

# The novel Anti-Cytomegalovirus Compound AIC246 Interferes with Processing of HCMV-progeny DNA

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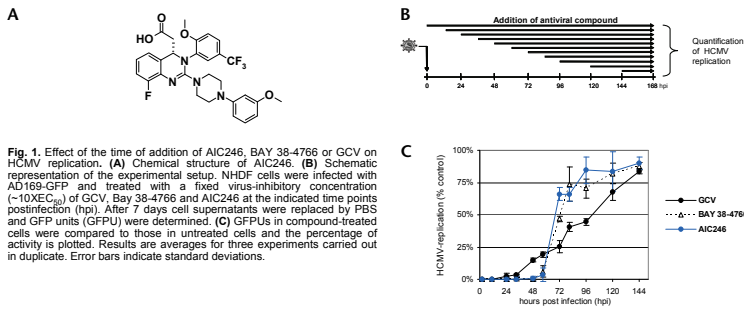
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## Summary

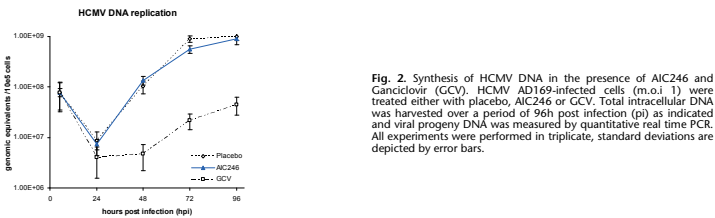
Human Cytomegalovirus (HCMV) remains the leading viral cause of birth defects and life-threatening disease in transplant-recipients. All approved drugs target the viral DNA-polymerase and are associated with severe toxicity issues and emergence of drug resistance. Attempts to identify improved anti-HCMV drugs led to the identification of the small-molecular-weight-compound AIC246 that is currently undergoing clinical Phase II testing. Previously we have shown that AIC246 exhibits an outstanding anti-HCMV activity in vitro and in vivo and demonstrates a superior antiviral profile compared to the current gold-standard Ganciclovir. Initial mode of action studies suggested that AIC246 acts late in the HCMV replication cycle via a mechanism distinct from that of polymerase inhibitors. Here we report on experiments aiming to elucidate the mode of action of AIC246. AIC246 was found to block viral replication without inhibiting the synthesis of progeny HCMV-DNA or viral proteins. Genotyping of mutant viruses that escaped AIC246 inhibition uncovered single point mutations in the UL56 subunit of the viral terminase complex. Marker transfer analyses confirmed that these mutations were sufficient to mediate AIC246 resistance. Mapping of drug resistance to the terminase subunit UL56 suggests that viral DNA processing and/or packaging is targeted by AIC246. In line with this, we found that AIC246 affects the formation of unit length genomes from viral DNA concatemers. Moreover, electron microscopy revealed that viral capsid maturation was disturbed in the presence of AIC246. Interestingly, experiments designed to further characterize AIC246 activity demonstrated that AIC246 resistant viruses do not exhibit cross-resistance to the published, chemically unrelated terminase inhibitors BDCRB and BAY-384766. Thus our data indicate that AIC246 interferes with viral DNA processing by interacting with the terminase subunit pUL56 and suggest that AIC246 acts via a molecular mechanism that is distinct from that of other compound classes known to target the viral terminase.

## Results

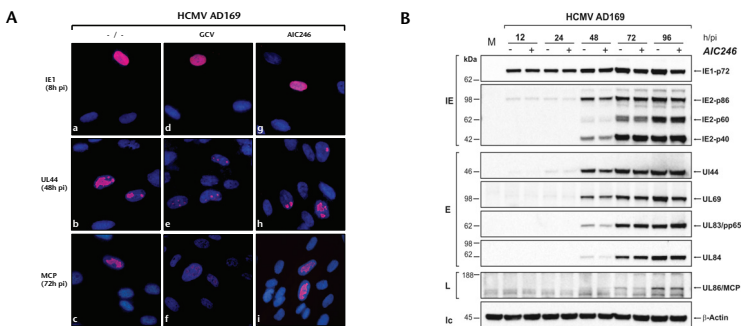
### AIC246 targets a late process in the HCMV replication cycle



### AIC246 does not interfere with HCMV DNA synthesis



### AIC246 does not interfere with HCMV protein expression



### Isolation and characterization of mutant viruses that escape AIC246 inhibition

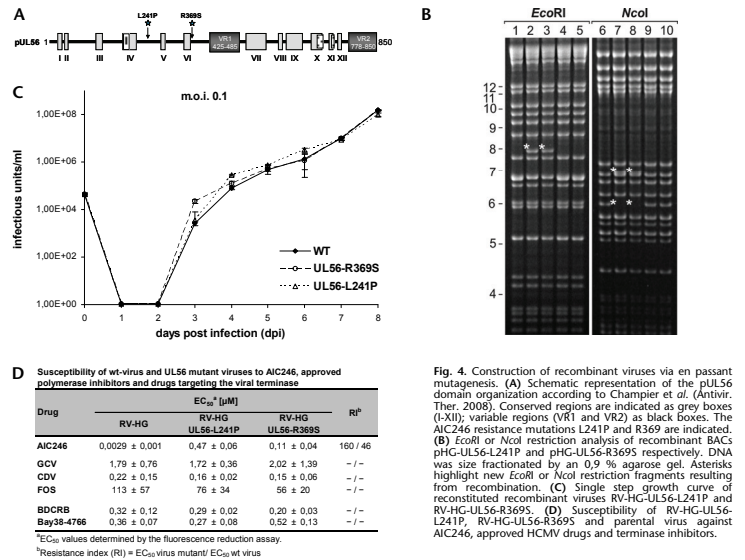
Isolation of AIC246-resistant HCMV strains			
HCMV strain	clone	EC <sub>50</sub> [pM] <sup>a</sup>	
		AIC246	GCV
AD169	-	0,0056 ± 0,0016	3,5 ± 1,5
rAIC246-1	5C9ppG9	1,24 ± 0,38	1,2 ± 0,2
rAIC246-2	M17E8	0,37 ± 0,07	4,0 ± 0,9

<sup>a</sup>EC<sub>50</sub> values determined by a CPE reduction assay.  
<sup>b</sup>AIC246 resistance index (RI) = EC<sub>50</sub> mutant virus/EC<sub>50</sub> wild-type virus.

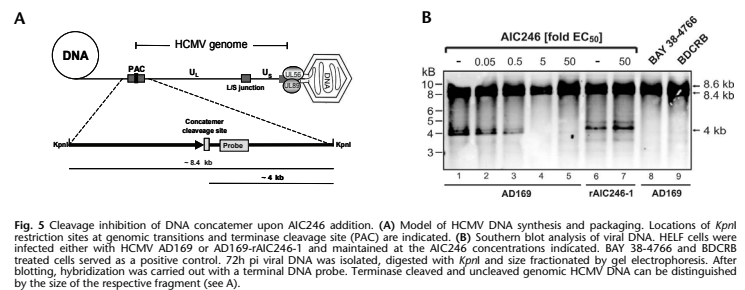
Genotyping of AIC246 resistant viruses					
HCMV strain	clone	UL56		UL89	UL104
		DNA <sup>a</sup>	AA <sup>b</sup>		
AD169	-	-	-	-	-
rAIC246-1	5C9ppG9	I723c	L241P	-	-
rAIC246-2	M17E8	g1107c	R369S	-	-

<sup>a</sup>nucleotide exchange  
<sup>b</sup>amino-acid exchange

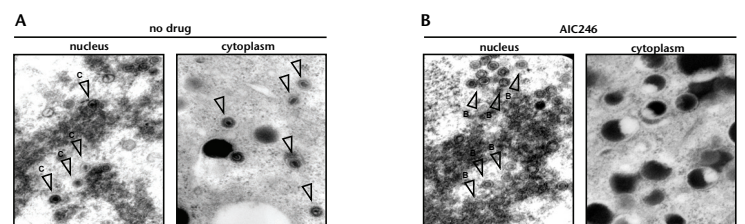
### Marker transfer experiments confirm UL56 mediated AIC246 resistance



### AIC246 interferes with cleavage / processing of genomic HCMV DNA



### AIC246 affects DNA packaging / nuclear egress



## Conclusion

AIC246 interferes with processing of viral DNA by targeting the HCMV terminase complex