Assessing Changes in Donor Derived cfDNA in Post Kidney Transplant Patients and its Correlation with Graft Survival

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Background

We aim to evaluate the efficacy of Holograft One assay for use in transplant laboratories for quicker and low-cost monitoring of donor derived cell free DNA (dd-cfDNA) in circulating blood of kidney transplant patients. dd-cfDNA has been shown to be associated with graft rejection in heart and kidney patients. However, there isn't enough data on how these levels change during the first 6 months following transplant. Therefore, dd-cfDNA level assessment during this period will serve as a baseline for detecting cell mediated/ antibody mediated rejection and monitoring graft survival and function.

Holograft One assay is being evaluated for its ease of use, cost and accuracy in monitoring dd-cfDNA in kidney patients post-transplant. With the target of 60 patients, 45 patients are currently enrolled in the *Sequential Measurement of Donor Derived cfDNA (dd-cfDNA) (Holograft) and Correlation with Patient Outcomes Post Kidney Transplant* study. Patient blood samples are collected in Streck Cell-Free DNA BCT® tubes at 6 timepoints post-transplant including: day 2; 1-, 2- and 6- weeks; 4- and 6- months. cfDNA is extracted from plasma using QIAamp MinElute ccfDNA Midi Kit. dd-cfDNA concentrations are measured in patient plasma using Omixon HoloGRAFT ONE kit and Qiagen QIAcuity One dPCR (digital Polymerase Chain Reaction).In HoloGRAFT assay's dPCR, the patient cfDNA is divided into thousands of partitions and Polymerase Chain Reaction (PCR) amplification occurs in each partition. Quantification of target DNA molecules in each partition makes it highly efficient in detecting lower concentrations of dd-cfDNA in patient blood. The assay provides absolute quantification of dd-cfDNA using 48 multiplexed copy number variant (CNV) markers (long stretches of DNA inherited in zero, one or two-copy forms).

Currently, 39 donor and patient pairs have been screened for the presence of unique CNVs in their genomic DNA (gDNA). Patient's plasma samples will then be screened for the presence and concentration of these unique donor specific markers. The results from each patient's dd-cfDNA% will be compared to clinically accepted markers of renal function including creatinine levels as well as tissue biopsies.

Assessment of dd-cfDNA in patient blood over 6 months will assess the efficacy of HoloGRAFT assay in predicting rejection of allograft in kidney patients, months before chronic rejection is confirmed by current gold standard of tissue rejection, tissue biopsy.

Methods

Consent 60 patients post kidney transplant

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Blood collection
(Day 2, weeks 1, 2, 6; 4 months and 6 months)

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Plasma separation from blood

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Isolation and quantification of dd-cfDNA using Omixon HoloGRAFT ONE and Qiagen QIAcuity ONE dPCR

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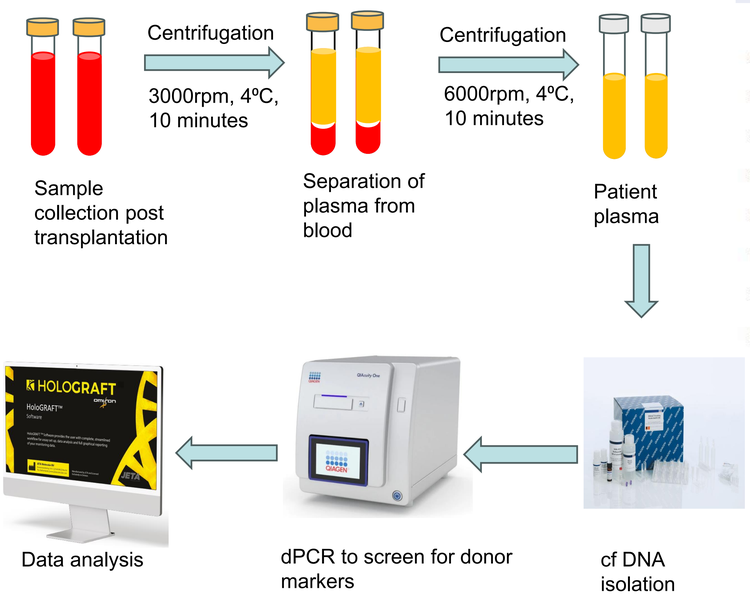
Comparison of dd-cfDNA levels with clinically accepted markers



Patient blood is collected in cell preparation Streck tubes followed by separation of plasma through centrifugation. cfDNA in the plasma samples is isolated using QIAamp MinElute ccfDNA kit. dd-cfDNA% in patient blood is measured using Omixon HoloGRAFT ONE kit and Qiagen QIAcuity ONE dPCR (digital Polymerase Chain Reaction). HoloGRAFT ONE software analyses the preliminary run for donor and recipient specific informative markers in their genomic DNA (gDNA), following which the successive cfDNA samples are only tested for the concentration of identified informative markers.

Results

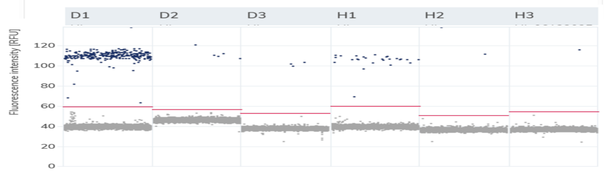
Each donor-recipient pair is screened for the presence of unique informative markers using their gDNA. For all the pairs screened, minimum of 5 donor-specific informative markers have been identified in each. Using the assay mixes positive for donor markers, sequentially collected patient samples are screened for the presence and concentration of these informative markers to quantify dd-cfDNA.

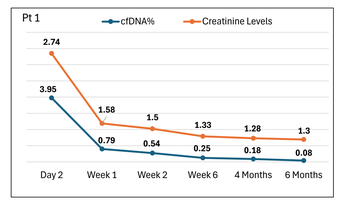
Each Assay Mix (M) contains 4 Informative Markers+ 1 Ref Control, making it possible for this assay to identify up to 48 different markers.

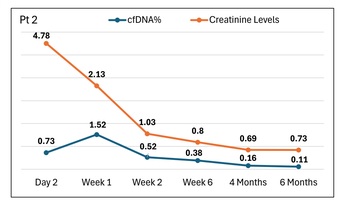
	Assay Mix 1	Assay Mix 2	Assay Mix 3	Assay Mix 4	Assay Mix 5	Assay Mix 6	Assay Mix 7	Assay Mix 8	Assay Mix 9	Assay Mix 10	Assay Mix 11	Assay Mix 12
Patient	-	-	-	+	+	-	+	-	+	+	-	+
Donor	+	+	+	+	-	-	-	-	+	-	+	-

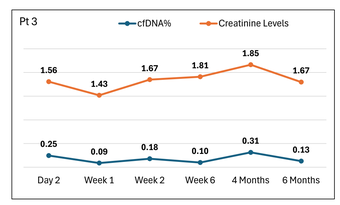
	1	2	3	4	5	6	7	8	9	10	11	12
Day2	HG-M 01	HG-M 02	HG-M 03								HG-M 11	
Week 1	HG-M 01	HG-M 02	HG-M 03								HG-M 11	
Week 2	HG-M 01	HG-M 02	HG-M 03								HG-M 11	
Week 6	HG-M 01	HG-M 02	HG-M 03								HG-M 11	
4Months	HG-M 01	HG-M 02	HG-M 03								HG-M 11	
6Months	HG-M 01	HG-M 02	HG-M 03								HG-M 11	

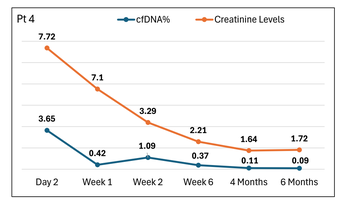
	1	2	3
A	01 Mix 1 Sample 1	02 Mix 1 Sample 2	03 Mix 1 Sample 3
B	01 Mix 2 Sample 1	02 Mix 2 Sample 2	03 Mix 2 Sample 3
C	01 Mix 3 Sample 1	02 Mix 3 Sample 2	03 Mix 3 Sample 3
D	01 Mix 11 Sample 1	02 Mix 11 Sample 2	03 Mix 11 Sample 3
E	04 Mix 1 Sample 4	05 Mix 1 Sample 5	06 Mix 1 Sample 6
F	04 Mix 2 Sample 4	05 Mix 2 Sample 5	06 Mix 2 Sample 6
G	04 Mix 3 Sample 4	05 Mix 3 Sample 5	06 Mix 3 Sample 6
H	04 Mix 11 Sample 4	05 Mix 11 Sample 5	06 Mix 11 Sample 6

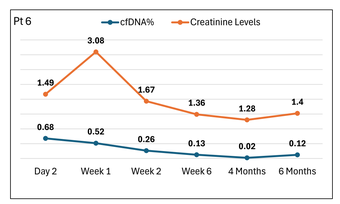


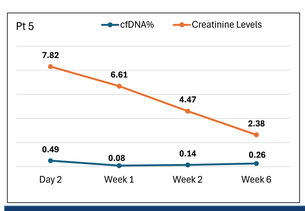


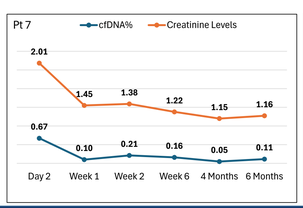


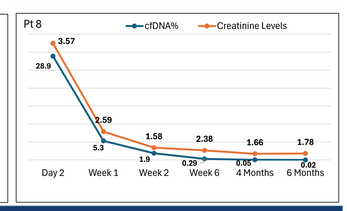












Conclusion

Allograft rejection remains a major challenge for patients after transplant, despite the use of immunosuppressants. Graft health assessment is important to monitor the progress of tissue acceptance. Changes in dd-cfDNA levels in patient blood, within first six months of transplantation might act as an important marker for early detection of tissue injury, which can help design immunotherapy specific to patient needs.

References: www.omixon.com/products/holograftone; www.qiagen.com

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