Brigham and Women's Hospital
Founding Member, Mass General Brigham

Monitoring Kidney Transplant Patients:
Beyond Creatinine

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Associate Professor of Medicine
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Director, Kidney and Pancreas Transplantation
Director, Renal Transplant Fellowship
Brigham and Women's Hospital

Jamil R. Azzi, MD, PhD

- Renal Fellowship @ BWH and MGH
- Transplant Fellowship @ BWH
- Post doctoral Fellowship @ BWH and HMS
- Associate Professor of Medicine @ HMS
  - Clinical focus: Transplant nephrology
  - Research focus: Basic immunology, cell and targeted therapies, and biomarker discovery

Jamil R. Azzi, MD PhD
Associate Professor of Medicine, Harvard Medical School

I have financial relationship(s) with:

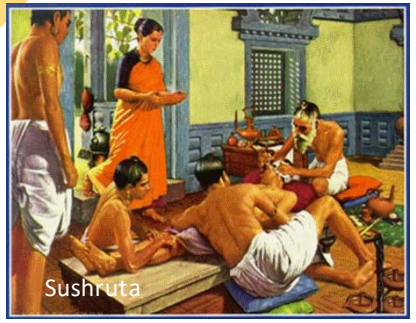
Disclosures:

- ***Intellectual properties:*** ExosomeDx (Biotechne-Brand), Thermo Fisher and Accrue inc.
- ***Founder:*** *Lybra Bio. ImmunNanoDx*
- ***Royalties:*** Accrue inc.
- ***Grants:*** ExosomeDx (Biotechne-Brand), CareDx, Moderna Inc., Alexion.
- ***Consultancy:*** Moderna Inc., CareDx, Trustech, Kezar Life Sciences, CSL Behring, Sanofi,
- ***Advisory Boards:*** Trustech, CareDx, Moderna Inc., Vertex.

AND

My presentation does include discussion of off-label or investigational use.

Early Transplantation and Immunosuppression



Burn Victims
U.K.

Skin Grafting

Immunology of Rejection
(Medawar)

Immunosuppression

Whole Body Irradiation
Adrenal Corticosteroids

Corticosteroids
Azathioprine

Crush Victims
The Netherlands

Artificial Kidney
(Brigham-Kolff)

Dialysis

Cadaveric Kidney Tx
(Boston)

Identical Twin
Kidney Tx
(Boston/Paris)

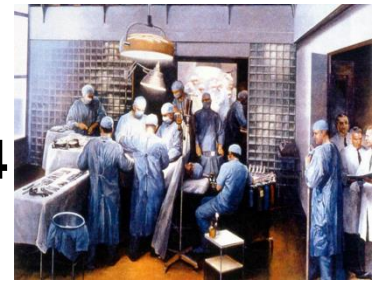
Cadaveric Kidney
Tx

WW II

1947

1954

1962



Balancing Immunosuppression, Infection And Malignancy

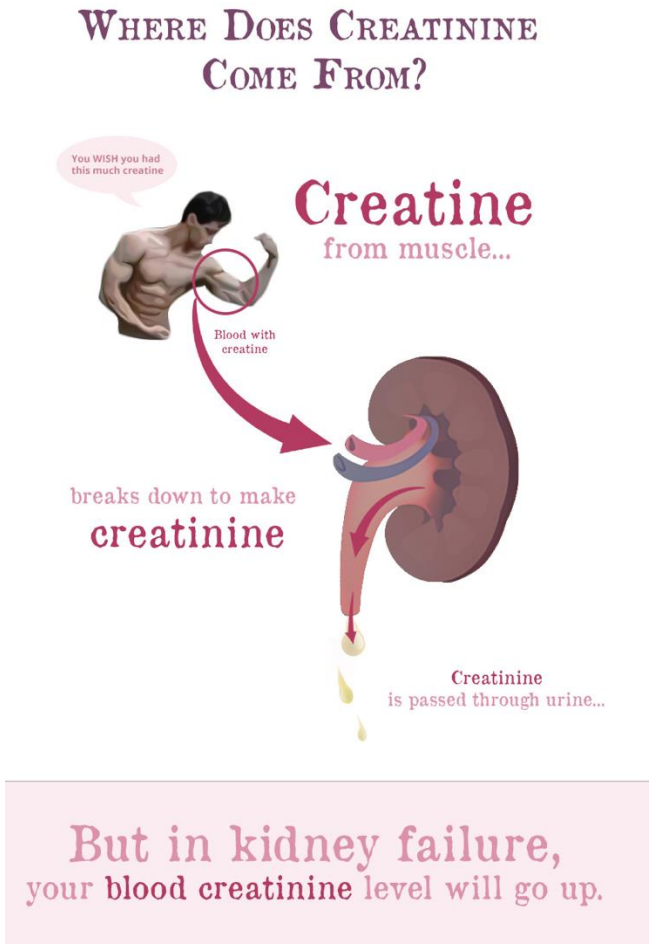
Too Much

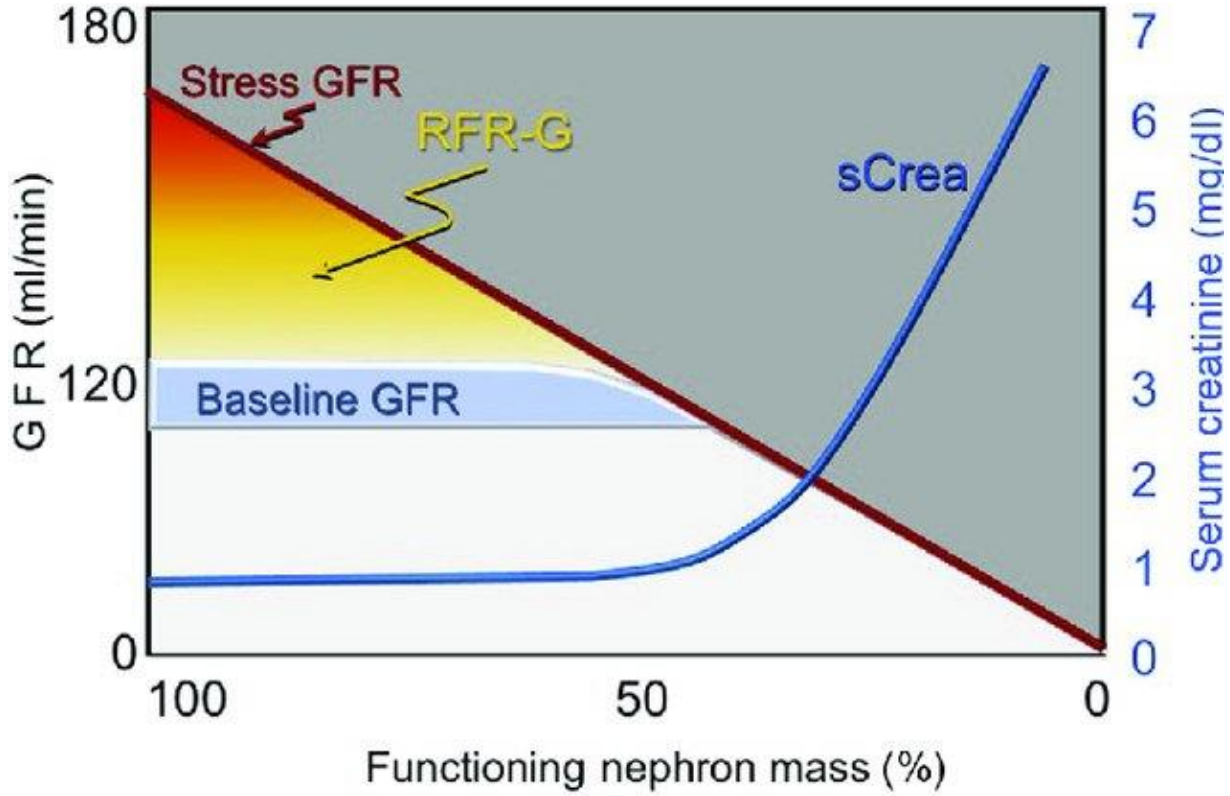
- *Infection*
- *Malignancy*

Too Little

- *Allograft Rejection*

Monitoring kidney function through serum creatinine





20 % Subclinical rejection

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Long-term outcomes following acute rejection in kidney transplant recipients

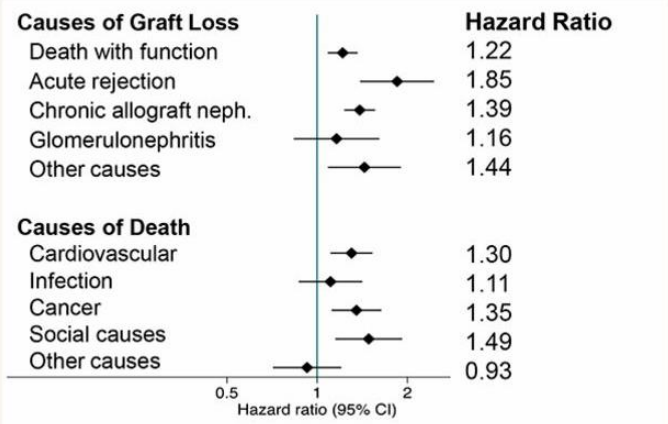
ANZ DATA
AUSTRALIA & NEW ZEALAND DIALYSIS & TRANSPLANT REGISTRY

13614 KTRs 1997-2017
2906 with early acute rejection (AR in 1st 6mth)

Comparison of AR vs no AR:
- Graft survival
  - Causes of graft loss
- Patient survival
  - Causes of death

Models adjusted for era, donor, recipient, immune risk and DGF

Association between early AR and late outcomes for KTRs



KTRs with early AR are at increased risks of death, from CV disease and cancer, and graft failure, from chronic allograft nephropathy and late AR.

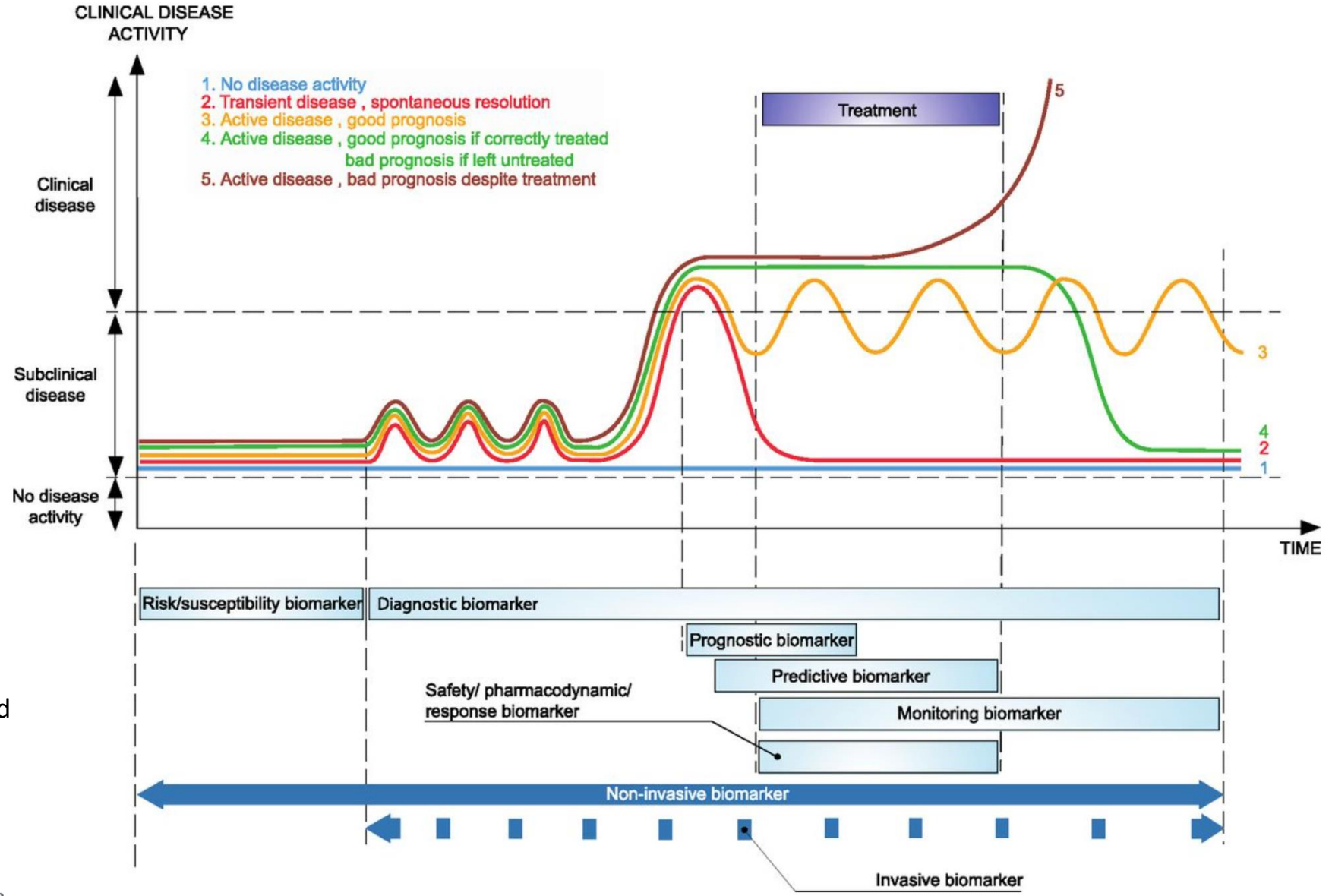
doi: 10.1681/ASN.2018111101

Philip A. Clayton et al. JASN 2019;30:1697-1707

JASN
JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY

Biomarkers subtypes

- **Prognostic biomarkers:**
  - predict clinical outcome regardless of treatment
- **Predictive biomarkers:**
  - Change in response to treatment
- **Surrogate end points:**
  - Substitute for a clinical end point.



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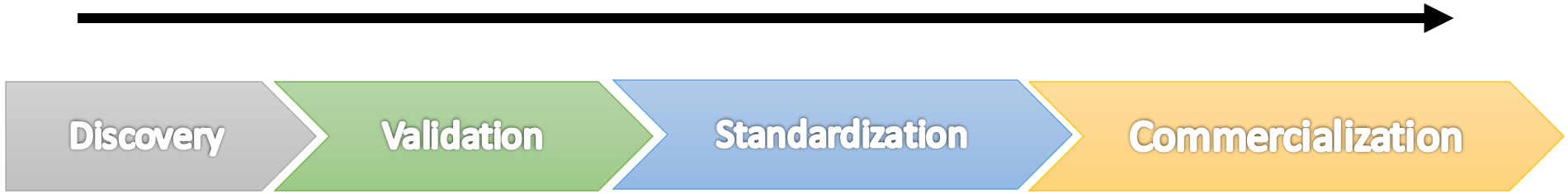
Maarten Naesens, JASN 29(1) 24-34, 2018

Phases of Biomarkers Discovery



- Targeted or non-targeted approaches
- High-throughput technologies
- Identify a panel

- Use independent samples
- Identify a refined set
- Prospective multicentral validation

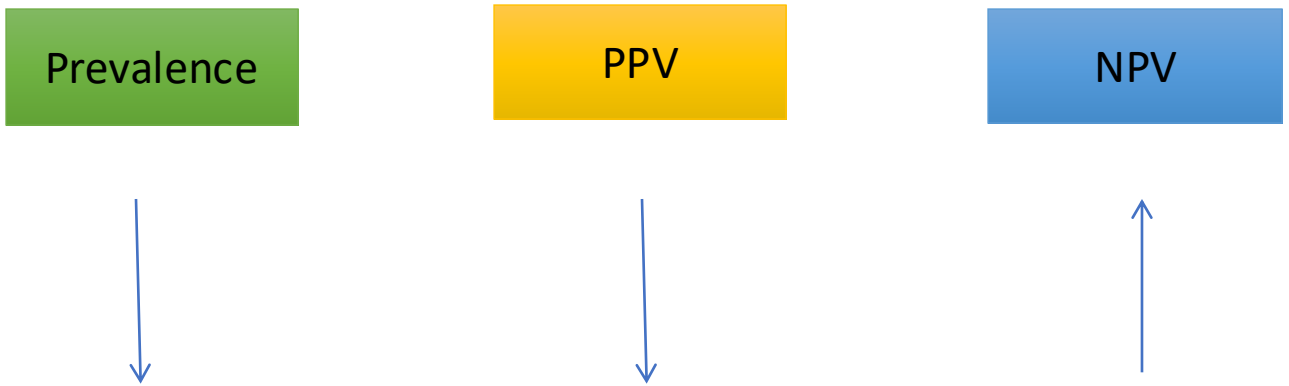
- Laboratory equipment
- Reagents
- Can the test be reproduced in different laboratories

Ideal Biomarker

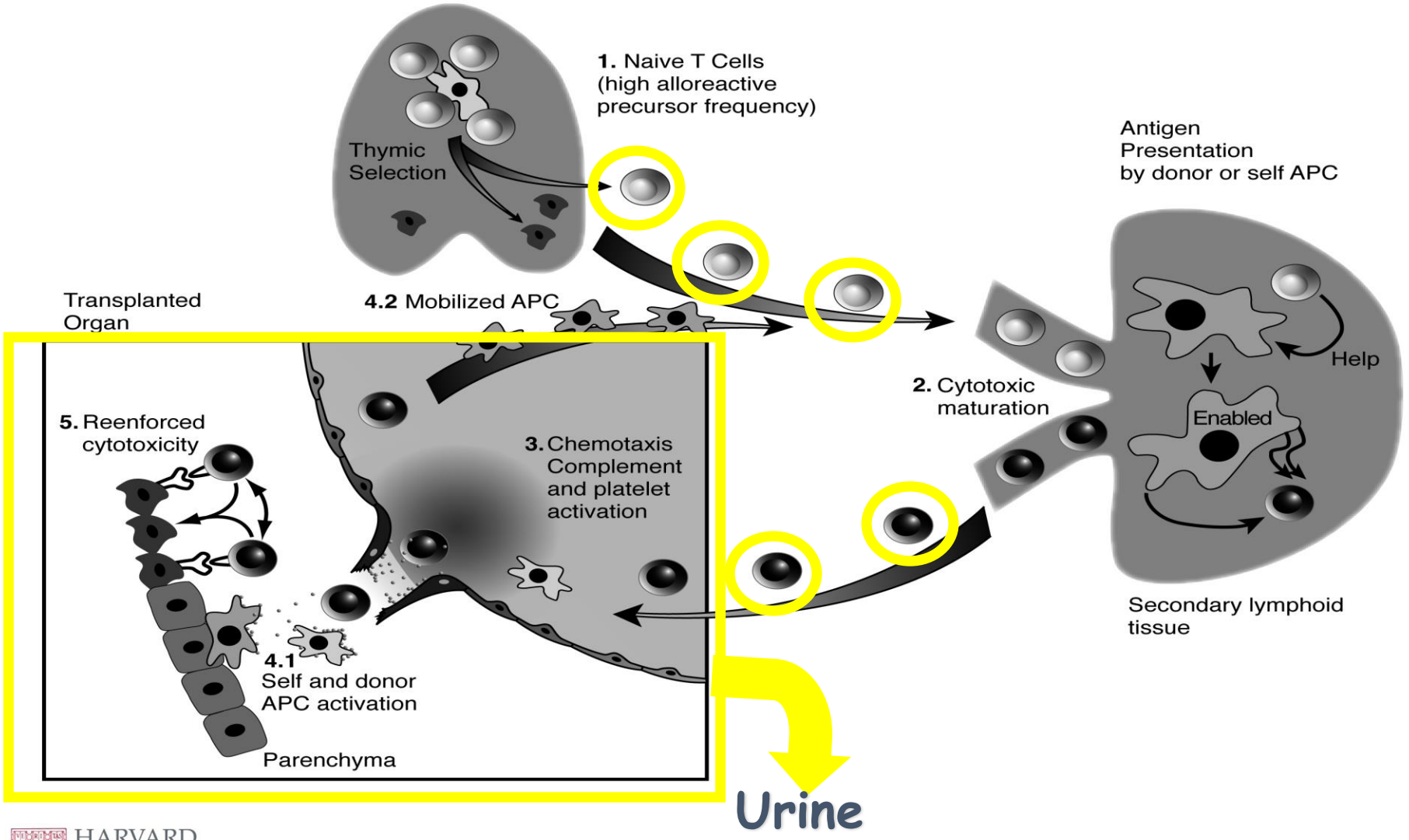
- Sample entire graft (avoid bias from sampling error)
- High positive and negative predictive value
- Diagnose rejection (acute and/or chronic) before its occurrence
- Uncover over-immunosuppressed/identify tolerant states
- Rapid
- Responsive
- Non-invasive
- Inexpensive

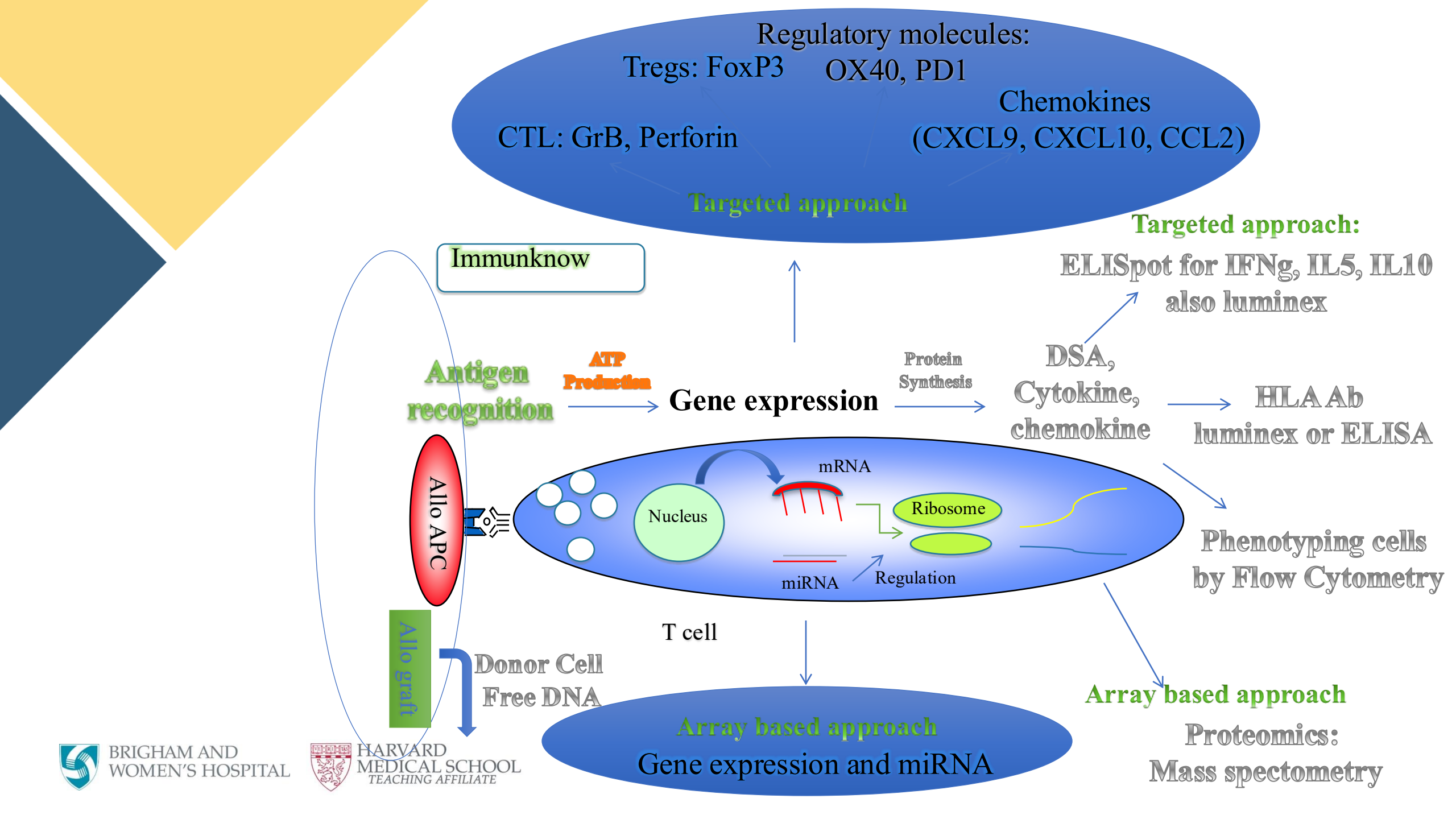
Impact of Prevalence on predictive values

- Positive predictive value (PPV) is the number of true positives if the test is positive
- Negative predictive value (NPV) is the number of true negatives if the test is negative



Mechanisms of Immune Monitoring
What's Available for Evaluation?





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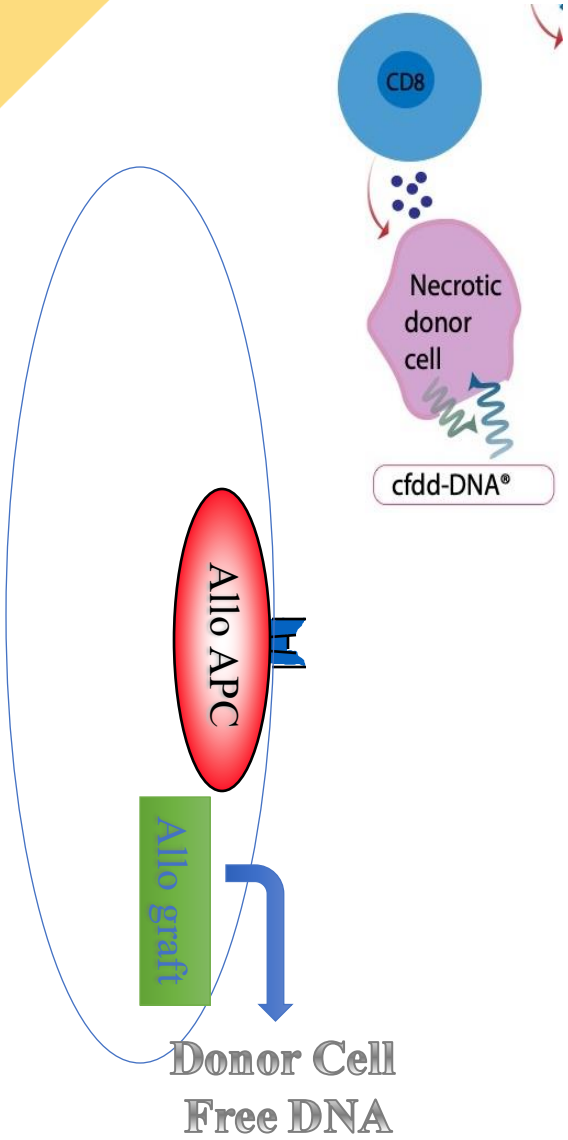
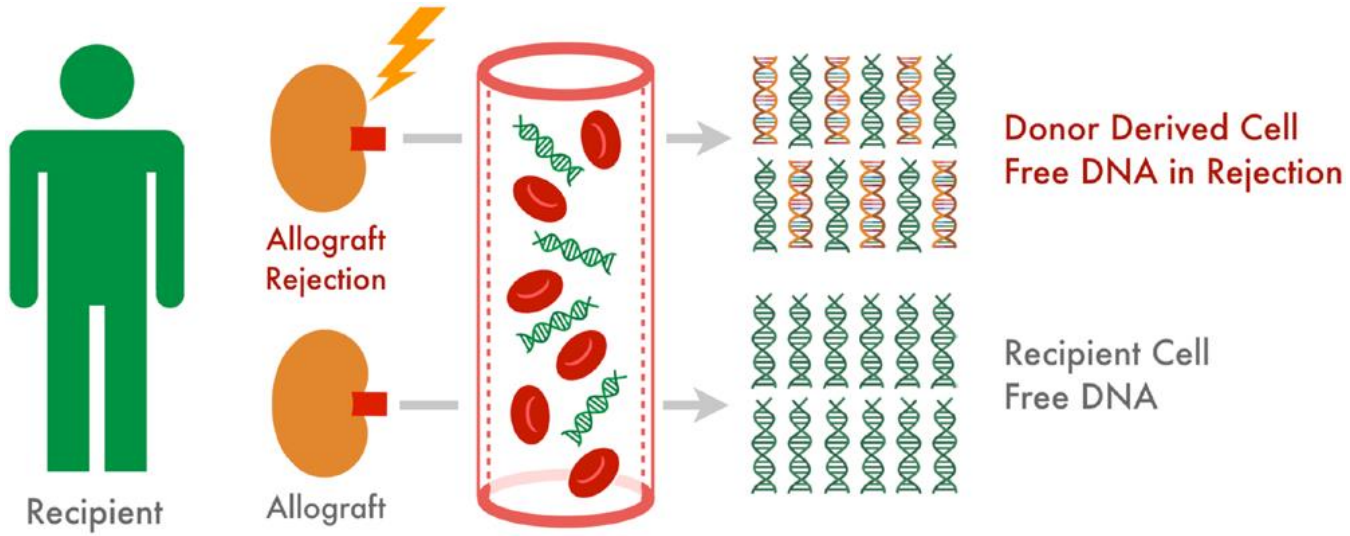
Clinically Available Biomarkers: Donor-derived Cell Free DNA





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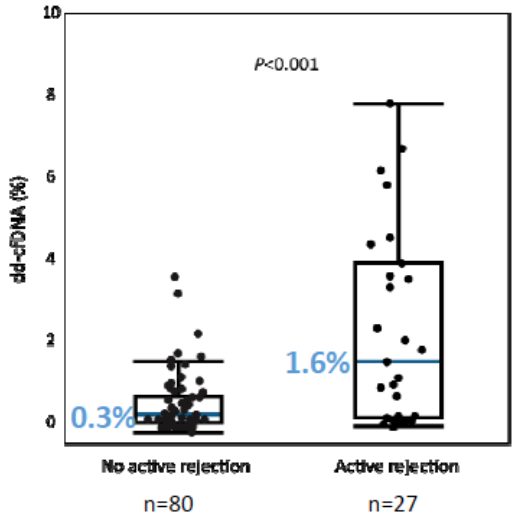
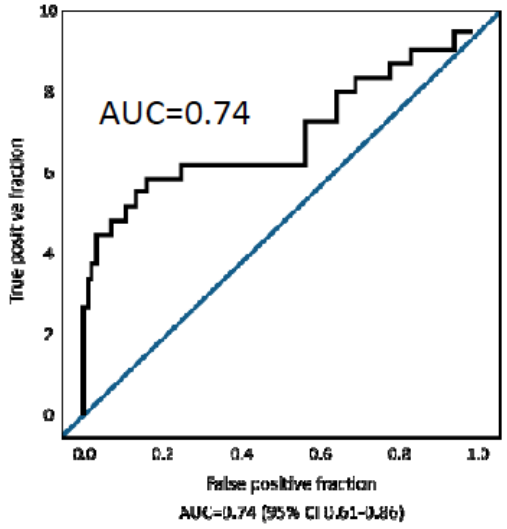
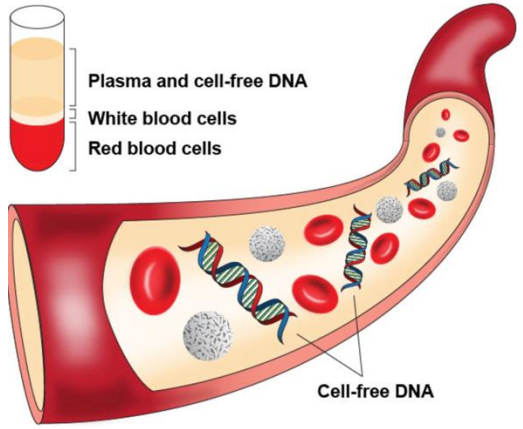
Paul et al., Kidney Transplantation, 2021

Clinically Available Biomarkers: Donor-derived Cell Free DNA

- DART study, Bloom et al, J Am Soc Nephrol, 28:2221-2232, 2017

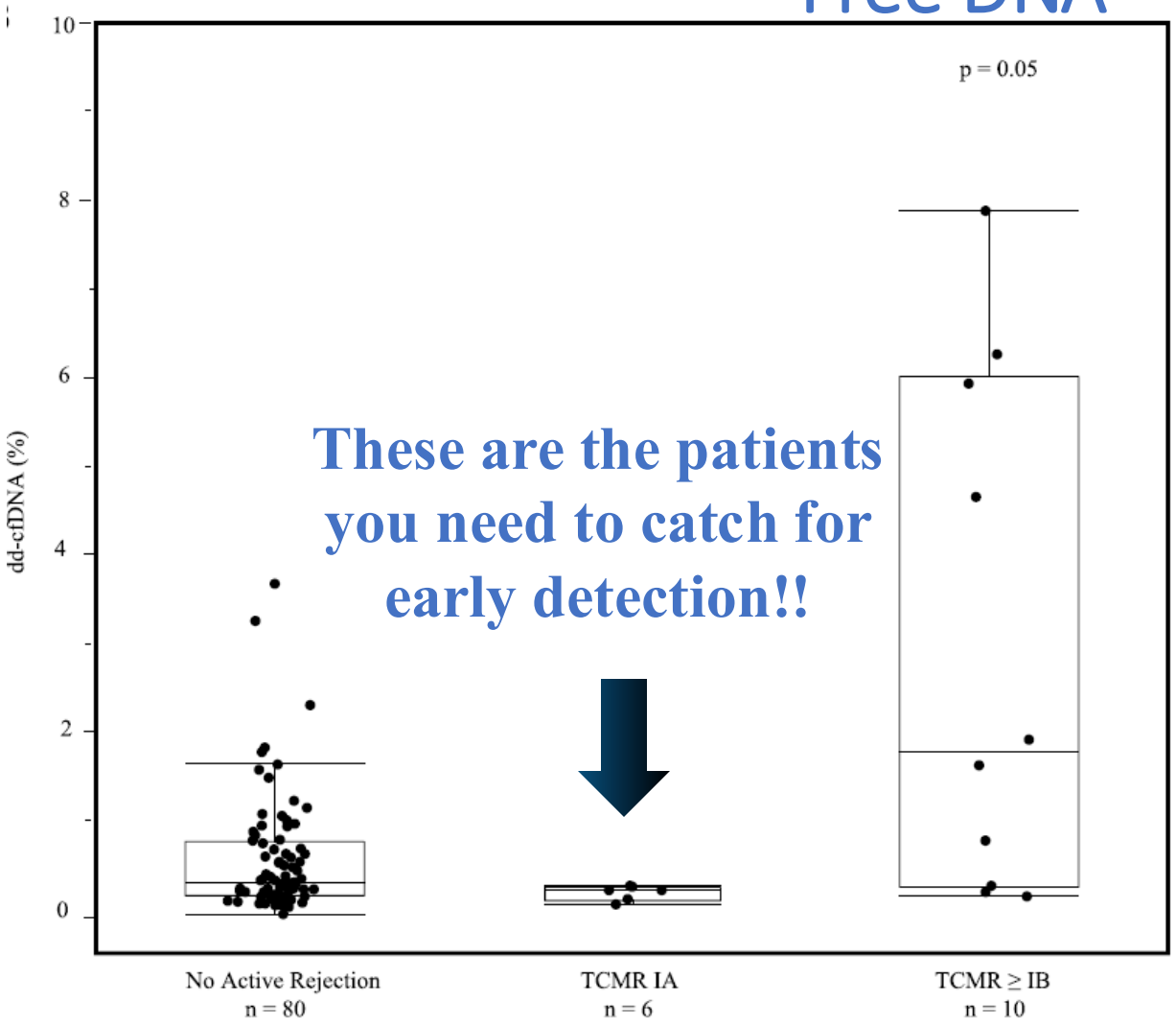


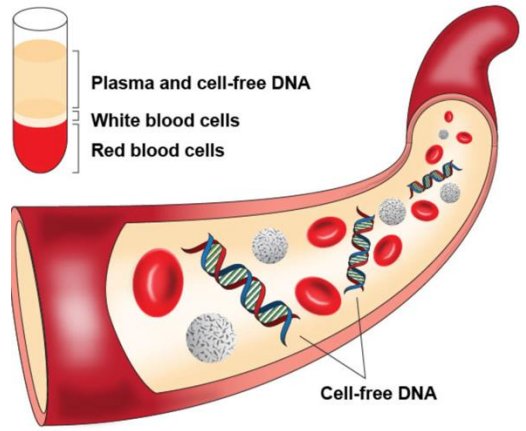




Performance metric	AlloSure test performance at 1% threshold
ROC/AUC	0.74 (95% CI 0.61-0.86)
Sensitivity	85%
Specificity	59%
NPV	84%
PPV	61%

Clinically Available Biomarkers: Donor-derived Cell Free DNA





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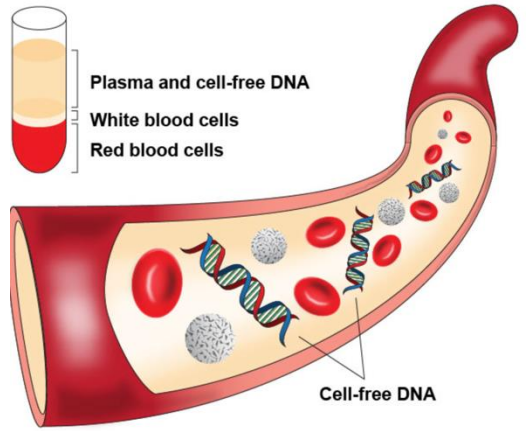
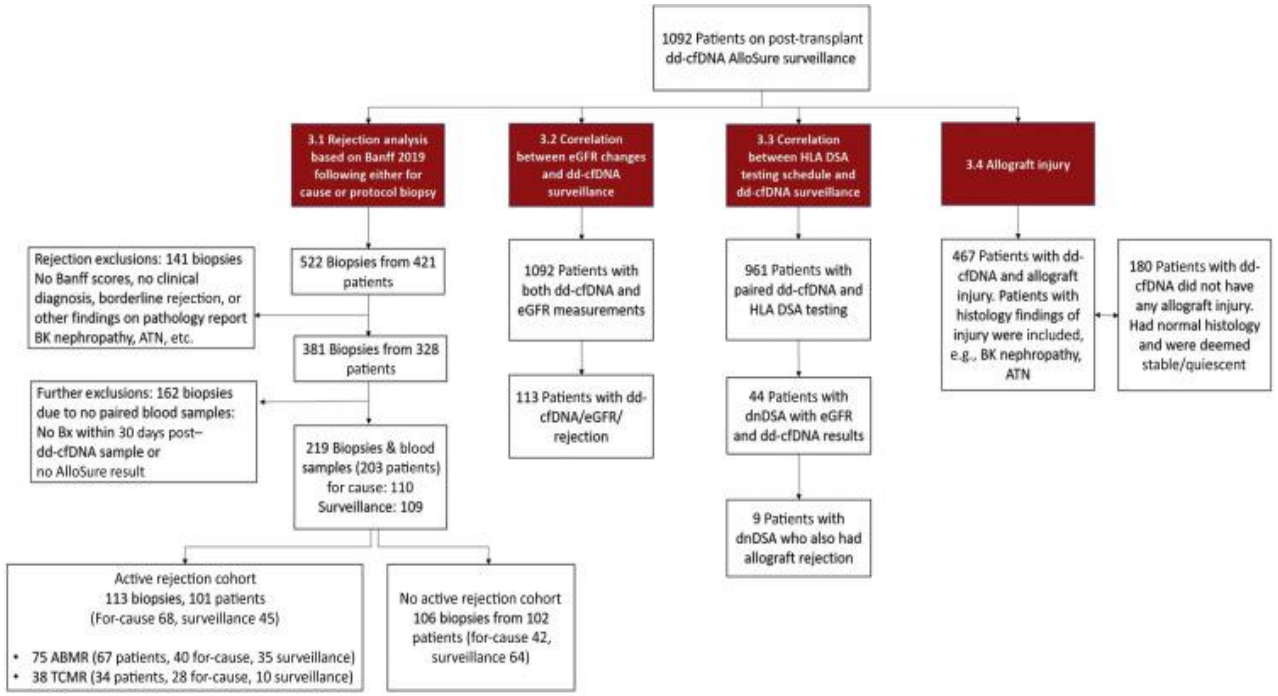
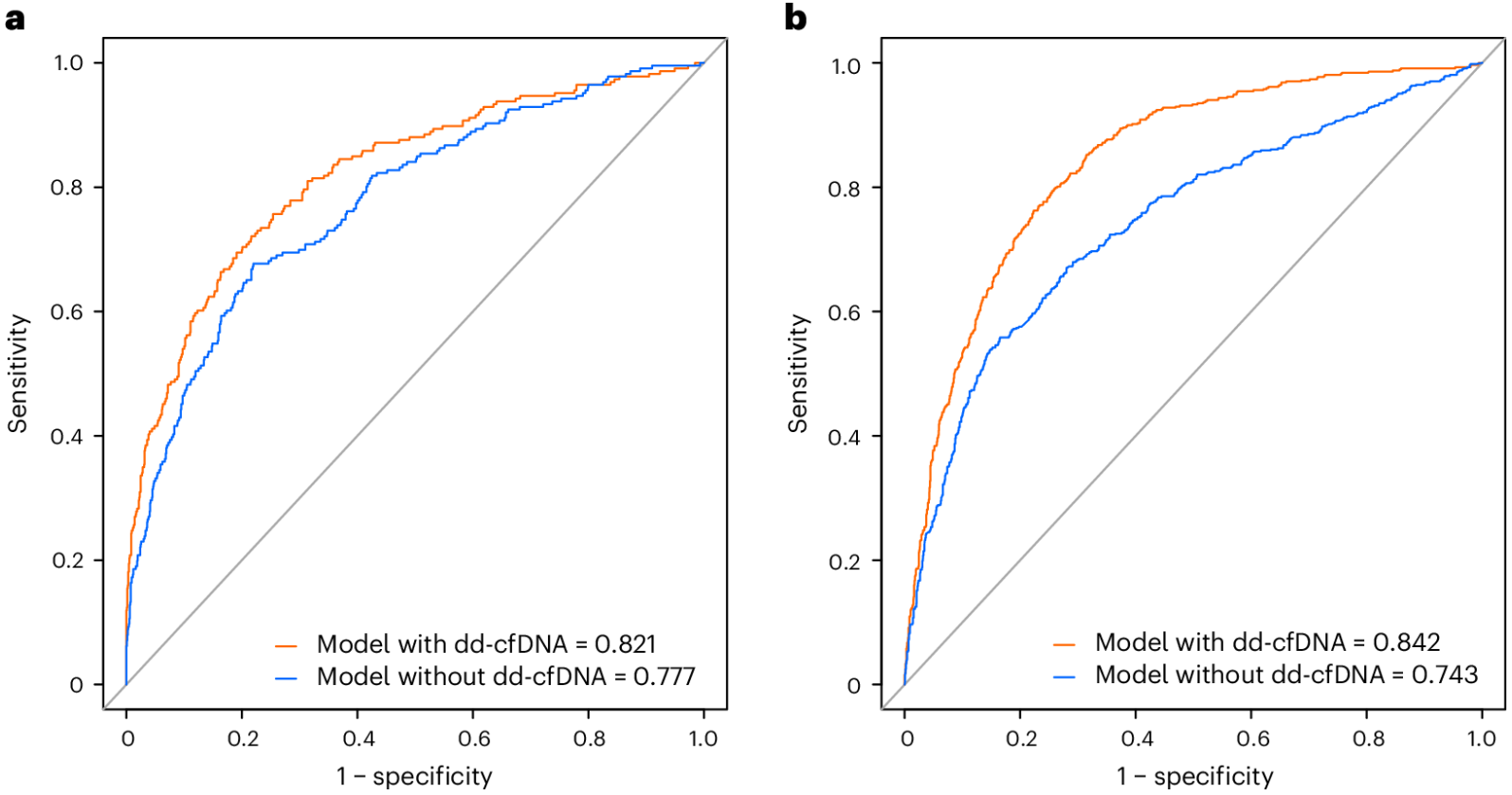




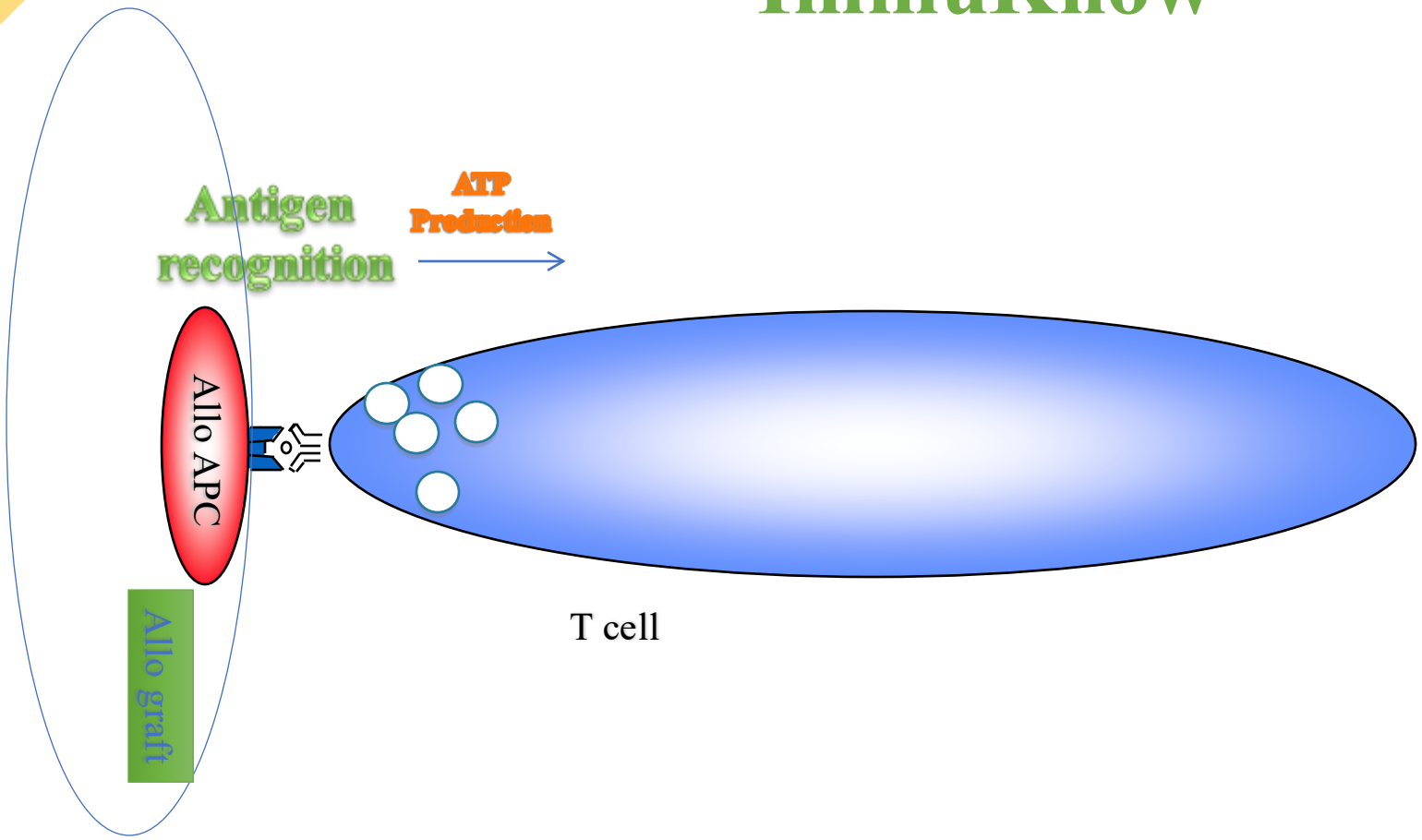
Data from 1092 kidney transplant recipients monitored for dd-cfDNA over a three-year period

Clinically Available Biomarkers: Donor-derived Cell Free DNA

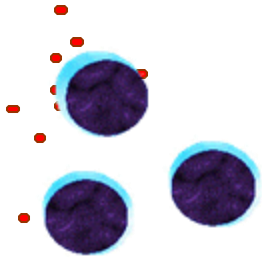


Aubert et al. Nature Medicine, 2004

Clinically Available Biomarkers:
ImmuKnow

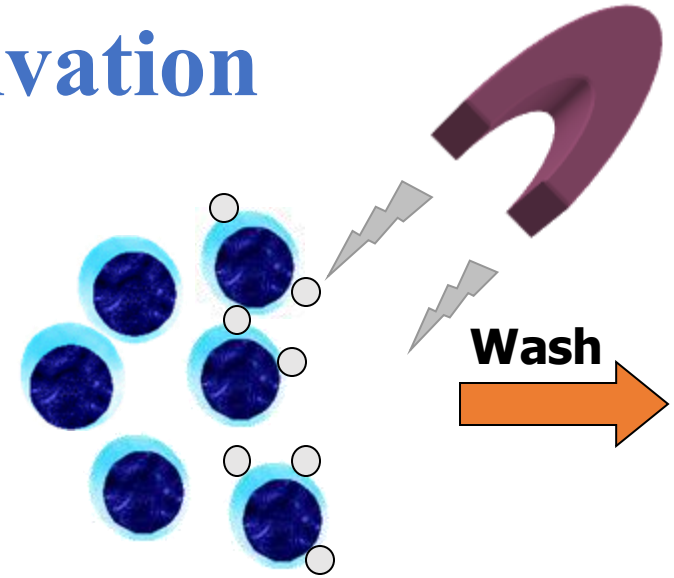


Lymphocyte activation



Lymphocyte Stimulation w/PHA

Incubate 15-18 hrs.



Magnetic separation of CD4 cells.

Wash

ATP ATP ATP

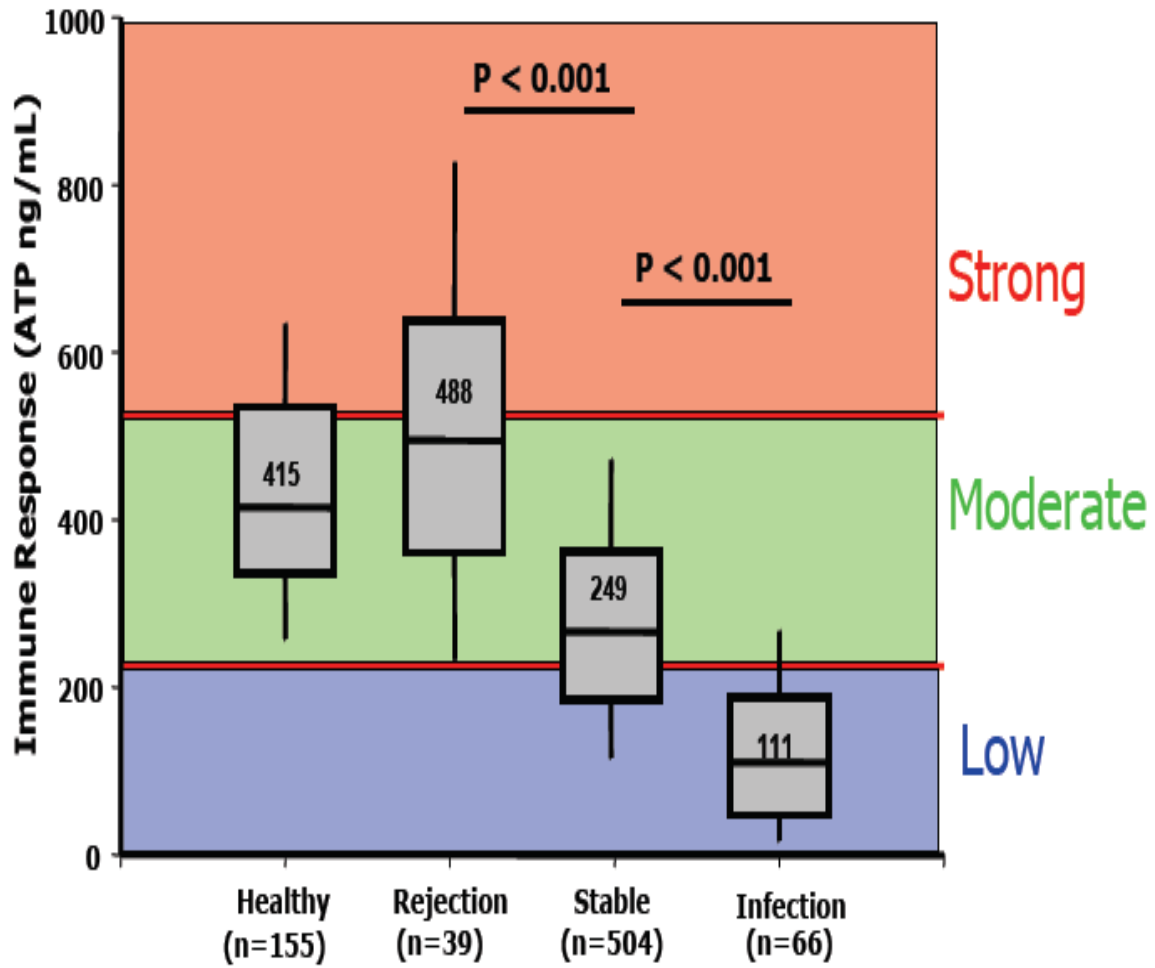
Cell lysis to release ATP

ATP detection reagents

Measure light intensity

J. Britz et. al., "In Vitro CMI: Rapid Assay for Measuring Cell-Mediated Immunity", CRC Press, 2002

Immune Function Correlates With Clinical Outcomes

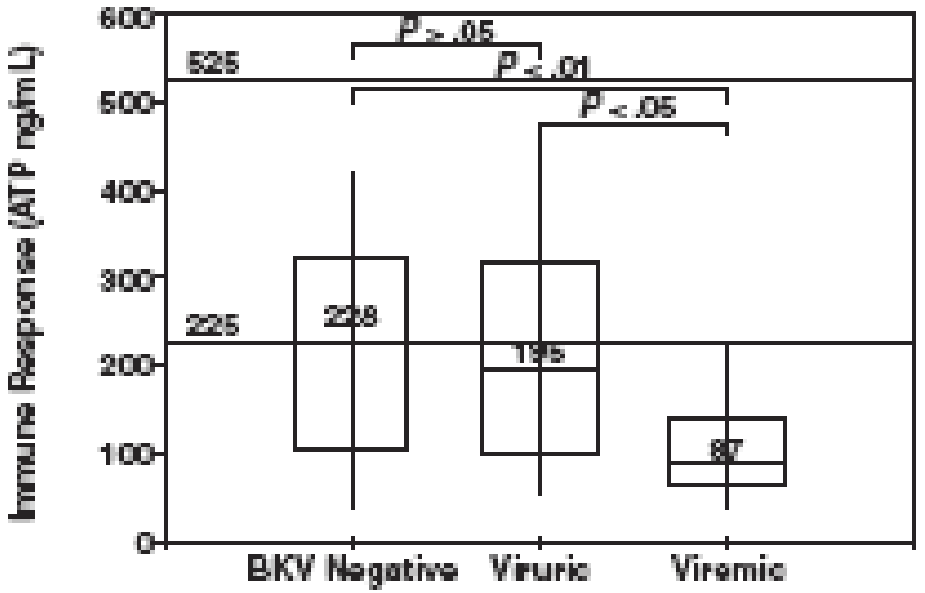


Multicenter Studies: NIDDK, UCLA, JHU, UPMC, NZTI, LifeLink, UAB, UMD, INOVA, UTMB

Measurements of Global Cell-Mediated Immunity in Renal Transplant Recipients With BK Virus Reactivation

Ibrahim Batal, MD,[1] Adriana Zeevi, PhD,[1] Amer Heider, MD,[1] Alin Girnita, MD,[1] Amit Basu, MD,[2] Henkie Tan, MD, PhD,[2] Ron Shapiro, MD,[2] and Parmjeet Randhawa, MD[1]

Key Words: BK virus; Immune cell function; Viremia; Viruria



Am J Clin Pathol 2008

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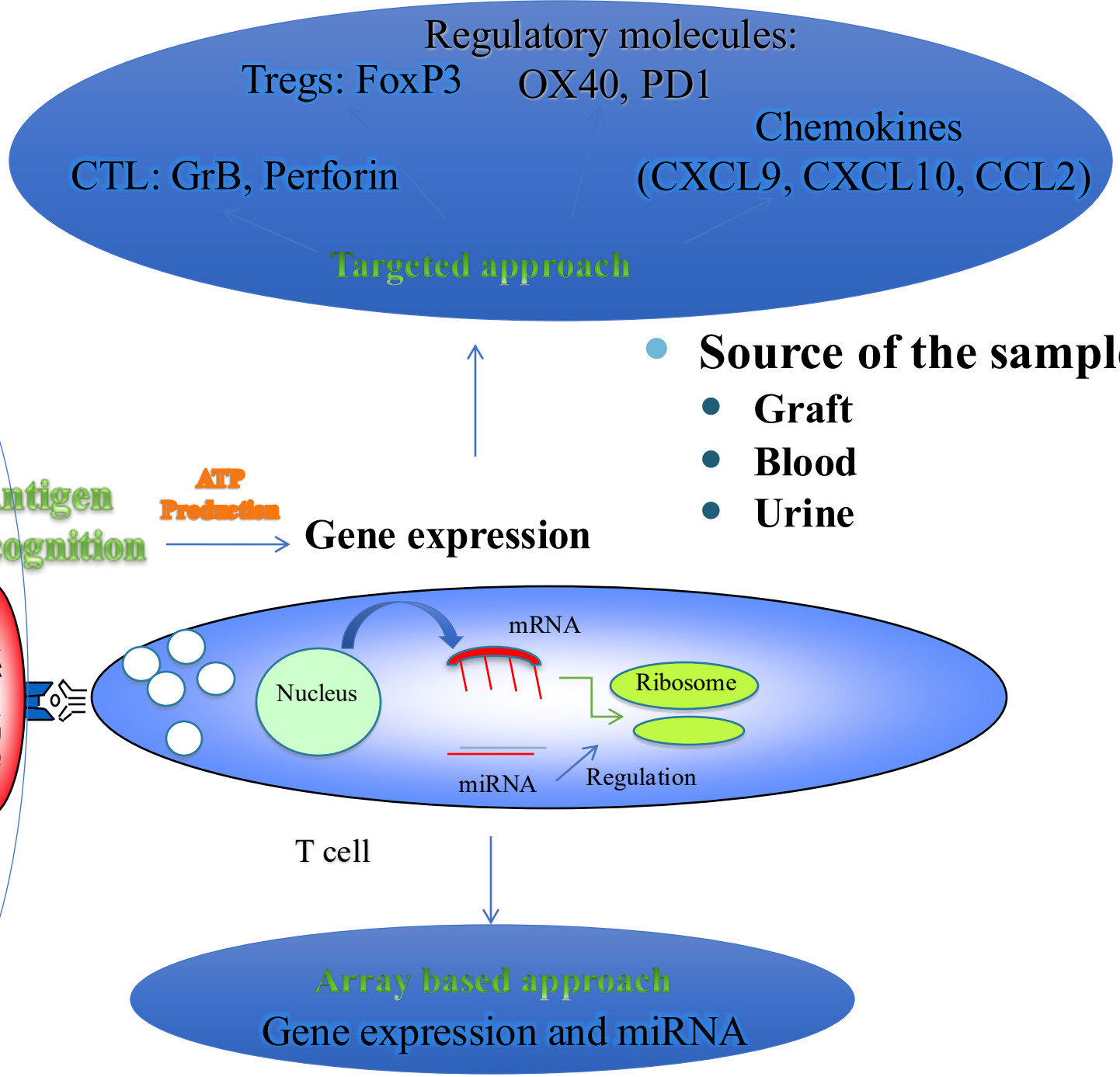

- **Source of the samples:**
  - **Graft**
  - **Blood**
  - **Urine**

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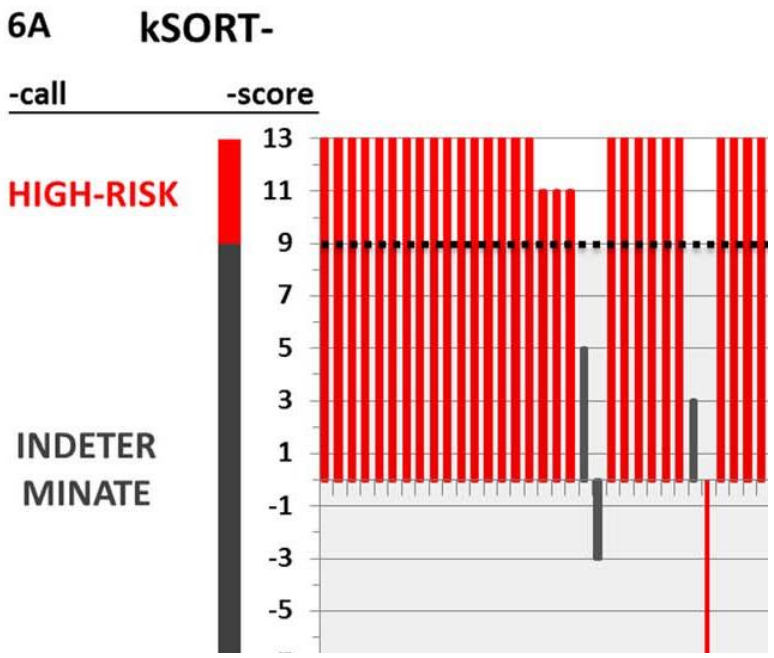
Clinically Available Biomarkers:

KSORT



- qPCR to measure mRNA expression levels of 17 genes
- Algorithm generates risk scores
- Multicenter study of 436 adults,
  - Predict patients at high risk of acute rejection
  - Sens 92%
  - Spec 93% Unable to distinguish between acute TCMR and ABMR.
- A subsequent large, multicenter study of 1134 patients (under real-world clinical conditions) was unable to validate the utility of the kSORT assay.

Roedder S, PLoS Med, 2014
Crespo E, Transplantation, 2017
Loon EV, AJT, 2021

Clinically Available Biomarkers: TruGraf

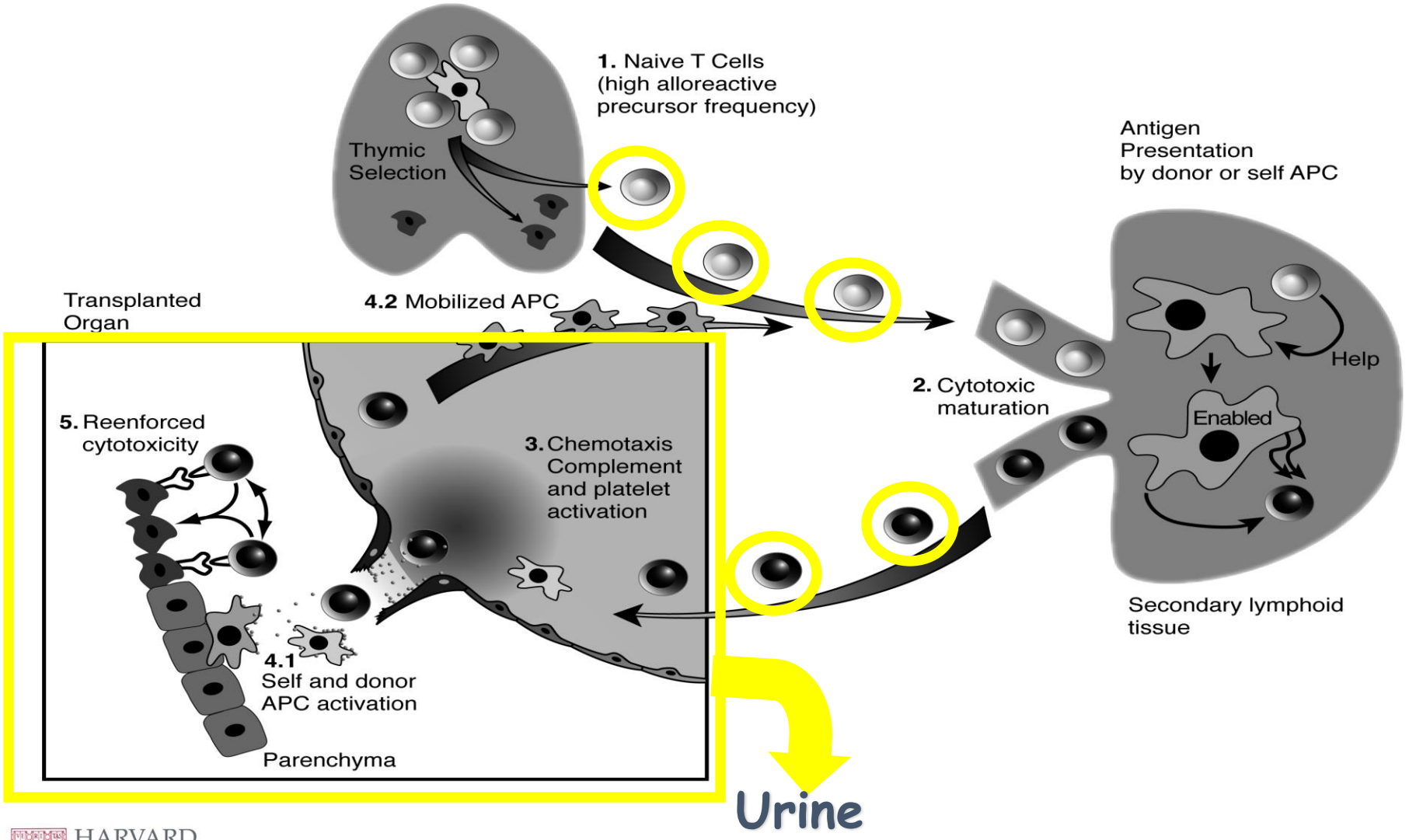
Table 1A. Results of TruGraf Blood Test and Comparison With Clinical Phenotype in All 192 Kidney Transplant Recipients with Stable Renal Function

	Clinical Phenotype not-TX	Clinical Phenotype TX
TruGraf Blood Test not-TX	26	42
TruGraf Blood Test TX	8	116

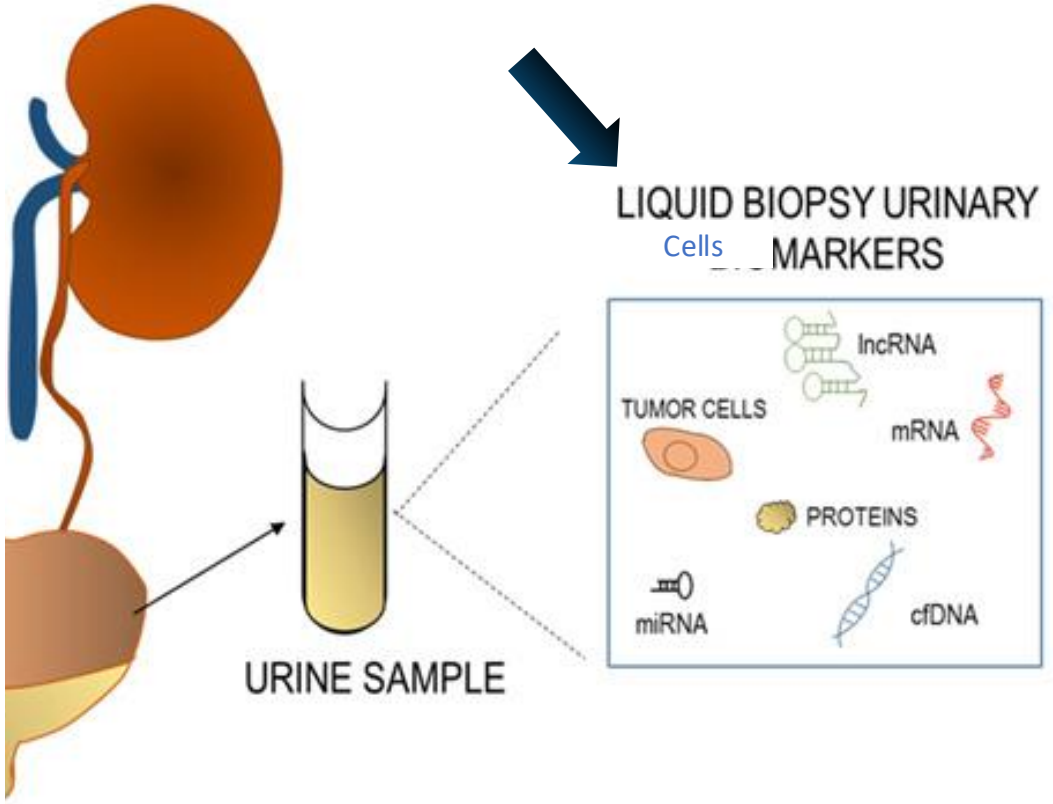
Accuracy = 142/192 (74%).
Accuracy of TruGraf TX result 116/124 (94%).
NPV = 91%.
PPV = 48%.
Sensitivity = 76%.
Specificity = 73%.

Marsh et al. Transplant P, 2019

Mechanisms of Immune Monitoring
What's Available for Evaluation?

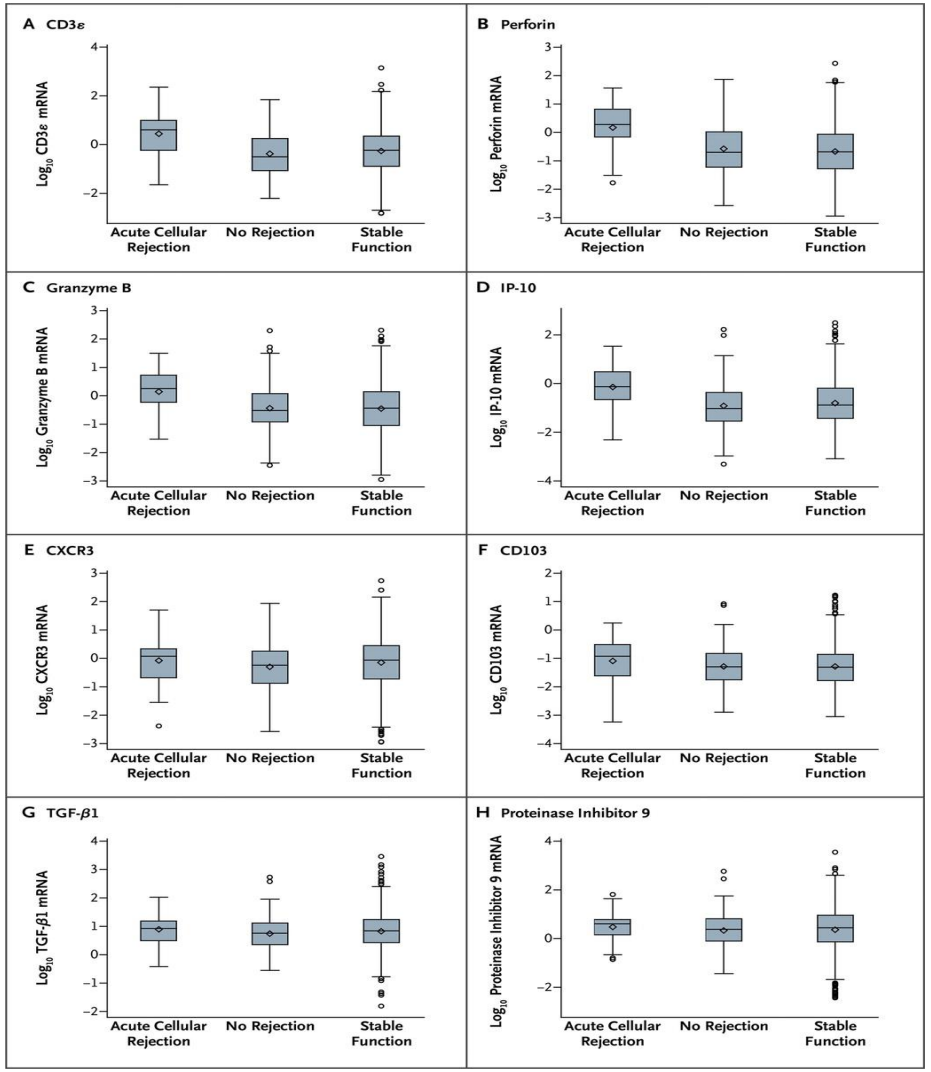


mRNA in Urinary Cells.



Li B et al. N Engl J Med 2001;344:947-954.
Suthanthiran M et al. N Engl J Med 2013;369:20-31.

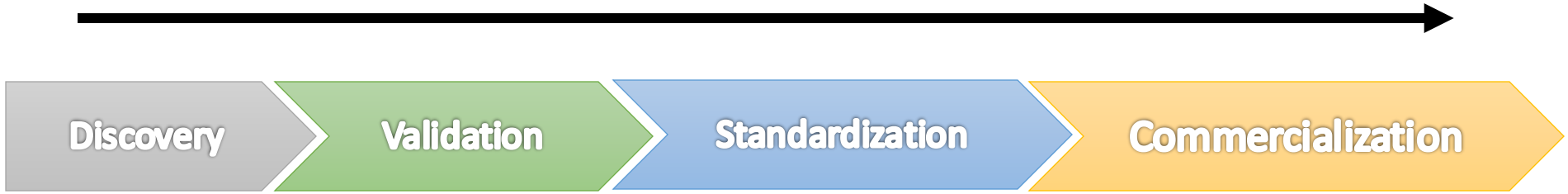
Levels of mRNA in Urinary Cells.



- Prospective collection
- 4300 urine specimens from 485 kidney-graft recipients
- Day 3 through month 12 after transplantation.
- CD3ε, IP-10 and 18S rRNA
  - 79% sensitivity and 78% specificity
  - [AUC], 0.85

Suthanthiran M et al. N Engl J Med 2013;369:20-31.

Phases of Biomarkers Discovery



- Targeted or non-targeted approaches
- High-throughput technologies
- Identify a panel

- Use independent samples
- Identify a refined set
- Prospective multicentral validation

- Laboratory equipment
- Reagents
- Can the test be reproduced in different laboratories

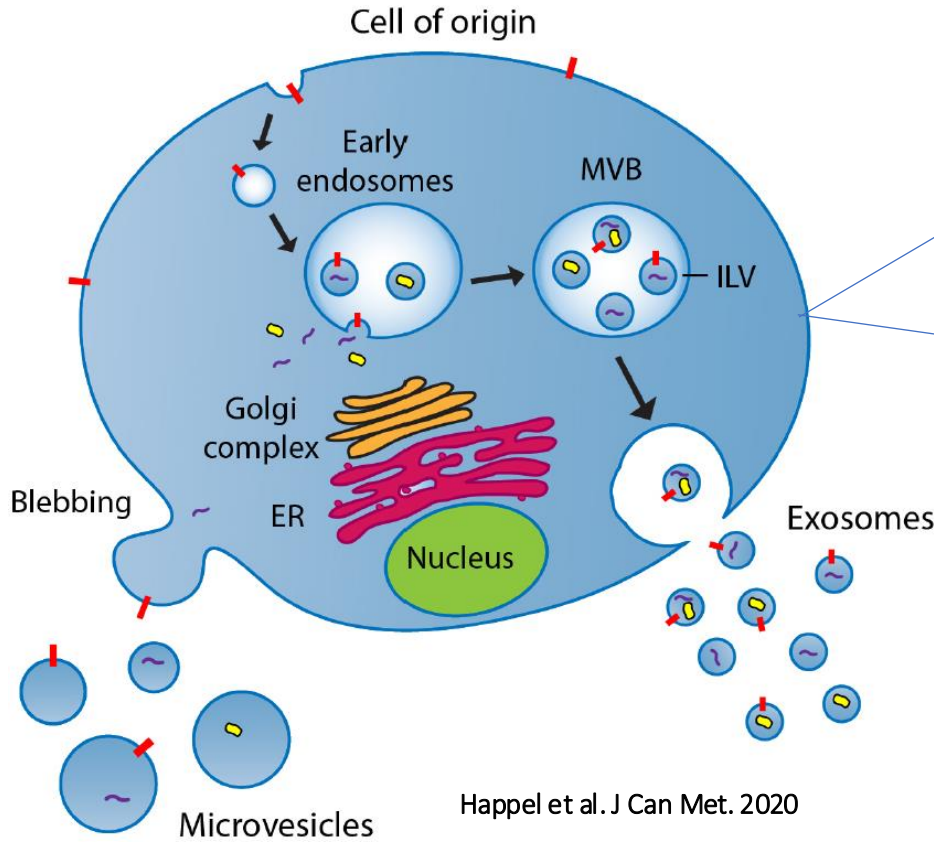
Extracellular vesicles as biomarkers

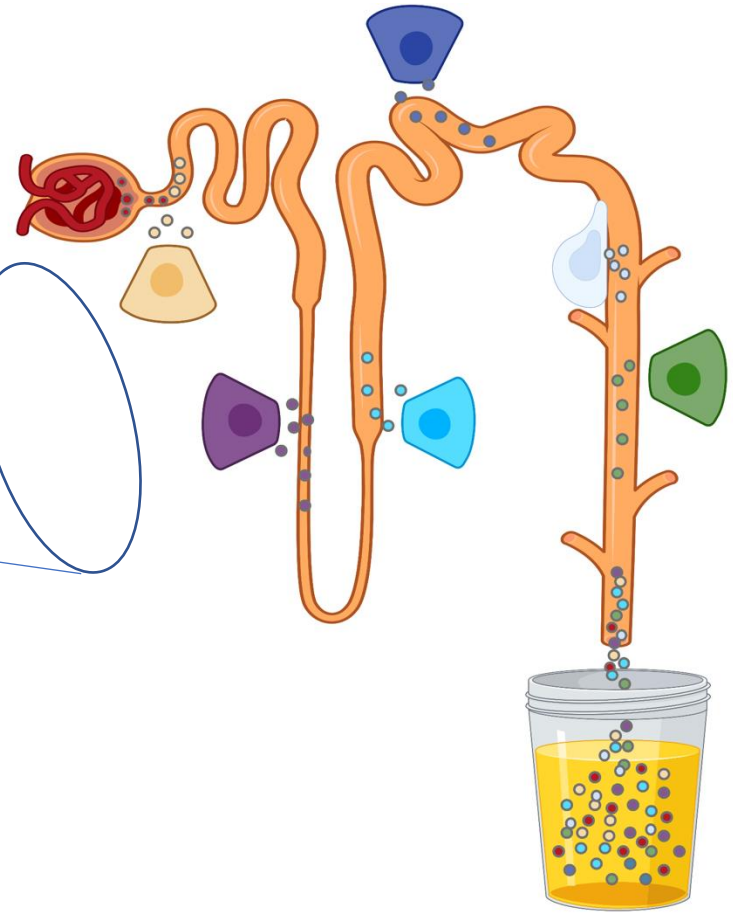
Cell-specific receptors

Proteins

Nucleic acids/exRNA

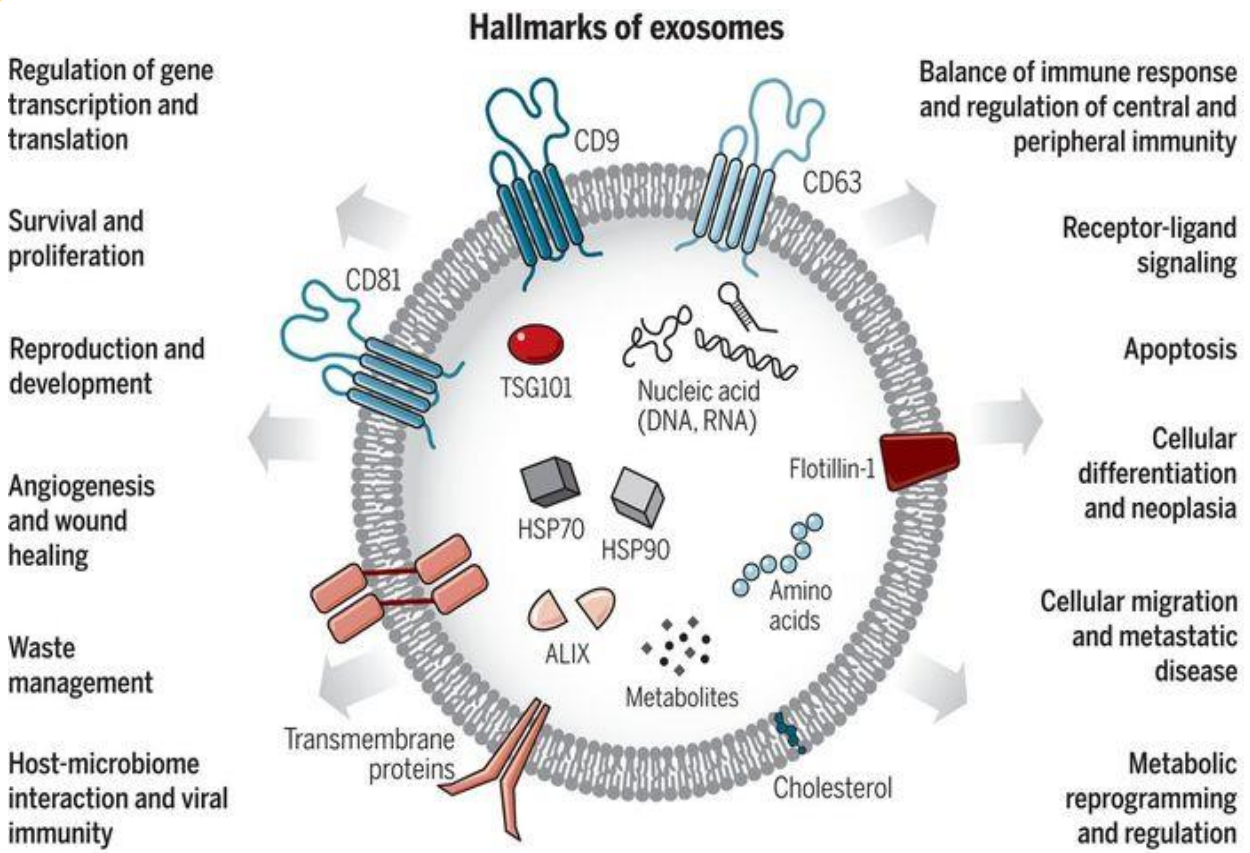
exRNA Types	
miRNA	piRNA
tRFs	snoRNA
Y RNA	lncRNA

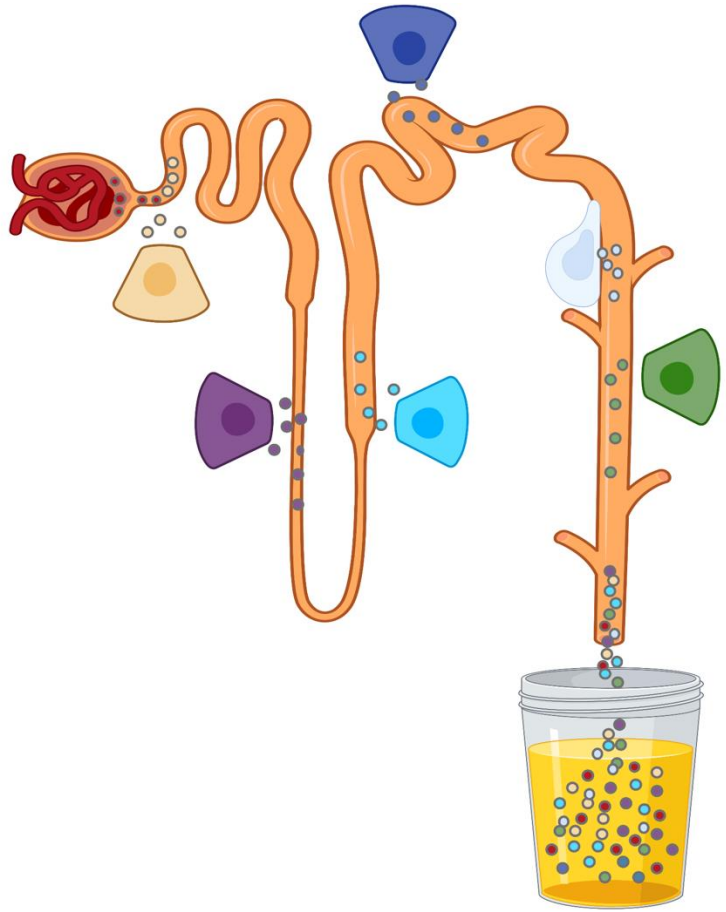




Happel et al. J Can Met. 2020

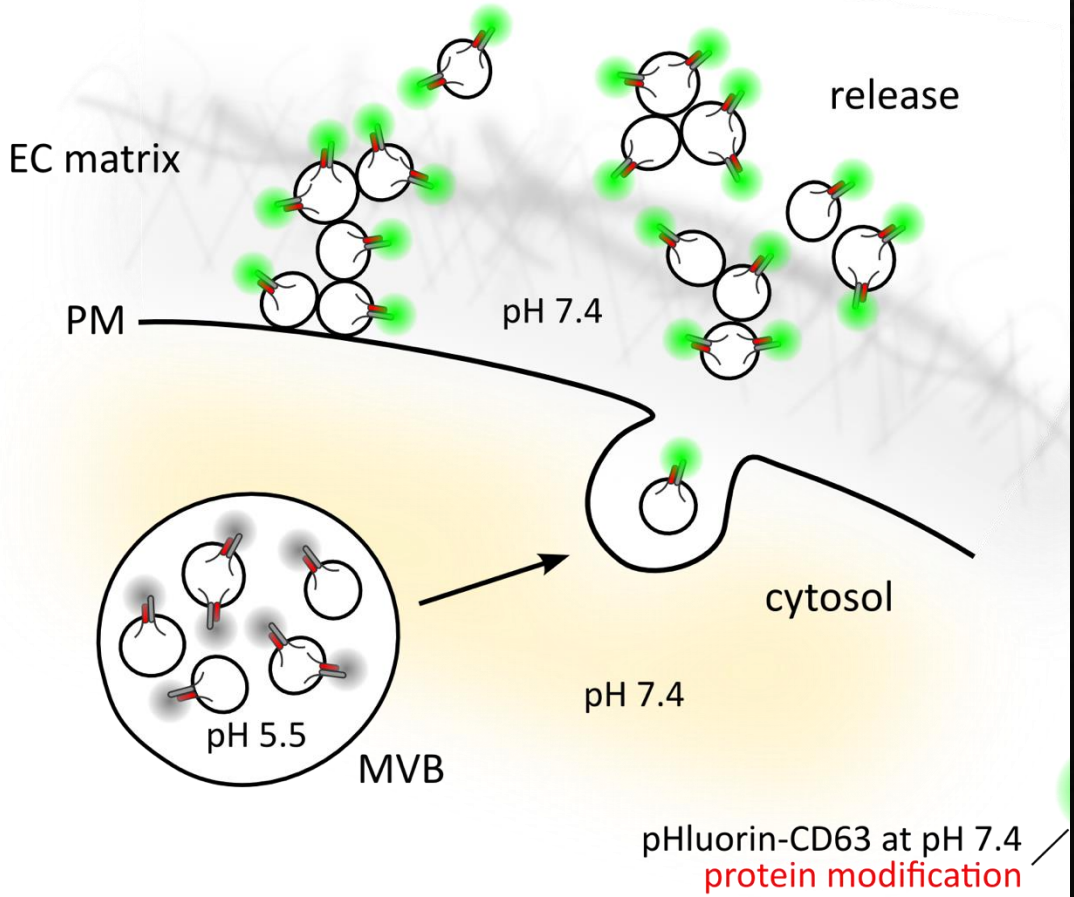
Hallmarks of exosomes

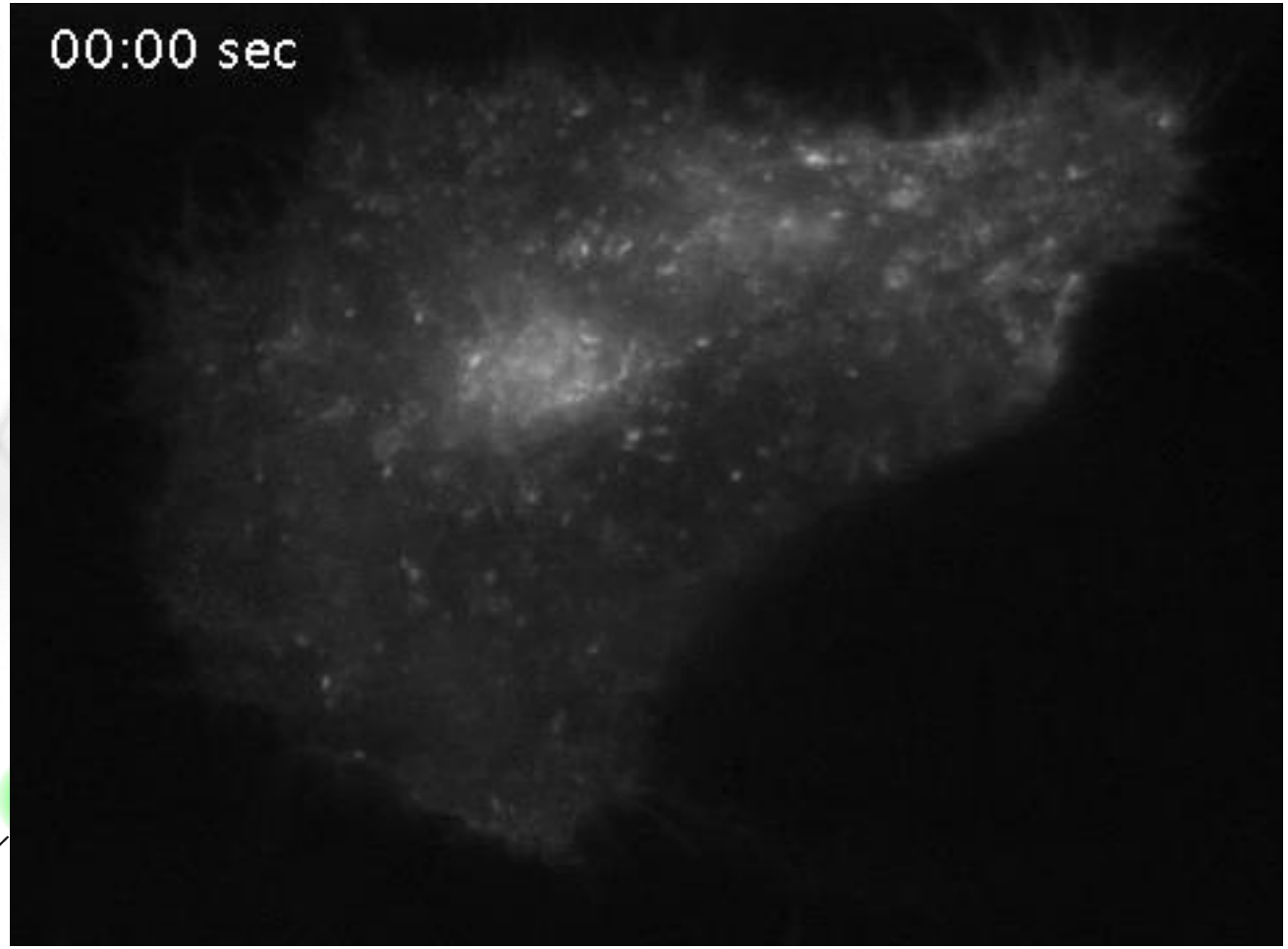




The biology, function, and biomedical applications of exosomes, Volume: 367, Issue: 6478, DOI: (10.1126/science.aau6977)

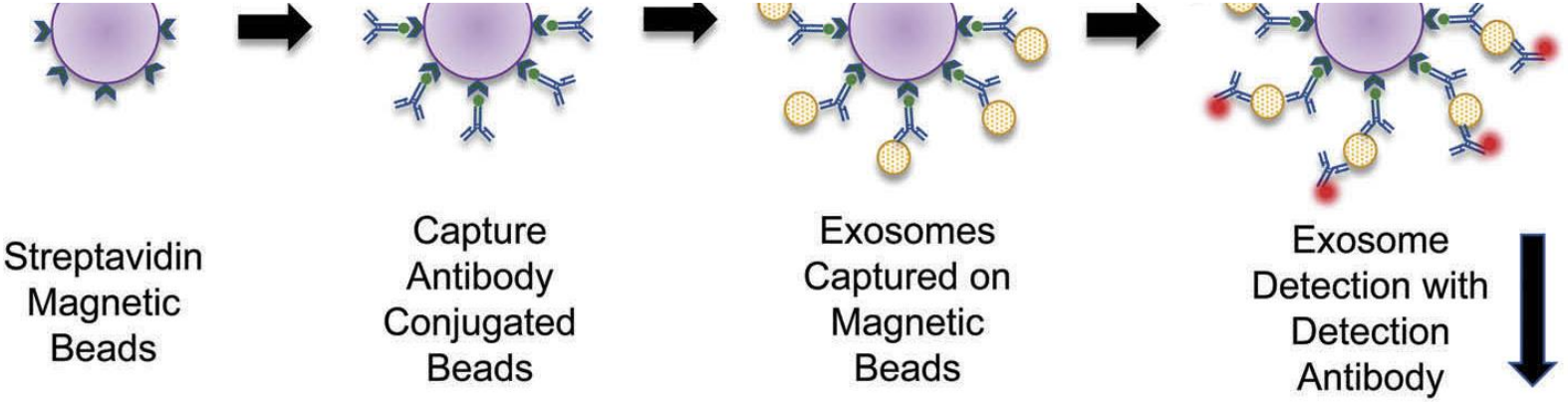
LIVE-IMAGING REVEALS EXOSOME SECRETION DYNAMICS FROM A SINGLE LIVING CANCER CELL

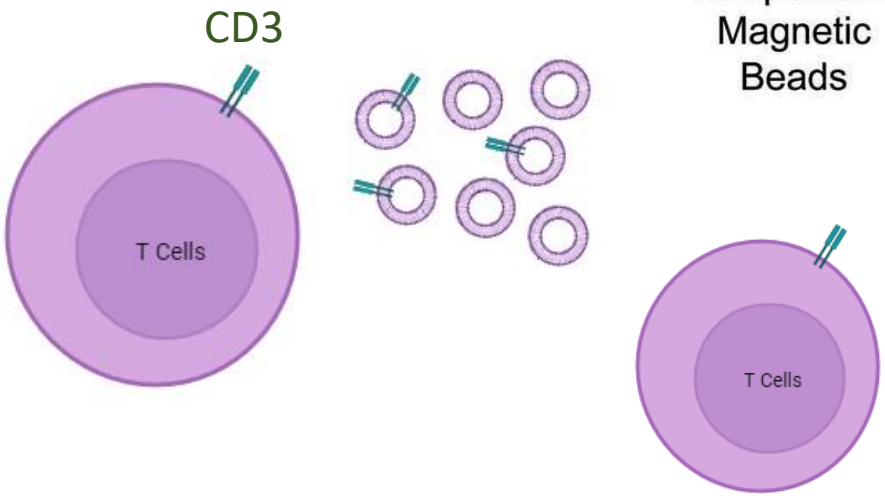


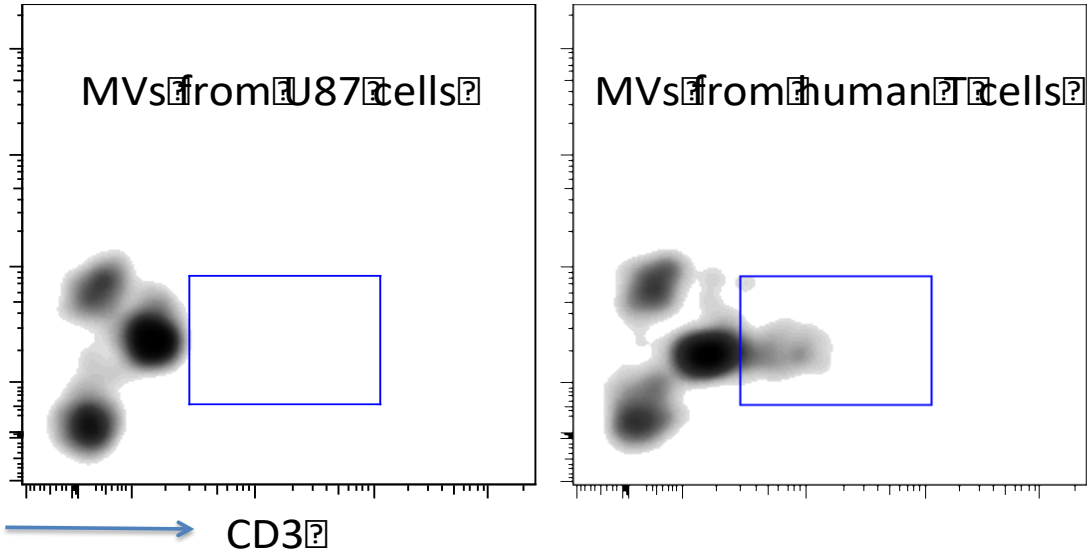


Verweij et al., J Cell Biol 2018

Immune cell specific EVs







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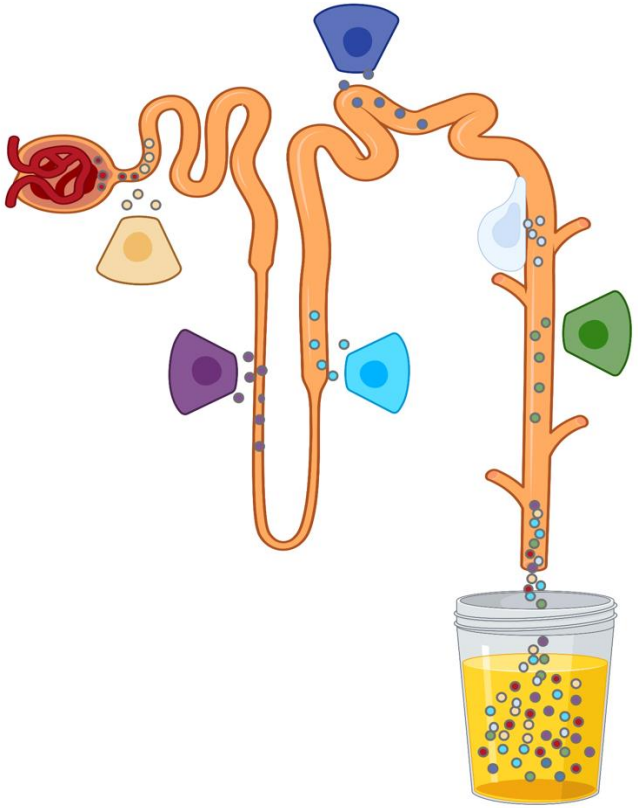
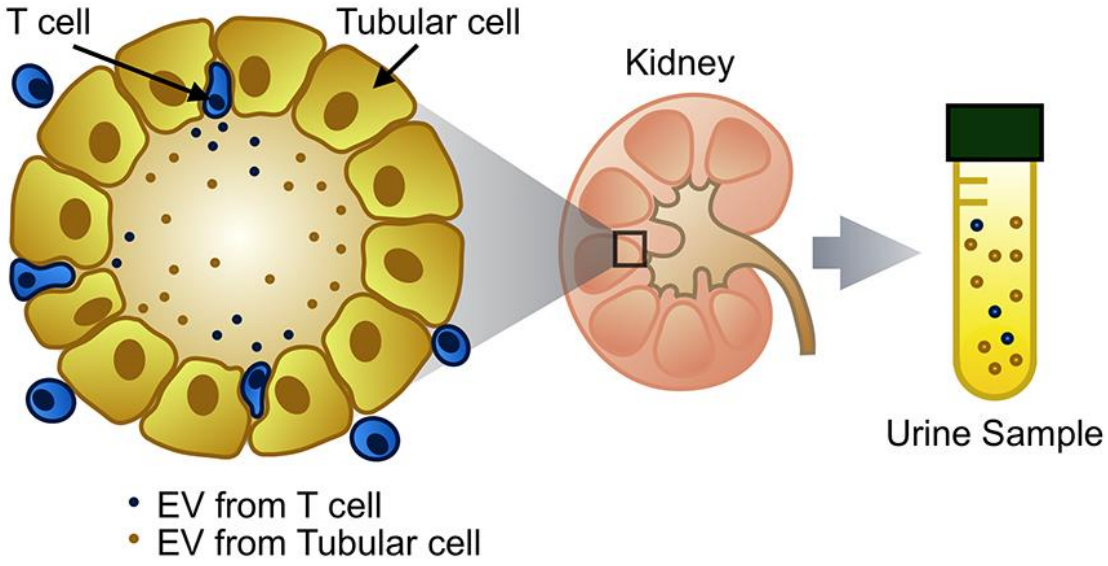
Immune cell specific EVs in urine of rejecting kidney allografts





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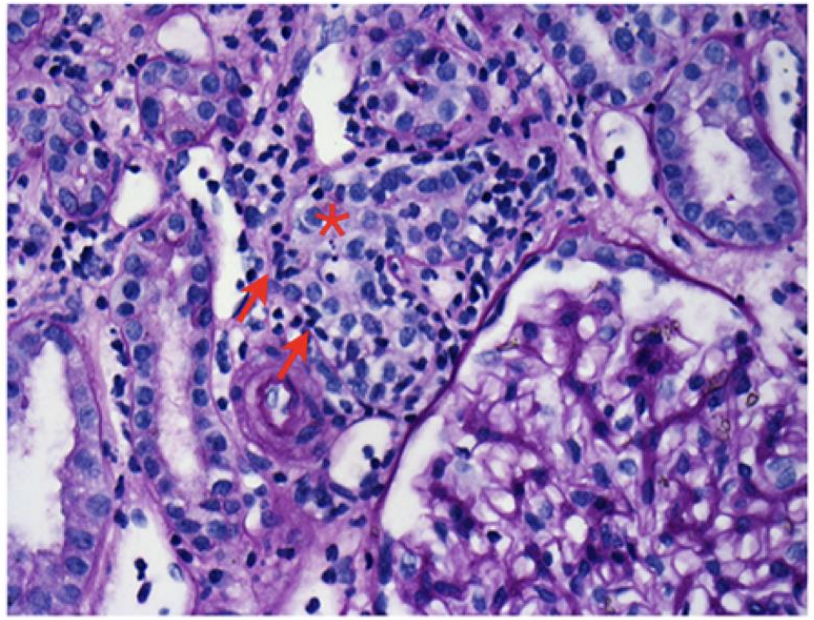

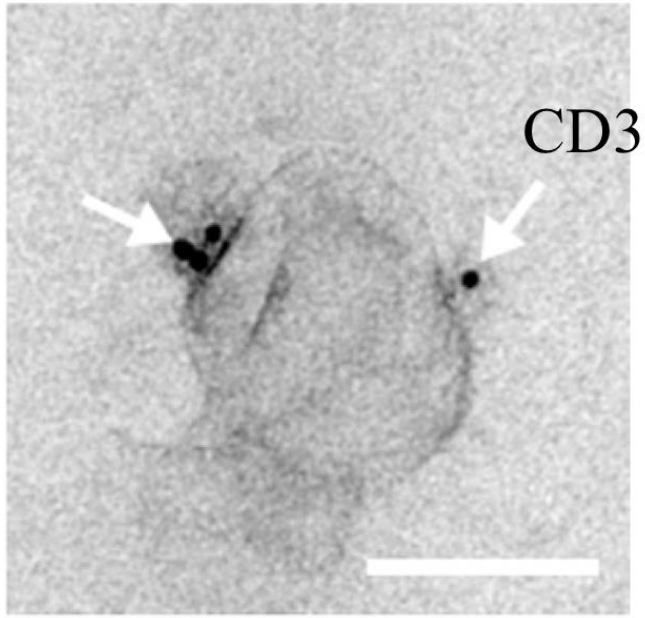

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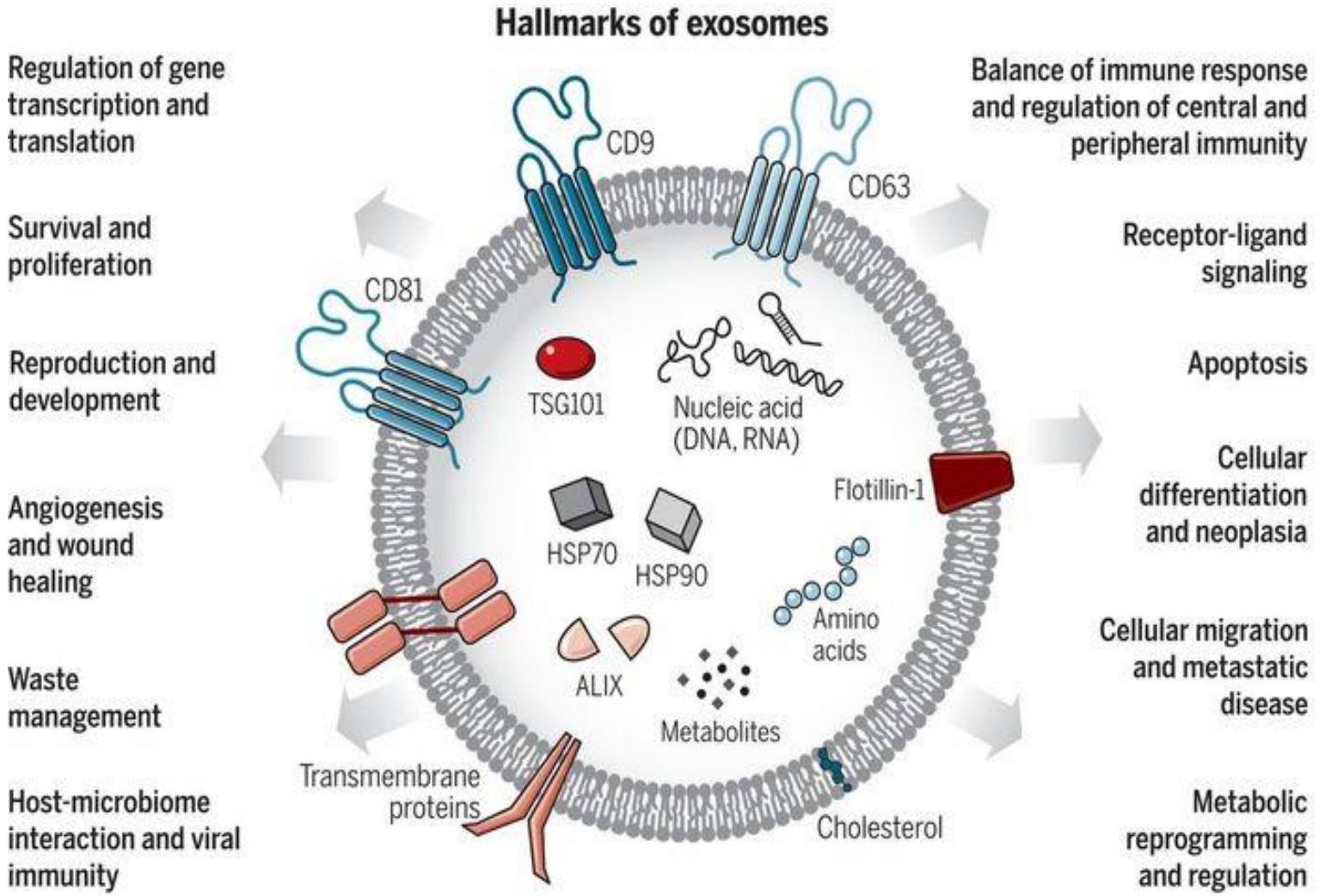
Park….Lee and Azzi et al. ACS Nano. 2017 11 28; 11(11):11041-11046.

High Throughput approach of discovery



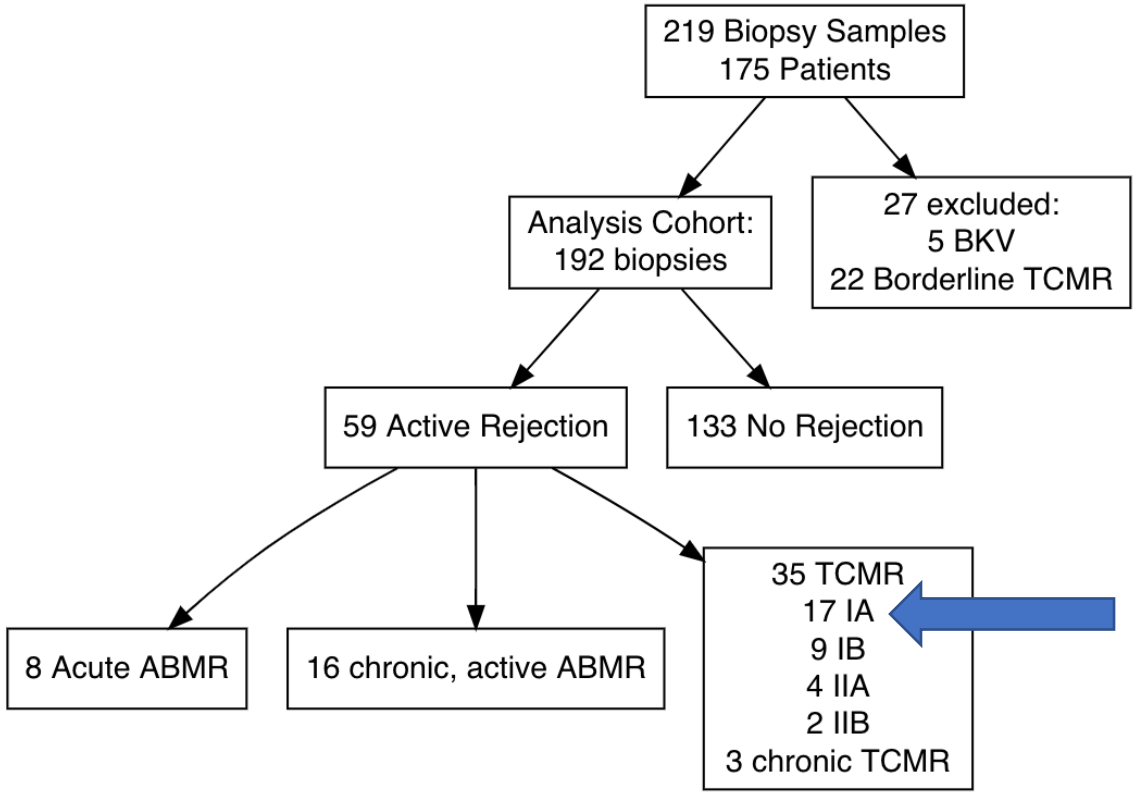
CLINICAL RESEARCH www.jasn.org

Discovery and Validation of a Urinary Exosome mRNA Signature for the Diagnosis of Human Kidney Transplant Rejection

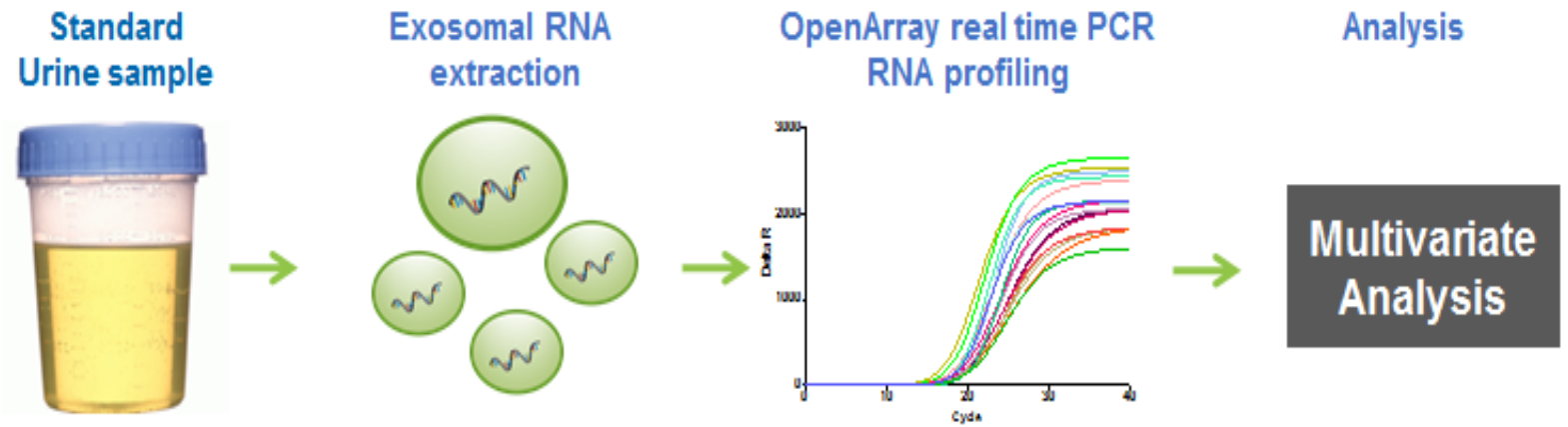
Rania El Fekih,[1] James Hurley,[2] Vasisht Tadigotla,[2] Areej Alghamdi,[1] Anand Srivastava,[1] Christine Coticchia,[2] John Choi,[1] Hazim Allos,[1] Karim Yatim,[1] Juliano Alhaddad,[1] Siawosh Eskandari,[1] Philip Chu,[1] Albana B. Mihali,[1] Isadora T. Lape,[1] Mauricio P. Lima Filho,[1] Bruno T. Aoyama,[1] Anil Chandraker,[1] Kassem Safa,[3] James F. Markmann,[3] Leonardo V. Riella,[1] Richard N. Formica,[4] Johan Skog,[2] and Jamil R. Azzi[1]

Due to the number of contributing authors, the affiliations are listed at the end of this article.

Consort Flow Diagram and histologic diagnosis of enrolled patients

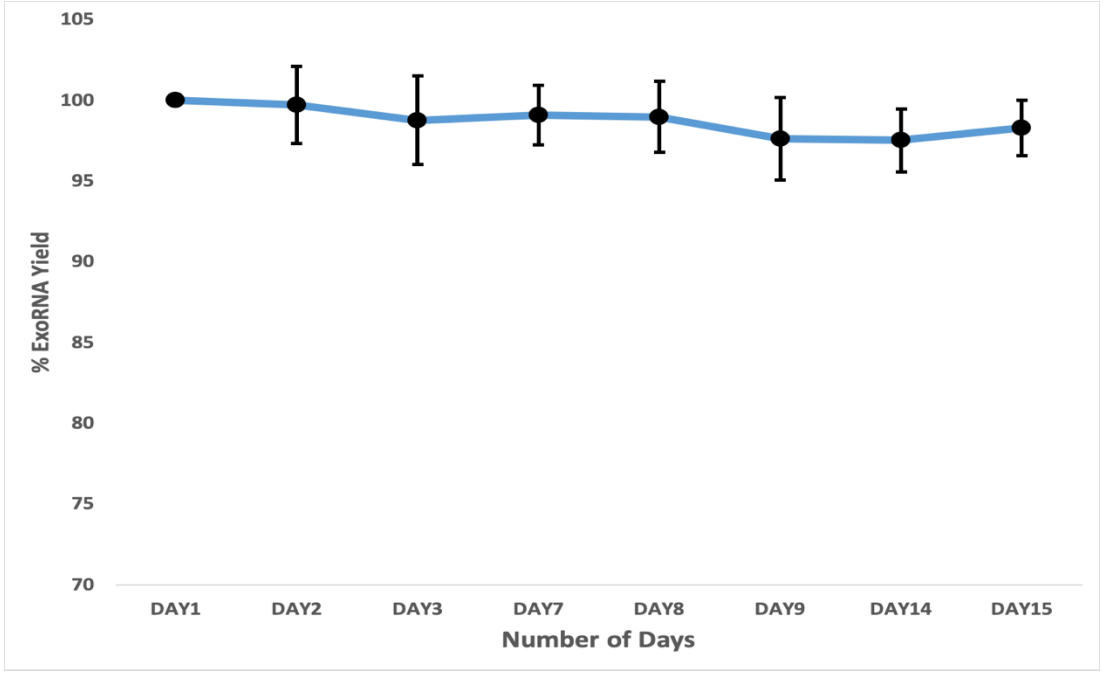


Sample processing workflow



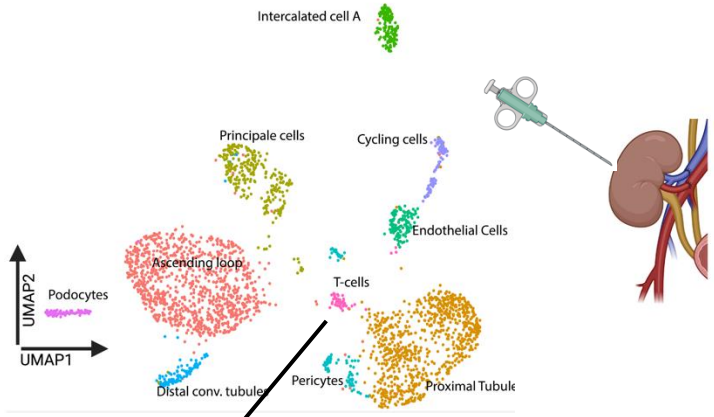
- The exosomes are isolated from the urine with EXOPRO (ExosomeDx)
- RNA reverse transcribed using the VILO cDNA synthesis kit (Thermo Fisher).
- Analyzed using the TaqMan® OpenArray® Human Inflammation

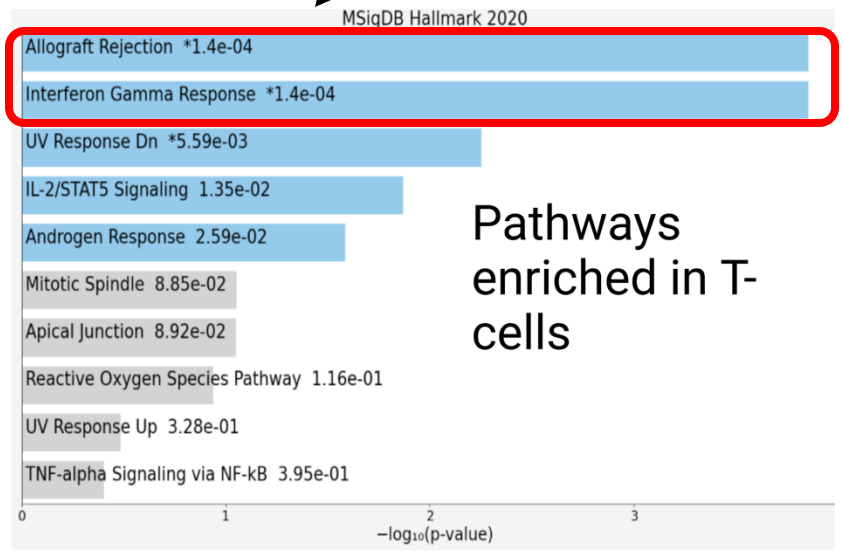
Urine exosome RNA stability



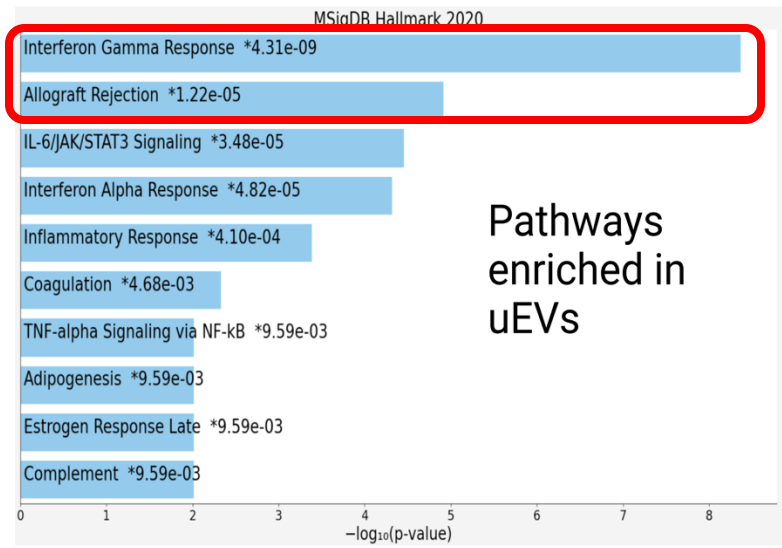
Upregulated Genes

Role in Immune activation	Gene signature	
	All cause rejection	ABMR vs TCMR
Pro-inflammatory cytokines	IL32 CXCL14 B2M	
TGFβ superfamily	BMP7	
Upregulated in acute allograft rejection	C3 CXCL11	C3 CXCL11
Inflammasome, different inflammatory processes	STAT1, TBP, NAMPT, IFNGR1, IRAK2, IL18BP, SERPINA1	IFNAR2
Regulating protein trafficking, dendritic cell migration and T lymphocytes homing	CD74	CD74 CD44



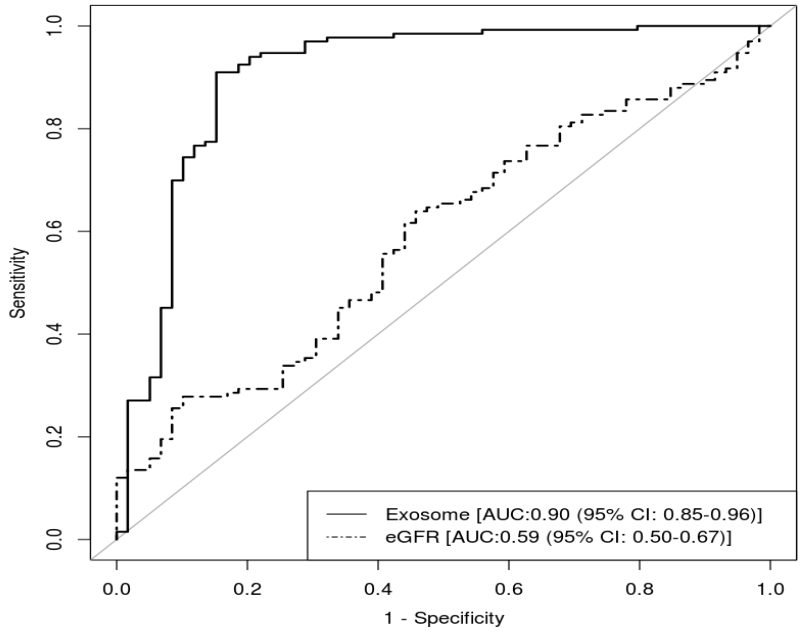


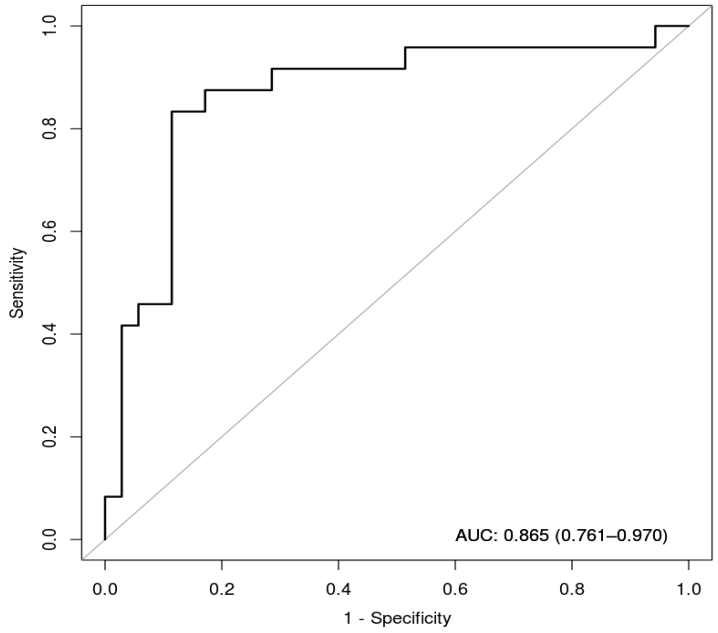
Pathways enriched in T-cells



Pathways enriched in uEVs

Receiver-Operating-Characteristic (ROC) curve for Diagnosis of any-cause Acute Rejection and ABMR

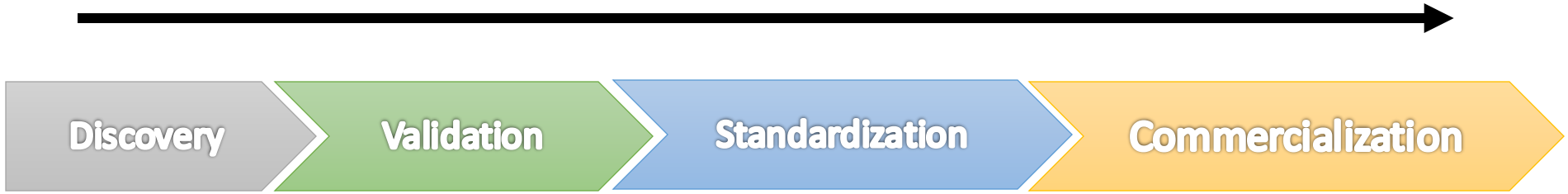




- Any cause rejection: Exosome RNA signature AUC =0.90 (95% CI 0.84-0.96) vs eGFR AUC= 0.59 (95%CI 0.5-0.67).

ABMR discriminating RNA signature AUC 0.87 (95%CI 0.76-0.97).

Phases of Biomarkers Discovery

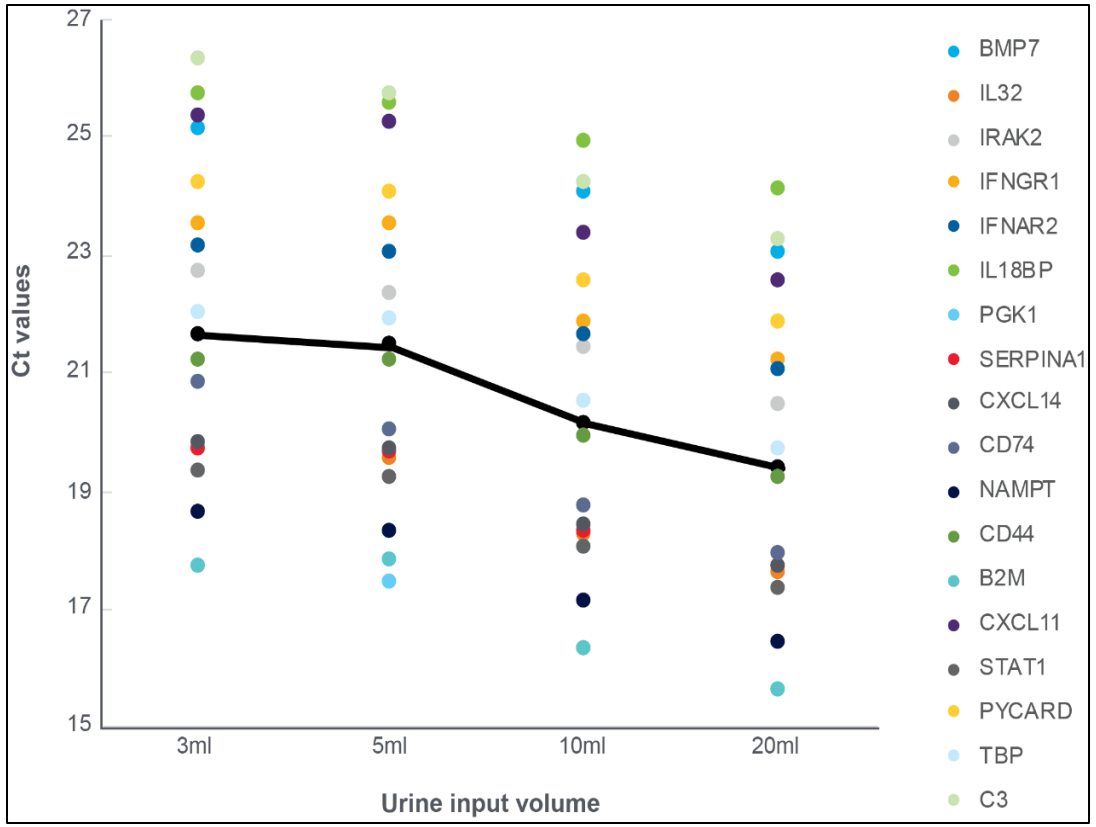


- Targeted or non-targeted approaches
- High-throughput technologies
- Identify a panel

- Use independent samples
- Identify a refined set
- Prospective multicentral validation

- Laboratory equipment
- Reagents
- Can the test be reproduced in different laboratories

Transfer the signature to a clinical platform



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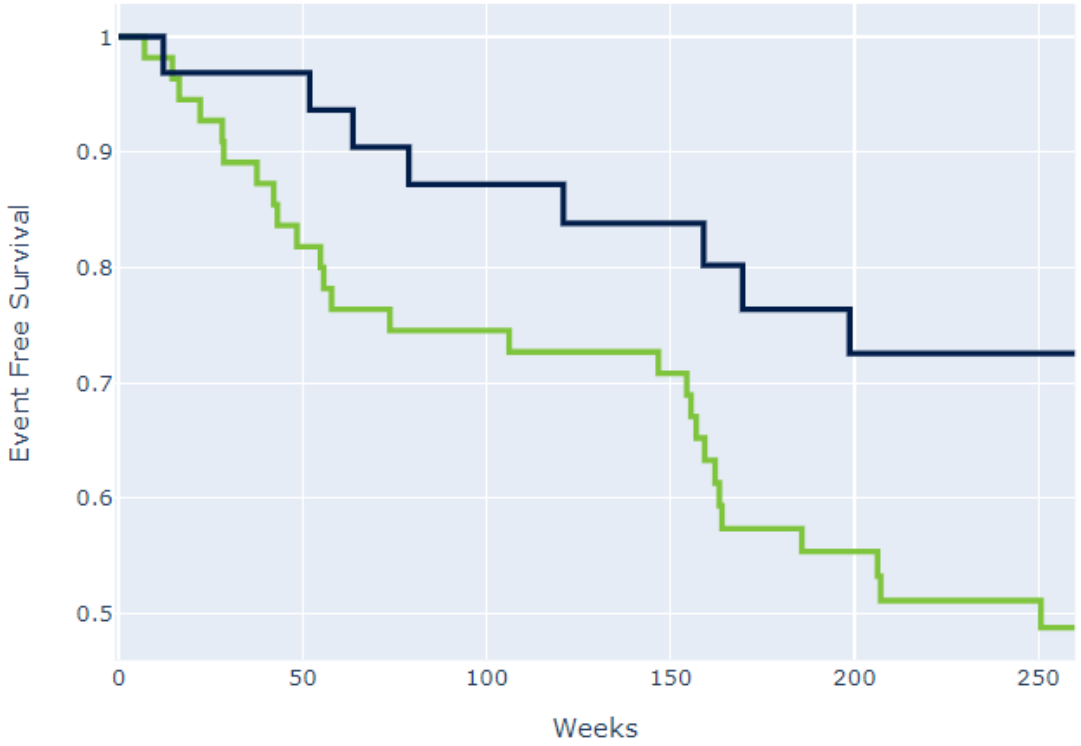
El Fekih et al. KI reports, 2025

Prognostic Potential:
ExoTRU score is associated with 5 years outcome

HR=2.23, p-val=0.023



Five-Year Composite Endpoints:

- >30% decrease in eGFR
- Subsequent rejection
- De novo DSA
- Death
- Loss of graft with either return to dialysis or re-transplant

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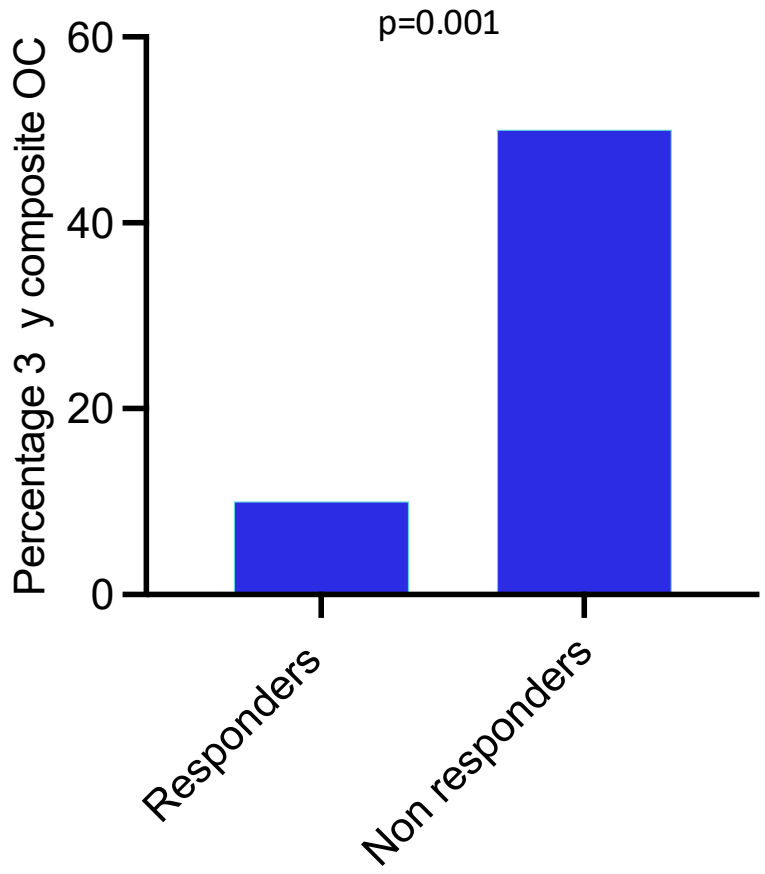
Predictive Potential:
ExoTRU scores correlate with response to treatment and 3 years outcome

23 samples from patients who have had a repeat ExoTRU score after being treated for rejection, we looked at the change in score after treatment.

Responders: patients who showed at least 30% decrease in the urinary score after treatment.

Non responders: score increased or was stable after treatment



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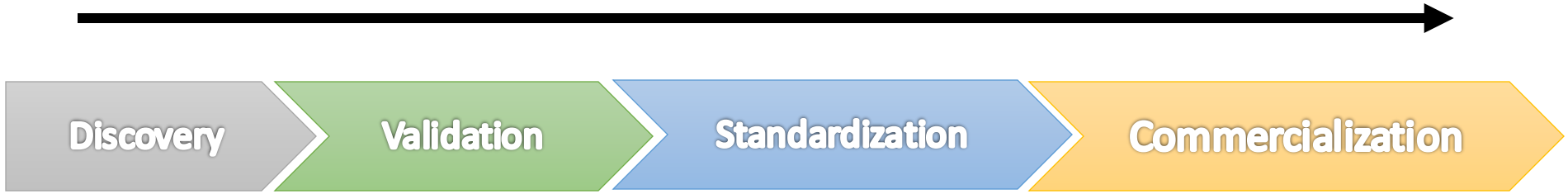

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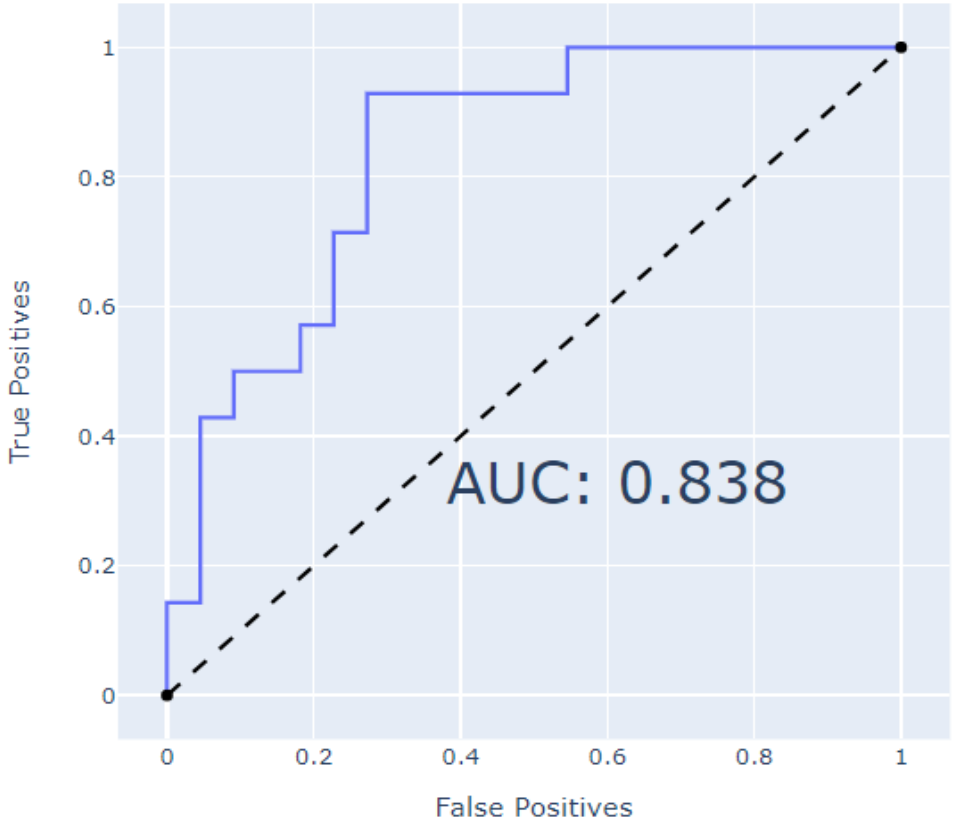
Independent Validation By Thermo Fisher



ExoTRU

↓

One Lambda Urinary Exosome Test

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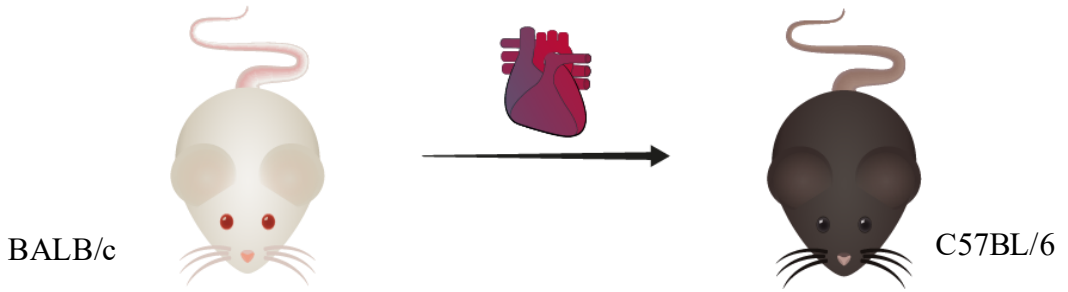
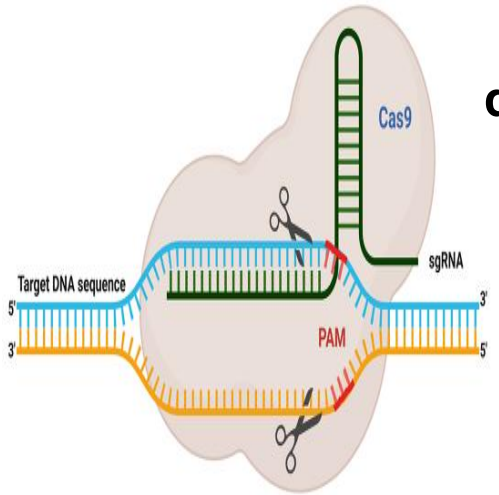
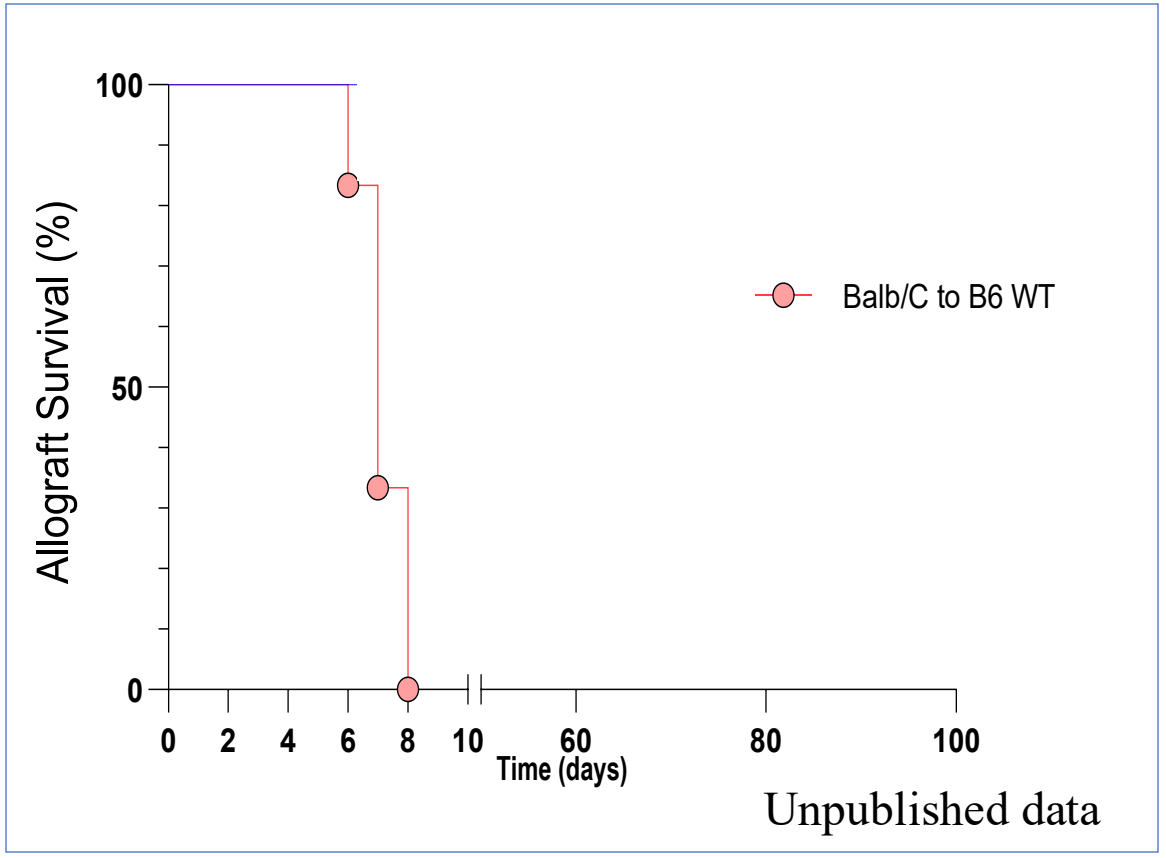
From humans back to mice





CD74 deletion



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Biomarkers in Tx: Challenges!

- Where to look: periphery versus graft tissue?
- Adequacy of sample collections
- Quality assurance and control, reproducibility, multicenter validation
- Handling massive amount of data
- Bioinformatics
- Statistical analysis
- Assay standardization and commercialization
- Incorporation into endpoints

Summary

- Different types of Biomarkers
- PPV and NPV
- Phases of Biomarker Discovery
- Clinically available tests
- Importance of molecular signatures

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TRANSPLANTATION RESEARCH CENTER

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