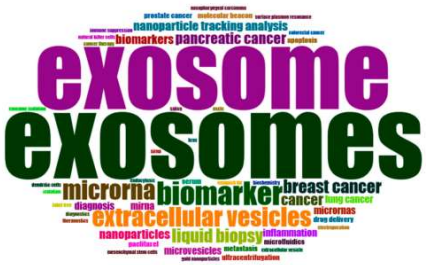
EXOSOMAS Y CÁNCER

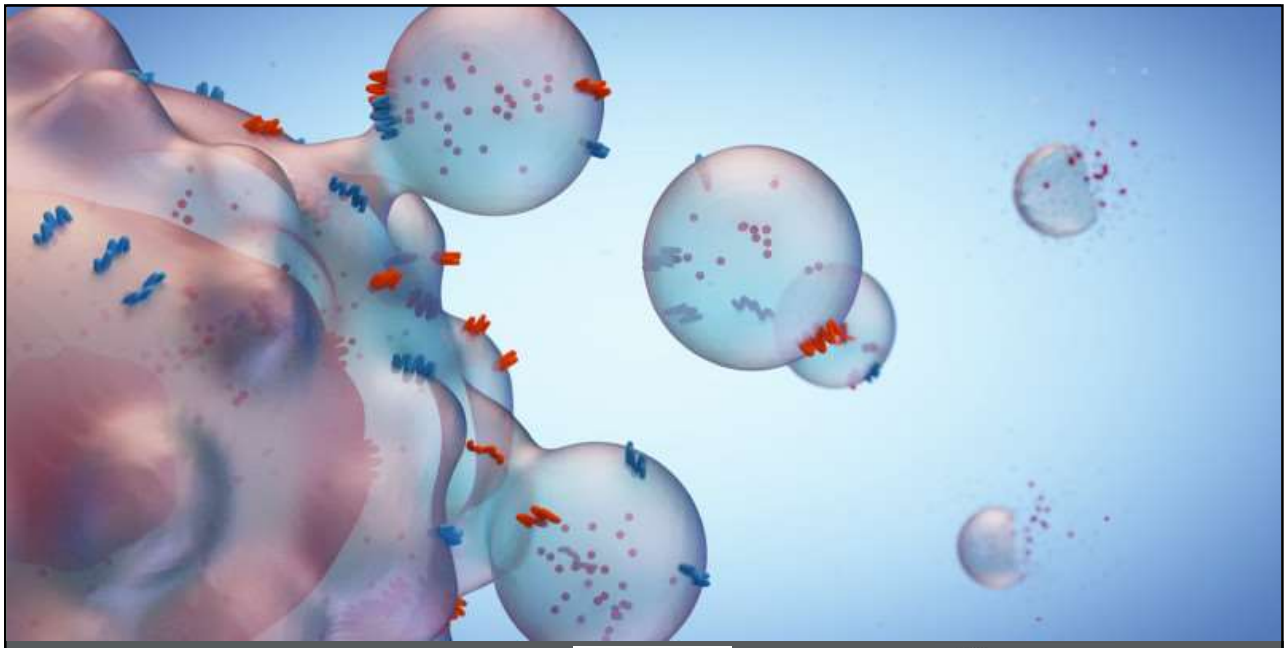


Neil Vásquez Araque
Biol. MSc. Dr en Biotecnología

Docente e Investigador UNAL – Med

EXOSOMAS

1



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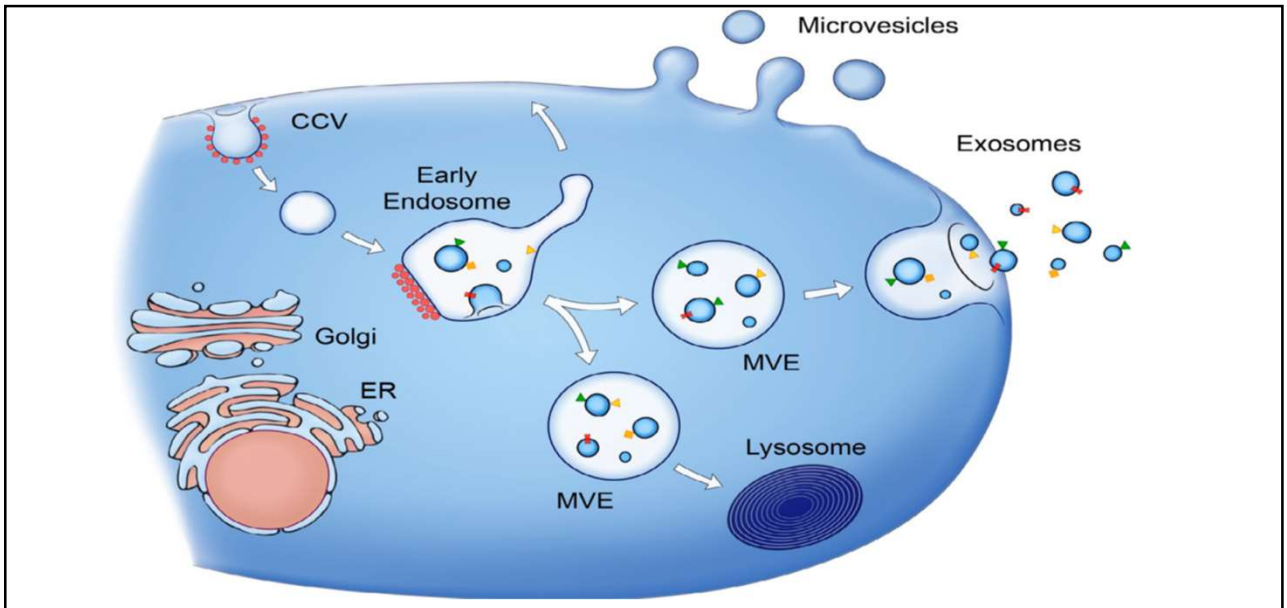
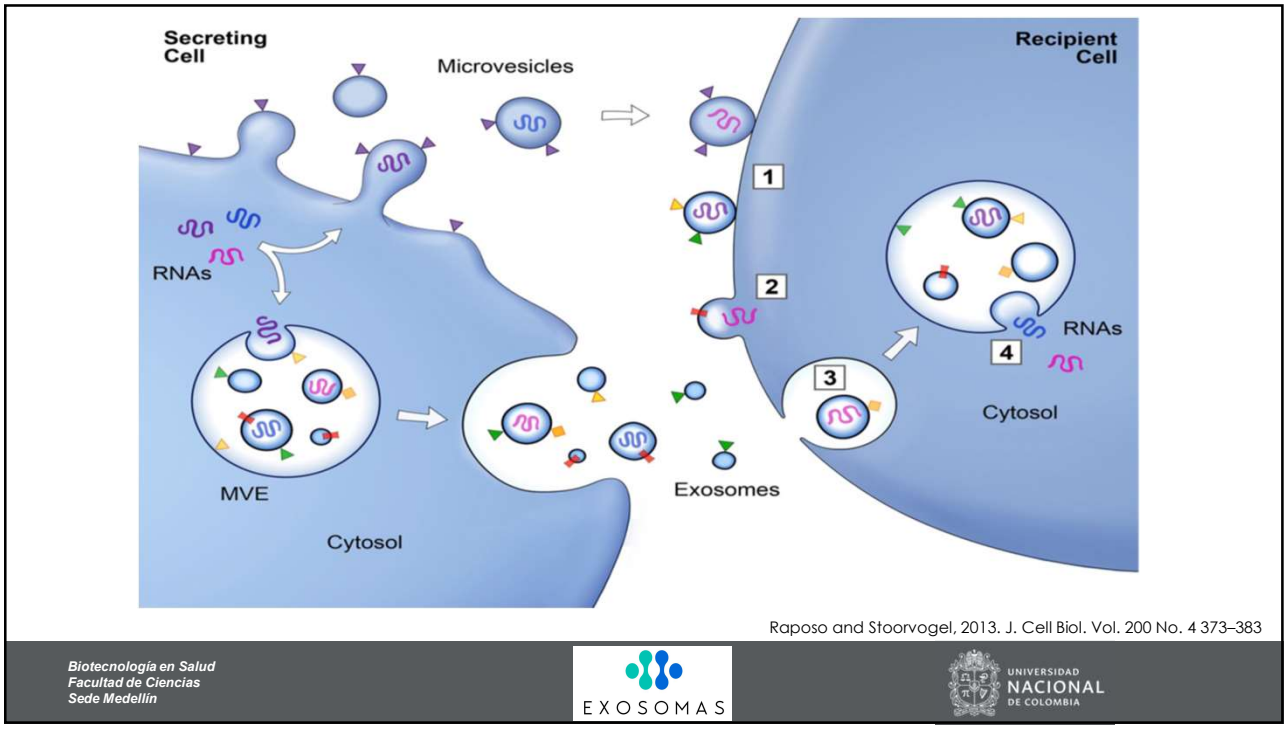


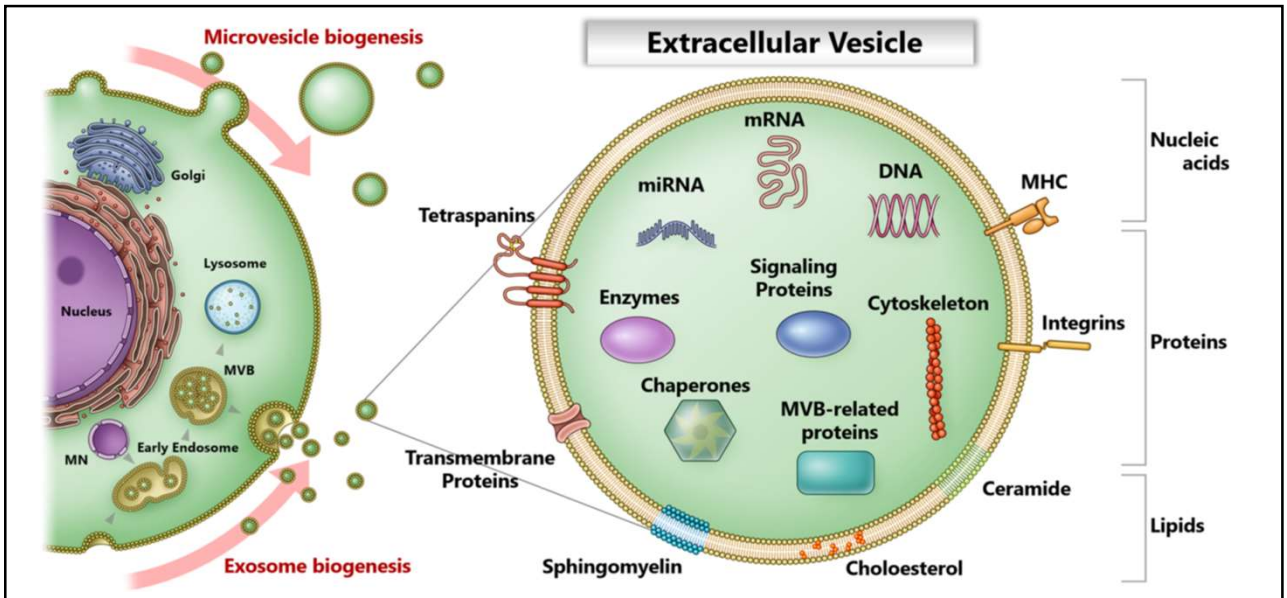
Raposo and Stoorvogel, 2013. J. Cell Biol. Vol. 200 No. 4 373–383

3



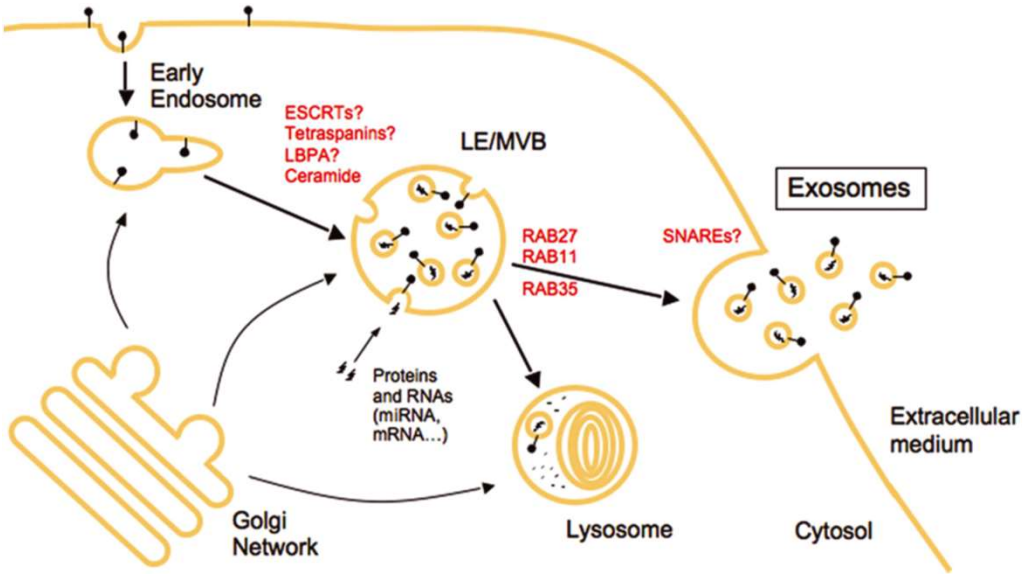
Raposo and Stoorvogel, 2013. J. Cell Biol. Vol. 200 No. 4 373–383

4



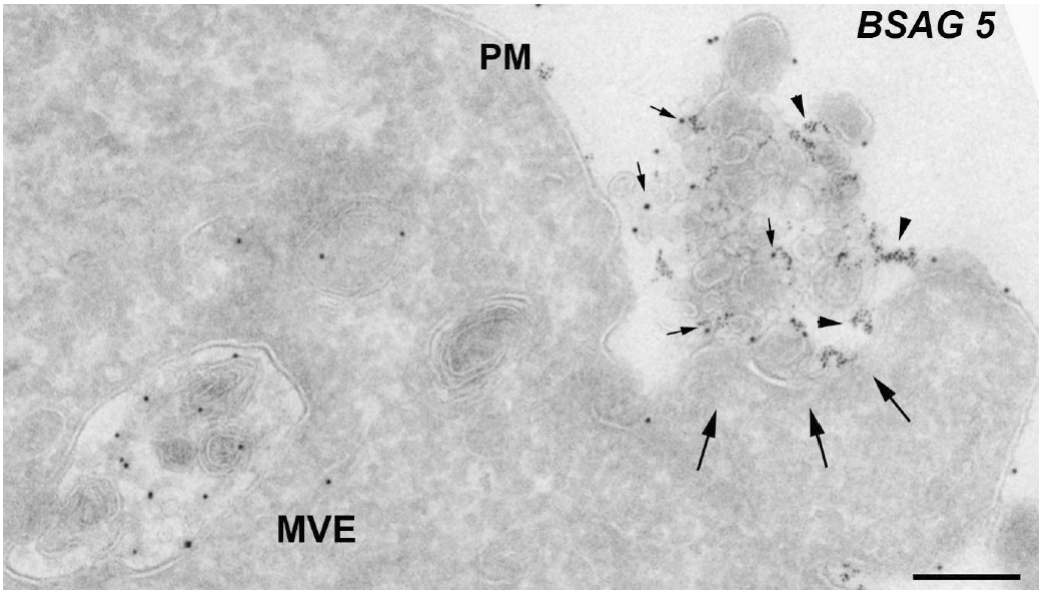
Yokoi A and Ochiya T, Seminars in Cancer Biology 74 (2021) 79–91

5



Bobrie et al., Traffic 2011; 12: 1659–1668

6

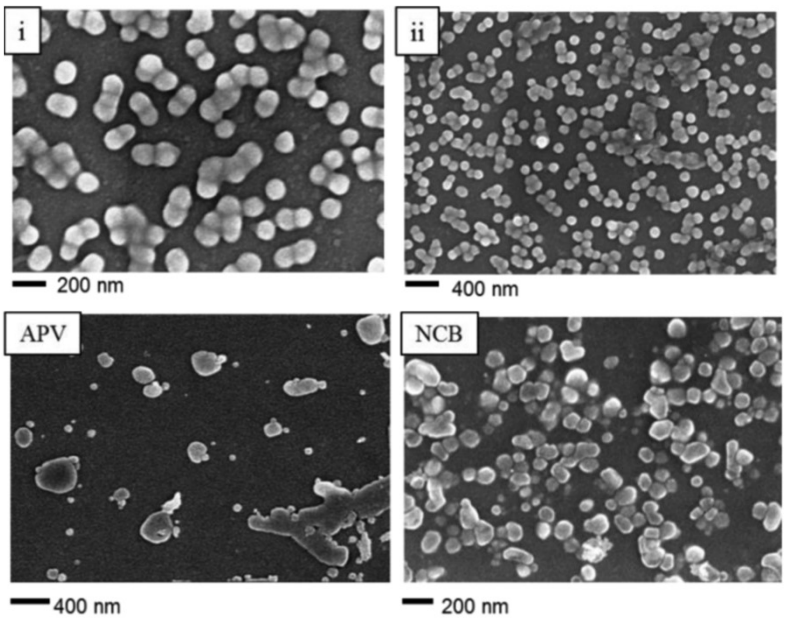


BSA coupled to 5-nm gold particles (BSAG 5)

Raposo and Stoorvogel, 2013. J. Cell Biol. Vol. 200 No. 4 373–383

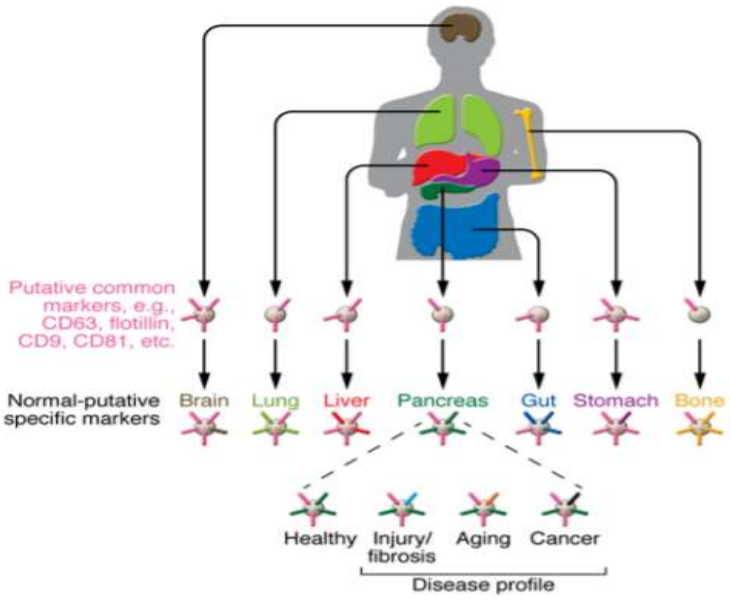
7

Vesículas extracelulares de células de melanoma



i, ii Exosomas. Vesículas Apoptóticas (apoptotic vesicles APV). Cuerpos Necróticos (Necrotic bodies -NCB)

8



9

Biomarcadores

Liquid biopsy analytes: features, extractable information, and clinical applications as biomarkers.

Traits	Liquid Biopsy Analyte				
	CTCs [1]	ctDNA [2]	Exosomes	ctRNA [3]	miRNA
Origin					
Viable cells	✔ [4]	✘ [5]	✔	? [6]	?
Apoptotic cells	✔	✔	?	?	?
Components					
DNA	✔	✔	✔	N.A. [7]	N.A.
RNA	✔	N.A.	✔	✔	✔
Proteins	✔	N.A.	✔	N.A.	N.A.
Metabolites	✔	N.A.	?	N.A.	N.A.
Extractable information					
Copy number variation	✔	✔	✔	✘	✘
Mutations	✔	✔	✔	✔	✘
Epigenetic information	✔	✔	✔	✘	✘
Fusion genes	✔	✔	✔	✔	✘
Splice variants	✔	✘	✔	✔	✘
Single-cell information	✔	✘	✘	✘	✘
Application in personalized medicine					
Diagnosis	✔	✔ [8]	✔	?	✔
Classification of molecular subtypes	✔	✔	?	?	✘
Clonal evolution tracking	✔	✔	?	✘	✘
Prognosis	✔	✔	✔	?	✔
Recurrence	✔	✔	✔	✔	✘
Predictive	✔	✔	✔	?	✘
Resistance prediction	✔	✔	✔	?	✘
Monitoring treatment	✔	✔	✔	?	?

[1] Circulating tumor cell; [2] circulating tumor DNA; [3] circulating tumor RNA; [4] yes; [5] no; [6] no data; [7] not applicable; [8] most probably.

Valencia and Montuenga. Exosomes in Liquid Biopsy Cancers 2021, 13, 2147 8

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Examples of exosomal-derived potential biomarkers with clinical significance published in the last three years.

Exosomal miRNAs as Cancer Biomarkers			
miRNA	Cancer type	Clinical value	Biofluid
Let-7b-5p, -122-5p, -146b-5p, -210-3p, -215-5p	Breast cancer	Diagnosis	Plasma
miR-224	Hepatocellular carcinoma	Diagnosis/Prognosis	Serum
miR-106b, miR-1269a	Lung cancer	Diagnosis/Prognosis	Serum
miR-375, -1307	Ovarian cancer	Diagnosis	Serum
Exosomal lncRNAs as Cancer Biomarkers			
lncRNA	Cancer type	Clinical value	Biofluid
PCAT-1, UBC1 and SNHG16	Bladder cancer	Diagnosis/Prognosis	Urine
MALAT-1	Lung cancer	Diagnosis	Serum
Exosomal mRNA as Cancer Biomarkers			
mRNA	Cancer type	Clinical value	Biofluid
BRAF, KRAS (mutant)	Colorectal cancer	Diagnosis	Serum
Exosomal mutated DNA as Cancer Biomarkers			
DNA	Cancer type	Clinical value	Biofluid
IDH1	Glioblastoma	Diagnosis/Prognosis	Plasma
EGFR	Lung cancer	Diagnosis/Prognosis	Plasma/Bronchioalveolar lavage
BRAF	Melanoma	Therapeutic monitoring	Plasma
KRAS, P53	Pancreatic cancer	Diagnosis/Prognosis	Serum/Plasma
MYC, P53, MLH1, PTEN, AR	Prostate cancer	Diagnosis/Prognosis	Plasma
Exosomal proteins as Cancer Biomarkers			
Protein	Cancer type	Clinical value	Biofluid
PDL-1	Melanoma	Prognosis	Plasma

Valencia and Montuenga. Exosomes in Liquid Biopsy Cancers 2021, 13, 2147 8

11

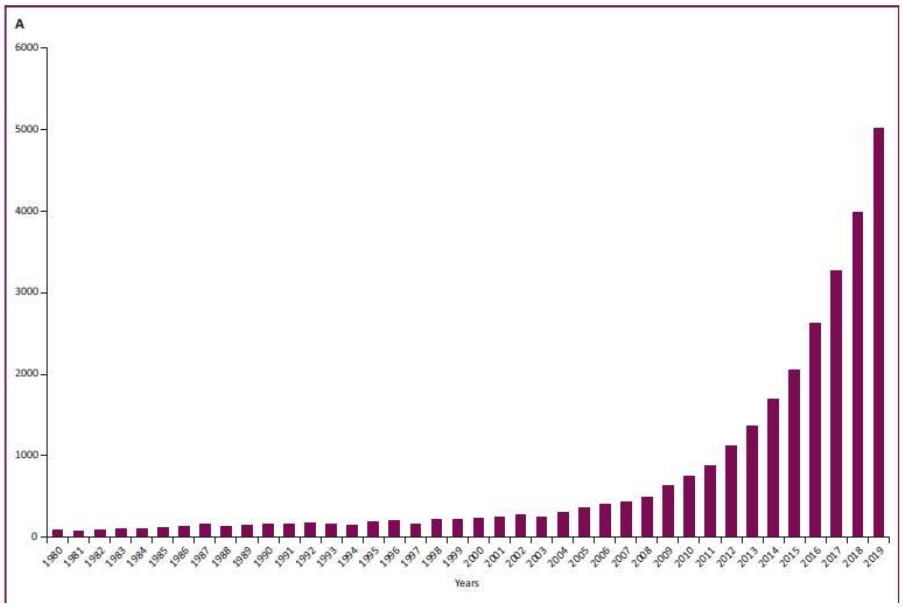
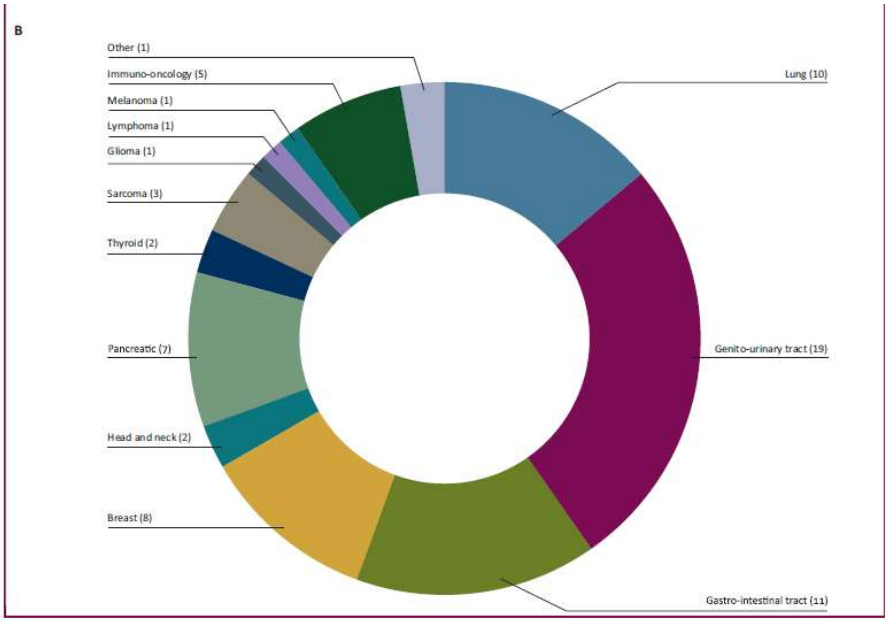


Figure 4. (A) Exosome-based research publications. The interest in exosomes for diagnostics, therapeutics and as an intricate part of intercellular communication has significantly increased in recent years. (B) Overview of a total of 71 exosome-based clinical trials registered worldwide in ClinicalTrials.gov (as of 22 October 2020) in diagnostics and translational biomarkers for various cancers, manually curated through searches with keywords (exosomes, extracellular vesicle, EVs, and microvesicles) with the elimination of both duplicate hits and those for therapeutic applications.

12

Clinical Trials - Cancer Exoxomes



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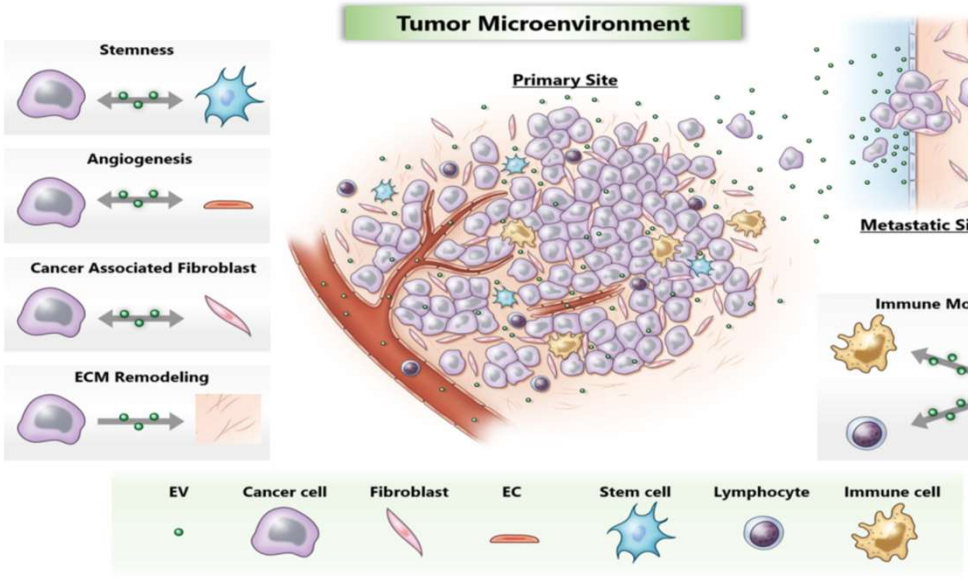
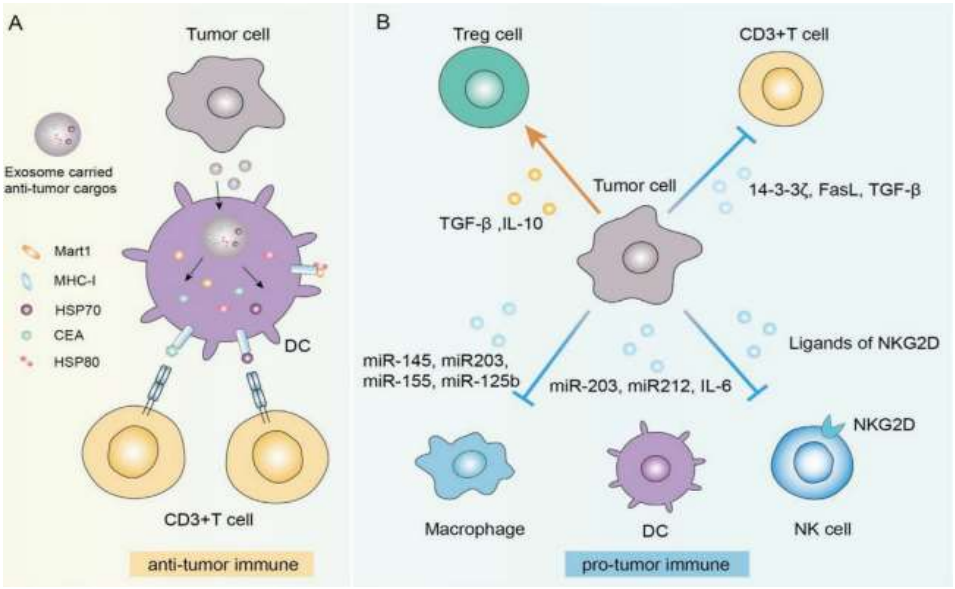


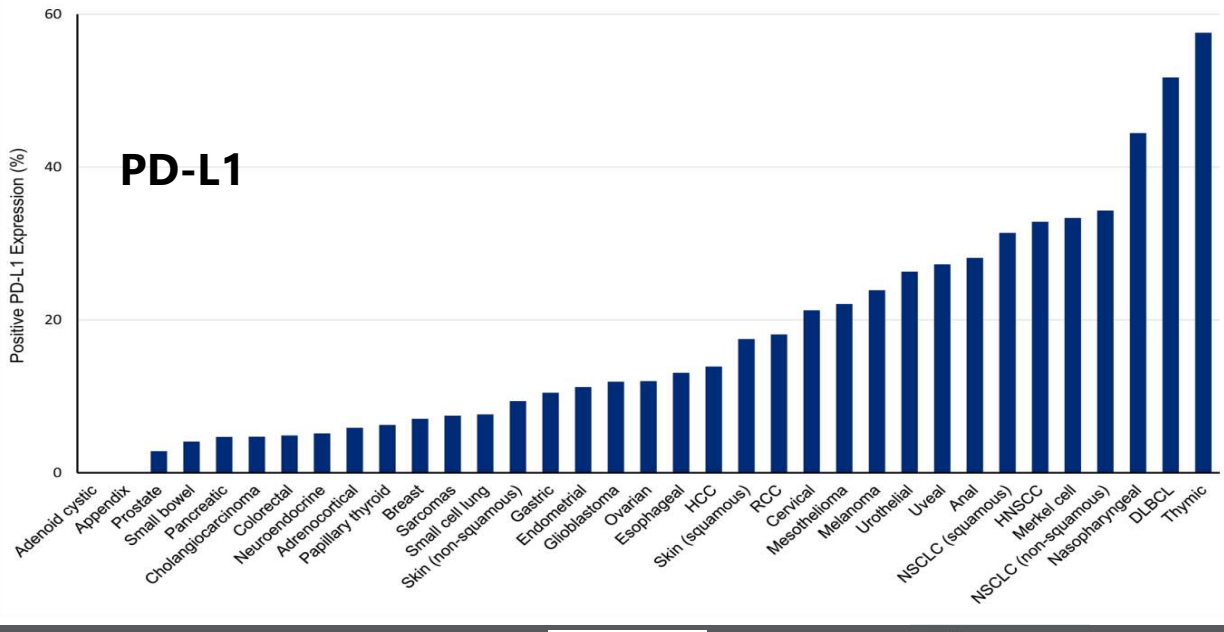
Yokoi A and Ochiya T, Seminars in Cancer Biology 74 (2021) 79–91

14



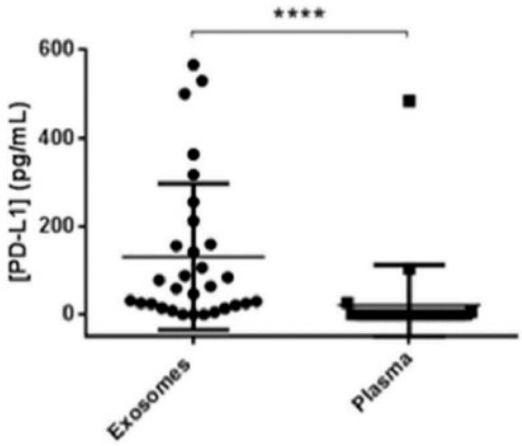
Wan Z-Adv. Biosys. 2020, 2000061(1-10)

15



16

Levels of PD-L1 in exosomes isolated from the plasma of melanoma patients

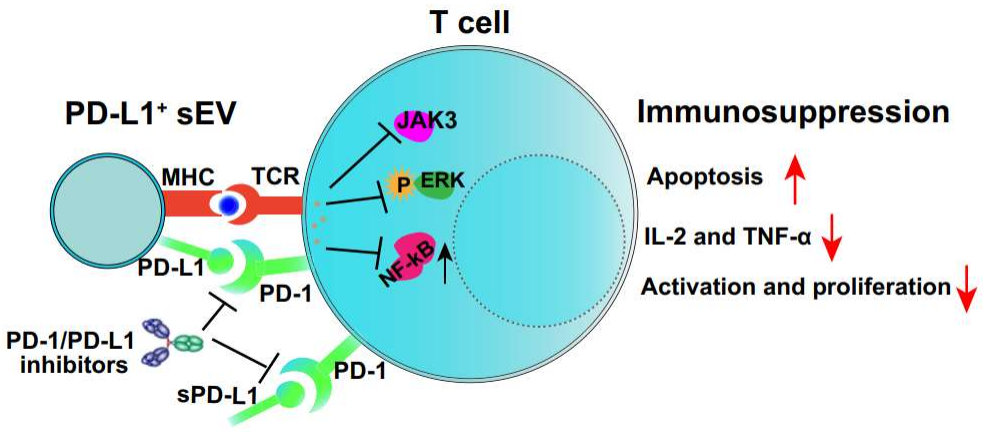


Levels of PD-L1 in exosomes isolated from the plasma of melanoma patients compared with PD-L1 levels free in the plasma (n = 30) (****p <0.0001), determined by ELISA

Tracking the evolution of circulating exosomal-PD-L1 to monitor melanoma patients, JOURNAL OF EXTRACELLULAR VESICLES2020, VOL. 9, 1710899

17

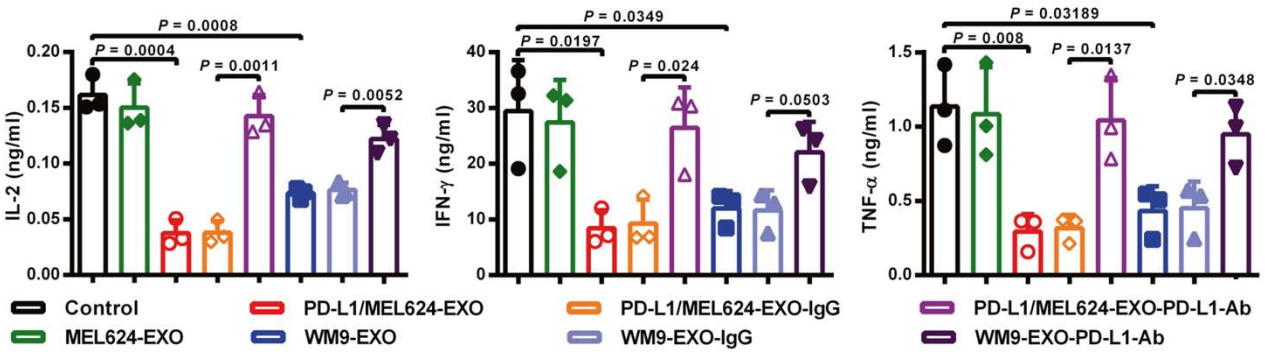
Efecto inmunosupresor de los exosomas tumorales



npj Precision Oncology (2022) 6:42

18

Efecto de los exosomas tumorales sobre la producción de citoquinas por células T de sangre periférica

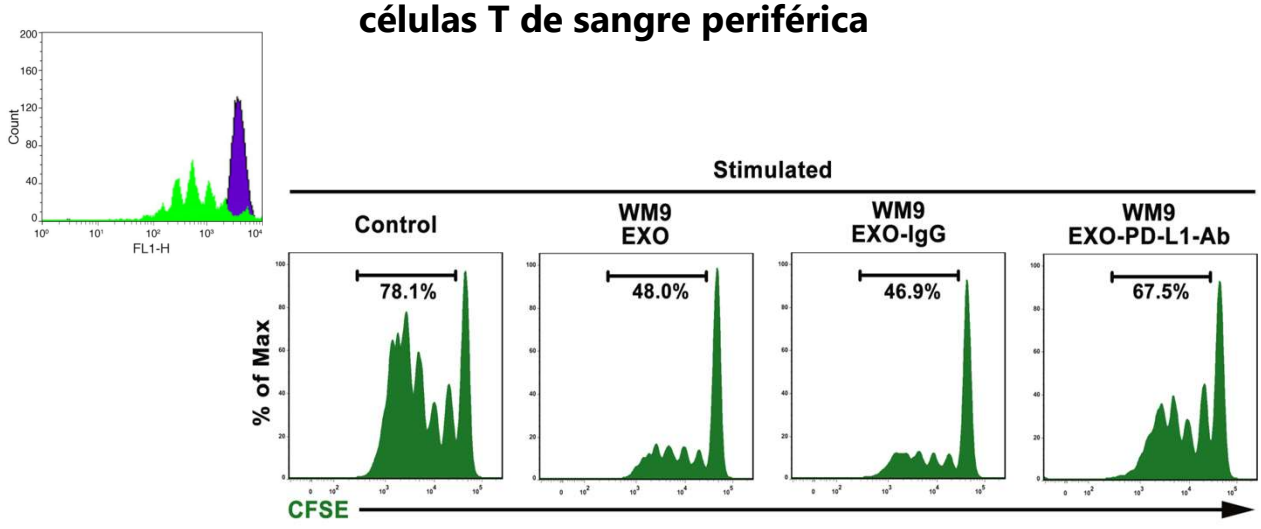


ELISA de IL-2, IFN-γ, y TNF de linfocitos T CD8 estimulados luego de tratamiento con exosomas tumorales.

Nature. 2018 August ; 560(7718): 382–386

19

Efecto de los exosomas tumorales sobre la proliferación de las células T de sangre periférica

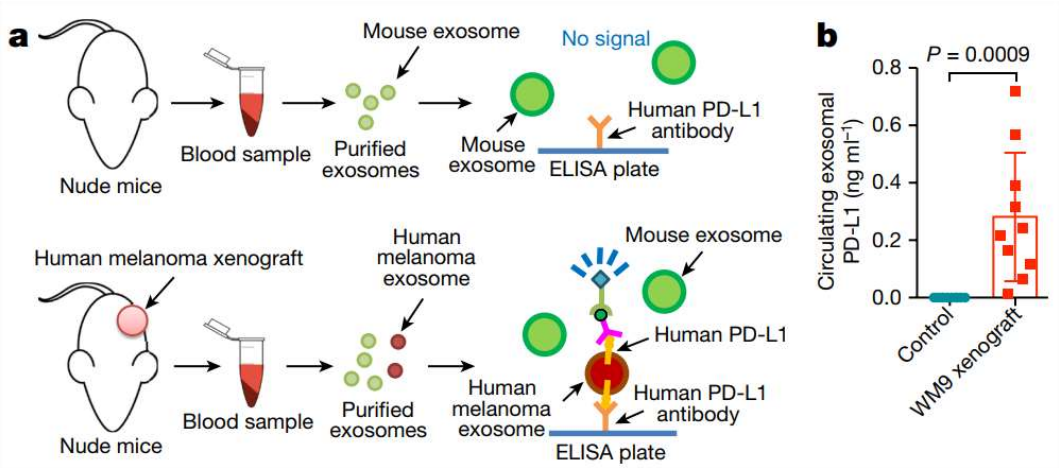


Histograma de linfocitos T CD8 estimulados luego de tratamiento con exosomas tumorales de WM9

Nature. 2018 August ; 560(7718): 382–386

20

Nivel de PD-L1 exosomal en sangre de ratón.

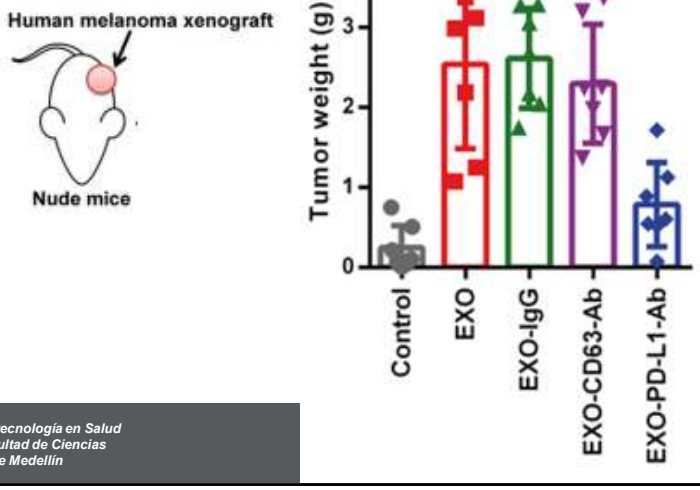


a. Esquema de purificación de exosomas y ELISA para medir PD-L1 exosomal.

b. Diferencia de exosomas provenientes de sangre en ratones de control y ratones con melanoma

Nature. 2018 August ; 560(7718): 382–386

Efecto de exosomas tumorales de ratón con el crecimiento tumoral *in vivo*



Nature. 2018 August ; 560(7718): 382–386

Exosomas de suero de pacientes con NSCLC

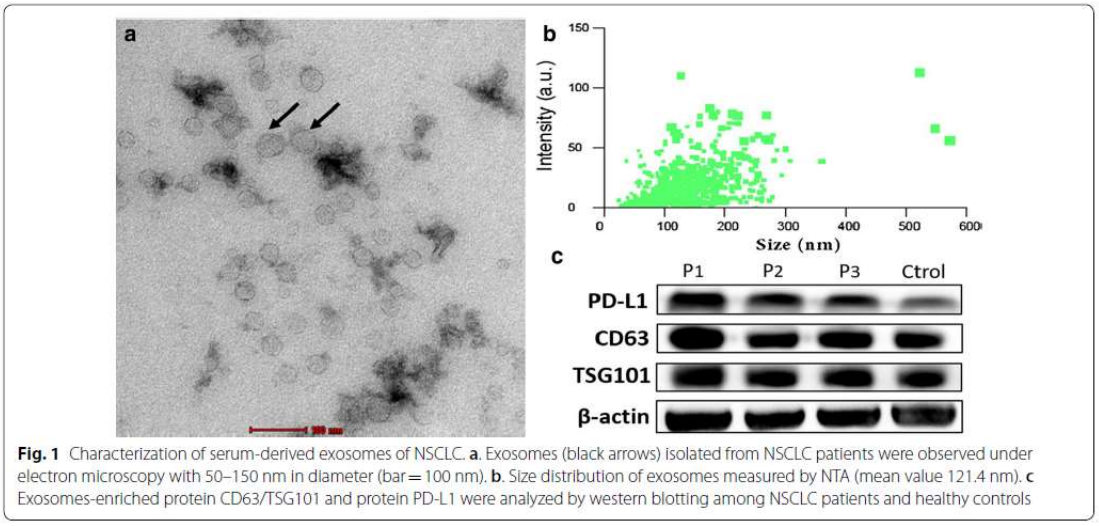


Fig. 1 Characterization of serum-derived exosomes of NSCLC. **a**. Exosomes (black arrows) isolated from NSCLC patients were observed under electron microscopy with 50–150 nm in diameter (bar = 100 nm). **b**. Size distribution of exosomes measured by NTA (mean value 121.4 nm). **c** Exosomes-enriched protein CD63/TSG101 and protein PD-L1 were analyzed by western blotting among NSCLC patients and healthy controls

Li et al. J Transl Med (2019) 17:355

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Niveles de PD-L1 exosomal vs PD-L1 soluble

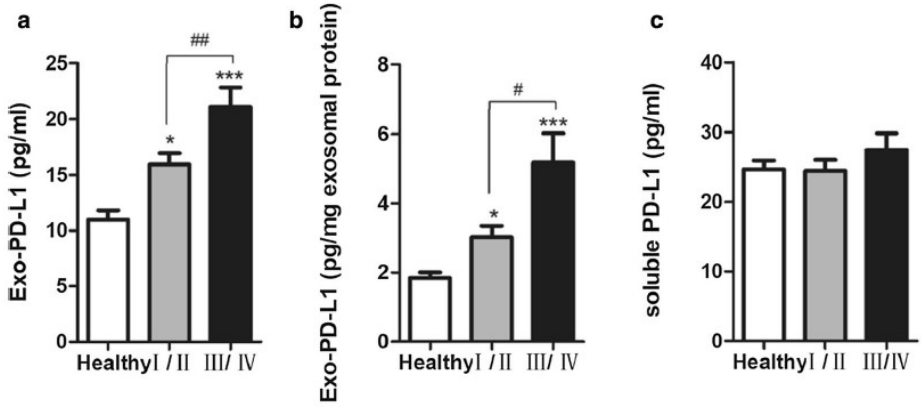

Fig. 3 Correlation between Exo-PD-L1 and sPDL1 profiles. **a**, **b** Quantitative analysis of Exo-PD-L1 levels (pg/ml serum) (**a**) and relative Exo-PD-L1 levels (pg/mg exosomal protein) (**b**) among healthy individuals (n = 27), stage I–II (n = 57) and III/IV (n = 28) NSCLC patients; $^{*}p < 0.05$; $^{***}p < 0.001$; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$. **c** sPDL1 levels were not statistically different in NSCLC patients from healthy donors.

Li et al. J Transl Med (2019) 17:355

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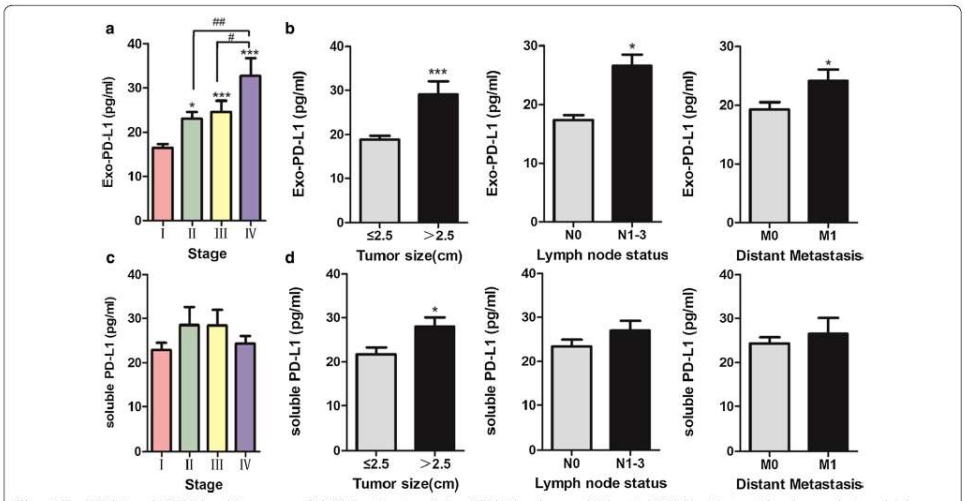
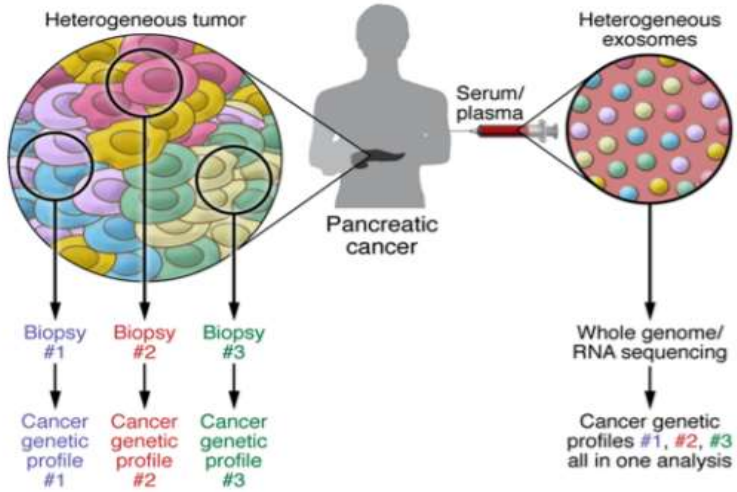
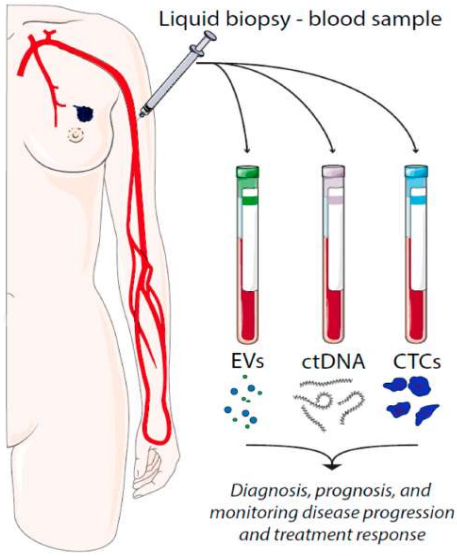


Fig. 4 Exo-PD-L1 and sPDL1 levels in serum of NSCLC patients. **a**, **b** Exo-PD-L1 levels were higher in NSCLC patients with advanced stage (**a**), larger tumor size (> 2.5 cm), positive lymph node status (N1–N3) and distant metastasis (M1) (**b**); $^{*}p < 0.05$; $^{***}p < 0.001$; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$. **c**, **d** sPDL1 levels were not significantly different in NSCLC patients stratified by stage (**c**), lymph node status and metastasis except for tumor size (> 2.5 cm) (**d**); $^{*}p < 0.05$

Li et al. J Transl Med (2019) 17:355

Biomarcador - Exosomal





Liquid biopsies in breast cancer and their utilities. EV = extracellular vesicles, ctDNA = circulating tumor DNA, CTCs = circulating tumor cells.

Tellez-Gabriel et al., Int. J. Mol. Sci. 2020, 21, 9457

27

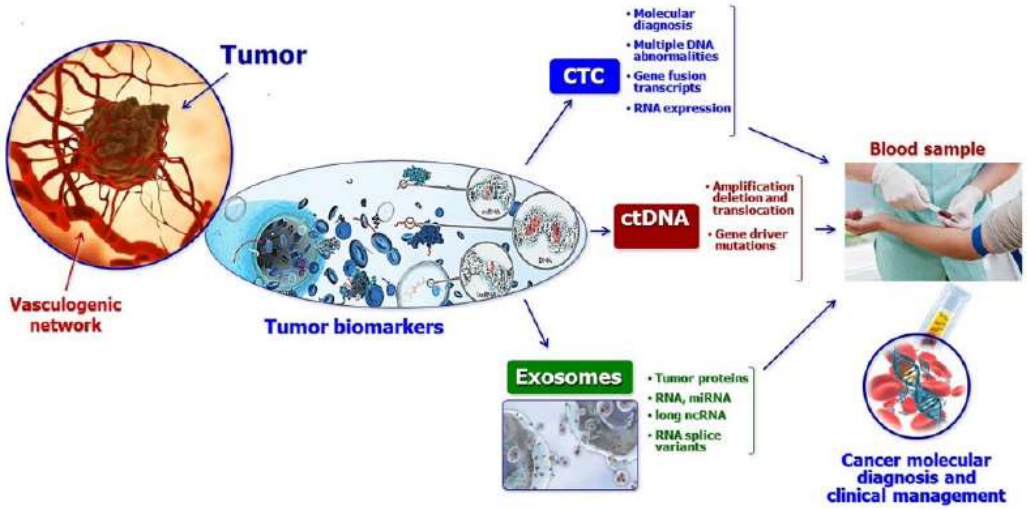


Figure 1. Molecular applications of circulating tumor cells (CTCs), circulating tumor DNA (ctDNA) and exosomes as liquid biopsy for personalized medicine.

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exoRNA → **cDNA** + **cf DNA** → **exoNA**

exoRNA y cfDNA Combinado **exoNA**
vs **cfDNA**

Concordance between tumor and plasma *EGFR* status

		Tissue Biopsy Result: Activating[a]	Tissue Biopsy Result: T790M		Activating	T790M
TIGER-X Representative Subgroup A ($n = 56$ total, 54 with valid tumor status)						
exoNA (EXO1000)[b]	+	53	44	Sensitivity (exoNA)	98%	90%
	−	1	5			
ctDNA (BEAMing)[b]	+	44	41	Sensitivity (ctDNA)	82%	84%
	−	10	8			

[a]All activating mutations were *EGFR* L858R or del19.
[b]*P*-value (exoNA versus ctDNA) is 0.004 for activating mutations and 0.25 for T790M.

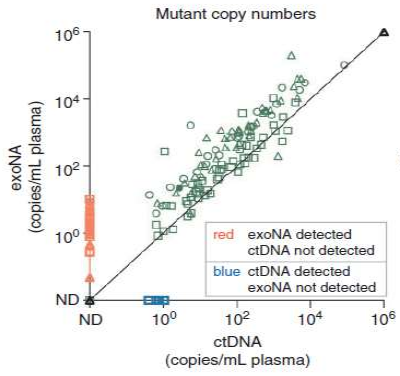
Annals of Oncology 29: 700–706, 2018

29

exoRNA y cfDNA Combinado **exoNA**
vs **cfDNA**

Falsos negativos

A



B

Tumor mutation-positives	Activating	T790M	
exoNA detected ctDNA not detected	11	5	**ctDNA**
ctDNA detected exoNA not detected	1	2	**exoNA**

C

Complete cohort		Mutant cps/mL: Median	Range	*P*-value
Activating *EGFR* MUT	exoNA	234	0-202092	<0.0001
	ctDNA	24	0-82406	
T790M *EGFR* MUT	exoNA	12	0-10642	<0.0001
	ctDNA	6	0-3867	

(A) EGFR mutant copies found in exoNA compared with copies in ctDNA within the complete patient cohort. The triangles represent del19, hollow circles L858R, full circle L861Q (activating mutations) and squares T790M mutations; identity line shows equal copies/mL plasma.
(B) Summary of EGFR detection in plasma within all tumor EGFR positives.
(C) Summary of mutant copies found in exoNA and ctDNA

Annals of Oncology 29: 700–706, 2018

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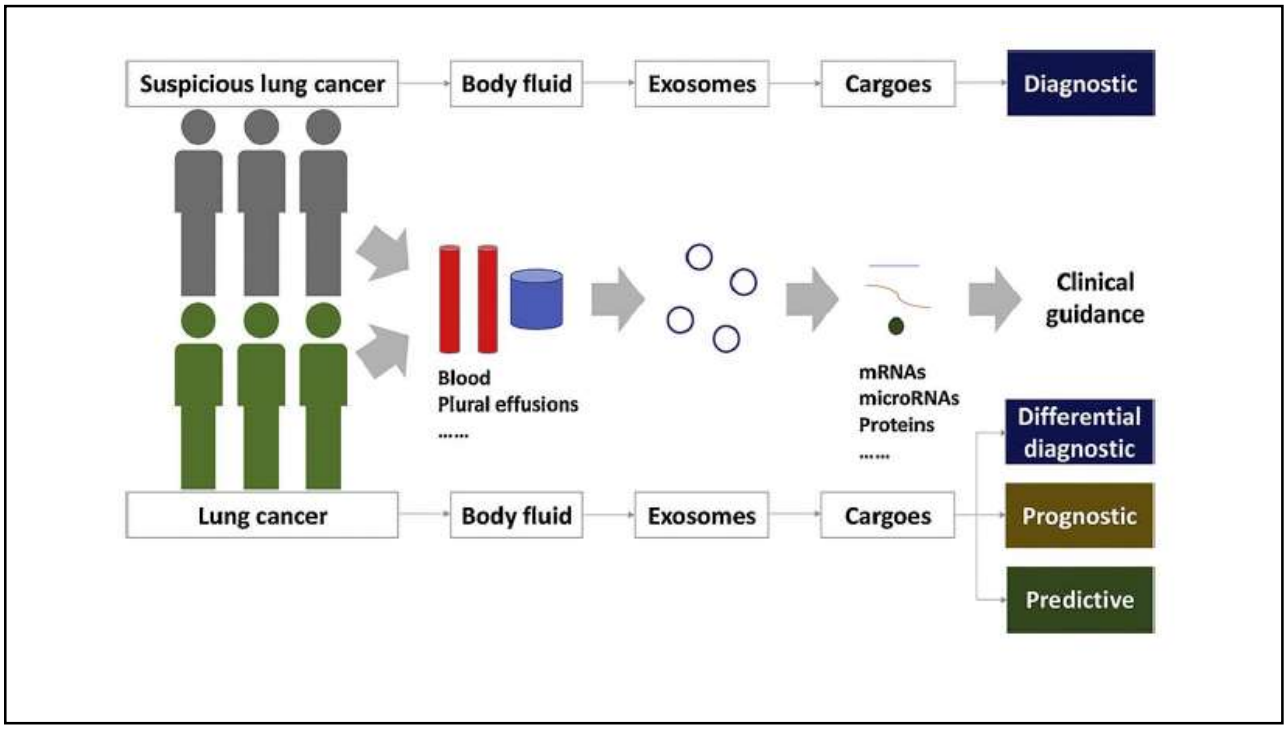
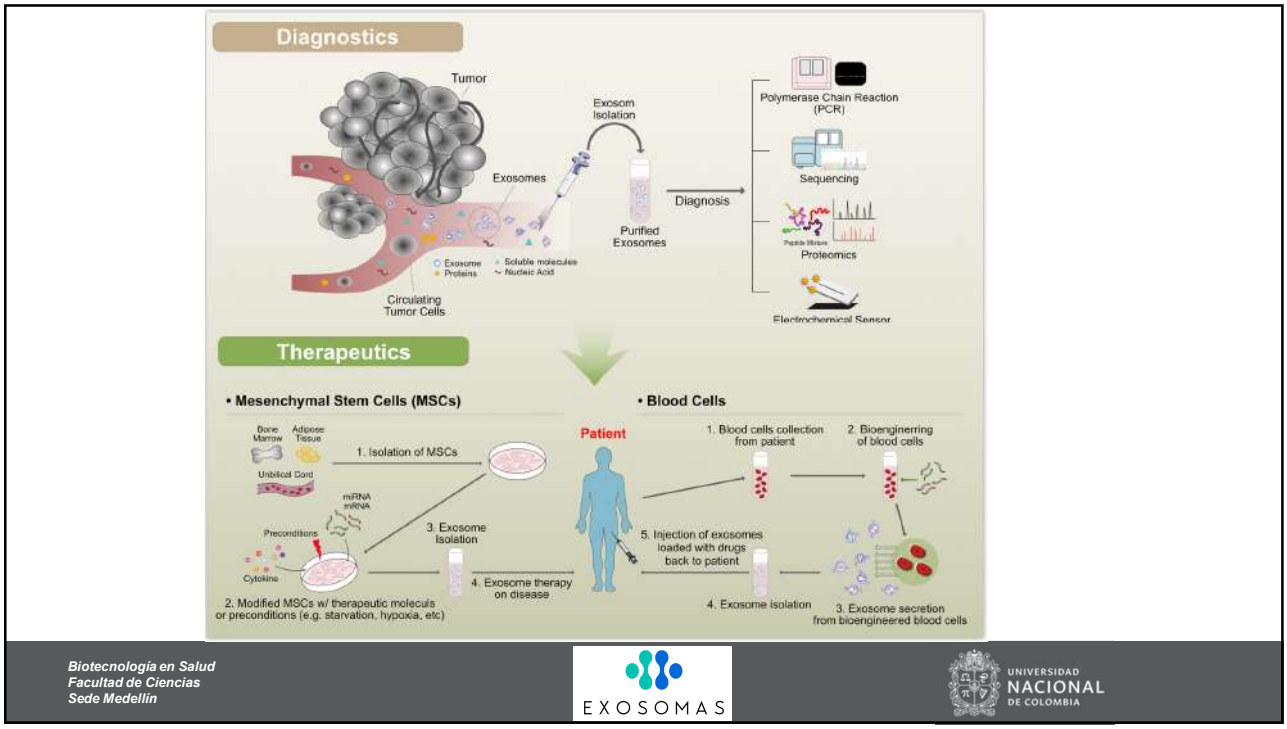

Suspicious lung cancer
Body fluid
Exosomes
Cargoes
Diagnostic
Blood
Plural effusions
.....
mRNAs
microRNAs
Proteins
.....
Clinical guidance
Differential diagnostic
Prognostic
Predictive
Lung cancer
Body fluid
Exosomes
Cargoes

31


Diagnostics
Tumor
Exosom Isolation
Exosomes
Purified Exosomes
Diagnosis
Polymerase Chain Reaction (PCR)
Sequencing
Proteomics
Circulating Tumor Cells
Therapeutics
• Mesenchymal Stem Cells (MSCs)
• Blood Cells
Patient
1. Isolation of MSCs
2. Modified MSCs w/ therapeutic molecuis or preconditions (e.g. starvation, hypoxia, etc)
3. Exosome Isolation
4. Exosome therapy on disease
1. Blood cells collection from patient
2. Bioenginerring of blood cells
3. Exosome secretion from bioengineered blood cells
4. Exosome isolation
5. Injection of exosomes loaded with drugs back to patient

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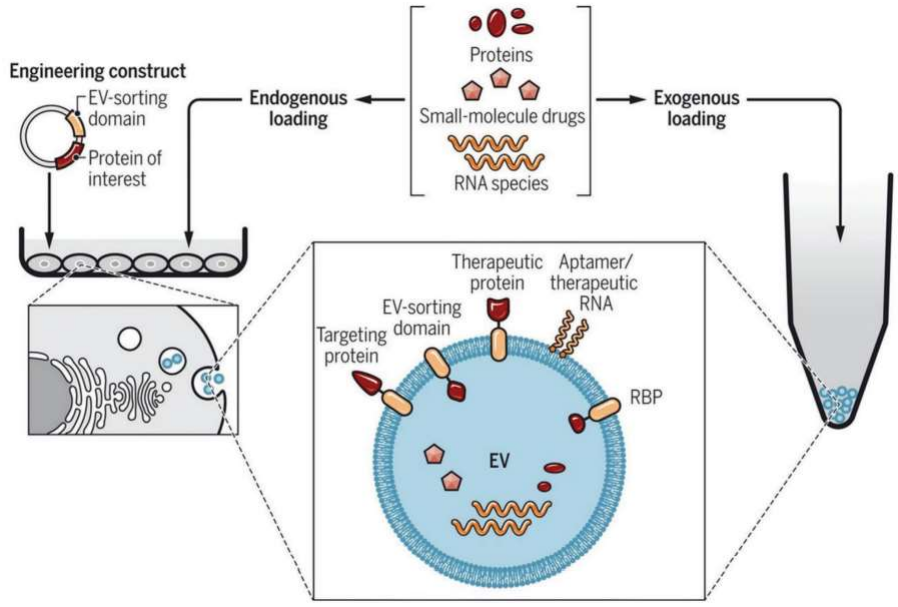

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EVs as drug delivery agents for cancer therapy.

Therapeutic Agents	Cancer Type	Ev Source	Target Cell	Remarks
			Small molecules	
Paclitaxel	Prostate	Prostate cancer cell lines, LNCaP and PC3	Prostate cancer cell lines, LNCaP and PC3	EVs isolated from the prostate cancer cells previously treated with Paclitaxel, increased the cytotoxicity of the drug in vitro against autologous prostate cancer cells.
	Pancreatic	Murine SR4987 MSCs	Human pancreatic cell line, CFPAC-1	EVs loaded with paclitaxel in vitro cancer cell proliferation.
Doxorubicin	Lung	Human lung cancer cell lines, H1299 and YRC9	Human lung cancer cell lines, H1299, A549, MRC9—lung fibroblast, HCASM—smooth muscle cells	Inhibited cancer cell growth in vitro.
	Breast and ovarian	Human breast cancer cell line, MDA-MB-231, and mouse ovarian cancer cell line, STOSE	MDA-MB-231 and STOSE (used for In Vitro experiments and also injected into mice)	In vitro: presented cytotoxicity against cancer cells. In vitro: reduced tumor volume and cardiotoxicity compared with free doxorubicin.
Paclitaxel and doxorubicin	Brain	Brain endothelial cells, bEND.3	Human brain neuronal glioblastoma—astrocytoma U-87MG xenograft in zebrafish	EVs delivered anticancer drug the blood-brain barrier to xenograft transplanted brain cancer cells. Reduced expression levels of VEGF RNAs compared with free drugs.
Cisplatin	Lung	Tumor cells previously treated	Hepatocarcinoma cells—resistant murine	Extracellular vesicles released from tumor cells containing cisplatin reversed drug resistance and

Jury et al., Cancers **2020**, *12*, 298

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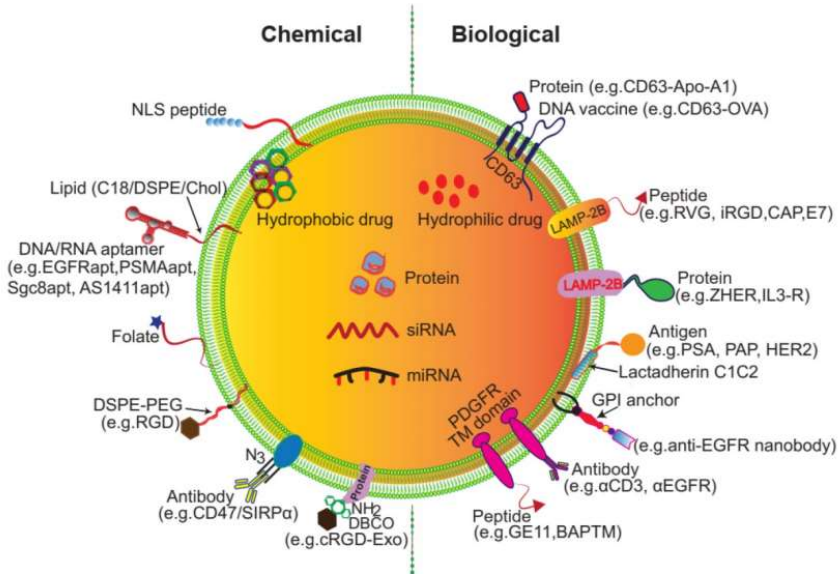
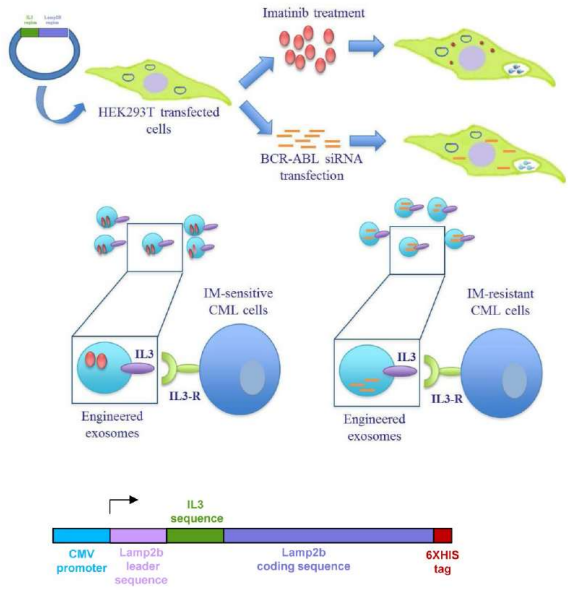
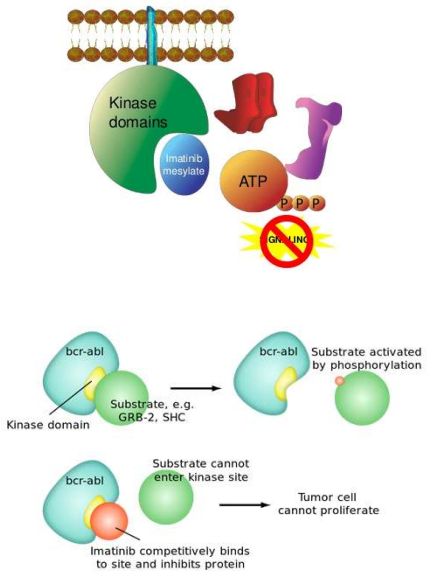
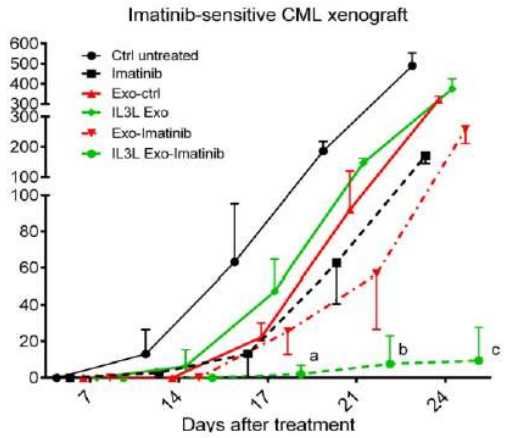


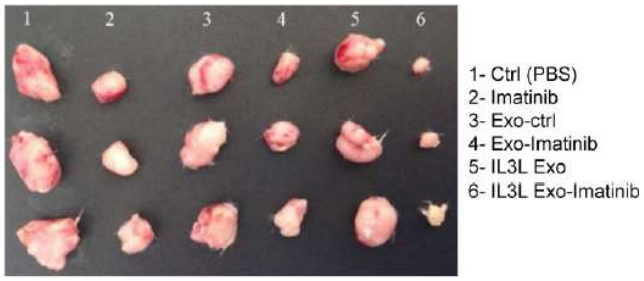
Theranostics 2021, Vol. 11, Issue 7





Theranostics 2017; 7(5):1333-1345





Theranostics 2017; 7(5):1333-1345

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Engineering strategies for targeted delivery of therapeutic exosomes to cancer cells.

Category	Method	Targeting Moiety	Target Cancer
Direct engineering of exosomes	Click chemistry	Neuropillin-1-targeting RGE peptide (RGERPPR)	Glioma
	PEGylation	Aminoethyl anisamide-PEG (AA-PEG)	Sigma receptor-overexpressing lung cancer
	Mixing with micelles	DMPE-PEG conjugated with anti-EGFR nanobody	EGFR-overexpressing tumor cells in vitro
Indirect engineering of exosomes	Conjugation with C1C2 domain	Anti-Her2 scFv	HER2-expressing breast cancer
	GPI anchorage	Anti-EGFR nanobody	EGFR-expressing breast cancer
	Conjugation with Lamp2b	α_v integrin-targeting iRGD peptide	Breast cancer cell line
		NSCLC-homing peptide Tlyp-1	Lung cancer cell line
		HER2 targeting DARPins	HER2-expressing breast cancer
	Conjugation with CD63	Apo-A1	Hepatocellular carcinoma
	Conjugation with CD47	U87-targeting CDX peptide, GL261-targeting CREKA peptide	U87 glioblastoma cell, GL261 glioma cell

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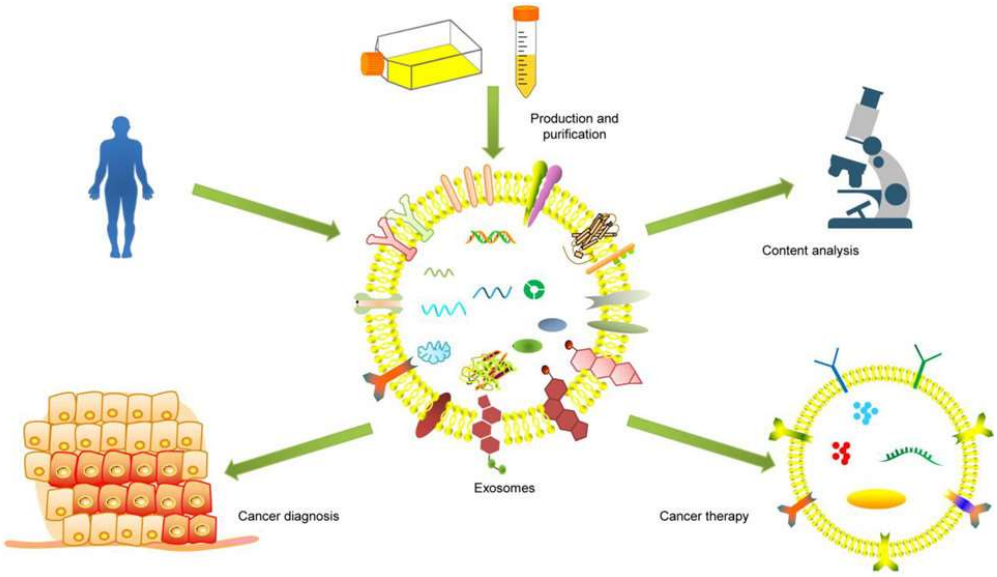

Mol. Pharmaceutics 2019, 16, 8, 3333–3349

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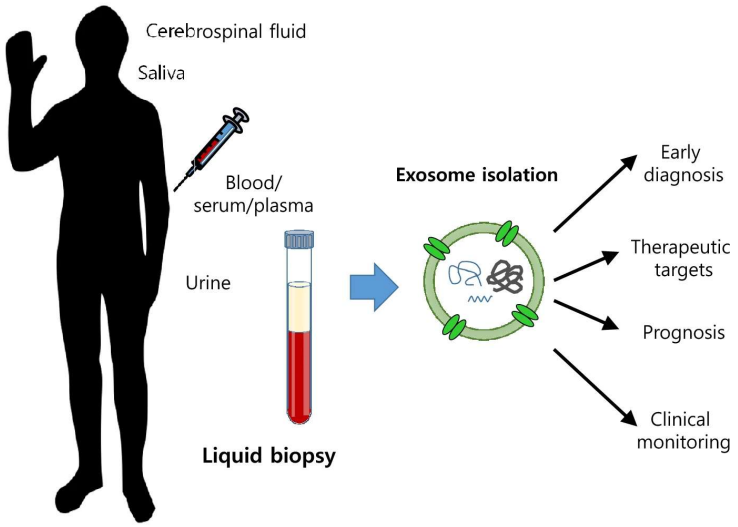

Biomarcadores

Proteicos

miRNA

DNA

Plasma

Orina

Cáncer de mama

NSCLC

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EXOSOMAS
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