Cell signalling and responses: Pathways to cure?

Meeting Summary of the 22nd Nikolas Symposium, Loutraki, 3-6 May 2012

The Nikolas Symposia

The Nikolas Symposium is an annual meeting aimed at finding a rational cure for Langerhans Cell Histiocytosis (LCH). This meeting was founded and continues to be sponsored by Paul and Elizabeth Kontoyannis whose son Nikolas developed LCH in infancy. The symposium is an interactive forum of basic scientists and clinicians who meet each year to discuss a different theme of biology related to improved knowledge and treatment of LCH. A summary of the 2012 meeting is presented here.

Introduction

The Nikolas Symposium 2012 brought together experts in the field of cell signalling, including 1) dysregulation of the RAF/MEK/ERK pathway in oncogenesis, 2) genetic control of dendritic cell (DC) development and 3) macrophage dysregulation in inflammation and neoplasia. The recent discovery and validation of mutated BRAF V600E in 60% of LCH biopsies has provided a possible etiologic basis of LCH and the potential for developing targeted therapeutic trials with BRAF inhibitors (Badalian-Very et al. 2010). The 22nd meeting also honoured the immense contribution of pathologist Dr Ron Jaffe in helping to define the pathology of LCH and other histiocytoses by presenting him with the Jon Pritchard award.

Pathogenesis of LCH

The 'cell of origin' of LCH continues to be a subject of investigation. One hypothesis is that neoplastic transformation LCH arises from neoplastic transformation of an immature DC-like progenitor. LCH is recognized as a 'dendritic cell-related disorder of varied biologic behaviour' according to WHO/Histiocyte Society nomenclature (Favara et al. 1997). The lesions of LCH contain mixtures of inflammatory cells including macrophages, eosinophils and lymphocytes and the reactive, cytokine-rich milieu is almost certainly important in prolonging the survival of LCH cells and promoting local destructive pathology. However, activation of the MEK/ERK pathway most frequently associated with heterozygous V600E mutation of BRAF, is likely to be a critical pathway in the development of LCH. From this perspective, LCH may be viewed as a dominant oncogene-driven neoplasm, but one that is highly dependent upon inflammation. It may therefore resolve with anti-inflammatory therapy, or even spontaneously regress through the action of intrinsic regulatory mechanisms, but will otherwise require some form of cyto-reductive chemotherapeutic treatment. This model accommodates most of the clinical features of LCH including a very heterogeneous presentation involving a wide diversity of sites and variable pathological appearances, the ability of many different anti-inflammatory and cytoreductive therapies to treat the disease, and the high rate of relapse in multi-system disease. Promotion of cancer by inflammation is a venerable concept in

oncology and recent research on the potential of macrophages to advance neoplasia was highlighted during the meeting. A significant problem remains, however, that no model offers a means of deciphering the pathogenesis of late neurological sequelae. Histologically, this process is distinct from primary LCH and affected neural tissue shows gliosis, CD8+ T infiltration but no LCH cells. The concept of a 'burnt out' LCH lesion is often cited in relation to this appearance. While it is clear that LCH lesions lose characteristic LCH cells and evolve to fibrosis in a number of other sites, a much better understanding of this process will be required to make sense of late neurological syndromes.

LCH in the thymus – Ron Jaffe

Haematopoietic organs, bone marrow, spleen and liver may be involved in LCH are all recognised as 'risk organs' in multi-system LCH. Dr Jaffe demonstrated that very few Langerin+ cells are found in normal thymus, and then usually only in association with Hassall corpuscles or as scattered medullary cells. A connection between the thymus and LCH had been proposed many years previously by Gordon Vawter who had advocated the use of thymic extract to treat LCH. While this idea remains speculative, Dr Jaffe demonstrated the prevalence of thymic LCH according to three patterns: 1) an incidental finding at cardiac surgery, usually a small focus, that is inconsequential; these incidental lesions are likened to a spectrum of small LCH-like lesions found in lymph nodes stimulated by lymphoma, leukaemia and other disorders; 2) Isolated solitary disease that behaves like LN only or bone only disease, is low grade and may regress spontaneously; occasionally this may leave a hard sclerotic residual mass that can be disconcerting clinically but represents a complete response; 3) thymus involvement in multi-system disease; this is typically a pattern of medullary infiltration that is common but under-recognised; radiology indicates thymic enlargement and calcification and these findings often resolve with systemic treatment and are effectively clinically silent.

Transcriptional profiling and proteomics – Ken McClain and Caroline Hutter

Dr McClain reviewed transcriptional profiling studies of the Houston group. Osteopontin, neuropilin, vannin-1 and CEACAM together with myeloid markers CD13, CD33, CD11b and CD11c were found to be upregulated in LCH cells compared with epidermal LCs in the initial study of Allen et al (Allen et al. 2010). Although this was important in defining the many differences between LCH and LC in an unbiased fashion, re-screening of the data had surprisingly not clustered lesions into two entities according to BRAF mutation status. Dr McClain presented a second larger series of transcriptional profiles in which BRAF mutation emerged as the strongest factor segregating the data. Promising leads were also seen in relation to a number of clinical variables that were not apparent in the previous array series. The Houston group also concur with Rollins and colleagues, finding that 64% of LCH specimens are BRAF mutated and that sequential biopsies remain true to BRAF mutational status. Intriguingly, the BRAF wild-type samples showed greater upregulation of MEK/ERK pathway genes, but Dr Rosen observed that this result is not inconsistent with kinase feedback mechanisms that vary according to the initiation point of pathway activation. Within limits, he argued, gene

expression also has very indirect bearing on protein activity and overall pathway flux, which are the critical biochemical factors. Dr McClain also described progress in proteomic analysis based on the Luminex platform from 110 serum analytes. He identified 7 clusters by unsupervised analysis and 4 clusters by k-means analysis that correlate with clinical variables, including disease stage and site of lesion. The goal of these studies is to identify biomarkers that may lead to more rapid and accurate recognition of high-risk disease, remission, re-activation and CNS risk.

Caroline Hutter, attending as the Pritchard Scholar, also presented a transcriptional profiling study from the Vienna group. Her approach compared LCH cells with normal LCs, blood myeloid DCs and pDCs. By principal component analysis (PCA), it was apparent that LCH cells were distinct from LCs and blood DCs and that either LCs or blood myeloid DCs would serve as useful comparator populations for gene expression studies, being approximately equidistant from LCH cells on a 2D PCA plot. This is an important difference with the Houston group who have derived most of their gene expression data relative to epidermal Langerhans Cells. Dr Hutter followed her global transcriptomic experiment with a more focused examination of the expression of Notch 1 and Jagged 2 by LCH cells. She verified expression of active intracellular Notch-1 by immunostaining and western blot and argued that the expression of both ligand and receptor by LCH might provide a survival signal circuit. Interestingly, Jagged 2 was able to replace IL-4 and cooperate with GM-CSF and TGF in the generation of Langerin+ E-cadherin- cells from monocytes. Together these data offer a differentiation and survival pathway that looks potentially relevant to the biology of LCH.

Molecular control of DC development – Li Wu and Boris Reizes

A variety of differentiation factors have been shown to be required for DC development, with specificity for single or multiple lineages. Gene dosage and the particular type of mutation (null, dominant negative, domain-specific) can exert distinct effects. Dr Wu reviewed her recent studies showing that expression of FLT3, a critical tyrosine kinase receptor in DC development, is under the control of master transcription factor PU.1 (Carotta et al. 2010). PU.1 was previously known principally for its role in inhibiting granulocytic potential, so directing granulocyte-macrophage progenitors towards monocyte/macrophage fate but a role in DC development had been difficult to interrogate partly because of early lethality in PU.1 knock-outs. FLT3 identifies subsets of both myeloid and lymphoid progenitors that give rise to DCs in human (Karsunky et al. 2003)and mouse models (D'Amico and Wu 2003) and adminstration of FLT3 ligand enhances DC development *in vivo* in humans and mice. FLT3 is expressed on all cells of the DC lineage from progenitors to mature cells. Dr Wu showed that PU.1 parallels this pattern and that inducible deletion of PU.1 prevents development of lymphocytes, monocytes and all DCs, including LCs in a cell-intrinsic fashion. Examination of the progenitor compartment in an *in vitro* inducible model indicates that PU.1 deletion at any stage of development from HSC to CDP prevents FL-driven DC differentiation. In contrast, GM-CSF-driven inflammatory DC generation from HSCs, CDPs or monocytes remains intact. Examination of PU.1 knockout mice during development indicates that FLT3 expression is absent and PU.1 binding sites are found upstream of the FLT3 gene.

Taken together, these results show a novel, potential, non-redundant function for PU.1 in controlling DC fate.

Dr Reizes reviewed the subsets of DCs found in steady state. Plasmacytoid DCs are the most easily recognised across several species including mouse and humans. In addition, recent studies have shown that classical or 'myeloid' DCs comprise two lineages with distinct functional capacities. A minor subset with superior cross-presenting capacity is defined by CD141 expression in humans and CD103 or CD8 expression in the mouse. The remaining and major population of myeloid DCs in humans is identified by CD1c; in mouse the parallel population bears CD11b. CD14+ human DCs, notable in dermis, represent a third myeloid DC lineage without an obvious murine counterpart. This puzzle was also addressed based on antigen markers that divided the murine CD11b DC subset into two fractions.

Dr Reizes reviewed his previous work on the development of pDCs as a model of molecular control of DC differentiation. The function of pDC is dual: innate protection against acute cytopathic viruses and enhancement of T cell memory responses against chronic or recurrent viral infections. E2-2 is most highly expressed in pDC in both mouse and human and is absolutely required for the development of pDCs in mice, as shown by knock-out experiments(Cisse et al. 2008). In humans, Pitt-Hopkins syndrome partly recapitulates this phenotype. Expression of E2-2 is required to maintain pDC differentiation, as developmentally delayed knock-down of E2-2 causes pDC to convert spontaneously into cDC (Ghosh et al. 2010). Id2 is an antagonistic factor of E2-2 and plays a key role in promoting classical or myeloid DC development although this remains formally untested in humans. Malignant transformation of pDC is described in humans in the form of a rare CD4+ CD56+ 'hematodermic lymphoma' sometimes associated with acute myeloid leukaemia. This might be a model of dysregulated growth applicable to LCH in terms of a skin-tropic neoplasm with DC phenotype. E2-2 is expressed at very high level in this disease as shown by the analysis of a number of cell lines derived from neoplasms.

A number of groups have used CD11c-cre to direct conditional expression or gene excision to DC. This is not completely DC specific as 6% T cells, 5% B cells and 12% NK cells also express a transgene controlled by this strategy. Dr Reizes showed that DC-restricted deletion of PTEN, a phosphatase that normally inhibits the PI3K/Akt results in acceleration and expansion of DC development. The most prominent effect is observed on the CD8+ cross-presenting DC subset and is appropriately reversed by mTOR, a downstream inhibitor of the PI3K/Akt pathway. This work is interesting in relation to the preliminary observation that PTEN deletion might play a role in LCH, providing activation of PI3K/Akt in patients with wild type BRAF (Barrett Rollins; HS meeting 2011). Mouse CD8+ tissue DC homologues also express Langerin but paradoxically this is probably not relevant per se, as Langerin is absent from the corresponding human CD141+ DC subset.

In addition to soluble factors, cell-contact signals are likely to be important in instructing DC fate. Dr Reizes has recently explored the role of Notch activation, which is seen in T

cell and B cell development when precursor cells reach their target destinations in the thymus (Notch 1) and follicle (Notch 2). In DC development, global interruption of Notch signalling or deletion of Notch 2 reduces cDC numbers in the spleen. This is specific to the Endothelial **cell**-selective adhesion molecule (**ESAM**)+,CX3CR1-CLEC12- subset of DC (Lewis et al. 2011). This subset is independent of monocytes and highly represented in the CD4+ fraction of splenic DCs. An important consequence of this work in relation to human DCs, is the identification of two subsets of CD11b+ mouse DCs that are likely to map to human tissue DCs: CD11b+ Esam+ appear to correspond to CD1c+ DCs while Esam- DCs share properties with CD14+ human DCs. Combined with the recent discovery that CD8+ mouse DCs are homologous with CD141+ human DCs, it is now possible to make cross-species comparison of the majority of DC subsets in the two species.

The molecular biology of kinase inhibition – Kevan Shokat and Neal Rosen

Two presentations focused on the chemistry and application of kinase inhibition to treat human neoplasia, by Kevan Shokat and Neal Rosen. Dr Shokat began by describing the human kinome and its 518 members. He illustrated the rapid kinetics of phosphorylation and de-phosphorylation with the shape changes governing chemotaxis of a neutrophil. Kinase inhibitors are chemically based on ATP, required by all kinases to deliver activated phosphoryl groups to their substrates. Initial scepticism that drugs with sufficient potency and specificity could be generated by modifying ATP was quashed by the invention of imatinib (Gleevec) to target the mutated BCR-ABL kinase. *In vitro* assays show that available kinase inhibitors block between 12-200 kinases, with staurosporine, a naturally-occurring compound the most promiscuous, and imatinib, among the most specific. Specificity alone is a poor indicator of clinical utility as next generation BCR-ABL inhibitors such as dasatinib are more potent but typically have a broader profile.

The two phosphorylation cascades RAF/MEK/ERK and PI3K/PTEN/AKT/mTOR that transduce signals from receptor tyrosine kinases (RTKs), are the most prevalently mutated kinase pathways in human neoplasia. MEK/ERK signalling upregulates transcription, while the AKT pathway controls ribosome biogenesis and translation. Dr Shokat described a powerful *in vivo* screening approach based on lethal transgenic oncogene activation in developing Drosophila (Knight and Shokat 2007). Drugs may be fed to pupae in a high-throughput screening format and successful inhibition of the mutated kinase is identified by rescue of the pupae to adulthood. This approach deals with bioavailability, potency and toxicity in a single round of screening and even allows combinatorial testing of novel agents. A number of potential pitfalls in inhibitor design have emerged from this work. Key among these is that kinases with negative feedback inhibition of a parallel pathway may make futile or even disadvantageous targets for inhibition. For example, mTOR is a potent inhibitor of MEK, such that inhibition of mTOR can lead to increased flux through the MEK/ERK cascade. Dr Shokat demonstrated that it was possible to develop more selective inhibitors that only block the forward flux and not the feedback inhibition (Justman et al. 2009).

Dr Rosen extended the theme of feedback inhibition in kinase pathways and how this shapes the clinical utility of kinase inhibitors. In the RAF/MEK/ERK pathway, a number of circuits inhibit flux through the pathway when activated by a mutated oncogene in the same manner as any homeostatic physiological system responding to perturbation (Solit and Rosen 2011). The spread of inhibition affects other survival pathways so that the cell becomes more dependent upon the mutated kinase; a process that has been dubbed 'oncogene addiction'. One of the consequences of inhibiting a mutated kinase is that the lateral inhibition of parallel pathways is suddenly relieved allowing tumour cells to adapt rapidly by utilising other survival signals. A specific example of this occurs with mutated RAF in which feedback inhibition of RAS-GTP (mediated by *sprouty* and other pathways) leads to a widespread inhibition of signal transduction through RTKs. Drugging mutated RAF causes a rebound in RAS-GTP and enhanced survival signals in response to extracellular growth factors binding to RTKs. A phenomenon like this might be directly relevant in LCH where inflammation provides a rich extracellular milieu. Dr Rosen showed specific examples in which inhibition of MEK/ERK signalling caused rebound activation of AKT which in some cases lead to clinical tumour progression. He postulated that an effective clinical strategy would be to biopsy a tumour, determine the dominantly acting oncogene, inhibit the appropriate pathway and rapidly re-biopsy the lesion to understand the principal adaptive mechanism to block with a second pathway inhibitor.

Dr Rosen also stressed the need to achieve rapid maximal pathway inhibition to effect tumour kill. This partly beats rebound phenomena but also minimises the risk of another Achille's heel of inhibitors targeted to kinases that dimerise during signal transduction, including BRAF (Poulikakos et al. 2010). Owing to steric effects, low concentration inhibitor binding to monomeric kinase may lead to dimerisation and signal transduction by the recruited but uninhibited second molecule of the dimer. Some mutated kinases (including BRAF V600E) are active in the monomeric form and are highly susceptible to inhibitors while tissues bearing native kinases may suffer pathway activation and toxicity. Genotyping of tumour tissue is therefore critical as tumour progression can also occur if the target kinase is wild-type. Pathway inhibition by Vemurafinib (PLX4720) in melanoma with BRAF V600E mutation results in near universal tumour responses, 70% clinical response and a median survival advantage of 5 months in patients compared to one month. The side effects in normal tissues (due to wild-type RAF activation) are reversible upon cessation of the drug. Dr Rosen postulated that ERK activation was almost certainly driving LCH and that RAF inhibitors would be safe and effective in patients with V600E mutation. He predicted that RAS-GTP levels would be low in the presence of mutated BRAF and that this combined with the low grade nature of LCH would further favour clinical responses to RAF inhibitors.

ERK activation in LCH and CSF-1 signalling mononuclear phagocytes – Barrett Rollins and Richard Stanley

Barrett Rollins reviewed the BRAF V600E mutation and confirmed its presence in approximately two-thirds of LCH cases in 2010 (Badalian-Very et al. 2010). His group used 'oncomap', a cancer-adapted version of mass-spectroscopy genotyping to

interrogate 983 mutations in 115 cancer-related genes. BRAF mutation was verified by pyrosequencing and confirmed to be a somatic mutation enriched in LCH cells, using laser capture microscopy. Controls included dermatopathic lymphadenopathy, JXG and RDD. The finding has since been independently confirmed by Geissman and colleagues on fixed tissue (Badalian-Very et al. 2010) and by Allen and McClain on sorted CD1a+ cells. Geissman also reported a novel activating mutation of BRAF in one patient (Satoh et al. 2012). Both Rollins and Allen reported that sequential sampling within one patient yields identical BRAF genotype, supporting the clonal models of LCH pathogenesis. Dr Rollins described gene set enrichment analysis that increases the statistical power of array profiling to detect families of dysregulated genes. By this approach, 'DC differentiation' genes are down-regulated by LCH and RAF and PTEN family members are both enriched. PTEN deletion has been explored in paraffin-fixed sections and pilot studies suggest monosomy in 30-40% of nuclei in some specimens. This analysis is complicated by a background truncation artefact that may account for up to monosomy in up to 10% of sectioned nuclei; PTEN loss is therefore being further tested by whole exome sequencing and copy number variation measurement.

Two potential mouse models of LCH have recently been developed using the BRAF-CA (conditional allele) mouse. This has an advantage over first generation BRAF LSL (lox-STOP-lox) knock-ins in that two copies of wild-type BRAF are expressed prior to activation by Cre recombinase. Two novel strains have been made, one in which BRAF activation is under the control of the human Langerin promoter (hu-Langerin-cre; Kaplan et al, 2007) and a second using the promiscuous Mx1 promoter (Mx1-cre). The human Langerin promoter in mouse directs highly specific transgene expression in epidermal LCs only and is not significantly transcribed in other Langerin+ DCs. BRAF activation in this context did not produce any histiocytic disease but only slight excess of Langerin+ LN cells and a wasting syndrome through some unknown mechanism. It may be argued that this model is evidence against direct transformation of epidermal LCs as the origin of LCH. The second model with Mx1-directed BRAF mutation resulted in leukocytosis and widespread histiocytosis, including giant cell formation. Dr Rollins confirmed that histiocytic lesions in this model contained many cells with CD11c+ CD207+ phenotype and Birbeck granules. Mutated BRAF expression in non-haematopoietic organs caused papillomas, adenomas and melanomas but an attempt to hone the phenotype by transplanting modified BM into wild-type recipients is proposed.

From immuno-fluorescence studies, it had been argued that MEK/ERK pathway activation is common to all LCH cells regardless of BRAF mutation status. The increase in pMEK expression in the cytoplasm in CD1a+ cells is particularly impressive; nuclear pERK also appears increased. Examination of other proteins in the MEK/ERK signalling pathway is underway, including upstream TKRs such as the CSF-1 (M-CSF) receptor. LCH cells appear to contain higher levels of CSF-1R and phospho CSF-1R than epidermal Langerhans cells.

Dr Stanley presented work relating to his long-standing exposition of the biology of CSF-1 and its receptor. He began by describing the role of CSF-1 in macrophage maturation. CSF-1 is widely produced by endothelium, stromal cells and placenta and is found as a

secreted glycoprotein, a surface-bound molecule and a proteoglycan-linked form. The suggestion that CSF-1R had a second ligand was first raised by the fact that the receptor knock-out mouse has a more severe phenotype than the ligand knock-out. IL-34 was subsequently identified as a second ligand in 2008 (Lin et al. 2008). (Geissmann et al. 2001; Lin et al. 2008)IL-34 has a more restricted pattern of expression and is only found as a secreted glycoprotein. It is expressed early in the developing nervous system and appears critical for recruitment and differentiation of yolk sac macrophages into microglia (Nandi et al. 2012). Activating mutations of CSF-1R in humans causes a condition known as hereditary diffuse leukoencephalopathy with spheroids (Rademakers et al. 2012). It is tempting to speculate about disorders of microglial homeostasis in relation to the late effects of LCH on the CNS but explicit mechanistic links remain elusive.

Another new piece of biology concerning signal transduction by CSF-1R revealed more insights into the regulation of macrophages and inflammatory disorders. The phosphatase PSTPIP2 negatively regulates the morphological and secretory response to CSF-1 and hypomorphic mutations of this protein cause inflammatory syndromes known as 'Lupo' or 'Chronic Multifocal Osteomyelitis' (Chitu et al. 2009). These are intriguing models in which dysregulation of myeloid cells causes chronic inflammatory lesions; a mode of pathogenesis that has significant resonance with LCH. Of further interest, the Plexxikon inhibitor of TKRs, CSF-1R and FLT3 (PLX3397) is able to reverse the phenotype of PSTPIP2 mutant mice.

Macrophage stories – Flavius Martin and Claire Lewis

The theme of spontaneous macrophage activation in the pathogenesis of inflammatory lesions was continued by Dr Flavius Martin. He presented a fascinating story of disorders caused by mutations in ENT3, a transporter molecule that mediates the recovery of nucleosides from phagolysosomes (Hsu et al. 2012). ENT3 mutation in humans causes a series of rare familial histiocytic syndromes including Faisalabad Histiocytosis, a disseminated Rosai-Dorman disease-like syndrome and H syndrome. Knockout mice have lymphadenopathy, splenomegaly and histiocytosis at an early stage of development. 70% develop histiocytic sarcoma between 16-26 weeks old. The phenotype is conferred by BM transplantation and is associated with increased proliferation of myeloid precursors and extra-medullary haematopoiesis. The expansion of macrophages is related to increased M-CSF, expression of M-CSFR and phosphorylation of M-CSFR and may be reversed by anti-M-CSFR antibodies. Regulation of signalling by TKRs such as M-CSFR depends of receptor-ligand dissociation and degradation in lysosomes, a process that is impaired in the poorly acidified lysosomes of ENT3 mutants. Consistent with this, upregulation of M-CSFR is seen when mutant macrophages are loaded with apoptotic cells *in vitro*. This mechanism has implications for a number of human diseases including histiocytoses and storage disorders. The occurrence of RDD-like disease in ENT3 mutation suggests that elevation of MCSF and its receptor may play a direct role in the pathogenesis of all forms of RDD. In addition, deregulated homeostasis of M-CSFR in macrophages may contribute to elevated M-CSF levels, macrophage expansion and giant cell formation in LCH and Gaucher disease.

Dr Claire Lewis presented her work on tumour-associated macrophages (TAMs). TAMs are found in more than 80% of human tumours and are probably derived from monocytes attracted by CCL2, SDF-1 and CSF-1 (Murdoch et al., 2008). Hypoxia is a significant factor in the biogenesis of TAMs and the relationship of TAMs phenotype to macrophages in LCH was discussed. From histopathology, neo-angiogenesis and necrosis are not notable features of LCH lesions, although intralesional haemorrhage and spontaneous involution may occur. TAMs are attracted to hypoxic regions, upregulate HIF1/2 and express many cytokines and growth factors implicated in tumour progression including IL-6, IL-10 , bFGF and VEGF. Dr Lewis's work has shown the importance of angiopoietin receptor (Tie-2) expression by TAMs and that targeting Tie-2 positive monocytes and macrophages enhances tumour sensitivity to hypoxia and necrosis(Welford et al. 2011). Finally she described the exciting possibility of delivering oncolytic virus to tumours via tumour-infiltrating macrophages. LCH lesions may be similarly susceptible to such an approach.

Targeting apoptosis in neoplastic lesions – Simone Fulda

Many cancer therapy agents trigger apoptosis and defects in the apoptosis pathway may cause treatment failure. Simone Fulda argues that exploration of the apoptotic pathway is therefore relevant to increasing the sensitivity of malignant cells to conventional and novel anti-cancer therapy. Three avenues may be exploited: 1) activation of pro-apoptotic proteins (caspase 8); 2) inhibition of anti-apoptotic proteins (XIAP); 3) inhibition of growth factor survival signals. Dr Fulda described a number of molecular targets in the apoptosis pathway including inhibition of anti-apoptotic pathways by Smac agonists and blockage of Inhibitory Apoptotic Programmes (IAP) to sensitize ALL samples to TRAIL and chemotherapy induced apoptosis (Fulda et al. 2002; Fulda 2012). Detailed dissection of these pathways using knock-out models has revealed new molecular targets including cIAPs, RIP1 and FADD (Löder et al. 2012). Her work has also shown that a number of agents already in clinical use including epigenetic modifiers valproate and azacytidine may increase caspase-8 expression and sensitivity to TRAIL agonists; similar effects are also observed with interferon-γ.

Summary and future directions

This meeting revealed that the excitement and anticipation generated by the discovery of mutated BRAF in LCH is further amplified by discoveries in a number of converging disciplines: signal transduction in myeloid cells, genetic control of dendritic cell development and molecular characterisation of the neoplasm itself. The application of BRAF inhibitors to selected patients with LCH cannot be far off. Many hard lessons have been learned about kinase inhibition in the field of solid malignancy but a very sophisticated level of understanding has now been reached. Although caution must be observed in the introduction of new drugs, especially to children, therapeutics in LCH will benefit enormously from the experience gained in the treatment of other BRAF mutated malignancies. The main challenges for the immediate future are to understand what other defects lead to LCH in the absence of BRAF mutation and how a common

signalling defect causes pathology in terms of the cell of origin and the nature of its transformation.

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