Cellular origins of Dendritic Cells: implications for Langerhans Cell Histiocytosis

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The Nikolas Symposia

The Nikolas Symposium is an annual gathering of 20 or so scientists and clinicians who meet to discuss a rational basis for curing the histiocytic disorders. The Symposium is named after Nikolas, the son of Paul and Elizabeth Kontoyannis, who developed Langerhans cell histiocytosis (LCH) in infancy. A different theme of biology related to histiocytosis is discussed every year; the 2103 meeting focussed on the cellular origins of dendritic cells. A summary is presented here.

Introduction

The Nikolas Symposium 2013 brought together experts in the field of myelopoiesis and dendritic cell development. The 23rd meeting also honoured the contribution of immunologist **Abul Abbas** to the Symposium and the understanding histiocytosis with the John Pritchard award. The meeting was opened by **Robert Arceci (Phoenix)** who welcomed participants and provided an overview of the symposium. **Vasanta Nanduri (London)** reviewed the history, clinical presentation and late complications of LCH. LCH is currently classified as a malignant histiocytosis by the 2008 4th edition of the Classification of Tumours of Haematopoietic and Lymphoid Tissues (Swerdlow et al., 2008). This definition anticipated the discovery of mutated BRAF in LCH lesions and should replace the 1997 WHO/Histiocyte Society working group classification of LCH as a 'non-malignant disorder of varied biologic behaviour' (Favara et al., 1997). In long term follow up studies of children with LCH, Dr Nanduri reported that approximately 50% of LCH patients require systemic therapy. Of these, 10%-20% are at risk of death and up to 50% of the survivors develop a range of long-term sequelae. Most common are bony deformities, restrictive lung disease, and central nervous system (CNS) damage leading to diabetes insipidus and hypothalamic syndrome (morbid obesity), cerebellar problems and learning difficulty. As these patients enter adulthood, there is an increasing need to identify age-appropriate clinical services.

Jim Whitlock (Toronto) reviewed current treatment of LCH. Treatment is graded according to stage, risk organ involvement and rate of initial response. The staging of LCH is determined by the involvement of single or multiple organ systems; risk organs including spleen, liver, bone marrow and lung; and the rate of initial response is determined after 6-8 weeks of therapy. International trials LCH I and II were extremely successful in defining the optimal empirical therapy of LCH and enrolled more than 1,000 patients over several decades. LCH III has been recently completed and stratified patients with multisystem (MS) LCH according to the involvement of risk organs, (Gadner et al., 2013). Approximately half of patients have high risk MS LCH and their overall survival is now 84%, a continual improvement since LCH I and II. MS LCH without risk organ involvement is now known to benefit from extended maintenance therapy with a 37% risk of relapse after 12 months vs 54% risk after conventional 6 months of treatment (Trial No. NCT00276757). Progress has also been made in the salvage of refractory high-risk patients with combined cladribine (2-CdA) and cytarabine (Ara-C) (Bernard et al., 2005) or single-agent clofarabine (Simko et al., 2014). LCH IV is still in the set up phase and again takes a stratified risk approach to ask multiple therapeutic questions including the optimal duration of therapy, salvage options including stem cell transplantation and CNS disease.

The risk of developing CNS lesions is increased in paediatric patients with multi-system LCH and bone lesions in certain sites. These 'CNS risk lesions' have been defined in paediatric patients by the Vienna group and include lesions in the skull base, in particular those adjacent to the eye and ear.

These patients have a risk of pituitary diabetes insipidus (DI) of almost 40 percent, as opposed to 4 percent in single-system patients without CNS-risk lesions (Grois et al., 2010). CNS disease in children may have long term degenerative sequelae; however, in adults, pituitary involvement is a common isolated or second site occurrence and is not thought to be associated with degenerative CNS disease.

Several new agents are in phase I/II studies of LCH including BRAF inhibitors, MEK and Akt inhibitors. Dabrafenib is available for children with BRAF V600E mutations in glioma, melanoma and LCH (Trial number NCT01677741). Trametinib, a MEK inhibitor also in trial in melanoma, apparently increases the efficacy and reduces side effects of BRAF inhibitors. The next generation of trials may be involved kinase inhibitors combined with immunomodulators (e.g. PD-1; CTLA-4 inhibitors).

Marian Malone (London), a paediatric pathologist who sees 20-30 new cases of LCH per year, outlined the pathological presentation of histiocytosis. The principal diagnostic antibodies used to identify and classify histiocytoses are:

	Antibody	**Target**
•	S-100	LCs and LCH cells
•	CD1a	LCs and LCH cells
•	Langerin	LCs and LCH cells
•	Fascin	maturing DCs, macrophages, JXG histiocytes and some LCH cells
•	Factor XIIIa	CD14+ dermal DCs, resident macrophages and JXG histiocytes
•	CD68; CD163	inflammatory and resident macrophages and JXG histiocytes

LCH lesions contain mixed inflammatory infiltrates of LCH cells, macrophages, eosinophils and T cells. The LCH cell itself is not dendritic in morphology and is very pleiotropic; some are multinucleate and may show haemophagocytosis. Giant cells staining with macrophage markers such as CD68 are frequently seen in LCH bone lesions but are also occur more rarely at other sites. They may express CD1a but not Langerin. LCH cells are uniformly CD1a positive but Langerin expression and Birbeck granules are more variable and absent in LCH lesions of the liver. The most common pathological samples are skin and bone biopsies. Superficial scaling and 'epidermotropism' (hugging the epidermal border) is typical of skin lesions. In contrast, Hashimoto-Pritzker disease, a congenital form of LCH that often spontaneously regresses, presents with deeper infiltration of the adnexal structures and is E-Cadherin positive (Geissmann et al., 1997). It was also noted that LCH lesions evolve from an active cellular form with frequent LCH cells and eosinophils to a 'burnt out' fibrotic lesion in which mainly CD1a negative and Langerin negative histiocytes remain. The T cell infiltrate of LCH is not clonal and shows frequent expression of FoxP3 (Senechal et al., 2007).

Developmental origins of the mammalian haematopoietic system

Marella De Bruijn (Oxford) began the session on the developmental origins of the mammalian haematopoietic system by introducing the early ontogeny of haematopoiesis. She outlined that there are two models of early haematopoiesis, a two-wave and a three-wave model. The two-wave model recognises primitive haematopoiesis in the yolk sac and definitive haematopoiesis in the aorta-gonad-mesonephros (AGM). In the three-wave model, the primitive yolk sac stage is subdivided into an early phase at mouse E7 producing erythroid, macrophage and megakaryocyte unipotential progenitors and a later phase at E8.5 in which bipotential and multipotential progenitors appear. These more advanced cells are also found in the para-aortic splanchnic tissues, endocardium and placenta. The critical functional difference between both primitive waves and the subsequent AGM wave at E11.5 is that only the AGM wave gives rise to transplantable haematopoietic stem cells (HSCs) that migrate to the foetal liver and are capable of definitive adult haematopoiesis. De Bruijn

and others have defined the genesis of definitive haematopoiesis in detail by analysing the haemogenic endothelium of the dorsal aorta as it undergoes a process of endothelial to haematopoietic transition (EHT). This remarkable fate change can now be visualised in real time as endothelial cells round up, acquire expression of HSC antigens VEcadherin and CD45 and bud off into the circulation (de Bruijn et al., 2000; Lancrin et al., 2009; Bertrand et al., 2010; Boisset et al., 2010; Swiers et al., 2010). The main questions that still drive the field are to define the spatio-temporal localisation of EHT, the intermediate cellular stages of development and the genetic regulation of these events. RUNX1, a core-binding factor alpha family member, is critical for definitive haematopoiesis. Knock-out of *Runx1* leads to a block in EHT at an early stage in the formation of haemogenic endothelium. Multiple enhancers control the expression of RUNX1 at different stages in haematopoiesis. De Bruijn and colleagues have shown a key role for the +23 Kb enhancer which is bound by GATA2, SCL, LMO2 and ETS. A GFP tag under control of the +23 enhancer identifies a transitional population of cells with both endothelial and haematopoietic transcripts. *Runx1* activation is preceded by GATA2 expression and single cell gene expression analysis indicates a progressive lineage commitment of hemogenic cells rather than an abrupt bipotential fate decision (Swiers et al., 2013a; Swiers et al., 2013b).

Hiroshi Kawamoto (Kyoto) expounded his work over the last decade calling into question the myeloid-lymphoid bifurcation that has been adopted by textbooks as the earliest haematopoietic fate decision since the era of Maximov c1935 (Lu et al., 2002; Wada et al., 2008). One of the limitations of cell fate modelling is that the potential ascribed to progenitor cells is limited by the ability of *in vitro* or even xenogeneic differentiation to capture a simultaneous read-out of multiple different lineages. Strictly speaking, potential can only be ruled in but not excluded by such assays. The existence of common lymphoid progenitors is now widely questioned because these populations are now found to have myeloid potential using improved assays. Kawamoto was one of the first to postulate a 'myeloid-based' model of differentiation and many others have now provided evidence of lymphoid-primed multipotent progenitors or multi-lymphoid progenitors that have lost erythroid and megakaryocyte potential but maintained granulocyte, monocyte and lymphoid potential. Together these data suggest that the earliest bifurcation in haematopoiesis separates erythroid and megakaryocytic potential from lympho-myeloid potential (Luc et al., 2008; Goardon et al., 2011; Doulatov et al., 2012; Sanjuan-Pla et al., 2013). Thymic haematopoiesis was one of the first models to demonstrate lympho-myeloid progenitors (Wada et al., 2008) and interesting recent data show that progenitors arising earlier than definitive HSC are able to contribute to thymic populations (Boiers et al., 2013). Kawamoto and colleagues have used a dual engineered stroma (TST-4/DLL) to demonstrate that the thymic DN1 population contains macrophage and T cell potential; the macrophages forming beneath the stroma while T cells bud from the apical surface (Bell and Bhandoola, 2008; Wada et al., 2008). In his model there is a progressive restriction of potential: MEMgTB – METB – MTB – MT – T. It is argued that the separation of B and T potential recapitulates a previously unappreciated early phylogenetic distinction between B and T functions that actually pre-dates the abrupt arrival of the Rag transposon and antigen receptor rearrangement in jawed fish (Agrawal et al., 1998). Studies of the molecular control of T cell differentiation support a model in which B cell fate is an early differentiation pathway that must be repressed by polycomb complexes (including genes *Ring1* and *Ring2*) mediating heritable histone modifications of genes such as *Ebf1 and Pax5*, in order for T cell differentiation to proceed. Loss of RING1/2 function in knockouts leads to reprogramming of T cells to B cell fate (Oguro et al., 2010). Kawamoto and colleagues have recently pioneered the use of iPSC derived from T cells in order to investigate T cell differentiation and as a route to the generation of antigen-specific cellular therapy (Vizcardo et al., 2013).

Modelling the development of monocytes and macrophages

Siamon Gordon (Oxford) reviewed the plasticity of monocytes and macrophages. An *in vitro* model system that has been developed and applied to *in vivo* macrophage populations is based on the M1

classical and M2 'alternative' activated macrophage phenotypes. A number of well established markers have been described for M1/M2 and potentially, this system may be expanded to encompass other phenotypes in parallel to the polarity of a T cell response:

Macrophage	**cytokine stimulus**	**features**
M1 or 'classical'	IFNγ	class II, iNOS, TNF
M2 or 'alternative'	IL-4; IL-13	MMR, Dectin-1, arginase
Inflammatory	IL-17	extracellular pathogens, acute inflammation
Regulatory	IL-10;	TGF would healing and repair

The functions of macrophages in different contexts may be defined by a range of receptor systems such as: opsonic; toll-like; lectin; scavenger; and, cytosolic recognition. A comprehensive gene expression comparison of mouse and human monocytes undergoing alternative activation reveals a common signature of 87 genes differentially expressed genes including up-regulated IRF4 and transglutaminase 2 (TGM2) and down-regulated CD14 and S100A8 (Martinez et al., 2013). The upregulation of TGM2 is of interest as it is functionally related to FXIIIA, a known marker of resident macrophages and pathological histiocytes in humans. IL-4 also induces macrophage fusion and a fusion screen was used to identify participating surface proteins including CD9 and CD36 and lipids such as phosphatidyl serine (Helming and Gordon, 2009). The presence of giant cells and eosinophils in LCH suggests that IL-4 signalling plays a role in pathogenesis. IL-4 expression has been noted to be up-regulated in lesional T cells, relative to peripheral blood T cells (Allen et al., 2010).

Frederic Geissmann (London) described his recent work on the ontogeny of tissue macrophages. Frederic highlighted the fact that the 'mononuclear phagocyte' concept in which tissue macrophages are continually replenished blood-borne precursors was derived only from experiments on the recruitment of inflammatory cells and largely ignored studies of resident tissue populations (van Furth and Cohn, 1968; van Furth et al., 1972). The latter provided an early indication that many resident cells such as Kupffer cells and alveolar macrophages are maintained in the absence of BM-derived progenitors and can respond to depletion by proliferating locally (Sawyer et al., 1982; Tarling et al., 1987; Yamamoto et al., 1996). A recent example of this was published using modern tools (Jenkins et al., 2013). Geissmann and colleagues have used knockout mice lacking definitive haematopoiesis *(Myb -/-)* to show that tissue macrophages arise from Myb-independent precursors (Schulz et al., 2012). At E10.5 it is possible to observe F4/80 bright yolk sac macrophages migrating and proliferating throughout the embryo. These primitive cells do not depend upon GATA-2, FLT3 or GM-CSFR. Their contribution to adult macrophage populations may be tracked by comparing *Myb*-/- mice (yolk sac present but HSC absent) with *Pu.1*-/- mice (no haematopoiesis present), *Flt3*-cre YFP mice (HSC marked) and *Csfr-1*-cre YFP (yolk sac and HSC marked). Using these approaches it appears that up to 50-60% of adult tissue macrophages are ultimately derived from MYB-independent precursors, the remaining 40-50% deriving from definitive haematopoiesis later in embryogenesis. So far it has not been possible to discern functional differences between cells arising from these two origins. Also genetic dissection does not permit an analysis of the anatomical location of the immediate precursor population so it is possible that MYB-independent progenitors from the yolk sac seed the developing liver and secondarily infiltrate the tissues, in parallel with macrophages derived from definitive haematopoiesis. The significance of these studies is yet to be fully realised. In Geissmann's view, the tissues develop in parallel with their resident macrophage populations and the renewal and homeostasis of tissue leukocytes is under local environmental control, not dictated by the bone marrow. It is predicted that epigenetic modification will distinguish between resident and recruited macrophages and it is possible that local somatic mutation within tissue macrophage populations could give rise to histiocytic disorders.

Florent Ginhoux (SIgN) presented complementary data to Geissmann. Ginhoux has taken a cellular rather than genetic approach to defining the origin of tissue macrophages. In steady-state models of macrophage homeostasis it is clear that monocytes are not required to maintain alveolar macrophages or kupffer cells, while they contribute variably to red pulp macrophages and to bone marrow macrophages. This can be demonstrated by the lack of labelling of tissue macrophages by fluorescent lineage markers expressed by monocytes, the lack of contribution of non-host cells to the tissue macrophages of parabionts and the failure of induced depletion with diphtheria toxin to recruit circulating precursors to tissue macrophage populations. Donor-derived cells are observed after lethal irradiation and transplantation but with slower kinetics than bone marrow repopulation. Resident tissue macrophage proliferation can be observed in all these models and is increased by depletion manoeuvres (Hashimoto et al., 2013; Yona et al., 2013). Ginhoux went on to describe a RUNX1-based fate mapping experiment which is able to induce a time-dependent labelling of RUNX1 expressing cells. Early time point labelling of yolk sac macrophages shows that they contribute to nearly 100% of microglia and initially to a high proportion of tissue macrophages and Langerhans cells. However, with time, foetal liver haematopoiesis populates and eventually dilutes out or replaces most of the tissue macrophage contingent derived from the yolk sac. Critically, although these macrophages are derived from foetal liver monocytes, they may still be MYB-independent remnants of primitive haematopoiesis, rather than products of definitive haematopoiesis. Thus the genetic model of Geissmann and the cellular studies of Ginhoux are not inconsistent but are reporting different facets of the same process. Both agree fully upon the key issue that the adult tissue macrophage population does not depend upon haematopoiesis and therefore that histiocytic disorders may have local precursor origins.

Nikolaus Romani (Innsbruck) reviewed the molecular pathology of a congenital LC deficiency and immunodeficiency caused by mutation of the endosomal adaptor protein p14 (Bohn et al., 2007). He developed a mouse knockout/reporter for DC-specific deletion of p14 and expression of tdTomato which has LCs at birth that rapidly dwindle and disappear (Sparber et al., 2014). The defect is intrinsic as labelled bone marrow cells are able to repopulate the epidermis upon transfer. Loss of LCs is associated with increased apoptosis and diminished proliferation in the neonatal epidermis. Molecular analysis shows that deletion of p14 (aka Lamtor2) disrupts the lysosomal membrane associated Ragulator (sic) complex and that this blocks MEK/ERK signalling, resulting in failure of homeostatic proliferation and survival. This does not appear to affect the homeostasis of DC populations but compromises the abilities of short term monocyte-derived and long-term homeostatic populations of LCs to reconstitute the epidermis (Sere et al., 2012; Sparber et al., 2014). The effect on the survival of tissue macrophages or their ability to reconstitute after injury is not known. It is an exciting possibility that intact p14 may also be required to maintain the proliferation of LCH cells and that drugs targeting this molecule, or the Lamtor complex, may be useful in treating LCH.

The cellular origins of LCH and recent advances in treatment

Ken McClain (Houston) presented recent data on the analysis of mutated BRAF in a large cohort of 100 paediatric patients (Berres et al., 2014). As previously reported (Badalian-Very et al., 2010), BRAF V600E was found in approximately two-thirds (64%) of LCH lesions. In the first report of a significant clinical association with genotype, mutated BRAF was associated with increased risk of recurrence although it did not define specific clinical risk groups (overall survival, risk organ positive/negative; single system/multisystem). The application of sensitive mutation detection by allele-specific PCR revealed that patients with active, high-risk LCH also carried BRAF V600E in circulating CD11c+ and CD14+ mononuclear cells and in bone marrow (BM) CD34+ hematopoietic cell progenitors, whereas only lesional LCH cells were positive in low-risk LCH patients. Overall, the detection of BRAF V600E in peripheral blood was 97% sensitive and 100% specific for active multisystem disease. These data suggest two different routes to the evolution of LCH depending upon whether mutated BRAF arises in the BM or in peripheral tissues. The ability to detect BRAF

V600E in peripheral blood is likely to be very useful clinically but will require prospective validation. It is hoped that this might be achieved through the international LCH IV study. The Houston group has made further inroads into identifying effective treatment for relapsed LCH and has recently shown that clofarabine is effective with a 61% complete response rate and 17/18 patients on treatment still alive (Simko et al., 2014).

Astrid van Halteren (Pritchard Fellow, Toronto) showed data that CXCR4 expression is associated with a poor disease outcome in LCH. Typically, LCH cells express CCR6, CCR7 and variable CXCR4 (Fleming et al., 2003). CXCR4 is of interest because its ligand CXCL12 is sometimes co-expressed in LCH lesions and because CXCR4 signals via MEK/ERK. The components have been implicated in the recruitment of LC precurors after injury and might constitute an autocrine circuit in higher risk LCH lesions (Charbonnier et al., 1999; Ouwehand et al., 2008). Other data were presented that CD1a+ cells could be detected in the peripheral blood of patients with LCH. These were a subset of CD11c+ cells that have been previously shown to be expanded in LCH, although several other studies have failed to detect CD1a expression in the blood. An antibody clone B-B5 that was previously used to identify CD1a+ 'LC precursors' in the blood of healthy individuals has subsequently been found to recognise CD1c (Ito et al., 1999).

Jennie Rowell (Ohio) described how canine models are useful in cancer genetics (Rowell et al., 2011). Pure breeds are isolated genetic populations with large regions of linkage disequilibrium that can be mapped to cancer phenotypes with relative low density 50,000 SNP arrays on as few as 100 animals. The Bernese mountain dog develops both LCH and histiocytic sarcoma and GWAS has identified a region of chromosome 11 containing MTAP and CDKN2A (Hedan et al., 2011). Mutation of CDKN2A is linked to histiocytic sarcomas arising in patients with ALL . A less well known canine model of histiocytosis is the flat-coated retriever which suffers from localised and disseminated forms of histiocytic sarcoma, each associated with discrete GWAS signals. It is relevant to LCH that histologically identical lesions appear to arise through distinct genetic mechanisms.

Robert Arceci (Phoenix) reviewed recent advances in the treatment of myeloid malignancies, using AML as an example (Pui et al., 2011). Conventional empirical chemotherapy reaches a limit at around 50% survivorship when toxic death in remission approximates relapse death. Targeted therapy has been developed on this background, attempting to open new windows in the 'chemotherapeutic principle' that the effect of a treatment on the disease should be more potent than the effect on the host (originally defined in the theory of anti-microbial therapy). However, anti-CD33 (*Myelotarg*), despite showing early promise, is not approved in the US although it remains licensed for low risk disease in Europe. Targeted therapy of the FLT3 internal tandem duplication, which confers high risk to AML, has also not been impressive; lesaurtinib (CEP701) is very active in blocking FLT3 phosphorylation in vitro but produced no benefit in early phase trials, possibly through failure to achieve adequate plasma levels. All trans retinoic acid (ATRA) and arsenic have been more successful in the treatment of acute promyelocytic AML (APL). Despite the difficulties in getting new targeted therapies through early phase trials, new impetus has been provided by the arrival of next generation sequencing and the potential to define the complete genetic landscape of a tumour (Schuback et al., 2013). In AML samples there are typically an order of 10^6 variants compared with the reference human genome, of which 10^4 are somatic mutations relative to constitutive DNA, 10^2 are non-synonymous and 10^1 are likely to be deleterious or functional. Many are private, appear to have arisen in the HSC pool prior to leukaemic transformation and to have been propagated as passenger mutations. A small number such as TET2, WT1 and ETV6 are highly recurrent (Welch et al., 2012). Greater diversity and evidence of clonal evolution is perhaps apparent in myelodysplastic syndrome (Papaemmanuil et al., 2013).

Molecular pathology and targeted therapy of LCH

Maarten Egeler (Toronto) presented a talk prepared by **Barrett Rollins (Boston)** who was unable to attend at short notice. Egeler and colleagues had previously attempted to identify somatic mutation in LCH by multiple technologies (da Costa et al., 2009). The application of mass spectrometry to DNA extracted from formalin fixed samples (OncoMap) enabled screening of up to 1000 common somatic mutations in LCH lesions. This technology coupled with independent validation by pyrosequencing led Rollins and colleagues to identify BRAF V600E in 57% of LCH lesions (Badalian-Very et al., 2010). The group has recently expanded genetic basis of LCH further with a description of ARAF mutation in 1 of 3 exomes form BRAF wild-type cases (Nelson et al., 2014). Geissmann and colleagues have also found new BRAF mutations including a constitutive mutation (Satoh et al., 2012). The ARAF mutation was not seen in another 19 cases suggesting that it does not constitute a second major genotype group in LCH. Importantly, mutated ARAF was found to transform fibroblasts in a classic anchorage independence assay and was inhibitable by BRAF inhibitor (vemurafinib) (Nelson et al., 2014).

Poulikos Poulikakos (New York) presented a talk on RAF kinase inhibitors. The RAF kinases a, b, and c have similar structures with regulatory N terminus and catalytic C terminus. The regulatory domain mediates dimerization that can occur between heteromers in addition to the formation of homodimers. Dimerisation is the initial step in kinase activation and phosphorylation occurs at residues 599T and 602S. Glutamate substitution (V) at 600E locks the kinase in an active conformation. It is not at all clear why certain oncogenic versions of RAF associate with cancers of different tissues. RAF inhibitors target the monomeric forms of RAF proteins but can induce dimerization and activation at low concentrations (Poulikakos et al., 2010). This effect explains some of the 'off-target' (actually on-target) effects of BRAF inhibitors upon tissues with wild-type BRAF, especially when there is high RAS state with basal RAF dimerisation. Mutants that spontaneously dimerize are resistant to inhibition (Poulikakos et al., 2011). The RAF-MEK-ERK pathway has been the subject of intense scrutiny in the search for therapeutic kinase inhibition. MEK inhibitors were the first drugs to be developed on the grounds that a downstream target might be more effective in pathway inhibition but have been clinically disappointing. In some cell lines with mutated RAF, complete inhibition of MEK resulting in absent phospho-ERK fails to inhibit cell growth. ERK inhibitors are in developmental phase but the complexity of signalling interactions and feedback inhibition makes it difficult to predict the outcome of inhibition of any single pathway member. Recent studies indicate that the combination of RAF and MEK inhibitors may be effective. Resistance to RAF inhibition may occur by several mechanisms (Poulikakos and Rosen, 2011): 1) intrinsic primary resistance (e.g. dimerization); 2) adaptive resistance over days and weeks probably due to biochemical processes such as the relief of feedback inhibition (Lito et al., 2012); 3) acquired over months to years probably due to escape mutations in other pathways e.g MAPK and PTEN mutation (Turajlic et al., 2014). It was argued that resistance to BRAF inhibitors such as vemurafinib should be anticipated in LCH.

Summary and conclusions

Impressive advances have been achieved since the discovery of BRAF V600E in LCH in 2010. The molecular calling card of LCH promises to reveal much more about the pathogenesis of LCH and to provide clinically useful tools in the near future. As with the 22nd Nikolas Symposium in 2012, much effort continues to be devoted to the integration of RAF kinase biology into the sphere of LCH knowledge. This field continues to challenge biologists and oncologists alike and is moving at a very fast pace. The 23rd Symposium also highlighted that venerable truths may be overthrown unexpectedly: the mononuclear phagocyte system now lies in tatters and new concepts about macrophage ontogeny have emerged. The impact of this on LCH biology is not yet fully realised but at least if the answer to the origin of LCH is not yet clear, the right questions are increasingly better defined.

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