In Health and Disease

11-12 October 2017 - Milano

Index Kick off meeting

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EV SEARCH

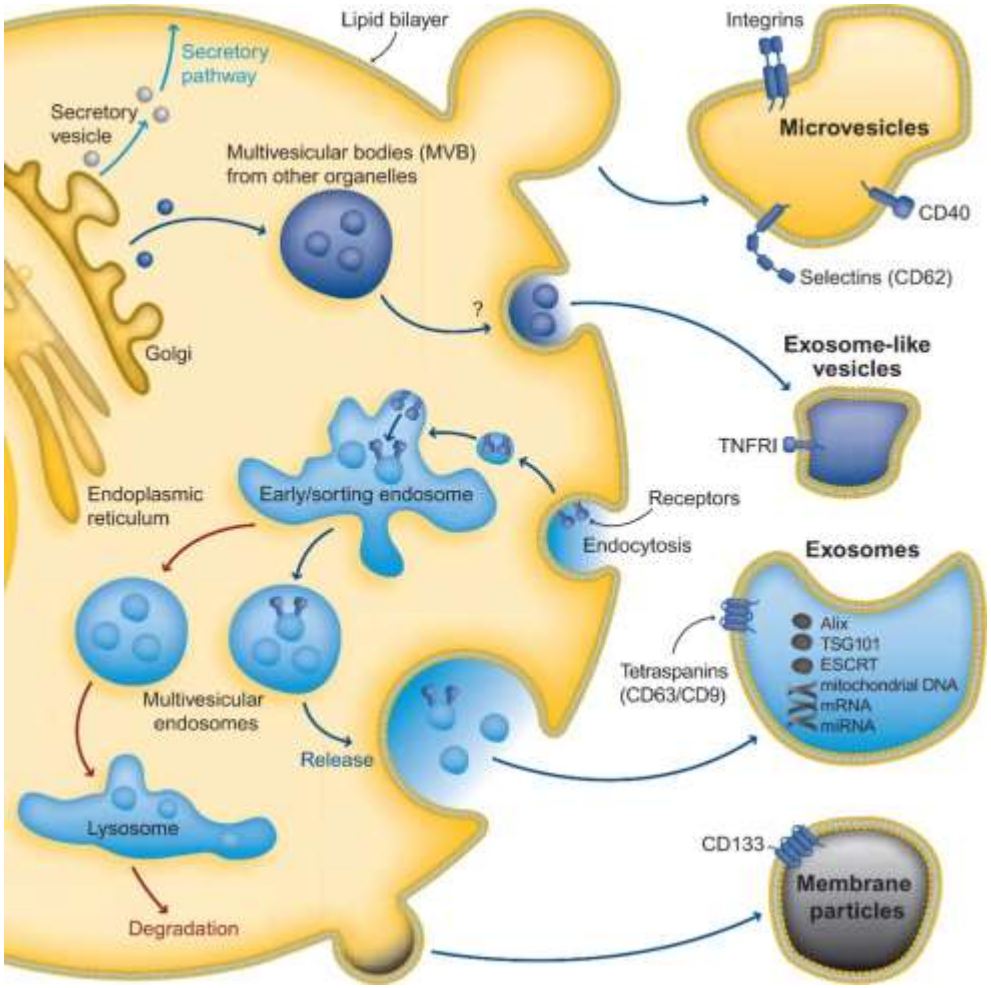
EXTRACELLULAR VESICLE RESEARCH CENTER DENMARK

NORTH DENMARK REGION

AALBORG UNIVERSITY HOSPITAL

WWW.EVSEARCH.DK

Extracellular Vesicles



Adapted from Abcam.com

Microvesicles

Size: 100-1000nm
Markers: Integrins, selectins, CD40 ligand

Exosome-like vesicles

Size: 20-50nm
Markers: TNF RI

Exosomes

Size: 50-100nm
Markers: Tetraspanins (CD9,CD63,CD81), Alix, TSG101

Membrane particles

Size: 50-80nm
Markers: CD133, no CD63

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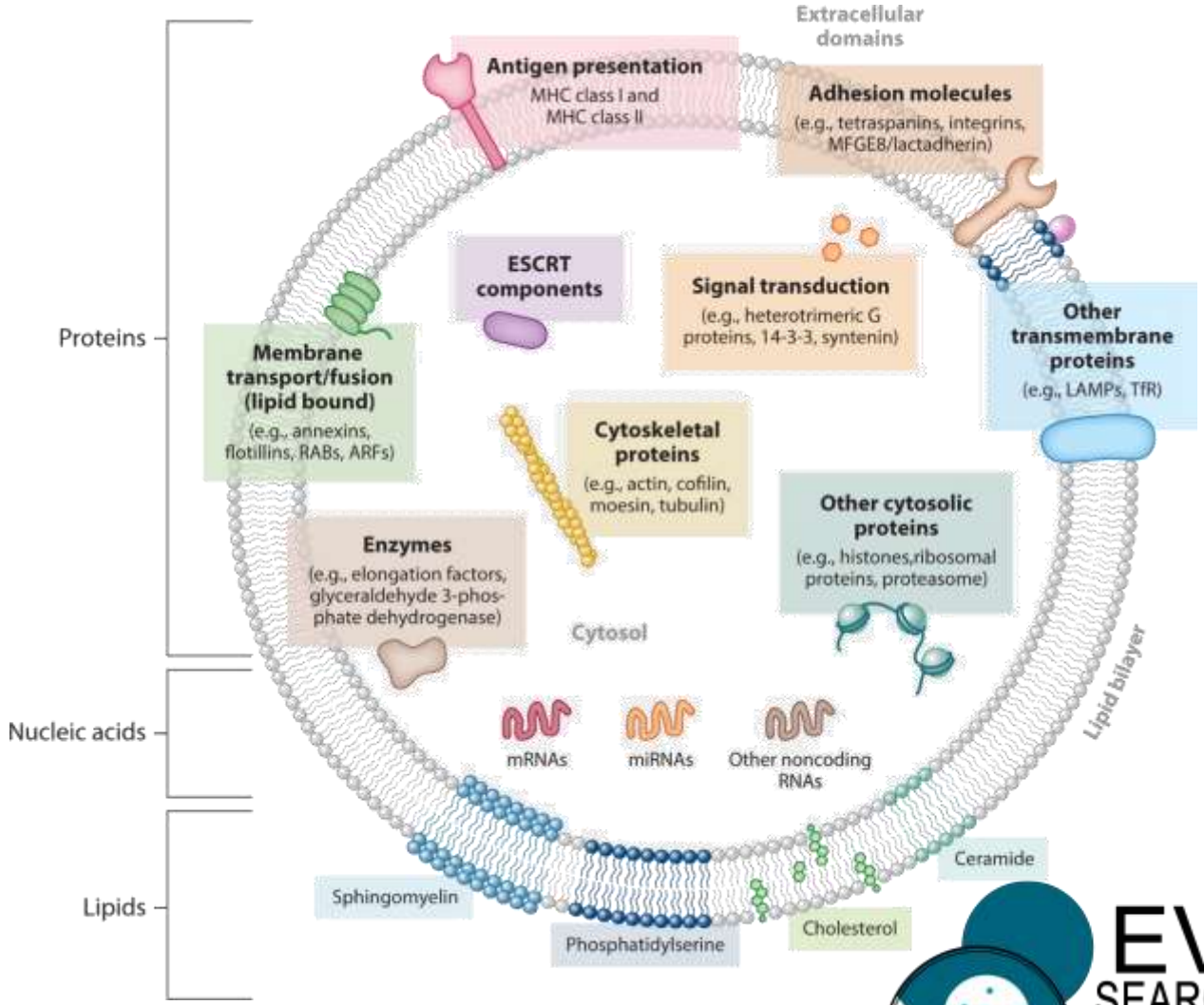
Overall Composition of Extracellular Vesicles



Adapted from Colombo et al., Ann Rev Cell Dev Biol (2014)

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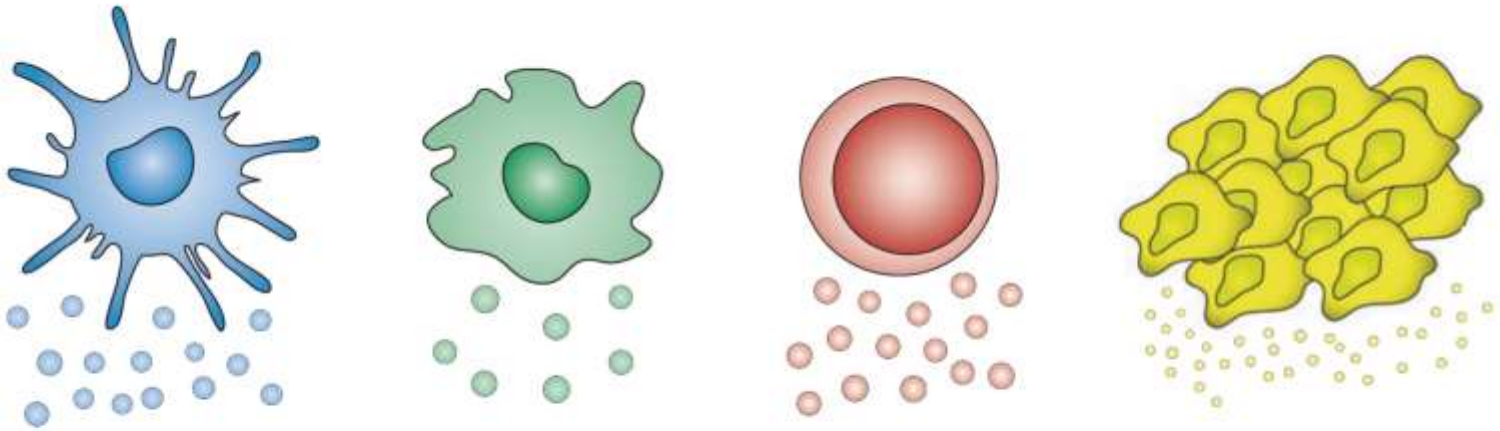
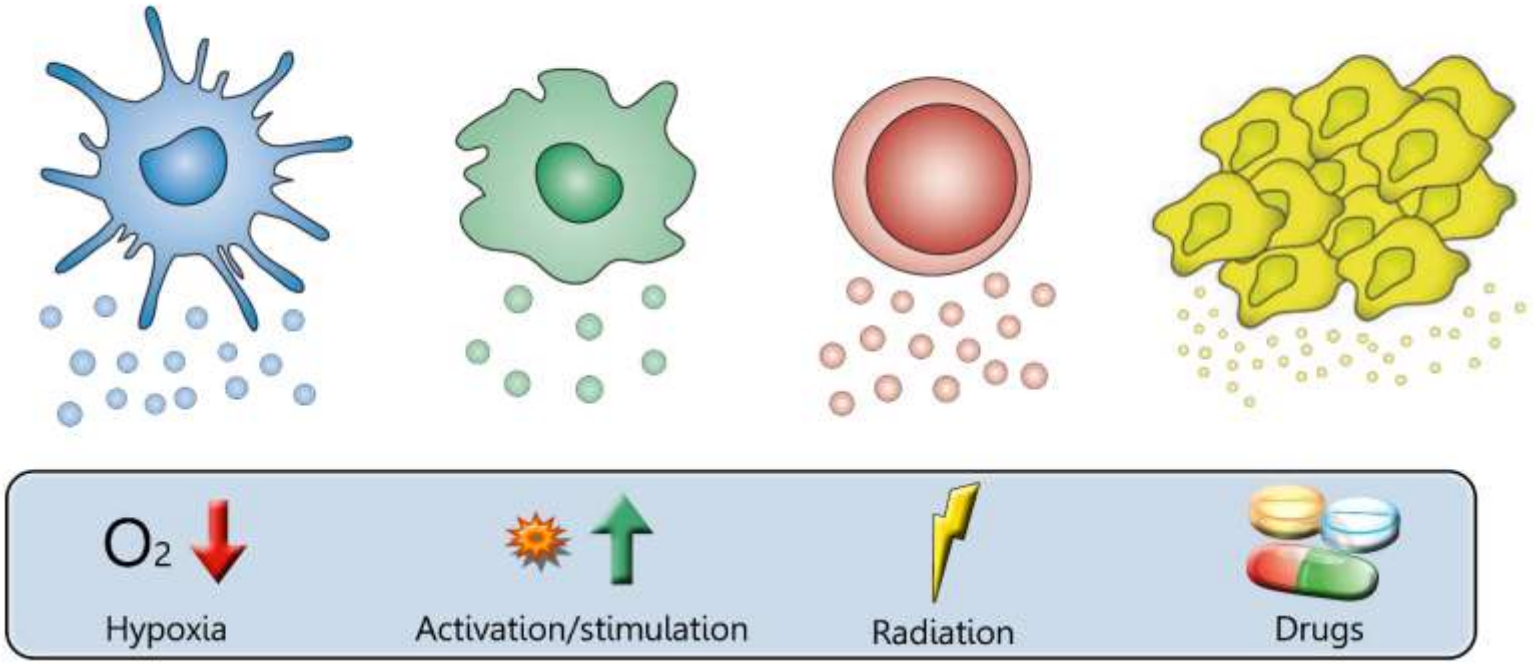
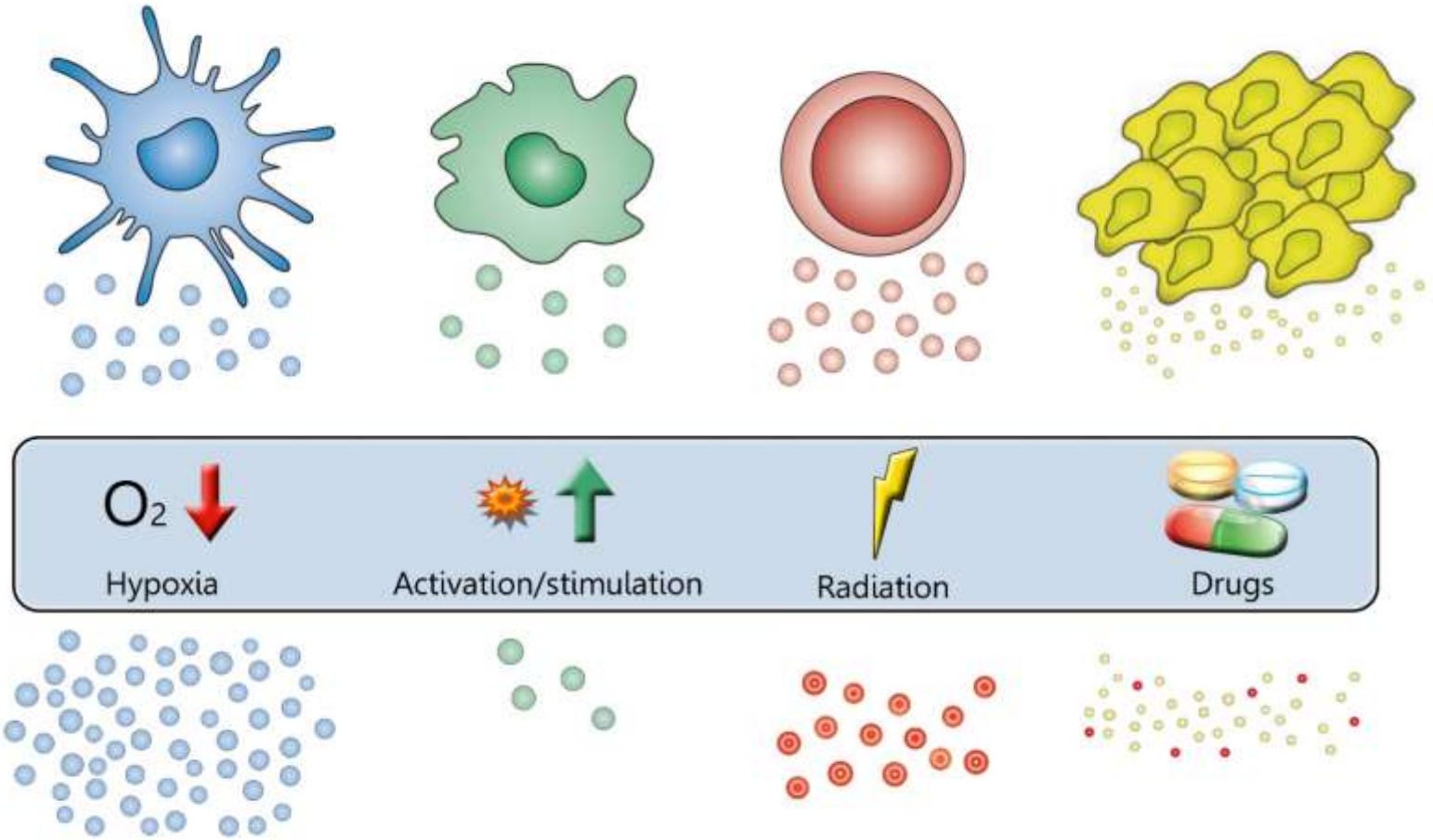


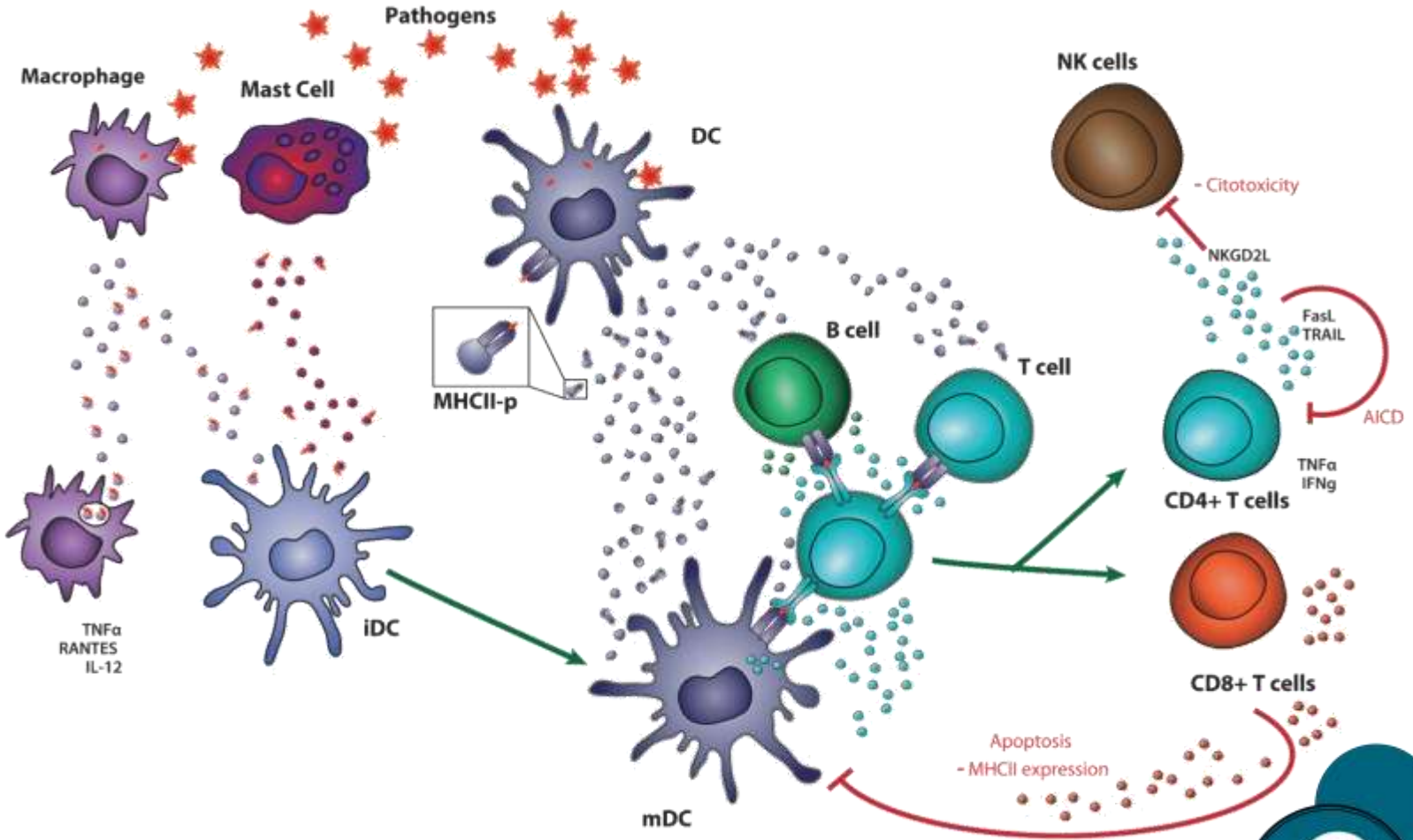
EV SEARCH



EV SEARCH


O2
Hypoxia
Activation/stimulation
Radiation
Drugs

EVs as Immune Regulators



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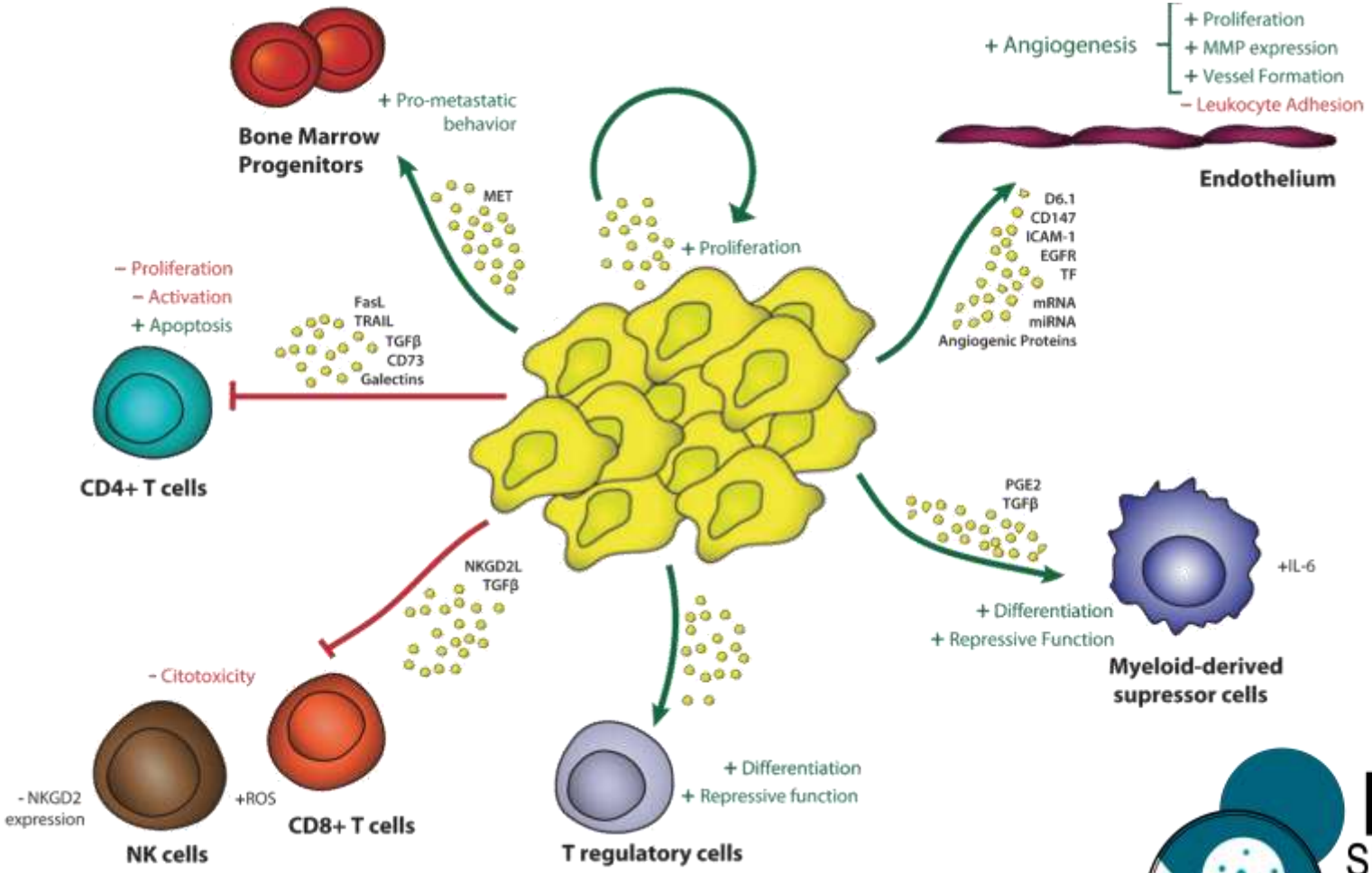
EVs in Cancer and Immune Respons



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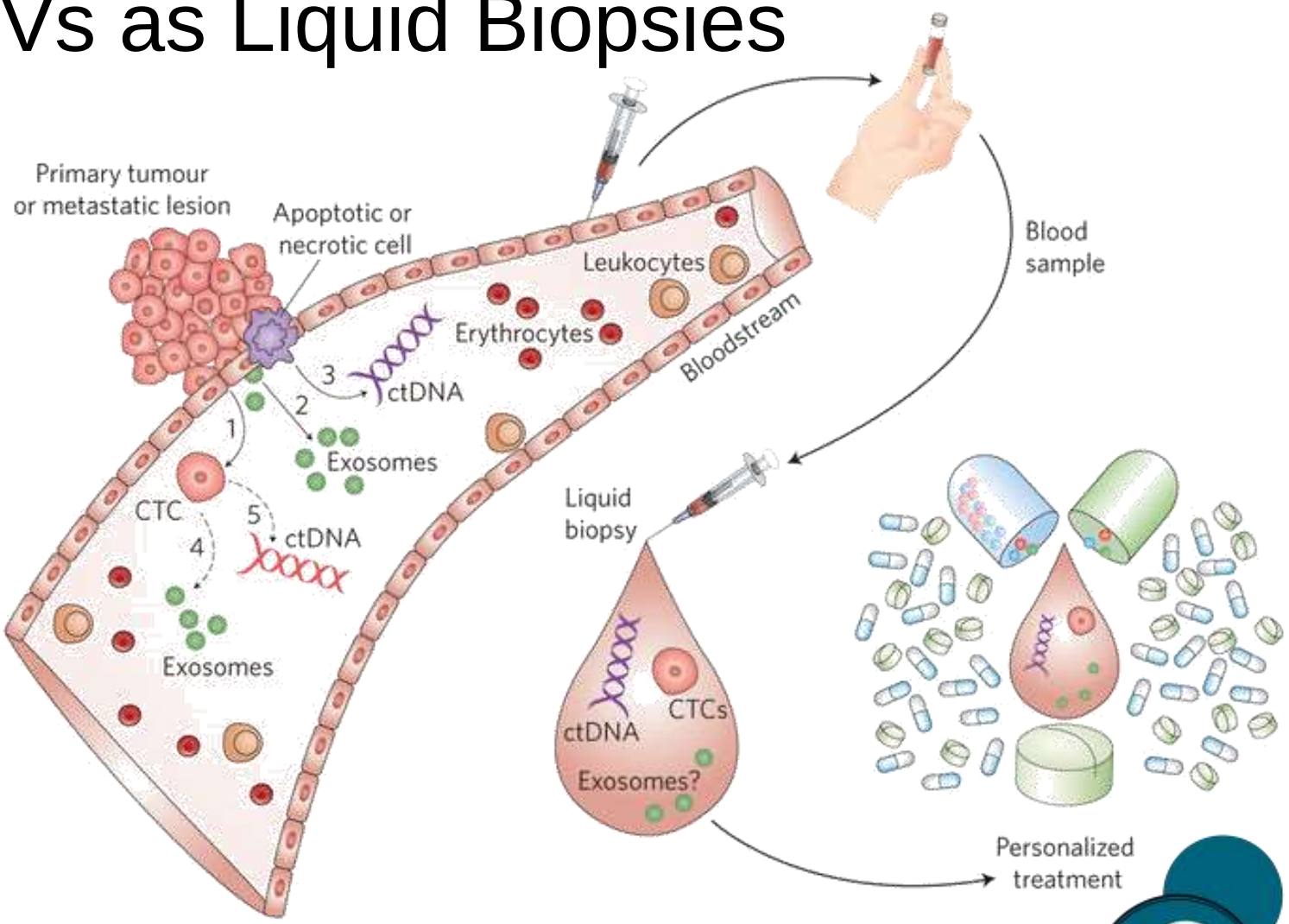
EVs as Liquid Biopsies



Alix-Panabiéres and Pantel, K. Cancer Discov. (2016)

EV SEARCH

Journal of Extracellular Vesicles

ORIGINAL RESEARCH ARTICLE

Extracellular Vesicle (EV) Array: microarray capturing of exosomes and other extracellular vesicles for multiplexed phenotyping

Malene Jørgensen[1]*, Rikke Bæk[1], Shona Pedersen[2], Evo K.L. Søndergaard[1], Søren R. Kristensen[2] and Kim Varming[1]

[1]Department of Clinical Immunology, Aalborg University Hospital, Aalborg, Denmark; [2]Department of Biochemical Chemistry, Aalborg University Hospital, Aalborg, Denmark

Journal of Extracellular Vesicles 2013, **2**

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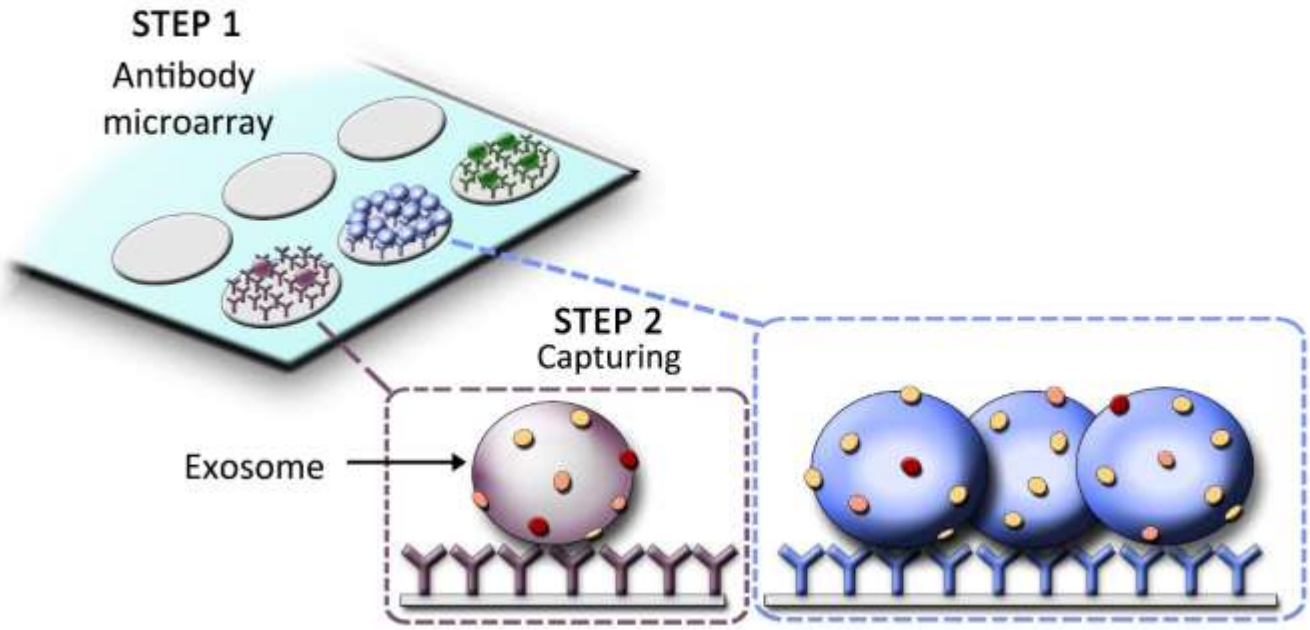
Principle of the EV Array



Bæk and Jørgensen (2017) Meth. Mol. Biol.; Jørgensen et al., JEV (2013, 2015)

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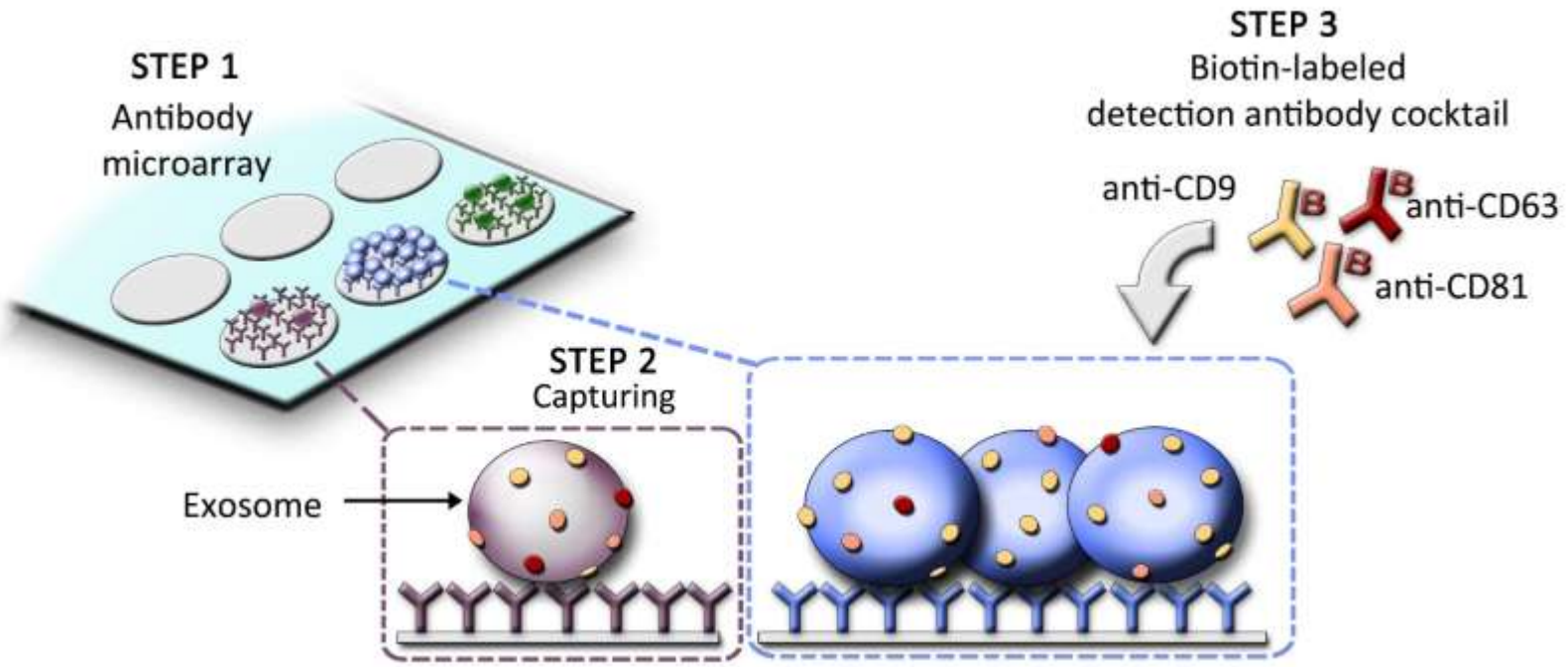
Principle of the EV Array



Bæk and Jørgensen (2017) Meth. Mol. Biol.; Jørgensen et al., JEV (2013, 2015)

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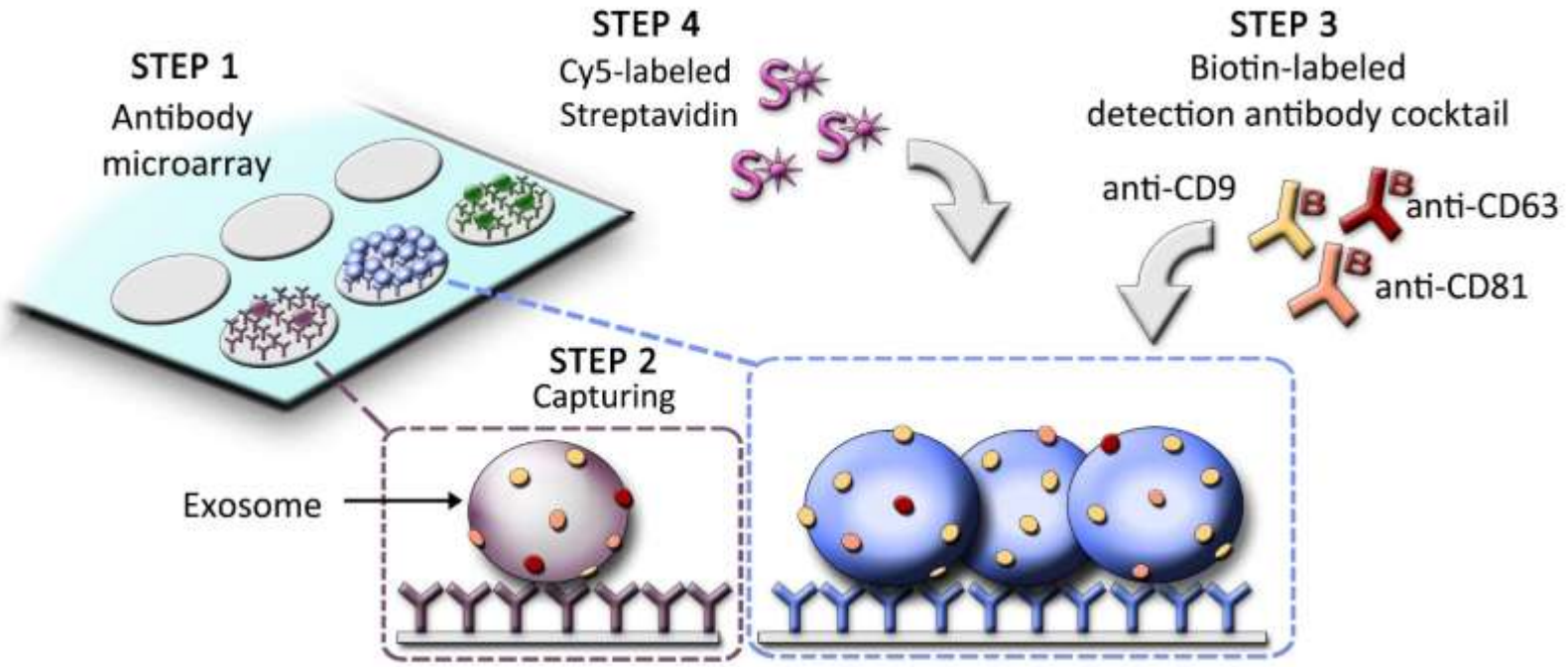
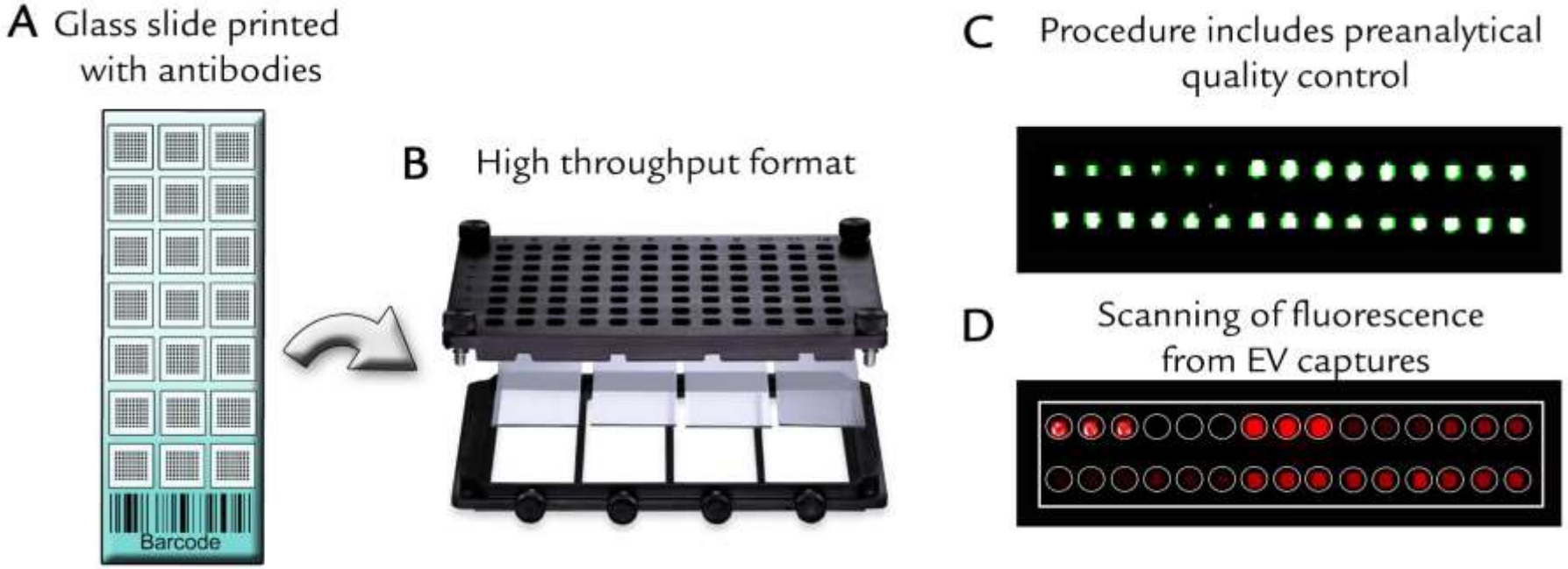
Principle of the EV Array



Bæk and Jørgensen (2017) Meth. Mol. Biol.; Jørgensen et al., JEV (2013, 2015)

EV SEARCH



Bæk and Jørgensen (2017) Meth. Mol. Biol.

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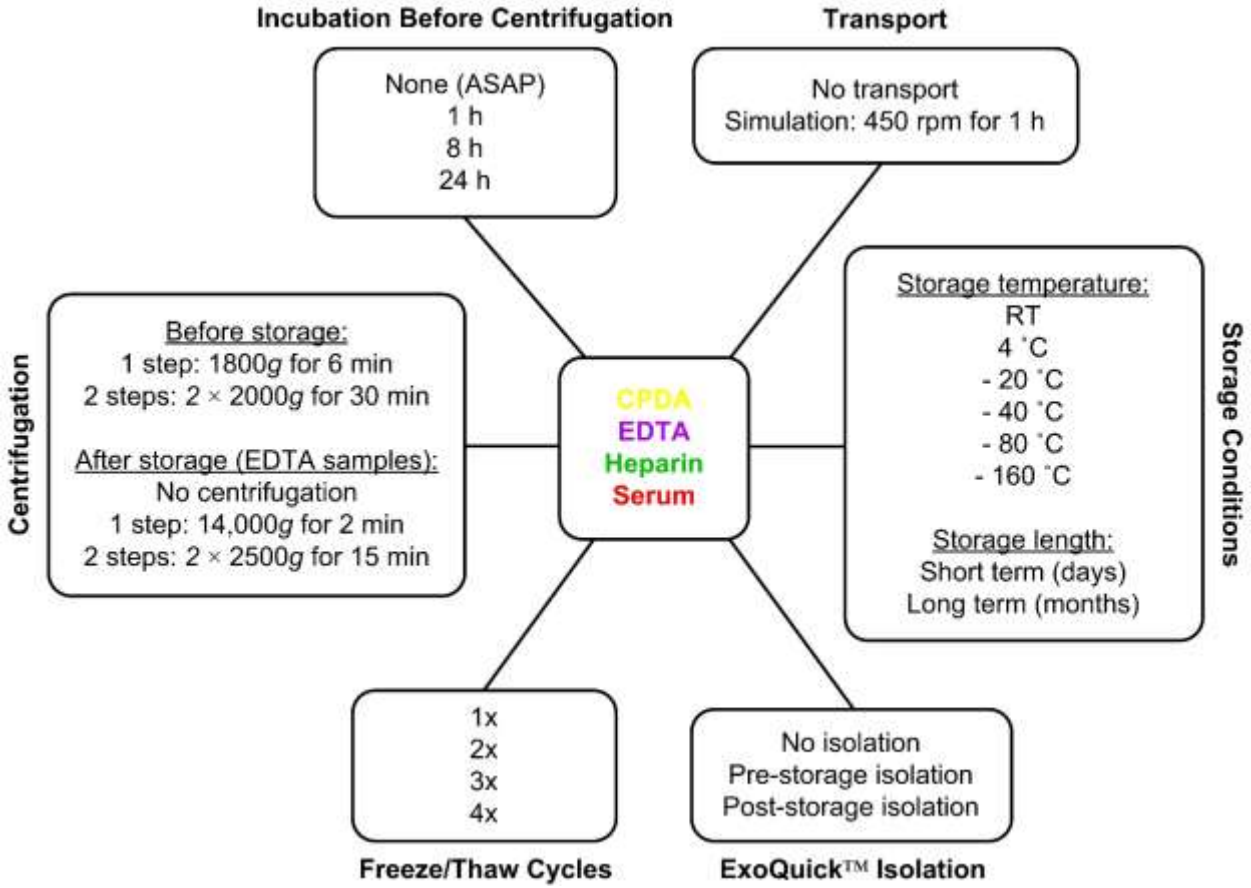
Clinical Robustness of the EV Array

The impact of various pre-analytical treatments of plasma



Bæk and Jørgensen, J. Imm. Meth. 2016

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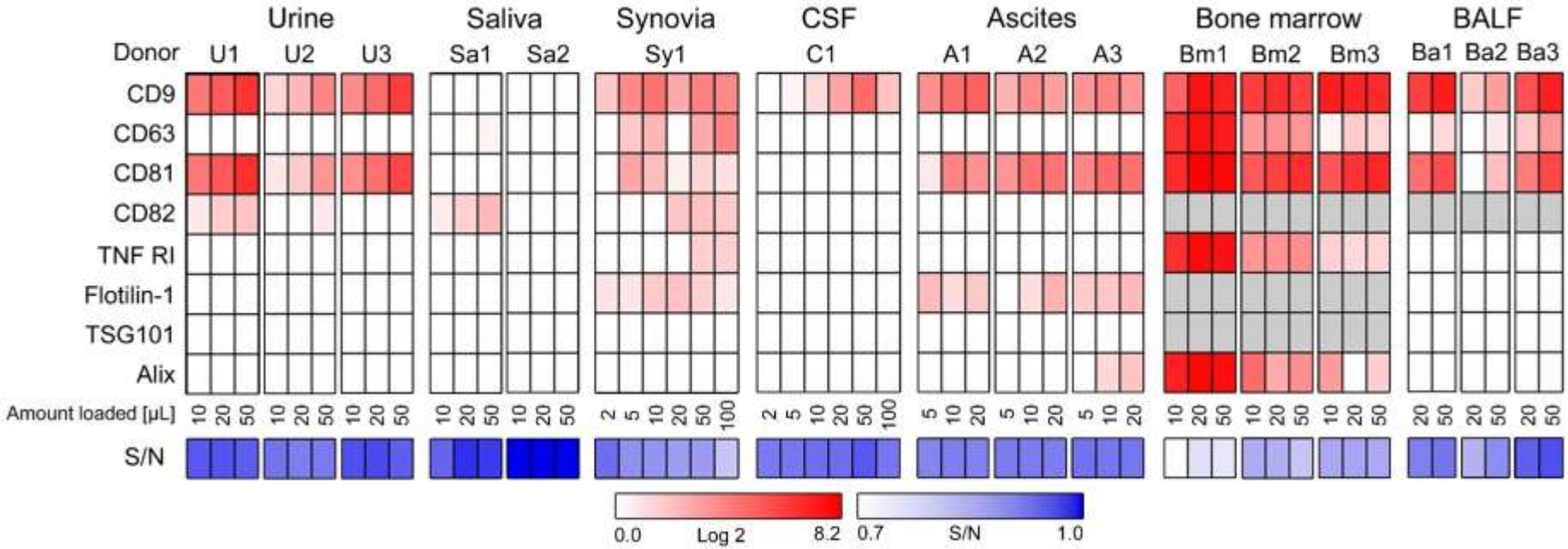
Clinically relevant body fluids



Jørgensen, et al., (2016), EV special issue, Nova Publishers.

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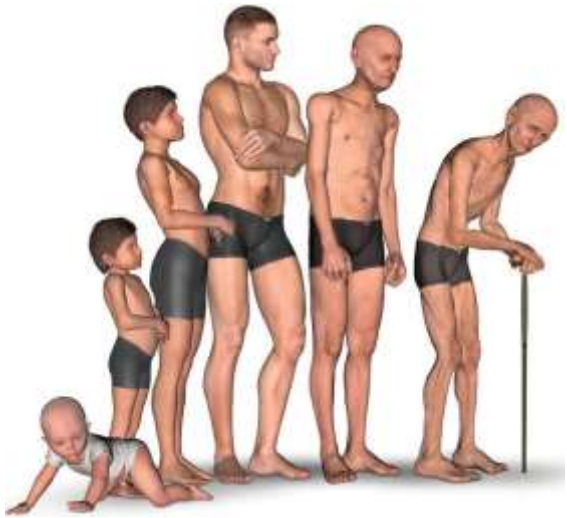
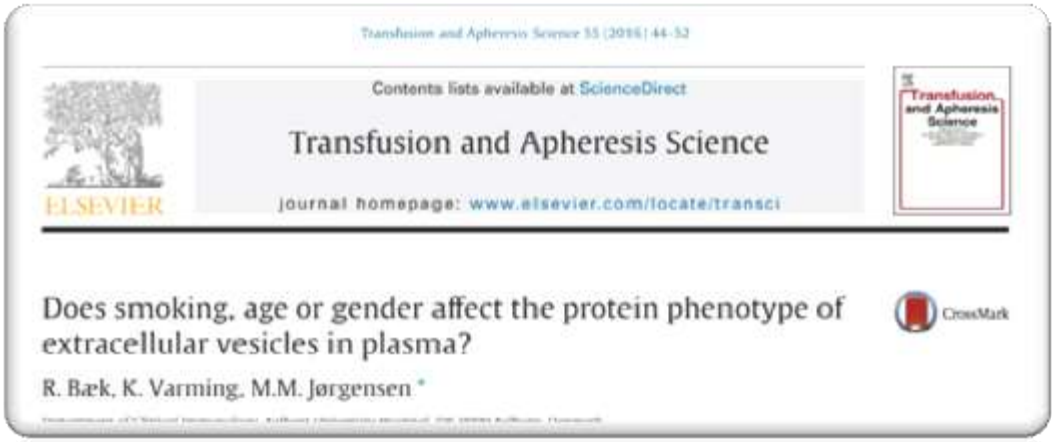
Aims of the studies….

In order to use EVs in a clinical setting, it is of great importance to know whether the characteristics of EVs in a healthy cohort are dependent on demographic parameters
such as race, gender, age or smoking status.



EV SEARCH



Transfusion and Apheresis Science 55 (2016) 44–52

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

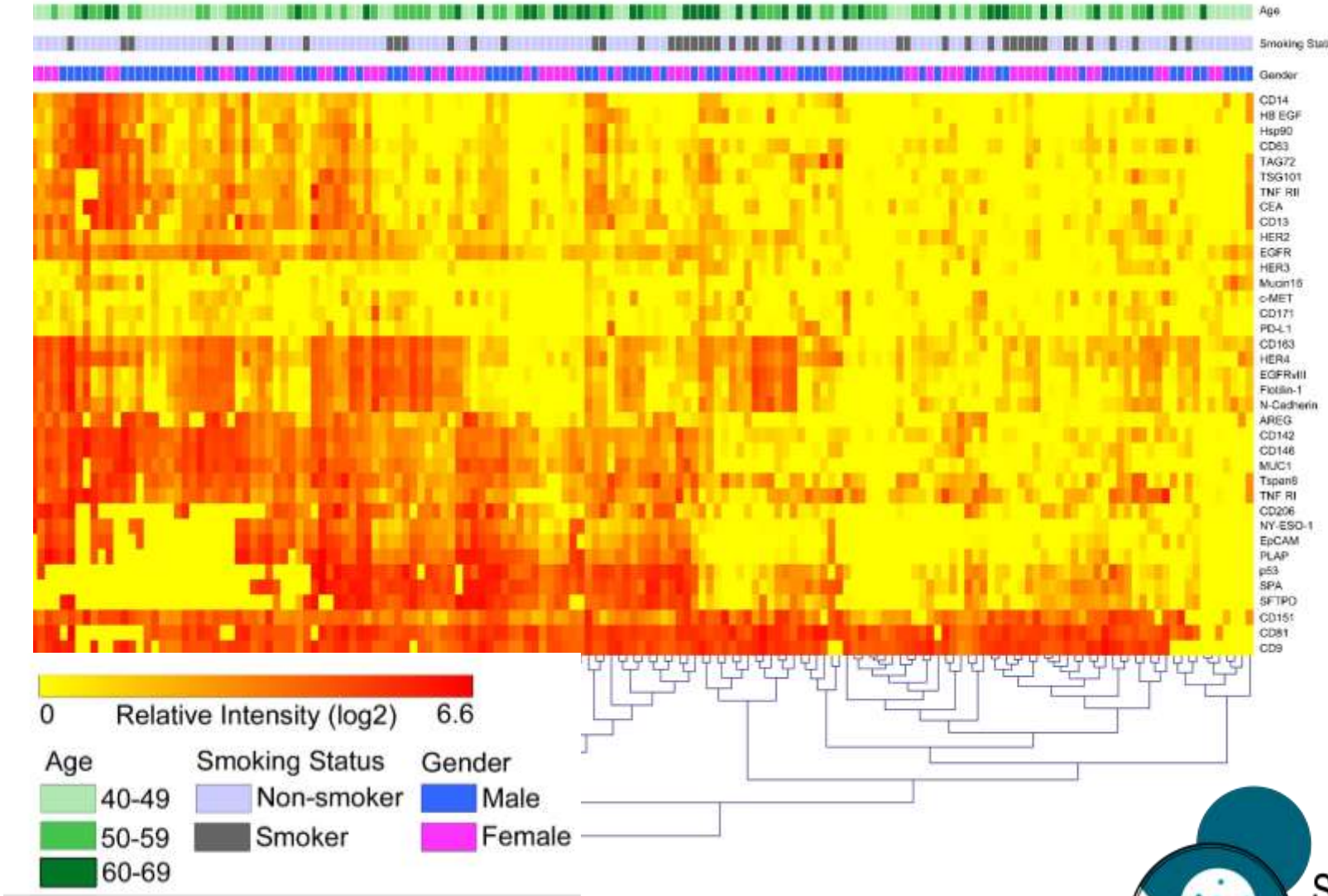
journal homepage: www.elsevier.com/locate/transci

Does smoking, age or gender affect the protein phenotype of extracellular vesicles in plasma?

R. Bæk, K. Varming, M.M. Jørgensen *

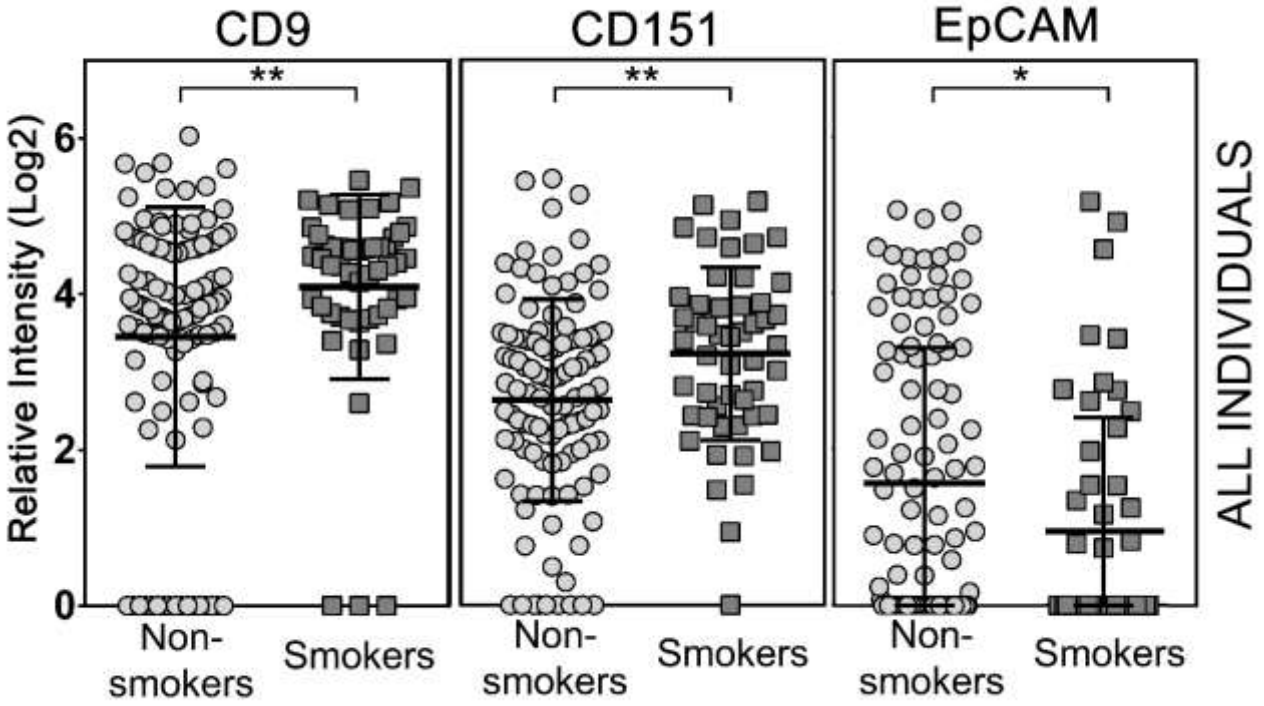
Table 1
Demographic characteristics of the 161 included individuals according to gender, smoking status and age.

	Female	Male
All individuals	**71**	**90**
Non-smokers	**48**	**62**
Age 40–49	19	25
Age 50–59	17	25
Age 60–69	12	12
Smokers	**23**	**28**
Age 40–49	10	8
Age 50–59	7	13
Age 60–69	6	7


Age
Smoking Status
Gender
CD14
HB EGF
Hsp90
CD63
TAG72
TSG101
TNF RII
CEA
CD13
HER2
EGFR
HER3
Mucin16
c-MET
CD171
PD-L1
CD163
HER4
EGFRvIII
Flotilin-1
N-Cadherin
AREG
CD142
CD146
MUC1
Tspan8
TNF RI
CD206
NY-ESO-1
EpCAM
PLAP
p53
SPA
SFTPD
CD151
CD81
CD9
0
Relative Intensity (log2)
6.6
Age
40-49
50-59
60-69
Smoking Status
Non-smoker
Smoker
Gender
Male
Female

EV SEARCH

Changes in markers with relation to smoking



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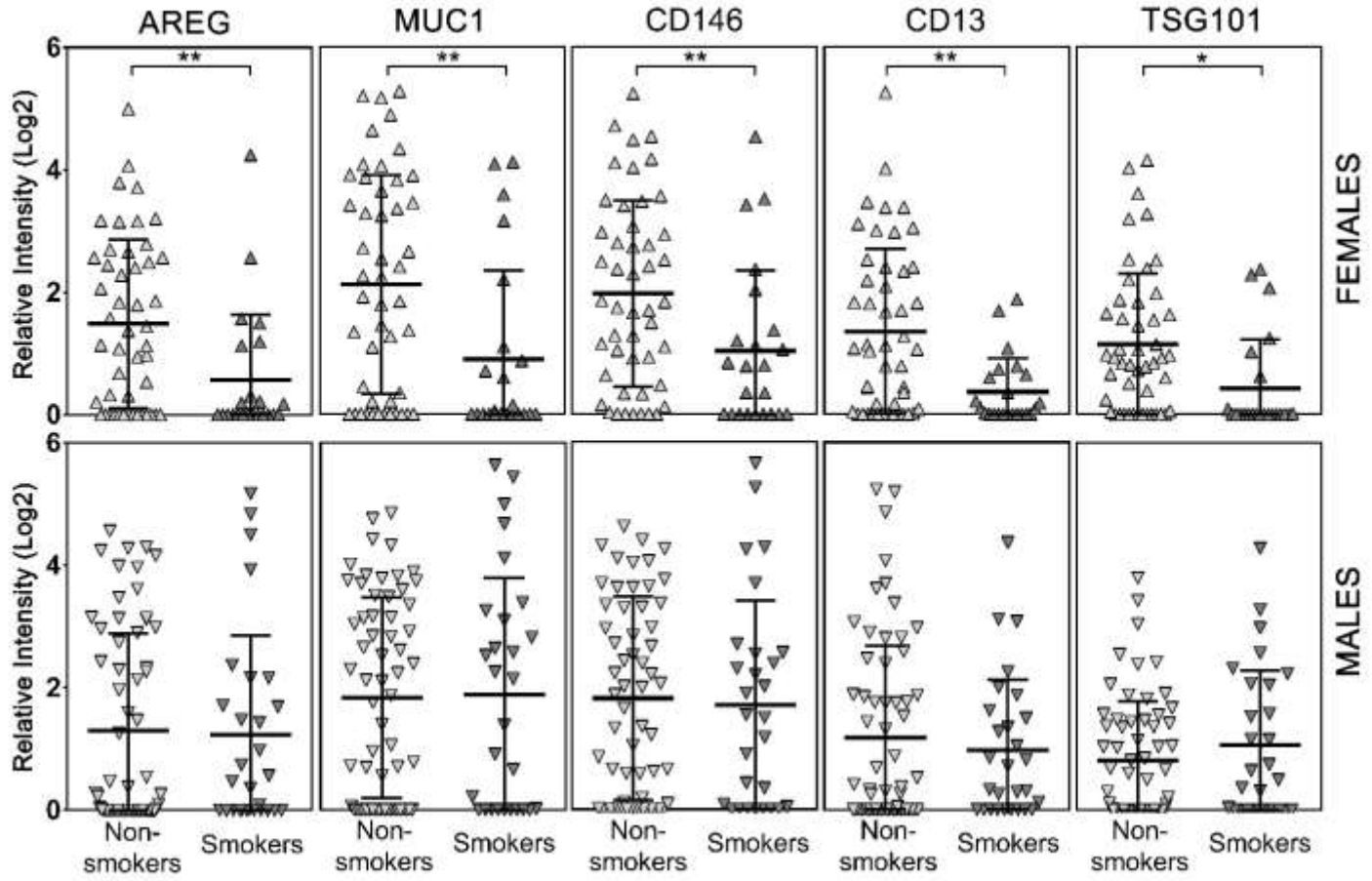
Changes in markers with relation to smoking



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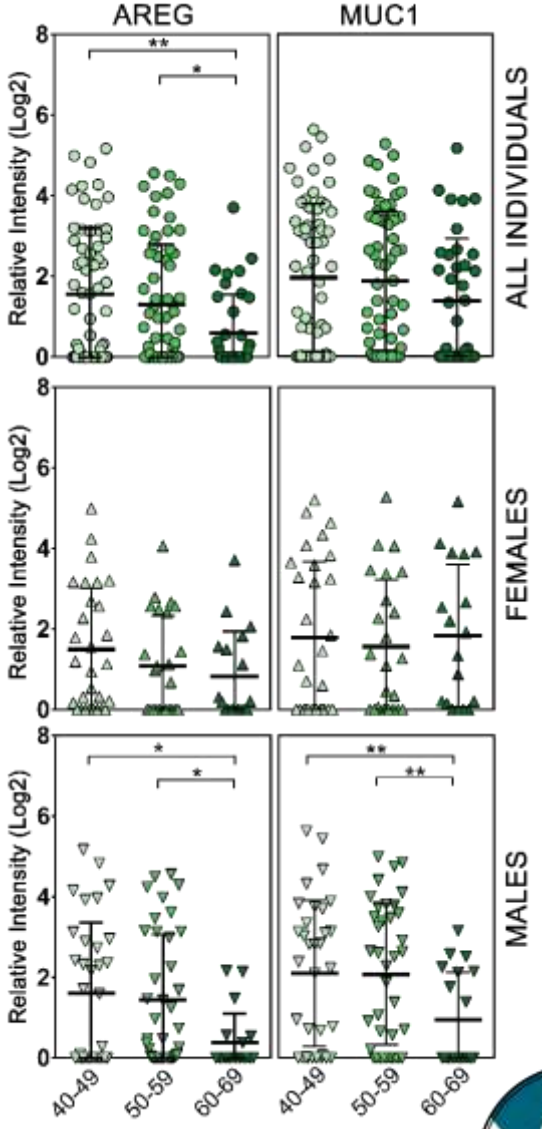
Changes in markers with relation to age



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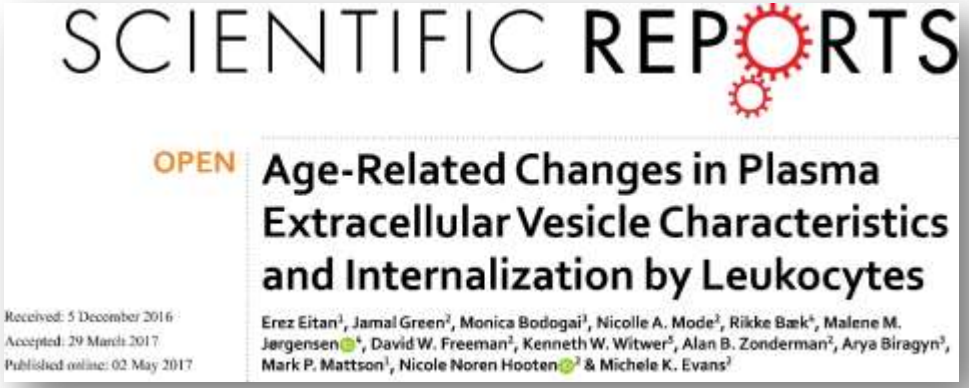


SCIENTIFIC REPORTS

OPEN

Age-Related Changes in Plasma Extracellular Vesicle Characteristics and Internalization by Leukocytes

Received: 5 December 2016
Accepted: 29 March 2017
Published online: 02 May 2017

Erez Eitan[1], Jamal Green[2], Monica Bodogai[1], Nicolle A. Mode[2], Rikke Bæk[4], Malene M. Jørgensen[4], David W. Freeman[2], Kenneth W. Witwer[5], Alan B. Zonderman[2], Arya Biragyn[3], Mark P. Mattson[1], Nicole Noren Hooten[2] & Michele K. Evans[2]

NIH National Institute on Aging
Turning Discovery Into Health

Healthy Aging in Neighborhoods of Diversity across the Life Span
HANDLS

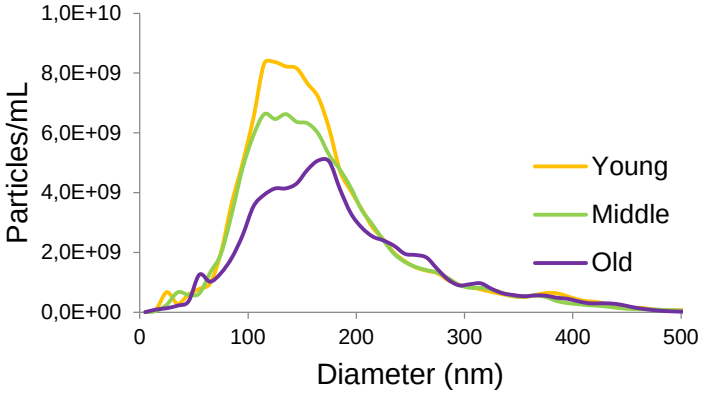
Healthy Aging in Neighborhoods of Diversity across the Life Span

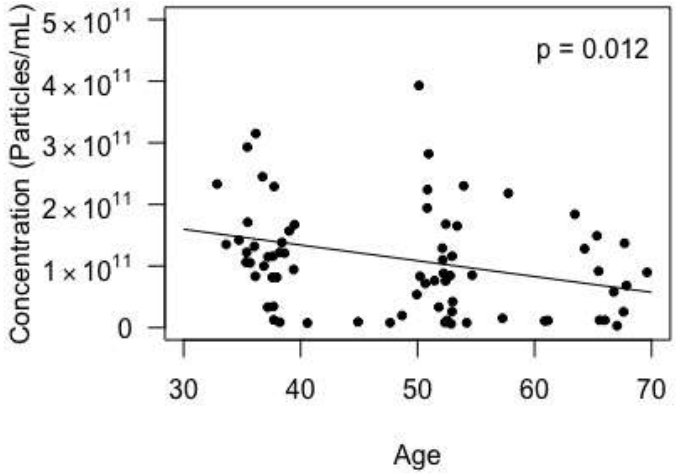
- HANDLS is planned as a 20-year longitudinal study.
- HANDLS participants are a fixed cohort of 3,720 community-dwelling African American and white adults aged 30-64.

74 persons (small subset)
– samples with a 5-year span

EV SEARCH

NTA (Nanoparticle Tracking Analysis)





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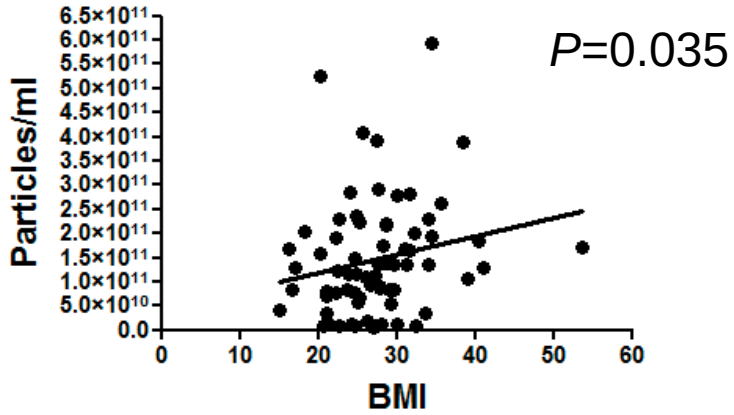
NTA (Nanoparticle Tracking Analysis)

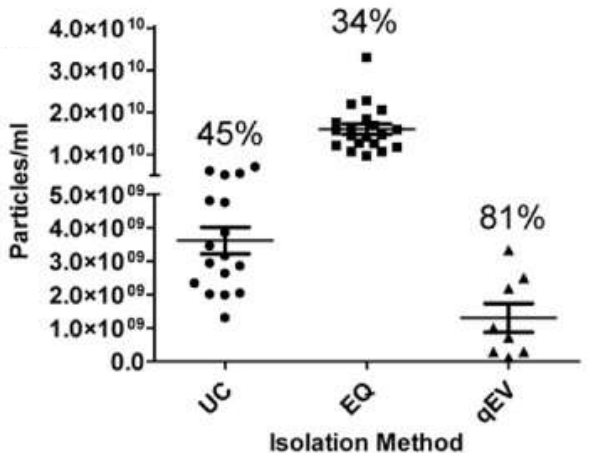
	Visit			P Value
		Male	Female	
Sex	1	1.10E + 11 ± 0.93E + 11	1.08E + 11 ± 0.89E + 11	0.928
	2	1.09E + 11 ± 0.87E + 11	1.09E + 11 ± 1.16E + 11	0.796
		Whites	African American	
Race	1	1.01E + 11 ± 0.92E + 11	1.17E + 11 ± 0.90E + 11	0.433
	2	1.05E + 11 ± 1.28E + 11	1.19E + 11 ± 0.69E + 11	0.542
		Smoker	Non-Smoker	
Smoking	1	1.20E + 11 ± 1.02E + 11	0.96E + 11 ± 0.79E + 11	0.287
	2	1.66E + 11 ± 1.30E + 11	0.89E + 11 ± 0.75E + 11	0.002



P=0.035

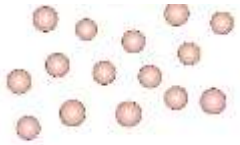
EV SEARCH

Which cells take up the vesicles?



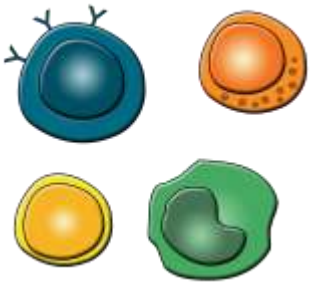
ExoQuick

Plasma



Isolated vesicles

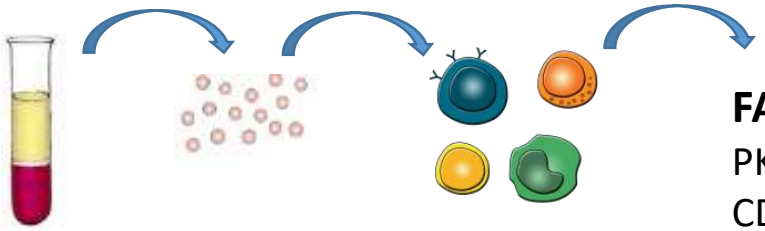
- Membrane staining (PKH26)
- Size column
- NTA



PBMC

FACS

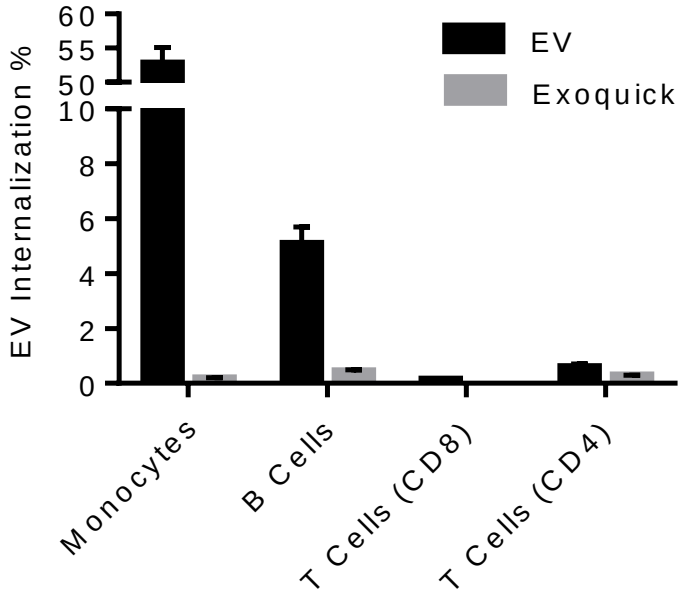
EV SEARCH

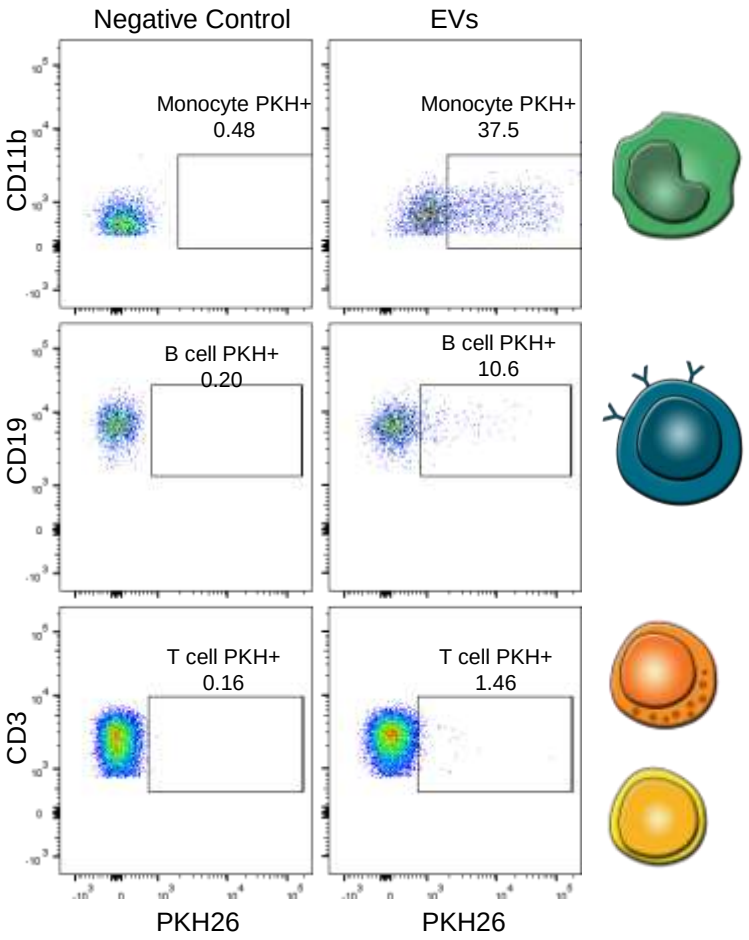


74 donors

PBMC
5 donors
24h incubation

FACS
PKH26
CD11b
CD14
CD15
CD19
CD3
CD4
CD8





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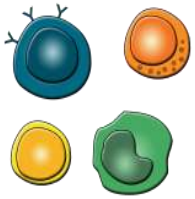
EV uptake by B-cells

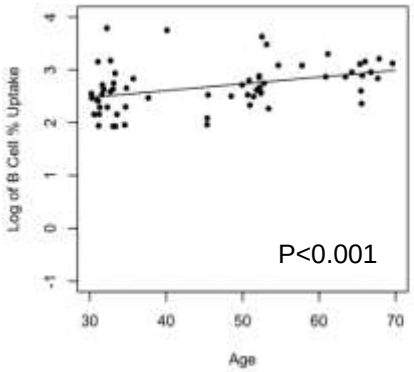
62 donors



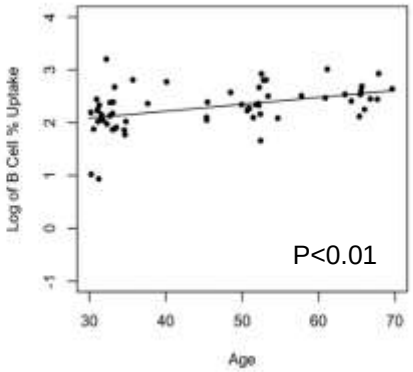
PBMC
4 donors

FACS

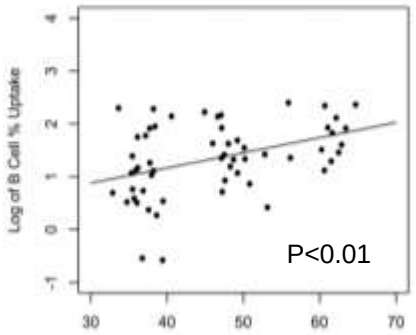
PBMC Recipient 1 Old



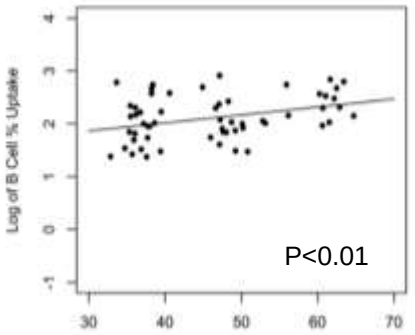
PBMC Recipient 2 Young



PBMC Recipient 3 Old



PBMC Recipient 4 Young



Conclusion:

EVs from older individuals were more readily internalized by B cells

EV SEARCH

Conclusions

- The level (numbers) of EVs in plasma decrease with advancing age.
- EV concentration was positively associated with BMI and smoking.
  - No differences with regards to gender or race
- Age, smoking and gender have an influence on the protein characteristics of the EVs in plasma.
- In healthy individuals a high percentage of monocytes internalized EVs.
- EVs from older individuals were more readily internalized by B cells.

Lung cancer

- To date lung cancer accounts for more deaths each year than any other type of cancer.
- The 5-year survival rate for non-small cell lung cancer (NSCLC) is currently 11 - 18%.
- Because the majority of NSCLC patients present the advanced stage disease, the outcome is often fatal.
- Still, diagnosing NSCLC can be challenging, and new methods to support the already used clinical tools are needed to secure a better overall outcome.

Journal of Extracellular Vesicles

ORIGINAL RESEARCH ARTICLE

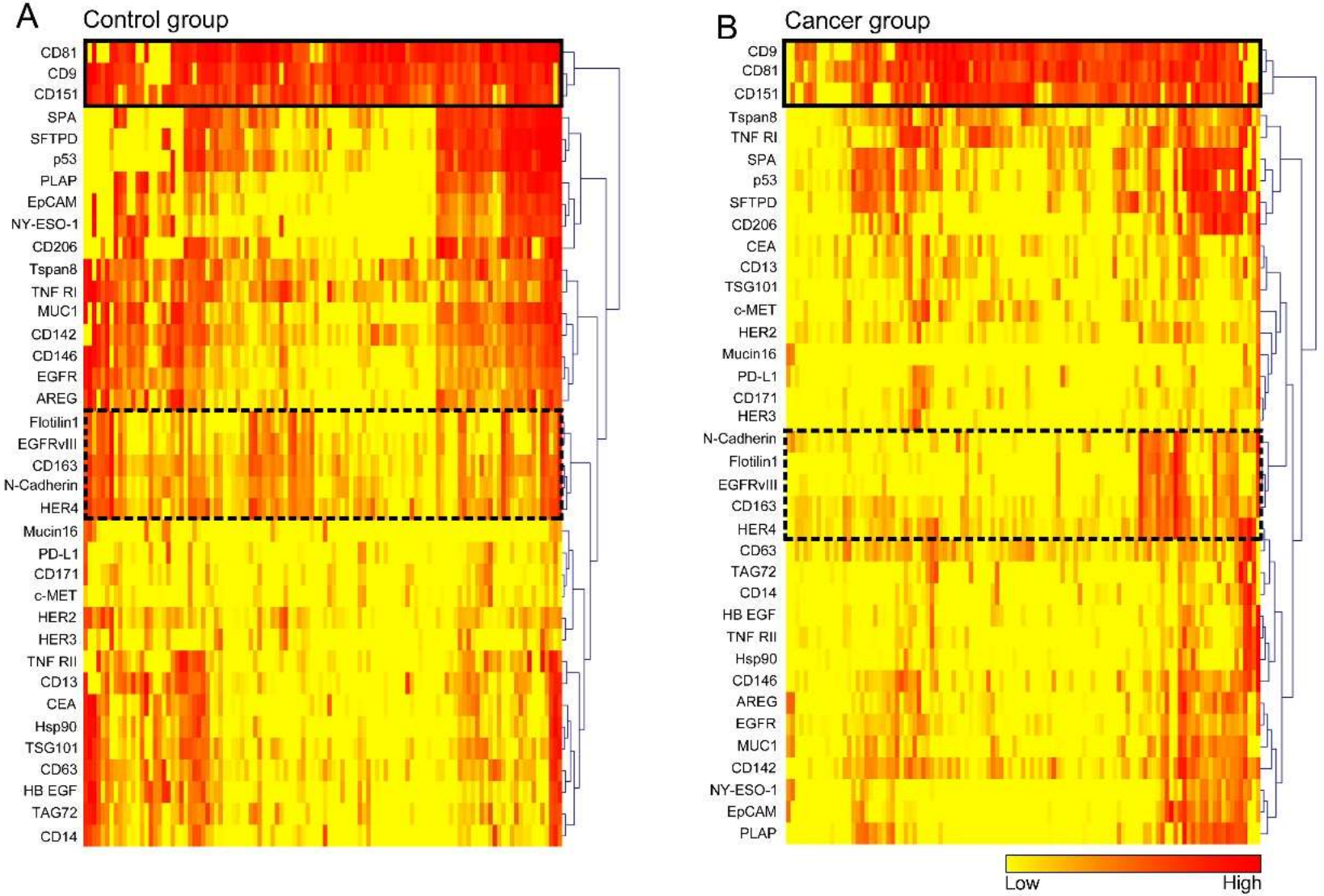
Exosomal proteins as potential diagnostic markers in advanced non-small cell lung carcinoma

Kristine R. Jakobsen[1,2], Birgitte S. Paulsen[1,3], Rikke Bæk[4], Kim Varming[4], Boe S. Sorensen[1] and Malene M. Jørgensen[4]*

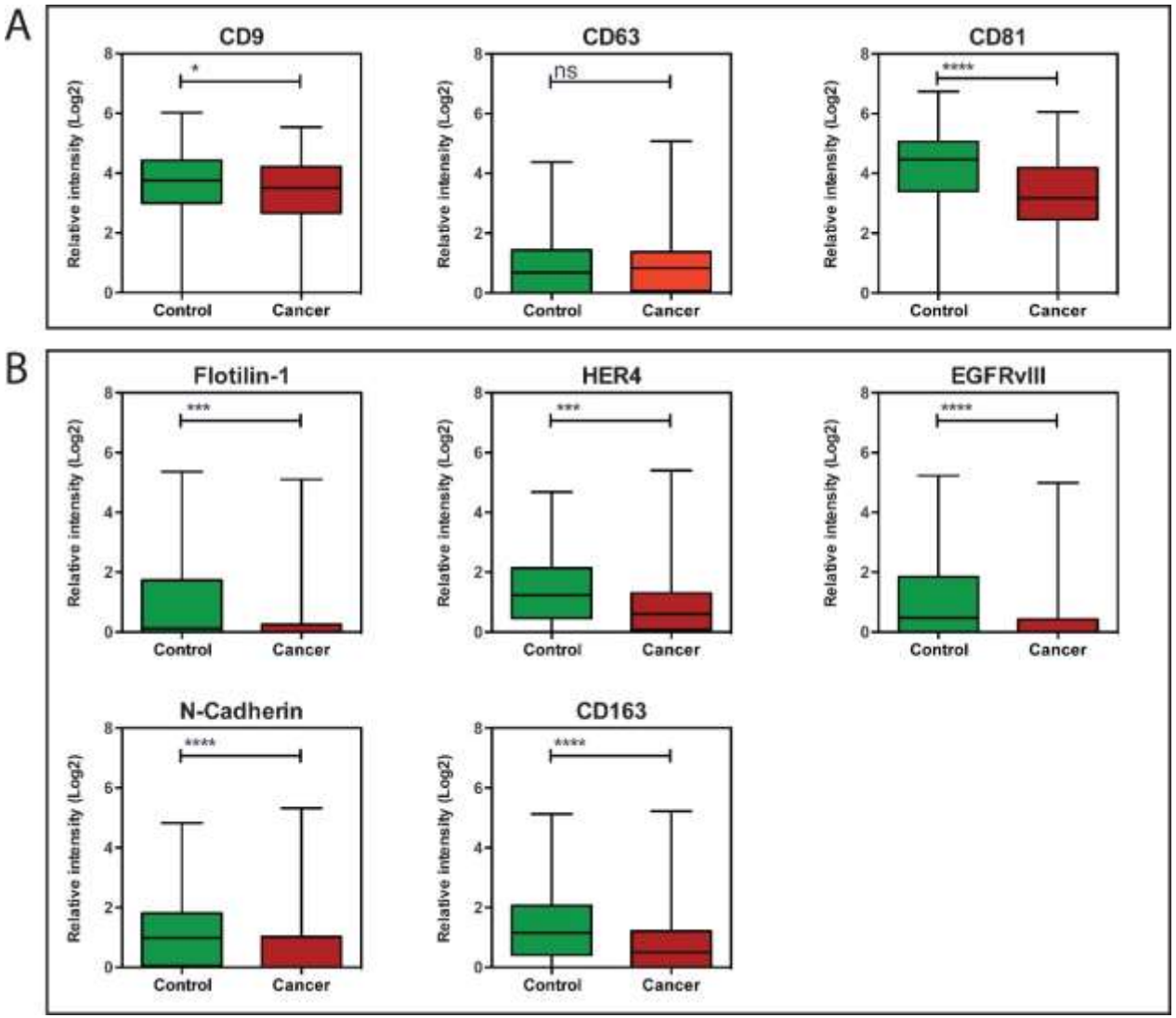
[1]Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark; [2]Department of Biomedicine, Aarhus University, Aarhus, Denmark; [3]Department of Oncology, Aarhus University Hospital, Aarhus, Denmark; [4]Department of Clinical Immunology, Aalborg University Hospital, Aalborg, Denmark

Characteristics	NSCLC group (N = 109)	Control group (N = 110)
	Number (%)	
Age	45–88	21–90
Median	66	65
Gender		
Male	56 (51.4)	64 (58.2)
Female	53 (48.6)	46 (41.8)
Stage		
IIIa	28 (25.7)	
IIIb	20 (18.3)	
IV	61 (56.0)	

RESULTS


A
Control group
CD81
CD9
CD151
SPA
SFTPD
p53
PLAP
EpCAM
NY-ESO-1
CD206
Tspan8
TNF RI
MUC1
CD142
CD146
EGFR
AREG
Flotilin1
EGFRvIII
CD163
N-Cadherin
HER4
Mucin16
PD-L1
CD171
c-MET
HER2
HER3
TNF RII
CD13
CEA
Hsp90
TSG101
CD63
HB EGF
TAG72
CD14
B
Cancer group
CD9
CD81
CD151
Tspan8
TNF RI
SPA
p53
SFTPD
CD206
CEA
CD13
TSG101
c-MET
HER2
Mucin16
PD-L1
CD171
HER3
N-Cadherin
Flotilin1
EGFRvIII
CD163
HER4
CD63
TAG72
CD14
HB EGF
TNF RII
Hsp90
CD146
AREG
EGFR
MUC1
CD142
NY-ESO-1
EpCAM
PLAP
Low
High

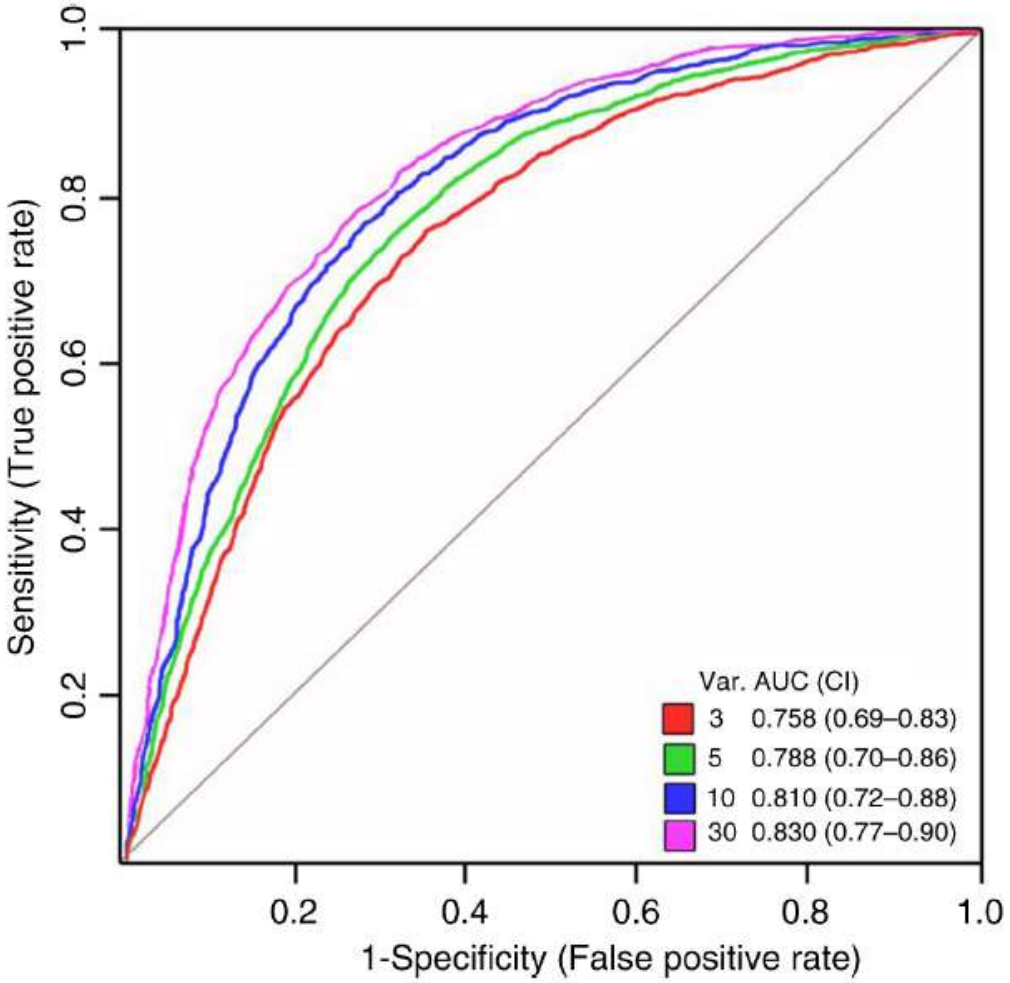
RESULTS



EV SEARCH

RESULTS

Multivariate Data Analysis



30-marker model

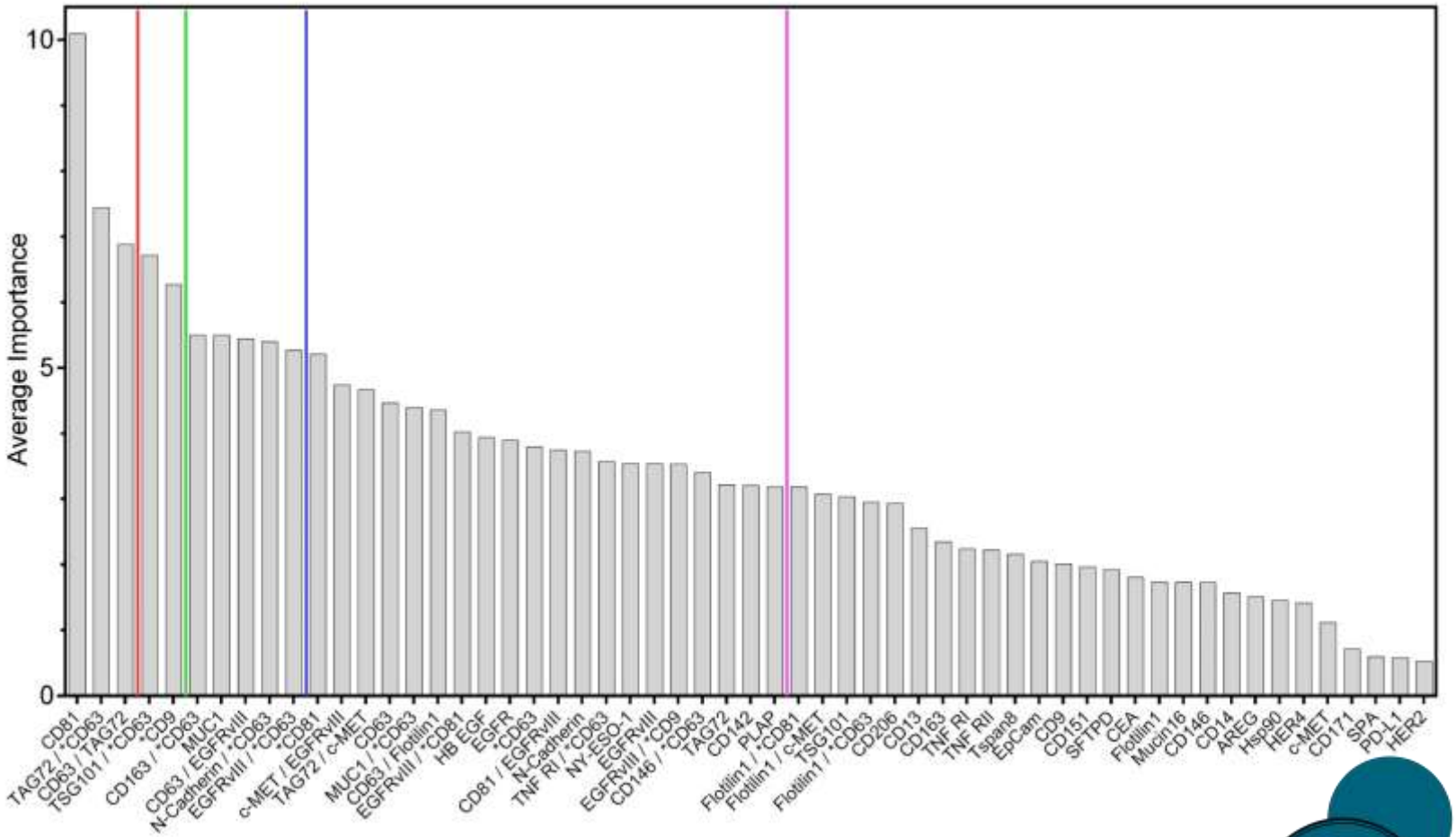
Sensitivity: 75%

Specificity: 76%

Accuracy: 75%

RESULTS

Multivariate Data Analysis / Importance to the model



EV SEARCH

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ORIGINAL ARTICLE

Exosomal Proteins as Diagnostic Biomarkers in Lung Cancer

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Molecular Oncology

Exosomal proteins as prognostic biomarkers in non-small cell lung cancer

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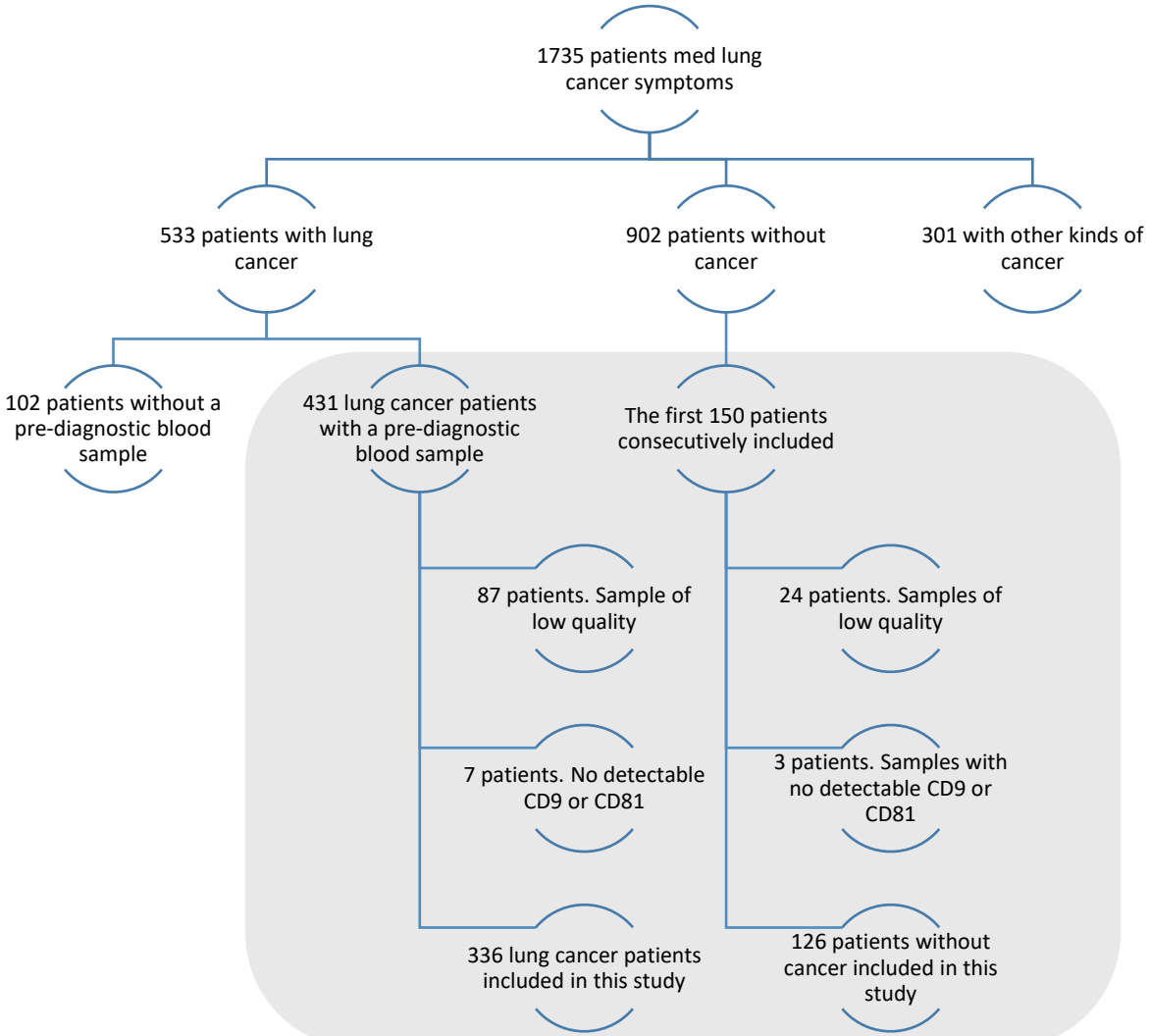
1735 patients med lung cancer symptoms
533 patients with lung cancer
902 patients without cancer
301 with other kinds of cancer
102 patients without a pre-diagnostic blood sample
431 lung cancer patients with a pre-diagnostic blood sample
The first 150 patients consecutively included
87 patients. Sample of low quality
24 patients. Samples of low quality
7 patients. No detectable CD9 or CD81
3 patients. Samples with no detectable CD9 or CD81
336 lung cancer patients included in this study
126 patients without cancer included in this study

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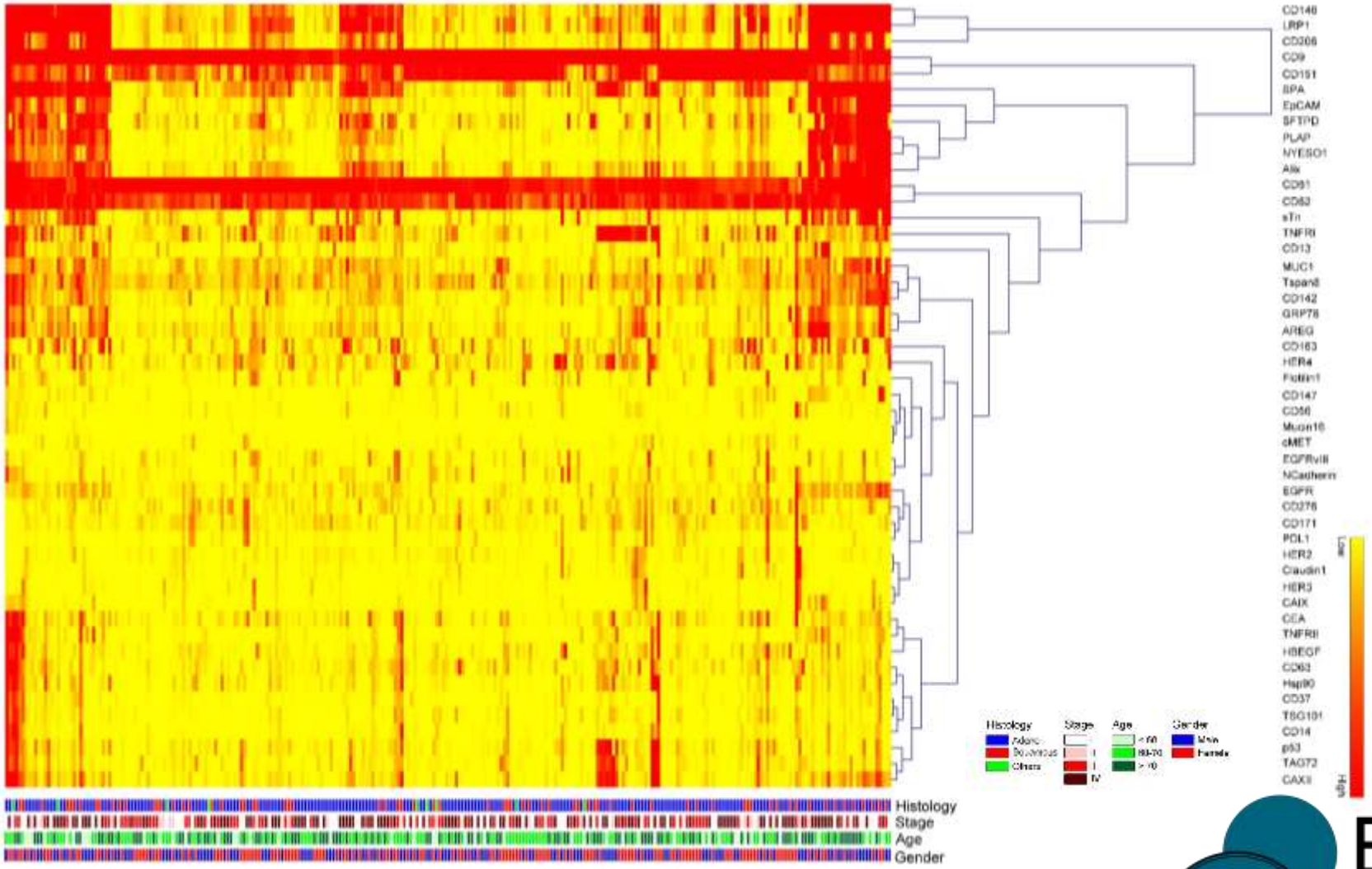
RESULTS



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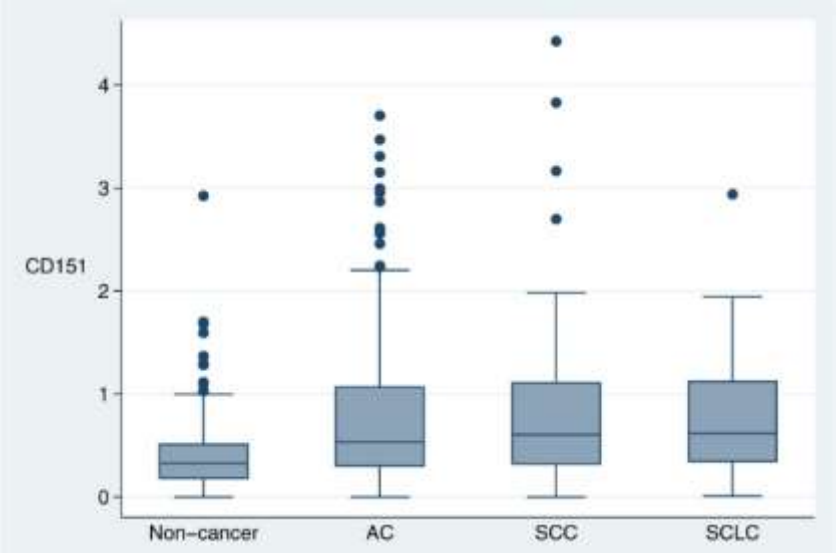
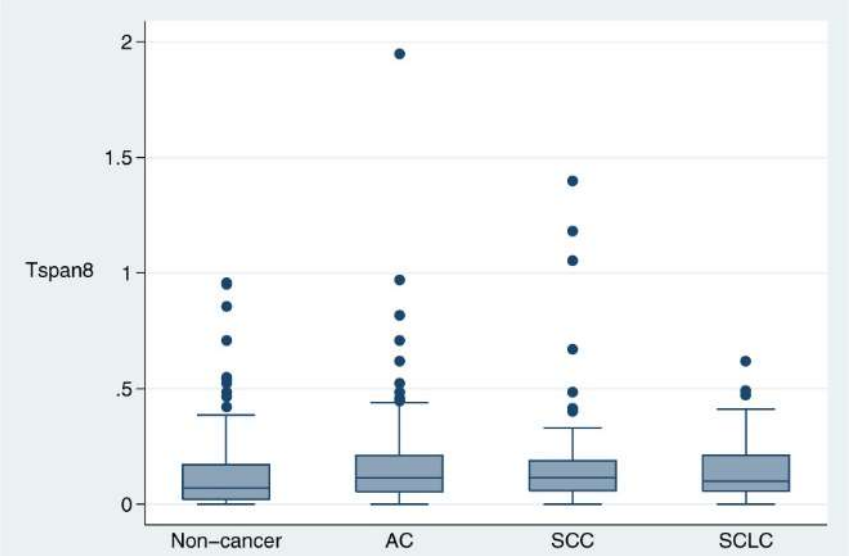
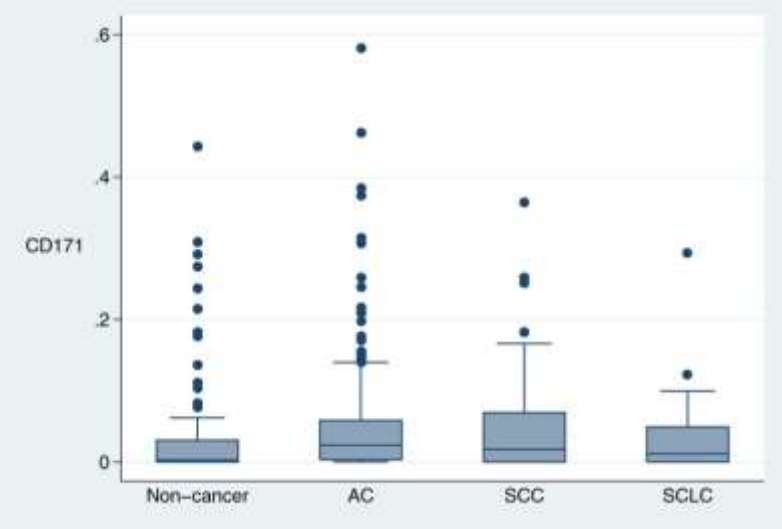
RESULTS







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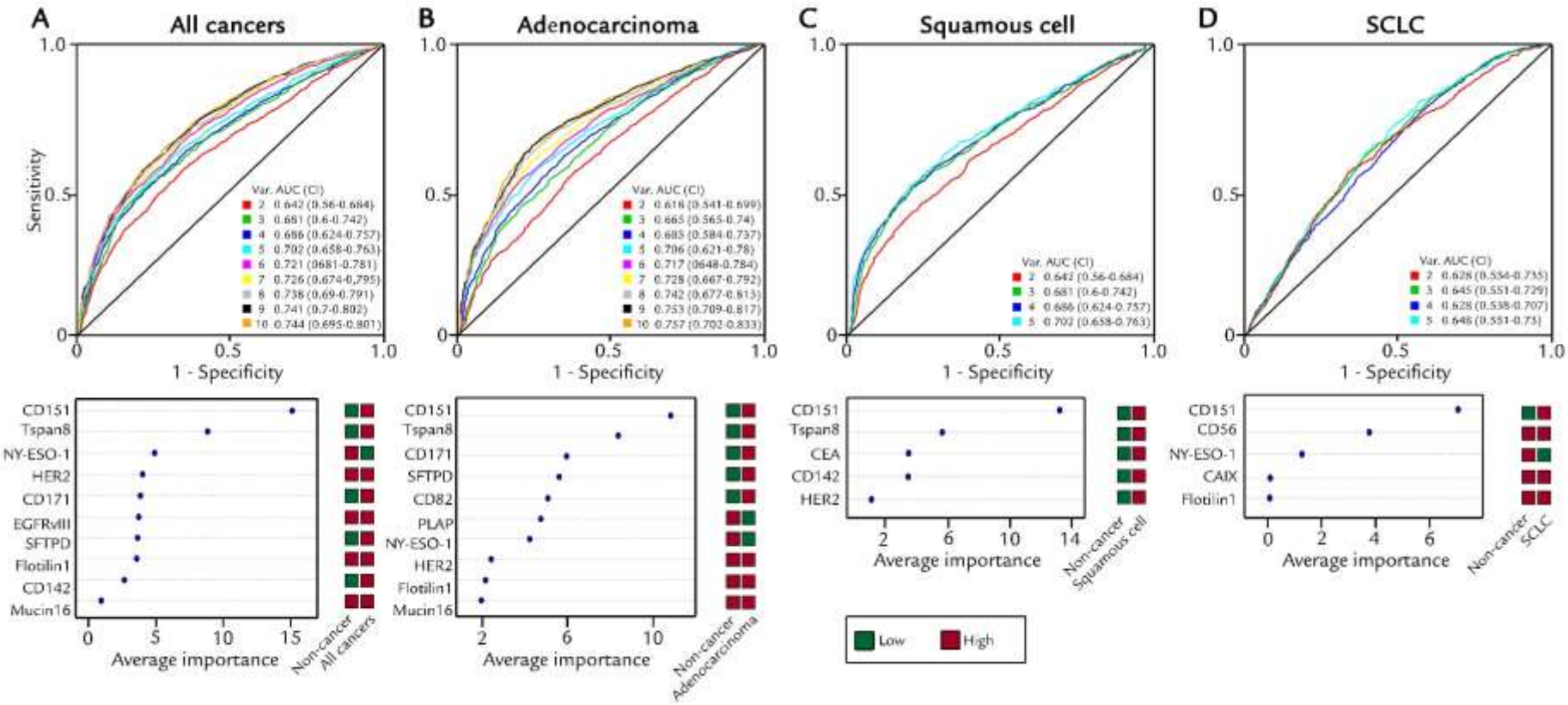
RESULTS

Table 2. Exosomal Proteins as Individual Diagnostic Biomarkers

	All Cancer			AC			SCC			SCLC		
Markers	*p* Value[a]	AUC	*p* Value[b]	*p* Value[a]	AUC	*p* Value[b]	*p* Value[a]	AUC	*p* Value[b]	*p* Value[a]	AUC	*p* Value[b]
CD151	0.00001	0.68	0.0002	0.00001	0.68	0.0008	0.00001	0.69	0.001	0.00001	0.71	0.0002
CD171	0.0003	0.61	0.0002	0.00001	0.63	0.00002	ns	0.57	ns	ns	0.54	ns
TSPAN8	0.001	0.60	0.0002	0.002	0.60	0.0004	0.0005	0.61	0.003	ns	0.58	ns
HER2	0.003	0.58	0.003	0.006	0.58	0.006	0.02	0.59	0.04	ns	0.57	ns
Flotilin[c]	0.004	0.58	0.003	0.003	0.59	0.002	ns	0.54	ns	0.03	0.59	0.05
SFTPD	0.008	0.58	0.004	0.02	0.57	0.007	0.05	0.58	ns	ns	0.57	ns
NY-ESO-1[c]	0.009	0.57	0.01	0.01	0.58	0.02	ns	0.56	ns	ns	0.58	0.09[d]
CD9	0.01	0.57	ns	ns	0.56	ns	0.005	0.61	ns	ns	0.57	ns
CD81[c]	0.01	0.57	ns	ns	0.56	ns	0.005	0.57	ns	ns	0.57	ns
CD82[c]	0.02	0.57	ns	0.005	0.59	0.009	ns	0.57	ns	ns	0.50	ns
Mucin16[c]	0.02	0.53	0.03	0.01	0.54	0.02	ns	0.52	ns	ns	0.54	ns
EGFRvIII	0.03	0.56	0.05	ns	0.55	ns	ns	0.56	ns	ns	0.57	ns
PLAP[c]	0.03	0.56	ns	0.03	0.57	0.05	ns	0.53	ns	ns	0.55	ns
CD142	ns	0.55	0.03	ns	0.54	ns	0.02	0.60	0.008	ns	0.55	ns
CD206	ns	0.55	ns	0.04	0.57	ns	ns	0.52	ns	ns	0.52	ns
CEA	ns	0.55	ns	ns	0.54	ns	0.009	0.60	0.03	ns	0.50	ns
CAIX	ns	0.52	ns	ns	0.53	ns	ns	0.52	ns	ns	0.55	0.09[d]
CD56	ns	0.52	ns	ns	0.50	ns	ns	0.53	ns	ns	0.55	0.01

RESULTS



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CONCLUSION

- We have demonstrated the potential of EV protein profiling by the EV Array in a prospective study including patients with symptoms of lung cancer.

- We found CD151, TSPAN8, and CD171 to be highly expressed in EVs from patients with lung cancer compared with those from patients without cancer.

- In a multimarker model including 10 EV markers, we were able to make a fair separation of adenocarcinoma (AC) and non-cancer, demonstrating the promising perspectives of EV protein profiling as a biomarker.

Prognostic

- To explore EV membrane-bound proteins as prognostic biomarkers in NSCLC, the presence of the individual marker was evaluated.
- Each marker was dichotomised based on its detection or non-detection.
- The impact of each marker on OS was estimated by univariate and multivariate analysis adjusting for the clinico-pathological characteristics of age, sex, PS, histology and stage.

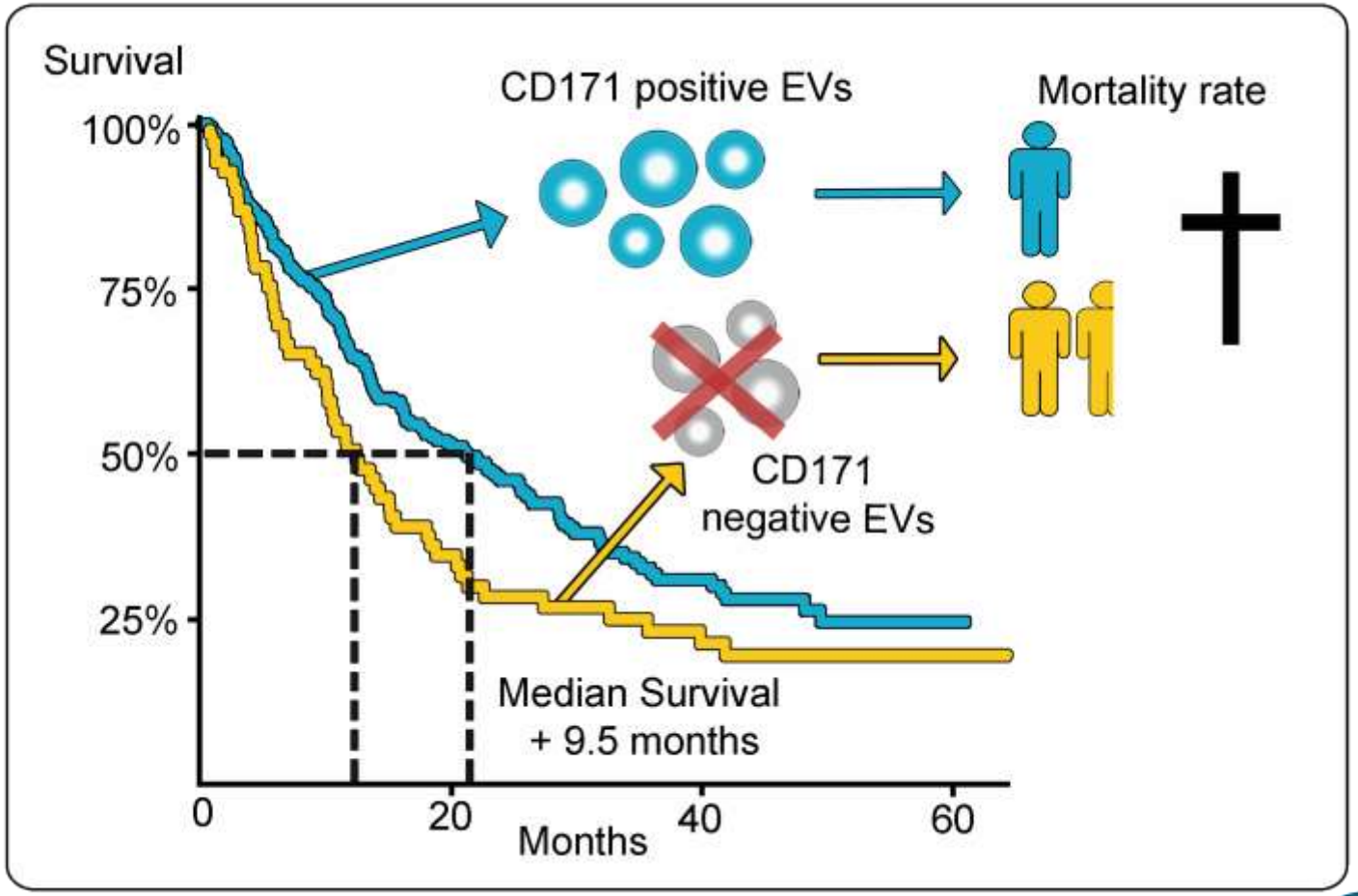
RESULTS (Absent or present)

	Univariate			Multivariate				
Marker	HR	95%CI	p	HR	95%CI	p	Bootstrap	Fraction (%) (n=276) **
CD171	0.71	0.52 – 0.98	0.04	0.56	0.41 – 0.79	0.001	*	75
Flotilin1	0.73	0.53 – 0.99	0.04	0.63	0.46 – 0.86	0.004	*	35
HER3	0.68	0.48 – 0.96	0.03	0.61	0.43 – 0.87	0.006	*	24
GRP78	0.75	0.56 – 0.99	0.05	0.69	0.51 – 0.91	0.01	*	57
cMET	1.11	0.79 – 1.57	0.53	1.41	1.00 – 2.01	0.05	-	22
sTn	1.66	1.06 – 2.59	0.03	1.51	0.93 – 2.43	0.07	-	53
Tspan8	2.37	0.97 – 5.76	0.06	2.22	0.91 – 5.47	0.08	-	96

Table 3. Membrane-bound exosomal proteins as prognostic biomarkers in NSCLC based on the presence of each marker. Only markers with a trending or significant ($p \leq 0.10$) impact on OS are presented * multivariate results verified by bootstrapping ** number of patients with the individual marker present as a fraction of the total number of patients

RESULTS (Absent or present)

CD171



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RESULTS (Concentration)

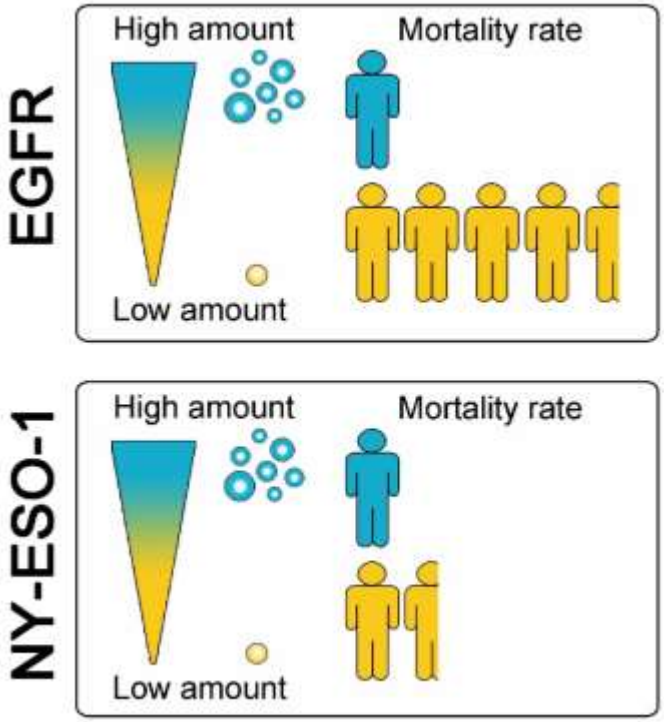
	Univariate			Multivariate			
Marker	HR	95%CI	p	HR#	95%CI	p	Bootstrap
NY-ESO-1	1.11	0.83 – 1.47	0.49	1.53	1.13 – 2.10	0.007	*
EpCam	1.03	0.83 – 1.28	0.80	1.31	1.04 – 1.65	0.02	-
CAIX	2.81	1.00 – 7.84	0.05	2.84	1.18 – 6.81	0.02	-
CD13	1.93	1.11 – 3.37	0.02	1.81	1.09 – 3.03	0.02	-
EGFR	1.21	0.25 – 5.79	0.81	4.43	1.18 – 16.68	0.03	*
PLAP	1.01	0.80 – 1.29	0.91	1.28	1.02 – 1.60	0.03	*
CD276	1.53	0.33 – 7.10	0.59	4.11	1.00 – 17.05	0.05	-
AREG	1.58	0.92 – 2.71	0.09	1.55	0.94 – 2.54	0.08	-
HER2	1.16	0.29 – 4.61	0.83	3.28	0.88 – 12.18	0.08	-

Table 4. Membrane-bound proteins as prognostic biomarkers based on the expression level. # Hazard ratio adjusted for stage, histology, PS, age and sex * multivariate results verified by bootstrapping. Only markers with a significant or a trending impact on OS ($p<0.10$) are included.

CONCLUSION

- Exploration of EV membrane-bound proteins as prognostic markers in NSCLC revealed a total of nine markers with potential impact on OS that were independent of known clinico-pathological factors.

- However promising, our results need to be validated in an independent cohort.



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More information on www.evarray.dk

Aknowledgement to INDEX PROJECT

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