

ANNELI SAMEL

Unveiling the characteristics  
of cancer-testis antigen MAGEA10





**ANNELI SAMEL**

Unveiling the characteristics  
of cancer-testis antigen MAGEA10



UNIVERSITY OF TARTU

Press

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 02.01.2024, by the Joint Council of the  
Doctoral Program of Engineering and Technology of the University of Tartu.

Supervisor: Prof. Reet Kurg, PhD  
Professor of Molecular Biomedicine  
Institute of Technology, University of Tartu, Estonia

Reviewer: Ivar Ilves, PhD  
Associate Professor of Biomedicine  
Institute of Technology, University of Tartu, Estonia

Opponent: Yegor Vassetzky, PhD, DSc  
Senior Scientist of the French National Centre for Scientific  
Research  
Institut Gustave Roussy, Villejuif, France

Commencement: Auditorium 121, Nooruse 1, Tartu, Estonia, at 14.15 on  
February 9<sup>th</sup>, 2024

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

ISSN 2228-0855 (print)  
ISBN 978-9916-27-454-5 (print)  
ISSN 2806-2620 (pdf)  
ISBN 978-9916-27-455-2 (pdf)

Copyright: Anneli Samel, 2024

University of Tartu Press  
[www.tyk.ee](http://www.tyk.ee)

# CONTENTS

LIST OF ORIGINAL PUBLICATIONS .....	7
ABBREVIATIONS.....	8
1. INTRODUCTION.....	10
2. LITERATURE REVIEW.....	11
2.1 Cancer-testis antigens .....	11
2.1.1 Expression of CTAs.....	12
2.1.1.1 Regulation of CTA expression.....	14
2.1.2 Most CTAs are intrinsically disordered proteins .....	15
2.1.3 Functions of CTAs.....	16
2.1.3.1 Functions in normal cells .....	16
2.1.3.2 Tumour-associated functions .....	17
2.1.4 Clinical implications and usage in cancer immunotherapy .....	18
2.2 MAGE superfamily.....	20
2.2.1 MAGE homologs and evolution .....	21
2.2.2 The MAGE homology domain .....	21
2.2.3 Functions of MAGE proteins.....	22
2.2.4 MAGEA subfamily.....	24
2.2.5 MAGEA10.....	25
2.3 Extracellular vesicles .....	25
2.3.1 Functions of EVs .....	26
2.3.2 Clinical potential of EVs .....	27
3. AIMS OF THE STUDY.....	28
4. MATERIALS AND METHODS .....	29
5. RESULTS AND DISCUSSION .....	30
5.1 The N-terminal region affects multiple MAGEA10 characteristics (Study I) .....	30
5.1.1 The first 7 aa influence the aberrant migration of MAGEA10 in SDS-PAGE .....	30
5.1.2 Amino acids K6 and R7 are required for the nuclear localisation of MAGEA10.....	30
5.1.3 The N-terminus has a stabilising effect on MAGEA10.....	34
5.2 MAGEA proteins are incorporated into EVs (study II and I).....	35
5.2.1 Endogenous MAGEA4 is also incorporated into EVs.....	35
5.2.2 MAGEA proteins are expressed on the surface of EVs.....	36
5.2.3 The C-terminal region is required for incorporation into EVs .....	36
5.2.4 MAGEA10 induces EV shedding.....	37
5.3 EVs with MAGEA proteins are stable (study III).....	37

6. CONCLUSIONS.....	38
SUMMARY IN ESTONIAN.....	39
REFERENCES.....	41
ACKNOWLEDGEMENTS.....	73
PUBLICATIONS.....	75
CURRICULUM VITAE.....	131
ELULOOKIRJELDUS.....	132

## LIST OF ORIGINAL PUBLICATIONS

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

- I. **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>
- II. **Kuldkepp, A.**, Karakai, M., Toomsoo, E., Reinsalu, O., & Kurg, R. (2019). Cancer-testis antigens MAGEA proteins are incorporated into extracellular vesicles released by cells. *Oncotarget*, 10(38), 3694–3708.  
<https://doi.org/10.18632/oncotarget.26979>
- III. Reinsalu, O., **Samel, A.**, Niemeister, E., & Kurg, R. (2021). MAGEA4 Coated Extracellular Vesicles Are Stable and Can Be Assembled In Vitro. *International Journal of Molecular Sciences*, 22(10), 5208.  
<https://doi.org/10.3390/ijms22105208>

The original publications have been reproduced in this dissertation with the permission of MDPI (I, III) and Impact Journals, LLC (II).

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

- I. The author is the main author of this paper, having done a large portion of the experiments and contributed to the writing of the manuscript.
- II. The author is the main author of this paper, having done a large portion of the experiments and contributed to the writing of the manuscript.
- III. The author did some of the experiments and contributed to the writing of the article.

## ABBREVIATIONS

5DC	– 5'-aza-2'-deoxycytidine
AMPK	– AMP-activated protein kinase
BAGE	– B melanoma antigen
BORIS	– brother of imprinted sites
C/TIC	– cancer/tumour initiating cell
CAR	– chimeric antigen receptor
CG	– cancer-germline
CHMP6	– charged multivesicular body protein 6
CMV	– cytomegalovirus
CNS	– central nervous system
CSC	– cancer stem cell
CT	– cancer-testis
CT45	– cancer-testis antigen family 45
CTA	– cancer-testis antigen
DNMT	– DNA methyltransferase
DNMTi	– DNA methyltransferase inhibitor
DSB	– DNA double-strand break
EMT	– epithelial-mesenchymal transition
ESCRT	– endosomal sorting complex required for transport
ETS	– erythroblast transformation specific
EV	– extracellular vesicle
GAGE	– G antigen
GCI	– GpC island
HDAC	– histone deacetylase
HDACi	– histone deacetylase inhibitor
hESC	– human embryonal stem cell
HLA	– human leukocyte antigen
HORMAD1	– HORMA domain-containing protein 1
HSP70-2	– heat shock protein 70-2
IDP	– intrinsically disordered protein
ILV	– intraluminal vesicle
LIN28B	– Protein lin-28 homolog B
MAGE	– melanoma-associated antigen
MAGEA	– melanoma-associated antigen A
MAGEB	– melanoma-associated antigen B
MHC	– major histocompatibility complex
MHD	– MAGE homology domain
MLV	– murine leukaemia virus

MSC	– mesenchymal stem cell
MW	– molecular weight
MVB	– multi-vesicular body
NLS	– nuclear localisation signal
NSE1	– non-structural maintenance of chromosomes element 1
NXF2	– nuclear RNA export factor 2
NY-ESO-1	– New York esophageal squamous cell carcinoma 1
PASD1	– PAS domain-containing protein 1
PBS	– phosphate-buffered saline
PDB	– Protein Data Bank
PIWI	– P-element induced wimpy testis
POTE	– prostate, ovary, testes, and embryo
POTEE	– POTE ankyrin domain family member E
POTEG	– POTE ankyrin domain family member G
PRAME	– preferentially expressed antigen in melanoma
RING	– really interesting new gene
SCP-1	– synaptonemal complex protein 1
SDS-PAGE	– sodium dodecyl sulfate-polyacrylamide gel electrophoresis
Sp1	– specificity protein 1
SPAG9	– sperm-associated antigen 9
SPANX	– sperm protein associated with the nucleus on the X chromosome
SSX	– synovial sarcoma breakpoint X
SV40	– simian virus 40
TAA	– tumour-associated antigen
TCR	– T cell receptor
TRIM	– tripartite motif-containing protein
TSA	– trichostatin A
WASH	– Wiskott-Aldrich syndrome protein and scar homolog
WH	– winged-helix
VLP	– virus-like particle
wt	– wild-type

# 1. INTRODUCTION

Cancer is one of the leading causes of death in the modern world (Roth et al., 2018; WHO, 2022). Despite decades of extensive research, treatment options rarely go beyond chemo- and radiotherapy and surgical interventions. While immunological approaches to cancer treatment exist, they are usually not a part of the first line of care. Unfortunately, irradiation and chemotherapeutic agents do not distinguish between cancerous and normal tissues and therefore patients must endure a wide array of side effects from treatment. Meanwhile, researchers are working towards developing diagnostic and therapeutic tools that are more specific and convey less risks to the patients, such as many different types of specifically targeted therapies and immunotherapies. Nonetheless, these approaches have difficulties reaching the general clinical setting due to the heterogeneous nature of tumours and the resulting absence of universal cancer biomarkers.

Cancer-testis antigens (CTA) comprise a large family of tumour-associated antigens that are associated with tumorigenesis, are capable of eliciting immune responses in cancer patients, and have a fairly tumour-restricted expression pattern. This makes CTAs very good targets for cancer immunotherapy and indicates their usage as diagnostic or prognostic biomarkers. Of these, the MAGEA family comprises a set of highly immunogenic CTAs that have been shown to contribute to various processes in tumour cells. Their expression has been associated with poor patient outcomes and advanced stages of cancer. Unfortunately, their clinical and biological significance remains poorly understood and due to this many clinical trials involving the MAGEA family have failed due to ineffectiveness and unexpected side effects.

Another novel approach to cancer treatment involves extracellular vesicles (EV), lipid bilayer-enclosed nanosized particles that are released by all types of cells. They contain proteins, nucleic acids, metabolites, etc. from their cell of origin and are involved in cell-to-cell communication. Similar to CTAs, EVs can also be used as therapeutic or diagnostic tools against cancer. EVs have been shown to participate in antigen recognition and can be purified from all types of serological fluids.

CTAs and EVs both have the potential to be crucial elements of future cancer treatment. This dissertation focuses on the biological characteristics of MAGEA10, a highly immunogenic CTA. It is discovered that some known features of MAGEA10 are determined by unique sequences within the N-terminal part of the protein. Another discovery is that MAGEA10 is incorporated into native EVs and shares this characteristic with another family member, MAGEA4. These findings may support wider research into targeted cancer therapies and less invasive diagnostics.

## 2. LITERATURE REVIEW

### 2.1 Cancer-testis antigens

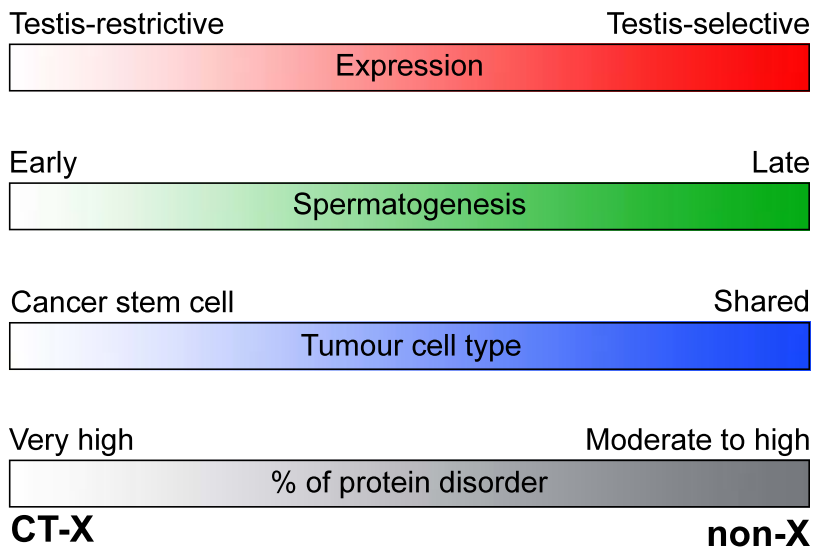
Cancer-testis (CT) antigens (Y.-T. Chen et al., 1997) (also referred to as cancer-germline (CG) antigens (Akers et al., 2010)) have been classified as a large family of tumour-associated antigens (TAAs), which are expressed in a variety of malignant tumours of different histological origin (Y.-T. Chen et al., 1997; Cho et al., 2002; Hofmann et al., 2008; Scanlan, Gordon, et al., 2002) as well as the germ cells of the testes (Bode et al., 2014; Y.-T. Chen et al., 2011), ovaries (Nelson et al., 2007), and placenta (Sahin et al., 1995; Scanlan et al., 2000). In most adult somatic tissues, the CTA genes are not expressed (Scanlan, Gure, et al., 2002). Another distinct feature of CT antigens is the ability to elicit immune responses in cancer patients, making them an attractive target for cancer immunotherapy and diagnostics (Sahin et al., 1995).

The first CT antigen was discovered in 1977, when two separate groups determined that antigen F9, previously found to be expressed in murine embryonal carcinoma cells, as well as early embryos and spermatozoa of different mammalian species, was also expressed in human teratocarcinomas (Hogan et al., 1977; Holden et al., 1977). However, wider research did not begin until van der Bruggen and colleagues managed to clone the first CT antigen MZ2-E or melanoma-associated antigen 1 (MAGE-1, now known as MAGEA1) and noted that it is responsible for the recognition of melanoma cell line MZ2-MEL by cytotoxic T lymphocytes (CTL) (Bruggen et al., 1991). Since then, over 250 CT antigens have been discovered and detailed records about most of them can be found in the CT database (<http://www.cta.lncc.br/>) (Almeida et al., 2009).

CTAs are divided into two groups, based on their chromosomal localisation: CT-X antigens, encoded by the X chromosome and non-X CTAs, encoded by the autosomes or the Y chromosome (Rajagopalan et al., 2011; Simpson et al., 2005). Interestingly, CT-X antigens make up about 10% of the X chromosome (Ross et al., 2005), whereas only one CTA is known to be encoded by the Y chromosome (Rajagopalan et al., 2011). CT-X genes form multigene families containing direct or inverted DNA repeats and are mainly distributed along the Xp11 and Xq26–28 regions, while non-X CT genes are usually single copy genes distributed throughout the genome (Ross et al., 2005; Simpson et al., 2005). There has been some debate about how many CT genes belong to either group. Initially, the CT Database has deemed that about half of the known CT antigens belong in the CT-X group (Almeida et al., 2009) but genome-wide analyses by Wang *et al* and Bruggeman *et al* have revealed a wide array of new potential CTAs of which only a small percentage (4% in the Bruggeman dataset and 105/1019 CT genes in the Wang dataset) turned out to be encoded by the X chromosome (Bruggeman et al., 2018; C. Wang et al., 2016).

### 2.1.1 Expression of CTAs

Different normal tissues express CT antigens during foetal development, but expression is constrained to immune-privileged sites after birth (Gjerstorff et al., 2010), whereas the adult testis is the only tissue which expresses all CTAs universally (Scanlan, Gure, et al., 2002). In adults, CTA mRNA expression is mainly confined to the germ cells of the testis and placenta (Caballero & Chen, 2009; Simpson et al., 2005). However, some CT antigens show expression in the adult brain, another site of immune privilege (Hofmann et al., 2008; Lifantseva et al., 2011), as well as other somatic tissues such as (but not limited to) the thyroid, spleen, liver, uterus, and pancreas although usually at mRNA levels less than 1% compared to the expression levels in the testis (Caballero & Chen, 2009; Y.-T. Chen et al., 1997; Crew et al., 1995; Lethé et al., 1998; Scanlan et al., 2004). Based on the differences in mRNA expression, Hofmann and colleagues categorised CTAs into three groups: a) testis-restrictive (genes that are expressed only in the adult testis and are silenced in all other normal adult tissues except for the placenta), b) testis/brain-restrictive (which show additional expression in all regions of the brain), and c) testis-selective (which have a more ubiquitous expression pattern) (Hofmann et al., 2008). Interestingly, there are noticeable differences between CT-X and non-X CT antigens (Fig. 1). CT-X antigens have a mostly testis-restricted expression pattern, while non-X CTAs tend to be in the testis-selective category (Hofmann et al., 2008). Additionally, CT-X antigens are expressed during the early stages of spermatogenesis, whereas non-X CTAs are expressed in the later stages (Simpson et al., 2005).



**Figure 1. The differences between CT-X and non-X CTAs.** (Hofmann et al., 2008; Rajagopalan et al., 2011; Simpson et al., 2005; Yamada et al., 2013)

CTA protein expression in normal tissues is restricted to the germ cells of the testis, ovaries, and placenta (dos Santos et al., 2000; Jungbluth et al., 2000, 2001), as well as the developing central nervous system (CNS) (Gjerstorff et al., 2010) and adrenal cortex (Gjerstorff et al., 2008). It is important to note that, while low levels of CTA mRNA expression is seen in some additional somatic tissues mentioned above, their expression has not been confirmed at a protein level (Caballero & Chen, 2009; Scanlan et al., 2004). However, during foetal development, CT antigen expression is initiated and maintained at the protein level in both male and female germ cells already at 5 weeks gestation, in sexually non-differentiated migrating primordial germ cells (Gjerstorff et al., 2007). A common denominator for CTA expression during foetal development is that the expression is shut down towards birth before the immune system is fully developed (Gjerstorff et al., 2010). The genes are expressed again when male germ cell proliferation is reinitiated during puberty (Gjerstorff et al., 2010). The only known exceptions to this are members of the GAGE family, whose expression is maintained in a subset of adult oocytes (Gjerstorff et al., 2006).

CT antigen expression in human malignancies tends to be largely heterogenous between different tumour types, different tumours of the same type (intertumour heterogeneity) as well as between different regions of the same lesion (intra-tumour heterogeneity) (Akers et al., 2010). However, it is common for CTAs to be co-expressed (Djureinovic et al., 2016; Güre et al., 2005; Sahin et al., 1998; Scanlan et al., 2000; Tajima et al., 2003). MAGE-A1, MAGE-A3, New York esophageal squamous cell carcinoma 1 (NY-ESO-1), synovial sarcoma X breakpoint 2 (SSX-2), and SSX-4 are some of the most frequently expressed CTAs, whereas B melanoma antigen (BAGE), G antigen A1 (GAGE-A1) and synaptonemal complex protein 1 (SCP-1) show very rare expression (Scanlan, Gure, et al., 2002). Cancers which frequently express CTAs include melanoma and cancers of the lung, bladder and breast (including triple-negative breast cancer) (Curigliano et al., 2011; Scanlan et al., 2004; Scanlan, Gure, et al., 2002). CT antigen expression is rarely seen in hematologic malignancies, as well as renal and colon cancers (Gjerstorff et al., 2010; Scanlan et al., 2004; Scanlan, Gure, et al., 2002).

Additionally, it has been found that CT antigens are expressed in both normal (Cronwright et al., 2005) and cancer stem cells (Gjerstorff et al., 2006; Sigalotti et al., 2008). Under normal circumstances, CTA expression can be seen in foetal and adult mesenchymal stem cells (MSCs) and bone marrow (Cronwright et al., 2005), undifferentiated human embryonic stem cells (hESCs) (Lifantseva et al., 2011), and differentiated hESCs from different lineages (Gjerstorff et al., 2007, 2008; Lifantseva et al., 2011). This type of CT antigen expression can be seen in both early human and mouse embryos, suggesting that CTAs play important roles in early embryo development (Gordeeva & Pochaev, 2017; Madissoon et al., 2014). However, it is more complicated in cancerous tissues. CTA expression has been found in teratomas (Gjerstorff et al., 2008), which are benign growths derived from all three embryonic germ layers (Lensch & Ince, 2007), and teratocarcinomas (Lifantseva et al., 2011), which are malignant germ cell tumours (Lensch & Ince, 2007). Furthermore, CTA expression and its heterogeneity in malignancies

can be linked to cancer stem cells (CSCs) (Gordeeva, 2018). CSCs, also referred to as cancer/tumour-initiating cells (C/TICs) or tumorigenic cells are described as a subpopulation of cancer cells that are resistant to chemo- and radiotherapy (Dean et al., 2005) and have the abilities of tumour-initiation, self-renewal, and differentiation (Clarke et al., 2006). This type of cell was first identified in hematologic malignancies in both mice (Bruce & Van Der Gaag, 1963; Park et al., 1971) and humans (Griffin & Lowenberg, 1986; Sabbath et al., 1985) over 50 years ago and also confirmed in solid tumours (Hamburger & Salmon, 1977; Southam & Brunschwig, 1961). CT antigen expression in CSCs has been confirmed in melanoma (Gedye et al., 2009; Sigalotti et al., 2008), glioblastoma (Low et al., 2010), and breast cancer (J. H. Lee et al., 2010), for example. An interesting aspect, found by Yamada *et al*, is that some CTAs show preferential expression in CSCs, compared to non-CSCs (CSC genes), whereas others have similar expression levels in both cell types (shared antigens) (Yamada et al., 2013). Curiously, a difference in expression was noted between CT-X and non-X CTAs (Fig. 1), as 67% of the CSC genes were located on the X chromosome compared to 20% of the shared antigens (Yamada et al., 2013).

### **2.1.1.1 Regulation of CTA expression**

CTA expression is primarily regulated by epigenetics – stably heritable gene expression states that do not directly depend on the DNA base sequence (Berger et al., 2009; De Smet et al., 1999; Gjerstorff et al., 2009). Epigenetic regulation has been shown to influence both the coexpression of CT genes (Yao et al., 2014) and their heterogeneous expression pattern in tumours (Fratta et al., 2010; Sigalotti et al., 2004; Woloszynska-Read et al., 2008). DNA methylation was the first epigenetic modification to be associated with CT antigen expression (De Smet et al., 1996; J. Weber et al., 1994). It was noted that DNA methyltransferase inhibitors (DNMTi), such as 5'-aza-2'-deoxycytidine (5DC), can upregulate existing and induce *de novo* CTA expression (Cho et al., 2002; De Smet et al., 1996, 1999; dos Santos et al., 2000; Lethé et al., 1998; Lurquin et al., 1997; Sigalotti, Coral, Altomonte, et al., 2002; Sigalotti, Coral, Nardi, et al., 2002; J. Weber et al., 1994). Most CT gene promoters contain CpG islands (CGI), DNA sites with a high CpG dinucleotide frequency (Illingworth & Bird, 2009), which are methylated in normal somatic tissues, thus silencing the genes (Akers et al., 2010; De Smet et al., 1999; Güre et al., 2002). These CGI become demethylated when global DNA hypomethylation occurs during gametogenesis (De Smet et al., 1999; Kimmins & Sassone-Corsi, 2005; Reik et al., 2001) and tumorigenesis (De Smet et al., 1996; Feinberg & Vogelstein, 1983; Gama-Sosa et al., 1983; Woloszynska-Read et al., 2008) and more so in the case of promoter-specific hypomethylation of CT genes (R. Kim et al., 2013; Woloszynska-Read et al., 2008). As described above, promoter methylation is an integral part of CTA expression regulation. However, it is not solely responsible for CT gene activity, since, for example, DNMTi treatments alone do not induce CT antigen expression in primary fibroblasts, normal mammary epithelial cells or melanocytes (Karpf et al., 2004; J. Weber et al., 1994).

Histone modifications, such as acetylation and methylation, also have an effect on CTA expression (James et al., 2006; Link et al., 2009; Sun et al., 2009; Tachibana et al., 2002; Wischniewski et al., 2006; Woloszynska-Read et al., 2007). Histone deacetylase inhibitors (HDACi), like trichostatin A (TSA) or depsipeptide FR901228 (DP), have been shown to have a minor effect on CT gene expression, but in combination with 5DC demonstrated a much stronger effect than either HDACi or DNMTi alone (Wargo et al., 2009; Weiser et al., 2001; Wischniewski et al., 2006). Similar effects have been observed when investigating the effect of histone methylation on CT gene expression (Link et al., 2009; Sun et al., 2009).

There is also a wide array of non-epigenetic agents that regulate CTA expression. Studies have shown that CTA expression can also be influenced by:

- cytokines (Z. Wang et al., 2006; Y. Zhang et al., 2009);
- tyrosine kinases (Hoei-Hansen et al., 2007; Kondo et al., 2007; B. Yang, Wu, et al., 2007; X. Zhu et al., 2008);
- erythroblast transformation specific (ETS) transcription factor sites (De Smet et al., 1995, 1996, 2004; Janssen et al., 1999; Loriot et al., 2008; Serrano et al., 1996);
- cAMP (Bai et al., 2008; Karpf et al., 2009);
- specificity protein 1 (Sp1) (Kang et al., 2007);
- p53 (Renaud et al., 2007);
- ncRNA (C. Wang et al., 2016);
- gamma radiation (Sharma et al., 2011).

Furthermore, it has been suggested that brother of the regulator of imprinted sites (BORIS), a CT antigen, can influence the expression of other CT genes (Hong et al., 2005; Kang et al., 2007; Loukinov et al., 2002; Vatolin et al., 2005) but there is contradicting evidence (Kholmanskikh et al., 2008).

### **2.1.2 Most CTAs are intrinsically disordered proteins**

Intrinsically disordered proteins (IDP) lack a rigid 3D structure in localised regions or along their entire length under physiological conditions (Uversky & Dunker, 2010). Due to this, they are structurally flexible and can undergo disorder-to-order transitions when binding to their biological target, which enables interactions with a broad range of binding partners (Tompa & Csermely, 2004). This, in turn, demonstrates why proteins with a higher disorder content tend to occupy hub positions (interacting with at least 5 partners) in protein interaction networks (Haynes et al., 2006; Patil et al., 2010). It has been determined that a significant majority (over 90%) of CT antigens can be classified as IDPs, whereas

CT-X antigens tend to be more disordered than non-X CTAs (Fig. 1) (Rajagopalan et al., 2011). Moreover, Rajagopalan and colleagues noted that, in line with previous understandings about IDPs, the more disordered CTAs were predicted to occupy hub positions in protein-protein interaction networks and to have a high probability of binding DNA (Rajagopalan et al., 2011).

### 2.1.3 Functions of CTAs

Despite decades-long research, the relevance of CT antigens in either germ cell development or tumorigenesis remains poorly understood, although it has been implied that CTAs may have important roles in both, based on their expression patterns and the fact that cancer cells share similar phenotypes with germ cells (Beard, 1905; Gjerstorff et al., 2010; Old, 2001). However, discerning individual CTA functions and whether CTAs are expressed in cancer because they contribute to tumorigenesis or as a side-effect of genomic instability has proven to be difficult (Gibbs & Whitehurst, 2018; Gjerstorff et al., 2010). The reasons for this include the disordered structures and lack of known motifs or domains in many CTAs (Rajagopalan et al., 2011) and a lack of model organisms for many CT-X antigens because of very rapid evolution of the genes (Stevenson et al., 2007). On the other hand, many non-X CT antigens do have orthologs in other species and therefore their functional roles are much better understood (Gibbs & Whitehurst, 2018).

#### 2.1.3.1 Functions in normal cells

Many CTAs, particularly non-X CTAs, have distinct functions in spermatogenesis and fertilisation. Members of the P-element induced wimpy testis (PIWI) and MAGE family are involved in germline stem cell self-renewal (Cox et al., 1998, 2000) and maintenance as well as protecting the germline from stress (Fon Tacer et al., 2019; A. K. Lee et al., 2020). Some CTAs participate in germ cell meiosis, having roles in homologous chromosome pairing, the synaptonemal complex and recombination (Hamer et al., 2006; Keeney et al., 1997; Meuwissen et al., 1992). Another CT antigen, BORIS plays a role in epigenetic reprogramming during spermatogenesis (Loukinov et al., 2002). It has also been determined that CTAs are involved in spermatid differentiation (Aoki & Matsui, 2019), acrosome development (Kanemori et al., 2013), chromatin compaction (Brower-Toland et al., 2007; Pivot-Pajot et al., 2003), sperm movement (Whitehurst, 2014), sperm-egg adhesion (H. Nishimura et al., 2001) and successful fertilisation (Depa-Martynów et al., 2007) and probably in many more germ cell processes.

CT antigens also have functions outside germ cells. For example, PLU-1 is a transcriptional corepressor (Tan et al., 2003), nuclear RNA export factor 2 (NXF2) exports RNA from the nucleus to the cytoplasm and is therefore thought to be a part of the translational control of gene expression (Herold et al., 2000), MAGEA1 is involved in BORIS promoter methylation (Zhao et al., 2019), MAGEB3 has a

role in X chromosome inactivation (W. Li et al., 2018), and PAS domain-containing protein 1 (PASD1) disrupts the circadian rhythm (Michael et al., 2015).

### 2.1.3.2 Tumour-associated functions

CTAs have many functions in tumours which express them. Since cancer cells acquire a germ-like state (Old, 2001), functions like promoting proliferation and migration are shared by both cell types. A brief overview of CT antigens participating in different tumour cell functions is given in Table 1. The functional categories were reported in (C. Fan et al., 2021).

The genome and everything surrounding it comprises a very delicate system within the cell. Genome integrity as well as epigenetic modulations are important in all types of cells and changes in either may lead to the cell becoming cancerous. Therefore, the aforementioned epigenetic functions of MAGEA1 and MAGEB3 in promoter methylation (Zhao et al., 2019) and X chromosome inactivation (W. Li et al., 2018), respectively, can have significant roles in tumours as well. Other CTAs have been linked to genomic stability, for example SSX2 induces DNA damage (Greve et al., 2015) and HORMA domain-containing protein 1 (HORMAD1) promotes DNA double-strand break (DSB) repair while compromising DNA mismatch repair (Y. Gao et al., 2018; Liu et al., 2020).

**Table 1. CTA functions in tumour cells.**

Genome-related	Genome instability	HORMAD1, SSX2
	Epigenetic modulation	MAGEA1, MAGEB3
Tumour proliferation	Mediating apoptosis/autophagy	CAGE, GAGE7C/B, MAGEA3, PASD1, POTEE, POTEG, SPAG6
	Promoting tumour growth	HSP70-2, MAGEA3, NY-ESO-1, POTEE, SPAG9, SPANX
	Tumour metabolism	LIN28B
Tumour progression	Resistance to treatment	GAGE, HORMAD1, LIN28B, MAGEA
	Inducing angiogenesis	CAGE, Hiwi, XAGE-1b
	Invasion and metastasis	HSP70-2, LEMD1, NY-ESO-1, POTEG, SPA17, SPAG9, SPANXN2

Proliferation is an integral part of cancer survival and development. Thus, many CTAs have been found to contribute to it. Protein lin-28 homolog B (LIN28B) has been shown to promote aerobic glycolysis and attenuate oxidative phosphorylation, thus influencing the Warburg effect (Warburg, 1956) on cell metabolism

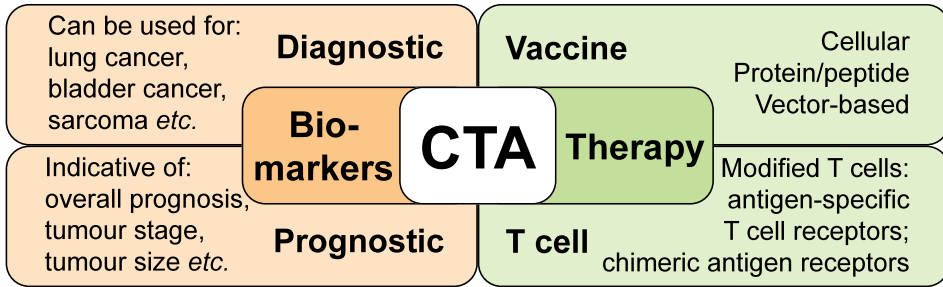
(Mizushima et al., 2020). CT antigens that positively affect tumour growth include, but are not limited to, sperm protein associated with the nucleus on the X chromosome (SPANX) (Lazar et al., 2020), sperm-associated antigen 9 (SPAG9) (C. Xiao et al., 2019), prostate, ovary, testes, and embryo (POTE) ankyrin domain family member E (POTEE) (Shen et al., 2019), NY-ESO-1 (F. Li et al., 2020), heat shock protein 70-2 (HSP70-2) (Gupta et al., 2017), MAGEA3 (X. Gao et al., 2020). It has recently been found that, in addition to CT antigens, tumour proliferation can also be influenced by CT long non-coding RNAs (Hosono et al., 2017).

Avoiding cell death is another key element in tumour survival. However, CTAs can have different effects on processes like autophagy and apoptosis. Many CT antigens inhibit apoptosis and autophagy, like SPAG6 (M. Zhang et al., 2020), POTEE (Shen et al., 2019), MAGEA3 (X. Gao et al., 2020), GAGE7C/B (Cilensek et al., 2002), PASD1 (R. Li et al., 2019), MAGEC2 (Lajmi et al., 2015). Meanwhile, CAGE enhances autophagosome formation (Yeon et al., 2019), POTE ankyrin domain family member G (POTEG) induces apoptosis (L. Wang et al., 2018) and MAGEA4 has been shown to both increase (Peikert et al., 2006) and inhibit (Bhan et al., 2012) apoptosis.

Lastly, CTAs also influence tumour progression by promoting tumour cell invasion (Gupta et al., 2017; F. Li et al., 2020; Sasahira et al., 2016; C. Xiao et al., 2019), epithelial-mesenchymal transition (EMT) (L. Chen et al., 2021; B. Shang et al., 2014) and metastasising (Y. Zhou et al., 2019), inducing angiogenesis (Y. Kim et al., 2013; S. Li et al., 2010; B. Zhou et al., 2013), and making tumours more resistant to chemo- and/or radiotherapy (Cilensek et al., 2002; Duan et al., 2003; Y. Gao et al., 2018; Mizushima et al., 2020; Monte et al., 2006; Suzuki et al., 2021). Interestingly, some CTAs can instead inhibit cell migration and EMT (L. Wang et al., 2018; F. Zhu et al., 2020).

#### **2.1.4 Clinical implications and usage in cancer immunotherapy**

Since their discovery, implications have been made that CTAs could be used as cancer biomarkers and/or targets for immunotherapy (Holden et al., 1977). Over the years, CT antigen expression has been proven as a probable useful diagnostic marker in hepatocellular carcinoma (Ren et al., 2017), sarcoma (Iura et al., 2017), and cancers of the bladder (Afsharipad et al., 2019), lung (Ren et al., 2017), thyroid (Melo et al., 2017), and stomach (Shida et al., 2020). Furthermore, CTAs could also be used as prognostic markers, as their expression has been associated with advanced tumour stage (Tarnowski et al., 2016; Velazquez et al., 2007; Yao et al., 2014), larger tumour size (Güre et al., 2005), progressive disease (Jäger et al., 1999), as well as both poor and better overall prognosis (da Silva et al., 2017; Freitas et al., 2013; Yao et al., 2014). Nevertheless, using these associations in a clinical setting may be cumbersome due to the large number of CTAs and their heterogeneous expression in cancers. A brief overview of the clinical uses of CTAs is given in Figure 2.



**Figure 2. Clinical uses of CTAs.**

As explained beforehand, CTA expression has been detected in a multitude of cancers, but the genes are mainly silenced in normal adult tissues. In most cases, their expression is limited to immune-privileged sites (Streilein, 1995), such as the testes, placenta, and brain, so that when the genes are activated in other somatic cells, their products can elicit both cellular and humoral immune responses (Jäger et al., 1998; Jäger, Nagata, et al., 2000). Thus, CTAs are regarded as very good targets for cancer immunotherapy. In this regard, NY-ESO-1 is known as the most immunogenic CT antigen (Stockert et al., 1998) and is therefore one of the best targets for all types of therapies. Other CTAs used for immunotherapeutic clinical trials include members of the MAGEA family, preferentially expressed antigen in melanoma (PRAME), BORIS, and cancer/testis antigen family 45 (CT45) (P. Yang et al., 2021).

Cancer immunotherapy can largely be divided into vaccine-based and T cell therapy. Tumour vaccines have three main types: cellular, protein- or peptide-based, and vector-based vaccines (Bolhassani et al., 2011). Cellular vaccines containing patient-derived dendritic cells (DC) pulsed with immunogenic peptides have been used for MAGEA vaccinations but have not shown significant responses (Andersen et al., 2001; Banchereau et al., 2001, 2005). The majority of clinical trials have been carried out using protein- or peptide-based vaccines. NY-ESO-1 (Davis et al., 2004; Jäger, Gnjatic, et al., 2000; Jäger, Nagata, et al., 2000) and members of the MAGEA family (Chianese-Bullock et al., 2005; Marchand et al., 1999; Pujol et al., 2015; Slingluff et al., 2016) have been tested in various malignancies and multiple vaccine combinations, but results have revealed little clinical significance. It has become clear that recombinant proteins or peptides have a greater therapeutic effect when they are accompanied by adjuvants (Atanackovic et al., 2004; Davis et al., 2004) or when the vaccines include multiple epitopes (Migliorini et al., 2019; Walter et al., 2012; Zeng et al., 2002). The last group, vector-based vaccines, comprises a diverse array of vectors ranging from plasmid DNA to oncolytic viruses that are used to deliver tumour-specific antigens (Bolhassani et al., 2011). DNA vaccines have shown promising results for both MAGEA (Duperret et al., 2018) and NY-ESO (Xue et al., 2016). Other promising vaccines include lentivirus- (Somaiah et al., 2019), cowpea mosaic virus (Patel et al., 2020) and mRNA-based vaccines (Sahin et al., 2017, 2020; Sebastian et al., 2019).

Adoptive T cell therapy includes isolation and *ex vivo* expansion of tumour-infiltrating lymphocytes or blood-derived genetically engineered T cells, which are then reintroduced into the patient (Met et al., 2019). T cells can be genetically modified to express antigen-specific T cell receptors (TCR) using viral vectors (Morgan et al., 2006) or to bear chimeric antigen receptors (CAR) by fusing the antigen-binding domain to domains that can initiate T cells without the help of the major histocompatibility complex (MHC) (Eshhar et al., 1993). TCR T cell therapy has shown promising results for NY-ESO-1 (Robbins et al., 2011) and MAGEA (Lu et al., 2017; Meng et al., 2021) and research is ongoing with other CTAs as well (Meng et al., 2021). CAR T cell therapy can easily be used for surface antigens but with the help of TCR mimic antibodies, these cells can identify intracellular targets as well (Jakobsen & Gjerstorff, 2020). Thus far, positive results have been obtained with MAGEA1 (Mao et al., 2019), but PRAME and other CT antigens may prove to be useful in the future (Jakobsen & Gjerstorff, 2020).

Although individual strategies seem promising, CTA-based immunotherapy faces many challenges regarding the heterogeneous tumour expression of CTAs, the presence of MHCs on cancer cells, the hostile tumour microenvironment *etc* (P. Yang et al., 2021). These can be overcome by combining different conventional and immunotherapeutic treatments. Cancer immunotherapy in combination with chemo- or radiotherapy, DNMT or HDAC inhibitors, or immune checkpoint inhibition, or a combination of different immunotherapeutic strategies have already shown and will continue to show better results than any of these alone (Gjerstorff et al., 2010, 2015).

## 2.2 MAGE superfamily

The *MAGE* family of genes has interested researchers since the discovery of the first member in the 1990s (Bruggen et al., 1991). It consists of over 40 genes in humans, including some classified as pseudogenes (Chomez et al., 2001). The family is divided into type I and type II based on the chromosomal localization, sequence homology, and tissue expression (Barker & Salehi, 2002). Type I comprises *MAGE* subfamilies *A*, *B*, and *C*, which are encoded by the X chromosome and follow the expression pattern of classical CTAs, whereas *MAGE-D*, *-E*, *-F*, *-G*, *-H*, *-L* and *NECDIN* genes, which are encoded by chromosomes X, 3, and 15 and have a more ubiquitous expression pattern make up type II (Barker & Salehi, 2002; Chomez et al., 2001; Fon Tacer et al., 2019; Gee et al., 2020). They can be further classified by their expression in normal tissues into types Ia (testis-restricted), Ib (testis/placenta/ovary), Ic (not restricted), IIa (ubiquitous), and IIb (brain-enriched) (Gee et al., 2020).

## 2.2.1 MAGE homologs and evolution

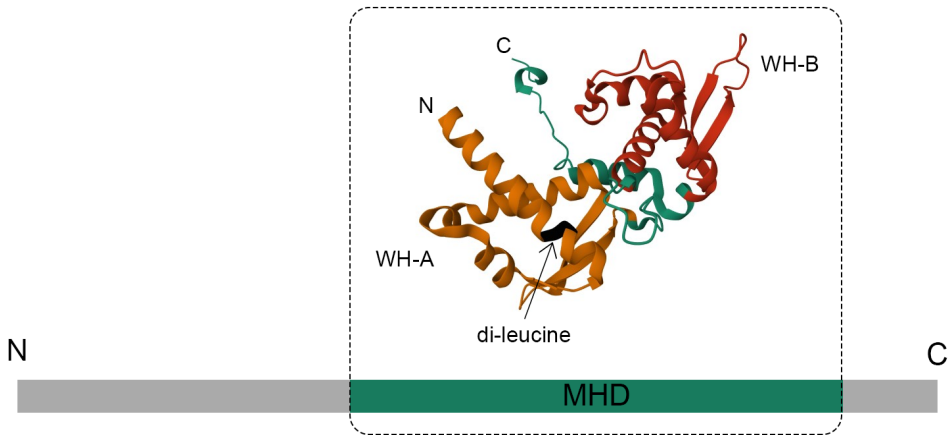
*MAGE* genes are found in a majority of eukaryotes, going all the way back to protozoa (López-Sánchez et al., 2007). Mammalian genomes have a variety of *MAGE* genes, divided into subfamilies, whereas lower eukaryotes typically have only one *MAGE* gene (Chomez et al., 2001; Katsura & Satta, 2011; López-Sánchez et al., 2007). The *MAGE* found in *Arabidopsis thaliana* has a 25% similarity to human *MAGEA8* (Katsura & Satta, 2011) and *MAGEG1* has homologs in *Drosophila melanogaster* (*DMAGE*) (I. Nishimura et al., 2007; Pöld et al., 2000) and *Danio rerio* (*Dre mage*) (Bischof et al., 2003). *CMage* from the chicken genome shares similarities with both *MAGED* and *MAGEG1* (López-Sánchez et al., 2007). The most researched homologs to human *MAGEs* are mouse *Mages*, in hopes of using mice as model organisms for CTA research (Fon Tacer et al., 2019). They share many similarities with their human counterparts in both genomic and functional aspects and therefore provide an abundance of information about the gene family (Gee et al., 2020).

It has been speculated that all *MAGE* genes are evolved from a single ancestral *MAGE* gene and that the rapid expansion of the superfamily began with the emergence of placental mammals (Chomez et al., 2001; Katsura & Satta, 2011; López-Sánchez et al., 2007). This idea is supported by the aforementioned fact that genomes of lower eukaryotes have only one *MAGE* gene, whereas mammals have multiple *MAGE* subfamilies (Chomez et al., 2001; Katsura & Satta, 2011). Although the idea of an ancestral *MAGE* gene is commonly accepted, it is not entirely clear whether *MAGED* or *MAGEG1* is more closely related to it (A. K. Lee & Potts, 2017). *MAGED* seems to be the closest match when looking at the genomic structure (Chomez et al., 2001; A. K. Lee & Potts, 2017) but *MAGEG1* appears to be functionally more related (López-Sánchez et al., 2007; I. Nishimura et al., 2007; Pebernard et al., 2004; Taylor et al., 2008). Regardless of which exact modern *MAGE* gene is the closest relative of the ancestral gene, it is clear that type II *MAGEs* are evolutionarily older than type I *MAGEs* (Katsura & Satta, 2011).

## 2.2.2 The MAGE homology domain

All *MAGE* proteins share a conserved domain known as the *MAGE* homology domain (MHD) (Barker & Salehi, 2002). It is about 170 amino acids long and can be found in proteins of many eukaryotic organisms, including *Drosophila melanogaster*, *Saccharomyces cerevisiae*, and *Arabidopsis thaliana* (Barker & Salehi, 2002; I. Nishimura et al., 2007; Pebernard et al., 2004). The MHD is highly conserved in the mammalian *MAGE* family, all human MHDs share 46% of amino acids and the similarities within subfamilies are even more prevalent, reaching 70 and 75% in *MAGEA* and *MAGED* subfamilies, respectively (Doyle et al., 2010). The position of the MHD within the protein is generally near the C terminus (**Fig. 3**) and it is flanked by non-conserved N- and C-terminal sequences

(Barker & Salehi, 2002; Chomez et al., 2001). Although rare, some MAGE proteins have a truncated MHD and a few type II MAGEs have two MHDs (Barker & Salehi, 2002; Chomez et al., 2001).



**Figure 3. Overall structure of MAGE proteins.** The 3D structure of the MHD is from MAGEA3 (PDB ID: 4V0P) (Newman et al., 2016). The image was constructed, using the Mol\* Viewer software (Sehnal et al., 2021) on the Protein Data Bank (PDB) website [www.RSCB.org](http://www.RSCB.org) (Berman et al., 2000).

The MHD consists of two winged-helix (WH) motifs (Fig. 3), termed WH-A and WH-B of which the former contains a di-leucine motif crucial for the biochemical functions of MAGE proteins (Doyle et al., 2010; Newman et al., 2016). Studies have shown that the overall structure of individual MHDs is similar, despite peptide sequence differences or the presence of disordered regions (Hagiwara et al., 2016; Newman et al., 2016). However, the disordered regions and a broad charge state distribution revealed by native mass spectrometry of MAGEA4 indicate that although MHDs are relatively conserved, they are dynamic and give unique features to individual MAGE proteins (Doyle et al., 2010; Hagiwara et al., 2016).

### 2.2.3 Functions of MAGE proteins

The most ubiquitous function of MAGE proteins is their ability to bind and interact with different E3 ligases (Gee et al., 2020). Both type I and type II MAGE proteins have been shown to assemble with E3 ligases containing the really interesting new gene (RING) domain to form MAGE-RING ligases (MRL) (Deshaies & Joazeiro, 2009; Doyle et al., 2010; Gee et al., 2020). Generally, each MAGE interacts with a specific ligase, however, highly homologous MAGEs tend to have the same binding partner (Doyle et al., 2010). For example, MAGEA2, -A3, -A6, and -C2 all bind E3 ligase tripartite motif-containing protein 28 (TRIM28) and MAGEF1 and -G1 both bind non-structural maintenance of chromosomes element 1 (NSE1) (Doyle et al., 2010). MAGEs bind to the RING ligases through

their MHD, whereas the binding region on the ligases varies across different MRLs, demonstrating the versatility of the MHDs (Doyle et al., 2010).

MAGE interactions with RING E3 ligases are known to alter the subcellular localisation, enhance the ubiquitination activity, and specify novel substrates of the ligases (A. K. Lee & Potts, 2017). It has been shown that MAGEL2 binds with TRIM27 and mediates its localisation to retromer-positive endosomes (Y.-H. Hao et al., 2013). On the other hand, enhancing the ubiquitination activity of the ligases seems to be the most common effect of the MAGEs (Gee et al., 2020). They have been shown to increase the activity of E3 ligases TRIM28 (Doyle et al., 2010; T. Z. Xiao et al., 2011, 2014), TRIM31 (Kozakova et al., 2015), and NSE1 (Doyle et al., 2010; Pebernard et al., 2004). MAGEs are also able to modify RING E3 ligases by specifying novel substrates for ubiquitination (Gee et al., 2020). For example, the MAGEL2-TRIM27 MRL is required for endosomal sorting of proteins, since it ubiquitinates the Wiskott-Aldrich syndrome protein and scar homolog (WASH) complex, thus activating it and allowing F-actin assembly on endosomes (Y.-H. Hao et al., 2013). Moreover, the presence of MAGEA3/6 determines the ubiquitination and subsequent degradation of AMP-activated protein kinase AMPK $\alpha$ 1 by TRIM28 and thus inhibits autophagy and promotes cell growth, since AMPK $\alpha$ 1 is the catalytic subunit of the AMPK heterotrimer responsible for maintaining energy homeostasis, which has a cytostatic effect when activated. (Hardie et al., 2012; Pineda et al., 2015). Another interesting example is that upon binding to MAGEF1, NSE1 targets methyl methane sulfonate-sensitive 19 (MMS19) for ubiquitination and degradation, which results in impaired DNA repair capacity due to reduced MMS19-mediated DNA repair enzyme activation (Weon et al., 2018). MRLs have also been shown to regulate transcription (T. Z. Xiao et al., 2011, 2014), inhibit p53 (Doyle et al., 2010; B. Yang, O'Herrin, et al., 2007), and influence cancer metabolism (Jin et al., 2017). MAGEs also affect Skp1-Cullin-F-box (SCF) cullin-RING ligases (CRL), a family of multicomponent E3 ligases, by interacting with individual elements of the SCFs and thus participate in the regulation of cyclins A and E (J. Hao et al., 2015; Petroski & Deshaies, 2005; Su et al., 2017). Lastly, MAGEA11 has been shown to bind HECT, UBA and WWE domain-containing protein 1 (HUWE1), a homologous to E6AP C-Terminus (HECT) E3 ligase, and to promote alternative mRNA polyadenylation and 3' untranslated region shortening in tumours (Rotin & Kumar, 2009; S. W. Yang et al., 2020).

Beyond functioning in tandem with E3 ligases, MAGEs are involved in transcription regulation (Askew et al., 2009; Laduron et al., 2004; Masuda et al., 2001), stress tolerance (A. K. Lee et al., 2020), neurogenesis (Kendall et al., 2005), and genetic disorders (Fon Tacer & Potts, 2017; Laghmani et al., 2016). MAGE involvement in tumour processes is briefly described in section 2.1.3.2.

## 2.2.4 MAGEA subfamily

The *MAGEA* subfamily is located on the q28 region of the X chromosome and is comprised of 12 genes, of which 11 are protein-coding and *MAGEA7* is a pseudo-gene (Plaen et al., 1994; Rogner et al., 1995). Members of the family share a DNA coding sequence similarity between 70–98%, with *MAGEA3* and *MAGEA6* being the most similar (Rogner et al., 1995). *MAGEA* protein lengths range from 124 (*MAGEA5*) to 429 (*MAGEA11*) amino acids, however most of them are 314–319 amino acids long (Meek & Marcar, 2012). The proteins show a high degree of sequence similarity as well, especially within the MHD, but also within both termini (Doyle et al., 2010; Meek & Marcar, 2012). A few members have interesting features, such as the truncated MHD of *MAGEA5* and additional inserts to the termini of *MAGEA10* and *MAGEA11* (Meek & Marcar, 2012).

*MAGEA* protein functions have mostly been described in previous sections of the paper, but a brief overview is given in Table 2. *MAGEA* proteins have been found to bind several other proteins. For example, *MAGEA4* binds to and suppresses the oncoprotein gankyrin (Nagao et al., 2003) and also Miz-1, leading to the transcriptional suppression of p21<sup>Cip1</sup> (Sakurai et al., 2004). *MAGEA2* binds the DNA binding surface of p53, inhibiting its interactions with chromatin (Marcar et al., 2010).

**Table 2. Biological functions of MAGEA proteins**

<b>MAGEA protein</b>	<b>Biological functions</b>
<i>MAGEA1</i>	Activating p-C-JUN directly or through ERK-MAPK pathways (D. Wang et al., 2016)
	Repressing transcription by binding to SKIP and recruiting HDAC1 (Laduron et al., 2004)
<i>MAGEA2</i>	Degradation of p53 (Doyle et al., 2010)
	Inhibition of MDM2 auto-ubiquitination (Marcar et al., 2015)
	Reduced degradation of MDM4 (Marcar et al., 2015)
	Increasing ER-dependent signalling (Wong et al., 2014)
	Repression of p53 activity (Marcar et al., 2010; Monte et al., 2006)
<i>MAGEA3/6</i>	Degradation of p53 (Doyle et al., 2010)
	Degradation of AMPK $\alpha$ 1 (Pineda et al., 2015)
	Degradation of ZNF382 (T. Z. Xiao et al., 2011)
	Enhancing TRIM28-dependent degradation of FBP1 (Jin et al., 2017)
<i>MAGEA4</i>	Suppressing the oncoprotein gankyrin (Nagao et al., 2003);
	Interaction with Miz-1 and down-regulating transcription of p21 <sup>Cip1</sup> (Sakurai et al., 2004)
<i>MAGEA5</i>	Not well characterised
<i>MAGEA8</i>	Not well characterised
<i>MAGEA9</i>	Not well characterised

**Table 2. Continuation**

<b>MAGEA protein</b>	<b>Biological functions</b>
<b>MAGEA10</b>	Not well characterised
<b>MAGEA11</b>	Increasing Skp2-mediated degradation of cyclin A (Su et al., 2017)
	Increasing Skp2-mediated degradation of p130 (Su et al., 2017)
	Decreasing Skp2-mediated degradation of E2F1 (Su et al., 2017)
	Decreasing Skp2 self-ubiquitination (Su et al., 2017)
	Increasing the AR transcriptional activity (Bai et al., 2005, 2008)
	Promoting alternative mRNA polyadenylation and 3'untranslated region shortening (S. W. Yang et al., 2020)
<b>MAGEA12</b>	Promoting the ubiquitination of p21 (Yanagi et al., 2017);
	Activating Akt (Y. Shang et al., 2021)

### 2.2.5 MAGEA10

MAGEA10 is an interesting member of the MAGEA family. It is probably the most immunogenic of the 11 MAGEA proteins, eliciting strong immune responses and leading to the creation of very persistent CD8+ T cells (Bricard et al., 2005; Groeper et al., 2007; Le Gal et al., 2007; Sartorius et al., 2008; Valmori et al., 2001). It is a nuclear protein with a length of 369 aa and an apparent molecular weight of 72 kDa (Carrel et al., 1996; Rimoldi et al., 1999; Schultz-Thater et al., 2011). However, it has an abnormal electrophoretic mobility, since other MAGEA family members have a molecular weight of about 45–50 kDa and MAGEA10 is only about 50 aa longer (Rimoldi et al., 1999). Unfortunately, the reasons behind this have remained undiscovered. Another difference between MAGEA10 and other MAGEA proteins can be seen in the N- and C-terminal regions of the proteins, where MAGEA10 has additional unique inserts after amino acid 37 and at the C terminus (Meek & Marcar, 2012).

### 2.3 Extracellular vesicles

The term “extracellular vesicles” (EV) is used to describe lipid bilayer-enclosed, cell-derived particles that do not contain a functional nucleus and therefore cannot replicate (György et al., 2011; Théry et al., 2018). The release of EVs from cells is conserved across all organisms from bacteria to mammals (Cui et al., 2020; Deathage & Cookson, 2012). Since the detection of “platelet dust” in the 1960s and the first description of EV secretion as a means for eliminating unnecessary compounds from cells, researchers have reached an understanding that EVs are a highly heterogeneous group of particles (Johnstone et al., 1987; van Niel et al., 2018; Wolf, 1967). Due to this, the general classification of EVs remains complicated and guidelines suggest to classify the particles according to physical characteristics (size and density), biochemical characteristics (surface markers), or cell

of origin or the conditions of their origin (apoptotic bodies, hypoxic EVs, podocyte EVs) (Théry et al., 2018). However, EVs are mostly divided into three biotypes – exosomes, microvesicles, and apoptotic bodies – according to their biogenesis (L. Cheng & Hill, 2022).

Exosomes, otherwise known as small EVs, are 50–150 nm in diameter and are further classified as “classical” and “non-classical” exosomes (L. Cheng & Hill, 2022). Classical exosomes are formed via the endosomal pathway, where a multivesicular body (MVB) is formed by the invagination of the late endosomal membrane (L. Cheng & Hill, 2022). This process is orchestrated by the proteins of the endosomal sorting complex required for transport (ESCRT), which influence the recruitment of cargo, the inward invagination of the late endosomal membrane and the subsequent formation of intraluminal vesicles (ILV) in the MVB (Wollert & Hurley, 2010). However, there are also ESCRT-independent pathways for the formation of ILVs (Verderio et al., 2018). Then, the MVB fuses with the cellular plasma membrane and the ILVs are released into the extracellular space, where they are referred to as exosomes (Harding et al., 1984; Pan et al., 1985). The difference between classical and non-classical exosomes is defined by the presence of classical exosome surface markers (CD63, CD9, CD81) (Kowal et al., 2016).

Microvesicles, also known as large EVs, have an average diameter of 250–400 nm but their size can range from 150 to over 1000 nm and they are generated by the outward budding and fission of the plasma membrane (L. Cheng & Hill, 2022; Koifman et al., 2017; Théry et al., 2018). The formation of microvesicles occurs at specific locations on the cell membrane, where phospholipid rearrangements and cytoskeletal processes influence the pinching and detachment of vesicles (Connor et al., 1992; Fox et al., 1990; Piccin et al., 2007).

Apoptotic bodies are 1–5  $\mu\text{m}$  in diameter and thus the largest class of EVs (L. Cheng & Hill, 2022). They are formed during apoptosis, when the cell membrane begins blebbing and forming microtubule spikes, apoptopodia, and beaded apoptopodia, which later disassemble into apoptotic bodies (Atkin-Smith et al., 2015; L. Cheng & Hill, 2022; Moss et al., 2006; Poon et al., 2014).

All EVs enclose cargo that represents their cell of origin (L. Cheng & Hill, 2022; Cossetti et al., 2014; Kalra et al., 2016; Valadi et al., 2007; van Niel et al., 2018). This cargo can be actively packaged into the EVs during their formation or just randomly included (L. Cheng & Hill, 2022; van Niel et al., 2018). The latter is more characteristic of apoptotic bodies, owing to their size and manner of formation (Atkin-Smith et al., 2015; L. Cheng & Hill, 2022).

### 2.3.1 Functions of EVs

EVs have a myriad of cellular functions. The initial hypothesis, that has been later proven to be true is that EVs facilitate the removal of harmful contents from cells (Takahashi et al., 2017). However, the most prominent function seems to be EV-mediated cell to cell interaction, which in turn mediates processes such as cell differentiation and tissue regeneration (Qin et al., 2016), immune responses

(Gebraad et al., 2018; Segura et al., 2005), and genetic exchange (Valadi et al., 2007). EVs have also been shown to be involved in blood coagulation (Mallat et al., 1999) and to have specific roles in neurodegenerative (Ngolab et al., 2017; Vella et al., 2007) and cardiovascular diseases (Mesri & Altieri, 1998; Yu et al., 2012) as well as cancer development (Al-Nedawi et al., 2009; Y. Chen et al., 2017; Skog et al., 2008). Another interesting fact is that EVs can cross and even assist in the destruction of the blood-brain barrier (Morad et al., 2019; Tominaga et al., 2015).

### 2.3.2 Clinical potential of EVs

Researchers are very interested in the clinical potential of EVs, since they are involved in many pathological processes and carry a vast array of biomolecules. One way of exploiting the knowledge on EVs in disease is to use the particles as therapeutic targets. Therapies targeting EVs mainly focus on inhibiting either EV release pathways or their uptake by recipient cells (L. Cheng & Hill, 2022). Inhibiting EV release has shown good results in both neurodegenerative disease (Dinkins et al., 2014, 2016) and cancer (Datta et al., 2018; Kosgodage et al., 2018; B. Wang et al., 2019). However, blocking EV uptake in the recipient cells has proven to be more difficult but initial results are promising (Kawamoto et al., 2012; Nanbo et al., 2013).

Another potential clinical use for EVs is their application as diagnostic biomarkers. A wide selection of glycans, lipids, proteins, and nucleic acids have been identified in EVs and catalogued, but few of them are used in a clinical setting (Kalra et al., 2012; Keerthikumar et al., 2016). So far, EV-based tumour diagnostic tests are available for prostate and lung cancer (Castellanos-Rizaldos et al., 2019; McKiernan et al., 2016) but work is ongoing to include pancreatic ductal adenocarcinoma (Allenson et al., 2017), melanoma (García-Silva et al., 2019), and other tumours with cancer-associated mutations (Domenyuk et al., 2017; Möhrmann et al., 2018) to the list of malignancies that can be diagnosed with the aid of EVs. EVs can also be used for neurodegenerative disease diagnostics. For example, both protein and RNA-based assays are being validated for Alzheimer's (Goetzl et al., 2018; T. T. Yang et al., 2018) and Parkinson's diseases (Cao et al., 2017; S. Wang et al., 2017).

Lastly, EVs can be used as therapeutic agents. For example, native EVs derived from MSCs can be used to enhance recovery from myocardial infarct (Lai et al., 2010) and stroke (Xin et al., 2013), plasma-derived EVs have shown to aid in wound healing (S.-C. Guo et al., 2017), and EVs from dendritic cells may be used for several vaccinations for infectious diseases (Beauvillain et al., 2007; Y. Cheng & Schorey, 2013; Choi et al., 2015) and cancer (Escudier et al., 2005; Morse et al., 2005). Native EVs can also be manipulated to contain additional cargo to enhance therapeutic performance (Jang et al., 2021), target them to a specific location (Alvarez-Erviti et al., 2011; Pi et al., 2018; J.-H. Wang et al., 2018), or to be used as a carrier for therapeutic biomolecules (Alvarez-Erviti et al., 2011; Banizs et al., 2014; Lamichhane et al., 2015).

### 3. AIMS OF THE STUDY

As cancer is one of the leading causes of death in the modern world (Roth et al., 2018; WHO, 2022), many efforts have been made to find better diagnostic and therapeutic tools to battle the disease. For the last few decades, CTAs have been regarded as potential targets for usage in both cancer diagnostics and immunotherapy due to their restrictive expression pattern and immunological properties. However, despite extensive research many aspects about them have remained elusive and thus CTAs are not a part of common oncology yet. To keep moving towards effective and safe therapeutical and diagnostic strategies involving CTAs, we must first understand their biology.

We have discovered that MAGEA proteins MAGEA4 and MAGEA10, both well-known CTAs are incorporated into vesicles (virus-like particles, VLPs) released by cells and exposed on the surface of said vesicles (Kurg et al., 2016). This prompted us to conduct a more detailed research project concerning MAGEA family members to further our understanding of these CT antigens.

This study focuses mainly on MAGEA10, a highly immunogenic CTA, which is an outlier in the MAGEA family, based on overall biological characteristics. We hypothesised that the individual characteristics of MAGEA proteins are determined by the sequences flanking the conserved MHD region, whereas the shared properties are determined by the MHD. The aims of the study are the following:

- Define the importance of the different regions of MAGEA10 in the context of its characteristics.
- Determine the region responsible for the nuclear localisation of MAGEA10.
- Determine whether MAGEA are incorporated into naturally occurring extracellular vesicles.

## 4. MATERIALS AND METHODS

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

MAGEA protein coding sequences were cloned into pQM expression vectors under the control of the cytomegalovirus (CMV) promoter to enable high expression levels. Deletions and point mutations were carried out using PCR. The expression plasmids were electroporated into COP5-EBNA mouse fibroblast cells to achieve transient MAGEA protein expression, since this cell line does not harbour human *MAGEA* genes and thus does not produce MAGEA proteins endogenously. U2OS human osteosarcoma cells were used to follow endogenous MAGEA expression to see whether the same characteristics apply to transiently and endogenously produced proteins (Study II).

For isolating EVs, COP5-EBNA cells were cultivated for 72 hours post-transfection and U2OS cells for 72 hours after reaching the desired confluency. EVs were isolated from the cell culture medium by differential centrifugation to achieve separation of different EVs by size. Firstly, the media was centrifuged at  $300 \times g$  to remove all cellular debris and dead cells. The next centrifugation step was carried out with the remaining supernatant at  $2000 \times g$  to sediment the large vesicles (2K fraction, large EVs), after that the remaining supernatant from the previous step was centrifuged at  $16\,500 \times g$  to sediment medium-sized vesicles (16K fraction, omitted in Study I), and finally the remaining supernatant was centrifuged at  $120\,000 \times g$  to sediment the small EVs (120K fraction, small EVs). All EVs were resuspended in phosphate-buffered saline (PBS) and subjected to an additional purification step by centrifuging the samples through PBS at either  $17\,000 \times g$  for the 2K and 16K fractions or  $120\,000 \times g$  for the 120K fraction. The samples were resuspended in fresh PBS and stored at  $4\text{ }^{\circ}\text{C}$ .

Protein expression in cells and EVs was analysed by immunofluorescent staining (cells only), Western blotting or flow cytometry 24 to 72 hours post-transfection.

## 5. RESULTS AND DISCUSSION

### 5.1 The N-terminal region affects multiple MAGEA10 characteristics (Study I)

#### 5.1.2 Amino acids K6 and R7 are required for the nuclear localisation of MAGEA10

It has been speculated that amino acids 5–11 of MAGEA10 harbour a NLS similar to the simian virus 40 (SV40) large T antigen (Rimoldi et al., 1999), however, it has not been experimentally confirmed in previous studies. The amino acid sequence at the N-terminus of MAGEA10 (PRAPKRQR, aa 2–9) contains the motif (P/R)XXKR(^DE)(K/R) (where ^DE refers to any aa except Asp or Glu), which is classified as an importin  $\alpha$ -binding class 2 NLS (Kosugi et al., 2009). It was therefore determined that the positively charged amino acid residues in the N-terminal region can be responsible for the nuclear localisation of MAGEA10, with K6 and R7 being the most probable candidates, since they are located in the middle of the (P/R)XXKR(^DE)(K/R) motif. To test it, these residues were replaced with alanines, individually and together (Fig 2A, Study I). Expression of all mutants was confirmed by western blotting (Fig 2B, 2C, Study I). Indirect immunofluorescence analyses with antibodies against MAGEA10 revealed that mutating residues K6 and R7 disrupts the nuclear localisation (Fig 2D, Study I), confirming the presence of a NLS in the N-terminus of MAGEA10. Furthermore, the nuclear localisation is not influenced by the availability of the N-terminus, since wt MAGEA10 is located in the cell nucleus, whereas the mutated construct P2/R3/P5/K6/R7A localises in the cytoplasm, regardless of the positioning of the epitope tag E2Tag (Fig. 5D, Study I), further confirming the existence of the NLS. The NLS sequence classification as an importin  $\alpha$ -binding class 2 NLS suggests that MAGEA10 may be imported into the nucleus via the importin  $\alpha/\beta$  pathway (Lange et al., 2007), similar to SV40 and *c-myc* (Saphire et al., 1998). However, some cells expressing the mutated proteins retained a minute signal in the nucleus, suggesting that other regions of MAGEA10 may have a role in directing its localisation as well.

#### 5.1.1 The first 7 aa influence the aberrant migration of MAGEA10 in SDS-PAGE

A defining characteristic of the MAGEA10 protein is that it migrates as a roughly 70 kDa protein in SDS-PAGE, whereas its calculated molecular weight (MW) is 42.3 kDa and other MAGEA proteins show sizes of about 45–50 kDa (Rimoldi et al., 1999; Schultz-Thater et al., 2011). The reasons behind this anomaly have remained elusive. It has been determined that proteins with certain characteristics, such as high net charge (Pitt-Rivers & Impiombato, 1968; K. Weber et al., 1972)

or an abundance of post-translational modifications (PTM) (Arndt & Berg, 1970; Pitt-Rivers & Impiombato, 1968) move aberrantly in SDS-PAGE. However, a certain reason for the abnormal mobility of MAGEA10 has not been confirmed.

To identify the region(s) responsible for this phenomenon, deletion mutants of MAGEA10 (Fig. 1A, 1B, Study I) were constructed. The N-terminal 120 amino acids (mutant 1–120) and the C-terminal region starting from amino acid 121 (mutant 121–369), which contains the MHD, were separated and linked to the EGFP protein or the epitope tag E2Tag. Furthermore, mutants 15–120 and 15–369, lacking the first 14 N-terminal amino acids, and mutant 121–327 encoding the MHD alone, were also constructed and fused with the aforementioned tags. All constructs had the epitope tag or EGFP attached to the C-terminus, thus the N-terminus remained unobstructed (Fig. 1A, 1B, Study I).

Mouse fibroblast COP5-EBNA cells were transfected with expression plasmids encoding for MAGEA10 wt and mutants and protein expression was analysed by immunoblotting. It was determined that wt MAGEA10 has an apparent MW of about 68–70 kDa (Fig. 1C, lane 2, Study I), consistent with previous data. The observed difference between the computational and apparent MW for wt MAGEA10 was 1.6-fold (Table 3). Similar results were seen with the MAGEA10-EGFP fusion protein (Fig 1D lane 3, Study I), where a 1.4-fold difference was noted (Table 3). Interestingly, both the 1–120 and 121–369 mutants migrated as 30–32 kDa proteins (Fig 1C, lanes 4 and 7, respectively, Study I), although only 121–369 is expected to be around that size, whereas 1–120 should have a MW of 14 kDa, thus demonstrating a massive 2.2-fold difference between the computational and apparent MWs (Table 3). Again, fusion proteins with EGFP showed the same tendencies (Fig 1D, lanes 5 and 8, Study I). It is interesting to note that the MAGEA10 protein has a serine-rich region within the 1–120 aa stretch, which holds many possible glycosylation sites. Glycosylation has been shown to decrease the electrophoretic mobility of proteins in SDS-PAGE (Scheller et al., 2021). Thus, the first assumption was that MAGEA10 might undergo PTM, where suitable amino acid residues undergo glycosylation. Therefore, MAGEA10 mutants where either four or eleven serine residues within the serine-rich stretch were substituted for glycine, were constructed and analysed. Nevertheless, no significant change in migration was observed (unpublished data) and, taking into account that each glycan is thought to add about 2.5 kDa of weight to a protein (Scheller et al., 2021), glycosylation is probably not the main reason behind the aberrant migration of MAGEA10. Although, there were still some serine residues left unchanged which might still be subjected to PTM and thus could influence the electrophoretic mobility.

**Table 3. Differences in MAGEA10 computational and apparent molecular weight.** The observed fold-differences are highlighted in shades of red, with the highest being the most vibrant.

	Protein length (aa)	Calculated MW (kDa)	Apparent MW in SDS-PAGE (kDa)	Apparent vs calculated MW (fold-difference)
MAGEA10-E2tag	383	42.3	68	1.6
15–369-E2tag	370	40.7	46	1.1
1–120-E2tag	133	13.9	30	2.2
121–327-E2tag	221	25.3	25	1.0
121–369-E2tag	264	30	32	1.1
MAGEA10-EGFP	620	69	98	1.4
15–369-EGFP	598	66.4	74	1.1
1–120-EGFP	369	40.4	64	1.6
15–120-EGFP	356	38.8	48	1.2
121–327-EGFP	457	51.8	52	1.0
121–369-EGFP	500	56.5	56	1.0

An interesting observation was that the deletion of the first 14 aa showed a significant change in protein mobility in SDS-PAGE. In both E2tag and EGFP constructs, 15–369 had a MW difference of over 20 kDa from the full-length MAGEA10 (Fig 1C, compare lanes 2 and 3; 1D, compare lanes 3 and 4, Study I), but remained around 5 kDa higher than expected (Table 3). In the case of 15–120, the E2tag construct showed no expression (Fig 1C, lane 5, Study I), but the EGFP fusion differed from 1–120 by over 10 kDa (Fig 1D, compare lanes 5 and 6, Study I), although the computational difference should be about 1.6 kDa (Table 3).

These results and an observation that the double-mutation of K6 and R7 also reduced the apparent MW on SDS-PAGE (Fig. 2B, lane 5, Study I) prompted further investigations into the significance of the first 14 aa residues in the aberrant migration of MAGEA10. Two prolines (P2 and P5), three positively charged residues (R3, K6, R7), and three negatively charged residues (E13, E14, D15) were changed to alanines in different combinations (Fig. 3A, 3C, 4A, Study I). Western blot analyses showed that single mutations of aa residues P2, R3, P5, K6, and R7 to alanines had no effect on the migration of wt MAGEA10 (Fig. 3B, Study I). However, mutants with two, three or five substitutions had differing effects based on the combination of substituted residues in both the wt and 1–120 deletion mutant. Both double mutants K6/R7A and P2/R3A resulted in a small shift in size (Fig. 3B, lanes 5 and 9; 3F, lanes 3 and 4, Study I). Triple mutant P5/K6/R7A produced two bands, one similar to the double mutants and the other with an additional approximate 7 kDa size shift (Fig. 3B, lane 10, Study I), and a similar result in 1–120, where the lower band was comparable in size to the 15–120 mutant (Fig. 3F, compare lanes 5 and 8, Study I). Interestingly, triple mutant E13/E14/D15 did not have an effect on the migration of 1–120 (Fig. 3F, lane 7,

Study I). Most notably, mutating five residues from the first seven (P2/R3/P5/K6/R7A) resulted in an approximate 8 kDa shift in both the wt and 1–120 proteins. Fascinatingly, inserting either the native or mutated stretch of the N-terminal 14 aa to the N-terminus of the EGFP protein did not affect the fusion-protein's mobility (Fig. 3C-D, Study I). This shows that the positively charged and proline residues within the first seven amino acids of MAGEA10 influence the SDS-PAGE mobility of the protein but do not account for the whole size shift and only work in the context of the intrinsically disordered N-terminal region of MAGEA10.

Another probable reason behind the aberrant migration of MAGEA10 is the ability to bind SDS. MAGEA10 contains an abundance of both positively and negatively charged amino acids, as well as many proline residues, with the latter being more prevalent in the 1–120 region. In the context of SDS-PAGE migration, basic amino acids bind SDS very well, proline does so at a lower affinity, and acidic amino acids do not bind SDS at all, but repel it due to both being negatively charged (Maley & Guarino, 1977). The effect of negatively charged amino acids on SDS-binding and subsequent decreased mobility has been well documented (Armstrong & Roman, 1993; Klenova et al., 1997; Moussa et al., 2004; Sang & Barbosa, 1992). However, our results state that the greatest effect on MAGEA10 migration was observed when mutating proline or basic residues, while substitution of the acidic residues had no effect. This constitutes yet another possibility regarding proline residues within the protein. It has been shown that proteins with a higher proline content migrate slower in SDS-PAGE than expected (Fayet et al., 2023; Hamauzu et al., 1975; Noelken et al., 1981). This may be due to rigid structures, that inhibit movement in the acrylamide gel. MAGEA10 has an overall proline content of 7,9%, whereas the proline content of the N-terminal region (1–120) is 12,5% and the rest of the protein contains only 5,6% proline. Some of the proline residues are in very close proximity to each other in the 1–120 region, with three being located within the first 14 aa residues. This suggests the possibility of proline-dependent rigid structures responsible for the aberrant movement of the N-terminal region of MAGEA10.

A further observation about the N-terminus of MAGEA10 in the context of SDS-PAGE mobility was that if the region is blocked by an epitope tag (Fig. 5A, Study I) or fused to another protein (Fig. 4A, Study I), its effect is significantly decreased and further manipulations, such as mutations, do not seem to influence the mobility any more (Fig. 4B, 5B, Study I). This suggests that the possible electrostatic or structural elements responsible for the aberrant SDS-PAGE migration of MAGEA10 only have an effect if the N-terminus is unobstructed. However, the exact mechanisms behind this phenomenon stay elusive.

### 5.1.3 The N-terminus has a stabilising effect on MAGEA10

While analysing the aberrant migration of MAGEA10, a difference in signal intensity between the deletion mutants was detected. Therefore, flow cytometric analyses were conducted with COP5-EBNA cells transiently expressing the EGFP fusion proteins. It was noted that the N-terminal region 1–120 had a much higher mean fluorescence intensity (MFI) than the C-terminal regions 121–327 and 121–369 (Fig. 1F, Study I). Furthermore, deletion of the first 14 amino acids from the N-terminus resulted in an approximately two-fold decrease of MFI in both the 1–120 region and the full-length protein (Fig. 1F, Study I). We also determined that the differences in expression rate emerge due to the ubiquitin-proteasome pathway, since treatment with the proteasome inhibitor MG-132 increased the expression of the C-terminal regions, while not affecting the N-terminal 1–120 segment (Fig. 1E, Study I), indicating that the N-terminus protects the MAGEA10 protein from degradation. To investigate the stabilising effect of the N-terminus further, additional constructs were made, where the epitope tag E2Tag (Fig. 5A, Study I) or EGFP (Fig. 4A, Study I) was inserted in front of the N-terminus. The epitope tag E2Tag should not sterically influence the structure of the protein, since it is a very small peptide (SSTSSDFRDR). It was evident that blocking the N-terminus had a detrimental effect on the expression level of MAGEA10 (Fig. 5B) or the truncated 1–120 region (Fig. 4B). However, this decreased expression was not the result of proteasomal degradation, since treatment with MG-132 had no effect on the expression of the N-terminal region, regardless of the position of the EGFP protein (Fig. 4D) and did not fully restore the expression of the N-terminally tagged full-length protein (Fig. 5C).

Both the N- and C-terminal tails of MAGEA10 have been predicted as intrinsically disordered regions (IDR) by AlphaFold, but the crystallised protein structure has not been confirmed. Although crystal structures of the MHDs other members of the MAGEA family are known (Newman et al., 2016), structures of the full-length proteins have only been predicted and these predictions also contain IDRs in the tails of the proteins. While IDRs are mostly known to negatively affect protein stability (Rogers et al., 2021; van der Lee et al., 2014), there is evidence that they can also stabilise their binding partners, whether it is another protein (L.-W. Guo & Ruoho, 2011) or a different region within the same protein (Hegde et al., 2013; Maurizio et al., 2011). Thus, it is possible that the N-terminal disordered tail interacts with other parts of the MAGEA10 protein, stabilising them. Another possibility lies within the amino acid sequence of the N-terminal region. Fishbain *et al* have demonstrated that disordered protein tails with sequence bias protect the proteins from proteasomal degradation and that one such bias includes proline-rich regions (Fishbain et al., 2015). The sequence of the N-terminal region 1–120 of MAGEA10 contains 12,5% proline, compared to 5,6% in the remaining protein (121–369). Additionally, the first 14 N-terminal amino acids include three proline residues in close proximity to each other. This suggests that MAGEA10 could possibly escape proteasomal degradation thanks to its N-terminal proline-rich sequences.

## 5.2 MAGEA proteins are incorporated into EVs (study II and I)

During research regarding virus-like particles (VLP), it was discovered that MAGEA proteins were incorporated into and exposed on the surface of VLPs (Kurg et al., 2016). This prompted research into whether this phenomenon is extended to EVs. To test this, COP5-EBNA cells were transfected with MAGEA-encoding expression plasmids and grown for 72 hours. Afterwards, the cell culture media was collected and differentially centrifuged as described by Kowal *et al* (Kowal et al., 2016) to precipitate EVs of different sizes (Fig. 1A, Study II). Three fractions of EVs were obtained: 2K, 16K, and 120K. Transfected cells were used as positive controls for protein expression.

Western blot analyses of the cells and EVs indicated that MAGEA10 was present in all EV fractions, whereas 2K and 120K had stronger MAGEA10 signals than 16K (Fig. 1B, Study II). It seemed that the intracellular localisation of the protein did not influence its overall inclusion into EVs, although comparison between MAGEA10 and -A4 revealed that MAGEA4, a cytoplasmic protein, was more prevalent in EVs than the nuclear MAGEA10 (Fig. 1C, Study II). Both the immunoblotting and particle analyses showed that the three EV fractions were well distinguishable and that each MAGEA family member was incorporated into mouse fibroblast-derived particles of various sizes (Fig 1B-F, Study II). Interestingly, since there were differences between the particle profiles of MAGEA10- and MAGEA4-positive EVs (Fig. 1F, Study II), it is possible that different mechanisms are behind each protein's incorporation into EVs.

### 5.2.1 Endogenous MAGEA4 is also incorporated into EVs

Since previous work had been conducted with cells transiently overexpressing MAGEA proteins, it was difficult to ascertain whether endogenously expressed MAGEA proteins behave in the same way. Not many cell lines are known to express MAGEA proteins, but human osteosarcoma cell line U2OS is a great tool for researching endogenous MAGEA expression. It expresses many MAGEA proteins, including MAGEA4 and MAGEA10, although the latter does not have a very high expression level (B. Li et al., 2014; Marcar et al., 2010). EVs produced by U2OS cells were shown to express MAGEA4, confirming the packing of endogenous MAGEA4 into EVs (Fig. 5B, Study II). This is probably the case for endogenous MAGEA10 as well but needs to be proven. Nevertheless, this could prove difficult, since very few cell lines express MAGEA10 and at low levels.

Packing of endogenous MAGE proteins has been shown in squamous cell carcinomas of the head and neck (Bergmann et al., 2009), glioma (Bu et al., 2011), and bladder carcinoma (Yazarlou et al., 2018). This may indicate that inclusion into EVs is a shared feature of MAGE proteins and can therefore be utilised in cancer therapy and diagnostics. The studies, which revealed MAGE proteins as components in tumour-derived EVs also indicated the immunological properties of the particles (Bu et al., 2011) and their possible usage as diagnostic tools (Yazarlou et al., 2018).

## 5.2.2 MAGEA proteins are expressed on the surface of EVs

To determine the possibility that MAGEA10 is exposed to the surface of EVs, flow cytometric analyses were conducted. The EVs were loaded onto aldehyde/sulphate latex beads (Kurg et al., 2016) and incubated with MAGEA-specific antibodies. Signals were detected for all three fractions (Fig. 2A, Study II), suggesting that MAGEA10 is exposed on the EV surface, at least partly. Like the western blot analyses, MAGEA10 produced a lower signal in the 16K fraction, compared to 2K and 120K (Fig. 2A, Study II). The surface expression was confirmed with immunoprecipitation (Fig. 3A, 3B, Study II). Analyses with MAGEA4 indicated that the C-terminal region of the protein is exposed on the surface, since the C-terminal epitope tag E2Tag was recognised by the corresponding antibody (Fig. 2B, 2C, Study II). Whether this is the case for MAGEA10, is yet to be determined.

Since it has been shown that MAGEA10 peptides are presented to the immune system by human leukocyte antigen (HLA) molecules (Jia et al., 2011; Valmori et al., 2001) and that EVs can contain MHC capable of inducing immune responses (Admyre et al., 2006; André et al., 2004; Buschow et al., 2009) it is possible that MAGEA proteins are presented to the surface of EVs via antigen presenting pathways. However, MAGEA proteins have not been fully structurally or functionally characterised and can thus harbour other possibilities of crossing the lipid bilayer and reaching the EV surface. Regardless of the specific mechanisms, the fact that MAGEA proteins are exposed on the surface of EVs indicates usage of MAGEA-EVs as cancer biomarkers. Therefore, this aspect of MAGEA proteins deserves to be investigated further.

## 5.2.3 The C-terminal region is required for incorporation into EVs

The notion that the C-terminus is exposed on the surface led to the question whether the C-terminal region influences the incorporation of MAGEA proteins into EVs. Truncated MAGEA4 proteins, where either 104 (MAGEA4-105) or 160 (MAGEA4-161) amino acids from the N-terminus were deleted, were constructed, and expressed in COP5-EBNA cells. MAGEA4-105 retains the entire MHD, whereas in MAGEA4-161 the MHD is disrupted (Fig. 4A, Study II). It was observed that a disrupted MHD prevented the protein from being included into EVs (Fig. 4C, Study II). In the case of MAGEA10, mutations in the stabilising N-terminus did not affect its incorporation into EVs (Fig 6C, D, Study I) and the truncated protein 1–120 was not incorporated into vesicles at all, further indicating that regions within the C-terminus of the protein are responsible for that function.

As other MAGE proteins have also been found in EVs (Bergmann et al., 2009; Bu et al., 2011; Yazarlou et al., 2018), it is possible that a shared mechanism is behind their incorporation into the particles. This may be facilitated by the MHD, which is known to be the driving force between the interactions of MAGE proteins and RING ubiquitin ligases (Doyle et al., 2010). However, since MAGEA10

and -A4 seem to occupy different types of EVs, it is also possible that multiple EV generation pathways are involved in the packaging of MAGEA proteins as EV cargo.

### 5.2.4 MAGEA10 induces EV shedding

While researching EVs containing MAGEA proteins, it was observed that MAGEA-expressing cells tend to produce more EVs than the negative controls. Analysing the total protein and particle yield demonstrated that MAGEA10 is capable of inducing EV formation and release (Fig. 6C, 6D, Study II) and showed an ability to induce EV shedding similar to the murine leukaemia virus (MLV) Gag protein, which is known to drive the formation of VLPs (Kurg et al., 2016). In addition to viral proteins inducing vesicle production, it seems to be a common phenomenon that the first regulators of EV formation are its cargoes (van Niel et al., 2018). For example, Fan *et al* demonstrated that a peptide from the charged multivesicular body protein 6 (CHMP6) is capable of promoting small EV production (J. Fan et al., 2022). This indicates that MAGEA10 may be an initiating factor in EV formation and secretion if it is packaged into the particles as cargo. This, however, needs further research as the mechanisms behind MAGEA incorporation into EVs and their fate in the particles is still elusive.

### 5.3 EVs with MAGEA proteins are stable (study III)

To further characterise EVs loaded with MAGEA proteins, the particles were subjected to long-term storage at +4 °C and –80 °C. The EVs were stored in PBS for three weeks at either temperature and showed no deterioration or loss of MAGEA signal (Fig. 1, Study III). It was determined that MAGEA-containing EVs can be stored at typical EV storing conditions (Kusuma et al., 2018), without having to consider vesicle concentration loss (Frank et al., 2018; Lőrincz et al., 2014), possible changes in size (Maroto et al., 2017; Sokolova et al., 2011) or loss of cargo (H. Zhou et al., 2006) reported in some cases of EV storage.

Another aspect of MAGEA proteins attached to EVs seems to be the tight binding of the protein to the particle. It seems that MAGEA are stably bound to the vesicles via hydrophobic forces, since disruptions of electrostatic bonds did not strip the EVs of MAGEA, whereas a non-ionic detergent or high pH did (Fig. 3C, 3D, Study III). It also seemed that the latter two tended to disintegrate the EVs themselves (Fig. 3E, Study III), so this might not fully reflect the mechanism behind MAGEA-EV interactions, as it has been shown that high pH can be detrimental to vesicles (Ban et al., 2015). Therefore, additional research is needed to determine the mechanisms behind MAGEA incorporation into and subsequent surface presentation on EVs.

## 6. CONCLUSIONS

Members of the MAGEA family of cancer-testis antigens are highly immunogenic and have the potential to be used in cancer diagnostics and immunotherapy. Nonetheless, many of their biological characteristics have remained elusive. In this study, several aspects of MAGEA10 and, to a lesser extent, MAGEA4 were investigated to further the understanding of their relevance in cancer biology.

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

- The distinct characteristics of MAGEA10, such as the nuclear localisation and slower SDS-PAGE movement are determined by the N-terminal region spanning amino acid residues 1–120, which contains sequences unique to this family member. Interestingly, the first 14 amino acid residues play a crucial role in how MAGEA10 acts. The MHD does not influence these aspects of the protein.
- The nuclear localisation of MAGEA10 is determined by the first seven amino acid residues, which contain an importin  $\alpha$ -binding class 2 NLS, like those of SV40 Large T antigen and human *c-myc*.
- MAGEA10 and MAGEA4 are incorporated into native EVs released by cells and this process may be facilitated by the MHD, since other MAGE proteins have been shown to be present in naturally occurring EVs as well.
- MAGEA10 and MAGEA4 are exposed on the surface of the EVs and the interactions between the protein and the particle are probably controlled by hydrophobic interactions.

These results showcase the similarities between MAGEA proteins as well as distinctive features of each individual family member. Both aspects should be taken into consideration when exploiting MAGEA in the clinical setting to achieve the best results.

## SUMMARY IN ESTONIAN

### Vähi-testise antigeen MAGEA10 omaduste paljastamine

Vähk on tänapäeval üks juhtivatest surmapõhjustest ning seetõttu otsitakse pidevalt efektiivsemaid ja kahjutumaid viise selle raviks. Olenemata aastakümnete pikkustest pingutustest hõlmab vähiravi siiski peamiselt kirurgilist sekkumist ja/või kiiritus- või keemiaravi, millest viimase kahega kaasnevad laiaulatuslikud kõrvalnähud, kuna ravi ei erista vähkkasvajat tervetest kudedest. Seetõttu on pandud rõhku vähispetsiifiliste sihtmärkide otsimisele, et ravi mõjuks vaid sellele ette nähtud kohas ning ei kahjustaks ülejäänud organismi.

Üheks uurimissuunaks on kujunenud vähi-testise antigeenid (ingl. k *cancer-testis antigens*, CTA) – valgud, mis on võimelised vähipatsientides esile kutsuma immuunvastuseid ning mida toodetakse enamasti vaid vähkkasvajates. Tava-kudedes on neid valke kodeerivad geenid enamjaolt vaigistatud, erandiks suure osa puhul vaid sperma tüvirakud. Üheks tuntumaks vähi-testise antigeenide alam-perekonnaks on MAGEA (melanoomi-seoseline antigeen A), kuhu kuuluvad valgud suudavad esile kutsuda väga tugevaid immuunvastuseid. Lisaks on MAGEA valke leitud väga eriilmelistest vähkkasvajatest. Seega on nende näol tegemist väga ahvatlevate sihtmärkidega, kasutamiseks nii diagnoosimisel kui ravis. Kahjuks ei ole siiani lõpuni kindlaks tehtud, milline on vähi-testise antigeenide roll rakkudes ning seetõttu ei ole nende kasutamine jõudnud igapäevasesse vähiravisse.

Teine spetsiifiline lähenemine vähiravile hõlmab endas rakuväliseid vesiikuleid (ingl. k *extracellular vesicle*, EV). EV-d on lipiidse kaksikkihiga ümbritsetud nanoosakesed, mida toodavad kõik elusrakud ning milles sisalduvad päritolurakust pärit nukleiinhapped, valgud, metaboliidid jms. Kuna on näidatud, et vähkkasvajatest eraldunud vesiikulites sisalduvad vähile omased molekulid ning et EV-d osalevad immuunsüsteemi töös, on ka EV-dest kujunenud potentsiaalsed sihtmärgid nii vähi diagnostikas kui ravis.

Antud doktoritöös on uuritud vähi-testise antigeeni MAGEA10 ning vähemal määral sama perekonna valgu MAGEA4 omadusi. Leiti, et MAGEA10 omadused, mis erinevad teistest perekonnakaaslastest, määravad ära valgu N-terminaalses osas asuvad unikaalsed aminohappelased järjestused. Avastati, et MAGEA10 tavapäratult aeglase liikuvuse eest akrüülamiidgeelis vastutavad valgu esiotsa aminohappejäägid 1–120. Selles regioonis sisaldub hulgaliselt proliinijääke, mis võivad tekitada jäiga struktuuri, mille puhul on akrüülamiidgeelist läbi liikumine raskendatud. Siiski, kindlat põhjust antud fenomenile ei leitud ning see vajab edasist uurimist.

Lisaks näidati, et MAGEA10 tuumalokalisatsiooni eest vastutavad valgu esimesed seitse aminohappejääki, milles peitub tuumalokalisatsioonisignaal. Sarnane signaal on olemas viiruse SV40 suurel T antigeenil ja valgul *c-myc* ning see viitab, et MAGEA10 transporditakse rakutuuma sama mehhanismi kaudu.

Veel tehti kindlaks, et MAGEA10 lülitatakse EV-de koostisesse ning et see omadus on olemas ka perekonnakaaslasel MAGEA4. Mõlema valguga puhul täheledata, et need eksponeeriti erineva suurusega vesiikulite pinnale, ning lisaks nähti, et MAGEA valgud on vesiikulite pinnale tugevalt kinnitunud. See kõik omakorda tähendab, et MAGEA valke kandvaid EV-sid oleks võimalik kasutada vähi biomarkeritena.

Kokkuvõttes võib öelda, et MAGEA perekonna esindajad on väga head sihtmärgid vähiraviks ning diagnostikaks, nii üksi kui vesiikulite koostises, kuid heade kliiniliste tulemuste saavutamiseks tuleb neid sügavuti uurida.

## REFERENCES

- Admyre, C., Johansson, S. M., Paulie, S., & Gabrielsson, S. (2006). Direct exosome stimulation of peripheral human T cells detected by ELISPOT. *European Journal of Immunology*, *36*(7), 1772–1781. <https://doi.org/10.1002/eji.200535615>
- Afsharpad, M., Nowroozi, M. R., Mobasheri, M. B., Ayati, M., Nekooresh, L., Saffari, M., Zendehtdel, K., & Modarressi, M. H. (2019). Cancer-Testis Antigens as New Candidate Diagnostic Biomarkers for Transitional Cell Carcinoma of Bladder. *Pathology & Oncology Research*, *25*(1), 191–199. <https://doi.org/10.1007/s12253-017-0313-4>
- Akers, S. N., Odunsi, K., & Karpf, A. R. (2010). Regulation of cancer germline antigen gene expression: Implications for cancer immunotherapy. *Future Oncology*, *6*(5), 717–732. <https://doi.org/10.2217/fon.10.36>
- Allenson, K., Castillo, J., San Lucas, F. A., Scelo, G., Kim, D. U., Bernard, V., Davis, G., Kumar, T., Katz, M., Overman, M. J., Foretova, L., Fabianova, E., Holcatova, I., Janout, V., Meric-Bernstam, F., Gascoyne, P., Wistuba, I., Varadhachary, G., Brennan, P., ... Alvarez, H. (2017). High prevalence of mutant KRAS in circulating exosome-derived DNA from early-stage pancreatic cancer patients. *Annals of Oncology*, *28*(4), 741–747. <https://doi.org/10.1093/annonc/mdx004>
- Almeida, L. G., Sakabe, N. J., deOliveira, A. R., Silva, M. C. C., Mundstein, A. S., Cohen, T., Chen, Y.-T., Chua, R., Gurung, S., Gnjjatic, S., Jungbluth, A. A., Caballero, O. L., Bairoch, A., Kiesler, E., White, S. L., Simpson, A. J. G., Old, L. J., Camargo, A. A., & Vasconcelos, A. T. R. (2009). CTdatabase: A knowledge-base of high-throughput and curated data on cancer-testis antigens. *Nucleic Acids Research*, *37*(suppl\_1), D816–D819. <https://doi.org/10.1093/nar/gkn673>
- 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>
- Alvarez-Erviti, L., Seow, Y., Yin, H., Betts, C., Lakhai, S., & Wood, M. J. A. (2011). Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nature Biotechnology*, *29*(4), Article 4. <https://doi.org/10.1038/nbt.1807>
- Andersen, M. H., Keikavoussi, P., Bröcker, E.-B., Schuler-Thurner, B., Jonassen, M., Søndergaard, I., Straten, P. thor, Becker, J. C., & Kämpgen, E. (2001). Induction of systemic CTL responses in melanoma patients by dendritic cell vaccination: Cessation of CTL responses is associated with disease progression. *International Journal of Cancer*, *94*(6), 820–824. <https://doi.org/10.1002/ijc.1536>
- André, F., Chaput, N., Scharltz, N. E. C., Flament, C., Aubert, N., Bernard, J., Lemonnier, F., Raposo, G., Escudier, B., Hsu, D.-H., Tursz, T., Amigorena, S., Angevin, E., & Zitvogel, L. (2004). Exosomes as Potent Cell-Free Peptide-Based Vaccine. I. Dendritic Cell-Derived Exosomes Transfer Functional MHC Class I/Peptide Complexes to Dendritic Cells 1. *The Journal of Immunology*, *172*(4), 2126–2136. <https://doi.org/10.4049/jimmunol.172.4.2126>
- Aoki, N., & Matsui, Y. (2019). Comprehensive Analysis of Mouse Cancer/Testis Antigen Functions in Cancer Cells and Roles of TEKT5 in Cancer Cells and Testicular Germ Cells. *Molecular and Cellular Biology*, *39*(17), e00154-19. <https://doi.org/10.1128/MCB.00154-19>
- Armstrong, D. J., & Roman, A. (1993). The Anomalous Electrophoretic Behavior of the Human Papillomavirus Type 16 E7 Protein Is Due to the High Content of Acidic

- Amino Acid Residues. *Biochemical and Biophysical Research Communications*, 192(3), 1380–1387. <https://doi.org/10.1006/bbrc.1993.1569>
- Arndt, D. J., & Berg, P. (1970). Isoleucyl Transfer Ribonucleic Acid Synthetase Is a Single Polypeptide Chain. *Journal of Biological Chemistry*, 245(3), 665–667. [https://doi.org/10.1016/S0021-9258\(18\)63381-2](https://doi.org/10.1016/S0021-9258(18)63381-2)
- Askew, E. B., Bai, S., Hnat, A. T., Minges, J. T., & Wilson, E. M. (2009). Melanoma Antigen Gene Protein-A11 (MAGE-11) F-box Links the Androgen Receptor NH2-terminal Transactivation Domain to p160 Coactivators\*. *Journal of Biological Chemistry*, 284(50), 34793–34808. <https://doi.org/10.1074/jbc.M109.065979>
- Atanackovic, D., Altorki, N. K., Stockert, E., Williamson, B., Jungbluth, A. A., Ritter, E., Santiago, D., Ferrara, C. A., Matsuo, M., Selvakumar, A., Dupont, B., Chen, Y.-T., Hoffman, E. W., Ritter, G., Old, L. J., & Gnjjatic, S. (2004). Vaccine-Induced CD4+ T Cell Responses to MAGE-3 Protein in Lung Cancer Patients. *The Journal of Immunology*, 172(5), 3289–3296. <https://doi.org/10.4049/jimmunol.172.5.3289>
- Atkin-Smith, G. K., Tixeira, R., Paone, S., Mathivanan, S., Collins, C., Liem, M., Goodall, K. J., Ravichandran, K. S., Hulett, M. D., & Poon, I. K. H. (2015). A novel mechanism of generating extracellular vesicles during apoptosis via a beads-on-a-string membrane structure. *Nature Communications*, 6(1), Article 1. <https://doi.org/10.1038/ncomms8439>
- Bai, S., Grossman, G., Yuan, L., Lessey, B. A., French, F. S., Young, S. L., & Wilson, E. M. (2008). Hormone control and expression of androgen receptor coregulator MAGE-11 in human endometrium during the window of receptivity to embryo implantation. *Molecular Human Reproduction*, 14(2), 107–116. <https://doi.org/10.1093/molehr/gam080>
- Bai, S., He, B., & Wilson, E. M. (2005). Melanoma Antigen Gene Protein MAGE-11 Regulates Androgen Receptor Function by Modulating the Interdomain Interaction. *Molecular and Cellular Biology*, 25(4), 1238–1257. <https://doi.org/10.1128/MCB.25.4.1238-1257.2005>
- Ban, J.-J., Lee, M., Im, W., & Kim, M. (2015). Low pH increases the yield of exosome isolation. *Biochemical and Biophysical Research Communications*, 461(1), 76–79. <https://doi.org/10.1016/j.bbrc.2015.03.172>
- Banchereau, J., Palucka, A. K., Dhodapkar, M., Burkeholder, S., Taquet, N., Rolland, A., Taquet, S., Coquery, S., Wittkowski, K. M., Bhardwaj, N., Pineiro, L., Steinman, R., & Fay, J. (2001). Immune and Clinical Responses in Patients with Metastatic Melanoma to CD34+ Progenitor-derived Dendritic Cell Vaccine1. *Cancer Research*, 61(17), 6451–6458.
- Banchereau, J., Ueno, H., Dhodapkar, M., Connolly, J., Finholt, J. P., Klechevsky, E., Blanck, J.-P., Johnston, D. A., Palucka, A. K., & Fay, J. (2005). Immune and Clinical Outcomes in Patients with Stage IV Melanoma Vaccinated with Peptide-Pulsed Dendritic Cells Derived From CD34+ Progenitors and Activated with Type I Interferon. *Journal of Immunotherapy*, 28(5), 505. <https://doi.org/10.1097/01.cji.0000171292.79663.cb>
- Banizs, A. B., Huang, T., Dryden, K., Berr, S. S., Stone, J. R., Nakamoto, R. K., Shi, W., & He, J. (2014). In vitro evaluation of endothelial exosomes as carriers for small interfering ribonucleic acid delivery. *International Journal of Nanomedicine*, 9, 4223–4230. <https://doi.org/10.2147/IJN.S64267>
- 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>

- Beard, J. (1905). The Cancer Problem. *The Lancet*, 165(4249), 281–283. [https://doi.org/10.1016/S0140-6736\(01\)42822-4](https://doi.org/10.1016/S0140-6736(01)42822-4)
- Beauvillain, C., Ruiz, S., Guiton, R., Bout, D., & Dimier-Poisson, I. (2007). A vaccine based on exosomes secreted by a dendritic cell line confers protection against *T. gondii* infection in syngeneic and allogeneic mice. *Microbes and Infection*, 9(14), 1614–1622. <https://doi.org/10.1016/j.micinf.2007.07.002>
- Berger, S. L., Kouzarides, T., Shiekhatar, R., & Shilatifard, A. (2009). An operational definition of epigenetics. *Genes & Development*, 23(7), 781–783. <https://doi.org/10.1101/gad.1787609>
- Bergmann, C., Strauss, L., Wieckowski, E., Czystowska, M., Albers, A., Wang, Y., Zeidler, R., Lang, S., & Whiteside, T. L. (2009). Tumor-derived microvesicles in sera of patients with head and neck cancer and their role in tumor progression. *Head & Neck*, 31(3), 371–380. <https://doi.org/10.1002/hed.20968>
- Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N., & Bourne, P. E. (2000). The Protein Data Bank. *Nucleic Acids Research*, 28(1), 235–242. <https://doi.org/10.1093/nar/28.1.235>
- Bhan, S., Chuang, A., Negi, S., Glazer, C., & Califano, J. (2012). MAGEA4 induces growth in normal oral keratinocytes by inhibiting growth arrest and apoptosis. *ONCOLOGY REPORTS*, 28(4), 1498–1502.
- Bischof, J. M., Ekker, M., & Wevrick, R. (2003). A MAGE/NDN-like gene in zebrafish. *Developmental Dynamics*, 228(3), 475–479. <https://doi.org/10.1002/dvdy.10398>
- Bode, P. K., Thielken, A., Brandt, S., Barghorn, A., Lohe, B., Knuth, A., & Moch, H. (2014). Cancer testis antigen expression in testicular germ cell tumorigenesis. *Modern Pathology*, 27(6), Article 6. <https://doi.org/10.1038/modpathol.2013.183>
- Bolhassani, A., Safaiyan, S., & Rafati, S. (2011). Improvement of different vaccine delivery systems for cancer therapy. *Molecular Cancer*, 10(1), 3. <https://doi.org/10.1186/1476-4598-10-3>
- 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 Carcinoma. *The Journal of Immunology*, 174(3), 1709–1716. <https://doi.org/10.4049/jimmunol.174.3.1709>
- Brower-Toland, B., Findley, S. D., Jiang, L., Liu, L., Yin, H., Dus, M., Zhou, P., Elgin, S. C. R., & Lin, H. (2007). Drosophila PIWI associates with chromatin and interacts directly with HP1a. *Genes & Development*, 21(18), 2300–2311. <https://doi.org/10.1101/gad.1564307>
- Bruce, W. R., & Van Der Gaag, H. (1963). A Quantitative Assay for the Number of Murine Lymphoma Cells capable of Proliferation in vivo. *Nature*, 199(4888), Article 4888. <https://doi.org/10.1038/199079a0>
- Bruggeman, J. W., Koster, J., Lodder, P., Repping, S., & Hamer, G. (2018). Massive expression of germ cell-specific genes is a hallmark of cancer and a potential target for novel treatment development. *Oncogene*, 37(42), Article 42. <https://doi.org/10.1038/s41388-018-0357-2>
- Bruggen, P. van der, Traversari, C., Chomez, P., Lurquin, C., Plaen, E. D., Eynde, B. V. den, 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>
- Bu, N., Wu, H., Sun, B., Zhang, G., Zhan, S., Zhang, R., & Zhou, L. (2011). Exosome-loaded dendritic cells elicit tumor-specific CD8+ cytotoxic T cells in patients with

- glioma. *Journal of Neuro-Oncology*, 104(3), 659–667. <https://doi.org/10.1007/s11060-011-0537-1>
- 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>
- Caballero, O. L., & Chen, Y.-T. (2009). Cancer/testis (CT) antigens: Potential targets for immunotherapy. *Cancer Science*, 100(11), 2014–2021. <https://doi.org/10.1111/j.1349-7006.2009.01303.x>
- Cao, X.-Y., Lu, J.-M., Zhao, Z.-Q., Li, M.-C., Lu, T., An, X.-S., & Xue, L.-J. (2017). MicroRNA biomarkers of Parkinson’s disease in serum exosome-like microvesicles. *Neuroscience Letters*, 644, 94–99. <https://doi.org/10.1016/j.neulet.2017.02.045>
- Carrel, S., Schreyer, M., Spagnoli, G., Cerottini, J.-C., & Rimoldi, D. (1996). Monoclonal antibodies against recombinant-MAGE-1 protein identify a cross-reacting 72-kDa antigen which is co-expressed with MAGE-1 protein in melanoma cells. *International Journal of Cancer*, 67(3), 417–422. [https://doi.org/10.1002/\(SICI\)1097-0215\(19960729\)67:3<417::AID-IJC17>3.0.CO;2-4](https://doi.org/10.1002/(SICI)1097-0215(19960729)67:3<417::AID-IJC17>3.0.CO;2-4)
- Castellanos-Rizaldos, E., Zhang, X., Tadigotla, V. R., Grimm, D. G., Karlovich, C., Raez, L. E., & Skog, J. K. (2019). Exosome-based detection of activating and resistance EGFR mutations from plasma of non-small cell lung cancer patients. *Oncotarget*, 10(30), 2911–2920. <https://doi.org/10.18632/oncotarget.26885>
- Chen, L., Wu, Q., Xu, X., Yang, C., You, J., Chen, F., & Zeng, Y. (2021). Cancer/testis antigen LDHC promotes proliferation and metastasis by activating the PI3K/Akt/GSK-3 $\beta$ -signaling pathway and the in lung adenocarcinoma. *Experimental Cell Research*, 398(2), 112414. <https://doi.org/10.1016/j.yexcr.2020.112414>
- Chen, Y., Zeng, C., Zhan, Y., Wang, H., Jiang, X., & Li, W. (2017). Aberrant low expression of p85 $\alpha$  in stromal fibroblasts promotes breast cancer cell metastasis through exosome-mediated paracrine Wnt10b. *Oncogene*, 36(33), Article 33. <https://doi.org/10.1038/onc.2017.100>
- Chen, Y.-T., Chiu, R., Lee, P., Beneck, D., Jin, B., & Old, L. J. (2011). Chromosome X-encoded cancer/testis antigens show distinctive expression patterns in developing gonads and in testicular seminoma. *Human Reproduction*, 26(12), 3232–3243. <https://doi.org/10.1093/humrep/der330>
- Chen, Y.-T., Scanlan, M. J., Sahin, U., Türeci, Ö., Gure, A. O., Tsang, S., Williamson, B., Stockert, E., Pfreundschuh, M., & Old, L. J. (1997). A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proceedings of the National Academy of Sciences*, 94(5), 1914–1918. <https://doi.org/10.1073/pnas.94.5.1914>
- Cheng, L., & Hill, A. F. (2022). Therapeutically harnessing extracellular vesicles. *Nature Reviews Drug Discovery*, 21(5), Article 5. <https://doi.org/10.1038/s41573-022-00410-w>
- Cheng, Y., & Schorey, J. S. (2013). Exosomes carrying mycobacterial antigens can protect mice against Mycobacterium tuberculosis infection. *European Journal of Immunology*, 43(12), 3279–3290. <https://doi.org/10.1002/eji.201343727>
- Chianese-Bullock, K. A., Pressley, J., Garbee, C., Hibbitts, S., Murphy, C., Yamshchikov, G., Petroni, G. R., Bissonette, E. A., Neese, P. Y., Grosh, W. W., Merrill, P., Fink, R., Woodson, E. M. H., Wiernasz, C. J., Patterson, J. W., & Slingluff, C. L., Jr. (2005). MAGE-A1-, MAGE-A10-, and gp100-Derived Peptides Are Immunogenic When Combined with Granulocyte-Macrophage Colony-Stimulating Factor and

- Montanide ISA-51 Adjuvant and Administered as Part of a Muropeptide Vaccine for Melanoma. *The Journal of Immunology*, 174(5), 3080–3086. <https://doi.org/10.4049/jimmunol.174.5.3080>
- Cho, B., Lim, Y., Lee, D.-Y., Park, S.-Y., Lee, H., Kim, W. H., Yang, H., Bang, Y.-J., & Jeoung, D.-I. (2002). Identification and Characterization of a Novel Cancer/Testis Antigen Gene CAGE. *Biochemical and Biophysical Research Communications*, 292(3), 715–726. <https://doi.org/10.1006/bbrc.2002.6701>
- Choi, S. J., Kim, M.-H., Jeon, J., Kim, O. Y., Choi, Y., Seo, J., Hong, S.-W., Lee, W.-H., Jeon, S. G., Gho, Y. S., Jee, Y.-K., & Kim, Y.-K. (2015). Active Immunization with Extracellular Vesicles Derived from *Staphylococcus aureus* Effectively Protects against Staphylococcal Lung Infections, Mainly via Th1 Cell-Mediated Immunity. *PLOS ONE*, 10(9), e0136021. <https://doi.org/10.1371/journal.pone.0136021>
- Chomez, P., Backer, O. D., Bertrand, M., Plaen, E. D., Boon, T., & Lucas, S. (2001). An Overview of the MAGE Gene Family with the Identification of All Human Members of the Family. *Cancer Research*, 61(14), 5544–5551.
- Cilensek, Z. M., Yehiely, F., Kular, R. K., & Deiss, L. P. (2002). A Member of the GAGE Family of Tumor Antigens is an Anti-Apoptotic Gene that Confers Resistance to Fas/CD95/APO-1, Interferon-g, Taxol and g-irradiation. *Cancer Biology & Therapy*, 1(4), 379–386. <https://doi.org/10.4161/cbt.1.4.11>
- Clarke, M. F., Dick, J. E., Dirks, P. B., Eaves, C. J., Jamieson, C. H. M., Jones, D. L., Visvader, J., Weissman, I. L., & Wahl, G. M. (2006). Cancer Stem Cells—Perspectives on Current Status and Future Directions: AACR Workshop on Cancer Stem Cells. *Cancer Research*, 66(19), 9339–9344. <https://doi.org/10.1158/0008-5472.CAN-06-3126>
- Connor, J., Pak, C. H., Zwaal, R. F., & Schroit, A. J. (1992). Bidirectional transbilayer movement of phospholipid analogs in human red blood cells. Evidence for an ATP-dependent and protein-mediated process. *Journal of Biological Chemistry*, 267(27), 19412–19417. [https://doi.org/10.1016/S0021-9258\(18\)41791-7](https://doi.org/10.1016/S0021-9258(18)41791-7)
- Cossetti, C., Iraci, N., Mercer, T. R., Leonardi, T., Alpi, E., Drago, D., Alfaro-Cervello, C., Saini, H. K., Davis, M. P., Schaeffer, J., Vega, B., Stefanini, M., Zhao, C., Muller, W., Garcia-Verdugo, J. M., Mathivanan, S., Bachi, A., Enright, A. J., Mattick, J. S., & Pluchino, S. (2014). Extracellular Vesicles from Neural Stem Cells Transfer IFN- $\gamma$  via Ifngr1 to Activate Stat1 Signaling in Target Cells. *Molecular Cell*, 56(2), 193–204. <https://doi.org/10.1016/j.molcel.2014.08.020>
- Cox, D. N., Chao, A., Baker, J., Chang, L., Qiao, D., & Lin, H. (1998). A novel class of evolutionarily conserved genes defined by piwi are essential for stem cell self-renewal. *Genes & Development*, 12(23), 3715–3727. <https://doi.org/10.1101/gad.12.23.3715>
- Cox, D. N., Chao, A., & Lin, H. (2000). Piwi encodes a nucleoplasmic factor whose activity modulates the number and division rate of germline stem cells. *Development*, 127(3), 503–514. <https://doi.org/10.1242/dev.127.3.503>
- Crew, A. j., Clark, J., Fisher, C., Gill, S., Grimer, R., Chand, A., Shipley, J., Gusteron, B. a., & Cooper, C. s. (1995). Fusion of SYT to two genes, SSX1 and SSX2, encoding proteins with homology to the Kruppel-associated box in human synovial sarcoma. *The EMBO Journal*, 14(10), 2333–2340. <https://doi.org/10.1002/j.1460-2075.1995.tb07228.x>
- 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>
- Cui, Y., Gao, J., He, Y., & Jiang, L. (2020). Plant extracellular vesicles. *Protoplasma*, 257(1), 3–12. <https://doi.org/10.1007/s00709-019-01435-6>
- Curigliano, G., Viale, G., Ghioni, M., Jungbluth, A. A., Bagnardi, V., Spagnoli, G. C., Neville, A. M., Nolè, F., Rotmensz, N., & Goldhirsch, A. (2011). Cancer–testis antigen expression in triple-negative breast cancer. *Annals of Oncology*, 22(1), 98–103. <https://doi.org/10.1093/annonc/mdq325>
- da Silva, V. L., Fonseca, A. F., Fonseca, M., da Silva, T. E., Coelho, A. C., Kroll, J. E., de Souza, J. E. S., Stransky, B., de Souza, G. A., & de Souza, S. J. (2017). Genome-wide identification of cancer/testis genes and their association with prognosis in a pan-cancer analysis. *Oncotarget*, 8(54), 92966–92977. <https://doi.org/10.18632/oncotarget.21715>
- Datta, A., Kim, H., McGee, L., Johnson, A. E., Talwar, S., Marugan, J., Southall, N., Hu, X., Lal, M., Mondal, D., Ferrer, M., & Abdel-Mageed, A. B. (2018). High-throughput screening identified selective inhibitors of exosome biogenesis and secretion: A drug repurposing strategy for advanced cancer. *Scientific Reports*, 8(1), Article 1. <https://doi.org/10.1038/s41598-018-26411-7>
- Davis, I. D., Chen, W., Jackson, H., Parente, P., Shackleton, M., Hopkins, W., Chen, Q., Dimopoulos, N., Luke, T., Murphy, R., Scott, A. M., Maraskovsky, E., McArthur, G., MacGregor, D., Sturrock, S., Tai, T. Y., Green, S., Cuthbertson, A., Maher, D., ... Cebon, J. S. (2004). Recombinant NY-ESO-1 protein with ISCOMATRIX adjuvant induces broad integrated antibody and CD4+ and CD8+ T cell responses in humans. *Proceedings of the National Academy of Sciences*, 101(29), 10697–10702. <https://doi.org/10.1073/pnas.0403572101>
- De Smet, C., Backer, O. D., Faraoni, I., Lurquin, C., Bresseur, F., & Boon, T. (1996). The activation of human gene MAGE-1 in tumor cells is correlated with genome-wide demethylation. *Proceedings of the National Academy of Sciences*, 93(14), 7149–7153.
- De Smet, C., Courtois, S. J., Faraoni, I., Lurquin, C., Szikora, J.-P., De Backer, O., & Boon, T. (1995). Involvement of two Ets binding sites in the transcriptional activation of the MAGE1 gene. *Immunogenetics*, 42(4), 282–290. <https://doi.org/10.1007/BF00176446>
- De Smet, C., Lorient, A., & Boon, T. (2004). Promoter-Dependent Mechanism Leading to Selective Hypomethylation within the 5' Region of Gene MAGE-A1 in Tumor Cells. *Molecular and Cellular Biology*, 24(11), 4781–4790. <https://doi.org/10.1128/MCB.24.11.4781-4790.2004>
- De Smet, C., Lurquin, C., Lethé, B., Martelange, V., & Boon, T. (1999). DNA Methylation Is the Primary Silencing Mechanism for a Set of Germ Line- and Tumor-Specific Genes with a CpG-Rich Promoter. *Molecular and Cellular Biology*, 19(11), 7327–7335. <https://doi.org/10.1128/MCB.19.11.7327>
- Dean, M., Fojo, T., & Bates, S. (2005). Tumour stem cells and drug resistance. *Nature Reviews Cancer*, 5(4), Article 4. <https://doi.org/10.1038/nrc1590>
- Deatherage, B. L., & Cookson, B. T. (2012). Membrane Vesicle Release in Bacteria, Eukaryotes, and Archaea: A Conserved yet Underappreciated Aspect of Microbial Life. *Infection and Immunity*, 80(6), 1948–1957. <https://doi.org/10.1128/iai.06014-11>
- Depa-Martynów, M., Kempisty, B., Lianeri, M., Jagodziński, P. P., & Jedrzejczak, P. (2007). Association between fertilin beta, protamines 1 and 2 and spermatid-specific linker histone H1-like protein mRNA levels, fertilization ability of human spermatozoa,

- and quality of preimplantation embryos. *Folia Histochemica et Cytobiologica*, 45(I), Article I.
- Deshaies, R. J., & Joazeiro, C. A. P. (2009). RING Domain E3 Ubiquitin Ligases. *Annual Review of Biochemistry*, 78(1), 399–434. <https://doi.org/10.1146/annurev.biochem.78.101807.093809>
- Dinkins, M. B., Dasgupta, S., Wang, G., Zhu, G., & Bieberich, E. (2014). Exosome reduction in vivo is associated with lower amyloid plaque load in the 5XFAD mouse model of Alzheimer's disease. *Neurobiology of Aging*, 35(8), 1792–1800. <https://doi.org/10.1016/j.neurobiolaging.2014.02.012>
- Dinkins, M. B., Enasko, J., Hernandez, C., Wang, G., Kong, J., Helwa, I., Liu, Y., Terry, A. V., & Bieberich, E. (2016). Neutral Sphingomyelinase-2 Deficiency Ameliorates Alzheimer's Disease Pathology and Improves Cognition in the 5XFAD Mouse. *Journal of Neuroscience*, 36(33), 8653–8667. <https://doi.org/10.1523/JNEUROSCI.1429-16.2016>
- Djureinovic, D., Hallström, B. M., Horie, M., Mattsson, J. S. M., La Fleur, L., Fagerberg, L., Brunnström, H., Lindskog, C., Madjar, K., Rahnenführer, J., Ekman, S., Stähle, E., Koyi, H., Brandén, E., Edlund, K., Hengstler, J. G., Lambe, M., Saito, A., Botling, J., ... Micke, P. (2016). Profiling cancer testis antigens in non-small-cell lung cancer. *JCI Insight*, 1(10), e86837. <https://doi.org/10.1172/jci.insight.86837>
- Domenyuk, V., Zhong, Z., Stark, A., Xiao, N., O'Neill, H. A., Wei, X., Wang, J., Tinder, T. T., Tonapi, S., Duncan, J., Hornung, T., Hunter, A., Miglarese, M. R., Schorr, J., Halbert, D. D., Quackenbush, J., Poste, G., Berry, D. A., Mayer, G., ... Spetzler, D. (2017). Plasma Exosome Profiling of Cancer Patients by a Next Generation Systems Biology Approach. *Scientific Reports*, 7(1), Article 1. <https://doi.org/10.1038/srep42741>
- dos Santos, N. R., Torensma, R., de Vries, T. J., Schreurs, M. W. J., de Bruijn, D. R. H., Kater-Baats, E., Ruiter, D. J., Adema, G. J., van Muijen, G. N. P., & van Kessel, A. G. (2000). Heterogeneous Expression of the SSX Cancer/Testis Antigens in Human Melanoma Lesions and Cell Lines1. *Cancer Research*, 60(6), 1654–1662.
- 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>
- Duan, Z., Duan, Y., Lamendola, D. E., Yusuf, R. Z., Naeem, R., Penson, R. T., & Seiden, M. V. (2003). Overexpression of MAGE/GAGE Genes in Paclitaxel/Doxorubicin-resistant Human Cancer Cell Lines. *Clinical Cancer Research*, 9(7), 2778–2785.
- Duperret, E. K., Liu, S., Paik, M., Trautz, A., Stoltz, R., Liu, X., Ze, K., Perales-Puchalt, A., Reed, C., Yan, J., Xu, X., & Weiner, D. B. (2018). A Designer Cross-reactive DNA Immunotherapeutic Vaccine that Targets Multiple MAGE-A Family Members Simultaneously for Cancer Therapy. *Clinical Cancer Research*, 24(23), 6015–6027. <https://doi.org/10.1158/1078-0432.CCR-18-1013>
- Escudier, B., Dorval, T., Chaput, N., André, F., Caby, M.-P., Novault, S., Flament, C., Leboulaire, C., Borg, C., Amigorena, S., Boccaccio, C., Bonnerot, C., Dhellin, O., Movassagh, M., Piperno, S., Robert, C., Serra, V., Valente, N., Le Pecq, J.-B., ... Zitvogel, L. (2005). Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: Results of the first phase I clinical trial. *Journal of Translational Medicine*, 3(1), 10. <https://doi.org/10.1186/1479-5876-3-10>
- Eshhar, Z., Waks, T., Gross, G., & Schindler, D. G. (1993). Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and

- T-cell receptors. *Proceedings of the National Academy of Sciences*, 90(2), 720–724. <https://doi.org/10.1073/pnas.90.2.720>
- Fan, C., Qu, H., Wang, X., Sobhani, N., Wang, L., Liu, S., Xiong, W., Zeng, Z., & Li, Y. (2021). Cancer/testis antigens: From serology to mRNA cancer vaccine. *Seminars in Cancer Biology*, 76, 218–231. <https://doi.org/10.1016/j.semcancer.2021.04.016>
- Fan, J., Pan, J., Zhang, X., Chen, Y., Zeng, Y., Huang, L., Ma, D., Chen, Z., Wu, G., & Fan, W. (2022). A peptide derived from the N-terminus of charged multivesicular body protein 6 (CHMP6) promotes the secretion of gene editing proteins via small extracellular vesicle production. *Bioengineered*, 13(3), 4702–4716. <https://doi.org/10.1080/21655979.2022.2030571>
- Fayet, M., Prybylski, N., Collin, M.-L., Peyretailade, E., Wawrzyniak, I., Belkorchia, A., Akossi, R. F., Diogon, M., El Alaoui, H., Polonais, V., & Delbac, F. (2023). Identification and localization of polar tube proteins in the extruded polar tube of the microsporidian *Anncaliia algerae*. *Scientific Reports*, 13(1), Article 1. <https://doi.org/10.1038/s41598-023-35511-y>
- Feinberg, A. P., & Vogelstein, B. (1983). Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature*, 301(5895), Article 5895. <https://doi.org/10.1038/301089a0>
- Fishbain, S., Inobe, T., Israeli, E., Chavali, S., Yu, H., Kago, G., Babu, M. M., & Matouschek, A. (2015). Sequence composition of disordered regions fine-tunes protein half-life. *Nature Structural & Molecular Biology*, 22(3), Article 3. <https://doi.org/10.1038/nsmb.2958>
- Fon Tacer, K., Montoya, M. C., Oatley, M. J., Lord, T., Oatley, J. M., Klein, J., Ravichandran, R., Tillman, H., Kim, M., Connelly, J. P., Pruett-Miller, S. M., Bookout, A. L., Binshtock, E., Kamiński, M. M., & Potts, P. R. (2019). MAGE cancer-testis antigens protect the mammalian germline under environmental stress. *Science Advances*, 5(5), eaav4832. <https://doi.org/10.1126/sciadv.aav4832>
- Fon Tacer, K., & Potts, P. R. (2017). Cellular and disease functions of the Prader–Willi Syndrome gene MAGEL2. *Biochemical Journal*, 474(13), 2177–2190. <https://doi.org/10.1042/BCJ20160616>
- Fox, J. E. B., Austin, C. D., Boyles, J. K., & Steffen, P. K. (1990). Role of the membrane skeleton in preventing the shedding of procoagulant-rich microvesicles from the platelet plasma membrane. *The Journal of Cell Biology*, 111(2), 483–493.
- Frank, J., Richter, M., de Rossi, C., Lehr, C.-M., Fuhrmann, K., & Fuhrmann, G. (2018). Extracellular vesicles protect glucuronidase model enzymes during freeze-drying. *Scientific Reports*, 8(1), Article 1. <https://doi.org/10.1038/s41598-018-30786-y>
- Fratta, E., Sigalotti, L., Colizzi, F., Covre, A., Nicolay, H. J., Danielli, R., Fonsatti, E., Altomonte, M., Calabrò, L., Coral, S., & Maio, M. (2010). Epigenetically regulated clonal heritability of CTA expression profiles in human melanoma. *Journal of Cellular Physiology*, 223(2), 352–358. <https://doi.org/10.1002/jcp.22040>
- Freitas, M. R. P., Malheiros, S. M. F., Stávale, J. N., Biassi, T. P., Zamunér, F. T., de Souza Begnami, M. D. F., Soares, F. A., & Vettore, A. L. (2013). Expression of Cancer/Testis Antigens is Correlated with Improved Survival in Glioblastoma. *Oncotarget*, 4(4), 636–646.
- Gama-Sosa, M. A., Slagel, V. A., Trewyn, R. W., Oxenhandler, R., Kuo, K. C., Gehrke, C. W., & Ehrlich, M. (1983). The 5-methylcytosine content of DNA from human tumors. *Nucleic Acids Research*, 11(19), 6883–6894. <https://doi.org/10.1093/nar/11.19.6883>

- Gao, X., Li, Q., Chen, G., He, H., & Ma, Y. (2020). MAGEA3 promotes proliferation and suppresses apoptosis in cervical cancer cells by inhibiting the KAP1/p53 signaling pathway. *American Journal of Translational Research*, *12*(7), 3596–3612.
- Gao, Y., Kardos, J., Yang, Y., Tamir, T. Y., Mutter-Rottmayer, E., Weissman, B., Major, M. B., Kim, W. Y., & Vaziri, C. (2018). The Cancer/Testes (CT) Antigen HORMAD1 promotes Homologous Recombinational DNA Repair and Radioresistance in Lung adenocarcinoma cells. *Scientific Reports*, *8*(1), Article 1. <https://doi.org/10.1038/s41598-018-33601-w>
- García-Silva, S., Benito-Martín, A., Sánchez-Redondo, S., Hernández-Barranco, A., Ximénez-Embún, P., Nogués, L., Mazariegos, M. S., Brinkmann, K., Amor López, A., Meyer, L., Rodríguez, C., García-Martín, C., Boskovic, J., Letón, R., Montero, C., Robledo, M., Santambrogio, L., Sue Brady, M., Szumera-Ciećkiewicz, A., ... Peinado, H. (2019). Use of extracellular vesicles from lymphatic drainage as surrogate markers of melanoma progression and BRAF V600E mutation. *The Journal of Experimental Medicine*, *216*(5), 1061–1070. <https://doi.org/10.1084/jem.20181522>
- Gebräad, A., Kornilov, R., Kaur, S., Miettinen, S., Haimi, S., Peltoniemi, H., Mannerström, B., & Seppänen-Kaijansinkko, R. (2018). Monocyte-derived extracellular vesicles stimulate cytokine secretion and gene expression of matrix metalloproteinases by mesenchymal stem/stromal cells. *The FEBS Journal*, *285*(12), 2337–2359. <https://doi.org/10.1111/febs.14485>
- Gedye, C., Quirk, J., Browning, J., Svobodová, S., John, T., Sluka, P., Dunbar, P. R., Corbeil, D., Cebon, J., & Davis, I. D. (2009). Cancer/testis antigens can be immunological targets in clonogenic CD133+ melanoma cells. *Cancer Immunology, Immunotherapy*, *58*(10), 1635–1646. <https://doi.org/10.1007/s00262-009-0672-0>
- Gee, R. R. F., Chen, H., Lee, A. K., Daly, C. A., Wilander, B. A., Tacer, K. F., & Potts, P. R. (2020). Emerging roles of the MAGE protein family in stress response pathways. *Journal of Biological Chemistry*, *295*(47), 16121–16155. <https://doi.org/10.1074/jbc.REV120.008029>
- Gibbs, Z. A., & Whitehurst, A. W. (2018). Emerging Contributions of Cancer/Testis Antigens to Neoplastic Behaviors. *Trends in Cancer*, *4*(10), 701–712. <https://doi.org/10.1016/j.trecan.2018.08.005>
- Gjerstorff, M. F., Andersen, M. H., & Ditzel, H. J. (2015). Oncogenic cancer/testis antigens: Prime candidates for immunotherapy. *Oncotarget*, *6*(18), 15772–15787.
- Gjerstorff, M. F., Burns, J., & Ditzel, H. J. (2010). Cancer–germline antigen vaccines and epigenetic enhancers: Future strategies for cancer treatment. *Expert Opinion on Biological Therapy*, *10*(7), 1061–1075. <https://doi.org/10.1517/14712598.2010.485188>
- Gjerstorff, M. F., Burns, J. S., Nielsen, O., Kassem, M., & Ditzel, H. (2009). Epigenetic Modulation of Cancer-Germline Antigen Gene Expression in Tumorigenic Human Mesenchymal Stem Cells: Implications for Cancer Therapy. *The American Journal of Pathology*, *175*(1), 314–323. <https://doi.org/10.2353/ajpath.2009.080893>
- Gjerstorff, M. F., Harkness, L., Kassem, M., Frandsen, U., Nielsen, O., Lutterodt, M., Møllgård, K., & Ditzel, H. J. (2008). Distinct GAGE and MAGE-A expression during early human development indicate specific roles in lineage differentiation. *Human Reproduction*, *23*(10), 2194–2201. <https://doi.org/10.1093/humrep/den262>
- Gjerstorff, M. F., Johansen, L. E., Nielsen, O., Kock, K., & Ditzel, H. J. (2006). Restriction of GAGE protein expression to subpopulations of cancer cells is independent of genotype and may limit the use of GAGE proteins as targets for cancer immunotherapy. *British Journal of Cancer*, *94*(12), Article 12. <https://doi.org/10.1038/sj.bjc.6603163>

- Gjerstorff, M. F., Kock, K., Nielsen, O., & Ditzel, H. J. (2007). MAGE-A1, GAGE and NY-ESO-1 cancer/testis antigen expression during human gonadal development. *Human Reproduction*, *22*(4), 953–960. <https://doi.org/10.1093/humrep/del494>
- Goetzl, E. J., Abner, E. L., Jicha, G. A., Kapogiannis, D., & Schwartz, J. B. (2018). Declining levels of functionally specialized synaptic proteins in plasma neuronal exosomes with progression of Alzheimer’s disease. *The FASEB Journal*, *32*(2), 888–893. <https://doi.org/10.1096/fj.201700731R>
- Gordeeva, O. F. (2018). Cancer-testis antigens: Unique cancer stem cell biomarkers and targets for cancer therapy. *Seminars in Cancer Biology*, *53*, 75–89. <https://doi.org/10.1016/j.semcancer.2018.08.006>
- Gordeeva, O. F., & PochaeV, V. A. (2017). Expression of cancer-testis antigens of the Mage family in mouse oocytes and early embryos. *Russian Journal of Developmental Biology*, *48*(4), 287–294. <https://doi.org/10.1134/S1062360417040051>
- Greve, K. B. V., Lindgreen, J. N., Terp, M. G., Pedersen, C. B., Schmidt, S., Mollenhauer, J., Kristensen, S. B., Andersen, R. S., Relster, M. M., Ditzel, H. J., & Gjerstorff, M. F. (2015). Ectopic expression of cancer/testis antigen SSX2 induces DNA damage and promotes genomic instability. *Molecular Oncology*, *9*(2), 437–449. <https://doi.org/10.1016/j.molonc.2014.09.001>
- Griffin, J., & Lowenberg, B. (1986). Clonogenic cells in acute myeloblastic leukemia. *Blood*, *68*(6), 1185–1195. <https://doi.org/10.1182/blood.V68.6.1185.1185>
- 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>
- Guo, L.-W., & Ruoho, A. E. (2011). N-terminal Half of the cGMP Phosphodiesterase  $\gamma$ -Subunit Contributes to Stabilization of the GTPase-accelerating Protein Complex \*. *Journal of Biological Chemistry*, *286*(17), 15260–15267. <https://doi.org/10.1074/jbc.M110.210567>
- Guo, S.-C., Tao, S.-C., Yin, W.-J., Qi, X., Yuan, T., & Zhang, C.-Q. (2017). Exosomes derived from platelet-rich plasma promote the re-epithelization of chronic cutaneous wounds via activation of YAP in a diabetic rat model. *Theranostics*, *7*(1), 81–96. <https://doi.org/10.7150/thno.16803>
- Gupta, N., Jagadish, N., Surolia, A., & Suri, A. (2017). Heat shock protein 70-2 (HSP70-2) a novel cancer testis antigen that promotes growth of ovarian cancer. *American Journal of Cancer Research*, *7*(6), 1252–1269.
- Güre, A. O., Chua, R., Williamson, B., Gonen, M., Ferrera, C. A., Gnjjatic, S., Ritter, G., Simpson, A. J. G., Chen, Y.-T., Old, L. J., & Altorki, N. K. (2005). Cancer-Testis Genes Are Coordinately Expressed and Are Markers of Poor Outcome in Non-Small Cell Lung Cancer. *Clinical Cancer Research*, *11*(22), 8055–8062. <https://doi.org/10.1158/1078-0432.CCR-05-1203>
- Güre, A. O., Wei, I. J., Old, L. J., & Chen, Y.-T. (2002). The SSX gene family: Characterization of 9 complete genes. *International Journal of Cancer*, *101*(5), 448–453. <https://doi.org/10.1002/ijc.10634>
- György, B., Szabó, T. G., Pásztoi, M., Pál, Z., Misják, P., Aradi, B., László, V., Pállinger, É., Pap, E., Kittel, Á., Nagy, G., Falus, A., & Buzás, E. I. (2011). Membrane vesicles, current state-of-the-art: Emerging role of extracellular vesicles. *Cellular and Molecular Life Sciences*, *68*(16), 2667–2688. <https://doi.org/10.1007/s00018-011-0689-3>

- Hagiwara, Y., Sieverling, L., Hanif, F., Anton, J., Dickinson, E. R., Bui, T. T. T., Andreeva, A., Barran, P. E., Cota, E., & Nikolova, P. V. (2016). Consequences of point mutations in melanoma-associated antigen 4 (MAGE-A4) protein: Insights from structural and biophysical studies. *Scientific Reports*, 6, 25182. <https://doi.org/10.1038/srep25182>
- Hamauzu, Z., Nakatani, M., & Yonezawa, D. (1975). Studies on Sodium Dodecyl Sulfate Complex of Reduced Gliadin in Relation to the Abnormality in SDS-Polyacrylamide Gel Electrophoresis. *Agricultural and Biological Chemistry*, 39(7), 1407–1410. <https://doi.org/10.1080/00021369.1975.10861789>
- Hamburger, A. W., & Salmon, S. E. (1977). Primary Bioassay of Human Tumor Stem Cells. *Science*, 197(4302), 461–463. <https://doi.org/10.1126/science.560061>
- Hamer, G., Gell, K., Kouznetsova, A., Novak, I., Benavente, R., & Höög, C. (2006). Characterization of a novel meiosis-specific protein within the central element of the synaptonemal complex. *Journal of Cell Science*, 119(19), 4025–4032. <https://doi.org/10.1242/jcs.03182>
- Hao, J., Song, X., Wang, J., Guo, C., Li, Y., Li, B., Zhang, Y., & Yin, Y. (2015). Cancer-testis antigen MAGE-C2 binds Rbx1 and inhibits ubiquitin ligase-mediated turnover of cyclin E. *Oncotarget*, 6(39), 42028–42039.
- 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>
- Hardie, D. G., Ross, F. A., & Hawley, S. A. (2012). AMPK: A nutrient and energy sensor that maintains energy homeostasis. *Nature Reviews Molecular Cell Biology*, 13(4), Article 4. <https://doi.org/10.1038/nrm3311>
- Harding, C., Heuser, J., & Stahl, P. (1984). Endocytosis and intracellular processing of transferrin and colloidal gold-transferrin in rat reticulocytes: Demonstration of a pathway for receptor shedding. *European Journal of Cell Biology*, 35(2), 256–263.
- Haynes, C., Oldfield, C. J., Ji, F., Klitgord, N., Cusick, M. E., Radivojac, P., Uversky, V. N., Vidal, M., & Iakoucheva, L. M. (2006). Intrinsic Disorder Is a Common Feature of Hub Proteins from Four Eukaryotic Interactomes. *PLOS Computational Biology*, 2(8), e100. <https://doi.org/10.1371/journal.pcbi.0020100>
- Hegde, M. L., Tsutakawa, S. E., Hegde, P. M., Holthausen, L. M. F., Li, J., Oezguen, N., Hilser, V. J., Tainer, J. A., & Mitra, S. (2013). The Disordered C-Terminal Domain of Human DNA Glycosylase NEIL1 Contributes to Its Stability via Intramolecular Interactions. *Journal of Molecular Biology*, 425(13), 2359–2371. <https://doi.org/10.1016/j.jmb.2013.03.030>
- Herold, A., Suyama, M., Rodrigues, J. P., Braun, I. C., Kutay, U., Carmo-Fonseca, M., Bork, P., & Izaurralde, E. (2000). TAP (NXF1) Belongs to a Multigene Family of Putative RNA Export Factors with a Conserved Modular Architecture. *Molecular and Cellular Biology*, 20(23), 8996–9008. <https://doi.org/10.1128/MCB.20.23.8996-9008.2000>
- Hoei-Hansen, C. E., Kraggerud, S. M., Abeler, V. M., Kærn, J., Rajpert-De Meyts, E., & Lothe, R. A. (2007). Ovarian dysgerminomas are characterised by frequent KIT mutations and abundant expression of pluripotency markers. *Molecular Cancer*, 6(1), 12. <https://doi.org/10.1186/1476-4598-6-12>
- Hofmann, O., Caballero, O. L., Stevenson, B. J., Chen, Y.-T., Cohen, T., Chua, R., Maher, C. A., Panji, S., Schaefer, U., Kruger, A., Lehvaslaiho, M., Carninci, P., Haya-shizaki, Y., Jongeneel, C. V., Simpson, A. J. G., Old, L. J., & Hide, W. (2008).

- Genome-wide analysis of cancer/testis gene expression. *Proceedings of the National Academy of Sciences*, 105(51), 20422–20427. <https://doi.org/10.1073/pnas.0810777105>
- Hogan, B., Fellous, M., Jacob, F., & Avner, P. (1977). Isolation of a human teratoma cell line which expresses F9 antigen. *Nature*, 270(5637), Article 5637. <https://doi.org/10.1038/270515a0>
- Holden, S., Bernard, O., Artzt, K., Whitmore, W. F., & Bennett, D. (1977). Human and mouse embryonal carcinoma cells in culture share an embryonic antigen (F9). *Nature*, 270(5637), Article 5637. <https://doi.org/10.1038/270518a0>
- Hong, J. A., Kang, Y., Abdullaev, Z., Flanagan, P. T., Pack, S. D., Fischette, M. R., Adnani, M. T., Loukinov, D. I., Vatolin, S., Risinger, J. I., Custer, M., Chen, G. A., Zhao, M., Nguyen, D. M., Barrett, J. C., Lobanenko, V. V., & Schrupp, D. S. (2005). Reciprocal Binding of CTCF and BORIS to the NY-ESO-1 Promoter Coincides with Derepression of this Cancer-Testis Gene in Lung Cancer Cells. *Cancer Research*, 65(17), 7763–7774. <https://doi.org/10.1158/0008-5472.CAN-05-0823>
- Hosono, Y., Niknafs, Y. S., Prensner, J. R., Iyer, M. K., Dhanasekaran, S. M., Mehra, R., Pichiaya, S., Tien, J., Escara-Wilke, J., Poliakov, A., Chu, S.-C., Saleh, S., Sankar, K., Su, F., Guo, S., Qiao, Y., Freier, S. M., Bui, H.-H., Cao, X., ... Chinnaiyan, A. M. (2017). Oncogenic Role of THOR, a Conserved Cancer/Testis Long Non-coding RNA. *Cell*, 171(7), 1559-1572.e20. <https://doi.org/10.1016/j.cell.2017.11.040>
- Illingworth, R. S., & Bird, A. P. (2009). CpG islands – ‘A rough guide’. *FEBS Letters*, 583(11), 1713–1720. <https://doi.org/10.1016/j.febslet.2009.04.012>
- Iura, K., Kohashi, K., Ishii, T., Maekawa, A., Bekki, H., Otsuka, H., Yamada, Y., Yamamoto, H., Matsumoto, Y., Iwamoto, Y., & Oda, Y. (2017). MAGEA4 expression in bone and soft tissue tumors: Its utility as a target for immunotherapy and diagnostic marker combined with NY-ESO-1. *Virchows Archiv*, 471(3), 383–392. <https://doi.org/10.1007/s00428-017-2206-z>
- Jäger, E., Chen, Y. T., Drijfhout, J. W., Karbach, J., Ringhoffer, M., Jäger, D., Arand, M., Wada, H., Noguchi, Y., Stockert, E., Old, L. J., & Knuth, A. (1998). Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: Definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. *The Journal of Experimental Medicine*, 187(2), 265–270. <https://doi.org/10.1084/jem.187.2.265>
- Jäger, E., Gnjatich, S., Nagata, Y., Stockert, E., Jäger, D., Karbach, J., Neumann, A., Rieckenberg, J., Chen, Y.-T., Ritter, G., Hoffman, E., Arand, M., Old, L. J., & Knuth, A. (2000). Induction of primary NY-ESO-1 immunity: CD8+ T lymphocyte and antibody responses in peptide-vaccinated patients with NY-ESO-1+ cancers. *Proceedings of the National Academy of Sciences*, 97(22), 12198–12203. <https://doi.org/10.1073/pnas.220413497>
- Jäger, E., Nagata, Y., Gnjatich, S., Wada, H., Stockert, E., Karbach, J., Dunbar, P. R., Lee, S. Y., Jungbluth, A., Jäger, D., Arand, M., Ritter, G., Cerundolo, V., Dupont, B., Chen, Y.-T., Old, L. J., & Knuth, A. (2000). Monitoring CD8 T cell responses to NY-ESO-1: Correlation of humoral and cellular immune responses. *Proceedings of the National Academy of Sciences*, 97(9), 4760–4765. <https://doi.org/10.1073/pnas.97.9.4760>
- Jäger, E., Stockert, E., Zidianakis, Z., Chen, Y.-T., Karbach, J., Jäger, D., Arand, M., Ritter, G., Old, L. J., & Knuth, A. (1999). Humoral immune responses of cancer patients against “Cancer-Testis” antigen NY-ESO-1: Correlation with clinical events. *International Journal of Cancer*, 84(5), 506–510. [https://doi.org/10.1002/\(SICI\)1097-0215\(19991022\)84:5<506::AID-IJC10>3.0.CO;2-6](https://doi.org/10.1002/(SICI)1097-0215(19991022)84:5<506::AID-IJC10>3.0.CO;2-6)

- Jakobsen, M. K., & Gjerstorff, M. F. (2020). CAR T-Cell Cancer Therapy Targeting Surface Cancer/Testis Antigens. *Frontiers in Immunology*, *11*. <https://www.frontiersin.org/articles/10.3389/fimmu.2020.01568>
- James, S. R., Link, P. A., & Karpf, A. R. (2006). Epigenetic regulation of X-linked cancer/germline antigen genes by DNMT1 and DNMT3b. *Oncogene*, *25*(52), Article 52. <https://doi.org/10.1038/sj.onc.1209678>
- Jang, S. C., Economides, K. D., Moniz, R. J., Sia, C. L., Lewis, N., McCoy, C., Zi, T., Zhang, K., Harrison, R. A., Lim, J., Dey, J., Grenley, M., Kirwin, K., Ross, N. L., Bourdeau, R., Villiger-Oberbek, A., Estes, S., Xu, K., Sanchez-Salazar, J., ... Sathyanarayanan, S. (2021). ExoSTING, an extracellular vesicle loaded with STING agonists, promotes tumor immune surveillance. *Communications Biology*, *4*(1), Article 1. <https://doi.org/10.1038/s42003-021-02004-5>
- Janssen, B. L., van de Locht, L. T., Fourkour, A., de Smet, C., Mensink, E. J., van Muijen, G. N., & de Vries, T. J. (1999). Transcription of the MAGE-1 gene and the methylation status of its Ets binding promoter elements: A quantitative analysis in melanoma cell lines using a real-time polymerase chain reaction technique. *Melanoma Research*, *9*(3), 213–222. <https://doi.org/10.1097/00008390-199906000-00002>
- Jia, Z.-C., Tian, Y., Huang, Z.-M., Wang, J.-X., Fu, X.-L., Ni, B., & Wu, Y.-Z. (2011). Identification of a new MAGE-A10 antigenic peptide presented by HLA-A\*0201 on tumor cells. *Cancer Biology & Therapy*, *11*(4), 395–400. <https://doi.org/10.4161/cbt.11.4.14100>
- Jin, X., Pan, Y., Wang, L., Zhang, L., Ravichandran, R., Potts, P. R., Jiang, J., Wu, H., & Huang, H. (2017). MAGE-TRIM28 complex promotes the Warburg effect and hepatocellular carcinoma progression by targeting FBP1 for degradation. *Oncogenesis*, *6*(4), Article 4. <https://doi.org/10.1038/oncsis.2017.21>
- Johnstone, R. M., Adam, M., Hammond, J. R., Orr, L., & Turbide, C. (1987). Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). *Journal of Biological Chemistry*, *262*(19), 9412–9420. [https://doi.org/10.1016/S0021-9258\(18\)48095-7](https://doi.org/10.1016/S0021-9258(18)48095-7)
- Jungbluth, A. A., Chen, Y.-T., Stockert, E., Busam, K. J., Kolb, D., Iversen, K., Coplan, K., Williamson, B., Altorki, N., & Old, L. J. (2001). Immunohistochemical analysis of NY-ESO-1 antigen expression in normal and malignant human tissues. *International Journal of Cancer*, *92*(6), 856–860. <https://doi.org/10.1002/ijc.1282>
- Jungbluth, A. A., Kolb, D., Iversen, K., Coplan, K., Old, L. J., Busam, K. J., Chen, Y. T., & Spagnoli, G. C. (2000). Expression of MAGE-antigens in normal tissues and cancer. *International Journal of Cancer*, *85*(4), 460–465. [https://doi.org/10.1002/\(SICI\)1097-0215\(20000215\)85:4<460::AID-IJC3>3.0.CO;2-N](https://doi.org/10.1002/(SICI)1097-0215(20000215)85:4<460::AID-IJC3>3.0.CO;2-N)
- Kalra, H., Drummen, G. P. C., & Mathivanan, S. (2016). Focus on Extracellular Vesicles: Introducing the Next Small Big Thing. *International Journal of Molecular Sciences*, *17*(2), Article 2. <https://doi.org/10.3390/ijms17020170>
- Kalra, H., Simpson, R. J., Ji, H., Aikawa, E., Altevogt, P., Askenase, P., Bond, V. C., Borràs, F. E., Breakefield, X., Budnik, V., Buzas, E., Camussi, G., Clayton, A., Cocucci, E., Falcon-Perez, J. M., Gabrielsson, S., Gho, Y. S., Gupta, D., Harsha, H. C., ... Mathivanan, S. (2012). Vesiclepedia: A Compendium for Extracellular Vesicles with Continuous Community Annotation. *PLOS Biology*, *10*(12), e1001450. <https://doi.org/10.1371/journal.pbio.1001450>
- Kanemori, Y., Ryu, J.-H., Sudo, M., Niida-Araida, Y., Kodaira, K., Takenaka, M., Kohno, N., Sugiura, S., Kashiwabara, S., & Baba, T. (2013). Two Functional Forms

- of ACRBP/sp32 Are Produced by Pre-mRNA Alternative Splicing in the Mouse. *Biology of Reproduction*, 88(4), 105, 1–8. <https://doi.org/10.1095/biolreprod.112.107425>
- Kang, Y., Hong, J. A., Chen, G. A., Nguyen, D. M., & Schrupp, D. S. (2007). Dynamic transcriptional regulatory complexes including BORIS, CTCF and Sp1 modulate NY-ESO-1 expression in lung cancer cells. *Oncogene*, 26(30), Article 30. <https://doi.org/10.1038/sj.onc.1210218>
- Karpf, A. R., Bai, S., James, S. R., Mohler, J. L., & Wilson, E. M. (2009). Increased Expression of Androgen Receptor Coregulator MAGE-11 in Prostate Cancer by DNA Hypomethylation and Cyclic AMP. *Molecular Cancer Research*, 7(4), 523–535. <https://doi.org/10.1158/1541-7786.MCR-08-0400>
- Karpf, A. R., Lasek, A. W., Ririe, T. O., Hanks, A. N., Grossman, D., & Jones, D. A. (2004). Limited Gene Activation in Tumor and Normal Epithelial Cells Treated with the DNA Methyltransferase Inhibitor 5-Aza-2'-deoxycytidine. *Molecular Pharmacology*, 65(1), 18–27. <https://doi.org/10.1124/mol.65.1.18>
- Katsura, Y., & Satta, Y. (2011). Evolutionary History of the Cancer Immunity Antigen MAGE Gene Family. *PLOS ONE*, 6(6), e20365. <https://doi.org/10.1371/journal.pone.0020365>
- Kawamoto, T., Ohga, N., Akiyama, K., Hirata, N., Kitahara, S., Maishi, N., Osawa, T., Yamamoto, K., Kondoh, M., Shindoh, M., Hida, Y., & Hida, K. (2012). Tumor-Derived Microvesicles Induce Proangiogenic Phenotype in Endothelial Cells via Endocytosis. *PLOS ONE*, 7(3), e34045. <https://doi.org/10.1371/journal.pone.0034045>
- Keeney, S., Giroux, C. N., & Kleckner, N. (1997). Meiosis-Specific DNA Double-Strand Breaks Are Catalyzed by Spo11, a Member of a Widely Conserved Protein Family. *Cell*, 88(3), 375–384. [https://doi.org/10.1016/S0092-8674\(00\)81876-0](https://doi.org/10.1016/S0092-8674(00)81876-0)
- Keerthikumar, S., Chisanga, D., Ariyaratne, D., Al Saffar, H., Anand, S., Zhao, K., Samuel, M., Pathan, M., Jois, M., Chilamkurti, N., Gangoda, L., & Mathivanan, S. (2016). ExoCarta: A Web-Based Compendium of Exosomal Cargo. *Journal of Molecular Biology*, 428(4), 688–692. <https://doi.org/10.1016/j.jmb.2015.09.019>
- Kendall, S. E., Battelli, C., Irwin, S., Mitchell, J. G., Glackin, C. A., & Verdi, J. M. (2005). NRAGE Mediates p38 Activation and Neural Progenitor Apoptosis via the Bone Morphogenetic Protein Signaling Cascade. *Molecular and Cellular Biology*, 25(17), 7711–7724. <https://doi.org/10.1128/MCB.25.17.7711-7724.2005>
- Kholmanskikh, O., Lorient, A., Brasseur, F., De Plaen, E., & De Smet, C. (2008). Expression of BORIS in melanoma: Lack of association with MAGE-A1 activation. *International Journal of Cancer*, 122(4), 777–784. <https://doi.org/10.1002/ijc.23140>
- Kim, R., Kulkarni, P., & Hannenhalli, S. (2013). Derepression of Cancer/Testis Antigens in cancer is associated with distinct patterns of DNA Hypomethylation. *BMC Cancer*, 13(1), 144. <https://doi.org/10.1186/1471-2407-13-144>
- Kim, Y., Park, D., Kim, H., Choi, M., Lee, H., Lee, Y. S., Choe, J., Kim, Y. M., & Jeoung, D. (2013). miR-200b and Cancer/Testis Antigen CAGE Form a Feedback Loop to Regulate the Invasion and Tumorigenic and Angiogenic Responses of a Cancer Cell Line to Microtubule-targeting Drugs \*. *Journal of Biological Chemistry*, 288(51), 36502–36518. <https://doi.org/10.1074/jbc.M113.502047>
- Kimmins, S., & Sassone-Corsi, P. (2005). Chromatin remodelling and epigenetic features of germ cells. *Nature*, 434(7033), Article 7033. <https://doi.org/10.1038/nature03368>
- Klenova, E. M., Nicolas, R. H., Sally, U., Carne, A. F., Lee, R. E., Lobanekov, V. V., & Goodwin, G. H. (1997). Molecular Weight Abnormalities of the CTCF Transcription Factor: CTCF Migrates Aberrantly in SDS-PAGE and the Size of the Expressed Protein is Affected by the UTRs and Sequences Within the Coding Region of the

- CTCF Gene. *Nucleic Acids Research*, 25(3), 466–473. <https://doi.org/10.1093/nar/25.3.466>
- Koifman, N., Biran, I., Aharon, A., Brenner, B., & Talmon, Y. (2017). A direct-imaging cryo-EM study of shedding extracellular vesicles from leukemic monocytes. *Journal of Structural Biology*, 198(3), 177–185. <https://doi.org/10.1016/j.jsb.2017.02.004>
- Kondo, T., Zhu, X., Asa, S. L., & Ezzat, S. (2007). The Cancer/Testis Antigen Melanoma-Associated Antigen-A3/A6 Is a Novel Target of Fibroblast Growth Factor Receptor 2-IIIb through Histone H3 Modifications in Thyroid Cancer. *Clinical Cancer Research*, 13(16), 4713–4720. <https://doi.org/10.1158/1078-0432.CCR-07-0618>
- Kosgodage, U. S., Mould, R., Henley, A. B., Nunn, A. V., Guy, G. W., Thomas, E. L., Inal, J. M., Bell, J. D., & Lange, S. (2018). Cannabidiol (CBD) Is a Novel Inhibitor for Exosome and Microvesicle (EMV) Release in Cancer. *Frontiers in Pharmacology*, 9. <https://www.frontiersin.org/articles/10.3389/fphar.2018.00889>
- Kosugi, S., Hasebe, M., Matsumura, N., Takashima, H., Miyamoto-Sato, E., Tomita, M., & Yanagawa, H. (2009). Six Classes of Nuclear Localization Signals Specific to Different Binding Grooves of Importin  $\alpha$  \*. *Journal of Biological Chemistry*, 284(1), 478–485. <https://doi.org/10.1074/jbc.M807017200>
- Kowal, J., Arras, G., Colombo, M., Jouve, M., Morath, J. P., Primdal-Bengtson, B., Dingli, F., Loew, D., Tkach, M., & Théry, C. (2016). Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. *Proceedings of the National Academy of Sciences*, 113(8), E968–E977. <https://doi.org/10.1073/pnas.1521230113>
- Kozakova, L., Vondrova, L., Stejskal, K., Charalabous, P., Kolesar, P., Lehmann, A. R., Uldrijan, S., Sanderson, C. M., Zdrahal, Z., & Palecek, J. J. (2015). The melanoma-associated antigen 1 (MAGEA1) protein stimulates the E3 ubiquitin-ligase activity of TRIM31 within a TRIM31-MAGEA1-NSE4 complex. *Cell Cycle*, 14(6), 920–930. <https://doi.org/10.1080/15384101.2014.1000112>
- Kurg, R., Reinsalu, O., Jagur, S., Õunap, K., Vösa, L., Kasvandik, S., Padari, K., Gilde-mann, K., & Ustav, M. (2016). Biochemical and proteomic characterization of retrovirus Gag based microparticles carrying melanoma antigens. *Scientific Reports*, 6. <https://doi.org/10.1038/srep29425>
- Kusuma, G. D., Barabadi, M., Tan, J. L., Morton, D. A. V., Frith, J. E., & Lim, R. (2018). To Protect and to Preserve: Novel Preservation Strategies for Extracellular Vesicles. *Frontiers in Pharmacology*, 9. <https://www.frontiersin.org/articles/10.3389/fphar.2018.01199>
- Laduron, S., Deplus, R., Zhou, S., Kholmanskikh, O., Godelaine, D., De Smet, C., Hayward, S. D., Fuks, F., Boon, T., & De Plaen, E. (2004). MAGE-A1 interacts with adaptor SKIP and the deacetylase HDAC1 to repress transcription. *Nucleic Acids Research*, 32(14), 4340–4350. <https://doi.org/10.1093/nar/gkh735>
- Laghmani, K., Beck, B. B., Yang, S.-S., Seaayfan, E., Wenzel, A., Reusch, B., Vitzhum, H., Priem, D., Demaretz, S., Bergmann, K., Duin, L. K., Göbel, H., Mache, C., Thiele, H., Bartram, M. P., Dombret, C., Altmüller, J., Nürnberg, P., Benzing, T., ... Kömhoff, M. (2016). Polyhydramnios, Transient Antenatal Bartter's Syndrome, and MAGED2 Mutations. *New England Journal of Medicine*, 374(19), 1853–1863. <https://doi.org/10.1056/NEJMoa1507629>
- Lai, R. C., Arslan, F., Lee, M. M., Sze, N. S. K., Choo, A., Chen, T. S., Salto-Tellez, M., Timmers, L., Lee, C. N., El Oakley, R. M., Pasterkamp, G., de Kleijn, D. P. V., & Lim, S. K. (2010). Exosome secreted by MSC reduces myocardial ischemia/

- reperfusion injury. *Stem Cell Research*, 4(3), 214–222. <https://doi.org/10.1016/j.scr.2009.12.003>
- Lajmi, N., Luetkens, T., Yousef, S., Templin, J., Cao, Y., Hildebrandt, Y., Bartels, K., Kröger, N., & Atanackovic, D. (2015). Cancer-testis antigen MAGEC2 promotes proliferation and resistance to apoptosis in Multiple Myeloma. *British Journal of Haematology*, 171(5), 752–762. <https://doi.org/10.1111/bjh.13762>
- Lamichhane, T. N., Raiker, R. S., & Jay, S. M. (2015). Exogenous DNA Loading into Extracellular Vesicles via Electroporation is Size-Dependent and Enables Limited Gene Delivery. *Molecular Pharmaceutics*, 12(10), 3650–3657. <https://doi.org/10.1021/acs.molpharmaceut.5b00364>
- Lange, A., Mills, R. E., Lange, C. J., Stewart, M., Devine, S. E., & Corbett, A. H. (2007). Classical Nuclear Localization Signals: Definition, Function, and Interaction with Importin  $\alpha^*$ . *Journal of Biological Chemistry*, 282(8), 5101–5105. <https://doi.org/10.1074/jbc.R600026200>
- Lazar, I., Fabre, B., Feng, Y., Khateb, A., Turko, P., Martinez Gomez, J. M., Frederick, D. T., Levesque, M. P., Feld, L., Zhang, G., Zhang, T., James, B., Shklover, J., Avitan-Hersh, E., Livneh, I., Scortegagna, M., Brown, K., Larsson, O., Topisirovic, I., ... Ronai, Z. A. (2020). SPANX Control of Lamin A/C Modulates Nuclear Architecture and Promotes Melanoma Growth. *Molecular Cancer Research*, 18(10), 1560–1573. <https://doi.org/10.1158/1541-7786.MCR-20-0291>
- Le Gal, F.-A., Widmer, V. M., Dutoit, V., Rubio-Godoy, V., Schrenzel, J., Walker, P. R., Romero, P. J., Valmori, D., Speiser, D. E., & Dietrich, P.-Y. (2007). Tissue Homing and Persistence of Defined Antigen-Specific CD8<sup>+</sup> Tumor-Reactive T-Cell Clones in Long-Term Melanoma Survivors. *Journal of Investigative Dermatology*, 127(3), 622–629. <https://doi.org/10.1038/sj.jid.5700580>
- Lee, A. K., Klein, J., Fon Tacer, K., Lord, T., Oatley, M. J., Oatley, J. M., Porter, S. N., Pruett-Miller, S. M., Tikhonova, E. B., Karamyshev, A. L., Wang, Y.-D., Yang, P., Korff, A., Kim, H. J., Taylor, J. P., & Potts, P. R. (2020). Translational Repression of G3BP in Cancer and Germ Cells Suppresses Stress Granules and Enhances Stress Tolerance. *Molecular Cell*, 79(4), 645–659.e9. <https://doi.org/10.1016/j.molcel.2020.06.037>
- 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., Jung, C., Javadian-Elyaderani, P., Schweyer, S., Schütte, D., Shoukier, M., Karimi-Busheri, F., Weinfeld, M., Rasouli-Nia, A., Hengstler, J. G., Mantilla, A., Soleimanpour-Lichaei, H. R., Engel, W., Robson, C. N., & Nayernia, K. (2010). Pathways of Proliferation and Antiapoptosis Driven in Breast Cancer Stem Cells by Stem Cell Protein Piwil2. *Cancer Research*, 70(11), 4569–4579. <https://doi.org/10.1158/0008-5472.CAN-09-2670>
- Lensch, M. W., & Ince, T. A. (2007). The terminology of teratocarcinomas and teratomas. *Nature Biotechnology*, 25(11), Article 11. <https://doi.org/10.1038/nbt1107-1211a>
- Lethé, B., Lucas, S., Michaux, L., De Smet, C., Godelaine, D., Serrano, A., De Plaen, E., & Boon, T. (1998). LAGE-1, a new gene with tumor specificity. *International Journal of Cancer*, 76(6), 903–908. [https://doi.org/10.1002/\(SICI\)1097-0215\(19980610\)76:6<903::AID-IJC22>3.0.CO;2-1](https://doi.org/10.1002/(SICI)1097-0215(19980610)76:6<903::AID-IJC22>3.0.CO;2-1)
- Li, B., Zhu, X., Sun, L., Yuan, L., Zhang, J., Li, H., & Ye, Z. (2014). Induction of a specific CD8<sup>+</sup> T-cell response to cancer/testis antigens by demethylating pre-treatment against osteosarcoma. *Oncotarget*, 5(21), 10791–10802.

- Li, F., Zhao, F., Li, M., Pan, M., Shi, F., Xu, H., Zheng, D., Wang, L., & Dou, J. (2020). Decreasing New York esophageal squamous cell carcinoma 1 expression inhibits multiple myeloma growth and osteolytic lesions. *Journal of Cellular Physiology*, 235(3), 2183–2194. <https://doi.org/10.1002/jcp.29128>
- Li, R., Guo, M., & Song, L. (2019). PAS Domain Containing Repressor 1 (PASD1) Promotes Glioma Cell Proliferation Through Inhibiting Apoptosis In Vitro. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research*, 25, 6955–6964. <https://doi.org/10.12659/MSM.916308>
- Li, S., Meng, L., Zhu, C., Wu, L., Bai, X., Wei, J., Lu, Y., Zhou, J., & Ma, D. (2010). The universal overexpression of a cancer testis antigen hiwi is associated with cancer angiogenesis. *Oncology Reports*, 23(4), 1063–1068. [https://doi.org/10.3892/or\\_00000733](https://doi.org/10.3892/or_00000733)
- Li, W., Hong, R., Lai, L.-T., Dong, Q., Ni, P., Chelliah, R., Huq, M., Ismail, S. N. B., Chandola, U., Ang, Z., Lin, B., Chen, X., Chen, L., & Zhang, L.-F. (2018). Genome-Wide RNAi Screen Identify Melanoma-Associated Antigen Mageb3 Involved in X Chromosome Inactivation. *Journal of Molecular Biology*, 430(17), 2734–2746. <https://doi.org/10.1016/j.jmb.2018.05.031>
- Lifantseva, N., Koltsova, A., Krylova, T., Yakovleva, T., Poljanskaya, G., & Gordeeva, O. (2011). Expression Patterns of Cancer-Testis Antigens in Human Embryonic Stem Cells and Their Cell Derivatives Indicate Lineage Tracks. *Stem Cells International*, 2011, e795239. <https://doi.org/10.4061/2011/795239>
- Link, P. A., Gangisetty, O., James, S. R., Woloszynska-Read, A., Tachibana, M., Shinkai, Y., & Karpf, A. R. (2009). Distinct Roles for Histone Methyltransferases G9a and GLP in Cancer Germ-Line Antigen Gene Regulation in Human Cancer Cells and Murine Embryonic Stem Cells. *Molecular Cancer Research*, 7(6), 851–862. <https://doi.org/10.1158/1541-7786.MCR-08-0497>
- Liu, K., Wang, Y., Zhu, Q., Li, P., Chen, J., Tang, Z., Shen, Y., Cheng, X., Lu, L.-Y., & Liu, Y. (2020). Aberrantly expressed HORMAD1 disrupts nuclear localization of MCM8–MCM9 complex and compromises DNA mismatch repair in cancer cells. *Cell Death & Disease*, 11(7), Article 7. <https://doi.org/10.1038/s41419-020-2736-1>
- López-Sánchez, N., González-Fernández, Z., Niinobe, M., Yoshikawa, K., & Frade, J. M. (2007). Single mage gene in the chicken genome encodes CMage, a protein with functional similarities to mammalian type II Mage proteins. *Physiological Genomics*, 30(2), 156–171. <https://doi.org/10.1152/physiolgenomics.00249.2006>
- Lőrincz, Á. M., Timár, C. I., Marosvári, K. A., Veres, D. S., Otrokocsi, L., Kittel, Á., & Ligeti, E. (2014). Effect of storage on physical and functional properties of extracellular vesicles derived from neutrophilic granulocytes. *Journal of Extracellular Vesicles*, 3(1), 25465. <https://doi.org/10.3402/jev.v3.25465>
- Loriot, A., Sterpin, C., Backer, O. D., & Smet, C. D. (2008). Mouse embryonic stem cells induce targeted DNA demethylation within human MAGE-A1 transgenes. *Epigenetics*, 3(1), 38–42. <https://doi.org/10.4161/epi.3.1.5411>
- Loukinov, D. I., Pugacheva, E., Vatolin, S., Pack, S. D., Moon, H., Chernukhin, I., Mannan, P., Larsson, E., Kanduri, C., Vostrov, A. A., Cui, H., Niemitz, E. L., Rasko, J. E. J., Docquier, F. M., Kistler, M., Breen, J. J., Zhuang, Z., Quitschke, W. W., Renkawitz, R., ... Lobanekov, V. V. (2002). BORIS, a novel male germ-line-specific protein associated with epigenetic reprogramming events, shares the same 11-zinc-finger domain with CTCF, the insulator protein involved in reading imprinting marks in the soma. *Proceedings of the National Academy of Sciences*, 99(10), 6806–6811. <https://doi.org/10.1073/pnas.092123699>

- Low, J., Dowless, M., Shiyanova, T., Rowlinson, S., Ricci-Vitiani, L., de Maria, R., Pallini, R., & Stancato, L. (2010). Knockdown of Cancer Testis Antigens Modulates Neural Stem Cell Marker Expression in Glioblastoma Tumor Stem Cells. *Journal of Biomolecular Screening*, *15*(7), 830–839. <https://doi.org/10.1177/1087057110374983>
- Lu, Y.-C., Parker, L. L., Lu, T., Zheng, Z., Toomey, M. A., White, D. E., Yao, X., Li, Y. F., Robbins, P. F., Feldman, S. A., van der Bruggen, P., Klebanoff, C. A., Goff, S. L., Sherry, R. M., Kammula, U. S., Yang, J. C., & Rosenberg, S. A. (2017). Treatment of Patients With Metastatic Cancer Using a Major Histocompatibility Complex Class II–Restricted T-Cell Receptor Targeting the Cancer Germline Antigen MAGE-A3. *Journal of Clinical Oncology*, *35*(29), 3322–3329. <https://doi.org/10.1200/JCO.2017.74.5463>
- 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 HumanMAGEB-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>
- Madissoon, E., Töhönen, V., Vesterlund, L., Katayama, S., Unneberg, P., Inzunza, J., Hovatta, O., & Kere, J. (2014). Differences in Gene Expression between Mouse and Human for Dynamically Regulated Genes in Early Embryo. *PLOS ONE*, *9*(8), e102949. <https://doi.org/10.1371/journal.pone.0102949>
- Maley, F., & Guarino, D. U. (1977). Differential binding of sodium dodecyl sulfate to amino acids as evidenced by elution from Sephadex. *Biochemical and Biophysical Research Communications*, *77*(4), 1425–1430. [https://doi.org/10.1016/S0006-291X\(77\)80138-1](https://doi.org/10.1016/S0006-291X(77)80138-1)
- Mallat, Z., Hugel, B., Ohan, J., Lesèche, G., Freyssinet, J.-M., & Tedgui, A. (1999). Shed Membrane Microparticles With Procoagulant Potential in Human Atherosclerotic Plaques. *Circulation*, *99*(3), 348–353. <https://doi.org/10.1161/01.CIR.99.3.348>
- Mao, Y., Fan, W., Hu, H., Zhang, L., Michel, J., Wu, Y., Wang, J., Jia, L., Tang, X., Xu, L., Chen, Y., Zhu, J., Feng, Z., Xu, L., Yin, R., & Tang, Q. (2019). MAGE-A1 in lung adenocarcinoma as a promising target of chimeric antigen receptor T cells. *Journal of Hematology & Oncology*, *12*(1), 106. <https://doi.org/10.1186/s13045-019-0793-7>
- 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>
- Marcar, L., MacLaine, N. J., Hupp, T. R., & Meek, D. W. (2010). MAGE-A Cancer/Testis Antigens Inhibit p53 Function by Blocking Its Interaction with Chromatin. *Cancer Research*, *70*(24), 10362–10370. <https://doi.org/10.1158/0008-5472.CAN-10-1341>
- Marchand, M., van Baren, N., Weynants, P., Brichard, V., Dréno, B., Tessier, M.-H., Rankin, E., Parmiani, G., Arienti, F., Humblet, Y., Bourlond, A., Vanwijck, R., Liénard, D., Beauduin, M., Dietrich, P.-Y., Russo, V., Kerger, J., Masucci, G., Jäger, E., ... Boon, T. (1999). Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene MAGE-3 and presented by HLA-A1. *International Journal of Cancer*, *80*(2), 219–230. [https://doi.org/10.1002/\(SICI\)1097-0215\(19990118\)80:2<219::AID-IJC10>3.0.CO;2-S](https://doi.org/10.1002/(SICI)1097-0215(19990118)80:2<219::AID-IJC10>3.0.CO;2-S)
- Maroto, R., Zhao, Y., Jamaluddin, M., Popov, V. L., Wang, H., Kalubowilage, M., Zhang, Y., Luisi, J., Sun, H., Culbertson, C. T., Bossmann, S. H., Motamedi, M., & Brasier, A. R. (2017). Effects of storage temperature on airway exosome integrity for diagnostic and functional analyses. *Journal of Extracellular Vesicles*, *6*(1), 1359478. <https://doi.org/10.1080/20013078.2017.1359478>

- Masuda, Y., Sasaki, A., Shibuya, H., Ueno, N., Ikeda, K., & Watanabe, K. (2001). Dlxin-1, a Novel Protein That Binds Dlx5 and Regulates Its Transcriptional Function\*. *Journal of Biological Chemistry*, 276(7), 5331–5338. <https://doi.org/10.1074/jbc.M008590200>
- Maurizio, E., Cravello, L., Brady, L., Spolaore, B., Arnoldo, L., Giacotti, V., Manfioletti, G., & Sgarra, R. (2011). Conformational Role for the C-Terminal Tail of the Intrinsically Disordered High Mobility Group A (HMGA) Chromatin Factors. *Journal of Proteome Research*, 10(7), 3283–3291. <https://doi.org/10.1021/pr200116w>
- McKiernan, J., Donovan, M. J., O'Neill, V., Bentink, S., Noerholm, M., Belzer, S., Skog, J., Kattan, M. W., Partin, A., Andriole, G., Brown, G., Wei, J. T., Thompson, I. M., Jr, & Carroll, P. (2016). A Novel Urine Exosome Gene Expression Assay to Predict High-grade Prostate Cancer at Initial Biopsy. *JAMA Oncology*, 2(7), 882–889. <https://doi.org/10.1001/jamaoncol.2016.0097>
- Meek, D. W., & Marcar, L. (2012). Mini-review: MAGE-A antigens as targets in tumour therapy. *Cancer Letters*, 324, 126–132. <https://doi.org/10.1016/j.canlet.2012.05.011>
- Melo, D. H., Mamede, R. C. M., Neder, L., Silva, W. A., Barros-Filho, M. C., Kowalski, L. P., Pinto, C. A. L., Zago, M. A., Figueiredo, D. L. A., & Jungbluth, A. A. (2017). Expression of cancer/testis antigens MAGE-A, MAGE-C1, GAGE and CTAG1B in benign and malignant thyroid diseases. *Oncology Letters*, 14(6), 6485–6496. <https://doi.org/10.3892/ol.2017.7072>
- Meng, X., Sun, X., Liu, Z., & He, Y. (2021). A novel era of cancer/testis antigen in cancer immunotherapy. *International Immunopharmacology*, 98, 107889. <https://doi.org/10.1016/j.intimp.2021.107889>
- Mesri, M., & Altieri, D. C. (1998). Endothelial Cell Activation by Leukocyte Micro-particles1. *The Journal of Immunology*, 161(8), 4382–4387. <https://doi.org/10.4049/jimmunol.161.8.4382>
- Met, Ö., Jensen, K. M., Chamberlain, C. A., Donia, M., & Svane, I. M. (2019). Principles of adoptive T cell therapy in cancer. *Seminars in Immunopathology*, 41(1), 49–58. <https://doi.org/10.1007/s00281-018-0703-z>
- Meuwissen, R. I., Offenberg, H. H., Dietrich, A. J., Riesewijk, A., van Iersel, M., & Heyting, C. (1992). A coiled-coil related protein specific for synapsed regions of meiotic prophase chromosomes. *The EMBO Journal*, 11(13), 5091–5100. <https://doi.org/10.1002/j.1460-2075.1992.tb05616.x>
- Michael, A. K., Harvey, S. L., Sammons, P. J., Anderson, A. P., Kopalle, H. M., Banham, A. H., & Partch, C. L. (2015). Cancer/Testis Antigen PASD1 Silences the Circadian Clock. *Molecular Cell*, 58(5), 743–754. <https://doi.org/10.1016/j.molcel.2015.03.031>
- Migliorini, D., Dutoit, V., Allard, M., Grandjean Hallez, N., Marinari, E., Widmer, V., Philippin, G., Corlazzoli, F., Gustave, R., Kreutzfeldt, M., Blazek, N., Wasem, J., Hottinger, A., Koka, A., Momjian, S., Lobrinus, A., Merkle, D., Vargas, M.-I., Walker, P. R., ... Dietrich, P.-Y. (2019). Phase I/II trial testing safety and immunogenicity of the multipeptide IMA950/poly-ICLC vaccine in newly diagnosed adult malignant astrocytoma patients. *Neuro-Oncology*, 21(7), 923–933. <https://doi.org/10.1093/neuonc/noz040>
- Mizushima, E., Tsukahara, T., Emori, M., Murata, K., Akamatsu, A., Shibayama, Y., Hamada, S., Watanabe, Y., Kaya, M., Hirohashi, Y., Kanaseki, T., Nakatsugawa, M., Kubo, T., Yamashita, T., Sato, N., & Torigoe, T. (2020). Osteosarcoma-initiating cells show high aerobic glycolysis and attenuation of oxidative phosphorylation mediated by LIN28B. *Cancer Science*, 111(1), 36–46. <https://doi.org/10.1111/cas.14229>
- Möhrmann, L., Huang, H. J., Hong, D. S., Tsimberidou, A. M., Fu, S., Piha-Paul, S. A., Subbiah, V., Karp, D. D., Naing, A., Krug, A., Enderle, D., Priewasser, T.,

- Noerholm, M., Eitan, E., Coticchia, C., Stoll, G., Jordan, L.-M., Eng, C., Kopetz, E. S., ... Janku, F. (2018). Liquid Biopsies Using Plasma Exosomal Nucleic Acids and Plasma Cell-Free DNA Compared with Clinical Outcomes of Patients with Advanced Cancers. *Clinical Cancer Research*, 24(1), 181–188. <https://doi.org/10.1158/1078-0432.CCR-17-2007>
- Monte, M., Simonatto, M., Peche, L. Y., Bublik, D. R., Gobessi, S., Pierotti, M. A., Rodolfo, M., & Schneider, C. (2006). MAGE-A tumor antigens target p53 transactivation function through histone deacetylase recruitment and confer resistance to chemotherapeutic agents. *Proceedings of the National Academy of Sciences*, 103(30), 11160–11165. <https://doi.org/10.1073/pnas.0510834103>
- Morad, G., Carman, C. V., Hagedorn, E. J., Perlin, J. R., Zon, L. I., Mustafaoglu, N., Park, T.-E., Ingber, D. E., Daisy, C. C., & Moses, M. A. (2019). Tumor-Derived Extracellular Vesicles Breach the Intact Blood-Brain Barrier via Transcytosis. *ACS Nano*, 13(12), 13853–13865. <https://doi.org/10.1021/acsnano.9b04397>
- Morgan, R. A., Dudley, M. E., Wunderlich, J. R., Hughes, M. S., Yang, J. C., Sherry, R. M., Royal, R. E., Topalian, S. L., Kammula, U. S., Restifo, N. P., Zheng, Z., Nahvi, A., de Vries, C. R., Rogers-Freezer, L. J., Mavroukakis, S. A., & Rosenberg, S. A. (2006). Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes. *Science*, 314(5796), 126–129. <https://doi.org/10.1126/science.1129003>
- Morse, M. A., Garst, J., Osada, T., Khan, S., Hobeika, A., Clay, T. M., Valente, N., Shreeniwas, R., Sutton, M. A., Delcayre, A., Hsu, D.-H., Le Pecq, J.-B., & Lyerly, H. K. (2005). A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. *Journal of Translational Medicine*, 3(1), 9. <https://doi.org/10.1186/1479-5876-3-9>
- Moss, D. K., Betin, V. M., Malesinski, S. D., & Lane, J. D. (2006). A novel role for microtubules in apoptotic chromatin dynamics and cellular fragmentation. *Journal of Cell Science*, 119(11), 2362–2374. <https://doi.org/10.1242/jcs.02959>
- Moussa, C. E.-H., Wersinger, C., Rusnak, M., Tomita, Y., & Sidhu, A. (2004). Abnormal migration of human wild-type  $\alpha$ -synuclein upon gel electrophoresis. *Neuroscience Letters*, 371(2), 239–243. <https://doi.org/10.1016/j.neulet.2004.09.004>
- Nagao, T., Higashitsuji, H., Nonoguchi, K., Sakurai, T., Dawson, S., Mayer, R., Itoh, K., & Fujita, J. (2003). MAGE-A4 interacts with the liver oncoprotein gankyrin and suppresses its tumorigenic activity. *JOURNAL OF BIOLOGICAL CHEMISTRY*, 278(12), 10668–10674.
- Nanbo, A., Kawanishi, E., Yoshida, R., & Yoshiyama, H. (2013). Exosomes Derived from Epstein-Barr Virus-Infected Cells Are Internalized via Caveola-Dependent Endocytosis and Promote Phenotypic Modulation in Target Cells. *Journal of Virology*, 87(18), 10334–10347. <https://doi.org/10.1128/JVI.01310-13>
- Nelson, P. T., Zhang, P. J., Spagnoli, G. C., Tomaszewski, J. E., Pasha, T. L., Frosina, D., Caballero, O. L., Simpson, A. J. G., Old, L. J., & Jungbluth, A. A. (2007). Cancer/testis (CT) antigens are expressed in fetal ovary. *Cancer Immunity Archive*, 7(1), 1.
- Newman, J. A., Cooper, C. D. O., Roos, A. K., Aitkenhead, H., Oppermann, U. C. T., Cho, H. J., Osman, R., & Gileadi, O. (2016). Structures of Two Melanoma-Associated Antigens Suggest Allosteric Regulation of Effector Binding. *PLOS ONE*, 11(2), e0148762. <https://doi.org/10.1371/journal.pone.0148762>
- Ngolab, J., Trinh, I., Rockenstein, E., Mante, M., Florio, J., Trejo, M., Masliah, D., Adame, A., Masliah, E., & Rissman, R. A. (2017). Brain-derived exosomes from dementia with Lewy bodies propagate  $\alpha$ -synuclein pathology. *Acta Neuropathologica Communications*, 5(1), 46. <https://doi.org/10.1186/s40478-017-0445-5>

- Nishimura, H., Cho, C., Branciforte, D. R., Myles, D. G., & Primakoff, P. (2001). Analysis of Loss of Adhesive Function in Sperm Lacking Cyritestin or Fertilin  $\beta$ . *Developmental Biology*, 233(1), 204–213. <https://doi.org/10.1006/dbio.2001.0166>
- Nishimura, I., Shimizu, S., Sakoda, J., & Yoshikawa, K. (2007). Expression of Drosophila MAGE gene encoding a necdin homologous protein in postembryonic neurogenesis. *Gene Expression Patterns*, 7(3), 244–251. <https://doi.org/10.1016/j.modgep.2006.09.008>
- Noelken, M. E., Wisdom, B. J., & Hudson, B. G. (1981). Estimation of the size of collagenous polypeptides by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Analytical Biochemistry*, 110(1), 131–136. [https://doi.org/10.1016/0003-2697\(81\)90123-8](https://doi.org/10.1016/0003-2697(81)90123-8)
- Old, L. J. (2001). Cancer/testis (CT) antigens—A new link between gametogenesis and cancer. *Cancer Immunity*, 1, 1.
- Pan, B. T., Teng, K., Wu, C., Adam, M., & Johnstone, R. M. (1985). Electron microscopic evidence for externalization of the transferrin receptor in vesicular form in sheep reticulocytes. *The Journal of Cell Biology*, 101(3), 942–948. <https://doi.org/10.1083/jcb.101.3.942>
- Park, C. H., Bergsagel, D. E., & McCulloch, E. A. (1971). Mouse Myeloma Tumor Stem Cells: A Primary Cell Culture Assay 2. *JNCI: Journal of the National Cancer Institute*, 46(2), 411–422. <https://doi.org/10.1093/jnci/46.2.411>
- Patel, B. K., Wang, C., Lorens, B., Levine, A. D., Steinmetz, N. F., & Shukla, S. (2020). Cowpea Mosaic Virus (CPMV)-Based Cancer Testis Antigen NY-ESO-1 Vaccine Elicits an Antigen-Specific Cytotoxic T Cell Response. *ACS Applied Bio Materials*, 3(7), 4179–4187. <https://doi.org/10.1021/acsabm.0c00259>
- Patil, A., Kinoshita, K., & Nakamura, H. (2010). Hub Promiscuity in Protein-Protein Interaction Networks. *International Journal of Molecular Sciences*, 11(4), Article 4. <https://doi.org/10.3390/ijms11041930>
- Pebernard, S., McDonald, W. H., Pavlova, Y., Yates, J. R., & Boddy, M. N. (2004). Nse1, Nse2, and a Novel Subunit of the Smc5-Smc6 Complex, Nse3, Play a Crucial Role in Meiosis. *Molecular Biology of the Cell*, 15(11), 4866–4876. <https://doi.org/10.1091/mbc.e04-05-0436>
- Peikert, T., Specks, U., Farver, C., Erzurum, S. C., & Comhair, S. A. A. (2006). Melanoma Antigen A4 Is Expressed in Non-Small Cell Lung Cancers and Promotes Apoptosis. *Cancer Research*, 66(9), 4693–4700. <https://doi.org/10.1158/0008-5472.CAN-05-3327>
- Petroski, M. D., & Deshaies, R. J. (2005). Function and regulation of cullin-RING ubiquitin ligases. *Nature Reviews Molecular Cell Biology*, 6(1), Article 1. <https://doi.org/10.1038/nrm1547>
- Pi, F., Binzel, D. W., Lee, T. J., Li, Z., Sun, M., Rychahou, P., Li, H., Haque, F., Wang, S., Croce, C. M., Guo, B., Evers, B. M., & Guo, P. (2018). Nanoparticle orientation to control RNA loading and ligand display on extracellular vesicles for cancer regression. *Nature Nanotechnology*, 13(1), Article 1. <https://doi.org/10.1038/s41565-017-0012-z>
- 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>
- 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>

- Pitt-Rivers, R., & Impiombato, F. S. A. (1968). The binding of sodium dodecyl sulphate to various proteins. *Biochemical Journal*, *109*(5), 825–830. <https://doi.org/10.1042/bj1090825>
- Pivot-Pajot, C., Caron, C., Govin, J., Vion, A., Rousseaux, S., & Khochbin, S. (2003). Acetylation-Dependent Chromatin Reorganization by BRDT, a Testis-Specific Bromodomain-Containing Protein. *Molecular and Cellular Biology*, *23*(15), 5354–5365. <https://doi.org/10.1128/MCB.23.15.5354-5365.2003>
- Plaen, E. D., Traversari, C., Gaforio, J. J., Szikora, J.-P., Smet, C. D., Brasseur, F., Bruggen, P. van der, Lethé, B., Lurquin, C., Chomez, P., Backer, O. D., 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>
- Pöld, M., Pöld, A., Ma, H. J., Sjak-Shie, N. N., Vescio, R. A., & Berenson, J. R. (2000). Cloning of the first invertebrate MAGE paralogue: An epitope that activates T-cells in humans is highly conserved in evolution. *Developmental & Comparative Immunology*, *24*(8), 719–731. [https://doi.org/10.1016/S0145-305X\(00\)00027-6](https://doi.org/10.1016/S0145-305X(00)00027-6)
- Poon, I. K. H., Chiu, Y.-H., Armstrong, A. J., Kinchen, J. M., Juncadella, I. J., Bayliss, D. A., & Ravichandran, K. S. (2014). Unexpected link between an antibiotic, pannexin channels and apoptosis. *Nature*, *507*(7492), Article 7492. <https://doi.org/10.1038/nature13147>
- Pujol, J.-L., Vansteenkiste, J. F., Pas, T. M. D., Atanackovic, D., Reck, M., Thomeer, M., Douillard, J.-Y., Fasola, G., Potter, V., Taylor, P., Bosquée, L., Scheubel, R., Jarnjak, S., Debois, M., de Sousa Alves, P., Louahed, J., Brichard, V. G., & Lehmann, F. F. (2015). Safety and Immunogenicity of MAGE-A3 Cancer Immunotherapeutic with or without Adjuvant Chemotherapy in Patients with Resected Stage IB to III MAGE-A3-Positive Non-Small-Cell Lung Cancer. *Journal of Thoracic Oncology*, *10*(10), 1458–1467. <https://doi.org/10.1097/JTO.0000000000000653>
- Qin, Y., Wang, L., Gao, Z., Chen, G., & Zhang, C. (2016). Bone marrow stromal/stem cell-derived extracellular vesicles regulate osteoblast activity and differentiation in vitro and promote bone regeneration in vivo. *Scientific Reports*, *6*(1), Article 1. <https://doi.org/10.1038/srep21961>
- Rajagopalan, K., Mooney, S. M., Parekh, N., Getzenberg, R. H., & Kulkarni, P. (2011). A majority of the cancer/testis antigens are intrinsically disordered proteins. *Journal of Cellular Biochemistry*, *112*(11), 3256–3267. <https://doi.org/10.1002/jcb.23252>
- Reik, W., Dean, W., & Walter, J. (2001). Epigenetic Reprogramming in Mammalian Development. *Science*, *293*(5532), 1089–1093. <https://doi.org/10.1126/science.1063443>
- 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>
- Renaud, S., Pugacheva, E. M., Delgado, M. D., Braunschweig, R., Abdullaev, Z., Loukinov, D., Benhattar, J., & Lobanenkova, V. (2007). Expression of the CTCF-paralogous cancer-testis gene, brother of the regulator of imprinted sites (BORIS), is regulated by three alternative promoters modulated by CpG methylation and by CTCF and p53 transcription factors. *Nucleic Acids Research*, *35*(21), 7372–7388. <https://doi.org/10.1093/nar/gkm896>
- Rimoldi, D., Salvi, S., Reed, D., Coulie, P., Jongeneel, V. C., De Plaen, E., Brasseur, F., Rodriguez, A.-M., Boon, T., & Cerottini, J.-C. (1999). cDNA and protein characterization of human MAGE-10. *International Journal of Cancer*, *82*(6), 901. edb.

- Robbins, P. F., Morgan, R. A., Feldman, S. A., Yang, J. C., Sherry, R. M., Dudley, M. E., Wunderlich, J. R., Nahvi, A. V., Helman, L. J., Mackall, C. L., Kammula, U. S., Hughes, M. S., Restifo, N. P., Raffeld, M., Lee, C.-C. R., Levy, C. L., Li, Y. F., El-Gamil, M., Schwarz, S. L., ... Rosenberg, S. A. (2011). Tumor Regression in Patients With Metastatic Synovial Cell Sarcoma and Melanoma Using Genetically Engineered Lymphocytes Reactive With NY-ESO-1. *Journal of Clinical Oncology*, 29(7), 917–924. <https://doi.org/10.1200/JCO.2010.32.2537>
- Rogers, E. M., Allred, S. C., & Peifer, M. (2021). Abelson kinase's intrinsically disordered region plays essential roles in protein function and protein stability. *Cell Communication and Signaling*, 19(1), 27. <https://doi.org/10.1186/s12964-020-00703-w>
- 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>
- Ross, M. T., Grafham, D. V., Coffey, A. J., Scherer, S., McLay, K., Muzny, D., Platzer, M., Howell, G. R., Burrows, C., Bird, C. P., Frankish, A., Lovell, F. L., Howe, K. L., Ashurst, J. L., Fulton, R. S., Sudbrak, R., Wen, G., Jones, M. C., Hurles, M. E., ... Bentley, D. R. (2005). The DNA sequence of the human X chromosome. *Nature*, 434(7031), 325. <https://doi.org/10.1038/nature03440>
- Roth, G. A., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., Abbatistabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R. S., Abebe, H. T., Abebe, M., Abebe, Z., Abejie, A. N., Abera, S. F., Abil, O. Z., Abraha, H. N., ... Murray, C. J. L. (2018). Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: A systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*, 392(10159), 1736–1788. [https://doi.org/10.1016/S0140-6736\(18\)32203-7](https://doi.org/10.1016/S0140-6736(18)32203-7)
- Rotin, D., & Kumar, S. (2009). Physiological functions of the HECT family of ubiquitin ligases. *Nature Reviews Molecular Cell Biology*, 10(6), Article 6. <https://doi.org/10.1038/nrm2690>
- Sabbath, K. D., Ball, E. D., Larcom, P., Davis, R. B., & Griffin, J. D. (1985). Heterogeneity of clonogenic cells in acute myeloblastic leukemia. *The Journal of Clinical Investigation*, 75(2), 746–753. <https://doi.org/10.1172/JCI11756>
- Sahin, U., Derhovanessian, E., Miller, M., Kloke, B.-P., Simon, P., Löwer, M., Bukur, V., Tadmor, A. D., Luxemburger, U., Schrörs, B., Omokoko, T., Vormehr, M., Albrecht, C., Paruzynski, A., Kuhn, A. N., Buck, J., Heesch, S., Schreeb, K. H., Müller, F., ... Türeci, Ö. (2017). Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature*, 547(7662), Article 7662. <https://doi.org/10.1038/nature23003>
- Sahin, U., Oehm, P., Derhovanessian, E., Jabulowsky, R. A., Vormehr, M., Gold, M., Maurus, D., Schwarck-Kokarakis, D., Kuhn, A. N., Omokoko, T., Kranz, L. M., Diken, M., Kreiter, S., Haas, H., Attig, S., Rae, R., Cuk, K., Kemmer-Brück, A., Breitkreuz, A., ... Türeci, Ö. (2020). An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. *Nature*, 585(7823), Article 7823. <https://doi.org/10.1038/s41586-020-2537-9>
- Sahin, U., Türeci, Ö., Chen, Y. T., Seitz, G., Villena-Heinsen, C., Old, L. J., & Pfreundschuh, M. (1998). Expression of multiple cancer/testis (CT) antigens in breast cancer and melanoma: Basis for polyvalent CT vaccine strategies. *International Journal of Cancer*, 78(3), 387–389. [https://doi.org/10.1002/\(SICI\)1097-0215\(19981029\)78:3<387::AID-IJC22>3.0.CO;2-2](https://doi.org/10.1002/(SICI)1097-0215(19981029)78:3<387::AID-IJC22>3.0.CO;2-2)

- Sahin, U., Türeci, O., Schmitt, H., Cochlovius, B., Johannes, T., Schmits, R., Stenner, F., Luo, G., Schobert, I., & Pfreundschuh, M. (1995). Human neoplasms elicit multiple specific immune responses in the autologous host. *Proceedings of the National Academy of Sciences*, *92*(25), 11810–11813. <https://doi.org/10.1073/pnas.92.25.11810>
- Sakurai, T., Itoh, K., Higashitsuji, H., Nagao, T., Nonoguchi, K., Chiba, T., & Fujita, J. (2004). A Cleaved Form of MAGE-A4 Binds to Miz-1 and Induces Apoptosis in Human Cells. *Journal of Biological Chemistry*, *279*(15), 15505–15514. <https://doi.org/10.1074/jbc.M310437200>
- Sang, B. C., & Barbosa, M. S. (1992). Single amino acid substitutions in ‘low-risk’ human papillomavirus (HPV) type 6 E7 protein enhance features characteristic of the ‘high-risk’ HPV E7 oncoproteins. *Proceedings of the National Academy of Sciences*, *89*(17), 8063–8067. <https://doi.org/10.1073/pnas.89.17.8063>
- Saphire, A. C. S., Bark, S. J., & Gerace, L. (1998). All Four Homochiral Enantiomers of a Nuclear Localization Sequence Derived from c-Myc Serve as Functional Import Signals \*. *Journal of Biological Chemistry*, *273*(45), 29764–29769. <https://doi.org/10.1074/jbc.273.45.29764>
- Sartorius, R., Pisu, P., D’Apice, L., Pizzella, L., Romano, C., Cortese, G., Giorgini, A., Santoni, A., Velotti, F., & De Berardinis, P. (2008). The Use of Filamentous Bacteriophage fd to Deliver MAGE-A10 or MAGE-A3 HLA-A2-Restricted Peptides and to Induce Strong Antitumor CTL Responses<sup>1</sup>. *The Journal of Immunology*, *180*(6), 3719–3728. <https://doi.org/10.4049/jimmunol.180.6.3719>
- Sasahira, T., Kurihara, M., Nakashima, C., Kirita, T., & Kuniyasu, H. (2016). LEM domain containing 1 promotes oral squamous cell carcinoma invasion and endothelial transmigration. *British Journal of Cancer*, *115*(1), Article 1. <https://doi.org/10.1038/bjc.2016.167>
- Scanlan, M. J., Altorki, N. K., Gure, A. O., Williamson, B., Jungbluth, A., Chen, Y.-T., & Old, L. J. (2000). Expression of cancer-testis antigens in lung cancer: Definition of bromodomain testis-specific gene (BRDT) as a new CT gene, CT9. *Cancer Letters*, *150*(2), 155–164. [https://doi.org/10.1016/S0304-3835\(99\)00385-7](https://doi.org/10.1016/S0304-3835(99)00385-7)
- Scanlan, M. J., Gordon, C. M., Williamson, B., Lee, S.-Y., Chen, Y.-T., Stockert, E., Jungbluth, A., Ritter, G., Jäger, D., Jäger, E., Knuth, A., & Old, L. J. (2002). Identification of cancer/testis genes by database mining and mRNA expression analysis. *International Journal of Cancer*, *98*(4), 485–492. <https://doi.org/10.1002/ijc.10276>
- 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>
- Scanlan, M. J., Simpson, A. J., & Old, L. J. (2004). The cancer/testis genes: Review, standardization, and commentary. *Cancer Immunity*, *4*, 1–1.
- Scheller, C., Krebs, F., Wiesner, R., Wätzig, H., & Oltmann-Norden, I. (2021). A comparative study of CE-SDS, SDS-PAGE, and Simple Western—Precision, repeatability, and apparent molecular mass shifts by glycosylation. *ELECTROPHORESIS*, *42*(14–15), 1521–1531. <https://doi.org/10.1002/elps.202100068>
- Schultz-Thater, E., Iezzi, G., Le Magnen, C., Zajac, P., Spagnoli, G. C., Piscuoglio, S., Carafa, V., Terracciano, L., & Tornillo, L. (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>

- Sebastian, M., Schröder, A., Scheel, B., Hong, H. S., Muth, A., von Boehmer, L., Zippe-  
lius, A., Mayer, F., Reck, M., Atanackovic, D., Thomas, M., Schneller, F., Stöhl-  
macher, J., Bernhard, H., Gröschel, A., Lander, T., Probst, J., Strack, T., Wiegand, V.,  
... Koch, S. D. (2019). A phase I/IIa study of the mRNA-based cancer immunotherapy  
CV9201 in patients with stage IIIB/IV non-small cell lung cancer. *Cancer Immuno-  
logy, Immunotherapy*, 68(5), 799–812. <https://doi.org/10.1007/s00262-019-02315-x>
- Segura, E., Nicco, C., Lombard, B., Véron, P., Raposo, G., Batteux, F., Amigorena, S.,  
& Théry, C. (2005). ICAM-1 on exosomes from mature dendritic cells is critical for  
efficient naive T-cell priming. *Blood*, 106(1), 216–223. <https://doi.org/10.1182/blood-2005-01-0220>
- Sehnal, D., Bittrich, S., Deshpande, M., Svobodová, R., Berka, K., Bazgier, V.,  
Velankar, S., Burley, S. K., Koča, J., & Rose, A. S. (2021). Mol\* Viewer: Modern  
web app for 3D visualization and analysis of large biomolecular structures. *Nucleic  
Acids Research*, 49(W1), W431–W437. <https://doi.org/10.1093/nar/gkab314>
- Serrano, A., García, A., Abril, E., Garrido, F., & Ruiz-Cabello, F. (1996). Methylated  
CpG points identified within MAGE-1 promoter are involved in gene repression.  
*International Journal of Cancer*, 68(4), 464–470. [https://doi.org/10.1002/\(SICI\)1097-0215\(19961115\)68:4<464::AID-IJC11>3.0.CO;2-5](https://doi.org/10.1002/(SICI)1097-0215(19961115)68:4<464::AID-IJC11>3.0.CO;2-5)
- Shang, B., Gao, A., Pan, Y., Zhang, G., Tu, J., Zhou, Y., Yang, P., Cao, Z., Wei, Q.,  
Ding, Y., Zhang, J., Zhao, Y., & Zhou, Q. (2014). CT45A1 acts as a new proto-onco-  
gene to trigger tumorigenesis and cancer metastasis. *Cell Death & Disease*, 5(6),  
Article 6. <https://doi.org/10.1038/cddis.2014.244>
- Shang, Y., Jiang, Y.-L., Ye, L.-J., Chen, L.-N., & Ke, Y. (2021). Resveratrol acts via  
melanoma-associated antigen A12 (MAGEA12)/protein kinase B (Akt) signaling to  
inhibit the proliferation of oral squamous cell carcinoma cells. *Bioengineered*, 12(1),  
2253–2262. <https://doi.org/10.1080/21655979.2021.1934242>
- Sharma, A., Bode, B., Wenger, R. H., Lehmann, K., Sartori, A. A., Moch, H., Knuth, A.,  
Boehmer, L. von, & Broek, M. van den. (2011).  $\gamma$ -Radiation Promotes Immunological  
Recognition of Cancer Cells through Increased Expression of Cancer-Testis Antigens  
In Vitro and In Vivo. *PLOS ONE*, 6(11), e28217. <https://doi.org/10.1371/journal.pone.0028217>
- Shen, Z., Feng, X., Fang, Y., Li, Y., Li, Z., Zhan, Y., Lin, M., Li, G., Ding, Y., & Deng, H.  
(2019). POTE drives colorectal cancer development via regulating SPHK1/p65  
signaling. *Cell Death & Disease*, 10(11), Article 11. <https://doi.org/10.1038/s41419-019-2046-7>
- Shida, A., Fukuyama, T., Futawatari, N., Ohmiya, H., Ichiki, Y., Yamashita, T., Nishi, Y.,  
Kobayashi, N., Yamazaki, H., Watanabe, M., & Takahashi, Y. (2020). Cancer/testis  
antigen, Kita-Kyushu lung cancer antigen-1 and ABCD stratification for diagnosing  
gastric cancers. *World Journal of Gastroenterology*, 26(4), 424–432. <https://doi.org/10.3748/wjg.v26.i4.424>
- Sigalotti, L., Coral, S., Altomonte, M., Natali, L., Gaudino, G., Cacciotti, P., Libener, R.,  
Colizzi, F., Vianale, G., Martini, F., Tognon, M., Jungbluth, A., Cebon, J., Maras-  
kovsky, E., Mutti, L., & Maio, M. (2002). Cancer testis antigens expression in  
mesothelioma: Role of DNA methylation and bioimmunotherapeutic implications.  
*British Journal of Cancer*, 86(6), Article 6. <https://doi.org/10.1038/sj.bjc.6600174>
- Sigalotti, L., Coral, S., Nardi, G., Spessotto, A., Cortini, E., Cattarossi, I., Colizzi, F.,  
Altomonte, M., & Maio, M. (2002). Promoter Methylation Controls the Expression  
of MAGE2, 3 and 4 Genes in Human Cutaneous Melanoma. *Journal of Immuno-  
therapy*, 25(1), 16.

- Sigalotti, L., Covre, A., Zabierowski, S., Himes, B., Colizzi, F., Natali, P. G., Herlyn, M., & Maio, M. (2008). Cancer testis antigens in human melanoma stem cells: Expression, distribution, and methylation status. *Journal of Cellular Physiology*, *215*(2), 287–291. <https://doi.org/10.1002/jcp.21380>
- Sigalotti, L., Fratta, E., Coral, S., Tanzarella, S., Danielli, R., Colizzi, F., Fonsatti, E., Traversari, C., Altomonte, M., & Maio, M. (2004). Intratumor Heterogeneity of Cancer/Testis Antigens Expression in Human Cutaneous Melanoma Is Methylation-Regulated and Functionally Reverted by 5-Aza-2'-deoxycytidine. *Cancer Research*, *64*(24), 9167–9171. <https://doi.org/10.1158/0008-5472.CAN-04-1442>
- 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>
- 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), Article 12. <https://doi.org/10.1038/ncb1800>
- Slingluff, C. L., Petroni, G. R., Olson, W. C., Smolkin, M. E., Chianese-Bullock, K. A., Mauldin, I. S., Smith, K. T., Deacon, D. H., Varhegyi, N. E., Donnelly, S. B., Reed, C. M., Scott, K., Galeassi, N. V., & Grosh, W. W. (2016). A randomized pilot trial testing the safety and immunologic effects of a MAGE-A3 protein plus AS15 immunostimulant administered into muscle or into dermal/subcutaneous sites. *Cancer Immunology, Immunotherapy*, *65*(1), 25–36. <https://doi.org/10.1007/s00262-015-1770-9>
- Sokolova, V., Ludwig, A.-K., Hornung, S., Rotan, O., Horn, P. A., Epple, M., & Giebel, B. (2011). Characterisation of exosomes derived from human cells by nanoparticle tracking analysis and scanning electron microscopy. *Colloids and Surfaces B: Biointerfaces*, *87*(1), 146–150. <https://doi.org/10.1016/j.colsurfb.2011.05.013>
- Somaiah, N., Block, M. S., Kim, J. W., Shapiro, G. I., Do, K. T., Hwu, P., Eder, J. P., Jones, R. L., Lu, H., ter Meulen, J. H., Bohac, C., Chen, M., Hsu, F. J., Gnjjatic, S., & Pollack, S. M. (2019). First-in-Class, First-in-Human Study Evaluating LV305, a Dendritic-Cell Tropic Lentiviral Vector, in Sarcoma and Other Solid Tumors Expressing NY-ESO-1. *Clinical Cancer Research*, *25*(19), 5808–5817. <https://doi.org/10.1158/1078-0432.CCR-19-1025>
- Southam, C. M., & Brunschwig, A. (1961). Quantitative studies of autotransplantation of human cancer. Preliminary report. *Cancer*, *14*(5), 971–978. [https://doi.org/10.1002/1097-0142\(196109/10\)14:5<971::AID-CNCR2820140510>3.0.CO;2-O](https://doi.org/10.1002/1097-0142(196109/10)14:5<971::AID-CNCR2820140510>3.0.CO;2-O)
- Stevenson, B. J., Iseli, C., Panji, S., Zahn-Zabal, M., Hide, W., Old, L. J., Simpson, A. J., & Jongeneel, C. V. (2007). Rapid evolution of cancer/testis genes on the X chromosome. *BMC Genomics*, *8*(1), 129. <https://doi.org/10.1186/1471-2164-8-129>
- Stockert, E., Jäger, E., Chen, Y.-T., Scanlan, M. J., Gout, I., Karbach, J., Arand, M., Knuth, A., & Old, L. J. (1998). A Survey of the Humoral Immune Response of Cancer Patients to a Panel of Human Tumor Antigens. *The Journal of Experimental Medicine*, *187*(8), 1349–1354.
- Streilein, J. W. (1995). Unraveling Immune Privilege. *Science*, *270*(5239), 1158–1158. <https://doi.org/10.1126/science.270.5239.1158>
- Su, S., Chen, X., Geng, J., Minges, J. T., Grossman, G., & Wilson, E. M. (2017). Melanoma antigen-A11 regulates substrate-specificity of Skp2-mediated protein degradation. *Molecular and Cellular Endocrinology*, *439*, 1–9. <https://doi.org/10.1016/j.mce.2016.10.006>

- Sun, F., Chan, E., Wu, Z., Yang, X., Marquez, V. E., & Yu, Q. (2009). Combinatorial pharmacologic approaches target EZH2-mediated gene repression in breast cancer cells. *Molecular Cancer Therapeutics*, 8(12), 3191–3202. <https://doi.org/10.1158/1535-7163.MCT-09-0479>
- Suzuki, I., Yoshida, S., Tabu, K., Kusunoki, S., Matsumura, Y., Izumi, H., Asanoma, K., Yagi, H., Onoyama, I., Sonoda, K., Kohno, K., Taga, T., Itakura, A., Takeda, S., & Kato, K. (2021). YBX2 and cancer testis antigen 45 contribute to stemness, chemoresistance and a high degree of malignancy in human endometrial cancer. *Scientific Reports*, 11(1), Article 1. <https://doi.org/10.1038/s41598-021-83200-5>
- Tachibana, M., Sugimoto, K., Nozaki, M., Ueda, J., Ohta, T., Ohki, M., Fukuda, M., Takeda, N., Niida, H., Kato, H., & Shinkai, Y. (2002). G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes & Development*, 16(14), 1779–1791. <https://doi.org/10.1101/gad.989402>
- Tajima, K., Obata, Y., Tamaki, H., Yoshida, M., Chen, Y.-T., Scanlan, M. J., Old, L. J., Kuwano, H., Takahashi, T., Takahashi, T., & Mitsudomi, T. (2003). Expression of cancer/testis (CT) antigens in lung cancer. *Lung Cancer*, 42(1), 23–33. [https://doi.org/10.1016/S0169-5002\(03\)00244-7](https://doi.org/10.1016/S0169-5002(03)00244-7)
- Takahashi, A., Okada, R., Nagao, K., Kawamata, Y., Hanyu, A., Yoshimoto, S., Takasugi, M., Watanabe, S., Kanemaki, M. T., Obuse, C., & Hara, E. (2017). Exosomes maintain cellular homeostasis by excreting harmful DNA from cells. *Nature Communications*, 8(1), Article 1. <https://doi.org/10.1038/ncomms15287>
- Tan, K., Shaw, A. L., Madsen, B., Jensen, K., Taylor-Papadimitriou, J., & Freemont, P. S. (2003). Human PLU-1 Has Transcriptional Repression Properties and Interacts with the Developmental Transcription Factors BF-1 and PAX9 \*. *Journal of Biological Chemistry*, 278(23), 20507–20513. <https://doi.org/10.1074/jbc.M301994200>
- Tarnowski, M., Czerewaty, M., Deskur, A., Safranow, K., Marlicz, W., Urańska, E., Ratajczak, M. Z., & Starzyńska, T. (2016). Expression of Cancer Testis Antigens in Colorectal Cancer: New Prognostic and Therapeutic Implications. *Disease Markers*, 2016, e1987505. <https://doi.org/10.1155/2016/1987505>
- Taylor, E. M., Copsey, A. C., Hudson, J. J. R., Vidot, S., & Lehmann, A. R. (2008). Identification of the Proteins, Including MAGEG1, That Make Up the Human SMC5-6 Protein Complex. *Molecular and Cellular Biology*, 28(4), 1197–1206. <https://doi.org/10.1128/MCB.00767-07>
- 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>
- Tominaga, N., Kosaka, N., Ono, M., Katsuda, T., Yoshioka, Y., Tamura, K., Lötvall, J., Nakagama, H., & Ochiya, T. (2015). Brain metastatic cancer cells release microRNA-181c-containing extracellular vesicles capable of destructing blood–brain barrier. *Nature Communications*, 6(1), Article 1. <https://doi.org/10.1038/ncomms7716>
- Tompa, P., & Csermely, P. (2004). The role of structural disorder in the function of RNA and protein chaperones. *The FASEB Journal*, 18(11), 1169–1175. <https://doi.org/10.1096/fj.04-1584rev>

- Uversky, V. N., & Dunker, A. K. (2010). Understanding protein non-folding. *Biochimica et Biophysica Acta (BBA) – Proteins and Proteomics*, 1804(6), 1231–1264. <https://doi.org/10.1016/j.bbapap.2010.01.017>
- 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), Article 6. <https://doi.org/10.1038/ncb1596>
- 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-A10254–262 in Melanoma. *Cancer Research*, 61(2), 509–512.
- 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), Article 4. <https://doi.org/10.1038/nrm.2017.125>
- van der Lee, R., Lang, B., Kruse, K., Gsponer, J., Sánchez de Groot, N., Huynen, M. A., Matouschek, A., Fuxreiter, M., & Babu, M. M. (2014). Intrinsically Disordered Segments Affect Protein Half-Life in the Cell and during Evolution. *Cell Reports*, 8(6), 1832–1844. <https://doi.org/10.1016/j.celrep.2014.07.055>
- Vatolin, S., Abdullaev, Z., Pack, S. D., Flanagan, P. T., Custer, M., Loukinov, D. I., Pugacheva, E., Hong, J. A., Morse, H., III, Schrupp, D. S., Risinger, J. I., Barrett, J. C., & Lobanenko, V. V. (2005). Conditional Expression of the CTCF-Paralogous Transcriptional Factor BORIS in Normal Cells Results in Demethylation and Derepression of MAGE-A1 and Reactivation of Other Cancer-Testis Genes. *Cancer Research*, 65(17), 7751–7762. <https://doi.org/10.1158/0008-5472.CAN-05-0858>
- Velazquez, E. F., Jungbluth, A. A., Yancovitz, M., Gnjatic, S., Adams, S., O’Neill, D., Zavilevich, K., Albukh, T., Christos, P., Mazumdar, M., Pavlick, A., Polsky, D., Shapiro, R., Berman, R., Spira, J., Busam, K., Osman, I., & Bhardwaj, N. (2007). Expression of the cancer/testis antigen NY-ESO-1 in primary and metastatic malignant melanoma (MM)—Correlation with prognostic factors. *Cancer Immunity*, 7(1), 11. <https://doi.org/10.1158/1424-9634.DCL-11.7.1>
- Vella, L., Sharples, R., Lawson, V., Masters, C., Cappai, R., & Hill, A. (2007). Packaging of prions into exosomes is associated with a novel pathway of PrP processing. *The Journal of Pathology*, 211(5), 582–590. <https://doi.org/10.1002/path.2145>
- Verderio, C., Gabrielli, M., & Giussani, P. (2018). Role of sphingolipids in the biogenesis and biological activity of extracellular vesicles. *Journal of Lipid Research*, 59(8), 1325–1340. <https://doi.org/10.1194/jlr.R083915>
- Walter, S., Weinschenk, T., Stenzl, A., Zdrojowy, R., Pluzanska, A., Szczylik, C., Staehler, M., Brugger, W., Dietrich, P.-Y., Mendrzyk, R., Hilf, N., Schoor, O., Fritsche, J., Mahr, A., Maurer, D., Vass, V., Trautwein, C., Lewandrowski, P., Flohr, C., ... Singh-Jasuja, H. (2012). Multi-peptide immune response to cancer vaccine IMA901 after single-dose cyclophosphamide associates with longer patient survival. *Nature Medicine*, 18(8), Article 8. <https://doi.org/10.1038/nm.2883>
- Wang, B., Wang, Y., Yan, Z., Sun, Y., & Su, C. (2019). Colorectal cancer cell-derived exosomes promote proliferation and decrease apoptosis by activating the ERK pathway. *International Journal of Clinical and Experimental Pathology*, 12(7), 2485–2495.
- Wang, C., Gu, Y., Zhang, K., Xie, K., Zhu, M., Dai, N., Jiang, Y., Guo, X., Liu, M., Dai, J., Wu, L., Jin, G., Ma, H., Jiang, T., Yin, R., Xia, Y., Liu, L., Wang, S., Shen, B., ... Hu, Z. (2016). Systematic identification of genes with a cancer-testis expression

- pattern in 19 cancer types. *Nature Communications*, 7(1), Article 1. <https://doi.org/10.1038/ncomms10499>
- Wang, D., Wang, J., Ding, N., Li, Y., Yang, Y., Fang, X., & Zhao, H. (2016). MAGE-A1 promotes melanoma proliferation and migration through C-JUN activation. *Biochemical and Biophysical Research Communications*, 473(4), 959–965. <https://doi.org/10.1016/j.bbrc.2016.03.161>
- Wang, J.-H., Forterre, A. V., Zhao, J., Frimannsson, D. O., Delcayre, A., Antes, T. J., Efron, B., Jeffrey, S. S., Pegram, M. D., & Matin, A. C. (2018). Anti-HER2 scFv-Directed Extracellular Vesicle-Mediated mRNA-Based Gene Delivery Inhibits Growth of HER2-Positive Human Breast Tumor Xenografts by Prodrug Activation. *Molecular Cancer Therapeutics*, 17(5), 1133–1142. <https://doi.org/10.1158/1535-7163.MCT-17-0827>
- Wang, L., Li, M., Zhan, Y., Ban, X., Zeng, T., Zhu, Y., Yun, J., Guan, X.-Y., & Li, Y. (2018). Down-regulation of POTEg predicts poor prognosis in esophageal squamous cell carcinoma patients. *Molecular Carcinogenesis*, 57(7), 886–895. <https://doi.org/10.1002/mc.22809>
- Wang, S., Liu, Z., Ye, T., Mabrouk, O. S., Maltbie, T., Aasly, J., & West, A. B. (2017). Elevated LRRK2 autophosphorylation in brain-derived and peripheral exosomes in LRRK2 mutation carriers. *Acta Neuropathologica Communications*, 5(1), 86. <https://doi.org/10.1186/s40478-017-0492-y>
- Wang, Z., Zhang, J., Zhang, Y., & Lim, S. H. (2006). SPAN-Xb expression in myeloma cells is dependent on promoter hypomethylation and can be upregulated pharmacologically. *International Journal of Cancer*, 118(6), 1436–1444. <https://doi.org/10.1002/ijc.21499>
- Warburg, O. (1956). On respiratory impairment in cancer cells. *Science*, 124(3215), 269–270.
- Wargo, J. A., Robbins, P. F., Li, Y., Zhao, Y., El-Gamil, M., Caragacianu, D., Zheng, Z., Hong, J. A., Downey, S., Schrupp, D. S., Rosenberg, S. A., & Morgan, R. A. (2009). Recognition of NY-ESO-1+ tumor cells by engineered lymphocytes is enhanced by improved vector design and epigenetic modulation of tumor antigen expression. *Cancer Immunology, Immunotherapy*, 58(3), 383–394. <https://doi.org/10.1007/s00262-008-0562-x>
- Weber, J., Salgaller, M., Samid, D., Johnson, B., Herlyn, M., Lassam, N., Treisman, J., & Rosenberg, S. A. (1994). Expression of the MAGE-1 Tumor Antigen Is Up-Regulated by the Demethylating Agent 5-Aza-2'-Deoxycytidine. *Cancer Research*, 54(7), 1766–1771.
- Weber, K., Pringle, J. R., & Osborn, M. (1972). Measurement of molecular weights by electrophoresis on SDS-acrylamide gel. In *Methods in Enzymology* (Vol. 26, pp. 3–27). Academic Press. [https://doi.org/10.1016/S0076-6879\(72\)26003-7](https://doi.org/10.1016/S0076-6879(72)26003-7)
- Weiser, T. S., Guo, Z. S., Ohnmacht, G. A., Parkhurst, M. L., Tong-On, P., Marincola, F. M., Fischette, M. R., Yu, X., Chen, G. A., Hong, J. A., Stewart, J. H., Nguyen, D. M., Rosenberg, S. A., & Schrupp, D. S. (2001). Sequential 5-Aza-2'-deoxycytidine-Depsipeptide FR901228 Treatment Induces Apoptosis Preferentially in Cancer Cells and Facilitates Their Recognition by Cytolytic T Lymphocytes Specific for NY-ESO-1. *Journal of Immunotherapy*, 24(2), 151.
- Weon, J. L., Yang, S. W., & Potts, P. R. (2018). Cytosolic Iron-Sulfur Assembly Is Evolutionarily Tuned by a Cancer-Amplified Ubiquitin Ligase. *Molecular Cell*, 69(1), 113-125.e6. <https://doi.org/10.1016/j.molcel.2017.11.010>

- Whitehurst, A. W. (2014). Cause and Consequence of Cancer/Testis Antigen Activation in Cancer. *Annual Review of Pharmacology and Toxicology*, 54(1), 251–272. <https://doi.org/10.1146/annurev-pharmtox-011112-140326>
- WHO. (2022). *Cancer*. <https://www.who.int/news-room/fact-sheets/detail/cancer>
- Wischniewski, F., Pantel, K., & Schwarzenbach, H. (2006). Promoter Demethylation and Histone Acetylation Mediate Gene Expression of MAGE-A1, -A2, -A3, and -A12 in Human Cancer Cells. *Molecular Cancer Research*, 4(5), 339–349. <https://doi.org/10.1158/1541-7786.MCR-05-0229>
- 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>
- Wollert, T., & Hurley, J. H. (2010). Molecular mechanism of multivesicular body biogenesis by ESCRT complexes. *Nature*, 464(7290), Article 7290. <https://doi.org/10.1038/nature08849>
- Woloszynska-Read, A., James, S. R., Link, P. A., Yu, J., Odunsi, K., & Karpf, A. R. (2007). DNA methylation-dependent regulation of BORIS/CTCF expression in ovarian cancer. *Cancer Immunity*, 7(1), 21. <https://doi.org/10.1158/1424-9634.DCL-21.7.1>
- Woloszynska-Read, A., Mhawech-Fauceglia, P., Yu, J., Odunsi, K., & Karpf, A. R. (2008). Intertumor and Intratumor NY-ESO-1 Expression Heterogeneity Is Associated with Promoter-Specific and Global DNA Methylation Status in Ovarian Cancer. *Clinical Cancer Research*, 14(11), 3283–3290. <https://doi.org/10.1158/1078-0432.CCR-07-5279>
- Wong, P.-P., Yeoh, C. C., Ahmad, A. S., Chelala, C., Gillett, C., Speirs, V., Jones, J. L., & Hurst, H. C. (2014). Identification of MAGEA antigens as causal players in the development of tamoxifen-resistant breast cancer. *Oncogene*, 33(37), Article 37. <https://doi.org/10.1038/onc.2014.45>
- Xiao, C., Li, M., Huang, Q., & Si-Tu, J. (2019). SPAG9 promotes prostate cancer proliferation and metastasis via MAPK signaling pathway. *American Journal of Translational Research*, 11(8), 5249–5260.
- Xiao, T. Z., Bhatia, N., Urrutia, R., Lomberk, G. A., Simpson, A., & Longley, B. J. (2011). MAGE I Transcription Factors Regulate KAP1 and KRAB Domain Zinc Finger Transcription Factor Mediated Gene Repression. *PLOS ONE*, 6(8), e23747. <https://doi.org/10.1371/journal.pone.0023747>
- Xiao, T. Z., Suh, Y., & Longley, B. J. (2014). MAGE proteins regulate KRAB zinc finger transcription factors and KAP1 E3 ligase activity. *Archives of Biochemistry and Biophysics*, 563, 136–144. <https://doi.org/10.1016/j.abb.2014.07.026>
- Xin, H., Li, Y., Liu, Z., Wang, X., Shang, X., Cui, Y., Zhang, Z. G., & Chopp, M. (2013). MiR-133b Promotes Neural Plasticity and Functional Recovery After Treatment of Stroke with Multipotent Mesenchymal Stromal Cells in Rats Via Transfer of Exosome-Enriched Extracellular Particles. *Stem Cells*, 31(12), 2737–2746. <https://doi.org/10.1002/stem.1409>
- Xue, W., Metheringham, R. L., Brentville, V. A., Gunn, B., Symonds, P., Yagita, H., Ramage, J. M., & Durrant, L. G. (2016). SCIB2, an antibody DNA vaccine encoding NY-ESO-1 epitopes, induces potent antitumor immunity which is further enhanced by checkpoint blockade. *Oncology*, 5(6), e1169353. <https://doi.org/10.1080/2162402X.2016.1169353>
- Yamada, R., Takahashi, A., Torigoe, T., Morita, R., Tamura, Y., Tsukahara, T., Kanaseki, T., Kubo, T., Watarai, K., Kondo, T., Hirohashi, Y., & Sato, N. (2013). Preferential expression of cancer/testis genes in cancer stem-like cells: Proposal of a

- novel sub-category, cancer/testis/stem gene. *Tissue Antigens*, 81(6), 428–434. <https://doi.org/10.1111/tan.12113>
- Yanagi, T., Nagai, K., Shimizu, H., & Matsuzawa, S.-I. (2017). Melanoma antigen A12 regulates cell cycle via tumor suppressor p21 expression. *Oncotarget*, 8(40), 68448–68459. <https://doi.org/10.18632/oncotarget.19497>
- Yang, B., O'Herrin, S. M., Wu, J., Reagan-Shaw, S., Ma, Y., Bhat, K. M. R., Gravekamp, C., Setaluri, V., Peters, N., Hoffmann, F. M., Peng, H., Ivanov, A. V., Simpson, A. J. G., & Longley, B. J. (2007). MAGE-A, mMage-b, and MAGE-C Proteins Form Complexes with KAP1 and Suppress p53-Dependent Apoptosis in MAGE-Positive Cell Lines. *Cancer Research*, 67(20), 9954–9962. <https://doi.org/10.1158/0008-5472.CAN-07-1478>
- Yang, B., Wu, J., Maddodi, N., Ma, Y., Setaluri, V., & Jack Longley, B. (2007). Epigenetic Control of MAGE Gene Expression by the KIT Tyrosine Kinase. *Journal of Investigative Dermatology*, 127(9), 2123–2128. <https://doi.org/10.1038/sj.jid.5700836>
- Yang, P., Meng, M., & Zhou, Q. (2021). Oncogenic cancer/testis antigens are a hallmark of cancer and a sensible target for cancer immunotherapy. *Biochimica et Biophysica Acta (BBA) – Reviews on Cancer*, 1876(1), 188558. <https://doi.org/10.1016/j.bbcan.2021.188558>
- Yang, S. W., Li, L., Connelly, J. P., Porter, S. N., Kodali, K., Gan, H., Park, J. M., Tacer, K. F., Tillman, H., Peng, J., Pruett-Miller, S. M., Li, W., & Potts, P. R. (2020). A Cancer-Specific Ubiquitin Ligase Drives mRNA Alternative Polyadenylation by Ubiquitinating the mRNA 3' End Processing Complex. *Molecular Cell*, 77(6), 1206–1221.e7. <https://doi.org/10.1016/j.molcel.2019.12.022>
- Yang, T. T., Liu, C. G., Gao, S. C., Zhang, Y., & Wang, P. C. (2018). The Serum Exosome Derived MicroRNA–135a, –193b, and –384 Were Potential Alzheimer's Disease Biomarkers. *Biomedical and Environmental Sciences*, 31(2), 87–96. <https://doi.org/10.3967/bes2018.011>
- Yao, J., Caballero, O. L., Yung, W. K. A., Weinstein, J. N., Riggins, G. J., Strausberg, R. L., & Zhao, Q. (2014). Tumor subtype-specific cancer-testis antigens as potential biomarkers and immunotherapeutic targets for cancers. *Cancer Immunology Research*, 2(4), 371–379. <https://doi.org/10.1158/2326-6066.CIR-13-0088>
- Yazarlou, F., Mowla, S. J., Oskooei, V. K., Motevaseli, E., Tooli, L. F., Afsharpad, M., Nekooesh, L., Sanikhani, N. S., Ghafouri-Fard, S., & Modarressi, M. H. (2018). Urine exosome gene expression of cancer-testis antigens for prediction of bladder carcinoma. *Cancer Management and Research*, 10, 5373–5381. <https://doi.org/10.2147/CMAR.S180389>
- Yeon, M., Lee, S., Lee, J.-E., Jung, H. S., Kim, Y., & Jeoung, D. (2019). CAGE-miR-140-5p-Wnt1 Axis Regulates Autophagic Flux, Tumorigenic Potential of Mouse Colon Cancer Cells and Cellular Interactions Mediated by Exosomes. *Frontiers in Oncology*, 9. <https://www.frontiersin.org/articles/10.3389/fonc.2019.01240>
- Yu, X., Deng, L., Wang, D., Li, N., Chen, X., Cheng, X., Yuan, J., Gao, X., Liao, M., Wang, M., & Liao, Y. (2012). Mechanism of TNF- $\alpha$  autocrine effects in hypoxic cardiomyocytes: Initiated by hypoxia inducible factor 1 $\alpha$ , presented by exosomes. *Journal of Molecular and Cellular Cardiology*, 53(6), 848–857. <https://doi.org/10.1016/j.yjmcc.2012.10.002>
- Zeng, G., Li, Y., El-Gamil, M., Sidney, J., Sette, A., Wang, R., Rosenberg, S. A., & Robbins, P. F. (2002). Generation of NY-ESO-1-specific CD4+ and CD8+ T Cells by a Single Peptide with Dual MHC Class I and Class II Specificities: A New Strategy for Vaccine Design. *Cancer Research*, 62(13), 3630–3635.

- Zhang, M., Luo, J., Luo, X., & Liu, L. (2020). SPAG6 silencing induces autophagic cell death in SKM-1 cells via the AMPK/mTOR/ULK1 signaling pathway. *Oncology Letters*, 20(1), 551–560. <https://doi.org/10.3892/ol.2020.11607>
- Zhang, Y., Wang, Z., Zhang, J., & Lim, S. H. (2009). Core promoter sequence of SEMG I spans between the two putative GATA-1 binding domains and is responsive to IL-4 and IL-6 in myeloma cells. *Leukemia Research*, 33(1), 166–169. <https://doi.org/10.1016/j.leukres.2008.05.021>
- Zhao, J., Wang, Y., Liang, Q., Xu, Y., & Sang, J. (2019). MAGEA1 inhibits the expression of BORIS via increased promoter methylation. *Journal of Cell Science*, 132(1), jcs218628. <https://doi.org/10.1242/jcs.218628>
- Zhou, B., Li, T., Liu, Y., & Zhu, N. (2013). Promoting effects on the proliferation and metastasis of ACC tumor cell with XAGE-1b overexpression. *Oncology Reports*, 30(5), 2323–2335. <https://doi.org/10.3892/or.2013.2719>
- Zhou, H., Yuen, P. S. T., Pisitkun, T., Gonzales, P. A., Yasuda, H., Dear, J. W., Gross, P., Knepper, M. A., & Star, R. A. (2006). Collection, storage, preservation, and normalization of human urinary exosomes for biomarker discovery. *Kidney International*, 69(8), 1471–1476. <https://doi.org/10.1038/sj.ki.5000273>
- Zhou, Y., Qiu, J., Wang, Y., Liu, P., Lv, Q., & Du, Z. (2019). Sperm Protein Antigen 17 Expression Correlates With Lymph Node Metastasis and Worse Overall Survival in Patients With Breast Cancer. *Frontiers in Oncology*, 9. <https://www.frontiersin.org/articles/10.3389/fonc.2019.00710>
- Zhu, F., Bo, H., Liu, G., Li, R., Liu, Z., & Fan, L. (2020). SPANXN2 functions a cell migration inhibitor in testicular germ cell tumor cells. *PeerJ*, 8, e9358. <https://doi.org/10.7717/peerj.9358>
- Zhu, X., Asa, S. L., & Ezzat, S. (2008). Fibroblast Growth Factor 2 and Estrogen Control the Balance of Histone 3 Modifications Targeting MAGE-A3 in Pituitary Neoplasia. *Clinical Cancer Research*, 14(7), 1984–1996. <https://doi.org/10.1158/1078-0432.CCR-07-2003>

## ACKNOWLEDGEMENTS

Firstly, I would like to extend my gratitude towards my supervisor, Prof. Reet Kurg. My journey from a second-year bachelor's student up to a PhD candidate has been amazing under your guidance. Thank you for accepting me into your group and teaching me about the academic world. You are an excellent example of a hard-working scientist and I look up to you.

My thanks are also extended to everyone who has worked in our group at the Institute of Technology during my time there: Eve Toomsoo, Margit Mutso, Baiba Brūmele, Olavi Reinsalu, Kristiina Kurg, Fred Väärtnõu, Evgeniia Serova, Magda Karakai, Kadri Õunap, and Lilian Telanne. Thank you for all your help at the lab, meaningful discussions about both our work and the state of the world, as well as all the fun we have had in and out of the lab.

I am also thankful for the students who have worked on their theses in our lab, especially Anjali Gyawali, whose supervisor I had the pleasure to be. You have kept me alert to the smallest details in laboratory work and I owe my meticulousness partly to you.

And last but definitely not least I would like to express my sincerest thanks to all my friends and family. I thank my parents, Katrin and Rudolf Kuldkepp, for supporting me through all my schooling, my sister, Helin Kuldkepp, for cheering me on, my husband, Leemet Samel, for always being there for me, no matter what, my son, Aare Samel, for being such a source of joy and laughter every day, my friends for listening to me and my sorority, korp! Amicitia, for inspiring me to always reach for the stars and excel at everything I do.

## **PUBLICATIONS**

## CURRICULUM VITAE

**Name:** Anneli Samel (née Kuldkepp)  
**Date of birth:** 7<sup>th</sup> of March 1995, Tartu, Estonia  
**Citizenship:** Estonia  
**Contact:** anneli.samel@gmail.com

### Education:

2019–... University of Tartu, PhD studies in Engineering and Technology  
2017–2019 University of Tartu, Gene technology, MSc  
2014–2017 University of Tartu, Gene technology, BSc  
2003–2014 Tallinn English College (graduated with honours)  
2002–2003 Tallinn Secondary School of Science

### Professional employment:

2022–2023 University of Tartu, Institute of Technology, junior research fellow in technology

### Main research areas:

Proteins, cancer-testis antigens, biotechnology

### Supervised theses:

Anjali Gyawali bachelor's thesis, 2022

### List of publications:

- Kuldkepp, A.**, Karakai, M., Toomsoo, E., Reinsalu, O., & Kurg, R. (2019). Cancer-testis antigens MAGEA proteins are incorporated into extracellular vesicles released by cells. *Oncotarget*, 10(38), 3694–3708. <https://doi.org/10.18632/oncotarget.26979>
- Reinsalu, O., **Samel, A.**, Niemeister, E., & Kurg, R. (2021). MAGEA4 Coated Extracellular Vesicles Are Stable and Can Be Assembled In Vitro. *International Journal of Molecular Sciences*, 22(10), 5208. <https://doi.org/10.3390/ijms22105208>
- 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>

## ELULOOKIRJELDUS

**Nimi:** Anneli Samel (sünd. Kuldkepp)  
**Sünniaeg:** 7. märts 1995, Tartu, Eesti  
**Kodakondsus:** Eesti  
**Kontakt:** anneli.samel@gmail.com

### Hariduskäik:

2019–... Tartu Ülikool, tehnika ja tehnoloogia, doktorant  
2017–2019 Tartu Ülikool, geenitehnoloogia, MSc  
2014–2017 Tartu Ülikool, geenitehnoloogia, BSc  
2003–2014 Tallinna Inglise Kolledž (lõpetatud kuldmedaliga)  
2002–2003 Tallinna Reaalkool

### Erialane teenistuskäik:

2022–2023 Tartu Ülikool, Tehnoloogiainstituut, tehnoloogia nooremteadur

### Uurimistöö põhisuunad:

Valgud, vähi-testise antigeenid, biotehnoloogia.

### Juhendatud lõputööd:

Anjali Gyawali bakalaureusetöö, 2022

### Ilmunud publikatsioonid:

- Kuldkepp, A.**, Karakai, M., Toomsoo, E., Reinsalu, O., & Kurg, R. (2019). Cancer-testis antigens MAGEA proteins are incorporated into extracellular vesicles released by cells. *Oncotarget*, 10(38), 3694–3708. <https://doi.org/10.18632/oncotarget.26979>
- Reinsalu, O., **Samel, A.**, Niemeister, E., & Kurg, R. (2021). MAGEA4 Coated Extracellular Vesicles Are Stable and Can Be Assembled In Vitro. *International Journal of Molecular Sciences*, 22(10), 5208. <https://doi.org/10.3390/ijms22105208>
- 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>

## DISSERTATIONES TECHNOLOGIAE UNIVERSITATIS TARTUENSIS

1. **Imre Mäger.** Characterization of cell-penetrating peptides: Assessment of cellular internalization kinetics, mechanisms and bioactivity. Tartu 2011, 132 p.
2. **Taavi Lehto.** Delivery of nucleic acids by cell-penetrating peptides: application in modulation of gene expression. Tartu 2011, 155 p.
3. **Hannes Luidalepp.** Studies on the antibiotic susceptibility of *Escherichia coli*. Tartu 2012, 111 p.
4. **Vahur Zadin.** Modelling the 3D-microbattery. Tartu 2012, 149 p.
5. **Janno Torop.** Carbide-derived carbon-based electromechanical actuators. Tartu 2012, 113 p.
6. **Julia Suhorutšenko.** Cell-penetrating peptides: cytotoxicity, immunogenicity and application for tumor targeting. Tartu 2012, 139 p.
7. **Viktoryia Shyp.** G nucleotide regulation of translational GTPases and the stringent response factor RelA. Tartu 2012, 105 p.
8. **Mardo Kõivomägi.** Studies on the substrate specificity and multisite phosphorylation mechanisms of cyclin-dependent kinase Cdk1 in *Saccharomyces cerevisiae*. Tartu, 2013, 157 p.
9. **Liis Karo-Astover.** Studies on the Semliki Forest virus replicase protein nsP1. Tartu, 2013, 113 p.
10. **Piret Arukuusk.** NickFects—novel cell-penetrating peptides. Design and uptake mechanism. Tartu, 2013, 124 p.
11. **Piret Villo.** Synthesis of acetogenin analogues. Asymmetric transfer hydrogenation coupled with dynamic kinetic resolution of  $\alpha$ -amido- $\beta$ -keto esters. Tartu, 2013, 151 p.
12. **Villu Kasari.** Bacterial toxin-antitoxin systems: transcriptional cross-activation and characterization of a novel *mqsRA* system. Tartu, 2013, 108 p.
13. **Margus Varjak.** Functional analysis of viral and host components of alpha-virus replicase complexes. Tartu, 2013, 151 p.
14. **Liane Viru.** Development and analysis of novel alphavirus-based multi-functional gene therapy and expression systems. Tartu, 2013, 113 p.
15. **Kent Langel.** Cell-penetrating peptide mechanism studies: from peptides to cargo delivery. Tartu, 2014, 115 p.
16. **Rauno Temmer.** Electrochemistry and novel applications of chemically synthesized conductive polymer electrodes. Tartu, 2014, 206 p.
17. **Indrek Must.** Ionic and capacitive electroactive laminates with carbonaceous electrodes as sensors and energy harvesters. Tartu, 2014, 133 p.
18. **Veiko Voolaid.** Aquatic environment: primary reservoir, link, or sink of antibiotic resistance? Tartu, 2014, 79 p.
19. **Kristiina Laanemets.** The role of SLAC1 anion channel and its upstream regulators in stomatal opening and closure of *Arabidopsis thaliana*. Tartu, 2015, 115 p.

20. **Kalle Pärn.** Studies on inducible alphavirus-based antitumour strategy mediated by site-specific delivery with activatable cell-penetrating peptides. Tartu, 2015, 139 p.
21. **Anastasia Selyutina.** When biologist meets chemist: a search for HIV-1 inhibitors. Tartu, 2015, 172 p.
22. **Sirle Saul.** Towards understanding the neurovirulence of Semliki Forest virus. Tartu, 2015, 136 p.
23. **Marit Orav.** Study of the initial amplification of the human papillomavirus genome. Tartu, 2015, 132 p.
24. **Tormi Reinson.** Studies on the Genome Replication of Human Papillomaviruses. Tartu, 2016, 110 p.
25. **Mart Ustav Jr.** Molecular Studies of HPV-18 Genome Segregation and Stable Replication. Tartu, 2016, 152 p.
26. **Margit Mutso.** Different Approaches to Counteracting Hepatitis C Virus and Chikungunya Virus Infections. Tartu, 2016, 184 p.
27. **Jelizaveta Geimanen.** Study of the Papillomavirus Genome Replication and Segregation. Tartu, 2016, 168 p.
28. **Mart Toots.** Novel Means to Target Human Papillomavirus Infection. Tartu, 2016, 173 p.
29. **Kadi-Liis Veiman.** Development of cell-penetrating peptides for gene delivery: from transfection in cell cultures to induction of gene expression *in vivo*. Tartu, 2016, 136 p.
30. **Ly Pärnaste.** How, why, what and where: Mechanisms behind CPP/cargo nanocomplexes. Tartu, 2016, 147 p.
31. **Age Utt.** Role of alphavirus replicase in viral RNA synthesis, virus-induced cytotoxicity and recognition of viral infections in host cells. Tartu, 2016, 183 p.
32. **Veiko Vunder.** Modeling and characterization of back-relaxation of ionic electroactive polymer actuators. Tartu, 2016, 154 p.
33. **Piia Kivipõld.** Studies on the Role of Papillomavirus E2 Proteins in Virus DNA Replication. Tartu, 2016, 118 p.
34. **Liina Jakobson.** The roles of abscisic acid, CO<sub>2</sub>, and the cuticle in the regulation of plant transpiration. Tartu, 2017, 162 p.
35. **Helen Isok-Paas.** Viral-host interactions in the life cycle of human papillomaviruses. Tartu, 2017, 158 p.
36. **Hanna Hõrak.** Identification of key regulators of stomatal CO<sub>2</sub> signalling via O<sub>3</sub>-sensitivity. Tartu, 2017, 260 p.
37. **Jekaterina Jevtuševskaja.** Application of isothermal amplification methods for detection of *Chlamydia trachomatis* directly from biological samples. Tartu, 2017, 96 p.
38. **Ülar Allas.** Ribosome-targeting antibiotics and mechanisms of antibiotic resistance. Tartu, 2017, 152 p.
39. **Anton Paier.** Ribosome Degradation in Living Bacteria. Tartu, 2017, 108 p.
40. **Vallo Varik.** Stringent Response in Bacterial Growth and Survival. Tartu, 2017, 101 p.

41. **Pavel Kudrin.** In search for the inhibitors of *Escherichia coli* stringent response factor RelA. Tartu, 2017, 138 p.
42. **Liisi Henno.** Study of the human papillomavirus genome replication and oligomer generation. Tartu, 2017, 144 p.
43. **Katrin Krõlov.** Nucleic acid amplification from crude clinical samples exemplified by *Chlamydia trachomatis* detection in urine. Tartu, 2018, 118 p.
44. **Eve Sankovski.** Studies on papillomavirus transcription and regulatory protein E2. Tartu, 2018, 113 p.
45. **Morteza Daneshmand.** Realistic 3D Virtual Fitting Room. Tartu, 2018, 233 p.
46. **Fatemeh Noroozi.** Multimodal Emotion Recognition Based Human-Robot Interaction Enhancement. Tartu, 2018, 113 p.
47. **Krista Freimann.** Design of peptide-based vector for nucleic acid delivery in vivo. Tartu, 2018, 103 p.
48. **Rainis Venta.** Studies on signal processing by multisite phosphorylation pathways of the *S. cerevisiae* cyclin-dependent kinase inhibitor Sic1. Tartu, 2018, 155 p.
49. **Inga Põldsalu.** Soft actuators with ink-jet printed electrodes. Tartu, 2018, 85 p.
50. **Kadri Künnapuu.** Modification of the cell-penetrating peptide PepFect14 for targeted tumor gene delivery and reduced toxicity. Tartu, 2018, 114 p.
51. **Toomas Mets.** RNA fragmentation by MazF and MqsR toxins of *Escherichia coli*. Tartu, 2019, 119 p.
52. **Kadri Tõldsepp.** The role of mitogen-activated protein kinases MPK4 and MPK12 in CO<sub>2</sub>-induced stomatal movements. Tartu, 2019, 259 p.
53. **Pirko Jalakas.** Unravelling signalling pathways contributing to stomatal conductance and responsiveness. Tartu, 2019, 120 p.
54. **S. Sunjai Nakshatharan.** Electromechanical modelling and control of ionic electroactive polymer actuators. Tartu, 2019, 165 p.
55. **Eva-Maria Tombak.** Molecular studies of the initial amplification of the oncogenic human papillomavirus and closely related nonhuman primate papillomavirus genomes. Tartu, 2019, 150 p.
56. **Meeri Visnapuu.** Design and physico-chemical characterization of metal-containing nanoparticles for antimicrobial coatings. Tartu, 2019, 138 p.
57. **Jelena Beljantseva.** Small fine-tuners of the bacterial stringent response – a glimpse into the working principles of Small Alarmone Synthetases. Tartu, 2020, 104 p.
58. **Egon Urgard.** Potential therapeutic approaches for modulation of inflammatory response pathways. Tartu, 2020, 120 p.
59. **Sofia Raquel Alves Oliveira.** HPLC analysis of bacterial alarmone nucleotide (p)ppGpp and its toxic analogue ppApp. Tartu, 2020, 122 p.
60. **Mihkel Örd.** Ordering the phosphorylation of cyclin-dependent kinase Cdk1 substrates in the cell cycle. Tartu, 2021, 228 p.
61. **Fred Elhi.** Biocompatible ionic electromechanically active polymer actuator based on biopolymers and non-toxic ionic liquids. Tartu, 2021, 140 p.

62. **Liisi Talas.** Reconstructing paleo-diversity, dynamics and response of eukaryotes to environmental change over the Late-Glacial and Holocene period in lake Lielais Svētiņū using sedaDNA. Tartu, 2021, 118 p.
63. **Livia Matt.** Novel isosorbide-based polymers. Tartu, 2021, 118 p.
64. **Koit Aasumets.** The dynamics of human mitochondrial nucleoids within the mitochondrial network. Tartu, 2021, 104 p.
65. **Faiza Summer.** Development and optimization of flow electrode capacitor technology. Tartu, 2022, 109 p.
66. **Olavi Reinsalu.** Cancer-testis antigen MAGE-A4 is incorporated into extracellular vesicles and is exposed to the surface. Tartu, 2022, 130 p.
67. **Tetiana Brodiazhenko.** RelA-SpoT Homolog enzymes as effectors of Toxin-Antitoxin systems. Tartu, 2022, 132 p.
68. **Georg-Marten Lanno.** Development of novel antibacterial drug delivery systems as wound scaffolds using electrospinning technology. Tartu, 2022, 175 p.
69. **Liubov Cherkashchenko.** New insights into alphaviral nsP2 functions. Tartu, 2023, 171 p.
70. **Kristina Kiisholts.** Peptide-based drug carriers and preclinical nanomedicine applications for endometriosis treatment. Tartu, 2023, 138 p.
71. **Kai Rausalu.** Alphaviral nsP2 protease: From requirements for functionality to inhibition. Tartu, 2023, 175 p.
72. **Laura Sandra Lello.** Unraveling the intricate nature of the alphavirus RNA replicase. Tartu, 2023, 219 p.
73. **Houman Masnavi.** Visibility Aware Navigation. Tartu, 2023, 180 p.
74. **Kadir Aktas.** Cosmic Ray Tomography based Object Reconstruction and Recognition. Tartu, 2023, 104 p.
75. **Egils Avots.** Brain abnormality detection using statistical analysis of individual structural connectivity networks and EEG signals. Tartu, 2023, 223 p.
76. **Sainan Wang.** Structure-guided insights into the functions of CHIKV nsP2. Tartu, 2024, 154 p.