Non-Invasive Metabolite-Based Urine Signature Detects Over-Immunosuppression in Renal Transplant Recipients

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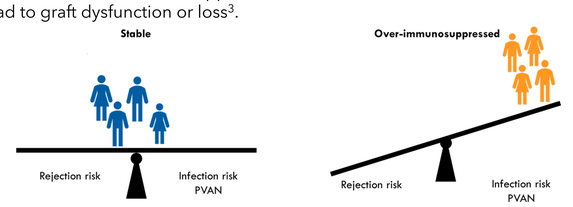
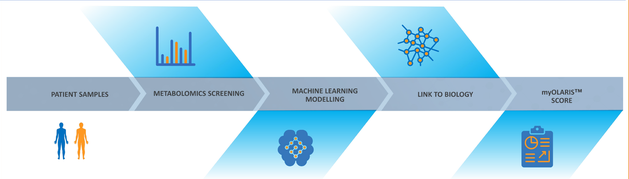
DETECTING OVERIMMUNOSUPPRESSION: THE MISSING GUIDE RAIL

Managing complications related to over-immunosuppression is a clinical challenge in post-transplant care, with **infections accounting for the second leading cause of death with functioning graft (DWFG)** in renal transplant recipients (RTRs) within the first year[1]. At present, there are no clinically validated biomarkers to detect over-immunosuppression[2]. **Polyomavirus-associated nephropathy (PVAN)** is the result of an opportunistic infection indicative of over-immunosuppression that occurs in 5-10% of RTRs, which can lead to graft dysfunction or loss[3].



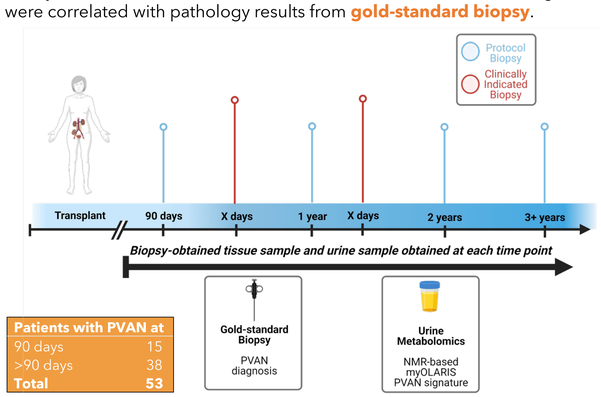
OLARIS CEREBRO PLATFORM: METABOLOMICS & MACHINE LEARNING



Altered metabolism has been linked to kidney function and transplant tolerance[4]. By measuring an individual's metabolome, it is possible to identify biomarkers that correlate with disease status and prognosis. Using the Olaris CEREBRO platform, we detect and quantify **metabolites** from hundreds of patient biofluid samples (blood and/or urine). Using **NMR-** and **MS-** based metabolomics and **machine learning,** we identify a biomarker-based myOLARIS™ score that can monitor patient disease status with a high degree of accuracy.

STUDY DESIGN

In a pilot study, we identified differential metabolites in the urine from RTRs with biopsy-confirmed PVAN from those with a stable graft and built a machine learning algorithm model to classify patients with high accuracy[5]. To expand upon these results, we examined metabolites from a larger cohort comprising 115 RTRs with longitudinal samples (N=371). Urine samples were collected from patients at ~90 days, 1 year, and 2 years post-transplant in conjunction with a protocol biopsy. Samples were also collected during clinically indicated biopsies. Urine metabolites were extracted and analyzed via 1D ^{1}H and 2D ^{1}H-^{13}C Heteronuclear Spectrum Quantum Coherence (HSQC) NMR spectroscopy. As previously described[5], spectra were processed using in-house processing and normalization tools and metabolite resonance peaks were assigned to metabolites based on chemical shift mapping to a library of known metabolites. Metabolite levels and machine learning models were correlated with pathology results from **gold-standard biopsy**.



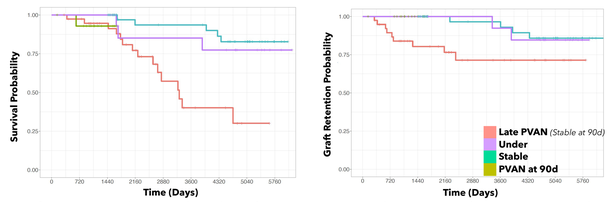
Patients with PVAN at	
90 days	15
>90 days	38
Total	**53**

90 DAYS POST-TRANSPLANT	Stable	Over	Under
Number of patients (N= 115 total)	86	15	14
Sex			
Male	55	10	8
Female	31	5	6
Age (years)	55 ± 12.8	57.4 ± 8.2	57.8 ± 13.5
Starting Body Weight (kg)	71.5 ± 14.5	65.5 ± 13.9	72.7 ± 11.7
Serum Creatinine (mg/dL)	1.61 ± 0.56	1.49 ± 0.55	1.54 ± 0.34
eGFR (mL/min)	49.35 ± 18.69	54.01 ± 19.80	47.22 ± 18.58
HLA Mismatches	2.9 ± 1.3	2.4 ± 1.3	3.4 ± 1.2
HLA-A	1.0 ± 0.8	0.8 ± 0.8	1.0 ± 0.8
HLA-B	1.2 ± 0.7	0.8 ± 0.8	1.4 ± 0.6
HLA-DR	0.7 ± 0.7	0.9 ±0.6	1.0 ± 0.7

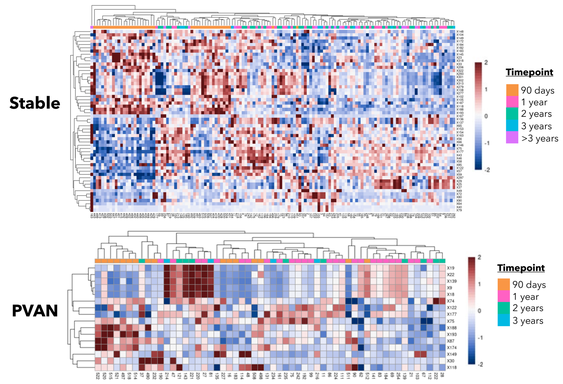
Values shown are mean ± SD where applicable.

Summary data for all patient groups at 90 days are shown. Demographic, biochemical, clinical, and immunosuppression-related metadata, including immunosuppressant(s) used and PCR results for BKV (not shown), were not found to improve the metabolite-based analysis of kidney transplant status.

FIRST 90 DAYS POST-TRANSPLANT IS CRITICAL AND HAS A DISTINCT METABOLIC PROFILE

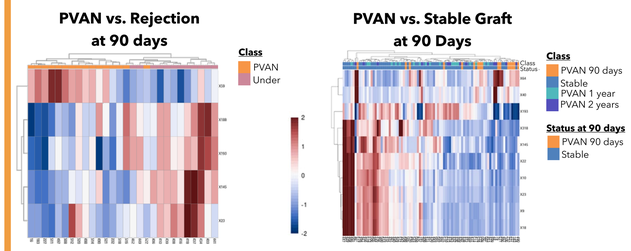


We first assessed survival and graft retention in each RTR population using Kaplan-Meier analysis. RTRs who were stable at 90 days but later experienced PVAN (N=39) were a discrete population with significantly lower survival (log-rank p<0.001) and graft retention (p=0.037) than those who were stable and did not experience rejection or PVAN over 2 years post-transplant (N=47), those who were under-immunosuppressed (N=14), and those who experienced PVAN at 90 days (N=15). Data for the analysis was right-censored at the last known record date, October 10, 2021.

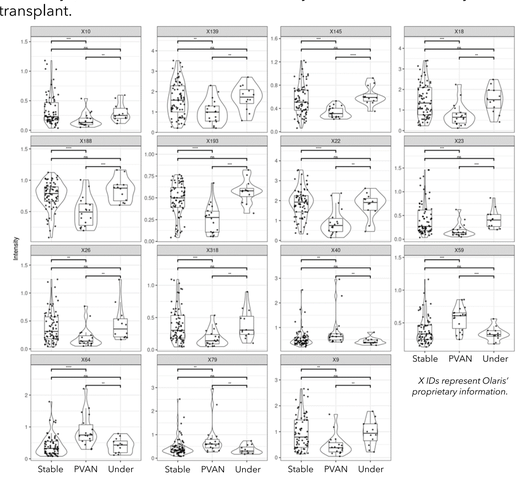


Using a Kruskal-Wallis (KW) non-parametric one-way analysis of variance, we identified **differential metabolite resonances** with fold-change >1.5 and p-value <0.05 between PVAN and Stable patients. Heatmaps show clustering of metabolite levels in Stable (top) and PVAN (bottom) patients at 90 days vs. later time points post-transplant.

90-DAY BIOPSY-CONFIRMED PVAN, REJECTION OR STABLE RTRS HAVE DISTINCT METABOLIC FINGERPRINTS



We next evaluated metabolites altered in patients with PVAN compared with those with under-immunosuppression or stable grafts at 90 days post-transplant. Heat maps show clustering of differential metabolite resonances identified using Kruskal-Wallis (KW) non-parametric one-way analysis of variance as above. Left, RTRs with PVAN vs. underimmunosuppression at 90 days post-transplant. Right, RTRs with PVAN vs. stable graft at 90 days, further stratified into continually stable vs. PVAN at 1 year or 2 years post-transplant.

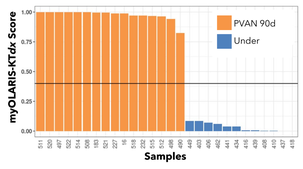


X IDs represent Olaris' proprietary information.

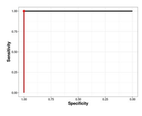
Violin plots show differential levels of the metabolites shown above, comparing patients with stable graft function, PVAN, or underimmunosuppression at 90 days post-transplant.

myOLARIS-KT*dx*™ DETECTS PVAN AND REJECTION AT 90 DAYS POST-TRANSPLANT

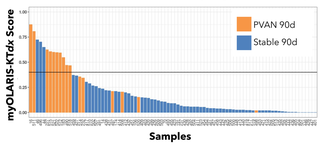
PVAN vs. Rejection at 90 days post-transplant



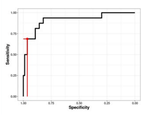
100% AUC
100% Accuracy
100% Sensitivity
100% Specificity
100% PPV
100% NPV



PVAN vs. Stable at 90 days post-transplant



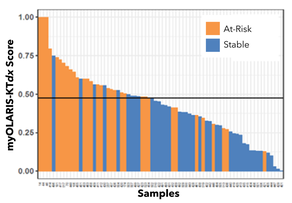
88% AUC
90% Accuracy
62% Sensitivity
96% Specificity
77% PPV
92% NPV



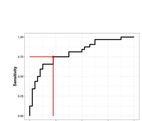
Differential metabolite resonances between **PVAN (at 90 days), Stable, and Under** samples at 90 days post-transplant were used to generate cross-validated machine learning models, including orthogonal partial least square discriminate analysis (OPLS-DA) and elastic net (ENET), with or without Boruta feature selection, which selects features based on the random forest (RF) importance of the unchanged vs. shuffled feature and can be used for feature reduction[6]. Based on the model with the highest stability and performance, we developed a scoring method (**myOLARIS-KT*dx* Score**) with high accuracy to differentiate RTRs with PVAN at 90 days from stable or under-immunosuppressed RTRs. Waterfall plots show visualization of scoring for different patient populations as indicated, with horizontal line depicting score cutoff for each analysis. Receiver operating characteristic (ROC) curves show sensitivity/specificity at score cutoff.

myOLARIS-KT*dx* DETECTS PVAN RISK AT 90 DAYS POST-TRANSPLANT

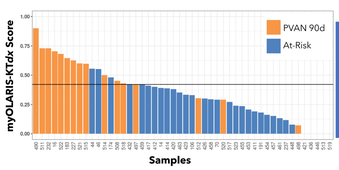
At-Risk for Late PVAN vs. Stable at 90 days post-transplant



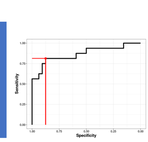
81% AUC
76% Accuracy
75% Sensitivity
78% Specificity
73% PPV
79% NPV



Early PVAN (90d) vs. At-Risk for Late PVAN at 90 days post-transplant



87% AUC
85% Accuracy
81% Sensitivity
88% Specificity
76% PPV
90% NPV



Similar analysis was used to generate **myOLARIS-KT*dx* Scores** with high accuracy to differentiate RTRs with high PVAN risk at 90 days (i.e., patients who later developed PVAN) from stable RTRs and those currently experiencing PVAN. Waterfall plots show visualization of scoring for different patient populations as indicated, with horizontal line depicting score cutoff for each analysis. Receiver operating characteristic (ROC) curves show sensitivity/specificity at score cutoff.

myOLARIS-KT*dx* DIFFERENTIATIES PATIENTS WITH PVAN, REJECTION, OR HIGH PVAN RISK FROM STABLE RTRS AT 90 DAYS

Ensemble Model Prediction of Patient Status at 90 days post-transplant

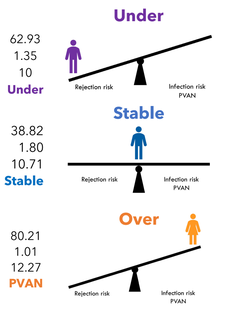
myOLARIS-KT*dx* Prediction	Reference (biopsy) PVAN	Under	Stable	At Risk
PVAN	**12**	0	1	3
Under	0	**11**	4	4
Stable	3	1	**34**	9
At Risk	1	0	1	**16**

	Accuracy	Balanced Accuracy	PPV	NPV	Sensitivity	Specificity
PVAN	0.9200	0.8512	0.7500	0.9524	0.7500	0.9524
Under	0.9100	0.9129	0.5789	0.9877	0.9167	0.9091
Stable	0.8100	0.8167	0.7234	0.8868	0.8500	0.7833
At Risk	0.8200	0.7353	0.8889	0.8049	0.5000	0.9706

The individual models above were stacked to create an ensemble model for identification of PVAN vs. stable graft vs. underimmunosuppression vs. at-risk for late PVAN. Confusion matrix shows the number of accurate predictions vs. biopsy, and performance metric table shows performance metrics for each patient subclass using the ensemble model. **myOLARIS-KT*dx* score correlates with biopsy in the majority of cases in each population.**

CLINICAL USE CASES 90 DAYS POST-TRANSPLANT

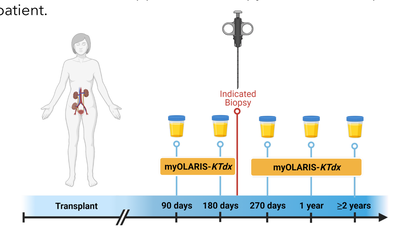
Patient Clinical Information			myOLARIS Prediction
Patient 72 Male, 44 years old, Body weight 69 kg, 2 HLA mismatches	**GFR** (mL/min)	62.93	Under
	Serum Creatinine (mg/dL)	1.35	
	Tac Trough (ng/mL)	10	
	Biopsy Status	Under	
Patient 101 Male, 64 years old, Body weight 111 kg, 2 HLA mismatches	**GFR** (mL/min)	38.82	Stable
	Serum Creatinine (mg/dL)	1.80	
	Tac Trough (ng/mL)	10.71	
	Biopsy Status	Stable	
Patient 65 Male, 60 years old, Body weight 84 kg, 3 HLA mismatches	**GFR** (mL/min)	80.21	Over
	Serum Creatinine (mg/dL)	1.01	
	Tac Trough (ng/mL)	12.27	
	Biopsy Status	PVAN	



Patient clinical information and biopsy results are compared to the myOLARIS-KT*dx* score for selected examples of underimmunosuppressed, stable, and overimmunosuppressed RTRs included in the study.

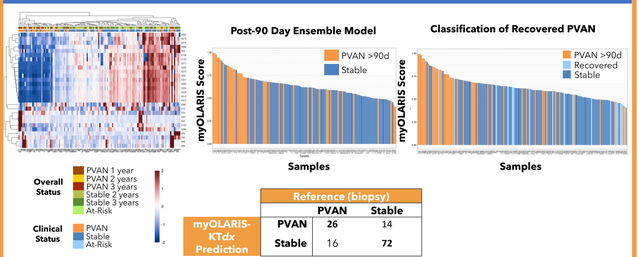
myOLARIS-KT*dx* VS ROUTINE CLINICAL TEST

Biopsy remains the gold standard method to confirm a PVAN diagnosis following detection of viremia and/or viruria, which is invasive, potentially dangerous, and costly. myOLARIS-KT*dx* offers a **noninvasive, urine-based metabolic analysis** to diagnose PVAN, differentiate PVAN from rejection, and identify RTRs with stable graft function. Collectively, myOLARIS-KT*dx* enables clinicians to confidently determine whether their patients' immunosuppressive therapy is within an optimal range for that individual patient.



- **Avoids invasive, potentially dangerous, and costly biopsy**
- **Confidence in immunosuppressant dose adaptations**
- **Indicates when more information is needed**

myOLARIS-KT*dx* IDENTIFIES PVAN DURING FIRST TWO YEARS AND BIOPSY-CONFIRMED RECOVERED PVAN CLASSIFY AS STABLE



myOLARIS-KT*dx* Prediction	Reference (biopsy) PVAN	Stable
PVAN	26	14
Stable	16	**72**

In a similar analysis, differential metabolites between patients with PVAN or stable grafts at >90 days (heat map) were used to generate a myOLARIS-KT*dx* Score to differentiate **RTRs with PVAN between 90 days and 2 years from biopsy-defined stable RTRs**. Waterfall plot (middle) shows visualization of scoring for PVAN vs. stable RTRS. Confusion matrix shows number of accurate predictions for each patient subclass. myOLARIS-KT*dx* scores from patients with **biopsy-confirmed recovery** from earlier PVAN resemble those of RTRs with stable grafts, as shown in waterfall plot (right).

CONCLUSIONS & FUTURE DIRECTIONS

By applying the Olaris CEREBRO platform to urine samples from RTRs, we identified **differential metabolite resonances** in RTRs experiencing PVAN vs. those with stable grafts or under-immunosuppression at 90 days post-transplant. Using machine learning, we developed the myOLARIS-KT*dx* Score to detect PVAN in RTRs at ≥90 days post-transplant with high accuracy and optimal NPV. This score has the potential to serve as a diagnostic tool to confidently rule out PVAN in RTRs with stable graft function **as well as distinguish from rejection**, providing critical information for clinicians to optimize immunosuppressant dosage to prevent infection as well as rejection. Future directions include extending these results to a larger, more diverse patient population as well as to additional infections and other complications of over-immunosuppression.

REFERENCES ~ DISCLOSURES

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C. Dong, J. Zhao, S. Raghavendra Rao, C. Honrao, A. Trimigno, K. Sheehan, L. Housman and **L.O. Rodrigues** are employees of Olaris, Inc. and have ownership and salary interest in the company. **E.M. O'Day** is CEO of Olaris, Inc. and has ownership and salary interest in the company. **D.R. Kuypers** is an Advisory Committee member for Olaris, Inc. and serves as a consultant for UCB, HANSA, Sangamo Tx, MSD, Boehringer, AZ, Astellas, and Novartis. He receives grant/research support from Astellas and Roche.
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