Report NCU grant

Report submission date: 15 March, 2012

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 (€): 30.000

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)**

   We employed 27K methylation arrays to compare the methylation profiles in 30 CLL samples, belonging to the poor-prognostic subset #1 and the good-prognostic subset #4, and 20 mantle cell lymphoma (MCL) samples. Unsupervised clustering could separate MCL from CLL cases based on methylation pattern, where MCL displayed a more homogenous profile (Halldorsdottir et al, American J of Hematology 2012). We also analyzed 3 different CLL subsets with distinct clinical course (i.e. subsets #1, #2 and #4) on the methylation array and were able to detect distinct methylation profiles between these stereotyped subsets (Marincevic et al, submitted).

   Using next generation RNA-sequencing, we have generated data from complete transcriptomes for eight CLL cases (four subset #4 and four subset #1). Analysis revealed that 156 genes (e.g. *WNT9A*) 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 *E2F4*) with a strong potential in CLL pathogenesis

(Mansouri et al., submitted). This is the first study of its kind in hematological malignancies.

DNA from 120 CLL cases from various prognostic subgroups has been prepared for targeted sequencing. After a thorough search, 180 genes which include the major players in B-cell receptor signaling, toll like receptor signaling, TGFβ signaling and NFκB signaling were chosen for sequencing analysis. The enrichment probes, using haloplex technology, were designed and the sequencing libraries for all cases were constructed. All libraries have now been sequenced using next-generation sequencing (Illumina) and the sequence data is currently being analyzed. Our initial results show an average depth coverage of 1228 reads per base pair per sample with an average specificity of 99.3%. Final results are expected in early spring.

We performed SNP-arrays on paired samples (from diagnosis and follow-up) from 59 CLL patients with and without progressive disease. Results indicate that patients presenting with poor-prognostic markers at diagnosis are far more likely to acquire additional genetic aberrations (Gunnarsson et al, Haematologica 2011). We also investigated the stability of four RNA-based prognostic markers (*LPL*, *TCL1*, *MCL1*, *ZAP70*) in 96 CLL patients over time with a median follow-up of 7 years and were able to demonstrate that *LPL* is the only stable marker over time making it useful for evaluating clinical outcome at any time point during disease (Sevov et al, manuscript).

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

Zainuddin N, Murray F, Kanduri M, Gunnarsson R, Smedby KE, Enblad G, Jurlander J, Juliusson G, Rosenquist R. TP53 Mutations are Infrequent in Newly-Diagnosed Chronic Lymphocytic Leukemia. Leukemia Research 2011 Feb;35(2):272-4.

Gunnarsson R, Mansouri L, Isaksson A, Göransson H, Cahill N, Jansson M, Rasmussen M, Lundin J, Norin S, Buhl AM, Ekström Smedby K, Hjalgrim H, Karlsson K, Jurlander J, Geisler C, Juliusson G, Rosenquist R. Array-based genomic screening at diagnosis and follow-up in chronic lymphocytic leukemia. Haematologica. 2011 Aug;96(8):1161-9.

Kaderi MA, Kanduri M, Buhl AM, Sevov M, Cahill N, Gunnarsson R, Jansson M, Ekström Smedby K, Hjalgrim H, Juliusson G, Jurlander J, Mansouri L*, Rosenquist R*. LPL is the strongest prognostic factor in a comparative analysis of RNA-based markers in chronic lymphocytic leukemia. Haematologica. 2011 Aug;96(8):1153-60.

Sevov M, Rosenquist R, Mansouri L. RNA-based markers as prognostic factors in chronic lymphocytic leukemia [review]. Expert Reviews Hematology 2012; 5: 69-79.

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. Accepted in Clinical Lymphoma, Myeloma and Leukemia.

Submitted manuscripts

Marincevic M, Kanduri M, Halldorsdottir A, Mansouri L, Göransson H, Isaksson A, Juliusson G, Ehrencrona H, Stamatopoulos K, Rosenquist R. Chronic lymphocytic leukemia subsets with stereotyped B cell receptors are distinguished by unique methylation patterns. Submitted.

Mansouri L, Gunnarsson R, Sutton LA, Ameur A, Hooper SD, Rasmussen M, Juliusson G, Isaksson A, Gyllensten U, Rosenquist R. Transcriptome sequencing reveals novel mutations and differential gene expression in stereotyped subsets of chronic lymphocytic leukemia. Submitted (revised manuscript re-submitted to American Journal of Hematology).

Manuscripts:

Mansouri L, Grabowski P, Degerman S, Svenson U, Gunnarsson R, Cahill N, Ekström Smedby K, Geisler C, Juliusson G, Rosenquist R, Roos G. Telomere length is a robust prognostic marker in early chronic lymphocytic leukemia. Manuscript.

Sevov M, Mansouri M, Kanduri M, Kaderi MA, Smedby KE, Jurlander J, Geisler C, Juliusson G, Rosenquist R. Longitudinal study of RNA-based prognostic markers in chronic lymphocytic leukemia. Manuscript.

Benner A, Mansouri L, Rossi D, Majid A, Willander K, Parker A, Bond G, Trbusek M, Nückel H, Merkel O, Rosenquist R, Gaidano G, Dyer M, Söderkvist P, Linderholm M, Oscier D, Pospisilova S, Dührsen U, Greil R, Döhner H, Stilgenbauer S, Zenz T. MDM2 promotor polymorphism and disease characteristics in CLL: Individual patient data meta-analysis of 2598 CLL patients. Manuscript.

Kaufman M, Yan XJ, Ghia EM, Neuberg D, Rassenti LZ, Stamatopoulos K, Ghia P, Oscier DG, Davi F, Rosenquist R, Belessi C, Davis Z, Agathangelidis A, Kay N, Byrd JC, Brown JR, Rai KR, Kipps TJ, Chiorazzi N. Chronic Lymphocytic Leukemia Patients with IGHV Genes Carrying Only Silent Mutations Have A Longer Time From Diagnosis to Initial Therapy Than Patients Expressing B-Cell Receptors with No Somatic Mutations. Manuscript.