

Langerhans cell histiocytosis in children: from the bench to bedside for an updated therapy

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Summary

Langerhans cell histiocytosis (LCH) is a rare disease, affecting subjects of any age, with extremely variable clinical manifestations. Although most patients with LCH have localized disease, requiring local or even no therapy, those patients with disseminated, 'multi-system' disease require specific therapy because they may be at risk for morbidity or even mortality. The current standard of care has developed empirically, based mainly on the experience of treating children with leukaemia and other haemo-proliferative disorders. At the time of writing, the combined use of vinblastine and prednisone remains the standard of care for children with multi-system LCH. The combination of cytarabine and cladribine is the current standard for second-line therapy of refractory cases with vital organ dysfunction. Recent advances in the knowledge of the pathogenesis of LCH may support a change in treatment strategy. Evidence of mutations that aberrantly activate RAF/MEK/ERK signalling in over two thirds of patients with LCH may direct a target therapy strategy. Vemurafenib, a small molecule widely used in the treatment of melanoma, is the main candidate for testing in prospective trials for patients with evidence of *BRAF*^{V600E} mutation on lesional tissue. Additional molecules, including the recently approved trametinib, could follow. Identification of mutations in other genes in the remaining multisystem LCH cases could contribute to define a scenario in which target therapy becomes the main therapeutic choice in this intriguing disorder. However, because the long-term risks and benefits of these agents in children are unknown, and other effective treatments exist for many LCH patients, the optimal indications for administering a tyrosine kinase inhibitor to children is an open question.

Keywords: histiocytosis, vemurafenib, RAF, MAP2K1.

Langerhans cell histiocytosis (LCH) is a rare disease that affects subjects of any age (Favara *et al*, 1997; Aricò & Egeler,

1998; Aricò *et al*, 2003). Its clinical manifestations are extremely variable, inasmuch as they may range from a solitary, asymptomatic osteolytic lesion (originally reported as 'eosinophilic granuloma') with propensity to spontaneously heal, to several osteolytic lesions of the skull, intriguingly associated with exophthalmos and diabetes insipidus, originally reported under the name 'Hand-Schuller-Christian' disease. Furthermore, in a minority of patients, more frequently toddlers, LCH may have a very aggressive clinical course: the disease presents when already disseminated to various organs other than bones (particularly skin, liver and spleen), rapidly becoming as life-threatening as childhood acute lymphoblastic leukaemia (ALL). This phenotype had been originally described as the 'Abt-Letterer-Siwe' disease. After the 'pioneer' phase, since 1953 the disease has been indicated as a unique entity, with a common disease spectrum, defined as Histiocytosis X (Lichtenstein, 1953). This name highlighted the pivotal role of histiocytic cell in the pathogenesis of the disease, in particular pointing to its dysregulated proliferation due to an unknown trigger. Finally, the denomination of Langerhans cell histiocytosis was adopted and commonly used (Favara *et al*, 1997).

Treatment of LCH: who deserves treatment?

Given that the individual granuloma at the basis of LCH has a propensity toward spontaneous healing, treatment should aim to limit its duration, or the damage which may be induced by the phase of granuloma activity. The damage may derive from either the destruction of an anatomic structure, e.g. a weight-bearing bone, or, less frequently, from the functional damage induced by disseminated granuloma in a tissue with vital function, such as the liver, lung or brain tissue. The combination of such concepts may build the individual need for LCH-directed therapy.

Treatment of localized LCH

Skin and bone are the most frequently involved sites in children with LCH (Aricò & Egeler, 1998; Weitzman & Egeler, 2005). This explains why, in most cases, childhood LCH is a disease with limited aggressiveness and minimal or no risk for life (Aricò & Egeler, 1998; Kim *et al*, 2014). As a rule, the

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intention-to-treat should never exceed the amount of discomfort caused by the disease itself. Thus, once the diagnosis has been assessed (which means, for instance, that the differential diagnosis versus bone tumour or dermatitis from another cause has been cleared), the attending clinician should consider watchful waiting as the first and main hypothesis. The parents should receive a wide explanation of the biology of the disease and its propensity to self-healing, thus supporting the choice not to treat the bone lesion or skin rash. Clinical follow-up should be the standing point, providing the parents with confidence that nothing very dangerous is expected to happen in the near future, and blood checks are not expected to provide trustful disease markers to monitor.

In a minority of cases the single bone lesion in a weight-bearing segment may represent a risk for future bone deformity or even morbidity (Haupt *et al*, 2004; Nanduri *et al*, 2006). This may be the case for isolated vertebral infiltrates, especially when peri-lesional, neoformed tissue is evident. In such cases, decompression or removal of the 'neoplastic' cuff may be considered or performed in the initial neuro/orthopaedic surgical approach. To this issue, it is important to remember that removal of a single bone lesion of the skull is usually not only unnecessary, but could be a cause for morbidity or even require the placement of a prosthesis. In most cases, that osteolytic lesion is expected to progressively heal within months; thus the surgeon should limit the intervention to collection of a diagnostic sample, rather than aiming at a 'surgical cure'.

Although it must be acknowledged that even biopsy itself may exert some therapeutic effect and speed-up the healing process, large osteolytic lesions in weight-bearing long bones have been treated with intralesional steroids (Egeler *et al*, 1992; Herwig *et al*, 2003; Putters *et al*, 2005; Ruggieri *et al*, 2009). This procedure, derived from other benign bone diseases by expert orthopaedic practitioners, still has value given that local effects of steroids may accelerate the healing process by inducing apoptosis of infiltrating inflammatory cells. The need for repeated infiltration is limited and time should be allowed for bone reconstitution. Orthopaedic aids should be provided during this time interval with the aim to reduce the risk of collapse of infiltrated vertebrae, which might displace and cause spinal damage. However, already collapsed vertebral bodies ('vertebra plana') usually do not need any further external support, as displacement is not a real risk. The prolonged use of unnecessary aids will cause undesired weakening of the regional muscles, thus complicating the healing process. Although radiotherapy is definitely effective in LCH, it must be considered that radiation-associated morbidity in children, whose tissues are expected to evolve and grow, may cause not only a limited risk of radiation-induced cancer, but also loss of growth and, sometimes, treatment-induced bone asymmetry (Aricò & Egeler, 1998; Haupt *et al*, 2004; Nanduri *et al*, 2006).

Differential diagnosis of skin isolated LCH from seborrhoeic dermatitis may be sometimes not easy. These

children usually do not require specific therapy, as spontaneous healing will occur in most cases. In exceptional cases, the use of topical nitrogen mustard has been effective in reducing the duration and the intensity of the disease course (Sheehan *et al*, 1991; Hoeger *et al*, 2000). Although this may be considered in rare cases of adults with long lasting deep skin lesions that prevent patients achieving the usual sitting position, thus limiting their working capacity, this treatment should not be considered as a common practice.

Sometimes, LCH affects only the bone tissue, thus being defined as a 'single system' disease; however, the number and distribution of osteolytic lesions, and also the symptoms, may induce the clinician to treat the child. In particular, retrospective analyses of such patients raised the hypothesis that children with multiples bone lesions, especially when affecting the cranio-facial region, may be at higher risk of sequelae, particularly of developing diabetes insipidus (DI) and multiple anterior pituitary hormone deficiency (Grois *et al*, 2006). On this basis, selected children with single-system but multiple bone lesions had access to limited intensity chemotherapy in the prospective LCH-II and III trials (see below). In a recent data collection from ten paediatric institutions across North America and Europe, 93 such children who were either treated with systemic therapy (chemotherapy, chemo-radiotherapy or radiotherapy; $n = 59$) or local therapy (biopsy, curettage and/or intralesional steroids; $n = 34$) were evaluated. The 5-year event-free survival (EFS) and overall survival were $80 \pm 5\%$ and $98 \pm 2\%$ in the systemic therapy group, *versus* $85 \pm 6\%$ and $95 \pm 5\%$ in the local therapy group, with no difference between the two treatment groups. (Chellapandian *et al*, 2015) Altogether these data, although not derived from a prospective randomized trial, suggest that systemic therapy is not effective in reducing the risk of recurrence or late sequelae in children with LCH and single-bone central nervous system (CNS)-risk lesions, as compared to local treatment, thus challenging the current indication. The use of anti-inflammatory monotherapy with indomethacin has been recently reported by Braier *et al* (2014) as effective and less toxic than other systemic agents.

Treatment of multisystem LCH

The minority of patients with LCH affecting vital organs offers a completely different landscape, because of their high risk of morbidity, and even mortality, due to end-organ dysfunction. The management of these patients was initially conducted by paediatric haematologist-oncologists on an empirical basis (Minkov, 2012). Thus, it reflected the different views and the uncertainty that have prevailed regarding the nature of the disease. Leukaemia-oriented chemotherapy was used in children with disseminated disease by the Austrian-German group during the 1980s, with favourable response (Gadner *et al*, 1987, 1994a). On the other hand,

based on the concept of an inflammatory disease, anti-inflammatory agents, especially steroids, were used by the British group (McLelland *et al*, 1990); although this enabled disease control in most cases, children with chronic/reactivating LCH developed unacceptable side effects of long-lasting steroid exposure (Nanduri *et al*, 2006). In the early 1990s, an international cooperative group of paediatric haematology-oncology specialists was formed that was able to conduct three consecutive randomized trials in this 'orphan disease' (Gadner *et al*, 2001, 2008, 2013). Overall, these studies provided meaningful lessons.

The prospective randomized clinical trials

In the first study, named LCH-I and conducted between 1991 and 1995, a 6-month therapy of vinblastine or etoposide, together with an initial 3-day pulse of prednisone, was compared in all patients with multisystem-LCH; the results were equivalent with respect to response, survival, disease reactivation, permanent consequences and toxicity (Gadner *et al*, 2001). However, the proportion of early response and prevention of disease reactivation appeared to be inferior to results obtained with the more aggressive (five drug combination) and longer (12 months duration) DAL-HX83/90 protocols conducted by the German-Austrian group (Gadner *et al*, 1987, 1994a; Minkov *et al*, 2000), suggesting a need to intensify treatment.

In the second study, LCH-II, conducted between 1996 and 2001, patients with multisystem-LCH were stratified by risk, with patients aged less than 2 years and/or involvement of 'risk organ' (i.e. liver, spleen, haematopoietic system or lung) being stratified as 'high risk'. Based on the finding of LCH-I, to achieve treatment intensification, they were treated with the standard combination of prednisone and vinblastine, and randomized to receive or not additional etoposide. This study was successful inasmuch, in both treatment arms, these patients showed faster disease resolution and a higher survival rate than those enrolled in LCH-I. However, an unacceptably high rate of patients, 44%, still reactivated. Remarkably, lack of advantage, together with its reported leukaemogenic potential, put etoposide outside the mainstream of therapeutic studies in multisystem LCH (Gadner *et al*, 1994b, 2008).

In the LCH-III study, run between 2001 and 2008, the efficacy of increasing intensity, achieved by adding methotrexate in risk organ patients (treated for 12 months) and by a prolonged initial intense therapy in patients with only partially response by 6 weeks, was tested. Nevertheless, it did not provide a better outcome compared to the previous studies. Otherwise, in patients with standard risk LCH, who did not require treatment intensification to prevent end-stage organ dysfunction, but rather a reduction of the disease reactivations, patients randomized to an extended duration of treatment of 12 months had a significantly better outcome than those treated for only 6 months, in terms of

reduced rate of disease reactivation once they had achieved disease control (Gadner *et al*, 2013).

Standard of care and open questions

The above prospective trial results showed that the combination of vinblastine and low-dose steroids proved to be extremely effective in the vast majority of patients at the disease onset and even at the time of disease reactivation. A large body of evidence supports the concept that children who receive weekly doses of vinblastine do not show limiting toxicity; also, low-dose steroids for 4 weeks, followed by short pulses three-weeks apart, do not induce undesired, cushingoid toxicity. Thus, this combination is still recommended as the standard of care for front-line therapy of multi-system LCH in children, aiming at limitation of the disease course and thus also of permanent consequences.

It may be worth mentioning that, in a recent retrospective review of mono-institutional experience, of 38 patients treated with regimens containing 3- to 4-week interval pulses of low dose cytarabine, including 16 patients treated for *de novo* LCH, 14 (88%) achieved non-active disease within one year of therapy (Simko *et al*, 2015).

Most of the treating paediatricians had experience on the definition of the prognostic value of early response to treatment in ALL patients. Similarly to that observed in ALL, *in vivo* response to front-line therapy identifies, by week 2–4, a small cohort of LCH patients. Patients with multisystem LCH and 'poor-response' are at the highest risk of treatment failure and fatal outcome (in up to 40% of cases), driven by disease progression in vital organs. (Gadner *et al*, 2001, 2008, 2013). They account for no more than 20% of multisystem LCH patients, and are characterized by involvement of liver, spleen and bone marrow.

Skeletal involvement is generally, but not universally, characteristic of LCH. In a recent analysis of 938 children with multisystem LCH, the presence of bone lesions at diagnosis was evaluated as a prognostic factor for survival. Risk organ involvement (RO+) was defined as: haematopoietic system (haemoglobin <100 g/l, and/or white blood cell (WBC) count <4.0 × 10⁹/l and/or platelet count <100 × 10⁹/l), spleen (>2 cm below the costal margin), liver (>3 cm and/or hypoproteinaemia, hypoalbuminaemia, hyperbilirubinaemia and/or increased transaminase). Given the general view that prognosis in LCH worsens with increasing extent of disease, the surprising finding was that in multisystem RO+ LCH the probability of survival with bone involvement of 74 ± 3% (*n* = 230, 56 events) was reduced to 62 ± 4% (*n* = 156, 55 events) if this was absent (*P* = 0.007). An even greater difference was seen in the subgroup of patients with both liver and either haematopoietic or spleen involvement: 61 ± 5% survival (*n* = 105; 52 events) if patients had bony lesions, vs. 47 ± 5% (*n* = 111; 39 events) if they did not (*P* = 0.014). This difference was retained in multivariate analysis (*P* = 0.048). Although as yet unexplained, these data suggest

that bone involvement at diagnosis is a previously unrecognized favourable prognostic factor in multisystem RO+ LCH. On the contrary, lack of bone lesions in patients with multisystem disease including liver and thrombocytopenia helps to profile patients with the worst prognosis (Aricò *et al*, 2015).

The French group has piloted an innovative experience of very intensive chemotherapy, similar to that used for acute myeloid leukaemia, which was shown to be useful in this subset of patients (Bernard *et al*, 2005). Based on this preliminary data, an international phase 2 study was run combining cladribine and cytarabine in patients with refractory, risk-organ-positive LCH. The protocol, comprising at least two 5-day courses of cytarabine (1 g/m²/day) plus cladribine (9 mg/m²/day) followed by maintenance therapy, was administered to 27 patients. At inclusion, all patients had liver and spleen involvement, and 25 patients had haematological cytopenia and were refractory after at least 1 course of vinblastine plus corticosteroid. After 2 courses of the investigated combination, the overall response rate was 92% and the median disease activity scores had decreased from 12 to 3 ($P < 0.0001$). All patients experienced severe toxicity, with World Health Organization grade 4 haematological toxicity and 6 documented severe infections. At a median follow-up of 5.3 years, the overall 5-year survival rate was 85%. There were four deaths; two were related to therapy toxicity and two were related to reactivation. Overall, six patients had developed disease reactivation: in two cases this was limited to the skin and was controlled by conventional therapy; in four cases it involved risk organs and required additional cladribine alone (in one case, who died soon thereafter) or in combination with cytarabine (one died and three are currently disease-free including one who received stem cell transplantation) (Donadieu *et al*, 2015). On the basis of these results, the combination of cladribine/cytarabine is effective for refractory multisystem LCH, although much caution must be paid to its high toxicity.

Recently, the use of clofarabine has been suggested as an alternative agent for second line therapy of refractory cases (Rodriguez-Galindo *et al*, 2008; Abraham *et al*, 2013; Simko *et al*, 2014).

Allogeneic haematopoietic stem cell transplantation (HSCT) has been performed in some patients with refractory LCH (Caselli & Aricò, 2008). A recent review explored the impact of the intensity of the conditioning regimen in 87 patients with high-risk LCH who were transplanted between 1990 and 2013. Prior to the year 2000, most patients underwent HSCT following myeloablative conditioning (MAC): only 5 of 20 patients (25%) survived with a high rate (55%) of transplant-related mortality. After the year 2000 an increasing number of patients underwent HSCT with reduced intensity conditioning (RIC): 49/67 (73%) patients survived, however, the improved survival was not overtly achieved by the introduction of RIC regimens, with similar 3-year probability of survival after MAC (77%) and RIC transplantation (71%). There was no significant difference in treatment-related mortality by conditioning regimen intensity. Nevertheless, the

relapse rates were significantly higher after RIC (28%) compared to MAC regimens (8%; $P = 0.02$), although most patients relapsing after RIC transplantation could be salvaged with further chemotherapy. Thus, HSCT may be a curative approach in three out of four patients with high risk LCH refractory to chemotherapy. The optimal conditioning regimen is not yet defined (Veys *et al*, 2015).

Additional unmet clinical needs lie ahead: about one third of patients who achieve complete control of the disease will suffer disease 'relapse' during the following months, most often within the same tissue/organ(s) type(s) involved at presentation. Fortunately, whereas in childhood leukaemia relapse is usually associated with very unfavourable prognosis, reappearance of LCH is usually amenable to treatment with the same agents used before, or even with anti-inflammatory agents (Minkov *et al*, 2008). For this reason, the term 'reactivation', initially proposed by Ladisch (1982), is now preferred to the term 'relapse'. Non-lethal disease reactivation in non-vital organs may cause additional morbidity for the LCH patient, and increase the anxiety for their families. The results of the prospective trials have documented that extended duration of the same, non-intensive therapy, may offer an advantage in the prevention of disease reactivation (Minkov *et al*, 2008). Thus, in keeping with the concept that bone repair requires extended time interval and that the immune system is probably able to progressively gain control over the exuberant inflammation/proliferation in LCH patients, children with LCH may have a better result when a non-aggressive, but long lasting, therapy is patiently administered over a period of not less than one year. This type of information should be promptly delivered to the family at the treatment start.

It is also important to explain that the main target of the treatment in LCH is to achieve initial disease control. Indeed, the main cause for treatment failure is the inability to achieve initial response. The analysis of the accumulated cohorts from the LCH prospective trials confirms that the risk of death is minimal in patients who have achieved a complete response, and that even patients with multisystem disease, once they have achieved disease control, do not have a worse prognosis than patients with multifocal bone disease, regardless that they may develop disease reactivations over the first 3 years from diagnosis (Minkov *et al*, 2008). This awareness has major implication in designing second-line treatment approaches, which never should expose the child to a major risk of treatment-related morbidity or even mortality.

Although non-lethal, a number of permanent consequences including DI, sclerosing cholangitis, pulmonary failure, neurodegeneration, reduced linear growth, other endocrine dysfunctions and bone deformities, may represent quite a heavy burden for the quality of life of patients cured of LCH (The French LCH Study Group, 1996; Willis *et al*, 1996; Haupt *et al*, 2004; Nanduri *et al*, 2006). DI is rare in children but not in those with multisystem LCH, in which it may reach a 12-20% prevalence (Maghnie *et al*, 2000; Grois *et al*, 2006; Minkov *et al*, 2008). It only appears when the vast majority of

cells in the supraoptic-paraventricular region of hypothalamus have been destroyed by LCH granuloma. This stage may be visualized as a bulging hypothalamus with thick pituitary stalk and lack of the posterior pituitary bright signal at magnetic resonance imaging. On this basis, it may be reasonable that, in the vast majority of patients, DI is not reversed by LCH-directed therapy, with very few exceptions. Those patients, even when cured of LCH, still need replacement therapy with desmopressin and are exposed to the risk of multiple pituitary hormone deficiency, affecting most often the linear growth (because of acquired growth hormone deficiency) and thyroid function. Both of these are amenable to effective treatment with replacement therapy, which should not thus be missed. In particular, there is no evidence of any adverse effect of growth hormone replacement therapy on the LCH disease course (Donadieu *et al*, 2004).

The inflammatory burst of active LCH may cause the destruction of lung or liver functional tissue and later replacement by fibrosis. This process drives the patient to progressive functional defect with oxygen-dependency or even to end-stage insufficiency (Aricò *et al*, 2000). A comparable picture may develop in patients with progressive liver infiltration causing 'cirrhosis' or, as we currently prefer to call it, 'sclerosing cholangitis'. None of the two above described clinical pictures may be reversed by any LCH-directed therapy; they are observed in patients with frequently inactive LCH, following specific therapies, which in some cases may have included haematopoietic stem cell transplantation. Solid organ – either lung or liver – transplant has been performed in those patients with some success (Melendez *et al*, 1996; Suri *et al*, 2012). However, this remains a very aggressive procedure that is not easily and promptly accessible to all patients, with enormous economic and social costs. Furthermore, LCH reactivation may occur in the engrafted organ (Hadzic *et al*, 2000). Thus, in those patients who still have signs of active LCH, a special effort should be made to achieve complete disease control before solid organ transplant (Scanzi *et al*, 2015).

A small number of patients with LCH, especially among those with DI, may develop bilateral symmetric alterations in the cerebellar grey matter, basal ganglia and brainstem. Analysis of lesional tissue from biopsies or necropsies revealed neuronal loss, axonal degeneration and a profound T-cell inflammation. The pathogenesis of this remains unsolved, and propagation from long-standing granulomatous lesions of the craniofacial bones to the intracranial space, with stimulation of chemokine/cytokine tissue damage or initiation of an autoimmune response to brain components has been suggested (Grois *et al*, 2005). Neurodegenerative (ND)-LCH may be devastating and, unfortunately, no effective therapy is available so far. The follow-up of eight Japanese children with ND-LCH treated with intravenous immunoglobulin (IVIG) for >3 years has been reported (Imashuku *et al*, 2015). After a median follow-up time of 11.6 years, the median Expanded Disability Status Scale (EDSS) score of the

eight patients was 4.0 (range 2.0–9.5). At the last follow-up as of March 2014, three patients have low EDSS scores (<3.0) and can walk without any assistance. Another three patients have EDSS scores of 3.5–4.5 and can walk by themselves, albeit occasionally with support. However, the remaining two patients are wheelchair bound or bed ridden. The academic performance of seven of the eight patients was below average. IVIG appeared to be most beneficial when it was administered soon after ND-CNS disease diagnosis when the EDSS scores were low (1.0–2.5). Based on this limited observation, to prevent progression of disease, the authors proposed that IVIG should be initiated early and continued for >3 years (Imashuku *et al*, 2015). Identification of patients in the earlier stage of ND-LCH could thus be beneficial. Recently, the combined use of somato-sensory evoked potentials and careful neurological evaluation has been proposed as a valuable, low-cost, well-tolerated and easily available methodology to monitor LCH patients from pre-symptomatic to symptomatic stages. This might identify a population target for future therapeutic trials (Sieni *et al*, 2015).

Target therapies in LCH

The precise chain of events driving lesional granuloma formation in LCH has remained elusive for many years (Lichtenstein, 1953; Coury *et al*, 2008; Delprat & Aricò, 2014). However, novel therapeutic approaches may result from the recent advances in this field.

The observation made by Badalian-Very *et al* (2010), that 57% of the archived specimens of LCH tissue carried the $BRAF^{V600E}$ mutation opened a novel research avenue in LCH. Haroche *et al* (2012) confirmed this finding but were unable to document $BRAF^{V600E}$ in peripheral blood cells of patients with LCH, confirming that it is a somatic mutation within the lesional cells. This picture was further clarified by a more recent study of 100 LCH lesions, of which 64 carried the $BRAF^{V600E}$ mutation within infiltrating CD207+ dendritic cells; patients with active, high-risk LCH were found to carry $BRAF^{V600E}$ in circulating CD11c+ and CD14+ fractions and in bone marrow CD34+ haematopoietic cell progenitors, while the mutation was restricted to lesional CD207+ dendritic cells in low-risk patients. In a new mouse model, the expression of conditional $BRAF^{V600E}$ enforced under the langerin promoter was sufficient to drive LCH-like disease (Berres *et al*, 2014). In summary, while expression of $BRAF^{V600E}$ in marrow dendritic cells progenitors was found to recapitulate the human high-risk LCH, $BRAF^{V600E}$ expression in differentiated dendritic cells resembled low-risk LCH. Based on these findings, the authors proposed that of LCH should be classified as a myeloid neoplasia.

Subsequently, Nelson *et al* (2014) showed, by whole exome sequencing, that *ARAF* may also be involved and the relative kinase activity of this mutant *ARAF* molecule is comparable to $BRAF^{V600E}$, resulting in the constitutive activation of *ARAF* kinase.

Nevertheless, at this stage, *BRAF* (and exceptional *ARAF*) mutations accounted for only a minority of LCH cases. Thus, with the aim of investigating other somatic mutation, different groups analysed available cases of LCH to screen other mutations in cases wild type. The role of *MAP2K1* mutation was established in two independent and simultaneous reports in September 2014. Brown *et al* (2014) reported that out of 40 cases of LCH, 18 (45%) had *BRAF* mutation while 11 (27%) had *MAP2K1* mutation. In their simultaneous paper, Chakraborty *et al* (2014) performed whole exome sequencing on samples from lesional and normal tissues obtained from 41 patients. In this series, 20/41 cases had somatic *BRAF* mutation, while seven cases harboured mutations in *MAP2K1*. Those last mutations were different and, when tested *in vitro*, were documented to induce phosphorylation of extracellular signal-regulated kinase (ERK). Furthermore, somatic mutations of the mitogen-activated protein kinase (MAPK) pathway genes *ARAF* and *ERBB3* were also detected in two cases, intriguingly both with the combination of LCH and Erdheim–Chester Disease (ECD) (Chakraborty *et al*, 2014). Similar results were reported a few months later by Nelson *et al*, (2015). Based on the above studies, mutations in *BRAF* or in *MAP2K1* are mutually exclusive, with *MAP2K1* being involved in a minority of LCH cases, accounting for one-third to one-quarter of the total (Chakraborty *et al*, 2014).

Overall, the current knowledge supports the concept that activation of the ERK pathway may result from mutation in genes encoding one of the upstream proteins, with pathogenic value in LCH. This may have biological and clinical implications. On one side, evidence of a recognizable pathogenic pathway in about two thirds of cases provides further impetus for comprehensive genomic analysis of the remaining one third of cases (Shannon & Hermiston, 2014). Indeed, performing whole exome sequencing of additional patients without *RAF* or *MAP2K1* mutations appears to be the logical next step toward unravelling the pathogenesis of LCH.

But even more important is the potential for a novel, targeted therapeutic approach to LCH. Rapid and dramatic

responses to vemurafenib of three adults with ECD or LCH with *BRAF*^{V600E} mutations opened the avenue to targeted therapy (Haroche *et al*, 2013). Héritier *et al* (2015) reported the off-label treatment with vemurafenib of an 8-month-old girl with disseminated *BRAF*^{V600E}LCH (affecting skin, bone, gut, node and spleen and with haematological dysfunction) who was refractory to first and second line therapy: the treatment was very effective with complete disease resolution. Treatment discontinuation was followed by disease reactivation, which was again sensitive to re-treatment with vemurafenib. At 5 months after the second discontinuation of vemurafenib therapy, the patient remained in complete remission without any sequelae (Héritier *et al*, 2015). The same French group reported very recently good results with vemurafenib as first line therapy in *BRAF*^{V600E}LCH and *BRAF*^{V600E}ECD (Haroche *et al*, 2015a,b), and in one case of neurohistiocytosis (Euskirchen *et al*, 2015).

In summary, recent advances in the knowledge of the pathogenesis of LCH may support a change in the treatment strategy (Allen *et al*, 2015). At the time of writing, the combination of vinblastine and prednisone remains the standard of care for children with multi-system LCH, either front-line but also in most cases of reactivation in non-vital organs. The combination of cytarabine and cladribine is the current standard for second-line therapy of refractory cases with vital organ dysfunction. In the meanwhile, a prospective phase II trial for evaluation of target therapy of patients with evidence of mutations that aberrantly activate RAF/MEK/ERK signalling appears warranted. While vemurafenib is the main candidate agent to be tested, the recently approved MEK inhibitor trametinib appears to be a potential alternative (Flaherty *et al*, 2012). The long-term effects of vemurafenib are quite well understood. However, because the long-term risks and benefits of these agents in children are unknown, and other effective treatments exist for many patients with LCH, the optimal indications for administering a tyrosine kinase inhibitor—particularly to children—is an open question.

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