Resistant Cytomegalovirus - actual management and future directions

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Disclosures

- Consulting fees:
  - Sunovion
  - Paladin
  - Merck
  - Basilea

- Clinical trials:
  - SHIRE: Maribavir for refractory or resistant CMV infection

Definition of CMV antiviral resistance

- **Definition:**
  - Genetic alteration that decreases the susceptibility to one or more antiviral drugs.

- **Manifestations of antiviral resistance:**

Persistent or increasing CMV viral load

OR

Persistent or worsening symptomatic disease

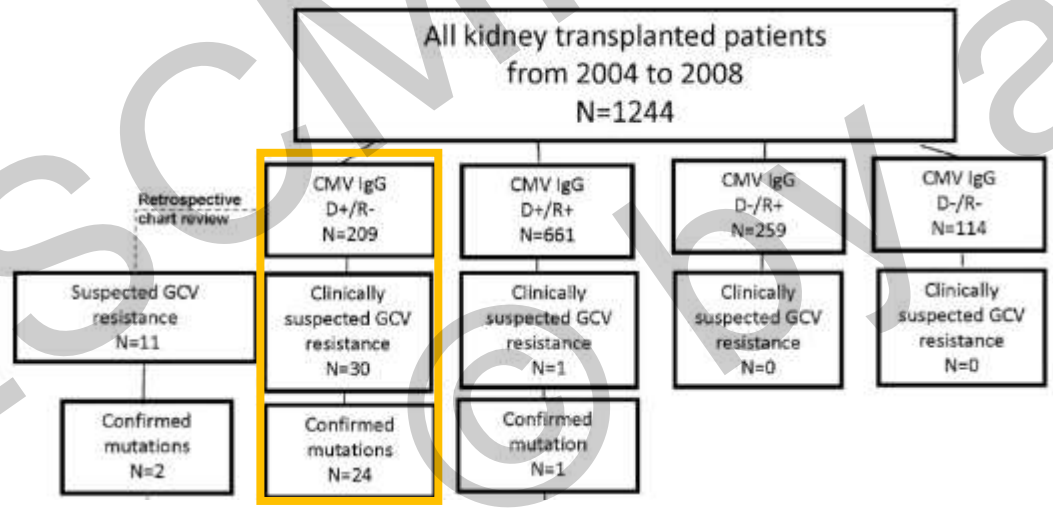
AND

Normally effective dosage and duration of antiviral therapy

Kotton et al. Transplantation 2018 (ahead of print)

Risk factors for resistant CMV

- **Risk factors:**
  - Prolonged antiviral exposure & ongoing active replication:
    - Lack of prior CMV immunity
    - Strong immunosuppressive therapy
    - Inadequate drug delivery
  - Preemptive management of CMV high-risk transplant recipients



CMV D+/R-: Suspected 14%; confirmed 11.5%
CMV D+/R+: Suspected 0.15%; confirmed 0.15%

Kotton et al. Transplantation 2018 (ahead of print)
Myhre HA, et al. Transplantation 2011

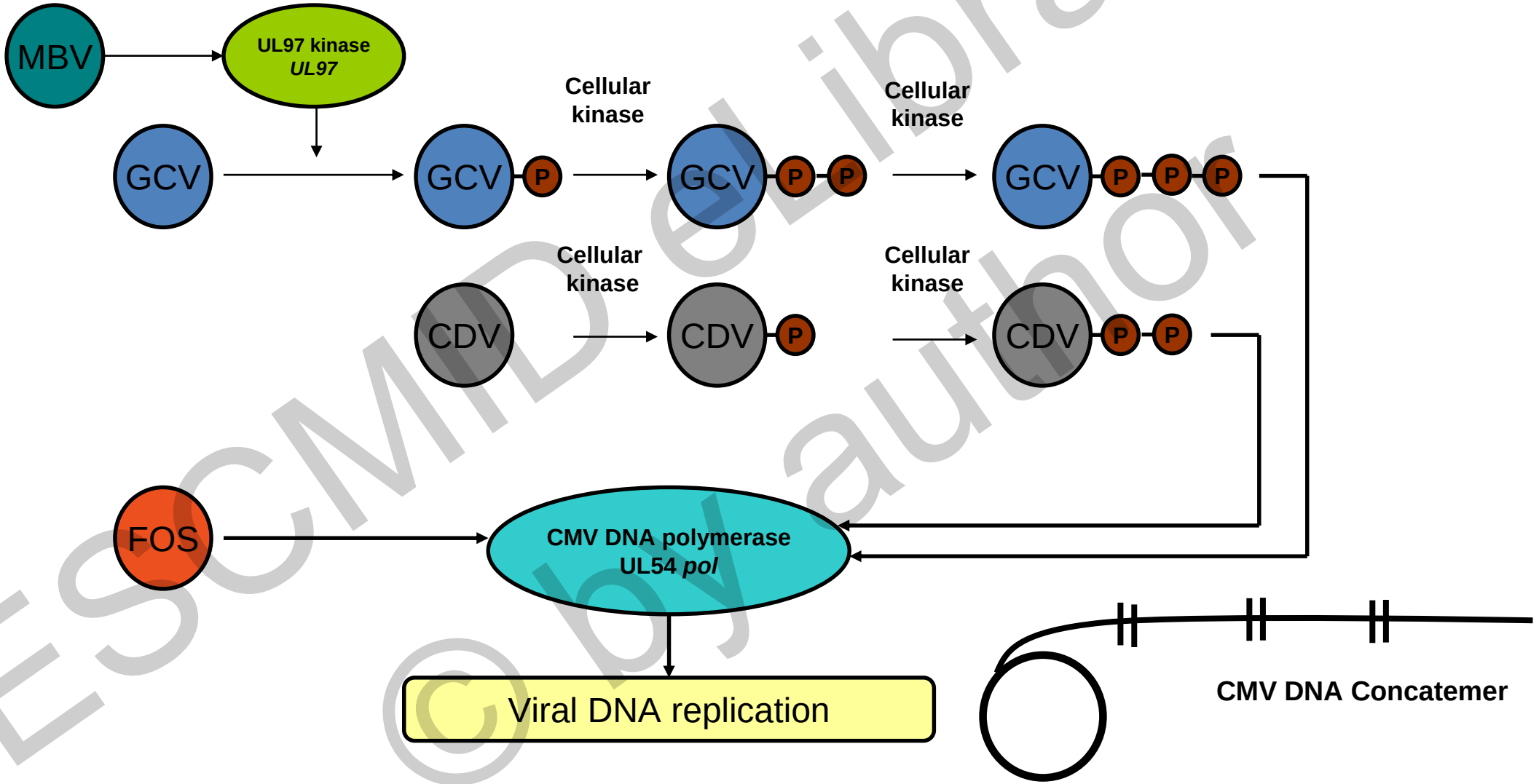
Consequences of resistant CMV

- Resistant CMV infection is associated with:
  - Delayed clearance of viremia
  - Worse kidney function and higher rater of rejection in kidney transplant patients
  - Less number of days alive and out of hospital
  - Increased mortality
- Compared to CMV infection with susceptible strains

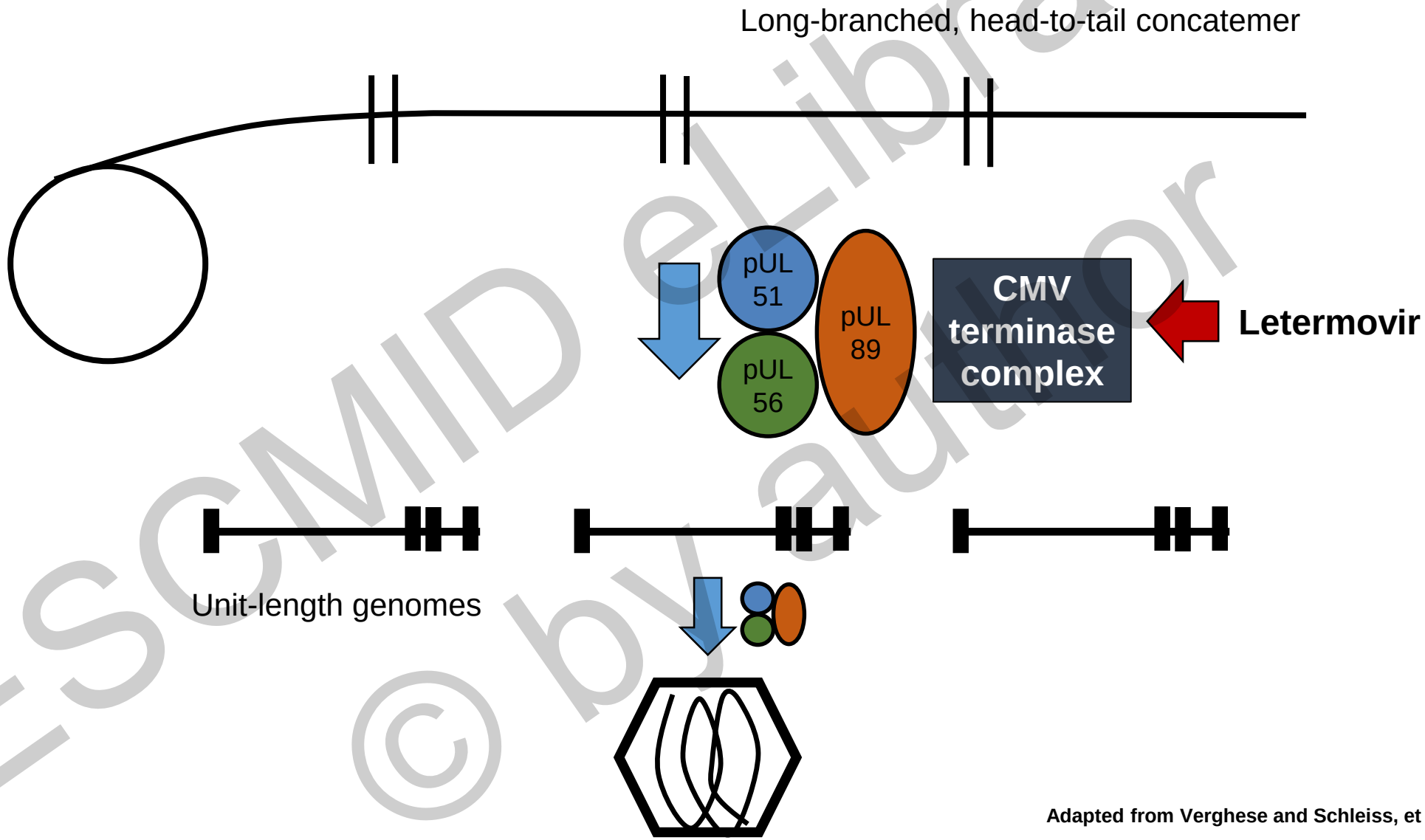
Outcome	Resistant CMV N=37	Susceptible CMV N=109	P
Days to clearance	113	53	0.006
≥20% decrease eGFR by 3 months	42%	19%	0.008
Days alive and outpatient by 3 months	72.7	81.0	0.039
Mortality			
• 3 months	11%	1%	0.004
• 12 months	16%	5.5%	0.032

Fisher et al. Clin Infect Dis 2017

Mechanism of action of antivirals for CMV



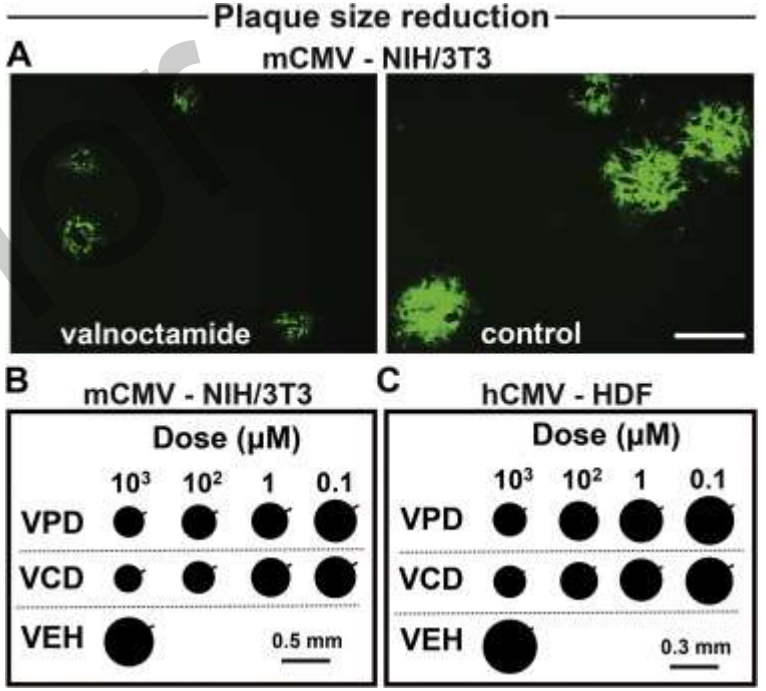
Mechanism of action of antivirals for CMV



Adapted from Verghese and Schleiss, et al. Drugs Future 2013

Phenotypic CMV resistance testing

- Cell culture & Reduction of plaque.
- Determination of the concentration of the drug needed to inhibit viral growth by 50% (EC50).
- Ratio EC50 compared to wild-type strains.
- Recombinant phenotyping: Introduction of specific mutations into a baseline CMV strain.



Ornaghi et al. Virology 2016

Genotypic CMV resistance testing

- Sequence analysis of PCR-amplified CMV DNA from clinical specimens for the presence of diagnostic mutations.
- In patients treated with ganciclovir/valganciclovir, UL97 kinase gene mutations appear first
- Specific regions cover most of resistance mutations:
  - UL97: Codons 450-650
  - UL54: Codons 300-1,000
- Some non-canonical mutations may appear after antiviral exposure. Recombinant phenotyping is the preferred strategy to clarify antiviral resistance.

Canonical UL97 mutations

- Seven “canonical” mutations account for over 80% of cases of GCV resistance

5-15x	2-5x
M460V	C592G
M460I	
H520Q	
A594V	
L595S	
C603W	

Kotton et al. Transplantation 2018 (ahead of print)

Management of resistant CMV infection

- **Antivirals**
  - High-dose ganciclovir / valganciclovir
  - Foscarnet
  - Cidofovir
- **Other drugs with antiviral activity**
  - IV immunoglobulins/CMV-specific Ig G
  - Leflunomide
  - mTOR inhibitors
  - Artesunate
- **CMV-specific CTLs**
- **Future directions: New antivirals**
  - Maribavir
  - Letermovir
  - Brincidofovir

Treatment of Resistant CMV according to phenotype

Kotton et al. 3rd international consensus. Transplantation 2018

EC50	Level of resistance	Usual mechanism	Most common SNPs	Action
< 2 fold	Insignificant	Single UL97		Continue GCV
2-5 fold	Low-grade	Single UL97	C592G	Continue GCV/Dose escalation
5-15 fold	Moderate	Single UL97	M460V/I H520Q A594V L595S C603W	Foscarnet
> 15 fold	High-level	Combined UL97 and UL54		Combination treatment

High-dose GCV/VGCV for Resistant CMV

Gracia-Ahufinger et al. Transplantation 2013

D/R	Type Tx	CMV prevention	Symptoms	Peak VL	Mutation	EC_{50}	Time to negative VL
+/-	K	Preemptive	None	5011	C603W	5-15X	7
+/-	K	Proph	None	1251	M460V	5-15X	14
+/-	SPK	Proph	Colitis	1398	A594T	2-5X	21
+/-	Liver	Proph	None	1794	L595S	5-15X	28
+/-	K	Proph	None	3981	T503I (UL54)	2-5X	42
-/+	K	Preemptive	None	1794	A594V	5-15X	21

- No Lung transplant recipients
- Mild disease
- Rate of neutropenia (<1,500): 4/6
- Complications: None

Foscarnet & Cidofovir for Resistant/Refractory CMV

Foscarnet

- N=39
- SOT: 22 (56%); HSCT: 17 (44%
- Indications:
  - Resistant/refractory
- GCV resistant: 38%
- Virological failure:
  - 6/22 (27%) SOT
  - 7/17 (41%) HSCT
- Renal failure (>20% ↓ eGFR)
  - 12/22 (55%) SOT
  - 8/17 (47%) HSCT

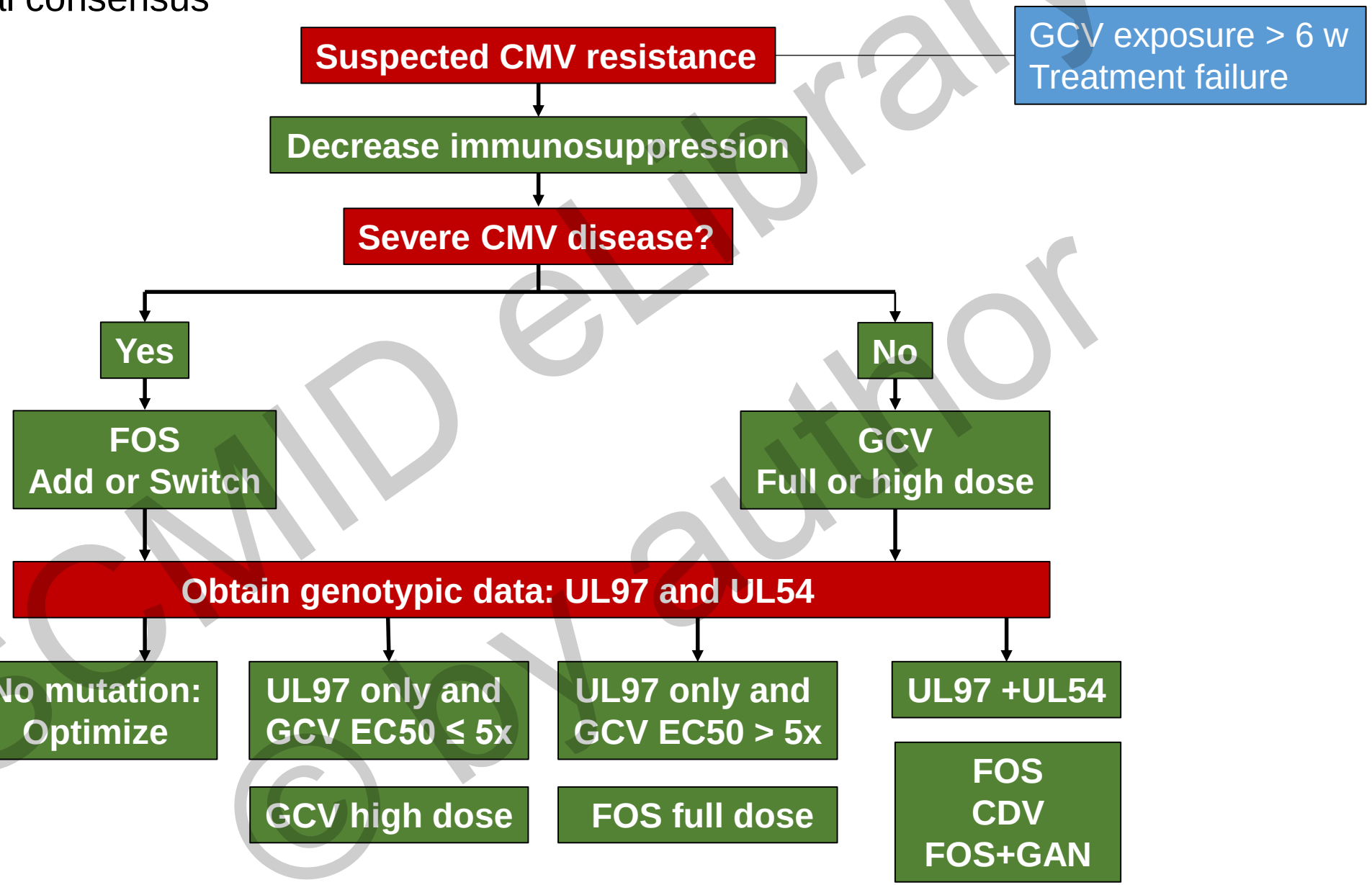
Cidofovir

- N=9
- SOT: 9 (100%)
- Indications:
  - Side effects: 5
  - Lack of response: 4
- GCV resistant: 22%
- Response:
  - Complete: 7
  - Partial 2
- Renal failure
  - 8/9 (89%). Transient 2

Avery et al. Transplantation 2016
Bonatti et al. Surg Infect 2017

Management of confirmed or suspected CMV resistance flowchart

3rd international consensus



CMV Immunoglobulins for Resistant CMV

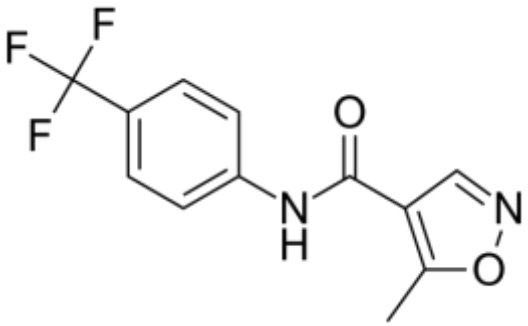
Thoracic Transplant Recipients

Study	N	Symptoms	CMV Ig G	Antiviral	Outcome
1	15	No	Cytotect	None	9/15 negative 1 Rx 5/15 negative 2 Rx 1/5 negative >2 Rx
2	1	Yes	Unknown	Valganciclovir	Negative NAT
3	1	Yes	Cytotect	None	Complete recovery
4	2	Yes	Unknown	None	Clinical recovery & persistent NAT
5	1	Yes	Cytotect	Foscarnet	Decrease viral load
6	4	Yes	Cytotect	Ganciclovir	Favorable outcome
7	1	Yes	Immuno-AG	Ganciclovir	Improvement

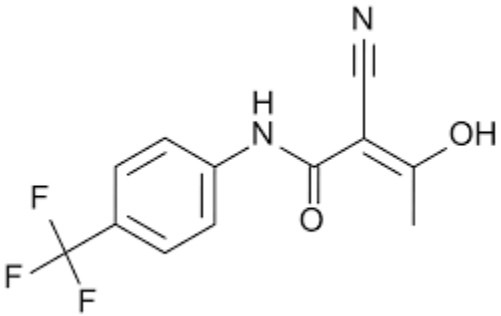
1. Schulz et al. Transplantation 2016;100: S5–S10
2. Rouphael et al. Am J Transplant 2011;11:1330-3
3. Chou et al. Zhonghua Yi Xue Za Zhi (Taipei). 1996;57:310–313
4. Cremer et al. Dtsch Med Wochenschr. 1988;113:18–20
5. Schulz et al. Transplantation 2016;100: S5–S10
6. Kneidinger et al. Int J Infect Dis 2014;28:140–142
7. López Garcia-Gallo et al. Transplant Proc 2005;37:4043–4045

Leflunomide

- Family: Malononitrile amides.
- Active metabolite: Teriflunomide
- Mechanism of action: Acts at a late stage of virion assembly
- Dosing:
  - Loading 100 mg PO daily for 3-5 days
  - Then 20-40 mg PO daily
- Teriflunomide: half-life of 5-15 days in kidney transplant patients
- Target trough levels teriflunomide 50-80 µg/mL (trough levels variable)
- Trough levels > 100 µg/mL are associated with side effects (25-30%).
  - Anemia
  - Diarrhea
  - Leukopenia
  - Polyneuropathy



Leflunomide



Teriflunomide

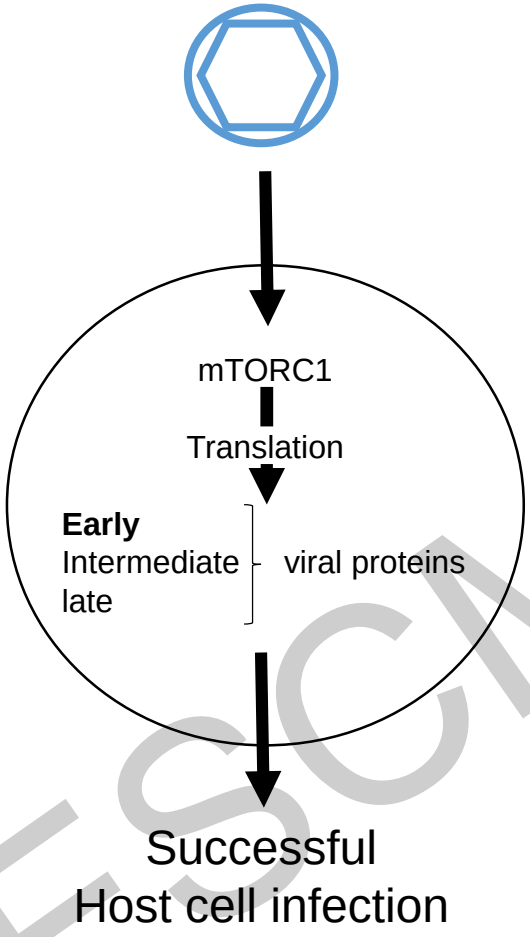
Leflunomide for resistant CMV infection

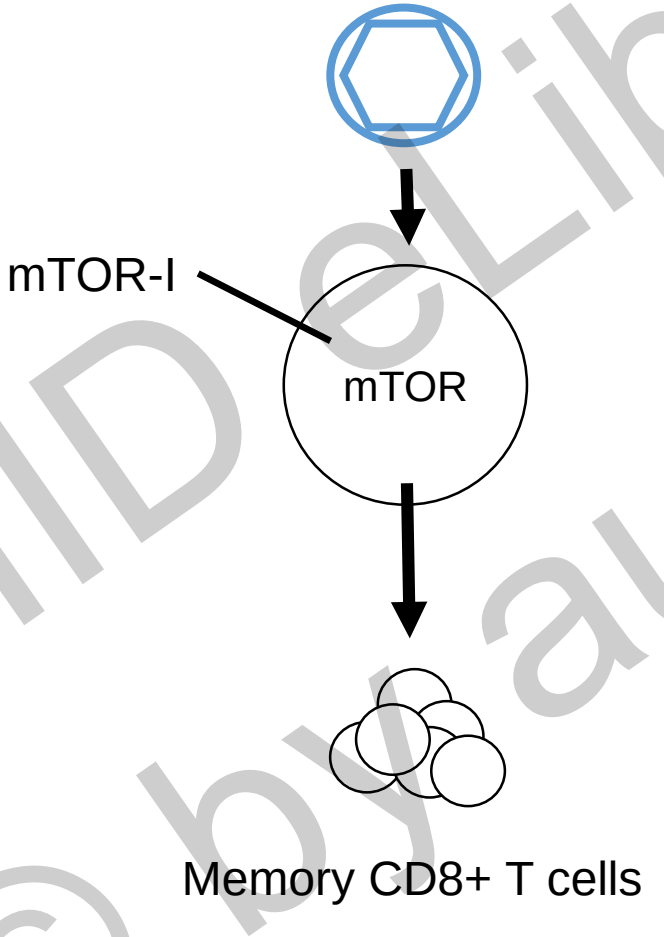
Avery RK, et al. Transplantation 2010;90:419-26

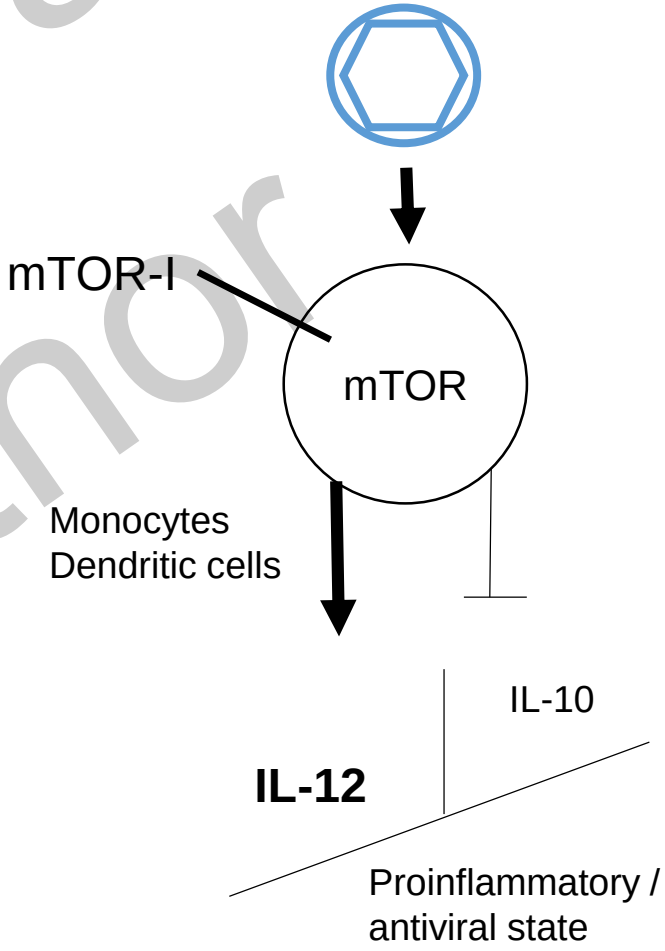
Patient	VL at start of LEF	Peak VL	VL at LEF d/c	Other agents	Adverse events	CMV—LEF outcome
1	4768	210,426	Undetectable	GCV[a]	Alk phos[b]	Suppressed
2	16,460	111,342	Undetectable	GCV, VGCV[c]	Diarrhea, leukopenia[b]	Suppressed
3	7085	105,971	Undetectable	GCV, IVIg[d]	None	Suppressed
4	522	91,291	Undetectable	VGCV[e]	None	Suppressed
5	826	174,677	Undetectable	VGCV[f]	Diarrhea, anemia	Suppressed
6	1665	27,575	Undetectable	None	None	Suppressed
7	2259	19,166	Undetectable	None	Anemia	Suppressed
8	9475	126,973	Undetectable	None	None	Suppressed
9	110,171	110,171	Undetectable	GCV[g]	Anxiety[b]	Suppressed
10	169,303	457,973	1307	VGCV, GCV, CDV, CMVIg, IVIg	Diarrhea[b]	Transient
11	26,182	166,841	3057	GCV, IVIg	Diarrhea, neuropathy[b]	Transient
12	238,039	238,039	Undetectable, then 56,702	Foscarnet	Bilirubin[b]	Transient
13	11,630	52,707	11,363	VGCV	Pancytopenia[b]	Transient
14	44,723	580,397	20,354	GCV, foscarnet, CMVIg	None	Transient
15	167,540	201,862	860,026	GCV, foscarnet, CDV, CMVIg	Diarrhea	Failed
16	5031	1,246,314	1636	VGCV, CMVIg	None	Failed
17	56,466	257,319	30,765	Foscarnet, CMVIg, maribavir	Anemia, diarrhea, increased creatinine	Failed

- Only 2/9 patients with ≥ 4 log CMV VL cleared viremia
- All except 1 patient who failed CMV DNA clearance had CMV VL ≥ 4 log at start of LEF.

Antiviral activity of mTOR inhibitors







Adapted from Nashan et al. Transplantation 2012

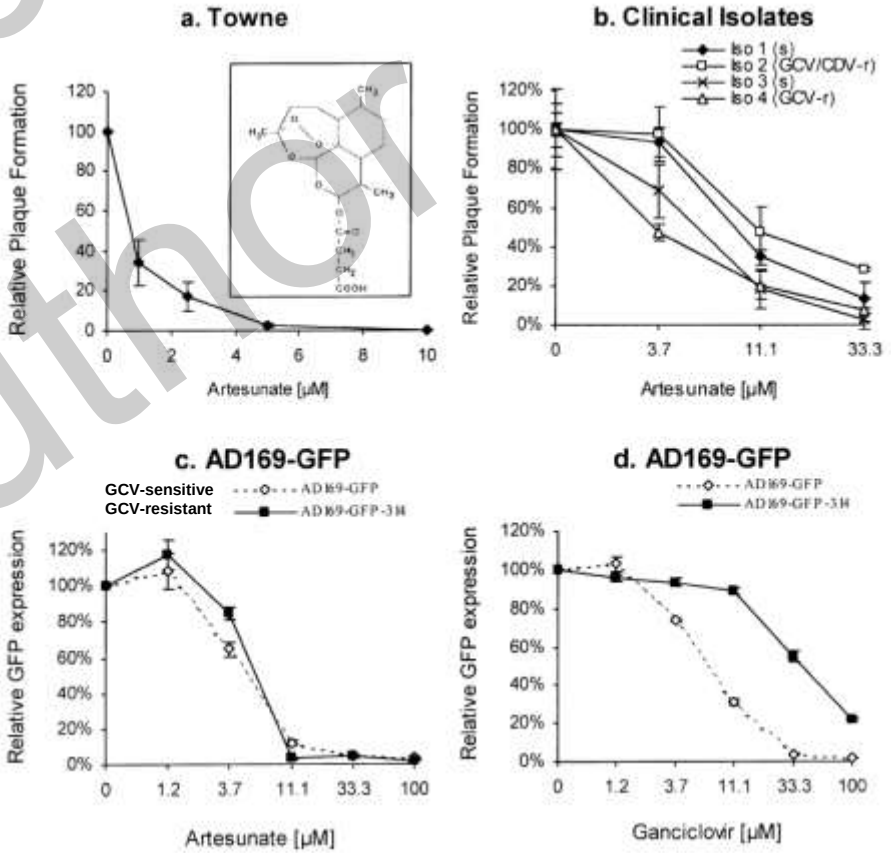
mTOR inhibitors switch for resistant CMV

Sabe N, et al. Transplant Int 2012;25:e78-82

Variable	Patient 1	Patient 2
Sex/age, years	Male/57	Male/49
Type of transplant	Heart	Kidney
Donor/recipient CMV sero-status	Positive/Negative	Positive/Positive
Previous valganciclovir	Yes	Yes
CMV infection manifestations	Viral syndrome	Viremia
Immunosuppressive regimen	Prednisone; cyclosporine; mycophenolate mofetil	Prednisone; tacrolimus; mycophenolate mofetil
CMV mutation	A594V in UL97 gene	A595S in UL97 gene
Antiviral treatment, before switching to mTOR inhibitor	Ganciclovir; foscarnet	Valganciclovir; ganciclovir
Drug-related adverse effects	Neutropenia; genital ulcers; encephalopathy; renal failure	Neutropenia
Switching from calcineurin inhibitors to mTOR inhibitor	Cyclosporine; everolimus	Tacrolimus; sirolimus
Outcome	Negativization of CMV antigenemia; cure	Negativization of CMV antigenemia; cure

Antiviral activity of Artesunate against CMV

- Artesunate (ART) is a semisynthetic derivative of artemisinin
- Strong anti-malarial activity
- Artesunate effects a strong inhibition of plaque formation of GCV sensitive and resistant CMV strains
- Mechanism of action:
  - Inhibition of CMV-induced DNA binding activities of NF-κB and Sp1
  - Inhibition of PI3-K activity, leading to decreased activity of a variety of kinases



Efferth et al. J Mol Med 2002

Clinical Efficacy of Artesunate for resistant CMV

Type Tx	D/R	Antivirals	Symptoms	Peak VL	Artesunate	Effect of Artesunate on CMV VL	Final outcome
HSCT	-/+	VACV, VGCV, GCV, CDV, FOS	Fever/neutropenia	>4 log	200 mg QD PO	Negative in 4 weeks	Alive
Lung	+/-	VGCV, GCV	Fever/neutropenia	> 4 log	200 mg QD PO	Favorable in 3 months	Alive
Lung	+/-	VGCV, GCV	Fever/neutropenia	> 4 log	200 mg QD PO	Favorable in 3 months	Alive
Kidney	+/-	VGCV, GCV, FOS	Pneumonitis	> 5 log	80 mg QD PO	Decrease > 2 log in 7 days	Death
HSCT	+/+	VGCV, GCV, FOS	Pneumonitis	> 7 log	120 mg QD	Decrease 1 log in 24 days	Death

Germi et al. Antiviral Res 2014

Adoptive immunotherapy with CMV-specific CTL

- Complex process of generating and/or expanding CMV-specific CTLs
- Expansion strategies:
  - Ex-vivo expansion of CMV-specific CTLs:
    - Manufacture time 10 days-3 months
    - Long-term persistence of the cells (>10 years)
    - Good memory response
  - Direct selection of donor T cells:
    - Restricted to HLA-type
    - Rapid manufacturing time

Published trials: Transplant donor-derived expanded single CMV-specific CTLs. HSCT recipients

N	CTL activator	GVHD	Antiviral effect
14	CMV virion proteins	3; mild disease	Reconstitution of CMV-specific immunity in all patients
8	CMV lysate	-	5 cleared after 1st dose of CTL 1 cleared after a 2nd dose 1 did not clear 1 not evaluable
16	DCs pulsed with CMV antigens	3 mild disease	Massive in vivo expansions of CMV-specific CTLs resulting in reconstitution of viral immunity
25	CMV antigen	1	7 CMV reactivation 5 CMV disease
9	DCs pulsed	3 severe GVHD	2 CMV reactivation 0 CMV disease
7	Peptide mixes	-	5 had increase CMV replication
16	Peptide related to pp65	-	14 cleared CMV viremia

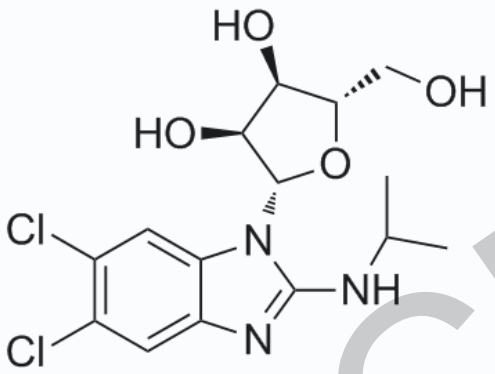
Adoptive treatment for resistant CMV in SOT recipients

	Case 1	Case 2	Case 3
Type of Tx	Kidney	Lung	Lung
Source of T cells	Healthy donor	Healthy donor	Autologous
Side effects	Mild fever	No	No
CMV VL at start of Rx	6 log	3 log	4 log
Evolution of CMV viral load	4 months: 682 12 months 73	Undectable after 1st infusion	Persistent CMV DNAemia
Outcome	Curation	Curation	Death: CMV pneumonitis Graft rejection

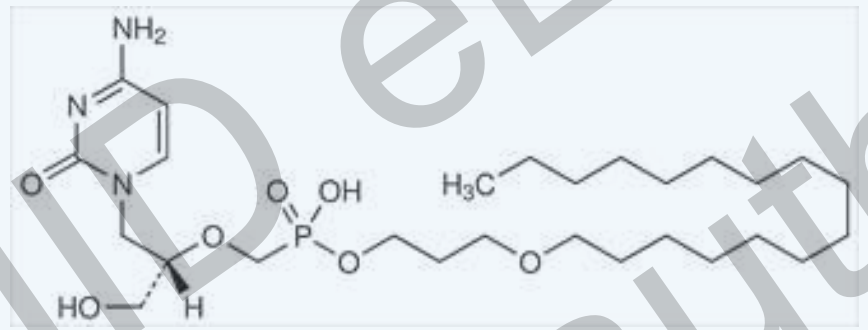
Macesic N, et al. Am J Transplant 2015;15(3):827-32
Holmes-Liew CL, et al. Clin Transl Immunology 2015:4(3):e35
Brestich G, et al. Am J Transplant 2009;9:1679-1684

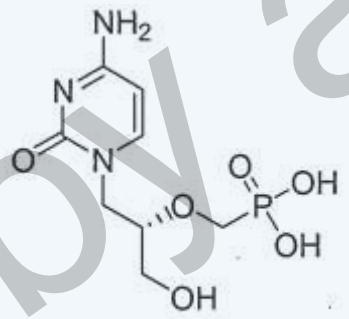
Upcoming drugs for the management of CMV

Maribavir

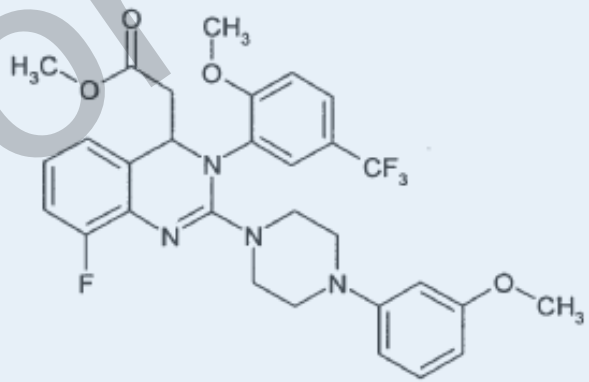


Brincidofovir





Letermovir



Maribavir

- Orally bioavailable benzimidazole riboside
- Binds to UL97 viral protein kinase
- Inhibit viral encapsidation and nuclear egress of viral particles
- Maribavir failed to prevent CMV disease in liver transplant patients and hematopoietic stem cell transplants
- The dose selected for clinical trials was 100 mg bid PO
- The most frequent side effect is dysgeusia
- Non myelotoxic or nephrotoxic

Maribavir for resistant CMV infection

Avery RK, et al. Transplant Infect Dis 2010;12:489-96

- Six patients with confirmed or suspected antiviral resistance were treated with MBV
- Dose of Maribavir: 400 mg twice daily

Type of Tx	Serology	Months post-Tx	Symptoms	Genotypic resistance	Baseline CMV DNA	Days on MBV until undetectable	Total days of MBV	MBV resistance
Kidney	D+/R-	12	Glomerulitis, retinitis	GCV	58,500	-	228	No
Lung	D+/R-	16	Pneumonitis	GCV	50,947	6	186	-
Lung	D+/R-	33	Duodenitis, retinitis	GCV+FOS+CDV	7200	41	147	No
Heart	D+/R-	17	None	GCV+FOS	1,811,171	-	376	T409M H411Y
Small intestine	D+/R-	5	Enteritis	Unknown	18,203	20	227	-
Allo-SCT	D-/R+	0.7	Pneumonitis	Unknown	8283	10	15	-

Maribavir for resistant CMV infection. Active clinical trial

- ClinicalTrials.gov Identifier: NCT02931539
- Randomized, parallel assignment, comparing:
  - Maribavir: 200 mg twice daily
  - Investigator-assigned treatment
- Sample size: 351
- Primary outcome measure: CMV viremia clearance at the end of study Week 8
- Rescue arm to maribavir if patients assigned to investigator-assigned treatment develop side effects

https://clinicaltrials.gov/ct2/show/NCT02931539

Letermovir vs Placebo to prevent CMV infection in HSCT

Marty FM, et al. N Engl J Med. 2017 Dec 21;377(25):2433-2444

- Allo-HSCT recipients CMV seropositive.
- Randomized 2:1 to receive Letermovir 480 mg QD or 240 QD if receiving concomitant cyclosporin or placebo.
- Evidence of engraftment not required.
- Absence of CMV replication at baseline.
- Prophylaxis through week 14 (aprox. 100 days).
- Primary end-point: Proportion of patients with clinically significant CMV infection* through week 24. Discontinuation of the trial before week 24 or had missing data were imputed as having a primary end-point.

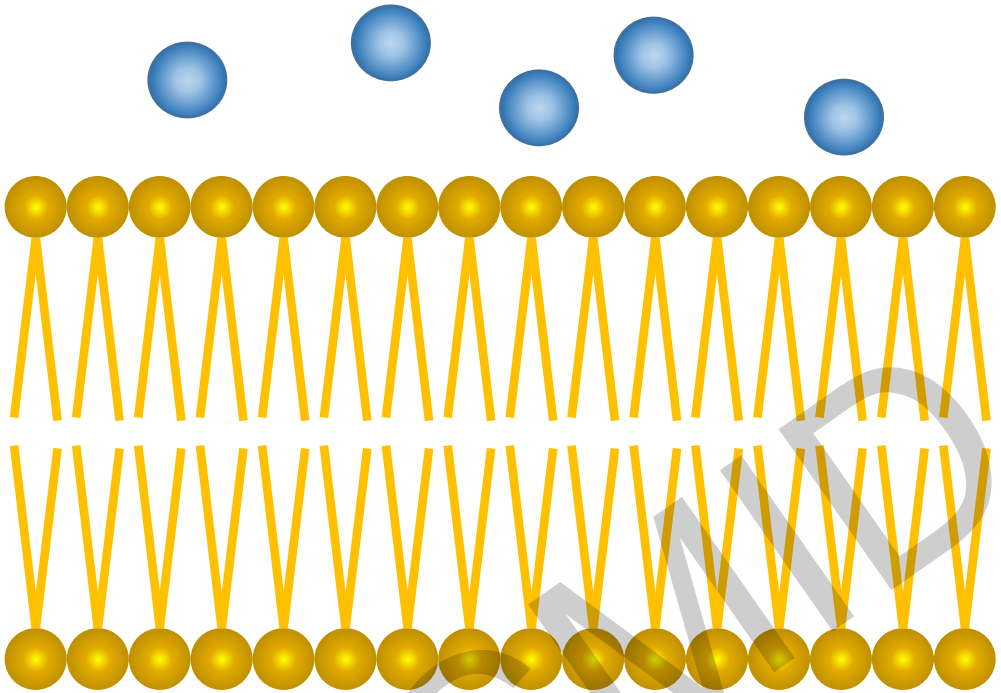
Letermovir. Primary efficacy end-point. Primary efficacy population

Marty FM, et al. N Engl J Med. 2017 Dec 21;377(25):2433-2444

	Letermovir (n=325)	Placebo (n=170)	Difference (95% CI)	P value
Key secondary endpoint at Week 14 after transplantation, n (%)	62 (19.1)	85 (50.0)	-31.3 (-39.9, -22.6)	<0.001
Clinically significant CMV infection, n (%)	25 (7.7)	67 (39.4)		
Initiation of PET	24 (7.4)	65 (38.2)		
CMV disease	1 (0.3)	2 (1.2)		
Discontinued study before Week 14, n (%)	33 (10.2)	16 (9.4)		
Owing to AE	5 (1.5)	1 (0.6)		
Owing to death without CMV	14 (4.3)	6 (3.5)		
Owing to other reasons	14 (4.3)	9 (5.3)		
Missing outcome in Week 14 visit window, n (%)	4 (1.2)	2 (1.2)		

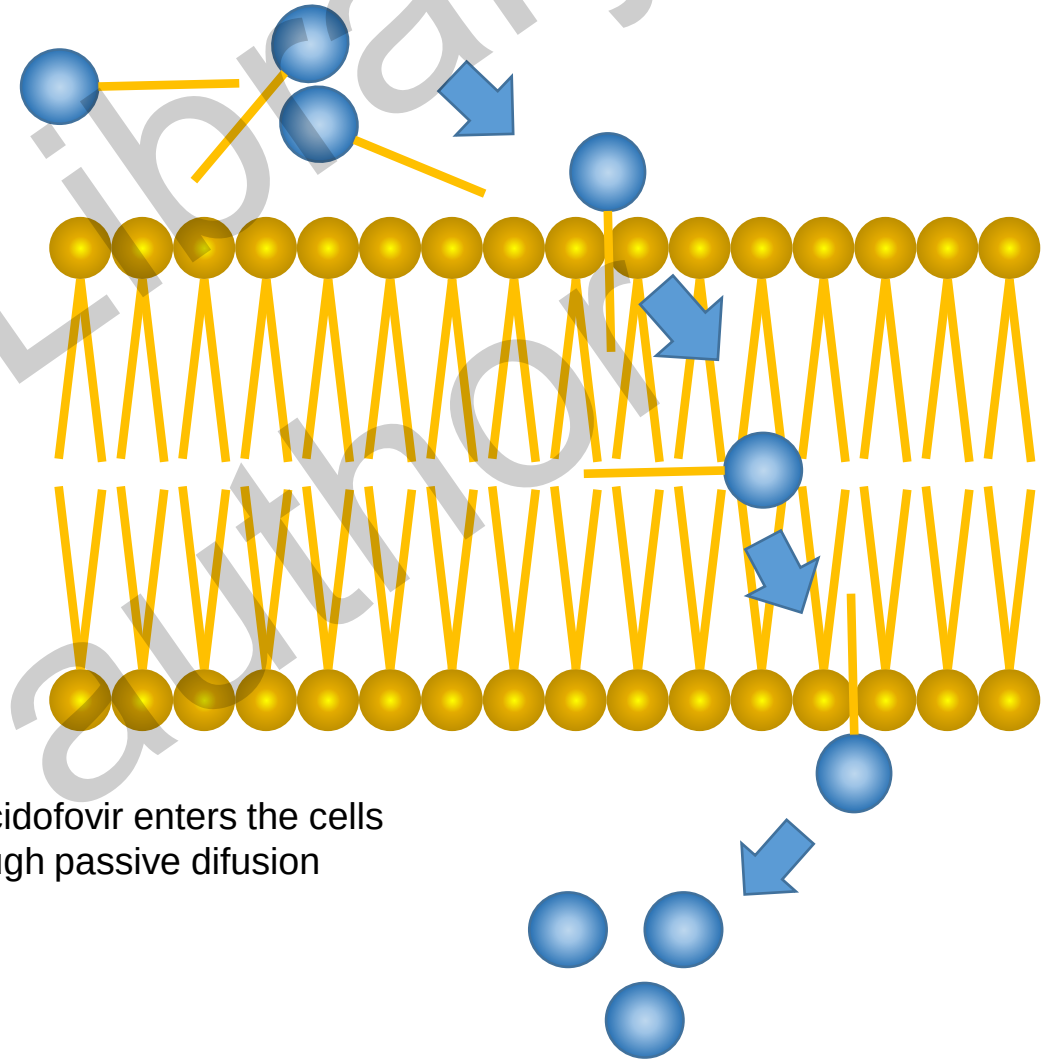
- 12 patients DC letermovir before the development of clinically significant CMV infection
- One patient had completed the trial regimen 6 days within the week 14 window before CMV
- The other 12 events occurred while patients were receiving letermovir for a median of 15 days
  - Central CMV DNA levels were less than 151 copies per milliliter in 10/12
  - 1/2 successfully sequenced specimens showed V236M mutation in UL56 gene

Pharmacodynamic. Cidofovir vs Brincidofovir



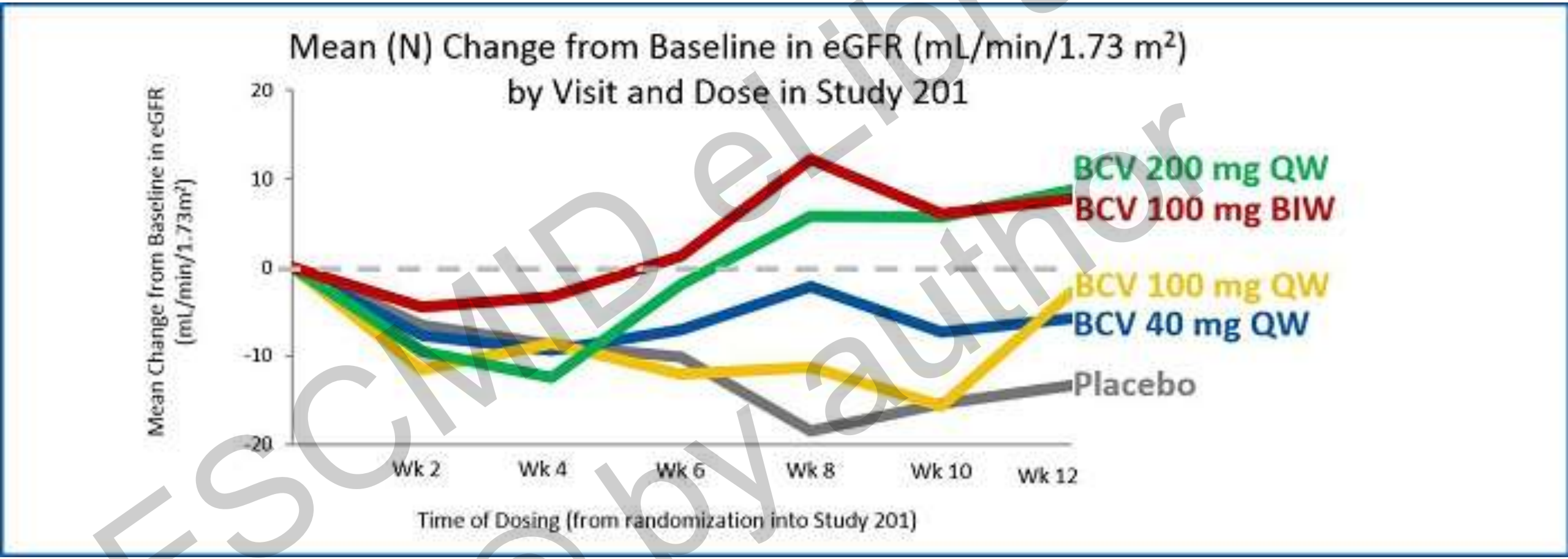
Endocitosis

In the kidney, the organic anion transporter recognize CDV as an organic anion and concentrate the compound (nephrotoxicity)



Brincidofovir enters the cells through passive difusion

Brincidofovir-associated nephrotoxicity



Brincidofovir vs Placebo to prevent CMV infection in HSCT

Marty FM, et al. N Engl J Med. 2013;369(13):1227-36

- Allo-HSCT seropositive for CMV randomized 3:1 to CMX001 vs placebo.
- Stratification according to baseline CMV VL and GVHD
- Dose escalation: 40, 100 or 200 mg weekly and 100 or 200 mg two times week.
- Length of Rx: 9-11 weeks to stop Rx at week 13 post-Tx
- Primary efficacy end-point: Failure to prevent progressive CMV infection:
  - CMV disease or a plasma CMV DNA greater than 200 copies/mL

Brincidofovir vs Placebo - HSCT

	Placebo 59 participants 22/59 (37%)	CMX001 40 mg weekly 13/25 (52%)	CMX001 100 mg weekly 6/27 (22%)	CMX001 200 mg weekly 12/39 (31%)	CMX001 200 mg 2xweek 7/30 (23%)	CMX001 100 mg 2xweek 5/50 (10%)
Primary efficacy end-point Risk difference	Reference	15 (-8 to 38) P=0.23	-15 (-35 to 5) P=0.22	-6 (-26 to 13) P=0.53	-14 (-34 to 6) P=0.24	-27 (-42 to -12) P=0.002
Secondary EP CMV disease	2 (3%)	3 (12%)	3 (11%)	0	0	1 (2%)
Diarrhea as AE ≥ 1 serious AE Acute GVHD Diarrhea Neutropenia	16 (27%) 27 (46%) 4 (7%) 1 (2%) 6 (10%)	3 (12%) 12 (48%) 1 (4%) 0 2 (8%)	8 (30%) 10 (37%) 2 (7%) 0 5 (19%)	13 (33%) 19 (49%) 6 (15%) 1 (3%) 9 (23%)	21 (70%) 21 (70%) 12 (40%) 10 (33%) 4 (13%)	26 (52%) 30 (60%) 15 (30%) 5 (10%) 13 (26%)

Take-home messages

- Resistant CMV is a serious infection in transplant patients associated with increased morbidity and mortality
- Genotyping is the preferred diagnostic tool in the clinical practice
- High-doses ganciclovir and foscarnet are still the cornerstone of treatment
- Adjunctive treatment with leflunomide is commonly used in the clinical practice, although the published evidence is weak
- The role of new antivirals in the management of resistant CMV infection need further investigation

Thank you for your attention