ORIGINAL ARTICLE

DNA copy number imbalances in primary cutaneous lymphomas

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Abstract

Background  Cutaneous lymphomas (CL) represent a clinically defined group of extranodal non-Hodgkin lymphomas harbouring heterogeneous and incompletely delineated molecular aberrations. Over the past decades, molecular studies have identified several chromosomal aberrations, but the interpretation of individual genomic studies can be challenging.

Objective  With a comprehensive meta-analysis, we aim to delineate genomic alterations for different types of CL and propose a more accurate classification in line with their various pathogenicity.

Methods  We searched PubMed and ISI Web of Knowledge for publications from 1996 to 2016 reporting the investigation of CL for genome-wide copy number alterations, by means of comparative genomic hybridization techniques and whole-genome sequencing and whole-exome sequencing. We then extracted and remapped the available copy number variation (CNV) data from these publications with the same pipeline and performed clustering and visualisation to aggregate samples of similar CNV profiles.

Results  For 449 samples from 22 publications, CNV data were accessible for sample based meta-analysis. Our findings illustrate structural and numerical chromosomal imbalance patterns. Most frequent CNAs were linked to oncogenes or tumour suppressor genes with important roles in the course of the disease.

Conclusion  Summary profiles for genomic imbalances, generated from case-specific data, identified complex genomic imbalances, which could discriminate between different subtypes of CL and promise a more accurate classification. The collected data presented in this study are publicly available through the ‘Progenetix’ online repository.

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Conflicts of interest

We have no conflict of interest to declare.

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Introduction

Cutaneous lymphomas (CL) represent a complex group of extranodal non-Hodgkin lymphomas for which the underlying molecular events are incompletely understood. So far, the classification of cutaneous lymphoma remains one of the most challenging areas of oncologic dermatopathology. The first widely accepted classification, which emphasizes the features of cutaneous lymphomas as primary tumours of the skin, was the ‘WHO/EORTC classification (2005)’, representing a consensus based on the European Organisation for Research and Treatment of Cancer (EORTC) and World Health Organization (WHO) classifications.1,2 All forms of primary CL found in the WHO/EORTC consensus have been adopted by the WHO classifications.3 Updates which integrate these nosological entities into a larger classification, containing both nodal and extranodal lymphomas as different categories, are made periodical, with currently active classification having been published in 2016.4 Since our meta-analysis is based on data spanning the period from 1996 to 2016, we decided to follow the 2008 classification to avoid discrepancies and ambiguities in code assignment.

CLs are represented by primary cutaneous T-cell lymphomas (CTCLs) in approximately 65% of cases and in a smaller percentage by primary cutaneous B-cell lymphomas (CBCL). Although a large number of clinicopathologic variants are being acknowledged in the WHO classification, a clear, biologically supported definition of specific entities is missing so far.
CTCL identifies a group of extranodal T-cell lymphomas characterized by the infiltration of malignant CD4+ T cells in the skin. Among those, mycosis fungoides (MF) represents the most common diagnosis (44% of CL). MFs are neoplasms of skin-homing T cells with CD4+ T-helper phenotype and Th2 pattern which commonly behave as a low-grade lymphoma with a protracted clinical evolution; however, a subset of patients progress rapidly to severe forms, refractory to current treatment modalities. For MF, a wide spectrum of subtypes has been described, such as folliculotropic, pagetoid reticulosis and granulomatous slack skin. In contrast to the frequently indolent MF, Sézary syndrome (SS) represents a rare aggressive subtype of CTCL defined by diffuse pruritic rash, lymphadenopathy and the early appearance of malignant T cells in the peripheral blood. The mechanisms underlying the proliferation of neoplastic CD4+ T cells in SS are not fully understood.

Lymphomatoid papulosis and primary cutaneous anaplastic large cell lymphoma (ALCL) are part of a spectrum of CD30+ cutaneous lymphoproliferative diseases, the second most common group of CTCL. They have similar morphologic and immunophenotypic characteristics, with differentiation relying predominantly on the clinical representation. Adult T-cell leukaemia/lymphoma is an aggressive peripheral T-lymphocytic neoplasm caused by a human retrovirus, human T-cell lymphotropic virus type 1 (HTLV-1). Skin involvement is generally a manifestation of disseminated disease, but a slowly progressive form which may have primary cutaneous involvement has been described (smouldering subtype). Other recognized CTCLs include subcutaneous panniculitis-like T-cell lymphoma, extranodal NK/T-cell lymphoma, and peripheral T-cell lymphomas, rare subtypes (cutaneous gamma/delta T-cell lymphoma, primary cutaneous CD4+ small-medium-sized pleomorphic T-cell lymphoma, primary cutaneous aggressive epidermotropic CD8+).

The class of CBCL have provided cause for debate for a long time and present a compelling reason for developing a new classification. Currently, recognized categories include primary cutaneous marginal zone B-cell lymphoma, primary cutaneous follicle centre lymphoma, primary cutaneous diffuse large B-cell lymphoma and primary cutaneous intravascular large B-cell lymphoma.

To date, little is known about the underlying molecular events in CL, both because of the limited ability to extrapolate lessons learnt from the nodal counterpart (similar pathological entities with different biological behaviour, prognosis and treatment) and also the difficulty to conduct studies addressing CL (overall low incidence together with high clinicopathologic heterogeneity). Over the past decade, molecular studies have identified several molecular events such as gene-specific mutations, aberrant gene expression profiles, upregulated microRNAs, telomere shortening and chromosomal aberrations. However, the aetiology remains unknown for the majority of cases, with a set of common pathways or initiating events in different stages of lymphocyte maturation to be identified. Importantly, the behaviour of a malignant clone of lymphocytes is not only the result of its uncontrolled proliferation but also may depend on factors and stimulants from the cutaneous microenvironment.

A number of studies have been investigating structural genome variants in CL, such as chromosomes copy number alterations, localized copy number variations (CNVs), deletions, amplifications and insertions. While these genome variants may be based on different rearrangement mechanisms, the common end point of somatic copy number variations/alterations (CNV) is a potential gene dosage effect, promoting over- or underrepresentation of the transcripts and proteins derived from genes in the affected regions. While large CNVs make it difficult to identify the possible target gene(s), ‘focal CNV’—an operational term based on a limited size up to 3 Mb and the inferred relevance of direct gene targeting—have the potential to point directly towards pathogenetic gene defects.

The increasing number of genomic studies and the advances in genome analysis technologies promise a better understanding of the molecular events responsible for clonal transformation and histopathological representation of CL. Also, genomic alterations have the potential to support a better classification of these diseases and can be supportive in prognostic evaluation and treatment response. However, the interpretation of individual genomic studies – especially in rare diseases – can be challenging due to limitations such as small sample sizes as well as differences in analysis methods and data processing. Here, we conduct a meta-analysis of published CL studies to identify genomic alterations and alteration patterns specific for different types of CL. The collected data presented in this study are publicly available through the ‘Progenetix’ repository for molecular-cytogenetic data.

**Methods**

**Search strategy**

We conducted a search of published research using PubMed and ISI Web of Knowledge from the year 1996 to 2016 with no language restrictions. Search terms included the following sets of keywords variably combined: ‘lymphoma’, ‘cutaneous lymphoma’, ‘skin lymphoma’, ‘CTCL’, ‘mycosis fungoides’, ‘sezary’, ‘leukaemia’, ‘leukemia’, ‘lymphomatoid papulosis’, and ‘cgh’ or ‘CGH’ or ‘aCGH’ or ‘comparative genomic hybridisation’ or ‘snp’ or ‘SNP’ or ‘array’ or ‘genomic array’ or ‘genome’ or ‘copy number’ or ‘dna microarray’ or ‘amplification’. Additional, we followed up on references from the selected articles for possibly relevant publications followed by evaluation of the abstracts. If more than one article was published using the same series of subjects, we chose the latest or the most complete study for this meta-analysis.
Inclusion and exclusion criteria

For selection of the studies, we followed the guidelines of the critical checklist proposed by the Dutch Cochrane Centre Meta-analysis of Observational Studies in Epidemiology (MOOSE). Articles were identified as eligible when they fit the following criteria: (i) they contain genomic copy number data with whole genome coverage and access to this information; (ii) diagnosis of primary cutaneous lymphoma (PCL) with equivalent terminology in EORTC-WHO (2005) and WHO (2008) classifications (Table 1); and (iii) matching available or convincingly inferred locus information.

The process of study retrieval is presented in Table 2 according to PRISMA (preferred reporting items for systematic reviews and meta-analyses) statement.

Data extraction and quality assessment

For each of the identified studies, the following information was extracted: first author, year of publication, population demographics, clinical characteristics, follow-up data where available, and genomic alterations for each patient individually. The techniques considered for the assessment of genomic copy number aberrations included both chromosomal and array-based comparative genomic hybridization (cCGH/aCGH).

Copy number aberration data were either mapped to the genomic base locations of a 862 band karyotype data (UCSC genome browser hg38 mapping; genome.ucsc.edu) or based on the genome mapped segmentation calls where available. For genomic array data without called regional gain/loss information, we used the arrayMap software tools to segment and visualize the probe specific data and derived regions of imbalances.

Data analysis

We used the complete DNA copy number alterations (CNAs) data to generate non-overlapping genomic segments, in order to

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### Table 1 WHO/EORTC (2005) and WHO (4th edn., 2008) classifications of CL

<table>
<thead>
<tr>
<th>WHO/EORTC</th>
<th>WHO classification (ICD-O code)</th>
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<tbody>
<tr>
<td>Cutaneous T-cell and NK-cell lymphomas</td>
<td>Mature T-cell and NK-cell neoplasms</td>
</tr>
<tr>
<td>Mycosis fungoides (MF)</td>
<td>Mycosis fungoides (MF) 9700/3</td>
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<tr>
<td>MF variants and subtypes</td>
<td>MF variants and subtypes</td>
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<td>Folliculotropic MF</td>
<td>Folliculotropic MF</td>
</tr>
<tr>
<td>Pagetoid reticulosis</td>
<td>Pagetoid reticulosis</td>
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<tr>
<td>Granulomatous slack skin</td>
<td>Granulomatous slack skin</td>
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<td>Sézary syndrome</td>
<td>Sézary syndrome 9701/3</td>
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<td>Adult T-cell leukaemia/lymphoma</td>
<td>Adult T-cell leukaemia/lymphoma 9627/3</td>
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<td>Primary cutaneous CD30+ lymphoproliferative disorders</td>
<td>Primary cutaneous CD30+ T-cell lymphoproliferative disorders</td>
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<td>Primary cutaneous anaplastic large cell lymphoma</td>
<td>Primary cutaneous anaplastic large cell lymphoma 9718/3</td>
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<tr>
<td>Lymphomatoid papulosis</td>
<td>Lymphomatoid papulosis 9718/3</td>
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<td>Subcutaneous panniculitis-like T-cell lymphoma</td>
<td>Subcutaneous panniculitis-like T-cell lymphoma 9708/3</td>
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<td>Extramodal NK/T-cell lymphoma, nasal type</td>
<td>Extramodal NK/T-cell lymphoma, nasal type 9719/3</td>
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<tr>
<td>Primary cutaneous peripheral T-cell lymphoma, unspecified</td>
<td>Primary cutaneous peripheral T-cell lymphoma, rare subtypes</td>
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<tr>
<td>Primary cutaneous aggressive epidermotropic CD8*</td>
<td>Primary cutaneous CD8* aggressive</td>
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<tr>
<td>T-cell lymphoma (provisional)</td>
<td>Epidermotropic cytotoxic T-cell lymphoma (provisional) 9709/3</td>
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<tr>
<td>Cutaneous c. d T-cell lymphoma (provisional)</td>
<td>Primary cutaneous γ/δ T-cell lymphoma 9726/3</td>
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<tr>
<td>Primary cutaneous CD4* small/medium-sized</td>
<td>Primary cutaneous CD4* small/medium</td>
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<tr>
<td>Pleomorphic T-cell lymphoma (provisional)</td>
<td>T-cell lymphoma (provisional) 9709/3</td>
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</table>

### Table 2 Study retrieval

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Full text</th>
<th>Open access</th>
<th>After excluding the doubles</th>
<th>After article review</th>
<th>After data review</th>
</tr>
</thead>
<tbody>
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<td>138 752</td>
<td>120 354</td>
<td>46 754</td>
<td>23 266</td>
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</table>
evaluate overall and subgroup specific frequency as well as sample based patterns of regional copy number changes. Case-specific CNA data were visualized and ordered by using data matrices containing imbalance status (gain/loss) mapped to 1 Mb genomic intervals. Hierarchical clustering of matrices was used to order the cases, and the obtained order then used for replotting of CNA in their original resolution. The relative genomic CNA coverage (number of bases with CNA compared to genome size) was used as a proxy for CNA complexity. The imbalance distribution was determined, by calculating the gain/loss frequencies, mapped to genomic intervals on a 1 Mb level. A heatmap of gain/loss distributions was generated to compare the copy number profiles between samples. For patients with clinical follow-up, a limited survival analysis was performed.

**Results**

Our search using the keywords with a logical 'OR' assertion returned a total of 138,752 abstracts. Title and abstract review resulted in the exclusion of 115,486 papers which were duplicates and another 23,234 which were not relevant to our study. Additionally, no full text was available for 18,398 publications, which were thus excluded. We reviewed 32 articles in full. Ten articles were excluded due to the lack of individual data (even after contacting the authors) or because they did not meet the inclusion criteria (secondary cutaneous etc.).

Finally, we included 22 articles in the present meta-analysis. The 22 studies included here comprised 449 samples in total (Table 3).

### Distribution of studies

Almost all of the studies included in our meta-analysis originated in Europe; one case was selected from a Chinese paper, and one additional study was the result of a collaboration between groups from the USA and the Netherlands (41 patients with T-cell lymphoma). No studies from other geographic regions could be included as they did not meet the inclusion criteria.

### CNAs in CL

The genomic abnormalities found in CTCL (376 samples) and CBCL (73 samples) are summarized in Fig. 1.

Reviewing the studies on CTCL included in our meta-analysis, we found that the most frequent CNAs, that is genomic regions with frequent imbalances, consisted of copy number gains on chromosomes 17q, 7 and 8q, while the most frequent losses presented themselves in regions on 17p, 6q, 9p21 and 13q.

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**Table 3** Cutaneous lymphoma samples included in meta-analysis

<table>
<thead>
<tr>
<th>WHO classification</th>
<th>Diagnostic text</th>
<th>ICD-O</th>
<th>No. Samples</th>
<th>PMID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycosis fungoides (MF)</td>
<td>Mycosis fungoides</td>
<td>9700/3</td>
<td>145 (32.2%)</td>
<td>18558289, 18832135, 26551667, 17584911, 15086538, 18663754, 12207585, 19759554</td>
</tr>
<tr>
<td>Sézary syndrome (SS)</td>
<td>Sézary syndrome</td>
<td>9701/3</td>
<td>142 (31.6%)</td>
<td>10084322, 10874879, 12207585, 12557225, 18413736, 18558289, 19843862, 25314094, 26551667, 15086538</td>
</tr>
<tr>
<td>Primary cutaneous anaplastic large cell lymphoma lymphoma (ALCL)</td>
<td>Anaplastic large cell lymphoma [cutaneous]; Anaplastic large cell lymphoma, cutaneous type</td>
<td>9718/3</td>
<td>73 (16.2%)</td>
<td>12648233, 12696066, 14595754, 15086538, 15111330, 19710685, 26551667</td>
</tr>
<tr>
<td>Primary cutaneous peripheral T-cell lymphoma, rare subtypes† (TCRS)</td>
<td>Peripheral T-cell lymphoma [cutaneous] (PTL-NOS)</td>
<td>9709/3</td>
<td>16 (3.5%)</td>
<td>19710685, 26551667</td>
</tr>
<tr>
<td>Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) (MZBL)</td>
<td>Extramedulary marginal zone B-cell lymphoma; Marginal zone lymphoma [cutaneous]</td>
<td>9699/3</td>
<td>16 (3.5%)</td>
<td>12203778</td>
</tr>
<tr>
<td>Primary cutaneous follicle centre lymphoma (FCBL)</td>
<td>Follicular lymphoma [primary cutaneous]; Follicular lymphoma [cutaneous]</td>
<td>9597/3</td>
<td>19 (4.2%)</td>
<td>12203778, 15175042, 16274455</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma‡ (DLBCL)</td>
<td>Diffuse Large B-cell Lymphoma [extranodal, skin]; Diffuse large B-cell lymphoma [primary cutaneous, leg type]; Diffuse large B-cell lymphoma [cutaneous]</td>
<td>9680/3</td>
<td>38 (8.4%)</td>
<td>16274455, 15175042, 12203778</td>
</tr>
</tbody>
</table>

Additionally, recurring deletions could be observed on 1p, 8p and chromosome 10q. Not surprisingly, each of these regions has been found to harbour oncogenes or tumour suppressor genes, respectively, with critical roles in the course and development of these oncologic entities. Karenko et al. evaluated the clinical evolution of CTCL for an average 54 months in comparison with chromosomal abnormalities. They analysed chromosomes 1, 6, 8, 9, 11, 13/21, 15 or 17 from five cases of large plaque parapsoriasis (LPP), eight MF and two SS, using G-banding and in situ hybridization techniques. Chromosomal aberrations from patients achieving complete clinical remission predominantly involved chromosomes 1, 6 and 11, while patients with active and progressing disease showed mutation on chromosomes 1, 6, 8, 11 and 17.22

In the histogram of CBCL, gains are harbouring loci like 12q, 18q and losses in 6qter. While the genomic copy number gains represented the more prevalent type of CNA in both B-cell- and T-cell-derived cutaneous lymphomas, the involved genomic regions differed in accordance with the lineage. The most frequent gains in T-NHL involved 17q and 8q, while the most frequent duplications in B-NHL occurred on 18q (involving the BCL2 and MALT1 locus) and as frequently focal amplification on 12q21 (around CDK4, oncogenes GLI1 and MDM2).

**Figure 1**: Frequency of genomic gains and losses in CBCL and CTCL. This histogram shows the frequency of genomic gains/amplifications (orange, up) and deletions (blue, down) along genomes of cutaneous B- (a) and T-CL (b) with “CBCL (a) and CTCL (b), ordered from chromosome 1–22. In this plot, a frequency of, for example, 25% for a gain means that in 25% of the selected cases, a genome duplication was observed in 25% of the samples, for the matching genomic interval. The same situation is also valid for deletions. In panel (c), the regional changes are compared using a heatmap style display, in which a high local frequency of copy number gains and losses results in predominantly yellow and blue colour, respectively.

**CNAs in MF**

Mycosis fungoides, a malignancy of skin-homing T cells, is the most common type of CTCL whose early diagnosis is often challenging. The use of genomic analyses has been studied, and its importance is undeniable. Gains in copy numbers were more often described than deletions in this type of CL. Recurrent alterations were highlighted as gains on chromosomes 1, 7, 8, 9qter, 17, 19, 22 and losses of 6q, 9pter, 13q and 17p.23

Figure 2a, generated from 145 samples of MF, highlights duplications on chromosome 1p, chromosome 7, 8 with (predominantly 8qter), 9qter, 17 predominant on the long arm, 19, 22; and deletions on 6q, 10, 13q, 16q, 19, 9p and q (two predominant regions, with narrow hot spot on 9p21.3) and 17p. The TP53 gene, also known as the guardian of the genome, is an important instruction for p53 protein, which acts as a tumour suppressor. This gene is located on 17p13.1, and its deletion is easily noticed on MF histoplot and also on the histoplots generated for CTCL and CBCL (Fig. 1). The deletion spike seen in 9p21.3 corresponds to CDKN2A, CDKN2B and MTAP genes, that encode tumour suppressor proteins p14 (role in p53 protection) and p16. Gains seen in the long arm of chromosome 8 on locus 8q24.21 are harbouring the MYC proto-oncogene, which is an functional target in numerous human cancers.24
ALK amplification in a case of MF

Reviewing the data from the van Doorn et al. paper, we observed in one case of MF (GSM325151) a gain of the distal part of chromosome 2p, with a breakpoint in or close to the 3′ end of the ALK proto-oncogene. Also, while the probe segmentation resulted in a single segment (2:29193223-29920658 DUP; hg38 coordinates), a cluster of five probes covering the ALK locus suggested for an additional copy number gain.

While typically ALK is implicated in the pathogenesis of ALCL (see Subsection CNAs in ALCL)25,26 or diffuse large B-cell lymphoma (DLBCL; see Subsection CNAs in DLBCL),27 this observation provides a rare report of ALK rearrangement in MF (Fig. 3).

In histological analysis, transformed MF presents with similarities to primary cutaneous ALCL, although ALK expression does not constitute a typical feature of MF. After Covington et al.28 identified a case of MF and concurrent nodal ALCL with the expression of ALK, they decided to analyse 103 biopsies of 96 MF patients for ALK expression. Out of all biopsies, only the case with MF and nodal ALCL association presented positive for ALK, leading to the conclusion that ALK is not routinely present in any stage or variant of MF. In another report, one case of CTCL with an aggressive evolution turned ALK positive.29 The authors concluded that most likely this case represented CD30+ transformed MF. There, ALK expression was accompanied by an aggressive course in contrast to the typical favourable prognosis of ALK+ ALCL. The rarity of such observations emphasizes the value of data accessibility for meta-analyses, thereby allowing the integration and proper evaluation of such rare genomic events, since multiple instances of single observations may support recurring pathogenetic mechanisms.

Figure 2 Comparison of CNAs in MF and SS. In (a) the distribution of local CNAs in MF is shown, and compared to those in SS in (b).

CNAs in SS

Sézary Syndrome, although clinically related to MF and sometimes genetic and molecular aberrations are described with similar patterns,30 it has a negative impact on the survival and shares distinct genetic properties. Figure 2b, generated from 142
samples of SS, shows gains on chromosomes 4, 7, 19 and predominant on chromosomes 8q and 17q and losses on 1p, 2p, 6q, 8p, 9, 13q with massive losses on chromosome 10 and a spike on 17p (TP53 locus).

**MF vs. SS CNAs**

We compared CNAs between MF and SS, to spot the differences and compare the data with literature reports. Figure 2c compares the distributions of CNAs in a nearly even set of total 287 samples, collected from 14 publications.

Main differences between MF and SS in our 287 samples are seen on chromosomes:

- 1p (MF gains, SS losses)
- 2 (losses on short arm SS)
- 4 (gains in some SS),
- 6q (losses predominant in MF),
- 7 (much higher number of gains in MF),
- 9pter (gains in MF),
- 10 (very high no. of losses in SS, overall a rare event in cancer apart from gliomas),
- 13 (higher number of deletions in MF),
- 17 (higher number of gains in q arm and losses in p arm in SS, probably reflecting i17q and TP53 involvement),
- 19 (gains in MF)

The loss in TP53 gene located on 17p13.1 is more prominent in SS than MF. The deletion spike seen in 9p21.3 – corresponding to the locus of the CDKN2A, CDKN2B and MTAP genes – is encountered in both MF and SS with a slightly higher rate in MF. Gains seen in 8q24.21, encoding the MYC proto-oncogene, are somewhat more frequent in SS.

Sézary Syndrome is characterized by highly recurrent alterations including gains on 17q23-25 and 8q24 as well as losses on 17p13, with frequencies higher than those in MF. In our CNA analysis of this locus, both SS and MF are affected by these CNA, but with higher numbers in SS. In the same paper, it was reported that gains in 7q36 rarely occur in SS, in contrast to MF. In our analysis, gains of mostly the whole chromosome 7 appear in both diseases but predominantly MF. Overall, in accordance with van Doorn et al.:

- MF characterized by gains on chromosomes 1 and 7 and loses on chr. 9
- SS characterized by gains on chromosomes 8 and 17 and loses on chr. 10

The differences in focal gains and losses between MF and SS highlight the unique character of these pathologies, substantiating a differentiated approach of their classification and clinical evaluation.

**CNAs in ALCL**

Cutaneous ALCL has an usual indolent clinical evolution and overall good prognosis. In our study, cALCL was characterized by gains on chromosomes 1, 2p, 5, 6p, 9 and high number of gains on chromosome 7. Frequent deletions are seen in chromosome 6 (mostly on the long arm), 13 but also some deletions on 1p, 3p, 16q, 17p, 18p. All can be observed on the graphic of 73 cALCL cases (Fig. 4b).
Oncogenes like NRAS (1p13.2), RAF1 (3p25), CBFA2 (21q22.3) and JUNB (19p13.2) are amplified more or less in the 73 samples of ALCL. Even if ALK gene rearrangements are rarely seen in cALCL, a subset of case reports describes this gene rearrangement. In our case, copy number gains are positive on the ALK locus gene 2p23.2-p23.1 (in four cases out of 73). Deletions in TP53 gene located on 17p13.1 and PRDM1 (6q21) are also seen in our cases, fact reported by other authors as often events in this type of lymphoma. This consistent copy number alteration is an important pawn for future studies in those cases with severe clinical evolution.

CNAs in TCRS (PTL-NOS)
In contrast with ALCL, primary cutaneous peripheral T-cell lymphoma not otherwise specified (PTL-NOS) shows an aggressive clinical evolution and bad prognosis. PTL-NOS shows gains on 6p, 7q and 17q, and was distinguished by high no. of gains on chromosome 8. Losses are encountered on 6q, 9q, chromosomes 10, 11, 13 and also some deletions on various regions of chromosome 8 (Fig. 4a). However, this characterization may be influenced by the low number of samples (16 cases).

CNAs in MALT lymphoma (MZBL)
Out of the 16 samples of MALT lymphoma (MZBL) accessible, six presented CNAs (only in gains, recurrently on chromosomes 7, 8q, 13q, 18p, 20p, 21; Fig. 5c). Gains on 1p22.3 corresponding to BCL10 apoptosis gene are present in two cases. This is reported as being associated with the development of extracutaneous disease but without importance in primary cutaneous disease.

CNAs in FCBL
Out of 19 samples of FCBL, six presented CNAs mostly represented by gains. Most often gain was on chromosomes 1p22, 1q21-1q31, 2p16 with a spike on 2p13, 2q21-q32, 3q25-pter, chromosome 4, chromosome 5, chromosome 7 with high number of gains in 7q31-q32, chromosomes 8, 12 (Fig. 5b). Literature reports reveal amplification in 2p16.1 in most cases of FCL with amplification of c-REL apoptotic gene, case confirmed by
our analysis. Deletions of 14q31.33 were also highlighted, situation not met in the current meta-analysis.33

**CNAs in DLBCL**
From all CBCL, DLBCL has the worst prognosis and survival. Genetic studies revealed recurrent amplifications in 18q21.31q21.33, which includes the MALT1 and BCL2 loci, as well as increased expression of proto-oncogenes PIM1 (6p21.2), WDR26 (1q42.11-q42.12), MYC (8q24.21) and IRF4 (6p25.3). Recurrent deletions were described in 9p21.3 (CDKN2A, CDKN2B, NSG-x genes).33

In Fig. 5a, in 38 DLBCL case analyses, we observe a large variety of copy number alteration in this type of lymphoma. CNAs are mostly covered by gains. They are encountered on chromosomes 1q, 2, 3, 6, 8q and 13 and a large number of amplifications in chromosomes 7, 12 and 18. Less common losses are observed on 1p, 6q with a spike on 6q22 and on chromosomes 17 and 19 with higher frequency on short arms.

Wiesner et al. reported the most frequent numerical aberrations in their 40 cases analysis. From the most frequent to the less frequent, gains affected chromosomes 12, 7, 3, 18q, 11 and losses affected chromosome 18. In our cohort of 38 samples, most frequent gains were on chromosome 18q (including BCL2 gene and MALT1 gene locus), 7q, 7p, 1q (including WDR26 gene amplifications), 12q, 3q, 2q, 13q, 6q, 8q (including MYC gene amplification). Most frequent losses were present on chromosomes 6q, 17p, 19p, 20q, 6p, 1p. Deletions on 9p21 locus corresponding to CDKN2A gene were not met in our patient group.

**Genomic imbalance patterns**
To quantitatively measure genomic imbalances, we derived the fractions of genomic regions with aberrant copy number per sample (abfrac) and determined their distributions among all the analysed CL entities (Fig. 6).

The lowest overall amount of CNA was observed in indolent cutaneous B-NHL (cFCL &cMZL), while higher rates of genomic imbalances were observed in cTNHL and especially DLBCL, with an overall agreement between clinical aggressiveness of disease type and amount of genomic abnormalities.

We want to point out that in the future the analysis of distribution and the fraction of genomic imbalances can be a novel prognostic factor to predict the clinical progression of the lymphoma and potentially direct the therapeutic approach.

**Survival data and prognoses**
In addition, we analysed the effect of existing copy number alteration on disease progression and survival. For 86 patients (90 samples) with cutaneous lymphomas of different histologies (Table 4), both follow-up and survival data were available. All of them were profiled using cCGH. A total of 15 patients were aged <50 years, and 71 patients were aged >50 years.

In a study of 20 cases of SS, Vermeer et al.\textsuperscript{17} reported a median survival time of 31 months. In addition to this, some authors demonstrated that a higher number of CNAs resulted in a shorter overall survival.\textsuperscript{34,35}

As shown in that Table 5, MF has the largest number of CNAs. The most affected chromosomes were 6, 7 and 17. On
We analysed CNAs in this group of patients to compare the results of survival with the reports from literature. We had 90 samples (86 patients; Fig. 7) from which 60 samples (57 patients) had CNAs and 30 samples (29 patients) are negative for CNAs.

As we can see in the Table 6, the overall survival in patients without detected CNAs is superior to those with pronounced genomic instability. In the group with CNAs (57 patients), 24 died (42.1% with average follow-up of 28.7 months). From the patients without CNAs (29 patients), two died (3.5%). We mention that from the one of the two CNA negative patients who died, a second sample showed a molecular progression with appearance of CNA 24 months after diagnosis. The survival curves obtained by using the Kaplan–Meyer method shown in Fig. 7 represent the clinical course (maximum observation time 186 months) in those 86 patients. After 70 months, the survival rates seem to stabilize for both groups.

Salgado et al.34 in a multicenter study of 41 cases of tumour stage MF using a high-resolution oligoarray comparative genomic hybridization platform demonstrated that three specific chromosomal imbalances were associated with poor prognosis: gains of 8q24.21, and deletion of 9p21.3 and 10q26qter. We analysed 30 MF cases metadata, and we can observe gains of 8q24 in six cases but preponderant on 8q24.3, loses on 9p21 in three cases, and homogenous loses in two cases on 10q26. In the case of 9p21 and 10q26, all five patients died, and in 8q24 gains, half of the patients survived.

Other factors that promoted a shorter survival rate were age (older than 60) and the presence of multiple cutaneous lesions (more than two sites).34 In our case, out of 86 patients, 57 were equal or older than 60 and 23 (40.3%) died. Prochazkova et al. in their study of common chromosomal imbalances in cutaneous CD30+ T-cell lymphoma demonstrated that the number of chromosomal abnormalities was higher in the cases of patients with relapsing disease (mean number of changes 6.29). The samples obtained from these patients presented in most cases gains in chromosome 9 while chromosomes 6 and 18 were mostly affected by loss (6q and 18p).36 Also, Fischer et al. in a study on 32 patients with CTCL showed that more aggressive tumours were presented with more pronounced chromosome imbalances (≥5 CI meant a shorter survival). They also demonstrated that not all CNAs lead to a poorer prognosis, for example the loss of 17p and the gain in chromosome 7 did not influence the prognosis. On the other hand, gains in 8q and loss of 6q and 13q were associated with a significantly shorter survival.35

In our 86 patient analyses, 12 had deletions on 17p, out of which 6 had also gains on 17q and 1 deletion of 17q.

From these 12 patients, four had MF, three SS, three ALCL and two DLBCL. Of these patients, eight died (66%) with an average survival of 35.2 months. We observe that the medium survival in these cases is better than the one calculated for all 86 patients.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Demographics and disease characteristics for 86 patients with CL</th>
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</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td>Number of patients (complete follow-up; n = 86)</td>
</tr>
<tr>
<td>Death (no. of patients)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>60 (69.7%)</td>
</tr>
<tr>
<td>1</td>
<td>26 (30.2%) (8 ALCL, 1 PTL-NOS, 3 SS, 9 MF, 5 DLBCL)</td>
</tr>
<tr>
<td>Medium survival Follow-up (months)</td>
<td>29.1 months</td>
</tr>
<tr>
<td>Max</td>
<td>186</td>
</tr>
<tr>
<td>Min</td>
<td>3</td>
</tr>
<tr>
<td>Mean</td>
<td>29.44</td>
</tr>
<tr>
<td>Age (mean; years)</td>
<td>63.96</td>
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<tr>
<td>CL type</td>
<td></td>
</tr>
<tr>
<td>MF</td>
<td>30 (34.8%)</td>
</tr>
<tr>
<td>SS</td>
<td>4 (4.65%)</td>
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<tr>
<td>PTL-NOS</td>
<td>1 (1.1%)</td>
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<tr>
<td>ALCL</td>
<td>29 (33.7%)</td>
</tr>
<tr>
<td>FCBL</td>
<td>9 (10.4%)</td>
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<tr>
<td>DLBCL</td>
<td>13 (15.1%)</td>
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<table>
<thead>
<tr>
<th>Table 5</th>
<th>Number of CNAs per chromosome per disease in 86 patients (90 samples)</th>
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<tbody>
<tr>
<td>CHR no</td>
<td>ALCL</td>
</tr>
<tr>
<td>1</td>
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</tr>
<tr>
<td>2</td>
<td>3</td>
</tr>
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</tr>
<tr>
<td>Total</td>
<td>100</td>
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</table>

chromosome 6, the most of CNAs are found in ALCL; whereas on chromosomes 7 and 17, a high number of CNAs are developed in MF.
When it comes to gains in chromosome 7 and survival, we noticed that out of 86 patients 23 had gains in chromosome 7. Five were DLBCL, seven were ALCL, nine were MF, one SS and one FCBL. Eleven died (47%) with a medium survival of 24.8 months. We also looked over for gains in 8q to identify its influence on survival. Thirteen patients presented this CNA; out of them, three were ALCL, two DLBCL, five MF and three SS, of which six patients (46%) died with an average survival of 20.8 months. As shown, the average survival is lower than in the cases above but is not significantly reduced. Gains in 6q were encountered in 16 cases out of 86, six MF, six ALCL and four DLBCL. From these patients, 10 died (62%) with a medium survival of 30.1 months. The survival in this case is greater than the mean for entire lot of patients (86) but the percentage of patients who did not survive vs. those who did is significant. In the case of 13q loss, we analysed 11 patients (four MF, five ALCL, one DLBCL, one SS) out of 86, who had this CNA from which seven died (63.6%) on 26.5 average month survival.

In Fig. 8, we can easily notice the difference in survival rate between patients with or without CNV. The number of CNAs in patients who did not survive is much higher with frequent gains in 1, 6p, 7, 8q, 9qter, 14q, 15q, 16p, 17q, 19, 20q and 22q and frequent deletions on 3, 4q, 5q, 6q, 10q, 13, 17p.

On the other hand, the surviving group are presented with fewer CNAs. We report less frequent gains on 7, 8q, 9, 17q and 18. We also correlate the average fraction of chromosomal imbalances with the survival. We observed more chromosomal imbalances in the patients who did not survive (average abfrac in deceased group 0.14 vs. in surviving group 0.06).

### Discussions

Our data support the findings of individual genomic studies that the different types of CL are characterized by partially complex chromosomal imbalances, including structural and numerical abnormalities. While the small number of cases included in a single study frequently limits the comparative interpretation of molecular observations, our meta-analysis represents an approach to increase patient numbers for such comparisons and provides an integrative analysis of genomic imbalance patterns in CL.

Among the cases of MF, the most frequent aberrations were represented by gains in chromosomes 7, 8, 17, 19 and 22. Previously, alterations of chromosomes 8 and 17 (detected by G-banding and FISH) have been associated with increased disease activity in MF. Gain of chromosome 8 has been used as a prognostic factor, together with deletions in chromosomes 9 and 10.34 While those alterations could also be observed in SS, gains on chromosomes 19 and 22 were less common in SS patients, in contrast to copy number gains in chromosome 4 and deletions...
in chromosomes 1, 10 (highly characteristics) and 22. A more recent study using WGS with nine MF samples observes large-scale gains on chromosomes 7, 18, 3q, 5p, 17q and deletions within 8q, 9q, 10q and 16q. The focal deletions at 16p13.13 and 9q21.32 involve JAK-STAT pathway regulator SOCS1 and p21 co-activator HNRNPK.38

Studies have demonstrated that activation of PI3K/AKT signalling pathway due to PTEN alterations is rarely attributed to abnormalities in PTEN, PI3K and AKT1 genes, but still the presence of mutations in the PTEN gene (10q23.31) has a negative impact on the patient prognosis.39 Prominent among deregulated genes are those encoding MYC, MYC-regulating proteins, mediators of MYC-induced apoptosis and IL-2 signalling pathway components.17

Previously, several cytogenetic studies report 12q aberrations as the most common alterations in CTCL, involving NAV 3 gene (POMFIL1), which may regulate IL-2 production of activated T cells and may be implicated in cellular signalling and cell cycle regulation.30,40–42 In addition, our study supports an important role of chromosome 10 deletions in the pathogenesis of aggressive CTCL, especially SS, and encourages new studies to analyse the role of tumour suppressor genes found on this chromosome (e.g. MGMT and EBF3).

Duplications in chromosomes 8q (contains MYC) and 17q (contains STAT and ERBB3), proposed as early clonal events in tumour development,17,43,44 seems to be markers for CTCL with very frequent alterations observed in MF and SS, but not in CBCL. At the same time, these molecular events distinguish Sezary Syndrome from erythrodermic inflammatory dermatoses. MYC gene gains and MNT gene losses were found as recurrent genetic alterations in SS.45 In Fig. 2b, we point out the important gains of 8q, locus for MYC gene, and losses of 17p13, loci for MNT (17p13.3) and TP53 (17p13.1) genes. So far, it is not known whether TP53 and MNT loss are common or specific events, but in a recent study, Yang and Hurlin demonstrated that both are important outcomes in SS evolution. Also, the antagonism relationship between MYC and MNT generates a balance of proliferation and apoptosis regulated by a poorly defined feedback system. This relationship seems to be favourable for disease evolution.46

ALCL presents with imbalances in all chromosomes, possibly reflecting a complex pathogenesis, but with most frequent CNA involving chromosome 7 (gains) as well as 6 and 13 (losses).

The most frequent abnormalities in CBCL are gains in chromosomes 1, 2, 3, 7, 12, 13 and 18. DLBCL is characterized by a wide spectrum of imbalances, including a large number of deletions in chromosome 6 (also found in nodal lymphomas)47,48 as well as gains in chromosome 18 which contains the BCL2, a well-established antiapoptotic effector involved in the pathogenesis of some nodal and extranodal lymphomas.59–53

Limitations of the study include that the original data were produced over several years, with analyses performed on different platforms and in many different laboratories and mostly not being based on the 'state-of-the-art’ technologies (i.e. high-resolution genotyping arrays or whole-genome sequencing). Regarding the metadata, some of the studies were published before establishment of the two main classifications (WHO/EORTC and WHO); and the original diagnostic assignments did not follow their standardized criteria. Another important limitation is the small number of cases for some entities and therefore the risk of their biased representation. Also, this study was severely limited in correlating CNAs to clinical parameters such as 'stage'
due to the small number of cases with such information. Moreover, some rare types of CL could not be included in the analysis simply due to the absolute lack of genomic studies. However, we will provide support for future analyses by periodically updating the CL database accessible through the Progenetix and arrayMap repositories, as such data become available.

The results demonstrate that copy number gains and losses detected by genomic copy number profiling could be used – at least as supporting information – when classifying lymphomas into biologically and clinically distinct diseases or subtypes. Genomic copy number alterations have the potential to help diagnose or classify different disease entities, tumour subtypes and even prognostic features.

In conclusion, CLs are characterized by varying and sometimes complex CNA profiles, reflecting their variable pathogenesis. The identification of specific genomic imbalances could yield the critical insights of these intriguingly subjacent molecular events. Moreover, genomic signatures could support more accurate classifications, in an area which still represents a matter of debate.

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References