

LUND UNIVERSITY

STAT3 in Prostate Cancer

Don-Doncow, Nicholas

2017

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

Link to publication

Citation for published version (APA): Don-Doncow, N. (2017). *STAT3 in Prostate Cancer*. [Doctoral Thesis (compilation), Department of Translational Medicine]. Lund University: Faculty of Medicine.

Total number of authors:

General rights

Unless other specific re-use rights are stated the following general rights apply:

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights. • Users may download and print one copy of any publication from the public portal for the purpose of private study

or research.

You may not further distribute the material or use it for any profit-making activity or commercial gain
You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: https://creativecommons.org/licenses/

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117 221 00 Lund +46 46-222 00 00

STAT3 in Prostate Cancer

Nicholas Don-Doncow



DOCTORAL DISSERTATION by due permission of the Faculty of Medicine, Department of Translational Medicine, Division of Urological Cancers, Lund University, Sweden

To be defended at Lilla Aulan Jan Waldenströms Gata 5

February 2nd 2017 at 13:00.

Faculty opponent Hing Leung MD, PhD

Professor of Urology and Surgical Oncology, Institute of Cancer Sciences, University of Glasgow, United Kingdom

Organization LUND UNIVERSITY	Do	ocument name			
	Da	ate of issue			
Author(s) Nicholas Don-Doncow	Sp	consoring organization			
Title and subtitle: STAT3 in Prostate	Cancer				
Abstract Prostate cancer is the second most of biomarkers to distinguish between in the disease treatment options are lin Recently the signal transducer and a target for treatment, and a biomarke In this work we explored the potentia We also investigated the mechanism combination treatments with chemot In a rapid autopsy cohort of patients pSTAT3 was present in all metastas microenvironment. A second cohort expression was higher in benign tiss predictive of shorter time to recurrent valuable at later stages of the diseas We also investigated galiellalactone, We found that galiellalactone binds of galiellalactone was used in combina was likely due to the observed down In conclusion, our results suggest th may lead to benefit when used in co	Prostate cancer is the second most common diagnosed cancer among men. Currently there are no reliable biomarkers to distinguish between indolent and aggressive disease. When men progress to advanced stages of the disease treatment options are limited and resistance to treatments available today is a growing problem. Recently the signal transducer and activator of transcription 3 (STAT3) has been suggested as both a potential target for treatment, and a biomarker in advanced prostate cancer. In this work we explored the potential of activated STAT3 (pSTAT3) as a tissue biomarker in two different cohorts. We also investigated the mechanism of action of the STAT3 inhibitor galiellalactone as well as its effect in combination treatments with chemotherapeutic agent docetaxel. In a rapid autopsy cohort of patients that have died of metastatic prostate cancer we found that expression of pSTAT3 was present in all metastases, with highest expression in the bone, likely an effect of the tumor microenvironment. A second cohort of hormone naïve patients with localized prostate cancer showed that pSTAT3 expression in tumor cells was predictive of shorter time to recurrent disease. These two cohorts suggest that targeting pSTAT3 would be valuable at later stages of the disease. We also investigated galiellalactone, a natural fungal compound, and its mechanism of action in inhibiting STAT3. We found that galiellalactone binds directly to STAT3, thus blocking the ability of STAT3 to bind to DNA. When galiellalactone was used in combination with docetaxel it was able to produce a synergistic inhibitory effect, which was likely due to the observed downregulation of genes involved in docetaxel resistance.				
Key words: prostate cancer, STAT3, biomarker, immunohistochemistry, drug resistance					
Classification system and/or index te	erms (if any)				
Supplementary bibliographical information		Language			
ISSN and key title 1652-8220		ISBN 978-91-7619-400-3			
Recipient's notes	Number of	pages	Price		
	Security classification				

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature

____Date _____

STAT3 in Prostate Cancer

Nicholas Don-Doncow



Coverphoto by Nicholas Don-Doncow

Copyright Nicholas Don-Doncow

Faculty of Medicine, Translational Medicine, Division of Urological Cancers

ISBN 978-91-7619-400-3 ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University Lund 2016









To Catja and Spencer

"Well I guess now is the time for me to say something profound...

Table of Contents

Table of Contents	8
List of Papers	11
Abbreviations	13
I. The Prostate	17
Structure	18
Cellular Structure	19
Prostate Cancer and Diagnosis	20
Androgen Receptor	20
Treatment Options	21
II. Biomarkers	23
Introduction	24
Immunohistochemistry	24
Prostate Cancer Biomarkers	25
Gleason Score	25
PSA	26
PCA3	27
TMPRSS2-ERG	27
AR-V7	
PIEN Other Piemerkers	
Other Biomarkers	28
III. STAT3	29
Introduction	30
STAT3 activation and signaling pathway	31
IL-6/STAT3 pathway	31
STAT3 signaling	33
STAT3 in cancer	
STAT3 in PCa	34
STAT3 and cell proliferation and apoptosis	34
STAT3 and metastases	35

STAT3 and angiogenesis	35
STAT3 and immune suppression	
Targeting STAT3	
Galiellalactone	
IV. Drug resistance	
Introduction	40
ADT	40
Docetaxel resistance	41
STAT3 and drug resistance	42
Combination Therapies	42
V. The Current Investigation	45
Paper I	47
Introduction	47
Results and Discussion	47
Paper II	49
Introduction	49
Results and Discussion	49
Paper III	51
Introduction	51
Results and Discussion	51
Paper IV	53
Introduction	53
Results and Discussion	53
Concluding Remarks	55
Popular Science Summary	57
Acknowledgements	59
References	63
VI. Papers	79

List of Papers

The thesis is based on the following papers:

- I. Expression of STAT3 in Prostate Cancer Metastases. **Don-Doncow N**, Marginean F, Coleman I, Nelson P, Ehrnström R, Krzyzanowska A, Morrissey C, Hellsten R and Bjartell A. Eur Urol, 2016. Available online 22 June 2016.
- II. Immunohistochemical expression patterns of activated STAT3 and androgen receptor in hormone-naïve prostate cancer. Don-Doncow N*, Krzyzanowska A*, Marginean F, Hellsten R, Bjartell A. Preliminary Manuscript
- III. Galiellalactone is a Direct Inhibitor of STAT3 in Prostate Cancer Cells. Don-Doncow N, Escobar Z, Johansson M, Kjellstrom S, Garcia V, Munoz E, Sterner O, Bjartell A and Hellsten R. J Bio Chem, 2014. 289(23): p. 15969-78.
- IV. The STAT3 inhibitor galiellalactone down-regulates genes involved in drug resistance and enhances the anti-proliferative effects of docetaxel in prostate cancer cells. **Don-Doncow N**, Bjartell A and Hellsten R. Preliminary Manuscript.
- * = Authors have equally contributed

Abbreviations

AA anti-androgen ABC adenosine triphosphate binding cassette ADT androgen deprivation therapy ALDH1 aldehyde dehydrogenase 1 ALK anaplastic lymphoma kinase AR androgen receptor ARE androgen-responsive elements AR-V7 and rogen receptor splice variant 7 AS active surveillance ASTRO American Society of Therapeutic and Radiology Oncology BAD Bcl-2-associated death promoter BCR biochemical recurrence BCL-2 B-cell lymphoma 2 BCL-XL B-cell lymphoma-extra large BCR-ABL breakpoint cluster region - abelson BCRP breast cancer resistance protein BPH benign hyperplasia of the prostate c-PARP cleaved poly(ADP-ribose) polymerase CDK cyclin dependent kinase CML chronic myeloid leukemia CRPC castration-resistant prostate cancer cT clinical tumor stage CZ central zone

DHT dihydrotestosterone

DNA deoxyribonucleic acid

DRE digital rectal examination

EBR external beam radiation

EGF epidermal growth factor

EGFR epidermal growth factor receptor

EMA European Medicines Agency

ERG erythroblast transformation-specific -related gene

ERSPC European Randomized Screening Study for Prostate Cancer

FAS apoptosis antigen 1

FDA Food and Drug Administration

gp130 glycoprotein 130

GS Gleason score

H&E hematoxylin and eosin

HER2 human epidermal growth factor receptor 2

HIF1 α hypoxia-inducible factor 1 α

HSP heat shock protein

IGF1R insulin-like growth factor 1 receptor

IHC immunohistochemistry

IL interleukin

IL-6R interleukin 6 receptor

JAK Janus kinase

LHRH Luteinizing Hormone-Releasing Hormone

LIF leukemia inhibitory factor

MCL-1 BCL2 family apoptosis regulator

MMP matrix metalloproteinase

MRP1 multidrug resistance-associated protein 1

 $NF\kappa B$ - Nuclear factor kappa-light-chain-enhancer of activated B cells

nRTK non-receptor tyrosine kinases

P-gp P-glycoprotein

PCa prostate cancer

PCA3 prostate cancer antigen 3

PIM1 proto-oncogene serine/threonine-protein kinase

PLCO Prostate, Lung, Colorectal and Ovarian Cancer Screening

PSA prostate-specific antigen

pT pathological tumor stage

PTEN phosphatase and tensin homolog

PZ peripheral zone

RAC1 Ras-related C3 botulinum toxin substrate 1

RP radical prostatectomy

RT radiation therapy

S727 serine 727

SH2 Src homology 2

SOCS3 suppressor of cytokine signaling 3

STAT3 signal transducer and activator of transcription 3

T testosterone

TAB total androgen blockade

TGF1β transforming growth factor-1 beta

Th17 T helper 17 cell

TMA tissue microarray

TMPRSS2 transmembrane protease serine 2

TRUS trans-rectal ultrasound

TZ transition zone

VEGF vascular endothelial growth factor

VEGFR vascular endothelial growth factor receptor

Y705 705 tyrosine

I. The Prostate

"The good thing about science is that it's true whether or not you believe in it."

Neil Degrasse Tyson

Structure

In males, located below the bladder, there exists a walnut sized exocrine gland: the prostate [1]. The prostate develops in men and reaches maturity at puberty. The prostates main function is to secrete about 30% of the fluid that makes up semen. This fluid is alkaline and rich in enzymes and simple sugars that allow the sperm to be maintained and flourish. The prostate is situated around the urethra and during ejaculation it squeezes the seminal fluid into the urethra. The fully formed prostate is made up of 3 different zones; the peripheral zone (PZ), the central zone (CZ) and the transition zone (TZ) [2] (Figure 1). The largest zone, the PZ, occupies around 70% of the total prostate and is most common location for tumors. The CZ makes up about 25% of the prostate and is the location of around 20% of tumors. [3, 4]. Finally, the smallest area of the prostate, the TZ makes up the remaining 5% of the prostate and it is the main source of benign prostate hyperplasia (BPH), an enlargement of the prostate. The TZ position around the urethra makes it troublesome since its enlargement may lead to a blockage of the urethra, causing problems urinating [5].



Figure 1. This is a depiction of the prostate showing three zones; peripheral zone (PZ), central zone (CZ) and transition zone (TZ) as well as the urethra and the connections to the penis, bladder and seminal vesicles.

Cellular Structure

The prostate is composed of glandular structures of epithelial cells at the microscopic level. These glands contain a lumen that allows for secretion through ducts into the urethra. A single layer of basal cells surrounds the epithelial cells. These basal cells are potentially stem cell-like cells that can differentiate into epithelial cells [6]. A striking difference between the two cells at the microscopic level is their different structure and shape and at the molecular level their reliance on the androgen receptor (AR) [7]. In a normal gland, the epithelial cells are tall and columnar in shape and basal cells are flat or cuboidal [8]. During cancer development a loss of the basal cell layer can be observed as well as a change in shape and size of epithelial cells and their nuclei. An example staining with hematoxylin and eosin (H&E) of both benign and cancerous prostate tissue is shown in Figure 2. H&E staining is a common method used in diagnosing cancer by the appearance of the glandular structure.



Figure 2. Progression from normal glandular structure (left) to a very aggressive tumor (right) with sample H&E stainings below.

Prostate Cancer and Diagnosis

Cancer of the prostate is the most commonly diagnosed cancer in men in the United States [9]. Most diagnoses of prostate cancer (PCa) are not life threatening, with most men dying of unrelated causes/diseases [10]. Risk for PCa increases with age and the majority of PCa diagnoses are in men over the age of 65, with an average age of diagnosis of 66 [9]. Other risk factors include; genetics, ethnicity and diet [11]. PCa is diagnosed via a digital rectal examination (DRE), where the physician examines the prostate with their finger, a rise in prostate specific antigen (PSA) levels, detected via a blood test, and a trans-rectal ultrasound (TRUS)guided biopsy [12]. PCa is driven by the expression of androgen receptor (AR), which is also responsible for the expression of PSA. PSA testing was introduced in the 90s and it relies on a blood test that determines the level of total PSA in the blood, with increased levels of PSA suggesting a possibility of PCa [13, 14]. Its introduction has vastly changed the landscape of PCa diagnosis and led to an increase in diagnosis of PCa. Multiplex tests measuring different forms of PSA are commercially available today, including prostate health index (PHI), four kallikrein panel (4K score) and STHLM3 and they all have been shown to increase the predictive accuracy in PCa diagnosis [15-17].

Androgen Receptor

The AR is a 110-kDa receptor protein found in the cytoplasm. It is held in an inactive state by a heat-shock protein complex (HSP) [18, 19]. The AR is made up of 3 important functional domains comprising a transcription regulation domain, found at the N-terminal region, a DNA binding domain, and a ligand binding domain [20]. Activation occurs when either testosterone (T) or dihydrotestosterone (DHT) bind to the AR, resulting in a conformational change that sheds the HSP chaperone protein complex [21] (Figure 3). Testosterone is produced in the testes and adrenal glands with cytochrome P450 and 5 α -reductase converting testosterone to DHT. Active AR then homo-dimerizes and translocates to the nucleus where it binds to androgen-responsive elements (ARE) and stimulates the transcription of a number of downstream targets including PSA [22]. AR is an important driver of normal prostatic growth and development but it is also a driver of carcinogenesis in the prostate. AR expression has been shown to be present at all stages of the disease and importantly overexpressed in castration-resistant PCa (CRPC) [23, 24].



Figure 3: Simplified androgen receptor (AR) signaling. Testosterone (T) is converted to dihydrotestosterone (DHT), which binds AR and releases it from its heat shock protein (HSP) chaperone complex. AR then dimerizes and goes to the nucleus where it binds to androgen-responsive elements (ARE) to induce transcription of specific genes.

Treatment Options

Treatment for PCa is dependent on the stage and progression of the disease. At diagnosis, patients can be stratified into the following groups based on outcome: low, intermediate or high risk. The first risk classification system was described by D'Amico and based on PSA level, clinical tumor stage (cT) (by DRE) and Gleason score (GS) [25]. Today, different classification systems have been suggested but they are usually based on the same three parameters as D'Amico's

system, with additional parameters taken into account [26, 27]. The majority of patients have localized PCa with low GS (explored further in Biomarkers chapter), low cT, and PSA level <10 ng/ml)[12]. These patients are considered low risk and treatment can be either active surveillance (AS) where a patient is monitored for PSA levels over time and with repeat biopsy, or they can have local treatment with curative intent. Local treatment for PCa is usually a radical prostatectomy (RP), with a complete removal of the prostate or radiation therapy (RT). RP is performed either via robotic, laparoscopic or open surgery but all of them have a similar rate of oncological outcome [28]. RPs are good at mitigating further risk for patients with local low-grade disease but the side effects incurred from this treatment are non-negligible, including impotence and incontinence. Today low risk patients are often recommended AS until disease progression is observed [29, 30]. Radiation therapy can be either full-dose external beam radiation (EBR), interstitial radiation by brachytherapy or a combination [12].

Once a primary tumor is treated, patients are monitored for any raise in PSA. In around 20-40% patients who have undergone RP and in about 30-50% of patients who have had adjuvant or salvage RT, biochemical recurrence (BCR) occurs within 10 years [31]. BCR for RP is defined as a "patient who has a measured PSA level of 0.2 ng/ml after RP, which is confirmed again after a few weeks". With patients who have undergone primary RT the definition is a bit more complex; according to the American society of therapeutic and radiology oncology (ASTRO), the BCR is defined as "PSA nadir and the first of 3 consecutive rises in PSA" [32]. After a patient has been diagnosed with BCR, the most common form of treatment is androgen deprivation therapy (ADT). Currently the standard of care is to use a Luteinizing Hormone-releasing Hormone (LHRH) agonist, which includes but is not limited to leuprorelin or goserelin. LHRH acts by indirectly inhibiting the secretion of hormones such as DHT and T. LHRH antagonists, like degarelix, which has a more rapid effect, are also available but not used to the same extent as agonists [33]. Orally administered anti-androgens (AA) like bicalutamide and flutamide may be given as monotherapy in the case of limited metastatic spread or in combination with LHRH agonists for total androgen blockade (TAB).

The treatment options for men who have failed ADT and moved onto CRPC are mainly novel AR signaling inhibitors, like abiraterone and enzalutamide, chemotherapeutic drugs such as docetaxel and cabazitaxel or radium-233, a radiotherapeutic drug [34-38]. A need exists to identify new targets for treatment of aggressive PCa as well as better diagnostic tools to at an early stage identify those patients who may develop more aggressive forms of cancer. A large number of new drugs and combinations, including immunotherapy, are currently tested in clinical studies (www.clinicaltrials.gov).

II. Biomarkers

"You want to know how I did it? This is how I did it, Anton: I never saved anything for the swim back."

Vincent, Gattaca

Introduction

Biomarkers are molecules, genes or other characteristics that are specific to a given disease and can help in its identification. Prognostic, diagnostic and predictive are the most common types of biomarkers. Prognostic biomarkers are intended to evaluate a patients overall outcome, for example in PCa the likelihood of BCR or developing metastases after localized treatment. Meanwhile predictive markers determine a possible benefit that a treatment or combination will have [39]. Diagnostic markers help a physician determine the presence of a disease; for example increased PSA values. Some biomarkers are also considered as potential drug targets. Biomarkers in cancer have been hotly pursued for over 50 years, the first genetic biomarker in cancer was discovered in the 1960's in Chronic myelogenous leukemia, called the Philadelphia chromosome [40]. Since then a number of different biomarkers have been discovered. Biomarkers can be present in a number of samples including blood, tissue, urine and saliva. The methodology for identifying biomarkers is broad and includes analysis of aberrations in germline and somatic DNA, epigenetics, transcriptome, proteome, metabolome and lipidome. In this summary, I will primarily focus on immunohistochemistry (IHC).

Immunohistochemistry

IHC is a method that is used on fixed tissue to stain for proteins of interest using antibodies specific to the target protein. IHC is one of the main current methods in trying to identify biomarkers. Biomarkers are easily quantifiable but the problem with IHC is that relies on qualitative scoring that can be subject to interpretation. Currently there are very few IHC tests that are approved for diagnostic and prognostic tests in cancer. The HER2 test in breast cancer, and detection of ALK protein in non-small-cell lung cancer are two of IHC tests currently used for diagnostic detection [41, 42]. The largest problem with IHC that exist today is the lack of standardization in tissue collection, preparation and staining. Samples are taken from patients and can be fixed in a number of different solutions, something that has been shown to affect the phosphorylation of proteins [43]. Another factor to consider is if tissue is affected during the process of removal from the patients. In prostate, contradictory results were found: one study claimed that tissue ischemia lead to changes in expression of 41 genes within 1 hour after the removal of the prostate [44], whilst another found that the ischemia did not affect the tissue for up to 2 hours [45]. Many attempts have been made to standardize the IHC procedure but to date no international standardization requirements exists. Most institutions have their own way of handling samples and staining. A study from 75

institutions in Canada in 2011 tested the most common IHC techniques in use at the time; pan-cytokeratin and low molecular weight cytokeratin. Their findings suggested that more than half of the participating institutions used incorrect methods [46]. Another problem in IHC is the need for positive and negative controls. There are multiple methods suggested for determining antibody specificity but most methods cannot give clear evidence that an antibody is specific for a given molecule [47]. A very useful method for determining antibody specificity is Western blot but even then it must be run in very similar conditions to the tissue that will be stained otherwise the information gleaned from it may not be valid due to antibodies reacting in different ways in different tissue/cell types [48]. Even with these problems IHC presents a useful tool for exploratory work into target proteins as biomarkers.

Prostate Cancer Biomarkers

One of the largest problems in PCa is the inability to distinguish between aggressive and indolent disease. Still, we are lacking a reliable biomarker of this kind. Below is a discussion of some of the current biomarkers used as well as those that are being researched for potential benefit. Recently, genetic tests came on the market (Oncotype DX, Prolaris, and others) but they all need further validation before they can be generally recommended [49].

Gleason Score

Currently the best prognostic marker in PCa continues to be GS[50]. GS was first introduced in the 1960s by pathologist Donald F Gleason [51]. It is based on the pattern that cells express when stained with H&E staining. The system has been amended a few times, most notably in 2005, but the basic method involved five different growth patterns (grades) (Figure 4). The tumor sample is graded on an addition of what the primary Gleason grade (most common) and secondary Gleason grade (second most common), this produces a final GS between 2-10. Originally if the secondary grade was less than 3% of the sample it was ignored and the primary grade was used for both [50]. At an international consensus meeting in 2005, the Gleason scoring system was upgraded with a removal of the score 1 and some modifications on how diagnosis worked for 3 and 4 [52]. More recently in 2014 a smaller modification has been suggested and approved involving the different patterns that may be apparent when looking at PCa, such as cribriform and glomeruloid glands and intraductal carcinoma [53]. With the current GS system the lowest grade given is a 6 (out of a 2-10 system) resulting in

an implication of intermediate cancer, therefore a new scoring system to be used in parallel with GS was devised giving a score from 1-5 instead [53].



Figure 4. The classical representation of the Gleason Grades 1-5 as drawn by Donald Gleason. Cells progress from an ordered and uniform structure to sheets of undifferentiated cancer. Image has been adapted from a public domain image available from the NIH [54].

PSA

PSA is used for diagnosis of PCa but since it can also be found in patients that have enlarged prostates due to benign disease, its use has been questioned. In 2012 the United States Preventive Services Task Force recommended against using PSA screening based on two large studies of PSA effectiveness [55-57].

Controversy has skirted the use of PSA, with opponents pointing to an overdiagnosis of non life threatening diseases that leads to overtreatment while proponents point to a decrease in mortality from PCa. There have been multiple studies conducted to check the effectiveness of screening PSA. The European Randomized Screening Study for Prostate Cancer (ERSPC) found that mortality was decreased with screening with reduction of 1.28 deaths per 1000 men [58]. The Prostate, Lung, Colorectal and Ovarian cancer screening trial (PLCO) found no significant difference in mortality between the screened and unscreened group



after 7-10 years of follow up, but it has been heavily criticized for contamination of PSA testing in the control group [55].

Due to the continuing controversy with PSA a need to find more robust markers for diagnosis is currently underway.

PCA3

Prostate cancer antigen 3 (PCA3) is a non-coding RNA that can be detected in the urine. PCA3 is highly expressed by PCa cells and has been correlated with higher chance of a positive biopsy if tested for directly after a DRE [59, 60]. PCA3 has also been suggested as a useful prognostic tool to determine outcome of treatment [61]. Although the Food and Drug Administration (FDA) have approved it, it is not widely used today.

TMPRSS2-ERG

Trans membrane protease serine 2-ETS-related gene (TMPRSS2-ERG) first described in 2005 by Tomlins et al. is a fusion gene between TMPRSS2 and ERG, which occurs via genetic rearrangement. Since the TMPRSS2 gene has androgen response elements, its fusion with ERG leads to an increased expression of the oncoprotein ERG directly driven by the AR [62]. Transfecting cells with the fusion gene has shown an increase in invasion-associated gene expression suggesting that TMPRSS2-ERG may play a role in metastases [63]. Studies in patient cohorts have not suggested a predictive role for TMPRSS2-ERG on its own but there have been suggestions that its combination with other markers may have clinical value [64-66]. There has been a controversy about the role of TMPRSS2-ERG as a prognostic biomarker in prostate cancer, but most studies have not been able to show a significant prognostic value [67].

AR-V7

AR mutants have been theorized to help PCa become castrate resistant [68]. More recently AR mutations have been explored as potential biomarkers for prognostic value. AR splice variant 7 (AR-V7) has recently been discussed due to its active transcriptional function without a functional ligand binding domain suggesting its constitutive activation. AR-V7 has recently been found to be associated with resistance to enzalutamide and abiraterone treatment, but confirmatory studies are needed [69-71].

PTEN

Phosphatase and tensin homolog (PTEN) is a well characterized tumor suppressing gene that acts by dephosphorylating PI3K, therefore deactivating it [72]. PTEN mutation or loss is a very common occurrence and an early event in a number of different cancers [73, 74]. In PCa PTEN loss has been correlated with worse GS and tumor stage but has not been found to be as predictive as GS [75, 76].

Other Biomarkers

Examples of other markers that have had more diverging results are interleukin 6 (IL-6) and transforming growth factor-1 beta (TGF1 β). Studying IL-6 in patient tissue using IHC has shown that PCa has significantly higher expression of IL-6 and its receptor, IL-6R [77]. In a study that evaluated circulating IL-6 and sIL-6R in the plasma of patients, elevated levels were found in patients with bone metastases [78]. Similarly to IL-6, TGF1 β has been shown to have increased expression on an IHC level in cancer tissue compared to normal tissue [79]. Also TGF1 β has been reported to be increased in plasma [80, 81] but another study suggested that plasma levels of TGF1 β were similar in patients who had cancer vs. those who did not [82]. Interestingly the same study found that urinary levels of TGF1 β were more predictive of cancer [82]. Downstream target of IL-6, the signal transducer and activator of transcription 3 (STAT3) has been suggested as a possible biomarker in PCa and will be the focus of papers I and II.

III. STAT3

"It is a capital mistake to theorize before one has data. Insensibly one begins to twist facts to suit theories, instead of theories to suit facts."

[Sherlock Holmes] Sir Arthur Conan Doyle A Scandal in Bohemia

Introduction

Signal transducer and activator of transcription 3 (STAT3) is a transcription factor that belongs to the STAT protein family. STAT3 consists of an N-terminal domain, a DNA binding domain, an SH2 domain involved in protein-protein interactions and a C-terminal domain that contains an area for transactivation [83, 84]. STAT3 can be activated by phosphorylation on two residues: the 705 tyrosine (Y705) and the 727 serine (S727) [83, 85]. The Y705 phosphorylation is essential for activation; the S727 is not necessary for activation but has been shown to enhance activation [86].



Figure 5. Overview of STAT3 signaling. Activation can occur via a number of different receptors; cytokine receptors (left), receptor tyrosine kinases (middle) and non-receptor tyrosine kinases (nRTK) (right). Activation of STAT3 occurs via phosphorylation and is followed by a homodimerization step before translocating to the nucleus and acting on transcription of a number of genes.

STAT3 activation and signaling pathway

STAT3 is a tightly regulated transcription factor with many upstream activators including different cytokine and growth factors. Most commonly STAT3 is activated by the Janus kinases (JAKs) by interaction with different cytokine receptors (IL-6R and IL-10R). It can also be activated by a number of receptor tyrosine kinases (e.g. EGFR, VEGFR, IGF-1R) and non receptor tyrosine kinases (e.g. Src, BCR-ABL) [87] (Figure 5).

IL-6/STAT3 pathway

The most common activation of STAT3 is via IL-6 [88, 89]. IL-6 is a small cell signaling protein, known as a cytokine, and has both pro- and anti-inflammatory effects [90]. IL-6 binds to its receptor (IL-6R). The IL-6R is made up of two different molecules; the IL-6Ra (also known as gp80) and gp130 (also known as IL-6R β) [91, 92]. The IL-6R α binds directly to the cytokine IL-6 but it has a relatively short cytoplasmic domain (82 kDA) [93], with very limited activation activity [89]. The gp130 receptor on the other hand has a larger cytoplasmic domain with multiple motifs for intracellular signaling, which allows binding to JAK [94]. Upon IL-6 binding to the IL-6Ra, the complex binds to gp130. This three-molecule complex then homodimerizes to create a hexamer with the resulting complex constituting its active state [95]. The hexamer does not directly activate STAT3 but instead activates JAKs via a JAK binding domain present on the gp130 subunits [96]. The JAKs and IL-6R interact and trans activate each other [88]. The activated JAK can then in turn phosphorylate and activate STAT3 [97]. Following this phosphorylation, STAT3 dimerizes and goes into the nucleus to activate downstream targets. It is important to note that there is also a soluble IL-6R (sIL-6R), which lacks the cytoplasmic component and is created via cleavage or alternative splicing [98]. This sIL-6R can also bind IL-6 and form the same hexameric structure with IL-6 and gp130 as the membrane-bound IL-6R α [88] (Figure 6). The gp130 protein is not specific for IL-6 signaling and is also found to complex with a number of other receptors including but not limited to; IL-27, LIF and IL-11 [99]. STAT3 activation is tightly regulated by a number of its own downstream targets leading to a negative feedback loop. Part of the common IL-6 pathway is its activation of transcription of the suppressor of cytokine signaling 3 (SOCS3), which inactivates the IL-6R and allows for tight control of the IL-6 signaling axis [100].



Figure 6. IL-6/JAK/STAT3 pathway. An illustration of the IL-6 pathway showing the binding of IL-6 to IL-6R α (1) and the formation of the hexameric structure with gp130 (2) that goes on to cross phosphorylate with the JAK (3). JAK then phosphorylates STAT3 (4) which homodimerizes (5) and goes to the nucleus where it binds to DNA and is involved in transcription of target genes (6). One of these targets the SOCS3 protein goes on to bind gp130 (7) and block its phosphorylation of JAK (8), which leads to a shut down of the STAT3 pathway. Illustrated to the left is the sIL-6R α which undergoes the same signaling process but without a cytoplasmic domain on the IL-6R α .

STAT3 signaling

STAT3 has been linked to a number of processes in normal cell function including embryogenesis, innate and adaptive immune function, regulation of cell differentiation, growth, and apoptosis. Through knockout of STAT3 in mouse embryos, it was determined that STAT3 is required for the embryo to come to term [101]. It has been shown that STAT3 is required in stem cell renewal and determination of stem cell phenotype [102, 103]. STAT3 is involved in immune response with both an inhibitory and promoting role. It has been shown that STAT3 expression can lead to T-cell tolerance, while loss of STAT3 expression was shown to lead to T-cell priming [104]. STAT3 is also needed to prevent the apoptosis of IL-6 stimulated T cells, via activation of BCL-2 [105].

STAT3 in cancer

Normal STAT3 signaling can become aberrantly activated, leading to an oncogenic state. The constitutive activation of STAT3 has been postulated to occur through a number of different abnormalities in upstream receptors of STAT3. The first evidence of constitutively activated STAT3 was published in 1995 and connected with the proto-oncogene tyrosine protein kinase Src [106]. This groundbreaking paper showed that infection of different cell lines with v-Src, a tyrosine kinase known to induce cancer in chickens, was able to activate STAT3. This association was later confirmed in a number of studies showing that v-Src transformation of cells was mediated by STAT3 and in the absence STAT3 the transformation would not occur [107, 108]. Studies of human samples in different cancer types found high expression of activated STAT3 [109-112]. Different methods of constitutive activation have been suggested in different cancer types. In lung carcinoma a mutant epidermal growth factor receptor (EGFR) was shown to be responsible for constitutive STAT3 activation [113]. Meanwhile in breast cancer the overexpression of different cytokines related to inflammation, including IL-6 have been linked to constitutive STAT3 activation [114]. Constitutive activation of STAT3 can also occur through STAT3 mutation, a common occurrence in cancers associated with the blood [115, 116] Constitutive STAT3 activation has been linked to oncogenesis due to its ability to activate a number of downstream targets that are involved in cell proliferation and apoptosis, metastatic potential, angiogenesis and immune evasion [117].

STAT3 in PCa

In PCa, STAT3 has been found to be more highly expressed in benign and tumor areas of PCa patients compared to healthy patients and STAT3 activation has also been observed in a number of cell lines [118, 119]. It has been found that activated STAT3 correlated with worse prognosis and higher GS in some studies [120-122]. Metastatic potential of DU145 and PC3 cells transfected with STAT3 was increased, with high expression of STAT3 observed in metastatic samples from patients [123]. STAT3 has been linked with direct interaction to the AR, one of the main drivers of prostate cell growth [124]. Meanwhile downregulation of AR was shown to induce a stem cell like phenotype that was driven by IL-6/STAT3[125]. There is evidence that anti-androgen treatment may lead to a more stem cell-like cell that is more likely to metastasize [126]. Profiling of PCa stem cells has revealed over 500 changes in gene expression with many of them linked to the JAK/STAT3 pathway [127].

STAT3 and cell proliferation and apoptosis

Cell proliferation and apoptotic escape are a hallmark of tumor cells, which are able to continuously proliferate without ever reaching senescence and escape programmed cell death. STAT3 has been shown to influence cell proliferation in a number of different cancer types [128, 129]. Cyclin D1 functions by regulating cyclin dependent kinases (CDK), CDK4 and CDK6 and allowing cells to progress through the G1 phase of the cell cycle. In cancer it has been linked to stimulating anchorage independent growth and cell proliferation [130]. Specifically in PCa cyclin D1 has been linked to proliferation via IL-6/STAT3 [131]. Another method of cell proliferation increase in PCa happens via upregulation of Wnt3a via the interference of TGF-β signaling promotes cell proliferation in a STAT3 dependent manner [132]. STAT3 increased cell proliferation has also been linked to presence of HSP90, which is specifically linked to the activation of both AR and STAT3 [133]. IL-6, in a STAT3 dependent manner, is involved in the expression of AR, the main driver of cell proliferation in the prostate [134]. Interestingly STAT3 was also shown to activate MMP-3 with suggested involvement in both metastases and cell proliferation [135].

Meanwhile apoptosis, a method of programmed cell death first discovered in the *Caenorhabditis elegans* model system [136] has been linked to STAT3 tumorigenic function. One of the most studied families of apoptosis is the BCL-2 family of proteins [137]. STAT3 has been linked to the upregulation of BCL-XL, MCL-1 and BCL-2, all anti-apoptotic members of the BCL-2 family [138-140].

Not only does STAT3 activate these anti-apoptotic genes but it is also known to downregulate p53, one of the main drivers of pro-apoptotic expression, indirectly leading to the downregulation of pro-apoptotic genes such as BAX, Fas and caspase 6 [141, 142].

STAT3 and metastases

Metastasis is an important process in a tumors progression to an aggressive form. Matrix metalloproteinase (MMPs) have been linked to cellular invasion in a number of different cancer types [143, 144]. STAT3 has been shown to regulate the expression of numerous MMPs in different cancer types: MMP7 in pancreatic cancer, MMP2 in melanoma, MMP9 in breast, and MMP1 in bladder cancer [145-148]. STAT3 has also been linked to cellular migration through regulation of a number of proteins including Ras-related C3 botulinum toxin substrate 1 (RAC1) [149] and stathmin [150]. In squamous cell carcinoma, overexpression of STAT3 was shown to lead to higher invasion [151]. Specifically, in PCa, it has been shown that STAT3 enhances migration via integrin β6 and MMP3 [152].

STAT3 and angiogenesis

Angiogenesis is the process of attracting and forming new blood vessels. In tumors angiogenesis is important for the uptake of nutrients, for continued growth, and for metastatic potential. One of the most important proteins involved in angiogenesis is the vascular endothelial growth factor (VEGF). It has been shown that VEGF is downstream of STAT3 and hyper activation of STAT3 can lead to increased expression of VEGF [153]. Not only is STAT3 involved directly in VEGF expression, there is evidence that AR can also be involved in VEGF expression through hypoxia inducible factor 1 α (HIF1 α) [154]. Interestingly STAT3 has been shown to induce HIF1 α expression [155]. This complex overlap, though well controlled in normal cells, is strongly affected when STAT3 becomes constitutively active and leads to angiogenic activation in tumors.

STAT3 and immune suppression

Immune evasion is key to a tumors success locally, in the blood stream and at metastatic sites. STAT3 is linked to immune evasion in a number of ways: with
involvement in the tumor cells, the microenvironment and the immune cells themselves. Evidence exists that STAT3 expression in the tumor cells can induce IL-6 and IL-10 that activate STAT3 in myeloid cells [156]. Upregulation of IL-10 has also been linked to a decrease in proliferation to a number of different immune cells [157, 158]. STAT3 has also been linked to the differentiation of T cells into Th17 cells via the expression of IL-6 and TGF β [159, 160]. STAT3's role in immune response is complex but many studies have pointed to STAT3 activation as key to the tumor cells ability to escape the immune system via it functions in different immune cells [161].

Targeting STAT3

The tumorigenic potential of the STAT3 pathway has made it an attractive target for potential therapeutic purposes. Multiple targeting methods have been explored that include targeting upstream, at the receptor or kinase level, as well as directly to STAT3.

Multiple cell surface receptors that are upstream of STAT3 have been studied as potential targets, among them EGFR, VEGFR and IL-6R. IL-6R and its binding cytokine have both been targeted with different drugs. In 2012 the results of a phase 2 study with siltuximab, an IL-6 chimeric monoclonal antibody, suggested treatment did not yield outcome benefit in CRPC [162]. Tocilizumab, an antibody targeting the IL-6R instead has also shown promise *in vitro* and *in vivo* but has not been clinically approved for any cancer treatments [163, 164]. Success has been seen *in vivo* and *in vitro* in oral squamous cell carcinoma and breast carcinoma in targeting the EGFR receptor, which led to a decrease in STAT3 phosphorylation [165, 166]. The most successful receptor target so far has been VEGFR with a number of drugs currently approved to be used in the clinic, including sorafenib, sunitinib and axitinib, all of which have been shown to affect STAT3 [167-172].

By targeting cell surface receptors upstream of STAT3 there is a clear reduction in STAT3 activity but there is a need for more directed therapies in order to reduce side effects. Therefore, there is more focus on the more direct approaches to target either the JAKs or STAT3 directly. Currently a large number of JAK inhibitors exist but only two, ruxolitinib and tofacitinib, have been approved for treatment in rheumatoid arthritis, but not in cancer. JAK inhibitors still pose a large amount of side effects with tofacitinib only being approved by the Food and Drug Administration (FDA) but not by the European Medicines Agency (EMA) due to its side effects.

Directly targeting the STAT3 protein has a high potential for therapeutic effect with fewer side effects. A large number of compounds are currently being tested with many targeting different parts of STAT3: disrupting translation, disrupting phosphorylation, disrupting protein-protein interaction, nuclear translocation or the DNA-binding of STAT3 [173, 174]. As previously mentioned, the SH2 domain of STAT3 is involved in protein-protein interaction, specifically its association with the JAKs for activation as well as its homodimerization and nuclear translocation. Targeting the SH2 domain of STAT3 was one of the first direct STAT3 targeting techniques and has proven to shutdown constitutive activation of STAT3 [175, 176]. One of the most successful SH2 targeting compounds is Stattic, which specifically targets the dimerization of STAT3 [177]. Due to STAT3s transcriptional activity another big target on STAT3 is its DNA binding domain. The DNA binding domain of STAT3 consists of three loops that interact with the DNA double helix at specific STAT3 consensus sequences [83]. By binding to the DNA binding domain on the STAT3 protein you can, in theory, disrupt the ability of STAT3 binding to DNA and therefore inhibit its transcriptional abilities. This direct effect on the DNA binding domain would likely not interfere with the phosphorylation and dimerization of STAT3. A number of options exist to target the DNA binding domain of STAT3. A common method is to introduce decoy oligonucleotides which mimic the response element of the target transcription factor [178]. Major problems exist in stabilizing the decoy oligonucleotide but a recent clinical trial was successful in modulating STAT3 expression with a decoy oligonucleotide in patients with head and neck cancer [179]. Another method for targeting the DNA binding domain is to use a small molecule inhibitor such as inS3-54A18 [180].

Galiellalactone

Small molecules present an attractive treatment option due to their many advantages such as; easy to get into the cell, stable, can bind directly to the target and can target specific interactions of the target [181].

We used a small molecule STAT3 inhibitor named galiellalactone in papers III and IV of the current investigation. Galiellalactone is a small molecule that can be found in the Galiella Rufa mushroom [182]. Galiellalactone has been successfully synthesized by Johansson and Sterner [182, 183]. First suggested as an inhibitor of the IL-6 pathway in 2000 by Weidler et al. it has since become an interest for study for its potential clinical benefits [184]. Our group has shown that galiellalactone is an effective treatment in castrate resistant cell lines that express constitutively active STAT3 [185]. Galiellalactone has also been shown to have a

potent effect on ALDH1+ cells, which are considered stem cell-like cells, and are present in small subpopulations in PCa cell lines [186]. Our group has also shown that galiellalactone inhibited metastases in a mouse model [187]. The current study focuses on the mechanism of action of galiellalactone in paper III and the use of galiellalactone in combination with docetaxel in paper IV.

IV. Drug resistance

"Quoth the Raven 'Nevermore." Excerpt from *The Raven* by Edgar Allan Poe

Introduction

The largest problem facing current cancer treatments is the tumors ability to overcome the effects of treatment and become resistant [188]. Resistance to drugs can fall into two categories: intrinsic and acquired [189]. Intrinsic resistance occurs when the resistance mechanism is already present in some or all cells in the tumor. Due to tumor heterogeneity it is unlikely that all cells are resistant meaning that treatment will still lead to a reduction in tumor size, but eventually the resistance occurs when cells initially respond to the treatment but due to mutation, differential expression of the drug target, or activation of an alternative pathway, become resistant.

ADT

As discussed in Chapter 1, when local therapy fails, a rise in PSA levels represents BCR. The most common treatment after the observance of BCR is ADT. ADT benefits last for up to 2-3 years before patients become resistant, progressing to CRPC [190, 191]. ADT focuses on blocking the AR signaling pathway. AR is the main target of ADT and resistance to ADT stems from modifications to the AR pathway. After ADT treatment, AR levels are commonly very low in patients but tumor cells are able to develop high sensitivity to the low levels of AR via amplification [192] or mutations that allow activation without native ligand, or via alternative ligands [193]. Some examples of these mutations that lead to higher AR activity are a mutation at the 877 alanine or the substitution of histidine 874 for tyrosine [194, 195]. AR can also be activated by a number of other signaling pathways such as IL-6, IGF-1 and EGF [134, 196].

Once ADT options have been exhausted, one of the main treatments relies on the chemotherapeutical agents, like taxanes. Docetaxel was approved in 2004 while cabazitaxel was developed later and approved in 2010 [197, 198]. Currently there is ongoing research to determine which taxane is more effective, with early evidence suggesting that in PCa, cabazitaxel is more effective than docetaxel but that this is not true in all cancer types [199, 200]. There have been a few clinical studies suggesting that patients that fail docetaxel are still responsive to cabazitaxel [201, 202]. Progression to resistance to both cabazitaxel and docetaxel is a major challenge today [203, 204]. Paper IV is about docetaxel resistance mechanisms and therefore the rest of this chapter will focus on docetaxel.

Docetaxel resistance

Docetaxel is a taxane that binds the β subunit of tubulin in microtubules; this causes a stabilization of microtubules. The stabilized microtubules are unable to depolymerize, which does not allow the cell to go through mitosis [205]. The resistance methods to docetaxel are not well elucidated but there are a number of possible mechanisms that are currently being studied. The most studied form of docetaxel resistance is through the multidrug transporters belonging to the adenosine triphosphate binding cassette (ABC) family. There are 49 members but the most studied across all cancers are P-glycoprotein (P-gp), MDR protein 1 (MRP1) and breast cancer resistance to chemotherapy [206] and worse clinical outcome [207]. MRP1 has been shown to be expressed in DU145 and PC3 PCa cell lines [208], and correlated with advanced disease and GS [209-211].

Docetaxel treatment has also been shown to increase the expression of a number of cytokines such as IL-6 and IL-10 possibly leading to inflammation or apoptotic escape [212, 213]. Patients with high serum levels of the cytokine IL-6 had worse outcome with docetaxel treatment [214, 215]. These increases in IL-6 expression may lead to higher activation of STAT3 and indeed a number of the mechanisms discussed further in this chapter are downstream targets of STAT3. Docetaxel is also known to induce apoptosis by indirectly leading to phosphorylation of the anti-apoptotic B-cell lymphoma 2 (BCL-2) causing BCL-2 to become inactive [216, 217]. Apoptotic escape has been a growing topic of interest in docetaxel resistance with multiple proteins involved being implicated in resistance including: BCL-2 and Proto-oncogene serine/threonine-protein kinase (PIM1).

The BCL-2 family of proteins has been extensively studied in PCa and many of its members have been shown to be highly expressed in aggressive PCas [218]. Recent focus for docetaxel resistance has focused on BCL-2 due to its indirect targeting by docetaxel, making its mutation or overexpression a possible resistance mechanism [219]. In PCa cells it has been shown that directly targeting BCL-2 resulted in higher sensitivity to docetaxel [220]. Upregulation of both BCL-2 and BCL-XL has been suggested as a possible resistance mechanism via activation of the glucocorticoid receptor [221]. Other BCL-2 family members such as MCL-1 and survivin have also been implicated in docetaxel resistance [222, 223]. Furthermore, upstream modulators of the BCL-2 family have also been suggested as possible docetaxel resistance mechanisms. One of these is PIM1; its main mechanism of action is phosphorylation of the BAD protein, another member of the BCL-2 family [224, 225]. It has been shown that PIM1 can be activated by docetaxel and possibly leads to docetaxel resistance via anti-apoptosis by

activation of NF κ B [226]. It has not only been linked to docetaxel resistance but to drug resistance in general [227].

Another suggested form of resistance is the chaperone protein clusterin. Clusterin exists in a number of different isoforms and interestingly the amount of specific isoforms of the proteins have different effects with higher nuclear clusterin associated with apoptosis and cell death while secreted clusterin has an opposing effect [228, 229]. In PCa cell lines, secreted clusterin is activated through AKT and can lead to a docetaxel resistant phenotype, with implications that the Src kinase is involved in the expression of secreted clusterin [230].

STAT3 and drug resistance

STAT3 has been implicated in multiple cancers as a resistance mechanism to a wide variety of drugs. In lung cancer, in cells that were addicted to EGFR, increased activation of STAT3 was observed in response to erlotinib treatment, an EGFR inhibitor, and conferred resistance [231]. Moreover in chronic myeloid leukemia (CML), treatment with an inhibitor of the tyrosine kinase, BCR-ABL, increased not only pSTAT3 expression but also its downstream targets MCL-1 and BCL-XL [232].

An increase of pSTAT3 and BCL-2 coupled with a reduction of p53 expression was observed in cisplatin-resistant gastric cancer cell lines compared to sensitive parental cell lines. When these resistant cells were treated with STAT3 inhibition their sensitivity to cisplatin increased [233]. In renal carcinoma, non-Hodgkin lymphoma and multiple myeloma, resistance to chemotherapy was linked to IL-6 and IL-10, two cytokines known to activate STAT3 [140, 234, 235].

In PCa STAT3 has been linked to resistance of both chemotherapy and antiandrogens. Activated STAT3 expression was shown to be associated with an enzalutamide-resistance in cell lines, with STAT3 inhibition leading to a resensitization to enzalutamide [236]. It has also been suggested that the PIM1 kinase involvement in docetaxel resistance is mediated in a STAT3 dependent manner and leads to the expression of NF κ B [226].

Combination Therapies

As resistance mechanisms have become more studied, the idea of combination therapies has arisen as a possible solution. Combination therapies focus on treatment of two different targets that would make adaptation for the cancer cells

harder than a single target. Due to its high expression in a number of cancers and the fact that many proteins implicated in resistance are downstream targets, STAT3 seems like a viable option for combination treatment with ADT and chemotherapy. Specifically combination of STAT3 inhibition with docetaxel seems promising with a number of the resistance mechanisms previously discussed in this chapter being linked to STAT3.

V. The Current Investigation

The aim of this work was to investigate the expression of STAT3 in tumor tissue from PCa patients from different stages of the disease and to investigate the potential therapeutic benefit that targeting STAT3 holds. STAT3 targeting was specifically investigated with the use of galiellalactone.

Specific Aims

- Determine the expression of STAT3 and pSTAT3 in metastases from CRPC patients and if the metastases have differential expression of STAT3 within and across patients
- Determine the expression of pSTAT3 in hormone naïve patients and if it correlates to disease progression and AR expression
- Study the molecular mechanism of galiellalactone's inhibitory effect on STAT3
- Investigate the feasibility of using galiellalactone in combination with the chemotherapeutic agent docetaxel

Paper I

Introduction

There is a need in PCa to find new treatment targets and new markers that can help understand PCa progression. Since STAT3 has been shown to be constitutively activated in a number of malignancies, and has been linked to metastases, we wanted to examine STAT3 and its main upstream receptor IL-6R in patients with metastatic CRPC [152, 237]. We collaborated with researchers from Washington University who provided us with metastatic samples in tissue microarray (TMA) format from multiple sites taken from patients who had died from CRPC (samples were taken at autopsy). We used IHC staining to determine the level of activated STAT3 (phosphorylated at the Y705) and IL-6R followed by an analysis of the mRNA expression of STAT3 and IL-6R. We were interested if the levels of expression varied between metastatic sites because a recent study has shown that visceral metastases have much worse prognosis than bone and lymph node metastases [238].

Results and Discussion

We found that all patients expressed both IL-6R and pSTAT3 in at least one of their tissue samples. The IL-6R expression was higher in bone compared with lymph node but not visceral metastases but this was not seen at the mRNA level. We then examined the expression of pSTAT3 where we found that bone metastases had higher expression than both lymph node and visceral metastases. In order to get a better understanding of the expression in a single patient we narrowed down our analysis to only patients with all three metastases available. When looking at only matched patients we still found significant differences between bone and visceral metastases for both pSTAT3 and IL-6R, showing that the metastases were highly heterogeneous. Our results suggest that pSTAT3 activation is a common event in PCa metastases. The high pSTAT3 expression may explain why downstream STAT3 targets have been shown to be more highly expressed in bone [239]. Low correlation between IL-6R and pSTAT3 suggests

that multiple pathways maybe involved in the activation of STAT3 in metastases. The high expression of pSTAT3 specifically in the bone may be due to presence of IL-6 in the microenvironment [240]. Our results suggest that pSTAT3 may be a viable target in CRPC metastatic patients. The role of STAT3 in metastases and progression needs to be further explored in patient samples with larger cohorts where samples from different stages of the disease are available.

Paper II

Introduction

To date, there is a lack of good biomarkers to indicate whether PCa is indolent or aggressive [241]. Since we observed in Paper I that metastases had high expression of pSTAT3 we were interested in examining the expression of pSTAT3 (phosphorylated at Y705) and AR in hormone-naïve patients to determine if they could add clinical value to diagnosis. Previously, smaller cohorts were shown to have some correlations between pSTAT3 and GS or between pSTAT3 and BCR [118, 122, 242]. More recently Pencik et al. have suggested the opposite, that loss of STAT3 expression is a more tell-tale sign for more aggressive disease, but these patients were also negative for PTEN [243].

Results and Discussion

We examined TMAs from 300 patients that had undergone RP but not been subjected to any pretreatment (ADT or RT). The intensity of the pSTAT3 IHC staining was scored using h-index, where both percent and intensity were taken into account. Most patients included in the study were still alive at time of analysis and therefore BCR was used as a surrogate marker for survival. Surprisingly, we found that pSTAT3 expression was higher in the benign regions compared to the tumor. We also found that lower pSTAT3 expression significantly correlated to higher risk of BCR but no correlation with GS was observed, unlike previous reports from other groups. This discrepancy most likely stems from a difference of methodology and cohort size. Previous publications did not use an h-index, instead opting to only score intensity or percentage stained. Our scoring methodology was stringent and only took into account epithelial cells ignoring any stromal staining and inflammatory staining, something not explored or mentioned in earlier studies. Some of the earlier studies included whole cores/tissue sections for analysis, without separating by structure nor taking into account whether the area was tumor or benign. Given the importance of stroma and inflammation in tumor progression, it would be crucial to investigate the expression of pSTAT3 in those areas in

future studies of our cohort. In order to better understand the finding of pSTAT3 levels significantly predicting BCR, we compared the concordance index of pSTAT3, GS and pathological tumor stage (pT) in predicting BCR. The expression of pSTAT3 was significantly predictive but was not as robust as GS and pT, it also did not add predictive power to either GS or pT.

Similar to previous studies, we found increased expression of AR in the cancerous tissue compared to benign, but AR did not provide any predictive benefit for BCR. Slight correlation was observed between AR and pSTAT3 in benign areas but not cancerous areas [244]. In conclusion we found that hormone-naïve patients had lower expression of pSTAT3 in their tumors compared with benign areas and lower pSTAT3 in the tumor tissue correlated with worse outcome in terms of BCR. This data suggests that STAT3 targeting therapy would not likely benefit hormone-naïve patients. Similar to Paper I more studies need to be done with larger cohorts.

Paper III

Introduction

STAT3 is known to be constitutively activated in prostate tumor samples from patients and PCa cell lines [119]. Previous studies have indicated that targeting the STAT3 pathway may be beneficial in PCa [174].

Galiellalactone is an inhibitor of STAT3 and has previously been shown to affect PCa cell growth both *in vivo* and *in vitro* in cells that have high expression of pSTAT3 [185, 186]. We were interested in exploring the exact mechanism of action of galiellalactone's STAT3 inhibitory effect. In order to study galiellalactone's interaction with STAT3, we used a biotinylated galiellalactone compound for pull down of STAT3 bound to galiellalactone and for confocal microscopy to visualize co-localization of STAT3 and galiellalactone. Mass spectrometry with recombinant STAT3 was used to determine the binding sites of galiellalactone on STAT3.

Results and Discussion

We used a biotinylated galiellalactone that had a similar effect on cell proliferation as galiellalactone. With the aid of mass spectrometry the binding areas of galiellalactone on STAT3 were determined to be three cysteines of which two (c367 and c468) were located in the DNA binding domain and one was located in the linker domain (c542). The cysteine residues that were modified by galiellalactone in STAT3 were not present in STAT1 or STAT5, indicating specificity for STAT3. Competition assays with galiellalactone and biotinylated galiellalactone and wash-out experiments indicated irreversible binding. Pulldown in LNCaP cells, which express an inactive STAT3, confirmed that galiellalactone binding was irrespective of STAT3 activation. No effect on phosphorylation was observed in DU145 cells treated with galiellalactone for 24 h. Confocal microscopy showed co-localization of biotinylated galiellalactone and STAT3 to bind its specific DNA response elements by using an electric motility

shift assay. In short, our paper showed that galiellalactone binds to cysteines in the STAT3 DNA binding region affecting its ability to bind to DNA. The covalent interaction with STAT3 and the inability of competition assays to displace the compound, coupled with effects seen even after removal of galiellalactone suggests that this targeting method can be useful in inhibiting STAT3 until it is degraded. Since STAT3 has been shown to be a vital protein in cancer metastases, cell proliferation and apoptotic escape targeting STAT3 with galiellalactone presents a potential treatment option with the added benefit of binding regardless of the activating method.

Paper IV

Introduction

Docetaxel is a common second-line treatment in CRPC but unfortunately many patients display inherent or aquired resistance [245]. Docetaxel is also highly toxic with a large amount of detrimental side effects reported. Due to high rates of resistance, combination therapies have been tested in a number of clinical trials, mostly with disappointing outcome. As discussed in the drug resistance chapter, cancer cells are very adept at overcoming single agent treatments; therefore targeting multiple targets at one time may stem resistance or increase the time to resistance with the added potential of lowering effective doses. Docetaxel treatment has been implicated in an upregulation of a number of downstream STAT3 targets, which have been linked to eventual docetaxel resistance. Therefore targeting STAT3 concurrently with docetaxel may in theory block certain resistance mechanisms. We used the PCa cell line LNCaP-IL6, a model of aggressive PCa that has constitutively activated STAT3, to carry out experiments of combination treatments between docetaxel and the STAT3 inhibitor galiellalactone.

Results and Discussion

Galiellalactone and docetaxel were administered to cells in varying concentrations as single agents and in combination to determine their IC₅₀ values and their combination index. Combination index was determined with the aid of CompuSyn that uses a previously published algorithm developed by Chou to determine synergy between treatments [246]. We found that galiellalactone and docetaxel showed synergy at a number of different concentrations. The gene expression of genes involved in docetaxel resistance (PIM1, BCL-2, IL-6 and clusterin) were investigated by qPCR analysis. Three of these genes PIM1, BCL-2 and IL-6 are known to be regulated by STAT3. We found that the gene expression of BCL-2, PIM1 and IL-6 were downregulated by galiellalactone alone and in combination with docetaxel compared to control while all three gene expressions were

upregulated under docetaxel treatment. Clusterin was unaffected by galiellalactone treatment but was upregulated by docetaxel. A slight downregulation of clusterin was observed in docetaxel and galiellalactone treated cells compared with docetaxel alone. Western blot analysis indicated an increase in cleaved poly(ADP-ribose) polymerase(s) (c-PARP) in both galiellalactone and combination treated samples. This data suggests that galiellalactone and docetaxel can be used in combination to synergistically inhibit cell proliferation and increase apoptosis by c-PARP. The synergistic effect likely stems via STAT3 targeting leading to a downregulation of BCL-2, PIM1 and IL-6. Combination therapy between STAT3 inhibitors and docetaxel may lead to an increase in effect while potentially decreasing the docetaxel dose resulting in reduced side effects.

Concluding Remarks

PCa is a common disease in the western world affecting a large number of men. Current detection methods are decent and lead to better treatment for many men but there is a need for more robust methods to identify the patients with aggressive disease. A need for new biomarkers has spawned a number of possible candidates but their validation has been spurred with controversy. Treatment options have heavily centered on the AR pathway and chemotherapy, with the latter causing detrimental side effects for short survival benefit. New targets and biomarkers must be found to increase survival and decrease unnecessary treatment.

STAT3 is here presented as both a potential biomarker and target for treatment. We found that in early stages of PCa STAT3 did not add predictive value to outcome as measured by BCR but we did find that in metastases, STAT3 was almost ubiquitously expressed suggesting its importance in later stages of the disease. We explored the STAT3 inhibitor galiellalactone and found that it bound directly to STAT3, blocking its DNA binding ability. It was also found that galiellalactone treatment in combination with chemotherapeutic docetaxel reduced expression of genes involved in docetaxel resistance. These results coupled with earlier publications on STAT3 targeting suggest that direct STAT3 targeting is a viable treatment in more aggressive PCas.

It is imperative that we continue to study STAT3 and other potential biomarkers in larger cohorts with as much clinical information as possible to validate or exclude their potential uses for predictive, diagnostic or prognostic benefit.

Popular Science Summary

Prostate cancer is the most diagnosed non-skin cancer in the world among men, it is also the second leading cause of death (due to cancer). Two problems face current patients diagnosed with prostate cancer: over diagnosis with the inability to distinguish between aggressive and non aggressive cancer and the cancers ability to gain resistance to current treatment options. This thesis is split into two parts consisting of four papers that deal with these two issues.

Patients being overdiagnosed with prostate cancer is directly linked to a shortage of valuable markers that can identify more aggressive tumors. Currently the only markers used for diagnosis are the assessment of Gleason Score of biopsy samples and measuring PSA levels in the blood. The screening of blood for PSA can detect whether a man has an increased androgen activity but it cannot distinguish between changes associated with cancer or benign hyperplasia. This results in the overtreatment of men that have non-life threatening disease. It is of great importance to find new markers that can identify which patients are in need of treatment and what treatment is beneficial. STAT3 is a protein involved in controlling the levels of a number of proteins, many of which have been linked to a cancers ability to metastasize, escape programmed cell death and escape the immune system. We explore the presence of STAT3 in different groups of patients in papers I and II. In paper I the levels of STAT3 were studied in various metastatic samples from patients who had died from prostate cancer. STAT3 expression was observed to be present in at least one sample of each patient, with bone metastases having the highest levels. Levels of a cell receptor, IL-6R, which can activate STAT3 were also studied and similar results to STAT3 levels were observed. Meanwhile paper II focused on a group of patients that had undergone a surgical removal of the prostate but had not had any other treatment. In this early stage of the disease it was found that STAT3 levels were lower in the tumor tissue compared to the normal tissue and the lower STAT3 levels in the tumor suggested a more severe disease.

Removal of the prostate is an effective treatment option in prostate cancer but has a number of high risk side effects including impotence and incontinence. Even when patients have their prostates removed there is a high likelihood that the cancer will come back. When the cancer comes back, treatment options are limited to chemotherapy, radiotherapy and targeted therapy focused on the androgen

receptor and its activating hormones. Many patients receiving these therapies gain resistance after a few years. There is a need to find new drug targets in prostate cancer. As mentioned earlier STAT3 has been shown to be involved in a number of cancer related processes and is therefore a good candidate for treatment.

Paper III focused on determining the mechanism of action of the STAT3 inhibitor galiellalactone. Galiellalactone was found to interact with STAT3, limiting its ability to bind to DNA. Since STAT3 controls the levels of a number of proteins linked to resistance to chemotherapy, paper IV explored the ability of galiellalactone to enhance the effects of docetaxel, a common chemotherapeutic agent. Galiellalactone treatment was shown to have synergistic effects when used in combination with docetaxel. The synergy may in part be due to galiellalactones ability to decrease production of genes that had increased levels when treated by docetaxel treatment alone.

The four papers in this thesis help to establish what patients are suitable for treatment with a drug that blocks STAT3 and how this drug interacts with the STAT3 protein.

Acknowledgements

"Always take a banana to a party, bananas are good!" Tenth Doctor, Doctor Who

First and foremost I would like to thank the funders, this work was supported by the Swedish Cancer Foundation, Swedish Scientific Council, Gunnar Nilsson Cancer Foundation, the BioCare program at Lund University, the Skåne University Hospital Research Foundations, Government Funding of Clinical Research with-in the National Health Services and Lund University (ALF) to Anders Bjartell. The funding for paper I was also supported by the Pacific Northwest Prostate Cancer SPORE program (P50CA97186), a PO1 NIH grant (PO1CA085859), R01 CA165573 and the Richard M. Lucas Foundation.

A big thanks goes out to my supervisor **Anders**, you challenged me with interesting projects and gave me the opportunity to pursue a PhD. I look up to you and your ability to handle so many different things at once. You always seem to be doing something but always have time for questions and discussions, thank you.

To my co-supervisor, **Rebecka**, thank you for always having an open door to discuss any problems with experiments. You also have given be invaluable insight into drug development research and I appreciate all of the help you have given me, whether it be with analysis or experimental design.

Agi, thank you for everything!!! You have helped me so much with so many different aspects, analysis, writing, etc., basically figuring out which direction is up. I know that at this point, especially as I sit writing this section you just cannot wait until this book is printed. I apologize to you for all the times I have sent you work texts late or on weekends. You have been a valuable mentor and I could not have done it without you.

Giacomo, I teased you that you wouldn't get an acknowledgment but you deserve huge thanks from me. You've taught me so many things, I still can't believe I can

find a mouse prostate. I have probably talked your ear off but thank you for being there to teach and listen.

Giuseppe, you helped me in the impossible sea that is statistical analysis, thank you.

To the rest of the group, **Aseem**, thanks for your suggestions, **Felicia**, thanks for your help with IHC, **Annica**, for putting the TMA database together, **Susan**, for teaching me some animal work and for being the other American, **Anna Stiehm** thank you for all of the assistance and **Anna Holst** thank you for all the work you've done behind the scenes.

Kiki and **Elise**, without your IHC expertise we would not have had the great stainings that have given us so much information, thank you.

I would like to thank all of the patients who made this work possible.

I am grateful to investigators **Drs. Robert Vessella**, **Celestia Higano**, **Bruce Montgomery**, **Evan Yu**, **Paul Lange**, **Martine Roudier**, and **Lawrence True** for their contributions to the University of Washington Medical Center Prostate Cancer Donor Rapid Autopsy Program.

To all my coauthors, thank you; Ilsa Coleman, Peter S. Nelson, Roy Ehrnström, Colm Morrissey, Zilma Escobar, Martin Johansson, Sven Kjellstrom, Victor Garcia, Eduardo Munoz and Olov Sterner.

To my office mates past and present **Krzysztof** (I still don't know how to spell your name without looking), **Roni**, **Lisa**, **Melly**, **Carro** and **Greta** thank you for the strange conversations, the useful conversations and most importantly with all the help you were able to give whether it be for a poster or a method.

To the rest of the CMP, Karin, Martin, Maite, Tamae, Johan, Totte, Ben, Camilla, Helen and Elisabet thank you for all the help.

Mom and **Dad**, you were always very supportive of me and helped me whenever I needed it. You gave me support and were always there in my time of need even across an ocean. You didn't even blink an eye when I said I was going to Sweden even though I know that you both have missed me as I have missed you. You always wanted what was best for me. I love you.

Tomas, what can I say? You are that person who is always there, we have spent many hours (I checked and you don't want to know) playing destiny (and other games of course). Its nice how we "hang-out" online its as if we are in the room together, its been tough not being home sometimes but I know that I can always reach you and the best part, our sleeping schedules are so insane that we end up having conversations at the oddest hours.

To my friends in Sweden, **Leandro** and **Johan** the game nights we had were a welcome distraction and all those times throwing the football around, **Pavle** always there to grab a beer and just talk, **Mike** always with some new idea did you ever think that when you met me in 2009, I would still be in Sweden in 2017?

To my friends in the US, **Bev** and **AJ**, I know at this point you are asking, "When is this guy coming home?", and no one knows the answer to that question, thanks for being there, the random texts we send each other never get old. To **Stephen**, its crazy to think that we spent high school and university together and now I'm hallway across the world, thanks for all the fun we've had.

To **George Cachianes** and **Maureen Boland**, the two teachers who set me on the path to molecular biology, thank you.

To Catjas family, **Daina**, **Louise**, **Mats**, **Torbjörn**, **Anne**, **Calle** and **Max** thank you for making me feel at home.

Catja, 6 years, did you ever think that you'd be together with that weird American for that long? What can I say, there is so much? You have helped me through some tough times but mainly we've had a lot of fun. I don't know how I would have done it without you. I love you. (I know you hate batman, but do you realize that without the joker, we wouldn't be here, now?)

Finally, one last acknowledgement remains, to **Spencer**, you wont be able to read this but maybe you can chew on one of the books. As a rabbit your life is simple; dig, sleep, bite Nick, rinse and repeat. Thank you for being cuddly (sometimes).

...Nothing comes to mind" Colonel Jack O'Neill Stargate SG-1

References

- 1. Wein, A.J., L.R. Kavoussi, and M.F. Campbell, Campbell-Walsh Urology. 2012: Elsevier Saunders.
- 2. Selman, S.H., The McNeal Prostate: A Review. Urology, 2011. 78(6): p. 1224-1228.
- 3. McNeal, J.E., et al., Zonal distribution of prostatic adenocarcinoma: correlation with histologic pattern and direction of spread. The American journal of surgical pathology, 1988. 12(12): p. 897-906.
- 4. Greene, D., et al., A comparison of the morphological features of cancer arising in the transition zone and in the peripheral zone of the prostate. The Journal of urology, 1991. 146(4): p. 1069-1076.
- 5. Kaplan, S.A., et al., Transition zone index as a method of assessing benign prostatic hyperplasia: correlation with symptoms, urine flow and detrusor pressure. The Journal of urology, 1995. 154(5): p. 1764-1769.
- 6. Robinson, E.J., D.E. Neal, and A.T. Collins, Basal cells are progenitors of luminal cells in primary cultures of differentiating human prostatic epithelium. Prostate, 1998. 37(3): p. 149-60.
- 7. Bonkhoff, H. and K. Remberger, Widespread distribution of nuclear androgen receptors in the basal cell layer of the normal and hyperplastic human prostate. Virchows Arch A Pathol Anat Histopathol, 1993. 422(1): p. 35-8.
- 8. Kumar, V. and P. Majumder, Prostate gland: structure, functions and regulation. International urology and nephrology, 1995. 27(3): p. 231-243.
- 9. Siegel, R.L., K.D. Miller, and A. Jemal, Cancer statistics, 2016. CA Cancer J Clin, 2016. 66(1): p. 7-30.
- Ballentine Carter, H., Differentiation of lethal and non lethal prostate cancer: PSA and PSA isoforms and kinetics. Asian Journal of Andrology, 2012. 14(3): p. 355-360.
- 11. Bostwick, D.G., et al., Human prostate cancer risk factors. Cancer, 2004. 101(S10): p. 2371-2490.
- Heidenreich, A., et al., EAU Guidelines on Prostate Cancer. Part 1: Screening, Diagnosis, and Treatment of Clinically Localised Disease. European Urology, 2011. 59(1): p. 61-71.
- 13. Catalona, W.J., et al., Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. N Engl J Med, 1991. 324(17): p. 1156-61.
- 14. Christensson, A., et al., Serum prostate specific antigen complexed to alpha 1antichymotrypsin as an indicator of prostate cancer. The Journal of urology, 1993. 150(1): p. 100-105.

- 15. Loeb, S. and W.J. Catalona, The Prostate Health Index: a new test for the detection of prostate cancer. Therapeutic Advances in Urology, 2014. 6(2): p. 74-77.
- 16. Braun, K., et al., A Four-kallikrein Panel Predicts High-grade Cancer on Biopsy: Independent Validation in a Community Cohort. Eur Urol, 2016. 69(3): p. 505-11.
- Grönberg, H., et al., Prostate cancer screening in men aged 50–69 years (STHLM3): a prospective population-based diagnostic study. The Lancet Oncology. 16(16): p. 1667-1676.
- Gelmann, E.P., Molecular Biology of the Androgen Receptor. Journal of Clinical Oncology, 2002. 20(13): p. 3001-3015.
- 19. Fang, Y., et al., Hsp90 Regulates Androgen Receptor Hormone Binding Affinity in Vivo. Journal of Biological Chemistry, 1996. 271(45): p. 28697-28702.
- Dehm, S.M. and D.J. Tindall, Androgen Receptor Structural and Functional Elements: Role and Regulation in Prostate Cancer. Molecular Endocrinology, 2007. 21(12): p. 2855-2863.
- Askew, E.B., et al., Modulation of Androgen Receptor Activation Function 2 by Testosterone and Dihydrotestosterone. Journal of Biological Chemistry, 2007. 282(35): p. 25801-25816.
- 22. Sharifi, N. and R.J. Auchus, Steroid biosynthesis and prostate cancer. Steroids, 2012. 77(7): p. 719-726.
- 23. Heinlein, C.A. and C. Chang, Androgen receptor in prostate cancer. Endocr Rev, 2004. 25(2): p. 276-308.
- Yuan, X. and S.P. Balk, Mechanisms mediating androgen receptor reactivation after castration. Urologic Oncology: Seminars and Original Investigations, 2009. 27(1): p. 36-41.
- 25. D'Amico, A.V., et al., Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. JAMA, 1998. 280(11): p. 969-74.
- Cooperberg, M.R., J.F. Hilton, and P.R. Carroll, The CAPRA-S score: A straightforward tool for improved prediction of outcomes after radical prostatectomy. Cancer, 2011. 117(22): p. 5039-46.
- 27. Joniau, S., et al., Stratification of high-risk prostate cancer into prognostic categories: a European multi-institutional study. Eur Urol, 2015. 67(1): p. 157-64.
- Gandaglia, G., et al., Comparative Effectiveness of Robot-Assisted and Open Radical Prostatectomy in the Postdissemination Era. Journal of Clinical Oncology, 2014. 32(14): p. 1419-1426.
- 29. Hayes, J.H., et al., Active surveillance compared with initial treatment for men with low-risk prostate cancer: a decision analysis. Jama, 2010. 304(21): p. 2373-2380.
- Tosoian, J.J., et al., Active Surveillance Program for Prostate Cancer: An Update of the Johns Hopkins Experience. Journal of Clinical Oncology, 2011. 29(16): p. 2185-2190.
- 31. Lee, B.H., et al., Are biochemical recurrence outcomes similar after radical prostatectomy and radiation therapy? Analysis of prostate cancer-specific mortality

by nomogram-predicted risks of biochemical recurrence. Eur Urol, 2015. 67(2): p. 204-9.

- 32. Cookson, M.S., et al., Variation in the definition of biochemical recurrence in patients treated for localized prostate cancer: the American Urological Association Prostate Guidelines for Localized Prostate Cancer Update Panel report and recommendations for a standard in the reporting of surgical outcomes. J Urol, 2007. 177(2): p. 540-5.
- 33. Shore, N.D., Experience with degarelix in the treatment of prostate cancer. Therapeutic Advances in Urology, 2013. 5(1): p. 11-24.
- Petrylak, D.P., et al., Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. New England Journal of Medicine, 2004. 351(15): p. 1513-1520.
- 35. Paller, C.J. and E.S. Antonarakis, Cabazitaxel: a novel second-line treatment for metastatic castration-resistant prostate cancer. Drug Des Devel Ther, 2011. 5(10): p. 117-124.
- 36. de Bono , J.S., et al., Abiraterone and Increased Survival in Metastatic Prostate Cancer. New England Journal of Medicine, 2011. 364(21): p. 1995-2005.
- 37. Scher, H.I., et al., Increased Survival with Enzalutamide in Prostate Cancer after Chemotherapy. New England Journal of Medicine, 2012. 367(13): p. 1187-1197.
- 38. Parker, C., et al., Alpha Emitter Radium-223 and Survival in Metastatic Prostate Cancer. New England Journal of Medicine, 2013. 369(3): p. 213-223.
- 39. Mehta, S., et al., Predictive and prognostic molecular markers for cancer medicine. Therapeutic Advances in Medical Oncology, 2010. 2(2): p. 125-148.
- 40. National Academy of Sciences. Science, 1960. 132(3438): p. 1488-1501.
- 41. Wolff, A.C., et al., American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch Pathol Lab Med, 2007. 131(1): p. 18-43.
- 42. Conde, E., et al., Profile of Ventana ALK (D5F3) companion diagnostic assay for non-small-cell lung carcinomas. Expert review of molecular diagnostics, 2016: p. 1-7.
- 43. Burns, J.A., et al., Choice of Fixative Is Crucial to Successful Immunohistochemical Detection of Phosphoproteins in Paraffin-embedded Tumor Tissues. Journal of Histochemistry & Cytochemistry, 2009. 57(3): p. 257-264.
- 44. Dash, A., et al., Changes in differential gene expression because of warm ischemia time of radical prostatectomy specimens. Am J Pathol, 2002. 161(5): p. 1743-8.
- 45. Best, S., et al., Integrity of prostatic tissue for molecular analysis after roboticassisted laparoscopic and open prostatectomy. Urology, 2007. 70(2): p. 328-32.
- 46. Copete, M., et al., Inappropriate calibration and optimisation of pan-keratin (pan-CK) and low molecular weight keratin (LMWCK) immunohistochemistry tests: Canadian Immunohistochemistry Quality Control (CIQC) experience. J Clin Pathol, 2011. 64(3): p. 220-5.

- 47. Hewitt, S.M., et al., Controls for Immunohistochemistry: The Histochemical Society's Standards of Practice for Validation of Immunohistochemical Assays. Journal of Histochemistry and Cytochemistry, 2014. 62(10): p. 693-697.
- 48. Saper, C.B., A Guide to the Perplexed on the Specificity of Antibodies. Journal of Histochemistry and Cytochemistry, 2009. 57(1): p. 1-5.
- 49. Kern, A. and A.W. Partin, Genetic Tests for Prostate Cancer. Reviews in Urology, 2013. 15(4): p. 208-209.
- 50. Humphrey, P.A., Gleason grading and prognostic factors in carcinoma of the prostate. Mod Pathol, 2004. 17(3): p. 292-306.
- Gleason, D.F., Classification of prostatic carcinomas. Cancer Chemother Rep, 1966. 50(3): p. 125-8.
- 52. Epstein, J.I., et al., The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Surg Pathol, 2005. 29(9): p. 1228-42.
- Epstein, J.I., et al., The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: Definition of Grading Patterns and Proposal for a New Grading System. Am J Surg Pathol, 2016. 40(2): p. 244-52.
- 54. NIH. Gleason Grade Image. December 2016]; Available from: https://training.seer.cancer.gov/prostate/abstract-code-stage/morphology.html.
- 55. Andriole, G.L., et al., Mortality Results from a Randomized Prostate-Cancer Screening Trial. New England Journal of Medicine, 2009. 360(13): p. 1310-1319.
- 56. Moyer, V.A. and U.S.P.S.T.F. on behalf of the, Screening for prostate cancer: U.s. preventive services task force recommendation statement. Annals of Internal Medicine, 2012. 157(2): p. 120-134.
- 57. Schröder, F.H., et al., Screening and Prostate-Cancer Mortality in a Randomized European Study. New England Journal of Medicine, 2009. 360(13): p. 1320-1328.
- Schröder, F.H., et al., The European Randomized Study of Screening for Prostate Cancer – Prostate Cancer Mortality at 13 Years of Follow-up. Lancet, 2014. 384(9959): p. 2027-2035.
- 59. Deras, I.L., et al., PCA3: A Molecular Urine Assay for Predicting Prostate Biopsy Outcome. The Journal of Urology, 2008. 179(4): p. 1587-1592.
- 60. Haese, A., et al., Clinical Utility of the PCA3 Urine Assay in European Men Scheduled for Repeat Biopsy. European Urology, 2008. 54(5): p. 1081-1088.
- 61. Wei, W., et al., High PCA3 scores in urine correlate with poor-prognosis factors in prostate cancer patients. International Journal of Clinical and Experimental Medicine, 2015. 8(9): p. 16606-16612.
- 62. Tomlins, S.A., et al., Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science, 2005. 310.
- 63. Tomlins, S.A., et al., Role of the TMPRSS2-ERG Gene Fusion in Prostate Cancer. Neoplasia (New York, N.Y.), 2008. 10(2): p. 177-188.
- 64. Gopalan, A., et al., TMPRSS2-ERG gene fusion is not associated with outcome in patients treated by prostatectomy. Cancer research, 2009. 69(4): p. 1400-1406.

- 65. Pettersson, A., et al., The TMPRSS2:ERG rearrangement, ERG expression, and prostate cancer outcomes: a cohort study and meta-analysis. Cancer Epidemiol Biomarkers Prev, 2012. 21(9): p. 1497-509.
- 66. Tomlins, S.A., et al., Urine TMPRSS2: ERG plus PCA3 for individualized prostate cancer risk assessment. European urology, 2016. 70(1): p. 45-53.
- 67. Boström, P.J., et al., Genomic predictors of outcome in prostate cancer. European urology, 2015. 68(6): p. 1033-1044.
- 68. Hu, R., et al., Ligand-Independent Androgen Receptor Variants Derived from Splicing of Cryptic Exons Signify Hormone-Refractory Prostate Cancer. Cancer Research, 2009. 69(1): p. 16-22.
- 69. Antonarakis, E.S., et al., AR-V7 and Resistance to Enzalutamide and Abiraterone in Prostate Cancer. New England Journal of Medicine, 2014. 371(11): p. 1028-1038.
- 70. Takeuchi, T., et al., Detection of AR-V7 mRNA in whole blood may not predict the effectiveness of novel endocrine drugs for castration-resistant prostate cancer. Research and Reports in Urology, 2016. 8: p. 21-25.
- Scher, H.I., et al., Association of AR-V7 on Circulating Tumor Cells as a Treatment-Specific Biomarker With Outcomes and Survival in Castration-Resistant Prostate Cancer. JAMA Oncol, 2016. 2(11): p. 1441-1449.
- 72. Maehama, T. and J.E. Dixon, The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3, 4, 5-trisphosphate. Journal of Biological Chemistry, 1998. 273(22): p. 13375-13378.
- 73. Saal, L.H., et al., PIK3CA mutations correlate with hormone receptors, node metastasis, and ERBB2, and are mutually exclusive with PTEN loss in human breast carcinoma. Cancer research, 2005. 65(7): p. 2554-2559.
- 74. Sos, M.L., et al., PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. Cancer research, 2009. 69(8): p. 3256-3261.
- 75. Pourmand, G., et al., Role of PTEN gene in progression of prostate cancer. Urol J, 2007. 4(2): p. 95-100.
- 76. Trotman, L.C., et al., Pten dose dictates cancer progression in the prostate. PLoS Biol, 2003. 1.
- 77. Giri, D., M. Ozen, and M. Ittmann, Interleukin-6 is an autocrine growth factor in human prostate cancer. Am J Pathol, 2001. 159(6): p. 2159-65.
- 78. Shariat, S.F., et al., Plasma levels of interleukin-6 and its soluble receptor are associated with prostate cancer progression and metastasis. Urology, 2001. 58(6): p. 1008-15.
- 79. Perry, K.T., C.T. Anthony, and M.S. Steiner, Immunohistochemical localization of TGF beta 1, TGF beta 2, and TGF beta 3 in normal and malignant human prostate. Prostate, 1997. 33(2): p. 133-40.
- 80. Adler, H.L., et al., Elevated levels of circulating interleukin-6 and transforming growth factor-beta1 in patients with metastatic prostatic carcinoma. J Urol, 1999. 161(1): p. 182-7.

- 81. Shariat, S.F., et al., Preoperative plasma levels of transforming growth factor beta(1) (TGF-beta(1)) strongly predict progression in patients undergoing radical prostatectomy. J Clin Oncol, 2001. 19(11): p. 2856-64.
- 82. Perry, K.T., et al., Transforming growth factor beta as a clinical biomarker for prostate cancer. Urology, 1997. 49(1): p. 151-5.
- 83. Becker, S., B. Groner, and C.W. Muller, Three-dimensional structure of the Stat3[beta] homodimer bound to DNA. Nature, 1998. 394(6689): p. 145-151.
- Zhang, T., et al., The Coiled-Coil Domain of Stat3 Is Essential for Its SH2 Domain-Mediated Receptor Binding and Subsequent Activation Induced by Epidermal Growth Factor and Interleukin-6. Molecular and Cellular Biology, 2000. 20(19): p. 7132-7139.
- 85. Wen, Z., Z. Zhong, and J.E. Darnell, Jr., Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. Cell, 1995. 82(2): p. 241-50.
- 86. Turkson, J., STAT proteins as novel targets for cancer drug discovery. Expert Opin Ther Targets, 2004. 8(5): p. 409-22.
- Bishop, J.L., D. Thaper, and A. Zoubeidi, The Multifaceted Roles of STAT3 Signaling in the Progression of Prostate Cancer. Cancers (Basel), 2014. 6(2): p. 829-59.
- Heinrich, P.C., et al., Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem J, 1998. 334 (Pt 2): p. 297-314.
- Heinrich, P.C., et al., Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J, 2003. 374(Pt 1): p. 1-20.
- Scheller, J., et al., The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 2011. 1813(5): p. 878-888.
- 91. Simpson, R.J., et al., Interleukin-6: structure-function relationships. Protein Sci, 1997. 6(5): p. 929-55.
- Yawata, H., et al., Structure-function analysis of human IL-6 receptor: dissociation of amino acid residues required for IL-6-binding and for IL-6 signal transduction through gp130. EMBO J, 1993. 12(4): p. 1705-12.
- Martens, A.S., et al., The cytoplasmic domain of the interleukin-6 receptor gp80 mediates its basolateral sorting in polarized madin-darby canine kidney cells. Journal of Cell Science, 2000. 113(20): p. 3593-3602.
- 94. Dittrich, E., et al., Identification of a region within the cytoplasmic domain of the interleukin-6 (IL-6) signal transducer gp130 important for ligand-induced endocytosis of the IL-6 receptor. J Biol Chem, 1994. 269(29): p. 19014-20.
- Boulanger, M.J., et al., Hexameric structure and assembly of the interleukin-6/IL-6 alpha-receptor/gp130 complex. Science, 2003. 300(5628): p. 2101-4.
- 96. Mihara, M., et al., IL-6/IL-6 receptor system and its role in physiological and pathological conditions. Clin Sci (Lond), 2012. 122(4): p. 143-59.
- Stahl, N., et al., Choice of STATs and other substrates specified by modular tyrosinebased motifs in cytokine receptors. Science, 1995. 267(5202): p. 1349.

- Mullberg, J., et al., The soluble interleukin-6 receptor is generated by shedding. Eur J Immunol, 1993. 23(2): p. 473-80.
- 99. Silver, J.S. and C.A. Hunter, gp130 at the nexus of inflammation, autoimmunity, and cancer. J Leukoc Biol, 2010. 88(6): p. 1145-56.
- 100. Croker, B.A., et al., SOCS3 negatively regulates IL-6 signaling in vivo. Nat Immunol, 2003. 4(6): p. 540-5.
- Takeda, K., et al., Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality. Proceedings of the National Academy of Sciences, 1997. 94(8): p. 3801-3804.
- 102. Niwa, H., et al., Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. Genes Dev, 1998. 12(13): p. 2048-60.
- 103. Raz, R., et al., Essential role of STAT3 for embryonic stem cell pluripotency. Proceedings of the National Academy of Sciences, 1999. 96(6): p. 2846-2851.
- Cheng, F., et al., A critical role for Stat3 signaling in immune tolerance. Immunity, 2003. 19(3): p. 425-36.
- 105. Takeda, K., et al., Stat3 activation is responsible for IL-6-dependent T cell proliferation through preventing apoptosis: generation and characterization of T cell-specific Stat3-deficient mice. The Journal of Immunology, 1998. 161(9): p. 4652-4660.
- 106. Yu, C.L., et al., Enhanced DNA-binding activity of a Stat3-related protein in cells transformed by the Src oncoprotein. Science, 1995. 269(5220): p. 81-3.
- 107. Turkson, J., et al., Stat3 activation by Src induces specific gene regulation and is required for cell transformation. Mol Cell Biol, 1998. 18(5): p. 2545-52.
- Bromberg, J.F., et al., Stat3 activation is required for cellular transformation by v-src. Mol Cell Biol, 1998. 18(5): p. 2553-8.
- 109. Abou-Ghazal, M., et al., The incidence, correlation with tumor-infiltrating inflammation, and prognosis of phosphorylated STAT3 expression in human gliomas. Clinical Cancer Research, 2008. 14(24): p. 8228-8235.
- 110. Lo, H.W., et al., Constitutively activated STAT3 frequently coexpresses with epidermal growth factor receptor in high-grade gliomas and targeting STAT3 sensitizes them to Iressa and alkylators. Clin Cancer Res, 2008. 14(19): p. 6042-54.
- 111. Garcia, R., et al., Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. Oncogene, 2001. 20(20): p. 2499-2513.
- 112. Grandis, J.R., et al., Constitutive activation of Stat3 signaling abrogates apoptosis in squamous cell carcinogenesis in vivo. Proceedings of the National Academy of Sciences, 2000. 97(8): p. 4227-4232.
- 113. Alvarez, J.V., et al., Signal Transducer and Activator of Transcription 3 Is Required for the Oncogenic Effects of Non–Small-Cell Lung Cancer–Associated Mutations of the Epidermal Growth Factor Receptor. Cancer Research, 2006. 66(6): p. 3162-3168.
- 114. Dethlefsen, C., G. Højfeldt, and P. Hojman, The role of intratumoral and systemic IL-6 in breast cancer. Breast Cancer Research and Treatment, 2013. 138(3): p. 657-664.

- 115. Ohgami, R.S., et al., STAT3 mutations are present in aggressive B-cell lymphomas including a subset of diffuse large B-cell lymphomas with CD30 expression. Haematologica, 2014. 99(7): p. e105-e105.
- 116. Jerez, A., et al., STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia. Blood, 2012. 120(15): p. 3048-3057.
- 117. Carpenter, R.L. and H.W. Lo, STAT3 Target Genes Relevant to Human Cancers. Cancers (Basel), 2014. 6(2): p. 897-925.
- Dhir, R., et al., Stat3 activation in prostatic carcinomas. Prostate, 2002. 51(4): p. 241-6.
- Mora, L.B., et al., Constitutive activation of Stat3 in human prostate tumors and cell lines: direct inhibition of Stat3 signaling induces apoptosis of prostate cancer cells. Cancer Res, 2002. 62(22): p. 6659-66.
- 120. Liu, X., et al., Correlation analysis of JAK-STAT pathway components on prognosis of patients with prostate cancer. Pathol Oncol Res, 2012. 18(1): p. 17-23.
- 121. Torres-Roca, J.F., et al., Activated STAT3 as a correlate of distant metastasis in prostate cancer: a secondary analysis of Radiation Therapy Oncology Group 86-10. Urology, 2007. 69(3): p. 505-9.
- 122. Tam, L., et al., Expression levels of the JAK/STAT pathway in the transition from hormone-sensitive to hormone-refractory prostate cancer. Br J Cancer, 2007. 97(3): p. 378-83.
- Abdulghani, J., et al., Stat3 promotes metastatic progression of prostate cancer. Am J Pathol, 2008. 172(6): p. 1717-28.
- 124. Ueda, T., N. Bruchovsky, and M.D. Sadar, Activation of the androgen receptor Nterminal domain by interleukin-6 via MAPK and STAT3 signal transduction pathways. J Biol Chem, 2002. 277(9): p. 7076-85.
- 125. Schroeder, A., et al., Loss of Androgen Receptor Expression Promotes a Stem-Like Cell Phenotype in Prostate Cancer through STAT3 Signaling. Cancer research, 2014. 74(4): p. 1227-1237.
- 126. Lee, S.O., et al., New therapy targeting differential androgen receptor signaling in prostate cancer stem/progenitor vs. non-stem/progenitor cells. Journal of Molecular Cell Biology, 2013. 5(1): p. 14-26.
- 127. Birnie, R., et al., Gene expression profiling of human prostate cancer stem cells reveals a pro-inflammatory phenotype and the importance of extracellular matrix interactions. Genome Biology, 2008. 9(5): p. R83.
- 128. Corvinus, F.M., et al., Persistent STAT3 Activation in Colon Cancer Is Associated with Enhanced Cell Proliferation and Tumor Growth. Neoplasia (New York, N.Y.), 2005. 7(6): p. 545-555.
- 129. Masuda, M., et al., Constitutive Activation of Signal Transducers and Activators of Transcription 3 Correlates with Cyclin D1 Overexpression and May Provide a Novel Prognostic Marker in Head and Neck Squamous Cell Carcinoma. Cancer Research, 2002. 62(12): p. 3351-3355.

- 130. Shintani, M., et al., Overexpression of cyclin DI contributes to malignant properties of esophageal tumor cells by increasing VEGF production and decreasing Fas expression. Anticancer Res, 2002. 22(2A): p. 639-47.
- Kurosaka, M. and S. Machida, Interleukin-6-induced satellite cell proliferation is regulated by induction of the JAK2/STAT3 signalling pathway through cyclin D1 targeting. Cell Prolif, 2013. 46(4): p. 365-73.
- 132. Li, X., et al., Prostate tumor progression is mediated by a paracrine TGF-β/Wnt3a signaling axis. Oncogene, 2008. 27(56): p. 7118-7130.
- 133. Kolosenko, I., D. Grander, and K.P. Tamm, IL-6 activated JAK/STAT3 pathway and sensitivity to Hsp90 inhibitors in multiple myeloma. Curr Med Chem, 2014. 21(26): p. 3042-7.
- 134. Culig, Z., G. Bartsch, and A. Hobisch, Interleukin-6 regulates androgen receptor activity and prostate cancer cell growth. Mol Cell Endocrinol, 2002. 197(1-2): p. 231-8.
- 135. Bohonowych, J.E., et al., Extracellular Hsp90 mediates an NF-κB dependent inflammatory stromal program: Implications for the prostate tumor microenvironment. The Prostate, 2014. 74(4): p. 395-407.
- 136. Horvitz, H.R., Genetic Control of Programmed Cell Death in the Nematode Caenorhabditis elegans. Cancer Research, 1999. 59(7 Supplement): p. 1701s-1706s.
- 137. Czabotar, P.E., et al., Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat Rev Mol Cell Biol, 2014. 15(1): p. 49-63.
- 138. Catlett-Falcone, R., et al., Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. Immunity, 1999. 10(1): p. 105-15.
- Epling-Burnette, P.K., et al., Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes and decreased Mcl-1 expression. J Clin Invest, 2001. 107(3): p. 351-62.
- 140. Alas, S. and B. Bonavida, Rituximab inactivates signal transducer and activation of transcription 3 (STAT3) activity in B-non-Hodgkin's lymphoma through inhibition of the interleukin 10 autocrine/paracrine loop and results in down-regulation of Bcl-2 and sensitization to cytotoxic drugs. Cancer Res, 2001. 61(13): p. 5137-44.
- 141. Haupt, S., et al., Apoptosis-the p53 network. Journal of cell science, 2003. 116(20): p. 4077-4085.
- 142. Niu, G., et al., Role of Stat3 in Regulating p53 Expression and Function. Molecular and Cellular Biology, 2005. 25(17): p. 7432-7440.
- 143. Foda, H.D. and S. Zucker, Matrix metalloproteinases in cancer invasion, metastasis and angiogenesis. Drug Discov Today, 2001. 6(9): p. 478-482.
- 144. Gialeli, C., A.D. Theocharis, and N.K. Karamanos, Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. FEBS J, 2011. 278(1): p. 16-27.
- 145. Fukuda, A., et al., Stat3 and MMP7 Contribute to Pancreatic Ductal Adenocarcinoma Initiation and Progression. Cancer cell, 2011. 19(4): p. 441-455.
- 146. Xie, T.-x., et al., Stat3 activation regulates the expression of matrix metalloproteinase-2 and tumor invasion and metastasis. Oncogene, 2004. 23(20): p. 3550-3560.
- 147. Dechow, T.N., et al., Requirement of matrix metalloproteinase-9 for the transformation of human mammary epithelial cells by Stat3-C. Proceedings of the National Academy of Sciences of the United States of America, 2004. 101(29): p. 10602-10607.
- 148. Itoh, M., et al., Requirement of STAT3 activation for maximal collagenase-1 (MMP-1) induction by epidermal growth factor and malignant characteristics in T24 bladder cancer cells. Oncogene, 2005. 25(8): p. 1195-1204.
- 149. Teng, T.S., et al., Stat3 promotes directional cell migration by regulating Rac1 activity via its activator betaPIX. J Cell Sci, 2009. 122(Pt 22): p. 4150-9.
- Verma, N.K., et al., STAT3-stathmin interactions control microtubule dynamics in migrating T-cells. J Biol Chem, 2009. 284(18): p. 12349-62.
- Suiqing, C., Z. Min, and C. Lirong, Overexpression of phosphorylated-STAT3 correlated with the invasion and metastasis of cutaneous squamous cell carcinoma. J Dermatol, 2005. 32(5): p. 354-60.
- 152. Azare, J., et al., Constitutively Activated Stat3 Induces Tumorigenesis and Enhances Cell Motility of Prostate Epithelial Cells through Integrin β 6. Mol Cell Biol, 2007. 27(12): p. 4444-53.
- 153. Niu, G., et al., Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. Oncogene, 2002. 21(13): p. 2000-8.
- 154. Boddy, J.L., et al., The Androgen Receptor Is Significantly Associated with Vascular Endothelial Growth Factor and Hypoxia Sensing via Hypoxia-Inducible Factors HIF-1a, HIF-2a, and the Prolyl Hydroxylases in Human Prostate Cancer. Clinical Cancer Research, 2005. 11(21): p. 7658-7663.
- 155. Niu, G., et al., Signal Transducer and Activator of Transcription 3 is required for hypoxia-inducible factor- 1α RNA expression in both tumor cells and tumor-associated myeloid cells. Molecular cancer research : MCR, 2008. 6(7): p. 1099-1105.
- 156. Deng, J., et al., S1PR1-STAT3 signaling is crucial for myeloid cell colonization at future metastatic sites. Cancer Cell, 2012. 21(5): p. 642-54.
- 157. Steinbrink, K., et al., Induction of tolerance by IL-10-treated dendritic cells. The Journal of Immunology, 1997. 159(10): p. 4772-4780.
- 158. Williams, L., et al., Signal transducer and activator of transcription 3 is the dominant mediator of the anti-inflammatory effects of IL-10 in human macrophages. J Immunol, 2004. 172(1): p. 567-76.
- 159. Yang, X.O., et al., STAT3 Regulates Cytokine-mediated Generation of Inflammatory Helper T Cells. Journal of Biological Chemistry, 2007. 282(13): p. 9358-9363.
- Wei, L., et al., IL-21 Is Produced by Th17 Cells and Drives IL-17 Production in a STAT3-dependent Manner. Journal of Biological Chemistry, 2007. 282(48): p. 34605-34610.

- 161. Rebe, C., et al., STAT3 activation: A key factor in tumor immunoescape. JAKSTAT, 2013. 2(1): p. e23010.
- 162. Fizazi, K., et al., Randomised phase II study of siltuximab (CNTO 328), an anti-IL-6 monoclonal antibody, in combination with mitoxantrone/prednisone versus mitoxantrone/prednisone alone in metastatic castration-resistant prostate cancer. Eur J Cancer, 2012. 48(1): p. 85-93.
- 163. Oguro, T., et al., Humanised antihuman IL-6R antibody with interferon inhibits renal cell carcinoma cell growth in vitro and in vivo through suppressed SOCS3 expression. Eur J Cancer, 2013. 49(7): p. 1715-24.
- 164. Ando, K., et al., Possible role for tocilizumab, an anti-interleukin-6 receptor antibody, in treating cancer cachexia. J Clin Oncol, 2013. 31(6): p. e69-72.
- 165. Ge, H., et al., Therapeutic and Preventive Effects of an Epidermal Growth Factor Receptor Inhibitor on Oral Squamous Cell Carcinoma. Journal of International Medical Research, 2012. 40(2): p. 455-466.
- 166. Buerger, C., et al., Sequence-specific Peptide Aptamers, Interacting with the Intracellular Domain of the Epidermal Growth Factor Receptor, Interfere with Stat3 Activation and Inhibit the Growth of Tumor Cells. Journal of Biological Chemistry, 2003. 278(39): p. 37610-37621.
- 167. Cheng, A.-L., et al., Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, doubleblind, placebo-controlled trial. The Lancet Oncology, 2009. 10(1): p. 25-34.
- 168. Huang, S. and F.A. Sinicrope, Sorafenib Inhibits STAT3 Activation to Enhance TRAIL-Mediated Apoptosis in Human Pancreatic Cancer Cells. Molecular Cancer Therapeutics, 2010. 9(3): p. 742.
- 169. Demetri, G.D., et al., Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. The Lancet. 368(9544): p. 1329-1338.
- 170. Xin, H., et al., Sunitinib Inhibition of Stat3 Induces Renal Cell Carcinoma Tumor Cell Apoptosis and Reduces Immunosuppressive Cells. Cancer Research, 2009. 69(6): p. 2506.
- 171. Rini, B.I., et al., Comparative effectiveness of axitinib versus sorafenib in advanced renal cell carcinoma (AXIS): a randomised phase 3 trial. The Lancet. 378(9807): p. 1931-1939.
- 172. Yuan, H., et al., Axitinib augments antitumor activity in renal cell carcinoma via STAT3-dependent reversal of myeloid-derived suppressor cell accumulation. Biomedicine & Pharmacotherapy, 2014. 68(6): p. 751-756.
- 173. Miklossy, G., T.S. Hilliard, and J. Turkson, Therapeutic modulators of STAT signalling for human diseases. Nature reviews. Drug discovery, 2013. 12(8): p. 611-629.
- 174. Furtek, S.L., et al., Strategies and Approaches of Targeting STAT3 for Cancer Treatment. ACS Chem Biol, 2016. 11(2): p. 308-18.
- 175. Turkson, J., et al., Novel peptidomimetic inhibitors of signal transducer and activator of transcription 3 dimerization and biological activity. Mol Cancer Ther, 2004. 3(3): p. 261-9.

- 176. Turkson, J., et al., Phosphotyrosyl peptides block Stat3-mediated DNA binding activity, gene regulation, and cell transformation. J Biol Chem, 2001. 276(48): p. 45443-55.
- 177. Schust, J., et al., Stattic: a small-molecule inhibitor of STAT3 activation and dimerization. Chem Biol, 2006. 13(11): p. 1235-42.
- 178. Ahmad, M.Z., et al., Application of decoy oligonucleotides as novel therapeutic strategy: a contemporary overview. Curr Drug Discov Technol, 2013. 10(1): p. 71-84.
- 179. Sen, M., et al., First-in-human trial of a STAT3 decoy oligonucleotide in head and neck tumors: implications for cancer therapy. Cancer discovery, 2012. 2(8): p. 694-705.
- Huang, W., et al., Small-molecule inhibitors targeting the DNA-binding domain of STAT3 suppress tumor growth, metastasis and STAT3 target gene expression in vivo. Oncogene, 2016. 35(6): p. 783-792.
- 181. Arkin, M.R. and J.A. Wells, Small-molecule inhibitors of protein-protein interactions: progressing towards the dream. Nat Rev Drug Discov, 2004. 3(4): p. 301-317.
- 182. Johansson, M., et al., Synthesis of (-)-pregaliellalactone, conversion of (-)pregaliellalactone to (-)-galiellalactone by mycelia of Galiella rufa. Tetrahedron, 2002. 58(13): p. 2523-2528.
- 183. Johansson, M. and O. Sterner, Synthesis of (+)-galiellalactone. Absolute configuration of galiellalactone. Org Lett, 2001. 3(18): p. 2843-5.
- 184. Weidler, M., et al., Inhibition of interleukin-6 signaling by galiellalactone. FEBS Lett, 2000. 484(1): p. 1-6.
- 185. Hellsten, R., et al., Galiellalactone is a novel therapeutic candidate against hormonerefractory prostate cancer expressing activated Stat3. Prostate, 2008. 68(3): p. 269-80.
- 186. Hellsten, R., et al., Galiellalactone inhibits stem cell-like ALDH-positive prostate cancer cells. PLoS One, 2011. 6(7): p. e22118.
- 187. Canesin, G., et al., The STAT3 Inhibitor Galiellalactone Effectively Reduces Tumor Growth and Metastatic Spread in an Orthotopic Xenograft Mouse Model of Prostate Cancer. European Urology.
- Gottesman, M.M., Mechanisms of cancer drug resistance. Annual review of medicine, 2002. 53(1): p. 615-627.
- 189. Holohan, C., et al., Cancer drug resistance: an evolving paradigm. Nat Rev Cancer, 2013. 13(10): p. 714-726.
- 190. Grayhack, J.T., T.C. Keeler, and J.M. Kozlowski, Carcinoma of the prostate. Hormonal therapy. Cancer, 1987. 60(3 Suppl): p. 589-601.
- 191. Bubendorf, L., et al., Metastatic patterns of prostate cancer: an autopsy study of 1,589 patients. Hum Pathol, 2000. 31(5): p. 578-83.
- 192. Visakorpi, T., et al., In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nat Genet, 1995. 9(4): p. 401-6.

- 193. Gregory, C.W., et al., Androgen receptor stabilization in recurrent prostate cancer is associated with hypersensitivity to low androgen. Cancer Res, 2001. 61(7): p. 2892-8.
- 194. Suzuki, H., et al., Codon 877 mutation in the androgen receptor gene in advanced prostate cancer: relation to antiandrogen withdrawal syndrome. Prostate, 1996. 29(3): p. 153-8.
- 195. Steketee, K., et al., Broadened ligand responsiveness of androgen receptor mutants obtained by random amino acid substitution of H874 and mutation hot spot T877 in prostate cancer. Int J Cancer, 2002. 100(3): p. 309-17.
- 196. Culig, Z., et al., Androgen Receptor Activation in Prostatic Tumor Cell Lines by Insulin-like Growth Factor-I, Keratinocyte Growth Factor, and Epidermal Growth Factor. Cancer Research, 1994. 54(20): p. 5474-5478.
- 197. Dagher, R., et al., Approval Summary. Docetaxel in Combination with Prednisone for the Treatment of Androgen-Independent Hormone-Refractory Prostate Cancer, 2004. 10(24): p. 8147-8151.
- 198. Oudard, S., TROPIC: Phase III trial of cabazitaxel for the treatment of metastatic castration-resistant prostate cancer. Future Oncology, 2011. 7(4): p. 497-506.
- 199. Madan, A., et al., Phase II study of a novel taxane (Cabazitaxel-XRP 6258) in previously treated advanced non-small cell lung cancer (NSCLC) patients. Cancer Chemotherapy and Pharmacology, 2016. 78(3): p. 509-515.
- 200. de Leeuw, R., et al., Novel Actions of Next-Generation Taxanes Benefit Advanced Stages of Prostate Cancer. Clinical Cancer Research, 2015. 21(4): p. 795-807.
- 201. Kotsakis, A., et al., A multicentre phase II trial of cabazitaxel in patients with advanced non-small-cell lung cancer progressing after docetaxel-based chemotherapy. Br J Cancer, 2016. 115(7): p. 784-788.
- 202. Massard, C., et al., Phase I/II trial of cabazitaxel plus abiraterone in patients with metastatic castration-resistant prostate cancer (mCRPC) progressing after docetaxel and abiraterone. Annals of Oncology, 2016.
- 203. Corcoran, C., et al., Docetaxel-resistance in prostate cancer: evaluating associated phenotypic changes and potential for resistance transfer via exosomes. PloS one, 2012. 7(12): p. e50999.
- 204. Duran, G.E., et al., Mechanisms of resistance to cabazitaxel. Molecular cancer therapeutics, 2015. 14(1): p. 193-201.
- 205. Yvon, A.M.C., P. Wadsworth, and M.A. Jordan, Taxol Suppresses Dynamics of Individual Microtubules in Living Human Tumor Cells. Mol Biol Cell, 1999. 10(4): p. 947-59.
- 206. Sanchez, C., et al., Expression of multidrug resistance proteins in prostate cancer is related with cell sensitivity to chemotherapeutic drugs. Prostate, 2009. 69(13): p. 1448-59.
- 207. Sissung, T.M., et al., ABCB1 genetic variation influences the toxicity and clinical outcome of patients with androgen-independent prostate cancer treated with docetaxel. Clin Cancer Res, 2008. 14(14): p. 4543-9.

- 208. Zalcberg, J., et al., MRP1 not MDR1 gene expression is the predominant mechanism of acquired multidrug resistance in two prostate carcinoma cell lines. Prostate Cancer Prostatic Dis, 2000. 3(2): p. 66-75.
- 209. Sullivan, G.F., et al., The expression of drug resistance gene products during the progression of human prostate cancer. Clin Cancer Res, 1998. 4(6): p. 1393-403.
- 210. Bhangal, G., et al., Expression of the multidrug resistance gene in human prostate cancer. Urol Oncol, 2000. 5(3): p. 118-121.
- 211. Van Brussel, J.P., et al., Expression of multidrug resistance related proteins and proliferative activity is increased in advanced clinical prostate cancer. J Urol, 2001. 165(1): p. 130-5.
- 212. Mahon, K.L., et al., Cytokine profiling of docetaxel-resistant castration-resistant prostate cancer. Br J Cancer, 2015. 112(8): p. 1340-8.
- 213. Kastl, L., I. Brown, and A.C. Schofield, miRNA-34a is associated with docetaxel resistance in human breast cancer cells. Breast Cancer Research and Treatment, 2012. 131(2): p. 445-454.
- 214. Visa, L., et al. Correlation of serum interleukin-6 (IL-6) levels and clinical outcome in hormone-independent (HI) prostate cancer (PC) patients (PTS) treated with docetaxel. in ASCO Annual Meeting Proceedings. 2009.
- 215. Domingo-Domenech, J., et al., Interleukin 6, a nuclear factor-kappaB target, predicts resistance to docetaxel in hormone-independent prostate cancer and nuclear factor-kappaB inhibition by PS-1145 enhances docetaxel antitumor activity. Clin Cancer Res, 2006. 12(18): p. 5578-86.
- Fabbri, F., et al., Mitotic catastrophe and apoptosis induced by docetaxel in hormonerefractory prostate cancer cells. Journal of Cellular Physiology, 2008. 217(2): p. 494-501.
- 217. Kramer, G., et al., Docetaxel induces apoptosis in hormone refractory prostate carcinomas during multiple treatment cycles. Br J Cancer, 2006. 94(11): p. 1592-8.
- 218. Krajewska, M., et al., Immunohistochemical analysis of bcl-2, bax, bcl-X, and mcl-1 expression in prostate cancers. Am J Pathol, 1996. 148(5): p. 1567-76.
- 219. Friedland, D., et al., A phase II trial of docetaxel (Taxotere) in hormone-refractory prostate cancer: correlation of antitumor effect to phosphorylation of Bcl-2. Semin Oncol, 1999. 26(5 Suppl 17): p. 19-23.
- 220. Tamaki, H., et al., Bcl-2 family inhibition sensitizes human prostate cancer cells to docetaxel and promotes unexpected apoptosis under caspase-9 inhibition. Oncotarget, 2014. 5(22): p. 11399-412.
- 221. Kroon, J., et al., Glucocorticoid receptor antagonism reverts docetaxel resistance in human prostate cancer. Endocrine-Related Cancer, 2016. 23(1): p. 35-45.
- 222. Kucukzeybek, Y., et al., Enhancement of docetaxel-induced cytotoxicity and apoptosis by all-trans retinoic acid (ATRA) through downregulation of survivin (BIRC5), MCL-1 and LTbeta-R in hormone- and drug resistant prostate cancer cell line, DU-145. Journal of Experimental & Clinical Cancer Research, 2008. 27(1): p. 37.

- 223. Hwang, J.J., et al., A novel histone deacetylase inhibitor, CG200745, potentiates anticancer effect of docetaxel in prostate cancer via decreasing Mcl-1 and Bcl-XL. Investigational new drugs, 2012. 30(4): p. 1434-1442.
- 224. Aho, T.L., et al., Pim-1 kinase promotes inactivation of the pro-apoptotic Bad protein by phosphorylating it on the Ser112 gatekeeper site. FEBS letters, 2004. 571(1-3): p. 43-49.
- 225. Lilly, M., et al., The PIM-1 serine kinase prolongs survival and inhibits apoptosisrelated mitochondrial dysfunction in part through a bcl-2-dependent pathway. Oncogene, 1999. 18(27): p. 4022-4031.
- 226. Zemskova, M., et al., The PIM1 kinase is a critical component of a survival pathway activated by docetaxel and promotes survival of docetaxel-treated prostate cancer cells. J Biol Chem, 2008. 283(30): p. 20635-44.
- 227. Xie, Y., et al., The 44-kDa Pim-1 kinase phosphorylates BCRP/ABCG2 and thereby promotes its multimerization and drug-resistant activity in human prostate cancer cells. Journal of Biological Chemistry, 2008. 283(6): p. 3349-3356.
- 228. Leskov, K.S., et al., Synthesis and Functional Analyses of Nuclear Clusterin, a Cell Death Protein. Journal of Biological Chemistry, 2003. 278(13): p. 11590-11600.
- 229. Zhang, H., et al., Clusterin inhibits apoptosis by interacting with activated Bax. Nat Cell Biol, 2005. 7(9): p. 909-15.
- 230. Zhong, B., et al., Induction of Clusterin by AKT—Role in Cytoprotection against Docetaxel in Prostate Tumor Cells. Mol Cancer Ther, 2010. 9(6).
- 231. Lee, H.J., et al., Drug resistance via feedback activation of Stat3 in oncogeneaddicted cancer cells. Cancer Cell, 2014. 26(2): p. 207-21.
- 232. Bewry, N.N., et al., Stat3 contributes to resistance toward BCR-ABL inhibitors in a bone marrow microenvironment model of drug resistance. Molecular Cancer Therapeutics, 2008. 7(10): p. 3169-3175.
- 233. Huang, S., et al., Inhibition of activated Stat3 reverses drug resistance to chemotherapeutic agents in gastric cancer cells. Cancer Letters, 2012. 315(2): p. 198-205.
- 234. Mizutani, Y., et al., Sensitization of human renal cell carcinoma cells to cisdiamminedichloroplatinum(II) by anti-interleukin 6 monoclonal antibody or antiinterleukin 6 receptor monoclonal antibody. Cancer Res, 1995. 55(3): p. 590-6.
- 235. Alas, S. and B. Bonavida, Inhibition of constitutive STAT3 activity sensitizes resistant non-Hodgkin's lymphoma and multiple myeloma to chemotherapeutic drug-mediated apoptosis. Clin Cancer Res, 2003. 9(1): p. 316-26.
- 236. Liu, C., et al., Inhibition of constitutively active Stat3 reverses enzalutamide resistance in LNCaP derivative prostate cancer cells. Prostate, 2014. 74(2): p. 201-9.
- 237. Devarajan, E. and S. Huang, STAT3 as a central regulator of tumor metastases. Curr Mol Med, 2009. 9(5): p. 626-33.
- 238. Halabi, S., et al., Meta-Analysis Evaluating the Impact of Site of Metastasis on Overall Survival in Men With Castration-Resistant Prostate Cancer. Journal of Clinical Oncology, 2016.

- 239. Akfirat, C., et al., Tumor Cell Survival Mechanisms in Lethal Metastatic Prostate Cancer Differ Between Bone and Soft Tissue Metastases. The Journal of pathology, 2013. 230(3): p. 291-297.
- 240. Lu, Y., et al., Osteoblasts induce prostate cancer proliferation and PSA expression through interleukin-6-mediated activation of the androgen receptor. Clinical & Experimental Metastasis, 2004. 21(5): p. 399-408.
- 241. Culig, Z., Distinguishing indolent from aggressive prostate cancer. Recent Results Cancer Res, 2014. 202: p. 141-7.
- 242. Horinaga, M., et al., Clinical and pathologic significance of activation of signal transducer and activator of transcription 3 in prostate cancer. Urology, 2005. 66(3): p. 671-5.
- 243. Pencik, J., et al., STAT3 regulated ARF expression suppresses prostate cancer metastasis. Nat Commun, 2015. 6.
- 244. Chen, T., L.H. Wang, and W.L. Farrar, Interleukin 6 Activates Androgen Receptormediated Gene Expression through a Signal Transducer and Activator of Transcription 3-dependent Pathway in LNCaP Prostate Cancer Cells. Cancer Research, 2000. 60(8): p. 2132-2135.
- 245. Mahon, K.L., et al., Pathways of chemotherapy resistance in castration-resistant prostate cancer. Endocrine-Related Cancer, 2011. 18(4): p. R103-R123.
- 246. Chou, T.C., Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev, 2006. 58(3): p. 621-81.

VI. Papers

"All of life in its complexity and beauty is forever minted in the gold of words."

[D-503] Yevgeny Zamyatin, We