MOLECULAR CYTOGENETICS OF PRIMARY CUTANEOUS T-CELL LYMPHOMAS : FROM CYTOGENETICS TO THE IDENTIFICATION OF SPECIFIC GENE-LEVEL ABERRATIONS

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Academic dissertation

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II Karenko L, Sarna S, Kähkönen M, Ranki A: Chromosomal abnormalities in relation to clinical disease in patients with cutaneous T-cell lymphoma: a 5-year follow-up study. *Br J Dermatol* 148:55-64, 2003

III Karenko L, Nevala H, Raatikainen M, Franssila K, Ranki A: Chromosomally clonal T-cells in the skin, blood or lymph nodes of two Sezary syndrome patients express CD45RA, CD45RO, CDw150, and interleukin-4, but no interleukin-2 or interferon-γ. *J Invest Dermatol* 116:188-193, 2001

IV Karenko L, Kähkönen M, Hyytinen E-R, Lindlöf M, Ranki A: Notable losses at specific regions of chromosomes 10q and 13q in the Sézary syndrome detected by comparative genomic hybridization. *J Invest Dermatol* 112:392-395, 1999

V Karenko L, Hahtola S, Karhu R, Syrjä S, Kähkönen M, Nedoszytko B, Kytölä S, Pesonen M, Nupponen N, Harri Sihto H, Poustka A, Krebs I, Nevala H Roszkiewicz J, Peterson P, Visakorpi T, Ranki A. Primary cutaneous T-cell lymphomas (CTCL) show a deletion or translocation affecting NAV3, the human UNC-53 homologue. Submitted

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2. ABBREVIATIONS

ATM: ataxia telangiectasia mutated

BER: base-excision repair or nucleotide-excision repair

CGH: comparative genomic hybridization

CIN: chromosomal instability

CISS: chromosome *in situ* suppression hybridization, CISS

CTCL: cutaneous T-cell lymphoma

EORTC: The European Organization for Research and Treatment of Cancer

FICTION: fluorescence immunophenotyping and interphase cytogenetics as a tool for investigation of neoplasms

FISH: fluorescence *in situ* hybridization

FM: follicular mucinosis

ISH: *in situ* hybridization

LINE: long interspersed nuclear element

LPP: large plaque parapsoriasis

LOH: loss of heterozygosity

MF: mycosis fungoides

MFISH: multifluor FISH, one form of 24-colour FISH e.g. multicolour FISH

MMR: mismatch repair

NER: nucleotide-excision repair

NHEJ: non homologous end joining

PPS: parapsoriasis en plaques

PUVA: Psoralen + UVA

SKY: spectral karyotyping, one form of 24-colour FISH e.g. multicolour FISH

SS: Sézary syndrome

3. ABSTRACT

Cutaneous T-cell lymphomas (CTCL) are a heterogenous group of non-Hodgkin-lymphomas (NHL), belonging to primary cutaneous lymphomas and show a growing incidence. The most common form of CTCL is mycosis fungoides (MF), and its leukaemic form with erythrodermia and affected lymph nodes is called Sézary syndrome (SS). Sézary syndrome may evolve from MF, or start directly as SS.

Clinically, MF usually develops slowly over years from eczema like patches resulting in tumours or erythroderma. In the skin lesion of MF malignant CD4-positive, atypical lymphocytes infiltrate towards the epidermis, surrounded by reactive (e.g. cytotoxic) T lymphocytes. At later stages, the malignant cells may be observed in the lymphoreticular system, blood, kidneys, lungs and brain. The cause, anatomical site and timing of malignant transformation are unknown. Early-stage MF is difficult to diagnose clinically and histologically, because it resembles benign, inflammatory eruptions e.g. alopecia mucinosa or large-plaque parapsoriasis (LPP). A controversy prevails, as to whether LPP is a premalignant or a fully malignant condition. T-cell gene rearrangement analysis has not provided decisive help beacuse of its relative lack of specificity, but has indicated early systemic spread of the disease. None of the treatments used is curative, but at early stages of the disease, long term remissions can be achieved.

Acquired, recurrent chromosomal aberrations in cancer associate with malignant transformation and disease progression. Such aberrations may reveal microscopically observable amplification of oncogenes, deletions of tumour suppressor genes or translocations producing functional fusion genes or disrupting tumour suppressor genes. Patients with CTCL commonly show a wide variety of clonal or non-clonal chromosomal aberrations in their blood or skin. The conventional cytogenetic studies of CTCL are difficult, as the malignant cells respond poorly to mitogens needed for inducing visible, analysable mitotic chromosomes for G-banding staining. Before the molecular cytogenetic studies presented here, no recurrent specific abnormality had been found in CTCL.

In the two first studies of this thesis, chromosomal aberrations in the peripheral blood were screened with both G-banding and *in situ* hybridizations (ISH) to interphase cells. Statistically significant differences between different diagnostic groups (healthy, parapsoriasis and CTCL) were found for aberrations of several chromosomes, that can together be applied in diagnostics. In the follow-up, patients with an active and progressing disease differed from patients in stable remission for aberrations of chromosomes 1,6,8,11,and 17. Remarkably, patients in stable remission also differed from healthy controls for chromosomes 1,6,11, indicating a persisting disease. Patients with active but stable disease differed from healthy controls for aberrations of the same chromosomes and chromosome 8, too. Healthy controls did not differ statistically from controls with non-malignant conditions treated with PUVA, the most commonly used treatment of the patients included in the studies. The presence of chromosomal clones were associated with active or progressive disease, e.g. preceded a relapse. The finding of chromosomal abnormalities or a clone in LPP was novel.

The phenotype of individual chromosomally clonal malignant cells in tissue samples was studied in Sézary syndrome using a new modification of the combination of immunohistology with fluorescent *in situ* hybridization (FICTION). With the phenotype CD45RA+, CD45RO+, CDw150±, IL-4+, IL-2-, IFN-γ-, and variation in IL-10 expression between the patients, the clonal cells seem to be intermediate forms between naive CD45RA+ and mature CD45RO+ Th2 cells. Cells representing the malignant chromosomal clone were detected in lymph nodes with dermatopathic lymphonoditis histology,

even several months prior to histologically malignant infiltrate in the skin. Thus, this study confirms the concept of the early systemic nature of CTCL.

Patient DNA-based comparative genomic hybridization (CGH), that reveals also potential aberrations in cells not inducible to division, showed losses (deletions) of DNA-regions most commonly in chromosomes 10q25-q26, 13q21-q22 and gains (amplifications) in chromosomes 8 and 17q21-q25 in Sézary syndrome. This was the first CGH study of CTCL, and the results have been confirmed by later studies. The chromosomal regions found by this method may harbour genes important for the aetiology or progression of CTCL. The method is not suitable for the study of balanced translocations with no visible loss of chromosomal material.

The origin of chromosomes involved in clonal translocations in blood lymphocytes was studied with 24-colour hybridizations to the blood lymphocyte metaphases of a group of consecutive CTCL-patients. The most commonly affected chromosome in this analysis was chromosome 12q. Locus-specific probes showed, that the break-point of a balanced translocation of one patient was in the middle of the 7 YAC's long minimal common region of a proximal and a distal deletion of two other patients. The translocation disrupted a 40 exon-long gene, neuron navigator 3 (NAV3). Using FISH with BAC-probes specific to parts of the gene, deletions of the gene were observed in the skin lesions of 4 of 8 (50%) patients with early MF and a deletion or a translocation was observed in 11 of 13 (85%) patients with advanced MF or SS. Of the three patiets studied above, the first patient having a distal deletion showed a point mutation in NAV3. NAV3 (POMFIL1), with possible location in the nuclear pore complex. It is expressed in neural tissue and by RT-PCR performed in this study, in normal lymphocytes. By structure, it is a helicase and may also have roles in cell signalling. NAV3 may also be a nonclassical tumour suppressor gene showing haploinsufficiency. The deletion of NAV3 is currently being developed for a new clinical diagnostic test.

4. INTRODUCTION

Cutaneous T-cell lymphomas (CTCL) are a heterogenous group of non-Hodgkin-lymphomas (NHL), belonging to primary cutaneous lymphomas, that first present in the skin (Willemze *et al.* 1997). During the past decades, the incidence has been increasing in the developed world including Finland; however the increasing trend in USA has possibly stabilized lately (Weinstock and Horm 1988, Weinstock and Gardstein 1999, Siegel *et al.* 2000, Väkevä *et al.* 2000; The incidence is higher in men, in Finland now about 2.5 /100 000 men (Väkevä *et al.* 2000).

The most common form of CTCL is mycosis fungoides (MF), which is difficult to diagnose in its early presentations since the skin lesions resemble benign, inflammatory conditions like eczema (Willemze 1987, Payne *et al.* 1992, Shapiro and Pinto 1994) or the premalignant large-plaque parapsoriasis (LPP, Parapsoriasis en plaques), that may evolve into MF or even already be MF (Burg *et al.* 1995, 1996, 2001, King-Ismael *et al.* 1992, Ackerman and Schiff 1996, MacKie 1998). T-cell gene rearrangement analyses have not provided decisive help for their unspecifity, and histologic examination still shows high rates of false negatives (up to 25%, Santucci *et al.* 2000).

In the early phase of CTCL, CD4-positive lymphocytes infiltrate towards the epidermis. The time and compartment of malignant transformation are not known (Veelken *et al.* 1995, MacKie 1998, Burg *et al.* 2001). In most cases, MF develops slowly over years from eczema-like patches, through plaques to tumours or erythroderma. The skin infiltrates of CTCL typically also consist of a dense infiltrate of reactive T lymphocytes. A cytotoxic T-cell response directed against the tumour cells seems to control the malignancy to some extent (Bagot *et al.* 1998, MacKie 1998, Bagot *et al.* 1998). At later stages, malignant cells may be observed in the lymphoreticular system and peripheral blood, and they invade kidneys, lungs and brain. The triad of erythroderma, with lymphoid and blood affision, showing malignant cells with cerebriform nuclei (Sézary cells), is called Sézary syndrome (SS; Sézary and Bouvrain 1938, Willemze *et al.* 1997), an aggressive form of CTCL. The latter may also evolve directly, without preceding MF.

The 5 year survival in MF is 87%, but prognosis varies with stage (Willemze *et al.* 1997). The 5-year survival of patients with Sézary syndrome is 11% (Willemze *et al.* 1997, Whittaker *et al.* 2003, review). About 20% of CTCL cases undergo rapid progression or transformation which cannot be predicted by any current means. The 5-year survival of these patients is less than 15% (Cerroni *et al.* 1992).

A conservative and stage-adjusted treatment approach is widely accepted. Commonly used treatments for early-stage CTCL include topical corticosteroids, mechlorethamine, carmustine (BCNU), electron beam irradiation, low-dose methotrexate, UVB and PUVA. Local electron beam irradiation therapy is used for stages IB-IIB, and whole body TSEB (total skin electron beam) is indicated for widespread infiltrated plaque and tumour stage disease. Interferon alpha has been used alone or in combination with PUVA. Retinoids may be valuable for early and moderately advanced CTCL and may be used in combination with IFN (Whittaker *et al.* 2003). Systemic disease (III-IVB) requires combination chemotherapy but responses are usually of short duration. The costly extracorporeal photopheresis (ECP) has been used for Sézary syndrome but the response rates vary widely among treatment centres and there are no randomized studies to clarify whether it has any effect on overall survival (Roupe *et al.* 1996, Willemze *et al.* 1997, Muche *et al.* 2000a, Whittaker *et al.* 2003). None of the therapies used is curative, but as treatment at early stages may result in long-lasting remissions (Roupe *et al.* 1996), more sensitive and accurate diagnostic methods are needed.

The aetiology and pathomechanism of CTCL are poorly understood. Studies of retroviruses, occupational or environmental factors have not revealed a consistent, causative agent (Ranki *et al.* 1990, Burg *et al.* 2001; Girardi 2004, review). Cytogenetic studies preceding the studies presented here, showed a large repertoire of chromosomal aberrations, clonal or non-clonal, but no specific or recurrent one, leading to a hypothesis of genetic instability (Whang -Peng *et al.* 1982; Kaltoft *et al.* 1992, 1994, Thestrup-Pedersen *et al.* 1994). The conventional G-banding method used, required mitotic cells

typically difficult to obtain in CTCL (Burg 1978). Gene-level abnormalities were unknown, except for the observations of roles or lacking of roles of some common oncogenes often in a small number of cases (p53, c-myc, lyt-10; Peris *et al.* 1991, Tosca *et al.* 1991, Ro *et al.* 1993, Garatti *et al.* 1995, Laurizen *et al.* 1995).

In the studies presented here, the new methodology of *in situ* hybridizations was used for studying interphase cells for diagnostic and follow-up purposes, for the study of the role of chromosomes in the pathogenesis and progression of the disease, for identification of individual malignant cells for the study of their phenotype, for screening the whole genome to detect DNA-copy number changes reflecting regions of amplified oncogenes or deleted tumour suppressor genes, and finally, for specifying previously unidentifiable chromosome parts in structural chromosome aberrations and for the identification of genes involved in them. All these approaches proved to be fruitful.

5. REVIEW OF THE LITERATURE

5.1. Clinical and pathologial aspects of cutaneous T-cell lymphoma (CTCL)

5.1.1. The classification of cutaneous T-cell lymphomas

Classification of primary cutaneous lymphomas The previously used classifications, such as the updated Kiel classification, the Working Formulation (of NIH) and the Revised European-American Lymphoma (REAL) classification were inadequate for classifying primary cutaneous lymphomas, as they e.g. could group together clinically different diseases and immunophenotyping was not used as a grouping criterion. Thus, The European Organization for Research and Treatment of Cancer (EORTC) Cutaneous Lymphoma Project Group published (Willemze *et al.* 1997) a new classification of primary cutaneous lymphomas, that is based on clinical, histological, and some immunophenotypical and genetic features (CD30 antigen positivity, T-cell receptor gene analysis). After that, WHO has published a new, general classification of lymphomas (Sander *et al.* 2001 reviewed in Girardi *et al.* 2004).

EORTC classification of primary cutaneous lymphomas The EORTC classification defines primary cutaneous lymphomas as non-Hodgkin lymphomas presenting in the skin, with no evidence of extracutaneous disease at the time of diagnosis and within the first 6 months after diagnosis. Exceptions to the 6 months' rule are classical mycosis fungoides (MF) presenting in the skin with peripheral lymph node involvement and Sézary syndrome (SS). Malignant lymphomas with secondary skin involvement, lymphomas in immunocompromised patients and HTLV-I-associated adult T-cell lymphoma leukaemias (ATLL) are excluded.

Primary cutaneous T-cell lymphomas in EORTC classification According to the EORTC classification, primary cutaneous T-cell lymphomas are mycosis fungoides (MF), MF-associated follicular mucinosis, variants of MF, pagetoid reticulosis, and granulomatous slack skin, Sézary syndrome (SS), lymphomatoid papulosis, CD30-positive large T-cell lymphoma (anaplastic, pleomorphic or immunoblastic), CD30-negative large T-cell lymphoma (pleomorphic large cell, or immunoblastic), pleomorphic (small/medium-sized) lymphoma and subcutaneous panniculitis-like T-cell lymphoma. The most common is MF, with a relative frequency of 44% of all primary cutaneous lymphomas, and a median 5-year survival of 87% depending on the stage (Willemze *et al.* 1997). The 5-year survival for stage IA patients has been reported to be nearly normal, and for stage IB, IIA, IIB, III, IVA and IVB patients has been reported to be 73-86%, 49-73%, 40-65%, 45-57%, 15-40%, and 0-15%, respectively (Whittaker *et al.* 2003, review). In contrast, Sézary syndrome, with a relative frequency of 2%, is an aggressive disease with 5-year survival of 11% (Willemze *et al.* 1997).

In the EORTC classification, MF is defined as an epidermotropic CTCL characterized by a proliferation of small or medium-sized neoplastic T lymphocytes with cerebriform nuclei. Classically, it evolves from patches, to plaques, and later tumours (Willemze *et al.* 1997). Histopathologically, papillary dermis shows epidermotropic, band-like infiltrates of small, medium-sized and occasionally large mononuclear cells with hyperchromatic, cerebriform nuclei and an admixture of inflammatory cells. Small groups of neoplastic cells colonize lower layers of the epidermis. Characteristic Pautrier's microabscesses (clusters of malignant cells in the epidermis) are seen in only a minority of cases. As MF progresses to tumour stage, the infiltrates become more diffuse, the proportion and size of tumour cells increases, and epidermotropism decreases (Willemze *et al.* 1997).

The malignant cells in MF are mature Th1 memory cells, and immunophenotypically usually $CD3^+$, $CD4^+$,$CD45RO^+$, $CD8^-$, and $CD30^-$, in rare cases $CD3^+$, $CD4^-$, and $CD8^+$ (Saed *et al.* 1994, Willemze *et al.* 1997).

Sézary syndrome (SS) is defined by erythroderma, generalized lymphadenopathy and the presence of neoplastic T cells, Sézary cells, with cerebriform nuclei, in the blood (Willemze *et al.* 1997 review). SS may be preceded by MF or

nondiagnostic dermatitis. SS patients characteristically have a pruritic erythroderma, and may have alopecia, onychodystrophy or palmoplantar hyperkeratosis (Willemze *et al.* 1997 review).

Histopathologically SS resembles MF, but the lymphocyte infiltrate may be more monotonous and epidermotropism may be absent. The same type of infiltrate of SS cells is seen in lymph nodes. A usual criterion for Sézary syndrome diagnosis is at least 1,000 Sézary cells per mm^3 of blood, but this level can been reached in benign erythrodermas as well, and EORTC has proposed an additional criterion the ratio CD4/CD8 >10 (Willemze *et al.* 1997).

The malignant cells in SS are mature Th2 memory cells, and immunophenotypically usually $CD3^+$, $CD4^+$, $CD45RO^+$, $CD8^-$, and $CD30^-$, in rare cases $CD3^+$, $CD4^-$, and $CD8^+$ (Vowels *et al.* 1992, Saed *et al.*1994, Dummer *et al.* 1996, Willemze *et al.* 1997).

Up to 20% of cases with advanced CTCL transform cytologically, developing the appearance of a large-cell lymphoma showing more than 25% of pleomorphic large cells with prominent nucleoli, CD30 positivity and an increase in the clinical aggressiveness of the diseases (Cerroni *et al.* 1990, 1992, Wolfe *et al.* 1995).

MF-associated follicular mucinosis is a special group of T-cell lymphomas in EORTC-classification. Follicular mucinosis is characterized by accumulation of mucin within hair follicles. The reaction may occur in a number of inflammatory, infectious and neoplastic conditions (Cerroni *et al.* 2002). A non-malignant, idiopathic FM (synonym alopecia areata) has been considered to occur preferentially in children and young adults, and FM associated with MF occur in older patients. However, according to recent reports, the two groups cannot be distinguished in a clear cut manner. There is overlapping of the age spectrum of the patients, locations of the lesions (often in head and neck), and histopathology, with dense lymphoid infiltrates in seen also the idiopathic form. The conditions cannot be distinguished immunohistologically either, and the frequency clonal TCR- gene rearrangements (see below) is similar (Mehregan *et al.* 1991; van Doorn *et al.* 2002, Cerroni *et al.* 2002, both including references to previous studies). As progression of idiopathic follicular mucinosis into CTCL has also been documented in several cases, it has been suggested, that idiopathic follicular mucinosis may represent a form of localized CTCL (Sentis *et al.* 1988, Cerroni *et al.* 2002, including references to previous studies).

WHO classification The classification of WHO resembles the EORTC classification, except that the CD30 positive large cell cutaneous T-cell lymphoma and the pleomorphic (small/medium sized) cutaneous T-cell lymphomas are grouped together into peripheral T-cell lymphoma, not otherwise specified (Willemze *et al.* 1997, Sander *et al.* 2001 reviewed in Girardi *et al.* 2004).

5.1.2. Staging of MF and Sézary syndrome

Cutaneous lymphomas can be staged according to the TNM-system (**Table Ia**) or a clinical staging system designed for CTCL and SS suggested by the North American MF Cooperative Group (**Table Ib**). The latter has been used in Helsinki University Central Hospital Skin and Allergy Hospital. The staging systems are based on skin, nodal, visceral and blood involvement (Bunn *et al.* 1979, 1980b, MacKie 1998, Girardi *et al.* 2004, review).

Table Ia. TNM-classification of CTCL[a]

T1	Limited patches/plaques (<10% of total skin surface)
T2	Extensive patches/plaques (>10% of total skin surface)
T3	Tumours
T4	Erythroderma
N0	No clinical lymphadenopathy
N1	Histologically uninvolved, enlarged lymph nodes
N2	Histologically involved, unenlarged lymph nodes
N3	Histologically involved, enlarged lymph nodes
M0	No visceral involvement
M1	Visceral involvement
B0	No peripheral Sézary cells (<5% of total lymphocyte count)
B1	Peripheral blood Sézary cells (>5% of total lymphocyte count)

[a]Bunn and Lamberg 1979

Table Ib. Staging of MF by North American MF Cooperative Group[a]

IA	T1, N0, M0
IB	T2, N0, M0
IIA	T1 or T2, N1, M0
IIB	T3, N0 or N1, M0
III	T4, N0 or N1[b]
IVA	T1 - T4, N2 or N3, M0
IVB	T1-T4, N0-N3, M1

[a]Bunn and Lamberg 1979. [b]Stage III can be further divided into IIIA (T4N0M0) and IIIB (T4N1M0) (Girardi et al. 2004, review).

5.1.3. Parapsoriasis en plaques

Parapsoriasis is a term including a group of uncommon but not rare inflammatory disorders, which are not necessarily related. The terminology was previously very variable, but the following groups have gained general practical acceptance (Lambert and Everett 1981, MacKie 1998).

Large plaque parapsoriasis (LPP; parapsoriasis en plaques, synonyms atrophic parapsoriasis, poikilodermatous parapsoriasis). The skin shows slighty indurated, red-blue scaly plaques with indistinct, irregular borders mainly on the buttocks, proximal extremities or in women, on the breasts. The size of most lesions is over 10 cm in diameter. Epidermal atrophy is often seen. The histology of early lesions is not diagnostic. Slight spongiosis with minimal exocytosis and slight upper dermal perivascular lymphocytic infiltrate may be seen (Lambert and Everett 1981). A lichenoid or interface reaction may be seen at the dermo-epidermal junction and a band-like infiltrate in the papillary dermis may be seen. The overall

pattern may be reminiscent of mycosis fungoides (MacKie 1998). About 10% of cases develop cutaneous lymphoma. Most cases progress to cutaneous lymphoma (Lambert and Everett 1981).

Small plaque parapsoriasis (Variants: digitate dermatosis and xanthoerythrodermia persistans). In skin, especially on the trunk, well-defined, round or ovoid, slightly scaly, nonatrophic, nonindurated, erythematous, yellow or blown macules or very thin plaques, mostly less than 5 cm in diameter, are seen. In digitate dermatosis, the lesions are elongated and tend to palisade, in xanthoerythrodermia persistans, a variant of digitate dermatosis, the colour of the lesions is yellow. The histology is not diagnostic. (Lambert and Everett 1981). Epidermis shows small focal areas of hyperkeratosis and parakeratosis, and in dermis, small aggregates of morphologically normal T-helper cells around the vasculature (MacKie 1998). Previously, progression to cutanenous lymphoma was not implicated (MacKie 1998), however, according to a recent Finnish study, a minority of patients may develop MF (Väkevä *et al.* 2004 submittted). The term guttate parapsoriasis has been used to denote pityriasis lichenoides, but some authors use it for small plaque parapsoriasis (Lambert and Everett 1981).

Pityriasis lichenoides (Synonyms: pityriasis lichenoides et varioliformis acuta, PLEVA, PLVA, Mucha-Habermann disease). All authors do not accept this disease into the group of parapsoriases (Lambert and Everett 1981). Clinically, the skin shows generalized erythematous or brown, often haemorrhagic, scaly papules and small macules that either persist for several months or recur periodically. Histologically, parakeratosis, epidermal necrosis, dilated and haemorrhagic small blood vessels in the papillary dermis and a wedge-shaped lymphohistiocytic inflammatory infiltrate is seen. Atypical lymphocytes and histiocytes may be present, and such cases are termed lymphomatoid papulosis. Pityriasis lichenoides tends to clear spontaneously after weeks to months (Lambert and Everett 1981). Lymphomatoid papulosis has usually a benign clinical course, but up 20% of patients develop a malignant lymphoma, e.g. mycosis fungoides, Hodgkin's disease or CD30+ large cell lymphoma (Willemze *et al.* 1987).

The term guttate parapsoriasis has been used to denote pityriasis lichenoides, but some authors use it for small plaque parapsoriasis (Lambert and Everett 1981).

A controversy prevails over, whether LPP is already an early stage MF (King-Ismael and Ackerman 1992, Burg *et al.* 1995, 1996, 2001, Ackerman and Schiff 1996). Like MF, LPP may show clonal TCR gene rearrangements (see below) and abnormal telomerase activity is present (see below), which would point to an existing neoplasia from the beginning of the skin lesion. However, early LPP can not be discerned histologically from non-malignant conditions, which would indicate a step-wise process occurring in the skin (Kikuchi *et al.* 1993, Burg 2001). A similar debate concerns small plaque parapsoriasis (Ackerman and Schiff 1996).

5.1.4. The biology and development of T-lymphocytes in relation to the development of CTCL

About the aetiology of CTCL Normally, precursors of T-cells proliferate in the thymus, where they select their antigen specificity, and specialize to naive cytotoxic or naive helper T- cells. Thereafter, they circulate between blood and secondary lymphoid organs, where they may be activated by antigen presenting cells, proliferate, and enter through blood their target tissue, like skin, where they polarize to e.g, Th1 or Th2-cells. The cause, compartment or timing of malignisation of T-cells in CTCL are not known. A continuous or longstanding polyclonal proliferation could give rise to malignant transformation. Such a proliferation has been suggested to be caused by infectious agents and occupational exposures, but no consistent evidence exists (Fischmann *et al.* 1979, Greene *et al.* 1979, Ranki *et al.* 1990, Zucker-Franklin *et al.* 1991, Heald *et al.* 1993, Lambert 1994, Kim *et al.* 1998, Abrams *et al.*1999, Burg *et al.* 2001; Girardi *et al.* 2004, review). A malignant clone may be present in the skin from the beginning of the infiltrative process (Veelken *et al.* 1995). The skin infiltrates of CTCL typically also consist of a dense infiltrate of reactive T lymphocytes. A cytotoxic T-cell response directed against the tumour cells seems to control the malignancy to some extent (Bagot *et al.* 1998). On the other hand, bcl-2 mediated apoptosis is weak (Dummer *et al.* 1995, Nevala *et al.* 2001).

Cell cycle Cell proliferation occurs in cycles consisting of four phases regulated by activation and degradation of cyclins associated with cyclin dependent kinases (CDK) (**Figure 1**.) The CDKs are activated by dephosphorylation of threonine and tyrosine residues by Cdc25s phophatases upregulated in the G1 to S transition and inhibited by several inhibitors, e.g. $p15^{INK4b}$, $p16^{INK4a}$, $p21^{CIP}$, and $p27^{KIP1}$ (reviewed in Fukada *et al.* 1998). T- cell proliferation or differentiation is influenced by molecules of their surroundings, for example, by cytokines including e.g. interleukins, which are soluble proteins influencing e.g. cell proliferation, differentiation, and inflammation (pro and anti-inflammatory cytokines, distinction not sharp; Del Prete *et al.* 1993, Lauw *et al.* 2000, Schreiber *et al.* 2000; Alam and Gorska 2003, Borish and Steinke 2003, reviews), and activate cells by specific receptors. The signal of cytokine receptors situated on the cell surface is commonly mediated by receptor-associated janus kinases (Jak) phosphorylating signal transducer and activator of transcription (STAT) molecules, that enter the cell nucleus, bind to DNA and regulate transcription (O'Shea *et al.* 2002 review). For example, activated STAT3 induced by interleukin (IL)- 6 receptor promotes G1 to S transition by upregulating cyclins D and A and cdc25 and inhibiting p21 and p27, inhibitory factors upregulated by IL-6 receptor, too (Fukada *et al.* 1998, Heinrich *et al.* 2003 review about IL-6-type cytokine signalling). Also, STAT5 regulates transcription of cyclins D1/D2 (Matsumura *et al.* 1999, Wen *et al.* 1999, de Groot *et al.* 2000). In some other cases, cyclins may regulate STATs (Drosophila; Chen *et al.* 2003).

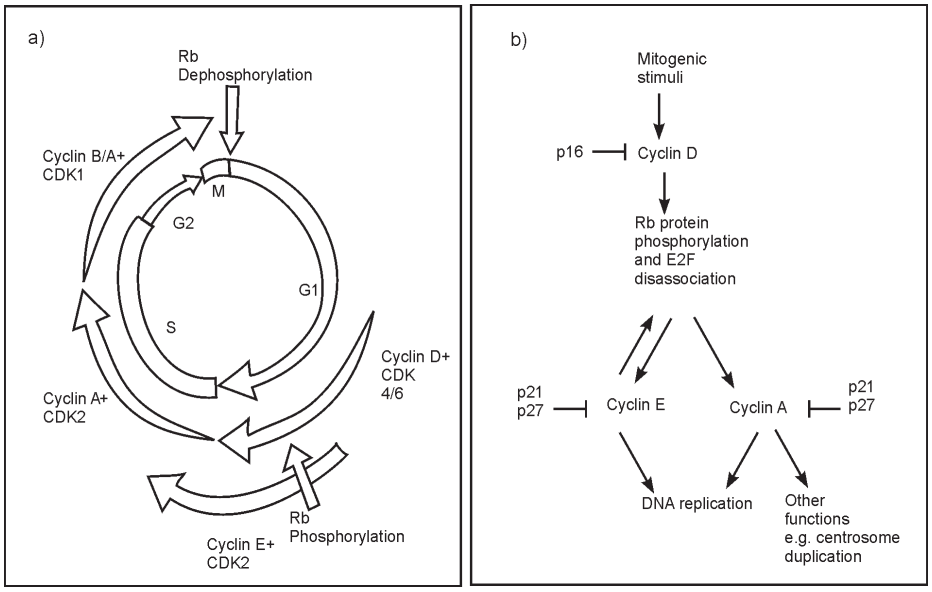

Figure 1 a and b. **Cell cycle** consists of four phases, G1, S (DNA-synthesis), G2 and M (mitosis). It is regulated by cyclin dependent kinases (CDKs) along with the increase or decrease of the levels of of cyclins, and phosphorylation or dephosphorylation of retinoblastoma protein regulating the level of free E2F transcription factor. P16, p17 and p21 are inhibitors of cell cycle progression. (Modified from Sherr 1993, Murray 2004, reviews).

T-cell development in thymus T-cells develop (lineage commitment) in the thymus, where they select their antigen specificity rearranging their TCR receptor gene structure by V(D)J recombination, where parts of the gene are cut off the sequence (Rothenberg and Dionne 2002 review). The process is analogous to the assembly of immunoglobulin genes (Jung and Alt 2004, review). Thymocytes expressing both CD8 and CD4 develop either to CD8 positive cytotoxic T-cells or to CD4 positive naive T-helper cells (Th).

T-cell development in secondary lymphoid organs In lymph nodes, the naive CD4+ T-cells proliferate activated by signals of TCR and the costimulatory CD28 molecule, and express interleukin (IL)-2. **(Figure 2)** After a few days the proliferation ends and cells enter an anergic state maintained by CTLA-4, an inhibitory ligand of CD28, and are transferred by the circulation to their target tissues. (Mohrs *et al.* 2003). Maturing of Th memory cells in secondary lymphoid tissues shifts expression of CD45 tyrosine phosphatase isoforms from CD45RA to CD45RO and cells become capable to responding by proliferation to recall antigens. However, in later proliferation processes cells coexpress CD45RO and RA isoforms during S, G2 and M phases of the cell cycle (LaSalle and Hafler 1991, Picker *et al.* 1993a).

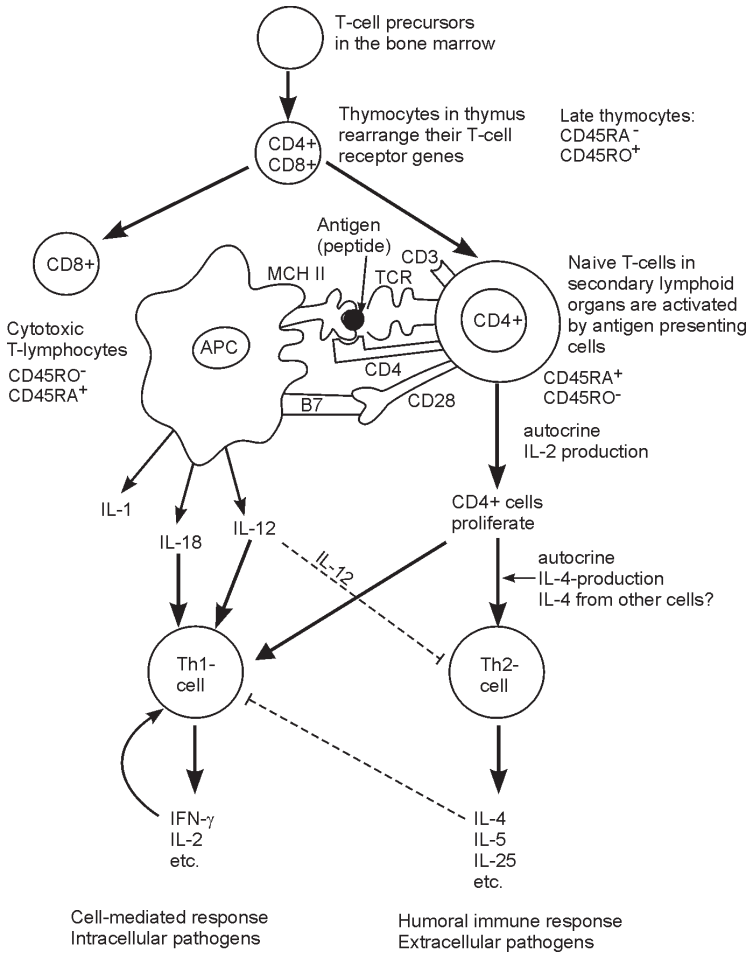

Figure 2. The development of Th1 and Th2 cells. After commitment to T-cell lineage and rearranging their TCR genes, naive CD4+ T-cells ciruclate in blood and secondary lymphoid organs, where they get in contact with antigen presenting cells (APC, e.g. dendritic cells derived from skin). The latter stimulate the TCR - CD3 -complex of naive T-cells with MCHII complex in association with the antigen. Several co-stimulatory mechanisms exist, the most important of them being CD28-B7-mediated co-stimulation (Drawn according to ideas and figures in Opal and DePalo 2000, Ho and Glimcher 2002, Murphy and Reiner 2002, reviews, Arstila and Hänninen 2003, textbook).

T-cell migration to skin The cause of proliferation of malignant T-cells in skin is not known. A superantigen could cause a polyclonal proliferation, followed by a malignant transformation. Alternatively, a malignant clone is present from the beginning of the infiltrative process. Skin keratinocytes respond to cellular injury or stress by releasing cytokines that up-regulate adhesion molecules on the surfaces of dermal endothelial cells and chemokines attracting lymphocytes characteristic to inflammation (Girardi *et al.* 2004, review). The migration of Th memory cells to different compartments of skin is dependent on the interactions of a large variety of molecules expressed on the cell surface of T-cell and endothelium of postcapillary venules, components of connective tissue in dermis and basement membrane, and keratinocyte membranes (Watson *et al.* 1996; Schön *et al.* 2003, review). During the virgin to memory cell transition in lymph nodes, virgin, maturing T-cells start expressing the cutaneous lymphocyte associated antigen (CLA, Picker *et al.* 1993b), that interacts with E-selectin and P-selectin during the first step of extravasation, cell rolling on the endothelium (Schön *et al.* 2003 review). CLA and CC chemokine receptor (CCR) 4, which interacts with its ligand TARC (thymus and activation regulated chemokine or CCL17) may together, and with CCR10, regulate the migration of Th memory cells to skin (Reiss *et al.* 2001, Homey *et al.* 2002, Ferenczi *et al.* 2002, Schön *et al.* 2003, review). Patients with erythrodermic CTCL have elevated levels of CD45RO+ ,CLA+ malignant cells in their blood, as defined by TCR receptor analysis (Heald *et al.* 1993), and skin infiltrates of early (patch or plaque) skin lesions also show a predominantly CLA+ T-cell phenotype (Picker 1990, Heald *et al.* 1993). CTCL patients with peripheral blood involvement show increased leves of cells coexpressing CLA and CCR4+. High levels of such cells and abundant expression of CCR4 ligands TARC/CCL17 and MDC/CCL22 can be found in the skin lesions (Ferenczi *et al.* 2002). The migration of CTCL cells to the epidermis may be further enhanced by their integrin adhesion molecules (e.g. $\alpha_E\beta_7$) and chemokine receptors (e.g. CCR4 and CXC) that bind ligands on endothelial dells, keratinocytes and Langerhans' cells (Girardi *et al.* 2004, review). In the skin lesion, malignant cells concentrate close to the skin surface, whereas non-malignant cells predominate in the dermal infiltrate (Bagot *et al.* 1992, Cerroni *et al.* 2000, Gellrich *et al.* 2000, Yazdi *et al.* 2003; Girardi *et al.* 2004, review). A dynamic communication between Langerhans' cells and CTCL-cells stimulating the latter to proliferate against their own tumour antigens has been suggested (Berger et al 2002).

Polarization In the target tissues, the anergic checkpoint of Th-cells is bypassed or released by signals from TCR/CD28 and cytokines activating STATs (Mohrs *et al.* 2003). Determined by their cytokine milieu, Th-cells polarize to type 1 (Th1) or type 2 (Th2) helper cells with distinct profiles of functions and cytokine production **(Figure 2.)** The development of Th1 cells requires interleukin 12 (IL-12) and interferon gamma (IFN-γ). Intracellularly, the activation of cytokine receptors is mediated by janus kinases (Jak), and STAT4 or STAT1 and T-bet, a member of T-box family of transcription factors. Th2 development requires IL-4, followed by intracellular activation of STAT6, and activation and autoactivation of GATA3, a zink finger protein. C-maf, a basic-leucine zipper protein, is induced by TCR signalling. Several factors involved in Th1 and Th2 development inhibit each other, and the intracellular pathways involved show multiple interactions with each other and other pathways (Kaplan *et al.* 1996, Takeda *et al.* 1996, Zheng and Flavell 1997, Szabo *et al.* 2000). After differentiation of Th cells, STAT3 and STAT 5 are selectively activated in Th1 but not in non-polarized or Th2 cells possibly having a role in the maintenance of the Th1 and Th2 phenotypes (Anderson *et al.* 2003). The polarization process possibly requires several cell divisions, after which the cytokine expression pattern is no longer dependent on the cytokine environment (reviewed by Mohrs *et al.* 2003). Overexpression of GATA3 and underexpression of STAT4 have been reported in Sézary syndrome (Kari *et al.* 2003).

Local growth factors possibly involved in cell proliferation in CTCL Interleukins IL-2, IL-7 and IL-15 are growth factors of T-cells, and they trigger mitogenesis, sustain growth and inhibit or promote (IL-2 after the clonal expansion of CD4+ cells in vivo) apoptosis. Their receptors are structurally related with partly common subunits associated with Janus kinases which phosphorylate STAT-transcription factors including STAT5 (Döbbeling *et al.* 1998, Qin *et al.* 1999, 2001, Eriksen *et al.* 2001, all including a review; Leroy *et al.* 2001). IL-15 is also a chemoattractant of T-cells (Wilkinson and Liew 1995). IL-2, IL-12, IL-15 and interferon gamma produced by antigen presenting cells induce expression of IL-2Rα, *c-myc* and *pim-1* genes in T-cells. (Matikainen *et al.* 1999). In normal skin, keratinocytes produce IL-7 (Matsue et *al.* 1993), and only small amounts of IL-15, the latter increasing after UVB exposure. IL-15 protein level is regulated at post-

transcriptional level (Mohamadzadeh *et al.* 1995, Leroy *et al.* 2001 including a review). IL-15 is also produced by antigen-presenting cells (Kanegane and Tosato 1996).

In parapsoriasis and CTCL, especially mycosis fungoides, the keratinocytes seem to express IL-15 protein (Asadullah *et al.* 2000, Leroy *et al.* 2001). IL-7 and IL-15, more than IL-2, promote growth and survival of CTCL cells in vitro and stimulate DNA-binding of JUND, activator of gene transcription (Dalloul *et al.* 1992, Döbbeling *et al.* 1998, Qin *et al.* 1999). Particularly, in later stages of CTCL, tumour cells may become independent of these three exogenous growth factors by several mechanisms including their own autocrine production of IL-15, production of new DNA-binding factors associating to the same sequences as IL-7 and IL-15-stimulated STATs, interleukins activating a larger spectrum of STATs than normally (loss of specificity of STATs), constitutive expression of STATs (1 to 6, observed in several cell lines), and constitutive phosphorylation and DNA-binding of e.g. STAT3 (Döbbeling *et al.* 1998, Asadullah *et al.* 2000, Eriksen *et al.* 2001, Qin *et al.* 1999, 2001). STAT3 may act as an oncogene (Sinibaldi *et al.* 2000, Bowman *et al* 2001), and it also mediates constitutive expression of suppressor of cytokine signaling 3 (SOCS-3) in CTCL (Brender *et al.* 2001). Constitutive STAT3 expression and a resulting IL-10 secretion in some tumours seems to induce T-cell tolerance toward the tumour cell by impairing the maturation and activation of dendritic cells (Wang *et al.* 2004). Stats 1 to 6 are also present in skin lesions in CTCL, most prominently STAT5 (Quin *et al.* 2001). STAT5a/b is constitutively activated in many neoplasias, including lymphomas (Weber-Nordt *et al.* 1996). In Sézary syndrome, a dysregulation of the balance between full-length and truncated forms of STAT5 leading to a predominant expression of the truncated form after mitogenic activation, has been observed (Mitchell *et al.* 2003). IL-2-induced cell cycle progression of peripheral T-cells is dependent on STAT5 signalling (Moriggl *et al.* 1999). Underexpression of STAT4 has been reported in Sézary syndrome (Kari *et al.* 2003).

TCR gene rearrangments in the study of CTCL The cause of proliferation of malignant T-cells in skin is an open question. A superantigen could cause a polyclonal proliferation, followed by a malignant transformation. Alternatively, a malignant clone is present from the beginning of the infiltrative process. The clonal origin of T-cells can be studied by analysis of TCR gene rearrangements. In contrast to the normally polyclonal occurence of cells with varying TCR-receptors, different tissues of CTCL-patients often show one or a few clones of cells with one kind of TCR receptor (Whittaker *et al.* 1991, Zelickson *et al.* 1991, Wood *et al.* 1994a, Vega *et al.* 2003). The receptor composition is individual, and a restricted use of Vβ segments (Jack *et al.* 1990), that would suggest one kind of a superantigen, has not been confirmed (Gorochov *et al.* 1995). However, a decrease in the complexity of the T-cell repertoire is seen comparable to that seen in HIV-infected patients. A decrease is normal T-cells occurs in a non-random fashion (Yawalkar *et al.* 2003).

The TCR clones in CTCL are observed with a frequency that is utterly dependent on the method used (Southern Blotting techiques, different PCR methods with low or very high sensitivity, microdissection; e.g. Wood *et al.* 1994b review; Cerroni *et al.* 2000, Gellrich *et al.* 2000, Costa *et al.* 2004). In some studies, clones are found more often in patients with an advanced than an early stage disease (Ralfkiaer *et al.* 1987, Fraser-Andrews *et al.* 2000), but with sensitive methods, TCR clones have also been found at the early stages (Wood *et al.* 1994a, Muche *et al.* 1997, Fraser-Andrews *et al.* 2000) even in morphologically normal extracutaneous tissues (Veelken *et al.* 1995). In CTCL, the TCR clones are considered to have prognostic significance (Fraser-Andrews *et al.* 2000, Muche *et al.* 2000b, Delfau-Larue *et al.* 2000), but in blood they may also be age-related (Delfau-Larue *et al.* 2000). TCR clones and chromosomal clones studied with the sensitive Genescan method coincide in the same patients, and in Sézary syndrome, they can be observed in the same cells (Muche *et al.* 2004).

TCR clones have also be detected in the skin lesions in large plaque parapsoriasis (LPP, Kikuchi *et al.* 1993, Simon *et al.* 2000, Klemke *et al.* 2002), primary follicular mucinosis and lymphomatoid papulosis (Zelickson *et al.* 1991), each of which may develop into CTCL (Wood *et al.* 1995, Willemze *et al.* 1997). According to Simon *et al.* (2000), TCR clones in skin lesions in LPP do not have prognostic significance, or allow distinction of the disease from early stage MF (Simon *et al.* 2000). They have also been found in blood (Muche *et al.* 1999) and in the skin lesions (Haeffner *et al.* 1995, Klemke *et al.*

2002) in small plaque parapsoriasis, that according to a recent epidemiological study, may develop into CTCL (Väkevä *et al.* 2004, in press). Solid tissue samples of some non-malignant diseases, like sarcoidosis (Sawabe *et al.* 2000) and lichen sclerosus et atrophicus (Lukowsky *et al.* 2000) also show clonal TCR rearrangements.

5.2. Chromosome aberrations observed in cancer and their origin

Cancer may be defined as a genetic disease due to accumulation of mutations causing the respective cells to lose sensitivity to growth control mechanisms (Sarasin 2003, review). According to the two hit-hypothesis based on studies of retinoblastoma, both alleles of one gene are affected, so that one mutation may be inherited, the other acquired, or both acquired (Knudson 1971). As the incidence of adult tumours increases exponentially with age, it has been estimated, that at least 4 to 7 different mutations in key genes are needed (Fearon and Vogelstein 1990). A mutator phenotype with an elevated mutation rate caused by an initial mutation affecting DNA synthesis or DNA repair (caretakers) or a gene regulating cell cycle or apoptosis (gatekeepers), and resulting in more mutations in the whole genome has been postulated (genetic instability). An alternative hypothesis suggests a normal mutation rate combined with clonal selection (Loeb *et al.* 1991, Rajagopalan *et al.* 2003, Sieber *et al.* 2003). This may be the case in e.g. many leukaemias, lymphomas and sarcomas characterized by solitary, specific translocations, and genetic instability emerging later during the disease as a possible late consequnce of mutations of a few key genes, e.g. TP53 preventing apoptosis of cells defective in DNA repair (Knudson 2001). Cutaneous T-cell lymphoma shows a large variety of both numerical and structural chromosomal abnormalities, and genetic instability has been suggested (Kaltoft *et al.* 1994).

The majority of cancers show genetic instability observable at chromosome or gene-level. Gene-level instability may be produced by mutations in DNA polymerase genes or overexpression of error-prone polymerases (Sarasin 20003, review) or defects in DNA- repair. Subtle sequence changes instabilities arise from defects in base-excision repair (BER) or nucleotide-excision repair (NER, NIN). Mutations in NER-genes cause diseases with sun sensitivity, like Xeroderma pigmentosum, that also includes proneness to cancer especially in skin (Hoeijmakers 2001). Mismatch repair deficiencies (defects in MMR genes) cause microsatellite instability (MIN) with point mutations and instability of repeat sequencies in microsatellites also inside exons. The wild type allele is often lost or methylated. MIN is seen especially in cancers of colon, endometrium and ovary (Leach et al 1993, Peltomäki *et al.* 1993; Hoeijmakers 2001, Sarasin 2003 reviews).

A special form of genetic instability is chromosomal instability (CIN), an increased rate of occurrence of chromosomal aberrations compared to normal cells. Tumours are characterized by microscopically observable, acquired numerical or structural chromosome aberrations and chromosomal instability (CIN). Often karyotypes vary slightly from cell to cell. The karyotype may remain fairly stable over long periods of time, or new clones with genomic changes conferring growth advantage emerge (Gollin 2004, review). The possible development of metastatic clones arising from genetically unstable cells at the same time as the primary tumour, has been suggested (Boveri 1929 reviewed in Gollin 2003, Kuukasjärvi *et al.* 1997, Chiba *et al.* 2000, Schmidt-Kittler *et al.* 2003).

Microscopically, chromosome aberrations are classified as numerical or structural. Numerical aberrations can be multiples of the haploid chromosome number or extra or missing chromosomes as compared to the nearest multiple of the haploid chromosome number of the specific cell (aneuploidy). Structural abnormalities include all aberrations, where the integrity of a chromosome is broken. They include for example deletions, translocations, inversions and multiplications of parts of the chromosome (duplications or higher order amplifications).

Chromosomal abnormalities cause loss or defects in tumour suppressor genes, amplification of oncogenes, and generation of fusion genes with altered function or enhanced expression, phenomenons observed in leukaemias and lymphomas (Nowell and Hungerford 1960, Benedict *et al.* 1983, Coquelle *et al.* 1997, Tanaka and Kamada 1998, MacLeod *et al.* 2000; Difilippantonino *et al.* 2002; Marculescu *et al.* 2002, Gladdy *et al.* 2003, Martín-Subero *et al.* 2003; Vega *et al.* 2002,

review). A wide variety of chromosomal abnormalities, both numerical and structural, is typical to CTCL (Whang-Peng *et al.* 1982) suggesting genetic instability (Kaltoft *et al.* 1992, 1994; Thestrup-Pedersen *et al.* 1994).

5.2.1. Numerical chromosome aberrations

Numerical chromosome aberrations are the most common cytogenetic changes observed in 20 000 malignancies studied (Heim and Mitelman 1995 referred in Krämer *et al.* 2002). In the early 1900s' Boveri suggested that cancer arises from a single cell with an abnormal genetic constitution resulting from defects in mitotic spindle apparatus (referred in Gollin, 2003). In contrast, the "somatic gene mutation hypothesis", DNA-sequence-level gene mutations activating cellular oncogenes or inactivating tumour suppressor genes, alone cause cancer, and chromosomal instability is a mere consequence of malignant transformation. However, new evidence suggests that aneuploidy is a distinct form of genetic instability in cancer (Sen 2000, review). For the separation of chromosomes during mitosis, see **Figure 3**.

Numerical abnormalities are caused by defective segregation of chromosomes, which may result from abnormalities in chromosome condensation, kinetochore-spindle interactions, premature chromatid separation, multipolar spindles, centrosome amplification or abnormal cytokinesis (Gebhart 1989, Hirano *et al.* 1994, Michaelis *et al.* 1997, Cahill *et al.* 1988, Pihan *et al.* 1998, Tatsuka *et al.* 1998, Carroll *et al.* 1999).

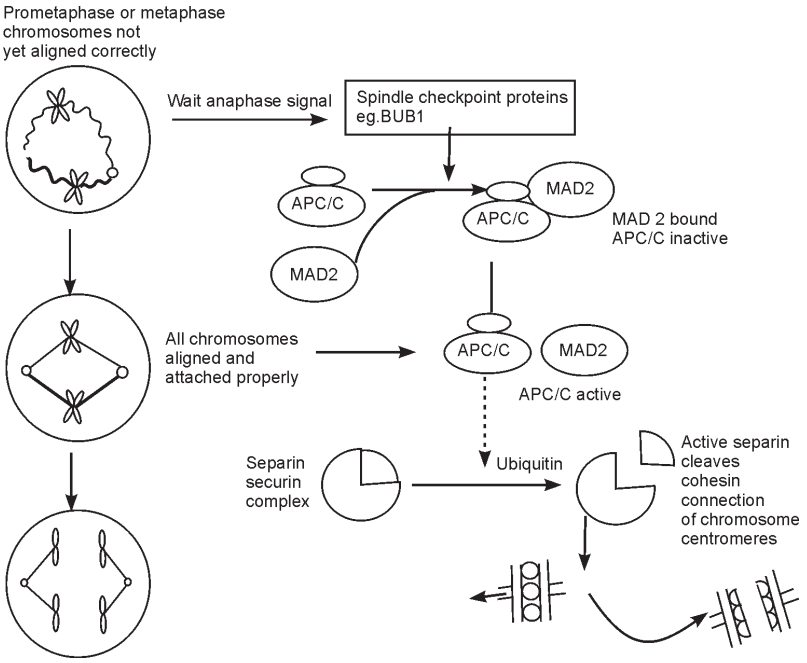

Figure 3. Regulation of chromosome separation in mitosis. Chromosomes unattached to the spindle generate a signal delaying progress to anaphase, transduced by spindle-checkpoint proteins (e.g. MAD/BUB), until all chromosomes are properly attached. Then MAD is released from the anaphase promoting complex (APC, attached to the cofactor CDC20). The latter is activated, separin-securin complex is degraded releasing active separin, a protease catalysing the cleavage of cohesin complexes that have held the sister chromatids together. The sister chromatids are separated and migrate towards the poles (Modified from Jallepalli and Lengauer 2001, review)

Recently, centrosome abnormalities have been under intensive study (Martín-Subero *et al.* 2003) Many solid human tumours, including brain, breast, lung, colon, prostate, pancreas, bile duct, and head and neck, show supernumerary centrosomes (Pihan et al 1998; Krämer *et al.* 2002, review). In acute myeloid leukaemia both numerical and structural aberrations of centrosome are seen (Neben *et al.* 2003). Malignant cells in non-Hodgkin's lymphomas have abnormally large centrosomes. Large centrosomes have been suggested to reflect clustering of centrosomes to create a bipolar spindle (Krämer *et al.* 2003). Increased levels of centrosome proteins found in tumours may lead to acentriolar assembly of spindle poles and aneuploidy, or functional defects of centrosomes (Pihan and Doxey 1999, review).

Centrosomes control chromosome segregation and cytokinesis and all other microtubule-related functions, such as cell shape, polarity, adhesion, motility, intracellular transport and positioning of organelles. The centrosome reproduction cycle is regulated by G1/S phase regulatory proteins, and both DNA replication and centrosome duplication are controlled by the Rb pathway (Krämer 2002, Nigg 2002, reviews). An abnormal number of centrosomes could arise by 1) formation of centrioles de novo, 2) overduplication of centrosomes within one cell cycle during inhibition of DNA replication for example in response to drugs, 3) if cells are fused, 4) if cell divison is delayed by spindle assembly checkpoint that allows anaphase onset only after all chromosomes are properly attached to the spindle (Nigg 2002, review). Consequently, a large number of genes involved in the control of the cell cycle e.g. cyclin E, p53 pathway, DNA-repair, protein degradation and mitosis, and several kinases are implicated in centrosome amplification and numbers (Fukasawa *et al.* 1996, Zhou *et al.* 1998, Carroll *et al.* 1999, Mussman *et al.* 2000, Meraldi *et al.* 2002, Anand *et al.* 2003; Krämer *et al.* 2002 Nigg 2002, Gollin 2003, Pihan 2003, reviews). A transient tetraploidization has been proposed to be a major step in the formation of numerical centrosome aberrations (Meraldi *et al.* 2002). A defective p53 pathway favours the proceeding of the abnormal cells to mitosis rather than apoptosis (Meraldi *et al.* 2002, Nigg 2002, review). P53 mutations also enhance the microtubule nucleation capacity of centrosomes (Lingle *et al.* 2002)

At present, different opinions about the timing of centrosome aberrations and their significance to the development of cancer prevail (Lingle *et al.* 2002, Meraldi *et al.* 2002, Nigg 2002, review, Pihan *et al.* 2003, Rajagopalan *et al.* 2003). Centrosome aberrations occur at a higher frequency in advanced than in early stage cancer (Pihan *et al.* 2001; Krämer *et al.* 2002, review), but centrosome and chromosome abnormalities are also observed in *in situ* lesions without p53 mutations, and centrosome defects and CIN have been suggested to contribute to the earliest stages of cancer development (Lingle *et al.* 2002, Pihan *et al.* 2003, Nowak *et al.* 2002, Rajagopalan *et al.* 2003).

Specific aneuploidies seem to have a role in malignant transformation or progression of cancer (Knauf *et al.* 1995). They may cause loss of one allele of a tumour suppressor gene (loss of heterozygosity LOH) or enhance the expression of an oncogene when several copies of the respective chromosome are present. Cells try to counteract these processes with functional dosage compensation, or by duplicating the remaining whole chromosome. If the remaining chromosome has a small deletion, the aberration may be hidden in a cytogenetic examination (Brat *et al.* 1997, Thiagalingam *et al.* 2001, McEvoy *et al.* 2003; Krämer *et al.* 2002, Rajagopalan *et al.* 2003, reviews). A common trisomy of chromosome 7 involving a non-random duplication of the chromosome with a mutant allele of the oncogene MET has been observed in hereditary papillary renal carcinoma (Zhuang *et al.* 1998). In addition, trisomy of one chromosome misregulates the expression of genes in other chromosomes (FitzPatrick *et al.* 2002). Numeric aberrations of specific chromosomes are seen in several malignancies or their subtypes, and they, as well as hypodiploidy as a whole, may be used as prognostic factors (Krämer *et al.* 2002, review, Raimondi *et al.* 2003). Aneuploid genomes are prone to chromosome breakage with erroneous rejoining producing structural chromosomal abnormalities, as recombination repair of DNA depends on the presence of the homologous chromosome and many genes involved in DNA repair are haploinsufficient (Hoeijmakers 2001, Matzke *et al.* 2003, reviews).

5.2.2. Structural chromosome aberrations

Structural abnormalities include all aberrations, where the integrity of a chromosome is broken. They include for example deletions, translocations, inversions and multiplications of parts of the chromosome (duplications or higher order amplifications). They cause losses of chromosomal regions with tumour suppressor genes, amplification of regions with oncogenes, and formation of fusion genes. Structural chromosomal abnormalities arise from erroneous repair of DNA double strand breaks, which may be caused by for example ionizing radiation or other genotoxic agents or replication of spontaneous single strand breaks (Hoeijmakers 2001, Obe *et al.* 2002), apoptotic endonucleases followed by cell recovery, and chemicals binding topoisomerase II, an enzyme active in replication (Greaves and Wiemels 2003, review).

Normally, double strand breaks are repaired in S and G2 by homologous recombination, which requires a sister chromatid to be used as a template, or by joining the broken ends (non homologous end joining, NHEJ), in G1, when no sister chromatid is available (Hoeijmakers 2001). Erroneus rejoining of DNA breaks may produce dicentric chromosomes, that in anaphase form a breaking bridge between two spindle poles (breakage-fusion-bridge-cycle, Coquelle *et al.* 1997, Gollin 2003, review) and mediate chromosomal instability. Certain DNA sequences near the breakpoints, like repetitive long interspersed nuclear elements (LINE), Alu repeats, intrachromosomal telomeric or subtelomeric sequences, homological subtelomeric sequences in two different chromosome pairs, or topoisomerase II DNA-consensus binding sites or viral integration sites may predispose to chromosomal rearrangements observed in cancer, including leukaemias and lymphomas (Rogers et al. 1985, Azzalin *et al.* 1997, Day *et al.* 1998, Busson *et al.* 2000, MacLeod *et al.* 2000, Padilla-Nash *et al.* 2001; Mefford and Trask 2002, Kolomietz *et al.* 2002, Oliveira and Fletcher 2003, reviews).

A break in DNA is followed by a damage response in several signalling pathways e.g. DNA-repair, cell cycle checkpoints and telomere maintenance or apoptosis (Gollin 2003), events controlled by many tumour suppressor genes or genes of chromosomal instability and cancer syndromes. One of the latter is ATM (ataxia telangiectasia mutated), the key gene at the beginning of DNA damage signalling pathways (Shiloh 2003). Mutations in ATM cause the recessive disorder ataxia telangiectasia with sensitivity to ionizing radiation and increased levels of chromosome rearrangements (Chan and Blackburn 2003, Shiloh 2003, review). Another early response gene is ATR (Rad3-related), that is required for stability of fragile sites in chromosomes and inhibition of gene amplification by breakage-fusion-bridge-cycles (Coquelle *et al.* 1997, Casper *et al.* 2002). Mutations in ReQ helicases, involved in homologous recombination, cause recessive disorders with chromosomal abnormalities, e.g. Bloom syndrome and Werner syndrome, that also show and increased risk of diverse malignancies (Prince *et al.* 2001, Hickson 2003 review, Sengupta *et al.* 2003).

Many malignant tumours appear to lack replicative senescence, e.g. their cells have an infinite replicative life span, a property explained with abnormalities in their telomeres, which may also explain microscopically visible chromosomal abnormalities. Chromosome ends are protected from shortening during replication (end replication problem) by telomeres, that consist of looping tandem TTAGGG repeats and binding proteins. Many of them are involved in NHEJ, homologous recombination or V(D)J recombination (Blasco 2002, 2003, Jung and Alt 2004, reviews), and function e.g. as negative regulators of telomere length, or in protecting or repairing telomeres. In somatic cells, the telomeric sequence is shortened by every cell division, leading to critically short telomeres leading to NHEJ-mediated telomere fusions, breakage of dicentric chromosomes in subsequent cell cycles and apoptosis (Artandi *et al.* 2000; Blasco 2002, Karlseder 2003, reviews). In germline and stem cells, telomere length is stablilized by telomerase enzyme. Most cancer cells show short telomeres, telomerase activity and chromosome abnormalities, interpreted as a sign of a period with telomere dysfunction in their past, which they have survived by activating telomerase and consequent regeneration of their short telomeres (Artandi *et al.* 2000, O'Hagan *et al.* 2002, Blasco 2002, 2003, Chan and Blackburn 2003, Sharpless and DePinho 2004). Expression of telomerase may create a state of limited chromosomal instability allowing healing of broken chromosomal ends with different mechanisms, like copying the end of another chromosome with a homologous region, translocation or creation of a new telomere (Gisselsson *et al.* 2001, Stellwagen et al 2003; Feldser *et al.* 2003, Cech 2004, reviews). Some cancer cells conserve their telomeres by an alternative pathway, alternate lengthening of telomeres (Chang *et al.* 2003; Neumann and Reddel 2002, Stewart *et al.* 2002, Meeker and de Marzo 2003 reviews). Skin biopsy-derived skin-homing T-cell lines and peripheral blood mononuclear cells (including lymphocytes) have a high level of telomerase activity and short telomeres. Both are observed already in parapsoriasis patients and have been suggested to be important in the tumorigenesis of CTCL (Wu *et al.* 1999).

Structural chromosome aberrations may promote carcinogenesis by causing losses of chromosomal regions with tumour suppressor genes and amplification of regions with oncogenes, with gene-level effects resembling those caused by numerical chromosome aberrations but with possible changes of position effects (Brown *et al.* 1999, Baur *et al.* 2001, Carvalho *et al.* 2001; Mefford and Trask 2002, review). Additionally, unlike numerical aberrations, structural chromosome aberrations may cause formation of fusion genes encoding fusion proteins (Vega *et al.* 2002, 2003, Scandura *et al.* 2002).

Many translocations associated with leukaemias and lymphomas, especially of B-cell origin, have been extensively studied (reviewed in Vega *et al.* 2002, 2003, Scandura *et al.* 2002, and Stilgenbauer 2002, Huntly *et al.* 2003), and occur also in some sarcomas (reviewed in Rego and Pandolfi, 2002). Most of these translocations are observed only in one or a few types of cancer. Their frequency varies from less than one percent of cases of the respective disease to the majority of patients (Rego and Pandolfi 2002, and Scandura *et al.* 2002, and Vega *et al.* 2002, reviews), and may be the only chromosome abnormality observed at early stages of the diseases, which have been regarded as "one hit" cancers (Knudson 1971, Knudson 2001 review). However, many of the tumour specific translocations are observed commonly in normal cell populations, with a low frequency, like in one cell of 10^4 to 10^8 cells for each translocation. Their significance awaits further research. They might be somatic, non-dividing cells, or their malignant potential is restrained by some yet unknown mechanism. The view has emerged, that translocations alone are not sufficient to cause overt cancer, but other mutations are needed (Limpens *et al.* 1995, Biernaux *et al.* 1995, Trümper *et al.* 1998, Maes *et al.* 2001, Marculescu *et al.* 2002, Vega *et al.* 2002, Bäsecke *et al.* 2002, Janz *et al.* 2003; Vega *et al.* 2002, Oliveira and Fletcher 2003, Greaves and Wiemels 2003, reviews). - In contrast to many non-Hodgkins lymphomas, Hodgkin's lymphoma shows mainly complex structural and numerical abnormalities, although rare cases with t(2;5)(p23;q35),(see below), have been reported (Weber-Matthiesen *et al.* 1993 a and b, 1995, Li *et al.* 1997, Barth *et al.* 2003; Re *et al.* 2002 review).

Interestingly, translocations may affect genes coding transcription factors, nucleoporins, protein tyrosine kinase genes, nucleoporins, or factors regulating cell cycle or apoptosis. One reciprocal translocation produces two fusion genes. Usually one of them has been considered to be decisive in carcinogenesis, but in some cases the other one also plays a role (reviewed in Vega *et al.* 2002, Rego and Pandolfi, 2002, Scheijen and Griffin 2002, Scandura *et al.* 2002). One gene may have several alternative translocation partners forming different fusion genes. In leukaemias, the function of the gene taking part in the fusion, is often changed through an alteration in its structure (Scandura *et al.* 2002, Vega *et al.* 2002, 2003, Greaves and Wiemels 2003). Alternatively, the translocation may put an oncogene under the control of regulatory elements of another gene, changing the expression of a structurally normal protein (Pekarsky *et al.* 2001, Vega *et al.* 2002, 2003, Greaves and Wiemels 2003). In lymphomas, such translocations often arise by errors during V(D)J recombination that place another gene under the control of an immunoglobulin gene. Such illegitimate V(D)J recombination may occur during selection of the variable region of immunoglobulin during class switch or light chain change called receptor editing. Analogically, illegitimate V(D)J recombinase activity can combine TCR genes with oncogenes, two genes others than TCR genes, or inactivate tumour suppressor genes by deletions (Aplan *et al.* 1990, Brown *et al.* 1990, Cayuela *et al.* 1997, Marculescu *et al.* 2002; Vega *et al.* 2002, 2003 reviews, Messier *et al.* 2003). Gesk *et al.* (2003) did not find breakpoints in TCRA/D or TCRB genes with locus-specific FISH among 12 patients with CTCL not showing cytogenetic evidence of translocations involving the respective cytogenetic regions.

Some translocations are accompanied by deletions in the translocated chromosomes spanning up to 1 Mb. They may be associated with sequences rich in Alu repeats, and cause haploinsufficiency (König *et al.* 2002, Kolomietz *et al.* 2001, review). For example, the translocation t(9;22)(q34;q11.2) producing Philadelphia chromosome (Nowell 1960, Rowley 1973), and fusing of genes BCR and ABL in chronic myeloid leukaemia (CML) shows deletions up to several hundred kilobases at the translocation breakpoint (Sinclair *et al.* 1997). The deletions arise during the translocation process, and correlate with a shorter survival (Sinclair et al 2000, Huntly *et al.* 2003, review, Lee *et al.* 2003). Apart from all patients with CML, the fusion gene is observed in 25% of patients with adult (ALL) and 5% of childhood acute lymphoblastic leukaemia. However, two thirds of cases with ALL show another breakpoint within the BCR gene than patients with CML, but in rare cases it may involve a deletion, too (Huntly *et al.* 2003, review). ALL cases without BCR/ABL translocation may rarely show a deletion in ABL (Lee *et al.* 2003).

Anaplastic large cell lymphomas (ALCL) of T-cell or null cell phenotype (Vega *et al.* 2002, 2003), and rarely primary CD30 positive CTCL (Beylot-Barry *et al.* 1996, 1998, show t(2;5)(p23;q35) with a fusion gene of nucleophosmin (NPM) and anaplastic lymphoma kinase (ALK) gene, a tyrosin kinase receptor (Vega *et al.* 2002, 2003). ALK shows also other fusion genes in alternative translocations and in an inversion of chromosome 2 (Pittaluga *et al.* 1997, Vega *et al.* 2002,

2003). The cytogenetic t(2;5), or the transcript with PCR or immmunoreactivity of the protein have been observed in a minority, at most 20% of cases with primary CD30 positive CTCL (Lopategui *et al.* 1995, Shiota *et al.* 1995, Beylot-Barry *et al.* 1996,1998,Wood 1998 review). Controversially, in many studies evidence of the translocation has not been observed in the patients (Wellman *et al.* 1995, DeCoteau *et al.* 1996, Sarris 1996, Wood *et al.* 1996, Wood 1998 review) but with PCR it has been observed in the blood of healthy persons, (Trümper *et al.* 1998). Its significance for the pathogenesis of the primary cutaneous CD30 positive lymphoma has been questioned (Wood 1998), and it has not been observed in transformed (Wolfe *et al.* 1995) large cell CD30 + CTCL (Li *et al.* 1997). It was observed, exceptionally, in a few cases with lymphomatoid papulosis and in 6/27 cases with CD30+ MF , by one group with a highly-sensitive nested PCR method, but no ALK1 immunoreactivity was seen (Beylot Barry *et al.* 1996, 1998; Wood 1998 review). By quantitative RT-PCR, Maes *et al.* (2001) observed a low level expression of ALK-fusion genes, not supported by cytogenetic or FISH-studies in ALK-immunohistologically negative ALCL, Hodgkin's disease and non-neoplastic cells suggesting the presence of transcripts in normal cells (Maes *et al.* 2001).

5.2.3. Previous chromosome studies in CTCL

The cytogenetic studies in MF or SS patients preceding the first publication of this thesis, were performed mostly on blood lymphocytes (Fukuhara *et al.* 1978, Edelson *et al.* 1979, Van Vloten *et al.* 1980, Nowell *et al.* 1982, 1986, Whang-Peng *et al.* 1982, Johnson *et al.* 1985, Gamperl 1986, Barbieri *et al.* 1986, Berger and Bernheim, 1987, Mecucci *et al.* 1988, Berger *et al.* 1988, Shapiro *et al.* 1987, D'Alessandro *et al.* 1990, 1992, Kaltoft *et al.* 1992, 1994), and revealed a large spectrum of chromosomal abnormalities, both numerical and structural. No specific abnormality could be detected, but some non-randomness was observed. According to a 41 patient study of Whang-Peng *et al.* (1982), e.g. the ten or nine chromosomes most often involved in structural abnormalities were chromosomes 1 (10 patients), 6,7 (9), 4, 9 (8),10, 12, 14, 15 and 17 (7), and in numerical abnormalities chromosomes 11,21,22 (15 patients) ,8,9 (14),15,16 and 17 (11), respectively, but the continuum went on involving all the chromosomes. Cytogenetic abnormalities were observed prior to histological malignancy, and were suggested to have a significant diagnostic and prognostic value (Whang-Peng *et al.* 1982).

5.3. Molecular cytogenetic methods

Conventional cytogenetics with G-banded chromosomes is time consuming and detects only spontaneously dividing cells, or cells inducible to division in cultivation, which in CTCL are often difficult to obtain (Burg *et al.* 1978, Bunn *et al.* 1980a, Dalloul *et al.* 1992, Abrams *et al.* 1993, Hindkjær *et al.* 1993, Berger *et al.* 2002). Additionally, G-banding does not allow identification of the chromosomal origins of all chromosome parts involved in chromosome aberrations. Many of these problems may be solved by use of molecular cytogenetic techniques.

Both interphase cells and metaphase chromosomes can be analysed by *in situ* hybridization with chromosome centromere specific probes for numerical aberrations of specific chromosomes. Locus-specific probes can be used analogically. Tumour DNA can be studied to show amplification or loss of chromosome regions by comparative genomic hybridization, in which tumour and reference DNA are hybridized to normal metaphases, in which they compete for their specific hybridization target regions (CGH, Kallioniemi *et al.* 1992b). In metaphases, whole chromosomes or their arms can be "painted", and the 24-colour methodology for identification of every chromosome pair, especially suitable for the study of translocations and origin of marker chromosomes, became available during the time course of the studies included in this thesis.

5.3.1. Non-radioactive *in situ* hybridization with centromere- or whole chromosome-specific probes, or locus-specific probes

In situ hybridization utilizes nucleic acid probes, that detect similar target sequences in tissue sections, cytological or chromosome preparations. *In situ* hybridization was originally developed for radioactive detection, which has largely been replaced by non-radioactive *in situ* hybridization (ISH) with no irradiation hazard, better spatial resolution, and allowing simultaneous multi-colour analysis of several targets and long-term storage of probes (Komminoth *et al.* 1992, Poddighe *et al.* 1992). Both DNA and RNA can be used as probes or detected (Gall and Pardue, 1969, John *et al.* 1969, Pardue and Gall, 1969, Raap *et al.* 1991, Komminoth *et al.* 1992, Poddighe *et al.* 1992, Werner *et al.* 1997). If not otherwise stated, *in situ* hybridization in the present text refers to non-radioactive DNA *in situ* hybridization using DNA probes. The probes can be made by cloning the sequences into cosmids (insert size 40 kb), phages (P1-phages, ~100kb), bacteria (P1-derived artificial chromosomes, PACs, ~100-150 kb; BACs, ~100-200kb) or yeasts (yeast artificial chromosomes YACs, 100-1000kb).

The number and localization of a specific chromosome centromere in a given interphase or metaphase cell may be studied using centromere-specific probes, that represent repetitive sequences, classical satellite, alpha-satellite or beta-satellite, present in the heterochromatin near the centromere of the given chromosome. The chromosome specificity of these probes is sensitive to hybridization conditions, and there is polymorphism of the size of the region detected. Nowadays, specific probes for each human chromosome centromere are available, except for 13 and 21 having a common probe (Devilee *et al.* 1986a,b).

Human chromosomes in somatic hybrid cell lines, flow-sorted chromosomes or microdissected chromomosomes have been used to create probes specific for individual chromosomes or chromosome arms (chromosome- or arm-specific painting probes, Cremer *et al.* 1988, Lichter *et al.* 1988, Lengauer *et al.* 1990, Carter *et al.* 1992, Guan *et al.* 1994a, 1994b, 1996), smaller parts of chromosomes (band-specific probes) specific chromosomal regions or chromosome aberrations (Carter *et al.* 1992, Meltzer *et al.* 1992, Guan *et al.* 1993, 1994a). These probes are used especially for detection of translocations or numerical aberrations in metaphases.

Labelling Labelling of the probes may be performed by several methods using nucleotides conjugated with a reporter molecule. In nick translation, the DNA template is nicked with DNAse I, DNA polymerase I extends the nicks to gaps by the 5'->3' exonuclease activity and replaces the missing nucleotides by a mixture of labelled and unlabelled ones (Rigby *et al.* 1977, Langer *et al.* 1981). Other common labelling methods are for example random primed labelling (Feinberg and Vogelstein 1984), Polymerase chain reaction labelling (PCR labelling, Lo *et al.* 1988) adding labelled nucleotides to the 5 or 3'-end of the probe (Kempe *et al.* 1985, Murasugi and Wallace 1984, Schmitz *et al.* 1991; Höltke *et al.* 1995 review to several methods). Diverse commercial methods based on chemical reactions are available.

The length of the probe affects its penetration, diffusion, hybrid formation and hybrid stability. After labelling, the optimal length for centromere-specific probes is 200–400bp, for CGH probes about 600-2000bp (Unger 1990, Kallioniemi *et al.* 1994a). In nick translation, the length of the probe is regulated by the relation of DNAse and polymerase as well as the reaction time. In random primed labelling, the length of the product depends on the DNA to be labelled.

Hybridization and detection In the hybridization process, the DNA double helix of the probe and the target DNA are unwound in a denaturation reaction, generally by heating, and the hybridization of the probe to the its complementary sequence in the target, is allowed to proceed in a warm, sub- melting point, moist chamber. Unhybridized probe is washed off, and the reporter molecules are detected either directly with fluorescence microscopy or enzymatic colour reactions, or indirectly, after enhancement by sandwiching with several layers of molecules. They may be for example avidin or streptavidin conjugated with a fluorescent colour or an enzyme, used with anti-avidin or anti-streptavidin antibodies conjugated with biotin, or antibodies to digoxigenin, conjugated with fluorescent colours or enzymes producing colour

reactions. The enzymes may be selected along with substrates producing a colour visible in fluorescent or bright light (Jablonski *et al.* 1986 with references to previous studies; Unger 1990, Poddighe *et al.* 1992, Raap *et al.* 1990, Holtke *et al.* 1995, reviews). For FISH, the colours must have different absorption and emission spectras, separable by the optical system (filters) of the microscope.

The hybridization of painting probes to repeated sequences as Alu and KpnI is suppressed (chromosome *in situ* suppression hybridization, CISS) using unlabelled total human DNA , usually of placental origin (e.g. Cot-1 DNA, placental DNA enriched for repetitive sequences) mixed with the probe (Cremer *et al.* 1988a, Lichter *et al.* 1988). Cot-1 DNA is also used for reducing unspecific hybridization of centromere-specific probes (e.g. Karenko *et al.* 1997).

Alternatively, hybridization may be performed with unlabelled, synthetic oligonucleotides, specific for the chromosomal region or gene studied, and the process is continued as a PCR reaction, where a thermostable polymerase incorporates labelled nucleotides (PRimed *In situ* labelling, PRINS, Koch *et al.* 1989). Compared to conventional FISH, its advantages are the simple production of the probes and speed, but only one signal colour is produced in one PCR reaction.

The preparations made for bright field microscopy are counterstained and mounted according to the requirements set by the colours used, even permanent preparations can be made. Preparations made for fluorescent microscopy tend to bleach with time, although they are mounted in fluids containing anti-fade agents. They are often mixed with a counterstain, for example 4',6-Diamidine-2'-Phenylindole Dihydrochloride (DAPI), that stains mitotic chromosomes with a band pattern resembling a reversed G-banding.

Imagining Video- or digital cameras, connected to the microscope, have replaced kinofilm cameras used in the beginning of these studies for documentation. Imagining FISH is often performed with a high-resolution black-and white camera taking one picture of every colour of one microscope field at the time. The computer assignes a different pseudocolour to every shot, and combines the images to one picture. Thus superimposed signals of different colours are discerned, and further computerized image analysis is possible (Viegas-Pequinot *et al.* 1989). Laser microscope enables the study of relative positions of chromosomes and even their banding pattern in interphase cells (Lichter *et al.* 1998, Carvalho *et al.* 2001, Müller *et al.* 2002, Lemke *et al.* 2002).

Factors affecting reliability Evaluation of hybridization signals should be performed only in well hybridized areas of the preparation. Good hybridization quality often requires the use of protein degrading enzymes, as proteinase K or pepsin, especially when the target is a tissue section. The hybridization conditions are usually optimized for each type of target material and probes, and may be calibrated by using normal tissue controls and internal contorols of different type of cells on the same slide (Pinkel *et al.*1986, Walt *et al.* 1989, Larsson et Hougaard, 1990, Hopman *et al.* 1989, 1991, Poddighe *et al.* 1992, van Dekken *et al.* 1993). For metaphase preparations in FISH-processes, the visual quality of DAPI-banding in microscopy after denaturation is used for optimization of hybridization conditions (Karhu *et al.* 1997).

The reliability of scoring of interphase cells with chromosome aberrations detected with *in situ* hybridization depends on the choice of the target material used. In preparations based on cells suspensions dropped on slides, overlapping cells are avoided. Split spots (paired arrangement) are counted as one (Poddighe *et al.* 1992). In tissue sections, the location of abnormal cells in relation to other tissue components is preserved and intratumoural heterogenity is better observable than by using single cell preparations. On the other hand, a part of the cell is often cut off, or cells are upon each other, thus affecting the number of signals designated for each cell. Therefore, in studies using tissue sections, diverse mathematical evaluation systems calibrated in relation to normal tissue or single cell suspension-based preparation of the same tumour have been used used. (Dhingra *et al.* 1992, Qian *et al.* 1996, Poddighe *et al.* 1992, van Dekken *et al.* 1993**).** Thick sections (20 m) have been used in confocal microscopy (Thompson *et al.* 1994), and also in bright-field microscopy (Looijenga *et al.* 1993). The abovementioned problems may also be resolved by using whole nuclei isolated from paraffin sections (Hyytinen *et al.* 1994). Biopsies snap-frozen with liquid nitrogen and mounted in a standard pathologic freeze preservative,

may be pressed gently against a slide to produce whole-cell touch preparations, which are easy and reliable to hybridize. Recently, FISH has been performed in suspension (S-FISH), which will open new possibilites for the study of interphase cell architechture, as the cell conserves its spherical form during microscopy (Steinhaeuser *et al.* 2002).

Resolution achievable with locus-specific probes Locus-specific probes are mostly used in combination with fluorescent detection. They can be localized to metaphase chromosome bands, and their order in metaphase or prometaphase chromosomes, or chromosomes streched mechanically in the cytocentrifuge, or interphase nuclei, can be discerned with the resolution of approximately 2-3 Mb, 1 Mb, 400-500kb, 50-1000 kb, respectively. (Trask *et al.* 1991, Laan *et al.* 1995, Haaf and Ward 1994b). Fiber-FISH, where the target of hybridization is DNA deposited on microscope slides, yields a resolution of 1-300kb (Heng *et al.* 1992, Wiegant *et al.* 1992, Parra and Windle 1993, Fidlerová *et al.* 1994, Haaf and Ward 1994a, Houseal *et al.* 1994, Senger *et al.* 1994, Heiskanen *et al.* 1995).

5.3.2. Combined DNA *in situ* hybridization and immunocytochemistry

Conventional cytogenetics, or in-situ hybridizations using conventional chromosome preparations, can not be used to study the phenotype of the abnormal cells, as cell membrane and cytoplasm have been removed by hypotonic treatment, fixation and dropping of the cells to the slides, all designed to spread the chromosomes and remove other cellular materials disturbing the analysis. Chromosome analysis with diverse standard cytogenetic stainings of immunophenotyped cells, a method called MAC (morphology, antibody, chromosomes) is possible by using a mild hypotonic treatment leaving the cytoplasm and cell membrane intact, cytocentrifugic spreading of cells on the slides, and avoiding of acidic or formaldehyde containig fixatives before immunocytochemistry (Stenman *et al.* 1975, Bernheim *et al.* 1981, Teerenhovi *et al.* 1984, Knuutila and Keinänen 1985, Perry and Thomson 1986, Pérez *et al.* 1991, Schlegelberger *et al.* 1994b; Knuutila *et al.* 1994a, review). The immunocytochemical staining and chromosomes can be analysed either in two separate, sequential steps, so that same cells are photographed twice, or simultaneously in a single step (Knuutila *et al.* 1994). The requirement of mitotic cells and difficulties in obtaining a good spreading and staining quality of the chromosomes limits the use of MAC method (Weber- Matthiesen *et al.* 1992).

The yield of MAC-technique may be improved by combining it with *in situ* hybridization (MACISH), but interphase cells offer a larger and more easily processable material for study, especially in diseases, where metaphases are difficult to obtain. Analogically with MAC, immunocytochemistry and *in situ* hybridization of interphase cells may be performed in two sequential steps or in one step with simultaneous detection of both (Wessman and Knuutila 1988, Tiainen *et al.* 1992, Knuutila et al, 1994a review, 1994b). Both bright field microscopy (Haas *et al.* 1987, Mullink *et al.* 1989, van den Brink *et al.* 1990, Looijenga *et al.* 1993, Strehl and Ambros 1993, Speel *et al.*1994a,b, Knuutila *et al.* 1994b, Kerstens *et al.* 1994), and fluorescent dyes (fluorescence immunophenotyping and interphase cytogenetics as a tool for investigation of neoplasms, FICTION, Weber-Matthiesen, 1992, 1993a, 1993b, Price *et al.* 1992) have been used. The antibodies used in immunohistochemistry must not cross-react with antibodies used for the detection of *in situ* hybridization, although the antigenic properties of proteins are destroyed to some extent during the hybridization procedure. The choice of colours is limited by the tendency of the fluorescence colours of immunohistochemistry to fade during the hybridization procedure. The colourigenic complexes on the cell surface or in the cytoplasm should not hinder probe penetrance. When bright field microscopy is used, particular attention must be paid to the transparency of immunohistochemical staining, which must not hide the hybridization signals. FICTION has been used for the study of several lymphoma-specific chromosome aberrations (Martín-Subero *et al.* 2002, Barth *et al.* 2003, Gesk *et al.* 2003).

5.3.3. Multicolour FISH, cross-species colour banding and bar coding methods

Structural rearrangements of the whole genome can be screened with multicolour FISH, that discerns also balanced translocations. Multicolour FISH is based on chromosome-specific painting probes, that are labelled (Nederlof *et al.* 1990, Dauwerse *et al.* 1992, Ried *et al.* 1992) with several colours, so that every chromosome has a specific colour combination. Translocations are observed as a change in the colour combination of the parts of the chromosomes involved.

The most commonly used, nowadays commercial methods of multicolour FISH are multifluor or multiplex FISH (MFISH, Speicher *et al.* 1996) and spectral karyotyping (SKY, Schröck *et al.* 1996). Their main difference is in the imaging, which in MFISH is done with one image for each six colours including DAPI, and the images are combined with the computer. In SKY, only one image is taken through a triple-band pass filter and analysed spectroscopically (Fourier analysis), which excludes the effect of changes in the intensity of the colour (Speicher *et al.* 1996, Schröck *et al.* 1996, Macville *et al.* 1997, Schröck and Padilla-Nash, 2000).

In MFISH and SKY, the probes are labelled in a binary fashion (combinatory labelling): each colour is present in or absent from each chromosome and usually at least six colours have to be detectable with the microscope and camera, if all probes are hybridized in the same time instead of sequential (Speicher *et al.* 1996, Schröck *et al.* 1996, Müller *et al.* 2002) hybridizations. In contrast, the colour-changing karyotyping (CCK) method is based on intensity differences, achieved using specific colours as direct or indirect labels, and analysable with a standard fluorescent microscope with only three filters (Henegariu *et al.* 1999). Other methods are based on delayed luminescence (Tanke *et al.* 1998), or mixing of ratio colours and binary colours (Combined Binary Ratio labelling, COBRA, Tanke *et al.* 1999).

Multicolour FISH defines the origins of marker chromosomes or partially identified chromosomes observed in G-banding, and may reveal aberrations not detectable by G-banding (Veldman *et al.* 1997, Rao *et al.* 1998, Sawyer *et al.* 1998, Zattara-Cannoni *et al.* 1998, Rowley *et al.* 1999, Uhrig *et al.* 1999, Nordgren *et al.* 2002). However, the sensitivity of multicolour FISH for detection of translocations depends on the condensation of the chromosomes and the colour combination of the chromosomes involved, and may be quite low, between 320kb to 2.6 Mb, especially in the subtelomeric regions (Schröck *et al.* 1996, Uhrig *et al.* 1999, Kearney 1999, Azofeifa *et al.* 2000). The standard MFISH can be improved with the use of arm-specific probes adding one more colour, that adds the capacity to distinguish the arms involved in translocations and to detect pericentric inversions (Sallinen *et al.* 2003). However, generally the colour system of multicolour FISH does not detect intrachromosomal events. Inversions, duplications or deletions must be approximated using the DAPI staining, the quality of which is quite variable.

Intrachromosomal rearrangements can be sought with bar code probes made of YACs (Lengauer *et al.* 1992, 1993) or radiation hybrids (Müller *et al.* 1997). YAC probes and subtelomeric probes have been combined to a modified MFISH (goldFISH) (Saracoglu *et al.* 2001). Cross-species colour banding (Rx-FISH, Müller *et al.* 1997, 1998, 2002) is a coarse whole-genome screening method based on probes made of primate chromosomes, the DNA of which hybridizes to different human chromosomes forming bands, and can be combined with other probes or with G-banding (Schröck and Padilla-Nash 2000, Teixeira *et al.* 2000). Multicolour banding can also be achieved with partially overlapping probes derived from microdissected normal human chromosomes (Chudoba *et al.* 1999, Lemke *et al.* 2002).

5.3.4. Comparative Genomic Hybridization (CGH)

Comparative genomic hybridization (Kallioniemi *et al.* 1992b), which is based on analysis of DNA of malignant cells and does not require metaphase preparations of malignant cells, has been most useful in the study of solid tumours (Kallioniemi *et al.* 1994b). Comparative genomic hybridization allows identification and localization of DNA copy-number changes of the whole genome in a single experiment. In short, the patient's DNA, labelled with a green fluorescent colour, for example, FITC (fluorescein isothiocyanate), is mixed with a reference DNA labelled with red fluorescent colour, for example, Texas Red, and hybridized to normal chromosomes, where they compete for suitable binding sites reflecting their relative concentrations. Each colour in the metaphasis as well as the DAPI background stain are detected separately with a UV-microscope and captured with a CCD-camera (charge coupled device). Computer software analyses the light intensities of each pixel along the axis of each chromosome, and subtracts the background noise. The result is shown as a green to red ratio profile, where the average normalized green to red intensity ratio is 1.0 for the entire metaphase. To reduce the noise, data from multiple metaphases is combined giving profiles for the mean ratio ± 1 S.D. A comparison between two normal DNA samples is always included to control hybridization quality, so that the normal variation (± 1 S.D) does not exceed 0.85 or 1.15 (Kallioniemi *et al.* 1994a).

The original method of CGH detects deletions of 10-20 Mb (reviewed in Kallioniemi *et al.* 1994a, Bentz *et al.* 1998). Amplifications (gains) of small sequences, even 1 Mb, including whole oncogenes, may be detected, if they are highly (5-10x) amplified (Kallioniemi *et al.* 1994a, Forozan *et al.* 1997, review). True polyploidy itself is not possible to detect, nor balanced structural chromosome aberrations. Normal cell contamination, increases the proportion of normal DNA in the sample, so that more than 50% of normal cells makes the analysis increasingly difficult; at least 35% of cells with a similar aberration should be present, but the sensitivity can be increased by selection of statistical thresholds taking into account the variation of normal chromosome regions (Kallioniemi *et al.* 1994a, du Manoir *et al.* 1995, Lichter *et al.* 2000, review). Heterogenity in the tumour is hidden and properties of small subclones possibly giving rise to metastases may go undetected (Lichter *et al.* 2000 review). The sensitivity for deletion detection depends on ploidy level, as the number of normal chromosomes in the malignant cell may exceed the number of chromosomes with a deletion. Repeat sequences, like in peri-centromeric and heterochromatic regions, cannot be analysed with CGH. They have to be blocked with Cot-1 DNA during the hybridization to avoid large ratio changes. Telomeres are often excluded from analysis for their weak staining intensity, and chromosomes 1p32-pter, 16p,19, and 22 may show false deletion (Kallioniemi *et al.* 1994a). Sensitivity of CGH for deletions can be increased to the range of 3 Mb by use of standard reference intervals, based on a series of normal samples (Kirchhoff *et al.* 1999). New matrix-based CGH, with hybridization of tumour DNA to arrays of large insert genomic clones (BACs or cDNA clones) or oligonucleotides on chips, allows analyses at single gene level, especially of high-level amplifications (Solinas-Toldo *et al.* 1997; Albertson and Pinkel 2003, Schwaenen *et al.* 2003, reviews), and has also been used in the study of CTCL (Mao *et al.* 2003a).

6. AIMS OF THE STUDY

The aims of this thesis were to detect and characterize chromosomal aberrations associated with the aetiology and progression of CTCL using cytogenetic and molecular cytogenetic methods, and to examine their association with clinical progression of the disease, in order to provide diagnostic and prognostic tools and investigate the aetiology of the disease. This was achieved by

1. investigating chromosome aberrations by cytogenetic and simple centromere-sepcific *in situ* hybridizations

2. by following-up of the clinical condition and changes in the above chromosomal aberrations

3. studying immunohistochemically the expression of molecules with functional or signal-transducing properties in malignant cells identifiable by *in situ* hybridizations

4. searching chromosomal regions with DNA amplified or lost, possibly harbouring oncogenes or tumour suppressor genes, using comparative genomic hybridization (CGH)

5. identifying the most common chromosomal abnormality observable with 24-colour *in situ* hybridization in blood metaphase cells, identifying the gene involved, and studying its ploidy level in skin lesion cells

7. MATERIAL AND METHODS

7.1. Material

7.1.1. Patients

Patients were from the Departments of Dermatology and Venereology of Helsinki University Central Hospital's Skin and Allergy Hospital, Helsinki, of Tampere University Hospital, Tampere (7 SS-patients in study I), Finland and of the Medical University of Gdansk (4 SS-patients in study V), Gdansk, Poland. The studies were approved by the ethical review boards of the respective Hospitals.

The diagnosis was based on clinical, histological and immunohistological findings (reviewed in Kuzel *et al.* 1991), the latter two in at least two consecutive biopsies according to the principles of the European Organization for Research and treatment of Cancer (EORTC, Willemze *et al.* 1997).

The patients were seen and samples were obtained in the context of the patients' regular hospital visits and examinations, and therefore the intervals between sampling of the individual patients were irregular. Also, while some patients were untreated, many had received different treatment modalities, e.g. psoralen and UVA (PUVA) as topical or systemic treatment, electron beam treatment or chemotherapy. All the patients had given informed consent for the additional samples obtained for this study.

For control purposes, peripheral blood of 38 healthy persons (for details see the studies I to V), 4 patients with psoriasis vulgaris or alopecia treated with PUVA (psoralen + UVA, studies I and II), frozen tissue biopsies of one patient with histopathologically confirmed lymphomatoid papulosis (study III) volunteering for this study were obtained. Also skin biopsies from 9 patients with histopathologically confirmed lupus erythematosus discoides or eczema, typically with non-malignant T lymphocyte infiltrates (study V), obtained after informed consent, were used.

The number and staging of patients are shown in **Table IIa**. Individual patients appearing in more than one study are shown in Table **IIb**.

Table IIa. The number of patients studied and the cytogenetic methods used

Diagnosis	LPP/FM	MF/Stage						SS/Stage	Methods
Study		IA	IB	IIA	IIB	III	IVA		
I	3/1	2	4		3	1		3/IIIB1	G-banding, centromere specific FISH
II	4/1	2	1	1	2			2/IVA	G-banding, centromere specific ISH
III								2/IVA	FICTION
IV			1		3			5/IVA;2/IIIB1	CGH
V		1	6	1	6		2	8/IVA	MFISH/SKY: 12 patients; CGH 5:patients; locus-specific FISH: 21patients

LPP: large plaque parapsoriasis, FM: follicular mucinosis, MF: mycosis fungoides, SS: Sézary syndrome

ISH: *in situ* hybridization, FISH: fluorescent ISH, MFISH: multifuor FISH, SKY: spectral karyotyping

FICTION: fluorescence immunophenotyping and interphase cytogenetics as a tool for investigation of neoplasms

CGH: comparative genomic hybridization

Table IIb. Cases appearing in multiple studies

	Case number of the specific patient in the respective studies				
Diagnosis/stage	Study I	Study II	Study III	Study IV	Study V
AlopM	1	4			
LPP	2	3			
LPP	3	1			
LPP	4	5			
MF/IA	5	13			8
MF/IA	6	9			
MF/IB	7	11			16
MF/IB	8				
MF/IB	9	7			15
MF IB->IIB	10				21
MF/IIA		12			9
MF/IIB	11	6		1	20
MF/IIB	12	10		4	
MF/IIB	13	8			
MF/III				2	23
MF III->SS IVA	14			7	
SS/IIIB1	15			9	
SS/IIIB1	16				
SS/IIIB1	17			8	
SS/IVA		14	1	6	1
SS/IVA		15	2	11	3

AlopM: alopecia mucinosa, LPP: large plaque parapsoriasis, MF: mycosis fungoides, SS: Sézary syndrome
For stages, see Table I.

7.1.2. Patient samples

Peripheral heparin and EDTA blood was sampled in studies I, II , IV and V. Lesional skin or lymph node biopsies were obtained in studies II to V, snap frozen in liquid nitrogen and stored in -70 C. In addition, in one case, post-mortem lesional skin and lymph node samples were studied (studies III and V).

7.2. Methods

7.2.1. Basic principles of the studies and methods used

Study I Conventional metaphase slides were prepared of T-cell mitogen stimulated blood lymphocytes cultures, and used for traditional G-banded metaphase analyses, and fluorescent *in situ* hybridizations (**Figure 4)** with probes selected on basis of findings of Whang-Peng *et al.* (1982). Chromosomally aberrant cells were scored, and analysed statistically. Some chromosomal findings were further studied with enzyme-detected *in situ* hybridizations (EDISH).

Study II As in Study I, conventional metaphase slides were preparared from T-cell mitogen stimulated blood lymphocyte cultures, and used for traditional G-banded metaphase analyses, and *in situ* hybridizations (**Figure 4),** which were all performed with an own version of the EDISH method, producing archivable preparations. Centromere-specific probes were selected on the basis of the findings of the first study (Karenko *et al.* 1997). Follow-up samples were taken. Chromosomally aberrant cells were scored and analysed statistically.

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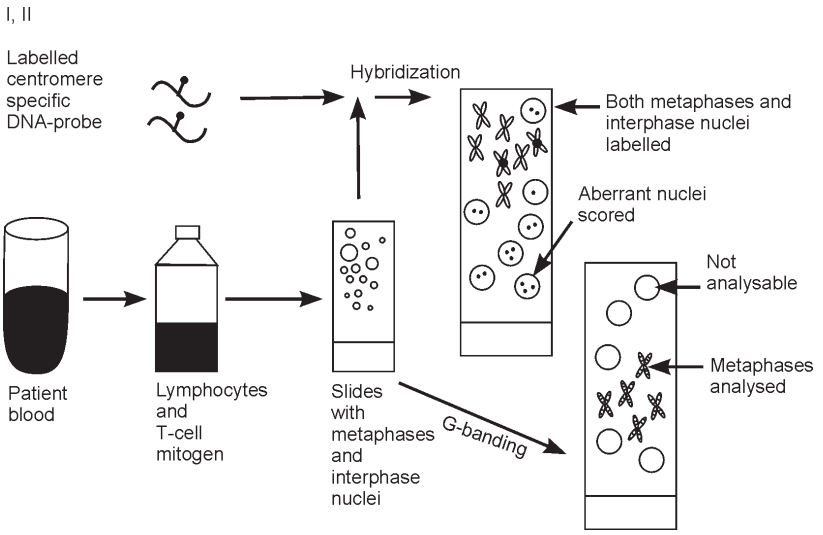

Figure 4. Principles of studies I and II Cultivated blood lymphocyte preparations with metaphases and interphase cells were used for G-banding studies of the mitotic chromosomes and in situ hybridization analyses of the interphase cells. Studies I and II.

Study III Phenotype and function-associated markers on interphase cells were detected with fluorescent immunohistochemistry, followed by *in situ* hybridizations allowing an individual identification of malignant cells (an own modification of the FICTION method of Weber-Matthiesen *et al.* 1992, 1993) **(Figure 5**).

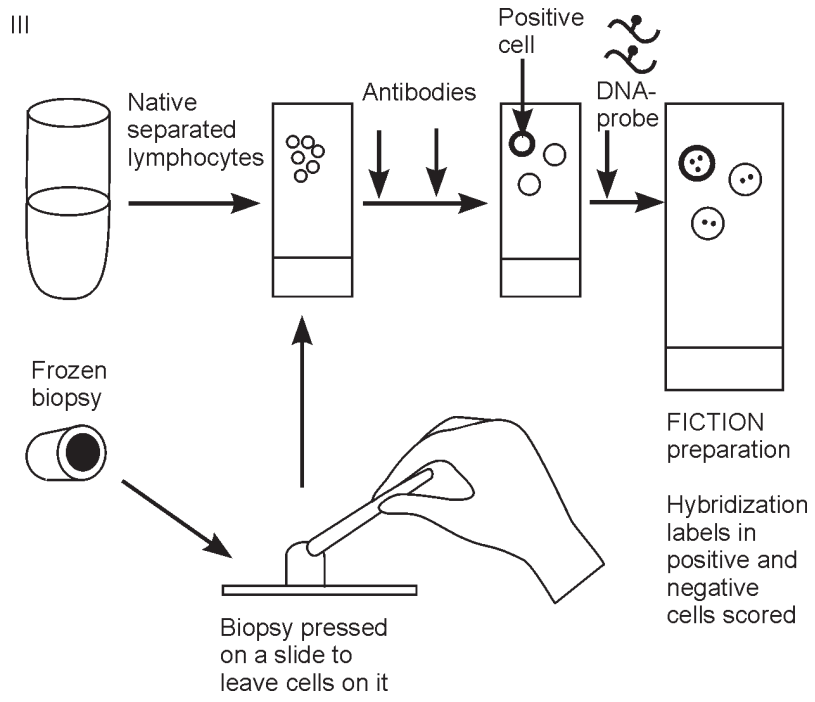

Figure 5. **Simultaneous immunolabelling and *in situ* hybridization** (Study III). Native separated blood lymphocytes or touch preparations of whole cells of frozen biopsies were immunolabelled and hybridized with chromosome centromere-specific probes allowing the individual identification of malignant cells showing aberrant numbers of chromosome centromeres.

Study IV Peripheral blood lymphocyte DNA of CTCL-patients was studied with comparative genomic hybridization in order to reveal DNA copy-number changes and to localize them to the respective chromosomal regions (**Figure 6**).

Study V Peripheral blood lymphocyte metaphases of seven consecutive patients with Sézary syndrome and four patients with mycosis fungoides were studied with 24-colour FISH (multicolour FISH, including spectral karyotyping, SKY, or multi-fluor FISH, MFISH) detecting every chromosome pair with a specific colour combination (**Figure 7**) in order to find the most common chromosome affected by structural abnormalities. The aberrations in the most commonly affected chromosome band were studied with locus-specific YAC- and BAC-probes revealing the gene affected. Mutations affecting the microscopically intact chromosome arm allele were sought with DHPLC and/or sequencing. Gene expression was studied with RT-PCR. The abnormality was further studied with locus-specific FISH in skin or lymph node biopsies of 21 CTCL patients at different stages of the disease.

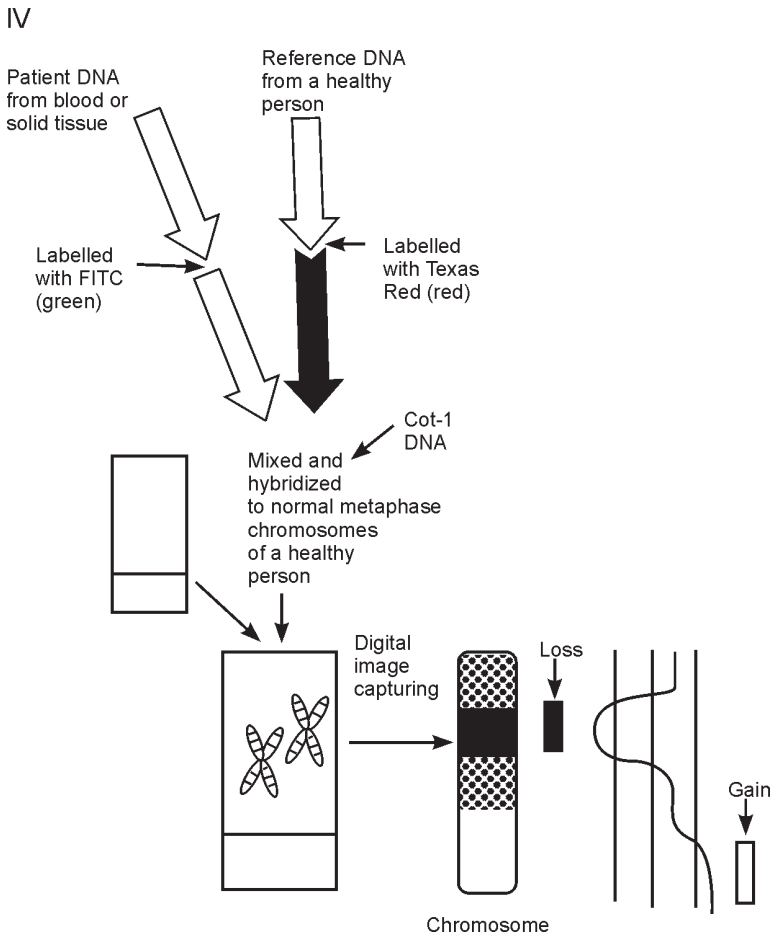

Figure 6. **Principle of CGH**. (Study IV) Patient blood or skin lesion DNA was labelled with a green colour and a reference DNA sample with red colour. They were mixed and hybridized to normal chromosomes of a healthy person. The DNAs compete from hybridization target sequences on the chromosomes, resulting in a banding pattern. Images were captured and analysed digitally creating a profile, that demonstrates regions of relative excess of patient DNA as green and relative loss of patient DNA as red.

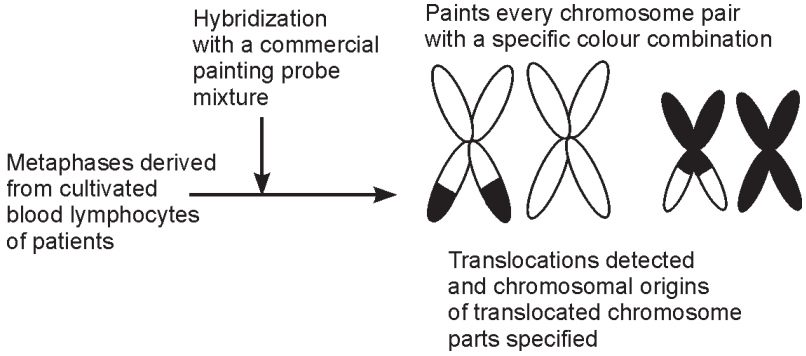

Figure 7. **Principle of 24-colour FISH (MFISH, SKY)** Every chromosome pair is detectable with a specific colour combination allowing the identification of chromosomal origin of chromosome fragments transferred to another chromosome. In the Figure, only one chromosome pair is shown as an example.

7.2.2. The purification of DNA

Lymphocytes were extracted from peripheral blood with Ficoll gradient centrifugation, and DNA was purified with phenol-chloroform extraction (Sambrook 1989) The skin tumour or lymph node samples were snap frozen in liquid nitrogen, ground in a mortar, and the DNA was extracted with phenol-chloroform (Sambrook 1989).

7.2.3. Metaphase preparations, G-banding and chromosomal analysis (Studies I, II, V)

Peripheral blood lymphocytes were stimulated with PHA, cultured for three to four days, and metaphases G-banded in the conventional way (Verma and Babu, 1989). In initial samples, 100 metaphases and in follow- up samples, 20 to 100 metaphases were analysed.

7.2.4. *In situ* hybridizations (all studies)

Probes and probe labelling DNA probes, 200-400 bp in size after labelling, and specific for the pericentromeric regions of chromosomes 1, 7, 8, 9, 11, 12, 17 and 18 (see **Table III**) were biotinylated (BioNick kit; Gibco BRL, Gaithersburg, MD USA) or labelled with with digoxigenin -11-dUTP (Boehringer Mannheim, Germany) by nick translation (BioNick kit). The commercial probes are indicated (Biotinylated: Oncor Inc. Gaithersburg, MD, USA, digoxigenin-labelled: Boehringer Mannheim).

Table III. Centromere-specific probes and labels used

Chromosome	Centromere-specific probe	label (study)
1	1q12[pUC177]	B(I,II,III);D(I,II),TxRX(III)
6	Commercial	D(I,II);B(II)
7	p7alphaTET[PUC99]	FITC,TxR
8	D8Z2[pJM128]	B(I,II);D(II);A488,A594(III)
9	pHuR98	B,D(II)
11	pSP65[pLC11A]	B(I;II);D(II)
12	pA12H8	D(I,V);B(V)
13 and 21	Commercial	B,D(II)
15	Commercial	B,D(II)
17	D17Z1[p17H8]	B,D(I);
18	p18R	D(I,V);B(V)

B: Biotin, D: Digoxigenin-11-dUTP, FITC: Fluorescein isothiocyanate-dUTP
TxR: Texas Red-dUTP, TxRX: Texas Red X, A488: Alexa 488, A594: Alexa 594

Microdissection painting probes specific for chromosomes 2, 4p, 4q, 5, 6q, 8q, 10q, 12q, 13q, 18q (kindly provided by Dr. X-Y. Guan, NIH, USA; Guan *et al.* 1996) were directly labelled with FITC-dUTP or Texas Red-dUTP (DuPont) and a PAC clone specific for 13q22 labelled with digoxigenin and biotin (Laan *et al.* 1996; kindly supplied by Dr. Tuomas Klockars, Institute of National Health, Helsinki, Finland) by nick translation as above, for validation of CGH results.

Chromosomes 12 and 18 were studied with locus-specific YAC- probes (obtained from Fondation Jean Dausset, France), BAC- and PAC-probes (obtained from Research Genetics Inc., Huntsville, AL, USA), which were selected with the help of NCBI databases (MapViewer program). The probe identities were confirmed using PCR with locus-specific primers according to NCBI's databases. The YAC-probes used for chromosome 12 are indicated in **Figure 8**. YACs and BACs for chromosome 18q are indicated in **Table IV**. BACs used for the study of 18p11.3 were RP11-683I23 (AP001005.5), RP11-705O1 (AP000845.4), RP11-683J11 (AP000900.3), RP11-720L2 (AP000915.5), RP11-778P8 (AC021474.3), and of 18p11.2, RP11-771B1 (AP000876.2).

The YAC, BAC, and PAC DNAs were isolated using routine techniques and labelled with FITC (Fluorescein-12-dUTP, NEN Life Science Products, Inc, Boston MA USA), Alexa 488®, Alexa 594® (both Molecular probes), biotin-14dATP (Gibco Invitrogen, Rockville, MD, USA) or digoxigenin-11-dUTP (Roche, Mannheim, Germany) using nick translation.

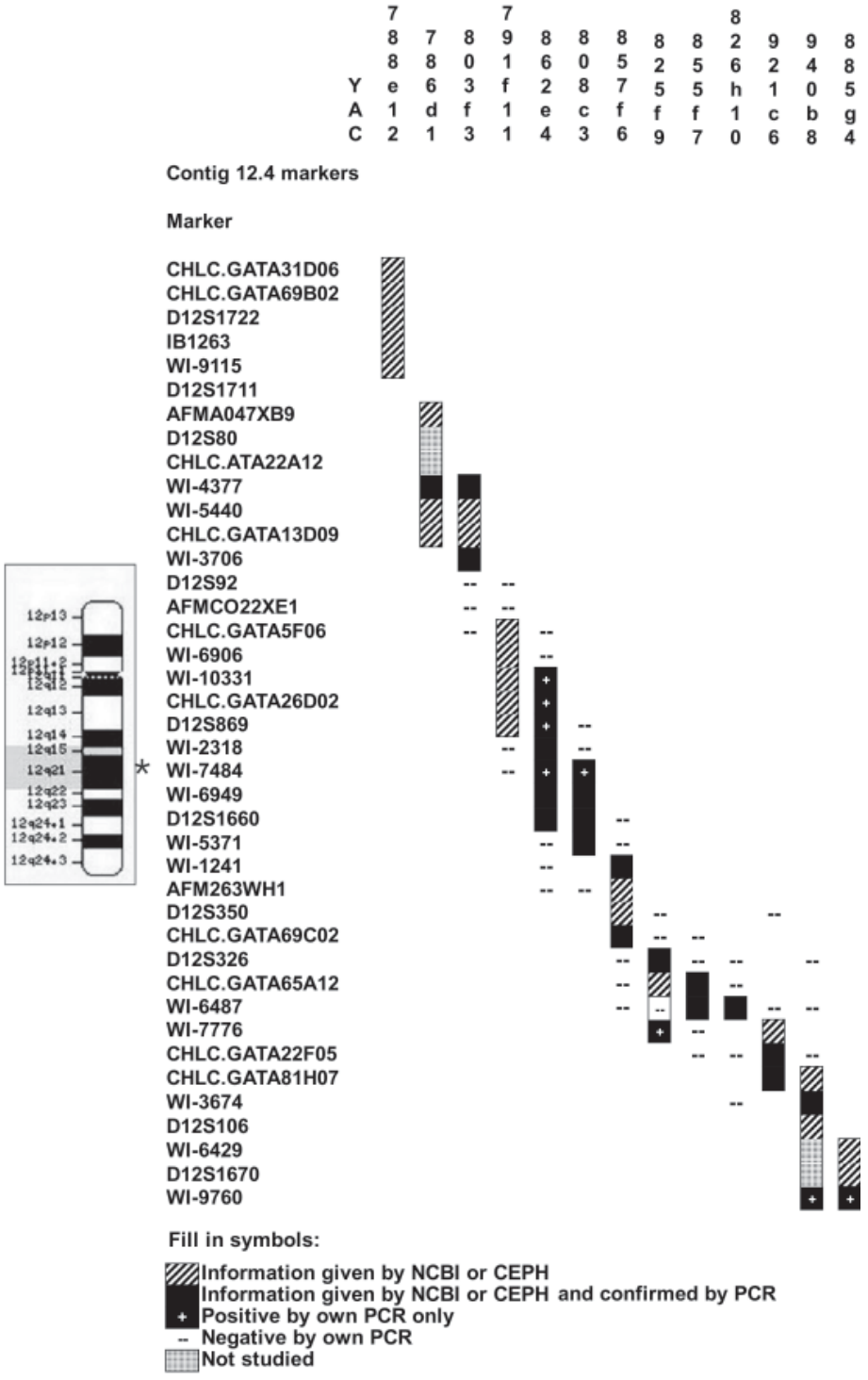

Figure 8. Breakpoint of translocation (12;18)(q15or21;q21) and deletions of 12q in SS (cases 1 and 2) were specified with part of the YAC contig 12.4. PCR confirmations of the localisation of the respective YACs are indicated with symbols below. Approximation region 12q15 to 12q21 is marked with grey in the chromosome 12 ideogram (NCBI), with an approximation of the distal end of 12q21.1 with an asterisk. Additionally, other YACs outside this region were studied: 893A3 (12q12, contig 12.1,WI-1851; 850H3, 765B4, 12q13, contig 12.4, markers D12S72 and AFMB303XC1, respectively, patient 2), 803C2, 745A10 (12q14-q15, contig 12.4, markers WI-3072 and D12S313, respectively), 939H2 and 825G7 (cases 1 and 3; Schoenmakers *et al.* 1995), 817H10, 829B5, 896H8, 823E12, 948E9 (12q22-12q23, contig 12.5, markers CHLC.GATA69F06, D12S318, D12S338, D12S78 Renault *et al.* 1995). For 12q24, PACS RP3-443K8, RP3-462E4, RP1-315L5 and BAC RP11-144J4 were used (AC005907, AC003029.3, AC002395.1, AC079406.6, respectively)

Table IV. Probes in 18q used for specifying the breakpoint in translocation t(12;18)(q12;q21), patient 3

Band	YAC or BAC		Accession nr of the BAC	Markers in the BAC or YAC	Genes in the probe
18q12.3	RP11-	687F6	AP002391.1	StSG46608	piasx-beta
18q21	RP11-	699C17	AP002393.1	WI-19692	MBD1
18q21	RP11-	839G9	AP001910.2	SHGC-58160	RAB27B, part, Se57-1, LOC3427765
18q21.1	RP11-	837M2	AC091111.4	StSG47676	TCF4, part
18q21	852H2			AFM357TD5, AFM191XCP9	
18q21	RP11-	619L19[a]	AC018994.7	AFM357TD5	TCF4, part
18q21	RP11-	397A16[a]	AC022031.8	RH123771	LOC350570, LOC 284256
18q21	RP11-	214L13[a]	AC027584.4	SHGC-79995	LOC 284256
18q21.2	RP11-	450M22[a]	AC016165.11	RH98615	LOC 284256
18q21	RP11-	822F4	AC090758.2	D18S69	LOC342769, LOC342770
18q21	RP11-	859C21	AC090408.2	SHGC-7237	LOC342772, TNXL,WDR7, part
18q21	RP11-	383D22	AC012301.5	SHCG-103952	WDR7, part
18q21	RP11-	700H19	AC090296.2	SHCG-103952	WDR7, part, LOC350571
18q21	RP11-	660C14	AP001772.2	D18S1245	LOC350572, LOC342773, SIAT8C, LOC342774
18q21	RP11-	248C13	AC084350.1	D18S1245	SIAT8C, LOC342774, Onecut, LOC342775
18q21	RP11-	837J4	AP002417	WI-20204	FECH, NARS, ATP8B1, part
18q21	RP11-	275K5	AC022724.8	SHGC-154413	ATP8B1 part, LOC342776, LOC284288, LOC342777 part
18q21	RP11-	693L9	AP001487.3	SHGC-154413	LOC284288, LOC342777
18q21	762D8			WI-5450, WI5827	
18q21	789F3			D18S1144, WI-5827	
18q21	817C16			WI2299, D18S1103	MALT1
18q21.3		LSI/IGH/BCL2[b]			BCL2

[a] Backs in the same region as YAC 852H2 (between AFM357TD5 and AFM191XCP9)

[b] LSI/IGH/BCL2 dual color, dual fusion translocation probe Vysis, Vysis Inc., Downers Grove, Illnois, USA

***In situ* hybridizations of centromere-specific probes to metaphase preparations** (Studies I, II, III, V) Centromere-specific probes were hybridized to metaphase preparations (studies I and II) essentially as previously described in Hyytinen *et al.* (1994). Target interphase cells and metaphases on slides were denatured in for 2 to 3 minutes in 70% formamide /2xSSC solution (pH 7.0) at 70 to 73 C, and dehydrated in 70%, 85%, and 100% ethanol, and treated with proteinase K (1µg/ml, Sigma Chemical Co, St Louis, MO, USA) in 20 mM Tris/2mM $CaCl_2$ (pH 7.5) buffer for 7.5 minutes at 37 C, and dehydrated as above. Hybridization mixture containing the labelled probe (1-5ng), dextran sulphate (10%, Sigma, St. Louis Mo, U.S.A), formamide (55% in SSC) and herring sperm DNA (0.5µg/ml in TE buffer, pH 8.0; Sigma) and optionally, Cot-1 DNA (e.g. 125ng; Gibco BRL, Gaithersburg, MD USA, or Boehringer Mannheim/Roche, Mannheim Germany) was denatured in 70 C for 5 minutes and applied on the pretreated slides, sealed under a coverslip with Rubber Cement (Starkey Chemical Co, LaGrange IL USA) and allowed to hybridize in a humid chamber (37 C) overnight.

In Study I, depending on the probe (FISH or EDISH), the procedure continued in two different ways. The slides for FISH were preblocked with 1% BSA (Sigma) in 4xSSC, incubated with avidin-FITC (avidin fuorescence isothiocyanate 5 µg/ml; Vector Laboratories, Burlingame, CA,USA), washed with 4xSSC and PN solutions (0,1M NaH_2HPO_4, 0,1M Na_2HPO_4/0,1%NP-40, pH 8,0). Further preblocking was done in PNM (5% Carnation dry milk/PN), prior to biotinylated anti-avidin antibody (5 µg/ml; Vector Laboratories) as described before (Hyytinen *et al.* 1994). Finally, the slides were stained anew with avidin-FITC, washed, and mounted in 10 µl propidium iodide (1µg/ml propidium iodide, Sigma) in an antifade solution (Vectashield, Vector Laboratories). In the EDISH method, digoxigenin-labelled probe was detected with mouse anti-digoxigenin antibody (Boehringer Mannheim) followed by biotinylated anti-mouse antibody, and avidin-biotin-peroxidase-mixture (Vectastain Elite mouse IgG kit; Vector Laboratories). Diaminobenzidine (DAB) with nickel was used as chromogen (DAB Substrate Kit, Vector Laboratories). The slides were counterstained with alum-kernechtrot ($Al_2(SO_4)_3.18H_20$, 10g, kernechtrot 0,2g, H_20 200ml, both Merck, Darmstadt, Germany) for 5 minutes, washed with tap water, dehydrated, and mounted in Entellan (Merck). The hybridization results were evaluated without knowledge of the patient's clinical diagnosis and the result of G-banding. Overlapping nuclei were not analysed. The signals had to be of equal intensity and clearly separate. At least a hundred interphase nuclei were analysed from each sample, with a few exceptions.

In Study II, hybridizations were performed as above with the modification of the simultaneous use of two probes, labelled with digoxigenin and biotin. The digoxigenin-labelled probe was detected with sheep anti-digoxigenin antibody (Boehringer Mannheim), followed by AFOS-conjugated donkey anti-sheep (Sigma) and goat anti-equine (Harlan Sera-lab, Crawley Down, Sussex, England) antibodies, and visualisation with Nitro blue tetrazolium chloride (NTB) mixed with 5-bromo-4-chloro-3-indolyl-phosphate, 4-toluidine salt (0.5% and 0.4%, respectively, in TRIS-buffer with 0.1 mol/l of NaCl and $MgCl_2$; Boehringer Mannheim). The biotin-conjugated probes were detected with two layers of avidin peroxidase (Vector Laboratories), biotinylated mouse anti-avidin (Sigma) between the layers, and visualised with a purple chromogen (Vector VIP®, Vector Laboratories). The preparations were washed and mounted in Pertex (Histolab, Göteborg, Sweden). The number of centromere signals of each chromosome studied was counted among 400 interphase cells in well hybridised areas with low background colour.

Hybridizations with painting probes and locus-specific probes to metaphase preparations (Studies IV and V) For hybridization with painting probes (Study IV), 8 µl of one probe labelled with FITC and another labelled with Texas Red were mixed and precipitated by adding 1/10 volume 3M sodium acetate and 2x volume 100% ethanol, and centrifuged. The supernatant was discarded, the pellet was allowed to dry, after which the DNA was dissolved in 10µl of a mixture consisting of 50% formamide, 10% dextran suphate, 2x SSC, pH 7. The hybridization was then performed as above. The slides were counterstained with DAPI. For enzyme detected ISH, the above probes for chromosomes 6q, 8q, 10q, 12q and 13q were labelled with digoxigenin-11-dUTP (Boehringer Mannheim). They were hybridized as other painting probes above, washed 3 times with 50% formamide in 2xSSC, pH 7, 4xSSC, and 0.1 xSSC, all at 45 C, and with 4xSSC, 2xSSC and PBS at room temperature. The hybridized probe was detected with mouse anti-digoxigenin antibody (Boehringer Mannheim), followed by biotinylated anti-mouse antibody (Vector laboratories), avidin peroxidase (Vector laboratories), biotinylated mouse anti-avidin (Sigma St. Louis, Missouri, USA) and another layer of avidin-peroxidase. VIP® (Vector® VIP Substrate Kit, Vector laboratories) was used as chromogen. The slides were washed with distilled water, dehydrated, and mounted in Pertex (Histolab Products AB, Västra Frölunda, Sweden), and evaluated with bright-field microscopy. The locus-specific probes (Study V) were precipitated and hybridized to metaphase slides as above. The biotin labelled probes were detected with one or two layers of avidin-Cy3 (ExtrAvidin-Cy3 conjugate, Sigma-Aldrich, St Louis, Missouri, USA) or avidin-FITC (Vector Laboratories). The digoxigenin labelled probes were detected either with sheep anti-digoxigenin antibody (Roche, Mannheim, Germany) followed by donkey anti-sheep antibody labelled with FITC (Jackson Immuno Research Laboratories) or by sheep anti-digoxigenin-rhodamine antibody (Roche, Mannheim). Digital images of the metaphases were taken and analysed as described above.

***In situ* hybridization of centromere-specific probes or locus-specific probes to touch preparations** (Studies III and V) In the study III, after immunohistological staining, the samples were fixed with 1% paraformaldehyde in PBS for 3 minutes or 1% paraformaldehyde for 4 minutes, washed in PBS or dehydrated and hybridized (see Study I above) with a chromosome 8 and 1 centromere-specific probes (**Table III**) labelled with colours or haptens to be detected with colours different from the colour used in the immunolabelling. In study V, two-color interphase fluorescence *in situ* hybridization (FISH) with BACs in the region of NAV3 was performed. The slides were pretreated modifying the method used in study III, the probes were precipited as painting probes above. To detect the deletions, digoxigenin labelled BACs 136F16 and 36P3 were cohybridized together with a centromere-specific probe labelled with biotin. The translocation was detected with digoxigenin labelled BACs 136F16 and P36P3 with biotin labelled BACs 786A1 and 494K17. After hybridization, the probes were detected with avidin-FITC and anti-digoxigenin rhodamine as described above and counterstained with DAPI.

Comparative genomic hybridization (Studies IV and V) CGH was performed essentially as described before (Kallioniemi *et al.* 1994a, Visakorpi *et al.* 1995). Briefly, the patient's DNA was labelled with fluorescein isothiocyanate-dUTP (FITC-dUTP, DuPont) using nick translation resulting in DNA fragments of 600-2000 bp. DNA of a healthy control was similarly labelled with Texas Red-dUTP (DuPont). About 400ng of each of the labelled DNAs and 10µg of Cot-1 DNA (Gibco BRL, Gaithersburg, MD USA) were mixed, ethanol precipitated and dissolved in 10µl of buffer containing 50% formamide and 10% dextran sulphate in 2xSSC, pH 7. The DNA mixture was denatured and hybridized to metaphase preparations of normal lymphocytes denatured and treated with proteinase K (concentration optimized, ad 0.1 mg/ml) as explained above.

The hybridization was allowed to take place at 37 C in a moist chamber for 16-40 hours. The slides were washed and counterstained with 1 M 4,6-diamidino-2-phenylindole 0.1 g/ml (DAPI, Boehringer Mannheim) in an antifade solution (Vectashield ®, Vector Laboratories).

Multicolour fluorescent *in situ* hybridization (Study V). Multicolour fluorescent *in situ* hybridization (multicolour FISH) was performed either as spectral karyotyping (SKY; Schröck *et al.* 1996), or as multifluor FISH (MFISH: Speicher *et al.* 1996). The SKY was performed according to the protocol recommended by the manufacturer (Applied Spectral Imaging, ASI, Migdal HaEmek, Israel). For MFISH, the metaphase preparations were postfixed with 0.1% paraformaldehyde and denatured as in Study I and II, and hybridized with probe mixture (24XCyte-MetaSystems' 24 colour kit, MetaSystems GmbH, Altlussheim, Germany) containing differently labelled painting probe combinations specific for each chromosome pair labelled with a chromosome -specific fluorochrome combination, that had been denatured in 76 C for 6 minutes and incubated in 37 C for 60 minutes, as recommended by the manufacturer. After hybridization for 3 to 5 days in 37C, and washes, the biotin labelled probes were detected with one or two layers of streptavidin-Cy5 (B-tect kit, MetaSystems GmbH), and the preparations were mounted in antifade and DAPI.

Immunolabelling and the preparations used (Study III). Cytospin preparations of Ficoll-enriched blood mononuclear cells or touch preparations of frozen skin or lymph node biopsies were fixed in ice-cold acetone, and an immunohistological staining was performed using antibodies to CD3, CD4, (Dako, Glostrup, Denmark), CD45RA (Caltag, Burlingame, California, USA), CD45RO, CD8 (Dako), granzyme B, IL-2, IL-4, IL-10 (Santa Cruz Biotechnology Inc., Santa Cruz, California, USA), IFN-γ (Neomarkers, Fremont, CA, USA) and signalling lymphocytic activation molecule (SLAM, CDw150; A12 antibody kindly provided by Dr. G. Aversa, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, California, USA). The primary antibodies were monoclonal mouse antibodies except for the polyclonal rabbit antibodies against CD3 and IL-2, and goat anti-bodies against granzyme B, IL-4, and IL-10. The secondary antibodies were anti-rabbit or anti-mouse goat conjugates of Texas Red X or Alexa 594 ® (Molecular Probes, Leiden, Netherlands), or anti-rabbit, anti-goat or anti-mouse donkey conjugates of Rhodamine Red X (Jackson Immuno Research Laboratories, West Grove, PA, USA). When necessary, for detection of weak mouse primary antibodies, Rhodamine Red X-conjugate was followed with anti-rhodamine rabbit (Molecular Probes) and an anti-rabbit donkey conjugate Rhodamine Red X (Jackson Immuno Research Laboratories). All antibody layers were preceded by a layer of normal serum from the animal species, in which the secondary antibody was raised. Through every step of the process, each slide was accompanied by a similar control slide with no primary antibody.

Alternative stainings were used to confirm the results. The immunohistology was performed by detecting the primary mouse antigen with biotinylated anti-mouse raised in horse, followed by avidin conjugated with FITC (Vector Laboratories). Alternatively, the primary mouse antibody was detected with rabbit anti-mouse antibody (Sigma), followed by anti-rabbit raised in swine (Dako), anti-swine raised in rabbit (Rockland, Gilbertsville, PA, USA), and finally goat anti-rabbit conjugated with a blue color (Alexa 350®, Molecular probes).

The formalin-fixed, paraffin-embedded skin biopsies of cases 1 and 2 were examined for CD30 and a skin biopsy of the third patient with lymphomatoid papulosis for granzyme B with standard immunoperoxidase technique (for CD30, StreptABC Complex/HRP kit, Dako followed by 3-amino-ethylcarbazole, for granzyme B (Nevala *et al.* 2001).

7.2.5. Analyses and imaging

G-banded chromosome preparations (Studies I, II, V) The chromosomal aberrations were classified according to ISCN 1995 (Mitelman 1995). As polyclonal, numerical chromosomal aberrations are common in CTCL, a chromosomal clone was defined as 3 or more metaphases with the same numerical aberration, or 2 or more metaphases with the same structural aberration (ISCN 1995) per 100 metaphases. In initial samples, 100 metaphases and in follow- up samples, 20 to 100 metaphases were analysed. Metaphase preparations were also used for *in situ* hybridizations (Studies I, II, V).

Analyses of *in situ* hybridizations *In situ* hybridizations with centromere specific probes, FICTION and locus specific hybridizations were analysed blinded for the diagnosis and identity of the patient. In two-colour EDISH preparations, 400 interphases and in touch preparations, at least 50 interphase cells were analysed. In SKY or in MFISH, 10 to 70 metaphases and in CGH at least four metaphases were analysed. Only good hybridization quality was accepted, and no overlapping nuclei were analysed. In CGH, relative DNA sequence copy number changes were detected as differences in the ratio of the green (FITC) to the red (Texas Red) fluorescence colour intensities along the length of all chromosomes from pter to qter in the metaphase spread. The results were displayed as a set of average profiles with +\- 1 SDs for each chromosome. Chromosomal regions with the mean ratio of +1 SD below 0.85 were considered as lost, and regions with the mean ratio of -1 SD exceeding 1.15 as gained (Kallioniemi *et al.* 1994a).

Microsocpy and imaging Microscopy was performed with a Leitz Dialux (Germany)(Studies I, II) or Olympus BH-2 Tokyo, Japan, equipped with a kinofilm camera (Olympus C-35AD-4, Tokyo, Japan (Study I) Olympus BX 50, Tokyo, Japan, equipped with equipped with filter set 8300 and tripleband exciter 83103x, Chroma Technology Corp., Brattleboro, VT, USA) and a cooled CCD camera (Sensi Cam, PCO, Computer Optics, Kelheim, Germany) combined to a computer (Dell, Limerick, Ireland) with software Cario Image (Immagini & Computer SNC, Milano, Italia) inserted in Image pro Plus (Media Cybernetics, Silver Spring, MD, USA) (Studies II, III); or Zeiss Axioplan 2 equipped with a CCD camera and a computer (Dell, Limerick, Irland) with software Ikaros or Isis of MetaSystems GmbH with MFISH-program module (Metasystems, Altlusheim Germany (Study V). For SKY (Study V), image acquisitions were preformed using a SD200 Spectracube system (ASI) mounted on a Zeiss Axioskop microscope with a custom-designed optical filter (SKY-1, Chroma Technology, Brattleboro, VT, USA). The conversion of emission spectra to the display colours was achieved by assigning blue, green, and red colours to specific sections of the emission spectrum.

Digital images in CGH (Study IV) were analysed with an epifluorescence microscope (Nikon SA, Nikon Corp. Tokyo, Japan) equipped with a camera (Xillix CCD, Xillix technologies Corp., Vancouver, BC, Canada) and a computer and a Sun LX workstation (Sun Microsystems Computer Corp., Mountain View, CA, USA; Visakorpi *et al.* 1995) or using an IPLab Spectrum Image acquisition system (Signal Analytics Corporation, Vienna, VA) and Quips 2.3 Software (Vysis Inc. Downers Grove, IL60515). Each fluorochrome was sequentially excited, and images of 3-6 adequate quality metaphases of every sample were stored and analysed. Relative DNA sequence copy number changes were detected as differences in the ratio of the green (FITC) to the red (Texas Red) fluorescence colour intensities along the length of all chromosomes from pter to qter in the metaphase spread. The results were displayed as a set of average profiles with +\- ISDs for each chromosome. Chromosomal regions with the mean ratio of +1 SD below 0.85 were considered as lost, and regions with the mean ratio of -1 SD exceeding 1.15 as gained (Kallioniemi *et al.* 1994a).

7.2.6. Statistical analyses

The statistical analyses. The statistical analyses were performed with the BMDP package (BMDP, Statistical Software) (Studies I and II). In the study I, The G-banding results were analysed with the Kruskall-Wallis nonparametric ANOVA continuing with the Dunn multiple comparison test. The FISH results were evaluated with the Mann-Whitney U-test. All p-values < 0.05 were considered statistically significant. As the number of patients studied was small, p-values 0.05-0.10 are also reported. The FISH- and G-banding results were compared with the Kappa test. An aberration rate higher than the highest observed individual aberration frequency in the healthy control group in FISH was considered abnormal.

The follow-up statistical analyses (Study II) For EDISH, all the chromosomes studied were tested separately. For G-banding, the metaphases with numerical aberrations (G_N) or numerical or structural aberrations (G_S) of the chromosomes 1,6,8,9,11,15,13, and 17 were counted and tested. In addition, the total percentage of aberrant metaphases of every sample was tested (G_{tot}).

To study the frequency of aberrations in different diagnostic groups (**Table V**), the patients were grouped 1) according to the diagnosis (SS, MF or LPP), 2) whether CTCL or LPP, and 3) all patients. In the groupings 1-3, every person was represented by the mean of aberrant interphases or metaphases weighted for the total amount of interphases or metaphases studied in his/her samples.

The effect of the activity of the disease was studied by dividing all patients' separate samples into two groups (**Table V**), those taken during active disease or those obtained during remission (grouping 4).

Both healthy individuals and non-cancer patients treated with PUVA were always included as controls (except for chromosome 13/21, with only healthy controls).

The relationship between chromosomal findings and disease outcome was studied by dividing the samples of grouping 4 (see above) further according to the clinical disease course after each sampling until the next sample or the end of the study (grouping 5, **Table V**). Stable disease was defined as neither complete regression of previous skin lesions (despite therapy) nor appearance of new lesions. Remission was defined as disappearance of all visible skin lesions and resolution of eventual lymphadenopathy (i. e. complete remission). Regarding SS, the presence of morphological Sézary cells in the peripheral blood was also taken into account.

All groupings were tested with Kruskall-Wallis, continued with paired comparisons corrected for the number of pairs (Dunns' test). As there were only two patients with Sézary's syndrome, they were excluded from the paired comparisons.

To evaluate the relationship between any individual chromosome EDISH, G_S or G_N finding and the development of the disease, the percentage of aberrant cells in each sample was compared to the normal distribution of percentage of aberrant cells in the healthy controls. For G-banding, the total percentage of aberrant metaphases ($G_{tot)}$ was also calculated for each patient sample and compared with the normal distribution of the aberration frequency in the healthy controls. The percentage of samples representing progressing or stable disease and showing aberration levels within or above the normal range (cut off-level 5% probability) was also calculated.

The agreement between both the change in the chromosomal finding and the change in the clinical condition in two consecutive samples of each patient, was studied by dividing the paired samples and chromosomal aberration findings into four groups: a) both the chromosomal finding and clinical disease had changed, b) both were unchanged, c) a change in clinical condition but not in chromosomal finding d) a change in chromosomal finding but not in clinical condition. The change of the chromosomal finding was defined as $|a-b|-2SDc > 0$, where a and b are the percentages of abnormal interphases (EDISH) or metaphases (G-banding, G_N, G_S, or G_{tot}) of the two patient samples, and SDc is the standard

deviation of the abnormal metaphases of the healthy controls. The agreement was tested with the Kappa-test. The statistical analyses were performed with BMDP (1992).

Table V. Grouping of patient samples for comparisons by Kruskall-Wallis tests

Gr 1	Gr2	Gr 3	Gr 4	Gr 5
LPP	LPP	All patients	Active disease	Active disease->progression
MF	CTCL			Active disease->remission
SS				Active disease->stable
			Remission	Remission->progression
				Remission->stable
PUVA-c	PUVA-c	PUVA-c	PUVA-c	PUVA-c
Healthy c	Healthy c	Healthy c	Healthy c	Healthy c

Gr: grouping, LPP: large plaque parapsoriasis, MF: mycosis fungoides,
CTCL: cutaneous T-cell lymphoma, SS: Sézary syndrome,
PUVA-c: PUVA-treated controls, Healthy c: healthy controls

7.2.7. Sequencing

All exons and one intron region (intron 20) of the NAV3 gene in two patient blood cell-derived DNA samples (cases 1 and 3) were amplified with primers specific for each exon or the intron, and subsequently sequenced with ABIPRISM 310 sequencer. The mutation and polymorphisms were subsequently sequenced in the reverse direction as well. To study the frequency of sequence variations in the normal population, all exons were amplified and sequenced from one healthy control sample and, in addition, DNA samples of 50 healthy volunteers were amplified and sequenced for exon 37 and intron 35.

7.2.8. Denaturing High-Performance Liquid Chromatography (DHPLC)

All exons of case 2 were studied with DHLPC. PCR products were denatured for 3 min at 95 C and then reannealed gradually over 30 min using a 95 C to 40 C temperature gradient. The optimal melting temperature for each PCR amplicon was obtained by analysis of the wild-type sequence, using an algorithm at the Stanford Denaturing High-Performance Liquid Chromatography (DHPLC) web site http://insertion.stanford.edu/melt.html). DHPLC heteroduplex analysis was performed using automated HPLC-instrumentation (Agilent Technologies) equipped with a Helix Analysis Column (3.0mm ID x 50mm length, Varian). The analytical gradient composed of Varian BufferPak A (100 mM TEAA and 0.1 mM EDTA, pH 7.0) and Varian Bufferpak B (100 mM TEAA, 0.10 mM EDTA, and 25% acetonitrile pH 7.0) with a flow rate of 0.450 ml/min. The injection volume of each PCR sample was 5-7 µl. The analysis time for each sample was 6 min, including an equilibration step. Exons showing abnormalities were were sequenced as above.

NAV3 expression by reverse transcriptase-PCR The expression of NAV3 mRNA was studied by reverse transcriptase polymerase chain reaction (RT-PCR) in Ficoll-purified and PHA-stimulated blood lymphocytes (Gibco Invitrogen, Rockville, MD, USA) of a healthy person on the third day of the culture, total skin lesion biopsies of case 15, and human foetal liver cDNA library (Clontech, Palo Alto, CA, USA). The human astrocyte-derived cell line, CCF-STTG1, (a generous gift from prof. Jorma Isola, University of Tampere), served as a reference. For comparison, a total skin lesion biopsy of an additional patient suffering from a CD30+ CTCL with translocation t(2;5)(p?23; q?21) (Karenko *et al.* unpublished observation), was also studied. The total RNA was purified with phenol-chloroform and precipitated in isopropanol. The cDNA was transcribed with Revert Aid TM First Strand cDNA Synthesis Kit (Fermentas, St.Leon-Rot, Germany), and PCR was then performed with Nav3A-EcoF and Nav3A-SalR primer pairs, fragment size 565bp. The amplification product was visualized in 1.5 % agarose gel.

8. RESULTS

8.1. The most common chromosome abnormalities in CTCL observable with conventional G-banding and centromere-specific *in situ* hybridizations can be used in diagnostic purposes (studies I and II)

Numerical and structural chromosome aberrations were detected by G-banding in PPS and all stages of CTCL and differences between the diagnostic groups were observed All CTCL and PPS patients in the Study I examined showed numerical and structural chromosome abnormalities so that numerical aberrations were more common in each group. In the Study I, the median of the percentage of chromosomally abnormal metaphases was highest in the MF-group (15%). As only two G-banded samples were available from the SS-group, it was thus not included in this statistical analysis. The difference of the total number of chromosomally abnormal metaphases between all the other groups was significant (p= 0.03, Kruskall-Wallis test). Significant differences between the diagnostic groups were observed for numerical abnormalities of chromosomes 6,13,15, and 17, and structural aberrations of chromosomes 3, 9, 13 and marker chromosomes, e.g. unidentifiable aberrant chromosomes (study I, **Figures 9 and 10)**. The existence of statistical differences between all diagnostic groups and healthy controls in G-banding for the chromosomes studied was confirmed by the Study II. The median percentage of aberrant metaphases for MF-patients was 17.6%, comparable to that observed in the the Study I, but higher for SS-patients (87%). Healthy controls and PUVA-treated controls had only non-clonal aberrations (medians 7.9%, and 9.8%, respectively). PUVA treatment did not significantly relate to the observed chromosomal abnormalities; In Study I the untreated CTCL or parapsoriasis patients showed a higher total percentage of chromosomally aberrant cells in G-banding than controls treated with PUVA. Contrasting with the findings in patients with PPS or CTCL, most aberrations in PUVA-treated controls were structural. Controls treated with PUVA did not differ from healthy controls in any statistical test (Study II).

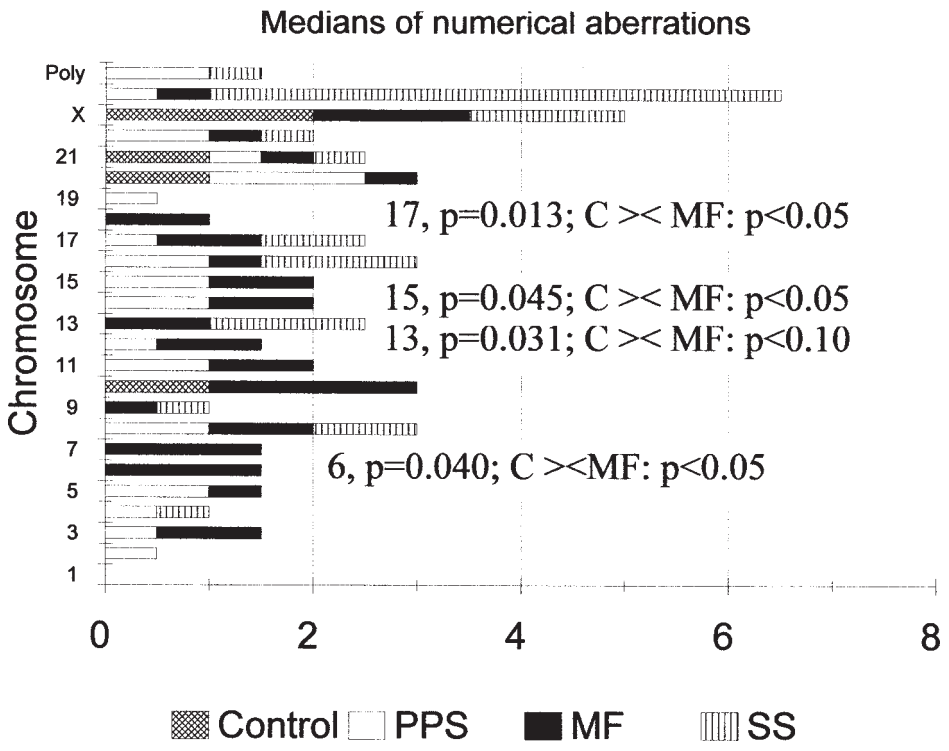

Figure 9. Parapsoriasis and CTCL patients had significantly more numerical chromosomal aberrations of individual chromosomes in G-banding than healthy controls. The total length of each column indicates the median percentage of aberrant metaphases for the specific chromosome. Significant differences between healthy controls, the parapsoriasis group and the MF-group are indicated as *p<0.05 and **p<0.02, Kruskall-Wallis. In paired comparisons there was a significant difference (p<0.05) between healthy controls and patients with MF for chromosomes 6, 13, 15, and 17.

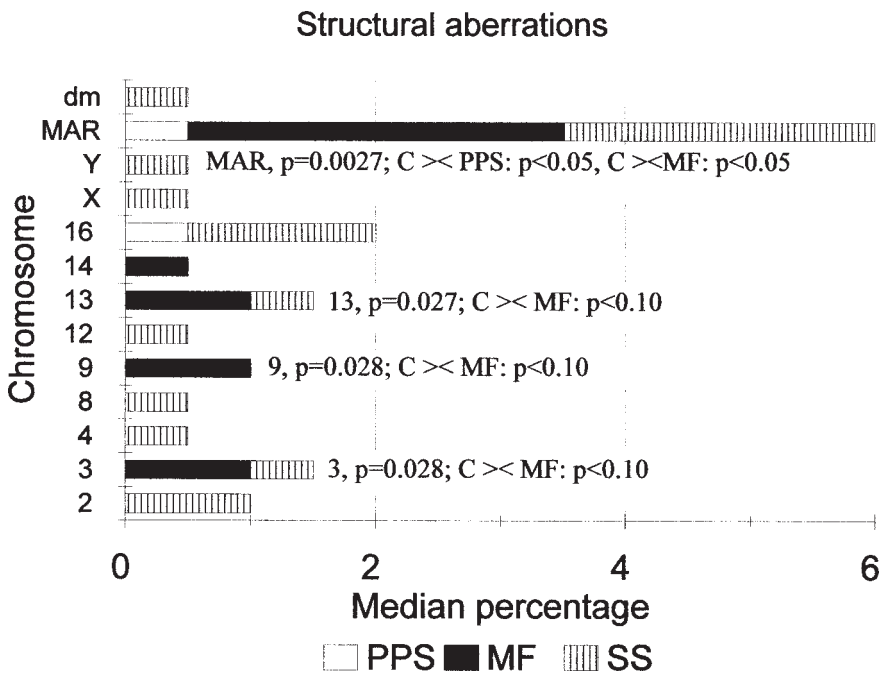

Figure 10. Parapsoriasis and CTCL patients had significantly more structural chromosomal aberrations in G-banding than healthy controls. The length of each bar indicates the median percentage of aberrant metaphases for the specific chromosome of each patient group. Median percentage of healthy controls was 0 for each chromosome. Significant differences between healthy controls, the parapsoriasis group and the MF-group are indicated as *p<0.05 and **p<0.01, Kruskall-Wallis. In paired comparisons for marker chromosomes, the difference between healthy controls and MF or PPS patients was also significant (p<0.05). The SS-group is not included in the statistics.

Also *in situ* hybridization showed significant differencies between patient groups and healthy controls In the Study I, patients with CTCL had more aberrations in FISH than healthy controls. Combining the results obtained in all chromosomes studied (1, 8, 11 and 17, biotinylated probes), the median percentage of abnormal interphase cells was 1.0% (range 0.0-8.0) in the healthy control group, 3.4% (range 0.0-8.3) in the PPS-group, 4.8% (range 0.0-11.5) in the MF-group and 7.0% (range 3.0-16.0) in the SS-group. The differences between the healthy control group and MF- or SS-group were significant (p<0.01). Also, the number of abnormal interphases in the SS- group significantly exceeded that in the PPS-group or in the MF- group (p<0.05). The significance of differences for individual chromosomes is given in **Figure 11**. For chromosome 11, the difference between healthy controls and the SS-group approached significance (p=0.06). The patient with follicular mucinosis (alopecia mucinosa) had the lowest percentage (5%) of metaphases with aberrations in G-banding in the statistical PPS-group, and not higher than the mean (7%) or median (8%) of the healthy control group. In EDISH, (Study II), healthy controls showed a slightly higher percentage of abnormal interphases than in FISH , e.g. for chromosome 1, the median percentage of aberrant interphases in healthy controls was 1.3% (range 0.3 to 2.3%) and in PUVA-treated controls 1.6% (range 0.6 to 2.0%), and for chromosome 8, 2.5% (range 1 to 6.5%) and 2.8% (range 1.8 to 4.25%), respectively. However, EDISH showed significant differencies between the patient and the control groups (groupings 1 to 3, **Table V**; p<0.01 to p<0.05) regarding aberrations of all chromosomes studied (1, 6, 8, 9, 11, 13/21, and 17). In paired comparisons, patients with CTCL, and also, all patients (CTCL and LPP together) differed significantly from healthy controls (p<0.01 to p<0.05) for all chromosomes with all methods used. Patients with LPP differed from healthy controls for chromosomes 11 and 13 and for chromosome 17 from patients with CTCL with the EDISH method. PUVA- treated controls did not differ from healthy controls by any test.

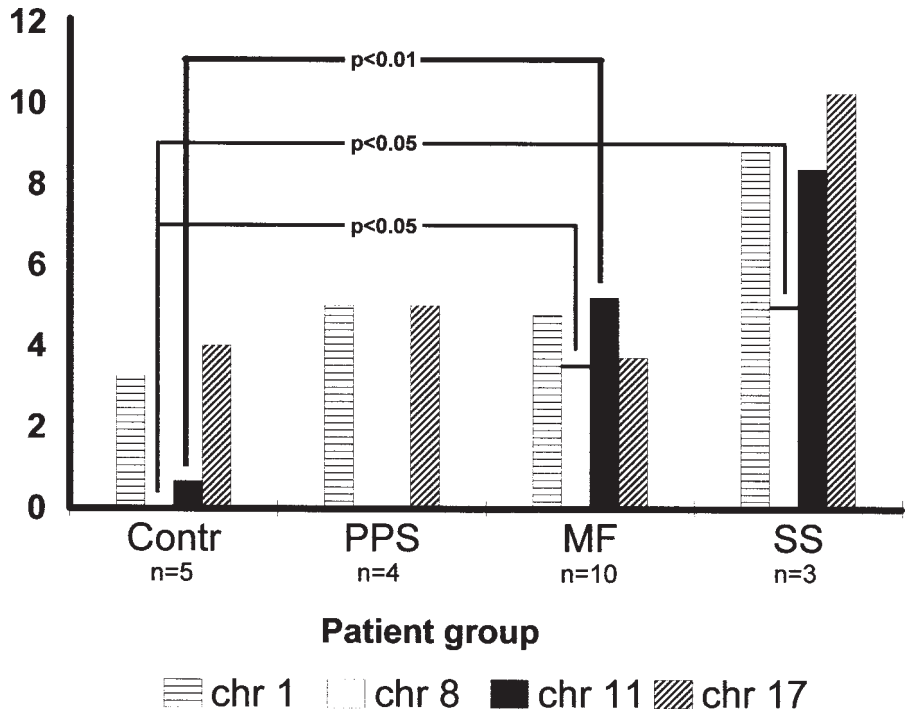

Figure 11. Chromosomally aberrant cells detected by FISH were significantly increased in CTCL patients. Significant differences between individual chromosomes are indicated in the Figure. (For chromosome 11 in PPS group n=1.)

Chromosomal aberrations and disease activity The presence of chromosomal abnormalities associated with the activity or progression of CTCL or large-plaque parapsoriasis (studies I and II). In study II, a significant difference was observed in paired comparisons between patients with active disease and healthy controls (groupings, see **Table V**) for all chromosomes studied with EDISH and the total percentage of aberrations in G-banding, except for chromosome 9 in EDISH ($p = 0.067$). In addition, statistical differencies were found between patients with active and progressing disease and patients in stable remission (chromosomes 1,6,8,11,17, G-banding or EDISH, $p<0.05$). Patients with active but stable disease or patients with active disease preceding later remission differed from healthy controls for chromosomes 1, 6, 8, and 11 or chromosomes 1 and 8, respectively (grouping 5). All patients in remission (grouping 4) differed from healthy controls for chromosomes 1, 6 and 11; and in addition to these chromosomes, patients in stable remission (grouping 5) differed for chromosome 8 from patients with active, progressive disease. Patients with LPP differed from healthy controls for chromosomes 1 and 13/21 (EDISH, $p<0.01$ and $p<0.05$, respectively).

Chromosomal clones In both studies chromosomal clones associated with active or progressing disease leading to death in 3 of 4 (Study I) or 4 of 7 cases (Study II) during 22 and 29 months of follow-up, respectively. Of the three surviving patients with a clone in Study II, one had active LPP, and other relapsing CTCL in the same restricted anatomical regions (IA) as previously. The three different clones of the third patient disappeared after treatment as remission was achieved. However, this patient later developed new chromosomal aberrations and relapsed (Muche *et al.* 2004). In both studies, no association with any treatment modalities or chromosomal clones could be observed, as clones were observed in patients with different treatment histories or without any preceding treatment.

The different cytogenetic and molecular cytogenetic methods showed agreement (Study I) The ability of FISH and G-banding to detect monosomy of chromosomes 8 and 17, and monosomy of chromosomes 1, 8, 11 and 17 studied collectively, agreed in 76-93% of tests performed, (κ=0.48-0.87) in all available samples. In other cases, the observed agreement rate was usually larger than that expected by chance, but not significant. FISH and EDISH were compared in four samples, and yielded comparable results, so that EDISH detected 2.4-4.5 % more interphase cells with one signal only than FISH, while less than 3% differencies in either direction were seen in interphase cells with 3 or more signals, or among signals in metaphases. The numerical chromosome aberrations observed with G-banding were also detected with EDISH.

8.2. Chromosomally clonal cells coexpressed CD45RA and CD45RO in the Sézary syndrome and were detected in lymph nodes histologically characterized as non-malignant

The immunophenotype of the chromosomally clonal cells revealed a coexpression of CD45RA and CD45RO in the Sézary syndrome and malignant cells in lymph nodes histologically characterized as non-malignant A similar and constant phenotype over more than 2 years of time was found in malignant cells with clonal chromosomal aberrations (i.e. supernumerary copies of chromosome 8 in near-diploid or near-tetraploid cells) in the blood, skin and lymph nodes of the two Sézary syndrome patients. The malignant cells were CD3- positive and CD4-positive but CD8-negative. Both CD45RO and CD45RA positivity was observed in the majority of clonally malignant cells in both patients, although in the blood of both patients and in the skin of case 2, the staining with CD45RA was of weaker intensity[1]. In the post-mortem lymph node of case 1, both antigens showed intense staining in the clonal cells. The lymph node biopsies of both patients obtained before histologically verified lymphoma involvement, showed chromosomally clonal cells in hybridisation (25 to 35% of the cells in the touch preparate in case 1 and 24 to 42%, respectively, in case 2. In both cases, these clonal cells expressed both CD45RO and CD45RA markers. The CDw150 (SLAM) antigen, characterising activated memory T cells (CD45RO high); was detected in all tissues of case 1. In the skin of both patients, the expression was observed in about half of the clonal cells. In contrast to positive control samples (blood lymphocytes of a healthy person, and the patient with Lymphomatoid papulosis), granzyme B-positive cells were observed only occasionally in the skin or lymph node samples of case 1, and none of these cells represented the malignant clone. A few (less than 10%) clonal skin cells of case 2 expressed granzyme B very weakly.

The majority of clonal T-cells expressed IL-4 typical to Th2 cells To assess the Th polarization, the cytokine expression in the skin lesion of both patients and a post-mortem lymph node of one of the patients was studied. The majority of clonal cells expressed IL-4. The staining intensity was variable with all colours used (Rhodamine Red X, Alexa 594®) showing very bright colour intensities in up to 50% of the clonal cells. At most 20% of the clonal cells were IL-4 negative. The majority of clonal cells of case 1 were IL-10 negative (95 to 98%), but the majority of the cells of case 2 were IL-10 positive (80%). The majority of the clonal cells of both patients were IL-2 negative (range 97% to 98%), and none expressed IFN-γ. Thus, the cytokine expression pattern of the clonal cells in skin and lymph node was IL-2 negative, IL-4 positive, varaible for IL-10, and IFN-γ negative.

8.3. Comparative genomic hybridization (CGH) revealed copy number changes and potential tumour suppressor or oncogene locations in chromosomes 10q and 13q

Copy number changes were observed in the blood of SS patients but not in the blood of MF patients Six of seven SS patients, but none of four MF cases, showed DNA copy number changes (see Table I in Study IV). Losses were more common than gains. In SS-patients 6, 7 and 11, G-banding confirmed complex chromosomal aberrations (data not shown). Two patients had near-tetraploid cells with the same rearrangements as their near-diploid clonal cells (case 6 and the follow-up sample of case 11).

The most common aberration was loss in 10q Losses of DNA copy number were most frequent at 10q. The losses were found in four of the seven SS-patients, with a minimal overlapping region at 10q25-q26. The disease of three of these patients (cases 6, 10 and 11, Table I in Study IV) clinically and histopathologically evolved from MF. Three patients also had losses of chromosome 13, with minimal overlapping region at 13q21-q22. These are potential oncogene or tumour suppressor gene locations. For other copy number changes, see the Table I in the original publication (Karenko *et al.* 1999).

Follow-up samples showed partly common changes with the first sample CGH changes, partly common with the first sample (Table I in Study IV), persisted in the two follow-up samples of cases 6 and 11 obtained during active disease, and

[1]There is a printing error in Table I in the original aricle denoting the skin of case 1 with dim, whereas the Results text is correct.

the tumour sample of case 11. Loss of chromosome 10 remained in all follow-up samples of the respective patients, despite of therapy. In the above two cases with progressive disease, the loss of chromosome 10 was associated with a gain of 8 or 8q.

The previous treatments did not explain the copy number changes observed Previous treatment did not explain the genetic changes, since untreated patients or patients with different treatments (cases 5, 6, 9, and 10) showed changes in CGH similar to those of electron beam-treated patients (cases 7 and 11). Also, two electron beam treated patients (cases 1 and 4) showed no changes in CGH.

The CGH changes associated with disease progression Most patients with changes in CGH had rapid disease progression, since five of six patients died within one year (including patient 6, who died after the writing of the Study IV, 11 months after the first sampling).

8.4. The first CTCL-specific chromosome aberration found and the gene affected identified

Aberrations of chromosome 12 are frequently found in CTCL patients The most often affected chromosome in the peripheral blood clones observed by MFISH or SKY was chromosome 12. Five of seven consecutive patients with Sézary syndrome, showed a clonal structural aberration of chromosome 12 and one (case 7) showed a non-clonal deletion of 12q with a clonal monosomy of chromosome 12 (**Table VI**). Five of the 6 MF patients studied with these methods showed non-clonal deletions of chromosome 12. All structural clonal aberrations of chromosome 12 involved bands q21 or 22, although in case 4, owing to the small size of the fragment of 12 translocated, the breakpoint was defined by CGH only. Additionally, case 13 had enh(12)(q15)(q21) in CGH of blood lymphocytes. Case 15 showed del(12)(q21q?23) in 4/100 metaphases in G-banding of blood lymphocytes. Structural aberrations of chromosome 17 were also detected in 5 SS-patients, but these aberrations could involve either p or q. Chromosome 12 aberrations were also detected in the skin lesions of cases 24 and 25, which showed a suspicion[2] of dim(12)(q15q21) and dim(12)(q15q21) by CGH, respectively.

Three cases (cases 1,3 and 4) showed a translocation with chromosome 18 in multi-colour FISH. One (case 3) had a balanced translocation with 18q , another showed a translocation with 18 p with loss of much of the 12q-arm (case 1) **(Table VI)**, and in the third one (case 4), the aberration involved also chromosome 22. In case 5, a translocation between chromosomes 4q, 10 and 12q was found.

Specification of the break point in chromosome 12 The aberrations of cases 1, 2 and 3, in which enough cell material was available, were studied with locus-specific FISH. Cases 1 and 2 showed large deletions of chromosome 12, del(12)(q15q15)(q21.1 q24) and del(12)(q12q21), respectively (**Figure 12).** The balanced translocation of case 3 was within the minimal common region of deletions in cases 1 and 2, and divided the signal of YAC 855F7 between chromosomes 12 and 18 **(Figure 12)**, enabling us to fine map the gene affected.

The YAC 855F7, is part of the YAC-contig WC12.4 (NCBI: www.ncbi.nlm.nih.gov) and spans the region between markers CHLC.GATA65A12 and WI-6487. Four overlapping BAC-probes, RP11-781A6, RP11-494K17, RP11-136F16, RP11-36P3, each with a marker represented in the YAC 855F7 by PCR-analysis (SHGC-155034, G62498, SHGC-79622, D12S2006, respectively) were further used. Signal division in FISH analyses indicated that the translocation breakpoint lies within BAC-probes RP11- 494K17 and 136F16 (**Figure 13),** which both contain parts of NAV3 gene (genomic contig NT_019546) disrupted by the translocation. No other mapped genes or ESTs were located in the translocation breakpoint. The breakpoint of 18q involved in the balanced translocation of case 3, splits YAC 852H2 (located between markers AFM357TD5 and AFM191XC9P) and BAC 450M22 (AC016165, included within YAC 852H2) into two parts, one

[2]A copy number below normal but not attaining the significance level

Table VI. Proportion of clonal cells and composite karyotypes of the clones observed in multicolour FISH of blood samples and specified with G- banding

Patient	Diagnosis	Proportion of clonal cells	Composite karyotype[a]	Special remarks
1	SS	10/10 (100%)	69-73,XY,-X[10],der(Y)t(Y;8)(?;?)[10], der(1)t(1;16)(q32;?)[10], +2[4],der(2)t(2;17)(p?21;q?21)[9], +3[7],der(4)t(4;1)(?;?)[10], der(5)t(4;5)(q26 or 28;?q21)x1-2[10],-7[10],+8[2],-10[9], **der(12)t(12;18)(q?22;p11)x2**[10], der(14)t(5;7;14)(q?13;q?11q36;p11)x2[10], der(16)t(1;16)(q32;p13),der(16)t(1;16)(?;?p)[10],-17[7], **der(18)t(12;18)(?;p?)x1-2**[10],+19[10],+22[9][cp10]	der(12) specified with locus specific probes as der(12)del(12)(q?21q24)t(12;18)(q24;p11.3). Other breakpoints specified with G-banding.
2	SS	20/22 (91%)	36-98,X,-Y[17],der(1)t(1;9)(1pter->?1q11.1::9?->9?)x1-2[20],+5[8], del(5)(q21)x2-4[20],+7[9],+7,+7[3], del(7)(q?32)[8],ins(7;17)(p14;q25q12)[2],+8[18], del(8)(q?)[2],+9[16],der(9)t(1;9)(q10;?q10)del(1)(q?)x1-4[20], der(9)t(1;9)(q?->?::q11.1->pter)x1-2[7],+10[7], del(10)(p11 or 12)x1-2[18],**del(12)(q?2)**[13],-14[3],-17[3],i(17)(q10)[7], ider(17)t(7;17)(?;q?25)[5], der(17)t(10;17)(?;p?11.2)[2],+21[6], -22[3][cp19]	del(12) specified with locus specific probes as del(12q11q21)
3	SS	11/11 (100%)	44-91,XY,der(1)t(1;22)(q?42;?)[2], der(1)t(1;10)(p?34;?)t(1;22)(q?42;?)[9], der(1)t(1;11)(q21;p?14)[3], der(1)t(1;11)(q21;p?14)t(10;11)(?;p15)[9], der(3)t(3;5)(q25;q?15)[10],der(3)t(3;6)(q25;q22)[11], der(5)t(5;7)(q?15;q?)[11],del(6)(q2)[3], der(6)t(1;6)(6pter->6q22::1q21->1q23)[6], del(7)(q31)[10],der(7)t(7;10)(?q31;?)[10],+8[11], der(8)t(8;17)(?p1;?q1)[11],del(9)(q13)[11],-10[8], der(10)t(10;11)(?;?)t(10;17)(?;?)x1-2[10], der(11)t(1;11)(q24;p14)[11],**t(12;18)(q15or21;q21)**[11], del(15)(q1~21)[11],der(16)t(16;20)(q?;?)[11],],-17[11],der(17)t(7;17)(q?;q?),der(20)t(X;20)(?;?)[11], der(20)t(16;20)(?;?)[11][cp11]	t(12;18) specified with locus specific probes as t(12;18)(q21;q21). Other breakpoints specified with G-banding. Additionally, one cell with der(10)t(11;10)(?;?)
4	SS	30/39 (77%)	44-46,XX[4]X[7],t(X;3)(q12;p11)[6], der(2)t(10;2;10;2;10;13;14;13;14)[30],t(2;11)(q21;q11)[8], der(4)t(4;13)(q22;?)t(4;13)(q31;?)[30],+7[11], +del(8)(q22)[2],del(9)(q11)[3],der(10)t(8;10)[5],-13[7], der(13)t(2;13)[10],i(17)(q10)[12],der(17)t(X;17)(?p;p?11.2)[7], **der(18)t(12;18)(?;?p)t(12;22)(?;?)**[30], der(19)t(19;21)(p13;q11)[30],-21[30][cp30]	Breakpoints specified partly with G-banding
5	SS	16/24 (67%)	39-48, XY[13], der(2)t(2;13)(p?21;?)[10], der(2)t(1;2)(?;q?35)t(2;13)(p16or21;q?)[3], der(3)t(3;9)(p21;p13)x1-2[14], der(4)t(4;12)(q31;?)[14],-5[3],del(8)(q?)[2],-9[12],-10[10], **der(12)t(10;12)(?;q21.3 or q22)**[13],-13[15], der(15)t(1;15)(?;p11.1)[9],der(16)t(10;16)(?;q24)[2], del(17)(p?)[9],-18[5],del(18)(p?)[3], der(19)t(15;19)(?;p13.3)[3],-20[3],-22[2][cp16]	Additionally, one cell with der(12)t(10;12)(?;q21.3 or22) t(10;12)(?;?)t(10;12)(?;?) and one other cell with del(12)(q12q21) One cell with sex chromosomes XXXY, two cells with -Y
6	SS	3/45 (6.7%)	86-92,XXYY,t(2;6)(q33;q24 or 25)[3], der(2)t(2;6)(q33;q24 or 25)[3],-11[3]	Two of the clonal cells showed -12
7	SS	5/24 (20.8%)	30-45,XY,**-12**[5]	
8	MF	2/70 (2.9%)	46,XY,dup(18)(q?;q?)[2]	Additionally 1 cell with karyotype 51X,i(Y)(q), +der(3),+del(5)(p11.1),+?del(12)(q21q24.1). iYq observed previously in G-banding as a clonal abnormality.
9	MF	2/25 (8.0%)	46,XY,del(9)(q11.1)[2]	Nonclonal aberrations of chromosome 12 In previous G-banded blood samples three different and one sideline clone, which diasappeared before the present sample (Study II)
10	MF	0/54 (0%)		Nonclonal aberrations of chromosome 12
11	MF	0/43 (0.0 %)		
12	MF	7/50 (14.0%)	46-49,XX,+10[7],del(10)(p?11.2)(q?11.2)[7]	Nonclonal aberrations of chromosome 12 TCR beta analysis showed different TCR clones In skin and blood (Muche *et al.* 2004)

[a]ISCN1995. For clonality of whole extra chromosomes, 3 cells required (Karenko *et al.* 1997, ISCN 1995, Muche *et al.* 2004). Derivative chromosomes originating from more than four chromosomes have been presented as a chromosome list.

giving a signal in 18q and the other in 12q. All BACs located in 18q proximal to 450M22 remain in 18q, whereas BACs and YACs below the break point distally move to chromosome 12q in the translocation **(Table IV)**. Although most of the material lost from the aberrant 12q in case 2, was totally deleted (for CGH see Study IV)**,** a small part of 12q24 was translocated to 18p (PAC 144J4**; Figure 12),** to the region of BAC 683L23, partly translocated to 12q24. BAC683L23 contains Hs18_11016_33_1_1, Hs18_11016_33_2_1, LOC284210, Hs_18_33_3_1, IL9RP4, ROCK1, and a small part of distal USP14. Other, more proximal BACs in 18p studied, remained in their respective locations in 18p.

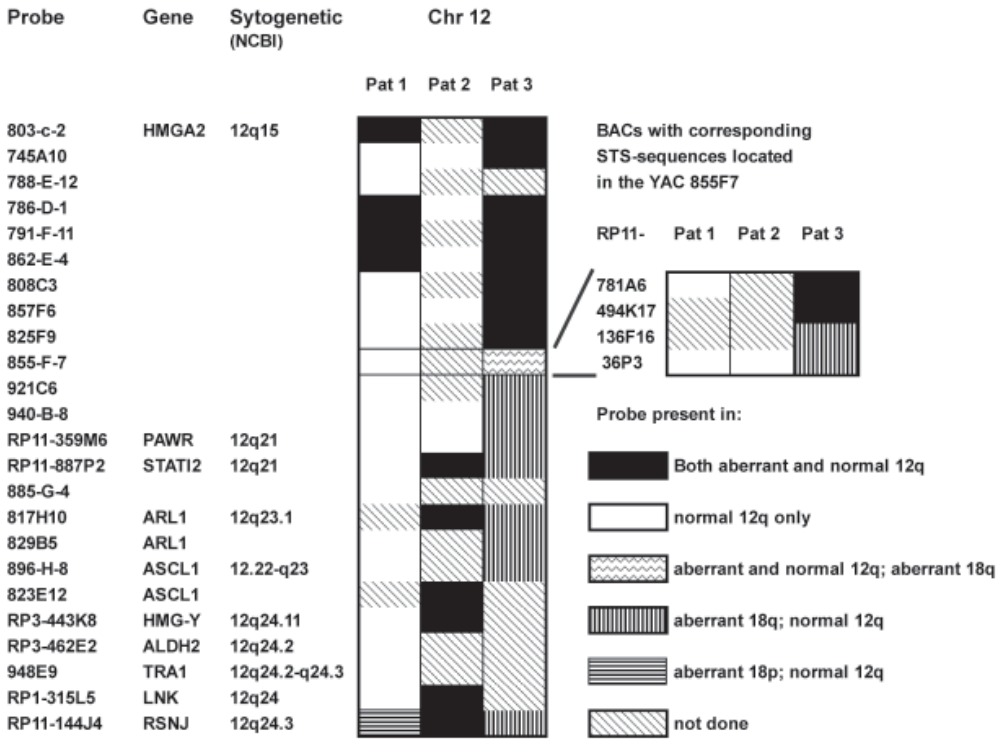

Figure 12. Two SS-patients had a deletion and one had a translocation in 12q21.1 in their blood lymphocyte as shown with YACs and BACs. Part of the chromosomes studied are shown as vertical columns. Fill-in symbols representing the hybridization results are explained in lower right.

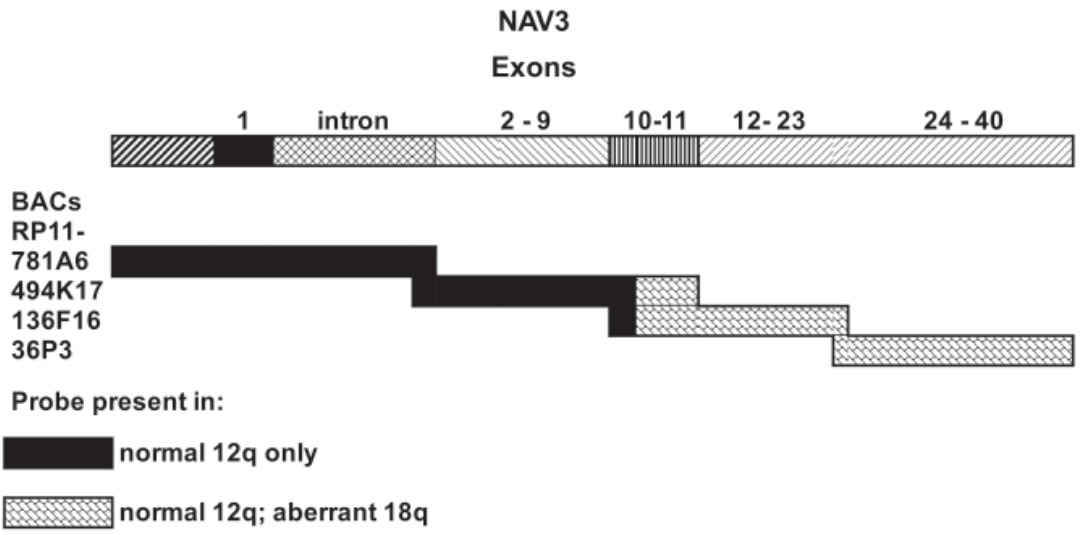

Figure 13. DNA represented in BACs 786A1, 494K17, 136F16 and 36P3 together comprise the NAV3 gene. Hybridization of BACs RP11-781A6, RP11-494K17, RP11-136F16, RP11-36P3 (AC073552.1, AC022268.5, AC073571.14, and AC073608.19, respectively) together spanning the whole NAV3 gene, indicated the translocation breakpoint as division of BAC-probes RP11- 494K17 and 136F16 between chromosomes 12q and 18q. The whole BAC 781A6 remained in chromosome 12 and the whole BAC 36P3 was translocated to chromosome 18q. Fill-in symbols of bars indicating BACs and their parts remaining in chromosome 12 or translocated to chromosome 18q are explained in lower left.

NAV3 deletion/translocation is found in interphase cells of skin lesions of CTCL patients The translocation observed in lymphocyte metaphases of one SS patient (case 3) was also observed in the locus-specific FISH to lesional skin touch preparation **(Table VII).** Deletions of the NAV3-gene were observed in solid tissue samples of the three other SS patients studied (case 1, lymph node and cases 2 and 13, skin), and in the lesional skin of 11 of 17 (65%) patients with various stages of MF (**Table VII**). The NAV3 deletions were found in the skin lesions of 4 of 8 (50%) patients with early MF (stages IA-IIA) and a deletion or a translocation was observed in 11 of 13 (85%) patients with advanced MF or SS with locus-specific FISH (**Table VII**). The deletion was equally well found in touch preparations from archival skin samples as in more recent samples. There was no consistent association between the NAV3 deletion or the type of previous therapy. All patients with NAV3 deletion or translocation had a frequently relapsing disease despite therapy or had died of CTCL. Of the 5 patients not showing NAV3 deletion in their skin lesions, 3 had an early stage disease. Two of them had received PUVA or electron beam therapy and one was untreated (case 17).

Demonstration of NAV3 mutation in the microscopically intact chromosome arm To detect mutations in the NAV3 gene, blood lymphocyte-derived DNA of cases 1 and 3 was sequenced (enough material available) and that of case 2 was analysed with DHPLC. In case 1, a point missense mutation G->A in exon 37 (cDNA nucleotide 1010 6643; NM_019403), resulting in amino acid change E2200K was found. No mutation changing amino acid was found in the NAV3 gene from cases 2 or 3. Seven polymorphic variations have been recorded in NAV3 coding region (NT_019546) and two of these changes (4509G->A and 4830C->T, NM_019403) were also observed in cases 1 and 3. Altogether, the NAV3 gene region, spanning approximately 381Kb of chromosomal sequence, contains 849 polymorphic sites.

NAV3 is expressed in normal human T lymphocytes With RT-PCR, NAV3 mRNA could be detected in polyclonally activated T-lymphocytes, as well as in human foetal liver cells and astrocytes**.**

Table VII. Result of FISH with NAV3 specific bacs 136F16 and 36P3 to skin lesion or lymph node and disease characteristics

Patient nr.1[a]	Diagnosis	Stage	Treatment[b]				Disease outcome[c]	Aberrant cells in FISH, % Deletion	Translocation
1	SS		PUVA	EB		Ch	DOD	68	
2	SS			EB		Ch	DOD	44	
3	SS[d]		PUVA				DOD		48
13	SS		UVA				DOD	50	
8	MF	IA	PUVA	EB			AR	32	
14	MF	IB	PUVA				AR	50	
15	MF	IB	PUVA				AR	55	
16	MF	IB[e]		EB			REM	8	
17	MF	IB					REM	3	
18	MF	IB[e]	PUVA				† Other	5	
19	MF	IB[f]	PUVA	EB		Ch	AR	32	
9	MF	IIA		EB			AR	10	
10	MF	IIB	PUVA	EB	I	Ch	AR	44	
11	MF	IIB	PUVA	EB	I	Ch	AR	8	
12	MF	IIB	PUVA	EB		Ch	DOD	44	
20	MF	IIB[e]	PUVA	EB			DOD	22	
21	MF	IIB[e]		EB			DOD	58	
22	MF	IIB[e]			I	R	DOD	4	
23	MF	III[e]				Ch	DOD	38	
24	MF	IVA	PUVA	EB		Ch	DOD	44	
25	MF	IVA	PUVA				DOD	28	
Control 1	DLE	DLE						10	
Control 2	ECZ	ECZ						6	
Control 3	DLE	DLE						8	
Control 4	ECZ	ECZ						6	
Control 5	ECZ	ECZ						8	
Control 6	ECZ	ECZ						7	
Control 7	ECZ	ECZ						4	
Control 8	ECZ	ECZ						0	
Control 9	ECZ	ECZ						10	

[a]Arranged according to the diagnosis. Cases 4 to 7 were not studied due to lack of frozen material.
[b]PUVA: Psoralen + ultraviolet A, EB: electron beam, I: Interferon-α, Ch: Chemotherapy, R: Retinoids
[c]DOD: Died of disease,†Other: Died of another cause than CTCL, REM: Clinical remission, AR: alive, relapsing disease
[d]Preceded by MF. [e]Biopsies of skin lesion obtained 5 ot 15 years earlier and stored in liquid nitrogen. [f]CD30-positive

9. DISCUSSION

Chromosomal aberrations in peripheral blood associate with the activity or progression of CTCL or large plaque parapsoriasis. G-banding, FISH or EDISH showed statistically increased aberration frequencies for chromosomes 1,3, 6, 8, 9, 11, 13/21, 15 and 17 between the diagnostic groups (Studies I and II). The aberration level of chromosomes 6, 8, 9, 11, 13/21, 15 and 17 (Study II) was elevated in active disease compared with healthy controls. Patients in complete clinical remission differed from healthy controls for aberrations in three different chromosomes (chromosomes 1, 6, 11) studied, and for the total percentage of aberrant metaphases in G-banding. On the other hand, as the aberration level of chromosomes 9, 8, 13/21, 15 and 17 was elevated in active disease but not in remission, the difference in chromosomal variety could reflect either increased chromosomal instability or increased number of malignant cells, both involving especially chromosomes 9, 8, 13/21, 15 and 17. The lack of statistically significant difference in paired comparisons between patients with active disease and in remission, could be due to the smaller number of patients in remission (8 versus 32). On the other hand, despite the small number non-CTCL PUVA-patients (4 samples), significant differences between them and the group of CTCL patients with active disease were observed.

It is noteworthy that patients with active but stable disease (neither complete regression of previous skin lesions nor appearance of new ones), as a group, show significant differences from healthy controls for the same chromosomes (1, 6, 11 and G_{tot}, see Table 3 in Study II, Karenko *et al.* 2003) as patients in remission. Thus, aberrations of those chromosomes might be a hallmark of existing disease, even sub-clinical, but aberrations of the other chromosomes could be more important in the progression of the disease. Patients with active, progressing disease showed significant differences from patients in stable remission, for chromosomes 1, 6, 8, 11 and 17 in EDISH or G-banding. Thus, the frequency of aberrations of chromosomes 1, 6 and 11, also aberrant in remission, seems to increase further with progression of the disease.

The increasing aberration level of chromosomes 8 and 17 along with increasing clinical activity of the disease, might reflect mutations of tumour suppressor gene p53, (17p13), or c-myc oncogene (8q24.12 –q24.13), not analysable with the methods used in this study. Mutations of p53 gene have been observed in advanced CTCL (tumour-stage or transformed; Garatti *et al.* 1995, McGregor *et al.* 1999, Marrogi *et al.* 1999), and the expression of both p53 (wild-type or mutated) and C-myc increases with advancing disease (Kanavaros *et al.* 1994, Li *et al.* 1998). On the other hand, in primary cutaneous T-cell lymphoma, no correlation between p53 expression and prognosis has been found (van Haselen *et al.* 1997). In this study, chromosome 8 aberrations were elevated in all samples representing a progressive disease. However, as the material is small, it does not exclude the existence of progressive cases with no chromosome 8 abnormalities.

Clonal versus non-clonal abnormalities In both studies chromosomal clones observed in G-banding associated with disease activity, and often death ensued in 3 of 4 (Study I) or 4 of 7 cases (Study II) with a clone during 22 and 29 months of follow-up, respectively, which confirms previous reports (Whang-Peng *et al.* 1982, Shapiro *et al.* 1987). The methods used in Studies I and II also revealed large karyotype variation typical to CTCL (e.g. Whang-Peng *et al.* 1982, Berger and Bernheim 1987) and the extent of non-clonal abnormalities, which may be a result of chromosomal instability. Based on studies of CTCL-derived cell lines, a hypothesis of polyclonal, "genotraumatic", genetically unstable cells has been presented (Kaltoft *et al.* 1992, 1994; Thestrup-Pedersen *et al.* 1994) stating that non-malignant, genetically unstable cells develop into tumour cells with chromosomal aberrations, and that a single patient may have (*in vitro*) several chromosomal clones grown out of the “genotraumatic”, chromosomally normal but unstable strain (Kaltoft *et al.* 1994). The studies concerned have the pitfalls of *in vitro* studies, where long-term cell cultivation *per se* can cause chromosomal abnormalities, but the theory would nicely fit the gradual development of CTCL from precursor lesions. The present study (I and II) showed for the first time elevated frequency of non-clonal chromosomal aberrations in LPP, and in the Study II, a chromosomal clone in a patient whose clinical and histopathological diagnosis was LPP. Thus, whether cells with non-clonal chromosomal aberrations really are malignant or represent a premalignant form, can not be decided by the Studies I or II, but certainly the aberrations associate with diagnosis and disease activity.

Thus, Studies I and II indicate, that chromosomal studies with G-banding and *in situ* hybridizations with centromere-specific probes may be used as an aid for diagnostic or prognostic purposes also in the difficult diagnostic procedure

(Willemze 1987, Payne *et al.* 1992, Shapiro and Pinto 1994) of the early stages of MF. As these studies confirmed the large variation of chromosomal abnormalities observed previously in CTCL (e.g. Whang-Peng *et al.* 1982), a combination of several different probes and G-banding should be applied.

Methodological aspects. Abnormal cells observed with G-banding were more common in MF-patients than in SS-patients (in only Study I), whereas *in situ* hybridization showed more abnormal cells in SS-patients than in MF-patients. This difference may reflect the well-known difficulties in propagating CTCL-cells in vitro (Burg *et al.* 1978 with references to earlier studies, Bunn *et al.* 1980a, Dalloul *et al.* 1992, Abrams *et al.* 1993, Hindkjær *et al.* 1993, Berger *et al.* 2002). Despite the small sizes of the groups tested, statistical concordances for both methods were achieved for several numerical aberrations and *in situ* hybridizations (Study I). EDISH and FISH showed comparable results (Study I). Thus, the application of EDISH developed (Study II) can be used without an expensive fluorescence microscope, and gives permanent, archival preparations. The concordant change in both the chromosomal and clinical status showed statistically significant agreement in EDISH only for chromosome 17, whereas in G-banding significant agreement for chromosomes 1, 8, 9, 15 and 17 were found. The difference in sensitivity between G-banging and EDISH might be caused by the higher aberration rate in healthy controls in EDISH versus healthy controls in G-banding, which lowers sensitivity of the EDISH for small differences in aberration levels. G-banding also shows structural abnormalities not detectable with with centromere-specific *in situ* hybridization. However, a rather high aberration rate in G-banding of some of the healthy laboratory workers used as controls (in Study II) was observed. It might reflect their past exposure to clastogenic agents, which cannot be totally excluded, and may have slightly reduced the statistical sensitivity of G-banding to only slightly elevated aberration rates in the patients. On the other hand, neither were the patients selected according to their past occupational exposures. Also for laboratory-methodological reasons, the patient data obtained by *in situ* hybridizations of centromere-specific probes should always be compared to controls.

The finding of abundant cells with chromosomally aberrant clones, stable over time, and well studied with other methods (Study II and IV), was the prerequisite for using FICTION, that enabled the study of the phenotype of the individual malignant cells in two SS-patients.

Chromosomal aberrations and previous treatments Since our patients were mostly not treatment-naive (for ethical reasons) when the sampling was performed during the follow-up it may be speculated that some treatment might have induced chromosomal aberrations. However, in any study, no association with any treatment modalities or chromosomal clones could be observed, as clones were observed in patients with different treatment histories or without any preceding treatment. The elevated frequencies of non-clonal aberrations in patients could neither be explained statistically (study II) by treatment with PUVA, the most common preceding therapy.

Cell maturity in the light of the phenotype expressed Phenotypically, Sezary cells have been considered to be CD4+,CD45RO+,CD45RA-, and functionally of Th2-type (Vowels *et al.* 1992, Saed *et al.* 1994, Dummer *et al.* 1996). However, the chromosomally clonal cells of our patients commonly expressed also CD45RA, normally observed in naive T-cells (Clement *et al.* 1988). They also expressed CDw150 (SLAM), which is normally expressed on CD45RO+ peripheral blood memory cells (Th0/Th1) and rapidly up-regulated on activated T cells (Cocks *et al.* 1995). CDw150 directs the immune response towards Th0-Th1 pathway (Aversa *et al.* 1997).

Normally, naive CD45RA+ cells express IL-2, and mature CD45RO+ cells express IL-4, IL-5, IL-10 (Th2), or IFN-γ (Th1). A strong coexpression of both isoforms occurs during transition from CD45RA+ to CD45RO+ (Dbright in flow cytometry), with expression of IL-2 and IFN-γ (LaSalle and Hafler 1991, Picker *et al.* 1993a, Hamann *et al.* 1996). In healthy persons, such cells are found in peripheral blood and in secondary lymphoid organs (Picker *et al.* 1993a, Hamann *et al.* 1996). Cells with weaker coexpression of CD45RA/RO (Ddull) express mainly RO+ type cytokines, IL-4, IL-5, IL-10 or IFN-γ, and have been suggested to represent some stage in T cell differentiation or resting primed T cells (Hamann *et al.* 1996). The differentiation of naive to Th1 or Th2 cells seems to go through a phase where naive cells express small amounts of IL-4 with IL-2 (Kamogawa *et al.* 1993, Bullens *et al.* 1999). The chromosomally clonal cells of our patients characteristically expressed IL-4, typical of RO+ type Th2 cells, but only in one case was IL-10 expression observed.

Despite the variable expression of CDw150, the clonal cells of our patients did not express IFN-γ, usually upregulated by CDw150 (Cocks *et al.* 1995). Taken together, the phenotype CD45RA+, CD45RO+, CDw150±, IL-4+, IL-2-, IFN-γ-, and with the variation of IL-10 expression between the patients, the clonal cells seem to be intermediate forms between naive CD45RA+ and CD45RO+ Th2 cells. Possibly, they might represent cells that have not attained complete maturity, or they could be mature Th2 cells partly reverted towards a more naive or resting T cell type (Hamann *et al.* 1996). The differences in the immunohistologic staining intensities in immunohistology of IL-4-positive cells must be interpreted with caution, and the interactions with other cells in the microenvironment were not studied. Our observation of malignant cells with the phenotype CD45RA+, CD45RO+ is in concordance with the heterogeneity of RA+, RA+/RO+, RO+ expression between four SS patients studied with flow cytometry by Urban and coworkers (1999, abstract) in cells rearranged for the TCR Vβ gene. The chromosomal clones with supernumerary copies of chromosome 8 in our patients represented the chromosomal clones and were stable over time.

Interestingly, the IL-4 positivity may contribute to the low frequency of apoptotic cells found in skin lesions of CTCL patients, as IL-4 blocks the action of caspase 3 (Manna and Aggarwal 1998), and IL-4 producing T cells have been shown to be resistant to activation-induced apoptosis (Carbonari *et al.* 2000). In contrast to Vermeer *et al.* (1999), who showed cytotoxic T-lymphocyte-associated granzyme B and cytotoxic-granule-associated TIA protein expression in morphologically identified malignant cells in Mycosis fungoides patients, we did not find notable granzyme B expression in the chromosomally clonal cells of our Sézary syndrome patients, although the reference sample of lymphomatoid papulosis showed clear positive staining.

Interpretations concerning timing and compartment of malignization Our finding of cells representing a malignant chromosomal clone in lymph nodes of both patients in Study III with dermatopathic lymphonoditis histology and obtained even several months prior to malignant infiltrate in the skin (case 1), can be interpreted in two ways. First, malignant clones may rise extracutaneously and they may invade lymph nodes early in the course of the disease, or they may arise in lymph nodes. In study V, one patient had different chromosomal clones in the skin and the blood, and the TCR clones in those tissues were equally different from each other (Study V; Muche *et al.* 2004). It is also noteworthy that even if the SS of case 2 (Study III) evolved from MF by clinical and histopathological criteria, the high percentage (30%) of Sézary cells in the blood during the patch stage skin lesion might be interpreted as systemic involvement. Secondly, malignant cells may arise in the skin, which is still histopathologically undiagnostic of CTCL. Heald *et al.* (1993), on the basis of the CD45RO and CLA positivity of blood lymphocytes defined malignant with TCR-family immunohistology, suggested that CTCL cells could rise from T-cells undergoing virgin-to memory transition in lymph nodes in a microenvironment upregulating CLA epitope (Picker *et al.* 1993b), or memory cells activated in the skin. The present study (III) now shows the partly virgin properties of the cells. As they may also result from partial reversion toward a more naive cell type, further studies are needed to firmly establish the initial compartment of the malignant transformation. The concept of early systemic nature of CTCL has gained evidence also from some other studies (Whang-Peng *et al.* 1982, Veelken *et al.* 1995, Dommann *et al.* 1996, Trotter *et al.* 1997, Tok *et al.* 1998, Muche *et al.* 2004) and early extracutaneous T-cell chromosomal aberrations or T-cell clonality has been verified even in large or small plaque parapsoriasis recently (Study I, Muche *et al.* 1999). Thus, lymph node biopsies should be obtained during the early phases of CTCL, as early as possible, to demonstrate the presence of malignant cells and to study their relationship to other cell types presenting or secreting cytokines.

Amplified or deleted chromosomal regions were found by CGH (Study IV, which was the first CGH study of CTCL published). Later studies (Fischer *et al.* 2001, 2004, Mao *et al.* 2002) have confirmed our findings concerning chromosome 10q loss and aberrations in chromosome 17 in German and British patients. Microsatellite instability in the tumour suppressor gene PTEN in 10q23 in CTCL was shown in 10/44 CTCL patients (23%), most with advanced disease (Scarisbrick *et al.* 2000). As the minimal common region in of the loss in the CGH studies both in the Study I and the study of Mao *et al.* (2002) was more distal, 10q25-q26 and 10q26, respectively, the finding of another important tumour suppressor gene in chromosome 10 more distally than PTEN is expectable.

The most common abnormality in the study of Mao *et al.* (2002) was gain in 1p36, which we have observed later and only in a few cases (Muche *et al.* 2004 and unpublished observations). In addition, abnormalities of chromosome 13 were more rare

in their study than in ours. As the observations of Fischer *et al.* (2001, 2004) in a German patient material show more resemblance with our results, there may be some underlying factor either in the causation of the disease or in the genetic background. In our study (Study IV), the DNA copy number changes did not differentiate between SS, whether the disease had evolved through MF or begun directly as SS. The CGH-findings were reproducible with only little variation between follow-up samples or samples taken from different tissues.

Multicolour FISH identified the most common structural chromosome aberration in CTCL Because previous studies (I and II) had shown a vast number of marker chromosomes, e.g. unidentifiable, structurally aberrant chromosomes in CTCL, multicolour FISH was used to reveal the chromosomal origins of structurally aberrant chromosomes. First, we found that a deletion or translocation of the q-arm of chromosome 12 was the most common recurrent change detected by multicolour FISH in the blood lymphocytes in 6 of 7 (86%) consecutively presenting SS patients. Three of them, studied with locus-specific FISH, showed a deletion or translocation of NAV3. A deletion of NAV3 region was subsequently observed, in the skin lesions of 11/17 (65%) randomly selected MF patients and one SS-patient whose blood was studied with CGH only.

Chromosome 12 abnormalities have been a frequent finding in CTCL in previous cytogenetic studies. Earlier cytogenetic studies have already suggested that aberrations of 12q are among the most common alterations in CTCL (Whang-Peng *et al.* 1982, Schlegelberger *et al.* 1994a) but the reported frequencies of chromosomal abnormalities are influenced by the detection methods used (Mao *et al.* 2003b). Mao and coworkers (2003b) recently reviewed 274 karyotypes, most of them G-banded, published in 27 articles, and found that aberration of 12q was among the eleven most commonly altered chromosome arms, with structural aberrations found in 7% of the CTCL cases. In comparison, the most commonly observed aberrations of 1p occurred in 11% of cases. The complex chromosomal alterations found in the present study would have been very difficult or impossible to be resolved without techniques such as MFISH or SKY, which made it possible to identify the origins of chromosome parts involved in rearrangements and to reveal the composition of aberrations designated only as markers in G-banding (Study I).

The distribution and similarity of chromosomal clones in the different tissues of CTCL patient as studied by chromosome analysis or locus-specific FISH Despite of the NAV3 deletion observed in skin, most MF patients had only non-clonal aberrations of chromosome 12 were in their blood samples. In analogy, Barbieri and coworkers (1986) found inv(12)(q15;q24) in combination with an additional chromosome 12 in the skin but not in the blood of an early stage MF-patient.This is in accordance with our previous results (Study I), in which G-banding of blood lymphocytes detected a clonal aberration of chromosome 12 in only one MF patient (47,XY,+12), whereas non-clonal aberrations of chromosome 12q occurred in 8/10 MF patients (data not shown). However, the skin lesions of 5 of the latter cases with non-clonal aberrations of chromosome 12 in the blood, were subsequently included in Study V and examined with locus specific FISH, and 4 of them showed a deletion of NAV3. The fifth patient with no NAV3 deletion (case 16) has remained in remission for over 10 years now after EB therapy (Study II). Due to the lack of contemporaneous blood samples and skin biopsies in the two other cases, it is not possible to conclude, whether the NAV3 deletion first occurred in the skin or in the blood. However, one of the latter cases (case 15) showed a clonal deletion in 12q in blood G-banding 3 years before the skin sample. Thus, G-banding of blood either does not always reveal subtle deletions of chromosome 12 observable with locus-specific FISH, or the frequency of malignant cells bearing chromosome 12 aberrations is too low in blood to be detected with cytogenetics, especially in the early stages of MF. Blood clones could represent different subclones than the clones in skin, as the blood clones of cases 20 and 21 studied with G-banding and showing marker chromosomes (4/100 and 2/100 metaphases, respectively, the latter contemporaneous with the skin sample) both included one clonal cell with monosomy of chromosome 12 as an additional aberration. Skin and blood clones may also be totally different clones, as depicted by case 12, whose blood MFISH showed a clonal aberration of chromosome 10, and non-clonal aberrations of 12q. This patient, with a deletion of NAV3 in skin, also had a different TCR gamma clone in skin compared to blood (Muche *et al.* 2004). On the other hand, some cases may belong to subgroups of CTCL without NAV3 aberrations, as there are cases showing other chromosomal abnormalities (Study II, Muche *et al.* 2004).

Chromosomal aberrations inactivating tumour suppressor genes and NAV3. The fact that the most common chromosomal aberration type we found in 12q was deletion, strongly suggests that the region harbours a tumour suppressor gene. The two SS-patients studied, with long deletions proximally and distally in the 12q showed the minimal common region in 12q21 covered

by 7 YAC-long contig, with approximate size of 6 Mb. This region may well contain tens or hundreds of genes. By serendipity, a third SS patient showed a balanced translocation with breakpoint right in the middle of the minimal region of deletion. Reciprocal translocations, even from one donor chromosome to several recipient chromosomes, have previously been used to pinpoint the location of target tumour suppressor genes, as was the case for example for the retinoblastoma gene (Davison *et al.* 1979, Higgins *et al.* 1989, Mitchell and Cowell 1989). In addition, inactivation of the well known tumour suppressor gene APC (adenomatous polyposis coli) by reciprocal translocation has also been reported (van der Luijit 1995). The mapping of translocation break-point in the above mentioned SS patient showed that the translocation disrupted a gene for the human homolog of *unc-53*, the NAV3 (also named POMFIL1; Coy *et al.* 2002, Maes *et al.* 2002).

The structure of NAV3 and cell signalling. The NAV3 gene has previously not been associated with any lymphoid malignancy and was thus an unexpected target of the recurrent aberration associated with CTCL. The NAV3 gene is large, spanning around 400 Kb of genomic sequence, and has only recently been cloned Coy *et al.* 2002, Maes *et al.* 2002. NAV3 is one of the three human homologues of *unc-53*, a gene involved in axonal elongation in *Caenorhabditis elegans* (Merrill *et al.* 2002, Frauwirth and Thompson 2002, review). NAV3 consists of 40 exons, and is known to be expressed in brain, placenta and colon. Three exons are differentially spliced. It is believed that NAV3 has arisen through duplication of NAV1 and NAV2 (HELAD1, RAINB1), situated in 1q32.1 and 11p15.1, respectively (Coy *et al.* 2002, Maes *et al.* 2002). Like *unc-53*, all three homologues have an AAA-domain characteristic of ATP-ases, and ATP/GTP-binding sites (P-loops). NAV3 shows a large number of phosphorylation sites, a leucine zipper domain, coiled-coil domains and PXXP motifs, binding sites for SH3-domain of Src (Coy *et al.* 2002). Unc-53 interacts with SEM-5, the nematode homologue of human GRB2, which is an intermediator in cell signalling e.g. by CD28 a costimulatory molecule involved in T-cell activation (Stringham *et al.* 2002; Frauwirth and Thompson 2002, Moghal and Sternberg 2003, reviews). GRB2 is also an inhibitory regulator of STAT3 transcription (Zhang *et al.* 2003). Interestingly, a constitutive activation of STAT 3 and an abnormal balance of STAT5 isoforms have been observed in Sézary syndrome Eriksen *et al.* 2001, Mitchell *et al.* 2003). NAV2 and NAV3 also have calponin-like (CH) domains 30 conferring actin binding to many cytoskeletal and signalling molecules (Frauwirth and Thompson 2002, Krawczyk and Penninger 2001 reviews). Recently, T-helper cells of most patients with Sézary syndrome have been shown to express T-plastin, an actin binding molecule normally not expressed in T-cells, that regulates cell structure and motility (Su *et al.* 2003).

Potential roles of NAV3 as a pore complex protein and helicase. Mouse NAV3/POMFIL1 was recently shown to locate in nuclear pore complexes (Coy *et al.* 2002), which may indicate a function in nucleocytoplasmic transport regulation (Linder and Stutz 2001). Nuclear pore complexes are also involved in cell cycle regulation, and kinetochore formation. In yeast, mutations in nucleoporins lead to defects in chromosome segregation (Fahrenkrog and Aebi 2003, review). Like NAV2, NAV3 also shows the properties of a helicase and exonuclease as predicted by its protein sequence (Ishiguro *et al.* 2002). NAV2 is upregulated in colorectal cancer, like ReQ helicases BLM and WRN, belonging to the superfamily II helicases (Ishiguro *et al.* 2002). If also NAV3 has helicase-like properties in the maintenance of the stability of chromosomes, its deficiency, like deficiencies of BLM and WRN, could cause a hyper-recombination phenotype, which includes formation of deletion mutants and possibly also loss of heterozygosity (LOH), and increase in sister chromatid exchanges, as has been observed in CTCL (Limon *et al.* 1995, Scarisbrick *et al.* 2000; Nakayama *et al.* 2002, review), too. Thus, a defective NAV3 might, with other possible defects contribute to the genomic instability observed in CTCL (Kaltoft *et al.* 1994, Schultz and Zakian 1994, Paz-y-Mino *et al.* 2002). The only other tumour association of the reduced or absent expression of NAV3/POMFIL1 has been reported in neuroblastoma cell lines (Coy *et al.* 2002).

The inactivation of the remaining allele of NAV3. If the NAV3 is a classical tumour suppressor gene important for aetiology or progression of CTCL, one would expect to find inactivation of the remaining allele of the gene. We were able to sequence only two of the SS cases and one was studied with DHLPC. One of them had a missense mutation showing, that both alleles were aberrant in this case. However, it is difficult to predict the functional consequence of the missense mutation. Thus, at this point we cannot be sure, that the mutation found is an inactivating one. In addition to mutations, the remaining allele of a tumour suppressor gene is frequently inactivated by epigenetic events, such as promoter hypermethylation, in cancer cells (Jones and Baylin 2002 review) . Hypermethylation is a frequent event silencing the $p16^{INK4a}$ gene in CTCL (Navas *et al.* 2000). Whether NAV3 is hypermethylated in CTCL, needs to be studied. Another possibility is that the loss of only one copy of the

gene could result in the inactivation of the gene. Such nonclassical tumour suppressor genes showing **haploinsufficient phenotype** are called nonclassical tumour suppressors. Well-known examples are mouse Cdk inhibitor $p27^{Kip1}$ and human PTEN (DiCristofano *et al.* 1998, Kwabi-Addo *et al.* 2001, Byun *et al.* 2003; Sherr 2004, review) and NKX3.1 (Bhatia-Gaur *et al.* 1999, Magee *et al.* 2003). They may also manifest haploinsufficent effects with collaborating mutations affecting other tumour suppressor genes or oncogenes. Humans heterozygous for the BLM^{Ash} have a slightly increased risk of colorectal cancer (Gruber *et al.* 2002). Consistently, mice heterozygous for BLM mutations have and increased risk of malignancies after infection with murine leukaemia virus. Mice heterozygous for both BLM mutations and APC (adenomatous polyposis coli) mutations developed intestinal tumours more quickly than mice heterozygous for APC alone - without viral challenge (Goss *et al.* 2002). Haploinsufficient tumour suppressor genes are more difficult to investigate than the classical ones, but new evidence of established or possible non-classical tumour suppressors is emerging (Sharpless *et al.* 2001, Kemkemer *et al.* 2002, Kucherlapati *et al.* 2002, Bai *et al.* 2003, Bassing *et al.* 2003, Bernert *et al.* 2003, Celeste *et al.* 2003, Deans *et al.* 2003, Dumon-Jones *et al.* 2003, Hauguel and Buntz 2003, Matsuno *et al.*2003, Moshous *et al.* 2003, Steinemann *et al.* 2003, Srivastava *et al.* 2003, Dai *et al.* 2004, McPherson et al. 2004; Sherr 2004 review). - NAV3 may well show haploinsufficiency in analogy to the gene-dosage effects in behavioral tests observed in mice with mutations in the mammalian analogue of Unc-53 (Peeters *et al.* 2004).

Deletion of NAV3 occurs early in comparison to other gene aberrations known in CTCL. The deletion of NAV3 seems to be a relatively early event during the pathogenesis of CTCL, since it detectable with locus-specific FISH in the skin of half of the patients with early MF (stages IA to IIB) whereas it was observed in 85% of cases with a later stage CTCL. In previous studies, genetic aberrations of some known tumour suppressor genes studied, like PTEN p15, p16, and p53, or overexpression of the latter, have been observed but each with lower frequencies than deletions of NAV3, especially at early stages of the disease (Garatti *et al.* 1995, Lauritzen *et al.* 1995, Li *et al.* 1998, Marrogi *et al.* 1999, McGregor *et al.* 1999, Navas *et al.* 2000, Scarisbrick *et al.* 2000, 2002). In a genomic microarray study of selected genes, not including NAV3, *JUNB* (19p13) along with *CTSB*, *RAF1* and *PAK1* showed amplifications in 5/7 CTCL cases studied (5 with SS, 2 with MF) whereas real-time-PCR showed amplification of *JUNB* in 6/14 (43%) MF and 4/22 SS (9%) cases Mao *et al.* 2003 . Also, in the present study, the aberrations in 12q were more common than aberrations of chromosomes 1, 10 or 19, associated with Sézary syndrome (Whang-Peng *et al.* 1982, present Study IV, Mao *et al.* 2002, 2003). The overexpression of JUNB was recently confirmed by a cDNA array of Sézary patients (Kari *et al.* 2003)

The deletion of 12q, with the putative target gene, NAV3, is the first chromosomal aberration found to be associated with the majority of the most common forms of CTCL. It is obvious that also other aberrations are required to explain the complex pathogenesis of CTCL, and various subgroups of CTCL are expected to be revealed. Studies providing information about the function of NAV3 in T cells and in CTCL are warranted and under way.

10. CONCLUSIONS

Cytogenetic and molecular cytogenetic *in situ* hybridizations were used to find the most common chromosomal abnormalities observed in CTCL. These methods can be used both in the diagnostics and follow-up of CTCL. The phenotype of individually identified malignant cells in Sezary syndrome was studied *in situ*, and found to be intermediate between naive CD45RA+ and mature CD45RO+ Th2 cells. The most common DNA level gains and losses were screened with CGH showing common deletions in 10q25q26 and 13q21q22, and gains in chromosome 8 and 17q21q25. These findings have paved the way to future studies of oncogenes and tumour suppressor genes affected in CTCL. Such information can be used for the future development of targeted therapy. The first chromosome aberration specific to CTCL, chromosome 12q deletions or translocations, were found with 24-colour hybridizations. The gene involved was identified with locus-specific hybridizations. The gene is NAV3, a putative tumour suppressor gene in CTCL. It is deleted in the majority of cases, also in the early stages, and the deletion can be used in diagnostics. In the future, studies concerning the role of NAV3 in signal transduction, nuclear transport, helicase properties and malignant transformation are warranted.

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12. LIST OF REFERENCES

Abrams JT, Ghosh SK, DeFreitas E: Sézary T-cell-activating factor induces functional interleukin 2 receptors on T-cells derived from patients with Sézary syndrome. Cancer Res 53: 5501-5506, 1993

Abrams JT, Vonderheid EC, Kolbe S, Appelt DM, Arking EJ, Balin BJ: Sézary T-cell activating factor is a *Chlamydia pneumoniae*-associated protein. Clin Diagn Lab Immunol 6: 895-905, 1999

Abrams T, Balin BJ, Vonderheid EC: Association between Sézary T cell-activating factor, C*hlamydia pneumoniae*, and cutaneous T cell lymphoma. Ann NY acad Sci USA 941: 69-85, 2001, review

Ackerman AB, Schiff TA: If small plaque (digitate) parapsoriasis is a cutaneous T-cell lymphoma, even an 'abortive' one, it must be mycosis fungoides! Arch Dermatol 132: 562-566, 1996

Agrawal S, Christodoulow C, Gait MJ: Efficient methods for attaching non-radioactive labels to the 5' ends of synthetic oligoreoxyribonucleotides. Nucleic Acids Res 14: 6227-6245, 1986

Alam R, Gorska M: Lymphocytes. J Allergy Clin Immunol 111: S476-4785, 2003, review

Albertson DG, Pinkel D: Genomic microarrays in human genetic disease and cancer. Hum Mol Genet 12 (Spec No 2): R145-152, 2003

Anand S, Penrhyn-Lowe S, Venkitaraman A: *Aurora-A* amplification overrides the mitotic spindle assembly checkpoint, including resistance to Taxol. Cancer Cell 3: 51-62, 2003

Anderson P, Sundstedt A, Li L, O'Neill EJ, Li S, Wraith DC, Wang P: Differential activation of signal transducer and activator of transcription (STAT)3 and STAT5 in induction of suppressors of cytokine signalling in T_h1 and T_h2 cells. International Immunology 15: 1309-1317, 2003

Aplan PD, Lombardi DP, Ginsberg AM, Cossman J, Bertness VL, Kirsch IR: Disruption of the human SCL locus by "illegitimate" V-(D)-J recombinase activity. Science 250: 1426-1429, 1990

Arstila P, Hänninen A: Soluvälittein en immuniteetti. In Huovinen P, Meri S, Peltola H, Vaara M, Vaheri A, Valtonen V (eds.) Mikrobiologia ja infektiosairaudet. Kustannus Oy Duodecim, Helsinki, Gummerus Kirjapaino Oy Jyväskylä, Vol 1 pp. 709-733, 2003, textbook

Artandi SE, Chang S, S-L Lee, Alson S, Gottlieb GJ, Chin L, DePinho RA: Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. - Nature 406: 641-645, 2000

Asadullah K, Haeußler-Quade A, Gellrich S, Hanneken S, Hansen-Hagge TE, Döcke W-D, Volk H-D, Sterry W: IL-15 and IL-16 overexpression in cutaneous T-cell lymphomas: stage dependent increase in mycosis fungoides progression. Exp Dermatol 9: 248-251, 2000

Aversa G, Carballido J, Punnonen J, Chang CCJ, Cocks BG, de Vries JE: SLAM and its role in T-cell activation and Th cell responses. Immunol Cell Biol 75: 202-205, 1997

Azofeifa J, Fauth C, Kraus J, Maierhofer C, Langer S, Bolzer A, Reichman J, Schuffenhauer S, Speicher MR: An optimized probe set for the detection of small intrachromosomal aberrations by 24-color FISH. Am J Hum Genet 66: 1684-1688, 2000

Azzalin CM, Mucciolo E, Bertoni L, Giulotto E: Fluorescence in situ hybridization with a synthetic $(T_2AG_3)_n$ polynucleotide detects several intrachromosomal telomere-like repeats on human chromosomes. Cytogenet Cell Genet 78: 112-115, 1997

Bagot M, Wechsler J, Lescs M-C, Revuz J, Farcet J-P, Gaulard P: Intraepithelial localization of the clone in cutaneous T-cell lymphoma. J Am Acad Dermatol 27: 589-593, 1992

Bagot M, Echchakir H, Mami-Chouaib F, Delfau-Larue M-H, Charue D, Bernheim A, Chouaib S, Boumsell L, Bensussan A: Isolation of tumor-specific cytotoxic CD4+ and CD4+CD8dim+ T-cell clones infiltrating a cutaneous T-cell lymphoma. Blood 91:4331-4341, 1998

Bai F, Pei XH, Godfrey VL, Xiong Y: Haploinsufficiency of p18(INK4c) sensitizes mice to carcinogen-induced tumorigenesis. Mol Cell Biol 23: 1269-1277, 2003

Barbieri D, Spanedda R, Castoldi GL: Involvement of chromosomes 12 and 14 in the cutaneous stage of mycosis fungoides: evidence for a multistep pathogenesis of the disease. Cancer Genet Cytogenet. 20: 287-292, 1986

Barth TFE, Martin-Subero JI, Joos S, Menz CK, Hasel C, Mechtersheimer G, Parwaresch RM, Lichter P, Siebert R, Möller P: Gains of 2p involving the REL locus correlate with nuclear c-REL protein accumulation in neoplastic cells of classical Hodgkin's lymphoma. Blood 101: 3681-3686, 2003

Bassing CH, Suh H, Ferguson DO, Chua KF, Manis J, Eckersdorff M, Gleason M, Bronson R, Lee C, Alt FW: Histone H2AX: a dosage-dependent suppressor of oncogenic translocations and tumors. Cell 114: 359-370, 2003

Baur JA, Zou Y, Shay JW, Wright WE: Telomere positioning effect in human cells. Science 292: 2075-2077, 2001

Benedict WF, Murphree AL, Banerjee A, Spina CA, Sparkes MC, Sparkes RS: Patient with 13 chromosome deletion: Evidence that the retinoblastoma gene is a recessive cancer gene. Science 286: 973-975, 1983

Bentz M, Plesch A, Stilgenbauer S, Döhner H, Lichter P: Minimal sizes of deletions detected by comparative genomic hybridization. Genes Chromosomes Cancer 21: 172-175, 1998

Berger R, Baranger L, Bernheim A, Valensi F, Flandrin G: Cytogenetics of T-cell malignant lymphoma. Report of 17 cases and review of the chromosomal breakpoints. Cancer Genet Cytogenet 36: 123-130, 1988

Berger R, Bernheim A Cytogenetic studies of Sézary Cells. Cancer Genet Cytogenet 27:79-87, 1987

Berger CL, Hanlon D, Kanada D, Dhodapkar M, Lombillo V, Wang N, Christensen I, Howe G, Crouch J, El-Fishawy P, Edelson R: The growth of cutaneous T-cell lymphoma is stimulated by immature dendritic cells. Blood 99: 2929-2939, 2002

Bernert H, Sekikawa K, Radcliffe RA, Iraqi F, You M, Malkinson AM: Tnfa and Il-10 defieciencies have contrasting effects on lung tumor susceptibility: gender-dependent modulation of IL-10 haploinsufficiency. Mol Carcinogen 38: 117-123, 2003

Bernheim A, Berger R, Preud'Homme JL, Labaume S, Bussel A, Barot-Ciorbaru R: Philadelphia chromosome positive blood B lymphocytes in chronic myelocytic leukemia. Leuk Res 5: 331-339, 1981

Beylot-Barry M, Vergier B, DeMascarel A, Beylot C, Merlio J-P: p53 oncoprotein expression in cutaneous lymphoproliferations. Arch Dermatol 131: 1019-1024, 1995

Beylot-Barry M, Lamant L, Vergier B, de Muret A, Fraitag S, Delord B, Dubus P, Vaillant L, Delaunay M, Mac Grogan G, Beylot C, de Mascarel A, Delsol G, Merlio J-P: Detection of t(2;5)(p23;q35) translocation by reverse transcriptase polymerase chain reaction and *in situ* hybridization in CD30-positive primary cutaneous lymphoma and lymphomatoid papulosis. Am J Pathol 149: 483- 492, 1996

Beylot-Barry M, Groppi A, Vergier B, Pulford K, Merlio JP: Characterization of t(2;5) reciprocal transcripts and genomic breakpoints in CD30+ cutaneous lymphoproliferations. Blood 91: 4668-4676, 1998

Bhatia-Gaur R, Donjacour AA, Sciavolino PJ, Kim M, Desai N, Young P, Norton CR, Gridley T, Cardiff RD, Cunha, GR, Abate-Shen C, Shen MM: Roles for Nkx3.1 in prostate development and cancer. Genes Dev13: 966-967, 1999

Biernaux C, Loos M, Sels A, Huez G, Stryckmans P: Detection of major bcl-abl gene expression at a very low level in blood cells of some healthy individuals. Blood 86: 3118-22, 1995

Blasco MA: Telomerase beyond telomeres. Nat Rev Cancer 2: 1-6, 2002, review

Blasco MA: Mammalian telomeres and telomerase: why they matter for cancer and aging. Eur J Cell Biol 82: 441-446, 2003, review

BMDP, Statistical Software Version 7.0. University of California Press, Los Angeles, 1992

Borish LC, Steinke JW: Cytokines and chemokines. J Allergy Clin Immunol 111:S460-475, 2003

Bowman T, Garcia R, Turkson J, Jove R: STATs in oncogenesis. Oncogene 19: 2474-2488, 2000

Bowman T, Broome MA, Sinibaldi D, Wharton W, Pledger WJ, Sedivy JM, Irby R, Yeatman T, Courtneidge SA, JoveR: Stat3-mediated myc expression is required for Src transformation and PDGF-induced mitogenesis. Proc natl Acad Sci USA: 98: 7319-7324, 2001

Brat DJ, Hahn SA, Griffin C, Yeo CJ, Kern SE, Hruban RH: The structural basis of molecular genetic lesions. An integration of classical cytogenetic and molecular analyses in pancreatic adenocarcinoma. Am J Pathol 150: 383-391, 1997

Brender C, Nielsen M, Kaltoft K, Mikkelsen G, Zhang Q, Wasik M, Billestrup N, Ødum N: STAT3-mediated constitutive expression of SOCS-3 in cutaneous T-cell lymphoma. Blood 97: 1056-1062, 2001

Brown L, Cheng JT, Chen Q, Siciliano MJ, Crist W, Buchanan G, Baer R: Site-specific recombination of the *tal-1* gene is a common occurrence in human T cell leukemia. EMBO J 9: 3343-3351, 1990

Brown KE, Baxter J, Graf D, Merkenschlager M, Fisher AG: Dynamic repositioning of genes in the nucleus of lymphocytes preparing for cell division. Mol Cell 3: 207-217, 1999

Bullens DMA, Rafiq K, Kasran A, van Gool SW, Ceuppens JL: Naive human T-cells can be a source of IL-4 during primary immune responses. Clin Exp Immunol 118. 384-391, 1999

Bunn PAJr, Lamberg SI: Report of the Committee on staging and classification cutaneous T-cell lymphomas. Cancer Treat Rep 63: 725-8, 1979

Bunn PAJr, Whang-Peng J, Carney DN, Schlam ML, Knutsen T, Gazdar AF: DNA Content Analysis by Flow Cytometry and Cytogenetic Analysis in Mycosis and Sézary Syndrome. J Clin Invest 65:1440-1448, 1980a

Bunn PAJr, Huberman MS, Whang-Peng J, Schechter GP, Guccion JG, Matthews MJ, Gazdar AF, Dunnick NR, Fischmann AB, Ihde DC, Cohen MH, Fossieck B, Minna JD: Prospective staging evaluation of patients with Cutaneous T-cell lymphomas. Ann Intern Med 93: 223-230, 1980

Burg G, Rodt H, Grosse-Wilde H, Braun-Falco O: Surface markers and mitogen response of cells harvested from cutaneous infiltrates in mycosis fungoides and Sézary's syndrome. J Invest Dermatol 70: 257-259, 1978

Burg G, Dummer R. Small plaque (digitate) parapsoriasis is an 'abortive cutaneous T-cell lymphoma' and is not mycosis fungoides. Arch Dermatol 131: 336-338,1995

Burg G, Dummer R, Nestle FO, Doebbeling U, Haeffner A: Cutaneous lymphomas consist of a spectrum of nosologically different entities including mycosis fungoides and small plaque parapsoriasis. Arch Dermatol 132: 567-572, 1996

Burg G, Dummer R, Haeffner A, Kempf W, Kadin M: From inflamation to neoplasia. Mycosis fungoides evolves from reactive inflammatory conditions (lymphoid infiltrates) transforming into neoplastic plaques and tumors. Arch Dermatol 137: 949-952, 2001

Busson M, Coniat L, Brizzard F, Smadja NV, Maarek O, Der Sarkissian H, Berger R: Interstitial telomere repeats in translocations of hematopoietic disorders. Leukemia 14: 1630-1633, 2000

Byun DS, Cho K, Ryu BK, Lee MG, Park JI, Chae KS, Kim HJ, Chi SG: Frequent monoallelic deletion of Pten and its reciprocal association with PIK3CA amplification in gastric carcinoma. Int J Cancer 104: 318-327, 2003

Bäsecke J, Griesinger F, Trümper L, Brittinger G: Leukemia- and lymphoma-associated genetic aberrations in healthy individuals. Ann Hematol 81: 64-75, 2002, review

Cahill DP, Lengauer C, Yu J, Riggins GJ, Willson JKV, Markowitz SD, Kinzler KW, Vogelstein B: Mutations of mitotic checkpoint genes in human cancers Nature 392:300-303, 1998

Caló V, Migliavacca M, Bazan V, Macaluso M, Buscemi M, Gebbia N, Russo A: STAT proteins: From normal control of cellular events to tumorigenesis. J Cell Phys 197: 157-168, 2003

Carbonari M, Tedesco T, Del Porto P, Paganelli R, Fiorilli M: Human T cells with a type-2 cytokine profile are resistant to apoptosis induced by primary activation: consecuences for immunopathogenesis. Clin Exp Immunol 120. 454-462, 2000

Carroll PE, Okuda M, Horn HF, Biddinger P, Stambrook PJ, Gleich LL, Li Y-Q, Tarapore P, Fukasawa K: Centrosome hyperamplification in human cancer: chromosome instability induced by p53 mutation and/or Mdm2 overexpression. Oncogene 18: 1935-1944, 1999

Carter NP, Ferguson-Smith MA, Perryman MT, Telenius H, Pelmear AH, Leversha MA, Glancy MT, Wood SL, Cook K, Dyson MH, Ferguson-Smith ME, Willatt LR: Reverse chromosome painting: a method for the rapid analysis of aberrant chromosomes in clinical cytogenetics. J Med Genet 29: 229-307, 1992

Carvalho C, Pereira HM, Ferreira J, Pina C, Mendonça D, Rosa AC, Carmo-Fonseca M: Chromosomal G-dark bands determine the spatial organization of centromeric heterochromatin in the nucleus. Mol Biol Cell 12: 3563-3572, 2001

Casper AM, Nghiem P, Arlt MF, Glover TW: ATR regulates fragile site stability. Cell 111: 779-789, 2002

Cayuela J-M, Gardie B, Sigaux F: Disruption of the multiple tumor suppressor gene *MTS1*/p16(INK4a)/*CDKN2* by illegitimate V(D)J recombinase activity in T-cell acute lymphoblastic leukemias. Blood 90: 3720-3726, 1997

Celeste A, Difilippantonio S, Difilippantonia MJ, Fernandez-Capetillo O, Pilch DR, Sedelnikova OA, Eckhaus M, Ried T, Bonner WM, Nussenzeig A: H2AX haploinsufficiency modifies genomic sability and tumor susceptibility. Cell 114: 371-383, 2003

Cerroni L, Hödl S, Rieger E, Soyer HP, Smolle J, Kerl H: Transformation of mycosis fungoides to large cell lymphoma. J Cutan Pathol 17: 290, 1990

Cerroni L, Rieger E, Hödl S, Kerl H: Clinicopathologic and immunologic features associated with transformation of mycosis fungoides to large cell lymphoma. Am J Surg Pathol 16: 543-552, 1992

Cerroni L, Arzberger E, Ardigò M, Pütz B, Kerl H: Monoconality of intraepidermal T lymphocytes in early mycosis fungoides detected by molecular analysis after laser-beam-based microdissection. J Invest Dermatol 114: 1154-1157, 2000

Cerroni L, Fink-Puches R, Bäck B, Kerl H: Follicular Mucinosis. A critical reappraisal of clinopathologic features and association with mycosis fungoides and Sézary syndrome. Arch Dermatol 138: 182-189, 2002

Chan S W-L, Blackburn EH: Telomerase and ATM/Tell p protect telomeres from nonhomologous end joining. Mol Cell 11: 1379-1387, 2003

Chang S, Khoo CH, Naylor ML, Maser RS, DePinho RA: Telomere-based crisis: functional differences between telomerase activation and ALT in tumor progression. Gen Dev 17: 88-100, 2003

Chen X, Oh S-W, Zheng Z, Chen H-W, Shin H-h, Hou SX: Cyclin D-Cdk4 and cyclin E-Cdk2 regulate the JAK/STAT signal transduction pathway in *Drosophila*. Dev Cell 4: 179-190, 2003

Chudoba I, Plesch A, Lörch T, Lemke J, Claussen U, Senger G: High resolution multicolour-banding: a new technique for refined FISH analysis of human chromosomes. Cytogenet Cell Genet 84: 156-160, 1999

Cech TR: Beginning to understand the end of the chromosome. Cell 116: 273-279, 2004, review

Chang S, Khoo CM, Naylor ML, Maser RS, DePinho RA: Telomerase-based crisis: functional differences between telomerase activation and ALT in tumor progression. Genes Dev 17: 88-100, 2003

Chiba S, Okuda M, Mussman JG, Fukasawa K: Genomic convergence and suppression of centrosome hyperamplification in primary p53-/- cells in prolonged culture. Exp Cell Res 258: 310-321, 2000

Clement LT, Yamashita N, Martin AM: The functionally distinct subpopulations of human CD4+ helper/inducer T lymphocytes defined by anti-CD45R antibodies derive sequentially from a differentiation pathway that is regulated by activation-dependent post-thymic differentiation. J Immunol 141:1464-1470, 1988

Cocks BG , Chang C-CJ, Carballido JM, Yssel H, de Vries JE, Aversa G: A novel receptor involved in T-cell activation. Nature 376:260-263, 1995

Coquelle A, Pipiras E, Toledo F, Buttin G, Debatisse M: Expression of fragile sites triggers intrachromosomal mammalian gene amplification and sets boundaries to early amplicons. Cell 89: 215-225, 1997

Coy JF, Wiemann, S, Bechmann I, Bächner, D, Nitsch R, Kretz O, Christiansen H., Poustka A: Pore membrane and/or filament interacting like protein 1 (POMFIL1) is predominantly expressed in the nervous system and encodes different protein isoforms. Gene 290: 73-94, 2002

Cremer T, Lichter P, Borden J, Ward DC, Manuelidis L: Detection of chromosome aberrations in metaphase and interphase tumor cells by *in situ* hybridization using chromosome-specific library probes. Hum Genet 80: 235-246, 1988

Costa C, Gallardo F, Pujol RM, Espinet B, Bellosillo B, Estrach T, Servitje O, Barranco C, Serrano S, Sole F: Comparative analysis of TCR-γ gene rearrangements by Genescan and polyacrylamide gel-electrophoresis in cutaneous T-cell lymphoma. Acta Derm Venereol 84: 6-11, 2004

D'Alessandro E, Paterlini P, Lo Re ML, Di Cola M, Ligas C, Quaglino D, Del Porto G: Cytogenetic follow-up in a case of Sézary syndrome. Cancer Genet Cytogenet 45: 231-236, 1990

D'Alessandro E, De Pasquale A, Ligas C, Lo Re ML, Di Cola M, Del Porto G, Quaglino D: Cytogenetic findings in terminal large cell transformation in a case of Sézary syndrome. Cancer Genet Cytogenet 58: 100-104, 1992

Dai W, Wang Q, Liu T, Swamy M, Fang Y, Xie S, Mahmood R, Yang Y-M, Xu M, Rao CV: Slippage of mitotic arrest and enhanced tumor development in mice with *BubRI* haploinsufficiency. Cancer Res 64: 440-445, 2004

Dalloul A, Laroche L, Bagot M, Mossalayi MD, Fourcade C, Thacker DJ, Hogge DE, Merle-Béral H, Debré P, Schmitt C: Interleukin-7 is a growth factor for Sézary lymphoma cells. J Clin Invest 90: 1054-1060, 1992

Dauwerse JG, Wiegant J, Raap AK, Breuning MH, van Ommen GJB: Multiple colors by fluorescence *in situ* hybridization using ratio-labelled DNA probes create a molecular karyotype. Hum Mol Genet 1: 593-598, 1992

Davison EV, Gibbons B, Aherne GE,Roberts DF Chromosomes in retinoblastoma patients. Clin Genet 15: 505-8, 1979

Day JP, Limoli CL, Morgan WF: Recombination involving interstitial telomere repeat-like sequences promotes chromosomal instability in Chinese hamster cells. Carcinogenesis 19: 259-265, 1998

Deans B, Griffin CS, O'Regan PO, Jasin M, Thacker J: Homologous recombination deficiency leads to profound genetic instability in cells derived from Xrcc2-knockout mice. Cancer Res 63: 8181-8187, 2003

DeCoteau JF Butmarc JR, Kinney MC, Kadin ME: The t(2;5) chromosomal translocation is not a common feature in cutaneous CD30+ lymphproliferative disordres: Comparison with anaplastic large-cell lymphoma of nodal origin. Blood 87: 3437-3441, 1996

de Groot RP, Raajimakers JAM, Lammers J-WJ, Koenderman L: STAT5-dependent cyclin D1 and Bcl-xL expression in Bcr-Abl-transformed cells. Mol Biol Res Commun 385: 299-305, 2000

Delfau-Larue M-H, Laroche L, Wechsler J, Lepage E, Lahet C, Asso-Bonnet M, Bagot M, Farcet J-P: Diagnostic value of dominant T-cell clones in peripheral blood in 363 patients presenting consecutively with a clinical suspicion of cutaneous lymphoma. Blood 96 : 2987-2992, 2000

Del Prete G, De Carli M, Almerigogna F, Giudizi MG, Biagiotti R, Romagnani S: Human IL-10 is produced by both type 1 helper (Th1) and type 2 helper (Th2) T cell clones and inhibits their antigen-specific proliferation and cytokine production. J Immunol 150: 353-360, 1993

Devilee P, Slagboom P, Cornelisse CJ, Pearson PL: Sequence heterogeneity within the human alphoid repetitive DNA family. Nucleid Acid Res 14: 2059-2073, 1986a

Devilee P, Cremer T, Slagboom P, Bakker E, Scholl HP, Hager HD, Stevenson AFG, Cornelisse CJ, Pearson PL: Two subsets of human alphoid repetitive DNA show distinct preferential localization in the pericentric regions of chromosomes 13, 18 and 21. Cytogenet Cell Genet 41: 193-201, 1986b

Dhingra K, Sahin A, Supak J, Kim SY, Hortobagyi G, Hittelman WN: Chromosome *in situ* hybridization on formalin-fixed mammary tissue using non-isotopic, non-fluorescent probes: technical considerations and biological implications. Breast Cancer Res Treatment 23: 201-210, 1992

DiCristofano A, Pesce B, Cordon-Cardo C, Pandolfi PP: Pten is essential for embryonic development and tumour suppression. Nat Genet19: 348-55, 1998

Difilippantonino MJ, Petersen S, Chen HT, Johnson R, Jasin M, Kanaar R, Ried T, Nussenzweig A: Evidence for replicative repair of DNA double-strand breaks leading to oncogenic translocation and gene amplification. J Exp Med 196: 469-480, 2002

Dommann SNW, Dommann-Scherrer CC, Dours-Zimmermann M-T, Zimmermann DR, Kural-Serbes B, Burg G: Clonal disease in extracutaneous compartments in cutaneous T-cell lymphomas. A comparative study between cutaneous T-cell lymphomas and pseudo lymphomas. Arch Dermatol Res 288:163-167,1996

Du Manoir S, Schröck E, Bentz M, Speicher MR, Joos S, Ried T, Lichter P, Cremer T: Quantitative analysis of comparative genomic hybridization. Cytometry 19: 27-41, 1995

Dummer R, Michie SA, Kell D, Gould JW, Haeffner AC, Smoller BR, Warnke RA, Wood GS: Expression of bcl-2 protein and Ki-67 nuclear proliferation antigen in benign and malignant cutaneous T-cell infiltrates. J Cutan Pathol 22: 11-17, 1995

Dummer R, Heald PW, Nestle FO, Ludwig E, Laine E, Hemmi S, Burg G: Sezary syndrome T-cell clones display T-helper 2 cytokines and express the accessory factor-1 (interferon-γ receptor -chain). Blood 88: 1383-1389, 1996

Dumon-Jones V, Frappart PO, Tong WM, Sajithlal G, Hulla W, Schmid G, Herceg Z, Digweed M, Wang ZQ: Nbn heterozygosity renders mice susceptible to tumor formation and ionizing-radiation induced tumorigenesis. Cancer Res 63: 7263-7269, 2003

Döbbeling U, Dummer R, Laine E, Potoczna N, Qin J-Z, Burg G: Interleukin-15 is an autocrine/paracrine viability factor for cutaneous T-cell lymphoma cells. Blood 92: 252-258, 1998

Edelson RL, Berger CL, Raafat J, Warburton D: Karyotype studies of cutaneous T cell lymphoma: evidence for clonal origin. J Invest Dermatol 73: 548-550, 1979

Eriksen KW, Kaltoft K, Mikkelsen G, Nielsen M, Zhang Q, Geisler C, Nissen MH, Röpke C, Wasik MA, Ødum N: Constitutive STAT3-activation in Sezary syndrome: tyrphostin AG490 inhibits STAT3-activation, interleukin-2 receptor expression and growth of leukemic Sezary cells. Leukemia 15: 787-793, 2001

Fahrenkrog B, Aebi U: The nuclear pore complex: nucleocytoplasmic transport and beyond. Nat Rev. Mol Cell Biol 4:757-766, 2003, review

Fearon ER, Vogelstein B: A genetic model for colorectal tumorigenesis. Cell 61: 759-767, 1990

Feinberg AP, Vogelstein B: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6-13, 1983, with Addendum Anal Biochem 137(1):266-227,1984, with addenum in Anal Biochem 137: 266-267, 1984

Feldser DM, Hackett JA, Greider CW: Telomere dysfunction and the initiation of genome instability. Nat Rev Cancer: 3: 1-5, 2003

Ferenczi K, Fuhlbrigge RC, Pinkus JL, Pinkus GS, Kupper TS: Increased CCR4 expression in cutaneous T-cell lymphoma. J Invest Dermatol 119: 1405-1410, 2002

Fidlerová H, Senger G, Kost M, Sanseau P, Sheer D: Two simple procedures for releasing chromatin from routinely fixed cells for fluorescence in situ hybridization. Cytogenet Cell Genet 65: 203-205, 1994

Fischer TC, Sterry W, Tönnies H: Chromosomal imbalances in mycosis fungoides analysed by comparative genomic hybridization (CGH). Meeting of EORTC European Organization for Research and Treatment of Cancer, Oxford, UK, 14-16. September 2001, abstract

Fischer TC, Gellrich S, Muche JM, Sherev T, Audring H, Neitzel H, Walden P, Sterry W, Tönnies H: Genomic aberrations and survival in cutaneous T cell lymphomas. J Invest Dermatol 122: 579-586, 2004

Fischmann AB, Bunn PAJr, Guccion JG, Matthews MJ, Minna JD: Exposure to chemicals, physical agents, and biologic agents in mycosis fungoides and the Sézary syndrome. Cancer Treat Rep 63: 591-596, 1979

FitzPatrick DR, Ramsay J, McGill NI, Shade M, Carothers AD, Hastie ND: Transcriptome analysis of human autosomal trisomy. Hum Mol Genet 11: 3249-3256, 2002

Forozan F, Karhu R, Kononen J, Kallioniemi A, Kallioniemi O-P: Genome screening by comparative genomic hybridization. Trends in Cell Biology: 13: 405-409, 1997, review

Frauwirth KA, Thompson G B: Activation and inhibition of lymphocytes by costimulation. J Clin Invest., 109: 295-299, 2002, review

Fraser-Andrews EA, Woolford AJ, Russell-Jones R, Seed PT, Whittaker SJ: Detection of a peripheral blood T cell clone is an independent prognostic marker in mycosis fungoides. J Invest Dermatol 114 :117-21, 2000

Fukada T, Ohtani T, Yoshida Y, Shirogane T, Nishida K, Nakajima K, Hibi M, Hirano T: STAT3 orchestrates contradictory signals in cytokine-induced G_1 to S cell-cycle transition. EMBO J 22: 6670-6677, 1998

Fukasawa K, Choi T, Kuriyama R, Rulong S, Van de Woude GF: Abnormal centrosome amplification in the absence of p53. Science 271:1744-1747, 1996

Fukuhara S, Rowley JD, Variakojis: Banding studies of chromosomes in a patient with mycosis fungoides. Cancer 42: 2262-2268, 1978

Gall JG, Pardue ML; Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc Natl Acad Sci USA 63: 378-383, 1969

Gamero AM, Young HA, Wiltrout RH: Inactivation of Stat3 in tumor cells: Releasing a brake on immune responses against cancer? Cancer Cell 5 (Feb) 2004: 111-112, 2004 review

Gamperl R: Clonal chromosomal aberrations in a case of cutaneous T-cell lymphoma. Cancer Genet Cytogenet 19: 341-344, 1986

Garatti SA, Roscetti E, Trecca, Fracciolla NS, Neri A, Berti E: bcl-1, bcl-2, p53,c-myc, and lyt-10 analysis in cutaneous T-cell lymphomas. Recent Results in Cancer Research 139: 249-261 1995

Gebhart ER: Patterns of early centromere separation and aneuploidy in human carcinoma cells. Prog Clin Biol Res 318: 129-135, 1989

Gellrich S, Lukowsky A, Schilling T, Rutz S, Muche JM, Jahn S, Audring H, Sterry W: Microanatomical compartments of clonal and reactive cells in mycosis fungoides: molecular demonstration by single cell polymerase chain reaction of T cell receptor gene rearrangements. J Invest Dermatol 115: 620-624, 2000

Gesk S, Martín-Subero JI, Harder L, Luhmann B, Schlegelberger B, Calasanz MJ, Siebert R: Molecular cytogenetic detection of chromosomal breakpoints in T-cell receptor gene loci. Leukemia 17: 738-745, 2003

Girardi M, Heald PW, Wilson LD: The pathogenesis of mycosis fungoides. N Engl J Med 350: 1978-1988, 2004, review

Gisselsson D, Jonson T, Peterson Å, Strömbeck B, Dal Cin P, Höglund M, Mitelman F, Mertens F, Mandahl N: Telomere dysfunction triggers extensive DNA fragmentation and evolution of complex chromosome abnormalities in human malignant tumors. Proc Natl Acad Sci USA 98: 12683-12688, 2001

Gladdy RA, Taylor MD, Williams CJ, Grandal I, Karaskova J, Squire JA, Rutka JT, Guidos CJ, DanskaJS: The RAG-1/2 endonuclease causes genomic instability and controls CNS complications of lymphoblastic leukemia in p53/Prkdc-deficient mice. Cancer Cell 3: 37-50, 2003

Gollin SM: Chromosomal instability. Current Opinion in Oncology 16: 25-31, 2003, review

Gorochov G, Bachelez H, Cayuela JM, Legac E, Laroche L, Duertret L, Sigaux F: Expression of Vβ gene segments by Sezary cells. J Invest Dermatol 105: 56-61, 1995

Goss KH, Risinger MA, Kordich JJ, Sanz MM, Straughen JE, Slovek LE, Capobianco AJ, James G, Boivin GP, Groden J: Enhanced tumor formation in mice heterozygous for *Blm* mutation. Science 297: 2051-2053, 2002

Greaves MF, Wiemels J: Origins of chromosome translocations in childhood leukaemia. Nature Reviews Cancer 3: 1-11, 2003

Greene MH, Dalager NA, Lamberg SI, Argyropoulos CE, Fraumeni JFJr: Mycosis fungoides: Epidemiologic observations. Cancer Treat Rep 63: 597-606, 1979

Gruber SB,Ellis NA, Rennert G, Offit K, Scott KK, Almog R, Kolachana P, Bonner JD, Kirchoff T, Tomsho LP, Nafa K, Pierce H, Low M, Satagopan J, Rennert H, Huang H, Greenson JK, Groden J, Rapaport B, Shia R, Johnson S, Gregersen PK, Harris CC: BLM heterozygosity and the risk of colorectal cancer. Science 297: 2013, 2002

Guan X-Y, Trent JM, Meltzer PS: Generation of band-specific painting probes from a single microdissected chromosome. Hum Mol Genet 2: 1117-1121, 1993

Guan X-Y, Meltzer PS, Dalton WS, Trent JM: Identification of cryptic sites of DNA sequence amplification in human breast cancer by chromosome microdissection. Nat Genet 8: 155-161, 1994a

Guan X-Y, Meltzer PS, Trent JM: Rapid generation of whole chromosome painting probes (WCPs) by chromosome microdissection. Genomics 22: 101-107, 1994b

Guan X-Y, Zhang H, Bittner M, Jiang Y, Meltzer P, Trent J: Chromosome arm painting probes. Nature Genetics 12: 10-11, 1996, correspondence

Haaf T, Ward DC: High resolution ordering of YAC contigs using extended chromatin and chromosomes. Hum Mol Genet 3: 629-633, 1994a

Haaf T, Ward DC: Structural analysis of α-satellite DNA and centromere proteins using extended chromatin and chromosomes. Hum Mol Genet 3: 697-709, 1994b

Haas OA, Köller U, Ambros P, Kornmüller R, Majdic O, Gadner H, Knapp W: Immunoenzymatic staining methods for simultaneous demonstration of chromosomes and cell surface markers. Cancer Genet Cytogenet 27: 229-240, 1987

Haddad BR, Schröck E, Meck J, Cowan J, Young H, Ferguson-Smith MA, du Manoir S, Ried T: Identification of de novo chromosomal markers and derivatives by spectral karyotyping. Hum Genet 103: 619-625, 1998

Haeffner AC, Smoller BR, Zepter K, Wood GS: Differentiation and clonality of lesional lymphocytes in small plaque parapsoriasis. Arch Dermatol 131: 321-328, 1995

Hamann D, Baars PA, Hooibrink B, van Lier RAW: Heterogeneity of the human CD4+ T-cell population: Two distinct CD4+ T-cell subsets characterized by coexpression of CD4RA and CD45RO isoforms. Blood 88:3513-3521, 1996

Hauguel T, Bunz F: Haploinsufficiency of hTERT leads to telomere dysfunction and radiosensitivity in human cancer cells. Cancer Biol ther 6: 679-684, 2003

Heald PW, Yan S-L, Edelson RL, Tigelaar R, Picker LJ: Skin-selective lymphocyte homing mechanisms in the pathogenesis of leukemic cutaneous T-cell lymphoma. J Invest Dermatol 101: 222-226, 1993

Heinrich PC, Behrmann I, Haan S, Hermanns HM, Müller-Newen G, Schaper F: Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 374: 1-20, 2003

Heiskanen M, Hellsten E, Kallioniemi O-P, Mäkelä TP, Alitalo K, Peltonen L, Palotie A: Visual mapping by fiber-FISH. Genomics 30: 31-36, 1995

Henegariu O, Heerema NA, Bray-Ward P, Ward DC: Colour-changing karyotyping: an alternative to M-FISH/SKY. Nature Genetics 23: 263-264, 1999

Heng HHQ, Squire J, Tsui LC: High resolution mapping of mammalian genes by *in situ* hybridization to free chromatin. Proc Natl Acad Sci USA 89: 9509-9513, 1992

Hickson I D: RecQ Helicases: Caretakers of the genome. Review. Nature Reviews Cancer 3: 169-178, 2003, review

Higgins M.J, Hansen MF, Cavenee WK., Lalande M: Molecular detection of chromosomal translocations that disrupt the putative retinoblastoma susceptibility locus. Mol Cell Biol 9: 1-5, 1989

Hindkjær J, Brandt CA, Kaltoft K: Aneuploid malignant T cells from a patient with Sézary syndrome can be visualized by in situ hybridization. Arch Dermatol 129: 1141-1145, 1993

Hirano T, Mitchison TJ: A heterodimeric coiled-coil protein required for mitotic chromosome condensation in vitro. Cell 79: 449-458, 1994

Ho I-C, Glimcher LH: Transcription: Tantalizing times for T cells. Cell 109: S109-S120, 2002, review.

Hoeijmakers JHJ: Genome maintenance mechanisms for preventing cancer. Nature 411: 366-374, 2001, review

Homey B, Alenius H, Müller A, Soto H, Bowman E, Yuan W, McEvoy L, Lauerma AI, Assmann T, Bünemann E, Lehto M, Wolff H, Yen D, Marxhausen H, To W, Sedgwick J, Ruzicka T, lehman P, Zlotnik A: CCL27-CCR10 interactions regulate T-cell mediated skin inflammation. Nat Med 8: 157-165, 2002

Hopman AHN, Poddighe PJ, Wim A, Smeets GB, Moesker O, Beck JLM, Vooijs GP, Ramaekers FCS: Detection of numerical chromosome aberrations in bladder cancer by *in situ* hybridization. Am j Pathol 135: 1105-1117, 1989

Hopman AHN, van Hooren E, van de Kaa CA,Vooijs PGP, Ramaekers FCS: Detection of numerical chromosome aberrations using *in situ* hybridization in paraffin sections of routinely processed bladder cancers. Modern Pathology 4: 503-513, 1991

Houseal TW, Dackowski WR, Landes GM, Klinger KW: High resolution mapping of overlapping cosmids by fluorescence in situ hybridization. Cytometry 15: 193-198, 1994

Huntly BJP, Bench A, Green AR: Double jeopardy from a single translocation: deletions of the derivative chromosome 9 in chronic myeloid leukemia. Blood 102: 1160-1168, review

Hyytinen E, Visakorpi T, Kallioniemi A, Kallioniemi O-P, Isola JJ: Improved technique for analysis of formalin-fixed, paraffin-embedded tumors by fluorescence in situ hybridization. Cytometry 16:93-99, 1994

Höltke HJ, Ankenbauer W, Muhlegger K, Rein R, Sagner G, Seibl R, Walter T: The digoxigenin (DIG) system for non-radioactive labelling and detection of nucleic acids - an overview. Cell Mol Biol (Noisy-Le-Grand) 41: 883-905, 1995

Ishiguro H, Shimokawa T, Tsunoda, T, Tanaka T, Fujii Y, Nakamura Y, FurukawaY: Isolation of HELAD1, a novel human helicase gene up-regulated in colorectal carcinomas. Oncogene 21: 6387-6394, 2002

Jablonski E, Moomaw EW, Tullis RH, Ruth JL: Preparation of oligonucleotide-alkaline phosphatase conjugates and their use as hybridization probes. Nucleic Acids Res 14: 6115-6129, 1986

Jack AS, Boylston AW, Carrel S, Grigor I: Cutaneous T-cell lymphoma cells employ a restricted range of a T-cell antigen receptor variable region genes. Am J Pathol 136: 17-21, 1990

Jallepalli PV, Lengauer C: Chromosome segregation and cancer: cutting through the mystery. Nature Rev Cancer 1: 109-117, 2001, review

Janz S, Potter M, Rabkin CS: Lymphoma- and leukemia-associated chromosomal translocations in healthy individuals. Gen Chrom Cancer: 36: 211-223, 2003

John HA, Birnstiel ML, Jones KW: RNA-DNA hybrids at the cytological level. Nature 223: 582-587, 1969

Johnson GA, Dewald GW, Strand WR, Winkelmann RK: Chromosome studies in 17 patients with the Sézary Syndrome. Cancer 55: 2426-2433, 1985

Jones PA, Baylin SB: The fundamental role of epigenetic events in cancer. Nature Rev Cancer 3: 415-428, 2002, review

Jung D, Alt FW: Unraveling V(D)J recombination: Insights into gene regulation. Cell 116: 299-311, 2004, review

Kallioniemi O-P, Kallioniemi A, Kurisu W, Thor A, Chen L-C, Smith HS, Waldman FM, Pinkel D, Gray JW: *ERBB2* amplification in breast cancer analyzed by fluorescence *in situ* hybridization. Proc Natl Acad Sci U S A 89:5321-5325, 1992a

Kallioniemi A, Kallioniemi O-P, Sudar D, Rutowitz D, Gray JW, Waldman F, Pinkel D: Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 258: 818-821, 1992b

Kallioniemi O-P, Kallioniem A, Piper J, Isola J, Waldman FM, Gray JW, Pinkel D: Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. Gens, Chromosomes & Cancer, 10: 231-243, 1994a

Kallioniemi O-P, Kallioniem A, Piper J, Tanner M, Stokke T, Chen L-C, Smith HS, Pinkel D, Gray JW, Waldman FM: Detection and mapping of amplified sequences in breast cancer by comparative genomic hybridization. Proc natl Acad Sci USA 91: 2156-2160, 1994b

Kaltoft K, Bisballe S, Dyrberg T, Boel E, Rasmussen PB, Thestrup-Pedersen K: Establishment of two continuous T-cell strains from a single plaque of a patient with mycosis fungoides. In Vitro Cell Dev Biol 28A:161-167, 1992

Kaltoft K, Hansen BH, Thestrup-Pedersen K: Cytogenetic findings in cell lines from cutaneous T-cell lymphoma. Dermatologic Clinics 12: 295-304, 1994

Kamogawa Y, Minasi LE, Carding SR, Bottomly K, Flavell RA: The relationship of IL-4 and INF gamma producing T-cells studied by lineage ablation of IL-4-producing cells. Cell 75: 985-995, 1993

Kanavaros P, Ioannidou D, Tzardi M, Datseris G, Katsantonis J, Delidis G, Tosca A: Mycosis fungoides: Expression of -myc p62, p53, bcl-2 and PCNA proteins and absence of association with Epstein-Barr virus. Path Res Pract 1994; 190 :767-74.

Kanegane H, Tosato G: Activation of naive and memory T cells by interleukin-15. Blood: 88:230-235, 1996

Kaplan MH, Schindler U, Smiley ST, Grusby MJ: Stat6 is required for mediating responses to IL-4 and for the develoment of Th2 cells. Immunity 4: 313-319, 1996

Kari L, Loboda A, Nebozhyn M, Rook AH, Vonderheid EC, Nichols C, Virok D, Chang C, Horng W-H, Johnston J, Wysocka M, Showe MK, Showe LC: Classification and prediction of survival in patients with the leukemic phase of cutaneous T cell lymphoma. J Exp Med 197: 1477-1488, 2003

Karhu R, Kähkönen M, Kuukasjärvi T, Pennanen S, Tirkkonen M, Kallioniemi O: Quality control of CGH: Impact of metaphase chromosomes and the dynamic range of hybridization. Cytometry 28: 198-205, 1997

Karlseder J: Telomere repeat binding factors: keeping the ends in check. Cancer Lett 194: 189-197, 2003

Kearney L: The impact of the new FISH technologies on the cytogenetics of haematological malignancies. Br J Haematol 104: 648-658, 1999, review

Kemkemer R, Schrank S, Vogel W, Gruler H, Kaufmann D: Increased noise as an effect of haploinsufficiency of the tumor-suppressor gene neurofibromatosis type 1 *in vitro*. Proc Natl Acad Sci USA: 99: 13783-13788, 2002

Kempe T, Sundquist WI, Chow F, Hsu SL: Chemical and enzymatic biotin-labelling of oligodeoxynucleotides. Nucleic Acids Res 13: 45-57, 1985

Kerstens, HMJ, Poddighe PP, Hanselaar AGJM: Double-target in situ hybridization in brightfield microscopy. J Histochem Cytochem 42: 1071-1077, 1994

Kikuchi A, Naka W, Harada T, Sakuraoka K, Harada R, Nishikawa T: Parapsoriasis en plaques: Its potential for progression to malignant lymphoma. J Am Acad Dermatol 29: 419-422, 1993

Kim JE, Huh J, Cho K, Kim CW: Pathologic characteristics of primary cutaneous T-cell lymphoma in Korea. J Korean Med Sci 13: 31-8, 1998

King-Ismael D, Ackerman AB: Guttate parapsoriasis/digitate dermatosis (small plaque parapsoriasis) is mycosis fungoides. Am J Dermatopathol 14(6): 518-530, 1992

Kirchhoff M, Gerdes T, Maahr J, Rose H, Bentz M, Döhner H, Lundsteen C: Deletions below 10 megabasepairs are detected in comparative genomic hybridization by standard reference intervals. Gen Chrom Cancer 25: 410-413, 1999

Klemke C-D, Dippel E, Dembinski A, Pönitz N, Assaf C, Hummel M, Stein H, Goerdt S: Clonal T cell receptor γ-chain gene rearrangement by PCR-based GeneScan analysis in the skin and blood of patients with parapsoriasis and early-stage mycosis fungoides. J Pathol 197: 348-354, 2002

Knauf WU, Knuutila S, Zeigmeister B, Thiel H: Trisomy 12 in B-cell chronic lymphocytic leukemia: Correlation with advanced disease, atypical morphology, high levels of sCD25, and with refractoriness to treatment. Leuk Lymph 19: 289-294, 1995

Knudson AG Jr, Mutation and cancer: Statistical study of retinoblastoma. Proc Natl Acad Sci USA 68: 820-823, 1971

Knudson AG: Two genetic hits (more or less) to cancer. Nature Rev Cancer 1: 157-162, 2001, review

Knuutila S, Keinänen M: Chromosome banding techniques for morphologically classified cells. Cytogenet Cell Genet 39: 70-72, 1985

Knuutila S, Nylund SJ, Wessman M, Larramendy ML: Analysis of genotype and phenotype on the same interphase or mitotic cell. A manual of MAC (Morphology Antibody Chromosomes) methodology. Cancer Genet Cytogenet 72: 1-15, 1994a, review

Knuutila S, Teerenhovi L, Larramendy ML, Elonen E, Franssila KO, Nylund SJ, Timonen T, Heinonen K, Mahlamäki E, Winqvist R, Ruutu T: Cell lineage involvement of Recurrent Chromosomal Abnormalities in hematologic Neoplasms. Genes Chromosomes Cancer 10: 95-102, 1994b

Koch JE, Kølvraa S, Petersen KB, Gregersen N, Bolund L: Oligonucleotide priming methods for the chromosome -specific labeling of alpha satelite DNA *in situ*. Chromosoma (Berl.) 98: 259-265, 1989

Kolomietz E, Al-Maghrabi J, Brennan S, Karaskova J, Minkin S, Lipton J, Squire JA: Primary chromosomal rearrangements are frequently accompanied by extensive submicroscopic deletions and may lead to altered prognosis. Blood 97: 3581-3588, 2001

Kolomietz E, Meyn MS, Pandita A, Squire JA: The role of Alu repeat clusters as mediators of recurrent chromosomal aberrations in tumors. Genes Chromosomes Cancer 35: 97-112, 2002

Komminoth P, Merk FB, Leav I, Wolfe HJ, Roth J: Comparison of ^{35}S- and digoxigenin-labeled RNA and oligonucleotide probes for *in situ* hybridization. Expression of mRNA of the seminal vesicle secretion protein II and androgen receptor genes in the rat prostate. Histochemistry 98: 217-228, 1992

Krawczyk C, Penninger J: M. Molecular controls of antigen receptor clustering and autoimmunity. Trends in Cell Biology., 11: 212-220, 2001, review

Krämer A, Neben K, Ho AD: Centrosome replication, genomic instability and cancer. Leukemia 16: 767-775, 2002, review

Krämer A, Schweizer S, Neben K, Giesecke C, Kalla J, Katzenberger T, Benner A, Müller-Hermelink HK, Ho AD, Ott G: Cetrosome aberrations as a possible mechanism for chromosomal instability in non-Hodgkin's lymphoma. Leukemia 17: 2207-2213, 2003

Krug U, Ganser A, Koeffler HP: Tumor suppressor genes in normal and malignant hematopoiesis. Oncogene 21: 3475-3495, 2002

Kucherlapati M, Yang K, Kuraguchi M, Zhao J, Lia M Heyer J, Kane MF, Fan KF, Russell R, Brown AMC, Kneitz B, Edelmann W, Kolodner RD, Lipkin M, Kucherlapati R: Haploinsufficiency of Flap endonuclease (Fen1) leads to rapid tumor progression. Proc Natl Acad Sci USA 99(15): 9924-9929, 2002

Kušek R: Molecular and genetic mechanisms of lymphomagenesis. Croat Med J : 43: 519-525, 2002, review

Kuukasjärvi T, Karhu R, Tanner M, Kähkönen M, Schäffer A, Nupponen N, Pennanen S, Kallioniemi A, Kallioniemi O-P, Isola J: Genetic heterogeneity and clonal evolution underlying development of asynchronous metastasis in human breast cancer. Cancer Res 57: 1597-1604, 1997

Kuzel TM, Roenigk HJJr, Rosen ST: Mycosis Fungoides and the Sézary Syndrome: A review of pathogenesis, diagnosis, and therapy. J Clin Oncol 9: 1298-1313, 1991

Kwabi-Addo B, Giri D, Schmidt K, Podsypanina K, Parsons R, Greenberg N, Ittmann M: Haploinsufficiency of the Pten tumor suppressor gene promotes prostate cancer progression: Proc Natl Acad Sci USA. 98: 11563-11568, 2001.

König M, Reichel M, Marschalek R, Haas OA, Strehl S: A highly specific and sensitive fluorescence in situ hybridization assay for the detection of t(4;11)(q21;q23) and concurrent submicroscopic deletions in acute leukaemias. Br J Haematol 2002 116: 758-764, 2002

Laan M, Kallioniemi O-P, Hellsten E, Alitalo K, Peltonen L, Palotie A: Mechanically streched chromosomes as targets for high resolution FISH-mapping. Genome Res 5: 13-20, 1995

Laan M, Isosomppi J, Klockars T, Peltonen L, Palotie A: Utilization of FISH in positional cloning: An example on 13q22. Genome Res 6:1002-1012, 1996

Lambert WC: The thymus bypass model. A new hypothesis for the etiopathogenesis of mycosis fungoides and related disorders. Dermatologic Clinics: 305-310, 1994

Lambert WC, Everett MA: The nosology of parapsoriasis. J Am Acad Dermatol 5: 373-395, 1981

Langer PR, Waldrop AA, Ward DA, Enzymatic synthesis of biotin-labeled polynucleotides: novel nucleic acid affinity probes. Proc Natl Acad Sci USA 78: 6633-6637, 1981

Larsson L-I, Hougaard DM: Optimization of non-radioactive in situ hybridization: image analysis of varying pretreatment, hybridization and probe labelling conditions. Histochemistry 93.347-354, 1990

LaSalle J and Hafler DA: The Coexpression of CD45RA and CD45RO isoforms on T cells during the S/G_2/M stages of cell cycle. Cellular Immunology 138: 197-206, 1991

Lauritzen AF, Vejlsgaard GL, Hou-Jensen K, Ralfkiaer E: p53 protein expression In cutaneous T-cell lymphomas. Brit J Dermatol 133: 32-36, 1995

Lauw FN, Pajkrt D, Hack CE, Kurimoto M, van Deventer SJH, van der Poll T: Proinflammatory effects of IL-10 during human endotoxemia. J Immunol 165: 2783-2789, 2000

Lawrence JB, Singer RH: Quantitative analysis of in situ hybridization methods for the detection of actin gene expression. Nucleic Acids Res 13: 1777-1799, 1985

Leach FS et al Mutations in MutS homolog in hereditary nonpolyposis colorectal cancer. Cell 75: 1215-1225, 1993

Lee DS, Lee Y-S, Yun Y-s, Kim Y-R, Jeong SS, Lee YK, She CJ, Yoon SS, Shin HR, Kim Y, Cho HI: A study on the incidence of *ABL* gene deletion on derivative chromosome 9 in chronic myelogenous leukemia by interphase fluorescence in situ hybridization and its association with disease progression. Genes Chrom Cancer 37: 291-299, 2003

Lemke J, Claussen J, Michel S, Chudoba I, Mühlig P, Westermann M, Sperling K, Rubtsov N, Grummt U-W, Ullmann P, Kromeyer-Hauschild K, Liehr T, Claussen U: The DNA-based structure of human chromosome 5 in interphase. Am J Hum Genet 71: 1051-1059, 2002

Lengauer C, Riethman H, Cremer C: Painting of human chromosomes with probes generated from hybrid cell lines by PCR with Alu and L1 primers. Hum Genet 86: 1-6, 1990

Lengauer C, Green ED, Cremer T: Fluorescence in situ hybridization of YAC clones after Alu-PCR amplification. Genomics 13: 826-828, 1992

Lengauer C, Speicher MR, Popp S, Jauch A, Taniwaki M, Nagaraja R, Riethman HC, Donis-Keller H, D'Urso M, Schlessinger D, Cremer T: Chromosomal bar codes produced by multicolour fluorescence *in situ* hybridization with multiple YAC clones and whole chromosome painting probes. Hum Mol Genet 2: 505-512, 1993

Leroy S, Dubois S, Tenaud I, Chebassier N, Godard A, Jacques Y, Dréno B: Interleukin-15 expression in cutaneous T-cell lymphoma (mycosis fungoides and Sézary syndrome). Br J Dermatol 144: 1016-1023, 2001

Li G, Chooback L, Wolfe JT, Rook AH, Felix CA, Lessin SR, Salhany K.E: Overexpression of p53 protein in cutaneous T cell lymphoma : Relationship to large cell transformation and disease progression: J Invest Dermatol 110: 767-770, 1998

Lichter P, Cremer T, Borden J, Manuelidis L, Ward DC: Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. Hum Genet 80:224-234, 1988

Lichter P, Joos S, Bentz M, Lampel S: Comparative genomic hybridization: Uses and limitations. Seminars in Hematology 37: 348-357, 2000, review

Li, G, Salhany KE, Rook AH, Lessin SR: The pathogenesis of large cell transformation in cutaneous T-cell lymphoma is not assocaciated with t(2;5)(p23;q35) chromosomal translocation. J Cutan Pathol 24: 403-408, 1997

Li G, Chooback L, Wolfe JT, Rook AH, Felix CA, Lessin SR, Salhany K, E: Overexpression of p53 protein in cutaneous T cell lymphoma : Relationship to large cell transformation and disease progression. J Invest Dermatol 110: 767-770, 1998

Linder P, Stutz F: mRNA export: Travelling with DEAD box proteins. Curr Biol 11: R961-963, 2001

Limon J, Nedoszytko B, Brozek I, Hellmann A., Zajaczek S, Lubinski J, and Mrózek, K: Chromosome aberrations, spontaneous SCE, and growth kinetics in PHA-stimulated lymphocyte of five cases with Sézary syndrome. Cancer Genet Cytogenet 83 :75-81, 1995

Limpens J, Stad R, Vos C, de Vlaam C, de Jong D, van Ommen G-JB, Schuuring E, Kluin PM: Lymphoma-associated translocation t(14;18) in blood B cells of normal individuals. Blood 85: 2528-2536, 1995

Lingle WL, Barrett SL, Negron VC, D'Assoro BD, Boeneman K, Liu W, Whitehead CM, Reynolds C, Salisbury JL: Centrosome amplification drives chromosomal instability in breast tumor development. Proc Natl Acad Sci USA 99: 1978-1983, 2002

Lo Y-MD, Mehal WZ, Fleming KA: Rapid production of vector-free biotinylated probes using the polymerase chain reaction. Nucleic Acids Res 16: 8719, 1988

Loeb LA, Loeb KR, Anderson JP: Multiple mutations and cancer. Proc Natl Acad Sci USA 100: 776-781, 2003, review

Loeb LA: Mutator phenotype may be required for multistage carcinogenesis. Cancer Res 51: 3075-3079, 1991

Looijenga LHJ, Gillis AJM, van Putten WLJ, Oosterhuis JW: In situ numeric analysis of centromeric regions of chromosomes 1, 12, and 15 of seminomas, nonseminomatous germ cell tumors, and carcinoma in situ of human testis. Lab Invest 68: 211-219, 1993

Lopategui JR, Sun L-H, Chan JKC, Gaffey MJ, Frierson HF, Glackin C, Weiss LM: Low frequency association of the t(2;5(p23;q35) chromosomal translocation with CD30+ lymphomas from American and Asian patients. Am J pathol 146: 323-328, 1995

Lukowsky A, Muche JM, Sterry W, Audring H. Detection of expanded T cell clones in skin biopsy samples of patients with lichen sclerosus et atrophicus by T cell receptor-g polymerase chain reaction assays. J Invest Dermatol115 :254-59, 2000

MacKie RM: Cutaneous lymphomas and lymphocytic infiltrates. In Champion RH, Burton JL, Burns DA, Breathnach SM (eds.). Rook, Wilkinson, Ebling: Textbook of dermatology. 5th edn, Blackwell Science Ltd, Oxford, 1998, pp. 2373-2402

MacLeod RAF, Spitzer D, Bar-Am I, Sylvester JE, Kaufmann M, Wernich A, Drexler HG: Karyotypic dissection of Hodgkin's disease cell lines reveals ectopic subtelomeres and ribosomal DNA at sites of multiple jumping translocations and genomic amplification. Leukemia 14: 1803-1814, 2000

Macville M, Veldman T, Padilla-Nash H, Wangsa D, O'Brien P, Schröck E, Ried T: Spectral karyotyping, a 24-colour FISH technique for the identification of chromosomal rearrangements. Histochem Cell Biol 108: 299-305, 1997, review

Maes B, Vanhentenrijk V, Wlodarska I, Cools J, Peeters B, Marynen P, De Wolf-Peeters C: The NPM-ALK fusion genes can be detected in nin-neoplastic cells. Am J Path: 158: 2185-2193, 2001

Maes T, Barceló A, Buesa C: Neuron navigator: A human gene family with homology to *unc-53*, a cell guidance gene from *Caenorhabiditis elegans*. Genomics 80: 21-30, 2002.

Magee JA, Abdulkadir SA, Milbrandt J: Haploinsufficiency at the Nkx3.1 locus. A paradigm for stochastic, dosage-sensitive gene regulation during tumor initiation. Cancer Cell 3: 273-83, 2003

Manna SK, Aggarwal B: Interleukin-4 down-regulates both forms of tumor necrosis factor receptor and receptor-mediated apoptosis, NK- B, AP-1, and c-Jun N-terminal kinase. J Biol Chem 273: 33333-33341, 1998

Mao X, Lillington D.,Scarisbrick JJ, Mitchell T, Czepulkowski B, Russell-Jones R, Young BD, Whittaker SJ: Molecular cytogenetic analysis of cutaneous T-cell lymphomas: identification of common genetic alterations in Sézary syndrome and mycosis fungoides. Br J Dermatol 147: 464-475, 2002

Mao X, Orchard G, Lillington DM., Russell-Jones R, Young BD, Whittaker SJ: Amplification and overexpression of *JUNB* is associated with primary cutaneous T-cell lymphomas. Blood 101: 1513-1519, 2003a

Mao X, Lillington DM, Czepulkowski B, Russell-Jones R, Young BD, Whittaker S: Molecular cytogenetic characterization of Sézary syndrome. Genes Chrom Cancer 36: 250-260, 2003b

Marculescu R, Le T, Simon P, Jaeger U, Nadel B: V(D)J-mediated translocations in lymphoid neoplasms. A functional assessment of genomic instability by cryptic sites. J Exp Med 195: 85-98, 2002

Marculescu R, Vanura K, Le T, Simon P, Jäger U, Nadel B: Distinct t(7;9)(q34;q32) breakpoints in healthy individuals and individuals with T-ALL. Nature Genetics 33: 342-344, 2003

Marrogi AJ, Khan M.A, Vonderheid EC, Wood GS, McBurney E: p53 tumor suppressor gene mutations in transformed cutaneous T-cell lymphoma: A study of 12 cases. J Cutan Pathol 26: 369-378, 1999.

Martín-Subero J, Chudoba I, Harder L, Gesk S, Grote W, Novo FJ, Calasanz MJ, Siebert R: Multicolor FICTION. Expanding the possibilities of combined morphologic immunophenotyping, and genetic single cell analyses. Am J Pathol 161: 413-420, 2002

Martín-Subero JI, Knippschild U, Harder L, Barth TFE, Riemke J, Grohmann S, Gesk S, Höppner J, Möller P, Parwaresch RM, Siebert R: Segmental chromosomal aberrations and centrosome amplifications: pathogenetic mechanisms in Hodgkin and Reed-Sternberg cells of classical Hodgkin's lymphoma? Leukemia 17: 2214-2219, 2003

Maser RS, DePinho RA: Take care of your chromosomes lest cancer takes care of you. Cancer Cell 3: 4-6, review

Matikainen S, Saraneva T, Ronni T, Lehtonen A, Koskinen PJ, Julkunen I: Interferon-α activates multiple STAT proteins and upregulates proliferation-associated *IL-2Rα*, *c-myc*, and *pim-1* genes in human T Cells. Blood 93: 1980-1991, 1999

Matsumura I, Kitamura T, Wakao H, Tanaka H, Hashimoto K, Albanese C, Downward J, Pestell RG, Kanakura Y: Transcriptional regulation of the cyclin D1 promoter by STAT5: its involvement in cytokine-dependent growth of hematopoietic cells. EMBO J 18: 1367-1377, 1999

Matsue H, Bergstresser PR, Takashima A: Keratinocyte-derived IL-7 serves as a growth factor for dendritic epidermal T cells in mice. J Immunol 151: 6012-6019, 1993

Matsuno N, Osato M, Yamashita N, Yanagida M, nNnri T, Fukushima T, Motoji T, Kusumoto S, Towatari M, Suzuki R, Naoe T, Nishii K, Shigesada K, Ohno R, Mitsuya H, Ito Y, Asou N: Dual mutations in the AML1 and FLT3 genes are associated with leukemogenesis in acute myeloblastic leukemia of the M0 subtype. Leukemia 17: 2492-2499, 2003

McEvoy CRE, Morley AA, Firgaira FA: Evidence for whole chromosome 6 loss and duplication of the remaining chromosome in acute lymphoblastic leukemia. Genes Chrom Cancer 37: 321-325, 2003

McGregor JM, Crook T, Fraser-Andrews EA, Rozycka M, Crossland S, Brooks L, Whittaker SJ: Spectrum of p53 gene mutations suggests a possible role for ultraviolet radiation in the pathogenesis of advanced cutaneous lymphomas. J Invest Dermatol 112: 317-321, 1999.

McPherson JP, Lemmers B, Chahwan R, Pamidi A, Migon E, Matysiak-Zabloki E, Moynahan ME, Essers J, Hanada K, Poonepalli A, Sanchez-Sweatman O, Khokha R, Kanaar R, Jasin M, Hande MP, Razqallah H: Involvement of mammalian Mus81 in genome integrity and tumor suppression. Science 304: 1822-1826, 2004

Mecucci C, Louwagie A, Thomas J, Boogaerts M, Van Den Berghe: Cytogenetic studies in T-cell malignancies. Cancer genet Cytogenet 30: 63-71, 1988

Meeker AK, De Marzo AM: Recent advances in telomere biology: implications for human cancer. Curr Opin Oncol 16: 32-38, 2003, review

Mefford HC, Trask BJ: The complex structure and dynamic evolution of human subtelomeres. Nature Rev Genet 3: 91-102, 2002. Review

Mehregan DA, Gibson LE, Muller SA: Follicular mucinosis: Histopathologic review of 33 cases. Mayo Clin Proc 66: 387-390, 1991

Meltzer PS, Guan X-Y, Burgess A, rent JM: Rapid generation of regions specific probes by chromosome microdissection and their application. - Nat Genet 1: 24-28, 1992

Meraldi P, Honda R, Nigg EA: Aurora-A overexpression reveals tetraploidization as a major route to centrosome amplification in $p53^{-/-}$ cells. The EMBO Journal 21: 483-492, 2002

Merrill RA, Plum LA, Kaiser ME, Clagett-Dame M.: A mammalian homolog of unc-53 is regulated by all-trans retinoic acid in neuroblastoma cells and embryos. Proc Natl Acad Sci 99 :3422-3427, 2002.

Messier TL, O'Neill JP, Hou S-M, Nicklas JA, Finette BA: *In vivo* transposition mediated by V(D)J recombinase in human T lymphocytes. EMBO J: 22: 1381-1388, 2003

Michaelis C, Ciosk R, Nasmyth K: Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell 91: 35-45, 1997

Mitchell CD, Cowell JK: Predisposition to retinoblastoma due to a translocation within the 4.7R locus. Oncogene 4: 253-257, 1989

Mitchell TJ, Whittaker SJ, John S: Dysregulated expression of COOH-terminally truncated Stat5 and loss of IL2-inducible Stat5-dependent gene expression in Sezary syndrome. Cancer Res 63: 9048-9054, 2003

Mitelman F (ed): ISCN (1995). An International system for Human Cytogenetic Nomenclature. S. Karger, Basel, 1995

Moghal N, Sternberg PW: The epidermal growth factor system in *Caenorhabditis elegans.* Exp Cell Res 284:150-159, 2003

Mohrs M, Lacy DA, Locksley RM: Stat signals release activated naive Th cells from an anergic chekpoint. J Immunol 170: 1870-1876, 2003

Mohamadzadeh M, Takashima A, Dougherty I, Knop J, Bergstresser PR, Cruz Pdjr: Ultraviolet B radiation up-regulates the expression of IL-15 in human skin. J Immunol 155: 4492-4496, 1995

Moriggl R, Topham DJ, Teglund S, Sexl V, McKay C, Wang D, Hoffmeyer A, van Deursen J, Sangster MY, Bunting KD, Grosveld CG, Ihle JN: Stat 5 is required for IL-2-induced cell cycle progression of peripheral T cells. Immunity 10: 249-259, 1999

Moshous D, Pannetier C, de Chasseval R, le Deist F, Cavazzana-Calvo M, Romana S, Macintyre E, Canioni D, Brousse N, Fischer A, Casanova J-L, Villartay J-P: Partial T and B lymphocyte immunodeficiency and predisposition to lymphoma in patients with hypomorphic mutations in Artemis. J Clin Invest 111: 381-387, 2003

Muche JM, Lukowsky A, Asadullah K, Gellrich S, Sterry W: Demonstration of frequent occurrence of clonal T cells in the peripheral blood of patients with primary cutaneous T-cell lymphoma. Blood 90: 1636-42, 1997

Muche M, Lukowsky A, Heim J, Friedrich M, Audring H, Sterry W: Demonstration of frequent occurrence of clonal T cells in the peripheral blood but not in the skin of patients with small plaque parapsoriasis. Blood 94: 1409-17, 1999

Muche JM, Gellrich S, Sterry W: Treatment of cutaneous T-cell lymphomas. Semin Cutan Med Surg 19: 142-148, 2000a

Muche JM, Lukowsky A, Ahnhudt C, Gellrich S, Sterry W: Peripheral Blood T cell clonality in Mycosis Fungoides - an independent prognostic marker? J Invest Dermatol 115: 504-05, 2000b

Muche JM, Karenko L, Gellrich S, Karhu R, Kytölä S, Kähkönen M, Lukowsky A, Sterry W, Ranki, A., Cellular coincidence of clonal T-cell receptor rearrangements and complex clonal chromosomal aberrations – a hallmark of malignancy in cutaneous T-cell lymphoma. J Invest Dermatology 122: 574-578, 2004

Mullink H, Walboomers JMM, Tadema TM, Jansen DJ, Meijer CJLM: Combined immuno- and non-radioactive hybridocytochemistry on cells and tissue sections: Influence of fixation, enzyme pre-treatment, and choice of chromogen on detection of antigen and DNA sequences. J Histochem Cytochem 37: 603-609, 1989

Murasugi A, Wallace RB: Biotin-labeled oligonucleotides: entzymatic synthesis and use as hybridization probes. DNA 3: 269-277, 1984

Murphy KM, Reiner SL: The lineage decisions of helper T cells. Nat Rev Immunol 2: 933-944, 2002

Murray AW: Recycling the cell cycle: Cyclins revisited. Cell 116: 221-234, 2004, review

Musio A, Montagna C, Zambroni D, Indino E, Barbieri O, Citti L, Villa A, Ried T, Vezzoni P: Inhibition of BUB1 results in genomic instability and anchorage-independent growth of normal human fibroblasts. Cancer Res 63: 2855-2863, 2003

Mussman JG, Horn HF, Carroll PE, Okuda M, Tarapore P, Donehower LA, Fukasawa K: Synergistic induction of centrosome hyperamplification by loss of p53 and cyclin E overexpression. Oncogene 19: 1635-1646, 2000

Müller S, Rocchi M, Ferguson-Smith MA, Wienberg J: Toward a multicolor chromosome bar code for the entire human karyotype by fluorescence in situ hybridization. Hum Genet 100: 271-278, 1997

Müller S, O'Brien PCM, Ferguson-Smith MA, Wienberg J: Cross-species colour segmenting: A novel tool in human karyotype analysis. Cytometry 33: 445-452, 1998

Müller S, Neusser M, Wienberg J: Towards unlimited colors for fluorescence *in-situ* hybridization (FISH). Chrom Res 10: 223-232, 2002

Nakayama H: RecQ family helicases: roles as tumor suppressor proteins. Oncogene 21: 9008-9021, 2002, review

Navas I., C, Ortiz-Romero PL, Villuendas R, Martinez P, Garcia C, Gómez E, Rodriguez JL, Garcia D, Vanaclocha F, Iglesias L, Piris MA, Algara P: $p16^{INK4a}$ gene alterations are frequent in lesions of mycosis fungoides. Am J Pathol 156: 1565-1572, 2000.

Neben K, Giesecke C, Schweizer S, Ho AD, Krämer A: Centrosome aberrations in acute myeloid leukemia are correlated with cytogenetic risk profile. Blood 101: 289-291, 2003

Nederlof PM, van der Flier S, Wiegant J, Raap AK, Tanke HJ, Ploem JS, van der Ploeg M: Multiple fluoresence in situ hybridization. Cytometry 11: 126-131, 1990

Nevala H, Karenko L, Ranki A. Proapoptotic and antiapoptotic markers in cutaneous T-cell lymphoma skin infiltrates and in comparison with lymphomatoid papulosis. Brit. J Dermatol 145: 928-937, 2001

Neumann AA, Reddel RR: Telomere maintenance and cancer - look, no telomerase. Nat Rev Cancer 2: 879-884, 2002, review

Nigg EA: Centrosome aberrations: Cause or consequence of cancer progression? Nature Rev Cancer 2: 1-11, 2002, review

Nordgren A, Heyman M, Sahlén S, Schoumans J, Söderhäll J, Nordenskjöld M, Blennow E: Spectral karyotyping and interphase FISH reveal abnormalities not detected by conventional G-banding. Eur J Haematol 68: 31-34, 2002

Nowell PC, Hungerford DA: A minute chromosome in human chronic granulocytic leukemia. Science 132: 1497, 1960

Nowell PC, Finan JB, Vonderheid EC: Clonal Characteristics of Cutaneous T Cell Lymphomas: Cytogenetic Evidence from Blood, Lymph Nodes, and Skin. J Invest Dermatol 78:69-75, 1982

Nowell PC, Vonderheid EC, Besa I, Hoxie JA, Moreau L, Finan JB: The most common chromosome change in 86 chronic B cell or T cell tumors: a 14q32 translocation. Cancer Genet Cytogenet 19: 219-227, 1986

Nowak MA, Komarova NL, Sengupta A, Jallepalli PV, Shih I-M, Vogelstein B, Lengauer C: The role of chromosomal instability in tumor initiation. Proc Natl Acad Sci USA: 99: 16226-16231, 2002

Obe G, Pfeiffer P, Savage JRK, Johannes C, Goedecke W, Jeppesen P, Natarajan AT, Martínez-López W, Folle GA, Drets ME: Chromosomal aberrations: formation, identification and distribution. Mutation Research 504: 17-36, 2002, review

O'Hagan R, Chang S, Maser RS, Mohan R, Artandi SE, Chin L, DePinho RA: Telomere dysfunction provokes regional amplification and deletion in cancer genomes. Cancer Cell 2: 149-155, 2002

O'Shea JJ, Gadina M, Schreiber RD: Cytokine signaling in 2002: New surprises in the Jak/Stat pathway. Cell 109: S121-S131, 2002, review.

Oliveira AM, Fletcher JA: Translocation breakpoints in cancer. In: Encyclopedia of the human genome (ed.) David Cooper, pp. 1-11 (1066.1-1066.32), Nature Publishing Group, 2003 (ISBN-0-333-80386-8)

Opal SM, DePalo VA: Anti-inflammatory cytokines. Chest 117: 1162-1172, 2000, review

Padilla-Nash HM, Heselmeyer-Haddad K, Wangsa D, Zhang H, Ghadimi BM, Macville M, Augustus M, Schröck E, Hilgenfeld E, Ried T: Jumping translocations are common in solid tumor cell lines and result in recurrent fusions of whole chromosome arms. Genes Chromosome Cancer 30: 349-363, 2001

Pardue ML, Gall JG: Molecular hybridization of radioactive DNA to the DNA of cytological preparations. Proc Natl Acad Sci USA 64: 600-604, 1969

Parra I, Windle B: High resolution visual mapping of stretched DNA by fluorescent hybridization. Nat Genet 5: 17-21, 1993

Payne M, Grogan TM, Spier CM: A multidisciplinary approach to the diagnosis of cutaneous T-cell lymphomas. Ultrastruct Pathol 16: 99-125, 1992

Paz-y-Mino C, Pérez JC, Fiallo BF, Leone PE: A polymorphism in the *hMSH2* gene(gIVS12-6T>C) is associated with non-Hodgkin lymphomas. Cancer Genet Cytogenet 133: 29-33, 2002

Peeters PJ, Baker A, Goris I, Daneels G, Verhasselt P, Luyten WH, ML, Geysen J J, GH, Kass SU, Moechars DWE: Sensory deficits in mice hypomorphic for a mammalian homologue of unc-53. Dev Brain Res 150: 89-101, 2004

Pekarsky Y, Hallas C, Croce CM: Molecular basis of mature T-cell leukemia. JAMA 286: 2308-2314, 2001, review

Peltomäki P, Aaltonen L, Sistonen P, Pylkkänen L, Mecklin J-P, Järvinen H, Green JS, Jass JR, Weber JL, Leach FS, Petersen GM, Hamilton SR, de la Chapelle A, Vogelstein B: Genetic mapping of a locus predisposing to human colorectal cancer. Science 260: 810-812, 1993

Perez A, Solé F, Caballín R, Tarrida N, Woessner S: G-banding improvement for the MAC method. Cancer Genet Cytogenet 51: 41-44, 1991

Peris K, Cerroni L, Kerl H, Radaszkiewicz T, Chimenti S, Höfler H: Expression of c-myc in cutaneous lymphomas and pseudolymphomas. Dermatologica 183. 1-6, 1991

Perry PE, Thomson EJ: Immunogold labeling of metaphase cells. Cytogenet Cell Genet 41:121-125, 1986

Picker LJ, Michie SA, Rott LS, Butcher EC: A unique phenotype of skin associated lymphocyes in humans. Am J. Pathol 136: 1053-1068, 1990

Picker LJ, Treer JR, Ferguson-Darnell B, Collins PA, Buck D, Terstappen LWMM: Control of lymphocyte recirculation in man. I. Differential regulation of the peripheral lymph node homing receptor L-selectin on T cells during the virgin to memory cell transition. J Immunol 150:1105-1121, 1993a

Picker LJ, Treer JR, Ferguson-Darnell B, Collins PA, Buck D, Terstappen LWMM: Control of lymphocyte recirculation in man. I. Differential regulation of the cutaneous lymphocyte-associated antigen, a tissue-selective homing receptor for skin-homing T cells. J Immunol 150:1105-1121, 1993b

Pihan GA, Purohit A, Wallace J, Knecht H, Woda B, Quesenberry P, Doxey SJ: Centrosome defects and genetic instability in malignant tumors. Cancer Res 58: 3974-3985, 1998

Pihan GA, Doxey SJ: The mitotic machinery as a source of genetic instability in cancer. Semin Cancer Biol 9: 289-302, 1999, review

Pihan GA, Wallace J, Zhou Y, Doxsey SJ: Centrosome abnormalities and chromosome instability occur together in pre-invasive carcinomas. Cancer Res 63: 1398-1404, 2003

Pinkel D, Straume T, Gray JW: Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 83: 2934-2938, 1986

Pittaluga S, Wlodarska I, Pulford K, Campo E, Morris SW, Van den Berghe H, De Wolf-Peeters C: The monoclonal antibody ALK1 identifies a distinct morphological subtype of anaplastic large cell lymphoma associated with 2p23/ALK rearrangements. Am J Pathol 151: 343-51, 1997

Poddighe PJ, Ramaekers FCS, Hopman AHN: Interphase cytogenetics of tumours. J Pathol 166: 215-224, 1992

Price CM, Kanfer EJ, Colman SM, Westwood N, Barrett AJ, Greaves MF: Simultaneous genotypic and immunophenotypic analysis of interphase cells using dual-color fluorescence: A demonstration of lineage involvement in polycythemia vera. Blood 80: 1033-1038, 1992

Prince PR, Emond MJ, Monnat RJ Jr: Loss of Werner syndrome protein function promotes aberrant mitotic recombination. Gen Dev 15: 933-938, 2001

Qian J, Bostwick DG, Takahashi S, Borell TJ, Brown JA, Lieber MM, Jenkins RB: Comparison of fluorescence *in situ* hybridization analysis of isolated nuclei and routine histological sections from paraffin-embedded prostatic adenocarcinoma specimens. Am J Pathol 149:1193-1199, 1996

Qin J-Z, Dummer R, Burg G, Döbbeling U: Constitutive and interleukin-7/Interleukin-15 stimulated DNA binding of myc, Jun, and novel myc-like proteins in cutaneous T-cell lymphoma cells. Blood 93: 260-267, 1999

Qin J-Z, Kamarashev J, Zhang C-L, Dummer R, Burg G, Döbbeling U: Constitutive and interleukin-7- and Interleukin-15-stimulated DNA binding of STAT and novel factors in cutaneous T-cell lymphoma cells. J Invest Dermatol 117: 583-589, 2001

Raap AK, Dirks RW, Jiwa NM, Nederlof PM, van der Ploeg M: In situ hybridization with hapten-modified DNA probes. In Racz P, Haase AT, Gluckman JC (eds): Modern Pathology of AIDS and other retroviral infections. Basel, Karger, 1990, pp 17-28

Raap AK, Van de Rijke FM, Dirks RW, Sol CJ, Boom R, Van der Ploeg M: Bicolor fluorescence *in situ* hybridization to intron- and exon mRNA sequences. Exp Cell Res 197: 319-322, 1991

Rajagopalan H, Nowak MA, Vogelstein B, Lengauer C: The significance of unstable chromosomes in colorectal cancer. Nature Reviews Cancer 3: 695-701, 2003, review

Ralfkiaer E, O'Connor NTJ, Crick J, Wantzin GL, Mason DY: Genotypic analysis of cutaneous T-cell lymphomas. J Invest Dermatol 88: 762-765, 1987

Ranki A, Niemi K-M, Nieminen P, Krohn K: antibodies against retroviral core proteins in relation to disease outcome in patients with mycosis fungoides. Arch Derm Res 282: 532-538, 1990

Rao PH, Cigudosa JC, Ning Y, Calasanz MJ, Iida S, Tagawa S, Michaeli JM, Klein B, Dalla-Favera R, Jhanwar SC, Ried T, Chaganti RSK: Multicolor spectral karyotyping identifies new recurring breakpoints and translocations in multiple myeloma. Blood 92: 1743-1748, 1998

Raimondi SC, Zhou Y, Mathew S, Shurtleff SA, Sandlund JT, Rivers GK, Behm FG, Pui CH: Reassessment of the prognostic significance of hypodiploidy in pediatric pateints with acute lymphoblastic leukemia. Cancer 98: 2715-2722, 2003

Re D, Zander T, Diehl V, Wolf J: Genetic instability in Hodgkin's lymphoma. Symposium article. Annals of Oncology 13 (Supplement 1): 19-22, 2002, review

Rego EM, Pandolfi PP: Reciprocal products of chromosomal translocations in human cancer pathogenesis: key players or innocent bystanders. TRENDS Mol Med 8: 396-405, 2002, review

Renault B, Lieman J, Ward D, Krauter K, Kucherlapati R: Localization of the human Achaete-Scute homolog gene (ASCL1) distal to phenylalanine hydroxylase (PAH) and proximal to tumor Rejection Antigene (TRA1) on chromosome 12q22-q23. Genomics 30: 81-83, 1995

Reiss Y, proudfoot AE, Power CA, Campbell JJ, Butcher EC: CC chemokine receptor (CCR) 4 and the CCR10 ligand cutaneous T cell-attracting chemokine (CTACK) in lymphocyte trafficking to inflamed skin. J Exp Med 194: 1541-1547, 2001

Ried T, Baldini A, Rand TC, Ward DC: Simultaneous visualization of seven different DNA probes by in situ hybridization using combinatorial fluorescence and digital imaging microscopy. Proc Natl Acad Sci USA 89: 1388-1392, 1992

Rigby PWJ, Dieckmann M, Rhodes C, Berg P: Labeling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase I. J Mol Biol 113: 237-251, 1977

Ro YS, Cooper PN, Lee JA, Quinn AG, Harrison D, Lane D, Horne CHW, Rees JL, Angus B: p53 protein expression in benign and malignant skin tumours. Br J Dermatol 128: 237-241, 1993

Rogers J: Oncogene chromosome breakpoints and alu sequences. Nature 317: 559- , 1985

Rothenberg EV, Dionne CJ: Lineage plasticity and commitment in T-cell development. Immunol Rev 187: 96-115, 2002

Roupe G, Sandström MH, Kjellström C: PUVA in early mycosis fungoides may give long-term remission and delay extracutaneous spread. Acta Derm Venereol (Stockh) 76: 475-478, 1996

Rowley JD: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature 243: 290-293, 1973

Rowley JD, Reshmi S, Carlson K, Roulston D: Spectral karyotype analysis of T-cell acute leukemia. Blood 93: 2038-2042, 1999

Saed G, Fivenson DP, Naidu Y, Nickoloff BJ: Mycosis fungoides exhibits a Th1-type cell-mediated cytokine profile whereas Sezary syndrome expresses a Th2-type profile. J Invest Dermatol 103: 29-33, 1994

Sallinen S-L, Sallinen P, Ahlstedt-Soini M, Haapasalo H, Helin H, Isola J, Karhu R: Arm-specific multicolor fluorescence in situ hybridization reveals widespread chromosomal instability in glioma cells lines. Cancer Genet Cytogenet 144: 52-60, 2003

Sambrook J, Fritsch EF, Maniatis T: Molecular cloning: a laboratory manual, 2d ed. Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, 1989
Sander CA, Flaig MJ, Jaffe ES: Cutaneous manifestations of lymphoma: a clinical guide based on the WHO classification. Clin Lymphoma 2: 86-100, 2001

Santucci M, Biggeri A, Feller AC, Burg G: Accuracy, concordance, and reproducibility of histologic diagnosis in cutaneous T-cell lymphoma. An EORTC cutaneous lymphoma project group study. Arch Dermatol 136: 497-502, 2000

Saracoglu K, Brown J, Kearlney L, Uhrig S, Azofeifa J, Fauth C, Speicher MR, Eils R: New concepts to improve resolution and sensitivity of molecular cytogenetic diagnostics by multicolor fluorescence in situ hybridization. Cytometry 44: 7-15, 2001

Sarasin A: An overview of the mechanisms of mutagenesis and carcinogenesis. Mut Res 544: 99-106, 2003, review

Sarris AH, Luthra R, Papadimitracopoulou V, Waasdorp M, Dimopoulos MA, McBride JA, Cabanillas F, Duvic M, Deisseroth A, Morris SW, Pugh WC: Amplification of genomic DNA demonstrates the presence of the t(2;5)(p23;q35) in anaplastic large cell lymphoma, but not in other non-Hodgkin's lymphomas, Hodgkin's disease, or lymphomatoid papulosis. Blood 88: 1771- 1779, 1996

Sawabe T, Shiokawa S, Sugisaki K, Tsuda T, Yamamoto K: Accumulation of common clonal T cells in multiple lesions of sarcoidosis. Mol Med 9 :793-802, 2000

Sawyer JR, Lukacs JL, Munshi N, Desikan KR, Singhai S, Mehta J, Siegel D, Shaughnessy J, Barlogie B: identification of new nonrandom translocations in multiple myeloma with multicolor spectral karyotyping. Blood 92: 4269-4278, 1998

Scandura JM, Boccuni P, Cammenga J, Nimer SD: Transcription factor fusions in acute leukemia: variations on a theme. Oncogene 21: 3422-3444, 2002

Scarisbrick JJ, Woolford AJ, Russell-Jones R, Whittaker SJ: Loss of heterozygosity on 10q and microsatellite instability in advanced stages of primary cutaneous T-cell lymphoma and possible association with homozygous deletion of PTEN. Blood 95 :2937-2942, 2000

Scarisbrick, JJ, Woolford AJ, Calonje E, Photiou A, Ferreira S, Orchard G, Russell-Jones R Whittaker SJ: Frequent abnormalities of the p15 and p16 genes in mycosis fungoides and Sezary syndrome. J Invest Dermatol 118: 493-499, 2002

Scheijen B, Griffin JD: tyrosine kinase oncogenes in normal hematopoiesis and hematological disease.Oncogene 21: 3314-3333, 2002, review

Schlegelberger B, Himmler A, Bartels H, Kuse R, Sterry W, Grote W: Recurrent chromosome abnormalities in peripheral T-cell lymphomas. Cancer Genet Cytogenet 78:15-22, 1994a

Schlegelberger B, Weber-Matthiesen K, Himmler A, Bartels H, Sonnen R, Kuse R, feller AC, Grote W: Cytogenetic findings and results of combined immunophenotyping and karyotyping in Hodgkin's disease. Leukemia 8: 72-80, 1994b

Schmidt M, Stolzmann M, Neumann E: Cytogenetic findings in a case of Sézary syndrome. Cancer Genet Cytogenet 16: 117-121, 1985

Schmidt-Kittler O, Ragg T, Daskalakis A, Granzow M, Ahr A, Blankenstein TFJ, Kaufmann M, Diebold J, Arnholdt H, Müller P, Bischoff J, Harich D, Schlimik G, Riethmûller G, Eils R, Klein CA: Proc Natl Acad Sci USA 100: 7737-7742, 2003

Schmitz GG, Walter T, Seibl R, Kessler C: Nonradioactive labeling of oligonucleotides *in vitro* with the hapten digoxigenin by tailing with terminal transferase. Anal Biochem 192: 222-231, 1991

Schoenmakers EFPM, Geurts JMW, Kools PFJ, Mols R, Huysmans C, Bullerdiek J, Van den Berghe, H, Van de Ven WJM: A 6-Mb yeast artificial chromosome contig and long-range physical map encompassing the region on chromosome 12q15 frequently rearranged in a variety of benign solid tumors. Genomics 29: 665-678, 1995

Schreiber S, Fedorak RN, Nielsen OH, Wild G, Williams CN, Nikolaus S, Jacyna M, Lashner BA, Gangl A, Rutgeerts P, Isaacs K, van Deventer SJH, Koningsberger JC, Cohard M, LeBeaut A, Hanauer SB: Safety and efficacy of recombinant human interleukin 10 in chronic and active Crohn's disease. Gastroenterology 119: 1461-1472, 2000

Schröck E, du Manoir S, Veldman T, Schoell B, Wienberg J, Ferguson-Smith MA, Ning Y, Ledbetter DH, Bar-Am I, Soenksen D, Garini Y, Ried T: Multicolor Spectral karyotyping of human chromosomes. Science: 273: 494-497, 1996

Schröck E, Padilla-Nash H: Spectral karyotyping and multicolor fluorescence in situ hybridization reveal new tumor-specific chromosomal aberrations. Seminars in Hematology 37:334-347, 2000

Schultz VP, Zakian VA.: The saccharomyces PIF1 DNA helicase inhibits telomere elongation and de novo telomere formation. Cell 76: 145-155, 1994.

Schwaenen C, Wessendorf S, Kestler HA, Döhner H, Lichter P, Bentz M: DNA microarray analysis in malignant lymphoma. Ann Hematol 82: 323-332, 2003. Review

Schön MP, Zollner TM, Boehncke W-H: The molecular basis of lymphocyte recruitment to the skin: Clues for pathogenesis and selective therapies of inflammatory disorders. J Invest Dermatol 121: 951-962, 2003. Review.

Schmid J, Weissmann CM: Introduction of mRNA for a serine protease and a beta-tromboglobulin-like protein in mitogen-stimulated human leukocytes. J Immunol 1: 250-256, 1987

Sen S: Aneuploidy and cancer. Curr Opin Oncol 12: 82-88, 2000, review

Senger G, Jones TA, Fidlerová H, Sanséau P, Trowsdale J, Duff M, Sheer D: Reselased chromatin: linearized DNA for high resolution fluorescence *in situ* hybridization. Hum Mol Genet 3: 1275-1280, 1994

Sengupta S, Linke SP, Pedeux R, Yang Q, Farnsworth J, Garfield SH, Valerie K, Shay JW, Ellis NA, Wasylyk B, Harris CC: BLM helicase-dependent transport of p53 to sites of stalled DNA replication forks modulates homologous recombination. EMBO J 22: 1210-1222, 2003

Sentis HJ, Willemze R, Scheffer E: Alopecia mucinosa progressing into mycosis funoides. A long term follow-up study of two patients. Am J Dermatopathol 10: 478-486, 1988

Sézary A, Bouvrain Y: Erythrodermie avec présence de cellules monstrueuses dans le derme et le sang circulant. Bull Soc Fr Dermatol Syph 45: 254-260, 1938

Shapiro PE, Pinto FJ: The histologic spectrum of mycosis fungoides/Sézary syndrome (Cutaneous T-cell lymphoma) Am J Surg Pathol 18(7): 645-667, 1994

Shapiro PE, Warburton D, Berger CL, Edelson RL: Clonal chromosomal abnormalities in cutaneous T-cell lymphoma. Cancer Genet Cytogenet 28:267-276, 1987

Sharpless NE, DePinho RA: Telomeres, stem cells, senescence and cancer. J Clin Invest 113: 160-168, 2004, review

Sharpless NE, Ferguson DO, O'Hagan R, Castrillon DH, Lee C, Paraskevi VA, Alson S, Fleming J, Morton C, Frank K, Chin L, Alt FW, DePinho RA: Impaired nonhomologous end-joining provokes soft tissue sarcomas harboring chromosomal translocations, amplifications, and deletions. Mol Cell 8: 1187-1196, 2001

Sherr CJ: Mammalian G_1 cyclins. Cell 73: 1059-1065, 1993, review

Sherr CJ, Roberts JM: CDK inhibitors:positive and negative regulators of G1-phase progression. Gen Devel 13: 1501-1512, 1999, review

Sherr CJ: Principles of tumor suppression. Cell 116: 235-246, 2004, review

Shiloh Y: ATM and related protein kinases: safeguarding genome integrity. Nat Rev Cancer 3: 155-168, 2003, review

Shiota M, Nakamura S, Ichinohasama R, Abe M, Akagi T, Takeshita M, Mori N, Fujimoto J, Miyauchi J, Mikata A, Nanba K, Takami Y, Yamabe H, Takano Y, Izumo T, Nagatani T, Mohri N, Nasu K, Satoh H, Katano H, Fujimoto J, Yamamoto T, Mori S: Anaplastic large cell lymphomas expressing the novel chimeric protein $p80^{NPM/ALK}$: A distinct clinocopathologic entity. Blood 86: 1954-1960, 1995

Sieber OM, Heinimann K, Tomlinson IPM: Genomic instability - the engine of tumorigenesis? Nat Rev Cancer 3: 701-708, 2003

Siegel R S, Pandolfino T, Guitart J, Rosen S, Kuzel T M: Primary cutaneous T-cell lymphoma: review and current concepts. J Clin Oncol 18: 2908-2925, 2000

Simon M, Flaig MJ, Kind P, Sander CA, Kaudewitz P: Large plaque parapsoriasis: clinical and genotypic correlations. J Cutan Pathol 27 :57-60, 2000

Sinclair P, Green A, Grace C, Nacheva E: Improved sensitivity of BCR-ABL detection: a triple probe three-color fluorescence in situ hybridization system. Blood 90: 1395-1402, 1997

Sinclair PB, Nacheva EP, Laversha M et al Large deletions at the t(9;22) breakpoint are common and may identify a poor prognosis subgroup of patients with chronic myeloid leukaemia. Blood 95: 738-744, 2000

Sinibaldi D, Wharton W, Turkson J, Bowman T, Pledger WJ, Jove R: Induction of $p21^{WAF1/CIP1}$ and cyclin D1 expression by the Src oncoprotein in mouse fibroblasts: role of activated STAT3 signaling. Oncogene 19: 5419-5427, 2000

Solinas-Toldo S, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, Döhner H, Cremer T, Lichter P: Matrix-based comparative genomic hybridization: Biochips to screen for genomic imbalances. Genes Chrom cancer 20. 399-407, 1977

Speel EJM, Herbergs J, Ramaekers, FCS, Hopman AHN: Combined immunocytochemistry and fluorescence in situ hybridization for simultaneous tricolor detection of cell cycle, genomic, and phenotypic parameters of tumor cells. J Histochem Cytochem 42: 961-966, 1994a

Speel EJM, Jansen MPHM, Ramaekers, FCS, Hopman AHN: A novel triple-color detection procedure for brightfield microscopy, combining in situ hybridization with immunocytochemistry. J Histochem Cytochem 42: 1299-1307, 1994b

Speicher MR, Gwyn Ballard S, Ward DC: Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat Genet 12: 368-375, 1996

Spit H: Development of $\alpha\beta$ T cells in the human thymus. Nature reviews Immunol 2: 760-772, 2002, review

Srivastava M, Montagna C, Leighton X, Glasman M, Naga S, Eidelman O, Ried T, Pollard HB: Haploinssufficiency of Anxt tumor suppressor gene and consequent genomic instability promotes tumorigenesis in the Anx7(+/-) mouse. Proc Natl Acad Sci ISA: 100: 14287-14292, 2003

Steinemann D, Gesk S, Zhang Y, Harder L, Pilarsky C, Hinzmann B, Martin-Subero JI, Calasanz, M.J, Mungall A, Rosenthal A, Siebert R, Schlegelberger B: Identification of candidate tumor-suppressor genes in 6q27 by combined deletion mapping and electronic profiling in lymphoid neoplasms. Genes Chrom Cancer, 37: 421-426, 2003.

Steinhaeuser U, Starke H, Nietzel A, Lindenau J, Ullman P, Claussen, Liehr T: Suspension (S)-FISH, a new technique for interphase nuclei. J Histochem Cytochem 50: 1697-1698, 2002

Stellwagen AE, Haimberger ZW, Veatch JR, Gottschling DE: Ku interacts with telomerase RNA to promote telomere addition at native and broken chromosome ends. Gen Dev 17: 2384-2395, 2003

Stenman S, Rosenqvist M, Ringertz NR: Preparation and spread of unfixed metaphase chromosomes for immunofluorescence staining of nuclear antigens. Exp Cell Res 90: 87-94, 1975

Stewart SA, Hahn WC, O'Connor BF, Banner EN, Lundberg AS, Modha P, Mizuno H, Brooks MW, Fleming M, Zimonjic DB, Popescu NC, Weinberg RA: Telomerase contributes to tumorigenesis by a telomere length-independent mechanism. Proc Natl Acad Sci USA 99: 12606-12611, 2002

Stilgenbauer S, Bullinger L, Lichter P, Döhner H, German CLL Study Group CLLSG: Genetics of chronic lymphocytic leukemia: genomic aberrations and V_H gene mutation status in pathogenesis and clinical course. Leukemia 16: 993-1007, 2002, review

Strehl S, Ambros PF: Fluorescence in situ hybridization combined with immunohistochemistry or highly sensitive detection of chromosome 1 aberrations in neuroblastoma. Cytogenet Cell Genet 63: 24-28, 1993

Stringham E, Pujol N, Vandekerckhove J, Bogaert T. unc53 controls longitudinal migration in C. elegans. Development 129:3367-3379, 2002

Su M-w, Dorocicz, I, Dragowska WH, Ho V, Li G, Voss N, Gascoyne R, Zhou Y: Aberrant expression of T-plastin in Sezary cells. Cancer Res 63: 7122-7127, 2003

Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman GC, Glimcher LH: A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell 100: 655-669, 2000

Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, Nakanishi K, Yoshida N, Kishimoto T, Akira S: Essential role of Stat6 in IL-4 signalling. Nature 380: 627-630, 1996

Tanaka K, Kamada N: Segmental jumping translocation in leukemia and lymphoma with a highly complex karyotype. Leukemia Lymphoma 29: 563-575, 1998

Tanke HJ, De Haas RR, Sagner G, Ganser M, van Gijlswijk RPM: Use of platinum coproporphyrin and delayed luminescence imaging to extend the number of targets FISH karyotyping. Cytometry 33: 453-459, 1998

Tanke HJ, Wiegant J, van Gijlswijk RPM, Bezrookove V, Pattenier H, Heetebrij RJ, Talman EG, Raap AK, Vrolijk J: New strategy for multi-colour fluorescence *in situ* hybridisation: COBRA: COmbined Binary RAtio labelling. Eur J Hum Genet 7: 2-11, 1999

Tatsuka M, Katayama H, Ota T, Tanaka T, Odashima S, Suzuki F, Terada Y: Multinuclearity and increased ploidy caused by overexpression of the aurora- and Ip11-like midbody-associated protein mitotic kinase in human cancer cells. Cancer Res 58: 4811-4816, 1998

Teerenhovi L, Knuutila S, Ekblom M, Rossi L, Borgström GH, Tallman JK, Andersson L, de la Chapelle A: A method for simultaneous study of the karyotype, morphology, and immunologic phenotype of mitotic cells in hematologic malignancies. Blood 64: 1116-1122, 1984

Teixeira MR, Micci F, Dietrich CU, Heim S: Detailed genome-wide screening for inter- and intrachromosomal abnormalities by sequential G-banding and Rx-FISH color banding of the same metaphase cells. Cancer Genet Cytogenet 119: 94-101, 2000

Thangavelu M, Finn WG, Yelawarthi KK, Roenigk HH jr, Samuelson E, Peterson LA, Kuzel TM, Rosen ST : Recurring structural chromosome abnormalities in peripheral blood lymphocytes of patients with mycosis fungoides/Sézary syndrome. Blood 89: 3371-3377, 1997

Thestrup-Pedersen K, Kaltoft K: Genotraumatic T cells and cutaneous T-cell lymphoma. A causal relationship? Arch Dermatol Res 287:97-101, 1994

Thiagalingam S, Laken S, Willson JKV, Markowitz SD, Kinzler KW, Vogelstein B, Lengauer C: Mechanisms underlying losses of heterozygosity in human colorectal cancers. Proc Natl Acad Sci USA: 2698-2702, 2001

Thompson CT, LeBoit PE, Nederlof PM, Gray JW: Thick-section fluorescence *in situ* hybridization on formalin-fixed paraffin-embedded archival tissue provides a histogenetic profile. Am J. Pathol 144:237-243, 1994

Tiainen M, Popp S, Parlier V, Emmerich P, Bellomo MJ, Ruutu T, Cremer T, Knuutila S: Chromosomal in situ suppression hybridization of immunologically classified mitotic cells in hematologic malignancies. Genes Chrom Cancer 4: 135-140, 1992

Tok J, Szabolcs MJ, Silvers DN, Zhong J, Matsushima AY: Detection of clonal T-cell receptor gamma chain gene rearrangements by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR/DGGE) in archival specimens from patients with early cutaneous T-cell lymphoma: correlation with histologic findings with PCR/DGGE. J Am Acad Dermatol 38: 453-460, 1998

Tosca A, Linandropoulos S, Malliri A, Hatziolou E, Nicolaidou A, Spandidos DA: Implication of the RAS and MYC oncoproteins in the pathogenesis of mycosis fungoides. Anticancer Res 11: 1433-1438, 1991

Trask BJ: Fluorescence in situ hybridization: applications in cytogenetics and gene mapping. Trends Genet 7: 149-154, 1991, review

Trotter MJ, Whittaker SJ, Orchard GE, Smith NP: Cutaneous histopathology of Sézary syndrome: a study of 41 cases with proven circulating T-cell clone. J Cutan pathol 24: 286-291, 1997

Trumper L, Pfreundschuh M, Bonin FV, Daus H: Detection of the t(2;5)-associated NPM/ALK fusion cDNA in peripheral blood cells of healthy individuals. Br J Haematol 103: 1138-1134, 1998

Uhrig S, Schuffenhauer S, Fauth C, Wirtz A, Daumer-Haas C, Apacik C, Cohen M, Müller-Navia J, Cremer T, Murken J, Speicher MR: Multiplex-FISH for pre- and postnatal diagnostic applications. Am J Hum Genet 65: 448-462, 1999

Unger E: In situ hybridization: principles and practice. Clin Immunol 10: 120-126, 1990, review

Urban A, Asadullah K, Friedrich M, Volk H-D, Sterry W, Döcke W-D: CD45RA-expressing malignant T-helper cells in Sezary-syndrome (SS). 29th annual meeting of the European Society for Dermatological Research, Montpellier 22-25. 9. 1999. J Invest Dermatol 113:474, 1999 (abstr.)

Van Dekken H, Bosman FT, Teijgeman R, Vissers CJ, Tersteeg TA, Kerstens HMJ, Voojis GP, Verhofstad AAJ: Identification of numerical chromosome aberrations in archival tumours by in situ hybridization to routine paraffin sections: evaluation of 23 phaeochromocytomas. J Pathol 171: 161-171, 1993

van den Brink W, van der Loos C, Volkers H, Lauwen R, van den Berg F, Houthoff H-J, Das PK: Combined β-galactosidase and immunogold/silver staining for immunohistochemistry and DNA in situ hybridization. J Histochem Cytochem 38: 325-329, 1990

van der Luijt, RB, Tops CM J, Khan PM., van der Klift HM, Breukel C, van Leeuwen-Cornelisse ISJ, Dauwerse HG, Beverstock GC, van Noort E, Snel P, Slors FJM., Vasen, H.FA.,Fodde, R: Molecular, cytogenetic, and phenotypic studies of a constitutional reciprocal translocation t(5;10)(q22;q25) responsible for familial adenomatous polyposis in a Dutch pedigree. Genes Chromosomes Cancer13: 192-202, 1995

van Doorn R, Scheffer E, Willemze R: Follicular mycosis fungoides, a distinct disease entity with or without associated follicular mucinosis. A clinopathologic and follow-up study of 51 patients. Arch Dermatol 138: 191-198, 2002

van Haselen CW, Vermeer MH, Toonstra J van der Putte SC, Mulder PG, van Vloten WA, Willemze R: P53 and bcl-2 expression do not correlate with prognosis in primary cutaneous large T-cell lymphomas. J Cutan Pathol 24 :462-67, 1997

van Vloten WA, Pet EA, Geraedts: Chromosome studies in mycosis fungoides. Br J Dermatol 102: 507-513, 1980

Veelken H, Wood GS, Sklar J: Molecular staging of cutaneous T-cell lymphoma: Evidence for systemic involvement in early disease. J Invest Dermatol 104: 889-894, 1995

Vega F, Orduz R, Medeiros LJ: Chromosomal translocations and their role in the pathogenesis of non-Hodgkin's lymphomas. Pathology 34: 397-409, 2002

Vega F, Medeiros LJ: Chromosomal translocations involved in non-Hodgkin lymphomas.Arch Pathol Lab Med 127: 1148-1160, 2003, review

Veldman T, Vignon C, Schröck E, Rowley JD, Ried T: Hidden chromosomal abnormalities in haematological malignancies detected by multicolour spectral karyotyping. Nat Genet 15: 406-410, 1997

Verma, RS, Babu, A: Human Chromosomes. Manual of basic techniques, 1st ed. Pergamon Press, New York, 1989, p 6, p 47

Vermeer MH, Geelen FAMJ, Kummer JA, Meijer CJLM, Willemze R: Expression of cytotoxic proteins by neoplastic T cells in mycosis fungoides increases with progression from plaque stage to tumor stage disease. Am J Pathol 154:1203-1210, 1999

Viegas-Pequinot E, Dutrillaux B, Magdelenat H, Coppey-Moisan M: Mapping of single-copy DNA sequences on human chromosomes by in situ hybridization with biotinylated probes: Enhancement of detection sensitivity by intensified-fluorescence digital-imaging microscopy. Proc Natl Acad Sci USA 86: 582-586, 1989

Visakorpi T, Kallioniemi AH, Syvänen AC, Hyytinen ER, Karhu R, Tammela T, Isola JJ, Kallioniemi O-P: Genetic changes in primary and recurrent prostate cancer by comparative genomic hybridization. Cancer Res 55: 342-347, 1995

Vowels BR, Cassin M, Vonderheid EC, Rook AH: Aberrant cytokine production by Sezary syndrome patients: Cytokine secretion pattern resembles murine Th2 cell. J Invest Dermatol 99:90-94, 1992

Väkevä L, Pukkala E, Ranki A: Increased risk of secondary cancers in patients with primary cutaneous T cell lymphoma. J Invest. Dermatol 115: 62-65, 2000

Väkevä L, Sarna S, Vaalasti A, Pukkala E, Kariniemi A-L, Ranki A: A retrospective study on the evolution of parapsoriasis en plaques into mycosis fungoides. Acta Dermato-Venereologica, in press

Walt H, Emmerich P, Cremer T, Hoffman M-C, Bannwart F: Supernumerary chromosome 1 in interphase nuclei of atypical germ cells in paraffin-embedded human seminiferous tubules. Lab Invest 61: 527-531, 1989

Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S, Bhattacharya R, Gabrilovich D, Heller R, Coppola D, Dalton W, Jove R, Rardoll D, Yu Hua: Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. Nat Med 10: 48-54, 2004

Watson C, Whittaker S, Smith N, Vora AJ, Dumonde DC, Brown KA: IL-6 acts on endothelial cells to preferentially increase their adherence for lymphocytes. Clin Exp Immunol 105: 112-119, 1996

Weber-Matthiesen K, Winkemann M, Müller-Hermelink A, Schlegelberger B, Grote W: Simultaneous fluorescence immunophenotyping and interphase cytogenetics: A contribution to the characterization of tumor cells. J Histochem Cytochem 40: 171-175, 1992

Weber-Matthiesen K, Pressl S, Schlegelberger B, Grote W: Combined immunophentoyping and interphase cytogenetics on cryostat sections by the new FICTION method. Leukemia 7: 646-649, 1993a

Weber-Matthiesen K, Deerberg J, Müller-Hermelink A, Schlegelberger B, Grote W: Rapid immunophenotypic characterization of chromosomally aberrant cells by the new fiction method. Cytogenet Cell Genet 63: 123-125, 1993b

Weber-Matthiesen K, Deerberg J, Poetsch M, Grote W, Schlegelberger B : Numerical chromosome aberrations are present within the CD30+ Hodgkin and Reed-Sternberg Cells in 100% of analyzed cases of Hodgkin's disease. Blood 86: 1464-1468, 1995

Weber-Nordt RM, Egen C, Wehinger J, Ludwig W, Gouilleux-Gruart V, Mertelsmann R, Finke J: Constitutive activation of STAT proteins in primary lymphoid and myeloid leukemia cells and Epstein-Barr Virus (EBV)-related lymphoma cell lines. Blood 88: 809-816, 1996

Weinstock MA, Horm JW: Mycosis fungoides in the United States. Increasing incidence and descriptive epidemiology. JAMA.260: 42-46, 1988

Weinstock M, Gardstein B: Twenty-year trends in the reported incidence of mycosis fungoides and associated mortality. Am J Publ Health 89: 1240-1244, 1999

Wellmann A, Otsuki T, Vogelbruch M, Clark HM, Jaffe ES, Raffeld M: Analysis of the t(2;5)(p23;q35) translocation by reverse transcription-polymerase chain reaction in CD30+ anaplastic large-cell lymphomas, in other non-Hodgkin's lymphomas of the T-cell phenotype, and in Hodgkin's disease. Blood 86: 2321-2328, 1995

Wen X, Lin HH, Shih H-M, Kung H-J, Ann DK: Kinase activation of the non-receptor tyrosine kinase Etk/BMX alone is sufficient to transactivate STAT-mediated gene expression in salivary and lung epithelial cells. J Biol Chem 274: 38204-38210, 1999

Werner M, Wilkens L, Aubele M, Nolte M, Zitzelsberger H, Komminoth P: Interphase cytogenetics in pathology: principles, methods, and applications of fluorescence in situ hybridization (FISH). Histochem Cell Biol 108: 381-390, 1997

Wessman M, Knuutila S: A method for the determination of cell morphology, immunologic phenotype and numerical chromosomal abnormalities on the same mitotic or interphase cancer cell. Genet (Life Sci Adv) 7: 127-130, 1988

Whang-Peng, J, Bunn PA, Knutsen T, Matthews MJ, Schechter G, Minna JD: Clinical Implications of Cytogenetic Studies in Cutaneous T-Cell Lymphoma (CTCL). Cancer 50: 1539-1553, 1982

Whittaker SJ, Smith NP, Russell Jones R, Luzzatto L: Analysis of β,γ, and δ T-cell receptor genes in mycosis fungoides and Sézary syndrome. Cancer 68: 1572-82, 1991

Whittaker SJ, Marsden JR, Spittle M, Russell Jones R: Joint British association of dermatologist and UK cutaneous lymphoma group guidelines for the management of primary cutaneous T-cell lymphomas. Br J Dermatol 149: 1095-1107, 2003

Wiegant J, Kalle W, Mullenders L, Brookes S, Hoovers JMN, Dauwerse JG, van Ommen GJB, Raap AK: High-resolution *in situ* hybridization using DNA halo preparations. Hum Mol Genet 1: 587-591, 1992

Wilkinson PC, Liew FY: Chemoattraction of human blood T-lymphocytes by interleukin-15. J Exp Med 181: 1255-1259, 1995

Willemze R: Recent development in the early diagnosis of mycosis fungoides and Sézary syndrome. Eur J Cancer Clin Oncol 23: 1581-1583, 1987, review

Willemze R, Kerl H, Sterry W, Berti E, Cerroni L, Chimenti S, Diaz-Perez JL, Geerts ML, Gos M, Knobler R, Ralfkiaer E, Santucci M, Smith N, Wechsler J, van Vloten WA: EORTC classification for primary cutaneous lymphomas: A proposal from the cutaneous lymphoma study group of the European Organization for Research and Treatment of Cancer. Blood 90:354-371, 1997

Wolfe JT, Chooback L, Finn DT, Jaworsky C, Rook AH, Lessin SR: Large-cell transformation following detection of minimal residual disease in cutaneous T-cell lymphoma: Molecular and in situ analysis of a single neoplastic T-cell clone expressing the identical T-cell receptor. J Clin Oncol 13: 1751-1757, 1995

Wood, GS, Tung RM, Haeffner AC, Crooks CF, Liao S, Orozco R, Veelken H, Kadin ME, Koh H, Heald P, Barnhill RL, Sklar J: Detection of clonal T-cell receptor gene rearrangements in early Mycosis Fungoides/Sézary syndrome by polymerase chain reaction and denaturing gradient gel electrophoresis (PCR/DGGE). J Invest Dermatol 103 : 34-41, 1994

Wood GS, Haeffner A, Dummer R, Crooks CF: Molecular biology of cutaneous T-cell lymphoma.Dermatologic Clinics 12: 231-241, 1994b, review

Wood GS, Crooks CF, Uluer AZ: Lymphomatoid papulosis and associated cutaneous lymphoproliferative disorders exhibit a common clonal origin. J Invest Dermatol 105: 51-55, 1995

Wood GS, Hardman DL, Boni R, Dummer R, Kim Y-H, Smoller BR, Takeshita M, Kikuchi M, Burg G: Lack of the t(2;5) or other mutations resulting in expression of anaplastic lymphoma kinase catalytic domain in CD30+ primary cutaneous lymphoproliferative disorders and Hodgkin's disease. Blood 88: 1765-1770, 1996

Wood GS: Analysis of the t(2;5)(p23;q35) translocation in CD30+ primary cutaneous lymphoproliferative disorders and Hodgkin's disease. Leuk Lymph 29: 93-101, 1998, review

Wu K, Lund M, Bang K, Thestrup-Pedersen K: Telomerase activity and telomere length in lymphocytes from patients with cutaneous T-cell lymphoma. Cancer 86: 1056-1063, 1999

Yazdi AS, Medeiros IJ, Puchta U, Thaller E, Flaig MJ, Sander CA: Improved detection of clonality in cutaneous T-cell lymphomas using laser capture microdissection. J Cutan Pathol 30: 486-491, 2003

Yawalkar N, Ferenczi K, Jones DA, Yamanaka K, Suh K-Y, Sadat S, Kupper TS: Profound loss of T-cell receptor repretoire complexity in cutaneous T-cell lymphoma. Blood 102: 4059-4066, 2003

Zattara-Cannoni H, Dufour H, Lepidi H, Chatel C, Grisoli F Vagner-Capodano AM: Hidden chromosomal abnormalities in a primary central nervous system lymphoma detected by multicolor spectral karyotyping. Cancer Genet Cytogenet 107: 98-101, 1998

Zelickson BD, Peters MS, Muller SA, Thibodeau SN, Lust JA, Quam LM, Pittelkow MR: T-cell receptor gene rearrangement analysis: Cutaneous T-cell lymphoma, peripheral T cell lymphoma, and premalignant and benign cutaneous lymphoproliferative disorders. J Am Acad Dermatol 25 :787-96, 1991

Zhang T, Ma J, Cao X: Grb2 regulates Stat3 activation negatively in epidermal growth factor signalling. Biochem J: 376: 457-464, 2003

Zheng W-p, Flavell RA: The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell 89: 587-596, 1997

Zhou H, Kuang J, Zhong L, Kuo W-l, Gray JW, Sahin A, Brinkley BR, Sen S: Tumour amplified kinase *STK15/BTAK* induces centrosome amplification, aneuploidy and transformation. Nat Genet 20: 189-193, 1998

Zhuang Z, Park W-S, Pack S, Schmidt L, Vortmeyer AO, Pak E, Pham T, Weil RJ, Candidus S, Lubensky IA, Linehan WM, Zbar B, Weirich G: Trisomy 7-harbouring non-random duplication of the mutant MET allele in hereditary papillary renal carcinomas. Nat Genet 20: 66-69, 1998

Zucker-Franklin D, Coutavas EE, Rush MG, zouzias DC: Detection of human T-lymphotropic virus-like particles in cultures of peripheral blood lymphocytes from patients with mycosis fungoides. Proc Natl Acad Sci USA 88: 7630-7634, 1991