# REVISITING EBV EXPRESSION PATTERNS IN LYMPHOMA

Amy Chadburn1[1], Ethel Cesarman[1]

[a] *Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY*
*achadbur@med.cornell.edu*

Three canonical expression patterns have been well recognized in EBV associated lymphoproliferative disorders, namely latency I, II and III (Lat I-III). These can be determined by RT-PCR based on promoter utilization, but this does not provide information at the single cell level. Previous classification of latency stages has also been done by immunohistochemistry using antibodies to LMP1 and EBNA2, together with EBER ISH. EBV+ cases with expression of neither of these viral proteins can be classified as Lat I, cases with LMP1 but not EBNA2 are Lat II, and cases with both LMP1 and EBNA2 are Lat III. Using this approach, several groups including our own have reported on these three specific latencies in AIDS-related or post-transplant lymphoproliferative disorders (PTLD). In probably the largest study of AIDS-related lymphoma (ARL) (N=212), we reported the latency pattern based on this approach, finding that diffuse large B cell lymphomas (DLBCLs) of the germinal center (GCB) cell subtype were mostly Lat 1, but in the activated B cell cases there was an approximately even distribution between Lat I, Lat II and Lat III cases [1]. Notably, there was a switch from many Lat III cases in the era before combination anti-retroviral therapy (cART), to many Lat I in cases in the era of cART.

Some studies have also reported use of double immunohistochemistry or immunofluorescence to LMP1 and EBNA2 in small numbers of patient specimens, including infectious mononucleosis [2-4], PTLD [5] and ARL [2], as well as in humanized mice [6]. Invariably, these reported the presence of cells with EBNA2 expression without LMP1, which would correspond to a Lat IIb expression pattern. Some of these studies have noted that in lymphomas, the cells with EBNA2 in the absence of LMP1 tend to be small, while cells expressing LMP1 tend to be large [2]. These Lat IIb cells have also been observed in humanized mice with EBV infection and cord blood lymphocyte populations infected with EBV *in vitro*, and have generally be interpreted to represent a transitory stage shortly after infection since in newly infected B cells expression of EBNA2 precedes expression of LMP1. The interpretation that there are cells with EBNA2 but no LMP1 is consistent with a study showing that Lat IIb is a real latency type, with truly no LMP1 expression (or consequent NFkB activation), and with a distinguishable transcriptional profile, which occurs at an early stage upon infection of B cells with EBV [7].

Given that many of these studies were conducted in the era before availability of antiretroviral therapy for the AIDS lymphomas, and when immunosuppressive treatment in transplant patients was more intensive, we examined cohort of 79 cases of ARL (38 EBV+) and 23 cases of PTLD (19 were EBV+), specifically focusing on latency subtypes using IHC for LMP1 and EBNA2, either alone or by double immunohistochemistry. We found there are cases with many numerous Lat IIb cells, but these are among the minority of cases. In fact, four ARLs contained many cells expressing EBNA2 in the absence of LMP1, all of which were diffuse large B cell lymphomas (DLBCLs) arising between 1992 and 1996, when cART was not available or limited, suggesting this pattern occurs primarily in highly immunodeficient individuals. One PTLD, from 2004, also showed many cells with a Lat 2b expression pattern. In contrast, Lat 2a was very common in the post-cART era, occurring in 55% of AIDS-related DLBCLs.

1. Arvey, A., et al. 2015, Blood, 20, e14-22.
2. Brink, A.A., et al. 1997, J Clin Pathol, 11, 911-8.
3. Kurth, J., et al. 2000, Immunity, 4, 485-95.
4. Niedobitek, G., et al. 1997, J Pathol, 2, 151-159.
5. Oudejans, J.J., et al. 1995, Am J Pathol, 4, 923-33.
6. Ma, S.D., et al. 2011, J Virol, 1, 165-77.
7. Messinger, J.E., et al. 2019, mBio, 4.