A.Kirchenstein Institute of Microbiology and Virology

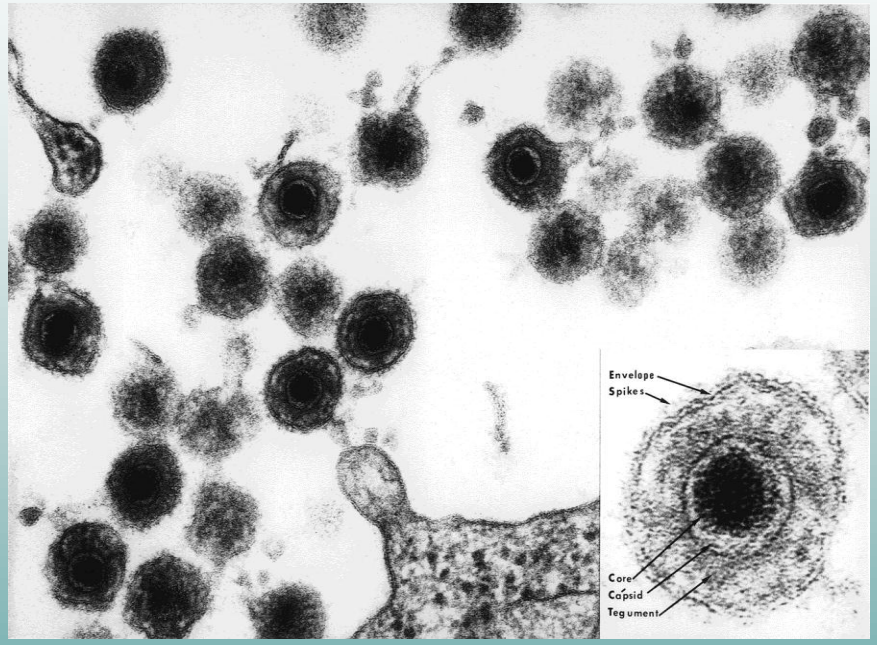
Presence of human herpes virus 6 (HHV6) and Epstein-Barr virus (EBV) DNA in peripheral blood cells of primary chronic lymphocytic leukemia (CLL) patients and its influence on B-cell subpopulation profile.

Artjoms Spaks, Rita Birkenfelde, Irina Spaka, Alla Rivkina, Madara Upmane, Ilona Sasoveca, Sandra Lejniece, Vaira Kalnina, Irina Holodnuka

"Promotion of International Cooperation
Activities of Riga Stradiņš University in Science and Technologies", agreement
No. 2010/0200/2DP/2.1.1.2.0/10/APIA/VIAA/006

INTRODUCTION

- Human herpesvirus 6 (HHV6) was first reported in 1986, as human B-lymphotropic virus
- Seroepidemiological surveys have shown that antibodies to HHV6 are highly prevalent in human populations in different geographical areas with prevalence varying between 70 and 100%*



*De Bolle L, Naesens L, De Clercq E: Update on Human Herpesvirus 6 Biology, Clinical Features, and Therapy. Clin Microbiol Rev 2005, 18:217-245.

INTRODUCTION

- EBV and HHV-6 have an ability to cause latent infection with reactivation during periods of immunosuppression[1,2]
- Multiple studies have suggested an association between both HHV6 and EBV and pathogenesis of lymphoma[1,2]

[1]Tailor PB, Saiki TK, Advani SH, Mukhopadhyaya R: Activation of HHV6 in lymphoproliferative disorders: a polymerase chain reaction-based study. Ann N Y Acad Sci 2004, 1022:282-285

[2]Diepstra A, Niens M, Vellenga E et al. Association with HLA class I in Epstein-Barr-virus-positive and with HLA class III in Epstein-Barr-virus-negative Hodgkin's lymphoma. Lancet 365: 2216-2224, 2005.

INTRODUCTION

- Chronic lymphocytic leukemia (CLL) is a chronic lymphoproliferative disorder characterized by the accumulation of monoclonal B cells in the blood, bone marrow, and secondary lymphoid tissues.*
- Traffic of the B-cell subsets through peripheral blood (PB) reflects the immune status of an individual and potentially also disorders of B-cell development.
- Circulating B-cell subsets have been poorly defined until recently, when ≥6-color flow cytometry became routinely available.**

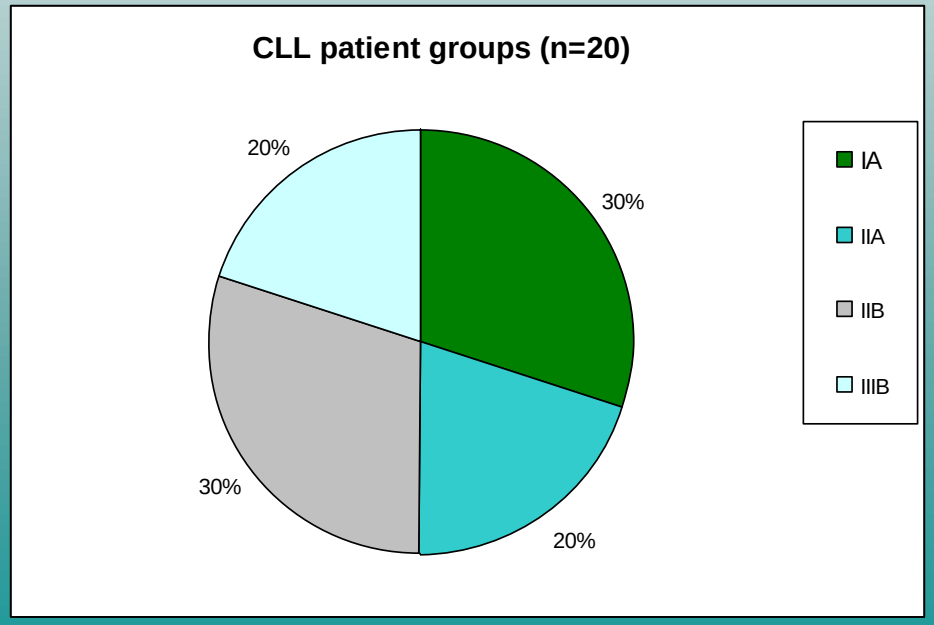
*Gribben J. G. How I treat CLL up front. Blood, 2010, Vol. 115.

**Caraux A, Klein B et al. Circulating human B and plasma cells. Age-associated changes in counts and detailed characterization of circulating normal CD138- and CD138+ plasma cells. Haematologica. 2010 Jun;95(6):1016-20.

THE AIM

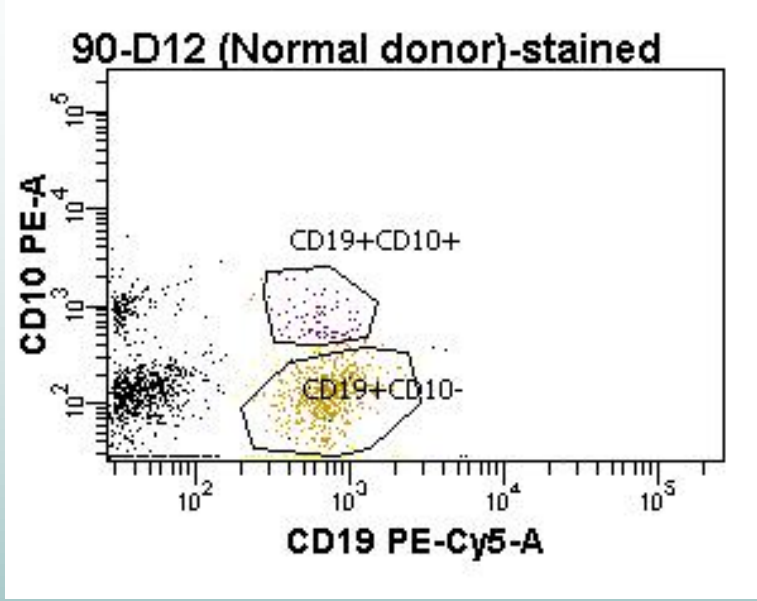
- Analyse and quantify B-cell sub-populations in PB samples of CLL patients in order to find, whether the data correlate with the clinical stage of the disease.
- Verify, whether the presence of HHV6 and/or EBV influence the PB B-cell subsets at the different clinical stages of CLL.

- Peripheral blood samples were collected from 20 patients diagnosed with CLL prior to treatment
- Combined Rai-Binet staging system was used to divide patients into groups.
- The median age of the 20 CLL patients in this study was 74.2 ± 7 years (range 66 – 86 years).
- Male/female ratio was 3:1.

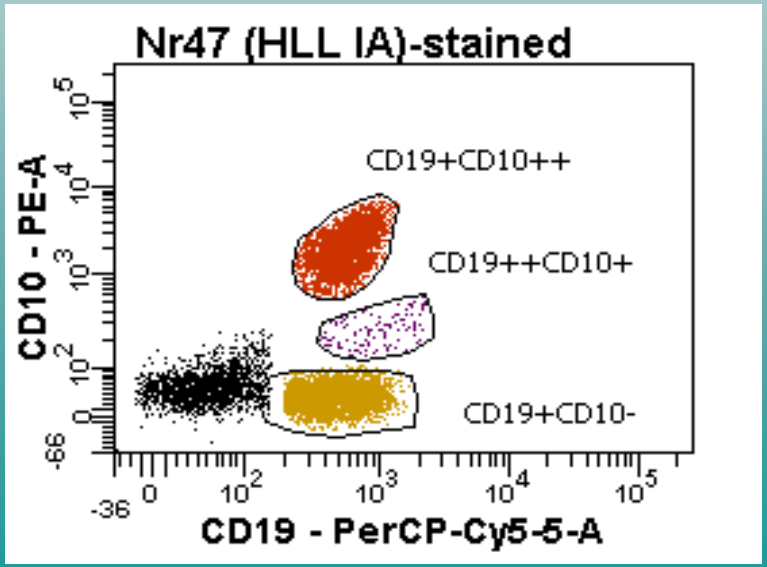


RESULTS

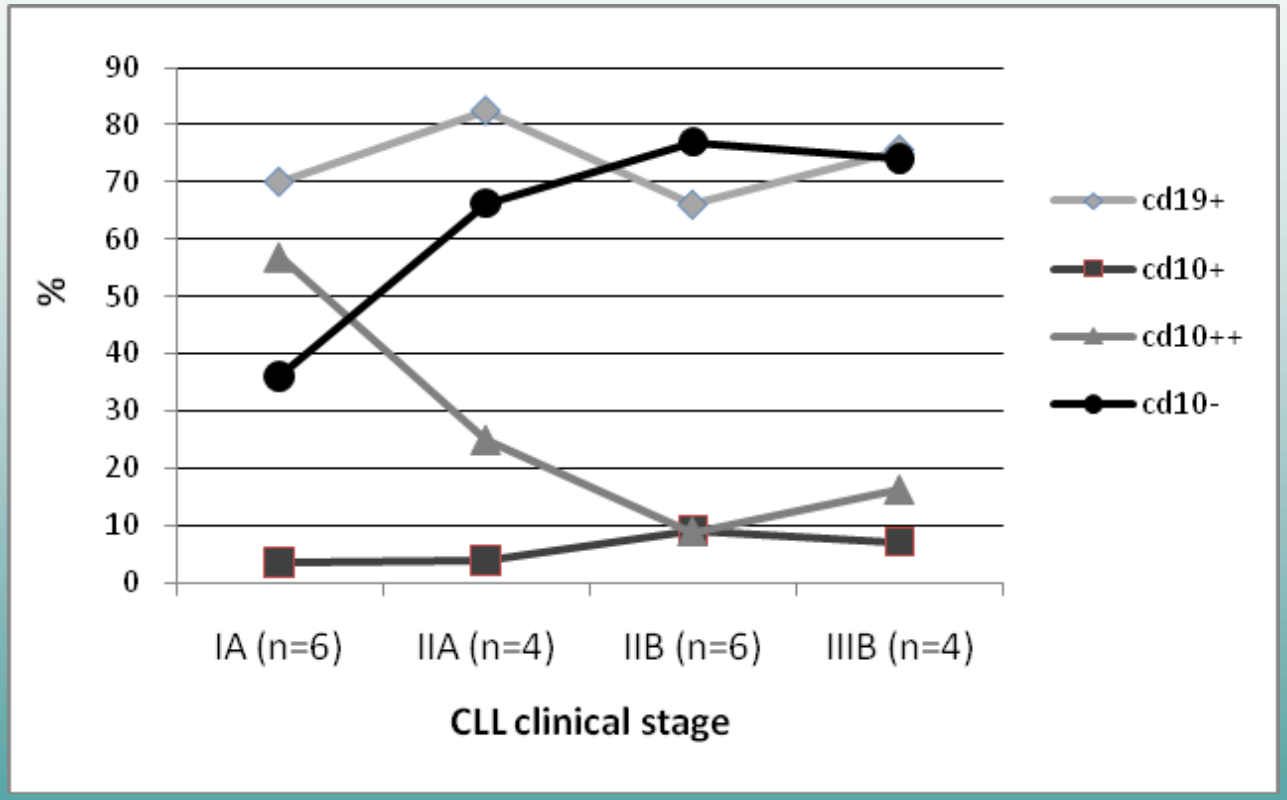
- Two subpopulations of B-cells were detected in peripheral blood of normal donors: CD19+/CD10+dim and CD19+/CD10-.



- Three subpopulations of B-cells were identified in peripheral blood of CLL patients: CD19+/CD10+dim, CD19+/CD10++bright and CD19+/CD10-.

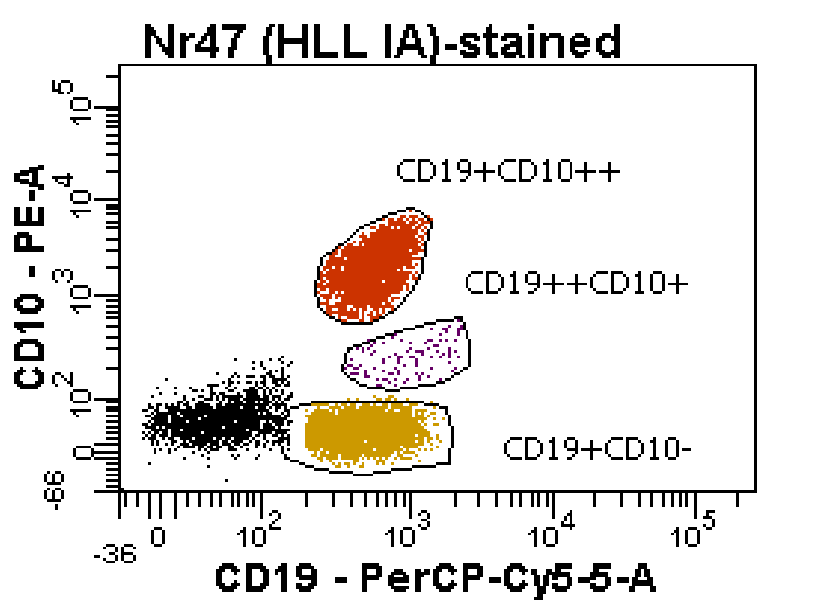


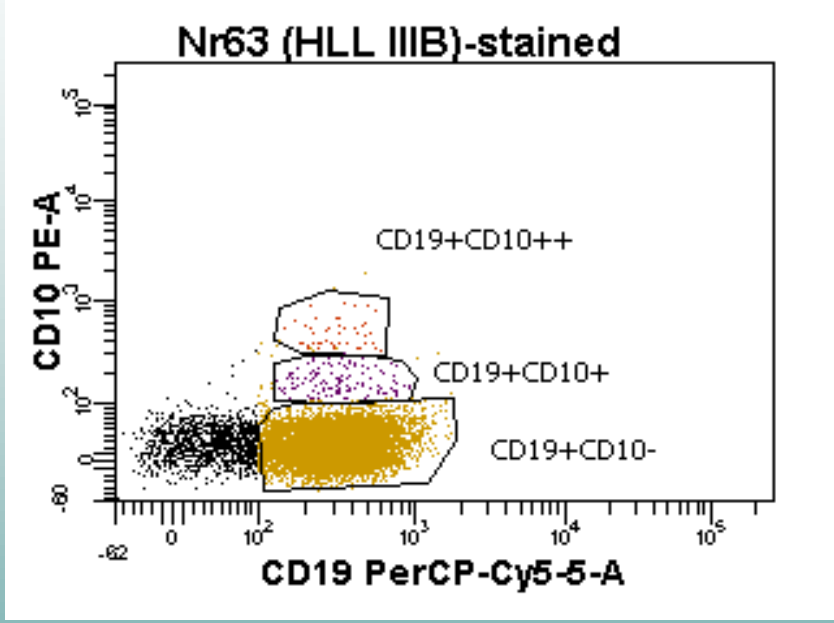
Distribution of B-cell surface receptors CD19 and CD10 according to the clinical stage of CLL



There is marked decrease of CD10++ (bright) B-cell subpopulation and increase of CD10- B-cell subpopulation during progression of disease. (p=0.05)

RESULTS

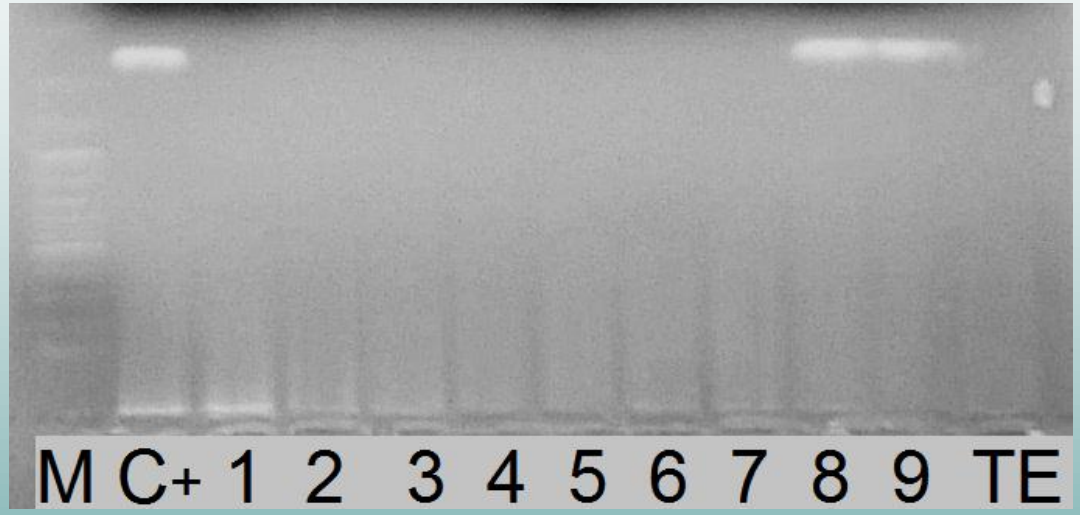




There is marked decrease of CD10++ (bright) B-cell subpopulation and increase of CD10- B-cell subpopulation during progression of disease. (p=0.05)

Presence of HHV6 DNA in PB samples of CLL patients

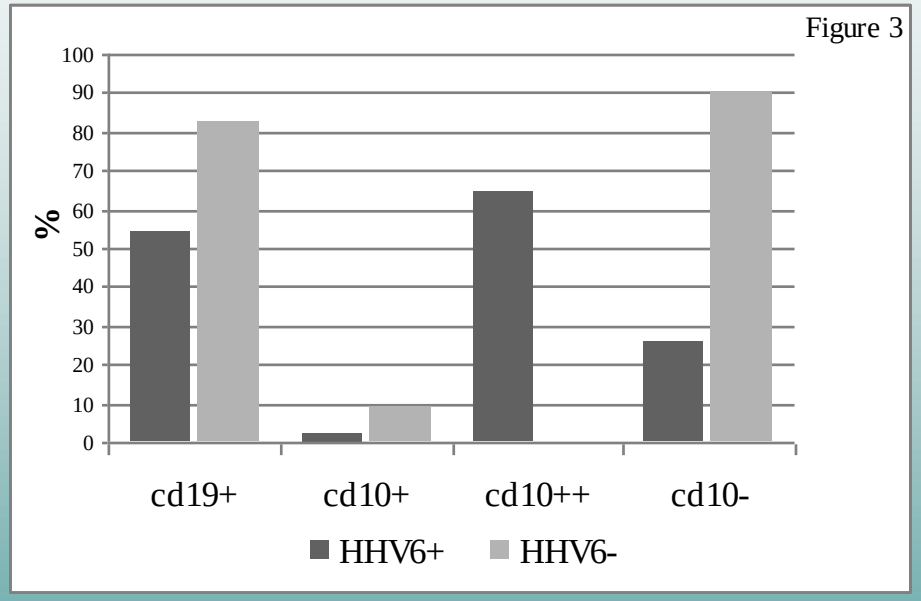
HHV6 nested PCR analysis of the leukocytes isolated from PB of CLL patients

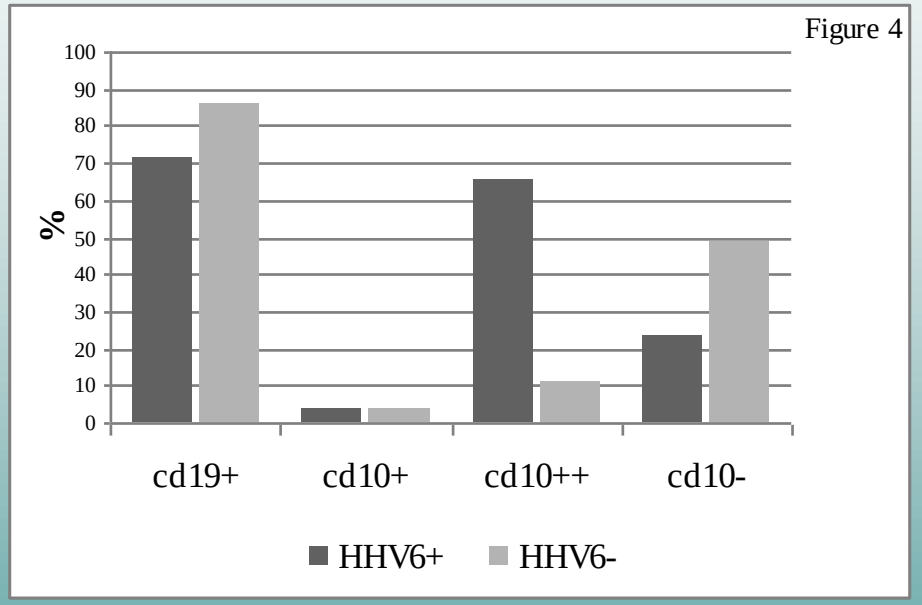


The HHV6-specific PCR-product is 258-bp in size. 1 - 9, number of the patient; C, HHV6 DNA control (HHV6A GA Quantitated Viral DNA, Advanced Biotechnoligies Inc) 10 copies per reaction; TE, TE-buffer as a negative control; M, DNA marker; 500 ng of DNA were used for nested PCR. The results were confirmed at least in two experiments.

EBV DNA (by PCR with sensitivity of 50 copies/reaction) was not detected in any of the patients

Changes of B-cell subpopulation profile in HHV6 positive patients.





Decrease in CD19+/CD10- subpopulation and increase in CD19+/CD10++ subpopulation

CONCLUSIONS

1. Our results suggest that the profile of two PB B-cell subsets - CD10+ and CD10- is characteristic for the clinical stages of CLL disease and distinguishes the early stages from advanced stages.

CONCLUSIONS

1. Our results suggest that the profile of two PB B-cell subsets - CD10+ and CD10- is characteristic for the clinical stages of CLL disease and distinguishes the early stages from advanced stages.

2. The monitoring of peripheral blood B-cell sub-populations during follow-up of CLL patients may be proposed as a disease progression indicator.

CONCLUSIONS

1. Our results suggest that the profile of two PB B-cell subsets - CD10+ and CD10- is characteristic for the clinical stages of CLL disease and distinguishes the early stages from advanced stages.

2. The monitoring of peripheral blood B-cell sub-populations during follow-up of CLL patients may be proposed as a disease progression indicator.

3. In all HHV-6 positive CLL patients (two out of the 20 patients prior the treatment) we have detected decrease of CD19+/CD10- subpopulation count and increase of CD19+/CD10++ subpopulation count.

Further prospective follow-up studies are required to identify a pathogenic role of HHV6 in each CLL patient case.

DISCUSSION

- Frequencies of positive HHV-6 DNA appear to vary widely among studies, and may depend on the differences in PCR sensitivity for each study.
- Quantitative analysis of HHV-6 in peripheral blood cells and serum will improve the interpretation of results.
- Positive PCR results do not necessarily indicate the presence of HHV-6 in neoplastic cells.
- Examination of HHV-6 antigens expression in PB circulating cells and lymphoid tissues in follow up studies is necessary for better understanding of the HHV6 impact on disease.

Potential involvement of HHV-6 in pathogenesis of CLL could be due to:

- Immunosuppression from both lymphatic malignancy and its treatment that may predispose to higher risk of co-infection.
- Immunomodulating effect of HHV6 since it can induce production of Il-1β and TNF-α, suppress T lymphocyte function due to reduced Il-2 synthesis.
- HHV6 can directly infect CD4+ T-cells and induce apoptosis, thus altering key immune activation molecular pathways and subsequently disturbing the cytokine network.
- HHV-6 can also infect thymic epithelial cells, hematopoietic stem cells, and natural killer cells, which are critical for immune maturation and protection against cancer and viral infections.

RĪGAS STRADIŅA UNIVERSITĀTE

ERAF EIROPAS REĢIONĀLĀS ATTĪSTĪBAS FONDS IEGULDĪJUMS TAVĀ NĀKOTNĒ EIROPAS SAVIENĪBA

"Promotion of International Cooperation Activities of Riga Stradiņš University in Science and Technologies", agreement No. 2010/0200/2DP/2.1.1.2.0/10/APIA/VIAA/006