetration of BCNU through the epidermal barrier is the potential for the drug to cause bone marrow suppression when utilized to treat extensive skin involvement. Consequently, blood counts need to be monitored every 4 weeks while on treatment.

For total skin applications, Zackheim recommends that 5 mL (10 mg) of BCNU from a 0.2% alcoholic stock solution (100 mg/50 mL) be dissolved in 60 mL of water and applied to the skin surface once daily, excluding the genital skin and other areas such as the head, body folds, palms and soles unless involved with disease.[30,49,50] If the patient tolerates treatment well, but the response is inadequate after 3 months, then the concentration may be increased to 20 mg in 60 mL of water. With more limited disease, the treatment is administered to the affected skin only. This differs somewhat with the author's recommendation for use of topical HN2 in which the entire skin surface is treated to avoid missing unapparent skin lesions. Nevertheless, with Zackheim's approach, the CCR rate of topical BCNU for patch/plaque phase MF is quite similar to that reported for topical HN2 in aqueous solutions (respectively, 66% versus about 70%), and the effect on disease-free survival is also comparable (Table 2).

The adverse effects of topical BCNU include skin irritation, telangiectasia, and allergy in addition to the potential for systemic toxicity from percutaneous absorption. Mild irritation during treatment is common and may persist for several months off treatment. Persistent telangiectasia may develop in patients with more severe irritant reactions, and the author has observed that patients who are treated with local radiotherapy after having been treated with topical BCNU are quite prone to develop permanent telangiectasia. Allergic contact dermatitis to topical BCNU occurs in less than 10% of patients treated with topical BCNU and cross reactions to HN2 do not occur so the drug is useful as an alter-

ative treatment for patients who are allergic to HN2. Although a carcinogen in animal models,[52] patients who have been treated with topical BCNU have not been reported to have an increased risk of skin cancers or internal malignancies. The frequency of bone marrow suppression attributed to topical BCNU (mostly mild leukopenia) with treatment to large areas of the body was about 5%. As with topical HN2, BCNU should be used cautiously or not at all in women planning to become pregnant.

Topical biological response modifiers

Bexarotene

Bexarotene, a drug recently approved by the Food and Drug Administration for oral and topical treatment of CTCL, is classified as a rexinoid, i.e., a ligand that selectively binds and activates retinoid X receptor (RXR) subtypes in the cell nucleus.[53] The RXR-rexinoid complex forms heterodimers with various other nuclear hormone receptors such as tyroxine receptor and vitamin D receptor, and these heterodimers function as transcription factors that regulate expression of genes that control cellular differentiation and proliferation. As with other biological response modifiers, the biological effects are complex and the mechanism of action in MF is not completely understood. Bexarotene appears to affect neoplastic T-cells directly by suppressing proliferation and promoting apoptosis, and this may be the main mechanism of action in MF.[53,54] In addition, the drug interferes with secretion of interleukin-2 and type 2 cytokines produced by activated neoplastic T-cells, which favor tumor cell growth.[53] Indirect effects may also be mediated via the effect of bexarotene on keratinocytes or Langerhans cells that change the local tissue environment.

Bexarotene is marketed in the United States for the topical therapy of MF as a 1% gel. The recommended dosing is to apply the ointment to lesions up to 4 times daily according to skin tolerance, but in practice this seldom exceeds twice daily applications.[55] The results of two company-sponsored studies of bexarotene gel in patients with patch/plaque MF are summarized in Table 3. Based on physician's global response scores, objective responses occurred in 64% of patients studied in the phase I/II study reported by Breneman with a CCR occurring in about one third of these patients.[56] The author's experience is similar to these results. However, in an open label, multicenter, phase III study with the 1% gel, the objective response and CCR rates were considerably less than those reported for the phase I/II study (Table 3; Product monograph, Ligand Pharmaceuticals, Inc.). The reason for this discrepancy may be related to the well-known problem of differentiating the local irritant effect of bexarotene from active disease. In this regard, the author often finds it necessary to discontinue applications of bexarotene gel temporarily and periodically (every few months) to allow the local irritant reactions of bexarotene to subside in order to judge the effectiveness of treatment. The most common adverse effect of bexarotene gel is local irritation at sites of application (erythema, dermatitis, pruritus, pain). This was treatment-limiting in 11 of 58 (19%) patients treated with the 1% gel.[56] Severe reactions were rare, but included leukocytoclastic vasculitis and vesiculobullous reaction. The chemical name of bexarotene is 4-[1-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)ethenyl] benzoic acid, and as such there may exist the potential for allergic cross reactivity with other retinoids and other benzoic acid deriviatives. However, no significant allergic sensitization occurred in the clinical trials. Low blood levels of bexarotene were detected in 21% of post-dose samples, and were associated with the extent of skin being treated. Therefore, percutaneous absorption might conceivably account for the frequency of widespread pruritus (36%) or maculopapular/exfoliative dermatitis (12%), headache (14%), hyperlipidemia (10%), leukopenia (6%), and mildly abnormal liver function tests (4%) observed in the multicenter phase III trial (Product monograph, Ligand Pharmaceuticals, Inc.).

Because of the potential of bexarotene to be absorbed through the skin, the same precautions

Table 2. Topical Carmustine (BCNU) in Patch/Plaque Mycosis Fungoides*.

Series	No. Pts.	OR (%)	CCR (%)
Zackheim, 1990	109	100 (92)	72 (66)
Ia	49	42 (86)	6 (12)
Ib	38	18 (47)	14 (37)
IIa	22	12 (55)	8 (36)

*Abbreviations: OR = objective response rate, i.e., partial plus complete responses; CCR = clinical complete response rate.

Table 3. Studies of bexarotene gel in patch/plaque mycosis fungoides.*

Series	No. Pts.	OR (%)	CCR (%)
Phase I-II‡	66	42 (64)	14 (21)
Ia	41	24 (58)	10 (24)
Ib	20	16 (80)	3 (15)
IIa	5	2 (40)	1 (20)
Phase III†	49	19 (39)	1 (2)

*Abbreviations: OR = objective response rate, i.e., partial plus complete responses; CCR = clinical complete response rate. ‡Study performed at 3 centers, gel concentrations ranged from 0.1% to 1.0%. †Study performed at 25 centers, gel concentration 1.0% (data from product monograph, Ligand Pharmaceuticals, Inc.).

Table 4. High potency topical corticosteroids (clobetasol) in patch/plaque mycosis fungoides.*

Series	*No. Pts.*	*OR (%)*	*CCR (%)*
Zackheim, 1998	79	71 (90)	39 (49)
T1	51	48 (94)	32 (63)
T2	28	23 (82)	7 (25)

*Abbreviations: OR = objective response rate, i.e., partial plus complete responses; CCR = clinical complete response rate.
†All but 4 patients had patch phase MF (3 patients with plaques at T1, 1 patient at T2).

and blood monitoring as those used for oral bexarotene should be followed, especially when large areas of the skin are being treated. The drug is metabolized in the liver through cytochrome P450 3A4 and excreted via the hepatobiliary system with negligible urinary excretion. Therefore, drugs that effect cytochrome 3A4 activity such as gemfibrozil, erythromycin, itraconazole and grapefruit juice may potentially influence the disposition of bexarotene. Also, animal studies indicate that the concomitant application of bexarotene gel and products that contain the insect repellent DEET (N,N-diethy-m-toluamide) may increase the risk of DEET toxicity. Photosensitivity may occur and patients should be advised to minimize exposure to the sun and sun lamps while on treatment. As with retinoids, women of childbearing potential need reliable contraception.

High potency corticosteroids

Similar to retinoids/rexinoids, the biological effects of corticosteroids are mediated through a cytosolic receptor (glucocorticoid receptor) that, upon activation, can regulate transcriptional activation or repression of responsive genes in the nucleus. The subsequent molecular events are complex and only partially understood, but result in the activation of caspases that mediate apoptosis of lymphocytes.[57] In addition, membrane-receptor mediated mechanisms may provide an additional effector pathway that is active at high doses of glucocorticoids.[58]

Topical corticosteroids have been utilized as an adjunctive therapy for MF for many years,[59] and lesions of MF may regress from its use, presumably because neoplastic T-cells are involved to undergo apoptosis. However, no formal clinical studies had been undertaken to evaluate the role of topical steroids as a primary treatment of MF until recently when high potency (class I) corticosteroids became available. A study that utilizes clobetasol 0.05% has been initiated by Zackheim at the University of California, San Francisco, as the first-line treatment for patients with patch phase MF.[50,60] Patients are instructed to apply the cream or ointment vigorously twice daily to lesions only with supplemental use of plastic film occlusion to lesions on the extremities. The minimal interval of treatment to evaluate effectiveness was 2 to 3 months, and patients continued treatment for an additional month after the lesions had cleared.

The objective response rates to intensive topical corticosteroids are presented in Table 4. Of 79 patients treated, 90% improved significantly on treatment, with nearly half achieving a CCR. Histologic clearing was demonstrated on post-treatment biopsy samples from 7 patients with complete responses. The study did not comment on the durability of the complete responses off treatment. However, it is worth noting that disease progression occurred in 2 of 51 (4%) patients with T1 and 3 of 28 (11%) patients with T2 disease during a short follow-up interval ranging from 3 to 36 months (median, 9 months). By comparison, in the Stanford experience with topical HN2 as the initial treatment, 8% and 17% of patients with T1 and T2 disease, respectively, had evidence of disease progression at 5 years.[33] This observation makes the author question whether topical corticosteroids provide only temporary benefit in MF. Long-term studies are needed to address this issue.

The sustained use of high potency topical corticosteroids in MF is surprisingly well tolerated. The most common side effect is purpura and mild irritant dermatitis that occurs in about 20% and 10% of patients, respectively.[50] Striae and atropy are uncommon and reversible. Percutaneous absorption of corticosteroids that depressed the hypothalamic-pituitary-adrenal occurred in 13% of patients, but this was temporary and without clinical consequence.

Other topical immune-modulating drugs

The experience with bexarotene and corticosteroids suggest that other agents that induce neoplastic T-cell apoptosis or alter the epidermal environment might be therapeutically effective. Optimally, the drug should have a high benefit-to-risk index.

Imiquimod and other imidazoquinolone compounds modulate immune responses by activating Langerhans' cells and macrophages via Toll receptor 7 binding to induce release of interferon alfa and pro-inflammatory T_H1 cytokines including interleukin (IL)-12.[61] This results in an increase in NK-cell activity and cell-mediated immune responses. Both interferon-α and IL-12 have been shown to have activity in MF when administered as single agents, and this effect is believed to be mediated by a tumor-specific cytotoxic response.[62] Topical application of imiquimod 5% cream once daily to a test site in a patient with patch phase MF resulted in intense erythema and vesiculation initially but then clinical and histologic clearing of MF after 4 months of treatment.[63] This single report indicates that top-

ical application of immunomodulatory drugs may be another useful treatment in the future. However, the local irritant properties of imiquimod may preclude application to large areas of skin.

The purine nucleoside phosphoylase inhibitor, peldesine (BCX-34), in a 1% cream was tested in a randomized, double-blind, placebo-controlled study that involved 43 patients with patch/plaque MF.[64] The objective response rate was 28% (12 patients with 1 CCR), but interestingly 24% of 46 patients treated with the vehicle cream also responded (11 patients with 1 CCR). The importance of this study is that potential new topical treatments should be evaluated with a placebo control, i.e., the cream vehicle *per se* may result in clinical improvement, perhaps through its moisturizing effect.

Cost issues

What are the costs of these treatments if the physician desires to treat most of the skin surface of the patient daily for 30 days? The author obtained the following quotes from a pharmacy that is equipped to prepare and dispense these drugs (*personal communication, February 2003*). For topical HN2 solution (10 mg in 50 mL of water), the cost of 30 commercially available 10 mg vials of HN2, which is marketed for intravenous administration, is $537. The cost to prepare and dispense a 30-day supply of HN2 in an ointment (2 lb ≈ 450 g) is $410 and $594 for the 10 mg/100 gram and 20 mg/100 gram formulations, respectively. The cost of 150 mL of the carmustine 0.2% alcoholic stock solution that allows administration of 10mg (5 mL) in 60 mL of water for 30 days is $682. The cost of a 30-day supply of clobetasol cream or ointment 0.05% is $288. For comparison, the cost of a similar amount of bexarotene gel 1% is $18,835. Thus, except for topical bexarotene and clobetasol which the author reserves for local use, the cost of treatment of topical HN2 and BCNU is similar in the United States.

Use of topical agents in clinical practice

The author believes that the goal of treatment of MF at all phases of disease evolution should be to attain a CCR when feasible, with the proviso that this may be unnecessary or unrealistic for some patients of advanced age, in poor general health, or with extensive advanced disease requiring highly toxic drug therapies.[65] The reason for this opinion is that patients who achieve a CCR from treatment have a more favorable prognosis than do patients who do not reach this response in any stage of disease.[43] When CCR to treatment is evaluated with stage in a multivariate model, it remains significantly associated with survival.[43,66] In addition, data from several centers suggest that between 30 to 50% of patients with patch/plaque MF in stage Ia and 10 to 15% of patients in stage Ib who are treated with various skin-directed therapies may achieve long-term relapse-free intervals off treatment suggesting that cure is likely.[65] These cures would never be realized unless a CCR is realized. Even when cure is unlikely or cannot be achieved, intermittent or continuous treatment to keep the tumor burden relatively low may theoretically decrease the risk of disease progression or transformation to a more aggressive lymphoma.

Therefore in this context, skin-directed therapies of all types that substantially reduce skin tumor burden and inhibit disease relapses may positively influence the natural history of MF. For clinically early MF (patch or early plaque phase MF, stage I to IIa), the author's preference is to administer treatment to as much of the skin surface as possible in order not to miss clinically occult disease and increase the chance of a true complete response as well as a sustained remission without the need for maintenance therapy, thereby reducing the risk of side effects related to chronic treatments. Of the topical therapies discussed in this review, only topical HN2 (aqueous preferred over ointment) and topical BCNU (aqueous) would fulfill this requirement although the potential for percutaneous absorption by BCNU may necessitate treatment interruptions. The use of bexarotene gel and high potency topical corticosteroids is reserved for local therapy when there is limited skin involvement, and therefore is not used by the author as a primary treatment. Of course, the author recognizes that local therapy may be equally effective to control disease progression, but controlled studies would be need to be conducted over many years to address this point.

For patients with advanced skin involvement (thick plaques, tumors, erythroderma), topical chemotherapy may be used in conjunction with other modalities. For example for tumor phase MF without evidence of transformation to large cell lymphoma, the author often arranges for the patient to receive a course of electron beam to clear the skin of disease, then administers topical chemotherapy to the entire skin surface (HN2 preferred over BCNU) along with a relatively well tolerated drug with systemic effects such a interferon α, methotrexate, oral bexarotene, or extracorporeal photopheresis depending on the clinical situation.[65] Duvic has adopted a similar strategy with interferon-α and topical HN2 used after electron beam or systemic chemotherapy.[67] The goal with advanced MF is to inhibit the recurrence of disease for as long as possible and to keep tumor burden low. In support of this concept, the use of topical HN2 following total skin electron beam was reported to provide a significantly longer freedom-from-relapse interval than electron beam alone for patients with MF at T2; however, no difference in overall survival was found.[68]

Unanswered questions and speculations

The author poses questions regarding the use of topical chemotherapy for MF. Does topical HN2 or BCNU induce apoptosis of neoplastic T-cells *in vivo*? This could be addressed today using the TUNEL assay.[69,70] What is the clinical significance when small numbers of neoplastic cells are identified in the blood, and does effective skin-directed therapy also remove these cells? Could mild, delayed hypersensitivity reactions from topical HN2 recruit circulating neoplastic T cells into the skin where they might be eliminated?[71] Could topical treatment alter the expression of some putative epidermal antigen such as *Chlamydia pneumoniae* that may play a role in the early pathogenesis of MF.[72] Could the fact that topical HN2 promotes lesion regression without inhibiting immune responses provide a therapeutic advantage over other modalities that suppress immune responses, e.g., phototherapy or photochemotherapy (PUVA)? These speculations might be worth investigating in the future.

Conclusions

Early MF can be effectively controlled, and sometimes cured with topical chemotherapy using HN2 or BCNU. The ideal topical chemotherapy should be well tolerated by skin with minimal short (contact dermatitis) or long-term side effects (carcinogenesis) on the skin, have minimal systemic side effects from percutaneous absorption when applied to a large area of skin, be highly effective in clearing skin lesions, be applicable to the to entire skin surface if desired, and penetrate the skin sufficiently to clear intradermal involvement. At present topical HN2 is closest to this ideal although cutaneous irritation and allergy and failure to clear deep seated disease remain as major problems. However, the search continues for new agents, and immunotherapeutic approaches seem promising.

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Photopheresis in the treatment of cutaneous T-cell lymphoma

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The initial positive experience with extracorporeal photopheresis (ECP) goes back to 1985. Since then, this therapeutic modality has become an important addition to our possibilities for treating cutaneous T-cell lymphoma (CTCL), the Sézary-syndrome variant in particular.

The results of the first multicenter (USA and Europe) clinical trial were published by R.L. Edelson and co-workers in 1987.[1] Subsequently other centers proceeded to explore the use of ECP both as monotherapy and in combination with more standard proven treatment modalities. Approval was obtained from the USA Food and Drug Administration (FDA) in 1988 to use this strategy for the palliative treatment of CTCL.

As of 2003, ECP is being administered in over 160 centers in the United States, Europe, Latin America, South Korea and the Near East for an expanded spectrum of T-cell mediated diseases.[2,3]

As is well known from psoralen and ultraviolet-A (PUVA) light therapy in dermatology, ECP involves the extracorporeal exposure of peripheral blood mononuclear cells (PBMC) to photoactivated 8-methoxypsoralen (8-MOP) followed by the return of these cells to the patient. As in normal PUVA the drug 8-MOP was administered orally in a dose previously determined to produce a minimum of 60 µg/mL (suggested range: 60-200 ng/mL) of drug in the plasma approximately 1.5 hr after ingestion. Presently, following approval by the FDA in 1999 and the European Community regulatory agency, the drug is added directly into the collected buffy-coat/plasma fraction prior to radiation and then returned to the patient. As previously described and in order to completely circumvent problems in obtaining reproducible psoralen levels in the to be irradiated extracorporeal blood fraction the newer generation of instruments use exclusively extracorporeal administrable 8-MOP, a modification introduced by Knobler *et al.*[4] In this manner the known side effects of oral 8-MOP administration as well as need for pre-medication and drug level monitoring are eliminated. In general the treatment is repeated on 2 successive days at 2 to 4-week intervals for a minimum period of 6 to 12 months prior to the decision whether to continue or not. It is estimated that during one treatment approximately 5 % to 10% of the total circulating T-cell pool is treated in this fashion; the total UVA dose delivered to the individual cell has been determined to be approximately 2 J/cm^2.[5]

Since the first clinical application of ECP in the treatment of CTCL, its efficacy and low side-effect profile has been confirmed in numerous studies for this as well as for other indications.[3,6-13]

The data presented in the original multicenter study performed by Edelson and co-workers[1] have been subsequently confirmed, such that ECP is considered by leading authorities as the first-line treatment for the erythrodermic stage disease of CTCL[7-13] either alone or in combination with other well established treatments. Many experimental studies have evaluated the effect of ECP on the immune system.[14-18] Under the assumption that ECP can suppress pathophysiologically relevant T-cell clones and relevant associated peptides, pilot trials have been performed in order to evaluate the efficacy of ECP in inflammatory diseases other than CTCL in which auto-reactive T-cells play a major role. Data collected over the last years seem to confirm that ECP may have a major impact on autoimmune diseases, in the treatment of acute and chronic rejection in organ transplantation, as well as in graft-versus-host disease (GvHD) after allogeneic bone marrow transplantation.[2] Ongoing prospective randomized multicenter trials are expected to be completed within the next two years and should help to pinpoint the precise further use of ECP outside of dermato-oncology.

It is expected that further developments in understanding the mechanisms involved in ECP should lead to greater efficacy and lower treatment costs. Shorter treatment times and improved drug delivery systems are also issues for even lower side-effects.[4] New research advances concentrating on identifying the molecular biological effects of ECP are hoped to contribute to a better understanding of the pathophysiology of the target diseases to be treated and improve the treatment strategies.[18,22]

Since CTCL was the first disease for which ECP was evaluated it is the indication for which the longest periods of observation are available. In the first study by Edelson and co-workers, published in 1987,[1] 27 of 37 patients responded with either a partial or a complete remission. In a follow-up study in which patients treated with ECP were compared to historical controls, ECP also appeared to increase duration of survival.[10] Even in the context of controversy and questioned statistical validity associated with comparing results with those of

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historical controls, the survival prolungation from 33 to 66 months appears to be important. Other publications seem to confirm the initial positive reports.[8,13,23,24] These studies in CTCL describe a response rate that can reach 75%, with possible complete remissions of up to 25%, and no response in 25% of treated patients. Based on clinical, immunological and laboratory data attempts have been made to better characterize those patients who are more likely to respond to ECP. As will be discussed later, and also summarized in Table 2, a normal CD4/CD8 ratio, a normal absolute count of CD8 positive cells in the peripheral blood and short disease duration are associated with chances of good therapeutic response.[12,13] An important and clinically relevant observation which has repeatedly been confirmed by all studies is the very low side-effect profile attributed to ECP.[25]

To improve the efficacy of ECP in patients with Sézary syndrome, clinical studies have been performed to evaluate synergistic effects with other treatments such as interferon-α, methotrexate, total skin electron beam therapy, PUVA, retinoids including bexarotene, and others.[26-28]

Extracorporeal photochemotherapy as monotherapy

ECP was first tried as a monotherapy in the palliative treatment of therapy-resistant CTCL Sézary syndrome.[1] A subsequent report suggested that ECP may improve quality of life as well as extend survival.[10] As previously reported and published, groups in both the USA and Europe have reported on over 450 patients (see Table 1).[29] The overall response rate reported by the groups from the USA on the use of ECP as monotherapy is 56% in 282 patients studied; the comparable overall response rate is 69% in 168 patients reported by the European groups.

In 1992, Heald and co-workers reported that the median survival of the patients originally treated by Edelson and co-workers is 60 months, 33 months from the date of diagnosis and 47.9 from the date of initiation of ECP.[10] In four of the six patients who went into complete remission this state has been maintained. In this report the best responders were found among patients with a lower CD4/CD8 ratio in the peripheral blood when ECP was initiated.[2] Criticism of this conclusion has often been voiced.[30,31] Gottlieb *et al.*[26] reported on a large, collective, 10-year retrospective study of 41 patients. They summarized their results on the value of ECP used as monotherapy or in synergism with interferon-α (12 patients) and other local or systemic medications. In their group of 41 patients, 31 underwent six or more cycles of ECP; 28 patients had ECP as monotherapy. Seventy-one percent of these patients were described as responders to ECP; seven patients (25%) had a partial remission as defined by more than 50% clearing of skin disease. Presence of Sézary cells in the peripheral blood was associated with an improved response, an observation later confirmed by others.[2] Gottlieb *et al.* also suggested that ECP may be associated with prolonged survival; in their study the median time to treatment failure was 18 months, the median survival from initiation of therapy was 77 months and that from the time of diagnosis was 100 months. These are longer than the times reported in previous studies of similar patient cohorts in which ECP combination therapies produced survival rates of 30 to 40 months.[2] In a similarly retrospective review Duvic *et al.*[24] reported on

Table 1. The largest series of patients treated with ECP in CTCL in North America and Europe.

Author (Ref.)	*Year*	*Pts#*	*OS§*	*PR§*	*CR§*
Edelson[1]	1987	37	27 (73%)	18	9 (24%)
Heald[9]	1989	32	17 (53%)	12	5 (14%)
Dall'Amico[55]	1991	37	27 /(73%)	n.d.	9 (24%)
Koh[46]	1994	34	18 (53%)	13	5 (15%)
Prinz[53]	1995	17	12 (71%)	6	0
Zic[25]	1996	20	10 (50%)	5	5 (25%)
Gottlieb[26]	1996	31	20 (65%)	13	7 (23%)
Duvic[24]	1996	34	17 (50%)	11	6 (18%)
Owsianowski[56]	1996	16	11 (69%)	7	4 (25%)
Konstantinow[58]	1997	12	8 (67%)	5	1 (8%)
Russell-Jones[32]	1997	19	10 (53%)	7	3 (16%)
Vonderheid[45]	1998	32	10 (31%)	6	4 (13%)
Zouboulis[57]	1998	20	13 (65%)	n.d.	n.d.
Jiang[51]	1999	25	20 (80%)	15	5 (20%)
Crovetti[54]	2000	30	22 (73%)	12	10 (33%)
Wollina[52]	2001	15	10 (67%)	3	5 (33%)
Knobler[59]	2002	20	10(50%)	7	3(15%)
Stevens[60]	2002	17	9(53%)	8	9(53%)
Totals		485	291 (60%)	153 (32%)	83 (17%)

*Reference: modified from #29. OS. overall survival; PR: partial response; CR: complete response. #Only patients who underwent 6 or more cycles of photopheresis or who were treated with ECP for at least 6 months are included. §defined heterogeneously by different authors.

Table 2. Characteristics of CTCL patients most likely to respond to ECP.

1. Disease duration less than 2 years.
2. No bulky adenopathy or major organ involvement.
3. White blood count less than 20,000/mm^3
4. Presence of a discrete number of Sézary cells (10% -20% of mononuclear cells).
5. Natural killer cell activity close to normal.
6. Cytotoxic T-lymphocytes close to normal ($CD8^+ > 15\%$)
7. Absence of prior intensive chemotherapy.
8. Plaque stage disease should not cover more than 10% to 15% of total skin surface.

34 patients who had an overall response rate of 50%; six patients (18%) achieved a CR and 11 (32%) were obtained a PR. Twenty-eight of these 34 patients had erythrodermic CTCL and a treatment schedule of at least two consecutive treatments at monthly intervals appeared optimal. Duvic *et al.* tried to improve on the original schedule reported by Edelson by increasing the number of collection cycles/treatment from six to nine and also by using an alternate anticoagulant – acid citrate dextrose-A instead of heparin. Non-responders were treated at intervals of two weeks. with no obvious advantage. A significant decrease in the absolute number of $CD4^+$ peripheral blood cells suggests that this may correlate with a drop in circulating malignant cells carrying this marker.[2]

A significantly increased survival duration was reported by Heald and co-workers in 1992.[10] In contrast, in 1997 a European group led by Russell Jones and co-workers reported on their experience with ECP as monotherapy in 19 patients[32] with Sézary syndrome. Their results showed minor responses (>25% improvement in skin score) in 10 patients (53%). Of the responding group, 16% had a complete response which was defined as greater than 90% improvement in their skin score. In this group only patients in whom a T-cell clone had been previously detected in the peripheral blood were treated. As pointed out in the original study by Edelson only 11 of the 37 patients included had been proven to have clonal disease. Unfortunately all the studies which included series of patients treated with ECP as monotherapy or in combination are easy targets for criticism: none was qualified as a truly prospective, randomized study using established and standardized elements of classification and diagnosis. Agreement among experts on these issues is still difficult and recent new classification attempts (such as that of the European Organization for Research and Treatment of Cancer – EORTC – Cutaneous Lymphoma Project Group)[33] ave yet to be universally accepted for organizing the appropriate clinical trials.

Extracorporeal photochemotherapy in combination with other treatment modalities

Unsatisfactory responses in patients with tumor stage disease (plaque, patch, tumor) as well as refractory Sézary patients formed the motivation behind combination therapies, although again appropriate protocols were not followed. The first combination to be reported on and the most widely used is interferon-α-2b (IFNα). Addition of a biological response modifier to ECP in refractory patients may be highly beneficial.[22,26,27,35-38] As early as 1991, Rook and co-workers reported that the combination of ECP and low dose IFNα-2b had a possible synergistic effect that could lead to complete remission with disappearance of the malignant T-cell from the peripheral blood, as documented by Southern blot analysis.[22]

The study by Dippel and co-workers gave support to possible synergistic effects between ECP and IFNα-2b.[39] In this study 19 patients with advanced disease were treated with ECP and interferon-α 2b. Their photopheresis treatment protocol set out treatment for two consecutive days every 2 to 4 weeks for an average of 16 months. This was an open, two-arm trial comparing ECP alone (10 patients) to a combination of drugs (9 patients). The dose range of IFNα-2b was 3-18 million units three times weekly. These researchers identified one complete remission in the ECP arm, one minor response, and eight patients with stable disease, the reduction in their own designed score (CTCL-SI) being 1.4%. In contrast, the combined therapy produced four complete remissions, two partial responses and two stable disease with a median reduction in the CTCL-SI score of 65.7%. This study has often been criticized for not including a third arm of subjects treated with interferon-α2b alone.[40] Zackheim points out other studies that document that IFNα-2b maybe be at least as good if not superior on its own.[36,40] Future studies are needed to provide clarity on this issue.

Similar reports of success, coming from refractory patients treated with a third agent such as interleukin 2[40,41] or interleukin 12 plus GMCSF,[2,42,43] need to be confirmed. Gottlieb *et al.*,[26] in their 10-year review series on 41 patients of whom 31 had at least 6 cycles of ECP, described 9 patients who had an enhanced response when IFNα-2b was added.

The most extensive series of patients documenting a synergistic therapeutic effect with increased long term survival are those in which total skin electron beam (TSEB) therapy has been used.[44-47] Wilson *et al.*[44] initially evaluated 163 patients with CTCL who received a complete dose of TSEB therapy (36 Gy at 1 Gy/day for 9 weeks and 6 MeV electrons) with curative intent. Patients with a clinical complete response or good partial responses to TSEB therapy were subsequently randomized to be treated with either adjuvant doxorubicin/cyclophosphamide chemotherapy or ECP. The authors retrospectively showed that at 3 years those patients who had been in a more advanced stage of disease, namely T3 and T4, experienced an improved overall survival. The 3-year survival rate in the group treated with combination chemotherapy was 75%, that in the ECP group 100% and that in patients who received neither treatment was 50%. Analysis of the overall survival curves showed that the increase in survival in the group treated with ECP approached statistical significance ($p < 0.06$), while a true sur-

vival benefit from the addition of chemotherapy was not observed. A report from the same group on 44 patients with erythrodermic (T4) mycosis fungoides who received ECP concurrently to TSEBT or TSEB only[47] was also positive. In this retrospective, non-randomized study all patients received TSEB obtaining a CR of 73% within 2 months of completion. Thirty-two patients with CR had a disease-free survival (DFS) of 63%. Within this group the DFS was 49% for the 17 patients who had not received concomitant ECP whereas the DFS was 81% among the 15 patients who had received TSEB + ECP. These data motivated the authors to conclude that the concurrent use of ECP with TSEB warrants further evaluation.

Photopheresis is an immuno-modulating therapy that requires a functional immune system with the potential to mount an immune response; it is therefore suggestive that it is applied in earlier stages of CTCL. Certainly, the very low side-effect profile of ECP makes its use as first line therapy inviting. Addition of biological response modifiers, such as interferon-α, as adjunctive therapy is recommended within the framework presented.[2] Based on available literature and studies, recommendations for selecting patients, using the characteristics shown in Table 2, can be followed.[29]

Characteristics of CTCL patients likely to respond to ECP

The clinical information that best describes responders to ECP, particularly as monotherapy, and which is agreed upon by a number of investigators[2,10,45,46] is presented in Table 2. These criteria can be used as guidelines in identifying best responders but are not mandatory; impressive responses in patients have been described who did not fit this scheme.[48] Still, these criteria do imply that the prospective patient must have an immune system capable of responding to immunomodulating mechanisms initiated by ECP. New reports confirm this hypothesis.[49,50] The notion that photopheresis can work as the first step in establishing a patient-specific tumor vaccine, without prior requirement of identifying the distinctive tumor antigen, is inviting; on-going research should identify approaches that will be successful in clinical medicine.

Summary

Extracorporeal photochemotherapy was conceived as a modified treatment for cutaneous T-cell lymphoma (CTCL) and other T-cell mediated diseases. The data presented since 1987 have clearly proven that this treatment approach can benefit some patients with CTCL, although it still remains controversial. Correctly designed, multicenter trials are currently being carried out so that a number of important issues will soon be clarified. In spite of the controversy surrounding it, ECP is an innovative approach for the treatment of T-cell mediated diseases and has certainly opened new perspectives of therapy in photoimmunology. The indication of choice is the erythrodermic variant of Sézary syndrome. When the mechanisms of action have been unraveled and correct trials evaluating various treatment schedules and different combinations have been completed, the proper value of photopheresis in the treatment CTCL will be established.

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Radiotherapy of primary cutaneous lymphomas

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Lymphoid tissue is among the most radiosensitive tissues and, as in the nodal form, radiotherapy plays a major role in the treatment of cutaneous lymphomas. Scholtz[1] was the first to employ ionizing radiation in the treatment of tumor lesions in mycosis fungoides (MF) at the beginning of the 20th century. Others then proposed roentgen irradiation to treat large areas of the skin affected by MF,[2] and described a technique of *roentgen irradiation at a distance* with berillium window tubes producing soft radiation.[3] The introduction of linear accelerators producing high energy electrons created the basis for modern radiation treatment of MF.

In the late 1970s the recognition of clinical entities due to neoplastic lymphoid proliferation in the skin that differed from MF gradually led to the distinction between B- and T-cell cutaneous lymphomas and finally to the recent *European Organization for Research and Treatment of Cancer* (EORTC) classification of primary cutaneous lymphomas (PCL),[4-6] which makes it easier to define the role of radiotherapy in their treatment. Treatment with radiation is often indicated as first line management for the forms with an indolent clinical behavior (Table 1).

Mycosis Fungoides

When the disease is limited to the skin, the treatment should be directed to the skin alone; therefore radiotherapy is widely employed in different stages of the disease[7] (Table 2), administered to localized fields or as total skin irradiation. In the range of stage IA the entity of *MF minimal disease*, defined as the picture presenting with a single lesion or no more than 2-3 lesions physically close to one another, is suitable for localized radiotherapy, and this appears to have a curative action[8-10] at total doses ranging from 16.25Gy[9] to 40 Gy,[8] administered with orthovoltage machines (55-280 kV) or with electron beam limited fields (6MeV). The overall ten-year survival has been 100% in all series (five-year survival was 100% in our series[9]) and relapse-free rates at ten years have been about 85% in the most representative series.

In total skin electron beam radiation (TSEB), ionizing radiation is administered to the entire skin surface. Developed in the sixties and implemented in the following years, this technique has recently been the object of a consensus report between the EORTC Cutaneous Lymphoma Group and experts from radiotherapy centers in North America.[11] The main indications remain stages IA-IB and IIA of newly diagnosed MF, in which a rate of cutaneous remission of about 95% is achieved. The 10-year progression-free survival (PFS) in stage IA patients is 50% and that of stage IB patients, 20%. Therefore in half of the patients diagnosed in stage IA, TSEB may be curative, while in stage IB it is a possible good primary therapy, but an adjuvant therapy may be considered. In the other stages of the disease TSEB has a palliative action and may be part of a combined treatment. Many problems have had to be resolved in order to use the technique of TSEB: it is necessary to reach the primary target volume, comprising epidermis, adnexal structures and the dermis, with a homogeneous distribution of the dose, and to reduce the photon contamination to bone marrow to less than 0.7 Gy. The technique of multiple beams (generally six with the dual field method) permits a shorter treatment time, so that the patient, who is standing, is able to maintain this position more correctly (with angled limbs and devices to unfold the skin). The dose prescription is 26 Gy at a depth of 4 mm (= 31-36 Gy at the surface), administered with an effective electron energy of about 4 to 5.5 MeV. The recommended fractionation is 30 to 36 fractions over 6 to 10 weeks, with small fractions of about 1.2 Gy per day, to reduce adverse effects, without compromising the effectiveness of the treatment. At the prescribed doses and fractions, the adverse effects, such as erythema, desquamation, depilation, nail stasis and impaired sweat gland function are temporary. Male infertility may occurr. The eyes must always be shielded and shielding may also be necessary for some fractions administered to regions where there may be overdosage because of overlapping fields (nose, ears, hands). Some areas of the skin that remain unexposed or shielded may require a regional patch treatment (soles, folds, head) to obtain the clinical objective of remission with lasting control of the disease. The dose prescribed is 26-28 Gy with localized electron beam fields, equal to that administered to the whole skin surface. Boost treatments, using direct electron fields (higher energy electrons up to 16 MeV with a dose not exceeding 20 Gy in 10 to 15 fractions) or orthovoltage irradiation (60 to 120 kV, 20 Gy in 6 or 10 fractions), may be decided for tumor or ulcerative lesions before beginning TSEB.

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Table 1. Indolent forms of primary cutaneous lymphoma.[4]

Cutaneous T-cell lymphomas (CTCL)
Mycosis fungoides
CTCL large cell CD30+
Lymphomatoid papulosis
Cutaneous B-cell lymphomas (CBCL)
Primary cutaneous follicle center cell lymphoma
Primary cutaneous immunocytoma/marginal zone B-cell lymphoma

Table 2. Clinical staging of MF according to skin involvement: stages of interest for radiotherapy.

Stage	*Skin involvement*
IA	Patches and plaques <10% skin surface
IB	Patches and plaques >10% skin surface
IIA	Patches and plaques (Lymphadenopathy)
IIB	Tumor ≥1
III	Erythroderma

TSEB must be practised in highly specialized centers endowed with specific experience.

Primary cutaneous CD30+ lymphoproliferative disorders

These disorders are the second most common group of CTCL, accounting for about 25% of all CTCL,[6,12] and include two conditions that represent a spectrum of the disease: primary cutaneous CD30+ large T-cell lymphoma (PCDC30+ LTCL) and lymphomatoid papulosis (LP). Both conditions have a favorable prognosis. PCCD30+ LTCL presents with one, solitary nodules or a few localized nodules, sometimes with spontaneous regression, but with a tendency to relapse in the skin. It rarely involves extracutaneous sites. Aggressive treatments are considered *over-treatments*, and when self-healing does not occur, radiotherapy is considered one of the best therapeutic choices.[13] In the course of LP, generally characterized by waxing and waning papular lesions, larger skin tumors may develop: when spontaneous resolution does not occur radiotherapy may be employed as an alternative to surgical excision.

Radiotherapy should be administered as orthovoltage or local electron beam irradiation at low doses (median dose 20 Gy in our series).[14]

Primary cutaneous B-cell lymphoma

The classification of cutaneous B-cell lymphomas according to EORTC system[4] has been the cause of some controversies, in particular because of the distinction of a clinical entity according to its location (primary cutaneous B-cell lymphoma of the leg). This topic does not, however, prevent the identification of forms of CBCL with *indolent clinical behavior*, for which radiotherapy is considered the first choice of treatment.[5,6,15,16]

Primary cutaneous immunocytoma/marginal zone B-cell lymphoma is characterized by the onset of solitary or multiple cutaneous tumors localized on extremities. The disease has a favorable course with an excellent prognosis: localized radiotherapy is recommended.[5,6]

Primary cutaneous follicle center cell lymphoma (PCFCCL) typically presents with nodular or large plaque skin lesions localized on the head or trunk, sometimes with multifocal skin manifestations. The dissemination to extracutaneous locations is rare, and the disease has a very good prognosis. Radiotherapy is considered the treatment of choice both in the case of localized lesions[17-20] and in some cases of multifocal skin involvement.[6,21] Techniques of radiologic treatment vary from localized orthovoltage radiotherapy,[17-20] electron beam localized fields,[17] to extended field irradiation with electrons,[18,21] with total doses ranging from a median of 20 Gy[19,20] to 40 Gy.[17,18] The response to the treatment is close to 100%, but skin relapses are not rare, so that the reported five-year relapse-free rates varied from 22.82%[20] to 75%[18] in the different series. Skin relapses can be managed with a new course of radiotherapy when of limited extent, but multiple disseminated skin lesions and an extracutaneous spread obviously require a chemotherapeutic approach. The higher relapse rate observed in patients with PCFCCL of the leg after standard radiotherapy treatment has been one of the reasons that has led this expression of lymphoma to be considered as a separate entity:[5,15] in these cases radiotherapy may be preferred only when a single tumor occurs.

The indications for radiotherapy reported here are those for which this treatment has a prominent role, but there are secondary reasons, no less important for the patients, for using radiological techniques, such as the reduction or care of tumoral or ulcerative lesions in large cell CTCL CD30– and stage IIB MF[6-8] or the reduction of erythroderma in stage III MF without blood involvement, with significant improvement of progression-free survival when TSEB is associated with extracorporeal photopheresis.[22] Finally, radiotherapy to lymph nodes involved by progressing disease should not to be forgotten.

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Interferons

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Interferons (IFNs) are a family of naturally occurring cellular proteins inducible by various foreign antigenic stimuli, such as viruses or tumor cells. IFNs are divided in two types: type I and type II.

Type I interferons comprise two serologically distinct groups of proteins. The first group, collectivelly called IFN α, is a family of about 20 structurally related polypeptides of approximately 18 kD, each encoded by a separate gene. The major cell source for the production of IFN-α is the mononuclear phagocyte, so IFN-α is sometimes called leukocyte interferon. The second serologic group of type I IFN consists of a single gene product, a 20 kD glycoprotein called IFN-β. The usual cell source for isolation of IFN-β is the cultured fibroblast, and IFN-β is sometimes called fibroblast interferon. The most potent natural signal that elicits type I IFN synthesis is viral infection. Both IFN-α and IFN-β are also secreted during immune responses to antigens. In this case, antigen-activated T-cells stimulate mononuclear phagocytes to synthesize type I IFNs.

IFN-α and IFN-β show little structural similarity to each other. Nevertheless, all type I IFN molecules bind to the same cell surface receptor and appear to induce a similar series of cellular responses. Type I IFN acts on target cells primarily by activating new gene transcription. The intracellular signaling pathway used by type I IFN is called the Janus kinase/signal transducer and activator of transcription (Jak/STAT) pathway.

Type I IFN inhibits viral replication. IFN causes cells to synthesize a number of enzymes, such as 2'-5' oligoadenylate synthetase, which collectively interfere with the replication of viral RNA or DNA, inducing in the cell that has responded to IFN, an antiviral state.

Type I IFN increases the lytic potential of NK cellsand also modulates MHC molecule expression. In general, type I IFN increases the expression of class I MHC molecules and inhibits the class II MHC molecule expression. It is widely known that most cytolytic T-lymphocytes (CTLs) recognize foreign antigens bound to class I MHC molecules. Type I IFN therefore boosts the effector phase of cell-mediated immune responses by enhancing the efficiency of CTL-mediated killing. At the same time, type I IFN may inhibit the recognition phase of immune responses by preventing the activation of class II MHC-restricted helper T-lymphocytes.

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Type I IFN inhibits cell proliferation and has a pro-apoptotic effect on cells. This may be due to the induction of the same enzymes that inhibit viral replication but may also involve other enzymes that prevent amino acid synthesis, especially essential amino acids such as tryptophan. It exerts a cytostatic activity by prolonging the tumor cell multiplication cycle and by modulating oncogenes.

Type II interferon-γ, also called immune interferon, is a homodimeric glycoprotein containing two 21 to 24 kD subunits. Each subunit contains an identical 18 kD polypeptide encoded by the same gene. IFN-γ is produced by activated $CD4^+$ and $CD8^+$ T-cells and by NK cells. IFN-γ has several properties related to immunoregulation that separate it functionally from type I IFN.

IFN-γ is a potent activator of mononuclear phagocytes. It is the principal macrophage-activating factor (MAF) and provides the means by which T-cells activate macrophages. It also increases class I MHC molecule expression and, in contrast to type I IFN, stimulates the expression of class II MHC molecules in a wide variety of cell types. Furthermore, IFN-γ acts on T lymphocytes to promote their differentiation, and stimulates the cytolytic activity of NK cells to a greater degree than type I IFN does.

IFNs induce immunomodulation that may be beneficial to patients with CTCL.

The patients with CTCL exhibit depressed cell-mediated immunity: their malignant lymphoid T-cells are deficient in interleukin (IL)-2 and IFN-γ production, and show an increased production of IL-4 and IL-5. This cytokine production pattern is consistent with that produced by Th2 T lymphocytes. Th2 cells are naturally involved in allergic conditions and parasitic disease and are known to suppress normal Th1 cytokine secretion and cell-mediated immunity. It is likely that these cytokine abnormalities play a crucial role in the observed immune alterations that occur with the progression of CTCL.

IFN-α alters the T-cell phenotype from a Th2 cytokine profile towards a Th1 profile. In some patients, together with the IFN-α–induced clinical remission and the disappearance of the malignant clone, the cytokine secretion pattern shifts to increased IFN-γ production and decreased IL-4 production.

Type I IFNs are important in the therapy of many diseases, including viral hepatitis, leukemia, Kaposi's sarcoma, melanoma and renal adenocarcinoma, as well as being the first immunomodulators to be used in the treatment of cutaneous T-cell lymphoma.

The interferons are detectable in blood and tissues for only a few hours after therapeutic injection, but the antiviral or antitumoral state they induce persists for 24-48 hours after they have been completely cleared from the plasma. Their plasma half-life is a few minutes but is prolonged by renal failure. In a few hours IFN α reaches its peak plasma concentration and is completely cleared within 24 hours. It diffuses very slowly in the central nervous system.

Recombinant IFN-α2a has been the most widely used IFN in the treatment of CTCL since 1984. The two commercially available forms, Roferon A and Intron A (the subtypes 2a and 2b), do not have different activities.

During more than 18 years, IFN-α has proven to be a clinically effective antiviral and antineoplastic therapeutic drug. During this time, evidence from *in vitro* laboratory studies and the clinical arena has supported the concept that IFN-α is an immunotherapeutic drug. By regulating a diverse set of cytokines and their receptors, IFN-α is uniquely positioned to prime the host immune response and provide an effective antineoplastic (and antiviral) immune response. IFN-α stimulates the innate cell-mediated response and then participates in the transition of the initial host innate response into an effective adaptative immune response. IFN-α also drives the adaptative cell-mediated $CD8^+$ T-cell response and helps to maintain a $CD4^+$ Th1 cell population balance for an effective antineoplastic and antiviral defense of the host.

Most data on the treatment of CTCL, particularly mycosis fungoides, have come from the use of recombinant IFN-α2a. The first trials used very high doses. The dosage was then reduced to at least half of the initial schedule because of the high grade side effects in about 90% of patients. In subsequent studies, doses as low as 3 MU per day or three times per week have been tolerated better, and have been shown to be as effective as the higher doses.

The combined data show an overall response rate of 57% with a complete response rate of 17%. The median duration of response, evaluable in 7/13 trials, is 5 months; the range is from 4 to 41.8 months (Table 1).

The maximally tolerated daily dose could be 18 MU. The therapeutic effect of IFN-α is visible at all stages of MF, but response depends upon the disease stage. IFN-α is more effective when the disease is not widely spread, producing higher rates of remission in stage I patients. The duration of the disease may also be a predictor of response. Disappointing results have been reported in patients who have had the disease longer and are heavily pretreated. The therapeutic effect of IFN-α is not dose-dependent.

Our experience is based on the available data of 289 CTCL patients (stage I-IV) treated in the *Istituto Dermopatico dell'Immacolata IDI-IRCCS* since 1986. Of these patients, 140 underwent IFN-α2a therapy, 86 as a monotherapy, the others as a treatment associated with PUVA, retinoids, extracorporeal photochemotherapy, chemotherapy, radiotherapy or systemic steroids. One hundred and three patients were evaluable at January 2001.

Table 1. Clinical trials: IFN α2 in cutaneous T-cells lymphoma.

	Patients	*Initial dose in MU*	*PR*	*CR*	*Median duration of response (months)*
Bunn	20	50	6	3	6
Lang	6	3-18	1	0	NS
Tura	15	3-18	8	3	NS
Vonderheid	5	5	0	0	NS
Thes. Peder	5	3-36	3	0	4
Estrach	9	5-10	3	0	NS
Nicolas	6	6	3	1	NS
Dreno	5	3-6	2	2	NS
Vegna	23	3-18	9	8	41,8
Kohn	24	50	6	1	8
Papa	23	3-18	9	8	14
Olsen	26	3-36	10	3	4-28
Simoni	12	3-18	6	5	5

Our data confirm the results published to date.

Although the optimal dose and frequency of administration are not defined, we start IFN-α at low dosages of 1 MU subcutaneously 3 times weekly in an effort to minimize the initial induction of side effects. Over several weeks, the dosage is slowly increased from 3 to 6 MU and to 4 to 6 times weekly. Usually the effect of IFN-α becomes evident after 3-5 months of treatment, but it may appear later. As there does appear to be a dose-response phenomenon, more frequent administration and higher dose regimens may be used before abandoning therapy. In cases of response neither the duration of the treatment nor the patients' need for maintenance therapy is known. Maintenance therapy is recommended for patients with continuing partial responses. It has been reported that relapses after complete response (CR) are not dependent on the continuation of IFN-α therapy. Many of our patients with advanced-stage disease who have achieved a complete response or an ongoing significant response have been maintained on IFN-α for years. Most of the relapses are seen in the first 18 months.

Intralesional injections of IFN-α, in addition to systemic administration, may be used successfully to clear MF plaques and reduce tumors, in addition to systemic administration.

The adverse effects of IFN are well characterized and include initial flulike symptoms consisting of fever, nausea, vomiting, diarrhea, chills, myalgias, headache and malaise. These symptoms appear dur-

ing the first days of therapy. Fever and flu-like symptoms may be controlled by paracetamol and usually disappear after two or three weeks of therapy. Some symptoms may continue during treatment occurring after each subcutaneous injection. Administering the dose of IFN with paracetamol before going to bed minimizes the flu-like symptoms.

IFN-α may also cause thyroid disfunction, more frequently hypothyroidism, in 6% of treated patients, especially in women with thyroid autoantibodies present at baseline.

Telogen effluvium alopecia has been reported, but we have never seen this. Mental changes, such as drowsiness, confusion or depression, also occur. Psoriasis worsens in patients with this condition and we have experience of IFN-α exacerbating unrecognized psoriasis.

At high doses the side effects include fatigue, anorexia and, in 2% of patients, the deleterious cardiovascular effects of orthostatic hypotension and ventricular arrhythmias. The high-dose side-effects are related to the dosage and patient age and are reversible. Dilated cardiomyopathy and myocardial infarction have been reported, but never seen in our patients.

In our experience the principal causes for treatment suspension are cardiovascular (3%) and psychiatric symptoms (2%).

The most frequent laboratory changes are leukopenia with relative neutropenia, thrombocytopenia and increasing hepatic enzyme values, but these disturbances are usually reversible.

The individual response to IFN-α therapy is unpredictable and depends on essentially unknown factors.

One of the causes for the failure of treatment is the presence of neutralizing anti-IFN antibodies, which may induce resistance to the drug.

The study by Stadler, the only randomized, controlled clinical trial, compared the use of IFN-α2a plus acitrein versus IFN-α2a plus PUVA. In this study high titers of neutralizing anti-IFN antibodies were observed, while in previous reports it was suggested that the immunosuppressive effects of the PUVA therapy might prevent the development of anti-IFN antibodies. Furthemore the data of this trial show that the presence of neutralizing anti-IFN antibodies has no clinical significance on the treatment results in CTCL patients.

Other studies show that antibodies tend to develop less frequently in patients treated with IFN-α 2b subtype rather than with IFN-α2a subtype.

In the cases of anti-IFN positivity associated with a low response, suggested options are: prolonging therapy, escalating the dose of IFN-α, changing the IFN-α subtype, using the purified natural lymphoblastoid form of interferon, or combining IFN with short cycles of intermediate doses of prednisone. In our experience the best results are obtained by changing the IFN and associating it with prednisone.

It is also possible that IFN-α resistance could be caused by mutations in the CTCL cell line.

In a paper recently published in the American Journal of Pathology, Tracey and his Spanish group used cDNA microarrays analysis, a powerful technique that allows the analysis of the expression of thousands of genes simultaneously, to show that resistance to IFN-α is associated with significant changes in the expression of a total of 39 genes involved in signal transduction, apoptosis, transcription regulation, and cell growth. Six genes are expressed at a higher level by the IFN-α resistant lymphoma cells, whereas the expression of a set of 33 genes is down-regulated in the resistant cell line. The most upregulated is *MAL*, a gene which codes for proteins involved in a variety of functions, including membrane transport and signal transduction. *MAL* was found to be overexpressed by tumor cells in a series of cutaneous T-cell lymphoma patients treated with IFN-α. *MAL* expression was associated with a longer response time. Its overexpression may be a negative predictive factor for the treatment outcome of IFN-α. In contrast with previous works Tracey did not find a significant difference in the levels of STAT 1 and STAT 3 expression between cell lines from responders and resistant patients. It seems plausible that a large number of genes needs to be taken in account to explain the clinical phenomenon of IFNα resistance fully.

IFN is a relatively safe treatment in selected and adequately monitored patients. Patients with important autoimmune phenomena, heart disease, old age, renal failure, and abnormal clearance must be treated with great care.

IFN-α is probably the most effective agent in monotherapy for CTCL. Combinations with others therapies may improve its activity. Psoralen-ultraviolet A photochemotherapy (PUVA) plus IFN is a

Table 2.

	Type I α	*Type I* β	*Type II* γ
Structure and weight	protein 18kD	glycoprotein 20kD	homodimeric glycoprotein 21-24kD
Subtypes	22	1	1 (2 subunits)
Producers	B lymphocytes and monocytes	Fibroblasts	T lymphocytes and NK

combination that allows the use of a lower dosage of both therapies, reducing the adverse effects associated with each. The above mentioned controlled clinical trial of Stadler found that this association produced a 93% overall response rate, with 77% CR and a 75% disease-free survival at 48 months in stage I and II CTCL patients. This therapy combination is more effective than PUVA alone, than IFN-α alone, or the combination of IFN plus retinoids.

IFN-α associated with extracorporeal photochemotherapy may act synergically, improving the survival and quality of life of patients with Sézary syndrome. Retinoid compounds, particularly low-dose bexarotene, can also be added to a regimen of photopheresis and IFN-α.

IFN-α has also been used in combination with topical chemotherapy and systemic chemotherapeutic agents, including deoxycoformycin (pentostatin), fludarabine, and vinblastine. These associations have resulted in response rates consistent with the results of treatment with chemotherapeutic agents alone, without real improvement, and with higher bone marrow toxicity.

Although IFN-γ and IFN-β are less well studied than IFN-α, there is no evidence to suggest that these classes of interferons are superior to IFN-α. Moreover IFN-γ is associated with more frequent and more severe side effects.

The new agent pegylated (polyethylenglycol) IFN, currently being tested in other diseases, may prove to be a more active and better tolerated form of interferon (Table 2).

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Retinoids: therapeutic applications and mechanisms of action in cutaneous T-cell lymphoma

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Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of lymphoproliferative disorders characterized by localization of clonal, malignant T lymphocytes to the skin at presentation. Mycosis fungoides (MF) is the most common and indolent variant but may progress to tumor stage, lymph nodes, or leukemia (Sézary syndrome). Retinoids are derivatives of vitamin A that serve as biological regulators of differentiation, proliferation, apoptosis, and immune responses. Retinoid acid receptor (RAR)-selective retinoids (e.g. all-trans retinoic acid, 13-cis-retinoic acid), as well as other synthetic analogs (isotretinoin, etretinate, and acitretin) have been used alone, or in combinations with other agents, for the treatment of CTCL since the early 1980s. Bexarotene is the first synthetic retinoid X receptor (RXR) selective retinoid and was approved by the FDA as an oral and topical treatment for cutaneous manifestations of CTCL. *In vitro* studies have shown that bexarotene treatment induces apoptosis of CTCL cells with downregulation of its cognate receptors and survivin, an inhibitor of apoptosis protein. Identification of new receptor suptype-selective retinoids, combinations of various receptor-selective retinoids or other agents, and new drug delivery systems may improve the clinical efficacy of retinoids.

Molecular basis of retinoid action

Retinoids are vitamin A derivatives, including naturally occurring vitamin A (retinol), its metabolites, and more recently, synthetic analogs. They are members of the steroid hormone family, physiologic regulators of essential biological processes including embryonic development, vision, reproduction, bone formation, metabolism, hematopoiesis, differentiation, proliferation, and apoptosis.[1] Retinoids belong to a class of anti-tumor agents known as *biological response modifiers.*[2] They can be administered orally and induce anti-tumor response including apoptosis of malignant T-cells.[3-5]

The biological effects of retinoids are mediated by two distinct families of intracellular receptors: retinoic acid receptors (RARs), for which the endogenous ligands are all-trans retinoic acid (ATRA) and 9-cis retinoic acid (9-cis RA), and retinoid X receptors (RXRs) for which the endogenous ligand is 9-cis RA. Each retinoid receptor has three major subtypes (α, β, and γ) with distinct amino- and carboxy-terminal domains.[6] Each RAR and RXR subtype includes several tissue specific isoforms, differing from one another in the A region, which arise from the differential usage of promoters and alternative splicing. Two major isoforms of RARα (α1 and α2) and also of RARγ (γ1 and γ2) and four major isoforms of RARβ (β1-β4) have been identified. Similarly, several isoforms distinguished by their amino-terminal region have been identified for RXRa (α1 and α2), RXRb (β1 and β2), and RXRg (γ1 and γ2).[6,7]

Retinoid receptors are ligand-activated, DNA-binding, trans-acting, transcription-modulating proteins, belonging to the superfamily of steroid hormone receptors.[6] RARs can form heterodimers with RXRs, which then bind to specific DNA sequence-RA response elements (RAREs). RAREs are characterized by direct repeats of (A/G)GGTCA separated by five nucleotides (DR5) (e.g. RARβ2 gene) or by one or two nucleotides (DR1 or DR2) (e.g. CRABP II and CRBP I genes), with RXR bound in the 5' and RAR in the 3' position.[6,7] RXRs form homodimers as well as heterodimers with most other nuclear receptors for thyroid hormone, vitamin D, peroxisomal proliferator-activators, farnesoid X, and liver X. RXR and RARs play a central role in hormonal signaling and transcriptional modulation and have the ability to modulate the expression of a wide range of genes.[8,9] The effects of topical retinoids on keratinocyte differentiation in skin is mediated by RXR-α/RAR-γ heterodimers.[5]

Nuclear receptor associated proteins (co-activators and co-repressors) also interact with DNA-bound unliganded and liganded receptor dimers to influence the transcription of target genes. In the absence of RAR ligand, the RXR/RAR heterodimer will recruit nuclear receptor co-repressor proteins N-CoR or SMRT, mSin3, and histone deacetylase.[10] Histone deacetylation induces an inactive chromatin structure, preventing gene transcription.[11] In contrast, ligand-receptors result in dissociation of co-repressor proteins and promote association of co-activators (e. g. CBP/p300 and ACTR), resulting in chromatin decondensation and activation of gene transcription.[12] Histone deacetylation inhibiting compounds also appear to be active in CTCL and may ultimately be found to synergize with retinoids (*Zhang and Duvic, unpublished data*).

Co-activators and co-repressors are shared by multiple signaling pathways. CBP has been implicated in AP-

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1, p53, STAT signaling among others and Sin3 and HDAC-1 are involved in Mad-Max signaling.[13,14] AP-1 activity, mediated by c-fos and c-jun, is influenced by retinoid action and controls a number of genes related to matrix degradation and cytokine action that are important in skin inflammation and photoaging processes.[15,16] This complex process of transcriptional activation or repression mediated by nuclear receptors and their co-factors may allow retinoids to influence multiple signaling pathways that are critical for cellular proliferation, differentiation, and apoptosis, and involve changes in the chromatin structure of target genes.

Historical perspective. Clinical use of retinoids for CTCL

Anecdotal reports of oral retinoids as single agents for the treatment of MF date back to 1983. Clinical trials using retinoids as monotherapy, as well as in combination, were reported in Europe in the late 1980s and in the United States in the 1990s, as previously reviewed by Zackheim.[17] However, RAR selective retinoids were not approved for treatment of CTCL. Oral bexarotene is an RXR selective retinoid (or rexinoid) and was approved as an oral treatment for CTLC in 1999. A topical gel formulation followed in 2000.

Topical retinoid therapy

Because of their potential for irritation, topical retinoids, unlike topical corticosteroids. have not been commonly used to treat mycosis fungoides. Beginning in the 1990s, new topical retinoids and reformulations of older retinoids were introduced with the hope of reducing the irritancy profiles. The RAR β/γ selective synthetic retinoid, tazarotene was approved for psoriasis and acne, and paved the way for testing other topical retinoids for activity in MF.[18] In the mid-1990s, two phase I-II dose-ranging studies were conducted to determine the safety and possible efficacy of alitretinoin (13-cis-retinoid acid) as well as bexarotene in MF and in Kaposi's sarcoma. Although both drugs were active in MF and resulted in reduction of the lymphocytic infiltrates within MF lesions by 8 weeks, bexarotene had a higher efficacy and was less irritant when used in MF and was selected for registration trials for MF.

Topical bexarotene gel was evaluated in a phase I-II dose-ranging study of concentrations of 0.01% - 1% involving 67 stage IA-IIA patients. Complete responses (CR) were reported in 21% and partial responses (PRs) in 42% of patients for an overall response rate of 63%.[19] The median time to response was approximately 20 weeks and a median duration of response was 99 weeks. Patients who had not previously been treated for MF with other agents had a higher response rate of 76%. Topical bexarotene displayed a dose response effect with greater efficacy at higher concentrations and frequencies of application.

In a second phase III, placebo-controlled trial of refractory stage IA, IB, and IIA MF patients, bexarotene gel 1% or placebo was applied topically every other day to lesions with increasing frequency up to four times a day as tolerated, and demonstrated a 44% overall response rate with an 8% CR rate. Bexarotene gel has also been used to treat refractory skin tumors, and is effective in combination with intralesional interferon or with phototherapy.[20]

Like other topical retinoids, bexarotene induces local irritation in approximately 70% of treated patients, with most cases being mild to moderate in severity. In MF patients, retinoid erythema may obscure the assessment of clinical response without a lapse in therapy. Irritation is easily managed by a dose reduction, decreasing the frequency of application, or by adding or alternating bexarotene with a low to mid-potency topical corticosteroid. Since topical corticosteroids are the most widely used treatment for early MF,[21] the combination of topical retinoid/corticosteroid treatment should allow steroid sparing, decrease atrophy, as well as counteracting the irritancy of using topical bexarotene.

Since tazarotene normalizes epidermal differentiation and reduces cytokines and adhesion molecules found in psoriasis and in hypertrophic MF lesions,[22,23] we decided to test this drug in the treament of MF. A six month pilot study of topical tazarotene gel 0.1% was conducted in 20 stage IA and IB mycosis fungoides patients with refractory MF lesions. The response rate was similar to that achieved by bexarotene gel and mild to moderate local irritation was relieved by local corticosteroids (*Apisarnthanarax N et al. in press, JAAD*). To date no comparison studies have been conducted and although other topical retinoids may be useful in MF lesions, they have not been systematically tested.

Oral systemic retinoid therapy

RAR retinoids. Three retinoids, isotretinoin, etretinate, and acitretin, work through RAR receptors, and were first tested for activity in CTCL. Cumulative data from studies of isotretinoin and etretinate therapy report a 19% CR and a 58% overall response with a median response duration ranging from 3 to 13 months.[17,24-29] Etretinate appears to be equally effective as isotretinoin in terms of response and toxicity[30] but has been largely replaced by acitretin because of its long half-life, long-term storage issues, and prolonged risk of teratogenicity in women. Acitretin has not been tested as monotherapy for MF, to our knowledge. However, all trans-retinoic acid (ATRA) has been studied in a small pilot studies as an oral tablet with

about a 20% response rate. In order to reach higher levels of drug and to avoid metabolism, we studied liposomal ATRA for patients with lymphoma. Eight CTCL patients responded including one patient with aggressive subcutaneous panniculitic CTCL who had failed to respond to CHOP and who obtained a durable complete response to ATRA.

RXR retinoids. Bexarotene was the first synthetic, highly selective RXR retinoid or *rexinoid* to be studied in humans. *In vitro* studies from our laboratory have shown that bexarotene treatment at clinically relevant concentrations causes apoptosis of CTCL cell lines with down-regulation of its cognate receptor RXR-α, RAR-α, and anti-apoptotic protein, survivin, and activation of caspase-3.[4] Oral Targretin® capsules were approved as a 75 mg oral formulation in 1999 for the treatment of the cutaneous manifestations of all stages of CTCL.

Two phase II-III, multicenter, open-label trials were conducted in 58 early-stage MF[31] and in 94 late-stage CTCL patients[32] who had failed or were refractory to other therapies. The drug, at three randomized dose levels (6.5, 300, and 650 mg/m^2/day) was administered to patients with earlier stages of MF and the lowest dose was dropped when a statistically lower response rate was seen. At doses of 6.5, 300, and 650 mg/m^2/day, the response rates were 20%, 54%, and 67%, respectively. Late stage patients were initially treated with 650 mg/m^2/day but an optimal dose of 300 mg/m^2 was then instituted. Response rates were 45% using the 300 mg/m^2/day dose and 55% with the 650 mg/m^2/day regimen. Overall, the 300 mg/m^2/day dose level displayed the optimal balance between response and tolerability, with an OR rate of 48% in the combined group of patients. Four percent of patients had complete responses, and 23% had a 75-100% improvement in disease, representing significant benefit. Futhermore, some of the patients who achieved CRs are still in remission after 5 years on a maintenance dose.[33]

Oral bexarotene was active even in heavily pretreated patients with later stages of CTCL whose disease manifestations included tumors with large cell transformation, lymph node enlargement, and erythroderma due to Sézary syndrome. Response rates were identical among patients who were retinoid naïve versus those who had received prior RAR therapy.[32] As recently reported, bexarotene reduced the number of abnormal CD4$^+$ cells in the blood and surrogate markers of disease such as soluble IL-2 receptors and lactate dehydrogenase levels.[33] Complete remissions of up to 5 years' duration have been seen in 5 patients with advanced disease (*Duvic, personal observation*).

The dose-limiting toxicity of bexarotene in the phase II studies was several cases of pancreatitis following the onset of hypertriglyceridemia. The most common side effects of bexarotene therapy include hypertriglyceridemia (82%), hypercholesterolemia (30%), central hypothyroidism (29%), and leukopenia (11%).[31,32] Side effects are dose-dependent, and are manageable without discontinuing the treatment. Hypertriglyceridemia is best managed with concomitant administration of fenofibrate and HMG-coA reductase inhibitors or *statins*.[33] Gemfibrozil, while effective for isotretinoin-induced hypertriglyceridemia, may induce higher bexarotene levels, hypertriglyceridemia, and increased risk of pancreatitis. Response rates are higher when the triglycerides are adequately managed allowing higher dose administration.[33] We have used two lipid-lowering agents, Tricor at 160 mg and Lipitor up to 80 mg daily, to control triglycerides in spite of the possibility of rhabdomyolysis.[33] Lipid-lowering agents should be studied in advance and treatment with bexarotene initiated at low doses (75-150 mg). Fasting triglyceride levels should be checked weekly, aiming for triglyceride levels up to 400 mg/dL. Pancreatitis was not seen under 1000 mg/dL and patients with pre-existing hyperlipidemia or diabetes appear to be most susceptible.

Bexarotene-induced central hypothyroidism results from suppressed thyrotropin secretion, and is manifested by a very low TSH value throughout therapy.[34] Patients on bexarotene will become hypothyroid, and their symptoms of fatigue improve on thyroid replacement and following normalization of free T4 values.[33] The condition is reversible upon discontinuation of therapy. Thyroid supplementation requires from 0.025 to 0.250 mg of synthroid, but results in more rapid lipid clearance and easier control of the triglyceride elevations.[33,34]

Retinoid combination therapies

With a response rate of around 50% and a short duration of response of 5-6 months, retinoids have been tried in combination with other biological response modifiers, especially PUVA or interferon α. The response rates of PUVA plus retinoids do not differ from those of PUVA alone (73% CR), however, RAR selective retinoids lowered the PUVA dosage requirements and produce longer remissions with retinoid maintenance therapy.[35] Bexarotene is also effective in combination with PUVA therapy with lower doses of each agent being necessary.

Etretinate was added to interferon α in patients who failed to respond after 3 months and appears to enhance response rates, especially in patients with less advanced disease.[36] Four of seven patients responded to low dose interferon α given three times weekly with 1 mg/kg/day of accutane. Accutane at 1 mg/kg and 3 million units of interferon α were compatible as induction therapy for a combined modality therapy regimen, which also includ-

ed total body electron beam radiation and in patients with advanced disease, six courses of combined chemotherapy.[37] The combination of interferon with bexarotene is currently under investigation. Leukopenia induced by interferon may be exacerbated by retinoid therapy.

Systemic chemotherapy plus retinoids did not produce a significantly different response rate from that of systemic chemotherapy alone.[38] Bexarotene has been combined with interferon, PUVA, extracorporeal photopheresis, and denileukin diftitox with favorable responses seen even in patients with prior treatment failures on one agent alone.[33] *In vitro*, bexarotene induces expression of the high affinity IL-2 receptor on T-cells, suggesting a rational for giving bexarotene prior to Ontak administration to increase target cells.[39] *In vitro* studies have shown that combination of bexarotene and CDDO, a PPAR-γ ligand, was superior to that of either agent alone in inducing apoptosis of CTCL cells.[40] Combinations of various receptor-selective retinoids or other members of the steroid hormone family can be utilized to induce apoptosis, differentiation and to inhibit proliferation of CTCL cells. For example, treatment of HL-60 cells with a combination of TTNPB, a RAR-selective retinoid, with LG100268, an RXR-selective retinoid, results in a greater than additive increase in cellular differentiation followed by apoptosis.[41,42] The development of mechanism-directed combinations of retinoids with other agents would be critical for improving the efficacy of retinoids as therapeutic agents in the future. Further evaluations of bexarotene should focus on determining the optimal efficacy of the drug in combination with existing therapeutic modalities, as maintenance therapy following irradiation or chemotherapy, and in combination with other newly developed classes of therapy currently under investigation.

Conclusions

Although retinoids have been used alone or in combination with other agents in CTCL, we have not yet optimized their efficacy. Their application in CTCL combination regimens may require strategies to decrease their side effects and to identify the most effective retinoids with few or no side effects. The structure-activity relationship studies of retinoic acid and its first generation analogs have resulted in the recent discovery of receptor subtype-selective retinoids such as bexarotene with a better therapeutic index. Combination with other agents may also enhance the clinical efficacy of retinoids. More mechanistic studies are needed to identify new target genes and clarify the molecular details of retinoid action that could be used as surrogate end-points to develop novel function-selective retinoids.

Funding

This work was supported in part by grants from National Institutes of Health (R21-CA 74117 & K24-CA 86815) (to M. D.), the M.D. Anderson Cancer Center Core Cancer Grant (CA 16672), the CTCL Patient Education Fund and the Sherry L. Anderson CTCL Research Funds, and an unrestricted educational grant by Ligand Pharmaceuticals, Inc.

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Purine analog therapy in cutaneous T-cell lymphomas

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The purine analogs are a group of agents which have demonstrated activity in a variety of lymphoid malignancies, including hairy cell leukemia, chronic lymphocytic leukemia, and the T- and B-cell non-Hodgkin's lymphomas. The first of these agents to be developed, pentostatin (2-deoxycoformycin) was demonstrated to be a transition state inhibitor of adenosine deaminase, thereby causing a rise in dATP and a reduction in ATP, leading to cell death.[1] In subsequent studies, the mechanism of lymphocytotoxicity of pentostatin was shown to be associated with induction of apoptosis via an adenosine-sensitive, NAD-dependent mechanism involving the activation of caspases.[2,3] Fludarabine (9-β-d-arabinofuranosyl-2-fluoroadenine (F-ara-A), and cladribine (2-chloro-2'-deoxyadenosine) are halogenated derivatives of purine nucleosides which resist deamination by adenosine deaminase. Both agents have been shown to be phosphorylated intracellularly to their mono-, di-, and triphosphate forms, which lead to inhibition of ribonucleotide reductase, DNA polymerases and DNA repair.[4,5] Gemcitabine, (2',2'-difluorodeoxycytidine, dFdC) is a nucleoside analog of deoxycytidine in which two fluorine atoms have been inserted into the deoxyribofuranosyl ring. Gemcitabine is phosphorylated by deoxycytidine kinase to gemcitabine triphosphate which, when incorporated on the end of the elongating DNA strand, inhibits DNA polymerase and subsequently DNA synthesis.[6] Despite similar mechanisms of action, the clinical activity and toxicities of the purine analogs in patients with cutaneous T-cell lymphoma (CTCL) is variable.

Pentostatin

A number of studies have reported activity of pentostatin in patients with refractory CTCL (Table 1). An early dose escalation study by Grever *et al.* demonstrated responses at doses up to 10 mg/m^2, which were associated with significant toxicity.[7] A subsequent phase II study was conducted by the South West Oncology Group (SWOG) using a lower dose of pentostatin 4 mg/m^2 daily for 3 days with cycles repeated every 21 days.[8] The dose was increased to a maximum of 8 mg/m^2 if no toxicity occurred. Of 21 enrolled patients, 20 were evaluable and 18 were eligible for response. The median age was 67 years, there was an equal sex distribution, and the median number of prior therapies was 3. Ten patients had had prior total skin electron beam (TSEB) ± psoralen ultraviolet-A (PUVA) treatment, and 4 had had local irradiation. Of 11 patients who had had prior combination chemotherapy, 6 had had one prior regimen and 5 had had 2 or more. Seven patients were untreated. Most of the patients (70%) were in clinical stage IV, with diffuse skin involvement in 15 (75%). Peripheral blood was involved in 4 patients. The median dose of pentostatin delivered in the study was 12 mg (range 7.9-102). Twelve patients received only one course of therapy, 4 received 2-3 courses, and 5 received 4 or more. There were 2 complete and 5 partial responses with a median time to progression (TTP) of 4.8 months (range 1.7-37), and a median survival of 3.1 months. One of the complete responders had received 7 courses of therapy and one of the partial responders had received 6. The TTP for the two complete responders was 37 and 13.5 months, respectively. Six of the responders had extensive plaque disease with adenopathy, one had erythroderma, and two had blood involvement.

The most significant adverse events seen in this study were nausea and vomiting in 5 patients, and nephrotoxicity in 6. Grade 3 or 4 hematologic toxicity occurred in 2 patients, keratoconjunctivitis in 2, and sepsis in 3. Three patients developed opportunistic infections, including *Candida albicans*, *Legionella pneumonia*, and *Herpes simplex* infections. Since these patients were heavily pretreated and in an advanced stage, it is unclear whether these infections were related to therapy or to the severity of the underlying disease.

A more dose-intensive phase II study was conducted by the ECOG using 5 mg/m^2 pentostatin daily for 3 days every 21 days.[9] This study accrued 25 patients with non-Hodgkin's lymphoma, 3 with Hodgkin's disease, and 8 with CTCL. Prior therapy was limited to no more than 2 chemotherapy regimens. Four of 8 eligible CTCL patients had a partial response, as did 3 nodular and 2 diffuse non-Hodgkin's lymphoma patients and one patient with Hodgkin's disease. One CTCL patient had a response lasting 1.6 years, and one responder had a prolonged survival (3.8 years). Overall, the median response duration in the study was 25.3 months with 5 patients in unmaintained response after one year. The median time to treatment failure (TTF) was 1.3 months (range:1-53.1) and the median survival was 2.7 months (range:1-63.2). The major drawback of this schedule was that toxicity was more significant than with lower dose regimens. Overall, 11 patients developed grade 3 or 4 hematologic toxicity, and 3 CTCL patients developed life-threatening infections, most likely related to poor skin integrity and further immunologic compromise. In addition, 4 patients experienced nausea and vomiting and 3 had confusion.

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Table 1. Pentostatin in refractory CTCL.

Study	*N*	*CR + PR (%)*
Grever *et al.*[7]	18	6 (33%)
Merceica *et al.*[27]	29	10 (62%)
Greiner *et al.*[11]	18	7 (39%)
Kurzrock *et al.*[12]	24	17 (71%)
Ho *et al.*[28]	43	24 (56%)

The EORTC treated a larger number of CTCL patients in a phase II study of pentostatin at a dose of 4mg/m^2 weekly for the first three weeks, followed by every other week.[10] Forty-four patients with CTCL were enrolled, 21 with Sézary syndrome and 23 with mycosis fungoides. Of this group, 30 had had prior extensive radiation to the skin, and 23 had had prior systemic chemotherapy, with 8 patients having received 2 or more prior chemotherapy regimens. More than half of the patients (51%) had extensive skin involvement, defined as involvement of 50% or more of the skin surface, and only 5 patients had skin disease limited to <10%. Circulating lymphoma cells were detected in low amounts (<5% of lymphocytes) in 22 patients, while 7 patients had extensive Sézary leukemia. Palpable nodes were present in 24 (56%). The median total dose of pentostatin delivered was 24 mg/m^2 and the median number of courses was 6 (range 1-13).

There was one complete response in a patient with Sézary syndrome and 15 partial responses (7 in patients with Sézary syndrome and 8 in patients with mycosis fungoides). The median response duration was 4.5 months for the Sézary syndrome patients and 8.3 months for the mycosis fungoides) patients. The median survival was 10.2 months, with median follow-up 8.1 months. There was no correlation between prior therapy and response. Two patients experienced grade 3 hematologic toxicity, 20 experienced weight loss during therapy, and 5 had infection, 2 with Herpesvirus.

Eighteen patients with stage I-IVB CTCL were treated at Duke by Greiner *et al.*, using different schedules.[11] One patient was treated with 4 mg/m^2 pentostatin weekly and 3 at 5 mg/m^2 $\times$ 3 every 28 days; all subsequent patients received 4 mg/m^2 every other week. Six of these patients had stage IVA disease and one had stage IVB. Two had circulating Sézary cells and four had tumor stage mycosis fungoides. Eight of the 18 had received more than two prior systemic therapies, and 8 had received at a least one systemic therapy.

The overall response rate among these patients was 39% with 2 CR and 5PR. The median response duration was 4 months (range:1.5-76), and the median pentostatin dose was 44.8 mg (range 21.6-307). The response durations for the CR were 19 and 76 months, and the PR were 2, 3, and 1.5 months. There was a significant association between disease duration (2 vs >4 years) and response (p=0.018).

Kurzrock *et al.* recently reported results using pentostatin in 28 patients who had relapsed cutaneous T-cell lymphoma or peripheral T-cell lymphoma with prominent cutaneous disease treated at MD Anderson Cancer Center.[12] In this dose escalation study, the starting dose was 3.75 mg/m^2/day intravenously for 3 days every 3 weeks, and doses were escalated to 6.25 mg/m^2. The median number of prior therapies in these patients was 3 (range 1-12). Of the 86 courses of pentostatin given, 39 were administered at doses of 5.0 mg/m^2/day and 30 at doses of 3. 75 mg/m^2/day. Dose escalation to 6.25 mg/m^2/day was possible in only five courses, and toxicity necessitated dose reduction to 2.8 mg/m^2/day in 12 courses. The most common side effects were granulocytopenia, nausea, and non-neutropenic fever. The CD4 counts lowered significantly in most patients. *Herpes zoster* was seen within 1 year after pentostatin use in five patients (19%).

The overall response rate was 71%. Of 14 patients with SS, 10 (71%) responded, and of 6 patients with tumor stage CTCL, 4 responded. One patient with blastic transformation did not respond. This study demonstrated that pentostatin is an effective therapy for refractory CTCL patients and suggests that alternative dosing may be explored in future trials.

Cladribine

Most of the studies using the other purine analogs to treat relapsed/refractory CTCL have produced less promising results, although the data are limited to small, single-center studies. Overall, responses to cladribine have been noted in about 30% of patients (Table 2).

Kong *et al.* treated 25 patients with relapsed or refractory cutaneous T-cell lymphoproliferative disorders (24 mycosis fungoides or Sézary syndrome, one Ki-1$^+$ anaplastic large cell lymphoma) with 2-CdA initially administered by continuous intravenous infusion at a dose of 0.1 mg/kg/day for 7 days (13 patients) or 5 days (9 patients) at 28-day intervals. The overall response rate was 24% with 3 (12%) complete responses, median duration of 4.5 months (range: 2.5 to 16), and 3 (12%) partial responses, median duration 2 months (range: 2 to 4). Significant hematologic toxicity occurred in 62% of patients, especially those treated for 7 days, and infectious complications occurred in 62%.[13,14] Other studies have reported similar response rates and response durations in smaller numbers of patients.[14–16]

Table 2. Cladribine, fludarabine and gemcitabine monotherapy in previously treated CTCL.

	Study	*N*	*CR (%)*	*OR (%)*
Cladribine	Saven *et al.*[29]	16	29	47
	O'Brien *et al.*[16]	22	18	41
	Kong *et al.*[13]	25	12	24
	Betticher *et al.*[15]	31	13	29
Fludarabine	Von Hoff *et al.*[17]	31	3	19
	Redman *et al.*[18]	5	0	40
Gemcitabine	Zinzani *et al.*[20]	44	11	70
	Sallah *et al.*[19]	10	20	60

*Subcutaneous cladribine.

Fludarabine

There has been even less experience with fludarabine monotherapy in CTCL (Table 2). Von Hoff *et al.* reported responses in 3 of 31 patients with advanced/refractory CTCL.[17] In another study, responses were reported in 40% of patients with mycosis fungoides treated with fludarabine at a dose of 25 mg/m^2 daily $\times$ 5 every 28 days.[18] The median response durations reported for patients in these studies was 4 months, similar to that seen with cladribine.

Gemcitabine

Gemcitabine administered at a dose of 1200 mg/m^2 on days 1, 8 and 15 every 28 days has demonstrated activity in a variety of relapsed/refractory T-cell malignancies.[19] In one study by Zinzani *et al.*, [44] previously treated patients with mycosis fungoides (n= 30) and peripheral T-cell lymphoma unspecified (PTCLU) (n = 14) were treated.[20] Of the 44 patients, five (11.5%) achieved complete responses, and 26 (59%) partial responses. Two of the CRs were histologically confirmed. The CR and PR rates were the same for patients with mycosis fungoides and those with PTCLU. No difference in terms of overall response rate was observed between relapsed and refractory patients. The median durations of CR and PR were 15 months (range, 6 to 22 months) and 10 months (range, 2 to 15 months), respectively.

In another study, a patient with the granulomatous slack skin variant of MF was treated after failure of conventional therapies. After 6 cycles of gemcitabine a partial remission of cutaneous lesions was noted with a duration of 12 months.[21]

Purine analogs in combination therapy

A number of studies have demonstrated potential synergy using purine analogs with alkylating therapies. In a small study, 12 patients with refractory CTCL received fludarabine and cyclophosphamide.[22] Nine patients had erythrodermic CTCL and three had tumor-stage mycosis fungoides. Patients received intravenous fludarabine and cyclophosphamide 3 days monthly for 3-6 months. The overall response rate was 42%, with 5 responses in the erythrodermic patients and one in a patient with MF. The mean duration of the response was 10 months. Six patients had treatment withdrawn, five due to bone marrow suppression and one due to progressive disease.

Combination therapy with purine analogs and interferon (IFN)-α was first used in the setting of hairy cell leukemia to determine whether there would be an improvement in response rates by combining 2 active agents. Based on these results, studies were initiated to explore the activity of interferon-alfa with both pentostatin and fludarabine in patients with refractory CTCL.[23,24] The first study used pentostatin 4 mg/m^2 daily for 3 consecutive days, alternating every 21 days with high dose IFN-α-2a (10×10^6 U on day 22 and 50×10^6 U on days 23 to 26). 23This study enrolled 41 refractory CTCL patients with advanced skin or visceral disease. Their median age was 59 years. Fifteen patients had cutaneous tumors and 13 had erythroderma. Seven patients had visceral involvement and 24 had blood involvement, defined as greater than 20% of lymphocytes appearing to be atypical with convoluted nuclear contours on peripheral smear. Most patients had failed to respond to multiple therapies, with 25 having failed both chemotherapy and total-skin electron-beam irradiation. Six patients had received only topical therapies and 6 were untreated.

The overall response rate was 41%, with 2 CRs and 15 PRs. Both patients who attained a CR had SS and diffuse erythroderma, and had disappearance of all skin lesions and circulating cells. A third CR, diagnosed at autopsy in a patient with extensive plaque disease, was initially scored as a PR based on persistent skin abnormalities. The patient died of gastrointestinal hemorrhage 3 months after completing therapy. Another CR occurred in a patient thought to have stable disease after 4 cycles of therapy; this patient had refused further treatment and was noted on routine follow-up 2 months later to have no evidence of disease.

Responses were noted in all sites of disease except in visceral sites. There was no correlation between response and skin stage (T1,2 v T3,4), presence of blood involvement, or lymph node stage (LN2,3 v LN4), although patients with erythroderma had a higher response rate (8 of 18) than those of any other skin stage, similar to studies with single-agent pentostatin. The correlation between prior therapy and response was not statistically significant (p=0.045), unlike prior studies with pentostatin, which had suggested a higher response rate in untreated patients. The overall survival duration was 15.8 months, with a trend toward improved survival in patients who had not had prior therapy (29

Table 3. Response durations for purine analogs and other systemic therapies in refractory CTCL.

Treatment	*Response duration (median) in months*
TSEB + CAPO[30]	13.7
IFN high dose[25]	8
Fludarabine[17]	3
2-CDA (Kuzel *et al.*, Saven *et al.*)[14,29]	4, 3
Pentostatin (Greiner *et al.*, Duke U)[11]	4
Pentostatin (ECOG)[8]	25.3
Pentostatin (SWOG)[9]	37, 13
Pentostatin (EORTC)[10]	8.3, 4.5
Gemcitabine[20]	10-15
Pentostatin + interferon[23]	13.1
Fludarabine + interferon[24]	6.6
EPOCH[31]	13
ONTAK[32]	7.3

months) compared with those who had (15 months). As seen in Table 3, the median progression-free survival duration was 13.1 months, similar to that achieved by single-agent IFN at this dose.[25]

The most frequently observed grade 3-4 toxicity was hematologic, with granulocytopenia in 15 of 41 patients. Although pentostatin therapy has been shown to be immunosuppressive, only 8 patients developed opportunistic infections. Seven had disseminated herpes zoster, and 1 had CNS toxoplasmosis and *cytomegalovirus* pneumonia. Six patients developed bacterial sepsis. Nausea occurred in 5 patients, and reversible cntral nervous system events including confusion and headache in 7.

In a subsequent study, patients with advanced or refractory CTCL were treated with the combination of fludarabine phosphate and low dose IFN.[24] Of the 35 evaluable patients, 21 had failed to benefit from prior chemotherapy or total skin electron beam irradiation, seven had had prior topical therapy, and 7 were untreated. Thirty-one patients had advanced skin disease (tumors or erythroderma), and 19 had circulating tumor cells in the peripheral blood. Ten patients had received prior pentostatin therapy. Fludarabine was administered at a dose of 25 mg/m^2 daily for 5 consecutive days, and IFN was given at a dose of 5×10^6 units/m^2 three times a week with a dose escalation to 7.5×10^6 units/m^2 on day 29 if no grade 3 toxicity was noted. The overall response rate was 51%, with 4 CR and 14 PR. Of the patients who received prior pentostatin, 5 (50%) had a PR. Univariate analysis showed no statistically significant association between skin stage, presence of blood involvement, or prior therapy and response, similar to the findings of the pentostatin-IFN study.

While response rates were similar between the two studies, the response duration with the fludarabine-IFN combination was significantly shorter (median time to progression 5.9 months). The time to progression for the responders who had received prior pentostatin ranged from 3-6 months. The response durations with the combination of fludarabine-IFN are similar to those of single agent fludarabine in phase II studies (range 2 to 17 months).[14]

The incidence of hematologic toxicity was significantly higher with the fludarabine-IFN combination than with the pentostatin-IFN one, perhaps due to the different spectrum of activity of fludarabine compared with pentostatin. The incidence of grade 3 or 4 neutropenia was 61%, with grade 4 anemia and thrombocytopenia in 11%, compared to 36% with pentostatin-IFN. Prolonged nadirs occurred early in this trial, than in trials using fludarabine alone, wherein treatment delays were noted after a median of 5 cycles of fludarabine.[15] One patient developed bone marrow aplasia and remained transfusion-dependent until death four months later. Six patients developed septicemia and 5 developed opportunistic infections, again similar to observations in the pentostatin-IFN study.

These results suggest different spectrums of activity and toxicity when pentostatin or fludarabine are combined with IFN. The higher incidence of hematologic toxicity in the fludarabine-IFN study suggests that the IFN may have unfavorable effects on bone marrow hematologic recovery after purine analog administration which occurs to a less significant degree with pentostatin than with fludarabine. The high incidence of infections in both studies may be attributable not only to myelosuppression but also to underlying immunologic deficits in the CTCL patients, as indicated by anergy studies which documented that over 80% of the patients entered on the fludarabine-IFN study were anergic at study entry.

While overall time to progression was significantly different between the two studies, it is notable that response durations of 37+ months for complete responders were noted in both studies. The differences in overall response durations cannot be attributed to differences in the populations of patients between the two studies, as they were similar, but rather, may reflect differences in biological activities between purine analogs. *In vitro* synergy studies in B-CLL cells by Zinzani *et al.* suggest a differential effect of the combination of IFN and purine analogs with respect to the induction of apoptosis, suggesting different mechanisms of action of these agents.[26] Further randomized studies are needed to explore the activity of combination therapies with purine analogs in patients with CTCL.

Conclusions

In summary, the purine analogs are a group of agents which have demonstrated activity in patients with advanced and refractory CTCL. While overall response rates are similar among these agents, the

overall response durations are disparate, with longer responses reported with pentostatin than with cladribine or fludarabine. Of particular interest is that the response duration of 13.1 months reported for the combination of pentostatin and IFN is similar to that of multi-agent chemotherapy, suggesting that this combination may be worthy of further investigation for patients requiring aggressive systemic therapy. Among these studies using purine analogs, the subset of patients consistently demonstrating the highest response rate are patients with Sézary syndrome. Further studies are warranted to explore the activity of purine analogs as first line systemic therapy in patients with advanced stage disease, as well as to explore combinations with other agents, including retinoids and targeted cytotoxic agents.

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Gemcitabine in cutaneous T-cell lymphomas

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Gemcitabine (2',2'-difluorodeoxycytidine, dFdC) is an analog of deoxycytidine. It is transformed to the active triphosphate (dFdCTP) after intracellular phosphorylization. Gemcitabine monophosphate is inserted into the DNA and inhibits DNA elongation as a false nucleotide. In contrast to other antimetabolites, an additional, altered nucleotide is inserted behind dFdC inhibiting repair mechanisms (masked chain termination).

In this way, *repair enzymes* (exonucleases) of the DNA are inhibited and repair mechanisms are prevented. This factor as well as enzymatic inhibition of gemcitabine diphosphate lead to high intracellular concentrations of gemcitabine and enforce the cytostatic effect. Gemcitabine is mostly inserted into DNA but partly also into RNA. Competition at the receptor with the nucleoside deoxycytidine phosphate (dCTP) leads to a competitive inhibition of DNA polymerases.[1]

The effects of gemcitabine on cellular metabolism therefore include:

- inhibition of ribonucleotide reductase with lowering/disruption of *de novo* deoxynucleotide synthesis (mostly dCTP);
- multiplication of the effect of deoxycitidine kinase by inhibition of the negative feedback on this enzyme, leading to an enhanced phosphorylization of gemcitabine;
- inhibition of the enzyme responsible for the elimination of gemcitabine (deoxycitidine monophosphate deaminase). By exhausting the dCTP pool and increasing the intracellular concentration of dFdCTP, the positive feedback mechanism of the enzyme is further inhibited;
- inhibition of cytidine triphosphate synthetase (CTP-synthetase) leading to a further exhaustion of the dCTP pool and inhibition of RNA synthesis.

Pharmacokinetics

After infusion of 1000 mg/m^2 of femcitabine over 30 min, maximal concentrations of 10 to 40 µg/mL are observed. The extracellular half life is approximately 30 minutes. Gemcitabine is metabolized to the cytostatically inactive metabolite 2'desoxy-2',2'-difluorouridine (dFdU) at a rate of 91-98%. Metabolization occurs in liver, kidneys, blood and other tissues via cytidine deaminases. After infusion of 1,000 mg/m^2, 92-98% of the dose is recovered in urine within one week. The excretion of the original substance and dFdU via the urinary tract is 99% with less than 1% being eliminated via feces. Cytostatically active metabolites of gemcitabine are not detectable in plasma or urine. The plasma protein binding of gemcitabine is only 10%.

So far, gemcitabine has been found to demonstrate a broad spectrum of activity in solid tumors, including pancreatic, ovarian, breast, lung and bladder cancers. In hematopoietic malignancies, gemcitabine has shown a high level of activity as a single agent in relapsed or refractory Hodgkin's disease and some degree of efficacy in aggressive and indolent non-Hodgkin's lymphoma.

T-cell disorders

Concerning the effect of gemcitabine on cutaneous T-cell lymphoma (CTCL), we conducted a phase II trial in 44 consecutive, previously treated patients with mycosis fungoides (MF) (30 cases) and peripheral T-cell lymphoma unspecified (PTCLU) (14 cases) with exclusive skin involvement.[2] Gemcitabine was given to all patients on days 1, 8, and 15 of a 28-day schedule at a dose of 1200 mg/m^2 for a total of three cycles. Of the 44 patients, five (11.5%) achieved complete responses, 26 (59%) partial responses, and the remaining 13 showed no benefit from the treatment. Two of the complete responses were histologically confirmed. The complete and partial response rates were the same for patients with MF and those with PTCLU, respectively. No difference in terms of overall response rate was observed between relapsed and refractory patients. The median durations of complete response and partial response were 15 months and 10 months, respectively. This report has confirmed our preliminary data on 13 patients,[3] who are also included here with a longer follow-up; in this study, gemcitabine-treated patients had a higher or at least comparable overall response rate to that published in the literature for patients with MF treated with other nucleoside analogs, such as fludarabine and pentostatin.

Recently, we started a phase IIb multicenter study with gemcitabine as primary chemotherapy in patients with advanced CTCL (possibly pretreated only with PUVA or radiotherapy). The patients will be recruited from the *Italian Cutaneous Lymphoma Study Group*.

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Sallah *et al.*[4] reported their experience in 10 patients with refractory and relapsed T-cell malignancies treated with gemcitabine. Two of the patients had CTCL, 2 had prolymphocytic leukemia (PLL), 2 had nodal PTCL, 2 had small lymphocytic lymphoma (SLL), 1 had anaplastic lymphoma and 1 angiocentric lymphoma. The drug dose was the conventional 1,200 mg/m^2 on days 1, 8 and 15 of each 28-day cycle. Of the 10 patients, two achieved a complete response (1 had PLL, the other had anaplastic lymphoma) and four had a partial response (2 cases of CTCL, 1 of angiocentric lymphoma and 1 PTCL) for an overall response rate of 60%. The median and mean duration of response was 13 and 16 months, respectively.

Toxicity

Hematologic toxicities. Anemia of WHO grade III was observed in 5-10% of patients, neutropenia of WHO grades III and IV in 20% and 10% of patients, respectively, WHO grade III and IV thrombocytopenia in 20% and 10% of patients, respectively.

Non-hematologic toxicity. Transient elevations in liver transaminases were observed in 5-10% of patients. Renal and pulmonary toxicity was very rare: WHO grade III-IV toxicity of these organs occurred in less than 1% of the patients. Flu-like symptoms with headache, fever, myalgias and fatigue occurred in up to 10% of patients. Alopecia does not usually occur during gemcitabine therapy. Neurotoxicity in connection with gemcitabine is rare. Peripheral edema occurs in 10% of patients. Toxicities were usually mild and reversible after the end of therapy.

Conclusions

Its modest toxicity profile and easy schedule of administration make gemcitabine an ideal agent for the development of chemotherapy regimens. It would be particularly interesting to evaluate the use of two different nucleoside analogs (fludarabine or pentostatin plus gemcitabine) in modulating the entry route into DNA and their action in terms of direct cytoxicity and apoptosis, respectively. Earlier investigations have demonstrated the possibility of potentiating fludarabine with low doses of gemcitabine.[5] In addition, administration of the Campath-1H antibody, which has demonstrated activity in T-PLL, in combination with gemcitabine may provide a unique mechanism of cell killing and prove to be an effective regimen in T-cell malignancies. Given the efficacy of regimens combining cytarabine and cisplatin in lymphoid malignancies, and the experience of combinations of Gemcitabine with platinum compounds in solid tumors, combinations of gemcitabine with other compounds should be investigated. The *Italian Group of Cutaneous Lymphomas* (GILC) are currently carrying out a multicenter national trial on gemcitabine as a first-line chemotherapy regimen for patients with CTCL who have been pretreated only with local radiation therapy or PUVA.

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The potential role of liposomal doxorubicin and daunorubicin in the treatment of advanced cutaneous T-cell lymphoma

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Primary cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of clinico-pathologic entities, mostly characterized by an indolent clinical course (mycosis fungoides, MF; $CD30^+$ CTCL) and more rarely by an aggressive behavior (Sézary syndrome, SS; $CD30^-$ CTCL; newly described entities, such as panniculitis-like CTCL and epidermotropic cytotoxic CTCL).[1,2] The treatment of advanced CTCL (MF stage IIB-IV), aggressive CTCL, and refractory or repeatedly relapsed CTCL is a great challenge, especially considering that no survival benefit has been demonstrated for CTCL patients treated with polychemotherapy regimens. The recent availability of new treatment modalities - such as novel retinoids (bexarotene), denileukin diftox (DAB389- IL2), interleukin-12, monoclonal antibodies, novel chemotherapeutic agents, vaccine therapy, and transplantation – have opened new perspectives in this regard.[3-5]

Liposomal doxorubicin

Among novel cytotoxic agents, pegylated liposomal doxorubicin (PEG-DOXO) – an encapsulated anthracycline with a much better therapeutic index, i.e., much lower toxicity and higher bioavailability in tumor cells - was preliminarily found to be effective in relapsing or resistant CTCL. Six patients with MF stage IB-IIB were treated at the dosage of 20 mg/m^2 once a month (cumulative dose 320 mg), with an overall response rate of 83% and temporary, mostly unremarkable side effects (mild anemia, lymphopenia).[6]

The results of this prospective, pilot study have recently been updated. A total of 10 patients (1 female, 9 males) aged 50-78 years (mean 66.7 years) with relapsing or resistant CTCL (MF stage I B-IVB) were treated with PEG-DOXO 20 mg/m^2 once a month, with an upper limit of 400 mg to induce a clinical response. In nine patients (one drop out after a single infusion because of a capillary leak syndrome), the best response was a complete response (CR) in 5 patients and a partial response (PR) in 4 patients. The final outcome was CR in 6, PR in 2, stable disease (SD) in 1, and progressive disease (PD) in 1 patient. The overall response rate (CR + PR) was 80%. The follow-up was 2-22 months (mean 12.8±7.1 months). The overall survival was calculated as 19.8±7.4 months, with 8/10 patients still alive. Response duration was 15.2±3.9 months, disease-free survival 13.3±6.1 months, event-free survival 16.7±9.0 months, and progression-free survival 18.2±6.5 months. The follow-up after the first course with PEG-DOXO was 2-22 months (mean 12.8±7.1 months). The survival rate after 12 months of follow-up was 80% (n = 5). The most frequent side effects of treatment were confirmed to be anemia and lymphopenia, without the need of supportive treatment or dose-reduction. Only one patient developed grade 4 toxicity (anemia).

A pilot, multicenter trial of the *Italian Group for Cutaneous Lymphomas* (GILC, *Gruppo Italiano Linfomi Cutanei*) with PEG-DOXO 20 mg/m^2 every 2-4 weeks is currently underway. To date 9 patients (2 females, 7 males), age range 29-79 years (median 65), with pretreated, advanced CTCL (stage IIA-IVA) have been enrolled and treated. In the 6 evaluable patients, there were 4 partial responses and 2 complete responses.

Liposomal daunorubicin

A recent phase II trial[8] showed that liposomal daunorubicin can be safely and quite effectively administered in relapsed or refractory non-Hodgkin's lymphoma of low grade, intermediate grade and mantle cell types at the dosage of 100 mg/m^2 i.v. every 3 weeks in a 2-hour infusion, with a median of 6 cycles (range 1-15). The overall response rate was 39% in 31 patients (2 CR and 10 PR), with a median duration of response of 19.5 months 4.3-41.1). Major toxicities were grade 3 or 4 neutropenia in 79% patients, mild to moderate nausea (67%) and fatigue (48%). Prior studies (reviewed in 8) had shown the efficacy and safety of a schedule of 40 mg/m^2 every 2 weeks in various hematologic malignancies and in AIDS-related Kaposi's sarcoma.

On the basis of the above preliminary studies, and considering the frequent occurrence of severe immunodeficiency in multiply relapsed CTCL patients, we initiated a pilot study with liposomal daunorubicin in 8 patients with repeatedly relapsed, heavily pretreated (at least one line of chemotherapy) CTCL patients (MF tumor stage, 5 patients; CD30—large cell CTCL, 2 patients; epidermotropic cytotoxic lymphoma, 1 patient). Six patients (2 females, 4 males; age 53-76 years, median 62) are currently evaluable. We used a schedule of 50 mg/m^2 i.v. every 2 weeks, with a switch to 100 mg/m^2 every 3 weeks in case of no response after 6 cycles. The median cumulative dose was 400

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mg/m^2. We had 1 CR, 2 PR, 2 SD and 1 PD, with a 50% overall response rate. The response duration was 3-11 months (median 7), with a time to treatment failure of 8-21 months (median 14). Grade 2 or 3 neutropenia occurred in 50% (3/6) and fatigue in 67% (4/6) of the patients.

Discussion

Liposomal doxorubicin can be considered a very promising cytotoxic agent in the treatment of CTCL. A wider stage- and type-oriented study (advanced and/or aggressive CTCL) and the recognition of the optimal schedule (classical every 4 weeks vs. every 2 weeks; this latter with the aim to accelerate remissions, with possible maintainace treatment every 4 weeks to follow) are the current goals of clinical trials. In this regard, based on a pilot experience in some European centers, a phase II trial of the *EORTC Cutaneous Lymphoma Task Force*, using a schedule of 20 mg/m^2 every 2 weeks in patients with stage IIB-IV CTCL, is ongoing.

Our preliminary experience with liposomal daunorubicin in repeatedly, heavily pretreated patients with advanced or aggressive CTCL does not exclude the possible role of this drug in the treatment of advanced/aggressive CTCL. Indeed, our results seem to indicate the utility of phase II trials in CTCL patients not previously treated with chemotherapy.

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Interleukin-12 for treatment of cutaneous T-cell lymphoma

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Cutaneous T cell lymphomas (CTCL), especially the most indolent form, mycosis fungoides (MF), usually presents as a chronic and persistant dermatitis that may go on for many years before a skin biopsy shows pathognomonic epidermal infiltration with abnormal CD4+ helper/memory T-cells ie, Pautrier's microabscesses.[1,2] More advanced disease is characterized by a vertical growth phase resulting in tumors, ulcers, and metastatic spread to regional lymph nodes. Sézary syndrome (SS) is the leukemic varant of MF, although a subset of CTCL/MF patients present de novo with generalized erythroderma, pruritus, and circulating malignant cerebriform T-cells.[3,4]

Immunology and cytokine profile of CTCL

The presence of interleukin (IL)-4 and IL-5 mRNA in the skin lesions of CTCL patients, as determined by polymersa chain reaction (PCR) amplification, supports the hypothesis that malignant T-cells arise from the Th2 subpopulation of CD4+ cells.[5] Peripheral blood mononuclear cells (PBMC) from patients with SS have been shown to produce increased levels of IL-4,[6] probably acting as an autocrine growth factor for these tumor cells. Finally, *in vitro* treatment of PBMC from SS patients with interferon (IFN) α or IFN-γ diminishes IL-4 production.[7] As a potent stimulator of IFN-γ secretion and an inhibitor of Th2 lymphocytes, ricombinant human IL-12 may function as an indirect inhibitor of CTCL cells as well as augment the anti-tumor immune response.

Rationale for use of IL-12 in CTCL

IL-12 is naturally produced *in vivo* by monocytes, B-cells, and other antigen-presenting cells. This cytokine acts a potent growth factor for activated T-cell and NK-cell proliferation, as well as enhancing cytolytic T- and NK-cell cytotoxicity. Interleukin 12 is a modulator of other cytokines, as it induces interferon-γ production, which in turn is thought to induce the expression of chemokines such as Interferon inducible protein 10.[8] Through induction of interferon γ, IL-12 facilitates the development of T-helper (Th-1) cell differentiation and blocks the differentiation of Th2 cells that produce IL-4 and IL-5.[9]

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Recent studies from the Rook laboratory have demonstrated a deficiency in production of IL-12 by peripheral blood cells from patients with SS.[9] Notably, culturing these patients' cells with recombinant IL-12 (rIL-12) *in vitro* leads to a restoration of IFN-γ production and a marked enhancement of cell-mediated cytotoxicity. Infiltrating cytolytic CD8+ T-cells have been demonstrated in early skin lesions of CTCL,[10] are correlated with a favorable response and are lost during progression of disease. The ability to enhance the generation of cytotoxic CD8+ T-cells via the administration of exogenous IL-12 was hypothesized to be important for the therapy of CTCL.[11] Moreover, the restoration of IFN-γ production and the enhancement of cell-mediated cytotoxicity are primary rationales for using IL-12 as therapy for this T-cell malignancy.

Clinical trials of IL-12 for CTCL patients

Trial design. To date there have been two small, open label, clinical trials evaluating the safety and efficacy of subcutaneous low dose recombinant IL-12 therapy in CTCL patients. The phase I and phase II clinical trials included 32 evaluable patients, and had an overall response rate of about 50%.[12] In these studies, a complete clinical response (CR) was defined as complete disappearance of all measurable and evaluable lesions for at least one month, documented by re-biopsy of a previously involved skin site and by re-evaluation of previously involved peripheral blood by gene rearrangement studies and Sezary count. A partial clinical response (PR) was defined as 25 to 49% disappearance of all CTCL skin lesions for at least one month, while stable disease was defined as less than 25% disappearance of all measurable and evaluable lesions or stabilization of all existent lesions for at least one month. Progressive disease was defined as at least a 50% increase from baseline in measured cutaneous disease burden.

Phase I Trial. The phase I trial was conducted in ten CTCL patients who had plaques (n=5), tumors (n=2), and erythroderma with SS (n=3) but who did not have visceral disease. The patients with tumors and SS had received a mean of three previous therapies. Exclusion criteria included seropositivity against HIV, HTLV-1, or hepatitis C, and a past history of gastrointestinal hemorrhage or serious autoimmune, cardiac, or renal disease. The patients received escalating doses of 50 ng/kg, 100 ng/kg, or 300 ng/kg twice weekly by subcutaneous injection for up to 24 weeks. In some cases, rhIL-12

was administered directly into discrete plaques or tumors for additional benefit.[12]

All of the patients with plaque disease had measurable clinical improvement while on IL-12. Two patients who received 100 ng/kg achieved CR at weeks seven and eight, with partial responses by five weeks of therapy. Two patients achieved PR at 12 weeks and 7 weeks of therapy. The former received 300 ng/kg, while the latter received 100 ng/kg. One patient had a minor response at week 12 while receiving 50 ng/kg, but elected to discontinue therapy at week 13 due to increasing pruritis which continued after the rhIL-12 had been stopped. Both tumor-stage patients had rapidly progressive disease with numerous skin tumors at the time of initiation of rhIL-12. Intralesional rhIL-12 injection into tumors was associated with tumor flattening and complete resolution of the several injected tumors. However, new lesions continued to develop beyond the injection sites, and the patients discontinued therapy after six and eight weeks. There were three patients with SS. One discontinued treatment after the first week for personal reasons; one discontinued at week 10 with stable skin and blood disease at a dose of 100 ng/kg; and one had a PR at week 13 with clearing of erythema from large areas of the trunk. The improvement was maintained throughout the remainder of the 24 weeks of therapy.[12]

Phase II Trial. The phase II trial was limited to patients with stage IA, IIA, or IB cutaneous disease who received a pre-dose of 100 ng/kg and were then dosed with 300 ng/kg twice weekly for up to 24 weeks. Twenty-three patients enrolled; fourteen (61%) had received three or more treatments prior to study entry. Ten of 23 (43%) patients experienced partial responses and 3 (13%) had minor responses during treatment with rhIL-12. Several patients had near-total clearing of skin lesions by the end of the 24 weeks of therapy but there were no complete responses. Three patients had disease progression while on rhIL-12.[12]

Side effects of IL-12 in the trials

Adverse effects of rhIL-12 during the phase I trial included fatigue, headache, and myalgias, similar to those experienced with interferon administration. Most of these effects were mild and short-lived, lasting 24 to 36 hours after the initial injection. Some patients had elevations in serum hepatic enzyme levels. One patient experienced severe depression after dose escalation from 50 ng/kg to 100 ng/kg at week 4. He discontinued rhIL-12 after 6 weeks of therapy, and the depression resolved within one week of discontinuation. He also experienced severe depression during treatment with recombinant IFN-α, which also resolved on discontinuation of this cytokine, suggesting that the adverse effect was not unique to IL-12.[11]

Adverse effects in the Phase II trial included fatigue, headache, malaise and myalgias. Dosing at 300 ng/kg was associated with more prolonged constitutional symptoms, with the potential for the fatigue to persist for several days. Elevations in serum hepatic enzymes were also noted at this dose. Other effects included neutropenia, diarrhea, depression, and anxiety.[12] One patient developed a sudden and fatal case of autoimmune hemolytic anemia after several months on therapy.

Future combination therapy with IL-2 may overcome refractoriness to IL-12

The previous trials of IL-12 for CTCL were associated with the development of apparent refractoriness to rhIL-12.[12] At least one-third of treated patients who exhibited initial clinical responses appeared to have their responses plateau by 12 to 14 weeks of therapy with no further improvement. Furthermore, patients with initial clinical responses developed evidence of lesion enlargement or new lesion formation at this time. Thus, they had become *refractory* to rhIL-12 with no further response.

Recombinant IL-2 may be a clinically efficacious biological agent in the treatment of CTCL. In a phase I study, 19 patients with advanced CTCL received IL-2 as a single agent in escalating doses up to eleven million units four times weekly. Responses were observed with minimal toxicity (*T Kuzel and F Foss, unpublished observations*). In addition, substantial *in vitro* data suggest that Il-2 and rhIL-12 can synergistically enhance cell-mediated immunity.[13] Similarly, laboratory observations also indicate that IL-2 may be able to overcome the refractoriness to rhIL-12 by enhancing IL-12 receptor expression,[13] thereby enhancing responses to rhIL-12.

A multicenter phase II open-label study of rhIL-12 with cross-over to phase I evaluation of escalating doses of IL-2 administered with rhIL-12 is currently ongoing. Patients will receive IL-12 at a dose of 100 ng/kg twice weekly for 12 weeks. All patients, except for those with progressive disease, will be eligible to receive IL-2 in addition to rhIL-12 for another 12 weeks and the dose of IL-2 will be escalated in 3 to 6 patients ranging from 0.5, 1, 2, 3, 4.5 to 6 million units three times weekly subcutaneously. Dose-limiting toxicity will be defined by two patients per group having toxicity. Clinical response will be correlated with studies of cellular immunity, including interferon γ production, natural killer cell activity, cytotoxic T-cells in skin biopsies and IL-12 receptor display on lymphocytes.

Conclusions

Biological response modifiers such as IL-12 and IL-2 offer the advantage of restoring normal immune function in patients and boosting cyto-

toxic lymphocytes within lesions; this may keep the tumor cells under control.[14] Interleukin 12 is a modulator of Th1 cytokine production, which is know to diminish with progression of CTCL, and can reverse Th2 cytokine production by Sézary cells *in vitro*. Combinations of various cytokines and other biological response modifiers including retinoids, may prove extremely beneficial for the control of this disease if strategies to overcome refractoriness can be developed.

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Rituximab therapy for primary cutaneous B-cell lymphomas

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Rituximab (Mabthera) is a chimeric monoclonal antibody with murine variable region directed against CD20 antigen and human constant regions IgG1κ. The binding of murine variable region to CD20 induces apoptosis and inhibits cell proliferation. The Fc part of the constant human region activates the host immune response to mediate cell lysis through antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).

The wide therapeutic use of rituximab, is justified by the large diffusion of this transmembrane protein on neoplastic cell surface in lymphoproliferative disorders and by the encouraging results so far obtained from treatment with this monoclonal antibody.

Almost 90% of B-cell lymphomas and chronic lymphocytic leukemias and 50% of preB-cell acute leukemias express the CD20 antigen on the neoplastic cell surface as do normal B-cells from pre-B to activated B-cell stages of differentiation. Hematopoietic stem cells and plasmacells are CD20 negative.

The efficacy and safety of rituximab therapy were first demonstrated in the treatment of relapsed indolent lymphomas in which it produced a response rate of 48% and the median time to progression in responding patients was 13 months.[1] In further studies, rituximab as a single agent showed the same efficacy in patients with a bulky disease[2] and in untreated patients.[3,4] In aggressive histologies, both in untreated and in pretreated patients, rituximab resulted effective and safe even in the elderly.[5] The association with chemotherapy (even in high doses as *in vivo* purging)[6,7] is possible for minimal overlapping toxicity.

Several studies are underway to improve the outcome of this treatment in small lymphocytic lymphoma subtypes[8,9] and in mantle cell lymphoma.[10] Furthermore, rituximab therapy has been investigated in Waldenström's disease, in cryoglobulinemia and in autoimmune disorders.

The first description of the efficacy of rituximab as intralesional therapy in 2 patients with a primary cutaneous B-cell lymphoma (PCBCL) was given by Heinzerling and colleagues.[11] These case reports were followed by other studies on intralesional administration of a variable number and dose of injections of rituximab in patients affected by different subtypes of PCBCL.[12] The duration of responses ranged from a few weeks to 12 months.

Sabroe and colleagues[13] reported the results of systemic administration of rituximab in two patients affected by diffuse large cell lymphoma (DLCL) in relapse and with widespread cutaneous nodules. The longest response observed was three months.

Heinzerling and colleagues[14] reported on ten patients treated with a variable number (from 1 to 4) of systemic infusions of rituximab at 375 mg/m^2.

According to the EORTC classification, 5 patients had a large B-cell lymphoma (four of whom had lymphoma of the leg), 3 patients had follicular lymphoma, 1 a diffuse large cell lymphoma and 1 an extranodal large cell lymphoma. Their median age was 71 years. The systemic treatment with rituximab resulted in two complete responses (CR), five partial responses (PR) and one mixed response (some nodules disappeared, whereas new lesions developed). Two patients did not respond. The two patients who obtained CR and the one with a PR were in remission after, respectively, 12, 5, and 4 months of follow-up. In relapsed patients the median time to disease recurrence was 4 months. No severe side effects were observed and CD19/CD20-positive B-lymphocytes reappeared in the peripheral blood 6 months after the end of the treatment.

We report our experience on systemic rituximab therapy in thirteen consecutive patients.

Patients and Methods

Table 1 lists the patients' age, sex, histology, status of disease and previous treatment. Thirteen patients (7 males, 6 females) were treated between February 1999 and June 2002. Their median age at presentation was 52 years (range 38 to 84).

Patients had either a single (large and deep) lesion or multiple and disseminated lesions over the trunk, scalp, and limbs. Two patients had progressive disease with lymph node involvement.

Nine patients were resistant to or in relapse after different treatment regimens. In six patients prior therapy included chemotherapy (CHT). Of these patients, one had received high doses of CHT with peripheral blood stem cell (PBSC) support and one was in relapse 18 months after a non-myeloablative PBSC allograft. Two patients had been treated with radiotherapy (RT), one with surgery alone. Two patients received rituximab after CHT: patient #8 had been previously treated for 12 weeks with MACOP-B therapy, patient #7 had received

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Table 1. Patients' characteristics.

Patient	*Age 8 yrs)*	*Sex*	*Histology (WHO)*	*Immunohistochemical data*	*Cutaneous lesions*	*Pre-treatment if relapsed/resistant patient*	*Associated Chemotherapy*
1 CN	48	M	DLCL	$Cd20^{+};Cd43;Bcl2^{+};catk^{+};Cd30^{-}$	Single	CHOP, HD-CHT +PBSC-Autograft	
2 PL	84	F	DLCL	$Cd20^{+};Cd43^{-};Cd30^{-/+}$	Single	CVP, Etoposide	
3 DVM	52	F	DLCL (leg)	$Cd20^{+};Cd43^{-};Cd30^{-}$	Single	CHOP, Gemcitabine, IEV, PBSC-Allograft	
4 DAN	43	M	Marg. zone	$Cd20^{+};Cd43^{-/+};Cd3^{-};Cd10^{-}$	Multiple	None	
5 BAM	77	F	Marg. zone	$Cd20^{+};Cd43^{-/+};Cd3^{-};Cd10^{-}$	Multiple	IFN, Chlorambucil+PDN	
6 MA	38	M	Marg. zone	$Cd20^{+};Cd3^{-};Cd10^{-}$	Multiple	IFN,VBM	
7 NR	54	F	Marg. zone/DLCL	$Cd20^{+};Cd3^{-};Cd10^{-}$	Multiple	RT	FLUC (6 cycles)
8 DBG	52	M	DLCL	$Cd20^{+};Cd43^{-};Cd30^{-}$	Single, LN	Surgery	MACOP-B
9 CA	49	M	DLCL	$Cd20^{+};Cd43^{-};Cd30^{+};Bcl2^{+}$	Single	None	
10 IE	60	M	Marg. zone	$Cd20^{+};Cd3^{-};Cd15^{-};Cd68^{-}$	Multiple	None	
11 SL	56	F	DLCL	$Cd20^{+};Cd79/A^{+};Cd3^{+};Cd30^{-}$	Single, LN	MACOP-B	FLUC (4 cycles)
12 BA	47	M	DLCL	$Cd20^{+};Cd3^{-};Cd10^{-};Bcl2^{+}$	Single	RT	
13 AM	59	F	DLCL	$Cd20^{+};Cd3^{-};Cd19^{-};Cd25^{-};Cd4^{-}$	Multiple	None	

CHOP: cyclophosphamide, doxorubicin, vincristine, prednisone; FLUC: fludarabin, cyclophosphamide; HD-CHT: high dose chemotherapy; PBSC: peripheral blood stem-cells; IEV: ifosfamide, epirubicin, etoposide; CVP: cyclophosphamide, vincristine, prednisone; IFN: α2-interferon; PDN: prednisone; RT: radiotherapy; MACOP-B: methotrexate, doxorubicin, cyclophosphamide,vincristine, prednisone bleomycin; LN: progressive disease with lymph nodes involved.

Table 2. Response to rituximab treatment.

Patient	*Histology*	*Response*	*Response duration (months)*	*Therapy at relapse*
1 CN	DLCL	CR	48^{+}	
2 PL	DLCL	CR	27^{+}	
3 DVM	DLCL (leg)	Resistant	Resistant	RT, IL2 (CR 13)
4 DAN	Marginal Zone	PR	8	Rituximab, IFN (CR 24^{+})
5 BAM	Marginal Zone	PR	6	Chlorambucil (PR 23^{+})
6 MA	Marginal Zone	PR	IFN (CR 23^{+})	
7 NR	Marg.Zone /DLCL*	CR	6	RT, IFN (CR 13^{+})
8 DBG	DLCL*	CR	27^{+}	
9 CA	DLCL	CR	28^{+}	
10 IE	Marginal Zone	CR	9^{+}	
11 SL	DLCL*	CR	8^{+}	
12 BA	DLCL	CR	8^{+}	
13 AM	DLCL	CR	6^{+}	

CR: complete response; PR: partial response; *chemotherapy was associated to Rituximab; $^{+}$response ongoing.

six months of FLUC therapy (fludarabine, cyclophosphamide). In one patient rituximab was administered before each of the four cycles of FLUC therapy, every three weeks.

All patients had a CD20 positive cutaneous lymphoma. The histologic distribution according to the WHO classification was as follows: 7 patients had a DLCL, 4 patients a marginal zone lymphoma (MZL), and 2 patients a follicular lymphoma. Only 1 patient, at the time of the first observation, had a DLCL recognizable in EORTC classification as a large cell lymphoma of the leg.

Pre-therapy evaluation included physical examination, bilateral bone marrow biopsies and radiographic studies with chest X-ray and computed tomography (CT) imaging of the head, neck, chest, abdomen and pelvis. Laboratory testing included routine hematology, serum chemistries, lymphocyte subpopulation measurement, Igs, β2-microglobulin and autoimmunity studies. Informed consent was required and obtained before treatment.

Rituximab dose was 375 mg/m^2 and was administered intravenously once a week for a total of four infusions (days 1, 8, 15 and 22) given on an outpatient basis.

Toxicity

None of the patients developed adverse effects during the rituximab infusion. A severe reduction of circulating B lymphocytes was observed for 7 months, on average, after the last administration, but no patients showed an increase of infectious diseases.

Results

The responses to rituximab treatment are shown in Table 2.

All 13 treated patients were evaluated. Nine out of ten patients treated with rituximab alone responded (6 CR and 3 PR). The patient with Large-cell lymphoma of the leg was resistant to treatment, obtaining only slight reduction in the lesion size after the second administration of rituximab. Patients treated with associated CHT obtained a CR. At a median follow-up of 23 months (range 6 to 48), all patients treated with rituximab alone and 2 with rituximab plus CHT maintained their CR. Three relapses occurred among patients with a lymphoma of marginal zone origin. One patient in PR after rituximab treatment received α2interferon (IFN) and obtained a prolonged continuous CR (23 months).

Discussion

PCBCLs are a group of neoplastic diseases heterogeneous for pathogenesis and biological and clinical features. Most of these lymphomas have a good prognosis after local therapy, considering the long survival and the very infrequent spread of the disease to other organs. RT alone or after surgical excision, when possible, is considered the first choice treatment for these patients. IFN and systemic doxorubicin-based CHT[15] are also used in a minority of patients. Cutaneous relapse of lymphoma can, however, require more aggressive treatment to obtain a longer duration of response.

Rituximab immunotherapy is effective in the treatment of systemic B-lymphoproliferative disorders particularly in indolent diseases.

In patients with PCBCL, rituximab is effective either as intralesional or as systemic therapy and the response rate and duration are currently the subject of research. Side effects are reported to be infrequent, and the therapy has little impact on the quality of life.

In our experience, the administration of dexamethasone as premedication, aided good tolerability of rituximab and, perhaps even afforded a synergistic effect with the monoclonal antibody.[16] The efficacy of rituximab in the treatment of different histological and biological subtypes of PCBCL remains to be defined. Synergistic effects of rituximab with interferon or other cytokines have not yet been studied in PCBCLs.

Conclusions

Our experience confirms the efficacy of rituximab in the treatment of PCBCL particularly in patients affected by DLCL and in patients with a lymphoma of follicular center.

The efficacy and low toxicity suggest that rituximab could be used for the treatment of PCBCLs in relapse after different, even aggressive, therapy and in elderly patients.

The high cost of rituximab is the most important factor limiting its prescription.

The study of a larger number of patients and a longer observation of patients in CR after rituximab therapy are necessary in order to be able to indicate rituximab as a front-line treatment for patients who are good responders to traditional treatments.

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Alemtuzumab (anti-CD52 monoclonal antibody, Campath-1H) in patients with advanced mycosis fungoides/Sézary syndrome

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Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of T-cell malignancies mainly affecting the skin. Mycosis fungoides (MF) with or without Sézary syndrome (SS) is the most common form of CTCL and represents 70% of all cases of CTCL.[1,2] The incidence of MF is approximately 0.4 per 100 000 per year.[3] The majority of patients are between 40 and 60 years old at diagnosis. The disease is twice as common in males as in females, and incidence rates are somewhat higher in black races than in whites.

The clinical course of MF/SS is usually indolent with pruritic erythematous areas slowly developing over long periods.[4] Eventually, however, the erythematous patches become progressively infiltrated, developing into plaques and finally to ulcerating tumors. Some patients may develop progressive, generalized erythema, frequently associated with severe itching. Other tissues and organs, such as peripheral blood, lymph nodes, or viscera, may also be involved. During disease progression, a defect in cell-mediated immunity becomes evident,[5,6] and septicemia and other infections are common causes of death in patients with advanced MF/SS. Transformation to a high-grade lymphoma may also occur during the course of the disease, and is associated with a poor prognosis.[7]

The traditional treatment of MF/SS includes both topical and systemic therapies, alone or in combination.[8] Once the disease becomes refractory to topical therapy, interferon-α, bexarotene, single-agent or combination chemotherapy may be given, but the duration of response is often < 1 year and ultimately all patients will relapse and become refractory.[8-12] There is, therefore, a great unmet need for novel treatment modalities for patients with advanced, symptomatic MF/SS. One such alternative may be monoclonal antibodies, having therapeutic advantages such as different mechanism(s) of action, no or only minor bone marrow toxicity, no hairloss/alopecia and delayed nausea/vomiting.

Trials have been conducted in patients with CTCL using the IgG2a murine monoclonal antibody T101 targeting CD5. HAMA were demonstrated in 5 of 10 CTCL patients and brief objective clinical responses were observed in 4/10 patients, but the effect was limited by antigenic modulation and the emergence of HAMA.[13]

A chimeric anti-CD4 monoclonal antibody was administered intravenously as a single dose to eight patients with mycosis fungoides.[14] Seven of eight patients responded to treatment with an average freedom from progression of 25 weeks (range, 6 to 52 weeks). Following treatment, there was significant suppression of peripheral blood CD4 counts in all patients, which lasted from 1 up to 22+ weeks.[14]

Alemtuzumab (CAMPATH-1H)

Alemtuzumab (Campath-1H) is a humanized IgG1 monoclonal antibody[15] directed against CD52, a glycosylated peptide antigen which is expressed on most malignant B- and T-cells[16] but not on hematopoietic stem cells.[17,18] The effector mechanisms of alemtuzumab and other Campath antibodies are not fully understood but may include antibody-dependent cellular cytotoxicity,[19,20] complement-mediated cell lysis,[15,21] and apoptosis.[22] Alemtuzumab has been developed primarily for the treatment of patients with B-cell chronic lymphocytic leukemia (B-CLL), in which response rates of 33–81%, depending on disease stage, have been reported.[23-25]

Malignant T-cells express particularly high numbers of CD52 cell-surface markers (approximately 500,000 molecules/lymphocyte[26]), and the intensity of CD52 expression seems to correlate with the clinical effects.[27] T-cell malignancies may therefore be particularly responsive to therapy with alemtuzumab. A high complete response rate was reported in patients with T-cell prolymphocytic leukemia (T-PLL) treated with alemtuzumab.[28] A phase II study 29 of 50 patients with advanced, heavily pretreated, low-grade, non-Hodgkin's lymphoma also included eight pilot patients with MF/SS; four of these eight patients responded to alemtuzumab therapy, but no further details were reported.

Alemtuzumab (anti-CD52 monoclonal antibody, Campath-1H) in patients with advanced mycosis fungoides/Sézary syndrome

A phase II, open-label study,[30] conducted in eight European centers, evaluated the safety and efficacy of alemtuzu-mab (Campath-1H) in 22 patients with advanced mycosis fungoides/Sézary syndrome (MF/SS) who had failed to respond adequately to therapy with at least PUVA and or radio/chemotherapy.[30] All patients had clinical symptoms or signs (pruritus, skin ulcers, B-symptoms, symptomatic lymphadenopathy, anemia, or thrombocytopenia) needing treatment. Alemtuzumab was adminis-

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tered intravenously using a rapidly-escalating initial dose regimen, followed by 30 mg three times a week for up to 12 weeks. The primary objective was to assess the overall response (OR) rate in these patients. Secondary objectives were to evaluate the safety profile of alemtuzumab and clinical benefit (i.e. relief from severe itching and time to progression in this population).

Patients received paracetamol (1 g orally) and an antihistamine (clemastine 2 mg iv) 30 minutes before the infusions. The use of corticosteroids (betamethasone 8 mg or hydrocortisone 100 mg iv) as secondary prophylaxis during week 1 in the case of flu-like, 'first-dose' reactions was optional. Once all 'first-dose' reactions had disappeared, clemastine and then paracetamol were gradually withdrawn. Patients also received prophylaxis with cotrimoxazole, twice daily, three times weekly, and valaciclovir 500 mg, twice daily, for a minimum of 2 months following discontinuation of alemtuzumab therapy. All patients received oral allopurinol 300 mg per day from days 1 to 28.

Most patients had stage III or IV disease, reduced performance status and severe itching. The overall response (OR) rate was 55%, with 32% having complete remission (CR) and 23% partial remission (PR). The effect was better in patients who had received 1–2 previous regimens (OR 80%) than in those who had received ≥ 3 prior regimens (OR 33%). In addition, itching, self-assessed on a 0–10 visual analog scale, was significantly reduced from a median score of eight before treatment to two at end of therapy. The median time to treatment failure was 12 months (range 5–32+). Cytomegalovirus (CMV) reactivation (causing fever without pneumonia and responding to ganciclovir) occurred in four patients (18%). Six more patients had other suspected or manifested infection: all these patients had received ≥ 3 prior regimens. These data show that alemtuzumab has promising clinical activity and an acceptable safety profile in patients with advanced MF/SS, particularly in patients with erythroderma and severe itching, and in those who are not heavily pretreated. The findings of this phase II, multicenter study therefore support the role of alemtuzumab as treatment for patients with MF/SS, requiring systemic therapy. These results also indicate that there is scope for investigating combination therapies with other modern drugs in the treatment of advanced CTCL.

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Stem cell transplantation in cutaneous T-cell lymphomas

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Mycosis fungoides (MF) and Sézary syndrome (SS) are the most frequent primary lymphomas involving the skin. The average age at diagnosis is about 50 years. Histopathological features, similar in both diseases, are marked epidermotropism of T-lymphocytes with convoluted pleomorphic or cerebriform nuclei. The malignant cells more commonly express a mature and memory T-helper phenotype ($CD45RO^+CD4^+$) and their clonality always emerges from T-cell receptor (TCR)-β-γ gene rearrangements by molecular analyses.[1]

Although the natural history of MF is usually indolent, many patients eventually progress to more advanced stages with an aggressive clinical course and shortened survival. In a large series of patients[2] the median survival of all patients with stage IV disease was 13 months from the date of first treatment; even shorter survival (1 year) was observed in patients aged 58 or more, who are generally not eligible for more aggressive therapies. The prognosis is even worse in SS patients, who have a median survival ranging between 2-4 years from diagnosis.

For patients with advanced MF/SS, single-agent or combination chemotherapy offers a high response rate, but does not confer any survival advantage in comparison to more conservative approaches, such as psoralen ultraviolet light A (PUVA), total electron beam irradiation (TSEB), extracorporeal photophoresis (EP), and/or interferon (α-IFN).[3] For this reason high dose therapies with the support of either autologous or allogeneic hematopoietic stem cells (HSC) have been explored.

Autologous HSC transplantation (AHSCT)

AHSCT is a well established treatment modality which has been able to significantly improve survival of patients with relapsed Hodgkin's and non-Hodgkin's lymphomas.[4] Singles case of AHSCT have been reported in the literature[5,6] and a larger series was published by Bigler *et al.*[7] in 1991. The majority of the cases reached a complete remission (CR) but this was invariably short, generally lasting a few months. Our experience in 3 patients with advanced CTCL was similar. All three patients were previously treated with chemotherapy to reduce tumor burden and 2 patients were *consolidated* after the transplant with either TSEB or α-IFN. However one patient died shortly after the transplant because of sepsis, and the other 2 relapsed within 5 and 7 months after the transplant and eventually died at 10 and 20 months after the transplant from disease progression (Table 1). None of these three patients had bone marrow involvement and bone marrow was used for the transplant, suggesting that recurrence of the disease arose from residual CTCL cells. This hypothesis was further confirmed by a recent and larger study by Olavarria *et al.*[8] who transplanted 8 patients with T-cell-depleted ($CD34^+$ and $CD4/CD8^-$) peripheral blood stem cells. At the time of Olavarria's report only one patient was still in CR, the median duration of CR having been 7 months and the median survival after the transplant 11 months. Taken together these data suggest that AHSCT is associated with high transplant-related mortality, produces only short-lasting remissions and, therefore, should not be recommended.

Allogeneic stem cell transplantation

Allogeneic stem cell transplantation (SCT) may, at least theoretically, be applicable for the treatment of cutaneous T-cell lymphomas for several reasons. Indirect effects of an immune recognition of MF/SS cells may be the presence of $CD8^+$ lymphoctes in the perilesional skin and the response to a variety of treatments including photopheresis,[9] systemic high dose interleukin-2[11] and interleukin-12,[12] which are known to selectively modulate immune responses or elicit cell-mediated cytotoxicity. An anti-MF activity through immune mechanisms was observed in phase I/II trials using systemic cytokines or fusion toxins.[12] Furthermore, the graft-versus-lymphoma effect is known to be associated with graft-versus-hosrt disease (GVHD) and the skin is the predominant target for both GVHD and MF. In addition conventional allogeneic bone marrow transplantation was already reported by different authors in at least 9 patients with MF/SS.[13-19] The majority of these patients achieved long-lasting remissions and in some cases a cutaneous relapse was completely resolved by discontinuing immunosuppression. All these data unequivocally prove the existence of a strong graft-versus-lymphoma effect towards CTCLs.

However, the feasibility of conventional allografting has been limited by many factors, most related to the clinical features of MF/SS. In particular the advanced median age at diagnosis, the indolent clinical course in early stages, but also the multiple skin lesions favoring

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Table 1: AHSCT for CTCL

Inits	Age	Diagnosis	Stage	Response	Relapse	Outcome
RE	32	CD8+ CTCL	IVA	RC	5m.	Died +10m.
AJ	31	pleom CD4+ CTCL	IVA	RC	7m.	Died +20m (leuk tr.)
BE	53	CD4 MF	IVA	died +12d for infection		

Table 2: NST in CTCL

	Age	sex	Diagnosis	Stage
1	56	M	MF	IVA T4-N3-B1
2	47	M	MF	IVA T4-N3-B1
3	37	M	MF	IVA T4-N3
4	54	F	MF	IVA T4-N3
5	50	F	MF/foll. mucinosis	IB

Table 3: NST in CTCL

Conditioning	*Graft*	*Response*	*Outcome*	*Complications*	*GVHD*
1. Flux/TBI200	PBSC	CR	Alive in CR +28m	EBV related LPD	no
2. Flux/TBI200	PBSC	CR	Alive in CR +24m	CNS EBV infection CMV react.	limited cGVHD
3. Flux/TBI200	PBSC	CR	died +76d	SA bacteremia, BK hem. Cystitis CMV react.	aGVHD grade II (skin)
4. Pentostatin/TBI200	PBSC	PR	died +33	multiple infections CMV react.	aGVHD grade II
5. Pentostatin/TBI200	PBSC	PR	Alive +100	no	no

systemic infections,[20] and the compromised immunity worsened by the prior lines of therapy[21] caution against an approach which carries a high treatment-related mortality. In these patients the recent introduction of allogeneic stem cell transplantation with reduced intensity conditioning[22] was, therefore, seen as a reasonable alternative. These regimens, which generally include flurarabine, have been designed to be immunosuppressive, rather than myeloablative (from which their name, non-myeloablative stem cell transplantation - NST), in order to facilitate donor engraftment with limited systemic toxicity.[23] We developed a regimen to suppress the recipient's immune system sufficiently to allow allogeneic engraftment without causing excessive regimen toxicity or GVHD. In addition, the use of fludarabine in combination with cyclophosphamide, whose efficacy in primary T-cell lymphomas have already been reported,[24] was aimed to debulk the tumor burden prior to transplantation. To further improve the anti-tumor effect of the conditioning regimen further, while still providing adequate immunosuppression, we have recently substituted fludarabine with pentostatin, a purine analog with proven efficacy in CTCL.[25,26] We have so far treated 5 patients with advanced CTCL with this protocol (Tables 2 and 3). All patients rapidly achieved full donor chimerism, already by day +14 after the transplant: this could have been facilitated by the severe immunodeficiency induced by both the underlying disease and the immunosuppresive regimen. Three patients achieved clinical, histologic and molecular remissions already between day +30 and +90 and two patients had a partial response and are not evaluable for complete response. It is unlikely that the remissions obtained in our patients were produced by the transplant regimen for several reasons. Radiaton therapy, as commonly delivered in reduced intensity transplants (TBI, 200 cGy at 7 cGy/min), is unlikely to be effective in controlling advanced cutaneous T-cell diseases, since conventional autologous bone marrow transplantation with total body irradiation was invariably associated with short-lived responses in all published cases. Purine analogs can obtain complete remission rates up to 30% but responses are of short duration and cyclosporine A (used in combination with myco-

phenolate for GVHD profilaxis) has been used in the treatment of the disease but with rapid recurrences after withdrawal.[27] On the other hand there was a close temporal correlation between the onset of full donor T-cell chimerism, modulation of immunosuppresion, disappearance of the MF/SS lesions (both clinically, histopathologically and molecularly) and development of skin GVHD. The interpretation of the skin lesions can be difficult because of the frequent overlapping of signs of MF and GVHD and generally requires regular and systematic biopsies. Nevertheless these data confirm the existence of a strong graft-versus-CTCL effect and indicate that allogeneic stem cell transplantation may be the only curative option in these patients.

While hematologic and general toxicity was very low and GVHD was always limited to the skin, a high incidence of bacterial and viral infections was observed: these infections were sometimes life-threatening and were fatal in two cases. Gram positive infections of the skin, blood and indwelling catheters have been documented in more than 80% of patients with CTCL, with *S. aureus* bacteremia being the most common cause of death.[1,20,28] It has been suggested that patients with CTCL and HLA-DR5 in association with HLA-DQ*03[29] have a genetic susceptibility to *S. aureus* colonization: patient #3 was indeed DR5, DQ*03 and probably succumbed to a *S. aureus* sepsis (and was particularly susceptible to *S. aureus* colonization, sepsis and cardiovascular complications associated with toxigenic staphylococcci). On the other hand the viral infections we observed were both atypical (peculiar, rare) and uncommonly frequent: Epstein-Barr virus (EBV)-related lymphoproliferative diseases have been seen only in patients transplanted with T-cell depleted grafts,[30] EBV-associated encephalopathies have been described only in pediatric patients with severe immunodeficiencies[31] and severe BK virus-associates cystitis[32] has been reported only in patients treated with conventional myeloablative stem cell transplantation. This suggests that these patients may have a profound defect in cell-mediated immunity, possibly induced by the underlying disease, by prior therapies and the pre- and post-transplant immunosuppression.

Although selection of patients, preparative regimens, as well as identification and treatment of infectious complications should be further improved, allogeneic stem cell transplantation, possibly with reduced intensity conditioning regimen, seems the only curative option in these patients.

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