Langerhans Cell Histiocytosis: The Cell of Origin and a Pathway to a Rationale Cure

Summary of the 27th Nikolas Symposium, Athens, May 18-21, 2017

The 27th Nikolas Symposium organized discussion around two major themes. The first focused on remaining unsolved questions regarding the development of LCH, returning to the question of cell (or cells) of origin. The second theme represents a major advance for the Nikolas Symposium by focusing on not just discovery, but implementation of a rationale cure for LCH. Recurrent activating mutations in *BRAF* and other genes in the MAPK signaling pathway in LCH lesion cells have been identified in almost all cases, and ERK activation is a universal feature of pathologic LCH dendritic cells (DC). While the last several years have brought major advances in understanding of LCH, the origin of LCH and mechanisms that lead to formation and progression of lesions remain largely undefined. Understanding how MAPK activation in specific blood cells lead to disease may identify additional opportunities for therapy. Early clinical studies in adults with LCH and related disorders demonstrate promising responses to drugs that block MAPK signaling. Now that we understand a piece of the puzzle that points toward rationale cure(s), a practical question is how to optimally test the safety and efficacy of MAPK inhibitor or other novel agents in children with LCH.

Session I. Introduction. The meeting was opened by **Dr. Maarten Egeler** (Toronto), who introduced the history of the Symposium and tasked the participants to commit to present provocative data, share ideas and argue. **Dr. Carl Allen** (Houston) presented the historical evolution of concepts of LCH, clinical overview, and discussed a biological framework form LCH in which the stage of differentiation of a white blood cell that acquires the MAPK mutation may impact extent of disease and response to therapy. According to the "Misguided Myeloid Differentiation" model, the state of differentiation of the myeloid cell in which activating MAPK pathway mutations arise determine the extent of disease.[1] Analysis of patients with LCH-associated neurodegenerative disorder (LCH-ND) was presented as an example of an application of this model. *BRAF*-V600E mutations in peripheral blood mononuclear cells (PBMC) in patients with BRAF-V600E$^+$ lesions BRAF-V600E$^+$ PBMC following therapy in the absence of other systemic lesions was associated with very high risk of LCH-associated neurodegeneration (LCH-ND). Analysis of brain biopsy specimens from patients with LCH-ND further identified perivascular BRAF-V600E$^+$ cells. Together, these data support a hematopoietic origin for LCH-ND that is clonal with systemic LCH lesion CD207$^+$ cells. This model is clinically significant in that LCH-ND has been regarded as an immune-mediated disorder. In a pilot series, 3 out of 4 patients with LCH-ND experienced significant clinical and radiologic responses to BRAF-V600E inhibition.[2] **Dr. Matthew Collin** (Newcastle) summarized the 2016 Nikolas meeting and highlighted advances in myeloid cell biology and genomics over the past year that impact understanding of LCH and frame the 2107 Symposium discussion.

Session II. Cell of Origin. **Dr. Jean-Francois Emile** (Paris) presented an overview of biology of LCH and related histiocytic disorders. Historically, these diseases have been characterized by comparisons of cellular features of the lesion cells to those of normal components of the immune system (e.g. "Langerhans" cell histiocytosis shares expression of the cell surface protein CD207 with epidermal Langerhans cells). LCH, with a "dendritic cell" phenotype has historically been categorized apart from ECD and JXG, with "macrophage" phenotype. Dr. Emile proposed reclassification of these conditions. The "L-group" includes LCH, Erdheim-Chester disease (ECD) and systemic juvenile xanthogranuloma (JXG) based on common features of somatic MAPK mutations, presence of circulating precursors, overlap in anatomic distribution of lesions (notably pituitary and LCH-ND), and the phenomenon of mixed histiocytic disease (one lesion with features of DC and macrophage or separate lesions with distinct features).[3]

Dr. Alan Mowat (Glasgow) discussed the intestinal immune system as an example where both origin and location of immune cells (dendritic cells and macrophages) determine character and function. The extreme infectious environment of the intestines provide a model to highlight the need for the immune system to be able to recruit cells and also differentiate resident cells in response to specific challenges. Tissue resident macrophages with limited scope of function are seeded during embryonic development, but may be replaced or supplemented by more dynamic monocyte-derived macrophages. However, resident intestinal macrophages share features with CCR2-dependent $Ly6C^{hi}$ monocytes which functionally differentiate in response to local stimuli. Similarly, monocyte-derived intestinal macrophages differentiate into anti-inflammatory scavengers with local cues from microbiota including TGFβ signaling. Intestinal macrophages do not prime naïve T cells, but may sustain a T cell response. By comparison intestinal DCs are heterogeneous, but all are derived from pre-DC, produce retinoic acid, migrate in lymph and prime naïve T cells.[4] The complexity demonstrated by the intestinal mononuclear phagocyte system demonstrates the potential for many cells of origin as well as a variety of cell fates based on local factors that influence terminal differentiation that may apply to LCH ontogeny.

Dr. Chloe Villani (Boston) and **Dr. Florent Ginhoux** (Singapore) presented independent projects with seminal findings taking advantage of single cell sequencing to demonstrate that human immune cells represent a far more complex network than previously appreciated. They are using these new techniques to determine pathways of differentiation of dendritic cells and macrophages and alterations that arise due to external cues such as tissue-specific factors or inflammation. Dr. Villani discussed the limitations of conventional immunophenotyping, which is based on known antigens, which is the basis for current classification of cells of the mononuclear phagocyte system. Applying a single-cell sequencing model, a new cDC population was identified in healthy individuals distinct from $CD1C^+$ and $CD141^+$ DC with superior ability to induce T cell proliferation. With conventional flow cytometry, these cells co-localize with pDC, which may account for previous reports where pDC were ascribed T cell stimulatory function that in fact may have been derived from "contaminating" "CD5" cDCs. In addition to characterizing steady-state populations in healthy individuals, single cell sequencing can characterize ontogeny of pathologic cells. For example, blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare, poorly characterized disorder that may be localized or disseminated. With single cell sequencing BPDCN clustered very closely with pDC, suggesting common origin or shared terminal differentiation.[5,6] Dr. Ginhoux also highlighted the ability of RNA sequencing (>1000s of genes) to characterize a cell with far more detail than flow cytometry (~17 antigens) or even CyTOF (~45 antigens). Applying single cell RNA sequencing with unsupervised classification and dimension reduction algorithms, 5 clusters were identified from $Lin^-HLA\text{-}DR^+FLT3^+$ DC: 2 cDC, 2 pDC and 1 intermediate. Further functional and phenotypic analysis identified "Cluster#4" as a pre-DC that gives rise to cDCs.[7] Given the examples of dynamic morphology and function intestinal dendritic cells and macrophages with many overlapping features and the increasing complexity of myelomonocytic cell populations, it is possible that LCH cells may in fact have several potential cells of origin, and single-cell sequencing is a powerful tool which could be used to identify the origins of LCH and mechanisms of pathogenesis.

Session III. Cell of Origin – Continued. Peter Campbell (Cambridge) presented fascinating data identifying mutations in human blood cells, then using computational tools to work backward to identify relationships between cells based on mutation burden. "Normal" adults have many more gene abnormalities than they might expect, with specific mutations or combinations of mutations with potential to cause cancers. For example, approximately 25% of physiologically normal skin cells in a normal adult harbors a "driver" cancer mutation. Skin may therefore be visualized as a patchwork of thousands of evolving clones. Cancer develops as a result of somatic mutation and clonal selection, but quantitative measures of selection in cancer evolution are lacking. Dr. Campbell's applied similar analysis of molecular evolution among thousands of tumors across 29 cancer

types. Unlike species evolution, positive selection outweighs negative selection during cancer development. On average, <1 coding base substitution/tumor is lost through negative selection, with purifying selection almost absent outside homozygous loss of essential genes. On average, tumors carry ~4 coding substitutions under positive selection, ranging from <1 to >10/tumor. Surprisingly, half of driver substitutions occur in yet-to-be-discovered cancer genes. With increasing mutation burden, numbers of driver mutations increase, but not linearly[8;9].

Jon Pritchard Fellow **Paul Milne** (Newcastle) presented mutation analysis of blood from adult patients with LCH, Erdheim-Chester Disease (ECD) and hairy cell leukemia (a B-cell leukemia). He was able to identify bone marrow stem cells with the *BRAF-V600E* mutation in each disease, which leaves a question of how one patient develops LCH, ECD, LCH/ECD, or hairy cell leukemia. The pattern of involvement of peripheral blood myeloid cells was indistinguishable between LCH and ECD, although the histiocytic disorders were distinct to HCL. The healthy counterparts of myeloid cells affected by *BRAF* mutation had a range of differentiation potentials depending on exogenous signals. $CD1c^+$ DCs acquired high langerin and CD1a with granulocyte-macrophage colony-stimulating factor and transforming growth factor β alone, whereas $CD14^+$ classical monocytes required additional notch ligation. Both classical and nonclassical monocytes, but not $CD1c^+$ DCs, made foamy macrophages easily in vitro with macrophage colony-stimulating factor and human serum. These findings support a common hematopoietic origins for LCH and ECD with potential for the lesion environment to influence terminal differentiation.[10] Pritchard Fellow **Matthias Papo** (Paris) studied adults with LCH, ECD and mixed LCH/ECD and found that they had a much higher prevalence of other myeloid neoplasias and malignancies than the general population, suggesting an association between risk of histiocytic disease and other disorders of myeloid cell growth and differentiation with progenitor cells that may share common predisposing mutations. A significant percentage (~10%) of patients with ECD have an overlapping myeloid neoplasm, most commonly occurring as a myeloproliferative neoplasm (MPN), myelodysplastic syndrome (MDS), or mixed MDS/MPN overlap syndrome (including chronic myelomonocytic leukemia). Consistent with this, molecular analysis frequently detected hallmark driver mutations of myeloid neoplasms (such as *JAK2*V617F and *CALR* mutations) coexisting with those characteristic of histiocytosis (such as *BRAF*-V600E and *MAP2K1* mutations). This series suggests the adult ECD (and LCH) mutation burden may be more complex than in children with histiocytic disorders where lesions were found to have a median of 1 mutation/exome.[11] **Dr. Anahita Rafiei** from Dr. Markus Manz's group (Zurich) presented a humanized MISTRG mouse model (expressing human M-CSF, IL-3, GM-CSF, thrombopoietin and SIRPα) where human bone marrow stem cells were engineered to express the BRAF-V600E protein.[12] Mice transplanted with human CD34+ HSPCs expressing BRAF-V600E developed anemia and reduced WBC and RBC counts. Analysis of lymphoid and non-lymphoid organs demonstrated the accumulation of "atypical" BRAFV-600E expressing dendritic cells in granuloma-like lesions. Remarkably, the percentage of bone marrow infiltrated by these cells was relatively low, though these mice developed aggressive disseminated LCH-like disease. The *BRAF*-V600E mutation seemed to increase monocytosis and generation of classical dendritic cells in short term liquid cultures, without affecting the proliferation potential of human HSPCs (Rafiei ASH 2017). No mice developed hairy cell leukemia. Together, these presentations demonstrate that cells constantly accumulate genetic errors over time, and that *BRAF*-V600E or other MAPK mutations in the right cell at the right time may lead to LCH. The role of additional mutations in modifying phenotype has not yet been defined.

Dr. Brandon Hogstad from Dr. Miriam Merad's group (New York) presented mechanistic studies of a mouse model of LCH. Where most cancer models demonstrate progressive migration and tissue invasion, in these mice the *BRAF-V600E* -mutated dendritic cells are trapped in place and do not exhibit increased proliferation. Sustained ERK activity induced by *BRAF*-V600E inhibits CCR7–mediated DC migration, trapping DCs in tissue lesions. Additionally, *BRAF*-V600E increases expression of BCL2-like protein 1 (BCL2L1) in DCs,

resulting in resistance to apoptosis. Pharmacological MAPK inhibition restores migration and apoptosis potential in a mouse LCH model, as well as in primary human LCH cells. MEK inhibitor-loaded nanoparticles have the capacity to concentrate drug delivery to phagocytic cells, significantly reducing off-target toxicity. These results indicate that MAPK tightly suppresses DC migration and augments DC survival, rendering DCs in LCH lesions trapped and resistant to cell death.[13] **Dr. Astrid van Halteren** (Leiden) discussed the role of MAPK signaling in differentiation of Langerhans cells and tumor formation in LCH. In her LCH biopsy series, abundant CXCR4 expression was identified in tumors and increased CD1a+CXCR4+ in circulation CXCR4 correlated with worse clinical outcomes. Characterization of the LCH lesion microenvironment identified both conventional and regulatory T cells, with increased IL-10 expression in lesions enriched with conventional T cells. Additionally, formation of "tertiary lymphoid structures" (immune aggregates) were identified in a large number of LCH lesions, most often in single bone lesions.[14-16] Mechanisms that regulate proliferation and survival of precursors, differentiation, and recruitment and activation of immune cells are largely unknown. These studies are beginning to address the functional implications of MAPK signaling in LCH, which may identify additional opportunities to "disarm" the ability of MAPK to drive LCH tumor formation and disease progression.

Session IV. Trial Design. When biology begins to point toward a rationale cure, how can it be tested and implemented? **Drs. David Solit** (New York) **and Dr. Barry Taylor** (New York) described innovative strategies to identify and test potential therapies for rare diseases with the a "precision" approach where a tumor is sequenced for disease-causing mutations, and drugs are tested based on mutation rather than the traditional approach of creating treatment based on tumor type. Patients with common mutations are treated in a "basket" cohort, allowing even patients with rare tumors to access to novel drugs and clinical trials. This study design led to a seminal publication of adults with LCH and/or ECD treated with vemurafenib.[17] This trial, and expansion cohort and the French ECD/LCH series clearly demonstrate a very high response rate for adults with BRAF-V600E+ ECD/LCH to BRAFV600E inhibition.[18;19] **Dr. Katherine Janeway** (Boston) presented the NCI-COG MATCH trial design where children with relapsed or refractory cancers have tumors sequenced, then become eligible for treatment on one of several arms based on potential for a particular mutation to respond to a MATCH drug. Children with LCH who relapse or have active disease after initial therapy are eligible to participate on the MATCH, which includes vemurafenib (BRAF-V600E inhibitor) and selumetinib (MEK inhibitor).[20] **Dr. Thomas Gross** (Bethesda) discussed the challenges (and opportunities) of international clinical research trials. Testing great ideas for rational cure also requires understanding of legal and political landscape of pharmacologic industry and governmental regulation. In the >30 years the Histiocyte Society has been leading international trials for children with histiocytic disorders, the international clinical trial landscape has become vastly more complex. **Dr. Makras** and **Dr. Papadakis** (Athens) presenting challenging cases, including an intriguing observation of responses to a RANKL inhibitor.[21] **Dr. Milen Minkov** (Vienna), President of the Histiocyte Society, moderated a panel (Dr. **Johannes Visser** (Cambridge), **Dr. Jean Donadieu** (Paris), **Dr. Jean-Francois Emile** (Paris)) to discuss paths forward to bring LCH care into the era of precision medicine. It seems clear that patients respond to MAPK inhibition, but defining the potential for cure and long-term toxicities will require prospective multi-center clinical trials.

After excited discussion in the 27th Nikolas around the explosive growth of knowledge in blood cell differentiation, LCH biology, and cancer immunology, Dr. Matthew Collin noted that despite everything we have learned, we still offer our patients vinblastine and prednisone as standard of care front-line therapy. Investigations of the cell of origin in LCH are leading to a model where every patient develops their personal version of LCH based on the specific mutation, the state of differentiation of the mutated cell, and other "host" factors (e.g. other inherited or acquired mutation). Our task over the next years may perhaps be redefined as identifying the rationale cures for LCH in order to predict optimal therapy for each patient.

Carl Allen MD, PhD
Houston, TX
May 1, 2018

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