Dipartimento di Biomedicina Comparata e Alimentazione

PTLD in primati non umani

21 Marzo 2013, Palazzo Marini Roma

Laura Cavicchioli DVM PhD

Università di Padova

PTLD (POST TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS)

gruppo eterogeneo di patologie che possono manifestarsi nei riceventi di trapianto di organi solidi (SOT) e di cellule emopoietiche

-**Insorgenza**: a parte i pazienti in età pediatrica, PTLD compaiono nella fase tardiva post TX (36-40 mesi post-SOT)

-Il **rischio** di linfoma è aumentato del 20-120% rispetto alla media della popolazione e in parte è legato all'immunosoppressione (IS)

-La maggior parte dei PTLD derivano dalle cellule B, il 10-15% dalle cellule T, raramente sono Linfomi Hodgkin

-Lo scopo della **terapia**: curare il PTLD preservando la funzione dell'organo trapiantato:

-riduzione IS

-rituximab, chemioterapia, **terapia antivirale** etc…

Jagadeesh et al Curr Treat Options Oncol. 2012 Mar;13(1):122-36

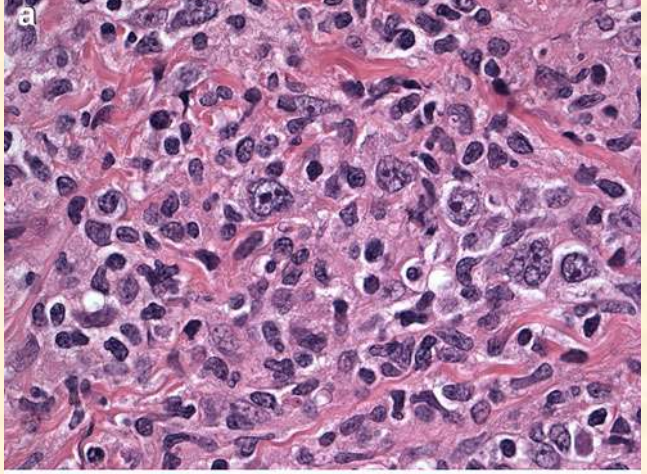
Classificazione (criteri morfologici, immunofenotipici, e molecolari)

Table 1. WHO classification of PTLD (2008)

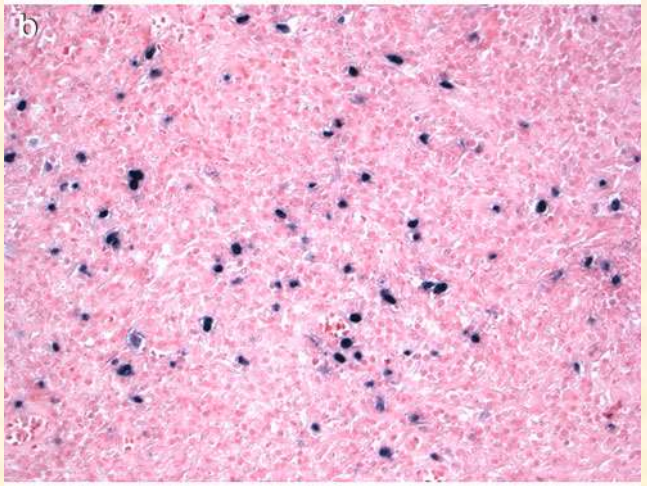
Category	Morphology and immunophenotype	Genetics
Early lesions[a]	Architecture intact; admixture of small polyclonal B- and T-cell immunoblasts and plasma cells; typically EBV+	Clonality not typical; small clonal populations may be present
Plasmacytic hyperplasia		
IM-like lesions		
Polymorphic PTLD	Architecture effaced; full spectrum of lymphoid maturation (immunoblasts, plasma cells and small to medium B-cell and T-cell lymphocytes); often EBV+	Typically with clonal Ig genes; non-clonal T-cells
Monomorphic PTLD[b]	Architecture effaced; fulfills classic WHO criteria for plasma cell neoplasm or NHL (other than indolent subtypes); phenotype dependent on subtype of NHL; EBV variably positive	Clonal B-cells and/or clonal T-cells present in virtually all cases
B-cell neoplasms		
DLBCL		
Burkitt lymphoma		
Plasma cell myeloma		
Plasmacytoma-like lesion		
Other subtypes[c]		
T-cell/NK neoplasms		
PTCL NOS		
Hepatosplenic		
Other subtypes		
Classic Hodgkin lymphoma-type PTLD	Architecture effaced; fulfills same criteria as WHO classical HL	Clonality not typical

Jagadeesh et al Curr Treat Options Oncol. 2012 Mar;13(1):122-36

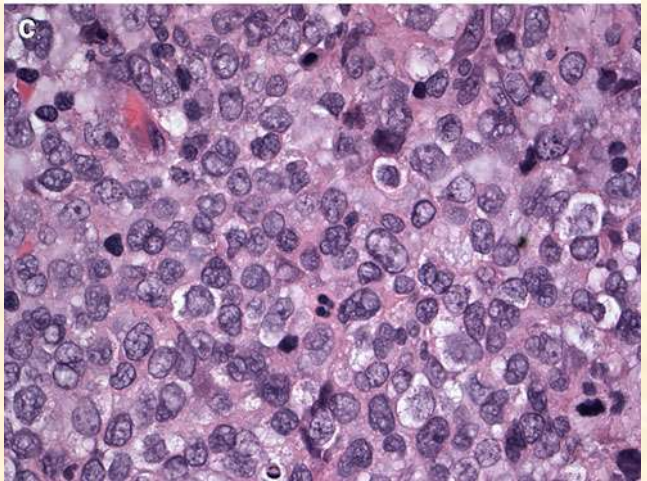
PTLD polimorfo: infiltrato composto da popolazione cellulare polimorfa (immunoblasti, istiociti, linfociti e plasmacellule). (HE 60X)



ISH per EBV virus sonda EBER: presenza di positività nucleare disseminata (20X)



PTLD monomorfo: tappeto di cellule linfoidi (diffuse large B-cell lymphoma basato su positività per CD20). (HE 60X)



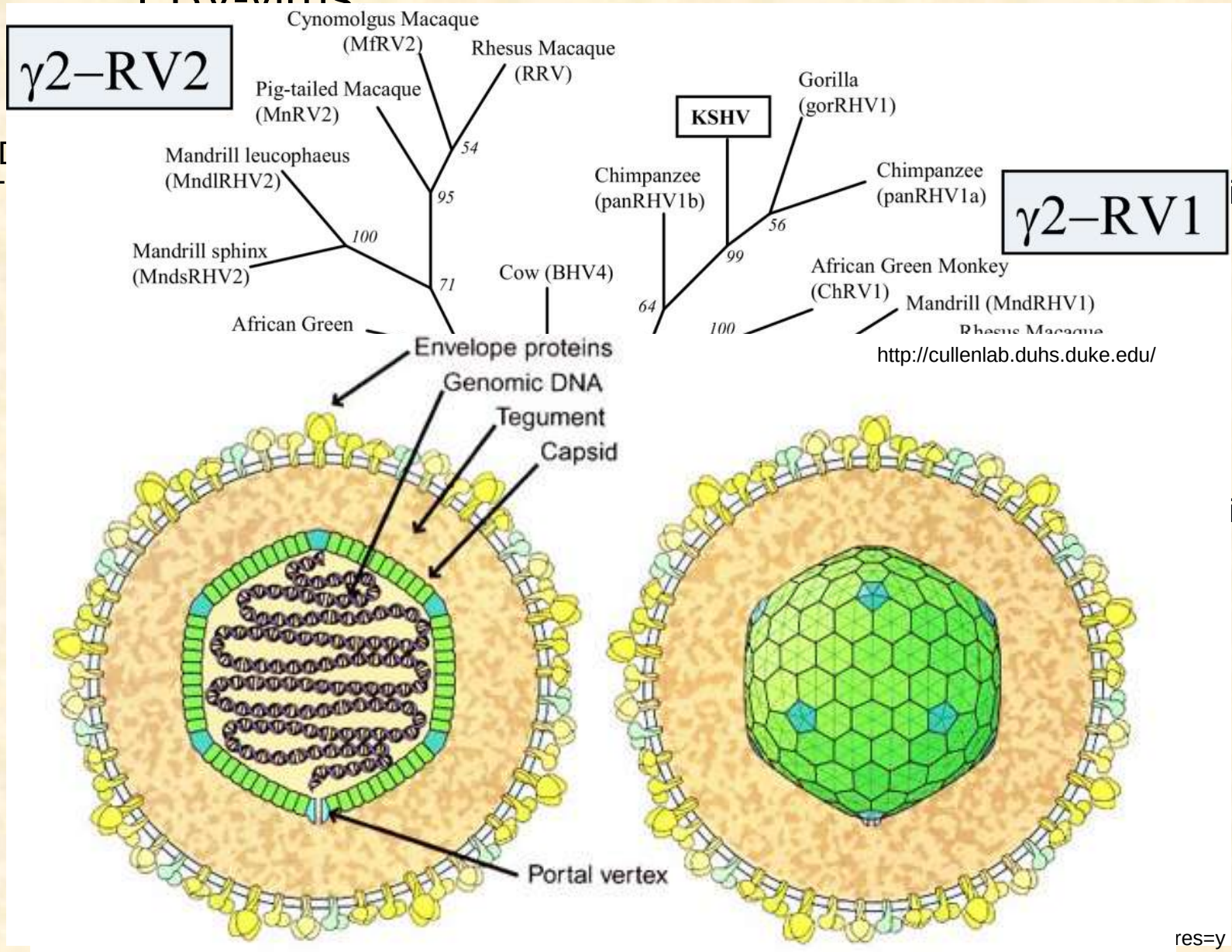
Evens et al Curr Oncol Rep. 2010 Nov;12(6):383-94.

EBV-virus

PTLD opo

SOT ici.

irus



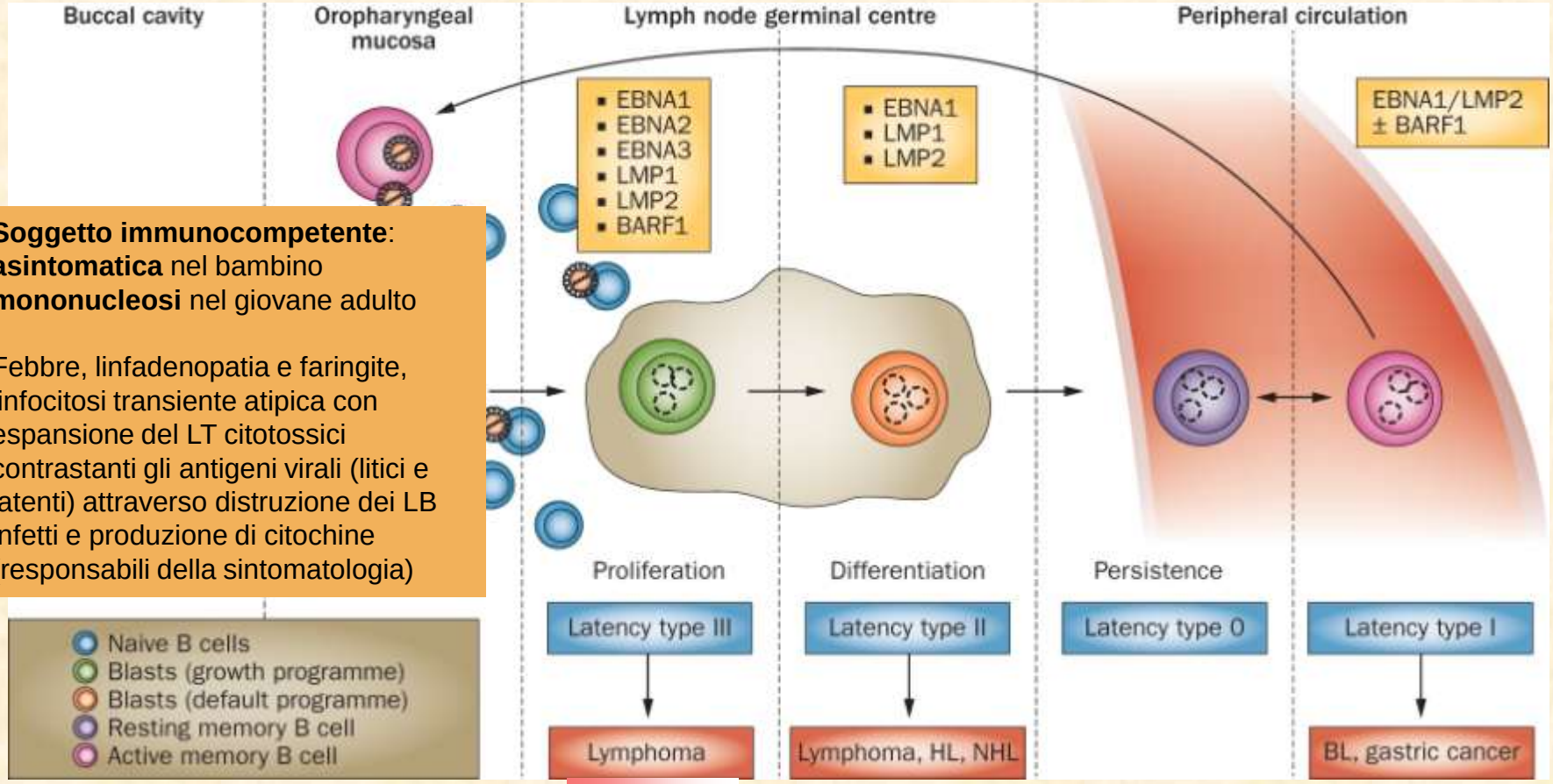
http://cullenlab.duhs.duke.edu/

res=y

Evens et al Curr Oncol Rep. 2010 Nov;12(6):383-94.

Jagadeesh et al Curr Treat Options Oncol. 2012 Mar;13(1):122-36

Virus EBV (HHV-4): ciclo, latenza e linfoma



Soggetto immunocompetente: **asintomatica** nel bambino **mononucleosi** nel giovane adulto

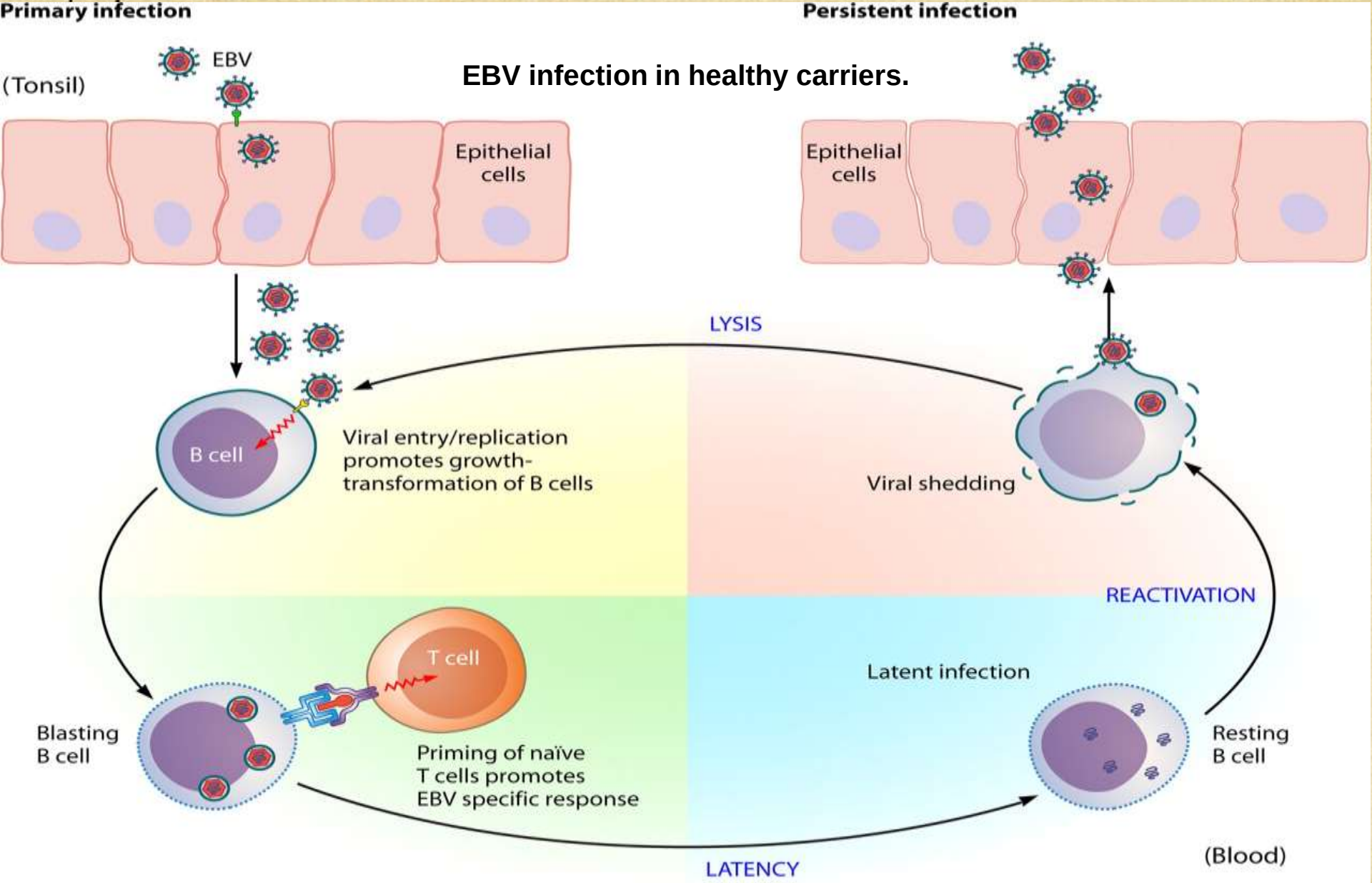
Febbre, linfadenopatia e faringite, linfocitosi transiente atipica con espansione del LT citotossici contrastanti gli antigeni virali (litici e latenti) attraverso distruzione dei LB infetti e produzione di citochine (responsabili della sintomatologia)

(PTLD)

Bollard, C. M. *et al.* (2012) T-cell therapy in the treatment of PTLD
Nat. Rev. Clin. Oncol

nature REVIEWS CLINICAL ONCOLOGY

EBV infection in healthy carriers.



Odumade O A et al. Clin. Microbiol. Rev. 2011;24:193-209

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Lymphocryptovirus dei primati

Rhesus Lymphocryptovirus (RhLCV) repertorio genetico quasi identico a EBV.
I neonati spesso sieropositivi per anticorpi materni fino a 4-6 mesi.
La sieroconversione in colonia avviene tra 6-12 mesi (>80%)

INDIVIDUO IMMUNOCOMPETENTE:
-Infezione primaria spesso asintomatica
-Linfocitosi atipica, linfoadenopatia periferica

INDIVIDUO IMMUNODEPRESSO:

-Linfoma non-Hodgkin (Macaca fascicularis SIV+) (Blaschke J Med Virol 2001, Marr-Belvin Vet Pathol 2008)
-Leucoplachia orale in SIV+ (Carville-Mansfield Comp Med 2008)
-Linfoma simil-Mycosis fungoides in Macaca nemestrina (Carville-Mansfield Comp Med 2008)
-**PTLD**

PTLD nel primate trapiantato



0041-1337/02/7309-1431/0
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POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER

0041-1337/02/7301-44/0
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Vol. 73, 44–52, No. 1, January 15, 2002
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J Med Primatol 2001: 30: 88–93
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JOURNAL OF MEDICAL PRIMATOLOGY
ISSN 0047-2565

Ultrasound detection of non-Hodgkin's lymphoma in three cynomolgus monkeys after renal transplantation and cyclosporine immunosuppression

Lorrie Gaschen,
Henk-Jan Schuurman

Prolonged Survival of Composite Facial Allografts in Non-Human Primates Associated With Posttransplant Lymphoproliferative Disorder

Rolf N. Barth,[1,6] Arthur J. Nam,[2] Matthew G. Stanwix,[2] Debra Kukuruga,[3] Cinthia I. Drachenberg,[4] Rachel Bluebond-Langner,[2] Helen Hui-Chou,[2] Steven T. Shipley,[5] Stephen T. Bartlett,[1] and Eduardo D. Rodriguez[2]

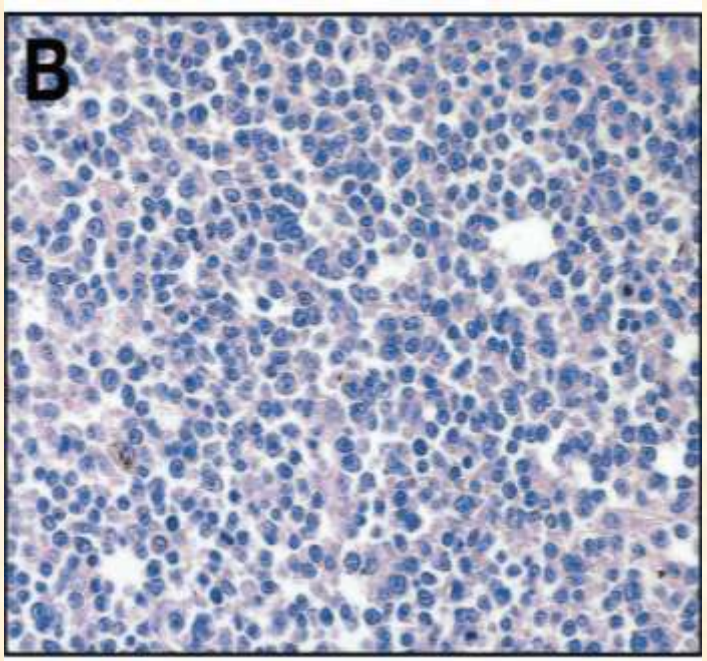
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POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER ASSOCIATED WITH AN EPSTEIN-BARR-RELATED VIRUS IN CYNOMOLGUS MONKEYS[1]

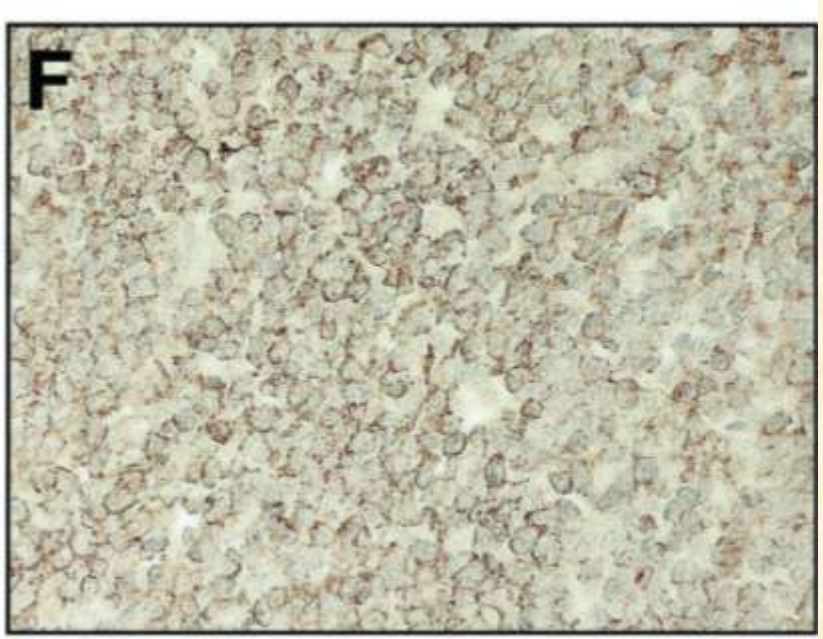
JAN SCHMIDTKO,[2] RUOJIE WANG,[2] CHIN-LEE WU,[2] SHAMILA MAUIYYEDI,[2] NANCY L. HARRIS,[2] PATRICIA DELLA PELLE,[2] NICOLE BROUSAIDES,[2] LUBA ZAGACHIN,[2] JUDITH A. FERRY,[2] FRED WANG,[4] TATSUO KAWAI,[3] DAVID H. SACHS,[3] A. BENEDICT COSIMI,[3] AND ROBERT B. COLVIN[2,5]

160 *Macaca fascicularis* sottoposti a trapianto di rene
-**IS**: irradiazione midollo osseo, deplezione linfociti T e CsA
-Casi PTLD 5,6% (9/160) da minimamente monomorfi a polimorfi
-Sopravvivenza con PTLD: 28-103 gg post TX
-Caratterizzazione: CD20+, IHC focalmente positivi per EBNA2 e ISH EBER+
-Localizzazione: linfonodi, fegato, milza, polmone, cuore

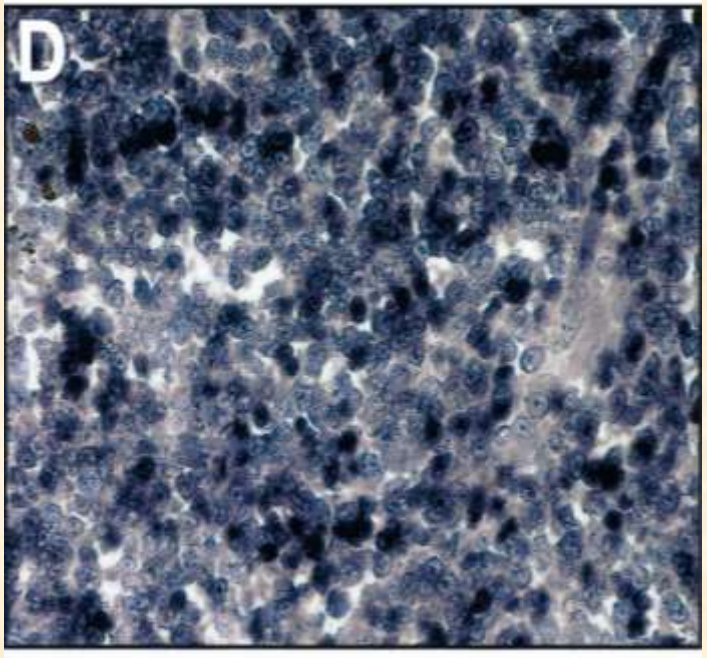




Linfonodo con **PTLD** diffuso **polimorfo** (HE 400X)



Linfonodo con PTLD: diffusa espressione **CD20** nelle cellule tumorali (IHC, 400)



Linfonodo con PTLD: segnale forte (nero) nelle cellule tumorali per EBER (ISH EBER 500X)

Schmidtko et al, 2002

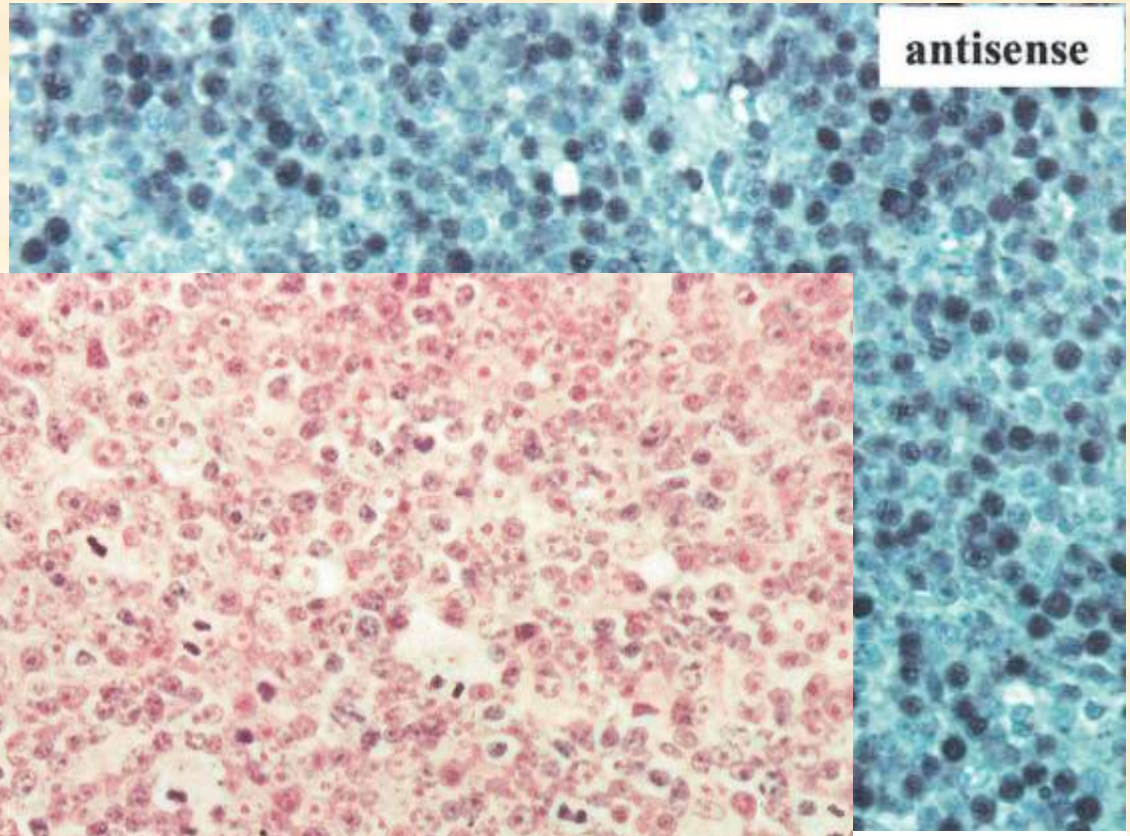
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TRANSPLANTATION
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POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER ASSOCIATED WITH PRIMATE GAMMA-HERPESVIRUS IN CYNOMOLGUS MONKEYS USED IN PIG-TO-PRIMATE RENAL XENOTRANSPLANTATION AND PRIMATE RENAL ALLOTRANSPLANTATION

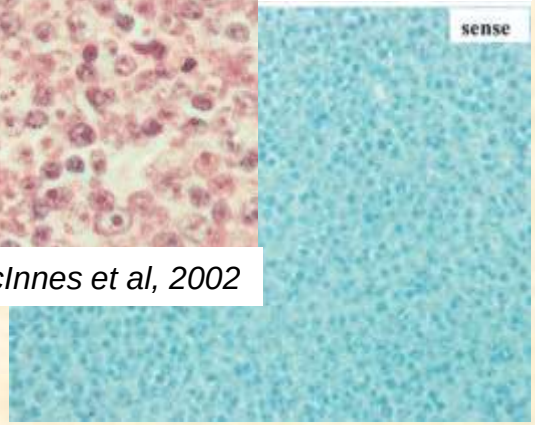
ELIZABETH F. MCINNES,[1] RUTH F. JARRETT,[2] GILLIAN LANGFORD,[1] CARL ATKINSON,[3] JO HORSLEY,[3] MARTIN J. GODDARD,[3] EMANUELE COZZI,[1] AND HENK-JAN SCHUURMAN[1,4]

-*Macaca fascicularis* tx di rene: 245 xenotx e 231 allotx
-**IS**: diversi farmaci (CsA, immunotossina CD3, steroidi, derivati della rapimicina, MMS)
-Sopravvivenza media con PTLD: xenotx 40 gg, allotx 104 gg
-PTLD Xenotx (4%): 4/10 iperplasia linfoide + 6/10 linfomi
-PTLD Allotx (3,8%): 9/9 linfomi
-Caratterizzazione: CD20+ e ISH EBER+
-Localizzazione Xenotx: tratto GI e linfonodi
Allotx: linfonodi, fegato, MALT

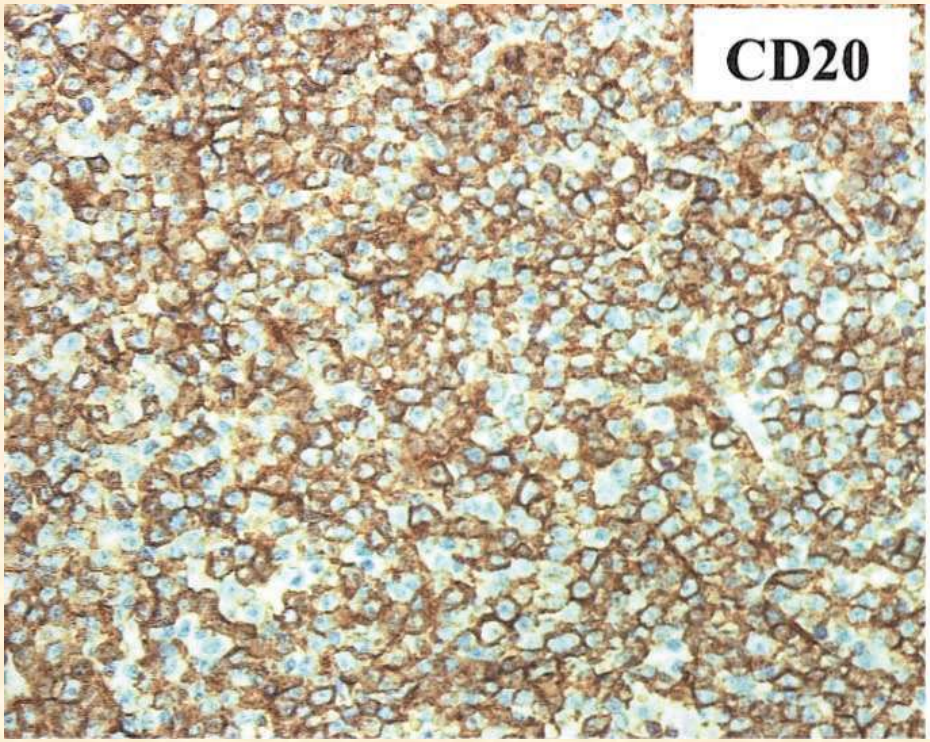


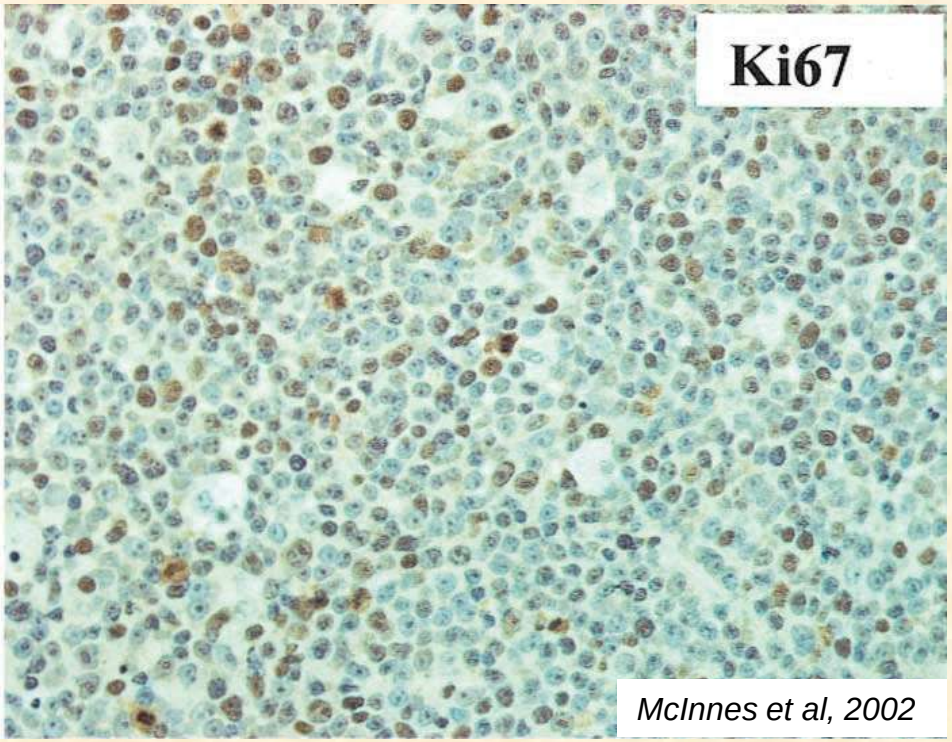


cInnes et al, 2002



McInnes et al, 2002


CD20


Ki67
McInnes et al, 2002

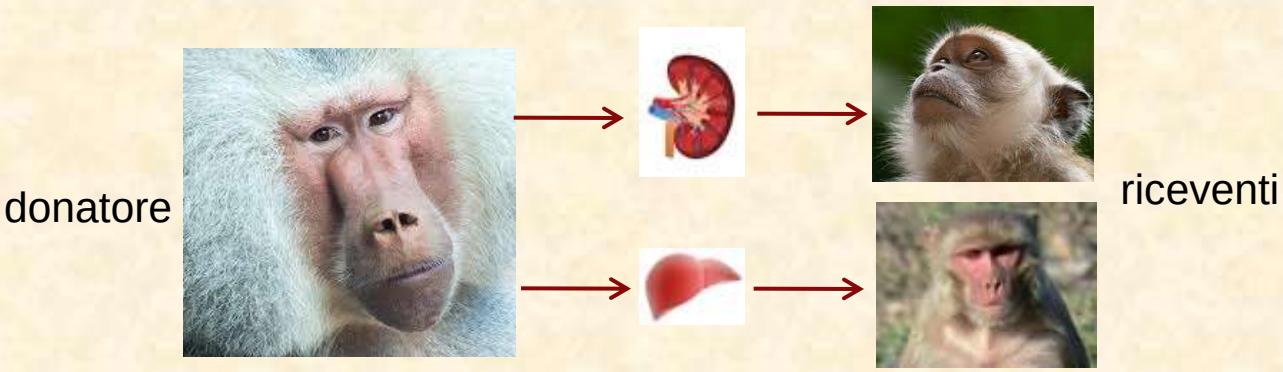
Xenotransplantation 2003: 10: 398–409
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XENOTRANSPLANTATION
ISSN 0908-665X

The long-term survival of baboon-to-monkey kidney and liver xenografts*

Zhong R, Tucker J, Zhang Z, Wall W, Grant D, Quan D, Garcia B, Gao Z, Asfar S, Sharpe M, Gelb A, Bailey M, Stiller



-**IS** baseline: CsA e MPS poi 3 gruppi con CyP, MMF, CyP+Rap
A dosi elevate di farmaci IS e per periodi prolungati (>6mo)
sviluppo di PTLD in tutti gli animali (**100%**)

-Caratterizzazione: linfomi B-cell

-Localizzazione: masse extranodali, spesso multiviscerali, anche organo tx

Transplantation 88 (11), 2009

Prolonged Survival of Composite Facial Allografts in Non-Human Primates Associated With Posttransplant Lymphoproliferative Disorder

Rolf N. Barth,[1,6] Arthur J. Nam,[2] Matthew G. Stanwix,[2] Debra Kukuruga,[3] Cinthia I. Drachenberg,[4] Rachel Bluebond-Langner,[2] Helen Hui-Chou,[2] Steven T. Shipley,[5] Stephen T. Bartlett,[1] and Eduardo D. Rodriguez[2]

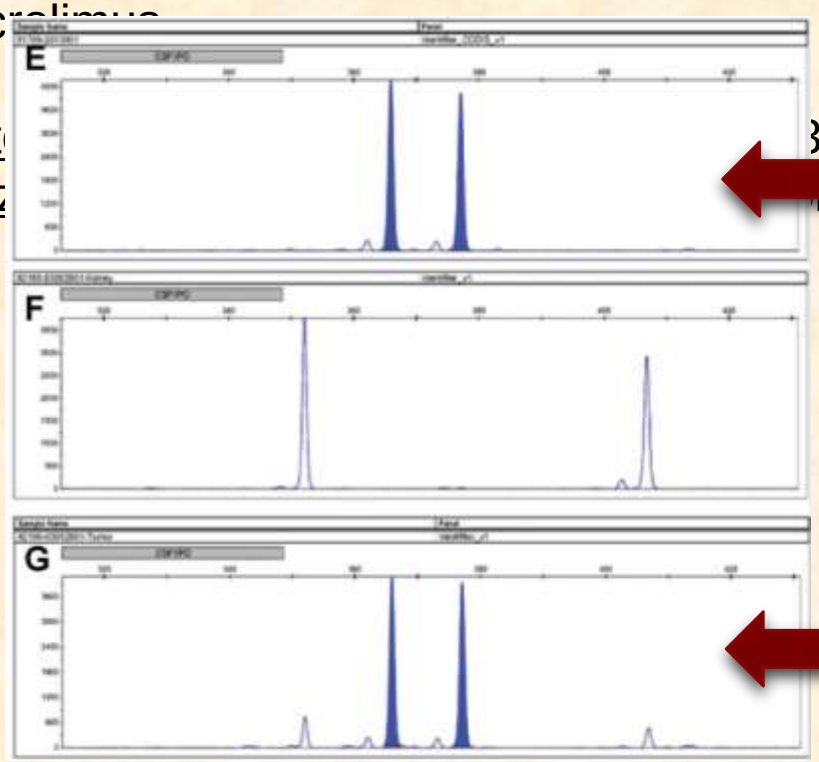
-16 *Macaca fascicularis* allotrapianto facciale composito (cute, muscolo, osso)

-**IS**: tac

-PTLD

-Caratt 3- CD20+, 1 CD3+

-Localiz



Short-tandem repeats (STR) del donatore (E), del ricevente (F), del PTLD (G): "**consistency with donor origin in 87% of cells calculated by the height of STR peaks**"



Progetto EU

28 *Macaca fascicularis* riceventi precursori neuronali da suini wyld-type o transgenici per hCTLA4Ig per trattare una forma di malattia di Parkinson indotta farmacologicamente (MPTP)
IS: CsA, MPS, MMF

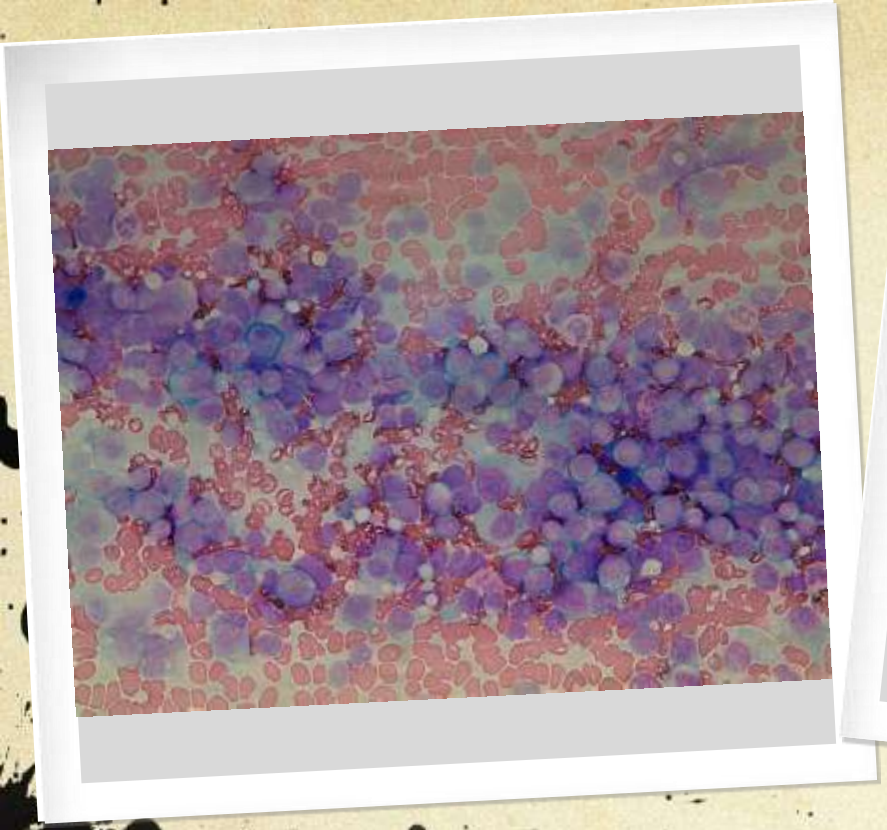
PTLD in 9 primati: 2 maschi e 7 femmine, età media 7 anni.

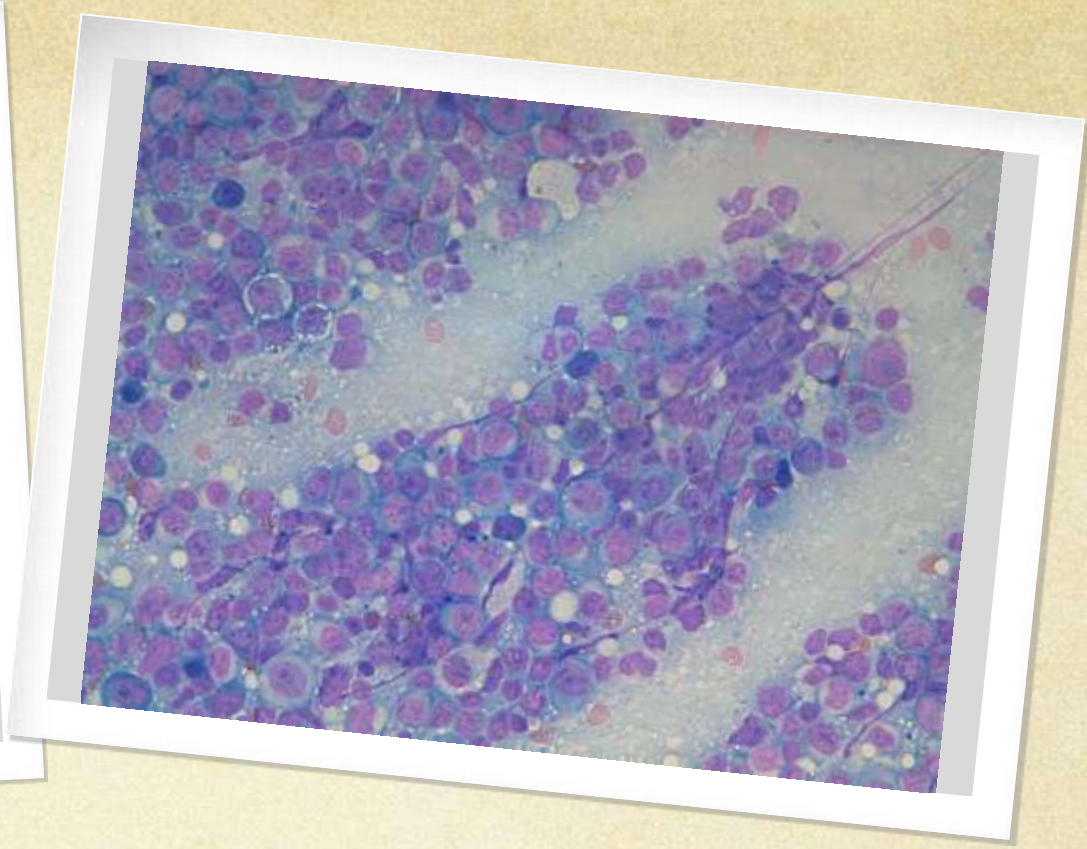
Tempo medio di insorgenza del sintomo di PTLD 172,7 giorni post tx (range 110-287) e tempo medio di sopravvivenza post tx 192,4 giorni (range 117-330).

Localizzazione: cavità nasale (3/9), tratto intestinale (4/9), 2/9 in entrambi. 50% dei casi con disseminazione linfonodale

22 centri di ricerca Europei:
-CORIT
-Università di Padova
-Ospedale di Padova







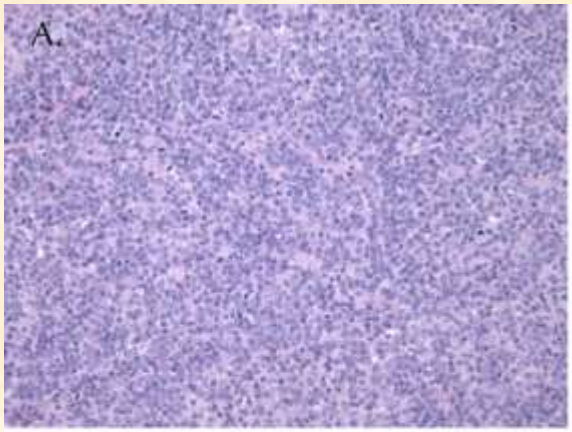
Esame citologico (e valutazione fluorimetrica):

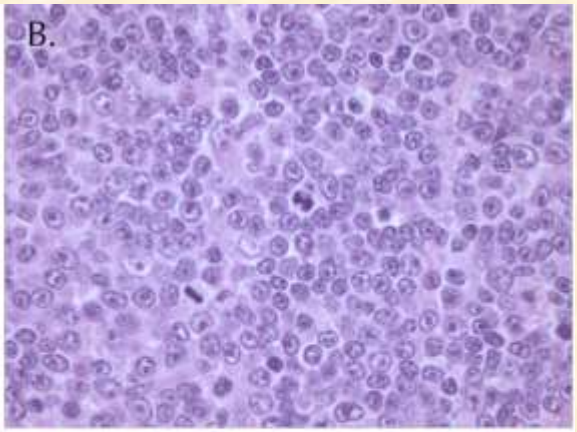
linfoma a cellule B, intermedie, di grado elevato



Esame istologico e profilo IHC (panel: anti-CD3, anti-CD5, anti-CD20, anti-CD79cyt, Ki-67) rivelano **un PTLD monomorfo**, in particolare **linfoma a grandi cellule B, di grado elevato**

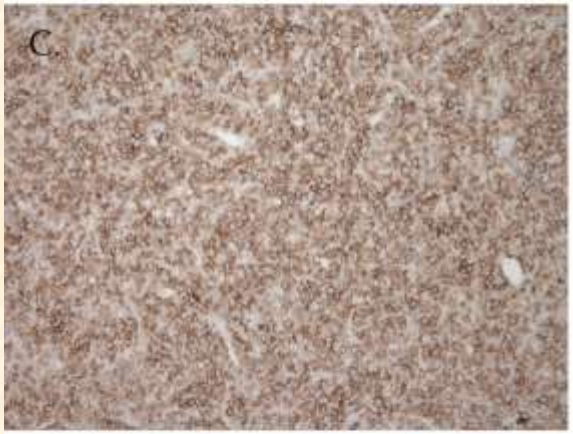
HE 4X

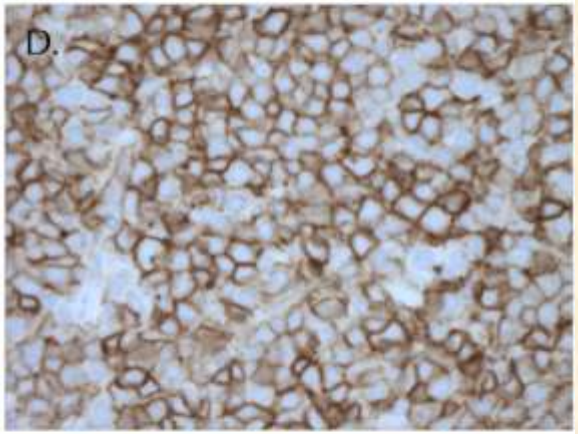




HE 20X

IHC CD20 4X

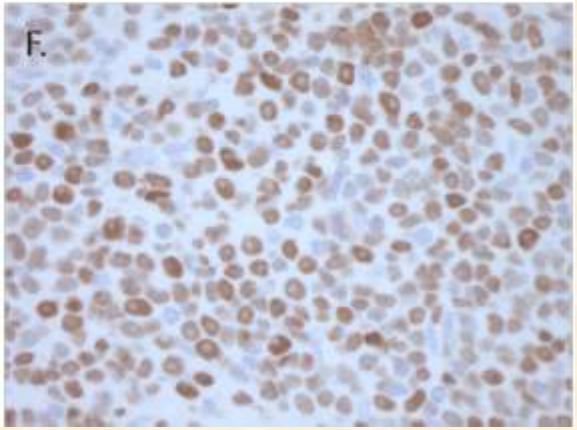




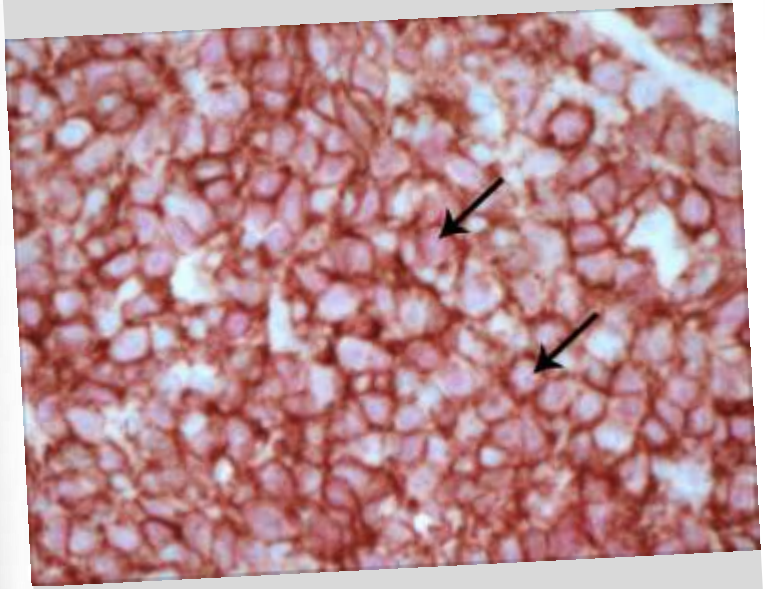
IHC CD20 20X

IHC EBNA2 4X





IHC EBNA2 20X



Double-labeled IHC per EBNA-2 e CD20 rivela diffusa doppia positività

Double-labeled IHC per EBNA-2 e CD3 non permette di osservare doppia positività

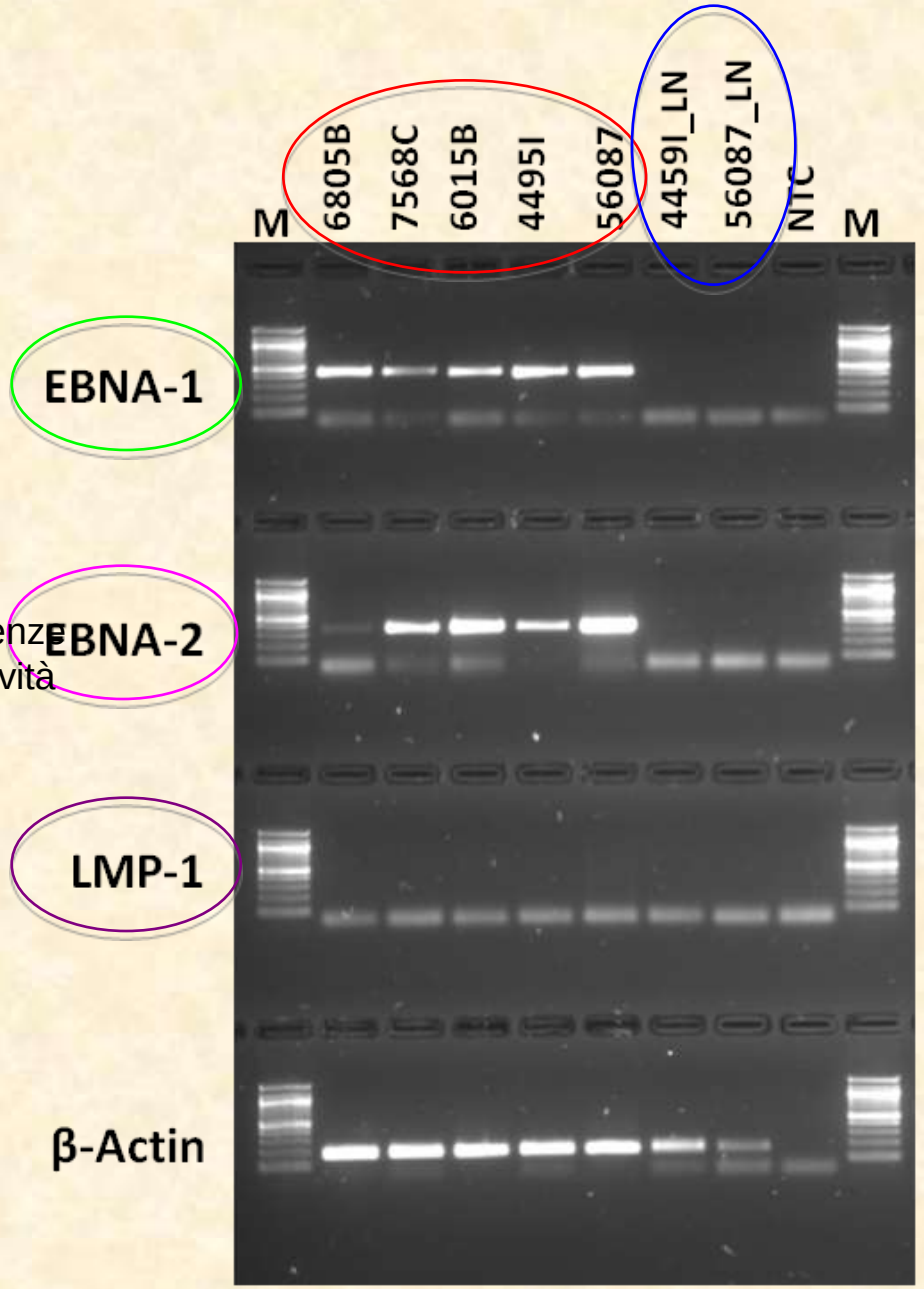
RT-PCR e sequenziamento dei trascritti di EBNA-1, EBNA-2 e LMP-1

Su **5/9 primati PTLD+** e su 2 linfonodi iperplastici di due primati con PTLD (**controllo**)

EBNA-1 presente in tutti i campioni PTLD+ con una identità del 98.6% con la sequenza di cynomolgus presente in GenBank. Nessuna espressione di EBNA1 nel controllo

EBNA2 (per confermare positività IHC per EBNA2 sequenza di cyno non presente in Genebank, sequenze conservate di Rhesus e Cercopiteco e uomo): positività nei PTLD+

Assenza di espressione di LMP1 in tutti i campioni

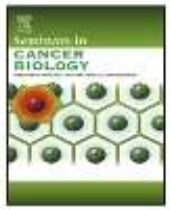


Seminars in Cancer Biology 20 (2010) 410–415

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journal homepage: www.elsevier.com/locate/semcancer



Review

Restricted expression of EBV encoded proteins in *in vitro* infected CLL cells

Eva Klein*, Noemi Nagy

A B S T R A C T

CLL is not associated with EBV. CLL cells separated from blood express CR2, the complement receptor that serves also as EBV receptor. Thus CLL cells can be infected *in vitro* with the virus, however, in contrast to normal B lymphocytes, only rare CLL clones yield transformed lines. This is due to a restricted EBV encoded protein expression in the CLL cells, they express EBNAs, the virus encoded proteins that are localized in the nucleus, but not the cell membrane associated LMP-1, that is also pivotal for the virus induced transformation of B lymphocytes. This expression pattern seems to be unique to a defined B cell maturation window that is represented by the CLL cells. We named this restricted viral expression as Type IIb. Such B lymphocytes have been encountered in lymphoid tissues of infectious mononucleosis (IM) and in post transplant lymphoproliferative disease (PTLD). Moreover, they were shown in tissues of EBV infected "humanized" mice.

The EBV encoded protein expression pattern may serve as a marker for the B cell differentiation stage from which CLL clones can develop.

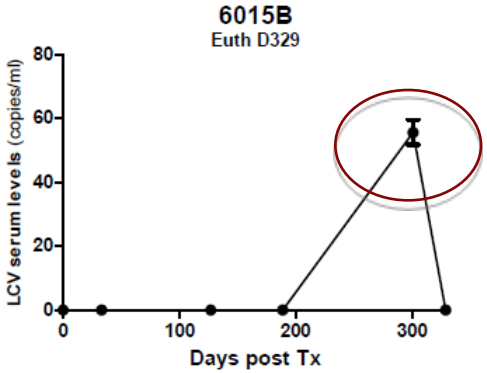
Screening sierologico

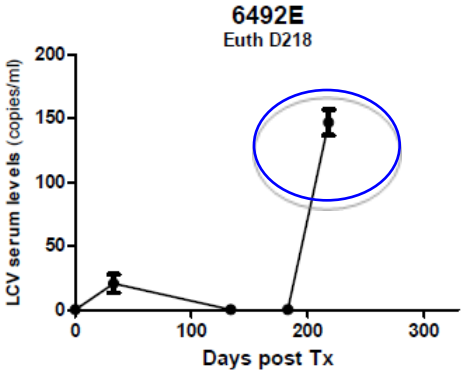
Porcyne lymphotropic HV (PLHV): PCR da PBMCs dei suini donatori
positività 44% dei donatori e 100% negatività dei riceventi

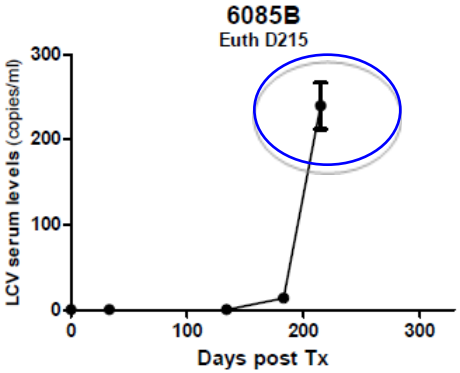
MacLCV: DNA virale isolato dai sieri dei riceventi
100% di positività dei trapiantati

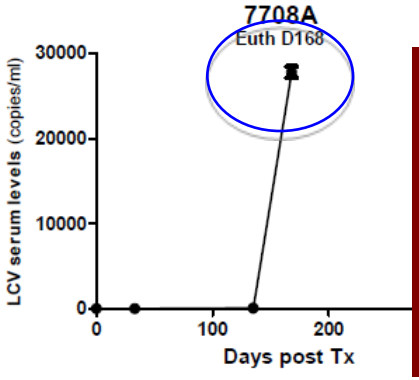
PCR quantitativa (per rivelare macLCV nel pre-Tx, tra TX e endpoint e all'eutanasia):

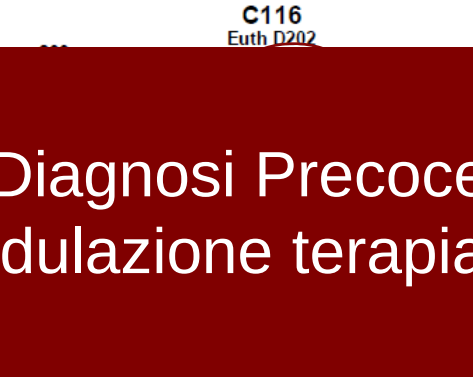
i primati PTLD+ presentano viremia tra 36-278 gg post tx (media 117 +/- 30gg)
e **circa 60 gg prima dei sintomi clinici**
Viremia assente nei primati PTLD- negli stessi tre tempi

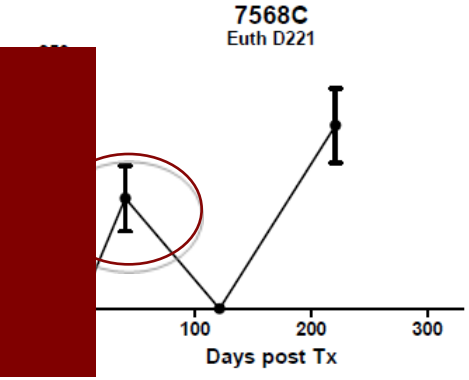




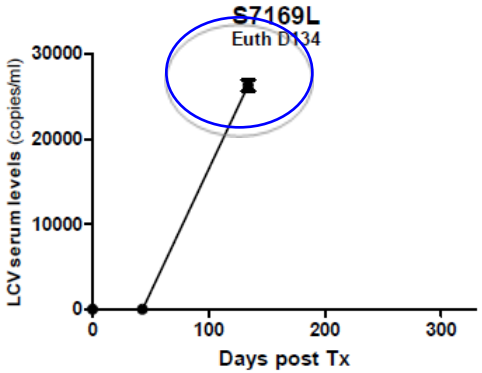


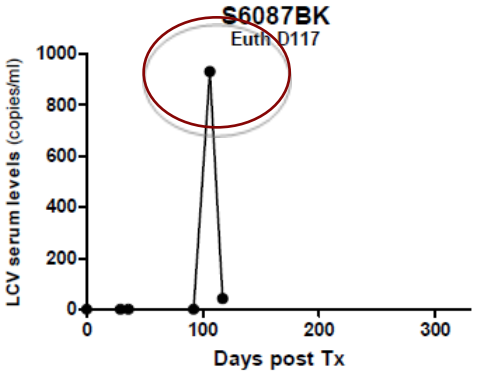


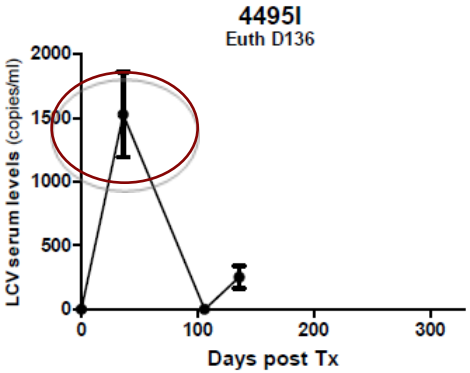




Diagnosi Precoce?
Modulazione terapia IS?







Courtesy from Claire Crossan, Glasgow Caledonian University

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