September 18, 2014
NIH, Bethesda, Maryland

ECD Global Alliance
Synopsis of the 2014 International Erdheim-Chester Disease Medical Symposium

On September 18th 2014, the second International ECD Global Alliance Medical Symposium was held at the NIH in Bethesda, Maryland. It was an opportunity for specialists in the field to come together and discuss the most recent data related to the diagnosis and treatment of ECD. The following is a summary of the meeting.

Dr. Eric Green, the Director of the National Human Genome Research Institute, gave a background introduction into the work carried out at the NIH. It is the largest institute in the world dedicated to genome research. It was founded to lead the US genome project. 85% of the research grants which are awarded in the US are from the NIH. They are currently studying how changes in the genome leads to disease specification, which in turn will allow better means for prevention, diagnosis and treatment.

Dr. Kenneth McClain, from the Children's Hospital in Houston, TX, spoke about advances in ECD and other forms of histiocytosis from 1930-to present. In 1930, "lipoid granulomatosis" was first described by Jakob Erdheim and William Chester, but in 1972, this became known as ECD. The number of papers published on ECD has greatly increased over the years (2011-2014: 183 papers). In 2011, the involvement of numerous cytokine and chemokine networks came to light (e.g. CCL4, RANTES, MCP1, MIP3α and MIP3β) and upregulation of interferon (IFN)γ-inducible protein, interleukin (IL)-6 and RANKL would suggest a trend towards a more Th1 inflammatory milieu. In 2012, Dr. Haroche found that 50% of patients displayed a mutation in the BRAFV600E gene and in 2013, treatment using the BRAF inhibitor Vemurafenib began. Immunohistochemical (ICH) analysis of 3 patients showed that monocytes secrete IL-6, IL-8 and TNF when stimulated. It has also been shown that monocytes express the inflammatory makers CRP, IL-1α, IL-1β, IL-6 and mIL-1α on their cell surface. The use of Anakinra has therefore also been suggested as a possible treatment, as it decreases C-reactive protein (CRP), IL-6 and TNFα levels, reducing the pro-inflammatory response and improving clinical symptoms. Contrary to original thoughts, ECD appears to be an accumulation of cells rather than a problem with excess cell proliferation. Regarding the BRAFV600E mutation, 18/18 cases have shown the mutation in blood and biopsies. This was confirmed by phospho-ERK+ in biopsies and the blood senescence marker P16lnk4a+. Haroche et al have also shown BRAF mutations in 19 out of 37 (51%) ECD cases, by using pyrosequencing and IHC. There is also a clear link evident between Langerhans cell histiocytosis (LCH) and ECD. There have been 23 cases of combined LCH-ECD reported. The research focus at present is on indentifying the varying BRAF, RAS, PIK3CA mutations and many of these overlap with other syndromes. The future of ECD biology will look at an expression array analysis of purified cells and further single cell ERK activation analysis. There is a need for clinical trials using BRAF and ERK inhibitors and the role of anti-IL-1 or IL-6 therapy.

Dr. Lorenzo Dagna, MD, from the Vita-Salute San Raffalele University and San Raffaele Scientific Institute, both in Milan, Italy, spoke about oncogene-induced senescence: the missing link between oncogenic mutations and chronic inflammation in ECD. There are a number of cytokines and chemokines expressed in ECD histiocytes, but no proliferation markers, confirming that ECD is an inflammatory disease. Two distinct, but possibly interconnected, pathogenetic events are now recognized in ECD: full-blown chronic inflammation and BRAF600E mutation in histiocytes. Indeed, plasma soluble TNF receptor and TNF-regulated cyto-chemokine levels are increased in ECD patients. Based on this over production of cytokines by histocytes, this has inspired the use of Th1 (pro-inflammatory) cytokine blockers. Previously published series showed that roughly 51% of ECD and 57% of LCH patients display the BRAF mutation. Using a highly sensitive (1:10,000 mutated:wildtype copies) locked nucleic acid (LNA) PCR to detect the BRAF mutation (pyrosequencing), Dr. Dagna's group showed that a BRAF mutation could be indeed found in virtually all studied patients in their cohort. BRAFV600E-mutated histiocytes from ECD lesions stained for phospho-ERK, confirming an active MAPK pathway. Dr. Dagna also referred to Dr. Diamond's recent publication which refers to a patient case which displayed a NRAS mutation but was BRAF-, since these data reiterate the importance of the MAPK pathway in ECD. ECD lesions display beta galactosidase activity and are constituted by an extremely heterogenous and complex network of cells. p16Ink4a (a senescence marker) is expressed in ECD lesions and these senescent cells display a typical secretory profile (e.g. IL-6, IL-1). The lesions likely originate from a circulating mutated precursor of the myeloid lineage that accumulates in tissues and undergoes to senescence in response to oncogenic mutations, i.e. oncogene-induced senescence. While discussing research data originated with his collaboration with Drs. Ferrarini ad Ferrero, both at San Raffaele, Dr. Dagna showed also that circulating ECD monocytes are not a major source of TNF-related cytokines/chemokines. Indeed, exudates from ECD lesions mirror the native microenvironment and can be used as a surrogate for in vitro pathogenic studies. Cytokines from ECD pericardial fluid (and TNFα in particular) affect endothelial functions and the morphology of endothelial cells. Dr. Dagna showed experiments demonstrating that the fluid had chemotactic and activating activity, i.e inflammatory cells are able to migrate through an endothelial transwell system, and get activated upon pericardial fluid exposure. Also, ECD pericardial fluid induces the conversion of monocyte-derived macrophages into foamy cells. Dr. Dagna also spoke about preclinical drug testing in an ex vivo 3-D dynamic culture system using a bioreactor. They're using this bioreactor to culture tissue explants over long-term, thus providing an optimal delivery of O2 and nutrients as required. The RCCS bioreactor can be exploited to assess the impact of pharmacological inhibitors on ECD lesions.

Dr. Elaine Ostrander, PhD from the Cancer Genetics Branch at the NIH spoke about the Genetics of Histocytic Sarcoma (HS) in Bernese mountain dog (BMD). 80 percent of canine HS cases are in the BMD's. Age of onset is about 6 years and there is rapid progression with a poor prognosis and currently no effective treatment. It manifests as visceral disease and is caused by an excess of white blood cells called histiocytes. The group carried out a DNA study on dogs with the mutation (n=244) and healthy controls (n=230). They found that there was a substantial difference between North American (NA) and European (French and Dutch) dogs, so they had to consider these groups separately. Chromosome 11 was identified as key player in NA dogs and although European dogs support the chromosome 11 result, chromosome 14 was also been identified as being important in these dogs. Telombin and 2 Hyaluronidases were

identified as being promising candidate genes. These results showed that there are also animal models with similar gene involvement which may shed light on the underlying genetic involvement in histocytic diseases.

Dr. Jean Francois Emile, MD, from Ambroise Pare Hospital in Boulogne, France spoke about the Recurrent RAS and PIK3CA mutations in ECD. The diagnosis of histiocytosis is difficult to perform but 483 histiocytosis samples have been analysed since 2011. The BRAF mutation has been shown in 47% of those with LCH and 51% in ECD. All patients have both a histological and clinical review (use of MySeq and pyrosequencing techniques). However, with regard to detecting mutations, more sensitive techniques are necessary if a biopsy is BRAF-. 3/80 patients displayed the NRAS mutation (via pyrosequencing) and this was associated with the activation of RAF-RAF-ERK pathway. The NRAS mutation was also detected in blood monocytes. A more recent PIK3CA mutation has also been detected in 7/58 (12%) ECD patients. Importantly, this mutation was identified in both BRAF+ and BRAF- patients and therefore is not dependent on the presence of the BRAF mutation. The PIK3CA mutation was only found in 1/54 LCH patients and therefore appears to be less frequent. Dr. Emile also spoke about the most effective method for BRAF analysis. He mentioned the limit of detection and % of histiocytes and confirmed that there is no correlation between the % of mutated alleles and % of tumour cells in ECD. Such a finding is not the case in melanoma as there is correlation present, which suggests a different mechanism in ECD. A combination of histology, phenotype, clinical involvement, imaging and somatic genetic alterations are all necessary for the classification of histiocytosis. Histological data alone is not enough and the identification of underlying molecular mechanisms is necessary. ECD is probably caused by mutations in a mix of pathways and not by a mutation is a single pathway. One should consider the somatic mutation when considering the classification of histiocytosis.

Dr. Giulio Cavalli, MD, from the San Raffaele University in Milan, Italy spoke about the molecular analysis of peripheral blood in ECD to complement histology and imaging techniques. ECD features a network of pro-inflammatory cytokines/chemokines. These are more than likely responsible for the recruitment and activation of histiocytes in tissues and may also contribute to systemic symptoms. Cytokine-blocking agents often induce stabilization or reduction in lesions, but these do not prevent disease progression. Infiltrating histiocytes in ECD exhibit markers of oncogene-induced senescence (OIS). Features of cellular senescence include irreversible cell-cycle arrest, large and flat vacuolated appearance. These senescence-associate secretory phenotypes upregulate pro-inflammatory molecules. Whether ECD should be considered an inflammatory or a neoplastic disease is still debatable, but OIS may help answer this question. Dr. Cavalli presented a case report of a 37 year old male with cardiac involvement who had the BRAF mutation (identified using LNA-pyro) in peripheral blood monocytes (PBMCs) (so circulating mutated cells). He was being treated with 960mg of Vemurafenib and the clearance of mutated PBMCs paralleled the efficacy on tissue infiltration. Although there was a fast clinical response with inflammatory markers normalizing after 4 months, secondary malignancies and treatment resistance still remain concerns. Vemurafenib treatment is associated with reversal of systemic inflammation and this provides hints towards disease pathogenesis. ECD should be carefully monitored with stringent surveillance of both diseases and treatment efficacy. BRAFV600E is almost invariably present in lesions and PBMCs Of ECD patients and its pivotal pathogenic role is demonstrated by the clinical efficacy of specific pharmacologic

inhibition with Venurafenib. OIS cells secrete inflammatory molecules which can recruit other monocytes and macrophages. This may be worth looking at further as OIS cells have a very unique phenotype which may provide the missing link between oncogenic mutation and inflammatory disease.

Dr. Eli Diamond, MD, from Memorial Sloan Kettering Cancer Center in New York, NY spoke about the detection of BRAF mutations in cell-free DNA(cfDNA) in ECD and LCH. ECD and LCH are rare multi-system disorders with around 60% of patients displaying a BRAFV600E mutation, in addition other MAPK pathway mutations. These have profound therapeutic implications. Apoptotic tumor cells shed DNA into blood and then through the kidneys into the urine. The BRAFV600E mutation can now be identified in cfDNA as an alternative diagnostic modality. The advantages are that it is non-invasive, provides potentially improved sensitivity, can be followed longitudinally and is quantitative. A prospective, blinded comparison of tissue- and cfDNA-determined BRAF mutational status was carried out in ECD and LCH patients. Serial quantitative monitoring of cfDNA allele burden was done during the course of treatment. 60-120ml of urine was collected in a study of 31 patients. Out of the 31 patients, 29 were untreated and 2 were on Venurafenib. 24 patients had ECD, 5 had LCH and 2 had a mix of ECD/LCH. It was found that urine-based cfDNA accurately identifies BRAFV600E mutations in ECD and LCH when compared to tissue-based diagnosis. This is especially true in untreated patients. This provides a dynamic biomarker in the setting of BRAF inhibitor treatment or immunomodulatory therapy. cfDNA may have utility for the pre-clinical detection of disease reactivation in the setting of surveillance of treatment.

Dr. Fillip Janku, MD, from MD Anderson Cancer Center in Houston, Texas, also spoke about the BRAF mutation testing of cfDNA, but this time from plasma samples of ECD patients. They're working on developing a novel, rapid and automated molecular diagnostics platform. BRAFV600E mutations can be detected in more than half of ECD patients. Early data suggests that PATIENTS WITH these mutations can benefit from BRAF inhibitors. IN CONTRARY DATA FROM SOME BRAF MUTANT CANCERS SUGGEST THAT IN PATIENTS without the BRAF mutation, BRAF inhibitors CAN accelerate disease progression. Molecular testing of plasma cfDNA has the potential to further advance personalized medicine in ECD, by offering a source of easily obtainable material for mutation analysis. Longitudinal monitoring of plasma cfDNA can be plausibly used for monitoring disease trajectory. The IdyllaTM platform (designed by a Biocartis) can provide fast and reproducible BRAF testing of plasma cfDNA. Testing time is about 50 minutes.

Dr. Susan Robertson, PhD, from the NIH in Bethesda, MD, spoke about the quality of life with patients with ECD and their caregivers. Dr. Robertson's findings showed that the majority of patients were Caucasian, aged 14 to 72 and had an age of onset of 24 to 51 years. The time prior to diagnosis varied from 1 to 18 years. Caregivers were mostly Caucasian females aged 53 to 72 years and most were wives (66%) and mothers (33%). When interviewed, caregivers were the dominant speakers. She looked at their reactions to uncertainty. They spoke of their hectic life schedule and difficulty with identifying the best and most effective way to help. Doctors who treat these patients help manage the effects of uncertainty.

Dr. Julien Haroche, MD, PhD, from Hospital Pitie-Salpêtrière, Paris 6 university, France, presented results from a monocentric series of 122 ECD patients with special focus on the phenotypic characterization of BRAFV600E mutated patients. The study consisted of 91 male and 31 female patients with 27 deaths

recorded. The mean age of patients was 56 with a range of 5 to 80. The mean diagnostic delay was 48 months. Presentation with diabetes insipidus (approximatively 16%) is usually the first sign as well as bone involvement/pain, but many other presenting signs can be seen. The percentage of patients displaying varying symptoms can be broken down as follows: increased levels of pressure of acute inflammatory protein (CRP) in 71%, bone pain in 39%, xanthelasmas in 25%, exophthalmos (retro-orbital infiltration) in 21% and diabetes insipidus in 26%. There was CNS involvement in 40% of patients and this is an independent predictor of death, of which 17% have cerebellar involvement. 34% have lung involvement, and 57% have "hairy kidneys" (good option for taking biopsy sample). 25% of patients have hydronephrosis and there is adrenal involvement in 16%. The thoracic aorta is involved in 55%, a "coated aorta" is seen in 43% and 57% have involvement of the abdominal aorta. There is frequent involvement of the aortic branches with overall little clinical consequences. 16% have reno-vascular hypertension and this should be checked for systemically in ECD patients with hyperstension. 30% of patients have pericardial symptoms (second most frequent cardiovascular symptom) and 31% have pseudo-tumor infiltration of the right atrium. The right coronary is affected in 23%, while the left coronary is sheathed in 21%. A PET/CT seems best to follow up the disease (along with clinical examination, CRP and assessment of various infiltrations with MRI and or/CT scan), especially in patients with CNS and/or heart involvement. First line of treatment is interferon-α (IFNα) or pegylated IFNα which has been shown to improve survival. The dose may be raised and administered 3 times per week for patients with CNS and/or cardiac involvement when the standard formulation of IFNα is chosen. Alternative treatments include anakinra, cladribine, tyrosine kinase inhibitors (such as glivec), tocilizumab, infliximab, but of all of the varying treatments tested in Paris, vemurafenib has shown to be the most effective (upcoming study in press in Journal of Clinical Oncology). Dr Haroche recommends 2 x 2 tablets of vemurafenib (which is half of melanoma regimen) and then to taper. Out of 19 patients treated with vemurafenib (n=11 between Oct 2013 – present), 2 presented with DRESS syndrome and 1 with squamous cell carcinoma. It is difficult to predict the long-term outcome following vemurafenib treatment, as so far, the follow-up time is too short. However, the LOVE study (long-term outcome after vemurafenib interruption) looks what happens after at least 6 months of vemurafenib treatment and put the patients of the drug and see if and when the patients relapse: this should provide greater insight and indeed help in future options strategy. The prognosis of ECD in 2014 is better. Previous statistics suggested that 60% of patients died after 3 years. Now the 5 year survival is 82% and 10 year is 70%. Dr Haroche believes all patients should be treated. They also have 1 patient on dabrafenib, but the response does not seem as convincing as vemurafenib. Vemurafenib is not as effective for those who have cerebellar involvement but is recommended for patients with severe heart/CNS involvements. The duration of treatment still remains an open question; however results of the LOVE study should shed some light on this. It seems more than likely that the disease will relapse after stopping vemurafenib treatment. The MEK inhibitor trametinib may be a possible alternative as well as combination with other BRAF inhibitors.

Dr. Ganzel Chezi, MD, from Shaare Zedek Medical Center, Jerusalem, Israel spoke about a conjoint use of cardiac MRI and CT angiography as a tool for distinction between cardiac ECD involvement and ischemic heart disease in ECD patients. Latest findings show that 64% of ECD patients display cardiac involvement. Dr. Ganzel described the coronary and cardiac effects from a case study of one male patient being treated with pegilated IFNα. At the beginning of treatment, they decided that they would only switch the patient

to Venurafenib if cardiac involvement worsened. By using a combination of cardiac MRI, CT angiography and PET CT they could prove that the ECD cardiac involvement is stable and that the cardiac infarct of the patient is related to the ischemic heart disease and not to the ECD. They therefore decided not to change the pegilated IFNα treatment. Dr Ganzel showed how each cardiac imaging modality has its strengths and weaknesses and that combination of methods contribute to the understanding of cardiac disease etiology. Cardiac MRI and CT angiography help to distinguish between IHD and ECD, and a precise diagnosis is important to prevent over/under treatment of ECD.

Dr. Roei D. Mazor, MD, from Sheba Medical Center in Tel Aviv, Israel, spoke about the clinical considerations and key issues in the management of patients with ECD. He has seen 9 patients so far in Israel. Patient #1 is a 39 year old with multi-systemic disease and increasing bone pain. The patient was treated with IFNα and Vinblastine. Rapidly progressing CNS and retro-orbital involvement, showed stabilization after 3 months. The patient was BRAF positive and was started on Venurafenib treatment (1920 mg/d). A dramatic neurological improvement was observed after 3 weeks. Patient #2 was a 50 year old male with multi-systemic disease symptoms and was BRAF negative. He was treated with a low dose of Cladribine and showed improvement after 36 months. Dr. Mazor and colleagues are currently exploring new research venues, in particular, the feasibility of various immunological treatments for ECD.

Dr. Talha Munir, MD, from St. James's Hospital Leeds, Leeds, UK, spoke about a single-centre experience of treating patients with LCH and ECD and the sustained response to oral Methotrexate (MTX) as a first line treatment. ECD has a distinct histologic, immunohistochemical and radiological pattern. The BRAFV600E mutation is detected in 50% to 60% of cases in both LCH and ECD suggesting a potential link between the two. First line treatment is IFNα/pegulated IFNα. Other options include Cladribine, IImatinib and other experimental treatments. A case presentation of a 52 year old man showed diplopia, unsteady gait and maculopapular rash on the back as well as pre-orbital swelling on MRI. He was initially treated with 6 cycles of Cladribine but displayed recurring symptoms. He was then treated with Prednisolone and was started on 20mg of MTX which was increased to 40mg a week. The patient continues to do well on MTX and his last PET showed further remission. MTX is used to treat a variety of autoimmune disorders as well as cancer. It is a dihydrofolate reductase inhibitor, inhibiting purine and pyrimidine synthesis. Adenosine mediated anti-inflammatory effects have been established (also used to treat rheumatoid arthritis) and MTX also has an effect on cytokine production. MTX was used as a first-line treatment in 7/8 patients with ECD in their centre in Leeds. The dose titrated from 10-15mg/week to a max dose of 40mg/week. Average duration of treatment was 19.8 months and there has been no deaths in this group. The main toxicity seen was de-arranged liver function tests. MTX is effective in attaining at least a stable response in a majority of ECD patients, however, it's role should be explored in larger trials.

Dr. Julien Haroche, MD, PhD, from Hospital Pitie-Salpêtrière, Paris, France, spoke about the variability in efficacy of the IL-1 receptor antagonist, anakinra, for treating ECD and their study consisted of a series of 12 patients. IFNα (or PEG IFNα) is still the first line treatment used in Paris, although tolerance may be poor is some patients. Anakinra tolerance has generally shown to be better than IFNα, but in the present series the response is variable and there is a lack of efficacy on CRP values and PET scan results. The median treatment length was 22 months with a dose of 100mg/day. In some cases, patients developed

new CNS localization and cardiac progression. Poor tolerance lead to treatment discontinuation in 4 patients. One patient developed a tamponade and was switched to vemurafenib. Anakinra could be discussed as a front-line therapy in patients without severe cardiac or CNS involvement and in patients with mild forms of the disease, with little biological inflammation. It may also be compared to IFNα therapy and/or perhaps to simple surveillance.

Dr. Juvianee I. Estrada-Veras, MD, from the NIH in Bethesda, gave an update on the ECD Natural History Study being carried out at the NIH. In order to participate, ECD patients should be aged between 2-80 and the study is open to both US and non-US patients. Patients visit the NIH for 1 week from Sunday to Saturday. From October 2011 to Sept 2014, there have been 50 patients enrolled and 47 seen at the NIH. There are 40 males and 10 females. The mean age of diagnosis is 45.6 with a range of 16 to 72 years. Bone involvement is seen in 88%, sinus involvement in 20%, CNS effects in 42%, pulmonary effects in 42% and cardiac involvement in 70%. Retroperitoneal fibrosis is seen with peri-aortic involvement in 26% and peri-renal in 24%. Thirty percent have xanthelasma and diabetes insipidus is seen in 42%. Hypogonadism is observed in 54% and suboptimal vitamin B and C complex is seen. ANA antibodies have been observed in 13 cases and anti-ENA antibodies in 3 cases. There is an increased CRP in 38%. 45 patients were tested for the BRAF mutation and 51% were BRAFV600 positive, 44% were BRAFV600E negative and 2 patients are still being tested. The survival following diagnosis is greater than or equal to 61 months in 21 cases. As a next step, the study aims to look at the CNS involvement in ECD using Diffusion tensor imaging MRI, CSF studies, neurocognitive testing as well as EMG, EEG and MRS. Dr. Estrada-Veras also expressed some interest into looking further into the autoimmune and metabolic profiles of ECD patients.

Additional remarks:

The ECD experts finished with a panel discussion on treatment availability for patients as well as side effects and rare complications. Dr. Haroche mentioned that they are looking at stopping Venurafenib treatment after a certain amount of time to try reduce the risk of resistance and also to see if patients relapse. The panel also discussed the recently identified RAS mutations. Interestingly, it was mentioned that patients who are BRAF negative but have the RAS mutation, may have a worsened prognosis if treated with Venurafenib. 50% of Dr. Haroche's patients are based outside of Paris. At Sloan Kettering, 5 patients have been diagnosed there and the rest are referrals. The panel spoke about referral centres and networks both overseas and within the US. They discussed the possibility of having designated specialty centres which would be able to provide patient treatment guidelines. The development of an International Classification of Disease (ICD) and a registry for ECD was also discussed.

The next ECD symposium and patient and family gathering has been scheduled to take place in Houston, Texas on October 8, 2015 and most likely in Paris, France in 2016.