

OULU 2018
D 1470

ACTA UNIVERSITATIS OULUENSIS

Hamid Bur

BIOLOGICAL PROGNOSTIC
AND PREDICTIVE MARKERS
IN HODGKIN LYMPHOMA

UNIVERSITY OF OULU GRADUATE SCHOOL;
UNIVERSITY OF OULU,
FACULTY OF MEDICINE;
MEDICAL RESEARCH CENTER OULU;
OULU UNIVERSITY HOSPITAL



ACTA UNIVERSITATIS OULUENSIS
D Medica 1470

HAMID BUR

**BIOLOGICAL PROGNOSTIC AND
PREDICTIVE MARKERS IN HODGKIN
LYMPHOMA**

Academic dissertation to be presented with the assent of
the Doctoral Training Committee of Health and
Biosciences of the University of Oulu for public defence in
Auditorium 7 of Oulu University Hospital, on 8 June
2018, at 12 noon

UNIVERSITY OF OULU, OULU 2018

Copyright © 2018
Acta Univ. Oul. D 1470, 2018

Supervised by
Docent Peeter Karihtala
Professor Taina Turpeenniemi-Hujanen
Docent Kirsi-Maria Haapasaari

Reviewed by
Professor Veli-Matti Kosma
Docent Sirkku Jyrkkiö

Opponent
Professor Timo Paavonen

ISBN 978-952-62-1944-8 (Paperback)
ISBN 978-952-62-1945-5 (PDF)

ISSN 0355-3221 (Printed)
ISSN 1796-2234 (Online)

Cover Design
Raimo Ahonen

JUVENES PRINT
TAMPERE 2018

Bur, Hamid, Biological prognostic and predictive markers in Hodgkin lymphoma.

University of Oulu Graduate School; University of Oulu, Faculty of Medicine; Medical Research Center Oulu; Oulu University Hospital

Acta Univ. Oul. D 1470, 2018

University of Oulu, P.O. Box 8000, FI-90014 University of Oulu, Finland

Abstract

Hodgkin lymphoma (HL) is among a heterogeneous group of lymphomas. Over 80% of all patients can be cured with chemo- and radiotherapy. HL has become a model to study long-term effects of radio- and chemotherapy, because of the excellent prognosis. There are a significant number of patients who suffer or die because of the treatment-related long-term toxicity. The aim of this work was to discover new possible biological factors to predict poor prognosis and offer new aspects to individualize patient treatment in a convenient manner in HL.

The retrospective study involved HL patients uniformly treated in 1997–2015. Immunohistochemistry was used to determine the expression of various biological markers, including oxidative stress markers 8-hydroxydeoxyguanosine (8-OHdG) and nitrotyrosine and the antioxidant enzymes manganese superoxide dismutase (MnSOD) as well as peroxiredoxins (Prx II, Prx III, Prx V, Prx VI) in HL patient samples. Using immunohistochemistry, we also evaluated expression of hypoxia-inducible factors (HIF-1 α , HIF-2 α), prolyl hydroxylase domain enzymes (PHD1, PHD2, PHD3), the epigenetic regulator lysine (K)-specific demethylase 4 (KDM4A, KDM4B, KDM4D) as well as sirtuins (SIRT1, SIRT4, SIRT6), the DNA-repair proteins Human Rap1 interacting factor 1 (Rif1) and O⁶-alkylguanine DNA alkyltransferase (MGMT) from representative classical Hodgkin lymphoma (cHL) patient samples.

Low-level expression of 8-OHdG was associated with poorer relapse-free survival (RFS) in advanced-stage HL and a high extent of MnSOD predicted early relapse in the whole HL cohort. Strong expression of PHD1, KDM4B and KDM4D predicted dismal RFS in radiotherapy-treated cHL patients. The results also showed that strong expression of HIF-1 α , SIRT6 and Rif1, and SIRT6 together with Rif1, were associated with prolonged RFS, especially in advanced-stage radiotherapy-treated cHL patients. In multivariate analysis, PHD1, MnSOD, 8-OHdG and Rif1 separately and together with SIRT6 were statistically significant predictors of RFS.

The results reflect the significance of the studied biomarkers in HL, especially in radiotherapy-treated patients. This might be beneficial when individualizing treatment strategies, avoiding overtreatment and controlling long-term treatment-related toxicity. Further research, however, is needed to confirm these preliminary findings.

Keywords: antioxidants, DNA repair, epigenetic, Hodgkin lymphoma, hypoxia, oxidative stress, radiotherapy

Bur, Hamid, Biologiset prognostiset ja prediktiiviset markkerit Hodgkinin lymfoomassa.

Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta; Medical Research Center Oulu; Oulun yliopistollinen sairaala

Acta Univ. Oul. D 1470, 2018

Oulun yliopisto, PL 8000, 90014 Oulun yliopisto

Tiivistelmä

Hodgkinin lymfooma (engl. HL) kuuluu heterogeeniseen imukudossyöpien eli lymfoomen ryhmään. Yli 80 % lymfoomapotilaista voidaan parantaa solunsalpaaja- ja sädehoidon avulla. Hyvän ennusteen takia HL- tutkimuksen tärkeä painopiste on säde- ja solunsalpaajahoidon pitkän ajan haittavaikutukset. Huomattava määrä potilaista kärsii tai jopa kuolee hoitoon liittyvistä pitkäaikaishaitoista johtuen. Tämän tutkimuksen tarkoituksena oli löytää uusia mahdollisia biologisia tekijöitä, jotka ennakoisivat taudin huonoa ennustetta ja samalla antaa uusia näkökulmia HL potilaiden hoidon yksilöllistämiseen.

Tämä retrospektiivinen tutkimus käsitti vuosina 1997-2015 samanlaisesti hoidettuja Hodgkinin lymfooma -potilaita. Immunohistokemiallisilla värjäyksillä määritettiin biologisten merkkiaineiden, mukaan lukien oksidatiivisen stressin markkereiden 8- hydroksideoksiguanosiiniin (8-OHdG) ja nitrotyrosiinin, sekä antioksidanttientsyymien mangaanisuperoksidi-dismutaasin (MnSOD) sekä peroksiredoksiinien (Prx II, Prx III, Prx V, Prx VI) ilmentymistä HL -potilasnäytteissä. Määrittelimme myös immunohistokemiallisilla värjäyksillä epigeneettisten säätelijöiden lysiinin spesifisen demetylaasientsyymien 4 (KDM4A, KDM4B, KDM4D) sekä sirtuiinien (SIRT1, SIRT4, SIRT6), hypoksiaa indusoivien tekijöiden (HIF-1 α , HIF-2 α), prolyylihydroksylaasientsyymien (PHD1, PHD2, PHD3) ja DNA:ta korjaavien proteiinien Rap1 vaikuttuvan tekijä 1 (Rif1) ja O⁶-metyyliguaaniini-DNA metyyli transferaasin (MGMT) ilmentymistä edustavissa klassista Hodgkinin lymfoomaa sairastavien potilaiden (engl. cHL) näytteissä.

Heikko 8-OHdG värjäytyminen ennusti ennen aikaista taudin uusiutumaa levinneessä HL:ssa ja korkea MnSOD ilmaantuvuus ennusti ennen aikaista taudin uusiutumaa koko HL -ryhmässä. Sädehoidetuilla cHL potilailla voimakas PHD1, KDM4B ja KDM4D värjäytyminen ennusti ennen aikaista taudin uusiutumaa. Tulokset osoittivat myös, että erityisesti sädehoidetuilla levinneen taudin cHL potilailla voimakas HIF-1 α , SIRT6, Rif1 ja SIRT6 yhdessä Rif1:n kanssa oli yhteydessä pidentyneeseen uusiutumavapaaseen aikaan. Monimuuttuja-analysissä PHD1, MnSOD, 8-OHdG ja Rif1 itsenäisenä ja yhdessä SIRT6 kanssa ennustivat tilastollisesti merkittävästi taudin ennen aikaista uusiutumaa.

Tulokset osoittavat näiden eri biomarkkereiden merkittävyyden HL:ssä, erityisesti sädehoitoa saaneilla potilailla. Tuloksista voi olla hyötyä, kun hoitokäytäntöjä yksilöidään, mikä voisi helpottaa välttämään liiallista hoitoa ja hallitsemaan pitkäaikaisiin hoitoihin liittyviä haittoja. Näiden alustavien havaintojen vahvistamiseksi tarvitaan kuitenkin lisätutkimuksia.

Asiasanat: antioksidantit, DNA:n korjaus, epigenetiikka, Hodgkinin lymfooma, hypoksia, oksidatiivinen stressi, sädehoito

To my family and Jukka Verkasalo

Acknowledgements

This work was carried out at the Department of Oncology and Radiotherapy Institute of Clinical Medicine, University of Oulu in 2012-2018.

I wish to thank my supervisors Docent Peeter Karihtala, M.D., Ph.D., Professor Taina Turpeenniemi-Hujanen M.D., Ph.D. and Docent Kirsi-Maria Haapasaari M.D., Ph.D., for endless support during the whole process. Thank you Peeter for leading me into the world of science and motivating me. You were continuous presence and helped me in the challenging situations. Peeter, without your support and help, this work would never have been published. Thank you Taina for your expertise and great ideas during this work. You always arranged time and helped me especially in the end of the process. I am pleased that you were my supervisor, you are an excellent scientific role model. Thank you Kirsi-Maria for supporting and encouraging me to start this work. You always found time to analyse our endless material. Thank you Kirsi-Maria for being my supervisor.

I want to thank all my co-authors. You all have great input in the original publications in this work and are greatly appreciated. Thank you Docent Outi Kuitunen M.D., Ph.D. from Oulu University Hospital and Professor Ylermi Soini, M.D., Ph.D., Professor Päivi Auvinen, M.D., Ph.D. and Katja Marin M.D. from Kuopio University Hospital for your skillful collaboration. Thank you also Raija Sormunen and Riitta Vuento for your valuable technical assistance and contribution to the original publications.

I also would like to thank my doctoral training follow-up members, Professor Tuomo Karttunen M.D., Ph.D., Docent Saila Kauppila, M.D., Ph.D. and Jenni Peltonen, M.D., Ph.D. I am thankful to my reviewers Docent Sirkku Jyrkkiö M.D., Ph.D. and Professor Veli-Matti Kosma M.D., Ph.D. for your comments and respectable expertise.

My warmest gratefulness goes to our incredible research group, especially Mrs. Anne Bisi. Anne, thank you for taking care of me and giving endless support during these years.

I would like to thank my close relatives; Veikko Kondelin, Itani family, Hussein Bur family, Hassan Bur family, Shahda Bur family, Fatma Bur family, Sayed Bur family, Alli Bur and Nael Bur for love, support and the memorable moments, which I have had with all of you. I would like to warmly thank teachers Sirkku Suutari and Sirkku Nisula-Voutilainen for motivating and supporting me. I would also like to thank my close friends; Mikko Tuomaala, Aleksii Ryhänen, Hannu Forstén, Ville Hautala, Johannes Perdahl, Ramin Akhi, Rasmus Valtonen, Santtu Liikala, Katja

Hukkanen-Reinilä, Ilpo Reinilä, Lauri Alasaarela, Jussi Vänskä, Jaakko Gummerus and Valjakka family for being around on my best and worst days. Last but not least, thank you my beloved partner Eeva Valjakka for understanding and supporting this work. Eeva, I would like to thank you for believing in me and always pushing me forward and finally for your love.

Finally, thank you to my mother Kerttu Kondelin-Bur and father Elshiekh Bur for being my parents. My whole life you have supported and loved me as I am. You both have motivated and encouraged me during this work especially at the moments when I was already giving up. I truly admire you both being unbelievable parents to me and my brothers. Of course I would also like to thank my beloved brothers Khalid and Hassan. Our warm relationship means a lot for me. Your support and love are something incredible

I would like to dedicate this book to my family and dear family friend Jukka Verkosalo.

This work was funded by Cancer Foundation Finland, The University of Oulu Scholarship Foundation, and the University Hospital of Oulu (KEVO funding) which I gratefully acknowledge.

February 2018

Hamid Bur

Abbreviations

53BP1	Tumour suppressor p53-binding protein 1
8-OHdG	8-hydroxy-2' -deoxyguanosine
ABVD	Doxorubicin bleomycin, vinblastine and dacarbazine
ADP	Adenosine diphosphate
AKT	Protein Kinase B
AP1	Activator protein 1
ATF4	Activating transcription factor 4
ATM	Ataxia-telangiectasia mutated
ATP	Adenosine triphosphate
BCR	B cell receptor
BEACOPP	Bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisolone
BER	Base excision repair
BRCA1	Breast cancer 1 gene
BV	Brentuximab vedotin
CCL	Chemokine ligand
CCL20	Chemokine ligand 20
CD	Cluster of differentiation
cHL	Classical Hodgkin Lymphoma
CI	Confidence interval
CR	Complete response
CSFR1	Colony-stimulating factor 1 receptor
CTL	Cytotoxic T lymphocyte
CTLA4	Cytotoxic T-lymphocyte-associated protein 4
DDR	DNA damage response
DDR1	Discoidin domain receptor tyrosine kinase 1
DLBCL	Diffuse large B-cell lymphoma
DNA	Deoxyribonucleic acid
DNA-PK	DNA-dependent protein kinase
DSBs	Double-strand breaks
E2F1	Transcription factor E2F1
EBNA1	EBV nuclear antigen 1
EBV	Epstein-Barr virus
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase

FasL	Fas ligand
FDG-PET	Fluorodeoxyglucose positron emission tomography
FGF	Fibroblast growth factor
FL	Follicular lymphoma
G2	Gap 2
GATA3	GATA-binding protein 3
GC	Germinal centre
GSH	Glutathione
H ₂ O ₂	Hydrogen peroxide
HDAC	Epigenetic histone deacetylase
HIF	Hypoxia-inducible factor
HIV	Human immunodeficiency virus
HL	Hodgkin lymphoma
HLA-A	Human leukocyte antigen-A
HR	Hazard ratio
HR	Homologous recombination
HRS	Hodgkin and Reed–Sternberg (cell)
ICAM-1	Intercellular adhesion molecule 1
ID2	DNA-binding protein inhibitor 2
IEM	Immunoelectronmicroscopy
IFRT	Involved-field radiation therapy
IHC	Immunohistochemistry
IKK β	I κ B kinase β
IL	Interleukine
IPS	International Prognostic Score
IR	Ionizing radiation
IRF	Interferon regulatory factor
JAK/STAT	Janus kinase/signal transducers and activators of transcription (signalling pathway)
KDM4	(K)-specific demethylase 4
LDCHL	Lymphocyte-depleted classical Hodgkin lymphoma
LMP	Latent membrane protein
LP	Lymphocyte-predominant
LRCHL	Lymphocyte-rich classical Hodgkin lymphoma
MAPK	Mitogen-activated protein kinase
MCCHL	Mixed cellularity classical Hodgkin lymphoma
MGMT	O6-alkylguanine DNA alkyltransferase

MMR	Mismatch repair
MnSOD	Manganese superoxide dismutase
NAD ⁺	Nicotinamide adenine dinucleotide
NER	Nucleotide excision repair
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NHEJ	non-homologous DNA end-joining
NHL	Non-Hodgkin lymphoma
NK	Natural killer (cell)
NLPHL	Nodular lymphocyte-predominant Hodgkin lymphoma
NO	Nitric oxide
NOTCH1	Notch homolog 1, translocation-associated
NSCHL	Nodular sclerosis classical Hodgkin lymphoma
O ₂	Oxygen
O ₂ ⁻	Superoxide anion
O ₂ ⁻	Superoxide
OH [•]	Hydroxyl radical
ONOO ⁻	Peroxynitrite
P38	Mitogen-activated protein kinase
PAX5	Paired box protein 5
PBS	Phosphate-buffered saline
PD-L1	Programmed death ligand 1
PDGF	Platelet-derived growth factor
PDGFA	Platelet-derived growth factor A
PDGFRA	Platelet-derived growth factor receptor A
PHD	Prolyl hydroxylase domain protein
PI3K	Phosphoinositide 3-kinase
PI3K	Phosphatidylinositol 3-kinase
Prx	Peroxiredoxin
RANK	Receptor activator of NF-κB
RCI	Reactive cellular infiltrate
RFS	Relapse-free survival
RIF1	Human Rap1-interacting factor 1
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RR	Risk ratio
RTK	Receptor tyrosine kinases
SIRT	Sirtuin

SOCS1	Suppressor of cytokine signalling 1
SOD	Superoxide dismutase
SSBs	Single-strand breaks
Th	Helper T (cell)
Th0	Naïve T (cell)
TNFR	Tumour necrosis factor receptor
TNFRSF	Tumour necrosis factor receptor superfamily
TNF α	Tumour necrosis factor alpha
Treg	Regulatory T (cell)
Trx	Thioredoxin
WHO	World Health Organization

Original publications

This thesis is based on the following publications, which are referred throughout the text by their Roman numerals:

- I Bur H, Haapasaari KM, Turpeenniemi-Hujanen T, Kuittinen O, Auvinen P, Marin K, Koivunen P, Sormunen R, Soini Y & Karihtala P (2014) Oxidative stress markers and mitochondrial antioxidant enzyme expression are increased in aggressive Hodgkin lymphomas. *Histopathology* 65(3):319-327.
- II Bur H, Haapasaari KM, Turpeenniemi-Hujanen T, Kuittinen O, Auvinen P, Marin K, Soini Y & Karihtala P (2018) Strong prolyl hydroxylase domain 1 expression predicts poor outcome in radiotherapy-treated patients with classical Hodgkin's lymphoma. *Anticancer Research* 38(1):329-336.
- III Bur H, Haapasaari KM, Turpeenniemi-Hujanen T, Kuittinen O, Auvinen P, Marin K, Soini Y & Karihtala P (2016) Strong KDM4B and KDM4D expression associates with radioresistance and aggressive phenotype in classical Hodgkin lymphoma. *Anticancer Research* 36(9):4677-4683.
- IV Bur H, Haapasaari KM, Turpeenniemi-Hujanen T, Kuittinen O, Auvinen P, Marin K, Soini Y & Karihtala P (2018) Low Rap1 interacting factor 1 and sirtuin 6 expression predict poor outcome in radiotherapy-treated Hodgkin lymphoma patients. *Leukemia & Lymphoma* 59(3):679-689.

Contents

Abstract	
Tiivistelmä	
Acknowledgements	9
Abbreviations	11
Original publications	15
Contents	17
1 Introduction	19
2 Review of the literature	21
2.1 Lymphoma	21
2.1.1 Epidemiology	21
2.1.2 Aetiology	21
2.1.3 Classification of lymphomas	22
2.1.4 Biology of classical Hodgkin lymphoma	24
2.1.5 The microenvironment in classical Hodgkin lymphoma	27
2.1.6 Clinical features and diagnostics of classical Hodgkin lymphoma	33
2.1.7 Treatment	36
2.1.8 Future aspects in the treatment of cHL	40
2.2 Oxidative stress and antioxidant enzymes	41
2.2.1 Oxidative stress	41
2.2.2 Antioxidant enzymes	42
2.2.3 Oxidative stress and antioxidant enzymes in cancer	43
2.3 Hypoxia	44
2.3.1 Hypoxia-inducible factor (HIF)	44
2.3.2 Prolyl hydroxylase domain (PHD) proteins	45
2.4 Epigenetics	46
2.4.1 KDM4	47
2.4.2 Sirtuins	49
2.5 DNA repair	50
2.5.1 Rif1	52
2.5.2 MGMT	52
3 Aims of the present study	55
4 Material and methods	57
4.1 Patient material	57
4.2 Immunohistochemistry	58

4.3	Immunoelectron microscopy.....	61
4.4	Sample evaluation.....	61
4.5	Statistical analyses.....	62
4.6	Ethical aspects.....	62
5	Results	65
5.1	Immunohistochemical staining patterns in HL.....	65
5.2	Associations between clinical parameters and biomarkers.....	68
5.3	Survival analysis.....	70
6	Discussion	75
6.1	Oxidative stress markers and antioxidant enzymes in HL.....	75
6.2	HIF and PHD enzymes in cHL.....	76
6.3	Epigenetic regulators in cHL.....	77
6.4	Sirtuins and DNA repair in cHL.....	78
6.5	Limitations of this study.....	79
6.6	Prospects for future research.....	80
7	Conclusions	81
	References	83
	Original publications	111

1 Introduction

Hodgkin lymphoma (HL) covers a group of lymphomas that is comparatively rare. The incidence rate of HL has changed minimally in recent decades. In Finland, 150 new cases of HL were diagnosed in 2015. World Health Organization (WHO) classification divides HL into two main types – classical Hodgkin lymphoma (cHL) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL). The former is further divided into four subgroups. The cHL portion represents 95% and the NLPHL type 5% of all cases of HL. The majority of patients are diagnosed between the ages of 15 and 30 and a second peak is seen in adults aged 55 years or more (Swerdlow *et al.*, 2016, Finnish Cancer Registry 2015).

The treatment of HL has improved as a result of multi-agent chemotherapy and advances in modern radiotherapy. Current chemo- and radiotherapy cures over 80% of all HL patients, both young and old (Stathis & Younes 2015). Although the prognosis of HL is generally favourable, there are still challenges in its treatment. Survival rates drop significantly if a complete response (CR) is not achieved with first-line treatment. The second main challenge is long-term overall survival, which is adversely affected because of the increased incidence of cardiovascular diseases and secondary malignancies arising from treatment-related long-term toxicity (van Nimwegen *et al.*, 2015, Schaapveld *et al.*, 2015).

Aerobic cells generate reactive oxygen species (ROS), which are highly reactive molecules. They have an unpaired electron in their outermost orbital. Immoderate production of ROS is able to cause damage to cellular macromolecules and lead cells to a state called oxidative stress. Antioxidant enzymes are situated in various locations in cells and their function is to reduce ROS to more harmless end-products (Finkel 2003, Karihtala & Soini 2007, Sies, Berndt & Jones 2017).

Hypoxia reflects inadequate oxygen in cells. Cancer cells must adapt to a hypoxic environment by initiating a specific DNA transcription programme to survive. Hypoxia-inducible factors (HIFs) are the most important transcription factors in hypoxia. Prolyl hydroxylase domain proteins (PHD1-3) can hydroxylate HIFs under normoxic conditions. PHD proteins also have HIF-independent roles (Semenza 2011).

DNA repair pathways are essential to defend against various forms of endogenous and environmental DNA damage. However, cancer cells can use up-regulated DNA repair pathways, leading to resistance to DNA-damaging chemotherapy and radiotherapy. Inhibiting or at least being conscious of these

pathways may be important as regards sensitizing cells to cancer treatment (Curtin 2012).

To evaluate treatment strategies better, there is a need for better predictive and prognostic biomarkers in cHL. Future biomarkers could open new aspects, focusing on molecular abnormalities in Hodgkin and Reed–Sternberg (HRS) cells or the microenvironment. These biomarkers could be used for individualizing treatment strategies, helping to eliminate the malignancy and also controlling long-term treatment-related toxicity. In the present study, the aim was to identify biological markers that could offer a new perspective in individualizing patient treatment. In further development of HL treatment it will be important to be conscious of therapeutic selection, using predictive biological markers to select patients at risk of refractory disease or relapse.

2 Review of the literature

2.1 Lymphoma

Lymphoma was first noted in 1666 by Dr. Malpighi, who found prominent splenic nodules in a young female at autopsy (Malpighi 1666). In 1832 Sir Thomas Hodgkin published a paper entitled “On some morbid appearances of the absorbent glands and spleen” (Hodgkin 1832) and later the disease was named after him. All lymphomas were subsequently divided into two main subgroups: Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). Diagnostic technology developed with great advances after 1832 and nowadays the World Health Organisation (WHO) recognises almost one hundred various subtypes of lymphoma (Swerdlow *et al.*, 2016).

2.1.1 Epidemiology

In 2015, a total of 1311 lymphomas were diagnosed in Finland. Of these cases, 150 were HL, which means that HL represented 11% of all lymphomas and 0.05% of all cancer cases in Finland in 2015. The incidence of HL worldwide shows a bimodal age distribution, first in 15- to 30-year-olds, with a second peak after 60 years of age in men and women. Men are diagnosed more often with HL than women in Western countries. The HL incidence rate was 2.7 per 100 000 in Finland in 2015 (Finnish Cancer Registry 2015). Globally the incidence rate of HL varies greatly; in developed countries it is much higher than in less developed regions. HL incidence has not increased in Finland or in other Western countries during the last decades. (Finnish Cancer Registry 2015, IARC, Globocan 2012).

2.1.2 Aetiology

In 1966 MacMahon suggested the hypothesis that an infectious aetiology may be behind HL in young patients (MacMahon 1966). Infectious mononucleosis has been known to be linked to HL since 1974 (Connelly & Christine 1974). The Epstein-Barr virus (EBV) has been linked to an elevated risk of HL in young adults. The prevalence of EBV in HRS cells varies greatly within cHL subtypes. In Western countries approximately 40% of cHL cases are EBV-positive, but in Central and South America the incidence is almost 90% (Shannon-Lowe,

Rickinson & Bell 2017, Swerdlow *et al.*, 2016). Although cHL patients may not be EBV-positive, there is evidence suggesting that EBV has a role in the pathogenesis of HRS cells (Vockerodt *et al.*, 2015). There are also researchers who believe that in EBV-negative cases another viral agent may be involved in the pathogenesis (zur Hausen & de Villiers 2005).

Viral infections do not explain the whole aetiology of HL; there are several risk factors which have been linked to HL, including genetic, selected lifestyle (e.g. tobacco, alcohol), environmental risk factors (e.g. ultraviolet radiation exposure) and moderate immunosuppression (e.g. Human immunodeficiency virus (HIV) infection, organ transplant) (Besson *et al.*, 2006, Ekström *et al.*, 2005, Crump, Sundquist, Sieh, Winkleby & Sundquist 2012, Shiels *et al.*, 2014, Clarke *et al.*, 2013).

2.1.3 Classification of lymphomas

Nowadays lymphomas can be classified according to the cell line from which they have originated. Most non-Hodgkin lymphomas originate from B cells. In NHL there are also subgroups originating from T cells and natural killer (NK) cells. HL is subdivided to classical Hodgkin lymphoma (cHL) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) (Swerdlow *et al.*, 2016). Classical Hodgkin lymphoma can be further sub-classified into nodular sclerosis cHL (NSCHL), mixed cellularity cHL (MCCHL) lymphocyte-depleted cHL (LDCHL) and lymphocyte-rich cHL (LRCHL). This classification is based on differences in morphology, histological picture and the immunophenotype of the tumour cells. Both cHL and NLPHL have neoplastic cell populations, which are mononucleated or multinucleated. In cHL these neoplastic cells are called Hodgkin and Reed–Sternberg (HRS) cells, and in NLPHL, lymphocyte-predominant (LP) cells.

Nodular Sclerosis Classical Hodgkin lymphoma

NSCHL is the most common subtype of cHL, representing approximately 70% of all HL cases, and its incidence continues to rise (Clavel Steliarova-Foucher, Berger, Danon & Valerianova 2006). NSCHL is found more often in patients of high socioeconomic status, in young adults, and less often in the elderly (Engert *et al.*, 2005). NSCHL gene expression profiling differs from that in other forms of cHL, and EBV infection is not as common in it as in other cHL subtypes. NSCHL is

clinically the most favourable histological subtype of cHL (Devillard *et al.*, 2002, Swerdlow *et al.*, 2016).

Histologically, NSCHL lymph nodes show thickened capsules and collagen bands surround a single nodulus. The number of HRS cells differs notably between NSCHL patient samples and also between lymph nodes (Swerdlow *et al.*, 2016).

Mixed cellularity classical Hodgkin lymphoma

MCCHL represents 20–25% of all HL cases. It is more common in low-socioeconomic-status patients. HIV infection increases the risk of development of MCCHL (Clarke Glaser, Keegan & Stroup 2005). EBV is linked to MCCHL HRS cells in almost 75% of cases (Swerdlow *et al.*, 2016). MCCHL is a more common subtype in men than in women and it is found in both paediatric and elderly patients (Eberle, Mani & Jaffe 2009). MCCHL occurs in the thymus gland or mediastinum. Unlike NSCHL, MCCHL involves peripheral lymph nodes and bone marrow. Usually MCCHL patients do not have B symptoms (Eberle, Mani & Jaffe 2009). Together with LDCHL patients, MCCHL patients have the worst prognosis among cHL subtypes (Allemani Sant, De Angelis, Marcos-Gragera & Coebergh 2006).

The microenvironment consists of small lymphocytes, neutrophils, eosinophils, plasma cells and histiocytes. The type of infiltration can be nebulous, nodular or diffuse. Characteristic features of other forms of cHL are absent (Swerdlow *et al.*, 2016).

Lymphocyte-depleted classical Hodgkin lymphoma

LDCHL is the most uncommon subtype of cHL – less than 1% of all HL cases. LDCHL is found mostly in elderly patients (Neiman & Rosen 1973). Similarly to MCCHL, HIV infection and low socioeconomic status are linked to LDCHL (Clarke, Glaser, Keegan & Stroup 2005). Typically, LDCHL involves peripheral lymph nodes and bone marrow, but not the thymus gland or mediastinum (Eberle, Mani & Jaffe 2009). EBV is closely linked to LDCHL; over 80% of cases are EBV-positive (Swerdlow *et al.*, 2016).

Histologically, there are plenty of HRS cells and/or a few small lymphocytes in the microenvironment of LDCHL. The histology varies greatly. In some cases HRS cells are anaplastic and in some cases the microenvironment reminds one of NSCHL, with extensive diffuse fibrosis (Swerdlow *et al.*, 2016).

Lymphocyte-rich classical Hodgkin lymphoma

LRCHL covers approximately 5% of HL cases. Most LRCHL patients are male. EBV-positive HRS cells vary greatly in LRCHL; they are found in approximately 40–80% of cases (Swerdlow *et al.*, 2016). At the time of diagnosis, LRCHL appears as peripheral lymphadenopathy, with no mediastinal involvement. Stage varies typically between stage I and II and B-symptoms are rare. The survival rate in cases of LRCHL is similar to that in NSCHL (Eberle, Mani & Jaffe 2009).

The microenvironment is known as lymphocyte-rich, which can be nodular or diffuse. Germinal centre (GC) shape is preserved with B cell follicles. HRS cells are found in mantle and marginal zones. Eosinophils and neutrophils are not present in LRCHL nodular infiltrates (Swerdlow *et al.*, 2016).

Nodular lymphocyte-predominant Hodgkin lymphoma

NLPHL is a unique lymphoma group covering around 5% of all HL cases. Most of the NLPHL patients are diagnosed with limited stage. Difference to cHL, NLPHL tends to transform into more aggressive NHL (Swerdlow *et al.*, 2016).

LP cells include one large single folded or polylobated vesiculated nucleus (Swerdlow *et al.*, 2016). The microenvironment around LP cells are a nodular or follicular background, where B lymphocytes are dominated. Discrimination of NLPHL from cHL and other lymphomas can be challenging. For correct diagnosis, immunophenotyping is key position (Swerdlow *et al.*, 2016).

2.1.4 Biology of classical Hodgkin lymphoma

Malignant cells, HRS cells, originate from germinal-centre (GC) B cells (Kanzler, Küppers, Hansmann & Rajewsky 1996). HRS cells rearrange immunoglobulin-chain genes in the GC (Küppers *et al.*, 1994). B cells gain favourable transformations in the GC, and high-affinity B cell receptors (BCRs). HRS cells have unfavourable mutations in the GC and have to escape apoptosis. There is not completely clear scientific knowledge of the transformation of B cells to HRS cells. At present the hypothesis is that it is a multistep process, where transforming events occur before naïve B cells enter the GC. The final transforming events take place when HRS cells exit the GC (Küppers, Engert & Hansmann 2012).

Origin of HRS cells

HRS cells retain representative markers of different hematopoietic lineages: B cells (paired box protein 5 (PAX5), CD20), T cells (CD3, notch homolog 1, translocation-associated (NOTCH1), GATA-binding protein 3 (GATA3)), dendritic cells (fascin, chemokine ligand 17 (CCL17)), NK cells (DNA-binding protein inhibitor 2 (ID2)), and myeloid cells (colony-stimulating factor 1 receptor (CSFR1)) (Schmitz, Stanelle, Hansmann, Küppers 2009). HRS cells express CD30 and CD40, in 75–80% of them, CD15, and in 30–40%, CD20 (Schwering *et al.*, 2003, Swerdlow *et al.*, 2016). Interferon regulatory factor 4 (IRF4) is also strongly expressed in HRS cells (Valsami *et al.*, 2007). Compared with other lymphomas HRS cells have a higher number of chromosome abnormalities (Weber-Matthiesen, Deerberg, Poetsch, Grote & Schlegelberger 1995).

The role of EBV in cHL pathogenesis

EBV-positive HRS cells express three proteins: EBV nuclear antigen 1 (EBNA1) and latent membrane proteins (LMPs) 1 and 2A. All EBV-infected cells express EBNA1 protein. EBNA1 is necessary for replication of the episomal EBV genome. EBNA1 down-regulates the tumour suppressor protein-tyrosine phosphatase. There is also evidence that it modifies regulatory T cells (Treg cells) in the microenvironment of cHL by upregulating CCL20 (chemokine ligand 20) (Flavell *et al.*, 2008, Baumforth *et al.*, 2008). LMP1 sticks to the HRS cell membrane. It imitates an active CD40 receptor, which causes activation of the NF- κ B pathway (Kilger, Kieser, Baumann & Hammerschmidt 1998). Furthermore, LMP1 brings about activation of AP1 (activator protein 1), p38 (mitogen-activated protein kinase), PI3K (phosphatidylinositol 3-kinase) and the JAK/STAT (Janus kinase/signal transducers and activators of transcription) signalling pathway (Young, Dawson & Eliopoulos 2000, Dawson, Tramountanis, Eliopoulos & Young 2003). LMP2A has an important role in HL lymphomagenesis. It mimics activated BCRs, which contributes to HRS cell survival, including activation of the PI3K/protein kinase B (AKT) pathway and anti-apoptotic functions (Portis & Longnecker 2004, Chaganti *et al.*, 2005, Shannon-Lowe, Rickinson & Bell 2017).

The NF- κ B pathway

In normal B cells nuclear factor kappa B (NF- κ B) is momentarily active when BCRs are positively chosen by interaction with T helper cells and signalling via the BCR (Bargou *et al.*, 1997). Activation of NF- κ B causes various changes in cellular processes, including cell survival, proliferation, cell adhesion and differentiation (Vallabhapurapu & Karin 2009).

In HRS cells the NF- κ B pathway is continuously active. There are two pathways that lead to NF- κ B pathway activation, the canonical and alternative pathways (de Oliveira *et al.*, 2016, Jost & Ruland 2007). Continuous activation of the NF- κ B pathway in HRS cells is created through receptor stimulation, but there are also mutations in the downstream pathway of NF- κ B (Horie *et al.*, 2002, Lake *et al.*, 2009, Liu *et al.*, 2010).

The JAK/STAT pathway

The JAK/STAT pathway is also significant in HRS cell pathogenesis. There are seven STAT and four JAK proteins in the human genome. This pathway is the most important signalling pathway for cytokines. Cytokine stimulation activates JAKs, which phosphorylate and further activate STAT transcription factors. These phosphorylated STAT proteins translocate into the nucleus and bring about the expression of target genes (Vainchenker & Constantinescu 2013). In HRS cells STAT3, STAT5 and STAT6 proteins are active all the time (Kube *et al.*, 2001, Skinnider *et al.*, 2002, Scheeren *et al.*, 2008). Cells in the microenvironment express interleukins, which activate STATs, but HRS cells can activate STAT6 by autocrine stimulation of IL-13 (Skinnider *et al.*, 2002). IL-21 and NF- κ B activity stimulate STAT3 and STAT5 (Lamprecht *et al.*, 2008, Hinz *et al.*, 2002).

SOCS1 (suppressor of cytokine signalling 1) is the most important inhibitor of STAT activity and it is affected by inactivating mutations in approximately 40% of cHL cases (Weniger *et al.*, 2006). In addition, HRS cells show genomic gains (9p24) of JAK2 in 20% of cases. JAK2 is an activator of STAT signalling (Joos *et al.*, 2000). The same genomic gains also up-regulate PD-L1 (programmed death ligand 1), PD-L2 and also the epigenetic regulator KDM4C (Green *et al.*, 2010, Rui *et al.*, 2010). Activation of the PD-1 pathway prevents T cell cytotoxic attack, and KDM4C participates in the epigenetic remodelling of HRS cells.

Other pathways

The PI3K/AKT pathway is the most important promoter of cell survival in HRS cells. This pathway is activated by signals transmitted through CD40, CD30, RANK (receptor activator of NF- κ B) and receptor tyrosine kinases (RTKs). HRS cells are driven to apoptosis if AKT is inhibited. Thus the PI3K/AKT pathway has a necessary role in the survival and growth of HRS cells (Dutton, Reynolds, Dawson, Young & Murray 2005, Georgakis *et al.*, 2006).

The mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway includes activation of ERK1, ERK2 and ERK5. In HRS cells, this pathway regulates apoptosis, proliferation and differentiation (Zheng *et al.*, 2003). MAPK/ERK pathway activity is important for HRS cell survival and proliferation, because inhibition of this pathway causes HRS cell apoptosis and cell-cycle arrest (Zheng *et al.*, 2003).

2.1.5 The microenvironment in classical Hodgkin lymphoma

The histology of cHL differs from that in other lymphomas, and it also differs within the group of cHLs. The major component cells of cHL are called reactive cellular infiltrate (RCI), or microenvironment. HRS cells comprise a minor portion of the tumour, only 0.1%–2% (Swerdlow *et al.*, 2016). The microenvironment differs within the subtypes of cHL (Table 1). EBV in HRS cells affects the structure of the microenvironment. EBV expression varies greatly in cHL subtypes (Table 1).

T cells are found in the microenvironment of all subtypes of cHL (Poppema, Bhan, Reinherz, Posner & Schlossman 1982). CD4⁺ T cells can be separated into memory (CD45RO⁺) or naïve (CD45RA⁺) cells. Most CD4⁺ T cells are helper T (Th) cells, which have a significant role in starting the potent immune response. Th cells can be subdivided into Treg, Th0 (naïve T cells), Th1 (cellular response), Th2 (humoral response) and Th17 (interleukin 17 [IL-17] production) types. CD4⁺ T cells are close to HRS cells, forming a tight rosette, especially Th cells and Treg cells, but T cells can also be widely spread around the microenvironment (Ma *et al.*, 2008, Aldinucci, Glohini, Pinto, Colombatti & Carbone 2012).

In addition to T cells there is remarkable variation in the composition of other cell types in the microenvironment, including eosinophils, histiocytes, plasma cells, fibroblasts, mast cells, B cells and neutrophils (Table 1). The structure of the lymph node is different from normal (including changes in the microenvironment) between the cHL subgroups (Table 1).

Table 1. Consistency of the microenvironment in classical Hodgkin lymphoma (cHL) subtypes. Table adapted from Visser *et al.*, 2015.

Subtypes	Background	T cells	Other cells	EBV (%)
Nodular sclerosis	Nodular and fibrosis	CD4 > CD8, Th2, Treg > Th	Eosinophils, histiocytes, fibroblasts, B cells, mast cells (neutrophils)	10–40
Mixed cellularity	Diffuse	CD4 > CD8, Th2, Treg > Th1	Eosinophils, histiocytes, plasma cells, B cells	75
Lymphocyte-rich	Nodular or diffuse	CD4 > CD8	Histiocytes	40–80
Lymphocyte-depleted	Diffuse	None	Histiocytes	80–100

EBV=Epstein-Barr virus

Communication between the microenvironment and HRS cells

HRS cells remodel the composition of the microenvironment of cHL. The microenvironment affects HRS cells in terms of survival, growth, immunosuppression and proliferation (Wein & Kuppers 2016). The microenvironment and HRS cells exhibit crosstalk, cytokines and chemokines being expressed by both. A representation of the relationship between the microenvironment and HRS cells is presented in Figure 1 and a histological picture of cHL in Figure 2.

Tumour growth and support of survival

HRS cells express CD40 (TNFRSF5/tumour necrosis factor receptor superfamily member 5) protein, which activates the NF-κB pathway and causes more effective HRS cell formation (Gruss *et al.*, 1994, Carbone, Gloghini, Gruss & Pinto 1995, Annunziata, Safiran, Irving, Kasid & Cossman 2000). At least Th2 cells and Treg cells in rosettes express CD40, which enables HRS to survive (Carbone, Gloghini, Gruss & Pinto 1995).

Th2 cells, mast cells and eosinophils can produce IL-3 and most HRS cells express IL-3 receptor (Aldinucci *et al.*, 2005). IL-3 activation causes cells to avoid apoptosis and also enhance proliferation (Aldinucci *et al.*, 2005). HRS cells produce IL-7, IL-9 and IL-13 and they also have receptors for the same interleukins. IL-7 promotes an anti-apoptosis effect and also increases proliferation (Cattaruzza *et al.*, 2009). IL-9 promotes tumour growth and IL-13 contributes to the

transformation of naïve T cells towards to the Th2 and Treg phenotypes (Kapp *et al.*, 1999, Glimelius *et al.*, 2006, Tanijiri *et al.*, 2007).

In addition to CD40, HRS cells have another TNFR (tumour necrosis factor receptor) on their membranes; CD30. Both mast cells and eosinophils have CD30 ligands, and they are found in the cHL microenvironment (Pinto *et al.*, 1996, Molin *et al.*, 2002). Activation of CD30 induces secretion of TNF α (tumour necrosis factor alpha) and IL6 and it may also participate in HRS cell NF- κ B activity (Pinto *et al.*, 1996, Molin *et al.*, 2002). Activation of CD30 also increases levels of lymphotoxin- α , ICAM-1 (Intercellular Adhesion Molecule 1) and B7 family proteins, such as CD80 and CD86. In cHL, activation of CD30 promotes tumour cell growth and survival (Deutsch Tadmor, Podack & Rosenblatt 2011).

Compared with B cells, HRS cells show abnormal activity and expression of multiple RTKs, which have a role in cell growth and survival. HRS cells can express tyrosine kinase ligands themselves, but they are also produced in the microenvironment. DDR1 (discoidin domain receptor tyrosine kinase 1) is produced by EBV-positive HRS cells and DDR2 is activated in a paracrine fashion (Cader *et al.*, 2013, Renné, Willenbrock, Küppers, Hansmann & Bräuninger 2005). Both DDR1 and DDR2 protect HRS cells from apoptosis by binding to collagen that is close to HRS cells (Renné, Willenbrock, Küppers, Hansmann & Bräuninger 2005, Cader *et al.*, 2013). PDGF (platelet-derived growth factor) is particularly involved in growth factor signalling pathways. PDGFRA (platelet-derived growth factor receptor A) is expressed in HRS cells and its ligand PDGFA (platelet-derived growth factor A) is produced by HRS cells. A therapeutic drug, Imatinib, is a PDGFRA inhibitor which decreases HRS cell proliferation (Renné, Willenbrock, Küppers, Hansmann & Bräuninger 2005).

HRS cells shape the microenvironment

Rosette formation between HRS cells and CD4⁺ T cells and Treg cells involves adhesion molecules CD54-CD11a and CD58-CD2 (Sanders *et al.*, 1988, Fromm, Kussick & Wood 2006). HRS cells create important connections to T cells by way of B7 family proteins (CD80, CD 86). These B7 family antigens bind to CD28 of T cells. Connection presumably enhances reciprocal stimulation of both HRS cells and T cells (Nozawa, Wakasa & Abe 1998).

Rosetting T cells support HRS cells by way of ligand-receptor interactions. HRS cells produce many specific chemokine ligands, to shape the microenvironment around the cells. They secrete CCL17 (chemokine ligand 17)

and CCL22 to bind CCR4-positive Th2 cells, Treg cells, monocytes and basophils in the microenvironment (van den Berg, Visser & Poppema 1999, Peh, Kim & Poppema 2001). IL-5, CCL5 and CCL2 induce eosinophils to remain in the microenvironment (Skinnider *et al.*, 2002, Hanamoto *et al.*, 2004), and IL-13, tumour necrosis factor alpha (TNF- α) and fibroblast growth factor (FGF) act on fibroblasts (Skinnider *et al.*, 2002). Fibroblasts also secrete CCL11 and IL-7 to infiltrate Th2 and proliferating Treg cells (Jundt *et al.*, 1999, Cattaruzza *et al.*, 2009). HRS cells are adversely affected by nearby CD26-positive cells because they inactivate HRS cell chemokines such as CCL5, CCL17 and CCL20 (Wolf, Albrecht & Märki 2008). As previously mentioned, HRS cells secrete interleukins (IL-7, IL-13) that stimulate differentiation of T cells.

In addition to HRS cells, “ordinary” cells also participate in shaping the microenvironment. Fibroblasts have an important function by producing eotaxin, which activates Th2 cell and eosinophil infiltration to the microenvironment (Jundt *et al.*, 1999).

Immunosuppression in the microenvironment

Treg cell rosette formation around HRS cells might explain its immunosuppressive role. Treg cells suppress CTLs (cytotoxic T lymphocytes) and NK cells. They strongly secrete immunosuppressive cytokine IL-10 and express CTLA4 (cytotoxic T-lymphocyte-associated protein 4) protein receptor (Marshall *et al.*, 2004, Chemnitz *et al.*, 2007). Treg cell CTLA-4 binds CTL cell CD28 and suppresses the cytotoxic effect. CTLA-4 also induces activated Th cells in the microenvironment, which might cause cytotoxic cells to repress an anti-HRS cell attack (Marshall *et al.*, 2004). HRS cells also directly secrete immunosuppressive cytokines such as TGF- β (transforming growth factor beta) and galectin-1 in addition to IL-10 (Skinnider *et al.*, 2002, Gandhi *et al.*, 2007).

Approximately 30–40% of cHL cases are infected by EBV and express viral antigens (Kapatai & Murray 2007). EBV-positive cases present HLA-A (human leukocyte antigen-A; included in HLA class I) and EBV-negative cHL cases present HLA class II (Poppema 2005). To escape from an effective anti-tumour immune response, HRS cells have to down-regulate HLA class II in EBV-negative cases and demonstrate polymorphism of HLA class I in EBV-positive cases, which allows HRS cells to escape from CD8-mediated cytotoxicity (Poppema 2005). Down-regulation of HLA class I usually activates NK cells. These cells contain HLA-G, which allows HRS cells to escape from NK cells. Also, HLA-G may induce Treg

cells and limit the cytotoxic T cell response (Diepstra *et al.*, 2008). HRS cells try to down-regulate MHC I and -II (major histocompatibility complex), in order not to be revealed by CTL or Th cells, which support the cytotoxic cells (Wein & Küppers 2016).

HRS cells express FasL (CD95L), and PD-L1 and -L2. HRS cells can bring about T-cell exhaustion by constant stimulation of the PD-1 receptor expressed on T cells by PD-L1 (Kim, Eow, Peh & Poppema 2003, Verbeke, Wenthe, Grobholz & Zentgraf 2001). T cells express PD1 and can be activated by HRS cell PD-L1/2, which causes exhaustion of T cells. This is reversible inhibition of T-cell proliferation and allows HRS cells to avoid an immune response (Keir, Butte, Freeman & Sharpe 2008, Weber 2010).

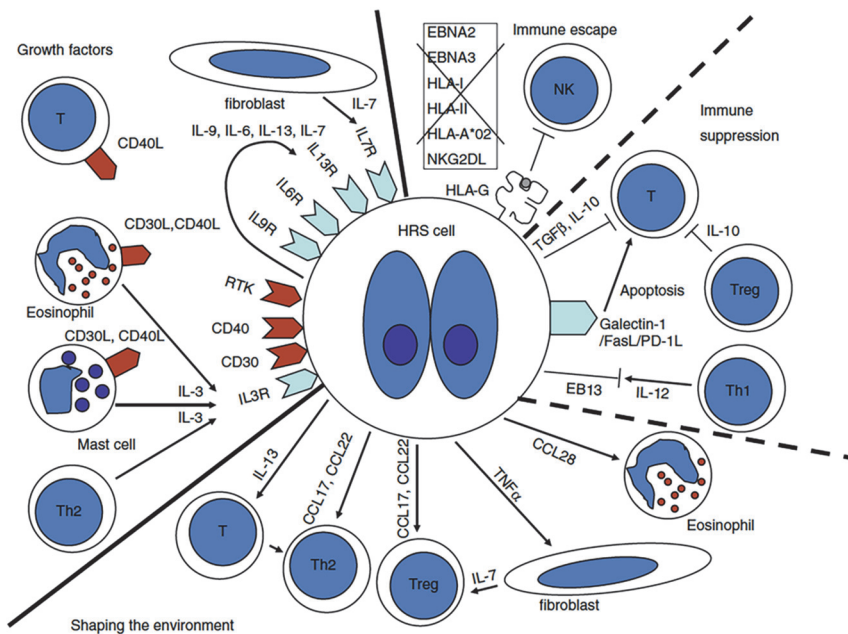


Fig. 1. Demonstrates the relationship between HRS cell and microenvironment. Arrows demonstrate the stimulating actions, two lines point out inhibitory actions. Figure published by permission of Visser *et al.*, 2015

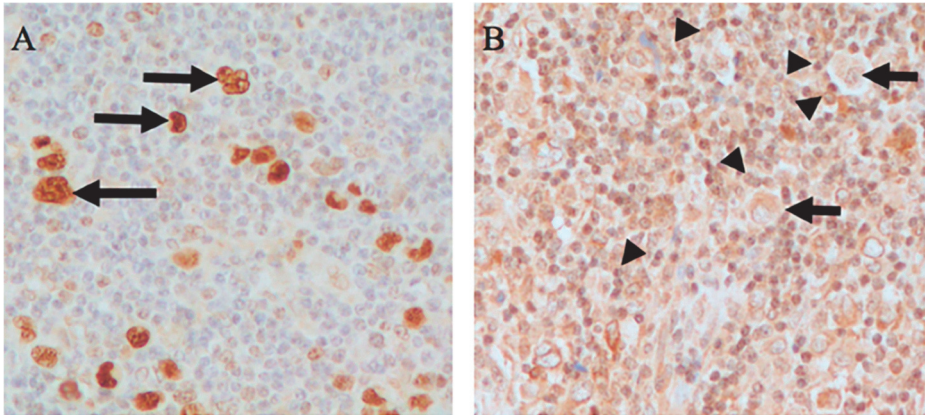


Fig. 2. (A) Positive immunohistochemical staining of Rif1 in HRS cells (arrows). (B) Positive immunohistochemical staining of SIRT6 in both HRS (arrows) cells and microenvironment/RCI (arrowheads)

Prognostic and predictive significance of the microenvironment

There is evidence that certain types of microenvironment are associated with prognosis. High amounts of mast cells or eosinophilia have been linked to a poor rate of failure-free survival (Pinto *et al.*, 1996, Molin *et al.*, 2002). The reason might be that mast cells and eosinophils express CD30L. Large amounts of Th2 cells in the microenvironment have been associated with prolonged disease-free survival (Wolf M, Albrecht S & Märki 2008). Similar results have been observed with Treg cells in two studies (Alvaro *et al.*, 2005, Schreck *et al.*, 2009). Treg cells seems to be in a significant role in immune evasion and most likely there will be tested Treg immune checkpoint inhibitor in cHL (Wein *et al.* 2017). High expression levels of active CTL cells are linked to poor clinical outcome and the same result is seen when low expression levels of Treg cell markers appear together with high-level expression of CTL markers (Oudejans *et al.*, 1997, Alvaro *et al.*, 2005). There is immune checkpoint inhibitor (ipilimumab), which activates the immune system by targeting CTLA-4. There is a clinical trial, where ipilimumab is combined with brentuximab vedotin (BV) has shown promising results (Diefenbach *et al.*, 2015).

The pathogenetic nature of the microenvironment is important for cHL and it has been a highlighted research topic for decades. Crosstalk significance is evident when trying to grow HRS cells in culture or in immunodeficient mice (Kapp *et al.*, 1993, Vockerodt *et al.*, 1998). There is more evidence of the crosstalk of HRS cells

with non-malignant cells, which enables new therapeutic strategies for treatment. Treatment does not need to involve targeting of HRS cells. For instance, alemtuzumab targets CD52-positive T cells in the microenvironment, ignoring CD52-negative HRS cells (Rodig *et al.*, 2006). Disturbing the PD1-PD-L1 system between HRS cells and T cells has shown promising results in refractory HL patients (Ansell *et al.*, 2015). Recent study showed that PD-1 blockade treatment predicts favourable outcome in those who have positive MHC class II on HRS. These results prove that PD-1 blockade treatment affects despite CD8+ T cell-mediated mechanisms also by CD4+ T cell-associated mechanism. (Roemer *et al.*, 2018) PD-1 inhibitors (nivolumab, pembrolizumab) have been approved by the US Food and Drug Administration and the European Medicines Agency for patients with refractory cHL.

The appearance of CD68+ or CD163+ tumour-associated macrophages (TAMs) in the microenvironment is linked to poor prognosis in HL (Steidl *et al.*, 2010, Tan *et al.*, 2012). In a recent meta-analysis, high expression levels of CD68+ and CD163+ TAMs in the microenvironment predicted poor overall survival (OS) and progression-free survival (PFS) in adult cHL patients and were also associated with EBV-positive HRS cells and certain clinical parameters (advanced stage, B-symptoms and IPS) (Guo, Cen, Tan & Ke 2016).

2.1.6 Clinical features and diagnostics of classical Hodgkin lymphoma

Patients with Hodgkin lymphoma can be diagnosed in different ways. Generally, the most common finding is an enlarged lymph node that is not painful. Typical enlarged lymph nodes can be found in supraclavicular, neck or mediastinal regions. HL is usually present only in lymph nodes; extranodal effusions are rare. A minority of HL patients also have B-symptoms, which are fever, notable weight loss, and night sweating. HL diagnosis is based on an adequate biopsy specimen, which should be evaluated by an experienced haematopathologist. For specific diagnosis the haematopathologist evaluates the morphology of the sample and uses immunohistochemical methods (Hoppe *et al.*, 2017).

After the haematopathologist has confirmed the diagnosis, the next step is to determine the extent and distribution of the disease by whole body CT scan, bone marrow biopsy and the assessment of risk factors of HL. Staging of HL is based on the Workshop on the Staging of Hodgkin's Disease at Ann Arbor in 1971 (Carbone,

Kaplan, Musshoff, Smithers & Tubiana 1971, Cheson *et al.*, 2014). The staging system is outlined in Table 2.

Table 2. Staging of Hodgkin lymphoma. Table adapted from Cheson *et al.*, 2014.

Stage	Involvement
Limited	
I	One node or a group of adjacent nodes
II	Two or more nodal groups on the same side of the diaphragm
Advanced	
III	Nodes on both sides of the diaphragm; nodes above the diaphragm with spleen involvement
IV	Additional non-contiguous extra-lymphatic involvement

Clinical Prognostic factors

Clinical prognostic factors can be used to evaluate the outcome of the disease and to choose the treatment strategy. These clinical prognostic factors are important for dividing patients into different groups as regards treatment strategy and they also aid in counselling (Gospodarowicz, O'Sullivan B & Koh 2006). Nevertheless, it is important to understand that clinical prognostic factors are more accurate in connection with patient groups than in individual patients. Clinical prognostic factors can be divided into three groups: tumour-related factors, host-related factors and environment-related factors (Gospodarowicz, O'Sullivan B & Koh 2006).

Limited-stage prognostic factors

In limited stage the most significant prognostic factor is still the combination of tumour-involved regions and the abundance of disease in individual regions (Specht 1988). There are so-called risk factors in limited stage, which are based on the definition of unfavourable prognostic groups in clinical trials (Table 3) (Henry-Amar *et al.*, 1991, Tubiana *et al.*, 1984). Functional imaging with fluorodeoxyglucose positron emission tomography (FDG-PET) is useful for assessing outcome and also the metabolic tumour volume during limited-stage treatment (Filippi *et al.*, 2013, Akhtari *et al.*, 2018). There are still false-positives during treatment, but a negative result after the early phase of treatment seems to have high predictive value and it also predicts good prognosis in limited-stage disease.

Table 3. Prognostic factors in limited-stage Hodgkin lymphoma.

Prognostic factors
Number of involved lymph node regions
Large tumour mass, particularly mediastinal
Tumour burden
B symptoms
Histological subtype
Age
Gender
ESR
Haemoglobin
Serum Albumin
(Early interim FDG-PET scan)

ESR= Erythrocyte sedimentation rate, FDG-PET = Fluorodeoxyglucose positron emission tomography

Advanced-stage prognostic factors

In advanced-stage HL the most important prognostic factor is still age (Peterson *et al.*, 1982). Co-morbidity increases with elderly patients, and with it the risk of treatment-related toxicity, mortality, and treatment reduction (Engert *et al.*, 2005, van Spronsen, Janssen-Heijnen, Lemmens, Peters, Coebergh 2005). Histological subtypes have a lesser role as prognostic factors. There are several studies where lymphocyte depletion and mixed cellularity subtypes have been found to be adverse prognostic factors (Allemani, Sant, De Angelis, Marcos-Gragera & Coebergh 2006, Ranson *et al.*, 1991). The most well-known variety of prognostic evaluation as regards advanced-stage disease involves use of “The International Prognostic Score” (IPS) (Table 4). The seven risk factors concerned are age ≥ 45 years, male gender, Ann Arbor Stage IV disease, haemoglobin < 10.5 g/dL, serum albumin < 40 g/L, white cell count $\geq 15 \times 10^9/L$ and lymphocyte count $< 0.6 \times 10^9/L$. (Hasenclever & Diehl 1998).

Table 4. The International Prognostic Score and its association with progression-free survival. Table adapted from Hasenclever & Diehl 1998.

Risk factors (n)	Freedom from disease progression in 5 years (%)
0	84
1	77
2	67
3	60
4	51
≥5	42

2.1.7 Treatment

Thomas Hodgkin started HL treatment by using cascarilla soda and iodine combination therapy in 1832 (Hodgkin 1832). In 1932 radiotherapy treatment was first confirmed as an effective treatment for HL patients in the Ontario Cancer Institute (Chevalier & Bernard 1932). Radiotherapy was the standard treatment for limited-stage patients for many decades (Duhmke *et al.*, 2001).

At present, standard chemotherapy in most cases consists of 4-drug combination therapy: doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) (Hoppe *et al.*, 2017). Doxorubicin affects DNA repair by disruption of topoisomerase-II, and free radicals are formed, which damage the cell (Gewirtz 1999). Bleomycin has a DNA-binding and an iron-binding region. It forms free radicals when binding with Fe²⁺ and these cause single- and double-strand DNA breaks (Burger, Peisach & Horwitz 1981). Vinblastine inhibits cell proliferation by disrupting microtubule function (Jordan, Thrower & Wilson 1991) and dacarbazine methylates DNA guanine, which complicates cell division (Pourahmad, Amirmostofian, Kobarfard & Shahraki 2009).

At the moment radiotherapy is part of the treatment programme for HL in some patients. Radiotherapy is more toxic in rapidly proliferating cancer cells than in normal cells (Bernier, Hall & Giaccia 2004). It produces free radicals, which appear from the ionization or excitation of the water component of the cells. They cause damage to cellular structures including DNA, ultimately leading to apoptosis (Hutchinson 1961). Over the last few decades radiotherapy techniques have improved greatly. Current radiotherapy treatments are carefully planned, taking advantage of pre- and post-chemotherapy imaging and giving accurate amounts of radiotherapy in precisely defined areas.

Guidelines for HL treatment vary globally. At the moment the principal treatment for limited-stage patients without risk factors is multidrug therapy, consisting of two to four cycles of ABVD chemotherapy. After two to three cycles of chemotherapy interim restaging should be carried out by positron emission tomography (PET). Responses to chemotherapy can be estimated in PET scanning by Deauville criteria (Table 5). After restaging, in patients with Deauville scores of 1 or 2, no further treatment is recommended. One additional ABVD cycle and radiotherapy is generally given if the Deauville score is 3 or 4. If the Deauville score is 5 a new biopsy is recommended (Hoppe *et al.*, 2017).

Limited-stage patients with risk factors are treated with ABVD given for four cycles, followed by interim restaging by means of PET. Patients having Deauville scores of 1 to 3 continue treatment with two additional cycles of ABVD plus radiotherapy, or radiotherapy alone, and a Deauville score of 4 indicates that treatment should be continued with two cycles of ABVD and followed by radiotherapy. If the Deauville score is 5, a new biopsy is recommended. If the biopsy sample is negative, then treatment continues with two cycles of ABVD with radiotherapy, and if the sample is positive, treatment continues and the disease is considered to be refractory (Hoppe *et al.*, 2017).

For advanced-stage patients chemotherapy is the main treatment. ABVD is the standard treatment for advanced-stage patients with an IPS of < 3 and escalated-dose BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisolone) for advanced-stage patients who are under 60 years old and have an IPS of ≥ 4 . For advanced stage patients with an IPS 3 the treatment strategy are decided case-by-case to either ABVD or escalated-dose BEACOPP (Hoppe *et al.*, 2017, Jyrkkiö, Mokka & Vasala 2014). The first restaging with PET is performed after the second cycle of ABVD chemotherapy, and with a Deauville score of 1 to 3 ABVD treatment is continued for an extra four cycles. At the moment, guidelines recommend that in cases with Deauville scores of 4 to 5, the treatment should be changed to escalated-dose BEACOPP (four cycles) after the first restaging (Hoppe *et al.*, 2017). After six cycles of ABVD chemotherapy it is recommended to carry out a second restaging. In the second restaging, a new biopsy is recommended in cases where the Deauville score is 4 to 5. A negative biopsy result leads to observation or radiotherapy if there was bulky tumour at the diagnosis, and a positive biopsy result is considered to indicate refractory disease (Hoppe *et al.*, 2017).

At the moment, there are several guidelines for treatment of relapsed or refractory HL. Treatment is chosen individually. It can be started with second-line

systemic therapy followed possibly by radiotherapy (depending on whether or not the patient has previously undergone irradiation). Another option is second-line systemic therapy combined with HDT/ASCR (high-dose therapy and autologous stem cell rescue) (Hoppe *et al.*, 2017).

Table 5. Deauville criteria. Table adapted from Barrington *et al.*, 2014

Deauville criteria scores	[¹⁸ F] FDG Uptake
1	no uptake
2	uptake ≤ mediastinum
3	uptake > mediastinum but ≤ liver
4	moderately ↑ uptake > liver
5	markedly ↑ uptake > liver and/or new sites of disease

[¹⁸F] FDG = Fludeoxyglucose

Challenges in the treatment of cHL

Currently, Hodgkin lymphoma is a very curable disease. Almost 90% of limited-stage- and 70% of advanced-stage patients can be cured with conventional treatment. On the other hand, up to 10% of advanced-stage patients will not achieve complete remission (CR) in first-line treatment. 10–15% of limited-stage- and 20–30% of advanced-stage HL patients will suffer from relapse after treatment. Only half of them can be cured with current salvage therapies (Evens, Hutchings & Diehl 2008, Kuruvilla 2009, Engert *et al.*, 2010).

As a result of good prognosis and also the young age of many patients, researchers have focused on the late effects of radio- and chemotherapy in HL. Survivors of HL are at an increased risk of cardiovascular diseases, pulmonary disease, secondary malignancies and treatment-related adverse effects. In one large cohort study it was found that 908 of 3905 HL patients were diagnosed with a secondary cancer, when the median follow-up time was over 19 years (van Nimwegen *et al.*, 2015, Schaapveld *et al.*, 2015). HL treatments are also associated with physical and psychosocial factors including infertility, sexual dysfunction and chronic fatigue (van der Kaaij, van Echten-Arends, Simons & Kluin-Nelemans 2010, Behringer *et al.* 2013, Daniëls, Oerlemans, Krol, Creutzberg & van de Poll-Franse 2014).

Limited-stage patients

In limited-stage HL one of most significant challenges is to recognize low-risk patients better and remove useless therapies. This helps to minimize long-term adverse consequences of the treatments (Schaapveld *et al.*, 2015). Bleomycin in older limited-stage HL patients (age over 60) has been shown to cause significantly more lung toxicity (including dyspnoea, interstitial pneumonitis and lung fibrosis), leucopenia and infection than in those patients who have been treated without it (ABVD vs. AVD). In addition, there are no significance differences in remission, progression rates or relapses, between treatments with and without bleomycin (Böll *et al.*, 2016).

When limited-stage patients with risk factors were treated with ABVD or ABVD plus radiotherapy, overall survival (OS) was better in the former group, but progression-free survival (PFS) was better in the latter (Behringer *et al.*, 2015). There are also clear links between secondary cancer and radiotherapy dose level and radiotherapy region. When patients were treated after chemotherapy with 20 Gy involved-field radiotherapy or extended-field radiotherapy of 35 Gy, secondary lung cancer and breast cancer risks were reduced significantly (57% and 77%, respectively) (Hodgson *et al.*, 2007).

Advanced-stage patients

In advanced-stage HL the challenge is to find the patients whose primary therapy would be unsuccessful (refractory disease) or who would suffer an early relapse. Of the two main types of chemotherapy BEACOPP is more efficient, but on the other hand it is more toxic than ABVD. BEACOPP is therefore used in high-risk patients and in salvage treatment (Jiang, Chen, Huang & Chen 2016).

The International Prognostic Score is often used for evaluation of advanced-stage treatment strategies. FDG-PET has been increasingly used to evaluate the final treatment outcome. Interim PET after two cycles of chemotherapy is now routine, since it has shown better prognostic value than the IPS (Hutchings *et al.*, 2006, Gallamini *et al.*, 2007). In advanced-stage patients, after chemotherapy treatment PET scanning is accurate in estimating disease progression and it can also be used to estimate the need of further radiotherapy (Engert *et al.*, 2012).

A new strategy in advanced-stage HL treatment is to add new drugs to the original form of combination chemotherapy (ABVD/BEACOPP). The purpose is to cut down the toxicity of BEACOPP and at the same time improve the efficacy of

ABVD. A potential chemotherapy drug may be BV, which is an anti-CD30 antibody–drug conjugate. CD30 is highly expressed on the surface of HRS cells and seldom expressed in normal cells (Katz, Janik & Younes 2011). Recent trial proved that BV in combination with AVD is more effective front-line treatment for advanced cHL than ABVD (Connors *et al.* 2018). Another trial, where the advanced stage cHL front-line treatment was BV in combination with etoposide, doxorubicin, cyclophosphamide, dacarbazine and dexamethasone (BrECADD), the results showed favourable toxicity profile and the studied treatment also effective. (Eichenauer *et al.* 2017) There is ongoing trial where BrECADD is compared directly with escalated BEACOPP.

2.1.8 Future aspects in the treatment of cHL

Multi-agent chemotherapy has been a great success in Hodgkin lymphoma treatment. Radiotherapy methods have also improved a lot. Nevertheless, there are still challenges in the treatment of refractory/relapsed or elderly cHL patients (Kuruvilla Keating & Crump 2011, Björkholm Svedmyr & Sjöberg 2011).

At the moment, both scientists and clinicians are interested in the pathogenesis of cHL, which enables new targets in treatment. Histologically, cHL is unique; it consists of only a small percentage of HRS tumour cells and is surrounded by non-neoplastic immune cells. The crosstalk between HRS cells and non-neoplastic immune cells is accurately regulated and it is a lifeline for tumour survival. This may open opportunities to develop novel targeted therapies. In addition to BV, there are encouraging new targeted therapy drugs for cHL (table 6), which utilize different perspectives of cHL biology, including Janus kinase (JAK), inhibitors, immune modulators, PI3K/Akt/mTOR inhibitors and also epigenetic histone deacetylase (HDAC) inhibitors (Kotla *et al.*, 2009, Johnston *et al.*, 2010, Younes *et al.*, 2012 Hao *et al.*, 2014, Gopal *et al.*, 2017). Use of PD1/PD-L1 is one of the most strongly emerging treatments, because of the impressive results with refractory/relapsed cHL patients (Ansell *et al.*, 2015). Also under development are new ways to use CD30+ cells in cHL. A novel strategy is to use CD30-specific non-neoplastic cytotoxic T cells in the treatment (Forero-Torres *et al.*, 2009, Ansell *et al.*, 2007). Another novel immune modulator in cHL is chimeric antigen receptor-modified T cells (CAR-T), which is has given encouraging results in other B cell malignancies (Levine, Miskin, Wonnacott & Keir 2016, Ramos *et al.* 2017).

Novel targeted therapies and immunotherapy are among new potential alternative treatments for HL. For refractory or relapsed patients these new

therapies can be combined with standard treatments or other novel therapies to improve prognosis. For advanced-stage patients, these novel therapies in first-line treatment with or without standard treatment enable more options in treatment strategies. New treatments are expected to reduce treatment-related toxicity, which could be especially useful for elderly and limited-stage patients (Mottok & Steidl 2018, Hoppe *et al.*, 2017).

Table 6. Novel drug their targets in cHL. Table adapted from Carbone *et al.* 2015.

Drug	Drug class	Target	Clinical use ¹	Reference
Brentuximab	ADC	CD30	Yes	Younes <i>et al.</i> , 2012
Vedotin				
Lenalidomide	Immunomodulator	T cells NK cells Tregs	No	Fehniger <i>et al.</i> , 2011
Nivolumab	MoAb	PD-1 receptor	Yes	Ansell <i>et al.</i> , 2015
Bendamustine	Chemotherapy	–	No	Moskowitz <i>et al.</i> , 2013
Rituximab	MoAb	CD20	No	Younes <i>et al.</i> , 2012
Galiximab	MoAb	CD80	No	Smith <i>et al.</i> , 2012
Panobinostat	HDACi	HDACs	No	Younes <i>et al.</i> , 2012
Mocetinostat	HDACi	HDACs	No	Younes <i>et al.</i> , 2011
Everolimus	mTOR inhibitor	mTORC1	No	Bennani <i>et al.</i> , 2017
CD30.CAR-Ts	Immunotherapy	CD30	No	Ramos <i>et al.</i> , 2017
Perifosine/sorafenib	AKT–MAPK inhibitor	AKT–MAPK	No	Guidetti <i>et al.</i> , 2014

ADC = antibody–drug conjugate, MoAb = monoclonal antibody, HDACi = histone deacetylase inhibitor, HDAC = histone deacetylase, mTOR = mammalian target of rapamycin, mTORC1 = mammalian target of rapamycin complex 1, PD-1 = programmed cell death protein 1; MAPK = mitogen-activated protein kinase, NK = natural killer, Tregs = regulatory T cells, CD30.CAR-Ts = CD30-specific chimeric antigen receptor.

¹European Medicines Agency has approved treatment for cHL

2.2 Oxidative stress and antioxidant enzymes

2.2.1 Oxidative stress

Reactive oxygen species (ROS) are produced in all aerobic organisms as a phenomenon of aerobic respiration, mostly as by-products of mitochondrial oxidative phosphorylation (Jabs 1999, Poyton, Ball & Castello 2009). Normal cells generate ROS such as hydroxyl radicals (OH^{*}), hydrogen peroxide (H₂O₂) and superoxide anions (O₂^{*-}), and hydrogen and organic peroxides from molecular oxygen in biological reduction (Fridovich 1978). Oxidative stress results from imbalance of ROS and ROS-suppressing protective mechanisms, mainly

antioxidant enzymes. Oxidative stress damages cells' biomolecules, and continued oxidative stress can promote carcinogenesis (Klaunig *et al.*, 1998, Sies, Berndt & Jones 2017).

There are several ways to evaluate oxidative stress in cells. Oxidative stress damage to DNA can be assessed by assay of 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is the end product of hydroxyl radical attack on DNA, and is considered to reflect oxidative stress-mediated damage with high precision (Kasai 1997, Evans, Dizdaroglu & Cooke 2004). Peroxynitrite (ONOO^-) is formed by the reaction between nitric oxide and superoxide ($\text{NO} + \text{O}_2^- = \text{ONOO}^-$), and nitrotyrosine is a stable end-product of peroxynitrite metabolism. It can be used for the evaluation of nitrosative/oxidative damage in proteins (Radi, Rodriguez, Castro & Telleri 1994).

2.2.2 Antioxidant enzymes

As a response to an increasing amount of ROS and oxidative stress, the cell has to have effective antioxidant systems. One member of the antioxidant system is the peroxiredoxin (Prx) group. Peroxiredoxins participate in cellular antioxidant defence by reducing alkyl hydroperoxides and hydrogen peroxide to (corresponding) alcohol and water (Rabilloud *et al.*, 2002). In total there are six different isoforms of Prx in mammals and they are found in the cytosol (Prxs I, II, III, V and VI), in peroxisomes (Prxs IV and V), in lysosomes (Prxs IV and VI), in the endoplasmic reticulum and Golgi apparatus (Prx IV), and also in mitochondria (Prxs III and V) (Kang, Baines & Rhee 1998, Okado-Matsumoto, Matsumoto & Fujii 2000, Declercq, Evrard, Clippe, Stricht & Bernard 2001, Kinnula *et al.* 2002).

The first antioxidant enzyme found was superoxide dismutase (SOD). The main function of SOD is catalyzing the dismutation of O_2^- to H_2O_2 (Valko, Rhodes, Moncol, Izakovic & Mazur 2006). In mammals, there are three isoforms of SOD. The differences in the isoforms are mostly in the active-site metal (Kohen & Nyska 2002). SOD2, known as manganese SOD (MnSOD) is found in the mitochondrial matrix and it has been discovered to be one of the most important antioxidant enzymes in mammalian tissues (VanRemmen *et al.*, 2003).

Thioredoxins (Trxs) are a group of antioxidant enzymes whose role is to regulate the redox state of the cell. Trx enzymes participate in many significant physiological actions, including inhibiting apoptosis and activating transcription factors such as NF- κ B. Trx itself is also an efficient growth factor (Hirota *et al.*, 1999, Powis & Montfort 2001, Karihtala & Soini 2007). Glutathione (GSH) is

another vital antioxidant enzyme. It is involved in DNA and protein synthesis and also DNA repair and proliferation systems (Ballatori *et al.*, 2009).

2.2.3 Oxidative stress and antioxidant enzymes in cancer

An imbalanced redox state damages cell structure and functions and may induce somatic mutations and neoplastic transformation (Karihtala & Soini 2007, Fang, Seki & Maeda 2009). Oxidative stress markers and redox state-regulating enzymes have been shown to be of prognostic significance in many solid malignancies (Reuter, Gupta, Chaturvedi & Aggarwal 2010). In oesophageal, ovarian and colorectal carcinoma high 8-OHdG levels have been associated with poor prognosis (Karihtala, Soini, Vaskivuo, Bloigu & Puistola 2009, Sheridan *et al.*, 2009, He, Zhao, Wang, Zhang & Wang 2014, Pylväs-Eerola, Karihtala & Puistola 2015), but then in breast and bladder cancers as well as in melanomas low levels of 8-OHdG are associated with poor prognosis and aggressive disease (Sova, Jukkola-Vuorinen, Puistola, Kauppinen, Karihtala 2010, Karihtala, Kauppila, Puistola, Jukkola-Vuorinen 2011, Soini *et al.*, 2011, Hintsala *et al.*, 2016). In diffuse large B-cell lymphoma (DLBCL), 8-OHdG has been involved with extranodal involvement, a high International Prognostic Index and poorer progression-free survival (Peroja *et al.*, 2012, Pasanen *et al.*, 2012). High expression levels of nitrotyrosine have been connected with poor prognosis in urinary bladder cancer, oesophageal carcinoma, DLBCL and melanoma (Soini *et al.*, 2011, Kato *et al.*, 2011, Peroja *et al.*, 2012, Hintsala *et al.*, 2016).

In cancer, Prxs have been linked to carcinogenesis and also partly to the development of drug resistance (Nicolussi, D'Inzeo, Capalbo, Giannini & Coppa 2017, Teppo, Soini & Karihtala 2017). The role of Prxs as prognostic markers in cancers is still uncertain. Low expression levels of Prxs have been linked to poor survival in pancreatic adenocarcinoma (Prx I, Prx VI), DLBCL (Prx VI), follicular lymphoma (FL) (total Prx I–VI), breast cancer (Prx III, VI) and melanoma (PrxI, IV) (Karihtala, Mäntyniemi, Kang, Kinnula & Soini 2003, Kuusisto *et al.*, 2015, Hintsala, Soini, Haapasaari & Karihtala 2015, Isohookana, Haapasaari, Soini & Karihtala 2016, Peroja *et al.*, 2016). On the other hand, strong Prx expression is associated with poor prognosis in hepatocellular carcinoma (Prx I), melanoma (Prx III) and breast and pancreatic cancer (Prx VI) (Karihtala, Mäntyniemi, Kang, Kinnula & Soini 2003, Sun *et al.* 2014, Hintsala, Soini, Haapasaari & Karihtala 2015, Cai, Zhai, Wu & Tang 2015). The role of Prxs in chemoresistance has been studied, and in particular, Prx II has been linked to breast cancer chemoresistance

(Wang, Diaz & Yen 2014). There is evidence that Prx II up-regulation is potentially linked to gefitinib resistance (Kwon *et al.*, 2015, Teppo, Soini, Karihtala 2017). Prxs are up-regulated in cancer cells, but the expression seems to vary widely in different malignancies (Karihtala & Soini 2007).

Early reports on MnSOD indicated low expression in cancers, and a role as a tumour suppressor (Zhong, Oberley, Oberley & St Clair 1997). At present, in several different cancers, MnSOD levels are known to be increased, and MnSOD expression has also been found to be increased at metastatic stage and during tumour progression in head and neck, pancreatic and gastric cancers, for example (Hempel, Carrico & Melendez 2011). Increased expression of MnSOD in several cancers is thought to be due to increased oxidative stress, boosting the redox system (Miriyyala *et al.*, 2012).

2.3 Hypoxia

Hypoxia is defined as a low level of oxygen (O₂). It appears in both non-pathological and pathological conditions and is caused by an imbalance between availability and consumption of O₂. Decreased oxygen availability causes alterations to metabolism (to a less oxygen-consuming state) by increasing the rate of glycolysis and decreasing oxidative phosphorylation. Cells within organisms adapt to hypoxia by remodelling cellular signalling pathways (Semenza 2012).

2.3.1 Hypoxia-inducible factor (HIF)

The main response to hypoxia in cells involves hypoxia-inducible factor (HIF). HIF consists of two main subunits, HIF α and HIF β (Wang & Semenza 1995). HIF β is continuously expressed in cells, but HIF α is stabilized only in a hypoxic environment. There are three HIF α isoforms altogether (HIF-1 α , HIF-2 α and HIF-3 α) (Ema *et al.*, 1997, Gu, Moran, Hogenesch, Wartman & Bradfield 1998). A hypoxic environment stabilizes HIF-1 α (and HIF-2 α) and brings about heterodimerization with HIF-1 β , which regulates the transcription of HIF-1 α target genes (Semenza 2012). The target genes include some of those involved in metabolism (Gordan, Thompson CB & Simon 2007), angiogenesis (Rey & Semenza 2010), apoptosis, proliferation (Khan *et al.*, 2011), suppression of immune reactivity (Yotnda, Wu & Swanson 2010), the DNA repair system (Bristow & Hill 2008) and ROS homeostasis (Guzy *et al.*, 2005).

The high growth rate of tumours outstrips expansion of the vasculature, which leads to deficient oxygen levels in the tumour area. To prevent lack of oxygen, tumour cells need to secrete profuse amounts of proangiogenic factors in order to promote revascularization (Casazza *et al.*, 2014). The oxygen tension in human tumours can reach extremely low levels compared with absolute oxygen tension (Brown & Wilson 2004). Tumours adapt to hypoxia mainly via the HIF pathway and its target genes play key roles in cancer biology, in tumour progression, cancer metastasis, and resistance to radiation therapy and chemotherapy (Hanahan & Weinberg 2011, Semenza 2012). HIF-1 α and HIF-2 α expression predict poor survival in several types of cancer (Johnson & Simon 2011, Sormendi S, Wielockx 2018). Overexpression of HIF-1 α is also associated with failure to achieve complete remission after radiation therapy in oropharyngeal cancer (Aebersold *et al.*, 2001). On the other hand, in renal cancer, DLBCL and head and neck cancers HIF-1 α is correlated to improved survival (Beasley *et al.*, 2002, Evens *et al.*, 2010, Lidgren *et al.*, 2005).

Hypoxia has been linked to the early-stage pathogenesis of cHL. Hypoxia affects HRS cell phenotype. HIF-1 α up-regulates ID2 and NOTCH1, which are hallmarks of HRS cells. HIF α stabilization also causes further epigenetic and/or genetic modifications, which lead to the final form of HRS cells (Wein & Kuppers 2015).

2.3.2 Prolyl hydroxylase domain (PHD) proteins

In mammalian cells, there are three isoforms of prolyl hydroxylase domain proteins (PHD1, PHD2 and PHD3). They control oxygen homeostasis by regulation of HIF stability (Epstein *et al.*, 2001). PHD protein is inhibited in hypoxic environments, which allows stabilization of HIF and induces target gene transcription. Besides regulation of HIF stability, PHD proteins control various cellular pathways independently of HIF. PHD isoforms are expressed in all tissues, albeit at different levels. PHD2 is considered to be the most important oxygen sensor as regards HIF stabilization (Berra *et al.*, 2003). PHD1 regulates HIF by suppressing HIF-1 α , especially in protracted hypoxia, and overexpression of PHD1 inhibits tumour growth via HIF-1 α (Appelhoffl *et al.*, 2004, Eres *et al.*, 2004). PHD1 and PHD3 inhibit NF- κ B formation by inhibiting I κ B kinase β (IKK β), which reduces NF- κ B signalling (Cummins *et al.*, 2006). PHD1 together with PHD3 represses transcriptional activity of activating transcription factor 4 (ATF4), which is highly expressed in hypoxic environments. ATF4 contributes to tumour growth by way of

many different mechanisms including regulation of tumour survival, and angiogenesis (Ameri & Harris 2008). PHD3 also suppresses cell growth by repressing EGFR (epidermal growth factor receptor) signalling (Garvalov *et al.* 2014).

PHD proteins have been proposed to be tumour suppressors, but they are also associated with chemoresistance and tumour growth (Klotzsche-von Ameln *et al.*, 2011, Fox *et al.*, 2011, Nguyen & Duran 2017). PHD1 has been linked to poorer survival in pancreatic endocrine tumours, prostate adenocarcinoma and non-small-cell lung carcinoma (Boddy *et al.*, 2005, Couvelard *et al.*, 2008, Andersen *et al.*, 2011). On the other hand, overexpression of PHD1 has also been linked to inhibition of tumour growth. The role of PHD2 in cancer progression is controversial. The expression of PHD2 has been shown to be inversely correlated to tumour-forming potential, but then when PHD2 has been completely inhibited, tumorigenesis is reduced (Lee *et al.*, 2008). PHD3 overexpression has been seen in several cancer types, including gastric, lung and breast cancer (Andersen *et al.*, 2011, Su *et al.*, 2012, Peurala, Koivunen, Bloigu, Haapasaari & Jukkola-Vuorinen 2012). In gastric, hepatocellular and breast cancers high PHD3 levels are linked with favourable prognosis (Su *et al.*, 2012, Peurala, Koivunen, Bloigu, Haapasaari & Jukkola-Vuorinen 2012, Ma *et al.*, 2017). In colorectal cancer PHD3 expression levels are lower than in normal tissue (Xue *et al.*, 2010).

2.4 Epigenetics

The definition of epigenetics is contentious and ambiguous, but the main concept is regulation of DNA-templated processes without remodelling the DNA sequence (Berger, Kouzarides, Shiekhhattar & Shilatifard 2009). For normal evolution and maintenance of tissue-specific gene expression, epigenetic mechanisms are essential for mammals (Sharma, Kelly & Jones 2010). When epigenetic mechanisms are disrupted, this can lead to changed gene function and further to malignant cellular transformation (Jones & Baylin 2002).

There are four main epigenetic mechanisms in cancer, these involving DNA methylation, histone modifications, nucleosome positioning, and non-coding RNAs. In DNA methylation the covalent cytosine residues in CpG dinucleotides are methylated, which leads to silencing of gene expression. There are three active DNA methyltransferases (DNMTs) in mammals and mutations in these DNMTs have been linked to cancer formation (Li, Bestor & Jaenisch 1992, Wang & Leung 2004, Robertson 2005). In histone modifications, histone N-tails can be modified

by methylation, acetylation, ubiquitylation, sumoylation and phosphorylation (Kouzarides 2007). Nucleosome positioning plays a significant role in chromatin structure and in gene regulation activity (Jiang & Pugh 2009). Non-coding RNAs participate in transcriptional and post-transcriptional gene silencing by way of base pairing in their targets (Wang & Chang 2011).

2.4.1 KDM4

Histone methylation is a reversible reaction. There are two main enzyme families found in humans that can demethylate histones, i.e. lysine-specific demethylase (LSD) 1 & 2 and Jumonji D2 proteins (JMJD2 proteins, known also as KDM4 proteins). KDM4 can demethylate histones through an oxidative reaction requiring Fe(II) and ketoglutarate (Tsukada *et al.*, 2006). There are four KDM4 family members in total (KDM4A–D). These demethylase enzymes can demethylate di- and trimethylated histone H3 lysine 9 (H3K9) and histone H3 lysine 36 (H3K36) and also trimethylated histone H1 isotype 4 lysine 26 (H1.4K26) (Figure 3) (Berry 2013). H3K9 and H1.4K26 trimethylation are linked to transcription repression and also to heterochromatin formation, while H3K36 methylation activates gene expression (Chi, Allis CD & Wang 2010, Dawson & Kouzarides 2012). Because of the capability of remodelling the epigenetic landscape, KDM4 family members have been linked to several tumour types (Berry & Janknecht 2013).

KDM4A is a transcription regulator that can either repress or stimulate gene transcription. It is involved in DNA damage response (DDR) by avoiding the recruitment of p53-binding protein 1 (53BP1) (Mallette *et al.*, 2012). KDM4A forms a complex with p53 and represses tumour suppressor p53 transcriptional activity. KDM4A expression is increased in several cancer types, including breast, lung and colorectal cancers (Shin & Janknecht 2007, Berry, Shin S, Lightfoot SA & Janknecht 2012, Kim, Shin, Berry, Oh & Janknecht 2012, Kogure *et al.* 2013).

Similarly to KDM4A, KDM4B is also linked to DDR. p53 regulates KDM4B gene expression by binding to it and inhibiting p53 transcriptional target genes: cyclin-dependent kinase inhibitor 1 (p21), p53-upregulated modulator of apoptosis (PUMA) and p53-inducible gene 3 (PIG3) (Li *et al.*, 2016, Castellini *et al.*, 2017). Under hypoxic conditions, HIF-1 α binds to KDM4B promoter, which up-regulates hypoxia-inducible genes including those involved in cell proliferation, the cell cycle, and invasion, and suppresses cell apoptosis genes (Tausendschon, Dehne & Brune 2011, Fu *et al.*, 2012). KDM4B is overexpressed in several cancers, including colorectal, breast, gastric, prostate, lung and bladder malignancies (Shi

et al., 2011, Toyokawa *et al.*, 2011, Fu *et al.*, 2012, Bjorkmann *et al.*, 2012, Zhao *et al.*, 2013).

The oncogenic role of KDM4D is still unclear and structurally it differs from the other KDM4 members (Berry & Janknecht 2013). KDM4D stimulates p53 gene expression, especially gene p21 (Kim, Oh, Shin & Janknecht 2012). On the other hand, in cell lines there is evidence that KDM4D is a pro-proliferative and pro-survival demethylating enzyme (Kim, Oh, Shin & Janknecht 2012). KDM4D demethylase activity promotes the repair of double-strand breaks (DSBs). KDM4D is regulated by poly (ADP-ribose) polymerase 1 (PARP1) -mediated poly-ADP ribosylation (PARylation). Deficiency of KDM4D attenuate a cell's DSB repair system and has been shown to sensitize cells to ionizing radiation (Khoury-Haddad *et al.*, 2014). TNF- α has been shown to induce KDM4D expression in cells of the microenvironment, including macrophages and dendritic cells. Also, KDM4D demethylates H3K9, which is involved in the TNF α response. KDM4D can influence tumorigenesis in both cancer cells and the microenvironment by way of TNF- α production (Zhu, Essen & Saccani 2012, Berry & Janknecht 2013).

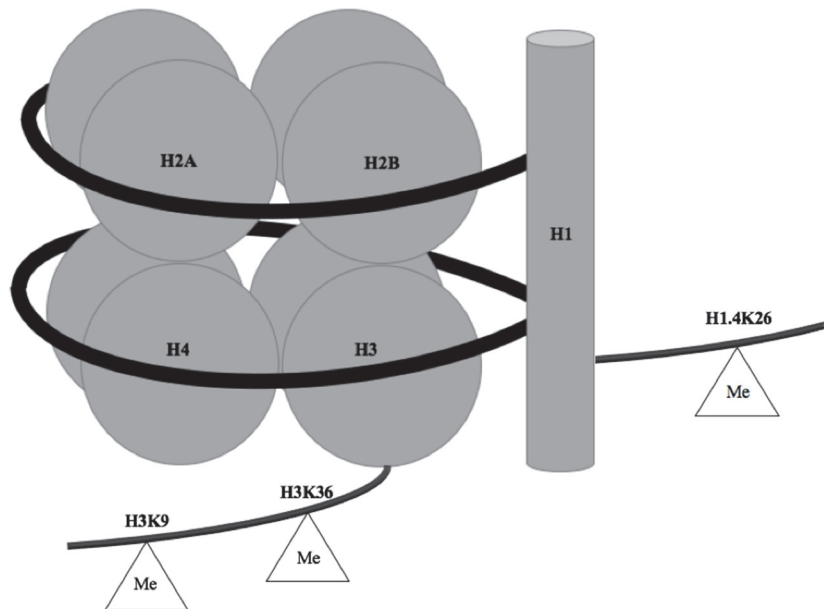


Fig. 3. Demonstrates the structure of nucleosome. Core histones (H2A, H2B, H4 and H3) form octamer and H1 is linker histone. DNA is packed around histones (black line). KDM4 modify histone tails by demethylating H3K9, H3K36 and H1.4K26

2.4.2 Sirtuins

Sirtuins make up the class III protein deacetylase family, which require nicotinamide adenine dinucleotide (NAD⁺) as a co - substrate in lysine deacetylase and mono(ADP-ribosyl)transferase activity (Imai 2000) There is a total of seven sirtuin proteins (SIRT1–7) in mammals, which all have their own unique subcellular localization and divergent functions (Finkel, Deng & Mostoslavsky 2009). Sirtuins have important biological roles, influencing genome stability, cellular metabolism and lifespan regulation in human biology and disease (Haigis & Sinclair 2010, Sebastian, Satterstrom, Haigis & Mostoslavsky 2012). The enzymatic activities of sirtuins and their effects on cancer have been widely studied. They can either suppress or promote cancer progression, depending on cellular and molecular contexts (Chalkiadaki & Guarente 2015).

SIRT1 is chiefly a nuclear deacetylase (Michishita, Park, Burneskis, Barrett & Horikawa 2005). It deacetylates the acetyl group from histones and non-histone proteins and further regulates target gene expression and protein activities. SIRT1 influences various essential cellular processes such as cell proliferation, differentiation, apoptosis, metabolism, and DNA damage and stress responses (Yeung *et al.*, 2004, Brunet *et al.*, 2004 Firestein *et al.*, 2005, Hallows, Lee S & Denu 2006, Li *et al.*, 2008, Kim *et al.*, 2009). Studies have shown that SIRT1 has both oncogenic and tumour-suppressive roles (Chalkiadaki & Guarente 2015). When DNA damage or oxidative stress occurs in the cell, SIRT1 overexpression suppresses p53 - mediated apoptosis (Cheng *et al.*, 2003). SIRT1 can also attenuate the action of transcription factor E2F1, which can result in apoptosis under DNA damage or oncogenic signalling (Wang *et al.*, 2006). It is considered that SIRT1 favours DNA repair and genomic stability, and it protects against initiation of cancers (Chalkiadaki & Guarente 2015). SIRT1 has been observed to be a disease-promoting factor in several cancers including breast, prostate, lung, colon and liver cancer (Huffman *et al.*, 2007, Jang *et al.*, 2008, Lee *et al.*, 2011, Chen *et al.*, 2012, Noh *et al.*, 2013). On the other hand, a tumour-suppressive role of SIRT1 has been detected in human head and neck squamous cell carcinoma (Noguchi *et al.*, 2013). SIRT1 deacetylates HIF-1 α and inhibits its transcription, thereby possibly protecting against vascular formation and tumour growth (Lim *et al.*, 2010).

SIRT4 is localized in the mitochondria and it is a NAD⁺- dependent protein adenosine diphosphate (ADP)-ribosyl transferase (Michishita *et al.*, 2005, Haigis *et al.*, 2006). It is closely linked to cellular metabolic functions such as insulin secretion and fatty acid oxidation (Ahuja *et al.*, 2007, Nasrin *et al.*, 2010). SIRT4

has been considered to be a tumour suppressor (Jeong *et al.*, 2013). SIRT4 expression in normal tissues has been found to be notably lower than in human cancers, including bladder, breast and lung cancer as well as leukaemia (Garber *et al.*, 2001, Blaveri *et al.*, 2005, Choi *et al.*, 2007, Jeong *et al.*, 2013). In a murine model, overexpression of SIRT4 has been shown to reduce cell proliferation and transformation, and also delay tumour progression (Csibi *et al.*, 2013). Another study on mice revealed that the loss of SIRT4 results in a tumorigenic phenotype, including increased glutamine-dependent cell proliferation and stress-induced genomic instability (Jeong *et al.*, 2013).

SIRT6 is primarily known as a nuclear protein, but in several studies cytoplasmic locations have also been noticed. SIRT6 has deacetylase and ADP-ribosyltransferase activity (Liszt Ford, Kurtev & Guarente 2005, Azuma *et al.*, 2015). It influences in transcription regulation, genome stability, metabolism and also lifespan (Kawahara *et al.*, 2009, McCord *et al.*, 2009, Kim *et al.*, 2010, Zhong *et al.*, 2010, Mao *et al.*, 2012). Like SIRT1, SIRT6 attenuates HIF1 α target genes and it also decreases transcriptional activity of the MYC gene (Zhong *et al.*, 2010). SIRT6 has an important role in regulating metabolism by controlling glucose homeostasis. It inhibits aerobic glycolysis, also known as the Warburg effect (Sebastian, Satterstrom, Haigis & Mostoslavsky 2012). SIRT6 is needed in the DSB repair system. It is involved in the non-homologous DNA end-joining (NHEJ) repair pathway, stabilizing DNA-dependent protein kinase (DNA-PK), which promotes DSBs to the pathway (McCord *et al.*, 2009). Under oxidative stress, SIRT6 activates PARP-1 by stimulating its mono- and poly-ADP-ribosylase activity, which results in enhanced NHEJ and homologous recombination (HR) repair pathways (Mao *et al.*, 2012).

The multifaceted role of SIRT6 in the cell is chiefly associated with tumour suppression (Chalkiadaki & Guarente 2015). Nevertheless, in specific cancer types, high expression levels of SIRT6 have been linked to poorer outcomes. (Desantis, Lamanuzzi & Vacca 2018) In breast cancer SIRT6 has been associated with resistance to chemotherapy and in pancreatic cancer cells SIRT6 contributes to cytokine production and migration, which causes inflammation, angiogenesis and metastasis (Khongkow *et al.* 2013, Bauer *et al.*, 2012).

2.5 DNA repair

To respond to DNA damage, cells have acquired effective repair mechanisms to defend against various forms of endogenous and environmental DNA damage. In

mammalian cells, there are at least five major DNA repair mechanisms: mismatch repair (MMR), nucleotide excision repair (NER), repair of single-strand breaks (SSBs), which includes base excision repair (BER), and repair of DSBs, which includes HR and NHEJ (Altieri, Grillo, Maceroni & Chichiarelli 2008).

The HR pathway, a form of DSB repair, is effective during synthesis (S) and gap 2 (G2) phases of the cell cycle. In HR, there are numerous proteins involved in the complex repair process (Shrivastav, De Haro & Nickoloff 2008). HR is important for preservation of genomic stability; if mutations occur they can compromise the integrity of genes (Evers, Helleday & Jonkers 2010). The Mre11-Rad50-NBS1 (MRN) complex binds to DNA ends and recruits nucleodepolymerases to remove damaged bases, and it initiates the HR process (Zhong *et al.*, 1999).

The NHEJ pathway is another DSB-repairing pathway. It is the most difficult way to repair DNA and is also cytotoxic. While HR repair occurs effectively in the S and G2 phases, NHEJ repairs DNA in all cell-cycle stages (Beucher *et al.*, 2009). NHEJ also competes efficiently for DSBs, although HR is also available (phases S and G2). The NHEJ process starts when two polypeptides, KU70 (XRCC6) and KU80 (XRCC5) recognize breaks and form heterodimers at the DNA ends. Heterodimer formation induces other NHEJ proteins, including DNA - PK catalytic subunits (DNA-PKcs) which form an active DNA - dependent protein kinase (DNA-PK). DNA-PK activation helps the recruitment of multiple proteins involved in limited DNA end-processing (Yoo & Dynan 1999).

DNA repair in cancer treatment

DNA continuously faces damage from agents within the cell, but also from external chemicals and radiation (Lindahl 1993). The main function of the DNA damage response (DDR) is to protect against genomic instability by recognizing DNA damage and mediating DNA repair. Dysregulation of the DDR is characteristic of cancer development (Hanahan & Weinberg 2011). However, the DDR also contributes to resistance to DNA-damaging chemotherapy and radiotherapy (Curtin 2012, Gavande *et al.*, 2016).

Reactive oxygen species create various oxidative DNA adducts, and bring about base modification, deoxyribose oxidation, single- and/or double-strand breakage and DNA-protein cross-links (Cadet, Berger, Douki & Ravanat 1997). In the case of ionizing radiation (IR) DNA is the main target. Direct action occurs when charged particles (ions or electrons) ionize DNA directly. Alternatively, in

indirect action, ionizing radiation causes water radiolysis, generating reactive OH• radicals, which react with DNA (Ward 1988). IR brings about DNA damage events that include changes in the DNA bases and sugars, which leads to the DDR (repair of single- and double-strand breaks) (Duncan Lyngdoh & Schaefer 2009).

There is evidence that malfunction of NHEJ may be linked to radioresistance, especially with an increase of DNA-PK function (Burma & Chen 2004). Of the DSB repair mechanisms, HR has been shown to have a crucial role in the development of radioresistance (Somaiah *et al.*, 2012, Somaiah, Yarnold, Lagerqvist, Rothkamm & Helleday 2013). Breast cancer 1 (BRCA1) deficiency blocks the HR pathway and it has also been shown to be linked to increased radiosensitivity in vivo and in vitro (Speit & Trenz 2004, Drost *et al.*, 2011). Cells have been shown to be most resistant to IR during the S phase, when HR is active (Hufnagl *et al.*, 2015). There are many tumour suppressors involved in the HR pathway, including BRCA1, BRCA2 and the ataxia-telangiectasia mutated (ATM) gene. At the same time, HR-defective proteins sensitize cancer to treatments, including radiotherapy, topoisomerase I poisons and cross-linking agents (cisplatin, carboplatin and nitrosoureas) (Evers, Helleday T & Jonkers 2010).

2.5.1 Rif1

Human Rap1 interacting factor 1 (Rif1) has an important role in DNA replication, DNA repair and the maintenance of genomic integrity (Xu *et al.*, 2010, Yamazaki, Hayano & Masai 2014, Escribano-Diaz *et al.*, 2013). In mammals, Rif1 protein binds aberrant telomeres at DNA damage sites (Silverman, Takai, Buonomo, Eisenhaber & de Lange 2004). When DSBs are detected, tumour suppressor 53BP1 accumulates by the chromatin at the break sites (Anderson, Henderson C, Adachi 2001). 53BP1 is a major promoter in the NHEJ pathway and Rif1 binds to 53BP1 in a DNA-damage-dependent manner (Silverman, Takai, Buonomo, Eisenhaber & de Lange 2004). Rif1 operates like an effector molecule of 53BP1, leading the DSBs into the NHEJ pathway and at the same time it suppresses BRCA1/CtIP, which facilitates the HR pathway.

2.5.2 MGMT

O⁶-alkylguanine DNA alkyltransferase (MGMT) is a DNA-repair protein. It removes the methyl or alkyl group at the O⁶ position of guanine and then returns the guanine to its normal shape without inducing DSBs (Liu & Gerson 2006). Lack

of MGMT can cause mutations that can lead to carcinogenesis (Soejima, Zhao & Mukai 2005). MGMT has been found to be epigenetically silenced in several human tumours, predicting a response to alkylating chemotherapeutics in tumours such as glioblastoma, low-grade glioma, and DLBCL (Hegi *et al.*, 2005, Everhard *et al.*, 2006, Uccella *et al.*, 2009). In an HL culture model, methylated MGMT promoter has been shown to predict more sensitivity to dacarbazine treatment (Kewitz, Stiefel, Kramm & Staeger 2014).

3 Aims of the present study

The aim of this study was to investigate the prognostic role of biomarkers related to both oxidative stress and factors connected to cellular stress responses in HL by using immunohistochemical methods. The biomarkers were evaluated in HRS cells and in the cHL microenvironment to explore their roles in HL. We hypothesized that it may be possible to offer new approaches to individualize patient treatment in a convenient way. It is important to design new strategies to avoid overtreatment-related toxicity among patients who could do well with less, and to aid therapeutic selection for those HL patients who are at risk of refractory disease or relapse.

More specifically, the aims of the present work were as follows:

- To evaluate the possible prognostic roles of several antioxidant enzymes and oxidative stress markers in HL.
- To investigate whether hypoxia-inducible factors and prolyl hydroxylase domains have a role in resistance to first-line treatment, or if they have a prognostic role in cHL.
- To characterize the expression of epigenetic regulators in cHL and to evaluate their predictive and prognostic significance.
- To find out if proteins involved in the DNA damage response are associated with treatment response or relapses.

4 Material and methods

4.1 Patient material

The material for this retrospective study included diagnostic biopsy samples from patients with HL, treated at Oulu University Hospital, Kuopio University Hospital and the Central Hospitals of Kainuu, Länsi-Pohja and Lapland. Diagnoses were carried out by a specialist haematopathologist (KMH), with histopathological examination and immunohistochemical studies of the tissue samples, which were taken from lymph nodes. All lymphomas were diagnosed and treated in 1997–2015 (Studies I and III: 1999-2012; study II: 1997-2015; study IV: 1997-2012). All patients were treated with ABVD chemotherapy in the first-line setting. Clinical and histological data concerning the four studies are presented in Table 7. In study I, there were six NLPHL patients, which were excluded from studies II-IV. In study III, the biopsy samples of two patients were missing and in study IV totally eight biopsy samples were missing (compared to study I). To the study II (the latest study), we collected the missing samples and also added twelve new cHL patients.

Table 7. Demographics of clinical and histological data.

Parameter	Study I		Study II		Study III		Study IV	
	n	%	n	%	n	%	n	%
Number of patients	99		115		91		85	
Histology (ICD-10 code)								
Nodular sclerosis (C81.1)	68	68.7	88	76.5	68	74.7	63	74.1
Mixed cellularity (C81.2)	14	14.1	18	15.7	14	15.4	14	16.5
Other (C81.7)	9	9.1	6	5.2	7	7.7	6	7.1
Unspecified (C81.9)	2	2.0	3	2.6	2	2.2	2	2.4
Nodular lymphocyte predominant (C81.0)	6	6.1	0		0		0	
Median age at diagnosis, years (range)	37	(16-85)	28	(11-85)	26	(16-85)	32	(16-85)
Male	47	47.5	54	47.0	45	49.5	42	49.4
Female	52	52.5	61	53.0	46	50.5	43	50.6
B-symptoms								
Absent	66	66.7	76	66.1	59	64.8	53	62.4
Present	33	33.3	39	33.9	32	35.2	32	37.6
Stage								
Limited	47	47.5	56	48.7	45	49.5	44	51.8
Advanced	52	52.5	59	51.3	46	50.5	41	48.2

Parameter	Study I		Study II		Study III		Study IV	
	n	%	n	%	n	%	n	%
Limited stage risk factors								
None	21	44.7	24	42.9	19	42.2	18	40.9
≥1	26	55.3	32	57.1	26	57.8	26	59.1
Limited stage risk factors								
None	21	44.7	24	42.9	19	42.2	18	40.9
≥1	26	55.3	32	57.1	26	57.8	26	59.1
International Prognostic Score								
0–2	40	78.4	50	84.7	35	76.1	31	75.6
3–7	11	21.6	9	15.3	11	23.9	10	24.4
WHO performance status ≥1	31	31.3	28	24.3	29	31.9	28	32.9
Number of ABVD cycles received								
2–3	10	10.1	6	5.2	6	6.6	6	7.1
4–5	29	29.3	36	31.3	28	30.8	26	30.6
6–7	42	42.4	48	41.7	39	42.9	37	43.5
8	18	18.2	25	17.6	18	19.8	16	18.8
Complete response with first-line ABVD								
No	31	31.3	34	29.6	30	33.0	29	31.9
Yes	68	68.7	81	70.4	61	67.0	61	67.8
Radiotherapy								
No	34	34.3	43	37.4	29	31.9	26	30.6
Yes	65	65.7	72	62.6	62	68.1	59	69.4
Complete response after radiotherapy								
No	7	10.8	6	8.3	7	11.3	6	10.2
Yes	58	89.2	66	91.6	55	88.7	53	89.8
Relapse								
No	81	81.8	96	83.5	75	82.4	76	89.4
Yes	18	18.2	19	16.5	16	17.6	15	17.6
Deaths								
Lymphoma-specific deaths	6	6.1	7	6.1	7	7.7	7	8.2
Deaths due to other causes	4	4.0	4	3.5	4	4.4	4	4.7

4.2 Immunohistochemistry

Lymphoma tissue samples were collected from patients at the time of diagnosis. Samples were all from lymph node biopsies and they were fixed in formalin and embedded in paraffin. Representative tumour areas from the paraffin blocks were

cut in approximately 3- μ m sections and placed on SuperFrostPlus glass slides (Menzel-Gläser, Braunschweig, Germany). The slides were incubated at 37 °C for 4 hours, deparaffinized in Histo-Clear (National Diagnostics, Atlanta, GA, USA) or xylene (Study IV), rehydrated in a descending ethanol series and rinsed in distilled water. Antigen retrieval was carried out in a microwave oven using Tris EDTA, pH 9 (Study III, and PHD1, PHD2, PHD3 and HIF-1 α in Study II), and sodium citrate buffer, pH 6 (Studies I and IV, and Hif-2 α in Study II) to retrieve the epitopes. Endogenous peroxide activity was blocked by incubation in a 3% H₂O₂ solution (Studies I–III) or in a peroxidase blocking solution (S2023, Dako, Glostrup, Denmark) (Study IV). Immunostaining continued according to the manufacturers' instructions, using the primary antibodies and staining methods presented in Table 7. Incubation with primary antibodies was carried out in a humidity chamber at room temperature for 1 hour (KDM4A, PHD1, PHD2), overnight at room temperature (HIF-1 α) or overnight at 4 °C (Studies I and IV, KDM4B, KDM4D, PHD3, HIF-2 α). Diaminobenzidine was used to detect the immunoreaction. Between all stages of the immunostaining procedure, slides were washed with phosphate-buffered saline (PBS). They were then counterstained with Mayer's haematoxylin (Reagens, Toivola, Finland), dehydrated and mounted.

Table 8. Antibodies and immunohistochemical staining methods.

Study	Primary antibody	Dilution	Source of primary antibody	Immunostaining method
I	Nitrotyrosine (06-284)	1:200	Millipore, New York, NY, USA	Histostain-Plus kit, HRP, broad spectrum (Camarillo, CA, USA)
I	8-OHdG (MOG-100P)	1:50	JaiCa, Fukuroi, Shizuoka, Japan	Envision+ System-HRP (DAB), mouse (Dako)
I	Prx II (LF-MA0144)	1:8000	AbFrontier, Seoul, South Korea	Novolink Polymer Detection System (Novocastra, Newcastle upon Tyne, UK)
I	Prx III (LF-MA0044)	1:500	AbFrontier, Seoul, South Korea	Novolink Polymer Detection System (Novocastra, Newcastle upon Tyne, UK)
I	Prx V (LF-PA0210)	1:200	AbFrontier, Seoul, South Korea	Novolink Polymer Detection System (Novocastra, Newcastle upon Tyne, UK)
I	Prx VI (LF-MA0018)	1:3000	AbFrontier, Seoul, South Korea	Envision+ System-HRP (DAB), mouse (Dako North America, Carpinteria, CA, USA)

Study	Primary antibody	Dilution	Source of primary antibody	Immunostaining method
I	MnSOD (S5069)	1:1000	Sigma-Aldrich, St Louis, MO, USA	Novolink Polymer Detection System (Novocastra, Newcastle upon Tyne, UK)
II	HIF-1 α (610958)	1:40	BD Trunduction Laboratories, Franklin Lakes, USA	Dako REAL™ EnVision™ Detection System (Dako Denmark A/S, Glostrup, DK)
II	HIF-2 α (ab8365)	1:100	Abcam, Cambridge, UK	Dako REAL™ EnVision™ Detection System (Dako Denmark A/S, Glostrup, DK)
II	PHD1 (NB100-310)	1:300	Novus Biologicals, Oxford, UK	Dako REAL™ EnVision™ Detection System (Dako Denmark A/S, Glostrup, DK)
II	PHD2 (NB100-138)	1:300	Novus Biologicals, Oxford, UK	Dako REAL™ EnVision™ Detection System (Dako Denmark A/S, Glostrup, DK)
II	PHD3 (NBP1-30440)	1:500	Novus Biologicals, Oxford, UK	Dako REAL™ EnVision™ Detection System (Dako Denmark A/S, Glostrup, DK)
III	KDM4A (ab104831)	1:2000	Abcam, Cambridge, UK	Dako REAL™ EnVision™ Detection System (Dako Denmark A/S, Glostrup, DK)
III	KDM4B (ab103129)	1:225	Abcam, Cambridge, UK	Dako REAL™ EnVision™ Detection System (Dako Denmark A/S, Glostrup, DK)
III	KDM4D (ab93694)	1:1500	Abcam, Cambridge, UK	Dako REAL™ EnVision™ Detection System (Dako Denmark A/S, Glostrup, DK)
IV	SIRT 1 (ab166821)	1:200	Abcam, Cambridge, UK	Vectastain ABC kit PK6100, Vector Laboratories Inc., CA
IV	SIRT4 (ab10140)	1:250	Abcam, Cambridge, UK	Vectastain ABC kit PK6100, Vector Laboratories Inc., CA
IV	SIRT6 (PA5-13225)	1:100	Thermo Fisher Scientific, Rockford, IL	Vectastain ABC kit PK6100, Vector Laboratories Inc., CA
IV	Rif1 (ab134812)	1:200	Abcam, Cambridge, UK	Vectastain ABC kit PK6100, Vector Laboratories Inc., CA
IV	MGMT (ab108630)	1:750	Abcam, Cambridge, UK	Vectastain ABC kit PK6100, Vector Laboratories Inc., CA

4.3 Immunoelectron microscopy

For immunoelectronmicroscopy (IEM) in Study I, the lymph node specimens were fixed in 4% paraformaldehyde with 2.5% sucrose in 0.1 M phosphate buffer for 2 h, immersed in 2.3 M sucrose in PBS at 4 °C, and frozen in liquid nitrogen. Thin cryosections were cut with a Leica EM FC7 ultramicrotome. The sections were first incubated in a blocking solution of 0.2% gelatin/PBS, and then in 0.1% glycine/PBS. Antibodies and gold conjugates were diluted in 1% BSA/PBS. All washing was performed in 1% BSA/ PBS. For single immunolabelling, the sections were exposed to the primary 8-OHdG antibody for 60 min, and then to rabbit anti-mouse IgG (Zymed, San Fran- cisco, CA, USA) as a bridging antibody for 30 min. After washing, incubation with protein A–gold complex (size 10 nm) was performed. Controls were prepared by carrying out the labelling procedure without the primary antibody. All sections were embedded in methylcellulose, and examined with a Tecnai Spirit transmission electron microscope (FEI Company, Eindhoven, the Netherlands). Images were captured using a Quemesa CCD camera (Olympus Soft Imaging Solutions, Munster, Germany).

4.4 Sample evaluation

Evaluation of immunostaining was performed by two independent observers; an experienced haematopathologist (KMH) together with another investigator (HB) blinded to the clinical data. In Study I, immunostaining was graded: (i) separately in HRS cells or lymphocyte predominance cells (in NLPHL cases) and in the surrounding RCI; (ii) separately in nuclei and cytoplasm; and (iii) separately according to intensity (0, no immunostaining; 1, weak immunostaining intensity; 2, moderate immunostaining intensity; 3, strong immunostaining intensity; 4, very strong immunostaining intensity) and extent (0–100%) of immunostaining. For statistical analyses, immunostaining was divided into two classed variables: for intensity, classification as either 0–1 or 2–4 (8-OHdG, MnSOD, Prx II and Prx III) or 0–2 and 3–4 (nitrotyrosine, Prx V and Prx VI), based on the distribution of immunostaining. Staining extent was divided into 0–19% and 20–100% groups.

In studies II–IV, immunostaining was graded (i) separately in HRS cells and in the surrounding RCI; (ii) separately in nuclei and cytoplasm; and (iii) separately according to the extent (0–100%) and intensity of immunostaining (1, weak intensity; 2, moderate intensity; 3, strong intensity; 4, very strong immunostaining intensity). For statistical analyses, the immunostaining results were divided into

two classed variables: intensity was multiplied by the degree of extent of immunostaining (0–100%), resulting in a continuous variable of 0–400, which was further divided into two classes (low expression and high expression), based on the median expression of each variable.

4.5 Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 21.0.–24.0. software (SPSS, Chicago, IL, USA). In Studies I and II, correlations between the expression levels of the different protein markers and clinicopathological parameters were determined using two-sided Fisher's exact test or the chi-square test. In Studies III and IV, associations between clinicopathological parameters and protein expression were determined by using continuous variable testing (Mann–Whitney test). Associations between levels of protein markers and patient survival were analysed by using the Kaplan–Meier method, and the statistical significance of differences was evaluated by using the log-rank test. Relapse-free survival (RFS) was calculated from the date of diagnosis to the date of confirmed relapse. Disease-specific survival (DSS) was calculated from the date of diagnosis to the date of lymphoma-specific death or to the last follow-up date. The cases dropped from follow-up free from disease were censored at the last date of follow-up. A complete response (CR) was defined as no detectable tumour after the first-line ABVD treatment. P-values of less than 0.05 were considered significant.

4.6 Ethical aspects

The studies were carried out with good ethical aspects in mind. They were approved by the regional Ethics Committee of Northern Ostrobothnia Hospital District (42/2010). Encoded markings without personal identification numbers were used in the patient samples and the personal identification numbers were stored in our locked laboratory in a sealed locker, with respect to patients' privacy. All data analyses were carried out without patients' personal identification numbers. Our studies have not influenced patient care or follow-up. Our study lymph node samples were collected for diagnostic purposes; hence patients were not compromised by an additional physical risk. Permission to use paraffin-embedded tissue samples for research was obtained from the National Supervisory Authority for Welfare and Health (Valvira 6622/05.01.00.06/2010). During the period of data

collection and management the principles of the Declaration of Helsinki were followed.

5 Results

5.1 Immunohistochemical staining patterns in HL

The expression levels of all studied markers are presented in Table 9 and immunohistochemistry figures of studied markers are presented in the original publications.

Table 9. Percentages of cases showing expression of the studied markers

Antibody (study)	Reed–Sternberg cells		Reactive cellular infiltrate	
	Nuclei	Cytoplasm	Nuclei	Cytoplasm
Nitrotyrosine (I)	93.9	*	97.0	*
8-OHdG (I)	76.8	76.8	84.8	0
Prx II (I)	0	51.5	0	97.0
Prx III (I)	0	99.0	0	99.0
Prx V (I)	0	100	0	100
Prx VI (I)	70.0	97.0	98.0	0
MnSOD (I)	0	94.0	0	33.3
HIF-1 α (II)	73.9	0	66.6	0
HIF-2 α (II)	0%	85.8	0	0
PHD1 (II)	0	9.6	0	100
PHD2 (II)	97.6	0	0	99.1
PHD3 (II)	86.5	0	0	86.5
KDM4A (III)	1.1	92.2	83.3	96.7
KDM4B (III)	3.3	93.4	20.9	93.4
KDM4D (III)	25.3	68.1	26.4	93.4
SIRT 1 (IV)	0	100	35.0	89.0
SIRT4 (IV)	0	95.6	13.0	95.6
SIRT6 (IV)	0	100	56.0	96.7
Rif1 (IV)	96.5	82.1	81.7	74.4
MGMT (IV)	74.7	13.2	98.9	0

*=Did not evaluate

Oxidative stress markers and antioxidant enzymes (I)

Nitrotyrosine immunostaining was found in the cytoplasm of both HRS cells and cells in the RCI, its intensity varying from weak to very strong. Expression of 8-OHdG was found in the cytoplasm and nuclei in HRS cells and nuclei in RCI. Its

intensity varied from weak to strong in nuclei and cytoplasm of HRS cells. In nuclei of the RCI, 8-OHdG intensity also varied from weak to strong.

Expression of MnSOD was found in the cytoplasm of HRS cells and RCI. Its intensity varied from moderate to very strong in both. Prx II expression was also found in the cytoplasm of HRS cells and RCI. Its intensity varied from weak to strong in both. Similarly to Prx II, Prx III was found in the cytoplasm of HRS cells and RCI. Prx III intensity varied from weak to very strong in both RS cells and RCI. Prx V appeared only in the cytoplasm of HRS cells and RCI. Its intensity varied from weak to very strong. Prx VI immunostaining was found in the cytoplasm and nuclei of HRS cells and in the cytoplasm of RCI. Prx VI intensity varied from moderate to strong in nuclei and from weak to strong in the cytoplasm of HRS cells. In RCI, cytoplasmic intensity varied from weak to strong.

Electron-microscopic expression patterns of 8-OHdG (I)

IEM analysis was performed on cHL samples with 8-OHdG staining. Electron microscopy showed the localization of 8-OHdG expression in lymphocytes, mainly in the chromatin, but also to some extent in the cytosol and in mitochondria.

Hypoxia regulators and prolyl hydroxylase domains (II)

HIF-1 α expression was found in nuclei of HRS and RCI cells. The intensity varied from moderate to strong in both. Cytoplasm was negative in HRS and RCI cells. HIF-2 α expression was seen only in cytoplasm of HRS cells; its intensity varied from moderate to very strong.

PHD1 expression was detected in the cytoplasm of HRS cells and also in RCI. Its intensity varied from weak to moderate in HRS cells and weak to very strong in the RCI. Expression of both PHD2 and PHD3 was found in nuclei of HRS cells and cytoplasm of the cells in the RCI. PHD2 intensity varied from weak to very strong in both nuclei of HRS cells and cytoplasm of the RCI. PHD3 intensity varied from weak to very strong in nuclei of HRS cells and weak to strong in cytoplasm of the RCI.

Histone demethylating enzymes (KDM4) (III)

HRS cells and RCI showed cytoplasmic KDM4A positivity; the intensity varied in both from weak to moderate. Nuclear KDM4A expression was detected in RCI; its

intensity varied from weak to moderate. KDM4B expression was found in nuclei and cytoplasm in HRS cells and in cells of the RCI. Its intensity in HRS cells varied from weak to moderate in nuclei and weak to very strong in cytoplasm. In reactive cellular infiltrate KDM4B intensity was weak or moderate in nuclei and weak to very strong in cytoplasm. KDM4D expression was seen in the cytoplasm and nuclei of HRS cells; its intensity varied from weak to strong in both. In cells of the RCI, KDM4D expression was also found in nuclei and cytoplasm. Its intensity varied from weak to very strong in the cytoplasm of HRS cells and in RCI.

Sirtuins (IV)

SIRT1 expression was found only in the cytoplasm of HRS cells, in all cHL samples. In RCI SIRT1 expression was found in both nuclei and cytoplasm. Its intensity varied from moderate to very strong in the cytoplasm of RS cells. In RCI, SIRT1 intensity varied in nuclei and cytoplasm from weak to strong. SIRT4 expression was found only in the cytoplasm of HRS cells. In cells of the RCI SIRT4 expression was seen mostly in cytoplasm, but also in a few samples in nuclei. Its intensity varied from weak to very strong in the cytoplasm of HRS cells and in RCI SIRT4 intensity varied in nuclei from moderate to strong and in cytoplasm from weak to strong. SIRT6 expression was found only in the cytoplasm of HRS cells, in all cHL samples. In RCI, it was seen in both nuclei and cytoplasm. Similarly to SIRT4, SIRT6 intensity varied from weak to very strong in the cytoplasm of HRS cells, and in RCI SIRT6 intensity varied in nuclei and cytoplasm from weak to strong.

MGMT and Rif1 (IV)

MGMT expression was found in nuclei and cytoplasm of HRS cells and in nuclei of the cells in RCI. Its intensity varied from weak to strong in the nuclei of HRS cells and from weak to moderate in cytoplasm of RS cells. MGMT intensity was moderate or strong in nuclei of RCI.

Rif1 expression was detected in both nuclei and cytoplasm of HRS cells and RCI. Rif1 intensity was moderate to very strong in nuclei and weak to strong in cytoplasm of the HRS cells. In RCI, intensity varied from weak to strong in both nuclei and cytoplasm of the cells.

5.2 Associations between clinical parameters and biomarkers

Associations between biomarkers and clinicopathological parameters are shown in Table 10.

Table 10. Associations between biomarkers and clinicopathological parameters.

Antibody (Study)	Immunostaining location (nuclear/cytoplasmic)	B-symptoms	Advanced stage	IPS >2	Limited- stage risk factor	No CR after ABVD	No CR after IFRT
Nitrotyrosine (I)	RCI (nuclear)	0.046 ↑					
8-OHdG (I)	HRS (nuclear)		0.006 ↑				
	HRS (cytoplasmic)			0.004 ↑			
Prx III (I)	RCI (cytoplasmic)	0.0006 ↑	0.002 ↑				
Prx V (I)	HRS (cytoplasmic)					0.04 ↑	
MnSOD (I)	HRS (cytoplasmic)		0.03 ↑				
	RCI (cytoplasmic)	0.002 ↑					
HIF-1α (II)	RCI (nuclear)					0.02 ↑ (LSP)	
HIF-2α (II)	HRS (cytoplasmic)						0.01 ↑
PHD1 (II)	HRS (cytoplasmic)				0.04 ↓		
	RCI (cytoplasmic)					0.002 ↓ (ASP)	
PHD3 (II)	HRS (nuclear)					0.002 ↓ (ASP)	
	RCI (cytoplasmic)						0.03 ↓ (ASP)
KDM4A (III)	HRS (nuclear)	0.0006 ↓					
KDM4B (III)	HRS (cytoplasmic)	0.007 ↑	0.02 ↑	0.001 ↑			
KDM4D (III)	HRS (cytoplasmic)		0.04 ↑				
	RCI (nuclear)	0.002 ↑	0.007 ↑				
SIRT 1 (IV)	RCI (nuclear)	0.01 ↑	0.01 ↑				
SIRT6 (IV)	HRS (cytoplasmic)			0.001 ↓			
	RCI (nuclear)	0.03 ↑	0.04 ↑				
	RCI (cytoplasmic)					0.007 ↓	
Rif1 (IV)	HRS (cytoplasmic)					0.04 ↑	
MGMT (IV)	HRS (cytoplasmic)	0.03 ↓	0.02 ↓				

HRS = Hodgkin and Reed–Sternberg (cell), RCI = Reactive cellular infiltrate, ↓ = Negative correlation, ↑ = positive correlation, ASP = advanced stage patients, LSP = limited stage patients

Oxidative stress markers and antioxidant enzymes (I)

In Study I, strong expression of 8-OHdG and expression of mitochondrial antioxidant enzymes (MnSOD, Prx III) were related to B-symptoms and advanced stage in HL. Strong 8-OHdG expression was associated with lower IPSs (0–2) in advanced disease. Strong Prx V expression was related to a low rate of CR after first-line ABVD-treatment. Expression levels of Prx II and Prx VI did not show any association with clinical parameters.

Hypoxia regulators and prolyl hydroxylase domains (II)

In Study II, strong HIF-2 α expression was associated with fewer CRs after radiotherapy. In limited-stage patients, low nuclear HIF-1 α expression was correlated with a low level of CR after first-line ABVD treatment. Expression levels of HIF-1 α and HIF-2 α did not show any association with other clinical parameters.

A low rate of cytoplasmic PHD1 staining in HRS cells was associated with limited-stage disease. Low levels of PHD1 and PHD3 were found to be associated with a low rate of CR to chemotherapy in advanced-stage patients. Low expression levels of PHD3 staining correlated with a reduced amount of CR after radiotherapy in advanced-stage patients. Expression levels of PHD1, PHD2 or PHD3 did not show any associations with the clinical parameters examined.

Histone demethylating enzymes (KDM4) (III)

In the KDM4 study (III) strong KDM4B and KDM4D expression levels were related to B-symptoms and an advanced stage of cHL. Strong KDM4B expression was also associated with higher IPSs (3–7). Low nuclear KDM4A expression was associated with the presence of B-symptoms. KDM4 enzymes were not associated with first-line treatment responses.

Sirtuins, MGMT and Rif1 (IV)

Study IV showed that strong SIRT1 and SIRT6 expression levels were associated with advanced stage and B-symptoms of cHL. Strong SIRT6 staining was also associated with low (0–2) IPSs. Strong SIRT6 expression was also associated with fewer CRs after ABVD chemotherapy. SIRT4 expression did not show any

association with clinical parameters. Low-level MGMT expression was related to B-symptoms and advanced stage and strong Rif1 expression was associated with a high rate of CR after first-line ABVD treatment.

5.3 Survival analysis

Table 11 summarizes all biomarkers associated with patient survival.

Oxidative stress markers and antioxidant enzymes (I)

Low 8-OHdG immunostaining intensity in the nuclei of HRS cells was a predictor of poorer RFS, but only in the patients with advanced disease ($p=0.038$). Cox regression analysis demonstrated this to be the most significant prognostic factor when the IPS score (0–2 or 3–7) and achievement of complete remission were taken into account.

An elevated extent of MnSOD expression was associated with poorer RFS ($p=0.022$). In Cox regression analysis, the MnSOD level was an independent prognostic factor when stage (limited or advanced) and achievement of complete remission were taken into account.

Hypoxia regulators and prolyl hydroxylase domains (II)

In Study II, strong nuclear HIF-1 α expression in RCI was associated with prolonged RFS in the advanced-stage patients who had received IFRT ($p=0.026$). When combined with the IPS in multivariate analysis, HIF-1 α expression in this subgroup appeared to have more prognostic power, although neither variable remained significant in this model (for HIF-1 α : risk ratio [RR] 0.223; 95% confidence interval [CI] 0.043–1.157; $p=0.074$, and for IPS: RR 1.301; 95% CI 0.251–6.730; $p=0.754$). Strong nuclear HIF-1 α expression in HRS cells was not, however, associated statistically significantly with prolonged RFS in advanced-stage patients who had received IFRT ($p=0.11$).

Strong cytoplasmic PHD1 expression in HRS cells was found to be associated with poor RFS among all patients treated with IFRT and among the advanced-stage patients who had received IFRT ($p=0.0028$, $p=0.0058$ respectively). In Cox regression analysis, the statistical significance of the predictive power exceeded that of the IPS in the patients treated with IFRT (for PHD1: RR 10.073; 95% CI 1.549–65.520; $p=0.016$, and for IPS: RR 0.340; 95% CI 0.387–1.388; $p=0.34$) and

also in the patients with both advanced-stage disease and IFRT (for PHD1: RR 18.383; 95% CI 1.521–222.246; $p=0.022$, and for IPS: RR 0.263; 95% CI 0.021–3.229; $p=0.297$).

Histone demethylating enzymes (KDM4) (III)

Strong cytoplasmic expression of KDM4B in HRS cells predicted poorer RFS in patients with limited-stage disease ($p=0.022$). Similarly, strong cytoplasmic KDM4B expression in the RCI predicted worse RFS in this patient group ($p=0.020$). Similarly to KDM4B, strong KDM4D expression in the cytoplasm of HRS cells was associated with dismal RFS among the limited-stage patients ($p=0.046$). Strong cytoplasmic KDM4D expression in HRS cells also predicted worse RFS in limited-stage patients who had received involved-field radiotherapy ($p=0.007$). As a result of the excellent prognosis and a limited number of relapses in this material, reliable multivariate analysis could not be performed. Strong cytoplasmic KDM4A expression in the RCI was associated nearly significantly with poorer RFS in the whole cohort ($p=0.054$).

Sirtuins and Rif1 (IV)

Strong cytoplasmic SIRT6 expression in the RCI was associated with prolonged RFS in the whole patient population ($p=0.040$). When the patients were divided according to stage (limited or advanced) and the administration of radiotherapy, SIRT6 was a prognostic factor only in those with advanced-stage disease who had received radiotherapy ($p=0.031$). However, these associations were not confirmed in multivariate analysis. In the subgroup of advanced-stage patients who had received radiotherapy, none of the seven patients with high-level SIRT6 expression experienced a relapse, compared with 7/14 (50%) patients with low-level expression. In other words, SIRT6 had a positive prognostic value of 50% and a negative prognostic value of 100% ($p=0.047$).

High-level nuclear Rif1 expression in HRS cells was found to be associated with prolonged RFS, but only in cases with advanced-stage disease (univariate analysis, $p=0.032$). In multivariate analysis, high-level nuclear Rif1 expression in cases with advanced-stage disease was a more significant predictor of favourable RFS (hazard ratio [HR] 8.596; 95% CI 1.604–46.073; $p=0.012$) than a high IPS (scores 0–2 versus scores 3–7, HR 5.207; 95% CI 1.108–19.351; $p=0.036$). When the patients were further divided according to therapy, nuclear Rif1 expression in

HRS cells had a prognostic value only among the advanced-stage-disease patients who had received radiotherapy ($p=0.0043$). Associations could not be confirmed by multivariate analysis, because the subgroup was too small. In this subgroup, 7/11 (63.6%) of the patients with low-level Rif1 expression suffered from relapse, compared with 0/9 (0%) patients with high-level Rif1 expression. Thus, Rif1 had a positive prognostic value of 63.6% and a negative prognostic value of 100% as regards developing a relapse in this subgroup ($p=0.0047$).

Sirtuin 6 together with Rif1 (IV)

Low-level expression of both Rif1 and SIRT6 predicted worse RFS according to the results of univariate analysis ($p=0.021$). However, in subgroups the significance remained only among those patients who had received radiotherapy ($p=0.0073$). Also, low-level expression of both Rif1 and SIRT6 predicted poor outcome in those with advanced-stage disease ($p=0.002$). Among the patients with both advanced-stage disease and radiotherapy received, the significance was even more pronounced ($p=0.000038$). In multivariate analysis this combined variable was still significant as regards the radiotherapy-treated patients (HR 8.521; 95% CI 1.714–42.358; $p=0.0088$) and when the stage was included in the analysis (HR 9.395; 95% CI 9.395–46.935). In line with the above, low-level Rif1/SIRT6 expression was associated with worse DSS in univariate analysis, but only in the patients treated with radiotherapy and with advanced-stage disease ($p=0.034$ for the whole population; $p=0.024$ for those with advanced-stage HL; $p=0.011$ for the patients treated with radiotherapy; $p=0.015$ for the patients with advanced-stage HL and radiotherapy). This observation could not be confirmed in multivariate analysis as a result of the low number of HL-related deaths.

Table 11. Biomarkers and patient survival.

Antibody (Study)	Immunostaining location (nuclear/cytoplasmic)	Subgroup	Endpoint	P-value (log-rank)	5-year survival in low expression group (%)	5-year survival in high expression group (%)
MnSOD (I)	HRS (cytoplasmic)	Whole cohort	RFS	0.022	97	77
8-OHdG (I)	HRS (nuclear)	ASP	RFS	0.038	67	85
HIF-1 α (II)	RCI (nuclear)	ASP, IRFT-treated	RFS	0.026	36	85
PHD1 (II)	HRS (cytoplasmic)	IFRT-treated	RFS	0.0028	88	50
	HRS (cytoplasmic)	ASP, IRFT-treated	RFS	0.0058	73	25
KDM4B (III)	HRS (cytoplasmic)	LSP	RFS	0.022	100	79
	RCI (cytoplasmic)	LSP	RFS	0.020	97	75
KDM4D (III)	HRS (cytoplasmic)	LSP	RFS	0.046	94	71
	HRS (cytoplasmic)	LSP, IRFT-treated	RFS	0.0071	97	60
SIRT6 (IV)	RCI (cytoplasmic)	Whole cohort	RFS	0.040	78	96
Rif1 (IV)	RCI (cytoplasmic)	ASP, IRFT-treated	RFS	0.031	50	100
SIRT6/Rif1 (IV)	HRS (nuclear)	ASP	RFS	0.032	67	89
	HRS (nuclear)	ASP, IRFT-treated	RFS	0.0043	36	100
	RCI (cytoplasmic)/HRS (nuclear)	Whole cohort	RFS	0.021	74	91
	RCI (cytoplasmic)/HRS (nuclear)	ASP, IRFT-treated	RFS	0.000038	13	100
	RCI (cytoplasmic)/HRS (nuclear)	Whole cohort	DSS	0.034	83	100
	RCI (cytoplasmic)/HRS (nuclear)	ASP	DSS	0.024	67	100
	RCI (cytoplasmic)/HRS (nuclear)	IFRT-treated	DSS	0.011	81	100
	RCI (cytoplasmic)/HRS (nuclear)	ASP, IRFT-treated	DSS	0.015	50	100

ASP = advanced stage patients, LSP = limited stage patients, IFRT = Involved-field radiation therapy

6 Discussion

In these studies, the potential predictive and prognostic roles of various proteins related to cellular stress-regulating mechanisms in HL were examined. These proteins were analysed immunohistochemically in both HRS cells and RCI. The RCI has an important impact on HRS cells and tumour behaviour, and the results presented in this study emphasize their significance in cHL.

6.1 Oxidative stress markers and antioxidant enzymes in HL

The roles of oxidative stress and antioxidant enzymes have not been studied in HL previously. In other (solid) cancers and lymphomas oxidative stress markers have shown predictive and prognostic roles (Karihtala & Soini 2007, Pasanen *et al.*, 2012, Peroja *et al.*, 2012, Kuusisto *et al.*, 2015). Encouraged by previous studies on cancer and especially lymphoma, the biological importance of oxidative stress and antioxidant enzymes were here studied in HL. Their roles were explored in HRS cells and tumour cellular infiltrate and their possible involvement in the development of chemoresistance and connection to survival was investigated.

ROS-mediated initiation of carcinogenesis may occur directly (including oxidation, nitration, halogenation of nuclear DNA, RNA, and lipids) or via the signalling pathways activated by ROS. For example, during aerobic respiration there is non-stop electron leakage to O₂ in adenosine triphosphate (ATP) synthesis in mitochondria. One example of ROS is O₂⁻, which is formed during aerobic metabolism. MnSOD is the main antioxidant enzyme to protect against this mitochondrial free radical (Karihtala & Soini 2007).

First-line treatment of cHL usually consists of ABVD chemotherapy. Most mechanisms of the drugs involved are based on ROS formation, especially as regards bleomycin (Burger, Peisach & Horwitz 1981). High levels of MnSOD in solid cancers reflect the aggressiveness of cancer and its metastatic potential and they have also been linked to poor prognosis (Malafa, Margenthaler, Webb, Neitzel & Christophersen 2000, Janssen *et al.*, 2000). In lymphoma, there is some evidence that elevated levels of antioxidant enzyme expression might be linked to chemoresistance (Kuusisto *et al.*, 2015, Tome *et al.*, 2012). In the present study, strong MnSOD expression was linked to advanced stage and B-symptoms and was associated with worse prognosis in multivariate analysis when stage (limited or advanced) and achievement of complete remission were taken into account. High-level MnSOD expression seemed to be particularly associated with relapses during

the first years of treatment. Furthermore, MnSOD mRNA levels have been recently studied in cHL and the researchers found that high levels of MnSOD mRNA in advanced-stage patients were linked to poor DSS (Karihtala, Porvari, Soini, Haapasaari 2017). These results, together with those presented here, indicate that MnSOD could be a significant biomarker as regards prediction of prognosis.

In our study we found no significant connection between MnSOD and chemoresistance. However, Prx V, which is primarily in cytosol and mitochondria, was associated with a reduced level of CR after ABVD treatment. Prx III is also found in the mitochondria and in the present study it was related to higher IPSs, advanced stage and B-symptoms. In follicular lymphomas high levels of total Prxs have been linked to favourable DSS and OS but not with progression-free survival (PFS), but on the other hand, in DLBCL, strong Prx VI expression has been associated with adverse DSS, OS and PFS (Peroja *et al.*, 2016, Kuusisto *et al.*, 2015). In our study Prx levels were not statistically significant in survival analysis.

In mammalian cells, the most injurious effects of ROS are brought about by OH, which causes DNA damage including the formation 8-OHdG (Valiko, Izakovic, Mazur, Rhodes & Telser 2004, Marnett 2000). In the present study strong 8-OHdG expression was related to advanced stage and B-symptoms, but on the other hand, to low IPSs and prolonged RFS in advanced-stage patients. Even though high levels of ROS have been linked to carcinogenesis, they can also cause detrimental oxidative stress that can lead to cell death.

6.2 HIF and PHD enzymes in cHL

To our knowledge, the associations between HIF and PHD proteins and clinical outcome have not previously been studied in cHL. Levels of HIFs are significantly increased in many human cancers and their association with metastasis formation has been confirmed (Semenza 2011). In the majority of cancers, HIF-1 α and HIF-2 α are linked to a poor outcome and other prognostic aspects (Semenza 2011). PHD proteins have been shown to act mainly as tumour suppressors (Klotzsche-von Ameln *et al.*, 2011).

In the present study, HIF-1 α and HIF-2 α were associated with a low rate of CR to first-line ABVD treatment, especially among limited-stage patients. In an HL cell line, hypoxic conditions cause resistance to cisplatin chemotherapy and in non-Hodgkin lymphomas HIF-1 α overexpression leads to chemoresistant disease (Hernandez-Luna, Rocha-Zavaleta, Vega & Huerta-Yepey 2013, Kewitz, Kurch, Volkmer & Staeger 2016). Hif-1 α causes chemoresistance by inhibiting apoptosis

and attenuating the rate of intracellular drug accumulation (Liu *et al.*, 2008). A hypoxic environment causes radioresistance by re-oxygenation and ROS formation, which enables stabilization of the DNA damage response. Stabilization of HIF also brings about target gene expression and epigenetic post-translational histone modifications, which further cause radioresistance (Beyer, Kristensen, Jensen, Johansen & Staller 2008).

In our material, increased HIF-1 α expression was associated with prolonged RFS in advanced-stage patients treated with IFRT. At first sight, our results seem to be inconsistent, because HIF-1 α has been linked to inferior survival in most cancers (Semenza 2012). Results consistent with ours have been observed in renal cell carcinoma, DLBCL and head and neck squamocellular carcinoma (Beasley *et al.*, 2002, Evens *et al.*, 2010, Lidgren *et al.*, 2005). One possible reason why HIF-1 α in RCI was correlated with prolonged RFS may be that HIF-1 α contributes to Treg cell differentiation. High numbers or proportions of Treg cells have been associated with a favourable prognosis (Alvaro *et al.*, 2005, Schreck *et al.*, 2009). On the other hand, a recent study showed that Treg cells in RCI were associated with poorer prognosis (Hollander *et al.*, 2018).

Our study showed that in advanced-stage patients, low levels of PHD1 and PHD3 immunostaining in RCI (PHD3 also in HRS cells) were associated with a low rate of CR. On the other hand, strong PHD1 expression in HRS cells was associated with poor RFS in advanced-stage patients, especially in the radiotherapy-treated patient population. It seems paradoxical that PHD1 expression has diverse roles in RCI and in HRS cells. PHD1 and PHD3 inhibit NF- κ B signalling, which has an important role in HRS cell survival (Bargou *et al.*, 1997, Cummins *et al.*, 2006). PHD3 also promotes growth inhibition through epidermal growth factor receptor and mediates alpha-ketoglutarate-induced apoptosis and tumour suppression (Tennant & Gottlieb 2010, Garvalov *et al.*, 2014). In patients with pancreatic endocrine tumours, prostate adenocarcinoma and non-small-cell lung carcinoma, PHD1 has also been linked to poorer survival (Couvelard *et al.*, 2008, Boddy *et al.*, 2005, Andersen *et al.*, 2011).

6.3 Epigenetic regulators in cHL

Epigenetic changes occur during the pathogenesis of cHL (Seitz *et al.*, 2011). There is also evidence that epigenetic changes in non-coding RNA are associated with clinical outcome in cHL (Cordeiro, Monzó & Navarro 2017). There is no previous research on KDM4 protein in relation to the clinical outcome of cHL.

Here, strong expression of KDM4B and KDM4D was associated with poor RFS in limited-stage patients. High KDM4D expression predicted dismal RFS most significantly in the patients who had received involved-field radiotherapy. This may be due to the participation of KDM4D in the repair of double-strand breaks, thereby maintaining genome integrity (Khoury-Haddad *et al.*, 2014). KDM4D also promotes DNA repair by way of enhanced expression of PARP1, which is known to be associated with radioresistance (Chen *et al.*, 2017). Similarly, in nasopharyngeal carcinoma, inhibition of PARP1 enhances radiotherapy responses (Chow *et al.*, 2013). Also in a recent study, strong KDM4D expression was associated with poor disease-free survival in pancreatic adenocarcinoma (Isohookana J, Haapasaari KM, Soini Y & Karihtala 2018). KDM4A, KDM4B and KDM4D did not show any association with treatment response in our material.

Our results showed that strong expression of KDM4B is linked to the traditional prognostic factors of HL. The effect is probably due to the fact that KDM4B participates in the DNA damage response and DSBs. Here, HIF-1 α was found to be expressed in cHL samples, and it promoted KDM4B protein expression. As earlier mentioned, strong expression of HIF-1 α was linked inversely to the achievement of CR after first-line ABVD chemotherapy. KDM4B might help HRS cells and RCI to survive and thrive in a hypoxic environment. For this reason, KDM4B may have an impact on resistance to chemotherapeutic agents, especially in short chemotherapy cycles (in limited-stage disease).

6.4 Sirtuins and DNA repair in cHL

Sirtuins have been studied widely in connection with several types of cancer. DNA repair proteins have been giving a new perspective in cancer research, especially in treatment response. There is no previous study in which sirtuins or the DNA-repair proteins Rif1 and MGMT have been investigated in cHL.

Sirtuins are involved in diverse biological functions, including cell division, differentiation, metabolism and survival. Our results showed that strong expression of SIRT1 and SIRT6 was associated with both B-symptoms and advanced stage. SIRT6 also predicted a poor benefit from first-line ABVD chemotherapy. SIRT1 is the most studied sirtuin in cancers. It has been linked to various cancer types. In DLBCL, SIRT1 is associated with shorter OS (Jang *et al.*, 2008). SIRT6 is a primary tumour suppressor protein, but its overexpression has been shown to lead to increased resistance to chemotherapy (paclitaxel and epirubicin) in breast cancer (Khongkow *et al.*, 2013). One explanation for this might be that SIRT6 is connected

to the DNA repair mechanism. Anthracyclines (including paclitaxel and epirubicin) cause DNA damage and create ROS (Chien & Moasser 2008, Jacobson 1996).

Low-level expression of nuclear Rif1 in HRS cells predicted early relapses in advanced-stage patients, especially in those who had received radiotherapy. Expression of Rif1 was statistically more significant in multivariate analysis than the IPS. One explanation for why high-level expression of Rif1 is a good prognosis marker is that it facilitates NHEJ and suppresses homologous recombination in BCRA1-mediated manner (Kumar & Cheok 2014). Of the two main DSB repair choices, NHEJ is thought to be more radiosensitive (Somaiah *et al.*, 2012, Somaiah, Yarnold, Lagerqvist, Rothkamm & Helleday 2013). On the other hand, a recent study showed that in cervical cancer cells Rif1 depletion sensitizes cells to cisplatin treatment (Mei *et al.* 2017).

In survival analysis, high-level expression of SIRT6 and Rif1 (new variant) was found to be linked to prolonged survival, particularly in advanced-stage patients who had received radiotherapy. There are no previous studies in which both biomarkers have been assessed in humans. SIRT6 and Rif1 have at least one common factor – they significantly stimulate the DSB repair mechanism, and there is evidence that inhibition of the HR repair mechanism could be a potential radiosensitizer (Mladenovic, Magin, Soni & Iliakis 2013).

6.5 Limitations of this study

There are some weaknesses in this study design, which are important to note. In study I, patient material was heterogeneous, including six NLRP1 patients. In the same study, the subgroup analysis was not done. There were a rather limited number of patients in this study, especially in the subgroup analysis there could be larger patient group. The protocol to estimate the treatment response assessment has been modified during the study (in years 1997-2015). Another weakness of this study is the study method, which was mainly based on immunohistochemistry assessing only protein levels. Immunoelectronmicroscopy was used only in study I.

The strengths of this study are strict documentation, uniformly treated patients from geographically small area, long follow-up time and representative HL samples with careful protein expression assessment in different compartments.

6.6 Prospects for future research

At the moment, the therapeutic decision making in HL is based on disease stage, risk factors (such as IPS, limited stage risk factors, patient age) and B-symptoms which have been used almost two decades. FDG-PET have given excellent results for interim treatment response assessment and adaptation of patient management. Nevertheless, these tools provide only very limited information about HL biology in individual patients. In this study, our aim was to identify new biomarkers for those patients who are at risk of poor treatment response or disease relapse. Secondly to find suitable biomarkers to those patients who could do well with less treatment and further reduce treatment-related toxicity and long-term morbidity.

The role of radiotherapy is under debate as regards HL as a result of its treatment-related side effects and excellent outcome of the patients. Epigenetic reulator KDM4D predicted worse outcome especially in limited stage patient, who had received radiotherapy. As well PHD1 and SIRT6 together with Rif1 predicted worse outcome especially in advanced stage patient, who had received radiotherapy. To confirm our findings these biomarkers should be further studied in a large cHL patient group.

Further studies have already been done in redox regulating enzymes in cHL (Karihtala, Porvari, Soini, Haapasaari 2017), but there are no studies where these markers evaluate the response to radiotherapy treatment. For future research is recommended to study redox status and oxidative stress, separately in cHL and NLPHL subgroups. Also the role of redox status and oxidative stress in radioresistance would be recommended to study in future.

In this study, hypoxia related factors (HIF-1 α , PHD1 and PHD3), antioxidant enzyme Prx V and DNA repairing related factors SIRT6 and Rif1 predicted chemoresistance. It is important to find those patients, who might get poor response to the first-line treatment, because survival rates drop significantly if CR is not achieved with first-line treatment. SIRT6 together with Rif1 have not been previously assessed in the same study in humans. To confirm our findings, these biomarkers should be confirmed in larger studies.

We hope to find biomarkers, which would help therapeutic decision making; to predict potential chemo- and radioresistance diseases and also reduce overtreatment.

7 Conclusions

The present work concerns the expression of various biological factors in uniformly treated HL patient samples. These biomarkers were evaluated in both HRS cells and RCI. The study methods included IHC. Our aim was to discover new biological factors possibly useful in the prediction of prognosis and to offer new aspects in individualizing patient treatment in a convenient manner in cases of HL.

The results indicate the significance of certain biomarkers in HL. We found that in both limited- and advanced-stage patient groups that several of the studied biomarkers predicted prognosis, especially in radiotherapy-treated patients.

The following conclusions were made based on the results of the present work:

- Low-level expression of 8-OHdG (in advanced stage patients) and a high extent of expression of MnSOD are linked to poor RFS, and they also have significant prognostic value independently of traditional prognostic factors of HL.
- Strong expression of HIF-1 α and low-level expression of PHD1 and PHD3 are linked to treatment resistance in cHL. Also strong expression of PHD1 is associated with adverse RFS in radiotherapy-treated cHL patients, especially in advanced-stage patients, and it also has significant prognostic value independently of traditional prognostic factors.
- Strong expression levels of KDM4D are linked to poor RFS, especially in radiotherapy-treated limited-stage patients. KDM4A, KDM4B or KDM4D do not have predictive significance in cHL.
- Low-level expression SIRT6 and Rif1 are associated with poorer RFS, and together, SIRT6 and Rif1 have prognostic significance in patients with advanced disease who have received radiotherapy. SIRT6 and Rif1 have also predictive significance in cHL.

References

- Aebbersold DM, Burri P, Beer KT, Laissue J, Djonov V, Greiner RH & Semenza GL (2001) Expression of hypoxia-inducible factor-1alpha: a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. *Cancer Res* 61(7):2911-2916.
- Ahuja N, Schwer B, Carobbio S, Waltregny D, North BJ, Castronovo V, Maechler P & Verdin E (2007) Regulation of insulin secretion by SIRT4, a mitochondrial ADP-ribosyltransferase. *J Biol Chem* 282(46): 33583–33592.
- Akhtari M, Milgrom SA, Pinnix CC, Reddy JP, Dong W, Smith GL, Mawlawi O, Abou Yehia Z, Gunther J, Osborne EM, Andraos TY, Wogan CF, Rohren E, Garg N, Chuang H, Khoury JD, Oki Y, Fanale M & Dabaja BS (2018) Reclassifying patients with early-stage Hodgkin lymphoma based on functional radiographic markers at presentation. *Blood* 131(1):84-94.
- Aldinucci D, Olivo K, Lorenzon D, Poletto D, Gloghini A, Carbone A, Pinto A (2005) The role of interleukin-3 in classical Hodgkin's disease. *Leuk Lymphoma* 46(3):303-311.
- Aldinucci, D, Gloghini A, Pinto A, Colombatti A & Carbone, A (2012) The role of CD40/CD40L and interferon regulatory factor 4 in Hodgkin lymphoma microenvironment. *Leuk. Lymphoma* 53(2): 195–201.
- Allemani C, Sant M, De Angelis R, Marcos-Gragera R & Coebergh JW (2006) Hodgkin disease survival in Europe and the U.S.: prognostic significance of morphologic groups. *Cancer* 107(2):352-360.
- Altieri F, Grillo C, Maceroni M, Chichiarelli S (2008) DNA damage and repair: from molecular mechanisms to health implications. *Antioxid Redox Signal* 10(5):891–937
- Alvaro T, Lejeune M, Salvadó MT, Bosch R, García JF, Jaén J, Banham AH, Roncador G, Montalbán C & Piris MA (2005) Outcome in Hodgkin's lymphoma can be predicted from the presence of accompanying cytotoxic and regulatory T cells. *Clin Cancer Res* 11(4):1467-1473.
- Ameri K, Harris AL (2008) Activating transcription factor 4. *Int J Biochem Cell Biol* 40(1):14-21.
- Andersen S, Donnem T, Stenvold H, Al-Saad S, Al-Shibli K, Busund LT & Bremnes RM (2011) Overexpression of the HIF hydroxylases PHD1, PHD2, PHD3 and FIH are individually and collectively unfavorable prognosticators for NSCLC survival. *PLoS One* 6(8): e23847.
- Anderson L, Henderson C & Adachi Y (2001) Phosphorylation and rapid relocalization of 53BP1 to nuclear foci upon DNA damage. *Mol Cell Biol* 21(5):1719-1729.
- Annunziata CM, Safiran YJ, Irving SG, Kasid UN & Cossman J (2000) Hodgkin disease: pharmacologic intervention of the CD40-NF kappa B pathway by a protease inhibitor. *Blood* 96(8): 2841-2848.
- Ansell SM, Horwitz SM, Engert A, Khan KD, Lin T, Strair R, Keler T, Graziano R, Blanset D, Yellin M, Fischkoff S, Assad A & Borchmann P (2007) Phase I/II study of an anti-CD30 monoclonal antibody (MDX-060) in Hodgkin's lymphoma and anaplastic large-cell lymphoma. *J Clin Oncol* 25(19):2764-2769.

- Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, Schuster SJ, Millenson MM, Cattray D, Freeman GJ, Rodig SJ, Chapuy B, Ligon AH, Zhu L, Grosso JF, Kim SY, Timmerman JM, Shipp MA & Armand P (2015) PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med* 372(4):311-319.
- Appelhoff RJ, Tian YM, Raval RR, Turley H, Harris AL, Pugh CW, Ratcliffe PJ & Gleadle JM (2004) Differential function of the prolyl hydroxylases PHD1, PHD2, and PHD3 in the regulation of hypoxia-inducible factor. *J Biol Chem* 279(37):38458-38465.
- Azuma Y, Yokobori T, Mogi A, Altan B, Yajima T, Kosaka T, Onozato R, Yamaki E, Asao T, Nishiyama M & Kuwano H (2015) SIRT6 expression is associated with poor prognosis and chemosensitivity in patients with non-small cell lung cancer. *J Surg Oncol* 112(2): 231-237.
- Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K & Hammond CL (2009) Glutathione dysregulation and the etiology and progression of human diseases. *Biol Chem* 390(3): 191-214.
- Bargou RC, Emmerich F, Krappmann D, Bommert K, Mapara MY, Arnold W, Royer HD, Grinstein E, Greiner A, Scheidereit C & Dörken B (1997) Constitutive nuclear factor-kappaB-RelA activation is required for proliferation and survival of Hodgkin's disease tumor cells. *J Clin Invest* 100(12): 2961-2969.
- Barrington SF, Mikhaeel NG, Kostakoglu L, Meignan M, Hutchings M, Müeller SP, Schwartz LH, Zucca E, Fisher RI, Trotman J, Hoekstra OS, Hicks RJ, O'Doherty MJ, Hustinx R, Biggi A & Cheson BD (2014) Role of imaging in the staging and response assessment of lymphoma: consensus of the International Conference on Malignant Lymphomas Imaging Working Group. *J Clin Oncol* 32(27):3048-3058.
- Bauer I, Grozio A, Lasigliè D, Basile G, Sturla L, Magnone M, Sociali G, Soncini D, Caffa I, Poggi A, Zoppoli G, Cea M, Feldmann G, Mostoslavsky R, Ballestrero A, Patrone F, Bruzzzone S & Nencioni A (2012) The NAD⁺-dependent histone deacetylase SIRT6 promotes cytokine production and migration in pancreatic cancer cells by regulating Ca²⁺ responses. *J Biol Chem* 287(49):40924-40937.
- Baumforth KR, Birgersdotter A, Reynolds GM, Wei W, Kapatai G, Flavell JR, Kalk E, Piper K, Lee S, Machado L, Hadley K, Sundblad A, Sjoberg J, Bjorkholm M, Porwit AA, Yap LF, Teo S, Grundy RG, Young LS, Ernberg I, Woodman CB & Murray PG (2008) Expression of the Epstein-Barr virus-encoded Epstein-Barr virus nuclear antigen 1 in Hodgkin's lymphoma cells mediates Up-regulation of CCL20 and the migration of regulatory T cells. *Am J Pathol* 173(1):195-204.
- Beasley NJ, Leek R, Alam M, Turley H, Cox GJ, Gatter K, Millard P, Fuggle S & Harris AL (2002) Hypoxia-inducible factors HIF-1alpha and HIF-2alpha in head and neck cancer: relationship to tumor biology and treatment outcome in surgically resected patients. *Cancer Res* 62(9):2493-2497.

- Behringer K, Goergen H, Hitz F, Zijlstra JM, Greil R, Markova J, Sasse S, Fuchs M, Topp MS, Soekler M, Mathas S, Meissner J, Wilhelm M, Koch P, Lindemann HW, Schalk E, Semrau R, Kriz J, Vieler T, Bentz M, Lange E, Mahlberg R, Hassler A, Vogelhuber M, Hahn D, Mezger J, Krause SW, Skoetz N, Böll B, von Tresckow B, Diehl V, Hallek M, Borchmann P, Stein H, Eich H, Engert A (2015) Omission of dacarbazine or bleomycin, or both, from the ABVD regimen in treatment of early-stage favourable Hodgkin's lymphoma (GHSG HD13): an open-label, randomised, non-inferiority trial. *Lancet* 385(9976):1418-1427.
- Behringer K, Müller H, Görgen H, Flechtner HH, Brillant C, Halbsguth TV, Thielen I, Eichenauer DA, Schober T, Nisters-Backes H, Fuchs M, Engert A & Borchmann P (2013) Sexual quality of life in Hodgkin Lymphoma: a longitudinal analysis by the German Hodgkin Study Group. *Br J Cancer* 108(1):49-57.
- Bennani NN, LaPlant BR, Ansell SM, Habermann TM, Inwards DJ, Micallef IN, Johnston PB, Porrata LF, Colgan JP, Markovic SN, Nowakowski GS, Macon WR, Reeder CB, Mikhael JR, Northfelt DW, Ghobrial IM & Witzig TE (2017) Efficacy of the oral mTORC1 inhibitor everolimus in relapsed or refractory indolent lymphoma. *Am J Hematol* 92(5):448-453.
- Berger SL, Kouzarides T, Shiekhattar R & Shilatifard A (2009) An operational definition of epigenetics. *Genes Dev.* 23(7):781-783
- Bernier J, Hall EJ & Giaccia A (2004) Radiation oncology: a century of achievements. *Nat. Rev. Cancer* 4(9): 737-747.
- Berra E, Benizri E, Ginouvès A, Volmat V, Roux D & Pouyssegur J (2003) HIF prolyl-hydroxylase 2 is the key oxygen sensor setting low steady-state levels of HIF-1alpha in normoxia. *EMBO J.* 22(16):4082-4090.
- Berry WL & Janknecht R (2013) KDM4/JMJD2 Histone Demethylases: Epigenetic Regulators in Cancer Cells. *Cancer Res* 73(10):2936-2942.
- Berry WL, Shin S, Lightfoot SA & Janknecht R (2012) Oncogenic features of the JMJD2A histone demethylase in breast cancer. *Int J Oncol* 41(5):1701-1706.
- Besson H, Brennan P, Becker, De Sanjosé SN, Nieters A, Font R, Maynadié M, Foretova L, Cocco PL, Staines A, Vornanen M, & Boffetta P (2006) Tobacco smoking, alcohol drinking and Hodgkin's lymphoma: a European multi-centre case-control study (EPILYMPH). *Br J Cancer* 95(3):378-384.
- Beucher A, Birraux J, Tchouandong L, Barton O, Shibata A, Conrad S, Goodarzi AA, Krempler A, Jeggo PA & Löbrich M (2009) ATM and Artemis promote homologous recombination of radiation-induced DNA double-strand breaks in G2. *EMBO J.* 28(21): 3413-3427.
- Beyer S, Kristensen MM, Jensen KS, Johansen JV, Staller P (2008) The histone demethylases JMJD1A and JMJD2B are transcriptional targets of hypoxia-inducible factor HIF. *J Biol Chem* 283(52): 36542-36552.

- Bjorkman M, Ostling P, Harma V, Virtanen J, Mpindi JP, Rantala J, Mirtti T, Vesterinen T, Lundin M, Sankila A, Rannikko A, Kaivanto E, Kohonen P, Kallioniemi O, Nees M (2011) Systematic knockdown of epigenetic enzymes identifies a novel histone demethylase PHF8 overexpressed in prostate cancer with an impact on cell proliferation, migration and invasion. *Oncogene* 31(29): 3444-3456.
- Björkholm M, Svedmyr E, Sjöberg J (2011) How we treat elderly patients with Hodgkin lymphoma. *Curr Opin Oncol* 23(5):421-428.
- Blaveri E, Simko JP, Korkola JE, Brewer JL, Baehner F, Mehta K, Devries S, Koppie T, Pejavar S, Carroll P & Waldman FM (2005) Bladder cancer outcome and subtype classification by gene expression. *Clin Cancer Res* 11(11):4044-4055.
- Boddy JL, Fox SB, Han C, Campo L, Turley H, Kanga S, Malone PR & Harris AL (2005) The androgen receptor is significantly associated with vascular endothelial growth factor and hypoxia sensing via hypoxia-inducible factors HIF-1a, HIF-2a, and the prolyl hydroxylases in human prostate cancer. *Clin Cancer Res* 11(21):7658-7663.
- Bristow RG & Hill RP (2008) Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. *Nat Rev Cancer* 8(3):180-192.
- Brown JM & Wilson WR (2004) Exploiting tumour hypoxia in cancer treatment. *Nat Rev Cancer* 4(6):437-447.
- Brunet A1, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW & Greenberg ME (2004) Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science* 303(5666):2011-2015.
- Burger RM, Peisach J & Horwitz SB (1981) Activated bleomycin. A transient complex of drug, iron, and oxygen that degrades DNA. *J Biol Chem* 256(22):11636-11644.
- Burma S & Chen DJ (2004) Role of DNA-PK in the cellular response to DNA double-strand breaks. *DNA Repair (Amst)* 3(8-9): 909–918.
- Böll B, Goergen H, Behringer K, Bröckelmann PJ, Hitz F, Kerkhoff A, Greil R, von Tresckow B, Eichenauer DA, Bürkle C, Borchmann S, Fuchs M, Diehl V, Engert A & Borchmann P (2016) Bleomycin in older early-stage favorable Hodgkin lymphoma patients: analysis of the German Hodgkin Study Group (GHSG) HD10 and HD13 trials. *Blood* 127(18):2189-2192.
- Cader FZ, Vockerodt M, Bose S, Nagy E, Brundler MA, Kearns P & Murray PG (2013) The EBV oncogene LMP1 protects lymphoma cells from cell death through the collagen-mediated activation of DDR1. *Blood* 122(26):4237-4245.
- Cadet J, Berger M, Douki T & Ravanat JL (1997) Oxidative damage to DNA: formation, measurement, and biological significance. *Rev Physiol Biochem Pharmacol* 131:1–87.
- Cai CY, Zhai LL, Wu Y & Tang ZG (2015) Expression and clinical value of peroxiredoxin-1 in patients with pancreatic cancer. *Eur J Surg Oncol* 41(2): 228–235.
- Carbone A, Ghoghini A, Castagna L, Santoro A & Carlo-Stella C (2015) Primary refractory and early-relapsed Hodgkin's lymphoma: strategies for therapeutic targeting based on the tumour microenvironment. *J Pathol* 237(1):4-13.

- Casazza A, Di Conza G, Wenes M, Finisguerra V, Deschoemaeker S & Mazzone M (2014) Tumor stroma: a complexity dictated by the hypoxic tumor microenvironment. *Oncogene* 33(14):1743–1754.
- Castellini L, Moon EJ, Razorenova OV, Krieg AJ, von Eyben R, Giaccia AJ (2017) KDM4B/JMJD2B is a p53 target gene that modulates the amplitude of p53 response after DNA damage. *Nucleic Acids Res* 45(7):3674-3692.
- Cattaruzza L, Gloghini A, Olivo K, Di Francia R, Lorenzon D, De Filippi R, Carbone A, Colombatti A, Pinto A & Aldinucci D (2009) Functional coexpression of Interleukin (IL)-7 and its receptor (IL-7R) on Hodgkin and Reed-Sternberg cells: Involvement of IL-7 in tumor cell growth and microenvironmental interactions of Hodgkin's lymphoma. *Int J Cancer*. 125(5):1092-1101.
- Chaganti S, Bell AI, Pastor NB, Milner AE, Drayson M, Gordon J & Rickinson AB (2005) Epstein–Barr virus infection in vitro can rescue germinal center B cells with inactivated immunoglobulin genes. *Blood* 106(13):4249-4252.
- Chalkiadaki A & Guarente L (2015) The multifaceted functions of sirtuins in cancer. *Nat Rev Cancer* 15(10):608-624.
- Chemnitz JM, Eggle D, Driesen J, Classen S, Riley JL, Debey-Pascher S, Beyer M, Popov A, Zander T & Schultze JL (2007) RNA fingerprints provide direct evidence for the inhibitory role of TGFbeta and PD-1 on CD4+ T cells in Hodgkin lymphoma. *Blood* 110(9): 3226–3233.
- Chen HC, Jeng YM, Yuan RH, Hsu HC & Chen YL (2012) SIRT1 promotes tumorigenesis and resistance to chemotherapy in hepatocellular carcinoma and its expression predicts poor prognosis. *Ann Surg Oncol* 19(6):2011-2019.
- Chen Y, Li Z, Dong Z, Beebe J, Yang K, Fu L, Zhang JT (2017) 14-3-3 σ Contributes to Radioresistance By Regulating DNA Repair and Cell Cycle via PARP1 and CHK2. *Mol Cancer Res*.15(4): 418-428
- Cheng HL, Mostoslavsky R, Saito S, Manis JP, Gu Y, Patel P, Bronson R, Appella E, Alt FW & Chua KF (2003) Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice. *Proc Natl Acad Sci U S A* 100(19):10794-10799.
- Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E & Lister TA (2014) Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol*. 2014 32(27):3059-3068.
- Chevalier P & Bernard J (1932), *La maladie de Hodgkin (lymphogranulomatose maligne)*, Masson, Paris.
- Chi P, Allis CD & Wang GG (2010) Covalent histone modifications-miswritten, misinterpreted and miserased in human cancers. *Nat Rev Cancer* 10(7):457-469.
- Chien AJ & Moasser MM (2008) Cellular mechanisms of resistance to anthracyclines and taxanes in cancer: intrinsic and acquired. *Semin Oncol* 35(2 Suppl 2):S1–S14.
- Choi YL, Tsukasaki K, O'Neill MC, Yamada Y, Onimaru Y, Matsumoto K, Ohashi J, Yamashita Y, Tsutsumi S, Kaneda R, Takada S, Aburatani H, Kamihira S, Nakamura T, Tomonaga M & Mano H (2007) A genomic analysis of adult T-cell leukemia. *Oncogene*. 26(8):1245-1255.

- Chow JP, Man WY, Mao M, Chen H, Cheung F, Nicholls J, Tsao SW, Li Lung M & Poon RY (2013) PARP1 is overexpressed in nasopharyngeal carcinoma and its inhibition enhances radiotherapy. *Mol Cancer Ther* 12(11): 2517-2528.
- Clarke CA, Glaser SL, Keegan TH & Stroup A (2005) Neighborhood socioeconomic status and Hodgkin's lymphoma incidence in California. *Cancer Epidemiol Biomarkers Prev* 14(6):1441-1447.
- Clarke CA, Morton LM, Lynch C, Pfeiffer RM, Hall EC, Gibson TM, Weisenburger DD, Martínez-Maza O, Hussain SK, Yang J, Chang ET & Engels EA (2013) Risk of lymphoma subtypes after solid organ transplantation in the United States. *Br J Cancer* 109(1):280-288.
- Clavel J, Steliarova-Foucher E, Berger C, Danon S & Valerianova Z (2006) Hodgkin's disease incidence and survival in European children and adolescents (1978-1997): report from the Automated Cancer Information System project. *Eur J Cancer* 42(13):2037-2049.
- Connelly RR, Christine BW (1974) A cohort study of cancer following infectious mononucleosis. *Cancer Res* 34(5):1172-1178.
- Connors JM, Jurczak W, Straus DJ, Ansell SM, Kim WS, Gallamini A, Younes A, Alekseev S, Illés Á, Picardi M, Lech-Maranda E, Oki Y, Feldman T, Smolewski P, Savage KJ, Bartlett NL, Walewski J, Chen R, Ramchandren R, Zinzani PL, Cunningham D, Rosta A, Josephson NC, Song E, Sachs J, Liu R, Jolin HA, Huebner D, Radford J (2018) Brentuximab Vedotin with Chemotherapy for Stage III or IV Hodgkin's Lymphoma. *N Engl J Med* 25;378(4): 331-344.
- Cordeiro A, Monzó M & Navarro A (2017) Non-Coding RNAs in Hodgkin Lymphoma. *Int J Mol Sci* 29;18(6).
- Couvelard A, Deschamps L, Rebours V, Sauvanet A, Gatter K, Pezzella F, Ruzsniowski P & Bedossa P (2008) Overexpression of the oxygen sensors PHD-1, PHD-2, PHD-3, and FIH Is associated with tumor aggressiveness in pancreatic endocrine tumors. *Clin Cancer Res* 14(20):6634-6639.
- Crump C, Sundquist K, Sieh W, Winkleby MA & Sundquist J (2012) Perinatal and family risk factors for Hodgkin lymphoma in childhood through young adulthood. *Am J Epidemiol* 176(12):1147-1158.
- Csibi A, Fendt SM, Li C, Pouligiannis G, Choo AY, Chapski DJ, Jeong SM, Dempsey JM, Parkhitko A, Morrison T, Henske EP, Haigis MC, Cantley LC, Stephanopoulos G, Yu J & Blenis J (2013) The mTORC1 pathway stimulates glutamine metabolism and cell proliferation by repressing SIRT4. *Cell* 153(4):840–854.
- Cummins EP1, Berra E, Comerford KM, Ginouves A, Fitzgerald KT, Seeballuck F, Godson C, Nielsen JE, Moynagh P, Pouyssegur J & Taylor CT (2006) Prolyl hydroxylase-1 negatively regulates I κ B kinase-beta, giving insight into hypoxia-induced NF κ B activity. *Proc Natl Acad Sci U S A* 103(48):18154-18159.
- Curtin NJ (2012) DNA repair dysregulation from cancer driver to therapeutic target. *Nat Rev Cancer* 12(12):801-817.
- Curtin NJ (2012) DNA repair dysregulation from cancer driver to therapeutic target. *Nat Rev Cancer* 12(12):801-817.

- Daniëls LA, Oerlemans S, Krol AD, Creutzberg CL & van de Poll-Franse LV (2014) Chronic fatigue in Hodgkin lymphoma survivors and associations with anxiety, depression and comorbidity. *Br J Cancer* 110(4):868-874.
- Dawson CW, Tramontanis G, Eliopoulos AG & Young LS (2003) Epstein-Barr virus latent membrane protein 1 (LMP1) activates the phosphatidylinositol 3-kinase/Akt pathway to promote cell survival and induce actin filament remodeling. *J Biol Chem* 278(6):3694-3704.
- Dawson MA & Kouzarides T (2012) Cancer epigenetics: from mechanism to therapy. *Cell* 150(1):12–27.
- Declercq JP, Evrard C, Clippe A, Stricht DV, Bernard A & Knoops B (2001). Crystal structure of human peroxiredoxin 5, a novel type of mammalian peroxiredoxin at 1.5 Å resolution. *J Mol Biol* 311(4):751-759.
- de Oliveira KAP, Kaergel E, Heinig M, Fontaine JF, Patone G, Muro EM, Mathas S, Hummel M, Andrade-Navarro MA, Hübner N & Scheiderei C (2016) A roadmap of constitutive NF-κB activity in Hodgkin lymphoma: Dominant roles of p50 and p52 revealed by genome-wide analyses. *Genome Med* 8: 28.
- Desantis V, Lamanuzzi A, Vacca A (2018) The role of SIRT6 in tumors. *Haematologica* 103(1):1-4.
- Deutsch YE, Tadmor T, Podack ER & Rosenblatt JD (2011) CD30: an important new target in hematologic malignancies. *Leuk Lymphoma* 52(9):1641-1654.
- Devillard E, Bertucci F, Trempat P, Bouabdallah R, Llorion B, Giaconia A, Brousset P, Granjeaud S, Nguyen C, Birnbaum D, Birg F, Houlgatte R & Xerri L (2002) Gene expression profiling defines molecular subtypes of classical Hodgkin's disease. *Oncogene* 21(19): 3095-3102.
- Diefenbach CS, Hong F, Cohen JB, Robertson MJ, Ambinder RF, Fenske TS, Advani RH, Kahl BS & Ansell S (2015) Preliminary safety and efficacy of the combination of brentuximab vedotin and ipilimumab in relapsed/refractory Hodgkin lymphoma: a trial of the ECOG-ACRIN Cancer Research Group (E4412). *Blood* 126(23):585.
- Diepstra A, Poppema S, Boot M, Visser L, Nolte IM, Niens M, Te Meerman GJ & van den Berg A (2008) HLA-G protein expression as a potential immune escape mechanism in classical Hodgkin's lymphoma. *Tissue Antigens* 71(3):219-226.
- Drost R, Bouwman P, Rottenberg S, Boon U, Schut E, Klarenbeek S, Klijn C, van der Heijden I, van der Gulden H, Wientjens E, Pieterse M, Catteau A, Green P, Solomon E, Morris JR & Jonkers J (2011) BRCA1 RING function is essential for tumor suppression but dispensable for therapy resistance. *Cancer Cell* 20(6):797-809.
- Duncan Lyngdoh RH & Schaefer HF (2009) Elementary lesions in DNA subunits: electron, hydrogen atom, proton, and hydride transfers. *Acc Chem Res* 42(4):563–572.
- Dutton A, Reynolds GM, Dawson CW, Young LS & Murray PG (2005) Constitutive activation of phosphatidylinositol 3 kinase contributes to the survival of Hodgkin's lymphoma cells through a mechanism involving Akt kinase and mTOR. *J Pathol* 205(4): 498–506.

- Dühmke E, Franklin J, Pfreundschuh M, Sehlen S, Willich N, Rühl U, Müller RP, Lukas P, Atzinger A, Paulus U, Lathan B, Rüffer U, Sieber M, Wolf J, Engert A, Georgii A, Staar S, Herrmann R, Beykirch M, Kirchner H, Emminger A, Greil R, Fritsch E, Koch P, Drochters A, Brosteanu O, Hasenclever D, Loeffler M & Diehl V (2001) Low-dose radiation is sufficient for the noninvolved extended-field treatment in favorable early-stage Hodgkin's disease: long-term results of a randomized trial of radiotherapy alone. *J Clin Oncol* 19(11):2905-2914.
- Eberle FC, Mani H & Jaffe ES (2009) Histopathology of Hodgkin's lymphoma. *Cancer J* 15(2):129-137.
- Eichenauer DA, Plütschow A, Kreissl S, Sökler M, Hellmuth JC, Meissner J, Mathas S, Topp MS, Behringer K, Klapper W, Kuhnert G, Dietlein M, Kobe C, Fuchs M, Diehl V, Engert A & Borchmann P (2017) Incorporation of brentuximab vedotin into first-line treatment of advanced classical Hodgkin's lymphoma: final analysis of a phase 2 randomised trial by the German Hodgkin Study Group. *Lancet Oncol* 18(12): 1680-1687.
- Ekström Smedby K, Hjalgrim H, Melbye M, Torräng A, Rostgaard K, Munksgaard L, Adami J, Hansen M, Porwit-MacDonald A, Jensen BA, Roos G, Pedersen BB, Sundström C, Glimelius B & Adami HO (2005) Ultraviolet radiation exposure and risk of malignant lymphomas. *J Natl Cancer Inst* 97(3):199–209.
- Ema M, Taya S, Yokotani N, Sogawa K, Matsuda Y & Fujii-Kuriyama Y (1997) A novel bHLH-PAS factor with close sequence similarity to hypoxia-inducible factor 1alpha regulates the VEGF expression and is potentially involved in lung and vascular development. *Proc Natl Acad Sci U S A* 94(9): 4273–4278.
- Engert A, Ballova V, Haverkamp H, Pfistner B, Josting A, Dühmke E, Müller-Hermelink K & Diehl V (2005) Hodgkin's lymphoma in elderly patients: a comprehensive retrospective analysis from the German Hodgkin's Study Group. *J Clin Oncol* 23(22): 5052–5060.
- Engert A, Haverkamp H, Kobe C, Markova J, Renner C, Ho A, Zijlstra J, Král Z, Fuchs M, Hallek M, Kanz L, Döhner H, Dörken B, Engel N, Topp M, Klutmann S, Amthauer H, Bockisch A, Kluge R, Kratochwil C, Schober O, Greil R, Andreesen R, Kneba M, Pfreundschuh M, Stein H, Eich HT, Müller RP, Dietlein M, Borchmann P & Diehl V (2012) Reduced-intensity chemotherapy and PET-guided radiotherapy in patients with advanced stage Hodgkin's lymphoma (HD15 trial): a randomised, open-label, phase 3 non-inferiority trial. *Lancet*. 379(9828): 1791-1799.
- Engert A, Plütschow A, Eich HT, Lohri A, Dörken B, Borchmann P, Berger B, Greil R, Willborn KC, Wilhelm M, Debus J, Eble MJ, Sökler M, Ho A, Rank A, Ganser A, Trümper L, Bokemeyer C, Kirchner H, Schubert J, Král Z, Fuchs M, Müller-Hermelink HK, Müller RP & Diehl V (2010) Reduced treatment intensity in patients with early-stage Hodgkin's lymphoma. *N Engl J Med* 363(7): 640-652.
- Epstein AC, Gleadle JM, McNeill LA, Hewitson KS, O'Rourke J, Mole DR, Mukherji M, Metzger E, Wilson MI, Dhanda A et al (2001) C. elegans EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* 107: 43-54.

- Erez N, Milyavsky M, Eilam R, Shats I, Goldfinger N & Rotter V (2003) Expression of prolyl-hydroxylase-1 (PHD1/EGLN2) suppresses hypoxia inducible factor-1 α activation and inhibits tumor growth. *Cancer Res* 63(24):8777-8783.
- Escribano-Díaz C, Orthwein A, Fradet-Turcotte A, Xing M, Young JT, Tkáč J, Cook MA, Rosebrock AP, Munro M, Canny MD, Xu D & Durocher D (2013) A cell cycle-dependent regulatory circuit composed of 53BP1-RIF1 and BRCA1-CtIP controls DNA repair pathway choice. *Mol Cell* 49(5):872-883.
- Evans MD, Dizdaroglu M & Cooke MS (2004) Oxidative DNA damage and disease: induction, repair and significance. *Mutat. Res* 567(1): 1-61.
- Evens AM, Hutchings M & Diehl V (2008) Treatment of Hodgkin lymphoma: The past, present, and future. *Nat Clin Pract Oncol* 5(9):543-556.
- Evens AM, Sehn LH, Farinha P, Nelson BP, Raji A, Lu Y, Brakman A, Parimi V, Winter JN, Schumacker PT, Gascoyne RD & Gordon LI (2010) Hypoxia-inducible factor-1 { α } expression predicts superior survival in patients with diffuse large B-cell lymphoma treated with R-CHOP. *J Clin Oncol* 28(6):1017-1024.
- Everhard S, Kaloshi G, Crinière E, Benouaich-Amiel A, Lejeune J, Marie Y, Sanson M, Kujas M, Mokhtari K, Hoang-Xuan K, Delattre JY & Thillet J (2006) MGMT methylation: a marker of response to temozolomide in low-grade gliomas. *Ann Neurol* 60(6):740-743.
- Evers B, Helleday T & Jonkers J (2010) Targeting homologous recombination repair defects in cancer. *Trends Pharmacol Sci* 31(8):372-80.
- Fang J, Seki T & Maeda H (2009) Therapeutic strategies by modulating oxygen stress in cancer and inflammation. *Adv Drug Deliv Rev* 61(4):290-302.
- Fehniger TA, Larson S, Trinkaus K, Siegel MJ, Cashen AF, Blum KA, Fenske TS, Hurd DD, Goy A, Schneider SE, Keppel CR, Wagner-Johnston ND, Carson KR & Bartlett NL (2011) A phase 2 multicenter study of lenalidomide in relapsed or refractory classical Hodgkin lymphoma. *Blood* 118(19):5119-5125.
- Filippi AR, Botticella A, Bellò M, Botto B, Castiglione A, Gavarotti P, Gottardi D, Parvis G, Bisi G, Levis A, Vitolo U & Ricardi U (2013) *Leuk Lymphoma* 54(6):1183-1187.
- Finkel T (2003) Oxidant signals and oxidative stress. *Curr. Opin. Cell Biol* 15:247–254.
- Finkel T, Deng CX & Mostoslavsky R (2009) Recent progress in the biology and physiology of sirtuins. *Nature* 460(7255):587-591.
- Finnish Cancer Registry - Institute for Statistical and Epidemiological Cancer Research (2015) URI: <https://syoparekisteri.fi/tilastot/tautitilastot/> Cited: 3/12/2017
- Firestein R, Blander G, Michan S, Oberdoerffer P, Ogino S, Campbell J, Bhimavarapu A, Luikenhuis S, de Cabo R, Fuchs C, Hahn WC, Guarente LP & Sinclair DA (2008) The SIRT1 deacetylase suppresses intestinal tumorigenesis and colon cancer growth. *PLoS One* 3(4):e2020.
- Flavell JR, Baumforth KR, Wood VH, Davies GL, Wei W, Reynolds GM, Morgan S, Boyce A, Kelly GL, Young LS & Murray PG (2008) Down-regulation of the TGF- β target gene, PTPRK, by the Epstein–Barr virus encoded EBNA1 contributes to the growth and survival of Hodgkin lymphoma cells. *Blood* 111(1):292–301.

- Forero-Torres A, Leonard JP, Younes A, Rosenblatt JD, Brice P, Bartlett NL, Bosly A, Pinter-Brown L, Kennedy D, Sievers EL & Gopal AK (2009) A Phase II study of SGN-30 (anti-CD30 mAb) in Hodgkin lymphoma or systemic anaplastic large cell lymphoma. *Br J Haematol* 146(2):171-179.
- Fox SB, Generali D, Berruti A, Brizzi MP, Campo L, Bonardi S, Bersiga A, Allevi G, Milani M, Aguggini S, Mele T, Dogliotti L, Bottini A & Harris AL (2011) The prolyl hydroxylase enzymes are positively associated with hypoxia-inducible factor-1 α and vascular endothelial growth factor in human breast cancer and alter in response to primary systemic treatment with epirubicin and tamoxifen. *Breast Cancer Res* 13(1): R16.
- Fridovich I. (1978) The biology of oxygen radicals. *Science* 201(4359):875–880.
- Fromm JR, Kussick SJ & Wood BL (2006) Identification and purification of classical Hodgkin cells from lymph nodes by flow cytometry and flow cytometric cell sorting. *Am. J. Clin. Pathol.* 126(5): 764–780.
- Fu L, Chen L, Yang J, Ye T, Chen Y & Fang J (2012) HIF-1 α -induced histone demethylase JMJD2B contributes to the malignant phenotype of colorectal cancer cells via an epigenetic mechanism. *Carcinogenesis* 33(9):1664-1673.
- Gallamini A, Hutchings M, Rigacci L, Specht L, Merli F, Hansen M, Patti C, Loft A, Di Raimondo F, D'Amore F, Biggi A, Vitolo U, Stelitano C, Sancetta R, Trentin L, Luminari S, Iannitto E, Viviani S, Pierrri I & Levis A (2007) Early interim 2-[18F] fluoro-2-deoxy-D-glucose positron emission tomography is prognostically superior to international prognostic score in advanced-stage Hodgkin's lymphoma: a report from a joint Italian-Danish study. *J Clin Oncol* 25(24): 3746-3752.
- Gandhi MK, Moll G, Smith C, Dua U, Lambley E, Ramuz O, Gill D, Marlton P, Seymour JF & Khanna R (2007) Galectin-1 mediated suppression of Epstein-Barr virus specific T-cell immunity in classic Hodgkin lymphoma. *Blood* 110(9): 1326-1329.
- Garber ME, Troyanskaya OG, Schluens K, Petersen S, Thaesler Z, Pacyna-Gengelbach M, van de Rijn M, Rosen GD, Perou CM, Whyte RI, Altman RB, Brown PO, Botstein D & Petersen I (2001) Diversity of gene expression in adenocarcinoma of the lung. *Proc Natl Acad Sci U S A* 98(24):13784-13789.
- Garvalov BK, Foss F, Henze AT, Bethani I, Gräf-Höchst S, Singh D, Filatova A, Dopeso H, Seidel S, Damm M, Acker-Palmer A & Acker T (2014) PHD3 regulates EGFR internalization and signalling in tumours. *Nat Commun* 5:5577.
- Gavande NS, VanderVere-Carozza PS, Hinshaw HD, Jalal SI, Sears CR, Pawelczak KS & Turchi JJ (2016) DNA repair targeted therapy: The past or future of cancer treatment? *Pharmacol Ther* 160:65-83.
- Georgakis GV, Li Y, Rassidakis GZ, Medeiros LJ, Mills GB & Younes A (2006) Inhibition of the phosphatidylinositol-3 kinase/Akt promotes G1 cell cycle arrest and apoptosis in Hodgkin lymphoma. *Br J Haematol* 132(4):503-511.
- Gewirtz DA (1999) A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. *Biochem Pharmacol.* 57(7):727-41.

- Glimelius I, Edström A, Amini RM, Fischer M, Nilsson G, Sundström C, Enblad G & Molin D (2006) IL-9 expression contributes to the cellular composition in Hodgkin lymphoma. *Eur J Haematol* 76(4):278-283.
- Gopal AK, Fanale MA, Moskowitz CH, Shustov AR, Mitra S, Ye W, Younes A & Moskowitz AJ (2017) Phase II study of idelalisib, a selective inhibitor of PI3K δ , for relapsed/refractory classical Hodgkin lymphoma. *Ann Oncol* 28(5):1057-1063.
- Gordan JD, Thompson CB & Simon MC (2007) HIF and c-Myc: sibling rivals for control of cancer cell metabolism and proliferation. *Cancer Cell* 12(2):108-113.
- Gospodarowicz MK, O'Sullivan B & Koh ES (2006) Prognostic factors: Principles and applications. in: MK Gospodarowicz, B O'Sullivan, L.H. Sobin (Eds.) *Prognostic factors in cancer*. Third ed: 23–38.
- Green MR, Monti S, Rodig SJ, Juszczynski P, Currie T, O'Donnell E, Chapuy B, Takeyama K, Neuberg D, Golub TR, Kutok JL & Shipp MA (2010) Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. *Blood* 116(17): 3268–3277.
- Gruss HJ, Hirschstein D, Wright B, Ulrich D, Caligiuri MA, Barcos M, Strockbine L, Armitage RJ & Dower SK (1994) Expression and function of CD40 on Hodgkin and Reed-Sternberg cells and the possible relevance for Hodgkin's disease. *Blood* 84(7): 2305–2314.
- Gu YZ, Moran SM, Hogenesch JB, Wartman L & Bradfield CA (1998) Molecular characterization and chromosomal localization of a third alpha-class hypoxia inducible factor subunit, HIF3alpha. *Gene Expr* 7(3): 205–213.
- Guidetti A, Carlo-Stella C, Locatelli SL, Malorni W, Mortarini R, Viviani S, Russo D, Marchianò A, Sorasio R, Doderò A, Farina L, Giordano L, Di Nicola M6 Anichini A, Corradini P & Gianni AM (2014) Phase II study of perifosine and sorafenib dual-targeted therapy in patients with relapsed or refractory lymphoproliferative diseases. *Clin Cancer Res.* (22): 5641-5651.
- Guo B, Cen H, Tan X & Ke Q (2016) Meta-analysis of the prognostic and clinical value of tumor-associated macrophages in adult classical Hodgkin lymphoma. *BMC Med.*14(1):159.
- Guzy RD, Hoyos B, Robin E, Chen H, Liu L, Mansfield KD, Simon MC, Hammerling U & Schumacker PT (2005) Mitochondrial complex III is required for hypoxia-induced ROS production and cellular oxygen sensing. *Cell Metab* 1(6):401-408.
- Haigis MC & Sinclair DA. *Mammalian Sirtuins: Biological Insights and Disease Relevance.* *Annu. Rev. Pathol. Mech. Dis.* 2010; 5:253–295.
- Haigis MC, Mostoslavsky R, Haigis KM, Fahie K, Christodoulou DC, Murphy AJ, Valenzuela DM, Yancopoulos GD, Karow M, Blander G, Wolberger C, Prolla TA, Weindruch R, Alt FW & Guarente L (2006) SIRT4 inhibits glutamate dehydrogenase and opposes the effects of calorie restriction in pancreatic beta cells. *Cell* 126(5):941–954.
- Hallows WC, Lee S & Denu JM (2006) Sirtuins deacetylate and activate mammalian acetyl-CoA synthetases. *Proc Natl Acad Sci U S A* 103(27):10230-10235.

- Hanahan D & Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144(5):646-674.
- Hanamoto, H., Nakayama T, Miyazato H, Takegawa S, Hieshima K, Tatsumi Y, Kanamaru A & Yoshie O (2004) Expression of CCL28 by Reed-Sternberg cells defines a major subtype of classical Hodgkin's disease with frequent infiltration of eosinophils and/or plasma cells. *Am. J. Pathol* 164(3): 997–1006.
- Hao Y, Chapuy B, Monti S, Sun HH, Rodig SJ & Shipp MA (2014) Selective JAK2 inhibition specifically decreases Hodgkin lymphoma and mediastinal large B-cell lymphoma growth in vitro and in vivo. *Clin Cancer Res* 20(10):2674-2683.
- Hasenclever D, Diehl VA (1998) Prognostic score for advanced Hodgkin's disease. International Prognostic Factors Project on Advanced Hodgkin's Disease. *N Engl J Med* 339(21):1506-1514.
- He H, Zhao Y, Wang N, Zhang L & Wang C (2014) 8-Hydroxy-2'-deoxyguanosine expression predicts outcome of esophageal cancer. *Ann Diagn Pathol* 18(6): 326-328.
- Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC & Stupp R (2005) MGMT gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 352:997-1003.
- Hempel N, Carrico PM & Melendez JA (2011) Manganese superoxide dismutase (Sod2) and redox-control of signaling events that drive metastasis. *Anticancer Agents Med Chem* 11(2): 191-201.
- Henry-Amar M, Friedman S, Hayat M, Somers R, Meerwaldt JH, Carde P, Burgers JM, Thomas J, Monconduit M & Noordijk EM (1991) Erythrocyte sedimentation rate predicts early relapse and survival in early-stage Hodgkin disease. The EORTC Lymphoma Cooperative Group. *Ann Intern Med* 114(5):361-365.
- Hernandez-Luna MA, Rocha-Zavaleta L, Vega MI & Huerta-Yepe S (2013) Hypoxia inducible factor-1 α induces chemoresistance phenotype in non-Hodgkin lymphoma cell line via up-regulation of Bcl-xL. *Leuk Lymphoma* 54(5):1048-1055.
- Hintsala HR, Jokinen E, Haapasaari KM, Moza M, Ristimäki A, Soini Y, Koivunen J & Karihtala P (2016) Nrf2/Keap1 Pathway and Expression of Oxidative Stress Lesions 8-hydroxy-2'-deoxyguanosine and Nitrotyrosine in Melanoma. *Anticancer Res* 36(4):1497-1506.
- Hintsala HR, Soini Y, Haapasaari KM, Karihtala P (2015) Dysregulation of redox-state-regulating enzymes in melanocytic skin tumours and the surrounding microenvironment. *Histopathology* 67(3): 348-357.
- Hinz M, Lemke P, Anagnostopoulos I, Hacker C, Krappmann D, Mathas S, Dörken B, Zenke M, Stein H & Scheiderei C (2002) Nuclear factor kappaB-dependent gene expression profiling of Hodgkin's disease tumor cells, pathogenetic significance, and link to constitutive signal transducer and activator of transcription 5a activity. *J Exp Med* 196(5):605-617.
- Hirota K, Murata M, Sachi Y, Nakamura H, Takeuchi J, Mori K & Yodoi J (1999) Distinct roles of thioredoxin in the cytoplasm and in the nucleus. A two-step mechanism of redox regulation of transcription factor NF-kappaB. *J Biol Chem* 274(39): 27891-27897.

- Hodgkin T (1832) On some morbid appearances of the absorbent glands and spleen. *Med. Chirurg. Trans* 17: 68-114.
- Hodgson DC, Koh ES, Tran TH, Heydarian M, Tsang R, Pintilie M, Xu T, Huang L, Sachs RK & Brenner DJ (2007) Individualized estimates of second cancer risks after contemporary radiation therapy for Hodgkin lymphoma. *Cancer* 110(11): 2576-2586.
- Hollander P, Rostgaard K, Smedby KE, Molin D, Loskog A, de Nully Brown P, Enblad G, Amini RM, Hjalgrim H & Glimelius I (2017) An anergic immune signature in the tumor microenvironment of classical Hodgkin lymphoma is associated with inferior outcome. *Eur J Haematol* 100(1):88-97.
- Hoppe RT, Advani RH, Ai WZ, Ambinder RF, Aoun P, Bello CM, Benitez CM, Bernat K, Bierman PJ, Blum KA, Chen R, Dabaja B, Forero A, Gordon LI, Hernandez-Ilizaliturri FJ, Hochberg EP, Huang J, Johnston PB, Kaminski MS, Kenkre VP, Khan N, Maloney DG, Mauch PM, Metzger M, Moore JO, Morgan D, Moskowitz CH, Mulrone C, Poppe M, Rabinovitch R, Seropian S, Smith M, Winter JN, Yahalom J, Burns J, Ogba N & Sundar H (2017) Hodgkin Lymphoma Version 1.2017, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 15(5):608-638.
- Horie R, Watanabe T, Morishita Y, Ito K, Ishida T, Kanegae Y, Saito I, Higashihara M, Mori S, Kadin ME & Watanabe T (2002) Ligand- independent signaling by overexpressed CD30 drives NF-kappaB activation in Hodgkin-Reed-Sternberg cells. *Oncogene* 21(16):2493-2503.
- Huffman DM1, Grizzle WE, Bamman MM, Kim JS, Eltoum IA, Elgavish A & Nagy TR (2007) SIRT1 is significantly elevated in mouse and human prostate cancer. *Cancer Res* 67(14):6612-6618.
- Hufnagl A, Herr L, Friedrich T, Durante M, Taucher-Scholz G & Scholz M (2015) The link between cell-cycle dependent radiosensitivity and repair pathways: A model based on the local, sister-chromatid conformation dependent switch between NHEJ and HR. *DNA Repair (Amst)* 27:28-39.
- Hutchings M, Loft A, Hansen M, Pedersen LM, Buhl T, Jurlander J, Buus S, Keiding S, D'Amore F, Boesen AM, Berthelsen AK & Specht L (2006) FDG-PET after two cycles of chemotherapy predicts treatment failure and progression-free survival in Hodgkin lymphoma. *Blood* 107(1):52-59.
- Hutchinson F (1961) Molecular basis for action of ionizing radiations. *Science* 134(3478): 533-538.
- International Agency for Research on Cancer, Lyon, France (2012) GLOBOCAN 2012: URI: <http://globocan.iarc.fr/>
- Isohookana J, Haapasaari KM, Soini Y & Karihtala P (2016) Loss of Peroxiredoxin Expression Is Associated with an Aggressive Phenotype in Pancreatic Adenocarcinoma. *Anticancer Res* 36(1):427-433.
- Isohookana J, Haapasaari KM, Soini Y & Karihtala P (2018) KDM4D Predicts Recurrence in Exocrine Pancreatic Cells of Resection Margins from Patients with Pancreatic Adenocarcinoma. *Anticancer Res* 38(4):2295-2302.
- Jabs T (1999) Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. *Biochem Pharmacol* 57(3):231-245.

- Jacobson MD (1996) Reactive oxygen species and programmed cell death. *Trends Biochem Sci* 21(3): 83–86.
- Jang KYI, Hwang SH, Kwon KS, Kim KR, Choi HN, Lee NR, Kwak JY, Park BH, Park HS, Chung MJ, Kang MJ, Lee DG, Kim HS, Shim H & Moon WS (2008) SIRT1 expression is associated with poor prognosis of diffuse large B-cell lymphoma. *Am J Surg Pathol* 32(10):1523–1531.
- Janssen AM, Bosman CB, van Duijn W, Oostendorp-van de Ruit MM, Kubben FJ, Griffioen G, Lamers CB, van Krieken JH, van de Velde CJ & Verspaget HW (2000) Superoxide dismutases in gastric and esophageal cancer and the prognostic impact in gastric cancer. *Clin Cancer Res* 6(8):3183-3192.
- Jeong SM, Xiao C, Finley LW, Lahusen T, Souza AL, Pierce K, Li YH, Wang X, Laurent G, German NJ, Xu X, Li C, Wang RH, Lee J, Csibi A, Cerione R, Blenis J, Clish CB, Kimmelman A, Deng CX & Haigis MC (2013) SIRT4 has tumor-suppressive activity and regulates the cellular metabolic response to DNA damage by inhibiting mitochondrial glutamine metabolism. *Cancer Cell* 23(4):450-463.
- Jiang C & Pugh BF (2009) Nucleosome positioning and gene regulation: advances through genomics. *Nat. Rev. Genet* 10(3): 161-172.
- Jiang Y, Chen Y, Huang R & Chen G (2016) Comparison of the efficiency of ABVD versus BEACOPP for Hodgkin lymphoma treatment: a meta-analysis. *Int J Hematol* 104(4):413-419.
- Johnston PB, Inwards DJ, Colgan JP, Laplant BR, Kabat BF, Habermann TM, Micallef IN, Porrata LF, Ansell SM, Reeder CB, Roy V & Witzig TE (2010) A Phase II trial of the oral mTOR inhibitor everolimus in relapsed Hodgkin lymphoma. *Am J Hematol* 85(5):320-324.
- Jones PA & Baylin SB (2002) The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 3(6):415-428.
- Joos S, Küpper M, Ohl S, von Bonin F, Mechttersheimer G, Bentz M, Marynen P, Möller P, Pfreundschuh M, Trümper L & Lichter P (2000) Genomic imbalances including amplification of the tyrosine kinase gene JAK2 in CD30+ Hodgkin cells. *Cancer Res* 60(3):549–552.
- Jordan MA, Thrower D, Wilson L (1991) Mechanism of inhibition of cell proliferation by Vinca alkaloids. *Cancer Res* 51(8): 2212-2222.
- Jost PJ & Ruland J (2007) Aberrant NF-kappaB signaling in lymphoma: mechanisms, consequences, and therapeutic implications. *Blood* 109(7):2700–2707.
- Jundt F, Anagnostopoulos I, Bommert K, Emmerich F, Müller G, Foss HD, Royer HD, Stein H & Dörken B (1999) Hodgkin/Reed- Sternberg cells induce fibroblasts to secrete eotaxin, a potent chemoattractant for T cells and eosinophils. *Blood* 94(6):2065–2071.
- Jundt, F. Anagnostopoulos I, Bommert K, Emmerich F, Müller G, Foss HD, Royer HD, Stein H & Dörken B (1999) Hodgkin/Reed- Sternberg cells induce fibroblasts to secrete eotaxin, a potent. *Blood* 94(6):2065-2071.
- Jyrkkiö S, Mokka M, Vasala K (2014) Hodgkinin lymfooma. *Lääketieteellinen Aikakauskirja Duodecim* 130(9):913-920.

- Kang SW, Baines IC & Rhee SG (1998) Characterization of a mammalian peroxiredoxin that contains one conserved cysteine. *J. Biol. Chem* 273(11):6303–6311.
- Kanzler H, Küppers R, Hansmann ML & Rajewsky K (1996) Hodgkin and Reed-Sternberg cells in Hodgkin's disease represent the outgrowth of a dominant tumor clone derived from (crippled) germinal center B cells. *J Exp Med* 184(4):1495-1505.
- Kapatai G & Murray P (2007) Contribution of the Epstein Barr virus to the molecular pathogenesis of Hodgkin lymphoma. *J. Clin. Pathol.* 60(12):1342-1349.
- Kapp U, Wolf J, Hummel M, Pawlita M, von Kalle C, Dallenbach F, Schwonzen M, Krueger GR, Müller-Lantzsch N & Fonatsch C (1993) Hodgkin's lymphoma-derived tissue serially transplanted into severe combined immunodeficient mice. *Blood* 82(4):1247-1256.
- Kapp U, Yeh WC, Patterson B, Elia AJ, Kägi D, Ho A, Hessel A, Tipsword M, Williams A, Mirtsos C, Itie A, Moyle M & Mak TW (1999) Interleukin 13 is secreted by and stimulates the growth of Hodgkin and Reed-Sternberg cells. *J. Exp. Med* 189(12): 1939-1946.
- Karihtala P & Soini Y (2007) Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. *APMIS* 115(2):81-103.
- Karihtala P, Kauppila S, Puistola U, Jukkola-Vuorinen A (2011) Divergent behavior of oxidative stress markers 8-hydroxydeoxyguanosine (8-OHdG) and 4-hydroxy-2-nonenal (HNE) in breast carcinogenesis. *Histopathology* 56(6):854-862.
- Karihtala P, Mäntyniemi A, Kang SW, Kinnula VL, Soini Y (2003) Peroxiredoxins in breast carcinoma. *Clin Cancer Res* 9(9):3418-3424.
- Karihtala P, Porvari K, Soini Y, Haapasaari KM (2017) Redox Regulating Enzymes and Connected MicroRNA Regulators Have Prognostic Value in Classical Hodgkin Lymphomas. *Oxid Med Cell Longev* 2017:2696071
- Karihtala P, Soini Y, Vaskivuo L, Bloigu R & Puistola U (2009) DNA adduct 8-hydroxydeoxyguanosine, a novel putative marker of prognostic significance in ovarian carcinoma. *Int J Gynecol Cancer* 19(6): 1047-1051.
- Kasai H (1997) Analysis of a form of oxidative DNA damage, 8- hydroxy-20-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutat. Res* 387(3): 147-163.
- Kato H, Miyazaki T, Yoshikawa M, Nakajima M, Fukai Y, Masuda N, Ojima H, Tsukada K, Nishida Y & Kuwano H (2001) Expression of nitrotyrosine is associated with angiogenesis in esophageal squamous cell carcinoma. *Anticancer Res* 21(5): 3323-3329.
- Katz J, Janik JE, Younes A. Brentuximab Vedotin (SGN-35) (2011) *Clin Cancer Res* 17(20):6428-6436.
- Kawahara TL, Michishita E, Adler AS, Damian M, Berber E, Lin M, McCord RA, Ongaigui KC, Boxer LD, Chang HY & Chua KF (2009) SIRT6 links histone H3 lysine 9 deacetylation to NF-kappaB-dependent gene expression and organismal life span. *Cell* 136(1):62-74.
- Keir ME, Butte MJ, Freeman GJ, Sharpe AH (2008) PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol* 26:677-704.

- Keith B, Johnson RS & Simon MC (2011) HIF1 α and HIF2 α : sibling rivalry in hypoxic tumour growth and progression. *Nat Rev Cancer* 12(1): 9-22.
- Kewitz S, Kurch L, Volkmer I & Staeger MS (2016) Stimulation of the hypoxia pathway modulates chemotherapy resistance in Hodgkin's lymphoma cells. *Tumour Biol* 37(6):8229-8237.
- Kewitz S, Stiefel M, Kramm CM & Staeger MS (2014) Impact of O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation and MGMT expression on dacarbazine resistance of Hodgkin's lymphoma cells. *Leuk Res* 38(1):138-143.
- Khan MN, Bhattacharyya T, Andrikopoulos P, Esteban MA, Barod R, Connor T, Ashcroft M, Maxwell PH & Kiriakidis S (2011) Factor inhibiting HIF (FIH-1) promotes renal cancer cell survival by protecting cells from HIF-1 α -mediated apoptosis. *Br J Cancer* 104(7):1151-1159
- Khongkow M, Olmos Y, Gong C, Gomes AR, Monteiro LJ, Yagüe E, Cavaco TB, Khongkow P, Man EP, Laohasinnarong S, Koo CY, Harada-Shoji N, Tsang JW, Coombes RC, Schwer B, Khoo US & Lam EW (2013) SIRT6 modulates paclitaxel and epirubicin resistance and survival in breast cancer. *Carcinogenesis* 34(7):1476-1486.
- Khoury-Haddad H, Guttmann-Raviv N, Ipenberg I, Huggins D, Jeyasekharan AD & Ayoub N (2014) PARP1-dependent recruitment of KDM4D histone demethylase to DNA damage sites promotes double-strand break repair. *Proc Natl Acad Sci U S A* 111(7): 728-737.
- Kilger E, Kieser A, Baumann M & Hammerschmidt W (1998) Epstein-Barr virus-mediated B-cell proliferation is dependent upon latent membrane protein 1, which simulates an activated CD40 receptor. *EMBO J* 17(6):1700-1709.
- Kim HS, Xiao C, Wang RH, Lahusen T, Xu X, Vassilopoulos A, Vazquez-Ortiz G, Jeong WI, Park O, Ki SH, Gao B & Deng CX (2010) Hepatic-specific disruption of SIRT6 in mice results in fatty liver formation due to enhanced glycolysis and triglyceride synthesis. *Cell Metab* 12(3): 224-236.
- Kim LH, Eow GI, Peh SC & Poppema S (2003) The role of CD30, CD40 and CD95 in the regulation of proliferation and apoptosis in classical Hodgkin's lymphoma. *Pathology* 35(5):428-435.
- Kim MJ, Ahn K, Park SH, Kang HJ, Jang BG, Oh SJ, Oh SM, Jeong YJ, Heo JI, Suh JG, Lim SS, Ko YJ, Huh SO, Kim SC, Park JB, Kim J, Kim JI, Jo SA & Lee JY (2009) SIRT1 regulates tyrosine hydroxylase expression and differentiation of neuroblastoma cells via FOXO3a. *FEBS Lett* 583(7):1183-1188.
- Kim TD, Oh S, Shin S & Janknecht R (2012) Regulation of tumor suppressor p53 and HCT116 cell physiology by histone demethylase JMJD2D/KDM4D. *PLoS One* 7(4):e34618.
- Kim TD, Shin S, Berry WL, Oh S & Janknecht R (2012) The JMJD2A demethylase regulates apoptosis and proliferation in colon cancer cells. *J Cell Biochem* 113(4):1268-1276.
- Kinnula VL, Lehtonen S, Sormunen R, Kaarteenaho-Wiik R, Kang SW, Rhee SG & Soini Y (2002) Overexpression of peroxiredoxins I, II, III, V, and VI in malignant mesothelioma. *J Pathol* 196(3):316-323.

- Klaunig JE, Xu Y, Isenberg JS, Bachowski S, Kolaja KL, Jiang J, Stevenson DE & Walborg EF Jr (1998) The role of oxidative stress in chemical carcinogenesis. *Environ Health Perspect* 106(Suppl 1):289–295.
- Klotzsche-von Ameln A, Muschter A, Mamlouk S, Kalucka J, Prade I, Franke K, Rezaei M, Poitz DM, Breier G and Wielockx B (2011) Inhibition of HIF prolyl hydroxylase-2 blocks tumor growth in mice through the antiproliferative activity of TGF β . *Cancer Res* 71(9): 3306-3316.
- Kogure M, Takawa M, Cho HS, Toyokawa G, Hayashi K, Tsunoda T, Kobayashi T, Daigo Y, Sugiyama M, Atomi Y, Nakamura Y & Hamamoto R (2013) Deregulation of the histone demethylase JMJD2A is involved in human carcinogenesis through regulation of the G(1)/S transition. *Cancer Lett* 336(1): 76-84.
- Kohen R & Nyska A (2002) Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol* 30(6): 620–650.
- Kotla V, Goel S, Nischal S, Heuck C, Vivek K, Das B & Verma A (2009) Mechanism of action of lenalidomide in hematological malignancies. *J Hematol Oncol.* 2:36.
- Kouzarides T (2007) Chromatin modifications and their function. *Cell* 128(4):693-705.
- Kube D, Holtick U, Vockerodt M, Ahmadi T, Haier B, Behrmann I, Heinrich PC, Diehl V & Tesch H (2001) STAT3 is constitutively activated in Hodgkin cell lines. *Blood* 98(3): 762–770.
- Kumar R & Cheok CF (2014) RIF1: a novel regulatory factor for DNA replication and DNA damage response signaling. *DNA Repair (Amst)* 15:54-59.
- Küppers R, Engert A & Hansmann ML (2012) Hodgkin lymphoma. *J Clin Invest* 122(10):3439-3447.
- Küppers R, Rajewsky K, Zhao M, Simons G, Laumann R, Fischer R & Hansmann ML (1994) Hodgkin disease: Hodgkin and Reed-Sternberg cells picked from histological sections show clonal immunoglobulin gene rearrangements and appear to be derived from B cells at various stages of development. *Proc Natl Acad Sci U S A* 91(23):10962-10966.
- Kuruvilla J (2009) Standard therapy of advanced Hodgkin lymphoma. *Hematology Am Soc Hematol Educ Program.* 2009:497-506.
- Kuruvilla J, Keating A & Crump M (2011) How I treat relapsed and refractory Hodgkin lymphoma. *Blood* 117(16):4208-4217.
- Kuusisto ME, Haapasaari KM, Turpeenniemi-Hujanen T, Jantunen E, Soini Y, Peroja P, Bloigu R, Karihtala P & Kuittinen O (2015) High intensity of cytoplasmic peroxiredoxin VI expression is associated with adverse outcome in diffuse large B-cell lymphoma independently of International Prognostic Index. *J Clin Pathol* 68(7):552-556.
- Kwon T, Rho JK, Lee JC, Park YH, Shin HJ, Cho S, Kang YK, Kim BY, Yoon DY & Yu DY (2015) An important role for peroxiredoxin II in survival of A549 lung cancer cells resistant to gefitinib. *Exp Mol Med* 47(5): e165.

- Lake A, Shield LA, Cordano P, Chui DT, Osborne J, Crae S, Wilson KS, Tosi S, Knight SJ, Gesk S, Siebert R, Hay RT & Jarrett RF (2009) Mutations of NFKBIA, encoding I κ B α , are a recurrent finding in classical Hodgkin lymphoma but are not a unifying feature of non-EBV-associated cases. *Int J Cancer* 125(6):1334-1342.
- Lamprecht B, Kreher S, Anagnostopoulos I, Johrens K, Monteleone G, Jundt F, Stein H, Janz M, Dörken B, Mathas S (2008). Aberrant expression of the Th2 cytokine IL-21 in Hodgkin lymphoma cells regulates STAT3 signaling and attracts Treg cells via regulation of MIP-3 α . *Blood* 112(8):3339-3347.
- Lee H, Kim KR, Noh SJ, Park HS, Kwon KS, Park BH, Jung SH, Youn HJ, Lee BK, Chung MJ, Koh DH, Moon WS & Jang KY (2011) Expression of DBC1 and SIRT1 is associated with poor prognosis for breast carcinoma. *Hum Pathol.* 42(2):204-213.
- Lee KA, Lynd JD, O'Reilly S, Kiupel M, McCormick JJ & LaPres JJ (2008) The biphasic role of the hypoxia-inducible factor prolyl-4-hydroxylase, PHD2, in modulating tumor-forming potential. *Mol Cancer Res* 6(5):829-842.
- Levine BL, Miskin J, Wonnacott K & Keir C (2016) Global Manufacturing of CAR T Cell Therapy. *Mol Ther Methods Clin Dev* 4:92-101.
- Li E, Bestor TH & Jaenisch R (1992) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* 69(6):915-926.
- Li H, Yang X, Wang G, Li X, Tao D, Hu J & Luo X (2016) KDM4B plays an important role in mitochondrial apoptosis by upregulating HAX1 expression in colorectal cancer. *Oncotarget* 7(36):57866-57877.
- Li K, Casta A, Wang R, Lozada E, Fan W, Kane S, Ge Q, Gu W, Orren D & Luo J (2008) Regulation of WRN protein cellular localization and enzymatic activities by SIRT1-mediated deacetylation. *J Biol Chem* 283(12):7590-7598.
- Lidgren A, Hedberg Y, Grankvist K, Rasmuson T, Vasko J & Ljungberg B (2005) The expression of hypoxia-inducible factor 1 α is a favorable independent prognostic factor in renal cell carcinoma. *Clin Cancer Res* 11(3):1129-1135.
- Lim JH1, Lee YM, Chun YS, Chen J, Kim JE & Park JW (2010) Sirtuin 1 modulates cellular responses to hypoxia by deacetylating hypoxia-inducible factor 1 α . *Mol Cell* 38(6):864-878.
- Lindahl T (1993) Instability and decay of the primary structure of DNA. *Nature.* 362(6422):709-715.
- Liszt G, Ford E, Kurtev M & Guarente L (2005) Mouse Sir2 homolog SIRT6 is a nuclear ADP-ribosyltransferase. *J Biol Chem* 280(22): 21313-21320.
- Liu L & Gerson SL (2006) Targeted modulation of MGMT: clinical implication. *Clin Cancer Res* 12(2):328-331.
- Liu L, Ning X, Sun L, Zhang H, Shi Y, Guo C, Han S, Liu J, Sun S, Han Z, Wu K & Fan D (2008) Hypoxia-inducible factor-1 α contributes to hypoxia-induced chemoresistance in gastric cancer. *Cancer Sci* 99(1):121-128.
- Liu X, Yu H, Yang W, Zhou X, Lu H & Shi D (2010) Mutations of NFKBIA in biopsy specimens from Hodgkin lymphoma. *Cancer Genet Cytogenet* 197(2):152-157.

- Ma M, Hua S, Li G, Wang S, Cheng X, He S, Wu P & Chen X (2017) Prolyl hydroxylase domain protein 3 and asparaginyl hydroxylase factor inhibiting HIF-1 levels are predictive of tumoral behavior and prognosis in hepatocellular carcinoma. *Oncotarget* 8(8):12983-13002.
- Ma Y, Visser L, Blokzijl T, Harms G, Atayar C, Poppema S & van den Berg A (2008) The CD4+CD262 T-cell population in classical Hodgkin's lymphoma displays a distinctive regulatory T-cell profile. *Lab. Invest* 88(5): 482-490.
- MacMahon B (1966) Epidemiology of Hodgkin' disease. *Cancer Res* 26(6):1189-1201.
- Malafa M, Margenthaler J, Webb B, Neitzel L, Christophersen M (2000) MnSOD expression is increased in metastatic gastric cancer *J Surg Res* 88(2):130-134.
- Mallette FA, Mattioli F, Cui G, Young LC, Hendzel MJ, Mer G, Sixma TK & Richard S (2012) RNF8- and RNF168-dependent degradation of KDM4A/JMJD2A triggers 53BP1 recruitment to DNA damage sites. *EMBO J.* 31(8):1865-1878.
- Malpighi M (1666) *De Viscerum Structura Exercitatio Anatomica*. J. Montij, Bononiae 125-156.
- Mao Z, Tian X, Van Meter M, Ke Z, Gorbunova V & Seluanov A (2012). Sirtuin 6 (SIRT6) rescues the decline of homologous recombination repair during replicative senescence. *Proc Natl Acad Sci U S A* 109(29):11800-11805.
- Marnett LJ. Oxyradicals and DNA damage. *Carcinogenesis* 21(3):361-370.
- Marshall NA, Christie LE, Munro LR, Culligan DJ, Johnston PW, Barker RN & Vickers MA (2004) Immunosuppressive regulatory T cells are abundant in the reactive lymphocytes of Hodgkin lymphoma. *Blood* 103(5):1755-1762.
- McCord RA, Michishita E, Hong T, Berber E, Boxer LD, Kusumoto R, Guan S, Shi X, Gozani O, Burlingame AL, Bohr VA & Chua KF (2009) SIRT6 stabilizes DNA-dependent protein kinase at chromatin for DNA double-strand break repair. *Aging (Albany NY)* 1(1):109-121.
- Mei Y, Peng C, Liu YB, Wang J & Zhou HH (2017) Silencing RIF1 decreases cell growth, migration and increases cisplatin sensitivity of human cervical cancer cells *Oncotarget* 8(63):107044-107051.
- Menssen A, Hydbring P, Kapelle K, Vervoorts J, Diebold J, Lüscher B, Larsson LG & Hermeking H (2012) The c-MYC oncoprotein, the NAMPT enzyme, the SIRT1-inhibitor DBC1, and the SIRT1 deacetylase form a positive feedback loop. *Proc Natl Acad Sci U S A* 109(4):E187-196.
- Michishita E, Park JY, Burneskis JM, Barrett JC & Horikawa I (2005) Evolutionarily conserved and nonconserved cellular localizations and functions of human SIRT proteins. *Mol Biol Cell* 16(10): 4623-4635.
- Miriyala S, Spasojevic I, Tovmasyan A, Salvemini D, Vujaskovic Z, St Clair D & Batinic-Haberle I (2012) Manganese superoxide dismutase, MnSOD and its mimics. *Biochim Biophys Acta* 1822(5):794-814.
- Mladenov E, Magin S, Soni A & Iliakis G (2013) DNA double-strand break repair as determinant of cellular radiosensitivity to killing and target in radiation therapy. *Front Oncol* 3:113.

- Molin D, Edström A, Glimelius I, Glimelius B, Nilsson G, Sundström C & Enblad G (2002) Mast cell infiltration correlates with poor prognosis in Hodgkin's lymphoma. *Br J Haematol* 119(1):122-124.
- Moskowitz AJ, Hamlin PA Jr, Perales MA, Gerecitano J, Horwitz SM, Matasar MJ, Noy A, Palomba ML, Portlock CS, Straus DJ, Graustein T, Zelenetz AD & Moskowitz CH (2013) Phase II study of bendamustine in relapsed and refractory Hodgkin lymphoma. *J Clin Oncol* 31(4): 456-460.
- Mottok A & Steidl C (In press) Biology of classical Hodgkin lymphoma: implications for prognosis and novel therapies. *Blood*.
- Nasrin N, Wu X, Fortier E, Feng Y, Bare' OC, Chen S, Ren X, Wu Z, Streeper RS & Bordone L (2010) SIRT4 regulates fatty acid oxidation and mitochondrial gene expression in liver and muscle cells. *J Biol Chem* 285(42):31995–32002.
- Neiman RS, Rosen PJ (1973) Lukes RJ. Lymphocyte-depletion Hodgkin's disease. A clinicopathological entity. *N Engl J Med*. 288(15):751–755.
- Nicolussi A, D'Inzeo S, Capalbo C, Giannini G & Coppa A (2017) The role of peroxiredoxins in cancer. *Mol Clin Oncol* 6(2):139-153.
- Noguchi A, Li X, Kubota A, Kikuchi K, Kameda Y, Zheng H, Miyagi Y, Aoki I & Takano Y (2013) SIRT1 expression is associated with good prognosis for head and neck squamous cell carcinoma patients. *Oral Surg Oral Med Oral Pathol Oral Radiol* 115(3):385–392.
- Noh SJ, Baek HA, Park HS, Jang KY, Moon WS, Kang MJ, Lee DG, Kim MH, Lee JH & Chung MJ (2013) Expression of SIRT1 and cortactin is associated with progression of non-small cell lung cancer. *Pathol Res Pract* 209(6):365-370.
- Nozawa, Y, Wakasa H & Abe M (1998) Costimulatory molecules (CD80 and CD86) on Reed-Sternberg cells are associated with the proliferation of background T cells in Hodgkin's disease. *Pathol. Int.* 48(1):10-14.
- Nguyen TL & Durán RV (2016) Prolyl hydroxylase domain enzymes and their role in cell signaling and cancer metabolism. *Int J Biochem Cell Biol* 80:71-80.
- Okado-Matsumoto A, Matsumoto A & Fujii J (2000) Peroxiredoxin IV is a secretable protein with heparin-binding properties under reduced conditions. *J Biochem* 127(3):493-501.
- Oudejans JJ, Jiwa NM, Kummer JA, Ossenkoppele GJ, van Heerde P, Baars JW, Kluin PM, Kluin-Nelemans JC, van Diest PJ, Middeldorp JM & Meijer CJ (1997) Activated cytotoxic T cells as prognostic marker in Hodgkin's disease *Blood* 89(4):1376-1382.
- Pasanen AK, Kuitunen H, Haapasaari K-M, Karihtala P, Kyllönen H, Soini Y, Turpeenniemi-Hujanen T & Kuittinen O (2012) Expression and prognostic evaluation of oxidative stress markers in an immunohistochemical study of B-cell derived lymphomas. *Leuk Lymphoma* 53(4):624-631.
- Peh SC, Kim LH & Poppema S (2001) TARC, a CC chemokine, is frequently expressed in classic Hodgkin's lymphoma but not in NLP Hodgkin's lymphoma, T-cell-rich B-cell lymphoma, and most cases of anaplastic large cell lymphoma. *Am J Surg Pathol* 25(7):925-929.

- Peroja P, Haapasaari KM, Mannisto S, Miinalainen I, Koivunen P, Leppä S, Karjalainen-Lindsberg ML, Kuusisto ME, Turpeenniemi-Hujanen T, Kuittinen O, Karihtala P (2016) Total peroxiredoxin expression is associated with survival in patients with follicular lymphoma. *Virchows Arch* 468(5):623-630.
- Peroja P, Pasanen AK, Haapasaari KM, Jantunen E, Soini Y, Turpeenniemi-Hujanen T, Bloigu R, Lilja L, Kuittinen O, Karihtala P (2012) Oxidative stress and redox state-regulating enzymes have prognostic relevance in diffuse large B-cell lymphoma. *Exp Hematol Oncol* 1:2.
- Peterson BA, Pajak TF, Cooper MR, Nissen NI, Glidewell OJ, Holland JF, Bloomfield CD & Gottlieb AJ (1982) Effect of age on therapeutic response and survival in advanced Hodgkin's disease. *Cancer Treat Rep* 66(4):889-898.
- Peurala E, Koivunen P, Bloigu R, Haapasaari KM & Jukkola-Vuorinen A (2012) Expressions of individual PHDs associate with good prognostic factors and increased proliferation in breast cancer patients. *Breast Cancer Res Treat.* 133(1):179-188.
- Pinto A, Aldinucci D, Gloghini A, Zagonel V, Degan M, Improta S, Juzbasic S, Todesco M, Perin V, Gattei V, Herrmann F, Gruss HJ & Carbone A (1996) Human eosinophils express functional CD30 ligand and stimulate proliferation of a Hodgkin's disease cell line. *Blood* 88(9):3299-3305.
- Poppema S (2005) Immunobiology and pathophysiology of Hodgkin lymphomas. *Hematology Am Soc Hematol Educ Program* 231-238.
- Poppema S, Bhan, AK, Reinherz EL, Posner MR & Schlossman SF (1982) In situ immunologic characterization of cellular constituents in lymph nodes and spleens involved by Hodgkin's disease. *Blood* 59(2): 226-232.
- Portis T & Longnecker R (2004) Epstein-Barr virus (EBV) LMP2A mediates B-lymphocyte survival through constitutive activation of the Ras/PI3K/Akt pathway. *Oncogene* 23(53):8619-8628.
- Pourahmad J, Amirmostofian M, Kobarfard F & Shahraki J (2009) Biological reactive intermediates that mediate dacarbazine cytotoxicity. *Cancer Chemother Pharmacol* 65(1):89-96.
- Powis G & Montfort WR (2001) Properties and biological activities of thioredoxins. *Annu Rev Biophys Biomol Struct* 30: 421-455.
- Poyton RO, Ball KA & Castello PR (2009) Mitochondrial generation of free radicals and hypoxic signaling. *Trends Endocrinol Metab* 20(7): 332-340.
- Pylväs-Eerola M, Karihtala P & Puistola U (2015). Preoperative serum 8-hydroxydeoxyguanosine is associated with chemoresistance and is a powerful prognostic factor in endometrioid-type epithelial ovarian cancer. *BMC Cancer* 15: 493.
- Rabilloud T, Heller M, Gasnier F, Luche S, Rey C, Aebersold R, Benahmed M, Louisot P, & Lunardi J (2002) Proteomics analysis of cellular response to oxidative stress. Evidence for in vivo overoxidation of peroxiredoxins at their active site. *J Biol Chem* 277(22):19396-19401.

- Ramos CA, Ballard B, Zhang H, Dakhova O, Gee AP, Mei Z, Bilgi M, Wu M, Liu H, Grilley B, Bollard CM, Chang BH, Rooney CM, Brenner MK, Heslop HE, Dotti G & Savoldo B (2017) Clinical and immunological responses after CD30-specific chimeric antigen receptor-redirected lymphocytes. *J Clin Invest* 127(9):3462-3471.
- Radi R, Rodriguez M, Castro L & Telleri R (1994) Inhibition of mitochondrial electron transport by peroxynitrite. *Arch Biochem Biophys* 308(1):89-95.
- Ranson MR, Radford JA, Swindell R, Deakin DP, Wilkinson PM, Harris M, Johnson RJ & Crowther D (1991) An analysis of prognostic factors in stage III and IV Hodgkin's disease treated at a single centre with MVPP. *Ann Oncol* 2(6):423-429.
- Renné C, Willenbrock K, Küppers R, Hansmann ML & Bräuninger A (2005) Autocrine- and paracrine-activated receptor tyrosine kinases in classic Hodgkin lymphoma. *Blood* 105(10):4051-4059.
- Reuter S, Gupta SC, Chaturvedi MM & Aggarwal BB (2010) Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med* 49(11):1603-1616.
- Rey S & Semenza GL (2010) Hypoxia-inducible factor-1-dependent mechanisms of vascularization and vascular remodelling. *Cardiovasc Res* 86(2):236-242
- Roemer MGM, Redd RA, Cader FZ, Pak CJ, Abdelrahman S, Ouyang J, Sasse S, Younes A, Fanale M, Santoro A, Zinzani PL, Timmerman J, Collins GP, Ramchandren R, Cohen JB, De Boer JP, Kuruvilla J, Savage KJ, Trneny M, Ansell S, Kato K, Farsaci B, Sumbul A, Armand P, Neuberg DS, Pinkus GS, Ligon AH, Rodig SJ & Shipp MA (2018) Major Histocompatibility Complex Class II and Programmed Death Ligand 1 Expression Predict Outcome After Programmed Death 1 Blockade in Classic Hodgkin Lymphoma. *J Clin Oncol* 36(10):942-950.
- Robertson KD (2005) DNA methylation and human disease. *Nat. Rev. Genet.* 6(8): 597-610.
- Rodig SJ, Abramson JS, Pinkus GS, Treon SP, Dorfman DM, Dong HY, Shipp MA & Kutok JL (2006) Heterogeneous CD52 expression among hematologic neoplasms: implications for the use of alemtuzumab (CAMPATH-1H). *Clin Cancer Res* 12(23):7174-7179.
- Rui L, Emre NC, Kruhlak MJ, Chung HJ, Steidl C, Slack G, Wright GW, Lenz G, Ngo VN, Shaffer AL, Xu W, Zhao H, Yang Y, Lamy L, Davis RE, Xiao W, Powell J, Maloney D, Thomas CJ, Möller P, Rosenwald A, Ott G, Muller-Hermelink HK, Savage K, Connors JM, Rimsza LM, Campo E, Jaffe ES, Delabie J, Smeland EB, Weisenburger DD, Chan WC, Gascoyne RD, Levens D & Staudt LM (2010) Cooperative epigenetic modulation by cancer amplicon genes. *Cancer Cell* 18(6):590-605.
- Sanders ME, Makgoba MW, Sussman EH, Luce GE, Cossman J & Shaw S (1988) Molecular pathways of adhesion in spontaneous rosetting of T-lymphocytes to the Hodgkin's cell line L428. *Cancer Res* 48(1):37-40.
- Schaapveld M, Aleman BM, van Eggermond AM, Janus CP, Krol AD, van der Maazen RW, Roesink J, Raemaekers JM, de Boer JP, Zijlstra JM, van Imhoff GW, Petersen EJ, Poortmans PM, Beijert M, Lybeert ML, Mulder I, Visser O, Louwman MW, Krul IM, Lugtenburg PJ, van Leeuwen FE (2015) Second Cancer Risk Up to 40 Years after Treatment for Hodgkin's Lymphoma. *N Engl J Med* 373(26):2499-2511.

- Scheeren FA, Diehl SA, Smit LA, Beaumont T, Naspetti M, Bende RJ, Blom B, Karube K, Ohshima K, van Noesel CJ & Spits H (2008) IL-21 is expressed in Hodgkin lymphoma and activates STAT5: evidence that activated STAT5 is required for Hodgkin lymphomagenesis. *Blood* 111(9):4706-4715.
- Schmitz R, Stanelle J, Hansmann ML, Küppers R (2009) Pathogenesis of classical and lymphocyte-predominant Hodgkin lymphoma. *Annu Rev Pathol* 4:151-174.
- Schreck S, Friebel D, Buettner M, Distel L, Grabenbauer G, Young LS & Niedobitek G. (2009) Prognostic impact of tumour-infiltrating Th2 and regulatory T cells in classical Hodgkin lymphoma. *Hematol Oncol* 27(1):31-39.
- Schwering I, Brauninger A, Klein U, Jungnickel B, Tinguely M, Diehl V, Hansmann ML, Dalla-Favera R, Rajewsky K, Küppers R (2003) Loss of the B-lineage-specific gene expression program in Hodgkin and Reed-Sternberg cells of Hodgkin lymphoma. *Blood* 101(4):1505-1512.
- Sebastian C, Satterstrom FK, Haigis MC & Mostoslavsky R (2012) From Sirtuin Biology to Human Diseases: An Update. *Journal of Biological Chemistry* 287(51):42444-42452.
- Seitz V, Thomas PE, Zimmermann K, Paul U, Ehlers A, Joosten M, Dimitrova L, Lenze D, Sommerfeld A, Oker E, Leser U, Stein H & Hummel M (2011) Classical Hodgkin's lymphoma shows epigenetic features of abortive plasma cell differentiation. *Haematologica* 96(6):863-870.
- Semenza GL (2011) Oxygen sensing, homeostasis, and disease. *N Engl J Med* 365(6): 537-547.
- Semenza GL (2012) Hypoxia-Inducible Factors in Physiology and Medicine. *Cell* 148(3): 399-408.
- Shannon-Lowe C, Rickinson AB & Bell AI (2017) Epstein-Barr virus-associated lymphomas. *Philos Trans R Soc Lond B Biol Sci* 372(1732).
- Sharma S, Kelly TK, Jones PA (2010) Epigenetics in cancer. *Carcinogenesis* 31(1): 27-36.
- Sheridan J, Wang LM, Tosetto M, Sheahan K, Hyland J, Fennelly D, O'Donoghue D, Mulcahy H & O'Sullivan J (2009) Nuclear oxidative damage correlates with poor survival in colorectal cancer. *Br J Cancer* 100(2): 381-388.
- Shi L, Sun L, Li Q, Liang J, Yu W, Yi X, Yang X, Li Y, Han X, Zhang Y, Xuan C, Yao Z & Shang Y (2011) Histone demethylase JMJD2B coordinates H3K4/H3K9 methylation and promotes hormonally responsive breast carcinogenesis. *Proc Natl Acad Sci U S A* 108(18):7541-7546.
- Shiels MS, Koritzinsky EH, Clarke CA, Suneja G, Morton LM & Engels EA (2014) Prevalence of HIV Infection among U.S. Hodgkin lymphoma cases. *Cancer Epidemiol Biomarkers Prev* 23(2):274-281.
- Sies H, Berndt C & Jones DP (2017) Oxidative Stress. *Annu Rev Biochem* 86:715-774.
- Shin S & Janknecht R (2007) Activation of androgen receptor by histone demethylases JMJD2A and JMJD2D. *Biochem Biophys Res Commun* 359(3):742-746.
- Shrivastav M, De Haro LP & Nickoloff JA (2008) Regulation of DNA double-strand break repair pathway choice. *Cell Res* 18(1):134-147.

- Silverman J, Takai H, Buonomo SB, Eisenhaber F & de Lange T (2004) Human Rif1, ortholog of a yeast telomeric protein, is regulated by ATM and 53BP1 and functions in the S-phase checkpoint. *Genes Dev* 18(17):2108-2119.
- Skinnider BF, Elia AJ, Gascoyne RD, Patterson B, Trumper L, Kapp U & Mak TW (2002) Signal transducer and activator of transcription 6 is frequently activated in Hodgkin and Reed–Sternberg cells of Hodgkin lymphoma. *Blood* 99(22):618-626.
- Smith SM, Schöder H, Johnson JL, Jung SH, Bartlett NL & Cheson BD (2013) The anti-CD80 primatized monoclonal antibody, galiximab, is well-tolerated but has limited activity in relapsed Hodgkin lymphoma. *Leuk Lymphoma* 54(7):1405-1410.
- Soejima H, Zhao W & Mukai T (2005) Epigenetic silencing of the MGMT gene in cancer. *Biochem Cell Biol* 83(4):429-437.
- Soini Y, Haapasaaari KM, Vaarala MH, Turpeenniemi-Hujanen T, Kärjä V, Karihtala P (2011) 8-hydroxydeguanosine and nitrotyrosine are prognostic factors in urinary bladder carcinoma. *Int J Clin Exp Pathol* 4(3):267-275.
- Somaiah N, Yarnold J, Daley F, Pearson A, Gothard L, Rothkamm K (2012) Helleday T. The relationship between homologous recombination repair and the sensitivity of human epidermis to the size of daily doses over a 5-week course of breast radiotherapy. *Clin Cancer Res* 18(19):5479-5488.
- Somaiah N, Yarnold J, Lagerqvist A, Rothkamm K & Helleday T (2013) Homologous recombination mediates cellular resistance and fraction size sensitivity to radiation therapy. *Radiother Oncol* 108(1):155-161.
- Sormendi S & Wielockx B (2018) Hypoxia Pathway Proteins As Central Mediators of Metabolism in the Tumor Cells and Their Microenvironment. *Front Immunol* 9:40.
- Sova H, Jukkola-Vuorinen A, Puistola U, Kauppinen S, Karihtala P (2010) 8-Hydroxydeoxyguanosine: a new potential independent prognostic factor in breast cancer. *Br J Cancer* 102(6):1018-1023.
- Specht L (1992) Tumour burden as the main indicator of prognosis in Hodgkin's disease. *Eur J Cancer* 28(12):1982-1985.
- Speit G & Trenz K (2004) Chromosomal mutagen sensitivity associated with mutations in BRCA genes. *Cytogenet Genome Res* 104(1-4):325-332.
- Spronsen DJ, Janssen-Heijnen ML, Lemmens VE, Peters WG & Coebergh JW (2005) Independent prognostic effect of co-morbidity in lymphoma patients: results of the population-based Eindhoven Cancer Registry. *Eur J Cancer* 41(7):1051-1057.
- Stathis A & Younes A (2015) The new therapeutical scenario of Hodgkin lymphoma. *Ann Oncol* 26(10):2026-2033.
- Steidl C, Lee T, Shah SP, Farinha P, Han G, Nayar T, Delaney A, Jones SJ, Iqbal J, Weisenburger DD, Bast MA, Rosenwald A, Muller-Hermelink HK, Rimsza LM, Campo E, Delabie J, Braziel RM, Cook JR, Tubbs RR, Jaffe ES, Lenz G, Connors JM, Staudt LM, Chan WC, Gascoyne RD (2010) Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. *N Engl J Med* 362(10):875-885.
- Su C, Huang K, Sun L, Yang D, Zheng H, Gao C, Tong J & Zhang Q (2012) Overexpression of the HIF hydroxylase PHD3 is a favorable prognosticator for gastric cancer. *Med Oncol* 29(4):2710-2715.

- Sun QK, Zhu JY, Wang W, Lv Y, Zhou HC, Yu JH, Xu GL, Ma JL, Zhong W & Jia WD (2014) Diagnostic and prognostic significance of peroxiredoxin 1 expression in human hepatocellular carcinoma. *Med Oncol* 31(1):786.
- Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles Ga, Zelenetz AD & Jaffe ES (2016) The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood* 127(20): 2375-2390.
- Tan KL, Scott DW, Hong F, Kahl BS, Fisher RI, Bartlett NL, Advani RH, Buckstein R, Rimsza LM, Connors JM, Steidl C, Gordon LI, Horning SJ & Gascoyne RD (2012) Tumor-associated macrophages predict inferior outcomes in classic Hodgkin lymphoma: a correlative study from the E2496 Intergroup trial. *Blood* 120(16):3280-3287.
- Tanjiri T, Shimizu T, Uehira K, Yokoi T, Amuro H, Sugimoto H, Torii Y, Tajima K, Ito T, Amakawa R & Fukuhara S (2007) Hodgkin's reed-sternberg cell line (KM-H2) promotes a bidirectional differentiation of CD4+CD25+Foxp3+ T cells and CD4+ cytotoxic T lymphocytes from CD4+ naive T cells. *J Leukoc Biol* 82(3):576-584.
- Tausendschon M, Dehne N & Brune, B (2011) Hypoxia causes epigenetic gene regulation in macrophages by attenuating Jumonji histone demethylase activity. *Cytokine* 53(2): 256-262.
- Tennant DA & Gottlieb E (2010) HIF prolyl hydroxylase-3 mediates alpha-ketoglutarate-induced apoptosis and tumor suppression. *J Mol Med (Berl)* 88(8): 839-849.
- Teppo HR, Soini Y, Karihtala P (2017) Reactive Oxygen Species-Mediated Mechanisms of Action of Targeted Cancer Therapy. *Oxid Med Cell Longev.* 2017:1485283.
- Tome ME, Frye JB, Coyle DL, Jacobson EL, Samulitis BK, Dvorak K, Dorr RT & Briehl MM (2012) Lymphoma cells with increased anti-oxidant defenses acquire chemoresistance. *Exp Ther Med* 3(5):845-852.
- Toyokawa G, Cho HS, Iwai Y, Yoshimatsu M, Takawa M, Hayami S, Maejima K, Shimizu N, Tanaka H, Tsunoda T, Field HI, Kelly JD, Neal DE, Ponder BA, Maehara Y, Nakamura Y & Hamamoto R (2011) The histone demethylase JMJD2B plays an essential role in human carcinogenesis through positive regulation of cyclin-dependent kinase 6. *Cancer Prev Res (Phila)*; 4(12): 2051-2061.
- Tsukada Y, Fang J, Erdjument-Bromage H, Warren ME, Borchers CH, Tempst P & Zhang Y (2006) Histone demethylation by a family of JmjC domain-containing proteins. *Nature* 439(7078): 811-816.
- Tubiana M, Henry-Amar M, Hayat M, Burgers M, Qasim M, Somers R, Sizoo W, Van der Schueren E (1984) Prognostic significance of the number of involved areas in the early stages of Hodgkin's disease. *Cancer* 54(5):885-894.
- Uccella S, Cerutti R, Placidi C, Marchet S, Carnevali I, Bernasconi B, Proserpio I, Pinotti G, Tibiletti MG, Furlan D & Capella C (2009) MGMT methylation in diffuse large B-cell lymphoma: validation of quantitative methylation-specific PCR and comparison with MGMT protein expression. *J Clin Pathol* 62(8):715-723.
- Vainchenker W, Constantinescu SN (2013) JAK/STAT signaling in hematological malignancies. *Oncogene* 32(21):2601-2613.

- Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J (2004) Role of oxygen radicals in DNA damage and cancer incidence. *Mol Cell Biochem* 266(1-2):37-56.
- Valko M, Rhodes CJ, Moncol J, Izakovic M & Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 160(1):1-40.
- Vallabhapurapu S & Karin M (2009) Regulation and function of NF-kappaB transcription factors in the immune system, *Annu. Rev. Immunol* 27: 693-733.
- Valsami S, Pappa V, Rontogianni D, Kotsiotti F, Papageorgiou E, Dervenoulas J, Karmiris T, Papageorgiou S, Harhalakis N, Xiros N, Nikiforakis E & Economopoulos TA (2007) clinicopathological study of B-cell differentiation markers and transcription factors in classical Hodgkin's lymphoma: a potential prognostic role of MUM1/IRF4. *Haematologica* 92(10):1343-1350.
- van den Berg A, Visser L & Poppema S (1999) High expression of the CC chemokine TARC in Reed-Sternberg cells. A possible explanation for the characteristic T-cell infiltrate in Hodgkin's lymphoma. *Am J Pathol* 154(6):1685-1691.
- van der Kaaij MA, van Echten-Arends J, Simons AH & Kluin-Nelemans HC (2010) Fertility preservation after chemotherapy for Hodgkin lymphoma. *Hematol Oncol* 28(4):168-179.
- van Nimwegen FA, Schaapveld M, Janus CP, Krol AD, Petersen EJ, Raemaekers JM, Kok WE, Aleman BM & van Leeuwen FE (2015) Cardiovascular disease after Hodgkin lymphoma treatment: 40-year disease risk. *JAMA Intern Med* 175(6):1007-1017.
- Van Remmen H, Ikeno Y, Hamilton M, Pahlavani M, Wolf N, Thorpe SR, Alderson NL, Baynes JW, Epstein CJ, Huang TT, Nelson J, Strong R, Richardson A (2003) Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging *Physiol Genomics* 16(1):29-37.
- Verbeke CS, Wenthe U, Grobholz R & Zentgraf H (2001) Fas ligand expression in Hodgkin lymphoma. *Am J Surg Pathol* 25(3):388-394.
- Visser L, van den Berg A, Poppema S & Diepstra A (2015) Microenvironment, Crosstalk, and Immune Escape Mechanisms. In: Engert A & Younes A. (eds), *Hodgkin Lymphoma. Hematologic Malignancies* (pp.65–75) Springer, Cham.
- Vockerodt M, Soares M, Kanzler H, Küppers R, Kube D, Hansmann ML, Diehl V & Tesch H (1998) Detection of clonal Hodgkin and Reed-Sternberg cells with identical somatically mutated and rearranged VH genes in different biopsies in relapsed Hodgkin's disease. *Blood*. 92(8):2899-2907.
- Vockerodt M, Yap LF, Shannon-Lowe C, Curley H, Wei W, Vrzalikova K & Murray PG (2015) The Epstein-Barr virus and the pathogenesis of lymphoma. *J Pathol* 235(2):312-322.
- Wang C, Chen L, Hou X, Li Z, Kabra N, Ma Y, Nemoto S, Finkel T, Gu W, Cress WD & Chen J (2006) Interactions between E2F1 and SirT1 regulate apoptotic response to DNA damage. *Nat Cell Biol* 8(9):1025-1031.
- Wang GL & Semenza GL (1995) Purification and characterization of hypoxia-inducible factor 1. *J Biol Chem* 270(3):1230-1237.
- Wang KC & Chang HY (2011) Molecular mechanisms of long noncoding RNAs. *Mol Cell* 43(6):904-914.

- Wang T, Diaz AJG & Yen Y (2014) The role of peroxiredoxin II in chemoresistance of breast cancer cells. *Breast Cancer (Dove Med Press)* 6:73-80.
- Wang Y & Leung FC (2004) An evaluation of new criteria for CpG islands in the human genome as gene markers. *Bioinformatics*, 20(7):1170-1177.
- Weber J (2010) Immune checkpoint proteins: a new therapeutic paradigm for cancer - preclinical background: CTLA-4 and PD-1 blockade. *Semin Oncol* 37(5):430-439.
- Weber-Matthiesen K, Deerberg J, Poetsch M, Grote W, Schlegelberger B (1995) Numerical chromosome aberrations are present within the CD30+ Hodgkin and Reed-Sternberg cells in 100% of analyzed cases of Hodgkin's disease. *Blood*. 86(4):1464-1468.
- Wein F & Küppers R (2016) The role of T cells in the microenvironment of Hodgkin lymphoma. *J Leukoc Biol* 99(1):45-50.
- Wein F, Weniger MA, Höing B, Arnolds J, Hüttmann A, Hansmann ML, Hartmann S & Küppers R (2017) Complex Immune Evasion Strategies in Classical Hodgkin Lymphoma. *Cancer Immunol Res* (12):1122-1132.
- Weniger MA, Melzner I, Menz CK, Wegener S, Bucur AJ, Dorsch K, Mattfeldt T, Barth TF & Möller P (2006) Mutations of the tumor suppressor gene SOCS-1 in classical Hodgkin lymphoma are frequent and associated with nuclear phospho-STAT5 accumulation. *Oncogene* 25(18): 2679-2684.
- Wolf, M, Albrecht, S & Märki, C (2008) Proteolytic processing of chemokines: implications in physiological and pathological conditions. *Int. J. Biochem. Cell Biol.* 40(6-7): 1185-1198.
- Xu D, Muniandy P, Leo E, Yin J, Thangavel S, Shen X, Ii M, Agama K, Guo R, Fox D 3rd, Meetei AR, Wilson L, Nguyen H, Weng NP, Brill SJ, Li L, Vindigni A, Pommier Y, Seidman M, Wang W (2010) Rif1 provides a new DNA-binding interface for the Bloom syndrome complex to maintain normal replication, *EMBO J.* 29(18):3140-315.
- Xue J, Li X, Jiao S, Wei Y, Wu G & Fang J (2010) Prolyl hydroxylase-3 is down-regulated in colorectal cancer cells and inhibits IKKbeta independent of hydroxylase activity. *Gastroenterology* 138(2):606-615.
- Yamazaki S, Hayano M & Masai H (2013) Replication timing regulation of eukaryotic replicons: Rif1 as a global regulator of replication timing, *Trends Genet.* 29(8): 449-460.
- Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA & Mayo MW (2004) Modulation of NF-kappaB- dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J* 23(12):2369-2380.
- Yoo S & Dynan WS (1999) Geometry of a complex formed by double strand break repair proteins at a single DNA end: recruitment of DNA-PKcs induces inward translocation of Ku protein. *Nucleic Acids Res* 27(24):4679-4686.
- Yotnda P, Wu D & Swanson AM (2010) Hypoxic tumours and their effect on immune cells and cancer therapy. *Methods Mol. Biol* 651:1-29.

- Younes A, Gopal AK, Smith SE, Ansell SM, Rosenblatt JD, Savage KJ, Ramchandren R, Bartlett NL, Cheson BD, de Vos S, Forero-Torres A, Moskowitz CH, Connors JM, Engert A, Larsen EK, Kennedy DA, Sievers EL & Chen R (2012) Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. *J Clin Oncol* 30(18):2183-2189.
- Younes A, Oki Y, Bociek RG, Kuruvilla J, Fanale M, Neelapu S, Copeland A, Buglio D, Galal A, Besterman J, Li Z, Drouin M, Patterson T, Ward MR, Paulus JK, Ji Y, Medeiros LJ & Martell RE (2011) Mocetinostat for relapsed classical Hodgkin's lymphoma: an open-label, single-arm, phase 2 trial. *Lancet Oncol* (13):1222-1228.
- Younes A, Oki Y, McLaughlin P, Copeland AR, Goy A, Pro B, Feng L, Yuan Y, Chuang HH, Macapinlac HA, Hagemester F, Romaguera J, Samaniego F, Fanale MA, Dabaja BS, Rodriguez MA, Dang N, Kwak LW, Neelapu SS & Fayad LE (2012) Phase 2 study of rituximab plus ABVD in patients with newly diagnosed classical Hodgkin lymphoma. *Blood* 119(18):4123-4128.
- Younes A, Sureda A, Ben-Yehuda D, Zinzani PL, Ong TC, Prince HM, Harrison SJ, Kirschbaum M, Johnston P, Gallagher J, Le Corre C, Shen A & Engert A (2012) Panobinostat in patients with relapsed/refractory Hodgkin's lymphoma after autologous stem-cell transplantation: results of a phase II study. *J Clin Oncol* 30(18): 2197-2203.
- Young LS, Dawson CW & Eliopoulos AG (2000) The expression and function of Epstein-Barr virus encoded latent genes. *Mol Pathol* 53(2):238-247.
- Zhao L, Li W, Zang W, Liu Z, Xu X, Yu H, Yang Q & Jia J (2013) JMJD2B promotes epithelial-mesenchymal transition by cooperating with β -catenin and enhances gastric cancer metastasis. *Clin Cancer Res* 19(23):6419-6429.
- Zheng B, Fiumara P, Li YV, Georgakis G, Snell V, Younes M, Vauthey JN, Carbone A & Younes A (2003) MEK/ERK pathway is aberrantly active in Hodgkin disease: a signaling pathway shared by CD30, CD40, and RANK that regulates cell proliferation and survival. *Blood* 102(4): 1019-1027.
- Zhong LD'Urso A, Toiber D, Sebastian C, Henry RE, Vadysirisack DD, Guimaraes A, Marinelli B, Wikstrom JD, Nir T, Clish CB, Vaitheesvaran B, Iliopoulos O, Kurland I, Dor Y, Weissleder R, Shirihai OS, Ellisen LW, Espinosa JM, Mostoslavsky R (2010) The histone deacetylase Sirt6 regulates glucose homeostasis via Hif1 α . *Cell* 140(2):280-293.
- Zhong Q, Chen CF, Li S, Chen Y, Wang CC, Xiao J, Chen PL, Sharp ZD & Lee WH (1999) Association of BRCA1 with the hRad50-hMre11-p95 complex and the DNA damage response. *Science* 285(5428):747-750.
- Zhong W, Oberley LW, Oberley TD & St Clair DK (1997) Suppression of the malignant phenotype of human glioma cells by overexpression of manganese superoxide dismutase. *Oncogene* 14(4):481-490.
- Zhu Y, van Essen D & Saccani S (2012) Cell-type-specific control of enhancer activity by H3K9 trimethylation. *Mol Cell* 46(4):408-423.
- zur Hausen H & de Villiers EM (2005) Virus target cell conditioning model to explain some epidemiologic characteristics of childhood leukemias and lymphomas. *Int J Cancer* 115(1):1-5.

Original publications

- I Bur H, Haapasaari KM, Turpeenniemi-Hujanen T, Kuittinen O, Auvinen P, Marin K, Koivunen P, Sormunen R, Soini Y & Karihtala P (2014) Oxidative stress markers and mitochondrial antioxidant enzyme expression are increased in aggressive Hodgkin lymphomas. *Histopathology* 65(3):319-327.
- II Bur H, Haapasaari KM, Turpeenniemi-Hujanen T, Kuittinen O, Auvinen P, Marin K, Soini Y & Karihtala P (2018) Strong prolyl hydroxylase domain 1 expression predicts poor outcome in radiotherapy-treated patients with classical Hodgkin's lymphoma. *Anticancer Research* 38(1):329-336.
- III Bur H, Haapasaari KM, Turpeenniemi-Hujanen T, Kuittinen O, Auvinen P, Marin K, Soini Y & Karihtala P (2016) Strong KDM4B and KDM4D expression associates with radioresistance and aggressive phenotype in classical Hodgkin lymphoma. *Anticancer Research* 36(9):4677-4683.
- IV Bur H, Haapasaari KM, Turpeenniemi-Hujanen T, Kuittinen O, Auvinen P, Marin K, Soini Y & Karihtala P (2018) Low Rap1 interacting factor 1 and sirtuin 6 expression predict poor outcome in radiotherapy-treated Hodgkin lymphoma patients. *Leukemia & Lymphoma* 59(3):679-689.

Reprinted with permission from John Wiley and Sons (I), International Institute of Anticancer Research (II, III) and Taylor & Francis (IV).

Original publications are not included in the electronic version of the dissertation.

- I454. Saarela, Ulla (2018) Novel culture and organoid technologies to study mammalian kidney development
- I455. Virtanen, Mari (2018) The development of ubiquitous 360° learning environment and its effects on students' satisfaction and histotechnological knowledge
- I456. Vuollo, Ville (2018) 3D imaging and nonparametric function estimation methods for analysis of infant cranial shape and detection of twin zygosity
- I457. Tervaskanto-Mäentausta, Tiina (2018) Interprofessional education during undergraduate medical and health care studies
- I458. Peroja, Pekka (2018) Oxidative stress in diffuse large B-cell lymphoma and follicular lymphoma, and TP53 mutations and translocations of MYC, Bcl-2 and Bcl-6 in diffuse large B-cell lymphoma
- I459. Bose, Muthiah (2018) Molecular and functional characterization of *ABRAXAS* and *PALB2* genes in hereditary breast cancer predisposition
- I460. Klemola, Tero (2018) Flexible hallux valgus : Results of a new surgical technique
- I461. Pasanen, Anu (2018) Genetic susceptibility to childhood bronchiolitis
- I462. Käkelä, Juha (2018) Family history of mental disorders and long-term outcome in schizophrenia
- I463. Xu, Qi (2018) Role of Wnt11 in kidney ontogenesis and development of renal organoid based models to identify candidate oncogenes
- I464. Lunkka, Nina (2018) Making sense of hospital change project actuality
- I465. Isojärvi, Henri (2018) Association of glucose metabolism, physical activity and fitness with peripheral nervous system function in overweight people
- I466. Matinolli, Hanna-Maria (2018) Nutrition and early life programming of health : Focus on preterm birth and infant feeding in relation to energy-balance and related traits in adulthood
- I467. Tikanmäki, Marjaana (2018) Preterm birth and parental and pregnancy related factors in association with physical activity and fitness in adolescence and young adulthood
- I468. Juvonen-Posti, Pirjo (2018) Work-related rehabilitation for strengthening working careers : A multiperspective and mixed methods study of its mechanisms
- I469. Palaniswamy, Saranya (2018) Vitamin D status and its association with leukocyte telomere length, obesity and inflammation in young adults : A Northern Finland Birth Cohort 1966 study

Book orders:

Granum: Virtual book store
<http://granum.uta.fi/granum/>

S E R I E S E D I T O R S

A
SCIENTIAE RERUM NATURALIUM
University Lecturer Tuomo Glumoff

B
HUMANIORA
University Lecturer Santeri Palviainen

C
TECHNICA
Postdoctoral research fellow Sanna Taskila

D
MEDICA
Professor Olli Vuolteenaho

E
SCIENTIAE RERUM SOCIALIUM
University Lecturer Veli-Matti Ulvinen

E
SCRIPTA ACADEMICA
Planning Director Pertti Tikkanen

G
OECONOMICA
Professor Jari Juga

H
ARCHITECTONICA
University Lecturer Anu Soikkeli

EDITOR IN CHIEF
Professor Olli Vuolteenaho

PUBLICATIONS EDITOR
Publications Editor Kirsti Nurkkala

