Summary of the 13th Nikolas Symposium "Langerhans cell histiocytosis (LCH): neoplasia or immune dysregulation?"

LCH: introduction. A Langerhans cell is a type of 'immune cell' that recognizes infectious organisms, such as bacteria, and starts the immune response directed against these organisms. Langerhans cells are normally present in the skin. After they have come into contact with bacteria, Langerhans cells move to the lymph nodes (also known as "lymph glands") where they instruct other types of white blood cells how to eliminate the invading bacteria. In patients with the rare disease, Langerhans cell histiocytosis (LCH), Langerhans cells accumulate in one or more organs of the body, including the bones, skin, lymph nodes, liver, lungs and brain, and form what are known as "lesions". Within LCH lesions, Langerhans cells multiply and secrete a variety of chemicals known as "chemokines" and "cytokines" that attract and activate additional white blood cells. Interestingly, the behaviour of LCH lesions varies widely between different patients. For example, certain lesions (usually those localized to one organ), often disappear spontaneously or require minimal treatment to induce healing. In contrast, other lesions (usually those occurring young patients or involving more than one organ), require treatment with corticosteroids or chemotherapy to make them regress or go away completely. In patients with this latter type of lesion, a delay in treatment, or use of the wrong medications, may lead to an increase in the size or number of lesions, which can result in organ damage and, rarely, in death.

To improve LCH treatment, and thereby, to increase the survival and quality of life for LCH patients, it is important to understand why 'lesions' develop. Unfortunately, and despite many research efforts, the cause of LCH remains unknown. In fact, one of the most fundamental controversies within the medical field centers around whether LCH is the result of an intrinsic defect in the Langerhans cells themselves (i.e. Is LCH a "neoplastic" disease? Please see below for definitions) or the result of external "triggers" that drive normal Langerhans cells to divide in an abnormal manner (i.e. Is LCH a "reactive" disease?). This year, the 13th Nikolas Symposium, sponsored by Paul and Elizabeth Kontoyonnis, the parents of a young man, Nikolas, who developed LCH in infancy but has survived, brought together clinicians and scientists from a variety of countries and medical specialties to address this very question. This summary will outline the arguments presented at the Symposium that support the suggestion that LCH is either on the one hand a "neoplastic" disease or on the other hand a "reactive" disorder.

Defining terms: what is a "neoplastic" versus a "reactive" disease? To understand this issue it is important, first, to define the term "neoplasm". Typically a "neoplasm" results from the accumulation of cells that proliferate more rapidly than normal cells. Unlike normal cells, however, neoplastic cells continue to proliferate even after the stimuli that initiated their growth have stopped. Because of their uncontrolled division, neoplastic cells build up and form what are known as "tumors", masses of cells that lack the structural organization characteristic of normal tissues. Tumors may be classified either as "benign" or "malignant", depending upon their appearance under the microscope and their behaviour in the patient. It has been well established that neoplastic tumors develop because one original cell has acquired abnormalities known as 'mutations' in its genetic material (also known as "genes"), thereby allowing it to divide in an unrestrained manner. This continued cell division leads to the accumulation of large numbers of genetically identical "daughter" cells (or "clones") that are capable of surviving on their own without any growth-sustaining 'signals' from the surrounding cells.

In contrast to "neoplastic" diseases in which the neoplastic cells themselves are genetically defective, "reactive" diseases are thought to result from "outside" forces (for example, environmental factors such as cigarette smoking, or infectious factors such as bacteria) that stimulate the proliferation of otherwise normal cells. Interestingly, "reactive" diseases may share many features with neoplastic diseases, which at times makes it difficult to distinguish between the two. For example, certain autoimmune diseases (such as systemic lupus erythematosis or autoimmune colitis) or diseases associated with an overly robust immune response to infection are characterized by the accumulation of activated white blood cells within certain organs. Occasionally, these white blood cells form lesions that resemble neoplastic tumors. Despite these similarities, there are particular features that separate "reactive" cells from their neoplastic counterparts. First, and most importantly, the accumulation of cells that results as a "reaction" to an external factor is generally due to the proliferation of a large population of precursor cells. Therefore, many of these cells are different from each other. This is in contrast to the identical "daughter" cells that make up a neoplastic tumor. Second, reactive cells are unlike neoplastic cells in that they do remain dependent upon 'growth' and 'survival' factors produced by other cells to stimulate their proliferation and continued existence within the body or in a laboratory dish.

The fundamental question: is LCH a "neoplastic" or a "reactive" disease? Over the years, various investigators have suspected that LCH may be neoplastic but interest in this possibility heightened when, in 1994, it was demonstrated by two separate groups that the Langerhans cells present within LCH lesions were "clonal" in nature. Using molecular methods, both groups showed that all the Langerhans cells isolated from the same LCH specimen demonstrated a similar pattern of "X-chromosome inactivation", a genetic marker indicating that they might be "daughter" cells derived from the same original cell. More recent investigations, summarized nicely at this year's symposium by *Dr. Pancras Hogendoorn (Department of Pathology, Leiden University Medical Center, the Netherlands)* and *Dr. Jean Gogusev (Institut National de la Sante et de la Recherce Medicale, Paris, France*, provided support for these findings. They demonstrated that "lesional" Langerhans cells (i.e. those obtained from the affected tissues of LCH patients) harbor additional genetic abnormalities, including gains or losses of chromosomal material (chromosomes are the structures that contain genes collectively known as DNA), translocations between different chromosomes, and upregulated expression of genes that promote cell division. Moreover, work presented by *Dr. Maurizio Arico (Director, Onco-Ematologia, Ospedale dei Bambini "G. Di Cristina", Palermo, Italy)*, demonstrates that there are rare families in which more than one member is affected by LCH, a finding that suggests a possible genetic or "hereditary" cause in these cases. Taken in combination with other factors including the clinically aggressive behavior of certain forms of LCH, the absence of a defined "external" factor driving the proliferation of Langerhans cells in childhood cases, at least, and pathology studies demonstrating that lesional Langerhans cells are arrested in their maturation process, these genetic data provide strong support for the argument that LCH might be a "neoplastic" disease.

However, and as pointed out by *Dr. Anthony Chu (Consultant Dermatologist, Imperial College School of Medicine, London)*, certain data also exist to support the notion that LCH might be, at least in subset of cases, a "reactive" disease. These data are strongest for adolescent or adult patients with 'isolated' pulmonary LCH (i.e. absence of LCH involvement of other organs). In this group of patients, there is a direct relationship between disease activity and cigarette smoking. For example, exposure to cigarette smoke initiates LCH, and cessation of smoking generally results in

disease regression. Recent genetic studies have also shown that the Langerhans cells obtained from patients with pulmonary LCH, unlike those obtained from patients with other forms of LCH, are not "clonal" in nature (they are not felt to originate from one original precursor cell). Currently, it is not known which chemical factors in cigarette smoke initiate pulmonary LCH, or why certain people are susceptible to developing this form of the disease.

As regards other forms of LCH (for example, bone lesions or LCH lesions involving multiple organs), several observations suggest they might be the result of a "reactive" expansion of normal Langerhans cells. First, it has been known for many years that LCH is capable of spontaneous remissions, a property not generally characteristic of "neoplastic" disorders. Second, it has been incidentally noticed that LCH can "flare" or re-occur when a patient develops a cold or other infectious process, suggesting that LCH cells may be "reactivated" by the same external stimuli that activate normal immune cells. In addition, milder forms of LCH may respond favorably to treatment with antibiotics, medications used treat bacterial infections, indicating that these cells are capable of "switching off" their activity in a manner similar to a normal white blood cell that is no longer needed. Third, it is extremely difficult to grow LCH cells in the laboratory, suggesting that these cells require essential growth and survival factors present in the body but absent outside it. By contrast, it is usually possible to grow neoplastic cells for long periods of time in the lab. Fourth and last, LCH lesional cells have never been shown to have abnormalities (also known as "mutations") in the particular genes that regulate cell division or survival. This is quite different from neoplastic cells, which almost always harbor mutations in one or more of these categories of genes.

Summary and conclusions: does it really matter whether one considers LCH a "neoplastic" or "reactive" disease? In an effort to address one of the most fundamental questions regarding LCH, this year's Nikolas Symposium provided indirect evidence to support the possible classification of LCH as both a "reactive" and a "neoplastic" disorder. Is such a dual classification for LCH possible? As pointed out by *Dr. Robert Arceci (Pediatric Oncology, Johns Hopkins Oncology Center, Baltimore, USA)* during his introduction to the symposium, and by *Dr. Jon Pritchard (Department of Haematology/Oncology, Royal Hospital for Sick Children, Edinburgh, UK)* who led the patient-parent session, LCH is an extremely heterogeneous disease that is characterized by a spectrum of clinical and pathological features. It may not be possible to generate one unifying model that sufficiently explains such a variable disease. Rather, the microscopic and clinical diagnosis of LCH may represent, a 'continuum' of disorders, with some patients developing lesions that appear "reactive" in nature, while others develop lesions that are more "neoplastic". A similar model was proposed by *Dr. Dorothy Crawford (School of Biomedical and Clinical Laboratory Sciences, University of Edinburgh, UK)* who used post-transplantation lymphoproliferative disease (PTLD) as an example of a human disease that, like LCH, has features of both immune dysregulation and cancer. PTLD is a disease that occurs in patients with suppressed immune systems and is associated with an abnormal expansion of white blood cells following infection with a virus known as Epstein-Barr virus (EBV) which causes 'Glandular Fever'. As in LCH, some patients with PTLD have a milder form of the disease that appears to be due to increased proliferation of otherwise normal white blood cells but there are others with more aggressive disease, in which the white blood cells have acquired genetic abnormalities and neoplastic features.

Does it really matter whether one classifies LCH as a neoplastic or a reactive disease? In reality, the lack of an answer does not significantly change how we treat LCH patients, given the limited range

of currently effective therapies. Ultimately, however, the answer to this question might provide additional insight into LCH, which could facilitate the development of newer and more effective treatments. It is the hope of Paul and Elizabeth Kontoyonnis, and all who are involved with the Nikolas Symposium, that the use of these therapies will cure all future LCH patients and prevent the long-term side effects of this disease. It is also anticipated that LCH research will increase our understanding of normal Langerhans cell biology, which may benefit a larger array of patients with other diseases caused by abnormal Langerhans cell migration, proliferation and/or activation. There is even hope that 'cracking' LCH will help sufferers from both common and rare forms of true cancer (examples). Dendritic cells – Langerhans cells belong to this 'cell family' – are now under study in several laboratories that are trying to produce 'cancer vaccines'. Thus, progress in LCH research could bring kinder and more effective treatments to the cancer sufferer more quickly than would otherwise be the case.