Treatment of Langerhans cell histiocytosis: role of BRAF/MAPK inhibition

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Langerhans cell histiocytosis (LCH) is a clonally derived neoplasm with a highly variable clinical course. Although LCH was once considered a disorder of immune regulation, the identification of activating mutations in the proto-oncogene *BRAF-V600E* in ~50%-60% of cases and MEK and ERK phosphorylation in 100% of examined cases, has changed the definition of LCH to a dendritic cell neoplasm with a strong inflammatory component. Current international LCH trials are focused on further improving the outcome of high-risk multisystem LCH patients, by decreasing the reactivation rate, optimizing early salvage regimens, and preventing late sequelae. Anecdotal responses to vemurafenib, a *BRAF-V600E* inhibitor, have been reported in a few cases of LCH and Erdheim–Chester disease. However, the development of resistance, as well as the potential risks of cutaneous and pancreatic cancers in patients with *BRAF-V600E*-mutated melanoma treated with single inhibitors, suggest the need for prospective trials with BRAF inhibitors, alone or in combination with other inhibitors of this pathway, for patients with refractory or multiply-relapsed LCH. The recent discovery of somatic mutations in ARAF and in MAP2K1, which lead to activation of the RAS-RAF-MEK–ERK pathway in the setting of wild-type BRAF, as well as the finding that activating mutation in MAP2K1 are relatively insensitive to MEK inhibitors, suggest that a more detailed understanding of this pathway in LCH may be necessary for the development of more effective targeted therapies.

Learning Objectives

- To gain an understanding of the advantages and disadvantages of current therapeutic options in children and adults with LCH
- To discuss mutations in genes of the RAS-RAF-MEK-ERK pathway in patients with LCH and the implications for targeted therapies

Langerhans cell histiocytosis (LCH) is a dendritic cell (DC) neoplasm defined by the presence in the lesion of pathologic Langerhans cells (LCH cells) that are positive for CD1a, CD207 (Langerin), and S100. Despite identical histopathologic features, LCH has a diverse clinical behavior ranging from benign single system disease (SS) that can regress spontaneously to multisystem (MS) disease that can be life-threatening or a chronically reactivating form of disease that is not life-threatening but has the potential to result in significant permanent sequelae, such as diabetes insipidus (DI), growth retardation, bone pain, hearing loss, sclerosing cholangitis, and CNS neurodegenerative disease. However, because LCH-associated DI may be the first manifestation of LCH, it is unclear whether therapy to prevent reactivations will entirely prevent the permanent sequelae.

LCH can occur at any age but is more common in children of whom two-thirds have SS-LCH predominantly in bone followed by skin. Children <2 years of age with MS-LCH commonly have involvement of "risk" organs (ROs) today defined as involvement of liver, spleen, and hematopoietic system, with "risk" being the risk of death.

In adults, the mean age at diagnosis is 35 years with 10% being older than 55 years. Sixty-nine percent of adults with LCH in the Histiocyte Society Adult Registry have MS-LCH with skin and lung involvement in 51% and 62%, respectively. Of the 31% of adult patients with SS disease, lung was involved in 51%, most of whom were smokers, followed by bone and skin in 38% and 14%.[1] The natural history of LCH in adults is less clear but it is thought that spontaneous regression, even in SS disease, is less likely to occur and that adult patients typically require some form of therapy depending on the extent of disease. Survival of adult patients with MS disease including skin is better than that seen in children, because of the lower number of organs involved.[2] But chronicity of disease, such as in skin-LCH, may pose a special problem in adults and this coupled with the greater toxicity of standard therapeutic protocols makes targeted therapies even more interesting in the adult patients.

Pathogenesis

Earlier pathogenetic theories of LCH suggested an inappropriate activation of epidermal Langerhans cells (LCs). The benign morphology of the pathologic LCs and the lesional expression of inflammatory chemokines and cytokines have historically deemed LCH an immune dysregulatory disorder. Further, the documented examples of spontaneous self-regression, the fact that limited disease responds well to mild treatment, and in particular, the fact that infants with RO+ LCH who respond to therapy have a 50% recurrence rate but the majority of those recurrences are in low risk organs, such as bone with a 100% survival, speaks strongly against a malignant etiology, at least from a clinical point of view. On the other hand, the

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Off-label drug use: Braf inhibitors as therapy for LCH.

detection of clonal LCs from nonpulmonary LCH, the telomere length shortening and the elevated expression of non-mutant TP53, *c-myc*, H-*ras*, Ki-67, and Bcl-2 were in favor of LCH being a neoplasm,[3] although not necessarily a malignancy. This hypothesis was confirmed by the discovery of recurrent somatic activating mutations of the *BRAF-V600E* gene in 57% of archived LCH lesions.[4] *BRAF* has an important function in the signaling cascade, which usually begins with activation of a receptor tyrosine kinase and proceeds by phosphorylation steps through Ras to Raf to MEK and the extracellular signal-regulated kinase (ERK), which ultimately leads to modulation of gene expression.[5] Evidence of *MEK* and *ERK* phosphorylation was found in 100% of examined cases in this study, regardless of *BRAF* mutation status, suggesting that activation of the pathway occurs in most or all LCH.[4]

In support of this, additional genetic drivers of ERK pathway activation have been since identified. Nelson et al, using whole-exome sequencing of DNA isolated from purified LCH cells of 3 patients with wild-type *BRAF*, documented the first somatic, activating mutations (F351L and Q347_A3438del) within the kinase-encoded domain of ARAF, leading to ARAF and MEK constitutive kinase activity,[6] which could also be inhibited by vemurafenib, a *BRAF* inhibitor. Brown et al,[7] using next-generation sequencing, found that 11 of 40 (27%) cases showed somatic *MAP2K1* mutations that occurred mutually exclusive of *BRAF* mutations, with 50% of wild-type BRAF cases showing *MAP2K1* mutation. Together with the studies by Badalian-Very et al[4] and Nelson et al,[6] these findings suggest that most LCH patients harbor a somatic activating mutation in critical signaling steps of the RAS-RAF-MEK-ERK pathway, supporting the potential for pursuing targeted therapeutic strategies in patients with wild-type BRAF, as well as those with BRAF mutations. Nelson et al went on to test 30 LCH samples for the presence of additional genetic alterations that might cause ERK pathway activation. In 20 BRAF wild-type samples, they found 3 somatic mutations in *MAP2K1 (MEK1)* all of which constitutively phosphorylated ERK in in vitro kinase assays. Importantly some of the variants were resistant to the MEK inhibitor trametinib in vitro, which has obvious implications for inhibitor therapy in LCH patients. Within the entire sample set, they also found 3 specimens with mutations in *MAP3K1 (MEKK1)*.[6]

These data were the first genetic evidence supporting the notion of LCH as a myeloid neoplasm and were rapidly confirmed by other investigators who found that LCH cells with different degrees of maturation, compatible either with myeloid cell or de-differentiated LC antigens, carry the *BRAF-V600E* mutation.[8] It remains unclear whether LCH derives from a bone marrow precursor or an abnormally reprogrammed LC. Recently, Berres et al reported that *BRAF-V600E* expression in tissue DCs was associated with a two-fold increased risk of recurrence but did not affect overall survival. However, patients with active high-risk LCH were found to harbor *BRAF-V600E* in circulating CD11c+ and CD14+ peripheral blood mononuclear cells and in bone marrow CD34+ progenitors, whereas in low-risk LCH patients the mutation was restricted to lesional CD207+ DCs.[9] This study suggested that high-risk LCH originates from somatic mutation of a hematopoietic progenitor whereas low-risk LCH originates from somatic mutation of tissue-restricted precursor DCs,[9] but this concept remains to be proven.

The *BRAF-V600E* mutations were also found to be present in 40% of the cases of adult pulmonary LCH[4] correlating reasonably well with the ~30% incidence of monoclonality found in these patients in earlier studies.[10] This might suggest 2 types of adult lung LCH, one of which is the traditional polyclonal inflammatory disease of adult smokers,[11] or as suggested by Bedalian-Very et al, smoking may induce BRAF V600E mutations at multiple sites throughout the lungs of susceptible smokers resulting in multiple clones.[12] The absence of *BRAF-V600E* from samples of other rare histiocytic disorders, such as Rosai-Dorfman disease (RDD) and juvenile xanthogranuloma adds to the specificity of this mutation to LCH.[4] However, *BRAF-V600E* mutations have been observed in 54% of patients with Erdheim-Chester disease (ECD), another rare histiocytosis, suggesting a possible common origin with LCH.[13]

Current standard treatment of pediatric LCH

The treatment of LCH depends on the extent and severity of disease at diagnosis. SS disease has an excellent survival rate and can be treated with either observation (isolated skin), curettage/intralesional steroids (unifocal bone), or indomethacin, bisphosphonates or low-dose systemic chemotherapy (multifocal bone).[3] MS-LCH, involving 2 or more systems, can be associated with a poor survival when there is RO^+ (liver, spleen, bone marrow) involvement. Patients with MS-LCH but without risk organ involvement (RO^-) have an excellent survival and are usually treated with systemic chemotherapy in an attempt to reduce reactivations and prevent permanent sequelae.[3] During the last 2 decades, a more aggressive chemotherapy approach has been used in RO^+ MS-LCH with 2 goals, first to decrease mortality and second to reduce the 50% reactivation rate in patients who respond to front-line therapy. The early prospective European trials, conducted in Italy (AIEOP-CNR-HX 83)[14] and Germany/Austria (DAL-HX 83/90),[15] as well as the Japanese[16] and Histiocyte Society randomized trials,[17-19] showed that mortality could be reduced for the whole group of patients to <10% with single-agent or multiple-agent chemotherapy, but that multi-agent chemotherapy given for a longer duration (ie, 12 months) reduced mortality in the highest-risk group, reduced the reactivation rate and reduced the diabetes insipidus rate. Other important findings were that the prolongation of induction therapy to 12 weeks in patients who did not achieve a complete response at week 6, and an early switch to salvage therapy for those with progressive disease by week 6 (or earlier if necessary) appears to significantly decrease mortality.[16,19] These findings form the basis for the currently open Histiocyte Society trial, LCH-IV, which has seven different strata. Stratum 1 will be testing in a randomized fashion whether prolonging (12 vs 24 months) and intensifying (±6-mercaptopurine) therapy for high-risk patients, and comparing 6 versus 12 month therapy for single-system disease (craniofacial bones or multifocal bone) will further reduce the rate of reactivation and the potentially devastating permanent consequences. Stratum 2 was designed for MS patients without risk organ involvement who fail first-line treatment or who initially respond but have a reactivation, and will include a 6 month re-induction with vincristine/prednisone/cytarabine followed by randomized 18 month maintenance of oral indomethacin versus oral 6-mercaptopurine and methotrexate. Stratum 3 will assess the efficacy of salvage with a cladribine/cytarabine combination in MS-LCH patients with risk organ involvement who fail to respond to first-line treatment. Stratum 4 will study the efficacy of a reduced intensity hematopoietic stem cell transplant as a salvage option for MS-LCH patients with risk organ involvement who fail stratum 1 and stratum 3 therapies. Stratum 5 will prospectively explore the effectiveness of cladribine in tumorous CNS-LCH and whether IVIg or cytarabine will impact the progression of neurodegenerative CNS-LCH. Stratum 6 will describe the natural history of single-system LCH treated by conservative methods ("wait and watch") or local therapy. Stratum 7 will monitor the long-term outcome and the incidence of

Table 1. Histiocyte society trials in Langerhans cell histiocytosis

	LCH I	LCH II	LCH III
Years	1991-1995	1996-2001	2001-2008
No. of patients	143	193	422
Protocol	3d MP pulse + randomized VBL vs VP16	Randomized Pred/VBL vs Pred/VBL/VP16; continuation: 6MP/Pred/VBL+/−VP16	RO+: MTX randomized; 12 wk induction if poor response at 6 wk
Duration	6 mon	6 mon	RO+: 12 mon RO$^-$: 6 vs 12 mon
Response	53% (equal)	63/71%; (56/68% if RO+)	71% in RO+
Overall survival	79% at 3 y (equal)	74/79% at 5 y; (64/73% if RO+)	84% at 5 years
Reactivations	58%	45%; (RO+ = RO$^-$)	27%
Sequelae	41%; 14% DI	40%; 21% DI	12% DI

late sequelae in all LCH patients. Details of the published Histiocyte Society trials are shown in Table 1.[17-19]

At the same time, colleagues at several institutions in the United States are testing in a non-randomized fashion the use of single-agent cytosine arabinoside,[20] but a longer follow-up period will be required to fully evaluate this strategy, particularly with respect to the rates of reactivation and permanent sequelae.

Treatment of relapsed/refractory MS-LCH

Approximately 50% of patients with LCH will be refractory to induction therapy or develop reactivation of disease within 5 years.[21] Patients with MS+LCH who progress early or who fail to respond after 2 courses of induction (week 12) have a very poor outcome, and their treatment has been challenging. Nucleoside analogs, such as cladribine and clofarabine, have activity against LCH because of their antiproliferative and immunomodulatory effects but also because of their efficacy against the immature myeloid precursors which have recently been suggested to be the precursor cells for LCH.[9] Data on relapsed LCH patients are largely derived from pilot studies, surveys or case series. Low-dose cladribine was effective in achieving a 22% response rate in RO+ patients and 66% in RO− patients refractory to front-line therapy. However, only 3% of all patients had "no active disease" by week 24 and prolonged myelosuppression was a limiting factor.[22] Clofarabine, a second-generation nucleoside analog, has shown significant activity as single-agent against disseminated LCH refractory to cladribine or cytarabine, and encouraging results with manageable toxicity have been reported by Abraham et al[23] and Simko et al.[24] The drug appears to be active in both high-risk and low-risk LCH and the major toxicity of myelosuppression appeared to be manageable with reduced doses and with filgrastim. Prolonged and cumulative cytopenias were not seen, possibly because of the relatively low dose of clofarabine required for therapy of LCH compared to the leukemic population. The authors concluded that the high cost of the drug could be justified by the potential to avoid hematopoietic stem cell transplantation in these refractory patients.[24]

Nonetheless, the safety and efficacy of clofarabine in relapsed LCH needs to be established in a larger prospective multicenter trial. For patients with high-risk refractory disease, the most successful published salvage regimen is a combination of higher doses of cladribine plus high-dose cytarabine. In the LCH-S-2005 protocol of the Histiocyte Society, a response rate of 92% was achieved in 27 very high-risk young patients (median age 0.7 months). Four patients relapsed, some of whom were salvaged by HSCT. There were 4 deaths, 2 from toxicity and 2 from disease. This regimen proved to be very effective but is associated with significant toxicity and requires excellent supportive care.[25] Rosso et al used the same combination of drugs and treated a series of 9 patients with progressive MS-LCH with a lower dose of cladribine and a much lower dose of cytarabine (100 mg/m^2/d for 4 days per cycle). Six patients achieved remission and 1 a partial response, 3 patients reactivated. The overall probability of survival at 3 years was 73% (standard error 16%).[26] The studies are not strictly comparable as only 5 of the 9 patients treated in the Rosso series had progressive disease at 6 weeks of LCH therapy, a known very poor prognosticator. However, these results are encouraging and suggest a worthwhile salvage strategy, particularly for sites that do not have the supportive care required for the high-dose therapy. A head-to-head comparison of the 2 strategies would be of interest, but considering the very slow accrual of LCH-S-2005, would be difficult to do.

Allogeneic hematopoietic stem cell transplant (HSCT) appears to be promising in LCH patients who fail multiple salvage regimens. Whether its beneficial effect is because of a graft-versus-LCH or the cytotoxic effect of high-dose therapy remains to be proven. The earlier published experience with myeloablative conditioning (MAC) regimens showed survival rates of ~50% in highly resistant patients, but a transplant-related mortality (TRM) which was unacceptably high.[27] Using reduced-intensity conditioning regimens (RIC), a 78% survival and a low TRM rates have been reported.[28] Kudo et al more recently published the Japanese experience of 15 refractory LCH patients treated with HSCT, 5 of whom had RIC regimens. Ten year overall and disease-free survival were 73% (11/15); 80% with myeloablative and 60% with RIC, although small numbers and patient variability precluded any definitive conclusions.[29] A recently published study from the United Kingdom retrospectively reviewed 87 LCH patients who underwent allogeneic transplant from 1990-2013. Prior to 2000, the TRM was 55%. Review of the patients transplanted after 2000, however, showed no significant difference in outcome between those receiving RIC compared with those receiving MAC transplants with overall survivals at 3 years of 71% and 77%, respectively ($p = 0.89$). The relapse rate was slightly higher after RIC transplants but most patients were salvaged with additional chemotherapy and the authors suggested that it was possible that more seriously ill patients might have received RIC. There was also no difference in GVHD rates or severity.[30] RIC-HSCT is currently being tested prospectively in the ongoing Histiocyte Society LCH-IV trial for refractory MS-LCH patients who fail more than one salvage regimen.

Treatment of adult LCH

There is no standard of care treatment for newly diagnosed adults with MS-LCH. The standard pediatric vinblastine/prednisone regimen has been associated with an increased neurotoxicity, more profound myelosuppression and more steroid toxicity in adults compared with children. A recent retrospective study of 58 adult patients with bone LCH showed an advantage for cytarabine monotherapy compared with vinblastine/prednisone and even to

cladribine, in terms of response and toxicity.[31] As a result, first-line monotherapy with cytarabine, etoposide, or cladribine is considered to be a preferable option in adult LCH in North America.[32] Vinblastine/prednisone remains the preferred first-line option in many European centers, however.[32] Other systemic therapy used in adult LCH patients, particularly those with skin-LCH, include weekly oral methotrexate, daily oral etoposide, oral azathioprine, and thalidomide.[32] More intensive combination regimens, such as methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, and bleomycin (MACOP-B), have been shown to be effective,[33] but should be reserved for rare aggressive forms of adult LCH.[32] Most adult experts recommend starting with cladribine in patients with risk organ or tumorous cerebral involvement, although cytarabine seems reasonable as well.[32] Bisphosphonates, such as zoledronic acid, have been shown to be effective in bone LCH in adults,[34] however, patients need to be counseled about risk of jaw osteonecrosis, renal and ocular adverse effects.

Adults with reactivated SS or MS-LCH can respond to single-agent cladribine, as was shown in a phase II trial.[35] Patients with CNS involvement may benefit from the combination of cladribine and cytarabine, as both these drugs cross the blood–brain barrier.[36] In the aggressive forms of adult LCH, RIC-HSCT has been performed successfully as well, but large studies are lacking.[37] The increased toxicity to standard front line pediatric therapies and the lack of agreement on the best therapy for adult LCH patients, as well as the failure to accrue patients to the 1 prospective adult trial that has been attempted (Histiocyte Society LCH-A1 study), suggest that targeted therapies may be at least as or even more important in this patient population.

Targeted therapies

Imatinib mesylate is a potent competitive inhibitor of tyrosine kinases associated with ABL, ARG, KIT, platelet derived growth factor receptors (PDGFRA and PDGFRB), and can inhibit differentiation of $CD34^+$ progenitors into dendritic cells. A recent study showed that a subset of patients with LCH were positive for PDGFRA, and suggested that they could potentially be treated with tyrosine kinase inhibitors.[38] Imatinib has been successfully used in few cases of refractory MS-LCH with cerebral and lung involvement,[39,40] but not all patients have responded. Imatinib has shown activity also in other histiocytic disorders such as ECD, and RDD, although again with mixed results.[39]

BRAF inhibition

The finding of the *A/BRAF* and *MAP2K1* mutations in LCH patients has raised the possibility of targeted therapies in histiocytic disorders, possibly through subsequent deactivation of the proliferative RAS/RAF/MEK/ERK pathway as demonstrated in *BRAF-V600E*/-driven melanoma treated with the *BRAF-V600E* inhibitor vemurafenib. The largest published series to date is by Haroche et al, who reported favorable responses to vemurafenib in 8 adult patients with refractory BRAF mutated ECD, 4 of whom had concurrent LCH. The responses were seen despite the reduction in vemurafenib dose to one-half, because of cutaneous adverse events seen in the first 3 patients. Of note, one patient developed squamous cell carcinoma. Importantly, all the others showed sustained response at a median of 10 (range, 10–16) months on verumafinib.[41]

Pediatric formulations are being developed, and phase I/II trials are ongoing in pediatrics for the first generation BRAF inhibitor, dabrafenib (NCT01677741). Preliminary results were presented at the American Society of Oncology (ASCO) meeting in June 2014. One child with refractory LCH, who was treated with oral dabrafenib, continued to show stable disease at week 16.[42] Anecdotal responses to vemurafenib have been reported in a 2-month-old girl with refractory MS-LCH,[43] a 3-year-old boy with progressive CNS neurodegenerative LCH,[44] a 45-year-old woman with refractory LCH,[45] and a 90-year-old woman with severely symptomatic refractory skin LCH who responded within a few days and achieved complete remission within 6 months of starting vemurafenib.[46]

The responses to *BRAF-V600E* inhibition are interesting for several reasons. First, there is no established standard of care for adults with severe LCH, and children with refractory LCH continue to have poor prognosis, especially those with progressive CNS neurodegeneration. Prospective clinical trials are clearly needed. However, a number of critical factors must be considered in the development of these trials for patients with histiocytic disorders. Treatment of melanoma patients with vemurafenib has been associated with the development of de novo squamous cell carcinoma in as many as 50% of treated patients,[47] and more recently, secondary pancreatic cancers in patients with melanoma and *BRAF-V600E* mutations treated with single inhibitors.[48] Although these can be perhaps tolerated in adults with life-threatening melanomas, their incidence is less acceptable in children with non life-threatening LCH. Second, several trials with BRAF inhibitors have shown that not all neoplasms with *BRAF-V600E* mutations respond similarly. This is not surprising in view of the results discussed earlier of some variants of MAP2K1 mutations being shown to result in resistance to MEK inhibition. These findings indicate the need to further define the molecular context in which the BRAF mutation exists.[6,49] Third, determination of the type of the RAF mutation is very important as not all mutations respond to inhibition, and some may get stimulated by inhibitors directed to the *BRAF-V600E* mutation potentially causing tumor progression,[50] such as has been seen in patients with mutations which result in high levels of RAS. Fourth, the duration of therapy has not been established and prolonged therapy may be needed, possibly even longer for the histiocytic disorders than for melanoma patients, and finally resistance mechanisms may rapidly develop to overcome initial sensitivity to BRAF inhibition.[51] Combination drug regimens, therefore, such as the combination of BRAF and other ERK pathway inhibitors or BRAF/MEK inhibitors with chemotherapy, may be more effective in preventing development of resistance and may additionally prove to be less toxic,[52] including less tumorigenic.

Conclusions

The finding that *BRAF-V600E* mutations were common in LCH lesional cells has led to an explosion of interest in the biology of LCH and other histiocytic disorders. However, the increased understanding of LCH biology has not yet lead to any change in risk stratification or front-line treatment strategies. More studies are needed to correlate *BRAF*, *ARAF*, or *MAP2K1* mutations with clinical risk status while awaiting the results of the ongoing clinical trials utilizing *BRAF* inhibitors. Experience with melanoma patients suggests that future clinical trials will most likely involve a combination of targeted agents or targeted agents combined with chemotherapy to ensure optimal efficacy, as well as safety, of this promising new approach.

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