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„Dose reductions in adults receiving VIDE chemotherapy for Ewing Sarcoma treatment“

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1. Abstract (German and English)

1.1 German Abstract

Das Ewing Sarkom (ES) ist der häufigste Knochenkrebs bei Jugendlichen und jungen Erwachsenen. In den vergangenen Jahren konnte die Überlebensrate vor allem bei Patienten mit einem lokal beschränkten Sarkom dank der Chemotherapie um ein Vielfaches gesteigert werden. Jedoch konnte bei primär metastasiertem ES noch kein Durchbruch erreicht werden.

Viele Studien und Forschungen wurden durchgeführt um die bestmögliche Behandlungsmethode aus einer Kombination von verschiedenen Chemotherapeutika, Operation und Radiotherapie für diese Erkrankung zu entwickeln. Die derzeitige Behandlung variiert zwischen den USA und Europa in Bezug auf die verabreichte Kombination an Chemotherapeutika. Der aktuelle europäische Behandlungsstandard wurde in einer multizentrischen Studie (EURO E.W.I.N.G. 99) entwickelt. Sie besteht aus einer neoadjuvanten Chemotherapie, einer darauffolgenden Operation oder Bestrahlung und einer adjuvanten Chemotherapie. In den letzten Jahren konnte jedoch beobachtet werden, dass es aufgrund von Hämatotoxizität selten möglich ist den Patienten über die komplette Dauer der Therapie die vollständige Dosis zu verabreichen. Bisher fehlen aber noch Daten in Bezug auf die Auswirkungen der Dosisreduktionen auf die Heilungsrate.

In dieser Arbeit konnten wir auf die Behandlungsdaten von 46 Patienten über 7 Jahre verteilt zurückgreifen. Unsere Studie unterscheidet sich vor allem durch das verhältnismäßig hohe Alter unserer Patientenkohorte, da es sich bei unserem Zentrum nicht um ein pädiatrisches Zentrum, wie in den meisten Zulassungsstudien, handelt. Dadurch lag das Durchschnittsalter bei unserer Kohorte über dem Alter der typischen ES-Patienten. Das Ziel dieser Studie war es,

Daten bezüglich Dosisreduktionen bei Erwachsenen Ewing Sarkom-Patienten unseres Zentrums zu objektivieren und darzustellen. Weiters sollte das Überleben der Patienten ohne oder mit später Dosisreduktion mit dem Überleben von Patienten mit früher Dosisreduktion verglichen werden, um die Sicherheit von Dosisreduktionen in diesem Studienkollektiv darzustellen.

1.2 English Abstract

Ewing sarcoma (ES) is a rare malignant, small cell tumor of the bone, which mainly affects young adults or children. Although considered as one of the most aggressive tumors of young people, within the last years, survival rates have been improved. On one hand, this was caused by introduction of novel chemotherapeutic combinational treatments and on the other hand patient outcome has been ameliorated due to interdisciplinary approaches of combining chemotherapy with local therapy such as irradiation and surgery. Nevertheless, although considered to be a chemosensitive tumor there is a huge difference in prognosis concerning patients with localized disease and patients with metastatic disease at diagnosis. Therefore several studies have tried to use molecular characteristics of ES to tailor treatment modalities, unfortunately without major improvements up to date.

In Europe the current therapy of ES includes neoadjuvant chemotherapy, local therapy and adjuvant chemotherapy, resulting from a variety of studies such as the EURO E.W.I.N.G. trial. Several chemotherapy regimes have been proposed, which all combine cytotoxic agents with different modes of action. Due to high toxicity of the chemotherapeutic substances observed in many patients resulting in neutropenia, thrombocytopenia and infection, dose adjustments

have to be performed in many patients. Until now, there are no data concerning time-points and extent of dose adjustments in relation to prognosis in adult patients.

In our study, we were able to collect data of 46 patients, all diagnosed at our center within seven years. Our cohort differs from other cohorts in studies concerning ES, since our center would not treat pediatric patients. Therefore, we present data of an older patient collective than in most previously published studies. Aims of this study were to objectify the prevalence of dose reductions at our center and to find potential correlations between patient outcome and dose reductions, in order to examine safety of dose adjustments in adult patients receiving the chemotherapy regime VIDE which is used in our department as induction chemotherapy.

2. Introduction

2.1 Cancer as a genetic disease of the cell cycle

Cancer is a genetic disease which is characterized by abnormal cell growth. The origins of cancer are dysregulated molecular mechanisms such as mutations, translocations, amplifications or epigenetic changes of genes. In particular, oncogenes, tumor-suppressor genes and caretaker genes have been characterized, playing different roles in cancer development and progression through either promoting or inhibiting carcinogenesis. Every mutation of the subtypes mentioned can lead to a deregulation of cell homeostasis (2-4), either by exhaustive cellproliferation through changes in the tumor cell cycle or through a lack of mechanisms causing cellular arrest.

The cell cycle consists of five phases, which are depicted in figure 1. In the S phase, as indicated by its name, DNA synthesis takes place and in the M or mitosis phase the cell divides into two daughter cells. In between these two phases there are the G1 and the G2 phases, in which neither cell division or a DNA synthesis occurs. The fifth phase is the G0 phase, in which the cell is in a quiescent state. Most cancer cells do not or only for a very short period of time resign in a G0 phase, although a small population of cancer cells, so called “cancer stem cells” were defined recently in most cancers (5), which are known to stay in a quiescent state and might therefore exhibit resistance to chemotherapy and radiotherapy. Between the phases of the cell cycle, there are specific checkpoints that, in a physiological state are control mechanisms, which are critical in the decision of continuation of mitosis or entering resting or apoptotic pathways. After passing one of those checkpoints a cell is going to the next phase after the activation of specific cyclin dependent kinase (CDK). If the cell has damaged DNA,

the cell is either going to be repaired or be led into apoptosis (6, 7). There are several checkpoints, but the most important checkpoint in the cell cycle, concerning the genesis of cancer is controlled by a protein called p53 (TP53) between M1 and S phase. The cell cycle in a cancer cell differs from a normal cell cycle in several points leading to an uncontrolled cell growth and genomic instability due to the failure of checkpoints. While the S and the M phase are of same length in cancer cells, the G1 phase is shorter than in normal cells, or even absent (7).

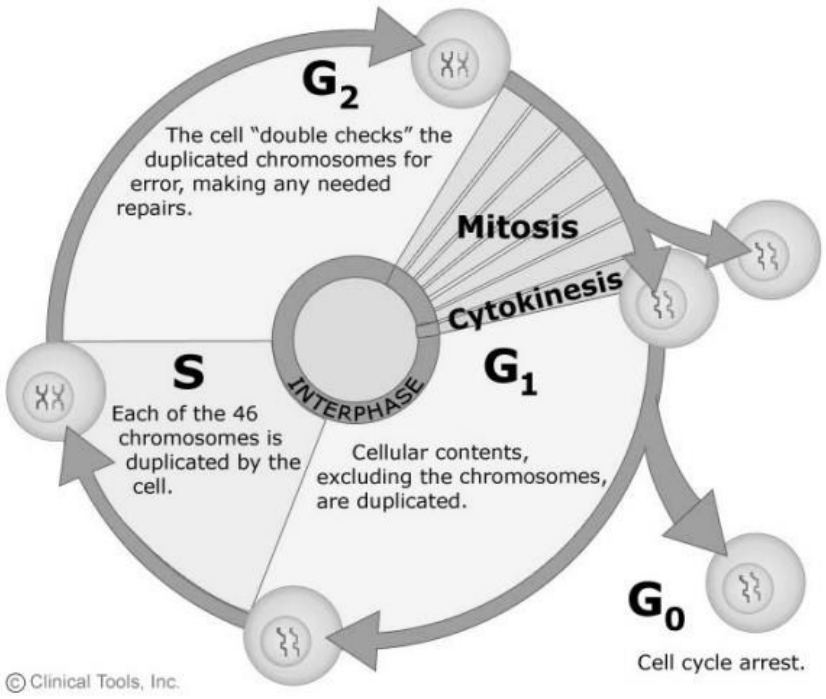

Figure 1: Schematic description of the cell cycle and its phases (8).

2.1.1 Tumor suppressor genes

The activity of tumor suppressor genes is of high relevance for the regulation of the cell cycle (9). Proteins like p53,Rb or Pten are encoded by Tumor suppressor genes. These proteins have either a function for the cell cycle checkpoints regulation themselves or are a part of the proliferative signaling pathways. Some of them, for instance p53, can detect damages in the DNA and either repair them or initiate cell death. If there is a mutation or a loss-of-function in a gene in a cell with nonfunctional p53 damaged DNA is not going to be repaired or apoptosis will not be initiated, which is estimated to take place in 30-50% of all human cancers (10, 11). Since every normal cell has two copies of the p53 gene it is necessary, that for full loss of function of p53 both alleles have to be inactivated (7).

Rb, as a further example, is a member of a cell cycle checkpoint proteins and its loss or inhibition can lead to initiation of S phase and therefore lead the cell to uncontrolled growth and proliferation (12).

Other tumor suppressor genes, like PTEN, inhibit proliferative cellular signaling pathways promoting the cancer phenotype of cells. Their loss hence leads to a change of the pathway into an “always active” state. For many tumor suppressor genes, functions are unknown or currently undergoing investigation.

2.1.2 Oncogenes

Oncogenes develop through mutation from proto-oncogenes. Proto-oncogenes encode for growth factors, growth factor receptors or transcription factors. A mutation or overexpression

of proto-oncogenes can lead to uncontrolled cell growth. Proto-oncogenes can exhibit fate-changing mutations in one of their alleles, causing severe changes in gene function, as has been shown for RAS, for instance. A mutated RAS pathway can then even stay active in absence of the right ligand of its upstream receptor (7).

As for some cancers, mutations in the sense of translocations that put proto-oncogenes in front of a new promoter or gene are of importance (e.g. EWS/FLI in ES or TMPRSS/ERG in prostate cancer) (13, 14).

2.2 Etiology of cancer

It is not possible to define one sole risk factor for cancer, but there are several factors which can either cause or influence the deregulated growth of cancer cells. Besides the inborn genetic risks which have already been mentioned there are several risk factors, causing somatic changes in genes resulting in tumor initiation or progression. For some of them, such as smoking, alcohol or unhealthy diet, a strong link between lifestyle and cancer prevalence of people can be observed. Alcohol and smoking do even have a synergistic effect for the genesis of particular cancer subtypes(7). Nearly 40% of cancers are caused by wrong behavior such as smoking, alcohol consumption or physical inactivity(15).It is estimated that 16% of cancers occur due to viral, bacterial or parasitic infections. Further reasons for genomic mutations can be chemical or radiation damage and excessive sun exposure (7).

Smoking

Tobacco consumption is one of the most dangerous human behaviors concerning the increase of cancer risk. The vast majority of lung cancers are connected to smoking habits (85% worldwide). More than 30 % of cancerous diseases in men are caused by smoking. Not only lung cancer is associated with the consumption of tobacco but also cancer of the esophagus, head and neck, bladder and pancreas (7). The risk of a cancer formation is proportional to the duration of the consumption and to the amount of cigarettes(16).

Alcohol

The consumption of alcohol favors the genesis of hepatocellular, esophagus and squamous cell cancer of the head and neck. Most of the time the excessive consumption of alcohol goes along with tobacco consumption, which is one of the reasons for the synergistic effect of them concerning the increasing risk of developing a cancer. Alcohol, in contrast to cigarette smoke, should be seen as a promoter of tumor growth but not carcinogenic by itself (7).

Diet

People with a balanced and healthy nutrition are less likely to develop cancer. Although several studies have been made, it is hard to proof the positive influence of vegetables and its ingredients such as antioxidant substances on cancer development. On the other hand it is a fact that obese people are at a higher risk to suffering from several types of cancer (7, 17).

Infections

Some cancers are known to develop as a result of an infection. There is a huge difference between developed and developing countries concerning the incidence of cancer due to infections. The occupation time between a viral infection and the actual cancer might be many years, depending on the viral vector and host factors such as comorbidities or medication. There are several cancers such as cervical cancer or hepatocellular cancer, which are associated with viruses, in the one case human papillomaviruses (HPV) or in the other case hepatitis B/C virus (HBV/HCV) infection. Another virus which can potentially lead to cancer is the Epstein-Barr virus (EBV). Especially Hodgkin´s disease in developed countries and the Burkitt´s lymphoma in Africa are known for their virus-dependent genesis. Other cancers, like Karposi´s sarcoma and lymphoma, are associated with immunodefiency developed due to HIV promoting HTLV1 infection. Besides viral infection, there are bacterial and parasitic infections known to be linked to cancer development. Helicobacter pylori infection of the stomach on the one hand is associated with several gastric cancers while Schistosoma haematobium is known for causing bladder cancer, especially in developing countries. For most patients, infection by itself would not cause tumor development. The reason for the development of cancer is a multifactorial combination of viral, genetic, immunological and environmental factors (7).

Sun exposure and radiation

Excessive sun exposure is associated with skin cancer. More than 90 % of melanomas are estimated to be caused by the sun’s ultraviolet light (UV light). People with white skin or red

hair and a high UV exposure are more likely to suffer from melanoma. Especially sunburns in the childhood increase risk.

The reason for the toxicity of radiation is a mutation of either tumor suppressor genes losing function or oncogenes gaining function. High radiation exposure, which means a dose of 500-200 mSv (Sievert) is known for causing several cancers such as acute leukemia and thyroid cancer (7).

Chemical exposure

There are three different sources of chemical agents that can cause cancer: pharmacological agents, industry and environment. Some workers in special fields are exposed to chemical substances like asbestos, naphythylamines or beryllium. Even therapeutic drugs like chemotherapeutics (alkylating agents) or immunosuppressive drugs can lead to a cancer. In several studies it was proven that immigrants would have the nearly the same distributions of incidences of cancers as the people native to the country after living in the examined area for a certain period of time. This leads to the conclusion that mostly environmental factors like air pollution do have an important role within the genesis of cancer (7).

2.3 Chemotherapy

2.3.1 History of chemotherapy

The very beginning of the expression “chemotherapy” was in the early 1910´s when german physician Paul Ehrlich started to fight parasites by using chemical substances. He also began

to screen chemicals using tumors in animals. Due to his success Paul Ehrlich is known as “the father of chemotherapy” although his main field were infectious diseases (18).

Throughout the World War I mustard gas was used as a weapon. As a result a huge amount of novel biological effects of the chemical compound, such as depletion of lymphoid elements, neutropenia and loss of function of the bone marrow were noticed. Due to these events mustard sulfur gas was expected to be a potential drug against cancer and other diseases. Alfred Gilman and Louis Goodman replaced a sulfur atom for a nitrogen atom to become the less toxic nitrogen mustard (18). In 1946 the first clinical trial about the use of nitrogen mustard in patients with tumors was published. Since the trial was successful the new therapy spread quickly around the world as a drug which would it make possible to cure cancer. Unfortunately the physicians had to realize that a total remedy was not possible with mustard gas (19, 20). Another substance which has been detected due to the world war was folic acid (21). Folic acid appears in vegetables and is important for bone marrow homeostasis and cell growth. Folate antagonists, which were successfully used in children with leukemia (19) are still a mainstay of chemotherapy today. At the same time penicillin was already used against infections and many other antibiotics were produced through fermentation. Especially Actinomycin D was very popular for use in children with tumors in the 1950´s and 1960´s. in the 1950´s, the cornerstone for targeted chemotherapy was found by Charles Heidelberger and his colleges. The drug (Fluoropyrimidine 5-fluorouracil) showed an improvement for patients suffering from solid tumors (19). In the early 1960s, new substances called vinca alkaloids were found (vincristine, vinblastine) (18). The alkaloids were extracted from a plant called Catharnthus roseus, which was already known for its medical potential (18, 22). The first combined therapies appeared in the late 1960s. A popular therapy back in this time was the combination VAMP (vincristine, methotrexate, 6-mercaptopurine, and prednisone) used

in children with leukemia and MOMP (melphalan, methotrexate, vincristine, and prednisone) or MOPP (methotrexate replaced by procarbazine) for Non-Hodgkin's lymphoma, where numbers of remissions were further improved (19).

2.3.2 Classes of chemotherapeutic agents

The most important difference between a cancer cell and a normal cell is the uncontrollable cell growth of cancer cells. Chemotherapeutics do inhibit the cell growth of normal and of cancer cells, which is the reason for the large amount of toxic side effects. Within the last years, new methods (targeted methods) were found, which show less side effects than usual chemotherapeutic agents. The side effects do affect in particular cells with a fast cell division, as also mentioned and further described in chapter XII.

Not every cancer can be treated with the same chemotherapeutic agents. Therefore it is important to distinguish between drugs which have their greatest toxicity in different phases of the cell cycle. Cell cycle specific drugs can either damage the DNA in the S phase or inhibit the mitotic spindle in the M phase. Besides the cell cycle specific drugs, there are also some agents that have high toxicity during each phase of the cell cycle. They are called cell cycle nonspecific agents (6).

It is useful to combine different classes of chemotherapeutics to optimize the cytotoxic effect. Today, six different classes of chemotherapeutics are known:

Antimetabolites

In all antimetabolites, the similarity of the structure with the building-blocks of the DNA such as purine or pyrimidin is very important. Due to that similarity, they are incorporated into the DNA, causing toxic effects on cell division and cell growth. Therefore they are used as anti-Tumor chemotherapeutics or in patients with autoimmune diseases (23).

- Antifolates

Folic acid is very important for cell proliferation. It is a carbon donor in the intracellular production of amino acids. Therefore antifolates, such as methotrexate (MTX), were designed as competitive inhibitors of folic acid by blocking dehydrofolat reductase (DHFR)(24), known to be of key importance for folate metabolism (25). As a consequence, antifolates would only work in the S-phase of the cell cycle (23).

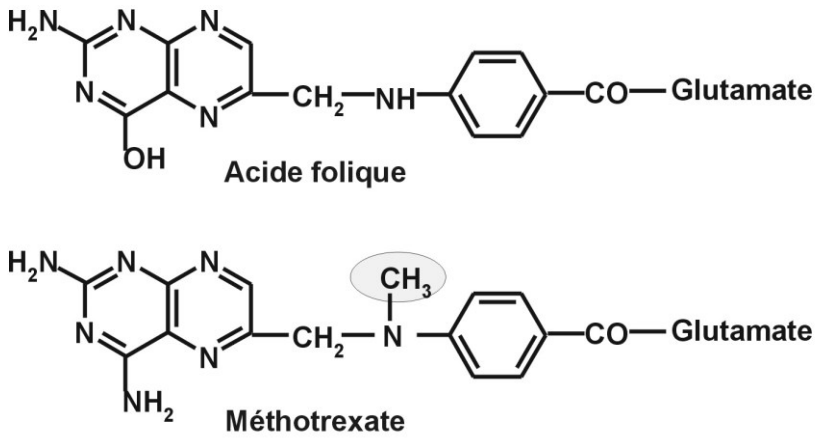

Figure 2: Structure of folic acid and the antifolate methotrexate (26)

Besides methotrexate, there are two other important drugs which are used as chemotherapeutics. Pemetrexed and pralatrexate have the same mechanism of action as

methrotrexate. Antifolates have shown to be clinically active and hence be of particular importance in patients with leukemia, lymphomas, osteosarcoma, teratoma, lung and bladder cancer and others (27). Besides their broad use in the oncologic field they are also used in patients with autoimmune disorders at lower dosage (23), where they would block proliferation of immune cells.

- Pyrimidine antagonists

Pyrimidine antagonists, as all other antimetabolite drug classes, interfere with DNA synthesis and would hence show greatest pharmacological activity in the S-phase of the cell cycle. They inhibit the synthesis of pyrimidine, which leads to impaired synthesis of both RNA and DNA(23). Four pyrimidine antagonists are widely used in cancer therapy: 5-fluorouracil (5-FU), capecitabine (nucleotide synthesis inhibitors) and cytarabine as well as gemcitabine (inhibitors of DNA synthesizing enzymes) (27). Cytarabine has high neurotoxic potential and its use is limited to hematological cancers (23).

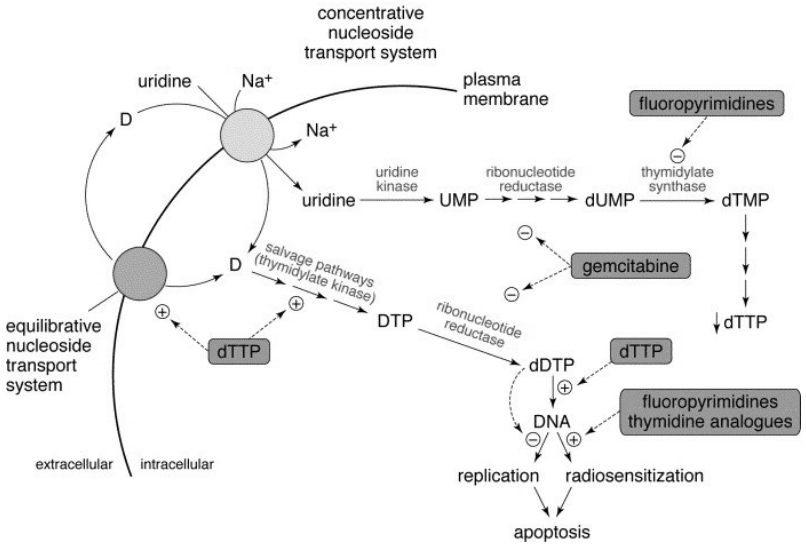

Figure 3: Molecular mechanism of antimetabolites used in cancer therapy (28)

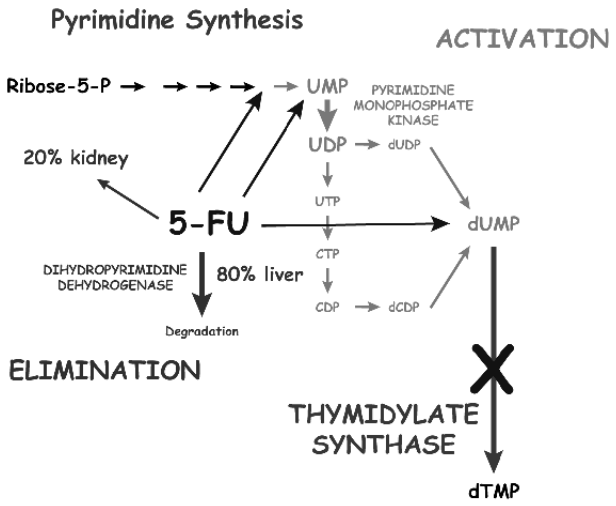

Figure 4: Inhibition of pyrimidine synthesis through 5-FU (29)

- Purine analogues

Purine analogues such as 6-mercaptopurine and azathioprine are incorporated into the DNA and as a result purine synthesis is inhibited. Azathioprine is a prodrug of 6-mercaptopurine. Nowadays they are mainly used in patients with autoimmune disorders. As with most cytostatic agents, the most important toxic adverse effect of azathioprine and 6-mercaptopurine is bone marrow suppression (23).

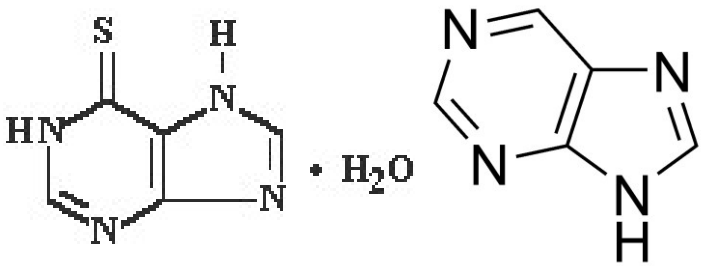

Figure 5: Structure of mercaptopurine (left) (30) Figure 6: Structure of purine (right) (31)

Topoisomerase interacting drugs

There are two different topoisomerase isoforms. These enzymes are important during the replication and transcription of the DNA, separating DNA strands. While tompoisomerase 2 is able to cut two strands at the same time, toposimoerase 1 only cuts single strands (23).

- Camptothecins

Main drugs in this class are irinotecan and topotecan. Those substances inhibit topoisomerase 1, hence blocking replication of DNA. As a consequence of the inhibition, a double-strand break is formed and the cancer cell stays in G2/M-phase arrest (23).

- Anthracyclines

There are four major substances within this group, namely daunorubicin, doxorubicin, epirubicin and idarubicin. The drugs intercalate directly with DNA. Besides, they destabilize topoisomerase 2 and DNA synthesis is further inhibited because of prevention of DNA strand fusion (32) mediated by this enzyme. The most severe adverse events of anthracyclines are acute or chronic heart failure due to their high cardiac toxicity (23).Between the different substances there are important differences concerning the metabolism, which leads to different toxicity profiles (33). Patients should therefore be monitored for deterioration of heart function when treated with anthracyclines.

- Epipodophyllotoxins

Podophyllotoxins were isolated from Podophyllum pelatum. Two important synthetic substances with therapeutic activity, etoposide and tenoposide, have been designed. These agents are interacting with topoisomerase 2 as well, by building a ternary complex. The result is an accumulation of DNA which leads to cell death (6, 32).

Mitotic inhibitors

Most drugs from this groups derive from natural products, such as vinca alkaloids, colchicine, taxanes, podophyllotoxin and epothilones. Common feature of these substances is there highest toxicity during the M-phase of the cell cycle, by blocking mitosis at the mitotic spindle. Nevertheless they can cause damage in each phase of the cells cycle(23).

- Vincaalkaloids

The potency of the Vinca catharanthus rosea or simply Vinca rosea has already been known for a long time and has been described in folklore. As all mitotic inhibitors the vinca alkaloids are substances, which have their highest toxicity in a specific phase of the cell cycle. That means they have their highest toxicity in the M-phase, by blocking the polymerization of alpha and beta tubulin in the microtubules. Due to the absence of a properly formed mitotic spindle, the chromosomes are not able to align properly at the cells equator prior to cell divison, resulting in immediate cell death and apoptosis (6, 23).

- Taxanes

Paclitaxel was isolated in 1971 from Taxus baccata. Just like vinca alkaloids, paclitaxel and docetaxel interact with the beta subunit of tubulin, but they bind to another side of beta tubulin so that formation of microtubules is promoted instead of blocked. The result is an arrest in M-phase. It is important to not combine taxanes with drugs which block the cell cycle before the M-phase because they would antagonize the taxanes (6, 23).

Estramustine

Estramustine is a combination of estradiol and nornitrogen mustard. The aim of this combination was to create a substance which was useful in treating estradiol-sensitive prostate cancer. Nevertheless clinical activity was modest in most cancers, so that the substance is rarely used in modern oncology (6).

Enzymes

In the early 1950, Kidd realized that a component from a guinea pig serum is very active against leukemia. Further on it was possible to produce L-Asparaginase, which proved to be a newly discovered antileukemic agent in guinea pig serum in Escherichia coli. L- Asparaginase is an enzyme which is essential for leukemic cell growth. Malignant cells, unlike normal tissue, are not able to synthesize L-asparagine but can acquire circulating asparagine. After the donation of L-Asparagin, the enzyme catalyzes hydrolysis from asparagin to aspartic acid. Due to the arising lack of L-asparagin, malignant lymphoid cells start to induce apoptotic

programs. It is often used in combination with methotrexate, doxorubicin, vincristine and prednisolone in hematological malignancies (6).

Targeted drugs

Due to increasing knowledge about molecular pathogenesis of cancer, new “targeted” drugs have been approved for cancer treatment. These new drugs exhibit high specificity, interacting with molecular targets of cancer growth. The substances can either block several deregulated molecular signaling pathways, interact with tumor angiogenesis or inhibit abnormal growth factor receptors. Chemically, these targeted drugs can be divided in two major classes: antibodies and small molecules(7, 23). Antibodies work by activating immune cells or deactivating the targets function by creating an antibody-antigen complex whereas small molecules may attack the same pathway but are further able to enter a cells cytosol. Inside the cell, they can inhibit particular enzymatic functions. Important targets already treated in clinical routine are kinases like receptor tyrosine kinases or BCR/ABL. There are several inhibitors of the epidermal growth factor receptor (EGFR), for instance, where both antibodies like cetuximab and small molecules like gefitinib are in use. Bevacizumab (antibody) and sunitinib (small molecule) are both inhibiting the tumor´s angiogenesis by inhibiting the vascular endothelial growth factor (VEGF) and its receptor (VEGFR). This may results in tumor shrinkage through the tumors incapability to create new blood vessels (6).

Alkylating agents

Alkylating drugs work in each phase of the cell cycle by adding alkyl groups to nucleic acids or proteins. Especially guanine is known for bonding with alkylating substances. After the addition of the alkyl group, guanine is becoming more acidic, mispairs with cytosine and loses its stability. Due to the second chloroethyl-group, bifunctional alkylating agents are able to alkylate two nucleic acids at the same time. This leads to a cross-linkage of the DNA (6, 23).

Further on, the DNA/RNA is destroyed and reproduction and physiological cell functions are no longer feasible. As nearly every chemotherapeutic agent, alkylating agents do not only effect cancer cells but all proliferative cells, which is the reason for their toxic adverse effects (23, 27). At the moment there are six different classes of alkylating substances in usage. Nitrogen mustards, such as cyclophosphamide and ifosfamide, which were both developed from mechlorethamine. Mechlorethamine was the first nitrogen mustard in clinical use.

A specialized group are ethyleneimines. Their molecular mechanism of action is yet to be fully understood. The other alkylating substances are alkyl sulfonates, nitrosoureas, triazenes and methylhydrazines.

Alkylating agents distinguish in side effects. Some of them, like ifosfamide and melphalan, exhibit very high bone marrow toxicity. Due to following suppression of humoral and cellular immunity they are also used in autoimmune diseases, which can lead to several opportunistic infections. Alkylating drugs are also very toxic to mucosal cells and neurotoxic, which might increase their emetogenic potential (6).

Platinum analogues

The three most important substances in this class are cisplatin, carboplatin and oxaliplatin. This group of chemotherapeutic agents works similar to alkylating agents, forming complexes with DNA. The difference between alkylating agents and platinum analogues is irreversibility of platinum-mediated DNA-complexes (27).

2.3.3 Practical use of chemotherapy – administration, dosage and limitations

Today, most cancer patients are not treated with just one modality. Most treatment modalities include regimes of several chemotherapeutics and combinations of pharmacotherapy with other treatment modalities such as surgery and radiotherapy, so-called interdisciplinary treatment.

It is important to treat every person individually, so that there is a balance between toxicity and efficacy of drugs or treatments used. Besides age, general performance status and body-mass-index (BMI) are other notable factors important for consideration prior to treatment administration. Not everybody has the same physical and emotional tolerances, which is a very critical fact regarding the adverse events of chemotherapeutics. Other medical conditions like renal or hepatic failure have to be noted as well. Comorbidities might be essential when it comes to specific adverse events such as cardiotoxicity, for instance with anthracyclins. Monitoring of laboratory values as well as organ function is hence essential to observe and secure patient health during treatment.

For most oral chemotherapeutics there is a uniform dosage regimen for all patients, mostly not regarding obesity or age. According to todays treatment standards, optimal doseage

should be calculated using adustments to body surface area. An example for a calculation formula can be seen in figure 6 an in figure 7 for children (6).

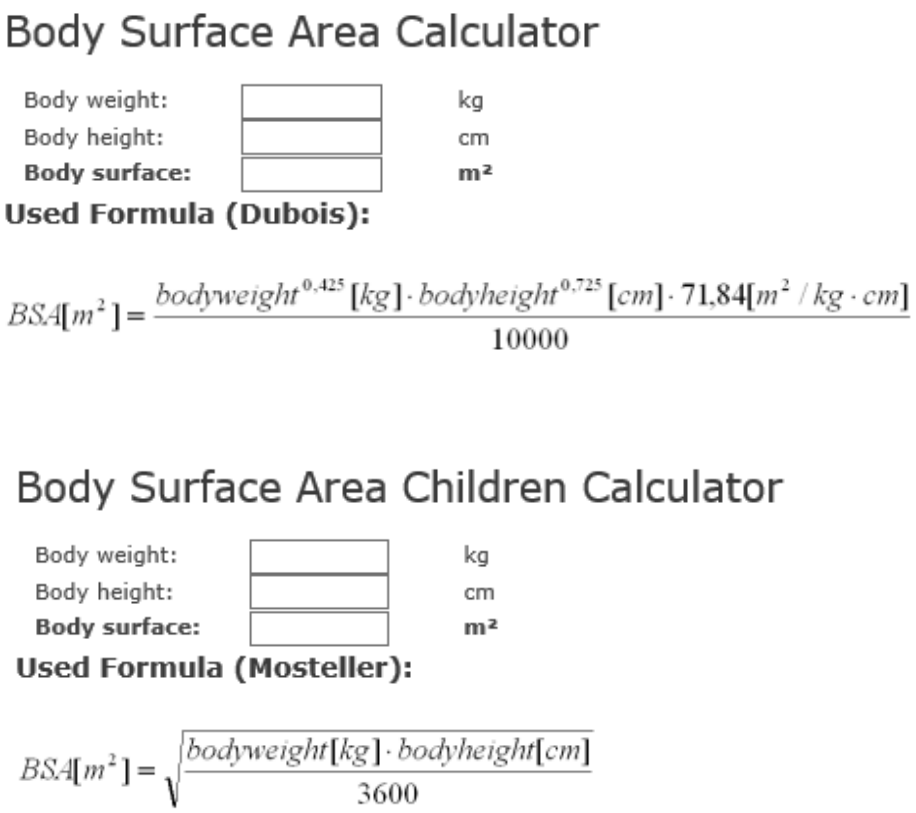

Figure 7: Body surface calculators for adults (upper) and children (lower panel) as used in clinical practice (34)

2.4 Ewing Sarcoma

Ewing sarcoma is the second most regular malignant bone tumour following osteosarcoma. This specific sarcoma can either have characteristics of ectodermal or mesodermal origin. For this reason it can be hard to classify ES (35). The uncommon disease is mostly developed by young adults, but sometimes the extrasceletal type of ES appears in adults. Nevertheless, the

average age at diagnosis is 15 (1). Although within the last years there was an improvement regarding the survival of patients with localized tumors, the survival of people with a highly malignant ES that has metastasized to regional lymph nodes and/or distant organs is still dissatisfying (36). The treatment of the aggressive sarcoma is neoadjuvant and adjuvant chemotherapy in combination with either surgery and radiotherapy or radiotherapy alone (37).

2.4.1 Epidemiology and risk factors

ES belongs to the Ewing sarcoma family of tumors (EFST). The EFST includes both classical ES the askin tumor and the peripheral primitive neuroectodermal tumor. All of them mostly appear in children. There is a higher risk developing this kind of cancer within the male white population. 25% off ES cancer cells are located in the soft tissues and three quarters initially develop in the bone. A quarter of ES are already metastasized at the time of diagnosis. Regarding prognosis there are three different groups of patients: patients with localized tumors, patients with lung metastases only and patients with multiple metastases (36).

Over the last 30 years the incidence of ES has not changed significantly (38). Young people are more likely to suffer from ES. The incidence in patients aged 10-19 years is 9 to 10 cases per one million and the incidence at all age is one case per one million people (0,1:100.000) (38).

It is known that white people are more likely to develop ES than black people. There is also a male predominance or “androtropism” described for ES (36). The paucity of cases of this specific cancer in black people could be explained as the result of a specific polymorphism in the EGR2 gene (39).

In several studies, different prognostic factors have been found, but there is currently no internationally accepted risk classification scheme for patients with this special malignant bone tumor. Factors as patient sex and age, tumor size, site, fever, serum lactate dehydrogenase concentration, anemia and many treatment variables appear in several studies as important factors concerning overall survival, but there is a huge variation between these studies, due to different criteria used (36). A consensus among all studies is the difference between the percentages of survival with or without metastases at the time of primary diagnosis. Survival rates in patients with localized disease is higher than in patients with metastatic ES (60-70% compared to 20-40%). Within the metastatic group at the time of diagnosis, there is also a significant difference in 5-year overall survival concerning the location of the metastases (bone metastases <20% 5y OS compared with lung/pleural metastases 20-40%) (1).

2.4.2 Clinical features

2.4.2.1 Diagnosis

The first signs of ES, just like with any other malignant bone tumor, are very unspecific. The patients which are affected by ES attend a doctor due to pain or a swelling in bone area. "Bone growth" or sport injuries are often mistaken for causes of pain (35). The median time from the first symptoms to the diagnosis is between two and five months (40). For exact staging, several different screenings and a biopsy is necessary. For extent of disease, ES patients may be examined using whole body magnet resonance (MR) or computer tomography (CT) scans. The exact specification of involved bones, bone marrow, soft tissues

and nerves or vessels is important for planning of local therapy (also see chapter “therapy”). The most effective way to precisely describe metastases in general was reported to be MR. Whilst positron emission tomography (PET) was better in detecting lymph node and bony metastases, CT was more advantageous for the detection of lung metastases (36).

2.4.3 EURO-E.W.I.N.G. 99

The EURO EWING 99 trial was a prospective, randomized multicenter study. The objectives of this study were the comparison of the administration of different chemotherapy regimes (VAI vs. VAC) in patients without metastases after VIDE chemotherapy or VIDE chemotherapy and radiotherapy. (EURO EWING 99) Further on, a high dose busulfan-mephalan chemotherapy followed by autologous stem-cell transplantation was performed. 281 patients with ES where included from 1999-2008. The mean age at diagnosis was 16,2 years. Primary endpoints were the overall survival (OS) and the event-free survival (EFS), which were 27% and 34% after 4 years. Further on, different prognostic factors have been investigated. Due to the outcome of the EURO EWING trial new guidelines, concerning the treatement of ES, have been developed.(41).

2.4.4 Prognostic factors

As already mentioned, survival rates do highly vary between patients with localized ES and patents wit primarily metastatic disease. The five-year survival in patients with localized tumors is 55 to 70% (42-44) (EURO EWING 99). Within the last years, the EFS has increased

rapidly (45). Several factors are influencing the survival prognosis, such as volume of the tumor, tumor site, histological response to chemotherapy and types of chromosomal rearrangements (46). In patients with primary metastatic ES, disease-free survival was shown to be 20-40% (44, 47). Prognosis highly varies between different sites of metastasis. The worst prognosis was shown for patients with bone marrow metastasis (3-year EFS of < 10%). As mentioned before, the outcome for patients with non-pulmonary metastasis is worse. Patients with single pulmonary metastasis have the best outcome within the metastatic group of patients (3-year EFS of 40%-50%) (41, 48, 49).

2.4.5 Molecular characterization

2.4.5.1 EWS/FLI and other oncogenes in Ewing Sarcoma

Typically, in most of ES a chromosomal translocation t(11;22)(q24;q12) is found resulting in a gene fusion concerning the EWS-FLI1 oncoprotein. FLI1 is a transcription factor of the ETS-family which, when overexpressed, can drive cell proliferation. In 85% of patients, the reason for the abnormal cell proliferation is the hence created fusion protein called EWS/FLI1 (13). The EWS/FLI1 fusion complex works as an oncogenic protein. Its function is not only important for activation of transcription of oncogenes but also for the repression of transcription of tumor suppressor genes (50).

Another pathway which is affected by EWS/FLI1 fusion is the RAS pathway. In about half of the tumors of patients with ES RASSF2, a gene interacting with members of the RAS superfamily of genes is methylated (51). Not only in ES but in several cancers, like prostate cancer, there is an overexpression of ETS transcription factors. It is proven that some

members of the ETS-family are even able to mimic RAS-pathway overexpression. Thus overexpression results in MAPK independent activation the RAS/MAPK pathway (52).

2.4.5.2 Tumor suppressor genes in Ewing Sarcoma

The chromosomal translocation and the resulting fusion protein is important step for the genesis of most of ES, but for an oncologic phenotype more mutations are required. Often, the tumor suppressor genes p53 and p16 are affected. In 10-20% of ES patients defect p53 and $p16^{INK4a}$ pathways were detected (53). P16 is encoded by the CDKN2A gene and p53 by the TP53 gene. P16 and p53 are both tumor suppressor genes and play important roles in the regulation of the cell cycle. In several studies, alterations of these two tumor suppressor genes were shown to be a prognostic factor. In a recent trial, on the other hand, there was no significant difference in patient outcome when correlated with loss-of-functions of the mentioned tumor suppressor genes (54-57). This reflects vast heterogeneity in ES carcinogenesis on a molecular level.

2.4.6 Treatment

2.4.6.1 Treatment plan

As ES is a rare but highly malignant disease, patients receive a complex multimodal treatment which in most cases includes chemotherapy, surgery and radiotherapy (1). Due to the combination of several chemotherapeutic agents survival of patients with localized ES has

improved from 10% to 75%. Unfortunately, there has not been a similar change within patients with metastatic ES (36, 38).

The treatment plan in the United States and the European countries is differing, especially concerning neoadjuvant chemotherapy (1). The current European treatment plan includes neoadjuvant chemotherapy with VIDE, local surgery and/or a radiotherapy and adjuvant chemotherapy with VAC or VAI. North America´s treatment standards of ES differs from the European regimen concerning the chemotherapeutic agents. Alternatively, the VDC-IE protocol (vincristine, doxorubicin, cyclophosphamide, ifosfamide and etoposide) is given every two weeks (dose-dense variant) or every three weeks for patients with localized disease, for a total of 14 cycles (36).

2.4.6.1.1 Local therapy

Local therapy includes either surgery, radiotherapy or both. When James Ewing first described ES, radiosensivity of ES was already known and appreciated in the clinical community. Within the last decades it became clear, that radiotherapy as a sole treatment would not be enough to significantly increase patient survival rates. Especially in large tumors there was a high risk for relapses. Nowadays, surgery or a combination of surgery and radiotherapy is always preferred compared to radiotherapy alone (1).

Surgeries are always advised when a marginal or a wide resection seems possible. Radiotherapy is only suggested for inoperable tumors. But also at this point of disease, there is some controversy concerning the different treatment plans. Postoperative radiotherapy is always advised for patients with incomplete resections (as defined as R1 or Rx in histological

reports). In Europe, patients with a completely resected tumor sometimes receive postoperative radiotherapy in the case of poor histologic response to the preoperative chemotherapy. In the EE99 trial and the Euro-Ewing 2012 trial, where radiotherapy has been broadened, it became clear that postoperative radiotherapy is useful even after good response to chemotherapy. Still, risks and benefits of high dosed radiotherapy have to be evaluated in each patient individually (1).

2.4.6.1.2 Chemotherapy

Chemotherapeutic agents in patients with ES where first used in the 1960s. Nowadays in Europe, there are four drugs which are given at once: Vincristine, ifosfamide, doxorubicin and etoposide (VIDE). This combination is considered the most effective treatment, although there have been studies with a combination of six different chemotherapeutic agents (VIDE + cyclophosphamide and actinomycin D) for patients with localized ES (58). The positive effect of etoposide depends on the spread of the tumor at diagnosis. Only patients with localized ES do profit from a therapy with etoposide (48).

Continuing chemotherapy after local therapy - VAI (vincristine, actinomycin, ifosfamide) for men or VAC (vincristine-actinomycin-cyclophosphamide) for women - is common in Europe. The currently used VIDE protocol emerged from VACA protocol (vincristine, doxorubicin, cyclophosphamide, actinomycin D) via VAIA (which uses ifosfamide instead of cyclophosphamide) to EVAIA (enclosing etoposide) to VIDE (without actinomycin D) (36). In recent studies, investigators tried to use the increased knowledge of the molecular biological characteristics of ES to tailor treatment. Further on, the aim is to develop targeted agents.

Especially the EWS/FLI1 fusion protein has been in the focus of investigation, but until now there was barely any clinically significant success in finding such novel and more specific treatments (59-61).

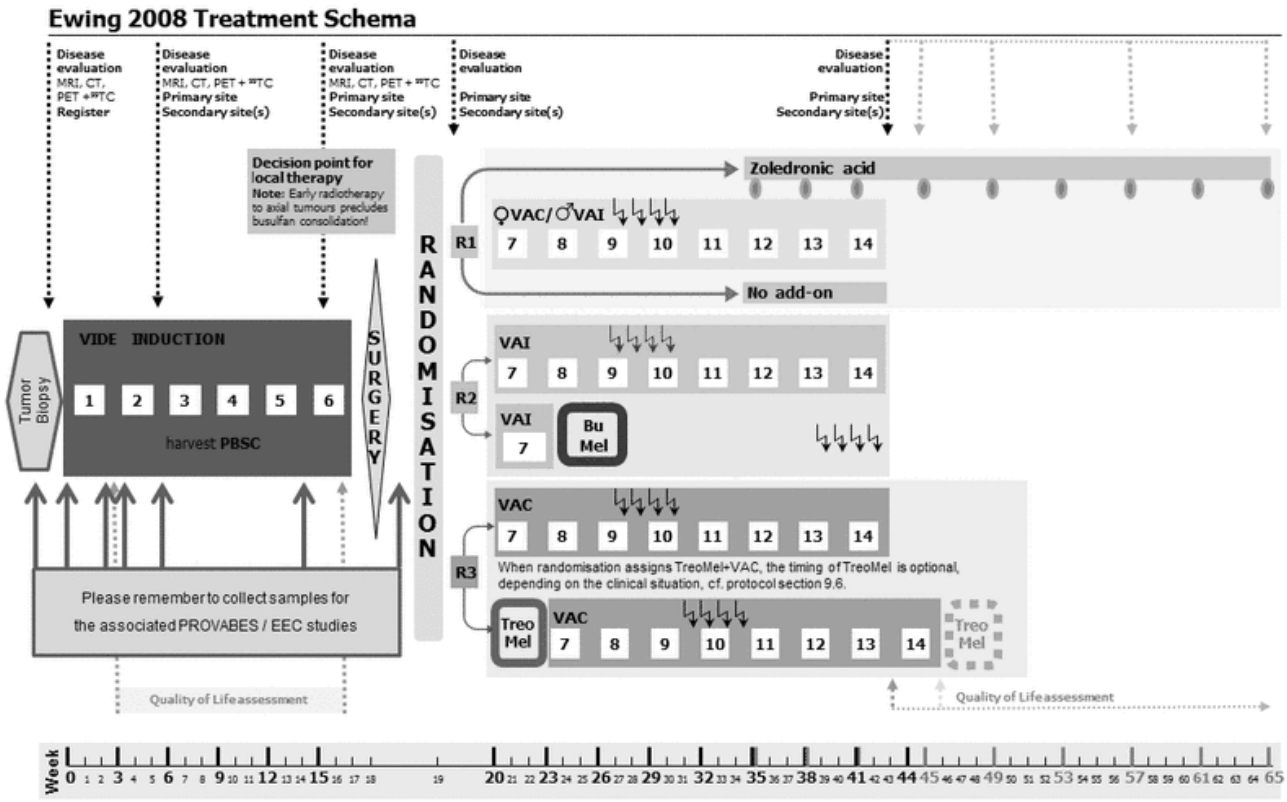

Figure 8: Flowchart of the VIDE Ewing treatment as used in clinical studies (62)

2.4.6.1.2.1 Vincristine

As mentioned before, vincristine is an alkaloid from catharantus roseus (vinca rosa). Vincrsitine has a high binding affinity to the beta–subunit of the tubulin protein. Due to its binding to tubulin, mitosis is stopped, potentially causing apoptosis of the whole cell. Most of the substance is excreted biliary. The half-life period of vincristine is 85 hours and its plasma

protein binding is 44% (63). Vincristine has two main indications. On the one hand it is used in combination with steroids in pediatric leukemia and on the other hand it is frequently used in combination with alkylating agents for childhood sarcomas. The key adverse event is considered neural toxicity. Beside neurological adverse events, vincristine also influences the bone marrow and hence leads to alopecia as well as nausea, obstipation and diarrhea (6).

2.4.6.1.2.2 Ifosfamide

Ifosfamide is an alkylating agent. Its structure is comparable to cyclophosphamide. Like all alkylating agents, it causes strand breaks in the DNA in each phase of the cell cycle by directly interacting its nucleotides. Ifosfamide is known for high neurotoxicity, nephrotoxicity, renal toxicity and toxicity due to platelet suppression (thrombocytopenia). The half-life ifosfamide is around 1,5 hours (6, 64, 65).

2.4.6.1.2.3 Etoposide

Etoposide is a chemical derivate of podophyllotoxin and a topoisomerase inhibitor. Due to the ternary DNA-etoposide-topoisomerase complex the relegation of the DNA is not possible (63). Etoposide has its highest toxicity in the S and G2 phases of the cell cycle. The common assumption is that the drugs sugar residues are binding to DNA, while the ABC-rings interact with topoisomerase enzyme. The whole mechanism leads to cell cycle arrest and apoptosis of rapidly dividing cells such as cancer cells but also mucosal, hair follicle or bone marrow cells, explaining most of the side-effects observed.

The elimination is mostly renal and its half-time period is 6,4 hours. The bioavailability is 48-72% whereas plasma protein binding is 94% (63). Due to high plasma protein binding, the dose should be reduced in patients with liver disease although the elimination via renal effusion is more important. Etoposide is used for testicular tumors, small cell carcinomas of the lung, non-Hodgkin´s lymphomas, nonlymphocytic leukemia and Kaposi sarcoma. The most frequent side effects of etoposide are myelosuppression. Other side effects like nausea and alopecia occur less often (6).

2.4.6.1.2.4 Doxorubicin

This anthracycline antitumor antibiotic is produced by bacterial species or more precisely by different serotypes of Streptomyces species. High toxicity of doxorubicin is not only caused by one mechanism but via several functions, which are essential for the cytotoxic effect as well.

Doxorubicin, as all anthracyclines, inhibits topoisomerase II. Doxorubicin stabilizes the DNA complex with topoisomerase II. The result is a double-strand break. Due to blocked reunion of two DNA strands, the cell will die.

Beside the DNA intercalation and the following inhibition of topoisomerase II, doxorubicin is also an alkylating and DNA cross-linking agent. Further on, doxorubicin interacts with lipids of the cell membrane ultimately destroying the integrity of the membrane. Last but not least, anthracyclines cause the production of reactive oxygen species (ROS). All these mechanisms lead to induction of apoptosis or necrosis.

The half-life period of Doxorubicin is 30-50 hours. It is mainly eliminated biliary. Doxorubicin has a high plasma protein binding of 79-85% (63). It is used for several cancerous diseases, like nephroblastoma, breast cancer, lymphoma and others but is also a mainstay chemotherapeutic agent for various sarcomas such as ES (32). Besides classical cytotoxic side-effects, the most important adverse event is acute and long term cardiotoxicity. Patients should hence be monitored by echocardiography and heart enzyme measurements (66).

2.4.6.1.3 Side effects of chemotherapy

Since the chemotherapeutics used in patients with ES, are not only affecting the cancer cells but also normal body cells there are several adverse events that patients have to know about. Some of them are associated with age or gender but most of them occur in all patients.

2.4.6.1.3.1 Neutropenia

Neutropenia and the resulting danger of infection is the most common side effect in patients tretated with VIDE (47). To decrease this problem the patients is given either filgrastim durng the days of leucopenia or prophylactic pegfilgrastim, but there were significantly reduced complications in patients which received the pegylated G-CSF (67). Leukopenia is mainly caused by etoposide, which has a nadir (from Arabic, meaning “counterpart”, usually referred to as time-point with lowest concentration of blood cells) at 10-14 days post treatment. Thrombocytopenia is a major side effect of etoposide as well but occurs less frequently (6).

2.4.6.1.3.2 Cardiac toxicity

Doxorubicin is known for its long term cardiac toxicity. The decreasing amount of PPARδ protein expression and cardiac troponin I phosphorylation has been described in a recent trial in rats, and is thought to be the main driver of cardiac toxicity (68). Since most of the patients treated for ES are very young, long term cardiac toxicity of doxorubicin may lead to chronic heart failure later in life (69).

2.4.6.1.3.3 Renal toxicity

The main substance which causes the renal toxicity within VIDE is ifosfamide. As stated before, ifosfamide is an alkylating agent which is activated in vivo. The active metabolite is nephrotoxic by inhibiting thymidine incorporation (70).

2.4.6.1.3.4 Hemoglobin, platelets & WBC

Ifosfamide is the major compound of VIDE combination therapy known for platelet suppression (6). Severe thrombocytopenia should be monitored closely using blood tests since it can cause life-threatening hemorrhages.

3. Aims and Rationale

The purpose of this retrospective study was the description of dose adjustments in adult patients receiving neoadjuvant chemotherapy (VIDE) for ES. Even though OS has improved within the past years, acute and long term toxicity is nevertheless a main problem or patients receiving VIDE (37). Two of the most critical side effects are a loss of function of the bone marrow and infection (47), which mostly has to result in dose adjustments (reduced dose or prolonged therapy interval). We created a database of young adults patients (mean age at diagnosis 29,84) receiving VIDE chemotherapy for ES. With the then analyzed data to objectivy dose reductions, patient characteristics and the hematotoxicity profile during VIDE chemotherapy. Further on, dose adjustments and their consequences towards patient outcome were investigated.

4. Materials and Methods

4.1 Database generation

Patients chemotherapy data were obtained retrospectively using the CATO® software (Becton Dickinson, Franklin Lakes, NJ, USA). CATO is a platform which allows pharmacist and medical doctors to work together closely by preparing the right dose of any pharmaceutical compound and by managing patient´s data on an easy-to-access shared platform. Usually, when admitted to treatment, medical oncologists would order chemotherapeutic agents in previously established fixed chemotherapy regimen protocols in the CATO software user interface. Pharmacists would then cross-check for right dosage, indication and start the production process (also see information provided at http://www.cato.eu/de/home.html).

4.2 Correlation with data from AKIM

Clinical data including laboratory results, radiology reports, patient letters, dates of patient visits, were collected using AKIM (IBM, Armonk, NY, USA), the central software of the general hospital of Vienna, Austria (Allgemeines Krankenhaus der Stadt Wien, Währinger Gürtel 18-20, 1090 Wien), by searching for patients names and corresponding birthdays. Patients (n=46) included in this study have been treated at our center within twelve years (2004-2016). Further on a descriptive analysis of the patients concerning sex, tumor size, metastasis at diagnosis, side of sarcoma, amount of VIDE cycles received, dose adjustment, time to progression, survival and infection profile (CRP>5) has been made. The most important step was the correlation of patient's characteristic with dose adjustments and prognosis.

After database generation, the database was anonymized using randomly assigned patient codes (numbers) with the prefix “E” for ES. The list with corresponding names was held secret from this time-point by prinicipal investigator of the clinic.

Lastly, data from pharmacy and oncology departments was merged to create an Excel Sheet (Microsoft Office Excel®, Microsoft, Redmond, WA, USA) and tables and figures were generated using this software.

4.3 Statistics

As mentioned above, statistical examination was performed using Excel. Standard deviations, means, correlations and significance were also calculated using this program.

5. Results

5.1 Descriptive analysis

In this retrospective study, a total number of 46 patients were identified, who received VIDE at our institution. 40 of those patients had confirmed histology of ES as diagnosed by an experienced and specialized pathologist of our institution. 39 of them received more than one cycle of VIDE at our institution and were followed up for further analysis. Hematotoxicity data was available for 38 of 39 patients, who were included in the study collective.

The mean age at diagnosis was 29,84 years, whereas the youngest patient was 15 and the oldest 65 years old. The mean body size was 176cm and the mean weight was 77,92kg, reflecting androtropism of the disease as reported in the literature (see table 1). In fact 3o (65,21 %) of the patients were male and 16 (34,79%) were female (see figure 9).

Table 1 - Patient characteristics

Patient characteristic (mean)	
Age at diagnosis (y) (n=46)	15-65 (average 29,84)
Size (cm)	151-192 (average 176)
Weight (kg)	46-110 (average 77,92)

Table 2 - Information about VIDE cycles administered

Characteristic	Number (Percentage) Total number of patients: 46
Patients who received VIDE w/o histology of ES	2 (4,34%)
Patients who received <2 cycles of chemotherapy at our center	5 (10,87%)
Mean number of VIDE cycles per patient	4,84(1-8)
Total cycles of VIDE administered	223
Cycles with reduced dose of VIDE	95 (42,60%)
Cycles (w/o 1st cycle) with reduction (n=177)	94 (53,11%)
Mean dose reduction	28% of full dose
Ewing patients who received dose reductions (full data for 39 of 46 patients with histology „Ewing“ and >1 cycle of chemotherapy at our center)	28 (71,79%)
Ewing patients w/o dose reductions	11 (28,21%)

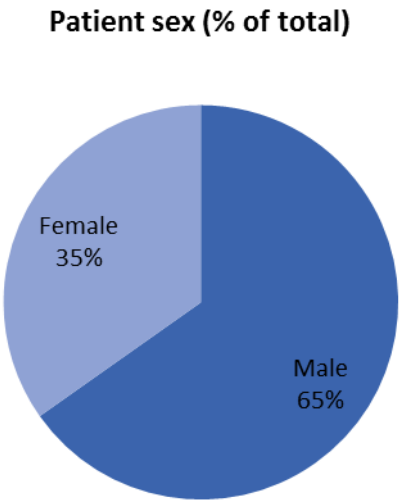

Figure 9: Patient sex

The mean number of administered VIDE cycles per patient was 4,84 (range x-y) and the total number of administered chemotherapy cycles of VIDE was 233. 95 cycles were administered with reduced dose. Not taking into account the first cycle of chemotherapy (n=177) 94 dose reduced cycles were observed, representing 53,11% of the total not first cycle therapies. Seven patients were excluded from final analysis. Two of them due to missing definitive histology of ES and five because they received less than two cycles of chemotherapy at our center and then left our institution. 28 of the 39 patients (71,80%) included for analysis had at least one dose reduction during their course of treatment. The mean dose reduction was 28% of full dose. Eleven patients received full dose of chemotherapy within all six cycles (see table 2).

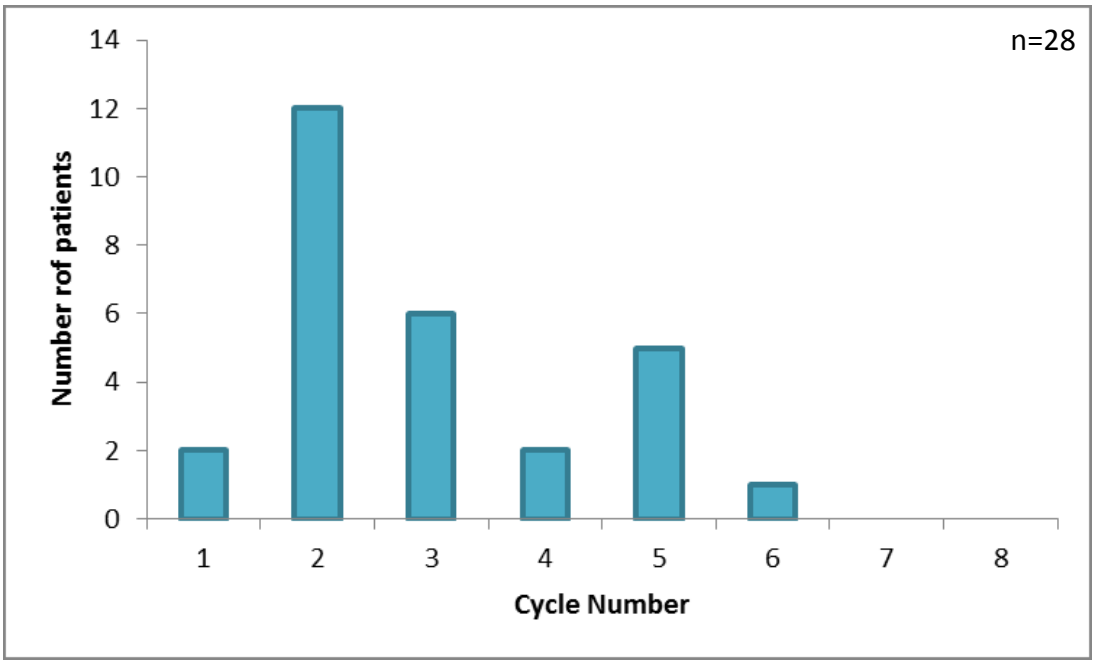

Figure 10: Dose adjustments

This figure shows at which cycle dose adjustment took place. Two patients started the chemotherapy with reduced dose from the beginning due to reduced performance status. In twelve patients the administered chemotherapy was first adjusted at the second cycle. Six patients received dose reductions at the third cycle, two at the forth. Six further patients got a reduction after the forth cycle. In those patients receiving a seventh and an eight cycle, the dose was remaining as it was in the sixth cycle. Dose reductions were maintained at the reduced level through the following cycles (see figure 10).

5.2 Side effect analysis

All patients treated at our institution routinely received bone marrow support with subcutaneously administered pegylated GCSF (e.g. Neulasta®) and antibiotic treatment with

ciprofloxacin and fluconazole during chemotherapy nadir (day 7 to 14 after 1st day of chemotherapy when lowest blood cell counts are expected) to prevent severe side effects.

Complete data of hematotoxicity with more than 1 cycle at our institution and histology of an ES were available of 38 patients. Regarding those 209 cycles of administered VIDE chemotherapy, there were 52 infections (24,88%) as determined by laboratory findings of elevated CRP in patient serum (>5mg/dl). In 74 of 209 cycles administered, VIDE caused thrombopenia of at least grade 1 (35,41%) defined as less than 100G/L of thrombocytes. In 48 (22,97%) of the cycles, patients where effected by severe anemia of grade 3 (hemoglobin <8mg/dL) and received blood transfusions. After 69 of the cycles (33,01%) administered, neutropenia of at least grade 1 was seen (Absolute neutrophil count (ANC) < 1,8G/L) (see table3).

Table 3 - Summarized laboratory data

Laboratory finding	Adverse events (209 in total)
CRP > 5mg/dL	52 (24,88%)
Thrombopenia < 100 G/L	74 (35,41%)
Anemia Hb < 8mg/dL	48 (22,97%)
Neutropenia < 1,8 G/L ANC	69 (33,01%)
	n=209 total cycles

Table 4 - Risks for side effect events per patient

Finding	Events per patient (total 38 patients)
Infections per patient (n=38)	1,37
Thrombopenia per patient	1,95
Anemia per patient	1,26
Neutropenia per patient	1,82

The most frequent adverse event per patient was thrombopenia with an amount of 1,95 events per patient. It was followed by neutropenia with 1,82 events found per patient. The amount of infections as defined before was 1,37 per patient and 1,26 anemias of grade 3 or more per patients (see table 4).

5.2.1 Gender analysis

Regarding the 171 administered VIDE cycles (without the first cycles) 59 were received by female patients and 112 by male patients. 36 (61,02%) cycles of female patients have been administered with a reduced dose whereas 50 (44,64%) cycles of male patients have been reduced. The most recent side effect in female patients was thrombopenia 34 (2,62 events per patient) followed by neutropenia with an amount of 29 (2,23 per patients) events and infections with an amount of 1,92 events per patients (25 in total). Anemia occurred in 20 cycles which is an amount of 1,54 events per patient. In male patients were less side effects observed than in female patients (8,31 per female patient vs 5,4 per male patient.) The most

frequent adverse event in male patients were neutropenia and thrombopenia (each 40 events in total and 1,6 events per patient) followed by anemia with am amount of 28 (1,12 per patient) and infections with an amount of 27 (1,08 per patient) (see table 5).

Table 5 - Gender analysis

Patient characteristic	Female	Male
Number (n=38)	13	25
Cycles administered (w/o first cycle)	59	112
Cycles with dose reductions	36 (61,02%)	50 (44,64%)
Thrombopenia	34 (2,62 per patient)	40 (1,6 per patient)
Anemia	20 (1,54 per patient)	28 (1,12 per patient)
Neutropenia	29 (2,23 per patient)	40 (1,60 per patient)
CRP>5mg/dl	25 (1,92 per patient)	27 (1,08 per patient)

5.2.2 Age analysis

13 patients were older than 35 years at the time of diagnosis and 25 patients were diagnosed before they have reached the 34th year of life. The percentage of dose reduced cycle was 42,75% (56 reduced cycles) in patients younger than 35 and 42,25% (30 reduced cycles) in

patients older than 35. Patients younger than 35 had an average of 7,08 adverse events (177 in total) and patients older than 35 had an average of 5,08 adverse events (66 in total). The most recent side effect was thrombopenia in both groups (2,12 per patient <35 and 1,62 per patient >35) followed by neutropenia (2,04 vs 1,38 events per patient) and infections with an amount of 1,52 per patient relative 1,08 in patient older than 35.In Patients younger than 35 anemia occurred in 35 administered cycles (1,4 per patient) and in 13 cycles (1 per patient) in patients older than 35 at diagnosis (see table 6).

Table 6 – Age group analysis

Patient characteristic	<35 years of age	>35 years of age
Number	25	13
Cycles administered (w/o first cycle)	138	71
Cycles with dose reductions	56 (42,75%)	30 (42,25%)
Thrombopenia	53 (2,12 per patient)	21 (1,62 per patient)
Anemia	35 (1,4 per patient)	13 (1 per patient)
Neutropenia	51 (2,04 per patient)	18 (1,38 per patient)
CRP>5mg/dl	38 (1,52 per patient)	14 (1,08 per patient)

5.3 Clinical outcome (Time to progression, overall survival)

At study inclusion, 21 of the patients (n=38) had a primary metastasized disease. 13 suffered from recurrence of disease after primary treatment. A total of 12 deaths was observed. For patients succumbing to disease, mean overall survival from diagnosis was 2,19 years (mean age at death 32,02 years). There was no significant difference between number of dose reductions in patients dying versus patients surviving (see table 7).

Table 7 – Clinical outcome analysis

Clinical outcome analysis	Events for total of 38 patients
Patients with metastases at diagnosis	21 (55,26%)
Patients with localized disease	17 (44,74%)
Patients with recurrent disease	13 (34,21%)
Age at death (y) (n=11)	32,02
Survival (y) (n=11)	2,19
Deaths observed	12

6. Discussion

In this retrospective, single center study, we created a database of 46 patients, which were treated with VIDE chemotherapy at our center, the general hospital of Vienna, from 2008 to 2015. The aim of this study was to report patients' outcome with varying dose reductions, since there are limited data or publications dealing with dose reductions of VIDE chemotherapy in patients with an ES.

Although in several studies the complications arising due to the high toxicity of the chemotherapeutic compounds are mentioned and well known, we sought to investigate the role of VIDE in adult patients suffering from ES (71). Most official guidelines for the treatment of ES are based on the "EURO E.W.I.N.G" trial, but the prescribed dosage is rarely administered due to adverse effects. This prospective, multicenter study started in 1999 and included 281 treated patients with a mean age of 16,2 years (41), which results in lower side effects than reported in this study. In our study, the mean age of the patients observed was higher. To our knowledge, a cohort of this age (mean 29,84 years) of patients suffering from ES was not reported in the literature to this date (44). This might be due to the fact that the disease has its peak in children or in younger adults (36, 40, 44). The reason for the older cohort treated at our institution is the nearby located hospital for pediatric ES patients (St. Anna Kinderspital). Due to close collaboration and specialization at this neighboring center, only adult patients were treated at our department and institution.

The oldest patient in our study was 64 years old at diagnosis. The most important difference between young adults or adults and children is the amount of side effects concerning the bone marrow, observed after the administration of chemotherapy. Neutropenia is more often

seen in younger patients (47, 72, 73). In a comparable study with a mean age of 19,6 years, there were two neutropenic events per adult and three per child (72). In our study, we were able to see a similar difference between patients older and younger than 35 years (5,08 vs 7,08 side effects per patient).

We were able to monitor hematoxicity in 209 cycles of VIDE chemotherapy with a total of 213 adverse events. 69 events of neutropenia were monitored, which reflected a somewhat higher amount of 1,82 events per patient.

Concerning gender balance, the study was quite similar to all other studies in younger patients with more male patients than female patients (39) suffering from the examined disease. Concerning the toxicity of VIDE, we observed a difference between male and female patients. In female patients, a higher amount of adverse events occurred than in male patients even though the administered chemotherapy was reduced more frequently in female patients (61,2% vs. 44,64%). Amongst others, one reason for the differing toxicity profiles might be a gender-dependent difference of enzymes playing a role in the metabolism of alkylating agents like ifosfamide (74).

From 46 patients which were diagnosed at our center, we had to exclude eight patients from final analysis. In six patients of the total 46, histology of ES could not be confirmed. These patients received VIDE for other soft tissue neoplasms. One of the patients had less than two cycles of chemotherapy and for 38 patients hematotoxicity data was available.

One could argue, that a patient number of 38 reflects a low number of patients for a retrospective study of this kind, but taking into account the rare cases of the disease,

especially in the not infant population we argue sample size was sufficient for analyses as performed in our study.

In 28 of the patients receiving more than one cycle of chemotherapy, the administered dose was reduced. Most patients got a reduction of dosage after the first cycle of VIDE. In total, 42,6% of cycles were administered with a dose reduction. Regarding the fact that the first cycle is started at full dose in most patients, it made sense to exclude patients not receiving more than one cycle. The result was a surprisingly high reduction rate of 80,34% within the 117 not first cycles.

Importance of this study lies in its ability to describe the amount of dose reductions in a young adult cohort of ES patients. Further on we created a database for patients suffering from ES with age of diagnosis at the end of their third decade of life. Most of the trials investigating ES took place in a pediatric setting so that our cohort is unique. The data hence created provides us with information about risks for patients treated with VIDE at our institution and helps us in treating future patients.

Due to the retrospective character of this study, some data concerning hematotoxicity and prognosis were missing. One of the reasons for missing data was the fact that some of the study patients originated from distant parts of Austria or foreign countries, especially eastern European countries and were hence lost for further follow up, when moving back to their home countries. Also, data concerning fever and hence febrile neutropenia could not be gathered in all cases due to restricted data entry into the patient administration software used at our institution.

In conclusion we present a retrospective study about hematotoxicity and dose reductions in young adults receiving VIDE as treatment for ES. Our results show that VIDE chemotherapy exhibits surprisingly high rates of hematotoxicity in a non infant patient population and hence needed dose reductions. In our subset of patients, we were not able to prove a significant correlation between poor patient survival and dose adjustments, so far. Further, prospective investigations with higher patient numbers are necessary to create age- or gender-specific dose adjustment guidelines.

7. Abbreviations

ES	Ewing sarcoma
DNA	Deoxyribonucleic acid
CDK	Cyclin dependent kinase
MTX	Methotrexate
DHFR	Dehydrofolate reductase
EGFR	Epidermal growth factor receptor
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
BMI	Body mass index
FEST	Family of ES tumors
MR/MRI/MRT	Magnetic resonance imaging
CT	Computed tomography
PET	Positron emission tomography
VAI	Vincristine, Actinomycine, Ifosfamide
VAC	Vincristine, Actinomycine, Cyclophosphamide
OS	Overall survival
EFS	Event-free survival
VIDE	Vincristine, Ifosfamide, Doxorubicine, Etoposide
ROS	Reactive oxygen species
G-CSF	Granulocyte-colony stimulating factor
PPARδ	Peroxisome proliferator-activated receptor delta
WBC	White blood cell
CRP	C-reactive protein

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