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### DEVELOPMENT OF A LUX-REAL TIME PCR FOR HUMAN HERPESVIRUS 7 (HHV7) IN PRIMARY CUTANEOUS T CELL LYMPHOMAS.

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**Introduction.** HHV7 is a CD4-positive T-lymphotropic herpesvirus, the transforming capacity of which has not been established. Few studies have investigated the possible role of HHV7 in Cutaneous T cell lymphomas (CTCL): mycosis fungoides (MF), Sezary syndrome (SS), primary cutaneous CD30+ CTCL, including Lymphomatoid papulosis (LyP). Aim of this study was to develop a real time PCR for quantification of HHV7-DNA and to investigate the role of HHV7 in the context of CTCL.
**Methods.** To determine sensitivity of the Real-time PCR a clone containing 311 bp of the HHV7-DNA was obtained. The plasmid was then serially diluted, the concentrations were 20000, 2000, 200, 20, 2, and 0.2 copies/$\mu$l, and a 5-$\mu$l aliquot of each was used as standard. LUX primers are designed with a fluorophore near the 3´end in a hairpin structure. PCR was used to quantify HHV7-DNA in 191 skin biopsies of 147 pts (99M/49F, mean age 63 years): 74 MF, 37 SS, 11 CD30+ CTCL, 25 LyP.
**Results.** The copy numbers of samples were calculated from the CT values by the software program, Sequence Detector version 1.6 (Applied Biosystems). The sensitivity of this PCR was estimated from the standard curve to be approximately 10 copies/reaction.. HHV7- DNA was detected in 24 tissue samples of 20/147 (13.6%) patients: 10/37 SS, 4/11 CD30+ CTCL, 6/25 LyP and 0/74 MF.
**Conclusions.** The LUX-Real-time assay described is highly sensitive and specific for the detection of HHV7-DNA and permits quantitative assessment of viral load. No definitive conclusion on HHV7 role in the pathogenesis of primary CTCL can be made, although it is to note that HHV7-DNA was not detected in any of the pts with MF, a less aggressive CTCL.

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### LYTIC CYTOMEGALOVIRUS GENE EXPRESSION ANALYSIS USING RT-PCR

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**Introduction.** The Human Cytomegalovirus (HCMV) is a widespread pathogen that remains latent after primary infection but it can reactivate in immunocompromised conditions such as transplant recipients. HCMV genome is a dsDNA encoding for several genes expressed in different phases of the viral life cycle: immediately early (IE), early (E), involved in viral replication, and late and true late (L,TL) encoding for structural proteins. The evaluation of transcriptional profile of HCMV genes could be utilized for determine the viral reactivation state with potential clinical and therapeutic implications.
**Method.** Positive controls were obtained from HCMV AD169 (ATCC VR-538) and a clinical isolate amplified on human embryonic lung fibroblasts (HELF). UL123 (IE, regulatory function), UL54 (E, DNA polymerase), UL65 (L, matrix tegument protein), and UL99 (TL, pp28 protein) were amplified by four RT-PCR assays on 29 clinical samples (polymorphonuclear leukocytes, PMNL) obtained from 14 renal transplant recipients. Reverse-transcription was evaluated by testing for cellular GAPDH transcript on positive control and clinical samples. Results were compared with those obtained with pp65-antigenaemia and viraemia test.
**Results.** Overall, 24/29 samples (82.8%) were positive for UL123 and UL54 transcripts; 11/29 (37.9%) for UL65, and 17/29 (58.6%) for UL99 transcript. Ten samples (34.5%) were positive for all the transcripts, while 4/29 (13.8%) samples were negative for all the transcripts. The pp65-antigaenemia assay was negative in 5 of 29 samples (17.2%), all of them viraemia-negative. Three of the pp-65 antigaenemia-negative samples (60%) were UL54 positive, one (20%) UL65 and UL99 positive.
**Conclusions.** Our results confirm that during the course of infection UL123, UL54, UL65 and UL99 transcripts are expressed *in vivo* in circulating PMNL and detection of such transcripts could be useful for discriminating between viral latency and active replication with potential clinical and therapeutic implications.