Eradicating the neoplastic clone

Summary of the 31st Nikolas Symposium, Athens, May 18-21, 2023

The Nikolas Symposium

The Nikolas Symposium is an annual meeting hosted by Paul and Elizabeth Kontoyannis, whose son Nikolas developed Langerhans Cell Histiocytosis (LCH) in infancy. Their motivation to initiate the Nikolas Symposium in 1989 was the lack of knowledge on the pathogenesis of this rare disease. The purpose of the meeting is to find a rational cure for LCH through fostering new collaborations between researchers in and outside the LCH field. Accordingly, the symposium is an interactive forum of basic scientists and clinicians who discuss a different topic each year related either to the clinical presentation of LCH, the spectrum of its complications (late effects), the neoplastic cells involved and new therapeutic targets (PMID 31831887). The meeting also provides an opportunity for Greek physicians to present difficult cases and to discuss results of their studies with experts in the field.

Introduction to the 31st symposium

The 2023 symposium brought together experts in the field of myeloid cell development, biological features of myeloid cells - including LCH cells and microglia cells of the brain - and therapeutic targeting of tissue resident histiocytes and their precursors. Two junior scientists active in the histiocytosis field - Dr. Paul Kemps (Leiden University Medical Center) and Dr. Camille Bigenwald (Gustave Roussy Cancer Institute) - received a Pritchard Fellowship, which provides an opportunity to awardees to present their research at the meeting. Traditionally, the scientific program was opened by Steering Committee members presenting the concept and history of the meeting (Dr. Maarten Egeler), clinical and pathophysiological features of LCH (Dr. Carl Allen) and the summary of the 30th Nikolas Symposium (Dr. Astrid van Halteren).

Myelopoiesis under different conditions

Tissue-accumulating histiocytes in LCH and other types of non-Langerhans cell histiocytosis are thought to be derived either from myeloid precursor cells produced during definitive hematopoiesis or from tissue-resident precursor cells that arise and seeded in various tissues during fetal hematopoiesis. After Dr. Jennifer Picarsic (Cincinnati Childrens Hospital Medical Center) presented a comprehensive overview on the pathology of the various histiocytosis subtypes, Dr. Daniel Lucas (Cincinnati Childrens Hospital Medical Center/PMID 38509363) presented his work on spatial organization of hematopoietic stem/progenitor cells in the bone marrow either under steady state or during an infection. Using different ways of cell imaging, he showed that myelopoiesis takes place in selective parts of the murine skeleton and is associated with recruitment of lineage-committed progenitor cells to blood vessels where they form microanatomical production sites composed of proliferating progenitors and immature cells derived thereof. The release of myeloid precursor cells like conventional dendritic cells into the blood takes place after downregulation of CSF-1 expression on endothelial cells lining these sinusoids. Dr. Siddhartha Jaiswal (Stanford Medicine Children's Health) discussed his studies on the aging hematopoietic system (PMID: 31672865). In elderly people, hematopoietic stem cells (HSC) express $1.4x10^6$ spontaneously acquired, coding mutations in their genome. Some of these mutations, so called clonal hematopoiesis of indeterminate potential (CHIP) mutations, provide a growth advantage, resulting in clonal expansion of one or more dominant clones. CHIP mutations typically concern genes which regulate the

expression pattern of other genes (epigenetic regulators like *TET2* or splicing factors like *SRSF2*. These dominant clones may acquire additional somatic mutations allowing them to transform into clones associated with clonal cytopenia of undetermined significance (CCUS) and even with myeloid neoplasia (PMID: 37483562). As discussed by Dr. Paul Kemps (Leiden University Medical Center), CHIP mutations occurring as a first mutational hit seem not a rare event in adult histiocytosis, which may explain why some ECD (PMID: 28566492 / PMID: 33067622 and LCH cases additionally develop a clonally related myeloid malignancy (PMID: 38213281).

Tools to study biological properties of (neoplastic) myeloid cells to find therapeutic targets

CHIP$^+$ myeloid clones generated in the bone marrow also infiltrate the human brain where they form a substantial population of microglia cells (PMID: 37322115). Microglia are specialized brain-resident macrophages that arise from primitive macrophages colonizing the embryonic brain. Microglia contribute to multiple aspects of brain development as well as to brain dysfunction for instance in the context of Alzheimer's disease. Dr. Chris Glass (University of California San Diego) zoomed in on mechanisms underlying the activation of macrophage- or microglia cell-specific enhancers that drive environmental signal-induced transitions in the their fate and biological state. He discussed how distinct gene expression/enhancer landscapes are associated with different microglia phenotypes which either protect against or actively promote brain pathology (PMID: 34464593). Dr. Florent Ginhoux (Gustave Roussy Cancer Institute) presented a new in vitro model to study human brain development. To this end, his team generated induced pluripotent stem cell (iPSC)-derived macrophage (iMac) and human brain organoids from the same donors. When cultured together, iMac differentiate into cells with microglia-like phenotype and function (iMicro) including the modulation of neuronal progenitor cell differentiation and axonogenesis (PMID: 37914940). Dr. Nicole Coufal (University of California San Diego) is also exploring iPSC technology combined with CRISPR-based introduction of MAPK pathway-activating mutations like BRAFV600E to generate iMicro from blood or bone marrow cells obtained from LCH patients. Reprogrammed LCH patient-derived iMicro express high levels of Iba1 (microglia marker), phosphorylated ERK and langerin, the latter being pathognomonic features of neoplastic histiocytes. When deprived of essential growthfactors, iMicro fail to upregulate caspase indicating that these cells are apopotosis-resistant. When injected into the brain, reprogrammed iMicro proliferate *in situ* and invade different parts of the brain (unpublished data). Dr. Camille Bigenwald (Gustave Roussy Cancer Institute) presented her studies in transgenic mice with enforced expression of BRAFV600E mutation in early hematopoietic progenitor cells. BRAFV600E overexpressing HSC are skewed towards generating mononuclear phagocytes (MNP) which invade different tissues like spleen, lung and liver where they mature into inflammation-promoting CD207$^+$ histiocytes which have turned on a senescence program. These LCH-like lesions resolve when the mice are orally treated with navitoclax, a pharmacological inhibitor of Bcl-2, Bcl-X_L and Bcl-w anti-apopotic proteins (PMID: 33958797).

Current and new therapeutic strategies

Based on excellent clinical results, targeted therapy (RAF or MEK inhibitors) has become a very popular strategy to treat histiocytic disorders, despite the earlier reported toxicity issues, particularly seen in adults. Dr. Mimi Bandopadhayay (Dana Farber Cancer Institute) presented her results of histological and molecular profiling of pediatric low-grade gliomas with prominent

presence of either BRAF duplications or the same BRAFV600E mutation as often found in histiocytosis patients (PMID: 25825052). Like histiocytosis patients with tumorous lesions in the brain, a BRAF inhibitor which can pass the blood-brain barrier would be an ideal drug to treat the various glioma subtypes. Dr. Samual Blackman (Day One Biopharmaceuticals) presented his efforts on drug development for childhood cancer through either accelerated testing of drugs approved for adults, the development of new drugs for pediatric cancers expressing specific molecular drivers or retargeting existing or discontinued drugs in adult oncology. The feasibility of the latter approach is evidenced by successful development of their drug tovorafenib (DAY101), an oral pan-RAF kinase inhibitor which is currently tested in 2 clinical trials enrolling patients with solid tumors – including low grade gliomas - expressing RAF activating mutations. Based on its advocated superior brain penetrance, tovorafenib would be an excellent addition to the currently available pharmacological inhibitors. As market authorization for the use of these agents in European patients has not yet been granted, Dr. Caroline Hutter (St. Anna's Children's Hospital) presented the set-up of a new ECHO/ITCC study for pediatric refractory / neurodegenerative LCH wherein a BRAF inhibitor will be combined with second line chemotherapy. She advocated the importance of incorporating a companion biological study wherein therapeutic efficacy is addressed in relation to measuring mutant BRAF alleles in fractionated blood cells. This will hopefully reveal whether the cell-of-origin that gives rise to circulating BRAF mutated cells in the blood (PMID: 32750121) has truly been eradicated. Finally, Dr. Anthony Latai (Dana Farber Cancer Institute) discussed how a cell's commitment to apoptosis can be actively regulated by exposure to BH3 mimetic drugs, which specifically target the anti-apoptotic Bcl-2, Bcl-X_L and MCL-1 proteins. His research team has developed an in vitro model wherein sensitivity of apoptosis-resistant cell lines to drug-induced apoptosis can be monitored through cytochrome C release (PMID: 37881837). Given the senescent state of histiocytes in human and murine LCH-like lesions, BH3 profiling on ex vivo or iPSC-induced LCH cells seems a logical next step.

Concluding remarks

Through efforts made by various research groups worldwide, more information has become available on biological features of neoplastic histiocytes and their precursor cells in the blood and bone marrow. Recently developed iPSC-based culture systems seem a promising new tool to study histiocyte differentiation trajectories as well as their susceptibility to drugs targeting distinct pathological features. One of these features (apoptosis-resistance) seems an interesting therapeutic target. Drug-induced apoptosis may be explored in combination with newly developed RAF inhibitors with optimal tissue penetrance. Rapid and complete elimination of the neoplastic cell-of-origin – most likely residing in the bone marrow - seems particularly relevant for adults presenting with histiocytosis, as additional CHIP mutations may lead to a proliferative advantage, which may set the stage for the development of an additional hematological malignancy.

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