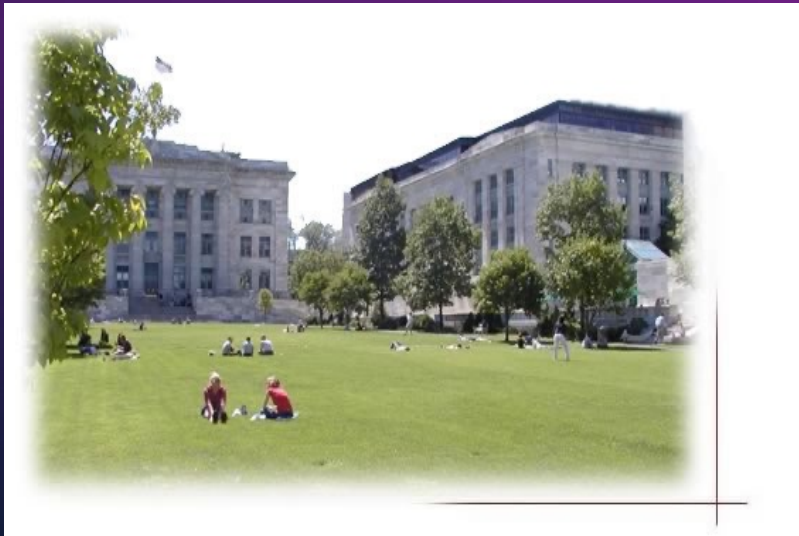




Monitoring Kidney Transplant Patients: Beyond Creatinine



J. Roumanos Azzi MD PhD

*John P. Merrill, MD, and Charles B. Carpenter, MD, Endowed Chair in
Transplantation Medicine*

Associate Professor of Medicine, Harvard Medical School

Director, Transplantation Research Center

Director, Kidney and Pancreas Transplant Program

Brigham and Women's Hospital

Mass General Brigham

Jamil R. Azzi, MD, PhD

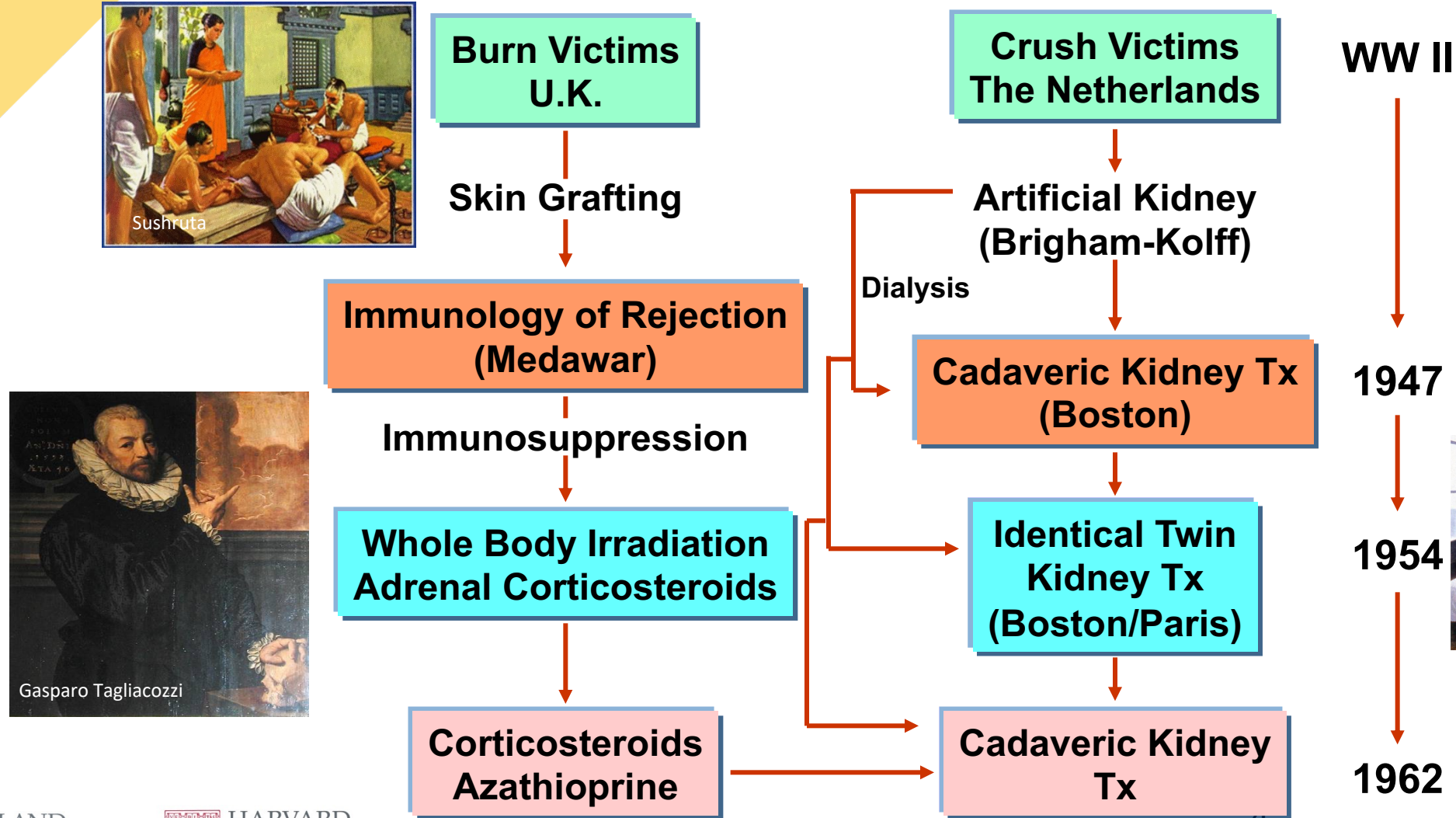


- ▶ Renal Fellowship @ BWH and MGH
- ▶ Transplant Fellowship @ BWH
- ▶ Post doctoral Fellowship @ BWH and HMS
- ▶ Associate Professor of Medicine @ HMS
 - Clinical focus: Transplant nephrology
 - Research focus: Basic immunology, cell and targeted therapies, and biomarker discovery

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.

Early Transplantation and Immunosuppression



Balancing Immunosuppression, Infection And Malignancy

Too Much

- *Infection*
- *Malignancy*

Too Little

- *Allograft Rejection*



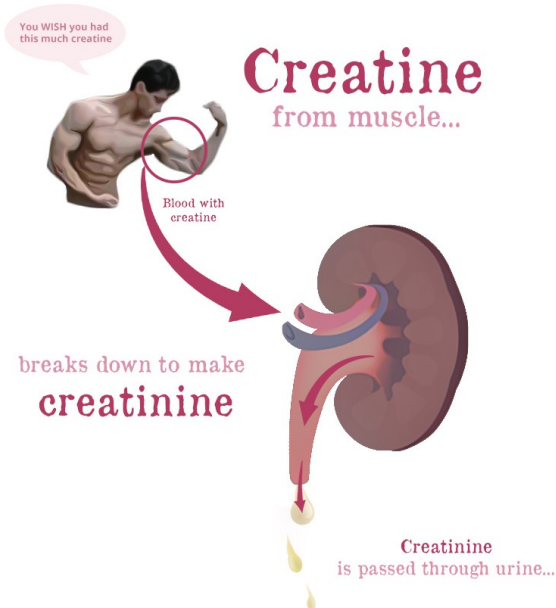
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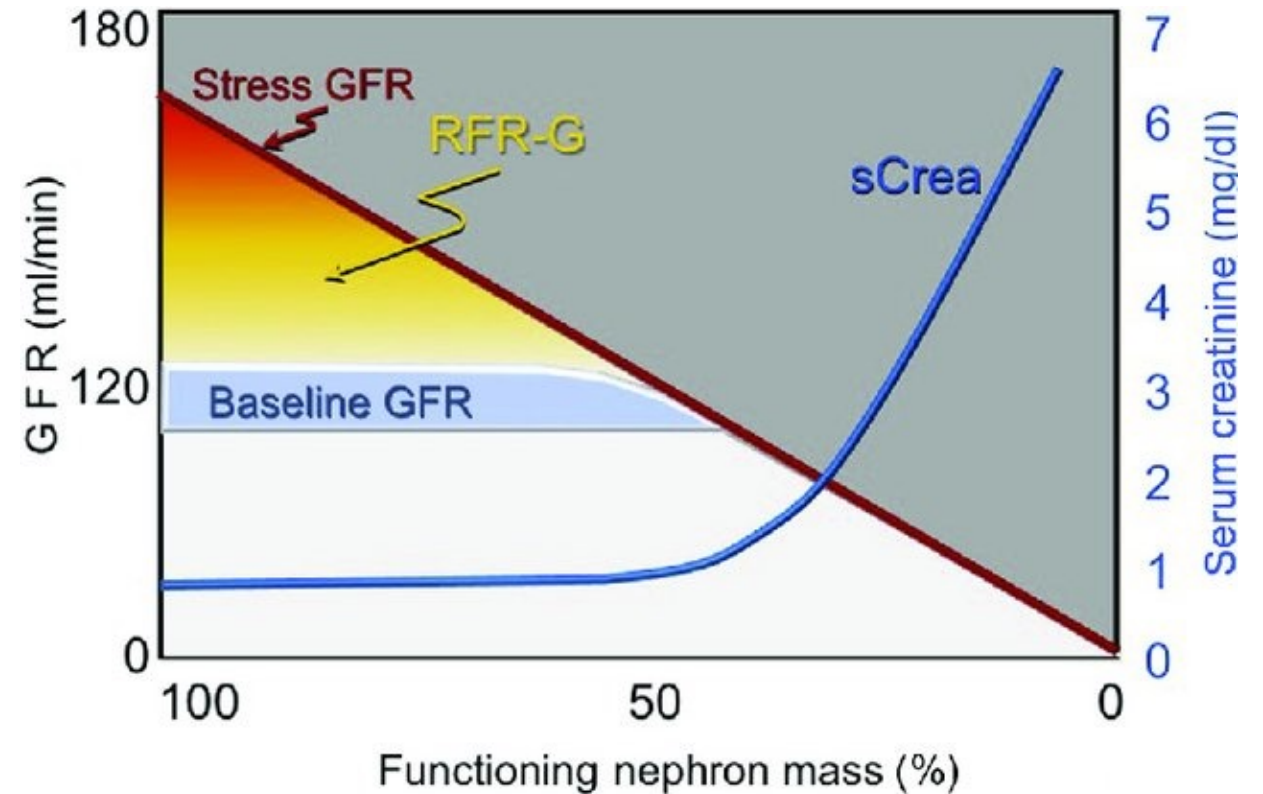
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Monitoring kidney function through serum creatinine

WHERE DOES CREATININE
COME FROM?



But in kidney failure,
your blood creatinine level will go up.



20 % Subclinical rejection



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Long-term outcomes following acute rejection in kidney transplant recipients



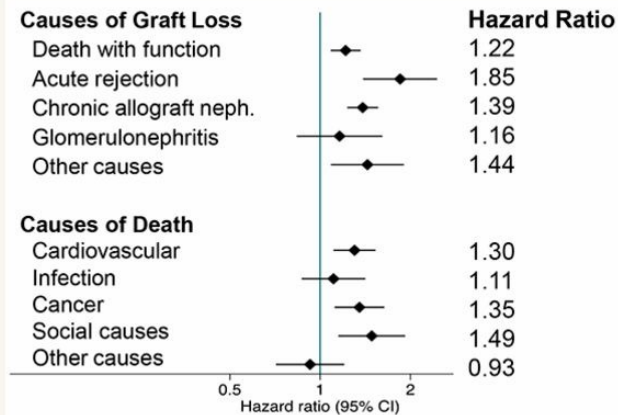
13614 KTRs 1997-2017
2906 with early acute rejection
(AR in 1st 6mth)

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.

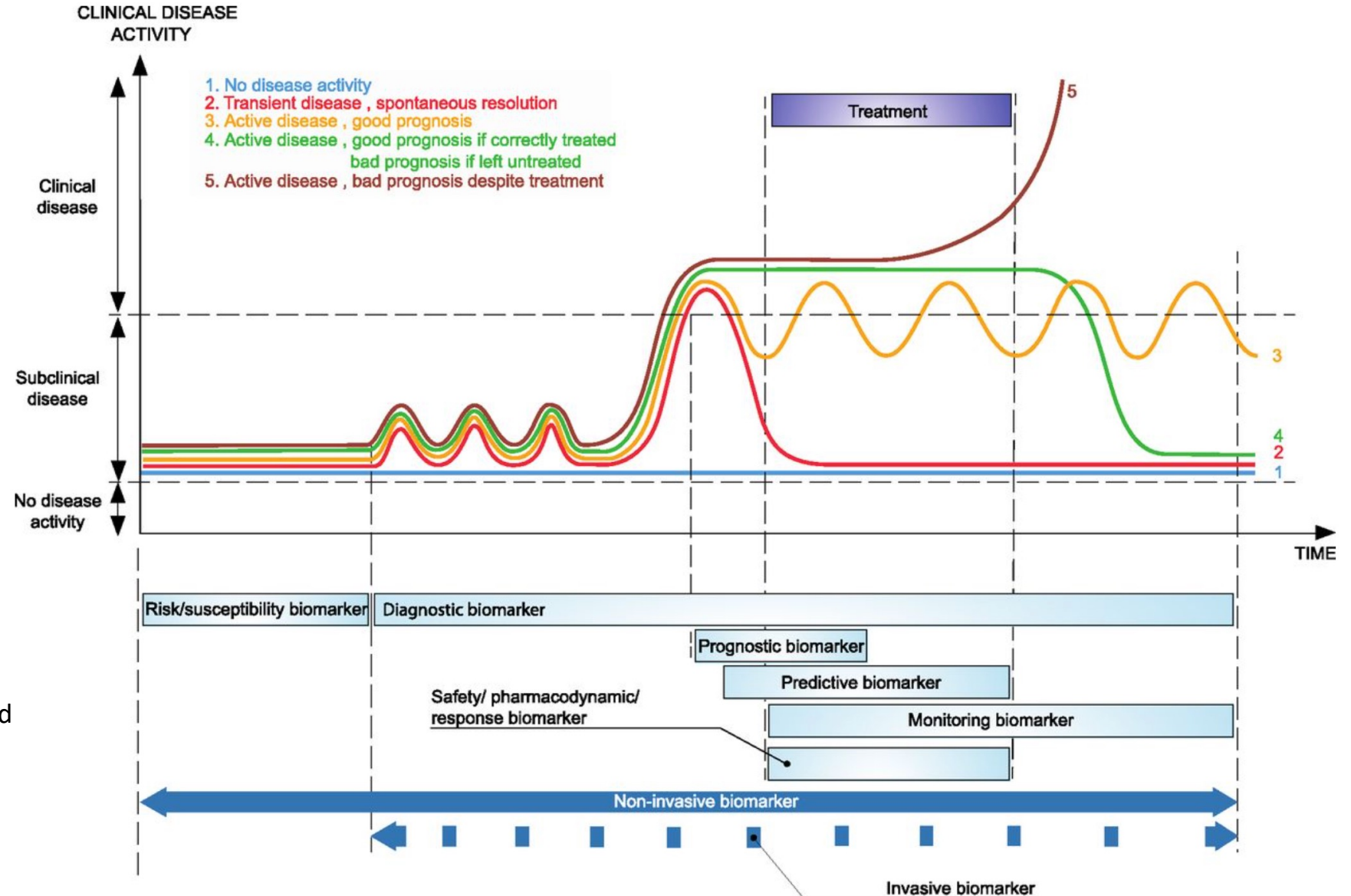
doi: 10.1681/ASN.2018111101

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

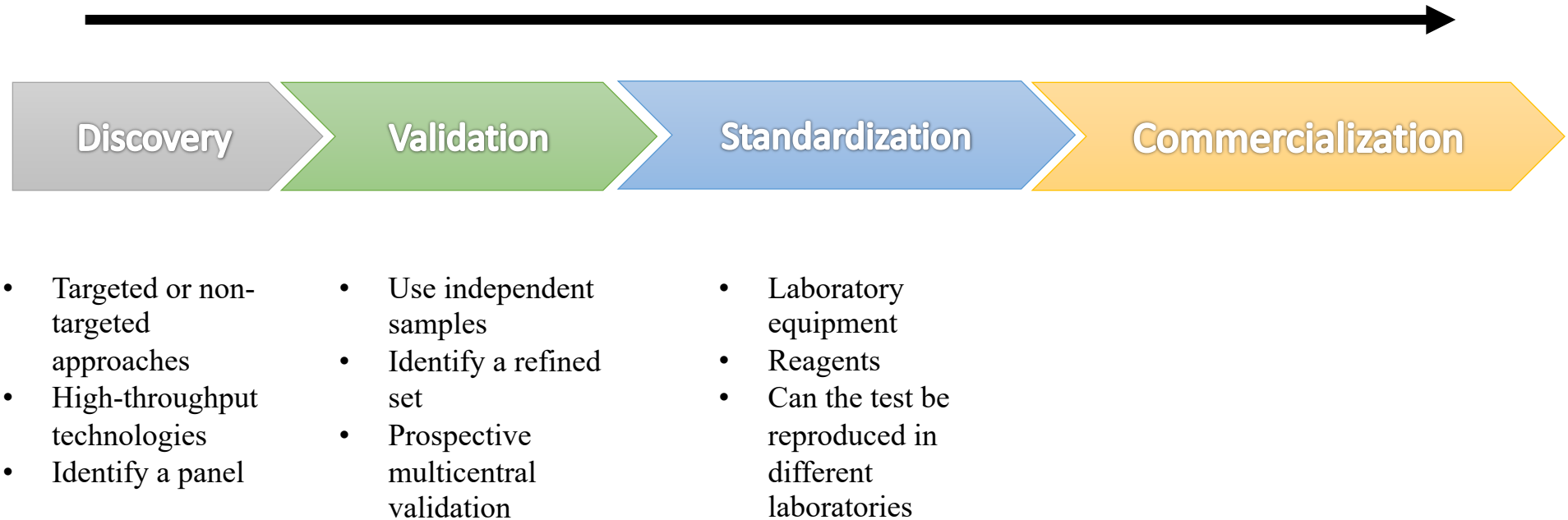
JASN
JOURNAL OF THE AMERICAN SOCIETY OF NEPHROLOGY

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.



Phases of Biomarkers Discovery



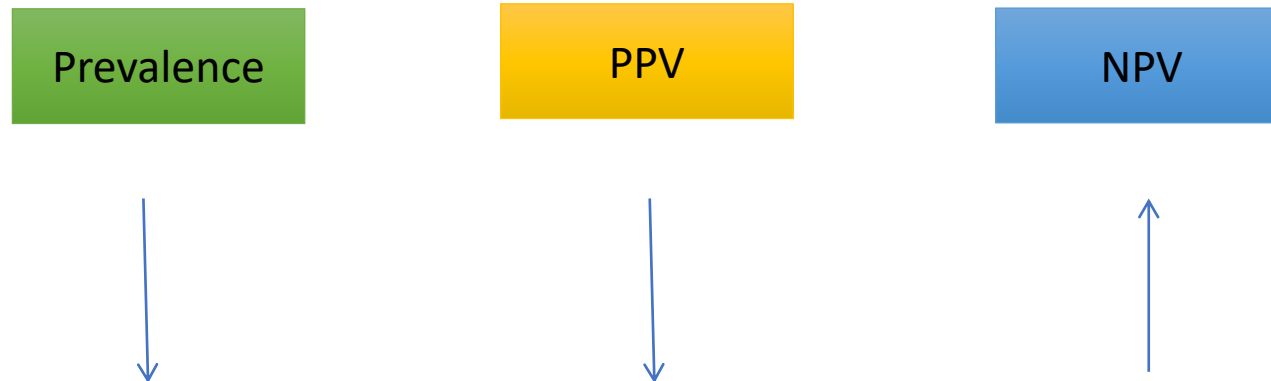
Ideal Biomarker

- 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



More than meets the eye

19 yo male with history of ESRD secondary to congenital kidney disease
S/P deceased kidney transplant 1/24/26 (KDPI 46%), 4 HLA MM, PRA 0%
induced with Thymo.

Had multiple GI symptoms and had Cellcept switched to Imuran and
dose reduced due to leukopenia and EBV viremia detected in a high-risk
patient.

Creatinine plateaued around 2.

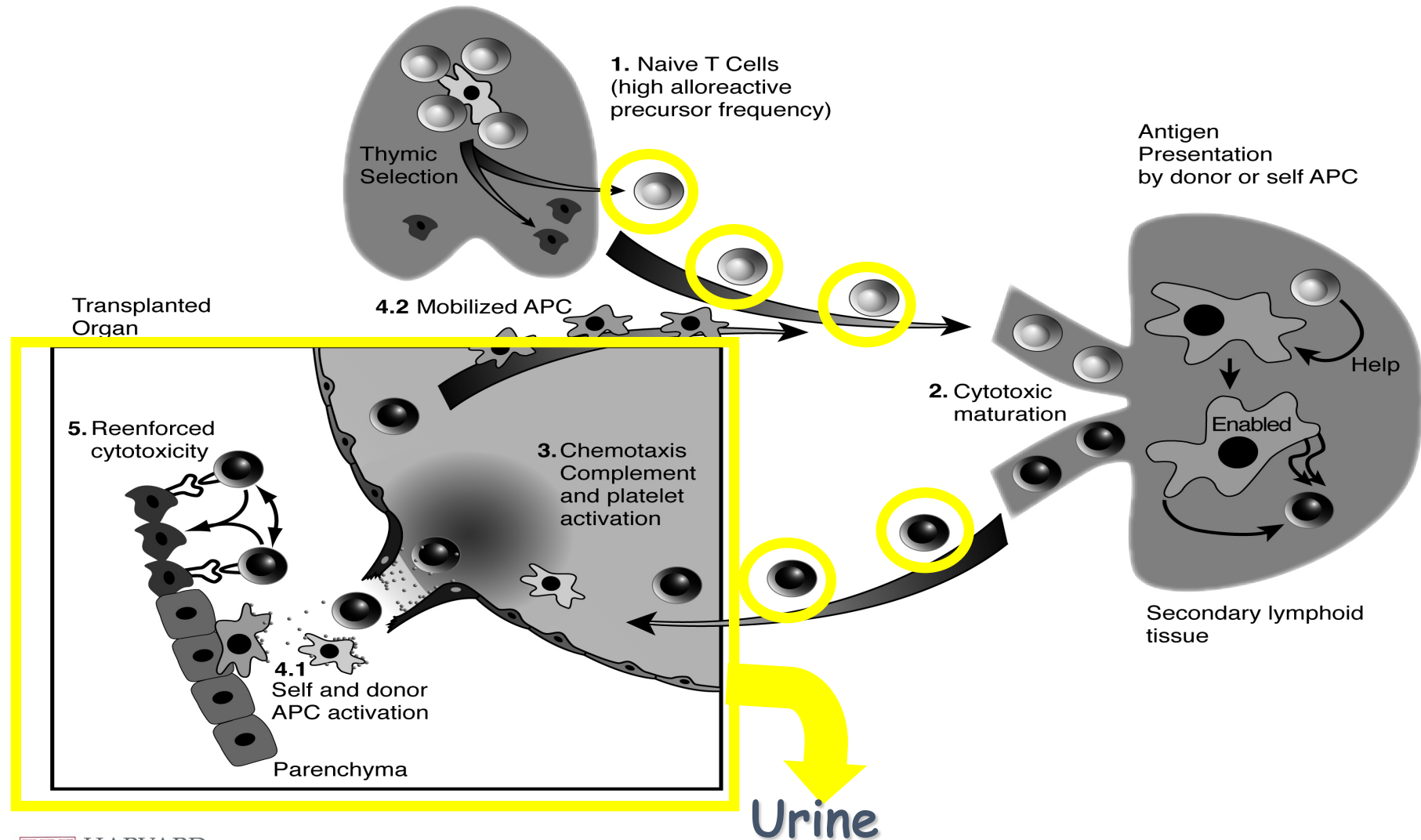
Biopsy done 4/6: No rejection

Creatinine worsened

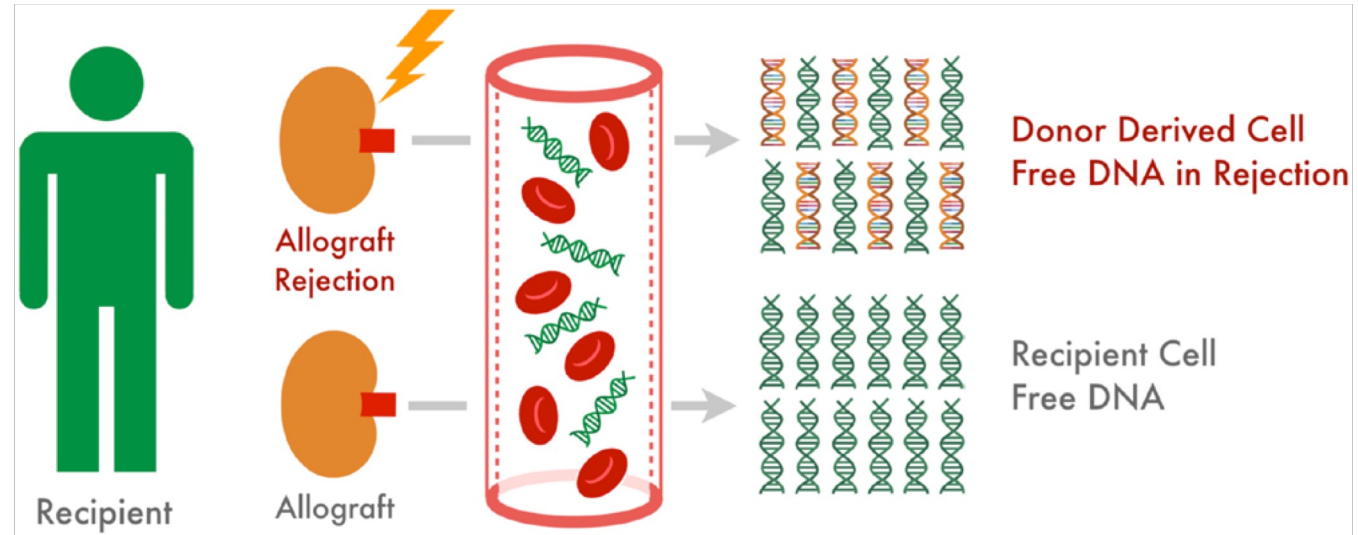
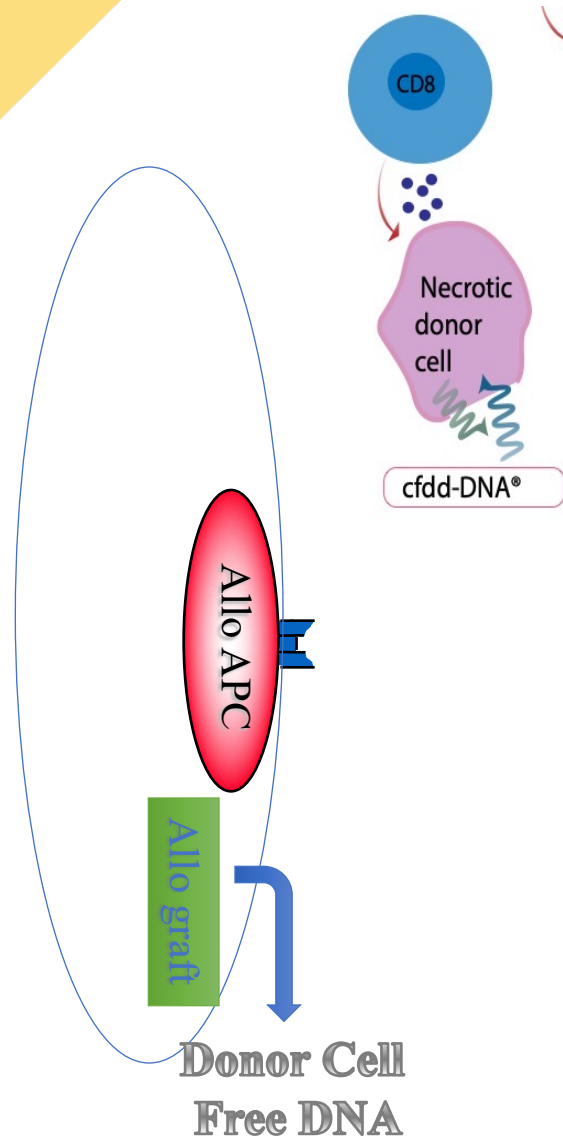
Biopsy done 4/28: ACR and AMR with de novo DSA.

Mechanisms of Immune Monitoring

What's Available for Evaluation?

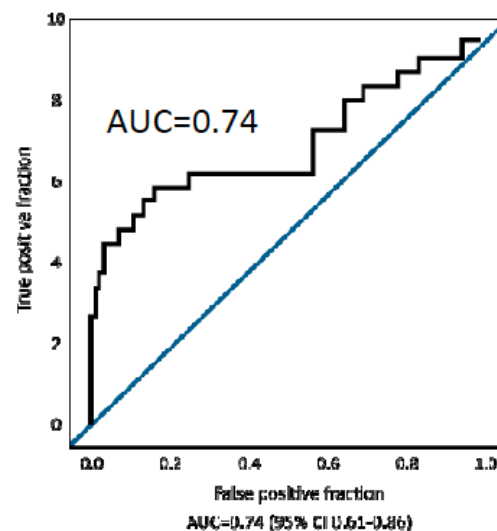
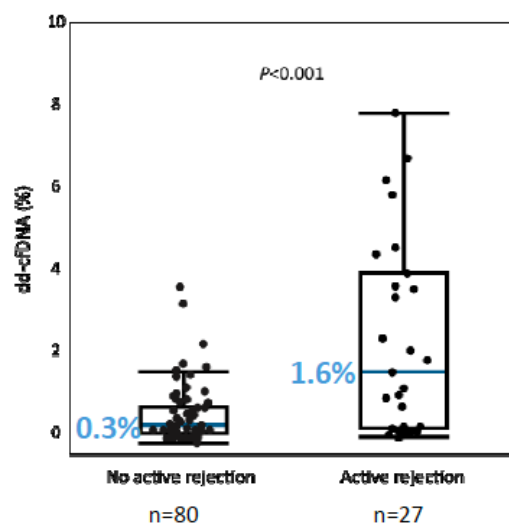
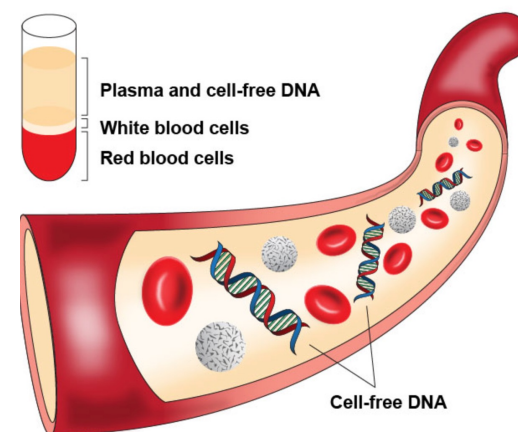


Clinically Available Biomarkers: Donor-derived Cell Free DNA



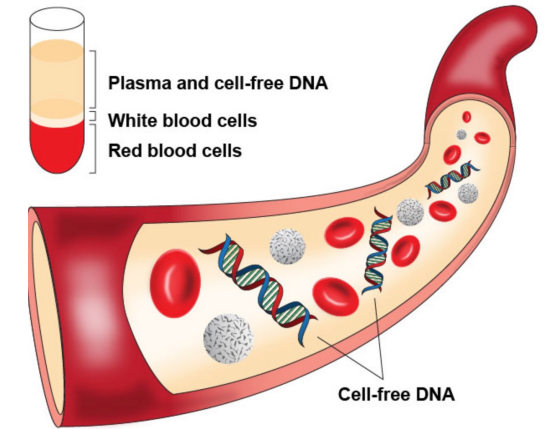
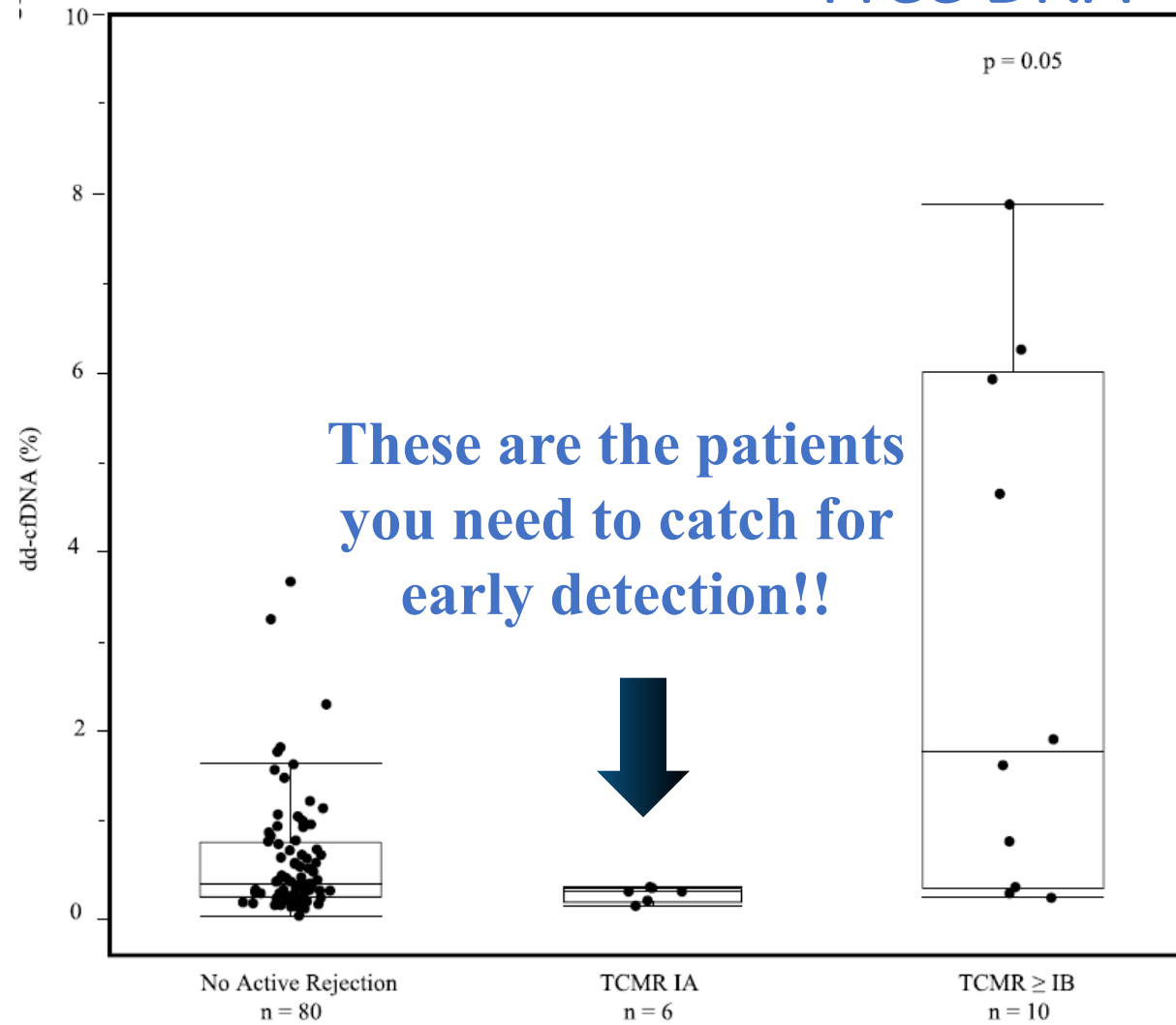
Clinically Available Biomarkers: Donor-derived Cell Free DNA

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



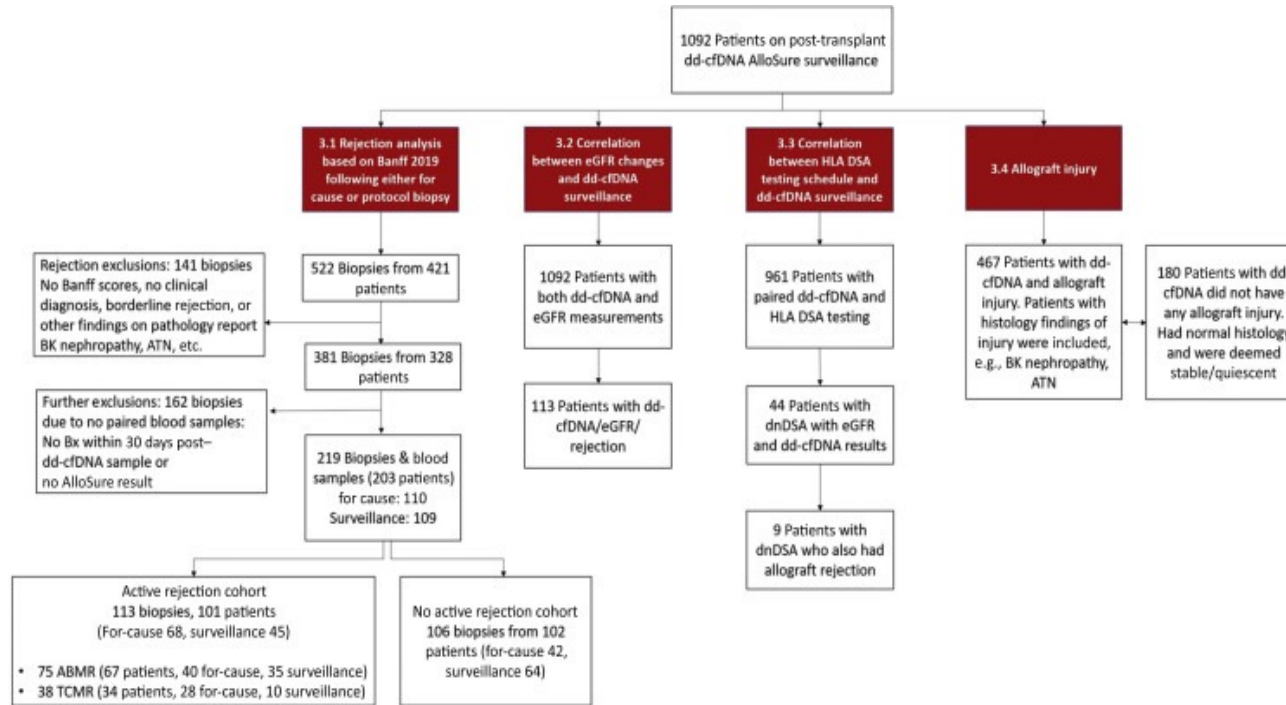
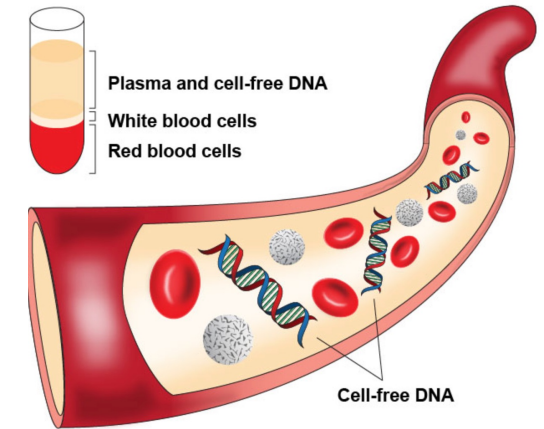
Performance metric	AlloSure test performance at 1% threshold
ROC/AUC	0.74 (95% CI 0.61-0.86)
Sensitivity	85%
Specificity	59%
NPV	84%
PPV	61%

Clinically Available Biomarkers: Donor-derived Cell Free DNA



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Clinically Available Biomarkers: Donor-derived Cell Free DNA



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

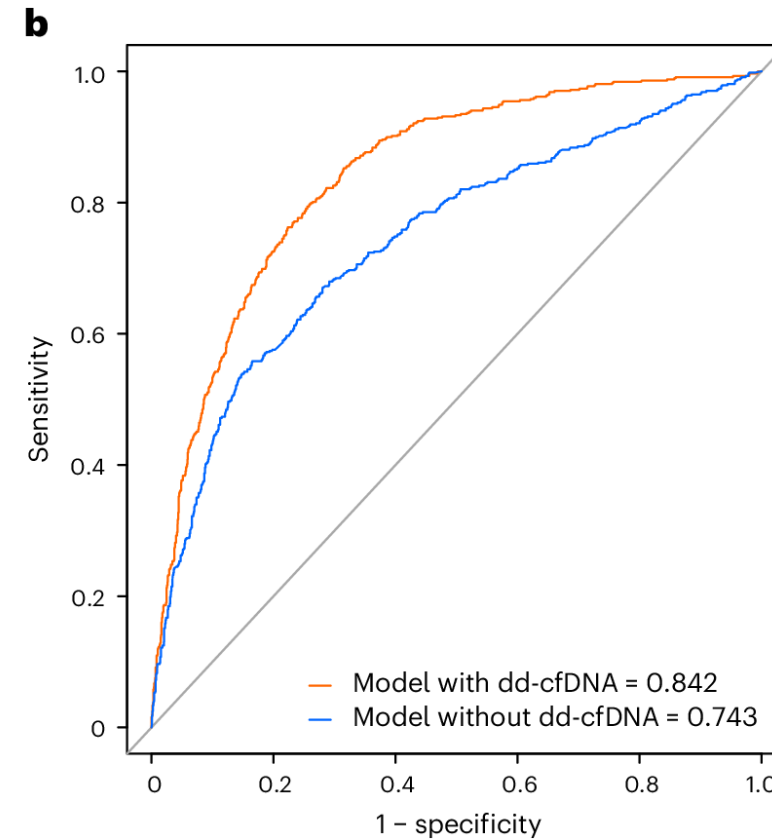
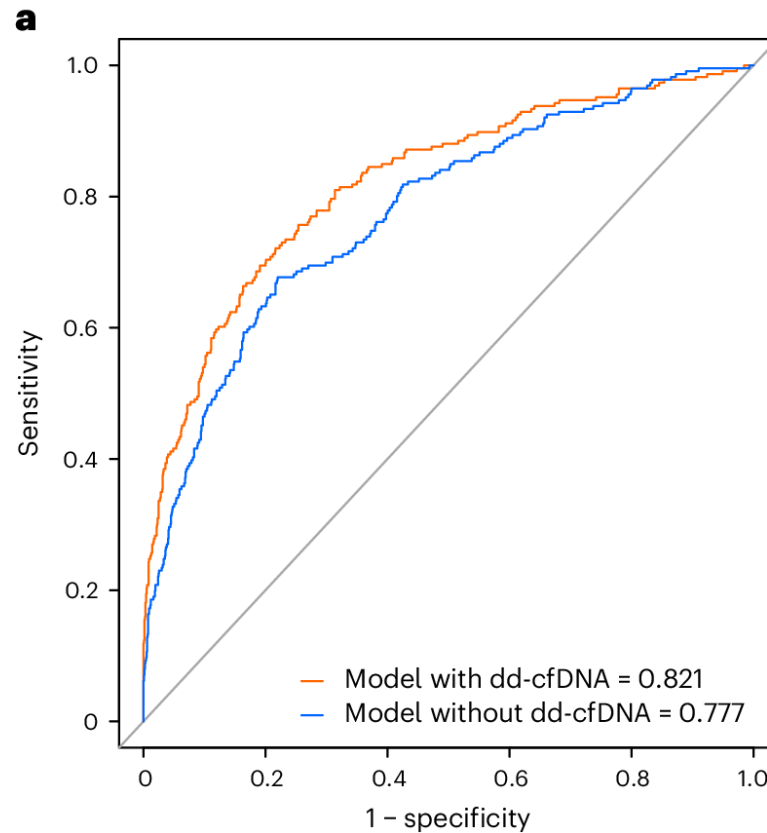


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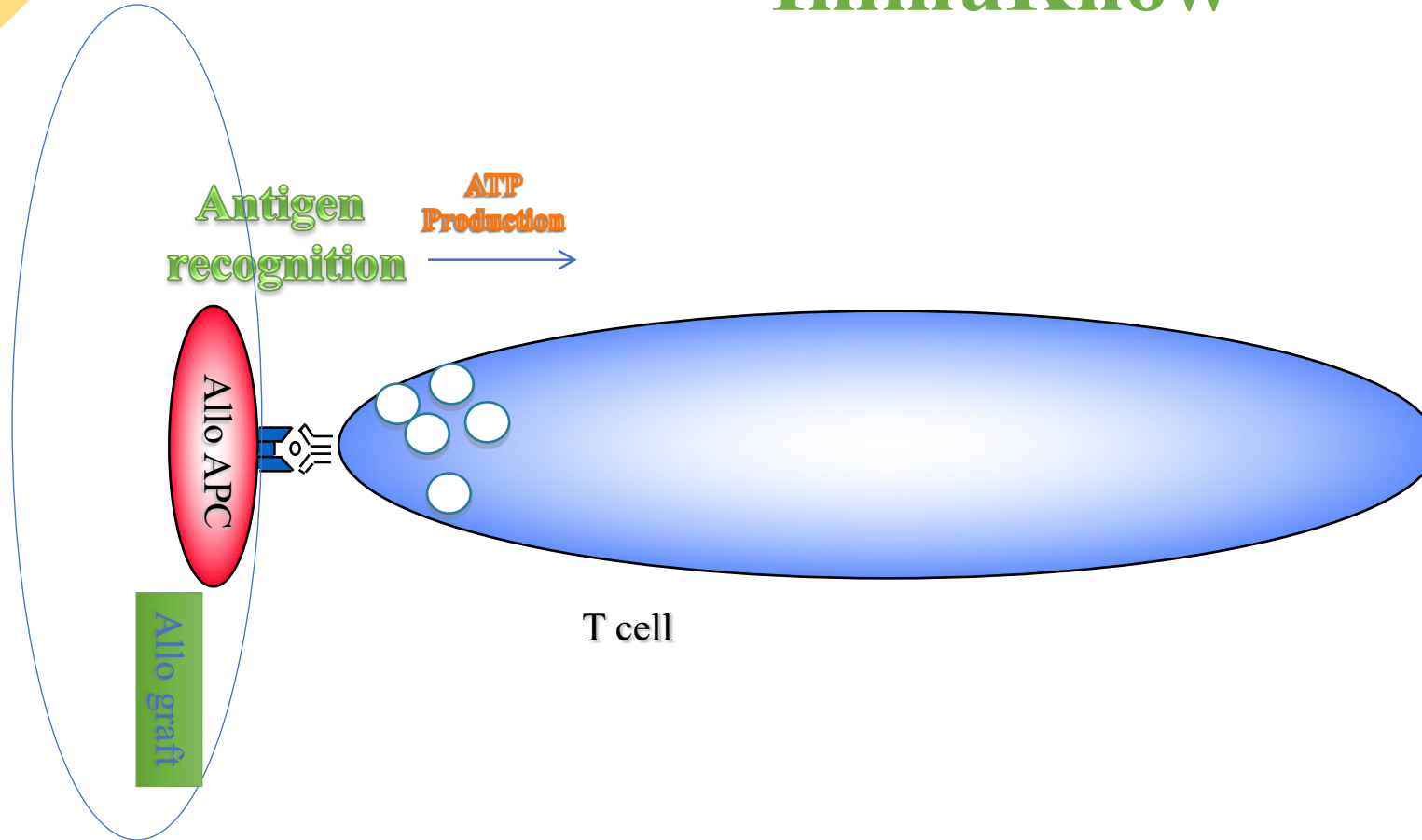
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Clinically Available Biomarkers: Donor-derived Cell Free DNA



Clinically Available Biomarkers:

ImmuKnow

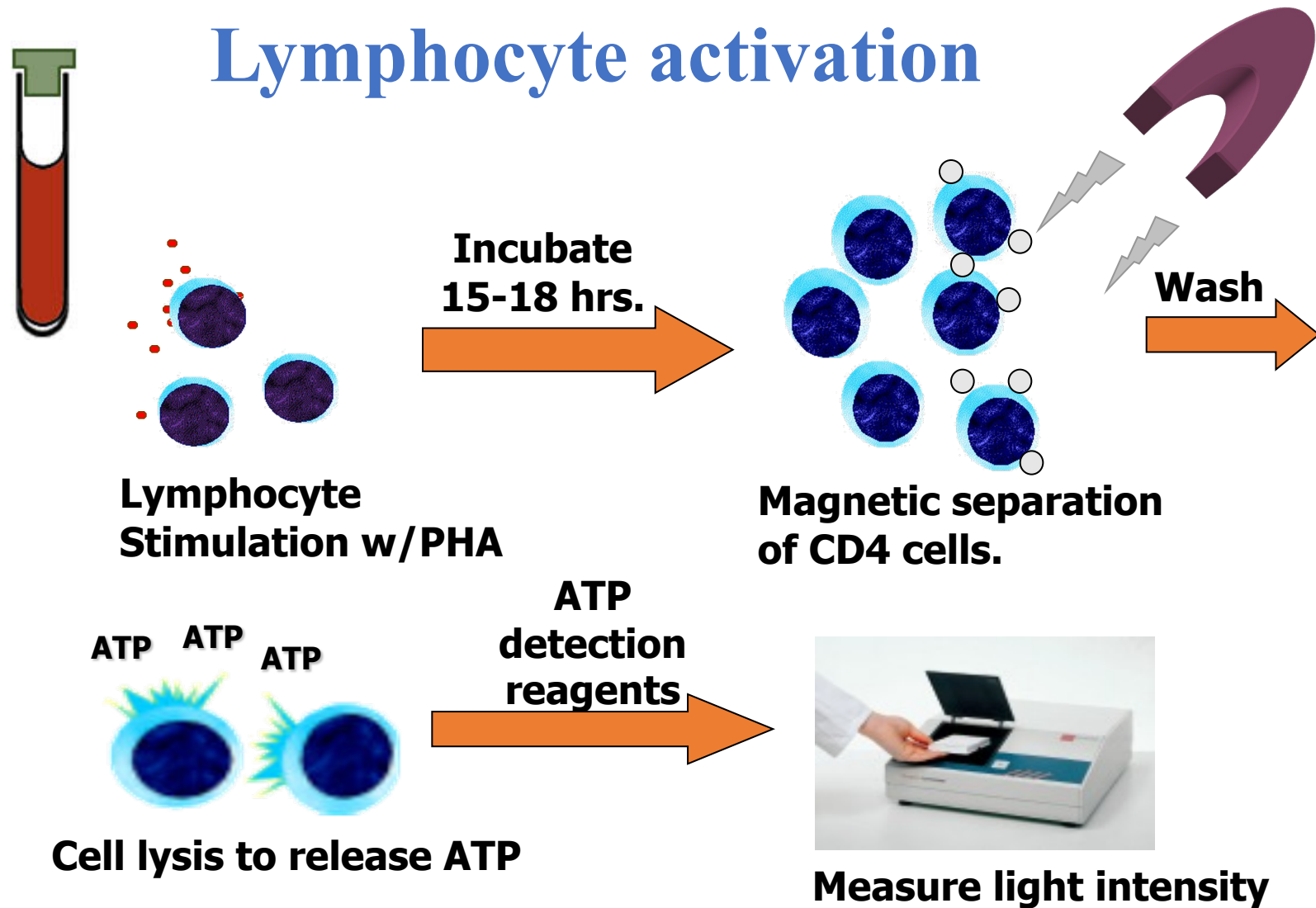


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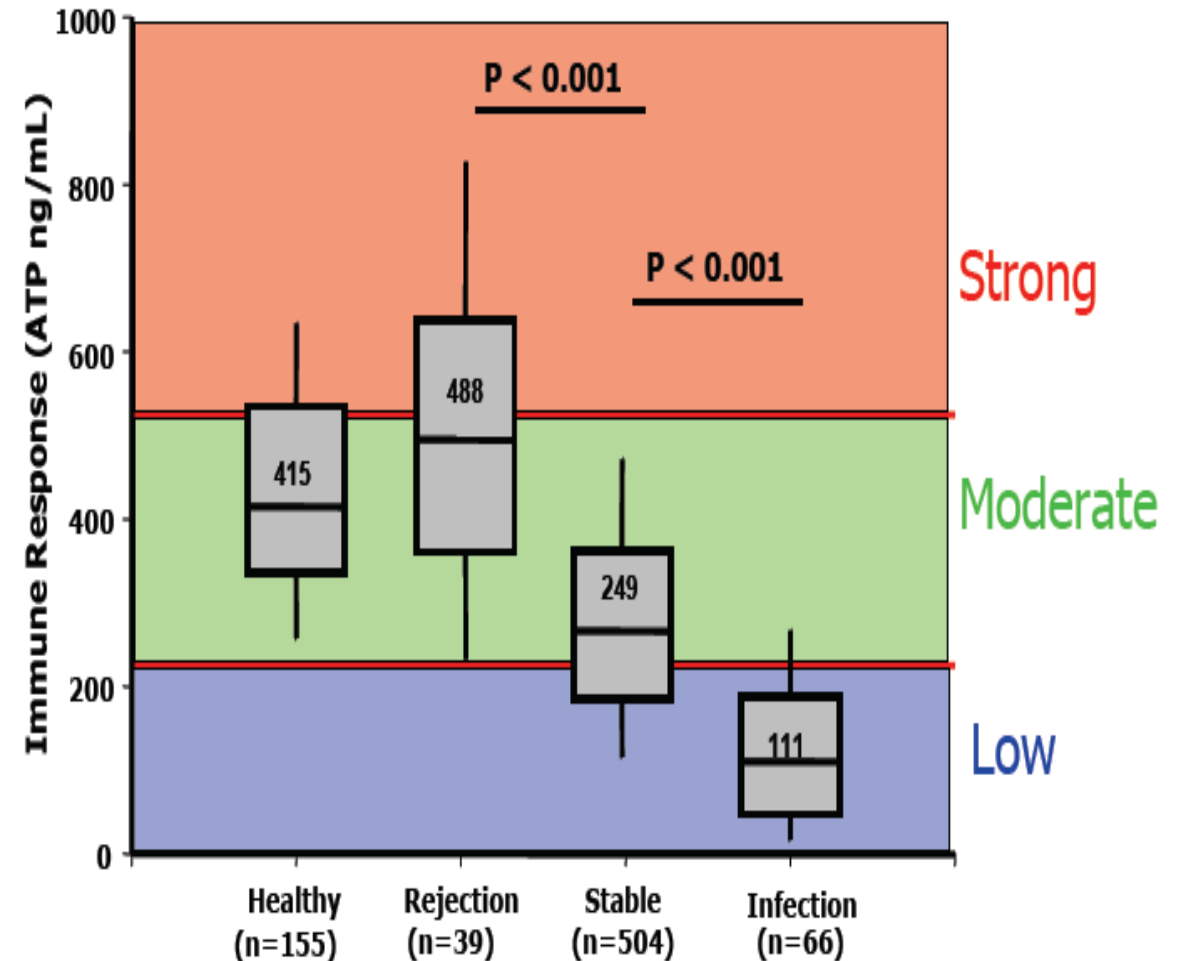


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Lymphocyte activation



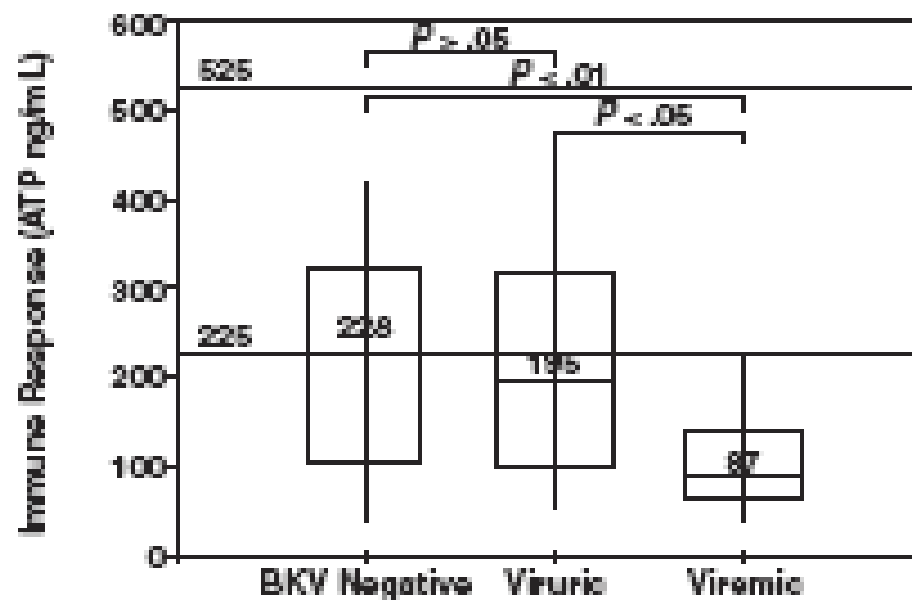
Immune Function Correlates With Clinical Outcomes



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 Girmata, MD,¹ Amit Basu, MD,² Henkie Tan, MD, PhD,² Ron Shapiro, MD,² and Parmjeet Randhawa, MD¹

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

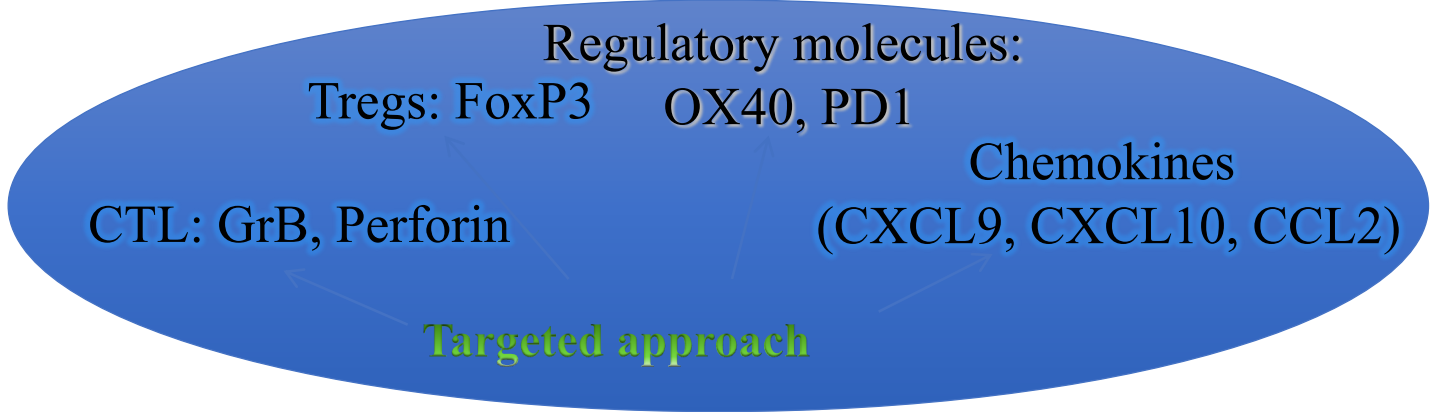


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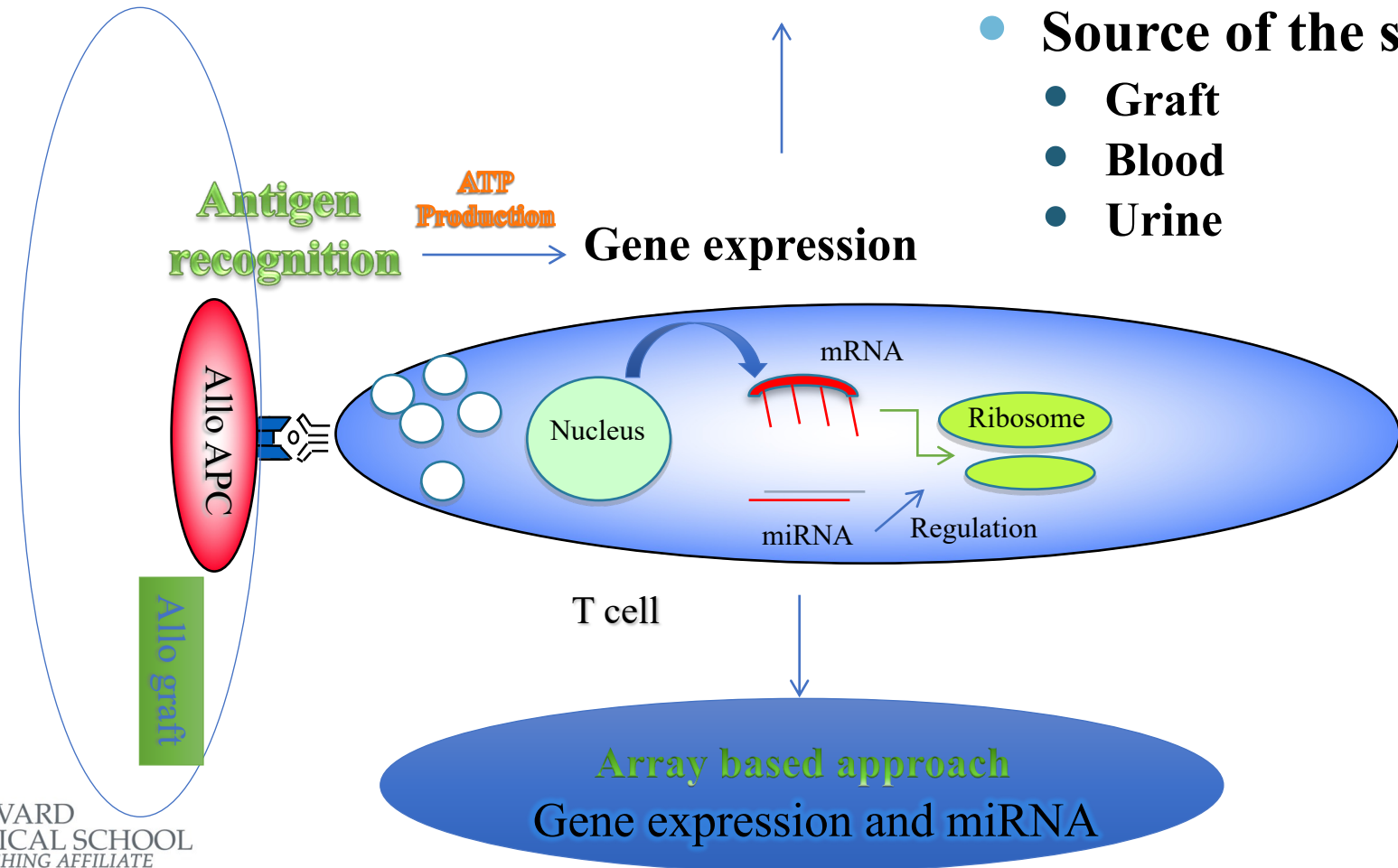
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Am J Clin Pathol 2008



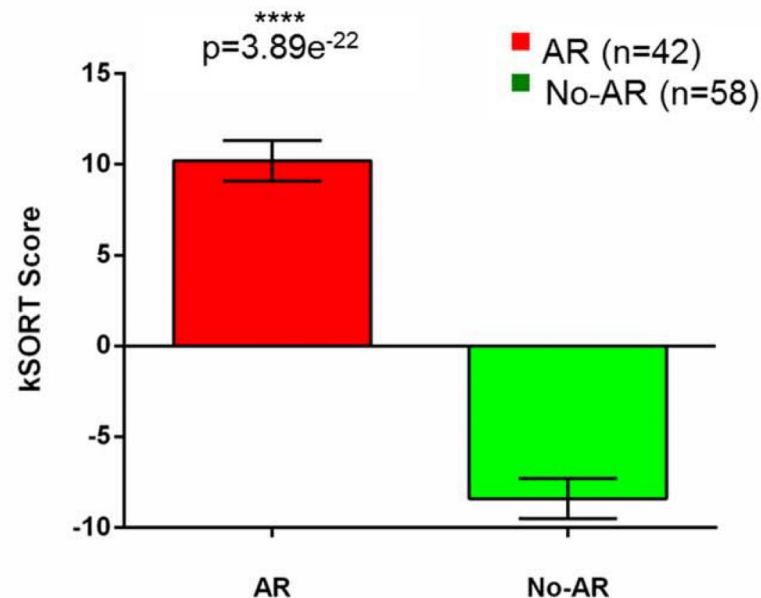
● **Source of the samples:**

- **Graft**
- **Blood**
- **Urine**



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.



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%.

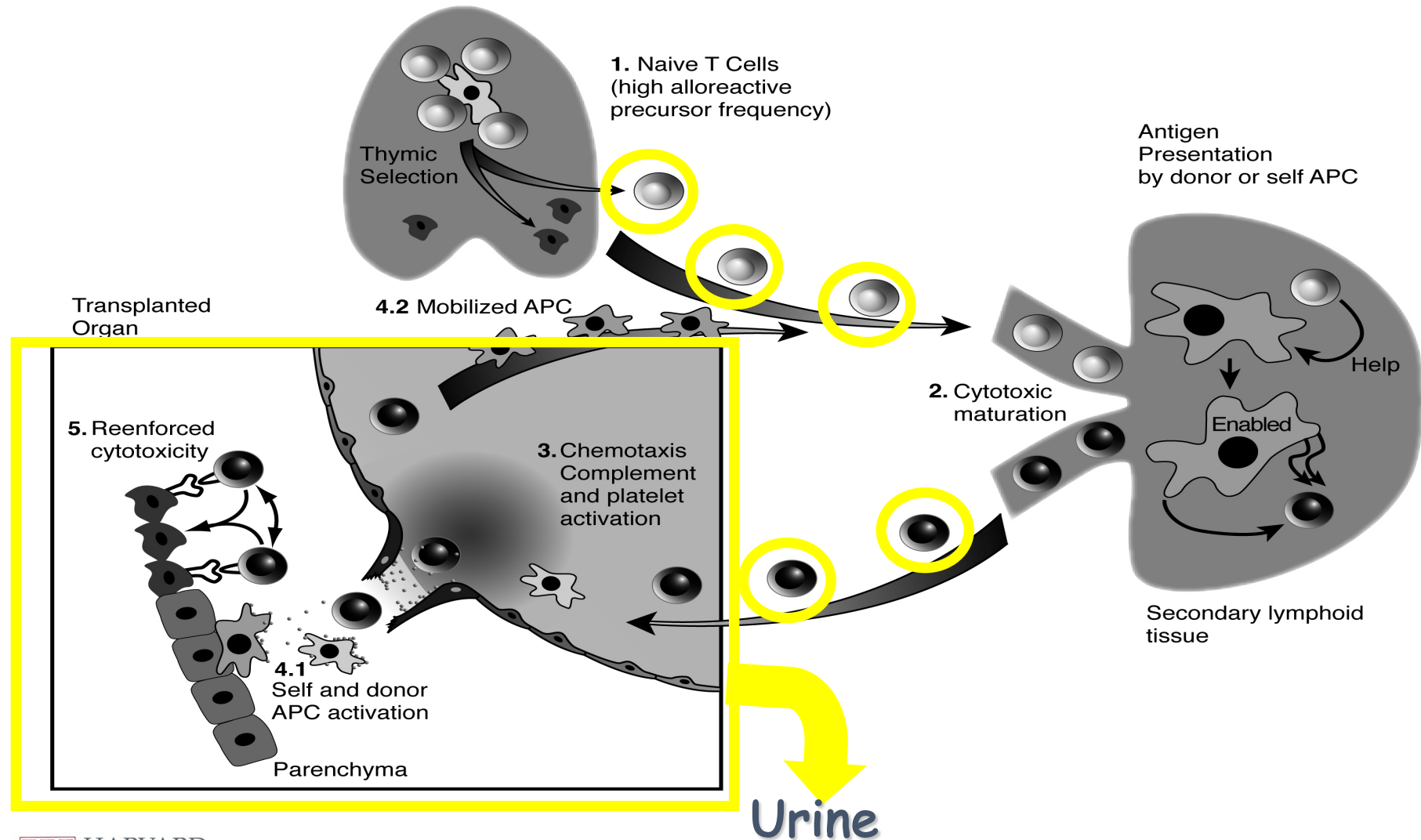
Sensitivity = 76%.

Specificity = 73%.

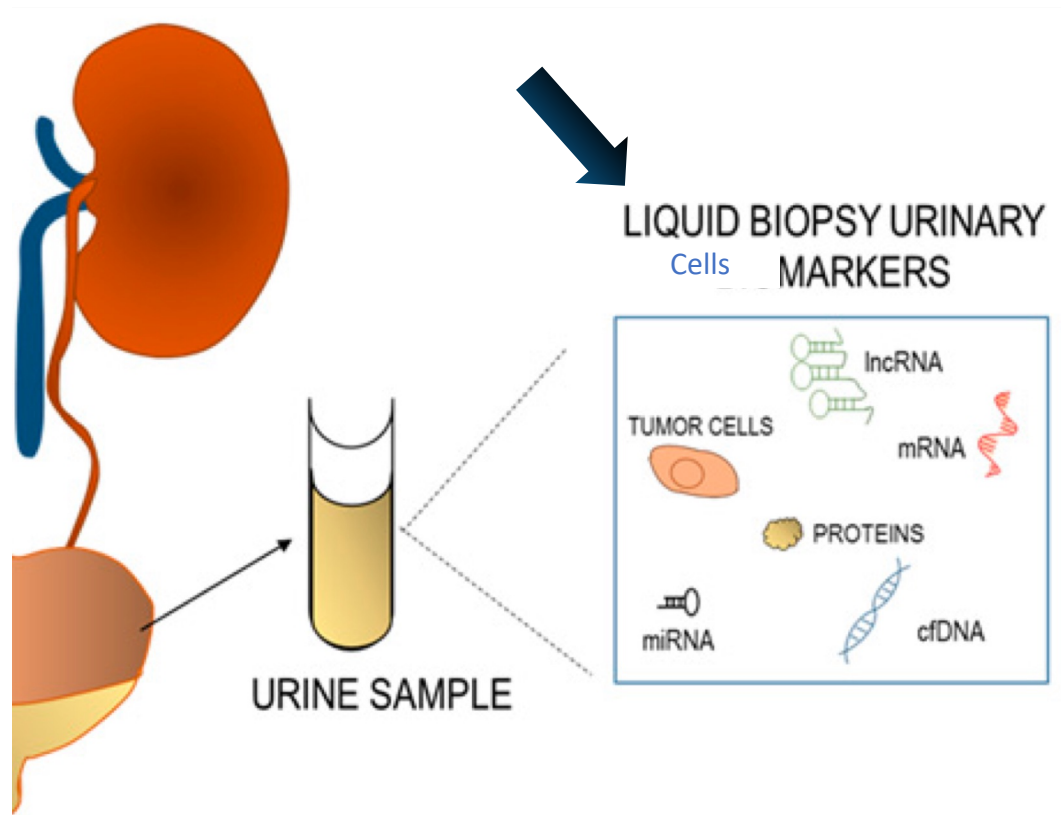


Mechanisms of Immune Monitoring

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.

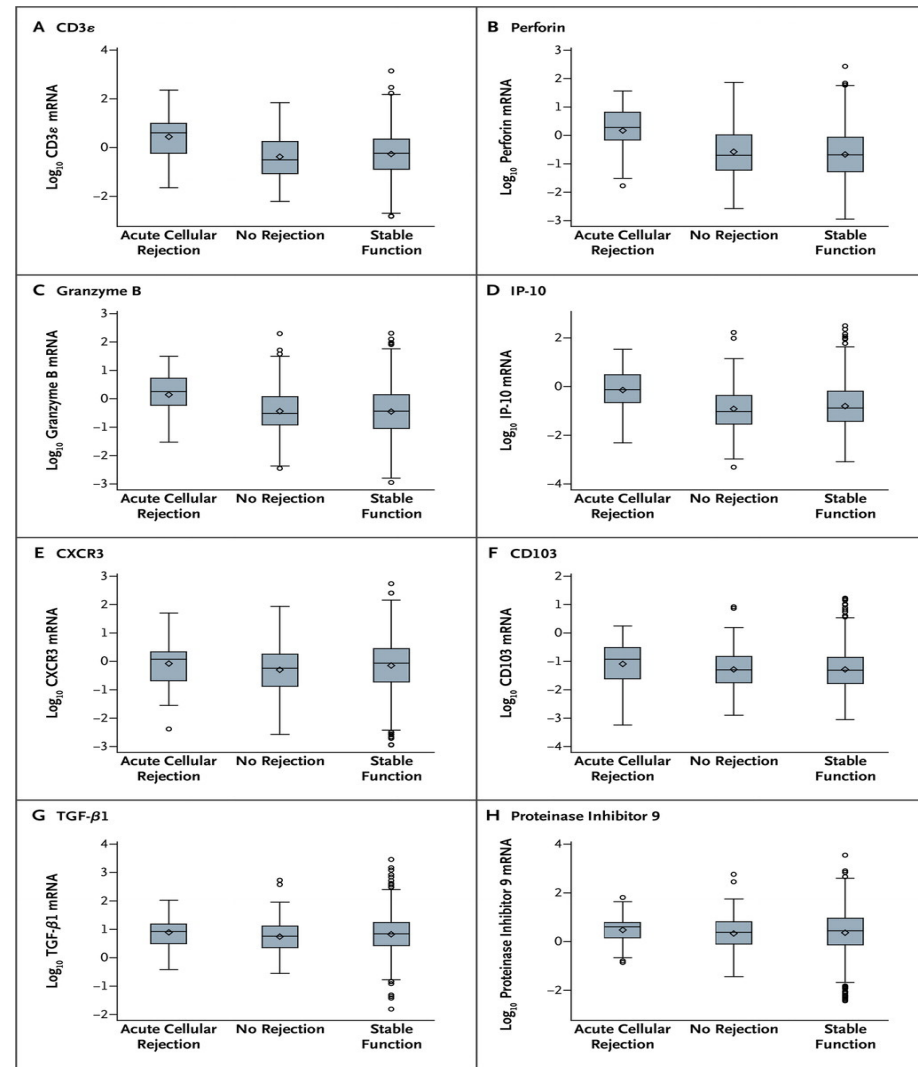


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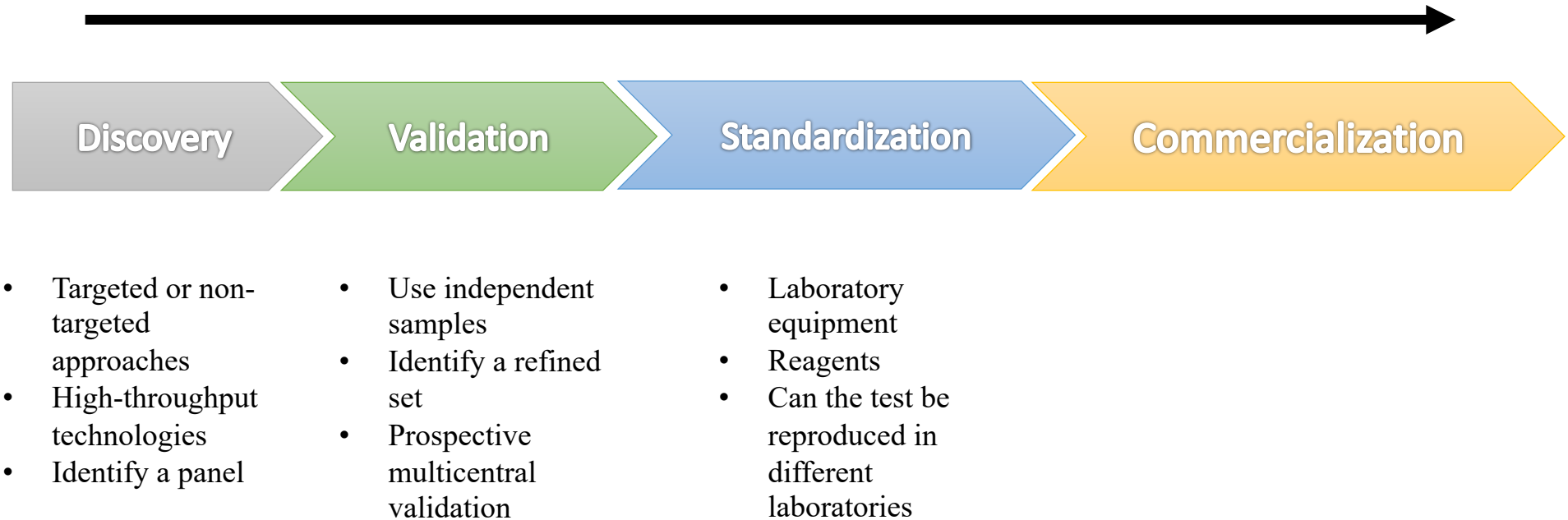
Levels of mRNA in Urinary Cells.



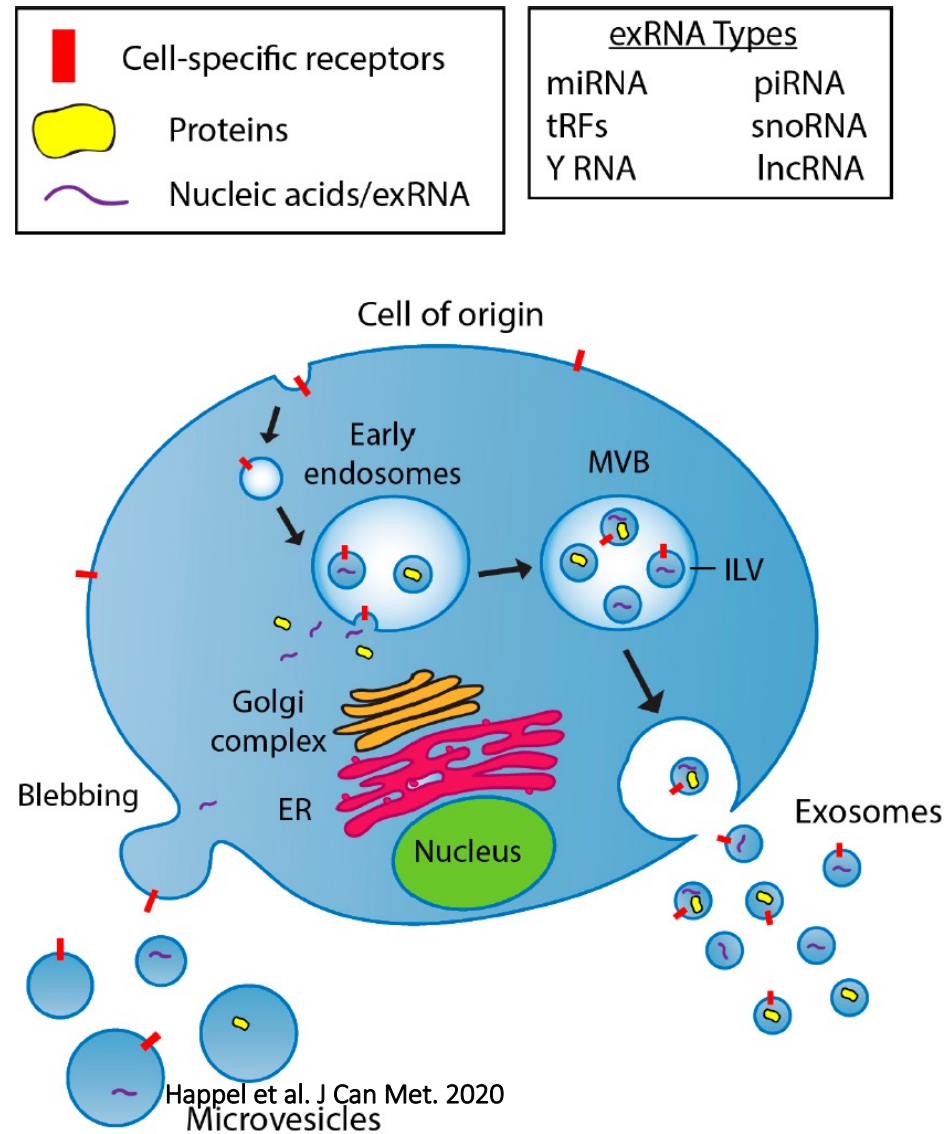
- Prospective collection
- 4300 urine specimens from 485 kidney-graft recipients
- Day 3 through month 12 after transplantation.
- CD3ε, IP-10 and 18S rRNA
 - 79% sensitivity and 78% specificity
 - [AUC], 0.85

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

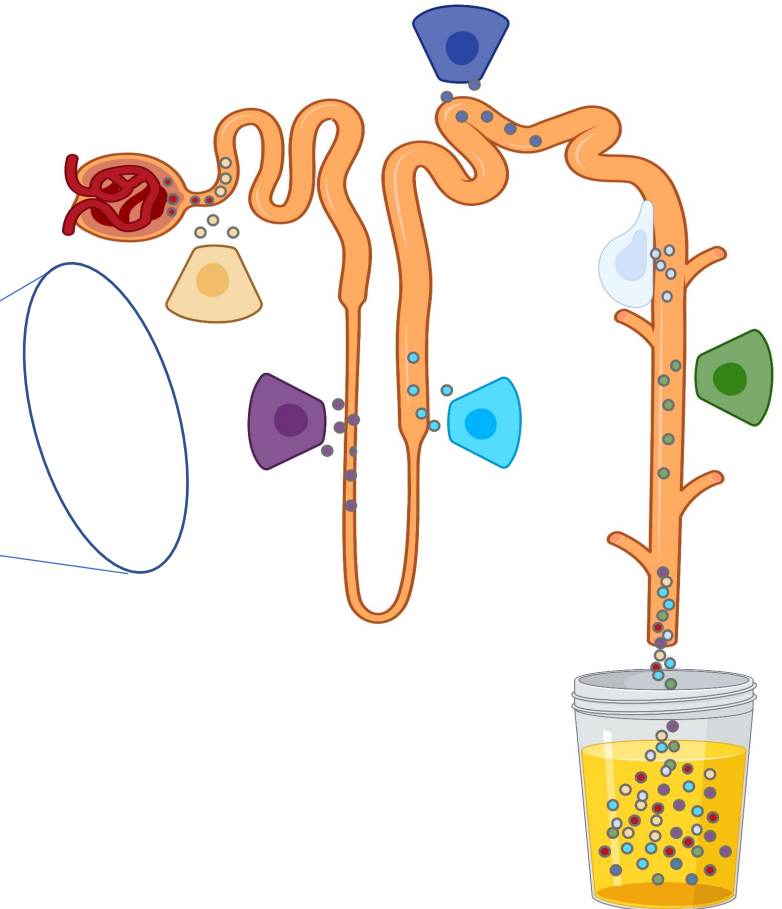
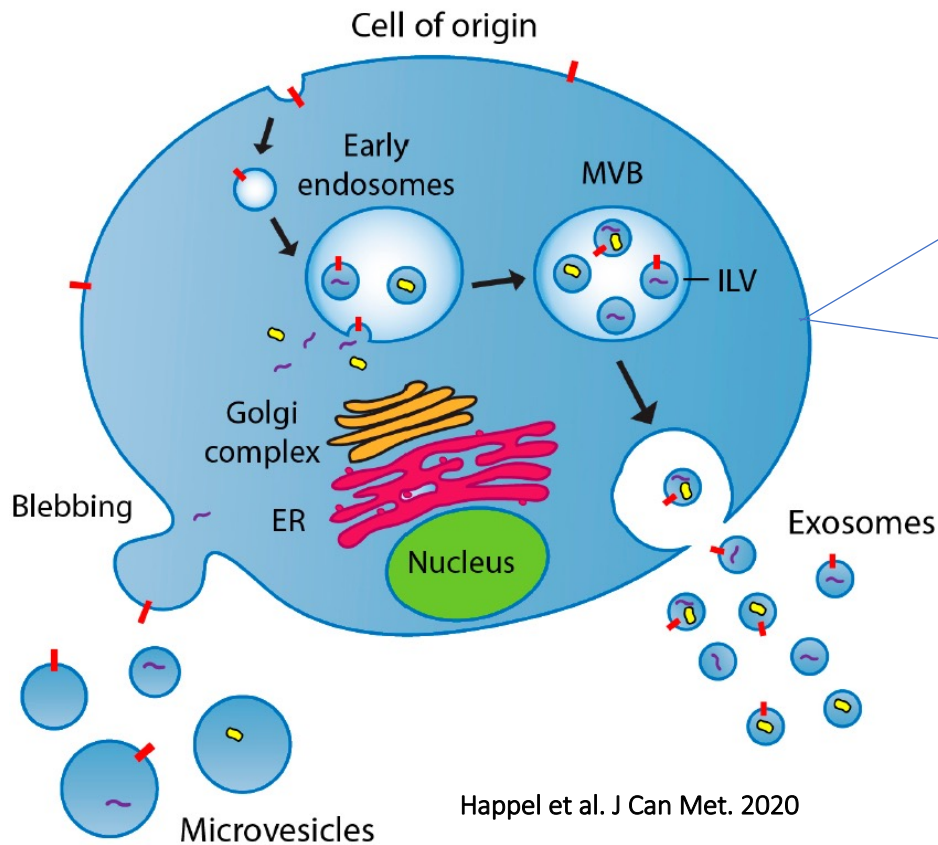
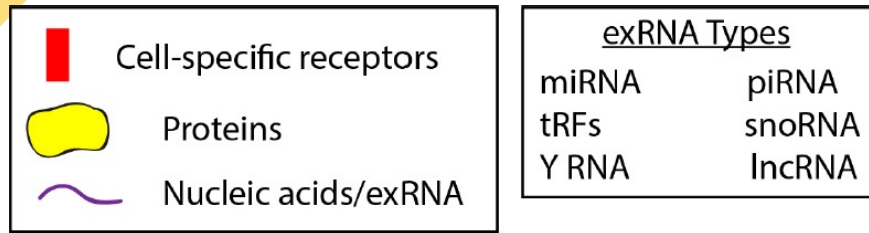
Phases of Biomarkers Discovery



Extracellular vesicles as biomarkers

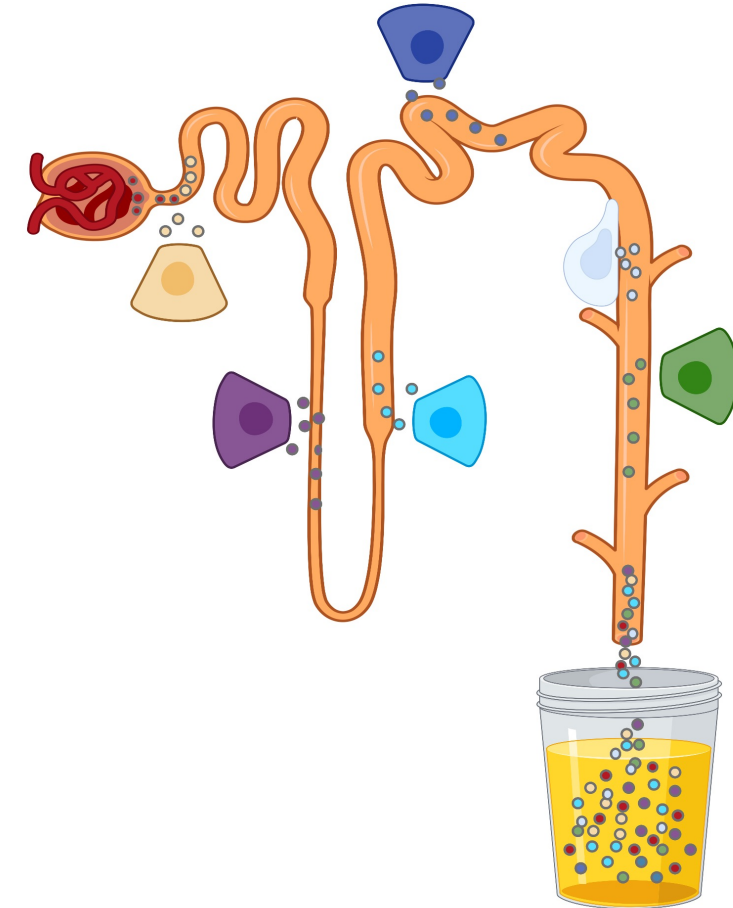
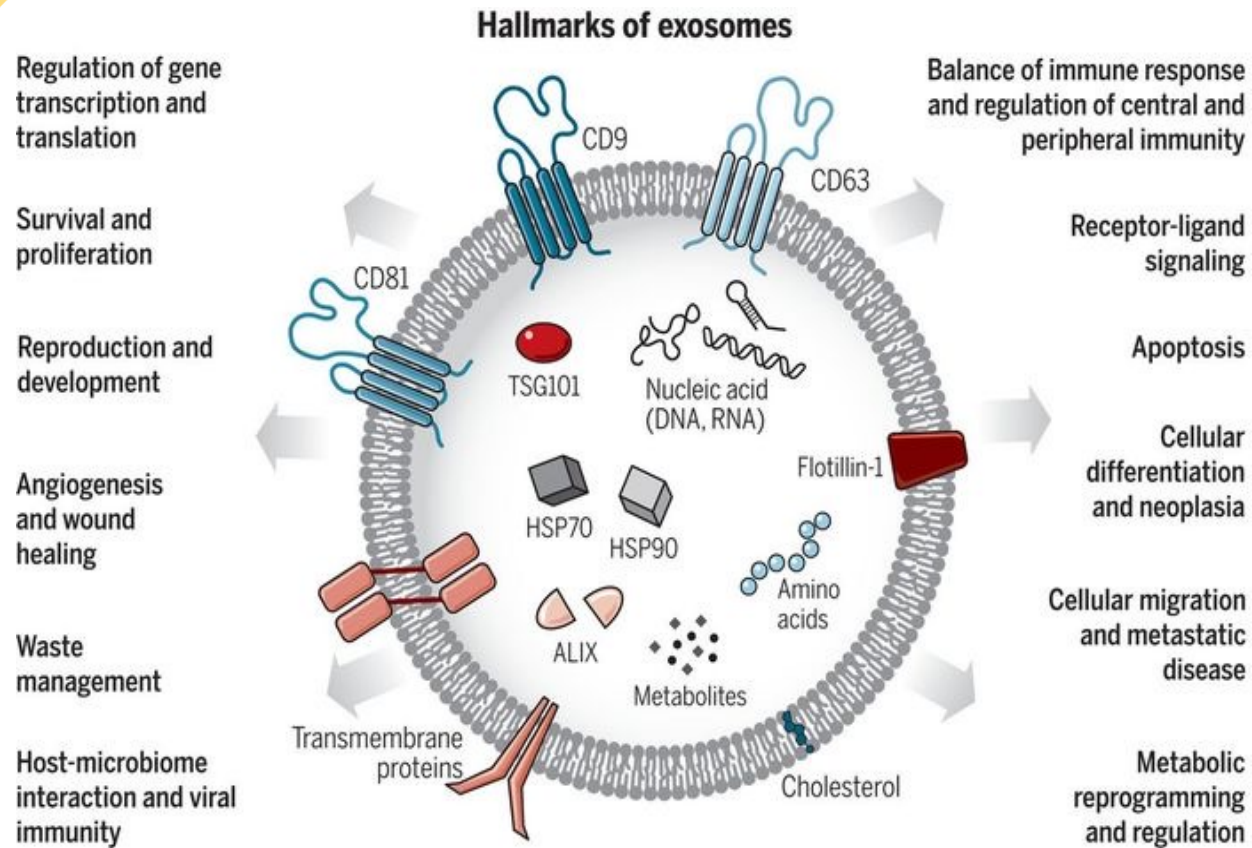


Extracellular vesicles as biomarkers



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)

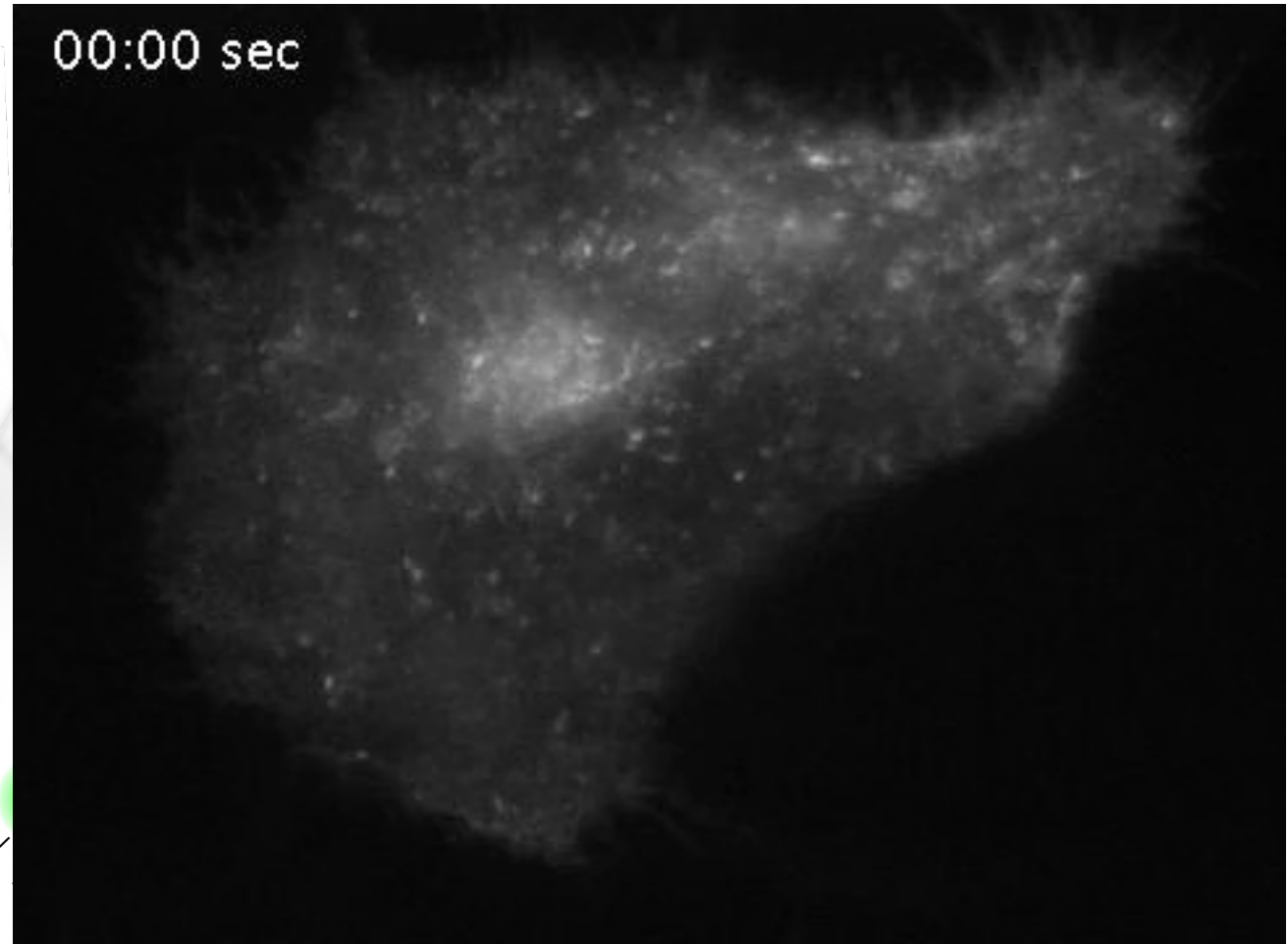
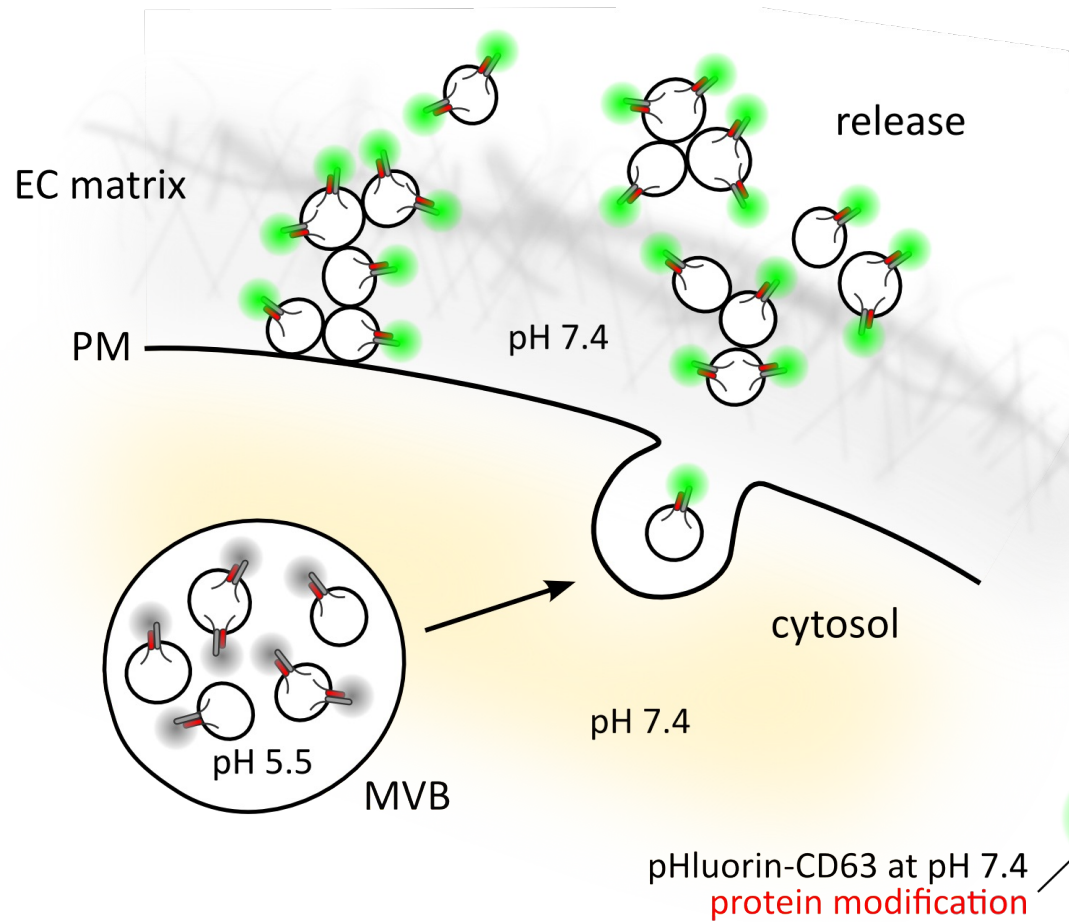


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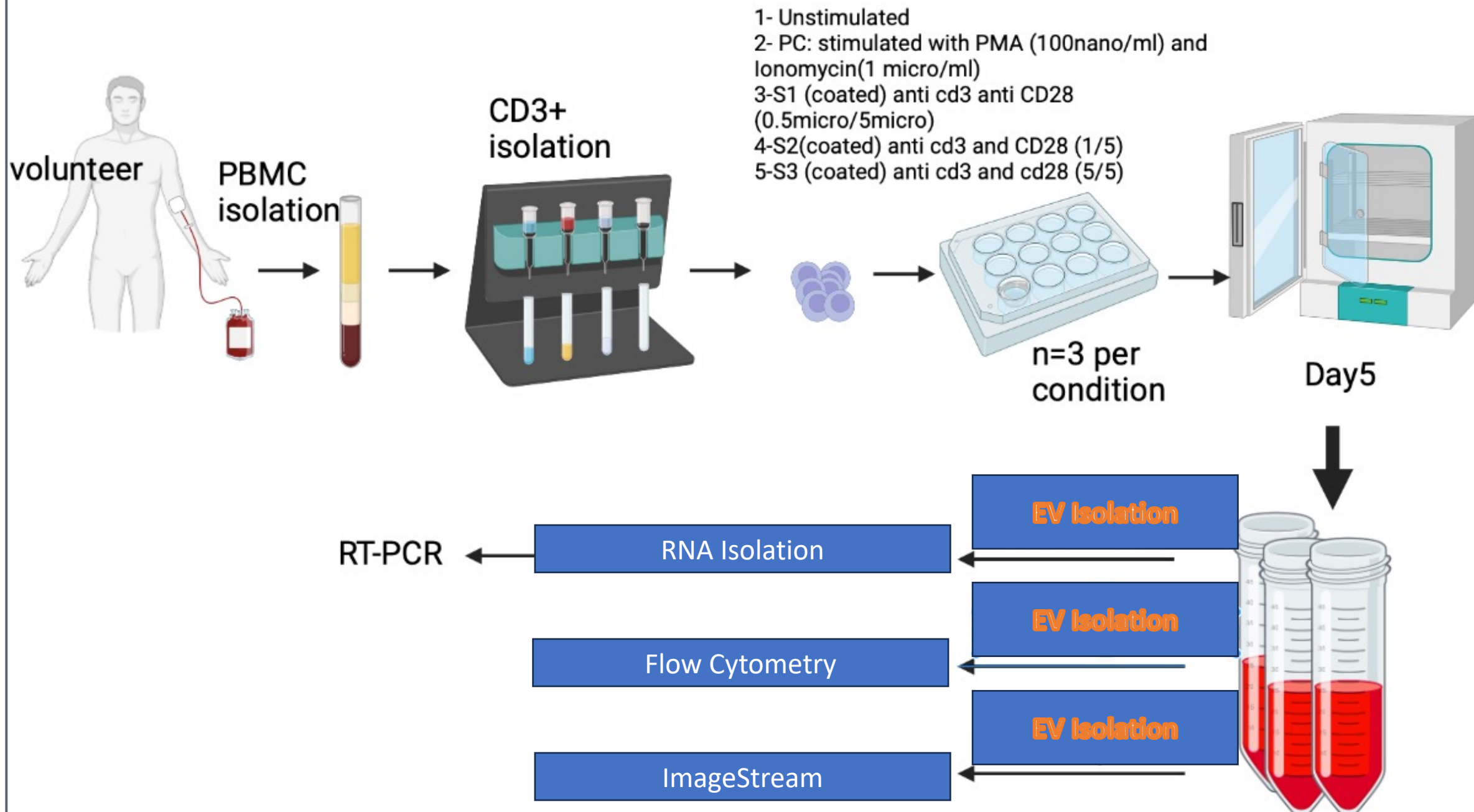


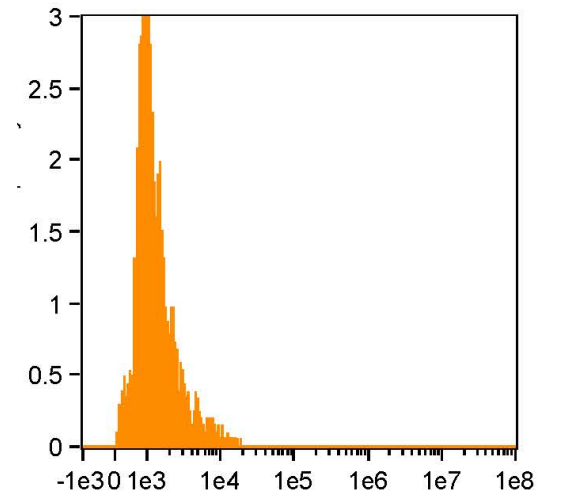
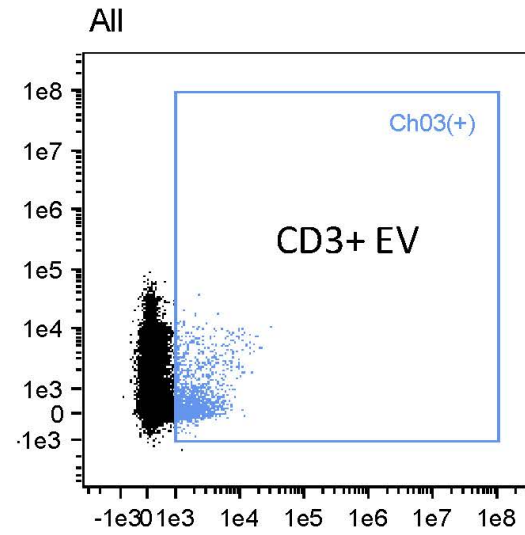
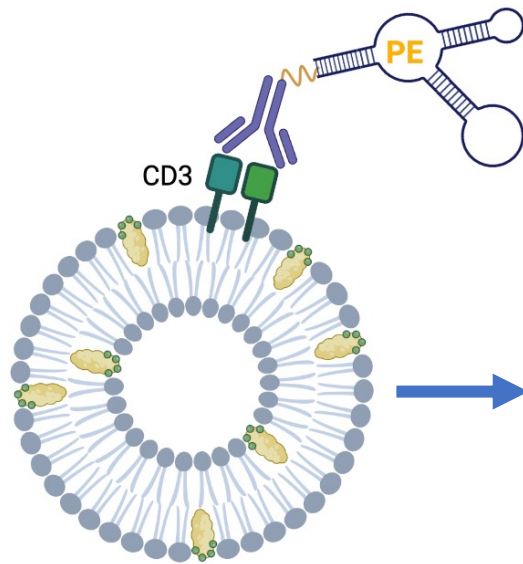
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LIVE-IMAGING REVEALS EXOSOME SECRETION DYNAMICS FROM A SINGLE LIVING CANCER CELL

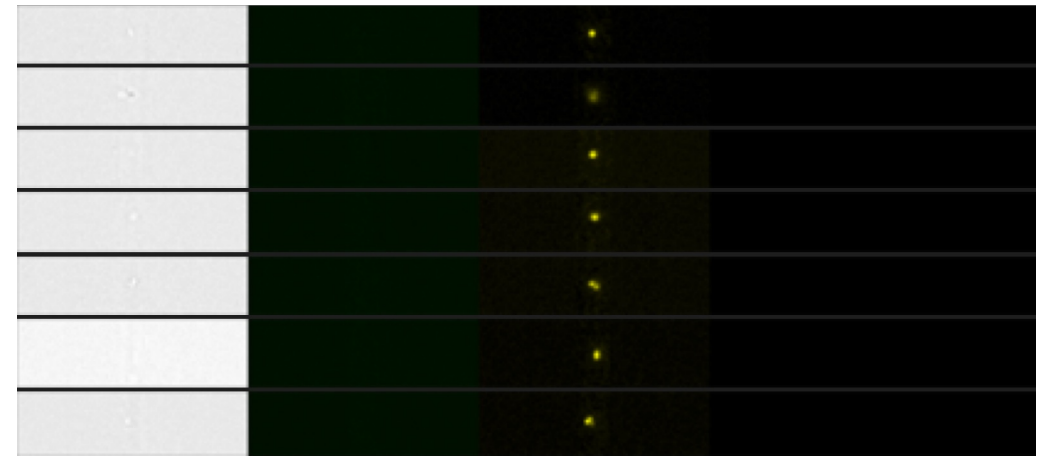


Verweij et al., J Cell Biol 2018





Single EV

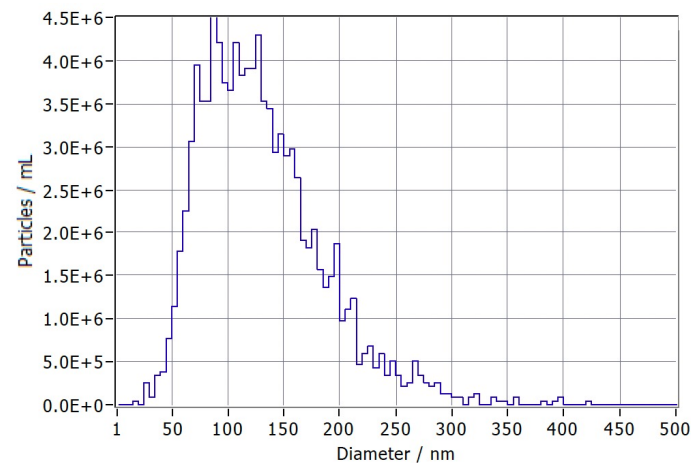
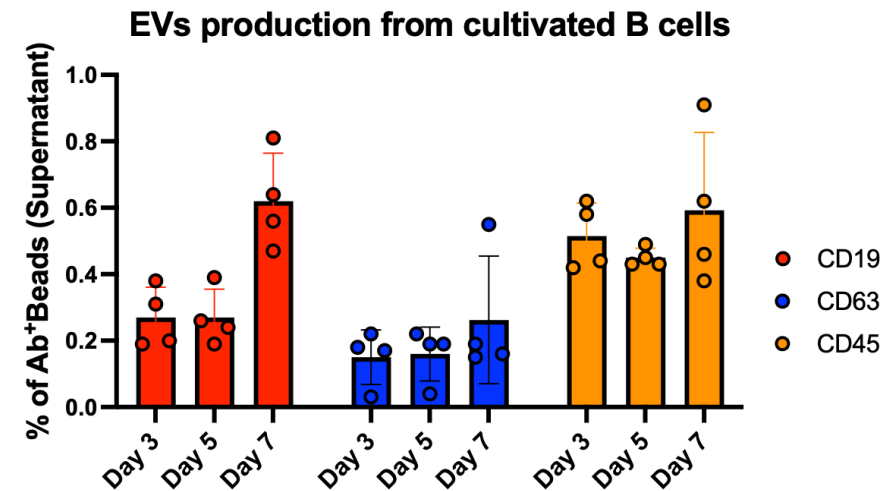
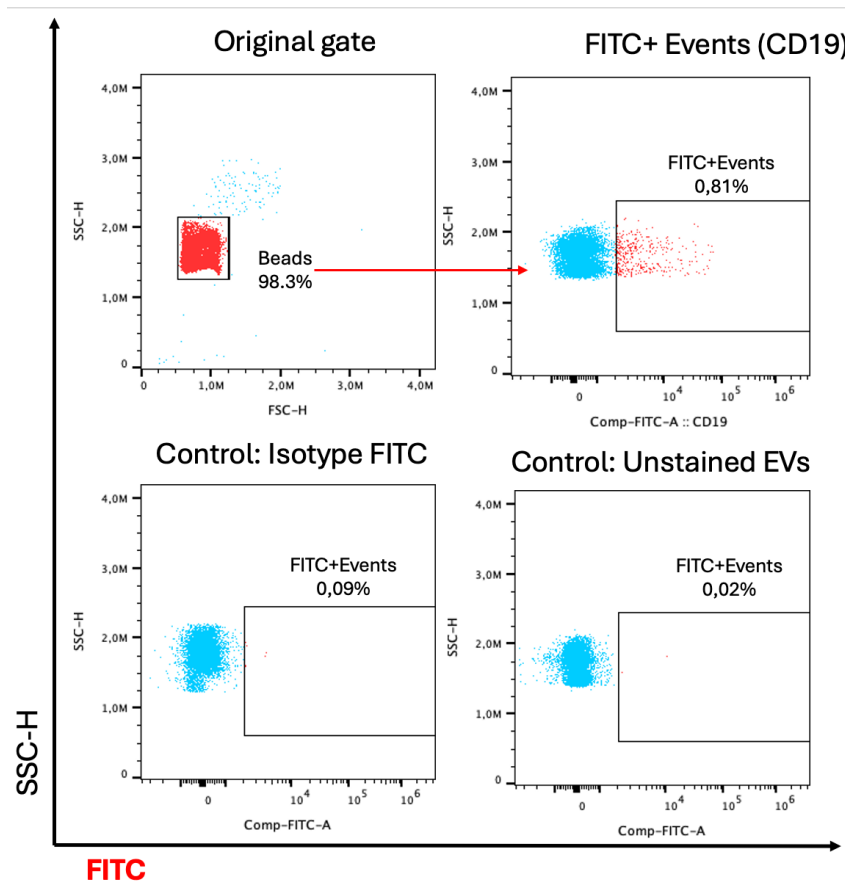


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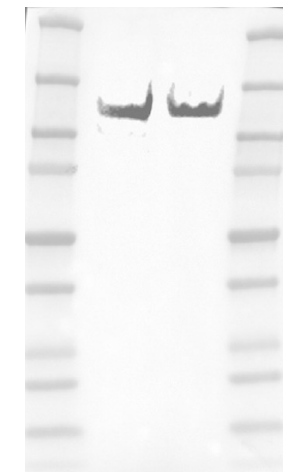
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B cell specific EV

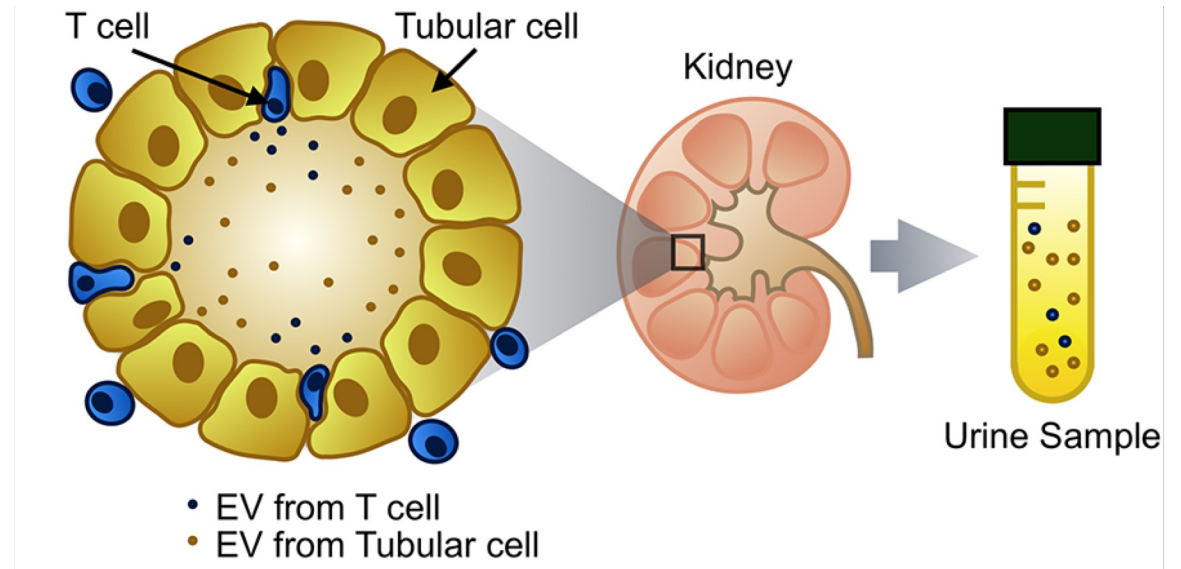
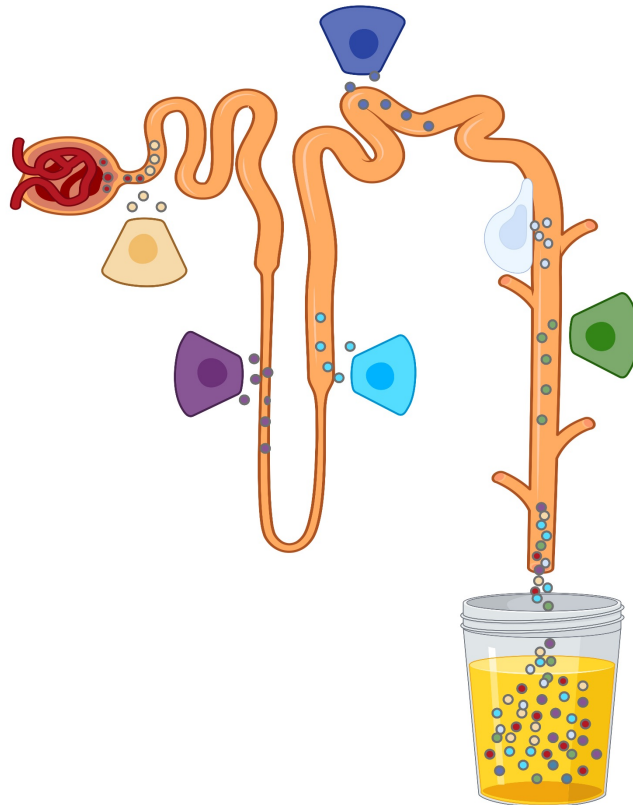


Mean diameter 119.4 ± 54.6 nm
 Concentration: 5.1×10^{10} particles/mL

CD45 150 kDa



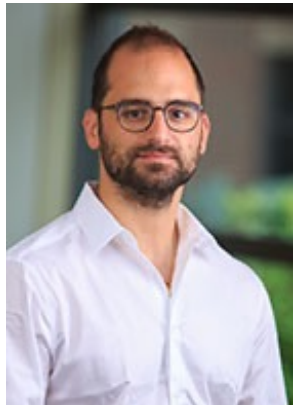
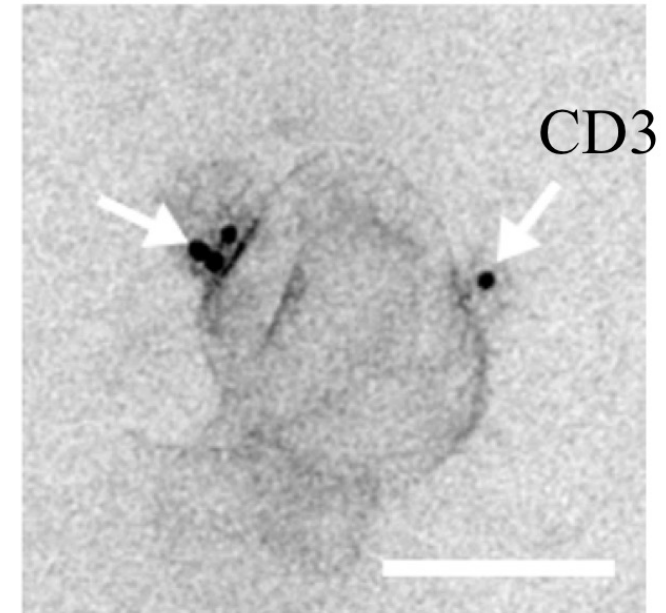
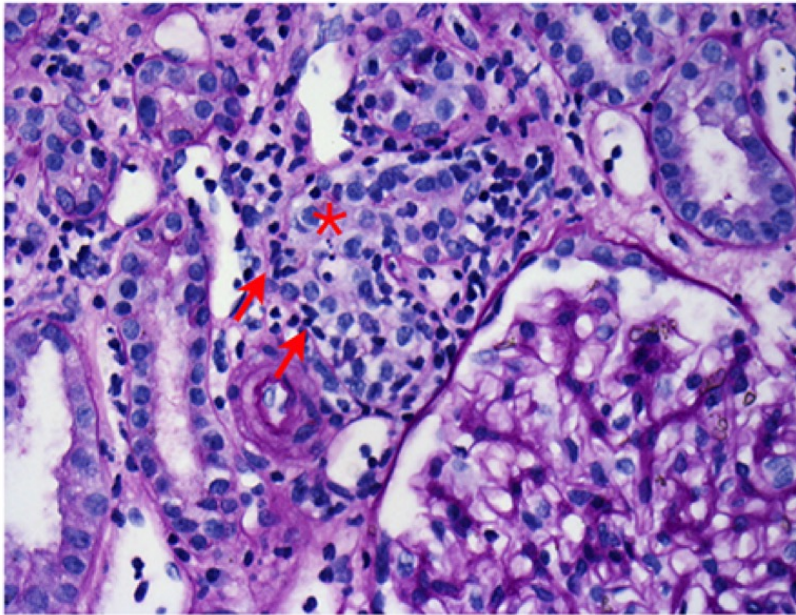
Immune cell specific EVs in urine of rejecting kidney allografts



Immune cell specific EVs in urine of rejecting kidney allografts



Jongmin Park



Jean Pierre Assaker



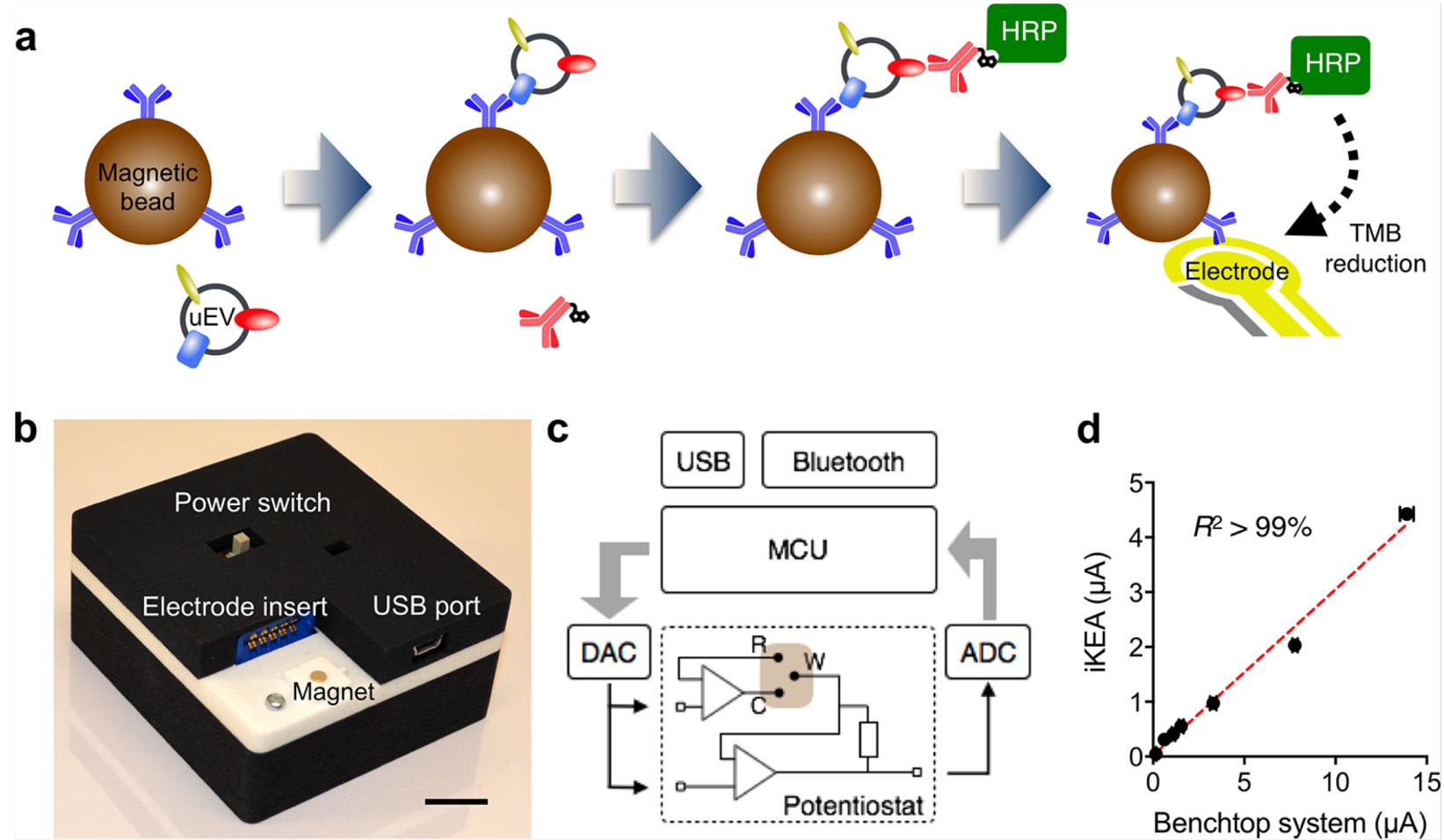
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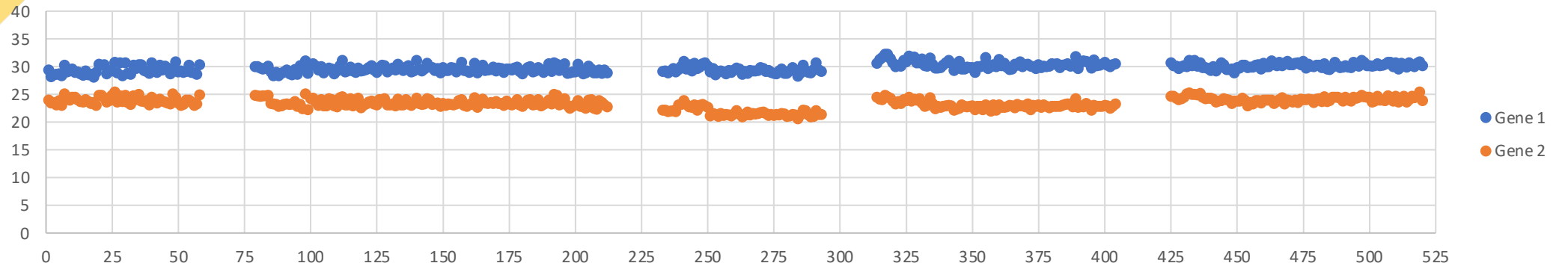
Park...Lee and Azzi et al. ACS Nano. 2017 11 28; 11(11):11041-11046.

Integrated Kidney Exosome Analysis (iKEA)



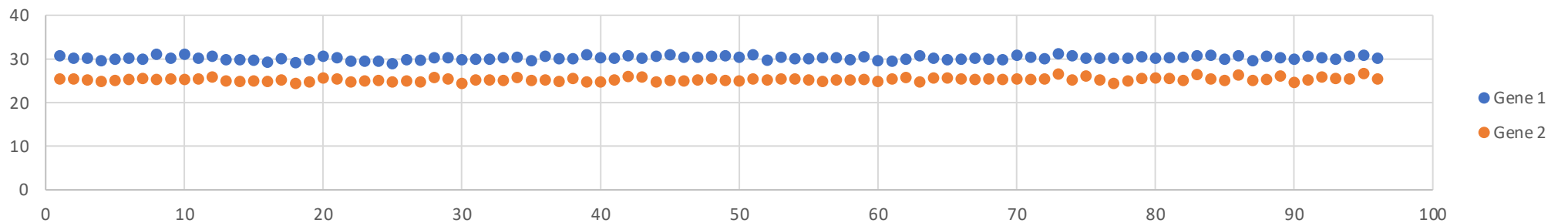
Urine exosome RNA stability

Reproducibility- Control Urine Batches



Extraction replicate

Batch 5 - Detail



Extraction replicate

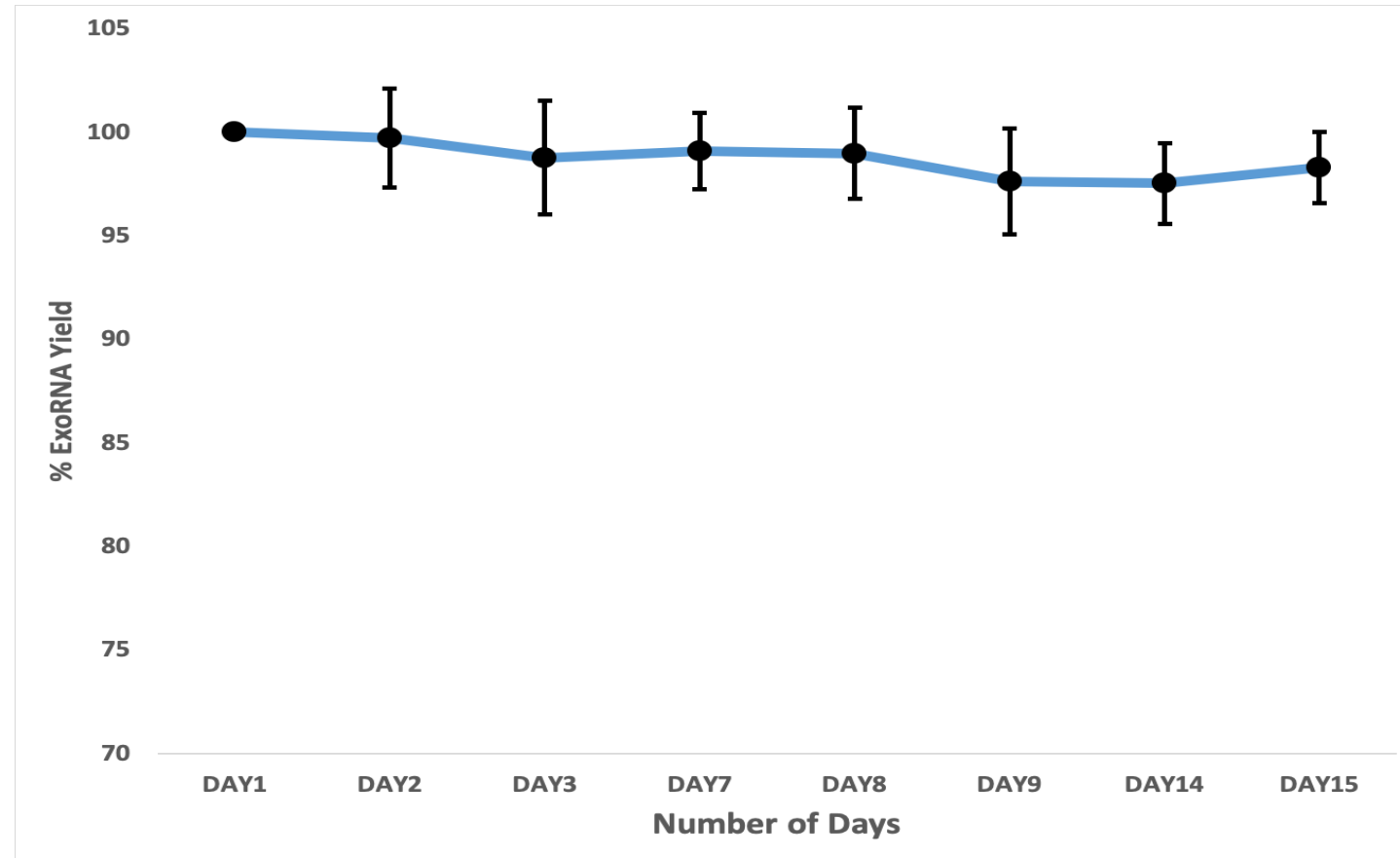


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Urine exosome RNA stability

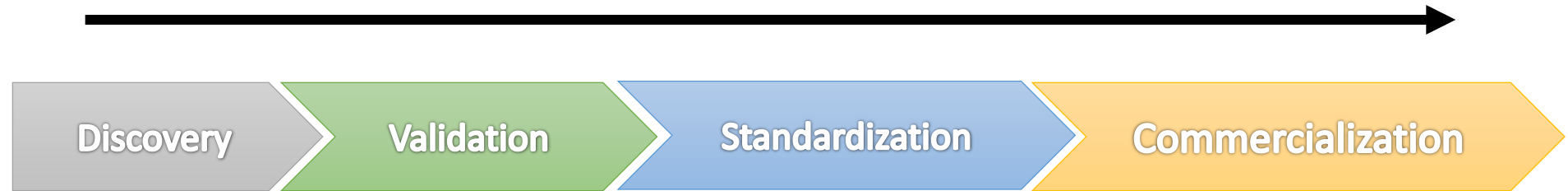


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Phases of Biomarkers Discovery



- 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

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^{ID},¹ Siawosh Eskandari,¹ Philip Chu,¹ Albana B. Mihali^{ID},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^{ID},¹ Richard N. Formica^{ID},⁴ Johan Skog,² and Jamil R. Azzi¹



Rania El Fekih



James Hurley



Ahmad Halawi

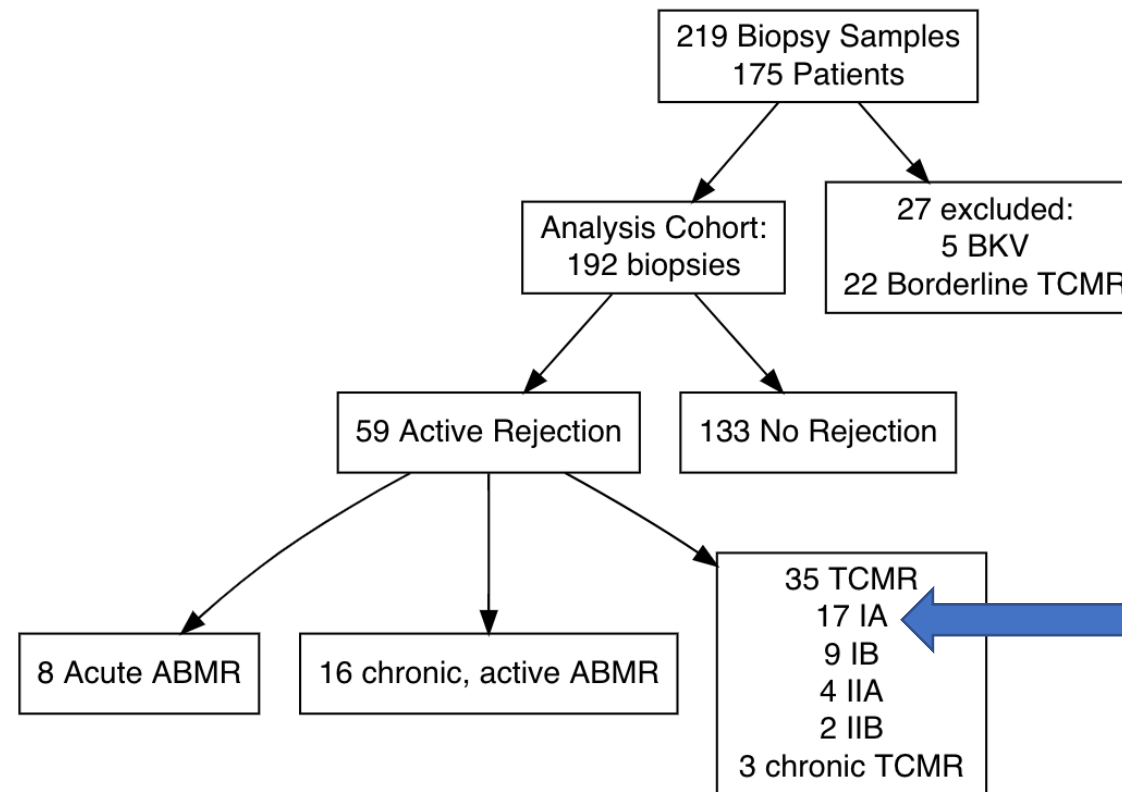


BRIGHAM AND
WOMEN'S HOSPITAL

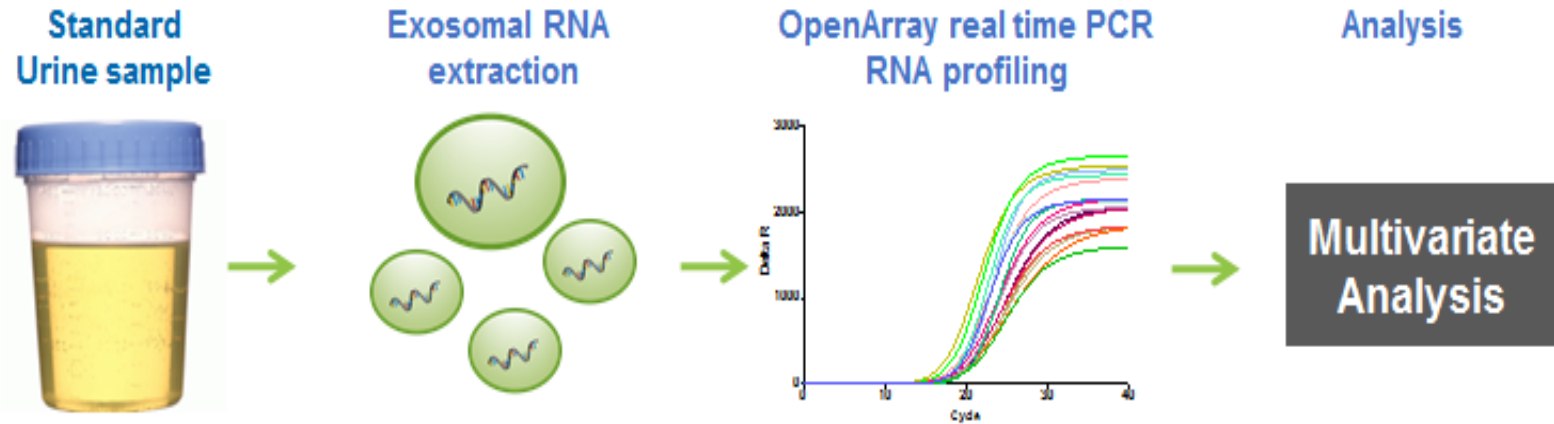


HARVARD
MEDICAL SCHOOL
TEACHING AFFILIATE

Consort Flow Diagram and histologic diagnosis of enrolled patients



Sample processing workflow

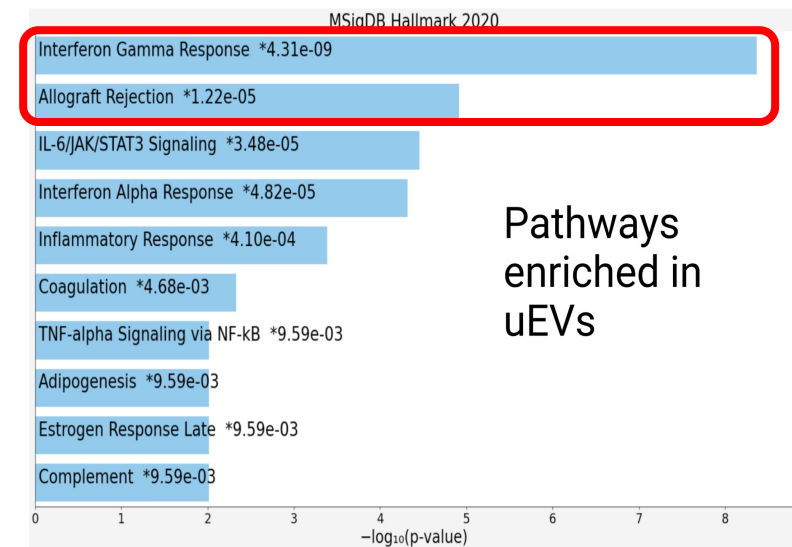
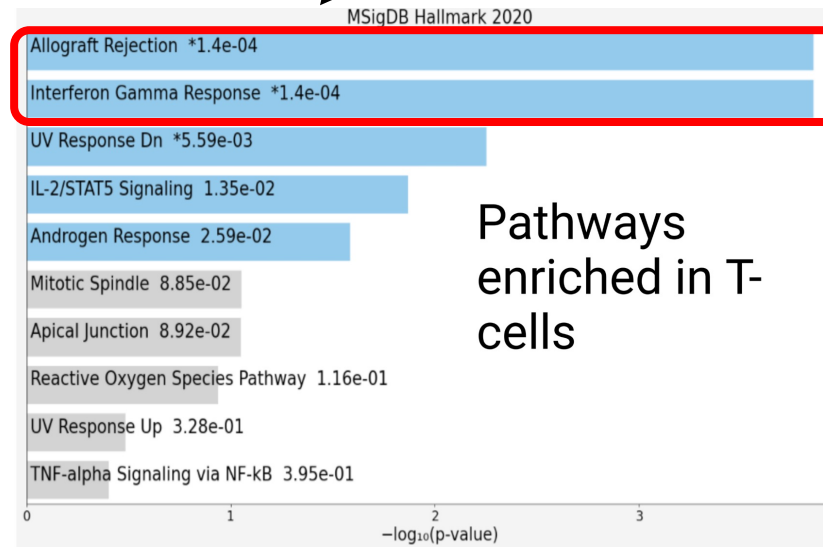
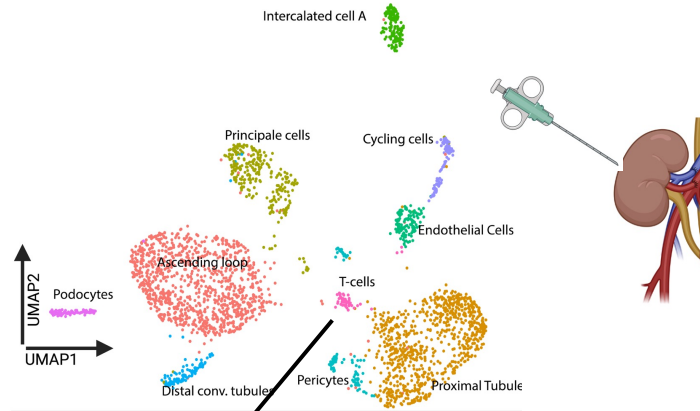


- The exosomes are isolated from the urine with EXOPRO (ExosomeDx)
- RNA reverse transcribed using the VILO cDNA synthesis kit (Thermo Fisher).
- Analyzed using the TaqMan® OpenArray® Human Inflammation

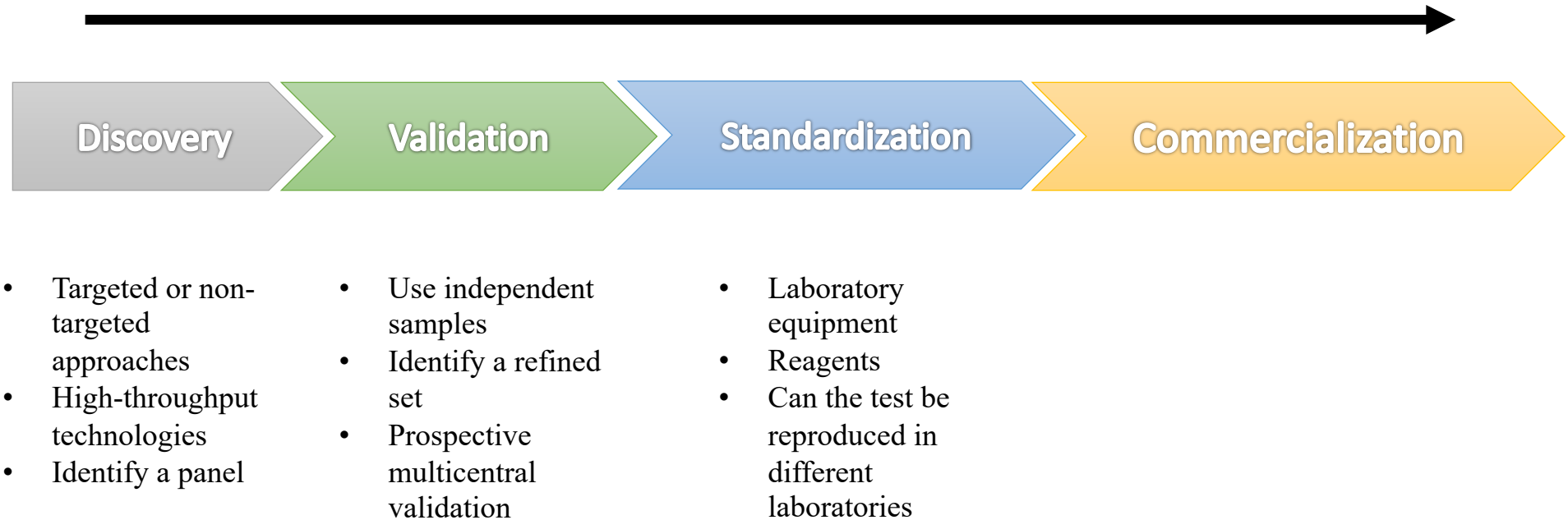
Upregulated Genes

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

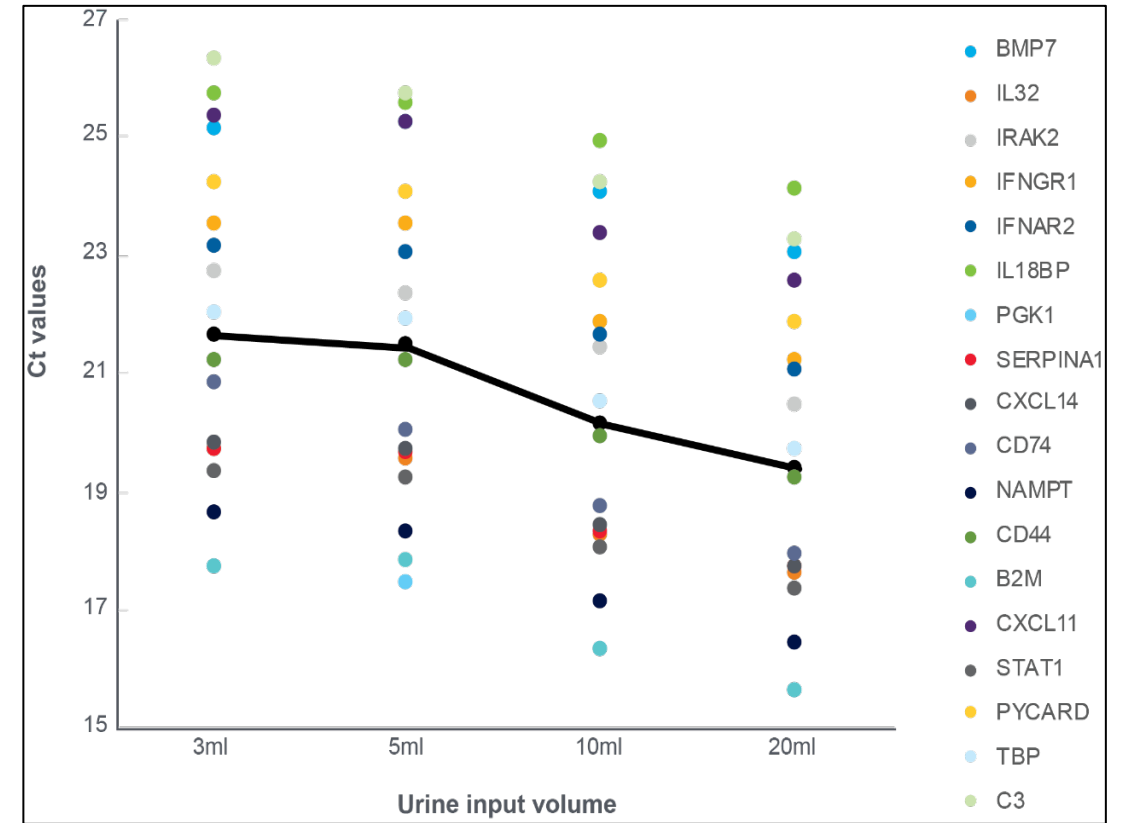




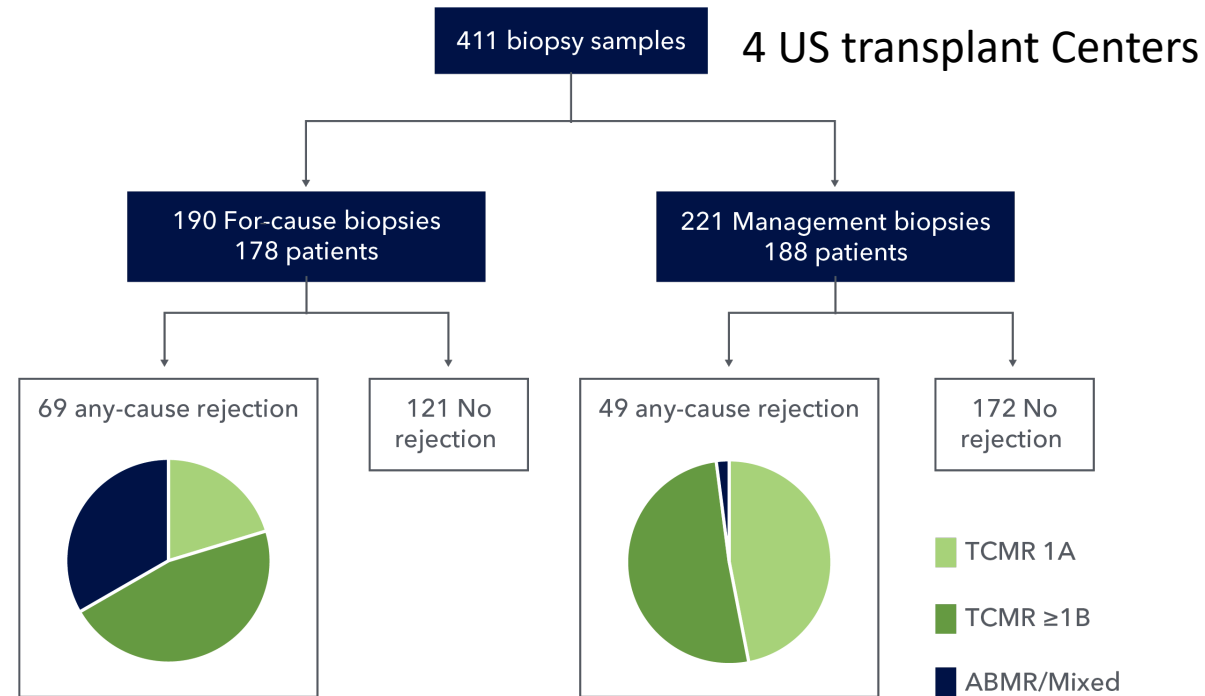
Phases of Biomarkers Discovery



Transfer the signature to a clinical platform

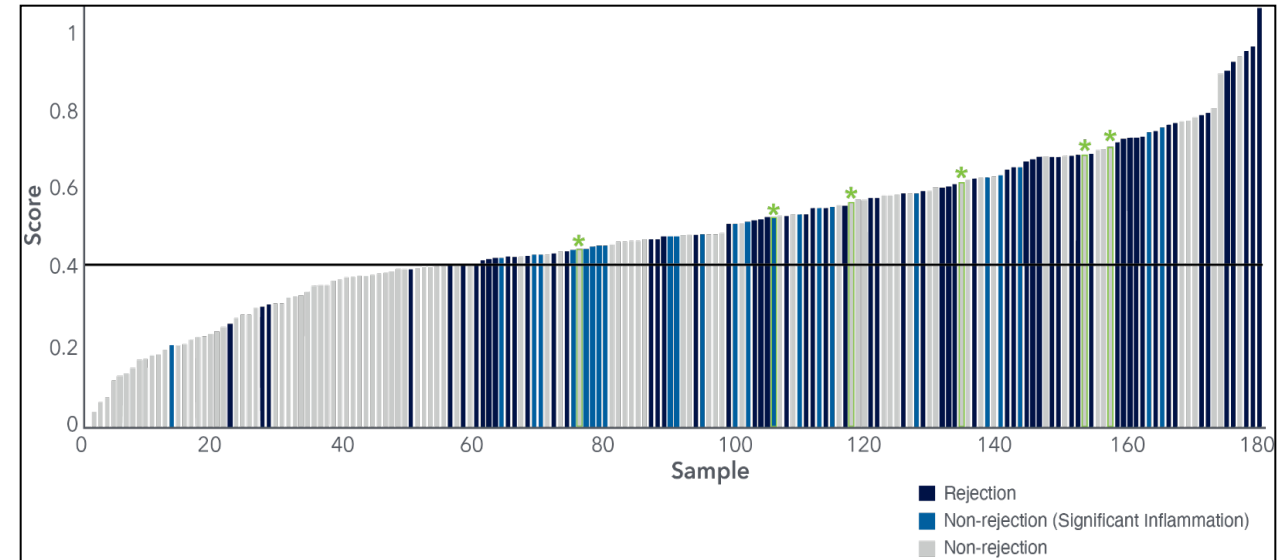
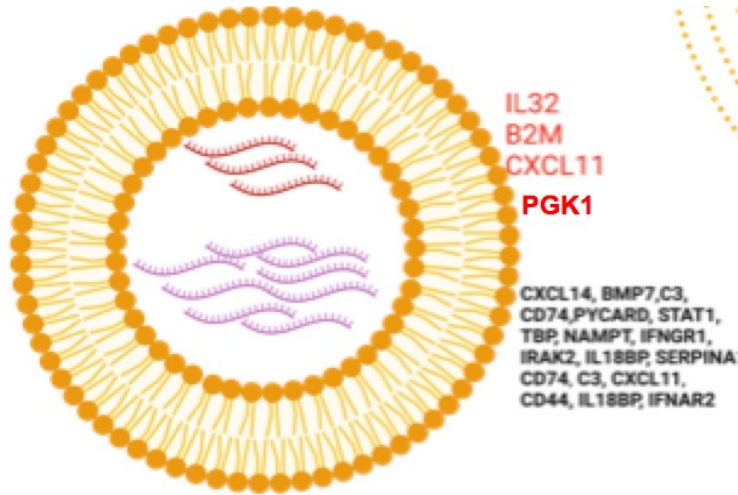


Validation study reflecting real-life



Biopsies with inflammation, such BKV associated nephropathy, Interstitial Nephritis, Recurrent Glomerulonephritis or IC deposition—were not excluded from analysis.

Role of Inflammation in Classification: Results for Clinically Indicated Biopsies



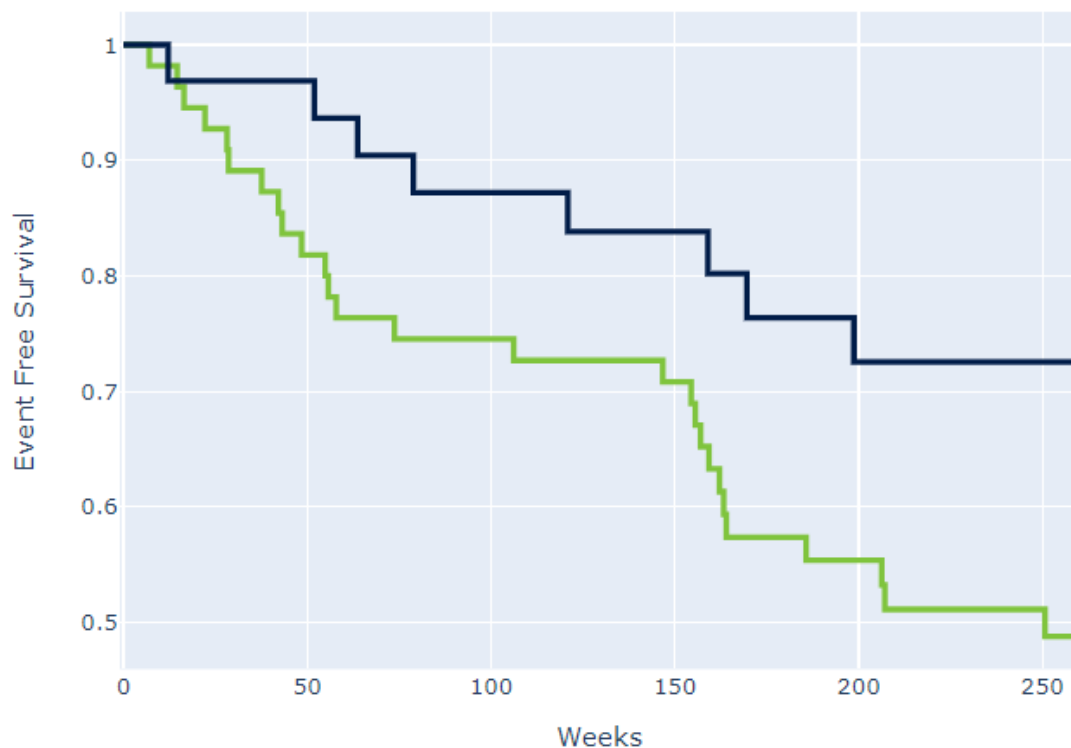
	<u>Any-cause rejection only</u>		<u>Any-cause rejection or significant inflammation</u>	
<u>Metrics</u>	<u>Point estimate</u>	<u>Confidence interval</u>	<u>Point estimate</u>	<u>Confidence interval</u>
Sensitivity	0.93	(0.841-0.982)	0.94	(0.868-0.981)
<u>Specificity</u>	0.43	(0.34-0.523)	0.53	(0.422-0.628)
PPV	0.45	(0.364-0.543)	0.63	(0.544-0.719)
NPV	0.93	(0.827-0.98)	0.91	(0.804-0.97)
<u>Unnecessary biopsies avoided</u>	43%	(34%-52%)	53%	(42%-63%)

Significant inflammation

- pathologic lymphoproliferative infiltrate
- moderate to severe lymphocytic infiltration
- interstitial nephritis (BKV nephritis or acute interstitial nephritis)
- glomerulopathy, or immune complex deposition)

Prognostic Potential: ExoTRU score is associated with 5 years outcome

HR=2.23, p-val=0.023



Five-Year Composite Endpoints:

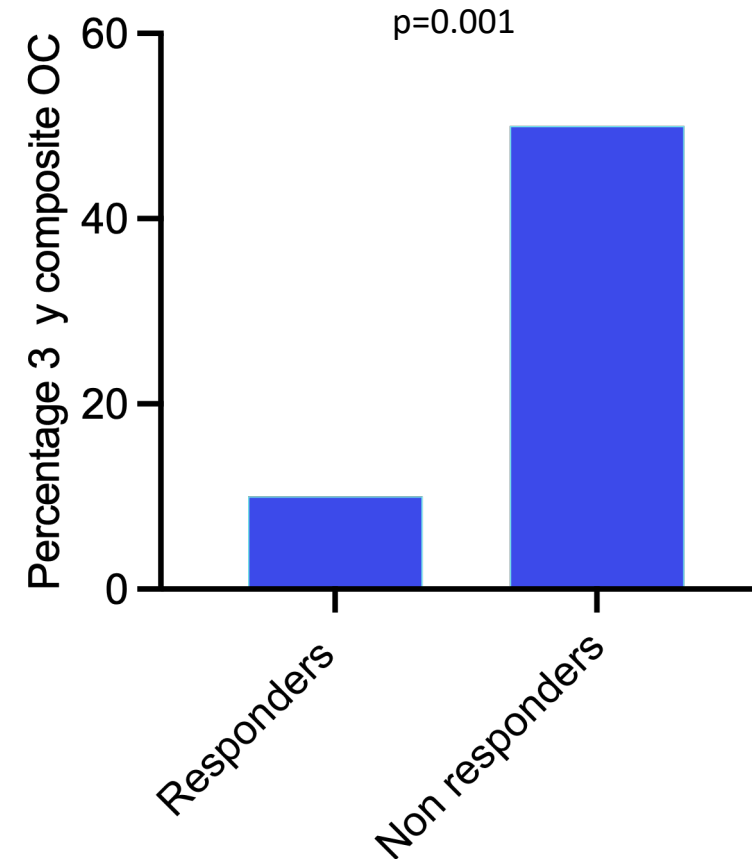
- >30% decrease in eGFR
- Subsequent rejection
- De novo DSA
- Death
- Loss of graft with either return to dialysis or re-transplant

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

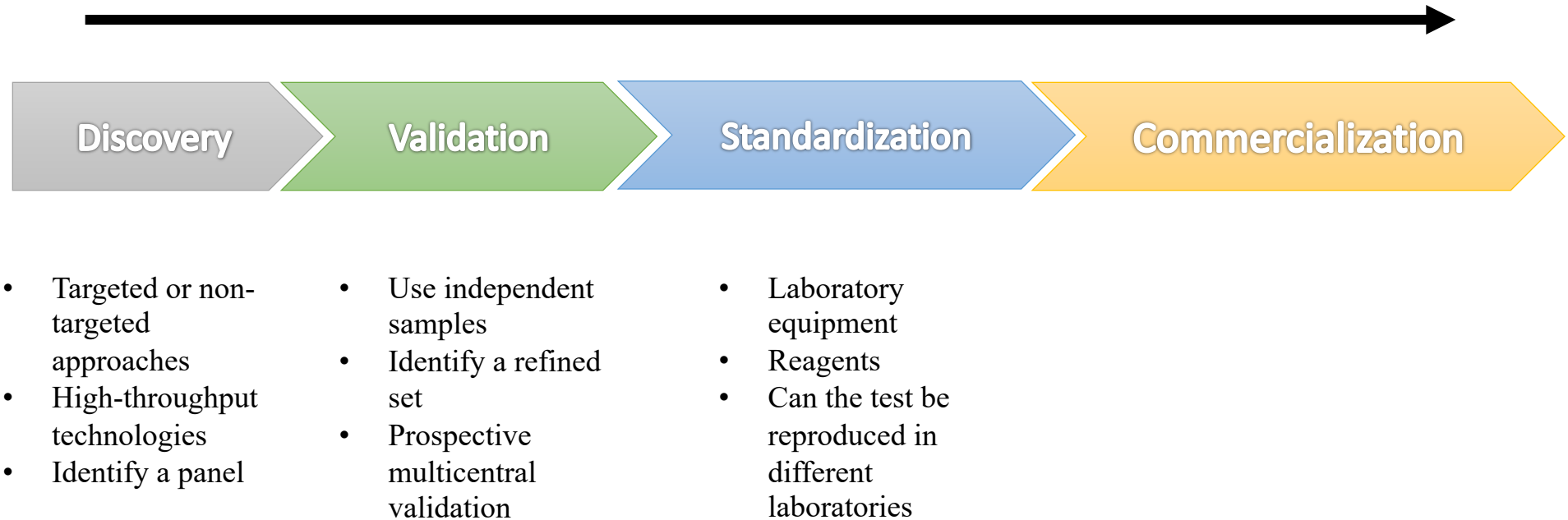
23 samples from patients who have had a repeat ExoTRU score after being treated for rejection, we looked at the change in score after treatment.

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

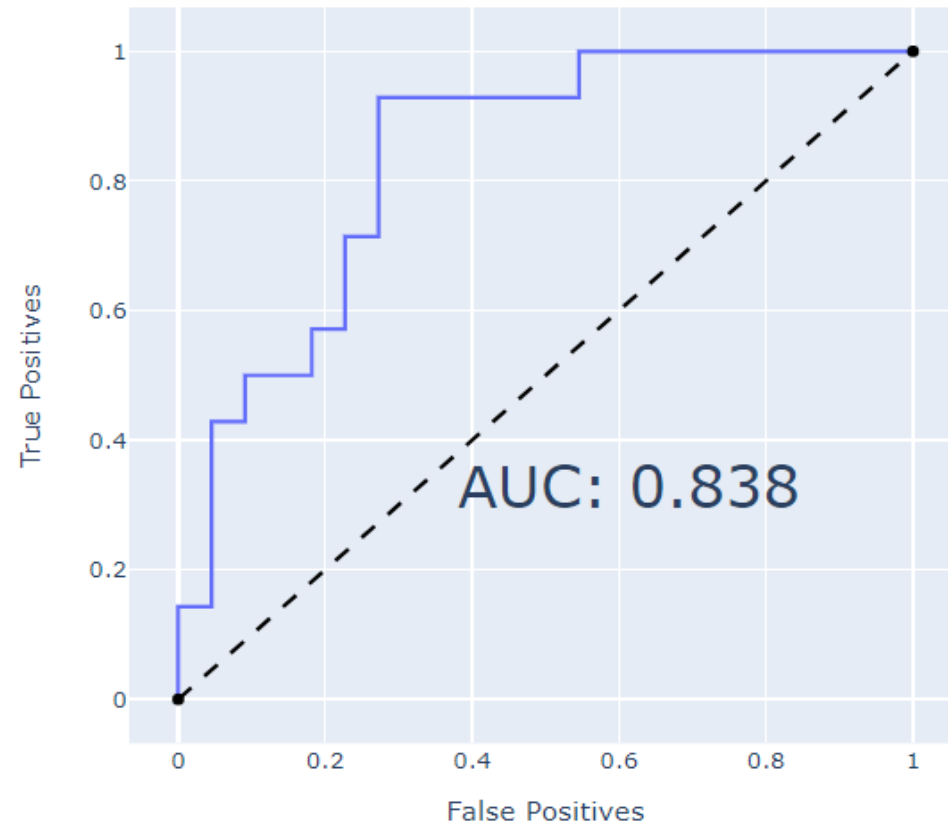
Non responders: score increased or was stable after treatment



Phases of Biomarkers Discovery



Independent Validation By Thermo Fisher



ExoTRU



One Lambda Urinary Exosome Test
EKTRA



One Lambda Pre-Transplant Risk Assessment Assay

A test leveraging a unique RNA signature pre-transplant that provides information about post-transplant graft function and immunosuppression status in deceased-donor kidney transplant recipients.

[Learn more](#)

MMDx for Molecular Gene Expression Biopsy Assessment

Innovative software leveraging gene expression data from biopsied heart and kidney tissue. This service can provide information that can supplement diagnoses of allograft injury via histopathology and other parameters.

[Learn more](#)

TacroType™ Pharmacogenetic Test

TacroType is a pharmacogenetic test designed to assess genetic variation in the CYP3A5 gene that affects how a transplant patient metabolizes tacrolimus. The test helps provide insights that transplant teams may use to inform dosing decisions from the very first dose.

[Learn more](#)

One Lambda Devyser Chimerism NGS Assay

Designed to provide accurate and sensitive detection of microchimerism levels in stem cell transplant samples, the One Lambda Devyser Chimerism NGS assay is an ideal choice for HLA and molecular core labs associated with HSCT Programs.

[Learn more](#)

One Lambda Devyser Accept cfDNA Assay

An NGS-based assay developed for clinicians and HLA laboratories to detect cell-free DNA in human plasma samples. This assay provides high sensitivity, with minimal hands-on time and results in as little as 24 hours.

[Learn more](#)

One Lambda CXCL10 Assay

Noninvasive urine-based test that can detect the CXCL10 chemokine in human urine samples for kidney transplantation. Multiple studies have shown that elevated CXCL10 is associated with inflammation and early graft dysfunction.

[Learn more](#)

Antibody monitoring assays

HLA donor specific antibodies (DSAs) are linked to transplant rejection. Our reliable, high-sensitivity single-bead assays offer optimal antibody monitoring for better transplant care.

[Learn more](#)

One Lambda Exosomes Kidney Transplant Assay

EkTRA is a rapid, non-invasive urinary assay utilizing qPCR technology to evaluate exosomal mRNA.

[Learn more](#)

More than meets the eye

19 yo male with history of ESRD secondary to congenital kidney disease S/P deceased kidney transplant 1/24/26 (KDPI 46%), 4 HLA MM, PRA 0% induced with thymo.

Had multiple GI symptoms and had Cellcept switched to Imuran and dose reduced due to leukopenia and EBV viremia detected in a high risk patient.

Creatinine plateaued around 2.

Biopsy done 4/6: No rejection

Creatinine worsened

Biopsy done 4/28: ACR and AMR.

EKTRA 3/30/26: 0.7 in favor of rejection

To raise or not to raise!!!

32 yo female with history of ESRD 2/2 MPGN type 1 and chronic immune complex GN with long history of treatment with IS, s/p LRKTx in 2017 cw EBV-negative PTLD, DLBCL type.

Completed 4 does of rituximab, followed by R-CHOP and she completed 4 cycles through 10/16/24.

PET CT 11/18/2024 No evidence of FDG avid disease.

Cr stable at baseline, 0.85 UPCR 0.11

On TAC 0.5 mg twice daily, level 5.3 and on prednisone 5 mg

-Discussed IS regimen option to switch from Tac to mTOR inh

-Patient anxious about her kidney function long-term. Should we reoptimize IS?

Lessons learned at BWH: EKTRA in the Clinic

- Excellent rule out test
- Stable kidney function and no proteinuria with EKTRA positive-> Many cases of ABMR and ACR from borderline to 2A
- cfDNA negative but EKTRA positive: Few cases showing biopsy with active disease from interstitial inflammation to ACR IB and partially treated ABMR.
- Patients with history of ESRD secondary to IgA nephropathy with positive EKTRA post transplant-> IgA nephropathy recurrence even in the absence of proteinuria and hematuria.
- EKTRA positive but biopsy normal: High Risk patients.
- Limitations: Preemptive Transplantation, urine infections...

Inflammation Precedes Injury

Inflammation Precedes Injury in Kidney Transplantation

