

Epstein-Barr Virus and Lymphomas

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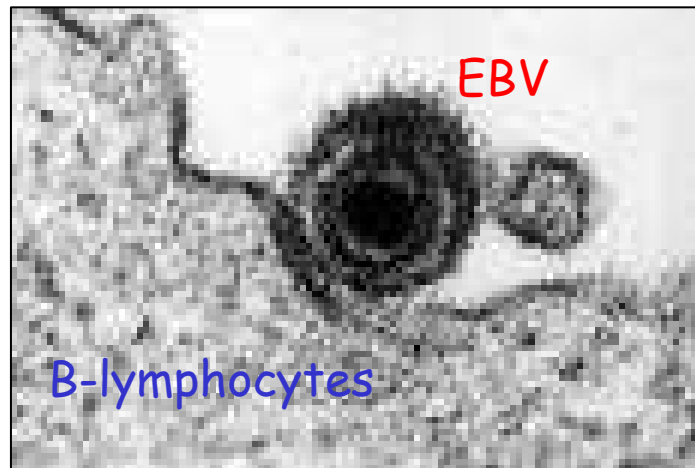
CHU Dupuytren et Faculté de Médecine,

Limoges, France

Key features of EBV (1)

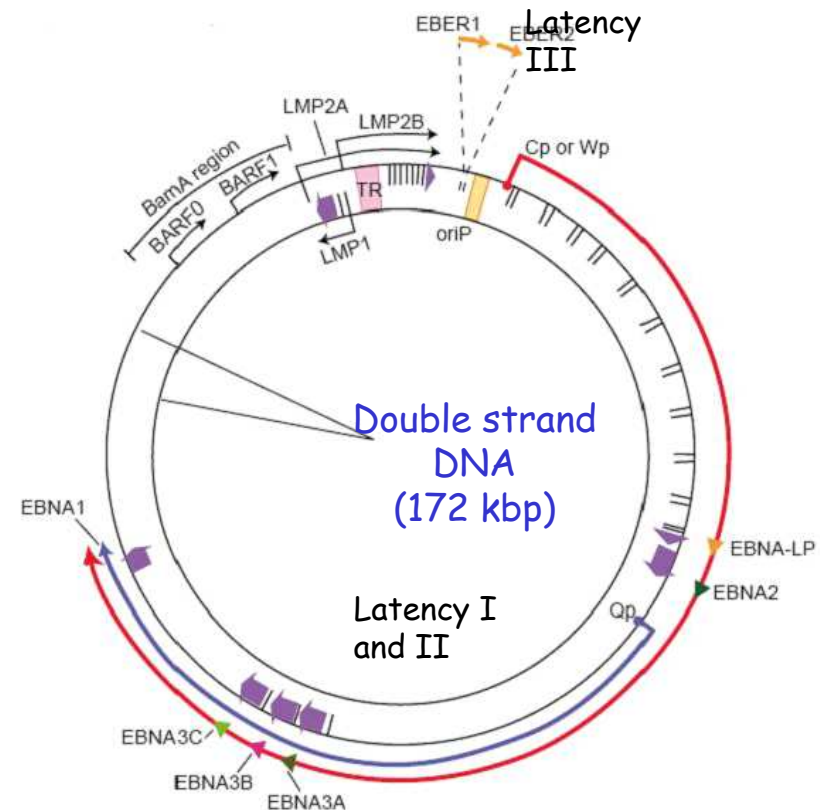
The virus

EBV is a gamma herpes virus



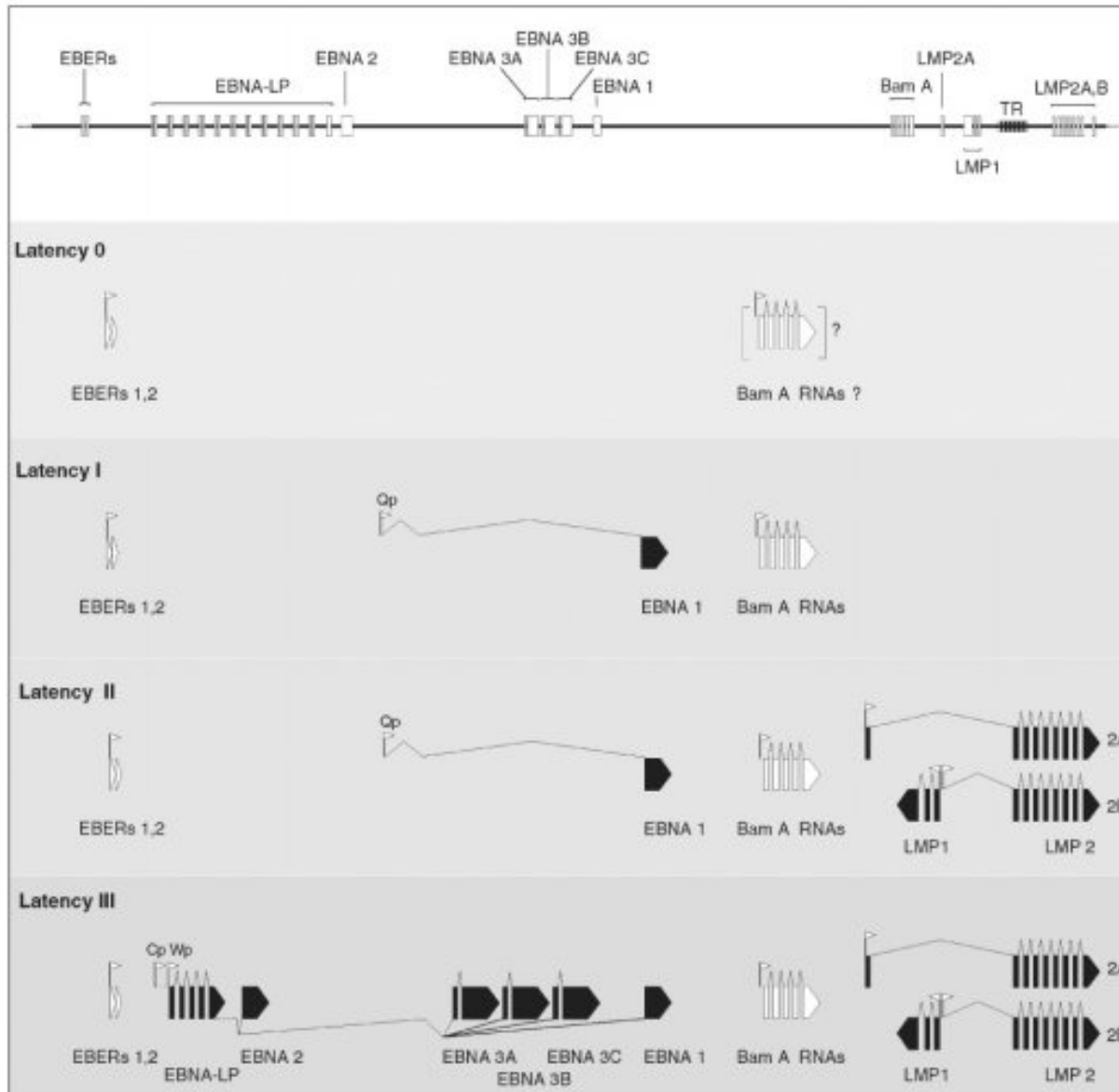
EBV attached to its CD21 receptor on a B lymphocyte.
Electronic Microscopy.

EBV Episome



(L Young, Nature Rev Cancer, 2004)

EBV latency programs



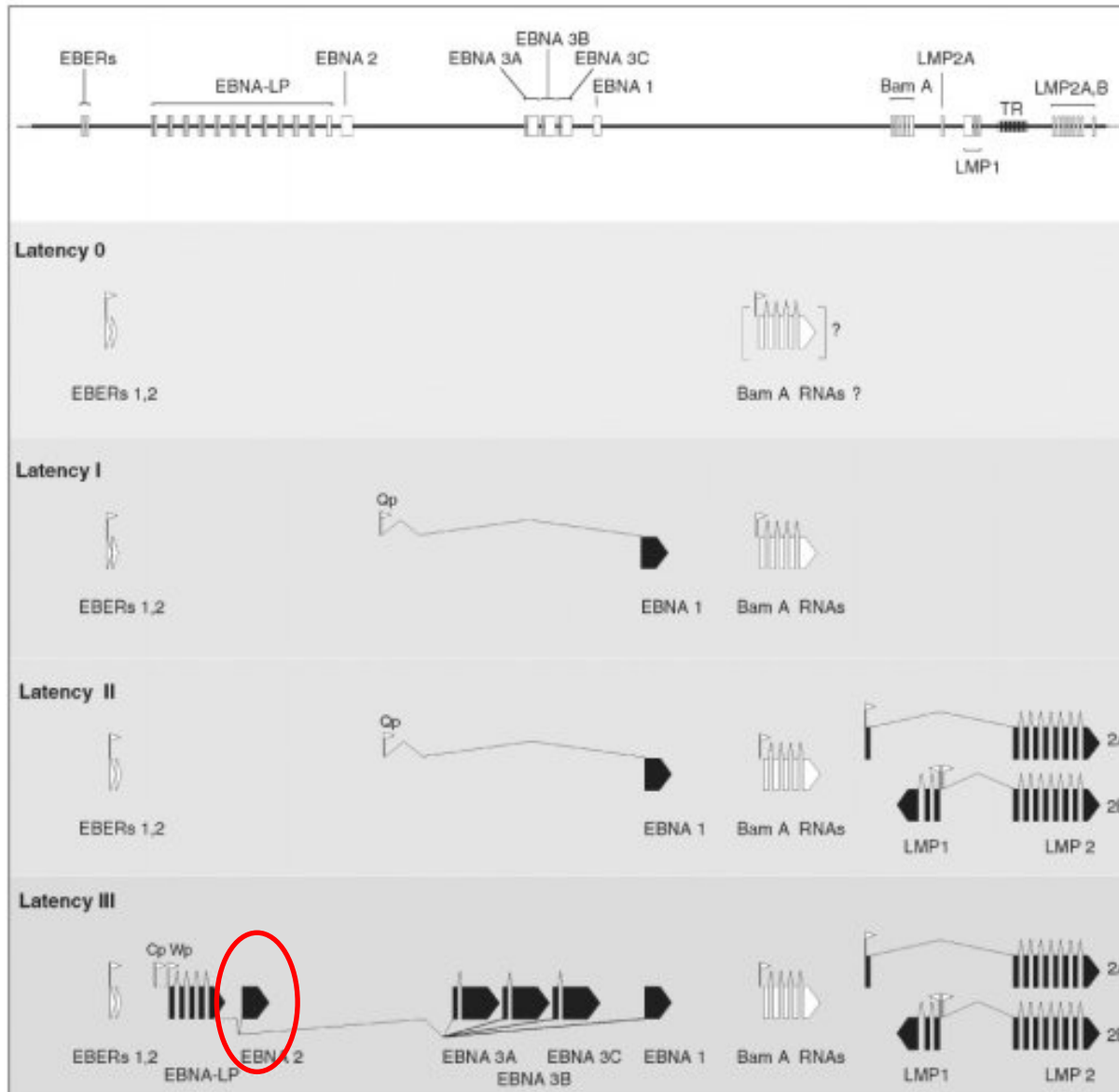
EBERS + BARTs

EBERS + BARTs + EBNA1

EBERS + BARTs + EBNA1
+ LMPs

EBERS + BARTs + EBNA1
+ LMPs + other EBNAs

EBV latency programs



EBERS + BARTs

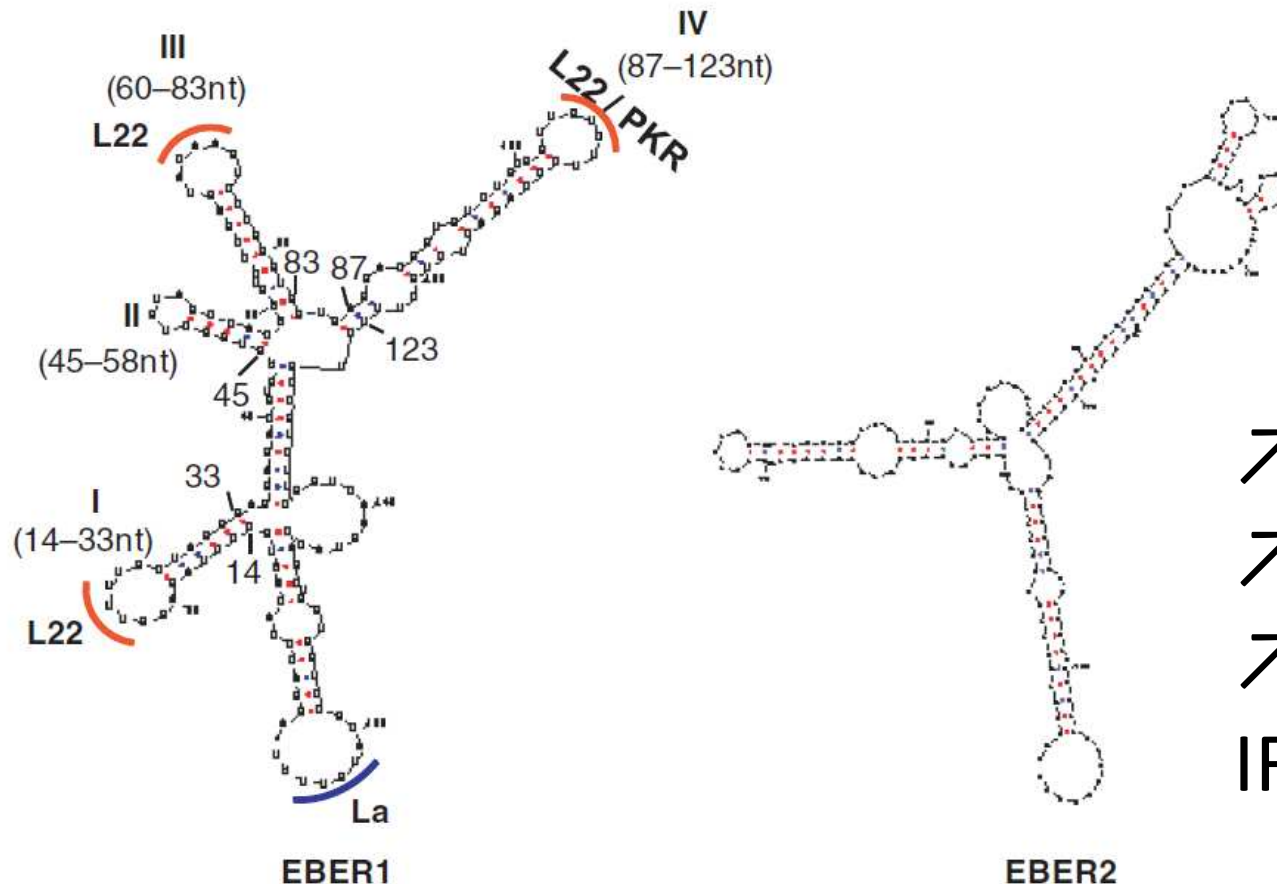
EBERS + BARTs + EBNA1

EBERS + BARTs + EBNA1
+ LMPs

EBERS + BARTs + EBNA1
+ LMPs + other EBNAs

The Epstein–Barr virus-encoded non-coding RNA

Both EBER1 and EBER2 form secondary structures with a number of short stem–loops.

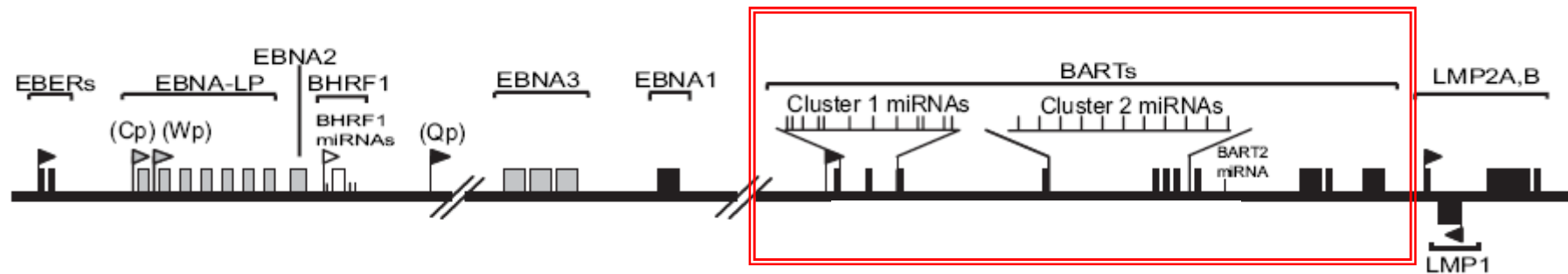


- ↗ Bcl2
- ↗ IL6, IL10, IGF1
- ↗ resistance to IFNs

BART non coding RNAs

BART RNAs = microRNAs = post-transcriptional regulators
(Pfeffer, Science, 2004)

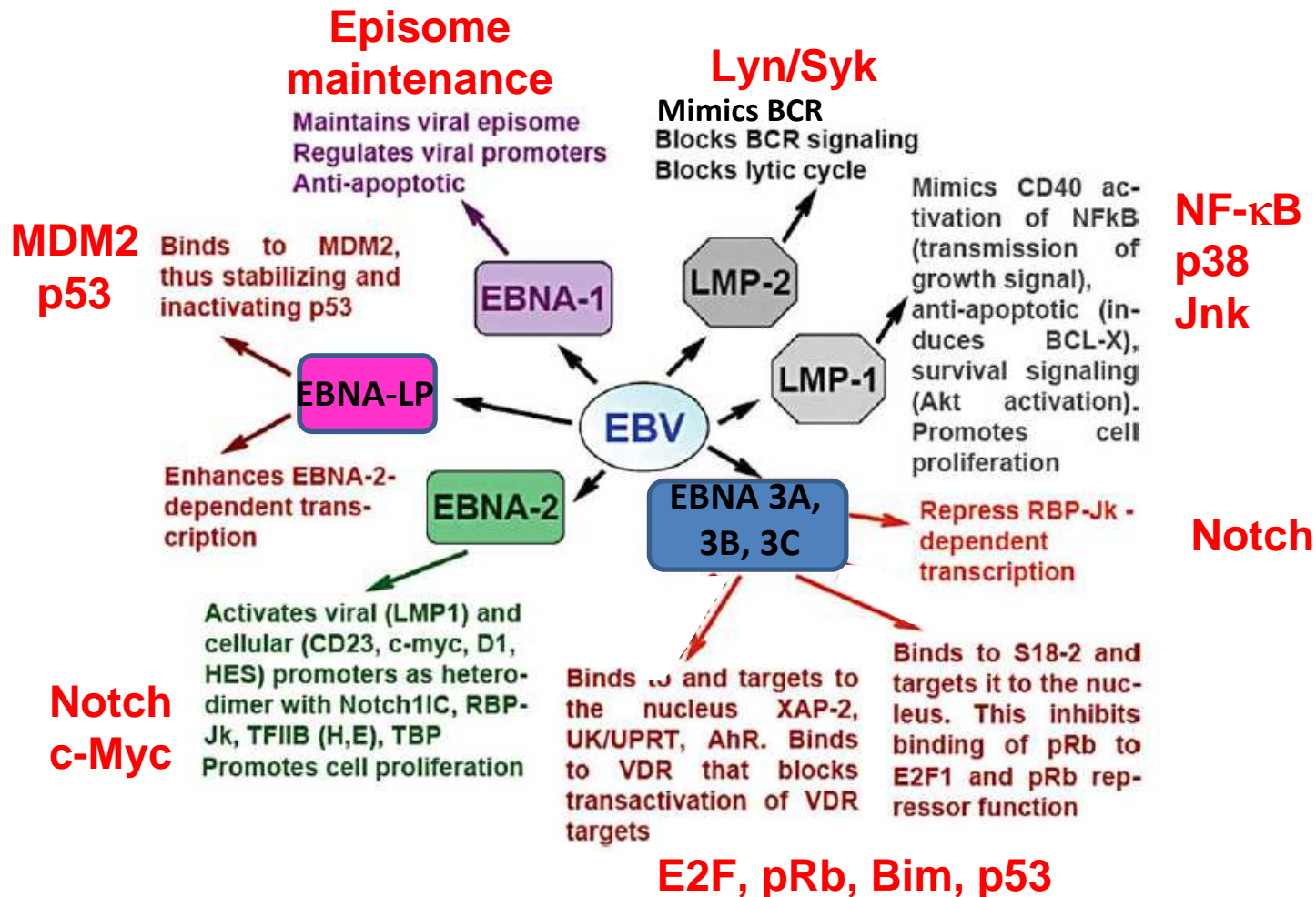
✓ 28 BART microRNAs, subdivided in 3 clusters : cluster 1 (12), cluster 2 (15) and miR-BART2



✓ Cluster 1 down-regulates LMP1 (Lo AK, PNAS 2007)

✓ miR-BART22 from cluster 2 down-regulates LMP2a (Lung RW., Neoplasia 2009)

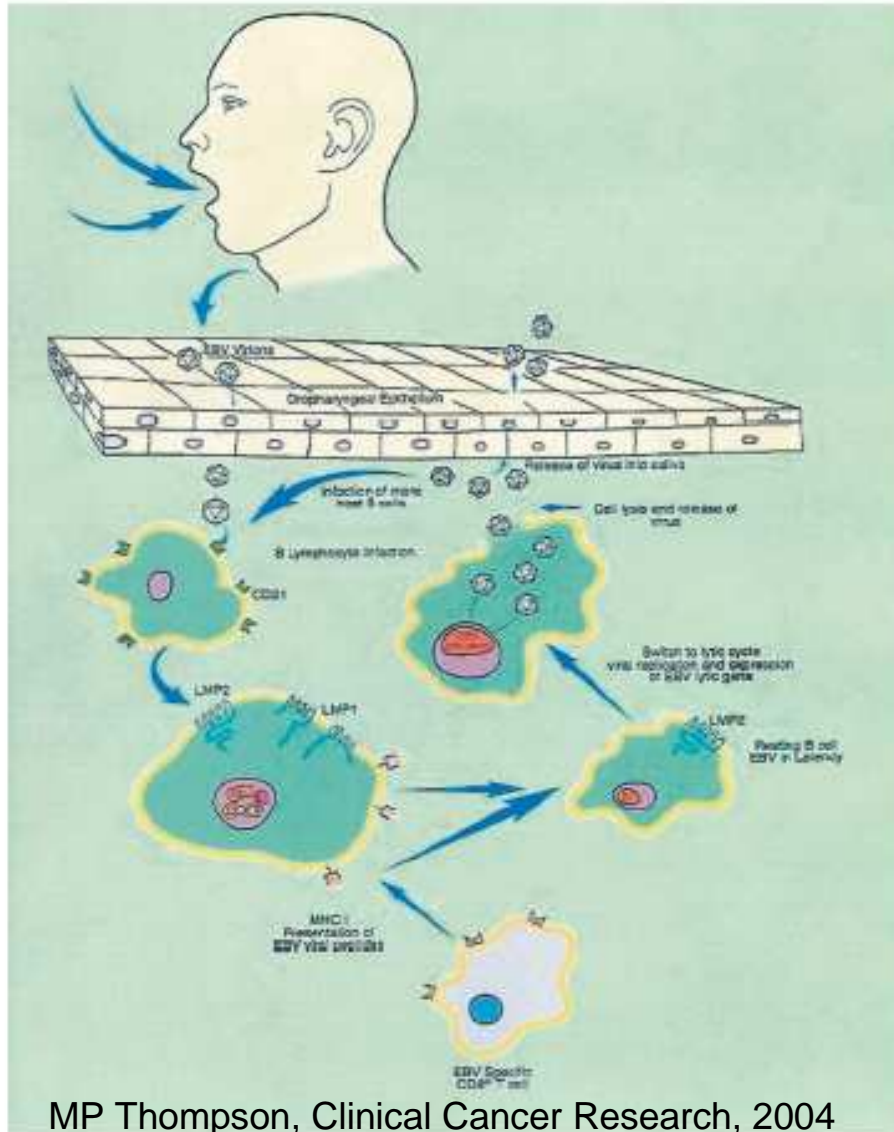
The latency III immortalizing program



Acting on key cellular players, EBV latency III viral proteins reprogram the quiescent B-lymphocyte to continuously proliferate

Key features of EBV (2)

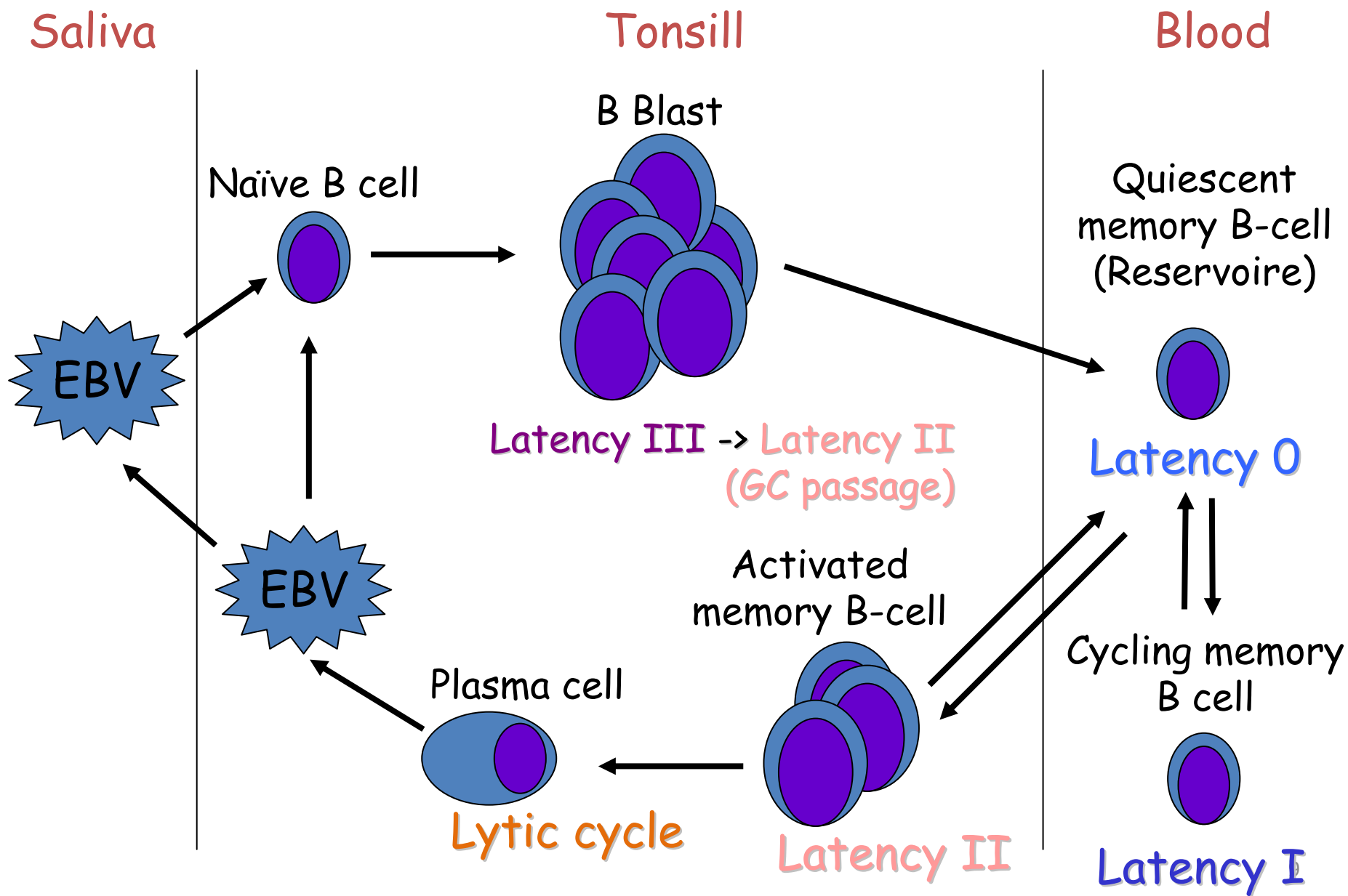
EBV in vivo



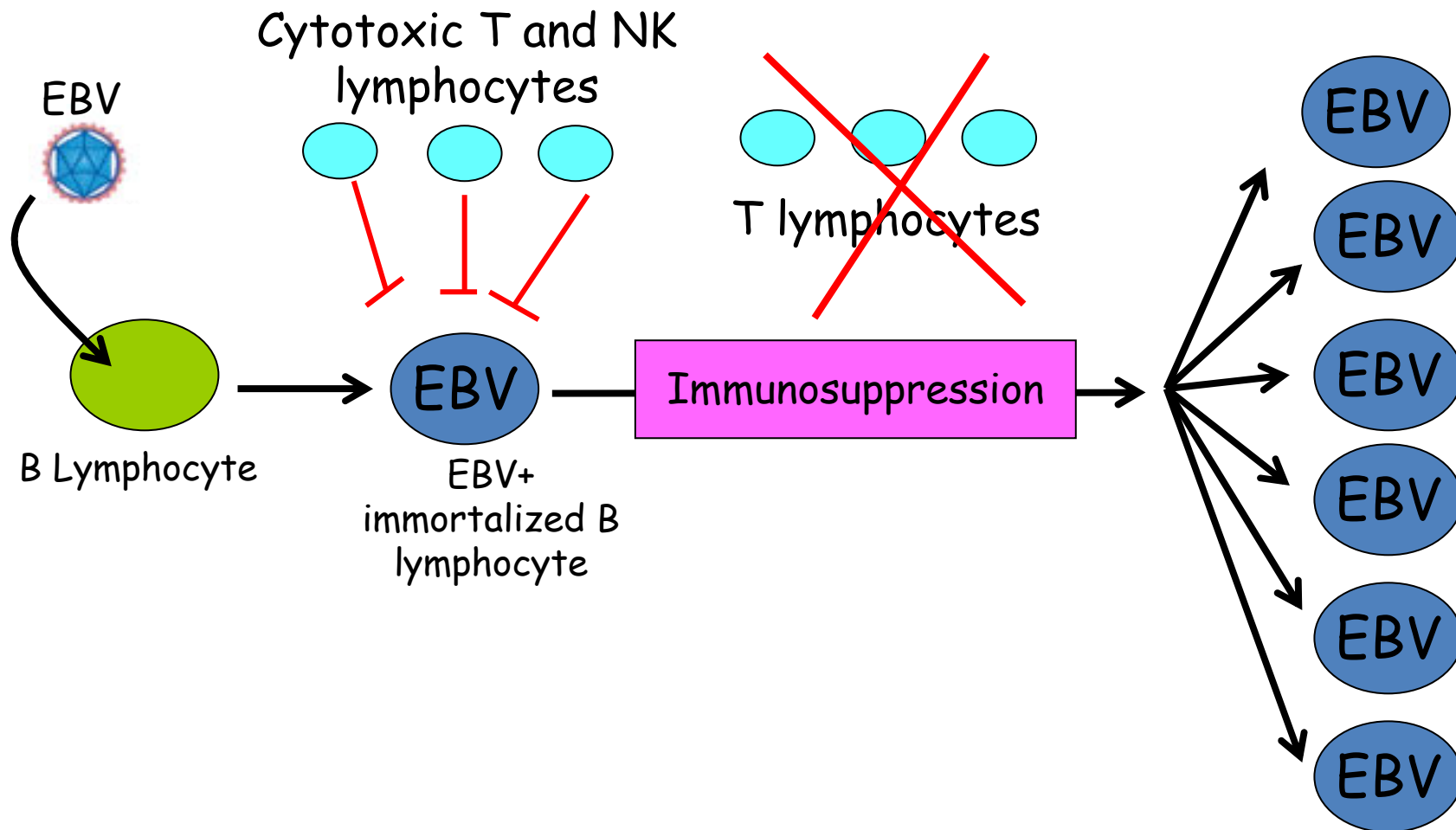
- EBV primary infection by oral contamination
- > 95% worldwide adults have been EBV infected in their past
- EBV persists in its host as an active virus and is continuously spread out in the saliva even at low levels.

EBV cycle

(adapted from D. Thorley-Lawson)



The EBV burden is under continuous control of the host immune system



The key features of EBV (3)

EBV and cancers

- EBV is an immortalizing virus for B-cells in vitro.
- EBV is the causative agent of post-transplant lymphoproliferative disorders.
- EBV is associated with various cancers of the B and T/NK cell lineage as well as with cancers of epithelial cells.

EBV-associated lymphomas

Table 2. EBV-associated lymphoproliferative disorders

EBV-associated B-cell lymphoproliferative disorders

Burkitt's lymphoma

Classic Hodgkin's lymphoma

Post-transplant lymphoproliferative disorders

HIV-associated lymphoproliferative disorders

Primary central nervous system lymphoma

Diffuse large B-cell lymphoma, immunoblastic

HHV-8-positive primary effusion lymphoma and its solid variant

Plasmablastic lymphoma

Other histotypes (rare)^a

^aOther histotypes include: lymphomatoid granulomatosis, pyothorax-associated lymphoma, senile EBV-associated B-cell lymphoproliferative disorders.

Abbreviations: EBV, Epstein-Barr virus; HHV-8, human herpesvirus 8.

Table 3. EBV-associated lymphoproliferative disorders

EBV-associated T/NK-cell lymphoproliferative disorders

Peripheral T-cell lymphoma, unspecified

Angioimmunoblastic T-cell lymphoma

Extranodal nasal T/NK-cell lymphoma

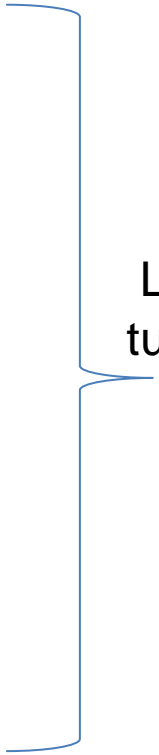
Other histotypes (rare)^a

^aOther histotypes include hepatosplenic T-cell lymphoma, nonhepatosplenic $\gamma\delta$ T-cell lymphomas, enteropathy-type T-cell lymphoma.

Abbreviations: EBV, Epstein-Barr virus; NK, natural killer.

Viral latencies and lymphomas

- Latency III (EBNAs + LMPs)
 - Lymphoblastoid cell lines
 - Post-transplant lymphoproliferative disorders
- Latency II (EBNA1 + LMPs)
 - Post-transplant lymphoproliferative disorders
 - Hodgkin's lymphoma
 - Peripheral T-cell lymphoma, unspecified
 - Extranodal nasal NK/T cell lymphoma
- Latency I (EBNA1)
 - Burkitt lymphomas
 - Primary Effusion Lymphoma



LMP1 expressing tumors with NF- κ B activation

When a tumour is associated with EBV, most tumor cells are EBV-infected

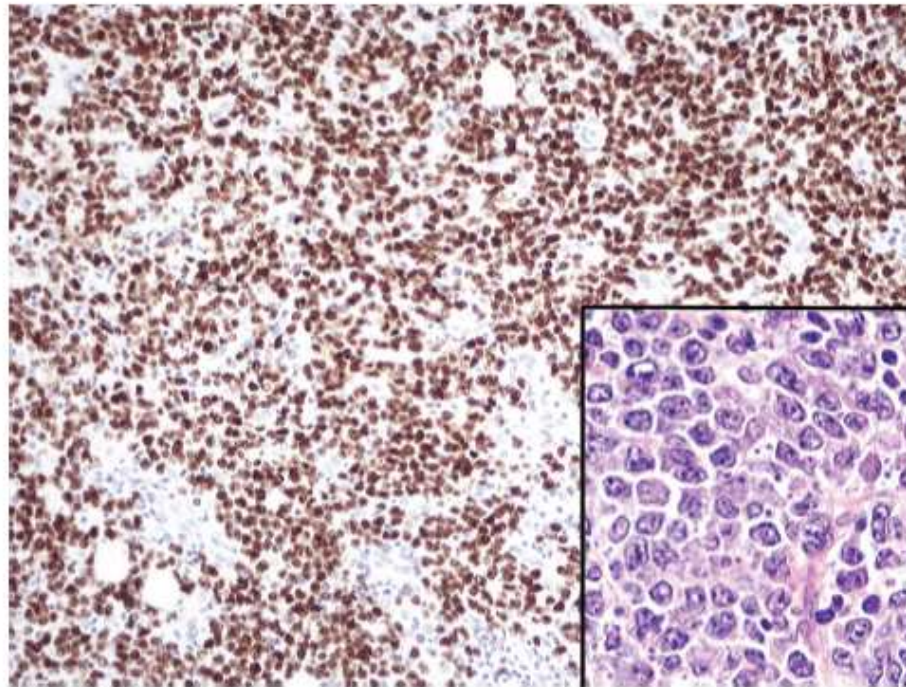


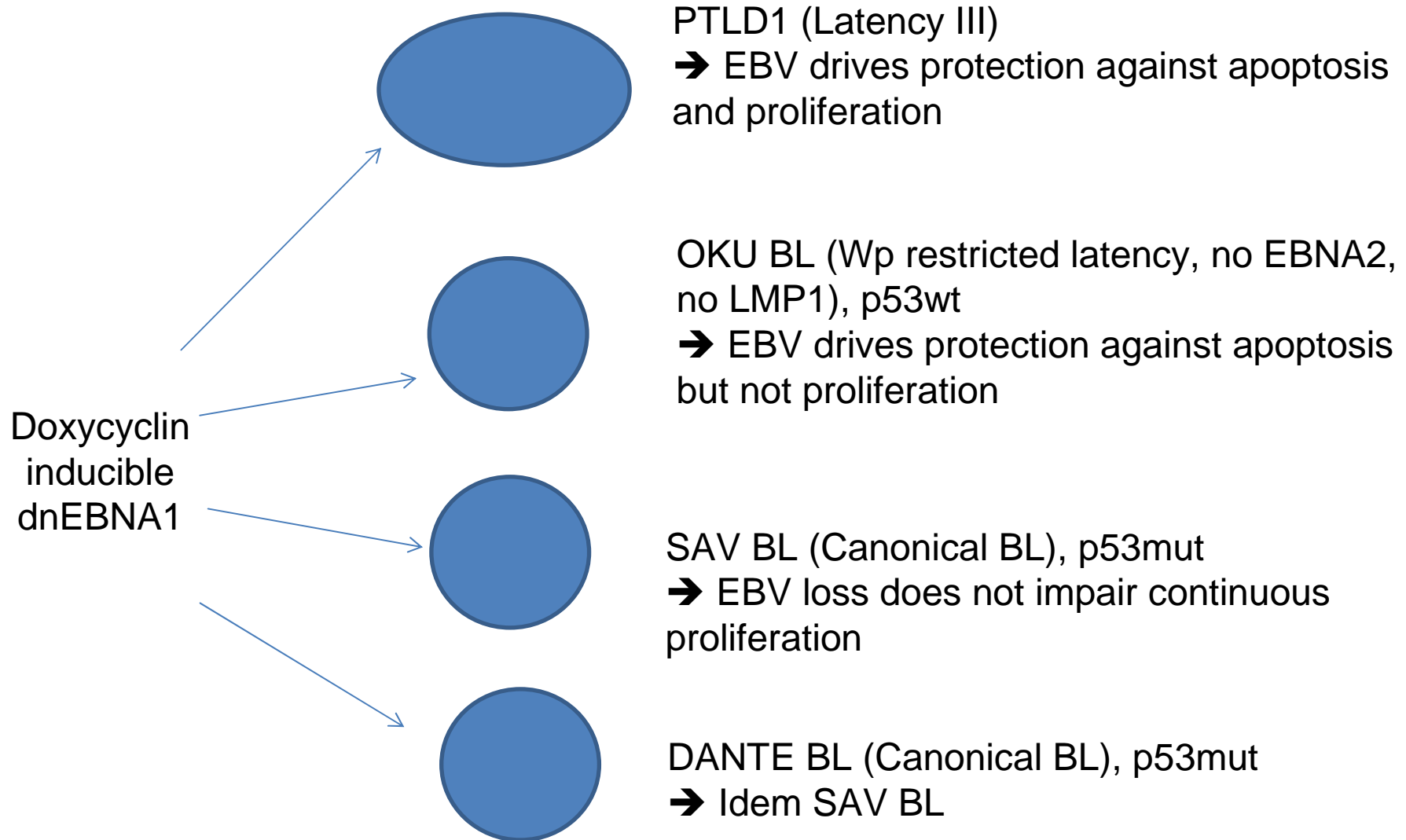
Figure 1. Burkitt's lymphoma. EBV is detected in almost all tumor cells. (**Inset**): Tumor consists of a homogeneous proliferation of medium-sized cells displaying a cohesive pattern. Tumor cells have round nuclei, multiple centrally located nucleoli, and limited cytoplasm. (EBER in situ hybridization, hematoxylin counterstain, original magnification $\times 200$; inset, hematoxylin-eosin, original magnification $\times 400$.)

Abbreviations: EBER, Epstein-Barr RNA; EBV, Epstein-Barr virus.

Has EBV a role in lymphomagenesis ?

- Asuka Nanbo, Arthur Sugden, and Bill Sugden. The coupling of synthesis and partitioning of EBV's plasmid replicon is revealed in live cells. *The EMBO Journal* (2007) 26, 4252–4262
- David Vereide and Bill Sugden, Proof for EBV's Sustaining Role in Burkitt's Lymphomas, *Semin Cancer Biol.* 2009 December ; 19(6): 389–393
- David T. Vereide and Bill Sugden, Lymphomas differ in their dependence on Epstein-Barr virus, *Blood* 2011 117: 1977-1985

David T. Vereide and Bill Sugden, Lymphomas differ in their dependence on Epstein-Barr virus, Blood 2011 117: 1977-1985



Interpretation

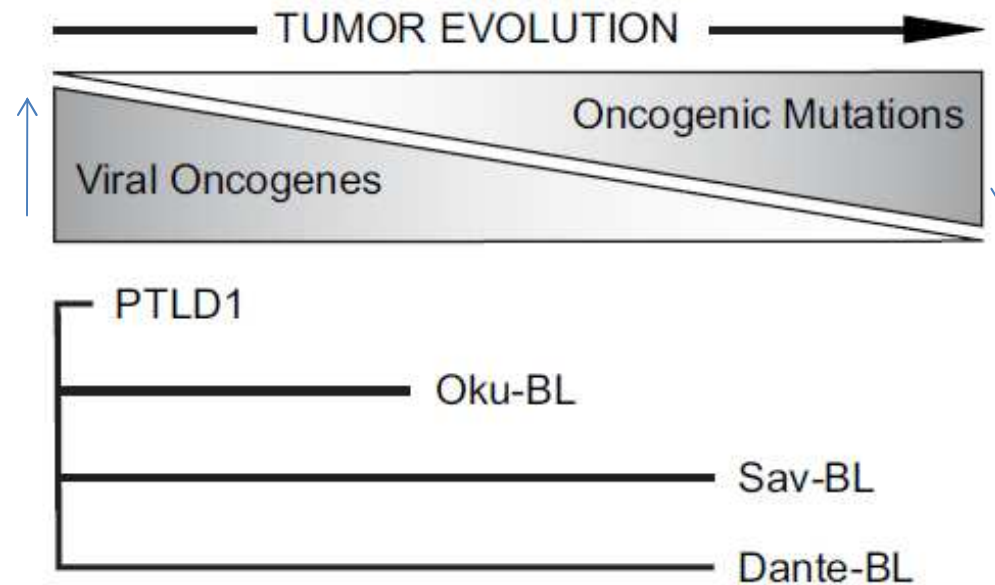
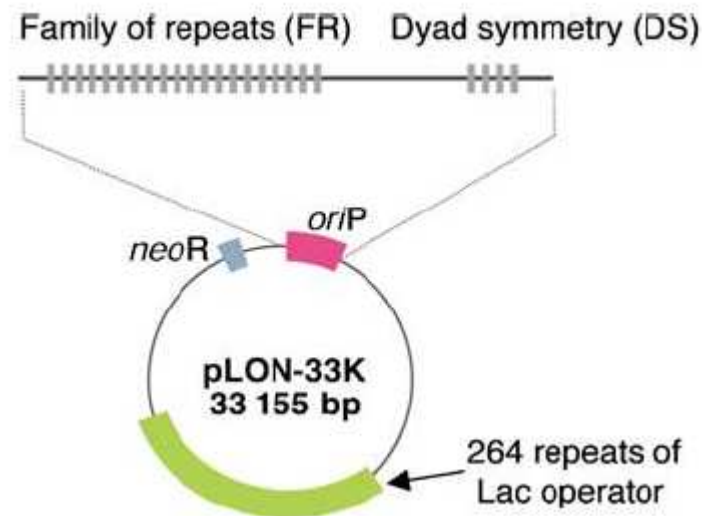


Figure 5. A hypothesis for EBV-induced lymphomagenesis. EBV transforms B lymphocytes, providing cells with much potentially oncogenic information. However, the viral genes these EBV-positive “proto” tumor cells express are immunogenic, placing the cells under strong negative selection by the immune system. In response, tumor cells evolve to express fewer viral genes by gaining cellular mutations that replace the functions of viral oncogenes. Different tumor cells express distinct sets of latent viral genes reflecting their in vivo evolution away from dependence on the virus and toward dependence on cellular mutations. The lengths of the lines for each tumor cell line reflect the hypothesized extent of this evolution.

David Vereide and Bill Sugden, Proof for EBV's Sustaining Role in Burkitt's Lymphomas, *Semin Cancer Biol.* 2009 December ; 19(6): 389–393

Aim: to follow the episome synthesis and partitioning



Nanbo, EMBO J 2007

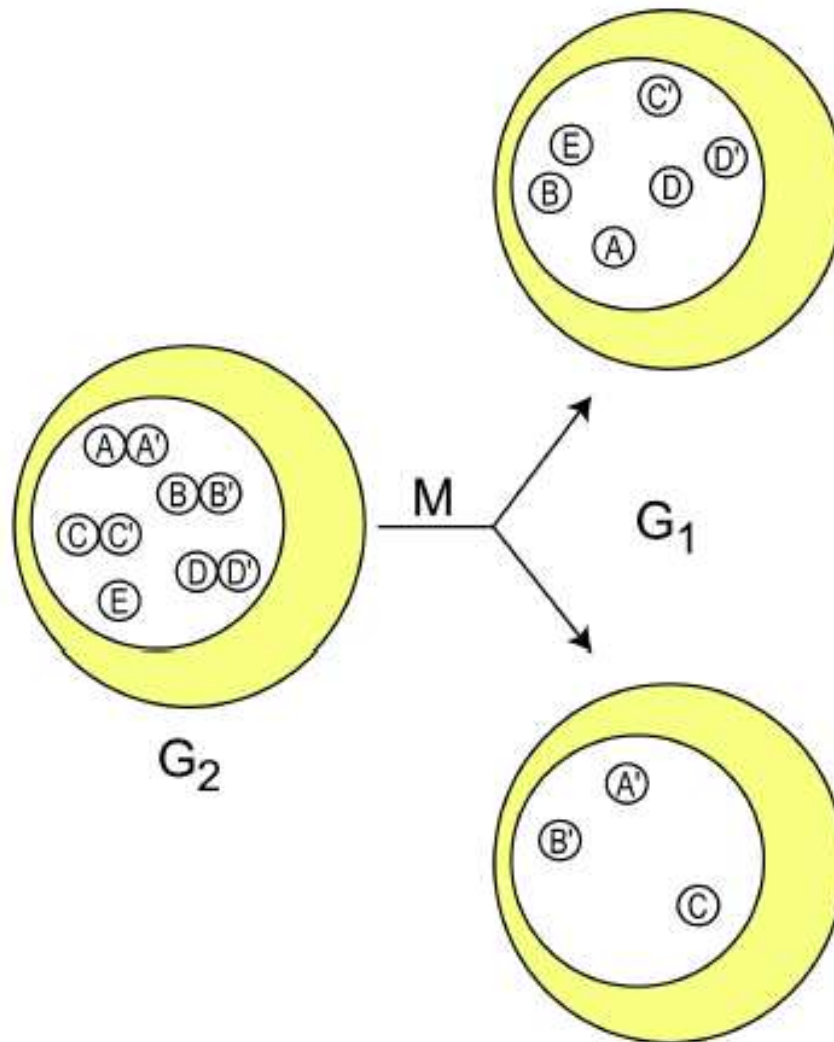
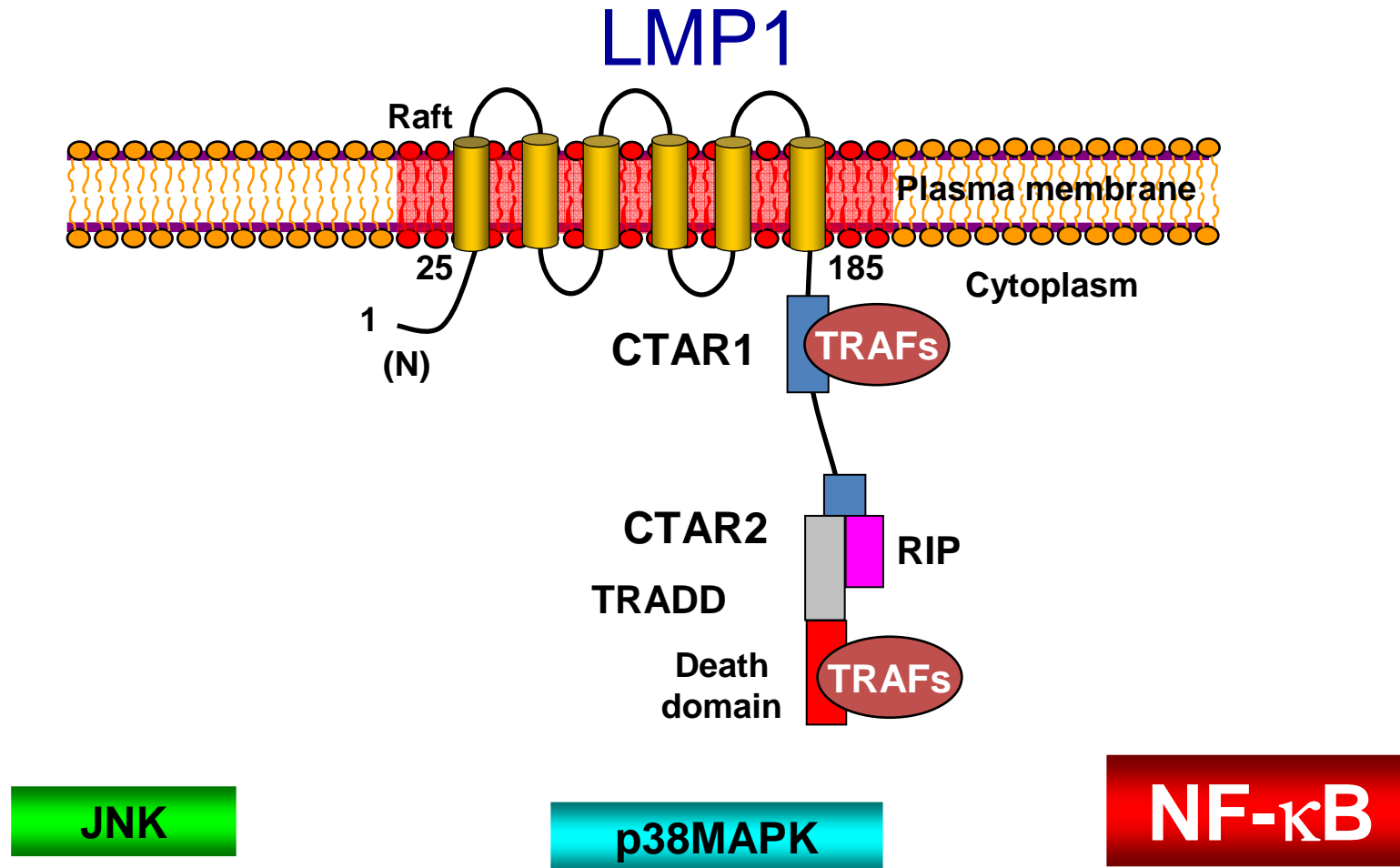


Figure 1. A model of the synthesis and partitioning of EBV plasmids
 Shown is one cell in the G₂ phase of the cell cycle with EBV plasmids identified with letters. Four plasmids were duplicated in the prior S phase to yield co-localized pairs of plasmids (AA', BB', CC', and DD'); one was not (E). Studies with EBV plasmids visualized in live cells have shown that 16% of the plasmids fail to be duplicated each S-phase [21]. These studies have also shown that during mitosis (M-phase) 88% of the co-localized plasmids are partitioned symmetrically. This partitioning is represented here as plasmids A and A', B and B', and C and C' each being distributed to separate daughter cells in G₁ phase. The 12% of the co-localized plasmids that do not partition symmetrically, represented by D and D', and the 16% of the plasmids that are not duplicated in S phase, represented by E, partition randomly. In the

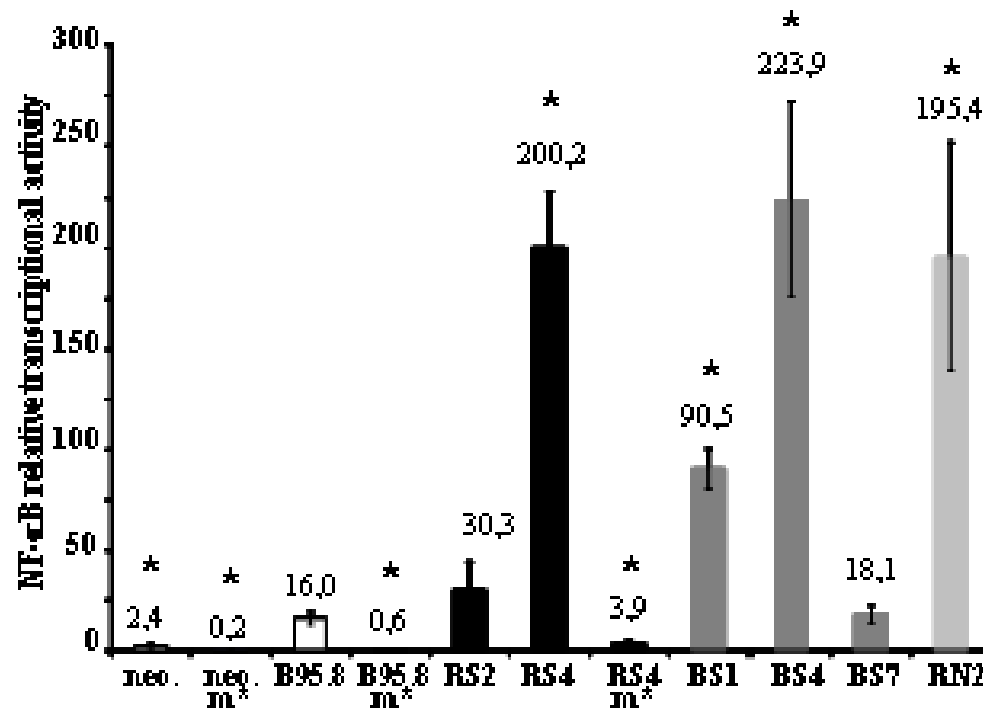
Results and interpretation

- In absence of selection
 - EBV-positive proliferating cells loses 8% of the viral genomes each cell cycle
 - after 8 cycles, only 50% of the viral DNA remain
 - after 50 cycles, only 1% of the viral DNA remain.
- Therefore,
 - When present in the cell, EBV is always useful (if not, it would be lost)
 - Whatever the EBV-associated cancer, if the EBV genome is present in tumor cells, it is because it has been positively selected (since all tumor cells are EBV positive, EBV confers an advantage).
 - During the life of an infected cell, EBV may be useful at the beginning (initial hit) and then not any more → theoretically, the EBV genome can be lost during lymphomagenesis.

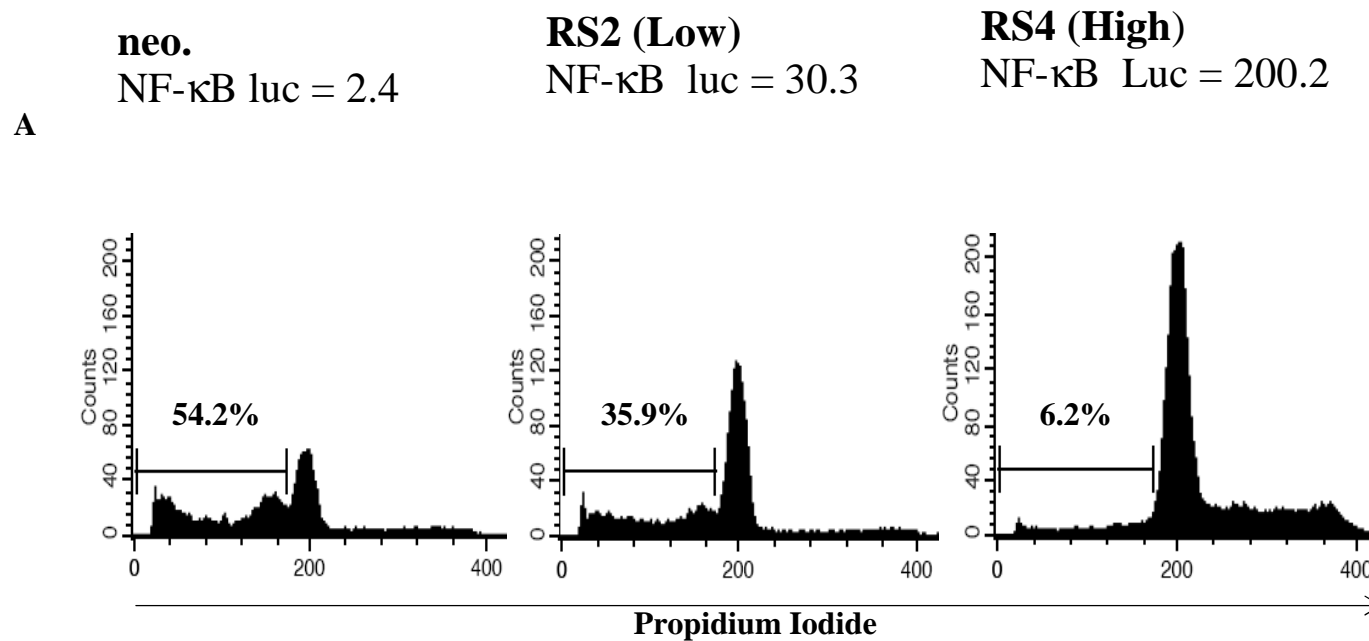
LMP1 (NF- κ B) is an ambivalent oncogene



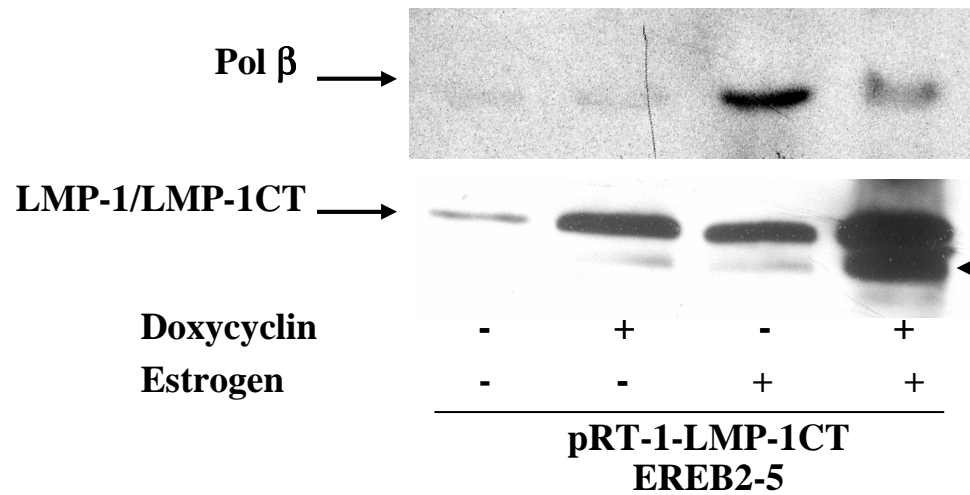
It exists a selection pressure to keep intact the ability of LMP1 to induce NF- κ B activation



The level of protection against apoptosis by LMP1 natural variants is related to the level of NF- κ B induction

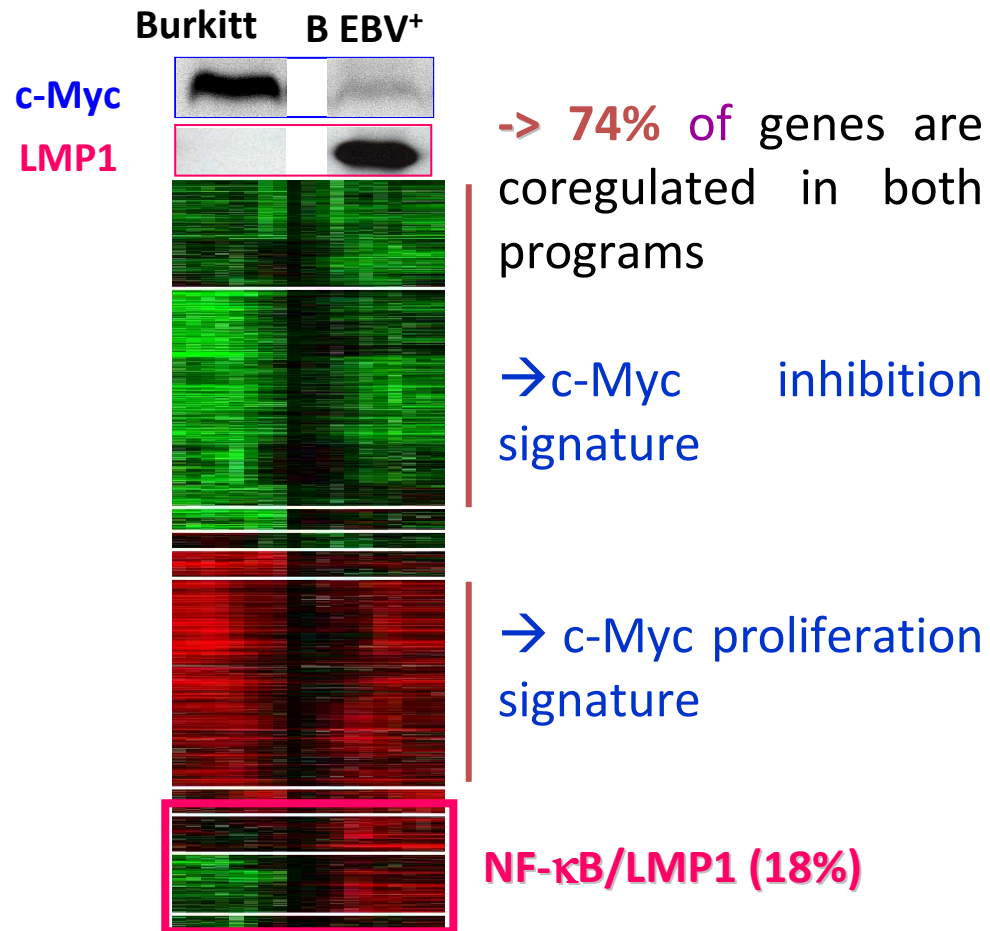


LMP1 induces expression of polymerase beta

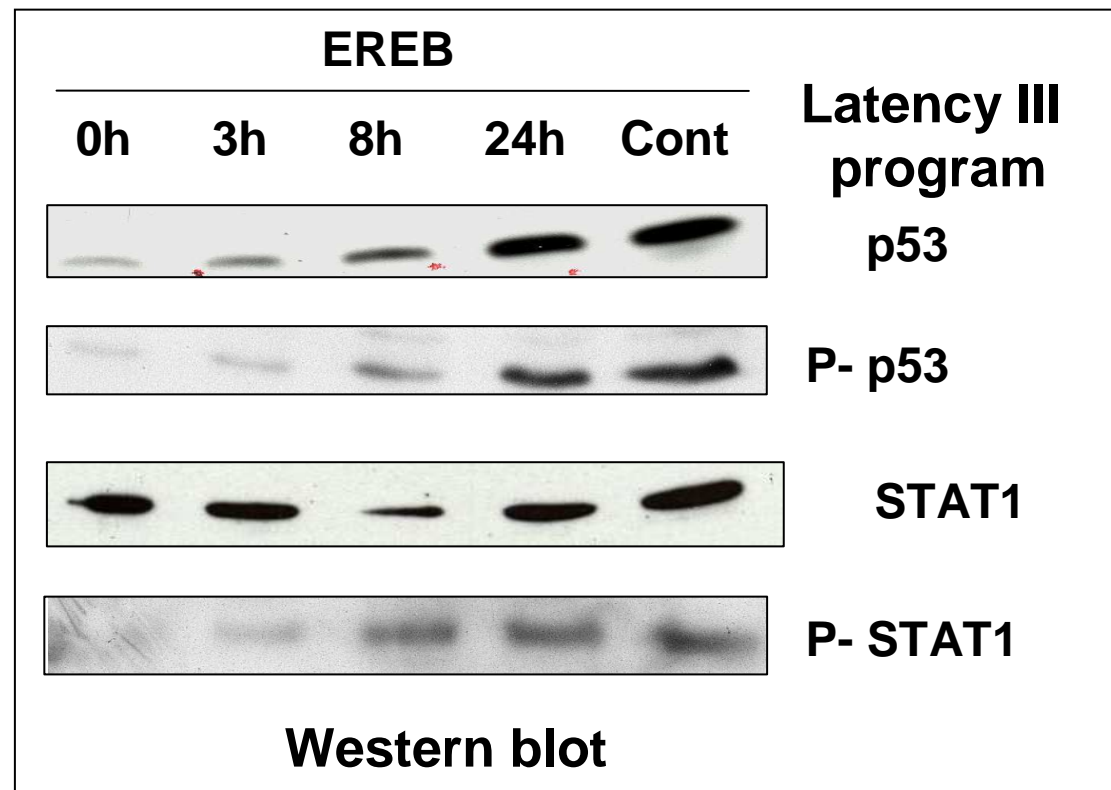
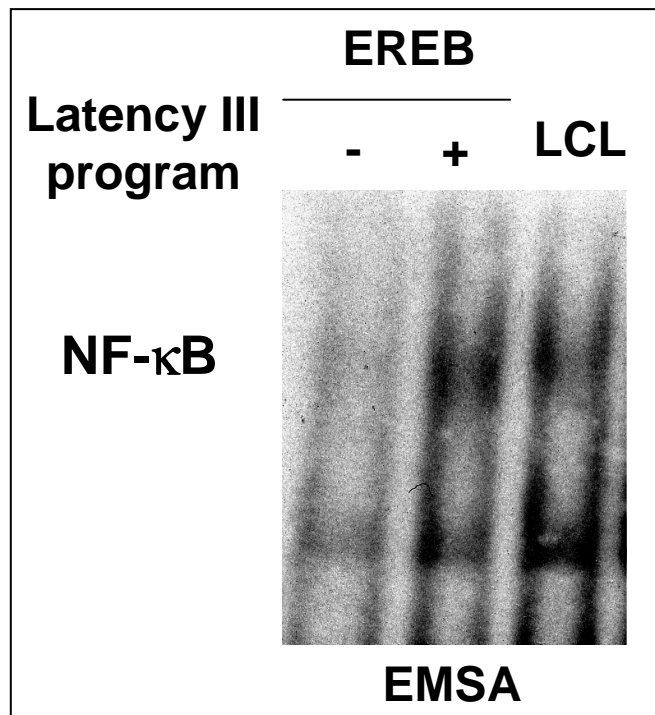


NF-κB and c-Myc are the master cellular transcriptional factors of EBV-latency III proliferating B-cells

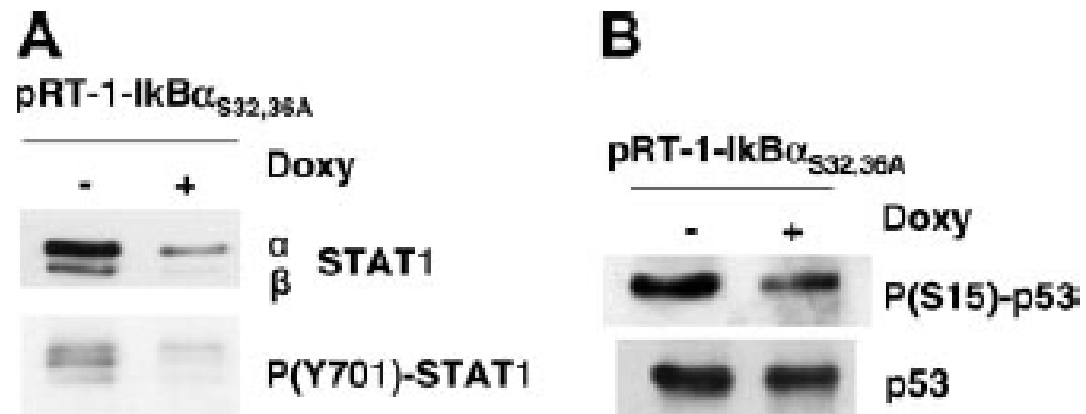
LymphoChip (12 000 gènes)



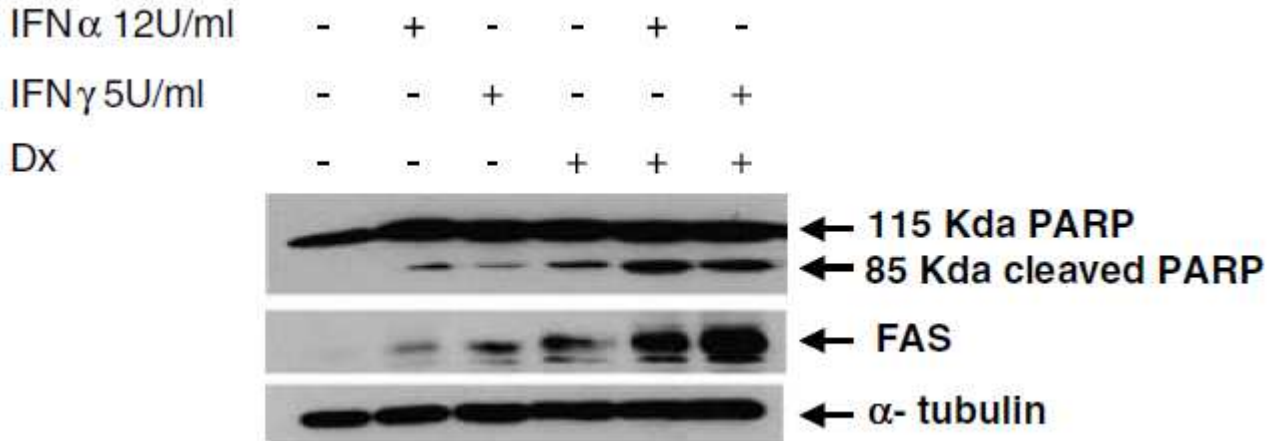
EBV latency III program not only induces NF- κ B and c-Myc



Induction of STAT1 and p53 is depending on NF- κ B

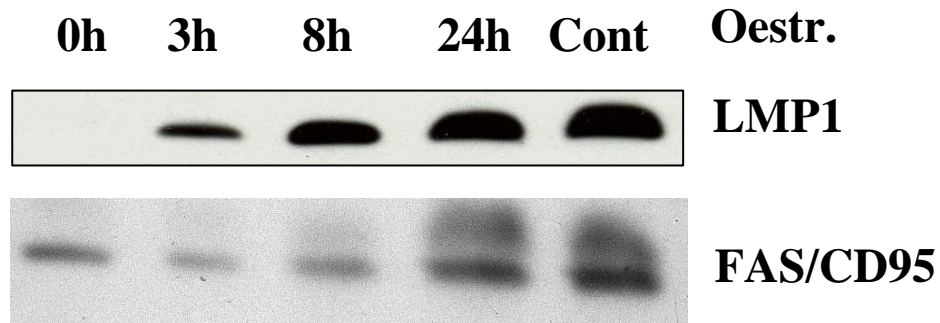


STAT1 and p53 cooperate in apoptosis induction



EBV latency III induces FAS that sensitizes EBV infected B-cells to T-cell killing

LCLs EREB 2-5



Control
BD29
BR17

+

-

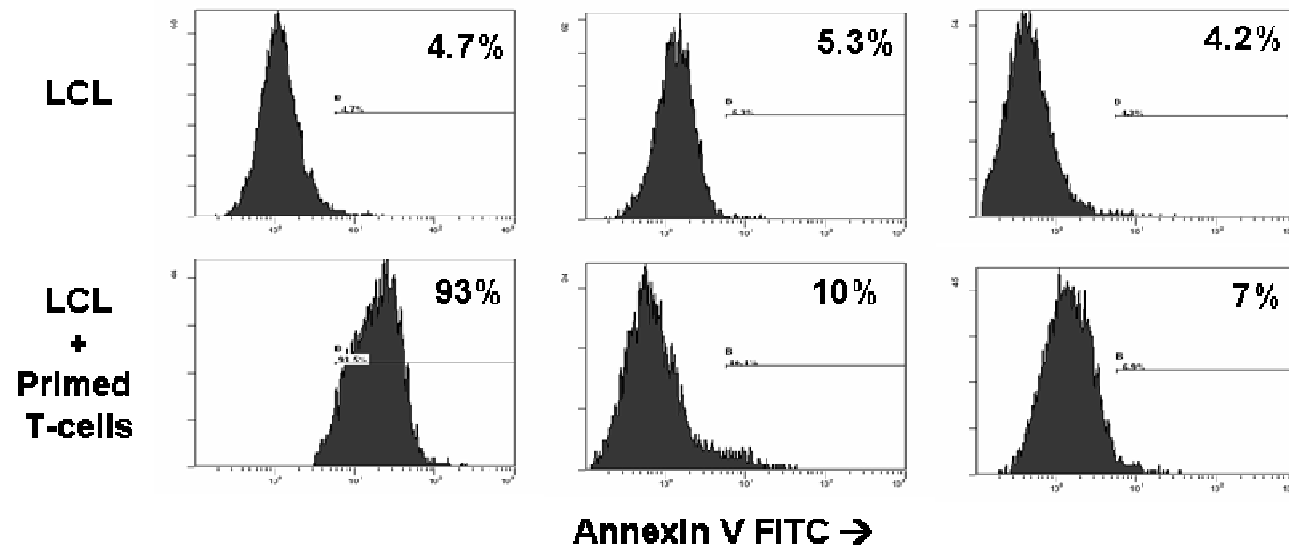
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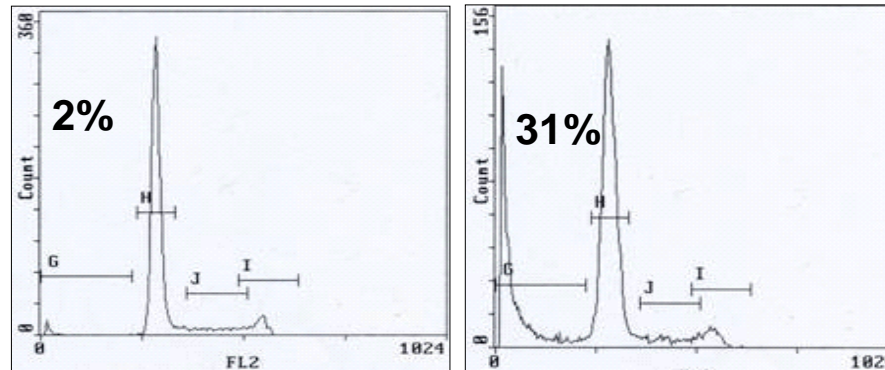


LMP1 is toxic for B-cells

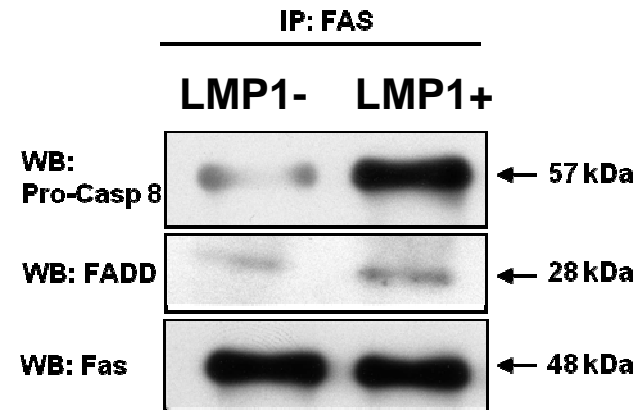
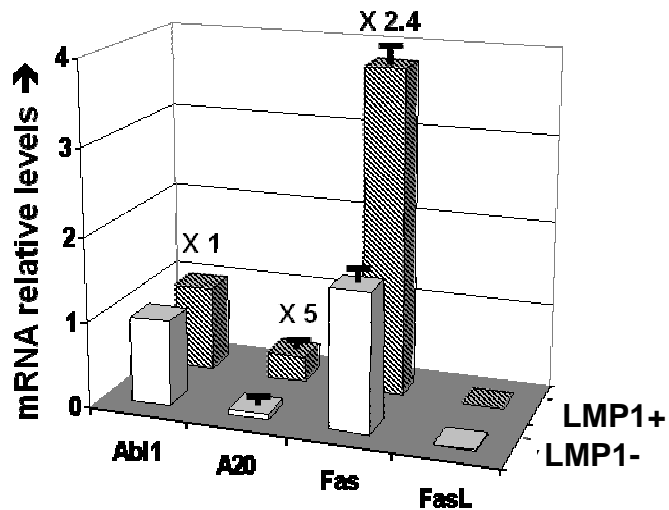
LMP1 induction

0

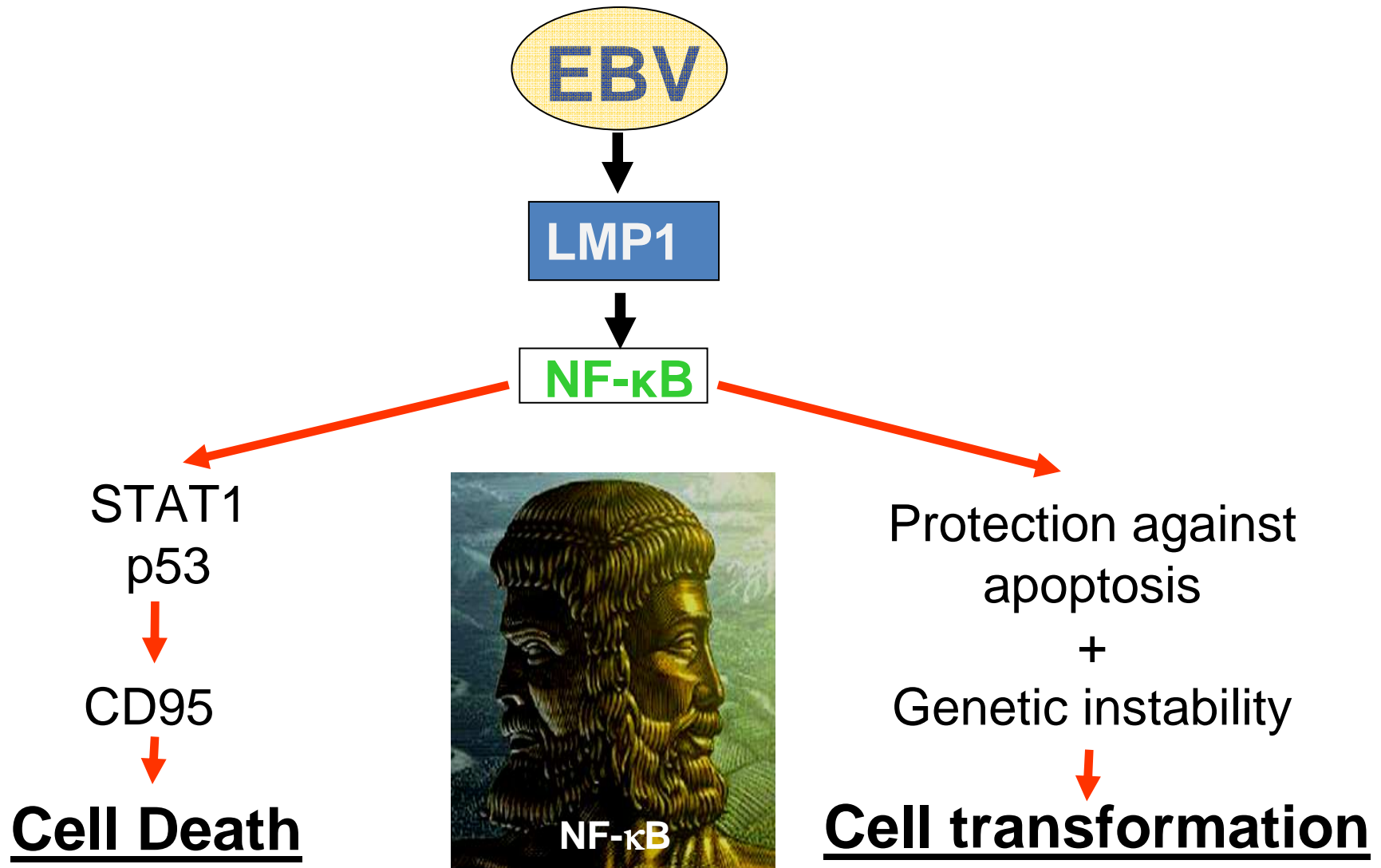
24h



ADN content →

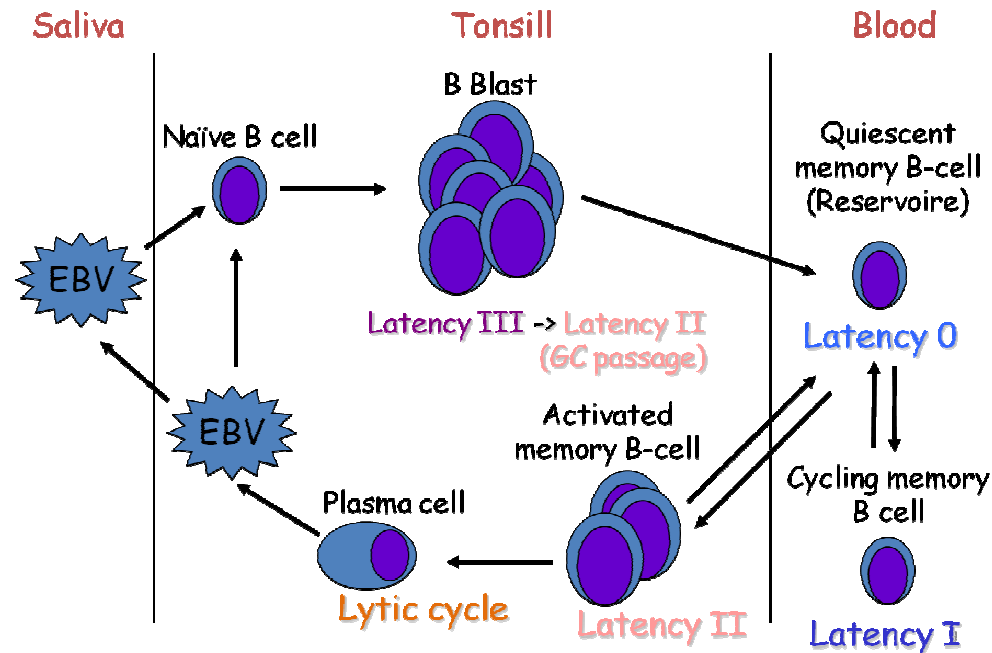


LMP1 (NF- κ B) is an ambivalent oncogene



Immunoregulatory role of LMP1 so that EBV is adapted to its host

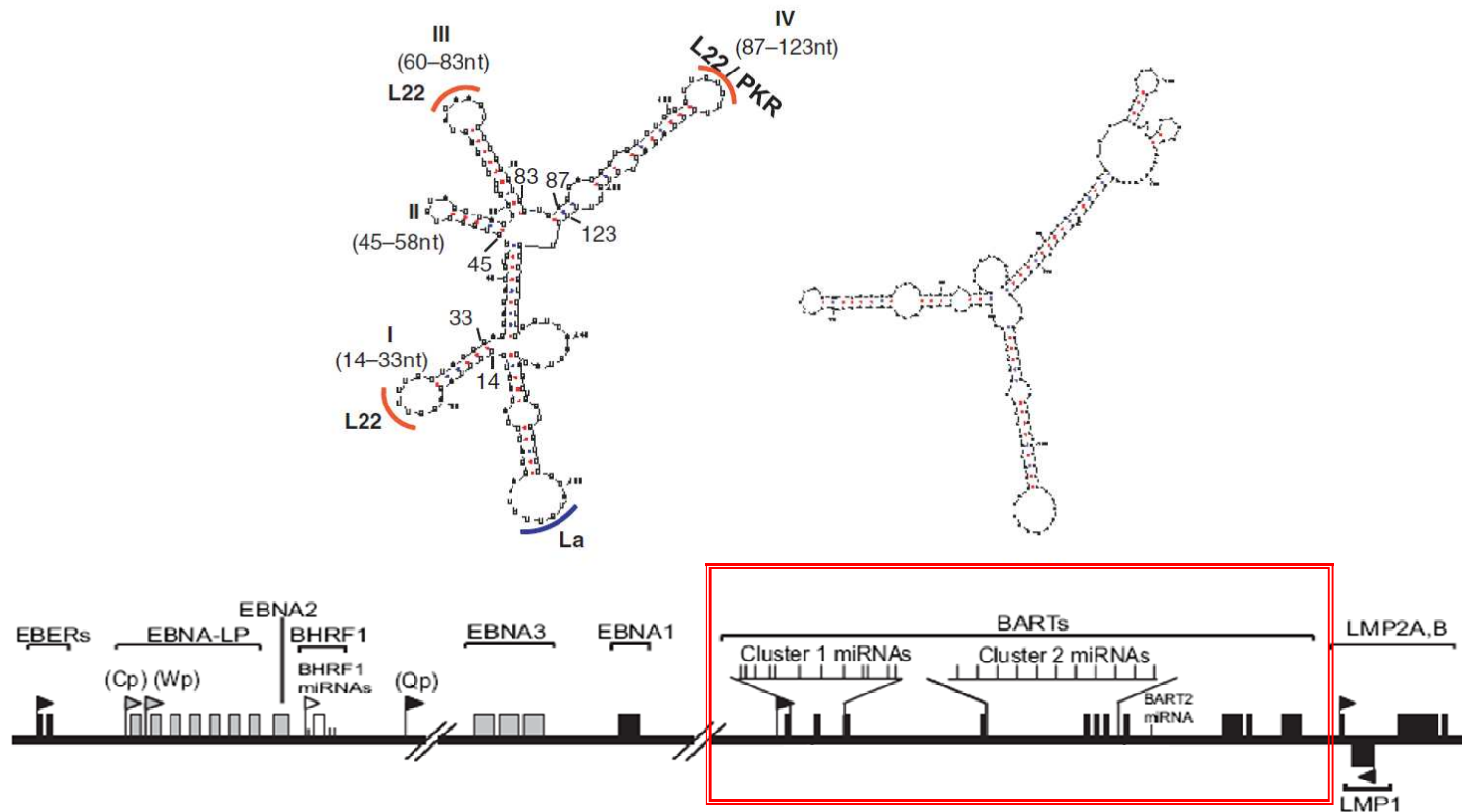
General conclusion



Frequency of EBV infected latency 0 B-cell in healthy adult = 10^{-6}

➔ Except for cells in latency 0, it is not a long term advantage for B-cells of the immunocompetent host to harbor the EBV genome (and thus to express LMP1).

Question : in immunocompetent humans, since only EBERS and BART RNAs are systematically expressed in EBV latency 0 infected B-cells, what is their role in the initial hit ?



Acknowledgements

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