ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI
SCIENZE MEDICHE E CHIRURGICHE

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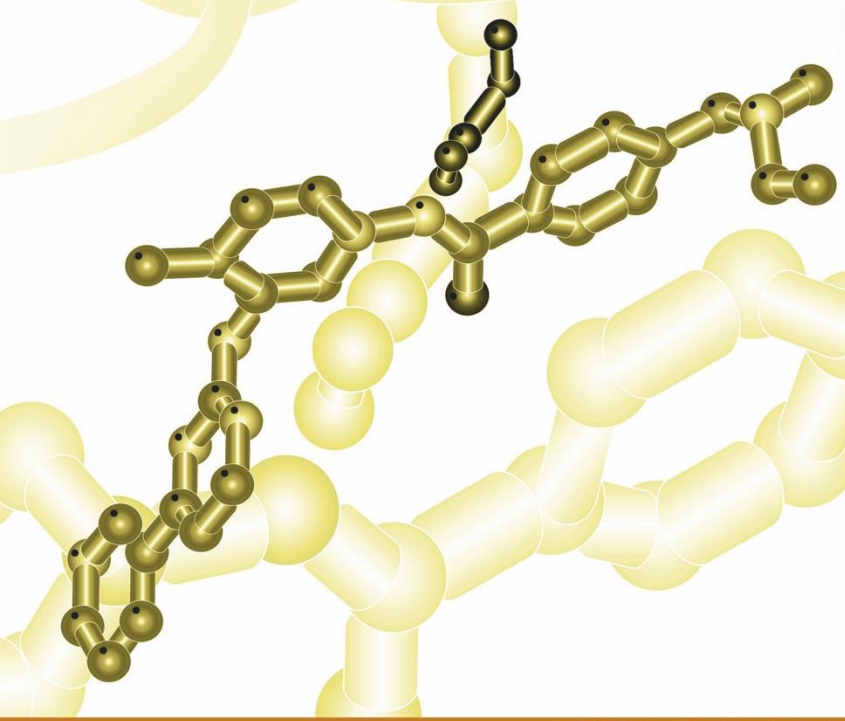

New Drugs in Hematology

President: Pier Luigi Zinzani
Co-President: Michele Cavo

Bologna,
Royal Hotel Carlton
January 15-17, 2024

BOLOGNA BOLOGNA, ROYAL HOTEL CARLTON

Improving outcomes of EBV^{+} PTLD with T-cell immunotherapy

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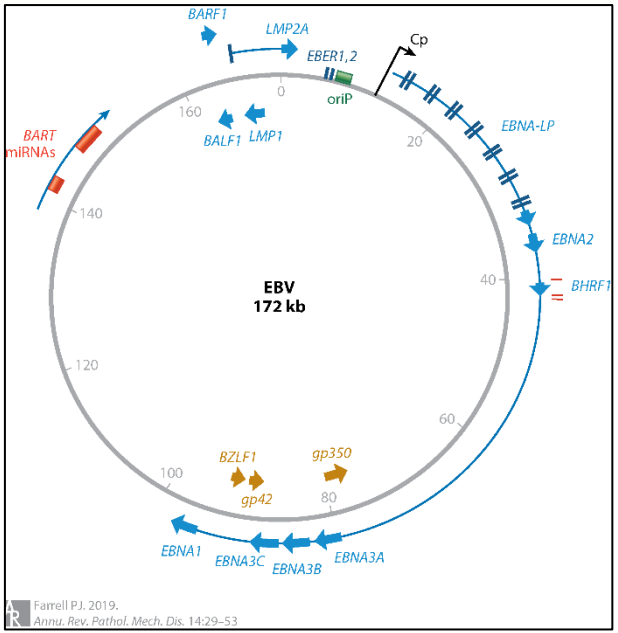
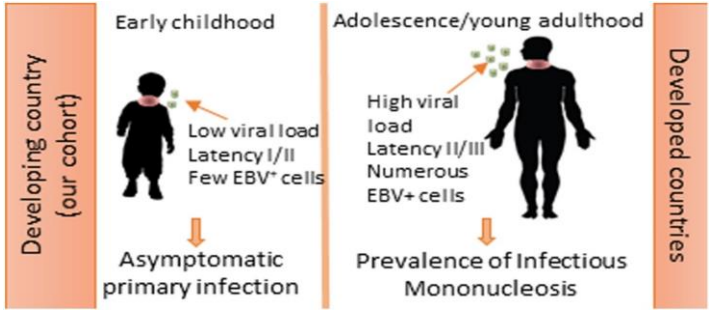
Disclosures of Daan Dierickx

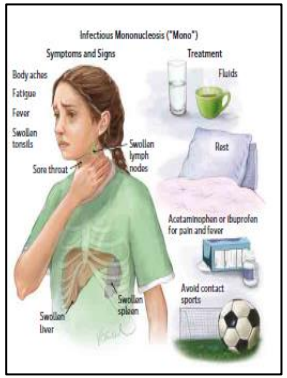
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Takeda			x			x	
Novartis					x	x	
Incyte						x	
Sanofi			x		x	x	
Amgen						x	
Atara			x			x	
Kite					x	x	
Pierre Fabre					x	x	

Introduction: EBV

Type HHV	Alternative common name	Viral subfamily
HHV-1	HSV-1	Alpha
HHV-2	HSV-2	Alpha
HHV-3	VZV	Alpha
HHV-4	EBV	Gamma
HHV-5	CMV	Beta
HHV-6	HHV-6	Beta
HHV-7	HHV-7	Beta
HHV-8	KSHV	Gamma



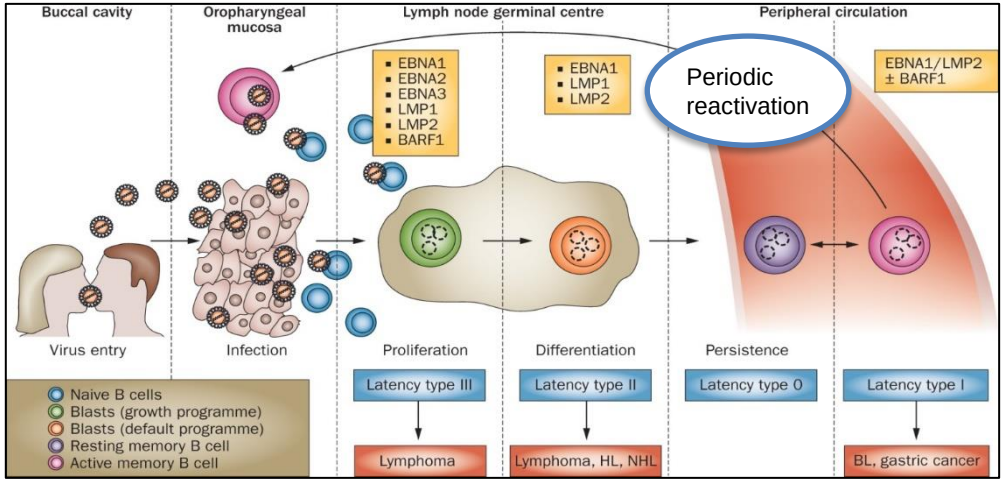




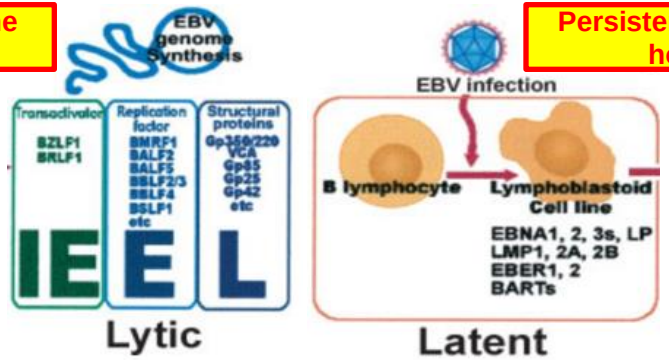
1. **Replication** of viral genome and production of new viral particles (lytic)
2. Allowing **long term persistence** in infected host (latent)
3. **Oncogenic** potential (latent)

Thompson AE. JAMA 2015;330:1180
Farrell PJ. Annu Rev Pathol 2019,14:29-53
Feressini Gerpe NM, et al. Int J Infect Dis 2020;93:139-45

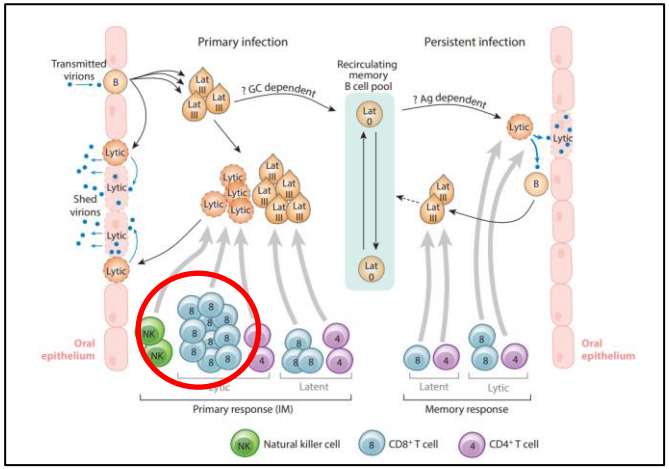
Introduction



Persistence in the population

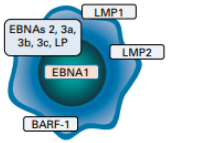


Persistence in the host



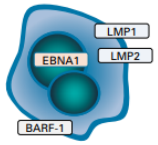
Tsurumi T, et al. Rev Med Virol 2005;15:3-15
Bollard CM, et al. Nat Rev Clin Oncol 2012;9:510-9
Taylor GS, et al. Annu Rev Immunol 2015;33:787-821

EBV and cancer



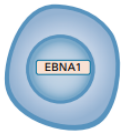
Latency III

Type of Lymphoma	EBV Frequency (%)
PTLD post HSCT	> 95
PTLD post SOT	95 in first year 70-80 after 1 year
NHL associated with primary immunodeficiency	> 95
Primary CNS lymphoma	> 95
Methotrexate-induced lymphoma	> 95



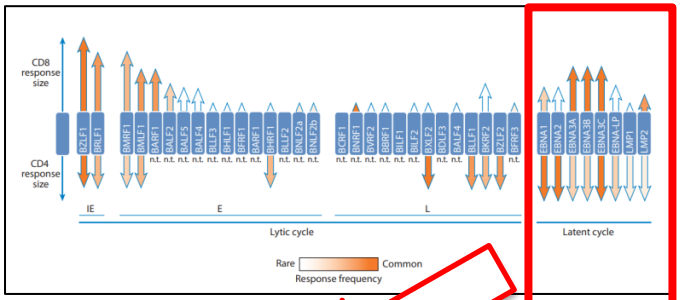
Latency II

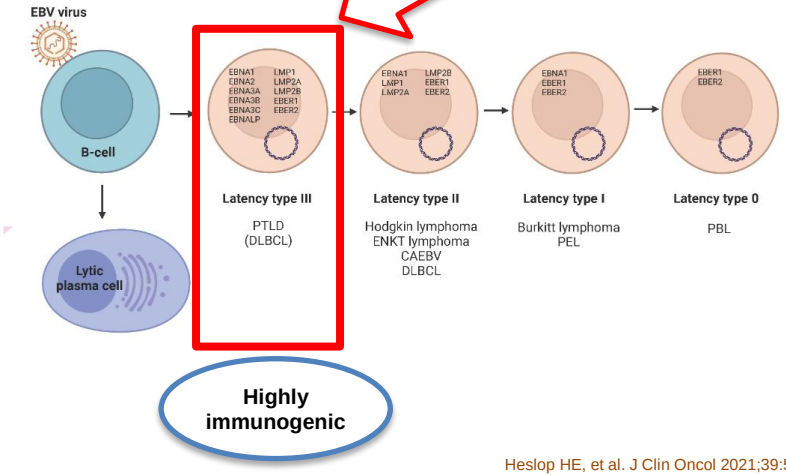
Type of Lymphoma	EBV Frequency (%)
Hodgkin lymphoma	30-40
NK-T lymphoma	> 95
PTLD post SOT (late)	70-80
Aggressive NK lymphoma	30-60
T, B, and NK cell CAEBV	> 95
Lymphatoid granulomatosis	80-95
Angioimmunoblastic lymphoma	> 80
T-cell rich B-cell lymphoma	20
DLBCL and ALCL	10-30



Latency I

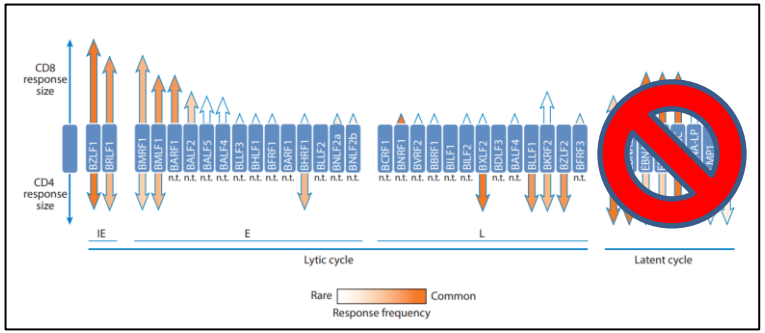
Type of Lymphoma	EBV Frequency (%)
Burkitt's	30-60
Primary effusion lymphoma	> 90

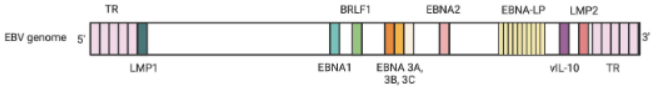




Heslop HE, et al. J Clin Oncol 2021;39:514-24
Bednarska K, et al. Br J Haematol 2023 Dec 28.[Online ahead of print]

EBV and cancer





Latent gene: *EBNA1*
Latency pattern: I, II, III
Function: Essential for the maintenance of EBV episomes and viral replication.
Enhances B-cell immortalization.
Suppresses spontaneous reactivation by decreasing *BZLF1* transcripts and protein.
Promotes lytic infection.

Latent gene: LMP1
Latency pattern: II, III
Function: Essential for B-cell transformation
Homologue of CD40 receptor. Acts as activated TNF receptor and activates cell-signalling pathways that are essential for transformation.

Latent gene: LMP2
Latency pattern: II, III
Function:LMP2A is a B-cell receptor (BCR) mimic that promotes survival of BCR-deficient B-cells.
Promotes B-cell growth Abrogates normal B-cell development.

Latent gene: *EBNA2*
Latency pattern: III
Function: Indispensable for immortalization.
Down-regulates *HLA II* transcription through the down-regulation of *CIITA*. Mediates tumourigenesis through *ATF4* activation and the modulation of lipid metabolism.

Latent gene: *EBNA3A*
Latency pattern: III
Function: Essential for *in-vitro* B-cell transformation and survival.

Latent gene: *EBNA3B*
Latency pattern: III
Function: Viral tumour suppressor.

Latent gene: *EBNA3C*
Latency pattern: III
Function: Essential for *in-vitro* B-cell transformation and survival.

Latent gene: *EBNA-LP*
Latency pattern: III
Function: Critical for B-cell transformation.
Enhances EBNA2-mediated transactivation.
Essential for the survival of EBV-infected naive B-cells.

Taylor GS, et al. Annu Rev Immunol 2015;33:787-821
Bednarska K, et al. Br J Haematol 2023 Dec 28.[Online ahead of print]

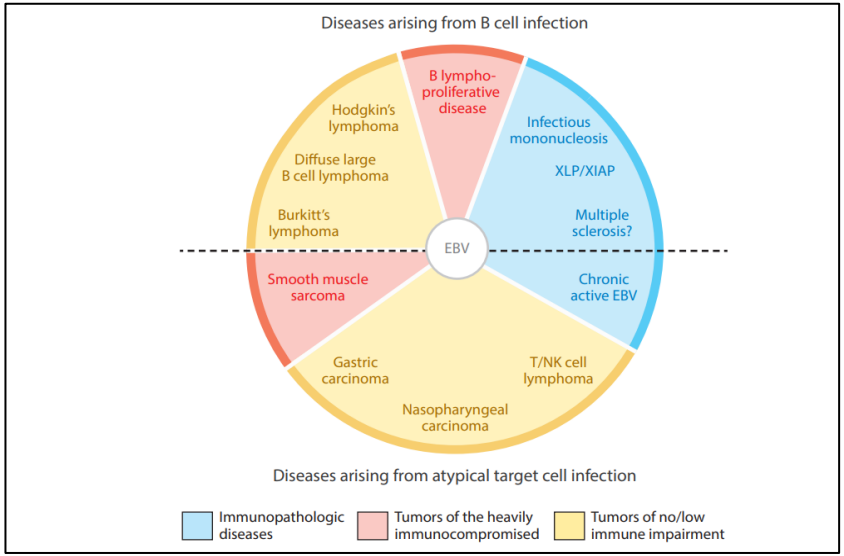
EBV and cancer

Group 1 agent	Cancers for which there is sufficient evidence in humans	Other sites with limited evidence in humans	Established mechanistic events
Epstein-Barr virus (EBV)	Nasopharyngeal carcinoma, Burkitt's lymphoma, immune-suppression-related non-Hodgkin lymphoma, extranodal NK/T-cell lymphoma (nasal type), Hodgkin's lymphoma	Gastric carcinoma,* lympho-epithelioma-like carcinoma*	Cell proliferation, inhibition of apoptosis, genomic instability, cell migration
Hepatitis B virus (HBV)	Hepatocellular carcinoma	Cholangiocarcinoma, non-Hodgkin lymphoma	Inflammation, liver cirrhosis, chronic hepatitis
Hepatitis C virus (HCV)	Hepatocellular carcinoma, non-Hodgkin lymphoma*	Cholangiocarcinoma*	Inflammation, liver cirrhosis, liver fibrosis
Kaposi's sarcoma herpes virus (KSHV)	Kaposi's sarcoma,* primary effusion lymphoma*	multicentric Castleman's disease*	Cell proliferation, inhibition of apoptosis, genomic instability, cell migration
Human immunodeficiency virus, type 1 (HIV-1)	Kaposi's sarcoma, non-Hodgkin lymphoma, Hodgkin's lymphoma,* cancer of the cervix,* anus,* conjunctiva*	Cancer of the vulva,* vagina,* penis,* non-melanoma skin cancer,* hepatocellular carcinoma*	Immunosuppression (indirect action)
Human papillomavirus type 16 (HPV-16)†	Carcinoma of the cervix, vulva, vagina, penis, anus, oral cavity, and oropharynx and tonsil	Cancer of the larynx	Immortalisation, genomic instability, inhibition of DNA damage response, anti-apoptotic activity
Human T-cell lymphotrophic virus, type-1 (HTLV-1)	Adult T-cell leukaemia and lymphoma	..	Immortalisation and transformation of T cells
Helicobacter pylori	Non-cardia gastric carcinoma, low-grade B-cell mucosa-associated lymphoid tissue (MALT) gastric lymphoma*	..	Inflammation, oxidative stress, altered cellular turn-over and gene expression, methylation, mutation
Clonorchis sinensis	Cholangiocarcinoma*	..	..
Opisthorchis viverrini	Cholangiocarcinoma	..	Inflammation, oxidative stress, cell proliferation
Schistosoma haematobium	Urinary bladder cancer	..	Inflammation, oxidative stress

*Newly identified link between virus and cancer. †For other types, see table 2.

Table 1: Biological agents assessed by the IARC Monograph Working Group

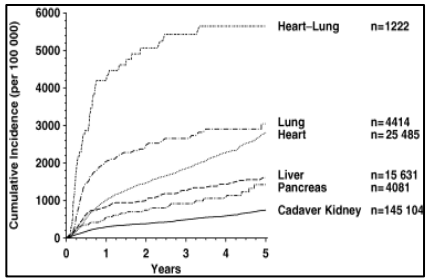
Cancer type	% estimated global EBV-related case proportion	Estimated incidence range of EBV-related cases	Estimated mortality range of EBV-related cases
NPC	84.6[1]	105,500–120,600	61,600–74,300
GC	7.7–10.4[2]	82,800–116,400	58,200–82,300
HL	45.8–58.3[2]	34,300–52,400	9400–17,400
BL	55[1]	6600[1]	3000–3200
DLBCL	3.6–12.8[2]	4900–27,000	2500–13,300
ENKTL-NT	100[3]	5500–34,700	3000–18,100
Cancer types combined	1.3–1.9[2]	239,700–357,900	137,900–208,700



Bouvard V, et al. Lancet Oncol 2009;10:321-2
Taylor GS, et al. Annu Rev Immunol 2015;33:787-821
Wong Y, et al. J Cancer Res Clin Oncol 2022;148:31-46

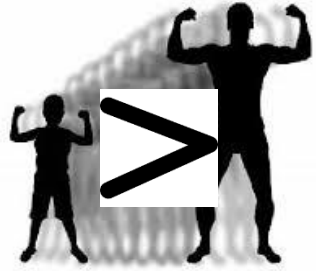
PTLD: risk factors

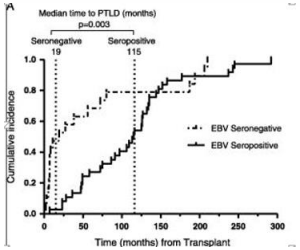
1. Organ type



Allogeneic HSCT
Selective T-cell depletion methods
Use of ATG therapy (prevention/therapy)
Two HLA antigen–mismatched siblings, or unrelated donors, accompanied by selective T-cell depletion methods or ATG therapy
Age 50 years or older at allogeneic HCT

2. EBV status at time of transplantation (R-/D+)





Opelz G, et al. Am J Transpl 2003;4:222-30
Landgren O, et al. Blood 2009;113:4992-5001
Shahinian VB, et al. Transplantation 2003;75:851-6
Dharnidharka VR, et al. Am J Transplant 2012;12:976-83
Morton M, et al. Transplantation 2013;95:470-80

PTLD: risk factors

3. Immune suppression

EBV

Increased risk associated with ATG, OKT3, tacrolimus, azathioprine, new agents (e.g., belatacept in EBV-negative transplant recipient)
Controversial degree of risk associated with alemtuzumab, cyclosporine, mTOR inhibitors
No increase in risk associated with mycophenolate mofetil, basiliximab, daclizumab

Early spike: Induction
EBV

Late wave:
Cumulative immune suppressive intensity

50-70%

Combination maintenance therapy

SOT

Induction regimen

Different dosesEpisodes of rejection

HSCT

Conditioning regimen

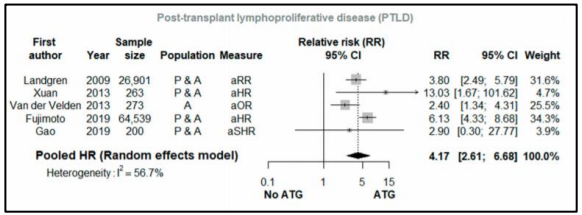
aGVHD

Maintenance therapy

cGVHD

Early spike:
Conditioning
EBV

> 95%



Dierickx D, Habermann TM. N Engl J Med 2018;378:549-62
Enok Bonong PR, et al. Vaccines (Basel) 2021;19:288

PTLD: classification

WHO 2017

- Non-destructive PTLD
  - Plasmacytic hyperplasia PTLD
  - Infectious mononucleosis-like PTLD
  - Florid follicular hyperplasia PTLD
- Polymorphic PTLD
- Monomorphic PTLD
  - B-cell neoplasms (DLBCL; BL; PCM; plasmacytoma-like lesions, other)
  - T-cell neoplasms (PTCL, NOS; HSTCL; other)
- Classic Hodgkin lymphoma PTLD

90% CD20+ DLBCL

"Precursors" EBV driven

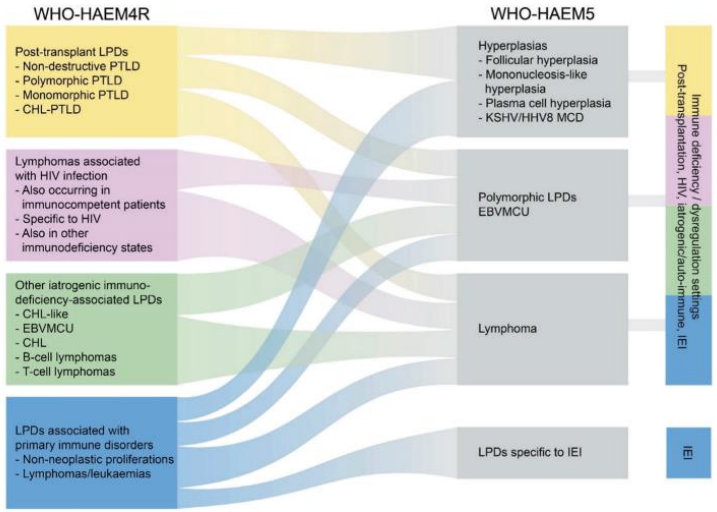
True lymphomas EBV+/ EBV-

Exclusion: indolent/small cell lymphomas (except EBV+ MALT lymphoma)

Swerdlow H, et al. Blood 2016;127:2375-90
Swerdlow H, et al. IARC Pres: Lyon. 2017

PTLD: classification

WHO 2022 (←→ ICC)



Histological diagnosis	Viral association	Immune deficiency/dysregulation setting
○ Hyperplasia (specify type) ○ Polymorphic lymphoproliferative disorder ○ Mucocutaneous ulcer ○ Lymphoma (classify as for immunocompetent patients)	○ EBV +/− ○ KSHV/HHV8 +/−	○ Inborn error of immunity (specify type) ○ HIV infection ○ Posttransplant (specify: solid organ/bone marrow) ○ Autoimmune disease ○ Iatrogenic/therapy-related (specify) ○ Immune senescence

EBER *in situ* hybridisation

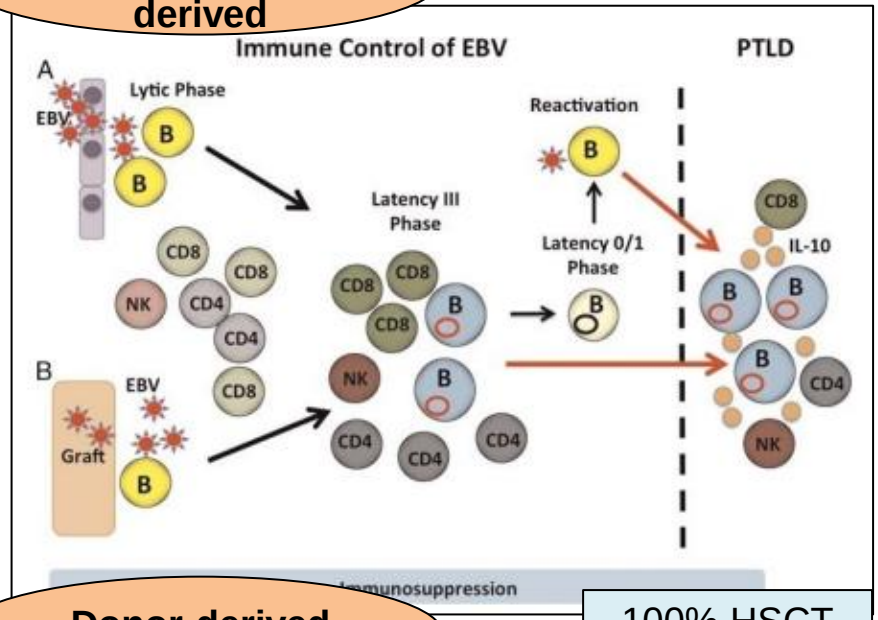
EBV-associated disease	Latency type	EBV viral gene expression
Healthy individuals (resting EBV-infected B cells)	0	EBERs, BARTs
Burkitt lymphoma (BL)	I	EBERs, BARTs, EBNA1
Gastric carcinoma	I or II	EBERs, BARTs, EBNA1
Hodgkin lymphoma (HL)	II	EBERs, BARTs, EBNA1, LMP1, LMP2
NK/T cell lymphoma (NKTL)	II	EBERs, BARTs, EBNA1, LMP1, LMP2
Nasopharyngeal carcinoma (NPC)	II	EBERs, BARTs, EBNA1, LMP1, LMP2
Diffused large B cell lymphoma (DLBCL)	II or III	EBERs, BARTs, EBNA1, EBNA2, EBNA3A,B,C, EBNA-LP, BHRF1 miRNAs
HIV-associated lymphomas	III	EBERs, BARTs, EBNA1, LMP1, LMP2, EBNA2, EBNA3A,B,C, EBNA-LP, BHRF1 miRNAs
Post-transplant lymphoproliferative disease (PTLD)	III	EBERs, BARTs, EBNA1, LMP1, LMP2, EBNA2, EBNA3A,B,C, EBNA-LP, BHRF1 miRNAs

Alaggio R, et al. Leukemia 2022;36:1720-48
Campo E, et al. Blood 2022;140:1229-53
Damania B, et al. Cell 2022;185:3652-70

PTLD: origin

Recipient-derived

85% SOT



Donor-derived

100% HSCT

15% SOT

Olagne J, et al. Am J Transplant 2011;11:1260-9
Kinch A, et al. Am J Transplant 2014;14:2838-45
Martinez O, Krams SM. Transplatation 2017;101:2009-16

PTLD: treatment

Restoring T cell function	• Reduction of immune suppression • Adoptive immunotherapy
Reducing tumor	• Local therapy • Rituximab and/or chemotherapy
Targeting EBV	• Inducers of lytic cycle • Antiviral agents

Gottschalk S, et al. Annu Rev Med 2005;56:29-44

PTLD: prognosis

SOT: RIS + R + chemotherapy

Figure 1. PTLD is the Most Common Reason for Death Among EBV⁺ PTLD Patients Post-SOT (n=63)

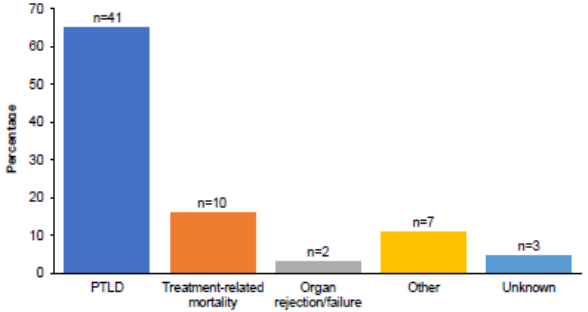
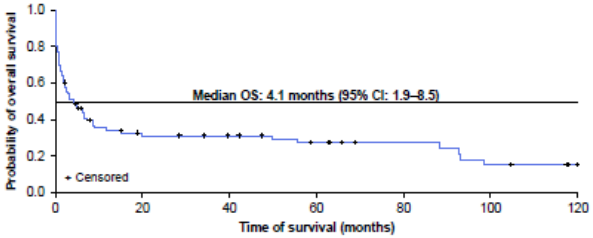


Figure 2. KM Plot for Overall Survival for Post-SOT EBV⁺ PTLD Patients Who Failed Rituximab Plus CT (n=86)



Number at risk (events)						
86 (0)	21 (57)	18 (57)	13 (59)	9 (59)	5 (63)	2 (63)
OS rate, % (95% CI):						
—	31.4 (21.5–41.7)	31.4 (21.5–41.7)	27.4 (17.8–38.0)	27.4 (17.8–38.0)	15.2 (6.6–27.2)	15.2 (6.6–27.2)

HSCT: RIS + R

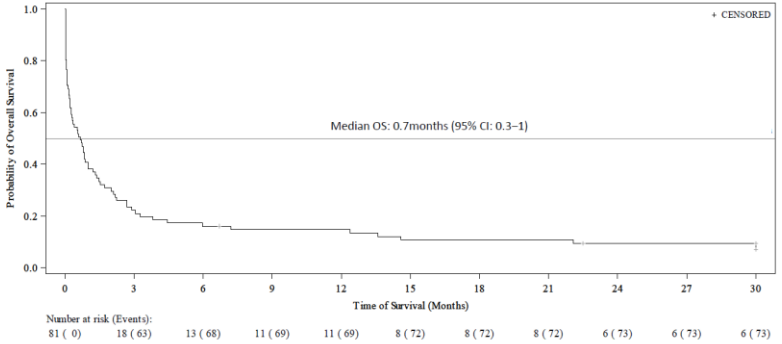


Figure 1: KM plot for overall survival for EBV⁺ PTLD pts who fail rituximab ± CT (n=81) from rituximab failure date

Dharnidharka V et al. Blood (2021) 138 (Supplement 1): 2528
Sanz J et al. Blood (2021) 138 (Supplement 1): 1454

PTLD: adoptive immunotherapy

Labor intensive

Availability

Cost

Transplantation cell type	AHSCT: donor origin	SOT: recipient origin
Donor lymphocytes	yes (GVHD risk)	no
Autologous EBV-CTLs	no (donor derived)	yes (often EBV-naive, ongoing IS)
Donor-derived EBV-CTLs	yes	no (mostly receptor derived)
Third party EBV-CTLs	yes	yes

Donor-derived (HSCT)

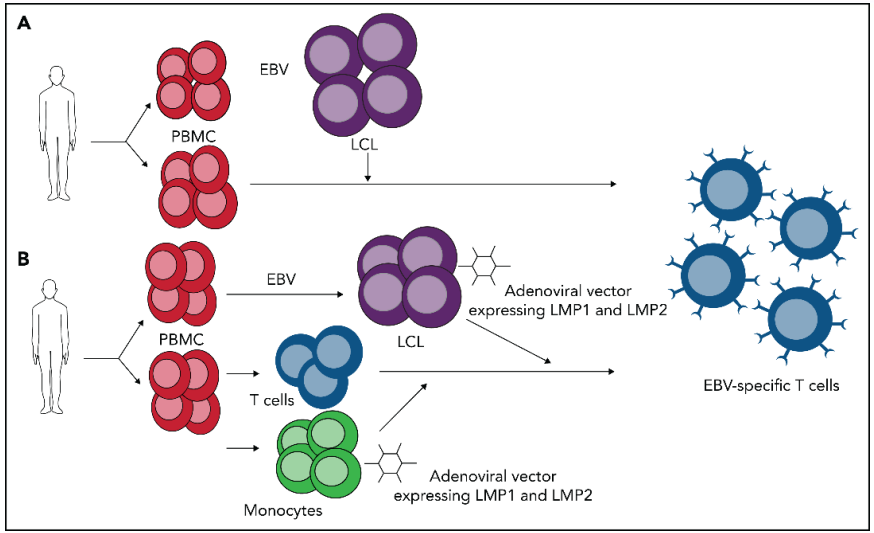
Autologous (SOT)

Third party (HSCT + SOT)

Merlo, A. et al. Haematologica 2010;95:1769-77
Roddie C, Peggs KS. J Clin Invest 2017;127:2513-22
Kaeuferle T, et al. J Hematol Oncol 2019;12:13
Heslop HE, et al. J Clin Oncol 2021;39:514-24
Toner K, Bollard CM. Blood 2022;139:983-94
Dierickx D, et al. Curr Opin Oncol 2022;34:413-21

PTLD: adoptive immunotherapy

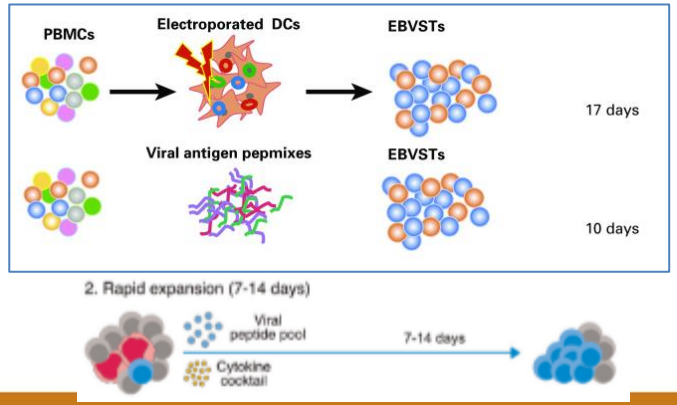
Ex vivo expansion

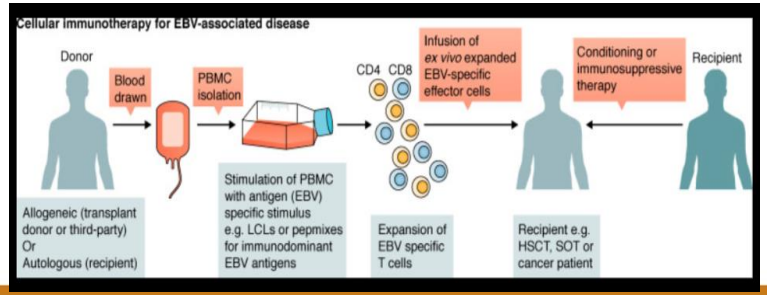


Activity against E lytic antigens and EBNA3(-6)s

Immunomagnetic selection

Activity against LMP1/2





Tangye SG, et al. J Exp Med 2017;214:269-83
Heslop HE, et al. J Clin Oncol 2021;39:514-24
Toner K, Bollard CM. Blood 2022;139:983-94
Walti CS, et al. Curr Opin Infect Dis 2022;35:302-11

Donor-derived: DLI

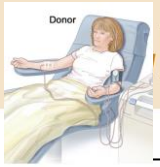


Table 1. Characteristics of the Patients and Outcome of Treatment.*

Patient No.	Sex/ Age (yr)	Diagnosis	Type of Graft	Conditioning Regimen†	Regimen of ATG Prophylaxis‡	EBV VCA IgG Titer		Onset of EBV-Associated LPD		Donor-Leukocyte Infusion	
						Patient	Donor		Day of Treatment	Dose	Outcome
								day		*CD3+ cells/kg*	
1	F/20	ALL	HLA-matched; unrelated donor	1	30 mg/kg/day on days −6, −5, −4	1:1280	1:80	90	105 118 121	0.55×10^6 0.35×10^6 0.12×10^6	Clinical and pathological response; 2nd biopsy on day 139 showed no evidence of disease; alive and well with limited chronic GVHD on day 300
2	M/29	CML	HLA-matched; related donor	1	30 mg/kg/day on days −5, −4, −3	1:80	1:160	74	87 93	1.0×10^6 1.0×10^6	Died of respiratory failure on day 94; autopsy showed no evidence of lymphoma
3	F/52	ANLL	HLA-matched; related donor	2	15 mg/kg every other day on days 5, 7, 9, 11, 13	1:80	1:160	107	113 129	1.0×10^6 1.0×10^6	Died of respiratory failure on day 130; autopsy showed no evidence of lymphoma
4	M/43	CML	HLA-matched; related donor	1	30 mg/kg/day on days −5, −4	1:10	1:20	113	121	0.8×10^6	Complete response; a 2nd biopsy on day 142 showed no evidence of disease; chronic GVHD of oral mucosa; alive and well 16 mo post-transplantation
5	F/33	ANLL	HLA-matched; related donor	1	None§	1:320	1:80	127	140	1.0×10^6	Complete response; chronic GVHD 16 mo post-transplantation

Table 4. Variables associated with response to cell therapy

Variable	Patients, n	Response CR/SD/PR	Response PD	Response NE	P
Overall association of number of sites involved and response (n = 49)					
1 site	14	14 (100%)	0 (0%)	0 (0%)	.01, .01*
2 sites	21	13 (60%)	4 (20%)	4 (20%)	
3+ sites	14	7 (50%)	6 (50%)	1 (0%)	
Overall association of rituximab use and response to cell therapy (n = 49)					
Patients failing Rituxan	19	11 (58%)	7 (37%)	1 (5%)	.07, .06*
Patient w/o prior Rituxan or in PR after Rituxan	30	23 (77%)	3 (11%)	4 (11%)	
Overall association of rituximab use and response (n = 49)					
Patients failing Rituxan by treatment	19	11	7	1	
DLI	9	4 (44%)	4 (44%)	1 (11%)	.47, .63*
EBV-CTL	10	7 (70%)	3 (30%)	0 (0%)	
Patients without prior Rituxan or in PR after Rituxan by treatment	30	23	3	4	
DLI	21	17 (82%)	1 (6%)	3 (12%)	.32, .22*
EBV CTL	9	6 (67%)	2 (22%)	1 (11%)	
Overall association of steroid use and response (n = 45)					
No steroid use	40	29 (72%)	8 (20%)	3 (7%)	.85, .99*
Steroid use	9	6 (66%)	2 (22%)	1 (11%)	
Overall association of use of steroids and/or cyclosporine or sirolimus (n = 49)					
No steroid use	35	26	6	3	.76, .44*
Steroid use	14	9	4	1	

GVHD

Table 3. Outcome following DLI according to presence or not of GVHD

	Cohort	*No GVHD-20*	*aGVHD-22*	*cGVHD-26*
	N = 68	*N* = 20 (29%)	*N* = 22 (32%)	*N* = 26 (38%)
Died	23 (34%)	5 (25%)	11 (50%)	7 (42%)
Alive	45 (66%)	15 (75%)	11 (50%)	19 (73%)
Relapse	25 (37%)	10 (50%)	8 (36%)	7 (27%)
Died relapse	16 (24%)	5 (25%)	6 (27%)	5 (19%)
Alive relapse	9 (13%)	4 (20%)	3 (1 with mild cGVHD)	2 (both without GVHD)
Alive remission	36 (53%)	11 (55%)	8 (2 with cGVHD)	17 (12 with cGVHD)
Alive cGVHD	15 (22%)	0	3 (14%)	12 (46%) (6 severe)

Papadopoulos EB, et al. N Engl J Med 1994;330:1185-91
Doubrovina E, et al. Blood 2012;119:2644-56
Scarisbrick JJ. Bone Marrow Transplant 2015;50:62-7

Donor-derived: EBV-CTL

Activity against E lytic antigens and EBNA3s

Prevention and treatment

Table 2. Clinical characteristics of 114 patients who received EBV-specific CTLs after transplantation

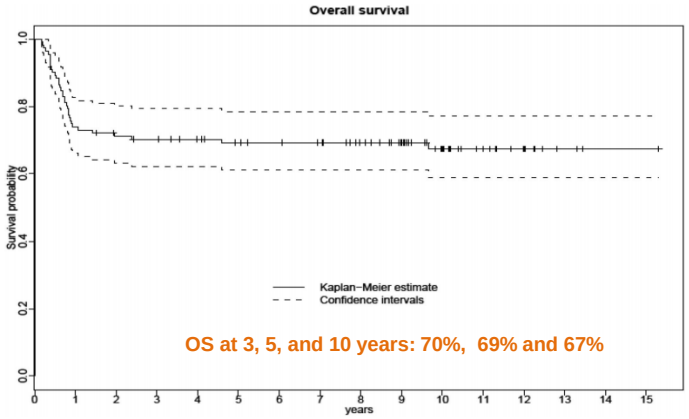
Characteristic	CTL recipients
Age at transplantation, y [mean (range)]	8.4 (0.5-38)
Male:female	69:45
Diagnosis	
Acute lymphoblastic leukemia	29
Acute myeloid leukemia	33
Myelodysplasia	11
Acute undifferentiated leukemia	1
Chronic myeloid leukemia	12
Hodgkin disease	1
Non-Hodgkin lymphoma	3
Aplastic anemia	1
Paroxysmal nocturnal hemoglobinuria	2
Histiocytic disorders	3
Hurler syndrome	2
X-linked lymphoproliferative disease	6
Wiskott-Aldrich syndrome	5
Osteogenesis imperfecta	3
Common immunodeficiency	2
Type of transplantation	
Unrelated donor	
10/10 match	3
9/10 match	4
6/6 match	63
5/6 match	30
Family member	
Syngeneic	1
5/6 match	6
4/6 match	4
3/6 match	3
HSC product	
Marrow	108
PBSC	6

Three centers
n = 114 (101: prevention)

Table 3. Severity and frequency of GVHD

GVHD	No. of patients
Acute GVHD (pre-CTL infusion)	
None	63
Grade 1	36
Grade 2	13
Grade 3	2
Grade 4	0
Total	114
Acute GVHD (post-CTL infusion)	
None	106
Grade 1	6
Grade 2	2
Grade 3	0
Grade 4	0
Total	114
Chronic GVHD	
None	95
Limited	11
Extensive	2
Total	108

Persistence of VST up to 105 months

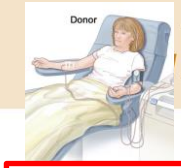


OS at 3, 5, and 10 years: 70%, 69% and 67%

Primary cause of death	No. of patients
Alive	79
Recurrence or persistence of primary disease	24
Motor vehicle accident	1
Infection	3
EBV-LPD	2
Regimen-related toxicity	5
Total	114

Heslop HE, et al. Blood 2010;115:925-35

Donor-derived: EBV-CTL



Activity against E lytic antigens and EBNA3s

Prevention and treatment

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Steroid use	14	9	4	1	

ORR DLI 72% - EBV-CTL 68%

GVHD DLI 17% - EBV-CTL 0%

Not available in UCB transplantation

Doubrovina E, et al. Blood 2012;119:2644-56

Autologous

Activity against E lytic antigens and EBNA3s

Prophylaxis and treatment

Table 1. Characteristics of the 7 transplant recipients treated with Epstein-Barr virus-specific cytotoxic T-lymphocyte lines

Patient	Sex/age	Treatment	Interval treatment/EBV DNA positivity (y)	Immunosuppression at time of cell harvest and infusion	Clinical effects of CTL infusion(s)
1	M/60	Heart	9	CsA (1.5 mg/Kg/d)	EBV DNA fell below detectable levels
					No evidence of rejection
2	M/51	Heart	3	FK506 (0.06 mg/Kg/d)*	EBV DNA fell below detectable levels
					No evidence of rejection
3	M/58	Heart	0.5	CsA (4 mg/Kg/d)	EBV DNA reduced > 2 logs
				Steroids (0.06-0.15 mg/Kg/d)	No evidence of rejection
4	M/7	Heart	1	CsA (10 mg/Kg/d)	EBV DNA levels still fluctuating
				Azathioprine (0.7 mg/Kg/d)	No evidence of rejection
5	F/7	Heart	5	CsA (7 mg/Kg/d)	EBV DNA levels reduced > 2 logs
					No evidence of rejection
6	F/4	Liver	1	CsA (4 mg/Kg/d)	EBV DNA levels still fluctuating
				Steroids (0.4 mg/Kg every other d)	No evidence of rejection
7	M/5	Kidney	2	CsA (5.8 mg/Kg/d)	EBV DNA levels reduced > 2 logs
				Steroids (0.2 mg/Kg/d)	No evidence of rejection

Prophylaxis

Table 3. Outcome of treatment

Patient	Before CTL evaluation: EBV DNA load	PTLD	CTL dose	Toxicity	Fold reduction in EBV load: 2 mo after	6 mo after	Outcome at 1 y: Clinical	EBV DNA load
1	4 156	No	$2 \times 10^7/m^2$	No	2.3	0.7	Well	1 500
2	136 000	No	$2 \times 10^7/m^2$	No	0.6	3	Well	180 000
3	4 506	No	$2 \times 10^7/m^2$	Transient rise in AST*	2.5	3.7	Well	400
4	16 204	No	$5 \times 10^7/m^2$	No	0.1	1	Well	15 000
5	10 172	No	$5 \times 10^7/m^2$	No	0.4	1.3	Well	3 900
6	6 704	No	$5 \times 10^7/m^2$	No	0.6	10	Well	1 100
7	4 938	No	$1 \times 10^8/m^2$	No	0.9	0.6	Well	3 900
8	14 002	No	$1 \times 10^8/m^2$	No	0.6	0.9	Well	7 200
9	2 500	No	$5 \times 10^7/m^2 \times 3$	No	1.7	10	Well	7 000
10	1 100	No	$5 \times 10^7/m^2 \times 3$	No	2.7	10	Well	< 400
11	1 500	No	$5 \times 10^7/m^2 \times 4$	No	0.4	1.2	Well	700
12	20 900	Eye	$5 \times 10^7/m^2 \times 4$	No	0.8	3	Well†	25 000

Prophylaxis + treatment

Challenges: ongoing immune suppression, often seronegativity

No development of PTLD

EBV VL: fluctuating

Comoli P, et al. Blood 2002;99:2592-8
Savoldo B, et al. Blood 2006;108:2942-9

Autologous

Activity against E lytic antigens and EBNA3s

cHL

Table I. *Patient Characteristics*

Patient ID	Age	Sex	Disease stage at diagnosis	Most recent chemo (time before CTL)	Gene marked	Dose level	Toxicity attributed to CTL	Response to CTL	Outcome
1	21	M	IA	SCT→relapse 5 mo later→CTL (8 mo)	Yes	1	None	Stable disease	DOD 13 mo after CTLs
2	18	M	IVB	3 wk hemipelvis RT (stopped 1 d before CTL no. 2)	Yes	1	None	Stable disease (hilar/mediastinal/ pulmonary)	DOD 10 mo after CTLs
3	24	M	IIIA	Vinblastine weekly (1 mo)	Yes	1	Transient malaise	PR	DOD 12 mo after CTLs
4	36	F	II_EA	Paraspinal RT and αIFN (1 mo)	Yes	1	None	NR	DOD 2 mo after CTLs
5	19	F	IIIA	Mantle/lung RT (1 mo)	Yes	1	None	Stable disease	DOD 10 mo after CTLs
6	36	M	IIIA	MOPP (2 mo)	Yes	1	None	Stable disease then allo BMT	In remission 56 mo after CTLs
7	40	F	IIIB	ABVD (2 mo)	No	2	None	Stable disease	DOD 20 mo after CTLs
8	24	M	IIA	MTX and 6TG (2 mo)	Yes	2	None	NR	DOD 7 mo after CTLs
9	20	M	IVB	SCT (10 mo)	No	1	None	Remains in remission	In remission 24 mo after CTLs then lost to follow-up
10	27	F	IIB	SCT (4 mo) RT (2 mo)	No	1	None	CRU[a]	In remission 24 mo after CTLs
11	16	M	IIB	SCT (2 mo) αIFN (1 wk)	No	1	None	CRU[a]	In remission 38 mo after CTLs
12	18	M	IIA	SCT (3 mo)	No	2	None	CR	In remission 27 mo after CTLs
13	29	F	IIB	SCT (3 mo)	No	2	None	NR	DOD 4 mo after CTLs
14	8	F	IA	RT (9 mo)	No	2	Transient swelling and pain in cervical lymph node	CR	In remission 9 mo after CTLs

NPC

Table 4. Toxicity and clinical response after CTL therapy

Patient no.	Toxicity	Clinical response	Outcome
Treated in remission			
729	None	N/A	Remains in remission > 27 mo
606	None	N/A	Remains in remission > 26 mo
697	None	N/A	Remains in remission > 25 mo
815	None	N/A	Remains in remission > 19 mo
Treated with relapsed or refractory disease			
845	Swelling at tumor site	No response then PR after chemotherapy	PR for 4 months then progressed and died at 12 mo
894	None	CR	Remains in remission > 23 mo after CTLs
389	None	CR	Remains in remission > 11 mo after CTLs
918	None	PR	PR for 12 mo after CTLs then relapsed
1042	None	Stable disease	Stable disease for > 14 mo
1046	None	No response	Died of disease at 3 mo

Patients (UPN)	Age (years)	Sex	Stage at diagnosis	Site(s) of tumor involvement at the time of cell therapy	Prior therapies	ECOG PS	Adverse events	Best response (duration)
1	19	F	IV (T4N2M0)	Liver, spleen	RT, three lines of CT	0	None	SD (4 months)
2	65	M	III (T3N1M0)	Primary tumor, skull base	Two lines of CT, RT, surgery	0	Inflammatory reaction at the disease site; fever and tremors after second infusion	PR (8 months)
3	21	M	III (T3N1M0)	Primary tumor, skull base	Two lines of CT, RT	0	None	PD
4	40	F	III (T2N2M0)	Skull base, neck	Three lines of CT, RT	1	None	SD (8 months)
5	48	M	IV (T2N2M1)	Primary tumor, skull base	Two lines of CT, RT, surgery	1	None	PD
6	64	M	III (T3N0M0)	Primary tumor	Two lines of CT, RT	0	None	SD (16+ months)
7	49	M	Unknown	Skull base, lung, lymph nodes, orbital cavity	Three lines of CT, RT, surgery	1	Orbital edema and visual field defects	PR (5 months)
8	40	M	Unknown	Primary tumor, skull base	Three lines of CT, RT	0	None	MR (12 months)
9	66	M	IV (TXN2M1)	Primary tumor, neck	Three lines of CT	0	None	PD
10	50	M	IV (T4N2M0)	Liver	Two lines of CT	1	None	PD
11	46	M	II (T2N1M0)	Lung, lymph nodes, liver	Two lines of CT, RT, surgery	1	None	PD

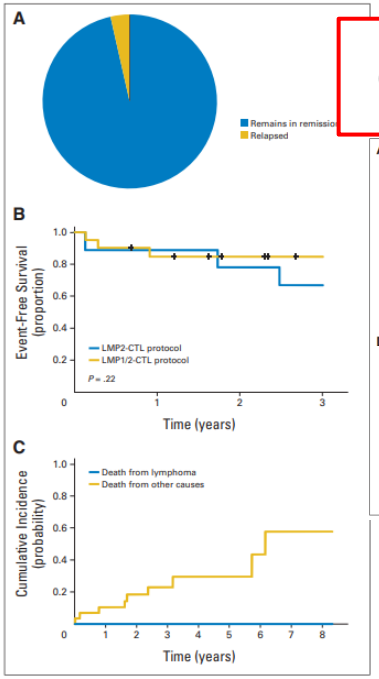
Bollard CM, et al. J Exp Med 2004;200:1623-33
Straathof KC, et al. Blood 2005;105:1898-904
Secondino S, et al. Ann Oncol 2012;23:435-41

Autologous

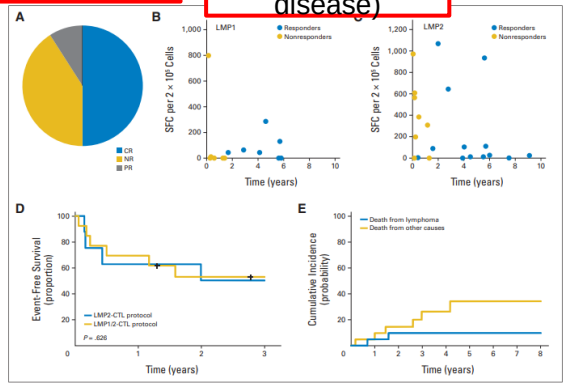
Activity against LMP1/2

Lymphoma

n=29 (adjuvant)

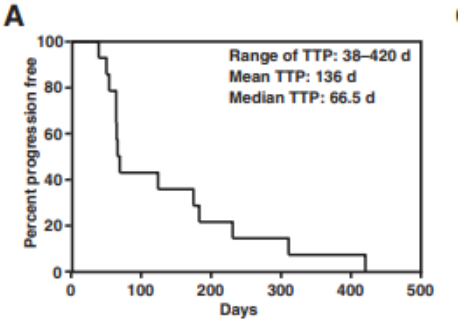


n=21 (R/R disease)



mNPC

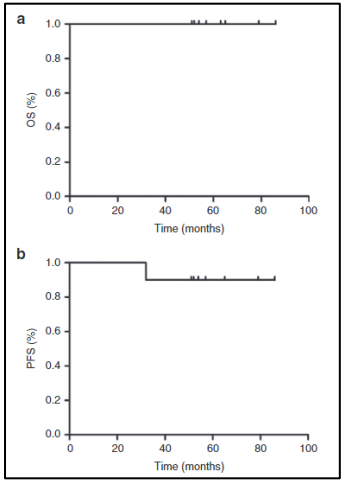
n=16



Median OS 523 days *vs* 220 days

NK/T-NHL

n=10

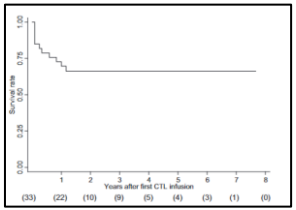


Postremission (CR)

Bollard CM, et al. J Clin Oncol 2014;798-808
Smith C, et al. Cancer Res 2012;72:1116-25
Secondino S, et al. Ann Oncol 2012;23:435-41
Cho SG, et al. Mod Ther 2015;23:1401-9

Third party

(Edinburgh)
HSCT: 33



ORR 52% at 6 months
OS 79% at 6 months
No significant toxicities

(MSKCC) HSCT: 33
SOT: 13

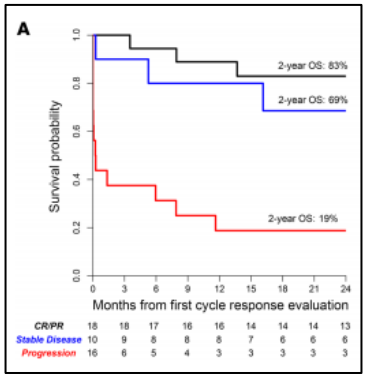
Table 3. Response to first and ultimate cycle of EBV-CTLs

Response to first cycle of EBV-CTLs

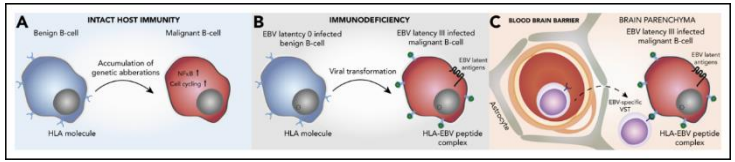
Cohort	N	CR	PR	SD	POD	CR + PR
HCT recipients	33	8	7	5	12	45%
SOT recipients	13	1	2	5	4	23%

Ultimate response to treatment

Cohort	N	CR	PR	SD	POD	CR + PR
HCT recipients	33	19	3	1	9	68%
SOT recipients	13	2	5	1	5	54%

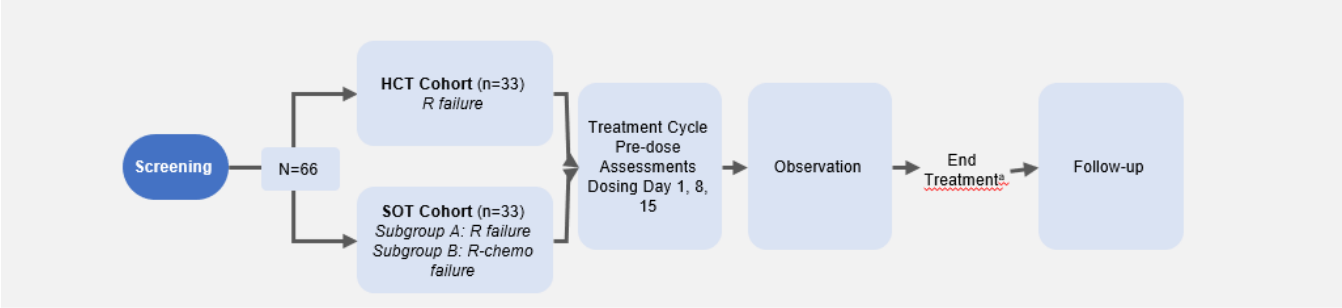


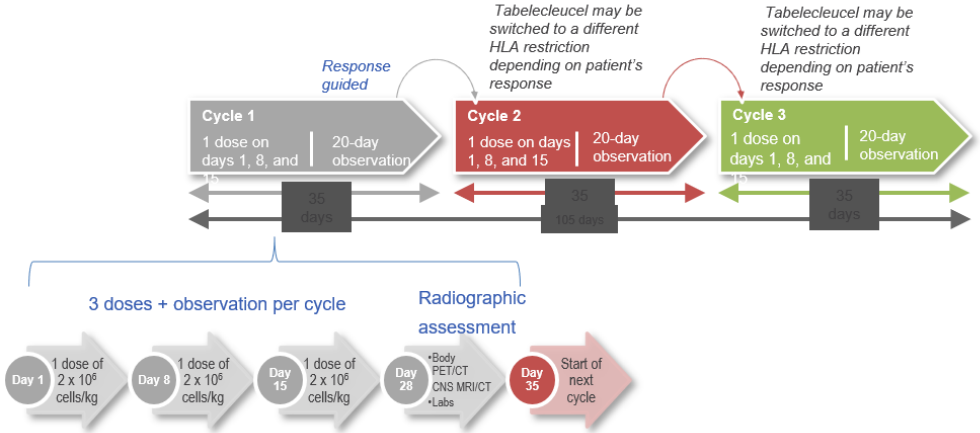
PCNSL
-PTLD



Haque T, et al. Blood 2007;110:1123-33
Prockop S, et al. J Clin Invest 2020;130:733-45
Gandhi MK, et al. Blood 2021;137:1468-77

ATA129-302 ALLELE Study: Tabelecleucel Phase 3 Clinical Trial

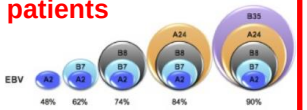




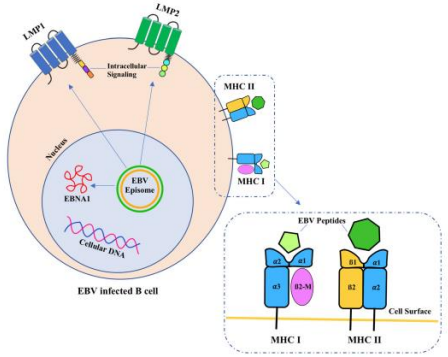
https://clinicaltrials.gov/ct2/show/NCT03394365

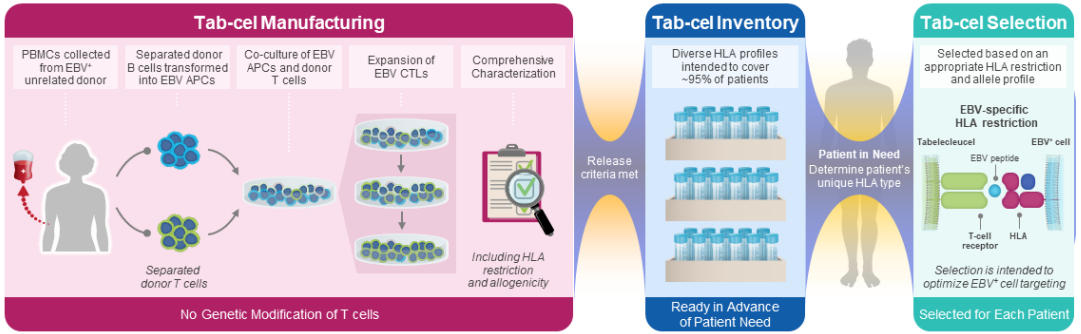
ATA129-302 ALLELE Study: Tabelecleucel Phase 3 Clinical Trial

- **Healthy EBV-positive donors**
- **Each T-cell line:**
  - Virus-specific
  - Extensive depletion of alloreactive T-cells
  - HLA (HR) typed
  - Identifying the restricting HLA allele (TCR recognises EBV peptide in complex with a specific HLA molecule- will increase the probability to eliminate tumor cells through cytotoxicity)
- **Cover ~ 95% of expected patients**



- **Selection based on:**
  - Specificity of the line for the target antigen through shared HLA alleles (HLA restriction)
  - Overall level of HLA match (selecting a lot that shares at least 2 HLA with the immunological profile of the patient will increase the probability of product acceptance and tolerance)
  - Possibility for restriction switch





Withers B, et al. Biol Blood Marrow Transplant 2018;24:2433-42
Gaballa MR, Ramos CA. Curr Treat Options Oncol 2020;21:21
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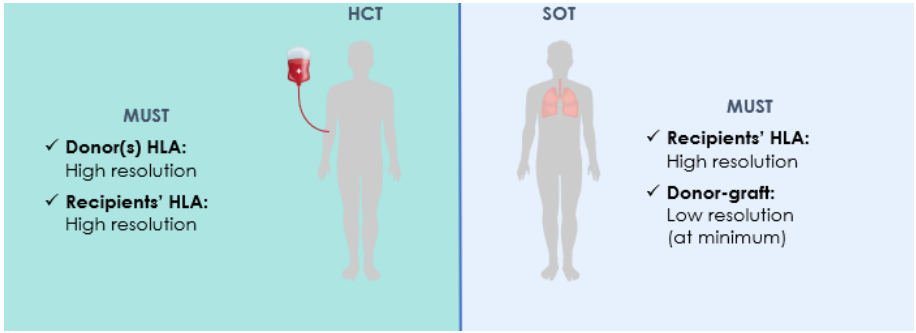
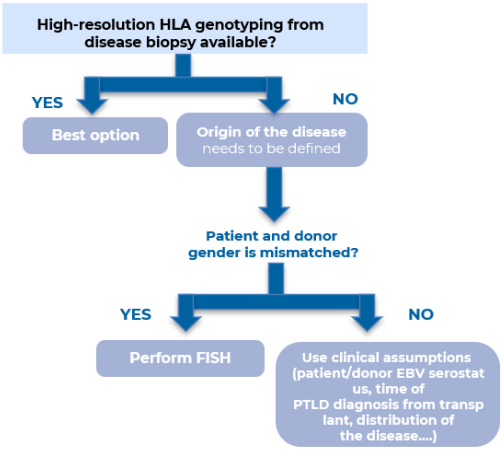
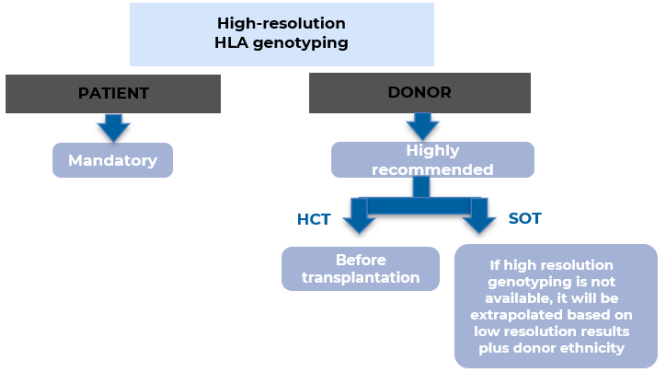


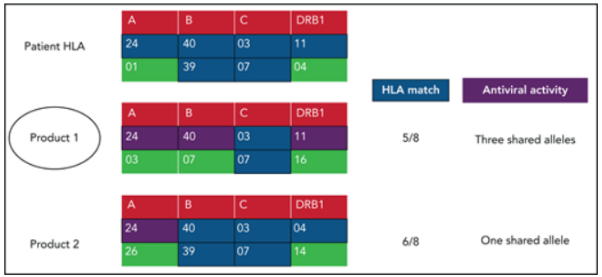




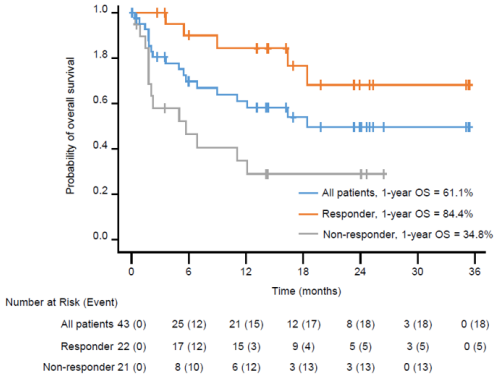
Withers B, et al. Biol Blood Marrow Transplant 2018;24:2433-42
https://clinicaltrials.gov/ct2/show/NCT03394365

ATA129-302 ALLELE Study: Tabelecleucel Phase 3 Clinical Trial

1 shared HLA restriction
+
≥ 1 other compatible HLA allele



	HCT (N=14)	SOT (N=29)	All (N=43)
Responders, n (%)	7 (50.0)	15 (51.7)	22 (51.2)
95% CI	23.0, 77.0	32.5, 70.6	35.5, 66.7
BOR, n (%)			
CR	6 (42.9)	6 (20.7)	12 (27.9)
PR	1 (7.1)	9 (31.0)	10 (23.3)
SD	3 (21.4)	2 (6.9)	5 (11.6)
PD	2 (14.3)	7 (24.1)	9 (20.9)
Not Evaluable	2 (14.3)	5 (17.2)	7 (16.3)
CBR, n (%) 95% CI	10 (71.4) 41.9, 91.6	17 (58.6) 38.9, 76.5	27 (62.8) 46.7, 77.0
TTR, months (range)	1.0 (1.0–4.7)	1.1 (0.7–4.1)	1.0 (0.7–4.7)
Median DOR, months (95% CI)	23.0 (15.9, not estimable)	15.2 (1.2, not estimable)	23.0 (6.8, not estimable)
Estimated median OS, months (95% CI)	NE (5.7, not estimable)	16.4 (5.0, not estimable)	18.4 (6.9, not estimable)
1-year OS rate, % (95% CI)	70.1 (38.5, 87.6)	56.2 (34.6, 73.2)	61.1 (43.7, 74.5)
Responders, n	7	15	22
1-year OS rate, % (95% CI)	100	75.2 (40.7, 91.4)	84.4 (58.9, 94.7)
Non-responders, n	7	14	21
1-year OS rate % (95% CI)	35.7 (5.2, 69.9)	33.6 (10.4, 59.1)	34.8 (14.6, 56.1)
Median follow-up, months (range)	14.1 (2.0–35.4)	6.0 (0.1–35.4)	11.0 (0.1–35.4)

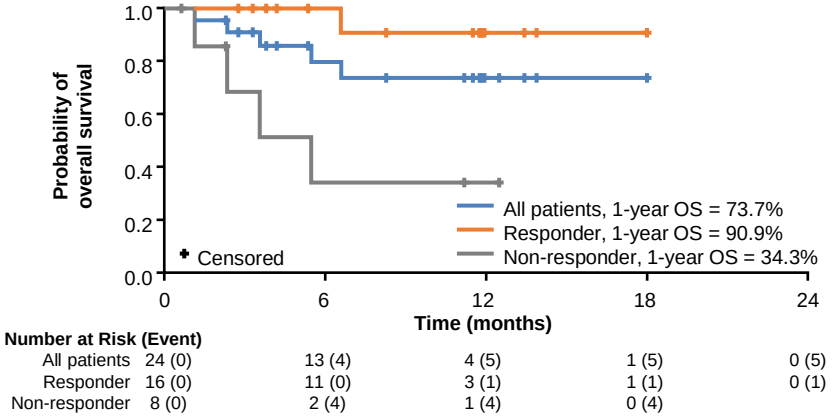


No reports of tumor flare reaction, infusion reactions, cytokine release syndrome, immune effector cell-associated neurotoxicity syndrome, graft rejection, GVHD, or transmission of infectious diseases.

Toner K, Bollard CM. Blood 2022;139:983-94
Mahadeo KM, et al. Blood 2022;140(Suppl1):10374-6

Expanded Access Program (EAP) Tabelecleucel in Europe

	EBV+ PTLD Post-HCT (n=8)	EBV+ PTLD Post-SOT (n=16)	All (N=24)
Responders*, n (%)	7 (87.5)	9 (56.3)	16 (66.7)
95% CI	47.3, 99.7	29.9, 80.2	44.7, 84.4
CR, n (%)	4 (50.0)	4 (25.0)	8 (33.3)
PR, n (%)	3 (37.5)	5 (31.3)	8 (33.3)
SD, n (%)	0	2 (12.5)	2 (8.3)
PD, n (%)	1 (12.5)	5 (31.3)	6 (25.0)
Median TTR, months (range)	1.0 (0.9–1.6)	1.0 (0.8–2.2)	1.0 (0.8–2.2)

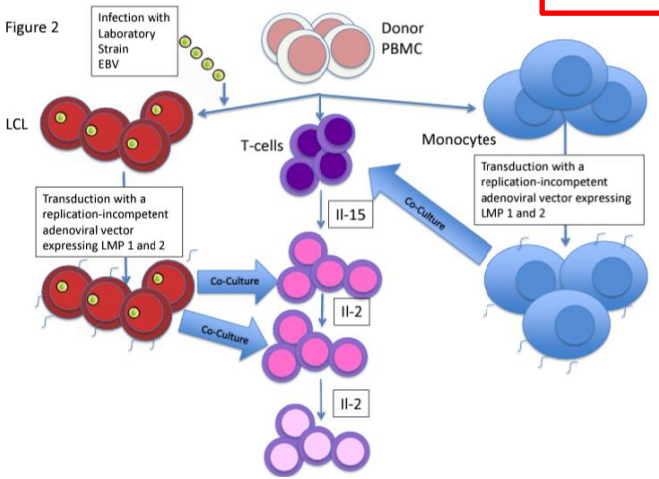


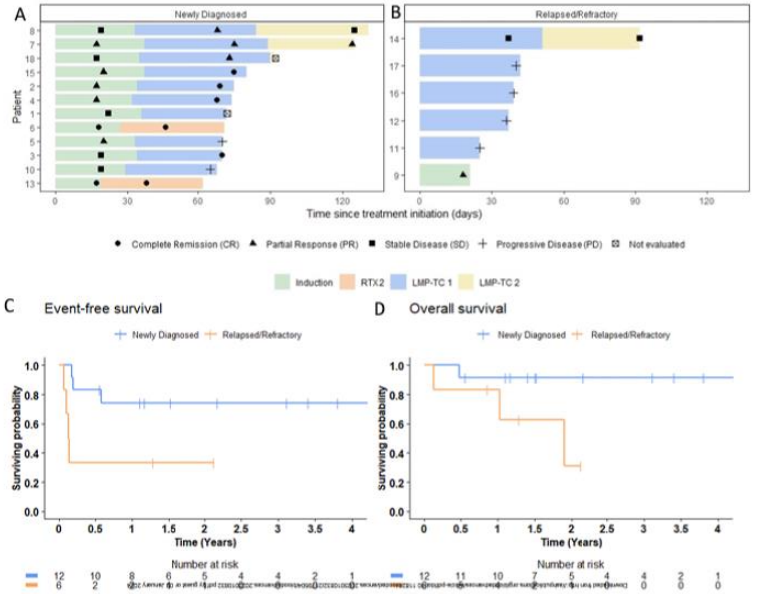
ORR of 75% among patients with EBV+ PCNSL-PTLD (n=4)

Choquet S, et al. J Clin Oncol 2023;41(Suppl 16):7521

ANHL1522: Durable Immunity to EBV post Rituximab and Third Party LMP-specific T-cells: A Children's Oncology Group Study

SOT

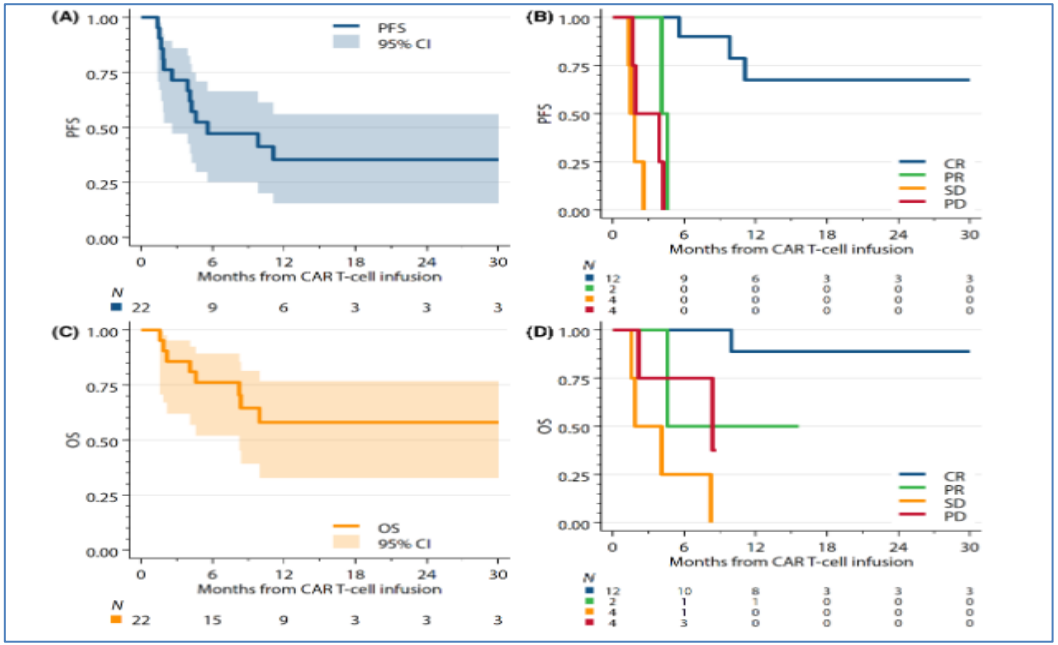




Persistence of VST up to 8 months

Wistinghausen B, et al. Blood Adv 2024.Jan 1 [Epub ahead of print]

Age at PTLD diagnosis, *N* (%)		Bulky disease (≥10 cm at CAR-T), *N* (%)	
Age <60	14 (64)	Yes	5 (23)
Age ≥60	8 (36)	No	17 (77)
Gender, *N* (%)		Bone marrow involvement, *N* (%)	
Male	16 (73)	Yes	4 (18)
Female	5 (22)	No	10 (45)
Unavailable	1 (5)	Unavailable	8 (36)
ECOG at PTLD relapse, *N* (%)		Extranodal sites present, *N* (%)	
0	9 (41)	≤1	17 (77)
1	12 (55)	>1	5 (23)
2	1 (5)		
IPI score prior to CAR-T, *N* (%)		CNS disease involvement, *N* (%)	
1	1 (5)	Yes	1 (5)
2	5 (23)	No	21 (95)
3	11 (50)		
4	2 (9)		
5	1 (5)		
Unavailable	2 (9)		
PTLD stage, *N* (%)		Organ transplant, *N* (%)	
I to II	2 (9)	Kidney	14 (64)
III to IV	20 (91)	Liver	3 (14)
LDH, *N* (%)		Heart	2 (9)
Elevated	18 (82)	Kidney, Pancreas	1 (5)
Normal	4 (18)	Intestine	1 (5)
		Lung	1 (5)
EBV tumour status, *N* (%)			
Positive	1 (5)		
Negative	18 (82)		
Unavailable	3 (14)		



McKenna M, et al. Br J Haematol 2023;202:248-55

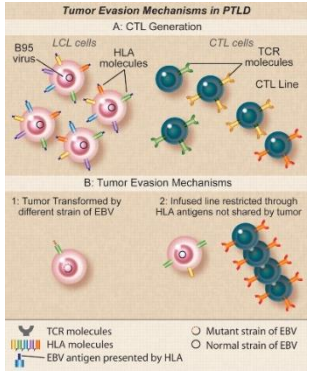
Conclusion

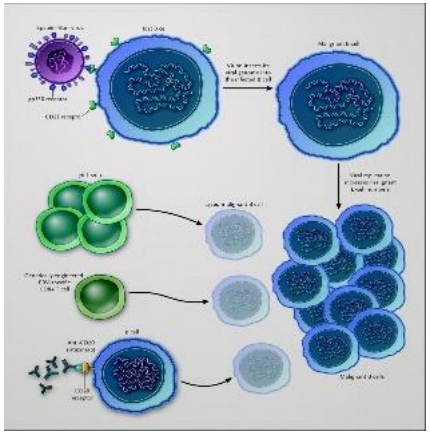
- Safe and effective therapy
- Persistence of EBV-CTLs: necessary for durable response?
  - ➔ up to 9 years following infusion
  - ➔ less long in third party CTLs
- Third party EBV-CTLs
  - ➔ SOT
  - ➔ HSCT
- Safety concerns?
  - ➔ minimal infusion-related toxicity
  - ➔ no CRS/ICANS
  - ➔ negligible GVDH/rejection

Heslop HE, et al. Blood 2010;115:925-35
Heslop HE. Blood 2012;119:2436-8
Doubrinova E, at al. Blood 2012;119:2644-56
Ricciardelli I, et al. Blood 2014;124:2514-22
Dharnidharka VR, Mohanakumar T. N Engl J Med 2015:372:569-71
Bollard CM, et al. J Clin Oncol 2018;36:1128-39
Sinha D, et al. J Immunother Cancer 2021; 9:e001608

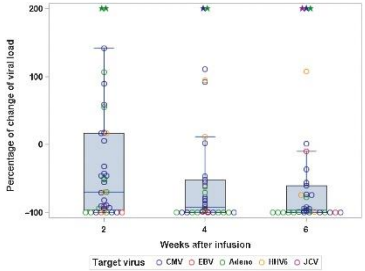
Conclusion

- Resistance





- Future:
  - Gene engineering of CTLs (CNI resistant – ITREC/NCT03131934)
  - Overcoming immunosuppressive properties of TME (dominant negative TGFβ receptor)
  - EBV-CTLs with CART / CARNK (receptors targeting lytic proteins)
  - Combination therapy (CPI, BV, BCL2-antagonists, DMAs,…)
  - Multivirus virus-specific T cells



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Bollard CM, et al. J Clin Oncol 2018;36:1128-39
Sinha D, et al. J Immunother Cancer 2021; 9:e001608
Pfeiffer T, et al. Clin Cancer Res 2023;29:324-30

Conclusion

Clinical trial identifier	Title of the study	Role in PTLD	Target population
NCT03266653	EBV-specific cytotoxic T-lymphocytes (CTLs) for refractory EBV infection	Preventive	Children and adults
NCT05183490	R-MVST cells for treatment of viral infections	Preventive	Adults
NCT04989491	Evaluation of the efficacy of a treatment by one single dose of rituximab (375mg/m^2) in the prevention of the EBV primary infection and posttransplant lymphoproliferative disorder in adult EBV seronegative patients who received an EBV seropositive kidney allograft (REPLY)	Preventive	Adults
NCT04507477	Ex-vivo delivery of rituximab to prevent PTLD in EBV mismatch lung transplant recipients: a pilot trial	Preventive	Adults
NCT02580539	A study of the safety and efficacy of EBV specific T-cell lines (EBV-TCL-01)	Preventive or frontline	Adults
NCT02900976	Rituximab and LMP-specific T-cells in treating pediatric solid organ recipients with EBV-positive, CD20-positive posttransplant lymphoproliferative disorder	Frontline	Children and adults
NCT04337827	Rituximab and acalabrutinib in newly diagnosed B-cell posttransplant lymphoproliferative disorder	Frontline	Adults
NCT04554914	A study to evaluate tabelecleucel in participants with EBV–associated diseases	Frontline	Children and adults
NCT05786040	Tafasitamab and rituximab for front-line treatment of posttransplant lymphoproliferative disorder	Frontline	Adults
NCT01192464	EBV CTLs expressing CD30 chimeric receptors for CD30$^+$ lymphoma (CARCD30)	Frontline or relapsed	Children and adults
NCT03131934	Immunotherapy with tacrolimus resistant EBV CTL for lymphoproliferative disease after solid organ transplant (ITREC)	Frontline or relapsed	Children and adults
NCT05011058	An open-label, phase 2 trial of nanatinostat in combination with valganciclovir in patients with EBV$^+$ relapsed/refractory lymphomas (NAVAL-1)	Relapsed	Adults
NCT03394365	Tabelecleucel for solid organ or allogeneic hematopoietic cell transplant participants with EBV-associated posttransplant lymphoproliferative disease (EBV$^+$ PTLD) after failure of rituximab or rituximab and chemotherapy (ALLELE)	Relapsed	Children and adults
NCT04664179	EBV-specific T-lymphocytes for treatment of EBV$^+$ lymphoma (CILESTE)	Relapsed	Children and adults
NCT04925544	Clinical trial of a novel small molecule EBNA1 inhibitor, VK 2019, in patients with EBV$^+$ nasopharyngeal cancer (NPC) and other EBV-associated cancers, with pharmacokinetic and pharmacodynamic correlative studies	Relapsed	Adults
NCT05714748	Application of mRNA immunotherapy technology in EBV-related refractory malignant tumors	Relapsed	Adults
NCT02287311	Most closely matched 3rd party rapidly generated LMP, BARF1 and EBNA1 specific CTL, EBV$^+$ lymphoma (MABEL)	Relapsed	Children and adults

Amengual JE, Pro B. Blood 2023;142:1426-37