Testicular cancer – response adapted treatment, prognostic markers and survivorship issues

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2015

Testicular cancer – response adapted treatment, prognostic markers and survivorship issues

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LUND
UNIVERSITY

DOCTORAL DISSERTATION
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To be defended at the Lecture Hall, Department of Radiotherapy, 3rd floor, Department of Oncology, Skane University Hospital, on Thursday, November 26 2015, at 1.00 PM.

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Organization:	Document name:
LUND UNIVERSITY, Department of Clinical sciences, Lund, Oncology and Pathology, Sweden	Doctoral dissertation
	Date of issue: 2015-11-26
Author: Sven-Erik Olofsson	Sponsoring organization:

Title: Testicular cancer – response adapted treatment, prognostic markers and survivorship issues

Abstract

In the US and most European countries, testicular cancer is the most common malignancy in young men aged 20-40 years. Since the introduction of cisplatin-based treatment in the 1970s, more than 95% of the patients are cured. The increasing incidence of testicular cancer and high survival rates, has led to a growing number of testicular cancer survivors (TCSs). As they have a long life expectancy it's important to minimize the treatment burden without compromising outcome in order to minimize the risk of late toxicity.

In the first study the aim was to evaluate the SWENOTECA IV (Swedish-Norwegian Testicular Cancer Group) treatment strategy for patients with metastatic NSGCT with respect to outcome. The protocol was designed for early identification of patients, in whom the response to two standard chemotherapy courses was inadequate and to provide intensified treatment to these individuals. Tumor marker decline and, for patients with marker negative disease, radiological assessment were to be used for response evaluation. The conclusion was that with detailed treatment protocols and a dedicated collaborative group of specialists, treatment results comparable to those reported from large single institutions can be achieved at a national level. The survival of intermediate risk patients is remarkable and close to that of good risk patients.

To investigate if testicular cancer survivors (TCSs) have a higher incidence of work loss compared with the general population, accounting for stage, treatment and relapse a cohort of TCSs was identified in the SWENOTECA register, and compared to matched population comparators. Prospectively recorded work loss data was obtained from national registers. Adjusted relative risks (RR) and 95% confidence intervals (CI) of sick leave and/or disability pension were calculated annually and overall with Poisson- and Cox regression, censoring at relapse. The mean number of annual work days lost was also estimated. The result indicated that extensively treated TCSs, but not those on surveillance or limited treatment, are at increased risk of work loss long-term, not explained by relapse. These patients may benefit from early rehabilitation initiatives.

Expression of the RNA-binding motif protein 3 (RBM3) has been shown to correlate with favourable clinicopathological parameters and prognosis in several cancer diseases. The aim of the study was to examine the expression and prognostic ability of RBM3 in patients with testicular non-seminomatous germ cell tumors (NSGCT). Low RBM3 expression was a predictor of treatment failure in metastatic NSGCT, in relation to the prognostic factors included in the International Germ Cell Consensus Classification (IGCCC). These findings suggest that RBM3 may be a potential biomarker for treatment stratification in patients with metastatic non-seminomatous germ cell tumors, and therefore merit further validation.

Key words testicular cancer, response adapted treatment, prognostic markers, survivorship, work ability, work loss, RBM3

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language English
ISSN and key title 1652-8220		ISBN 978-91-7619-131-6
Recipient's notes	Number of pages 101	Price
	Security classification	

Signature _____Date _____

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DOCTORAL DISSERTATION

Department of Clinical Sciences, Division of Oncology and Pathology
Lund University, Faculty of Medicine Doctoral Dissertion Series 2015:52
ISBN 978-91-7619-131-6
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2015


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Thesis at a glance

Paper	Aim	Method	Results and conclusions
I	To evaluate the outcome for 603 adult patients from Sweden and Norway with metastatic testicular NSGCT who were included prospectively from 1995 to 2003 in the population-based SWENOTECA IV study.	The strategy was to individualize treatment by using a response adapted strategy by evaluating tumor marker decline and radiological response after two courses of standard BEP and then select further therapy.	- 99% of all eligible patients during the period were included in the protocol. - Median follow up was 8.2 years. 10-year overall survival according to IGCCCG: - Good prognosis 94.7% - Intermediate prognosis 90.0% - Poor prognosis 67.4% With a detailed treatment protocol and a dedicated collaborative group of specialists, treatment results were comparable to those reported from large single institutions.
II	To investigate if testicular cancer survivors have a higher incidence of work loss compared with the general population, accounting for stage, treatment and relapse.	A cohort of Swedish testicular cancer survivors was identified in the SWENOTECA database and matched to population comparators. Prospectively recorded work loss data (both before and after diagnosis) were obtained from national registers	- Extensively treated TCSs have an increased risk of work loss. - Patients on surveillance or receiving limited treatment have no increased risk of work loss. The extensively treated TCSs may benefit from early rehabilitation initiatives.
III	To examine the expression and prognostic ability of RBM3 in patients with testicular non-seminomatous germ cell tumors.	Immunohistochemical RBM3 expression was analysed in tissue microarrays. Associations between RBM3 expression and clinico-pathological parameters and prognostic ability were evaluated.	Significant associations were found between: - RBM3 expression and clinical stage in the entire cohort. - RBM3 expression and prognostic group for patients with metastatic disease. - FFS was significantly inferior for patients with low RBM3 expression. RBM3 may be a potential biomarker for patients with metastatic NSGCT.

List of papers

Papers included in this thesis

I. Population-based study of treatment guided by tumor marker decline in patients with metastatic non-seminomatous germ cell tumor: a report from the Swedish-Norwegian Testicular Cancer Group

Olofsson SE, Tandstad T, Jerkeman M, Dahl O, Ståhl O, Klepp O, Bremnes R M, Cohn-Cedermark G, Langberg C W, Laurell A, Solberg A, Stierner U, Wahlqvist R, Wijkström H, Anderson H, Cavallin-Ståhl E

J Clin Oncol 2011; 29: 2032-2039.

II. Sick leave and disability pension among Swedish testicular cancer survivors according to clinical stage and treatment.

Nord C*, **Olofsson SE***, Glimelius I, Cohn Cedermark G, Ekberg S, Cavallin-Ståhl E, Neovius M, Jerkeman M, Smedby KE

Acta Oncol April 2, 2015.

III. Low RBM3 protein expression correlates with clinical stage, prognostic classification and increased risk of treatment failure in testicular non-seminomatous germ cell cancer.

Olofsson SE, Nodin B, Gaber A, Eberhard J, Uhlén M, Jirström K, Jerkeman M

PLOS One March 26, 2015.

* Shared first authorship

Selected abbreviations

AFP	Alpha fetoprotein
ASR	Age-standardized incidence rate
ATM	Ataxia-telangiectasia mutated
ATR	Ataxia telangiectasia and Rad3-related
BEP	Bleomycin-Etoposide-Cisplatin
BEP-if	Bleomycin-Etoposide-Cisplatin-Ifosfamide
BIP	Bleomycin-induced pneumonitis
CE	Carboplatin, Etoposide
CI	Confidence intervals
CiS	Cancer in situ = Intratubular germ cell neoplasia
CR	Complete response
CS	Clinical stage
CSS	Cancer specific survival
CT	Computed tomography
CVD	Cardiovascular disease
DDR	DNA damage response system
EAU	European Association of Urology
ED	Erectile dysfunction
EDCs	Endocrine disrupting chemicals
EGCCCG	European Germ Cell Cancer Collaboration Group
EGCT	Extragonadal germ cell tumor
EMACO	Etoposide, methotrexate, actinomycin D, cyclofosfamide, vincristine
EORTC	European Organization for Research and Treatment of Cancer
EP	Etoposide, cisplatin
ESMO	European Society of Medical Oncology
FSH	Follicle-stimulating hormone
GFR	Glomular filtration rate
GOP	Gemcitabin, oxaliplatin, paclitaxel
β-HCG	Human chorionic gonadotropin-β
HDCT	High-dose chemotherapy with autologous stem cell support
HR	Hazard ratio
IGCCCG	International Germ Cell Cancer Collaboration Group
LBB	Liver, bone, brain
LH	Luteinizing hormone

LDH	Lactate dehydrogenase
LISA	Longitudinal Database on Education, Income and Occupation
MiDAS	Micro Data Analysis of Social Insurance database
miRNA	microRNA
Mk	Marker (tumor marker)
Mk-	Marker negative
Mk+	Marker positive
MRC	Medical Research Council
MRI	Magnetic resonance imaging
NI	Nuclear staining intensity
NS	Nuclear score
NSGCT	Non-seminomatous germ cell tumor
NPVM	Non-pulmonary visceral metastasis
OS	Overall survival
PEI	Cisplatin, etoposide, ifosfamide
PET	Positron emission tomography
PFAS	Perflourated substances
PFNA	Perfluorononanoic acid
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctanesulfonic acid
PGCs	Primordial germ cells
PLAP	Placental-like alkaline phosphatase
PFS	Progression-free survival
QoL	Quality of life
RBM3	RNA binding motif protein 3
RPLND	Retroperitoneal lymph node dissection
RR	Relative risk
RT	Radiotherapy
SWENOTECA	Swedish Norwegian Testicular Cancer
TC	Testicular cancer
TCSs	Testicular cancer survivors
TDS	Testicular dysgenesis syndrome
TGCT	Testicular germ cell tumors
TIP	Paclitaxel, ifosfamide, cisplatin
TMA	Tissue micro array
WHO	World Health Organization

Testicular germ cell tumors

This thesis is based upon three papers illustrating various aspects of testicular germ cell tumors (TGCT). The first paper reports the outcome for patients with metastatic non seminomatous germ cell tumor (NSGCT) treated according to the management program SWENOTECA IV. In the second paper, long term side effects of treatment are investigated in terms of sick leave and disability pension among testicular cancer survivors (TCSs). In the third article the expression of RNA binding motif protein 3 (RBM3) in testicular NSGCT is investigated and its prognostic ability evaluated.

Epidemiology

Testicular cancer (TC) is a rare disease but is the most common malignancy in young men aged 20-40 years, with a variation in incidence worldwide from below 1 to 12 per 100 000 males and year [1, 2]. The incidence is estimated to have doubled in the last 4 decades and there is a substantial variation between countries. High-income countries have a higher annual increase in testicular cancer incidence in comparison to low-income countries. While other cancers show an increasing incidence with age, the increase for TC starts around adolescence and peaks in the early thirties. NSGCT occurs earlier in life, with a peak between 20-35 years, compared to seminomatous germ cell tumors (SGCT) witch have a peak between 30-45 years.

In contrast to the increasing incidence, the mortality rate has declined dramatically over the last four decades mostly due to the introduction of cisplatin-based chemotherapy regimens [3, 4]. The decline in mortality varies and has been more pronounced in high-income countries and seems now been stabilized at a low level.

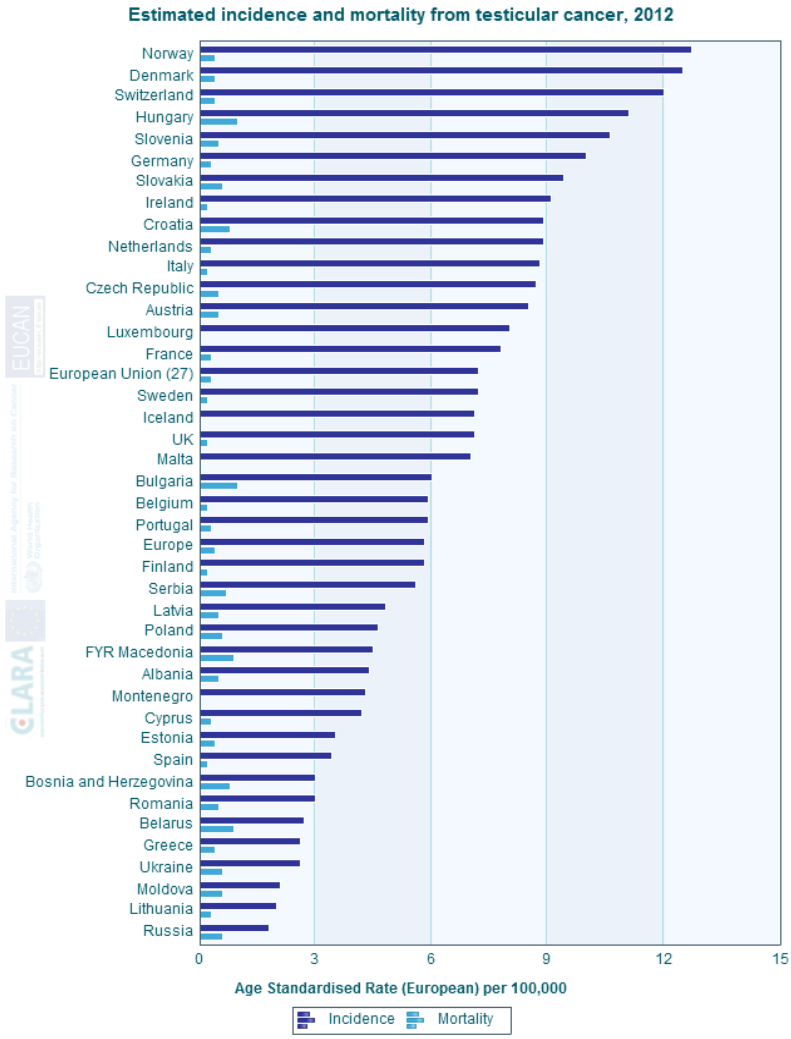


Figure 1. Age standardised incidence rate (European) per 100000 men and mortality 2012 [5].

Other factors that have helped to improve outcome are better diagnostic tools, standardized treatment guidelines, collaborative work groups specialized in testicular cancer and general improved quality of health care.

In Europe there was a wide variation in incidence in 2012, with an average incidence of 5.8 per 100 000 men (Figure 1) [5]. The incidence has stabilized for most high incidence countries (Sweden, Denmark and Norway not included) and is increasing in low and middle income countries [5, 6].

For the Nordic countries, the age-standardized incidence rate (ASR) (European) in 2013 was highest in Norway with 12.9 cases a year per 100.000 males, followed by Denmark 10.1, Sweden 7.8 and Finland 6.7 [6].

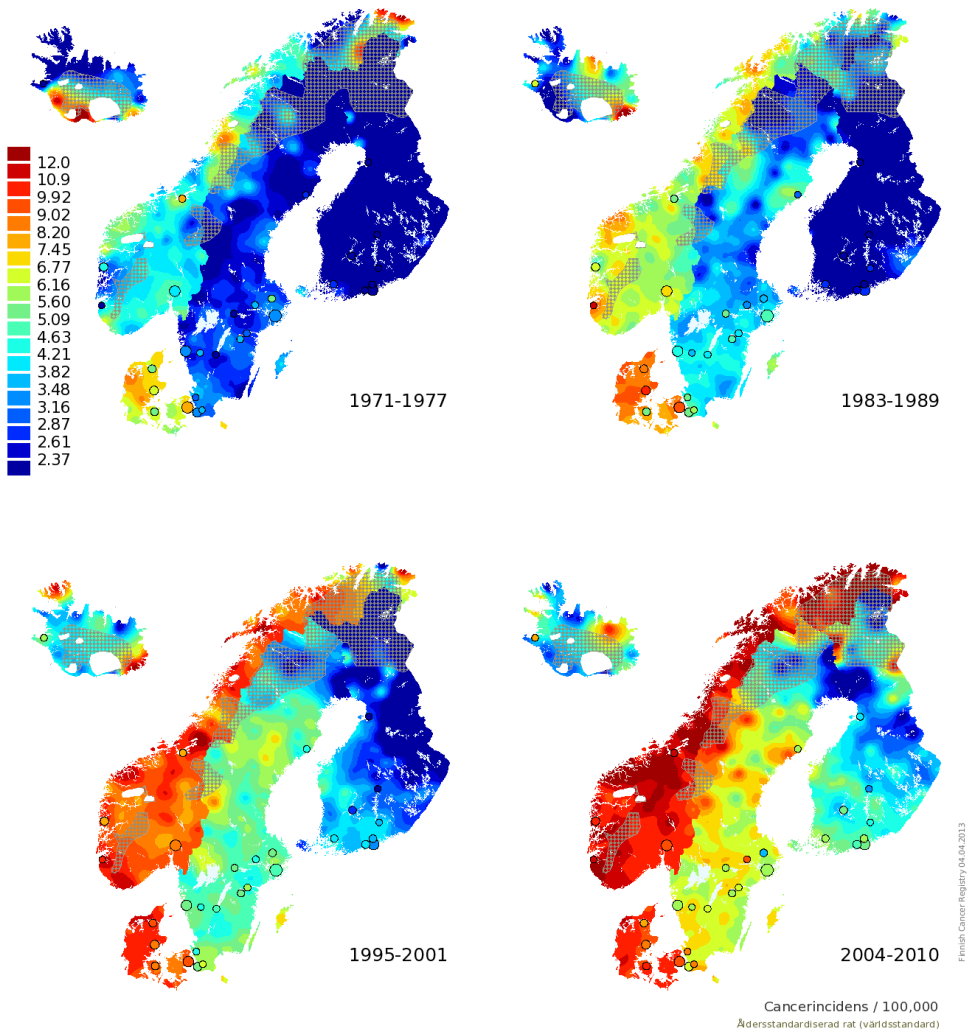


Figure 2. Testicular cancer incidence in the Nordic countries year 1971-1977, 1983-1989, 1995-2001 and 2004-2010 [7].

Figure 2 shows the geographical spread in incidence in the Nordic countries during 1971 to 2010 [7]. In Sweden TC represents approximately 1 percent of all new cancer cases and the increase during the last 10 years has been 2.3 % (Figure 3) [6].

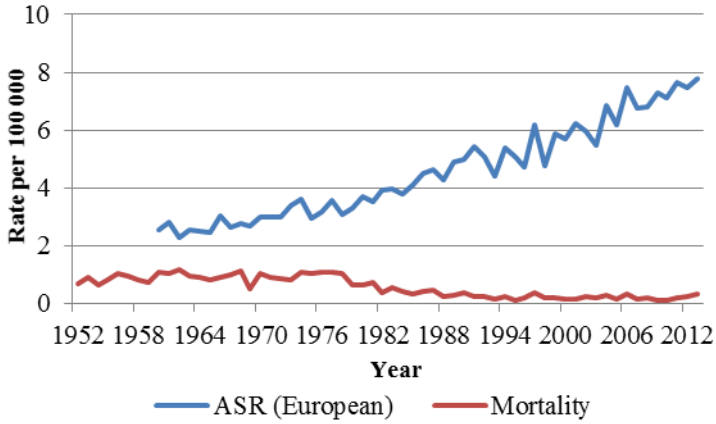


Figure 3. Age-standardized incidence per 100000 men (European) and mortality in Sweden from 1952 to 2013 [6].

The mortality rate has decreased with -3.2 % during the last decade (Figure 3) and the relative 5 year survival was 95% for patients diagnosed in the period of 2009-2013 [6]. Along with the increase in incidence, and the fact that more patients survive, the number of TCSs increases (prevalence) every year and in 2013, the number was 7712. The annual increase was during the years 1980 to 1990 between five and seven percent, but has now stabilized at around 3.5 percent during the last years (Figure 4) [6].

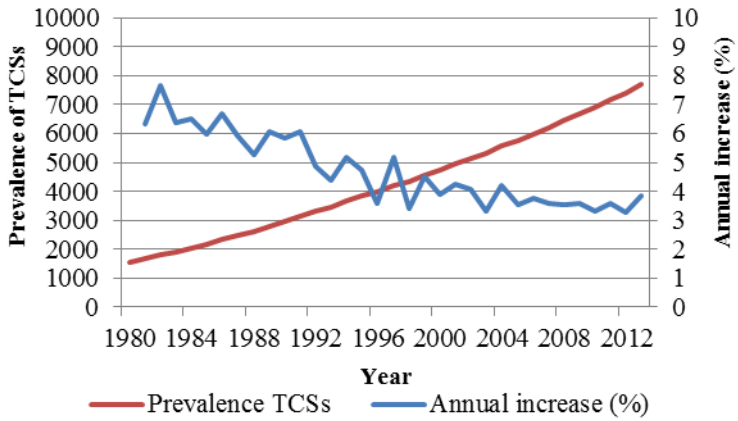


Figure 4. Prevalence of TCSs between 1980 and 2013 and the annual increase (%) [6].

Pathogenesis

Normal development of gonocytes

Primordial germ cells (PGCs) arise early in embryogenesis and migrate to the genital ridges that will develop into testes. The PGCs is now called gonocytes and will further maturate and lose their embryonic pluripotency markers (NANOG, SOX2, OCT3/4) and finally enter mitotic arrest and mature to pre-spermatogonia. (Figure 5) [8, 9]. At onset of puberty, the pre-spermatogonia undergo further maturation and enter meiosis to produce spermatozoa.

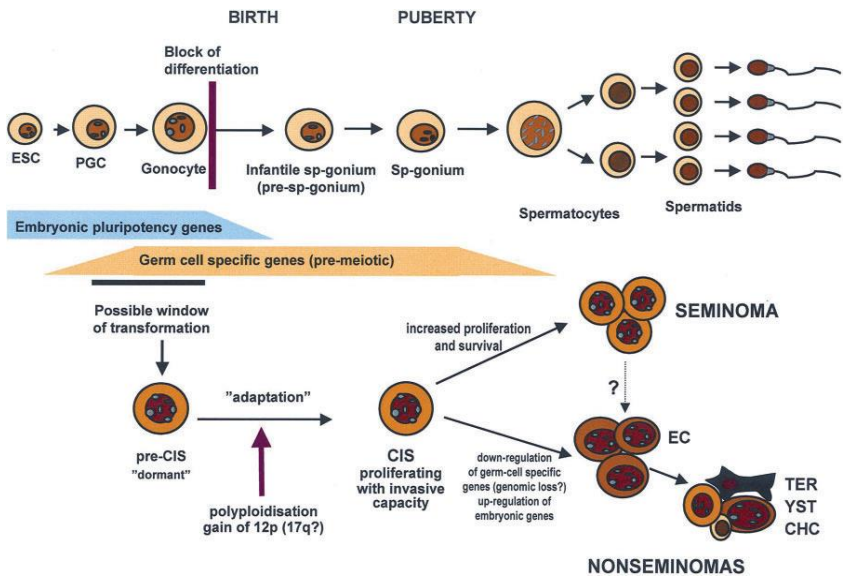


Figure 5. A scheme illustrating of the pathogenesis of testicular carcinoma in situ (CiS) in relation to germ cell differentiation. EC, embryonal carcinoma; TER, teratoma; YST, yolk sac tumor; CHC, choriocarcinoma. From [10], with permission from Oxford University Press

Development of CiS and invasive cancer

Most GCTs are believed to originate during the fetal period, with the development of intratubular germ cell neoplasia (CiS) that may lead to further malignant transformation. Fetal factors like environmental and genetic defects interfering with the differentiation of germ cells and development of the primordial germ cell are considered to be important factors for this transformation to occur.
CiS is found in approximately 5% to 9 % of the contralateral testicle in men with

TGCT [10-12]. If CiS is left untreated, 50 % of the cases will develop to invasive cancer within 5 years and there is a possibility that all of the cases will do so eventually [13, 14].

Studies indicate that CiS cells are gonocytes, which have failed to differentiate properly and then have underwent genomic alterations promoting survival and later invasive progression. The expression of embryonic genes can explain the pluripotency to differentiate to germ cell lineage (seminoma) or embryonal stem cell components (embryonal carcinoma), with possible further differentiation to choriocarcinoma, yolk sac tumors or teratoma (Figure 5) [10].

The development of CiS probably occurs during the process when PGC matures to gonocytes and prespermatogonia due to environmental and genetic factors or a combination of both. These disturbances may also lead to impaired fertility, cryptorchidism and hypospadias (gonadal dysgenesis).

The CiS cells display over expression of genes found in gonocytes [10]. The alterations often involve the amplification of 12p and thereby up-regulation of these genes, gain of genetic material with increased ploidy and genomic instability. These changes may promote proliferation, suppress apoptosis and facilitate the remaining steps for malignant transformation. The malignant transformation involves self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis [15, 16].

Several potential biomarkers detected by immunohistochemical analysis have been reported to discriminate CiS from invasive cancer such as HMGA1/2, OCT3/4, SOX2/17, NANOG, PATZ1, CKIT and Aurora-B [17-23]. Two markers, p53 and MIB-1, were shown to have prognostic ability and could predict the occurrence of progressive disease with approximately 50-60% sensitivity and 75-85% specificity [24].

DNA damage may be caused by oxygenation, methylating agents, UV-light, ionizing radiation and during normal processes like replication and recombination. In order to maintain genomic stability (preventing mutations to propagate and accumulate) the DNA has to be repaired by the DNA damage response system (DDR).

The p53 gene is a tumor suppressor gene that can hold the cell cycle on recognition of DNA damage and activate the DDR system (Fig 6).

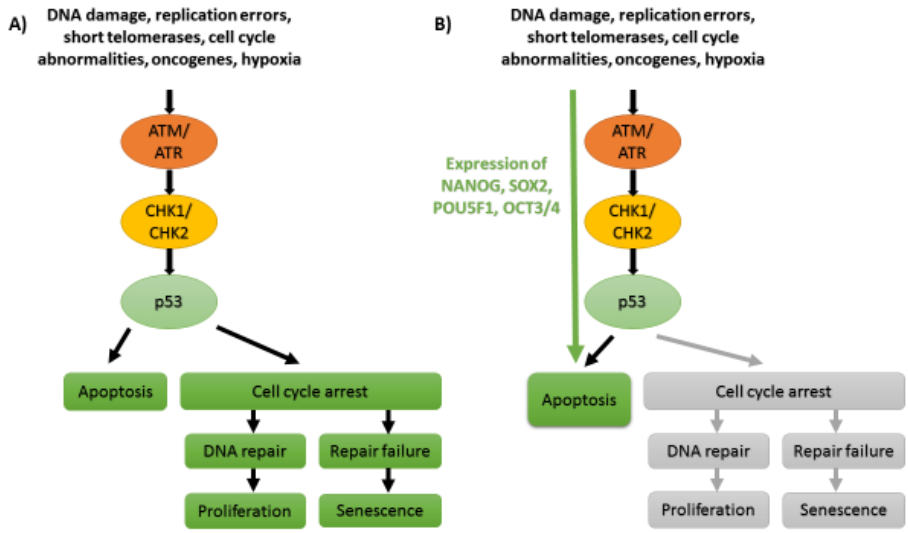


Figure 6. TP53 signalling. (A) The normal TP53 signalling – upon genotoxic stress, all the main TP53 executive pathways are activated, cells get arrested at cell cycle check points; after DNA repair the cells re-enter the cell cycle or in case of irreparable DNA damage they are directed to apoptosis. (B) The hypersensitive TP53 signalling in embryonic, germ cells and TGCTs - these cells lack the cell cycle arrest that would enable recognition and repair of DNA damage; pro-apoptotic factors are up-regulated and after its activation by genotoxic stress, p53 drives the cells directly to apoptosis. Modified from [9], with permission from Elsevier.

The DDR system activates cell functions leading to cell cycle arrest and increasing expression of DNA repair genes. DNA repair pathways differ depending on the type of DNA damage. The activated DDR pauses the cell cycle in the intra-S, G1/S and G2/M checkpoints in order to repair the DNA before continuing cell division. Mostly if the DNA repair is unsuccessful, the cells go into senescence and apoptotic pathways are initiated. The DNA damage checkpoint kinases ATM (ataxia-telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3-related) respond to different DNA alterations (Fig 6A). ATM is activated by double strand breaks and disruptions in chromatin structure and ATR by deviant replication structures. ATR activates Chk1 and ATM Chk2. Downstream, p53 is activated which induces apoptosis. The replication-associated damage induces apoptosis or senescence in case of repair failure or cell cycle arrest (Figure 6A) [25, 26] [27, 28].

When the DDR system has repaired the damage, the cell may pass through the checkpoints. In case of irreparable damage, p53 can initiate apoptosis and thereby eliminate cells that may harbour oncogenic changes or DNA damage [29]. A mutated inactivated p53 is present in about half of all malignant tumors and is associated with rapid tumor growth [9].

Another tumor suppressor is the Rb protein that suppresses gene transcription necessary for the cell to continue the cell cycle [30]. Dysregulation of the Rb gene may lead to accumulation of genetic changes involved in tumorigenesis. Studies indicate that Rb is down regulated due to transcriptional regulation changes both in CiS, TGCT (not teratoma) and normal gonocytes, which may enhance the pathway towards apoptosis [9].

As a barrier against cancer progression in pre-malignant lesions of several other types of solid tumors the DDR system is constitutively activated, but during progression into invasive cancer, the genes involved in DDR system are usually deregulated or inactivated [25, 31, 32]. The activated ATR⁄ATM system may serve as a barrier to constrain tumor development but over time there may be a selection for mutations in checkpoint genes like p53, Chk 2 and ATM [33]. The mutations may lead to the avoidance of senescence and reduction of apoptosis and there by leading to more genomic instability and tumor progression [28, 31, 32].

In germ cells there is modified activity of the DDR-system and apoptotic pathways such as altered ATM–CHK2–p53–p21 and ATM–CHK2–CDC25 pathways, lack of Rb-regulated G1 checkpoint arrest and hyper activation of apoptotic pathways (Fig 6 B) [34-36]. CiS lesions and TGCT have extremely low or completely deactivated DDR [33]. The events that drive the progression of CiS to TGCT is proposed to be related to the increased protein expression from chromosome 12p promoting gonocyte pluripotency, invasiveness and cell survival [37-40]. TGCT cells may keep the characteristics of p53-independent and p53-dependent DDR leading preferably to apoptosis and suppressing pathways leading to G1 or G2 arrests (Figure 6 B). In TGCT mutations of p53 are rare (up to 1–5%) and found mostly in seminomas [41-44]. Inactive wild-type p53 protein is often overexpressed and can accumulate in high levels in TGCT cells but can be reactivated in response to DNA damage [42-44]. Several proteins are involved in activation (Nutlin-3) and inactivation (MDM2, different micro RNAs) of p53 [45-51]. The upregulated p53 apoptotic pathway disappears with loss of stem cell gene expression (Figure 6B) [52].

Aetiology

The aetiology of TGCT is not fully understood. However, a birth cohort effect is seen associated with the increasing trends in incidence implicating that the risk is linked to the time period of birth [2]. The hypothesis is that maternal lifestyle factors (smoking, obesity and delayed childbearing) and environmental exposures (pollutions, food and chemicals) affecting the hormonal balance during the intrauterine period are linked to the increase in incidence.

Risk factors

Cryptorchidism

The prenatal exposure to estrogens may increase the risk of cryptorchidism, which is a risk factor with strong evidence for developing TGCTs, especially for seminoma, with an elevated risk of four times [53-55]. If an undescended testicle is corrected early, before the age 10 to 11, with orchiopexy, the risk decreases, indicating an environmental influence in testicular carcinogenesis [56, 57].
The role of infertility as a risk factor itself is uncertain but is linked to other risk factors such as cryptorchidism and gonadal dysgenesis.

Testicular dysgenesis syndrome

Testicular dysgenesis syndrome (TDS) is a concept including developmental disorders of the testis proposed by Skakkebæk in 2001 [58]. It suggests that impaired development of the fetal testes increases the risk of hypospadia, reduced spermatogenesis, cryptorchidism and development of TGCT. The risk of TDS is proposed to be related to factors in the fetal intrauterine environment and genetic predisposition separately or in combination. The increase in TDS has been suggested to be more related to environmental and maternal lifestyle factors than accumulation of genomic changes. The individual sensibility to environmental exposures may vary according to genomic polymorphisms in genes involved in steroid metabolism [9]. The clinical presentation may vary from minimal lowered spermatogenesis to having all symptoms.

Family history

There is a genetic predisposition for TGCT and approximately 2% of patients has a family history of TGCT [59-62]. A Swedish study showed that the RR of developing TGCT is 3.8 for sons to fathers with TGCT, and 8.3 for brothers to TCGT patients [63].

Another study has shown that first generation immigrants to Sweden have the same risk as the risk in their origin countries [64], whereas their sons have the same risk as native Swedes, supporting the hypothesis that environmental and lifestyle factors are linked to the risk of developing TGCT. Race has been a suggested risk factor since Caucasian American men have a five times higher incidence than African American men [54].

Environmental exposures

Endocrine disrupting chemicals (EDCs) like bisphenol A, phthalates, metals, polychlorinated biphenyls, and organochlorines interact with hormonal pathways and have been investigated for their linkage to testicular cancer. No firm conclusions can be drawn, due to lack of exposure assessment and to the difficulty in adjusting for possible prenatal factors [65]. A WHO/UNEP report identified EDCs as a global problem that will require global solutions as there are several hundreds of them and that they have contaminated the world via the natural flow of air and water [66]. EDCs have been measured in humans and wildlife, in remote places such as the Arctic, making it hard to examine an unexposed population anywhere on Earth.

Other studies have suggested that men that work in electrical occupations (electromagnetic radiation) and shift work (chronodisruption of hormonal cycles) have an increased risk for TGCT [65].

Exposure to the solvent dimethylformamide, has been suggested to be associated with testicular cancer, but most studies are based on job title only and not on specific assessment of solvent exposure [65].

PFAS are a group name for perflourated substances (PFOA, PFNA, PFOS) used in the industry since the beginning of the 1950-s, used in a large number of consumer products like Teflon treated products, impregnation for shoes and clothes, cleaning substances, fire extinguishing foam and surface treatment of food containers. One study found an increased risk of testicular cancer to PFOA exposure. The risk tended to increase according to the level of exposure to PFOA [67]. Fire fighters are also exposed to for respirable particulate matter and for several carcinogens, like benzene, benzopyrene, 1,3-butadiene, and formaldehyde. Epidemiologic studies of firefighters have noted increased cancer risks compared with the general population

for three types of cancer: testicular cancer, prostate cancer and non-Hodgkin lymphoma [68].

Other risk factors

Other factors associated with early development of TGCT are neonatal jaundice and low and high birth weights, trauma, orchitis, rural residence and higher socioeconomic status [69-72].

Genome-wide association studies have revealed three loci on chromosome 5, 6, and 12 that gives about a four-fold risk of developing TGCT [73, 74].

Patients previously treated for TGCT have an increased risk of a metachronous contralateral tumor, with a cumulative incidence at 20 to 25 years of 1.9–5.2% [75]. The risk is further elevated if the contralateral testis is atrophic or the patient is treated for cryptorchidism.

Summary

The major risk factors for TGCTs include a family history of testicular tumor (relative risk (RR) 4 to 6 for sons and 8 to 10 for brothers), cryptorchism (RR 4 to 8), previous testicular tumor (RR approximately 25) and sub/infertility (RR up to 20) [22, 76-78].

Biomarkers

The serum tumor markers alpha-fetoprotein (AFP), beta human chorionic gonadotropin (β-HCG), placental alkaline phosphatase (PLAP) and lactate dehydrogenase (LDH) are used in the management of TCGT. Approximately 80% of patients with NSGCT have elevated AFP and/or β-HCG at the time of diagnosis before orchiectomy and for patients with SGCT, β-HCG is elevated in 20-30% [79, 80]. Serum tumor markers levels at diagnosis and after orchiectomy are used in staging, for risk grouping, monitoring treatment efficacy and during follow up (FU) for detection of relapses. In a retrospective study including 2,483 CSI patients of which 19 % had a relapse [81], the relapse was detected by serum tumor marker elevation in 48 % of the NSGCT and in 3% of the SGCT cases. They are also used in the investigation of a cancer with unknown primary.

AFP

AFP is produced in yolk sac tumors and in 10%–20% of embryonal carcinomas but not by pure choriocarcinoma or pure seminoma. The half-life in serum is between five to seven days. AFP is elevated in 50-70 % of the cases with NSGCT [82, 83]. AFP levels are not elevated in SGCT, and in case an increased level of AFP is found, it should be classified and treated as a NSGCT.

Increased serum concentrations of AFP can also exist in hepatocellular carcinomas, gastric, pancreatic, colon and lung cancer. Other non-malignant causes of increased AFP levels are hepatitis or liver damage that can for example be induced by chemotherapy [83, 84]. It is the regeneration process in the liver that causes the elevation. Few patients constitutionally have a slightly elevated AFP level (≤ 1.5 upper limit).

β-HCG

Syncytiotrophoblastic cells produce β-HCG, and all patients with choriocarcinoma and 40% to 60% of patients with embryonal cell carcinoma have elevated serum levels of β-HCG [85]. Approximately 14% of the patients with stage I pure seminoma prior to orchiectomy and 15-20% of the patients with metastatic seminoma have elevated serum β-HCG and the level is typically below 500 U/l [85-88]. Increased levels of β-HCG is found in 20% of patients with NSGCT in clinical stage 1 (CS1) and in 40% of those with metastatic disease [84]. The serum half-life for β-HCG is 1.5 to three days [84].

Other cancers that have been associated with elevations in serum HCG levels in men are neuroendocrine tumors, bladder, kidney, prostate, lung, head and neck, and gastrointestinal cancer and haematological malignant disorders [89].

LDH

LDH is produced in all cells and leaks into the blood stream from dying and dead cells. LDH consists of five isoenzymes of which GCT usually express LDH isoenzyme1 [85]. LDH is elevated in 40 to 60 % of patients with TGCT but is a less specific marker than AFP and β-HCG [84]. Elevated values are correlated to tissue injury and may reflect growth rate and tumor burden [84, 90]. It can be elevated in several other tumors and due to non-malignant causes like liver failure, congestive heart failure, pancreatitis, haemolytic anemia, infections and muscular dystrophies etc. [85].

PLAP

PLAP is produced by the seminomatous component in TGCT and is elevated in 60 to 70 % of patients with SGCT [85]. Smokers may have increased levels of PLAP giving it a low sensitivity and specificity in these patients. PLAP is not routinely used at all treatment centres [85].

Other biomarkers

Measuring of circulating microRNA (miRNA) in the sera of patients with testicular cancer has been shown to be a non-invasive biomarker for detecting and monitoring disease [91, 92]. One study has shown a trend with higher levels in cases with more advanced disease [93]. Using a combination of four miRNAs, a sensitivity of 98% was found for patients with TGCT in a series of 80 patients and 40 controls [93].

In a study by Nastaly et al., circulating tumor cells were detected in 18% of 143 patients with GCT in all clinical stages (CS I 68%) [94]. Detection was correlated with higher tumor stage, tumor histology, increased tumor markers in serum and early relapses [94].

Diagnosis

In a series of 434 patients diagnosed with TGCT, half of the patients sought medical attention for a painless lump in the testicle and in 20 % of the cases the first clinical symptom was scrotal pain [80]. Severe testicular pain is rare but may occur in case of concurrent orchioepididymitis or tumor bleeding [95]. Approximately 25 % of the cases with metastatic disease present with symptoms from the involved sites. Retroperitoneal metastasis may give rise to back pain and vast pulmonary engagement can cause respiratory symptoms. Gynecomastia, caused by β-HCG elevation, as a presenting first symptom occurs in 2-4 % and 5-7% have gynecomastia at diagnosis [96].

Differential diagnoses for processes in the testis are hydrocele, spermatocele, benign cysts and hematomas. Sarcoidosis may be mistaken for lung metastasis and mediastinal lymphadenopathy [97]. An association between TGCT and sarcoidosis has been reported with an approximately 100 fold increase in incidence of sarcoidosis in patients with TGCT [97, 98].

Table 1. Clinical staging according to Royal Marsden

Clinical stage	Criteria
CS I	No evidence of metastases
CS Mk+	Tumor markers AFP/ß-HCG persistently elevated (not declining according to half-life), but no macroscopic metastatic disease demonstrated
CS II	Metastatic disease restricted to abdominal nodes: A Maximal transverse diameter <2 cm B Maximal transverse diameter 2–5 cm C Maximal transverse diameter >5–10 cm *D Maximal transverse diameter >10 cm
CS III	Supradiaphragmatic node involvement For abdominal lymph-nodes: 0 No metastases; A-D According to CS II.
CS IV	Extra-lymphatic metastases For abdominal lymph-nodes: 0 No metastases; A-D According to CS II. *Lung substage: L1 <3 metastases, no metastases >2 cm; L2 >3 – <20 metastases, no metastases >2 cm; L3 ≤20 metastases, >2 cm; L4 >20 metastases. For abdominal lymph-nodes: 0 No metastases; A-D According to CS 2. H+ Liver metastases, Br+ Brain metastases, Bo+ Bone metastases

*Modifications in the Royal Marsden Hospital system.

Standard procedures in investigating a testicular tumor include clinical examination, ultrasound of the testes, and analysis of tumor markers in serum. Orchiectomy is obligatory in case of suspected testicular tumor and the standard procedure is an inguinal incision. The presence of CiS in the contralateral testicle is seen in 5% to 9 % and for a subset of patients, with risk factors such as subfertility, heredity, atrophic testis and history of cryptorchism, the incidence can be up to 20 % [11, 12, 99, 100]. Many centres perform a biopsy of the contralateral testicle, either routinely or through a risk adapted approach.

Staging

The most common site for metastatic disease is the retroperitoneum below the renal vessels, with initial lymph node involvement from the left testicle typically seen in the left para-aortic nodes and from the right testicle in the interaortocaval nodes [101]. From there further antegrade and retrograde spread in the lymphatic system can occur. The lungs is the most common site for haematogenous dissemination and more seldom liver, bone and CNS metastases are seen.

The most commonly used staging system is the modified Royal Marsden Hospital system (Table 1) [102]. Staging procedures typically include physical examination, serum tumor marker concentrations before and after orchiectomy and CT scan of the thorax, abdomen, and pelvis [102]. Stage I disease is disease confined to the testicle only, and stage Mk+-IV defines the extent of disseminated disease (Table 1).

Risk grouping

Several prognostic classifications have been used, but since 1997 the International Germ Cell Consensus Classification (IGCCC) is the consensus prognostic index and is used world-wide for metastatic GCT. The IGCCC is based on clinical parameters present post orchiectomy prior to further therapy: histology, location of primary tumor, location of metastases, and tumor marker levels of AFP, β-HCG, and LDH [103]. It discriminates patients in to the three prognostic groups: good, intermediate and poor risk (Table 2).

The 5-year overall survival (OS) of patients in the IGCCCG population was 92% in good, 80% in intermediate and 48% in poor risk patients, respectively. A study by Sonneveld et al. from 2001 compared patients treated during the periods 1977–1986 and 1987–1996 according to the IGCCCG grouping [104]. The 10-year survival rates of patients with good, intermediate, and poor prognoses were 95%, 74%, and 37%, respectively, during the period 1977–1986 and 94%, 87%, and 66%, respectively, during the period 1987–1996.

Post orchiectomy treatment is based on the histological type of tumor and the prognostic classification.

Table 2. IGCCCG prognostic staging system. International Germ Cell Cancer Collaborative Group prognostic staging system for metastatic seminomatous and non-seminomatous germ cell cancer

Seminoma	Criteria	5-year OS (%)	Patients (%)
Good prognosis	any primary site and normal AFP, any β-HCG, any LDH and no non-pulmonary visceral metastasis	**86**	**60**
Intermediate prognosis	any primary site and normal AFP, any β-HCG, any LDH and non-pulmonary visceral metastasis	**72**	**10**
Poor prognosis	No seminoma with poor prognosis	**Na**	**Na**
Non-seminoma	**Criteria**	**5-year OS (%)**	**Patients (%)**
Good prognosis	Testicular or retroperitoneal primary site and no non-pulmonary visceral metastasis AFP < 1000 µg/l and β-HCG < 5000 IU/l and LD < 1.5 x ULN	**92**	**56**
Intermediate prognosis	Testicular or retroperitoneal primary site and no non-pulmonary visceral metastasis AFP 1000-10000 µg/l or β-HCG 5000-50000 IU/l or LD 1.5 -10x ULN	**80**	**28**
Poor prognosis	Mediastinal primary tumor or non-pulmonary visceral metastasis AFP > 10000 µg/l or β-HCG > 50000 IU/l or LD > 10 x ULN	**48**	**16**

SWENOTECA

SWENOTECA (Swedish Norwegian testicular cancer), initiated in 1981, is a collaboration between Swedish and Norwegian health services, providing mutual cancer care programs for germ cell cancer, clinical research projects and quality assurance. The first management program for NSGCT, SWENOTECA I, was introduced in 1981 and for SGCT, the first protocol was launched in 2000. The SWENOTECA register holds detailed information on clinical staging procedures, prognostic classification, treatment modality, short- as well as long- term treatment toxicity and FU for up to 10 years after diagnosis, for non-seminoma patients since 1995 and seminoma patients since 2000. All Swedish cancer centers report to the SWENOTECA database, which is then regularly cross-checked with the Swedish Cancer Register through the personal national registration number assigned to each resident in Sweden at birth or permanent residency. Completeness of the Norwegian data is ensured by thorough searches made in hospital databases and the findings are crosschecked with the SWENOTECA database. The completeness of the register is close to 100%.

Treatment results are continuously evaluated and compared to recent studies and contemporary guidelines and the management programs are updated accordingly. The results give feedback to the treating clinics and may help in identifying potential areas of improvement. The databases have been used in several research projects with different aims [105].

The SWENOTECA collaboration group of physicians have regular meetings to ensure up-to-date management programs and continuous research. The group is also the natural discussion forum for especially challenging patient cases.

Seminoma

Seminomas represents about 60% of all TGCT cases, typically presenting in the mid to late thirties. The majority of patients are classified as CSI (77 - 86 %) or CS II, and only few patients present with more advanced disease. Between 2009 and 2013, 92 % of all patients with metastatic SGCT (n =131) reported to the SWENOTECA database belonged to the good prognosis group, whereas the remaining 8 % were classified as intermediate risk [106] (SGCT is never classified as poor prognosis according to the IGCCCG classification). Patients diagnosed with SGCT have a long term survival of close to 100 % for the whole group [107]. Patients in the intermediate prognosis group have a worse 5-year OS compared to patients with NSGCT in the same risk group with a 5 year OS of 50-72% [107, 108].

Treatment CS I

The prognosis for SGCT CS I is excellent with close to 100 % survival. Without adjuvant treatment, 13-20 % will have a relapse and almost all of these patients will survive. The most relapses will occur within the first 3 years after diagnosis and there are few late relapses [81, 109, 110].

Radiotherapy (RT) to the retroperitoneal lymph nodes is a treatment option for patients in CS I. The RT doses used are 20 -24 Gy and will give a relapse rate of 1-3% [82]. A randomized trial compared one course of single-agent carboplatin with RT, with doses 20 to 30 Gy, as adjuvant treatment for patients in CS I, and the outcome was similar. However, the risk of toxicity and secondary malignancies was in favour of carboplatin [111]. Most centers now use carboplatin and the recommendation is not to use RT in patients younger than 40 years [82].

In a study of men with CS I disease, tumor size was shown to be a prognostic factor for relapse, with four-year relapse-free survival 94% for tumors < 3 cm, 82% between 3 and 6 cm and 64%, ≥ 6 cm [112]. Another study with men presenting with CS I identified primary tumor size > 4 cm and rete testis invasion as significant prognostic factors for relapse [113], but this could not be confirmed in a follow up study [114]. However other studies have reported that patients without any these risk factors have a low risk of relapse [109, 115, 116].

The treatment strategies varies between centers, where some use surveillance for all patients and some use a risk adapted approach. Treatment results after relapse are as good as for good risk patients with metastatic disease at diagnosis [81].

Treatment of metastatic disease

For patients with metastatic disease in stage CS IIA-B, the treatment recommended has been RT to the para-aortic and ipsilateral nodes with the dose for IIA 30 Gy (2 Gy x 15) and IIB 36 Gy (2 Gy x 18). This approach gives an overall survival of close to 100 % and a relapse free survival at 6 years for stage IIA 95% and for IIB 89% [117]. As in patients in CSI treated with RT the risk for late toxicity is a concern, and studies have shown that chemotherapy with 3 cycles of bleomycin, etoposide, cisplatin (BEP) or 4 cycles of etoposide, cisplatin (EP) are as effective as RT [82]. EAU guidelines recommend RT or chemotherapy with 3 x BEP or 4 x EP in cases of contraindications to bleomycin [82].

Patients with stages over IIB are treated with chemotherapy where patients with good risk are recommended 3 x BEP or 4 x EP and patients at intermediate risk 4 x BEP or etoposide, cisplatin, ifosfamide (VIP) in case of contraindications to bleomycin [82].

Overall, treatment results are favourable and one report presented a 5-year cancer-specific survival (CSS) of 97% for patients classified as good prognosis [107]. The intermediate prognosis group represented 0.4% of all patients treated and the 5-year OS was 50% [107].

Non Seminoma

Non-seminomatous tumors can consist of one or several of the histological subtypes: seminoma, choriocarcinoma, embryonal carcinoma, yolk sac tumor and teratoma (mature or immature). Tumors with more than one histological subtype is classified as a mixed tumor. NSGCT typically present at 25-35 years of age. More than half of the patients present with stage I disease, and 40-45 % are disseminated at diagnosis [82, 106]. According to the SWENOTECA protocol for patients with NSGCT with CSI disease at initial staging, a restaging is performed after six weeks in order to verify the stage. For patients without obvious metastases but with elevated tumor markers after orchiectomy, the markers are measured weekly, and definitive staging is postponed until marker levels normalize or are considered to be definitively increased (Mk^{+}). Few patients are in CS Mk+ (1-5%). Among all Swedish patients diagnosed between 2009-2013, 75 % patients with metastatic disease were classified as good prognosis, 11 % as intermediate prognosis and 13% as poor prognosis [106]. In a series from Spain, the distribution for patients diagnosed between 1994 -2001 was 63 % good prognosis, 19 % intermediate prognosis and 18 % poor prognosis [80].

Treatment of CS I

About 30% of the patients with stage I disease at staging have subclinical disseminated disease and will have a relapse if no adjuvant treatment is given [118, 119]. The strongest predictive risk factor for subclinical metastases is invasion of tumor cells in blood or lymphatic vessels (VASC+). Approximately 50 % of patients with VASC+ will relapse, compared to a relapse risk of 10 % without vascular invasion (VASC-) [120, 121].

The EAU guidelines recommend that patients with CSI NSGCT should be informed about [82]:

- adjuvant treatment options after orchiectomy are surveillance, adjuvant chemotherapy or RPLND
- treatment-specific recurrence rates
- acute and long-term side effects of treament.

RPLND is to-day mostly used only if there are conditions against surveillance and chemotherapy.

All the above described treatment options give almost 100% long term survival but the acute and long term side effects vary. The choice of treatment is debated and therefore it varies between countries and institutions.

In the current management program from SWENOTECA, a risk adapted approach is used, in which one cycle of adjuvant BEP is recommended to patients with VASC+ disease, and patients with VASC- disease are able to choose either surveillance or one course of BEP. All patients receive both oral and written information.

Treatment for metastatic disease

The first major breakthrough in treatment for patients with metastatic NSGCT was reported by Li et al. in 1960, when the combination of chlorambucil, methotrexate, and dactinomycin resulted in a response rate (RR) of 30% [122]. A combination of vinblastine plus bleomycin as first line treatment for 50 patients resulting in 39% complete response (CR) was reported by Samuels and Howe in 1975 [123]. Cisplatin was later the same decade found to inhibit cell division [124], and in 1977 Einhorn et al. reported results from a combination therapy where he added cisplatin to vinblastin and bleomycin (CVB) given to patients with advanced TGCT where 74% had CR and 26% partial response (PR) [125]. Patients with PR had their residual tumors removed and in total, 85% became disease free. There were also other studies indicating the importance of post chemotherapy surgery [126, 127].

Peckham et al. reported in 1983 the combination of bleomycin, etoposide and cisplatin (BEP) which was later shown to be more effective, especially for patients with advanced disease, and less toxic compared to CVB [128, 129]. Other studies had the aim to reduce the toxicity of the treatment for favourable prognosis patients. In one randomized study cisplatin was replaced by carboplatin, but the regimen containing cisplatin was found to be superior [130]. Another trial compared 3 courses of BEP to 3 courses of EP showing that BEP was associated with higher frequency of CR and fewer relapses [131]. A study by Einhorn et al., showed that three courses of BEP was equivalent to four courses in favourable prognosis patients [132].

Several prognostic classifications have been used during these years, but since 1997, the International Germ Cell Consensus Classification (IGCCC) is the consensus

prognostic index for metastatic GCT. The IGCCCG was created 1991 and constituted of groups treating GCT from several countries with the aim to make a simple prognostic factor-based staging classification in order to aid with appropriate risk-based decisions about therapy and to facilitate collaborative trials. The groups contributed with clinical data to a database with 5202 patients with metastatic NSGCT and 660 patients with metastatic SGCT that were treated between 1975 to 1990. The IGCCC, based on clinical parameters present prior to therapy, classifies the patients in three groups with good, intermediate or poor risk.

Side effects of treatment

In view of the excellent prognosis and that TC survivors have a nearly normal life expectancy after their diagnosis, there has been much focus on long term side effects of the disease and its treatment.

Renal

The acute renal toxicity caused by cisplatin may in up to 20- 30% cause subclinical permanent renal damage, with a reduction of glomular filtration rate (GFR) up to 30%. The chronic nephrotoxicity is primarily due to tubular damage [108, 133]. The risk of renal damage is linked to the cumulative dose of cisplatin. Tubular damage may cause hypomagnesemia due to tubular salt wasting which is a common finding during cisplatin-based chemotherapy but may persist may persist in some cases [134].

Abdominal RT used for treatment of seminoma patients may also induce renal damage that is delayed by 3–5 years after radiotherapy, in contrast to chemotherapy where the damage occurs immediately. The damage caused by RT is probably due to direct parenchymal damage, small vessel sclerosis, and renal artery stenosis [135]. Abdominal RT alone is shown to be less nephrotoxic than the combination of radiotherapy and chemotherapy. One study found 8% impaired renal function in patients treated with radiotherapy alone, compared with a 14% reduction in men treated with chemotherapy with or without RT [134]. The risk for renal damage at surgery is low.

Raynaud’s phenomenon

Bleomycin is believed to cause Raynaud’s phenomenon, which is a transient vasoconstriction of arteries that gives symptoms of discomfort and whitening of skin in hands and feet on exposure to cold [136]. The condition is proposed to be caused by direct vascular injury giving endothelial dysfunction. Another theory is altered sympathetic arterial vasoconstrictor response due to nerve damage. The risk of

developing Raynaud´s phenomenon is believed to increase with the cumulative dose of bleomycin and the incidence is reported to be between 10% and 49% [135-137].

Paraesthesia

Paraesthesia is probably caused by cisplatin that induces a direct nerve damage. Another hypothesis is that cisplatin causes endothelial cell apoptosis with microvascular disruption and ischemia [136]. In a report 22% of patients receiving 3-4 cycles BEP had significant symptoms after 2 years [138]. Another study has shown that the predictors for persistent paraesthesia were the cumulative dose of cisplatin and also the age of patient [136].

Ototoxicity

Cisplatin may cause ototoxicity and the mechanism is presumed to be hair cells damage in the organ of Corti. The clinical symptoms may be tinnitus and/or high-frequency hearing loss [136, 137, 139]. It has been reported that persisting ototoxicity was seen in 20 % of patients receiving standard dose cisplatin and in more than 50% of patients receiving a cumulative dose of cisplatin >600 mg/m2 [140]. Risk factors for ototoxicity are existing hearing impairment, previous significant noise exposure and hypomagnesemia [135].

Endocrine

There are reports of an increased incidence of metabolic syndrome in survivors after treatment for TGCC occurring in 20%–30% and starting much earlier than what is seen in the general population [108]. In one study with TCSs treated with chemotherapy for metastatic disease, 25 % were afflicted at a median follow-up of 5 years, with an odds ratio (OR) of 2.2 compared with controls [141]. The prevalence of hypogonadism after treatment for TC varies from 11 % to 34 % depending on the type of treatment and is a condition that can lead to metabolic syndrome, cardiovascular disease, type II diabetes, osteoporosis and decreased QoL [142-144]. Hypogonadism is thought to be the main cause of the metabolic syndrome [141, 145]. It has been shown that low grade systemic inflammation is present in men with hypogonadism even before any signs of the metabolic syndrome are present [146]. In an interventional study, some parts of the metabolic syndrome were improved and also a some inflammatory markers were reduced with

testosterone substitution [147]. Other studies have also shown improvement in lipid status and insulin resistance by substitution, but no risk reduction for cardiovascular disease (CVD) [148].

Cardiovascular

Cisplatin is thought to cause endovascular damage which leads to accelerated atherosclerosis syndrome. and an increased cumulative risk up to 18 % over 20 years for having coronary heart disease, myocardial infarction, congestive heart failure and stroke [108]. Studies has shown that there is no increased risk compared to the general population for patients treated with surgery alone [149-151]. In a study of seminoma patients treated with RT, an increased risk of death due to cardiac disease was found beyond 15 years after diagnosis, irrespective if mediastinal nodes were included or not, showing a RR of 2.1-2.4 [149-152]. Patients treated with chemotherapy have a RR of 1.9-2.6 and if RT is added, the RR is 2.3-4.8 [149-151]. The proposed factors influencing the increased risk are direct vascular damage by treatment and development of known risk factors for CVD like hypertension, diabetes, dyslipidaemia that all are part of the metabolic syndrome [153]. In addition, one study has shown an association between low testosterone levels and increased risk of coronary heart disease [154].

Fertility and sexuality

In a study by Eberhard et al. TCSs were found to have an increased risk for low sexual desire and erectile dysfunction (ED) independent of treatment and the presence of hypogonadism [155]. ED which has been reported in 12–40% of TCSs seems to respond well to phosphodiesterase type 5 inhibitor treatment [156].

The spermatogenesis capacity after treatment is dependent on the gonadal function before orchiectomy, and additional treatment given [157]. Brydoy et al. has shown that paternity-rate was associated with the number of cisplatin-based chemotherapy cycles and the overall 15-year actuarial post-treatment paternity rate (without assisted reproduction) was 71% [158, 159]. Patients on surveillance had a post treatment paternity rate of 92% and those receiving more than 850 mg cisplatin of 48%. The majority who attempt paternity after treatment will achieve success but more will need medical assistance [158, 160, 161].

Retrograde ejaculation after bilateral RPLND can lead to impaired infertility but if nerve-sparing technique is used, the risk is low [162].

A modest increase in the risk of congenital abnormalities among offspring of males with a history of cancer has been found independent of natural conception or assisted [163].

Pulmonary toxicity

Bleomycin induced pneumonitis occurs in up to 10 % and is fatal in 1-2%. The pneumonitis can lead to pulmonary fibrosis, but in most cases it a reversible condition with corticosteroid treatment [164] . The risk of inducing pneumonitis can be reduced by identifying risk factors like cumulative dose of bleomycin, age at diagnosis, smoking status, renal dysfunction, mediastinal RT, and oxygen administration [165]. A large study reported an increased risk of mortality from respiratory disease with standard mortality ratio 1.15 for TCSs compared to the general population [166]. In another study by Haugnes et al., evaluating pulmonary function in TCSs, high doses of cisplatin-based chemotherapy and combined chemotherapy and pulmonary surgery were found to be significantly associated with decreased pulmonary function several years after treatment [167]. The cumulative cisplatin dose was the factor that had the greatest impact on long-term pulmonary function, not bleomycin where the median dose was 300 mg and only two patients received more than 360mg.

In the same study they found signs of restrictive lung disease in 6.7-7.5% of the patients treated with surgery only, RT only and cisplatin $\leq$ 850 mg. Among patients treated with cisplatin > 850 mg, 17.7% had restrictive lung disease. Most patients were without symptoms.

Second malignancies

Second malignancies occur more often in TCSs, with a risk elevation of 65-90%, compared to age-matched controls [135]. Statistically significantly increased risks have been reported for several solid tumors, like malignant melanoma, cancer in lung, thyreoid, oesophagus, pleura, stomach, pancreas, colon, rectum, kidney, bladder, and connective tissue for at least 35 years after treatment [168]. The risk decreases with increasing age at testicular cancer diagnosis [168]. The increased risk appears to be similar for both SGCT and NSGCT patients, but a lower risk is observed for NSGCT patients treated after 1975, probably due to more effective chemotherapy leading to a lower treatment burden, less use of RT and the increased use of surveillance in CS I disease [168]. Chemotherapy for TC has been associated with myelodysplastic syndrome and secondary leukaemia [135]. The cumulative

dose of etoposide <2g/m2 and >2g/m2 increases the cumulative risk of secondary leukaemia with 0.5% and 2% respectively [108]. The risk is highest within 10 years from treatment and after that it decreases to the level of the general population. There are several reports showing an increased risk of second cancers and death for TC patients cured with radiotherapy [152, 169]. These second tumors most often occur within the RT fields [169]. During the follow-up period the TCSs are exposed to repeated imaging during 5 to 10 years. It has been estimated that the low ionizing doses received with a diagnostic abdominal CT-scan 13 to 16 times gives a 1.2-1.9% lifetime risk of second malignancy with a lower risk at an older age at diagnosis [169].

Relative survival

In an article in press by Kvammen et al., the authors have analysed long-term trends in relative survival, RS, among 8862 TC patients diagnosed in Norway from 1953 to 2012 with a matched comparison group with patients treated for malignant melanoma [170]. The result showed that RS was declining even after 30 years after diagnosis for the whole group and was more pronounced for all stages of seminomas. For the comparison group, there was no continuous decline. The main cause to the increase of non-TC deaths was assumed to be associated to treatment given.

Quality of life

Studies have found that chronic fatigue is more common in survivors of several cancers compared to the non-cancer population. A Norwegian study on TCS reported a prevalence of chronic cancer-related fatigue (CRF) of 17.1% in TCSs and 9.7% in the general population. CRF was associated to psychosocial problems, somatic complaints, and poor quality of life (QoL) [171]. A higher prevalence of anxiety has also been reported among TCSs compared to the general population 19.2% vs. 13.5%, while the rate of depression was similar to that in the general population [172].

One study found that a subgroup of TCSs experienced symptoms of cognitive dysfunction. These complaints were not associated with objective findings of cognitive impairment, but were more linked to anxiety and fatigue [173]. A finding in the same study was that cognitive dysfunction were more common in patients receiving chemotherapy compared to those treated with surgery. Another study could not verify the finding of a cognitive dysfunction in TCSs treated with

chemotherapy [174]. One Swedish study showed that TCSs that received more than four cycles of cisplatin-based chemotherapy had increased incidence of compromised language, which was more evident among men with a low level of education [175].

Psychosocial implication

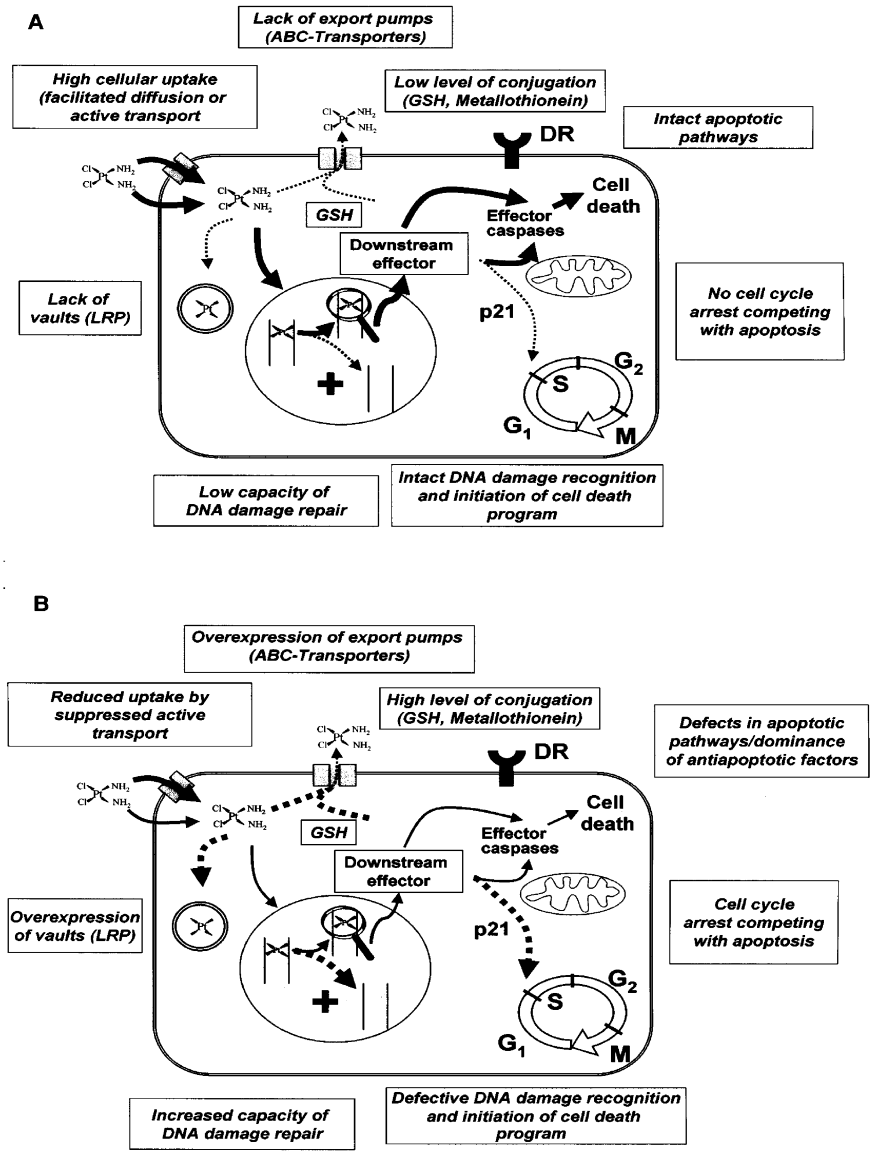
Cancer survivors in general are more likely to suffer from impaired health, leading to loss of work ability in comparison to healthy individuals, especially during the year of diagnosis and the year after [176, 177]. Previous studies have mostly not reported any significantly increased risks of work loss among TCSs [177-180]. In a study by Taskila et al. who studied work ability of survivors from an early-stage of breast cancer, lymphoma, testicular or prostate cancer, showed no difference from that of their referents [181]. Impaired work ability was more often reported by TCSs with comorbidities and by survivors after chemotherapy. In contrast, survivors with a strong commitment to their work organization or a good social climate at work reported less impairment. TCSs had the highest mean value for work ability. A meta-analysis of cancer survivors and unemployment with 20,366 cancer survivors and 157,603 comparators from the US, Canada, Europe, Taiwan, Korea and Australia, showed that cancer survivors were more likely to be unemployed than comparators (33.8% vs 15.2%; pooled relative risk [RR], 1.37; 95% confidence interval [CI], 1.21-1.55) [182]. The unemployment was higher for survivors from breast cancer, gastrointestinal cancers and cancers of the female reproductive organs. For survivors of blood cancers, prostate cancers and testicular cancer there was no increased risk in comparison to comparators.

Chemotherapy resistance

Mechanisms of chemotherapy resistance

TGCT (except teratomas) are most often very sensitive to cisplatin - the reason for the excellent treatment results. Resistance usually develops over time, during treatment, but may more seldom be present at the time of diagnosis. It is assumed that the initial oncogenic events may not cause sufficient damage to alert the DDR system. Due to the lack of activation during the development of TGCTs, there may be less pressure for selection of mutations in the DDR system. This would then preserve the DDR machinery largely intact and therefore capable of responding to DNA damaging insults like chemotherapy or radiotherapy [183] with a higher predisposition to apoptosis due to an intact p53 pathway (Figure 6 B) [24, 41, 184, 185]. This difference in the DDR system in TGCT compared to other solid tumors, is proposed to be a reason for the high sensitivity to chemotherapy.

The expression of the stem cell-associated genes and the pluripotent and embryonal characteristics of the TGCT cells is thought to be another cause of the high sensitivity to chemotherapy. It is the loss of these characteristics, in combination with over-expression of genes involving activation of progression through checkpoints, which probably leads to resistance to platinum-based chemotherapy [54, 186, 187]. In most subtypes of TGCTs, with the exception of teratomas, lack of p21 protein expression has been associated to cisplatin resistance [45, 188, 189]. Moreover, an increased level of Bcl-2 in mature teratoma has been related to cisplatin resistance in this subtype [190-192]. Other proposed mechanisms for chemotherapy resistance in TGCT is insufficient DNA binding due to low uptake or early inactivation of the drug or poor cytotoxic effect due to alterations in the DDR system and apoptotic pathway [193-195]. It has also been shown that there are miRNAs that can alter the p53 function [49-51]. Mechanisms for the high sensitivity to cisplatin are shown in Figure 7A and possible changes leading to reduced sensitivity to cisplatin in Figure 7B.



ABC, ATP binding cassette; DR, death receptor; GSH, glutathione; LRP, lung resistance protein

Figure 7. Mechanisms potentially influencing cisplatin activity. (A) Model of a germ-cell tumor cell showing sensitivity to cisplatin. (B) Model of a germ-cell tumor cell with induced mechanisms of chemotherapy resistance highlighted. Modified from [188], with permission from Oxford University Press.

RNA-binding motif protein 3 – RBM3

The gene for RBM3 is located on the X chromosome which harbours several genes encoding proteins with the ability to bind to mRNAs [196].

Some of the capacities for RBM3 that have been shown are:

- Binding to DNA with the proposed function to initiate transcription and also binding to encoded RNA to affect translation [197].
- Increased RBM3 expression is seen in highly proliferating benign cells as compared to resting cells [198].
- RBM3 is a cold-shock protein, showing increased translation during hypothermia, oxidative stress and if exposed to protein synthesis inhibitors [199-201].
- *In vitro*, RBM3 promotes proliferation, prevents apoptosis and stimulates angiogenesis [198, 202-204].
- Knockdown of RBM3 in colon cancer cell lines led to apoptosis and induced mitotic catastrophe [204].

The function of RBM3 is thought to be to protect cells during harsh conditions by facilitating translation of mRNAs for maintenance of protein synthesis and avoiding apoptosis [198, 201]. RBM3 has been shown to be a prognostic biomarker in several cancers, e.g. urothelial cancer, breast cancer, colorectal cancer, prostate cancer, esophageal and gastric adenocarcinoma and ovarian cancer [202, 205-209]. In ovarian cancer *in vitro*, RBM3 expression has also been demonstrated to correlate with sensitivity to cisplatin treatment [202, 210]. Moreover, a negative correlation was shown between RBM3 and the DNA damage checkpoint kinases Chk1 and Chk2, kinases in the DNA damage response (DDR) system that are able to drive the cell into cell cycle arrest or apoptosis. This could suggest that RBM3 expression in the cell is an indicator of an intact silenced DDR system, thereby influencing the response to cisplatin. As mentioned before, Bartkova et al. have shown that, in contrast to other solid tumors, the DDR system is virtually not activated and therefore intact in TGCT and CiS [183]. The intact DDR responds when the TGCT cell is exposed to supra-threshold DNA damage insults, such as radiotherapy or chemotherapy, which may explain its exceptional sensitivity to these modalities [183].

One hypothesis is that tumors with high RBM3 expression have a less active anti-cancer barrier, that during cell division fails to respond with proper validation of DNA integrity due to low levels of Chk1 and Chk2, which in turn will lead to the accumulation of mutations, but also to a lower pressure for selection of mutant clones with altered DDR system. Tumors with low RBM3 expression would then

have a more intact and active anti-cancer barrier, making them more prone to select mutations with the ability to avoid an active DDR. Supporting this hypothesis is a study where RBM3 expression was found to decrease with progression of malignant melanoma [211].

Background and aim of the present investigation

Paper I

The rationale behind the SWENOTECA IV protocol for metastatic NSGCT activated 1995 was that clinical stage and the risk factors recognized at that time, such as stage, tumor burden and location of metastases, present at staging (MRC) [212] were not considered specific enough to be used for treatment intensification and decision-making for patients with metastatic NSGCT. Tumor marker decline during chemotherapy had at the time for the protocol design, in retrospective analyses, demonstrated a strong impact on outcome [213-217]. Hence, this SWENOTECA IV protocol was designed for early identification of patients, in whom the response to two standard chemotherapy courses was inadequate and to provide intensified treatment to these individuals. Tumor marker decline and, in patients with marker negative tumors, radiological assessment were to be used for response evaluation.

In 1995 several risk grouping system were proposed but none generally accepted. The IGCCC prognostic classification, reported 1997, was accepted and is used worldwide since then. Patients included in the SWENOTECA protocol before the IGCCC classification was accepted could retrospectively be categorized to good, intermediate or poor risk group.

The SWENOTECA IV treatment strategy is evaluated with respect to prognostic group and outcome.

Paper II

Cancer survivors in general are more likely to suffer from impaired health, leading to loss of work ability in comparison to healthy individuals, especially during the year of diagnosis and the year after [176-179, 218]. In many previous investigations, however, researchers have had limited ability to consider potential variation in work

loss by cancer site and especially by treatment [178, 179, 218]. Previous studies have mostly not reported any significantly increased risks of work loss among TCSs, but the studies have been limited in size (n=<600) [177-180]. Also, few previous studies have investigated short- or long-term effects of work loss for testicular cancer survivors in relation to treatment modality [177, 179], or in comparison to the general population [177, 180] and none have considered the impact of cancer recurrence.

The aim was to investigate short- and long-term incidence of sick leave and disability pension among testicular cancer patients compared with the general population, separating the patients by stage and treatment intensity and taking recurrence into consideration.

Paper III

The IGCCC for NSGCT is used to classify patients with metastatic disease into three groups with good, intermediate or poor prognosis, based on clinical parameters present prior to therapy [103]. In a meta-analysis, performed in 2005, 5-year survival estimates for patients with good prognosis were 94 %, intermediate prognosis 83% and 71% in the poor prognosis group [219]. The majority of patients are young at diagnosis and it is therefore important to avoid long term morbidity that may result from TC treatment. Hence, although certain clinicopathological factors have proven to be of value to guide treatment, there is a clear need for additional prognostic markers to better discriminate between patients with high-risk and low-risk disease [117].

Low expression of RBM3 has been demonstrated to correlate with a worse prognosis in several major cancer forms, e.g. breast, ovarian, prostate, colorectal, bladder cancer and malignant melanoma [202, 205-208, 220]. RBM3 expression has also been demonstrated to correlate with sensitivity to platinum-based chemotherapy in ovarian cancer in vivo and in vitro [202, 210]. In gonads, RBM3 has been found to be expressed in Sertoli cells, but not germ cells, of the seminiferous tubules [221].

In this study, the aim was to examine the expression and prognostic significance of RBM3 in NSGCT.

Patients

Paper I

Adult patients diagnosed with a metastatic testicular NSGCT between July 1995 and December 2003 in Sweden and Norway, with the exception of one Norwegian center, were included. Exclusion criteria were extragonadal primary tumor and previous treatment for contralateral testicular tumor. Patients with retroperitoneal lymph node metastases but no tumor in the ablated testicle, but only a scar, were included. All participating centres report to the SWENOTECA database which is cross-checked with the Swedish national cancer registry once a year. Completeness of the Norwegian data is ensured by thorough searches made in hospital databases and the findings cross-checked with the SWENOTECA database. The survival status of all patients was checked against the national population registry in Sweden as of May 1, 2009 and in Norway September 1, 2009.

Paper II

TCSs were identified from the Swedish part of the SWENOTECA database and men diagnosed in the period July 1st 1995 (seminomas from July 1st 2000) to December 31st 2007 were included. TCS that died (Causes of Death Register) [222] or emigrated (Statistics Sweden) [223] within two years from diagnosis, or those with a previous cancer diagnosis according to the Swedish Cancer Register [224] (except previous non-melanoma skin cancer), were excluded.

The patients were divided into two main groups and six subgroups based on treatment: 1) no or limited treatment including: 1a) surveillance, 1b) radiotherapy (adjuvant for clinical stage I disease (20 Gy/10 fractions) or metastatic disease (27Gy/15 fractions), 1c) adjuvant chemotherapy 1 course (EP, BEP or carboplatin single), 1d) chemotherapy 2-3 courses (BEP, PEI or TIP) (SWENOTECA treatment Guidelines listed in Table 3), and 2) extensive treatment: 2 a) chemotherapy 4 courses and 2b) chemotherapy >4 courses (with any of the above mentioned regimens and in some instances also high-dose chemotherapy with stem-cell support).

A comparison cohort was used with four male cancer-free comparators for each TCS that were randomly sampled from the National population register kept at Statistics Sweden [223], matched on birth year and calendar year of diagnosis of the patient. Comparators who died or emigrated within the first two years of follow-up were excluded.

Paper III

Adult patients diagnosed with testicular NSGCT in the Southern Sweden Health Region between July 1, 1995 and December 31, 2007, in whom clinical data were available, were included in this study. The survival status of all patients was checked against the national population registry in 2012. The patients were treated according to the protocols SWENOTECA IV (metastatic disease) and SWENOTECA III and VI (non-metastatic disease).

Study design

Paper I

SWENOTECA IV is a prospective population-based non-randomized study in metastatic NSGCT. The individually response-adapted therapy was based on tumor marker decline and, in marker negative patients, on radiological assessment. An overview of the treatment principles is shown in Figure 8.

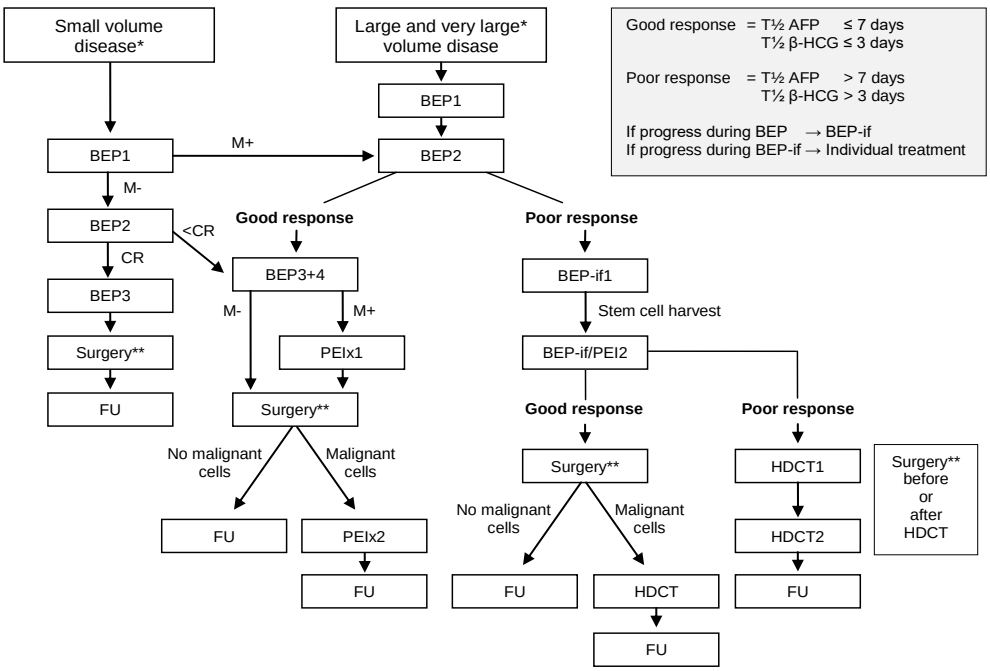


Figure 8. Treatment principles.
Abbreviations: M±: tumor markers increased/normal; CR: complete remission on CT or MRI; RPLND: retroperitoneal lymph node dissection; FU: follow up; HDCT: high dose chemotherapy with stem cell rescue; BEP, BEP-if, PEI, HDCT: see Table 3.
*according to MRC[212] , **see "Post chemotherapy surgery"

The study was approved by the Swedish and Norwegian Medical Ethics Committees. Written informed consent was not demanded.

Staging was performed according to the Royal Marsden Hospital system [102]. Staging procedures included physical examination, serum tumor marker concentrations (AFP, β-HCG, LDH) before and after orchiectomy and CT of the thorax, abdomen and pelvis. For patients without obvious metastases, but with elevated tumor markers after orchiectomy, the markers were followed weekly and definitive staging was postponed until normalized or definitively increased. Initially, risk grouping was performed according to the Medical Research Council system (MRC) [212] and since 1997, also according to the IGCCC[103]. Patients registered before 1997 were retrospectively classified according to IGCCC. Response evaluation was performed by analyzing AFP and β-HCG days 1, 5/6, and 15/16 in each chemotherapy cycle. The values were plotted in a semi logarithmic graph for AFP and β-HCG, respectively (Figure 9). Two consecutive marker values demonstrating a prolonged marker decline (i.e. β-HCG T½ > 3 days, AFP T½ > 7 days), was regarded as poor response. For the first marker evaluation, values from day 15/16 of the first course and days 1, 5/6 and 15/16 of the second cycle were used, taking into account a possible surge. The marker status was followed through all treatment cycles. For marker-negative patients post-orchiectomy, the evaluation was based on tumor volume reduction calculated by the product of two perpendicular axial diameters on CT. A shrinkage of >25% after the two initial chemotherapy cycles was considered a good response.

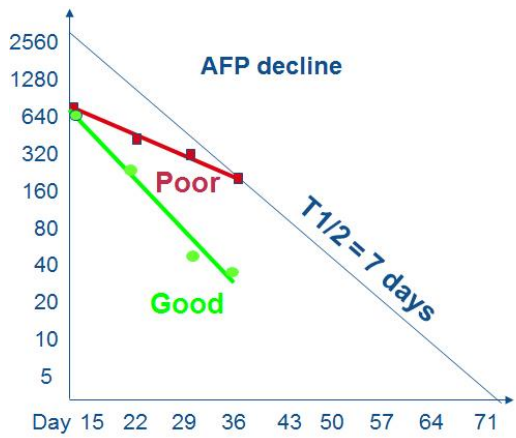


Figure 9. Example of poor and good AFP decline plotted in a semi logarithmic graph.

All patients were initially treated with 2 cycles of BEP. In case of severe renal impairment, not due to tumor obstruction, cisplatin was replaced by carboplatin (JEB).

Table 3. Chemotherapy regimens

	Regimen	Drug	Daily Dosing (mg/m^2)	Day
Initial therapy	BEP	Bleomycin*	30 000 IE§	1,5,16
		Etoposide	100	1-5
		Cisplatin	20	1-5
	JEB†	Bleomycin*	30 000 IE§	1,5,16
		Etoposide	100	1-5
		Carboplatin	AUC=7‡	1
Step 1	BEP-if	Bleomycin*	30 000 IE§	1,5,16
		Etoposide	75	1-5
		Cisplatin	20	1-5
		Ifosfamide	1200	1-5
		Mesna	240	1-5
		Mesna (po)	480	1-5
	PEI	Cisplatin	100	1-5
		Etoposide	20	1-5
		Ifosfamide	1200	1-5
		Mesna	240	1-5
		Mesna (po)	480	1-5
Step 2	HDCT cycle 1↑	Cyclophosfamide	1500	1-4
		Etoposide	440	1-4
		Carboplatin	AUC=7§‡	1-4
	HDCT cycle 2↓	Cyclophosfamide	1500	1-4
		Thiotepa	60x2	1-4
		Carboplatin	AUC=7§‡	1-4

Abbreviations: HDCT: high dose chemotherapy with stem cell support

* Bleomycin was omitted after a cumulative dose of 300000 IU

§ Total dose

† In case cisplatin was replaced by carboplatin

‡ According to Calverts formula [225]

↑ Modification of the regimen described by Ibrahim [226]

↓ Modification of the regimen described by Rodenhuis29, given 6-7 weeks after HDCT cycle 1 [227]

In patients with impaired pulmonary function, bleomycin was replaced by ifosfamide, PEI. Response evaluation was performed after 2 BEP. In case of satisfying response, BEP was continued. Patients with small volume disease,

normalized tumor markers after 1 cycle of BEP, and CR on CT after 2 cycles of BEP received a total of 3 cycles. The remaining patients who responded satisfactorily, received 4 cycles of BEP. After 4 cycles, a second evaluation was performed with CT and tumor markers. If tumor markers still were elevated, but declining according to their respective T½, one cycle of PEI was given before surgery. For marker-negative patients with stable or progressive disease after 2 BEP cycles, surgery was recommended.

In patients with poor response to initial treatment, the therapy was intensified by addition of ifosfamide. After the first cycle of BEP-if, a stem cell harvest was performed. A second evaluation was performed after two cycles BEP-if and patients with good response received in total two to three cycles before surgery. In case of poor response, the treatment was intensified by high dose chemotherapy with stem cell support (HDCT). Two cycles were recommended.

For patients with retroperitoneal lymph node metastases > 2 cm at staging, a RPLND was recommended post chemotherapy even if complete remission. Residual tumors in all locations should be resected if possible. When the resected specimen contained only necrotic debris/fibrosis or teratoma, no further treatment was given. Patients with remaining vital cancer in the resected tissue received adjuvant chemotherapy with two cycles of PEI. Patients intensified to step 1 (BEP-if/PEI) pre-surgery received one cycle of adjuvant HDCT.

Acute toxicities, gastrointestinal, hematological, renal, pulmonary, neurological and infectious were graded according to the WHO toxicity criteria [228].
After completion of treatment, patients were followed according to a standardized schedule with visits every two months the first two years, every three months the third year, every six months year four to five and every 12 months years 6 to 10.

Paper II

In Sweden, sick leave and disability pension, part- or full time, are tax-funded and open to everyone. The first day of sick leave is not compensated and day 2 until day14 is paid by the employer. The Swedish social insurance system compensates and the Swedish Social Insurance Office (Försäkringskassan) administrates the compensation from the 14th day and onwards (except between Jan 1997 to March 1998 when 28 days was the limit). Disability pension is compensated from day 1. The number of lost work days is registered in the Social Insurance Agency in the Micro Data Analysis of Social Insurance database (MiDAS). Usually retirement takes place at 65 years of age but can be offered from the age of 61 or later up to the age of 67 years.

For all study participants, exact dates were retrieved of all periods of sick leave (>14 days) and disability pension (from day one) from two years prior to diagnosis until end of follow-up September 30th 2013, as well as the number of days per year of sick leave and/or disability pension, from the Social Insurance Agency database [229].

Table 4. Databases used in paper II

Databases	Information retrieved
SWENOTECA	TCSs diagnosed in the period July 1st 1995 (seminomas from July 1st 2000) to December 31st 2007 were included.
Swedish Cancer Register	Patients with a previous cancer diagnosis (except previous non-melanoma skin cancer), were excluded.
Statistics Sweden	A comparison cohort with four male cancer-free comparators for each TCS, randomly sampled, matched on birth year and calendar year of diagnosis of the patient.
The Causes of death register, Sweden	Cause of death
LISA	Data for patients and comparators on educational level (≤9, 10-12, >12 years) and unemployment status (unemployed >50% of the time: yes/no) within the year before diagnosis.
MiDAS	The number of lost work days as registered by the Social Insurance Agency.

Abbreviations: LISA: Longitudinal Database on Education, Income and Occupation; MiDAS: Micro Data Analysis of Social Insurance

"0" days represented individuals with no episodes longer than 14 days of sick leave absence per year (and no disability pension) since the first 14 days are not registered in the Social Insurance Agency database. A new sick-leave episode occurring within 5 days of the previous episode was paid directly by the social security agent, and thus recorded. Disability pension is granted if work capacity is reduced by at least 25%. There are three levels of disability pension, 25%, 50% or 100%. Periods of sick leave can occur during the time not covered by the disability pension. In case of recovery or decline of work capacity, the degree of disability pension could be changed. The terminology disability pension was used 1992-2002, but was changed to sickness compensation (30-64 years of age) or activity compensation (19-29 years

of age) in 2003. The term disability pension was used to describe disability pension, sickness compensation or activity compensation
From the LISA-database (database for health insurance and labour market studies) held at Statistics Sweden [223], information for patients and comparators on educational level (≤9, 10-12, >12 years) and unemployment status (unemployed >50% of the time: yes/no) within the year before diagnosis (data available on all Swedish residents 16 years or older) were gathered. The databases used are listed in Table 4.

Paper III

Patients were identified in the SWENOTECA database and the corresponding slides and formalin-fixed paraffin-embedded tissue blocks were retrieved from the pathology archives. Prior to tissue micro array (TMA) construction, all available haematoxylin and eosin stained slides from each case were re-evaluated by a board-certified pathologist (KJ). The total number of histological subtypes as well as their estimated proportions was denoted. Irrespective of the number of histological components, a standard set of 4x1mm cores were taken from each invasive tumor in a proportional fashion, covering up to 3 different components. In addition, 2x1 mm cores were sampled from areas with CiS from 127 cases and adjacent, benign-appearing testis from 49 cases. A semi-automated arraying device was used (TMArrayer; Pathology Devices, Inc, Westminster, MD, USA). For immunohistochemical analysis of RBM3, 4 μm TMA-sections were automatically pre-treated (deparaffinization, rehydration and epitope retrieval) using the PT-link system (DAKO, Copenhagen, Denmark) and then stained in a Techmate 500 (DAKO, Copenhagen, Denmark) with the mouse monoclonal RBM3 antibody, clone CL0296, product ID AMAb90655, Atlas Antibodies AB, Stockholm, Sweden (formely known as AAb030038, Atlas Antibodies, Stockholm, Sweden) diluted 1:5000. The antibody has been validated in previous studies [202, 206]. For assessment of nuclear RBM3 expression, both the fraction of positive cells and staining intensity were taken into account for each tissue core using a semiquantitative scoring system, whereby the estimated percentage of cells with nuclear RBM3 expression was recorded as nuclear fraction (NF) and categorized into four groups, namely 0 (0–1%), 1 (2–25%), 2 (26–50%) , 3 (51-75%), 4 (>75%) and the nuclear staining intensity (NI) denoted as 0 = negative, 1 = mild, 2 = intermediate and 3 = strong intensity. A combined nuclear score (NS) of NFxNI, which had a range of 0 to 12, was then constructed. Cytoplasmic staining intensity was denoted as 0 = negative, 1 = mild and 2 = moderate-strong, and the fraction of positive cells not taken into account. The staining was evaluated by two independent observers (KJ, SEO) who were blinded to clinical and outcome data

Results

Outcome SWENOTECA IV

From 1995-2003 a total of 610 patients were diagnosed with metastatic testicular NSGCT (418 Swedish, 192 Norwegian). For one patient medical records were lost, four patients were diagnosed at autopsy and two patients were neither staged nor treated (severe mental retardation; 91 years and dementia). These seven patients (1.1%) are not included in the analyses. Patient characteristics, therapy-related data and survival data are presented in Table 5. Ninety four percent were considered tumor-free after primary treatment, comprising conventional dose chemotherapy (BEP only or with the addition of ifosfamide) +/- surgery in 95% of the patients and high dose chemotherapy with stem cell support (HDCT) +/- surgery in 5% (Figure 8). Survival data are presented in Table 6.

The median FU for surviving patients was 99 months (range 24-162). The 10-year OS was 89.6%. The cumulative relapse incidence was 7.1% (95% CI, 5.3-9.6%) and 9.6% (95% CI, 7.3-12.6%) at 2 and 10 years after end of treatment, respectively. In all 51 patients (9%) had a relapse of which 11 were late relapses (LR), occurring >2 years after treatment, corresponding to a 10-year cumulative incidence of 2.7% (95%CI, 1.3-4.9). Only one patient with LR died. The 10-year OS post relapse was 74.7% (95% CI 60.5-84.4%). Using the IGCCC prognostic group classification at relapse the majority was good risk (66%), 19 % were classified as intermediate risk and 16% as poor risk.

Good risk

All but nine patients (97.7%) became tumor-free after primary treatment. The cumulative relapse incidence at two years was 6.0%. There were 32 relapses in all, of which 22 (69 %) were located in retroperitoneal nodes. Seventeen were mature teratomas and treated with surgery only, while the rest were treated with pre- or post surgical chemotherapy. None received HDCT. Four patients with relapse died. There were nine LR of which all were cured, 56% with surgery alone. In total twenty (5.1%) patients died, 14 due to NSGCT/treatment, six due to other causes. Treatment results are presented in Table 6 and Figure 10.

Table 5. Characteristics and treatment of 603 patients with metastatic non-seminomatous testicular cancer

	All	Good risk	Intermediate risk	Poor risk
N (%)	603 (100)	395 (65.5)	114 (18.9)	94 (15.6)
Age				
median	28	28	28	28
Range	(16-70)	(16-69)	(16-70)	(16-62)
AFP* µg/l				
median	19	12	127	321
range	(1-159207)	(1-890)	(1-7037)	(1-159207)
ß-HCG† IU/L				
median	18	5	240	21677
range	(0-974000)	(0- 4735)	(0- 43883)	(0- 974000)
Marker negative				
Pre orchiectomy N (%)	92 (15)	80 (20)	8 (7)	3 (3)
Post orchiectomy N (%)	152 (26)	137 (35)	10 (10)	5 (6)
Clinical stage‡				
Mk+/II/III/IV (%)	9/50/5/36	13/60/4/23	1/45/10/45	0/15/2/83
Primary treatment				
BEP N (%)	435 (77)	345 (89)	83 (77)	7 (10)
BEP+BEP-if/PEI N (%)	99 (18)	38 (10)	22 (20)	39 (56)
HDCT N (%)	30 (5)	3 (0.8)	3 (3)	24 (34)
<= 3 cycles N (%)	122 (22)	116 (30)	6 (5.6)	0
<= 3 cycles+HDCT N (%)	1 (0.2)	0	1 (0.9)	0
4 cycles N (%)	325 (58)	243 (63)	76 (70)	6 (8.6)
4 cycles+HDCT N (%)	14 (2.5)	2 (0.5)	0	12 (17)
>= 5 cycles N (%)	87 (15)	24 (6.2)	23 (21)	40 (57)
>= 5 cycles+HDCT N (%)	15 (3)	1 (0.3)	2 (1.8)	12 (17)
Abd nodes>2cm %**	65	56	89	79
Abd postchemotherapy surgery performed %	75	73	74	87

Abbreviations: AFP, α-fetoprotein; β-HCG, β–human chorionic gonadotropin; BEP, bleomycin-etoposide-cisplatin; BEP-if, bleomycin-etoposide-cisplatin-ifosfamide; PEI, cisplatin-etoposide-ifosfamide; HDCT, high dose chemotherapy with stem cell rescue; Abd, abdominal

*** missing, good/intermediate/poor: 1/0/2**

† missing, good/intermediate/poor: 1/0/0

‡ According to MRC [102]

**** Patients in whom the protocol prescribed post chemotherapy abdominal surgery**

Intermediate risk

One-hundred-eight (95%) patients were disease free after primary treatment and 10 patients relapsed (9%, 2 LR). The cumulative relapse incidence at two years was 7.4%. Eleven (9.7%) patients died, nine due to NSCGT/treatment and two due to other causes. Treatment results (Table 6 and Figure 10) were slightly inferior compared to corresponding figures in the good risk group.

Table 6. Relapse and survival of 603 patients with metastatic non-seminomatous testicular cancer.

	All	Good risk	Intermediate risk	Poor risk
Tumor-free primary therapy N (%)	567 (94)	386 (98)	108 (95)	70 (74)
Relapse N (%)	51 (9.0)	32 (8.3)	10 (9.3)	9 (12.9)
Cumulative incidence of relapse (95% CI)				
2-year %	7.1 (5.3-9.6)	6.0 (4.0-8.9)	7.4 (3.8-14.3)	12.9 (6.9-23.3)
10-year %	9.6 (7.3-12.6)	9.1 (6.4-12.8)	9.5 (5.2-17.0)	12.9 (6.9-23.3)
Survival (95%CI)				
10-year OS %↑	89.6 (86.8-91.8)	94.7 (91.9-96.6)	90.0 (82.8-94.4)	67.4 (56.7-76.1)
10-year CSS %↓	91.1 (88.5-93.2)	96.4 (94.0-97.8)	91.7 (84.8-95.6)	68.5 (57.5-76.8)
10-year PFS %↕	83.2 (79.8-86.0)	87.4 (83.4-90.3)	84.9 (76.6-90.3)	63.8 (53.2-72.6)

Abbreviations: CI, confidence interval; OS, overall survival, CSS, cancer specific survival; PFS, progression free survival
↑ Pairwise comparison: Good vs. Intermediate p=0.066, Good vs. Poor p<0.001, Intermediate vs. Poor p<0.001
↓ Pairwise comparison: Good vs. Intermediate p=0.045, Good vs. Poor p<0.001, Intermediate vs. Poor p<0.001
↕ Pairwise comparison: Good vs. Intermediate p=0.446, Good vs. Poor p<0.001, Intermediate vs. Poor p<0.001

Poor risk

The poor prognostic factor was "poor tumor markers" only (AFP ≥10,000 µg/l or β-HCG ≥50,000 mU/ml or LDH ≥10 X ULN) in 38 patients while 56 had non-pulmonary visceral metastases (NPVM) +/- "poor markers". Seventy patients (74%) became tumor-free after primary treatment. Nine patients (13%) relapsed, and the cumulative relapse incidence at two years was 12.9%.Thirty patients (32%) died, 29 due to NSGCT/treatment, one due to other causes. Treatment results are presented

in Table 6 and Figure 10. The survival is inferior compared to the good- and intermediate risk groups (p<0.001). The 10-year OS for patients with ”poor markers” only and patients with non-pulmonary visceral metastases were 81.6% and 58.1% respectively (p=0.032).

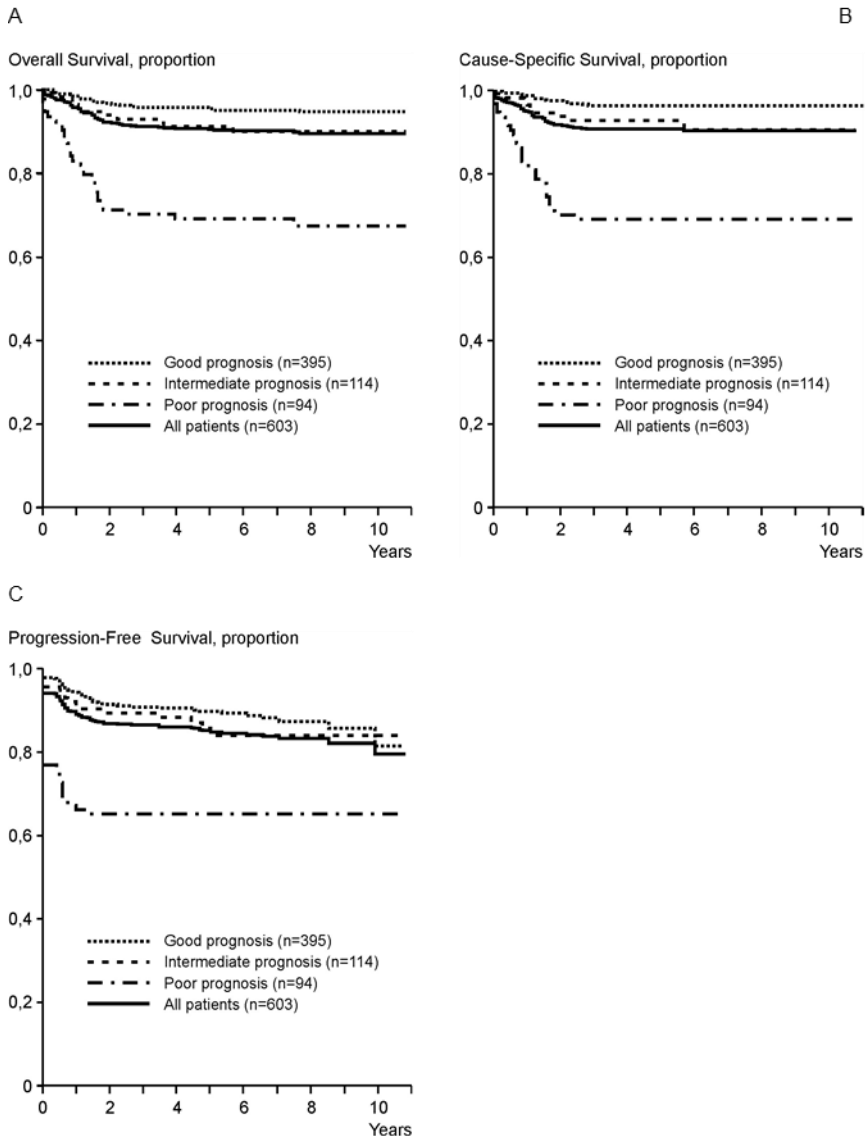


Figure 10. Survival of all 603 metastatic NSGCT and according to the IGCC classification. Panel A, Overall survival; Panel B, Cause-specific survival; Panel C, Progression-free survival

Eleven patients died due to treatment related complications; sepsis (n=3), myocardial infarction (1), intracerebral hemorrhage (2), kidney failure (1), liver cirrhosis (1), complications to surgery (1) and two not further specified . Seven patients died during standard BEP, two during BEP-if, and 2/31 patients who received HDCT died due to treatment.

Work loss after testicular cancer diagnosis

The patient cohort included 2 146 TCSs and the comparator cohort 8448 men with a median follow-up of 10 years (range 2 to 19 years). The majority of the TCSs were diagnosed with non-seminoma (57%), and most were diagnosed with stage I disease (seminoma 88%, non-seminoma 57%). A total of 29% TCSs were followed with surveillance only. Treatment with radiotherapy only was restricted to patients with seminoma (32%). Patients with non-seminoma more often received >4 courses of chemotherapy (5% vs. 0.3% in patients with seminoma). High-dose chemotherapy was given to 1% of the non-seminoma patients and to one of the seminoma patients. Relapse was diagnosed in 6% of all TCSs, and was more common among patients with non-seminoma (7%) than seminoma (4%).

TCSs were at a modestly increased annual risk of work loss up to the 3rd year of follow up (RR3rd year 1.25, 95% CI 1.08, 1.43), attributed to a more pronounced risk among extensively treated patients (4 chemotherapy courses: RR3rd year 1.60, 95% CI 1.19, 2.15; >4 courses: RR3rd year 3.70, 95% CI 2.25, 6.11). Patients on surveillance or limited treatment (radiotherapy, 1-3 chemotherapy courses) had no increased risk of work loss beyond the first year. TCSs receiving >4 chemotherapy courses had higher mean number of annual days of work loss up to the 10th year post-diagnosis (Figure 11), and a 5-fold risk of disability pension (RR 5.16, 95% CI 2.00, 10.3).

No at risk:															
1 courses (621) – · –	608	618	619	603	601	594	591	568	477	372	282	237			
2-3 courses (118) – – –	113	117	118	114	114	114	113	109	101	87	79	65			
4 courses (384) – · –	368	381	382	365	360	353	351	343	303	263	232	192			
>4 courses (102) – · –	97	101	102	92	91	90	90	88	81	73	66	60			
Comparators (8467) ——	8109	8344	8418	8448	8381	8302	8243	8006	6993	6064	5248	4312			
Mean difference (95% CI):															
1 course vs comp	0	0	**35**	0	-4	-4	-2	-4	-3	-3	-1	-1			
	(-5,6)	(-5,6)	**(28,43)**	(-5,7)	(-10,2)	(-10,2)	(-9,3)	(-9,2)	(-9,5)	(-10,3)	(-9,6)	(-10,9)			
2-3 courses vs comp	-4	-3	**105**	14	6	3	-1	2	2	4	12	3			
	(-16,8)	(-14,8)	**(87,124)**	(-2,32)	(-11,22)	(-11,19)	(-15,13)	(-13,16)	(-14,18)	(-12,21)	(-10,33)	(-16,23)			
4 courses vs comp	-1	0	**150**	**28**	**13**	**12**	10	10	8	7	7	4			
	(-8,6)	(-6,7)	**(138,161)**	**(17,38)**	**(3,22)**	**(2,22)**	(0,19)	(0,20)	(-2,18)	(-2,17)	(-4,18)	(-8,16)			
>4 courses vs comp	-2	6	**232**	**96**	**46**	**19**	16	**24**	18	**23**	**31**	**30**			
	(-15,10)	(-7,18)	**(208,256)**	**(66,126)**	**(21,70)**	**(0,38)**	(-4,36)	**(2,45)**	(-2,40)	**(0,46)**	**(4,57)**	**(2,59)**			

Abbreviations: Comp = Comparators

Figure 11. Mean annual days of sick-leave or disability pension from 2 years before, up to maximum 10 years after diagnosis (Dx) among TCSs treated with chemotherapy and population comparators. The numbers at risk and differences in mean annual days between TCSs and comparators, with 95% confidence intervals (CI), are displayed below the graph.

RBM3 expression and prognostic ability in NSGCT

A total number of 300 adult patients were diagnosed with testicular NSGCT in the Southern Sweden Health Region within the specified time frame. In 219 patients, complete clinical data were available in the SWENOTECA database at the time of data extraction. Thirteen (6%) cases were excluded from the study; four patients who started chemotherapy before orchiectomy, three cases with missing tissue blocks and six cases with an insufficient amount of tissue available for analysis.

Table 7. Characteristics, treatment, relapse and survival of 206 patients with non-seminomatous testicular cancer.

	All	**CS I**	**Good risk**	**Intermediate risk**	**Poor risk**
	N (%)	*N* (%)	*N* (%)	*N* (%)	*N* (%)
N (%)	206 (100)	118 (57)	64 (31)	13 (6)	11 (5)
Primary treatment					
CS I adjuvant		85 (72)			
1 regimen *N* (%)	64 (31)		53 (83)	10 (77)	1 (9)
2 regimens *N* (%)	21 (10)		11 (17)	2 (15)	8 (73)
>=3 regimens *N* (%)	4 (2)		1 (2)	1 (8)	2 (18)
HDCHT *N* (%)	6 (3)		0 (0)	0 (0)	6 (54)
Surgery *N* (%)	61 (30)		39 (61)	13 (100)	9 (82)
PAD-cancer *N* (%)	15 (25)		10 (16)	0 (0)	5 (56)
Relapse (*N* %)	14 (7)	8 (7)	3 (5)	1 (8)	2 (18)
Dead *N* (%)	13 (6)	7 (6)	2 (3)	1 (8)	3 (27)
NSGCT *N* (%)	6 (46)	1 (14)	1 (50)	1 (100)	3 (100)
Treatment *N* (%)	2 (15)	1 (14)	1 (50)	0	0
Other *N* (%)	5 (38)	5 (71)	0	0	0
TF↑ *N* (%)	23 (11)	3 (2)	13 (20)	1 (8)	6 (54)
Survival					
FU (m) median (range)	76 (3-167)	75 (3-142)	72 (9-167)	98 (57-125)	103 (50-134)
5-year OS %	96.0	98.2	96.8	92.3	72.7
5-year CSS %	96.6	99.1	96.8	92.3	72.7
5-year FFS %	89.6	97.4	80.3	92.3	34.1

↑ Relapse after treatment for metastatic disease, finding of active cancer at surgery post chemotherapy, or death from NSGCT.
Abbreviations: TF, treatment failure; HDCT, high-dose chemotherapy; OS, overall survival; CSS, cancer-specific survival; FFS, failure-free survival.

Characteristics of the 206 included patients are presented in Table 7.

In normal testis, RBM3 was expressed in weak to moderate intensity in spermatogonia and was not expressed in spermatocytes. In CiS, RBM3 was expressed with strong intensity in the vast majority of neoplastic cells. In TGCT, RBM3 was expressed in various intensities and fractions, mainly in the nucleus but also in the cytoplasm, with a particularly strong expression in seminomatous components.

Low RBM3 expression was associated with a significantly worse FFS [79.3% versus 90.4% (p=0.019)] (Figure 12B) and CSS [87.5% versus 97.3% (p=0.047)]. In the entire cohort, low RBM3 expression (NS <= 0.5) was observed in 16 (7.8%) cases and there was a significant association between clinical stage (p=0.044) and RBM3 expression. No significant associations were found between age, CS I vs >CS I and RBM3 expression.

For patients with metastatic disease, significant association was found between RBM3 expression and IGCCC group (p=0.007). The FFS was significantly inferior for patients with low RBM3 expression [59.3% versus 79.0% (p=0.013)] (Figure 12A), and this association remained significant in a multivariable model; including tumor marker level and the presence of NPVM or not (HR=3.67; 95% CI 1.14, 11.89). However, when tumor markers were categorized using the cut-points in the IGCCC, only AFP level remained as a significant prognostic marker. When combined with IGCCC groups in a multivariate model, RBM3 expression was not significantly associated with higher risk for treatment failure (HR=2.62; 95% CI 0.67, 10.31).

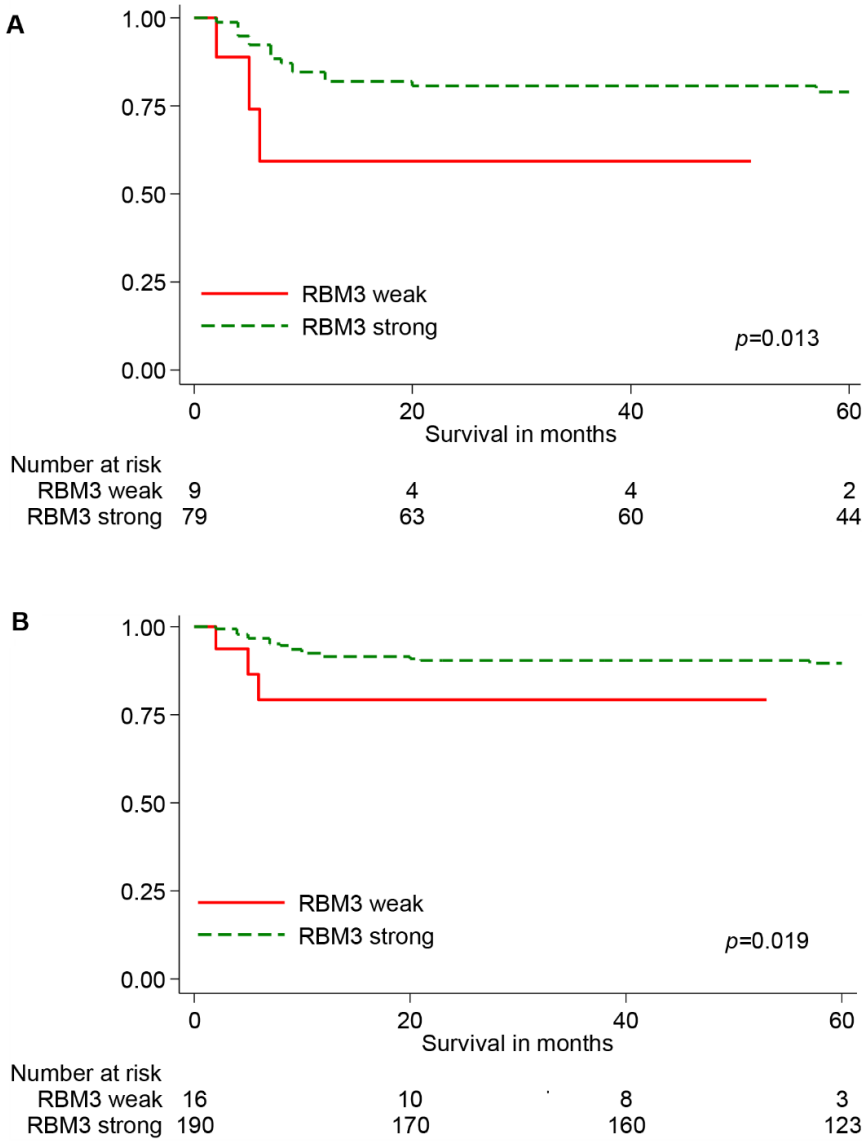


Figure 12. Failure-free survival of patients with non-seminomatous testicular cancer according to RBM3 expression. (A) 88 patients with metastatic disease and (B) 206 patients with clinical stage I to IV and Mk+.

Discussion

In the first paper of this thesis the outcome for 603 patients with metastatic non-seminomatous germ cell tumor (NSGCT) treated according to the management protocol SWENOTECA IV is reported. The SWENOTECA IV protocol was the first to apply the principle of early treatment intensification in case of therapy failure indicated by a slow tumor marker t1/2 or radiologically inadequate tumor response in a large prospective study. A weak point is that it was a non-randomized study which might be counterbalanced by the fact that it is population based and includes almost all patients diagnosed with metastatic NSGCT in Norway and Sweden during the period and that the follow up is long-term, median > 8 years. Four patients, three good risk and one poor risk, left the country and were lost to follow up, three patients five years and one good risk patient one year after diagnosis.

The strategy of early evaluation of tumor marker t1/2 has been shown to give prognostic information In a large retrospective study, evaluating the prognostic relevance of marker decline-rate using predicted ”time to marker normalization”, calculated from two marker values, pre and post first chemotherapy cycle, was found a strong and independent impact on OS and PFS of marker decline especially in patients with poor-risk [230]. Recently two randomized studies showed that intensification of treatment in patients with slow marker decline increased cure rate for patients with intermediate or poor prognosis. In a randomized phase III trial reported by Motzer et al., patients in the intermediate- or poor-risk group received either 4 x BEP or 2 x BEP followed by 2 x HDCT containing carboplatin. The addition of HDCT in first line treatment improved outcome only for the patients with slow marker decline during initial chemotherapy. The outcome for patients with satisfactory decline was superior in general, but primary HDCT was of no advantage in this group [231]. In a study by Fizazi et al., including poor prognosis patients, the tumor marker decline was measured before and after the first chemotherapy cycle and if unfavourable decline the patient was randomised to either continue BEP x 3 or to 4 cycles of an intensified dose-dense regimen with the addition of paclitaxel, oxaliplatin and ifosfamide [232]. The 3-year progression free survival was significantly better in the dose-dense group and the authors conclude that treatment with chemotherapy intensification reduces the risk of progression or death in patients with poor prognosis germ-cell tumors and an unfavourable tumor marker decline.

Taken all together, results from these studies support the hypothesis that there may be a chemotherapy resistance manifested by slow tumor marker decline during initial chemotherapy that could be overcome by treatment intensification.

And as a result of these studies, including SWENOTECA IV, the new EAU guidelines recommend intensification of treatment in case of slow t1/2 after one cycle of BEP in patients with poor prognosis [82].

In the SWENOTECA IV study, ninety eight percent of the good risk patients became tumor free, whereof 87% with standard BEP treatment. The results in this group are consistent with previous reports and the survival rate has been rather constant during the last 20 years [103, 104, 219]. In the SWENOTECA IV protocol, it was recommended that patients with small volume disease (CS Mk+, II-IV A_B, L1-L2) and optimal response on 2 BEP cycles should receive BEP x 3 as standard treatment and later studies has established that treatment with BEP x 3 is as effective as 4 courses for all patients classified as good prognosis [233]. In this series, the majority of good prognosis patients were treated with 4 courses, which, in the light of current knowledge, might represent over-treatment. Current SWENOTECA guidelines recommend BEP x 3 for good prognosis patients.

For the patients in the intermediate risk group, 95% became tumor free following primary therapy and three out of four of these patients with standard BEP. The outcome was excellent with results comparable with those reported from highly specialized single institutions [219], and to a recent randomized trial [234]. Furthermore, the survival figures from the SWENOTECA IV study are 10-year OS whereas most other studies present 3-year OS.

Frequently intermediate- and poor-prognosis patients are pooled together and treated with similar protocols [234, 235]. In our series, the outcome for patients in these two groups was fundamentally different and a 10-year OS of 90% indicate that the use of poor-risk protocols, which most often include at least one cycle of high dose chemotherapy with stem cell rescue, in patients belonging to the intermediate risk group would imply overtreatment. Actually, there was no significant difference in OS and PFS between the good and intermediate risk groups.

In the poor prognosis group with 95 patients, most patients received intensification of any level but approximately 10% became tumor free with standard BEP treatment only and 65 % with conventional dose chemotherapy. The long-term survival rate, 67%, compares well with other reports [231, 236, 237]. It should be noted that primary extragonadal germ cell tumors (EGCT), accounting for 2-5% of all germ cell tumors, were not included in SWENOTECA IV. The majority (60-70%) of EGCTs are located in the mediastinum, all categorized as poor-prognosis in the IGCCC. These are biologically distinct from and have a worse prognosis than

primary testicular GCT [238]. The exclusion of this poor prognostic group may have contributed to a more favourable outcome in our poor-prognosis population. Interestingly, the outcome for patients with marker elevation only was significantly superior to patients with non-pulmonary visceral metastasis, an observation which finds support in two studies using a regression-tree model (CART) as a method in order to find subgroups in patients with poor prognosis [239, 240]. This finding may be a result of our treatment strategy directed at early detection of slow marker decline, but could also reflect an underlying difference in tumor biology between these two patient populations.

In the 1997 report defining the IGCCCG grouping, the poor-prognosis group had an expected 5-year OS of 48%. In general, survival has increased over time, most likely due to improved treatment strategies and improvement of supportive care (intensive care and the usage of granulocyte-colony stimulating factor etc.) [241, 242]. The poor prognosis patients in the IGCCCG database were treated with cisplatin-based chemotherapy, but patients from the late 1970s and early 1980s could be treated with cisplatin–vinblastine– bleomycin regimens rather than BEP. There have been several attempts to improve the treatment results with changing to more dose intense regimens and also adding HDCT to initial treatment, but no improvement in efficacy has been shown in comparison to standard BEP [231, 232, 236, 237, 243]. The best survival rates have been reported from a randomized phase III EORTC trial comparing BEP x 4 with standard VIP x 1 plus Hd VIP x 3 finding a 2-year overall survival of 65% in the BEP treated group compared to 73% in the high-dose group. [236] However, the study was prematurely closed due to slow accrual and the difference between the two group did not reach statistical significance.
So, to improve the long-term survival of 50-65 % with today´s standard treatment in poor risk patients new treatment such as new regimens, new drugs, new principles for giving the regimens are needed.

A factor that may influence treatment results is the size of the treating institution. Two studies have shown that patients with advanced disease have a better prognosis if they are treated at an institution with a high volume [244, 245]. In SWENOTECA protocols it is postulated that patients with poor prognosis and those patients that do not respond well to initial chemotherapy should be referred to highly specialized care units.

Resection of residual tumors after chemotherapy is an essential part of treatment for advanced NSGCT. There are no good prediction models for evaluating the likelihood of finding viable cancer or teratoma after chemotherapy. In a study by Albers et al. with 193 non seminoma patients undergoing post chemotherapy retroperitoneal residual tumor resection, 35% of the patients had necrosis only, 31% viable cancer and 34% teratoma [246]. Multivariable analysis could not find any accurate model for selecting patients for surgery and therefore the recommendation

was to resect any residual disease. A newer study evaluating a new prediction model included 1024 patients [247]. They found that even in residual tumors < 1 cm, the finding was viable cancer or mature teratoma in 28%. The risk factors for these findings was teratoma in primary tumor, elevated serum tumor markers and small reduction of mass size after chemotherapy. Most guidelines and reports recommend active surveillance as a safe option if the residual mass is < 1 cm, with an expected relapse rate of 6-9% [82, 117, 248, 249]. Even if an relapse occurs, most patients are cured making surveillance an safe option not to compromise survival and to minimize overtreatment [250].

In SWENOTECA IV, RPLND was recommended for all patients with initial retroperitoneal lymph node enlargement >20 mm, but the recommendation was not followed during the last years of the study as data from more modern series emerged indicating that RPLND in patients in CR after initial chemotherapy safely could be abandoned. In the current SWENOTECA VIII protocol, RPLND is not recommended for patients with residual tumors < 1 cm post chemotherapy.

The increase in cancer incidence and an improvement in treatment of cancer leads to a rising prevalence of cancer survivors [251, 252]. After treatment long-lasting side effects may appear shortly after its completion or many years later. The cancer incidence is higher in the elderly for most cancers but still a large proportion are diagnosed before they are in their retiring age. For testicular cancer most survivors are in ages below 65 (Figure 13). A cancer diagnosis may affect the future work ability and quality of life. There has been a growing attention and research in this field as public health policies are needed to define and direct services to these cancer survivors [253]. It has been shown that most cancer survivors come back to work but there is a group with a higher risk of reduced work capacity [254]. Impaired work ability is mostly associated with the type of cancer, treatment, comorbidity, level of education and having physically strenuous work.

In the second paper, long term side effects of treatment in terms of sick leave and disability pension was investigated among TCSs. It is, to the best of my knowledge, the first nationwide investigation of work loss in cancer survivors with detailed clinical data, such as stage and treatment data and with a long follow-up. The Swedish population-based health care and labour market registries with prospectively recorded lost workdays provide a unique opportunity for a longitudinal investigation of work loss following a cancer diagnosis. For most TCSs there was no increased risk of work loss beyond the first year. The small group that received > 4 chemotherapy courses had higher mean number of annual days of work loss during the whole 10 year observation time, and a 5-fold risk of disability pension.

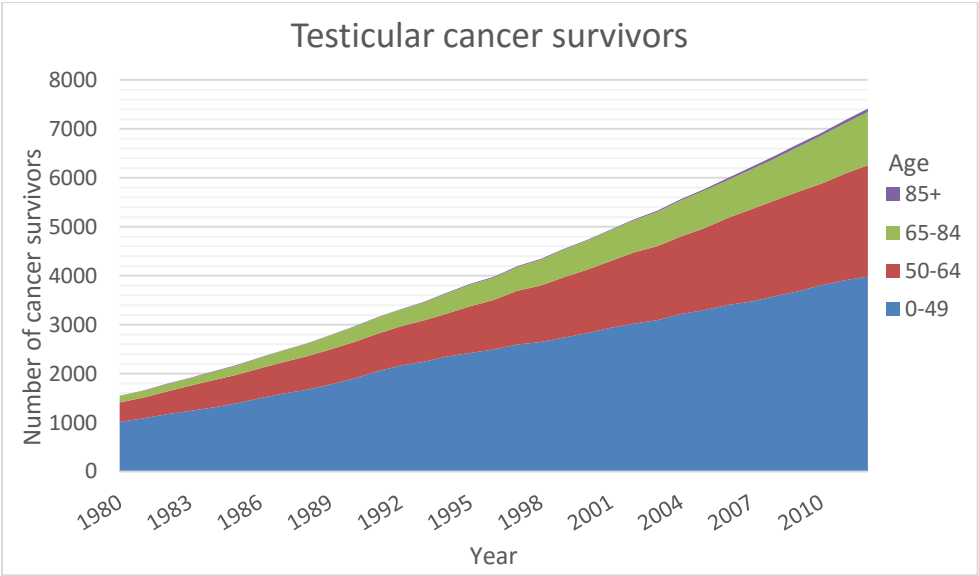


Figure 13. Prevalence of testicular cancer survivors in Sweden from 1980 to 2010 by age group [6].

Subgroups of TCSs were identified with a high risk of work loss, but we also noted the lack of an increase among patients receiving limited treatment. Among the most extensively treated patients, the results showed an increased risk of sick leave and/or disability pension up to the 5th year after diagnosis, with more mean days of sick leave and/or disability pension up to the 10th year. Even when excluding patients with relapse or HD chemo the difference remained. A reasonable explanation for the increased risk in the most extensively treated TCSs are the long-term treatment side effects [149, 153, 255, 256]. Studies reporting on dose dependent long term toxicity are presented in Table 8. Heavily treated TCSs are at increased risk of developing somatic complications such as gonadal dysfunction [255-257], oto- and neurotoxicity [258] and pulmonary toxicity [153], but it remains unclear to what extent these complications interfere with work ability.

In patients with other cancer types, chemotherapy has been associated with reduced cognitive function [259]. This has also been reported in TCSs [175], with possible effects on work ability.

The most severe long-term effects such as a second cancer and cardiovascular disease after TC treatment have been reported to mostly occur more than 10 years after treatment, and are thus probably not the reason for the observed risks up to 10 years [149, 168].

Table 8. Studies reporting on dose dependent long term toxicity.

Side effect	First author	Year	No of patients	Comments
Fertility	Brydøy et al. [158]	2005	1433	The paternity rate at 11 years after Dx was 65% total, 81% in the surveillance group, 77% RPLND, 65% RT, 62% Cisplatin ≤ 850 mg, 38% Cisplatin > 850 mg
Hypogonadism	Nord el al. [256]	2003	1235	After a median FU of 11 years the OR for hypogonadism was 3.7 total, 1.8 survillance, 3.6 RT , 4.4 Cisplatin ≤ 850 mg, 7.0 Cisplatin > 850 mg
Ototoxicity	Bokemeyer et al. [140]	1997	182	Persisting ototoxicity was seen in 20 % of patients receiving standard dose cisplatin and in more than 50% of patients receiving a cumulative dose of cisplatin >600 mg/m2
Fertility	Brydøy et al. [158]	2005	1433	Paternity rate (without assisted reproduction) for all TCSs was 71% (median 11.1 years FU time) treatment, 92% surveillance, RPLND 77%. 65% RT, 62% Cisplatin ≤ 850 mg,, 48% Cisplatin > 850 mg.
CVD	Haugnes et al. [149]	2010	990	Increased risks (HR) for atherosclerotic disease were compared with surgery only 2.3 RT, 2.6 chemotherapy, 4.8 RT/chemotherapy.
Pulmonary	Haugnes et al. [167]	2009	1049	Restrictive lung disease in 6.7-7.5% of the patients treated with surgery, RT and cisplatin ≤ 850 mg. Among patients treated with cisplatin > 850 mg 17.7% had restrictive lung disease. Most patients did not have any symptoms.
Cognitive	Skoogh et al. [175]	2012	1173	TCSs that received more than four cycles of cisplatin-based chemotherapy had increased incidence of compromised language, which was more evident among men with a low level of education.

Abbreviations: Dx, year of diagnosis; RPLND, retroperitoneal lymph node dissection; RT, radio therapy; FU, follow up; TCSs, testicular cancer survivors; HR, hazard ratio

For the extensively treated group it is probably hard to reduce the treatment burden and it has been shown that most long-term side effects are dose dependent. Focus should therefore be on early rehabilitation initiatives for this group. As late toxicity such as the metabolic syndrome, cardiovascular disease or a second cancer probably

will give more clinical symptoms beyond 10 years, there is a probability that the risk will increase again as indicated with the declining RS even after 30 years after diagnosis.

In order to establish individualized risk-adapted treatment strategies, minimize treatment burden, improve survival and restrict more intensive treatment to those who are most in need, it is necessary to further refine the established prognostic factors and to identify novel ones.

The risk factors used today are not good enough to distinguish subgroups in the poor prognosis patients. An improved risk classification could in the future possibly add molecular and genetic markers to the currently used IGCCC model. This could guide the treating clinician to better separate those cases that are likely to respond well to standard therapy from those that are at high risk of treatment failure. Some of these later patients may benefit from HDCT or novel therapies. Improved knowledge on new and existing markers may enlighten the natural development and progression of TGCT, improve the treatment and better predict the outcome. The question of a revised prognostic classification has been raised recently [260, 261].

New promising non-invasive methods for monitoring and diagnosing of disease are, as mentioned before, measurement of circulating mitochondrial DNA and CTCs [91, 93, 94, 262]. These methods will potentially be of use in treating patients with Mk- disease and maybe also aid in risk stratifying in all patients. Korkola et al. identified a gene expression signature that could predict outcome in men with metastatic NSGCT [263]. Signatures associated with immune function and repression of differentiation were associated with good outcome, whereas signatures associated with active differentiation, particularly into neural lineages, and loss of the pluripotent genotype, were associated with poor outcome. Adaptation of subsets of these genes for use in clinical assays could result in improved outcome prediction and risk stratification. New prognostic models including old and new markers may aid in risk grouping and treatment decisions.

Regarding new therapies, there are case reports where treatment with antibodies and targeted therapy has shown efficacy in chemotherapy resistant GCT, but this has not been verified in clinical studies. Imatinib has induced CR in a patient having a seminoma with over expression of CKIT [264]. In a phase II study with six patients expressing cKIT receiving imatinib only, one patient had temporary stable disease while the other progressed [265]. Other multikinase inhibitors have been tested with low response rates and short duration of responses [9, 266]. In terms of antibody therapy, trastuzumab has shown activity in one patient with a HER2 positive tumor and bevacizumab showed signs of activity in a patient with growing teratoma [9].

In the third paper, the expression of RBM3 in testicular NSGCT was investigated, with particular reference to its prognostic ability in risk stratification of patients. IHC and the TMA technique were used to evaluate the expression of RBM3. IHC is used in daily practice in routine diagnostics and classification of several diseases and cancers, and gives information on subcellular antigen localization. A drawback to IHC is that the staining evaluation is based on subjective assessment by individual observers, often lacking reference standards, which leads to difficulties in comparing different study results. Other differences that may affect the outcome are, for example, the handling of tissue and the antibody characteristics. Various automated image analysis tools have been introduced, but their availability is limited and they are generally not used in routine practice [267, 268]. Prompted by these difficulties, there are guidelines in order to standardize and improve the quality of biomarker studies [269]. A potential limitation in our study is that sampling bias cannot be excluded due to the heterogeneity of the tumors and that analysed tissue samples only represent a limited fraction of the tumor. On the other hand, a study analysing the prognostic markers (ER, PgR and p53) with four cores in breast cancer demonstrated that tissue heterogeneity did not negatively influence the predictive power of the TMA results[270]. The conclusion was that the high number of samples from each tumor that can be included in the TMA study may well compensate for false negative or positive tissue cores [270].

RBM3 has been shown to be a prognostic biomarker in several cancers (Table 9), e.g. urothelial cancer[205], breast cancer [220], colorectal cancer [206], prostate cancer[208], esophageal and gastric adenocarcinoma [209] and ovarian cancer [202]. Moreover, RBM3 expression has been demonstrated to correlate with sensitivity to cisplatin treatment in ovarian cancer *in vitro* [202]. A recent study analysing RBM3 expression by IHC on a tissue microarray containing 11,152 prostate cancers, found high RBM3 expression to be linked to advanced tumor stage, high Gleason score, positive nodal involvement and positive surgical margin status [271]. RBM3 staining was also related to early biochemical recurrence. These results were in contrast to the study by Jonson et al., wherein high nuclear RBM3 expression was found to be significantly associated with a prolonged time to biochemical recurrence and clinical progression [208]. Speculatively, these discrepant results may be due to differences in experimental procedures, patient selection or issues of cohort size [271]. Another study on prostate cancer found that RBM3 overexpression attenuated stem cell-like properties *in vitro* as well as tumorigenic potential *in vivo* [272]. Moreover, RBM3 expression was significantly increased in the primary tumors and relatively decreased in the metastatic prostate cancer samples, a finding in line with the study by Jonson et al. [208, 272].

In the present series, low expression of RBM3 was a predictor of an increased risk of treatment failure in patients with metastatic NSGCT, and one could hypothesize that RBM3 may be a predictor of sensitivity to cisplatin-based treatment also in this

type of cancer. However, if RBM3 expression would be related to cisplatin sensitivity only, one would have expected a higher risk of relapse after adjuvant BEP in CSI patients with tumors having low RBM3 expression, which was not observed.

A proposed mechanism of action for RBM3 is that it may promote tumorigenesis in early stages of cancer by affecting DDR (DNA damage repair) and checkpoint integrity, thereby lowering the threshold for the selection of more malignant clones [202, 210]. Once an invasive tumor is established, high levels of RBM3 may influence genomic stability and, hence, chemotherapy sensitivity. In order to personalize treatment, there is a need for new biomarkers in order to more accurately choose the appropriate therapy upfront. Our data suggest that RBM3 expression may provide additional prognostic information to conventional parameters, i.e. tumor marker levels and pattern of metastasis. When combined with the IGCCC in a multivariable model, RBM3 failed to add significant prognostic information. This may be due to the fact that plasma tumor marker levels (HCG, AFP and LDH) are categorical in the IGCCC, and used as continuous variables in our analysis. For the optimal use of RBM3 in a prognostic model, other cut-off limits for plasma tumor markers may need to be applied. Taken together, our findings suggest that RBM3 may be a potential biomarker for treatment stratification of patients with metastatic non-seminomatous germ cell tumors, and therefore merit further validation. The optimal cut-off between weak and strong RBM3 expression has to be established in forthcoming studies, since the classification and regression tree (CRT) method may lead to an overfitting of the model, and therefore needs to be cross-validated in a different data set.

The basis for the three papers in this thesis are clinical data collected in the SWENOTECA register and so much of the work are due to the foresighted people creating and maintaining the register and management programs. The SWENOTECA collaboration with its database over virtually all patients diagnosed with TC in Sweden and Norway and the prerequisites to link information from other quality registers with the personal ID gives unique opportunities to perform population-based studies. The collected data are used to evaluate the outcomes of the management programs and are the basis for updated comprehensive versions. The strength of using this method is that the results are from treatment in a community based setting in two countries and all treated patients are included. Therefore the data from the treatment protocol are robust and makes the probability of reproducibility in another community setting high [273].

Table 9. Studies on RBM3 as a prognostic marker in various cancers.

Diagnosis	First author	Year	No of patients	Comments
Urothelial	Boman et al. [205]	2013	343	Reduced nuclear RBM3 expression was significantly associated with more advanced tumor (T) stage ($p < 0.001$) and high grade tumors ($p=0.004$). Negative RBM3 expression was associated with a significantly shorter DSS (HR=2.55) and 5-year OS (HR=2.10).
Esophageal and gastric	Jonsson et al. [209]	2014	173	Low RBM3 expression was significantly associated with a shorter OS in cases with radically resected tumors (R0) (HR 2.19) and RFS in curatively treated patients with R0 resection/distant metastasis-free disease (HR = 3.21).
Malignant melanoma	Jonsson et al. [207]	2011	215	High nuclear RBM3 expression in the primary tumor was significantly associated with favourable clinicopathological parameters and a prolonged RFS (RR = 0.50) and OS (RR = 0.36).
Colorectal	Hjelm et al. [206]	2011	270 Cohort I 305 Cohort II	Tumors with high nuclear RBM3 staining had significantly prolonged overall survival in both cohorts in multivariate analysis. High tumor-specific nuclear expression of RBM3 was an independent predictor of good prognosis.
	Melling et al. [274]	2015	1800	Loss of RBM3 expression was linked to advanced tumor stage, right-sided tumor localization and poor prognosis. Multivariable analysis only tumor stage and nodal status proved to be independent prognostic markers.
Prostate	Jonsson et al. [208]	2011	88	High nuclear RBM3 expression was significantly associated with a prolonged time to biochemical recurrence (HR 0.56) and clinical progression (HR 0.09).
	Grupp et al. [271]	2014	11152	High RBM3 expression was linked to advanced tumor stage, high Gleason score, positive nodal involvement and positive surgical margin status. RBM3 staining was related to early biochemical recurrence
Ovarian	Ehlén et al. [202]	2010	267 Cohort I 154 Cohort II	In both cohort I and II increased RBM3 mRNA expression was associated with a prolonged and OS (HR = 0.64 and 0.53 respectively). RBM3 levels were significantly higher in the cisplatin sensitive cell line compared to the cisplatin resistant. siRNA-mediated silencing of RBM3 expression in the cisplatin sensitive cells resulted in a decreased sensitivity.

Abbreviations: DSS, disease specific survival; R0, radically resected; HR, hazard ratio; RFS, recurrence-free survival; OS, overall survival; RR, relative risk

Conclusions

Paper I

Observing the favourable treatment results in both the intermediate-prognosis group and in the poor-prognosis patients with marker elevation only, the strategy of early detection of slow t1/2 as an early indicator of treatment failure and subsequent treatment intensification seems to be advantageous for this subset of patients. The good treatment results makes it the standard treatment strategy for patients with advanced NSGCT in Sweden and Norway.

In the poor prognostic group where improvement in outcome is needed, two subgroups were identified, the outcome for patients with marker elevation only was significantly superior to patients with non-pulmonary visceral metastasis. This finding awaits conformational studies.

Paper II

In conclusion, we have shown that most TCSs, receiving no or limited treatment, do not have an increased risk of work loss in comparison to the general population beyond the first year after diagnosis. However, extensively treated TCSs have a prolonged increased risk of sick leave and/or disability pension, especially those who have received more than 4 cycles of chemotherapy. This increased risk cannot be explained by cancer relapse. Further studies are needed to investigate underlying reasons for the increased risk of work loss among the most heavily treated patients. Physicians should be aware of this risk in order to provide optimized support and early work-related rehabilitation.

Paper III

Low RBM3 expression is an independent predictor of treatment failure in metastatic NSGCT, in relation to the prognostic factors included in the IGCCC. These findings suggest that RBM3 may be a potential biomarker for treatment stratification in patients with metastatic non-seminomatous germ cell tumors, and therefore merit further validation.

Future perspectives

The excellent treatment results obtained today in patients with GCT is partly due to the dedicated work by the SWENOTECA collaboration and shows the importance of consistent structural work with continuous assessment and adaption of management programs according to new important findings and comprehensive knowledge.

Despite that more than 95% of all patients diagnosed with TGCT are cured, TGCTs are the most common cause of death in cancer in young men. For the patient with poor prognosis, only small advances have been made during the last years and it is this group that mostly needs novel treatment strategies and new therapies. The introductions of HDCT, taxanes and gemcitabine have given small improvements in survival. Newer agents like TKI and antibodies has shown to give hopeful results in only a subset of patients. Maybe individually tailored treatment based on molecular and genetic markers in addition to currently used risk factors can help improve the outcome in this high risk group. Another group of patients in need of novel strategies is patients with seminoma categorized in the intermediate prognosis group.

The finding of two subgroups, characterized by marker elevation only or by non-pulmonary visceral metastases, in the poor prognostic group needs to be validated. A project has been started where patients treated according to SWENOTECA IV principles between 1995 until 2012 are planned to be included in the analysis.

In order to further investigate the prognostic ability of RBM3, it is necessary to perform further functional studies. It would also be of interest to examine RBM3 expression in metastases after postchemotherapy surgery, where a low RBM3 expression would indicate reduced cisplatin sensitivity. Due to the heterogeneity of NSGCT, it is also necessary to investigate the expression in the different tumor components and to include this variable in the analysis.

As circulating mitochondrial DNA and also circulating tumor cells in the peripheral blood of patients with testicular cancer have been shown to be non-invasive biomarkers for monitoring of disease, the addition of a biobank with patient blood samples during diagnosis, treatment and FU to the SWENOTECA registry would be of tremendous value for future studies. Such a study has recently been initiated

in Norway in which blood samples from patients with GCT will be collected for analysis of the expression of a subset of miRNAs in serum.

It is clear that treatment may cause long term morbidity and it would be helpful to further investigate the reasons for the increased work loss in the heaviest treated group in order to provide adequate preventive measures. The standard FU after treatment is up to 10 years and after that it is important to provide thorough information to the primary care giver, emphasizing the kind of complications that may occur. Special efforts should be made for the heaviest treated patients with other risk factors for work loss.

There are also possibilities to prevent long-term toxicity through pharmacological measures. One example is a meta-analysis investigating cardio-protective therapy [275]. The authors found that antioxidants (Dexrazoxane), beta-blockers, statins or angiotensin antagonists appeared to have similar efficacy for reducing cardiotoxicity [275]. More studies involving patients diagnosed with TC, focusing at early interventions for protection of long term toxicity are needed.

Other long-term side effects may be prevented by alternative follow-up strategies. Studies have shown an increased risk of second cancers due to repeated CT scans, indicating that MRI should be used as the imaging choice the during the follow up period.

Possibly, in the future the use of RBM3 and other novel markers can be used in refining treatment selection and duration for patients and thereby avoiding overtreatment for patients with highly chemo sensitive tumors and giving intensified treatment only to those with a truly poor prognosis. This would then minimize late toxicity and potentially improve quality of life and work ability for a proportion of patients with testicular cancer.

Populärvetenskaplig sammanfattning

I Sverige är testikelcancer den vanligaste cancerformen hos unga män. Antalet som insjuknar ökar varje år, från 2 per 100000 män år 1958, 4,5 år 1987 och 7 st 2012. Trots mycket hög frekvens (mellan 5-45% beroende på undergrupp) av spridd sjukdom vid diagnos är prognosen väldigt god jämfört med andra cancersjukdomar. Svenska behandlingsresultat är bland de bästa i världen, men de kan förbättras ytterligare.

Varje år registreras ca 250 nya fall i Sverige respektive Norge. Antalet fall ökar i båda länderna. Testikelcancer utgör endast en procent av totala antalet nya cancerfall hos svenska män, men är den vanligaste cancerformen hos män i åldrarna 20–40 år. I de nordiska länderna, Tyskland och Nya Zeeland finns den högsta förekomsten. I Asien och Afrika är den däremot ovanlig.

Den direkta orsaken till testikelcancer är inte känd men det förmodas att någon påverkan tidigt i fosterlivet kan vara av betydelse, som till exempel rubbningar i den hormonella miljön. Ungefär 10% av de som insjuknar tidigare har opererats för en testikel som inte har vandrat ner i pungen på normalt sätt. Även vid missbildningar i könsorganen och vid infertilitet finns en ökad risk. Ärftlighet beräknas stå för 1–3% av alla insjuknanden.

Vid slutet av sjuttiotalet i Sverige var överlevnaden 5 år efter diagnos cirka sjuttio procent men har ökat till 95% i dag. De förbättrade resultaten beror troligen på att behandling med cisplatin (ett läkemedel) var ytterst verksamt, men även förbättringar av kirurgi och röntgenologiska metoder har bidragit.

Vid en kartläggning av testikelcancer i Europa 1995 var överlevnaden i de flesta länder över 90 %. Sverige och Norge toppade överlevnadslistan med över 95 %. Vid en ny genomgång i Europa 2004 låg fortfarande Sverige och Norge i topp.

SWENOTECA är ett samarbetsprojekt mellan svenska och norska sjukvårds-regioner för att skapa en kunskapsbank gällande testikelcancer. SWENOTECA är ett nätverk för utbyte av kunskaper, erfarenheter och kvalitetssäkring och har sedan 1981 erbjudit vård- och behandlingsprogram samt forskningsstudier för de deltagande klinikerna.

Sannolikt är SWENOTECA-samarbetet orsaken till dagens goda behandlingsresultat genom att det fortlöpande utarbetas moderna behandlingsprotokoll. Dessutom finns ett fungerande nätverk för diskussion och rådgivning vid bland annat svårdiagnostiserade och svårbehandlade fall.

Även om resultaten är bra så dör trots det ett antal unga män i sjukdomen. För överlevarna kan behandlingen ge långtidsbiverkningar. Som till exempel hjärtkärlsjukdom, övervikt, infertilitet, nedsatt hörsel och låga testosteronnivåer.

Forskning pågår för att optimera den individuella behandlingen genom att hitta bättre uttryck i tumörvävnad och blod för att styra behandlingen. Därmed kan man undvika att ge onödigt tung cytostatikabehandling till patienter som inte behöver det, och redan i tidigt skede intensifiera behandlingen för gruppen med sämst prognos. Detta för att försöka förbättra överlevnaden ytterligare.

I delarbete I analyserades behandlingsresultaten mellan 1995 till 2003 hos patienter som hade spridd testikelcancer av en typ som heter non -seminom. Behandlingen styrdes av hur tumör-proteiners koncentration (tumör markörer) i blodet sjönk under de två första behandlingsomgångarna. Vid otillfredsställande resultat så gavs en mer intensiv behandling. Patienterna delades in i tre prognosgrupper (god, intermediär och dålig) efter kända faktorer, som nivåer av tumörmarkörer och i vilka organ dottertumörerna spridits till.

Resultaten var mycket goda och överlevnaden var 90% efter 10 år för hela gruppen. Indelat i prognosgrupper var överlevnaden för patienter med bra prognos 95%, intermediär 90% och dålig 67%. Internationellt står sig resultaten mycket bra, framförallt för intermediärgruppen. Analyser visade att det inom gruppen med dålig prognos fanns två undergrupper med olika behandlingsresultat. De patienter som var klassificerade som dålig prognos bara genom höga nivåer av tumörmarkörer hade en bättre överlevnad på 82%, jämfört med dom som hade spridning till organ utanför lunga och lymfkörtlar 58%. Detta resultat måste verifieras i nya studier för att kunna användas i val av behandling för dessa två undergrupper.

Då flertalet testikelcancerpatienter är unga vid diagnos och de flesta överlever så kommer många testikelcanceröverlevare att finnas i samhället. Det har framkommit i andra studier att biverkningar av behandling kan vara nedsatt ork, låga testosteronnivåer, ökad risk för hjärtkärlsjukdom, lungfunktionsnedsättning och uppkomst av tumörer orsakade av behandling. Målet med den andra studien var att se hur arbetsförmågan påverkas efter att ha behandlats för testikelcancer. Materialet omfattade svenska patienter som diagnostiserats med testikelcancer från 1995 till 2007 och som lever två år efter behandling. En jämförelsegrupp identifierades ur befolkningsregistret, och både patienter och jämförelsegrupp länkades ihop med olika register för att få fram sjukfrånvaro och annan nödvändig information. Analys

av data genomfördes med undersökning av sjukfrånvaro i relation till tumörtyp, sjukdomsstadium, behandling samt andra karakteristika. Resultatet visade att patienter som fått mer än 4 behandlingar med cellgifter hade en ökad risk för sjukfrånvaro upp till tio år efter testikelcancerdiagnosen, samt ökad risk till förtidspension. Patienter som inte fått någon efterbehandling med cellgifter eller strålbehandling, samt de som fått mindre än 4 behandlingar med cellgifter, hade ingen ökad risk efter det första året.

RBM3 är ett protein som kan påverka vilka andra proteiner som ska tillverkas i cellen, men dess fulla funktion är inte helt kartlagd. I andra studier där man undersökt tumörer från bland annat bröstcancer, tjocktarmscancer, blåscancer, prostatacancer samt äggstockscancer visade resultaten att patienter vars tumörer uttryckte höga nivåer av RBM3 hade bättre överlevnad, jämfört med patienter vars tumörer hade låga nivåer. I delarbete 3 analyserades hur proteinet RBM3 uttrycktes i testikelcancerceller, och det genomfördes bland annat analyser för att se om nivån av RBM3 kunde förutse hur tumören svarade på cellgiftsbehandling. Resultatet visade att nivåerna av RBM3 var högt i förstadier till testikelcancer och att uttrycket var lägre i de tumörer där patienterna hade mer spridd sjukdom vid diagnostillfället. Det fanns också en koppling där patienter med lågt uttryck av RBM3 i tumören hade större risk att inte svara bra på behandling. Resultaten tyder på att RBM3 skulle kunna användas för att välja ut de patienter som kanske skulle ha nytta av en tyngre behandling redan från början, men ytterligare studier behövs för att bekräfta detta.

Acknowledgements

There are lot of people making this thesis possible that I want to thank:

Mats Jerkeman my supervisor that always been there for support with great knowledge, patience and encouragement.

Eva Cavallin-Ståhl and Olof Ståhl my co-supervisors, who have shared all their knowledge and support and making it a family effort to teach me all about testicular cancer without losing hope in me.

Karin Jirström, Björn Nodin, Alexander Gaber that guided me into the world of immunohistochemistry and tissue-micro arrays and making me one of the TMA-team.

Karin Ekström-Smedby, Carina Nord, Sara Ekberg and Ingrid Glimelius that aided in the making of paper II, sharing their wisdom in epidemiology and coding in various statistical softwares.

Gabriella Cohn Cedermark, Torgrim Tandstad, Olav Dahl, Olbjørn Klepp, Ulrika Stierner, Arne Solberg, Roy Bremnes, Carl Wilhelm Langberg, Anna Laurell, Rolf Wahlqvist, Hans Wijkström, and all other members of SWENOTECA, for all the work you do in order to keep a high quality register and management programs.

Monika Andersson, for excellence in data management and secretarial assistance during these years.

To all other co-authors of the papers. Thank you for your effort and valuable contributions.

My support in statistics Harald Andersson and Oskar Hagberg.

Mihalj Seke, my clinical supervisor during residency, for support and three questions a day.

Current and former colleagues at the Department of Oncology, Växjö Hospital for support and giving me the time to do this research.

The colleagues who introduced me to the field of urologic oncology during my working period in Lund; Magdalena Cwikiel, Per Flodgren and René Blom.

The funders of the research: Cancerstiftelsen i Kronobergs län, FoU Kronoberg, Region Skånes FoU, Lund University Faculty of Medicine and University Hospital Research Grants.

And finally my beloved family; my wife Fereshteh and our wonderful children Sara, Jacob and Hanna.

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