bjh review

Progress in understanding the pathogenesis of Langerhans cell histiocytosis: back to Histiocytosis X?

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Summary

Langerhans cell histiocytosis (LCH), the most common histiocytic disorder, is characterized by the accumulation of $CD1A^+/CD207^+$ mononuclear phagocytes within granulomatous lesions that can affect nearly all organ systems. Historically, LCH has been presumed to arise from transformed or pathologically activated epidermal dendritic cells called Langerhans cells. However, new evidence supports a model in which LCH occurs as a consequence of a misguided differentiation programme of myeloid dendritic cell precursors. Genetic, molecular and functional data implicate activation of the ERK signalling pathway at critical stages in myeloid differentiation as an essential and universal driver of LCH pathology. Based on these findings, we propose that LCH should be re-defined as an *inflammatory myeloid neoplasia*. Increased understanding of LCH pathogenesis will provide opportunities to optimize and personalize therapy through improved risk-stratification, targeted therapy and assessment of therapy response based on specific molecular features and origin of the pathological myeloid cells.

Keywords: Langerhans cell histiocytosis, *BRAF* V600E, extracellular signal-regulated kinase signalling pathway, misguided myeloid differentiation, inflammatory myeloid neoplasia.

Clinical overview

Histiocytic disorders include diseases involving aberrant 'histiocytes'. While *histiocyte* translates to 'tissue cell', histiocytic disorders include all diseases associated with cells of the mononuclear phagocyte system, which is comprised of haematopoietic cells with mononuclear morphology and phagocytic activity (van Furth & Cohn, 1968; Favara *et al*, 1997). Langerhans cell histiocytosis (LCH) is the most common histiocytic disorder, arising in approximately five children per million, similar in frequency to paediatric Hodgkin lymphoma and acute myeloid leukaemia (AML) (Guyot-Goubin *et al*, 2008; Stalemark *et al*, 2008; Salotti *et al*, 2009). The median age of presentation is 30 months, though LCH is reported in adults in approximately one adult per million, both as unrecognized chronic paediatric disease and *de novo* disease (Baumgartner *et al*, 1997). There are occasional reports of affected non-twin siblings and multiple cases in one family, though it is not clear if this is significantly more frequent than one would expect by chance (Arico *et al*, 2005).

The clinical presentations of LCH vary from clinically trivial lesions that resolve spontaneously or with curettage to life-threatening disseminated disease that requires systemic chemotherapy and, in some cases, haematopoietic stem cell transplantation. The wide spectrum of clinical manifestations that overlap with more common paediatric conditions make diagnosis of LCH challenging. In an institutional study, we found that the time from clinical symptoms of skin LCH lesions to diagnostic biopsy was a median of 3 months, and in some cases diagnosis was delayed by over 5 years (Simko *et al*, 2014a). However, once biopsy is performed, diagnosis of LCH is straightforward: LCH is defined by $CD1A^+/CD207^+$ histiocytes with an inflammatory background, including variable numbers of lymphocytes (enriched for regulatory T cells), macrophages and eosinophils (Favara *et al*, 1997; Chikwava & Jaffe, 2004; Senechal *et al*, 2007).

Traditionally, therapy for patients with LCH is determined by the extent and location of the lesions. The optimal therapeutic approaches for LCH remain incompletely defined as patients with skin-limited lesions, single-bone lesions and isolated diabetes insipidus have not been studied on prospective clinical trials. Standard of care for LCH has evolved from serial clinical trials based on empirically-derived therapeutic strategies. Patients with liver, bone marrow and/or spleen involvement are at highest risk for mortality. In the most recent Histiocyte Society trial, LCHIII, 5-year overall survival for patients with risk-organ disease was 84%, compared to 99% for patients without risk-organ disease (Gadner *et al*,

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2013). The current standard of care, based on LCHIII, is 1 year of therapy with vinblastine and prednisone, plus mercaptopurine for patients with high-risk disease. Response to initial therapy is also an important prognostic factor for patients with high-risk LCH: Survival was 95% for patients with good response after the first 6 weeks of treatment on LCHIII, 83% for patients with intermediate response and 57% for patients who progressed on therapy (Gadner *et al*, 2013).

Langerhans cell histiocytosis may involve the central nervous system (CNS) with mass lesions (brain tumours), pituitary lesions resulting in diabetes insipidus (DI) and/or a neurodegenerative syndrome. A large international series observed that risk of development of DI was decreased in patients with bone lesions in mastoid, temporal, orbital and skull base who received systemic chemotherapy (Grois *et al*, 2006). Therefore, systemic therapy is now considered standard of care for patients with 'CNS risk' skull and pituitary lesions. A devastating consequence of LCH is development of a progressive CNS neurodegenerative syndrome that may develop years after the apparent resolution of LCH (Grois *et al*, 2010). Reports of treatment for the CNS neurodegenerative syndrome have been limited to case series and pilot studies, and include corticosteroids, cladribine, all-*trans* retinoic acid, intravenous immunogobulin and cytarabine (Idbaih *et al*, 2004; Dhall *et al*, 2008; Imashuku *et al*, 2008; Allen *et al*, 2010a).

While mortality in patients with high-risk LCH is improving, and survival is near universal in patients with low-risk LCH, 'reactivation' (a term for relapse intended to remain neutral on LCH as immune *versus* a malignant disorder) remains a significant problem. Over half of all patients are refractory to vinblastine/prednisone or develop recurrent lesions (Minkov *et al*, 2008; Gadner *et al*, 2013). Optimal 'salvage' strategies have not been defined. In a prospective Histiocyte Society trial, cladribine (5 mg/m^2 daily × 5 days per month) had high response rates, but rarely resulted in cure in a prospective trial (Weitzman *et al*, 2009). A much more intense strategy with cladribine (9 mg/m^2) and cytarabine (1 g/m^2 daily × 5 days per month) was tested in a small trial and resulted in a high rate of cure, but also a very high rate of treatment-related mortality (Bernard *et al*, 2005). Clinical series suggest that clofarabine, when dosed ~50% below the maximum tolerated dose, used in AML and other paediatric malignancies (25–52 mg/m^2/day × 5 days per month) may be effective with modest toxicity in patients with disease refractory to other salvage regiments (Rodriguez-Galindo *et al*, 2008; Abraham *et al*, 2013; Simko *et al*, 2014b). This efficacy might further be improved by higher doses of clofarabine in refractory cases, but clinical experiences are limited to single cases (Simko *et al*, 2014b).

Permanent consequences and late effects of disease as well as therapy remain significant challenges, arising in the majority of patients with LCH. The risk of developing complications is related to extent of disease at presentation as well as the duration of active, uncontrolled disease (Willis *et al*, 1996; Haupt *et al*, 2013). Complications include endocrinopathies (posterior and anterior pituitary), neurodegenerative syndrome, decreased pulmonary function, orthopaedic problems and liver failure due to progressive sclerosing cholangitis.

Historical concepts of LCH pathogenesis (1900s–2010)

Langerhans cell histiocytosis has suffered from several historical identity crises, many of which persist today. We hypothesize that difficulty in defining the biology of LCH relative to other neoplastic disorders has left it (and patients with LCH) on the sidelines of scientific and haematology/oncology clinical research cooperative group agendas.

Evolution of the LCH brand

The first reports of patients now recognized as having LCH were reported in the early 1900s, describing children with bone lesions, mucosal lesions and diabetes insipidus (Hand-Schüller-Christian disease) or infants with hepatosplenomegaly and histiocytic infiltration of the bone marrow (Letterer-Siwe disease) (Arceci, 1999). Hashimoto-Pritzker syndrome describes spontaneously-resolving skin lesions in infants (Hashimoto & Pritzker, 1973). 'Eosinophilic granuloma' is also a frequently used nomenclature for bone LCH. Despite the spectrum of clinical presentations, the histological appearance of an LCH lesion is relatively consistent. In 1953, Dr. Lichtenstein proposed that the various clinical conditions with the shared histopathology probably represent a common condition, which he proposed to be collectively named 'Histiocytosis X', with the 'X' as an indication of incomplete understanding of the cell of origin (Lichtenstein, 1953). Twenty years later, the Birbeck granule, a cytoplasmic structure associated with langerin (CD207), thought to have some function in antigen processing, was discovered by electron microscopy in the pathological mononuclear phagocytic cells of LCH lesions. As epidermal Langerhans cells (LCs) were the only cells at that time known to contain Birbeck granules, 'Histiocytosis X' was hypothesized to arise from epidermal LCs, and has since been rebranded as 'Langerhans cell histiocytosis' (Nezelof *et al*, 1973). For the next four decades, models of LCH were primarily based on the assumption that LCH arises from epidermal LC, with the ongoing question: Does LCH arise from a pathological activation or neoplastic transformation of the epidermal LC?

Inflammation/immune dysregulation in LCH

Langerhans cell histiocytosis lesions have a median of only 8% pathological 'LCs' (Berres *et al*, 2014). The remainder of the lesion is composed of a diverse inflammatory infiltrate. The impressively inflammatory character of LCH lesions led to the investigation of infection and immune dysregulation

as mechanisms of pathogenesis. Many viruses have been hypothesized to play roles in LCH pathogenesis, but none have been convincingly validated (Jeziorski *et al*, 2008). Most recently, an association with the relatively ubiquitous Merckel cell polyomavirus and LCH has been reported (Murakami *et al*, 2014), however the significance of this observation remains uncertain.

Immunologically, the lesional cells share some features with resting epidermal LCs, including high levels of $CD207^+$ and $CD1A^+$ expression, but also possess features of activated LCs, including expression of T cell co-stimulatory molecules and pro-inflammatory cytokines, creating a 'cytokine storm' (Geissmann *et al*, 2001; Laman *et al*, 2003; Allen *et al*, 2010b). Interestingly, $CD4^+$ $CD25^+$ $CTLA4^+$ regulatory T cells are enriched in LCH lesions, and patients with LCH may have impaired skin delayed-type hypersensitivity responses (Senechal *et al*, 2007; Allen *et al*, 2010b). Moreover, a causal role for LCH pathogenesis from elevated IL17a expression by LCH cells has also been proposed, but these results have not been confirmed by other groups (Coury *et al*, 2008; Allen & McClain, 2009; Peters *et al*, 2011; Makras *et al*, 2012).

Despite the inflammatory character of LCH lesions, infectious or autoimmune causes for LCH pathogenesis remain to be proven. Furthermore, the contributions of inflammation to clinical manifestations of LCH remain to be defined.

LCH as a neoplastic disorder

The alternative model to LCH pathogenesis caused by dysfunctional immune activation has traditionally been neoplastic transformation of epidermal LCs (Nezelof *et al*, 1973). Langerhans cell histiocytosis lesions do have many classical features of malignancy (Hanahan & Weinberg, 2011). Based on non-random X-inactivation, the $CD1A^+$ cells of LCH lesions were determined to be clonal (Willman *et al*, 1994; Yu *et al*, 1994). Moreover, lesions display malignancy-associated mechanisms, e.g., immune evasion with enriched regulatory T cell populations, tumour-promoting inflammation with increased local and systemic pro-inflammatory cytokines, expression of metalloproteases potentially promoting invasion and metastasis (Hayashi *et al*, 1997; da Costa *et al*, 2005; Allen *et al*, 2010b) and overexpression of *BCL2L1*, which may contribute to resistance to cell death (Schouten *et al*, 2002; Amir & Weintraub, 2008; Allen *et al*, 2010b). However, despite clonality and other malignant hallmarks, LCH cells are characterized by a 'benign' morphology and mitoses are observed at very low and similar rates as in normal epidermal LCs. Clonality is essential for malignancy, but physiological LCs in mice may also have regional clonality, arising from a common precursor (Merad *et al*, 2002; Waskow *et al*, 2008). Karyotypes from LCH biopsies are typically normal and gross chromosomal lesions have not been described (da Costa *et al*, 2009). Langerhans cell histiocytosis cells do not survive long in cell culture, and no successful xenograft model has been reported, further supporting a malignant character of LCH.

Redefining LCH in the molecular era

Somatic gene mutations/MAPK activation in LCH

Analysis of genomic alterations in LCH had traditionally been challenging due to the cellular heterogeneity of the lesions. The progress in sequencing technology amplifying the depth of sequencing coverage by next-generation sequencing techniques recently allowed the identification of a recurrent somatic mutation in the gene encoding for the protein kinase BRAF (Badalian-Very *et al*, 2010). The high prevalence of the *BRAF* V600E point mutation in LCH has been validated in several independent cohorts and attributed to the histiocytes within the lesions (Haroche *et al*, 2012; Sahm *et al*, 2012; Satoh *et al*, 2012; Berres *et al*, 2014). A recent meta review comprising 653 patient samples assessed an overall frequency of 48·5% for the *BRAF* V600E point mutation in LCH (Bubolz *et al*, 2014). However, we speculate the actual frequency is higher as methods other than quantitative polymerase chain reaction may be insufficiently sensitive to identify *BRAF* V600E in cases of lesions with sparse infiltration by pathological LCH cells.

BRAF is a central kinase of the RAS/RAF/MEK pathway, which is essentially involved in numerous cell functions including cell proliferation and migration and is frequently mutated in various cancer cells (Davies *et al*, 2002). The *BRAF* V600E mutation results in a constitutive, RAS-independent activation of the downstream kinases extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK)/ERK kinase (MEK) (Maurer *et al*, 2011). Of note, both downstream kinases are highly activated in LCH cells with *BRAF* V600E mutation, supporting the potential functional relevance of the mutation in LCH (Badalian-Very *et al*, 2010; Chakraborty *et al*, 2014). Interestingly, ERK signalling has been highly implicated in myeloid cell differentiation and maturation under physiological conditions. While hyperphosphorylation of ERK drives differentiation of dendritic cell (DC) progenitors (Miranda *et al*, 2005; Hamdorf *et al*, 2011), sustained phosphorylation inhibits DC maturation upon stimulation with Toll-like receptor ligands or tumour necrosis factor, as recently demonstrated (Puig-Kroger *et al*, 2001; Aguilera-Montilla *et al*, 2013). This role of ERK activation in myeloid cell differentiation and maturation during homeostatic conditions might also have valuable implications on the origin and functional alterations of histiocytes in LCH.

Besides the frequent *BRAF* V600E mutation, single case reports have described additional mutations/polymorphisms within the *BRAF* gene locus with potential functional consequences, including the somatic mutations *BRAF* V600D, *BRAF* 600DLAT and the germline mutation/poylmorphism *BRAF* T599A (Satoh *et al*, 2012; Kansal *et al*, 2013). Moreover, a complex compound somatic mutation in *ARAF* with enhanced kinase activity *in vitro* has also been described in a single patient (Nelson *et al*, 2014). However, extended

studies are necessary to elucidate the frequency and significance of these additional RAF mutations in LCH.

Using whole exome sequencing on matched LCH lesions and peripheral blood tissue samples obtained from 41 patients, we recently set out to analyse the broader genomic landscape of LCH. The overall rate of somatic mutations was remarkably low, at 0·03 mutations per Mb, or a median of 1 somatic mutation within exons per patient. In this study, we identified a second recurrent mutated gene locus in LCH, *MAP2K1* (encoding mitogen-activated protein kinase kinase 1 [MAP2K1, alternatively termed MEK1], also a member of the RAS/RAF/ERK signalling pathway) with a frequency of 33% in lesions with wild-type *BRAF* (Chakraborty *et al*, 2014). A second study identified mutations through targeted sequencing of the second and third exons of *MAP2K1,* in 50% of wild-type *BRAF* cases (Brown *et al*, 2014). Interestingly, both independent studies could only identify mutually exclusive mutation of *BRAF* and *MAP2K1*. Functionally, all mutations described within the *MAP2K1* gene resulted in the expression of a hyperactive kinase and subsequent constitutive phosphorylation of the downstream targets MAPK3 (also termed ERK1) and MAPK1 (also termed ERK2) *in vitro* comparable to the effects observed by the expression of *BRAF* V600E (Chakraborty *et al*, 2014). In addition to recurrent somatic mutations in *BRAF* and *MAP2K1*, we observed single cases of mutations of other genes that transcribe MAPK pathway proteins, specifically in *ERBB3* and again within the *ARAF* locus (Chakraborty *et al*, 2014) in our study, though their frequency and functional significance remains to be proven in larger series (Fig 1).

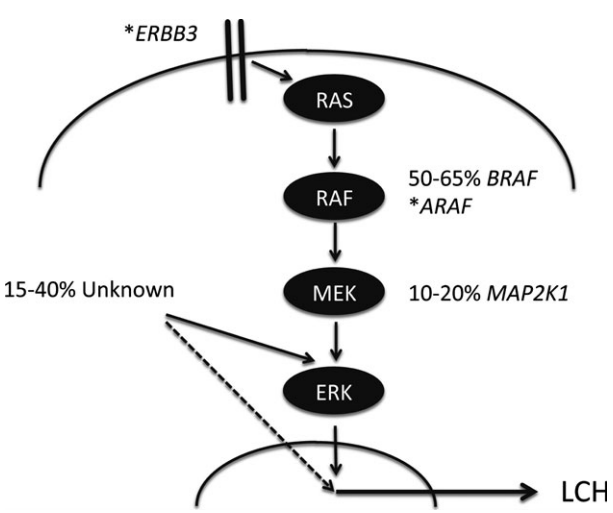

Fig 1. Routes to ERK activation in LCH. Model of MAPK pathway activation resulting from serial phosphorylation from cellular receptors through RAS, RAF, MEK and, ultimately, ERK. Estimates of frequency of somatic mutations of *BRAF* and *MAP2K1* are illustrated. (*) indicates genes with individual case reports of somatic mutations. 'Unknown' indicates ERK activation by mechanisms that have not yet been defined. While activated ERK has been identified in all lesions studied to date, there remains the possibility (dashed line) that Langerhans cell histiocytosis (LCH) may arise from alternative mechanisms in some cases.

In addition to genetic analysis, imaging flow cytometry and Western blotting was used to analyse activation of MEK and ERK proteins in $CD207^+$ cells from LCH lesions. MAP2K1 was highly phosphorylated in samples with *BRAF* V600E and *MAP2K1* mutations. However, MAP2K1 phosphorylation was minimal in cases with no detectable mutation in the MAPK pathway. In every case tested, MAPK3/MAPK1 was highly phosphorylated independent of the mutation or MAP2K1 phosphorylation status (Chakraborty *et al*, 2014). Similarly, the original *BRAF* V600E report by Badalian-Very *et al* (2010) also demonstrated ERK activation in BRAF wild-type lesions by immunohistochemistry. These reports support a common critical role of hyperactivation of the ERK signalling pathway in LCH pathology independent of the specific underlying mutation. Deciphering the mechanisms that drive ERK activation in the cases with undiscovered MAPK pathway mutations will require extended studies including complimentary methods (e.g. whole genome sequencing, RNA sequencing and assessment of epigenetic modifications) to fully assess the causative spectrum of ERK activation in LCH.

Activation of ERK may be universal to LCH pathogenesis, but like other diseases driven by MAPK pathway hyperactivity, it is likely that specific somatic mutations will be associated with certain unique clinical features. Although genotype-based risk assessment will require prospective studies in several independent cohorts, data so far suggest that the genotype does not correlate with extent of disease (high *versus* low-risk) or survival (Badalian-Very *et al*, 2010; Berres *et al*, 2014; Bubolz *et al*, 2014; Chakraborty *et al*, 2014). However, in our institutional series, the *BRAF* V600E mutation status correlated significantly with increased risk of initial treatment failure e.g. refractory disease or recurrence (Berres *et al*, 2014). In addition to the potential relevance of the genotype for clinical risk stratification, it might also have therapeutic implications given that mutations within the MAPK pathway predicted response to specific MAPK pathway inhibitors *in vitro*. While $CD207^+$ cells with *BRAF* V600E and *MAP2K1* mutation had predictable responses to BRAF and MEK inhibition, responses of BRAF wild-type/MAP2K1 wild-type lesional cells were highly variable (Chakraborty *et al*, 2014). Therefore, assessment of the mutation status will probably become an important clinical feature of LCH therapy to personalize and optimize therapeutic regimens.

LCH as an inflammatory myeloid neoplasia

The identification of the frequent *BRAF* V600E mutation in 2010 tipped the scales of the historical LCH debate to favour the classification of LCH as a neoplastic disorder. The mutation can be detected in various neoplastic diseases and seems to play a pivotal role in their pathology. The impact of *BRAF* V600E appears thereby to depend on the cellular context as it is frequently observed in more benign conditions, such as

epidermal nevi and colon polyps, as well as in highly aggressive malignancies, such as malignant melanoma (Cantwell-Dorris *et al*, 2011; Pratilas *et al*, 2012).

Discovery of *BRAF* V600E within the lesions provided an important foothold from which to understand pathogenesis of LCH, however, the specific impact of *BRAF* V600E on LCH pathogenesis remained elusive. To analyse the potential function of *BRAF* V600E mutation as an essential driver of LCH pathology, we recently crossed mice expressing the conditional *BRAF* V600E allele with transgenic mice expressing the cre-recombinase under control of the langerin promoter to enforce expression of *BRAF* V600E in langerin-expressing DC (differentiated epidermal LCs and langerin-expressing lymphoid and non-lymphoid DCs). To determine the significance of the stage during DC differentation at which the somatic mutation occurs, we also crossed mice with the conditional *BRAF* V600E allele to mice expressing cre-recombinase under the control of CD11c, which is already expressed in DC progenitors and immature DCs (for further details please see (Berres *et al*, 2014). Mice of both strains spontaneously developed classical granuloma-like lesions characterized by the accumulation of $CD207^+$ histiocytes by 2–6 months of age. The accumulation of histiocytes was associated with increased expression of specific cytokines and chemokines and the recruitment of additional inflammatory cell e.g. macrophages, multi-nucleated giant cells, B cells and T cells (including T regulatory cells), comparable to human LCH lesions. Interestingly, induction of *BRAF* V600E expression in circulating blood and bone marrow resident DC progenitors using the CD11c promoter system resulted in a much more severe and accelerated course, approximating the human high-risk LCH phenotype. Taken together, these results provide evidence that expression of oncogenic *BRAF* V600E in cells within the mononuclear phagocytes system is sufficient to induce LCH-like disease in mice, leading not only to the accumulation of $CD207^+$ histiocytes, but also to the recruitment of additional inflammatory cells and subsequent formation of granuloma with highly active cytokine expression. These data support the ability of MAPK activation in myeloid DC precursors to drive myeloid differentiation and granuloma formation in mice, similar to pathology observed in LCH. We further propose that LCH should be defined as an *inflammatory myeloid neoplasia* characterized by the formation of highly active, inflammatory granuloma induced by transformed DC clones.

LCH as consequence of misguided myeloid differentiation

Revisiting the cell of origin

As discussed above, it has been assumed that aberrant or transformed epidermal LCs embody the cellular origin of the histiocytes in LCH, which was mainly based on certain structural similarities of both cells types, including intracytoplasmatic Birbeck granules and especially the common expression of the antigen CD1A and the C-type lectin langerin (CD207) (Nezelof *et al*, 1973). Both features have traditionally been considered to be exclusive to LCs.

Langerhans cells represent a specific subpopulation of cells of the mononuclear phagocyte system residing in the outer layer of the skin, the epidermis. They are characterized by lower major histocompatibility complex (MHC)-II expression levels, intermediate CD11c levels as compared to other DCs and high expression of langerin (Merad *et al*, 2008). LCs arise from embryonic precursors – mostly fetal liver-derived monocytes (Hoeffel *et al*, 2012) that populate the skin prior to birth and maintain themselves locally throughout life in steady state conditions (Merad *et al*, 2002). Following severe inflammatory injury, such as ultraviolet (UV) light exposure, a transient population of LCs can be identified in the skin that derive from circulating Gr-1^{hi} blood monocytes (Ginhoux *et al*, 2006; Sere *et al*, 2012). However, it remains unclear if these inflammation-associated, monocyte-derived LCs residing in the epidermis after UV light exposure are equivalent to steady-state LCs in terms of function and tissue homeostasis.

In contrast to a primary embryonic origin of LCs, most other classical DCs residing in lymphoid as well as the non-lymphoid tissue arise from common circulating precursors–the pre-DC (Naik *et al*, 2007; Onai *et al*, 2007; Liu *et al*, 2009). Pre-DC share a common progenitor with plasmocytoid DCs (pDC), which is thus termed common DC precursor or CDP. CDPs are localized in the bone marrow and originate from a multipotent bone marrow resident myeloid precursor named macrophage-DC progenitor (MDP), which gives rise to monocytes and DC but has lost granulocyte potential as compared to the granulocyte-monocyte progenitor (GMP) and thereby marks the stage of commitment to the mononuclear phagocyte systems during development (reviewed in Merad *et al*, 2013).

The concept that LCH cells represent transformed cells of LC origin has been recently challenged by the observation that the expression of langerin (CD207) and the formation of Birbeck granules is not exclusive to LC but can also be identified in other subpopulations of the mononuclear phagocyte system. These $CD207^+$ DCs constitutively reside in all lymphoid and non-lymphoid tissues where LCH lesions can be found in patients (Chikwava & Jaffe, 2004; Ginhoux *et al*, 2007; Poulin *et al*, 2007; Segerer *et al*, 2008; Helft *et al*, 2010). This is in striking contrast to the restricted tropism of LCs to the epidermis and skin-draining lymph nodes. Gene expression profiling studies demonstrated that human $CD207^+$ DCs within LCH lesions only display a minimal profile overlap with differentiated human LCs, while their profile is more consistent with those of immature myeloid DC precursors (Allen *et al*, 2010b). Additionally, the maturation status of LCH cells within the lesions is heterogenous with variable $CD1A^+/CD207^-$ subpopulations (Chikwava &

Jaffe, 2004; Coury *et al*, 2008; Peters *et al*, 2011) and extended immunohistological studies were able to identified expression of the common *BRAF* V600E mutation not only in $CD207^+$ cells but also in $CD207^-$ cells as well as in $CD14^+$ monocytes (Sahm *et al*, 2012).

These new findings open up the possibility that LCH might not, as tradionally speculated, result from the accumulation of dysregulated or transformed mature fetal liver-derived epidermal LCs, but may rather arise from dysregulated differentiation and/or recruitment of precursor cells from the bone marrow-derived myeloid lineage. A dysregulated differentiation of early multipotent myeloid progenitors as origin of the disease is also consistent with reports of concurrent or serial lesions of more than one histiocytic phenotype in individual cases with overlapping features of LCH, juvenile xanthogranuloma (JXG) and Erdheim-Chester disease (ECD) (Hoeger *et al*, 2001; Patrizi *et al*, 2004; Tsai *et al*, 2010; Haroche *et al*, 2013; Berres *et al*, 2014; Chakraborty *et al*, 2014; Hervier *et al*, 2014).

Misguided myeloid differentiation translate into disease severity and dissemination

Langerhans cell histiocytosis is characterized by a broad spectrum of clinical presentations ranging from single lesions to multiple lesions within a single organ system to multisystemic disease. As discussed above, the involvement of liver, spleen and bone marrow is associated with higher risk of mortality. Histologically, lesions of high risk patients are indistinguishable from those with low risk disease. Additionally, with the exception of disease recurrence after therapy, all studies fail to correlate the presence of certain mutation with disease outcome or stage.

So, which mechanisms regulate disease severity and tropism in LCH? Assessing the *BRAF* V600E mutational status in circulating cells of patients with LCH, we recently observed that in patients with high risk multi-system disease and *BRAF* V600E mutation in lesional DCs, *BRAF* V600E was also identified in circulating cells and resident bone marrow cells. Contrarily, circulating cells with *BRAF* V600E mutation were absent in the blood and bone marrow of all patients with single-system low risk disease (Berres *et al*, 2014). Lineage analysis further localized the *BRAF* V600E mutation to $CD11c^+$ DC as well as $CD14^+$ monocytes in high-risk patients. These results point to a somatic mutation of a specific common DC and monocyte progenitor in these cases. Indeed, when bone marrow aspirates of patients with circulating *BRAF* V600E positive cells were analysed, cells carrying the mutation were identified within the $CD34^+$ haematopoietic stem cell (HSC) compartment, and clonal potential for *BRAF* V600E positive cells was further confirmed by colony-forming-unit assays *in vitro*. Interestingly, in single cases where *BRAF* V600E was identified in $CD34^+$ cells, it was also detectable in the $CD19^+$ B cells as well as the $CD11c^+$ and $CD14^+$ fractions, but was absent from $CD3^+$ T cells (Berres *et al*, 2014).

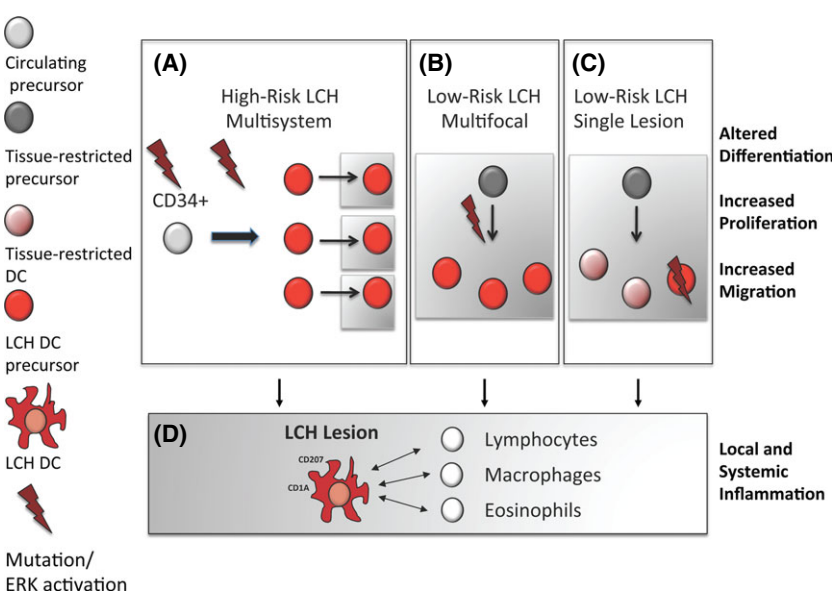

Fig 2. Developmental stage of pathological DC precursor defines extent of disease. (A) Somatic mutation of *BRAF* or other inciting event in $CD34^+$ haematopoietic stem cells or early DC progenitors induces proliferation, maturation and migration of pathological DCs in multiple tissues that results in high-risk multisystem LCH. (B) Somatic mutation of *BRAF* or other inciting event in a tissue-restricted DC precursor induces proliferation, maturation and tissue-limited migration of pathological DCs that results in low-risk multi-system LCH. (C) Somatic mutation of *BRAF* or other inciting event in mature DC results in proliferation and maturation of pathological DCs leading to low-risk single lesion LCH. (D) Regardless of cell of origin, the pathological DCs recruit 'bystander' immune cells in an inflammatory lesion characteristic of LCH. Cells in white areas indicate cells in circulation; cells in grey areas indicate cells that have migrated to tissue targets. LCH, Langerhans Cell Histiocytosis; DC, dendritic cell.

The association of disease severity with detection of *BRAF* V600E in HSC in the bone marrow is in line with the murine data presented above showing that expression of the mutation already at the stage of bone marrow resident DC progenitors resulted in an aggravated and accelerated phenotype with increased mortality as compared to langerin-driven expression, which is limited to the stage of differentiated DC (Berres *et al*, 2014).

We propose that the *misguided myeloid differentiation* model of LCH pathogenesis would account for the range of clinical manifestations in LCH. In this model, the extent of disease severity and dissemination is not defined by genomic or functional alteration at the histiocyte level but by the stage at which pathological ERK activation occurs during myeloid differentiation (Fig 2). Based on the data discussed above, we hypothesize that if the mutation occurs at the stage of a tissue-restricted precursor or even tissue-resident precursor/DC, disease will be limited to specific organs (multisystem low-risk) or even a single location (single lesion low risk). On the contrary, mutations impacting early myeloid progenitors with high proliferative capacity and multipotent potential, the disease will be highly aggressive and disseminate to various organs resulting in high-risk multisystemic disease. In all cases, the pathological DC seed to the tissue and follow a common path of terminal differentiation in which they acquire the expression of CD207 and CD1A and recruit and activate additional inflammatory cells, e.g., macrophages, eosinophils, B cells and T cells, to the lesion, mediated by altered cytokine/chemokines expression. This will then result in the formation of complex LCH granuloma. However, the relative contributions of these recruited 'innocent bystander' cells to LCH pathogenesis are uncertain.

While ERK activiation appears to be universal in LCH, the mechanisms through which ERK activation drives pathogenesis in myeloid precursors in LCH remain to be defined. Interestingly, *BRAF* V600E has been identified in $CD34^+$ haematopoeitic cells in patients with hairy cell leukaemia (Chung *et al*, 2014). Similarly, some patients display the *BRAF* V600E mutation in hybrid, synchronous or serial LCH/JXG or LCH/ECD lesions (Berres *et al*, 2014; Hervier *et al*, 2014). Furthermore, mutations in *NRAS*, upstream from RAF, have been described in ECD, but not LCH (Fig 1) (Diamond *et al*, 2013; Emile *et al*, 2014). The terminal phenotype of cells in which ERK is pathologically activated may depend on the state of differentiation of the original cell, the somatic mutation, or additional genetic or epigenetic factors that remain to be defined.

Clinical Implications of the misguided myeloid differentiation model

If future studies support the early observation that *BRAF* V600E confers increased risk of refractory/recurrent disease, these patients may benefit from prolonged or intensified chemotherapy. Furthermore, once the genetic lesion is established, the presence of circulating cells that carry the mutation may become a valuable diagnostic tool to define high *versus* low-risk disease. Finally, in patients in whom circulating cells bearing the somatic mutation are identified, persistence of circulating cells with the mutation may be a marker with which to follow residual disease (Berres *et al*, 2014).

While the Histiocyte Society and other cooperative group trials have contributed tremendously to the understanding and treatment of LCH, overall outcomes remain suboptimal. Due to the scientific progress on LCH pathology in the last years, we now have the opportunity to move beyond empiricism to rationale strategies. The relative efficacy of the second generation nucleoside analogue, clofarabine, in patients refractory to other therapies may be consistent with its known activity against immature myeloid cells in AML and myelodysplastic syndrome (Ghanem *et al*, 2013).

In addition to refining the therapeutic profile of chemotherapy strategies, recurrent somatic mutations in LCH offer an opportunity to develop targeted therapeutic strategies. Mutations in the MAPK pathway have been identified in approximately 75% of patients with LCH, and drugs that specifically inhibit kinase activity of RAF, MEK and ERK proteins have been developed and are in various stages of therapeutic evaluation for paediatric patients (Belden & Flaherty, 2012). Unlike melanoma or other aggressive malignancies associated with MAPK activation, the overall genomic landscape of LCH lesions is relatively quiet, which may make the efficiacy of these agents more durable in LCH than in tumours that can rapidly develop resistance through multiple mechanisms including acquisition of new mutations (Poulikakos & Rosen, 2011; van Allen *et al*, 2014). An early report of vemurafanib, which has activity against BRAFV 600E, in patients with mixed ECD and LCH, demonstrated promising early responses (Haroche *et al*, 2013). However, the side effect profile of BRAF inhibitors, which includes high rates of *de novo* squamous cell carcinoma among other toxicities, makes the the risk-benefit profile of these agents in LCH uncertain relative to rapidly fatal *BRAF* V600E-associated malignancies such as melanoma. Dabrafenib, another agent with activity against activated BRAF, is being evaluated in a paediatric phase I/II study that includes patients with LCH (clinicaltrials.gov NCT01677741). Efficacy, tolerability and optimal role for targeted therapies along with or instead of chemotherapeutic agents will be defined in future clinical trials.

Conclusions

Langerhans cell histiocytosis is a disease caused by somatic driver mutations at critical stages of myeloid differentiation that result in cellular transformation. An important implication of the new understanding of LCH biology is the re-definition of LCH as an inflammatory myeloid neoplasia. Like other neoplastic myeloid disorders, we believe LCH should be considered under the umbrella of cancer research. Patients

with LCH will ultimately benefit from inclusion in portfolios of cooperative cancer networks to be considered for research funding and clinical trial support. The recent advances in the understanding of LCH pathogenesis also question if 'Langerhans cell histiocytosis' is the appropriate nomenclature, given that pathological lesional cells arise from variable points along the myeloid/monocytic lineage. 'Histiocytosis X' may be more accurate after all. While ERK activation appears to be universal in LCH, pathways of ERK activation and cellular context in which pathological ERK activation arises are variable. Increased understanding of LCH pathogenesis will provide opportunities to optimize and personalize therapy through improved risk-stratification, targeted therapy and assessment of therapy response based on specific molecular features and origin of the pathological cells.

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Author contributions

All author's wrote substantial parts of the manuscript and revised all final versions.

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