THE MACROWORLD OF MICRORNAs

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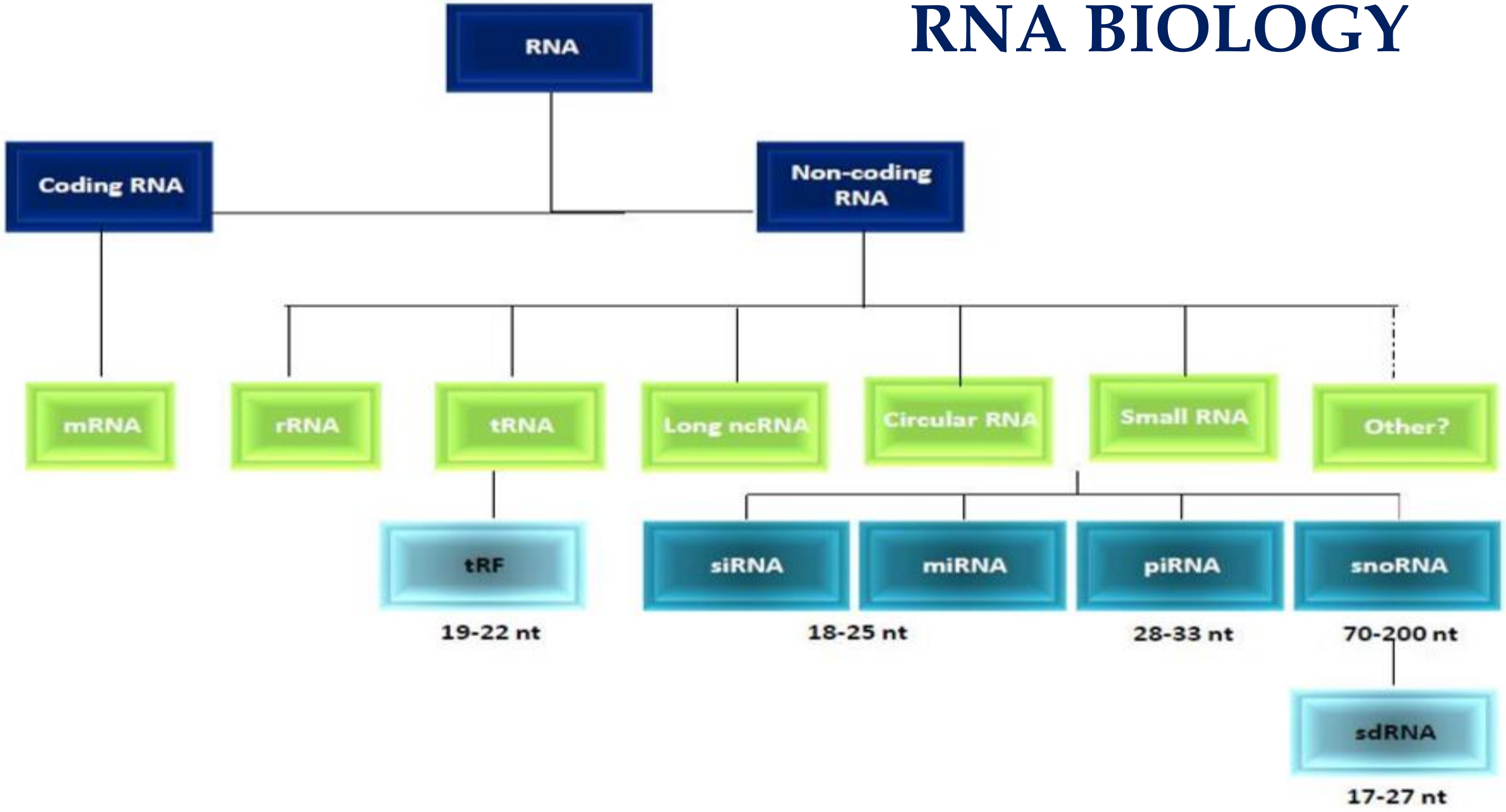
ASSISTANT SPECIALISE

UNIVERSITY OF NIMES MONTPELLIER, FRANCE

- microRNA basics
- miR as biomarker in transplantation

RNA BIOLOGY


RNA
Coding RNA
Non-coding RNA
mRNA
rRNA
tRNA
Long ncRNA
Circular RNA
Small RNA
Other?
tRF
19-22 nt
siRNA
miRNA
18-25 nt
piRNA
28-33 nt
snoRNA
70-200 nt
sdRNA
17-27 nt

GENE SILENCING

GENE KNOCK OUT/KNOCK DOWN

EPIGENETIC MECHANISMS

DNA methylation

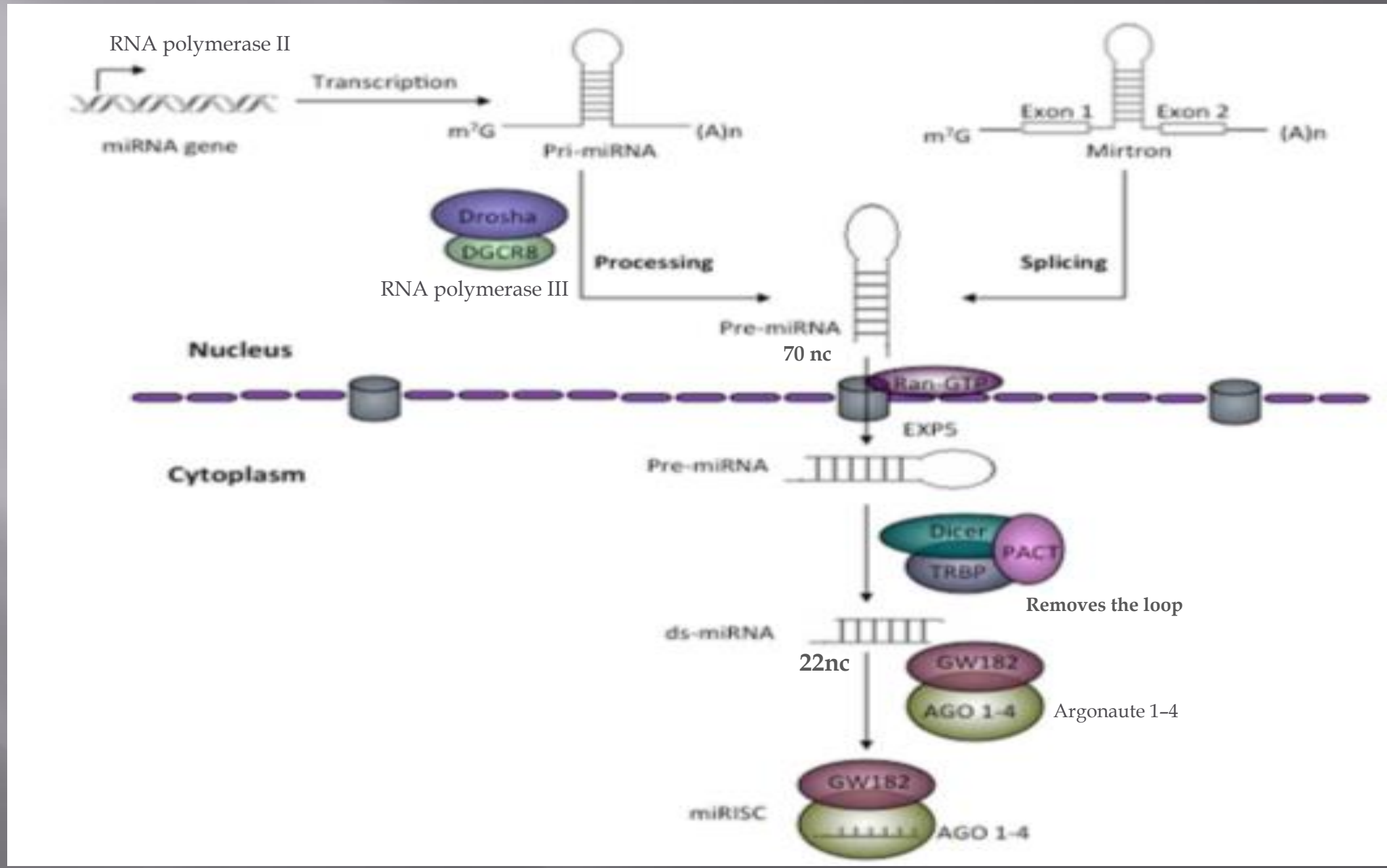
Histone modifications

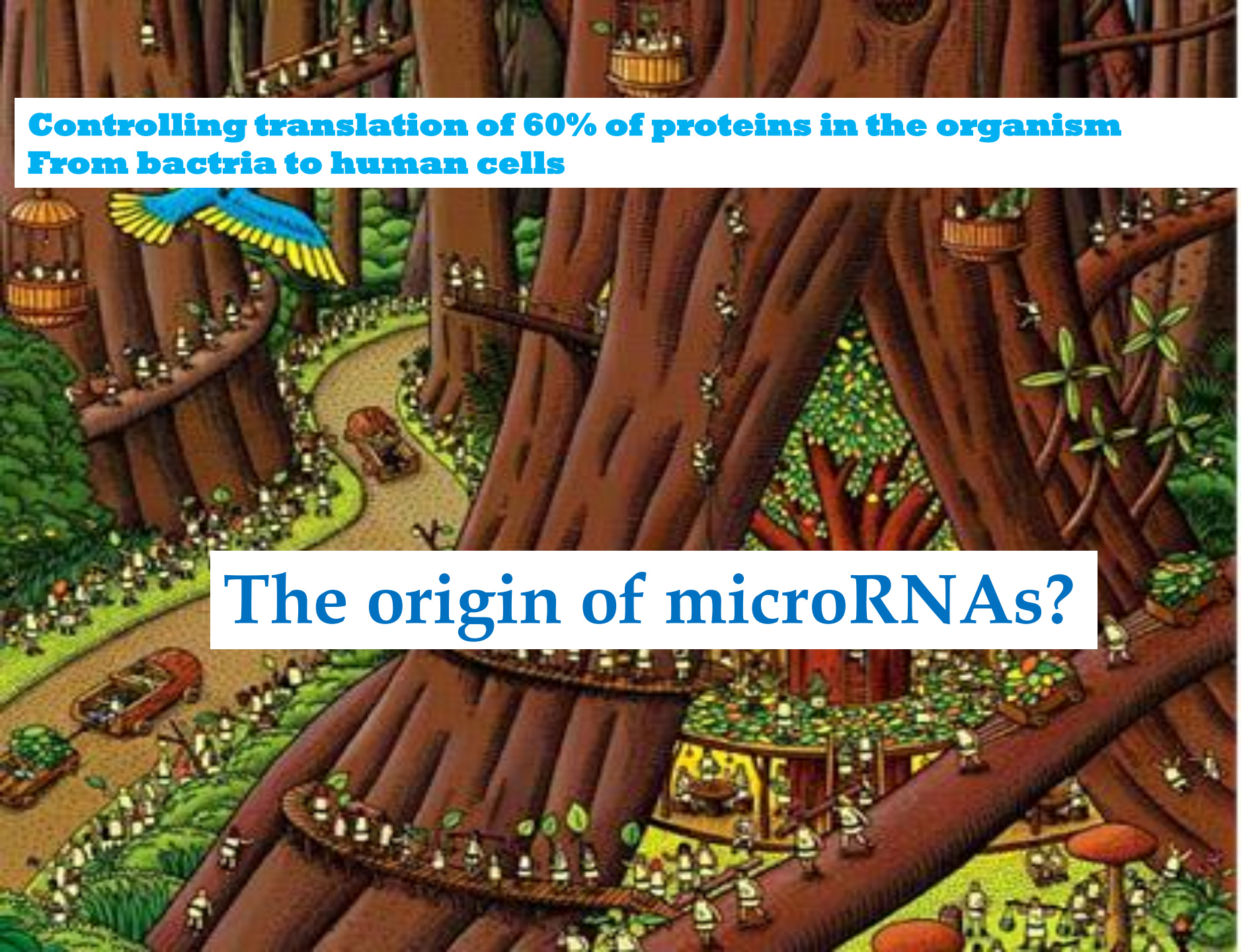
RNA mediated silencing (silencing at m RNA level)

- **Anti sense RNA (via RNA ase H)**
- **PIWI RNA**
- **RNA INTERFERENCE (via RISC)**

  SMALL INTERFERING RNA(double strand RNA)

  MICRORNAs (single strand)


RNA polymerase II
Transcription
miRNA gene
m7G
Pri-miRNA
(A)n
Exon 1
Exon 2
m7G
Mirtron
(A)n
Drosha
DGCR8
RNA polymerase III
Processing
Splicing
Pre-miRNA
70 nc
Nucleus
Ran-GTP
EXP5
Cytoplasm
Pre-miRNA
Dicer
PACT
TRBP
Removes the loop
ds-miRNA
22nc
GW182
AGO 1-4
Argonaute 1–4
GW182
miRISC
AGO 1-4



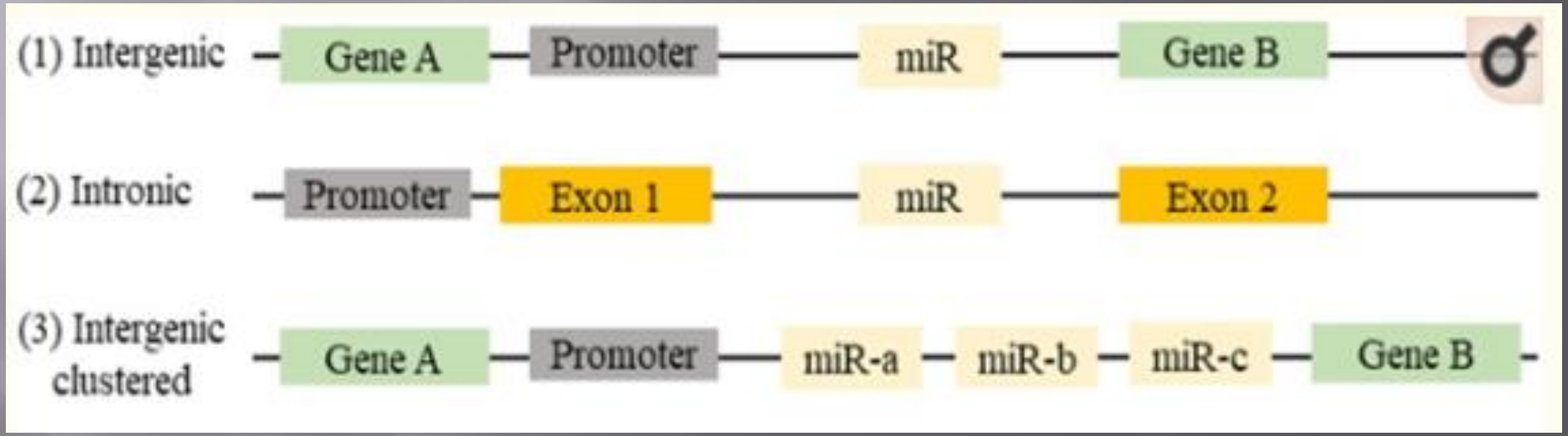
Controlling translation of 60% of proteins in the organism
From bactria to human cells

The origin of microRNAs?

The Macro World of MicroRNA

Short stretches of "junk DNA" are surprisingly influential in preventing or limiting disease—so influential, they are now high on the agendas of many drug companies.

Where do these noncoding small RNAs come from?



- simplified

NOMENCLATURE

- MIR = The gene
- mir = pre microrna or pri micro rna
- miR = mature microrna
- a and b … as suffix:

  closely related except one or 2 nucleotides miR 124a VS 124b

Suffix 3p or 5p = originated from opposite arm of same pre microrna ; may have opposite effects (sense vs antisense)

miRBase

Home | Search | Browse | Genomics | Help | Download | Submit

Search

miRBase has moved to http://www.mirbase.org/ - please update your links.

News - release 14

The miRBase database has moved to a new location at http://www.mirbase.org/, hosted in the Faculty of Life Sciences, University of Manchester. All pre-existing URLs should forward to their new locations. Please update your links, and note the new contact email address (mirbase@manchester.ac.uk).

With release 14, the miRBase sequence database has broken through the 10000 entries barrier!

miRNA count: 10883 entries

Release 14: Sept 2009

Search by miRNA name or keyword

Go | Example

Download published miRNA data

Download page | FTP site

This site is featured in:

NetWatch - *Science* 303:1741 (2004)

Highlights, Web watch - *Nature Reviews Genetics* 5:244 (2004)

miRBase: the microRNA database

miRBase provides the following services:

- The miRBase database is a searchable database of published miRNA sequences and annotation. Each entry in the miRBase Sequence database represents a predicted hairpin portion of a miRNA transcript (termed mir in the database), with information on the location and sequence of the mature miRNA sequence (termed miR). Both hairpin and mature sequences are available for searching and browsing, and entries can also be retrieved by name, keyword, references and annotation. All sequence and annotation data are also available for download.
- The miRBase Registry provides miRNA gene hunters with unique names for novel miRNA genes prior to publication of results. Visit the help pages for more information about the naming service.
- The miRBase Targets database and pipeline has been rebranded as microCosm, and is now hosted at the EBI. The microCosm resource continues to be maintained by the Enright group. miRBase currently links miRNAs to targets predicted by microCosm, TargetScan and Pictar, and aims to provide a more extensive target prediction aggregation service in the future.

To receive email notification of data updates and feature changes please subscribe to the miRBase announcements mailing list. Any queries about the website or naming service should be directed at mirbase@manchester.ac.uk.

miRBase is hosted and maintained in the Faculty of Life Sciences at the University of Manchester with funding from the BBSRC, and was previously hosted and supported by the Wellcome Trust Sanger Institute.

Not secure | www.mirbase.org

miRBase

Rapid evolution

MANCHESTER 1824

Home | Search | Browse | Help | Download | Blog | Submit | submit

Latest miRBase blog posts

MicroRNA Gene Ontology annotations — By sam (June 7, 2018)

You might have noticed some additional information on the mature miRNA pages in the last few weeks. See for example: http://mirbase.org/cgi-bin/mature.pl?mature_acc=MIMAT0000123 http://mirbase.org/cgi-bin/mature.pl?mature_acc=MIMAT0000069 The new section "QuickGO function" contains a set of high quality manual annotations of Gene Ontology terms for mature miRNAs, the vast majority of which come from the work of Rachael Huntley et [...]

miRBase 22 release — By sam (March 12, 2018)

miRNA count: 38589 entries

Release 22.1: October 2018

Search by miRNA name or keyword

Go | Example

In human around 2500 but most of the regulation is done by about 600 miR

miRBase: the microRNA database

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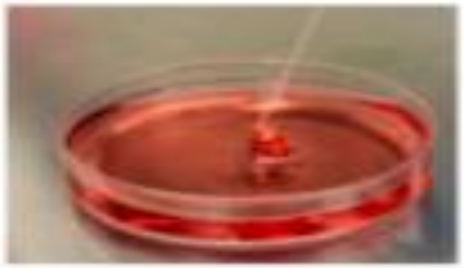
miRBase is managed by the Griffiths-Jones lab at the Faculty of Biology, Medicine and Health, University of Manchester with funding from the BBSRC. miRBase was previously hosted and supported by the Wellcome Trust Sanger Institute.

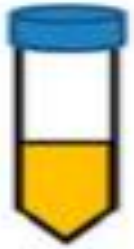
microRNA detection methods

Cancer cell culture



Fresh tumor tissue

FFPE tumor tissue



CSF

mirVana™ miRNA Isolation Kit

miRNA extraction

miRNeasy Mini Kit – Qiagen

mirPremier™ miRNA Isolation Kit

quantification

Bioanalyzer

Profiling

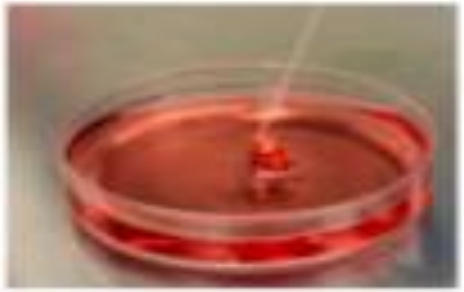
Microarray

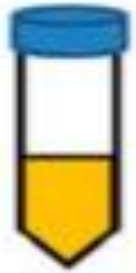
q-RT-PCR

Northern blot

ISH

Sequencing





Cancer cell culture

Fresh tumor tissue

FFPE tumor tissue

CSF

miRNA extraction

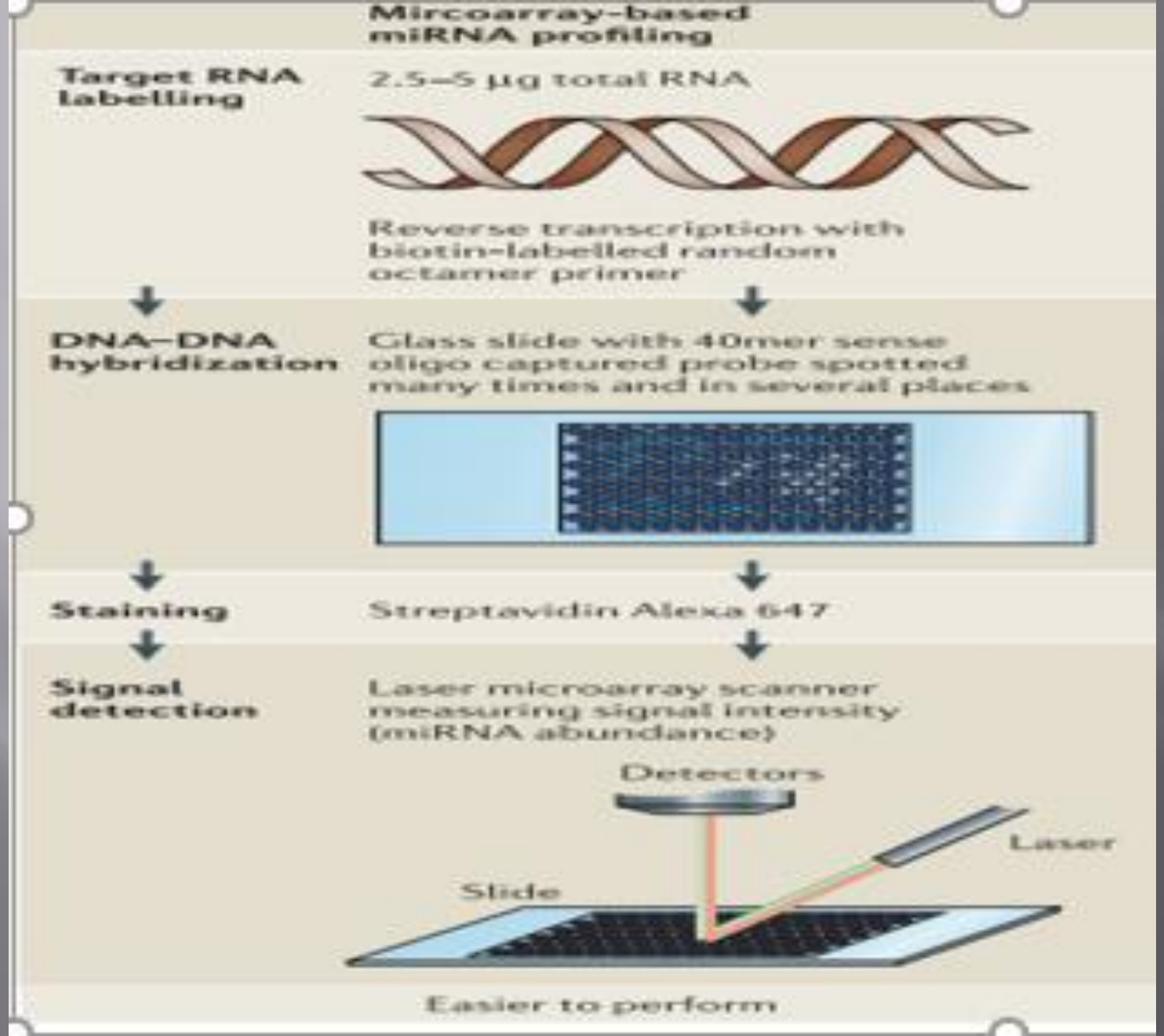
- mirVana™ miRNA Isolation Kit
- Genesid miRNA Isolation Kit
- miRNeasy Mini Kit – Qiagen
- mirPremier™ miRNA Isolation Kit

quantification

- Spectrophotometry
- Bioanalyzer

Profiling

- Microarray
- q-RT-PCR
- Northern blot
- ISH
- Sequencing



Ability to detect Hundreds of miRs

Next-Generation Sequencing

Print Bookmark Share

Maximize the yield of your miRNA sequencing

Our QIAseq solutions are designed to maximize the yield of your miRNA sequencing experiments. The QIAseq miRNA Library Kit virtually eliminates adapter dimerization and unwanted RNA species. The reaction chemistry facilitates preparation of robust, miRNA-specific libraries with a gel-free workflow from as little as 1 ng of total RNA. Plus, Unique Molecular Indices (UMIs) tag each miRNA at an early stage, eliminating PCR and sequencing bias.

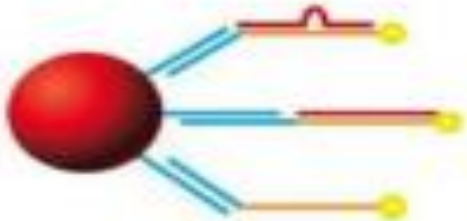
xMAP® INSIGHTS

Luminex® miRNA Analysis Made Easy with a Multiplex miRNA Probe Design Tool

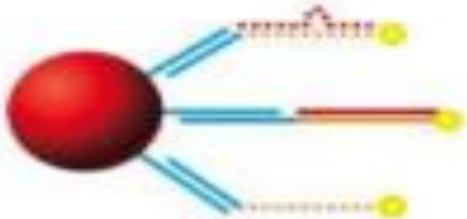
October 12th, 2015 / Stephen Angeloni

Figure 1. Schematic of Luminex miRNA assay.

Step-down Probe Hybridization – DNA/RNA chimeric probes hybridize to target miRNAs during incremental reductions in annealing temperature.
2 hours



Microsphere Hybridization – miRNA-chimeric probe complexes are hybridized to microspheres.
30 minutes



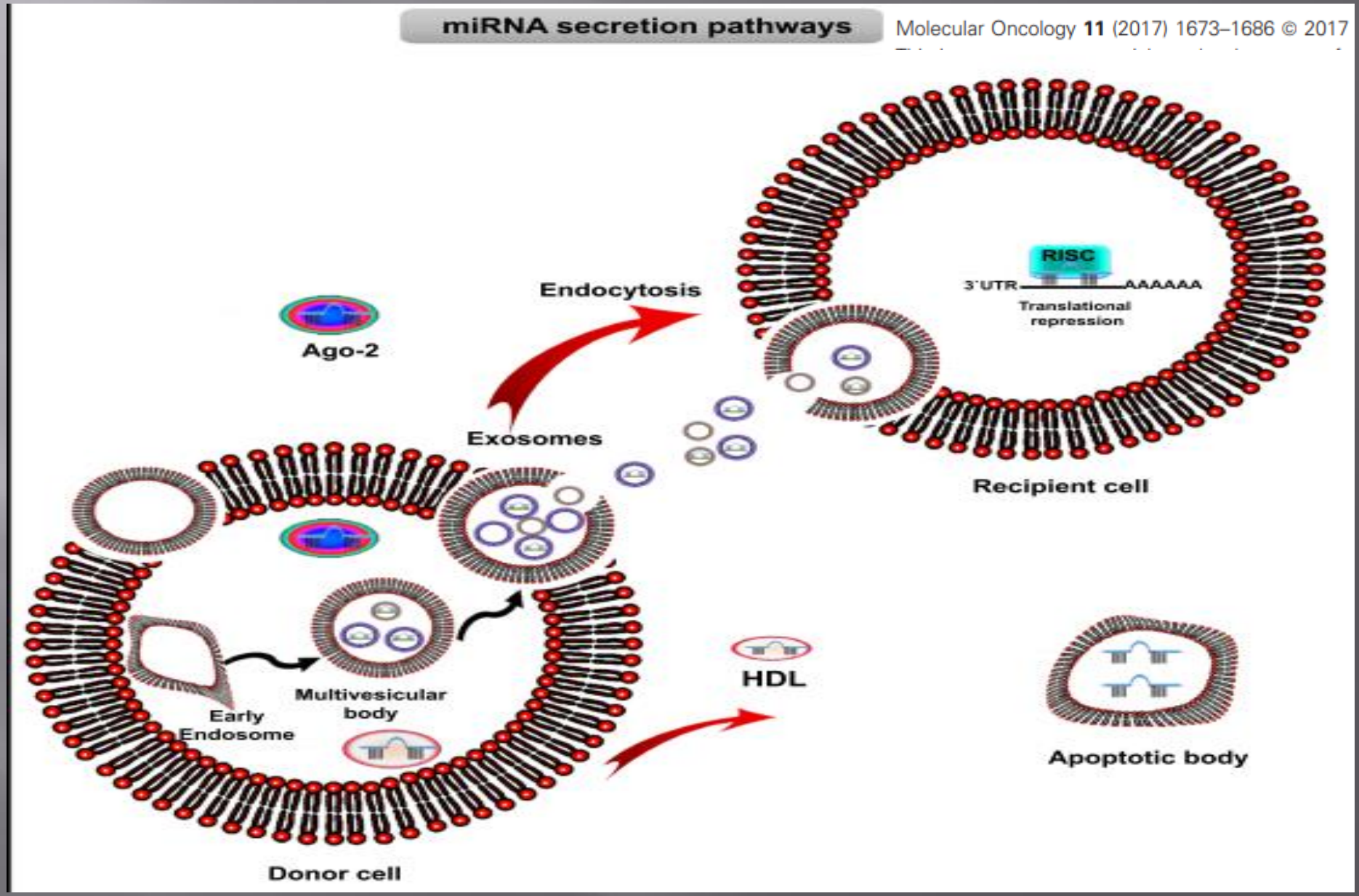
RNase Digestion – Excess probes, single-stranded RNAs and mismatched probes are digested. Only perfectly-matched probes are protected.
30 minutes

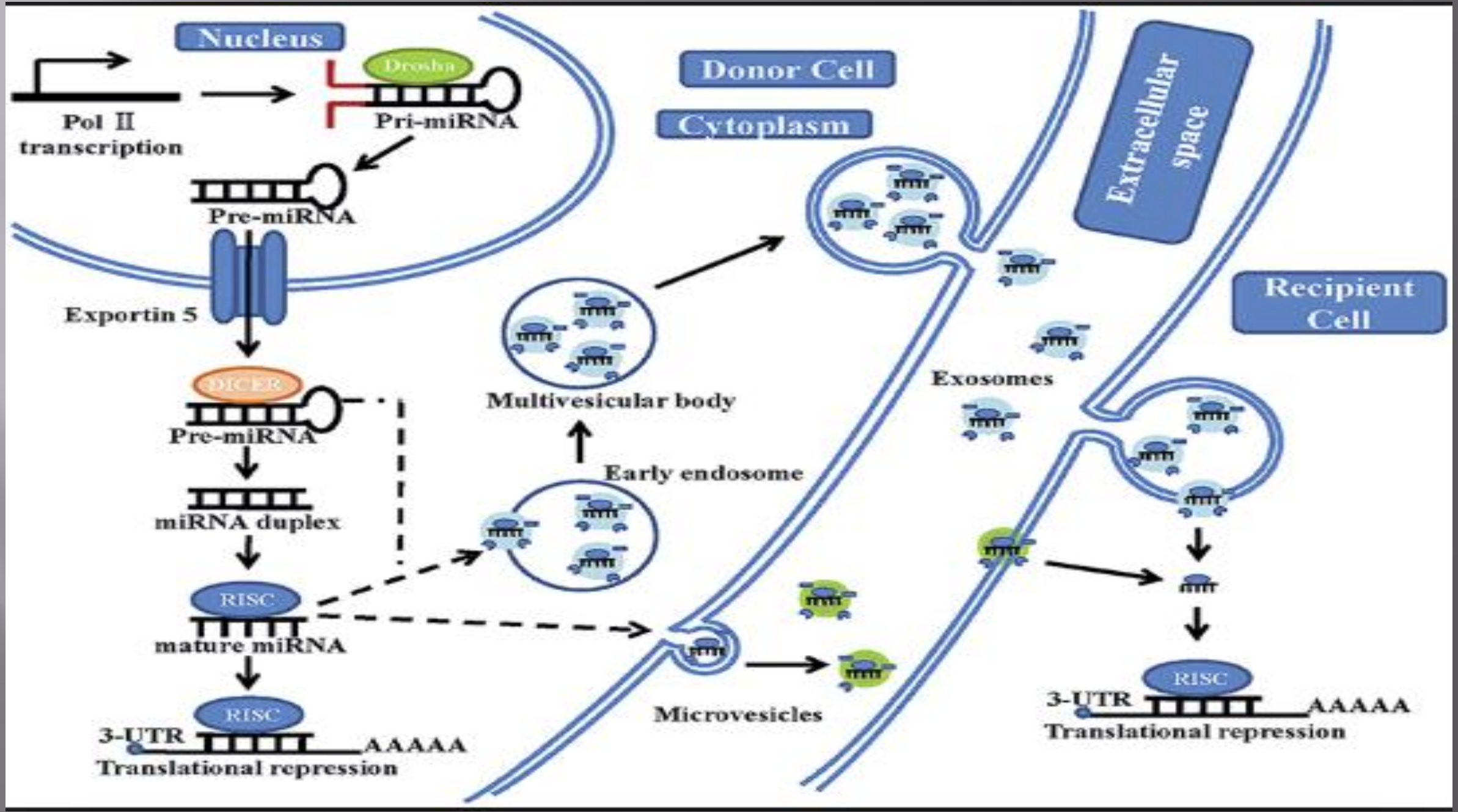
SAPE Incubation – A brief incubation with streptavidin-conjugated R-Phycoerythrin (SAPE) incorporates reporter molecules.
30 minutes

Detection –Targets of interest are quantified on a Luminex instrument.
< 5 hours total to results

Cell-to-cell communication: microRNAs as hormones

Recep Bayraktar[1], Katrien Van Roosbroeck[1] and George A. Calin[1,2,3]




Nucleus
Pol II transcription
Drosha
Pri-miRNA
Pre-miRNA
Exportin 5
DICER
Pre-miRNA
miRNA duplex
RISC
mature miRNA
RISC
3-UTR
AAAAA
Translational repression
Donor Cell
Cytoplasm
Multivesicular body
Early endosome
Microvesicles
Extracellular space
Exosomes
Recipient Cell
RISC
3-UTR
AAAAA
Translational repression

Box 1. The Potential Clinical Applications of miRNAs in Kidney Diseases

Disease diagnosis

- Early detection of cancer (through noninvasive urine and serum test)
- Early detection of glomerular disease and nephropathies (through urine testing)
- Cancer subclassification
- Confirming the tissue of cancer of unknown primary

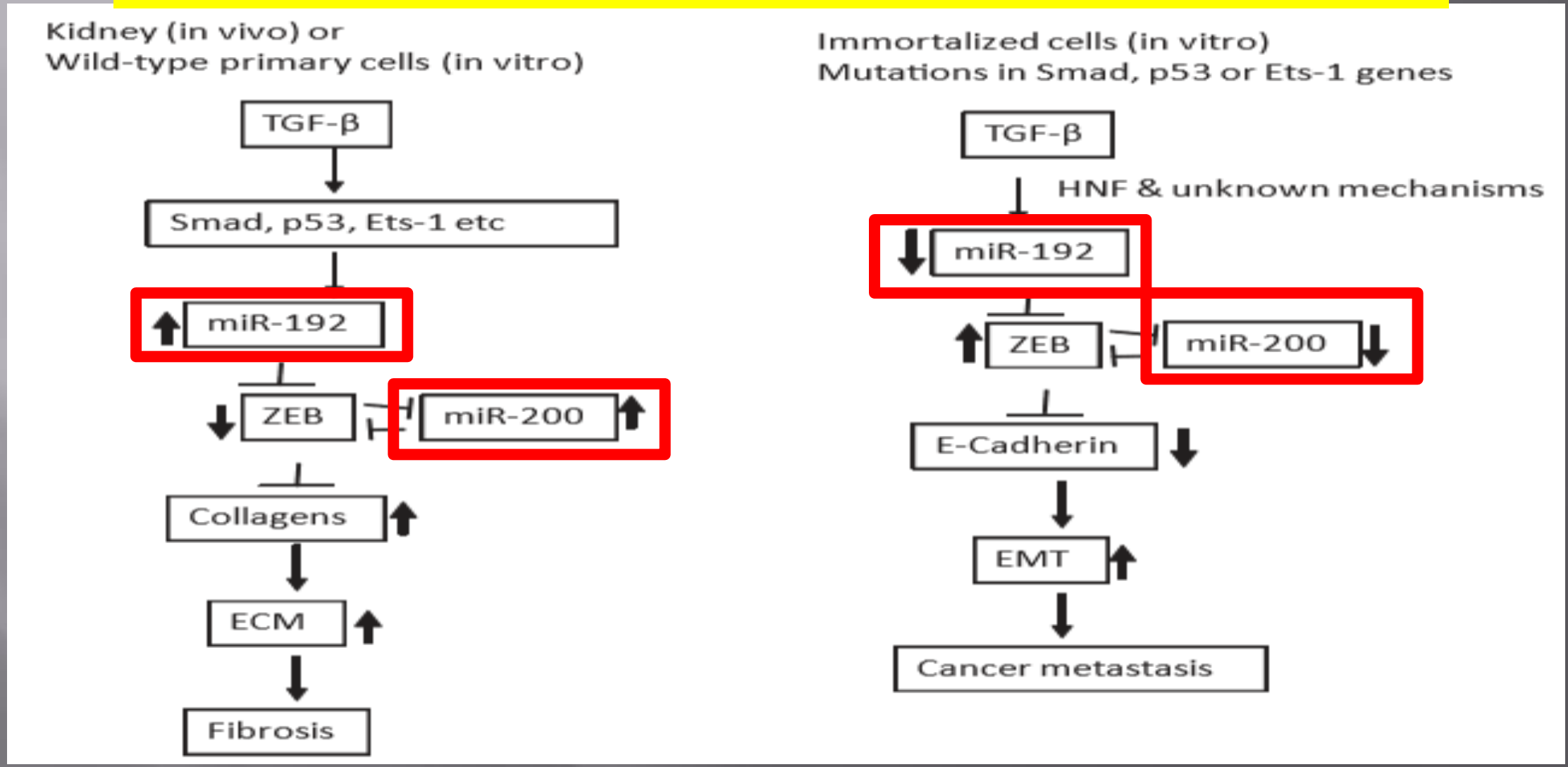
Disease prognostic marker (predicting the natural outcome of the kidney disease and its degree of aggressiveness)

Predictive markers (predicting treatment efficiency, especially for molecular-targeted therapies)

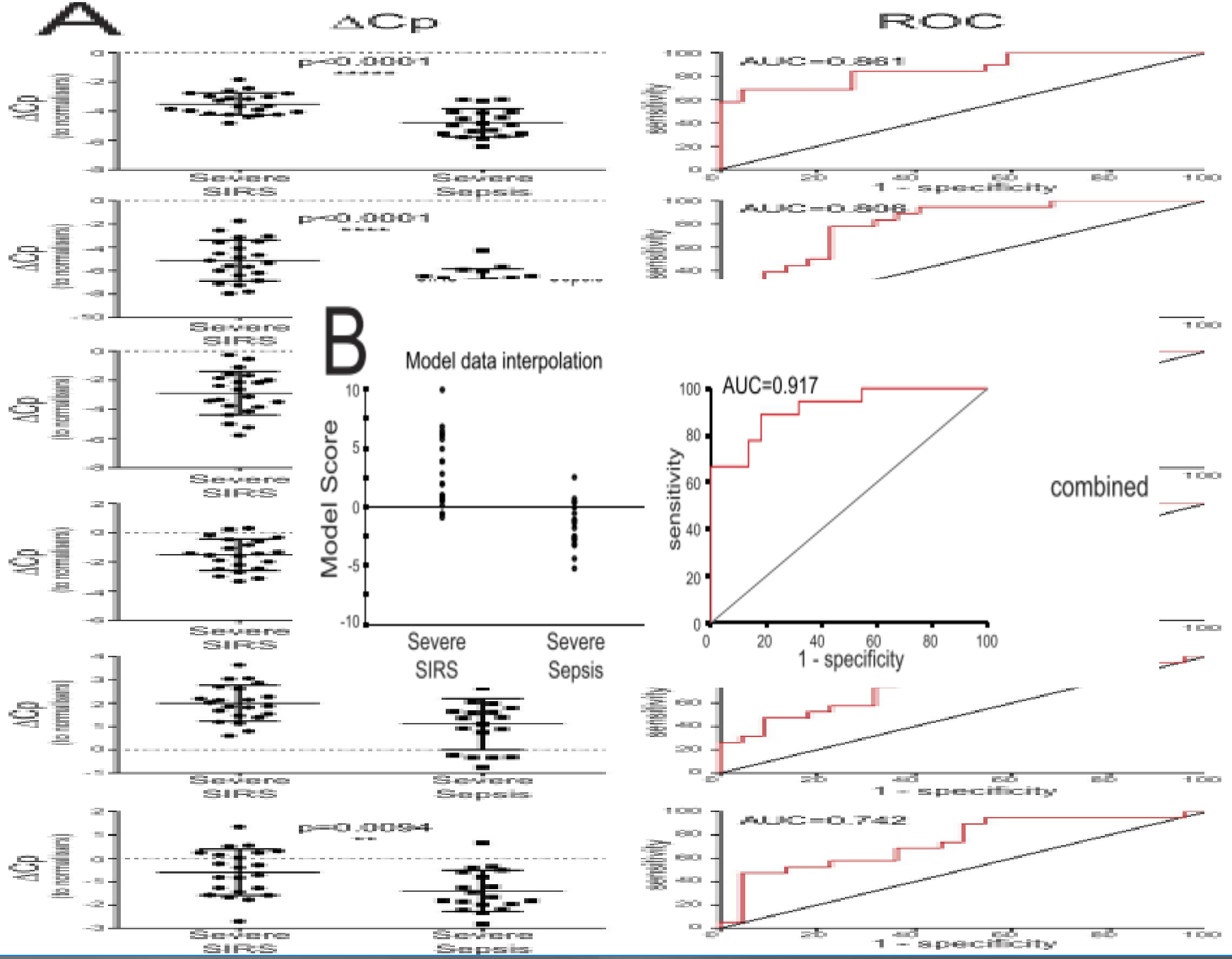
Therapeutic applications

- Overexpression of a downregulated miRNA
- Reducing the expression of an miRNA that is overexpressed in kidney disease
- Arresting disease progression through altering the level of candidate miRNAs
- Selecting patients who are ideal candidates for clinical trials

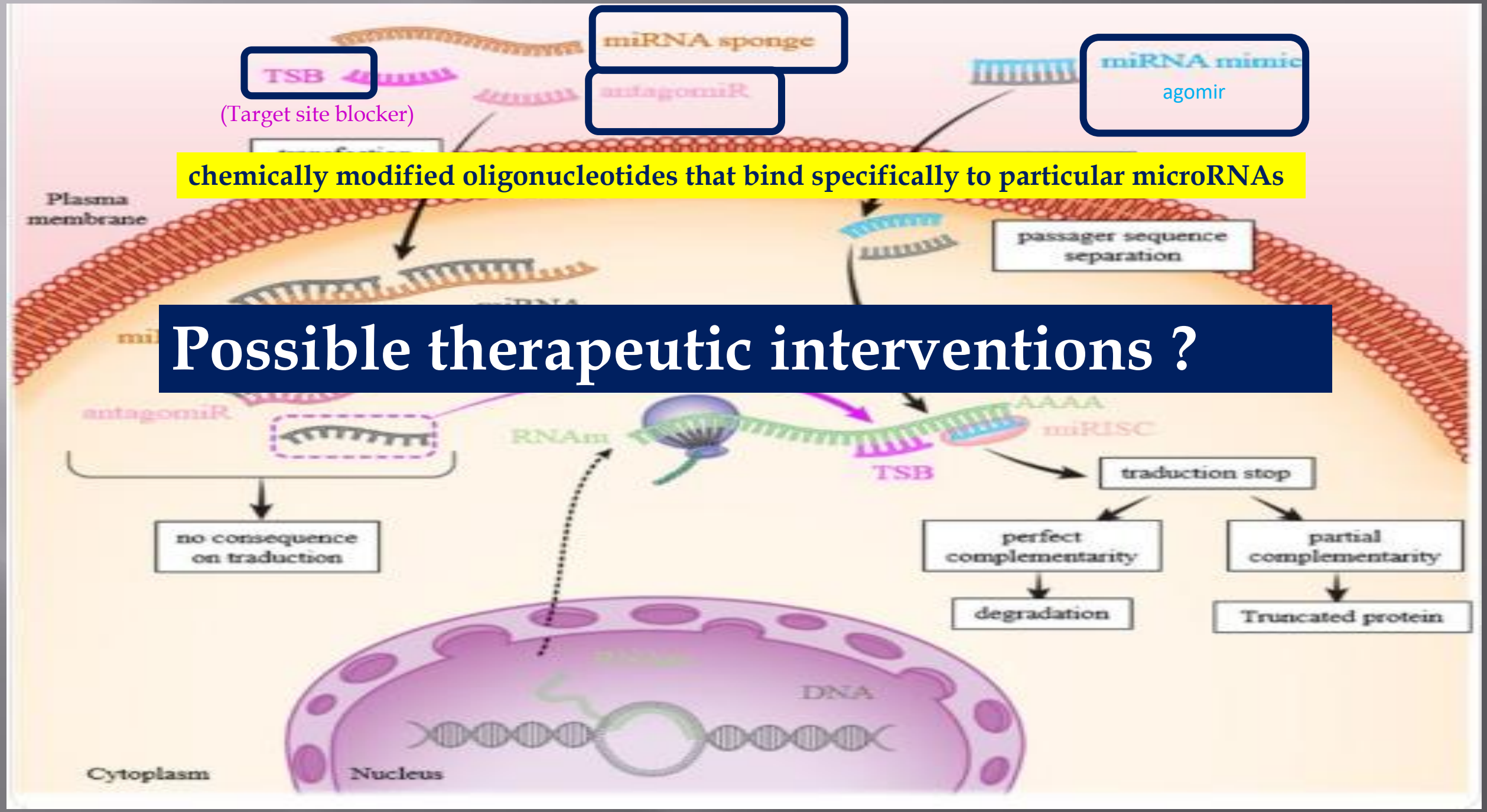
New insights in Differentiating ambiguous situations



Circulating Plasma microRNAs can

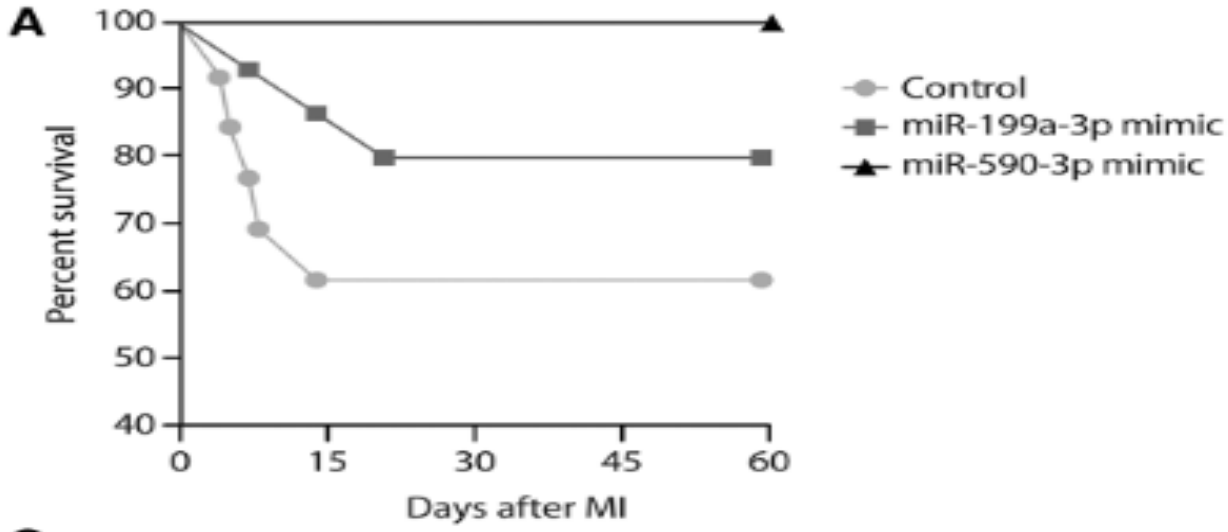


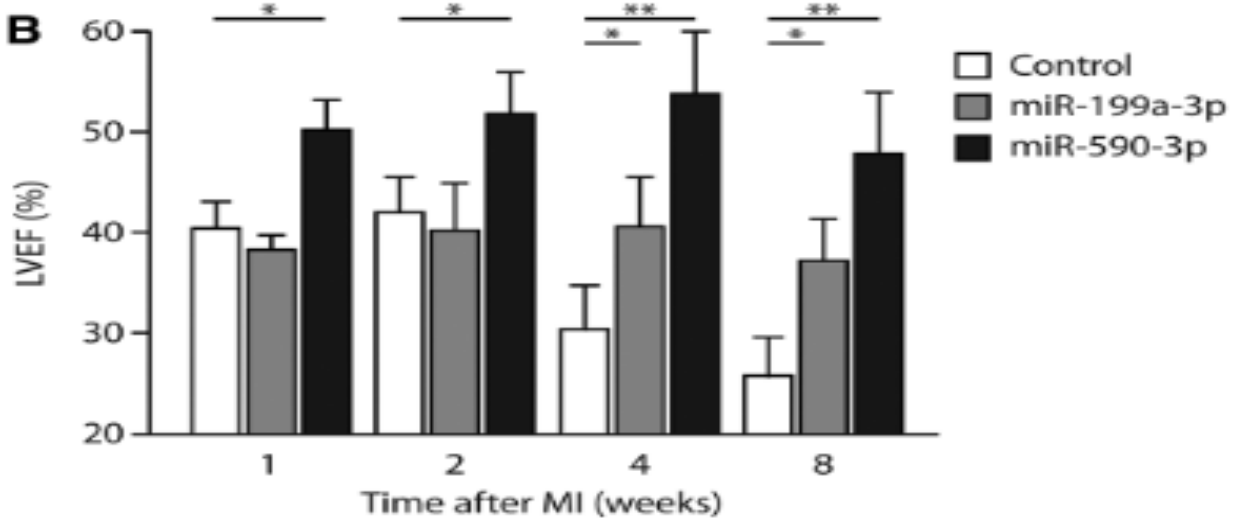
TREATMENT STRATEGIES

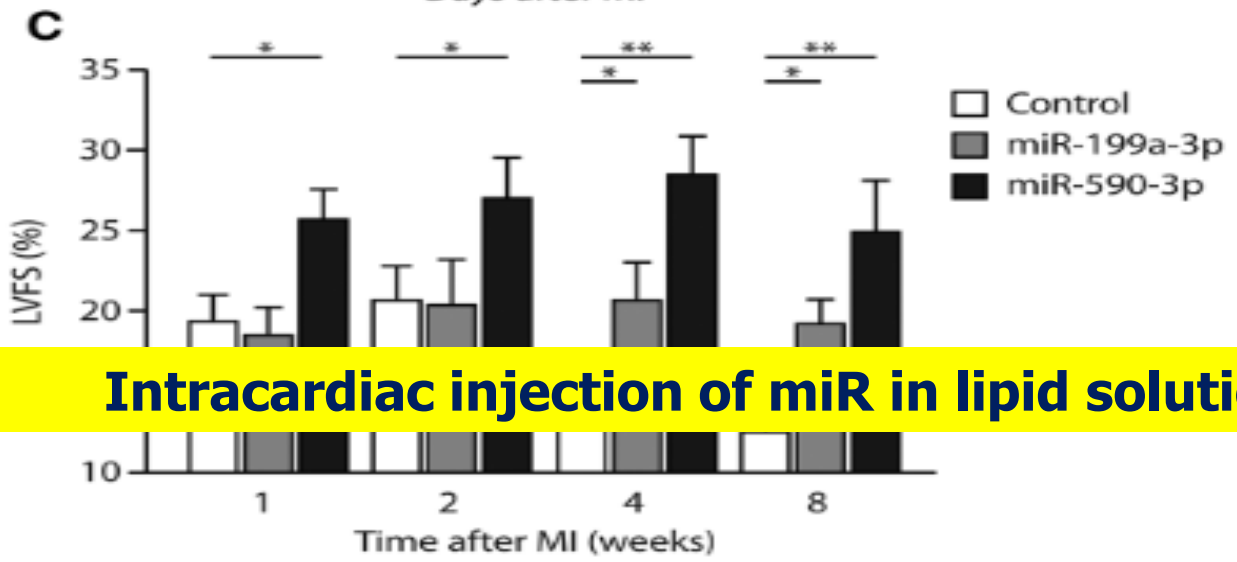

TSB
(Target site blocker)
miRNA sponge
antagomiR
miRNA mimic
agomir
chemically modified oligonucleotides that bind specifically to particular microRNAs
Plasma membrane
passager sequence separation
Possible therapeutic interventions ?
antagomiR
RNAm
AAAA
miRISC
TSB
traduction stop
no consequence on traduction
perfect complementarity
partial complementarity
degradation
Truncated protein
DNA
Cytoplasm
Nucleus

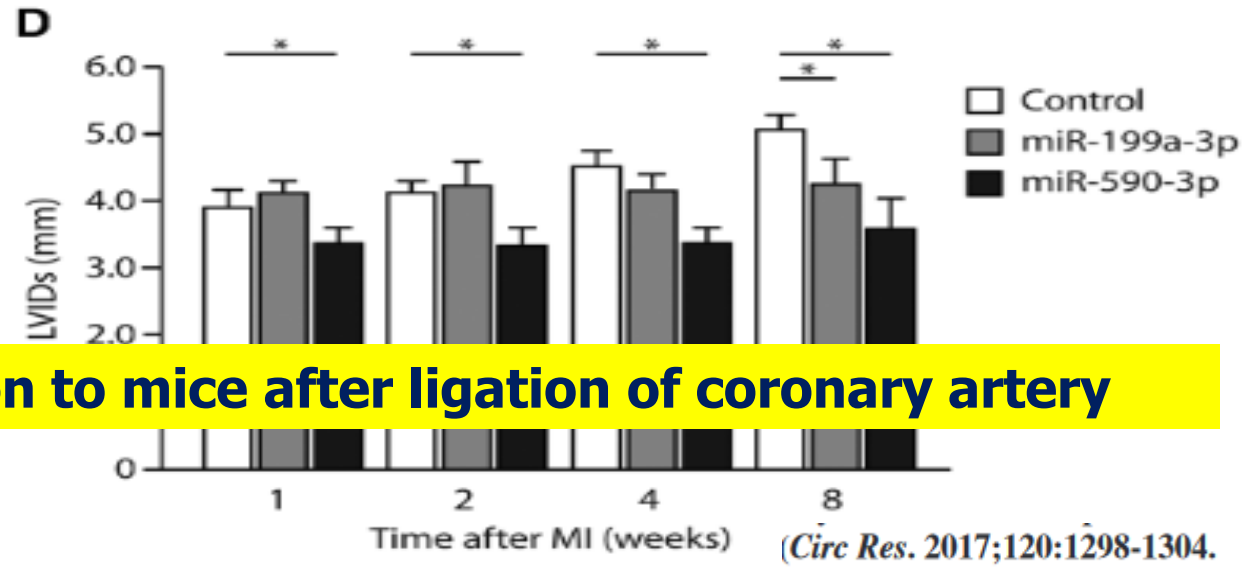
Single-Dose Intracardiac Injection of Pro-Regenerative MicroRNAs Improves Cardiac Function After Myocardial Infarction

Pierluigi Lesizza, Giulia Prosdocimo, Valentina Martinelli, Gianfranco Sinagra, Serena Zacchigna, Mauro Giacca









Intracardiac injection of miR in lipid solution to mice after ligation of coronary artery

(*Circ Res.* 2017;120:1298-1304.

INDICATION (target)	TARGET ORGAN	COMPOUND	PRECLINICAL	PHASE 1	PHASE 2	PHASE 3

Stopped ; 2 cases of cholestasis in HD pts BUT it was mostly a financial pharmaceutical war

INDICATION (target)	TARGET ORGAN	COMPOUND	PRECLINICAL	PHASE 1	PHASE 2	PHASE 3
HCV (miR-122)	Liver	RG-101	GSK Collaboration			
Alport Syndrome – (miR-21) ® *Orphan Disease*	Kidney	RG-012	Partner: Sanofi			
NASH – *Type 2 Diabetes/ Pre-diabetes* (miR-103/107)	Liver	AZD4076 (RG-125)	Partner: AstraZeneca			
Cholestatic Diseases (miR-27) (Multiple Undisclosed Targets)	Liver	RGLS5040				
ADPKD – (miR-17)	Kidney	RGLS4326				
Glioblastoma Multiforme – (miR-10b)	CNS					
NASH – (undisclosed targets)	Liver					
AKI/CKD – (undisclosed targets)	Kidney					

Condition or disease	Intervention/treatment	Phase
Alport Syndrome	Drug: RG-012 Drug: Placebo	Phase 2

Detailed Description:

This is a randomized, double-blind, placebo-controlled, multi-center, Phase 2 study of RG-012 in male subjects with Alport syndrome. Eligible subjects will be randomized in a 1:1 ratio to receive subcutaneous (SC) injections of RG-012 or placebo every other week for 48 weeks. After completion of this double-blind treatment period, subjects will have the opportunity to receive RG-012 in a 48 week open-label extension period.

Male subjects with a confirmed diagnosis of Alport syndrome and a baseline GFR between 40 and 90 mL/min/1.73m2 will be eligible for enrollment. Subjects may enter this study directly or may enroll after participation in the RG012-01 ATHENA Natural History Study.

Subjects may continue treatment with angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs), but must be on a stable dosing regimen for the 30 days prior to screening.

Study Design

Go to

Study Type :	Interventional (Clinical Trial)
Estimated Enrollment :	40 participants
Allocation:	Randomized
Intervention Model:	Parallel Assignment
Masking:	Triple (Participant, Care Provider, Investigator)
Primary Purpose:	Treatment
Official Title:	A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety, Efficacy, Pharmacodynamics, and Pharmacokinetics of RG-012 for Injection Administered Every 2 Weeks in Patients With Alport Syndrome
Actual Study Start Date :	November 7, 2017
Estimated Primary Completion Date :	December 2019
Estimated Study Completion Date :	December 2019

The NEW ENGLAND JOURNAL of MEDICINE

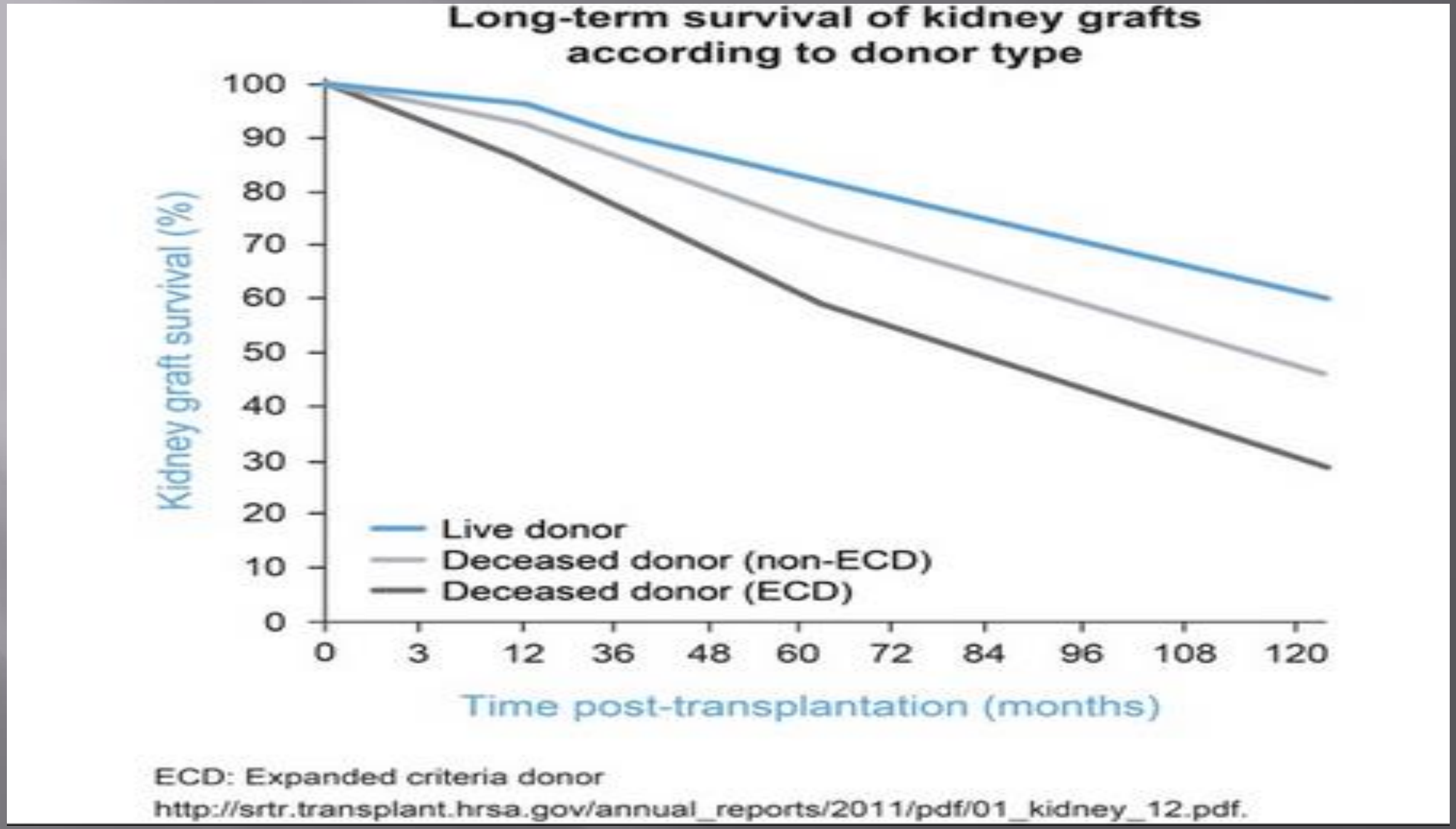
ORIGINAL ARTICLE

Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis

David Adams, M.D., Ph.D., Alejandra Gonzalez-Duarte, M.D., William D. O'Riordan, M.D., Chih-Chao Yang, M.D.,

These are probably our next decade drugs

- microRNA basics
- miR as biomarker in transplantation



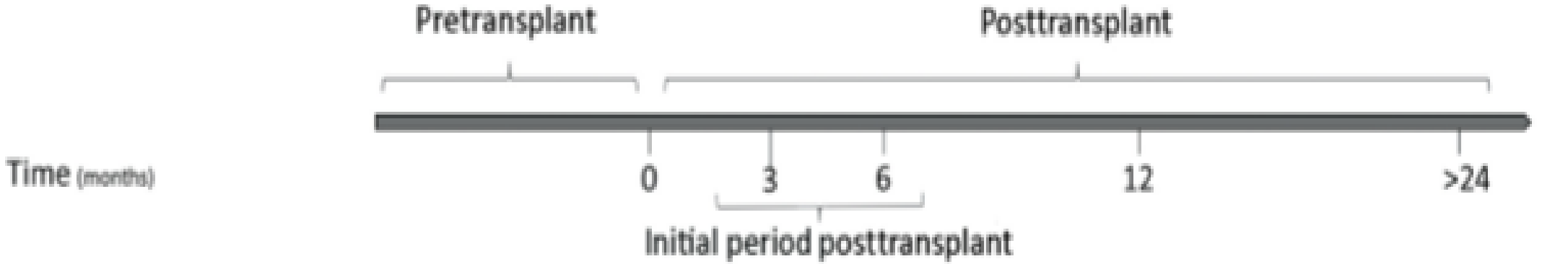
ECD: Expanded criteria donor

http://srtr.transplant.hrsa.gov/annual_reports/2011/pdf/01_kidney_12.pdf.

Strategies for long-term preservation of kidney graft function

Thomas Wekerle, Dorry Segev, Robert Lechler, Rainer Oberbauer

www.thelancet.com Vol 389 May 27, 2017



Assay	d-sp ELISPOT DSA Epitope mismatch	d-sp ELISPOT CXCL9/CXCL10	d-sp ELISPOT DSA CXCL9/CXCL10	DSA CXCL9/CXCL10
Change in management/ Immunosuppression (in case of positive tests)	Depleting antibody Induction		Closer clinical monitoring Surveillance graft biopsy Increase immunosuppressive doses	

CXCL-9/10, Chemokine (C-X-C motif) ligand 9/10; DSA, donor-specific antibodies; d-sp, donor-specific; ELISPOT, enzyme-linked immunosorbent spot.

Figure 6 | Proposed algorithm for tailoring clinical monitoring and immunosuppression based on the results of 6-month donor-specific interferon-γ enzyme-linked immune spot assay (d-sp IFN-γ ELISPOT). CXCL9, chemokine (C-X-C motif) ligand 9; CXCL10, chemokine (C-X-C motif) ligand 10; DSA, donor-specific antibody

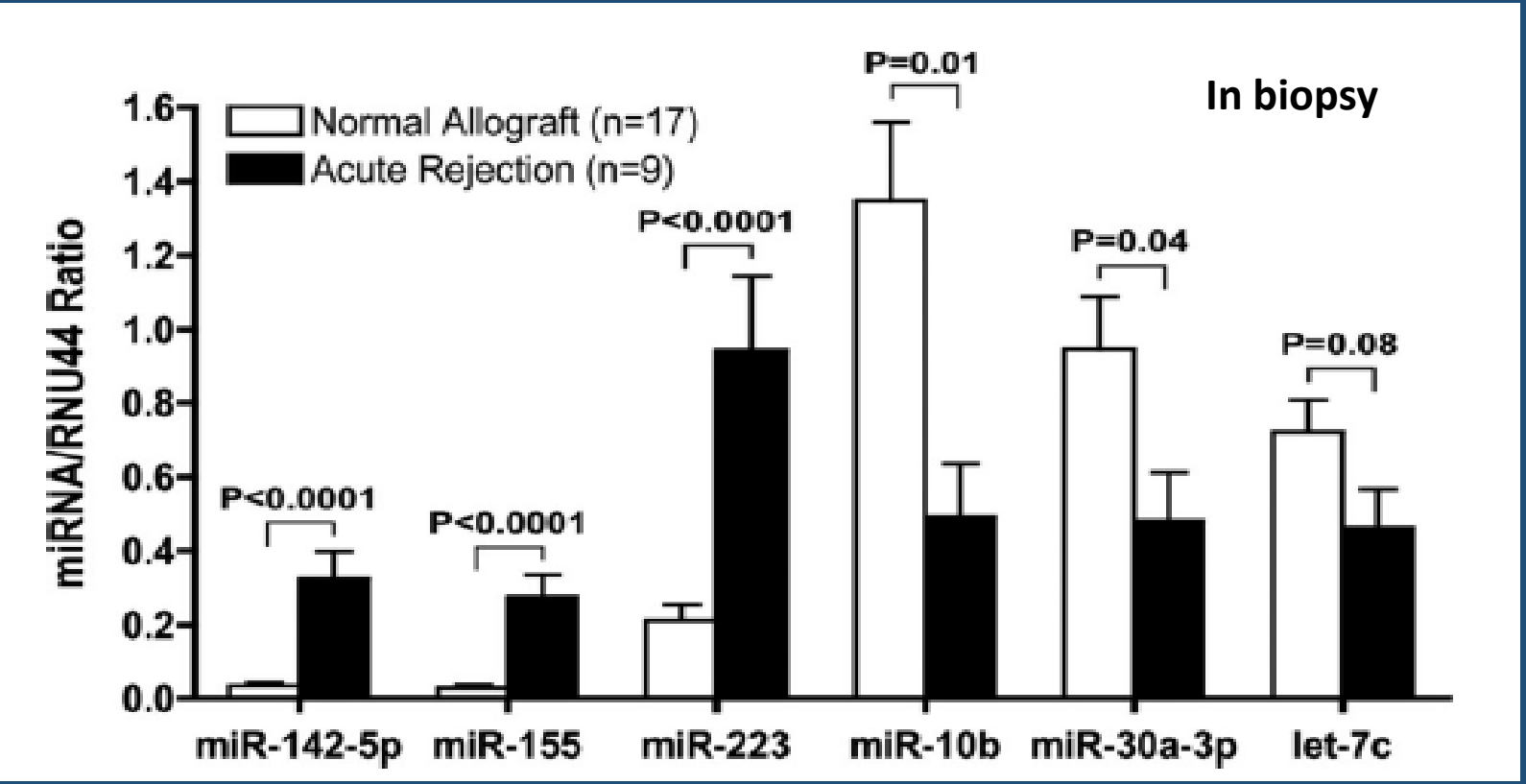
Table 4 Selected promising molecules and pathways evaluated as biomarkers in acute rejection[7]

World J Transplant 2017 June 24; 7(3): 161-178

Biomarker	Sample (assay method)	Patients/ samples	Rejection/no rejection	Sensitivity/ specificity (%)	PPV/NPV(%)	AUC
Granzyme B, perforin and FasL[74]	PBL (PCR)	25/31	11/20	100/95	100/95	NA
FOXP3[88]	PBL, urine (PCR)	65/78	20/58	94-100/ 95/100	94-100/ 95/100	0.95-1.00
Granzyme B, perforin[75]	Urine (PCR)	85/151	24/127	79-83/77-83	NA	NA
OX40, OX40L, PD-1 and FOXP3[76]	Urine (PCR)	46/46	21/25	95/92	NA	0.98
CD3ε,CXCL10, 18S rRNA[77]	Urine (PCR)	485/4300	43/1,70	79/78 (71/72)	NA	0.85 (0.74)
TIM-3[81]	PBL, urine (PCR)	115/160	65/95	87-100/95-100	87-100/93-100	0.96-1.00
CXCL9, CXCL10[78]	Urine (multiplex bead assay)	156/156	25/131	80-86/76-80	NA	0.83-0.87
CXCL9 mRNA and protein[79]	PBL, urine (PCR, ELISA, SELDI-TOF-MS	280/2770	37/113	66.7-85.2/ 79.6/80.7	61.5/67.6/83-92	0.78-0.85
miR-142-5p miR-155 miR-223[83]	Biopsy sample (PCR)	32/33	12/21	92-100/90-95	NA	0.96-0.99
miR-210[85]	Urine (PCR)	81/88	68/20	52/74	NA	0.7
IFNγ-producing memory T cells[...]	PBL (ELISPOT)	23/23	12/10	80/83	NA	0.8

MicroRNA expression profiles predictive of human renal allograft status

Dany Anglicheau[a,b,c], Vijay K. Sharma[a], Ruchuang Ding[a], Aurélie Hummel[a], Catherine Snopkowski[a], Darshana Dadhania[a,d], Surya V. Seshan[e], and Manikkam Suthanthiran[a,d,1]



Acute cellular rejection
Out of 365 different microRNAs

Differential expression of microRNAs in renal transplant patients with acute T-cell mediated rejection☆

Transpl Immunol. 2015 Sep;33(1):1-6

Ehsan Soltaninejad [a,b], Mohammad Hossein Nicknam [b,c], Mohsen Nafar [d], Pedram Ahmadpoor [d], Fatemeh Pourrezagholi [d], Mohammad Hossein Sharbafi [b], Morteza Hosseinzadeh [b], Farshad Foroughi [c], Mir Saeed Yekaninejad [e], Tayyeb Bahrami [c], Ehsan Sharif-Paghaleh [b], Aliakbar Amirzargar [b,c,*]

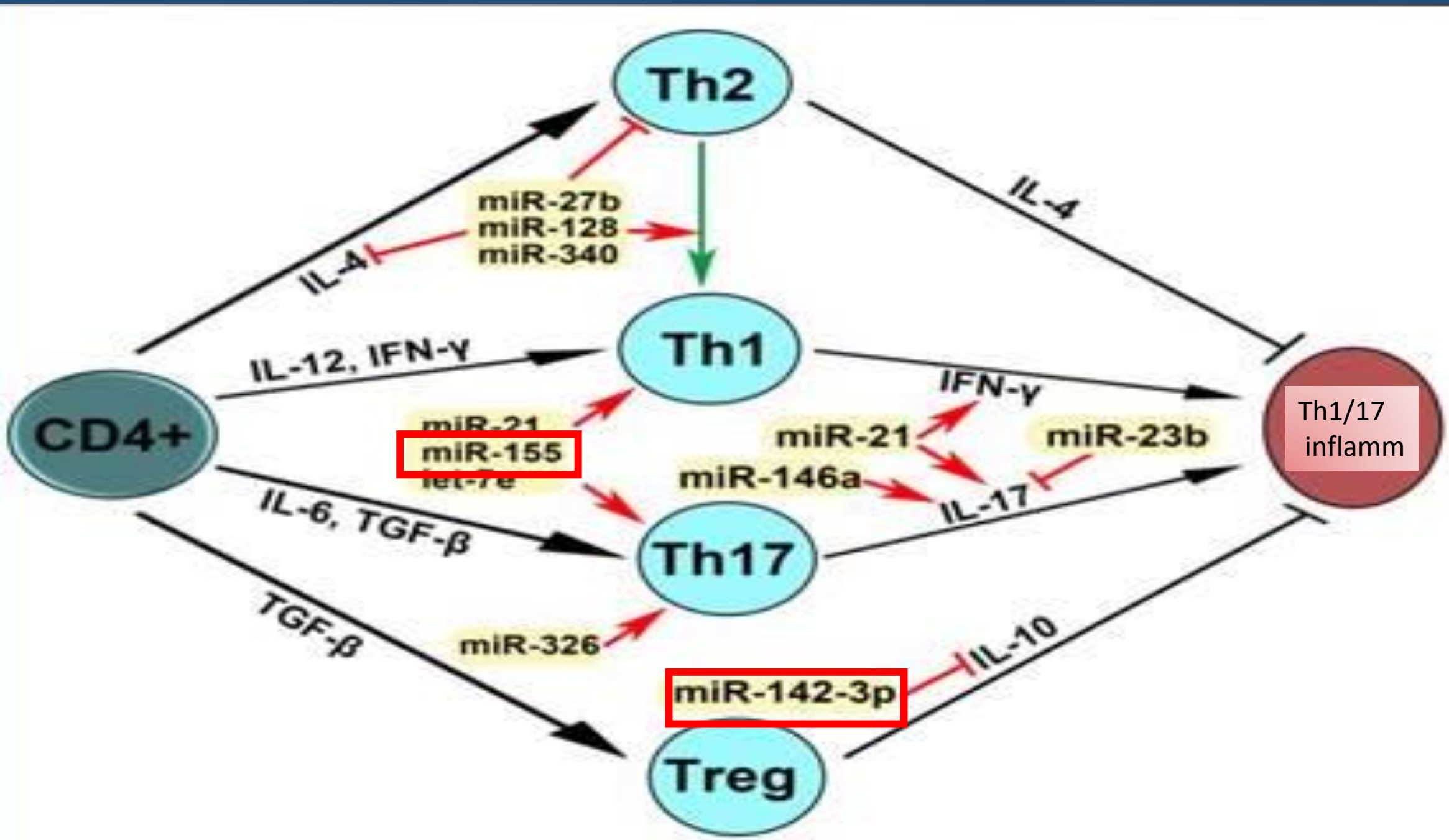
Table 2

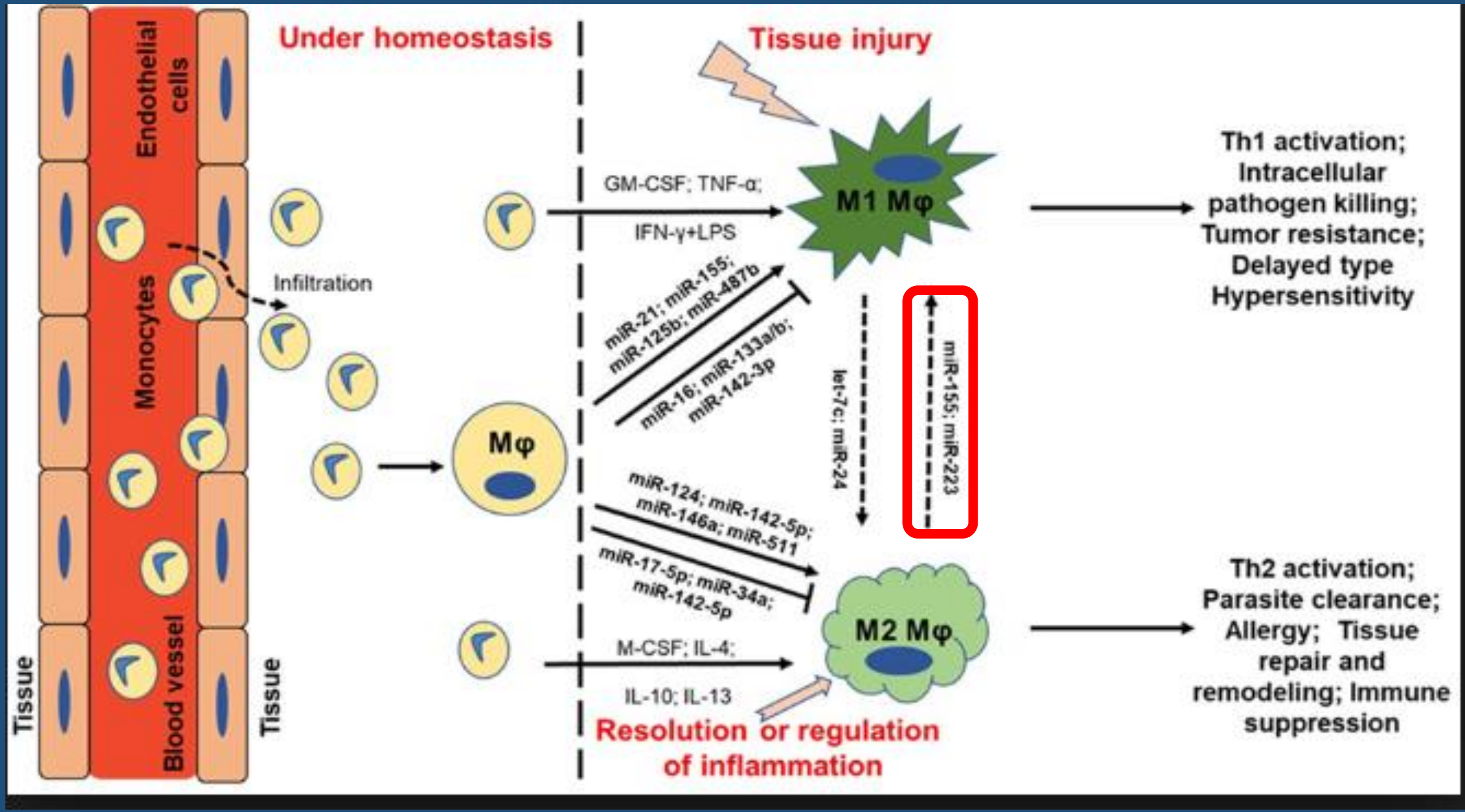
Expression levels of individual miRNA measured in biopsy and PBMC samples.

		Normal allograft FC[a] median (IQR[b])	Acute T-cell mediated rejection FC median (IQR)	P-value
Biopsy	miR-142-5p			
	miR-142-3p			
	miR-155			
	miR-223			
PBMCs	miR-142-5p			
	miR-142-3p			
	miR-155			
	miR-223			

But also in peripheral blood

[a] Fold change.
[b] Interquartile range.


Th2
IL-4
miR-27b
miR-128
miR-340
IL-4
IL-12, IFN-γ
Th1
IFN-γ
CD4+
miR-21
miR-155
miR-21
miR-23b
miR-146a
IL-17
IL-6, TGF-β
Th17
Th1/17
inflamm
TGF-β
miR-326
miR-142-3p
IL-10
Treg


Under homeostasis
Tissue injury
Endothelial cells
Monocytes
Blood vessel
Tissue
Tissue
Infiltration
Mφ
GM-CSF; TNF-α;
IFN-γ+LPS
M1 Mφ
Th1 activation; Intracellular pathogen killing; Tumor resistance; Delayed type Hypersensitivity
miR-21; miR-155; miR-125b; miR-487b
miR-16; miR-133a/b; miR-142-3p
let-7c; miR-24
miR-155; miR-223
miR-124; miR-142-5p; miR-146a; miR-511
miR-17-5p; miR-34a; miR-142-5p
M-CSF; IL-4;
IL-10; IL-13
M2 Mφ
Th2 activation; Parasite clearance; Allergy; Tissue repair and remodeling; Immune suppression
Resolution or regulation of inflammation

Differential expression of microRNAs in renal transplant patients with acute T-cell mediated rejection☆

Transpl Immunol. 2015 Sep;33(1):1-6

Ehsan Soltaninejad [a,b], Mohammad Hossein Nicknam [b,c], Mohsen Nafar [d], Pedram Ahmadpoor [d], Fatemeh Pourrezagholi [d], Mohammad Hossein Sharbafi [b], Morteza Hosseinzadeh [b], Farshad Foroughi [c], Mir Saeed Yekaninejad [e], Tayyeb Bahrami [c], Ehsan Sharif-Paghaleh [b], Aliakbar Amirzargar [b,c,*]

Table 2

Expression levels of individual miRNA measured in biopsy and PBMC samples.

		Normal allograft FC[a] median (IQR[b])	Acute T-cell mediated rejection FC median (IQR)	P-value
Biopsy	miR-142-5p	0.971 (0.801–1.779)	6.958 (6.635–7.867)	<0.0001
	miR-142-3p	1.123 (0.398–2.760)	4.906 (3.249–8.183)	<0.0001
	miR-155	1.103 (0.657–2.072)	5.013 (4.171–6.505)	<0.0001
	miR-223	0.961 (0.675–1.359)	3.115 (2.443–4.794)	0.001
PBMCs	miR-142-5p	1.100 (0.884–1.630)	1.554 (1.367–1.734)	0.112
	miR-142-3p	0.820 (0.660–1.870)	2.161 (1.396–3.231)	0.023
	miR-155	1.105 (0.948–1.654)	1.658 (1.467–1.992)	0.059
	miR-223	1.049 (0.694–1.565)	2.441 (1.527–3.706)	0.003

[a] Fold change.

[b] Interquartile range.

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[a] Fold change.
[b] Interquartile range.

PBMCs	miR-142-5p	1.364	80	70	0.71 (0.46-0.96)	0.112
	miR-142-3p	0.955	100	65	0.80 (0.59-1.00)	0.023
	miR-155	1.246	100	62	0.75 (0.52-0.98)	0.059
	miR-223	1.273	100	76	0.89 (0.75-1.00)	0.003

ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
December 2015; 14(6):615-623.

Altered Expression of MicroRNAs Following Chronic Allograft Dysfunction with Interstitial Fibrosis and Tubular Atrophy

Ehsan Soltaninejad[1,2], Mohammad Hossein Nicknam[2,3], Mohsen Nafar[4,5], Mohammad Hossein Sharbafi[2], Sanaz Keshavarz Shahbaz[2], Mehri Barabadi[2], Mir Saeed Yekaninejad[6], Tayyeb Bahrami[7], Pedram Ahmadpoor[4], and Aliakbar Amirzargar[2,3]

Table 1. Demographic and clinical characteristics of subjects

Topics	Normal allogra
Number of patients	17
Male (n;%)	11
Female (n;%)	6
Age (years; min-max)	52 (34-67)
Types of allografts	
Deceased	
Living	
Types of donors	
Related	
Unrelated	
Recipient CMV*	
CMV disease	
Donor CMV(pos)	3
Donor HCV(pos)	0
Donor HBS Ag (pos)	0
Donor HBC Ab (pos)	2
Donor HIV(pos)	0
Blood creatinine level (mg/dl)	1.23±0.20
Date of biopsy (months post-transplant)	14.11±3.87
	3.44±1.30
	56.37±22.97

No IF/TA on protocol bx

The exclusion criteria:
BK virus infection
histopathological evidence of CNI nephrotoxity
urinary tract obstruction
second transplantation

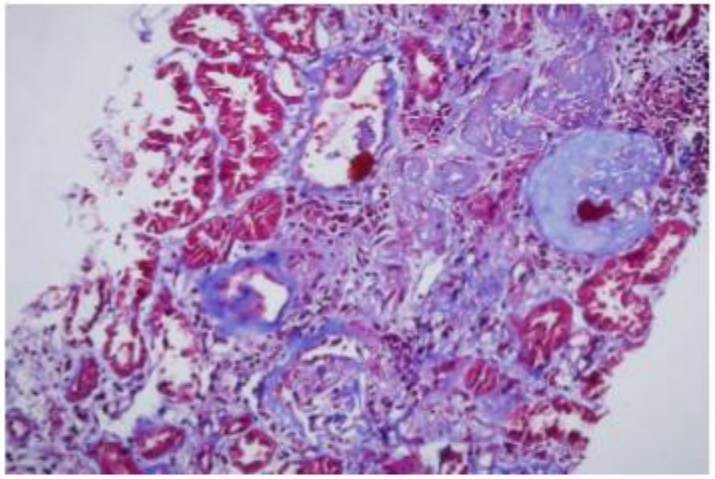
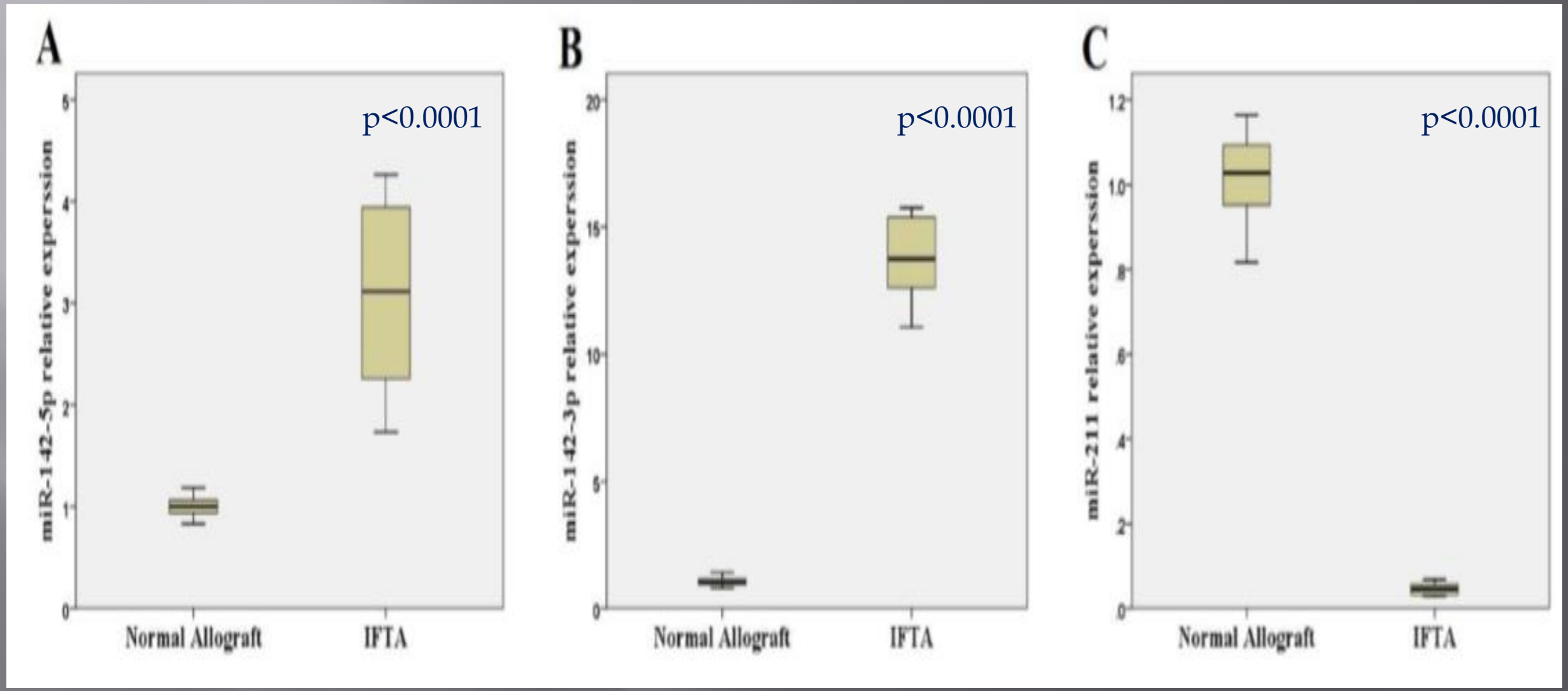


Figure 1. Interstitial fibrosis and tubular atrophy (IFTA) which previously known as chronic allograft nephropathy. This figure is an example of Grade III based on Banff IFTA grade which is characterized by a severe interstitial fibrosis. In this trichrome stain blue colored areas are fibrotic regions (collagen deposition). Grade II are defined as moderate interstitial fibrosis and tubular atrophy.

Expression in allograft biopsy



Taqman® MicroRNA Assays

Table 3. ROC curve analysis of miRNAs in biopsy and PBMC samples

Sample	miRNAs	Cutoff point	Sensitivity	1 - Specificity	AUC[a] (95% CI[b])	*P* value
Biopsy	miR-142-5p	1.45	1.00	0	1.00 (1.00-1.00)	<0.0001*
	miR-142-3p	6.29	1.00	0	1.00 (1.00-1.00)	<0.0001*
	miR-211	0.44	1.00	0	1.00 (1.00-1.00)	<0.0001*
PBMC	miR-142-5p	1.17	0.50	0.11	0.64 (0.44-0.83)	0.171
	miR-142-3p	1.54	0.93	0	0.99 (0.97-1.00)	<0.0001*
	miR-211	0.66	1.00	0	1.00 (1.00-1.00)	<0.0001*

MicroRNAs in Acute Kidney Injury and Kidney Transplantation

Kristien J. Ledeganck,[1] Els M. Gielis,[1] Daniel Abramowicz,[1,2] Peter Stenvinkel,[3] Paul G. Shiels,[4] and Amaryllis H. Van Craenenbroeck[1,2,3]

CJASN Clinical Journal of the American Society of Nephrology

www.cjasn.org Vol 14 February, 2019

Phenotype	Study	Study Population	Sample	miR	Internal Validation	Overlap with Other Studies: Upregulation	Overlap with Other Studies: Downregulation
Acute T cell–mediated rejection (Banff I–II)	Wilflingseder *et al.* (19)	T-cell mediated rejection ($n=30$) normal PBX ($n=10$)	Biopsy	↑ 4 miRs ↓ 18 miRs	—	↑ miR-155 (24–26) ↑ miR-150–3p (27)	↓ miR-125a (24,27) ↓ miR-27b (24) ↓ miR-193b (24) ↓ miR-181a (29) ↓ miR-23b–3p (27) ↓ miR-99b–5p (27)
Acute rejection	Oghumu *et al.* (27)	AR (heterogeneous) ($n=5$) normal TxBX ($n=4$)	Biopsy	↑ 13 miRs ↓ 16 miRs	—	↑miR-142–3p (24,25,29) ↑ miR-223–3p (24–26) ↑ miR-342–3p (24,29) ↑ miR-150–5p (19)	↓ miR-30c–5p (24,26) ↓ miR-125a–5p (19,24) ↓ miR-204–3p (24,29) ↓ miR-23b–3p (19) ↓ miR-30a–5p (24) ↓ miR-30d–5p (24) ↓ miR-99b–5p (19) ↓ miR-99a–5p (24) ↓ miR-100–5p (24) ↓ miR-125b–5p (24) ↓ miR-126–3p (24) ↓ miR-130a–3p (24)
Acute rejection	Liu *et al.* (26)	AR (n.o.s) ($n=15$) normal TxBX ($n=15$)	Biopsy	75 Differentially expressed miRs (fold changes not reported)	—	↑ miR-155 (19,24,25) ↑ miR-223 (24,25,27) ↑ miR-21 (24) ↑ miR-125a (28) ↑ miR-146a (24) ↑ miR-602 (28) ↑ miR-628 (28) ↑ miR-629 (28) ↑ miR-650 (24)	↓ miR-30c (24,27) ↓ miR-10b (24) ↓ miR-17–3p (28) ↓ miR-30a–3p (24) ↓ miR-32 (24) ↓ miR-330 (28) ↓ miR-483 (28) ↓ miR-611 (28) ↓ miR-663 (28)
Acute T cell–mediated rejection	Blijlevens *et al.* (34)	Discovery cohort: stable Tx (clinical) ($n=4$) Validation cohort: stable Tx (clinical) ($n=13$) T cell–mediated rejection ($n=13$)	Plasma	Not all differentially expressed miRs reported	↑ miR-17 ↑ miR-140–3p ↑ miR-130b ↑ miR-122 ↑ miR-192 ↓ miR-135a ↓ miR-199a–3p ↓ miR-15a		
Acute T cell–mediated rejection	Vitalone *et al.* (29)	T cell–mediated rejection ($n=29$) normal TxBX ($n=68$)	Biopsy	↑ 3 miRs miR-142–3p miR-342–3p miR-25 ↓ 6 miRs miR-181a miR-192 miR-204 miR-215 miR-106b–3p miR-615–3p	—	↑miR-142–3p (24,25,27) ↑ miR-342–3p (24,27)	↓ miR-204 (24,27) ↓ miR 181a (19)
Acute T cell–mediated rejection (Banff I)	Soltanirejad *et al.* (25)	T cell–mediated rejection ($n=17$) normal TxBX ($n=18$)	Biopsy	↑ 4 miR miR-142–5p miR-155 miR-142–3p miR-223	—	↑ miR-155 (19,24,26) ↑miR-142–3p (24,27,29) ↑ miR-223 (24,26,27) ↑ miR-142–5p (24)	—

MicroRNAs in Acute Kidney Injury and Kidney Transplantation

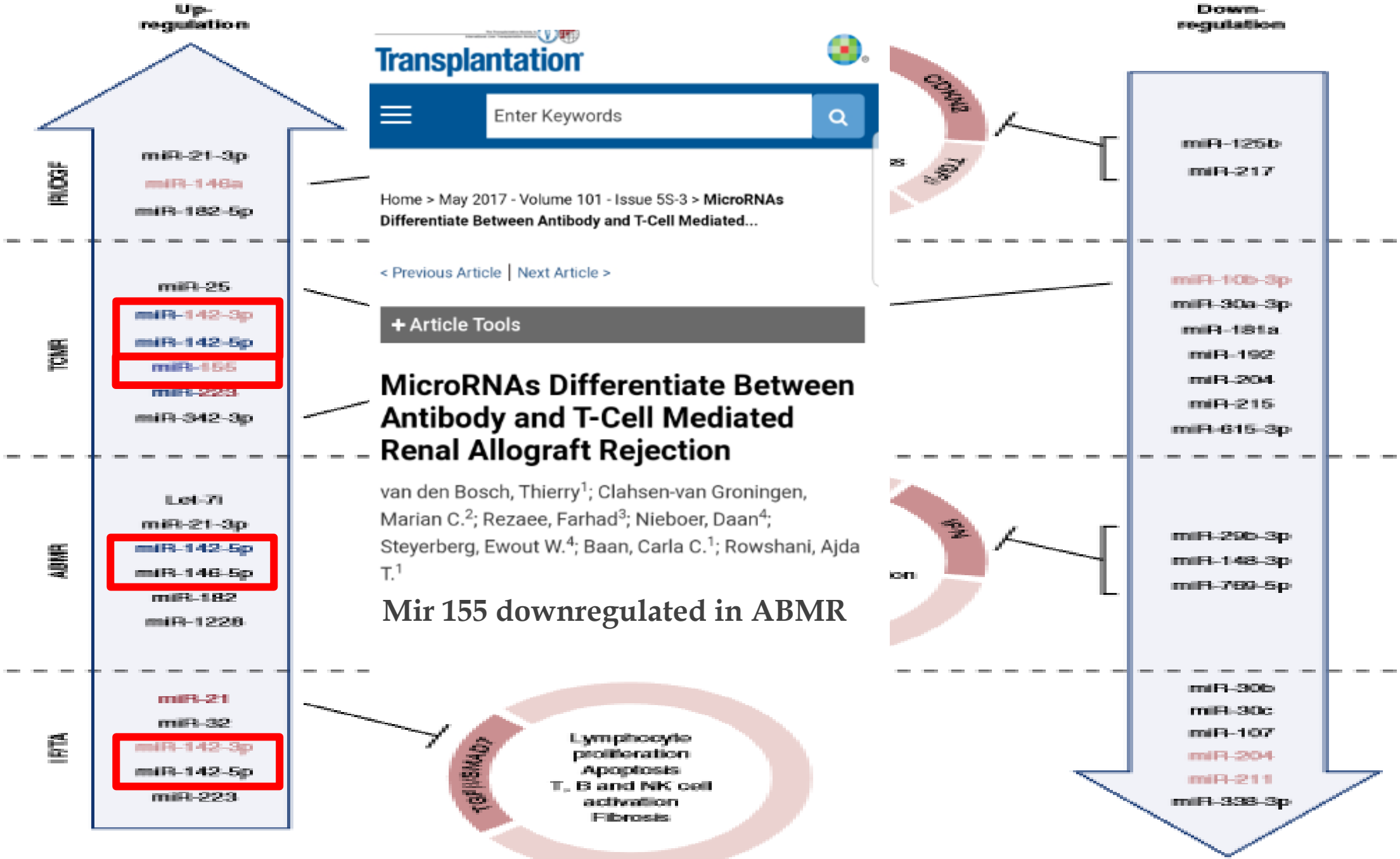
Kristien J. Ledeganck,[1] Els M. Gielis,[1] Daniel Abramowicz,[1,2] Peter Stenvinkel,[3] Paul G. Shiels,[4] and Amaryllis H. Van Craenenbroeck[1,2,3]

www.cjasn.org Vol 14 February, 2019

Table 2. (Continued)

Phenotype	Study	Study Population	Sample	miR	Internal Validation	Overlap with Other Studies: Upregulation	Overlap with Other Studies: Downregulation
Acute antibody-mediated rejection	Wilflingseder *et al.* (19)	Morphologic antibody-mediated rejection (n=11) normal PBX (n=10)	Biopsy	↑ 6 miRs	—	—	—
Chronic antibody-mediated rejection	Danger *et al.* (30)	Chronic antibody-mediated rejection (n=18) Stable Tx (clinical) (n=30) AR (heterogeneous) (n=9) Chronic antibody-	PBMC biopsy	Not all differentially expressed miRs reported ↑ miR-142-5p	↑ miR-142-5p	—	—
						—	—
IF/TA	Sciarra *et al.* (34)	AR (heterogeneous) (n=5) Discovery cohort: IF/TA (n=13) normal PBX (n=5) Validation cohort: IF/TA (n=19) normal PBX (n=8)	Biopsy	↓ 1 miR 56 Differentially expressed miRs (fold changes not reported)	↑ miR-142-3p ↑ miR-32 ↓ miR-204 ↓ miR-107 ↓ miR-211	↑ miR-142-3p (32,33)	↓ miR-211 (33)
IF/TA	Ben-Dov *et al.* (32)	Discovery cohort: n=4 IF/TA n=4 normal PBX Validation cohort: n=10 IF/TA n=8 normal PBX	Biopsy	↑ 28 miRs ↓ 7 miRs	↑ miR-142-3p ↑ miR-142-5p ↑ miR-21-3p ↑ miR-21-3p ↑ miR-223 ↓ miR-30b ↓miR-30c ↓ miR-338-3p	↑ miR-142-3p (33,34) ↑ miR-21-3p (35) ↑ miR-142-3p (33)	—
IF/TA	Glowacki *et al.* (35)	Severe graft fibrosis (explant) (n=11) nonpathologic parenchyma of urologic cancer (kidney/urinary tract) (n=12)	Biopsy	↑ miR-21	—	↑ miR-21 (32)	—
IF/TA	Soltaninejad *et al.* (33)	IF/TA (n=16) Normal TxBX (n=17)	Biopsy	↑ miR-142-3p ↑ miR-142-5p ↓ miR-211	—	↑ miR-142-3p (32,34) ↑ miR-142-5p (32)	—
Ischemia/reperfusion injury	Amrouche *et al.* (21)	LD (n=16) DD (n=35)	Urine pellet	↑ miR-146a	—	—	—

Although AUC of 1 or close to 1 is quite interesting but it is not clinically relevant



MicroRNAs Differentiate Between Antibody and T-Cell Mediated Renal Allograft Rejection

van den Bosch, Thierry[1]; Clahsen-van Groningen, Marian C.[2]; Rezaee, Farhad[3]; Nieboer, Daan[4]; Steyerberg, Ewout W.[4]; Baan, Carla C.[1]; Rowshani, Ajda T.[1]

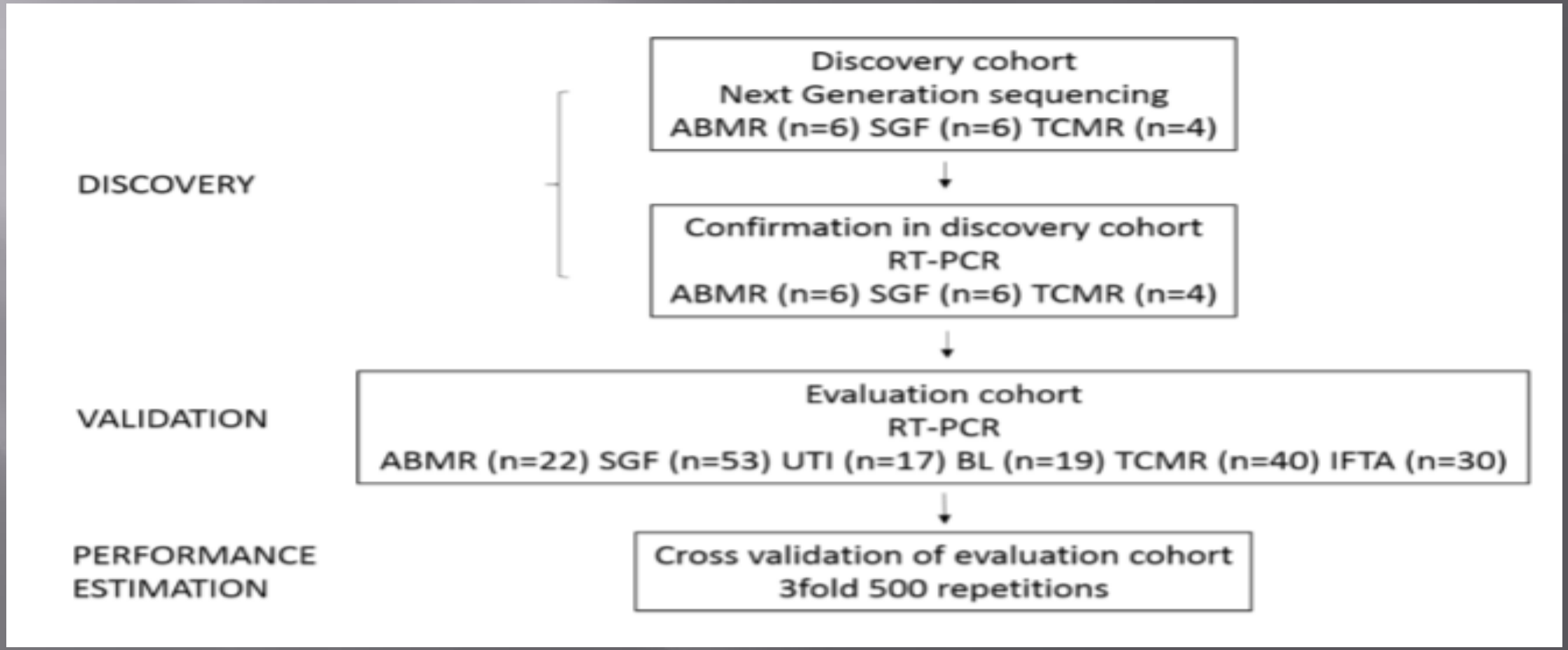
Mir 155 downregulated in ABMR

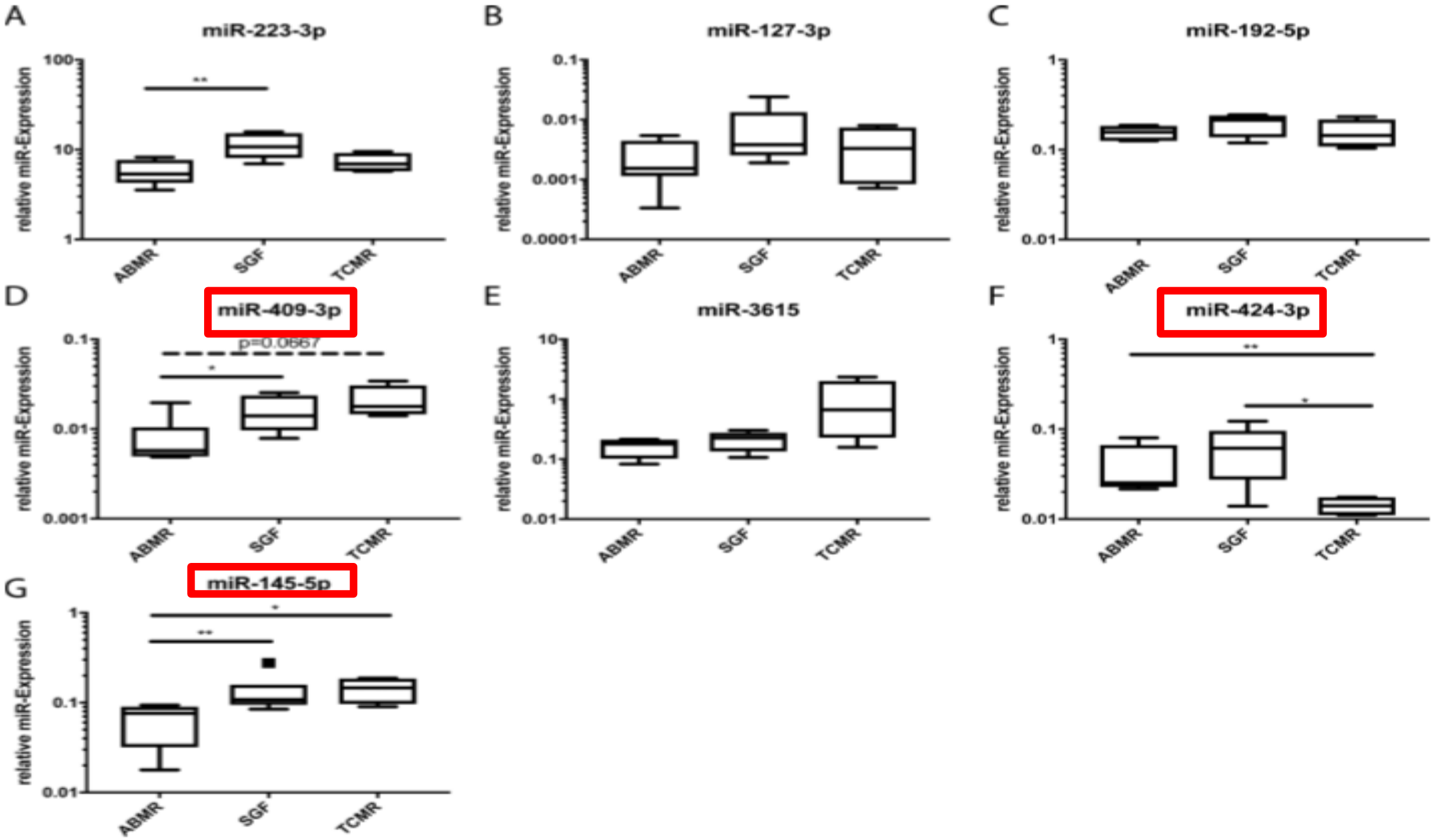
RESEARCH ARTICLE

MicroRNA regulation in blood cells of renal transplanted patients with interstitial fibrosis/ tubular atrophy and antibody-mediated rejection

PLOS ONE | https://doi.org/10.1371/journal.pone.0201925 August 13, 2018

Mareen Matz[1]*, Frederik Heinrich[2], Christine Lorkowski[1], Kaiyin Wu[3], Jens Klotsche[2], Qiang Zhang[1], Nils Lachmann[4], Pawel Durek[2], Klemens Budde[1☯], Mir-Farzin Mashreghi[2☯]




A
miR-223-3p
relative miR-Expression
100
10
1
**
ABMR
SGF
TCMR
B
miR-127-3p
relative miR-Expression
0.1
0.01
0.001
0.0001
ABMR
SGF
TCMR
C
miR-192-5p
relative miR-Expression
1
0.1
0.01
ABMR
SGF
TCMR
D
miR-409-3p
p=0.0667
*
relative miR-Expression
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0.01
0.001
ABMR
SGF
TCMR
E
miR-3615
relative miR-Expression
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0.01
ABMR
SGF
TCMR
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miR-424-3p
**
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relative miR-Expression
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G
miR-145-5p
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relative miR-Expression
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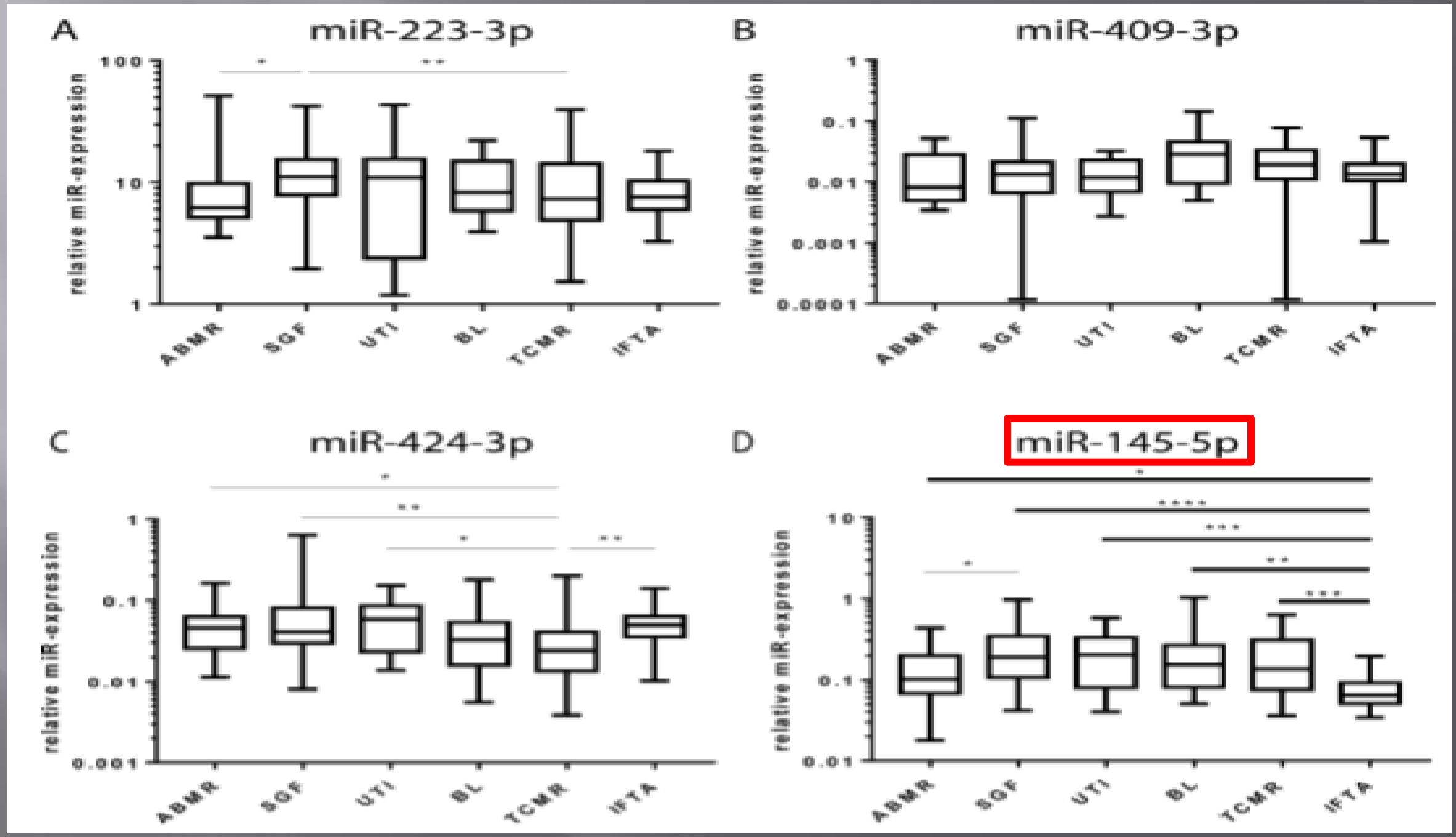
SGF
TCMR


A
miR-223-3p
relative miR-expression
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UTI
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miR-145-5p
relative miR-expression
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0.1
0.01
ABMR
SQF
UTI
BL
TCMR
IFTA

conclusion

- Microrna; A rapidly evolving player

- Probably our next decade game changer in
  DIAGNOSIS,
  PREDICTION,
  PROGNOSIS &
  TREATMENT


Thanks for your attention
CHU
NIMES
CHU de Nîmes
SOINS - ENSEIGNEMENT - RECHERCHE

Table 1. Diagnostic performance of miR-145-5p expression for IFTA.

A	cuttoff	AUC (95% CI)	Youden Index	Sensitivity* (95% CI)	Specificity* (95% CI)	LR+* (95% CI)	LR-* (95% CI)
IFTA vs SGF	0.111	0.891 (0.821–0.961)	0.664	0.933 (0.779–0.992)	0.731 (0.595–0.844)	3.467 (2.193–5.480)	0.091 (0.024–0.353)
IFTA vs all other	0.111	0.835 (0.773–0.896)	0.600	0.933 (0.779–0.992)	0.667 (0.597–0.731)	2.800 (2.252–3.481)	0.100 (0.026–0.383)

YOUDEN index:

To account for the effect of a Limit Of Detection

J = 1: there are no false positives or false negatives, i.e. the test is perfect.

J= VALIDITY OF A LAB TEST= J ranges from –1 to 1

perfect, 1;

excellent 0.9-1;

good 0.8-0.9;

moderate 0.6-0.8;

Fair 0.4-0.6;

Slight 0.2-0.4;

useless 0-0.2.

A negative value indicates an invalid test.

	Mircoarray-based miRNA profiling	Bead-based miRNA profiling
Target RNA labelling	2.5–5 µg total RNA  Reverse transcription with biotin-labelled random octamer primer	0.5–10 µg small RNAs fractionated on PAA gels  Adaptor ligation, then reverse transcription with adaptor complementary primer and PCR with a biotin-labelled primer
DNA–DNA hybridization	Glass slide with 40mer sense oligo captured probe spotted many times and in several places 	Polystyrene beads labelled with antisense oligo probes in a 96-well microtitre plate 
Staining	Streptavidin Alexa 647	Streptavidin-phycoerythrin (SAPE)
Signal detection	Laser microarray scanner measuring signal intensity (miRNA abundance) 	Flow cytometer measuring bead colour (miRNA identity) and SAPE intensity (miRNA abundance) 
	Easier to perform ?	Higher specificity