# Report NCU grant

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**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 (€): 40.000**

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

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## 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)

As a result of a collaborative effort, we investigated genetic variants in CLL and identified four novel variants on 2q, 8q, 15q, and 16q that increased the risk for CLL (Crowther-Swanepoel D et al, Nature Genetics 2010). We have also analysed six previously published risk variants and could confirm five of these to be associated with CLL (Crowther-Swanepoel D et al, British Journal of Hematology 2010).

We applied SNP-arrays to study genomic aberrations in stereotyped IGHV3-21 (subset #2) and stereotyped IGHV4-34 (subset #4). Genomic aberrations were more common in subset #2 compared to subset #4. In particular, high frequency of del(11q) was detected in subset #2 which may be linked to the adverse outcome reported for these patients (Marincevic et al, Haematologica 2010).

Global gene expression analysis was performed on 25 IGHV4-34 samples, where distinct gene expression profiles were revealed for subset #4 vs. subset #16. The differentially expressed genes, down-regulated in subset #4 patients, are involved in important regulatory pathways, which may explain the favorable outcome observed in subset #4 patients (Marincevic et al, Haematologica 2010).

We performed methylation arrays on subset #1 cases, subset #4 cases and subset #2 cases. Results showed unique differences in methylation patterns between the three subsets, including genes involved in immune response and apoptosis (Marincevic et al, manuscript).

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, submitted).

We generated complete transcriptome data for 8 CLL cases (4 subset #4 cases and 4 subset #1 cases). The sequencing has resulted in at least 76-128 million 50bp reads from each sample. We have detected significant differences in gene expression of coding/non-coding RNA between the two groups as well as found novel splice variants and mutations. We believe that our approach has identified novel aberrations of importance in CLL pathogenesis (Gunnarsson et al, manuscript).

Using a systems biology approach, gene expression data for a set of CLL patients has been analyzed to identify genes that correlated with the IGHV mutational status, which revealed a subset of eight genes, including the known prognostic marker *LPL*. Furthermore, we integrated the expression data with protein-protein interaction data and identified differences in expression of protein complexes or pathways that may partly explain the clinical differences observed between the two patient groups.

**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

Crowther-Swanepoel D, Broderick P, Di Bernardo MC, Dobbins SE, Torres M, Mansouri M, Ruiz-Ponte C, Enjuanes A, Rosenquist R, Carracedo A, Jurlander J, Campo E, Juliusson G, Montserrat E, Smedby KE, Dyer MJS, Matutes E, Dearden C, Sunter NJ, Hall AG, Mainou-Fowler T, Jackson GH, Summerfield G, Harris RJ, Pettitt AR, Allsup DJ, Bailey JR, Pratt G, Pepper C, Fegan C, Parker A, Oscier D, Allan JM, Catovsky D, Houlston RS. Common 2q37.3, 8q24.21, 15q21.3, and 16q24.1 variants influence chronic lymphocytic leukemia risk. Nature Genetics 2010; 42: 132-6.

Crowther-Swanepoel D, Mansouri M, Vega A, Enjuanes A, Smedby KE, Ruiz-Ponte C, Jurlander J, Juliusson G, Catovsky D, Campo E, Carracedo A, Rosenquist R, Houlston RS. Verification that common variation at 2q37.1, 6p25.3, 11q24.1, 15q23, and 19q13.32 influences chronic lymphocytic leukemia risk. Br J Haematol. 2010 Aug;150(4):473-9.

Marincevic M, Cahill N, Gunnarsson R, Isaksson I, Mansouri M, Göransson H, Rasmussen M, Jansson M, Ryan F, Karlsson K, Adami HO, Davi F, Jurlander J, Juliusson G, Stamatopoulos K, Rosenquist R. High-density screening reveals a different spectrum of genomic aberrations in chronic lymphocytic leukemia patients with 'stereotyped' IGHV3-21 and IGHV4-34 B cell receptors. Haematologica 2010;95:1519-25.

Gunnarsson R, Isaksson A, Mansouri M, Göransson H, Jansson M, Cahill N, Rasmussen M, Staaf J, Lundin J, Norin S, Buhl AM, Ekström-Smedby K, Hjalgrim H, Karlsson K, Jurlander J, Juliusson, Rosenquist R. Large but not Small Copy-Number Alterations Correlate to High-Risk Genomic Aberrations and Survival in Chronic Lymphocytic Leukemia: A High-Resolution Genomic Screening of Newly Diagnosed Patients. Leukemia. 2010;24:211-5.

Marincevic M, Mansouri M, Kanduri M, Isaksson A, Göransson H, Smedby KE, Jurlander J, Juliusson G, Davi F, Stamatopoulos K, Rosenquist R. Distinct gene expression profiles in stereotyped subsets utilizing the IGHV4-34 gene in chronic lymphocytic leukemia. Haematologica 2010;95:2072-9.

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. Leuk Res. 2011;35:272-4.

Manuscripts:

Kaderi MA, Kanduri M, Mansouri M, Buhl AM, Sevov M, Cahill N, Gunnarsson R, Jansson M, Smedby KE, Hjalgrim H, Karlsson K, Juliusson G, Jurlander J, Rosenquist R. LPL is the strongest prognostic factor in a comparative analysis of RNA-based markers in chronic lymphocytic leukemia. Under revision in Haematologica.

Gunnarsson R, Mansouri M, Isaksson A, Göransson H, Cahill N, Jansson M, Rasmussen M, Lundin J, Norin S, Buhl AM, Smedby KE, Hjalgrim H, Karlsson K, Jurlander J, Juliusson G, Rosenquist R. Genome-wide array-based screening at diagnosis and follow-up in chronic lymphocytic leukemia. Under revision in Haematologica.

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.

Marincevic M, Kanduri M, Halldorsdottir AM, 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. Manuscript.

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.