Diploma Thesis

WILMS TUMOUR GENE IN HAEMATOONCOLOGY OF THE YOUNG - A DENTRITIC CELL THERAPY APPROACH

SUBMITTED BY:

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TO BE AWARDED THE DEGREE:

DOCTOR MEDICINAE UNIVERSAE

(DR. MED. UNIV.)

AT THE

MEDICAL UNIVERSITY OF GRAZ

CARRIED OUT IN

INSTITUTE OF PATHOPHYSIOLOGY AND IMMUNOLOGY

UNDER THE KIND SUPERVISON OF

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Graz, 17.04.2018

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I declare that this diploma is on my own account, based upon work actually carried out by me, and that all sources of material have been clearly indicated. Furthermore, I confirm that no part of the work incorporated in the diploma is a quotation from published or unpublished sources, except where this has been clearly acknowledged as such, and that any specific direction or advice received is also properly acknowledgment.

Graz, 17.04.18 *Benedikt Ferch eh*

Acknowledgment

At this point I would like to thank all the people who motivated and supported me during the preparation of the diploma thesis:
Above all, I would like to thank Univ.-Ass. Mag. Dr. Nassim Ghaffari Tabrizi-Wizsy, who has agreed to take over the supervision of my thesis. During the whole process of finding a suitable thesis-topic, searching for literature and writing this thesis she was helpful to me. Furthermore, my thanks go to Univ.-Prof. Dr. med.univ. Herbert Strobl, who was willing to take over the second supervision of the thesis. I also owe a thanks to all the people, who were involved in prove reading my writings. Last but not least, special thanks go to my parents, who made my studies possible with their support.

Zusammenfassung

Hämato-onkologische Erkrankungen sind die häufigsten malignen Erkrankungen bei jungen Menschen und Krebs ist die zweithäufigste Todesursache bei europäischen Kindern. In den letzten Jahrzehnten wurden hocheffiziente Therapien für hämato-onkologische Erkrankungen entwickelt. Einige dieser Krebsarten zeigen jedoch immer noch eine schlechte Prognose und die Behandlung ist oft mit einer hohen Toxizität verbunden. Dies erfordert die Entwicklung neuerer, sicherer und effektiverer Therapien. Es gibt viele immunologische Ansatzpunkte, um Krebs anzugreifen; Einer zielt auf tumorassoziierte Antigene (TAA). Von 75 TAAs wurde das Wilms Tumor 1 (WT1) Gen-Produkt mit der höchsten Priorität eingestuft. WT1 ist ein Transkriptionsfaktor, der hauptsächlich in der fetalen Nephrogenese und Hämatopoese aktiv ist. Es wurde gezeigt, dass WT1 in vielen Tumoren einschließlich Lymphomen und Leukämien hochreguliert ist. WT1-mRNA kann sogar als Tumormarker bei einigen Leukämien eingesetzt werden. In-vitro- und Maus-Modelle haben die Möglichkeit gezeigt, WT1 mit Peptid- oder Dendritischen Zell-(DC)-basierten Krebsimpfstoffen anzugreifen. Klinische Studien mit diesen neuartigen Therapieschemata haben die Möglichkeit gezeigt, die Bildung von WT1-spezifischen zytotoxischen T-Lymphozyten (CTLs) anzuregen und klinische Wirkungen, wie die Abnahme von Tumorgröße, Blasten oder Tumormarker wie WT1-mRNA, wurden beobachtet. Bemerkenswerterweise konnte bei einigen PatientInnen mit minimaler Resterkrankung (MRD) eine lang anhaltende Remission nur durch den Einsatz Peptid- oder DC-Impfung erreicht werden. Mit der Ausnahme von lokalen Erythemen, scheinen diese Therapien für die meisten PatientInnen sicher zu sein. Nur bei PatientInnen mit Myelodysplastischem Syndrom (MDS) wurde eine Leukozytopenie beobachtet. Nichtsdestotrotz gibt es immer noch große Nachteile. Peptidimpfungen wurden nur für bestimmte HLA-Typen entwickelt. Dies könnte durch die Erzeugung neuer WT1-verwandter Peptide oder durch die Verwendung von DCs überwunden werden, aber es ist nicht klar, welcher DC-Subtyp für eine therapeutische Verwendung am besten geeignet ist. Darüber hinaus zeigen einige klinische Studien eine Diskrepanz zwischen immunologischem und klinischem Ansprechen. Zusammenfassend scheinen diese neuartigen therapeutischen Ansätze vielversprechend zu sein. Wenn sie optimiert werden können, könnten sie zu einem Durchbruch in der Behandlung von hämatoonkologischen Erkrankungen

von Kindern sowie Erwachsenen führen. Krebsimpfstoffe gegen WT1 haben bereits einige PatientInnen geheilt und scheinen meist sicher zu sein. Nichtsdestoweniger sind noch große Hindernisse zu überwinden und die wahre Stärke dieser Therapien für ein breites Spektrum an PatientInnen könnte nur nach großen klinischen Phase-III-Studien beurteilt werden.

Abstract

Haemato-oncological diseases are the most common malignant diseases in the young and cancer is the second most common reason for death in European children. Over the last decades highly efficient therapies for haemato-oncolgical diseases have been developed. However, some of these cancers still show poor prognosis and the treatment often comes with high toxicity. Both these facts call for development of new, safer and more effective therapies. There are several immunological strategies to target cancer; one is targeting tumor-associated antigens (TAA). Out of 75 TAAs Wilms Tumor 1 (WT1) gene product was ranked of highest priority. WT1 is a transcription factor that is mainly active in fetal nephrogenesis and haematopoiesis. It has been shown up-regulated in many tumours, including lymphomas and leukaemias. WT1-mRNA even can be used as tumour marker in some leukaemias. In vitro- and murine-models have shown the possibility to target WT1 using peptide- or dendritic cell-(DC)-based cancer vaccines. Clinical studies with these novel therapy regimes have shown the possibility to trigger generation of WT1 specific Cytotoxic T-Lymphocytes (CTLs) and clinical response, like reduction of tumor size, blast cells or tumor markers such as WT1-mRNA. Remarkably, in a few patients with minimal-residual disease (MRD) long lasting remission could be achieved only due to peptide- or DC-vaccination. Additionally, except from local erythema these therapies seem save for most patients. Only in some patients with myelodysplastic syndrome (MDS) leukocytopenia has been observed. Nonetheless, there are still huge drawbacks to over come. Peptide vaccinations have been developed only for some HLA-types. This could be overcome by generation of new WT1-related peptides or by use of DCs, but jet it is not clear which DC-subtype is most suitable for therapeutic use. Furthermore, some clinical trials show a discrepancy between immunological response and clinical response. In conclusion, these novel therapeutic approaches seem very promising. If they can be optimised, they might become a breakthrough in treatment in hemato-oncological diseases of children as well as adults. Cancer vaccines against WT1 already cured a few patients and seem to be mostly safe. Nonetheless, huge obstacles remain to over come and the true power of these therapies for a broad spectrum of patients could only be estimated after big clinical phase III trials.

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Glossary and abbreviations

ALL	Acute lymphoblastic leukaemia
AMH	Anti-Müllerian hormone
AML	Acute myelogenous leukaemia
APC	Antigen presenting cell
ATP	Adenosine triphosphate
BCG	Bacillus Calemette-Guérin
Bcl-2	B-cell lymphoma 2 oncogene
c-Myc	Myelocytomatosis oncogene
CD	Cluster of differentiation
Cdh1	Epithelial cadherin
CLL	Chronic lymphoblastic leukaemia
CML	Chronic myelogenous leukaemia
CRT	Calreticulin
CTL	Cytotoxic T-Lymphocyt
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
DNA	Deoxyribonucleic acid
DNAX1	DNAX accessory molecule 1
EBV	Ebstein-Barr-Virus
EGF-R	Epidermal growth factor receptor
EMT	Epidermal-mesenchymal transition
ER	Endoplasmic reticulum
FasL	Fas antigen ligand
FoxP3	Forkhead box protein 3
GM-CSF	Granulocyte-macrophages colony-stimulating factor
GvL	Graft versus leukaemia
HLA	Human-leukocyte-antigen
HMGB1	High-Mobility-Group-Protein B1
HPV	Human papilloma virus
HSCT	Haematopoietic stem cell transplantation
HSP70	Heat shock protein 70
HTERT	Human telomerase reverse transcriptase
IDO	Indoleamine 2,3-dioxygenase
IFN	Interferon
IGF-2	Insulin-like growth factor 2
IL	Interleukin
LC	Langerhans Cell
MAB	Monoclonal antibody
MAC	Membrane attack complex
MAGE-A3	Melanoma antigen A3
MALT	Mucosa-associated lymphoid tissue
MHC	Major-histocompatibility-complex
MPL	Monophosphoryl lipid A
MRD	Minimal residual disease

mRNA	Messenger ribonucleic acid
Nf	Nuclear factor
NK cell	Natural killer cell
NKT cell	Natural Killer T cell
p53	p53 tumour suppressor gene
PAP	Prostatic acid phosphatase
PD-1	Programmed Cell Death 1
PGE2	Prostaglandin E2
PR-1	Pathogenesis-related protein 1
PRAME	Preferentially expressed antigen in melanoma
PRR	Pattern recognition receptor
SOX9	Sex-determining region box 9
STING	Stimulator of interferon genes
Survin	Tumour suppressor gene survivin
TAA	Tumor-associated-antigen
TGF-β	Transforming growth factor-β
TH	T-helper cell
TLR4	Toll-like receptor 4
TRAIL	TNF-related apoptosis-inducing ligand
VEGF	Vascular endothelial growth factor
WAGR	Wilms tumor, aniridia, genitourinary abnormally & mental retardation
WT1	Wilms Tumor 1

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1 Introduction

Since the first development of cancer therapies in the second half of the 20th century the mortality of cancers dropped dramatically. Overall, the 5-years-survival of cancer patients increased from 50% (1970s) to over 65% (2014). (Anon 2014) This progress is due to constant improvements of methods in prevention and screening, which are leading to early detection of cancer and due to the development of innovative treatments. Nowadays there are a variety of therapy options including: Surgery, chemotherapy, radiotherapy, hormone therapy, small molecule drugs & immune therapy.

In my thesis I will focus on Wilms Tumour 1 (WT1) gene product as a target for dendritic cell-based immune therapy, which is an innovative and promising approach for treatment of haemato-oncological diseases in children. I will provide general background information behind this therapy approach before focusing on its status in today's clinical research.

2 Haemato-oncological diseases in Children – State of the art

Oncological diseases account for 1% of all diseases in children and adolescents. With the exception of infants under one year, cancer is the second most common cause for death of children in Europe. (Tallen & Grüneberg 2015) Oncological diseases can be divided into solid tumours and haematological tumours. In respect to their prevalence, their occurrence varies from age and gender. Including all children, 55% of cancers account for solid tumours and 45% account for haematological cancers, which consist of leukaemia and lymphomas. Leukaemias account for 31%, lymphomas for 14% of all cancers. (Figure 1) (Tallen & Grüneberg 2015) This Chapter provides an overview on haemato-oncological diseases in children.

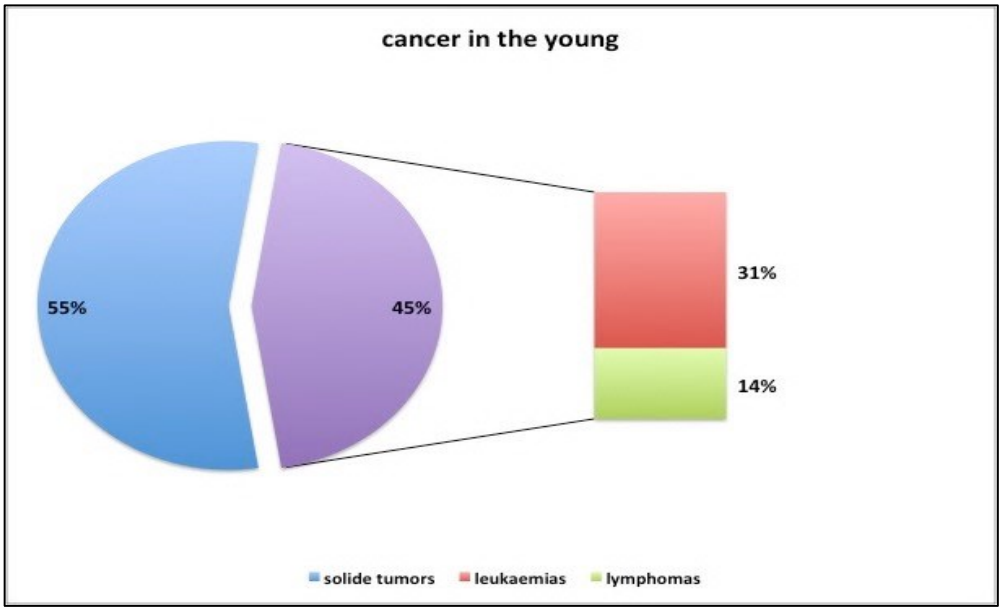

Figure 1 distribution of cancers in paediatric patients (data acquired from: (Gadner et al. 2006))

2.1 Classification

Haemato-oncological diseases can be divided into leukaemias and lymphomas. Leukaemias may be further classified in two ways: (A) by the cell line the mutated cell derived from as lymphoblastic or myelogenous or (B) by their behaviour and progression into acute or chronic. Therefore, four different groups of leukaemias are to be distinguished:

- Acute lymphoblastic leukaemia (ALL)
- Acute myelogenous leukaemia (AML)
- Chronic lymphoblastic leukaemia (CLL)
- Chronic myelogenous leukaemia (CML)

In children and amongst all types of leukaemias, ALL accounts the biggest part with 82,3% and is also the most common cancer. AML accounts for 15%, whereas chronic leukaemias are rare. Still, these percentages vary from age to age. Due to the fact that some early progenitor cells do not express specific characteristics of the cell line, the classification may be challenging. Therefore, some leukaemias cannot be classified as lymphoblastic or myelogenous. (Figure 2) Leukaemias can further be classified by the differentiation-stage the mutated progenitor cell has derived from. (Gadner et al. 2006)

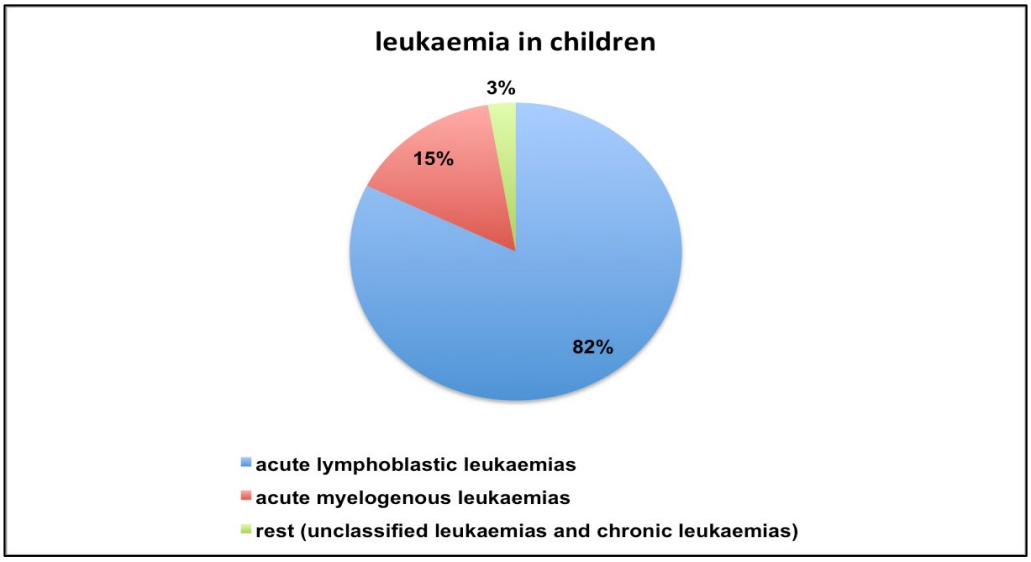

Figure 2 distribution of leukaemia in children (data acquired from: (Gadner et al. 2006))

Lymphomas are most commonly classified as either Hodgkin- or Non-Hodgkin-Lymphomas, occurring in children in 41% and 51% respectively. Additionally, 7% allot to Burkitt-Lymphomas, which are seen as a separate group. (Figure 3) (Gadner et al. 2006) (Figure 3) (Gadner et al. 2006)

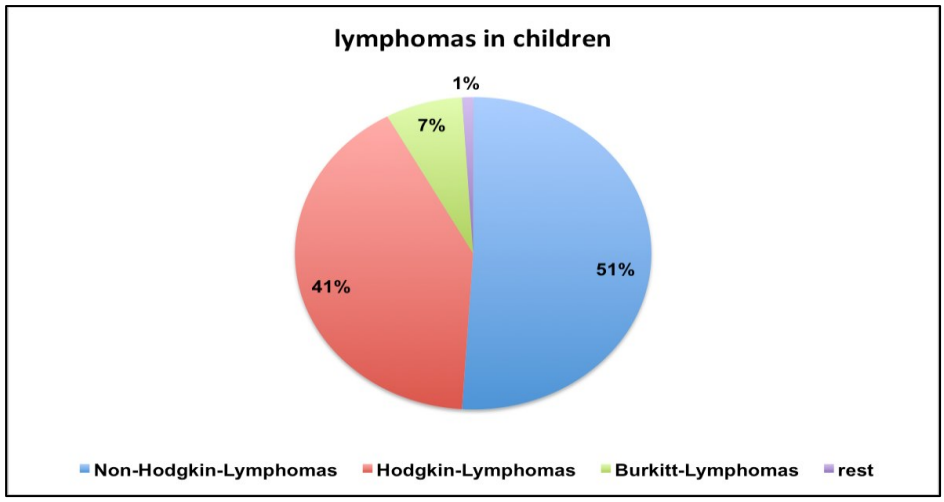

Figure 3 distribution of lymphomas in children (data acquired from: (Gadner et al. 2006))

2.2 Aetiology & Pathogenesis

The cause of Leukaemia and Lymphoma in children still remains largely unclear. Only very few risk factors have been surely identified. (Greaves 1997) In aetiology of Lymphomas, infections seem to be the most important factor and could also play a part in the development of leukaemia. (Gadner et al. 2006) Epstein-Barr-

Virus (EBV) RNA can be recognized in some Hodgkin- and Non-Hodgkin-Lymphomas. (Karajannis et al. 2003) One subtype of Burkitt-Lymphoma, the endemic Burkitt-Lymphoma of Central Africa, even shows 100% positivity for EBV-RNA. (Burkitt 1970) Helicobacter Pylori is strongly associated with development of mucosa-associated lymphoid tissue (MALT)-Lymphoma in the bowel. (Du 2007)

Ionizing radiation in high doses has been shown able to cause ALL or AML. (Bhatia & Robison 1999; Löning et al. 2000) Children suffering from hereditary syndromes like Fanconi-Anaemia, Neurofibromatosis & Bloom-Syndrome are at higher risk of ALL & especially of AML. (Li & Bader 1993) Children with Trisomy 21 show a 14-20 times higher risk for leukaemia. (Creutzig et al. 1996; Hasle 2001) Monozygotic twin of AML or ALL patients show a higher risk to develop leukaemia in contrast to non-monozygotic siblings, which do not share higher risk. The exposition of Nitrosamines and Benzoyl either intrauterine or after birth, may also be of aetiological importance. (Gadner et al. 2006) Many other risk factors may come to account. The mentioned risk factors show the most consistent data.

During the development of lymphatic system, on the one hand genes that enable the lymphatic progenitors to migrate to their determinate destination are active, and on the other hand the cells have a very high proliferation rate; both factors seem to result in a high vulnerability of the genome, which could be the explanation for the relatively high prevalence of lymphatic cancers in children. (Gadner et al. 2006)

In ALL some mutations can already be found in utero or in healthy monozygotic twins of diseased children. In blood samples taken at time of birth TEL-AML1- and MLL-AF4-fusiongenes could be detected in some patients, who later developed leukaemia; suggesting that a second event - also called “second hit” - is needed to start the disease. (Gale et al. 1997; Ford et al. 1998; Alexander et al. 2001)

In Reed-Sternberg-Cells, which is the typical cell of Hodgkin-Disease and account on average for 1% of the tumour mass, activity of Nf**κ**B, which can inhibit apoptosis, could be detected. (See also chapter 7.2.1). (Bargou et al. 1997)

2.3 Diagnosis

It is indispensable to pathologically analyse the bone marrow or a biopsy of lymph node to clarify the diagnosis of a haemato-malignant disease in children with

suspicious symptoms. Symptoms that can be part of leukaemia may include abnormal fatigue, anaemia, fever, susceptibility to infection, swollen lymph nodes, tendency to bleed, which can result in petechial bleeding and haematoma, stomach pain, joint and bone pain, headache and swollen testis. (Yiallouros 2015a) Using haematoxylin and eosin an aspirate of bone marrow or a biopsy of a suspicious lymph node are stained and then micrographically evaluated. (See figure 4 & 5)

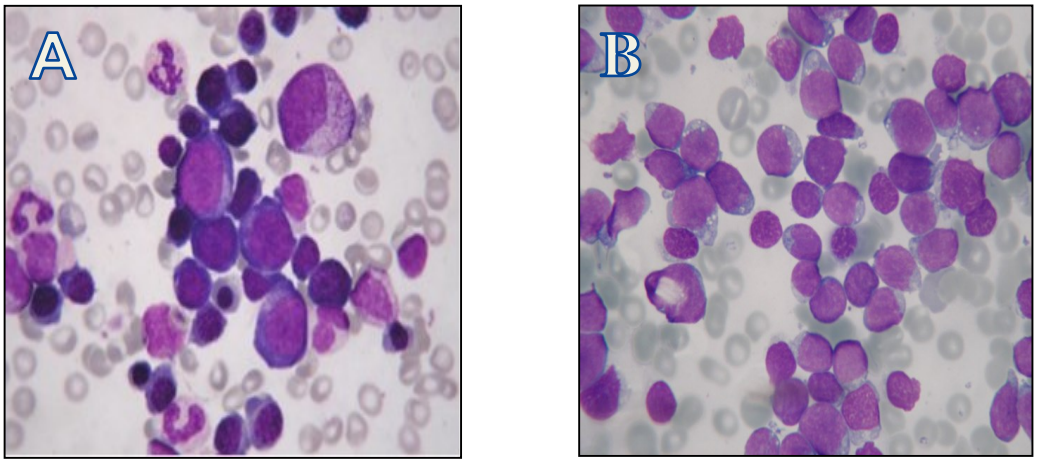

Figure 4 Light micrographics of bone marrows. *A* Healthy Bone Marrow with diverse cells and progenitors. *B* ALL diseased bone marrow, only one clone of progenitor cell is visible (with kind permission of Hämatologie Labor LKH Salzburg)

Lymphomas often come with very general symptoms like fever, night sweat, loss of weight and pruritus. More specific symptoms include swollen, pain-free and matted Lymph nodes. Most of the time they are located either at the head, neck, armpit or groin. Other Symptoms include anaemia, petechial bleeding, haematoma, stomach pain, headache, joint and bone pain. (Yiallouros 2015b) Reed-Sternberg cell are the typical cells of Hodgkin-Disease. (Figure 5)

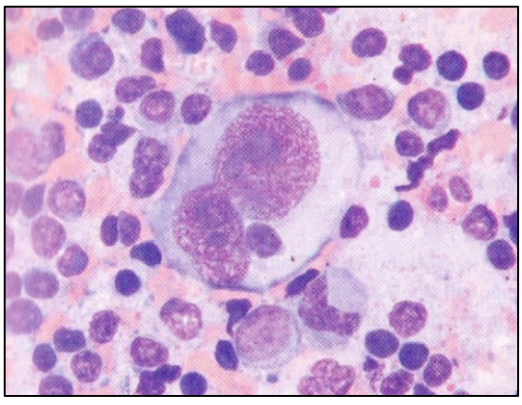

Figure 5 Light micrograph of Lymph node. Sternberg-Reed Cell - big cell with multiple cores and visible nucleoli (Gadner et al. 2006)

2.4 Therapy

Cancer therapy generally can include surgery, radiation, hormone-therapy, chemotherapy, small molecule drugs and immunotherapy.

In current therapy of haemato-oncological diseases in children there is barely use of surgery, merely to obtain biopsy material in lymphomas, but it remains doubtful whether there is an advantage in radically resecting localized lymphomas. (Gadner et al. 2006)

Radiation is used in therapy of leukaemia as well as of lymphoma. In leukaemia full body radiation is used before performing bone marrow transplantation. Head radiation in patients with invasion of tumour cells in central nervous system is used in AML and sometimes in ALL. However, due to its long-term side effects data for benefit of head radiation in leukaemia and especially in ALL is not totally compelling. Therefore, other methods like intrathecal injection of medication like Methotrexate may be more suitable in these cases. (Gadner et al. 2006) In lymphoma the use of radiation is dependent on the stage, response to treatment and subtype of lymphoma. In Non-Hodgkin-Lymphomas the benefit of mediastinal radiation, which brings a variety of risks during development like coronary aneurisms, cardiomyopathy, secondary AML or breast cancer, is not totally clear. (Gadner et al. 2006) Especially in localized early stage Non-Hodgkin-Lymphomas the disadvantages seem to outweigh the advantages. (Link et al. 1990) In Hodgkin-Lymphomas the data suggest that only in very few early stages and only if chemotherapy achieves full remission, patients should not undergo radiation. In all other Hodgkin-Lymphomas it is indicated to radiate the lymphoma with the use of so-called “involved-field-radiation”. (Gadner et al. 2006) (Dörffel et al. 2003)

Chemotherapy is the most important part of therapy in all haemato-oncological diseases. However, the strategy and therapy scheme vary quite differently in these diseases. For instance, both ALL and AML need to be treated with long exposure to chemotherapy. Therefore, the whole therapy of ALL and AML can last up to two years. In this time different intensity of chemotherapy is performed. However, generally speaking AML needs more intense therapy than ALL. (Gadner et al. 2006) Lymphoblastic lymphomas should generally be treated like ALL with long exposition to chemotherapy, whereas non-lymphoblastic lymphomas like the Burkitt-Lymphoma, which has an extremely high growth rate, should be exposed

to chemotherapy in very short and high dosed manner. Nearly all lymphomas should receive a combination of chemotherapy and radiation. (Gadner et al. 2006)
Tyrosine Kinase Inhibitors are small molecule drugs, which are used to treat CML in adults. Philadelphia-Chromosome, a translocation between chromosome 9 and 22, is the typical mutation in CML. This mutation leads to formation of fusion-gen BCR-ABL, which encodes a Tyrosine Kinase Receptor. (O'Brien et al. 2003) In some children development of acute leukaemia also underlies the Philadelphia-Chromosome. In these patients additional use of Tyrosine Kinase Inhibitors to chemotherapy has shown a more favourable outcome. (Schultz et al. 2009) (Burke et al. 2008)
Immune therapy is mainly used in ALL and AML in form of bone marrow transplantation. During this process the patient's bone marrow is completely destroyed through chemo- and radiotherapy, then bone marrow of a suitable donor is implanted. The new bone marrow should fulfil two tasks: Firstly, replacing the destroyed bone marrow and overtaking all its physiological tasks; Secondly, detecting remaining leukaemia cells and attacking them, which is called graft-vs-leukaemia and is the actual immunological principal behind this treatment. Bone marrow transplantation bares high risks therefore only relapses of leukaemia and lymphomas or leukaemias with poor prognosis should be treated this way. (Gadner et al. 2006)
This thesis is focused on immunological therapy. Beside bone marrow transplant no immunotherapies have been approved for leukaemias or lymphomas. However, in other types of cancers immunotherapies have been developed. In the next chapter the most important approaches in cancer immunotherapy will be outlined.

3 Immunotherapy in cancer treatment

There are several approaches of cancer treatment based on immunological mechanisms, some of which are already used in daily clinical practice. Generally speaking, immune therapies can be classified as active or passive immunotherapy.

3.1 Passive Immune Therapy

Immunotherapy is classified as passive if it does not initiate a response in the host's immune system. Mechanisms behind passive immune therapies are:

- Adoptive cell transfer
- Oncolytic viruses
- Non-immune stimulating monoclonal antibodies (MABs).

Adoptive cell transfer is performed by collecting lymphocytes, modelling them ex vivo and re-infusing them to the patient.

Oncolytic viruses infect tumour cells and unfold their cytotoxic potential in them; both principals currently play a minor role in the clinical treatment of cancer, but may have an impact in future. (Galluzzi, Vacchelli, Bravo-San Pedro, et al. 2014)

Of most interest are MABs. There are already several clinical approved uses of MABs in cancer treatment. These MABs either attack the neoplasm directly by targeting tumour-associated-antigens or indirectly by targeting various growth factors. Table 1 provides examples of targets for MABs currently in use. (Scott et al. 2012) (Galluzzi, Vacchelli, Bravo-San Pedro, et al. 2014)

Table 1 Targets for monoclonal Antibodies. (Scott et al. 2012)

Antigen category	Examples of antigens	Tumor types expressing antigen
Cluster of differentiation (CD) antigens	CD20	non-Hodgkin lymphoma
	CD30	Hodgkin lymphoma
	CD33	Acute myelogenous leukemia
	CD52	Chronic lymphocytic leukemia
Glycoproteins	EpCAM	Epithelial tumors (breast, colon, lung)
	CEA	Epithelial tumors (breast, colon, lung)
	gpA33	Colorectal carcinoma
	Mucins	Epithelial tumors (breast, colon, lung, ovarian)
	TAG-72	Epithelial tumors (breast, colon, lung)
	Carbonic anhydrase IX	Renal cell carcinoma
	PSMA	Prostate carcinoma
	Folate binding protein	Ovarian tumors
Glycolipids	Gangliosides (e.g., GD2, GD3, GM2)	Neuroectodermal tumors, some epithelial tumors
Carbohydrates	Lewis-Y[2]	Epithelial tumors (breast, colon, lung, prostate)
Vascular targets	VEGF	Tumor vasculature
	VEGFR	Epithelium-derived solid tumors
	αVβ3	Tumor vasculature
	α5β1	Tumor vasculature
Growth factors	ErbB1/EGFR	Glioma, lung, breast, colon, head and neck tumors
	ErbB2/HER2	Breast, colon, lung, ovarian, prostate tumors
	ErbB3	Breast, colon, lung, ovarian, prostate tumors
	c-MET	Epithelial tumors (breast, ovary, lung)
	IGF1R	Lung, breast, head and neck, prostate, thyroid, glioma
	EphA3	Lung, kidney, colon, melanoma, glioma, hematological malignancies
	TRAIL-R1, TRAIL-R2	Solid tumors (colon, lung, pancreas) and hematological malignancies
	RANKL	Prostate cancer and bone metastases
Stromal and extracellular matrix antigens	FAP	Epithelial tumors (colon, breast, lung, head and neck, pancreas)
	Tenascin	Glioma, epithelial tumors (breast, prostate)

3.2 Active immune therapy

Mechanisms behind active immunotherapeutic approaches are:

- Immune-stimulatory cytokines
- Immune-modulatory MABs
- Inhibitors of immunosuppressive metabolism
- Pattern recognition receptor (PRR) agonists
- Vaccination of peptides, DNA or antigen presenting cells (APCs).

There are already several immune-stimulatory cytokines in use. Most of them are used as an adjuvant to other cancer therapies. Notable cytokines in use include interferon-(IFN)-α2b and IFN-α2a, Interleukin (IL)-2, tumour necrosis factor α (TNFα) and granulocyte-colony stimulating factor (G-CSF). (Galluzzi, Vacchelli, Pedro, et al. 2014)

Immune-modulatory MABs are also used in cancer treatment. Ipilimumab for instance targets the cytotoxic T-lymphocyte-associated protein 4 (CTLA4), which usually inactivates cytotoxic T-cells. Pembrolizumab, approved in 2014 in the US for advanced unrespectable melanoma, targets the programmed cell death 1 (PD-1), which usually can trigger apoptosis in T-lymphocytes. Hence, blocking the function of CTLA4 or PD-1 results in higher levels of activated T-lymphocytes. (Scott et al. 2012) (Galluzzi, Vacchelli, Pedro, et al. 2014)

Ways to block immunosuppressive metabolism are being researched, but yet there are no medications approved. (Galluzzi, Vacchelli, Pedro, et al. 2014)

Pattern recognition receptors (PRRs) ignite pathways that can mediate the activation of nuclear factor κB (NF-κB), secretion of cytokines and maturation of dendritic cells DC. For instance bacillus Calemette-Guérin (BCG) is used against bladder carcinomas. Monophosphoryl lipid A (MPL) mediates a better response to vaccine against Human Papilloma Virus (HPV) 16 & 18, which are the main cause of cervical carcinomas. Other PPRs in use are imidazoquinoline and picibanil. (Galluzzi, Vacchelli, Pedro, et al. 2014)

While there are no approved vaccinations of peptides, the use of DC-based vaccine for treatment in prostate cancer, namely Sipuleucel-T, has been approved in the US since 2010. (Galluzzi, Vacchelli, Pedro, et al. 2014) The use of DCs seems to be a promising approach in cancer treatment and there seem to be many possible tumour antigens to target, one of high interest is the Wilms Tumour

1 (WT1) gene-product. Figure 6 provides an overview on the above-mentioned cancer immune therapies.

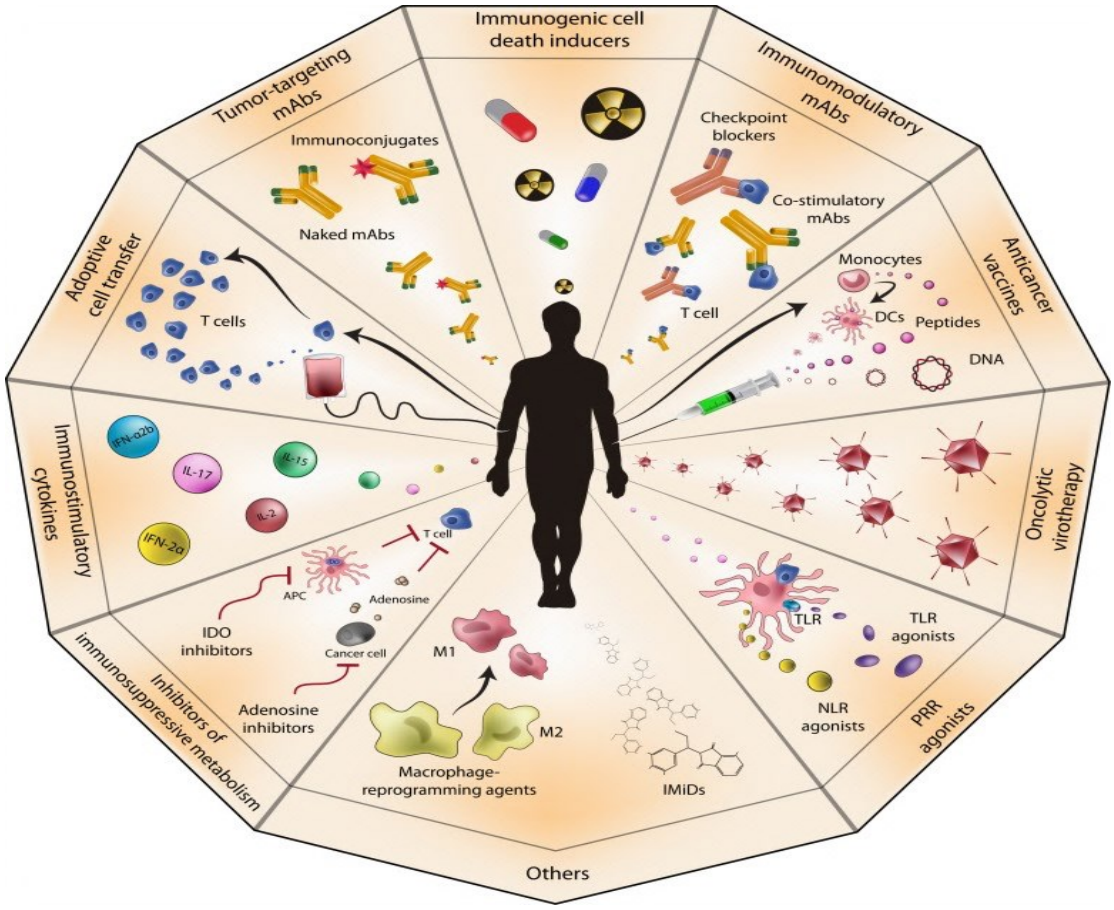

Figure 6 Immune therapies for cancer. Including immune-modulatory and tumour-targeting monoclonal antibodies, adoptive cell transfer, immune-stimulatory cytokines, inhibitors of immunosuppressive metabolism, pattern recognizing receptors and vaccination of peptides, DNA or Dendritic Cells. (Galluzzi, Vacchelli, Bravo-San Pedro, et al. 2014)

This thesis investigates WT1 gene-product as a target for DC-based therapy in haemato-oncological diseases of children and adolescents. To understand the principal of this approach, basic understanding of cancer immunology is needed and will be provided in the next chapter.

4 Cancer immunology – theoretical background

Generally speaking, cancer immunology can be divided into three phases: Elimination, Equilibrium and Escape. (Figure 7) (Dunn et al. 2002)

During elimination process, tumour surveillance by the host's immune system occurs. In equilibrium process, the evolutionary pressure of the immune system selects only the most evasive cells. These evolutionary changes can happen quickly because of the high mitotic rate and the genetic instability. During escape

phase, tumour cells that survived the equilibrium process begin to expand. (Abbas et al. 2015) (Dunn et al. 2002) (Kim et al. 2007)

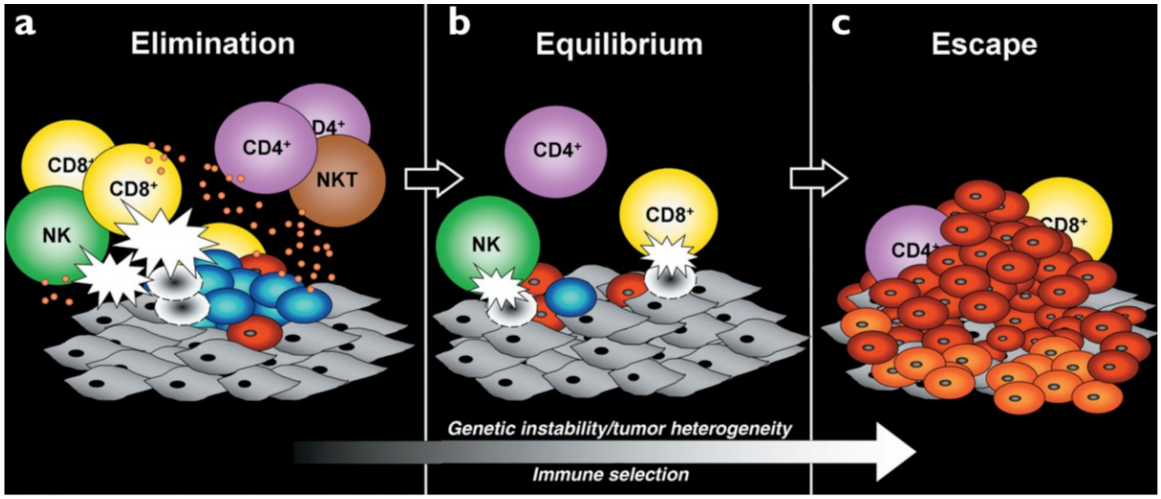

Figure 7: Phases in cancer immunology: a) Elimination. b) Equilibrium. c) Escape (Dunn et al. 2002)

There are multiple complex mechanisms, how immune surveillance and immune escape can occur. The most important are outlined in this chapter.

4.1 Immune surveillance of tumours

4.1.1 Cytotoxic T-Lymphocytes

Many cancers express antigens that can be recognized by T cells. In some patients occurrence of CD8+ Cytotoxic T-Lymphocytes (CTLs) against these antigens can be measured. (Woo et al. 2015) More detailed information on tumour antigens will be provided in chapter 6. CTLs have been shown able to infiltrate tumour mass, recognizing tumour-associated-antigens (TAAs) and killing malignant cells. (Abbas et al. 2015) Tumour microenvironment infiltrated by CTLs show better prognosis than non-infiltrated microenvironment. (Harlin et al. 2009) (Gajewski et al. 2010) CTLs seem to be the most important cells in cancer immune surveillance and the linkage between innate and adaptive immune system - and therefore the generation of anti-tumour CTLs – seems to be of essential role for sufficient tumour control. (Abbas et al. 2015) (Woo et al. 2015)

4.1.2 Natural Killer cells, Natural-Killer T cells & γδ T cells

Natural Killer, Natural Kill T and γδ T cells seem to be especially important in early detection of malignant cells and ignition of immune responses. (Woo et al. 2015)

Natural Killer (NK) cell infiltration of tumour microenvironment can be measured in some cancer patients and shows a more favourable outcome. (Villegas et al. 2002) (Ishigami et al. 2000) Additionally, deletion of NK cells in mouse-models has shown an increased incidence of neoplastic diseases. (Smyth et al. 2000) Furthermore, NK cells express DNAX accessory molecule 1 (DNAX 1) on their cell surface. Ligands of DNAX1 can be found on some tumour cells. (Bottino et al. 2003) (Masson et al. 2001) (Lakshmikanth et al. 2009) Activated NK cells directly kill tumour cells by realising cytotoxic granules or by inducing apoptosis via TNF-related apoptosis-inducing ligand (TRAIL) or via Fas antigen ligand (FasL). (Cheng et al. 2013) Activation of NK cells can be induced due to DNA damage or cellular stress of malignant cells, which results in higher expression of DNAX 1-, NKG2D- and other NK cell activating-ligands. (Gasser et al. 2005) (Soriani et al. 2009) Cytokines like IFNs, IL-12, IL-18 and IL-15 enhance NK cell activation. (Waldhauer & Steinle 2008) Also, NK cells are able to secret IFNs, which induces DC activation and maturation. (Mocikat et al. 2003) (Kelly et al. 2002) Furthermore, NKs are able to detect cells, which have lost type I major histocompatibility complex (MHC) to escape immunological detection. (Abbas et al. 2015) However, in some contexts NK cells may unfold immune regulatory functions. (Crome et al. 2013)

Similarly, Natural Killer T (NKT) cells and γδ T cells seem to be of importance in early tumour immune defence and their absence results in increased tumour rates in murine models. (Smyth et al. 2000) (Kabelitz et al. 2004) Some tumours have been shown to express surface-molecules that can be recognized by NKT cells and γδ T cells. (Wu et al. 2003) (Gober et al. 2003) After activation, both gain cytotoxic abilities, release cytokines, like IFN-γ and TNF-α and promote DC activation. (Matsuda et al. 2008) (Fujii et al. 2003) (Kondo et al. 2008) (Tokuyama et al. 2008) (Do & Min 2009)

4.1.3 Antigen Presenting Cells

Generally speaking, Antigen Presenting Cells (APCs) express class II MHC and are able to stimulate $CD4^+$ helper T cells, which promote the differentiation of $CD8^+$ T cells. The most specialised APCs are the heterogeneous group of

dendritic cells and will be discussed in detail in chapter 5. APCs can process tumour antigens and present them via class I MHC to initiate $CD8^+$ CTLs. This process is called cross presentation and is also used to display antigens of virally infected cells, which is shown in figure 8. (Abbas et al. 2015)

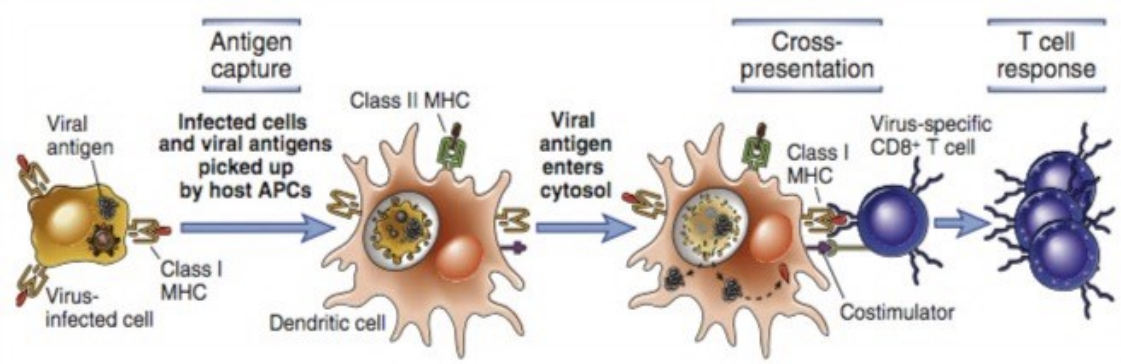

Figure 8 Antigen cross-presentation. Showcased by Dendritic Cell presenting viral antigen. The infected cell is ingested by the DC then processed and displayed via class I MHC to $CD8^+$ T Cell. The T Cell is now able to recognise the viral antigen (Abbas et al. 2015)

Another important mechanism in tumour surveillance is the secretion of type I IFNs by APCs. (Lou et al. 2007) (den Haan et al. 2000)

The activation of APCs seems to be triggered via recognition of damage-associated molecular patterns (DAMPs), like High-Mobility-Group-Protein B1 (HMGB1), Calreticulin (CRT), extracellular ATP and extracellular tumour-DNA. (Woo et al. 2015) (Figure 9)

HMGB1 is released from dying tumour cells and seems to be recognized by Toll-like receptor 4 (TLR4) of APCs. (Apetoh et al. 2007)

CRT is normally resident in the endoplasmic reticulum (ER). In some cancers CRT is translocated to the cell membrane and can be recognized by CD91 of APCs. (Obeid et al. 2007) (Garg et al. 2012) (Gardai et al. 2005)

Extracellular ATP is released from damaged cancer cells and can bind to P2Y2-receptors of APCs. (Elliott et al. 2009)

The uptake of tumour extracellular DNA into APCs and the following immune activation via secretion of type I IFNs is not understood in full detail yet. However, it seems that necrotic or apoptotic tumour cells release DNA in form of vesicles that can be uptake by APCs and trigger type I IFN secretion via stimulator of interferon genes (STING) pathway. (Woo et al. 2015)

Notably, in some patients immune reaction follows after chemotherapy or radiation, which seems to be triggered due to recognition of DAMPs (Obeid et al.

2007) (Mattarollo et al. 2011) (Deng et al. 2014) (Woo et al. 2015) In radiation, this even seems to be curtail for clinical effectives. (Deng et al. 2014)

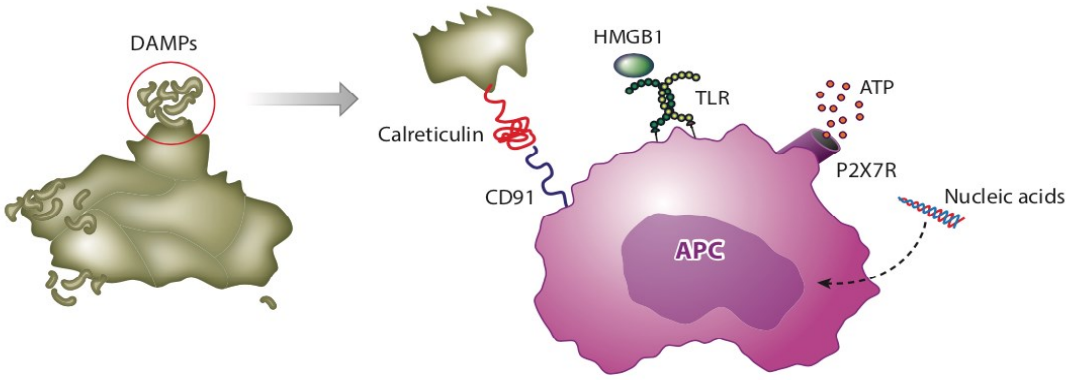

Figure 9 Four types of DAMPs seem to be involved in detection of cancer cells by APCs: Calreticulin, HMGB1, free ATP & free DNA (Woo et al. 2015)

4.1.4 Tumour associated macrophages

Macrophages in tumour environment come in two different phenotypes: M1 and M2. M1 macrophages have high expression IL-12 and low expression of IL-10 and contribute to tumour control. M2 macrophages, on the other hand, have high expression IL-10 and low expression of IL-12 and contribute to tumour growth. (Woo et al. 2015) Overall, clinical studies show that infiltration of macrophages at tumours comes with worse clinical outcome (Bingle et al. 2002) (Ryder et al. 2008) (Zhang et al. 2012) They are able to secret vascular growth factor (VEGF) and transforming growth factor-β (TGF-β). (Abbas et al. 2015) However, M2 macrophages can be switch into M1 macrophages via IFN-y and other cytokines. (Duluc et al. 2009)

4.1.5 Interferon

Type I interferons (IFNs) have been shown to be a crucial stimulator for cross-presentation of $CD8\alpha^+$ DCs to generate $CD8^+$ T cells and for production of IFN-γ in γδ T cells. (Fuertes et al. 2011) Also, type I IFNs contribute to immune surveillance via stimulation of NK cells. (Swann et al. 2007) Thus, type I IFNs seem to be vital for the linkage between innate and adaptive immune response to cancer. (Figure 10) Additionally, there is evidence that type I IFNs can inhibit maturation of tumour-associated macrophages. (U'Ren et al. 2010)

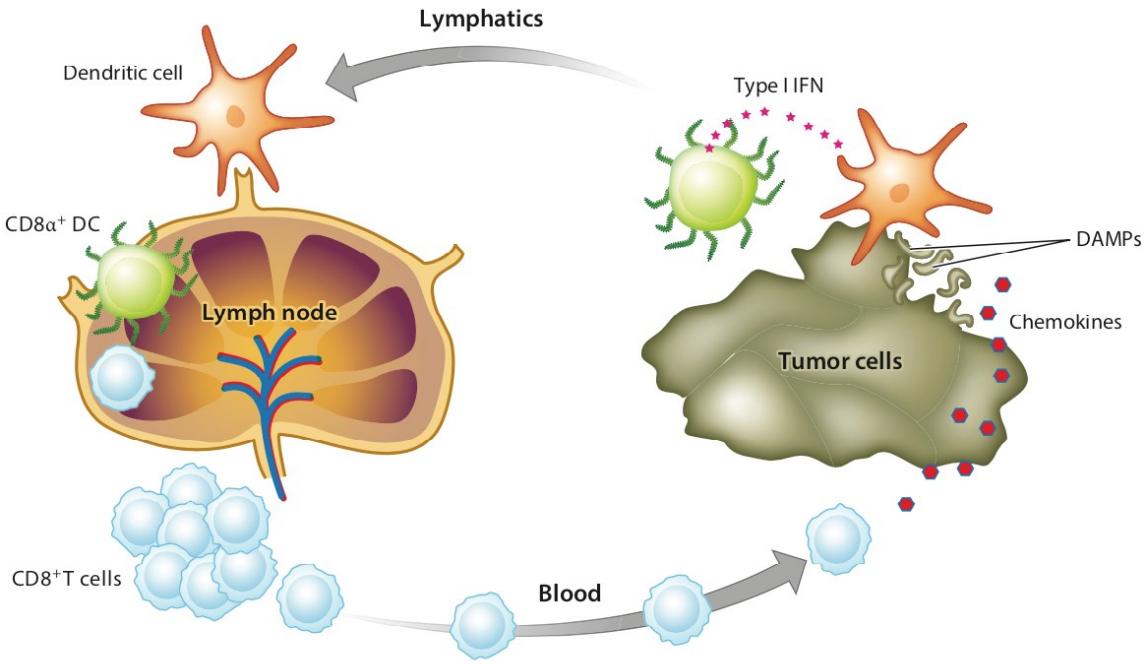

Figure 10: Type I interferons seem vital for linkage between innate and adaptive immunity in reaction to cancer. (Woo et al. 2015)

4.1.6 Complement

The complement system could be shown activated on surface of some cancer cells. (Gasque et al. 1996) (Lucas et al. 1996) (Niculescu et al. 1992) It consists of three different pathways: The alternative, classical and lectin pathway. They have different initial mechanisms, but all result in activation of C3 and therefore, trigger membrane attack complex (MAC). (Woo et al. 2015) However, long-term activation of complement can result in increased growth and metastasis of cancer (Markiewski et al. 2008) (Vadrevu et al. 2014) Some factors of complement system also show immunosuppressive activity. (Sohn et al. 2003) Overall, complement has been shown to promote tumor progression. (Woo et al. 2015)

4.2 Evasion of Immune response by tumours

There are two main principals how tumours evade the immune system: Loss of surface targets and active immunosuppression. (Figure 11)

Many tumours show loss or decreased expression of class I MHC or tumour antigens. Hence, the tumour becomes unrecognisable for CTLs.

Some tumour cells gain the ability to actively supress immune response against them, which seems to be the more important mechanism, because cancer cells

often come with multiple targetable antigens and some of these arise from proteins necessary for tumour progression. (Oka et al. 2007) (Weber et al. 2013) (Woo et al. 2015) There are various mechanisms how immune suppression can take place: Some cancers are able to secret programmed cell death protein-1 (PD-1) and Forkhead box protein 3 (FoxP3), which are ligands for a T cell inhibitory receptor. (Spranger et al. 2013) Blockage of NKG2D receptors via secretion of soluble NKG2D receptor ligands also has been reported. (Raulet et al. 2013) (Groh et al. 2002) Other immune suppressive factors that can be secreted by cancer cells include: Transforming growth factor β (TGF-β), Prostaglandin E2 (PGE2), Indoleamine 2,3-dioxygenase (IDO). (Martinet, Fleury-Cappellesso, et al. 2009) (Martinet, Poupot, et al. 2009) (Abbas et al. 2015)

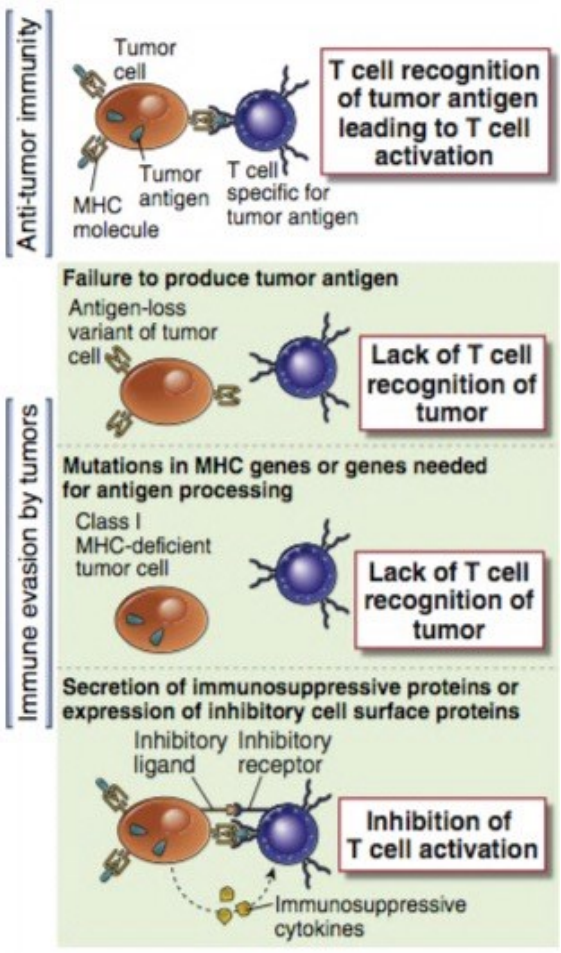

Figure 11 Immune response to tumours & evasion by tumours. Cytotoxic T Cells can recognise and attack tumour cells. Tumours are able to escape immune attack either by loss of recognisable antigens or by actively suppressing the immune response (Abbas et al. 2015)

To overcome immune escape many attractive immunotherapy models are being researched. (Woo et al. 2015) (Galluzzi, Vacchelli, Bravo-San Pedro, et al. 2014)

The linkage between innate and adaptive Immune system seems to vital may be achieved by the use of DC-therapy models.

5 Dendritic Cells

In 1868 Paul Langerhans described the first dendritic cells. Due to their morphology, containing multiple dendritic spines, Langerhans cells (LCs) have been mistaken for neurons. (Figure 12) (Jolles 2002)

It was not until the second half of the 20th century that Ralph Steinman discovered dendritic cells and their function as APCs. (Banchereau & Steinman 1998)

Nowadays, DCs are classified in several subsets. All of them undertake different tasks, which are not all completely understood yet. (Klechevsky 2015)

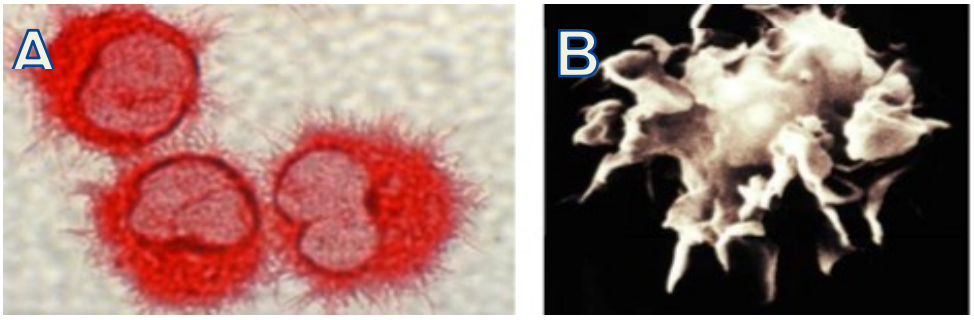

Figure 12 Micrographics of Dendritic Cells. *A* light micrograph. *B* electron micrograph. The dendrites of the cells can be examined in both (Abbas et al. 2015)

5.1 Derivation of DCs

Most of DCs develop from haematopoietic stem cells and share precursors with monocytes. Some can also develop directly from monocytes. Other DCs, like the LCs in the skin, probably develop from completely different embryonic stem cells. (Figure 13) (Abbas et al. 2015)

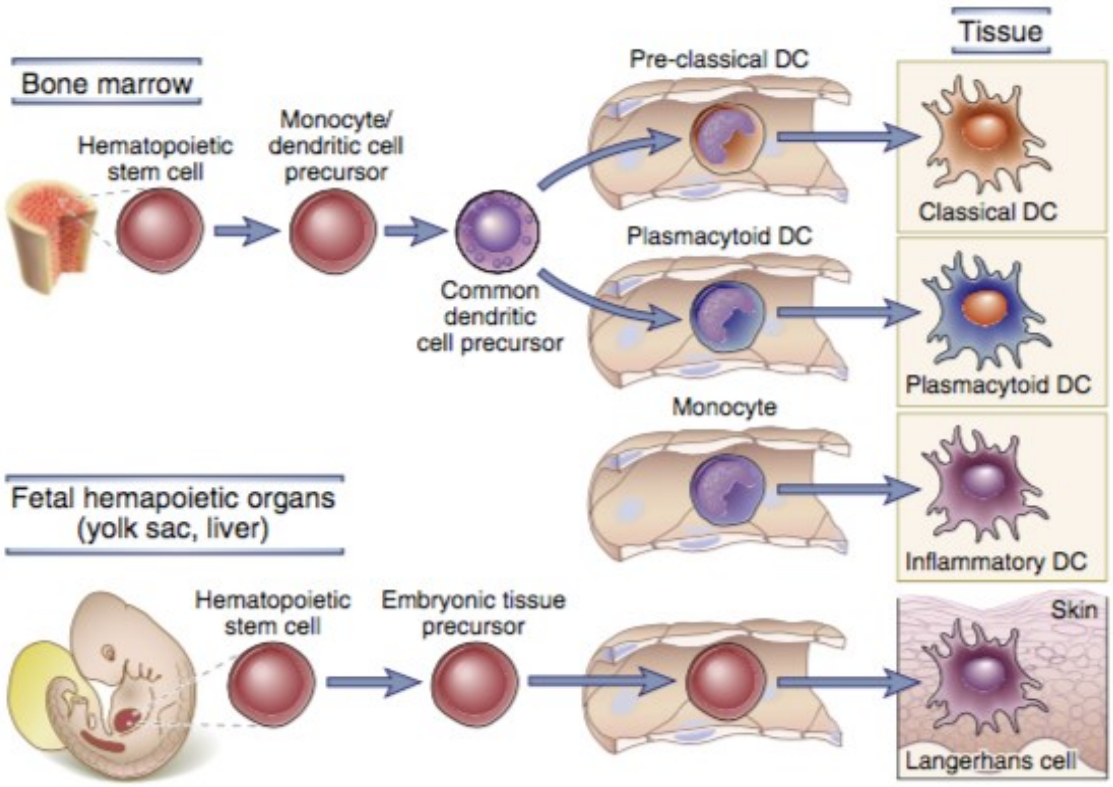

Figure 13 Derivation of dendritic cells. Some DC develop from common myeloid precursor cell into classical DC or plasmacytoid DCs. Inflammatory DCs may develop directly from Monocytes. Langerhans cells may derivate from precursors cells of the fetal yolk sac. (Abbas et al. 2015)

5.2 Function and subsets

Generally speaking, DCs are the most specialised APCs. This heterogeneous group of cells expresses the largest variety of receptors dedicated to detect external antigens. They are highly present in all body parts that are exposed to the environment, such as the skin, gastro intestine & lung. Their dendrites build a dense net. In the intestine some dendrites even reach the lumen. In the skin LCs send dendrites even through tight junctions into the stratum corneum to catch pathogens. (Lüllmann-Rauch & Asan 2015) (Abbas et al. 2015) During infection DCs are capable of phagocytosis, lysing the in-taken pathogen, processing it, leaving their original tissue, migrating to lymph nodes and presenting the processed antigens to lymphocytes via Major-Histocompatibility Complex I & II (MHC I & II) in combination with cofactors like CD80 & CD86. For stimulation via MHC I CD8 is needed as a co-receptor; for stimulation via MHC II CD4 is needed as a co-receptor.

By secretion of a large variety of cytokines, DCs can induce inflammation; moreover, DCs play a crucial role in linking the inherent and the adaptive immune

system. Of note is their contribution to immune tolerance: DCs are able to intake healthy cells from surrounding tissue and present their surface markers. While presenting these markers they are looking for lymphocytes, which react to them; and therefore would be auto aggressive. If one is detected, they induce inactivation or apoptosis in this lymphocyte. During this process they do not produce any inflammatory cytokines, making them an important step in immune tolerance. (Abbas et al. 2015; Klechevsky 2015)

Classical DCs, also called Conventional DCs, are the most common type of DCs. They migrate to lymph nodes where they present antigens to T-Lymphocytes. Depending on the desired effect, they then either induce inflammatory immune response or supress immune response to maintain immune tolerance.

Plasmacytoid DCs morphologically look similar to antibody-producing plasma cells. They are specialized in detecting virally infected cells. When a virus is detected, they secret type I interferons, which have a variety of antiviral functions.

Follicular DCs. This cell type is found in the follicular region of lymph nodes, mucosal lymph follicle and the spleen. They display antigens to B cells, but do not share precursors nor are related to DCs that display antigens to T cells. (Abbas et al. 2015)

5.3 DCs in cancer defence

A vital step in cancer defence underlies the activation of CD8+ Cytotoxic T-Lymphocytes (CTLs). (See also Chapter 4.1.1) To initiate these cells cross-presentation and secretion of type I IFNs are needed. (Woo et al. 2015) Figure 14 provides an overview on DCs in cancer immunology.

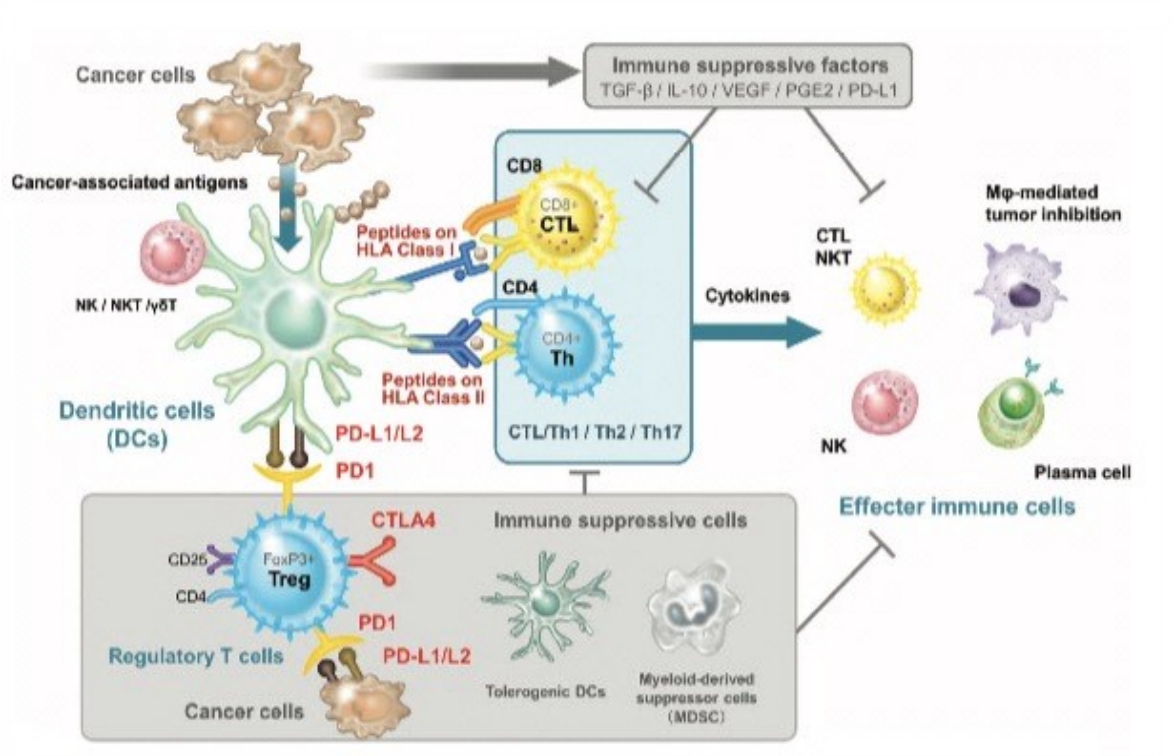

Figure 14 Dendritic Cells in cancer-immunity. Dendritic cell modulates cancer antigen and presents it via class I MHC to CD8+ Cytotoxic T cell and via class II MHC to CD4+ T-helper cell. On the other hand tolerogenic DC can supress immune reaction. (Shimodaira et al. 2016)

Plasmocytoid DCs have been shown to be able to generate anti-tumour response in murine models. (Liu et al. 2008) However, sometimes they can unfold immune regulatory properties, which could be due to immunosuppressive mediators released by the tumour. In some studies infiltration of pDCs in tumour microenvironment seems to have poor prognosis. (Wei et al. 2005) (Labidi-Galy et al. 2012)

CD8α+ DCs, a subtype of classical DCs, are most efficient at phagocytizing dead cells and cross presenting class I MHC-restricted antigens to induce CD8+ T cell reaction. (Woo et al. 2015)

Their essential importance in tumour surveillance has been shown in mouse models with deficient CD8α+ DCs, which lead to no generation of CD8+ T cells and abolished immunological tumour rejection. (Hildner et al. 2008) (Fuertes et al. 2011)

Another important contribution of DCs to immune reaction against cancer is the production of type I IFNs, which has several anti-tumour effects. (See Chapter 4.1.4) Hence, DCs play an important role in the body`s own cancer defence and

have already been used in clinical practice against advanced prostate carcinoma. (See also Chapter 3.2) In this therapy model DCs are primed against the tumour antigen prostatic acid phosphatase (PAP) and stimulated with granulocyte-macrophage colony-stimulating factor (GM-CSF). (Abbas et al. 2015) PAP is only one of many possible targets for DC-based cancer immunotherapy. In the following chapter I will provide a short overview on tumour antigens.

6 Tumour Antigens

The immune system is able detect and attack certain surface patterns of tumours. These so-called tumour antigens can either be tumour-specific or tumour-associated-antigens (TAAs). There are several origins tumour antigens can develop from. On the one hand they can be products of mutated oncogenes or tumour suppressor genes, on the other hand they can also be products of randomly mutated gens that play no role in cancer development. Additionally, they can be abnormally overexpressed normal proteins or proteins, that are normally only expressed during fetal development. Furthermore, tumour antigens can also be products of oncogenic virus and tumours can also express altered surface glycoproteins and glycolipids. Table 2 gives examples of tumour antigens. (Abbas et al. 2015)

Table 2 Examples of tumour antigens (Abbas et al. 2015)

TABLE 18-1 Tumor Antigens	
Type of Antigen	**Examples of Human Tumor Antigens**
Products of mutated oncogenes, tumor suppressor genes	Oncogene products: Ras mutations (~10% of human carcinomas), p210 product of Bcr/Abl rearrangements (CML) Tumor suppressor gene products: mutated p53 (present in ~50% of human tumors)
Unmutated but overexpressed products of oncogenes	HER2/Neu (breast and other carcinomas)
Mutated forms of cellular genes not involved in tumorigenesis	Various mutated proteins in melanomas recognized by CTLs
Products of genes that are silent in most normal tissues	Cancer/testis antigens expressed in melanomas and many carcinomas; normally expressed mainly in the testis and placenta
Normal nononcogenic proteins overexpressed in tumor cells	Tyrosinase, gp100, MART in melanomas (normally expressed in melanocytes)
Products of oncogenic viruses	Papillomavirus E6 and E7 proteins (cervical carcinomas) EBNA-1 protein of EBV (EBV-associated lymphomas, nasopharyngeal carcinoma)
Oncofetal antigens	Carcinoembryonic antigen on many tumors, also expressed in liver and other tissues during inflammation α-Fetoprotein
Glycolipids and glycoproteins	GM_2, GD_2 on melanomas
Differentiation antigens normally present in tissue of origin	Prostate-specific antigen in prostate carcinomas CD20 on B cell lymphomas

CML, chronic myelogenous leukemia; *CTL*, cytotoxic T lymphocyte; *EBNA*, Epstein-Barr nuclear antigen; *EBV*, Epstein-Barr virus; *MART*, melanoma antigen recognized by T cells.

6.1 TAAs as Target for Immune therapy

There is a vast amount of TAAs that could be promising targets for Immunotherapy. In 2009 Cheever et. al analysed 75 TAAs with the purpose to prioritize them. In their study they looked at nine predefined and pre-weighted criteria. They focused on: therapeutic function, immunogenicity, role of antigen in oncogenicity, specificity, expression level and percentage of antigen-positive cells, stem cell expression, number of patients with antigen-positive cancers, number of antigen epitopes, cellular location of antigen expression.

Looking at all criteria, WT1 was ranked of highest priority and will be discussed in the next chapter. (Cheever et al. 2009) Figure 15 shows their ranking.

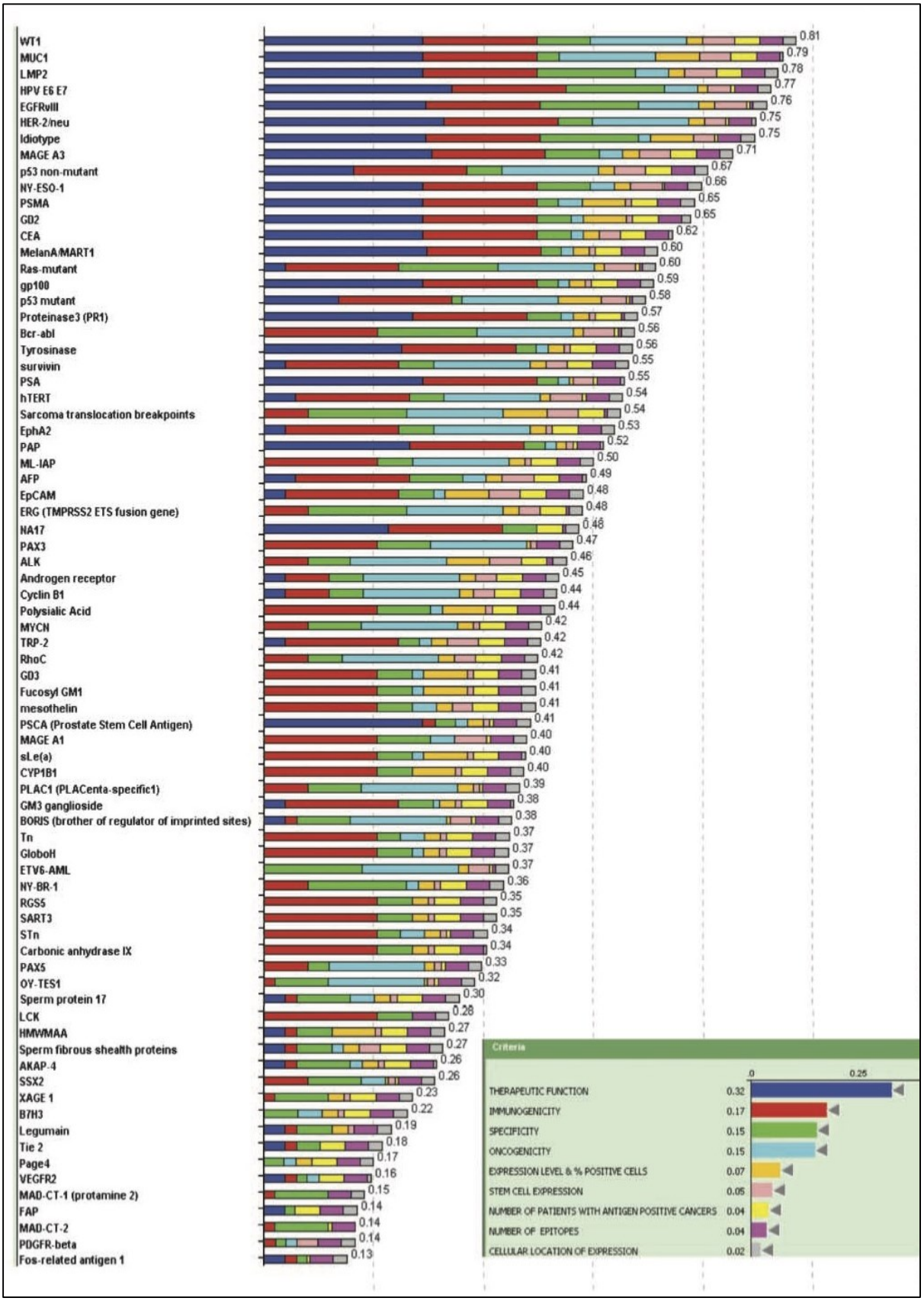

Figure 15 Prioritization of tumour-associated-antigens. (Cheever et al. 2009)

7 Wilms Tumour 1 (WT1)

WT1 is a transcription factor of the zinc-finger family that plays a crucial role during development of the urogenital system. It has also been shown that WT1 contributes to the development of various tumours and therefore may be a suitable target for DC-based Immunotherapy.

7.1 Gene and Structure

Wilms Tumour 1 Protein is encoded in the WT1 gene located at short arm of chromosome 11 and is about 50 kbps long. (Figure 16) (Call et al. 1990)

Figure 16 Location of WT1 on chromosome 11p13 (adapted from National Center for Biotechnology Information (NCBI))

WT1 is encoded by ten exons, leading to up to 24 different isoforms. Most studied are four isoforms, which result from including or excluding exon 5 and parts of exon 9 and 10 during alternative splicing. (Huff 2011) Exon 5 encodes 17 amino acids. The replaceable parts of exon 9 and 10 encode three amino acids (lysine, threonine, serine – KTS). These four isoforms are also labelled: WT1+/+, WT1+/-, WT1-/+, WT1-/-. All isoforms have four zinc finger domains. (Figure 17) (Strauss et al. 2003)

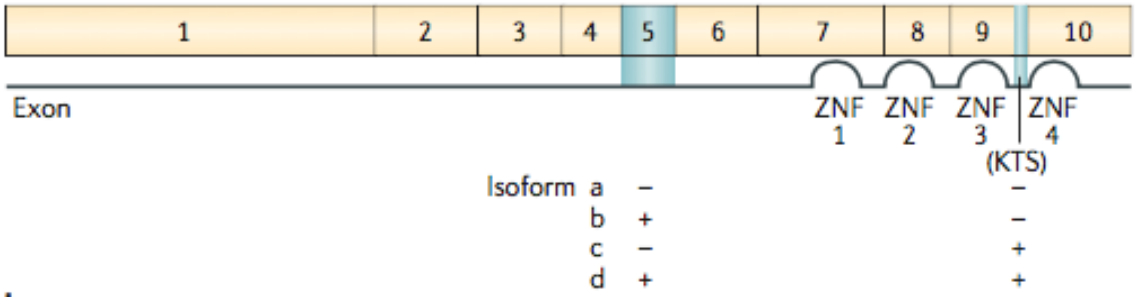

Figure 17 WT1 gene and protein structure. Alternative spliced domains are highlited blue, which results in four possible isoforms. (Huff 2011)

Zinc finger domains are loop shaped extensions, stabilized by four amino acid residues binding a zinc ion and are able to interact with DNA. (Figure 18) (Horn 2013) All isoforms share tasks, but some individual characteristics of them are known as well. These differences can be of pathological importance in some diseases and may also be important for WT1's role in cancer. (See also chapter 7.2.3) (Huff 2011) (Strauss et al. 2003)

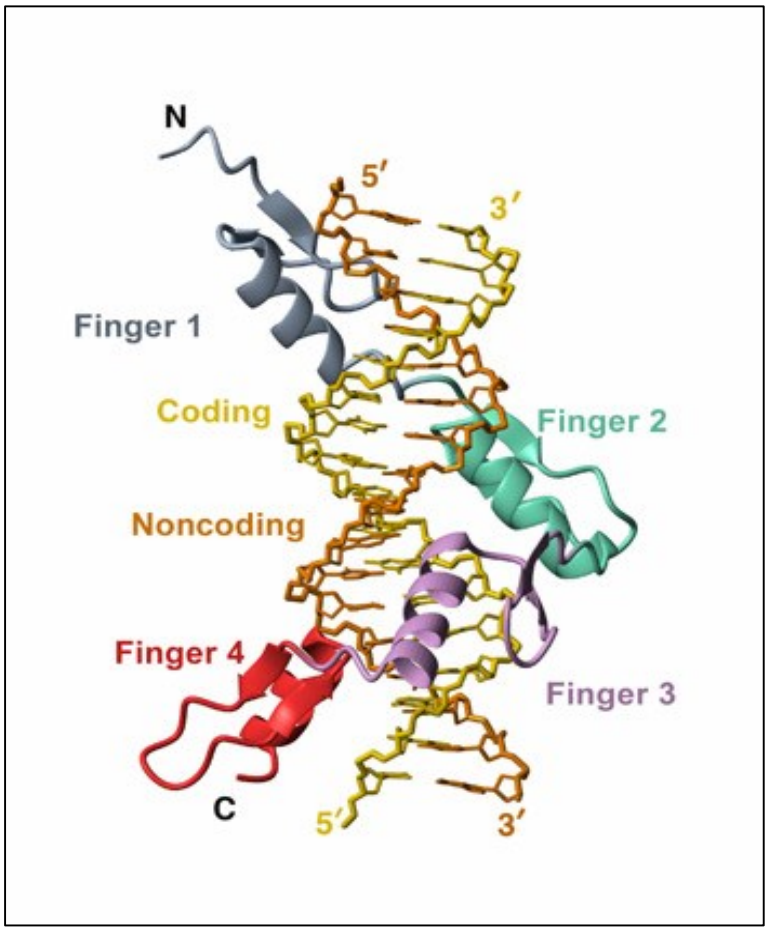

Figure 18 Wilms Tumour1 protein structure. The 4 zinc finger domains are interacting with DNA (Stoll et al. 2007)

7.2 Function

WT1 acts as a transcript factor and has influence on a variety of genes. It fulfils task during embryogenesis and is especially important during nephrogenesis and haematopoiesis. Among many others, WT1 takes part in regulation of the following genes: p53, Bcl-2, c-Myc, HSP70, HTERT, IGF-2 & EGF-R. (Scharnhorst et al. 2001) (Strauss et al. 2003) Furthermore, WT1-knockout mice showed a dysregulation in Sox-9, Snail & Cdh1. (Huff 2011)

7.2.1 Downstream genes

Understanding of the proteins coded by WT1 downstream genes is important to develop basic sense of WT1's function:

p53, also known as "guardian of genome", inhibits cells with damaged DNA from dividing and can induce apoptosis in them. Bcl-2 prevents apoptosis by blocking the release of cytochrome-c. c-MYC is a transcription factor, which mainly activates genes dedicated for cell growth. (Horn 2013) HSP70 (Heat-shock protein 70) is also a suppressor of apoptosis. (Beere et al. 2000) HTERT (Human Telomerase Reverse Transcriptase) is capable of catalysing telomerase to regain the original length of telomeres after cell division. (Kirkpatrick & Mokbel 2001) IGF-2 (Insulin like Growth Factor 2) is a growth factor, which is especially important during embryogenesis. (Horn 2013) EGF-R (Epithelial Growth Factor Receptor) is a receptor for epithelial growth factor, which can promote growth in epithelial cells. (Gadner et al. 2006) SOX9 (Sex-Determining Region Y Box 9) is involved in the suppression of Anti-Müller-Hormone (AMH), which induces male sex-determination during urogenital development and is active during chondrosynthesis. (De Santa Barbara et al. 1998) It also has been shown that SOX9 is highly active in a variety of tumours. (Matheu et al. 2012) Snail is a family of zinc finger containing transcription factors. They suppress transcription of E-cadherin and furthermore promote epithelial to mesenchymal transition (EMT). (Villarejo et al. 2014) This is a vital process during early embryogenesis and organ development. Additionally, EMT gives tumours the ability to migrate into tissues. (Thiery et al. 2009) Cdh1 encodes E-cadherin, a glycoprotein that is responsible for cell adhesion. Loss of it enables cells to escape their cell-to-cell contact. (Beavon 2000)

All mentioned interaction partners of WT1 take part in regulation of differentiation, growth or apoptosis of cells. Hence, it can be expected WT1 regulative tasks lie in similar areas.

7.2.2 WT1 in embryogenesis

WT1 has been shown highly expressed in metanephritic mesoderm during nephrogenesis. As kidney development proceeds, WT1 becomes increasingly restricted. In mature kidney it is only expressed in podocytes. WT1 is also up-regulated during myeloid differentiation. And similarly to nephrogenesis, WT1 becomes downregulated in later stages of myeloid differentiation and only very few multipotent haematological stem cells continue to express WT1. In both, nephrogenesis and haematoid differentiation, the moment when WT1 is downregulated may be of pathophysiological importance. (Huff 2011)

7.2.3 WT1 in disease and cancer

Mutation in WT1 gene can result in urogenital malformation. Relative reduction of KTS-positive isoform can result from mutation in one of WT1's alleles, which can lead to XY gonadal dysgenesis, gonadoblastoma and kidney failure. This is also known as Frasier syndrome. Therefore, Frasier syndrome shows the vital importance in all of WT1's isoforms. (Wang et al. 2005) (Strauss et al. 2003) Several missense mutations in WT1 are known to cause Denys-Drash syndrome, which is defined as dysgenesis, nephropathy and Wilms tumour. (Mueller 1994) However, occasionally the same mutations that cause Denys-Drash syndrome can result in Frasier syndrome and vice versa; indicting that both might be different phenotypical variants of each other. (Huff 2011)

WAGR syndrome is caused by a deletion of WT1 gene on chromosome 11p13. (Scharnhorst et al. 2001) This syndrome is characterised by Wilms tumour, aniridia, genitourinary anomaly and mental retardation. (Huff 2011)

While Wilms tumour is not common in Frasier syndrome, both WAGR and Deny-Drash syndrome come with Wilms tumour per definition. (Wang et al. 2005) In both, loss of WT1s function can be observed, additionally, up to 30% of all Wilms tumours also show down regulation of WT1. (Huff 2011) (Gadner et al. 2006) These findings lead to the assumption WT1 acts as tumour suppressor gene.

Moreover, loss of WT1 has been demonstrated in acute myeloid leukaemia of patients with pre-existing WGAR syndrome.

However, in vitro leukaemia cell lines have shown high expression of WT1. (Scharnhorst et al. 2001) Furthermore, WT1 overexpression has been detected in patients with acute myeloid leukaemia, acute lymphocytic leukaemia, during blast crisis of chronic myeloid and myelodysplastic syndrome. (Strauss et al. 2003) Moreover, Naitoh et al. immunhistochemicaly analysed 738 cancer patients and showed WT1 expression in 25,3% of them. Expression has been detected in a huge variety of solid cancers including colorectal carcinoma, lung cancer, mamma carcinoma, prostate carcinoma and many more. (Figure 19) (Naitoh et al. 2016)

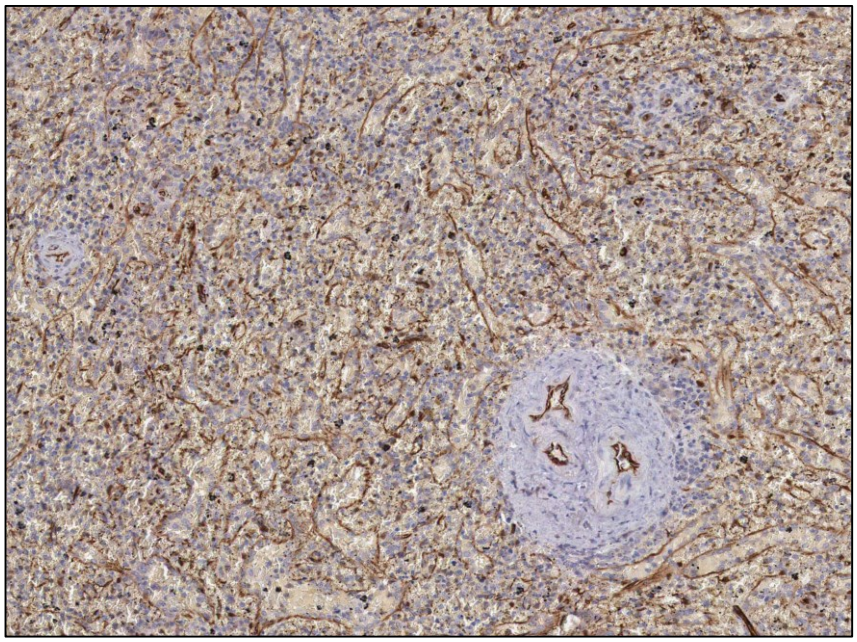

Figure 19 Micrograph of a Non-Hodgkin-Lymphoma of a 70-old patient immunehistochemically stained against WT1 (Uhlen et al. 2017; Human Protein Atlas)

After all, these findings implicate WT1 may also be able to act as oncogene. Therefore it is supposed WT1 can play both growth enhancing and suppressing roles. Conditions under which either function is taken, is not understood clearly yet. It has been shown that different stages of cell differentiation react differently to WT1 expression. (Figure 20) Other factors that could be of influence may be tissue specific functions of WT1, diverse functions of isoforms, other regulation of WT1 downstream genes or yet unknown additional factors. (Huff 2011)

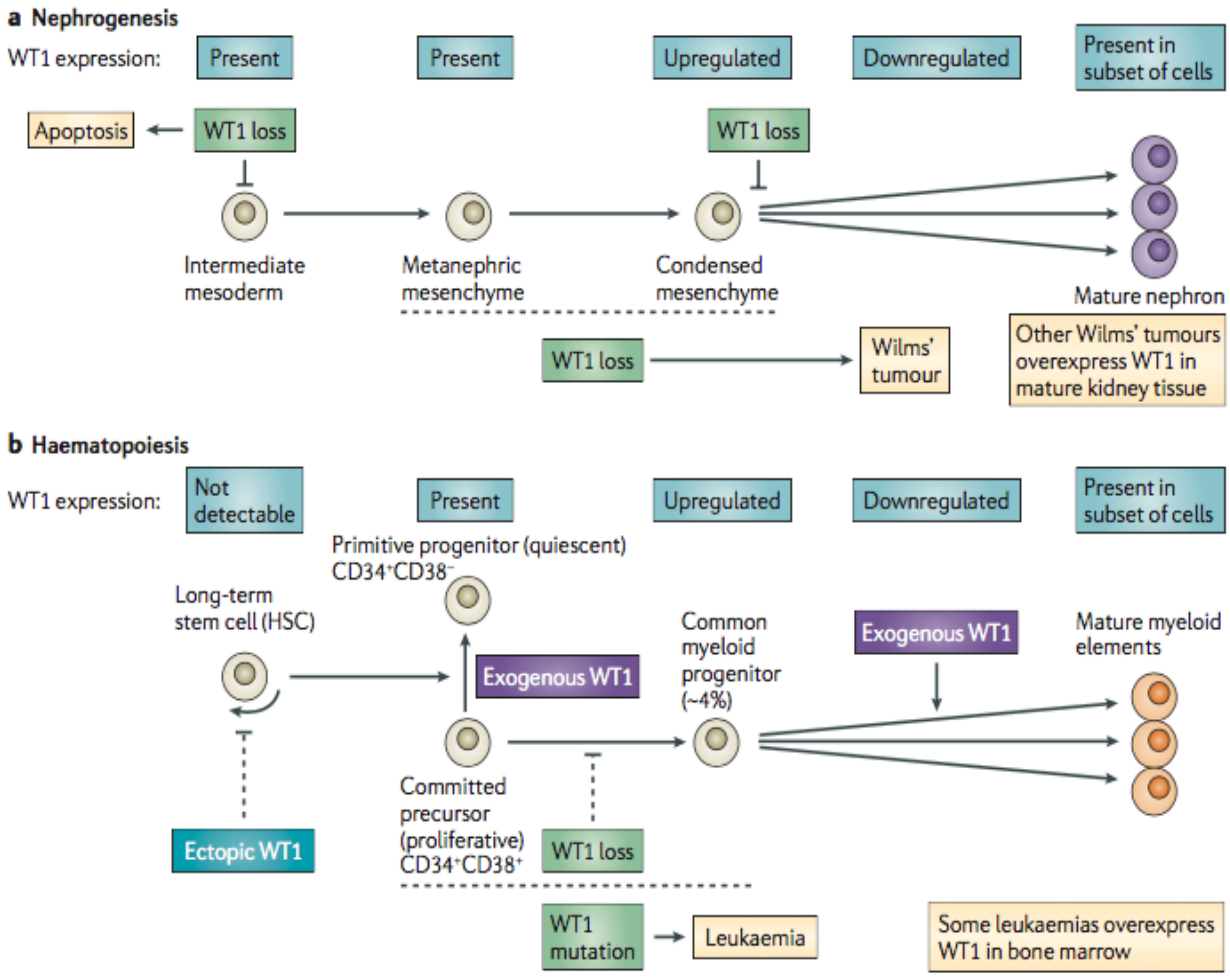

Figure 20 WT1 impact on Nephrogenesis and Haematopoesis. *a* In early Nephrogenesis WT1 expression is present, then becomes up-regulated and in the end down-regulated. Loss of WT1 in early Nephrogenisis can lead to Apoptosis, in later stages it can lead to generation of Wilms' tumour. However, in other Wilms' tumours WT1 is usually up-regulated. *b* In early haematopoesis WT1 expression is not present. In later stages, expression begins, is then being up-regulated and in the end it is shut down in most cells. Loss of WT1 damages prolifferation and differentation. Mutation of WT1 leads to generation of leukaemia. (Huff 2011)

Overall, WT1 could be a promising target for immunotherapy since it is expressed in a lot of tumours, especially in leukaemias, but has very low expression levels in healthy tissue. (Strauss et al. 2003)

8 WT1 as a target in haemato-oncological diseases

WT1 is expressed in many haemato-oncological diseases. Ogawa et al. have even shown that the WT1-mRNA can be a useful tool to measure relapse in some acute leukaemia. (Ogawa et al. 2003) Also, it is known that WT1 naturally can be targeted by the immune system. In people suffering from WT1 expressing leukaemia, generation of antibodies against WT1 or subunits of WT1 can naturally

occur. (Gaiger et al. 2001) (Oka et al. 2000) The therapeutic efficacy of graft-vs-leukaemia (GvL) following allogeneic stem cell transplantation (SCT) correlates with the production of anti-tumour CTLs (Kapp et al. 2009). There is evidence that immune attack against WT1 contributes to the GvL. Rezvani et al. have described the emerge of anti-WT1 CTLs in leukaemia patients after allo-SCT. Furthermore, they have shown that generation of anti-WT1 CTLs was linked to decrease of WT1 expression and vice versa; Loss of anit-WT1 CTLs was associated with reappearance of WT1 expression, consistent with a leukaemia relapse. (Rezvani et al. 2007)

8.1 Delivery system

To induce the production of CTLs against WT1 different delivery systems have been developed. CTL-inducing peptides and Dendritic Cells, which induce CTLs, are the most commonly used delivery systems to target WT1.

WT1-126 and WT1-235, two modified peptides of WT1 have been synthesised. Both have been shown able to induce CTLs in vitro and in vivo of HLA-A*0201- and HLA-A*2402-positive patients, which are commonly occurring HLA class I types and are especially frequent in Japanese people.(Oka et al. 2000) (Ohminami et al. 2000) (Oka et al. 2017) Recently, OCV-501 (WT1-332) was reported to successfully induce CTLs in vitro and in vivo. Compared to the previously mentioned, OCV-501 is a helper-peptide that induces $CD4^+$ T-Helpercells via HLA type II receptors, which leads to production of $CD8^+$ CTLs. Additionally, OCV-501 showed binding and/or activation of T Cells in at least 15 different HLA class II types. (Kobayashi et al. 2017)

Another approach to induce WT1-specific CTLs is the use of Dendritic Cells., DCs presenting WT1 are being generated ex vivo. This can be achieved by passively pulsing DCs with modified WT1-peptides or transfecting DCs. Transfecting DCs with mRNA to induce WT1 specific CTLs by using electroporation has been shown possible. (Van Tendeloo et al. 2001) Additionally, DCs have been transfected using viruses. However, this technique may bear more risks in clinical settings then other transfecting methods. (Van Tendeloo et al. 2001)

In the dendritic cell therapy approach the selection of subtype of DCs also comes into consideration. Most commonly, monocyte derived DCs are generated.

(Shimodaira et al. 2016) However, recently Fromm et al. postulated that the use of peripheral blood DCs, which consist of plasmocytoid and conventional DCs, has advantages compared to the use of monocyte derived DCs: Firstly, peripheral Blood DCs might be superior in antigen-presenting. Secondly, their potential to migrate seems to be superior. Thirdly, their preparation might be more cost efficient. (Fromm et al. 2016)

DCs can be obtained autologous - from patient's own blood - or similarly to HSCT, they can be obtained from suitable donors. (Figure 21) Additionally, modified DCs can be administered together with HSCT to enhance GvL-Effect. (de Haar et al. 2015) (Kitawaki et al. 2008)

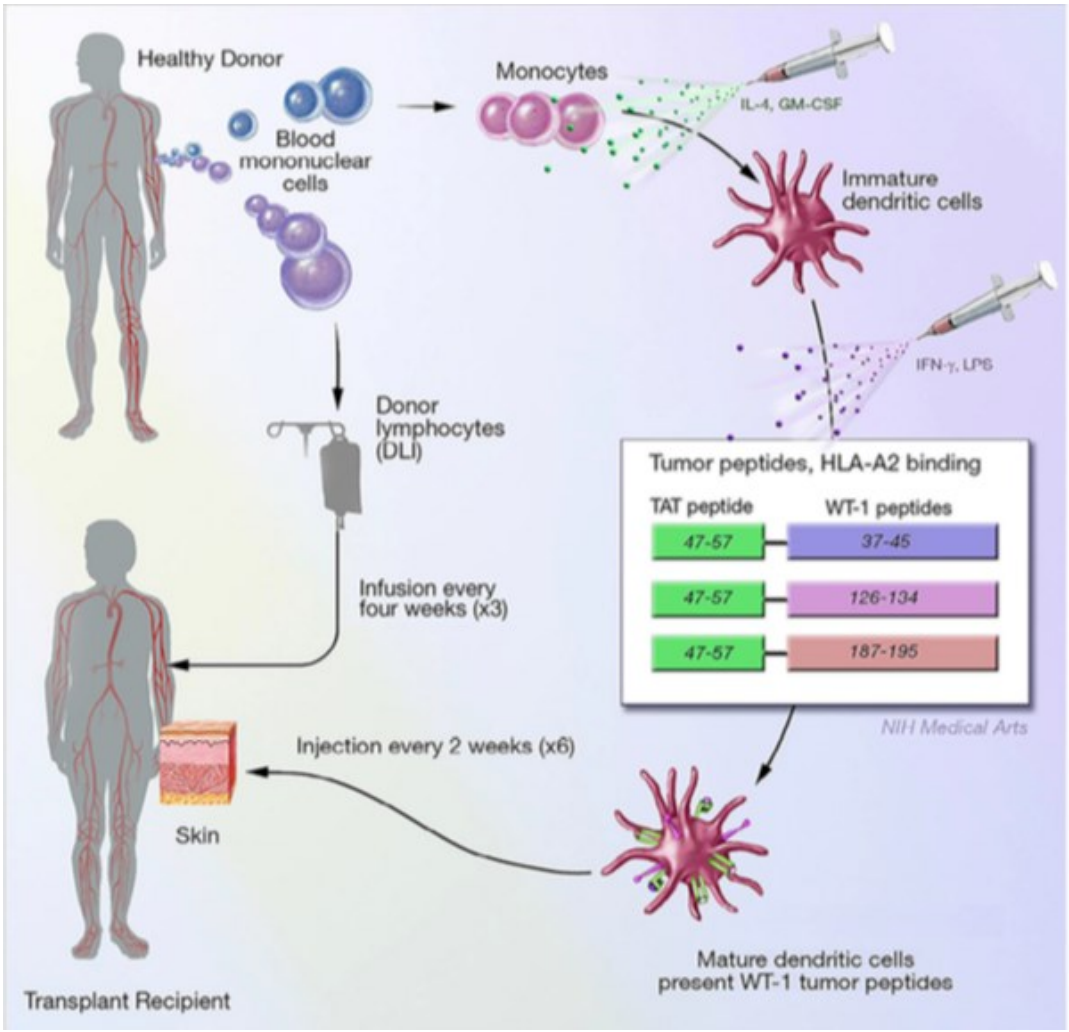

Figure 21 Protocol schema of clinical trial performed by Shah et al. Dendritic cells are generated from monocytes of healthy donors. Then they processed ex vivo to display WT1. The DCs are injected intradermal every 2 weeks. Additionally, donor derived Lymphocytes are administered intravenous every 4 weeks while (Shah et al. 2016)

Recently, Shirakawa and Kitagawa developed a novel delivery system. Recombinant bifidobacterium longum is used to deliver WT1 protein to gut immune system. They have shown its anti-tumour efficacy in murine models. Additionally, they hypothesized that this delivery system is superior because it contains multiple binding epitopes of WT1 to immunize $CD8^+$ CTLs as well as $CD4^+$ THs. (Shirakawa & Kitagawa 2017)

8.2 Preclinical prove of concept

It has been shown possible to generate anti-TAA CTLs, including anti-WT1 CTLs. Also, these anti-WT1 CTLs are able to attack WT1 expressing tumour cells and reduce tumour cell counts in vitro. However, these findings were restricted to cells of humans with certain types of HLA. (Oka et al. 2007) (Kobayashi et al. 2017) WT1-126 & WT1-235 are able to prime CTLs specifically against WT1 in cells of HLA-A*2402 & HLA-A*0201. (Ohminami et al. 2000) (Oka et al. 2000) Weber et al. were able to generate CTLs by using autologous DCs of children with ALL, pulsed them against WT1 and 3 other TAAs, namely Survivin, MAGE-A3 and PRAME, without the restriction to certain HLA-types. (Weber et al. 2013) In murine models, tumour rejection due to peptide induced WT1-specific CTLs has been investigated by Oka et al. Furthermore, the mice did not show any signs of auto immune reactions. (Oka et al. 2000)

8.3 Clinical Studies

In 2003 Oka et al treated two patients, who were suffering from myelodysplastic syndrome (MDS) derived leukaemia, with modified WT1 peptide (WT1-235m) combined with the immune adjuvant Monotanide ISA51. Both patients were HLA-A*2402-positive. After intradermal application of the peptide adjuvant emulsion, reduction of leukaemia cells and WT1-mRNA was observed in both patients. Increase in WT1-specific CTLs was detected in one patient. (Figure 22) (Oka et al. 2003)

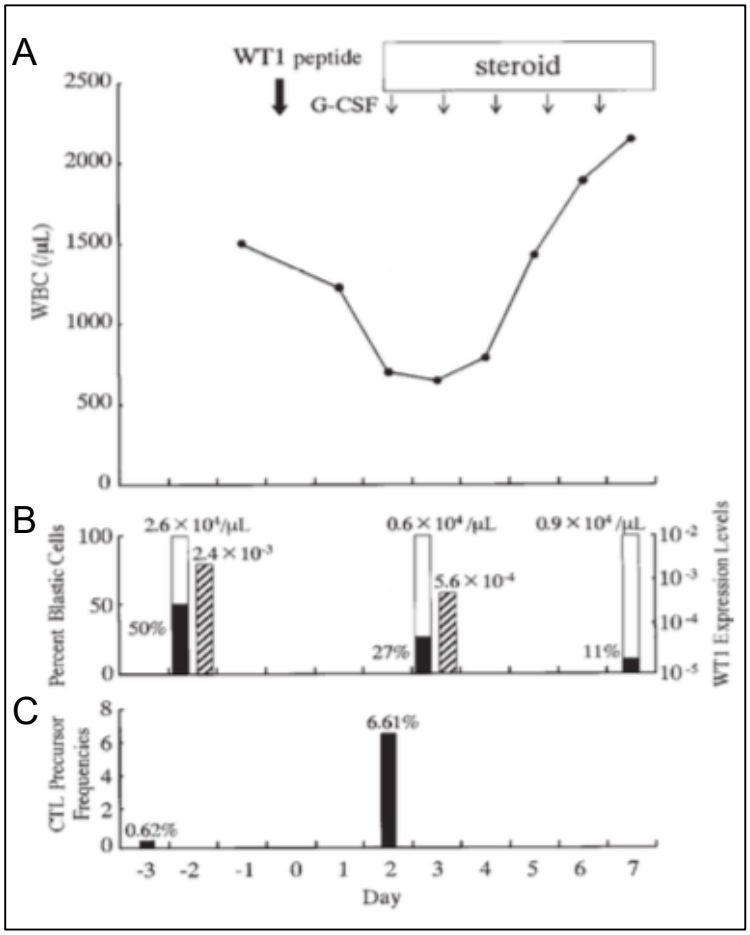

Figure 22 The clinical and immunological response of one case to WT1-235m from Oka et al. 2003 trial. *A* white blood cell (WBC) counts of peripheral blood. WBCs decreased after vaccination, three days after start of G-CSF they increased. *B* shows percentages of leukaemic blast cells in black bars and WT1 expression levels in shaded bars in the bone marrow; reduction of blasts and WT1-mRNA followed vaccination. *C* shows WT1-specific cytotoxic T-lymphocyte (CTL), which increased after vaccination. (Oka et al. 2003)

Mailänder et al. observed an increase of WT1-specific CTLs, reduction of blast cells as well as a durable complete remission in a HLA-A*0201 positive patient with AML. They used WT1-126 combined with keyhole limpet hemocyanin adjuvant and additionally injected granulocyte-stimulating factor (GM-CSF). (Mailänder et al. 2004)

In a clinical trial Saito et al. vaccinated a 15-year-old HLA-A*2402-positive patient after her third relapse of ALL with previously generated WT1-peptide pulsed DCs from her haematopoietic-stem-cell donor. She received a total of 14 vaccinations after which immune response against WT1 was detectable. Nevertheless, 44 months after her third SCT she relapsed again and died. (Saito et al. 2015)

Shah et al. administered monocyte derived DCs pulsed against WT1 from healthy donors in 5 paediatric patients, aged from 9 to 17 years, who were suffering from

ALL, AML or Non-Hodgkin Lymphoma. Their results have shown immunological response in 3 out of 5 patients, but unfortunately disease progression was shown in all of them. Nevertheless, their study indicated the safety and feasibly of their approach. (Shah et al. 2016)

Kitawaki et al. have shown neither benefit nor induction of anti-WT1 CTLs in a patient who suffered from AML. They collected mononuclear peripheral blood cells of allogeneic stem cell donors to generate peptide-pulsed DCs. These were given to the HLA-A*2402 positive patient, who already had relapsed twice. In the treatment she received SCT from the donor whose DCs had been generated, followed by intradermal administration of the peptide-pulsed DCs every second week for a total of five starting 6 months after transplantation. No adverse events, except local erythema was observed. (Kitawaki et al. 2008)

Rezvani et al. also have reported safety and clinical response of their vaccination, which targeted WT1 as well as pathogenesis-related protein 1 (PR1). All of the eight patients, who suffered from AML, CML or MDS have shown immunological response. Additionally, in three of them WT1-mRNA could be reduced. (Rezvani et al. 2008)

Tsuboi et al have shown a reduction of myeloma cells and reduction of M-protein[1] in a HLA-A*2402-positive patient, who suffered from advanced stage multiple myeloma after immunologically responding to WT1-235m vaccination. (Figure 23) (Tsuboi et al. 2007)

[1] *M-Protein represents overproduction of monoclonal antibodies by multiple myeloma in serum electrophoresis*

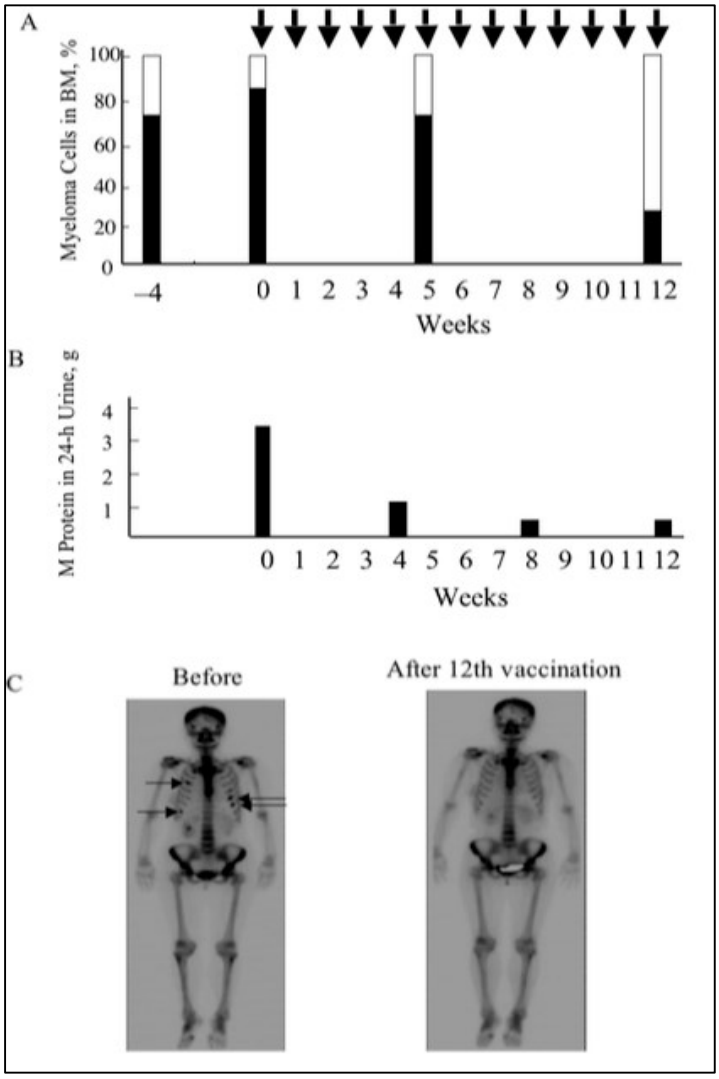

Figure 23 Clinical responses to WT1-235m vaccine in a patient with multiple myeloma. *A* Reduction of myeloma cells in bone marrow. *B* Reduction of M-Protein in 24h-Urine. C Bone scintigraphy: Left before, right after 12 vaccinations; reduction of bone lesions can be observed. (Tsuboi et al. 2007)

Narita et al. reported reduction of minimal residual disease (MRD) in a HLA-A*2402-positive patient with CML due to WT1-235m vaccination. Previously the patient has been treated with Imatinib, but MRD remained detectable. (Narita et al. 2010)

Sawada et al. also used WT1-235m in 24 HLA-A*2402 positive children with different cancers. They have shown an immunological response in some of them. Out of 13 patients who completed the vaccination, 11 showed some kind of clinical response. Furthermore, they reported that one patient who suffered from ALL with high risk of relapse sustained long-lasting remission. (Sawada et al. 2016)

Also in other paediatric cancers WT1-vaccination has been tested. Shimodaira et al. have shown safety and feasibility of vaccination of DCs pulsed with WT1-235m

peptide in a 6-year old child with Wilms tumour and a 14-year old child with neuroblastoma. In the patients, who both were HLA-A*2402 positive, immunological response was observed and in the patient with Wilms tumour also reduction of tumour markers occurred. However, eventually disease progressed in both patients. (Shimodaira et al. 2016)

These promising phase I studies have shown the feasibility of anti-WT1 vaccines. Furthermore, in all of them no adverse events except from local erythema at injection side were reported. These findings led to strong arguments for performing phase II clinical trials.

In 2004 Oka et al. tested WT1 peptide in 11 patients with AML and 2 patients with MDS, as well as 12 patients with either breast or lung cancer. All tumours overexpressed WT1 and all patients were HLA-A*2402 positive. In 20 of the 26 patients efficacy of WT1 vaccination could be measured, 12 of them showed clinical response like tumour size reduction or reduction of tumour markers. (Figure 24) Also the frequency of WT1-specific CTLs clearly correlated with clinical response. Furthermore, no adverse events expect from local Erythema was shown in AML, breast- and lung cancer patients. However, in patients with MDS WT1 severe leukocytopenia occurred. (Oka et al. 2004)

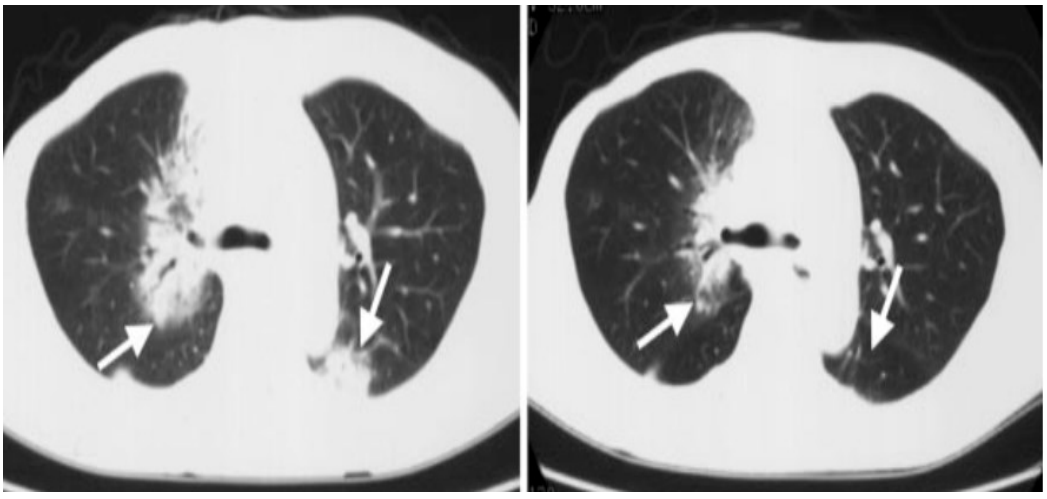

Figure 24 Reduction of tumour-mass in computer tomography of breast cancer patient. On the left before WT1 peptide treatment and on the left after treatment (Oka et al. 2004)

Similar results have been achieved in a phase II study by Kleinholz et al. in HLA-A*0201-positive patients with MDS and AML. Vaccination of WT1-126m and additional subcutan injection of GM-CSF showed clinical response such as

reduction of leukaemia cells. However, a correlation between immunological response and clinical response could not be shown. (Keilholz et al. 2009)

Tsuboi et al. have reported long-term survival of three out of eight patients only due to continuous intradermal vaccination of WT1-235m in 2012. The patients were HLA-A*2402-positive and five of the eight patients had clinical response. However, two relapsed later, but three patients showed long-term reduction of WT1-mRNA. Remarkably, all of these long-term survivors had detectable MRD before vaccination was started and therefore would have been destined to relapse without further treatment. At date of publication they had survived for 7 years only by use of vaccination. (Tsuboi et al. 2012) (Figure 22)

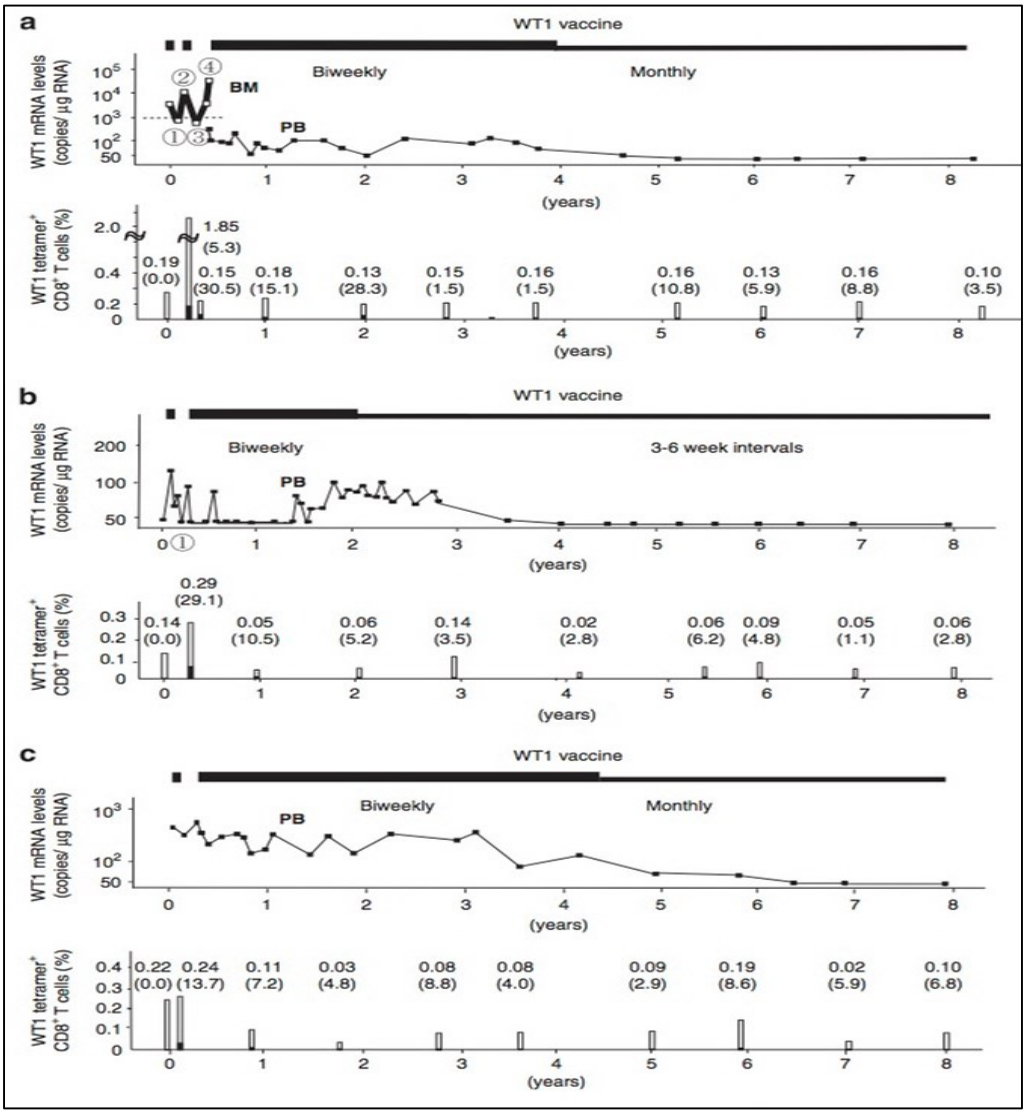

Figure 25 Immune-monitoring and clinical response of three AML patients to vaccination of WT1-235m. *a* Case 1. *b* Case 2. *c* Case 3. *a b c* On top: WT1mRNA in bone marrow (BM) in white squares and peripheral blood (PB) black squares. Long lasting remission was achieved in all three patients. On bottom: frequency of WT1-specific CD8+ T cells, which shows an increase in all three patients after beginning of vaccination. (modified from: Tsuboi et al. 2012)

Similarly, Nakata et al. have shown favourable outcome in high risk AML vaccinated with WT1-126 or WT1-235m. The patients were HLA-A*0201 or HLA-A*2402 positive and suffered from AML with high risk of relapse and some additionally had MRD. Their results have shown superior outcome compared to the estimated risk of this patient collective. (Nakata et al. 2017)

Hashii et al. tested WT1-235m in three HLA*A2402 positive children with WT1-expressing acute leukaemia after allo-HSCT. All three patients had high risk of relapse, however long lasting complete remission was achieved in two of them. (Hashii et al. 2012)

In 2010, Van Tendeloo et al. have published results of their trial in which they tested the efficacy of anit-WT1 DC-vaccine. Previously they have shown to be able to sufficiently transfect DCs using electroporation. (Van Tendeloo et al. 2001) They tested the vaccine in 10 AML patients independent of their HLA-type. Production of autologous anti-WT1 DCs was successfully obtained in all patients and in five patients clinical response was observed. Notably, in two patients who were only in partial remission and had uncontrolled AML refractory to chemotherapy, complete remission was achieved after vaccination. However, one of them relapsed nine months later and suffered from mild thrombocytopenia after vaccination. (Figure 26) In two other patients with primary complete remission WT1-mRNA expression in peripheral blood normalised due to chemotherapy. Notably, after mRNA expression re-increased equivalent to a molecular relapse, it has been managed to normalise mRNA expression again by only using vaccination. (Figure 27) Overall, long-term remission was achieved in three patients, one of which was a patient with multiple molecular relapses. (Van Tendeloo et al. 2010)

Table 3 and Table 4 give an overview of these clinical studies.

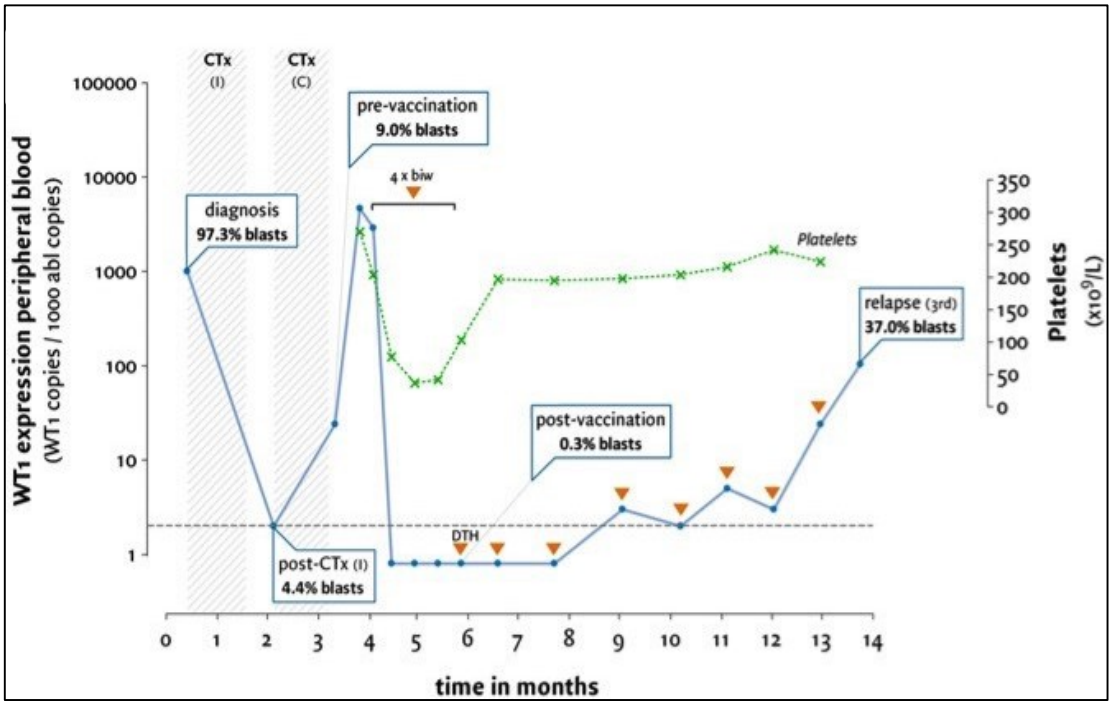

Figure 26 WT1 expression in peripheral blood in an AML patient who was treated with dendritic cell vaccination. High expression at diagnosis was successfully treated with chemotherapy, later expression re-increased. At start of vaccination complete remission could be achieved over a period of 8 months, after which the patient relapsed. After initial vaccination mild thrombocytopenia occurred. (Van Tendeloo et al. 2010)

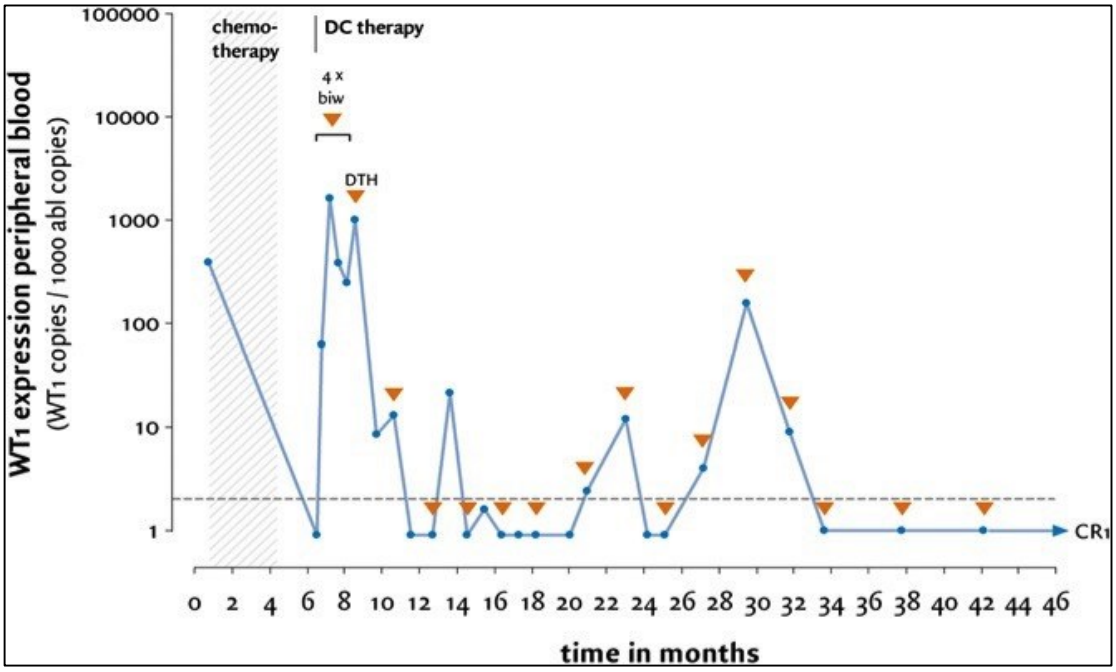

Figure 27 WT1 expression in peripheral blood in an AML patient who was treated with dendritic cell vaccination. WT1 expression re-increased multiple times and could be supressed only by the use of vaccination. (Van Tendeloo et al. 2010)

Table 3 Overview on clinical studies with cancer vaccines against WT1 in haemato-oncological diseases. (Continuation in Table 4 next page)

Date	Author	patients	HLA	paediatric patients	diseases	delivery system
PHASE I STUDIES						
2003	Oka et al.	2	A*2402	no	MDS	peptide
2004	Mailänder et al.	1	A*0201	no	AML	peptide
2015	Saito et al.	1	A*2402	yes	ALL	DC
2016	Shah et al.	5	A*2	yes	ALL, AML, Lymphoma	DC
2008	Kitawaki et al.	1	A*2402	no	AML	DC
2008	Rezvani et al.	8	A*0201	no	AML, CML, MDS	peptide (WT1+PR1)
2007	Tsuboi et al.	1	A*2402	no	MM	peptide
2010	Narita et al.	1	A*2402	no	CML	peptide
2016	Sawada et al.	24	A*2402	yes	ALL, AML, Lymphoma, solid tumours	peptide
PHASE II STUDIES						
2004	Oka et al.	26	A*2402	no	AML, MDS, solid cancers	peptide
2009	Keilholz et al.	19	A*0201	no	AML, MDS	peptide
2012	Tsuboi et al.	3	A*2402	no	AML	peptide
2017	Nakata et al.	20	A*2402 A*0201	no	AML	peptide
2012	Hashi et al.	2	A*2402	yes	ALL, AML	peptide
2010	Van Tendeloo et al.	10	various	no	AML	DC
	TOTAL	124			ALL, AML, MDS, Lymphoma, solid tumours	peptide or DC

Table 4 Overview on clinical studies with cancer vaccines against WT1 in haemato-oncological diseases.

Date	Author	immunological response	clinical response	clinical specifics	adverse events
PHASE I STUDIES					
2003	Oka et al.	2/2	2/2	reduction of blasts, WT1-mRNA reduction	erythema
2004	Mailänder et al.	1/1	1/1	reduction of blasts	erythema
2015	Saito et al.	1/1	n.a.	probably longer relapse-free interval	erythema
2016	Shah et al.	3/5	0/5	disease progression	erythema
2008	Kitawaki et al.	0/1	0/1	disease progression	erythema
2008	Rezvani et al.	8/8	3/7	WT1-mRNA reduction	erythema
2007	Tsuboi et al.	1/1	1/1	reduction of myeloma cells, reduction of M-gradient	erythema
2010	Narita et al.	1/1	1/1	reduction of MRD, reduction of bcr-abl	erythema
2016	Sawada et al.	n.a.	11/13	sustainable complete remission in high risk ALL patient	erythema
PHASE II STUDIES					
2004	Oka et al.	13/20	12/20	reduction of tumourmarkes, reduction of tumoursize	erythema, leucocyto-penia
2009	Keilholz et al.	8/18	14/18	reduction of blasts, immunological & clinical responses were incoherent	erythema
2012	Tsuboi et al.	5/8	5/8	suppression of MRD over 7 years in 3 patients	erythema
2017	Nakata et al.	n.a.	n.a.	superior outcome than estimated, complete remission in patients with MRD	n.a.
2012	Hashi et al.	3/3	3/3	sustainable remission after HSCT	erythema, skin ulcer
2010	Van Tendeloo et al.	5/10	4/7	WT1-mRNA reduction, long-term remission	erythema
	TOTAL	51/71	57/87		

9 Discussion

The aim of this thesis was to give a theoretical background of dendritic cell vaccination against Wilms Tumour 1 protein in children with haemato-oncological diseases and to review existing studies for clinical use of this innovative and promising novel immunotherapy approach. A brief overview on haemato-oncological diseases of the young, as well as general cancer immunotherapy has been given. The main characteristics of cancer immunology, dendritic cells, tumour-associated antigens and Wilms Tumour 1 have been shown before the focus was set on clinical uses of DC- or peptide-based vaccines to target WT1 in haematological cancers.

Although haemato-oncological diseases account for most of paediatrics malignancies, only few clinical studies on targeting WT1 have been performed in paediatric patients.

WT1, a transcription factor, plays a crucial role during development of the urogenital and haematopoietic system. Its importance as a possible target was outlined in this thesis. It has been shown that WT1 is naturally a target for immune reaction against cancer. (Gaiger et al. 2001) (Oka et al. 2000) However, the role of WT1 in cancer is not completely understood yet. Nevertheless, overexpression of WT1 has been shown in different types of cancer. Moreover, this zinc-finger protein is not expressed in most healthy tissue; making it one of the most promising tumour associated antigens. (Cheever et al. 2009)

Even though this thesis was intended to focus on DC-based vaccination, I found more papers with the use of peptide vaccination to target WT1. Both delivery systems still have some disadvantages that must be overcome to be suitable therapies for a broader spectrum of patients. Peptide vaccination is limited to certain HLA-types, which might be overcome due to development of new peptides or use of DCs. (Kobayashi et al. 2017) (Van Tendeloo et al. 2010) However, it is not known which subtype of DCs should be selected for optimal clinical outcome. (Fromm et al. 2016) Additionally, function of DCs is not understood in all details, which brings the concern, that they could potentially unfold their immunosuppressive character. In this case, immune checkpoint inhibitors combined with DCs vaccination might increase therapeutic benefits. (MacKeon et al. 2015) Also, in peptide vaccinations the use of immune stimulatory adjuvants, like GM-CSF, could result in better therapeutic efficacy. (Oka et al. 2017)

Additionally, maybe completely novel systems of delivery like use of bifidobacteria to orally vaccinate immune system will turn out superior. (Shirakawa & Kitagawa 2017)

Preclinical studies outlined the therapeutic potential of immune reaction against WT1 in vitro and in mice. It was possible to generate CTLs in vivo and in murine models; showing the feasibility of anti-WT1 vaccinations. Furthermore, in both immunological rejection of tumour cells have been observed. (Oka et al. 2000)

Human phase I studies indicate the safety and feasibility of this novel therapeutic regime. With the exception of local Erythema, peptide as well as DC vaccination has been shown safe for most patients. (Shah et al. 2016) (Oka et al. 2003) However, in some patients vaccination could lead to rapid and strong destruction of oncological cells before recovery of normal haematopoiesis, which could lead to tumour-lysis syndrome and/or pancytopenia. (Oka et al. 2007) For instance, in patients suffering from myelodysplastic syndrome (MDS), severe leukocytopenia has been reported. (Oka et al. 2004) Therefore, it might be mandatory to reduce dosage in these patients. Additionally, since data only exists for a relatively small number of patients, assumptions on rare side effects cannot be made.

Most groups have been able to induce production of WT1-specific CTLs in the majority of patients, demonstrating the feasibility of both DC- and peptide-vaccinations. (Shah et al. 2016) (Oka et al. 2003) In some patients clinical response like reduction of minimal residual disease (MRD), reduction of M-Protein or long lasting remission in high risk leukaemia were reported. (Tsuboi et al. 2007) (Sawada et al. 2016)

In phase II trials, clinical response could be shown in patients treated with peptide- as well as DC-vaccination. Moreover, a clear correlation between frequency of WT1-specific CTLs and clinical response could be demonstrated. (Oka et al. 2004) In high-risk AML patients treated with peptide-vaccination a more favourable outcome than statistically expected could be shown. (Nakata et al. 2017) Furthermore, in some patients with high risk of relapse, who were undergoing allo-HSCT combined with peptide vaccination, long lasting remission could be induced. (Hashii et al. 2012) Remarkably, Tsuboi et al. could prevent relapse in 3 patients with MRD by only using peptide vaccination. (Tsuboi et al. 2012) Similarly, Van Tendeloo et al. were able to show clinical response to autologous DC-vaccination like reduction of MRD and normalisation of WT1-mRNA expression. Notably, the

patients were selected independently from HLA-type. Moreover, they also achieved long-lasting remission in 3 patients, one of which had multiple molecular relapses. (Van Tendeloo et al. 2010)

These clinical trials seem very promising, though there are still a lot of drawbacks to overcome. A lot of studies have shown the power of WT1 as immunological target in preclinical setting, however the effectiveness of anti-WT1 vaccination in clinical studies remains very diversified between the different trials. Furthermore, most of the time anti-WT1 reaction can be achieved in patients, but in some, therapeutic response still remains low. This discrepancy between immunological and therapeutic effectiveness could be due to imperfect therapeutic modality, tumour progression, immune escape or other unknown factors. (Hashii et al. 2012) (Shimodaira et al. 2016) In terms of therapeutic modality, it is not clear which delivery system is superior and which adjuvants could maximize the therapeutic effects. Also there is no evidence for optimal timing of vaccination. A lot of studies were done in patients with highly progressed diseases with high tumour mass. This could limit the potential of immune therapy. (Van Tendeloo et al. 2010) Therefore it could be advisable to test the therapeutic potential in patients, who achieved remission or test it as an enhancement of HSCT, like some groups already did. (de Haar et al. 2015) (Kitawaki et al. 2008) (Kohrt et al. 2011) Immunological escape could be another problem to face. However, due to its essential role in tumour progression, down-regulation or loss of WT1 is unlikely to occur. (Oka et al. 2007) (Weber et al. 2013) But other mechanisms of tumour evasion could remain a problem and multiple vaccinations of WT1 could even lead to immune tolerance. (Di Stasi et al. 2015)

As for today, I was not able to find any phase III studies. To evaluate the true power of these novel therapies, double-blinded randomised trials with a high number of patients and previously determined clinical endpoints, would be needed.

In conclusion, WT1 could be a promising target in haemato-oncology. DC-vaccination as well as peptide-vaccination have shown clinical effectiveness. Nonetheless, there are still huge obstacles to overcome. If this novel therapy could be optimised, it could become a breakthrough in modern therapy of haemato-oncological diseases. Its low adverse effects could be a great adjuvant - maybe even an alternative – to aggressive chemotherapy. Additionally, for patients with

high risk or poor therapy response it could also provide survival advantage, especially combined with HSCT. Furthermore, as the vaccination can induces anti WT1 CTLs, someday an optimised form may even be suitable as a prophylaxis against WT1 overexpressing tumours, which has already been indicated in murine models (Kohrt et al. 2011)

10 Reference

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