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Apport de la biologie moléculaire dans la prise en charge des histiocytoses langerhansiennes de l'enfant

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Abstract Langerhans cell histiocytosis (LCH) is characterized by inflammatory lesions containing abundant CD1a+ CD207+ histiocytes that lead to the destruction of affected tissues. This disease has a remarkable pleiotropic clinical presentation and most commonly affects young children. Although the current mortality rate is very low for childhood LCH patients (<2%), reactivation frequently occurs after a long period of disease control and the rates of permanent complications and sequelae remain high. Advances in genomic sequencing technologies in this past decade have highlighting somatic molecular alterations responsible for the disease in around 80% of childhood LCH cases. More than half these cases harbored the *BRAF*^{V600E} mutation, and most other mutations also concerned proteins involved in the MAPKinase pathway. In addition to improving what is known about the LCH pathology, this molecular knowledge provides opportunities to optimize patient management. The *BRAF*^{V600E} mutation is associated with more severe presentations of the disease, a high reactivation rate, and a high permanent complications rate; thus, this mutation paves the way for future stratified management approaches. These therapies would be based on the patient's molecular status as well as other clinical characteristics of the disease that are independently associated with undesired events. Moreover, as observed in patients with solid tumors, the *BRAF*^{V600E} allele can be detected in the circulating cell-free DNA of patients with severe *BRAF*^{V600E}-mutated LCH. Quantification of the plasmatic *BRAF*^{V600E} load for this group of patient can precisely monitor response to therapy. Finally, targeted therapies, such as BRAF inhibitors, are new therapeutic options especially for refractory multi-systemic LCH involving risk organs. However, the long-term efficacy, long-term tolerance, optimal protocol scheme, and appropriate modalities of administration for these innovative therapies for children still need to be defined and this is a huge challenge.

Keywords: Langerhans cell histiocytosis, BRAFV600E, targeted therapy, circulating cell-free DNA, biomarker, histiocytoses

1. Introduction

Langerhans cell histiocytosis (LCH) is a relatively rare disease, but is of interest to a large number of pediatricians because of the wide clinical expression of this disease and its pediatric predominance. The first "revolution" in histiocytosis was the unification of various clinical conditions with shared histopathologic characteristics under the ‘Histiocytosis X’ name, secondly rebranded as LCH by Nezelof et al. in 1973. The next 30 years were spent empirically developing guidelines and protocols for the optimal management of patients. Since 2010, the growing molecular knowledge of histiocytosis opened a new chapter for this disease that could be considered a second "revolution". The purpose of this review is to present these latest discoveries and show how they can benefit patient care.

2. Langerhans cell histiocytosis diagnosis and clinical overview

2.1 Pathological considerations

Histiocytoses refer to rare disorders characterized by the accumulation of cells of the mononuclear phagocyte system in various tissues and organs. The classification of histiocytoses was recently revised [1], and LCH is the most common subtype. In LCH, the phenotypic features of the pathological histiocytes resemble epidermal Langerhans cells (surface expression of CD207 [Langerin] and CD1a, detection of Birbeck granules by electron microscopy), and this has led to the disease name; although, the precise origin of the pathological histiocytes in LCH is unclear. The current hypotheses is that these pathological cells were more likely to arise from dysregulated differentiation or recruitment of bone marrow–derived precursor cells or yolk-sac progenitors than from epidermal Langerhans cells [2,3].

In the lesional tissue, pathological CD1a+ CD207+ histiocytes were associated with an inflammatory background, including variable numbers of eosinophils, macrophages,

multinucleated giant cells, and lymphocytes (enriched for regulatory T cells). Basically, CD1a+ CD207+ histiocytes showed low mitotic activity and did not have the cytonuclear features of malignant cells. The combined staining CD1a and CD207 is recommended for differentiating LCH from indeterminate cell histiocytosis (ICH) which is associated with a specific translocation (*ETV3-NCOA2*) [1]. The LCH diagnosis should always be based on the histological and immunophenotypic examination of lesional tissue taken from the most easily accessible, yet representative lesion. Rare patients without a histologically confirmed diagnosis, because of the risk of the biopsy or an inconclusive result, should be closely monitored and treatment with systemic chemotherapy should be avoided [4].

2.2 Clinical presentation

LCH most commonly affects children with annual incidences of approximately five children per million [5] and one adult per million. This disease is considered sporadic, without genetic susceptibility, and the only risk factor clearly identified is smoking in adults with lung LCH.

The clinical behavior of LCH is remarkably heterogeneous with some cases being limited, indolent, and self-regressive; while, other cases are multi-systemic, refractory to standard therapy, and even life threatening. In childhood LCH (age at diagnosis < 18 years), the disease affects mostly young children as the median age at diagnosis is 3.2 years and there is a slight male predominance (56% of cases). LCH can affect nearly all organ systems, but those more frequently affected in childhood LCH are the bones (80% of cases), skin (35%), and the pituitary gland (25%), followed by the liver, spleen, hematopoietic system, or lungs (15% each), lymph nodes (8%), and central nervous system (CNS) (2-4% excluding the pituitary) [6,7].

2.3 Management of LCH patients: progress and remaining issues

After initial screening tests [4], minimal therapy (symptomatic treatment, local therapy, and/or a wait and see strategy) without systemic chemotherapy is the recommended option for many patients (45%) [6,7]. Other patients benefit from first line chemotherapy with vinblastine and steroid therapy, which is well tolerated in children and has a response rate around 85% [7]. For each patient, the extension of the disease is established by the classification of the international medical society of histiocytoses, the "Histiocyte Society", which considers the number of organs affected, lung involvement, and the involvement of risk organs (ROs), which are the liver, spleen, and hematological system. In childhood LCH, 60% of patients have single-system (SS) LCH, i.e. one organ system affected and no lung or RO involvement; 20% of patients have multi-system (MS) RO- LCH, i.e. multiple systems involved, but no lung or RO involvement; 5% of patients have Lung+ LCH, i.e. lung involvement, but no RO involvement. Finally, 15% of patients have MS RO+ LCH, i.e. multiple systems affected and at least one RO involved [6,7]. This last presentation typically affects young children before 2 years of age and was considered a high risk disease because of the lower response rate to chemotherapy [8,9] and the up to 50% mortality rates reported by historic cohorts [10].

Nowadays, the 10-year survival rate of childhood LCH is estimated to be higher than 98% [6], thanks to progress in the management of childhood LCH in the past two decades, obtained through collective international efforts and prospective multi-center trials conducted by the International Histiocyte Society [8,9,11–13]. The efficacy of the combined cladribine and cytarabine chemotherapy has greatly improved the prognosis, especially for refractory MS RO+ patients, [13]. However, this therapy has extremely high treatment-related toxicity.

Other issues were the persistent high rate of reactivation and permanent sequelae. The current 5-year reactivation rate is between 30 – 40% [6,7], with reactivation defined as the

reappearance of signs and symptoms of active disease after either complete disease resolution or a period of disease control that persisted for more than 3 months on maintenance therapy. This high reactivation rate was similarly reported in all published pediatric LCH cohorts [14,15]; although the reactivation rate appears to be lower in SS unifocal disease [15] and has been reduced by prolonging initial chemotherapy for patient with MS disease [12]. Little progress has been made in the reduction of the rate of permanent complications or sequelae [6], which affect 20 to 50% of children with LCH, depending on the cohort recruitment process and the follow-up of published cohorts [6,16]. Pituitary hormone deficiency is the most frequent permanent sequelae, affecting 15–30% of patients, and it is irreversible. This pituitary involvement is almost always responsible of diabetes insipidus, and is associated with anterior pituitary hormone deficiency in half those cases [6,16]. Orthopedic sequelae, esthetic sequelae, or hearing impairments were variably reported and respiratory insufficiency is very unusual in childhood LCH [6,10,16]. Neurodegenerative LCH is rare (2–8% of patients) [17] but remains a particularly feared late onset complication of LCH disease because of the associated severe disability. Manifestations of neurodegenerative LCH are radiological along with clinical symptoms of varying intensity: magnetic resonance imaging shows signal changes in the brain stem, basal ganglia, and cerebellum and patients progressively develop clinical symptoms such as cerebellar ataxia, cognitive disorders, motor involvement, and pseudobulbar palsy. The mechanisms responsible for these neurodegenerative lesions are unclear.

Also, sclerosing cholangitis secondary to damage of the bile ducts is very rare (~2% of patients) [6], but challenging to manage and often results in liver failure with the need for transplantation. Thus, in 2018, childhood LCH should no longer be considered a lethal disease but the following challenges persist: i) finding new therapeutic options for refractory MS RO+ patients with lower toxicity; ii) determining predictive markers for reactivation,

permanent complications, and sequelae to develop tailored strategies that are preventive, preemptive, and more effective early on.

3. Recent advances in molecular knowledge of LCH

Until 2010, debates on LCH pathogens revolved around the neoplastic nature or the immunoreactive nature of LCH. In the first hypothesis, LCH was considered a neoplasia caused by the clonal proliferation of genetically defective, intrinsically abnormal cells. In the second hypothesis, LCH was considered an immune disorder where "normal" cells proliferate and accumulate in response to an environmental stimulus. However, there was no solid argument for either hypothesis because no exogenous trigger was identified, the pathological histiocytes had a benign morphology, and there was no cytogenetic or recurrent somatic molecular alteration initially highlighted. Also, the cellular heterogeneity and the low proportion of pathological histiocytes within LCH lesions hampered the search for genomic alterations in LCH due to the low sensitivity of conventional sequencing techniques.

Advances in genomic sequencing technologies have allowed to greatly improve the sequencing depth along with the simultaneous studies of multiple targeted genomic regions per sample. In 2010, Badalian-Very et al. reported that the pathological CD1a+ CD207+ histiocytes of 57% of LCH patients bear the oncogenic somatic *BRAF*^{V600E} mutation [18]. This unexpected finding was obtained by simultaneously studying hotspot somatic oncogenic mutations in LCH samples using a mass spectrometry-based allelotyping platform. Subsequently, the high prevalence of this point mutation was confirmed in several independent LCH cohorts [19,19–22]. In 2016, the prevalence of *BRAF*^{V600E} was found to be 54% in the largest representative cohort of childhood LCH patients [7]. This discovery represented a powerful argument for the neoplastic nature of LCH and led to the definition of LCH as an inflammatory myeloid neoplasia [2].

The heterozygous *BRAF*^{V600E} somatic mutation is a well-known mutation associated with cancer. *BRAF*^{V600E} encodes for a mutant protein with the substitution of glutamate for valine at amino acid 600. This substitution disrupts the hydrophobic interaction, destabilizing the conformation that maintains the activation segment of the protein in its inactive orientation. Thus, the protein remains in a constitutively active state, and activates the MAPKinase RAS-RAF-MEK-ERK cell signaling pathway, which transmits extracellular growth signals from the cytoplasmic membrane to the nucleus. Finally, ERK activation leads to the activation of downstream transcription factors which regulates cell differentiation, proliferation and apoptosis.

Oncogenic somatic mutations in this RAS-RAF-MEK-ERK signaling pathway are reported in about 30% of all cancers, with *RAS* and *BRAF* mutations found in 15% and 7% of human cancers, respectively [23,24]. *BRAF* mutations are common in melanomas (~50%) and thyroid papillary carcinomas (~40%), are well represented in many subtypes of brain tumors, and less frequent (≤10%) in other solid cancers such as colorectal and lung cancers. In these *BRAF* mutant neoplasms, the *BRAF*^{V600E} mutation is largely predominant. The *BRAF*^{V600E} mutation is also present in almost all cases of hairy cell leukemia [25]. Whereas germline mutations of *BRAF* have been described in a RASopathy (i.e. cardiofaciocutaneous syndrome), these are different from the somatic oncogenic mutations reported in cancer. It was also shown that the *BRAF*^{V600E} mutation was lethal in embryos [26].

The identification of the somatic *BRAF*^{V600E} mutation launched explorations of the genomic landscape of childhood LCH using targeted and whole exome next-generation sequencing (NGS) approaches. Recurrent mutations (mainly deletions) in exons 2 and 3 of the *MAP2K1* gene, which encodes MEK1, the BRAF downstream protein in the MAPKinase pathway, was reported in 15–20% of LCH cases [27,28]. Also, small in-frame *BRAF* deletions or insertions localized to exon 12, which encodes for the small N-terminal lobe of

the BRAF kinase domain, were reported in 5–10% of LCH cases [27,29]. Finally, single cases of mutations of other genes that transcribe MAPKinase pathway proteins have been reported, such as a *FAM73A-BRAF* fusion [27], a *MAP3K1* mutation [28], and an *ARAF* mutation [30]. Importantly, these mutations in the MAPKinase pathway were mutually exclusive. We reported on one childhood LCH case with a somatic oncogenic *PIK3CA*^{E542K} mutation involved in the PIK3-AKT pathway [31], and mutations of *PIK3CA* have been reported in adult non-Langerhans cell histiocytosis (Erdheim-Chester disease) [32]. Yet, no other LCH case with a mutated *PIK3CA* has been reported. In this review, we did not report potential somatic mutations that have been identified in single cases by massive parallel sequencing studies without functional analyses, because their significance remains unknown.

Thus, to date, somatic mutations of the MAPKinase pathway are identified in more than ¾ of childhood LCH samples **(Figure 1)**. These mutations were mutually exclusive and whole-exome sequencing analyses showed a remarkably low frequency of somatic mutations per pediatric LCH sample [33], suggesting that only one somatic driver mutation may be found per sample. This hypothesis will have to be confirmed in future studies.

In cases for whom no mutation is highlighted (≈20% of LCH cases), copy number changes or epigenetic alterations may be additional mechanisms for MAPKinase pathway activation. Also, translocations involving *BRAF* may concern a more important proportion of cases than previously reported because they have been poorly investigated to date.

Beyond improving what is known about the LCH pathology, these recent advances in the molecular understanding of LCH provide an opportunity to develop rationale strategies that benefit patient management.

4. Targeted therapy in LCH

4.1 Detection of somatic mutations in LCH

Detection of somatic mutations within tumors is currently performed in many laboratories throughout the world, and *BRAF*^{V600E} is one of the most frequent mutations in human tumors. However, testing for mutations of genes of the MAPKinase pathway in LCH should be performed in laboratories with specific experience in this disease (i.e. analyze at least three cases of histiocytosis/month). DNA is extracted from formalin-fixed paraffin-embedded samples, after review of the histology by an experienced pathologist and micro-dissection of the areas with the highest infiltration by CD1a+ histiocytes. When samples are over-fixed (i.e. more than 72 h in formalin) or decalcified, the quality of DNA may be inadequate and a frozen sample is required.

The method used for detection should have a high sensitivity, because poorly sensitive sequencing methods may fail to highlight a low level of mutant alleles. Indeed, we recently showed, in a large series of adult patients with histiocytoses, that the variant allelic frequency (VAF relative frequency of the oncogenic mutant allele) was < 5% in 24.8% of cases and < 2% in 8.1% of cases [34]. This is related to the low infiltration rate of pathological histiocytes (which bear the oncogenic mutation) in lesional tissues as previously reported in LCH [2,7]. In our experience, droplet digital PCR is the best method to detect *BRAF*^{V600E}, which is present in more than half of the patients.. Detection of indels within *BRAF* or *MAP2K1* can be performed with targeted NGS, even when the VAF is as low as 0.5%, when the depth of sequencing is important (at least five altered reads). When the VAF is very low, confirmation with targeted methods should be considered. In contrast, the detection of single base pair substitutions, such as p.(Lys57Asn) in *MAP2K1*, is still an issue because it is difficult to differentiate from background on NGS files when the VAF is < 5%.

4.2 Specific RAF inhibitors

The discovery that the oncoprotein BRAF is frequently activated due to genetic alterations in a subset of human tumors has intensified the development of RAF inhibitors to be used as potential therapeutics. Numerous clinical studies were published since 2010 [35], first on metastatic melanoma, and have showed dramatic responses and prolonged the survival of patients whose tumors harbor mutationally activated *BRAF*^{V600}. These studies have led to the approval of two second generation RAF-inhibitors [36], vemurafenib (PLX4032) and dabrafenib (GSK2118436) by the EMA and FDA for the treatment of *BRAF*^{V600E}-mutated melanoma. However, their effectiveness varied among *BRAF*^{V600E}-mutated cancer subsets, and secondary resistance is frequently observed by activation of alternative signaling pathways as well as reactivating the MAPKinase pathway through alternative means.

Importantly, these second generation RAF inhibitors are ineffective for many other BRAF mutations (BRAF fusion with partner gene, insertion/deletion of exon 12) [29,36,37]. These other mutant proteins retain their ability to form a dimer with another RAF protein and then steric restraints hamper the activity of the second generation RAF inhibitors [36]. Also, second generation RAF inhibitors are ineffective in activating mutations of genes encoding proteins that act upstream of RAF (such as RAS), and a paradoxical transactivation mechanism is responsible for cutaneous side effects (keratoacanthomas and squamous-cell carcinomas) [38]. To avoid these drawbacks, a number of new-generation RAF inhibitors have entered preclinical and clinical development recently and have potential as future therapeutics [36]. Also, RAF inhibitors are ineffective for activating mutations of genes encoding proteins that act downstream of RAF (such *MAP2K1*, which encodes MEK1); for such cases, selective MEK inhibitors may be a valuable therapeutic option.

4.3 Effect of specific BRAF inhibitors in *BRAF*^{V600E} mutated LCH

The discovery of the *BRAF*^{V600E} mutation in pathological histiocytes led to the hypothesis that the presence of this oncogenic somatic mutation generates a proliferative and survival advantage in the mutated histiocytes that is responsible for the accumulation of these pathological cells. Berres et al. first reported in 2014 that induction of the activating *BRAF*^{V600E} mutation in myeloid dendritic cells drives an LCH-like phenotype in mice [22]. However, in other models such as nevi, the *BRAF*^{V600E} mutation was insufficient to cause a pathological process and represented an oncogene-induced senescence model [39]. The definitive answer about the causal driver status of this mutation in LCH was the study on the effects of selective BRAF inhibitors in this pathology.

The first efficacy data came from the off-label clinical use of vemurafenib (selective BRAF inhibitor) in two refractory adult patients with *BRAF*^{V600E} mutations treated for mixed histiocytosis (Erdheim-Chester and LCH disease association) [40], and in a young infant with a multisystem presentation that was "high-risk" and refractory [41]. Rapid treatment efficacy and good short-term tolerance were noted. After these reports, an Orphan Drug Designation status was obtained for vemurafenib in the European Union in 2016. Other infants also benefited from this treatment in Europe with an excellent response rate, but frequent recurrences were noted after discontinuation of the treatment [42–44]. Importantly, in cases of sclerosing cholangitis and advanced neurodegenerative LCH, no clear efficacy was noted [42]; although, one group reported improvement in neurologic symptoms for neurodegenerative LCH patients when the treatment was initiated early [45].

4.4 Progress towards a targeted therapy protocol in LCH

The therapeutic target *BRAF*^{V600E} mutation appears to be a very important advancement, especially for the care of infants with refractory high risk MS RO+ LCH. However, the

optimal protocol scheme and the appropriate modalities of administration for children remain to be defined for these innovative therapies and this is a huge challenge. Until recently, targeted therapies in childhood LCH have mainly been prescribed off-label. Clinical trials dedicated to pediatric patients are underway (https://clinicaltrials.gov): i) dabrafenib in children and adolescents (12 months – 17 years) with advanced *BRAF*^{V600} mutation positive solid tumors (BRAF inhibitor, NCT01677741); ii) vemurafenib in patients (12 months – 21 years) with relapsed or refractory advanced solid tumors, non-hodgkin lymphoma, or histiocytic disorders with *BRAF*^{V600} mutations (BRAF inhibitor, NCI-COG Pediatric MATCH, NCT03220035); iii) trametinib ± dabrafenib in children and adolescents (1 month – 17 years) with cancers harboring *BRAF*^{V600} mutations (MEK inhibitor ± BRAF inhibitor, NCT02124772); iv) cobimetinib in pediatric and young adult participants (6 months – 30 years) with previously treated solid tumors (MEK inhibitor, iMATRIXcobi, NCT02639546). It should be pointed out that none of these trials is dedicated only to cases with a histiocytic disorder. Also, the dilution of a few cases of pediatric LCH among many cases of solid pediatric cancers that share few of LCH's characteristics may be an important limitation in the analysis of data from these studies. Moreover, studies for which an age <12 months is an exclusion criterion will not enroll young infants, who are the most vulnerable for refractory high-risk MS RO+ LCH. A large international cooperative study of targeted therapy was discussed by the Euro-Histio-Net group and more widely within the International Histiocyte Society and will very likely lead towards a tailored protocol for pediatric LCH patients in the future.

5. *BRAF*^{V600E} genotype/phenotype correlation in LCH

In oncology, the stratified therapeutic approach aims to improve outcomes by identifying groups of patients who appear more likely to benefit from intensified therapy or new

therapeutic agents, while reducing unnecessary toxicity in patients without these markers. The recognition of such predictive markers represents an important step in better defining therapeutic strategies. In childhood LCH, traditional risk stratification criteria are sites of disease and response to initial therapy. Patients with lesions in ROs (liver, spleen, and hematological system) have a higher risk of mortality, especially if they are non-responders during the first 6 weeks of vinblastine-steroid combination therapy [12].

The recent discovery of a recurrent oncogenic mutation in LCH has afforded a new potential molecular marker. Although, the first studies that reported the *BRAF*^{V600E} mutation failed to enroll sufficient and representative numbers of LCH patients to highlight a significant correlation **(Table 1)**. In 2014, in a cohort of 100 LCH patients, the *BRAF*^{V600E} mutation was associated with an approximately two-fold risk of reactivation [22]. All of these studies included childhood and adult LCH patients. Based on a well-structured French network of pediatric oncologists and pathologists, our group reported in 2016 on a cohort of 315 children with LCH for whom the *BRAF*^{V600E} status (n=315) was known [7]. This cohort was representative of the full spectrum of childhood LCH diseases. We showed that patients with *BRAF*^{V600E} manifested more severe disease than those without this mutation. Eighty-eight percent of patients with MS RO+ LCH had the *BRAF*^{V600E} mutation. In addition, patients with *BRAF*^{V600E} were more commonly resistant to combined vinblastine-steroid therapy (22% if *BRAF*^{V600E} was present compared to 3% if it was absent, *P*=0.001), showed a higher 5-year reactivation rate (43% if *BRAF*^{V600E} was present compared to 28% if it was absent, *P*=0.006), and had more long-term sequelae or complications (28% if *BRAF*^{V600E} was present compared to 13% if it was absent, *P*=0.001). However, our study was limited by the unknown genotype among LCH patients without the *BRAF*^{V600E} mutation.Also, we showed that the *BRAF*^{V600E} status does not display an absolute dichotomous clinical and prognostic presentation of the disease, and others factors are needed. For instance, Berres et al. suggested

that the severity and extension of the disease may be driven by the degree of differentiation in cells containing the somatic oncogenic mutation (precursor versus differentiated dendritic cells) [22].

Future correlation studies should enroll a sufficient number of LCH patients who are representative of the full spectrum of the disease. Very importantly, this study must use sensitive genotyping methods to detect the *BRAF*^{V600E} mutation, with exclusion of samples with very little infiltration by pathological histiocytes unless a highly sensitive method is used (i.e. droplet digital PCR). Future studies should also determine the impact of *MAP2K1* mutations and small indels of *BRAF* localized in exon 12. From our national level in France, we recommend that molecular analysis must be performed at least once for the following LCH presentations with the initial biopsy: i) LCH with RO involvement, ii) LCH diagnosis before 2 years of age, iii) LCH with pituitary involvement or skull base or orbit bone lesions, iv) resistance to 1st line chemotherapy, and v) severe complications or sequelae. Those data may increment the number of genotyped LCH patients and, through a collaborative international effort, will better define the genotype/phenotype correlation and inclusion of this molecular marker in future prospective trials.

Very recently, our group showed that for patients considered susceptible for neurodegenerative LCH (those with pituitary, skull base, or orbit bone involvement), the *BRAF*^{V600E} mutation increases the risk to develop this late onset complication (10-year risk at 33.1% if *BRAF*^{V600E} was present compared to 2.9% if it was absent, P=0.002) [17]. This finding allows a more precise identification of patients at risk for neurodegenerative LCH, tailored screening with imaging and neurocognitive assessment, and evaluation of the benefits of BRAF inhibitor treatment in the early stages of this late-onset complication.

6. Free circulating *BRAF*^{V600E} alleles as a biomarker in LCH

Conventional imaging (radiography, CT scanning, MRI) is most often used to follow the activity of LCH disease with bone, soft tissue tumor, and lung involvement [4]. However, it may be suboptimal for defining disease remission and for guiding decisions about therapy modifications because lesions can appear to be stable for prolonged periods of time despite the response to intralesional therapy. Moreover, for patients with RO+ LCH, cytopenia and hypoalbuminemia are usually present and may be relevant biomarkers of disease activity, but they are limited by their lack of specificity, especially after cytotoxic chemotherapy [13]. The methods for assessing global response in LCH are important, but are hampered by the wide clinical expression of this disease. Our group previously published the Disease Activity Score (DAS), a quantitative score based on clinical and biological evaluations that can reliably segregate high-risk patients and assess treatment efficiency for them [48].

To date, international Histiocyte Society protocols have assessed the disease response using qualitative categories [8,12]. The four categories were: i) non-active disease (NAD), resolution of all signs/symptoms of the disease; ii) active disease better (ADB), regression of signs/symptoms, no new lesions; iii) active disease stable (ADS), persistence of signs or symptoms, no new lesions; and iv) active disease worse (ADW), progression of signs or symptoms and/or appearance of new lesions. This qualitative-based evaluation may generate biases due to inaccurate thresholds for defining a regression or a progression, and each investigating center may arbitrarily classify patients with their own subjective/variable thresholds. Also, this qualitative discontinuous categorization response assessment has an intrinsic limitation by converting a continuous variable into distinct categories.

In patients with solid tumors, circulating cell-free DNA (ccfDNA) in the peripheral blood contains cancer-derived DNA and has been used as a non-invasive diagnostic procedure

(liquid biopsy) and for patient management and cancer disease assessment during therapy [49].

In 2017, our study group reported the feasibility of circulating cell-free (ccf) *BRAF*^{V600E} allele detection for follow-up monitoring in our French cohort of children with MS RO+ LCH and LCH refractory to vinblastine-steroid first line chemotherapy [50]. Using a highly sensitive droplet digital PCR assay, we were able to quantify the ccf *BRAF*^{V600E} alleles (ccf *BRAF*^{V600E} load) from plasma samples of LCH patients with a threshold of sensitivity between 10^{-3} and 10^{-4}. We showed that the positive *BRAF*^{V600E} load was higher for RO+ patients (mean, 2.90%) than for RO- patients (mean, 0.16%). We also followed the kinetics of the ccf *BRAF*^{V600E} load for 17 patients. This allowed us to better understand the kinetics of the ccf *BRAF*^{V600E} load in LCH after various therapies.

Thus, the droplet digital PCR method could represent an objective marker of disease activity. The very short half-life of ccfDNA (approximately 2 h) makes this quantification almost a real-time measurement. and afford a new interpretative parameter for the understanding of the natural history of LCH, as was shown in recently published LCH cases that included follow-up of the *BRAF*^{V600E} load [43,44]. Of course, this method concerns only *BRAF*^{V600E}-mutated LCH cases, which have the most severe presentation of the disease. Also, this method is sensitive enough to detect ccf *BRAF*^{V600E} only for LCH cases with a “sufficient tumor burden”, but may not be helpful for the initial diagnosis of LCH for small and difficult-to-biopsy locations (base of the skull, vertebrae, pituitary, etc.). Future studies will more accurately evaluate dynamic changes in the ccf *BRAF*^{V600E} load for LCH patients and the use of this new blood biomarker should be considered in upcoming clinical trials.

7. Conclusion

Advances in genomic sequencing technologies in this past decade have advanced the molecular study of LCH. In a few years, knowledge about the mutational spectrum of LCH was considerably expanded, resulting in many applications that benefit the management and treatment of patients. Collaborative efforts should be sustained to improve those applications and extend them to more patients in more clinical centers using a defined consensus protocol. This new “molecular-based management” of childhood LCH represents tremendous progress.

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CONFLICT-OF-INTEREST STATEMENTS

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FIGURE AND TABLE LEGENDS

Figure 1: Estimated distribution of somatic gene alterations in childhood LCH.

The asterisk indicates single cases reported. We did not report potential somatic mutations that have been identified in single cases by NGS studies without functional analyses because their significance has not been proven.

Table 1: Main studies reporting *BRAF*^{V600E} mutations in a pediatric LCH cohort (> 40 patients)

_, unavailable data; SS, single system LCH; MS multiple systems LCH, RO, risk organ affected; LCH, Langerhans cell histiocytosis

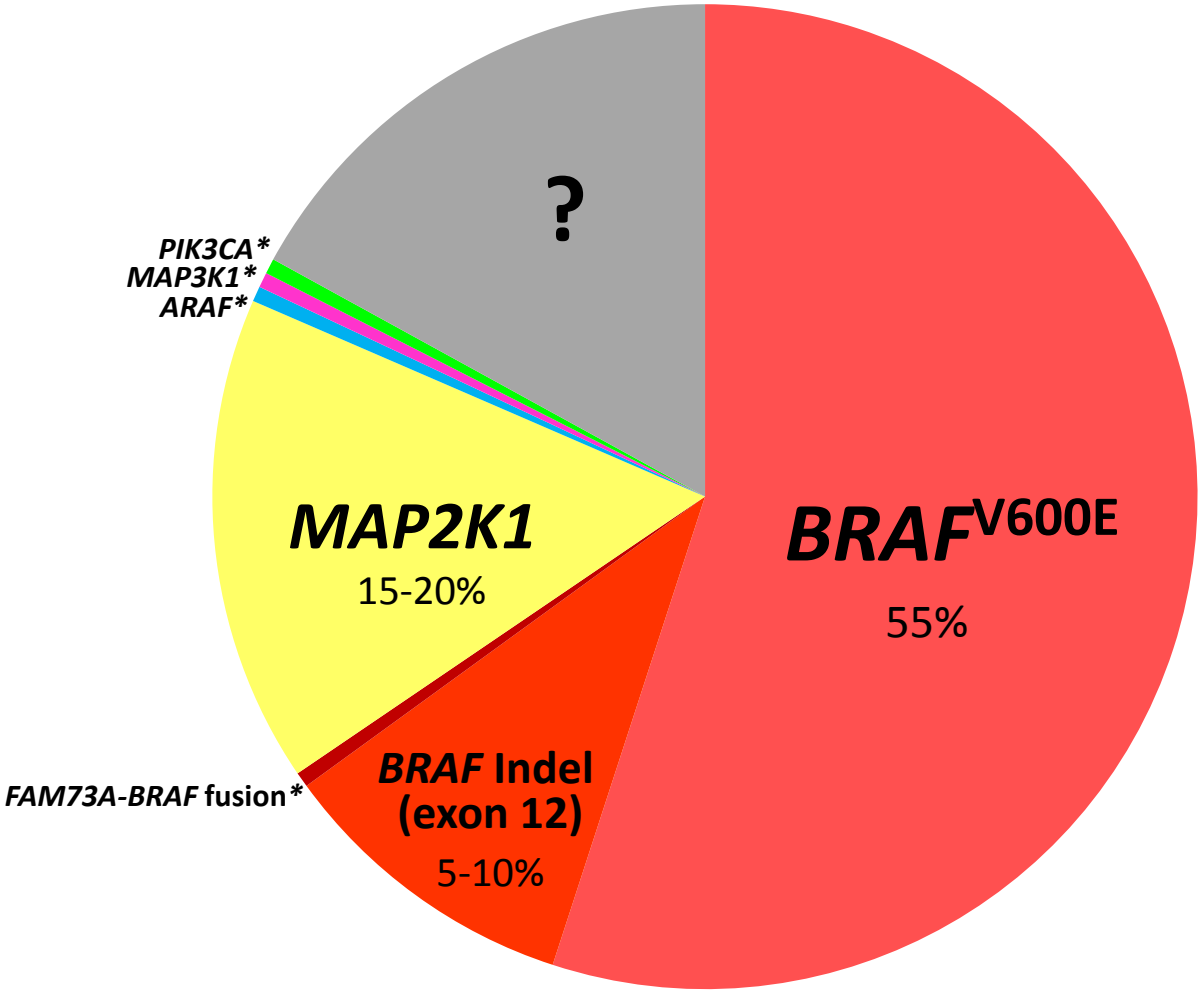
?
PIK3CA*
MAP3K1*
ARAF*
MAP2K1
15-20%
BRAFV600E
55%
FAM73A-BRAF fusion*
BRAF Indel
(exon 12)
5-10%

Table 1: Main studies reporting *BRAF*^{V600E} mutations in a pediatric LCH cohort (> 40 patients)

Publication	Cohort size	Median age at diagnosis	SS / MS OR- / MS OR+ (number of patients)	% of *BRAF* V600E	Phenotype/outcome associated with *BRAF*^{V600E} status
Badalian-Very, Blood 2010 [18]	n=61	12 [0.7-61]	47 / 8 / 0	57%	Younger age at diagnosis
Sahm, Blood 2012 [20]	n=46	22.5 [1-71]	_	41%	None
Wei, Biomed rep. 2013 [46]	n=50	15.4 [1-42]	39 / 9 / 2	56%	None
Bubolz, Oncotarget 2014 [47]	n=42	13 [0.6-65]	25 / 13 / 4	52%	None
Berres, JEM 2014 [22]	n=100	3.7 [0.3-47.3]	66 / 17 / 17	64%	Reactivation risk
Héritier, J Clin Oncol. 2016 [7]	n=315	3.2 [0-17.9]	196 / 70 / 49	55%	Younger age at diagnosis MS RO+ LCH Reactivation risk Resistance to first-line therapy Permanent sequelae

_, unavailable data; SS, single system LCH; MS multiple systems LCH, RO, risk organ affected; LCH, Langerhans cell histiocytosis