

L Scarfò^{1,2,3}, S Zibellini⁴, A Tedeschi⁵, F Maura⁶, A Neri⁶,
 P Bertazzoni⁷, B Sarina⁸, G Nalli⁹, M Motta¹⁰, F Rossini¹¹,
 A Cortelezzi⁶, M Montillo⁵, E Orlandi⁴ and P Ghia^{1,2,12,13}
 on behalf of Rete Ematologica Lombarda (REL)– CLL workgroup
¹Laboratory of B cell Neoplasia, Division of Molecular Oncology,
 Istituto Scientifico San Raffaele, Milano, Italy;
²Lymphoma Unit, Department of Onco-Hematology, Istituto
 Scientifico San Raffaele, Milano, Italy;
³Institute of Hematology, Università degli Studi di Ferrara,
 Ferrara, Italy;
⁴Department of Onco-Hematology, Hematology Unit,
 Fondazione IRCCS Policlinico San Matteo, Pavia, Italy;
⁵Department of Oncology/Hematology, Division of Hematology,
 Niguarda Ca' Granda Hospital, Milano, Italy;
⁶Ematologia 1-CTMO, Dipartimento di Scienze Mediche
 Università di Milano, Fondazione Ca' Granda IRCCS Ospedale
 Maggiore Policlinico, Milano, Italy;
⁷Division of Hematology, Istituto Europeo di Oncologia,
 Milano, Italy;
⁸Department of Onco-Hematology, Istituto Clinico Humanitas,
 Milano, Italy;
⁹Department of Internal Medicine, Presidio Ospedaliero di Lodi,
 Lodi, Italy;
¹⁰Division of Hematology, Spedali Civili, Brescia, Italy;
¹¹Divisione di Ematologia e Centro TMO,
 Ospedale San Gerardo, Monza, Italy;
¹²Università Vita-Salute San Raffaele and Istituto Scientifico
 San Raffaele, Milano, Italy and
¹³MAGIC (Microenvironment and Genes in Cancers of the blood)
 Interdivisional Research Program, Istituto Scientifico San Raffaele,
 Milano, Italy
 E-mail: marco.montillo@OspedaleNiguarda.it

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Clonal relationship between langerhans cell histiocytosis and myeloid sarcoma

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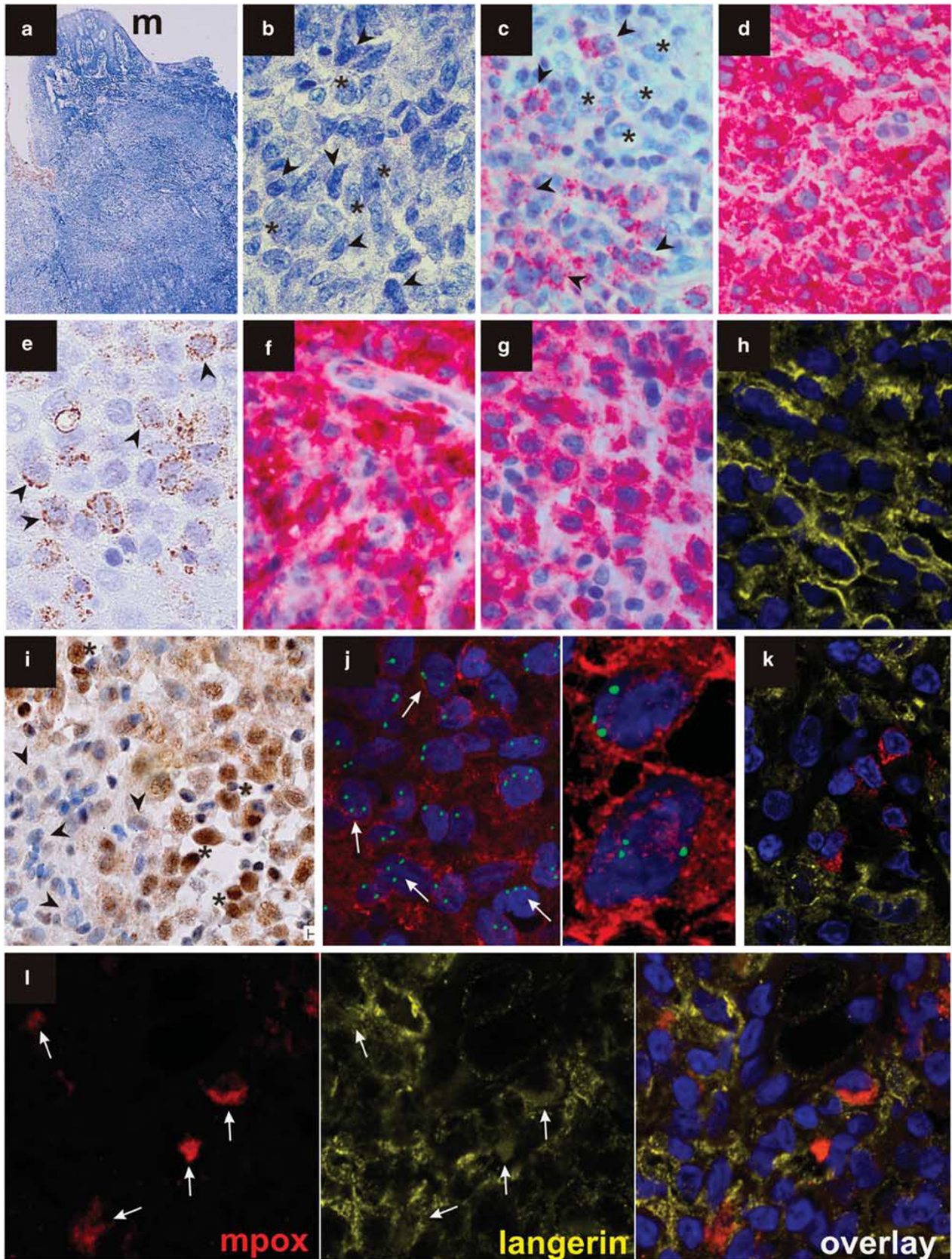
Langerhans cells (LCs) represent the main antigen capturing, processing and presenting dendritic cells (DCs) resident in epithelial surfaces including the skin or the oral mucosa, thus leading to either the induction of immune responses or tolerance. They are classically considered as myeloid-derived DCs sharing initial steps of their differentiation with monocytes. LC histiocytosis (LCH) and the more aggressive variant LC sarcoma (LCS) are considered as the neoplastic counterparts of this DC cell type. The immunoreactivity for macrophage-associated antigens in conjunction with CD1a, S-100 protein and langerin (CD207) represents a useful tool to establish the diagnosis of LCH in paraffin-embedded material.¹ Rare cases of neoplasms with LC differentiation features developing in patients previously diagnosed with follicular lymphoma or acute

lymphoblastic lymphoma/leukaemia (ALL) of T- and B-cell lineage have been reported. In these cases, identical clonal IgH- or TCR γ -gene rearrangements were detected in the H/DC neoplasms and the lymphomas consistent with a common clonal origin.^{2–5} The development of the histiocytic dendritic/cell (H/DC) neoplasm was associated with progressive disease in most of these cases. Thus, it has been suggested that these tumours may represent a part of the clonal evolution of the underlying lymphomas.⁴ The development of a LCS in a patient with an acute B-ALL in complete remission for 9 years has been attributed to a transcription factor-induced transdifferentiation.⁵ Both neoplasms showed an identical IgH rearrangement, but different expressions of PAX5 and ID2.⁵

Only rarely, an association of LCH and acute myeloid leukaemia (AML) has been reported. In most of these cases, the AML developed subsequently to LCH and this was considered as a therapy-related

leukaemia.⁶ Here, we describe a concomitant occurrence of LCH and a myeloid sarcoma (MS) characterized by the presence of the same chromosomal aberration (trisomy 8) in both populations.

A 61-year-old woman was seen by her dental surgeon because of an enlarging gingival mass. An excisional biopsy measuring 1.3 cm in diameter was performed at region 26 and



submitted by the referring pathologist for consultation to the Berlin Reference Centre for Lymphoma and Hematopathology. The patient had been well without a significant past medical history until approximately 3 months earlier when fatigue and anaemia developed. A bone marrow aspirate with 7% myeloblasts and an isolated trisomy 8 were consistent with a refractory anaemia with blast excess-1 (RAEB-1). As her clinical and haematological conditions appeared stable, she was monitored without therapeutic intervention. The formalin-fixed paraffin-embedded biopsy of the oral mucosa (Figure 1a) showed a tumour-forming infiltration by mononuclear cells with heterogeneous morphological and immunophenotypic features. Sheets of medium- to large-sized cells with grooved nuclei and a pale cytoplasm (Figure 1b, asterisks) with a low mitotic activity were intermingled with smaller monomorphic blast cells with basophilic cytoplasm and oval-shaped or slightly folded nuclei (Figure 1b, arrowheads). On immunohistological examination, the smaller blasts had higher mitotic counts and were positive for myeloperoxidase (Figure 1c), CD33 (Figure 1d), CD68 in a finely granular staining pattern (Figure 1e), partially for CD34 and showed a trisomy 8 when examined by FISH. The larger-sized cells (asterisks) were negative for myeloperoxidase (Figure 1c), CD34, CD3, CD20 and CD30 but strongly expressed CD1a (Figure 1f), protein S100, langerin (CD207, Figures 1g and h) and Id2 (Figure 1i). Combined immunolabelling (FICTION) with a langerin antibody (Novocastra, Newcastle Upon Tyne, UK) and interphase cytogenetics by FISH with a CEP 8 spectrum green direct-labelled fluorescent DNA probe kit (Abbott Laboratories, Abbott Diagnostics, Abbott Park, IL, USA) was performed. FICTION provided evidence that trisomy 8 and langerin expression were both harboured by identical cells (Figure 1j). In addition, confocal laser-scanning microscopy (CLSM) revealed the presence of myeloperoxidase-positive myeloid blasts (red) among the langerin-positive LCH cells (LCHC; yellow; Figures 1k, l). A subpopulation of neoplastic cells coexpressed myeloperoxidase and langerin suggestive of a mixed myeloid/LC phenotype (Figure 1l). The findings are consistent with a simultaneous manifestation of LCH and MS of myelomonocytic type. Interestingly, we observed a strong nuclear immunolabelling for the Id2 protein in the LCH component of the lesion, whereas the myeloid blasts appeared largely negative (Figure 1i). At this time, the patient's peripheral blood showed anaemia (hemoglobin 8.2 g/dL), a white cell count of $4 \times 10^9/l$ without evidence of blasts and a normal platelet count of $275 \times 10^9/l$. However, 1 month later, overt AML developed with 39% bone marrow blasts and peripheral hyperleucocytosis ($78 \times 10^9/l$). On flow cytometric analysis, the bone marrow blasts expressed HLA-DR, CD11b, CD13, CD14 and CD33 and partially CD34, which was consistent with a myelomonocytic phenotype. In contrast to the strongly CD1a-positive LCHC population observed in the gingival mass, bone marrow blasts were CD1a negative. Immunophenotyping for langerin was not performed on the bone marrow sample. Cytogenetics revealed the karyotype (46 XY, +8) both in the myelodysplastic syndrome (MDS) and the AML. Trisomy 8 was confirmed by FISH. No additional molecular genetic changes were detected. Induction chemotherapy with cytarabine, idarubicin and etoposide resulted in a transient cytorreduction, whereas bone marrow blasts rapidly increased and malignant

pleural and pericardial effusions developed. A buccal swab was suggestive of malignant cells. Despite 5-azacytidine treatment the patient died of progressive disease 4 months after the initial manifestation of MS. No autopsy was performed.

An MS is defined as a mass of myeloid cells occurring in an extramedullary site previously, simultaneously or subsequently to an AML or a myeloproliferative neoplasm. It may be preceded by a MDS, as seen in our patient. In rare cases, an MS may represent the unique manifestation of a myeloid neoplasm without bone marrow involvement at any time of the disease. The evidence of a trisomy 8 in both the components of the oral mass lesion of our patient strongly supports the conclusion that these neoplasms were clonally related. In a large cohort of 92 cases of MS, Pileri *et al.*⁷ did not describe any LCH but identified foci of plasmacytoid monocyte differentiation within an obvious myeloid population in four cases. In two of these cases, both the cellular components showed an opposite phenotype but belonged to the same clone. An inversion 16 was detected in the tumour-forming accumulations of plasmacytoid monocytes and in the myeloid population. This observation was in accordance with the hypothesis that the occurrence of plasmacytoid monocytes in the course of myeloid disorders may represent a differentiation of the underlying disorder. CLSM imaging of our case showed myeloperoxidase in conjunction with CD1a and langerin in a subpopulation of trisomy 8-positive tumour cells. This coexpression represents a phenotypic aberrancy consistent with a differentiation of myeloid blasts towards LCHCs *in vivo*. DCs can be generated from AML blasts *in vitro* but do not display typical morphological and immunophenotypic features of LCs.

The clonality of LCH has only rarely been assessed based on the on X-chromosomal inactivation mosaicism in somatic tissues and polymorphism of the androgen receptor gene in female patients.⁸ To our knowledge, this is the first report demonstrating a focus of LCH within an MS developing in the buccal mucosa where both neoplastic populations displayed the same cytogenetic abnormality, that is, trisomy 8, as the pre-existing myeloid disorder. Interestingly, trisomy 8 has been detected in 10.4% of MS studied by FISH in the series of Pileri *et al.*,⁷ suggesting that this aberrancy may be recurrently associated with the development of a MS. Noteworthy, FISH and cytogenetic analyses provided corresponding results in this study, confirming that FISH is a useful tool in cases where only formalin-fixed, but no fresh material is available. Unfortunately, our patient rapidly succumbed to her disease before allogeneic haematopoietic stem cell transplantation could be performed. This adverse outcome is in concordance with the data of Pileri *et al.*,⁷ suggesting that all of their MS patients with positive cytogenetics died of their disease irrespectively of their age or the type of their MS. By contrast to the unfavourable prognosis of MS, long-lasting responses have been obtained in adult LCH by multi-drug chemotherapy. Thus, rapid detection of phenotypic heterogeneity of tumour cells in biopsy specimens is mandatory for the choice of the best treatment modality. The factors responsible for the induction of a dichotomous phenotype in the trisomy 8-positive neoplastic cells of our patient are not yet understood. The high Id2 expression levels that we observed in the LCH component of our case suggests that a deregulation of this dominant negative helix-loop-helix transcription factor of the

Figure 1. (a) Histologic aspect of the Giemsa-stained gingival biopsy characterized by a broad-based mass located beneath the oral surface mucosa (m). (b) Large LCHCs with grooved nuclei and pale cytoplasm (asterisks) as well as smaller blasts with oval or delicately folded nuclei and indistinct nucleoli (arrowheads) were observed (Giemsa). The blasts (arrowheads) expressed myeloperoxidase (c), CD 33 (d) and showed a finely granular CD68 staining (e). While LCHCs were negative for myeloperoxidase (c, asterisks) they were labelled by CD1a (f), langerin (g, h, confocal image) and Id2 (i) antibodies. By FISH analysis using a DNA probe for trisomy 8 (green) and immunostaining for langerin (red), a trisomy 8 (arrows) was clearly detected in LCHCs (j). CLSM imaging of double stains for myeloperoxidase (red) and langerin (yellow) showed the presence of scattered myeloperoxidase-positive blasts within the sheets of langerin-positive LCHCs (k). There was evidence of a rare coexpressions of both antigens in identical cells, which were stained both in red and in yellow (l; arrows).

ID family may contribute to the commitment of haematopoietic precursors to an LC phenotype. An acquisition of Id2 and loss of PAX5 have been regarded as key factors in the transdifferentiation from a B-ALL to a LC/dendritic phenotype.⁵ Little is known about the mechanisms by which Id2 regulates myeloid development. It is conceivable that transcription factors of the bHLH protein and Ets protein families may act downstream of ID2 and modulate myeloid development and proliferation. The divergent differentiation patterns reported here represent another example of the plasticity of haematopoietic cells that could, at least in part, be mediated by transcription factors including ID2 and its target genes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AH Schmitt-Graeff¹, H Duerkop², B Vollmer-Kary¹, S Haxelmans³,
R Nitschke³, P Fisch¹, U Germing⁴ and H Stein²

¹Institute of Pathology, Universityhospital Freiburg, Freiburg, Germany;

²Berlin Reference Centre for Lymphoma and Hematopathology and Pathodiagnostik, Berlin, Germany;

³Life Imaging Center, Center for Biological Systems Analysis (ZBSA) Albert-Ludwigs-University, Freiburg, Germany and

⁴Department of Hematology, Oncology and Clinical Immunology, Heinrich-Heine-University of Duesseldorf, Duesseldorf, Germany
E-mail: annette.schmitt-graeff@uniklinik-freiburg.de

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Germline copy number variation associated with Mendelian inheritance of CLL in two families

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Chronic lymphocytic leukemia (CLL) shows significant familial predisposition, yet few genes have been associated with disease risk. We have used high-resolution single nucleotide polymorphism (SNP) arrays to study familial CLL and lymphoproliferative disorders (LPDs), focusing on a cohort of 44 families that includes 58 individuals with CLL (Supplementary Table 1). Thirty-one of these families met the classic definition of familial CLL, namely at least two first degree relatives with CLL, whereas the remaining thirteen families had a proband with CLL and at least one first degree relative with another LPD.¹ A parallel experiment was undertaken in 103 sporadic CLL patients enrolled on our prospective CLL natural history study and whose data were available for comparison. All subjects on both studies signed written informed consent before participation. Gene expression profiling was also performed on the CLL tumor samples when RNA could be obtained.

The SNP array data from the CLL tumors were analyzed initially using the GISTIC method² (Supplementary Figure 1) and showed 13q deletion in 48% of these familial CLLs, trisomy 12 in 15.5%, 11q deletion in only 3.4% and 17p deletion in 6.9% (Supplementary Table 2 and Supplementary Figure 1). 11q deletion was seen in only 2 of 58 samples (Supplementary Figure 1, Supplementary Table 2), suggestive of a low frequency in familial CLL as we previously reported.³ Otherwise, the pattern of somatic abnormalities was similar in familial and sporadic CLLs (Supplementary Table 2).

Detailed manual review of the 13q deletion region common in CLL revealed that family A in Supplementary Table 1 carries a germline deletion located telomeric to the commonly defined minimal region of deletion around *miR-15a* and *miR-16-1*, and

centered instead on the *DLEU7* gene. The affected family includes a father with CLL (generation 1) and his four children, three of whom also developed CLL (generation 2, Figure 1a). The three unaffected children of individual A-2A were assessed for evidence of monoclonal B cell lymphocytosis (MBL)⁴ with four-color flow cytometry and PCR to detect monoclonal IgH rearrangement (Supplementary Figure 2). These individuals had no evidence of MBL as yet but are young. The germline deletion at 13q in this family extends 525 kb on chromosome 13, from position 49935 614 to 50461 103, and includes the *DLEU7* and *RNASEH2B* genes (Figures 1b and c). A germline copy number variant (CNV) was not seen at this location in any other individual studied in this experiment (Figure 1b), in our parallel sporadic experiment, or in 143 other normal DNAs from individuals with solid tumors. This deletion excludes the commonly recognized minimal region of deletion around *DLEU2*, *miR-15a* and *miR-16-1* (Figures 1b and c), loss of which has been shown in knockout animals to result in a CLL-like disease.⁵ However, recent work has suggested that *DLEU7* can function as a tumor suppressor gene in CLL,⁶ and that the minimal region of 13q deletion in CLL may also include *DLEU7*.⁷ Furthermore, a mouse model carrying conditional deletion of *DLEU7*, *RNASEH2B* and the *miR-15a/miR-16-1* region in B cells develops CLL preferentially to MBL or NHL.⁸ These data taken together support a role for this genomic region in the pathogenesis of CLL.

We found further support for *DLEU7* acting as a tumor suppressor gene in CLL when we examined the affected children in generation 2, A-1A and A-2A, both of whom carry this deletion in their germline saliva samples and show deletion of the other allele in their tumor (Figure 1c). In the tumors, A-1A had a