Harnessing Immunology and Inflammation in Neoplasms: Relevance to LCH and Histiocytic Disorders

Summary of the 24th Nikolas Symposium, Athens May 8-11, 2014

The 24th Nikolas symposium was organised to capture the theme of inflammation and neoplasia. The histiocytic disorders exemplify the close synergy between these two processes and exploring the mechanisms involved is likely to generate new insights into pathogenesis and opportunities for therapy

Dr Robert Arceci (Phoenix, USA) introduced the meeting and **Dr Marian Malone** (London, UK) gave an overview of the pathology of LCH and other histiocytoses, emphasizing the inflammatory component of these conditions. She reminded participants that the inflammatory infiltrate of LCH is one of the key features that of LCH lesions, containing lymphocytes, macrophages, eosinophils, multinucleated giant cells and neutrophils. The reason that these many inflammatory cells are present is unknown but similar infiltration is seen also in Hodgkin Lymphoma and to a lesser degree in almost all cancers. **Dr Carlos Rodriguez-Galindo** (Boston, US), president of the Histiocyte Society, summarized the clinical presentation of LCH and gave an overview of LCH trial results. In multi-system (MS) LCH, patients with risk organ involvement (BM, liver or spleen) still have a 16% risk of death and endure frequent reactivations (Gadner et al., 2013). Patients without risk organ involvement nearly all survive but prolonged maintenance therapy is required and the risk of reactivation remains above 30%. It was noted by the participants that LCH trials including the newly open LCH IV trial still rely upon vinblastine and prednisolone as first-line treatment in MS-LCH and there was renewed speculation that more intense myeloid-targeted induction therapy might reduce the rate of reactivation. Furthermore, the terminology of 'reactivation' rather than 'relapse' is becoming difficult to reconcile with the view that LCH is a neoplastic disorder (Swerdlow et al., 2008).

Harnessing inflammation and immunity for worse or for better

It is widely accepted that tumours use inflammation to gain growth advantages and that suppressing interactions between cancer and the immune system may offer new therapeutic potential. **Dr Jeffrey Pollard** (New York, USA and Edinburgh, UK) summarised his recent work demonstrating the key role of macrophages in assisting metastasis from a primary tumour site to the lungs. Macrophages in the primary site promote tumour growth by a synergistic feedback loop involving tumour production of M-CSF and macrophage elaboration of tumour promoting factors such as EGF and angiopoietin. Macrophages directly assist the intravasation of malignant cells into the blood circulation, the first step in metastatic spread. Seeding of new sites is very inefficient but tumour cells are protected by platelet micro-aggregates which in turn attract inflammatory monocytes by CCR2/CCL2 and CCR1/CCL3 dependent mechanisms to become metastasis associated macrophages (MAMs). The pathway has been elegant dissected using knock-out mice and is expertly reviewed in several recent publications (Qian and Pollard, 2012; Noy and Pollard, 2014). From the LCH perspective this work highlights the potential role of lesional macrophages in promoting LCH cell survival and even migration to multiple sites.

Evading the adaptive immune response is another capacity that tumours must evolve in order to survive. Immune checkpoints that regulate effector cells may be subverted by tumours to avoid detection. **Dr Theodore Johnson** (Augusta, USA) described the role of IDO (indoleamine di-oxygenase) in allowing tumour cells to evade the cytotoxicity of chemo-radiotherapy. He showed that an IDO inhibitor markedly enhanced the effect of treatment on a glioma model and that this effect was contingent upon a local inflammatory response. In the presence of the inhibitor, there was more marked peri-vascular recruitment of lymphocytes and tumour

regression (Johnson and Munn, 2012). Although LCH is frequently treated with immunosuppressive drugs, it is also conceivable that the balance of inflammation and tolerance within lesions could be disturbed by IDO inhibitors and trigger a resolution.

Dr Ken Shortman (Melbourne, Australia) described his work dissecting role of dendritic cells in promoting either tolerance or immunity (Caminschi and Shortman, 2012). Sterile inflammation, in the absence of pathogen-associated molecular patterns may induce tolerogenic DCs with high MCH class II expression but a lack of co-stimulatory molecules. Tumours naturally induce this state so that the host often develops tolerance to tumour associated antigens (TAA). Successful cancer immunotherapy must overcome this problem and a conventional approach is to provide adjuvants to stimulate an inflammatory milieu. However, specific subsets of DCs have distinct functions and those that expresses XCR1 and the lectin CLEC9A are specialised to recognise necrotic cells and to cross-present associated antigens(Shortman and Heath, 2010). This DC subset expresses CD8 in mice but is conserved in all mammals corresponding to the minor population of CD141+ myeloid DCs in humans, recently classified as 'DC1'(Collin et al., 2013; Guilliams et al., 2014). Recent work by Dr Shortman and others has shown that the lectin CLEC9A recognises naked actin, exposed when cells undergo necrotic death (Zhang et al., 2012; Ahrens et al., 2012). Coupling antigens to anti-CLEC9A antibodies has a unique effect in inducing adaptive immune responses in the absence of inflammation or adjuvants (Li et al., 2015). This route of inoculation therefore offers the promise of targeted immunisation with TAA. BRAF V600E is potentially a neo-epitope that might be used to stimulate LCH-specific immunity but this possibility has not yet been explored.

The theme of immune system control was continued by **Dr William Decker** (Houston, USA) who described an intricate mechanism by which dendritic cells make the most potent responses when loaded with homologous protein and RNA sequences of the same antigen. This increases the potency of simultaneous MHC class I and class II mediated antigen presentation by stimulating production of AIMp1/p43, a pro-inflammatory mediator (Decker et al., 2009). The mechanism whereby RNA and peptide sequences are compared is thought to involve a giant aminoacyl-tRNA synthetase multi-enzyme complex that effectively mediates a reverse translation process, adding tRNA to amino acid residues and matching them to RNA sequence. Conceptually, this mechanism is a kind of 'chip and PIN' for unleashing the full potential of cellular immunity and controlling unwanted autoreactive responses.

Dr Rupert Handgretinger (Tubingen, Germany) reviewed the specific targeting of immune responses in cancer beginning with the history of immunotherapy using streptococcus, first recognised in Germany by Brun, several decades before the seminal and more widely known studies of Coley. His presentation summarised work on haplo-identical bone marrow transplantation with NK alloreactivity, bi-specific antibodies, chimeric antigen receptor T cells and tumour-specific immunisation. Potentially, all these approaches are applicable to LCH.

BRAF V600E relatives: hairy cell leukaemia and Erdheim Chester disease.

Dr Omar Abdel-Wahab (New York, USA) presented work describing the haematopoietic origin of hairy cell leukaemia (HCL), which arises due to BRAF V600E mutation in more than 95% of cases. HCL has a mature B cell phenotype with class-switched IgG and somatic hypermutation. This 'post-germinal centre' phenotype is said to indicate that neoplastic transformation occurs late in B cell development. However, Dr Abdel-Wahab demonstrated that BRAF V600E is mutated in the haematopoietic stem cell (HSC) at a frequency of 1-5% and that purified HSC are able to generate HCL-like cells with 100% V600E mutation in xenotransplantation (Chung et al., 2014). The parallel with MS-LCH is very close: a low frequency of V600E in the progenitor compartment is able to generate an expanded clone of

mature cells in the periphery. The occurrence of fibrosis in both HCL and ECD is also intriguing. A significant challenge remains to understand how the same mutation present in HSC can generate multiple distinct disease phenotypes. The occurrence of fibrosis in both HCL and ECD is also intriguing. Mice engineered to express BRAF V600E in the HSC (mx1-cre x V600E fl/+) had multiple cytopenias and extramedullary haematopoiesis. This was mooted as evidence that cytopenia in HCL may be cell-intrinsic rather than secondary. However, the model does not reproduce the low frequency of V600E mutation in HSC that occurs in HCL nor explain the relative lack of cytopenias in MS-LCH and ECD. Finally, Dr Abdel-Wahab showed the benefit of treating refractory HCL patients with vemurafinib in inducing remission and restoring cytopenias. Together with the responses seen in ECD, this makes compelling evidence for the use of BRAF inhibitors in LCH.

Dr Julien Haroche (Paris, France) gave a comprehensive description of ECD a histiocytic disease typically occurring in older males with a 54% incidence of BRAF V600E mutation and well documented overlap with LCH (Hervier et al., 2014; Haroche et al., 2014). The pathological diagnosis of ECD is arrived at by excluding LCH and is very variable from florid lymphohistiocytic infiltration to bland hypocellular fibrosis. Characteristic Touton giant cells and lipid-laden macrophages are not universal findings. The most common clinical findings are CNS involvement (50%), bone pain (50%) and xanthelasma (30%). The main radiographic findings are long bone sclerosis (95%) peri-nephric and peri-aortic fibrosis (70%). Dr Haroche also described his ground-breaking work in treating BRAF-mutated ECD with BRAF inhibitors such as Vemurafinib. He has now treated 9 patients with an average to 10 months follow up and is advising first line therapy in those patients with high risk cardiac or neurological disease (Haroche et al., 2015).

MAP2K1 (MEK1) mutations and mechanisms of pathogenesis in LCH

Dr Barrett Rollins (Boston) and Dr Carl Allen (Houston) presented data describing new mutations in BRAF V600E wild-type LCH. Both have discovered MAP2K1 (MEK1) mutations and demonstrated that they confer kinase activation (Chakraborty et al., 2014; Nelson et al., 2015). This important work strengthens the view that ERK activation is a unifying feature of LCH generated by convergent upstream kinase pathway mutations. Dr Rollins also uncovered mutations in MAP3K1 in some patients but theses were either loss of function or germline. Altogether, V600E and MAP2K1 mutation accounts for nearly 80% of LCH and efforts continue to identify the remaining genetic aetiologies. Deep sequencing also reveals only a small number of variants per patient showing that the genetic landscape of LCH is relatively quiescent compared with other haematopoietic malignancies. Observations from Houston also show a genotype correlation with overall outcome namely that BRAF-mutated disease relapses more frequently than MAP2K1-mutated disease. Dr Allen also reminded the meeting of his seminal finding that high risk MS-LCH has detectable BRAF in HSCs and in circulating myeloid cells, in contrast to low risk MS-LCH or SS-LCH in which BRAF is only detectable in lesions (Berres et al., 2014). This important finding has prognostic significance but also suggests a model in which the risk of disease is determined by the point in the haematopoietic hierarchy in which a mutation arises. Mutation arising anywhere downstream of a self-renewing progenitor will produce low risk disease involving single lesions or at most a wave of tainted myeloid precursors that may seed multiple peripheral sites but will eventually 'grow out'. The possibility still exists that a second stimulus is required to form an LCH lesion but this may be inflammatory or immune rather than genetic. Many studies have suggested viral infection, including a recent work on Merkel cell polyomavirus (Murakami et al, 2014).

The demonstration of BRAF V600E in stem cells is conceptually very important in helping to understand the links between LCH and other V600E-mutated hematopoietic malignancies. **Mr**

Paul Milne (Pritchard Fellow) described a BRAF allele-specific PCR study in which he had purified fractions of myeloid cells from bone marrow and blood from adults with HCL, LCH and ECD. He was able to demonstrate mutated BRAF in the blood of adults with MS-LCH, in parallel to the findings of Dr Allen in paediatric LCH, and also in ECD. Comparison of the three conditions showed that there was a distinct distribution of mutated alleles in each disease either in B cells (HCL), monocytes and myeloid DCs (LCH) or in the HLA-DR-negative compartment in ECD. Although this does not provide a mechanistic explanation of how mutated BRAF generates these patterns arising from the HSC it suggests that the pattern of affected organs and nature of lesions may be determined by the output of the bone marrow. The surprising lack of mutation in macrophage precursors in ECD is hard to reconcile with existing models of ECD as a primary macrophage disorder but it is notable that fibrosis also occurs in HCL, which is presumably secondary to transformation of the B cell lineage. Thus the pathological fibrotic lesion of ECD could arise indirectly and recruit unmutated inflammatory (rather than neoplastic) macrophages. Direct staining with antibody VE1, however suggest that lesional macrophages do contain BRAF mutation (Haroche et al., 2012).

Insights into how V600E mutation of BRAF gives rise to three clinically distinct syndromes may be derived form animal models in which BRAF V600E is expressed in a lineage-restricted fashion. **Dr Sergio Lira** (New York, US) presented data from mouse models in which lineage-specific using the cre-lox system targeted V600E to either Langerin+, CD11c+ or CX3CR1+ cells. All three models generate abnormal collections of Langerin+ histiocyte but activation of V600E earlier in the DC lineage in CD11c-expressing cells causes a more severe multisystem disorder than targeting V600E only to Langerin+ cells (Berres et al., 2014). Interestingly, the CD11c+ model activates all DCs to express Langerin including the CD11b+ DC (DC2; equivalent to the major CD1c+ myeloid DC in humans) that does not express langerin in healthy mice. This is consistent with human biology in which CD1c+ DCs can easily be induced to express Langerin and may contribute to LCH lesion formation (Bigley et al., 2015). Intriguingly, expression of V600E in CX3CR1+ monocytes led to ataxia and spastic paralysis associated with expansion and clustering of microglia in the brain. Microglial activation was evident through increased expression of IL-1, TNF, TGFβ, CCL2, iNOS and MMP12 by Q-PCR and T cells were also recruited. Clusters of langerin+ cells and multi-nucleated giant cells also appeared in the liver and lung. In addition to targeting the DC lineage, these experiments suggest that monocytes transformed by V600E can also cause a histiocytic disorder. These models are very useful to investigate the mechanism of BRAF activation abnormal DC development. The involvement of the CNS in 'monocyte-LCH' may be especially valuable in exploring neurological disease. One potential shortcoming is that V600E is continually generated as cells express the linage-specific cre. Thus they do not account for the generation of abnormal precursors from a single progenitor cell as is likely to occur in the natural history of human LCH.

Overcoming the problem of adaptation to BRAF inhibitors

Dr Piro Lito (New York, USA) gave a comprehensive update on drugs that inhibit BRAF and the MAP kinase signalling pathway. The RAF proteins normally dimerise to signal downstream and BRAF V600E is active as a monomer. Two significant problems are recognised with the current generation of BRAF inhibitors. First, at low dose they cause wild-type BRAF to dimerise thus increasing ERK activation and leading to hypertrophy and even neoplasia in healthy tissues. Second, ERK activation causes profound feedback inhibition of ras proteins that blocks upstream RTK signalling the presence of BRAF V600E (Lito et al., 2013). When BRAF is blocked, feedback inhibition is relieved resulting in restoration of RTK/ras activation and a partial restoration of ERK signalling (Lito et al., 2012). This process of adaptation occurs within 24 hours and is the principal cause of resistance to BRAF inhibitors. Using a cDNA screen, Dr Lito identified RTKs mediating adaptation in response to a number of extracellular signals

including FGF, MCSF, TGF, TNF, IL4, IL2, LTA that might be future adjunctive therapeutic targets. He also described an RNAi screen for intracellular pathways mediating adaptation that identified a critical role for CRAF in restoring ERK activity in the presence of BRAF inhibitors (Lito et al., 2014). The problem of feedback inhibition is critically dependent upon the level of activation of a signalling pathway by a mutation. Combination treatment with RAF and MEK inhibitors may surmount the issue. Alternatively, it may be possible to develop 'smarter' drugs that maintain feedback inhibition but still block forward signalling. The development of MEK and ERK inhibitors is also clearly important in providing therapeutic options for patients lacking BRAF V600E mutation.

Dr George Demetri (New York, USA) also summarised his experience in treating Gastro Intestinal Stromal Tumour (GIST) with the RTK inhibitor imatinib. GIST is the most common of all sarcomas (19%) and arises from the interstitial cells of Cajal due to gain of function mutations in c-kit (CD117) or PDGFR. Although rapidly fatal once disseminated, pioneer studies in 2000 demonstrated dramatic efficacy of RTK inhibition of c-kit mutation such that the first patient treated is still alive. However, PDGFR and certain c-kit mutations show primary resistance and patients fare less well (Corless et al., 2014). Also continuous therapy is required and resistance may emerge through second mutations. Dr Demetri described studies showing that paediatric GIST is due to succinate dehydrogenase mutations and showed that sunitinib can work for imatinib-resistant tumours (Reichardt et al., 2015). Finally he suggested that a broad specificity TKI pasopanib, may have efficacy in the malignant sarcoma follicular dendritic cell sarcoma, a mesenchymal rather than haematopoietic tumour. Overall these studies illustrate mechanisms of resistance to targeted therapy that have direct relevance to the next generation of LCH patients who may receive such treatment.

In summary, there have been significant achievements in the 4 years since mutated BRAF was first reported in LCH. These include advances in genetics, immunology, cell biology and the understanding and treatment of HCL and ECD, closely related disorders. Scientific interest in the histiocytosis is at an apogee and new discoveries are having a wide impact on the fields of inflammation, haematopoiesis and cancer biology. For patients with histiocytosis, the rational search for a cure has entered a new phase that promises further improvements in therapy that are already reaching the clinic.

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References

Ahrens, S., Zelenay, S., Sancho, D., Hanc, P., Kjaer, S., Feest, C., Fletcher, G., Durkin, C., Postigo, A., Skehel, M., Batista, F., Thompson, B., Way, M., Reis e Sousa, C., and Schulz, O. (2012). F-actin is an evolutionarily conserved damage-associated molecular pattern recognized by DNGR-1, a receptor for dead cells. Immunity *36*, 635–645.

Berres, M.L., Lim, K.P., Peters, T., Price, J., Takizawa, H., Salmon, H., Idoyaga, J., Ruzo, A., Lupo, P.J., Hicks, M.J., Shih, A., Simko, S.J., Abhyankar, H., Chakraborty, R., Leboeuf, M., Beltrao, M., Lira, S.A., Heym, K.M., Bigley, V., Collin, M., Manz, M.G., McClain, K., Merad, M., and Allen, C.E. (2014). BRAF-V600E expression in precursor versus differentiated dendritic cells defines clinically distinct LCH risk groups. J Exp Med *211*, 669–683.

Bigley, V., McGovern, N., Milne, P., Dickinson, R., Pagan, S., Cookson, S., Haniffa, M., and Collin, M. (2015). Langerin-expressing dendritic cells in human tissues are related to CD1c+ dendritic cells and distinct from Langerhans cells and CD141high XCR1+ dendritic cells. J Leukoc Biol *97*, 627–634.

Caminschi, I., and Shortman, K. (2012). Boosting antibody responses by targeting antigens to dendritic cells. Trends Immunol *33*, 71–77.

Chakraborty, R., Hampton, O.A., Shen, X., Simko, S.J., Shih, A., Abhyankar, H., Lim, K.P., Covington, K.R., Trevino, L., Dewal, N., Muzny, D.M., Doddapaneni, H., Hu, J., Wang, L., Lupo, P.J., Hicks, M.J., Bonilla, D.L., Dwyer, K.C., Berres, M.L., Poulikakos, P.I., Merad, M., McClain, K.L., Wheeler, D.A., Allen, C.E., and Parsons, D.W. (2014). Mutually exclusive recurrent somatic mutations in MAP2K1 and BRAF support a central role for ERK activation in LCH pathogenesis. Blood *124*, 3007–3015.

Chung, S.S., Kim, E., Park, J.H., Chung, Y.R., Lito, P., Teruya-Feldstein, J., Hu, W., Beguelin, W., Monette, S., Duy, C., Rampal, R., Telis, L., Patel, M., Kim, M.K., Huberman, K., Bouvier, N., Berger, M.F., Melnick, A.M., Rosen, N., Tallman, M.S., Park, C.Y., and Abdel-Wahab, O. (2014). Hematopoietic Stem Cell Origin of BRAFV600E Mutations in Hairy Cell Leukemia. Sci Transl Med *6*, 238ra71.

Collin, M., McGovern, N., and Haniffa, M. (2013). Human dendritic cell subsets. Immunology *140*, 22–30.

Corless, C.L., Ballman, K.V., Antonescu, C.R., Kolesnikova, V., Maki, R.G., Pisters, P.W., Blackstein, M.E., Blanke, C.D., Demetri, G.D., Heinrich, M.C., von Mehren, M., Patel, S., McCarter, M.D., Owzar, K., and DeMatteo, R.P. (2014). Pathologic and molecular features correlate with long-term outcome after adjuvant therapy of resected primary GI stromal tumor: the ACOSOG Z9001 trial. J Clin Oncol *32*, 1563–1570.

Decker, W.K., Xing, D., Li, S., Robinson, S.N., Yang, H., Steiner, D., Komanduri, K.V., and Shpall, E.J. (2009). Th-1 polarization is regulated by dendritic-cell comparison of MHC class I and class II antigens. Blood *113*, 4213–4223.

Gadner, H., Minkov, M., Grois, N., Potschger, U., Thiem, E., Arico, M., Astigarraga, I., Braier, J., Donadieu, J., Henter, J.I., Janka-Schaub, G., McClain, K.L., Weitzman, S., Windebank, K., and Ladisch, S. (2013). Therapy prolongation improves outcome in multi-system Langerhans cell histiocytosis. Blood

Guilliams, M., Ginhoux, F., Jakubzick, C., Naik, S.H., Onai, N., Schraml, B.U., Segura, E., Tussiwand, R., and Yona, S. (2014). Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. Nat Rev Immunol *14*, 571–578.

Haroche, J., Arnaud, L., Cohen-Aubart, F., Hervier, B., Charlotte, F., Emile, J.F., and Amoura, Z. (2014). Erdheim-Chester disease. Curr Rheumatol Rep *16*, 412.

Haroche, J., Charlotte, F., Arnaud, L., von Deimling, A., Helias-Rodzewicz, Z., Hervier, B., Cohen-Aubart, F., Launay, D., Lesot, A., Mokhtari, K., Canioni, D., Galmiche, L., Rose, C., Schmalzing, M., Croockewit, S., Kambouchner, M., Copin, M.C., Fraitag, S., Sahm, F., Brousse, N., Amoura, Z., Donadieu, J., and Emile, J.F. (2012). High prevalence of BRAF V600E mutations in Erdheim-Chester disease but not in other non-Langerhans cell histiocytoses. Blood *120*, 2700–2703.

Haroche, J., Cohen-Aubart, F., Emile, J.F., Maksud, P., Drier, A., Toledano, D., Barete, S., Charlotte, F., Cluzel, P., Donadieu, J., Benameur, N., Grenier, P.A., Besnard, S., Ory, J.P., Lifermann, F., Idbaih, A., Granel, B., Graffin, B., Hervier, B., Arnaud, L., and Amoura, Z. (2015). Reproducible and sustained efficacy of targeted therapy with vemurafenib in patients with BRAF(V600E)-mutated Erdheim-Chester disease. J Clin Oncol *33*, 411–418.

Hervier, B., Haroche, J., Arnaud, L., Charlotte, F., Donadieu, J., Neel, A., Lifermann, F., Villabona, C., Graffin, B., Hermine, O., Rigolet, A., Roubille, C., Hachulla, E., Carmoi, T., Bezier, M., Meignin, V., Conrad, M., Marie, L., Kostrzewa, E., Michot, J.M., Barete, S., Taly, V., Cury, K., Emile, J.F., and Amoura, Z. (2014). Association of both Langerhans cell histiocytosis and Erdheim-Chester disease linked to the BRAFV600E mutation. Blood *124*, 1119–1126.

Johnson, T.S., and Munn, D.H. (2012). Host indoleamine 2,3-dioxygenase: contribution to systemic acquired tumor tolerance. Immunol Invest *41*, 765–797.

Li, J., Ahmet, F., Sullivan, L.C., Brooks, A.G., Kent, S.J., De Rose, R., Salazar, A.M., Reis, E.S.C., Shortman, K., Lahoud, M.H., Heath, W.R., and Caminschi, I. (2015). Antibodies targeting Clec9A promote strong humoral immunity without adjuvant in mice and non-human primates. Eur J Immunol *45*, 854–864.

Lito, P., Pratilas, C.A., Joseph, E.W., Tadi, M., Halilovic, E., Zubrowski, M., Huang, A., Wong, W.L., Callahan, M.K., Merghoub, T., Wolchok, J.D., de Stanchina, E., Chandarlapaty, S., Poulikakos, P.I., Fagin, J.A., and Rosen, N. (2012). Relief of profound feedback inhibition of mitogenic signaling by RAF inhibitors attenuates their activity in BRAFV600E melanomas. Cancer Cell *22*, 668–682.

Lito, P., Rosen, N., and Solit, D.B. (2013). Tumor adaptation and resistance to RAF inhibitors. Nat Med *19*, 1401–1409.

Lito, P., Saborowski, A., Yue, J., Solomon, M., Joseph, E., Gadal, S., Saborowski, M., Kastenhuber, E., Fellmann, C., Ohara, K., Morikami, K., Miura, T., Lukacs, C., Ishii, N., Lowe, S., and Rosen, N. (2014). Disruption of CRAF-mediated MEK activation is required for effective MEK inhibition in KRAS mutant tumors. Cancer Cell *25*, 697–710.

Murakami I, Matsushita M, Iwasaki T *et al.* Merkel cell polyomavirus DNA sequences in peripheral blood and tissues from patients with Langerhans cell histiocytosis. *Hum. Pathol.* 2014; **45**: 119–26].

Nelson, D.S., van Halteren, A., Quispel, W.T., van den Bos, C., Bovee, J.V., Patel, B., Badalian-Very, G., van Hummelen, P., Ducar, M., Lin, L., MacConaill, L.E., Egeler, R.M., and Rollins, B.J. (2015). MAP2K1 and MAP3K1 mutations in langerhans cell histiocytosis. Genes Chromosomes Cancer *54*, 361–368.

Noy, R., and Pollard, J.W. (2014). Tumor-associated macrophages: from mechanisms to therapy. Immunity *41*, 49–61.

Qian, B.Z., and Pollard, J.W. (2012). New tricks for metastasis-associated macrophages. Breast Cancer Res *14*, 316.

Reichardt, P., Kang, Y.K., Rutkowski, P., Schuette, J., Rosen, L.S., Seddon, B., Yalcin, S., Gelderblom, H., Williams, C.C.J., Fumagalli, E., Biasco, G., Hurwitz, H.I., Kaiser, P.E., Fly, K., Matczak, E., Chen, L., Lechuga, M.J., and Demetri, G.D. (2015). Clinical outcomes of patients with advanced gastrointestinal stromal tumors: Safety and efficacy in a worldwide treatment-use trial of sunitinib. Cancer *121*, 1405–1413.

Shortman, K., and Heath, W.R. (2010). The CD8+ dendritic cell subset. Immunol Rev *234*, 18–31.

Swerdlow, S.H., Campo, E., NL, H., and, E. (2008). WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC

Zhang, J.G., Czabotar, P.E., Policheni, A.N., Caminschi, I., Wan, S.S., Kitsoulis, S., Tullett, K.M., Robin, A.Y., Brammananth, R., van Delft, M.F., Lu, J., O'Reilly, L.A., Josefsson, E.C., Kile, B.T., Chin, W.J., Mintern, J.D., Olshina, M.A., Wong, W., Baum, J., Wright, M.D., Huang, D.C., Mohandas, N., Coppel, R.L., Colman, P.M., Nicola, N.A., Shortman, K., and Lahoud, M.H. (2012). The dendritic cell receptor Clec9A binds damaged cells via exposed actin filaments. Immunity *36*, 646–657.