UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI FISIOPATOLOGIA MEDICO-CHIRURGICA E DEI TRAPIANTI

FONDAZIONE IRCCS CA' GRANDA
OSPEDALE MAGGIORE POLICLINICO
Sistema Sanitario Regione Lombardia

55° Congresso AINPeNC Associazione Italiana
Neuropatologia e Neurobiologia Clinica

45° Congresso AIRIC Associazione Italiana
Ricerca Invecchiamento Cerebrale

Bologna, 23-25 Maggio 2019

PLASMA DERIVED EXOSOMAL MICRORNAS PROFILE: NEW POTENTIAL BIOMARKER FOR ALZHEIMER'S DISEASE (AD) DIAGNOSIS

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BIOMARKERS FOR AD

Many proposed, but only few accurate

- Structural imaging (MRI)
- Functional imaging (PET-SPECT)
- Cerebrospinal fluid biomarkers (β-amyloid, total tau and p-tau)
- Genetics (PSEN1, PSEN2, APP, APOE)

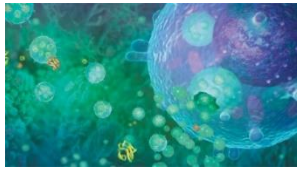
PERIPHERAL BIOMARKERS

- Cytokines
- Chemokines
- Receptors
- Growth factors
- Adhesion molecules
- Amyloid beta

Proteomics-based peripheral biomarkers of AD patients

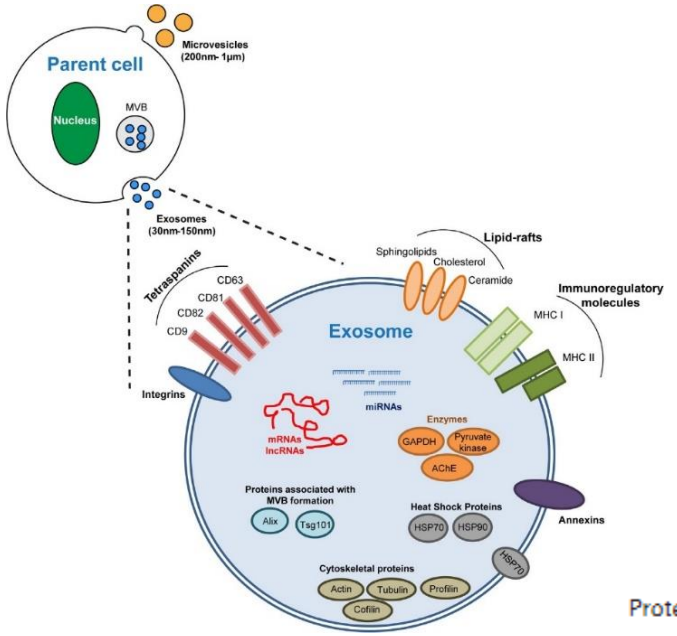
Protein identified	Method of detection	Comments	References
All ↓ in AD: TNF-α; PDGF-BB; M-CSF; G-CSF; CCL5; CCL7; CCL15; EGF; GDNF; IL-1α; IL-3	Proteomics antibody array. Patient groups: Non-AD dementia ($n=11$); AD ($n=86$); MCI ($n=47$); Control ($n=21$); Rheumatoid arthritis ($n=16$)	Immune-responsive analytes and cytokines. High accuracy for detecting AD.	[41]
All ↑ in AD: Ang-2; ICAM-1; CCL18; CXCL8; IGFBP-6; IL-11; Trail-R4			
All ↓ in AD: APOC3; TTR; ICAM-1; RANTES; Cystatin.	Flow cytometry-based immunoassay.	One of the largest multi-center validation studies. Predicted conversion of MCI to AD with an accuracy of 87%.	[44]
All ↑ in AD: PEDF; CC4; A1AcidG; Clusterin	Patient groups: AD ($n=476$); Control ($n=452$); MCI ($n=220$)		
All ↓ in AD: IL-17; EGFPR	Multiplex immunoassay. Patient groups: AD ($n=207$); Control ($n=754$)	Fold change between AD and control groups was not high.	[47]
All ↑ in AD: Insulin-like growth factor binding protein 2; pancreatic polypeptide; Ang-2; Cortisol; Beta-2-microglobulin			
Clusterin ↑ in AD	LC-MS Total subjects ($n=744$)	Has a role in atrophy in AD pathogenesis. Significantly ($p<0.001$) associated with the rate of progression of AD.	[45]
All ↓ in AD: Creatine MB; G-CSF; S-100B; IL-10; IL-1ra; Prostatic acid phosphatase; C-reactive protein; TNF-α; Stem cell factor; MIP1α.	Multiplex Immunoassay Patient groups: AD ($n=197$); Control ($n=203$)	Specific algorithm in data analysis provided high specificity and sensitivity.	
All ↑ in AD: Thromboprotein; Alpha-2-macroglobulin; Tenascin; TNF-β; Beta-2-microglobulin; Eotaxin; Pancreatic polypeptide; von Willebrand factor; IL-15; VCAM-1; IL-8; IGFBP2; Fas ligand; Prolactin Resistin.			[46]
Aβ ↓ in AD	$A\beta_{1-40}$ and $A\beta_{1-42}$ by immunoassay	No significant difference between AD and controls	[20]

Khan and Alkon, JAD, 2015

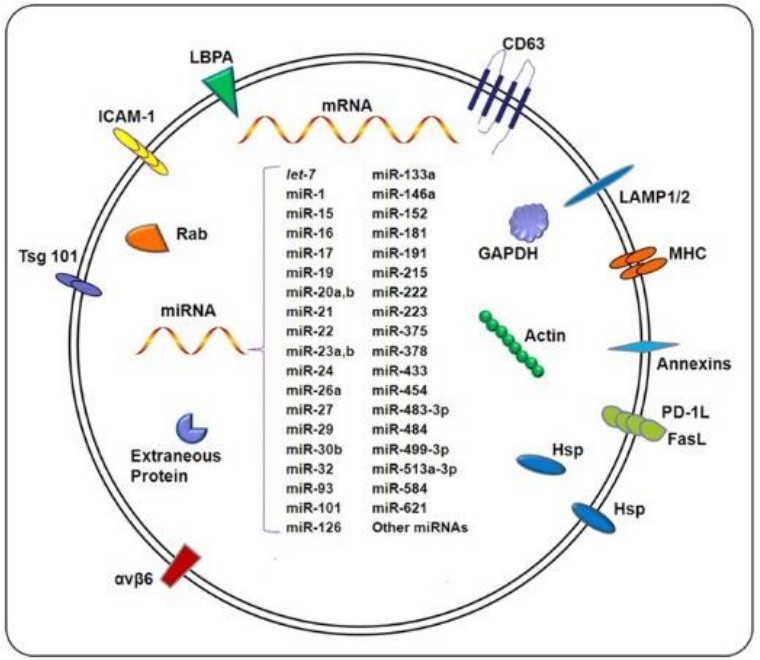


EXOSOMES: WHAT ARE THEY?

- **Vesicles** nano-sized (30-150nm) and cup-shaped used by cells for intercellular communication
- **Present** in many **biological fluids**; serum, plasma, urine, cerebral spinal fluid (CSF)
- Deliver their contents to cells in **neighborhood** as well as to cells **more distant**
- **Cell recognition molecules on surface** (**CD81** general marker, **L1CAM** neuron specific “cargo”)
- Able to **cross BBB** providing information on the CNS
- **Reflect** the **cell’s content** from which they are secreted (lipids, proteins, mRNAs and miRNAs)

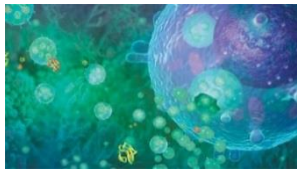


Proteomics Clin. Appl. 2015, 9, 358–367



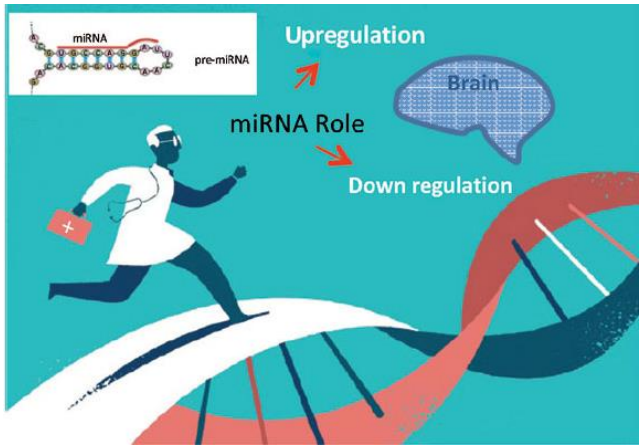
9769 proteins
1116 lipids
3408 mRNA
2838 miRNAs

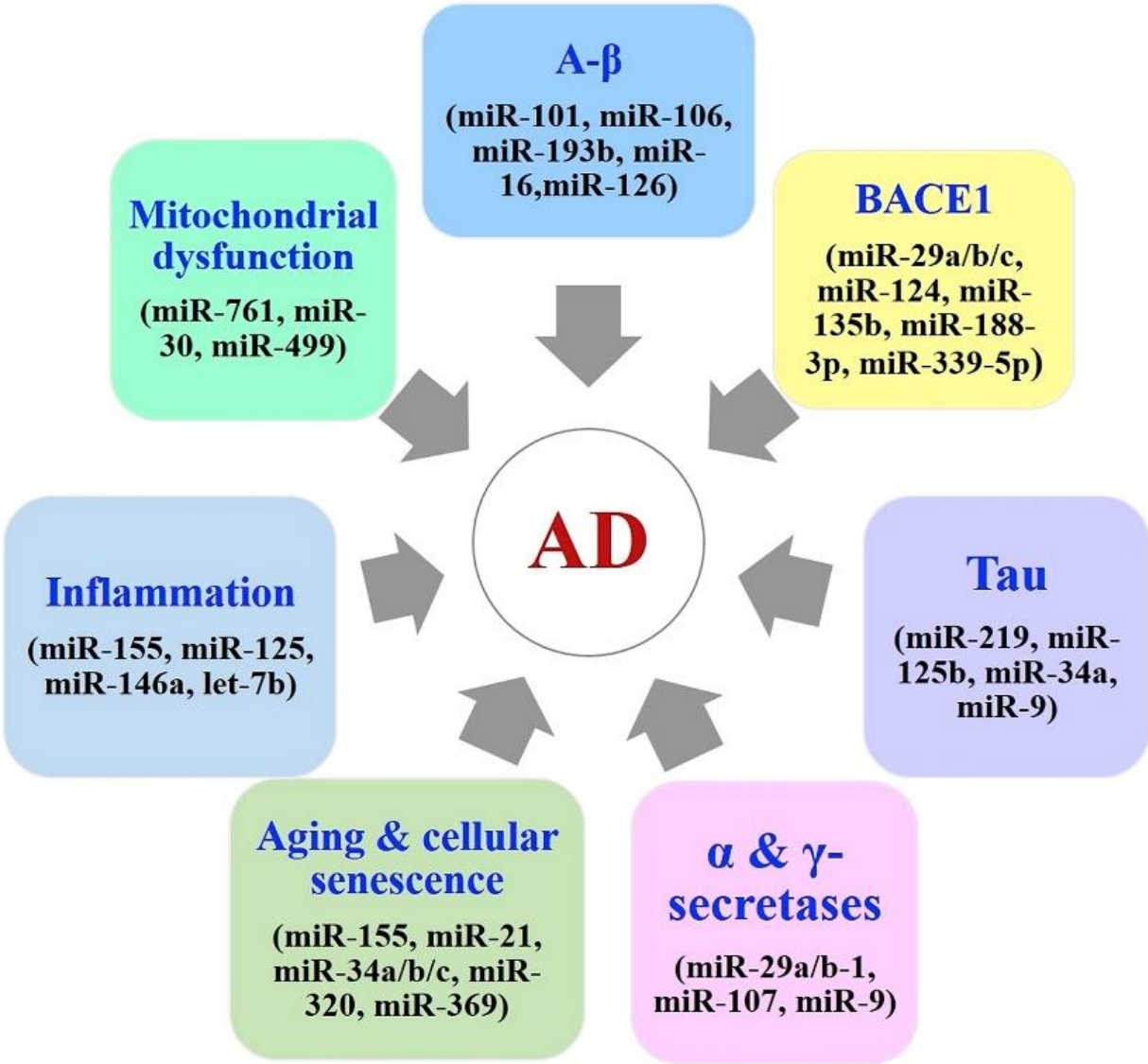
MIRNAS AND AD



miRNAs

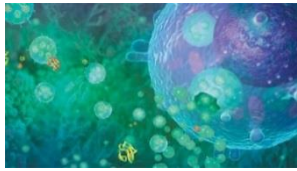
- short regulatory RNAs (19–24 nt) regulating the translation of up to 60% of protein-coding genes
- mainly target the 3′-UTRs of mRNAs, also the 5′-UTRs, or even the coding region
- Downregulation and upregulation of gene expression





Reddy et al., Biochem Biophys Res Commun., 2017

EXOSOMES: FROM THE BRAIN TO THE BLOOD



❖ Exosomes are cell specific thanks to molecular markers on membrane, L1CAM (neural adhesion protein)

Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: A case-control study

Massimo S. Fiandaca[a,1], Dimitrios Kapogiannis[b,1], Mark Mapstone[c,1], Adam Boxer[d], Erez Eitan[b], Janice B. Schwartz[e], Erin L. Abner[f], Ronald C. Petersen[g], Howard J. Federoff[a], Bruce L. Miller[d], Edward J. Goetzl[e,*]

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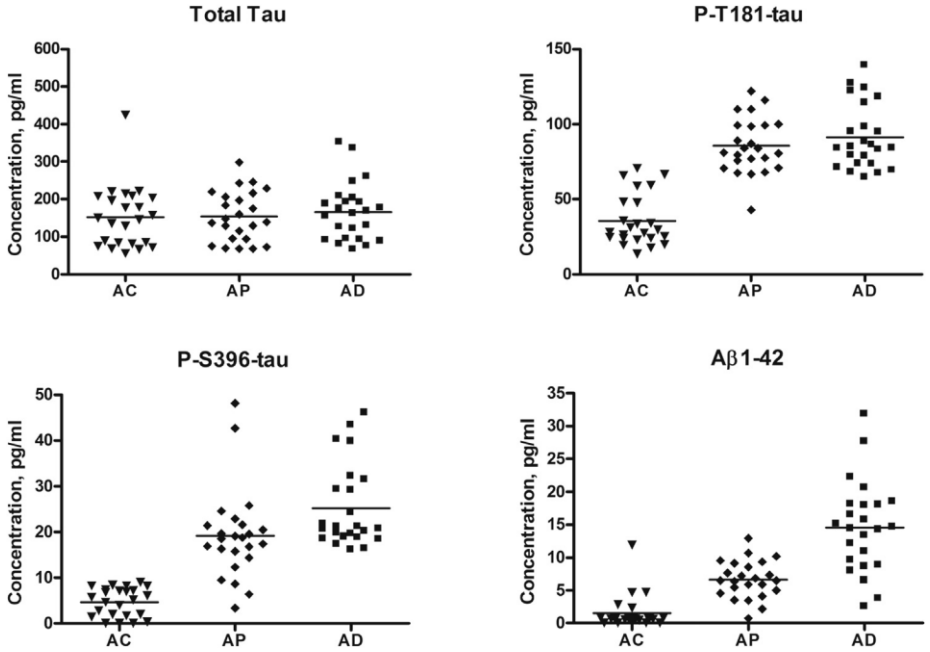
THE JOURNAL OF THE ALZHEIMER'S ASSOCIATION

Volume 11, Issue 6, June 2015, Pages 600–607.



AD-pathogenic exosome proteins from neurally-derived blood exosomes extracted and quantified by ELISA to develop biomarkers for staging of sporadic AD.

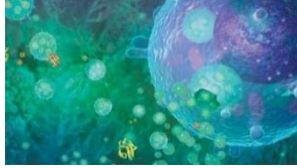
Levels of P-S396-tau, P-T181-tau, and Ab1–42 in extracts of neurally-derived blood exosomes predict the development of AD up to 10 years before clinical onset.

AIM OF THE STUDY

- isolate and characterize total and neural derived exosomes (NDEs) from plasma of AD patients and healthy controls in order to detect specific **microRNAs signature** as potentially peripheral AD biomarkers

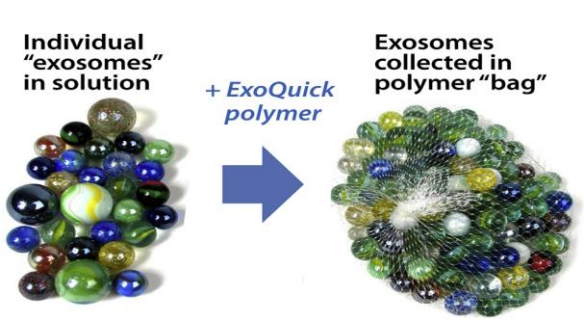
CHARACTERISTICS OF SUBJECTS ENROLLED IN THE STUDY

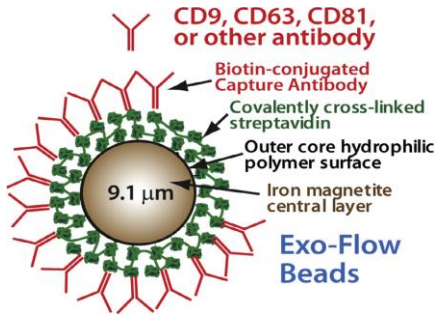
	AD	CTRLs
Number of subjects	20	20
Gender (M:F)	8:12	10:10
Mean Aβ1-42 (CSF)	510.96 pg/ml	804.98 pg/ml
Mean h-tau (CSF)	648.97 pg/ml	548.22 pg/ml
Mean P-tau (CSF)	86.54 pg/ml	74 pg/ml
Mean age, years	72.77± 0,22	66.54± 0,65



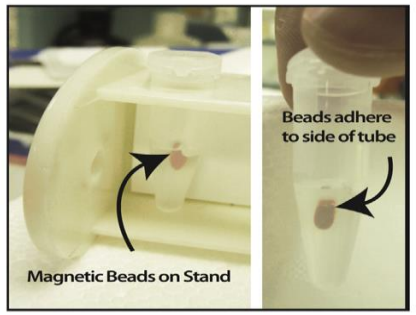
METHODS I
Isolate and characterize exosomes from plasma

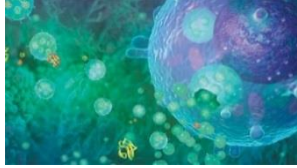
- Isolation of total exosomes and immuno-affinity purification of NDEs using anti-L1CAM antibody
- Sorting by flow cytometry, analysis by transmission electron microscopy (TEM) and Nanoparticle Tracking Analysis (NTA)
- Extraction of total RNA and analysis of the integrity and purity with Bioanalyzer





TEM/ NTA and extraction of total RNA



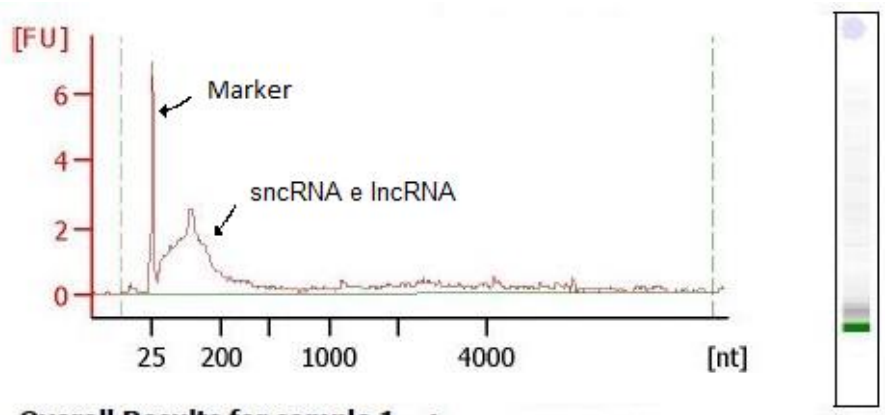


RESULTS I

Isolate and characterize exosomes from plasma: TEM analysis

- Detection and characterization of total and neural exosomes (L1CAM)
- The cup-shaped morphology is not affected by storage condition

Bioanalyzer electropherogram



Overall Results for sample 1 :

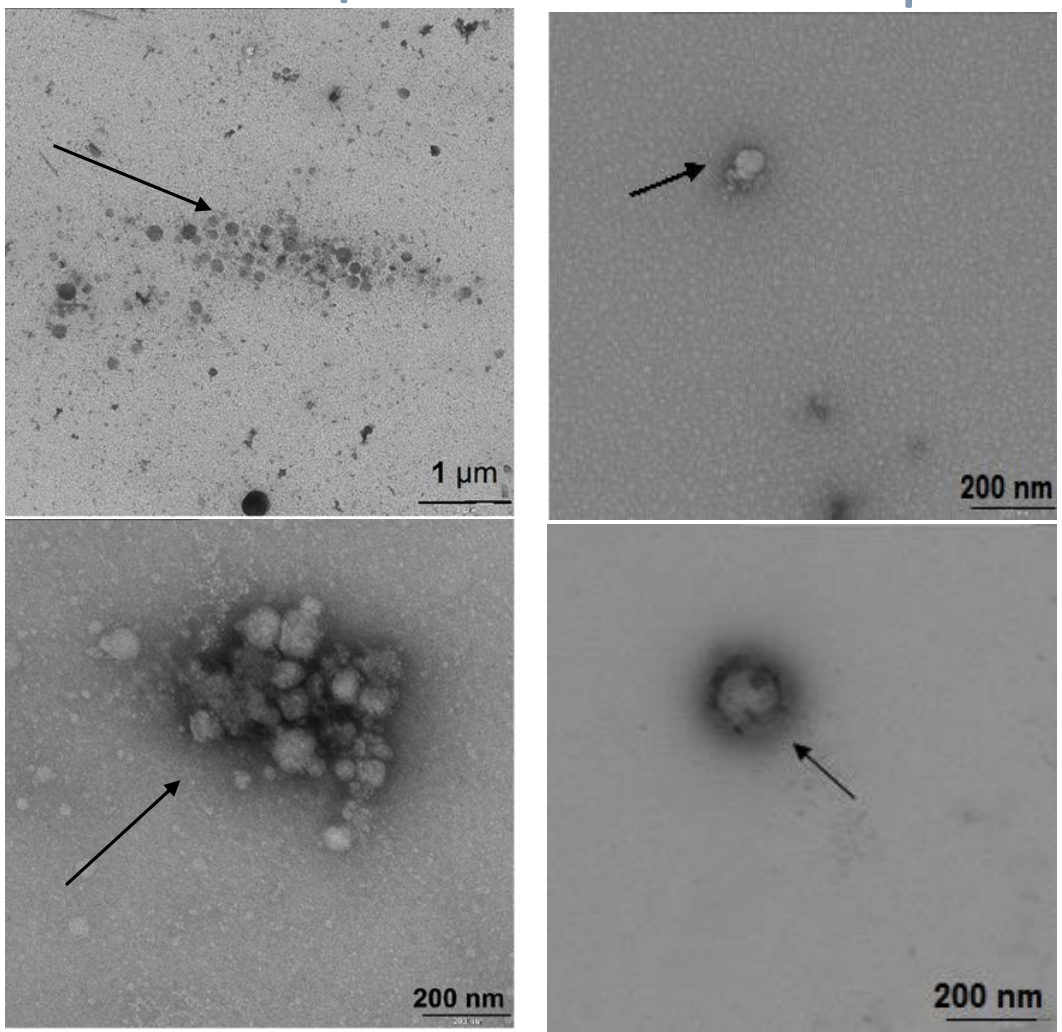
RNA Area:	48,0
RNA Concentration:	168 pg/µl
rRNA Ratio [28s / 18s]:	0,0
RNA Integrity Number (RIN):	2.5 (B.02.07)
Result Flagging Color:	
Result Flagging Label:	RIN: 2.50

TEM analysis

Fresh sample | Frozen sample

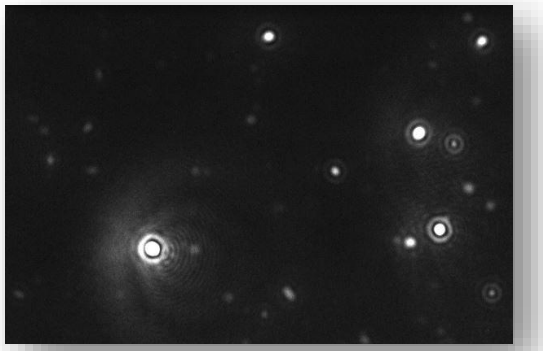
Total exosomes

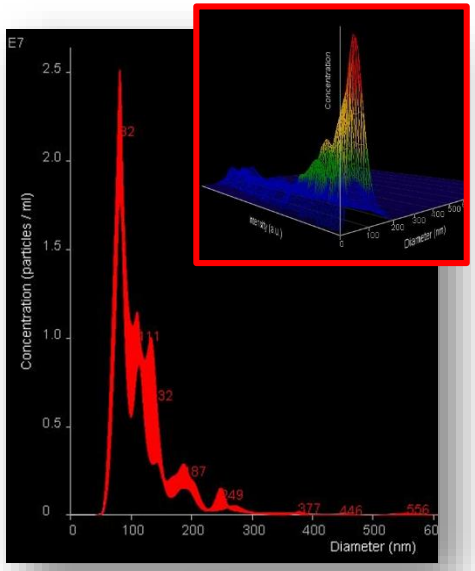
L1CAM



RESULTS I

Isolate and characterize exosomes from plasma: NTA analysis



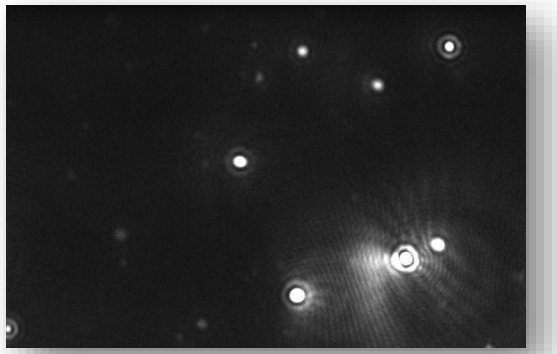


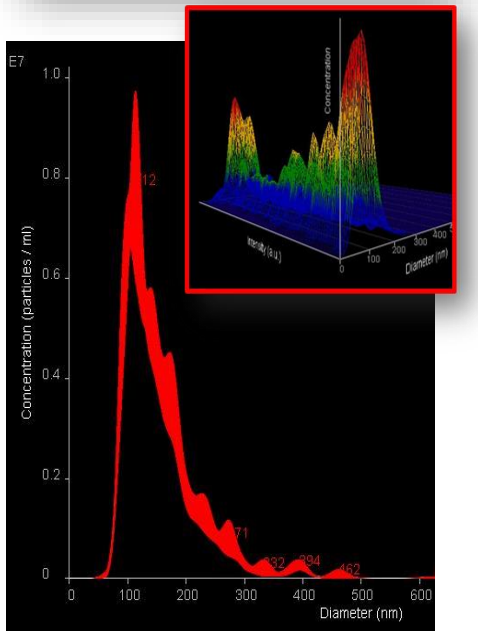
Total Completed Tracks: 37414
Total Valid Tracks: 13155

Concentration info: Mean +/- Standard Error
Particles per frame : 50.9 +/- 9.5
Particles per ml : (5.0 +/- 0.9) x 10e11
Dilution : 5.0 x 10e2

Stats: Merged Data	Stats: Mean +/- Standard Error
Mean : 117 nm	Mean : 118 +/- 2.2 nm
Mode : 81 nm	Mode : 79 +/- 1.5 nm
SD : 58 nm	SD : 57 +/- 5.3 nm
D10 : 72 nm	D10 : 73 +/- 1.1 nm
D50 : 100 nm	D50 : 100 +/- 2.3 nm
D90 : 183 nm	D90 : 187 +/- 4.4 nm

Total exosomes





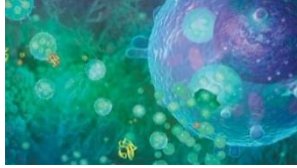
Total Completed Tracks: 30859
Total Valid Tracks: 7724

Concentration info: Mean +/- Standard Error
Particles per frame : 36.1 +/- 1.2
Particles per ml : (1.2 +/- 0.0) x 10e10
Dilution : 1.7 x 10e1

Stats: Merged Data	Stats: Mean +/- Standard Error
Mean : 156 nm	Mean : 156 +/- 3.7 nm
Mode : 112 nm	Mode : 114 +/- 7.3 nm
SD : 71 nm	SD : 70 +/- 6.1 nm
D10 : 93 nm	D10 : 94 +/- 2.8 nm
D50 : 136 nm	D50 : 135 +/- 3.4 nm
D90 : 239 nm	D90 : 239 +/- 7.3 nm

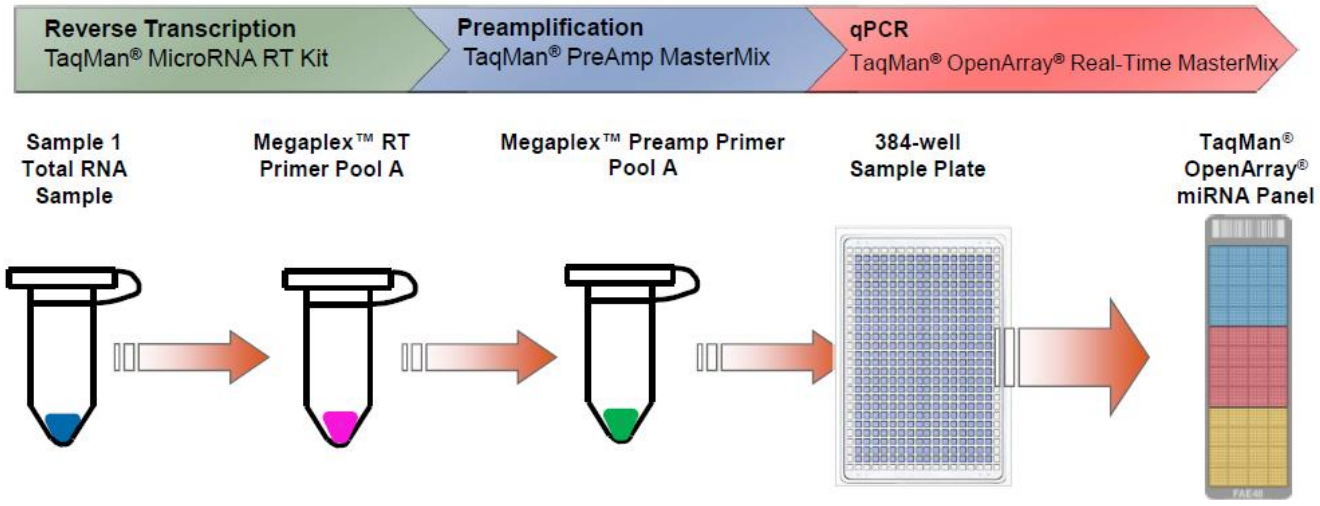
L1CAM

METHODS II: miRNAs expression analysis by high-throughput Real-Time PCR and OpenArray® (OA) Technology



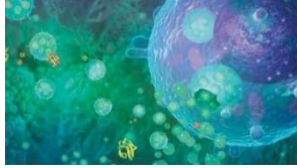
OpenArray® Instrumentation

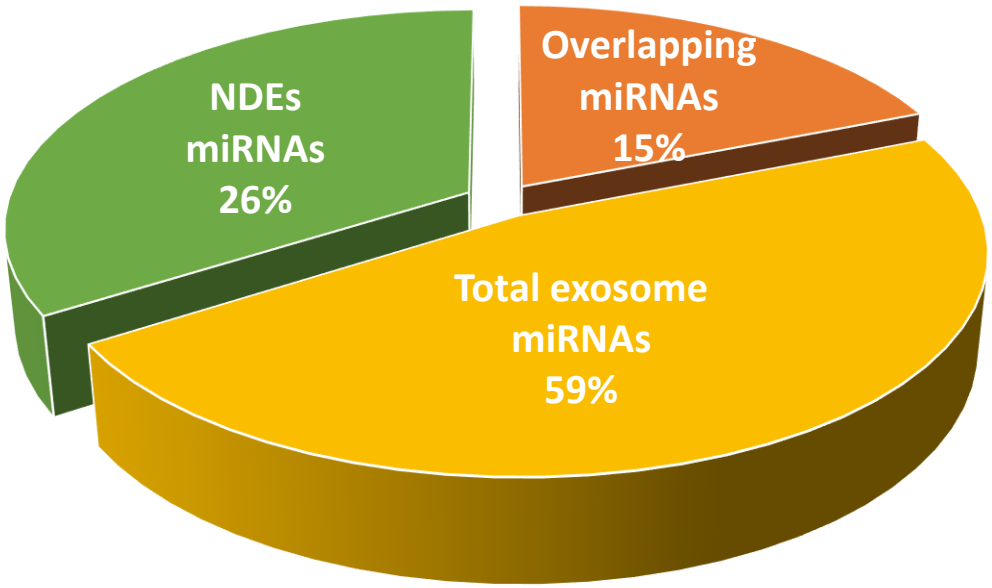
TaqMan® OpenArray® miRNA Panel

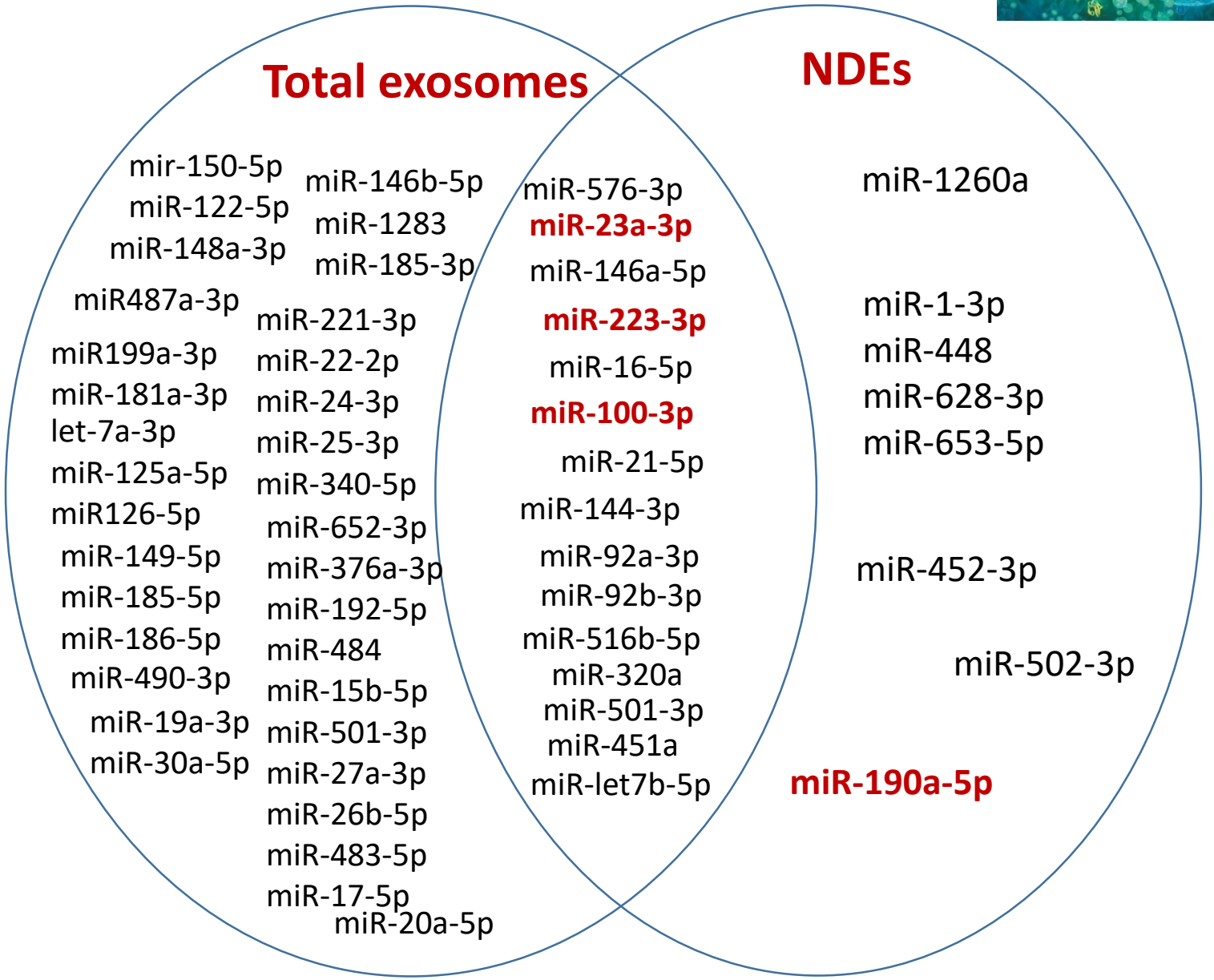


754 targets each sample

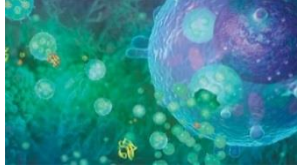
RESULTS II: miRNA distribution in exosomes from plasma through technology OA

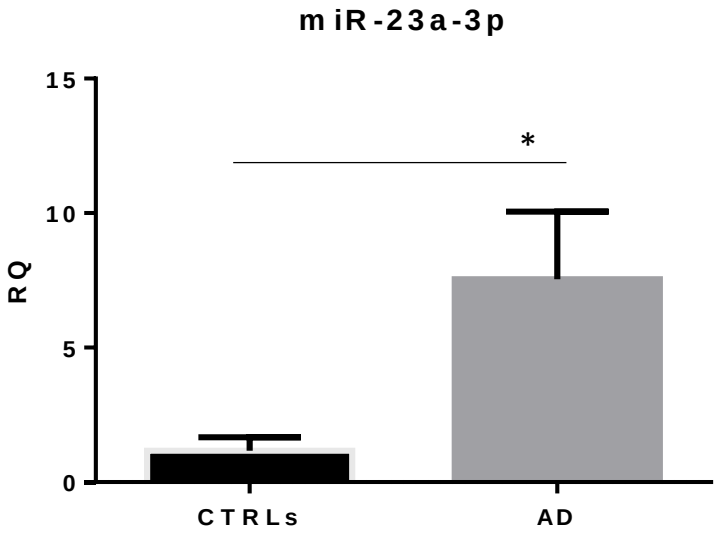




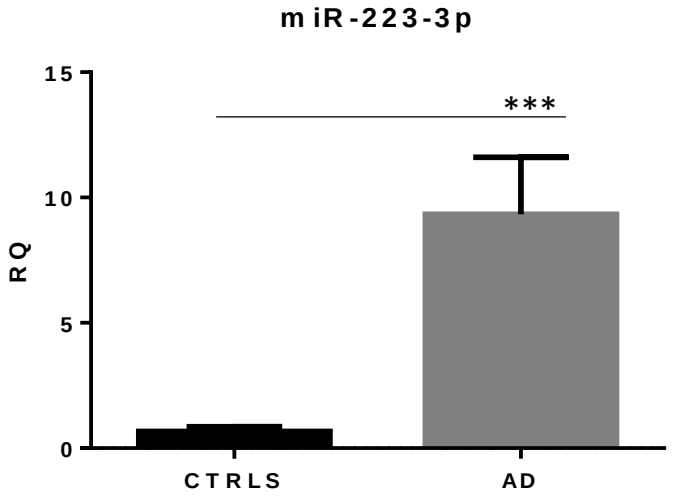


RESULTS II: miRNAs statistically significant in both exosomes population



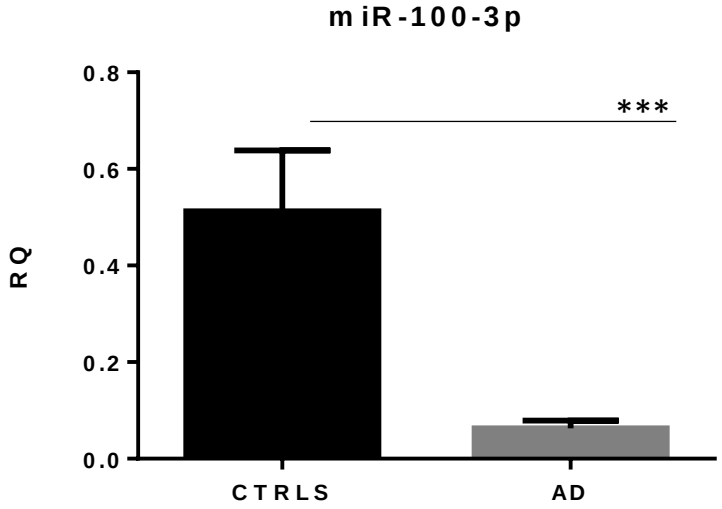


P= 0.026



P< 0.0001

AD patients= 20 subjects
CNTRLs= 20 subjects

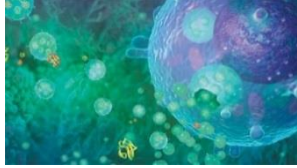


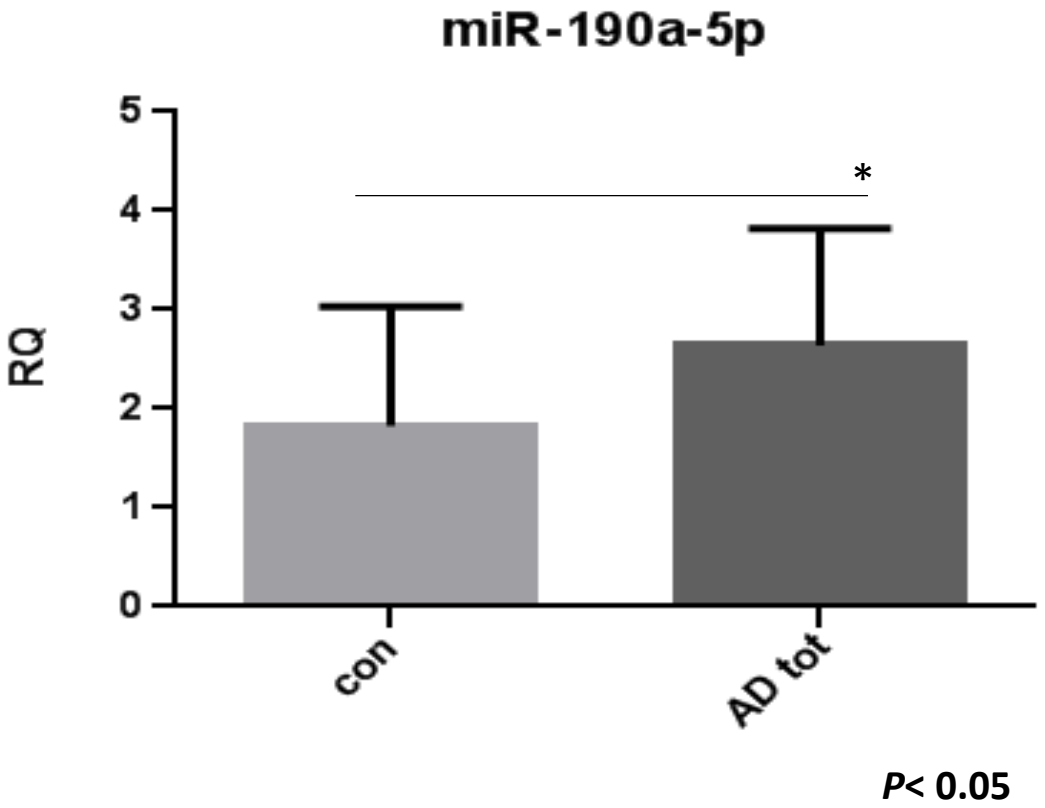
P< 0.0001

Predicted Target Genes

ADAM 9 (α-secretase of APP)
ADAM 17 (α-secretase of APP)
CXCL8 (IL8)
ACTBL2 (PD)

RESULTS II: miRNAs statistically significant in L1CAM exosome population



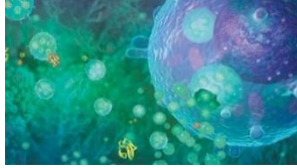


P< 0.05

AD patients= 20 subjects
CNTRLs= 20 subjects

Predicted Target Genes

NLGN1 (Neuroligin-1, post-synapse assembly)
SMAD2/4A (neuroinflammation)
NRG3 (Neuregulin-3, BACE1 substrate)
SYNJ1 (Synaptojanin-1, synaptic vesicle cycle)
NFASC (Neurofascin)
NCAM1 (Neural cell adhesion molecule-1)



WHAT'S NEXT

- Validation NDEs miRNAs expression from plasma with **Droplet Digital PCR technology**
- miRNA isolation **from exosomes of CSF** using miRCURY Exosomes Isolation Kit (Exiqon)
- Replicate the study on MCI subjects vs AD and healthy controls

PREDICTED TARGET GENES

miR-1260a:
NRXN2
NEDD8
ADAM7
NSG1
BACE1

miR-653-5p:
NEUROD6

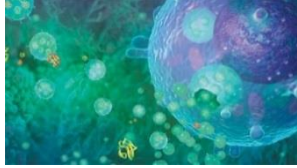
miR-452-3p:
TBK1
LRP1
LRP2
PFN2
CHCHD1

miR-190a-5p:
NLGN1
SMAD2
SMAD4A
NRG3
SYNJ1
NFASC
NCAM1

miR-223-3p:
SYNGR2
CACNG8
VAMP2

miR-100-3p:
VAMP3
NEUROD1

miR-23a-3p:
ADAM9
ADAM17
SOD2
SMAD3



CONCLUSIONS

- These data demonstrate that **we set up a good method** to isolate NDEs from plasma and CSF.
- Consistent **dysregulations of miRNAs** in AD patients compared to controls were found.
- 57 miRNAs detected in plasma exosomes, particularly miR-223-3p, miR-146a-5p, and miR-23a-3p, already found to be dysregulated in neurodegenerative diseases.

The investigation of miRNAs **specific signature** from NDEs could have a **great potential** in the field of **clinical biomarkers discovery** and could also contribute to clarify the molecular mechanisms underneath AD and other neurodegenerative diseases.

ACKNOWLEGEMENTS

Neurology Unit, Dept. of Pathophysiology and Transplantation, “Dino Ferrari" Center, University of Milan, Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico, Milan

Prof. Elio Scarpini
Daniela Galimberti

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THANK YOU FOR YOUR ATTENTION

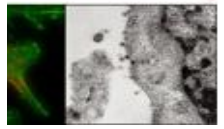
idoni
si

Tiziana Carandini

Consiglio Nazionale delle Ricerche

Claudia Verderio

mbic
microscopy bio-imaging center



Maria Carla Panzeri

Clinical Chemistry and Microbiology Laboratory, Flow Cytometry and Experimental Hepatology Service

Laura Porretti
Alessandra Cattaneo
Valentina Trunzo