Coagulation and other aspects of the cardiovascular system in small cell lung cancer

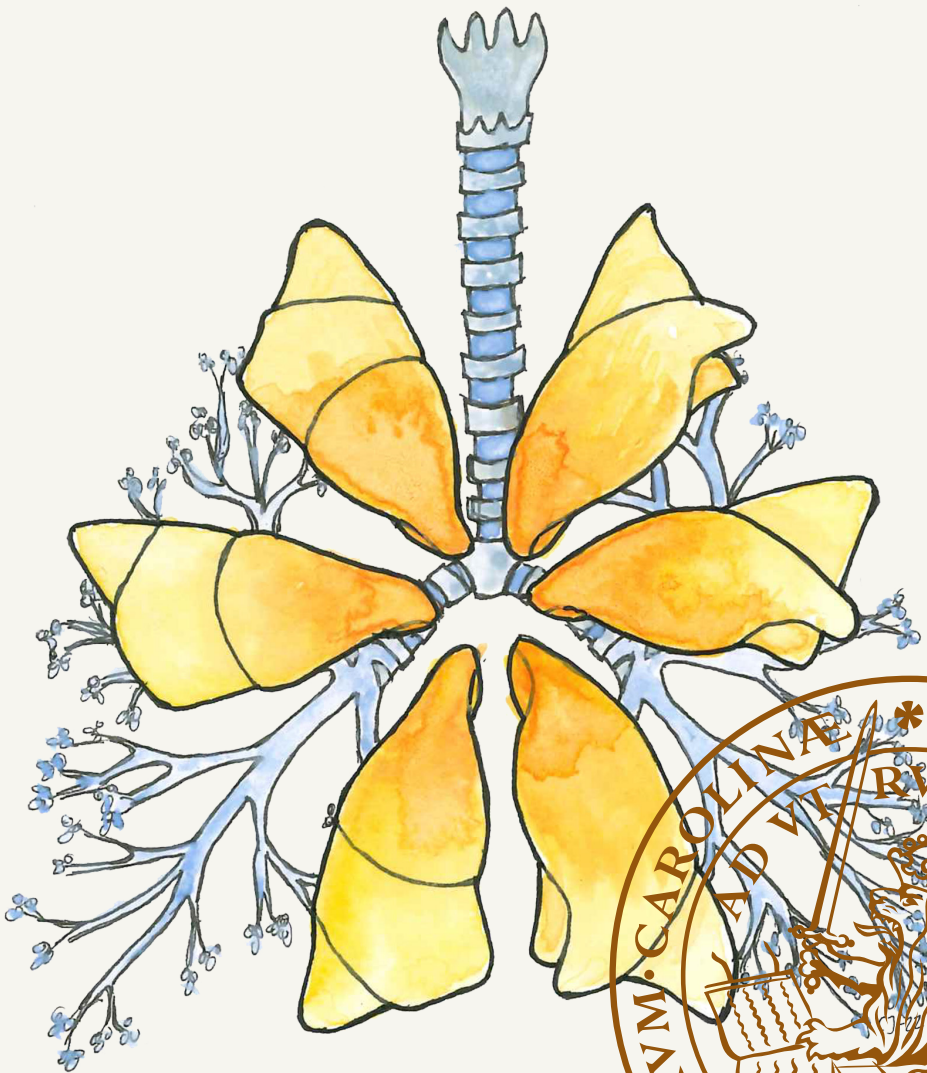
Gezelius, Emelie

2022

Document Version:
Publisher's PDF, also known as Version of record

Coagulation and other aspects of the cardiovascular system in small cell lung cancer

EMELIE GEZELIUS
DEPARTMENT OF CLINICAL SCIENCES, LUND | FACULTY OF MEDICINE | LUND UNIVERSITY



EMELIE GEZELIUS is a graduate of University of Birmingham Medical School, United Kingdom. After returning to Lund, Sweden, she specialised in medical oncology at Skåne University Hospital. Emelie has subspecialised in thoracic oncology and is working at the Thoracic Oncology unit, Department of Respiratory medicine.

Printed by Media-Tryck, Lund 2022 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical Sciences, Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2022:58
ISBN 978-91-8021-219-9
ISSN 1652-8220

Coagulation and other aspects of the cardiovascular system in small cell lung cancer

Emelie Gezelius

LUND
UNIVERSITY

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at the Lecture Hall of the Radiotherapy building, 3rd floor
Department of Oncology, Skåne University Hospital, Lund
Friday April 29, 2022, at 9.00 am.

Faculty opponent
Professor Bjørn Henning Grønberg
Department of Clinical and Molecular Medicine
Norwegian University of Science and Technology, Trondheim, Norway

Organization LUND UNIVERSITY	Document name Doctoral dissertation
	Date of issue April 29, 2022
Author Emelie Gezelius	Sponsoring organization

Title and subtitle
Coagulation and other aspects of the cardiovascular system in small cell lung cancer

Abstract
Cancer is associated with a hypercoagulable state and an increased risk of venous thromboembolism (VTE). Pre-clinical evidence suggests that some of the key coagulation factors are not only involved in the development of VTE but also contribute to tumour-promoting processes such as angiogenesis and metastasis. Anticoagulant agents, including low-molecular-weight heparin (LMWH), have been found to exert tumour-inhibiting effects *in vivo* and *in vitro*, and early clinical trials demonstrated improved survival specifically in patients with small cell lung cancer (SCLC), a particularly aggressive lung cancer subtype. These and other facts established the hypothesis that LMWH, independently of its anticoagulant activity, may exert direct tumour-inhibiting activity.

Paper I describes the randomised phase-III trial, RASTEN, investigating standard treatment with or without the addition of enoxaparin, a LMWH, in SCLC, with the primary aim to improve survival. In total, 377 patients were included in the final analysis. Although there was a significant reduction in VTE incidence, we found no difference in survival when comparing patients receiving LMWH to patients in the control arm.

In Paper II, we discerned if the results of Paper I could be explained by inadequate adherence to LMWH medication. Levels of LMWH were objectively assessed using the established anti-factor Xa assay in conjunction with the experimental Heparin Red assay. Adherence rates were considered acceptable (85% and 68% based on the anti-factor Xa and Heprin Red assays, respectively) but no survival benefits were seen in patients defined as adherent as compared with non-adherent or with patients in the control arm.

In Paper III we investigated various aspects of coagulation activity in the translational RASTEN cohort, with the aim to identify patients at risk of VTE and reduced survival, and ultimately, to identify patients that might benefit from prophylactic LMWH. The results did not reveal a distinct hypercoagulable profile, and we found that low levels of tissue factor bound to extracellular vesicles predicted a negative response to enoxaparin.

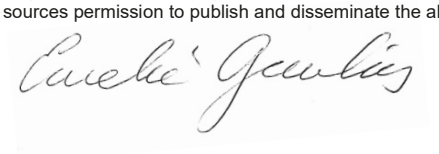
Paper IV explores the correlation between plasma biomarkers of cardiovascular stress and survival in SCLC, using the translational RASTEN cohort. The aim was to investigate a potential association between increased cardiovascular vulnerability and worse prognosis in SCLC patients that frequently display cardiovascular comorbidity. In particular, we found ST2, an FDA-approved cardiac biomarker, and adrenomedullin, a vasoactive peptide, to strongly correlate to mortality, independent of established prognostic factors.

To summarise, LMWH did not improve survival in SCLC despite a significant reduction in VTE, and this cannot be explained by inadequate adherence. Profiling of coagulation activity did not distinguish a subgroup of patients where LMWH would be beneficial. Based on these findings, the use of prophylactic LMWH cannot be recommended in the general management of SCLC. However, biomarkers of cardiovascular disease are potential prognostic factors that deserve to be explored further in prospective studies.

Key words
Small cell lung cancer; venous thromboembolism; low-molecular-weight heparin; coagulation; cardiovascular biomarkers

Classification system and/or index terms (if any)

Supplementary bibliographical information		**Language** English
ISSN 1652-8220 Lund University, Faculty of Medicine Doctoral Dissertation Series 2022:58		**ISBN** 978-91-8021-219-9
Recipient's notes	**Number of pages** 102	Price
	Security classification	

Signature  Date 2022-03-18

Coagulation and other aspects of the cardiovascular system in small cell lung cancer

Emelie Gezelius

LUND
UNIVERSITY

Cover picture by Catharina Jönsson, 2022

Emelie.gezelius@med.lu.se

Faculty of Medicine
Department of Clinical Sciences, Lund

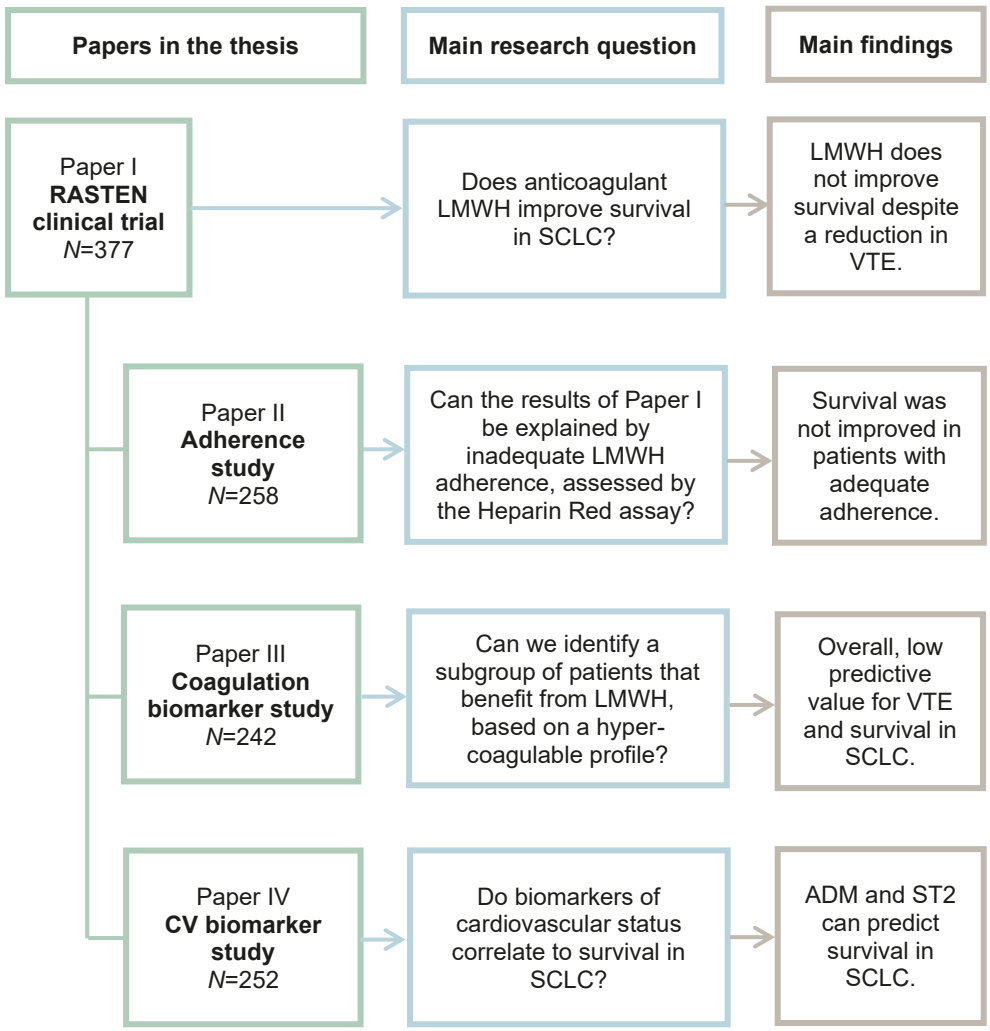
Lund University, Faculty of Medicine Doctoral Dissertation Series 2022:58
ISBN 978-91-8021-219-9
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2022

The more you know,
the more you know that you don't know.
Aristotle

Table of Contents

Thesis at a glance



Overview of research questions in focus in this thesis.
RASTEN = randomised phase-3 trial of standard treatment with or without the addition of enoxaparin in small cell lung cancer; SCLC = small cell lung cancer; LMWH = low-molecular-weight heparin; VTE = venous thromboembolism; CV = cardiovascular; ADM = adrenomedullin; ST2 = suppression of tumorigenicity 2.

List of papers included in the thesis

This thesis is based on the following original papers, referred to in the text by their roman numerals as indicated below:

I. L. Ek, **E. Gezelius**, B. Bergman, P-O. Bendahl, H. Andersson, J. Sundberg, M. Wallberg, M. Belting. Randomized Phase III Trial of Low Molecular Weight Heparin Enoxaparin in Addition to Standard Treatment in Small Cell Lung Cancer: the RASTEN Trial. *Ann Oncol.* 2018; 29(2):398–404

II. **Gezelius E**, Bendahl PO, Gonçalves de Oliveira K, Ek L, Bergman B, Sundberg J, Strandberg K, Krämer R, Belting M. Low-molecular-weight heparin adherence and effects on survival within a randomised phase III lung cancer trial (RASTEN). *Eur J Cancer*. 2019 Sep;118:82-90

III. **Gezelius E***, Flou Kristensen A*, Bendahl PO, Hisada Y, Risom Kristensen S, Ek L, Bergman B, Wallberg M, Falkmer U, Mackman N, Pedersen S, Belting M. Coagulation biomarkers and prediction of venous thromboembolism and survival in small cell lung cancer: A sub-study of RASTEN - A randomized trial with low molecular weight heparin. *PLoS One*. 2018 Nov 9;13(11). *Contributed equally

IV. **E. Gezelius**, P. O. Bendahl, W. Gallo, K. Gonçalves de Oliveira, L. Ek, B. Bergman, J. Sundberg, O. Melander, M. Belting. Circulating levels of the cardiovascular biomarkers ST2 and adrenomedullin predict outcome within a randomized phase III lung cancer trial (RASTEN). *Cancers*. 2022 Mar 3;14(5):1307

Abbreviations

ADM	Adrenomedullin
ANP	Atrial natriuretic peptide
Anti-FXa	Anti-factor Xa
AVP	Arginine vasopressin
bFGF	Basic fibroblast growth factor
BNP	Brain natriuretic peptide
CI	Confidence interval
CTCAE	Common terminology criteria for adverse events
CV	Cardiovascular
CVD	Cardiovascular disease
DOAC	Direct oral anticoagulant
ED	Extensive disease
ETP	Endogenous thrombin potential
EV	Extracellular vesicle
EV-TF	Tissue factor associated with extracellular vesicles
FIIa	Factor IIa, thrombin
FDA	Food and drug administration
GAG	Glycosaminoglycan
HIF	Hypoxia-inducible factor
HR	Hazard ratio
HS	Heparan sulfate
HSPG	Heparan sulfate proteoglycan
LD	Limited disease
LMWH	Low-molecular-weight heparin
MR-proADM	Midregional pro-adrenomedullin
MR-proANP	Midregional pro-atrial natriuretic peptide
NK-cell	Natural killer cell
NSCLC	Non-small cell lung cancer
OS	Overall survival
PAR	Protease-activated receptor
PD1	Programmed cell death-1
PDGF	Platelet-derived growth factor
PD-L1	Programmed cell death-1 ligand
PEA	Proximity extension assay
PFS	Progression-free survival
PG	Proteoglycan
PPL	Procoagulant phospholipid
RASTEN	A randomised phase-III trial of standard treatment with or without the addition of enoxaparin in small cell lung cancer

RCT	Randomised controlled trial
ROC	Receiver operating characteristics
SCLC	Small cell lung cancer
ST2	Suppression of tumorigenicity 2
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TGA	Thrombin generation assay
VEGF	Vascular endothelial growth factor
VTE	Venous thromboembolism
WHO	World health organisation

Preface

In spring 2021, as I was writing the fourth paper of this thesis, exploring the prognostic impact of the cardiovascular biomarkers adrenomedullin and ST2, I met a patient with newly diagnosed small cell lung cancer. She had a centrally growing tumour compressing pulmonary vessels and oesophagus, causing cardiac insufficiency and swallowing difficulties. The patient was admitted to the intermediate care unit where we planned to start first-line chemotherapy. However, she went into cardiac failure, requiring respiratory support, and the administration of intravenous chemotherapy became a balance act between potentially life-prolonging, or abruptly life-shortening, treatment.

> *I wished that I could measure her levels of adrenomedullin and ST2, to guide me in the possibly futile, possibly effective, management. I imagined the levels to be sky-high which, according to our study results, would signify a poor prognosis. Would I, in this case, do more harm by giving her active treatment? Would the toxicity of chemotherapy override the potential benefit? What would be most valuable in terms of quality of life?*

Due to her fulminant heart failure, only 50% of the first dose of chemotherapy was administered. As it turned out, 50% was enough to start shrinking the tumour, and three weeks later she came walking back to the clinic for a second dose.

> *What would be the role of prognostic biomarkers in this context? Withholding first-line treatment in a patient with raised levels may be difficult to justify, knowing that a majority of patients will experience at least a partial response and, importantly, clinical improvement. Perhaps these biomarkers become useful at the time of relapse, when the likelihood of a clinically meaningful response to treatment is lower? Would I then withhold treatment and focus on symptom control and best supportive care?*

Despite the initial response, the patient experienced an early disease progression and started oral topotecan only two months after the first-line treatment was completed. This time chemotherapy was futile, and she passed away exactly six months after receiving the diagnosis of small cell lung cancer.

> *In the biomarker study, the median survival time in patients with high levels of adrenomedullin and ST2 was six months. I hope that, in a few years, we will know the answers to some of the questions above.*

Emelie Gezelius,
March 2022

Introduction

Cancer is a major worldwide cause of death, and with a growing and ageing population the incidence is expected to rise by 47% from 2020 to 2040[1], making this a global health concern. With the successful implementation of screening programmes, some tumour types are diagnosed at an earlier stage with better chances of cure, and the recent therapeutic improvements involving immune checkpoint inhibitors and targeted therapies offer long-term disease control in patients with metastatic cancer. This is reflected in falling mortality rates over the last decades[2], but poses new demands on the management of long-term consequences of anticancer treatment, such as the development of secondary cardiac toxicity.

Still, in a significant proportion of patients the disease is beyond control and prognosis is dismal. Even if the tumour itself originates from a specific organ, cancer should be viewed as a systemic disease. Fatigue, cachexia, general inflammation or a prothrombotic state, are all signs of the systemic impact of cancer[3,4]. This thesis focuses on the roles and consequences of coagulation activation and other aspects of cardiovascular stress in patients with small cell lung cancer (SCLC), a lung cancer subtype with a particularly poor prognosis.

Cancer, coagulation, and clots – a complex relationship

The association between cancer and coagulation activation has intrigued scientists for more than a century. Already in the 1860's, the French physician Armand Trousseau (1801-1867) described a link between thrombosis and malignancy. In his famous collection of medical lectures *Clinique Medicale de l'Hôtel-Dieu,* Trousseau reports several cases of thrombophlebitis (thrombotic inflammation of a vein) in visceral malignancy and refers to the condition as '*an affection quite special, and well deserving attention from the numerous circumstances under which it is observed*'[5]. Further, Trousseau postulated that the cause of the thrombosis could not purely be explained by direct tumour invasion or compression of vasculature, he quite aptly suspected a state of hypercoagulability in cancer. Subsequently, the term 'Trousseau's syndrome' refers to migratory thrombophlebitis as a sign of visceral malignancy but may also be used to describe any case of thrombosis preceding a diagnosis of cancer [6].

Cancer-associated venous thromboembolism

Venous thromboembolism (VTE) is the pathological formation of a blood clot in a vein, typically either in a pulmonary vessel (pulmonary embolism) or in a deep vein in the upper or lower extremities (deep vein thrombosis). VTE can give rise to a range of clinical presentations, depending on site and severity. A deep vein thrombosis usually leads to a painful, swollen limb whereas a pulmonary embolism can manifest as an incidental finding on a routine computerised tomography scan, as a gradual or sudden onset of respiratory symptoms, or may even be acutely fatal[7].

Several factors are known to increase the risk of developing a VTE, including recent surgery, hospitalisation and, importantly, active malignancy[8]. In fact, 20% of all patients developing a VTE have a current or recent diagnosis of cancer[9]. Cancer patients have a 4 – 9-fold increased risk of developing a VTE compared to the general population, and ongoing chemotherapy further potentiates this risk[8,10,11]. Additionally, newer anti-tumoral agents such as tyrosine kinase inhibitors and immune checkpoint inhibitors have also been shown to enhance the risk of VTE[11]. The VTE incidence varies with different cancer types, of which cancer of the pancreas, stomach, ovary and brain, as well as haematological malignancies, are among those considered to be at highest risk[9,10,12]. In pancreatic cancer, the 1-year incidence of VTE per 100 patient-years is 14.0%, compared to 2.0% and 0.9% in colorectal and breast cancer, respectively[9]. With a 1-year rate of 4.3% per 100 patient-years, the VTE risk in lung cancer can be considered intermediate. Notably, in lung cancer the association to VTE is stronger with adenocarcinomas than other histological subtypes, including SCLC[13].

VTE is associated with more advanced cancer stages and reduced survival compared to cancer patients without a VTE, particularly in the first few months after VTE diagnosis[9,14]. Focusing on lung cancer, the effects on survival appear to vary with disease stage and subtype. Across all histological subtypes, having a VTE is associated with significantly reduced 2-year survival compared to lung cancer patients without a VTE, with a hazard ratio (HR) of 2.3 for non-small cell lung cancer (NSCLC) and 1.5 for SCLC[13]. Consistently, White *et al.* reported increased 1-year mortality in lung cancer patients with VTE, with HRs of 3.1, 2.9 and 2.5 by localised, regional, and distantly spread disease, respectively[9]. Patients with active cancer are more likely to develop recurrent VTE and to experience major haemorrhages during anticoagulant therapy[15,16], making the management even more challenging.

Anticoagulant therapy

Anticoagulant therapy used for treating VTE in cancer patients has traditionally evolved around low-molecular-weight heparins (LMWH), administered as daily subcutaneous injections, and the oral vitamin K antagonist, warfarin. The LMWHs have been preferred when treating cancer patients for practical and safety reasons,

and importantly, the risk of VTE recurrence is significantly reduced with LMWH compared to the vitamin K antagonist[17]. Several LMWH agents are available with comparably efficacy and safety profiles[18]. In addition, a more novel group of drugs referred to as direct oral anticoagulants (DOAC), targeting either coagulation factor Xa or thrombin, have recently proved to be a safe alternative in selected cancer patients[19].

Prediction of VTE – who is at risk?

Several risk scores have been developed aiming to identify a high risk of VTE in ambulatory cancer patients, where thromboprophylaxis may be indicated to prevent VTE. The most widely used risk assessment tool, and so far the only model recommended by international guidelines, is the so-called Khorana score[20,21], which incorporates cancer site, body mass index and routine laboratory parameters, as shown in Table 1. Despite being based on easily accessible variables, the Khorana score has some limitations. In a large meta-analysis[22] the predictive value could not be confirmed in patients with lung cancer (*N*=3,744), and the majority of patients developing a VTE were not identified by the Khorana score. In fact, only 23.4% of VTE patients were pre-defined as high-risk and potentially eligible for thromboprophylaxis. Other proposed risk assessment models include d-dimer, P-selectin or type of chemotherapy agent, but none of these have been thoroughly validated or implemented[23].

Table 1: Khorana score for prediction of cancer-associated venous thromboemblism in ambulatory patients[20].
Original risk categories by scores: Low (0); intermediate (1-2); high (≥3). BMI = body mass index.

Patient characteristic	Risk score
Cancer site: Pancreas, stomach	2
Cancer site: Lung, gynaecological, bladder, testicular, lymphoma	1
Pre-chemotherapy platelet count ≥350 x10^9/L	1
Haemoglobin level <100 g/L or use of red cell growth factors	1
Pre-chemotherapy leukocyte count >11 x10^9/L	1
BMI ≥35 kg/m^2	1

Coagulation in malignancy – a double-edged sword

Various factors may contribute to cancer-associated thrombosis, including the direct compression of veins caused by the tumour itself or metastases, which may impede blood flow and promote clot formation. As mentioned above, several anti-cancer therapies are prothrombotic, such as certain chemotherapy agents, surgery, angiogenesis inhibitors and tyrosine kinase inhibitors. But, perhaps most importantly, cancer is associated with a hypercoagulable state, which will be covered in the following section.

The coagulation cascade

In order to understand the complexities of the coagulation system in malignancy, we need to start by understanding its role in health. Tissue factor (TF), the main initiator of the extrinsic coagulation pathway, is normally expressed by adventitial fibroblasts in vessel walls where it is shielded from exposure to plasma, and only small amounts are present in the circulation[24,25]. In response to vascular injury, subendothelial TF is exposed to coagulation factors in plasma, triggering a cascade of downstream events that subsequently lead to clot formation. In brief, TF activates and forms a complex with the coagulation factor VII (FVIIa) which converts factor X into its active form (FXa). Subsequently, FXa leads to the generation of thrombin (FIIa) which in turn converts fibrinogen into its active form fibrin. In addition, thrombin activates platelets via cleavage of protease-activated receptors (PAR), which together with fibrin constitute the haemostatic plug which is essential to stop bleeding[24,26,27]. Tissue factor pathway inhibitor (TFPI) is the primary endogenous inhibitor of the coagulation cascade, binding to and blocking both the TF/FVIIa complex and FXa directly[25]. Other regulators with anticoagulant properties include antithrombin, protein C and heparin cofactor II[28].

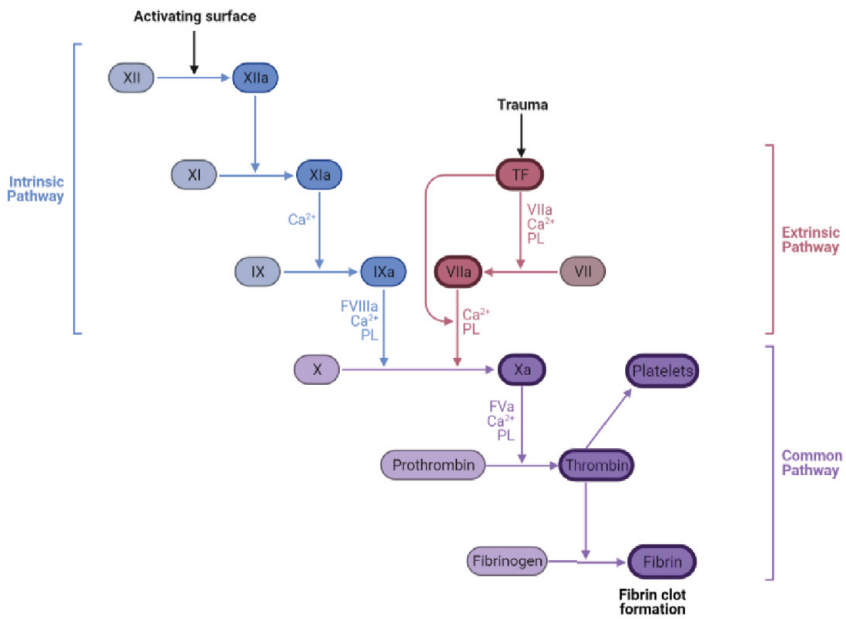


Figure 1: The coagulation cascade consists of an intrinsic, extrinsic and common pathway.
Tissue factor initiates the extrinsic coagulation pathway in response to vascular injury. Coagulation factors are indicated by roman numerals and the letter 'a' denotes the activated form. TF = tissue factor. Created with Biorender.com.

Description of tissue factor

Tissue factor is a transmembrane protein consisting of three components: an extracellular, a transmembrane and a cytoplasmic domain. The procoagulant function is mainly exerted by the extracellular domain which binds to FVIIa as part of the coagulation pathway, whereas the cytoplasmic domain is thought to be involved in signal transduction[24,29]. In the circulation, TF can be present either in a truncated, alternatively spliced isoform, which lacks the transmembrane and intracellular domains and has reduced procoagulant properties, or as a full-length isoform bound to extracellular vesicles (EV), with efficient procoagulant activity. These vesicles are membrane-derived microparticles, ranging from 0.1 to 1 μm in size[30], that can be secreted from any cell. The shedding is accentuated in conditions associated with cellular stress, such as inflammation, sepsis and cancer[31,32]. EVs carry surface proteins from the cell of origin, thus reflecting the parental cell and are thought to be involved in intercellular communication[30,33]. In healthy individuals, EV-associated TF (EV-TF) is largely derived from platelets and has been shown to contribute considerably to the stimulation of downstream coagulation factors and the generation of fibrin[34]. Historically, different nomenclature has been used depending on the size and origin of the EV which has been a source of confusion. In line with international consensus guidelines[35], the term EV will be used as a collective name for microparticles, microvesicles and exosomes in this thesis, and TF associated with EVs will be referred to as EV-TF.

Tissue factor in cancer

Malignancy is associated with a state of hypercoagulability, and this can largely be explained by an increased expression of TF. Induced TF expression has been reported in several types of cancer, including pancreatic, hepatocellular, colorectal, ovarian and lung cancer[36-41], and is associated with more advanced disease stages and a poor prognosis. Apart from being activated following vascular injury, the expression of TF can be induced by hypoxia, oncogenic mutations and signalling pathways[42-44], which may account for the upregulation seen in malignancy.

The presence of circulating EV-TF has also been correlated to survival in pancreatic cancer[45], and a study demonstrating a 4-fold increased risk of VTE in cancer patients with detectable EV-TF, supports the notion of EV-TF bearing procoagulant activity[46]. This was first described in early *in vitro* studies, showing that cancer cells can shed membrane-derived vesicles carrying procoagulant activity, indicating that the EV-TF originates from the actual tumour cells[47,48].

How does TF contribute to tumour development?

The association between TF and reduced survival in cancer cannot purely be explained by the prothrombotic consequences of an activated coagulation system. In fact, a substantial amount of evidence indicates that several of the most prominent clotting factors are actively involved in carcinogenic processes.

TF and its downstream coagulation proteases may contribute to tumour development by enhancing tumour growth, metastasis and angiogenesis[24,42] (Figure 2). The exact underlying mechanisms are not completely understood, but several processes have been suggested. The protease-activated receptors, particularly PAR-1 and PAR-2, are important mediators of these biological pathways. PARs are a group of G-protein-coupled transmembrane receptors which are activated through proteolytic cleavage in response to various proteases, including certain coagulation factors[49]. For example, PAR-1 and -2 are activated by FXa and the TF/FVIIa complex, whereas thrombin activates PAR-1, -3 and -4, of which PAR-1 and -4 are involved in platelet activation[27]. Expression of PAR-1 and PAR-2 have been noted in several cancer cell lines, and especially PAR-2 has been associated with downstream signalling through activation of the MAPK/ERK1/2 pathways, leading to cell growth and proliferation[29,49].

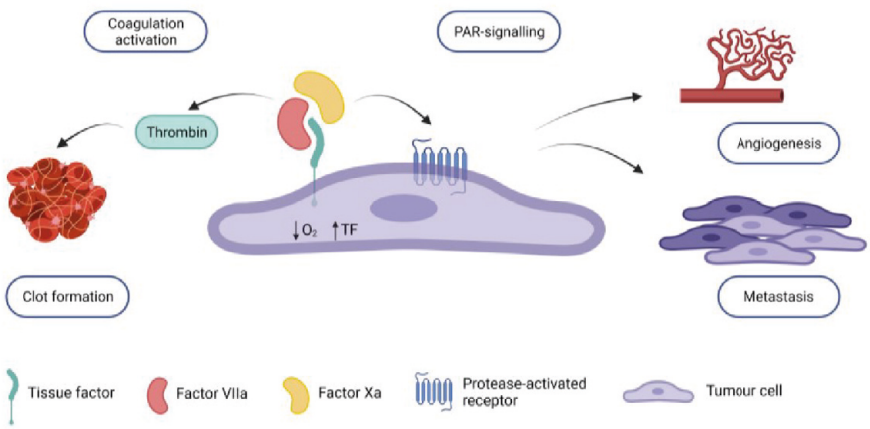


Figure 2: The TF/FVIIa/FXa complex contributes to coagulation activation and promotes tumour progression via PAR-signalling.
TF expression on cancer cells is upregulated by hypoxia, leading to downstream activation of the coagulation pathway. The TF/FVIIa/FXa complex also mediates PAR-signalling, contributing to angiogenesis and metastasis. TF = tissue factor; PAR = protease-activated receptor. Created with Biorender.com.

Angiogenesis, the formation of new vasculature, is an essential process in tumour development, ensuring the delivery of oxygen and nutrients to the proliferating cancer cells. TF has been shown to enhance angiogenesis both through TF/FVIIa/PAR2 signalling and by increasing the expression of vascular endothelial growth factor (VEGF), the principal inducer of angiogenesis[29,42,44]. In a study by Khorana *et al*.[36] TF expression on pancreatic tumour cells was found to correlate to reduced survival, but notably, also to increased VEGF expression and microvessel density, as evidence of enhanced neovascularisation. This has also been noted in hepatocellular carcinoma[38].

Finally, the role of TF in cancer is further illustrated by the promising reports of clinical trials investigating TF as a potential therapeutic target. Based on encouraging results from a phase-II trial, tisotumab vedotin, an antibody-drug conjugate targeting TF, was recently granted accelerated approval for the use in advanced cervical cancer[50]. Antibody-drug conjugates consist of a monoclonal antibody directed at a specific target, in this case TF which is aberrantly expressed on the surfaces of malignant cells, combined with a cytotoxic compound[51]. Following antibody binding, the target-antibody-drug complex is internalised, enabling the intracellular delivery of cytotoxic agents to specific target cells. Of note, tisotumab vedotin has shown minimal effect on coagulation, emphasising the importance of TF as a mediator of coagulation-independent tumorigenic pathways.

Thrombin, fibrin and platelets - further links between coagulation and cancer

Thrombin is, in normal conditions, bound to the inhibitory co-factor thrombomodulin, which is expressed on the surface of endothelial cells and transforms thrombin from a procoagulant to an anticoagulant[52]. Thrombin may promote metastasis through PAR-1 signalling and augment angiogenesis by various mechanisms, *e.g.* by increasing VEGF expression and upregulating the VEGF receptor on endothelial cells[52,53].

Fibrin, the principal end-product in the clotting cascade, provides a scaffold which promotes migration and facilitates haematological metastasis[54-56]. In addition, the fibrin matrix within the tumour microenvironment is able to sequester growth factors, including VEGF and basic fibroblast growth factor (bFGF), and protect them from degradation, thus sustaining pro-angiogenic signalling[55,57]. Another mechanism whereby the coagulation system is thought to contribute to metastasis is by limiting the clearance of micrometastases by natural killer cells (NK-cells) in a fibrin- and platelet-dependent manner. Evidence indicates that fibrin together with platelet aggregation, as a consequence of TF-activation, creates a protective sheath around metastatic cells, safeguarding against NK-cell attack[58-60].

Platelets play an important part in metastatic processes and, as previously stated, are activated via thrombin-mediated PAR-signalling, leading to aggregation and clot formation, further contributing to the protective shield enveloping circulating tumour cells. Platelets contain a vast number of granules, providing storage for mitogenic and proangiogenic factors such as VEGF, bFGF and platelet-derived growth factor (PDGF)[61-63], which are released into the circulation upon activation. Moreover, platelets express numerous cell adhesion molecules, mainly integrins and selectins, facilitating tumour cell migration[64]. Consistently, the effect of therapeutic platelet inhibition on cancer growth has been investigated both in preclinical and clinical settings, but despite initial positive trials[65,66], more recent studies have failed to demonstrate reduced cancer mortality with aspirin[67,68], an inhibitor of platelet aggregation.

The hypoxic tumour microenvironment

Causes and consequences

Hypoxia, defined as a reduction in the normal level of tissue oxygen tension, is a feature of aggressive tumours[69]. Hypoxia develops as rapidly expanding tumours out-grow their blood supply, limiting the delivery of oxygen and nutrients, and eventually leading to apoptotic and necrotic cell death. To overcome this, a range of adaptive mechanisms are triggered to help cancer cells to survive in their harsh surroundings (Figure 3). In addition, hypoxia is associated with increased resistance to conventional treatment modalities, most importantly radiotherapy[70], further contributing to an aggressive phenotype.

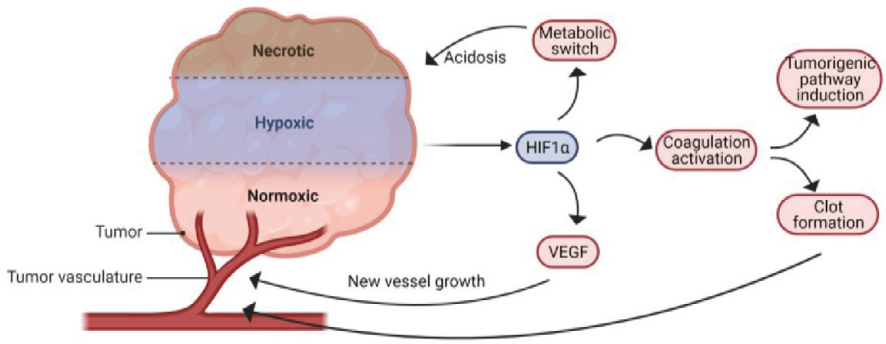


Figure 3: Adaptive responses to tumour hypoxia.
The oxygen delivery diminishes as tumour cells grow further away from their original blood supply, leading to necrosis. The subsequent hypoxia activates hypoxia-inducible factor (HIF) which induces numerous adaptive mechanisms, including upregulation of vascular endothelial growth factor (VEGF), a key pro-angiogenic factor. Other processes involve the metabolic switch to oxygen-independent glycolysis, and activation and extravasation of coagulation factors. Created with Biorender.com.

The adaptive pathways are mainly regulated by hypoxia-inducible factors (HIF) 1α and 2α, transcription factors that are stabilised and dimerised with the HIF-1β subunit under hypoxic conditions[71]. Consequently, HIFs activate the transcription of numerous genes that contribute to the hypoxic response. HIF expression may also be induced by oncogenic signalling in the absence of hypoxia[72,73], and has been shown to correlate to metastasis and mortality in several solid tumours[74-77]. Clinical trials are currently exploring the utility of HIF inhibitors in the treatment of various types of cancer, and the HIF-2α inhibitor belzutifan was recently approved by the US Food and Drug Administration (FDA) for the treatment of tumours with constitutive HIF activation due to von Hippel-Lindau gene inactivation[78].

The adaptive responses to hypoxia are vast and include:

a. The metabolic switch

Following diminished oxygen supply, cancer cells shift from aerobic to anaerobic metabolism via upregulation of the glycolytic pathway and downregulation of oxidative phosphorylation. This is mediated by HIF-1/2α, which induces the expression of genes required for glycolysis[79]. Glycolysis leads to an accumulation of acidic metabolites, such as lactate, carbon dioxide and protons. To avoid intracellular acidosis, pH-regulatory mechanisms are required, encompassing specific ion exchangers and lactate transporters, thus generating an acidic tumour microenvironment[80]. The ability to modulate the intra- and extracellular pH provides a survival advantage to tumour cells, over the less adaptable, non-malignant cells in the tumour microenvironment[81].

b. Angiogenesis

One of the essential hypoxic adaptations is the formation of new vasculature, so-called angiogenesis, which is fundamental for tumour growth as it restores the supply of nutrients and oxygen[82]. The vessels that are formed during malignant angiogenesis are aberrant in structure and more permeable than normal vessels[83]. This enables the extravasation of plasma proteins and coagulation factors, with resultant fibrin deposition in tissues that might facilitate the spread of metastatic cells.

VEGF, one of the key regulators of angiogenesis, is strongly induced by hypoxia and has been found to be upregulated in several types of cancer[84]. Other HIF-induced pro-angiogenic factors include adrenomedullin (ADM) and fibroblast growth factor (FGF)[69]. Pioneering work by Judah Folkman in the early 1970's acknowledged angiogenesis as a crucial component in tumour growth and described VEGF as a potential target to inhibit angiogenesis[85,86], which led to the development of VEGF-targeting therapies. Bevacizumab, a monoclonal antibody directed against VEGF, was the first approved anti-angiogenesis agent, and today there are several groups of drugs aimed at inhibiting angiogenesis, both antibodies and tyrosine kinase inhibitors. In SCLC, as in other types of cancer, increased levels of VEGF have been associated with a poor prognosis[87-89] and this has been subject to investigations with various angiogenesis inhibitors. However, results have been conflicting and despite numerous trials, there is currently only one approved angiogenesis-targeting drug, but only for use in Chinese populations[90].

c. Coagulation

Moreover, there is evidence suggesting that HIFs can activate the coagulation system through the induction of TF, thereby linking hypoxia and cancer-associated hypercoagulation[91]. However, it remains unknown whether the degree of tumour hypoxia directly correlates with VTE risk. Some of the pro-angiogenic and proliferative potential of hypoxia may also be related to PAR-signalling, as hypoxia

has been shown to upregulate PAR-2 in endothelial cells, stimulating proliferation and angiogenesis. Furthermore, hypoxia has been found to stimulate the release of procoagulant EVs carrying TF/FVIIa, with the potential to induce PAR-2 signalling[92].

Hence, hypoxia is a central event leading to several tumour-promoting processes including angiogenesis, coagulation activation and metabolic adaptations, contributing to a more aggressive and treatment-resistant phenotype.

The wound that does not heal

Cancer is sometimes referred to as the wound that does not heal, as it employs similar mechanisms to induce coagulation, new vessel formation and supportive stroma proliferation as in wound healing[83,93]. For example, the upregulation of VEGF resulting in angiogenesis and increased vascular permeability is as central in the angiogenesis of wound healing as it is in cancer development. A major difference, however, is that the proliferative pathways in normal healing terminate when the healing is completed, whereas the tumour is insatiable, with sustained hypoxia and coagulation activation driven by constitutively expressed mitogenic signalling[83].

Anticoagulants as tumour-inhibiting agents

Bearing in mind that coagulation factors contribute to angiogenesis and metastasis, it is conceivable that anticoagulants, used to treat thrombosis, also have tumour-inhibiting effects. The pre-clinical and clinical evidence of this will be discussed in the following sections.

Heparin and heparan sulfate – a diverse duo

Heparin

Endogenous heparin is produced by mast cells, where it aids the packaging of proteases and histamines in mast cell granules[94]. Heparin belongs to the family of glycosaminoglycans (GAG) and consists of a highly sulphated polysaccharide chain. Notably, the chemical characterisation of heparin and the closely related heparan sulfate (HS) was pioneered by the Finnish-Swedish chemists Erik Jorpes, at Karolinska Institute, and Sven Gardell, for many years professor of medicinal chemistry at Lund University[95].

The uniform distribution of sulphated regions gives heparin its high negative charge, which is decisive for its biological profile[94]. More specifically, the active site of heparin is formed by a distinct pentasaccharide sequence with the ability to bind the protease inhibitor antithrombin[96]. This causes a conformational change to antithrombin, which enhances the inhibition of thrombin and FXa. In addition, heparin releases the potent TF-inhibitor, TFPI, from the vascular endothelium, further preventing TF-dependent coagulation activation[97]. The clinical indications for unfractionated heparin include prevention and treatment of thrombotic events, but its therapeutic usage has largely been replaced by low-molecular-weight heparins (LMWH), which are depolymerised derivatives of heparin[18]. With a more predictable bioavailability and clinical efficacy, LMWH is often preferred over unfractionated heparin. The subcutaneous once-daily LMWH injections imply a clear clinical advantage compared to the more frequent, often intravenous, injections of unfractionated heparin that additionally, requires regular monitoring. With regards to toxicity, the risk of major haemorrhage is comparable between LMWH and unfractionated heparin, but the latter is also associated with heparin-induced thrombocytopenia[18].

Heparan sulfate

HS is a less sulphated GAG as compared with heparin, and is conjugated with a core protein to form a proteoglycan (PG). HSPGs are present in virtually all cells in the body, either on cell surfaces as *e.g.* syndecans (1-4) or glypicans (1-6), or in the extracellular matrix conjugated to *e.g.* perlecan, agrin or collagen XVIII[94]. The biological functions of HS are vast and partly dependent on localisation, cell of

origin and sequence of the GAG chain[98,99]. Numerous other GAGs and PGs have been identified, with various biological activities, but for the purpose of this thesis we will focus on heparin and HS.

HS is an important component in the extracellular matrix and in cell signalling pathways. HS can provide a scaffolding for presenting or carrying specific proteins and may serve as a co-receptor for numerous growth factors[100]. Thus, HS has been proposed to contribute to tumour cell proliferation and angiogenesis, for example by potentiating the binding of VEGF, FGF and PDGF, all of which are known mitogenic or pro-angiogenic factors[98,101]. Consistently, Fuster *et al*. demonstrated that genetically altered HSPGs *in vivo* resulted in reduced tumour growth and vascularisation due to an attenuated angiogenic response to FGF and VEGF[102]. However, the roles of HS in tumour biology appear somewhat conflicting. HS can either act to promote or inhibit carcinogenesis, and this may be context-dependent[98,99]. For example, the HSPG glypican-1 is associated with an increased metastatic potential in mouse models and in patients[103] and shedding of syndecan-1 has been shown to promote breast cancer growth[104,105]. On the contrary, down-regulation of syndecan-1 expression has been found in several types of cancer[99], and HSPGs have been associated with suppressed proliferation in neuroblastoma[106].

Antitumoral mechanisms of heparin

Several mechanisms have been proposed for the potential tumour-inhibiting effects of heparin and its low-molecular-weight derivatives, as illustrated in Figure 4, and these include:

a. Inhibition of tumour-promoting coagulation factors

By directly inhibiting FXa and thrombin via antithrombin potentiation, heparin may impede the tumorigenic effects of coagulation activation, in particular fibrinogen-dependent metastasis[54,107]. Moreover, TFPI, a potent inhibitor of TF, is released from the vascular endothelium in response to heparin. TFPI has been found to exert antiangiogenic effects[108] in addition to the inhibition of TF-mediated coagulation activation and PAR-signalling, further reducing metastasis and angiogenesis. Chemically modified heparins with limited or no anticoagulant activity have also been shown to reduce tumour growth, metastasis and angiogenesis, implying that the antitumoral effects of heparin may be partly independent of its anticoagulative properties[109-112].

b. Inhibition of co-receptor function

HS contributes to proliferation and angiogenesis by acting as a co-receptor, facilitating the binding of growth factors to their respective receptors. For example, HS plays a mandatory role in bFGF binding its cell surface receptor, leading to enhanced proliferation, differentiation and new vessel formation[113,114]. Pre-clinical

experiments have demonstrated that heparin competitively inhibits growth factors from binding the HS co-receptor[115]. For example, both unfractionated and low-molecular-weight heparins can interfere with the interactions between bFGF and HSPG, thereby reducing FGF-induced angiogenesis[116]. Also, smaller heparin fragments of less than octadecasaccharides have been shown to reduce VEGF activity[117].

c. Inhibition of heparanase

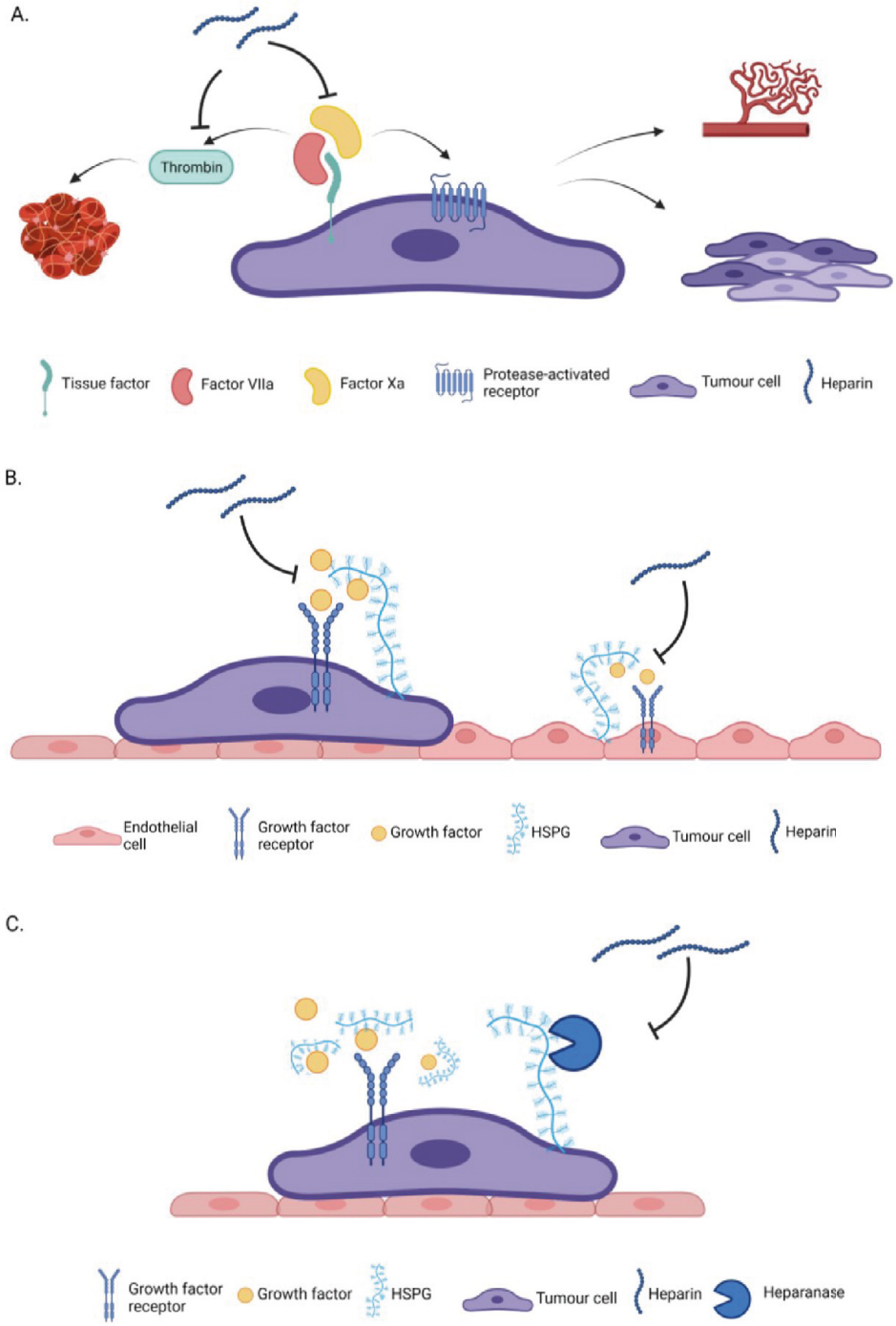
Heparanase is an enzyme that degrades and cleaves HS chains into shorter fragments. Heparanase is rarely expressed in normal tissues but can be secreted by tumour cells, and is associated with invasive cancer phenotypes and metastasis[118]. The HS fragments generated by heparanase cleavage may be even more potent mediators of growth factor signalling[107,119], and consistently, heparanase has been found to promote angiogenesis, metastasis and cell adhesion[120,121]. Several studies have demonstrated that heparin reduces the metastatic potential *in vitro* through inhibition of heparanase[122-124], providing yet another possible mechanism for the antitumoral effects of heparin.

d. Modulating cell adhesion

Selectins and integrins are important cell adhesion molecules, normally involved in cell-cell interactions, cell motility and anchorage to the extracellular matrix. In malignancy, several of the selectins and integrins contribute to metastatic spread. One mechanism whereby heparin may exert tumour-inhibiting effects, is by interacting with the selectin- and integrin-mediated cell adhesion, which is crucial for metastasis[64,125].

e. Exposure of cancer cells to immune cell surveillance

Platelets and fibrin can provide a protective sheath covering tumour cells in the circulation, helping the cancer cells evade clearance by NK-cells. It is conceivable that heparin breaks up the protective sheath primarily by interfering with platelet-associated selectins, thereby rendering the tumour cells more vulnerable to NK-cell clearance[58,59,126].


A.
Thrombin
Tissue factor
Factor VIIa
Factor Xa
Protease-activated receptor
Tumour cell
Heparin
B.
Endothelial cell
Growth factor receptor
Growth factor
HSPG
Tumour cell
Heparin
C.
Growth factor receptor
Growth factor
HSPG
Tumour cell
Heparin
Heparanase

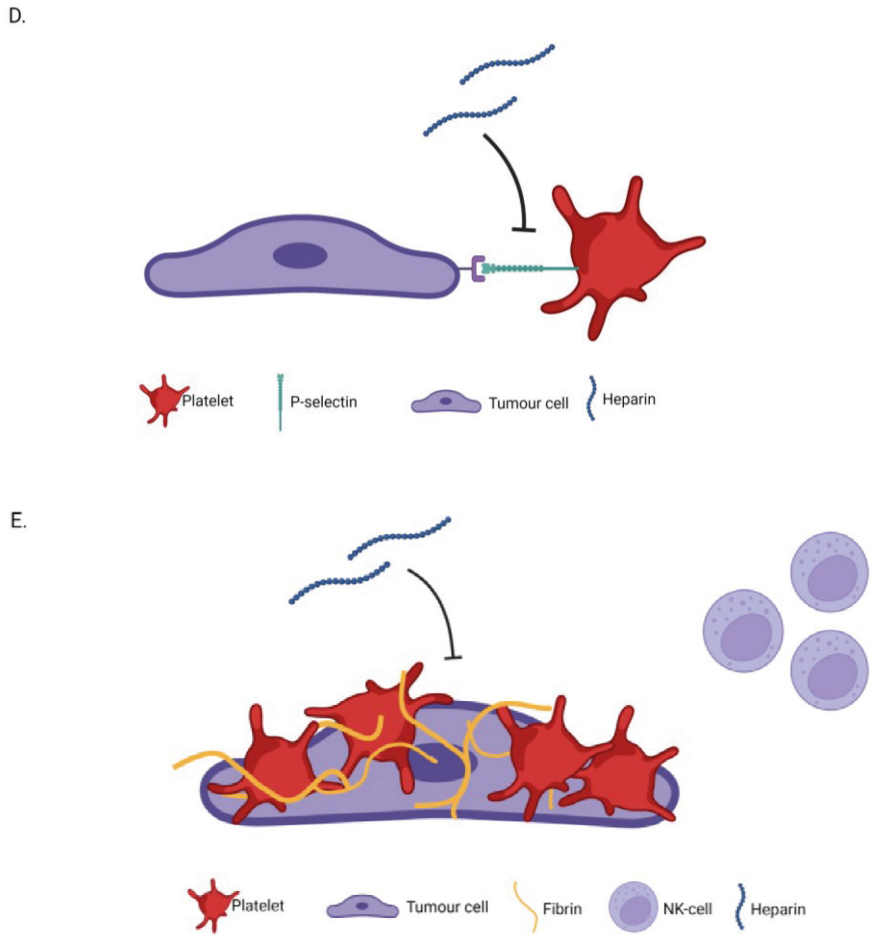


Figure 4: Potential antitumoral mechanisms of heparin.
a) Inhibition of tumorigenic effects of coagulation activation by blocking coagulation factors FXa and thrombin; b) interference of heparan sulfate co-receptor function, thereby blocking growth factor binding to their respective receptor; c) inhibition of heparanase activity; d) impeding tumour cell interaction with cell-adhesion molecules; e) disrupting the protective sheath consisting of platelets and fibrin, exposing the tumour cells to immune cell clearance. HSPG = heparan sulfate proteoglycan; NK-cell = natural killer-cell. Created with Biorender.com.

Clinical trials

Considering that VTE is a major contributor to cancer-associated morbidity and mortality, that several coagulation factors are involved in tumour development, and the large amount of preclinical evidence indicating that anticoagulants exert tumour-inhibiting effects, the next question to ask is how this translates into clinical situations. Do anticoagulants actually prolong survival in patients with cancer?

The first, pivotal trial to address this was the Veterans Administration Cooperative Study #75 published by Zacharski *et al*. in 1984[127]. Here, the addition of warfarin improved survival specifically in patients with SCLC (*N*=50), from 23 to 49 weeks. No effect was seen in patients with other types of cancer. Ten years later, unfractionated heparin was shown to increase survival from 261 to 317 days in SCLC (*N*=277) and the association was more pronounced in patients with limited disease[128]. Yet another ten years down the line, Altinbas *et al*.[129] demonstrated survival rates of 13 and 8 months, respectively, in SCLC patients receiving chemotherapy with addition of the LMWH dalteparin, or chemotherapy alone (*N*=84).

In contrast, other studies with more heterogenous cancer populations have only shown improvements by anticoagulants in patients with an already favourable prognosis or non-metastatic disease[17,130,131]. Based on results from the trials above and others, early review articles concluded that the adjunctive use of anticoagulants reduced mortality uniquely in patients with SCLC[132,133]. However, more recent studies have failed to show any survival benefit with prophylactic anticoagulants[134-136]. Of particular interest is the FRAGMATIC trial from 2016, in which 2,202 lung cancer patients received standard therapy with or without prophylactic dalteparin for 24 weeks[134]. Intriguingly, there was no difference in outcome between the two study arms, and subgroup analysis of the 392 patients with small cell histology did not reveal any improved survival.

A potential drawback with self-administered medication is the issue of adherence. Contrary to warfarin which is routinely monitored by assessing the international normalised ratio (INR) in plasma, there is no method to objectively measure adherence to LMWH, as accessible tools are lacking. This problem has been approached in different ways, for example, in the FRAGMATIC study patients were instructed to bring empty syringes to the follow-up appointments. Still, this is no guarantee that the medication has been administered, and in the vast majority of studies the issue of adherence has been somewhat neglected.

To summarise, the role of anticoagulants as tumour-inhibiting agents has been unclear to say the least, and the early, positive studies were either small or did not use modern chemotherapy regimens. This prompted the design of RASTEN, a phase-III trial where patients with SCLC were randomised to receiving standard therapy with or without the addition of enoxaparin, a LMWH. The trial was conducted between 2008-2016, with the aim to investigate whether enoxaparin at a supra-prophylactic dose would improve survival in a large, homogenous population of SCLC patients. A detailed description of the RASTEN trial follows in the Patients and methods section.

Small cell lung cancer – a dynamic disease

Lung cancer is the leading cause of death from cancer, worldwide and in Sweden[137,138]. Small cell lung cancer, accounting for 13% of all lung cancer cases, is a particularly aggressive disease with 5-year survival rates of <7%[138-140]. Typically, SCLC follows a dynamic course and is characterised by symptomatic patients at time of diagnosis, due to the rapid cancer growth and early metastatic spread. However, owing to the treatment-sensitive nature of the disease, a majority of patients will experience initial tumour shrinkage and relief of symptoms. Still, most patients will relapse, and the chances of responding to subsequent lines of therapy are substantially diminished[141]. Until very recently, there were no major therapeutic improvements into the care of SCLC patients, hence other treatment strategies, such as the use of prophylactic anticoagulants to prolong survival, were greatly sought after. Today, some therapeutic advances have been made with the emergence of immunotherapy, and hopefully there is more to come in the next few years. This section will briefly cover the current standard of care and other clinical considerations in SCLC.

Prognostic and predictive factors

Disease stage

Disease extent is one of the main factors affecting prognosis in SCLC[142], and correct staging is essential to guide clinicians in making the most appropriate treatment decisions. Most solid tumours are staged according to the TNM classification, which encompasses tumour size (T), nodal status (N) and distant metastasis (M), generating four stages ranging from I (local disease) to IV (metastatic disease)[143]. In contrast, SCLC has by tradition been classified as limited disease (LD) or extensive disease (ED) using a simplified system proposed by the Veterans Administration Lung Study Group (VALSG)[144,145]. Limited disease, corresponding to stage I-III, is defined as tumours confined to one hemithorax, whereas extensive disease, equivalent to stage IV, is any spread beyond that (Figure 5). Prognosis is particularly dismal in ED with 2-year survival rates of 5%, compared to 24% in LD[140]. The TNM classification is generally preferred in SCLC, however, as most clinical trials still apply the VALSG staging system, the terms LD and ED will be used throughout this thesis.

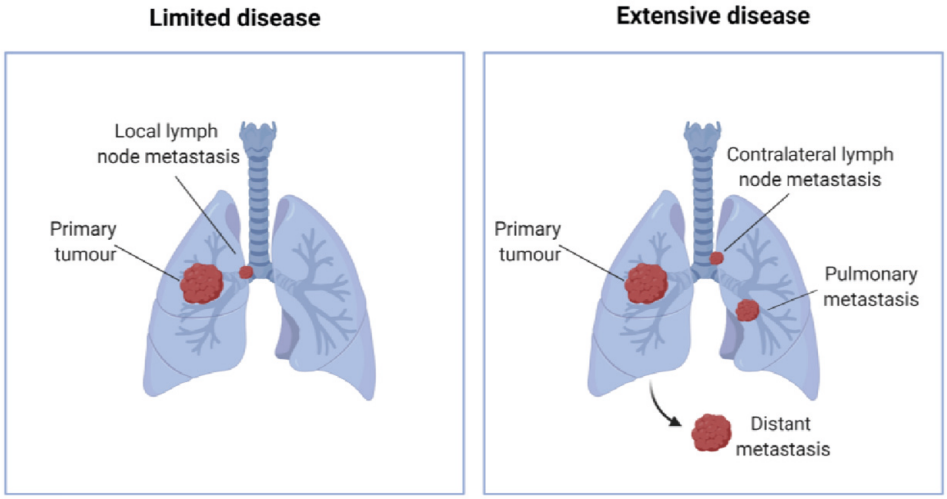


Figure 5: Staging of small cell lung cancer, according to the Veterans Administration Lung Study Group
Limited disease is confined to one hemithorax while extensive disease is any spread beyond that. Illustration created with Biorender.com.

The notion that LD is associated with a better outcome than ED, yet, in most patients the disease has already disseminated at diagnosis[138], raises the issue of screening. Trials aimed to elucidate the value of lung cancer screening using low-dose computerised tomography scans have demonstrated earlier detection and significantly reduced lung cancer mortality, compared to control arms[146,147]. However, with regards to SCLC, the results are unclear. In the Dutch-Belgian NELSON trial[146], most of the SCLC cases in the screening group were, in fact, not detected through the screening programme. This highlights that rapidly growing tumours may be difficult to capture at a predetermined time-point for radiographic assessment, but further discussions are needed regarding the implementation of screening programmes to reduce lung cancer mortality in high-risk populations.

Performance status and other clinical prognostic factors

When discussing treatment options, it is essential to consider the patient's functional level, as this will influence treatment tolerability and outcome. Assessment of performance status is a way to evaluate the daily activities a person is able to carry out. In clinical trials, the most widely used scale is the European Cooperative Oncology Group score[148], also referred to as the World Health Organisation (WHO) score, with grades ranging from 0 (asymptomatic and fully active) to 5 (dead). Performance status is a strong prognostic indicator, as illustrated by data extracted from the Swedish Lung Cancer Registry showing a median OS of 9.6 months in SCLC patients with a WHO performance status of 0-2, compared to a median OS of 1.2 months in patients with WHO 3-4[149], which is consistent with international

literature[142,150]. There is some evidence that laboratory parameters such as hyponatremia, leukocytosis and high levels of lactate dehydrogenase may affect outcome[142,150,151], but data is inconclusive, and these variables rarely influence treatment decisions.

Genetic and molecular characteristics

Unlike NSCLC where several key driver mutations have been identified, to date there are no known targetable genetic alterations in SCLC. Two mutations are almost universal, the loss of the tumour suppressor genes TP53 and RB1, but they are neither predictive, prognostic nor, so far, hold potential as therapeutic targets[152].

Recently however, four molecularly distinct subtypes of SCLC have been identified with differential response to treatment[153-155]. The two main subtypes, SCLC-A and SCLC-N, are defined by their predominant expression of the neuroendocrine transcription factors ASCL1 and NEUROD1, respectively. The third subtype, SCLC-P, expresses the non-neuroendocrine marker POU2F3, whereas the fourth type is lacking in all of the above markers, and is characterised by an inflammatory profile including the upregulation of cytokines and cytotoxic T-cells. Consequently, this is referred to as the inflamed subtype, SCLC-I [155]. In an exploratory subset of the IMpower-133 trial (see below), the combination of chemotherapy and the immune checkpoint inhibitor atezolizumab was associated with improved survival in patients with SCLC-I, as compared to the other subtypes[155], suggesting a predictive role in immunotherapy.

Treatment of SCLC

Chemotherapy

For several decades, treatment of SCLC has been based on chemo- and radiotherapy. The chemotherapy backbone normally consists of a platinum-compound, either cisplatin or carboplatin, in combination with a topoisomerase inhibitor, usually etoposide. The regimen is given in 3-weekly cycles, for 4-6 cycles. Despite being an aggressive and fast-growing disease, patients often respond quickly to initial therapy, with complete or partial responses achieved in 60% with ED-SCLC, illustrating the treatment-sensitive nature of the disease[156,157]. However, most patients will relapse and further therapeutic options are limited. Topotecan, another topoisomerase inhibitor, is the only approved drug for second-line treatment in SCLC. Re-challenge with carboplatin and etoposide is another possibility, particularly in patients with a treatment-free interval of at least 3 months, and this may be repeated as long as there is evidence of clinical benefit. Response rates in the second-line setting range between 15-30%, and are particularly poor in patients with a short treatment-free interval[141]. This rapid development of secondary resistance is a specific feature of SCLC, and one might wonder why this could be.

Highly proliferative cells are generally deemed more sensitive to therapy, which may explain the initial response to chemotherapy. Then why do we not see the same effects in second- or third-line settings? Multi-clonality is often put forward as an explanation of treatment resistance, providing survival advantage to clones with *e.g.* acquired mutations or more favourable phenotypes based on non-genetic mechanisms, including adaptive responses to hypoxia and metabolic stress[158-160]. Hopefully, further studies of genetic and epigenetic characteristics will provide more insight into this issue.

Radiotherapy

Radiotherapy is administered based on disease extent, response to systemic therapy and patient symptoms. Adding thoracic radiotherapy to chemotherapy improves local control and prolongs survival[161-163]. With modern dosing schedules and techniques, concurrent radiotherapy in LD is associated with 2-year survival rates of 56%[164], when given as 45 Gy in 30 twice-daily fractions, according to current international guidelines[165]. However, this recommendation is challenged by a Scandinavian randomised phase-II trial, demonstrating superior 2-year survival rates of 74.2% with accelerated, hyperfractionated radiotherapy administered as 60 Gy in 40 twice-daily fractions, compared to the standard dosing of 45 Gy in 30 fractions, twice-daily[166]. Toxicity was comparable between the arms, and this accelerated regimen is now being proposed in the Swedish guidelines for the treatment of LD-SCLC.

In patients with ED responding to systemic therapy, consolidating thoracic radiotherapy has been shown to increase PFS and improve 2-year survival rates from 3% to 13% as compared to patients receiving chemotherapy only[163], and may be an option in patients with a good performance status where the extrathoracic disease is under control.

Due to the high incidence of brain metastasis in SCLC, prophylactic cranial irradiation is recommended in patients with a favourable performance status who have responded to first-line therapy[165]. Prophylactic cranial irradiation reduces the incidence of brain metastasis and prolongs survival in both LD and ED[167,168] but it needs to be considered carefully due to the risk of neurocognitive toxicity, especially in the elderly[169]. As in other malignancies, radiotherapy is important in the palliative setting and may relieve symptoms caused by metastatic lesions for example in the brain and bones, or to reduce occlusions of large vessels or airways caused by bulky tumours.

Immunotherapy in SCLC

In oncology, immunotherapy refers to agents that activate the antitumour response of the host immune system. Evading immune surveillance is one of the hallmarks of cancer, and one mechanism whereby this is achieved is through the PD-1/PD-L1 pathway[170]. The binding of Programmed cell death-1 (PD-1) expressed on T cells,

to its ligand (PD-L1) found on the surface of tumour cells, can impede the cytotoxic T cell activity[171,172]. Inhibitory antibodies targeting PD-1 or PD-L1, often referred to as immune checkpoint inhibitors, have revolutionised medical oncology and have undoubtedly improved the care of lung cancer patients.

Still, in SCLC the role of immunotherapy has been uncertain, as trials in the second- or third-line settings have failed to show convincing effects on outcome in an unselected population[173-175]. Only recently, two large trials showed improved survival in patients with previously untreated extensive disease with the addition of atezolizumab (IMpower-133)[156] or durvalumab (CASPIAN)[157] to standard chemotherapy. The PD-L1 inhibitors prolonged median OS by 2.0 and 2.7 months in the IMpower-133 and CASPIAN trials respectively, and although the benefit is modest, atezolizumab and durvalumab are now considered to be part of the standard of care in patients with ED-SCLC. In contrast, a trial of the PD-1 inhibitor pembrolizumab in combination with first-line chemotherapy in ED demonstrated significantly improved PFS and prolonged duration of response[176], but as the study did not meet its pre-specified endpoint for OS, pembrolizumab is not yet recommended in this setting. The overall modest responses to immune checkpoint inhibitors have been unexpected considering the high tumour mutational burden (TMB) seen in SCLC[152,177], likely reflecting a high exposure to smoking-related carcinogens. TMB is a surrogate marker of neoantigen load and has been shown to predict response to immunotherapy, in *e.g.* NSCLC and malignant melanoma[178,179]. Still, in the IMpower-133 trial, response to atezolizumab was independent of TMB status[180]. Perhaps T cell infiltration is not as high as we think, or other immune checkpoints predominate.

Best supportive care

Despite current therapeutic improvements, a large group of patients will face symptomatic disease that significantly affects their quality of life. Furthermore, patients with a poor performance status may not be amenable to intense antitumour treatment. Best supportive care should always be considered when managing patients with SCLC, in particular patients with metastatic or relapsed disease. The American Society of Clinical Oncology recommends the early integration of palliative care in advanced cancer, preferably as an adjunct to active treatment[181].

What's in the pipeline?

Several new agents are under investigation for their use in SCLC, and other indications for immunotherapy are being explored. In 2020, lurbinectedin, an alkylating drug which selectively inhibits oncogenic transcription, was granted accelerated FDA approval as second-line therapy following the results of a phase-II trial, demonstrating objective response rates of 35%[182]. A subsequent phase-III trial of lurbinectedin combined with doxorubicin, compared to standard of care, did not meet its primary endpoint for OS[183], but numerous other studies are ongoing.

Lurbinectedin is not yet approved in Sweden, pending results from confirmatory studies.

Another target of interest is the Delta-like ligand-3 (DLL-3) which is an atypical Notch ligand highly expressed by neuroendocrine cancer cells. The antibody-drug conjugate rovalpituzumab teserine (Rova-T) targeting DLL-3 initially showed promising results[184], but two recent phase-III trials of Rova-T as second-line treatment or as maintenance following standard chemotherapy, were discontinued as patients receiving Rova-T experienced inferior OS and increased toxicity compared to the control arms[185,186]. Nonetheless, DLL3 remains an interesting target, and is subject to further drug developments.

Cardiovascular comorbidity in SCLC

Smoking is a major risk factor for developing SCLC, in fact only 2% of patients are never-smokers[187]. Smoking contributes to a range of other conditions, which is reflected by the high frequency of comorbidities in this patient group. In the late 1990's, the presence of one or more comorbidity was reported in 55% of SCLC patients[188]. In 2011-2012 this figure had risen to 76%, indicating that comorbidities are common and increasing. Cardiovascular and respiratory diseases are among the most prevalent, with cardiovascular disease (CVD) being reported in 40-48% of patients[189-191].

Comorbidity – a prognostic factor?

It is unclear to what extent comorbidities affect survival in lung cancer as some studies have reported negative associations with prognosis[188,190-192] whereas others have not [142,189,193-195]. A possible explanation for the discrepancies in outcome between the studies above is the use of different definitions and comorbidity scoring systems. For example, hypertension is classified as a CVD in the Simplified Comorbidity Score developed by Colinet et al[196], while the more commonly used Charlson Comorbidity Index[197] defines hypertension as a separate condition. What has been shown is that known comorbidities appear to predict the choice of anti-cancer treatment, suggesting an indirect effect on survival[188,189,198]. A Dutch study reported that LD-SCLC patients with CVD, hypertension or diabetes mellitus were less likely to receive thoracic radiotherapy[194]. Consistently, Ferris *et al.* found a higher incidence of prior cardiac events in patients receiving chemotherapy-only compared to patients receiving combined chemo- and radiotherapy[191], which indicates that physicians are influenced by co-existing disease when making treatment decisions.

The challenges of assessing cardiovascular disease

It is important to bear in mind that CVD encompasses a wide spectrum of conditions, with varying degrees of systemic impact. This highlights one of the

challenges in managing coexisting medical conditions, especially CVD which is particularly difficult to assess and grade in view of a recently diagnosed malignancy. Other organ systems, such as respiratory, renal and haematological, are easily monitored by spirometry and routine laboratory parameters, but cardiovascular (CV) status is rarely assessed as part of lung cancer investigations. Functional imaging of the heart is not prioritised in a patient with newly diagnosed SCLC where rapid initiation of treatment is crucial, and the value of monitoring established cardiac biomarkers such as troponin and brain natriuretic peptide (BNP) has not yet been determined[199].

Treatment-induced cardiotoxicity

Another aspect is that of the potentially cardiotoxic effects exerted by some of our more commonly used anticancer treatments, including thoracic radiotherapy, immunotherapy and certain chemotherapy agents. This is a growing concern in several types of cancer, such as breast cancer where there is an increasing population of long-term survivors having received anthracycline-based chemotherapy, sometimes combined with trastuzumab and radiotherapy, all of which can cause cardiac complications[200].

The main systemic therapies with cardiotoxic effects that are relevant to SCLC are cisplatin and immunotherapy[200]. Cisplatin mainly affects the vascular system, leading to arterial vascular disease and VTE, whereas myocarditis is the most common cardiac toxicity seen with immune checkpoint inhibitors, with a reported incidence of 0.27-1.14%[201]. Cases of pericarditis, vasculitis and takotsubo-like syndrome have also been described[200]. Other potentially cardiotoxic agents that may be used in later lines include doxorubicin, cyclophosphamide and paclitaxel, but the effects may not have time to develop due to the generally poor prognosis.

With regards to thoracic radiotherapy, limiting the radiation dose to the heart is essential to minimize the risk of subsequent cardiac toxicity[202]. Radiation activates acute inflammatory pathways eventually leading to chronic inflammation and fibrosis of cardiac structures. This can result in cardiomyopathy, pericardial disease, atherosclerosis and arrhythmias[200,202]. Interestingly, a large study of 7,060 SCLC patients reported baseline rates of cardiac events in 40% of the population. Adding thoracic radiotherapy to chemotherapy increased the absolute risk of further cardiac events at 5 years by 5% in all patients, and 10% in LD. The risk was significantly higher in patients with a prior history of cardiac events, with 1-year rates of 55% *vs* 28% in patients with and without baseline cardiac events, respectively[191]. The prediction and prevention of radiation-induced cardiotoxicity is currently addressed in several prospective lung cancer trials; NCT04305613, NCT03978377, and NCT03645317, but only the latter is enrolling patients with SCLC.

To summarise, cardiovascular disease is common and may affect treatment tolerability. CV status needs to be taken into consideration when managing patients with SCLC but comorbidity in general, and CVD in particular, may be challenging to assess. Hence, objective biomarkers could be a useful tool when making individual treatment decisions.

Rationale

This thesis is based on the RASTEN study, a randomised phase-III trial investigating the effects of enoxaparin, a low-molecular-weight heparin, in addition to standard therapy in patients with small cell lung cancer, with the purpose of prolonging survival. The rationale behind the trial is formed by the following key statements:

1. Venous thromboembolism is common in cancer and contributes to morbidity and mortality.
2. Cancer is associated with increased coagulation activation and several key coagulation factors contribute to tumour progression.
3. Anticoagulants have demonstrated tumour-inhibiting effects experimentally *in vitro* as well as *in vivo*, and several clinical trials have shown improved survival specifically in patients with small cell lung cancer, receiving prophylactic anticoagulants.

The role of anticoagulants as anti-cancer agents had been widely debated for decades, but the early clinical trials were either small or did not use standard chemotherapy, which prompted the design and initiation of RASTEN.

Aims

The overall aim of this thesis is to explore the role of coagulation activation in tumour development in small cell lung cancer, and to identify biomarkers that are prognostic or predictive of the development of venous thromboembolism and response to low-molecular-weight heparin.

The specific aims are:

Paper I — To investigate if the addition of LMWH to standard treatment improves survival in SCLC. The secondary outcomes include PFS, the incidence of VTE and haemorrhage.

Paper II — To objectively assess adherence to LMWH therapy using an experimental assay in conjunction with an established method for monitoring LMWH, and to determine if survival is improved in patients that are considered to be adherent.

Paper III — To identify SCLC patients with increased coagulation activity and investigate the correlation to survival and VTE. Ultimately, the objective is to define a subgroup of patients that benefit from prophylactic anticoagulants based on a hypercoagulable profile.

Paper IV — To explore the prognostic impact of circulating cardiovascular biomarkers in SCLC.

Patients and methods

The thesis is based on the RASTEN trial, reported in Paper I. The consecutive Papers (II-IV) use translational cohorts that stem from the clinical study population.

Clinical trial design and methodology

The gold standard when determining the efficacy of a new therapy is to perform a randomised controlled trial (RCT)[203]. In an RCT, patients are randomly allocated to one of two, or sometimes three, study groups, either the control arm encompassing the established standard therapy, or the intervention arm, receiving either a newly developed agent or a known treatment with a new indication. RCTs are always prospective trials, and the aim is either to show superiority or non-inferiority of the new therapy as compared to standard of care. The randomisation procedure is computerised, and patients are usually stratified by a few baseline characteristics to minimise systematic differences between the arms and limit bias by confounding effects[203].

Common outcome measures in oncological RCTs include overall survival (OS) and progression-free survival (PFS), but may also be organ-specific *e.g.* brain metastasis-free survival. In cancer types with a generally poor prognosis and limited treatment options after relapse, such as SCLC, OS may be more valuable than PFS. On the contrary, in studies focusing on localised disease where patients are treated with curative intent, PFS is often more informative. Apart from reporting efficacy of an intervention, safety and toxicity needs to be described in a clinical trial, to allow for the correct judgement of the potential benefits and harm of the therapy. Many trials also incorporate patient-reported outcomes such as quality of life, changes in symptom presentation and psychological effects, which are other important aspects[204].

The RASTEN trial

RASTEN is a *ra*ndomised phase-III trial of *st*andard treatment with or without the addition of *en*oxaparin, a low-molecular-weight heparin, in small cell lung cancer (ClinicalTrials.gov: NCT00717938). This international study was initiated and

conducted by the Swedish Lung Cancer Study Group, and enrolled patients at 23 different sites; 17 in Sweden, 5 in Canada and 1 in Denmark, between 2008-2016. The main study site was Skåne University Hospital, Lund, and funding was primarily academic with limited support from Sanofi-Aventis.

Patient selection and randomisation procedure

Patients with previously untreated SCLC of any stage were included if the following additional criteria were fulfilled: ≥ 18 years of age, WHO performance status 0-3, platelet count > 100 x 10^9/L and standard coagulation parameters within normal ranges. Key exclusion criteria were previous systemic chemotherapy, concomitant anticoagulant treatment except for acetylsalicylic acid or clopidogrel, active bleeding or high risk of clinically significant bleeding.

RASTEN is an open-labelled study where patients were randomised 1:1 between the control arm receiving standard treatment, and the intervention arm receiving enoxaparin in addition to standard therapy. The randomisation procedure was conducted at the Clinical Research Unit at Skåne University Hospital, Sweden, using a computerised algorithm, and patients were stratified according to study site, gender, age, disease stage and performance status.

Treatment

Standard systemic therapy included a platinum-compound and a topoisomerase inhibitor, administered every 3 weeks for 4-6 cycles. Radiotherapy was given according to local protocol, depending on disease extent and response to chemotherapy. Patients in the intervention arm received additional enoxaparin administered as subcutaneous injections at the dose of 1 mg/kg once daily. This is higher than the usual prophylactic dose set at 40 mg once daily, and slightly lower than the therapeutic dose of 1.5 mg/kg once daily. The injections were started on the first day of systemic therapy and continued until day 21 of the final chemotherapy cycle.

Outcome

RASTEN was designed as a superiority study with the primary aim to show improved overall survival with the addition of enoxaparin. OS was defined as the date of randomisation to the date of death from any cause. The secondary outcomes included progression-free survival, incidence of VTE and major haemorrhagic events.

Safety

In accordance with Good Clinical Practice guidelines, any adverse events that arose during the trial period were reported and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0, established by the National Cancer Institute, USA[205]. Based on the CTCAE classification, toxicity was

graded as mild (grade 1), moderate (grade 2), severe (grade 3), life-threatening (grade 4) or fatal (grade 5). The LMWH therapy was permanently stopped in case of major haemorrhage, intracranial haemorrhage, persistent thrombocytopenia or a VTE requiring therapeutic anticoagulation, or any other reason at the investigator's discretion.

Adherence to LMWH treatment

In order to monitor adherence, patients allocated to the enoxaparin arm were requested to register every injection in a diary, and to bring their empty syringe boxes to each appointment. Time and cause of any temporary disruptions were recorded. For further details regarding the study design of RASTEN, please see the Methods section of Paper I.

Statistical considerations: Paper I

Power calculations

When designing an RCT, a power calculation is required to estimate the sample size needed in the trial, in order to achieve the expected outcome with sufficient statistical power to be able to draw conclusions. With RASTEN, it was hypothesized that the addition of LMWH would improve OS with a hazard ratio of 0.75, corresponding to a median OS of 7.9 and 10.6 months in the control *vs* experimental arm, respectively, and an improvement in 1-year survival rates from 35% to 46%. Assuming exponentially distributed survival times, an accrual period of 78 months and an additional follow-up of 12 months, 195 patients per arm would be required to have 80% power to detect the expected increase in 1-year survival rate in the intervention arm at the alpha level 5% with a two-sided log rank test. Due to a slower accrual rate than initially expected, the study period was extended after approval from the local ethics committee and the Swedish Medical Products Agency.

All patients initiating the first treatment cycle were included in the statistical analysis according to intention to treat. Patients who withdrew from the study were included in the intention to treat analyses, and their follow-up times were censored at the date of withdrawal of consent.

Survival estimation

To estimate the primary outcome, OS, the Kaplan-Meier method was used with a log rank test to compare the two curves. Cox regression analysis was performed to quantify the effect of treatment on survival, reported as HR with 95% confidence interval (CI). A subsequent multivariable analysis was adjusted for stage, age, gender, performance status and study site. In addition, a subgroup analysis by disease extent was carried out.

Venous thromboembolism and haemorrhagic events

The cumulative incidence of first VTE was calculated per treatment group, using a standard method which accounts for death without VTE as a competing event. Haemorrhagic events were reported numerically but statistical testing was not possible due to the limited number of clinically relevant events.

Ethical considerations

The RASTEN trial was conducted according to ICH/GCP guidelines and in agreement with the Declaration of Helsinki. Approval was obtained from the Swedish Medical Products Agency (Läkemedelsverket) and the Regional Ethics Committee at Lund University, Sweden. All participants gave their written, informed consent, including a specific consent allowing for collection, storage and analysis of blood samples as part of the translational biobank. Questions regarding ethical considerations were discussed continuously within the study group.

Translational study designs and assay methods

During the clinical trial, blood samples were collected at three different time-points; at baseline prior to treatment start, before chemotherapy cycle 3, and at follow-up appointment 2 months after the end of treatment. The samples were stored as serum, EDTA- and citrate-plasma, in a -80°C freezer at the Clinical Research Unit, Skåne University Hospital Lund, Sweden, until use. This has enabled translational studies where plasma biomarkers have been correlated to clinical parameters such as OS, disease stage, the use of LMWH and VTE incidence.

Translational cohorts

Translational cohort I consists of all patients for which samples taken prior to chemotherapy cycle 3 were available. In total, 258 patients were included. The second translational cohort (Cohort II) was established at the cut-off date of November 1st 2013, and consists of the first 292 consecutively enrolled patients in the study. In paper III we took advantage of the serial blood collections and included plasma samples from all three time-points, *i.e.* at baseline, at cycle 3 and at 2-month follow-up. In paper IV we analysed plasma samples collected at baseline. Due to varying sample quality such as haemolysis and insufficient volume, and other assay-specific technical limitations, for each assay there was a small number of samples for which a result could not be obtained.

Table 2 outlines the assays used in the translational studies, which will be briefly discussed in the section below. For detailed descriptions of the methods, see the respective papers.

Table 2. Overview of the translational studies (Papers II-IV).

	Cohort	Plasma samples	Assays	Endpoints
Paper II Adherence study	Cohort I *N*=258	At cycle 3	Anti-FXa assay Heparin Red	Anti-FXa activity Heparin Red fluorescence, Enoxaparin concentration
Paper III Coagulation biomarker study	Cohort II *N*=242	At baseline, cycle 3, 2-month follow-up	Proximity extension assay Thrombin generation assay PPL assay EV-TF assay	TF TG-ETP, TG-peak,TG-ttPeak PPL EV-TF
Paper IV CV biomarker study	Cohort II *N*=252	At baseline	Immunoluminometric assay Proximity extension assay	ADM, ANP, Copeptin ADM, ST2

Anti-FXa = anti-factor Xa; PPL = procoagulant phopholipids; EV-TF = tissue factor associated with extracellular vesicles; TF = tissue factor; TG = thrombin generation; ETP = endogenous thrombin potential; ttPeak = time to peak; CV = cardiovascular; ADM = adrenomedullin; ANP = atrial natriuretic peptide; ST2 = suppression of tumorigenicity 2.

Assay methods: Paper II

Anti-factor Xa assay – gold standard for monitoring LMWH activity

In contrast to unfractionated heparin and vitamin K antagonists, which require continuous monitoring of global coagulation parameters to ensure that appropriate therapeutic levels are achieved, the efficacy of LMWH is not routinely measured in clinical practice. However, monitoring of LMWH may be valuable in selected patients where the anticoagulant effect is more unpredictable, *e.g.* in patients with extreme body weight, severe renal insufficiency or in pregnancy[18,206]. Assessment of anti-factor Xa activity is currently considered to be 'gold standard' and is available in most larger hospitals. In paper II we wanted to determine levels of LMWH in plasma as an objective measure of adherence to medication. For this we used the established anti-FXa assay in conjunction with the experimental Heparin Red assay (see below).

The anti-FXa assay is based on the inhibitory effects of LMWH, or heparin, on FXa. Excess FXa is added to the plasma sample, where LMWH accelerates the binding of endogenous antithrombin to the FXa reagent. The remaining FXa cleaves a chromogenic substrate, releasing a colour that can be quantified by a change in absorbance[207]. The level of absorbance is inversely proportional to the amount of LMWH, and the results are reported as the corresponding LMWH concentration. A limitation of the assay is that it only measures the anti-FXa activity without taking into account other anticoagulant effects exerted by LMWH, such as the inhibition of thrombin (FIIa), the release of TFPI and inhibition of other heparin-binding proteins[18,208]. This may be of importance as the relative contribution of FXa and FIIa

inhibition depends on the mean molecular weight of the heparin chain. The proportion of anti-FIIa activity is gradually reduced with shorter heparin fragments, while the FXa inhibition increases[209]. For example, enoxaparin, with the mean molecular mass of 4.2 kDa, is a stronger inhibitor of FXa, with a FXa/FIIa ratio of 3.9, compared to dalteparin which has a mean molecular weight of 6.0 kDa and a FXa/FIIa ratio of 2.5. For reference, unfractionated heparin has a FXa/FIIa ratio of 1, and a mean mass of 15 kDa[209]. Another potential limitation is that the assay is dependent on the availability of antithrombin in plasma[206], which may vary on an individual basis.

The Heparin Red assay - an experimental assessment of LMWH concentration

Heparin Red is a fluorescent probe assay developed to directly determine the plasma levels of heparin or its derivatives, independent of their anticoagulant activity. The fluorescent probe forms a supramolecular complex with its target heparin structure, which results in contact quenching of fluorescence (Figure 6)[210,211]. This generates an inverse relationship between Heparin Red fluorescence and concentrations of heparin in plasma.

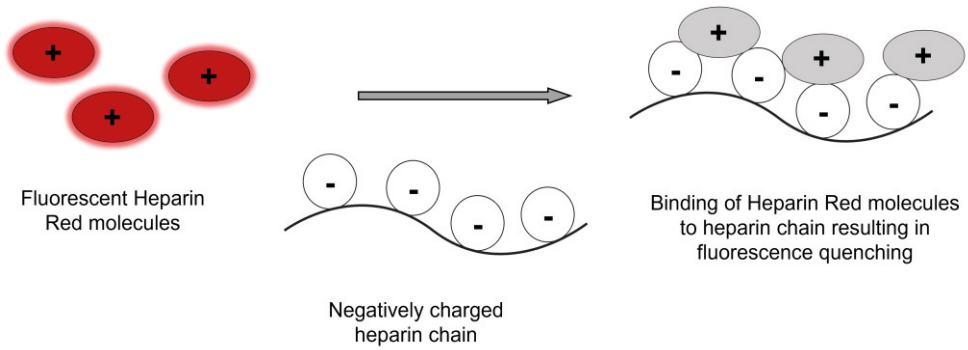


Figure 6: Heparin Red fluorescence quenching assay.
Heparin Red added to heparin-containing plasma will aggregate at the anionic heparin chains, resulting in quenching of fluorescence. The emitted fluorescence is detected by a fluorescence reader, and is inversely proportional to the heparin concentration. Author's own illustration, adapted from Warttinger et al; *Anal Bioanal Chem;* 2016[211].

When the adherence study was initiated, the Heparin Red assay had only been performed in heparin spiked plasma from healthy donors, and not in clinical samples[211]. Hence, the first step was to evaluate if Heparin Red could detect LMWH in patient samples and if this correlated to the anti-FXa assay. Secondly, the aim was to define adherence to enoxaparin based on anti-FXa and Heparin Red values and to correlate this to patient outcome.

The assay was established in-house using a 'research-use-only' Heparin Red® kit, provided by Redprobes UG, Münster, Germany. Due to the experimental nature of

the assay, the samples were analysed in two batches, 3 months apart; an initial batch consisting of the first 199 cases, which was later extended to include the remaining 59 participants. To limit technical bias, the assays were conducted by the same personnel each time. A standard curve was obtained using pooled plasma from healthy donors spiked with enoxaparin in the concentration range of 0-10 µg/ml. Of note, the standard curves were comparable between the two batches. Due to skewed distributions with negative concentrations, the mean fluorescence was used in the statistical analysis rather than the corresponding concentrations, unless otherwise stated.

Assay methods: Paper III

The aim of Paper III was to map various aspects of the coagulation profile in participants of the RASTEN trial and define a subgroup of patients with a high coagulation activity. The hypothesis was that hypercoagulability would correlate to an increased risk of VTE and reduced survival, and ultimately identify a group of patients that might benefit from prophylactic anticoagulants. To achieve this, four different coagulation parameters were assessed.

Proximity extension assay – Total tissue factor

As a key initiator of the coagulation cascade, TF has been identified as a major contributor of cancer-associated VTE and is associated with a poor prognosis in several types of cancer[24]. Therefore, TF was a highly interesting biomarker to study in the context of RASTEN.

Total TF was determined in plasma using a proximity extension assay (PEA) technology performed by Olink Bioscience, Uppsala, Sweden, as described by Assarsson *et al.*[212]. The assay is based on two oligonucleotide-labelled antibody probes binding to their specific target protein (Figure 7). When the antibody pair is within close proximity, the oligonucleotides will bind to each other and are further extended by DNA polymerase. This generates a protein-specific DNA template which is detected and quantified by real-time qPCR, and is proportional to the initial antigen concentration. The values are reported as normalised protein expression (NPX) in arbitrary units. The PEA method reduces cross-reactivity and provides a high specificity and sensitivity. The Proseek Multiplex Oncology 1-v2^{96x96} and CVD 1^{96x96} panels were used in our studies, generating 148 unique proteins relating to tumour biology, the hypoxic tumour microenvironment and CV.

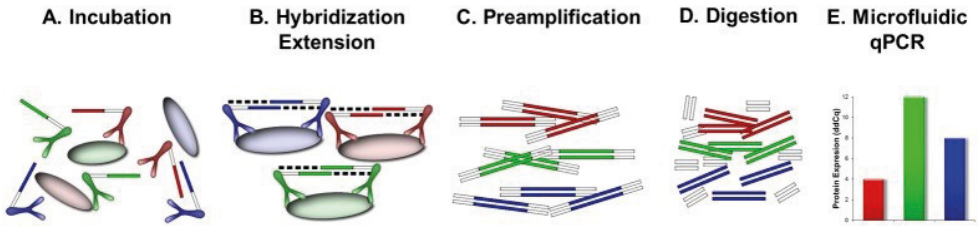


Figure 7: Proximity extension assay.
The assay is based on hybridisation and amplification of paired antibody probes, ultimately detected by real-time qPCR. Reproduced with permission under the Creative Commons Attribution License, originally published by Assarsson *et al*, *PLoS ONE*; 2014[212].

A limitation of the PEA is that it does not provide absolute concentrations, which makes it difficult to translate the findings into clinical practice. As such, PEA should rather be viewed as an exploratory analysis and may point out the direction for future biomarker studies. In addition, PEA does not distinguish between the different isoforms of TF that are present in the circulation.

Tissue factor activity associated with extracellular vesicles

As a complement to the assessment of total TF, we were interested in studying the more specific contribution of TF bound to extracellular vesicles, EV-TF. EV-TF activity was measured by an 'endpoint assay', as described previously[213]. Briefly, EVs are separated from plasma and pelleted by centrifugation, after which FVIIa, FX, and an anti-human TF antibody or a control antibody are added to trigger TF-independent and total activation of FXa, respectively. Ultimately, TF-dependent FXa generation was determined by subtracting the values obtained in the TF-antibody wells from values in control antibody wells. There is currently no standardised method for assessing EV-TF activity. Various factors can affect outcome, such as plasma preparation and type of assay, hence it may be difficult to make direct comparisons between studies[214]. The analyses in Paper III were performed by external collaborators with extensive knowledge within the field[42].

Thrombin generation assay

The generation of thrombin is a central step in the coagulation cascade as it directly leads to the activation of platelets and fibrin generation, resulting in final clot formation. The clotting time, *i.e.* the time it takes for a clot to form, can be measured as activated partial thromboplastin time (aPTT) or prothrombin time (PT). Both aPTT and PT are used in the routine monitoring of heparin and warfarin therapy, respectively, but none of the assays quantify the amount of thrombin formed. Instead, the preferred method for assessing thrombin generating capacity is by the calibrated automated thrombogram, referred to as the thrombin generation assay (TGA)[215,216]. TGA measures total and physiologically relevant amounts of thrombin[208]. The thrombogram yields a thrombin generation curve, of which the three main parameters include endogenous thrombin potential (TG-ETP), peak

height (TG-Peak), and time to peak (TG-ttPeak) (Figure 8). ETP is the most widely used parameter as it incorporates both the concentration of thrombin and the time it is active.

Of note, lag time, which corresponds to clotting time, is an early event in thrombin generation and precedes the main burst of thrombin[216], as illustrated in Figure 8. Hence, PT and aPTT are poor markers of thrombin generation.

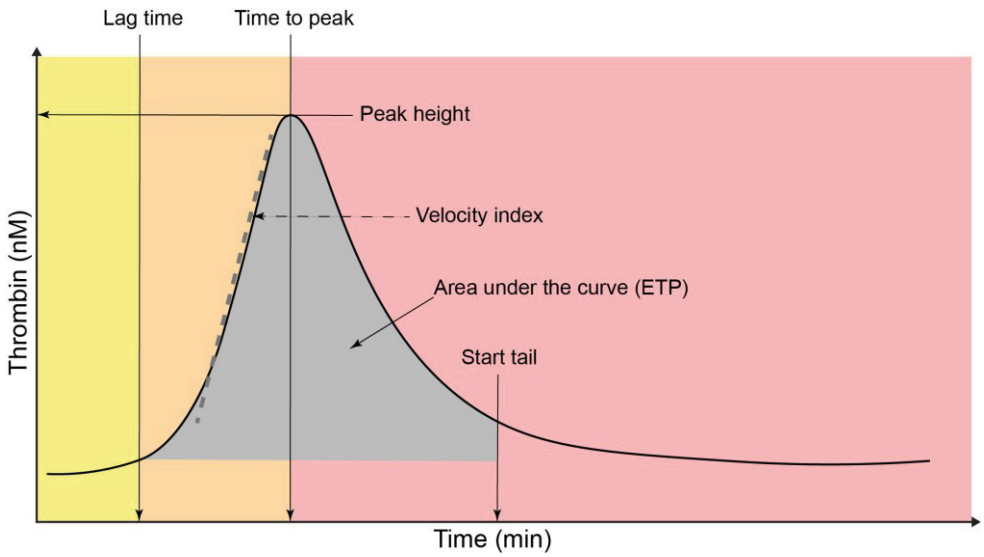


Figure 8: Thrombin generation curve obtained by calibrated automated thrombogram.
ETP = endogenous thrombin potential. Reproduced with permission under the Creative Commons Attribution License, originally published by Depasse et al, *J Thromb Haemost*; 2021 [217].

LMWH affects thrombin generation in two ways, indirectly through the inhibition of FXa and directly via the inhibition of thrombin. As such, ETP has been proposed as a superior method to monitor the effect of LMWH, compared to anti-FXa assays[206,208]. However, the purpose of Paper III was to assess the potentially predictive value of coagulation activity, hence the analysis at cycle 3 included patients in the control arm only, to limit LMWH interference.

Procoagulant phospholipid assay

In addition to expressing TF, EVs may also express procoagulant phospholipids (PPL) on the surface, mainly the anionic phosphatidyl serine[218]. Due to their negative charge, PPLs contribute to the procoagulant activity by assembling coagulation factors and promoting the formation of thrombin. PPLs are largely derived from platelets, but the role in haemostasis and thrombosis has been inconclusive[219], warranting further studies. Thus, the PPL assay was included in

Paper III to try to elucidate the potential contribution of PPL to overall hypercoagulability. The assay measures PPL clotting time, reported in seconds, using a method where FXa and calcium chloride are added to trigger PPL activity. Notably, a short clotting time indicates an increased PPL activity.

Assay methods: Paper IV

Cardiovascular status is not routinely assessed in patients with SCLC, despite a high frequency of cardiovascular disease in this patient group. In Paper IV we wanted to explore plasma biomarkers of CV stress in relation to survival in SCLC. Are CVD biomarkers raised in patients with a poor prognosis? What would the implications be? We examined the vasoactive peptides adrenomedullin (ADM), atrial natriuretic peptide (ANP) and copeptin, together with the FDA-approved cardiac failure biomarker, suppression of tumorigenicity 2 (ST2). Each biomarker will be described briefly below.

Adrenomedullin

ADM is present in most tissues in the body and is involved in numerous biological processes including the regulation of vascular tone, cell growth, hormone secretion and natriuresis[220]. ADM production is regulated by various factors including oxidative stress, inflammatory cytokines and hypoxia[69,220-222]. ADM is thought to exert a cardioprotective effect in conditions such as heart failure, hypertension and myocardial infarction[223] and reflects the neurohumoral activation, a hallmark of cardiac failure[224]. Apart from being a CV biomarker, there is evidence indicating that ADM is actively involved in malignant processes, particularly angiogenesis[225-228]. Overexpression of ADM has been reported in several malignant conditions, including pancreatic, colorectal, and renal cancer[225,229,230] and ADM has been correlated to tumour progression in neuroendocrine carcinomas of various origin[231].

Mature ADM has a short half-life in plasma, limiting its use in clinical settings. Thus, the stable peptide precursor midregional pro-ADM (MR-proADM) is generally preferred[232] and measured here using an immunoluminometric assay. In addition, ADM is also included in the proximity extension assay provided by Olink, allowing for comparisons between the assay methods.

Atrial natriuretic peptide

ANP is produced in the cardiac atria and exerts natriuretic, diuretic and vasodilator effects. ANP is a biomarker of myocardial stretch, and closely resembles BNP, a well-established marker of congestive heart failure. ANP seems to contribute to hyponatraemia in SCLC[233-235], but its role in malignancy appears otherwise to be protective rather than promoting[236-238]. As with ADM, the stable precursor midregional pro-ANP (MR-proANP) is the preferred target for analysis[239].

Copeptin – a surrogate marker for arginine vasopressin

Arginine vasopressin (AVP), also termed antidiuretic hormone (ADH), is produced by the hypothalamus and released from the posterior pituitary[240]. AVP is important in maintaining osmotic and CV homeostasis by inducing vasoconstriction and renal water retention in response to hypovolemia[241]. The syndrome of inappropriate ADH secretion (SIADH) is a common cause of hyponatremia in SCLC and is often attributed to ectopic secretion of vasopressin, but the prognostic value in SCLC is unclear[151,235,242]. Another uncertainty is whether AVP is actively involved in carcinogenesis. A small study of 34 patients with SCLC found increased levels of mature serum AVP to correlate to reduced survival[243]. In contrast, the synthetic AVP analogue [V^4Q^5] dDAVP has shown inhibitory effects on metastasis, tumour growth and angiogenesis in colorectal and breast cancer models[244,245].

Again, the short plasma half-life limits the use of AVP. Instead, the C-terminal fragment of the precursor molecule pre-provasopressin, called copeptin, has evolved as a stable and useful marker[240]. Copeptin has been shown to be a potent predictor of mortality in heart failure and has even been suggested as a superior marker in comparison to the established BNP[224,246,247].

Suppression of tumorigenicity 2

ST2 is a member of the interleukin 1 (IL-1) receptor superfamily, and more specifically, a receptor for IL-33[248]. The soluble isoform (sST2), which is measurable in plasma, is an FDA-approved biomarker in heart failure as it predicts mortality and morbidity in several CV conditions[224,248]. The membrane-bound isoform, ST2L, promotes an inflammatory response when bound to IL-33, and has been shown to exert a cardioprotective effect. On the contrary, sST2 may act as a decoy receptor and diminishes the inflammatory and cardioprotective effects of IL-33[249,250]. Whether ST2 contributes to tumour development is not clear as evidence is conflicting[251-253] and, to our knowledge, ST2 has not yet been studied in lung cancer patients. Here, the plasma levels of sST2 were determined by the Olink proximity extension assay.

Quantification of vasoactive peptides using an immunoluminometric assay

Absolute levels of stable fragments of the peptide precursors MR-proADM, MR-proANP and copeptin were measured in EDTA-plasma using a standardised, commercial immunoluminometric sandwich assay, as previously described (KRYPTOR, Thermo Fisher Scientific, Hennigsdorf/Berlin, Germany)[232,239,240]. In short, an antibody labelled with a luminescent tracer detects and binds a specific peptide sequence on the precursor molecule (Figure 9). The emitted light is measured by a luminometer and converted to absolute peptide concentrations as calculated from a calibration curve.

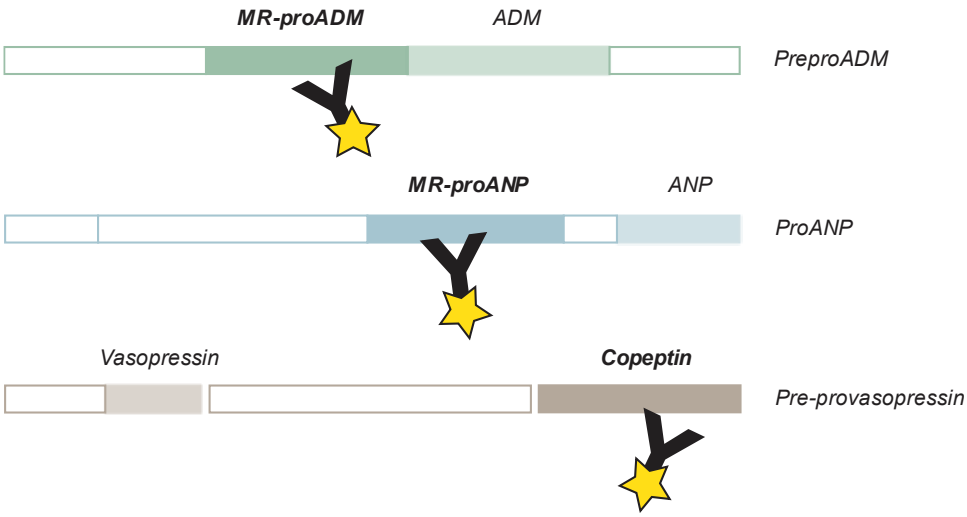


Figure 9. Precursor molecules of the vasoactive peptides targeted by the immunoassay.
An antibody labelled with a luminescent tracer (depicted by a star) detects stable fragments of the respective precursor molecules, for quantification via a luminometer. MR-proADM = midregional pro-adrenomedullin; MR-proANP = midregional pro-atrial natriuretic peptide. Adapted from Morgenthaler *et al*[232,239,240].

Copeptin levels can change rapidly in response to exercise and excessive fluid intake, reflecting its role in maintaining salt and water balance[240,254], but in general, the assays are considered robust.

Statistical considerations: Papers II-IV

General statistics

Non-parametric tests – comparisons and correlations

For biomarker correlations and comparisons between groups, non-parametric tests were performed. These are based on ranks rather than true values and should be used when normal distribution cannot be assumed, as was the case in the translational studies. Hence, Spearman rank correlation was employed, as well as the Mann-Whitney, Kruskal-Wallis and Wilcoxon tests for comparisons between different groups and time-points.

Survival estimation

Similarly to Paper I, survival in the translational studies was calculated using Kaplan-Meier curves and Cox regression. Since most of the studied biomarkers were exploratory, there were no predefined reference ranges for normal values.

Hence, each biomarker was split into groups based on tertiles or quartiles. Of note, the subgroups were defined in advance, to limit data-driven analysis. The following test groups were assessed:

Paper II: The control arm was compared to the adherent subgroup in the LMWH arm, as defined by anti-FXa activity and Heparin Red fluorescence. Patients receiving therapeutic LMWH due to a VTE diagnosis prior to cycle 3 were excluded from the survival analysis.

Paper III: The biomarkers were divided into tertiles (low/intermediate/high), except for EV-TF where the distribution was particularly skewed with a large proportion of values below or near the limit of detection. Therefore, EV-TF was dichotomised at the 75th percentile.

Paper IV: All biomarkers were split into quartiles. After having identified ST2 and MR-proADM as particularly strong predictors of mortality, a combined biomarker score, ranging between 2-8 points, was generated by adding the quartile scores 1-4 for ST2 and MR-proADM, respectively. A low combined score was defined as 2-5 points and a high combined score as 6-8 points.

Univariable Cox regression analysis was carried out in all studies. An additional multivariable model was used in Paper IV, adjusted for the recognised prognostic factors age, disease stage, gender, performance status, leukocytosis and hyponatremia.

Specific statistical analyses

Adherence calculations

In order to define patients that were considered to be adherent to LMWH therapy, cut-off values were determined for anti-FXa activity and Heparin Red mean fluorescence respectively. The values were obtained at the point maximising the product of sensitivity and specificity, and a Receiver Operating Characteristics (ROC) analysis was performed. The adherent and non-adherent patients of the LMWH arm were referred to as the $LMWH_{adh}$ and $LMWH_{non\text{-}adh}$ subgroups, respectively.

Predictive value of enoxaparin

One of the key questions in the coagulation paper, Paper III, was to examine if patients with hypercoagulability would derive a greater benefit from LMWH therapy. To assess the potential effect of enoxaparin on survival based on biomarker expression, a multivariable Cox model with enoxaparin as an interaction term was performed.

Prediction modelling – imputation and stepwise backwards linear regression

In Paper IV we were interested in investigating how the CV biomarkers compared to established prognostic factors. A prediction model was set up based on stepwise backward logistic regression with survival at 1 year as outcome. All experimental biomarkers were evaluated in this stepwise modelling procedure, as well as disease stage, age, performance status, gender, and routine laboratory parameters including haemoglobin levels, white blood cell count, platelet count, creatinine and sodium concentrations. Because of missing data for some of the candidate variables, 10 complete datasets were constructed using multiple imputation with chained equations. Linear regression imputation models were used for all variables with missing data, and the complete set of predictors evaluated in the study were used as predictors in these models.

A short note on the value of P

In medical literature, a *P*-value <0.05 is generally considered to be statistically significant. It indicates that the probability of obtaining the actual result is less than 5%, if the null hypothesis is true (*i.e.* if there is no difference)[203]. The lower the *P*-value, the lower is the probability that the results are obtained by chance, which means that we can trust the results to represent a true correlation. However, we need to bear in mind that a significant *P*-value does not necessarily reflect clinical relevance, and it should be interpreted with caution. A *P*-value of 0.06 is considered borderline significant. Why then, is a *P*-value of 0.04 not considered borderline insignificant? The use and misuse of the *P*-value is an ongoing debate among scientific statisticians[255,256] and the question of moving away from the significance threshold has been raised multiple times. Rather, it could be viewed as a continuous measure. Nevertheless, with regards to re-evaluating the importance of *P*-values, in this respect, we are no pioneers. The results in this thesis are reported as they are, with *P*-values ranging from high to low. And a value of 0.03 makes us significantly happier than a value of 0.06.

Confidence intervals (CI) are often more informative as they not only tell you if a value is significant, but also provides a range. For example, a 95% CI gives you the range within which you can expect to find the true value, in 95 out of 100 cases.

Results

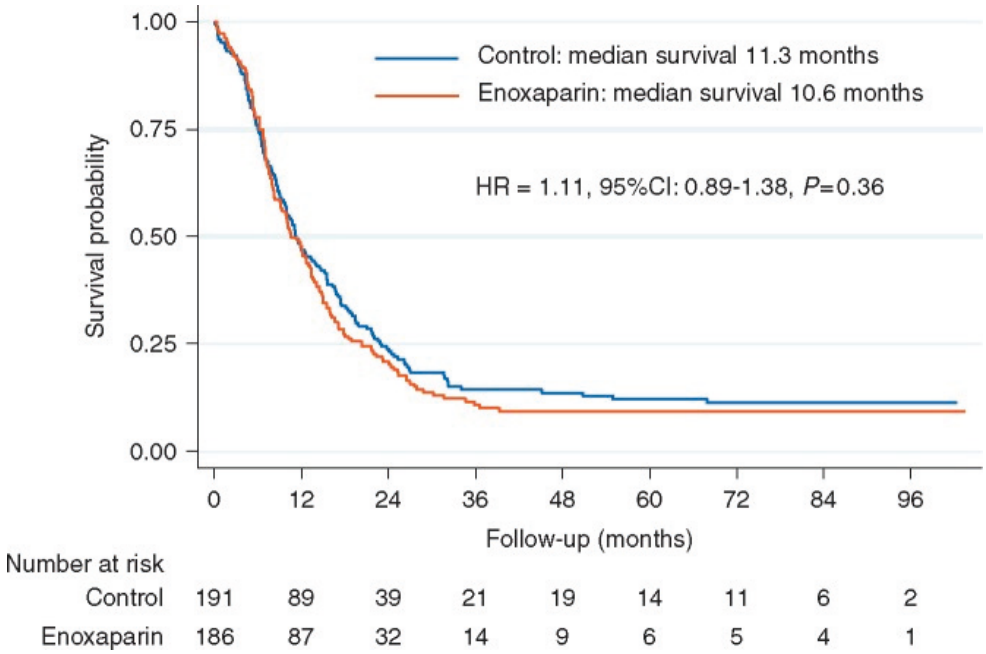
The main findings will be summarised in the following section. For a more detailed description of results, baseline characteristics of the study populations and supplementary material, see the original papers.

Paper I

Aim: To investigate if the addition of LMWH to standard treatment improves overall survival in SCLC. The secondary outcomes include progression-free survival, the incidence of VTE and haemorrhage.

In total, 377 patients were included in the final analysis, of which 186 were in the LMWH arm and 191 were in the control arm.

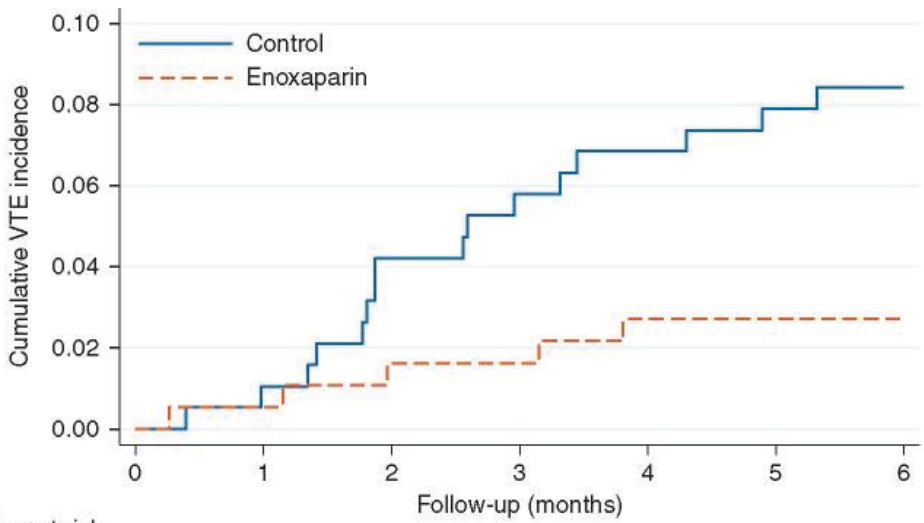
- Median OS was 10.6 months in the LMWH group and 11.3 months in the control group, with a HR of 1.11 (95% CI 0.89–1.38; *P*=0.36), as presented in Figure 10. Adjustment for age, gender, disease stage and performance status, and stratification for study centre yielded a HR of 1.14 (95% CI 0.91–1.45; *P*=0.26).
- Subgroup analysis did not reveal any improved survival based on disease stage, with HR 1.17 (95% CI 0.80–1.70; *P*=0.41) in limited disease and HR 1.07 (95% CI 0.82–1.40; *P*=0.63) in extensive disease.
- PFS was 5.8 and 6.9 months in the LMWH and control arms, respectively (HR 1.18; 95% CI 0.95–1.46; *P*=0.14).
- In the control arm, 16 patients (8.4%) developed a VTE, including two fatal pulmonary emboli. In comparison, 5 patients (2.7%) in the LMWH group developed VTE, none of which were fatal. The cumulative incidence of VTE at 6 months was 2.5% and 8.5% in the LMWH and control arms, respectively (HR 0.31; 95% CI 0.11–0.84; *P*=0.02), see Figure 11.



Number at risk

Control	191	89	39	21	19	14	11	6	2
Enoxaparin	186	87	32	14	9	6	5	4	1

Figure 10. Overall survival by treatment arm.



Number at risk

Control	191	180	169	165	157	141	131
Enoxaparin	186	178	170	166	159	151	139

Figure 11. Cumulative incidence of first venous thromboembolism by treatment arm.
VTE = venous thromboembolism.

- The rate of haemorrhagic events was higher in the LMWH group, but most were considered clinically non-relevant (CTCAE grades 1-2). Fatal pulmonary haemorrhages were reported in both study arms, three in the LMWH group and one in the control group.

In summary, the addition of the LMWH enoxaparin did not improve survival in SCLC, despite a significant reduction in VTE events.

Paper II

Aim: To objectively assess adherence to LMWH therapy using an experimental assay in conjunction with an established method for monitoring LMWH, and to determine if survival was improved in patients that were considered adherent.

Plasma samples collected prior to start of chemotherapy cycle 3 were available for 258 patients. Five patients had initiated therapeutic anticoagulation due to a diagnosis of VTE before cycle 3 and were only included in the assay validation analysis. The final adherence analysis included 253 patients; 125 and 128 in the LMWH and control arms, respectively.

- The presence of LMWH in plasma was detected and quantified by the Heparin Red assay, corresponding to enoxaparin concentrations of 0-8.1 μg/mL. The Heparin Red fluorescence strongly correlated to anti-FXa activity in an inverse relationship (Spearman's rho = -0.724; $P<0.001$).
- The distribution of anti-FXa activity and Heparin Red fluorescence in patients receiving prophylactic LMWH, therapeutic LMWH and patients in the control arm receiving no LMWH, revealed clear dose-response relationships with significant differences between the subgroups ($P<0.001$ by the Kruskal-Wallis test), as shown in Figure 12.
- To estimate adherence for the respective assays, ROC analyses were performed. For anti-FXa, the cut-off value of 0.082 identified the point maximising sensitivity (84.8%) and specificity (91.4%). Using this cut-off value, 106 (85%) patients were considered adherent and 19 (15%) were considered non-adherent in the LMWH arm.
- For the Heparin Red assay, the fluorescence cut-off value of 45,913 gave a sensitivity of 68.0% and specificity of 82.0%. Notably, this fluorescence value corresponded to the enoxaparin concentration of 0 μg/mL. Eighty-five (68%) patients were defined as adherent and 40 (32%) were defined as non-adherent, based on the Heparin Red assay.

- Survival analysis comparing the control arm to the adherent subgroup in the LMWH arm, did not reveal any differences in outcome, with HRs of 1.26 (95% CI: 0.95-1.67; $P = 0.105$) and 1.19 (95% CI: 0.89-1.60; $P = 0.248$), as defined by anti-FXa and Heparin Red, respectively (Figure 13).

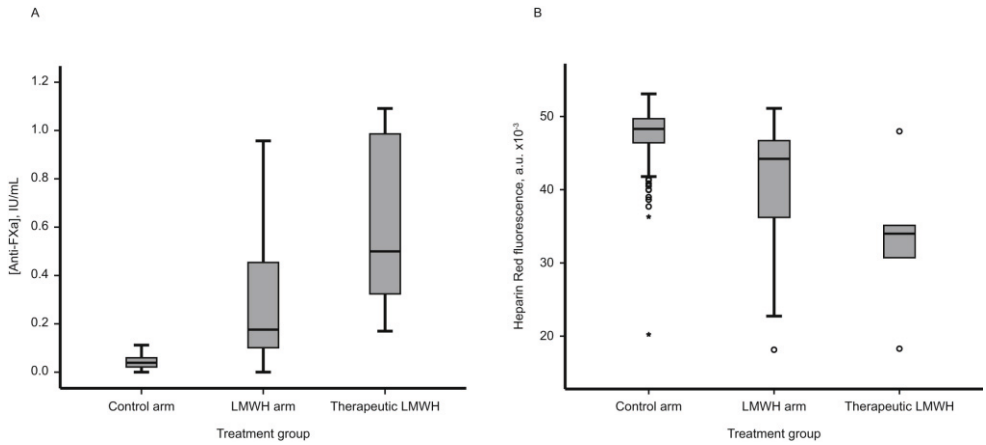


Figure 12. Dose-dependent distributions of anti-FXa activity and Heparin Red fluorescence.
Boxplots illustrating dose-response relationship of anti-FXa activity (A) and Heparin Red fluorescence (B) based on treatment groups: control arm (not receiving any LMWH, *N*=128), LMWH arm (receiving prophylactic LMWH, *N*=125), therapeutic LMWH (receiving therapeutic dosages of LMWH due to prior VTE, *N*=5). LMWH = low-molecular-weight heparin; anti-FXa = anti-factor Xa.

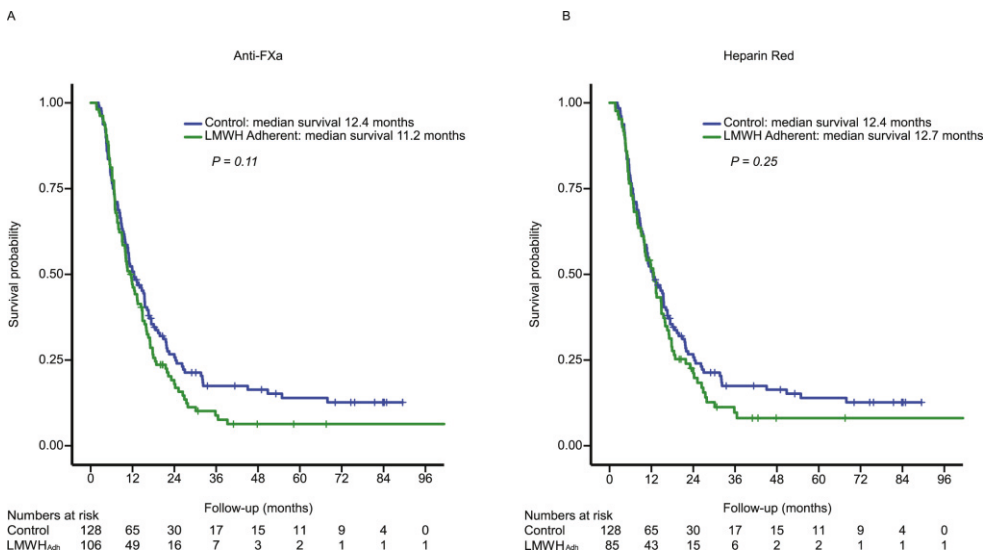


Figure 13. Overall survival in the control arm and the $LMWH_{adh}$ subgroup.
Adherence defined by anti-FXa activity (A) and Heparin Red fluorescence (B). LMWH = low-molecular-weight heparin; anti-FXa = anti-factor Xa.

To conclude, both the Heparin Red and the anti-FXa assays were able to provide an estimation of enoxaparin levels in patient samples and could potentially be used to assess adherence to LMWH. The adherence rates as defined by anti-FXa were considered acceptable, and the predictive value appeared higher than for Heparin Red. Survival was not improved in patients defined as adherent by either of the assays, thus we can conclude that the negative results of the RASTEN trial cannot be explained by inadequate adherence.

Paper III

Aim: To identify SCLC patients with increased coagulation activity and investigate the correlation to survival and VTE. Ultimately, the objective is to identify a subgroup of patients that benefit from prophylactic anticoagulants based on a hypercoagulable profile.

A total of 242 patients were included in any of the coagulation assays in Paper III, 127 and 115 in the control and LMWH arms, respectively. Twelve patients in the control arm and three in the LMWH arm developed a VTE during the study period.

- Baseline EV-TF, but none of the other biomarkers, was significantly higher in patients with ED as compared with LD ($P = 0.04$).
- A modest, but significant, increase in baseline TF was noted in control patients subsequently diagnosed with a VTE (P=0.03). None of the other biomarkers correlated to VTE incidence.
- High levels of TG-Peak were significantly associated with a reduced OS, particularly in patients with ED (HR 1.69; 95% CI 1.11–2.57; $P = 0.01$). For improved readability, Figure 14 shows Kaplan-Meier curves of 1-year survival based on TG-Peak. For OS curves, please see the original paper.
- With the exception of EV-TF, none of the other biomarkers showed a predictive value with regards to LMWH therapy. However, rather than predicting a positive response to anticoagulants, we found low levels of EV-TF at baseline to predict a reduced survival in patients receiving LMWH, compared to patients in the control arm (HR 1.42; 95% CI 1.04–1.95; $P = 0.03$).

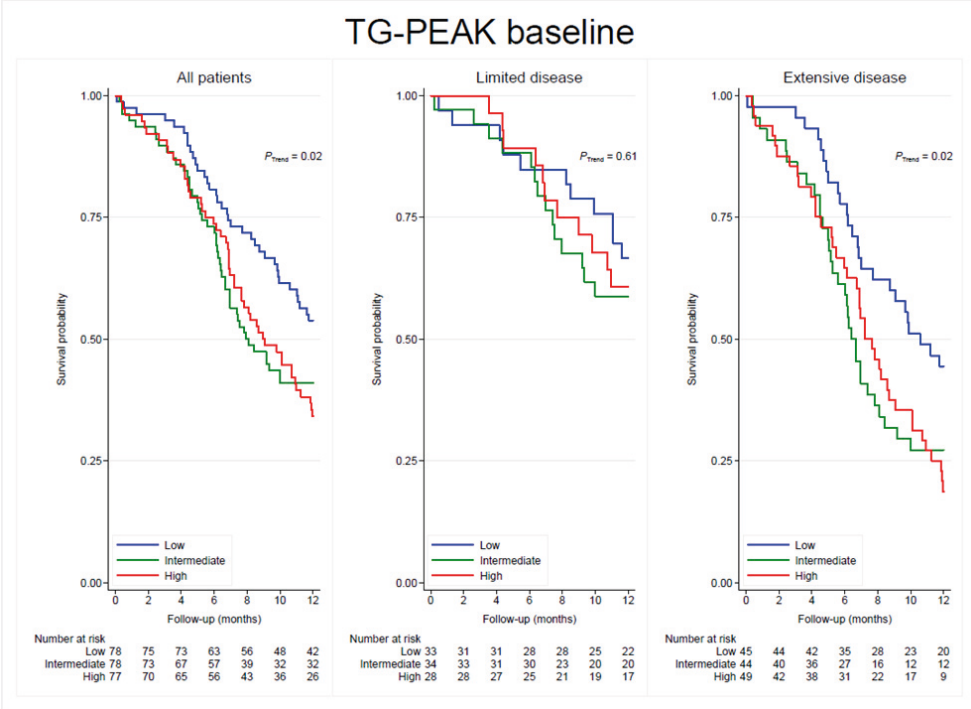


Figure 14. 1-year survival for all patients and by disease extent, based on TG-Peak values at baseline (tertiles). TG-Peak denotes the peak height of the thrombin generation curve obtained by the thrombin generation assay. TG = thrombin generation.

In all, we were not able to identify a distinct patient population with a particularly high coagulation activity. The results indicate a rather scattered picture, with total TF correlating to VTE risk, TF bound to EVs predicting a negative response to LMWH, and TG-Peak, but none of the other thrombin generation parameters, as a poor prognostic factor. Importantly though, this study supports the conclusion from the RASTEN trial, in that prophylactic LMWH should not be recommended in the general management of patients with SCLC.

Paper IV

Aim: To explore the prognostic impact of circulating biomarkers related to cardiovascular function in SCLC.

For this study, 252 patients were included in the final analysis, of which 104 patients had LD and 148 had ED at diagnosis.

- All measured biomarkers showed a correlation to survival in the unadjusted Cox regression analysis, particularly in patients with ED. The effect remained for all except MR-proANP in the multivariable model.
- Based on MR-proADM, median OS was 6.7 and 17.1 months in the quartiles with the highest and lowest MR-proADM levels, respectively (adjusted HR: 2.18; 95% CI 1.35-3.51; P=0.001). Subgroup analysis by disease extent gave an adjusted HR for OS of 3.49 (95% CI 1.84-6.60; P<0.001) in patients with ED.
- Similarly, comparison of the top and bottom quartiles based on ST2 yielded an adjusted HR for OS of 2.40 (95% CI 1.44-3.98; P=0.001) in the entire cohort, and 3.43 (95% CI 1.73-6.79; P<0.001) in ED patients.
- Prediction modelling incorporating the CV biomarkers as well as clinical factors and routine laboratory parameters identified three variables, MR-proADM, ST2 and tumour stage, as the main prognostic factors with respect to 1-year survival (P<0.01).
- Furthermore, combining the quartile scores for ST2 and MR-proADM revealed a significantly reduced median OS of 7.0 months compared to 14.9 months (P<0.001), in patients with a high *vs* low combined score, respectively. Notably, in LD, a low quartile score corresponded to a median OS of 22.9 months (N=75) compared to 9.8 months in patients with a high score (N=24) (unadjusted HR: 2.43; 95% CI 1.46-4.04; P=0.001). Accordingly, at two years of follow-up, 41% were alive in the LD low-scoring subgroup, compared to 8% in the LD high-scoring subgroup. Figure 15 illustrates the 1-year and OS curves based on the combined biomarker score.
- Neither MR-proADM nor ST2 were predictive of response to enoxaparin (P=0.44 and P=0.29, respectively), as assessed by multivariable regression model with enoxaparin as an interaction term.

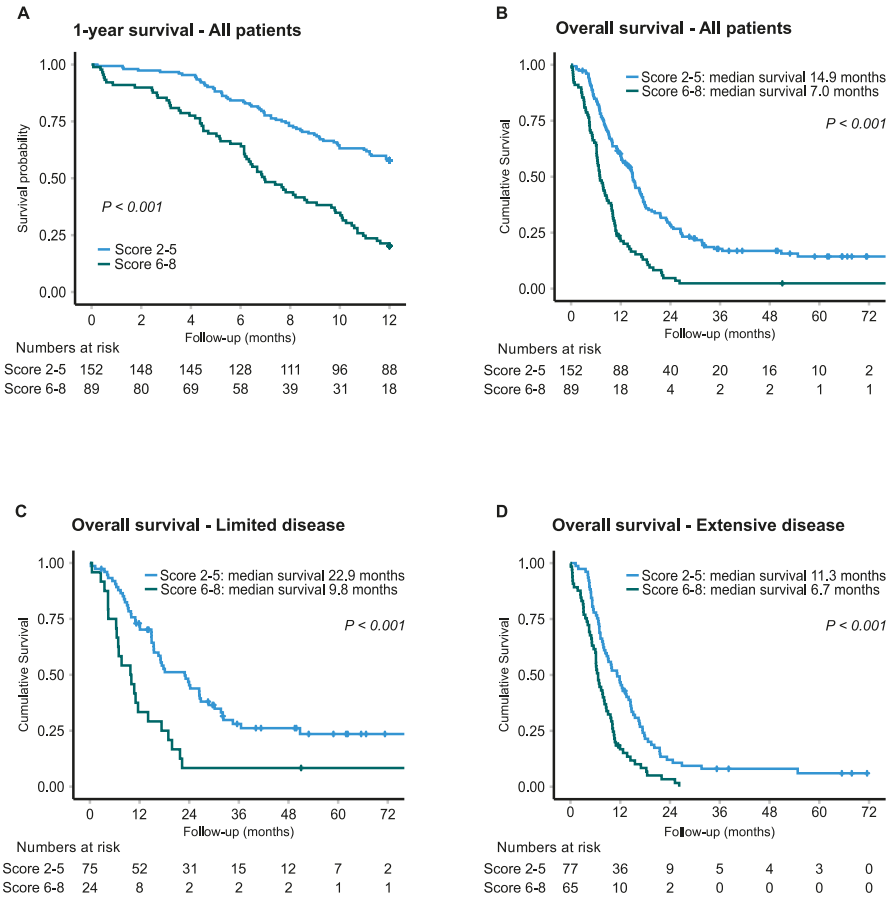


Figure 15. Kaplan-Meier analysis of survival by combined biomarker score of MR-proADM and ST2.
a) 1-year survival, all patients; b) overall survival, all patients; overall survival in c) limited and d) extensive disease.

To summarise, in Paper IV we demonstrate that biomarkers of CV function, ADM and ST2 in particular, strongly correlate to survival, independently of established prognostic factors.

Discussion

Papers I-III

Contrary to our hypothesis, RASTEN did not demonstrate an improved survival with the addition of enoxaparin. Our findings are supported by results from other, contemporary RCTs investigating the effects of prophylactic LMWH in cancer of the lung[134,135,257] and at other sites[136,258]. Yet, a negative trial does not necessarily have to be considered a failure, as long as the research question in itself is relevant. At the time when RASTEN was initiated, there was an apparent knowledge gap, reflected by the numerous other trials that were run in parallel. The question was widely debated, and anticoagulants were used on broad indications, assuming positive effects on survival. Hence, it may be argued that the results of RASTEN and the other trials, although negative, provide direct benefit to cancer patients by sparing them ineffective treatment with potential side-effects. Accordingly, the findings of the RASTEN study contributed to the "2019 international clinical practice guidelines for the treatment and prophylaxis of venous thromboembolism in patients with cancer", published by the International Initiative on Thrombosis and Cancer (ITAC) advisory panel[259].

Nonetheless, we do need to ask ourselves the critical question - why? Why did RASTEN fail to show a survival benefit? Let us start by revisiting the key statements forming the rationale behind the trial, and keep these in mind during the discussion:

1. VTE is common in cancer and contributes to morbidity and mortality.
2. Cancer is associated with increased coagulation activation and several key coagulation factors contribute to tumour progression.
3. Anticoagulants have demonstrated tumour-inhibiting effects experimentally *in vitro* as well as *in vivo*, and several clinical trials have shown improved survival specifically in patients with SCLC, receiving prophylactic anticoagulants.

Is venous thromboembolism less fatal than we previously thought?

Despite a significant reduction of VTE events by LMWH, RASTEN did not show an overall survival benefit. This is consistent with results from the previously mentioned trials by Macbeth *et al.*[134] and Pelzer *et al.*[136], both demonstrating a reduced VTE risk but no effect on survival, which raises the question to what extent VTE affects mortality. It is conceivable that we today are able to detect and treat thromboembolism at an earlier stage than previously, thereby preventing fatal pulmonary emboli. Our diagnostic tools have certainly improved in comparison to the clinical eye of Trousseau, and a significant part of all pulmonary emboli are incidental findings on routine computerised tomography scans[260,261], which are widely used today. It may also be argued that in clinical trials, such as RASTEN that have VTE as an important end-point, surveillance of early VTE-associated symptoms is increased, resulting in even earlier diagnosis and intervention. This illustrates the potential negative bias of a trial cohort as opposed to retrospective population-based cohorts in the reflection of the real-world setting.

Another point to consider is that some of the cancer types with the highest rates of VTE, such pancreatic, gastric, brain or lung cancer[262] are associated with a particularly poor prognosis due to tumour aggressiveness *per se*. It is plausible that the invasive tumour behaviour overrides the potential effect on mortality caused by VTE, which thereby contributes to morbidity rather than mortality. Perhaps VTE should be regarded as a marker of aggressive disease, rather than a cause of increased mortality. Prophylactic LMWH should then be considered in the context of morbidity and improved quality of life rather than survival (see below).

Tumour-promoting coagulation factors – is it a myth?

In Paper III, we focused on what we perceived to be the principal coagulation factors involved in carcinogenesis, namely TF and thrombin. Surprisingly, only one of the thrombin generation parameters, TG-Peak, showed any correlation to survival. In other words, we were not able to demonstrate that high levels of total TF, EV-associated TF, or an overall hypercoagulable profile in plasma corresponded to worse outcome. This suggests that the involvement of key coagulation factors in the development and progression of SCLC is not as prominent as we expected. In comparison, Thaler *et al.*[45] found circulating EV-TF to correlate to mortality in pancreatic cancer, but the relationship was not seen with gastric, brain or colorectal cancer. This may indicate that the contribution of coagulation factors in tumour biology varies depending on cancer type. Still, in pancreatic cancer, which is extensively studied with regards to the high incidence of VTE and expression of TF, anticoagulants have not been proved to prolong survival[136].

Does this imply that coagulation factors are not involved in carcinogenesis? This is a rather provocative question considering the extensive amount of research in the

field, and the answer may be of a more speculative nature. Most of the mechanistic studies demonstrating tumour-promoting effects exerted by coagulation factors, have been conducted *in vitro* or *in vivo*[125]. Importantly, these studies have addressed the role of TF and other coagulation factors expressed by cancer cells in the tumour microenvironment, as opposed to our studies that explored circulating levels in patient plasma. Accordingly, a large body of studies have shown that increased expression of TF in patient tumours correlates with worse prognosis in several cancers, including in breast, lung, and pancreatic cancer[37,263,264]. Hence, the controlled laboratory assessment of plasma coagulant activity may not reflect the complexity and dynamics of coagulation-dependent events in the tumour ecosystem. Future studies should address whether intratumoral coagulation activity may correlate with patient survival and VTE risk in SCLC. Nevertheless, mouse models as compared to humans may exhibit enhanced signalling pathways that would otherwise have been inhibited by negative feedback loops, normally triggered by activated coagulation proteases. Indeed, TFPI has been shown to exert antiangiogenic and antimetastatic effects in preclinical studies[265], and a limitation of our study was that it did not assess the contributions of TFPI and other endogenous inhibitors.

Why did enoxaparin not improve survival?

Assuming that VTE and coagulation activation are important in tumour biology, what are the possible reasons for the lack of survival benefit with LMWH? In an attempt to answer this, the following discussion points are divided into subsections covering aspects of administration, distribution, target, action and agents.

Administration - Was enoxaparin administered correctly?

Could the negative results of RASTEN, and possibly other RCTs with LMWH, be explained by inadequate adherence? That is what we set out to answer in Paper II. Adherence is challenging to assess, but it deserves our attention in clinical trials as well as in the real-world setting, especially in the case of self-injected agents. Adherence is often defined as the extent to which patients take the medication as prescribed by their health care provider, and can be influenced by numerous factors, such as patients' perception of the disease or potential benefit of the treatment, side-effects of the medication and the presence of psychological or cognitive impairment[266]. Patient-reports, diaries and counting syringe boxes, as in RASTEN, are simple ways to measure adherence, but can easily be altered and provide no guarantee that the medication has indeed been administered as prescribed[266]. Therefore, we were interested in exploring adherence to enoxaparin by objectively assessing plasma levels of LMWH using the established anti-FXa assay, in conjunction with the experimental Heparin Red assay.

Adherence rates in the RASTEN cohort were considered adequate, particularly based on the anti-FXa measurements, and in line with previous literature where rates of ≥80% are generally regarded as acceptable[267]. Importantly, survival was not improved in the subgroup defined as adherent. Thus, it is unlikely that the negative results of RASTEN were due to inadequate adherence, or that outcome would have been improved with perfect adherence rates.

A drawback of our study is the lack of information regarding the timing of the enoxaparin injections and the collection of blood samples. Thus, the assays provide a snapshot of the LMWH levels, but cannot inform us of long-term adherence. There have been reports of the so-called 'white-coat adherence' phenomenon[268,269], where patients improve their medication-taking behaviour during the days preceding a health care visit. If this was the case in RASTEN, the estimated adherence might be overrated. On the contrary, the half-life of enoxaparin is relatively short, which may underestimate the adherence rates. When preparing for the coagulation study (Paper III), the question of adherence had not yet been raised. Since enoxaparin interferes with the thrombin generation assay performed in Paper III, patients in the LMWH arm were excluded from analysis at cycle 3. However, it has been proposed that endogenous thrombin potential is superior in monitoring LMWH compared to anti-FXa activity[208], and in hindsight, it might have been of interest to perform the TGA in the LMWH patients as well, as part of the adherence study to further validate the assay methods.

Distribution – Did enoxaparin reach its target?

Aggressive tumours are characterised by hypoxia, which develops as the cancer cells outgrow their blood supply. Hypoxia is associated with increased resistance to treatment[70], partly explained by reduced vascularisation leading to impaired distribution of the therapeutic agents. Notably, hypoxia has been shown to induce TF expression, thus linking hypercoagulability to the hypoxic tumour niche[44]. Hence, it is possible that enoxaparin, a highly charged macromolecule, did not reach the most procoagulant and invasive tumour cells, due to poor circulation and limited distribution within the hypoxic tumour niche. In contrast, in animal studies investigating tumour-inhibiting effects of anticoagulants, LMWH is often injected at a time when cancer cells are known to be present in the circulation, making them more vulnerable to the effects of heparin as compared to later stages when the cancer cells have homed into a specific site and are less exposed to LMWH[125]. Following this line of thought, the ideal time for prophylactic anticoagulants would be at the time of metastatic seeding, which is, of course, impossible to foresee, unless we could be guided by detection of *e.g.* circulating tumour cells (CTCs) or ctDNA in plasma? Notably, experimental studies have linked increased expression of TF in CTCs to epithelial-mesenchymal transition and enhanced colonising potential in the lungs. This was supported by the identification of CTCs overexpressing TF in the

blood of metastatic breast cancer patients[270]. Could it even be possible that TF-expressing CTCs may act as embolus for distant VTE formation?

Target – Was the target correct?

The anticoagulant effects of LMWH are mainly exerted through the ability to directly inhibit FXa and thrombin. In contrast, TF, the main initiator of the coagulation cascade, which is put forward as a principal tumour-promoting coagulation factor, is indirectly inhibited via the release of TFPI (Figure 16). As described in the introduction, PAR-signalling is another important aspect of TF-dependent coagulation activation, and it may be argued that coagulation-induced tumorigenesis is primarily linked to TF-mediated PAR activation that occurs up-stream of FXa and thrombin generation. Specifically, the TF/FVIIa initiator complex and the TF/FVIIa/FXa tertiary complex have been shown to trigger PAR signalling, promoting metastasis and angiogenesis. Hence, direct targeting of TF may be a more successful approach, as supported by recent antibody-drug-conjugate trials, leading to the accelerated approval in the USA for the treatment of recurrent or metastatic cervical cancer[50,271].

Action – Was the action correct?

It is possible that other factors relating to the administration of LMWH may have influenced the outcome, such as the choice of dosing. There is some evidence indicating that the actions of heparin may be dose-dependent, as relatively low heparin concentrations have been found to bind and stabilise growth factor ligands, such as FGF, to potentiate their functional activity, whereas a higher concentration may compete with ligand interactions and inhibit down-stream functional effects on, *e.g.* angiogenesis and metastasis[272]. Thus, according to these findings, heparin has a narrow therapeutic window with regards to anti-cancer activity.

Consistently, in RASTEN, patients in the intervention arm received enoxaparin at the supraprophylactic dose of 1 mg/kg once daily, with the aim to optimise the tumour-inhibiting effect whilst limiting the risk of major haemorrhage. Three patients in the LMWH arm developed fatal pulmonary haemorrhages, compared to one patient in the control arm, illustrating the problematic toxicity profile seen with higher dosages. Intriguingly, there were two events of fatal pulmonary emboli in the control arm but none in the intervention arm. Hence, it could be argued that LMWH may have prevented two fatal thrombotic events whilst causing two fatal haemorrhages.

Agents – Would other anticoagulants have achieved different results?

The affinity for blocking thrombin in relation to FXa varies with the different LMWH compounds as it is dependent on the size of the heparin fragments. As antithrombotic agents, the compounds are considered comparable[18], but it is conceivable that the anti-metastatic and anti-angiogenic potentials differ between

the drugs. On the other hand, various clinical trials have studied the effects of dalteparin as well as tinzaparin and nadroparin amongst others, without demonstrating any clear benefit of one specific agent[134,135,258]. Thus, it seems unlikely that we would have achieved dramatically different results if we had chosen an alternative LMWH.

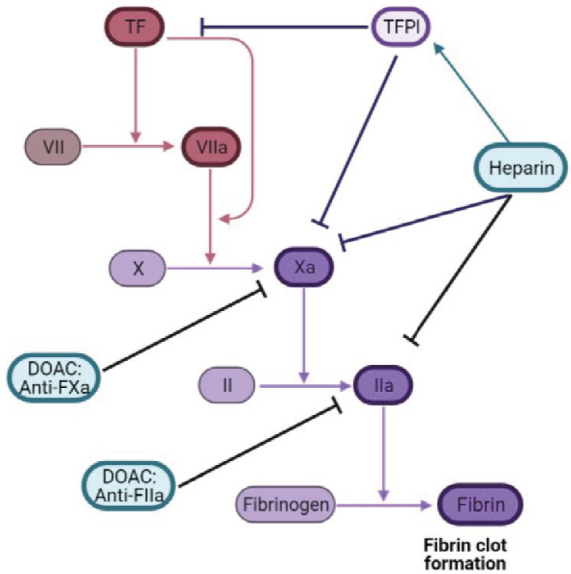


Figure 16: Targets of anticoagulants
A schematic summary of the coagulation factors targeted by commonly used anticoagulants. Heparin and low-molecular-weight heparins target FXa and thrombin directly and TF indirectly via the release of TFPI. DOACs target either FXa or thrombin. TF = tissue factor; TFPI = tissue factor pathway inhibitor; DOAC = direct oral anticoagulant; FIIa = thrombin. Created with Biorender.com.

As DOACs are emerging as an alternative to LMWH for the treatment and prevention of cancer-associated VTE, it is reasonable to consider the potential role of DOACs as antitumoral agents. DOACs are small molecule inhibitors targeting either FIIa (thrombin) or FXa (Figure 16). It is plausible that the direct FXa inhibitors have better chances of attenuating cancer growth compared to FIIa inhibitors, as they may target the TF/FVIIa/FXa complex, affecting downstream coagulation as well as PAR-mediated signalling[273]. So far, however, results from preclinical studies have been inconclusive[274]. Both pro- and anticarcinogenic effects have been demonstrated and the outcome appears to be dependent on the type of animal model and timing of drug administration, with respect to cancer cell inoculation[274].

In line with this, in two recent RCTs investigating the safety and efficacy of direct anti-FXa inhibitors as thromboprophylaxis in cancer patients, with intermediate-high risk of VTE based on the Khorana score, mortality was reported as secondary outcome[275,276]. The VTE rates were significantly reduced with active thromboprophylaxis, but there were no differences in survival when comparing patients with and without anticoagulants. In other words, patients considered at high risk of VTE based on clinical parameters, did not benefit from DOACs with regards to mortality. Patients were not stratified by markers of coagulation activity, but overall, the results are discouraging, and to my knowledge there are no ongoing trials investigating the effects of DOACs on survival as the primary endpoint.

Why did previous studies show positive effects on survival?

The three main trials demonstrating survival benefits with anticoagulants were conducted in the late 1970's-1990's[127-129]. This was before a consensus was reached regarding the combination of platinum compounds and topoisomerase inhibitors as the recommended first-line regimen in SCLC. Thus, the patients in the trials received a range of various therapies. The regimens were comprised of three, or even four, cytotoxic agents, including cyclophosphamide, anthracyclines or vinca alkaloids. Such chemotherapy agents are associated with a high degree of general toxicity, but more specifically, the risk of cardiac toxicity is particularly increased with cyclophosphamide and anthracyclines[200].

This could imply two things. Firstly, it is possible that patients in the early trials were at higher risk of developing CV complications, which were prevented by the prophylactic anticoagulants. On the other hand, it seems unlikely that the contribution of CV events on mortality can fully explain the survival benefit seen with anticoagulants. Secondly, it is conceivable that the discrepancy in outcome between the early, positive studies and the more recent, negative studies, is due to a general improvement in the management of SCLC, with more favourable chemotherapy combinations, improved radiographic monitoring and, as previously mentioned, earlier detection of VTE.

To further emphasise this point, the response rates in the trial by Altinbas *et al.*[129] were remarkably low. Patients receiving chemotherapy alone exhibited an objective response rate of 42.5%, and in ED-patients as little as 13% achieved a partial or complete response. In comparison, the objective response rates in patients with ED receiving platinum and etoposide range between 58-64% in large, modern trials[156,157,176]. So, even if major improvements have been lacking, there may have been small steps forward into the care of SCLC.

Finally, the SCLC cohorts in some of the early trials were small, with *N*=50 and *N*=84 in the trials by Zacharski and Altinbas *et al.*[127,129], respectively, which may have affected the statistical power and generalisability of the results.

Could there still be a role for prophylactic anticoagulants?

The results of this thesis provide strong evidence against the general use of prophylactic anticoagulants with the purpose of prolonging survival in cancer. Indeed, in view of the recent trials, RASTEN included, current international guidelines clearly state that anticoagulants should not be used in the absence of an established VTE to improve survival[19]. Still, we must not forget that a significant part of cancer patients will develop VTE, which may be prevented by anticoagulants. Thus, current guidelines recommend the use of thromboprophylaxis in high-risk patients, as defined by the Khorana score. However, the Khorana VTE risk score could be improved, as less than 25% of patients developing VTE are identified as high-risk, and the predictive value has not been confirmed in patients with lung cancer[22,277]. Therefore, it is still justified to continue the efforts to optimise the prediction models.

For this purpose, EV-TF has received attention due to its association to VTE in cancer patients[46,278], although other studies have not been able to demonstrate a correlation[45,279]. In a phase-II trial, cancer patients were stratified based on their levels of EV-TF and those with high levels were randomised to receiving prophylactic enoxaparin or no prophylaxis. Although not statistically confirmed, the study found higher rates of VTE in the control group, whereas enoxaparin reduced the thromboembolic events to the magnitude of patients with low levels of EV-TF[280]. The study was not accurately powered to detect differences in mortality.

Consistently, in Paper III we were hoping to find high coagulation activity to predict a beneficial response to LMWH, but as previously described, the results were not clear-cut. Indeed, rather than enoxaparin prolonging survival in patients with a hypercoagulable profile, we found low coagulation activity to be a negative predictor of response to LMWH. This supports the notion that prophylactic LMWH should not be recommended in the general management of patients with SCLC.

Paper IV

What are the implications of cardiovascular biomarkers in SCLC?

The results of Paper IV show that the cardiovascular biomarkers, ADM and ST2 in particular, strongly correlate to survival in SCLC, which raises a number of questions. What are the implications of these findings? What do the biomarkers represent, and how could it affect management? What follow-up studies should be performed?

What do the biomarkers represent?

A major drawback in our study is the lack of information regarding CV status, as this was not reported as part of the clinical trial. Because of this, correlations between measured biomarkers and known CVD were not possible. Therefore, we cannot conclude that the increased mortality in patients with raised biomarker levels is due to the presence of CVD.

If we presume that the biomarkers are cardiac-derived, do the results imply that patients with high levels die due to CV comorbidity? CVD is reported as the cause of death in six patients in the cohort, with the corresponding combined biomarker scores ranging between 2-8 points, making such conclusions difficult to draw. Another possibility is that mortality is increased because of poor tolerance to therapy, leading to suboptimal management. Further, it is conceivable that the oncological therapy unmasks or potentiates a pre-existing CV vulnerability.

If, on the other hand, the biomarkers are tumour-derived, do they merely reflect a particularly aggressive phenotype, or are they actively involved in tumorigenesis? Would that suggest their potential use as therapeutic targets? Pre-clinical evidence suggests that ADM, as a vasoactive peptide, contributes to angiogenesis and tumour growth[222,225]. In addition, as ADM is induced by hypoxia it is plausible that it can be expressed by cells in the hypoxic tumour microenvironment. The role of ST2 in cancer is, so far, inconclusive as there is evidence of both pro- and anti-tumoral effects[251-253].

Acknowledging that plasma ADM and ST2 can be raised in other conditions such as sepsis, endocrine and respiratory disease[220,249], we cannot rule out that the biomarkers are influenced by a third, unknown factor. Furthermore, it would have been valuable to correlate biomarker levels to smoking status, as there is some evidence that both ADM and ST2 may be up-regulated by cigarette smoke[281,282], representing a possible confounder. Overall, additional, longitudinal analyses of ADM and ST2 in the RASTEN cohort, *i.e.* at treatment cycle 3 and at 2 month follow-up, as well as assessment of tumoral ADM and ST2 expression, could bring increased insight into some of these questions.

How does this affect management of SCLC patients?

Based on circulating levels of ADM and ST2, we have been able to identify patients with a favourable *vs* a poor prognosis, independent of established prognostic factors. How can we use this information? Do we need yet another prognostic indicator? The implications depend on what the biomarkers represent.

If they reflect a subclinical vulnerability, this may imply that patients with high biomarker levels are prone to developing CV toxicity and should be managed conservatively, with modified chemo- and radiotherapy to avoid severe cardiac events, whereas patients with low levels are more likely to tolerate ambitious regimens. Perhaps optimisation of CV status could help improve outcome in the high-scoring patients. Yet again, if the biomarkers represent an aggressive disease, it could indicate that this patient group has more to gain from intense treatment, possibly involving immunotherapy.

Concluding remarks

In contrast to other organ systems, such as respiratory, haematological, renal and hepatic, which are routinely monitored as part of lung cancer management, CV status is rarely assessed, despite a high prevalence of CV comorbidity, especially in SCLC patients. Regardless of whether ADM and ST2 reflect cardiovascular stress or not, it is highly justified to pay more attention to aspects of the cardiovascular status in SCLC, potentially allowing for improved, more individualised treatment decisions.

Strengths and limitations

The strengths and limitations are summarised in the table below. Considerations of specific assay methods are described in the Methods section.

Tabel 3: Summary of strengths and limitations of the papers in the thesis
CV = cardiovascular; CVD = cardiovascular disease; ADM = adrenomedullin.

	Strengths	Limitations
Paper I	Randomised controlled trial. Large cohort with a homogenous patient population.	Long enrollment period. Open-label design
Paper II	Novelty of research question and design. Validation of an experimental assay in relation to an established assay.	Assays performed in two batches, with the risk of technical bias. Lack of information regarding timing of injections in relation to blood withdrawal.
Paper III	Covers several aspects of coagulation activation. Cohort based on a randomised controlled trial.	Assays measuring inhibitory mechanisms were not included. Relatively few thrombotic events.
Paper IV	Covers several markers of CV status. Concordance between MR-proADM and ADM by Olink giving strength to the results. Cohort based on a randomised controlled trial.	Information not available regarding CV comorbidities, limiting the correlations between biomarkers and CVD. Lack of information regarding patients' smoking status, a possible confounder.

Conclusions

Based on the findings of the papers in this thesis, we conclude that:

- LMWH does not improve survival in SCLC, despite a significant reduction in venous thromboembolic events.
- The negative results of the clinical trial cannot be explained by inadequate adherence to LMWH.
- Profiling of coagulation activity in plasma has not identified a subgroup of patients where LMWH would be beneficial.
- **Hence, the use of prophylactic LMWH cannot be recommended in the general management of SCLC.**
- Biomarkers of cardiovascular disease are potential prognostic factors that deserve to be explored further in prospective studies.

Future perspectives

One of the greatest challenges in oncology today is to select the right treatment for the right patient. This is usually referred to as tailored, or personalised, medicine and is often used in the context of therapies targeting oncogenic driver mutations, where we are constantly being equipped with new tools. Great efforts have also been made in an attempt to identify which patients will benefit from immunotherapy, although the perfect predictive marker is yet to be found. This thesis has touched upon another kind of personalised medicine, with the aim to identify patients that might benefit from thromboprophylaxis, either to prevent VTE or to improve survival. Additionally, using biomarkers of CV stress we have identified patients with a poor *vs* a favourable prognosis, which holds potential as guidance in individualised decision-making.

The implications of the latter findings are still unclear as there is much left to discover regarding the roles of CV biomarkers in SCLC. This warrants further, prospective studies encompassing the dynamic monitoring of ADM and ST2 in conjunction with established CV parameters, such as plasma BNP and cardiac function, for example evaluated by echocardiogram. An interesting aspect to explore in the future would be the biomarker dynamics during treatment and at time of progression. Do the levels increase during therapy, reflective of cardiotoxicity, or do they decrease, in response to reduced tumour burden? Could changes in biomarker levels predict time to progression? Furthermore, the incorporation of radiation doses to cardiac structures would enhance our knowledge of the potential predictive value of the biomarkers in terms of radiation-induced cardiotoxicity. Pre-clinical studies focusing on the expression of ADM and ST2 in various tissues and cell-lines under *e.g.* hypoxic conditions, may also shed a light onto the origins and actions of the biomarkers, bearing in mind that soluble ST2, present in plasma, and membrane-bound ST2, do not have the same functions.

With regards to anticoagulants as anti-cancer agents, either LMWH or DOACs, it seems unlikely that they would have clinically relevant tumour-inhibiting effects if they were administered in a different setting, not previously investigated. However, the prediction of VTE is still important as thromboembolism contributes to morbidity, sometimes with incomplete resolution of symptoms and psychological consequences. Thus, further studies to optimise current risk assessment models are warranted. It is possible that the coagulation factors measured at a systemic level do not reflect the local events leading to a thrombosis. To assess coagulation factors at

the site of a future VTE is near impossible for obvious reasons, but perhaps the presence of coagulation factors in tumour tissue or the surrounding microenvironment could give us a clue. We must not forget the coagulation-independent actions of TF in cancer, including PAR-signalling events. The TF-targeting antibody-drug conjugate tisotumab vedotin is currently being investigated in several trials, the results of which are eagerly awaited.

Finally, the recent advances regarding the molecular subtyping of SCLC are indeed promising. What features distinguish the different subtypes? What pathways are upregulated? Are there any distinct profiles in the tumour microenvironment? I hope that this will be explored in current and future clinical trials to give us tools to overcome the recalcitrance of SCLC.

Populärvetenskaplig sammanfattning

Går det att bromsa cancer med blodförtunnande läkemedel? Hur vet vi att patienterna verkligen har tagit sin medicin? Hur väljer vi rätt behandling till rätt patient? Det här är några av de frågor som vi har försökt besvara i detta avhandlingsarbete, som är baserat på en klinisk studie med lungcancerpatienter, kallad RASTEN-studien.

Redan på 1860-talet beskrev en läkare i Paris, Armand Trousseau, ett samband mellan cancer och en ökad risk för att utveckla venösa tromboser. Venösa tromboser är blodproppar som antingen kan sätta sig i lungorna, där de kan orsaka andfåddhet och bröstsmärtor, eller i armar och ben, där de kan leda till smärta och svullnad. I vissa fall ger de inga besvär alls, men ibland kan proppar i lungorna till och med vara dödliga. För att blodproppar ska bildas, krävs det att olika koagulationsfaktorer aktiveras i blodet. Koagulationsfaktorer är de ämnen som får blodet att levra sig, något som är nödvändigt när vi exempelvis har skadat oss. Några av de viktigare faktorerna är vävnadsfaktor, trombin och fibrin. Forskning har visat att dessa ämnen inte bara bidrar till en ökad risk för blodproppar, utan även kan driva cancercellers tillväxt och spridning.

Oavsett om man har cancer eller inte, behandlas venösa tromboser med blodförtunnande läkemedel, som antingen ges som tablett eller i spruta. Ofta har sprutor varit att föredra hos cancerpatienter då det är förknippat med mindre risk för biverkningar och interaktioner med andra läkemedel. Så, om koagulationsfaktorer leder till blodproppar och ökad cancerväxt, och blodförtunnande läkemedel kan motverka proppbildning, kan det samtidigt hämma tillväxten av cancercellerna?

Denna fråga har gäckat forskare i flera decennier, och ett antal studier från 1980- och 90-talen har visat att tillägg av blodförtunnande behandling faktiskt har förlängt överlevnaden, specifikt hos patienter med en typ av lungcancer som kallas för småcellig lungcancer (SCLC). Småcellig lungcancer är en särskilt aggressiv form av lungcancer med hög dödlighet. Den behandlas främst med cellgifter och strålbehandling, och trots att många patienter har god effekt av behandlingen i början, får de flesta patienterna återfall inom en kort period. Den förbättrade överlevnaden som man antydde med blodförtunnande läkemedel sågs därför som ett välkommet tillskott i behandlingsarsenalen.

Problemet med de tidiga studierna var att de baserades på ett litet antal patienter eller använde sig av omodern behandling i övrigt, vilket har gjort det svårt att dra

slutsatser från resultaten. Detta ledde till att RASTEN-studien startades, en klinisk studie där patienter med SCLC fick sedvanlig cancerhämmande behandling, med eller utan tillägg av det blodförtunnande läkemedlet enoxaparin (Klexane), som gavs dagligen under 3–4 månaders tid. Syftet med studien var att undersöka om tillägget av blodförtunnande enoxaparin kunde förbättra överlevnaden i denna patientgrupp.

I *delarbete I* beskrivs den kliniska delen av RASTEN-studien. Totalt inkluderades 377 patienter som lottades mellan att ingå i den så kallade 'kontrollarmen', där man fick standardbehandling (cellgifter, eventuellt i kombination med strålbehandling), eller i 'enoxaparin-armen' där patienterna erhöll standardbehandling med tillägg av blodförtunnande läkemedel. Resultaten visade ingen skillnad mellan grupperna – patienterna som fick blodförtunnande levde lika länge som patienterna som inte fick blodförtunnande, men däremot var det betydligt färre som fick blodproppar i enoxaparin-armen. Att överlevnaden inte förbättrades var tvärtemot vad vi hade hoppats på när studien startade 2008, men intressant nog har det publicerats flera, liknande studier på senare år som har visat samma resultat som RASTEN, dvs att blodförtunnande inte har förbättrat prognosen, och alltså inte verkar kunna bromsa cancercellernas tillväxt.

Detta fick oss och andra i forskningsfältet att ställa frågan – varför? Varför fick vi de här resultaten? Skulle det kunna bero på att patienterna inte har tagit sitt läkemedel som ordinerat? Enoxaparin ges dagligen som en spruta under huden, vilket kan orsaka obehag och blåmärken runt instickssstället. Patienters följsamhet med en läkemedelsbehandling är sällan 100%, vare sig i studier eller utanför, men det är ofta svårt att mäta följsamhet på ett objektivt sätt. I *delarbete II* bestämde vi oss därför för att mäta nivåerna av enoxaparin i blodet på patienterna i RASTEN-studien. Vi använde två olika metoder, dels en etablerad analys som finns på de flesta större sjukhus, och dels en experimentell metod som inte tidigare har testats på blodprov från patienter. Resultaten visade att båda analyser återspeglar nivåerna av enoxaparin, och efter statistiska beräkningar uppskattades att ca 65–80% av patienterna, beroende på analysmetod, hade tagit det blodförtunnande läkemedlet som de skulle. När överlevnadsanalyserna upprepades med de 'följsamma' patienterna jämfört med patienterna i kontrollarmen, sågs fortfarande ingen förbättrad överlevnad. Med andra ord kunde vi konstatera att de negativa resultaten i RASTEN inte beror på otillräcklig följsamhet hos patienterna.

Kan det ändå finnas en liten grupp patienter som kan ha nytta av blodförtunnande läkemedel? I *delarbete III* undersökte vi om vissa patienter hade extra höga nivåer av koagulationsfaktorer i blodet, som skulle kunna bidra dels till venösa tromboser och dels till en mer aggressiv cancersjukdom och sämre prognos. Det är tänkbart att dessa patienter faktiskt skulle kunna ha en positiv effekt av blodförtunnande medicin, baserat på vad blodproverna visar. Resultaten visade dock ingen entydig bild. Av sex olika sätt att mäta koagulationsaktiviteten på var det endast en som

visade en koppling till överlevnad. Dessutom såg vi att patienter med låga nivåer av vävnadsfaktor levde kortare tid om de fick blodförtunnande behandling.

Slutligen, i *delarbete IV* har vi studerat markörer i blodet som vanligtvis är kopplade till hjärt- och kärlsjukdomar. Majoriteten av alla patienter med SCLC är aktiva eller före detta rökare, vilket både bidrar till utveckling av cancer och av hjärt- och kärlsjukdomar. En bakomliggande hjärtsjukdom kan påverka hur väl en patient tål den intensiva cancerbehandlingen, då både cellgifter och strålbehandling kan försämra hjärtfunktionen. Tyvärr vet vi ofta för lite om patienternas hjärt- och kärlfunktion när de precis har blivit diagnosticerade med lungcancer, eftersom det saknas enkla metoder att kartlägga detta på. I det fjärde delarbetet mätte vi ämnen i blodet som kan stiga vid bland annat hjärtsvikt. Särskilt två av ämnena, adrenomedullin och ST2, var starkt kopplade till dödlighet, dvs höga nivåer i blodet förutspådde en kortare överlevnad, och *vice versa*.

Än så länge vet vi inte om blodproverna återspeglar en bakomliggande hjärt- och kärlsjukdom som påverkar patienternas överlevnad, eller om det är cancercellerna i sig som utsöndrar dessa ämnen. Kan de till och med bidra till ökad tumörväxt? Vi har mycket mer att lära oss om vilken roll adrenomedullin och ST2 har vid lungcancer, och eventuellt även vid andra cancerformer där hjärtsjuklighet är vanligt förekommande. Resultaten behöver bekräftas i nya studier där man även kan mäta hjärtfunktionen på andra sätt, tex med ultraljud av hjärtat, men i förlängningen skulle det kunna ha betydelse för hur vi väljer rätt behandling, till rätt patient.

Sammanfattningsvis kan vi konstatera att förebyggande behandling med blodförtunnande läkemedel inte ökar överlevnaden hos patienter med SCLC. Genom att mäta nivåerna av enoxaparin i patienternas blod har vi kunnat utesluta att resultaten i RASTEN beror på att patienterna har låtit bli att ta medicinen. Vi har inte kunnat identifiera en mindre grupp patienter som skulle kunna ha nytta av blodförtunnande läkemedel, baserat på koagulationsaktivitet i blodet. Däremot har vi sett ett starkt samband mellan överlevnad och äggviteämnen som är kopplade till hjärtsvikt, vilket leder till många nya frågeställningar.

Acknowledgements

I would like to express my sincere gratitude to everyone who has supported and encouraged me throughout this work, and I am grateful to all the patients who participated in the RASTEN trial, thus contributing vastly to research.

In particular, I would like to thank:

My main supervisor Mattias Belting, for taking me onboard on this scientific adventure, and for always challenging me to think one step further. I really appreciate getting the opportunity to work with you and your group.

My co-supervisor Hans Brunnström, your calmness is invaluable to a PhD student often in stress. I know this thesis contains less immunohistochemistry than you would have preferred, but I look forward to continuing projects with you.

The RASTEN study group, especially Lars Ek and Martin Wallberg, for trusting me to continue the work with the trial. Pär-Ola Bendahl, with your never-ending enthusiasm for medical statistics and thoroughness when solving problems, and Jan Sundberg, for practical support.

To all members, past and present, of the Belting lab group for great scientific discussions, excellent social events, friendship, laughter, and for giving me an insight into the pre-clinical world. A special thanks to Maria Johansson for all the problems you have helped me solve, Eva Lindqvist and Ann-Sofie Månsson for all the hours you have spent sorting and aliquoting samples, Kelin Gonçalves de Oliveira for help with graphics that I never seem to learn how to manage, and Anna Bång-Rudenstam for help with projects to be.

Kerstin Andersson and Håkan Griph, with a few years apart, both of you have inspired me to become a thoracic oncologist, with your compassionate approach to caring for your patients. Maria Planck, for your invaluable advice on how to navigate as a scientist, and clinician, in the field of lung cancer research.

My former colleagues at the oncology department for providing a great learning environment, laughter and for teaching me to become an oncologist.

My colleagues at the department of respiratory medicine, especially the fantastic team at the Thoracic Oncology Unit for your wisdom, willingness to discuss and friendship.

All staff at the Thoracic Oncology Unit – nurses, secretaries, social counsellor – for always doing your best for our patients, and for even making Mondays fun.

My previous Heads of the Department of Oncology and current Head of the Department of Respiratory medicine and Allergology, Johan Svahn, for giving me the opportunity to combine clinical work with research.

My dear housemates from Birmingham, for always being ready to solve my linguistic conundrums.

My parents, my brothers, other close family members and friends, for encouragement and support. Henrik, Elin and Wilhelm for making my everyday life full of joy and surprises.

Funding:

We also wish to thank those who have financially supported this research: Region Skåne, ALF, Fru Berta Kamprads stiftelse, Cancerfonden, Vetenskapsrådet.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**(3): 209-49.
2. Ervik M, Lam F, Laversanne M, Ferlay J, Bray F. Global Cancer Observatory: Cancer Over Time. 2021. https://gco.iarc.fr/overtime (accessed 09/03/2022).
3. Biswas AK, Acharyya S. Understanding cachexia in the context of metastatic progression. *Nat Rev Cancer* 2020; **20**(5): 274-84.
4. Diakos CI, Charles KA, McMillan DC, Clarke SJ. Cancer-related inflammation and treatment effectiveness. *The Lancet Oncology* 2014; **15**(11): e493-503.
5. Trousseau A. Lectures on Clinical Medicine at the Hotel-Dieu, Paris. London: New Sydenham Society; 1868.
6. Khorana AA. Malignancy, thrombosis and Trousseau: the case for an eponym. *Journal of thrombosis and haemostasis : JTH* 2003; **1**(12): 2463-5.
7. Kline JA. Diagnosis and Exclusion of Pulmonary Embolism. *Thromb Res* 2018; **163**: 207-20.
8. Heit JA, Silverstein MD, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ, 3rd. Risk factors for deep vein thrombosis and pulmonary embolism: a population-based case-control study. *Arch Intern Med* 2000; **160**(6): 809-15.
9. White RH, Chew H, Wun T. Targeting patients for anticoagulant prophylaxis trials in patients with cancer: who is at highest risk? *Thromb Res* 2007; **120 Suppl 2**: S29-40.
10. Blom JW, Vanderschoot JP, Oostindier MJ, Osanto S, van der Meer FJ, Rosendaal FR. Incidence of venous thrombosis in a large cohort of 66,329 cancer patients: results of a record linkage study. *Journal of thrombosis and haemostasis : JTH* 2006; **4**(3): 529-35.
11. Mulder FI, Horvath-Puho E, van Es N, et al. Venous thromboembolism in cancer patients: a population-based cohort study. *Blood* 2021; **137**(14): 1959-69.
12. Sorensen HT, Mellemkjaer L, Steffensen FH, Olsen JH, Nielsen GL. The risk of a diagnosis of cancer after primary deep venous thrombosis or pulmonary embolism. *N Engl J Med* 1998; **338**(17): 1169-73.
13. Chew HK, Davies AM, Wun T, Harvey D, Zhou H, White RH. The incidence of venous thromboembolism among patients with primary lung cancer. *Journal of thrombosis and haemostasis : JTH* 2008; **6**(4): 601-8.
14. Sorensen HT, Mellemkjaer L, Olsen JH, Baron JA. Prognosis of cancers associated with venous thromboembolism. *N Engl J Med* 2000; **343**(25): 1846-50.

15. Prandoni P, Lensing AW, Piccioli A, et al. Recurrent venous thromboembolism and bleeding complications during anticoagulant treatment in patients with cancer and venous thrombosis. *Blood* 2002; **100**(10): 3484-8.
16. Weitz JI, Haas S, Ageno W, et al. Cancer associated thrombosis in everyday practice: perspectives from GARFIELD-VTE. *J Thromb Thrombolysis* 2020; **50**(2): 267-77.
17. Lee AY, Rickles FR, Julian JA, et al. Randomized comparison of low molecular weight heparin and coumarin derivatives on the survival of patients with cancer and venous thromboembolism. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2005; **23**(10): 2123-9.
18. Gray E, Mulloy B, Barrowcliffe TW. Heparin and low-molecular-weight heparin. *Thromb Haemost* 2008; **99**(5): 807-18.
19. Key NS, Khorana AA, Kuderer NM, et al. Venous Thromboembolism Prophylaxis and Treatment in Patients With Cancer: ASCO Clinical Practice Guideline Update. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2020; **38**(5): 496-520.
20. Khorana AA, Kuderer NM, Culakova E, Lyman GH, Francis CW. Development and validation of a predictive model for chemotherapy-associated thrombosis. *Blood* 2008; **111**(10): 4902-7.
21. Lyman GH, Carrier M, Ay C, et al. American Society of Hematology 2021 guidelines for management of venous thromboembolism: prevention and treatment in patients with cancer. *Blood Adv* 2021; **5**(4): 927-74.
22. Mulder FI, Candeloro M, Kamphuisen PW, et al. The Khorana score for prediction of venous thromboembolism in cancer patients: a systematic review and meta-analysis. *Haematologica* 2019; **104**(6): 1277-87.
23. Gervaso L, Dave H, Khorana AA. Venous and Arterial Thromboembolism in Patients With Cancer: JACC: CardioOncology State-of-the-Art Review. *JACC CardioOncol* 2021; **3**(2): 173-90.
24. Kasthuri RS, Taubman MB, Mackman N. Role of tissue factor in cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2009; **27**(29): 4834-8.
25. Osterud B. Tissue factor/TFPI and blood cells. *Thromb Res* 2012; **129**(3): 274-8.
26. Dahlback B. Blood coagulation. *Lancet* 2000; **355**(9215): 1627-32.
27. Mackman N. Role of tissue factor in hemostasis, thrombosis, and vascular development. *Arterioscler Thromb Vasc Biol* 2004; **24**(6): 1015-22.
28. Gray E, Hogwood J, Mulloy B. The anticoagulant and antithrombotic mechanisms of heparin. *Handb Exp Pharmacol* 2012; (207): 43-61.
29. Belting M, Ahamed J, Ruf W. Signaling of the tissue factor coagulation pathway in angiogenesis and cancer. *Arterioscler Thromb Vasc Biol* 2005; **25**(8): 1545-50.
30. Tkach M, Thery C. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. *Cell* 2016; **164**(6): 1226-32.
31. Bang-Rudenstam A, Cerezo-Magana M, Belting M. Pro-metastatic functions of lipoproteins and extracellular vesicles in the acidic tumor microenvironment. *Cancer Metastasis Rev* 2019; **38**(1-2): 79-92.

32. Belting M, Christianson HC. Role of exosomes and microvesicles in hypoxia-associated tumour development and cardiovascular disease. *Journal of internal medicine* 2015; **278**(3): 251-63.
33. Cerezo-Magana M, Bang-Rudenstam A, Belting M. The pleiotropic role of proteoglycans in extracellular vesicle mediated communication in the tumor microenvironment. *Semin Cancer Biol* 2020; **62**: 99-107.
34. Muller I, Klocke A, Alex M, et al. Intravascular tissue factor initiates coagulation via circulating microvesicles and platelets. *FASEB J* 2003; **17**(3): 476-8.
35. Thery C, Witwer KW, Aikawa E, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. *J Extracell Vesicles* 2018; **7**(1): 1535750.
36. Khorana AA, Ahrendt SA, Ryan CK, et al. Tissue factor expression, angiogenesis, and thrombosis in pancreatic cancer. *Clin Cancer Res* 2007; **13**(10): 2870-5.
37. Nitori N, Ino Y, Nakanishi Y, et al. Prognostic significance of tissue factor in pancreatic ductal adenocarcinoma. *Clin Cancer Res* 2005; **11**(7): 2531-9.
38. Poon RT, Lau CP, Ho JW, Yu WC, Fan ST, Wong J. Tissue factor expression correlates with tumor angiogenesis and invasiveness in human hepatocellular carcinoma. *Clin Cancer Res* 2003; **9**(14): 5339-45.
39. Sakurai M, Matsumoto K, Gosho M, et al. Expression of Tissue Factor in Epithelial Ovarian Carcinoma Is Involved in the Development of Venous Thromboembolism. *Int J Gynecol Cancer* 2017; **27**(1): 37-43.
40. Sato T, Tsujino I, Ikeda D, Ieko M, Nishimura M. Trousseau's syndrome associated with tissue factor produced by pulmonary adenocarcinoma. *Thorax* 2006; **61**(11): 1009-10.
41. Seto S, Onodera H, Kaido T, et al. Tissue factor expression in human colorectal carcinoma: correlation with hepatic metastasis and impact on prognosis. *Cancer* 2000; **88**(2): 295-301.
42. Hisada Y, Mackman N. Tissue Factor and Cancer: Regulation, Tumor Growth, and Metastasis. *Semin Thromb Hemost* 2019; **45**(4): 385-95.
43. Yu JL, May L, Lhotak V, et al. Oncogenic events regulate tissue factor expression in colorectal cancer cells: implications for tumor progression and angiogenesis. *Blood* 2005; **105**(4): 1734-41.
44. Ruf W, Disse J, Carneiro-Lobo TC, Yokota N, Schaffner F. Tissue factor and cell signalling in cancer progression and thrombosis. *Journal of thrombosis and haemostasis : JTH* 2011; **9 Suppl 1**: 306-15.
45. Thaler J, Ay C, Mackman N, et al. Microparticle-associated tissue factor activity, venous thromboembolism and mortality in pancreatic, gastric, colorectal and brain cancer patients. *Journal of thrombosis and haemostasis : JTH* 2012; **10**(7): 1363-70.
46. Zwicker JI, Liebman HA, Neuberg D, et al. Tumor-derived tissue factor-bearing microparticles are associated with venous thromboembolic events in malignancy. *Clin Cancer Res* 2009; **15**(22): 6830-40.

47. Dvorak HF, Quay SC, Orenstein NS, et al. Tumor shedding and coagulation. *Science* 1981; **212**(4497): 923-4.
48. Dvorak HF, Van DeWater L, Bitzer AM, et al. Procoagulant activity associated with plasma membrane vesicles shed by cultured tumor cells. *Cancer Res* 1983; **43**(9): 4434-42.
49. Wojtukiewicz MZ, Hempel D, Sierko E, Tucker SC, Honn KV. Protease-activated receptors (PARs)--biology and role in cancer invasion and metastasis. *Cancer Metastasis Rev* 2015; **34**(4): 775-96.
50. Markham A. Tisotumab Vedotin: First Approval. *Drugs* 2021; **81**(18): 2141-7.
51. Breij EC, de Goeij BE, Verploegen S, et al. An antibody-drug conjugate that targets tissue factor exhibits potent therapeutic activity against a broad range of solid tumors. *Cancer Res* 2014; **74**(4): 1214-26.
52. Snyder KM, Kessler CM. The pivotal role of thrombin in cancer biology and tumorigenesis. *Semin Thromb Hemost* 2008; **34**(8): 734-41.
53. Catar R, Moll G, Hosp I, et al. Transcriptional Regulation of Thrombin-Induced Endothelial VEGF Induction and Proangiogenic Response. *Cells* 2021; **10**(4).
54. Palumbo JS, Kombrinck KW, Drew AF, et al. Fibrinogen is an important determinant of the metastatic potential of circulating tumor cells. *Blood* 2000; **96**(10): 3302-9.
55. Rickles FR, Patierno S, Fernandez PM. Tissue factor, thrombin, and cancer. *Chest* 2003; **124**(3 Suppl): 58S-68S.
56. Palumbo JS, Potter JM, Kaplan LS, Talmage K, Jackson DG, Degen JL. Spontaneous hematogenous and lymphatic metastasis, but not primary tumor growth or angiogenesis, is diminished in fibrinogen-deficient mice. *Cancer Res* 2002; **62**(23): 6966-72.
57. Sahni A, Baker CA, Sporn LA, Francis CW. Fibrinogen and fibrin protect fibroblast growth factor-2 from proteolytic degradation. *Thromb Haemost* 2000; **83**(5): 736-41.
58. Palumbo JS, Talmage KE, Massari JV, et al. Tumor cell-associated tissue factor and circulating hemostatic factors cooperate to increase metastatic potential through natural killer cell-dependent and-independent mechanisms. *Blood* 2007; **110**(1): 133-41.
59. Palumbo JS, Talmage KE, Massari JV, et al. Platelets and fibrin(ogen) increase metastatic potential by impeding natural killer cell-mediated elimination of tumor cells. *Blood* 2005; **105**(1): 178-85.
60. Nieswandt B, Hafner M, Echtenacher B, Mannel DN. Lysis of tumor cells by natural killer cells in mice is impeded by platelets. *Cancer Res* 1999; **59**(6): 1295-300.
61. Heldin CH, Westermark B, Wasteson A. Platelet-derived growth factor. Isolation by a large-scale procedure and analysis of subunit composition. *Biochem J* 1981; **193**(3): 907-13.
62. Kaplan DR, Chao FC, Stiles CD, Antoniades HN, Scher CD. Platelet alpha granules contain a growth factor for fibroblasts. *Blood* 1979; **53**(6): 1043-52.

63. Mohle R, Green D, Moore MA, Nachman RL, Rafii S. Constitutive production and thrombin-induced release of vascular endothelial growth factor by human megakaryocytes and platelets. *Proc Natl Acad Sci U S A* 1997; **94**(2): 663-8.
64. Bendas G, Borsig L. Cancer cell adhesion and metastasis: selectins, integrins, and the inhibitory potential of heparins. *Int J Cell Biol* 2012; **2012**: 676731.
65. Baron JA, Cole BF, Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas. *N Engl J Med* 2003; **348**(10): 891-9.
66. Logan RF, Grainge MJ, Shepherd VC, Armitage NC, Muir KR, uk CAPTG. Aspirin and folic acid for the prevention of recurrent colorectal adenomas. *Gastroenterology* 2008; **134**(1): 29-38.
67. Gaziano JM, Brotons C, Coppolecchia R, et al. Use of aspirin to reduce risk of initial vascular events in patients at moderate risk of cardiovascular disease (ARRIVE): a randomised, double-blind, placebo-controlled trial. *Lancet* 2018; **392**(10152): 1036-46.
68. McNeil JJ, Nelson MR, Woods RL, et al. Effect of Aspirin on All-Cause Mortality in the Healthy Elderly. *N Engl J Med* 2018; **379**(16): 1519-28.
69. Harris AL. Hypoxia--a key regulatory factor in tumour growth. *Nat Rev Cancer* 2002; **2**(1): 38-47.
70. Vaupel P, Thews O, Hoeckel M. Treatment resistance of solid tumors: role of hypoxia and anemia. *Med Oncol* 2001; **18**(4): 243-59.
71. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc Natl Acad Sci U S A* 1995; **92**(12): 5510-4.
72. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. *Mol Cell Biol* 2001; **21**(12): 3995-4004.
73. Zhong H, Chiles K, Feldser D, et al. Modulation of hypoxia-inducible factor 1alpha expression by the epidermal growth factor/phosphatidylinositol 3-kinase/PTEN/AKT/FRAP pathway in human prostate cancer cells: implications for tumor angiogenesis and therapeutics. *Cancer Res* 2000; **60**(6): 1541-5.
74. Jin Y, Wang H, Ma X, Liang X, Liu X, Wang Y. Clinicopathological characteristics of gynecological cancer associated with hypoxia-inducible factor 1alpha expression: a meta-analysis including 6,612 subjects. *PloS one* 2015; **10**(5): e0127229.
75. Matsuo Y, Ding Q, Desaki R, et al. Hypoxia inducible factor-1 alpha plays a pivotal role in hepatic metastasis of pancreatic cancer: an immunohistochemical study. *J Hepatobiliary Pancreat Sci* 2014; **21**(2): 105-12.
76. Ping W, Sun W, Zu Y, Chen W, Fu X. Clinicopathological and prognostic significance of hypoxia-inducible factor-1alpha in esophageal squamous cell carcinoma: a meta-analysis. *Tumour Biol* 2014; **35**(5): 4401-9.
77. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene* 2010; **29**(5): 625-34.

78. Jonasch E, Donskov F, Iliopoulos O, et al. Belzutifan for Renal Cell Carcinoma in von Hippel-Lindau Disease. *N Engl J Med* 2021; **385**(22): 2036-46.
79. Denko NC. Hypoxia, HIF1 and glucose metabolism in the solid tumour. *Nat Rev Cancer* 2008; **8**(9): 705-13.
80. Becker HM. Carbonic anhydrase IX and acid transport in cancer. *Br J Cancer* 2020; **122**(2): 157-67.
81. Swietach P, Patiar S, Supuran CT, Harris AL, Vaughan-Jones RD. The role of carbonic anhydrase 9 in regulating extracellular and intracellular ph in three-dimensional tumor cell growths. *J Biol Chem* 2009; **284**(30): 20299-310.
82. Carmeliet P. VEGF as a key mediator of angiogenesis in cancer. *Oncology* 2005; **69 Suppl 3**: 4-10.
83. Dvorak HF. Tumors: wounds that do not heal-redux. *Cancer Immunol Res* 2015; **3**(1): 1-11.
84. Kerbel RS. Tumor angiogenesis. *N Engl J Med* 2008; **358**(19): 2039-49.
85. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971; **285**(21): 1182-6.
86. Folkman J, Merler E, Abernathy C, Williams G. Isolation of a tumor factor responsible for angiogenesis. *J Exp Med* 1971; **133**(2): 275-88.
87. Fontanini G, Faviana P, Lucchi M, et al. A high vascular count and overexpression of vascular endothelial growth factor are associated with unfavourable prognosis in operated small cell lung carcinoma. *Br J Cancer* 2002; **86**(4): 558-63.
88. Salven P, Ruotsalainen T, Mattson K, Joensuu H. High pre-treatment serum level of vascular endothelial growth factor (VEGF) is associated with poor outcome in small-cell lung cancer. *Int J Cancer* 1998; **79**(2): 144-6.
89. Zhan P, Wang J, Lv XJ, et al. Prognostic value of vascular endothelial growth factor expression in patients with lung cancer: a systematic review with meta-analysis. *J Thorac Oncol* 2009; **4**(9): 1094-103.
90. Montanino A, Manzo A, Carillio G, et al. Angiogenesis Inhibitors in Small Cell Lung Cancer. *Front Oncol* 2021; **11**: 655316.
91. Evans CE, Bendahl PO, Belting M, Branco C, Johnson RS. Diverse roles of cell-specific hypoxia-inducible factor 1 in cancer-associated hypercoagulation. *Blood* 2016; **127**(10): 1355-60.
92. Svensson KJ, Kucharzewska P, Christianson HC, et al. Hypoxia triggers a proangiogenic pathway involving cancer cell microvesicles and PAR-2-mediated heparin-binding EGF signaling in endothelial cells. *Proc Natl Acad Sci U S A* 2011; **108**(32): 13147-52.
93. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med* 1986; **315**(26): 1650-9.
94. Lindahl U, Kjellen L. Pathophysiology of heparan sulphate: many diseases, few drugs. *Journal of internal medicine* 2013; **273**(6): 555-71.
95. Jorpes JE, Gardell S. On heparin monosulfuric acid. *J Biol Chem* 1948; **176**(1): 267-76.

96. Choay J, Petitou M, Lormeau JC, Sinay P, Casu B, Gatti G. Structure-activity relationship in heparin: a synthetic pentasaccharide with high affinity for antithrombin III and eliciting high anti-factor Xa activity. *Biochem Biophys Res Commun* 1983; **116**(2): 492-9.

97. Mousa SA, Petersen LJ. Anti-cancer properties of low-molecular-weight heparin: preclinical evidence. *Thromb Haemost* 2009; **102**(2): 258-67.

98. Knelson EH, Nee JC, Blobe GC. Heparan sulfate signaling in cancer. *Trends Biochem Sci* 2014; **39**(6): 277-88.

99. Sasisekharan R, Shriver Z, Venkataraman G, Narayanasami U. Roles of heparan-sulphate glycosaminoglycans in cancer. *Nat Rev Cancer* 2002; **2**(7): 521-8.

100. Blackhall FH, Merry CL, Davies EJ, Jayson GC. Heparan sulfate proteoglycans and cancer. *Br J Cancer* 2001; **85**(8): 1094-8.

101. Belting M. Glycosaminoglycans in cancer treatment. *Thromb Res* 2014; **133 Suppl 2**: S95-101.

102. Fuster MM, Wang L, Castagnola J, et al. Genetic alteration of endothelial heparan sulfate selectively inhibits tumor angiogenesis. *J Cell Biol* 2007; **177**(3): 539-49.

103. Aikawa T, Whipple CA, Lopez ME, et al. Glypican-1 modulates the angiogenic and metastatic potential of human and mouse cancer cells. *J Clin Invest* 2008; **118**(1): 89-99.

104. Maeda T, Desouky J, Friedl A. Syndecan-1 expression by stromal fibroblasts promotes breast carcinoma growth in vivo and stimulates tumor angiogenesis. *Oncogene* 2006; **25**(9): 1408-12.

105. Yang Y, Macleod V, Miao HQ, et al. Heparanase enhances syndecan-1 shedding: a novel mechanism for stimulation of tumor growth and metastasis. *J Biol Chem* 2007; **282**(18): 13326-33.

106. Knelson EH, Gaviglio AL, Nee JC, et al. Stromal heparan sulfate differentiates neuroblasts to suppress neuroblastoma growth. *J Clin Invest* 2014; **124**(7): 3016-31.

107. Niers TM, Klerk CP, DiNisio M, et al. Mechanisms of heparin induced anti-cancer activity in experimental cancer models. *Crit Rev Oncol Hematol* 2007; **61**(3): 195-207.

108. Holroyd EW, Delacroix S, Larsen K, et al. Tissue factor pathway inhibitor blocks angiogenesis via its carboxyl terminus. *Arterioscler Thromb Vasc Biol* 2012; **32**(3): 704-11.

109. Lapierre F, Holme K, Lam L, et al. Chemical modifications of heparin that diminish its anticoagulant but preserve its heparanase-inhibitory, angiostatic, anti-tumor and anti-metastatic properties. *Glycobiology* 1996; **6**(3): 355-66.

110. Ono K, Ishihara M, Ishikawa K, et al. Periodate-treated, non-anticoagulant heparin-carrying polystyrene (NAC-HCPS) affects angiogenesis and inhibits subcutaneous induced tumour growth and metastasis to the lung. *Br J Cancer* 2002; **86**(11): 1803-12.

111. Sciumbata T, Caretto P, Pirovano P, et al. Treatment with modified heparins inhibits experimental metastasis formation and leads, in some animals, to long-term survival. *Invasion Metastasis* 1996; **16**(3): 132-43.

112. Yoshitomi Y, Nakanishi H, Kusano Y, et al. Inhibition of experimental lung metastases of Lewis lung carcinoma cells by chemically modified heparin with reduced anticoagulant activity. *Cancer Lett* 2004; **207**(2): 165-74.
113. Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. Cell surface, heparin-like molecules are required for binding of basic fibroblast growth factor to its high affinity receptor. *Cell* 1991; **64**(4): 841-8.
114. Rapraeger AC, Krufka A, Olwin BB. Requirement of heparan sulfate for bFGF-mediated fibroblast growth and myoblast differentiation. *Science* 1991; **252**(5013): 1705-8.
115. Colin S, Jeanny JC, Mascarelli F, et al. In vivo involvement of heparan sulfate proteoglycan in the bioavailability, internalization, and catabolism of exogenous basic fibroblast growth factor. *Mol Pharmacol* 1999; **55**(1): 74-82.
116. Collen A, Smorenburg SM, Peters E, et al. Unfractionated and low molecular weight heparin affect fibrin structure and angiogenesis in vitro. *Cancer Res* 2000; **60**(21): 6196-200.
117. Soker S, Goldstaub D, Svahn CM, Vlodavsky I, Levi BZ, Neufeld G. Variations in the size and sulfation of heparin modulate the effect of heparin on the binding of VEGF165 to its receptors. *Biochem Biophys Res Commun* 1994; **203**(2): 1339-47.
118. Vlodavsky I, Friedmann Y. Molecular properties and involvement of heparanase in cancer metastasis and angiogenesis. *J Clin Invest* 2001; **108**(3): 341-7.
119. Kato M, Wang H, Kainulainen V, et al. Physiological degradation converts the soluble syndecan-1 ectodomain from an inhibitor to a potent activator of FGF-2. *Nat Med* 1998; **4**(6): 691-7.
120. Vlodavsky I, Friedmann Y, Elkin M, et al. Mammalian heparanase: gene cloning, expression and function in tumor progression and metastasis. *Nat Med* 1999; **5**(7): 793-802.
121. Bendas G, Borsig L. Heparanase in Cancer Metastasis - Heparin as a Potential Inhibitor of Cell Adhesion Molecules. *Adv Exp Med Biol* 2020; **1221**: 309-29.
122. Naggi A, Casu B, Perez M, et al. Modulation of the heparanase-inhibiting activity of heparin through selective desulfation, graded N-acetylation, and glycol splitting. *J Biol Chem* 2005; **280**(13): 12103-13.
123. Vlodavsky I, Mohsen M, Lider O, et al. Inhibition of tumor metastasis by heparanase inhibiting species of heparin. *Invasion Metastasis* 1994; **14**(1-6): 290-302.
124. Irimura T, Nakajima M, Nicolson GL. Chemically modified heparins as inhibitors of heparan sulfate specific endo-beta-glucuronidase (heparanase) of metastatic melanoma cells. *Biochemistry* 1986; **25**(18): 5322-8.
125. Borsig L. Heparin as an inhibitor of cancer progression. *Prog Mol Biol Transl Sci* 2010; **93**: 335-49.
126. Gorelik E, Bere WW, Herberman RB. Role of NK cells in the antimetastatic effect of anticoagulant drugs. *Int J Cancer* 1984; **33**(1): 87-94.
127. Zacharski LR, Henderson WG, Rickles FR, et al. Effect of warfarin anticoagulation on survival in carcinoma of the lung, colon, head and neck, and prostate. Final report of VA Cooperative Study #75. *Cancer* 1984; **53**(10): 2046-52.

128. Lebeau B, Chastang C, Brechot JM, et al. Subcutaneous heparin treatment increases survival in small cell lung cancer. "Petites Cellules" Group. *Cancer* 1994; **74**(1): 38-45.
129. Altinbas M, Coskun HS, Er O, et al. A randomized clinical trial of combination chemotherapy with and without low-molecular-weight heparin in small cell lung cancer. *Journal of thrombosis and haemostasis : JTH* 2004; **2**(8): 1266-71.
130. Kakkar AK, Levine MN, Kadziola Z, et al. Low molecular weight heparin, therapy with dalteparin, and survival in advanced cancer: the fragmin advanced malignancy outcome study (FAMOUS). *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2004; **22**(10): 1944-8.
131. Klerk CP, Smorenburg SM, Otten HM, et al. The effect of low molecular weight heparin on survival in patients with advanced malignancy. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2005; **23**(10): 2130-5.
132. Akl EA, Gunukula S, Barba M, et al. Parenteral anticoagulation in patients with cancer who have no therapeutic or prophylactic indication for anticoagulation. *Cochrane Database Syst Rev* 2011; (1): CD006652.
133. Zhang J, Zhang YL, Ma KX, Qu JM. Efficacy and safety of adjunctive anticoagulation in patients with lung cancer without indication for anticoagulants: a systematic review and meta-analysis. *Thorax* 2013; **68**(5): 442-50.
134. Macbeth F, Noble S, Evans J, et al. Randomized Phase III Trial of Standard Therapy Plus Low Molecular Weight Heparin in Patients With Lung Cancer: FRAGMATIC Trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2016; **34**(5): 488-94.
135. Meyer G, Besse B, Doubre H, et al. Anti-tumour effect of low molecular weight heparin in localised lung cancer: a phase III clinical trial. *Eur Respir J* 2018; **52**(4).
136. Pelzer U, Opitz B, Deutschinoff G, et al. Efficacy of Prophylactic Low-Molecular Weight Heparin for Ambulatory Patients With Advanced Pancreatic Cancer: Outcomes From the CONKO-004 Trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2015; **33**(18): 2028-34.
137. Ferlay J, Ervik M, Lam F, et al. Global Cancer Observatory: Cancer Today Sweden. March, 2021 2020. https://gco.iarc.fr/today/data/factsheets/populations/752-sweden-fact-sheets.pdf (accessed 13/03/2022).
138. Govindan R, Page N, Morgensztern D, et al. Changing epidemiology of small-cell lung cancer in the United States over the last 30 years: analysis of the surveillance, epidemiologic, and end results database. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2006; **24**(28): 4539-44.
139. Howlader N, Noone A, Krapcho M, et al. SEER Cancer Statistics Review, 1975-2017, National Cancer Institute. Bethesda, MD. April 2020. 2020. https://seer.cancer.gov/csr/1975_2017/ (accessed 29/12/2021).
140. Gaspar LE, McNamara EJ, Gay EG, et al. Small-cell lung cancer: prognostic factors and changing treatment over 15 years. *Clin Lung Cancer* 2012; **13**(2): 115-22.

141. Owonikoko TK, Behera M, Chen Z, et al. A systematic analysis of efficacy of second-line chemotherapy in sensitive and refractory small-cell lung cancer. *J Thorac Oncol* 2012; **7**(5): 866-72.
142. Fiegl M, Pircher A, Waldthaler C, et al. Small steps of improvement in small-cell lung cancer (SCLC) within two decades: a comprehensive analysis of 484 patients. *Lung cancer* 2014; **84**(2): 168-74.
143. Nicholson AG, Chansky K, Crowley J, et al. The International Association for the Study of Lung Cancer Lung Cancer Staging Project: Proposals for the Revision of the Clinical and Pathologic Staging of Small Cell Lung Cancer in the Forthcoming Eighth Edition of the TNM Classification for Lung Cancer. *J Thorac Oncol* 2016; **11**(3): 300-11.
144. Zelen M. Keynote address on biostatistics and data retrieval. *Cancer Chemother Rep 3* 1973; **4**(2): 31-42.
145. Stahel RA, Ginsberg R, Havemann K, et al. Staging and Prognostic Factors in Small Cell Lung Cancer: a Consensus Report *Lung cancer* 1989; **5**: 119-26.
146. de Koning HJ, van der Aalst CM, de Jong PA, et al. Reduced Lung-Cancer Mortality with Volume CT Screening in a Randomized Trial. *N Engl J Med* 2020; **382**(6): 503-13.
147. National Lung Screening Trial Research T. Lung Cancer Incidence and Mortality with Extended Follow-up in the National Lung Screening Trial. *J Thorac Oncol* 2019; **14**(10): 1732-42.
148. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 1982; **5**(6): 649-55.
149. Regional Cancer Centres S. Swedish Lung Cancer Registry, interactive database. 2016-2020. www.rcc.incanet.se [authorised access] (accessed 11/03/2022).
150. Brueckl WM, Herbst L, Lechler A, et al. Predictive and prognostic factors in small cell lung carcinoma (SCLC)--analysis from routine clinical practice. *Anticancer Res* 2006; **26**(6C): 4825-32.
151. Hansen O, Sorensen P, Hansen KH. The occurrence of hyponatremia in SCLC and the influence on prognosis: a retrospective study of 453 patients treated in a single institution in a 10-year period. *Lung cancer* 2010; **68**(1): 111-4.
152. Peifer M, Fernandez-Cuesta L, Sos ML, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. *Nature genetics* 2012; **44**(10): 1104-10.
153. Rudin CM, Poirier JT, Byers LA, et al. Molecular subtypes of small cell lung cancer: a synthesis of human and mouse model data. *Nature Reviews Cancer* 2019; **19**(5): 289-97.
154. Baine MK, Hsieh MS, Lai WV, et al. SCLC Subtypes Defined by ASCL1, NEUROD1, POU2F3, and YAP1: A Comprehensive Immunohistochemical and Histopathologic Characterization. *J Thorac Oncol* 2020; **15**(12): 1823-35.
155. Gay CM, Stewart CA, Park EM, et al. Patterns of transcription factor programs and immune pathway activation define four major subtypes of SCLC with distinct therapeutic vulnerabilities. *Cancer Cell* 2021; **39**(3): 346-60 e7.

156. Horn L, Mansfield AS, Szczesna A, et al. First-Line Atezolizumab plus Chemotherapy in Extensive-Stage Small-Cell Lung Cancer. *N Engl J Med* 2018; **379**(23): 2220-9.
157. Paz-Ares L, Dvorkin M, Chen Y, et al. Durvalumab plus platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial. *Lancet* 2019; **394**(10212): 1929-39.
158. Marine JC, Dawson SJ, Dawson MA. Non-genetic mechanisms of therapeutic resistance in cancer. *Nat Rev Cancer* 2020; **20**(12): 743-56.
159. Dagogo-Jack I, Shaw AT. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol* 2018; **15**(2): 81-94.
160. Navin NE. Tumor evolution in response to chemotherapy: phenotype versus genotype. *Cell Rep* 2014; **6**(3): 417-9.
161. Warde P, Payne D. Does thoracic irradiation improve survival and local control in limited-stage small-cell carcinoma of the lung? A meta-analysis. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 1992; **10**(6): 890-5.
162. Pignon JP, Arriagada R, Ihde DC, et al. A meta-analysis of thoracic radiotherapy for small-cell lung cancer. *N Engl J Med* 1992; **327**(23): 1618-24.
163. Slotman BJ, van Tinteren H, Praag JO, et al. Use of thoracic radiotherapy for extensive stage small-cell lung cancer: a phase 3 randomised controlled trial. *Lancet* 2015; **385**(9962): 36-42.
164. Faivre-Finn C, Snee M, Ashcroft L, et al. Concurrent once-daily versus twice-daily chemoradiotherapy in patients with limited-stage small-cell lung cancer (CONVERT): an open-label, phase 3, randomised, superiority trial. *The Lancet Oncology* 2017; **18**(8): 1116-25.
165. Dingemans AC, Fruh M, Ardizzoni A, et al. Small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up(). *Ann Oncol* 2021; **32**(7): 839-53.
166. Gronberg BH, Killingberg KT, Flotten O, et al. High-dose versus standard-dose twice-daily thoracic radiotherapy for patients with limited stage small-cell lung cancer: an open-label, randomised, phase 2 trial. *The Lancet Oncology* 2021; **22**(3): 321-31.
167. Auperin A, Arriagada R, Pignon JP, et al. Prophylactic cranial irradiation for patients with small-cell lung cancer in complete remission. Prophylactic Cranial Irradiation Overview Collaborative Group. *N Engl J Med* 1999; **341**(7): 476-84.
168. Slotman B, Faivre-Finn C, Kramer G, et al. Prophylactic cranial irradiation in extensive small-cell lung cancer. *N Engl J Med* 2007; **357**(7): 664-72.
169. Edelman MJ. Prophylactic Cranial Irradiation for Small-Cell Lung Cancer: Time for a Reassessment. *Am Soc Clin Oncol Educ Book* 2020; **40**: 24-8.
170. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer* 2012; **12**(4): 252-64.

171. Dong H, Strome SE, Salomao DR, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* 2002; **8**(8): 793-800.
172. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000; **192**(7): 1027-34.
173. Antonia SJ, Lopez-Martin JA, Bendell J, et al. Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): a multicentre, open-label, phase 1/2 trial. *The Lancet Oncology* 2016; **17**(7): 883-95.
174. Pujol JL, Greillier L, Audigier-Valette C, et al. A Randomized Non-Comparative Phase II Study of Anti-Programmed Cell Death-Ligand 1 Atezolizumab or Chemotherapy as Second-Line Therapy in Patients With Small Cell Lung Cancer: Results From the IFCT-1603 Trial. *J Thorac Oncol* 2019; **14**(5): 903-13.
175. Spigel DR, Vicente D, Ciuleanu TE, et al. Second-line nivolumab in relapsed small-cell lung cancer: CheckMate 331(). *Ann Oncol* 2021; **32**(5): 631-41.
176. Rudin CM, Awad MM, Navarro A, et al. Pembrolizumab or Placebo Plus Etoposide and Platinum as First-Line Therapy for Extensive-Stage Small-Cell Lung Cancer: Randomized, Double-Blind, Phase III KEYNOTE-604 Study. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2020; **38**(21): 2369-79.
177. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. *Nature* 2013; **500**(7463): 415-21.
178. Johnson DB, Frampton GM, Rioth MJ, et al. Targeted Next Generation Sequencing Identifies Markers of Response to PD-1 Blockade. *Cancer Immunol Res* 2016; **4**(11): 959-67.
179. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 2015; **348**(6230): 124-8.
180. Liu SV, Reck M, Mansfield AS, et al. Updated Overall Survival and PD-L1 Subgroup Analysis of Patients With Extensive-Stage Small-Cell Lung Cancer Treated With Atezolizumab, Carboplatin, and Etoposide (IMpower133). *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2021; **39**(6): 619-30.
181. Ferrell BR, Temel JS, Temin S, et al. Integration of Palliative Care Into Standard Oncology Care: American Society of Clinical Oncology Clinical Practice Guideline Update. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2017; **35**(1): 96-112.
182. Trigo J, Subbiah V, Besse B, et al. Lurbinectedin as second-line treatment for patients with small-cell lung cancer: a single-arm, open-label, phase 2 basket trial. *The Lancet Oncology* 2020; **21**(5): 645-54.
183. IASLC. Lurbinectedin Does Not Improve Overall Survival In SCLC-- Some Positive Action in Combination with Doxorubicin after Chemotherapy (Press release). 09/09/2021. https://www.iaslc.org/iaslc-news/press-release/lurbinectedin-does-not-improve-overall-survival-sclc-some-positive-action (accessed 14/03/2022).

184. Rudin CM, Pietanza MC, Bauer TM, et al. Rovalpituzumab tesirine, a DLL3-targeted antibody-drug conjugate, in recurrent small-cell lung cancer: a first-in-human, first-in-class, open-label, phase 1 study. *The Lancet Oncology* 2017; **18**(1): 42-51.
185. Blackhall F, Jao K, Greillier L, et al. Efficacy and Safety of Rovalpituzumab Tesirine Compared With Topotecan as Second-Line Therapy in DLL3-High SCLC: Results From the Phase 3 TAHOE Study. *J Thorac Oncol* 2021; **16**(9): 1547-58.
186. Johnson ML, Zvirbule Z, Laktionov K, et al. Rovalpituzumab Tesirine as a Maintenance Therapy After First-Line Platinum-Based Chemotherapy in Patients With Extensive-Stage-SCLC: Results From the Phase 3 MERU Study. *J Thorac Oncol* 2021; **16**(9): 1570-81.
187. Thomas A, Mian I, Tlemsani C, et al. Clinical and Genomic Characteristics of Small Cell Lung Cancer in Never Smokers: Results From a Retrospective Multicenter Cohort Study. *Chest* 2020; **158**(4): 1723-33.
188. Aarts MJ, Aerts JG, van den Borne BE, Biesma B, Lemmens VE, Kloover JS. Comorbidity in Patients With Small-Cell Lung Cancer: Trends and Prognostic Impact. *Clin Lung Cancer* 2015; **16**(4): 282-91.
189. Ball D, Thursfield V, Irving L, et al. Evaluation of the Simplified Comorbidity Score (Colinet) as a prognostic indicator for patients with lung cancer: a cancer registry study. *Lung cancer* 2013; **82**(2): 358-61.
190. Kuo YW, Jerng JS, Shih JY, Chen KY, Yu CJ, Yang PC. The prognostic value of the simplified comorbidity score in the treatment of small cell lung carcinoma. *J Thorac Oncol* 2011; **6**(2): 378-83.
191. Ferris MJ, Jiang R, Behera M, Ramalingam SS, Curran WJ, Higgins KA. Radiation Therapy Is Associated With an Increased Incidence of Cardiac Events in Patients with Small Cell Lung Cancer. *Int J Radiat Oncol Biol Phys* 2018; **102**(2): 383-90.
192. Islam KM, Jiang X, Anggondowati T, Lin G, Ganti AK. Comorbidity and Survival in Lung Cancer Patients. *Cancer Epidemiol Biomarkers Prev* 2015; **24**(7): 1079-85.
193. Halvorsen TO, Sundstrom S, Flotten O, et al. Comorbidity and outcomes of concurrent chemo- and radiotherapy in limited disease small cell lung cancer. *Acta Oncol* 2016; **55**(11): 1349-54.
194. Janssen-Heijnen ML, Lemmens VE, van den Borne BE, Biesma B, Oei SB, Coebergh JW. Negligible influence of comorbidity on prognosis of patients with small cell lung cancer: a population-based study in the Netherlands. *Crit Rev Oncol Hematol* 2007; **62**(2): 172-8.
195. Noguchi T, Mochizuki H, Yamazaki M, et al. A retrospective analysis of clinical outcomes of patients older than or equal to 80 years with small cell lung cancer. *J Thorac Oncol* 2010; **5**(7): 1081-7.
196. Colinet B, Jacot W, Bertrand D, et al. A new simplified comorbidity score as a prognostic factor in non-small-cell lung cancer patients: description and comparison with the Charlson's index. *Br J Cancer* 2005; **93**(10): 1098-105.
197. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; **40**(5): 373-83.

198. Sarfati D, Koczwara B, Jackson C. The impact of comorbidity on cancer and its treatment. *CA Cancer J Clin* 2016; **66**(4): 337-50.
199. Zamorano JL, Lancellotti P, Rodriguez Munoz D, et al. 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). *Eur Heart J* 2016; **37**(36): 2768-801.
200. Herrmann J. Adverse cardiac effects of cancer therapies: cardiotoxicity and arrhythmia. *Nat Rev Cardiol* 2020; **17**(8): 474-502.
201. Hu JR, Florido R, Lipson EJ, et al. Cardiovascular toxicities associated with immune checkpoint inhibitors. *Cardiovasc Res* 2019; **115**(5): 854-68.
202. Banfill K, Giuliani M, Aznar M, et al. Cardiac Toxicity of Thoracic Radiotherapy: Existing Evidence and Future Directions. *J Thorac Oncol* 2021; **16**(2): 216-27.
203. Kirkwood BR, Sterne JAC. Essential medical statistics: Blackwell Science; 2003.
204. Basch E, Abernethy AP, Mullins CD, et al. Recommendations for incorporating patient-reported outcomes into clinical comparative effectiveness research in adult oncology. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2012; **30**(34): 4249-55.
205. NCI. Common Terminology Criteria for Adverse Events v3.0 (CTCAE). 2006. https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf (accessed 14/03/2022).
206. Greaves M, Control of Anticoagulation Subcommittee of the S, Standardization Committee of the International Society of T, Haemostasis. Limitations of the laboratory monitoring of heparin therapy. Scientific and Standardization Committee Communications: on behalf of the Control of Anticoagulation Subcommittee of the Scientific and Standardization Committee of the International Society of Thrombosis and Haemostasis. *Thromb Haemost* 2002; **87**(1): 163-4.
207. Sanfelippo MJ, Tillema VB. Automated assay for fondaparinux (Arixtra) on the Dade Behring BCS XP. *Am J Clin Pathol* 2009; **132**(4): 608-12.
208. Thomas O, Lybeck E, Strandberg K, Tynngard N, Schott U. Monitoring low molecular weight heparins at therapeutic levels: dose-responses of, and correlations and differences between aPTT, anti-factor Xa and thrombin generation assays. *PloS one* 2015; **10**(1): e0116835.
209. Gerotziafas GT, Petropoulou AD, Verdy E, Samama MM, Elalamy I. Effect of the anti-factor Xa and anti-factor IIa activities of low-molecular-weight heparins upon the phases of thrombin generation. *Journal of thrombosis and haemostasis : JTH* 2007; **5**(5): 955-62.
210. Szelke H, Schubel S, Harenberg J, Kramer R. A fluorescent probe for the quantification of heparin in clinical samples with minimal matrix interference. *Chem Commun (Camb)* 2010; **46**(10): 1667-9.
211. Warttinger U, Giese C, Harenberg J, Holmer E, Kramer R. A fluorescent probe assay (Heparin Red) for direct detection of heparins in human plasma. *Anal Bioanal Chem* 2016; **408**(28): 8241-51.

212. Assarsson E, Lundberg M, Holmquist G, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PloS one* 2014; **9**(4): e95192.
213. Khorana AA, Francis CW, Menzies KE, et al. Plasma tissue factor may be predictive of venous thromboembolism in pancreatic cancer. *Journal of thrombosis and haemostasis : JTH* 2008; **6**(11): 1983-5.
214. Hisada Y, Alexander W, Kasthuri R, et al. Measurement of microparticle tissue factor activity in clinical samples: A summary of two tissue factor-dependent FXa generation assays. *Thromb Res* 2016; **139**: 90-7.
215. Hemker HC, Giesen P, Al Dieri R, et al. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiol Haemost Thromb* 2003; **33**(1): 4-15.
216. Al Dieri R, de Laat B, Hemker HC. Thrombin generation: what have we learned? *Blood Rev* 2012; **26**(5): 197-203.
217. Depasse F, Binder NB, Mueller J, et al. Thrombin generation assays are versatile tools in blood coagulation analysis: A review of technical features, and applications from research to laboratory routine. *Journal of thrombosis and haemostasis : JTH* 2021; **19**(12): 2907-17.
218. Lacroix R, Dignat-George F. Microparticles as a circulating source of procoagulant and fibrinolytic activities in the circulation. *Thromb Res* 2012; **129 Suppl 2**: S27-9.
219. Owens AP, 3rd, Mackman N. Microparticles in hemostasis and thrombosis. *Circ Res* 2011; **108**(10): 1284-97.
220. Hinson JP, Kapas S, Smith DM. Adrenomedullin, a multifunctional regulatory peptide. *Endocr Rev* 2000; **21**(2): 138-67.
221. Garayoa M, Martinez A, Lee S, et al. Hypoxia-inducible factor-1 (HIF-1) up-regulates adrenomedullin expression in human tumor cell lines during oxygen deprivation: a possible promotion mechanism of carcinogenesis. *Mol Endocrinol* 2000; **14**(6): 848-62.
222. Larrayoz IM, Martinez-Herrero S, Garcia-Sanmartin J, Ochoa-Callejero L, Martinez A. Adrenomedullin and tumour microenvironment. *J Transl Med* 2014; **12**: 339.
223. Wong HK, Cheung TT, Cheung BM. Adrenomedullin and cardiovascular diseases. *JRSM Cardiovasc Dis* 2012; **1**(5).
224. Savic-Radojevic A, Pljesa-Ercegovac M, Matic M, Simic D, Radovanovic S, Simic T. Novel Biomarkers of Heart Failure. *Adv Clin Chem* 2017; **79**: 93-152.
225. Keleg S, Kayed H, Jiang X, et al. Adrenomedullin is induced by hypoxia and enhances pancreatic cancer cell invasion. *Int J Cancer* 2007; **121**(1): 21-32.
226. Nikitenko LL, Fox SB, Kehoe S, Rees MC, Bicknell R. Adrenomedullin and tumour angiogenesis. *Br J Cancer* 2006; **94**(1): 1-7.
227. Oehler MK, Hague S, Rees MC, Bicknell R. Adrenomedullin promotes formation of xenografted endometrial tumors by stimulation of autocrine growth and angiogenesis. *Oncogene* 2002; **21**(18): 2815-21.
228. Vazquez R, Riveiro ME, Berenguer-Daize C, et al. Targeting Adrenomedullin in Oncology: A Feasible Strategy With Potential as Much More Than an Alternative Anti-Angiogenic Therapy. *Front Oncol* 2020; **10**: 589218.

229. Deville JL, Bartoli C, Berenguer C, et al. Expression and role of adrenomedullin in renal tumors and value of its mRNA levels as prognostic factor in clear-cell renal carcinoma. *Int J Cancer* 2009; **125**(10): 2307-15.

230. Nouguerede E, Berenguer C, Garcia S, et al. Expression of adrenomedullin in human colorectal tumors and its role in cell growth and invasion in vitro and in xenograft growth in vivo. *Cancer Med* 2013; **2**(2): 196-207.

231. Pavel ME, Hoppe S, Papadopoulos T, et al. Adrenomedullin is a novel marker of tumor progression in neuroendocrine carcinomas. *Horm Metab Res* 2006; **38**(2): 112-8.

232. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Measurement of midregional proadrenomedullin in plasma with an immunoluminometric assay. *Clin Chem* 2005; **51**(10): 1823-9.

233. Campling BG, Sarda IR, Baer KA, et al. Secretion of atrial natriuretic peptide and vasopressin by small cell lung cancer. *Cancer* 1995; **75**(10): 2442-51.

234. Chute JP, Taylor E, Williams J, Kaye F, Venzon D, Johnson BE. A metabolic study of patients with lung cancer and hyponatremia of malignancy. *Clin Cancer Res* 2006; **12**(3 Pt 1): 888-96.

235. Sun NH, Wang SH, Liu JN, et al. The productions of atrial natriuretic peptide and arginine vasopressin in small cell lung cancer with brain metastases and their associations with hyponatremia. *Eur Rev Med Pharmacol Sci* 2017; **21**(18): 4104-12.

236. Kong X, Wang X, Xu W, et al. Natriuretic peptide receptor a as a novel anticancer target. *Cancer Res* 2008; **68**(1): 249-56.

237. Nojiri T, Hosoda H, Tokudome T, et al. Atrial natriuretic peptide prevents cancer metastasis through vascular endothelial cells. *Proc Natl Acad Sci U S A* 2015; **112**(13): 4086-91.

238. Vesely BA, Song S, Sanchez-Ramos J, et al. Five cardiac hormones decrease the number of human small-cell lung cancer cells. *Eur J Clin Invest* 2005; **35**(6): 388-98.

239. Morgenthaler NG, Struck J, Thomas B, Bergmann A. Immunoluminometric assay for the midregion of pro-atrial natriuretic peptide in human plasma. *Clin Chem* 2004; **50**(1): 234-6.

240. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. *Clin Chem* 2006; **52**(1): 112-9.

241. Kam PC, Williams S, Yoong FF. Vasopressin and terlipressin: pharmacology and its clinical relevance. *Anaesthesia* 2004; **59**(10): 993-1001.

242. Hermes A, Waschki B, Reck M. Hyponatremia as prognostic factor in small cell lung cancer--a retrospective single institution analysis. *Respir Med* 2012; **106**(6): 900-4.

243. Umemura S, Segawa Y, Ueoka H, et al. Serum level of arginine-vasopressin influences the prognosis of extensive-disease small-cell lung cancer. *J Cancer Res Clin Oncol* 2007; **133**(8): 519-24.

244. Garona J, Pifano M, Orlando UD, et al. The novel desmopressin analogue [V4Q5]dDAVP inhibits angiogenesis, tumour growth and metastases in vasopressin type 2 receptor-expressing breast cancer models. *Int J Oncol* 2015; **46**(6): 2335-45.

245. Garona J, Sobol NT, Pifano M, et al. Preclinical Efficacy of [V4 Q5]dDAVP, a Second Generation Vasopressin Analog, on Metastatic Spread and Tumor-Associated Angiogenesis in Colorectal Cancer. *Cancer Res Treat* 2019; **51**(2): 438-50.
246. Neuhold S, Huelsmann M, Strunk G, et al. Comparison of copeptin, B-type natriuretic peptide, and amino-terminal pro-B-type natriuretic peptide in patients with chronic heart failure: prediction of death at different stages of the disease. *J Am Coll Cardiol* 2008; **52**(4): 266-72.
247. Stoiser B, Mortl D, Hulsmann M, et al. Copeptin, a fragment of the vasopressin precursor, as a novel predictor of outcome in heart failure. *Eur J Clin Invest* 2006; **36**(11): 771-8.
248. Dudek M, Kaluzna-Oleksy M, Migaj J, Straburzynska-Migaj E. Clinical value of soluble ST2 in cardiology. *Adv Clin Exp Med* 2020; **29**(10): 1205-10.
249. Mueller T, Jaffe AS. Soluble ST2--analytical considerations. *Am J Cardiol* 2015; **115**(7 Suppl): 8B-21B.
250. Pascual-Figal DA, Januzzi JL. The biology of ST2: the International ST2 Consensus Panel. *Am J Cardiol* 2015; **115**(7 Suppl): 3B-7B.
251. Akimoto M, Maruyama R, Takamaru H, Ochiya T, Takenaga K. Soluble IL-33 receptor sST2 inhibits colorectal cancer malignant growth by modifying the tumour microenvironment. *Nat Commun* 2016; **7**: 13589.
252. Lu DP, Zhou XY, Yao LT, et al. Serum soluble ST2 is associated with ER-positive breast cancer. *BMC Cancer* 2014; **14**: 198.
253. Van der Jeught K, Sun Y, Fang Y, et al. ST2 as checkpoint target for colorectal cancer immunotherapy. *JCI Insight* 2020; **5**(9).
254. Enhorning S, Tasevska I, Roussel R, et al. Effects of hydration on plasma copeptin, glycemia and gluco-regulatory hormones: a water intervention in humans. *Eur J Nutr* 2019; **58**(1): 315-24.
255. Begg CB. In Defense of P Values. *JNCI Cancer Spectr* 2020; **4**(2): pkaa012.
256. Gao J. P-values - a chronic conundrum. *BMC Med Res Methodol* 2020; **20**(1): 167.
257. Groen HJM, van der Heijden E, Klinkenberg TJ, et al. Randomised phase 3 study of adjuvant chemotherapy with or without nadroparin in patients with completely resected non-small-cell lung cancer: the NVALT-8 study. *Br J Cancer* 2019; **121**(5): 372-7.
258. van Doormaal FF, Di Nisio M, Otten HM, Richel DJ, Prins M, Buller HR. Randomized trial of the effect of the low molecular weight heparin nadroparin on survival in patients with cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2011; **29**(15): 2071-6.
259. Farge D, Frere C, Connors JM, et al. 2019 international clinical practice guidelines for the treatment and prophylaxis of venous thromboembolism in patients with cancer. *The Lancet Oncology* 2019; **20**(10): e566-e81.
260. Bach AG, Schmoll HJ, Beckel C, et al. Pulmonary embolism in oncologic patients: frequency and embolus burden of symptomatic and unsuspected events. *Acta Radiol* 2014; **55**(1): 45-53.

261. Singh R, Sousou T, Mohile S, Khorana AA. High rates of symptomatic and incidental thromboembolic events in gastrointestinal cancer patients. *Journal of thrombosis and haemostasis : JTH* 2010; **8**(8): 1879-81.
262. Moik F, Ay C, Pabinger I. Risk prediction for cancer-associated thrombosis in ambulatory patients with cancer: past, present and future. *Thromb Res* 2020; **191 Suppl 1**: S3-S11.
263. Ryden L, Grabau D, Schaffner F, Jonsson PE, Ruf W, Belting M. Evidence for tissue factor phosphorylation and its correlation with protease-activated receptor expression and the prognosis of primary breast cancer. *Int J Cancer* 2010; **126**(10): 2330-40.
264. Koomagi R, Volm M. Tissue-factor expression in human non-small-cell lung carcinoma measured by immunohistochemistry: correlation between tissue factor and angiogenesis. *Int J Cancer* 1998; **79**(1): 19-22.
265. Amirkhosravi A, Meyer T, Amaya M, et al. The role of tissue factor pathway inhibitor in tumor growth and metastasis. *Semin Thromb Hemost* 2007; **33**(7): 643-52.
266. Osterberg L, Blaschke T. Adherence to medication. *N Engl J Med* 2005; **353**(5): 487-97.
267. Nieuwlaat R, Wilczynski N, Navarro T, et al. Interventions for enhancing medication adherence. *Cochrane Database Syst Rev* 2014; (11): CD000011.
268. Feinstein AR. On white-coat effects and the electronic monitoring of compliance. *Arch Intern Med* 1990; **150**(7): 1377-8.
269. Cramer JA, Scheyer RD, Mattson RH. Compliance declines between clinic visits. *Arch Intern Med* 1990; **150**(7): 1509-10.
270. Bourcy M, Suarez-Carmona M, Lambert J, et al. Tissue Factor Induced by Epithelial-Mesenchymal Transition Triggers a Procoagulant State That Drives Metastasis of Circulating Tumor Cells. *Cancer Res* 2016; **76**(14): 4270-82.
271. Coleman RL, Lorusso D, Gennigens C, et al. Efficacy and safety of tisotumab vedotin in previously treated recurrent or metastatic cervical cancer (innovaTV 204/GOG-3023/ENGOT-cx6): a multicentre, open-label, single-arm, phase 2 study. *The Lancet Oncology* 2021; **22**(5): 609-19.
272. Jayson GC, Gallagher JT. Heparin oligosaccharides: inhibitors of the biological activity of bFGF on Caco-2 cells. *Br J Cancer* 1997; **75**(1): 9-16.
273. Featherby S, Madkhali Y, Maraveyas A, Ettelaie C. Apixaban Suppresses the Release of TF-Positive Microvesicles and Restrains Cancer Cell Proliferation through Directly Inhibiting TF-fVIIa Activity. *Thromb Haemost* 2019; **119**(9): 1419-32.
274. Najidh S, Versteeg HH, Buijs JT. A systematic review on the effects of direct oral anticoagulants on cancer growth and metastasis in animal models. *Thromb Res* 2020; **187**: 18-27.
275. Carrier M, Abou-Nassar K, Mallick R, et al. Apixaban to Prevent Venous Thromboembolism in Patients with Cancer. *N Engl J Med* 2019; **380**(8): 711-9.
276. Khorana AA, Soff GA, Kakkar AK, et al. Rivaroxaban for Thromboprophylaxis in High-Risk Ambulatory Patients with Cancer. *N Engl J Med* 2019; **380**(8): 720-8.

277. Kuderer NM, Poniewierski MS, Culakova E, et al. Predictors of Venous Thromboembolism and Early Mortality in Lung Cancer: Results from a Global Prospective Study (CANTARISK). *Oncologist* 2018; **23**(2): 247-55.

278. Tesselaar ME, Romijn FP, Van Der Linden IK, Prins FA, Bertina RM, Osanto S. Microparticle-associated tissue factor activity: a link between cancer and thrombosis? *Journal of thrombosis and haemostasis : JTH* 2007; **5**(3): 520-7.

279. Hernandez C, Orbe J, Roncal C, et al. Tissue factor expressed by microparticles is associated with mortality but not with thrombosis in cancer patients. *Thromb Haemost* 2013; **110**(3): 598-608.

280. Zwicker JI, Liebman HA, Bauer KA, et al. Prediction and prevention of thromboembolic events with enoxaparin in cancer patients with elevated tissue factor-bearing microparticles: a randomized-controlled phase II trial (the Microtec study). *Br J Haematol* 2013; **160**(4): 530-7.

281. Kearley J, Silver JS, Sanden C, et al. Cigarette smoke silences innate lymphoid cell function and facilitates an exacerbated type I interleukin-33-dependent response to infection. *Immunity* 2015; **42**(3): 566-79.

282. Portal-Nunez S, Shankavaram UT, Rao M, et al. Aryl hydrocarbon receptor-induced adrenomedullin mediates cigarette smoke carcinogenicity in humans and mice. *Cancer Res* 2012; **72**(22): 5790-800.