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Exploring the potential of a liquid biopsy approach for melanoma diagnostics and the role of extracellular vesicles in atherosclerosis development

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UNIVERSITY OF TARTU
Press
1632

Institute of Technology, Faculty of Science and Technology, University of Tartu, Estonia

The dissertation was accepted for the commencement of the degree of Doctor of Philosophy in Biomedical Engineering on 06.01.2025, by the Joint Council of the Doctoral Program of Engineering and Technology of the University of Tartu.

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Commencement: Auditorium 121, Nooruse 1, Tartu, Estonia, at 13.15 on January 28th, 2025

Publication of this thesis is granted by the Institute of Technology, Faculty of Science and Technology, University of Tartu.

ISSN 2228-0855 (print)
ISBN 978-9916-27-783-6 (print)
ISSN 2806-2620 (pdf)
ISBN 978-9916-27-784-3 (pdf)

University of Tartu Press
www.tyk.ee

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to by the Roman Numerals I–III

I. Õunap, K., Kurg, K., Võsa, L., Maiväli, Ü., Teras, M., Planken, A., Ustav, M., & Kurg, R. (2018). Antibody response against cancer-testis antigens MAGEA4 and MAGEA10 in patients with melanoma. Oncology Letters, 16(1), 211–218. https://doi.org/10.3892/ol.2018.8684

II. Kurg, K., Planken, A., & Kurg, R. (2022). Proteomic and Biochemical Analysis of Extracellular Vesicles Isolated from Blood Serum of Patients with Melanoma. Separations, 9(4), 4. https://doi.org/10.3390/separations9040086

III. Žėkas, V., Kurg, R., Kurg, K., Bironaitė, D., Radzevičius, M., Karčiauskaitė, D., Matuzevičienė, R., & Kučinskienė, Z. A. (2022). Oxidative Properties of Blood-Derived Extracellular Vesicles in 15 Patients After Myocardial Infarction. Medical Science Monitor, 28, 0–0. https://doi.org/10.12659/MSM.935291

The author's contribution to each article is as follows:

I. The author is one of the co-authors of this paper, who did a large portion of the experiments

II. The author is the main author of this paper, having done most of the experiments and wrote the manuscript

III. The author did data analysis and contributed to the writing of the article

ABBREVIATIONS

AA	acetaldehyde
ADAMP10	a disintegrin and metalloproteinase domain-containing protein 10
AFM	atomic force microscopy
AJCC	American Joint Committee on Cancer
Akt B	protein kinase B
ALM	acral lentiginous melanoma
AMPK	a master cellular energy sensor and regulator
AngII	angiotensin II
ANOVA	analysis of variance
apo	apolipoprotein
apoBD	apoptotic bodies
apoExo	apoptotic exosomes
apoMV	apoptotic microvesicles
ARF6	protein ADP-ribosylation factor 6
AUC	area under the curve
BDNF	brain-derived neurotrophic factor
CA125	cancer antigen-125
CAF	cancer associated fibroblast
Cav-1	caveolin-1
CD	cluster of differentiation
cfDNA	cell free DNA
cfRNA	cell free RNA
Chol	cholesterol
CI	credible intervals
CMC	circulating melanoma cells
CRP	C-reactive protein
CSF	cerebrospinal fluid
CTA	cancer-testis antigen
CTC	circulating tumor cells
ctDNA	circulating tumor DNA
CTLA-4	cytotoxic T lymphocyte antigen 4
ctRNA	circulating tumor RNA
CV	coefficient of variations

CVD	cardiovascular disease
CXCL12	C-X-C motif chemokine 12
DAPI	4′,6-diamidino-2-phenylindole
DGC	density gradient centrifugation
DLS	dynamic light scattering
DM	desmoplastic melanoma
DNA	deoxyribonucleic acid
dsDNA	double-stranded DNA
EBV	Epstein-Barr virus
ECL	chemiluminescent Western blot reagent
ECM	extracellular matrix
EGFRvIII	epidermal growth factor receptor vIII
ELISA	enzyme-linked immunosorbent assay
EMT	epithelial-mesenchymal transition
EpCAM	epithelial cell adhesion molecule
ERK	extracellular signal-regulated kinases
ESCRT	endosomal-sorting complex required for transport
EV	extracellular vesicle
FN1	fibronectin1
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GPC3	glypican-3
GTP	guanosine-5'-triphosphate
H&E	hematoxylin and eosin
H2DCFDA	2′,7′-dichlorodihydrofluorescein diacetate
HDL	high-density lipoprotein
HDL-Chol	high-density lipoprotein cholesterol
HMG-CoA	hydroxymethylglutaryl coenzyme A reductase
HPLC	high-performance liquid chromatography
hs-CRP	high-sensitivity C-reactive protein
Hsp	heat shock proteins
HUVEC	human umbilical vein endothelial cell
IBD	inflammatory bowel disease
ICAM-1	intercellular adhesion molecule 1
IHC	immunohistochemistry
IL	interleukin

ILV	intraluminal vesicles
LASA-P	lipid-bound sialic acid
LDH	lactate dehydrogenase
LDL	low-density lipoprotein
LDL-Chol	low-density lipoprotein cholesterol
LGALS3BP	galectin-3-binding protein
LIMK	LIM kinase
LMM	lentigo maligna melanoma
LO	large oncosomes
MAA	malondialdehyde-acetaldehyde
MAGE	melanoma-associated antigen
MDA	malondialdehyde
MHC	major histocompatibility complex
MHD	MAGE homology domain
MI	myocardial infarction
MIA	melanoma inhibitory activity
miRNA	microRNA
MLCK	myosin light-chain kinase
MMP	matrix metalloproteinase
mRNA	messenger RNA
MS	multiple sclerosis
MT1MMP	membrane-type 1 matrix metalloproteinase
MV	microvesicle
MVB	multivesicular bodies
NGS	next-generation sequencing
NM	nodular melanoma
NO	nitric oxide
NOS	nitric oxide synthase
NOX	NADPH oxidases
NSE	serum neuron-specific enolase
NSF	N-ethylmaleimide sensitive fusion protein
nSMase 2	neutral sphingomyelinase 2
NTA	nanoparticle tracking analysis
OD	optical density
p42/44 MAPK	p42/44 mitogen-activated protein kinase

PA	phosphatidic acid
PBS	phosphate buffered-saline
PC	protein corona
PCR	polymerase chain reaction
PD-1	programmed cell death receptor 1
PDL2	phospholipase D2
PEG	polyethylene glycol
PI	propidium iodide
PI3K-AKT	phosphatidylinositol-3-kinase
PS	phosphatidylserine
PSA	prostate-specific antigen
PVDF	polyvinylidene difluoride
RA	rheumatoid arthritis
RGP	radial growth phase
RNA	ribonucleic acid
ROC	receiver operating characteristic
ROCK	Rho-associated coiled-coil containing protein kinase
ROS	reactive oxygen species
RT-PCR	reverse transcriptase PCR
SD	standard deviation
SEM	scanning electron microscopy
SERPINA3	alpha-1-antichymotrypsin
sIL-2R	soluble IL-2 receptor
SLE	systemic lupus erythematosus
SNARE	soluble NSF-attachment protein receptor complex
SRY	sex-determining region Y
ssDNA	single-stranded DNA
SSM	superficial spreading melanoma
sVCAM-1	soluble intercellular adhesion molecule 1
T1D	type 1 diabetes
TAA	tumor-associated antigen
TEM	transmission electron microscopy
TF	tissue factor
TG	triglycerides
TGF-β1	transforming growth factor beta1

TGRL	triglyceride-rich lipoproteins
Timp	tissue inhibitor of metalloproteinases
TRAIL	TNF-related apoptosis-inducing ligand
TrpC5	transient receptor potential channel 5
TSA	tumor-specific antigen
TSG101	tumor susceptibility gene 101
tTG	tissue transglutaminase
TYRP-2	tyrosinase-related protein-2
UC	ultracentrifugation
UV	ultraviolet
VAMP3	vesicle-associated protein 3
VAMP7	Ca^{2+}-regulated vesicle-associated membrane protein 7
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor
VGP	vertical growth phase
VLA-4	very late antigen-4
VLDL	very-low-density lipoprotein
WB	Western Blot

1. INTRODUCTION

'Liquid biopsy' is an all-encompassing term used to describe the testing of body fluids, for example blood. It can be used to identify a wide range of biomolecular features and has the potential to give an indication of disease status (Connal et al., 2023). For liquid biopsy, circulating extracellular nucleic acids (cell free DNA, cfDNA; circulating tumor DNA, ctDNA; cell free RNA, cfRNA; circulating tumor RNA, ctRNA), extracellular vesicles, autoantibodies and protein detection could be used (Nikanjam et al., 2022; Connal et al., 2023; Elkon & Casali, 2008; Landegren & Hammond, 2021). Blood is a uniquely ubiquitous intersecting tissue that interacts with all other tissues and organs. It contains a diverse set of proteins that reached here by secretion, apoptosis or enzymatic cleavage (Yurkovich & Hood, 2019). Plasma, the fluid portion of blood, contains electrolytes, nutrients, metabolic products, vitamins, gases and proteins. When blood clots, fibrinogen in plasma is used up and the remaining fluid is referred to as 'serum' (Silbernagl & Lang, 2009).

Melanoma is skin cancer that is caused by malignant melanocytes (Ahmed et al., 2020). Currently, when the incidence of many tumor types is decreasing, melanoma incidence continues to increase (MacKie et al., 2009). Melanoma diagnosis is made through clinical assessment. Architectural features of malignant melanoma include asymmetry, confluence of growth, marked cellularity, and poor circumscription by histopathology (Ahmed et al., 2020). Reliable detection of melanoma is a challenge due to the diversity of surface marker expression and thus inspires the use of different detection methods (Xu et al., 2016). These include different liquid biopsy markers such as autoantibodies and extracellular vesicles.

Cancer-testis antigens (CTAs) are a group of proteins that have restricted expression to testis, ovaries and placenta, and are aberrantly re-expressed in cancer where they have potential to be immunogenic (Simpson et al., 2005). Autoantibodies have been found as a response to over-expressed, mutated, misfolded, or aberrantly degraded tumor self-proteins. These autoantibodies could serve as pioneer reporters of tumorigenesis as their formation occurs several months or years earlier than the onset of clinical symptoms of cancer (Yao et al., 2012). Serum profiling of circulating auto-antibodies is considered an attractive method in the diagnosis of cancer at early stages (Chen et al., 2012). The Melanoma Antigen Gene (MAGE) protein family is a large, highly conserved group of proteins that share a common MAGE homology domain (MHD) (Weon & Potts, 2015). MAGE proteins comprise a super-family of more than 60 genes in humans with a subset of these >40 human proteins are classified as cancer-testis antigens (Chomez et al., 2001; Simpson et al., 2005).

Extracellular vesicles (EVs) are a group of heterogeneous nano-sized cell-derived vesicles, which have attracted great interest as a target for liquid biopsy. EVs inherit bioactive components from parent cells and are able to transfer their contents to recipient cells (Huang et al., 2021). Many EV subtypes tend to overlap

in size, surface proteome, and cargo, making EVs broadly heterogeneous in nature. Additionally, biofluids of interest for EV isolation contain varying compositions of proteins and non-EV lipid particles that contribute to the heterogeneity, complexity, and viscosity of the biofluid. Both low cell-type abundance and low basal EV secretion rates make some cell-type specific EVs difficult to isolate using conventional technologies due to their low relative concentrations in biofluids (Shami-shah et al., 2023).

Atherosclerosis is the most frequent underlying cause of coronary, carotid, and peripheral arterial disease. It is a multifocal, smoldering, immunoinflammatory disease of medium-sized and large arteries fuelled by lipids with endothelial cells, leukocytes, and intimal smooth muscle cells being the major players in its development (Falk, 2006). Despite effective interventions for the control of LDL, blood pressure and other traditional risk factors, a considerable residual risk remains for atherosclerotic cardiovascular disease (Ridker, 2017). Circulating microvesicles (MVs) are small phospholipid-rich vesicles that contribute to the atherothrombotic process. MVs can be used as biomarkers of cardiovascular disease (CVD) burden and progression (Chiva-Blanch et al., 2020).

This dissertation focuses on the possibility of using an autoimmune response against MAGEA4 and MAGEA10 as well as extracellular vesicles purified from melanoma patient's blood serum as markers for melanoma diagnostics. It also aims to show that extracellular vesicles themselves can take part in disease development by comparing healthy controls and patients after myocardial infarction extracellular vesicle count and properties.

2. LITERATURE REVIEW

2.1 Liquid Biopsy

Liquid biopsies are minimally invasive methods that can be used to obtain cell-derived information from body fluids (Nikanjam et al., 2022). 'Liquid biopsy' is an all-encompassing term used to describe testing body fluids including blood, urine, cerebrospinal fluid (CSF), and saliva. It can identify a wide range of biomolecular features and has the potential to give an indication of disease status (Connal et al., 2023). Over the last decade, liquid biopsy has gained much attention as a powerful tool in personalized medicine, since it enables, for example, monitoring of cancer evolution and follow-up of cancer patients in real time (Markou et al., 2022).

Liquid biopsy testing is a straightforward process requiring no more than a blood draw and transportation to the local laboratory or central testing center with a shorter turnaround time than that for tumor tissue genotyping. In some cases, liquid biopsy results can return while a specimen is still undergoing diagnostic workup or can inform an established nonspecific diagnosis (Sholl et al., 2020).

2.1.1 Liquid biopsy versus classical biopsy

Surgical tissue biopsies are considered the gold standard for solid tumor diagnosis (Corcoran & Chabner, 2018). A biopsy can be obtained in several ways depending on tumor location and surgical treatment. In excisional biopsy, the entire area of abnormal cells is removed, whereas in incisional biopsy just a part of the abnormal area is removed. Open surgical biopsies enable more precise resections, but carry increased risk of complications, such as infections or bleeding (Connal et al., 2023). The surgical biopsy procedure is also constrained by accessibility, repeatability, patient age, cost, time, and some surgical biopsies might even cause harmful clinical complications (Yu et al., 2021). For some cancers, surgical tissue biopsies are not attainable due to the high risk associated with the procedure. One of the main issues related to tissue biopsies is the inability to capture tumor heterogeneity and its clonal evolution (Connal et al., 2023). Furthermore, tumor tissue handling is an operationally complex process requiring infrastructure for sample acquisition and histologic processing that often adds a week or more to the molecular test turnaround time (Sholl et al., 2020).

There are several benefits of liquid biopsy over conventional surgical tissue biopsy such as lower procedural costs, easy repeatability and increased reliability. Liquid biopsies are also not contaminated from the use of preservatives, whereas tissue sections are generally preserved for immunohistochemistry by processes such as fixation, embedding and freezing (Connal et al., 2023). As such, they provide a fresh source of reliable tumor-derived components and materials (Arneth, 2018). Furthermore, liquid biopsies can be carried out rapidly, provide genomic, proteomic and metabolomics information, and are less invasive (Adashek et al., 2021; Arneth, 2018).

Although liquid biopsy has the potential to disrupt the field of medical diagnosis, it is met by various challenges such as limited tumor-derived components, lower specificity, and inadequate advancement in methods to isolate biomarkers (Adhit et al., 2023). The Accelerating Anticancer Agent Development Workshop expert panel has outlined some these issues in a session entitled "Liquid Biopsy: State of the Science and Future Directions." The panel identified challenges such as standardization of liquid biopsy assessments and analyte validation, as well as regulatory considerations for their use as a biomarker in clinical trials (Narayan et al., 2020).

2.1.2 Different marker types measured in liquid biopsy

For liquid biopsy, circulating extracellular nucleic acids (cell free DNA, cfDNA; circulating tumor DNA, ctDNA; cell free RNA, cfRNA; circulating tumor RNA, ctRNA), extracellular vesicles, autoantibodies and proteins detection could be used (Connal et al., 2023; Elkon & Casali, 2008; Landegren & Hammond, 2021; Nikanjam et al., 2022).

2.1.2.1 Nucleic acid-based biomarkers

Circulating cell-free DNA (cfDNA) was first described by Mandel and Metais in 1948 (Mandel & Metais, 1948). CfDNA can be found in many body fluids, such as blood plasma (Metais & Mandel, 1948), serum, urine, saliva and cerebrospinal fluid, and is present in both healthy and diseased patients (Connal et al., 2023). It is found at low levels in plasma from healthy cells ,~ 10–15 ng/mL; however, it has been reported that cfDNA concentration can increase upon tissue stress induced by inflammation, surgery and acute trauma (Wan et al., 2017). Also, cfDNA presence can also result from physiological cell functions, such as secretion, apoptosis, or necrosis (Stroun et al., 2001). It forms a protein-complex and its length ranges from 18 to 10,000 bp (Zhang et al., 2019a).

Since its discovery, cfDNA has become an appealing biomarker, and the analysis of cfDNA has been utilized in a range of medical technologies, such as prenatal testing, detecting immune diseases, monitoring the effect tiveness of an organ transplants, and detecting the presence of cancer (Yan et al., 2021). It has been found to be a good source of information about specific mutations and gene alterations (Kamińska et al., 2021).

Cancer-derived cfDNA has been referred to as circulating tumor DNA (ctDNA) and cancer patients exhibit a higher level of this type of cfDNA in blood than healthy controls. As such, ctDNA has attracted significant research attention (Kamińska et al., 2021). Compared to cfDNA, ctDNA is shorter, being around 134–144 bp (Underhill et al., 2016). Usually it is fragmented and appears as nucleosomal single, double, or triple complexes (Kamińska et al., 2021).

Tumor-DNA is released into the bloodstream through apoptosis, necrosis, and active cellular secretion (Diaz & Bardelli, 2014). In cancer patients, ctDNA

accounts for around 1 to 2% of the overall cfDNA (Wyatt et al., 2017; Zhao et al., 2021). As blood collection is minimally invasive, ctDNA samples could be collected to examine changes in their quantity and composition over time, being a useful tool for cancer detection and monitoring (Phallen et al., 2017). The biggest hurdle is that ctDNA is quickly degraded by nucleases, with a half-life of less than 2 h (Sacher et al., 2016).

CtDNA can be distinguished from normal cfDNA fragments through the presence of epigenetic or genetic alterations including tumor-specific methylation markers and somatic mutations (Ramirez-Garrastacho et al., 2022). It could be used as a marker for treatment selection, to estimate prognosis, identify residual disease, and indicate potential risk of relapse. One study reported that ctDNA assays were able to detect residual disease faster than radiologic imaging by several weeks (Chen & Zhao, 2019).

Cell free RNA (cfRNA) refers to RNA fragments that are degraded and released into the bloodstream mainly by necrotic or apoptotic cells (Martinez-Dominguez et al., 2021). **Circulating tumor RNA (ctRNA)** refers to the fraction of circulating cell-free RNA derived from cancer cells (Connal et al., 2023). RNA, in comparison to DNA, is regarded as an unstable molecule with a 'naked' half-life in plasma of approximately only 15 s (De Rubis et al., 2019). The lack of stability is one of the major limitations associated with ctRNAs, and an optimal extraction method has yet to be identified (Connal et al., 2023).

Cell–free messenger RNA (mRNA) was first confirmed in the bloodstream of cancer patients in 1999, leading to mRNA being identified as a potential cancer biomarker with prognostic and diagnostic value (Martinez-Dominguez et al., 2021). **MicroRNAs (miRNAs)** are a large family of small noncoding RNAs that regulate post-transcriptional target gene expression. They play an important role in various diseases (Zeng et al., 2021). MicroRNAs have gained the most interest due to their stability. Moreover, in most human cancers the miRNA levels are altered, and its expression is tissue specific (Drokow et al., 2019). MiRNAs are able to regulate the expression of multiple targets by binding to the 3′-untranslated regions of genes. Emerging evidence suggests that miRNAs are involved in critical biological processes, including development, differentiation, apoptosis, proliferation and antiviral defense (Bartel, 2004). Most importantly, aberrant expression of miRNAs appears to be causatively linked cancer pathogenesis (Cho, 2007). MicroRNA can be detected not only in tissue samples but also in serum and urine using minimally invasive techniques (Drokow et al., 2019). Thus, miRNAs have potential as risk biomarkers, particularly following therapeutic intervention (Pfeffer et al., 2015).

However, there **are some limitations** of liquid biopsy markers based on nucleic acid detection. E.g ctDNA/cfDNA can be shed in only small amounts and not all patients will have detectable levels, especially with low tumor burden. Because of the small amount of material shed in the circulation, sequencing can be difficult and expensive. Standardization across laboratories and vendors is needed to ensure reproducibility. Moreover, not all ctDNA/cfDNA is equally shed from the primary tumor and metastases, so it is unclear if the alterations

detected accurately represent tumor heterogeneity (Nikanjam et al., 2022). In addition, not all detectable cfDNA alterations are cancer-related. For example, in invasive gliomas post treatment, cfDNA may be confounded by the mutations derived from clonal hematopoiesis of indeterminate potential, especially in elder people (Okamura et al., 2021). In addition, shedding ctDNA can be suppressed by treatment and may be limited at certain disease sites (Adashek et al., 2021).

2.1.2.2 Proteins

Liquid biopsies based on the detection of protein biomarkers have great potential for cancer detection and monitoring of disease progression (Landegren & Hammond, 2021). Proteins carry out many cellular functions within cells, therefore proteomic data may be able to aid novel biomarker identification and clinical implementation (Ding et al., 2022). However, current protein assays fail to reach the required diagnostic accuracy. Research into different methods to enhance the diagnostic accuracy and subsequently reduce the number of false positives and negatives include the use of panels or biosignatures comprising of more than one protein (De Rubis et al., 2019) or using a combination of protein and DNA biomarkers (Landegren & Hammond, 2021).

For example, prostate-specific antigen (PSA) is a protein biomarker, which is currently used for the identification of prostate cancer. There are several factors such as age, race, body mass index, medication as well as others which must be considered before determining what ‘elevated’ PSA levels are (Connal et al., 2023). Elevated PSA levels are not specific to prostate cancer; common conditions such as prostatitis and benign prostatic hyperplasia can impact the levels observed (Lin et al., 2008). As another example, cancer antigen-125 (CA125) is a tumor biomarker, which has been utilized as the primary ovarian cancer biomarker during the last four decades. Techniques used to detect CA-125 lack the sensitivity and specificity required to be used in a general-population screening program for detection of ovarian cancer (Charkhchi et al., 2020). CA125 is also not specific to ovarian cancer with elevated serum CA-125 levels also observed in menstruation, endometriosis and pregnancy, so false positive for cancer remains an issue (Dochez et al., 2019).

Natural antibodies or autoantibodies are antibodies that react with self-molecules. These self-antigens can be exclusive for a specific cell type or can be found against proteins found in all cell types, such as chromatin or the centromere. They have been shown to be useful disease biomarkers that give information about inflammation in autoimmune diseases patients (Elkon & Casali, 2008). Research around tumor-associated autoantibodies is still a developing field and more understanding surrounding their complex molecular response against cancer antigens is required (de Jonge et al., 2021).

2.1.2.3 Circulating tumor cells

Circulating tumor cells (CTCs) were first described by Ashworth in 1869 (IJzerman et al., 2021). CTCs are cells that are shed into the blood from tumors and usually last only for 1–2.5 h in the circulation prior to destruction by the immune system. However, a small fraction can survive and seed distant metastatic sites (Nikanjam et al., 2022). CTCs occur at very low concentrations, < 10 CTCs per mL of blood, even in patients with metastatic cancer (Wu et al., 2020) and have different molecular markers depending on the type of cancer (Lin et al., 2021). Nevertheless, since most cancers are of epithelial origin, there is a 'universal' epithelial molecular marker, EpCAM, which can be used for CTCs detection. The expression of EpCAM varies with different cancer types and is mainly applied to cancers which strongly express EpCAM such as breast and prostate cancer (Connal et al., 2023).

Utility of CTCs as a method for early detection of cancer is limited. The number of CTCs present in blood samples has been correlated with clinical staging, with the highest numbers generally found in patients with late-stage aggressive cancer, but the CTCs amount can still be very low (Mohan et al., 2017). CTCs could be protected in the bloodstream by platelets, which are considered to play an important role in the systemic and local responses to tumor growth (Markou et al., 2022).

Clinical significance of CTC detection has been evaluated in many types of solid tumors (Lianidou et al., 2014). But CTCs are not yet established in the daily clinical practice even if their enumeration and molecular characterization provide valuable information to guide therapeutic strategy and management of patients (Markou et al., 2022). In addition, the isolation of CTCs remains technologically challenging, and the number of CTCs isolated can be method dependent. Surface markers may be downregulated in certain tumors, which can limit the ability to detect CTCs (Su & Nieva, 2017). Also, it is unclear if the CTCs are uniformly shed from all areas of the primary tumor and metastases; thus, the CTCs isolation may not provide a full portfolio of tumor heterogeneity (Plaks et al., 2013).

2.1.2.4 Extracellular vesicles

Extracellular vesicles (EVs) are small membranous particles, which can be found in the majority of body fluids. They are fundamental mediators of intercellular communication as well as regulators of pathological and physiological processes (Connal et al., 2023). EVs have advantages over ctDNA and CTCs as a tool for liquid biopsy as they have a double-layered membrane structure, which makes them less easily degradable than nucleic acids. As such, they maintain the original source of cellular biological information (Xie et al., 2019).

EVs can work either for or against cancer. They have the ability to promote the spread of cancer cells, to create a suitable environment for cancer metastasis, and to aid cancer development and progression (Xie et al., 2019). However, assisting in the occurrence and spread of cancer also reveals the existence of

cancer and so EVs have become an effective way for both diagnosing and treating the disease. EV-based blood biomarker classifiers based on EV protein profiles have already been used to detect stage I and II pancreatic, ovarian and bladder cancer (Hinestrosa et al., 2022).

Limitations surrounding the clinical suitability of EVs are related to the lack of standardized protocols and the variability between different isolation techniques (Connal et al., 2023). Moreover, obtaining blood derived EVs with high purity is difficult, as they can be obscured by other components in blood such as cells, lipoproteins and cfDNA (Ramirez-Garrastacho et al., 2022).

2.1.3 Diagnostic tests

To make clinical decisions and guide patient care, providers must comprehend the likelihood of a patient having a disease (Bartol, 2015). Sensitivity and specificity are essential indicators of test accuracy and allow healthcare providers to determine the appropriateness of the diagnostic tool (Glaros & Kline, 1988).

Sensitivity is the proportion of true positive tests out of all patients with a condition (Bolin & Lam, 2013). As such, it shows that the subject that has that disease (Glaros & Kline, 1988). Specificity is the percentage of true negative tests out of all subjects who do not have a disease or condition (Bolin & Lam, 2013). In essence, it shows that if a patients test results are within the normal range, they do not have a disease (Glaros & Kline, 1988). Sensitivity and specificity are inversely related – as sensitivity increases, specificity tends to decrease, and vice versa (Parikh et al., 2008). Highly sensitive tests will lead to positive findings for patients with a disease, whereas highly specific tests will show patients without a finding having no disease (Naeger et al., 2013). Sensitivity and specificity should always merit consideration together to provide a holistic picture of a diagnostic test (Obuchowski & Bullen, 2018).

2.2 Blood and blood constituents

Blood is a uniquely ubiquitous intersecting tissue that interacts with all other tissues and organs. It is home to vital immune cells like lymphocytes, macrophages, and leukocytes; and plays host to a variety of small molecules, from metabolites to cell-free DNA and RNA. Blood contains a diverse set of proteins that get there by secretion, apoptosis or enzymatic cleavage. These proteins can serve as sensors for the state of their organs of origin. Overall, blood contains far more information than can be extrapolated from the proteome alone. In addition to immune cells and secreted proteins, blood plays host to cell-free nucleic acids, many types of vesicles, and metabolites. These metabolites arise from tissues and cells all over the body, as well as the microbiota in the gut (Yurkovich & Hood, 2019).

Total blood volume correlates with the fat free body mass and averages 3.6 L in women and 4.5 L in men. Blood contains both a cell and a fluid fraction. The blood cell fraction can be divided into red blood cells, white blood cells and

platelets. The ratio of blood cell volume to total blood volume is called hematocrit. More than 99% of hematocrit is made up of red blood cells, also known as erythrocytes, which are responsible for O_2 and part of CO_2 transport as well as pH buffering. Among the white blood cells, also called leukocytes, neutrophils are responsible for nonspecific immune defenses, and monocytes and lymphocytes for specific immune reactions. Eosinophils and basophils are involved in allergic reactions. The third blood cell subtype, platelets, also known as thrombocytes, are important for hemostasis (Silbernagl & Lang, 2009).

Plasma, the fluid portion of blood, contains electrolytes, nutrients, metabolic products, vitamins, gases and proteins. Plasma proteins take part in the humoral immune defense, maintenance of colloidal osmotic pressure, transport of water insoluble materials, and protection of various substances against their breakdown in blood and their excretion by kidneys. Coupling of hormones, drugs and toxins to plasma proteins reduces their signaling, therapeutic, or toxic actions; while at the same time prevent their rapid excretion. Numerous plasma proteins participate in blood clotting and fibrinolysis. During blood clotting, fibrinogen in plasma is used up and the remaining fluid is referred to as 'serum' (Silbernagl & Lang, 2009).

In a cohort of 5457 individuals >65 years of age whose complete genome sequence had been determined, a total of 4137 blood serum proteins were profiled. This blood protein network consisted of 27 coregulatory modules that included 85% of the measured proteins. Several of these modules – representing functional protein groups – were enriched for tissue-specific signatures and were found to be associated with cardiovascular and metabolic disease (Emilsson et al., 2018). Complex diseases are not the result of a single change in the network, but rather a coordinated change in ≥1 biological networks that gives rise to differing functions. These changes may to stem from a single change to a node involved in multiple networks, or from multiple changes to nodes in multiple networks. Proteins primarily synthesized in one organ and released into blood serve as direct reporters for network changes in their originating organ (Yurkovich & Hood, 2019).

2.3 Extracellular vesicles

Extracellular vesicles (EVs), are a group of heterogeneous nano-sized cell-derived vesicles, which have attracted great interest as targets used for liquid biopsy. Because EVs inherit bioactive components from parent cells and are able to transfer their contents to recipient cells, EVs also hold great promise as potential cell-free therapeutics and drug delivery systems. However, the development of EV-based diagnostics, therapeutics or drug delivery systems has been challenging due to the diversity of EVs in biogenesis, size and cellular origin, the lack of standardized isolation and purification methods, as well as low yield (Huang et al., 2021). As a generalization, EVs are small, heterogenous, phospholipid-rich vesicles that are secreted by all cells into the extracellular space (Shamishah et al., 2023).

Wolf first observed extracellular vesicles in plasma in 1960s. At first, they were considered to be “platelet dust” (Wolf, 1967). By now, all biological fluids that have been tested have shown to contain EVs. Also, in vitro grown cell lines have also been shown to release EVs (Hessvik & Llorente, 2018). These nano-scale vesicles are involved in various biological processes in virtually all organisms including bacteria, plants and mammals (Huang et al., 2021).

Recently, all types of vesicles found in the extracellular space have been recommended to be termed as “extracellular vesicles” by the International Society for Extracellular Vesicles (Yin et al., 2015). At first, different EV subtypes had been characterized based on their diverse sizes spanning the range of 20–2000 nm, such as apoptotic bodies (500–2000 nm), microvesicles (100–1000 nm), and exosomes (20–100 nm) (Minciacchi et al., 2015a). In recent years, multiple other EV subtypes with unique formation pathways have also been described (Ma et al., 2015; Minciacchi et al., 2015a). An overview of different extracellular vesicle subtypes is shown in figure 1. Although live cells were initially thought to shed exosomes and microvesicles, recent developments have identified novel EV subtypes including exomeres (secretory nanoparticles that are less than 50 nm in size with their mode of biogenesis currently unknown) and migrasomes (referred to as migarsomes in the figure), whereas cancer cells also partake in secretion of large oncosomes. On the contrary, apoptotic cells which were long though to shed only apoptotic bodies (apoBD), are now known to secrete other EV subtypes including apoptotic microvesicles (apoMV) and apoptotic exosomes (apoExo). Various EV subtypes shed by live or apoptotic cells are distinct in their biogenesis, cargo, origin, and size (Sanwlani & Gangoda, 2021).

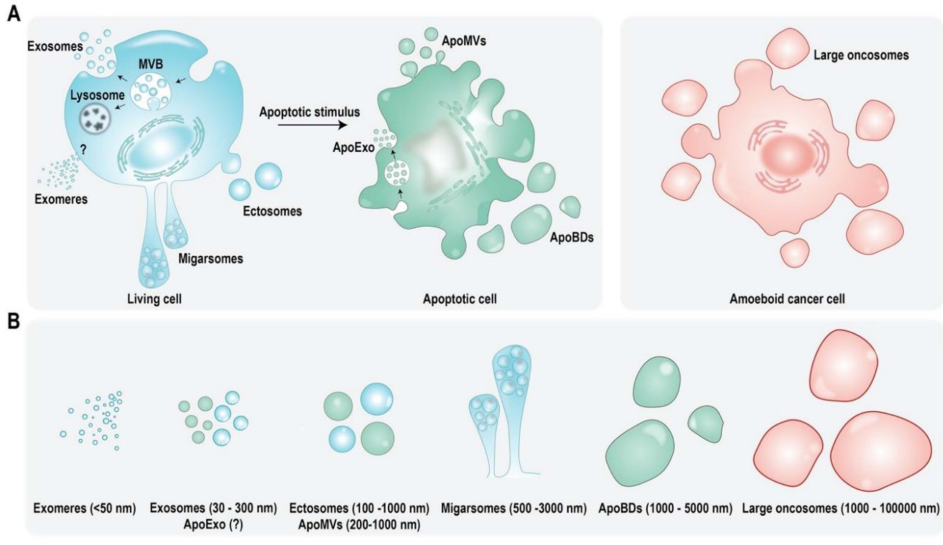

Figure 1. Schematic representation of EV subtypes identified in eukaryotic cells (Sanwlani & Gangoda, 2021)

EVs play a key role in intercellular communication as they can transport a variety of biomolecules, such as proteins, lipids, and nucleic acids, between cells (Shami-shah et al., 2023). Vesicles mediate the exchange of intricate intercellular messages comprised of classical soluble and insoluble signaling factors, structural proteins, nucleic acids, and lipids (Minciacchi et al., 2015a). EVs can travel through body fluids, thus conveying functional information to distant sites in vivo (Peinado et al., 2012) and also can have a role in immunity as microvesicles. Exosomes released by immune cells can elicit an immune response by presenting antigens to other immune cells and initiating immune infiltration (Robbins & Morelli, 2014). Extracellular vesicles that originate from cancer cells are emerging as important regulators of tumor progression (Redzic et al., 2014). For example, exosomes have been shown to play a role in the spread of cancer cells, as they can transport oncogenic proteins and RNA to other cells leading to tissue invasion and metastasis (Shami-shah et al., 2023).

Despite many challenges in EV isolation and characterization, it is now clear that several types of EVs can be released from a single cell. Currently it is not possible to selectively differentiate one EV population from another to fully study them individually. Current methods of purification often result in mixtures of particles (Minciacchi et al., 2015a).

Despite the above limitations, the notion that EVs are products of many – and possibly all – cells, and are actively shed in a finely regulated manner, has completely ruled out the possibility that they represent an artifact of purification (Cocucci et al., 2009). As such, EVs can be isolated from different biological fluids such as blood, urine, and cerebrospinal fluid, and their biomolecular content can be analyzed to monitor the progression of certain diseases (Shami-shah et al., 2023).

2.3.1 Extracellular vesicle subtypes

2.3.1.1 Exosomes

Exosomes are the smallest type of EVs that usually range from 30–100 nm. These EVs are formed by endosomal cell sorting pathways in a carefully orchestrated packing, budding, and fusion process (Shami-shah et al., 2023). Multivesicular bodies (MVBs) and late endosomes are a subset of specialised endosomal compartments rich in intraluminal vesicles (ILVs), which sequester specific proteins, lipids and cytosolic components (Gurung et al., 2021). ILVs are generated by the inward budding of endosomal membranes (Pan & Johnstone, 1983). MVBs are transported to the plasma membrane via the cytoskeletal and microtubule network (Colombo et al., 2014). After that, MVBs containing ILVs can either fuse with the plasma membrane for exosome release into extracellular space or fuse with lysosomes for degradation (Buschow et al., 2009; Minciacchi et al., 2015a). This last step, the fusion of the MVBs with the plasma membrane with consequent release of the EVs into the extracellular space, is the least characterized (Minciacchi et al., 2015a). The soluble NSF-attachment protein receptor complex

(SNARE) has been shown to take part in this process; and the Ca^{2+}-regulated vesicle-associated membrane protein 7 (VAMP7) seems to be necessary for MVB fusion with the plasma membrane in leukemic cells (Fader et al., 2009). After MVBs fuse with the cell surface, ILVs are secreted as exosomes (Colombo et al., 2014).

The endosomal-sorting complex required for transport (ESCRT) is responsible for the accumulation and sorting of molecules channeled into the ILVs (Adell et al., 2014; Morvan et al., 2012). Two proteins, called Alix and the tumor susceptibility gene 101 (Tsg101) are involved in this mechanism (Kowal et al., 2014). Other membrane bound and cytosolic proteins that are incorporated into exosomes are members of the tetraspanin family (CD9, CD63 and CD81), integrins, heat shock proteins (Hsp), actin and flotillins (Gurung et al., 2021).

Proteins frequently found involved in exosome biogenesis, such as Tsg101, have been used as exosome markers in benign and cancer models (Minciacchi et al., 2015a). Nevertheless, the ESCRT machinery is not the only mediator of ILV cargo sorting and formation. Other ESCRT-independent processes also seem to functionally participate in exosome biogenesis (Marsh & van Meer, 2008). Involvement of sphingomyelinase activity has been shown both by the high levels of ceramide found within purified exosomes, and by the reduction of EVs release upon inhibition of sphingomyelinases (Trajkovic et al., 2008). Two lipids, cholesterol (Chol) and phosphatidic acid (PA), have also been shown to participate in this process (Minciacchi et al., 2015a). Furthermore, evidence suggests that syntenin can promote exosome formation in collaboration with the GTP-binding protein ADP-ribosylation factor 6 (ARF6) and its effector phospholipase D2 (PLD2) (Baietti et al., 2012; Ghossoub et al., 2014). The involvement of the Rab family of small GTPases in vesicle trafficking and fusion with the plasma membrane suggests that there is also a role for these proteins in exosome release (Minciacchi et al., 2015a). While the importance of these proteins as a class in the regulation of exosome shedding is undenied, specific roles for each of the Rab family members in the process are still unclear (Ostrowski et al., 2010). For example, Rab27a appears to play a more specific function than Rab27b in regulating exosome release from metastatic tumor cells (Bobrie et al., 2012).

Exosome cargoes can include proteins, nucleic acids and metabolites (Bobrie et al., 2011) reflecting the nature of donor cell and its physiological state (Zamani et al., 2019). They can be selectively taken up by neighboring or distant cells far from their release, reprogramming the recipient cells upon their bioactive compounds (Zhang et al., 2019b). It is now evident that exosomes can be produced by most organisms, including bacteria, and can be identified in diverse ecosystems, including in the ocean (Biller et al., 2014).

Totally, in the human body, exosomes can be produced by all cell types examined so far (Minciacchi et al., 2015a). They are found in large quantities in most body fluids (Rashed et al., 2017; Vlassov et al., 2012). Exosomes and other EVs play important functions dictated by their cell of origin and their content (Cocucci et al., 2009) and are continuously released by all living cells (Skog et al., 2008). They are a natural carrier system, which transports mRNA, miRNA, protein,

and DNA from donor to recipient cells, where they target gene expression regulation (Lu et al., 2019).

Exosomes have appeared to play important roles in normal physiological intercellular communication, for example in cardiac development and myocardia angiogenesis (Emanueli et al., 2015). They are also involved in vascular leakage in pre-metastatic sites, which plays an important role in the formation of the pre-metastatic niche (Isola et al., 2016). Although the distinction between tumor derived and physiological exosomes is very hard, it is believed that cancer patients present higher content of exosomes, which could serve as a reliable biomarker for tumor management (Nanou et al., 2020). Additional studies have indicated that the release of small EVs containing genomic DNA is driven by specific oncogenes. This functionally active DNA is transferred to target cells via EVs (Lee et al., 2014).

2.3.1.2 Migrasomes

Migrasomes are extracellular vesicles, which transport multivesicular cytoplasmic content during cell migration. During cell migration, vesicles with diameters up to 3 μm grow on the tips or at the intersections of retraction fibers. These vesicles, which have been named "migrasomes", contain numerous smaller vesicles, with diameters of about 50–100 nm. During migrasome biogenesis, there is an initial phase of rapid growth, followed by a relatively stable period. Eventually, retraction fibers break and migrasomes are released into the medium or directly taken up by surrounding cells. As such, migracytosis is cell migration-dependent and the formation of migrasomes is dependent on both migration and actin polymerization. Migracytosis and migrasomes are present in many cell types and can occur in cells grown in a 3D matrix or on top of other cells. Transmission electron microscopy (TEM) analysis has revealed that migrasome-like structures are present in various tissues (Ma et al., 2015).

2.3.1.3 Microvesicles

Microvesicles (MVs) are 100–1000 nm sized cell blebs that are rich in phospholipids. They are shed from almost all cell types. This process is enhanced when cells are injured or activated, therefore reflecting the state of the cell from which they originate (Badimon et al., 2018). MVs contribute to coagulation and thrombus formation through the externalization of the procoagulant anionic phospholipid phosphatidylserine (PS), provide binding sites for the assembly of coagulation enzymes, induce tissue factor CD142 activity, and promote thrombin generation and thrombus formation (Chiva-Blanch et al., 2020). The surface of some MVs contains tissue factor (TF) which is one of the main triggers of coagulation (Suades et al., 2012). Overall, MVs exposing PS may have up to 50– to 100-fold higher specific procoagulant activity than activated platelets (Sinauridze et al., 2007).

Unlike exosomes, MVs seem to originate directly from the plasma membrane, and they are often classified as ectosomes (Minciacchi et al., 2015a). Their formation starts from the formation of outward buds in specific sites of the membrane followed by fission and subsequent release of the vesicle into the extracellular space (Muralidharan-Chari et al., 2010; Ratajczak et al., 2006). The plasma membrane undergoes several molecular rearrangements at the sites of MVs origin, which result in membrane budding, including changes in lipid and protein composition (Piccin et al., 2007) and in Ca^{2+} levels (Pap et al., 2009). The altered levels of Ca^{2+} result in the recruitment and activation of calcium-dependent enzymes like scramblase and floppase with subsequent modification of the plasma membrane lipid composition (Piccin et al., 2007). Externalization of PS appears to be one of the main features of MVs (Lima et al., 2009) but the secretion of PS-negative MVs has also been reported (Barteneva et al., 2013). Moreover, lipid raft domains seem to be abundant in MVs, and MV formation can be impaired by cholesterol depletion (del Conde et al., 2005).

In addition to rearrangements in the plasma membrane composition, proteins responsible for cell shape maintenance may be involved in MVs biogenesis by regulating actin dynamics (D'Souza-Schorey & Di Vizio, 2014; Minciacchi et al., 2015a). RhoA, a member of the small GTPases family, has been identified together with its downstream targets Rho-associated coiled coil containing protein kinase (ROCK) and the LIM kinase (LIMK) as a regulator of MVs release (Li et al., 2012). Calpain, a calcium dependent enzyme, which regulates cytoskeletal proteins, has also been reported as a component of the MVs biogenesis machinery in platelets (Crespin et al., 2009). In addition to this, the D'souza-Schorey group has demonstrated that ARF6 is a key protein in MVs formation and shedding (Muralidharan-Chari et al., 2009) and the ARF6-regulated endosomal complex seems to play an important role in the selective incorporation of molecular cargo into MVs (D'Souza-Schorey & Chavrier, 2006).

ARF6 downstream targets include extracellular signal-regulated kinases (ERK) and myosin light-chain kinase (MLCK), which are two key regulators of actin polymerization and myosin activity. As a result, MVs release in the extracellular space can be reduced by the inhibition of either ARF6 activity or activity of ARF6 targets (Muralidharan-Chari et al., 2009). In the case of MVs, an important role in the cargo selection seems to be directed by the ARF6-regulated recycling pathway (Muralidharan-Chari et al., 2009, 2010). This process can regulate the inclusion of proteins such as major histocompatibility complex (MHC) class I, β1 integrin receptors, vesicle associated protein 3 (VAMP3), and membrane type 1 matrix metalloproteinase (MT1MMP) (Muralidharan-Chari et al., 2009).

2.3.1.4 Apoptotic bodies

Apoptotic bodies (apoBD) are a EV type sized 1–5 μm which are formed during apoptosis when the plasma membrane of dying cells becomes blebbed and forms vesicles (van Niel et al., 2018). This kind of blebbing during apoptosis has long been known to produce microvesicles in the form of apoptotic bodies (Hristov et al., 2004).

The analysis of platelets with identical settings of the FACScan has shown that apoptotic bodies are of similar size as platelets at 1–4 μm. Apoptotic bodies, as well as microparticles, stain positively with annexin V, which demonstrates their origin from apoptotic cells. Furthermore, apoptotic bodies contain DNA as demonstrated by staining with DAPI or PI (Hristov et al., 2004). Data has also shown that somatic cells may rescue and reuse DNA from apoptotic bodies. For example, apoptotic bodies derived from EBV-carrying B lymphocytes may serve as the source of viral transfer to cells that lack receptors for the EBV virus in vivo (Holmgren et al., 1999).

2.3.1.5 Oncosomes

Large oncosomes (LO) are cancer-derived extracellular vesicles that are atypically large at a size range of 1–10 μm diameter. They originate from the shedding of membrane blebs and associate with advanced disease (Minciacchi et al., 2015b). Janus Rak's group first used the term 'oncosome' in 2008 to describe, in the context of brain tumors, the existence of EVs released from glioma cells and expressing EGFRvIII. These vesicles were shown to be capable of transferring the oncoprotein EGFRvIII to the membrane of tumor cells lacking this receptor, thus propagating tumor-promoting material and inducing transformation (Al-Nedawi et al., 2008). These 'oncosomes' are capable of transferring oncogenic signals and protein complexes across cell boundaries (Di Vizio et al., 2009).

Oncosome exchange is markedly different from paracrine effects induced by soluble ligands (Al-Nedawi et al., 2008). Although this process resembles paracrine signaling, it involves inter-cellular transfer of a membrane-bound micro-organelle rather than a soluble protein such as a growth factor or cytokine. Oncosomes might also be vectors of certain cancer progression markers that are detectable in the circulation (Di Vizio et al., 2009).

Vizio et al. demonstrated that prostate cancer cells shed membrane-bound vesicles in response to signal transducers. These structures were large, in a size range of 500 to 5000 nm, originated from non-apoptotic blebs in response to signaling cues, and had biological activity in their free-floating state. They identified the formin homology protein, DRF3/Dia2, as a protein that appeared to functionally inhibit oncosome formation. It was shown that oncosomes transfer between tumor cells, or between tumor and stroma, could play a role in propagation of aggressive behavior within the tumor microenvironment (Di Vizio et al., 2009). It has also been shown that oncosomes isolated from prostate cancer cells and patient plasma are an EVs population that is enriched in chromosomal DNA, including large fragments up to 2 million base pair long. While oncosomes and exosomes isolated from the same cells contained similar amounts of protein, the DNA was more abundant in oncosomes, despite exosomes being more numerous (Vagner et al., 2018).

Large oncosomes harbor abundant bioactive molecules, including signaling factors involved in cell metabolism, mRNA processing and cell growth and motility (Di Vizio et al., 2009). They also contain miRNAs, metalloproteinases,

and caveolin-1 (Cav-1) (Di Vizio et al., 2012). Cav-1 is a serum biomarker of metastatic prostate cancer (Tahir et al., 2003). Interestingly the presence of large oncosomes containing Cav-1 discriminates patients with metastatic disease from patients with organ-confined prostate cancer (Morello et al., 2013). Oncosomes also potently stimulate expression of metastasis-associated factors, such as brain-derived neurotrophic factor (BDNF), C-X-C motif chemokine 12 (CXCL12), and osteopontin, in stromal cells (Di Vizio et al., 2012). Larger EVs including large oncosomes might contain more abundant miRNA molecules, thus serving as an enriched source of biomarkers and for the identification of functionally relevant EVs cargo (Minciacchi et al., 2015a).

2.3.2 Content of extracellular vesicles

EV content is highly heterogeneous as EVs can accommodate proteins, nucleic acids and lipids, and protein cargo that includes both surface molecules and intravesicular molecules (D'Asti et al., 2012). EV cargo is not merely a reflection of the donor cell composition but rather the result of a regulated, but still largely unresolved, sorting mechanism (Minciacchi et al., 2015a). What seems to be clear, is that there is some kind of selection mechanism, which allows the discrimination between molecules meant to be included in EVs to be delivered and those that are not (Valadi et al., 2007).

The nature and abundance of the molecular cargo is often influenced by the type and physiological or pathological condition of the donor cell, the stimuli that modulate EV production and release, and, most likely, the pathways that lead to the formation of different EV types. These different layers of regulation explain, at least in part, how the EV message can be precisely modulated. EVs can carry and consequently transfer into recipient cells tumor-derived molecules, for example epidermal growth factor receptor vIII (EGFRvIII) and mutant Ras family members or c-Met, a receptor tyrosine kinase (RTK) that consists of an extracellular alpha chain and a beta subunit. Also, other proteins or transcripts with oncogenic functions that are currently being proposed as biomarkers in cancer (Minciacchi et al., 2015a).

Large-scale profiling experiments are generating valuable information on different types of EV populations originating from single cell systems and on single classes of EVs from different cell types. These include mass spectrometry, miRNA arrays and sequencing. On the other hand, lipidomics characterizations are still lagging behind. However, the resulting datasets have been collected into three main databases: Exocarta, Vesiclepedia and EVpedia. These resources contain information on protein, mRNA and miRNA identified in at least one EV population. EVpedia contains integrated datasets from prokaryotic and eukaryotic EVs. This information can be used as a means of comparison with new datasets and as a valuable resource for computational studies to identify new candidate markers that can facilitate the understanding of EVs origin and function (Minciacchi et al., 2015a).

2.3.2.1 Proteins

A recent report that analyzed 16 different mass spectrometry studies identified almost 800 common vesicular proteins in exosomes (Choi et al., 2015; D'Souza-Schorey & Di Vizio, 2014). Another report that looked at over 110 human plasma samples showed the presence of 4500 proteins and 829 lipids in small extracellular vesicles (Rai et al., 2024).

The selection of the proteins exported in EVs is not only affected by the status of the donor cell but also depends on the subcellular compartment of origin. This choice influences both the intercellular interactions and the message delivered by these EVs. Proteins, which are frequently used as exosome markers, are often also involved in exosome biogenesis. These include Alix, Tgs101, ceramide, flotillin, Rab and tetraspanin family members (Minciacchi et al., 2015a). In particular, CD9, CD81 and CD63 have been shown to participate in endosomal vesicle trafficking (Abache et al., 2007; Pols & Klumperman, 2009). A panel of 182 proteins including ADAM10, STEAP23, STX7, and 52 lipids including PS, PIPs, Hex2Cer, PAS, have been shown to be intrinsically linked to EVs. ADAM10 and PS (36:1) have been named as conserved EV biological markers that precisely differentiate between EV and non-EV particles (Rai et al., 2024).

2.3.2.2 Nucleic acids

Exosomes can export miRNAs, long non-coding and other non-coding RNAs, and mRNA. Importantly, EVs might represent a vehicle in which these nucleic acids can be preserved and analyzed in biological fluids, as well as delivered to their target cells without being degraded in the extracellular space. This is particularly important for mRNA, which is sensitive to RNAses. Once taken up by recipient cells, mRNAs could play specific functions upon translation into protein products (Minciacchi et al., 2015a).

The first demonstration that exosomes can carry single-stranded DNA (ssDNA) and retrotransposones was reported by Balaj et al. in 2011 (Balaj et al., 2011). More recently, two independent studies have shown that exosomes also contain double-stranded DNA (dsDNA) which can be profiled in circulation using next generation sequencing (NGS) technologies (Kahlert et al., 2014; Thakur et al., 2014). However, whether the DNA contained in EVs is representative of the originating tumor cells has not been fully demonstrated. This finding opens up the opportunity to use the dsDNA derived from exosomes and potentially other EVs as an alternative, more concentrated and better preserved source of cancer-derived genomic material, than circulating DNA (Minciacchi et al., 2015a).

2.3.3 Main functions of EVs

The interaction of EVs with their target cells is not only mediated by membrane-membrane contact, but often results in EV uptake with subsequent transfer of EV cargo. Both, fusion and active endocytosis, have been proposed as mechanisms

for exosome uptake (Chen et al., 2014a; Minciacchi et al., 2015a; Tadokoro et al., 2013). Exosome and MV binding and internalization can be regulated by adhesion molecules (Morelli et al., 2004). For example, the interaction between diverse combinations of tetraspanin complexes, highly represented on exosome membranes, and integrins on the target cell, might influence the selection of the recipient cell (Wei et al., 2014). EV-cell interactions can also be mediated by the PS and TIM4 expressed on EVs (Feng et al., 2010). Finally, a relevant role in EV internalization seems to be played by heparan sulfate proteoglycans residing on the plasma membrane of the target cell (Christianson et al., 2013). Whether these processes are truly selective or can occur randomly is still largely unclear (Minciacchi et al., 2015a).

The EV-target cell interaction is the first step of EV uptake, and seems to be unavoidably followed by fusion or endocytosis (Mulcahy et al., 2014). Fusion is considered a more passive event in which the membrane of the exosome and the membrane of the recipient cell melt together, thereby forming a continuous structure after the merging of the two distinct lipid bilayers. Endocytosis is an active process but the regulatory features of this active uptake is still largely undefined, although phagocytosis (Feng et al., 2010) as well as a lipid raft-dependent endocytosis, positively regulated by ERK1/2 and inhibited by Cav-1, might occur (Svensson et al., 2013). Once inside the recipient cell, exosomes can either release their content through fusion with the endosome membrane or can be targeted to lysosomes for degradation (Minciacchi et al., 2015a).

EVs participate in **cell-to-cell communication**. They may mediate the transfer of nucleic acids, including mutated genetic material (Minciacchi et al., 2015a). This might occur through direct transfer of DNA (Lee et al., 2014). Oncogene transcripts can be propagated through EV transfer and then translated into proteins in the recipient cells (Skog et al., 2008). Analogously, EV enclosed miRNAs can regulate gene expression, thus altering the behavior of the recipient cells (Chen et al., 2014a; Josson et al., 2015; Tadokoro et al., 2013).

EVs play a part in **extracellular matrix (ECM) remodeling**. The matrix metalloproteinase (MMP) family of proteins are implicated in ECM remodeling and in cancer cell protease-dependent migration and invasion. MT1-MMP, MMP9 and MMP2 or a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10) have been identified in different EV types (Minciacchi et al., 2015a). MMPs can be found inside or on the EV membrane and they are also functionally active. For example, the MT1-MMP ability to remodel the ECM has been shown in melanoma derived exosomes (Hakulinen et al., 2008). Because MT1-MMP is a proteolytic activator of MMP9 and MMP2, and these three proteins are not always identified in the same type of EV, it might be possible that different EVs release different molecules that then regulate each other's activity in the extracellular milieu (Minciacchi et al., 2015a).

EVs modify **fibroblast functionality**. Tumor derived EVs can functionally modify fibroblasts by reprogramming them to cancer associated fibroblasts (CAFs) that exhibit a myofibroblastic differentiation (Castellana et al., 2009). Webber et al. demonstrated that exosomes can release an EV specific form of transforming

growth factor beta1 (TGF-β1) that induces myofibroblast differentiation and actively promotes tumor progression (Webber et al., 2010, 2015). Disruption of EV mediated interactions of tumor cells with the surrounding stroma in vivo significantly reduces tumor growth by impairing either EV dependent signaling activation in target cells or exosome production (Webber et al., 2015).

Antonyak et al. have demonstrated in multiple studies that MVs released from breast cancer or glioblastoma cell lines can induce transformation of fibroblasts. This process is mediated by fibronectin1 (FN1) and tissue transglutaminase (tTG) (Antonyak et al., 2011). Along with the notion that CAFs might release soluble factors that induce epithelial-mesenchymal transition (EMT) or stemness in cancer cells, it is becoming evident that these processes might be also regulated by CAF-derived EVs (Minciacchi et al., 2015a). Wrana's group has demonstrated the ability of exosomes released by fibroblasts to enhance breast cancer cell invasion (Luga et al., 2012). Cancer cell motility is also induced by exosomes released from CAFs silenced for tissue inhibitor of metalloproteinases (Timp). In this system, Timp knock down resulted in increased expression of ADAM10 in the exosomes (Shimoda et al., 2014). Fibroblast-derived exosome miRNA has also been shown to induce EMT in cancer cells (Josson et al., 2015).

EVs have roles in **angiogenesis**. Cancer cell-derived EVs contain interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF) which are both potent pro-angiogenic factors, as well as other molecules able to enhance endothelial cell invasion and organization in tubule-like structures (Skog et al., 2008); (Thompson et al., 2013). Exosomes expressing the neutral sphingomyelinase 2 (nSMase 2) can induce tubule formation and migration of HUVEC cells and promote tumor progression through in vivo stimulation of angiogenesis (Kosaka et al., 2013). Angiogenesis is strongly stimulated by hypoxia, in which an increased release of exosomes stimulates tubule formation in different tumor types (Tadokoro et al., 2013). In addition to the direct transfer of pro-angiogenic proteins, recent studies are identifying novel EV-enclosed molecules that participate in angiogenesis (Minciacchi et al., 2015a). These include EGFR (Al-Nedawi et al., 2009), miR-210 (Tadokoro et al., 2013) and miR-9 (Zhuang et al., 2012).

EVs are involved in **immune response modification**. Tumor cells can acquire immunotolerance either through induction of T regulatory cell proliferation or cytotoxic T cell death. Both of these mechanisms can be mediated by EVs (Taylor & Gercel-Taylor, 2011). Ovarian cancer-derived EVs can enhance regulatory T cell proliferation and activity (Szajnik et al., 2010). EVs originating from tumor cell lines can induce a FasL or TNF-related apoptosis-inducing ligand (TRAIL) dependent cell death in CD8+ T cells (Abusamra et al., 2005; Huber et al., 2005). EV-mediated immunotolerance can also be seen as EVs containing TGF-β can impair Natural Killer and T cells activation (Clayton et al., 2008).

EVs take part in **drug resistence**. Breast cancer cell EVs as well as EVs from other tumor types can transfer resistance to docetaxel in cells that are sensitive to the drug (Choi et al., 2014; Lv et al., 2014). In breast cancer, this process seems to be specifically mediated by P-glycoprotein (Lv et al., 2014), or by its activator transient receptor potential channel 5 (TrpC5) (Ma et al., 2014). Transfer of the

prosurvival Akt/mTOR complex in EVs can propagate resistance to gefitinib in non-small cell lung carcinoma cells (Choi et al., 2014).

EVs are involved in creating the **metastatic niche**. Peinado et al. have shown that melanoma-derived exosomes can condition a metastatic niche within the bone marrow (Peinado et al., 2012). CD105 positive MVs, which can promote angiogenesis in vitro, may stimulate lung metastasis in vivo in renal cancer (Grange et al., 2011).

2.3.4 Purification of EVs

EVs can be isolated from biological fluids such as blood, cerebrospinal fluid, urine, saliva, and breast milk (Doyle & Wang, 2019). They can also be isolated from conditioned media of cultured cells as well as blood derivatives, and other fluids such as ascites and bronchoalveolar lavage (Minciacchi et al., 2015a). To be effectively utilized for biomedical applications, high-quality EV isolation techniques are required. The heterogeneity of EV size and their relatively low abundance compared to other contaminants in biological fluids make high-quality EV isolation a difficult challenge (Doyle & Wang, 2019). Despite the numerous reports published on comparative methods for EV isolation, we are still far from having standard protocols applicable to clinical practice (Minciacchi et al., 2015a).

Many techniques have been developed for the isolation and characterization of EVs, including reduced solubility approaches, ultracentrifugation, density gradient separation, size-exclusion chromatography, microfluidics, and magnetic bead-based methods (Konoshenko et al., 2018).

Overview of some of the different isolation techniques used has been shown in figure 2, which is modified from the work of Carnino et al. EVs can be isolated via several methods including ultrafiltration, size-exclusion chromatography, differential and density gradient ultracentrifugation, precipitation and immuno-affinity capture. Often, samples are first centrifuged to remove cells and cellular debris. For ultrafiltration and size-exclusion chromatography, EVs are isolated through nanomembrane filters based on their sizes. In ultracentrifugation and density gradient centrifugation, different EV types are sequentially isolated based on physical properties such as density and sedimentation rate. For precipitation-based methods, the addition of water-insoluble polymers force and concentrate the EVs from the other components in the samples. Immunoaffinity capture method involves isolating particular EV types based on the use of magnetic beads coupled with antibodies that bind specifically to a known EV-specific surface marker for separation (Carnino et al., 2021).

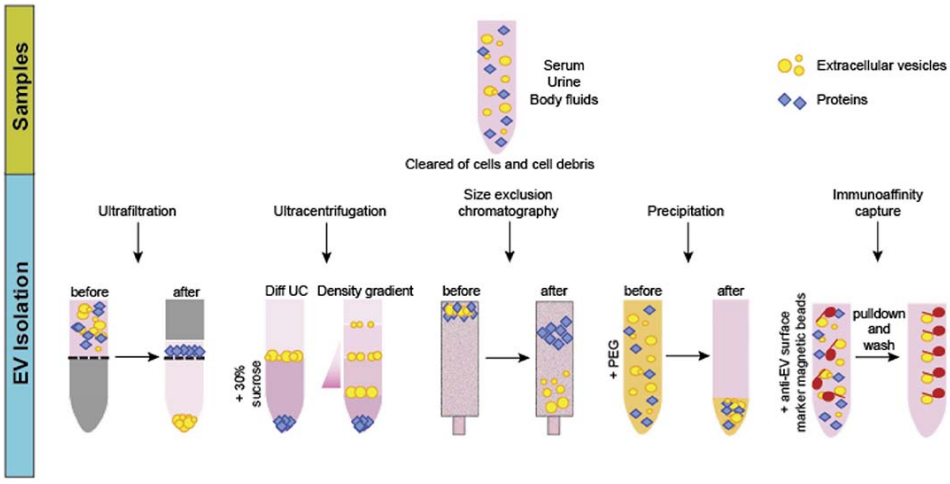

Figure 2. EV isolation, modified from Carnino et al (Carnino et al., 2021).

2.3.4.1 EV isolation challenges

2.3.4.1.1 Overall challenges

Many EV subtypes tend to overlap in size, surface proteome, and cargo, making EVs broadly heterogeneous in nature. Additionally, biofluids of interest for EV isolation contain varying compositions of proteins and non-EV lipid particles that contribute to the heterogeneity, complexity, and viscosity of the biofluid. For instance, analysis of blood serum and plasma estimates that there are roughly 10^{11} EVs and up to 10^{16} lipoproteins per mL of blood plasma. Furthermore, many biofluids, including blood plasma, serum, and cerebrospinal fluid, have a high abundance of albumin and matrix proteins. The lower relative abundance of EVs in these complex matrices can make high-purity EV isolation difficult, as both proteins and lipoproteins can co-isolate with the EVs (Shami-shah et al., 2023).

Many technical challenges persist that can also impact the overall yield and purity of EVs (Lee, 1971). EV losses can reach (51 ± 3%) when cell-culture-derived EVs are stored for 48 h at +4 °C in polypropylene Eppendorf tubes. Such significant EV loss could be reduced to 18–21% by using Eppendorf Protein LoBind tubes or surface blocking with protein blockers (Evtushenko et al., 2020). Although EV counts seem to remain consistent over time in undisturbed samples, mild agitation designed to simulate blood handling during transportation results in a notable and artificial discharge of EVs derived from platelets (Ramirez et al., 2018). Moreover, EVs tend to form EV-blood cell clusters over time, which can further impact EV isolation (Shami-shah et al., 2023).

Purification effectiveness can be evaluated by the presence of markers specific for endosomal plasma membrane and cytoplasmic components enclosed within vesicles, as well as a simultaneous absence of contaminants like albumin, A1/2 and B apolipoproteins (Théry et al., 2018).

2.3.4.1.2 Challenges to isolate cell specific EVs

One of the major barriers to cell-type specific EV isolation is the rare and very low abundance of cell- and tissue-type specific EVs in human biofluids as blood plasma is a repository of EVs from many different cells and tissues (Shami-shah et al., 2023). Totally, around 99% of EVs in healthy blood plasma originate from platelets, red blood cells and white blood cells. Less than 1% originates from solid tissues. There are 0.13 ± 0.1 erythrocyte-derived EVs per erythrocyte to $(1.9 \pm 1.3) \times 10^3$ monocyte-derived EVs per monocyte (Auber & Svenningsen, 2022). EVs originating from tissues have been estimated to account for less than 1% of all EVs, as opposed to the 99.8% generated from hematopoietic cells (Li et al., 2020).

Given that every cell type in the human body secretes EVs into biofluids, relative proportions of cell-type specific EVs can depend partly on the abundance of that cell type (Shami-shah et al., 2023). Additionally, EV secretion rate has been shown to differ drastically between cell types in cultured cells (Auber & Svenningsen, 2022). Both low cell-type abundance and low basal EV secretion rates make some cell-type specific EVs difficult to isolate using conventional technologies due to their low relative concentrations in biofluids (Shami-shah et al., 2023).

2.3.4.2 EV isolation techniques

2.3.4.2.1 Ultracentrifugation

Ultracentrifugation (UC) has been one of the most widely used methods for EV isolation from complex biological samples (Gudbergsson et al., 2016). UC is based on the principle of sedimentation, where EVs are separated from other biomolecules based on their shape, size, and density (Konoshenko et al., 2018).

Ultracentrifugation involves first centrifuging a sample at a low speed of up to 2,000 xg to remove large debris and dead cells (Gudbergsson et al., 2016). To purify MVs, apoptotic bodies and large oncosomes, centrifugation at 10,000–20,000 × g is needed (Cvjetkovic et al., 2014). This is why the resulting supernatant from the first low speed centrifugation is then centrifuged at 16,500 xg or less (Gudbergsson et al., 2016). After that, the supernatant is placed in an ultracentrifuge and spun at high speeds, typically around 100,000 xg or greater, for several hours (Kowal et al., 2017). This step is meant to gather exosomes which require 100,000–120,000 xg to be separated from other EV subtypes (Cvjetkovic et al., 2014). Overall, the high g-force generated from the centrifugation separates different components of the biofluid with the EVs pelleting at the bottom of the tube. The pellet can be washed and resuspended in a buffer for downstream analyses or long-term storage (Shami-shah et al., 2023).

Overall, ultracentrifugation has been a broadly used technique for EV isolation due to several advantages – particularly its adaptability to large volumes of samples. This helps to increase EV yield and makes the approach well suited for studies where large sample volumes are available, such as cell culture media or

easily accessible biofluids. Ultracentrifugation is also relatively inexpensive compared to other methods, such as immunoaffinity isolation (Shami-shah et al., 2023).

There are also several limitations to this method. Many studies have highlighted that this kind of differential centrifugation is known to result in heterogeneous preparations and is not sufficient to isolate pure populations of EVs (Tauro et al., 2013). In addition, ultracentrifugation may result in partial EV aggregation and degradation as the high centrifugal force required may lead to artificial fusion of smaller EVs and fission of larger EVs (Shami-shah et al., 2023). This not only limits the ability of ultracentrifugation to isolate EVs of uniform size, but can also lead to the loss of some of their original biomolecular contents (Momen-Heravi, 2017). Co-sedimentation of non-EV biomolecules, such as lipoproteins and protein aggregates with similar buoyancy, can cause yield and purity problems (Konoshenko et al., 2018). These disadvantages are only amplified when attempting to isolate EVs from sample matrices with higher viscosities, making ultracentrifugation a less viable option in some biofluids such as plasma. Moreover, lengthier spin times also make this technique less efficient and translatable to a clinical setting (Shami-shah et al., 2023).

2.3.4.2.2 Density gradient

Density gradient centrifugation (DGC) is a method that is commonly used in conjunction with ultracentrifugation (Shami-shah et al., 2023). It involves the use of a density gradient, which is created by layering solutions with a range of different densities, such as sucrose or iodixanol (OptiPrep, which contains 60% water), in a tube (Onódi et al., 2018). EVs can be purified and cleared from free proteins and protein complexes or other contaminants better. DGC has been typically used to separate different intracellular organelles based on their sedimentation coefficient (Tauro et al., 2013).

The process starts by filtering or centrifuging the samples to remove debris and large particles that could interfere with EV isolation. This process also dilutes the samples allowing them to pass through the thick gradient during prolonged centrifugation times. The sample is then layered on top of the density gradient and centrifuged at high speed for many hours, depending on the type of biofluid, to separate the different components of the samples (Shami-shah et al., 2023). Post centrifugation, samples are collected from each density fraction of the gradient and EVs are found in the denser fractions (Norman et al., 2021). For example, exosomes have a characteristic buoyant density of 1.10–1.19 g/mL on sucrose density gradients (Rosado et al., 2019).

However, DGC does have some limitations for EV isolation (Konoshenko et al., 2018). DGC requires specialized equipment and can be expensive, low throughput, and time intensive with more than 18-hour spin needed to reach density equilibrium (Shami-shah et al., 2023). Because of the long time required, it is not highly applicable to use in a clinical setting or efficient enough for biomarker discovery (Ter-Ovanesyan et al., 2021). While DGC yields EVs with a relatively high purity compared to ultracentrifugation, higher-order protein

aggregates and lipoproteins with similar densities to EVs can be found as contaminants (Shami-shah et al., 2023). For example, while potassium bromide-density gradient ultracentrifugation on plasma successfully removes very-low-density lipoproteins (VLDLs) from EV fractions, while high density lipoproteins (HDLs) persist. The ratio of EVs to HDLs has been estimated to be as high as 1:100 by TEM (Yuana et al., 2014). Impurities can also arise due to the improper setting up of the gradient, causing intermixing of the gradient interfaces and fractions. All these issues can contribute to an overall low yield and EV contamination, which should be considered when optimizing DGC isolation (Konoshenko et al., 2018).

2.3.4.2.3 Filtration

Size exclusion methods based on the use of filters with specific pore size are often used in combination with other isolation techniques. This is frequently the first step in exosome purification from serum and plasma (Witwer et al., 2013). Filters can also be combined with other methods. To improve enrichment via ultracentrifugation it can be preceded by a filtration step using a 0.22 μm filter. This will remove cell debris, protein aggregates, and lipoproteins before downstream methods are used (Sidhom et al., 2020).

2.3.4.2.4 Size exclusion chromatography

Size Exclusion Chromatography (SEC), also referred to as gel filtration, is a method for isolating EVs based on their hydrodynamic radius. EVs can be separated from soluble proteins based on their ability to be excluded or to pass through pores of different sizes in a chromatography column. To isolate EVs using SEC, sample is passed through the stationary phase of a chromatography column that is packed with beads of a porous material, such as agarose (Sepharose), allyl dextran (Sephacryl), or cross-linked dextran (Sephadex). The beads have pores of different sizes, and as the sample passes through the column with the flow of the mobile phase, the EVs are separated based on their size. Smaller EVs are trapped in the pores of the resin for longer times, so they are eluted from the column later. Larger EVs avoid the pores of the resin and mostly pass through the larger channels, so they are eluted earlier. The separated EVs are collected in fractions and can then be analyzed or used for further experimentation (Shami-shah et al., 2023).

The separation of EVs from various biological fluids, including CSF and plasma, can be optimized based on the column length, size, type of resin, and flow rate of the mobile phase (Ter-Ovanesyan et al., 2021). These parameters can further influence the yield and purity of the isolated EVs (Shami-shah et al., 2023). For example, SEC has been adapted as a single-step isolation system and modified over the years for greater purity and yield (Sidhom et al., 2020). Overall, SEC aids in separating small from large EVs and from non-EV soluble protein contaminants in samples. With an average 20 min processing time, it results in time and cost-effective, pure, intact, and functional EV retrieval. SEC can also be

scaled up and automated, making it a high-throughput method for EV isolation. It can be performed with varying sample volumes, making it useful for isolating EVs from samples with limited volume availability. Additionally, due to the use of gravity as an isolation principle for SEC rather than high g-forces for ultracentrifugation or DGC, the isolated EVs are of superior integrity with intact vesicular properties and higher quality (Shami-shah et al., 2023).

A key drawback to this technique is that SEC cannot effectively differentiate between microvesicles and exosomes of similar size (Sidhom et al., 2020). Furthermore, limitations include the need for specialized resins, columns, and co-elution of similarly sized lipoproteins and large protein aggregates (Taylor & Shah, 2015). Additionally, the dilution of sample created by the mobile phase of SEC makes isolating subpopulations of EVs from low-concentration and small-volume samples a challenging task (Shami-shah et al., 2023).

Despite this limitation, SEC has been shown to outperform the ultracentrifugation and precipitation method in terms of purity and yield in both plasma and CSF, making it a powerful isolation technique for EV research (Ter-Ovanesyan et al., 2021). Shu et al. combined ultrafiltration and size exclusion chromatography, which allowed more exosomes to be isolated than in any of the individual approaches (La Shu et al., 2020).

2.3.4.2.5 Immunoaffinity

Another isolation method is based on immunoaffinity. This method directly retrieves exosomes expressing different markers on their surface, for example CD9, CD63, or CD81. Reagents for such isolation are currently available as commercial kits using columns or microbeads, for example ExoTEST®. Obtained exosomes can be subjected to further research. However, its disadvantages include a small test sample volume used and that its effectiveness is dependent on the presence of specific markers (Zhang et al., 2017a).

For example, microbeads coated with glycoprotein A33 and epithelial cell adhesion molecule precursor, EpCAM, have been successfully used to immunocapture different EVs from colon cancer cells (Minciacchi et al., 2015a). However, several markers that have been considered exosome-specific, including CD63, CD81, CD9 are now being identified in other types of EVs. At the same time, proteins such as Alix and Tsg101 appear to be more consistent exosome markers (Yoshioka et al., 2013). PS, ARF6 and Rho family members have been proposed as MV markers (Minciacchi et al., 2015a). All of this can significantly limit the utility of this approach, as EVs have been found to be heterogeneous, with particular surface markers shared among various EVs subtypes (Kalluri & LeBleu, 2020). This especially undermines the use of a single marker (Kang et al., 2019).

Some methods combine immunoaffinity marker capture with magnetic beads. There are several magnetic bead-based approaches for EV isolation, including magnetic bead-based immunoaffinity chromatography and magnetic bead-based ligand affinity chromatography. In magnetic bead-based immunoaffinity

chromatography, magnetic beads are coated with antibodies that bind to specific proteins or other biomolecules on the surface of the EVs (Shami-shah et al., 2023). The sample is then incubated with the magnetic beads, and the beads, through specific binding interactions, capture the EVs. For instance, antibodies against generic EV surface markers, specifically the tetraspanins CD9, CD63, and CD81, have been used conventionally to perform bulk immunoaffinity EV isolation (Sidhom et al., 2020). In magnetic bead-based ligand affinity chromatography, the magnetic beads are coated with ligands that specifically bind to receptors or lipids, such as PS, on the surface of the EVs. The sample is incubated with the magnetic beads, and the EVs are affinity captured by the beads through ligand-receptor interactions. In both cases, the magnetic beads containing the captured EVs are then separated from the rest of the biomolecules in the sample using a magnetic field. The beads are then washed with buffers to reduce nonspecific interactions and the EVs on the beads can be subjected to further downstream analysis (Shami-shah et al., 2023).

There are several advantages to using magnetic bead-based methods for isolating EVs. Magnetic bead-based immunoaffinity isolation allows for the capture of EVs based on specific biomolecules or receptors, which is useful for isolating subpopulations of cell-type specific EVs (Shami-shah et al., 2023). Additionally, magnetic bead-based methods may be automated to achieve high throughput EV isolation, and can also be useful for isolating EVs from a low sample volume because the approach is highly targeted (Greening et al., 2015).

However, these methods can have some limitations including the requirement for specialized equipment; the high cost of antibodies; and the requirement for high-affinity antibodies or ligands to immunocapture certain populations of EVs. Additionally, because this is a targeted approach, a lack of high-quality antibodies could be a bottleneck for the successful use of this method. Nonspecific binding is another major drawback of magnetic bead capture. Both the adhesion of sample contents to the magnetic bead's surface and off-target antibody binding can result in impurities and can lead to false conclusions about the contents of EVs in downstream analyses (Shami-shah et al., 2023). Especially, as lipids on the EV surface tend to adhere to surfaces nonspecifically (Evtushenko et al., 2020).

Additionally, there are many challenges associated with verifying the low of abundance internal cargo biomolecules detected after immunocapture of EVs. Hence, nonspecific binding is especially problematic when attempting to isolate low-abundance EV subpopulations. Therefore, careful reagent validation and optimization of bead surface chemistry is a crucial step for bead-based immunocapture of EVs (Shami-shah et al., 2023).

2.3.4.2.6 PEG percipitation

EV precipitation by attenuating solubility is one of the earliest conventional approaches used to isolate EVs from a variety of complex biofluids. One such method includes using hydrophilic polymers, such as dextran or polyethylene glycol (PEG) (Weng et al., 2016). When added to a sample containing EVs, these

polymers will form a complex with EVs due to their negative charge. The complex can then be precipitated by adding a neutralizing agent. The EVs can then be recovered from the precipitate by centrifugation at a low speed, <1500 xg, or by filtration (Shami-shah et al., 2023).

There are several commercial PEG-based EV precipitation kits available, including ExoQuick®, ExoQuick® ULTRA (System Biosciences), ExoPrep™ (HansaBioMed), Total Exosome Isolation Kit (Invitrogen), and miRCURY™ (Qiagen) that can be used based on the application, type, and volume of biofluid being used as a starting material (Konoshenko et al., 2018).

When utilizing these reduced solubility approaches, bulk sample yields may be higher relative to other isolation techniques but purity is often compromised (Shami-shah et al., 2023). For example, PEG-based EV precipitation methods have led to the highest yield for both precipitated EVs and proteins (García-Romero et al., 2019). PEG-based polymers are nonspecific reagents for EV precipitation, so they are often accompanied by other non-EV precipitants such as albumin, lipoproteins, and immunoglobulins. These limitations make reduced solubility approaches ineffective for cell-type specific EV isolation. However, efficient processing time, simplicity, and low cost make this method particularly attractive for crude and rapid EV isolation (Shami-shah et al., 2023).

2.3.5 Visualization methods

A range of imaging methodologies have been applied to EVs, contributing to the conclusion that EVs are discrete, particulate structures with a lipid bilayer (Minciacchi et al., 2015a). In the nanoparticle tracking analysis system (NTA) light scattered by the particles can be captured and analyzed by computer software, resulting in a measurement of the size distribution and concentration of the EVs in the samples (Wright, 2012). This method is not suitable for quantitative analysis of EVs larger than 400 nm (Minciacchi et al., 2015a). On the other hand, dynamic light scatter (DLS) is a newer system, based on dynamic as well as electrophoretic and static light scattering, which seems to allow quantitative analysis of EVs of several microns in diameter (Kang et al., 2014).

Flow cytometry analysis is often employed for EV detection, although most instruments cannot analyze particles smaller than 500 nm (Minciacchi et al., 2015a). However, it has been demonstrated that nano-sized particles can be accurately quantified (van der Vlist et al., 2012). Recent studies have further demonstrated that nano-sized EVs can be enumerated by flow cytometry with the support of antibody-coated beads larger than exosomes (Minciacchi et al., 2015a).

To be visualized, nano-sized EVs and MVs require the resolution power of electron microscopy (Minciacchi et al., 2015a). The most common methods used are scanning or transmission electron microscopy (SEM or TEM), or atomic force microscopy (AFM) (Rupert et al., 2017). In SEM, the EV surface is scanned with a focused beam of electrons, and EV topography image is generated (Ma et al., 2019). TEM has higher resolution compared to SEM. In TEM a focused beam of electrons are transmitted through samples to generate images (Hakulinen et al.,

2008; Ma et al., 2019). In AFM, a metal probe is used for scanning EV surface to provide surface topography, local stiffness and adhesion information (Sharma et al., 2010).

Larger EVs, such as large oncosomes, can be visualized by confocal or optical microscopy in tissue plasma membranes, and measured using imaging software (Minciacchi et al., 2015a). Immunofluorescence imaging has also allowed the identification of large oncosomes in cell media and body fluids (Di Vizio et al., 2012; Morello et al., 2013).

2.4 Autoimmunity

Immune tolerance is a state of immune system unresponsiveness to self-tissues that the same time retains the ability to identify and respond against non-self and dangerous antigens. Specialized cell subsets, such as regulatory T and B cells, tolerogenic dendritic cells, and M2 macrophages, participate in keeping the balance between tolerance and activation. However, genetic predispositions and epigenetic modifications combined with exposure to environmental factors can disrupt this status, resulting in the development of autoimmunity (Sakowska et al., 2022).

2.4.1 Autoimmune diseases

Autoimmune diseases are caused by dysregulated immune responses against self-antigens. Close to 5% of the general population in Western countries develops some form of autoimmunity. These diseases have common mechanisms and are caused by both genetic and non-genetic risk factors (Alriyami & Polychronakos, 2021).

Antibodies have a central role in immune response. They represent a key defense mechanism against infectious diseases, recognizing molecules of exogenous microorganisms as non-self (de Jonge et al., 2021). Natural antibodies or autoantibodies are antibodies that react with self-molecules (Elkon & Casali, 2008). This kind of autoantibody effect is detrimental when they start targeting host tissues. In these pathological conditions, auto-antibodies can cause inflammation in joints, such as in rheumatoid arthritis (RA) or can affect the lungs, blood cells, nerves, and kidneys for example in systemic lupus erythematosus (SLE), or intestines, such as in inflammatory bowel disease (IBD) (de Jonge et al., 2021). A dysregulated inflammatory response against self-antigens, e.g. against the pancreatic insulin-producing beta cells causes type 1 diabetes (T1D), against the myelin sheath causes multiple sclerosis (MS), and against chromatin leads to SLE (Alriyami & Polychronakos, 2021).

It is worth noting that patients affected by these diseases have a significantly modified risk to develop cancer (Vlagea et al., 2018). This risk is often increased in SLE, RA, Sjögren syndrome, IBD, and systemic sclerosis, resulting in frequent occurrence of several types of cancers (Valencia et al., 2019).

2.4.2 Immunity and cancer development

For years, autoimmunity and cancer have been considered as two separate fields of research that do not have a lot in common. Autoimmune disease results from an immune response against self-antigens, while cancer develops when the immune system does not respond to malignant cells. One of the hallmarks of cancer is attracting tolerogenic cell subsets while evading the immune response. Malignant cells express immune checkpoint proteins to show impaired antigen presentation, undergo epithelial-to-mesenchymal transition, or present alterations in RNA editing. However, the discovery of immune checkpoints and the development of anti-cancer drugs targeting programmed cell death receptor 1 (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4) pathways proved that studying autoimmune diseases can be extremely helpful in the development of novel anti-cancer drugs (Sakowska et al., 2022).

Multiple approaches have been made to break cancer tolerance and awaken the immune system to fight against it. These strategies have been based on monoclonal antibodies, adoptive cell therapies, or therapeutic anti-cancer vaccines. Nevertheless, there is still a lack of full understanding of the complex network of mechanisms leading to tolerance induction or its breakdown (Sakowska et al., 2022). It is possible that pre-existing auto-antibodies could directly contribute to promote or suppress cancer progression (de Jonge et al., 2021).

2.4.3 Autoimmunity against cancer

Numerous studies have demonstrated that metabolites and other proteins released from tumor cells can trigger humoral immune responses in patients (Finn, 2005). Tumors themselves consist of a complex mixture of both germline-encoded and novel somatically generated antigens. Germline encoded antigens typically derive from proteins that are not antigenic in normal cells. However, when tumor cells start expressing them well above normal levels, or in places where they become exposed to the immune system, they can become so called tumor-associated antigens (TAAs). Somatically generated novel antigens are derived from normal genes by somatic mutation, deletion, or epigenetic modifications and are called tumor-specific antigens (TSAs) (de Jonge et al., 2021). Classification of tumor antigens has been shown in figure 3 (Feola et al., 2020).

One promising approach for early detection of cancer is to look for the immune response against cancer. The immune system is especially well adapted to detect very low levels of antigen and to respond to these minute amounts by generating very-high-affinity T cells and antibodies. This kind of immune response is generated locally after small amounts of TSA or TAA proteins, which originate in only a few tumor cells, are processed by antigen-presenting cells. After processing, they are displayed to lymphocytes in the lymph node that drains the site of a developing tumor. As a response, antibodies and T cells, that can enter circulation, are generated (Finn, 2005). These autoantibodies could serve as pioneer reporters of tumorigenesis as their formation occurs several months or

years earlier than the onset of clinical symptoms of cancer (Yao et al., 2012). Autoantibodies against cancer could be detected even at early stages of cancer development (Chen et al., 2014b). However, the presence of a TSA or TAA does not elicit immune responses to malignant cells themselves (Bates et al., 2018).

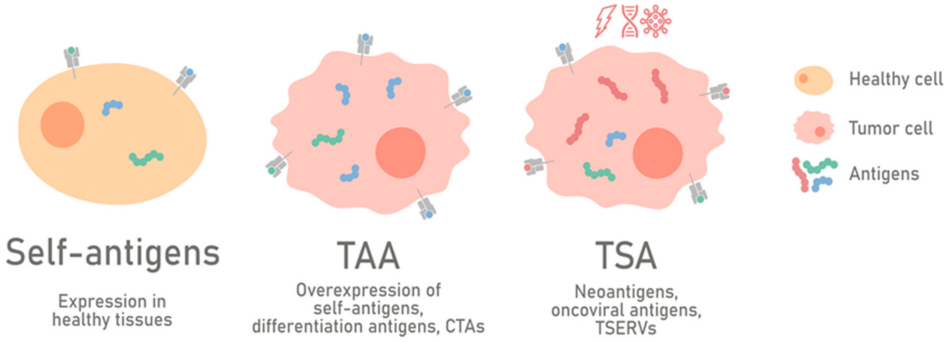

Figure 3. Classification of tumor antigens. Tumor antigens can generally be categorized into tumor-associated antigens (TAAs) and tumor-specific antigens (TSAs) based on the expression pattern of the parental gene. TAAs are self-proteins expressed in cancer cells that appear upon malignant transformation due to the overexpression of normal proteins (gene overexpressed), the expression of proteins with tissue-specific gene patterns (differentiation antigens), or the expression of proteins derived from gene expression restricted to the testes (cancer testis antigens, CTAs). TSAs are proteins expressed by tumor cells and can arise from mutations (neoantigens), from viruses being involved in the oncogenic transformation (oncoviral antigens), or from the expression of tumor-specific endogenous retroviruses (Feola et al., 2020).

One set of antibodies may tell us that a tumor is developing (diagnosis), whereas another set might tell us that the tumor has been or is likely to be destroyed (prognosis) (Mintz et al., 2003). Autoantibodies circulate for a longer time than other polypeptides, since they are stable in the serum and are often produced in large amounts. Therefore, serum profiling of circulating autoantibodies is considered an attractive method in the diagnosis of cancer at early stages (Chen et al., 2012).

2.4.4 Cancer testis antigens (CTA)

Cancer-testis antigens (CTAs) are a group of proteins that have restricted expression to testis, ovaries and placenta, and are aberrantly re-expressed in cancer where they have the potential to be immunogenic (Simpson et al., 2005). CTAs commonly share multiple characteristics including a highly tissue-restricted expression profile, existence as multigene families, frequent mapping to chromosome X, heterogeneous protein expression in cancer, likely correlation with tumor progression, induction of expression by hypomethylation and/or histone acetylation, and immunogenicity in cancer patients. By 2002, 20 CTA genes or gene families had been identified (Scanlan. et al., 2002). By 2020, there were over 270 distinguished CTAs (Danilova et al., 2020).

Some antigens, including MAGE antigens, are represented by multi-antigen families of related antigens. Each gene in the gene family is located in the same chromosome locus and is governed by the same enhancer. As such, members of one family are almost always co-expressed with the same level of expression. Moreover, there is high gemology of gene family members in the structure and function, and they also share immunogenic properties. On the other hand, some families consist of only one member, for example SCP1 (Danilova et al., 2020).

CTAs play a role during embryonic development and in stem cell self-renewal (Costa et al., 2007). For example, N-RAGE, NY-ESO, MAGE-A1, and SSX are expressed in human bone marrow mesenchymal stem cells (Cronwright et al., 2005). CTA expression is dependent on the maintenance of an undifferentiated phenotype in stem cells. In tumor tissues, they are heterogeneously expressed in only a few cells within the tumor mass (Costa et al., 2007).

Emerging evidence shows that a number of CTAs promote epithelial mesenchymal transition and genesis of cancer stem like cells. This escalates tumorigenesis, invasion, and metastasis. CTAs such as SSX, MAGE-D4B, CAGE, Piwil2, and CT45A1, upregulate EMT and metastatic genes, promoting tumor dissemination. In addition, certain members of CTAs, including Piwil2, DNAJB8, CT45A1, MAGE-A, GAGE, and SPANX, are implicated in the initiation or maintenance of cancer stem-like cells, promote tumorigenesis and malignant progression. Clinically CTAs are closely associated with poor prognosis in cancer patients (Yang et al., 2015).

CTAs are generally upregulated in response to DNA hypomethylation, which is a common feature of cancer cells. Treating cells with the DNA methyltransferase inhibitor decitabine, also known as 5-aza-2′-deoxycytidine, in vitro results in a dramatic upregulation of most, if not all, CTAs. A correlation between CTA expression and promoter methylation status in tissue samples has been shown in several different types of cancer (Kulkarni et al., 2012).

2.4.4.1 MAGE-A family

The Melanoma Antigen Gene (MAGE) protein family is a large, highly conserved group of proteins that share a common MAGE homology domain (MHD) (Weon & Potts, 2015). MAGE proteins comprise a super-family of more than 60 genes in humans (Chomez et al., 2001; Simpson et al., 2005). The MAGE family has garnered growing interest as biomarkers in cancer as well as immunotherapy targets. A subset of these >40 human proteins are classified as cancer-testis antigens (Simpson et al., 2005).

First member of the human MAGE family was identified as a gene encoding a tumor-specific antigen in 1991 (van der Bruggen et al., 1991). This gene was later found to belong to a cluster of 12 MAGE-A genes located in the q28 region of the X chromosome (De Plaen et al., 1994; Rogner et al., 1995). Sequencing the human Xp21 region led to the discovery of a second cluster that was named MAGE-B (Lucas et al., 1998; Lurquin et al., 1997; Muscatelli et al.,

1995). At the end of 1990s, the MAGE-C cluster was identified in Xq26–27 (Lucas et al., 1998, 2000).

Overall, MAGE family members can be divided into two categories based on tissue expression pattern. Type I MAGEs are considered CTAs and include MAGE-A, -B, and -C subfamilies (Barker & Salehi, 2002; Simpson et al., 2005). Genes belonging to the MAGE-A, -B, and -C subfamilies are characterized by a large terminal exon encoding the entire protein (Rosenberg, 2000). Excluding pseudo-genes, there are eleven genes in the MAGE-A family [termed MAGE-A1 to -A12 (MAGE-A7 is a pseudogene)], nine in MAGE-B and four in MAGE-C (Chomez et al., 2001). Type II MAGEs include MAGE-D, -E, -F, -G, -H, -L subfamilies and Necdin, which are expressed throughout many tissues and are not restricted to the X chromosome (Barker & Salehi, 2002).

Both type I and type II MAGEs contain a MAGE homology domain that is approximately 170 amino acids long and on average 46% conserved amongst all human MAGE proteins (Weon & Potts, 2015). The sequence encoding the MAGE conserved domain is entirely in a single exon in MAGE-A, -B, and -C genes (Chomez et al., 2001). Other regions of the proteins, especially N-termini, are completely different from one sub-family to another and could therefore reflect differences in functional specificity. In the MAGE-A family, the MHD encompasses as much as 70% of the protein. The high conservation of the MHD suggests that MAGE proteins may share common structure and function that can contribute to both their normal physiology and to the development of cancer (Meek & Marcar, 2012).

The large number of MAGE genes that can be expressed in cancers means that it is extremely difficult to assess the expression of all the relevant genes in all cancers. However, cancer cells generally tend to co-express combinations of two or more MAGE-A, or other CTAs, antigens in a manner that is unlikely to be random (Meek & Marcar, 2012). For example, combinations frequently include MAGE-A3 or MAGE-C2 suggesting a possible selection for these proteins. Additionally, MAGE-A expression is observed mainly in cancers that have acquired malignant phenotypes, for example invasiveness or metastasis (Simpson et al., 2005). MAGE detection also correlates with poor prognosis in cancer patients, underpinning the idea that MAGE proteins may contribute actively towards malignancy (Meek & Marcar, 2012).

2.4.4.1.1 MAGE-A in cancer development

MAGE family proteins function during embryonic development. After that, these genes are subsequently deactivated, perhaps by the epigenetic mechanisms including DNA methylation and histone deacetylation. During neoplastic transformation, these genes are re-activated, expressed, and may become antigenic targets that are recognized and attacked by the immune system (Sang et al., 2011).

A defining biochemical function of MAGE proteins is their ability to bind to specific E3 RING ubiquitin ligases through their MHDs (Doyle et al., 2010). As such, MAGE proteins can regulate the ubiquitination of proteins. This includes

enhancing general ligase activity, binding to and specifying novel substrates for ubiquitination by the E3 ligase complex, and altering the subcellular localization of E3 ligases to dictate substrates (Doyle et al., 2010; Hao et al., 2013; Pineda et al., 2015). As such, aberrant expression of MAGE proteins in tumor cells can lead to alterations in cellular processes and signaling pathways through ubiquitination and potentially other activities to contribute to tumorigenesis (Weon & Potts, 2015). MAGE-A proteins are also established regulators of certain cancer-associated transcription factors, for example p53 (Marcar et al., 2015).

As another mechanism, degradation of a master cellular energy sensor and regulator (AMPK) by MAGEA-TRIM28 results in significantly reduced autophagy and changes in cellular metabolism. Overall, expression of MAGE-A3 (or MAGE-A6) and degradation of AMPK is sufficient to induce transformation of normal cells and promote multiple hallmarks of cancer (Pineda & Potts, 2015).

2.4.4.1.2 MAGE-A in cancer diagnostics

Due to a high degree of homology of many MAGE family members and the lack of antibodies recognizing specific MAGE family members, the expression of MAGEs in tumors has only been analyzed for a few MAGE antigens by immunohistochemistry and Western-blot analysis (Sang et al., 2011). One of the best tools to determine MAGE-A protein expression has been the anti-MAGE-A1 antibody 6C1, which cross-reacts with MAGE-A1, -A2, -A3, -A4, -A6, -A10, and -A12; and the anti-MAGE-A3 antibody 57B, which cross-reacts with MAGE-A1, -A4, -A6, and -A12. Both antibodies are regarded as multi-MAGE antibodies (Busam et al., 2000; Rimoldi et al., 2000). Another diagnostic problem, is that due to the high similarity in the sequences of MAGE-A1, -A2, -A3, -A4 and -A6, it is difficult to design a unique primer to detect the different genes in different polymerase chain reaction (PCR) based applications (Liu et al., 2020a).

Cancers also show significant variation in nuclear versus cytoplasmic distribution of MAGE-A (Sang et al., 2011). This could be either due to different MAGE-A family members exhibiting differential localization, or that the mechanisms controlling localization are tumor cell-specific. Localization could be a factor in defining the outcome of MAGE-A expression. At the same time, MAGE-A expression depends on the localization of relevant MAGE-A target- or interacting proteins as well (Meek & Marcar, 2012). Most investigations have showed that higher frequency of MAGE expression has often been associated with poor outcome. However, there are some exceptions reported concerning MAGE-A4 (Bandić et al., 2006). Higher grade and metastatic tumors have also been found to have more frequent MAGEs expression than primary tumors (Bergeron et al., 2009; Brasseur et al., 1995). As another diagnostic complication, MAGE-A proteins are expressed only in a portion of cancer cells; different works have shown that the amount of expressing cells is between 25 and 50% (Barrow et al., 2006; Busam et al., 2000).

Overexpression of MAGE-A subfamily members has been linked to poor prognosis in multiple cancers. In 2020, the first comprehensive meta-analysis

evaluating the prognostic utility of MAGE-A members in different cancers, was conducted. It included 44 eligible studies consisting of 7428 patients from 11 countries. Univariate and multivariate analysis for overall survival, progression-free survival, and disease-free survival showed a significant association between high MAGE-A expression and various cancers. Additionally, subgroup analysis demonstrated that high MAGE-A expression was significantly associated with poor prognosis for lung, gastrointestinal, breast, and ovarian cancer (Poojary et al., 2020). However, tumor cells very often express more than one MAGE-A protein. For example, simultaneous expression of five or more MAGE-A proteins occurs in more than half of oral squamous cell carcinomas (Brisam et al., 2016). Simultaneous expression of MAGE-A1 and MAGE-A4 expression occurs in 60-70% of melanomas (Barrow et al., 2006).

Expression of these antigens may be highly heterogeneous in a variety of tumors of different histological origin, with percentages of positive cells ranging between 5 and 60% (Schultz-Thater et al., 2011). For example, MAGE-A10 has been shown to be expressed in 38% of malignant melanomas (Schultz-Thater et al., 2011). In the case of MAGE-A10, it has also been suggested to be the most immunogenic antigen of the MAGE-A family (Bricard et al., 2005; Groeper et al., 2007; Valmori et al., 2001).

2.4.4.1.3 MAGE-A and cancer progression

Several studies have shown that MAGE-A proteins are associated with or contribute to solid malignancies. As such, MAGE-A expression is considered to be an important predictor of malignant transformation (Laban et al., 2014). MAGE-A4 is expressed in 9% of primary tumors, but reaching to 44% in distant metastasis (Barrow et al., 2006). In case of melanomas, MAGE-A1 expression has been found in 16% of primary melanomas and 48% of metastatic melanomas (Brasseur et al., 1995b; Tan et al., 2019; Xu et al., 2019a). However, in another study no correlation was observed, and MAGE-A3/4 protein was present in 25% of primary invasive and metastatic tumors, but not in in situ melanomas (Busam et al., 2000). Previous studies have shown that MAGE-A4 is rarely lost when once acquired. Barrow et al showed no loss of MAGE-A4 in the 2006 study, where MAGE-A4 expression was studied in primary melanomas, lymph node, subcutaneous and distant metastases. Only 2% of patients showed its presence to be lower in subsequent samples than in the original one (Barrow et al., 2006).

2.4.4.1.4 MAGE-A in liquid biopsy

The expression of MAGE-A genes could be used as a CTC biomarker in colorectal, breast and gastric cancer. In colorectal cancer patients, positive expression levels of MAGE-A1/A6 were significantly higher in stages T3 and T4 compared with in stages T1 and T2 (Kim et al., 2017), where T stage stands for tumor stage (Mohr et al., 2009). Expression of serum and serum exosome MAGE-A3 and MAGE-A4 mRNA have been shown to be significantly higher in lung adenocarcinoma patients than in healthy donors. MAGE-A3 mRNA has been associated

with tumor diameter, TMN stage, and NSE, whereby MAGE-A3 mRNA has been correlated with N stage in serum-derived exosomes. Serum MAGE-A3 protein levels have been found to be elevated in lung adenocarcinoma patients, and were closely related to stage and NSE levels (Gan et al., 2024).

2.4.5 Autoantibodies in cancer

Autoantibodies against different cancers have been investigated in multiple studies. When looking at different cancer types, antigens have been shown to be immunogenic in multiple cancers and multiple antigens are immunogenic in each cancer type. For example, when looking at blood antibodies against a panel of more than 8000 human antigens, 202 antigens were identified to be immunogenic in ovarian cancer and 29 in pancreatic cancer (Gnjatic et al., 2010). In some studies a lower antibody level has also been related to cancer development (Liu et al., 2020b; Tan et al., 2019; Xu et al., 2019a).

Some tumor antigens are expressed in such a high percentage of tumors that they are called "universal antigens". Examples of antigens expressed in more than 50% of tumor types that are able to induce an immune response are p53, NY-ESO-1, survivin, and MART-1 (de Jonge et al., 2021). For example, serum p53 antibody has been shown to be a valuable prognostic factor for carcinomas that correlates with nodal involvement, TNM stage, histological type in lung cancer or tumor size in gastric cancer (Yu et al., 2011). Adding anti-p53 antibodies to conventional markers has also improved the overall detection rates of esophageal and colorectal cancers (Yajima et al., 2021).

However, the diagnostic performance of different autoantibody markers can vary greatly across studies, even for the markers examined in more than one study. The discrepancy could be explained by diverse definition of the cutoff value for blood autoantibody levels as a lower cutoff values result in higher sensitivity and lower specificity, and vice versa. In addition, diverse detection methods used with different study designs and diverse study populations could lead to differences between studies. Also as another problem, sample sizes of many studies have also been rather small (Chen et al., 2014b).

Even though most individual autoantibody markers lack the sensitivity and specificity required for cancer screening and diagnosis, promising performance has been reported for several marker combinations. For example, in colorectal cancers, the low sensitivity of individual autoantibody markers most likely reflects the diversity of colorectal cancers, and combinations of multiple markers could ensure detection of major subtypes. Sensitivity might be further enhanced by combination with other blood based markers, such as CEA, microRNAs, or metabolic markers (Chen et al., 2014b)

Table 1. Literature overview of different autoantibodies used in cancer diagnostics.

Cancer type	Autoantibody against	Significance	Reference
Gastric cancer	RalA	Presence of anti-RalA antibodies, in combination with CEA and/or CA19-9, was associated with poor survival in patients with gastric cancer	(Nanami et al., 2020)
	c-Myc, p16, HSPD1, PTEN, p53, NPM1, ENO1, p62, HCC1.4	Presence was detected	(Qin et al., 2019)
	p62, c-Myc, NPM1, 14-3-3ζ, MDM2, and p16	Optimal TAAs for a prediction model	(Wang et al., 2018)
	CTAG1B/CTAG2, DDX53, IGF2BP2, P53, and **MAGE-A3**	Panel detected in 13% of patients	(Meistere et al., 2017)
	ALDH1B1, UQCRC1, CTAG1, CENPF, ALDH1B1	Panel of four markers, or only ALDH1B1 alone, were found to be good early predictors of the presence of gastric cancer	(Wang et al., 2020a)
Colorectal cancer	p53	Able to predict the likelihood to develop colorectal cancer within 6 years	(Teras et al., 2018)
	p53, RalA, HSP70, Galectin1, KM-HN-1, **NY-ESO-1**, p90, Sui1, HSP40, Cyclin B1, HCC-22-5, c-myc, PrxVI, VEGF, HCA25a, p62, and Annexin	Autoantibody positive rates were seen for p53 (20%), RalA (14%), HSP70 (12%), and Galectin1 (11%). Positive rates increased to 56%, 62%, 66%, 71%, and 73% using panels of 6, 9, 11, 14, and 17 antibodies, respectively. Moreover, these autoantibodies showed relatively high positive rates even during stage 0/I disease (55% and 70% with 6 and 17 antibodies, respectively)	(Ushigome et al., 2018)
Pancreatic cancer	EID3	Antibody levels against the EID3 antigen were significantly higher in the patient group than in the healthy donor group, and high levels were related with shorter disease-free survival	(Hontani et al., 2017)

Cancer type	Autoantibody against	Significance	Reference
Liver cancer	HCC1, P16, P53, P90, and Survivin	Predictive autoantibody panel	(Koziol et al., 2018)
	SPAG9	Antibodies were significantly higher in hepatocellular carcinoma patients than those in patients with hepatitis or cirrhosis or healthy controls	(Ren et al., 2017)
	NY-ESO-1	In intrahepatic cholangiocarcinoma, autoantibodies were detected in 18.4% of patients. Antibodies were positively correlated with tumor differentiation, lymphatic metastasis, cTNM stage, and abdominal pain. Among stage III or IV patients, there was a higher cumulative survival rate in patients with serum anti-NY-ESO-1 positivity than in those with serum negativity.	(Zhang et al., 2017b)
Esophagus	p53	Monitoring of autoantibodies against p53 in post-surgical patients is predictive for residual tumor cells and recurrence	(Takashi et al., 2021)
	TOP48	Autoantibodies were found to be significantly higher in patients with esophageal carcinoma	(Zhang et al., 2018)
	NY-ESO-1	Autoantibodies are significantly sensitive and specific. They can be considered good early-stage biomarkers; increased levels of anti-NY-ESO-1 is associated with an advanced cancer stage	(Oshima et al., 2016)
	HCCR, C-myc, MDM2	A panel consisting of three autoantibodies and three miRNAs (miR-21, miR-223, and miR-375) attained great diagnostic value for esophageal squamous cell carcinoma, with a sensitivity of 69% and a specificity of 90%	(Xu et al., 2019b)
	Hsp70	Highest levels of anti-Hsp70 are found in esophageal carcinomas and are associated with poor prognosis	(Perisetti et al., 2020)
	Fascin	High levels of serum anti-fascin antibodies in esophageal cancers related to poor prognosis	(Zhang et al., 2006)
	MMP-7	High serum levels of anti-MMP-7 are found in esophageal cancer patients and are connected to staging and invasiveness	(Miao et al., 2015)

Cancer type	Autoantibody against	Significance	Reference
Thyroid	Tg	Anti-Tg antibodies appear in 10% of normal population and in 15–30% of differentiated thyroid carcinoma patients. Patients with a high level of anti-Tg at diagnosis have a higher probability of disease recurrence. Stable or increasing concentrations of anti-Tg during follow-up is significantly related with disease persistence and recurrence. A decrease in anti-Tg after surgery is a sign of good prognosis	(Reverter et al., 2020)
Lung cancer	EarlyCDT-Lung panel containing: p53, **NY-ESO-1**, CAGE, GBU4-5, Annexin 1, SOX2	This panel has been used to stratify patients into four risk classes and as such provided a 25-fold difference in lung cancer probability between the highest and lowest group	(Healey et al., 2013)
		Since 2013, the EarlyCDT-Lung test has become a relevant complementary tool to a Computer Tomography (CT) scan for detection of early lung cancer and, in 2017, a positive autoantibody test result was found to reflect a significant increased risk for malignancy in lung nodules of 4 to 20 mm in diameter	(Massion et al., 2017)
	IgA against BCL7A, TRIM33, and MTERF4; IgG against CTAG1A, DDX4, and **MAGEC2**	Diagnosis of early-stage lung cancer with 73.5% sensitivity at >85% specificity	(Pan et al., 2020)
	HE4	Auto-antigen useful for lung cancer diagnosis in high-risk groups, distinguished the affected group from the control group with a 54.76% sensitivity	(Yang et al., 2020)
	p53, PGP9.5, SOX2, **GAGE7**, GBU4-5, **MAGE A1**, **CAGE**	Tested to aid early diagnosis of lung adenocarcinoma with ground-glass nodules and/or solid nodules. The sensitivity and specificity of the auto-antibody assay was 48.6% and 92.7%, respectively	(Ren et al., 2018)
	CD25, MUC1, VEGFR1	Levels of autoantibodies against these antigens have been found to be systematically lower in non-small cell lung cancer patients than control subjects	(Liu et al., 2020b)
	GREM1, HMGB3, PSIP1	Effectively identify cancer cases compared to controls	(Jiang et al., 2020)

Cancer type	Autoantibody against	Significance	Reference
	NY-ESO-1	Significantly increased in the group of patients with non-small cell lung cancer compared with controls. Seropositivity was associated with an active smoking history in cancer patients	(Myšíková et al., 2017)
	p53, **NY-ESO-1**, **CAGE**, GBU4-5, SOX2, HuD, **MAGE- A4**	Lung cancer diagnostic panel	(Chapman et al., 2012)
Breast cancer	HER-2/neu	Presence of antibodies to HER-2/neu correlated with the presence of breast cancer	(Disis et al., 1997)
	HSP60	Detected in 31% cases of early-stage breast cancer and in 32.6% cases of ductal carcinoma in situ compared to only 4.5% detection in healthy control patients	(Desmetz et al., 2008)
	p53	Autoantibodies have also been shown to be associated with aggressiveness of breast cancer	(Tokunaga et al., 2018)
Ovarian cancer	p53	Circulating autoantibodies were found in patients	(Kobayashi et al., 2020)
		Only 20% to 25% of patients with invasive epithelial ovarian cancer showed elevated levels of autoantibodies against p53. P53 autoantibody levels could complement CA125 at the time of diagnosis	(Yang et al., 2017)
	ICAM3, CTAG2, p53, STYXL1, PVR, POMC, NUDT11, TRIM39, UHMK1, KSR1, NXF3	45% sensitivity and 98% specificity for serous ovarian cancer	(Fortner et al., 2017)
Cervical cancer	GAPDH	Lower anti-GAPDH IgG levels could discriminate between no cervical lesions and progressive stages of cervical lesions that often lead to cervical cancer	(Xu et al., 2019a)
	CA15-3, CEA, CA19-9	Reliably discriminated cervical intraepithelial neoplasia and cancer from normal cases, suggesting that this combination assay could be useful for primary screening of cervical cancer	(Jin et al., 2017)

Cancer type	Autoantibody against	Significance	Reference
Bladder cancer	PPP1CA	Anti-PPP1CA IgG were found to be higher in patients than in healthy individuals, with a specificity of 64.2% and a sensitivity of 65.7%. Seropositivity was associated with muscular invasion, a higher tumor grade, and poorer prognosis	(Chen et al., 2018)
Prostate cancer	SPARC, fetuin-a	Antibodies were detected in the serum, with significantly lower levels in prostate cancer patients compared to healthy controls	(Tan et al., 2019)
Testicular seminoma	Ta	High levels of anti-Ta antibodies are associated with testicular seminoma diagnosis	(Prüss et al., 2007)
	Kelch-like protein 11	Associated with a poor response to treatment of testicular seminoma	(Maudes et al., 2020)
Lymphoma	Scl-70, Jo-1, RF	Could be used in both diagnosis and staging	(Bilici et al., 2012)
	RNP, SM	High levels in non-Hodgkin lymphoma patients that were not detected in most of the control group	(Swissa et al., 1992)
CNS	APA, AHA	Detectable levels of APA and AHA antibodies were found in patients, but not in healthy controls	(Patti et al., 2020)
Angiosarcoma	p53	Used for early diagnosis, disease staging	(Kiyohara et al., 2020)
Melanoma	BPAG1	Autoantibodies were significantly higher in the sera of melanoma patients than in those of the healthy volunteers, and were detected in melanoma patients at the early and advanced stages of disease	(Shimbo et al., 2010)
	ZBTB7B, PRKCH, TP53, PCTK1, PQBP1, UBE2V1, IRF4, MAPK8_tv2, MSN, TPM1	Sensitivity of 79% and specificity of 84% for primary melanoma detection	(Zaenker et al., 2018)

2.5 Melanoma

Melanoma is a type of skin cancer that is caused by malignant melanocytes. It can be cutaneous, ocular, gastrointestinal, mucosal, leptomeningeal, genitourinary or lymphatic. It is the fifth most common type of cancer in men and the sixth most common in women in the world. Melanoma diagnosis is made through clinical assessment. Architectural features of malignant melanoma include asymmetry, confluence of growth, marked cellularity, and poor circumscription (Ahmed et al., 2020). In 1985, the ABCDE acronym was adopted to characterize the early clinical presentation of melanoma (Rastrelli et al., 2014).

2.5.1 Epidemiology

Currently, when the incidence of many tumor types is decreasing, melanoma incidence continues to increase (MacKie et al., 2009). The incidence of malignant melanoma has resulted in an important socio-economic problem (Rastrelli et al., 2014). From being a rare cancer one century ago, the average lifetime risk for melanoma has now reached 1 in 50 in many Western populations (Meyle & Guldberg, 2009). Currently, 1 in 63 Americans will develop melanoma during their lifetime (Markovic et al., 2007).

In Europe, there is a gradient in incidence rates with the highest rates in Northern countries and the lowest in the Southern countries. The incidence of melanoma in Italy is equal to 5–7 cases per 100,000 inhabitants per year (Lasithiotakis et al., 2006). Starting from 1960s, the incidence has increased in Caucasian populations and, thus, melanoma has become one of the most frequent cancers in fair-skinned populations (Caini et al., 2009). The white population has an approximately 10-fold greater risk of developing cutaneous melanoma than black, Asian or Hispanic populations (Lasithiotakis et al., 2006).

Unlike other solid tumors, melanoma mostly affects young and middle-aged people. The median age at the time of diagnosis is 57 years. Incidence of melanoma increases linearly after age 25 until age 50 and then slows (Rastrelli et al., 2014). From 2003 to 2007, the median age of death for malignant melanoma was 68 years. There is a greater mortality rate in men compared to women of the same age in the U.S. (Rigel, 2010). The distribution of favored sites of occurrence is sex-dependent: the most common areas are the back for men and the arms and legs for women (Markovic et al., 2007). Although the 5-year survival rate of patients with early stage localized melanoma is greater than 90%, survival rates drop to less than 20% once the melanoma has metastasized to distant sites (Jemal et al., 2008). A schematic overview of melanoma progression from benign nevus to metastatic stage is shown in figure 4 (Puglisi et al., 2021).

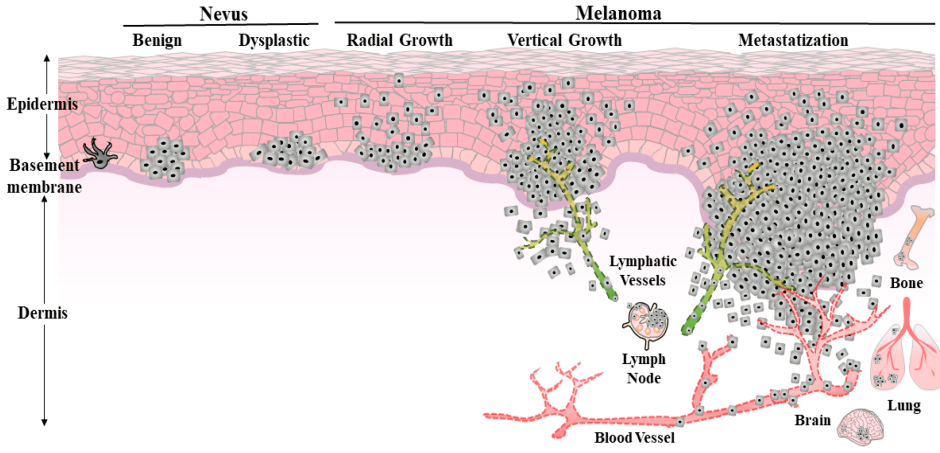

Figure 4. Schematic picture of melanoma progression from benign nevus to metastatic stage, modified from the work of Puglisi et al (Puglisi et al., 2021).

2.5.2 Risk factors

2.5.2.1 Clinical parameters

Clinical parameters such as age, sex, skin color, pigmentation status of the tumor and site of the primary tumor play an important role for the outcome of patients. Particularly high-risk sites include the back, upper arm, neck, and scalp. Known risk factors are multiple atypical nevi, positive family and/or personal history, immune suppressive diseases or treatments, and fair skin phenotype (Dimitriou et al., 2018). Certain phenotypic characteristics such as red hair, fair skin, numerous freckles, light eyes, sun sensitivity and an inability to tan, raise the risk of developing melanoma by approximately 50% (Titus-Ernstoff et al., 2005). Patients with an underlying genetic predisposition to develop melanoma usually show occurrence at a younger age, under 40 years, multiple primary melanomas or a history of precursor lesions such as dysplastic nevi, and are more likely to have tumors that are superficially invasive and have a better prognosis (Rastrelli et al., 2014).

2.5.2.2 Moles

Melanocytic nevi, also known as moles, are benign accumulations of melanocytes or nevus cells and may be congenital or acquired (Rastrelli et al., 2014). Approximately 25% of melanoma cases occur in conjunction with a pre-existing nevus (Bevona et al., 2003). Moreover, the total nevus count is positively correlated with melanoma risk and it varies on the basis of number, size and type of nevi (Rastrelli et al., 2014). Patients with more than 100 nevi have a 7-fold increased risk for melanoma (Friedman et al., 1985; Gandini et al., 2005). Regarding the size, larger (>5 mm) and giant (>20 cm) nevi are associated with a significantly higher risk of melanoma (Watt et al., 2004).

In addition, the presence of even a single nevus with atypical features enhances the risk. The presence of five atypical nevi give a six-fold increase for melanoma development (Rastrelli et al., 2014). Melanomas, which develop in the setting of previous nevi, are usually located on the trunk in younger patients and belong to the superficial spreading variety (Purdue et al., 2005).

2.5.2.3 UV radiation

The most important and potentially modifiable environmental risk factor for developing malignant melanoma is the exposure to ultraviolet (UV) rays (Rastrelli et al., 2014). Intermittent sun exposure and sunburn history have been shown to play considerable roles as risk factors for melanoma (Gandini et al., 2005). Sunburn history may be a marker of intense intermittent sun exposure. A history of sunburns in childhood is associated with the highest risk (Elwood & Jopson, 1997). Artificial UV exposure may play a role in the development of melanoma. The amount of UVA occurring in a typical tanning bed session is significantly higher in comparison to the exposure during ordinary outdoor activities or even during sunbathing [The International Agency for Research on Cancer Working Group on artificial ultraviolet (UV) light and skin cancer, 2007].

2.5.3 Classification

Majority of cutaneous melanomas arise from melanocytes in the epidermis. Most of them evolve through two major stages of tumor progression, the radial growth phase (RGP) and the vertical growth phase (VGP) (Bobos, 2021). The RGP melanoma lesions may be recognized as pigmented patches or plaques, which expand more or less along the radius of an imperfect circle in the horizontal axis within the skin. In the next stage of progression, in VGP, a tumor is formed and it may infiltrate the dermis or elevate the epidermis to form a nodule whose net direction of growth includes the vertical axis (Elder et al., 2020).

Based on the presence or absence of the RGP (Elder et al., 2018), as well as clinical and histological features (Rastrelli et al., 2014) three major categories of melanoma are recognized: nodular melanoma (NM), superficial spreading melanoma (SSM), and lentigo maligna melanoma (LMM).

Superficial spreading melanoma is the most common type of melanoma accounting for approximately 70% of cases. It is related to intermittent sun exposure and localizes most often on the back of the legs of women and on the backs of men. SMMs may arise de novo or in association with a nevus. Nodular melanoma accounts for 5% of melanomas and most often occurs on the trunk and limbs of patients in the fifth or sixth decade of life. It is more common in males than females. NMs are often ulcerated. It does not have a radial growth phase; it only has a vertical growth phase correlated with more rapid growth and higher rate of metastasis. Lentigo maligna melanoma accounts for 4% to 15% of cutaneous melanomas and, unlike NM and SSM, correlates with long-term sun exposure

and increasing age. This cancer may evolve for decades before invading into the dermis (Markovic et al., 2007).

In addition, acral lentiginous melanoma (ALM) can be found. ALM is uncommon, accounting for 5% of melanomas in white people but it is the most common type of melanoma among Asian, Hispanic and African patients. Typically, it affects elderly patients, with a female predominance. ALM is mainly localized on skin and adjacent skin of digits, palms and soles. It usually involves the nail bed of the great toe or thumb (Markovic et al., 2007). As a rarer subtype, desmoplastic melanoma (DM) exists. Desmoplastic melanoma often occurs in individuals between the age of 60 and 70 years. It rises on the head and neck but can occur on a variety of cutaneous and mucosal areas. It is slightly more common in men. Clinically DM may be amelanotic and it can present as an erythematous, pale, or flash-colored nodule or plaque arising in sun-damaged skin. This cancer is positive for S100 and it may be difficult to differentiate desmoplastic melanoma from scars tissue as S100-positive cells can also be seen in dermal scars (Markovic et al., 2007).

2.5.4 Stages

Most cancers can be classified according to the stage of disease. Stages are a measure of how widely cancer has spread in the primary organ and beyond. Stages 0-I-II are localized disease, where cancer is contained in the place where it started with no signs of spreading. Stages II–III is regional disease, where cancer has spread to nearby organs, tissues or lymph nodes. Stage IV refers to distant disease, also known as metastatic cancer. Cancer metastasis is the spread of cancerous cells to organs and tissues beyond the primary tumor site leading to the possible formation of secondary tumors (Connal et al., 2023).

Metastatic lesions are the leading cause of death in cancer patients, accounting for 90% of all cancer-related deaths (Guan, 2015). Other factors such as tumor size, location, type, and number of metastatic lesions, also impact survival. The general trend shows a decreasing survival rate with increasing cancer stage (Connal et al., 2023).

For melanoma, staging of tumors is done according to the American Joint Committee on Cancer (AJCC) guidelines (table 2 and 3). These take into account tumor thickness (Breslows thickness), ulceration, microsatellites, regional lymph node metastasis, extranodal extensions, distant metastatic sites, primary tumor mitotic rate, anatomic level of invasions (Clark level of invasion), tumor infiltrating lymphocytes, lymphovascular invasion, neurotropism and melanoma tumor burden in sentinel nodes to divide melanomas into five different stages with multiple substages (Amin et al., 2017).

Table 2. Tumor classification system. Based on Mohr et al., 2009.

Tumour classification (T)		
Tx		Primary tumor which cannot be assessed
Tis		Melanoma in situ
T1		≤1.00 mm tumor size
	T1a	Without ulceration or level II/III invasion
	T1b	With ulceration or level IV or V invasion
T2		1.01–2.00 mm tumor size
	T2a	Without ulceration
	T2b	With ulceration
T3		2.01–4.00 mm tumor size
	T3a	Without ulceration
	T3b	With ulceration
T4		>4.00 mm tumor size
	T4a	Without ulceration
	T4b	With ulceration
Node classification (N)		
N1		One lymph node contains tumor cells
	N1a	Micrometastases (clinically not seen), diagnosed after elective or sentinel lymphadenectomy
	N1b	Macrometastases (clinically apparent)
N2		Two to three lymph nodes contain tumor cells
	N2a	Micrometastases (clinically not seen), diagnosed after elective or sentinel lymphadenectomy
	N2b	Macrometastases (clinically apparent)
	N2c	In-transit satellite without metastatic lymph nodes
N3		Tumor cells in four or more lymph nodes, metastatic, or in-transit satellite without metastatic lymph nodes
Metastasis classification (M)		
M0		No metastasis
M1a		Distant skin, subcutaneous or lymph node metastases, normal LDH
M1b		Lung metastases, normal LDH
M1c		All other visceral metastases, normal LDH; any distant metastases, elevated LDH

Table 3. Cutaneous melanoma staging system. Referenced from Mohr et al., 2009.

Stage		Description	Stage grouping
0		Melanoma in situ, no lymph node involvement, no metastasis	Tis, N0, M0
I		Patients with low-risk primary melanomas without evidence of regional or distant metastases	
	Ia	Primary lesions ≤1 mm thick, without ulceration of the overlying epithelium or invasion	T1a, N0, M0
	Ib	Primary lesions ≤1 mm thick with epithelial ulceration or invasion	T1b or T2a, N0, M0
II		High-risk primary tumors, without evidence of lymphatic disease or distant metastases	
	IIa	Lesions >1 mm and ≤2 mm thick with ulceration; and >2 mm and ≤4 mm thick without epithelial ulceration	T2a or T3a, N0, M0
	IIb	Thickness between >2 mm and ≤4 mm with epithelial ulceration or >4 mm without ulceration	T3b or T4a, N0, M0
	IIc	Thickness >4 mm with epithelial ulceration	T4b, N0, M0
III		Lesions with pathologically documented involvement of regional lymph nodes or presence of in-transit or satellite metastases	
	IIIa	One to three microscopically involved lymph nodes and with a non-ulcerated primary tumor	T1 to 4a, N1a or N2a, M0
	IIIb	One to three microscopically involved lymph nodes with an ulcerated primary tumor	T1-4a to 4b, N1a to N2c, M0
	IIIc	Four or more affected lymph nodes or metastases or satellite lesions or ulceration	T1 to 4b, N2b or N3, M0
IV		presence of distant metastases	any T, any N, any M

2.5.5 Genetic markers

Cutaneous melanoma is regarded as a tumor of high tumor mutation burden (Alexandrov et al., 2013). Genes subjected to somatic mutation testing include BRAF, CDK4, GNAQ, JAK2, KRAS, MAP2K1, NF1, NRAS, and STAT1 (Forschner et al., 2019) with most commonly detected mutations affecting BRAF (V600E/K/R, L597R/S), NRAS (Q61K/L/R, G12D), and KIT (L576P). Among them, the most common is the BRAF oncogenic driver V600 mutation, that is found in 40–50% of cutaneous melanomas (Hodis et al., 2012).

The most common mutations in melanoma arising due to high cumulative sun damage are associated with NF1, NRAS, BRAF, or KIT genes. In turn, BRAF mutations are the dominating genetic causative factor in superficial spreading melanoma (Shain & Bastian, 2016). For acral melanoma, typical mutations can be found in the BRAF, NRAS, KIT, and NF1 genes (Hall & Rapini, 2024).

Hypermethylation of MGMT, RASSF1A, and DAPK has been shown to be significantly lower in primary melanomas compared to metastatic melanomas. However, hyper methylation of RAR-beta2 has been shown 70% in both primary and metastatic melanomas (Hoon et al., 2004).

2.5.6 Melanoma and the immune system

In 1953 Sumner first described regression in melanoma (Sumner, 1953) and it remains the most contentious of all dermatopathologic and clinical features in melanoma diagnosis (Ribero et al., 2016a). Histological regression in primary cutaneous melanoma occurs in 10–35% of cases (Blessing et al., 1990). Regression has traditionally been considered as a marker of poor prognosis, because it hampers the real evaluation of the initial thickness of the melanoma (Ribero et al., 2016a). However, the presence of histological regression has also been demonstrated to improve the patients' prognosis (Ribero et al., 2016b). It has been suggested that a host immunological response to tumor could be the basis of regression. This phenomenon could reflect the role of immunologic system against primary tumor and its presence should be considered prognostically favourable (Ribero et al., 2016a). For example, Ma et al. showed that the immune profile of the primary melanoma predicts metastatic involvement and that the presence of primary tumor regression results from a T-cell immune response (Ma et al., 2012).

2.5.7 Current diagnostics

Early detection of malignant melanoma remains the key factor in lowering mortality. In melanoma diagnostics, timely recognition, detection, and rapid treatment of melanoma remain critical. Malignant melanoma, compared to other cancers, has the advantage of the cutaneous location, which permits its early detection through non-invasive approaches (Rastrelli et al., 2014). The "gold

standard" for melanoma diagnosis continues to be histopathology, in conjunction with clinical characteristics. Sometimes immunohistochemistry is also needed (Bobos, 2021).

2.5.7.1 Skin examination

Skin self-examination has great potential as a simple, convenient method of screening for melanoma and precancerous lesions (Rastrelli et al., 2014). Before the 1980s, melanomas were often recognized by identifying clinically macroscopic features. Melanomas were often detected in an advanced stage when they appeared large, ulcerated and fungating (Montella et al., 2009).

Since then, there has been a need to educate physicians and the public to recognize melanoma in its early clinical presentation and the "ABCD" criteria was developed in 1985. The ABCD acronym stands for Asymmetry, Border irregularity, Color variation, Diameter >6 mm. Later the letter "E" was added for Evolving, which is especially important for the diagnosis of nodular melanomas (Friedman et al., 1985; Robinson & Turrisi, 2006). With the ABCD(E) criteria, the sensitivity of self-skin examination ranges from 57% to 90% (Rastrelli et al., 2014). Other clinical approaches have been developed to enhance early diagnosis, such as the Glasgow 7-point checklist, which includes 3 major criteria: change in size, shape, color; and 4 minor criteria: sensory change, diameter of 7 mm or greater, the presence of inflammation, and crusting or bleeding (MacKie, 1990).

Dermatoscopy is a non-invasive diagnostic technique for in vivo observation of the skin. A dermatoscope uses optic magnification to permit visualization of morphological structures that are not visible to the naked eye. Dermatoscopy has increased the accuracy of melanoma detection since this approach renders early signs of the disease visible in the pigmented lesions much earlier. The dermatoscopic criteria for melanoma diagnosis are atypical pigment network, irregular dots/globules, irregular streaks, irregular pigmentation, regression structure, blue-whitish veil, and changed vascular pattern (Neila & Soyer, 2011).

2.5.7.2 Histopathology

A biopsy must be taken to diagnose a melanoma lesion (Kaiser et al., 2014). Tissue biopsies are regarded as the "gold standard" for tumor profiling in cancer diagnostics (Corcoran & Chabner, 2018). First, a hematoxylin and eosin (H&E) stain will be done. H&E stain is essential for recognizing various tissue types and the morphologic changes (Fischer et al., 2008).The cytological features of malignant melanoma include an irregular and thick nuclear membrane and prominent nucleoli (Ahmed et al., 2020).

After an H&E stain, immunohistochemistry (IHC) is done. IHC is the most common ancillary technique used by pathologists to assist melanoma diagnostics, as it is readily available in most laboratories, relatively inexpensive, reliable, and reproducible. The use of IHC for the diagnosis of melanoma has significantly increased over the last 20 years (Kim & Meehan, 2017). There are several

melanocytic markers that provide robust support for melanocytic origin. These include Melan-A/MART-1, HMB-45, MITF, tyrosinase, S100 and SOX10 (Davis et al., 2019; Mohamed et al., 2013; Ohsie et al., 2008). Proliferation markers such as Ki-67 are also looked at. Proliferation markers are used to determine the number of cells in the cell cycle in a given lesion, with benign melanocytic tumors expected to have low proliferative indices (Davis et al., 2019).

2.5.7.3 Biochemistry

2.5.7.3.1 S100 and LDH

The S100 protein family consists of 21 structurally similar but functionally different proteins whose abnormal expression is characteristic for melanoma (Mandel & Metais, 1948). The presence of S100 proteins confirms cancer cells participating in malignant invasion, making S100 proteins useful in disease monitoring and prognosis (Cayrefourcq et al., 2019). Serum S100 levels are correlated with clinical stage of disease: the proportion of patients with serum S100 levels above a set cut-off increases from AJCC stage I–II patients, 0–9%; through stage III patients, 5–98%; to be highest in stage IV patients, 40–100%. Serum S100 levels have also been found to correlate with the extent of metastatic spread, disease activity, active disease/symptoms, disease relapse/progression and survival. The highest S100 levels have been observed in patients with liver and/or skeletal metastasis, while the proportion of S100-positive patients is lower in those with cutaneous metastases. Some authors have found that S100 levels are of prognostic significance independent of clinical stage (Brochez & Naeyaert, 2000).

Lactate dehydrogenase (LDH) is a crucial enzyme in glycolysis, facilitating the conversion of pyruvate to lactate. It also serves as an indicator of tissue and organ hypoperfusion (Tian et al., 2020). Elevated serum LDH levels are assumed to reflect tumor cell turnover and tumor burden. Several studies have reported serum LDH levels as an independent prognostic factor in patients with stage IV disease (Brochez & Naeyaert, 2000).

Serum S100β and LDH levels have been highly correlated. LDH has the highest value to discriminate between progressive and non-progressive disease in stage IV patients after treatment, while S100β levels before therapy have been shown to be most discriminative in predicting outcome (Deichmann et al., 1999).

2.5.7.3.2 Other serum markers

Some other proteins have also been shown to have relevance in melanoma diagnosis. Elevated serum levels of several cytokines such as interleukins IL-6, IL-8, IL-10; the soluble IL-2 receptor (sIL-2R), soluble cell adhesion molecules such as soluble intercellular adhesion molecule 1 (sVCAM-1) and P-selectin have been demonstrated to be elevated in melanoma patients (Brochez & Naeyaert, 2000).

Increased serum levels of IL-6 and IL-8 have been observed in stage IV patients and have been shown to correlate with tumor burden. Serum levels of

IL-6 were also higher in patients who were non-responsive to therapy, regardless of tumor burden (Brochez & Naeyaert, 2000). In a group of stage I–III melanoma patients, serum sIL-2R levels were observed to be elevated in over half of patients developing distant metastatic disease (Boyano et al., 2009). It has been found that sVCAM-1 could be an independent prognostic factor. Based on the presence or absence of elevated sVCAM-1 and LDH levels patients could be classified into different risk categories (Franzke et al., 1998). Glypican-3 (GPC3) has also been shown to be overexpressed in melanoma and its serum concentration could serve as an early stage melanoma diagnostic marker (Shimbo et al., 2010).

A correlation between serum neuron-specific enolase (NSE) and tumor burden has been reported in melanoma patients, especially in stage IV patients. However, elevated NSE levels have low sensitivity, and hence are of limited value in the individual patient (Brochez & Naeyaert, 2000). Serum NSE levels were observed to correlate with S100 levels, especially in stage III and IV melanoma patients. The overall sensitivity of both markers in melanoma patients is comparable, but elevated serum S100 levels have been shown to have a higher sensitivity in stage IV patients. NSE seems to be less specific to discriminate stage III and IV patients from stage I and II (Brochez & Naeyaert, 2000). Lipid-bound sialic acid (LASA-P) is a sialo glycolipid bound to the membranes of a variety of tumor cells. Sensitivity of LASA-P exceeds that of NSE but not that of S100β, while the specificity is comparable with that of NSE (Hauschild, 2009).

2.5.8 Liquid biopsy in melanoma diagnostics

Reliable detection of melanoma is a challenge due to the diversity of surface marker expression and thus inspires the use of different detection methods (Xu et al., 2016). These include different liquid biopsy markers such as circulating tumor cells, circulating DNA and extracellular vesicles.

Circulating melanoma cells (CMCs) were first described in the early 1990s (Cayrefourcq et al., 2019). Like all circulating tumor cells, CMCs descend from the primary tumor (Weight & Viator, 2014). CMCs occur mostly individually in melanoma patient bloodstream (De Giorgi et al., 2010) and express a variety of surface antigens, including melanoma cell markers, like MCAM, also known as MUC18/CD146, (Rapanotti et al., 2017), MART-1 (Ma & Frank, 2015), MAGE-A3, PAX3, HMW-MAA (Campoli et al., 2004), and GM2/GD2 (Bennaceur et al., 2006).

A disadvantage for using CMCs is the relatively low number of CMCs in peripheral blood, with 1–3 CMCs corresponding to 5 billion blood cells (Rapanotti et al., 2017). Thus, an enrichment stage increasing the concentration is highly recommended for an optimized CMCs detection (Alix-Panabières et al., 2012). Antigen-based detection technologies carry the risk of non-specific staining, and results need to be analyzed in relation to clinical data (Kamińska et al., 2021).

After purifying CMCs, reverse transcriptase PCR (RT-PCR) methods are frequently used for indirect detection of CMCs, through amplification of tyrosi-

nase, Melan-A, MART-1, PAX mRNA (Rodic et al., 2014). For example, tyrosinase expression is typical for melanoma cells and melanocytes that is not found in healthy patients' blood (Kamińska et al., 2021). DNA sequencing is another option that gives the possibility to detect genetic changes characteristic for melanoma. Certain abnormalities occur solely in cancer cells and might therefore be considered tumor markers, with their tracking enabled via PCR or next-generation sequencing (NGS) methods (Forthun et al., 2019); (Perkins et al., 2017).

The amount of **ctDNA** present in the circulation of melanoma patients at the onset of disease is small and accompanied by DNA from non-cancerous cells. As such, proper identification of disease in its early stage is often hampered (Postel et al., 2018). A higher concentration of ctDNA is often detected in the plasma of patients with progressive disease. Destruction of cancer cells during initiation of therapy may also lead to elevated ctDNA levels (Spindler et al., 2014).

Apart from total ctDNA concentration, specific mutation analysis in ctDNA can also be performed (Haselmann et al., 2018). Following tumor characterization and pre-identification of gene targets, isolated ctDNA can be subjected to PCR followed by targeted and nontargeted sequencing methods (O'Leary et al., 2019). Currently developed approaches are focused on the successful detection of mutations and epigenetic changes in ctDNA of low concentration (Kamińska et al., 2021).

Importantly, ctDNA as a time-dependent variable has been shown to be superior to LDH testing predicting 12-month survival (Lee et al., 2017). CtDNA has also been found to be a better prognostic factor than LDH (Chang et al., 2016) as well as S100 (Váraljai et al., 2021).

Epigenetic changes in cfDNA may also serve as diagnostic markers in melanoma (Eslami-S et al., 2020). CtDNA can be applied for non-typical melanoma mutation detection (Váraljai et al., 2021), tumor-specific gene methylation detection and be used for early diagnosis of melanoma (Diefenbach et al., 2019). Analysis of preoperative melanoma patients' plasma has demonstrated circulating hypermethylated MGMT, RAR-beta2, and RASSF1A DNA for at least one of the markers in 29% of the patients (Hoon et al., 2004).

In melanoma, **widely distributed mutations** in BRAF and NRAS genes make most technologies suitable for analysis. However, in the case of patients harboring BRAF and NRAS wild-types, analysis is far more complicated (Kamińska et al., 2021).

Since BRAF mutations are present in the majority of cutaneous melanoma patients, ctDNA analysis can be of great value in this context, representing a reliable alternative to the tissue biopsy (Haselmann et al., 2018). It has been found that the accordance between tissue and plasma BRAF(V600E) ranges from 75% (Schreuer et al., 2016) to 84% (Sanmamed et al., 2015). There is also a relation between baseline and follow-up BRAF(V600E) ctDNA levels and tumor burden; response to treatment with BRAF inhibitors; prediction of disease progression prior to CT scan; and progression-free survival (Ascierto et al., 2013). Furthermore, an abundance of BRAF(V600E) ctDNA in treatment-naïve patients has been related to tumor burden, with lower concentration reflecting longer overall

survival and progression-free survival compared to higher concentrations (Sanmamed et al., 2015). Quantification of BRAF(V600E) ctDNA prior to and during the course of treatment with B-Raf enzyme inhibitors has mirrored its outcome. BRAF(V600E) ctDNA decreases significantly at the moment of the strongest response to treatment while increasing significantly in the event of progression (Sanmamed et al., 2015).

2.5.8.1 Extracellular vesicles

Melanoma can modulate its microenvironment through various kinds of factors, promoting growth and formation of metastasis. Among direct cellular interactions, secretion of signaling factors and shedding of extracellular vesicles, are among the most promising sources of cancer information (Hood, 2019). There is evidence from cell culture experiments that melanoma exosomes can actively communicate with nearby melanocytes (Xiao et al., 2016). Molecular cargo, consisting of unique mRNA, miRNA, and proteins, has been found both in melanoma cell lines (Xiao et al., 2012) and blood of advanced-stage melanoma patients (Peinado et al., 2012).

Since exosomes carry a molecular "fingerprint" of the cell of origin, they could deliver invaluable information about the cancer status, making them prospective biomarkers for melanoma diagnosis or prognosis (Eisenstein et al., 2018). They have also been shown to carry information about drug resistance to other cells. For example, exosomes have been shown to transfer PDGFRβ to melanoma cells, activating the phosphatidylinositol-3-kinase (PI3K-AKT) signaling pathway. This pathway is involved in the growth and survival of cancer cells, and activating it lowers the sensitivity to BRAF inhibitors (Vella et al., 2017).

2.5.8.1.1 Proteins

The protein content of exosomes has been shown to be higher in melanoma patients serum than in healthy individuals, irrespectively of active or not evident disease (Sharma et al., 2020). Exosomal PD-L1 level has been shown to be elevated in the plasma of melanoma patients compared to healthy controls (Cordonnier et al., 2020). Melanoma stage does not influence the concentration of exosomes, but patients with advanced stages have a higher content of S100B and melanoma inhibitory activity (MIA) proteins per particle, which could reflect the stage, progression, and metastases (Alegre et al., 2016). A protein set, consisting of tyrosinase-related protein-2 (TYRP-2), very late antigen-4 (VLA-4), heat shock protein 70 (Hsp70), heat shock protein 90 (Hsp90) and MET oncoprotein, has been termed as an exosome-specific melanoma signature. It has been found to be increased in stage IV patients, affecting survival and metastatic spread (Peinado et al., 2012).

2.5.8.1.2 miRNA

Although limited, some data already exists showing that the tumor stage is represented by specific exosomal miRNAs content. It has been observed that there is a difference between exosomal miRNAs, specifically a melanocytic marker miR-211-5p, in early- versus late-stage patients (Margue et al., 2015). MiR-17, miR-19a, miR-21, miR-126, and miR-149 have been shown to be expressed at higher levels in patients with metastatic sporadic melanoma then in familial melanoma patients or unaffected control subjects (Pfeffer et al., 2015). Also, a lower level of miR-125b has been attributed to advanced melanoma, probably as a result of a disturbance in tumor cells (Alegre et al., 2014). For uveal melanoma, higher content of miR-146a has been described to be present in serum, suggesting its potential to become a non-invasive biomarker (Ragusa et al., 2015).

In the serum of patients harboring the BRAF(V600E) mutation, exosomal miRNA content differs before and after therapy with BRAF/MEK inhibitors. Particularly, an increase of let-7g-5p and miR-497-5p miRNAs has been noted during therapy, corresponding to treatment response and prolonged progression free survival (Svedman et al., 2018).

2.6 Atherosclerosis

Atherosclerosis is the most frequent underlying cause of coronary, carotid, and peripheral arterial disease. It is a multifocal, smoldering, immunoinflammatory disease of medium-sized and large arteries fuelled by lipids with endothelial cells, leukocytes, and intimal smooth muscle cells being the major players in its development. Atherosclerosis alone is rarely fatal; it is thrombosis, superimposed on a ruptured or eroded atherosclerotic plaque, that precipitates life-threatening clinical events such as acute coronary syndromes and a stroke (Falk, 2006). Prognosis and disease management can be greatly improved by early diagnosis. Diagnostic specificity and sensitivity for different biomarkers is often confounded by age, gender and some comorbidities such as renal dysfunction (Huang et al., 2021).

2.6.1 Development

Elevated plasma cholesterol level is sufficient to drive the development of atherosclerosis, even in the absence of other known risk factors (Glass & Witztum, 2001). The other risk factors, such as hypertension, diabetes, smoking, male gender, and inflammation, appear to accelerate this disease driven by atherogenic lipoproteins (Falk, 2006). Overview of atherosclerosis development is shown in figure 5, modified from the work of Figueiredo et al (Figueiredo et al., 2023).

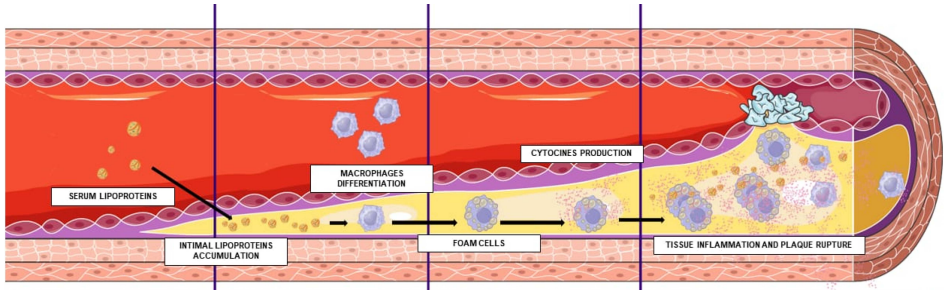

Figure 5. Pathophysiology of atherosclerosis. The formation of atheromatous plaque initially depends on the accumulation of lipids in the intima of the vessels. After oxidation and the phagocytosis of these molecules by macrophages, an intense inflammatory cascade occurs, causing a cycle of the recruitment of inflammatory cells and the release of cytokines, culminating in tissue damage. Finally, when the plaque ruptures, it obstructs blood flow and that leads to clinical complications (Figueiredo et al., 2023).

2.6.1.1 Cholesterol

Cholesterol (Chol) is biosynthesized by all animal cells and is an essential structural component of animal cell membranes. While most cells are capable of synthesizing it, the majority of cholesterol is ingested from food or synthesized by hepatocytes, after that transported in the blood to peripheral cells. The levels of cholesterol in peripheral tissues is dictated by a balance of uptake and export (Luo et al., 2020). The liver, which is the main site of cholesterol biosynthesis, delivers both endogenously synthesized and exogenously acquired cholesterol to the bloodstream as very-low-density lipoproteins (VLDLs). After processing in the bloodstream, the VLDLs generate circulating low-density lipoproteins (LDLs), which can be taken up by peripheral cells via receptor-mediated endocytosis (Goldstein & Brown, 2009).

Surplus cholesterol can be exported to lipid-free or lipid-poor apolipoprotein A-I (apoA-I) produced by the liver, intestine and pancreas via passive or active mechanisms to generate high-density lipoproteins (HDLs) (Phillips, 2014). Excess cholesterol is esterified by acyl-coenzyme A:cholesterol acyltransferase to cholesteryl esters, which are either stored as a cholesterol reservoir in cytosolic lipid droplets or released as a major constituent of plasma lipoproteins. HDLs are finally transported from peripheral tissues back to the liver and intestine, where cholesterol is recycled or eliminated. It is also transported to steroidogenic organs, where cholesterol is used to generate steroid hormones (Luo et al., 2020).

In clinical medicine, standard lipid analysis includes measuring serum or plasma total cholesterol (Chol), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-Chol) after an overnight fast. Low-density lipoprotein cholesterol (LDL-Chol) is then calculated based on subtracting HDL-Chol and TG values from total cholesterol values (Schaefer et al., 2000). Elevated Chol, LDL-Chol, TG, and non-high-density lipoprotein cholesterol and low high-density lipoprotein cholesterol (HDL-Chol) concentrations correlate with atherosclerotic cardiovascular disease and mortality (Duncan et al., 2019).

2.6.1.2 Endothelial dysfunction

In lesion-prone areas, atherosclerotic lesions begin to develop under an intact, but activated, dysfunctional endothelium. Later, endothelial cells may vanish and de-endothelialized areas appear over advanced lesions, with or without platelets adhering to the exposed subendothelial tissue (Davies et al., 1988). Depending on size and concentration, plasma molecules and lipoprotein particles go through leaky and defective endothelium into the subendothelial space, where potentially atherogenic lipoproteins are retained and oxidized. These lipoproteins, mainly LDL, become cytotoxic, proinflammatory, chemotaxic, and proatherogenic. The mechanisms responsible for the atherogenic modification of LDL are unknown but could include oxidation mediated by myeloperoxidase, 15-lipoxygenase, and/or nitric oxide synthase (NOS) (Glass & Witztum, 2001).

As such, endothelial dysfunction is defined as a decreased production and availability of nitric oxide (NO), with or without an imbalance between endothelium-derived contracting and relaxing factors (Scioli et al., 2020). NO is a potent oxidant produced by both endothelial cells and macrophages that appears to exert both protective and atherogenic effects (Falk, 2006). Decreased production and availability of NO leads to a pro-inflammatory and prothrombotic status (Scioli et al., 2020). Generally, it is assumed that this form of endothelial dysfunction equates with endothelial activation in which atheroprotective mechanisms are lost and atherothrombosis promoted (Willerson & Kereiakes, 2003).

2.6.1.3 Oxidative stress

Reactive oxygen species (ROS) are subcellular messengers in signal transduction pathways with both beneficial and deleterious roles. ROS are generated as a by-product of mitochondrial respiration or metabolism or by specific enzymes such as superoxide dismutases, glutathione peroxidase, catalase, peroxiredoxins, and myeloperoxidases. Under physiological conditions, low levels of ROS production are equivalent to their detoxification, playing a major role in cellular signaling and function. In pathological situations, particularly atherosclerosis or hypertension, the release of ROS exceeds endogenous antioxidant capacity, leading to cell death. At cardiovascular levels, oxidative stress is highly implicated in myocardial infarction, ischemia, reperfusion, or heart failure (Dubois-Deruy et al., 2020). In subclinical atherosclerosis, oxidative stress can induce endothelial cell activation, permeability changes and recruitment of various inflammatory cells (Burtenshaw et al., 2019).

At the cardiac level, the main sources of ROS are the mitochondrial electron transport chain, the xanthine oxidase, the NADPH oxidases (NOX), and the nitric oxide synthases (NOS). Dioxygen, O2, is the starting point for the formation of ROS. These are the most abundant ROS in cells and are responsible for the formation of all other types of ROS, notably hydroxyl (•OH) and hydroperoxyl (•HO2) radicals (Zorov et al., 2014).

2.6.1.4 NADPH oxidases (NOX)

NADPH oxidases (NOX) are a family of membrane enzymatic complexes whose primary function is the production of ROS. NOX catalyze the reduction of dioxygen to superoxide anion using NADPH or NADH as an electron donor. Seven NOX family members were initially described, with five occurring neutrophils, NOX 1–5, and two more called dual oxidase, DUOX 1–2. Only NOX1, 2, 4, and 5 are particularly expressed in the cardiovascular system. Human aortic smooth muscle cells express NOX1, 4, and 5. NOX2 and 4 are also expressed in several cardiac cell types, such as cardiomyocytes, fibroblasts, endothelial cells, or smooth muscle cells. NOX2 is activated during cardiovascular stress induced by angiotensin II (AngII), endothelin-1, growth factors, cytokines, or mechanical forces, whereas NOX2 is constitutively active but increases with hypoxia, ischemia, or pressure overload (Dubois-Deruy et al., 2020). AngII, that increases blood pressure, regulates all the vascular NOX types. NOX-dependent ROS in turn activate the AngII receptor type 1 with an auto amplificatory effect (Santillo et al., 2015).

NOX activity is increased in patients with a metabolic syndrome (Fortuño et al., 2006). A correlation between NOX mRNA expression and the severity of atherosclerotic lesions has been shown in human coronary arteries (Sorescu et al., 2002).

2.6.1.5 Nitric oxide synthase (NOS)

Nitric oxide synthase (NOS) is able to produce superoxide anions. Three NOS have been identified: type I or neuronal NOS (NOS1 or nNOS), type II or inducible NOS (NOS2 or iNOS), and type III or endothelial NOS (NOS3 or eNOS). NOS1 and NOS3 isoforms are constitutively expressed in the heart and possess calcium-dependent activity. NOS2 isoform has calcium-independent activity (Dubois-Deruy et al., 2020). When NOS are uncoupled, they switch from NO to superoxide anion and peroxynitrite production, leading to reduced bioavailability of NO and to vasoconstriction (Santillo et al., 2015).

Nitric oxide produced by endothelial NOS has vasodilator function and is potentially atheroprotective. In contrast, NO produced in macrophages is potentially proatherogenic. After macrophage production of NO, the endothelium becomes activated by atherogenic and proinflammatory stimuli, and the expression of adhesion molecules, primarily vascular cell adhesion molecule-1 (VCAM-1), is up regulated. At the same time monocytes and T cells are recruited which leads to atherosclerosis (Falk, 2006).

On the other hand, when the switch from NO to peroxynitrite has occurred, peroxynitrite is always atherogenic. Peroxynitrite can be involved in atherosclerosis progression by inhibiting vasorelaxation, decreasing the beneficial effects of NO on platelet aggregation and vascular smooth muscle cell proliferation, and oxidating of DNA and lipids (Cai & Harrison, 2000).

2.6.1.6 ERK1/2

Extracellular signal-regulated kinases 1/2 (ERK1/2), also known as p42/44 mitogen-activated protein kinase, (p42/44 MAPK), are enzymes involved in various biological processes (Roskoski, 2012). ERK1/2 are the specific substrates for MAPK kinase 1 and 2, MEK1/2. Regulation of ERK1/2 is implicated in cardiovascular disease (Zeng et al., 2021). For example, reduced ERK1/2 activity by inhibitors increases elastin synthesis and aortic elastin content (Lannoy et al., 2014). Also, calcification in human aortic valves has been positively correlated to ERK1/2 activity (Zeng et al., 2021). On the other hand, anti-atherogenic properties of ERK1/2 inhibition have been confirmed by demonstrating protection against hypercholesterolemia and atherosclerosis (Yu et al., 2019).

2.6.1.7 PI3K/Akt B pathway

PI3K/Akt signaling has been demonstrated to be involved in several physiological and pathological processes, including cell proliferation, cell cycle, cell apoptosis, inflammation, ischemic injury and tumor progression. Many studies have reported about the critical role of PI3K/Akt signaling in TLR4/NF-κB pathway-mediated inflammatory pathway (Wang et al., 2020b).

2.6.1.8 Lipoproteins

Endothelial cell dysfunction is an initial step in atherosclerotic lesion formation and is more likely to occur at arterial curves and branches that are subjected to low shear stress and disturbed blood flow (Davies, 1995; Gimbrone et al., 2000). In atherosclerosis susceptible regions, reduced expression of eNOS and SOD leads to compromised endothelial barrier integrity. This leads to increased accumulation and retention of subendothelial atherogenic apolipoprotein B (apoB)-containing lipoproteins such as LDL, remnants of VLDL and chylomicrons as shown in animal experiments (Gerrity et al., 1979; Schwenke & Carew, 1989). Endothelial cell activation leads to increased production of reactive oxygen species (Ungvari et al., 2006) that can cause oxidative modification of apoB-containing lipoproteins (Steinbrecher, 1988). In contrast, the atheroprotective function of HDL is to prevent endothelial activation and enhance NO production to maintain barrier integrity (Kratzer et al., 2014).

2.6.1.9 Extracellular vesicles

In cardiovascular disease patients' blood, EVs can be released by blood cells, such as platelets, erythrocytes, and leukocytes; and by heart and blood vessel cells such as cardiomyocytes, cardiac fibroblasts, and endothelial cells. These EVs have different types of effects. For example, EVs have been closely associated with damaging ROS signaling to the endothelium (Saeed-Zidane et al., 2017).

On the other hand, a systematic review comparing studies on the cardioprotective effect of EVs, has shown that EVs mediate this kind of protection using different pathways by carrying proteins or nucleic acids responsible for cell survival. For instance, in an oxidative stress environment, EVs from cardiac progenitor cells were found in greater quantity and at a higher count and carrying different cargo than controls. At the same time, only donor EVs had positive protective effects in preventing cell death. Overall, the authors of the aforementioned systemic review showed that EVs cardioprotective effects observed in different studies from different sources are inconsistent. It would seem that EVs, in general, are protective, but it is nearly impossible to properly compare the studies because of differences in purification methods and the EV experiments themselves (Wendt et al., 2018).

2.6.2 Current diagnostics

Despite effective interventions for the control of LDL, blood pressure and other traditional risk factors, considerable residual risk remains for atherosclerotic cardiovascular disease (Ridker, 2017). Atherosclerosis risk factor profile has shifted as LDL levels, blood pressure and smoking have decreased among the population (Libby, 2021).

Non-traditional drivers of atherosclerosis – such as disturbed sleep, physical inactivity, the microbiome, air pollution and environmental stress – have also gained attention. Inflammatory pathways and leukocytes link traditional and emerging risk factors alike to the altered behavior of arterial wall cells. Obesity and its attendant dysmetabolism, often manifested by insulin resistance and diabetes, now drives an increasing proportion of cardiovascular disease risk worldwide (Libby, 2021). Adipose tissue abounds with inflammatory cells and produces proinflammatory mediators. Inflammation contributes mechanistically to the link between obesity, insulin resistance and atherosclerotic risk (Ross et al., 2020).

2.6.2.1 Lipids

Low-density lipoprotein (LDL), which is encircled by its signature apolipoprotein B component, causes atherosclerosis (Libby, 2021). Rather than elevated LDL cholesterol, an elevation in triglyceride-rich lipoproteins (TGRL) and low high-density lipoprotein (HDL) now comprise the major pattern of lipid abnormality in many patients who are treated for atherosclerotic cardiovascular disease (Nordestgaard & Varbo, 2014). Highly effective and inexpensive therapies for lowering LDL have contributed to an overall drop in LDL, whereas obesity, along with insulin resistance and a high-carbohydrate diet, favor a rise in the prevalence of the cluster of conditions referred to as the ‘metabolic syndrome’. This syndrome is characterized in part by an elevation in TGRL (Libby, 2021).

2.6.2.2 Endothelial inflammation

Beyond dyslipidemia, inflammation also participates fundamentally in atherogenesis and in the pathophysiology of ischemic events (Libby & Hansson, 2019). Inflammation does not supplement or demote lipid risk. Rather, inflammatory responses provide a series of pathways that link lipids and other traditional risk factors to atherosclerosis (Libby, 2021).

Human biomarker studies have shown that indicators of inflammation predict risk of cardiovascular disease in individuals with or without cardiovascular disease, and independently of all traditional risk factors (Ridker et al., 2018). The acute phase reactant C-reactive protein (CRP) can be measured with a highly sensitive assay. When done so, it is known as hs-CRP and has a measurement range of 1–3 mg/L (Berk et al., 1990). Hs-CRP is a validated and clinically useful gauge of the atherosclerotic risk of an individual (Ridker, 2016).

2.6.2.3 Oxidative stress

When tissues are exposed to higher levels of oxidative stress, there is increased lipid peroxidation, which in turn results in the formation of malondialdehyde (MDA) which degrades into acetaldehyde (AA); together, MDA and AA then react with proteins to form the more stable protein malondialdehyde-acetaldehyde (MAA) (Tuma et al., 2001). MAA-adducted proteins are highly immunogenic and are found in the serum of patients with various systemic inflammatory diseases (Lomzenski et al., 2022). Elevated concentrations of anti-MAA antibodies in the serum are significantly associated with coronary artery disease in the general population (Anderson et al., 2014).

2.6.3 Liquid biopsy in atherosclerosis

2.6.3.1 Microvesicles

Circulating microvesicles (MV) are small phospholipid-rich vesicles that contribute to the atherothrombotic process. MV can be used as biomarkers of cardiovascular disease burden and progression (Chiva-Blanch et al., 2020). Several studies suggest the use of cell-specific MV subpopulations as predictive biomarkers for cardiovascular diseases at different stages and degrees of severity (Badimon et al., 2020). MV are released when cells suffer stress or a perturbation in the form of activation, injury or apoptosis. Interestingly, MV with different phenotypes, representing their parental origin, are released during chronic CVD progression and at the onset of acute ischemic events (Chiva-Blanch et al., 2020).

MV are created by cell activation and, in turn, act as triggers for further cell activation. As such, they contribute to both the initiation and progression of cardiovascular disease by increasing leukocyte activation and chemotaxis, enhancing lipid and cholesterol accumulation in macrophages and stimulating

foam cell formation, and increasing endothelial activation and platelet aggregation (Chiva-Blanch et al., 2020). MV are increased after an acute myocardial infarction (Chiva-Blanch et al., 2017a) and fatal CVD (Chiva-Blanch et al., 2017b).

2.6.3.2 Exosomes

Potential clinical significance of exosome-derived contents reside in possible cardiovascular disease risk prediction, as well finding biomarkers for atherosclerosis management (Ailawadi et al., 2015). Clinical symptoms often take years to develop atherosclerosis, but functional biomarkers allow to detect pathological changes earlier, which may guide therapeutic interventions in advance (Yin et al., 2015).

Sex-determining region Y, **SRY, gene in plasma EVs** could accelerate atherosclerosis both in vitro and in vivo. The gene copy number of SRY gene in plasma EVs were notably amplified in patients having coronary artery diseases compared to control healthy individuals. Transferred SRY gene-containing EVs or plasmids to monocytes and human umbilical vein endothelial cells (HUVECs) enhance adhesion function between the two cells, which indicates that SRY gene-containing EVs may be a novel class of biomarker in atherosclerosis (Cai et al., 2015).

Circulating miRNAs such as miRNA-133a, miRNA-143/145, miRNA-150, miRNA-155, miRNA-214, miRNA-223, and miRNA-320b in exosomes could be used as diagnostic biomarkers to predict the outcome of vascular inflammation and atherosclerosis (Cervio et al., 2015). For example, miR-133a has been demonstrated as a diagnostic biomarker of myocardial infarction (Cheng et al., 2014). Numerous pathologies, such as atherosclerosis and arterial calcification, have been associated with miR-133a downregulation (Lu et al., 2019). Increased levels of miR-150 have also been found in plasma exosomes isolated from atherosclerosis patients (Zhang et al., 2010a).

A significant role in atherosclerosis is played by exosomes derived from platelets via a dynamic interaction with neutrophils, monocytes, and vascular endothelial cells. Under inflammatory conditions platelets release large numbers of MVs containing abundant miRNAs (Lu et al., 2019). For example, activated platelets shed miRNA-320b that can regulate intercellular adhesion molecule 1 (ICAM-1) gene expression (Gidlöf et al., 2013). Vascular endothelial cell apoptosis could be remotely modulated by platelets through releasing miRNA-223 containing exosomes (Pan et al., 2014).

Exosomal proteins could also be used in liquid biopsy. Macrophage foam cell-derived exosomes can enter vascular smooth muscle cells and transport proteins into them. They can also activate ERK1/2 and PI3K/Akt pathways in these cells. These pathways promote progression of atherosclerosis by vascular smooth cell migration (Niu et al., n.d.). Blood exosomes containing PSMA6, PSMA7, and annexin A2 have been shown to be different in the atherosclerosis patient compared to healthy patient (Lu et al., 2019). Plasma endothelial cell-

derived exosome cargo levels of functional proteins involved in atherosclerosis, like VCAM-1 and eNOS, were significantly higher in patients having cerebrovascular disease when compared to controls (Goetzl et al., 2017).

2.6.4 Atherosclerosis and myocardial infarction

Myocardial infarction (MI), also known as a heart attack, is a leading cause of death and disability in the developed world and a major socioeconomic burden (Benjamin et al., 2019). It is typically the culmination of a long and complex process where the formation of an occlusive thrombus within a coronary artery leads to cardiac ischemia and infarction. Atherosclerosis, which is the primary underlying disease process that leads to MI, begins in early adulthood and is driven by lipid accumulation in the arterial wall, inflammation, and vascular injury. As it progresses, some plaques evolve to take on a more unstable phenotype with greater degrees of inflammation. Eventually, plaque rupture can occur and contact of blood with the exposed subendothelial matrix and plaque content causes the formation of occlusive thrombi. This cascade of events leads to the clinical manifestation of MI with chest pain, heart muscle cell death, and ultimately, impaired cardiac function (Palasubramaniam et al., 2019).

Once it occurs, MI can lead to potentially devastating consequences for patients. Three-year mortality rates following MI range from 5% to 10%, with the overall rate of major adverse cardiac events being as high as 30% (Palasubramaniam et al., 2019). Myocardial necrosis is irreversible and leads to progressive structural changes in a process known as ventricular remodeling (Pfeffer & Braunwald, 1990). Currently there are no clinically proven effective therapies for the regeneration of infarcted myocardial tissue. As such, the core focus of early therapy is to minimize infarct size through the prompt restoration of blood flow. Revascularization is accomplished through a combination of percutaneous coronary intervention and pharmacological therapy. Percutaneous coronary intervention, which is also referred to as coronary catheterization and angioplasty, serves to rapidly restore blood flow via mechanical expansion of a metal stent into the diseased arterial segment (Palasubramaniam et al., 2019).

2.6.4.1 Use of statins after myocardial infarction

Better secondary prevention after myocardial infarction (MI) could prevent over 30 000 deaths a year in England and Wales (Dondo et al., 2017). Most patients will need dual antiplatelet therapy for 12 months, an angiotensin-converting enzyme inhibitor, a beta-blocker, and a statin, all of which have been shown to reduce the risk of coronary death (Surveillance Report 2017 – Myocardial Infarction, 2017). A statin should be commenced in all patients post-MI immediately, regardless of lipid profile. A lipid profile should be performed at 4–6 weeks post-MI. Additional lipid-lowering agents should be introduced in cases of refractory dyslipidaemia (LDL-Chol ≥1.8 mmol/L) (Ibanez et al., 2018).

Statins, also known as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors, are a class of bioactive small molecules that efficiently reduce the levels of cholesterol. With respect to their original medical indications, statins are currently in the group of the most prescribed drugs worldwide (Markowska et al., 2020).

2.6.4.2 Statins and extracellular vesicles

There are currently seven statins approved in the United States for lowering cholesterol levels. Three statins are derived from fungi (lovastatin, simvastatin, and pravastatin) and four statins are synthesized (atorvastatin, rosuvastatin, fluvastatin, and pitavastatin). Which particular statin one elects to use may depend on the degree of cholesterol lowering needed and the potential of drug-drug interactions (Feingold, 2000). Different statins can change extracellular vesicle secretion in different ways. It has been shown that atorvastatin can lower EV secretion but enforce the release of cholesterol-enriched EVs (Chen et al., 2022). However, treatment with one statin, called pravastatin, has been shown to significantly reduce plasma levels of large extracellular vesicles from platelets, leukocytes, monocytes, endothelial cells, activated endothelial cells, and syn-cytiotrophoblast cells in pregnant women (Santoyo et al., 2023). Another statin, simvastatin, has also been found to reduce the secretion of exosome from various cell-types (Kulshreshtha et al., 2019).

3. AIMS OF THE STUDY

Over the last decade, liquid biopsy has gained attention as a powerful tool in personalized medicine, since it enables, for example, monitoring of cancer evolution and follow-up of cancer patients in real time (Markou et al., 2022). Liquid biopsy testing is a straightforward process requiring no more than a blood draw and transportation to the local laboratory (Sholl et al., 2020). As such, liquid biopsy is a valuable approach in diagnostics that we examined further in this work.

The aims of the study are the following:

- Map the potential of autoantibodies against cancer-testis antigens MAGEA4 and MAGEA10 as liquid biopsy markers in melanoma cancer diagnostics
- Map the potential of extracellular vesicles purified from melanoma patients blood serum as liquid biopsy markers in melanoma cancer diagnostics
- To compare the properties of extracellular vesicles purified from post myocardial infarction patients' blood plasma to extracellular vesicles purified from the control group.

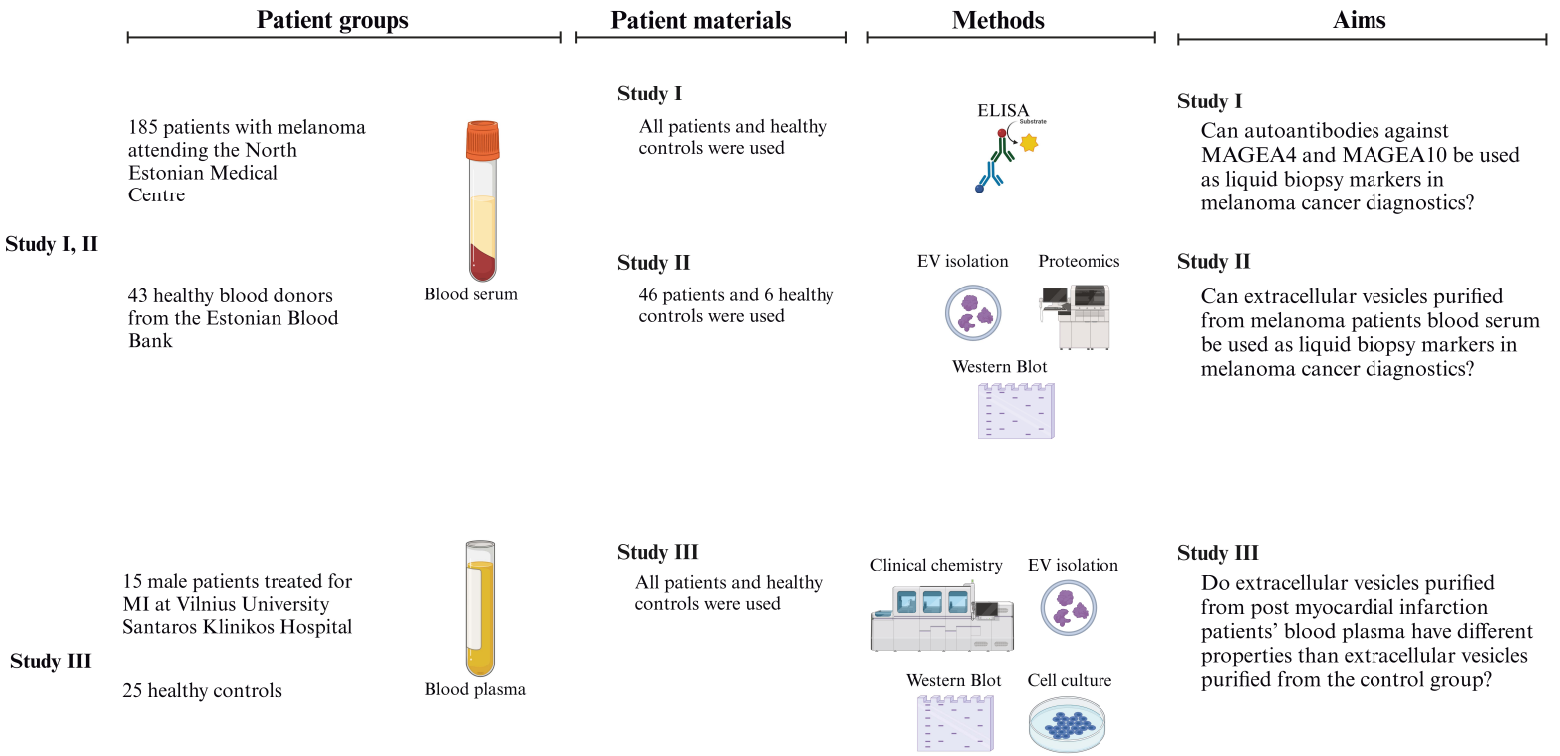

Figure 6. Dissertation overview (Created in BioRender.com)

4. MATERIALS AND METHODS

All materials and methods used in this dissertation are described in greater detail in the corresponding publications. This section provides a brief overview of the materials and methods used.

4.1 Patient groups

Two different patient groups were used; one group for study I and II, and another for study III. For study I, human sera were obtained from 185 patients with melanoma attending the North Estonian Medical Centre within two years (2013–2014). Melanoma stage was assigned based on tumor thickness, ulceration and the involvement of lymph nodes or organs. As controls, 43 sera of healthy blood donors from the Estonian Blood Bank were included. There was no data about the gender nor age of blood bank controls. All samples were handled by standard procedures and stored at -80˚C. For study II, a random selection of 48 patients out of the 185 used in study I, as well as 6 random patients from the 43 healthy blood donors.

For study III, 15 men who had been treated for myocardial infarction either at the Cardiac Intensive Care or at the Intensive Care hospital units, or the first cardiology ward of Vilnius University Santaros Klinikos Hospital, were included. Patients were enrolled between February and May of 2018 and were 40 to 60 years old, have had myocardial infarction (MI) for the first time in their life, and coronary angiography confirmed coronary occlusion of more than 50% in at least 2 arteries. Coronary catheterization and angioplasty were performed on all patients. At the time of blood collection, patients were under antihypertensive treatment and received aspirin and statins. Twelve patients had primary arterial hypertension. Altogether, only one patient had systemic inflammation, diabetes mellitus, and primary arterial hypertension at the same time. As a control group, healthy 40- to 60-year-old men were included between December 2015 and July 2017. Healthy subjects had no prior history of acute cardiovascular disease and were not under any treatment for cardiovascular diseases. For these study III patient groups, sodium citrate plasma was used.

4.2 Methods used

4.2.1 ELISA

For autoantibody detection in study I, enzyme linked immunosorbent assay (ELISA) with recombinant MAGEA4 or MAGEA10 proteins was used. ELISA was performed using MAGE-A4 and MAGE-A10 proteins, which were purified from E. coli, immobilized on micro titer plates, and subsequently probed with 1:200 to 1:800 human sera dilutions. After adding sera, the plates were incubated

for one hour at room temperature on the shaker. Horseradish peroxidase-conjugated goat anti-human IgG was used as a secondary antibody for 45 min. After washing four times, the reaction was developed with the TMB Peroxidase E1A substrate kit. Absorbance was read at 450 nm using the ELISA plate reader Sunrise™. For quality control, three reference sera were included that were analyzed on every ELISA plate. The coefficient of variations (CV) of their optical densities (ODs) did not exceed 20%. Serums that exhibited high OD values, indicating the presence of anti-MAGE-A antibodies, were tested at least three times on separate ELISA plates and the mean OD value was used in further analysis. OD values obtained from 1:400 diluted serums were used in statistical analysis.

4.2.2 Clinical chemistry and MDA measurements

In study III, the concentrations of C-reactive protein (CRP), glucose, total cholesterol (Chol), triglycerides (TG), high-density lipoprotein cholesterol (HDL-Chol), and low-density lipoprotein cholesterol (LDL-Chol) were measured from patients' and healthy subjects' blood using routine techniques of a clinical chemistry laboratory. Malondialdehyde (MDA) was measured to represent the level of oxidative stress in biological samples. For this, 50 µl of thawed serum was mixed with 750 µl of 0.44 M phosphoric acid solution, 250 µl of 42 mM thiobarbituric acid solution, and 450 µl of deionized water. The prepared samples were incubated at 100 °C for 60 min. After incubation, samples were rapidly cooled in an ice bath, and 500 µl of the sample was diluted in a methanol solution (1: 1). The blood serum sample was mixed by shaking and then centrifuged for 3 min at 10 000 g. After centrifugation, 500 µl of the centrifuged serum sample was added to the chromatographic vial, and serum sample was analyzed via the high-performance liquid chromatography (HPLC) method. Malondialdehyde concentration was measured by a Shimadzu Nexera X2 UHPLC system. Data was collected and processed using LabSolutions software.

4.2.3 Extracellular vesicle isolation and detection

For extracellular vesicle purification, two methods were used: ultracentrifugation (UC), and precipitation with 10% of polyethylene glycol (PEG). Both methods were used in study II; in study III only the ultracentrifugation method was used. For ultracentrifugation, up to a 100 µL of each blood serum sample was diluted to a total volume of 1 mL with PBS and samples were centrifuged at 1200 g for 30 min at 4 °C. Then, 3 mL of PBS was added to the supernatant, and it was further centrifuged at 120,000 g for 90 min at 4 °C using the Optima™ L-90K Ultracentrifuge with rotor SW55Ti. EVs were resuspended in 300 µL of Dulbecco's PBS. For the PEG method, precipitation with 10% of polyethylene glycol was used. First, up to 100 µL of each sample was centrifuged at 10,000 g for 10 min at 4 °C to remove insoluble material. Then, 20 µL of 50% PEG MW 6000

and 1.5 µL of 5M NaCl was added per 100 µL of sample and precipitated overnight (at least 8 hours) at 4 °C. EVs were centrifuged at 10,000 g for 10 min and the pellet was resuspended in 200 µL of PBS. 100 µL of the sample, which was diluted in 4 mL of PBS, was ultracentrifuged at 120,000 g for 1.5 h with the Beckman-Coultier SW55Ti rotor. The pellet was resuspended in 200 µL of Dulbecco's PBS. For both methods, the EV sample concentrations were measured with the Bradford Protein Assay using bovine serum albumine as a standard.

After extracellular vesicle isolation, nanoparticle tracking analysis (NTA) was performed with a ZetaView nanoparticle analyzer in study II and III. Eleven measurements were recorded for each sample twice and the results were averaged. ZetaView Software 8.04.02 was used to analyze images. Each sample was measured 3 times; extracellular vesicle concentration was expressed as the number of EVs per 1 ml of PBS.

4.2.4 Protein detection with Western Blot, proteomics and flow cytometry

In both study II and III, Western blot (WB) was used to analyze extracellular vesicle protein content. EVs were lysed and proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred by a semidry blotting method to a polyvinylidene difluoride (PVDF). After electrophoresis, the proteins were transferred onto a PVDF membrane and blocked for 30 min to overnight with 2% non-fat dry milk in buffer. In study II, the primary antibodies used were anti-GELSOLIN, anti-SERPINA3 and anti-LGALS3BP. In study III, the primary antibodies used were anti-TSG101 and anti-thioredoxin. In both studies, the secondary antibody was goat anti-rabbit. After incubation, the signal was visualized by adding the electrochemiluminescence (ECL) substrate for one minute and the films were exposed for either 10 (SERPINA3, TSG101, thioredoxin) or 60 min (GELSOLIN, LGALS3BP), after which they were scanned using Epson Expression 1680. In study II, after reading the image, western blot images were measured with Image J software, where each sample was given an arbitrary value based on blot intensity minus background intensity multiplied by blot size. To further normalize the measured signals, all Image J arbitrary values were divided by protein concentration obtained with Bradford assay, and a relative value was used in statistical analysis. In study III, two random healthy control samples and two post-MI samples were analyzed with Western Blot with the primary antibody being anti-NOX2, anti-NOX5, anti-NOX1, anti-Akt B, and anti-ERK 1/2.

In study II, proteomics was also performed. For this, a random selection of 20 PEG and 18 UC samples were selected for proteomics analysis from the study II patient group. Based on sample protein concentration, UC samples were pooled into stages containing 260 µg of protein per pool. 4 mL of PBS was added, and the mixture was ultracentrifuged at 120,000 g for 90 min at 4 °C using the Optima™ L-90K Ultracentrifuge with rotor SW55Ti. The pellet was resuspended

with 100 µL of PBS and sent for analysis. For PEG samples, 20 µL of each sample was pooled into a stage pool based on cancer stage, 4 mL of PBS was added, and the mixture was ultracentrifuged at the same conditions to remove the serum albumin. The pellet was resuspended in 100 µL of PBS and 10 µg of sample was sent for analysis. Proteomic analysis was performed in the Proteomics Core Facility of Institute of Technology, University of Tartu. Proteins from EVs were precipitated and digested with trypsin, and obtained peptides were detected with an LTQ Orbitrap XL mass-spectrometer. Mass-spectrometric raw data was analyzed with MaxQuant 1.4.0.8. Data were searched against UniProtKB (accessed on 15 September 2020, www.uniprot.org) sequences. Criteria for identification were specified as following: one peptide, minimum length of seven residues, and false discovery rate of <1% using a target decoy approach.

In study III, fluorescent flow cytometry was performed to identify EVs surface antigens. First, EV sample volume was adjusted to the total protein concentration. A vial containing only PBS was used as a negative control. 5 µl of latex beads was added to all EV samples and the negative control and left overnight at 4 °C. In the morning, 1 µl of 2M glycine was added and kept for 45 min at room temperature. Then, two washing steps were performed using a 0.5% PBS/BSA blocking solution, and EV samples were incubated for 20 min at room temperature. EV samples were incubated with CD9 antibody for 1 h at room temperature and centrifuged. The supernatant was removed until 100 µl was left in the tubes; then, 400 µl of PBS was added and the pellet was carefully re-suspended. This solution was tested on an LSRII flow cytometer.

In study III, oxidative stress in cell culture was also measured. EVs were added to the pooled HUVEC cells, which were at up to the 5th passage and incubated for 20 h. EV concentration was equalized in each sample of HUVEC cells by using NTA data. After the incubation, every sample was stained with 5 µM 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA) in the dark for 30 min. After staining, cells were lifted with trypsin, washed once with complete medium EGM-2 BulletKit, and centrifuged. After washing, samples were measured with flow cytometer FACS Canto II using FITC channel at excitation 490 nm and emission 520 nm. Collected data was analyzed using FACS Diva software.

4.3 Statistics

In study I, the data was analyzed in R (version 3.3.0). Parameter estimates and corresponding credible intervals (CI) were calculated using the BayesFirstAid package. Tukey post-test was also done in R to calculate analysis of variance (ANOVA). The pROC package was used to calculate the receiver operating characteristic (ROC) curve.

In study II, statistical analysis was carried out in R Studio using the Kruskal–Wallis rank sum test to find potential significant differences between different populations. If the Kruskal–Wallis was below 0.2, the Wilcoxon rank sum test

with continuity correction was used to screen through all population pairs to find statistically important differences (p-value under 0.05).

In study III, variables were tested by the paired non-parametric Wilcoxon test and Spearman test for correlation coefficient. The confidence level for the test was set at 0.05; all p-values were two-sided. Statistical analysis was carried out using R statistical software version 1.0.136 and SPSS version 21.

5. RESULTS AND DISCUSSIONS

5.1 Study I – MAGE-A4 and MAGE-A10 expression in melanoma

The aim of this study was to determine the presence of naturally occurring antibodies against two MAGE-A subfamily proteins, MAGE-A4 and MAGEA-10, in 185 patients with melanoma at different stages of disease and to compare their response with 43 healthy controls from the Northern Estonian blood bank.

5.1.1 The mean immune responses of patients

The mean OD value of the sera of melanoma patients was 0.59 (standard deviation, SD, 0.31) for MAGE-A4 and 0.73 (0.38) for MAGE-A10. For blood bank controls, the mean OD value was 0.67 (0.26) for MAGE-A4 and 0.70 (0.28) for MAGE-A10. We could not find evidence of elevated mean effects of melanoma patients over blood bank controls. On the other hand, the patients had higher variability at their anti-MAGE-A4 and anti-MAGE-A10 responses than controls. The failure of patients to exhibit aggregate effects over the controls is likely due to the voluntary blood donors, who make up the control group. We have no data about their age and gender, but they are probably younger than the melanoma patients. This limits the usefulness of blood donors as controls. Higher variability of immune response in patients could mean that in some patients the antibody response is induced, while in others it is not.

5.1.2 A strong antibody response against the MAGE-A4 and/or MAGE-A10 protein

Tumor cells can often express more than one MAGE-A protein. Simultaneous expression of five or more MAGE-A proteins occurs in more than half of oral squamous cell carcinomas (Brisam et al., 2016) and simultaneous expression of MAGE-A1 and MAGE-A4 expression occurs in 60–70% of melanomas (Barrow et al., 2006). MAGE-A10 has been shown to be expressed in 38% of malignant melanomas (Schultz-Thater et al., 2011). MAGE-A4 is expressed in 9% of primary tumors, but reaching to 44% in distant metastasis (Barrow et al., 2006). MAGE-A1 expression has been found in 16% of primary melanomas and 48% of metastatic melanomas (Brasseur et al., 1995). However, in another study no correlation was observed, and MAGE-A3/A4 protein was present in 25% of primary invasive and metastatic tumors, but not in in situ melanomas (Busam et al., 2000).

Overall, our study revealed that 8% of patients had strong antibody responses against the MAGE-A4 or/and MAGE-A10 protein. Two patients (M38 and M111) had a strong response against both proteins, which means in total there were 17 strong responses. Out of this 17, 12 were anti-MAGE-A10 and 5 were

anti-MAGE-A4 responses. Some patients had antibodies against both MAGE-A proteins, while others against only either MAGE-A4 or MAGE-A10. Five patients of 15 (33%) had a statistically significant higher OD value ($p<0.01$) against MAGE-A10 than MAGE-A4, while only one patient had a better immune response against MAGE-A4 (figure 7). This data shows that among strongly responding patients, there are more anti-MAGE-A10 than anti-MAGE-A4 responses. Thus, our work is consistent with the studies, which have suggested that MAGE-A10 is the most immunogenic antigen of the MAGE-A family (Bricard et al., 2005; Groeper et al., 2007; Valmori et al., 2001).

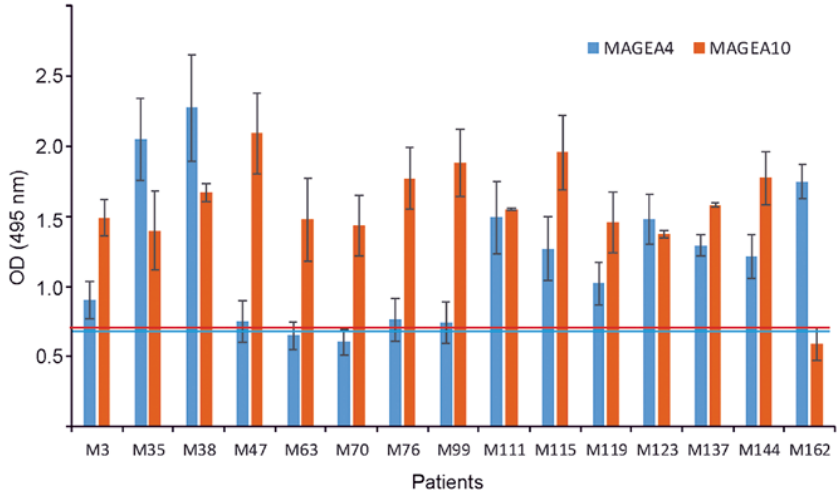

Figure 7. Comparison of OD values of MAGE-A4 and MAGE-A10 among the strongly responding patients. Lines correspond to mean values of ELISA assay for MAGE-A4 (blue) and MAGE-A10 (orange), respectively. Error bars show the SD of at least three different experiments performed on separate ELISA plates

However, it is well known that obtaining antibodies against one specific MAGE-A protein may be challenging; we cannot rule out the possibility that antibodies detected in our assay are formed against some other member of the family. MAGE-A proteins are highly similar to each other, with half of the amino acids identical between MAGE-A4 and MAGE-A10. The MAGEA subfamily consists of 11 MAGE-A proteins and in addition, there are MAGE-B, MAGE-C, MAGE-D etc. families which all share the MHD (MAGE homology) domain (Lee & Potts, 2017). All these proteins are to some extent similar to each other as MHD domains has similarities from 25 to 80% and may give some cross-reactivity.

Among the 15 patients of our study with the positive status for MAGE-A antibodies, only one has died and one has disease progression during the 2-year post-study follow-up period. As such, the majority of patients with strong antibody response have the disease under control. However, this cohort is too small to make long-term conclusions about the prognosis.

5.1.3 Positive autoantibody responses in stages I–III, especially in stage II melanoma patients

When patients were grouped according to the level of the disease, then 7 of 43 (16.3%) sera were positive among stage II patients, and 3 of 29 (10.3%) in stage III patients. When comparing MAGE-A10 autoantibody response between stages, there was a single contrast between stage 0 and stage II patients, which had a significant difference in mean OD values ($p = 0.047$). The mean OD values between stage II and stage IV patients were slightly different ($p = 0.10$), but no difference was observed between stage II and III patients ($p=0.78$). However, when looking at MAGE-A4 autoantibody response between stages, there was no clear preference to any stage. Strongly responding sera belonged to patients of stages I, II and III (figure 8). We did not observe statistically significant differences in mean values between patients with different stages of disease ($p = 0.74$ for stage 0 vs. stage II; $p = 0.18$ for stage II vs. stage IV and $p=0.47$ for stage II vs. stage III). No strong response from the blood samples of melanoma patients with stage 0 and IV were detected.

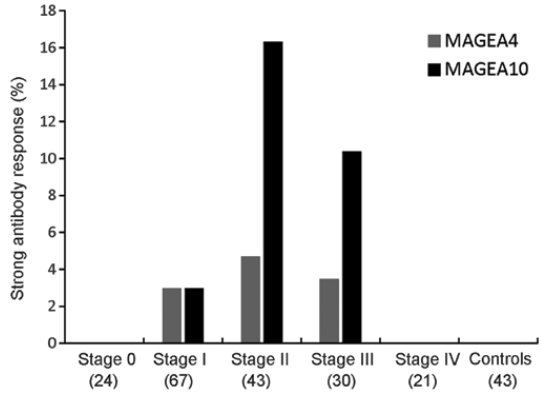

Figure 8. The fraction of strongly responding patients in relation to the melanoma stage. The number of sera is shown in the parenthesis.

The prevalence of MAGE-A antibodies was highest in stage II and lowest in stage 0 and stage IV patients. We have not determined the expression of MAGE-A proteins in tissue samples of our patients, but it is very unlikely that MAGE-A expression declines in advanced stages. Stage II melanoma patients might have a better immune response than patients with more advanced stages of disease. This is consistent with the immune evasion seen in metastatic cancer (Vinay et al., 2015). Interestingly, there were also very few responses amongst in situ and stage I melanoma patients. This can be explained by the localization of the primary tumor. Stage 0, also known as melanoma in situ, and stage I melanoma are found mostly on the outer layer of the skin, in epidermis. Stage II melanoma has spread to the lower part of the inner layer of skin (dermis), but not yet into the tissue below the dermis or into nearby lymph nodes. The dermis contains many antigen-presenting cells, which may help to boost the immune response.

The existence of strongly responding patients in stage II suggests that their immune system has been activated and has started to generate antibodies against the primary tumor. As such, our data support the hypothesis that the immune system is involved in the control of melanoma, at least in the early stages. Several studies have shown spontaneous regression of primary melanomas, but regression of metastatic tumors is very rare. A good antibody response at early stages can stop the growth of primary tumor and further spreading to the lymph nodes and other organs.

5.1.4 anti-MAGE-A antibodies as potential diagnostic biomarkers

To explore the potential diagnostic value of anti-MAGE-A antibodies, we classified all stage 0 versus pooled stage I and II patients using an additive logistic regression model that included both MAGE-A proteins, age, and sex. We summarized the model performance in a ROC curve where we plotted the sensitivity (true positive rate) values against 1-specificity (false positive rate) values for each possible cut-point. The area under the curve (AUC) was 0.74, suggesting that anti-MAGE-A antibodies could be treated as potential diagnostic biomarkers (figure 9).

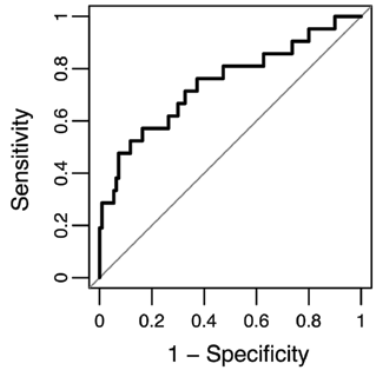

Figure 9. ROC curve for anti-MAGE antibody detection. ELISA determined antibody levels among 185 melanoma patients and 43 blood bank controls.

Discussion. A healthy immune system enables the creation of antibodies against cancer antigens that are expressed by tumor cells. The link between MAGE-A antigens and cancer is widely known and accepted; several works have shown a good cellular and humoral response against MAGE-As (Lee & Potts, 2017; Meek & Marcar, 2012; Sang et al., 2011). One of the limitations for use in clinics is that MAGE-A proteins are expressed only in a portion of cancer cells – different works have shown that the amount of expressing cells is between 25 and 50% (Barrow et al., 2006; Busam et al., 2000). When we assume that, only half of these people have a strong immune response, then the expected percentage of strongly responding patients will be 12 to 25%. On the other hand, these

antibodies are so-called early markers and there is a great need for early cancer markers. As such, if the presence of strong antibody response correlates with good prognosis, then they will be useful for clinical diagnostics. In addition, from the clinical aspect, the longitudinal detection of MAGE-A antibody levels could be utilized for profiling of disease status or of effectiveness of novel immunotherapies. There exists a great need for biomarkers, which could assist in discrimination of patients suitable for immunotherapy or for monitoring therapy.

Overall, these results indicate that the anti-MAGE-A4/MAGE-A10 immune response in melanoma patients was heterogeneous, with only ~8% of patients having a strong response. Comparing the number of strongly responding patients between different stages of disease revealed that the highest number of strong responses was detected among stage II melanoma patients. These findings support the model that the immune system is involved in the control of melanoma in the early stages of disease. MAGE-A expression has been looked in other cancer types as well, for example ovarian cancer, bladder cancer, gastric and lung cancer. In all of these studies, the expression of MAGE-A itself has been studied and its presence has been linked to negative effects, for example shorter survival times and worse patient outcomes.

In epithelial ovarian cancer tissues, at least five MAGE-A family members have been shown to be expressed in approximately 78% of patients. Compared with benign disease or healthy controls, MAGE-A has been shown to be highly expressed in tissues and serum of patients (Zhao et al., 2022). In case of MAGE-A4, serum levels of MAGE-A4 in ovarian cancer patients have been shown to be significantly higher than in patients with benign diseases. MAGE-A4 protein was expressed in nearly 22% of primary patients (Kawagoe et al., 2000). Zhang et al. reported that the expression of MAGE-A1 and MAGE-A3 was related to the degree of tumor differentiation and clinical stage (Zhang et al., 2010b). Daudi et al. found that the expression of MAGE-A1 and MAGE-A10 was significantly correlated with shorter progression-free survival (Daudi et al., 2014). Also, MAGE-A expression has been shown to be related to the pathological type and cancer stage of epithelial ovarian cancer and preoperative serum CA125 levels. Compared with patients that are MAGE-A-negative, the overall survival with expression of the MAGE-A family was significantly shorter (Sang et al., 2017). In high-grade serous ovarian cancer, MAGE-A4 expression has been shown to be present in 57% of cancer tissues. No staining was detected in serous cystadenoma or normal ovary. MAGE-A4 expression was negatively correlated with survival and multivariate analysis showed that the expression of MAGE-A4 is an independent risk factor for outcomes (Yakirevich et al., 2003).

In the case of bladder cancer, Patard et al. analyzed the expression of MAGE-A1, MAGE-A2, MAGE-A3, and MAGE-A4. Out of 57 samples of primary transitional-cell carcinomas of the bladder, 21% expressed MAGE-A1 and 35% expressed MAGE-A3. MAGE-A2 and MAGE-A4 genes were expressed by 30% and 33%. MAGE expression was more frequent in advanced tumor stages: 61% of invasive tumors were positive for expression of at least one of the four genes, whereas only 28% of superficial tumors expressed these genes (Patard et al.,

1995). MAGE-A4 and MAGE-A9 were observed in 38% and 63% of nonmuscle-invasive tumors, 48% and 57% of muscle-invasive tumors, 65% and 84% of carcinomas in situ, and in 73% and 85% of lymph node metastases. Expression was associated with higher grade of cancer. MAGE-A9 expression was associated with recurrence and MAGE-A4 expression was associated with progression to muscle-invasive cancer (Sang et al., 2017). In another study, MAGE-A4 protein was expressed at significantly higher levels in transitional cell carcinomas, its positivity was correlated with an invasive phenotype and high-grade tumors. When retrospectively evaluating transitional cell carcinomas of the bladder, patients who demonstrated strong MAGE-A4 staining had decreased tumor-specific survival (Kocher et al., 2002).

In gastric cancer, protein expression of MAGE-A10 was related to high MAGE-A10 mRNA expression, high cumulative incidences of hepatic recurrence, and poor relapse-free survival. However, overall survival did not differ significantly between patients who were positive or negative for MAGE-A10 (Fujiya et al., 2021). In the case of MAGE-A11, it was highly expressed in gastric cancer tissues and associated with poor patient prognosis. Additionally, MAGE-A11 functioned as an independent prognostic factor in gastric cancer and its expression showed significant correlation with both tumor immune cell infiltration and responsiveness to immunotherapy. It has been indicated that MAGE-A11 regulates gastric cell proliferation and migration and may operate as a prognostic factor, having potential as an immunotherapy target (Wang et al., 2024). In lung cancer, the relative expression levels of MAGE-A proteins and mRNA in non-small cell lung cancer tissues were significantly higher than those in adjacent tissues. Furthermore, MAGE-A protein expression was significantly higher in stage III–IV lung cancer when compared to stage I–II. Patients with high MAGE-A mRNA expression had a significantly shorter median overall survival compared to those with low MAGE-A mRNA expression. However, no significant difference was observed in median overall survival between patients with high and low MAGE-A protein expression (Zhu et al., 2024).

Autoimmune response against MAGE-A has been less studied than the expression of MAGE-A in cancer. However, anti-MAGE-A family protein autoantibodies have been looked at in esophageal squamous cell carcinoma. The levels of seven autoantibodies, including MAGE-A4-IgG and MAGE-A10-IgG, were significantly higher in cancer patients than in healthy controls or in benign esophageal diseases (Sun et al., 2023). Some studies have focused on looking at the biological roles of MAGE-As in the immune system, their potential in DNA vaccines and their link to immune system inhibitor therapies. In mice pancreatic ductal adenocarcinoma models, MAGE-A6 has been shown to suppress autophagy. Inhibition of autophagy is critical for tumor initiation (Tsang et al., 2020). In the same model, MAGE-A has been established as a regulator of tumor-stromal crosstalk. MAGE-A2, MAGE-A3 and MAGE-A10 expression has been shown to be up-regulated in chemotherapy resistant pancreatic ductal adenocarcinoma patient derived organoids and cancer cell lines. However, immunization with a DNA vaccine, that targets multiple MAGE-A antigens, has been shown to elicit

robust immune responses against the growth of gemcitabine resistant tumors (Qin et al., 2023). In the case of immune system inhibitor therapies, MAGE-A expression in non-small cell lung cancer and urothelial cancer has been shown to not significantly change before and after checkpoint inhibitor therapy. There is stable expression of MAGE-A3/A6 pre and post checkpoint inhibitor treatment in these cancer types (Faiena et al., 2024).

In conclusion, even though there have been more studies that look at MAGE-A expression in tumors and link them to negative patient survival statistics more often than not, work into MAGE-A immune effects is ongoing and has had its first positive breakthroughs. This study focused on using an autoantibody response against MAGE-A as diagnostic marker, which is a field that has not been explored thoroughly yet. At the same time, MAGE-A proteins themselves also have effects in tumor development and biological effects through immune system regulation, which means they are interesting targets with further research needed into their effects and diagnostical potential.

5.2 Study II – Extracellular vesicles produced by melanoma cells and use for diagnostics

The aim of this study was to purify EVs from the blood serum of 56 melanoma patients and 6 healthy controls and to determine the diagnostic potential of the EV proteome, in which the EVs have been purified with two different methods.

5.2.1 Purification of extracellular vesicles by ultracentrifugation and polyethylene glycol precipitation

Our study shows that EVs can be purified by both methods – ultracentrifugation (UC), which is the classical method for purification of EVs; and polyethylene glycol (PEG) precipitation, which is a more rapid and robust way to isolate EVs from biological fluids. As EVs purified with both methods had similar median size (figure 10), further EV analysis was done based on UC samples. EVs purified with UC were divided into subgroups according to the melanoma stage, and their amount and median diameter were compared between stages.

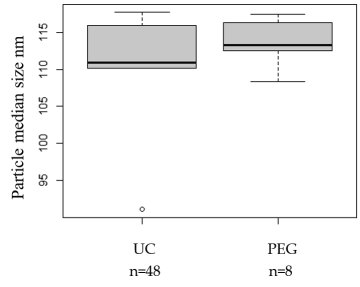

Figure 10. The particle median size measured by nanotracking analysis (NTA) for both ultracentrifugation (UC) and polyethylene glycol (PEG) precipitation samples

5.2.2 There are differences in EV amount and size in different cancer stages

The lowest particle concentration was seen in stage IV patients, 5.4×10^{10}/mL, while the peak was in stage II at 38.4×10^{10}/mL. Stage 0 had an average particle concentration of 10×10^{10}/mL, stage I 24.3×10^{10}/mL, and stage III 26.8×10^{10}/mL. Healthy controls had a similar concentration to stage 0 at 10.1×10^{10}/mL. There were statistically significant variabilities between stage 0 and III (p=0.05), between stage II and IV (p=0.03), and between stage III and IV (p=0.01).

When comparing the median diameter of EVs between different stages, the largest particles were detected in healthy controls at 120.6 nm. The second largest were in stage 0 at 117 nm, followed by stage I at 114.4 nm. The smallest particles were detected in stage III patients, at 103.7 nm, followed by stage II at 104.4 nm. There were statistically important differences between particle sizes between healthy controls and stage I (p=0.01), stage 0 (p=0.05), stage II patients (p=0.0005), and stage IV (p=0.007). Stage 0 melanoma in situ patients had also a statistically different particle size from stage I patients (p=0.0002), stage III (p=0.003), and stage IV (p=0.01). There was also a significant difference in median diameters of stage II and IV (p=0.01) (figure 11).

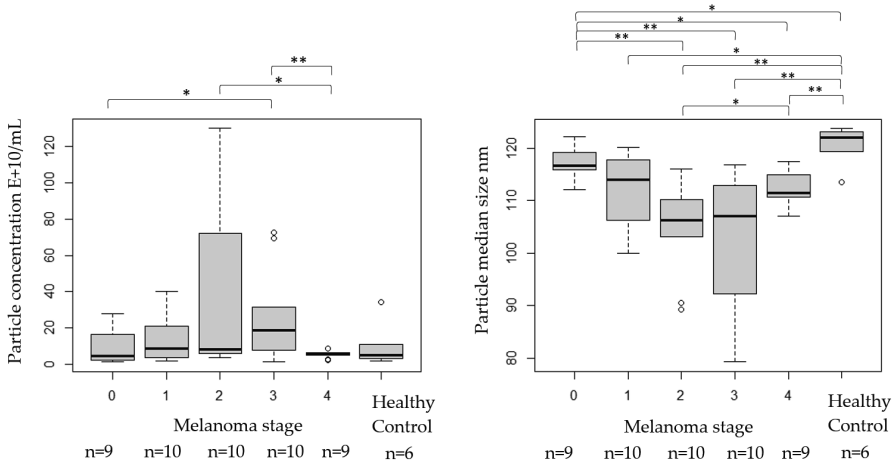

Figure 11. Particle concentration and median size measured by NTA in UC purified samples grouped by melanoma stages

The material obtained from stage II and III patients was the most heterogenous and differed the most from that of healthy controls. EVs obtained from stage II and III patients were on average smaller and more abundant than others. It has been shown that EVs purified by UC are a mixture of different vesicles and co-purified proteins, including exosomes, microvesicles, apoptotic bodies, and oncosomes (van der Meel et al., 2014; Ciardiello et al., 2016) which might explain the size differences. The differences could also reflect on the status of disease. Depending on the cancer stage, the proportion of different vesicles and their

relative amount in EVs may be different. Malignant cells secrete more EVs than nonmalignant cells of the same type and tumor microenvironment characteristics can further increase EV secretion rates (Hu et al., 2021; Keller et al., 2011).

5.2.3 Unique proteins (N=585) were found in EVs by proteomics and different proteins were found with different purification methods

Out of 585 unique proteins, 334 proteins were detected in PEG-precipitated samples and 515 in UC-purified EVs. Identified proteins were divided into twelve groups according to their biological and cellular function using the UniProt database, which was accessed on 15 September 2020, www.uniprot.org. A graphical representation of different proteins found with the two purification methods used is presented with figure 12.

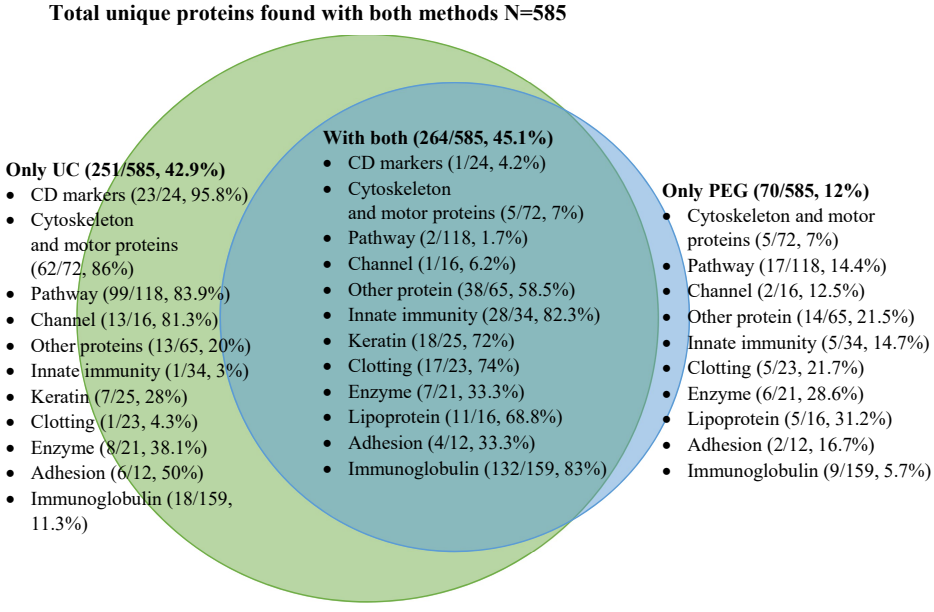

Figure 12. Different proteins found with the two purification methods

In UC-purified samples, significantly more CD markers, cytoskeleton, motor proteins, and pathway proteins, were detected. Extracellular surface markers such as CD31, CD151; and different MHC-A, B, and C molecules, were also present. All of these markers have been previously used to purify EVs by surface markers in microarray settings (Buzás et al., 2018). This suggests that our UC-purified samples contained a considerable amount of EVs. Also, different EV subpopulations were included as we found CD9 and WNT2 for exosomes, CD31 for microvesicles, as well as the exosome-associated epithelial cell adhesion markers CA125 and CD41b (Ciardiello et al., 2016; Sunkara et al., 2016; van Niel et al.,

2018). Multiple proteins involved in exosome formation, such as RAB7A, RAB11, and RAB27A, were also found (van Niel et al., 2018; Jaé et al., 2015). On the other hand, PEG-precipitated samples were enriched in immunoglobulins, lipoproteins, and clotting or innate immunity proteins, which could indicate co-purification of serum proteins and lipoproteins (Shami-shah et al., 2023).

Overall, the isolation and purification of EVs from serum has its challenges due to high serum viscosity and high abundance of serum proteins and non-EV lipid particles (Brennan et al., 2020). Consistent with previous studies, we also identified co-purified serum proteins such as apoB as well as a large amount of immune and inflammatory response proteins (Smolarz et al., 2019). In addition, a wide range of innate immunity complement proteins as well as complement regulatory protein CD59, TF, and clotting factors were detected. All of these might suggest that some microvesicles are platelet-derived and appeared in the sample during pre-analytical sample taking procedures, as our material was serum where no platelets should be present (van der Meel et al., 2014; Buzás et al., 2018; Smolarz et al., 2019).

5.2.4 Proteins (N=159) linked to melanoma. SERPINA3, gelsolin and LGALS3BP in EVs as diagnostic markers

We performed extensive literature review in 2021 to find which of these identified proteins have been linked to melanoma. In total, 159 proteins previously linked to melanoma in vivo experiments, immunohistochemistry, melanoma cell lines, murine models, in silico proteomics analysis, or gene expression studies were found at the time of writing study II. We detected multiple proteins shown to be upregulated in melanoma cell-derived EVs such as NOTCH2, TLN1, PGK1, SERPINF2, WDR, CSGP4, and YWHAE, as well as proteins linked with progressive disease such as ITHI3, MSN, THBS1, and TUBB (Pietrowska et al., 2021). In addition, EV markers linked to other tumors were also found. PIGR has been shown in EVs from ultracentrifuged cholangiocarcinoma patient sera (Arbelaiz et al., 2017) and ALK1, CD151, and ECM1 have been found in non-small cell lung cancer patient EVs as potential protein biomarkers, while CD91 has been found to be a powerful surface biomarker for the same disease (Ma et al., 2019; Ueda et al., 2014).

The expression of three potential biomarkers – SERPINA3, LGALS3BP, gelsolin – in UC purified EVs was analysed with Western Blot. In summary, the expression of SERPINA3 and LGALS3BP was higher in melanoma patients than healthy controls, and gelsolin exhibited higher expression in healthy controls compared to melanoma patients.

The first biomarker looked at was SERPINA3. SERPINA3, also called α-1-antichymotrypsin, is a serine protease inhibitor. As an acute-phase protein secreted into the plasma by liver cells, it plays an important role in the anti-inflammatory and antiviral response. Elevated levels of SERPINA3 have been

observed in heart failure and neurological diseases, such as Alzheimer's disease or Creutzfeldt-Jakob disease. Many studies have shown increased expression levels of the SERPINA3 gene in various types of cancer, such as glioblastoma, colorectal cancer, endometrial cancer, breast cancer, or melanoma (de Mezer et al., 2023). When delving into the EV world, osteoblast-derived extracellular vesicles have been shown to exert osteoblastic and tumor-suppressive functions via SERPINA3 in osteoblastic prostate cancer (Ito et al., 2023).

In this study, the lowest protein expression of alpha-1-antichymotrypsin (SERPINA3) was detected in EVs from healthy controls and the highest in stage II and III patients, followed by stage IV patients. There were statistically significant differences in signal strength between healthy controls and stage IV (p=0.002), stage II (p=0.005) and stage 0 patients (p=0.05). The upregulation of SERPINA3, has been shown to correlate with worse patient outcomes, as it has pro-migration and pro-invasion functions in melanoma cells (Zhou et al., 2016). Our results were consistent with previous findings as healthy controls had a significantly lower SERPINA3 signal when compared to stage II, III, and IV melanoma patients (figure 13). The highest signal of SERPINA3 was found in stage II patients when the invasion of melanoma cells starts and small nests of melanoma start to form in the papillary dermis, the lowest level of the epidermal skin (Davis et al., 2019).

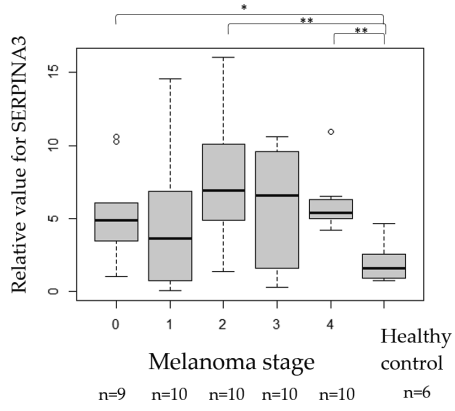

Figure 13. The relative values of the SERPINA3 signal obtained from the immunoblot analysis of EVs grouped by melanoma stages.

The second biomarker looked at was LGALS3BP. Galectin-3-binding protein (LGALS3BP) is a secreted multifunctional glycoprotein that is expressed by several cancerous specimens, but undetectable or poorly present in normal tissues (Cela et al., 2024). It is involved in immunity, angiogenesis, cellular adhesion, migration and tumor microenvironment crosstalk (Capone et al., 2021). LGALS3BP has been indicated as one of the most abundant glycoproteins on the surface of extracellular vesicles derived from ovarian and endometrial cancer, pancreatic ductal adenocarcinoma, glioblastoma, and neuroblastoma. It is shown to take part in cargo delivery in these vesicles (Cela et al., 2024).

In this study, the expression of LGALS3BP in EVs showed a similar pattern to SERPINA3; however, EVs from both healthy control and stage 0 patients showed very low expression. At the same time, the highest signals were detected for stage I and IV patients (figure 14). Statistically significant changes were seen between stage I and healthy controls (p=0.003), and between stage I and III patients (p=0.05). The expression of LGALS3BP is altered in the serum of patients with a variety of human carcinomas, and a down regulation of the protein it binds to, galectin-3, is related to increased aggressiveness of tumors. It promotes integrin-mediated cell adhesion and may stimulate host defense against viruses and cancer cells (van den Brûle et al., 1995). In melanoma, galectin-3 protein overexpression correlates with metastatic progression and a negative clinical outcome (Prieto et al., 2006).

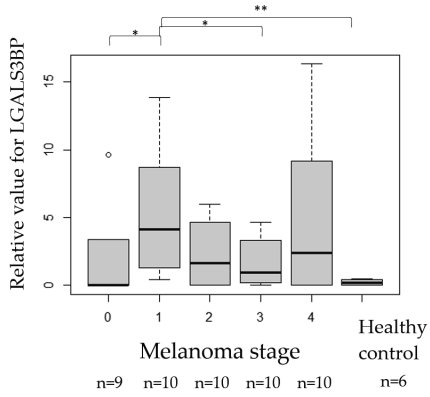

Figure 14. The relative values of the LGALS3BP signal obtained from the immunoblot analysis of EVs grouped by melanoma stages

The third biomarker looked at was gelsolin. Gelsolin is a calcium-activated actin filament severing and capping protein, which is found in many cell types. It also exists as a secreted form in the plasma of vertebrates. Tight regulation of gelsolin by calcium is crucial for its physiological role and constitutive activation leads to apoptosis (Spinardi & Witke, 2007). The protein expression of gelsolin has been found to be significantly decreased in several pathological conditions including neurodegenerative diseases, inflammatory disorders, and cancers. Its extracellular isoform, called plasma gelsolin, is one of the most abundant plasma proteins in circulation, and has emerged as a novel diagnostic biomarker for early disease detection. Current evidence reveals that gelsolin can function as either an oncoprotein or a tumor suppressor depending on the carcinoma type. Plasma gelsolin is also involved in immunomodulation (Hsieh & Wang, 2022). Overexpression of plasma gelsolin has been shown to be a key contributing factor to ovarian cancer chemoresistance and immunosuppression. Exosomes containing plasma gelsolin have been said to contribute to ovarian cancer progression (Onuma et al., 2022).

In this study, gelsolin showed a completely different expression pattern with the highest median signal detected in healthy controls (figure 15). Healthy controls had a significantly higher gelsolin signal when compared to stage I (p=0.003), stage II (p=0.002), stage III (p=0.005), and stage IV patients (p=0.04). There was also a significant difference between stage II and stage IV patient signals (p=0.03). According to previous studies, gelsolin occurs in acidic exosomes and its expression is highest in metastatic melanoma patients (Boussadia et al., 2018). In our study, the highest occurrence was seen in healthy patients instead.

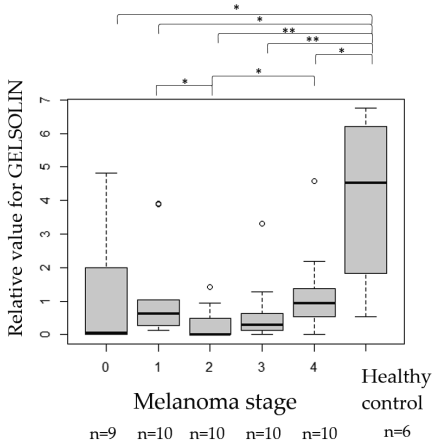

Figure 15. The relative values of gelsolin signal obtained from the immunoblot analysis of EVs grouped by melanoma stages.

In summary, in this study EVs were purified from the blood serum of melanoma patients and healthy controls. The total yield of EVs purified from patients varied in their size and concentration in different individuals. 585 unique proteins were found with 334 proteins in PEG-precipitated samples and 515 in UC-purified EVs. Detailed analysis of three potential biomarkers – SERPINA3, LGALS3BP, and gelsolin – revealed that the expression of SERPINA3 and LGALS3BP was higher in melanoma patient EVs than in healthy controls, while gelsolin exhibited higher expression in healthy controls.

Discussion. While working on this study, the underlying assumption was that proteins are either inside or on top of EVs. However, there remains a third possibility that we did not pay attention to – that we were looking at proteins in the protein corona (PC). With the emerging advances in utilizing nano carriers for biomedical applications, including synthetic particles as well as EVs, a molecular-level understanding of the in vivo fate of nano carriers is becoming more important. After administration into human fluids, nano carriers can attract proteins onto their surfaces, forming an assembled adsorption layer called protein corona (PC). The formed PC can influence the physicochemical properties and subsequently determine nano carriers' biological behaviors (Xiao et al., 2022). Numerous investigations have revealed two distinct layers – an inner hard and

outer loose layer (Kari et al., 2020). It is suggested that the entity of the PC is changed over time because of alterations in the composition of the hard PC (Pinals et al., 2020). The composition and levels of proteins in the hard corona layer can reflect the identity of biomolecules under physiological and pathological conditions (García-Álvarez et al., 2018). For example, the inner hard PC can lead to the activation of the reticuloendothelial system cells and the elimination of nano particles faster than expected. This effect is simultaneously intensified by the accumulation of misfolded proteins and NP aggregation. Interestingly, the protein–protein interaction is mainly involved with the soft corona formation because the surface of the nanoparticles pre-occupied with the hard PC layer (Heidarzadeh et al., 2023). In-situ investigations have indicated the axis role of soft corona compositions on the stealth properties of liposomes (Kari et al., 2020).

In EVs, the protein corona might be involved in the surface interactions of EVs and may regulate EV organo- and cellular tropism, immune recognition, and cellular uptake (Buzás et al., 2018). For example, plasma protein-coated EVs have a higher density compared to the nascent ones and carry numerous associated proteins. Nine shared EV corona proteins have been identified, which appear to be common corona proteins among EVs, viruses and artificial nanoparticles in blood plasma. There is a high overlap of the composition of the protein corona with blood plasma protein aggregates (Tóth et al., 2021).

The protein corona itself can change based on physiological conditions it encounters. It has been found that the stability of PC, especially the soft corona layer, can be changed based on environmental properties like blood flow velocity from capillaries to arteries. The occurrence of pathological conditions in the vascular wall such as aneurysms can alter the entity of the soft corona layer (Heidarzadeh et al., 2023). On the other hand, the coupling of proteins into the protein corona might change EV transit time. Certain plasma protein subsets such as complement factors 3 and 4B can attach to the exosomal surface, resulting in robust phagocytosis of opsonized exosomes by immune cells. This phenomenon can lead to a reduction of exosomal transit time through the blood (Tóth et al., 2021). The opposite mechanism might also be involved in some immune disease development. In the case of systemic lupus erythematous, exosomes can harbor large contents of C3d-opsonized immune complexes while the levels of C3b and C3ib are diminished. It seems that this can reduce phagocytosis of exosomes by immune cells and increase transit times, leading to prolonged chronic inflammatory conditions (Winberg et al., 2017). The existence of CD47 on the exosome surface can also lead to the transmission of “do not eat me” signals toward immune cells (Kamerkar et al., 2017).

This protein corona itself might have different effects and trigger cellular uptake into cells. For example, in the case of therapy-grade human placental-expanded stromal cells EVs, protein corona removal cancelled EVs positive effects on enhancing angiogenesis (Wolf et al., 2022). In liver disease, there is a problem with the rapid clearance of exogenous EVs by phagocytic cells. Liam-

Or et al have suggested that bound albumin creates an EV signature that can re-target EVs from hepatic macrophages, which results in markedly improved cellular uptake by hepatocytes, liver sinusoidal endothelial cells and hepatic stellate cells (Liam-Or et al., 2024). It has also been shown that the presence of a protein corona increases the uptake of EVs in human monocytes (Dietz et al., 2023).

For diagnostics, the protein corona, also sometimes referred to as biomolecular corona may enrich specific biomolecules or outline-specific biomolecular patterns hidden in the biological fluid. This striking feature makes the PC extraordinarily appealing for diagnostics, opening the possibility to single out low abundant biomarkers, which would be otherwise missed, and to follow their evolution over time and space. In view of this, explorative studies have leveraged the scavenging features of biomolecular corona to search for novel diagnostic and prognostic biomarkers: thus, unique fingerprints could be related to specific diseases. For example, different hydrophobic and hydrophilic synthetic nanoparticles have been incubated with plasma of patients with different diseases or conditions revealing alteration of the PC, mainly the hard corona, composition in respect to healthy samples (Radeghieri & Bergese, 2023). For example, specific glycoforms of the anticoagulant protein antithrombin are specifically and differently enriched in the PC of EVs separated from the plasma of healthy subjects and patients affected by qualitative antihtrombin deficiency (Radeghieri et al., 2022). In rheumatic arthritis, when EVs were incubated with EV-depleted plasma of patients and relative controls, the capability to differentiate patients from healthy subjects was demonstrated (Tóth et al., 2021).

Leaving the protein corona to the side and focusing on the EVs themselves, this study has fallen in line with others that also show the diagnostic potential of EVs in skin cancer diagnostics. In standard non-melanoma skin cancer, EVs are integral to carcinogenesis, and are found to release mediators influencing tumor progression. For example, circular RNA CYP24A1 holds promise as a diagnostic biomarker for this disease (Seretis et al., 2024). There have also been multiple other studies with melanoma EVs as diagnostic biomarkers for melanoma. For example, plasma exosome-derived Cx43 levels could be a potential biomarker. These levels have been shown to be substantially downregulated as opposed to the levels in healthy controls. Overall survival and disease-free survival have also been shown to be poorer in patients with melanoma who exhibited lower levels of plasma exosome-derived Cx43 (Shen et al., 2023). Three plasma exosome miRNAs, namely hsa-miR-200c-3p, hsa-miR-144-3p and hsa-miR-221-3p, have also been shown to be differentially expressed in plasma-derived exosomes from melanoma patients and controls (De Martino et al., 2023). EVs have even been used in rarer melanoma subtype diagnostics. For example, small extracellular vesicles have been found in the vitreous humor, the fluid that fills eyeballs, in uveal melanoma patients. Two subpopulations were increased after internal radiation therapy, which may suggest radiation-induced release of these particles (Sirivolu et al., 2024).

Some works have also focused on DNA mutations and immune checkpoint inhibitor therapy response. In one study, mutations in six genes in human metastatic melanoma tissue EVs were detected, and at least one mutation was detected in all melanoma EV samples. The mutant allele frequency was higher in DNA isolated from tumor-derived EVs compared to total DNA extracted directly from plasma DNA, supporting the potential role of tumor EVs as future biomarkers in melanoma (Crescitelli et al., 2022). When isolating EVs from metastatic melanoma patients, it was showed that circulating PD1 carrying EVs are a driver of resistance to anti-PD1 treatments and this method could be a promising tool to stratify metastatic melanoma patients for immunotherapy (Serratì et al., 2022).

In conclusion, this study falls in line with others in showing that melanoma EVs have potential in the diagnostic field yet much more work remains before anything concrete can be used. These EVs themselves can be involved in the development of the disease or act as biomarkers, which are released due to disease development. However, what complicates the field of proteomic studies is the lack of understanding about what exactly we are looking at and from where – are the proteins in or on the EVs or part of the protein corona that it accumulates in the body? Many questions remain; however, the first steps have been made in using EVs as diagnostic markers in melanoma patients.

5.3 Study III – EVs composition and biochemical characteristics difference between post heart attack and healthy controls

In this study, the yield and composition of extracellular vesicles (EVs) derived from 40- to 60-year-old healthy male controls and post-myocardial infarction (post-MI) patients' blood samples was investigated, and their pro-inflammatory and oxidative-related properties were assessed. This study aimed to determine the EV yield and composition differences between both groups and to find out if there were differences between EV-mediated oxidative stress reactions.

5.3.1 Low-grade inflammation, an altered blood lipid profile and high level of oxidative stress in post-myocardial infarction patients

Post-myocardial infarction (MI) patients had a significantly elevated concentration of blood serum MDA ($p<0.001$), lower levels of low-density lipoprotein cholesterol ($p<0.001$), high-density lipoprotein cholesterol ($p=0.03$), and total cholesterol concentrations ($p<0.001$) compared to healthy controls. In this study, the post-MI group had a hsCRP range of 0.15–11.2 mg/L and control group 4.2–8.1 mg/L. Hence, the post-MI group patients were in an atherosclerosis range as CRP is also used as an inflammation marker where the positive values start at above 5 mg/L. MDA concentration in blood plasma is an effective oxidative

stress biomarker and it has been shown to correlate with tissue damage in both acute and chronic diseases (Papac-Milicevic et al., 2016).

5.3.2 Low EV yield in post-MI patients' blood samples

There was a statistically significant difference in extracellular vesicle concentration between the healthy control group and the post-MI group ($p<0.001$). However, the total protein level measured by the Bradford method was higher in post-MI patient samples ($p=0.001$), which might be related to the higher level of protein cargo carried in the post-MI blood EVs (figure 16). In addition, healthy groups' blood EVs showed a positive correlation ($R=0.55$) between Bradford measurements and NTA, while post-MI EVs did not ($R=–0.15$). Comparing CD9 median fluorescence intensity and the percentage of positive events revealed that healthy human blood samples had a higher number of CD9-positive exosomes than post-MI group individuals ($p<0.001$). There was quite a strong correlation in both groups between CD9 MFI and the percentage of CD9-positive events ($R=0.74$ and $R=0.6$, respectively). CD9 levels found in the isolated EV fraction confirmed the presence of exosomes in the EV samples. The presence of exosomes was also confirmed by TSG101 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in both healthy and post-MI EVs samples. GAPDH testing showed EVs from patients had more proteins when compared to controls.

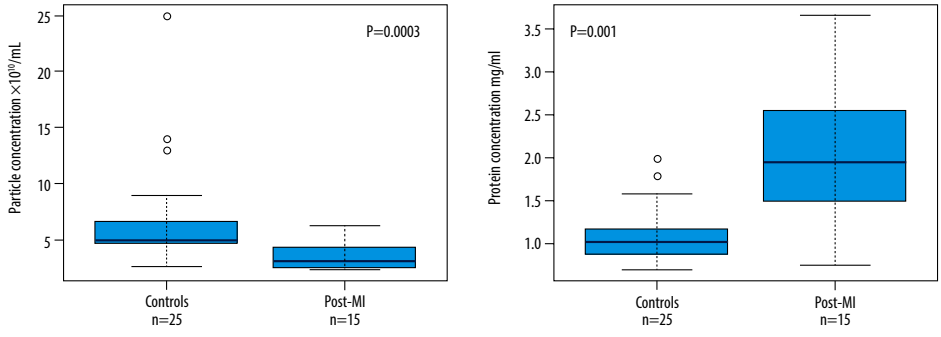

Figure 16. Comparison between healthy controls and post-MI (myocardial infarction) patient's particle concentration and total protein concentration

Looking at EV counts, our data showed that post-MI patients' blood samples had a significantly lower EV yield when compared to the healthy group. However, use of medications such as anti-atherogenic statins or the timing of blood collection (3 months post-MI) could have an impact on the post-MI EV yield in the current study. Patients with atherothrombotic diseases and atherosclerotic lesions have been shown to have high levels of circulating EVs derived from endothelial cells, vascular smooth cells, platelets, and erythrocytes (Rautou et al., 2011). The release of platelet-derived EVs has been shown to be increased in conditions such as MI (Puhm et al., 2021; Davidson et al., 2019). The level of circulating EVs starts to increase 1 h after myocardial infarction, with a significant increase

occurring in 24 h after MI or ischemia/reperfusion (Deddens et al., 2016; Ge et al., 2019).

Hydroxymethyl glutaryl coenzyme A reductase (HMG-CoA) inhibitors, statins, therapy has significant benefits for both primary and secondary prevention of cardiovascular disease (Ramkumar et al., 2016). The decreased number of circulating EVs derived from the endothelium, platelets, and inflammatory cells after lipid-lowering therapies with statins has also been observed (Suades et al., 2013). Simvastatin has been shown to reduce the secretion of EVs from various cell types (Kulshreshtha et al., 2019). While atorvastatin, along with other hypertensive treatments, increases the number of endothelium-derived EVs (Mobarrez et al., 2012; Zu et al., 2016). These controversial data reveal that the effects of statins on EV release are not fully clear.

5.3.3 Oxidative stress reactions between post-MI and control groups

To determine anti-oxidative EV properties, thioredoxin level was identified by western blotting. Thioredoxin acts as electron donors to peroxidases and ribonucleotide reductase (Arnér & Holmgren, 2000). Based on mice experiments, endogenous thioredoxin is an essential component of the cellular antioxidant mechanisms and plays a critical role in regulating oxidative stress in the heart (Yamamoto et al., 2003). In our study, it was detected in almost all samples, but at a noticeably higher level in post-MI EV samples than in healthy individuals. Similar results were obtained with the cell survival-regulating enzymes: extracellular signal kinases 1/2 (ERK 1/2) and protein kinase B (Akt) were detected in all EV samples, but were found at higher levels in post-MI EVs samples. The investigation of pro-oxidative NADPH oxidases (NOX1, NOX2, and NOX5 isoforms) in EV samples showed a strong upregulation of all 3 NOX isoforms in post-MI EVs, but only NOX1 and NOX5 isoforms were obtained in the healthy EVs. After this, oxidative stress testing of isolated EVs was done by adding them to the endothelial cell culture (HUVEC) and measuring the production of ROS through H2DCFDA fluorescence intensity (figure 17). Data showed that post-MI patient exosomes gave a lower oxidative stress response than the EVs of the healthy group ($p<0.001$).

Data from our study shows that exosomes isolated from post-MI patients' blood were of inflammatory origins as shown by their surface marker thioredoxin, and carry both pro- and anti-oxidative enzymes, with a higher predominance of antioxidant effects. This finding was confirmed in the experiments with HUVEC cell culture, since exosomes isolated from the post-MI patients' blood induced lower oxidative stress when compared to the healthy group. However, in the control group the EVs had fewer inflammation markers, yet at the same time caused a higher oxidative stress reaction.

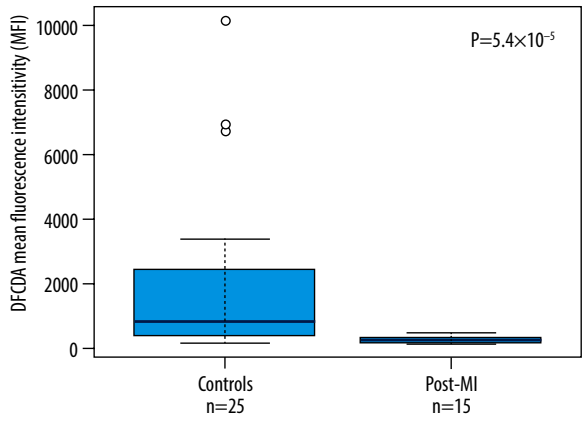

Figure 17. Comparison between healthy controls and post-MI (myocardial infarction) patients oxidative stress response through measuring ROS production using H2DCFDA fluorescence intensity

Recent studies have indicated the positive effect of cell-released exosomes on injured tissues (Wendt et al., 2018). It has been shown that treatment using exosomes can reduce systemic inflammation in mice after myocardial ischemia/reperfusion (Arslan et al., 2013). Those exosomes were found to induce phospho-inositol-3-kinase/protein kinase B (PI3K/Akt B) signaling, which is known to reduce oxidative stress and help protect cells and tissues (Tan et al., 2022). Results from our study show that exosomes from post-MI patients carry Akt and ERK1/2 at higher levels compared to EVs from healthy controls. Post-MI patients also carried higher levels of thioredoxin. All of this shows that post-MI EVs had antioxidant effects. This also matches previous findings as it has been shown that exosome-like structures could transfer anti-oxidant thioredoxin, which is up-regulated as a response to increased oxidative stress (Madrigal-Matute et al., 2015).

An association between pro-oxidant NOX2 and anti-oxidant thioredoxin levels have also been shown: in macrophages, the downregulation of NOX2 also decreased thioredoxin levels (Madrigal-Matute et al., 2015). The link between NOX2 and thioredoxin was observed in our study as well; NOX2 was found only in post-MI patients' EVs, which also had a higher thioredoxin level according to western blot data. NAPDH oxidases, except for NOX4, are usually described as enzymes responsible for increased atherogenesis (Poznyak et al., 2020). NOX2 isoform is undoubtedly an important enzyme in oxidative stress, yet its function in atherosclerosis remains enigmatic. NOX2 usually has a negative effect on the endothelium (Fan et al., 2014). However, NOX2 deficiency has been shown to cause the loss of survival signaling through ERK and Akt in neonatal rat myocytes (Rosc-Schlüter et al., 2012).

Discussion. Data from this study suggest that exosomes in post-MI patients' blood might have anti-oxidative properties that could serve as an additional factor in controlling the post-MI condition. This might indicate that EVs from a lower inflammation origin mediate oxidative stress reactions before atherosclerotic

events occur, and after their occurrence, they are produced from a higher inflammation origin but are less involved in mediating oxidative stress reactions. This suggest that exosomes in post-MI patients' blood might have anti-oxidative properties that could serve as an additional factor in controlling the post-MI condition.

However, extracellular vesicles can also have effects on diseases that are driven by atherosclerosis or are involved in the development of atherosclerosis itself. For example, extracellular vesicles could also be used as biomarkers for cerebral ischemic diseases. Cerebral ischemia is a mechanism of acute brain injury that results from impaired blood flow to the brain and is often caused by secondary events on top of atherosclerosis in brain vessels. The EV-surface antigen profile appears to be different in patients with transient symptoms compared with patients whose symptoms were less likely to be due to brain ischemia (Burrello et al., 2021). A link between non-alcoholic fatty liver disease and atherosclerosis through EVs has also been shown. Steatotic hepatocyte-derived small extracellular vesicles promote foam cell formation and facilitate atherogenesis. Reducing EVs secretion by steatotic hepatocytes might be a potential therapeutic approach to slow the progression of non-alcoholic fatty liver disease-driven atherosclerosis (Chen et al., 2023).

EVs released due to lifestyle choices might also influence atherosclerosis development both in a negative or positive way. According to functional studies, EVs from the serum of smokers have been shown to be significantly increased and to take part in endothelial dysfunction. Moreover, CD14 levels, which represent EVs from monocytes, have been shown to be significantly increased on EVs from smokers. This indicates that monocytes might be an important source of smoker-EVs (Wang et al., 2023a). Sleep deprivation might also be involved in atherosclerosis development. Isolated plasma exosomes from sleep-deprived humans have been shown to be potent inducers of endothelial inflammation and atherogenesis (Li et al., 2023). In mice, obesity-induced exosomal miR-27b-3p has also been shown to promote endothelial inflammation and facilitate atherogenesis (Tang et al., 2023). On the other hand, mice experiments have been shown that exercise remodels metabolism towards beneficial cardiovascular outcomes and does this at least partially via skeletal muscle secreted EVs (Wang et al., 2023b).

In addition, extracellular vesicles from viruses and bacteria can be involved in atherosclerosis development. As a more extreme example, people living with HIV are at a higher risk of having cerebrocardiovascular diseases compared to HIV-negative individuals. It has been hypothesized that HIV infection results in modified miRNA content in plasma extracellular vesicles, which modulate the functionality of vascular repairing cells and vascular wall cells (Da Fonseca Ferreira et al., 2023). Some studies have suggested that bacterial infections may contribute to the development of both atherogenesis and inflammation in atherosclerotic lesions. In particular, the participation of bacterial extracellular membrane nanovesicles have been shown to take part in atherosclerosis pathogenesis (Lusta et al., 2024).

In conclusion, not only atherosclerotic extracellular vesicles themselves have pathogenic effects as shown in this study, but also the overall human condition of comorbidities, lifestyle choices and even the surrounding environment might have a role in atherosclerosis development and its end negative endpoints such as myocardial infarction.

6. CONCLUSIONS

Based on the results of the studies reviewed in this dissertation, the following conclusions can be made:

- Autoimmune response against MAGE-A10 and MAGE-A4 could be used in liquid biopsy tests to detect stage I to III melanoma
- Extracellular vesicles from melanoma patients can be purified and their proteomic profile, for example SERPINA3, could be used in melanoma diagnostics
- Post myocardial infarction patients extracellular vesicles have different properties than healthy control extracellular vesicles and could be directly contributing to disease development.

These results show that autoantibodies and extracellular vesicles could be used in liquid biopsy tests as diagnostic tools for melanoma. In addition, extracellular vesicles themselves can also have biologically active functions in disease development as shown in post myocardial infarction patients.

SUMMARY IN ESTONIAN

Vedelbiopsia potentsiaali uurimine melanoomi diagnostikas ja ekstratsellulaarsete vesiikulite roll ateroskleroosi arengus

„Vedelbiopsia“ on lai termin, mida kasutatakse kehavedelike testimise kirjeldamiseks. Vedelbiopsia meetodeid saab kasutada paljude biomolekulaarsete tunnuste tuvastamiseks ning saadud info võib anda märku haiguste esinemise kohta. Vedelbiopsiat võib kasutada ekstratsellulaarsete vesiikulite, autoantikehade ja valkude tuvastamiseks

Melanoom on pahaloomulistest melanotsüütidest arenenv nahavähk. Kuigi hetkel paljude kasvajatüüpide esinemissagedus väheneb, siis melanoomi esinemissagedus kasvab. Melanoomi diagnoositakse kliinilise hindamise teel – pahaloomulise melanoomi tunnuste hulka kuuluvad asümmeetria, hüpertsellulaarsus ja halb piiritlus. Antud kasvaja usaldusväärne tuvastamine on väljakutse pinnamarkerite ekspressiooni mitmekesisuse tõttu. Seetõttu otsitakse uudseid tuvastamismeetodeid, milleks võiksid olla vedelbiopsia materjalis leiduvad markerid nagu autoantikehad ja ekstratsellulaarsed vesiikulid.

Autoantikehi leitakse kehas vastusena üleekspresseeritud, muteerunud, valesti pakitud või lagunenud kasvaja valkudele, mis võivad olla esimesed vihjed kasvaja arengust ning avalduda märkimisväärselt, mitmeid kuid või aastaid, varem kui ilmnevad kasvaja kliinilised sümptomid. Tsirkuleerivate autoantikehade seerumiprofiilide koostamist peetakse atraktiivseks meetodiks varases staadiumis vähi diagnoosimiseks. Melanoomi antigeeni geeni, MAGE, valkude perekond omab ühist MAGE homoloogi domeeni. Antud valkude superperekonda kuulub üle 60 valgu, millest >40 klassifitseeritakse kasvaja-testis antigeenideks. Kasvaja-testis antigeenid (CTA-d), on rühm valke, mille ekspressioon on seotud eelkõige munandite, munasarjade ja platsentaga. Inimese arengu käigus nende esinemine kaob, kuid kasvajate korral võivad CTA-d taastekkida ja põhjustada autoantikehade moodustumist.

Ekstratsellulaarsed ehk rakuvälised vesiikulid (EV-d) on rakust pärinevad nanosuurustes vesiikulid, mida on võimalik kasutada vedelikbiopsias. EV-d pärivad vanemrakkudest bioaktiivsed komponendid, mida on võimelised kandma retsipientrakkudesse. Paljud EV alatüübid kattuvad suuruse, pinnavalkude esinemise ja nende sees olevate valkude poolest, mistõttu EV-d on oma olemuselt mitmekesised. Mõnede rakutüübispetsiifiliste EV-de isoleerimine tavapäraste tehnoloogiate abil on keeruline nii kindla rakutüübi madala arvukuse, kui ka madala sekretsioonimäära tõttu.

Ateroskleroos on koronaar-, unearteri- ja perifeersete arterite haiguse kõige sagedasem põhjus. See on keskmiste ja suurte arterite multifokaalne immuunpõletikuline haigus, mille arengus on peamised osalejad endoteelirakud, leukotsüüdid ja sisekesta silelihasrakud. Vaatamata tõhusatele sekkumistele madala tihendusega lipoproteiini (LDL-i), vererõhu ja muude traditsiooniliste riski-

tegurite kontrolli all hoidmiseks, on siiski ateroskleroотiliste südame-veresoonkonna haiguste tekkimise risk märkimisväärne. Tsirkuleerivad mikrovesiikulid (MV) on väikesed vesiikulid, mis aitavad kaasa aterotrombootilisele protsessile. MV-d saab kasutada kardiovaskulaarsete haiguste olemasolu ja arenemise biomarkeritena.

Antud doktoritöö eesmärgiks oli kaardistada MAGE-A4 ja MAGE-A10 vastaste autoantikehade ning melanoomiga patsientide seerumist puhastatud rakuväliste vesiikulite potentsiaali vedelbiopsia markeritena melanoomidiagnostikas. Lisaks sellele oli eesmärgiks leida, kas müokardiinfarkti põdenud patsientide seerumist eraldatud rakuvälistel vesiikulitel on erinevad omadused kui kontrollrühma patsientide omadel.

Töö raames leiti, et MAGE-A4 ja MAGE-A10 vastast autoimmuunvastust saab kasutada vedelbiopsia testides, et tuvastada I kuni III staadiumi melanoomi. Kuigi patsientide keskmine immuunvastus ei olnud verepanga kontrollidega võrreldes suurem, oli siiski 15 patsiendil 185-st (8,2%) tugev autoimmuunvastus MAGE-A4 ja/või MAGE-A10 valgu vastu. Kõik autoimmuunvastused ilmnesid melanoomi I, II ja III staadiumis ning kõige enam tuvastati tugevaid vastuseid II staadiumi melanoomiga patsientidel seas. MAGE-A-vastaseid antikehi saab käsitleda potentsiaalsete diagnostiliste biomarkeritena.

Samuti avastati töö käigus, et melanoomiga patsientide rakuväliseid vesiikuleid saab puhastada ja nende valgulist profiili kasutada melanoomi diagnostikas. EV-sid saab puhastada nii ultratsentrifuugimise (UC) kui ka polüetüleenglükooli sadestamise (PEG) meetodi abil. Samuti leiti, et erinevates melanoomi staadiumides on EV kogus ja vesiikulite suurus erinev. Proteoomika meetodit kasutades leiti EV-des üle 585 unikaalse valgu, millest varasemalt on näidatud, et 159 neist on melanoomiga seotud ja kolme neist – SERPINA3, gelsoliin ja LGALS3BP – võib EV-des leidmisel kasutada diagnostiliste markeritena.

Lisaks tuvastati, et müokardiinfarkti (MI) põdenud patsientide ekstratsellulaarsetel vesiikulitel on erinevad omadused võrreldes tervete inimeste kontrollrühma rakuväliste vesiikulite omadustega. MI-järgselt patsientide vereproovidest saadud EV saagikus oli oluliselt väiksem võrreldes kontrollgrupi patsientidega. Leiti, et MI-järgse grupi EV-d olid põletikulise päritoluga, kuid samal ajal vahendasid nad vähem oksüdatiivse stressi teket. Samas võrdlevalt oli kontrollrühma EVd vähemal määral põletikulise päritoluga põhjustades rohkem oksüdatiivse stressi reaktsioone.

Antud doktoritöö tulemused näitavad, et autoantikehi ja ekstratsellulaarseid vesiikuleid saab kasutada vedelbiopsia testides melanoomi diagnoosimiseks. Lisaks võivad ekstratsellulaarsed vesiikulid ise omada bioloogiliselt aktiivseid funktsioone erinevate haiguste, antud juhul ateroskleroosi, arengus.

REFERENCES

Abache, T., Le Naour, F., Planchon, S., Harper, F., Boucheix, C., & Rubinstein, E. (2007). The transferrin receptor and the tetraspanin web molecules CD9, CD81, and CD9P-1 are differentially sorted into exosomes after TPA treatment of K562 cells. *Journal of Cellular Biochemistry*, *102*(3), 650–664. https://doi.org/10.1002/jcb.21318

Abusamra, A. J., Zhong, Z., Zheng, X., Li, M., Ichim, T. E., Chin, J. L., & Min, W.-P. (2005). Tumor exosomes expressing Fas ligand mediate CD8+ T-cell apoptosis. *Blood Cells, Molecules, and Diseases*, *35*(2), 169–173. https://doi.org/10.1016/j.bcmd.2005.07.001

Adashek, J. J., Janku, F., & Kurzrock, R. (2021). Signed in Blood: Circulating Tumor DNA in Cancer Diagnosis, Treatment and Screening. *Cancers*, *13*(14), 14. https://doi.org/10.3390/cancers13143600

Adell, M. A. Y., Vogel, G. F., Pakdel, M., Müller, M., Lindner, H., Hess, M. W., & Teis, D. (2014). Coordinated binding of Vps4 to ESCRT-III drives membrane neck constriction during MVB vesicle formation. *Journal of Cell Biology*, *205*(1), 33–49. https://doi.org/10.1083/jcb.201310114

Adhit, K. K., Wanjari, A., Menon, S., & K, S. (2023). Liquid Biopsy: An Evolving Paradigm for Non-invasive Disease Diagnosis and Monitoring in Medicine. *Cureus*, *15*(12), e50176. https://doi.org/10.7759/cureus.50176

Ahmed, B., Qadir, M. I., & Ghafoor, S. (2020). Malignant Melanoma: Skin Cancer & minus; Diagnosis, Prevention, and Treatment. *Critical Reviews™ in Eukaryotic Gene Expression*, *30*(4). https://doi.org/10.1615/CritRevEukaryotGeneExpr.2020028454

Ailawadi, S., Wang, X., Gu, H., & Fan, G.-C. (2015). Pathologic function and therapeutic potential of exosomes in cardiovascular disease. *Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease*, *1852*(1), 1–11. https://doi.org/10.1016/j.bbadis.2014.10.008

Alegre, E., Sanmamed, M. F., Rodriguez, C., Carranza, O., Martín-Algarra, S., & González, Á. (2014). Study of Circulating MicroRNA-125b Levels in Serum Exosomes in Advanced Melanoma. *Archives of Pathology & Laboratory Medicine*, *138*(6), 828–832. https://doi.org/10.5858/arpa.2013-0134-OA

Alegre, E., Zubiri, L., Perez-Gracia, J. L., González-Cao, M., Soria, L., Martín-Algarra, S., & González, A. (2016). Circulating melanoma exosomes as diagnostic and prognosis biomarkers. *Clinica Chimica Acta*, *454*, 28–32. https://doi.org/10.1016/j.cca.2015.12.031

Alexandrov, L. B., Nik-Zainal, S., Wedge, D. C., Aparicio, S. A. J. R., Behjati, S., Biankin, A. V., Bignell, G. R., Bolli, N., Borg, A., Børresen-Dale, A.-L., Boyault, S., Burkhardt, B., Butler, A. P., Caldas, C., Davies, H. R., Desmedt, C., Eils, R., Eyfjörd, J. E., Foekens, J. A., … Stratton, M. R. (2013). Signatures of mutational processes in human cancer. *Nature*, *500*(7463), 415–421. https://doi.org/10.1038/nature12477

Alix-Panabières, C., Schwarzenbach, H., & Pantel, K. (2012). Circulating tumor cells and circulating tumor DNA. *Annual Review of Medicine*, *63*, 199–215. https://doi.org/10.1146/annurev-med-062310-094219

Al-Nedawi, K., Meehan, B., Kerbel, R. S., Allison, A. C., & Rak, J. (2009). Endothelial expression of autocrine VEGF upon the uptake of tumor-derived microvesicles containing oncogenic EGFR. *Proceedings of the National Academy of Sciences*, *106*(10), 3794–3799. https://doi.org/10.1073/pnas.0804543106

Al-Nedawi, K., Meehan, B., Micallef, J., Lhotak, V., May, L., Guha, A., & Rak, J. (2008). Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. *Nature Cell Biology*, *10*(5), 619–624. https://doi.org/10.1038/ncb1725

Alriyami, M., & Polychronakos, C. (2021). Somatic Mutations and Autoimmunity. *Cells*, *10*(8), 8. https://doi.org/10.3390/cells10082056

Amin, M. B., Greene, F. L., Edge, S. B., Compton, C. C., Gershenwald, J. E., Brookland, R. K., Meyer, L., Gress, D. M., Byrd, D. R., & Winchester, D. P. (2017). The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA: A Cancer Journal for Clinicians*, *67*(2), 93–99. https://doi.org/10.3322/caac.21388

Anderson, D. R., Duryee, M. J., Shurmur, S. W., Um, J. Y., Bussey, W. D., Hunter, C. D., Garvin, R. P., Sayles, H. R., Mikuls, T. R., Klassen, L. W., & Thiele, G. M. (2014). Unique Antibody Responses to Malondialdehyde-Acetaldehyde (MAA)-Protein Adducts Predict Coronary Artery Disease. *PLOS ONE*, *9*(9), e107440. https://doi.org/10.1371/journal.pone.0107440

Antonyak, M. A., Li, B., Boroughs, L. K., Johnson, J. L., Druso, J. E., Bryant, K. L., Holowka, D. A., & Cerione, R. A. (2011). Cancer cell-derived microvesicles induce transformation by transferring tissue transglutaminase and fibronectin to recipient cells. *Proceedings of the National Academy of Sciences*, *108*(12), 4852–4857. https://doi.org/10.1073/pnas.1017667108

Arbelaiz, A., Azkargorta, M., Krawczyk, M., Santos-Laso, A., Lapitz, A., Perugorria, M. J., Erice, O., Gonzalez, E., Jimenez-Agüero, R., Lacasta, A., Ibarra, C., Sanchez-Campos, A., Jimeno, J. P., Lammert, F., Milkiewicz, P., Marzioni, M., Macias, R. I. R., Marin, J. J. G., Patel, T., … Banales, J. M. (2017). Serum extracellular vesicles contain protein biomarkers for primary sclerosing cholangitis and cholangiocarcinoma. *Hepatology*, *66*(4), 1125. https://doi.org/10.1002/hep.29291

Arnér, E. S. J., & Holmgren, A. (2000). Physiological functions of thioredoxin and thioredoxin reductase. *European Journal of Biochemistry*, *267*(20), 6102–6109. https://doi.org/10.1046/j.1432-1327.2000.01701.x

Arneth, B. (2018). Update on the types and usage of liquid biopsies in the clinical setting: A systematic review. *BMC Cancer*, *18*(1), 527. https://doi.org/10.1186/s12885-018-4433-3

Arslan, F., Lai, R. C., Smeets, M. B., Akeroyd, L., Choo, A., Aguor, E. N. E., Timmers, L., van Rijen, H. V., Doevendans, P. A., Pasterkamp, G., Lim, S. K., & de Kleijn, D. P. (2013). Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. *Stem Cell Research*, *10*(3), 301–312. https://doi.org/10.1016/j.scr.2013.01.002

Ascierto, P. A., Minor, D., Ribas, A., Lebbe, C., O'Hagan, A., Arya, N., Guckert, M., Schadendorf, D., Kefford, R. F., Grob, J.-J., Hamid, O., Amaravadi, R., Simeone, E., Wilhelm, T., Kim, K. B., Long, G. V., Martin, A.-M., Mazumdar, J., Goodman, V. L., & Trefzer, U. (2013). Phase II Trial (BREAK-2) of the BRAF Inhibitor Dabrafenib (GSK2118436) in Patients With Metastatic Melanoma. *Journal of Clinical Oncology*, *31*(26), 3205–3211. https://doi.org/10.1200/JCO.2013.49.8691

Auber, M., & Svenningsen, P. (2022). An estimate of extracellular vesicle secretion rates of human blood cells. *Journal of Extracellular Biology*, *1*(6), e46. https://doi.org/10.1002/jex2.46

Badimon, L., Suades, R., Crespo, J., Padro, T., & Chiva-Blanch, G. (2018). Diet, microparticles and atherothrombosis. *Frontiers in Bioscience-Landmark*, *23*(3), 3. https://doi.org/10.2741/4598

Badimon, L., Suades, R., Vilella-Figuerola, A., Crespo, J., Vilahur, G., Escate, R., Padro, T., & Chiva-Blanch, G. (2020). Liquid Biopsies: Microvesicles in Cardiovascular Disease. *Antioxidants & Redox Signaling*, *33*(9), 645–662. https://doi.org/10.1089/ars.2019.7922

Baietti, M. F., Zhang, Z., Mortier, E., Melchior, A., Degeest, G., Geeraerts, A., Ivarsson, Y., Depoortere, F., Coomans, C., Vermeiren, E., Zimmermann, P., & David, G. (2012). Syndecan–syntenin–ALIX regulates the biogenesis of exosomes. *Nature Cell Biology*, *14*(7), 677–685. https://doi.org/10.1038/ncb2502

Balaj, L., Lessard, R., Dai, L., Cho, Y.-J., Pomeroy, S. L., Breakefield, X. O., & Skog, J. (2011). Tumour microvesicles contain retrotransposon elements and amplified oncogene sequences. *Nature Communications*, *2*(1), 180. https://doi.org/10.1038/ncomms1180

Bandić, D., Juretić, A., Šarčević, B., Šeparović, V., Kujundžić Tiljak, M., Hudolin, T., Spagnoli, G. C., Čović, D., & Šamija, M. (2006). Expression and Possible Prognostic Role of MAGE-A4, NY-ESO-1, and HER-2 Antigens in Women with Relapsing Invasive Ductal Beast Cancer: Retrospective Immunohistochemical Study. *Croatian Medical Journal*, *47*(1), 32–41.

Barker, P. A., & Salehi, A. (2002). The MAGE proteins: Emerging roles in cell cycle progression, apoptosis, and neurogenetic disease. *Journal of Neuroscience Research*, *67*(6), 705–712. https://doi.org/10.1002/jnr.10160

Barrow, C., Browning, J., MacGregor, D., Davis, I. D., Sturrock, S., Jungbluth, A. A., & Cebon, J. (2006). Tumor Antigen Expression in Melanoma Varies According to Antigen and Stage. *Clinical Cancer Research*, *12*(3), 764–771. https://doi.org/10.1158/1078-0432.CCR-05-1544

Bartel, D. P. (2004). MicroRNAs: Genomics, Biogenesis, Mechanism, and Function. *Cell*, *116*(2), 281–297. https://doi.org/10.1016/S0092-8674(04)00045-5

Barteneva, N. S., Fasler-Kan, E., Bernimoulin, M., Stern, J. N., Ponomarev, E. D., Duckett, L., & Vorobjev, I. A. (2013). Circulating microparticles: Square the circle. *BMC Cell Biology*, *14*(1), 23. https://doi.org/10.1186/1471-2121-14-23

Bartol, T. (2015). Thoughtful use of diagnostic testing: Making practical sense of sensitivity, specificity, and predictive value. *The Nurse Practitioner*, *40*(8), 10. https://doi.org/10.1097/01.NPR.0000470366.64804.35

Bates, J. P., Derakhshandeh, R., Jones, L., & Webb, T. J. (2018). Mechanisms of immune evasion in breast cancer. *BMC Cancer*, *18*(1), 556. https://doi.org/10.1186/s12885-018-4441-3

Benjamin, E. J., Muntner, P., Alonso, A., Bittencourt, M. S., Callaway, C. W., Carson, A. P., Chamberlain, A. M., Chang, A. R., Cheng, S., Das, S. R., Delling, F. N., Djousse, L., Elkind, M. S. V., Ferguson, J. F., Fornage, M., Jordan, L. C., Khan, S. S., Kissela, B. M., Knutson, K. L., … null, null. (2019). Heart Disease and Stroke Statistics – 2019 Update: A Report From the American Heart Association. *Circulation*, *139*(10), e56–e528. https://doi.org/10.1161/CIR.0000000000000659

Bennaceur, K., Popa, I., Portoukalian, J., Berthier-Vergnes, O., & Péguet-Navarro, J. (2006). Melanoma-derived gangliosides impair migratory and antigen-presenting function of human epidermal Langerhans cells and induce their apoptosis. *International Immunology*, *18*(6), 879–886. https://doi.org/10.1093/intimm/dxl024

Bergeron, A., Picard, V., LaRue, H., Harel, F., Hovington, H., Lacombe, L., & Fradet, Y. (2009). High frequency of MAGE-A4 and MAGE-A9 expression in high-risk bladder cancer. *International Journal of Cancer*, *125*(6), 1365–1371. https://doi.org/10.1002/ijc.24503

Berk, B. C., Weintraub, W. S., & Alexander, R. W. (1990). Elevation of C-reactive protein in "active" coronary artery disease. *The American Journal of Cardiology*, *65*(3), 168–172. https://doi.org/10.1016/0002-9149(90)90079-G

Bevona, C., Goggins, W., Quinn, T., Fullerton, J., & Tsao, H. (2003). Cutaneous Melanomas Associated With Nevi. *Archives of Dermatology*, *139*(12), 1620–1624. https://doi.org/10.1001/archderm.139.12.1620

Bilici, A., Yapici, H. S., Ercan, S., Seker, M., Ustaalioglu, B. B. O., Salman, T., Orcun, A., & Gumus, M. (2012). The prevalence and significance of autoantibodies in patients with non-Hodgkin's lymphoma: Are they correlated with clinicopathological features? *Journal of B.U.ON.: Official Journal of the Balkan Union of Oncology*, *17*(3), 502–507.

Biller, S. J., Schubotz, F., Roggensack, S. E., Thompson, A. W., Summons, R. E., & Chisholm, S. W. (2014). Bacterial vesicles in marine ecosystems. *Science (New York, N.Y.)*, *343*(6167), 183–186. https://doi.org/10.1126/science.1243457

Blessing, K., McLAREN, K. m., McLEAN, A., & Davidson, P. (1990). Thin malignant melanomas (< 1.5 mm) with metastasis: A histological study and survival analysis. *Histopathology*, *17*(5), 389–395. https://doi.org/10.1111/j.1365-2559.1990.tb00757.x

Bobos, M. (2021). Histopathologic classification and prognostic factors of melanoma: A 2021 update. *Italian Journal of Dermatology and Venereology*, *156*(3), 300–321. https://doi.org/10.23736/S2784-8671.21.06958-3

Bobrie, A., Colombo, M., Raposo, G., & Théry, C. (2011). Exosome Secretion: Molecular Mechanisms and Roles in Immune Responses. *Traffic*, *12*(12), 1659–1668. https://doi.org/10.1111/j.1600-0854.2011.01225.x

Bobrie, A., Krumeich, S., Reyal, F., Recchi, C., Moita, L. F., Seabra, M. C., Ostrowski, M., & Théry, C. (2012). Rab27a supports exosome-dependent and –independent mechanisms that modify the tumor microenvironment and can promote tumor progression. *Cancer Research*, *72*(19), 4920–4930. https://doi.org/10.1158/0008-5472.CAN-12-0925

Bolin, E., & Lam, W. (2013). A Review of Sensitivity, Specificity, and Likelihood Ratios: Evaluating the Utility of the Electrocardiogram as a Screening Tool in Hypertrophic Cardiomyopathy. *Congenital Heart Disease*, *8*(5), 406–410. https://doi.org/10.1111/chd.12083

Boussadia, Z., Lamberti, J., Mattei, F., Pizzi, E., Puglisi, R., Zanetti, C., Pasquini, L., Fratini, F., Fantozzi, L., Felicetti, F., Fecchi, K., Raggi, C., Sanchez, M., D'Atri, S., Carè, A., Sargiacomo, M., & Parolini, I. (2018). Acidic microenvironment plays a key role in human melanoma progression through a sustained exosome mediated transfer of clinically relevant metastatic molecules. *Journal of Experimental & Clinical Cancer Research*, *37*(1), 245. https://doi.org/10.1186/s13046-018-0915-z

Boyano, M. D., García-Vázquez, M. D., Gardeazabal, J., de Galdeano, A. G., Smith-Zubiaga, I., Cañavate, M. L., Raton, J. A., Bilbao, I., & Díaz-Pérez, J. L. (2009). Serum-Soluble IL-2 Receptor and IL-6 Levels in Patients with Melanoma. *Oncology*, *54*(5), 400–406. https://doi.org/10.1159/000227726

Brasseur, F., Rimoldi, D., Liénard, D., Lethé, B., Carrel, S., Arienti, F., Suter, L., Vanwijck, R., Bourlond, A., Humblet, Y., Vacca, A., Conese, M., Lahaye, T., Degiovanni, G., Deraemaecker, R., Beauduin, M., Sastre, X., Salamon, E., Dréno, B., …

Marchand, M. (1995). Expression of MAGE genes in primary and metastatic cutaneous melanoma. *International Journal of Cancer*, *63*(3), 375–380. https://doi.org/10.1002/ijc.2910630313

Brennan, K., Martin, K., FitzGerald, S. P., O'Sullivan, J., Wu, Y., Blanco, A., Richardson, C., & Mc Gee, M. M. (2020). A comparison of methods for the isolation and separation of extracellular vesicles from protein and lipid particles in human serum. *Scientific Reports*, *10*(1), 1039. https://doi.org/10.1038/s41598-020-57497-7

Bricard, G., Bouzourene, H., Martinet, O., Rimoldi, D., Halkic, N., Gillet, M., Chaubert, P., MacDonald, H. R., Romero, P., Cerottini, J.-C., & Speiser, D. E. (2005). Naturally Acquired MAGE-A10- and SSX-2-Specific CD8+ T Cell Responses in Patients with Hepatocellular Carcinoma1. *The Journal of Immunology*, *174*(3), 1709–1716. https://doi.org/10.4049/jimmunol.174.3.1709

Brisam, M., Rauthe, S., Hartmann, S., Linz, C., Brands, R. C., Kübler, A. C., Rosenwald, A., & Müller-Richter, U. D. (2016). Expression of MAGE-A1-A12 subgroups in the invasive tumor front and tumor center in oral squamous cell carcinoma. *Oncology Reports*, *35*(4), 1979–1986. https://doi.org/10.3892/or.2016.4600

Brochez, L., & Naeyaert, J. (2000). Serological markers for melanoma. *British Journal of Dermatology*, *143*(2), 256–268. https://doi.org/10.1046/j.1365-2133.2000.03649.x

Burrello, J., Bianco, G., Burrello, A., Manno, C., Maulucci, F., Pileggi, M., Nannoni, S., Michel, P., Bolis, S., Melli, G., Vassalli, G., Albers, G. W., Cianfoni, A., Barile, L., & Cereda, C. W. (2021). Extracellular Vesicle Surface Markers as a Diagnostic Tool in Transient Ischemic Attacks. *Stroke*, *52*(10), 3335–3347. https://doi.org/10.1161/STROKEAHA.120.033170

Burtenshaw, D., Kitching, M., Redmond, E. M., Megson, I. L., & Cahill, P. A. (2019). Reactive Oxygen Species (ROS), Intimal Thickening, and Subclinical Atherosclerotic Disease. *Frontiers in Cardiovascular Medicine*, *6*. https://doi.org/10.3389/fcvm.2019.00089

Busam, K. J., Iversen, K., Berwick, M., Spagnoli, G. C., Old, L. J., & Jungbluth, A. A. (2000). Immunoreactivity with the Anti-MAGE Antibody 57B in Malignant Melanoma: Frequency of Expression and Correlation with Prognostic Parameters. *Modern Pathology*, *13*(4), 459–465. https://doi.org/10.1038/modpathol.3880078

Buschow, S. I., Nolte-'t Hoen, E. N. M., Van Niel, G., Pols, M. S., Ten Broeke, T., Lauwen, M., Ossendorp, F., Melief, C. J. M., Raposo, G., Wubbolts, R., Wauben, M. H. M., & Stoorvogel, W. (2009). MHC II in Dendritic Cells is Targeted to Lysosomes or T Cell-Induced Exosomes Via Distinct Multivesicular Body Pathways. *Traffic*, *10*(10), 1528–1542. https://doi.org/10.1111/j.1600-0854.2009.00963.x

Buzás, E. I., Tóth, E. Á., Sódar, B. W., & Szabó-Taylor, K. É. (2018). Molecular interactions at the surface of extracellular vesicles. *Seminars in Immunopathology*, *40*(5), 453–464. https://doi.org/10.1007/s00281-018-0682-0

Cai, H., & Harrison, D. G. (2000). Endothelial dysfunction in cardiovascular diseases: The role of oxidant stress. *Circulation Research*, *87*(10), 840–844. https://doi.org/10.1161/01.res.87.10.840

Cai, J., Guan, W., Tan, X., Chen, C., Li, L., Wang, N., Zou, X., Zhou, F., Wang, J., Pei, F., Chen, X., Luo, H., Wang, X., He, D., Zhou, L., Jose, P. A., & Zeng, C. (2015). SRY gene transferred by extracellular vesicles accelerates atherosclerosis by promotion of leucocyte adherence to endothelial cells. *Clinical Science*, *129*(3), 259–269. https://doi.org/10.1042/CS20140826

Caini, S., Gandini, S., Sera, F., Raimondi, S., Fargnoli, M. C., Boniol, M., & Armstrong, B. K. (2009). Meta-analysis of risk factors for cutaneous melanoma according to anatomical site and clinico-pathological variant. *European Journal of Cancer*, *45*(17), 3054–3063. https://doi.org/10.1016/j.ejca.2009.05.009

Campoli, M. R., Chang, C.-C., Kageshita, T., Wang, X., McCarthy, J. B., & Ferrone, S. (2004). Human high molecular weight-melanoma-associated antigen (HMW-MAA): A melanoma cell surface chondroitin sulfate proteoglycan (MSCP) with biological and clinical significance. *Critical Reviews in Immunology*, *24*(4), 267–296. https://doi.org/10.1615/critrevimmunol.v24.i4.40

Capone, E., Iacobelli, S., & Sala, G. (2021). Role of galectin 3 binding protein in cancer progression: A potential novel therapeutic target. *Journal of Translational Medicine*, *19*(1), 405. https://doi.org/10.1186/s12967-021-03085-w

Carnino, J. M., Hao Kwok, Z., & Jin, Y. (2021). Extracellular Vesicles: A Novel Opportunity for Precision Medicine in Respiratory Diseases. *Frontiers in Medicine*, *8*. https://doi.org/10.3389/fmed.2021.661679

Castellana, D., Zobairi, F., Martinez, M. C., Panaro, M. A., Mitolo, V., Freyssinet, J.-M., & Kunzelmann, C. (2009). Membrane Microvesicles as Actors in the Establishment of a Favorable Prostatic Tumoral Niche: A Role for Activated Fibroblasts and CX3CL1-CX3CR1 Axis. *Cancer Research*, *69*(3), 785–793. https://doi.org/10.1158/0008-5472.CAN-08-1946

Cayrefourcq, L., De Roeck, A., Garcia, C., Stoebner, P.-E., Fichel, F., Garima, F., Perriard, F., Daures, J.-P., Meunier, L., & Alix-Panabières, C. (2019). S100-EPISPOT: A New Tool to Detect Viable Circulating Melanoma Cells. *Cells*, *8*(7), 7. https://doi.org/10.3390/cells8070755

Cela, I., Caponio, V. C. A., Capone, E., Pinti, M., Mascitti, M., Togni, L., Lo Muzio, L., Rubini, C., De Laurenzi, V., Lattanzio, R., Perrotti, V., & Sala, G. (2024). LGALS3BP is a potential target of antibody-drug conjugates in oral squamous cell carcinoma. *Oral Diseases*, *30*(4), 2039–2050. https://doi.org/10.1111/odi.14719

Cervio, E., Barile, L., Moccetti, T., & Vassalli, G. (2015). Exosomes for Intramyocardial Intercellular Communication. *Stem Cells International*, *2015*, e482171. https://doi.org/10.1155/2015/482171

Chang, G. A., Tadepalli, J. S., Shao, Y., Zhang, Y., Weiss, S., Robinson, E., Spittle, C., Furtado, M., Shelton, D. N., Karlin-Neumann, G., Pavlick, A., Osman, I., & Polsky, D. (2016). Sensitivity of plasma BRAFmutant and NRASmutant cell-free DNA assays to detect metastatic melanoma in patients with low RECIST scores and non-RECIST disease progression. *Molecular Oncology*, *10*(1), 157–165. https://doi.org/10.1016/j.molonc.2015.09.005

Chapman, C. J., Healey, G. F., Murray, A., Boyle, P., Robertson, C., Peek, L. J., Allen, J., Thorpe, A. J., Hamilton-Fairley, G., Parsy-Kowalska, C. B., MacDonald, I. K., Jewell, W., Maddison, P., & Robertson, J. F. R. (2012). EarlyCDT®-Lung test: Improved clinical utility through additional autoantibody assays. *Tumor Biology*, *33*(5), 1319–1326. https://doi.org/10.1007/s13277-012-0379-2

Charkhchi, P., Cybulski, C., Gronwald, J., Wong, F. O., Narod, S. A., & Akbari, M. R. (2020). CA125 and Ovarian Cancer: A Comprehensive Review. *Cancers*, *12*(12), 12. https://doi.org/10.3390/cancers12123730

Chen, H., Werner, S., Tao, S., Zörnig, I., & Brenner, H. (2014b). Blood autoantibodies against tumor-associated antigens as biomarkers in early detection of colorectal cancer. *Cancer Letters*, *346*(2), 178–187. https://doi.org/10.1016/j.canlet.2014.01.007

Chen, M., Wan, L., Zhang, J., Zhang, J., Mendez, L., Clohessy, J. G., Berry, K., Victor, J., Yin, Q., Zhu, Y., Wei, W., & Pandolfi, P. P. (2018). Deregulated PP1α phosphatase activity towards MAPK activation is antagonized by a tumor suppressive failsafe mechanism. *Nature Communications*, *9*(1), 159. https://doi.org/10.1038/s41467-017-02272-y

Chen, M., & Zhao, H. (2019). Next-generation sequencing in liquid biopsy: Cancer screening and early detection. *Human Genomics*, *13*(1), 34. https://doi.org/10.1186/s40246-019-0220-8

Chen, W., Liu, X., Lv, M., Chen, L., Zhao, J., Zhong, S., Ji, M., Hu, Q., Luo, Z., Wu, J., & Tang, J. (2014a). Exosomes from Drug-Resistant Breast Cancer Cells Transmit Chemoresistance by a Horizontal Transfer of MicroRNAs. *PLOS ONE*, *9*(4), e95240. https://doi.org/10.1371/journal.pone.0095240

Chen, X., Chen, S., Pang, J., Huang, R., You, Y., Zhang, H., Xiao, J., Xue, H., & Ling, W. (2023). Hepatic steatosis aggravates atherosclerosis via small extracellular vesicle-mediated inhibition of cellular cholesterol efflux. *Journal of Hepatology*, *79*(6), 1491–1501. https://doi.org/10.1016/j.jhep.2023.08.023

Chen, X., Dong, K., Long, M., Lin, F., Wang, X., Wei, J., Ren, J., & Zhang, H. (2012). Serum anti-AEG-1 auto-antibody is a potential novel biomarker for malignant tumors. *Oncology Letters*, *4*(2), 319–323. https://doi.org/10.3892/ol.2012.734

Chen, Y., Xu, Y., Wang, J., Prisinzano, P., Yuan, Y., Lu, F., Zheng, M., Mao, W., & Wan, Y. (2022). Statins Lower Lipid Synthesis But Promote Secretion of Cholesterol-Enriched Extracellular Vesicles and Particles. *Frontiers in Oncology*, *12*, 853063. https://doi.org/10.3389/fonc.2022.853063

Cheng, C., Wang, Q., You, W., Chen, M., & Xia, J. (2014). MiRNAs as Biomarkers of Myocardial Infarction: A Meta-Analysis. *PLOS ONE*, *9*(2), e88566. https://doi.org/10.1371/journal.pone.0088566

Chiva-Blanch, G., Bratseth, V., Ritschel, V., Andersen, G. Ø., Halvorsen, S., Eritsland, J., Arnesen, H., Badimon, L., & Seljeflot, I. (2017a). Monocyte-derived circulating microparticles (CD14+, CD14+/CD11b+ and CD14+/CD142+) are related to long-term prognosis for cardiovascular mortality in STEMI patients. *International Journal of Cardiology*, *227*, 876–881. https://doi.org/10.1016/j.ijcard.2016.11.302

Chiva-Blanch, G., Laake, K., Myhre, P., Bratseth, V., Arnesen, H., Solheim, S., Badimon, L., & Seljeflot, I. (2017b). Platelet-, monocyte-derived and tissue factor-carrying circulating microparticles are related to acute myocardial infarction severity. *PLOS ONE*, *12*(2), e0172558. https://doi.org/10.1371/journal.pone.0172558

Chiva-Blanch, G., Sala-Vila, A., Crespo, J., Ros, E., Estruch, R., & Badimon, L. (2020). The Mediterranean diet decreases prothrombotic microvesicle release in asymptomatic individuals at high cardiovascular risk. *Clinical Nutrition*, *39*(11), 3377–3384. https://doi.org/10.1016/j.clnu.2020.02.027

Cho, W. C. (2007). OncomiRs: The discovery and progress of microRNAs in cancers. *Molecular Cancer*, *6*(1), 60. https://doi.org/10.1186/1476-4598-6-60

Choi, D.-S., Kim, D.-K., Kim, Y.-K., & Gho, Y. S. (2015). Proteomics of extracellular vesicles: Exosomes and ectosomes. *Mass Spectrometry Reviews*, *34*(4), 474–490. https://doi.org/10.1002/mas.21420

Choi, D.-Y., You, S., Jung, J. H., Lee, J. C., Rho, J. K., Lee, K. Y., Freeman, M. R., Kim, K. P., & Kim, J. (2014). Extracellular vesicles shed from gefitinib-resistant nonsmall cell lung cancer regulate the tumor microenvironment. *PROTEOMICS*, *14*(16), 1845–1856. https://doi.org/10.1002/pmic.201400008

Chomez, P., De Backer, O., Bertrand, M., De Plaen, E., Boon, T., & Lucas, S. (2001). An Overview of the MAGE Gene Family with the Identification of All Human Members of the Family1. *Cancer Research*, *61*(14), 5544–5551.

Christianson, H. C., Svensson, K. J., van Kuppevelt, T. H., Li, J.-P., & Belting, M. (2013). Cancer cell exosomes depend on cell-surface heparan sulfate proteoglycans for their internalization and functional activity. *Proceedings of the National Academy of Sciences*, *110*(43), 17380–17385. https://doi.org/10.1073/pnas.1304266110

Ciardiello, C., Cavallini, L., Spinelli, C., Yang, J., Reis-Sobreiro, M., De Candia, P., Minciacchi, V. R., & Di Vizio, D. (2016). Focus on Extracellular Vesicles: New Frontiers of Cell-to-Cell Communication in Cancer. *International Journal of Molecular Sciences*, *17*(2), 2. https://doi.org/10.3390/ijms17020175

Clayton, A., Mitchell, J. P., Court, J., Linnane, S., Mason, M. D., & Tabi, Z. (2008). Human Tumor-Derived Exosomes Down-Modulate NKG2D Expression1. *The Journal of Immunology*, *180*(11), 7249–7258. https://doi.org/10.4049/jimmunol.180.11.7249

Cocucci, E., Racchetti, G., & Meldolesi, J. (2009). Shedding microvesicles: Artefacts no more. *Trends in Cell Biology*, *19*(2), 43–51. https://doi.org/10.1016/j.tcb.2008.11.003

Colombo, M., Raposo, G., & Théry, C. (2014). Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annual Review of Cell and Developmental Biology*, *30*, 255–289. https://doi.org/10.1146/annurev-cellbio-101512-122326

Connal, S., Cameron, J. M., Sala, A., Brennan, P. M., Palmer, D. S., Palmer, J. D., Perlow, H., & Baker, M. J. (2023). Liquid biopsies: The future of cancer early detection. *Journal of Translational Medicine*, *21*, 118. https://doi.org/10.1186/s12967-023-03960-8

Corcoran, R. B., & Chabner, B. A. (2018). Application of Cell-free DNA Analysis to Cancer Treatment. *New England Journal of Medicine*, *379*(18), 1754–1765. https://doi.org/10.1056/NEJMra1706174

Cordonnier, M., Nardin, C., Chanteloup, G., Derangere, V., Algros, M.-P., Arnould, L., Garrido, C., Aubin, F., & Gobbo, J. (2020). Tracking the evolution of circulating exosomal-PD-L1 to monitor melanoma patients. *Journal of Extracellular Vesicles*, *9*(1), 1710899. https://doi.org/10.1080/20013078.2019.1710899

Costa, F. F., Le Blanc, K., & Brodin, B. (2007). Concise Review: Cancer/Testis Antigens, Stem Cells, and Cancer. *Stem Cells*, *25*(3), 707–711. https://doi.org/10.1634/stemcells.2006-0469

Crescitelli, R., Filges, S., Karimi, N., Urzì, O., Alonso-Agudo, T., Ståhlberg, A., Lötvall, J., Lässer, C., & Olofsson Bagge, R. (2022). Extracellular vesicle DNA from human melanoma tissues contains cancer-specific mutations. *Frontiers in Cell and Developmental Biology*, *10*, 1028854. https://doi.org/10.3389/fcell.2022.1028854

Crespin, M., Vidal, C., Picard, F., Lacombe, C., & Fontenay, M. (2009). Activation of PAK1/2 during the shedding of platelet microvesicles. *Blood Coagulation & Fibrinolysis*, *20*(1), 63. https://doi.org/10.1097/MBC.0b013e32831bc310

Cronwright, G., Le Blanc, K., Götherström, C., Darcy, P., Ehnman, M., & Brodin, B. (2005). Cancer/Testis Antigen Expression in Human Mesenchymal Stem Cells: Down-regulation of SSX Impairs Cell Migration and Matrix Metalloproteinase 2 Expression. *Cancer Research*, *65*(6), 2207–2215. https://doi.org/10.1158/0008-5472.CAN-04-1882

Cvjetkovic, A., Lötvall, J., & Lässer, C. (2014). The influence of rotor type and centrifugation time on the yield and purity of extracellular vesicles. *Journal of Extracellular Vesicles*, *3*(1), 23111. https://doi.org/10.3402/jev.v3.23111

Da Fonseca Ferreira, A., Wei, J., Zhang, L., Macon, C. J., Degnan, B., Jayaweera, D., Hare, J. M., Kolber, M. A., Bellio, M., Khan, A., Pan, Y., Dykxhoorn, D. M., Wang, L., & Dong, C. (2023). HIV Promotes Atherosclerosis via Circulating Extracellular Vesicle MicroRNAs. *International Journal of Molecular Sciences*, *24*(8), 7567. https://doi.org/10.3390/ijms24087567

Danilova, A., Misyurin, V., Novik, A., Girdyuk, D., Avdonkina, N., Nekhaeva, T., Emelyanova, N., Pipia, N., Misyurin, A., & Baldueva, I. (2020). Cancer/testis antigens expression during cultivation of melanoma and soft tissue sarcoma cells. *Clinical Sarcoma Research*, *10*(1), 3. https://doi.org/10.1186/s13569-020-0125-2

D'Asti, E., Garnier, D., Lee, T. H., Montermini, L., Meehan, B., & Rak, J. (2012). Oncogenic extracellular vesicles in brain tumor progression. *Frontiers in Physiology*, *3*. https://doi.org/10.3389/fphys.2012.00294

Daudi, S., Eng, K. H., Mhawech-Fauceglia, P., Morrison, C., Miliotto, A., Beck, A., Matsuzaki, J., Tsuji, T., Groman, A., Gnjatic, S., Spagnoli, G., Lele, S., & Odunsi, K. (2014). Expression and immune responses to MAGE antigens predict survival in epithelial ovarian cancer. *PloS One*, *9*(8), e104099. https://doi.org/10.1371/journal.pone.0104099

Davidson, S. M., Andreadou, I., Barile, L., Birnbaum, Y., Cabrera-Fuentes, H. A., Cohen, M. V., Downey, J. M., Girao, H., Pagliaro, P., Penna, C., Pernow, J., Preissner, K. T., Ferdinandy, P., & on behalf of the EU-CARDIOPROTECTION COST Action (CA16225). (2019). Circulating blood cells and extracellular vesicles in acute cardioprotection. *Cardiovascular Research*, *115*(7), 1156–1166. https://doi.org/10.1093/cvr/cvy314

Davies, M. J., Woolf, N., Rowles, P. M., & Pepper, J. (1988). Morphology of the endothelium over atherosclerotic plaques in human coronary arteries. *Heart*, *60*(6), 459–464. https://doi.org/10.1136/hrt.60.6.459

Davies, P. F. (1995). Flow-Mediated Endothelial Mechanotransduction. *Physiological Reviews*, *75*(3), 519–560.

Davis, L. E., Shalin, S. C., & Tackett, A. J. (2019). Current state of melanoma diagnosis and treatment. *Cancer Biology & Therapy*, *20*(11), 1366–1379. https://doi.org/10.1080/15384047.2019.1640032

De Giorgi, V., Pinzani, P., Salvianti, F., Panelos, J., Paglierani, M., Janowska, A., Grazzini, M., Wechsler, J., Orlando, C., Santucci, M., Lotti, T., Pazzagli, M., & Massi, D. (2010). Application of a Filtration- and Isolation-by-Size Technique for the Detection of Circulating Tumor Cells in Cutaneous Melanoma. *Journal of Investigative Dermatology*, *130*(10), 2440–2447. https://doi.org/10.1038/jid.2010.141

de Jonge, H., Iamele, L., Maggi, M., Pessino, G., & Scotti, C. (2021). Anti-Cancer Auto-Antibodies: Roles, Applications and Open Issues. *Cancers*, *13*(4), 4. https://doi.org/10.3390/cancers13040813

De Martino, E., Gandin, I., Azzalini, E., Massone, C., Pizzichetta, M. A., Giulioni, E., Javor, S., Pinzani, C., Conforti, C., Zalaudek, I., & Bonin, S. (2023). A group of three miRNAs can act as candidate circulating biomarkers in liquid biopsies from melanoma patients. *Frontiers in Medicine*, *10*, 1180799. https://doi.org/10.3389/fmed.2023.1180799

de Mezer, M., Rogaliński, J., Przewoźny, S., Chojnicki, M., Niepolski, L., Sobieska, M., & Przystańska, A. (2023). SERPINA3: Stimulator or Inhibitor of Pathological Changes. *Biomedicines*, *11*(1), 156. https://doi.org/10.3390/biomedicines11010156

De Plaen, E., Traversari, C., Gaforio, J. J., Szikora, J.-P., De Smet, C., Brasseur, F., van der Bruggen, P., Lethé, B., Lurquin, C., Chomez, P., De Backer, O., Boon, T., Arden, K., Cavenee, W., & Brasseur, R. (1994). Structure, chromosomal localization, and expression of 12 genes of the MAGE family. *Immunogenetics*, *40*(5), 360–369. https://doi.org/10.1007/BF01246677

De Rubis, G., Rajeev Krishnan, S., & Bebawy, M. (2019). Liquid Biopsies in Cancer Diagnosis, Monitoring, and Prognosis. *Trends in Pharmacological Sciences*, *40*(3), 172–186. https://doi.org/10.1016/j.tips.2019.01.006

Deddens, J. C., Vrijsen, K. R., Colijn, J. M., Oerlemans, M. I., Metz, C. H. G., van der Vlist, E. J., Nolte-'t Hoen, E. N. M., den Ouden, K., Jansen Of Lorkeers, S. J., van der Spoel, T. I. G., Koudstaal, S., Arkesteijn, G. J., Wauben, M. H. M., van Laake, L. W., Doevendans, P. A., Chamuleau, S. A. J., & Sluijter, J. P. G. (2016). Circulating Extracellular Vesicles Contain miRNAs and are Released as Early Biomarkers for Cardiac Injury. *Journal of Cardiovascular Translational Research*, *9*(4), 291–301. https://doi.org/10.1007/s12265-016-9705-1

Deichmann, M., Benner, A., Bock, M., Jäckel, A., Uhl, K., Waldmann, V., & Näher, H. (1999). S100-Beta, Melanoma-Inhibiting Activity, and Lactate Dehydrogenase Discriminate Progressive From Nonprogressive American Joint Committee on Cancer Stage IV Melanoma. *Journal of Clinical Oncology*, *17*(6), 1891–1891. https://doi.org/10.1200/JCO.1999.17.6.1891

del Conde, I., Shrimpton, C. N., Thiagarajan, P., & López, J. A. (2005). Tissue-factor–bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. *Blood*, *106*(5), 1604–1611. https://doi.org/10.1182/blood-2004-03-1095

Desmetz, C., Bibeau, F., Boissière, F., Bellet, V., Rouanet, P., Maudelonde, T., Mangé, A., & Solassol, J. (2008). Proteomics-Based Identification of HSP60 as a Tumor-Associated Antigen in Early Stage Breast Cancer and Ductal Carcinoma in situ. *Journal of Proteome Research*, *7*(9), 3830–3837. https://doi.org/10.1021/pr800130d

Di Vizio, D., Kim, J., Hager, M. H., Morello, M., Yang, W., Lafargue, C. J., True, L. D., Rubin, M. A., Adam, R. M., Beroukhim, R., Demichelis, F., & Freeman, M. R. (2009). Oncosome Formation in Prostate Cancer: Association with a Region of Frequent Chromosomal Deletion in Metastatic Disease. *Cancer Research*, *69*(13), 5601–5609. https://doi.org/10.1158/0008-5472.CAN-08-3860

Di Vizio, D., Morello, M., Dudley, A. C., Schow, P. W., Adam, R. M., Morley, S., Mulholland, D., Rotinen, M., Hager, M. H., Insabato, L., Moses, M. A., Demichelis, F., Lisanti, M. P., Wu, H., Klagsbrun, M., Bhowmick, N. A., Rubin, M. A., D'Souza-Schorey, C., & Freeman, M. R. (2012). Large Oncosomes in Human Prostate Cancer Tissues and in the Circulation of Mice with Metastatic Disease. *The American Journal of Pathology*, *181*(5), 1573–1584. https://doi.org/10.1016/j.ajpath.2012.07.030

Diaz, L. A., & Bardelli, A. (2014). Liquid Biopsies: Genotyping Circulating Tumor DNA. *Journal of Clinical Oncology*, *32*(6), 579–586. https://doi.org/10.1200/JCO.2012.45.2011

Diefenbach, R. J., Lee, J. H., & Rizos, H. (2019). Monitoring Melanoma Using Circulating Free DNA. *American Journal of Clinical Dermatology*, *20*(1), 1–12. https://doi.org/10.1007/s40257-018-0398-x

Dietz, L., Oberländer, J., Mateos-Maroto, A., Schunke, J., Fichter, M., Krämer-Albers, E., Landfester, K., & Mailänder, V. (2023). Uptake of extracellular vesicles into immune cells is enhanced by the protein corona. *Journal of Extracellular Vesicles*, *12*(12), e12399. https://doi.org/10.1002/jev2.12399

Dimitriou, F., Krattinger, R., Ramelyte, E., Barysch, M. J., Micaletto, S., Dummer, R., & Goldinger, S. M. (2018). The World of Melanoma: Epidemiologic, Genetic, and Anatomic Differences of Melanoma Across the Globe. *Current Oncology Reports*, *20*(11), 87. https://doi.org/10.1007/s11912-018-0732-8

Ding, Z., Wang, N., Ji, N., & Chen, Z.-S. (2022). Proteomics technologies for cancer liquid biopsies. *Molecular Cancer*, *21*(1), 53. https://doi.org/10.1186/s12943-022-01526-8

Disis, M. L., Pupa, S. M., Gralow, J. R., Dittadi, R., Menard, S., & Cheever, M. A. (1997). High-titer HER-2/neu protein-specific antibody can be detected in patients with early-stage breast cancer. *Journal of Clinical Oncology*, *15*(11), 3363–3367. https://doi.org/10.1200/JCO.1997.15.11.3363

Dochez, V., Caillon, H., Vaucel, E., Dimet, J., Winer, N., & Ducarme, G. (2019). Biomarkers and algorithms for diagnosis of ovarian cancer: CA125, HE4, RMI and ROMA, a review. *Journal of Ovarian Research*, *12*(1), 28. https://doi.org/10.1186/s13048-019-0503-7

Dondo, T. B., Hall, M., Timmis, A. D., Gilthorpe, M. S., Alabas, O. A., Batin, P. D., Deanfield, J. E., Hemingway, H., & Gale, C. P. (2017). Excess mortality and guideline-indicated care following non-ST-elevation myocardial infarction. *European Heart Journal. Acute Cardiovascular Care*, *6*(5), 412–420. https://doi.org/10.1177/2048872616647705

Doyle, J. M., Gao, J., Wang, J., Yang, M., & Potts, P. R. (2010). MAGE-RING Protein Complexes Comprise a Family of E3 Ubiquitin Ligases. *Molecular Cell*, *39*(6), 963–974. https://doi.org/10.1016/j.molcel.2010.08.029

Doyle, L. M., & Wang, M. Z. (2019). Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. *Cells*, *8*(7), 7. https://doi.org/10.3390/cells8070727

Drokow, E. K., Sun, K., Ahmed, H. A. W., Akpabla, G. S., Song, J., & Shi, M. (2019). Circulating microRNA as diagnostic biomarkers for haematological cancers: A systematic review and meta-analysis. *Cancer Management and Research*, *11*, 4313–4326. https://doi.org/10.2147/CMAR.S199126

D'Souza-Schorey, C., & Chavrier, P. (2006). ARF proteins: Roles in membrane traffic and beyond. *Nature Reviews Molecular Cell Biology*, *7*(5), 347–358. https://doi.org/10.1038/nrm1910

D'Souza-Schorey, C., & Di Vizio, D. (2014). Biology and proteomics of extracellular vesicles: Harnessing their clinical potential. *Expert Review of Proteomics*, *11*(3), 251–253. https://doi.org/10.1586/14789450.2014.874290

Dubois-Deruy, E., Peugnet, V., Turkieh, A., & Pinet, F. (2020). Oxidative Stress in Cardiovascular Diseases. *Antioxidants*, *9*(9), 9. https://doi.org/10.3390/antiox9090864

Duncan, M. S., Vasan, R. S., & Xanthakis, V. (2019). Trajectories of Blood Lipid Concentrations Over the Adult Life Course and Risk of Cardiovascular Disease and All-Cause Mortality: Observations From the Framingham Study Over 35 Years. *Journal of the American Heart Association*, *8*(11), e011433. https://doi.org/10.1161/JAHA.118.011433

Eisenstein, A., Gonzalez, E. C., Raghunathan, R., Xu, X., Wu, M., McLean, E. O., McGee, J., Ryu, B., & Alani, R. M. (2018). Emerging Biomarkers in Cutaneous Melanoma. *Molecular Diagnosis & Therapy*, *22*(2), 203–218. https://doi.org/10.1007/s40291-018-0318-z

Elder, D. E., Bastian, B. C., Cree, I. A., Massi, D., & Scolyer, R. A. (2020). The 2018 World Health Organization Classification of Cutaneous, Mucosal, and Uveal Melanoma: Detailed Analysis of 9 Distinct Subtypes Defined by Their Evolutionary Pathway. *Archives of Pathology & Laboratory Medicine*, *144*(4), 500–522. https://doi.org/10.5858/arpa.2019-0561-RA

Elder, D. E., Massi, D., Scolyer, R. A., & Willemze, R. (2018). *WHO Classification of Skin Tumours* (4th ed., Vol. 11). IARC. https://publications.iarc.fr/Book-And-Report-Series/Who-Classification-Of-Tumours/WHO-Classification-Of-Skin-Tumours-2018

Elkon, K., & Casali, P. (2008). Nature and functions of autoantibodies. *Nature Clinical Practice. Rheumatology*, *4*(9), 491–498. https://doi.org/10.1038/ncprheum0895

Elwood, J. M., & Jopson, J. (1997). Melanoma and sun exposure: An overview of published studies. *International Journal of Cancer*, *73*(2), 198–203. https://doi.org/10.1002/(SICI)1097-0215(19971009)73:2<198::AID-IJC6>3.0.CO;2-R

Emanueli, C., Shearn, A. I. U., Angelini, G. D., & Sahoo, S. (2015). Exosomes and exosomal miRNAs in cardiovascular protection and repair. *Vascular Pharmacology*, *71*, 24–30. https://doi.org/10.1016/j.vph.2015.02.008

Emilsson, V., Ilkov, M., Lamb, J. R., Finkel, N., Gudmundsson, E. F., Pitts, R., Hoover, H., Gudmundsdottir, V., Horman, S. R., Aspelund, T., Shu, L., Trifonov, V., Sigurdsson, S., Manolescu, A., Zhu, J., Olafsson, Ö., Jakobsdottir, J., Lesley, S. A., To, J., … Gudnason, V. (2018). Co-regulatory networks of human serum proteins link genetics to disease. *Science*, *361*(6404), 769–773. https://doi.org/10.1126/science.aaq1327

Eslami-S, Z., Cortés-Hernández, L. E., Cayrefourcq, L., & Alix-Panabières, C. (2020). The Different Facets of Liquid Biopsy: A Kaleidoscopic View. *Cold Spring Harbor Perspectives in Medicine*, *10*(6), a037333. https://doi.org/10.1101/cshperspect.a037333

Evtushenko, E. G., Bagrov, D. V., Lazarev, V. N., Livshits, M. A., & Khomyakova, E. (2020). Adsorption of extracellular vesicles onto the tube walls during storage in solution. *PLOS ONE*, *15*(12), e0243738. https://doi.org/10.1371/journal.pone.0243738

Fader, C. M., Sánchez, D. G., Mestre, M. B., & Colombo, M. I. (2009). TI-VAMP/VAMP7 and VAMP3/cellubrevin: Two v-SNARE proteins involved in specific steps of the autophagy/multivesicular body pathways. *Biochimica Et Biophysica Acta*, *1793*(12), 1901–1916. https://doi.org/10.1016/j.bbamcr.2009.09.011

Faiena, I., Adhikary, S., Schweitzer, C., Astrow, S. H., Grogan, T., Funt, S. A., Bot, A., Dorff, T., Rosenberg, J. E., Elashoff, D. A., Pantuck, A. J., & Drakaki, A. (2024). Gene and Protein Expression of MAGE and Associated Immune Landscape Elements in Non–Small-Cell Lung Carcinoma and Urothelial Carcinomas. *Journal of Immunotherapy*, *47*(9), 351. https://doi.org/10.1097/CJI.0000000000000538

Falk, E. (2006). Pathogenesis of Atherosclerosis. *Journal of the American College of Cardiology*, *47*(8, Supplement), C7–C12. https://doi.org/10.1016/j.jacc.2005.09.068

Fan, L. M., Douglas, G., Bendall, J. K., McNeill, E., Crabtree, M. J., Hale, A. B., Mai, A., Li, J.-M., McAteer, M. A., Schneider, J. E., Choudhury, R. P., & Channon, K. M. (2014). Endothelial Cell–Specific Reactive Oxygen Species Production Increases Susceptibility to Aortic Dissection. *Circulation*, *129*(25), 2661–2672. https://doi.org/10.1161/CIRCULATIONAHA.113.005062

Feingold, K. R. (2000). Cholesterol Lowering Drugs. In K. R. Feingold, B. Anawalt, M. R. Blackman, A. Boyce, G. Chrousos, E. Corpas, W. W. de Herder, K. Dhatariya, K. Dungan, J. Hofland, S. Kalra, G. Kaltsas, N. Kapoor, C. Koch, P. Kopp, M. Korbonits, C. S. Kovacs, W. Kuohung, B. Laferrère, … D. P. Wilson (Eds.), *Endotext*. MDText.com, Inc. http://www.ncbi.nlm.nih.gov/books/NBK395573/

Feng, D., Zhao, W.-L., Ye, Y.-Y., Bai, X.-C., Liu, R.-Q., Chang, L.-F., Zhou, Q., & Sui, S.-F. (2010). Cellular Internalization of Exosomes Occurs Through Phagocytosis. *Traffic*, *11*(5), 675–687. https://doi.org/10.1111/j.1600-0854.2010.01041.x

Feola, S., Chiaro, J., Martins, B., & Cerullo, V. (2020). Uncovering the Tumor Antigen Landscape: What to Know about the Discovery Process. *Cancers*, *12*(6), 6. https://doi.org/10.3390/cancers12061660

Figueiredo, C. S., Roseira, E. S., Viana, T. T., Silveira, M. A. D., de Melo, R. M. V., Fernandez, M. G., Lemos, L. M. G., & Passos, L. C. S. (2023). Inflammation in Coronary Atherosclerosis: Insights into Pathogenesis and Therapeutic Potential of Anti-Inflammatory Drugs. *Pharmaceuticals*, *16*(9), 9. https://doi.org/10.3390/ph16091242

Finn, O. J. (2005). Immune Response as a Biomarker for Cancer Detection and a Lot More. *New England Journal of Medicine*, *353*(12), 1288–1290. https://doi.org/10.1056/NEJMe058157

Fischer, A. H., Jacobson, K. A., Rose, J., & Zeller, R. (2008). Hematoxylin and Eosin Staining of Tissue and Cell Sections. *Cold Spring Harbor Protocols*, *2008*(5), pdb.prot4986. https://doi.org/10.1101/pdb.prot4986

Forschner, A., Battke, F., Hadaschik, D., Schulze, M., Weißgraeber, S., Han, C.-T., Kopp, M., Frick, M., Klumpp, B., Tietze, N., Amaral, T., Martus, P., Sinnberg, T., Eigentler, T., Keim, U., Garbe, C., Döcker, D., & Biskup, S. (2019). Tumor mutation burden and circulating tumor DNA in combined CTLA-4 and PD-1 antibody therapy in metastatic melanoma – results of a prospective biomarker study. *Journal for ImmunoTherapy of Cancer*, *7*(1), 180. https://doi.org/10.1186/s40425-019-0659-0

Forthun, R. B., Hovland, R., Schuster, C., Puntervoll, H., Brodal, H. P., Namløs, H. M., Aasheim, L. B., Meza-Zepeda, L. A., Gjertsen, B. T., Knappskog, S., & Straume, O. (2019). CtDNA detected by ddPCR reveals changes in tumour load in metastatic malignant melanoma treated with bevacizumab. *Scientific Reports*, *9*(1), 17471. https://doi.org/10.1038/s41598-019-53917-5

Fortner, R. T., Damms-Machado, A., & Kaaks, R. (2017). Systematic review: Tumor-associated antigen autoantibodies and ovarian cancer early detection. *Gynecologic Oncology*, *147*(2), 465–480. https://doi.org/10.1016/j.ygyno.2017.07.138

Fortuño, A., San José, G., Moreno, M. U., Beloqui, O., Díez, J., & Zalba, G. (2006). Phagocytic NADPH oxidase overactivity underlies oxidative stress in metabolic syndrome. *Diabetes*, *55*(1), 209–215. https://doi.org/10.2337/diabetes.55.01.06.db05-0751

Franzke, A., Probst-Kepper, M., Buer, J., Duensing, S., Hoffmann, R., Wittke, F., Volkenandt, M., Ganser, A., & Atzpodien, J. (1998). Elevated pretreatment serum levels of soluble vascular cell adhesion molecule 1 and lactate dehydrogenase as predictors of survival in cutaneous metastatic malignant melanoma. *British Journal of Cancer*, *78*(1), 40–45. https://doi.org/10.1038/bjc.1998.439

Friedman, R. J., Rigel, D. S., & Kopf, A. W. (1985). Early detection of malignant melanoma: The role of physician examination and self-examination of the skin. *CA: A Cancer Journal for Clinicians*, *35*(3), 130–151. https://doi.org/10.3322/canjclin.35.3.130

Fujiya, K., Terashima, M., Ohshima, K., Aizawa, D., Sugino, T., Serizawa, M., Nakamura, K., Nagashima, T., Hatakeyama, K., Urakami, K., Akiyama, Y., Tsubosa, Y., Kitagawa, Y., & Yamaguchi, K. (2021). MAGEA10 expression is a predictive marker of early hepatic recurrence after curative gastrectomy for gastric and gastroesophageal junction cancer. *Gastric Cancer*, *24*(2), 341–351. https://doi.org/10.1007/s10120-020-01123-y

Gan, Y., Kang, Y., Zhong, R., You, J., Chen, J., Li, L., Chen, J., & Chen, L. (2024). Cancer testis antigen MAGEA3 in serum and serum-derived exosomes serves as a promising biomarker in lung adenocarcinoma. *Scientific Reports*, *14*(1), 7573. https://doi.org/10.1038/s41598-024-58003-z

Gandini, S., Sera, F., Cattaruzza, M. S., Pasquini, P., Abeni, D., Boyle, P., & Melchi, C. F. (2005). Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. *European Journal of Cancer*, *41*(1), 28–44. https://doi.org/10.1016/j.ejca.2004.10.015

García-Álvarez, R., Hadjidemetriou, M., Sánchez-Iglesias, A., Liz-Marzán, L. M., & Kostarelos, K. (2018). In vivo formation of protein corona on gold nanoparticles. The effect of their size and shape. *Nanoscale*, *10*(3), 1256–1264. https://doi.org/10.1039/c7nr08322j

García-Romero, N., Madurga, R., Rackov, G., Palacín-Aliana, I., Núñez-Torres, R., Asensi-Puig, A., Carrión-Navarro, J., Esteban-Rubio, S., Peinado, H., González-Neira, A., González-Rumayor, V., Belda-Iniesta, C., & Ayuso-Sacido, A. (2019). Polyethylene glycol improves current methods for circulating extracellular vesicle-derived DNA isolation. *Journal of Translational Medicine*, *17*(1), 75. https://doi.org/10.1186/s12967-019-1825-3

Ge, X., Meng, Q., Zhuang, R., Yuan, D., Liu, J., Lin, F., Fan, H., & Zhou, X. (2019). Circular RNA expression alterations in extracellular vesicles isolated from murine heart post ischemia/reperfusion injury. *International Journal of Cardiology*, *296*, 136–140. https://doi.org/10.1016/j.ijcard.2019.08.024

Gerrity, R. G., Naito, H. K., Richardson, M., & Schwartz, C. J. (1979). Dietary induced atherogenesis in swine. Morphology of the intima in prelesion stages. *The American Journal of Pathology*, *95*(3), 775–792.

Ghossoub, R., Lembo, F., Rubio, A., Gaillard, C. B., Bouchet, J., Vitale, N., Slavík, J., Machala, M., & Zimmermann, P. (2014). Syntenin-ALIX exosome biogenesis and budding into multivesicular bodies are controlled by ARF6 and PLD2. *Nature Communications*, *5*(1), 3477. https://doi.org/10.1038/ncomms4477

Gidlöf, O., van der Brug, M., Öhman, J., Gilje, P., Olde, B., Wahlestedt, C., & Erlinge, D. (2013). Platelets activated during myocardial infarction release functional miRNA, which can be taken up by endothelial cells and regulate ICAM1 expression. *Blood*, *121*(19), 3908–3917. https://doi.org/10.1182/blood-2012-10-461798

Gimbrone, M. A., Topper, J. N., Nagel, T., Anderson, K. R., & Garcia-Cardeña, G. (2000). Endothelial dysfunction, hemodynamic forces, and atherogenesis. *Annals of the New York Academy of Sciences*, *902*, 230–239; discussion 239–240. https://doi.org/10.1111/j.1749-6632.2000.tb06318.x

Glaros, A. G., & Kline, R. B. (1988). Understanding the accuracy of tests with cutting scores: The sensitivity, specificity, and predictive value model. *Journal of Clinical Psychology*, *44*(6), 1013–1023. https://doi.org/10.1002/1097-4679(198811)44:6<1013::AID-JCLP2270440627>3.0.CO;2-Z

Glass, C. K., & Witztum, J. L. (2001). Atherosclerosis: The Road Ahead. *Cell*, *104*(4), 503–516. https://doi.org/10.1016/S0092-8674(01)00238-0

Gnjatic, S., Ritter, E., Büchler, M. W., Giese, N. A., Brors, B., Frei, C., Murray, A., Halama, N., Zörnig, I., Chen, Y.-T., Andrews, C., Ritter, G., Old, L. J., Odunsi, K., & Jäger, D. (2010). Seromic profiling of ovarian and pancreatic cancer. *Proceedings of the National Academy of Sciences*, *107*(11), 5088–5093. https://doi.org/10.1073/pnas.0914213107

Goetzl, E. J., Schwartz, J. B., Mustapic, M., Lobach, I. V., Daneman, R., Abner, E. L., & Jicha, G. A. (2017). Altered cargo proteins of human plasma endothelial cell–derived exosomes in atherosclerotic cerebrovascular disease. *The FASEB Journal*, *31*(8), 3689–3694. https://doi.org/10.1096/fj.201700149

Goldstein, J. L., & Brown, M. S. (2009). The LDL receptor. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *29*(4), 431–438. https://doi.org/10.1161/ATVBAHA.108.179564

Grange, C., Tapparo, M., Collino, F., Vitillo, L., Damasco, C., Deregibus, M. C., Tetta, C., Bussolati, B., & Camussi, G. (2011). Microvesicles Released from Human Renal Cancer Stem Cells Stimulate Angiogenesis and Formation of Lung Premetastatic Niche. *Cancer Research*, *71*(15), 5346–5356. https://doi.org/10.1158/0008-5472.CAN-11-0241

Greening, D. W., Xu, R., Ji, H., Tauro, B. J., & Simpson, R. J. (2015). A protocol for exosome isolation and characterization: Evaluation of ultracentrifugation, density-gradient separation, and immunoaffinity capture methods. *Methods in Molecular Biology (Clifton, N.J.)*, *1295*, 179–209. https://doi.org/10.1007/978-1-4939-2550-6_15

Groeper, C., Gambazzi, F., Zajac, P., Bubendorf, L., Adamina, M., Rosenthal, R., Zerkowski, H.-R., Heberer, M., & Spagnoli, G. C. (2007). Cancer/testis antigen expression and specific cytotoxic T lymphocyte responses in non small cell lung cancer. *International Journal of Cancer*, *120*(2), 337–343. https://doi.org/10.1002/ijc.22309

Guan, X. (2015). Cancer metastases: Challenges and opportunities. *Acta Pharmaceutica Sinica B*, *5*(5), 402–418. https://doi.org/10.1016/j.apsb.2015.07.005

Gudbergsson, J. M., Johnsen, K. B., Skov, M. N., & Duroux, M. (2016). Systematic review of factors influencing extracellular vesicle yield from cell cultures. *Cytotechnology*, *68*(4), 579–592. https://doi.org/10.1007/s10616-015-9913-6

Gurung, S., Perocheau, D., Touramanidou, L., & Baruteau, J. (2021). The exosome journey: From biogenesis to uptake and intracellular signalling. *Cell Communication and Signaling*, *19*(1), 47. https://doi.org/10.1186/s12964-021-00730-1

Hakulinen, J., Sankkila, L., Sugiyama, N., Lehti, K., & Keski-Oja, J. (2008). Secretion of active membrane type 1 matrix metalloproteinase (MMP-14) into extracellular space in microvesicular exosomes. *Journal of Cellular Biochemistry*, *105*(5), 1211–1218. https://doi.org/10.1002/jcb.21923

Hall, K. H., & Rapini, R. P. (2024). Acral Lentiginous Melanoma. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK559113/

Hao, Y.-H., Doyle, J. M., Ramanathan, S., Gomez, T. S., Jia, D., Xu, M., Chen, Z. J., Billadeau, D. D., Rosen, M. K., & Potts, P. R. (2013). Regulation of WASH-Dependent Actin Polymerization and Protein Trafficking by Ubiquitination. *Cell*, *152*(5), 1051–1064. https://doi.org/10.1016/j.cell.2013.01.051

Haselmann, V., Gebhardt, C., Brechtel, I., Duda, A., Czerwinski, C., Sucker, A., Holland-Letz, T., Utikal, J., Schadendorf, D., & Neumaier, M. (2018). Liquid Profiling of Circulating Tumor DNA in Plasma of Melanoma Patients for Companion Diagnostics and Monitoring of BRAF Inhibitor Therapy. *Clinical Chemistry*, *64*(5), 830–842. https://doi.org/10.1373/clinchem.2017.281543

Hauschild, A. (2009). The Use of Serological Tumor Markers for Malignant Melanoma. *Onkologie*, *20*(6), 462–465. https://doi.org/10.1159/000219008

Healey, G. F., Lam, S., Boyle, P., Hamilton-Fairley, G., Peek, L. J., & Robertson, J. F. R. (2013). Signal stratification of autoantibody levels in serum samples and its application to the early detection of lung cancer. *Journal of Thoracic Disease*, *5*(5). https://doi.org/10.3978/j.issn.2072-1439.2013.08.65

Heidarzadeh, M., Zarebkohan, A., Rahbarghazi, R., & Sokullu, E. (2023). Protein corona and exosomes: New challenges and prospects. *Cell Communication and Signaling : CCS*, *21*, 64. https://doi.org/10.1186/s12964-023-01089-1

Hessvik, N. P., & Llorente, A. (2018). Current knowledge on exosome biogenesis and release. *Cellular and Molecular Life Sciences*, *75*(2), 193–208. https://doi.org/10.1007/s00018-017-2595-9

Hinestrosa, J. P., Kurzrock, R., Lewis, J. M., Schork, N. J., Schroeder, G., Kamat, A. M., Lowy, A. M., Eskander, R. N., Perrera, O., Searson, D., Rastegar, K., Hughes, J. R., Ortiz, V., Clark, I., Balcer, H. I., Arakelyan, L., Turner, R., Billings, P. R., Adler, M. J., … Krishnan, R. (2022). Early-stage multi-cancer detection using an extracellular vesicle protein-based blood test. *Communications Medicine*, *2*(1), 1–9. https://doi.org/10.1038/s43856-022-00088-6

Hodis, E., Watson, I. R., Kryukov, G. V., Arold, S. T., Imielinski, M., Theurillat, J.-P., Nickerson, E., Auclair, D., Li, L., Place, C., DiCara, D., Ramos, A. H., Lawrence, M. S., Cibulskis, K., Sivachenko, A., Voet, D., Saksena, G., Stransky, N., Onofrio, R. C., … Chin, L. (2012). A Landscape of Driver Mutations in Melanoma. *Cell*, *150*(2), 251–263. https://doi.org/10.1016/j.cell.2012.06.024

Holmgren, L., Szeles, A., Rajnavölgyi, E., Folkman, J., Klein, G., Ernberg, I., & Falk, K. I. (1999). Horizontal Transfer of DNA by the Uptake of Apoptotic Bodies. *Blood*, *93*(11), 3956–3963. https://doi.org/10.1182/blood.V93.11.3956

Hontani, K., Tsuchikawa, T., Hiwasa, T., Nakamura, T., Ueno, T., Kushibiki, T., Takahashi, M., Inoko, K., Takano, H., Takeuchi, S., Dosaka-Akita, H., Kuwatani, M., Sakamoto, N., Hatanaka, Y., Mitsuhashi, T., Shimada, H., Shichinohe, T., & Hirano, S. (2017). Identification of novel serum autoantibodies against EID3 in non-functional pancreatic neuroendocrine tumors. *Oncotarget*, *8*(63), 106206–106221. https://doi.org/10.18632/oncotarget.22175

Hood, J. L. (2019). Natural melanoma-derived extracellular vesicles. *Seminars in Cancer Biology*, *59*, 251–265. https://doi.org/10.1016/j.semcancer.2019.06.020

Hoon, D. S. B., Spugnardi, M., Kuo, C., Huang, S. K., Morton, D. L., & Taback, B. (2004). Profiling epigenetic inactivation of tumor suppressor genes in tumors and plasma from cutaneous melanoma patients. *Oncogene*, *23*(22), 4014–4022. https://doi.org/10.1038/sj.onc.1207505

Hristov, M., Erl, W., Linder, S., & Weber, P. C. (2004). Apoptotic bodies from endothelial cells enhance the number and initiate the differentiation of human endothelial progenitor cells in vitro. *Blood*, *104*(9), 2761–2766. https://doi.org/10.1182/blood-2003-10-3614

Hsieh, C.-H., & Wang, Y.-C. (2022). Emerging roles of plasma gelsolin in tumorigenesis and modulating the tumor microenvironment. *The Kaohsiung Journal of Medical Sciences*, *38*(9), 819–825. https://doi.org/10.1002/kjm2.12578

Hu, T., Wolfram, J., & Srivastava, S. (2021). Extracellular Vesicles in Cancer Detection: Hopes and Hypes. *Trends in Cancer*, *7*(2), 122–133. https://doi.org/10.1016/j.trecan.2020.09.003

Huang, C., Neupane, Y. R., Lim, X. C., Shekhani, R., Czarny, B., Wacker, M. G., Pastorin, G., & Wang, J.-W. (2021). Chapter Two – Extracellular vesicles in cardiovascular disease. In G. S. Makowski (Ed.), *Advances in Clinical Chemistry* (Vol. 103, pp. 47–95). Elsevier. https://doi.org/10.1016/bs.acc.2020.08.006

Huber, V., Fais, S., Iero, M., Lugini, L., Canese, P., Squarcina, P., Zaccheddu, A., Colone, M., Arancia, G., Gentile, M., Seregni, E., Valenti, R., Ballabio, G., Belli, F., Leo, E., Parmiani, G., & Rivoltini, L. (2005). Human Colorectal Cancer Cells Induce T-Cell Death Through Release of Proapoptotic Microvesicles: Role in Immune Escape. *Gastroenterology*, *128*(7), 1796–1804. https://doi.org/10.1053/j.gastro.2005.03.045

Ibanez, B., James, S., Agewall, S., Antunes, M. J., Bucciarelli-Ducci, C., Bueno, H., Caforio, A. L. P., Crea, F., Goudevenos, J. A., Halvorsen, S., Hindricks, G., Kastrati, A., Lenzen, M. J., Prescott, E., Roffi, M., Valgimigli, M., Varenhorst, C., Vranckx, P., Widimský, P., & ESC Scientific Document Group. (2018). 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *European Heart Journal*, *39*(2), 119–177. https://doi.org/10.1093/eurheartj/ehx393

IJzerman, M. J., de Boer, J., Azad, A., Degeling, K., Geoghegan, J., Hewitt, C., Hollande, F., Lee, B., To, Y. H., Tothill, R. W., Wright, G., Tie, J., & Dawson, S.-J. (2021). Towards Routine Implementation of Liquid Biopsies in Cancer Management: It Is Always Too Early, until Suddenly It Is Too Late. *Diagnostics*, *11*(1), 1. https://doi.org/10.3390/diagnostics11010103

Isola, A. L., Eddy, K., & Chen, S. (2016). Biology, Therapy and Implications of Tumor Exosomes in the Progression of Melanoma. *Cancers*, *8*(12), 12. https://doi.org/10.3390/cancers8120110

Ito, K., Yamamoto, T., Hayashi, Y., Sato, S., Nakayama, J., Urabe, F., Shimasaki, T., Nakamura, E., Matui, Y., Fujimoto, H., Kimura, T., Egawa, S., Ochiya, T., & Yamamoto, Y. (2023). Osteoblast-derived extracellular vesicles exert osteoblastic and tumor-suppressive functions via SERPINA3 and LCN2 in prostate cancer. *Molecular Oncology*, *17*(10), 2147–2167. https://doi.org/10.1002/1878-0261.13484

Jaé, N., McEwan, D. G., Manavski, Y., Boon, R. A., & Dimmeler, S. (2015). Rab7a and Rab27b control secretion of endothelial microRNA through extracellular vesicles. *FEBS Letters*, *589*(20PartB), 3182–3188. https://doi.org/10.1016/j.febslet.2015.08.040

Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., & Thun, M. J. (2008). Cancer Statistics, 2008. *CA: A Cancer Journal for Clinicians*, *58*(2), 71–96. https://doi.org/10.3322/CA.2007.0010

Jiang, D., Wang, Y., Liu, M., Si, Q., Wang, T., Pei, L., Wang, P., Ye, H., Shi, J., Wang, X., Song, C., Wang, K., Dai, L., & Zhang, J. (2020). A panel of autoantibodies against tumor-associated antigens in the early immunodiagnosis of lung cancer. *Immunobiology*, *225*(1), 151848. https://doi.org/10.1016/j.imbio.2019.09.007

Jin, Y., Kim, S. C., Kim, H. J., Ju, W., Kim, Y. H., & Kim, H.-J. (2017). Use of auto-antibodies against tumor-associated antigens as serum biomarkers for primary screening of cervical cancer. *Oncotarget*, *8*(62), 105425–105439. https://doi.org/10.18632/oncotarget.22231

Josson, S., Gururajan, M., Sung, S. Y., Hu, P., Shao, C., Zhau, H. E., Liu, C., Lichterman, J., Duan, P., Li, Q., Rogatko, A., Posadas, E. M., Haga, C. L., & Chung, L. W. K. (2015). Stromal fibroblast-derived miR-409 promotes epithelial-to-mesenchymal transition and prostate tumorigenesis. *Oncogene*, *34*(21), 2690–2699. https://doi.org/10.1038/onc.2014.212

Kahlert, C., Melo, S. A., Protopopov, A., Tang, J., Seth, S., Koch, M., Zhang, J., Weitz, J., Chin, L., Futreal, A., & Kalluri, R. (2014). Identification of Double-stranded Genomic DNA Spanning All Chromosomes with Mutated *KRAS* and *p53* DNA in the Serum Exosomes of Patients with Pancreatic Cancer*. *Journal of Biological Chemistry*, *289*(7), 3869–3875. https://doi.org/10.1074/jbc.C113.532267

Kaiser, S., Vassell, R., Pinckney, R. G., Holmes, T. E., & James, T. A. (2014). Clinical impact of biopsy method on the quality of surgical management in melanoma. *Journal of Surgical Oncology*, *109*(8), 775–779. https://doi.org/10.1002/jso.23580

Kalluri, R., & LeBleu, V. S. (2020). The biology, function, and biomedical applications of exosomes. *Science*, *367*(6478), eaau6977. https://doi.org/10.1126/science.aau6977

Kamerkar, S., LeBleu, V. S., Sugimoto, H., Yang, S., Ruivo, C. F., Melo, S. A., Lee, J. J., & Kalluri, R. (2017). Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. *Nature*, *546*(7659), 498–503. https://doi.org/10.1038/nature22341

Kamińska, P., Buszka, K., Zabel, M., Nowicki, M., Alix-Panabières, C., & Budna-Tukan, J. (2021). Liquid Biopsy in Melanoma: Significance in Diagnostics, Prediction and Treatment Monitoring. *International Journal of Molecular Sciences*, *22*(18), 18. https://doi.org/10.3390/ijms22189714

Kang, M. H., Park, M. J., Yoo, H. J., hyuk, K. Y., Lee, S. G., Kim, S. R., Yeom, D. W., Kang, M. J., & Choi, Y. W. (2014). RIPL peptide (IPLVVPLRRRRRRRRC)-conjugated liposomes for enhanced intracellular drug delivery to hepsin-expressing cancer cells. *European Journal of Pharmaceutics and Biopharmaceutics*, *87*(3), 489–499. https://doi.org/10.1016/j.ejpb.2014.03.016

Kang, Y.-T., Purcell, E., Palacios-Rolston, C., Lo, T.-W., Ramnath, N., Jolly, S., & Nagrath, S. (2019). Isolation and Profiling of Circulating Tumor-Associated Exosomes Using Extracellular Vesicular Lipid–Protein Binding Affinity Based Microfluidic Device. *Small*, *15*(47), 1903600. https://doi.org/10.1002/smll.201903600

Kari, O. K., Ndika, J., Parkkila, P., Louna, A., Lajunen, T., Puustinen, A., Viitala, T., Alenius, H., & Urtti, A. (2020). In situ analysis of liposome hard and soft protein corona structure and composition in a single label-free workflow. *Nanoscale*, *12*(3), 1728–1741. https://doi.org/10.1039/c9nr08186k

Kawagoe, H., Yamada, A., Matsumoto, H., Ito, M., Ushijima, K., Nishida, T., Yakushiji, M., & Itoh, K. (2000). Serum MAGE-4 protein in ovarian cancer patients. *Gynecologic Oncology*, *76*(3). https://doi.org/10.1006/gyno.1999.5701

Keller, S., Ridinger, J., Rupp, A.-K., Janssen, J. W., & Altevogt, P. (2011). Body fluid derived exosomes as a novel template for clinical diagnostics. *Journal of Translational Medicine*, *9*(1), 86. https://doi.org/10.1186/1479-5876-9-86

Kim, D.-D., Yang, C.-S., Chae, H.-D., Kwak, S.-G., & Jeon, C.-H. (2017). Melanoma antigen-encoding gene family member A1-6 and hTERT in the detection of circulating tumor cells following CD45- depletion and RNA extraction. *Oncology Letters*, *14*(1), 837–843. https://doi.org/10.3892/ol.2017.6226

Kim, R. H., & Meehan, S. A. (2017). Immunostain use in the diagnosis of melanomas referred to a tertiary medical center: A 15-year retrospective review (2001–2015). *Journal of Cutaneous Pathology*, *44*(3), 221–227. https://doi.org/10.1111/cup.12867

Kiyohara, M., Aoi, J., Kajihara, I., Otuka, S., Kadomatsu, T., Fukushima, S., & Ihn, H. (2020). Serum anti-p53 autoantibodies in angiosarcoma. *The Journal of Dermatology*, *47*(8), 849–854. https://doi.org/10.1111/1346-8138.15416

Kobayashi, M., Katayama, H., Irajizad, E., Vykoukal, J. V., Fahrmann, J. F., Kundnani, D. L., Yu, C.-Y., Cai, Y., Hsiao, F. C., Yang, W.-L., Lu, Z., Celestino, J., Long, J. P., Do, K.-A., Lu, K. H., Ladd, J. J., Urban, N., Bast Jr., R. C., & Hanash, S. M. (2020). Proteome Profiling Uncovers an Autoimmune Response Signature That Reflects Ovarian Cancer Pathogenesis. *Cancers*, *12*(2), 2. https://doi.org/10.3390/cancers12020485

Kocher, T., Zheng, M., Bolli, M., Simon, R., Forster, T., Schultz-Thater, E., Remmel, E., Noppen, C., Schmid, U., Ackermann, D., Mihatsch, M. J., Gasser, T., Heberer, M., Sauter, G., & Spagnoli, G. C. (2002). Prognostic relevance of MAGE-A4 tumor antigen expression in transitional cell carcinoma of the urinary bladder: A tissue microarray study. *International Journal of Cancer*, *100*(6), 702–705. https://doi.org/10.1002/ijc.10540

Konoshenko, M. Y., Lekchnov, E. A., Vlassov, A. V., & Laktionov, P. P. (2018). Isolation of Extracellular Vesicles: General Methodologies and Latest Trends. *BioMed Research International*, *2018*, e8545347. https://doi.org/10.1155/2018/8545347

Kosaka, N., Iguchi, H., Hagiwara, K., Yoshioka, Y., Takeshita, F., & Ochiya, T. (2013). Neutral Sphingomyelinase 2 (nSMase2)-dependent Exosomal Transfer of Angiogenic MicroRNAs Regulate Cancer Cell Metastasis*. *Journal of Biological Chemistry*, *288*(15), 10849–10859. https://doi.org/10.1074/jbc.M112.446831

Kowal, E. J. K., Ter-Ovanesyan, D., Regev, A., & Church, G. M. (2017). Extracellular Vesicle Isolation and Analysis by Western Blotting. *Methods in Molecular Biology (Clifton, N.J.)*, *1660*, 143–152. https://doi.org/10.1007/978-1-4939-7253-1_12

Kowal, J., Tkach, M., & Théry, C. (2014). Biogenesis and secretion of exosomes. *Current Opinion in Cell Biology*, *29*, 116–125. https://doi.org/10.1016/j.ceb.2014.05.004

Koziol, J. A., Imai, H., Dai, L., Zhang, J.-Y., & Tan, E. M. (2018). Early detection of hepatocellular carcinoma using autoantibody profiles from a panel of tumor-associated antigens. *Cancer Immunology, Immunotherapy*, *67*(5), 835–841. https://doi.org/10.1007/s00262-018-2135-y

Kratzer, A., Giral, H., & Landmesser, U. (2014). High-density lipoproteins as modulators of endothelial cell functions: Alterations in patients with coronary artery disease. *Cardiovascular Research*, *103*(3), 350–361. https://doi.org/10.1093/cvr/cvu139

Kulkarni, P., Shiraishi, T., Rajagopalan, K., Kim, R., Mooney, S. M., & Getzenberg, R. H. (2012). Cancer/testis antigens and urological malignancies. *Nature Reviews Urology*, *9*(7), 386–396. https://doi.org/10.1038/nrurol.2012.117

Kulshreshtha, A., Singh, S., Ahmad, M., Khanna, K., Ahmad, T., Agrawal, A., & Ghosh, B. (2019). Simvastatin mediates inhibition of exosome synthesis, localization and secretion via multicomponent interventions. *Scientific Reports*, *9*(1), 16373. https://doi.org/10.1038/s41598-019-52765-7

La Shu, S., Yang, Y., Allen, C. L., Hurley, E., Tung, K. H., Minderman, H., Wu, Y., & Ernstoff, M. S. (2020). Purity and yield of melanoma exosomes are dependent on isolation method. *Journal of Extracellular Vesicles*, *9*(1), 1692401. https://doi.org/10.1080/20013078.2019.1692401

Laban, S., Atanackovic, D., Luetkens, T., Knecht, R., Busch, C.-J., Freytag, M., Spagnoli, G., Ritter, G., Hoffmann, T. K., Knuth, A., Sauter, G., Wilczak, W., Blessmann, M., Borgmann, K., Muenscher, A., & Clauditz, T. S. (2014). Simultaneous cytoplasmic and nuclear protein expression of melanoma antigen-A family and NY-ESO-1 cancer-testis antigens represents an independent marker for poor survival in head and neck cancer. *International Journal of Cancer*, *135*(5), 1142–1152. https://doi.org/10.1002/ijc.28752

Landegren, U., & Hammond, M. (2021). Cancer diagnostics based on plasma protein biomarkers: Hard times but great expectations. *Molecular Oncology*, *15*(6), 1715–1726. https://doi.org/10.1002/1878-0261.12809

Lannoy, M., Slove, S., Louedec, L., Choqueux, C., Journé, C., Michel, J.-B., & Jacob, M.-P. (2014). Inhibition of ERK1/2 phosphorylation: A new strategy to stimulate elastogenesis in the aorta. *Hypertension (Dallas, Tex.: 1979)*, *64*(2), 423–430. https://doi.org/10.1161/HYPERTENSIONAHA.114.03352

Lasithiotakis, K., Leiter, U., Krüger-Krasagakis, S., Tosca, A., & Garbe, C. (2006). Comparative analysis of incidence and clinical features of cutaneous malignant melanoma in Crete (Greece) and southern Germany (central Baden-Württemberg). *British Journal of Dermatology*, *154*(6), 1123–1127. https://doi.org/10.1111/j.1365-2133.2006.07163.x

Lee, A. K., & Potts, P. R. (2017). A Comprehensive Guide to the MAGE Family of Ubiquitin Ligases. *Journal of Molecular Biology*, *429*(8), 1114–1142. https://doi.org/10.1016/j.jmb.2017.03.005

Lee, J. H., Long, G. V., Boyd, S., Lo, S., Menzies, A. M., Tembe, V., Guminski, A., Jakrot, V., Scolyer, R. A., Mann, G. J., Kefford, R. F., Carlino, M. S., & Rizos, H. (2017). Circulating tumour DNA predicts response to anti-PD1 antibodies in metastatic melanoma. *Annals of Oncology*, *28*(5), 1130–1136. https://doi.org/10.1093/annonc/mdx026

Lee, K. Y. (1971). Loss of lipid to plastic tubing. *Journal of Lipid Research*, *12*(5), 635–636. https://doi.org/10.1016/S0022-2275(20)39484-0

Lee, T. H., Chennakrishnaiah, S., Audemard, E., Montermini, L., Meehan, B., & Rak, J. (2014). Oncogenic *ras*-driven cancer cell vesiculation leads to emission of double-stranded DNA capable of interacting with target cells. *Biochemical and Biophysical Research Communications*, *451*(2), 295–301. https://doi.org/10.1016/j.bbrc.2014.07.109

Li, B., Antonyak, M. A., Zhang, J., & Cerione, R. A. (2012). RhoA triggers a specific signaling pathway that generates transforming microvesicles in cancer cells. *Oncogene*, *31*(45), 4740–4749. https://doi.org/10.1038/onc.2011.636

Li, X., Cao, Y., Xu, X., Wang, C., Ni, Q., Lv, X., Yang, C., Zhang, Z., Qi, X., & Song, G. (2023). Sleep Deprivation Promotes Endothelial Inflammation and Atherogenesis by Reducing Exosomal miR-182-5p. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *43*(6), 995–1014. https://doi.org/10.1161/ATVBAHA.123.319026

Li, Y., He, X., Li, Q., Lai, H., Zhang, H., Hu, Z., Li, Y., & Huang, S. (2020). EV-origin: Enumerating the tissue-cellular origin of circulating extracellular vesicles using exLR profile. *Computational and Structural Biotechnology Journal*, *18*, 2851–2859. https://doi.org/10.1016/j.csbj.2020.10.002

Liam-Or, R., Faruqu, F. N., Walters, A., Han, S., Xu, L., Wang, J. T.-W., Oberlaender, J., Sanchez-Fueyo, A., Lombardi, G., Dazzi, F., Mailaender, V., & Al-Jamal, K. T. (2024). Cellular uptake and in vivo distribution of mesenchymal-stem-cell-derived extracellular vesicles are protein corona dependent. *Nature Nanotechnology*, *19*(6), 846–855. https://doi.org/10.1038/s41565-023-01585-y

Lianidou, E. S., Strati, A., & Markou, A. (2014). Circulating tumor cells as promising novel biomarkers in solid cancers. *Critical Reviews in Clinical Laboratory Sciences*, *51*(3), 160–171. https://doi.org/10.3109/10408363.2014.896316

Libby, P. (2021). The changing landscape of atherosclerosis. *Nature*, *592*(7855), 524–533. https://doi.org/10.1038/s41586-021-03392-8

Libby, P., & Hansson, G. K. (2019). From Focal Lipid Storage to Systemic Inflammation: JACC Review Topic of the Week. *Journal of the American College of Cardiology*, *74*(12), 1594–1607. https://doi.org/10.1016/j.jacc.2019.07.061

Lima, L. G., Chammas, R., Monteiro, R. Q., Moreira, M. E. C., & Barcinski, M. A. (2009). Tumor-derived microvesicles modulate the establishment of metastatic melanoma in a phosphatidylserine-dependent manner. *Cancer Letters*, *283*(2), 168–175. https://doi.org/10.1016/j.canlet.2009.03.041

Lin, D., Shen, L., Luo, M., Zhang, K., Li, J., Yang, Q., Zhu, F., Zhou, D., Zheng, S., Chen, Y., & Zhou, J. (2021). Circulating tumor cells: Biology and clinical significance. *Signal Transduction and Targeted Therapy*, *6*(1), 1–24. https://doi.org/10.1038/s41392-021-00817-8

Lin, K., Lipsitz, R., Miller, T., & Janakiraman, S. (2008). Benefits and Harms of Prostate-Specific Antigen Screening for Prostate Cancer: An Evidence Update for the U.S. Preventive Services Task Force. *Annals of Internal Medicine*, *149*(3), 192–199. https://doi.org/10.7326/0003-4819-149-3-200808050-00009

Liu, S., Zhang, X., Jiang, Q., & Liang, T. (2020b). Detection of circulating natural antibodies against CD25, MUC1, and VEGFR1 for early diagnosis of non-small cell lung cancer. *FEBS Open Bio*, *10*(7), 1288–1294. https://doi.org/10.1002/2211-5463.12878

Liu, S., Zhao, Y., Xu, Y., Sang, M., Zhao, R., Gu, L., & Shan, B. (2020a). MAGE-A genes as predictors of the outcome of laryngeal squamous cell carcinoma. *Oncology Letters*, *20*(4), 1–1. https://doi.org/10.3892/ol.2020.11920

Lomzenski, H. E., Thiele, G. M., Duryee, M. J., Chen, S.-C., Ye, F., Anderson, D. R., Mikuls, T. R., & Ormseth, M. J. (2022). Serum anti-malondialdehyde-acetaldehyde IgA antibody concentration improves prediction of coronary atherosclerosis beyond traditional risk factors in patients with rheumatoid arthritis. *Scientific Reports*, *12*(1), 10547. https://doi.org/10.1038/s41598-022-14954-9

Lu, M., Yuan, S., Li, S., Li, L., Liu, M., & Wan, S. (2019). The Exosome-Derived Biomarker in Atherosclerosis and Its Clinical Application. *Journal of Cardiovascular Translational Research*, *12*(1), 68–74. https://doi.org/10.1007/s12265-018-9796-y

Lucas, S., De Plaen, E., & Boon, T. (2000). MAGE-B5, MAGE-B6, MAGE-C2, and MAGE-C3: Four new members of the MAGE family with tumor-specific expression. *International Journal of Cancer*, *87*(1), 55–60. https://doi.org/10.1002/1097-0215(20000701)87:1<55::AID-IJC8>3.0.CO;2-J

Lucas, S., De Smet, C., Arden, K. C., Viars, C. S., Lethé, B., Lurquin, C., & Boon, T. (1998). Identification of a new MAGE gene with tumor-specific expression by representational difference analysis. *Cancer Research*, *58*(4), 743–752.

Luga, V., Zhang, L., Viloria-Petit, A. M., Ogunjimi, A. A., Inanlou, M. R., Chiu, E., Buchanan, M., Hosein, A. N., Basik, M., & Wrana, J. L. (2012). Exosomes Mediate Stromal Mobilization of Autocrine Wnt-PCP Signaling in Breast Cancer Cell Migration. *Cell*, *151*(7), 1542–1556. https://doi.org/10.1016/j.cell.2012.11.024

Luo, J., Yang, H., & Song, B.-L. (2020). Mechanisms and regulation of cholesterol homeostasis. *Nature Reviews Molecular Cell Biology*, *21*(4), 225–245. https://doi.org/10.1038/s41580-019-0190-7

Lurquin, C., De Smet, C., Brasseur, F., Muscatelli, F., Martelange, V., De Plaen, E., Brasseur, R., Monaco, A. P., & Boon, T. (1997). Two Members of the Human*MAGEB*Gene Family Located in Xp21.3 Are Expressed in Tumors of Various Histological Origins. *Genomics*, *46*(3), 397–408. https://doi.org/10.1006/geno.1997.5052

Lusta, K. A., Summerhill, V. I., Khotina, V. A., Sukhorukov, V. N., Glanz, V. Y., & Orekhov, A. N. (2024). The Role of Bacterial Extracellular Membrane Nanovesicles in Atherosclerosis: Unraveling a Potential Trigger. *Current Atherosclerosis Reports*, *26*(7), 289–304. https://doi.org/10.1007/s11883-024-01206-6

Lv, M., Zhu, X., Chen, W., Zhong, S., Hu, Q., Ma, T., Zhang, J., Chen, L., Tang, J., & Zhao, J. (2014). Exosomes mediate drug resistance transfer in MCF-7 breast cancer cells and a probable mechanism is delivery of P-glycoprotein. *Tumor Biology*, *35*(11), 10773–10779. https://doi.org/10.1007/s13277-014-2377-z

Ma, C., Jiang, F., Ma, Y., Wang, J., Li, H., & Zhang, J. (2019). Isolation and Detection Technologies of Extracellular Vesicles and Application on Cancer Diagnostic. *Dose-Response*, *17*(4), 1559325819891004. https://doi.org/10.1177/1559325819891004

Ma, J., & Frank, M. H. (2015). Isolation of Circulating Melanoma Cells. *Methods in Molecular Biology (Clifton, N.J.)*. https://doi.org/10.1007/7651_2015_300

Ma, L., Li, Y., Peng, J., Wu, D., Zhao, X., Cui, Y., Chen, L., Yan, X., Du, Y., & Yu, L. (2015). Discovery of the migrasome, an organelle mediating release of cytoplasmic contents during cell migration. *Cell Research*, *25*(1), 24–38. https://doi.org/10.1038/cr.2014.135

Ma, M. W., Medicherla, R. C., Qian, M., Vega-Saenz de Miera, E., Friedman, E. B., Berman, R. S., Shapiro, R. L., Pavlick, A. C., Ott, P. A., Bhardwaj, N., Shao, Y., Osman, I., & Darvishian, F. (2012). Immune response in melanoma: An in-depth analysis of the primary tumor and corresponding sentinel lymph node. *Modern Pathology*, *25*(7), 1000–1010. https://doi.org/10.1038/modpathol.2012.43

Ma, X., Chen, Z., Hua, D., He, D., Wang, L., Zhang, P., Wang, J., Cai, Y., Gao, C., Zhang, X., Zhang, F., Wang, T., Hong, T., Jin, L., Qi, X., Chen, S., Gu, X., Yang, D., Pan, Q., … Yao, X. (2014). Essential role for TrpC5-containing extracellular vesicles in breast cancer with chemotherapeutic resistance. *Proceedings of the National Academy of Sciences*, *111*(17), 6389–6394. https://doi.org/10.1073/pnas.1400272111

MacKie, R. M. (1990). Clinical recognition of early invasive malignant melanoma. *BMJ : British Medical Journal*, *301*(6759), 1005–1006.

MacKie, R. M., Hauschild, A., & Eggermont, A. M. M. (2009). Epidemiology of invasive cutaneous melanoma. *Annals of Oncology*, *20*, vi1–vi7. https://doi.org/10.1093/annonc/mdp252

Madrigal-Matute, J., Fernandez-Garcia, C.-E., Blanco-Colio, L. M., Burillo, E., Fortuño, A., Martinez-Pinna, R., Llamas-Granda, P., Beloqui, O., Egido, J., Zalba, G., & Martin-Ventura, J. L. (2015). Thioredoxin-1/peroxiredoxin-1 as sensors of oxidative stress mediated by NADPH oxidase activity in atherosclerosis. *Free Radical Biology and Medicine*, *86*, 352–361. https://doi.org/10.1016/j.freeradbiomed.2015.06.001

Mandel, P., & Metais, P. (1948). [Nuclear Acids In Human Blood Plasma]. *Comptes Rendus Des Seances De La Societe De Biologie Et De Ses Filiales*, *142*(3–4), 241–243.

Marcar, L., Ihrig, B., Hourihan, J., Bray, S. E., Quinlan, P. R., Jordan, L. B., Thompson, A. M., Hupp, T. R., & Meek, D. W. (2015). MAGE-A Cancer/Testis Antigens Inhibit MDM2 Ubiquitylation Function and Promote Increased Levels of MDM4. *PLOS ONE*, *10*(5), e0127713. https://doi.org/10.1371/journal.pone.0127713

Margue, C., Reinsbach, S., Philippidou, D., Beaume, N., Walters, C., Schneider, J. G., Nashan, D., Behrmann, I., & Kreis, S. (2015). Comparison of a healthy miRNome with melanoma patient miRNomes: Are microRNAs suitable serum biomarkers for cancer? *Oncotarget*, *6*(14), 12110–12127. https://doi.org/10.18632/oncotarget.3661

Markou, A., Tzanikou, E., & Lianidou, E. (2022). The potential of liquid biopsy in the management of cancer patients. *Seminars in Cancer Biology*, *84*, 69–79. https://doi.org/10.1016/j.semcancer.2022.03.013

Markovic, S. N., Erickson, L. A., Rao, R. D., McWilliams, R. R., Kottschade, L. A., Creagan, E. T., Weenig, R. H., Hand, J. L., Pittelkow, M. R., Pockaj, B. A., Bardia, A., Vachon, C. M., Schild, S. E., Laman, S. D., Maples, W. J., Pulido, J. S., & Cameron, J. D. (2007). Malignant Melanoma in the 21st Century, Part 1: Epidemiology, Risk Factors, Screening, Prevention, and Diagnosis. *Mayo Clinic Proceedings*, *82*(3), 364–380. https://doi.org/10.4065/82.3.364

Markowska, A., Antoszczak, M., Markowska, J., & Huczyński, A. (2020). Statins: HMG-CoA Reductase Inhibitors as Potential Anticancer Agents against Malignant Neoplasms in Women. *Pharmaceuticals*, *13*(12), 422. https://doi.org/10.3390/ph13120422

Marsh, M., & van Meer, G. (2008). No ESCRTs for Exosomes. *Science*, *319*(5867), 1191–1192. https://doi.org/10.1126/science.1155750

Martinez-Dominguez, M. V., Zottel, A., Šamec, N., Jovčevska, I., Dincer, C., Kahlert, U. D., & Nickel, A.-C. (2021). Current Technologies for RNA-Directed Liquid Diagnostics. *Cancers*, *13*(20), 20. https://doi.org/10.3390/cancers13205060

Massion, P. P., Healey, G. F., Peek, L. J., Fredericks, L., Sewell, H. F., Murray, A., & Robertson, J. F. R. (2017). Autoantibody Signature Enhances the Positive Predictive Power of Computed Tomography and Nodule-Based Risk Models for Detection of Lung Cancer. *Journal of Thoracic Oncology*, *12*(3), 578–584. https://doi.org/10.1016/j.jtho.2016.08.143

Maudes, E., Landa, J., Muñoz-Lopetegi, A., Armangue, T., Alba, M., Saiz, A., Graus, F., Dalmau, J., & Sabater, L. (2020). Clinical significance of Kelch-like protein 11 antibodies. *Neurology Neuroimmunology & Neuroinflammation*, *7*(3), e666. https://doi.org/10.1212/NXI.0000000000000666

Meek, D. W., & Marcar, L. (2012). MAGE-A antigens as targets in tumour therapy. *Cancer Letters*, *324*(2), 126–132. https://doi.org/10.1016/j.canlet.2012.05.011

Meistere, I., Werner, S., Zayakin, P., Siliņa, K., Rulle, U., Pismennaja, A., Šantare, D., Kikuste, I., Isajevs, S., Leja, M., Kupčinskas, L., Kupčinskas, J., Jonaitis, L., Wu, C.-Y., Brenner, H., Linē, A., & Kalniņa, Z. (2017). The Prevalence of Cancer-Associated Autoantibodies in Patients with Gastric Cancer and Progressive Grades of Pre-malignant Lesions. *Cancer Epidemiology, Biomarkers & Prevention*, *26*(10), 1564–1574. https://doi.org/10.1158/1055-9965.EPI-17-0238

Metais, P., & Mandel, P. (1948). Les acides nucléiques du plasma sanguin chez l'homme [Nuclear Acids In Human Blood Plasma]. *C R Seances Soc Biol Fil.*, *142*(3–4), 241–243.

Meyle, K. D., & Guldberg, P. (2009). Genetic risk factors for melanoma. *Human Genetics*, *126*(4), 499–510. https://doi.org/10.1007/s00439-009-0715-9

Miao, S., Zhou, S.-Y., Han, C.-S., Zhang, L.-N., Sun, H.-B., & Yang, B. (2015). Clinicopathological significance of matrix metalloproteinase-7 protein expression in esophageal cancer: A meta-analysis. *Drug Design, Development and Therapy*, *9*, 3729–3740. https://doi.org/10.2147/DDDT.S85987

Minciacchi, V. R., Freeman, M. R., & Di Vizio, D. (2015a). Extracellular Vesicles in Cancer: Exosomes, Microvesicles and the Emerging Role of Large Oncosomes. *Seminars in Cell & Developmental Biology*, *40*, 41–51. https://doi.org/10.1016/j.semcdb.2015.02.010

Minciacchi, V. R., You, S., Spinelli, C., Morley, S., Zandian, M., Aspuria, P.-J., Cavallini, L., Ciardiello, C., Sobreiro, M. R., Morello, M., Kharmate, G., Jang, S. C., Kim, D.-K., Hosseini-Beheshti, E., Guns, E. T., Gleave, M., Gho, Y. S., Mathivanan, S., Yang, W., … Vizio, D. D. (2015b). Large oncosomes contain distinct protein cargo and represent a separate functional class of tumor-derived extracellular vesicles. *Oncotarget*, *6*(13), 11327–11341. https://doi.org/10.18632/oncotarget.3598

Mintz, P. J., Kim, J., Do, K.-A., Wang, X., Zinner, R. G., Cristofanilli, M., Arap, M. A., Hong, W. K., Troncoso, P., Logothetis, C. J., Pasqualini, R., & Arap, W. (2003). Fingerprinting the circulating repertoire of antibodies from cancer patients. *Nature Biotechnology*, *21*(1), 57–63. https://doi.org/10.1038/nbt774

Mobarrez, F., Egberg, N., Antovic, J., Bröijersen, A., Jörneskog, G., & Wallén, H. (2012). Release of endothelial microparticles in vivo during atorvastatin treatment; a randomized double-blind placebo-controlled study. *Thrombosis Research*, *129*(1), 95–97. https://doi.org/10.1016/j.thromres.2011.09.027

Mohamed, A., Gonzalez, R. S., Lawson, D., Wang, J., & Cohen, C. (2013). SOX10 Expression in Malignant Melanoma, Carcinoma, and Normal Tissues. *Applied Immunohistochemistry & Molecular Morphology*, *21*(6), 506. https://doi.org/10.1097/PAI.0b013e318279bc0a

Mohan, S., Chemi, F., & Brady, G. (2017). Challenges and unanswered questions for the next decade of circulating tumour cell research in lung cancer. *Translational Lung Cancer Research*, *6*(4). https://doi.org/10.21037/tlcr.2017.06.04

Mohr, P., Eggermont, A. M. M., Hauschild, A., & Buzaid, A. (2009). Staging of cutaneous melanoma. *Annals of Oncology*, *20*, vi14–vi21. https://doi.org/10.1093/annonc/mdp256

Momen-Heravi, F. (2017). Isolation of Extracellular Vesicles by Ultracentrifugation. *Methods in Molecular Biology (Clifton, N.J.)*, *1660*, 25–32. https://doi.org/10.1007/978-1-4939-7253-1_3

Montella, A., Gavin, A., Middleton, R., Autier, P., & Boniol, M. (2009). Cutaneous melanoma mortality starting to change: A study of trends in Northern Ireland. *European Journal of Cancer*, *45*(13), 2360–2366. https://doi.org/10.1016/j.ejca.2009.03.004

Morelli, A. E., Larregina, A. T., Shufesky, W. J., Sullivan, M. L. G., Stolz, D. B., Papworth, G. D., Zahorchak, A. F., Logar, A. J., Wang, Z., Watkins, S. C., Falo, L. D., Jr, & Thomson, A. W. (2004). Endocytosis, intracellular sorting, and processing of exosomes by dendritic cells. *Blood*, *104*(10), 3257–3266. https://doi.org/10.1182/blood-2004-03-0824

Morello, M., Minciacchi, V., de Candia, P., Yang, J., Posadas, E., Kim, H., Griffiths, D., Bhowmick, N., Chung, L., Gandellini, P., Freeman, M., Demichelis, F., & DiVizio, D. (2013). Large oncosomes mediate intercellular transfer of functional microRNA. *Cell Cycle*, *12*(22), 3526–3536. https://doi.org/10.4161/cc.26539

Morvan, J., Rinaldi, B., & Friant, S. (2012). Pkh1/2-dependent phosphorylation of Vps27 regulates ESCRT-I recruitment to endosomes. *Molecular Biology of the Cell*, *23*(20), 4054–4064. https://doi.org/10.1091/mbc.e12-01-0001

Mulcahy, L. A., Pink, R. C., & Carter, D. R. F. (2014). Routes and mechanisms of extracellular vesicle uptake. *Journal of Extracellular Vesicles*, *3*(1), 24641. https://doi.org/10.3402/jev.v3.24641

Muralidharan-Chari, V., Clancy, J., Plou, C., Romao, M., Chavrier, P., Raposo, G., & D'Souza-Schorey, C. (2009). ARF6-Regulated Shedding of Tumor Cell-Derived Plasma Membrane Microvesicles. *Current Biology*, *19*(22), 1875–1885. https://doi.org/10.1016/j.cub.2009.09.059

Muralidharan-Chari, V., Clancy, J. W., Sedgwick, A., & D'Souza-Schorey, C. (2010). Microvesicles: Mediators of extracellular communication during cancer progression. *Journal of Cell Science*, *123*(10), 1603–1611. https://doi.org/10.1242/jcs.064386

Muscatelli, F., Walker, A. P., De Plaen, E., Stafford, A. N., & Monaco, A. P. (1995). Isolation and characterization of a MAGE gene family in the Xp21.3 region. *Proceedings of the National Academy of Sciences*, *92*(11), 4987–4991. https://doi.org/10.1073/pnas.92.11.4987

Myšíková, D., Adkins, I., Hradilová, N., Palata, O., Šimonek, J., Pozniak, J., Kolařík, J., Skallová-Fialová, A., Špíšek, R., & Lischke, R. (2017). Case-Control Study: Smoking History Affects the Production of Tumor Antigen–Specific Antibodies NY-ESO-1 in Patients with Lung Cancer in Comparison with Cancer Disease–Free Group. *Journal of Thoracic Oncology*, *12*(2), 249–257. https://doi.org/10.1016/j.jtho.2016.09.136

Naeger, D. M., Kohi, M. P., Webb, E. M., Phelps, A., Ordovas, K. G., & Newman, T. B. (2013). Correctly Using Sensitivity, Specificity, and Predictive Values in Clinical Practice: How to Avoid Three Common Pitfalls. *American Journal of Roentgenology*, *200*(6), W566–W570. https://doi.org/10.2214/AJR.12.9888

Nanami, T., Hoshino, I., Ito, M., Yajima, S., Oshima, Y., Suzuki, T., Shiratori, F., Nabeya, Y., Funahashi, K., & Shimada, H. (2020). Prevalence of autoantibodies against Ras-like GTPases, RalA, in patients with gastric cancer. *Molecular and Clinical Oncology*, *13*(4), 1–1. https://doi.org/10.3892/mco.2020.2098

Nanou, A., Miller, M. C., Zeune, L. L., de Wit, S., Punt, C. J. A., Groen, H. J. M., Hayes, D. F., de Bono, J. S., & Terstappen, L. W. M. M. (2020). Tumour-derived extracellular vesicles in blood of metastatic cancer patients associate with overall survival. *British Journal of Cancer*, *122*(6), 801–811. https://doi.org/10.1038/s41416-019-0726-9

Narayan, P., Ghosh, S., Philip, R., Barrett, J. C., McCormack, R. T., Odegaard, J. I., R Oxnard, G., Pracht, L. J., Williams, P. M., Kelloff, G. J., & Beaver, J. A. (2020). State of the Science and Future Directions for Liquid Biopsies in Drug Development. *The Oncologist*, *25*(9), 730–732. https://doi.org/10.1634/theoncologist.2020-0246

Neila, J., & Soyer, H. P. (2011). Key points in dermoscopy for diagnosis of melanomas, including difficult to diagnose melanomas, on the trunk and extremities. *The Journal of Dermatology*, *38*(1), 3–9. https://doi.org/10.1111/j.1346-8138.2010.01131.x

Nikanjam, M., Kato, S., & Kurzrock, R. (2022). Liquid biopsy: Current technology and clinical applications. *Journal of Hematology & Oncology*, *15*(1), 131. https://doi.org/10.1186/s13045-022-01351-y

Niu, C., Wang, X., Zhao, M., Cai, T., Liu, P., Li, J., Willard, B., Zu, L., Zhou, E., Li, Y., Pan, B., Yang, F., & Zheng, L. (n.d.). Macrophage Foam Cell–Derived Extracellular Vesicles Promote Vascular Smooth Muscle Cell Migration and Adhesion. *Journal of the American Heart Association*, *5*(10), e004099. https://doi.org/10.1161/JAHA.116.004099

Nordestgaard, B. G., & Varbo, A. (2014). Triglycerides and cardiovascular disease. *The Lancet*, *384*(9943), 626–635. https://doi.org/10.1016/S0140-6736(14)61177-6

Norman, M., Ter-Ovanesyan, D., Trieu, W., Lazarovits, R., Kowal, E. J. K., Lee, J. H., Chen-Plotkin, A. S., Regev, A., Church, G. M., & Walt, D. R. (2021). L1CAM is not associated with extracellular vesicles in human cerebrospinal fluid or plasma. *Nature Methods*, *18*(6), 631–634. https://doi.org/10.1038/s41592-021-01174-8

Obuchowski, N. A., & Bullen, J. A. (2018). Receiver operating characteristic (ROC) curves: Review of methods with applications in diagnostic medicine. *Physics in Medicine and Biology*, *63*(7), 07TR01. https://doi.org/10.1088/1361-6560/aab4b1

Ohsie, S. J., Sarantopoulos, G. P., Cochran, A. J., & Binder, S. W. (2008). Immunohistochemical characteristics of melanoma. *Journal of Cutaneous Pathology*, *35*(5), 433–444. https://doi.org/10.1111/j.1600-0560.2007.00891.x

Okamura, R., Piccioni, D. E., Boichard, A., Lee, S., Jimenez, R. E., Sicklick, J. K., Kato, S., & Kurzrock, R. (2021). High prevalence of clonal hematopoiesis-type genomic abnormalities in cell-free DNA in invasive gliomas after treatment. *International Journal of Cancer*, *148*(11), 2839–2847. https://doi.org/10.1002/ijc.33481

O'Leary, B., Hrebien, S., Beaney, M., Fribbens, C., Garcia-Murillas, I., Jiang, J., Li, Y., Huang Bartlett, C., André, F., Loibl, S., Loi, S., Cristofanilli, M., & Turner, N. C. (2019). Comparison of BEAMing and Droplet Digital PCR for Circulating Tumor DNA Analysis. *Clinical Chemistry*, *65*(11), 1405–1413. https://doi.org/10.1373/clinchem.2019.305805

Onódi, Z., Pelyhe, C., Terézia Nagy, C., Brenner, G. B., Almási, L., Kittel, Á., Manček-Keber, M., Ferdinandy, P., Buzás, E. I., & Giricz, Z. (2018). Isolation of High-Purity Extracellular Vesicles by the Combination of Iodixanol Density Gradient Ultracentrifugation and Bind-Elute Chromatography From Blood Plasma. *Frontiers in Physiology*, *9*. https://doi.org/10.3389/fphys.2018.01479

Onuma, T., Asare-Werehene, M., Yoshida, Y., & Tsang, B. K. (2022). Exosomal Plasma Gelsolin Is an Immunosuppressive Mediator in the Ovarian Tumor Microenvironment and a Determinant of Chemoresistance. *Cells*, *11*(20), 3305. https://doi.org/10.3390/cells11203305

Oshima, Y., Shimada, H., Yajima, S., Nanami, T., Matsushita, K., Nomura, F., Kainuma, O., Takiguchi, N., Soda, H., Ueda, T., Iizasa, T., Yamamoto, N., Yamamoto, H., Nagata, M., Yokoi, S., Tagawa, M., Ohtsuka, S., Kuwajima, A., Murakami, A., & Kaneko, H. (2016). NY-ESO-1 autoantibody as a tumor-specific biomarker for esophageal cancer: Screening in 1969 patients with various cancers. *Journal of Gastroenterology*, *51*(1), 30–34. https://doi.org/10.1007/s00535-015-1078-8

Ostrowski, M., Carmo, N. B., Krumeich, S., Fanget, I., Raposo, G., Savina, A., Moita, C. F., Schauer, K., Hume, A. N., Freitas, R. P., Goud, B., Benaroch, P., Hacohen, N., Fukuda, M., Desnos, C., Seabra, M. C., Darchen, F., Amigorena, S., Moita, L. F., & Thery, C. (2010). Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nature Cell Biology*, *12*(1), 19–30. https://doi.org/10.1038/ncb2000

Palasubramaniam, J., Wang, X., & Peter, K. (2019). Myocardial Infarction – From Atherosclerosis to Thrombosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *39*(8), e176–e185. https://doi.org/10.1161/ATVBAHA.119.312578

Pan, B.-T., & Johnstone, R. M. (1983). Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: Selective externalization of the receptor. *Cell*, *33*(3), 967–978. https://doi.org/10.1016/0092-8674(83)90040-5

Pan, J., Yu, L., Wu, Q., Lin, X., Liu, S., Hu, S., Rosa, C., Eichinger, D., Pino, I., Zhu, H., Qian, J., & Huang, Y. (2020). Integration of IgA and IgG Autoantigens Improves Performance of Biomarker Panels for Early Diagnosis of Lung Cancer*. *Molecular & Cellular Proteomics*, *19*(3), 490–500. https://doi.org/10.1074/mcp.RA119.001905

Pan, Y., Liang, H., Liu, H., Li, D., Chen, X., Li, L., Zhang, C.-Y., & Zen, K. (2014). Platelet-Secreted MicroRNA-223 Promotes Endothelial Cell Apoptosis Induced by Advanced Glycation End Products via Targeting the Insulin-like Growth Factor 1 Receptor. *The Journal of Immunology*, *192*(1), 437–446. https://doi.org/10.4049/jimmunol.1301790

Pap, E., Pállinger, É., Pásztói, M., & Falus, A. (2009). Highlights of a new type of intercellular communication: Microvesicle-based information transfer. *Inflammation Research*, *58*(1), 1–8. https://doi.org/10.1007/s00011-008-8210-7

Papac-Milicevic, N., Busch, C. J.-L., & Binder, C. J. (2016). Chapter One – Malondialdehyde Epitopes as Targets of Immunity and the Implications for Atherosclerosis. In F. W. Alt (Ed.), *Advances in Immunology* (Vol. 131, pp. 1–59). Academic Press. https://doi.org/10.1016/bs.ai.2016.02.001

Parikh, R., Mathai, A., Parikh, S., Chandra Sekhar, G., & Thomas, R. (2008). Understanding and using sensitivity, specificity and predictive values. *Indian Journal of Ophthalmology*, *56*(1), 45–50.

Patard, J. J., Brasseur, F., Gil-Diez, S., Radvanyi, F., Marchand, M., François, P., Abi-Aad, A., Van Cangh, P., Abbou, C. C., & Chopin, D. (1995). Expression of MAGE genes in transitional-cell carcinomas of the urinary bladder. *International Journal of Cancer*, *64*(1), 60–64. https://doi.org/10.1002/ijc.2910640112

Patti, G., Calandra, E., De Bellis, A., Gallizia, A., Crocco, M., Napoli, F., Allegri, A. M. E., Thiabat, H. F., Bellastella, G., Maiorino, M. I., Garrè, M. L., Parodi, S., Maghnie, M., & di Iorgi, N. (2020). Antibodies Against Hypothalamus and Pituitary Gland in Childhood-Onset Brain Tumors and Pituitary Dysfunction. *Frontiers in Endocrinology*, *11*. https://doi.org/10.3389/fendo.2020.00016

Peinado, H., Alečković, M., Lavotshkin, S., Matei, I., Costa-Silva, B., Moreno-Bueno, G., Hergueta-Redondo, M., Williams, C., García-Santos, G., Ghajar, C. M., Nitadori-Hoshino, A., Hoffman, C., Badal, K., Garcia, B. A., Callahan, M. K., Yuan, J., Martins, V. R., Skog, J., Kaplan, R. N., … Lyden, D. (2012). Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nature Medicine*, *18*(6), 883–891. https://doi.org/10.1038/nm.2753

Perisetti, A., Bellamkonda, M., Konda, M., Edwards, S., Ali Khan, S., Bansal, P., Hu, Z.-D., & Goyal, H. (2020). Tumor-associated antigens and their antibodies in the screening, diagnosis, and monitoring of esophageal cancers. *European Journal of Gastroenterology & Hepatology*, *32*(7), 779. https://doi.org/10.1097/MEG.0000000000001718

Perkins, G., Lu, H., Garlan, F., & Taly, V. (2017). Chapter Three - Droplet-Based Digital PCR: Application in Cancer Research. In G. S. Makowski (Ed.), *Advances in Clinical Chemistry* (Vol. 79, pp. 43–91). Elsevier. https://doi.org/10.1016/bs.acc.2016.10.001

Pfeffer, M. A., & Braunwald, E. (1990). Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. *Circulation*, *81*(4), 1161–1172. https://doi.org/10.1161/01.CIR.81.4.1161

Pfeffer, S. R., Grossmann, K. F., Cassidy, P. B., Yang, C. H., Fan, M., Kopelovich, L., Leachman, S. A., & Pfeffer, L. M. (2015). Detection of Exosomal miRNAs in the Plasma of Melanoma Patients. *Journal of Clinical Medicine*, *4*(12), 12. https://doi.org/10.3390/jcm4121957

Phallen, J., Sausen, M., Adleff, V., Leal, A., Hruban, C., White, J., Anagnostou, V., Fiksel, J., Cristiano, S., Papp, E., Speir, S., Reinert, T., Orntoft, M.-B. W., Woodward, B. D., Murphy, D., Parpart-Li, S., Riley, D., Nesselbush, M., Sengamalay, N., … Velculescu, V. E. (2017). Direct detection of early-stage cancers using circulating tumor DNA. *Science Translational Medicine*, *9*(403), eaan2415. https://doi.org/10.1126/scitranslmed.aan2415

Phillips, M. C. (2014). Molecular mechanisms of cellular cholesterol efflux. *The Journal of Biological Chemistry*, *289*(35), 24020–24029. https://doi.org/10.1074/jbc.R114.583658

Piccin, A., Murphy, W. G., & Smith, O. P. (2007). Circulating microparticles: Pathophysiology and clinical implications. *Blood Reviews*, *21*(3), 157–171. https://doi.org/10.1016/j.blre.2006.09.001

Pietrowska, M., Zebrowska, A., Gawin, M., Marczak, L., Sharma, P., Mondal, S., Mika, J., Polańska, J., Ferrone, S., Kirkwood, J. M., Widlak, P., & Whiteside, T. L. (2021). Proteomic profile of melanoma cell-derived small extracellular vesicles in patients' plasma: A potential correlate of melanoma progression. *Journal of Extracellular Vesicles*, *10*(4), e12063. https://doi.org/10.1002/jev2.12063

Pinals, R. L., Yang, D., Rosenberg, D. J., Chaudhary, T., Crothers, A. R., Iavarone, A. T., Hammel, M., & Landry, M. P. (2020). Quantitative Protein Corona Composition and Dynamics on Carbon Nanotubes in Biological Environments. *Angewandte Chemie (International Ed. in English)*, *59*(52), 23668–23677. https://doi.org/10.1002/anie.202008175

Pineda, C. T., & Potts, P. R. (2015). Oncogenic MAGEA-TRIM28 ubiquitin ligase downregulates autophagy by ubiquitinating and degrading AMPK in cancer. *Autophagy*, *11*(5), 844–846. https://doi.org/10.1080/15548627.2015.1034420

Pineda, C. T., Ramanathan, S., Fon Tacer, K., Weon, J. L., Potts, M. B., Ou, Y.-H., White, M. A., & Potts, P. R. (2015). Degradation of AMPK by a Cancer-Specific Ubiquitin Ligase. *Cell*, *160*(4), 715–728. https://doi.org/10.1016/j.cell.2015.01.034

Plaks, V., Koopman, C. D., & Werb, Z. (2013). Cancer. Circulating tumor cells. *Science (New York, N.Y.)*, *341*(6151), 1186–1188. https://doi.org/10.1126/science.1235226

Pols, M. S., & Klumperman, J. (2009). Trafficking and function of the tetraspanin CD63. *Experimental Cell Research*, *315*(9), 1584–1592. https://doi.org/10.1016/j.yexcr.2008.09.020

Poojary, M., Jishnu, P. V., & Kabekkodu, S. P. (2020). Prognostic Value of Melanoma-Associated Antigen-A (MAGE-A) Gene Expression in Various Human Cancers: A Systematic Review and Meta-analysis of 7428 Patients and 44 Studies. *Molecular Diagnosis & Therapy*, *24*(5), 537–555. https://doi.org/10.1007/s40291-020-00476-5

Postel, M., Roosen, A., Laurent-Puig, P., Taly, V., & Wang-Renault, S.-F. (2018). Droplet-based digital PCR and next generation sequencing for monitoring circulating tumor DNA: A cancer diagnostic perspective. *Expert Review of Molecular Diagnostics*, *18*(1), 7–17. https://doi.org/10.1080/14737159.2018.1400384

Poznyak, A. V., Grechko, A. V., Orekhova, V. A., Khotina, V., Ivanova, E. A., & Orekhov, A. N. (2020). NADPH Oxidases and Their Role in Atherosclerosis. *Biomedicines*, *8*(7), 7. https://doi.org/10.3390/biomedicines8070206

Prieto, V. G., Mourad-Zeidan, A. A., Melnikova, V., Johnson, M. M., Lopez, A., Diwan, A. H., Lazar, A. J. F., Shen, S. S., Zhang, P. S., Reed, J. A., Gershenwald, J. E., Raz, A., & Bar-Eli, M. (2006). Galectin-3 Expression Is Associated with Tumor Progression and Pattern of Sun Exposure in Melanoma. *Clinical Cancer Research*, *12*(22), 6709–6715. https://doi.org/10.1158/1078-0432.CCR-06-0758

Prüss, H., Voltz, R., Flath, B., Rudolph, B., Klingebiel, R., Zschenderlein, R., & Prass, K. (2007). Anti-Ta-associated paraneoplastic encephalitis with occult testicular intratubular germ-cell neoplasia. *Journal of Neurology, Neurosurgery & Psychiatry*, *78*(6), 651–652. https://doi.org/10.1136/jnnp.2006.101964

Puglisi, R., Bellenghi, M., Pontecorvi, G., Pallante, G., Carè, A., & Mattia, G. (2021). Biomarkers for Diagnosis, Prognosis and Response to Immunotherapy in Melanoma. *Cancers*, *13*(12), 12. https://doi.org/10.3390/cancers13122875

Puhm, F., Boilard, E., & Machlus, K. R. (2021). Platelet Extracellular Vesicles. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *41*(1), 87–96. https://doi.org/10.1161/ATVBAHA.120.314644

Purdue, M. P., From, L., Armstrong, B. K., Kricker, A., Gallagher, R. P., McLaughlin, J. R., Klar, N. S., Marrett, L. D., & for the Genes, E., and Melanoma Study Group. (2005). Etiologic and Other Factors Predicting Nevus-Associated Cutaneous Malignant Melanoma. *Cancer Epidemiology, Biomarkers & Prevention*, *14*(8), 2015–2022. https://doi.org/10.1158/1055-9965.EPI-05-0097

Qin, H., Chen, J., Bouchekioua-Bouzaghou, K., Meng, Y.-M., Griera, J. B., Jiang, X., Kong, X., Wang, M., Xu, Q., & Wong, P.-P. (2023). Immunization with a multi-antigen targeted DNA vaccine eliminates chemoresistant pancreatic cancer by disrupting tumor-stromal cell crosstalk. *Journal of Translational Medicine*, *21*(1), 702. https://doi.org/10.1186/s12967-023-04519-3

Qin, J., Wang, S., Shi, J., Ma, Y., Wang, K., Ye, H., Zhang, X., Wang, P., Wang, X., Song, C., Dai, L., Wang, K., Jiang, B., & Zhang, J. (2019). Using recursive partitioning approach to select tumor-associated antigens in immunodiagnosis of gastric adenocarcinoma. *Cancer Science*, *110*(6), 1829–1841. https://doi.org/10.1111/cas.14013

Radeghieri, A., Alacqua, S., Zendrini, A., Previcini, V., Todaro, F., Martini, G., Ricotta, D., & Bergese, P. (2022). Active antithrombin glycoforms are selectively physiosorbed on plasma extracellular vesicles. *Journal of Extracellular Biology*, *1*(9), e57. https://doi.org/10.1002/jex2.57

Radeghieri, A., & Bergese, P. (2023). The biomolecular corona of extracellular nanoparticles holds new promises for advancing clinical molecular diagnostics. *Expert Review of Molecular Diagnostics*, *23*(6), 471–474. https://doi.org/10.1080/14737159.2023.2215927

Ragusa, M., Barbagallo, C., Statello, L., Caltabiano, R., Russo, A., Puzzo, L., Avitabile, T., Longo, A., Toro, M. D., Barbagallo, D., Valadi, H., Di Pietro, C., Purrello, M., & Reibaldi, M. (2015). miRNA profiling in vitreous humor, vitreal exosomes and serum from uveal melanoma patients: Pathological and diagnostic implications. *Cancer Biology & Therapy*, *16*(9), 1387–1396. https://doi.org/10.1080/15384047.2015.1046021

Rai, A., Huynh, K., Poh, Q. H., Fang, H., Claridge, B., Cross, J., Duong, T., Duarte, C., Marwick, T. H., Meikle, P., & Greening, D. W. (2024). Multi-omics discovery of hallmark protein and lipid features of circulating small extracellular vesicles in humans. *BioRxiv*, 2024.03.16.585131. https://doi.org/10.1101/2024.03.16.585131

Ramirez, M. I., Amorim, M. G., Gadelha, C., Milic, I., Welsh, J. A., Freitas, V. M., Nawaz, M., Akbar, N., Couch, Y., Makin, L., Cooke, F., Vettore, A. L., Batista, P. X., Freezor, R., Pezuk, J. A., Rosa-Fernandes, L., Carreira, A. C. O., Devitt, A., Jacobs, L., … Dias-Neto, E. (2018). Technical challenges of working with extracellular vesicles. *Nanoscale*, *10*(3), 881–906. https://doi.org/10.1039/C7NR08360B

Ramirez-Garrastacho, M., Bajo-Santos, C., Line, A., Martens-Uzunova, E. S., de la Fuente, J. M., Moros, M., Soekmadji, C., Tasken, K. A., & Llorente, A. (2022). Extracellular vesicles as a source of prostate cancer biomarkers in liquid biopsies: A decade of research. *British Journal of Cancer*, *126*(3), 331–350. https://doi.org/10.1038/s41416-021-01610-8

Ramkumar, S., Raghunath, A., & Raghunath, S. (2016). Statin Therapy: Review of Safety and Potential Side Effects. *Acta Cardiologica Sinica*, *32*(6), 631–639. https://doi.org/10.6515/ACS20160611A

Rapanotti, M. C., Campione, E., Spallone, G., Orlandi, A., Bernardini, S., & Bianchi, L. (2017). Minimal residual disease in melanoma: Circulating melanoma cells and predictive role of MCAM/MUC18/MelCAM/CD146. *Cell Death Discovery*, *3*(1), 1–10. https://doi.org/10.1038/cddiscovery.2017.5

Rashed, M., Bayraktar, E., Helal, G., Abd-Ellah, M. F., Amero, P., Chavez-Reyes, A., & Rodriguez-Aguayo, C. (2017). Exosomes: From Garbage Bins to Promising Therapeutic Targets. *International Journal of Molecular Sciences*, *18*(3), 3. https://doi.org/10.3390/ijms18030538

Rastrelli, M., Tropea, S., Rossi, C. R., & Alaibac, M. (2014). Melanoma: Epidemiology, Risk Factors, Pathogenesis, Diagnosis and Classification. *In Vivo*, *28*(6), 1005–1011.

Ratajczak, J., Wysoczynski, M., Hayek, F., Janowska-Wieczorek, A., & Ratajczak, M. Z. (2006). Membrane-derived microvesicles: Important and underappreciated mediators of cell-to-cell communication. *Leukemia*, *20*(9), 1487–1495. https://doi.org/10.1038/sj.leu.2404296

Rautou, P.-E., Vion, A.-C., Amabile, N., Chironi, G., Simon, A., Tedgui, A., Boulanger, C. M., Weber, C., & Mause, S. (2011). Microparticles, Vascular Function, and Atherothrombosis. *Circulation Research*, *109*(5), 593–606. https://doi.org/10.1161/CIRCRESAHA.110.233163

Redzic, J. S., Balaj, L., van der Vos, K. E., & Breakefield, X. O. (2014). Extracellular RNA mediates and marks cancer progression. *Seminars in Cancer Biology*, *28*, 14–23. https://doi.org/10.1016/j.semcancer.2014.04.010

Ren, B., Zou, G., Xu, F., Huang, Y., Xu, G., He, J., Li, Y., Zhu, H., & Yu, P. (2017). Serum levels of anti-sperm-associated antigen 9 antibody are elevated in patients with hepatocellular carcinoma. *Oncology Letters*, *14*(6), 7608–7614. https://doi.org/10.3892/ol.2017.7152

Ren, S., Zhang, S., Jiang, T., He, Y., Ma, Z., Cai, H., Xu, X., Li, Y., Cai, W., Zhou, J., Liu, X., Hu, X., Zhang, J., Yu, H., Zhou, C., & Hirsch, F. R. (2018). Early detection of lung cancer by using an autoantibody panel in Chinese population. *Oncoimmunology*, *7*(2), e1384108. https://doi.org/10.1080/2162402X.2017.1384108

Reverter, J. L., Rosas-Allende, I., Puig-Jove, C., Zafon, C., Megia, A., Castells, I., Pizarro, E., Puig-Domingo, M., & Granada, M. L. (2020). Prognostic Significance of Thyroglobulin Antibodies in Differentiated Thyroid Cancer. *Journal of Thyroid Research*, *2020*, e8312628. https://doi.org/10.1155/2020/8312628

Ribero, S., Moscarella, E., Ferrara, G., Piana, S., Argenziano, G., & Longo, C. (2016a). Regression in cutaneous melanoma: A comprehensive review from diagnosis to prognosis. *Journal of the European Academy of Dermatology and Venereology*, *30*(12), 2030–2037. https://doi.org/10.1111/jdv.13815

Ribero, S., Osella-Abate, S., Pasquali, S., Rossi, C. R., Borgognoni, L., Piazzalunga, D., Solari, N., Schiavon, M., Brandani, P., Ansaloni, L., Ponte, E., Silan, F., Sommariva, A., Bellucci, F., Macripò, G., & Quaglino, P. (2016b). Prognostic Role of Multiple Lymphatic Basin Drainage in Sentinel Lymph Node-Negative Trunk Melanoma Patients: A Multicenter Study from the Italian Melanoma Intergroup. *Annals of Surgical Oncology*, *23*(5), 1708–1715. https://doi.org/10.1245/s10434-015-4973-6

Ridker, P. M. (2016). A Test in Context: High-Sensitivity C-Reactive Protein. *Journal of the American College of Cardiology*, *67*(6), 712–723. https://doi.org/10.1016/j.jacc.2015.11.037

Ridker, P. M. (2017). How Common Is Residual Inflammatory Risk? *Circulation Research*, *120*(4), 617–619. https://doi.org/10.1161/CIRCRESAHA.116.310527

Ridker, P. M., Koenig, W., Kastelein, J. J., Mach, F., & Lüscher, T. F. (2018). Has the time finally come to measure hsCRP universally in primary and secondary cardiovascular prevention? *European Heart Journal*, *39*(46), 4109–4111. https://doi.org/10.1093/eurheartj/ehy723

Rigel, D. S. (2010). Epidemiology of melanoma. *Seminars in Cutaneous Medicine and Surgery*, *29*(4), 204–209. https://doi.org/10.1016/j.sder.2010.10.005

Rimoldi, D., Salvi, S., Schultz-Thater, E., Spagnoli, G. C., & Cerottini, J.-C. (2000). Anti-MAGE-3 antibody 57b and anti-MAGE-1 antibody 6C1 can be used to study different proteins of the MAGE-A family. *International Journal of Cancer*, *86*(5), 749–751. https://doi.org/10.1002/(SICI)1097-0215(20000601)86:5<749::AID-IJC24>3.0.CO;2-O

Robbins, P. D., & Morelli, A. E. (2014). Regulation of immune responses by extracellular vesicles. *Nature Reviews Immunology*, *14*(3), 195–208. https://doi.org/10.1038/nri3622

Robinson, J. K., & Turrisi, R. (2006). Skills Training to Learn Discrimination of ABCDE Criteria by Those at Risk of Developing Melanoma. *Archives of Dermatology*, *142*(4), 447–452. https://doi.org/10.1001/archderm.142.4.447

Rodic, S., Mihalcioiu, C., & Saleh, R. R. (2014). Detection methods of circulating tumor cells in cutaneous melanoma: A systematic review. *Critical Reviews in Oncology/ Hematology*, *91*(1), 74–92. https://doi.org/10.1016/j.critrevonc.2014.01.007

Rogner, U. C., Wilke, K., Steck, E., Korn, B., & Poustka, A. (1995). The Melanoma Antigen Gene (MAGE) Family Is Clustered in the Chromosomal Band Xq28. *Genomics*, *29*(3), 725–731. https://doi.org/10.1006/geno.1995.9945

Rosado, M., Silva, R., G. Bexiga, M., G. Jones, J., Manadas, B., & Anjo, S. I. (2019). Chapter Four – Advances in biomarker detection: Alternative approaches for blood-based biomarker detection. In G. S. Makowski (Ed.), *Advances in Clinical Chemistry* (Vol. 92, pp. 141–199). Elsevier. https://doi.org/10.1016/bs.acc.2019.04.003

Rosc-Schlüter, B. I., Häuselmann, S. P., Lorenz, V., Mochizuki, M., Facciotti, F., Pfister, O., & Kuster, G. M. (2012). NOX2-derived reactive oxygen species are crucial for CD29-induced pro-survival signalling in cardiomyocytes. *Cardiovascular Research*, *93*(3), 454–462. https://doi.org/10.1093/cvr/cvr348

Rosenberg, S. (2000). *Principles and Practice of the Biologic Therapy of Cancer* (3rd ed.). Lippincott Williams & Wilkins. https://www.wolterskluwer.com/en/solutions/ovid/principles-and-practice-of-the-biologic-therapy-of-cancer-1779

Roskoski, R. (2012). ERK1/2 MAP kinases: Structure, function, and regulation. *Pharmacological Research*, *66*(2), 105–143. https://doi.org/10.1016/j.phrs.2012.04.005

Ross, R., Neeland, I. J., Yamashita, S., Shai, I., Seidell, J., Magni, P., Santos, R. D., Arsenault, B., Cuevas, A., Hu, F. B., Griffin, B. A., Zambon, A., Barter, P., Fruchart, J.-C., Eckel, R. H., Matsuzawa, Y., & Després, J.-P. (2020). Waist circumference as a vital sign in clinical practice: A Consensus Statement from the IAS and ICCR Working Group on Visceral Obesity. *Nature Reviews Endocrinology*, *16*(3), 177–189. https://doi.org/10.1038/s41574-019-0310-7

Rupert, D. L. M., Claudio, V., Lässer, C., & Bally, M. (2017). Methods for the physical characterization and quantification of extracellular vesicles in biological samples. *Biochimica et Biophysica Acta (BBA) – General Subjects*, *1861*(1, Part A), 3164–3179. https://doi.org/10.1016/j.bbagen.2016.07.028

Sacher, A. G., Paweletz, C., Dahlberg, S. E., Alden, R. S., O'Connell, A., Feeney, N., Mach, S. L., Jänne, P. A., & Oxnard, G. R. (2016). Prospective Validation of Rapid Plasma Genotyping for the Detection of EGFR and KRAS Mutations in Advanced Lung Cancer. *JAMA Oncology*, *2*(8), 1014–1022. https://doi.org/10.1001/jamaoncol.2016.0173

Saeed-Zidane, M., Linden, L., Salilew-Wondim, D., Held, E., Neuhoff, C., Tholen, E., Hoelker, M., Schellander, K., & Tesfaye, D. (2017). Cellular and exosome mediated molecular defense mechanism in bovine granulosa cells exposed to oxidative stress. *PLOS ONE*, *12*(11), e0187569. https://doi.org/10.1371/journal.pone.0187569

Sakowska, J., Arcimowicz, Ł., Jankowiak, M., Papak, I., Markiewicz, A., Dziubek, K., Kurkowiak, M., Kote, S., Kaźmierczak-Siedlecka, K., Połom, K., Marek-Trzonkowska, N., & Trzonkowski, P. (2022). Autoimmunity and Cancer – Two Sides of the Same Coin. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.793234

Sang, M., Wang, L., Ding, C., Zhou, X., Wang, B., Wang, L., Lian, Y., & Shan, B. (2011). Melanoma-associated antigen genes – An update. *Cancer Letters*, *302*(2), 85–90. https://doi.org/10.1016/j.canlet.2010.10.021

Sang, M., Wu, X., Fan, X., Lian, Y., & Sang, M. (2017). MAGE-A family serves as poor prognostic markers and potential therapeutic targets for epithelial ovarian cancer patients: A retrospective clinical study. *Gynecological Endocrinology: The Official Journal of the International Society of Gynecological Endocrinology*, *33*(6), 480–484. https://doi.org/10.1080/09513590.2017.1291611

Sanmamed, M. F., Fernández-Landázuri, S., Rodríguez, C., Zárate, R., Lozano, M. D., Zubiri, L., Perez-Gracia, J. L., Martín-Algarra, S., & González, A. (2015). Quantitative Cell-Free Circulating BRAFV600E Mutation Analysis by Use of Droplet Digital PCR in the Follow-up of Patients with Melanoma Being Treated with BRAF Inhibitors. *Clinical Chemistry*, *61*(1), 297–304. https://doi.org/10.1373/clinchem.2014.230235

Santillo, M., Colantuoni, A., Mondola, P., Guida, B., & Damiano, S. (2015). NOX signaling in molecular cardiovascular mechanisms involved in the blood pressure homeostasis. *Frontiers in Physiology*, *6*. https://doi.org/10.3389/fphys.2015.00194

Santoyo, J. M., Noguera, J. A., Avilés, F., Hernández-Caselles, T., de Paco-Matallana, C., Delgado, J. L., Cuevas, S., Llinás, M. T., & Hernández, I. (2023). Pravastatin reduces plasma levels of extracellular vesicles in pregnancies at high risk of term preeclampsia. *Frontiers in Pharmacology*, *14*, 1166123. https://doi.org/10.3389/fphar.2023.1166123

Sanwlani, R., & Gangoda, L. (2021). Role of Extracellular Vesicles in Cell Death and Inflammation. *Cells*, *10*(10), 10. https://doi.org/10.3390/cells10102663

Scanlan., M. J., Gure., A. O., Jungbluth., A. A., Old., L. J., & Chen., Y.-T. (2002). Cancer/testis antigens: An expanding family of targets for cancer immunotherapy. *Immunological Reviews*, *188*(1), 22–32. https://doi.org/10.1034/j.1600-065X.2002.18803.x

Schaefer, E. J., Tsunoda, F., Diffenderfer, M., Polisecki, E., Thai, N., & Asztalos, B. (2000). The Measurement of Lipids, Lipoproteins, Apolipoproteins, Fatty Acids, and Sterols, and Next Generation Sequencing for the Diagnosis and Treatment of Lipid Disorders. In K. R. Feingold, B. Anawalt, M. R. Blackman, A. Boyce, G. Chrousos, E. Corpas, W. W. de Herder, K. Dhatariya, K. Dungan, J. Hofland, S. Kalra, G. Kaltsas, N. Kapoor, C. Koch, P. Kopp, M. Korbonits, C. S. Kovacs, W. Kuohung, B. Laferrère, … D. P. Wilson (Eds.), *Endotext*. MDText.com, Inc. http://www.ncbi.nlm.nih.gov/books/NBK355892/

Schreuer, M., Meersseman, G., Van Den Herrewegen, S., Jansen, Y., Chevolet, I., Bott, A., Wilgenhof, S., Seremet, T., Jacobs, B., Buyl, R., Maertens, G., & Neyns, B. (2016). Quantitative assessment of BRAF V600 mutant circulating cell-free tumor DNA as a tool for therapeutic monitoring in metastatic melanoma patients treated with BRAF/MEK inhibitors. *Journal of Translational Medicine*, *14*(1), 95. https://doi.org/10.1186/s12967-016-0852-6

Schultz-Thater, E., Piscuoglio, S., Iezzi, G., Le Magnen, C., Zajac, P., Carafa, V., Terracciano, L., Tornillo, L., & Spagnoli, G. C. (2011). MAGE-A10 is a nuclear protein frequently expressed in high percentages of tumor cells in lung, skin and urothelial malignancies. *International Journal of Cancer*, *129*(5), 1137–1148. https://doi.org/10.1002/ijc.25777

Schwenke, D. C., & Carew, T. E. (1989). Initiation of atherosclerotic lesions in cholesterol-fed rabbits. II. Selective retention of LDL vs. Selective increases in LDL permeability in susceptible sites of arteries. *Arteriosclerosis (Dallas, Tex.)*, *9*(6), 908–918. https://doi.org/10.1161/01.atv.9.6.908

Scioli, M. G., Storti, G., D'Amico, F., Rodríguez Guzmán, R., Centofanti, F., Doldo, E., Céspedes Miranda, E. M., & Orlandi, A. (2020). Oxidative Stress and New Pathogenetic Mechanisms in Endothelial Dysfunction: Potential Diagnostic Biomarkers and Therapeutic Targets. *Journal of Clinical Medicine*, *9*(6), 6. https://doi.org/10.3390/jcm9061995

Seretis, K., Boptsi, E., & Boptsi, A. (2024). Extracellular Vesicles as Novel Diagnostic and Therapeutic Agents for Non-Melanoma Skin Cancer: A Systematic Review. *International Journal of Molecular Sciences*, *25*(5), 2617. https://doi.org/10.3390/ijms25052617

Serratì, S., Guida, M., Di Fonte, R., De Summa, S., Strippoli, S., Iacobazzi, R. M., Quarta, A., De Risi, I., Guida, G., Paradiso, A., Porcelli, L., & Azzariti, A. (2022). Circulating extracellular vesicles expressing PD1 and PD-L1 predict response and mediate resistance to checkpoint inhibitors immunotherapy in metastatic melanoma. *Molecular Cancer*, *21*(1), 20. https://doi.org/10.1186/s12943-021-01490-9

Shain, A. H., & Bastian, B. C. (2016). From melanocytes to melanomas. *Nature Reviews Cancer*, *16*(6), 345–358. https://doi.org/10.1038/nrc.2016.37

Shami-shah, A., Travis, B. G., & Walt, D. R. (2023). Advances in extracellular vesicle isolation methods: A path towards cell-type specific EV isolation. *Extracellular Vesicles and Circulating Nucleic Acids*, *4*(3), 447–460. https://doi.org/10.20517/evcna.2023.14

Sharma, P., Diergaarde, B., Ferrone, S., Kirkwood, J. M., & Whiteside, T. L. (2020). Melanoma cell-derived exosomes in plasma of melanoma patients suppress functions of immune effector cells. *Scientific Reports*, *10*(1), 92. https://doi.org/10.1038/s41598-019-56542-4

Sharma, S., Rasool, H. I., Palanisamy, V., Mathisen, C., Schmidt, M., Wong, D. T., & Gimzewski, J. K. (2010). Structural-Mechanical Characterization of Nanoparticle Exosomes in Human Saliva, Using Correlative AFM, FESEM, and Force Spectroscopy. *ACS Nano*, *4*(4), 1921–1926. https://doi.org/10.1021/nn901824n

Shen, Y., Li, M., Liao, L., Gao, S., & Wang, Y. (2023). Plasma exosome-derived connexin43 as a promising biomarker for melanoma patients. *BMC Cancer*, *23*(1), 242. https://doi.org/10.1186/s12885-023-10705-9

Shimbo, T., Tanemura, A., Yamazaki, T., Tamai, K., Katayama, I., & Kaneda, Y. (2010). Serum Anti-BPAG1 Auto-Antibody Is a Novel Marker for Human Melanoma. *PLOS ONE*, *5*(5), e10566. https://doi.org/10.1371/journal.pone.0010566

Shimoda, M., Principe, S., Jackson, H. W., Luga, V., Fang, H., Molyneux, S. D., Shao, Y. W., Aiken, A., Waterhouse, P. D., Karamboulas, C., Hess, F. M., Ohtsuka, T., Okada, Y., Ailles, L., Ludwig, A., Wrana, J. L., Kislinger, T., & Khokha, R. (2014). Loss of the Timp gene family is sufficient for the acquisition of the CAF-like cell state. *Nature Cell Biology*, *16*(9), 889–901. https://doi.org/10.1038/ncb3021

Sholl, L. M., Oxnard, G. R., & Paweletz, C. P. (2020). Traditional Diagnostics versus Disruptive Technology: The role of the pathologist in the era of liquid biopsy. *Cancer Research*, *80*(16), 3197–3199. https://doi.org/10.1158/0008-5472.CAN-20-0134

Sidhom, K., Obi, P. O., & Saleem, A. (2020). A Review of Exosomal Isolation Methods: Is Size Exclusion Chromatography the Best Option? *International Journal of Molecular Sciences*, *21*(18), 18. https://doi.org/10.3390/ijms21186466

Silbernagl, S., & Lang, F. (2009). *Color atlas of pathophysiology* (2nd ed.). Thieme.

Simpson, A. J. G., Caballero, O. L., Jungbluth, A., Chen, Y.-T., & Old, L. J. (2005). Cancer/testis antigens, gametogenesis and cancer. *Nature Reviews Cancer*, *5*(8), 615–625. https://doi.org/10.1038/nrc1669

Sinauridze, E. I., Kireev, D. A., Popenko, N. Y., Pichugin, A. V., Panteleev, M. A., Krymskaya, O. V., & Ataullakhanov, F. I. (2007). Platelet microparticle membranes have 50- to 100-fold higher specific procoagulant activity than activated platelets. *Thrombosis and Haemostasis*, *97*(3), 425–434.

Sirivolu, S., Peng, C.-C., Neviani, P., Xu, B. Y., Berry, J. L., & Xu, L. (2024). Comparative Single Vesicle Analysis of Aqueous Humor Extracellular Vesicles before and after Radiation in Uveal Melanoma Eyes. *International Journal of Molecular Sciences*, *25*(11), 11. https://doi.org/10.3390/ijms25116035

Skog, J., Würdinger, T., van Rijn, S., Meijer, D. H., Gainche, L., Curry, W. T., Carter, B. S., Krichevsky, A. M., & Breakefield, X. O. (2008). Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nature Cell Biology*, *10*(12), 1470–1476. https://doi.org/10.1038/ncb1800

Smolarz, M., Pietrowska, M., Matysiak, N., Mielańczyk, Ł., & Widłak, P. (2019). Proteome Profiling of Exosomes Purified from a Small Amount of Human Serum: The Problem of Co-Purified Serum Components. *Proteomes*, *7*(2), 2. https://doi.org/10.3390/proteomes7020018

Sorescu, D., Weiss, D., Lassègue, B., Clempus, R. E., Szöcs, K., Sorescu, G. P., Valppu,L., Quinn, M. T., Lambeth, J. D., Vega, J. D., Taylor, W. R., & Griendling, K. K. (2002). Superoxide Production and Expression of Nox Family Proteins in Human Atherosclerosis. *Circulation*, *105*(12), 1429–1435. https://doi.org/10.1161/01.CIR.0000012917.74432.66

Spinardi, L., & Witke, W. (2007). Gelsolin and diseases. *Sub-Cellular Biochemistry*, *45*, 55–69. https://doi.org/10.1007/978-1-4020-6191-2_3

Spindler, K.-L. G., Appelt, A. L., Pallisgaard, N., Andersen, R. F., Brandslund, I., & Jakobsen, A. (2014). Cell-free DNA in healthy individuals, noncancerous disease and strong prognostic value in colorectal cancer. *International Journal of Cancer*, *135*(12), 2984–2991. https://doi.org/10.1002/ijc.28946

Steinbrecher, U. P. (1988). Role of superoxide in endothelial-cell modification of low-density lipoproteins. *Biochimica Et Biophysica Acta*, *959*(1), 20–30. https://doi.org/10.1016/0005-2760(88)90145-2

Stroun, M., Lyautey, J., Lederrey, C., Olson-Sand, A., & Anker, P. (2001). About the possible origin and mechanism of circulating DNA: Apoptosis and active DNA release. *Clinica Chimica Acta*, *313*(1), 139–142. https://doi.org/10.1016/S0009-8981(01)00665-9

Su, D. W., & Nieva, J. (2017). Biophysical technologies for understanding circulating tumor cell biology and metastasis. *Translational Lung Cancer Research*, *6*(4), 473–485. https://doi.org/10.21037/tlcr.2017.05.08

Suades, R., Padró, T., Alonso, R., Mata, P., & Badimon, L. (2013). Lipid-lowering therapy with statins reduces microparticle shedding from endothelium, platelets and inflammatory cells. *Thrombosis and Haemostasis*, *110*(8), 366–377. https://doi.org/10.1160/TH13-03-0238

Suades, R., Padró, T., Vilahur, G., & Badimon, L. (2012). Circulating and platelet-derived microparticles in human blood enhance thrombosis on atherosclerotic plaques. *Thrombosis and Haemostasis*, *108*(12), 1208–1219. https://doi.org/10.1160/TH12-07-0486

Sumner, W. C. (1953). Spontaneous regression of melanoma. *Cancer*, *6*(5), 1040–1043. https://doi.org/10.1002/1097-0142(195309)6:5<1040::aid-cncr2820060525>3.0.co;2-5

Sun, G., Chen, H., Xia, J., Li, T., Ye, H., Li, J., Zhang, X., Cheng, Y., Wang, K., Shi, J., & Wang, P. (2023). Diagnostic performance of anti-MAGEA family protein auto-antibodies in esophageal squamous cell carcinoma. *International Immunopharmacology*, *125*(Pt A), 111041. https://doi.org/10.1016/j.intimp.2023.111041

Sunkara, V., Woo, H.-K., & Cho, Y.-K. (2016). Emerging techniques in the isolation and characterization of extracellular vesicles and their roles in cancer diagnostics and prognostics. *Analyst*, *141*(2), 371–381. https://doi.org/10.1039/C5AN01775K

Surveillance report 2017 – Myocardial infarction: Cardiac rehabilitation and prevention of further cardiovascular disease (2013) NICE guideline CG172. (2017). National Institute for Health and Care Excellence (NICE). http://www.ncbi.nlm.nih.gov/books/NBK552077/

Svedman, F. C., Lohcharoenkal, W., Bottai, M., Brage, S. E., Sonkoly, E., Hansson, J., Pivarcsi, A., & Eriksson, H. (2018). Extracellular microvesicle microRNAs as predictive biomarkers for targeted therapy in metastatic cutaneous malignant melanoma. *PLOS ONE*, *13*(11), e0206942. https://doi.org/10.1371/journal.pone.0206942

Svensson, K. J., Christianson, H. C., Wittrup, A., Bourseau-Guilmain, E., Lindqvist, E., Svensson, L. M., Mörgelin, M., & Belting, M. (2013). Exosome Uptake Depends on ERK1/2-Heat Shock Protein 27 Signaling and Lipid Raft-mediated Endocytosis Negatively Regulated by Caveolin-1*. *Journal of Biological Chemistry*, *288*(24), 17713–17724. https://doi.org/10.1074/jbc.M112.445403

Swissa, M., Cohen, Y., & Shoenfeld, Y. (1992). Autoantibodies in the Sera of Patients with Lymphoma. *Leukemia & Lymphoma*, *7*(1–2), 117–122. https://doi.org/10.3109/10428199209053610

Szajnik, M., Czystowska, M., Szczepanski, M. J., Mandapathil, M., & Whiteside, T. L. (2010). Tumor-Derived Microvesicles Induce, Expand and Up-Regulate Biological Activities of Human Regulatory T Cells (Treg). *PLOS ONE*, *5*(7), e11469. https://doi.org/10.1371/journal.pone.0011469

Tadokoro, H., Umezu, T., Ohyashiki, K., Hirano, T., & Ohyashiki, J. H. (2013). Exosomes Derived from Hypoxic Leukemia Cells Enhance Tube Formation in Endothelial Cells*. *Journal of Biological Chemistry*, *288*(48), 34343–34351. https://doi.org/10.1074/jbc.M113.480822

Tahir, S. A., Ren, C., Timme, T. L., Gdor, Y., Hoogeveen, R., Morrisett, J. D., Frolov, A., Ayala, G., Wheeler, T. M., & Thompson, T. C. (2003). Development of an immuno-assay for serum caveolin-1: A novel biomarker for prostate cancer. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, *9*(10 Pt 1), 3653–3659.

Takashi, S., Satoshi, Y., Akihiko, O., Naoya, Y., Yusuke, T., Kentaro, M., Yu, O., Yasuaki, N., Koichi, Y., Takashi, F., Ryo, O., Isamu, H., Chikara, K., Kosuke, N., Yasuhiro, T., Kazuhiko, Y., & Hideaki, S. (2021). Clinical impact of preoperative serum p53 antibody titers in 1487 patients with surgically treated esophageal squamous cell carcinoma: A multi-institutional study. *Esophagus*, *18*(1), 65–71. https://doi.org/10.1007/s10388-020-00761-6

Tan, S.-H., Martinez, A., Rastogi, A., Huang, W., Banerjee, S., Ravindranath, L., Young, D., Ali, A., Kohaar, I., Chen, Y., Cullen, J., Petrovics, G., Dobi, A., McLeod, D. G., Kagan, J., Srivastava, S., Sesterhenn, I. A., Rosner, I. L., Srivastava, S., & Srinivasan, A. (2019). Abstract 3296: Tumor antigens Fetuin-A and Secreted Protein Acidic and Rich in Cysteine (SPARC) Autoantibodies as diagnostic and prognostic biomarkers in prostate cancer. *Cancer Research*, *79*(13_Supplement), 3296. https://doi.org/10.1158/1538-7445.AM2019-3296

Tan, Y., Cheng, H., Su, C., Chen, P., & Yang, X. (2022). PI3K/Akt Signaling Pathway Ameliorates Oxidative Stress-Induced Apoptosis upon Manganese Exposure in PC12 Cells. *Biological Trace Element Research*, *200*(2), 749–760. https://doi.org/10.1007/s12011-021-02687-1

Tang, Y., Yang, L.-J., Liu, H., Song, Y.-J., Yang, Q.-Q., Liu, Y., Qian, S.-W., & Tang, Q.-Q. (2023). Exosomal miR-27b-3p secreted by visceral adipocytes contributes to endothelial inflammation and atherogenesis. *Cell Reports*, *42*(1), 111948. https://doi.org/10.1016/j.celrep.2022.111948

Tauro, B. J., Greening, D. W., Mathias, R. A., Mathivanan, S., Ji, H., & Simpson, R. J. (2013). Two Distinct Populations of Exosomes Are Released from LIM1863 Colon Carcinoma Cell-derived Organoids*. *Molecular & Cellular Proteomics*, *12*(3), 587–598. https://doi.org/10.1074/mcp.M112.021303

Taylor, D. D., & Gercel-Taylor, C. (2011). Exosomes/microvesicles: Mediators of cancer-associated immunosuppressive microenvironments. *Seminars in Immunopathology*, *33*(5), 441–454. https://doi.org/10.1007/s00281-010-0234-8

Taylor, D. D., & Shah, S. (2015). Methods of isolating extracellular vesicles impact down-stream analyses of their cargoes. *Methods*, *87*, 3–10. https://doi.org/10.1016/j.ymeth.2015.02.019

Teras, L. R., Gapstur, S. M., Maliniak, M. L., Jacobs, E. J., Gansler, T., Michel, A., Pawlita, M., Waterboer, T., & Campbell, P. T. (2018). Prediagnostic Antibodies to Serum p53 and Subsequent Colorectal Cancer. *Cancer Epidemiology, Biomarkers & Prevention*, *27*(2), 219–223. https://doi.org/10.1158/1055-9965.EPI-17-0407

Ter-Ovanesyan, D., Norman, M., Lazarovits, R., Trieu, W., Lee, J.-H., Church, G. M., & Walt, D. R. (2021). Framework for rapid comparison of extracellular vesicle isolation methods. *ELife*, *10*, e70725. https://doi.org/10.7554/eLife.70725

Thakur, B. K., Zhang, H., Becker, A., Matei, I., Huang, Y., Costa-Silva, B., Zheng, Y., Hoshino, A., Brazier, H., Xiang, J., Williams, C., Rodriguez-Barrueco, R., Silva, J. M., Zhang, W., Hearn, S., Elemento, O., Paknejad, N., Manova-Todorova, K., Welte, K., … Lyden, D. (2014). Double-stranded DNA in exosomes: A novel biomarker in cancer detection. *Cell Research*, *24*(6), 766–769. https://doi.org/10.1038/cr.2014.44

The International Agency for Research on Cancer Working Group on artificial ultraviolet (UV) light and skin cancer. (2007). The association of use of sunbeds with cutaneous malignant melanoma and other skin cancers: A systematic review. *International Journal of Cancer*, *120*(5), 1116–1122. https://doi.org/10.1002/ijc.22453

Théry, C., Witwer, K. W., Aikawa, E., Alcaraz, M. J., Anderson, J. D., Andriantsitohaina, R., Antoniou, A., Arab, T., Archer, F., Atkin-Smith, G. K., Ayre, D. C., Bach, J.-M., Bachurski, D., Baharvand, H., Balaj, L., Baldacchino, S., Bauer, N. N., Baxter, A. A., Bebawy, M., … Zuba-Surma, E. K. (2018). Minimal information for studies of extracellular vesicles 2018 (MISEV2018): A position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *Journal of Extracellular Vesicles*, *7*(1), 1535750. https://doi.org/10.1080/20013078.2018.1535750

Thompson, C. A., Purushothaman, A., Ramani, V. C., Vlodavsky, I., & Sanderson, R. D. (2013). Heparanase Regulates Secretion, Composition, and Function of Tumor Cell-derived Exosomes*♦. *Journal of Biological Chemistry*, *288*(14), 10093–10099. https://doi.org/10.1074/jbc.C112.444562

Tian, C., Kim, Y. J., Hali, S., Choo, O.-S., Lee, J.-S., Jung, S.-K., Choi, Y.-U., Park, C. B., & Choung, Y.-H. (2020). Suppressed expression of LDHB promotes age-related hearing loss via aerobic glycolysis. *Cell Death & Disease*, *11*(5), 1–13. https://doi.org/10.1038/s41419-020-2577-y

Titus-Ernstoff, L., Perry, A. E., Spencer, S. K., Gibson, J. J., Cole, B. F., & Ernstoff, M. S. (2005). Pigmentary characteristics and moles in relation to melanoma risk. *International Journal of Cancer*, *116*(1), 144–149. https://doi.org/10.1002/ijc.21001

Tokunaga, E., Takizawa, K., Masuda, T., Ijichi, H., Koga, C., Tajiri, W., Tanaka, J., Nakamura, Y., Taguchi, K., & Ishida, M. (2018). Tumor-infiltrating lymphocytes and serum anti-p53 autoantibody in HER2-positive breast cancer treated with neoadjuvant chemotherapy. *Journal of Clinical Oncology*, *36*(15_suppl), e12648–e12648. https://doi.org/10.1200/JCO.2018.36.15_suppl.e12648

Tóth, E. Á., Turiák, L., Visnovitz, T., Cserép, C., Mázló, A., Sódar, B. W., Försönits, A. I., Petővári, G., Sebestyén, A., Komlósi, Z., Drahos, L., Kittel, Á., Nagy, G., Bácsi, A., Dénes, Á., Gho, Y. S., Szabó-Taylor, K. É., & Buzás, E. I. (2021). Formation of a protein corona on the surface of extracellular vesicles in blood plasma. *Journal of Extracellular Vesicles*, *10*(11), e12140. https://doi.org/10.1002/jev2.12140

Trajkovic, K., Hsu, C., Chiantia, S., Rajendran, L., Wenzel, D., Wieland, F., Schwille, P., Brügger, B., & Simons, M. (2008). Ceramide Triggers Budding of Exosome Vesicles into Multivesicular Endosomes. *Science*, *319*(5867), 1244–1247. https://doi.org/10.1126/science.1153124

Tsang, Y. H., Wang, Y., Kong, K., Grzeskowiak, C., Zagorodna, O., Dogruluk, T., Lu, H., Villafane, N., Bhavana, V. H., Moreno, D., Elsea, S. H., Liang, H., Mills, G. B., & Scott, K. L. (2020). Differential expression of MAGEA6 toggles autophagy to promote pancreatic cancer progression. *ELife*, *9*(e48963). https://doi.org/10.7554/eLife.48963

Tuma, D. J., Kearley, M. L., Thiele, G. M., Worrall, S., Haver, A., Klassen, L. W., & Sorrell, M. F. (2001). Elucidation of Reaction Scheme Describing Malondialdehyde–Acetaldehyde–Protein Adduct Formation. *Chemical Research in Toxicology*, *14*(7), 822–832. https://doi.org/10.1021/tx000222a

Ueda, K., Ishikawa, N., Tatsuguchi, A., Saichi, N., Fujii, R., & Nakagawa, H. (2014). Antibody-coupled monolithic silica microtips for highthroughput molecular profiling of circulating exosomes. *Scientific Reports*, *4*(1), 6232. https://doi.org/10.1038/srep06232

Underhill, H. R., Kitzman, J. O., Hellwig, S., Welker, N. C., Daza, R., Baker, D. N., Gligorich, K. M., Rostomily, R. C., Bronner, M. P., & Shendure, J. (2016). Fragment Length of Circulating Tumor DNA. *PLoS Genetics*, *12*(7), e1006162. https://doi.org/10.1371/journal.pgen.1006162

Ungvari, Z., Wolin, M. S., & Csiszar, A. (2006). Mechanosensitive production of reactive oxygen species in endothelial and smooth muscle cells: Role in microvascular remodeling? *Antioxidants & Redox Signaling*, *8*(7–8), 1121–1129. https://doi.org/10.1089/ars.2006.8.1121

Ushigome, M., Nabeya, Y., Soda, H., Takiguchi, N., Kuwajima, A., Tagawa, M., Matsushita, K., Koike, J., Funahashi, K., & Shimada, H. (2018). Multi-panel assay of serum autoantibodies in colorectal cancer. *International Journal of Clinical Oncology*, *23*(5), 917–923. https://doi.org/10.1007/s10147-018-1278-3

Vagner, T., Spinelli, C., Minciacchi, V. R., Balaj, L., Zandian, M., Conley, A., Zijlstra, A., Freeman, M. R., Demichelis, F., De, S., Posadas, E. M., Tanaka, H., & Di Vizio, D. (2018). Large extracellular vesicles carry most of the tumour DNA circulating in prostate cancer patient plasma. *Journal of Extracellular Vesicles*, *7*(1), 1505403. https://doi.org/10.1080/20013078.2018.1505403

Valadi, H., Ekström, K., Bossios, A., Sjöstrand, M., Lee, J. J., & Lötvall, J. O. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nature Cell Biology*, *9*(6), 654–659. https://doi.org/10.1038/ncb1596

Valencia, J. C., Egbukichi, N., & Erwin-Cohen, R. A. (2019). Autoimmunity and Cancer, the Paradox Comorbidities Challenging Therapy in the Context of Preexisting Autoimmunity. *Journal of Interferon & Cytokine Research*, *39*(1), 72–84. https://doi.org/10.1089/jir.2018.0060

Valmori, D., Dutoit, V., Rubio-Godoy, V., Chambaz, C., Liénard, D., Guillaume, P., Romero, P., Cerottini, J. C., & Rimoldi, D. (2001). Frequent cytolytic T-cell responses to peptide MAGE-A10(254-262) in melanoma. *Cancer Research*, *61*(2), 509–512.

van den Brûle, F. A., Buicu, C., Sobel, M. E., Liu, F. T., & Castronovo, V. (1995). Galectin-3, a laminin binding protein, fails to modulate adhesion of human melanoma cells to laminin. *Neoplasma*, *42*(5), 215–219.

van der Bruggen, P., Traversari, C., Chomez, P., Lurquin, C., De Plaen, E., Van den Eynde, B., Knuth, A., & Boon, T. (1991). A Gene Encoding an Antigen Recognized by Cytolytic T Lymphocytes on a Human Melanoma. *Science*, *254*(5038), 1643–1647. https://doi.org/10.1126/science.1840703

van der Meel, R., Krawczyk-Durka, M., van Solinge, W. W., & Schiffelers, R. M. (2014). Toward routine detection of extracellular vesicles in clinical samples. *International Journal of Laboratory Hematology*, *36*(3), 244–253. https://doi.org/10.1111/ijlh.12247

van der Vlist, E. J., Nolte-'t Hoen, E. N. M., Stoorvogel, W., Arkesteijn, G. J. A., & Wauben, M. H. M. (2012). Fluorescent labeling of nano-sized vesicles released by cells and subsequent quantitative and qualitative analysis by high-resolution flow cytometry. *Nature Protocols*, *7*(7), 1311–1326. https://doi.org/10.1038/nprot.2012.065

van Niel, G., D'Angelo, G., & Raposo, G. (2018). Shedding light on the cell biology of extracellular vesicles. *Nature Reviews Molecular Cell Biology*, *19*(4), 213–228. https://doi.org/10.1038/nrm.2017.125

Váraljai, R., Elouali, S., Lueong, S. s., Wistuba-Hamprecht, K., Seremet, T., Siveke, J. t., Becker, J. c., Sucker, A., Paschen, A., Horn, P. a., Neyns, B., Weide, B., Schadendorf, D., & Roesch, A. (2021). The predictive and prognostic significance of cell-free DNA concentration in melanoma. *Journal of the European Academy of Dermatology and Venereology*, *35*(2), 387–395. https://doi.org/10.1111/jdv.16766

Vella, L. J., Behren, A., Coleman, B., Greening, D. W., Hill, A. F., & Cebon, J. (2017). Intercellular Resistance to BRAF Inhibition Can Be Mediated by Extracellular Vesicle–Associated PDGFRβ. *Neoplasia*, *19*(11), 932–940. https://doi.org/10.1016/j.neo.2017.07.002

Vinay, D. S., Ryan, E. P., Pawelec, G., Talib, W. H., Stagg, J., Elkord, E., Lichtor, T., Decker, W. K., Whelan, R. L., Kumara, H. M. C. S., Signori, E., Honoki, K., Georgakilas, A. G., Amin, A., Helferich, W. G., Boosani, C. S., Guha, G., Ciriolo, M. R., Chen, S., … Kwon, B. S. (2015). Immune evasion in cancer: Mechanistic basis and therapeutic strategies. *Seminars in Cancer Biology*, *35*, S185–S198. https://doi.org/10.1016/j.semcancer.2015.03.004

Vlagea, A., Falagan, S., Gutiérrez-Gutiérrez, G., Moreno-Rubio, J., Merino, M., Zambrana, F., Casado, E., & Sereno, M. (2018). Antinuclear antibodies and cancer: A literature review. *Critical Reviews in Oncology/Hematology*, *127*, 42–49. https://doi.org/10.1016/j.critrevonc.2018.05.002

Vlassov, A. V., Magdaleno, S., Setterquist, R., & Conrad, R. (2012). Exosomes: Current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *1820*(7), 940–948. https://doi.org/10.1016/j.bbagen.2012.03.017

Wan, J. C. M., Massie, C., Garcia-Corbacho, J., Mouliere, F., Brenton, J. D., Caldas, C., Pacey, S., Baird, R., & Rosenfeld, N. (2017). Liquid biopsies come of age: Towards implementation of circulating tumour DNA. *Nature Reviews Cancer*, *17*(4), 223–238. https://doi.org/10.1038/nrc.2017.7

Wang, C., Liu, C., Shi, J., Li, H., Jiang, S., Zhao, P., Zhang, M., Du, G., Fu, S., Li, S., Wang, Z., Wang, X., Gao, F., Sun, P., Tian, J., Wang, C., Liu, C., Shi, J., Li, H., … Tian, J. (2023a). Nicotine exacerbates endothelial dysfunction and drives atherosclerosis via extracellular vesicle-miRNA. *Cardiovascular Research*, *119*(3), 3.

Wang, H., Zhang, B., Li, X., Zhou, D., Li, Y., Jia, S., Qi, S., Xu, A., Zhao, X., Wang, J., Bai, Z., Cao, B., Li, N., Dai, M., Chen, H., & Huang, J. (2020a). Identification and Validation of Novel Serum Autoantibody Biomarkers for Early Detection of Colorectal Cancer and Advanced Adenoma. *Frontiers in Oncology*, *10*. https://doi.org/10.3389/fonc.2020.01081

Wang, N., Zhang, X., Ma, Z., Niu, J., Ma, S., Wenjie, W., & Chen, J. (2020b). Combination of tanshinone IIA and astragaloside IV attenuate atherosclerotic plaque vulnerability in ApoE(–/–) mice by activating PI3K/AKT signaling and suppressing TRL4/NF-κB signaling. *Biomedicine & Pharmacotherapy*, *123*, 109729. https://doi.org/10.1016/j.biopha.2019.109729

Wang, S., Qin, J., Ye, H., Wang, K., Shi, J., Ma, Y., Duan, Y., Song, C., Wang, X., Dai, L., Wang, K., Wang, P., & Zhang, J. (2018). Using a panel of multiple tumor-associated antigens to enhance autoantibody detection for immunodiagnosis of gastric cancer. *OncoImmunology*, *7*(8), e1452582. https://doi.org/10.1080/2162402X.2018.1452582

Wang, Y., Liu, Y., Zhang, S., Li, N., Xing, C., Wang, C., Wang, J., Wei, M., Yang, G., & Yuan, L. (2023b). Exercise Improves Metabolism and Alleviates Atherosclerosis via Muscle-Derived Extracellular Vesicles. *Aging and Disease*, *14*(3), 952–965. https://doi.org/10.14336/AD.2022.1131

Wang, Z.-W., Yu, Q.-Y., Xu, M.-J., Zhou, C.-Y., Li, J.-P., & Liao, X.-H. (2024). MAGE-A11 is a potential prognostic biomarker and immunotherapeutic target in gastric cancer. *Aging*, *16*(1), 285–298. https://doi.org/10.18632/aging.205368

Watt, A. J., Kotsis, S. V., & Chung, K. C. (2004). Risk of Melanoma Arising in Large Congenital Melanocytic Nevi: A Systematic Review. *Plastic and Reconstructive Surgery*, *113*(7), 1968. https://doi.org/10.1097/01.PRS.0000122209.10277.2A

Webber, J. P., Spary, L. K., Sanders, A. J., Chowdhury, R., Jiang, W. G., Steadman, R., Wymant, J., Jones, A. T., Kynaston, H., Mason, M. D., Tabi, Z., & Clayton, A. (2015). Differentiation of tumour-promoting stromal myofibroblasts by cancer exosomes. *Oncogene*, *34*(3), 290–302. https://doi.org/10.1038/onc.2013.560

Webber, J., Steadman, R., Mason, M. D., Tabi, Z., & Clayton, A. (2010). Cancer Exosomes Trigger Fibroblast to Myofibroblast Differentiation. *Cancer Research*, *70*(23), 9621–9630. https://doi.org/10.1158/0008-5472.CAN-10-1722

Wei, Y., Lai, X., Yu, S., Chen, S., Ma, Y., Zhang, Y., Li, H., Zhu, X., Yao, L., & Zhang, J. (2014). Exosomal miR-221/222 enhances tamoxifen resistance in recipient ER-positive breast cancer cells. *Breast Cancer Research and Treatment*, *147*(2), 423–431. https://doi.org/10.1007/s10549-014-3037-0

Weight, R. M., & Viator, J. A. (2014). Detection of circulating tumor cells by photoacoustic flowmetry. *Methods in Molecular Biology (Clifton, N.J.)*, *1102*, 655–663. https://doi.org/10.1007/978-1-62703-727-3_35

Wendt, S., Goetzenich, A., Goettsch, C., Stoppe, C., Bleilevens, C., Kraemer, S., & Benstoem, C. (2018). Evaluation of the cardioprotective potential of extracellular vesicles – a systematic review and meta-analysis. *Scientific Reports*, *8*(1), 15702. https://doi.org/10.1038/s41598-018-33862-5

Weng, Y., Sui, Z., Shan, Y., Hu, Y., Chen, Y., Zhang, L., & Zhang, Y. (2016). Effective isolation of exosomes with polyethylene glycol from cell culture supernatant for in-depth proteome profiling. *Analyst*, *141*(15), 4640–4646. https://doi.org/10.1039/C6AN00892E

Weon, J. L., & Potts, P. R. (2015). The MAGE protein family and cancer. *Current Opinion in Cell Biology*, *37*, 1–8. https://doi.org/10.1016/j.ceb.2015.08.002

Willerson, J. T., & Kereiakes, D. J. (2003). Endothelial Dysfunction. *Circulation*, *108*(17), 2060–2061. https://doi.org/10.1161/01.CIR.0000099580.72044.83

Winberg, L., Nielsen, C., & Jacobsen, S. (2017). Surface complement C3 fragments and cellular binding of microparticles in patients with SLE. *Lupus Science & Medicine*, *4*, e000193. https://doi.org/10.1136/lupus-2016-000193

Witwer, K. W., Buzás, E. I., Bemis, L. T., Bora, A., Lässer, C., Lötvall, J., Nolte-'t Hoen, E. N., Piper, M. G., Sivaraman, S., Skog, J., Théry, C., Wauben, M. H., & Hochberg, F. (2013). Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. *Journal of Extracellular Vesicles*, *2*(1), 20360. https://doi.org/10.3402/jev.v2i0.20360

Wolf, M., Poupardin, R. W., Ebner-Peking, P., Andrade, A. C., Blöchl, C., Obermayer, A., Gomes, F. G., Vari, B., Maeding, N., Eminger, E., Binder, H.-M., Raninger, A. M., Hochmann, S., Brachtl, G., Spittler, A., Heuser, T., Ofir, R., Huber, C. G., Aberman, Z., … Strunk, D. (2022). A functional corona around extracellular vesicles enhances angiogenesis, skin regeneration and immunomodulation. *Journal of Extracellular Vesicles*, *11*(4). https://doi.org/10.1002/jev2.12207

Wolf, P. (1967). The Nature and Significance of Platelet Products in Human Plasma. *British Journal of Haematology*, *13*(3), 269–288. https://doi.org/10.1111/j.1365-2141.1967.tb08741.x

Wright, M. (2012). Nanoparticle tracking analysis for the multiparameter characterization and counting of nanoparticle suspensions. *Methods in Molecular Biology (Clifton, N.J.)*, *906*, 511–524. https://doi.org/10.1007/978-1-61779-953-2_41

Wu, T.-M., Liu, J.-B., Liu, Y., Shi, Y., Li, W., Wang, G.-R., Ma, Y.-S., & Fu, D. (2020). Power and Promise of Next-Generation Sequencing in Liquid Biopsies and Cancer Control. *Cancer Control*, *27*(3), 1073274820934805. https://doi.org/10.1177/1073274820934805

Wyatt, A. W., Annala, M., Aggarwal, R., Beja, K., Feng, F., Youngren, J., Foye, A., Lloyd, P., Nykter, M., Beer, T. M., Alumkal, J. J., Thomas, G. V., Reiter, R. E., Rettig, M. B., Evans, C. P., Gao, A. C., Chi, K. N., Small, E. J., & Gleave, M. E. (2017). Concordance of Circulating Tumor DNA and Matched Metastatic Tissue Biopsy in Prostate Cancer. *JNCI Journal of the National Cancer Institute*, *109*(12), djx118. https://doi.org/10.1093/jnci/djx118

Xiao, D., Barry, S., Kmetz, D., Egger, M., Pan, J., Rai, S. N., Qu, J., McMasters, K. M., & Hao, H. (2016). Melanoma cell–derived exosomes promote epithelial–mesenchymal transition in primary melanocytes through paracrine/autocrine signaling in the tumor microenvironment. *Cancer Letters*, *376*(2), 318–327. https://doi.org/10.1016/j.canlet.2016.03.050

Xiao, D., Ohlendorf, J., Chen, Y., Taylor, D. D., Rai, S. N., Waigel, S., Zacharias, W., Hao, H., & McMasters, K. M. (2012). Identifying mRNA, MicroRNA and Protein Profiles of Melanoma Exosomes. *PLOS ONE*, *7*(10), e46874. https://doi.org/10.1371/journal.pone.0046874

Xiao, Q., Zoulikha, M., Qiu, M., Teng, C., Lin, C., Li, X., Sallam, M. A., Xu, Q., & He, W. (2022). The effects of protein corona on in vivo fate of nanocarriers. *Advanced Drug Delivery Reviews*, *186*, 114356. https://doi.org/10.1016/j.addr.2022.114356

Xie, C., Ji, N., Tang, Z., Li, J., & Chen, Q. (2019). The role of extracellular vesicles from different origin in the microenvironment of head and neck cancers. *Molecular Cancer*, *18*(1), 83. https://doi.org/10.1186/s12943-019-0985-3

Xu, M. J., Dorsey, J. F., Amaravadi, R., Karakousis, G., Simone, C. B., II, Xu, X., Xu, W., Carpenter, E. L., Schuchter, L., & Kao, G. D. (2016). Circulating Tumor Cells, DNA, and mRNA: Potential for Clinical Utility in Patients With Melanoma. *The Oncologist*, *21*(1), 84–94. https://doi.org/10.1634/theoncologist.2015-0207

Xu, M. L., Kim, H. J., Kim, S. C., Ju, W., Kim, Y. H., Chang, K.-H., & Kim, H.-J. (2019a). Serum anti-GAPDH autoantibody levels reflect the severity of cervical lesions: A potential serum biomarker for cervical cancer screening. *Oncology Letters*, *18*(1), 255–264. https://doi.org/10.3892/ol.2019.10326

Xu, Y.-W., Chen, H., Guo, H.-P., Yang, S.-H., Luo, Y.-H., Liu, C.-T., Huang, X.-Y., Tang, X.-M., Hong, C.-Q., Li, E.-M., Xu, L.-Y., & Peng, Y.-H. (2019b). Combined detection of serum autoantibodies as diagnostic biomarkers in esophagogastric junction adenocarcinoma. *Gastric Cancer*, *22*(3), 546–557. https://doi.org/10.1007/s10120-018-0894-y

Yajima, S., Suzuki, T., Oshima, Y., Shiratori, F., Funahashi, K., Kawai, S., Nanki, T., Muraoka, S., Urita, Y., Saida, Y., Okazumi, S., Kitagawa, Y., Hirata, Y., Hasegawa, H., Okabayashi, K., Murakami, M., Yamashita, T., Kato, R., Matsubara, H., … Shimada, H. (2021). New Assay System Elecsys Anti-p53 to Detect Serum Anti-p53 Antibodies in Esophageal Cancer Patients and Colorectal Cancer Patients: Multi-institutional Study. *Annals of Surgical Oncology*, *28*(7), 4007–4015. https://doi.org/10.1245/s10434-020-09342-4

Yakirevich, E., Sabo, E., Lavie, O., Mazareb, S., Spagnoli, G. C., & Resnick, M. B. (2003). Expression of the MAGE-A4 and NY-ESO-1 cancer-testis antigens in serous ovarian neoplasms. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, *9*(17), 6453–6460.

Yamamoto, M., Yang, G., Hong, C., Liu, J., Holle, E., Yu, X., Wagner, T., Vatner, S. F., & Sadoshima, J. (2003). Inhibition of endogenous thioredoxin in the heart increases oxidative stress and cardiac hypertrophy. *Journal of Clinical Investigation*, *112*(9), 1395–1406. https://doi.org/10.1172/JCI200317700

Yan, Y., Guo, Q., Wang, F., Adhikari, R., Zhu, Z., Zhang, H., Zhou, W., Yu, H., Li, J., & Zhang, J. (2021). Cell-Free DNA: Hope and Potential Application in Cancer. *Frontiers in Cell and Developmental Biology*, *9*. https://doi.org/10.3389/fcell.2021.639233

Yang, B., Ren, N., Guo, B., Xin, H., & Yin, Y. (2020). Measuring serum human epididymis secretory protein autoantibody as an early biomarker of lung cancer. *Translational Cancer Research*, *9*(2). https://doi.org/10.21037/tcr.2019.11.50

Yang, P., Huo, Z., Liao, H., & Zhou, Q. (2015). Cancer/Testis Antigens Trigger Epithelial-Mesenchymal Transition and Genesis of Cancer Stem-Like Cells. *Current Pharmaceutical Design*, *21*(10), 1292–1300.

Yang, W.-L., Gentry-Maharaj, A., Simmons, A., Ryan, A., Fourkala, E. O., Lu, Z., Baggerly, K. A., Zhao, Y., Lu, K. H., Bowtell, D., Jacobs, I., Skates, S. J., He, W.-W., Menon, U., Bast, R. C., Jr, & for the AOCS Study Group. (2017). Elevation of TP53 Autoantibody Before CA125 in Preclinical Invasive Epithelial Ovarian Cancer. *Clinical Cancer Research*, *23*(19), 5912–5922. https://doi.org/10.1158/1078-0432.CCR-17-0284

Yao, Y., Fan, Y., Wu, J., Wan, H., Wang, J., Lam, S., Lam, W. L., Girard, L., Gazdar, A. F., Wu, Z., & Zhou, Q. (2012). Potential application of non-small cell lung cancer-associated autoantibodies to early cancer diagnosis. *Biochemical and Biophysical Research Communications*, *423*(3), 613–619. https://doi.org/10.1016/j.bbrc.2012.06.050

Yin, M., Loyer, X., & Boulanger, C. M. (2015). Extracellular vesicles as new pharmacological targets to treat atherosclerosis. *European Journal of Pharmacology*, *763*, 90–103. https://doi.org/10.1016/j.ejphar.2015.06.047

Yoshioka, Y., Konishi, Y., Kosaka, N., Katsuda, T., Kato, T., & Ochiya, T. (2013). Comparative marker analysis of extracellular vesicles in different human cancer types. *Journal of Extracellular Vesicles*, *2*(1), 20424. https://doi.org/10.3402/jev.v2i0.20424

Yu, D.-H., Li, J.-H., Wang, Y.-C., Xu, J.-G., Pan, P.-T., & Wang, L. (2011). Serum anti-p53 antibody detection in carcinomas and the predictive values of serum p53 antibodies, carcino-embryonic antigen and carbohydrate antigen 12–5 in the neoadjuvant chemotherapy treatment for III stage non-small cell lung cancer patients. *Clinica Chimica Acta*, *412*(11), 930–935. https://doi.org/10.1016/j.cca.2011.01.028

Yu, H., Rimbert, A., Palmer, A. E., Toyohara, T., Xia, Y., Xia, F., Ferreira, L. M. R., Chen, Z., Chen, T., Loaiza, N., Horwitz, N. B., Kacergis, M. C., Zhao, L., BIOS Consortium, Soukas, A. A., Kuivenhoven, J. A., Kathiresan, S., & Cowan, C. A. (2019). GPR146 Deficiency Protects against Hypercholesterolemia and Atherosclerosis. *Cell*, *179*(6), 1276-1288.e14. https://doi.org/10.1016/j.cell.2019.10.034

Yu, W., Hurley, J., Roberts, D., Chakrabortty, S. K., Enderle, D., Noerholm, M., Breakefield, X. O., & Skog, J. K. (2021). Exosome-based liquid biopsies in cancer: Opportunities and challenges. *Annals of Oncology : Official Journal of the European Society for Medical Oncology*, *32*(4), 466–477. https://doi.org/10.1016/j.annonc.2021.01.074

Yuana, Y., Levels, J., Grootemaat, A., Sturk, A., & Nieuwland, R. (2014). Co-isolation of extracellular vesicles and high-density lipoproteins using density gradient ultracentrifugation. *Journal of Extracellular Vesicles*, *3*(1), 23262. https://doi.org/10.3402/jev.v3.23262

Yurkovich, J. T., & Hood, L. (2019). Blood Is a Window into Health and Disease. *Clinical Chemistry*, *65*(10), 1204–1206. https://doi.org/10.1373/clinchem.2018.299065

Zaenker, P., Lo, J., Pearce, R., Cantwell, P., Cowell, L., Lee, M., Quirk, C., Law, H., Gray, E., & Ziman, M. (2018). A diagnostic autoantibody signature for primary cutaneous melanoma. *Oncotarget*, *9*(55), 30539–30551. https://doi.org/10.18632/oncotarget.25669

Zamani, P., Fereydouni, N., Butler, A. E., Navashenaq, J. G., & Sahebkar, A. (2019). The therapeutic and diagnostic role of exosomes in cardiovascular diseases. *Trends in Cardiovascular Medicine*, *29*(6), 313–323. https://doi.org/10.1016/j.tcm.2018.10.010

Zeng, P., Yang, J., Liu, L., Yang, X., Yao, Z., Ma, C., Zhu, H., Su, J., Zhao, Q., Feng, K., Yang, S., Zhu, Y., Li, X., Wang, W., Duan, Y., Han, J., & Chen, Y. (2021). ERK1/2 inhibition reduces vascular calcification by activating miR-126-3p-DKK1/LRP6 pathway. *Theranostics*, *11*(3), 1129–1146. https://doi.org/10.7150/thno.49771

Zhang, H., Xu, L., Xiao, D., Xie, J., Zeng, H., Cai, W., Niu, Y., Yang, Z., Shen, Z., & Li, E. (2006). Fascin is a potential biomarker for early-stage oesophageal squamous cell carcinoma. *Journal of Clinical Pathology*, *59*(9), 958–964. https://doi.org/10.1136/jcp.2005.032730

Zhang, J., Cao, M., Chen, J., Ye, S., Xie, K., He, X., Ma, X.-L., Zhang, J., & Yie, S. (2018). Serum anti-TOPO48 autoantibody as a biomarker for early diagnosis and prognosis in patients with esophageal squamous cell carcinoma. *Clinics and Research in Hepatology and Gastroenterology*, *42*(3), 276–284. https://doi.org/10.1016/j.clinre.2017.09.007

Zhang, L., Liang, Y., Li, S., Zeng, F., Meng, Y., Chen, Z., Liu, S., Tao, Y., & Yu, F. (2019a). The interplay of circulating tumor DNA and chromatin modification, therapeutic resistance, and metastasis. *Molecular Cancer*, *18*(1), 36. https://doi.org/10.1186/s12943-019-0989-z

Zhang, S., Zhou, X., Yu, H., & Yu, Y. (2010b). Expression of tumor-specific antigen MAGE, GAGE and BAGE in ovarian cancer tissues and cell lines. *BMC Cancer*, *10*(1), 163. https://doi.org/10.1186/1471-2407-10-163

Zhang, W., Xia, W., Lv, Z., Ni, C., Xin, Y., & Yang, L. (2017a). Liquid Biopsy for Cancer: Circulating Tumor Cells, Circulating Free DNA or Exosomes? *Cellular Physiology and Biochemistry*, *41*(2), 755–768. https://doi.org/10.1159/000458736

Zhang, Y., Liu, D., Chen, X., Li, J., Li, L., Bian, Z., Sun, F., Lu, J., Yin, Y., Cai, X., Sun, Q., Wang, K., Ba, Y., Wang, Q., Wang, D., Yang, J., Liu, P., Xu, T., Yan, Q., … Zhang, C.-Y. (2010a). Secreted Monocytic miR-150 Enhances Targeted Endothelial Cell Migration. *Molecular Cell*, *39*(1), 133–144. https://doi.org/10.1016/j.molcel.2010.06.010

Zhang, Y., Liu, Y., Liu, H., & Tang, W. H. (2019b). Exosomes: Biogenesis, biologic function and clinical potential. *Cell & Bioscience*, *9*(1), 19. https://doi.org/10.1186/s13578-019-0282-2

Zhang, Z., Li, F.-F., Lu, M.-D., Zhang, S.-X., & Li, Y.-X. (2017b). Anti-NY-ESO-1 autoantibody may be a tumor marker for intrahepatic cholangiocarcinoma. *Oncotarget*, *8*(61), 103283–103289. https://doi.org/10.18632/oncotarget.22464

Zhao, J., Xu, Z., Liu, Y., Wang, X., Liu, X., Gao, Y., & Jin, Y. (2022). The expression of cancer-testis antigen in ovarian cancer and the development of immunotherapy. *American Journal of Cancer Research*, *12*(2), 681–694.

Zhao, X., Dai, F., Mei, L., Huang, D., Shen, X., Zhang, H., She, X., & Ma, Z. (2021). The Potential Use of Dynamics Changes of ctDNA and cfDNA in the Perioperative Period to Predict the Recurrence Risk in Early NSCLC. *Frontiers in Oncology*, *11*. https://doi.org/10.3389/fonc.2021.671963

Zhou, J., Cheng, Y., Tang, L., Martinka, M., & Kalia, S. (2016). Up-regulation of SERPINA3 correlates with high mortality of melanoma patients and increased migration and invasion of cancer cells. *Oncotarget*, *8*(12), 18712–18725. https://doi.org/10.18632/oncotarget.9409

Zhu, W., Xie, H.-Q., Xie, Y.-Q., & Lv, X.-D. (2024). Expression and Clinical Significance of MAGE-A Proteins and mRNA in Lung Cancer Patients: A Retrospective Study. *Alternative Therapies in Health and Medicine*, *30*(2), 131–135.

Zhuang, G., Wu, X., Jiang, Z., Kasman, I., Yao, J., Guan, Y., Oeh, J., Modrusan, Z., Bais, C., Sampath, D., & Ferrara, N. (2012). Tumour-secreted miR-9 promotes endothelial cell migration and angiogenesis by activating the JAK-STAT pathway. *The EMBO Journal*, *31*(17), 3513–3523. https://doi.org/10.1038/emboj.2012.183

Zorov, D. B., Juhaszova, M., & Sollott, S. J. (2014). Mitochondrial Reactive Oxygen Species (ROS) and ROS-Induced ROS Release. *Physiological Reviews*, *94*(3), 909–950. https://doi.org/10.1152/physrev.00026.2013

Zu, L., Ren, C., Pan, B., Zhou, B., Zhou, E., Niu, C., Wang, X., Zhao, M., Gao, W., Guo, L., & Zheng, L. (2016). Endothelial microparticles after antihypertensive and lipid-lowering therapy inhibit the adhesion of monocytes to endothelial cells. *International Journal of Cardiology*, *202*, 756–759. https://doi.org/10.1016/j.ijcard.2015.10.035

ACKNOWLEDGEMENTS

First, I would like to extend my gratitude towards my supervisor, Prof. Reet Kurg, for her continued support through all my years of working in her lab, both during my PhD program as well as before that. She has always held a dual role in my life yet has always balanced all her roles perfectly. My journey from a third-year medical school student up to a PhD student has been amazing under her guidance. Thank you for accepting me into your group, even though I came from another world, and letting me be part of this kind of science as well. You are an excellent example of a hard-working scientist and I look up to you.

I would also like to thank my co-supervisor prof. Mart Ustav and the Icosagen team, including my clinical partner dr. Anu Ustav, who provided access to the patient cohorts used in my work, with whom I had the priviledge to work with during the start of my PhD studies. The start of that experience coincided with the covid19 pandemic in 2020, but during my time with them, I learned valuable skills that have benefitted me in both my medical, scientific and teaching work.

My thanks are also extended to everyone who has worked in our Kurglab science group at the Institute of Technology during my time there: Eve, Margit, Baiba, Mihkel, Aleksandra; as well as to those who have already graduated or left – Kadri, Olavi, Fred, Jane and Anneli. Thank you for all your help in the lab, meaningful discussions about both our work as well as all the fun. I am also thankful for the students who have worked on their theses in our lab, especially whose supervisor I have had the pleasure to be – Kwame and Teivi, and whose supervisor, in both official and unofficial capacity, I still am – Liina, Marleen and Kerstin. As well as all the students, I met but whose work I did not participate in – Ülleri, Mihai, Rando and Adiya to name a few. Sharing my knowledge with you keeps me rethinking what I know and rechecking every little fact to make sure I part it to you in a correct meaningful way.

Last, but definitely not least, I would like to express my sincerest thanks to all my friends and family. Both to new friends like Kaia Grete, who has not been in my life for long, yet has already made her impact, as well as everyone else who has been here for a while already. Such as my medical school classmates and medical school adjacent friends – Aileen, Cärolin, Jana, Mari, Martin, Ekaterina, Mari-Ann and Mati; high school friends Heli, Helena, Laura and Lille; school friends Sigrid and Linda-Marie; old gymnastics training group members Annaliis and Kirsika, and friends I have found through them like Katri; coworkers in the medical faculty, headed by prof. Kalle Kisand, and hospital laboratory, headed by dr. Anu Tamm, in Tartu – Anne, Kaja, Katrin, Mailis, Aivar, Anneli, Ain, Signe, Agnes, Piret, Mikk, Pille, Tiina, Kai, Krista and Tuuli; co-laboratory medicine residents both contemporary and the ones who started after I finished – Triin, Maarit, Ingrid, Signe, Anne, Merit and Evelina; all the lovely people from laboratory medicine departments in Tallinn, who I have worked with while there – Liisa, Piret, Ave, Riin, Gulara, Karel, Marge and Marika; all my co-workers in Basel Biozentrum headed by prof. Dirk Bumann – Vishwachi, Bea, Beatrice,

Jiagui, Jasmin, Jasmin, Franzi, Fabi, Minia and Reto; and my previous personal life partners AJ, Daniel and Koopa.

I thank my parents, Reet and Ants, for supporting me through all my schooling, and my aunt Raili and grandparents, Ene and Kalju. Also, my sister, Katrin, and her husband Harri for cheering me on, and my adorable little nieces, Kirke and Marta, who will not be able to read this thesis for another decade or so. Overall, I am very thankful for everyone in my circle who has surrounded me and always inspired me to always reach for the stars and excel at everything I do.

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Kurg, K., Planken, A., & Kurg, R. (2022). Proteomic and Biochemical Analysis of Extracellular Vesicles Isolated from Blood Serum of Patients with Melanoma. Separations, 9(4), 4. https://doi.org/10.3390/separations9040086

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Samel, A., Väärtnõu, F., Verk, L., **Kurg, K**., Mutso, M., & Kurg, R. (2023). How the Intrinsically Disordered N-Terminus of Cancer/Testis Antigen MAGEA10 Is Responsible for Its Expression, Nuclear Localisation and Aberrant Migration. Biomolecules, 13(12), 1704. https://doi.org/10.3390/biom13121704

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Kwame Boateng bakalaureuse töö, 2021

Ilmunud publikatsioonid:

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Kurg, K., Planken, A., & Kurg, R. (2022). Proteomic and Biochemical Analysis of Extracellular Vesicles Isolated from Blood Serum of Patients with Melanoma. Separations, 9(4), 4. https://doi.org/10.3390/separations9040086

Žėkas, V., Kurg, R., **Kurg, K**., Bironaitė, D., Radzevičius, M., Karčiauskaitė, D., Matuzevičienė, R., & Kučinskienė, Z. A. (2022). Oxidative Properties of Blood-Derived Extracellular Vesicles in 15 Patients After Myocardial Infarction. Medical Science Monitor, 28, 0–0. https://doi.org/10.12659/MSM.935291

Samel, A., Väärtnõu, F., Verk, L., **Kurg, K**., Mutso, M., & Kurg, R. (2023). How the Intrinsically Disordered N-Terminus of Cancer/Testis Antigen MAGEA10 Is Responsible for Its Expression, Nuclear Localisation and Aberrant Migration. Biomolecules, 13(12), 1704. https://doi.org/10.3390/biom13121704

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1. **Imre Mäger**. Characterization of cell-penetrating peptides: Assessment of cellular internalization kinetics, mechanisms and bioactivity. Tartu 2011, 132 p.
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