Report NCU grant

Report submission date: 15 March, 2013

Main applicant: Richard Rosenquist

Project title: Comprehensive Molecular Screening and Large-Scale Data Integration in a Population-Based Cohort of Chronic Lymphocytic Leukemia

NCU grant received (€): 50.000

Project commencement and completion dates: Started 2009 – still ongoing.

Please e-mail report to: ncu@kreftforeningen.no

---

1. **Brief description of the project, written in a language understandable to non-scientists (Maximum length: 100 words)**

   Chronic lymphocytic leukemia (CLL) is the most common leukemia in the Western world. The etiology is unknown and the disease is clinically and biologically diverse. This study has taken advantage of a large population-based material to analyse the molecular characteristics of CLL with the final aim to perform epidemiological analysis. Our specific aims are: 1) To obtain detailed molecular data to identify important genetic variants/events in CLL development. 2) To evaluate clonal evolution at follow-up. 3) To integrate high-resolution data using a systems biology approach. 4) To combine molecular, clinical and epidemiological data to further our understanding of etiological factors in this disease.

2. **Summarize the major findings of the project (Maximum length: 400 words)**

   Screening for NOTCH1 and SF3B1 mutations has revealed mutations at a much lower level than the initial reports. Nevertheless, both NOTCH1 and SF3B1 mutations were associated with considerably shorter time to treatment and overall survival, similar to patients with TP53 aberrations. We have now proposed that these two novel genes should be added to the hierarchal model for prognostication of CLL (Mansouri et al, Leukemia 2013).

   We also performed telomere length analysis and could show that patients with NOTCH1/SF3B1/TP53 mutations had significantly shorter telomeres. Furthermore, patients could be subdivided both regarding time to treatment and overall survival with short telomeres indicating a worse prognosis. In multivariate analysis, TL was also shown to be an independent prognostic factor in CLL (Mansouri et al, American Journal of Hematology 2013).

   Using RNA-sequencing, we generated data from complete transcriptomes for eight CLL cases (four subset #4 and four subset #1). Analysis revealed that 156 genes and 76 non-coding RNAs were differentially expressed between the two subsets. In addition, we

identified more than 400 novel splice variants which were predominantly expressed in the poor-prognostic subset #1. Moreover, we detected 16-30 missense mutations per sample and mutations were found in genes (e.g. ATM and NOTCH2) with a strong potential in CLL pathogenesis (Mansouri et al, American Journal of Hematology 2012).

We employed 27K methylation arrays to compare the methylation profiles of 3 different stereotyped CLL subsets with distinct clinical course (i.e. subsets #1, #2 and #4) and were able to detect distinct methylation profiles between these stereotyped subsets (Kanduri et al, Epigenetics 2012). Importantly, genes involved in immune response were found to be differentially methylated between the subsets.

Using the 450K methylation array, we identified a large number of genes differentially methylated between good-prognostic IGHV-mutated and poor-prognostic IGHV-unmutated CLL Importantly, many CLL prognostic genes, epigenetic regulators, B cell signaling and TGF-ß and NF-κB/TNFR1 pathway genes were found alternatively methylated between subgroups. Furthermore, we demonstrate that the methylation profile is rather stable over time as well as between different anatomical compartments (Cahill et al, Leukemia 2013).

We have nearly completed a first library of clinical and epidemiological data and the most important molecular aspects of CLL. We will shortly be able to start with statistical analyses of potential risk or protective factors of CLL in relation to established CLL subgroups defined by IGHV mutation status and major genomic aberrations. We will also evaluate the potential role of host characteristics such as co-morbidity, family history and lifestyle for the prognosis of CLL.

3. Describe how the project has increased our knowledge of the prevention, cause and/or cure for cancer (Maximum length: 150 words)

By applying the latest available technologies, both in the laboratory and through computational analysis, we will learn more about how this incurable disorder is initiated and how it develops. This knowledge is invaluable and will help us to improve existing protocols for making the correct diagnosis, estimating prognosis and providing efficient treatment for patients. Finally, epidemiological analysis of this well-characterized cohort will help in understanding the etiology of the disease which can be used to study disease prevention. This integrated analysis of epidemiological data in relation to modern molecular markers will be the first of its kind in CLL.

4. Outline how Nordic cooperation has added value to this project (Maximum length 100 words)

Our large collaborative network of basic, epidemiological and clinical researchers in Sweden and Denmark and the unique population-based cohort have been absolutely vital for this project. Not only has each collaborative partner contributed with their expertise towards completing our goals, but the resulting large collection of CLL cases has enabled us to study frequencies of different genetic aberrations as well as the predictive capacity of new prognostic markers in relation to epidemiological data. We thus believe this new type of synergistic interaction between Sweden and Denmark will significantly contribute to generate clinically useful new knowledge within the CLL field.

5. Publications resulting from this grant

Ibbotson R, Athanasiadou A, Sutton LA, Davis Z, Gardiner A, Baliakas P, Gunnarsson R, Anagnostopoulos A, Juliusson G, Rosenquist R, Oscier D, Stamatopoulos K. Co-existence of trisomies 12 and 19 in chronic lymphocytic leukemia occurs exclusively in the rare IgG-positive variant. Leukemia 2012; 26: 170-2.

Agathangelidis A, Darzentas N, Hadzidimitriou A, Brochet X, Murray F, Yan XJ, Davis Z, van Gastel E, Tresoldi C, Chu C, Cahill N, Giudicelli V, Tichy B, Bredo Pedersen L, Foroni L, Francia di Celle P, Janus A, Smedby K, Anagnostopoulos A, Merle-Beral H, Laoutaris N, Juliusson G, Pospisilova S, Jurlander J, Tsaftaris A, Lefranc MP, Langerak AW, Oscier DG, Chiorazzi N, Belessi C, Davi F, Rosenquist R, Ghia P, Stamatopoulos K. Stereotyped B-cell receptors in one third of chronic lymphocytic leukemia: toward a molecular classification with implications for targeted therapeutic interventions. Blood 2012; 119: 4467-75.

Halldórsdóttir AM, Kanduri M, Marincevic M, Mansouri L, Isaksson A, Göransson H, Axelsson T, Agarwal P, Jernberg-Wiklund H, Stamatopoulos K, Sander B, Ehrencrona H, Rosenquist R. Mantle cell lymphoma displays a homogenous methylation profile: A comparative analysis with chronic lymphocytic leukemia. Am J Hematol. 2012; 87: 361-7.

Cahill N, Sutton LA, Jansson M, Murray F, Mansouri L, Gunnarsson R, Ryan F, Smedby KE, Geisler C, Juliusson G, Rosenquist R. IGHV3-21 gene frequency in a Swedish cohort of patients with newly diagnosed chronic lymphocytic leukemia. Clinical Lymphoma, Myeloma and Leukemia 2012; 12: 201-6.

Mansouri L, Gunnarsson R, Sutton LA, Ameur A, Hooper SD, Mayrhofer M, Juliusson G, Isaksson A, Gyllensten U, Rosenquist R. Next generation RNA-sequencing in prognostic subsets of chronic lymphocytic leukemia. American Journal of Hematology 2012; 87: 737-40.

Kanduri M, Marincevic M, Halldorsdottir AM, Mansouri L, Junevik K, Ntoufa S, Göransson H, Isaksson A, Juliusson G, Anderson PO, Ehrencrona H, Stamatopoulos K, Rosenquist R. Distinct transcriptional control in major immunogenetic subsets of chronic lymphocytic leukemia exhibiting subset-biased global DNA methylation profiles. Epigenetics 2012; 7: 1435-42.

Cahill N, Bergh AC, Kanduri M, Göransson-Kultima H, Mansouri L, Isaksson A, Ryan F, Smedby KE, Juliusson G, Sundström C, Rosén A, Rosenquist R. 450K-array analysis of chronic lymphocytic leukemia cells reveals global DNA methylation to be relatively stable over time and similar in resting and proliferative compartments. Leukemia 2013; 27: 150-8.

Mansouri L, Cahill N, Gunnarsson R, Smedby KE, Tjönnfjord E, Hjalgrim H, Juliusson G Geisler C, Rosenquist R. NOTCH1 and SF3B1 mutations can be added to the hierarchical prognostic classification in chronic lymphocytic leukemia. Leukemia. 2013; 27: 512-4

Sava GP, Speedy HE, Di Bernardo MC, Deaglio S, Karabon L, Frydecka I, Rossi D, Gaidano G, Mansouri L, Smedby KE, Juliusson G, Rosenquist R, Catovsky D, Houlston RS. rs2072135, a low-penetrance variant for chronic lymphocytic leukaemia? British Journal of Hematology, in press.

Mansouri L, Grabowski P, Degerman S, Svenson U, Gunnarsson R, Cahill N, Smedby KE, Geisler C, Juliusson G, Roos G, Rosenquist R. Short telomere length is associated with NOTCH1/SF3B1/TP53 aberrations and poor outcome in newly diagnosed chronic lymphocytic leukemia patients.. Am J Hematol. 2013 Apr 26. doi: 10.1002/ajh.23466. [Epub ahead of print].