A novel prospective validation trial of blood-based RNA signature assay to predict rejection post kidney transplant

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Abstract

The accuracy and utility of clinically defined and analytically validated predictive biomarkers in kidney transplantation is limited. We leveraged prior feasibility blood-RNA studies to develop a highly sophisticated next-generation sequencing (NGS) platform which interrogates the immunologic profile of kidney transplant patients. The approach has been established in a highly regulated CLIA environment to provide clinical grade assays. By applying algorithm-based clinical decision trees, two discrete gene sets predicting the likelihood of both pre- and post-transplant rejection, respectively were developed. The outcome of each assay is the generation of a risk score and a machine learning algorithm derived cut-off which classifies patients' risk of early acute or clinical / subclinical rejection.

To clinically validate both assays we designed a novel prospective multi-site, (14 transplant centers), multi-country (i.e. US, Spain, France, Italy, Australia) observational trial which correlates the 2 unique RNA biomarker signatures with the histopathology and BANFF criteria of either a protocol or for cause kidney biopsy as the gold standard for evidence of transplant rejection (clinical / subclinical acute rejection).

To date, 322 patients have been enrolled which completes both pre- and post-transplant assay requirements. A target time for acute clinical and subclinical rejection is 6 months; however, all patients will be followed for 24 months to validate a third assay for predictive risk of kidney fibrosis. After 24 months participants will be monitored through registry data. To harmonize the microscopic attributes of the kidney tissue specimens, a central pathology review of all diagnostic biopsies will be incorporated both independently and in conjunction with site-specific diagnoses.

The overall approach including an all-comers, multi-center, multi-country design coupled with the rigor of an NGS assay allows for the generation of performance characteristics, including accuracy and precisions, which should better inform medical management of kidney transplant patients in a more personalized and predictive manner. This unique design of correlating a quantitative blood-based transcriptomic signature with a rejection phenotype based on histopathology in the kidney biopsy represents a level of evidence which currently does not exist in biomarker transplant biology.

Test Development

Transcriptomic RNA from peripheral blood from an independent 112-member training set[1] of kidney transplant recipients was sequenced using Illumina NextSeq 550Dx technology to 25M read-depth. This cohort data was analyzed for genes of interest exhibiting differential expression using DESeq2[2] and correlating to outcome of clinical and subclinical rejection as defined by central pathology from renal biopsy. From this analysis, a 17 gene signature was identified, and a machine-learning logistic regression algorithm was developed and locked with pre-defined cutpoint for interpretation as a risk score, called Tuteva, correlated to the presence of acute rejection.

A prospective international all-comers clinical trial (fig. 1 and table 1) was conducted to validate the test performance in an independent cohort using correlation to histopathologic microscopic attributes of renal pathology assessed centrally according to current BANFF criteria of either a protocol or for-cause kidney biopsy taken within the first 6 months post transplant as the gold standard for evidence of acute transplant rejection.

Study Design

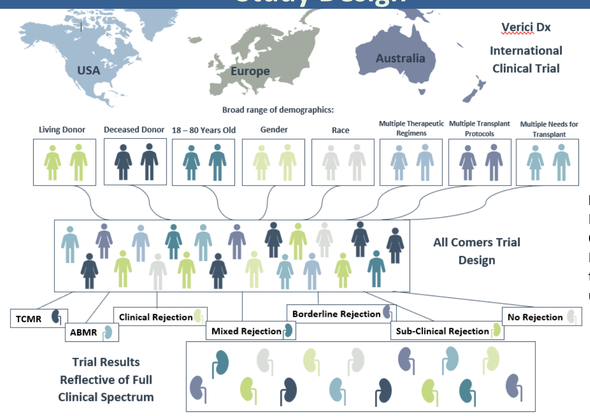

Figure 1. Importance of Clinical Study Design on translational utility.

Table 1. Study Population and Demographics

Tuteva™ Clinical Validation Study Demographics			
151 Unique Study Participants		**Living Donor Recipient**	**51 Total**
Mean/Median Recipient Age	53/53 Years	Living Related Donor	28
Mean/Median Donor Age	47/46 Years	Living Unrelated Donor	23
Male	97 (64%)	**Deceased Donor Recipient**	**100 Total**
Female	54 (36%)	Standard Criteria Donor	51
Donor Specific Antibodies		Expanded Criteria Donor	17
Anti HLA AB Class I	11%	Donors after Cardiac Death	31
Anti HLA AB Class II	12%	Not Answered (Deceased)	1
Race		**Underlying Causality**	**Percentage of Cohort**
Asian	5	Diabetes	21%
Black	31	Hypertension	74%
Native American	0	Glomerulonephritis	19%
Pacific Islander	3	Polycystic Kidney Disease	12%
White	108	Congenital Abnormality	3%
Not Answered	4	Family History of Kidney Disease	4%
Pathology Phenotype	**151 Total**	**Participation Location**	
Rejection	46 (30%)	USA	84
No Rejection	105 (70%)	Europe/Australia	57/10

Results

All evaluable participants (n = 151, table 1) enrolled at 13 centers in 5 countries were included in the Tuteva validation. Each study participant had blood collected at the time of for-cause (n = 44) or protocol biopsy (n = 107) within the first 6 months following transplant.

Each biopsy was read centrally by an expert in renal pathology to identify and characterize rejection phenotypes. In 151 unique patients, 46 (30%) rejection events were characterized (table 2) according to current (2019) BANFF criteria, including borderline TCMR[3]. Mean time to rejection was 61.1 days; range: 6 - 175d. Additionally, BK Nephropathy was identified in 8 patients and pyelonephritis in 3 patients.

Tuteva risk scores are calculated continuously from 0-100; for interpretation, a pre-determined risk cut-point of 50 was established based on the independent training set[1]. The risk score distribution (fig. 2) resulted in 26.5% of patients with a high risk (>50) score result in which 60% were shown to correlate with rejection on histopathology; low risk score results in 73.5% of patients correlated with an absence of rejection on histopathology findings in 80% of patients. The receiver operating characteristic curve AUC was 0.693, P<0.001 (fig. 3).

A subset of 12 participants had multiple biopsies for which differentiated results of rejection and non-rejection were found and for which there was a correlated blood sample from which Tuteva results were calculated, allowing a limited assessment of Tuteva signature response to changing pathology phenotypes, table 3.

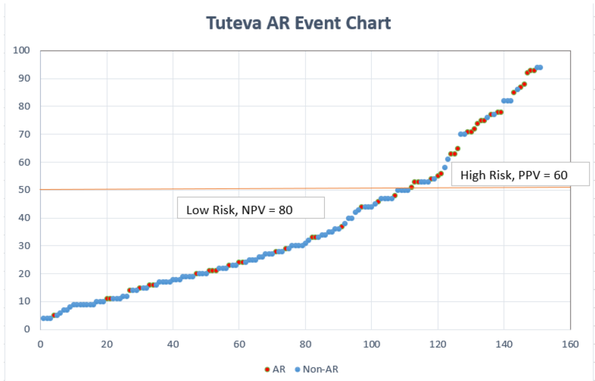

Figure 2. Tuteva AR event chart

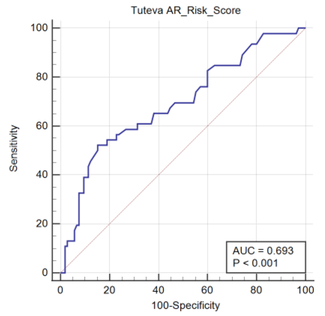

Figure 3. Receiver Operating Characteristic Curve

Table 2. Pathology Findings and Related AR Scores

BANFF 2019[2]	# Detected/ # Cases
Borderline	5/12
TCMR IA or higher	3/13
ABMR	7/11
Mixed	9/10
BKN Negative	7/8
Pyelonephritis Negative	1/3

Table 3. Multi-sample Follow-up Testing

Patient	Tuteva Risk Score	Risk Score Interpretation	Days between Biopsies	Biopsy	Biopsy Histopathology Phenotype
1	93	**High**		For-cause	**Rejection**
	7	Low	71	Protocol	No Rejection
2	99	**High**		For-cause	**Rejection**
	75	**High**	73	Protocol	No Rejection
3	71	**High**		For-cause	**Rejection**
	25	Low	73	Protocol	No Rejection
4	72	**High**		For-cause	**Rejection**
	48	Low	21	For-cause	No Rejection
5	71	**High**		For-cause	**Rejection**
	7	Low	64	Protocol	No Rejection
6	23	Low		Protocol	No Rejection
	5	Low	34	For-cause	**Rejection**
7	77	**High**		For-cause	**Rejection**
	48	Low	87	Protocol	No Rejection
8	74	**High**		For-cause	**Rejection**
	90	**High**	92	Protocol	No Rejection
9	54	**High**		For-cause	**Rejection**
	30	Low	75	Protocol	No Rejection
10	50	Low		For-cause	No Rejection
	37	Low	29	For-cause	**Rejection**
11	63	**High**		For-cause	**Rejection**
	38	Low	95	Protocol	No Rejection
12	93	**High**		For-cause	**Rejection**
	60	**High**	117	Protocol	No Rejection

Discussion

- The rigor of an NGS assay allows for the generation of performance characteristics, including accuracy and precisions, which better inform medical management of kidney transplant patients in a more personalized and predictive manner while accounting for full clinical continuum.
- The clinical trial continues for new validations of other pre and post-transplant tests which are included in trial objectives. Moreover, patients will be followed in-study at local sites for 2 years.
- Further study is needed to assess longitudinal testing capability and to assess future prospects of integration of varying pre and post-transplant tests to inform subsequent test interpretations.

Conclusions

- Validation in an all-comers cohort that is independent of the training set and representing all forms and levels of rejection is essential to assessment of expected test performance in translational clinical care settings to support medical management decision making.
- Prospective, multicenter, international studies allow for the inclusion of diverse populations and broad diversity in care management practices while allowing blinding to protect from introduction of biases; thereby producing robust and reliable results for clinical integration.
- Tuteva is a quantitative blood-based transcriptomic signature able to correlate an acute rejection risk score with rejection phenotype based on histopathology in the kidney biopsy, representing a level of evidence which has not yet not existed in biomarker transplant biology.

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