Set up of New Generation Sequencing (NGS) for the detection of Ganciclovir Resistance in the Molecular Biology Laboratory of the Hospital de Clínicas.

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Introduction: The incorporation of NGS in clinical analysis laboratories (LACs) has accelerated in recent years in the world. In Uruguay, the use of this tool is very recent, and its use is limited by the cost associated with processing and trained human resources (HR). Our laboratory is an academic reference center where HR are trained for LACs work, so training in NGS which has great expansion, is essential for their academic training.

Our objective is to set up NGS in the Molecular Biology area of the central laboratory of the Hospital de Clínicas

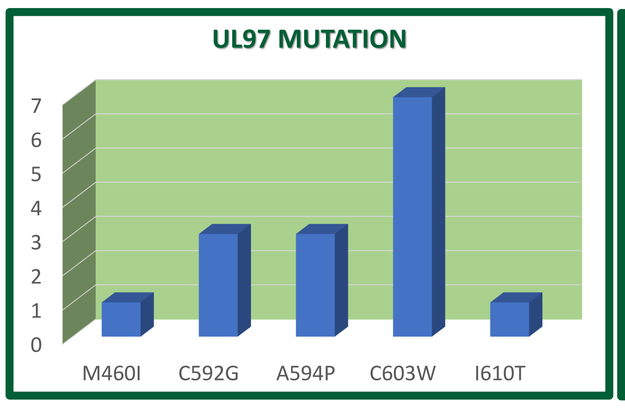
Methodology:

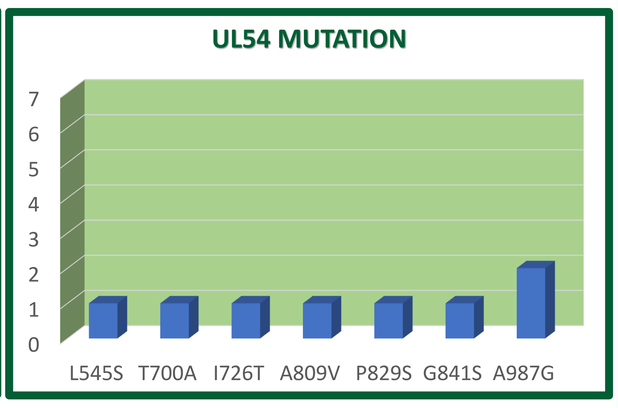
Theoretical training was carried out in 6 instances with support provided by a specialist from Biko-Illumina company. 24 samples were collected which has CMV loads greater than 1500 IU/ml in the 2020-2022 period. PCR was performed for UL54 and UL97 (ABL®) fragments. Agarose gel (0.5%) was performed to corroborate amplification. Purification with magnetic beads and quantification of products by fluorometry was performed. For Library preparation we used enzymatic fragmentation, end modification (3´-5´) and incorporation of adapters/sequencing indexes. The library was then amplified with magnetic bead purification to obtain 500bp fragments. A study of fragment quality and quantification and generation of sequencing pools was carried out. The sequencing was carried out on i-seq and the analysis was executed on ABL software.

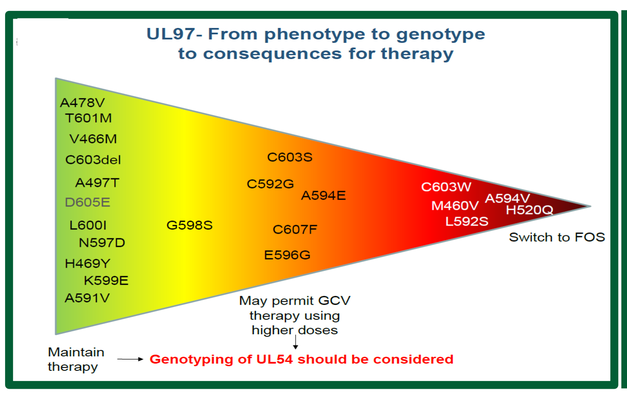
Results.

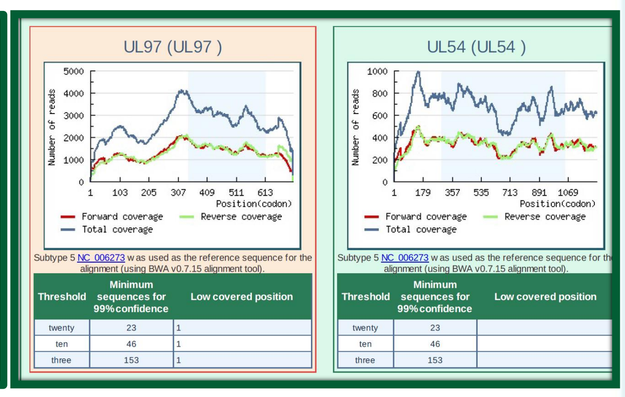
24 samples were processed but 2 did not achieve the minimum volume requirement so were discarded. In the fluorometric measurement, DNA concentrations between 0.117 and 101.85ng/ul were obtained with a mean of 10.2. For the library product, an average of 533 bp fragments were obtained for the measurable samples for a range expected of 450-600. 19 of 22 samples could be analyzed with a good coverage. 11 of 19 presented some type of resistance, all of them with Ganciclovir resistance and four combined with cidofovir or foscarnet resistance. 7/15 mutations located in UL97 were C603W which show high resistance to Ganciclovir. 5 samples showed combined UL97 and UL54 mutation.

N°	GANCICLOVIR			CIDOFOVIR			FOSCANET			BRINCIDOFOVIR			Maribavir		
	20	10	3	20	10	3	20	10	3	20	10	3	20	10	3
1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S
4	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S
5	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S
6	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S
7															
8	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
9	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
11	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
13	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S
14	S	S	I	S	S	S	S	S	S	S	S	S	S	S	S
15	R	R	R	S	S	S	S	S	R	S	S	S	S	S	S
16	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
17	R	R	R	S	S	R	S	S	R	S	S	S	S	S	S
18	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
19	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
20															
21	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S
22	R	R	R	S	S	R	S	S	S	S	S	S	S	S	S
23	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S
24															









Discussion:

This is the first report of CMV resistance in Uruguay. The sample preparation and library generation process steps were successfully executed. The results obtained in the evaluation of the library for concentration and size of fragments were acceptable. We emphasize the importance of having the necessary equipment and the technical team trained in this new and promising technology. The search for mutations associated with resistance to antiviral drugs against CMV is becoming increasingly important in drug selection, especially in countries like ours where the drug supply is scarce.

Bibliography:

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