Evaluation of Ganciclovir resistance mutations in cytomegalovirus UL97 gene in kidney transplant patients Urmia - Iran

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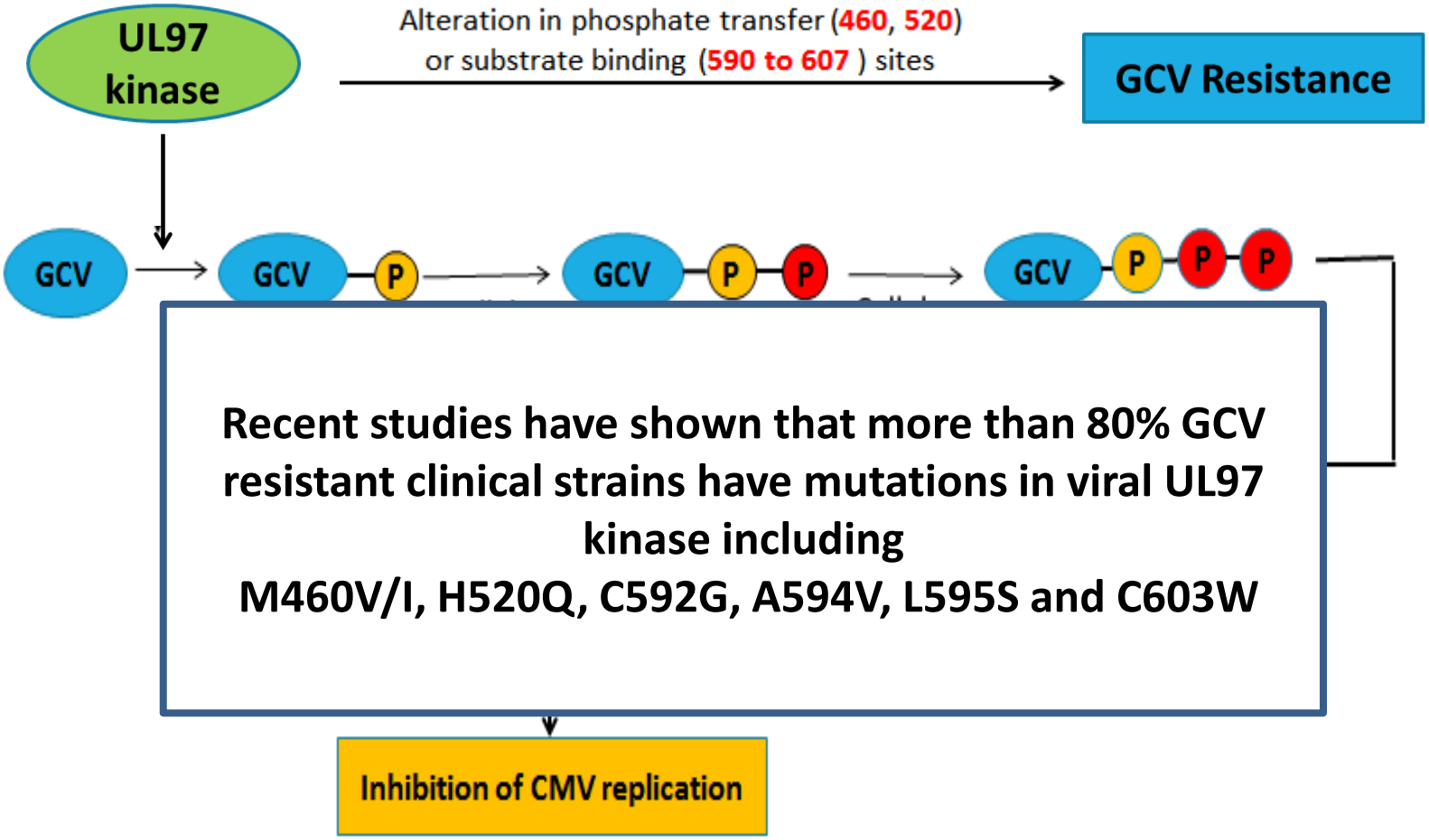
introduction

- CMV is a double-stranded DNA virus, 220 kb
- Member of the beta class of human herpesviruses
- Easily transmitted,
- Common: with 30–70% seroprevalence in developed countries
- Usually asymptomatic in immunocompetents
- **CMV infection is the leading viral cause of morbidity and mortality in patients who receive transplant**

Introduction

- Ganciclovir (GCV) is widely used for treatment of systemic CMV disease.
- However, Mutations associated with resistance to GCV have become an important problem (0-12%).
- Resistance to GCV arises from mutations in either the UL97 or the UL54 genes.
- Sequencing represents as standard approach to genotypic detection of drug resistance.

Anabolism of GCV



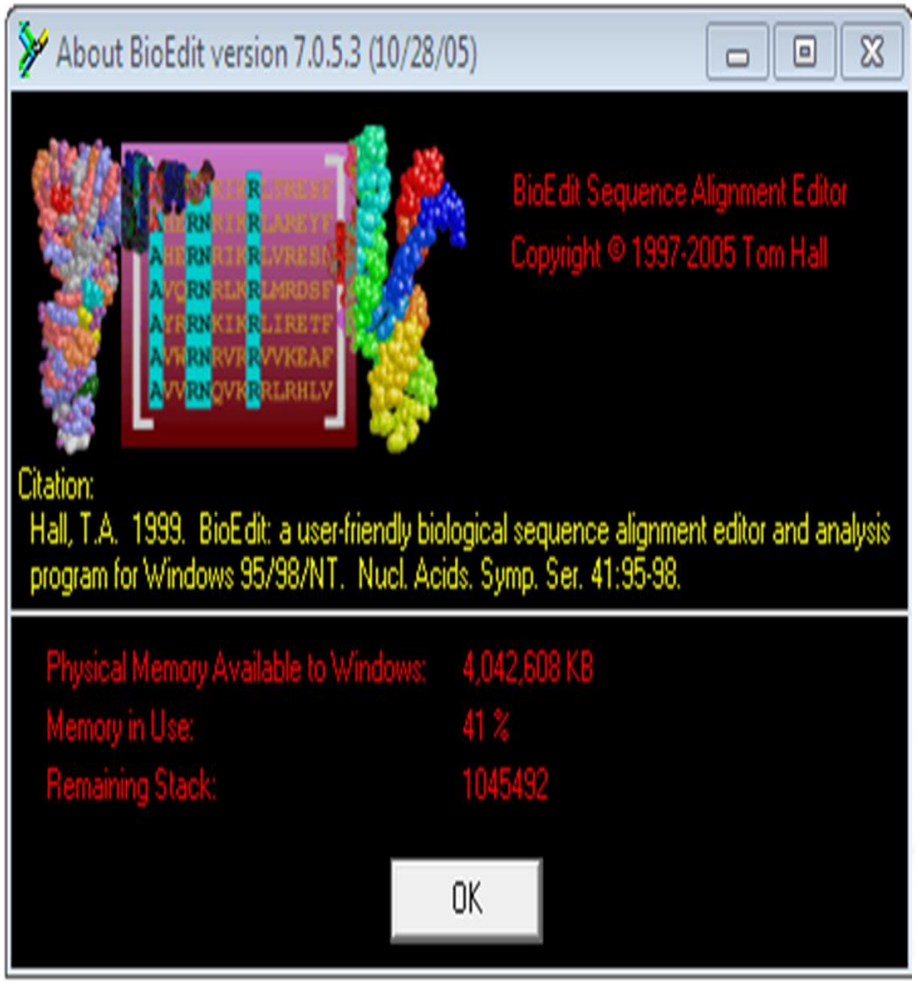
Materials and methods

Sera samples
Emam Hospital,
Urmia

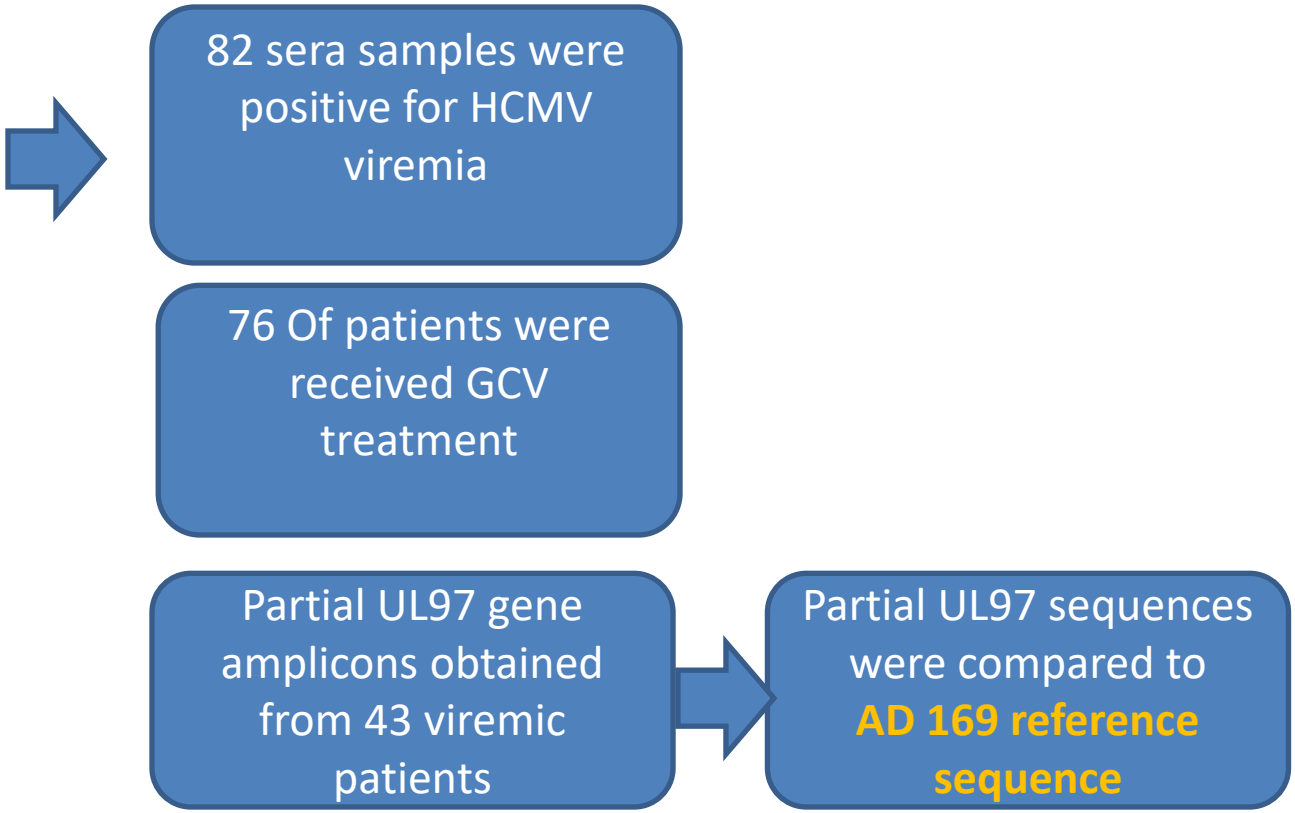
Extraction of DNA

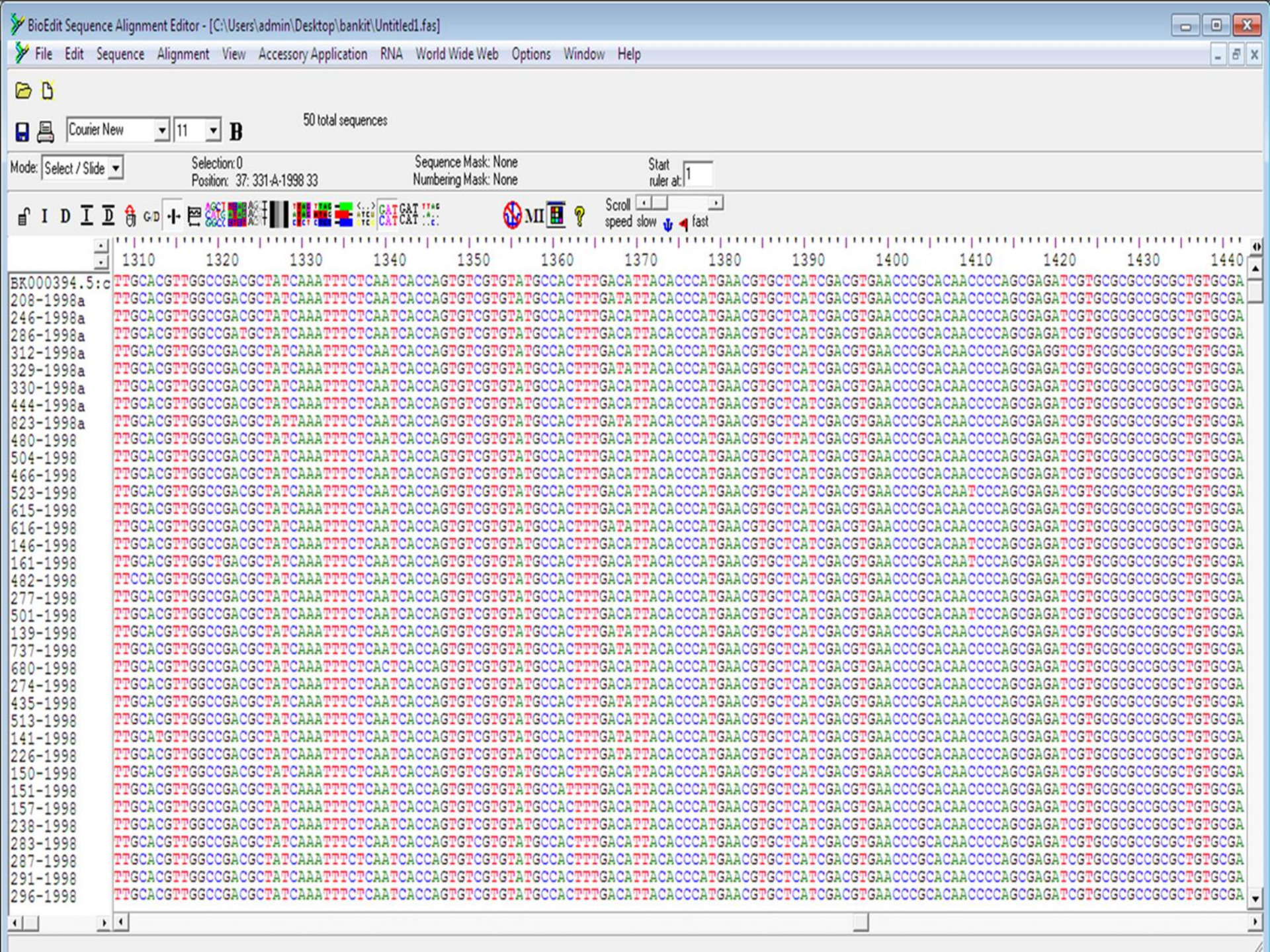
Detection of UL97
gene by nested-PCR

Sequencing of UL97
gene and analysis



Results


82 sera samples were positive for HCMV viremia
76 Of patients were received GCV treatment
Partial UL97 gene amplicons obtained from 43 viremic patients
Partial UL97 sequences were compared to AD 169 reference sequence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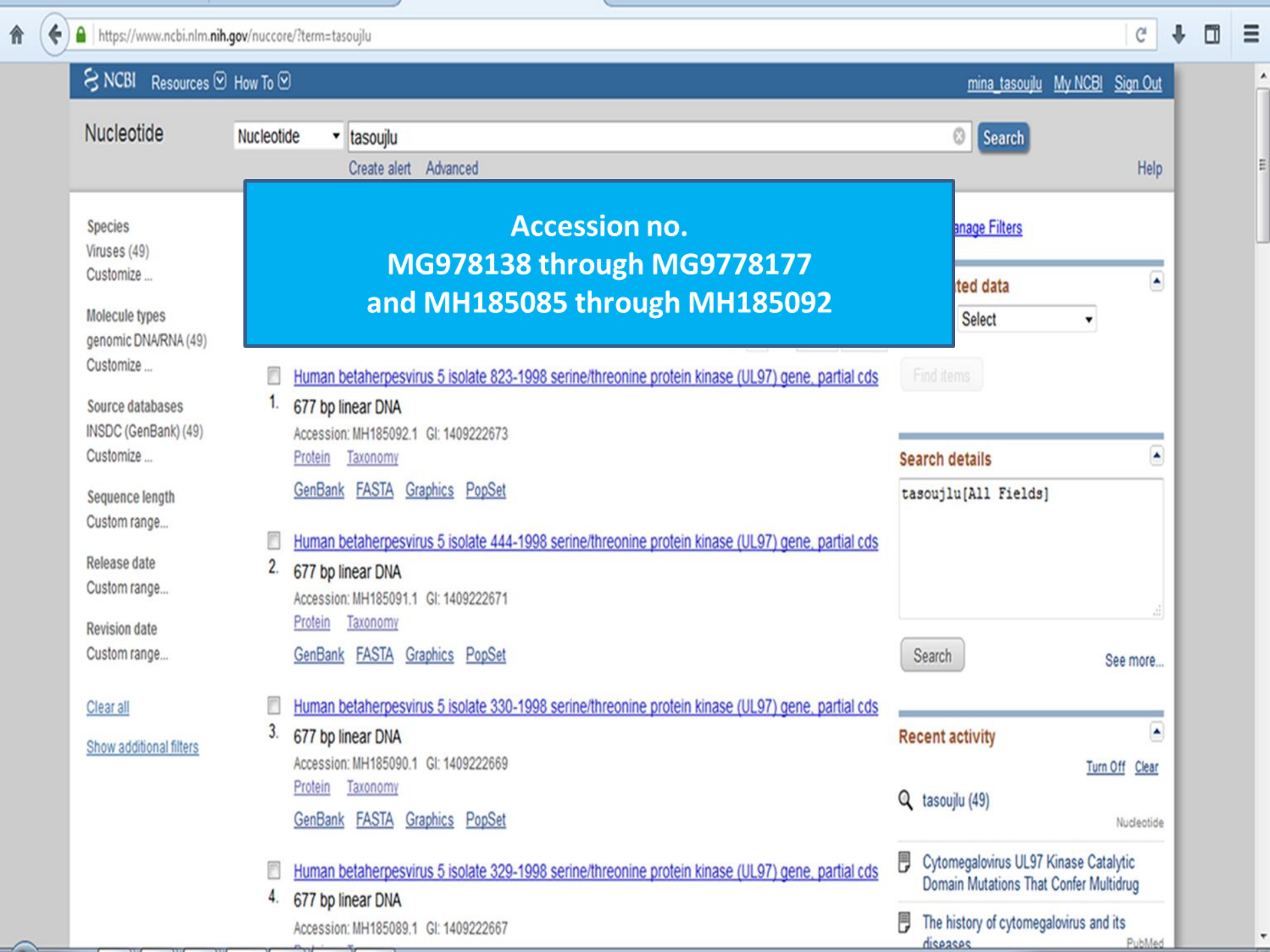
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Human betaherpesvirus 5 isolate 823-1998 serine/threonine protein kinase (UL97) gene, partial cds
1. 677 bp linear DNA
Accession: MH185092.1 GI: 1409222673
Protein Taxonomy
GenBank FASTA Graphics PopSet
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2. 677 bp linear DNA
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3. 677 bp linear DNA
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Protein Taxonomy
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Human betaherpesvirus 5 isolate 329-1998 serine/threonine protein kinase (UL97) gene, partial cds
4. 677 bp linear DNA
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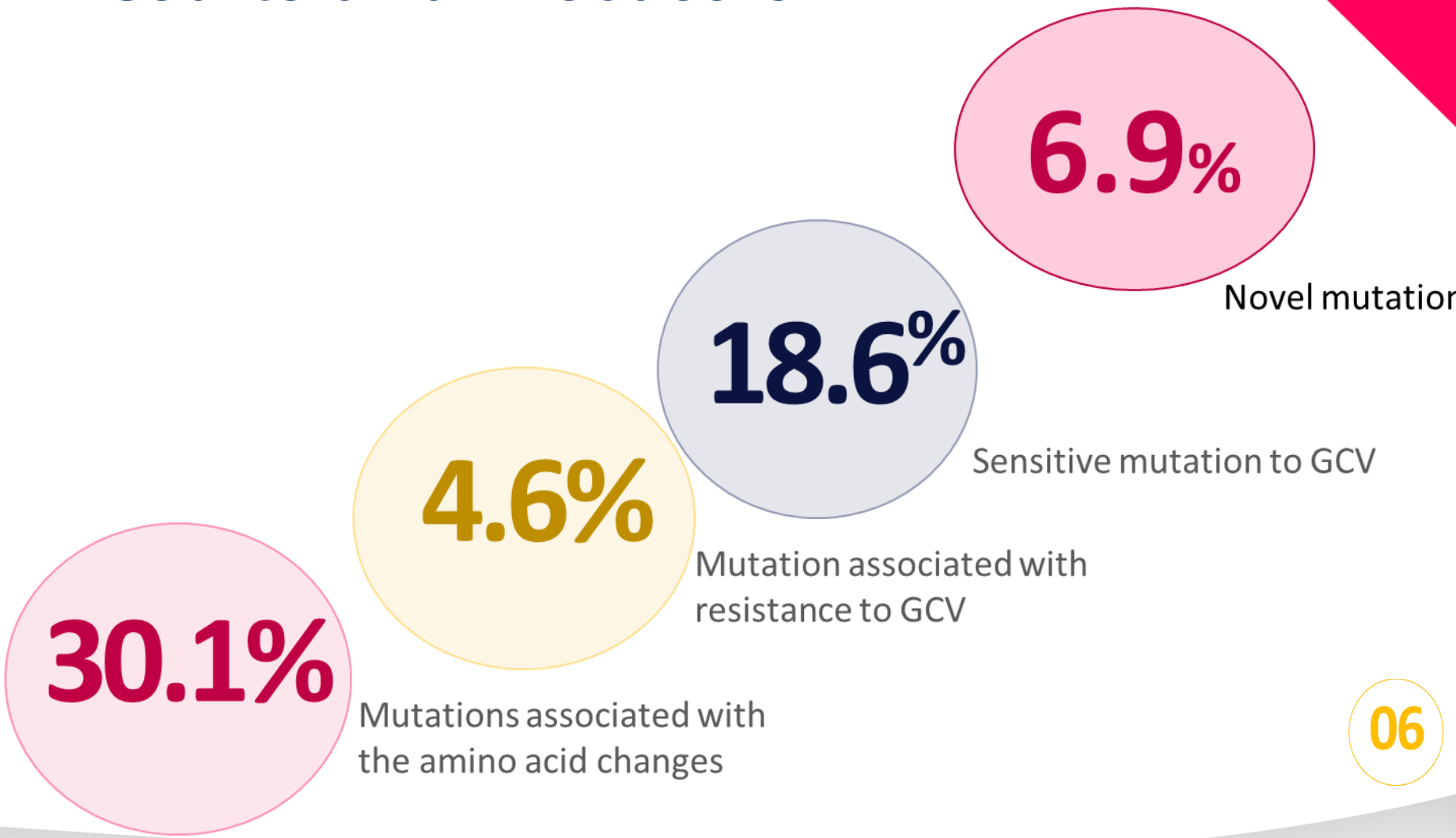
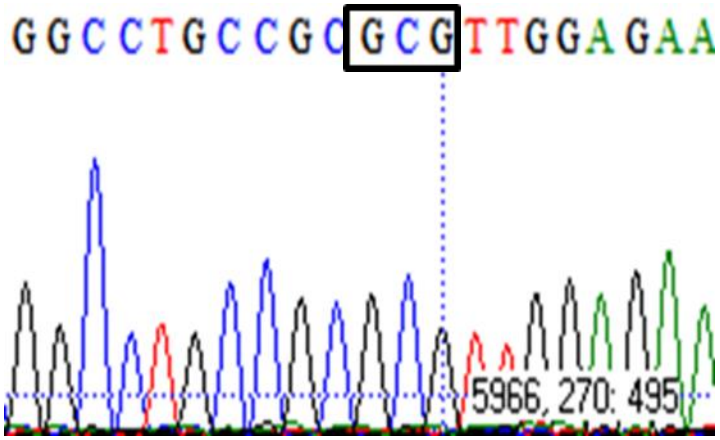
Nucleotide
Cytomegalovirus UL97 Kinase Catalytic Domain Mutations That Confer Multidrug
The history of cytomegalovirus and its diseases
PubMed

Results and Discussion

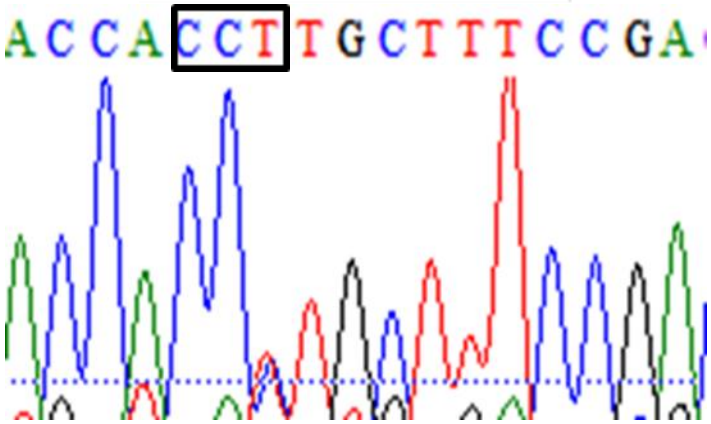


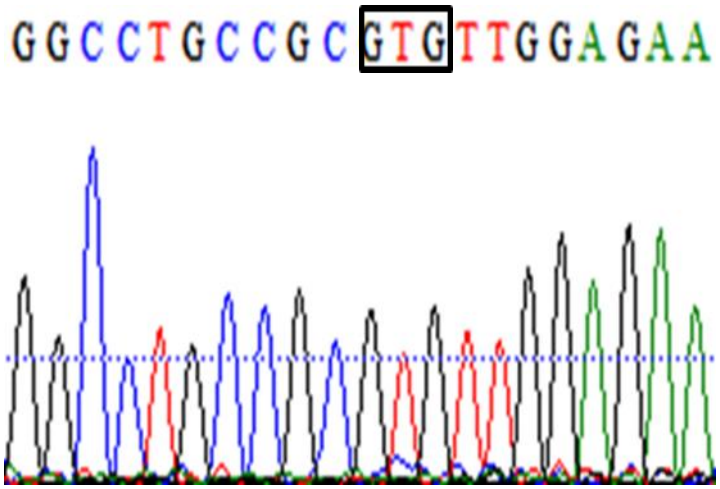
Amino acid mutation	Amino acid changes	No. observed	Sex/Age	Symptoms	Resistance status
A594V	Alanine → Valine	1 (2.3%)	M / 63	ulcer in the colon neutropenia	**Resistance mutation**
P521L	Proline →Leucine	1 (2.3%)	F / 43	Fever, neutropenia	**Resistance mutation**
D605E	Aspartate →glutamate	8 (18.6%)	M/45 M/52 M/60 M/28 M/52 M/51 F/60 F/61	Diarrhea Diarrhea Diarrhea None None None Pneumonia None	Sensitive mutation
T438M	Threonine → Methionine	1 (2.3%)	F/43	None	Uncertain impact
I474V	Isoleucine → Valine	1 (2.3%)	M / 55	None	Uncertain impact
N492S	Asparagine → Serine	1 (2.3%)	M/ 18	None	Uncertain impact

A594V Mutant

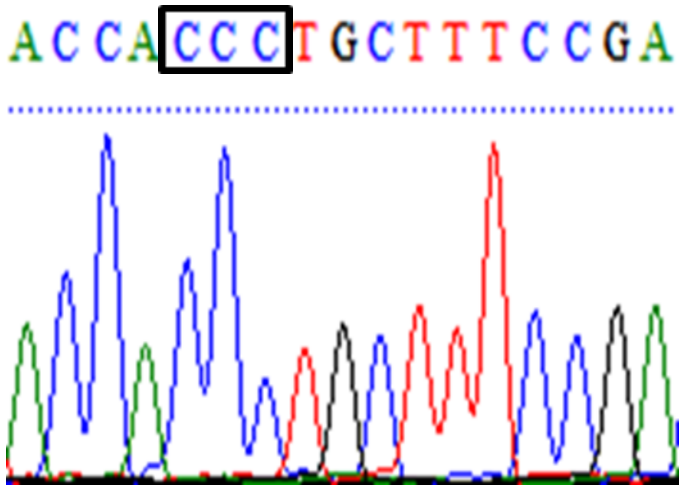


P521L Mutant





AD169 Strain



AD169 Strain

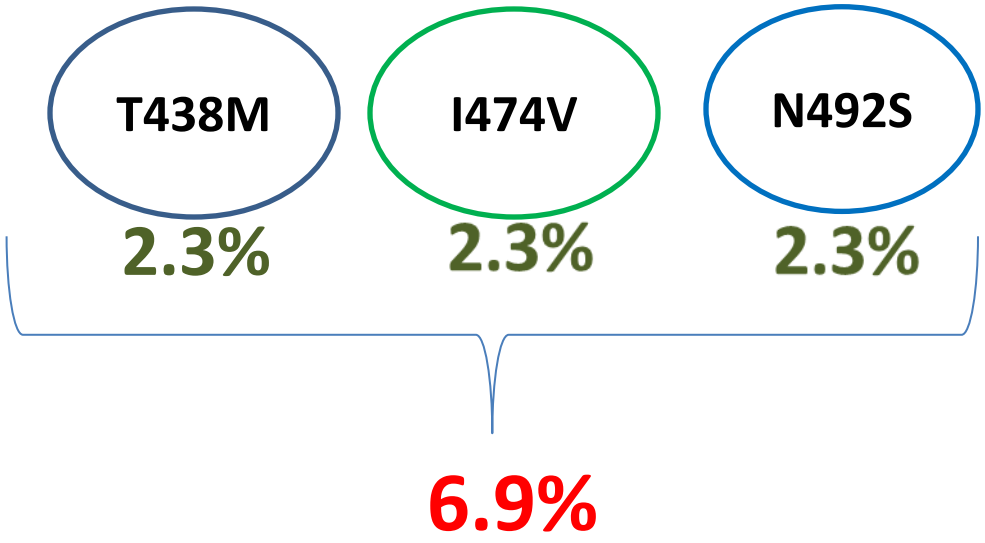
D605E mutation

- **Aspartate to glutamate substitution Were detected in 8 receipients (18.6%)**

- Its frequency is higher in Asian countries than Europe(91% in Japan, Korea)
- Its exact contribution to GCV resistance still remains unknown
- May be a natural variant?
- OR a molecular marker of CMV evolution in East Asian countries

Results and Discussion

❖ **Novel mutations**



Outside of resistance region
(codons 460,520 and 590 to 607)

phenotypic tests

Conclusion

- These findings suggest that incidence of GCV known resistant mutants were not prevalent in our renal recipients and only 1 out of 25 kidney recipients in our study represented resistant isolates.
- Genotypic diagnosis of UL97mutants can provide early detection of the emergence of CMV-resistant strains and subsequent adjustment of therapy.