STUDY PROTOCOL
in accordance with AMG

A pilot study of peroral Vorinostat (SAHA) in patients with refractory histone deacetylase-positive uterine sarcoma

SAHA-Pilot-2016

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Running head: SAHA-Pilot-2016

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Synopsis

Sponsor	Medical University of Graz
Name	A pilot study of peroral Vorinostat (SAHA) in patients with refractory histone deacetylase-positive uterine sarcoma
Running head	SAHA-Pilot- 2016
Target population (or indication)	Patients with refractory, histone deacetylase-positive, pre-treated **uterine sarcomas** (endometrial stromal sarcoma <ESS>, undifferentiated sarcoma <UUS, leiomyosarcoma <LMS>), adenosarcoma <AS> and carcinosarcoma <CS>) after hysterectomy
Study design and phase	Phase II pilot study
Version of protocol	1
Aims of the clinical trial	Primary aim of the trial The main purpose of this single-arm phase II proof-of-principle- pilot study is to test the **efficacy** of the hydroxamic acid-based HDAC inhibitor Vorinostat (SAHA) as monotherapy in patients with histone deacetylase-positive, progressive, metastatic uterine sarcomas (ESS, UUS, LMS) and mixed epithelial and mesenchymal tumors (CS, AS) after prior anti-proliferative therapy. Secondary aims of the trial Safety/toxicity
Outcome measures (endpoints) of the clinical trial	Primary outcome measure Progression-free survival (PFS) at 3, 6 and 9 months Secondary outcome measures Safety/toxicity
Number of patients	**8**
Total duration of the trial	4 years including follow-up, recruitment period of 3 years
Time schedule	With reference to the trial Recruitment period: 2016-2018 Planned start (FPFV): 06.2016 Planned end (LPLV): 07.2019 With reference to patients Duration of treatment: 3 to 9 months depending on clinical benefit

Main inclusion criteria	• Histologically confirmed metastatic uterine sarcoma (endometrial stromal sarcoma <ESS>, undifferentiated uterine sarcoma <UUS, leiomyosarcoma <LMS>; adenosarcoma <AS> and carcinosarcoma <CS>) • High HDAC-positivity of the tumor determined by immunohistochemistry • Patients must have received prior systemic antineoplastic therapy • Patient is not amenable for curative therapy • Age ≥ 18 years • Estimated life expectancy > 3 months • Measurable disease on CT/MRI (at least one measurable lesion ≥1cm) or chest X-ray (at least one measurable lesion ≥2cm) • Karnofsky performance status of 60-100 • Adequate hematologic, renal and hepatic function • Subject is able to swallow and retain oral medication and does not have uncontrolled emesis • No fertility preserved • Written informed consent
Main exclusion criteria	• Lack of or low expression of HDAC (see 4.1 “Pre-Screening”) • Significant cardiac disease • Other invasive malignant tumor diagnosed within the last 5 years (e.g. metastases from breast cancer in the last 3 years) • Significant bowel obstruction • Severe uncontrolled infection • Known HIV-positivity • Symptomatic brain metastasis or leptomeningeal disease • Pre-existing significant liver disease, severe hepatic impairment (Bilirubin no greater than 1.5 times upper limit of normal (ULN) and/or AST/ALT greater than 2.5 times ULN) • Known history of allergic reaction to vorinostat or similar medications • Systemic therapy or an investigational agent within 21 days prior to study inclusion • Uncontrolled hypertension (sustained systolic blood pressure > 150 mmHg or diastolic pressure > 100 mmHg despite optimal medical management) • Major surgery within 3 weeks of enrollment when diagnosed at an early stage • Symptomatic congestive heart failure • Unstable angina pectoris or cardiac arrhythmia • Myocardial infarction within last 6 months • Known active hepatitis B or hepatitis C

	• Psychiatric illness/social situations that would limit compliance with study requirements • Prior history of thrombotic or thromboembolic events, unless adequately controlled by anticoagulant therapy
Study medications	Active substance: Vorinostat (superoylanilide hydroxamic acid, SAHA) Commercial name: Zolinza Manufacturer: Merck Sharp and Dohme (MSD)
Treatment plan	Vorinostat, 400 mg (4 capsules á 100mg of Zolinza) orally once daily with food for the first 14 days of a 21 day cycle Treatment will be continued for 4 cycles (treatment period 1) Patients with a response or stable disease after 4 cycles will be continued on vorinostat therapy at the tolerated schedule and dosage until disease progression, unacceptable toxicity or patients' withdrawal of the consent. At the maximum, a total of 12 cycles will be administered over a 9 months period (treatment periods 2 and 3).

1 Scientific Background / Introduction

1.1 Study Diseases

Uterine sarcomas are rare tumors and comprise <5% of all primary uterine malignancies. (D'Angelo and Prat 2010, Kobayashi et al. 2013). The common variants of these mesenchymal tumors are leiomyosarcoma, endometrial stromal and related tumors as well as mixed epithelial and mesenchymal malignancies.

1.1.1 Leiomyosarcoma

Clinical features: **Leiomyosarcoma (LMS)** is the most common subtype of uterine sarcoma, since carcinosarcoma are currently classified as metaplastic carcinomas (see below). It accounts for 63% of all cases. LMS usually occur in women over 40 years of age and usually presents with abnormal vaginal bleeding, palpable pelvic mass and pelvic pain (D'Angelo and Prat 2010).

Pathological features of **LMS** are severe nuclear atypia and high mitotic rate exceeding 15 mitotic figures per 10 high-power fields (>15 MF per 10 HPF).

Immunohistochemistry and molecular pathology: Most tumors are positive for desmin, smooth muscle actin, h-caldesmon und HDAC8. They are often immunoreactive for CD10 and cytokeratins. Conventional LMS express estrogen- (ER), progesterone- (PR) and androgen receptors in about 30-40% of the cases.(De Fusco et al. 1989, Oliva et al. 2002, Kurman et al. 2014).

Prognosis: LMS are very aggressive tumors with poor prognosis even if confined to the uterus and even if diagnosed at an early stage (Abeler et al. 2009, D'Angelo and Prat 2010). The recurrence rate is around 71% (Major et al. 1993). Overall 5 year survival rates range from 15 to 25% (Pelmus et al. 2009).

1.1.2 Endometrial stromal tumors

Clinical features: Endometrial stromal tumors belong to the rarest uterine neoplasms accounting for less than 1% thereof (Puliyath and Nair 2012). Patients typically present with abnormal uterine bleeding and pelvic pain. The uterus may be enlarged or there may be a pelvic mass (Kurihara et al. 2008, Kurman et al. 2014). The pathogenesis of ESS is unknown, but exposure to tamoxifen, unopposed estrogens, and conditions such as polycystic disease of ovary are implicated (Cohen 2004, Puliyath and Nair 2012).

Pathological features: The histopathologic classification of endometrial stromal tumors from 2003 was revised by the WHO in 2014. According to this 2014 WHO classification (Kurman et al. 2014, Ali and Rouzbahman 2015) endometrial stromal tumors are divided into:

1) non-invasive endometrial stromal nodule (ESN), a well-circumscribed benign mesenchymal tumor consisting of uniform cells closely resembling the uterine stromal cells of normal proliferative-phase endometrium;

2) low-grade endometrial stromal sarcoma **(LG-ESS),** an infiltrative tumor with stromal cells cytologically almost identical to those observed in the ESN but associated with low aggressive malignant behaviour,

3) high–grade endometrial stromal sarcoma **(HG-ESS**), malignant tumors of endometrial stromal derivation with round cell morphology sometimes associated with a low- grade spindle cell component and

4) undifferentiated endometrial <uterine> sarcomas (**UES, UUS**). The heterogeneous group of **UES** often lacks specific differentiation and usually bears no morphological resemblance to endometrial sarcoma. (Kurman et al. 2014). They can be subdivided into groups with either uniform or pleomorphic nuclei. (Jakate et al. 2013).

Immunohistochemistry and molecular pathology: Typically but not always, **LG-ESS** are diffusely and strongly positive for CD10, often positive for smooth-muscle actin and occasionally for desmin, but they are negative for h-caldesmon and HDAC8. ESS frequently contain ER and PR (Reich and Regauer 2007). **HG-ESS**: the high-grade round cell areas are CD10, ER and PR negative but show strong diffuse cyclinD1 positivity whereas the low-grade spindle cell component is typically strongly and diffusely CD10, ER and PR positive and shows variable, heterogeneous cyclin D1 expression (>50%) (Lee et al. 2012). **UES** are variably CD10 positive and ER and PR negative or weakly positive. Cyclin D1 can be diffusely positive (Kurihara et al. 2008).

HG-ESS are usually necrotic and express a high mitotic activity (typically >10MF per 10 HPF). The overall 5-year survival rate of patients with low-grade ESS ranges from 68% to 100% whereas that of UES patients is markedly lower - patients usually die within two years (Kurihara et al. 2008, Tanner et al. 2012).

Prognosis: In comparison to LG-ESS, patients with HG-ESS have earlier and more frequent recurrences (often>1 year), (Kurman et al. 2014).

1.1.3 Mixed epithelial and mesenchymal tumors

Clinical features: **Adenosarcoma (AS)** and **carcinosarcoma (CS)** of the uterus (also referred to as malignant mixed mullerian tumor, MMMT) are biphasic neoplasm composed of epithelial and mesenchymal elements. Most women present with vaginal bleeding and uterine enlargement. In AS there may also be a mass pertruding into the vagina (Kurman et al. 2014).

In **AS** the epithelial component is benign or atypical and the stromal component is low-grade malignant. AS with a high-grade sarcomatous component >25%, are classified as “AS with sarcomatous overgrowth”. Around two third of AS occur in postmenopausal women, 30% are found in pre-menopausal patients (Kurman et al. 2014).

Carcinosarcomas typically also occur in post-menopausal women and account for almost one half of all uterine sarcomas. On the basis of the clonal origin of both tumor components, CS are currently thought to be metablastic carcinomas rather than uterine sarcoma (D'Angelo and Prat 2011). They are customarily separated into two types harboring either heterologous or homologous mesenchymal elements. The homologous components of CSs are usually spindle cell sarcoma without obvious differentiation many resemble pleomorphic sarcomas. The most common heterologous elements are malignant skeletal muscle or cartilage resembling either rhabdomyosarcoma (D'Angelo and Prat 2011).

Immunohistochemistry and molecular pathology: In most **AS** without sarcomatous overgrowth, the immunophenotype of the stromal component resembles that of an ESS. Cases with sarcomatous overgrowth show immunoreaction for Ki67 and p53 and loss of CD10, ER and PR expression (Gallardo and Prat 2009, D'Angelo and Prat 2011). **Carcinosarcomas** show only a low expression or absence of steroid receptors (ER, PR) and CD10. ER and PR are only expressed in the carcinomatous component, CD10 only in the sarcomatous elements (Kobayashi et al. 2013).

Prognosis: Carcinosarcomas are highly aggressive tumors. Its 5 year overall survival rate is less than 35% (Ferguson et al. 2007). These tumors are associated with a particularly poor outcome and have a pattern of spread similar to that of high-grade endometrial carcinoma (Ferguson et al. 2007). The prognosis of **AS** is far more favorable than that of CS except when associated with myometrial invasion or sarcomatous overgrowth.

Genetic alterations in uterine sarcomas

Chromosomal and cytogenetic studies have shown some heterogeneous genetic aberrations in **endometrial stromal tumors**. One of the most frequent genetic aberrations found is the t(7;17)(p15;q21) chromosomal translocation (Hennig et al. 1997), mainly present in **LG-ESS** (Dal Cin et al. 1992, Pauwels et al. 1996). At the sites of the 7p15 and 17q21 breakpoints fusion of two zinc-finger proteins, the so-called *JAZF1/JJAZ1* gene fusion was found, quite distinctive for ESS (Koontz et al. 2001, Hrzenjak et al. 2005, Jakate et al. 2013). **High-grade ESS** typically harbour the YWHAE-FAM22 genetic fusion as a result of t(10;17) (q22;p13) translocation (Lee et al. 2012, Lee et al. 2012) These tumors are associated with high-grade morphology and aggressive clinical behaviour which is important for prognostic and therapeutic purposes (Lee et al. 2012, Ried and Gaiser 2012). **Undifferentiated uterine sarcoma** have complex chromosomal changes, including gains of 2q, 4q, 6q, 7p, 9q, 20q and losses of 3q, 10p, 14q (Halbwedl et al. 2005). Although detection of these genetic alterations is undoubtedly an improvement in diagnostics for differentiation between LG-ESS, HG-ESS and UES, clinical utility and potential benefit for therapy needs to be established.

Uterine leiomyosarcomas have both complex numerical and structural chromosomal aberrations and it is suggested that genomic instability is a hallmark of malignancy in uterine smooth muscle cell tumors (Fletcher et al. 1990, Sandberg 2005, Kurman et al. 2014). In particular, frequent losses of 13q, 10q, 16Q, 12p, and 2p as well as occasional gain of 17p, Xp, and 1q were observed (Hu et al. 2001).

In general, in **carcinosarcoma**, gains (85%) are observed more frequently, than losses (30%). Chromosomal amplification is not random and is closely associated with chromosomes 8q and 20q (42 and 70%), (Schipf et al. 2008). The two chromosomes contain oncogenes, c-myc and ZNF 217, which are relevant to various types of tumor. Amplification of c-myc correlates with distant metastases and is a factor of poor prognosis (Kobayashi et al. 2013). The genetic and epigenetic aberrations of As remain largely unknown (Kobayashi et al. 2013).

Up-regulation of nuclear p53 expression which occurs as an early event of tumorigenesis, was associated with carcinosarcoma (73%), LMS (38%) and ESS (27%) (Nordal et al. 1998).

1.2 Therapeutic approaches for uterine sarcomas

For **all types of uterine sarcoma,** patients with operable tumors undergo primary surgery including abdominal hysterectomy ± bilateral salpingo-oophorectomy. In CS usually pelvic lymph node dissection is performed also (Kobayashi et al. 2013). Radiotherapy is uncommonly used as an increased pelvic control rate. In ESS, radiotherapy may be useful in controlling local recurrncies (Reed 2008). In the adjuvant treatment setting, chemotherapy is not established. Its use is mainly palliative (Kobayashi et al. 2013, Serrano and George 2013).

The most frequently used chemotherapeutic options for uterine sarcoma are summarized in Table 1.

Table1: Possible systemic regimens in uterine sarcoma

	Monotherapy	**Combination therapy**
LMS	Trabectedin, Pazopanib	Gemcitabine + Docetaxel Doxorubicin + Ifosfamide
LG-ESS	Progestin/aromatase inhibitors, Doxorubicin	
HG-ESS,UES	Ifosfamide, Doxorubicin	Doxorubicin+Ifosfamide
CS, AS	Cisplatin, Ifosfamide, Doxorubicin, Paclitaxel, Carboplatin	Ifosfamide + Cisplatin, Ifosfamide + Paclitaxel

(Hensley et al. 2002, Hensley et al. 2008, Hensley et al. 2008, Powell et al. 2010), (Imesch et al. 2014, Imesch et al. 2014), (Amant et al. 2015) (Sutton et al. 1996) (Thanopoulou et al. 2015) (Reed 2013, Serrano and George 2013)

Because of tumor rarity, one can hardly expect that novel molecularly targeted therapies will be specifically developed against uterine sarcoma. Therefore, therapies used for other solid tumors, have been investigated with regard to their efficacy for treatment of uterine sarcomas based on research focussed on molecular pathophysiology of these malignancies.

Chemotherapy regimens for **LMS** often include **gemcitabine + docetaxel** (Hensley et al. 2008) as well as **doxorubicin + ifosfamide** (Amant et al. 2015). **Trabectedin** (ET-743), a synthesized molecule, is a marine-derived antineoplastic agent that disrupts the cell cycle and was efficient in phase ll trials for LMS (Demetri et al. 2009, Kobayashi et al. 2013, Amant et al. 2015). Molecular targeted therapies have been conducted to assess the efficacy of pazopanib, mTOR, COX-2 and angiogenesis inhibitors. However, clinical trials with **imatinib**

and sunitinib failed to show improved outcomes as second or third line LMS treatment (Hensley et al. 2009, Kobayashi et al. 2013). A randomized, double-blinded, placebo-controlled phase III trial with the angiogenesis inhibitor **pazopanib** showed a consistent prolongation of progression-free survival of 4.6 vs 1.6 months (van der Graaf et al. 2012). Almost all patients with unresectable metastatic LMS are considered incurable; hence, in almost all cases, the treatment intension for systemic disease is palliative, with the goal of decreasing tumor bulk, diminishing symptoms, improving quality of life, and prolonging survival (Serrano and George 2013).

Therapeutic options for **ESS/UES** have been summarized in-depth in various reviews (Amant et al. 2009, Ioffe et al. 2009, Thanopoulou and Judson 2012) Current primary therapy for endometrial stromal sarcoma is surgery, mainly abdominal hysterectomy. The role of bilateral salpingo-oophorectomy and ovary preservation remain controversial (Amant et al. 2007, Reich and Regauer 2007, Chan et al. 2008, Feng et al. 2013). Lymphadenectomy does not have an effect on survival (Chan et al. 2008).
The expression of molecular targets **for tyrosine kinase inhibitors (TKI)** in ESS and UES was reported (Geller et al. 2004, Moinfar et al. 2005, Park et al. 2013). However, only a few cases of responses to **imatinib** in patients with uterine sarcomas expressing at least one target of TKI have been described. A single case of UES with EGFR expression temporarily responded to imatinib (Mitsuhashi et al. 2007). Furthermore, a complete metabolic response to imatinib mesylate in a patient with a low-grade ESS has been reported (Kalender et al. 2009). In contrast, a retrospective immunohistochemical and molecular analysis of potential targets of TKI in 52 ESS and 13 UES highly question the use of TKI in endometrial stromal tumors (Sardinha et al. 2013, Hrzenjak et al. 2014).

In some studies, a therapeutic use of **progestins** and aromatase inhibitors in the treatment of low-grade ESS has been shown. **Aromatase inhibitors** such **as letrozole** block the enzyme aromatase, which turns androgen into estrogen, thus reducing estrogen production in postmenopausal women. As a consequence, the amount of body estrogen for stimulation of estrogen-receptor positive tumor cells is decreased. Progestins bind to progesterone receptors and downregulate gene transcription. This is especially true for estrogen receptors, leading to the reduction of circulating estrogens and decrease in endometrial gland and stromal proliferation. (Puliyath and Nair 2012) In their review, Thanopolou et al. summarized, among other results, data of 18 patients with recurrent/metastatic ESS treated with aromatase inhibitors. Five complete responses and eleven partial responses were seen. In addition, a negative impact of hormonal replacement therapy in ESS was demonstrated (Thanopoulou and Judson 2012). A high percentage of ESS cases express hormone

receptors, especially estrogen (40-100%) and progesterone (60-100%) receptors (Chu et al. 2003, Pink et al. 2006, Thanopoulou and Judson 2012). On the other hand, contradictory data were published regarding the expression of androgen receptors in uterine sarcomas (Moinfar et al. 2004, Koivisto-Korander et al. 2011). Hormonal therapies seem to have differential efficacy in ESS and UES. ESS tumors frequently express estrogen and progesterone receptors and usually show better response to hormonal therapies. Although hormonal therapy has been shown to be able to stabilize disease or to induce a remission, it must be stressed that this effect depends on the receptor status (Amant et al. 2009). On the other side, **UES** tumors usually do not express hormone receptors and, therefore, are not susceptible to hormonal therapy. When the hormonal armentarium is exhausted, in the absence of hormone receptors or when progression into a high-grade malignancy occurs combination of **doxorubicin and ifosfamide** seem to be active cytotoxic drugs (Sutton et al. 1996, Amant et al. 2009).

Patients with higher stage of **CS**s may be considered for **ifosfamide plus pacitraxel** combination chemotherapy (Homesley et al. 2007, Powell et al. 2010). Adjunctive **ifosfamide and cisplatin** chemotherapy may also improve progression-free survival. Nevertheless, the incidence of tumor recurrence is high and the added toxicity may not justify the use of the combination (Sutton et al. 2000, Kobayashi et al. 2013). Compared to other uterine sarcoma, CSs exhibit a higher response rate to monotherapies (Imesch et al. 2014). There is no adjuvant or palliative chemotherapy-regimen existing for AS treatment. Recurrences are usually treated in the same way as CS (Petru et al. 2014, 4th Edition).

In general, it can be summarized that responses to various chemotherapeutic agents have been poor. There exist only limited treatment options for uterine sarcomas (D'Angelo and Prat 2010, Kobayashi et al. 2013, Serrano and George 2013).

1.3 Study medication

Vorinostat (superoylanilide hydroxamic acid, SAHA), Zolinza

Manufacturer: Merck Sharp and Dohme (MSD)

Histonedeacatylase inhibitor, new approach of targeted therapy

Vorinostat (superoylanilide hydroxamic acid, SAHA), Zolinza manufactured by Merck Sharp and Dohme (MSD), a subsidiary of Merck Co, Inc is a potent, orally active, competitive inhibitor of histone deacetylases (HDAC), prescribing information, see appendix 2. Zolinza was approved by the FDA in 2006 for the treatment of patients with advanced cutaneous T-cell lymphoma (CTCL) who have progressive, persistent, or recurrent disease on or following two systemic therapies (Mann et al. 2007). In addition to activity in hematologic malignancies, vorinostat has demonstrated promising efficacy in clinical trials with wide range of solid malignancies as breast cancer, lung cancer and prostate cancer. These studies, with vorinostat in monotherapy, have been summarized by us in a review (Hrzenjak et al. 2014) and are shown in table 2 (chapter 1.3.4 “Clinical experience”).

Storage and handling: Zolinza can be stored at 20-25°C, excursions permitted between 15-30°C (see prescribing information, Appendix 2). The capsules should not be opened or crushed. Direct contact of the powder in Zolinza capsules with the skin or mucous membranes should be avoided. If such contact occurs it should be thoroughly washed.

1.3.1 Mechanism of Action

Histone deacetylases exert their action during post-translational acetylation of core nucleosomal histones, which affects chromatin structure and thus regulates gene expression. Many cancer types, including uterine sarcoma, are characterized by epigenetic alterations (Boumber and Issa 2011, Ho et al. 2013). Acetylation and deacetylation of histone proteins are chemical modifications controlled by two groups of enzymes – histone acetylases (HATs) and histone deacetylases (HDACs). These enzymes regulate the acetylation level of histone proteins, thereby influencing the chromatin condensation and chromatin susceptibility for different transcription factors. Moreover, not only histone proteins, but also many other cellular proteins, are prone to those epigenetic modifications. Thus, the potential influence of de/acetylation on different cellular events goes far beyond our present knowledge. So far, 18 different isoforms of HDACs are known. The majority of them are zinc-dependent metalloproteases grouped into class I, II and IV, respectively. Class III HDACs are not zinc-

dependent, but NAD^+-dependent enzymes (Verdin et al. 2003, Bieliauskas and Pflum 2008). Because of their localisation both in cell nucleus and in cytoplasm, class II HDACs (HDAC 4, 5, 6, 7, 9 and 10) have a very broad range of substrates making their inhibition of special interest.

Histone deacetylases might be of particular relevance for ESS and UES since HDAC2 and 3 were found to be expressed in all ESS samples with strong immunohistological positivity indicating overexpression in approximately 80% of samples (Hrzenjak et al. 2014). Despite >80% homology on the protein level between HDAC1 and HDAC2, which are both constitutively expressed class I HDACs, HDAC1 was not overexpressed in analysed tumor samples when compared to non-neoplastic endometrial tissue (Hrzenjak et al. 2006).

Further clues on the role of HDAC in ESS and UES-related cell lines have been obtained in studies using vorinostat which is a potent inhibitor of both class I and II HDACs (Marks et al. 2004, Marks et al. 2004, Marks 2007). Vorinostat binds directly to the active centre of histone deacetylases, thereby acting as chelator for zinc ion and blocking the catalytic site of those enzymes (Finnin et al. 1999). In cell culture, vorinostat efficiently inhibits cell growth of various tumor cell types in a micromolar range. These inhibitory effects are mainly based on the activation of apoptosis, inhibition of cell growth or the activation of autophagic mechanisms and seem to be, at least to some degree, cell-type specific (Hrzenjak et al. 2014).

1.3.2 Nonclinical activity

Inhibition of HDAC2 expression by the HDAC-inhibitor sodium valproate resulted in increased inhibition of cell-growth which has been accompanied by G1 arrest and by reduction of the number of cells in the S-phase of the cell cycle, as determined by fluorescence activated cell sorting (FACS). Interestingly, in the endometrial stromal sarcoma cell line ESS-1 (Gunawan et al. 1998), increased growth inhibition was primarily based on arrested cell proliferation and not on apoptosis. The effects of valproate on ESS-1 cells were attributed to the inhibition of HDAC2 which has been verified by comparative studies using cells transfected with HDAC2-specific siRNA.

Experiments of our group and others showed that vorinostat efficiently inhibited growth of endometrial stromal sarcoma (ESS-1) and uterine sarcoma cell lines (MES-SA), (Harker et al. 1983) by influencing different HDAC members (HDAC 2, 3 and 7) (Hrzenjak et al. 2008, Hrzenjak et al. 2010). In MES-SA cells the growth inhibition was based on increased

activation of apoptosis. The cell lines differed in expression of CD10, Cyclin D1, P53 and PTEN-mutation. In contrast to MES-SA cells, ESS-1 cells are positive for CD10, cyclinD1and PTEN mutation but negative for P53. Other *in vitro* studies in ESS-1 with vorinostat showed similar results, indicating inhibition of G1/S transition based on down-regulation of HDAC7 expression. These inhibitory effects were not apoptosis-based, but relied on vorinostat-based activation of autophagy (Hrzenjak et al. 2008). In nude mice xenografts of MES-SA cells 50 mg/kg/day vorinostat administered over three weeks resulted in a more than 50% reduction of tumor size, as compared to a control group (Hrzenjak et al. 2010). This reduction of tumor size was rather based on vorinostat-based activation of apoptosis than on diminished tumor-cell proliferation. Based on these results, the main mechanism of cell growth inhibition by vorinostat seems to depend on the cell-type. On the other hand, these differences might also be dependent on duration of vorinostat treatment and/or the time-interval between treatment and final cell proliferation analysis (Hrzenjak et al. 2014).

1.3.3 Pharmacocinetics/ pharmacodynamics/ toxicology

A pharmacological-toxicological expert report as well as pharmacokinetics and pharmacodynamics are summarized in the "EMA Withdrawal assessment report" for vorinostat from 2008/2009 (Appendix 1) in the "Full prescribing information of Zolinza" by Merck USA (Appendix 2) and by Iwamoto in 2013 (Iwamoto et al. 2013)
http://www.ema.europa.eu/docs/en_GB/document_library/Application_withdrawal_assessment_report/2010/01/WC500063049.pdf.)
https://www.merck.com/product/usa/pi_circulars/z/zolinza/zolinza_pi.pdf
The main reason for the withdrawal of the application for marketing authorisation in the European Union was based on the provisional view of the CHMP (Committee for Medicinal Products for Human Use) that the available data were not sufficient to permit a treatment licence. The main points of criticism were the absence of comparative data and concerns about thromboembolic events.

Since that time much effort has been put into many clinical trials with vorinostat in mono- or combination- therapy of various hematologic malignancies and solid tumors (see next chapter).

1.3.4 Clinical experience

Both clinical trials and post-trial clinical experience have confirmed the efficacy and tolerability of vorinostat.

Searching for “Vorinostat AND solid tumors” currently 5 open clinical trials recruiting patients can be found on http://www.clinicaltrials.gov (see Table 2)

Table 2: Open clinical trials with vorinostat in solid tumors

Title	Phase	Clinical Trials.gov identifier
Hydroxychloroquine + Vorinostat in Advanced Solid Tumors	1	NCT01023737
Vorinostat in Combination With Paclitaxel and Carboplatin in Treating Patients With Metastatic or Recurrent Solid Tumors and HIV Infection	1	NCT01249443
Clinical Study of Vorinostat in Combination With Etoposide in Pediatric Patients < 21 Years at Diagnosis With Refractory Solid Tumors	1,2	NCT01294670
Vorinostat in Children	1,2	NCT01422499
MLN9708 and Vorinostat in Patients With Advanced p53 Mutant Malignancies	1	NCT02042989

Duvic et al describe the long-term tolerability of vorinostat administered to heavily pretreated patients with CTCL (Duvic et al. 2009). Fifteen of the initial 74 patients entered the extension phase after 6 months of basic treatment. Six of these patients continued on vorinostat therapy for more than two years. One patient continued to respond over 1445 days (4 years).

A survey of completed clinical trials with HDAC inhibitor monotherapies in solid tumors was reviewed by out group last year (Table 3)

Table 3: A survey of completed clinical trials with HDAC inhibitor monotherapies in solid tumors (Hrzenjak et al. 2014)

Drug/ Tumor type	Trial phase	Number of patients	Response	First author, year
A) vorinostat				
gastrointestinal cancer	I	16	7 SD (>8 weeks)	Doi et al., 2013
advanced solid tumors	I	57	12 SD, 1 PR	Ramalingam et al., 2010
advanced prostate cancer	II	27	2 SD (84 and 135d)	Bradley et al., 2009
solid tumors	I	18	9 SD, 9 PD	Fujiwara et al., 2009
glioblastoma multiforme GBM	II	66	PFS 6 m 9/52..	Galanis et al., 2009
advanced cancer	I	25	No prolongation of QTs	Munster et al., 2009
relapsed NSCLC	II	16	8 SD (TTP: median 2.3 m)	Traynor et al., 2009
thyroid cancer	II	19	9SD (TTP: median 24w)	Woyach et al., 2009
recurrent/metastatic head and neck cancer	II	12	3SD, 1PR (unconfirmed)	Blumenschein et al., 2008
metastatic breast cancer	II	12	4 SD (4-14m)	Luu et al., 2008
epithial ovarian or peritoneal carcinoma	II	27	1PR, 9 SD (2 PFS >6m)	Modesitt et al., 2008
relapsed or refractory breast, colorectal or NSCLC	II	16	8 SD (TTP: median 33.5d)	Vansteenkiste et al., 2008
mesothelioma	I	13	2 PR, 4SD	Krug et al., 2006
advanced cancer	I	23	PR, 1/14, SD 2/14	Rubin et al., 2006
advanced cancer(solid/hematological: 50/23)	I	73	1 CR, 5 PR (2 unconfirmed) 16 SD	Kelly et al., 2005
B) romidepsin				
thyroid carcinoma	II	20	13 SD	Sherman et al., 2013
head and neck cancer	II	14	2SD	Haigentz et al., 2012
recurrent glioma	I/II	8/35	PFS median 8w, 1 PFS of 6m	Iwamoto et al. 2011
prostate cancer	II	25	2 PR >or=6m	Molife et al., 2010
SCLC	II	16	3SD, PFS median 1.8m	Otterson et al., 2010
colorectal cancer	II	25	4SD	Whitehead et al., 2009
lung cancer	II	18	9SD	Schrump et al., 2008
renal cell cancer	II	25	1 CR	Stadler et al., 2006
C) belinostat				
unresectable hepatocellular carcinoma	I/II	54	PFS (median: 2.64m), SD:45.2%	Yeo et al.,2012
recurrent or refractory thymic epithelial tumors	II	41	PFS at 6m: 46%	Giaccone et al., 2011
epithelial ovariancancer	II	32	PFS (median: 2.3), SD 9/15	Mackay et al., 2010
/micropapillary ovarian tumors			PFS (median: 13.4m), SD 2/12	
advanced malignant pleural mesothelioma	II	13	2SD, PFS (median: 1m)	Ramalingam et al., 2009
advanced refarctory solid tumors	I	46	18SD	Steele et al., 2008
D) panabinostat				
refractory metastatic renal cell carcinoma	II	20	none	Hainsworth et al., 2011
E) entinostat				
pretreated metastatic melanoma	II	28	7SD(4/3), (TTP median: 55/51d)	Hauschield et al., 2008

Abbreviations:

FL: follicular lymphoma; MZL: marginal zone lymphoma; MCL: mantle cell lymphoma; NSCLC: non-small cell lung cancer

CR, complete response; PR, partial response; SD, stable disease; ORR, overall response rate;PFS, progression free survival;

TTP, time to progression; d,days; m, months

Furthermore, Lee and McGuire present a case study of woman with LMS who had progressed through multiple chemotherapeutic agents who achieved a partial response to vorinostat treatment (Lee and McGuire 2012). Stable disease was observed after 6 weeks and shrinkage in both her liver and lung metastases at 3 months. This patient remained on vorinostat for 18 months when she developed progressive disease. Before, she had exhausted all treatment options for effective chemotherapy including first and second line combination chemotherapies. The best and longest duration of response, overall stable disease (OSD) for over 1.5 years, was achieved with vorinostat (400mg/day), with only initial mild nausea which resolved, and with metastases shrinkage.

1.3.5 Benefits and risks to clinical trial participants

All patients enrolled in this study will receive an oral vorinostat treatment as second line therapy after operation and after a prior hormone- or chemotherapy. One advantage of vorinostat is the ease of administration since oral application does not require outpatient treatment. Tumors of all patients included in this study must reveal a high expression of HDAC in IHC (see section 4.1) to have the potential for clinical benefit.

Preclinical data demonstrate that vorinostat is active not only in hematologic malignancies but also in a variety of solid tumors (see last section) and data from early-phase clinical studies (completed or preliminary) are consistent with these observations. This study proposes to establish improved clinical outcomes for patients with advanced uterine sarcoma

Treatment with vorinostat is expected to be well tolerated and it is hypothesized that it will result in delayed disease progression by 3 or more months.

The safety of multiple daily doses of Zolinza was evaluated in hematologic patients as well as patients with non-CTCL cancers.
Due to the significant role of metabolism in elimination of vorinostat, the impact of hepatic dysfunction was studied in patients with varying degrees of hepatic impairment (Ramalingam et al. 2010). The highest dose studied in mild, moderate and severe hepatic impairment was 400, 300 and 200 mg daily respectively. The incidence of Grade 3 or 4 adverse reactions was similar among the hepatic function groups. The most common grade 3 or 4 adverse reaction in this study was thrombocytopenia.

Patients in our study will be carefully monitored for key toxicities that have been observed with vorinostat (see Tables 4 and 5) as described in chapter 4.

Table 4: Summary of safety outcomes of patients with non-CTCL malignancies

	Solid Tumors				Non-CTCL Hematologic Malignancies				Combination Therapy	
	400 mg once daily continuous (N = 49)		All Patients (N=129)		400 mg once daily continuous (N=11)		All Patients (N=105)		All Patients (N=78)	
Number (%) of patients:	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Clinical Adverse Experiences										
With one or more adverse experiences	49	(100)	129	(100.0)	11	(100.0)	105	(100.0)	77	(98.7)
With no adverse experience	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	1	(1.3)
With drug-related adverse experiences‡	46	(93.9)	125	(96.9)	10	(90.9)	103	(98.1)	75	(96.2)
With serious adverse experiences	11	(22.4)	50	(38.8)	6	(54.5)	51	(48.6)	32	(41.0)
With serious drug-related adverse experiences‡	4	(8.2)	18	(14.0)	5	(45.5)	16	(15.2)	16	(20.5)
Who died	2	(4.1)	11	(8.5)	0	(0.0)	10	(9.5)	6	(7.7)
Discontinued due to adverse experiences	7	(14.3)	23	(17.8)	1	(9.1)	5	(4.8)	24	(30.8)
Discontinued due to drug-related adverse experiences‡	5	(10.2)	16	(12.4)	1	(9.1)	4	(3.8)	17	(21.8)
Discontinued due to serious adverse experiences	1	(2.0)	9	(7.0)	1	(9.1)	2	(1.9)	16	(20.5)
Discontinued due to serious drug-related adverse experiences‡	1	(2.0)	5	(3.9)	1	(9.1)	1	(1.0)	11	(14.1)

Source:
http://www.ema.europa.eu/docs/en_GB/document_library/Application_withdrawal_assessment_report/2010/01/WC500063049.pdf

Risk will be further minimized by adherence to inclusion/exclusion selection criteria (see section 3), close safety monitoring (see section 5), dose adjustment guidelines (see section 4.2) and Zolinza prescribing information (Appendix 2). Safety, efficacy and trial data will be reviewed by an independent monitor as outlined in the protocol. Patients will be further monitored for the first signs of deep vein thrombosis and pulmonary embolism at each study visit.

The most common drug-related adverse experiences in patients with solid tumors treated with vorinostat 400 mg once daily were: gastrointestinal (diarrhea, nausea, anorexia, weight decrease, vomiting, constipation, appetite decrease); constitutional symptoms (fatigue, chills); hematologic abnormalities (thrombocytopenia, anemia) and taste disorders (dysgeusia, dry mouth), (Tables 4 and 5). Hyperglycemia and creatinine increase were the most common laboratory adverse experiences.

To assess potential of cardiac rhythm and electrocardiogram alterations and the propensity of vorinostat to prolong QTc interval, a QT assessment study of vorinostat was conducted in advanced-stage cancer patients (Munster et al. 2009). Single supratherapeutic doses of 800 mg vorinostat were generally well tolerated and not associated with prolongation of the QTc interval.

At clinically relevant concentrations, vorinostat is neither an inhibitor nor an inducer of CYP enzymes and is not eliminated via CYP pathways (package insert, see Appendix 2)

Table 5: Vorinostat monotherapy. Summary of specific clinical or laboratory adverse experiences by preferred term (incidence ≥10% in1 or more dose levels)

	CTCL Patients				Solid Tumor Patients				Non-CTCL Hematologic Malignancy Patients			
	400mg QD continuous (N=86)		Total Patients (N=107)		400mg QD continuous (N=54)		Total Patients (N=129)		400mg QD continuous (N=16)		Total Patients (N=105)	
	n	%	n	%	n	%	n	%	n	%	n	%
Diarrhoea	45	(52.3)	57	(53.3)	18	(33.3)	56	(43.4)	9	(56.3)	76	(72.4)
Fatigue	45	(52.3)	66	(61.7)	38	(70.4)	100	(77.5)	12	(75.0)	67	(63.8)
Nausea	35	(40.7)	52	(48.6)	32	(59.3)	91	(70.5)	6	(37.5)	62	(59.0)
Dysgeusia	24	(27.9)	39	(36.4)	10	(18.5)	14	(10.9)	0	(0.0)	12	(11.4)
Thrombocytopenia	22	(25.6)	34	(31.8)	13	(24.1)	24	(18.6)	1	(6.3)	32	(30.5)
Anorexia	21	(24.4)	28	(26.2)	35	(64.8)	85	(65.9)	4	(25.0)	57	(54.3)
Weight Decreased	18	(20.9)	26	(24.3)	16	(29.6)	46	(35.7)	1	(6.3)	26	(24.8)
Alopecia	17	(19.8)	18	(16.8)	6	(11.1)	12	(9.3)	1	(6.3)	12	(11.4)
Muscle Spasms	17	(19.8)	17	(15.9)	6	(11.1)	10	(7.8)	0	(0.0)	9	(8.6)
Blood Creatinine Increased	14	(16.3)	20	(18.7)	20	(37.0)	51	(39.5)	12	(75.0)	35	(33.3)
Chills	14	(16.3)	18	(16.8)	2	(3.7)	8	(6.2)	2	(12.5)	17	(16.2)
Dry Mouth	14	(16.3)	22	(20.6)	5	(9.3)	11	(8.5)	0	(0.0)	10	(9.5)
Constipation	13	(15.1)	15	(14.0)	12	(22.2)	47	(36.4)	5	(31.3)	32	(30.5)
Dizziness	13	(15.1)	15	(14.0)	7	(13.0)	16	(12.4)	4	(25.0)	22	(21.0)
Vomiting	13	(15.1)	21	(19.6)	24	(44.4)	67	(51.9)	3	(18.8)	41	(39.0)
Anaemia	12	(14.0)	17	(15.9)	14	(25.9)	24	(18.6)	0	(0.0)	31	(29.5)
Decreased Appetite	12	(14.0)	16	(15.0)	1	(1.9)	1	(0.8)	0	(0.0)	3	(2.9)
Oedema Peripheral	11	(12.8)	14	(13.1)	5	(9.3)	10	(7.8)	3	(18.8)	17	(16.2)
Headache	10	(11.6)	11	(10.3)	4	(7.4)	13	(10.1)	2	(12.5)	18	(17.1)
Pruritus	10	(11.6)	10	(9.3)	0	(0.0)	3	(2.3)	2	(12.5)	8	(7.6)
Cough	9	(10.5)	17	(15.9)	7	(13.0)	27	(20.9)	6	(37.5)	28	(26.7)
Pyrexia	9	(10.5)	16	(15.0)	6	(11.1)	30	(23.3)	5	(31.3)	23	(21.9)
Upper Respiratory Tract Infection	9	(10.5)	12	(11.2)	3	(5.6)	9	(7.0)	3	(18.8)	14	(13.3)
Abdominal Pain	7	(8.1)	8	(7.5)	6	(11.1)	26	(20.2)	1	(6.3)	17	(16.2)
Dyspnoea	7	(8.1)	9	(8.4)	13	(24.1)	39	(30.2)	9	(56.3)	36	(34.3)
Hyperglycaemia	7	(8.1)	11	(10.3)	19	(35.2)	54	(41.9)	15	(93.8)	57	(54.3)
Aspartate Aminotransferase Increased	6	(7.0)	6	(5.6)	13	(24.1)	32	(24.8)	6	(37.5)	20	(19.0)
Insomnia	6	(7.0)	11	(10.3)	8	(14.8)	15	(11.6)	0	(0.0)	7	(6.7)
Alanine Aminotransferase Increased	5	(5.8)	5	(4.7)	7	(13.0)	24	(18.6)	7	(43.8)	23	(21.9)
Hypokalaemia	5	(5.8)	7	(6.5)	8	(14.8)	21	(16.3)	2	(12.5)	26	(24.8)
Carbon Dioxide Decreased	4	(4.7)	4	(3.7)	3	(5.6)	8	(6.2)	3	(18.8)	5	(4.8)
Dyspepsia	4	(4.7)	5	(4.7)	4	(7.4)	8	(6.2)	0	(0.0)	16	(15.2)
Neutropenia	4	(4.7)	6	(5.6)	0	(0.0)	0	(0.0)	0	(0.0)	11	(10.5)
Blood Lactate Dehydrogenase Increased	3	(3.5)	5	(4.7)	6	(11.1)	11	(8.5)	0	(0.0)	6	(5.7)
Hyperkalaemia	3	(3.5)	3	(2.8)	4	(7.4)	17	(13.2)	1	(6.3)	8	(7.6)
Hypermagnesaemia	3	(3.5)	3	(2.8)	4	(7.4)	10	(7.8)	3	(18.8)	8	(7.6)
Leukopenia	3	(3.5)	4	(3.7)	3	(5.6)	3	(2.3)	0	(0.0)	12	(11.4)
Pain	3	(3.5)	5	(4.7)	2	(3.7)	7	(5.4)	1	(6.3)	15	(14.3)
Pollakiuria	3	(3.5)	4	(3.7)	3	(5.6)	16	(12.4)	1	(6.3)	6	(5.7)
Rash	3	(3.5)	5	(4.7)	0	(0.0)	2	(1.6)	1	(6.3)	13	(12.4)
White Blood Cell Count Decreased	3	(3.5)	3	(2.8)	3	(5.6)	23	(17.8)	8	(50.0)	24	(22.9)
Back Pain	2	(2.3)	4	(3.7)	5	(9.3)	21	(16.3)	1	(6.3)	9	(8.6)
Hypophosphataemia	2	(2.3)	2	(1.9)	2	(3.7)	5	(3.9)	5	(31.3)	21	(20.0)
Muscular Weakness	2	(2.3)	6	(5.6)	1	(1.9)	5	(3.9)	2	(12.5)	8	(7.6)
Asthenia	1	(1.2)	4	(3.7)	4	(7.4)	10	(7.8)	0	(0.0)	18	(17.1)
Blood Albumin Decreased	1	(1.2)	2	(1.9)	7	(13.0)	9	(7.0)	0	(0.0)	7	(6.7)
Blood Alkaline Phosphatase Increased	1	(1.2)	4	(3.7)	9	(16.7)	27	(20.9)	5	(31.3)	24	(22.9)
Blood Glucose Increased	1	(1.2)	1	(0.9)	4	(7.4)	15	(11.6)	0	(0.0)	10	(9.5)
Blood Urea Increased	1	(1.2)	2	(1.9)	8	(14.8)	8	(6.2)	0	(0.0)	7	(6.7)
Dehydration	1	(1.2)	9	(8.4)	6	(11.1)	19	(14.7)	5	(31.3)	26	(24.8)
Hypocalcaemia	1	(1.2)	3	(2.8)	3	(5.6)	23	(17.8)	7	(43.8)	31	(29.5)
Hypoglycaemia	1	(1.2)	1	(0.9)	3	(5.6)	5	(3.9)	2	(12.5)	4	(3.8)
Hyponatraemia	1	(1.2)	1	(0.9)	10	(18.5)	26	(20.2)	5	(31.3)	21	(20.0)
Neutrophil Count Decreased	1	(1.2)	1	(0.9)	2	(3.7)	13	(10.1)	3	(18.8)	15	(14.3)
Prothrombin Time Prolonged	1	(1.2)	2	(1.9)	4	(7.4)	21	(16.3)	11	(68.8)	18	(17.1)
Staphylococcal Infection	1	(1.2)	2	(1.9)	1	(1.9)	1	(0.8)	2	(12.5)	2	(1.9)

A patient is counted only once within a specific preferred term, even if more than 1 adverse experience with specific preferred term occurred.
Adverse experience terms are from MedDRA Version 9.1

Source:

http://www.ema.europa.eu/docs/en_GB/document_library/Application_withdrawal_assessment_report/2010/01/WC500063049.pdf

2 Aim of the trial

Uterine sarcoma is a rare but usually deadly orphan disease. The prognosis of patients has not changed in the last 20 years with an overall five-year survival between 17 und 54% (Trope et al. 2012). Uterine sarcoma patients with extra-uterine disease have a particularly poor prognosis and are candidates for new clinical trials (Trope et al. 2012, Reed 2013, Ledermann and Ray-Coquard 2014).

Surgical excision of the primary tumor is the treatment of choice. Due to the heterogeneity of uterine sarcomas and the limited efficacy of currently available treatment options in general, there exists no uniform treatment pattern for these rare malignancies (Dizon and Birrer 2014). There is currently no standard therapy for patients with recurrent metastatic disease (Puliyath and Nair 2012, Serrano and George 2013).

Histone acetylation plays a central role in the control of gene expression, influencing transcriptional control of many genes, including tumor suppressor genes. Mechanisms that involve hypoacetylation of core nucleosome histone proteins and DNA methylation have been reported to lead to the tight coiling of chromatin, thereby silencing the expression of a variety of genes, including those implicated in the regulation of cell survival, proliferation, differentiation, and apoptosis.

Histone deacetylase inhibitors belong to a novel, powerful class of drugs which are promising for treatment of different hematologic and solid malignancies (Qiu et al. 2013). They have been shown to restore expression of silenced genes by remodeling the tightly coiled chromatin, which leads to the induction of differentiation, and subsequent apoptosis. So far, three different HDAC inhibitors have been approved by the US Food and Drug Administration (FDA): Vorinostat (SAHA, Zolinza) (Marks and Breslow 2007), romidepsin (FK-228, Istodax), (Grant et al. 2010) and panobinostat (LBH589, Farydak), (Richardson et al. 2015).

The HDAC-inhibitor vorinostat (Zolinza) is already approved in the United States, Canada, Australia and Japan for treatment of a specific type of lymphoma. Zolinza is not yet approved for its use in Europe but has been used worldwide in numerous clinical trials (see previous section) in mono- and combination therapy of hematologic malignancies and various solid tumors. Safety and long-term tolerability (>2years) of Zolinza is already evaluated (Duvic et al. 2009)

As we could show, HDAC overexpression is a characteristic feature of uterine sarcoma (Hrzenjak et al. 2006, Hrzenjak et al. 2008, Hrzenjak et al. 2010, Hrzenjak et al. 2014) and uterine sarcoma cell lines (Quan et al. 2014). Therefore, a high HDAC - positivity was elected

as an inclusion criterion in this clinical trial. The selective reduction of tumor cell growth in pre-clinical in vitro studies and tumor xenografts by HDAC-inhibitors confirm the rationale for their therapeutic use in uterine sarcoma (Hrzenjak et al. 2010).

Since HDAC inhibitors including vorinostat represent a potential targeted therapy for the treatment of uterine sarcoma, the current pilot study investigates the use of vorinostat in pre-treated uterine sarcomas.

3 Recruitment and selection of patients

Number of patients: 8

Duration of the trial: 2016-2019

3.1 Inclusion Criteria

- Histologically confirmed diagnosis of metastatic uterine sarcoma (endometrial stromal sarcoma <ESS>, undifferentiated sarcomas <UUS, leiomyosarcoma <LMS>; adenosarcoma <AS> and carcinosarcoma <CS>)
- HDAC-positivity of the tumor determined by immunohistochemistry
- Prior systemic antineoplastic therapy
- Patient is not amenable for acurative therapy
- Women, age ≥ 18 years
- Life expectancy > 3 months
- Measurable (> 1cm) or non-measurable (but radiologically evaluable) disease per RECIST version 1.1 on computed tomography (CT) scan or MRT scan
- Karnofsky performance status of 60-100
- No fertility preserved
- Subject is able to swallow and retain oral medication and does not have uncontrolled emesis.
- Adequate hematologic, renal and hepatic function as follows (within 28 days of enrollment):
    - Bone Marrow: Absolute neutrophil count (ANC) ≥ 1500/mm3 (1.5 × 109/L); Platelets ≥ 100,000/mm3 (100 × 109/L); Hemoglobin ≥ 9.5 g/dL;
    - Renal Function: Serum creatinine ≤ 1.5 × upper limit of normal (ULN) range OR creatinine clearance ≥ 50 mL/min/1.73 m2 (according to local

assessment method) for subjects with creatinine levels above institutional normal;

- Hepatic function: Aspartate aminotransferase (AST) ≤ 2.5 × upper limit of normal; alanine transaminase (ALT) ≤ 2.5 × upper limit of normal; bilirubin ≤ 1.5 × the ULN range. For subjects with liver metastases, AST < 5 × ULN range; ALT < 5 × ULN range. Subjects with Gilbert's Syndrome may have a bilirubin ≥ 1.5 × the ULN range although no evidence of biliary obstruction exists; in this case the AST/ALT values have to be normal.

- Written informed consent

3.2 Exclusion criteria.

- Lack of or low expression of HDAC (tumor material tested by immunohistochemistry, see “pre-screening” in the next section)
- Significant cardiac disease
- Other invasive malignant tumor diagnosed within the last 5 years (e.g. metastases from breast cancer in the last 3 years)
- Significant bowel obstruction
- Severe uncontrolled active infection
- Known HIV-positivity
- Symptomatic brain metastasis or leptomeningeal disease
- Pre-existing liver disease, severe hepatic impairment (Bilirubin no greater than 1.5 times upper limit of normal (ULN)and or AST/ALT greater than 2.5 times ULN
- Known history of allergic reaction to vorinostat or similar medications
- Received systemic therapy or an investigational agent within 21 days prior to study inclusion
- Uncontrolled hypertension (sustained systolic blood pressure > 150 mmHg or diastolic pressure > 100 mmHg despite optimal medical management);
- Major surgery within 3 weeks of enrollment when diagnosed at an early stage.
- Prior history of thrombotic or thromboembolic events, unless adequately controlled by anticoagulant therapy
- Clinically significant uncontrolled condition(s):
    - Symptomatic congestive heart failure;
    - Unstable angina pectoris or cardiac arrhythmia;
    - Myocardial infarction within last 6 months;
    - Known active hepatitis B or hepatitis C

- Psychiatric illness/social situations that would limit compliance with study requirements

4 Study specific activities

Table 6 lists all of the assessments and indicates with an “X” the visits when they are performed. All data obtained from these assessments will be supported in the patient’s source documentation (CRFs). The study procedures outlined in table 6 are described in detail in this section.

4.1 Pre-screening (Visit I)

Signed and dated **Informed Consent** will be obtained prior to the initiation of any screening or study-specific procedures. Subjects will be considered screen failures if the informed consent has been signed but the subject does not meet eligibility criteria (e.g. if there is no high expression of HDAC). The reason for screen failure will be documented in the source document and will be captured in the CRF.

Pre-Screening: All patients considered to be treated in this study will be **pre-screened** for HDAC-expression. The archived tumor material of each patient (all patients underwent surgery before entering the clinical trial) will be examined for the expression of HDAC1, HDAC2 and 3. Only patients with a high HDAC expression fulfilling all other inclusion parameters will enter in the clinical trial. A “high expression” means that at least 1 out of 3 HDACs react highly positive in immunohistochemistry. Tumor specimens will be considered “highly positive” for HDAC if more than 50% of tumor cells reveal an intense staining, determined by two independent observers.

Medical history: The following information will be collected during the Pre-Screening visit:

Complete medical history, including documentation of any clinical significant medical condition, presence and severity of any symptoms and toxicity associated with metastatic malignancy and/or previous treatment.

In addition a detailed oncologic history will be recorded:
- Date of primary diagnosis of malignancy
- Pathology of primary tumor
- Surgical history
- Anticancer and radiation treatment administered (including dates and type of modality);

At each visit, the subject's medical history will be reviewed and clinically significant changes from baseline will be recorded in the source documents and in the adverse event section of the CRF.

Table 6a: Flow chart of study activities (treatment period 1: cycles 1-4)

Period	**Screening period**		**Treatment period 1 (TP1)**				**Final visit or Start of TP2**
Visit (V)	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**
Cycle			**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	
Study week	**-8 until -4**	**- 4 until 0**	**1**	**4**	**7**	**10**	**13**
Activity	**Pre-Screening**	**Screening**	**Start of treatment**				**Decision for Prolongation**
Informed Consent	**X**						
Medical history	**X**						
HDAC of tumor	**X**						
Physical examination		**X**	**X**	**X**	**X**	**X**	**X**
Vital signs		**X**	**X**	**X**	**X**	**X**	**X**
Body height		**X**					
Body weight		**X**	**X**	**X**	**X**	**X**	**X**
Karnofsky p.status		**X**	**X**	**X**	**X**	**X**	**X**
QLQ C30 questionnaire		**X**	**X**	**X**	**X**	**X**	**X**
12- lead- ECG		**X**		**X**[1]	**X**[1]		**X**[1]
Tumor-assessment: CT Chest, abdomen, pelvis		**X**					**X**[4]
Hematology		**X**	**X** **X**[2] **X**[2]	**X** **X**[3]	**X** **X**[3]	**X** **X**[3]	**X**
Chemistry		**X**	**X**	**X**	**X**	**X**	**X**
Urinalysis		**X**	**X**	**X**	**X**	**X**	**X**
Blood coagulation parameters		**X**					
Adverse events (AE/SAE) assessment		**X**	**X**	**X**	**X**	**X**	**X**
Drug dispense/			**X**	**X**	**X**	**X**	**X**
Drug accountability				**X**	**X**	**X**	
Concomitant medication			**X**	**X**	**X**	**X**	**X**

1 Can be done by the patients' practicioner or internist in the previous week
2 Should be done at the study center or by the patients' practicioner or internist in each of the following two weeks
3 Should be done at the study center or by the patients' practicioner or internist in one of the following two weeks
4 Should be done in the clinic in the previous 2 weeks

Table 6b: Flow chart of study activities (treatment period 2: cycles 5-8)

Period	Treatment period 2(TP2)				Final visit or Start of TP3
Visit (V)	VII	VIII	IX	X	XI
Cycle	Cycle 5	Cycle 6	Cycle 7	Cycle 8	
Study week	13	16	19	22	25
Activity					Decision for Prolongation
Physical examination	X	X	X	X	X
Vital signs	X	X	X	X	X
Body weight	X	X	X	X	X
Karnofsky p.status	X	X	X	X	X
QLQ C30 questionnaire	X	X	X	X	X
12- lead- ECG	X[1]		X[1]		X[1]
Tumor-assessment: CT Chest , abdomen, pelvis	X[3]				X[3]
Hematology	X	X X[2]	X X[2]	X X[2]	X
Chemistry	X	X	X	X	X
Urinalysis	X	X	X	X	X
Adverse events (AE incl SAE) assessment	X	X	X	X	X
Drug dispense/	X	X	X	X	X
Drug accountability		X	X	X	
Concomitant medication	X	X	X	X	X

1 Can be done by the patients' practicioner or internist in the previous week
2 Should be done at the study center or by the patients' practicioner or internist in one of the following two weeks
3 Should be done at the clinic in the previous 2 .weeks

Table 6c: Flow chart of study activities (treatment period 3: cycles 9-12)

Period	**Treatment period 3 (TP3)**				**Final visit**	**30±7d Day Follow-up visit**
Visit (V)	**XI**	**XII**	**XIII**	**XIV**	**XV**	**XVI**
Cycle	**Cycle 9**	**Cycle 10**	**Cycle 11**	**Cycle 12**		
Study week	**25**	**28**	**31**	**34**	**37**	**41**
Activity						
Physical examination	X	X	X	X	X	X
Vital signs	X	X	X	X	X	X
Body weight	X	X	X	X	X	X
Karnofsky p.status	X	X	X	X	X	X
QLQ C30 questionnaire	X	X	X	X	X	X
12- lead- ECG	X[1]		X[1]		X[1]	X[1]
Tumor-assessment: CT Chest , abdomen, pelvis	X[3]				X[3]	X[3]
Hematology	X	X X[2]	X X[2]	X X[2]	X	X
Chemistry	X	X	X	X	X	X
Urinalysis	X	X	X	X	X	X
Adverse events (AE incl SAE) assessment	X	X	X	X	X	X
Drug dispense/	X	X	X	X		
Drug accountability	X	X	X	X	X	
Concomitant medication	X	X	X	X	X	X

1 Can be done by the patients' practicioner or internist in the previous week
2 Should be done at the study center or by the patients' practicioner or internist in one of the following two weeks
3 Should be done at the clinic in the previous 2 weeks

4.2 Screening (Visit II)

A complete **physical examination** will be performed at the screening visit and before each cycle. **Vital signs** (heart rate, sitting blood pressure, and body temperature) will be recorded in addition. Height will be recorded at screening visit only. Since all patients underwent hysterectomy prior to the study, no pregnancy test is needed.

Physical exams will be done during all study visits to rule out disease progression. If progression is suspected, subsequent confirmation by CT will follow (see chapter 4.3).

A resting **12-lead electrocardiography (ECG)** will be performed at the screening visit, once during cycles 1 and 2 and before every second study visit by the patients' practitioner/internist or at the study site. In case of clinical significant abnormalities, the clinical evaluation will be performed by a cardiologist who will determine whether any pathologic or clinically significant signs have occurred.

Table 7: Karnofsky performance status

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

Patient reported outcomes: A determination of the Karnofsky status (Table 7 and Appendix 3) (Karnofsky and Buchenal 1949) and of the **quality of life** will be determined at the screening visit and the starting visit of each cycle via QLQ C30 questionnaire (Appendix 4). If the investigator feels that the quality of life of an individual patient is impaired, a dose reduction may be performed at the discretion of the investigator. The dose reduction has to be stated in the CRF.

Clinical Laboratory Tests

Hematology, laboratory chemistry and urinalysis will be collected at the screening visit and all visits before each subsequent cycle and at the final and follow-up visit. All subjects will undergo the laboratory assessments as outlined in Table 8. Blood coagulation will be only determined at the screening visit, afterwards only on the decision of the principal investigator. In the context of this routine blood testing, additional 10ml of blood (5ml of plasma) will be obtained for future biomarker studies.

Table 8: Clinical Laboratory Tests

Hematology	Clinical Chemistry	Urinalysis
Hemoglobin	Sodium	Ketones
Mean corpuscular volume	Potassium	pH
Mean corpuscular hemoglobin concentration	Chloride	Protein
	Blood Urea Nitrogen (BUN)	Blood
Hematocrit	Serum creatinine	Glucose
Red Blood Cell (RBC) count	Glucose	Urobilinogen
White Blood Cell (WBC) count	Calcium	Nitrate
Neutrophils	Magnesium	
Granulocytes	Total protein	
Lymphocytes	Albumin	
Monocytes	Total bilirubin	
Basophils	Serum glutamic-pyruvic transaminase (SGPT/ALT)	
Eosinophils		
Platelet count	Serum glutamic-oxaloacetic transaminase (SGOT/AST)	
(Blood coagulation)		
	Alkaline phosphatase	
	Uric acid	
	Lactate dehydrogenase (LDH)	
	Serum or urine β-HCG*	
	Glomerular filtration rate	
	C-reactive protein (CRP)	

4.3 Treatment

4.3.1 Vorinostat administration

Dispense of the study drug vorinostat will start at visit III. Patients will receive 64 capsules of Zolinza at the starting visit of each cycle. Vorinostat, 400 mg (4 capsules á 100mg of Zolinza) will be administered orally once daily with food for the first 14 days of a 21 day cycle on an outpatient basis. Patients will be instructed to drink at least 2 L/day of fluid to prevent dehydration and should promptly report excessive vomiting or diarrhea and any evidence of deep vein thrombosis to their physician.

Duration of therapy: Patients with a response or stable disease on therapy will be continued on vorinostat therapy at the same schedule and dosage until disease progression, unacceptable toxicity or withdrawal of the consent for up to 9 months (12 cycles at the maximum). A follow- up- visit will be after 30 +/- 5 days after the last day of the last cycle.

4.3.2 Dose modification and delays

If a patient is intolerant to therapy (significant fatigue etc.), the dose will be reduced to 300 mg orally once daily. Any changes from screening assessments, observed prior to dosing, will be recorded in the subject's CRF.

It will be acceptable for individual chemotherapy doses to be delivered within a **“24h”** window, for example: ”Day 7 chemotherapy“ may be delivered on Day 6, Day 7, or Day 8, respectively.

4.3.3 Supportive Care

Antiemetics, antibiotics, blood-transfusions, nutritional support, non-radiation palliative treatment for pain will be used according to institutional policy. Patients will receive 5-HT3-antagonists, according to the investigator’s discretion. Bisphosphonate or denosumab therapy will be used in the case of bone metastases

Erythrocyte-stimulation (e.g.erythropoietin, darbepoetin alpha) and colony stimulating factors (e.g. G-CSF) may be administered as supportive care or to maintain dose intensity or to avoid dose delays according to standard practice, institutional or clinical practice guidelines.

4.3.4 Prior and concomitant therapy

Due to potential interactions of vorinostat with other drugs coadministered, the case report form will capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. Prior therapy with biologic agents including vaccines and immunostimulants are allowed. Concomitant agents are not allowed.

Radiation: Prior treatment with radiation is allowed as long as it was performed no later than 28 days prior to vorinostat treatment.

4.4 Measurement of effect

The measurement of effect for tumor response will be performed at the end of each treatment period (weeks 12, 24, 36) and in the week before the follow-up visit.

RECIST (Version 1.1) for Tumor Response

Response criteria will be assessed using RECIST version 1.1 (Eisenhauer et al. 2009). Changes in the target and non-target lesions over the course of therapy must be evaluated using the criteria listed below.

Eligibility

Subjects with measurable or non-measurable (but radiologically evaluable) disease with at least one lesion outside previously irradiated areas at baseline can have objective tumor response evaluated by RECIST (version 1.1). Measurable disease is defined by the presence of at least one measurable lesion in at least one site which has not received prior radiotherapy. Lesions that have been previously irradiated will be considered non-target lesions. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology, if possible.

Measurability

Measurable Lesions: Lesions accurately measured in at least one dimension with a minimum size of: Longest diameter ≥ 10 mm (CT scan slice thickness no greater than 5 mm) 10-mm caliper measurement by clinical exam

Non-Measurable Lesions All other lesions, including small lesions (longest diameter < 10 mm) as well as truly non-measurable lesions. Lesions considered truly non-measurable include leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast

disease, lymphangitis cutis/pulmonis, and abdominal masses that are not confirmed and followed by imaging techniques.

Measurable Malignant Lymph Nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At Baseline and in follow-up, only the short axis will be measured and followed.

Non-Measurable Malignant Lymph Nodes: Pathological lymph nodes with ≥ 10 to < 15 mm short axis.

Special Considerations Regarding Lesion Measurability

Bone lesions - Bone lesions are considered non-target lesions.

Cystic lesions -Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

"Cystic lesions" thought to represent cystic metastases can be considered measurable lesions if they meet the definition of measurability described above. However, if noncystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment

Tumor lesions situated in a previously irradiated area will be considered non-target lesions.

All baseline evaluations should be performed as closely as possible to the beginning of treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥ 10 mm diameter as assessed using calipers. In the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesion is recommended. All measurements should be taken using calipers and recorded in metric notation, if clinically assessed. When lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken, since it is more objective and may also be reviewed at the end of the study.

Methods of Measurement

Conventional CT should be performed with contiguous axial cuts of 5 mm or less in slice thickness for tumors of the chest, abdomen and pelvis. A scale should be incorporated into

all radiographic measurements. MRI can be performed if required by local law, but must have sponsor/central imaging center approval. Non-axial slices may be of value in the interpretation of paraspinal lesions findings and other lesions that are better appreciated in non-axial planes. Lesions followed on non-axial imaging should be assessed qualitatively (i.e., as CR, Non-CR/Non-PD, unequivocal PD, unequivocal new, NE). Lesions only visible on non-axial imaging are not considered suitable as target lesions.

For accurate objective response evaluation, ultrasound or bone scan should not be used to measure tumor lesions.

The utilization of endoscopy and laparoscopy for objective tumor evaluation is not advised. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.

Cytology and histology can be used to differentiate between benign and malignant fluid collections in cases of new and/or enlarging pleural effusion and/or ascites in which the response will be based on other target or non-target lesions. New effusions or ascites should be considered unknown until cytology confirms whether they are benign or malignant. If cytology is available and suggesting malignancy, the data must be entered into the CRF and will be considered in the determination of progression. While fluid collections are present, the response determination cannot be considered CR.

Baseline Documentation of "Target" and "Non-Target" Lesions

All measurable lesions, up to a maximum of 2 lesions per organ and 5 lesions in total representative of all involved organs should be identified as target lesions and recorded and measured at Baseline. Tumor lesions situated in a previously irradiated area or in an area subjected to other loco-regional therapy are considered non-target lesions.

Lymph nodes merit special mention, since they are normal anatomical structures, which may be visible by imaging, even if not involved by tumor. Pathological nodes, which are defined as measurable and may be identified as target lesions (no more than 2 may be selected), must meet the criterion of a short axis of ≥ 15 mm by CT scan. Only the short axis of these nodes will contribute to the baseline sum. The short axis of the node is the diameter normally used by radiologists to judge whether a node is involved by solid tumor. Nodal size is normally reported as 2 dimensions in the plane in which the image is obtained (for CT scan this is almost always the axial plane). The smaller of these measures is the short axis. For example, an abdominal node, which is reported as being 20 mm × 30 mm has a short axis of 20 mm and qualifies as a malignant, measurable node. In this example, 20 mm should be recorded as the node measurement. All other pathological nodes (those with short axis ≥ 10

mm but < 15 mm) should be considered non-target lesions. Nodes that have a short axis < 10 mm are considered non-pathological and should not be recorded or followed.

A sum of the diameters (SOD) for all target lesions will be calculated and reported as the baseline sum SOD. If lymph nodes are to be included in the sum, then, as noted above, only the short axis is added into the sum. The baseline SOD will be used as reference by which to characterize the objective tumor response.

All other lesions (or sites of disease), including pathological lymph nodes, CNS metastases and skin lesions, should be identified as non-target lesions and should also be recorded at Baseline. Measurements of these lesions are not required, but the presence (stable, increasing, or decreasing) or absence of each should be noted throughout follow-up.

Evaluation of Target Lesions

Complete Response (CR)

The disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.

Partial Response (PR)

At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD)

At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest SOD recorded since the treatment started (baseline or after) or the appearance of one or more new lesions. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.

Stable Disease (SD)

Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest SOD since the treatment started (baseline or after).

Assessment of Target Lesions

Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10 mm on study. This means that when

lymph nodes are included as target lesions, the "sum" of lesions may not be zero, even if complete response criteria are met, since a normal lymph node is defined as having a short axis of < 10 mm. For PR, SD, and PD, the actual short axis measurement of the nodes is to be included in the sum of target lesions. All lesions (nodal and non-nodal) recorded at Baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (< 5 mm). However, sometimes target lesions or lymph nodes become too small to measure. If it is in the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the radiologist believes that the lesion is present, but too small to measure, a default value of 5 mm should be assigned (as derived from the 5-mm CT slice thickness). The measurement of these lesions is potentially non -reproducible; therefore, providing this default value will prevent false responses or progression based upon measurement error.
If interventions occur during the study that affect disease burden, such as surgery, the lesion(s) affected will typically be considered non-evaluable (NE) from that point forward and subsequent time points will be either NE or PD (if evidence of progression is available).

Evaluation of Non-Target Lesions

Complete Response (CR)

The disappearance of all non-target lesions. All lymph nodes must be non-pathological in

size (< 10 mm short axis).

Non-CR/Non-PD

Persistence of one or more non-target lesion(s).

Progressive Disease (PD)

Unequivocal progression of existing non-target lesions.

In this setting, to achieve "unequivocal progression" on the basis of non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in the presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest "increase" in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status. The designation of overall progression solely on the basis of change in non –target disease in the face of SD or PR of target disease, therefore, will be extremely rare.

New Lesions

The appearance of new malignant lesions denotes disease progression. While there are no specific criteria for the identification of new radiographic lesions, the findings of a new lesion should be unequivocal, i.e., not attributable to differences in scanning technique, timing of scanning, phase of contrast administration, change in imaging modality, or possibly representing something other than tumor (e.g., some "new" bone lesions may be simply healing or flare of pre-existing lesions). A lesion identified on a follow-up study in an anatomical location that was not scanned at Baseline is considered a new lesion and will indicate disease progression.

If a new lesion is equivocal (e.g., too small to measure), continued therapy and follow-up evaluation will clarify whether it truly represents new disease. If repeat scans confirm there is a new lesion, then progression should be declared using the date of the initial scan

Calculating Final Response:

Final response will be calculated according to Table 9 and 10 respectively.

Table 9: Response of combined lesion type

Response of Combined Lesion Type			
Target Lesion	**Non-Target Lesion**	**Unequivocal New Lesion***	**Overall Response**
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not evaluated	No	PR
SD	Non-PD or not evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

* Equivocal new lesions will not allow for CR but will otherwise not impact the overall response.

Table 10: Calculating final response for non-measurable disease

Non-Target Lesion	New Lesion	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/Non-PD
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

Definition of Disease Progression

Disease progression will be defined as progression of disease by RECIST (version 1.1). Clinical data that support progression will be collected and submitted for central review.
If the subject experiences symptomatic deterioration and clinical progression is determined by the investigator, every effort will be made to document radiographic or clinical evidence of progression for analysis of the primary endpoint, even after discontinuation of treatment.

Antitumor efficacy

Patients with a response or stable disease after every 4th cycle will be continued on vorinostat therapy at the tolerated schedule and dosage until disease progression, unacceptable toxicity or patients' withdrawal of the consent. At the maximum, a total of 12 cycles will be administered within a 9 months period. If the principal investigator decided that a subject should discontinue the study, a **Final Visit** will be conducted.

4.5 Follow up

All subjects will have one **Follow-up Visit** approximately 30 days after the last dose of study drug.

Post treatment information
will be collected on an individual basis with special emphasis given to overall survival.

5 Safety monitoring and reporting

5.1 Adverse Events

An **adverse event (AE)** is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not the event is considered causally related to the use of the product. Such an event can result from use of the drug as stipulated in the protocol or labeling, as well as from accidental or intentional overdose, drug abuse, or drug withdrawal. Any worsening of a pre-existing condition or illness is considered an adverse event.
Laboratory abnormalities and changes in vital signs are considered to be adverse events only if they result in discontinuation from the study, necessitate therapeutic medical intervention and/or if the Investigator considers them to be adverse events.

The investigator will monitor each subject for clinical and laboratory evidence of adverse events on a routine basis throughout the study. The investigator will assess and record any adverse event in detail including the date of onset, event diagnosis (if known) or sign/symptom, severity, time course (end date, ongoing, intermittent), relationship of the adverse event to study drug, and any action(s) taken. For serious adverse events considered as having "no reasonable possibility" of being associated with study drug, the investigator will provide another cause of the event. For adverse events to be considered intermittent, the events must be of similar nature and severity. Adverse events, whether in response to a query, observed by site personnel, or reported spontaneously by the subject will be recorded. All adverse events will be followed to a satisfactory conclusion.

5.2 Serious Adverse Events

A **serious adverse event (SAE)** is an adverse event that is fatal or life-threatening (independent of the dose), necessitates in-hospital treatment or its prolongation, leads to permanent or serious disability or incapacity or a congenital abnormality or birth defect.

A suspected unexpected serious adverse reaction (SUSAR) is designated as such according to Guideline 2001/20/EG. A serious adverse reaction is deemed unexpected when it is not listed in the corresponding basic document (summary of product characteristics, prescribing information, see Appendix 2).

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

5.3 Reporting obligations (in accordance with the AMG)

Principal investigator: AE, SAE and SUSAR to the sponsor

Reports about adverse events

> **AMG (Austrian Act on Pharmaceutical Products) § 41d.** (1) The principal investigator must inform the sponsor immediately about any serious adverse events, except for those events that, according to the study protocol or the investigator's brochure, need not be reported immediately. The immediate report must be followed by detailed written reports. In immediate reports and subsequent reports, the study participants must be designated by code numbers.
>
> (2) Undesirable events and abnormal laboratory values that are specified as critical circumstances for safety evaluations in the study protocol must be reported to the sponsor in accordance with the reporting requirements within the time limits mentioned in the study protocol.
>
> (3) In the event of the death of a study participant, the investigator must provide the sponsor with all required additional information.
>
> (4) The sponsor must document in detail and in writing all adverse events passed on to him by the investigators. These documents must be presented, upon request, to the appropriate authorities of all contracting parties of the European Economic Area in whose territories the clinical trial is being conducted.

Sponsor:

> **AMG §41e (Austrian Act on Pharmaceutical Products §41e).** (1) The sponsor must ensure that all important information about suspected serious adverse events that occur in the same clinical trial within the country or abroad and were either life-threatening or led to death are recorded and sent to the Federal Agency for Safety in Healthcare as well as the appropriate authorities of all contracting parties of the European Economic Area in whose territories the clinical trial is performed, and are communicated to the competent ethics committees as early as possible, but in any case within seven days after the sponsor received notice of the concerned case and that, subsequently, appropriate information about further measures is passed on within a renewed time period of eight days.
>
> (2) All other suspected serious adverse events that occur in the same clinical trial within the country or abroad must be communicated to the authorities mentioned in paragraph 1 as well as the competent ethics committees as early as possible, but definitely within 15 days from the time the sponsor was first notified of the event(s).
>
> (3) The sponsor must also inform all other investigators of the same clinical trial in accordance with paragraphs 1 and 2. For the entire duration of the clinical trial, once every year, the sponsor must send a list of all suspected severe adverse events that occurred during the entire period of the trial to the authorities named in paragraph 1 and the competent ethics committee, as well as a report about the safety of the study participants.

Since the information about adverse reactions in the prescribing information for the treatment of CTCL with Zolinza is logically restricted primarily to those obtained from clinical trials of CTCL/ hematologic malignancies, the information given in the EMA withdrawal assessment report about the results obtained in clinical trials with solid tumors is used in this protocol (http://www.ema.europa.eu/docs/en_GB/document_library/Application_withdrawal_assessment_report/2010/01/WC500063049.pdf) (Appendix1).

5.4 Termination of the trial

5.4.1 Premature termination of the trial for a proband (drop-out)

One or several of the following circumstances will lead to the premature termination of the trial for a single proband (this proband shall be regarded as a drop-out):

- The proband's withdrawal of her consent
- Intolerable adverse effects
- Occurrence of an exclusion criterion
- Other circumstances that would endanger the health of the proband if she were to continue her participation in the trial
- Non-compliance of the patient

5.4.2 Premature termination of the entire trial

Premature termination of the clinical trial will be considered when the risk-benefit ratio changes markedly for the patient, the use of the study medication is no longer justifiable, the sponsor believes it is necessary to terminate the clinical trial for safety reasons, or when the clinical trial proves to be impracticable.

For the benefit of, and in the interest of the probands, the principal investigator / sponsor may terminate the trial at any time prematurely due to relevant medical or ethical concerns, lack of feasibility or if serious adverse effects or other unforeseen circumstances occur.

In case of premature termination of the trial, the responsible ethics committee and the authorities (BASG) must be informed within 15 days; the reasons for termination must be clearly stated herein.

5.4.3 Termination of the entire trial

The principal investigator / sponsor is responsible for providing all documents required for the notification of the regular or premature termination of the trial to the competent authority (BASG) and the Ethics Committee.

6 Monitoring and Audit – Assurance of data quality

Monitoring and audits will be performed during the clinical trial for the purpose of quality assurance.
The investigator consents to data evaluation being performed by the person in charge of monitoring in order to ensure satisfactory data collection and adherence to the study protocol.
Furthermore, the investigator states that he/she is willing to cooperate with this person and will provide this person with all required information whenever necessary. This includes access to all documents associated with the trial, including study-relevant medical files of patients in original form. The tasks of the investigator include maintenance of these patients' medical files as comprehensively as possible; this includes information concerning medical history, accompanying diseases, inclusion in the trial, data about visits, results of investigations, dispensing medication, and adverse events. The monitor will also be permitted to perform data evaluation and draw comparisons with the relevant medical files in accordance with the SOPs and ICH-GCP guidelines at pre-determined intervals, in order to ensure adherence to the study protocol and continuous registration of data. All original medical reports required as sources for the information given in the CRF or the database will be inspected. The study participants will have given their consent to such inspection by signing the consent form.
The person in charge of monitoring is obliged to treat all information as confidential, and to preserve the basic claims of the study participants in respect of integrity and protection of their privacy.

7 Biometry

This clinical trial will be conducted in a limited number of highly selected and pre-treated patients suffering from recurrent uterine sarcomas which are very rare tumors within a **pilot study.** It is based on a high expression of HDAC in the tumor irrespective of the particular type of the uterine sarcoma.

In a simulation based on 15 cases, a 3 months PFS rate of about 0.2 (20%) and a 6 months PFS rate of about 0.1 (10%), the standard error for the estimation is ~ 0.13 or 0.16 respectively (Kaplan-Meier estimation, standard error: Greenwood), yielding a confidence interval of ~ ±0.25.

Because of the rarity of these tumors, we will only be able to treat 4-8 patients (see below) in a pilot study in a proper time. It might be expected that in case of success vorinostat would possibly qualify for an orphan drug status for uterine sarcomas. Testing of this novel targeted therapy in a pilot study might act as a door–opener for further multi-centre and/or multi-national clinical trials to improve the therapy of these very rare gynecologic malignancies. This pilot study should provide the scientific basis for a further industry-sponsored clinical trial.

To evaluate 4 patients treated per protocol for at least 6 months a total of 8 patients will be included in this study. Reasons for a premature end of treatment will be tumor progression, withdrawal of consent, or toxicity and/or death because of the severity of the underlying disease.

The objective of this pilot study is to assess if the study drug is active with respect to tumor response. This will be evaluated after 3, 6 and 9 months of treatment (at the end of each treatment period) and 1 month after the end of the treatment at the follow-up visit. The statistical evaluation will be descriptive.

Subjects will be considered screen failures if the informed consent has been signed but the subject does not meet eligibility criteria (e.g., immunohistochemical HDAC analysis). The reason for screen failure will be documented in the source document and will be captured in the CRF.

Data handling and record keeping

Case report forms (CRFs) will be provided on plain paper. Records and CRFs will be kept for a minimum of 15 years.

8 Ethical and legal matters

During the implementation of the trial, the (current versions of) following guidelines and laws must be followed in addition to the Declaration of Helsinki (such as):

- Current version of AMG (Austrian Act on Pharmaceutical Products)
- ICH-GCP guideline
- EU directives 2001/20/EC and 2005/28/EC

Vote of the ethics committee / Informed Consent

In accordance with § 40 AMG (Austrian Act on Pharmaceutical Products), the clinical trial will be started only after the competent ethics committee has issued its statement of approval and the appropriate authorities (BASG) have provided their non-prohibition/approval.

Signed informed consent will be obtained from the subject in order to participate in this study. The informed consent, approved by the local Ethics Committee must be signed and dated by each subject prior to undergoing any study procedures or before any prohibited medications are withheld from the subject in order for the subject to participate in this study.

Insurance

During the clinical trial, a no-fault insurance (personal injury protection insurance in accordance with § 47 of the Austrian Act on Medicinal Products and § 32 of the Austrian Act on Pharmaceutical Products) shall be concluded for the patient on behalf of the sponsor. Exact contact data of the insurance company and the policy number must be mentioned in the patient informed consent.

Principal investigator

By signing the study protocol the principal investigator confirms that he has read and understood the study protocol, and will work in accordance with the protocol. The principal investigator guarantees the confidentiality of all information.

Storage and data protection

The registration, transfer, storage and evaluation of personal data in this clinical trial will be subject to legal regulations (AMG - Austrian Act on Pharmaceutical Products - and data protection law). A prerequisite for this purpose is the participant's voluntary consent in the consent form, given prior to her participation in the clinical trial. The participants will be informed of the following as part of the information about this clinical trial:

1. Data obtained in the course of this clinical trial will be recorded on paper forms or electronic data storage devices, treated as strictly confidential, and passed on exclusively to the following persons without mentioning names (pseudonymised):

- the sponsor of the trial for scientific evaluation and assessment of adverse events.

2. If required for the inspection of the clinical trial, persons authorised by the sponsor and committed to secrecy (monitoring, auditing), authorised persons in national and foreign health authorities, and authorised persons of the competent ethics committee may inspect personal data at the study centre.

3. The participant will be informed that she may terminate her participation in the clinical trial at any time without stating reasons and with no subsequent disadvantages. However, due to the legal obligation of documentation (AMG; Austrian Act on Pharmaceutical Products), authorised persons committed to secrecy may be permitted to inspect personal data for test purposes, for a period of time specified by law.

9 Modification of the study protocol

The vote of the ethics committee applies solely to the information contained in the application; it does not include extensions or modifications of the research project undertaken at a later point in time. In case of any modification, an amendment of the study protocol signed by the principal investigator is required. Any modification of the study protocol must be attached as an amendment to all study protocols in circulation. The ethics committee must be informed of all modifications in the study protocol.
Substantial amendments must also be reported to the authorities (BASG).

10 Publication of study results

The clinical trial results will be published by the principal investigator and the scientists involved whether or not the results are favorable in a renowned magazine.

11 Financing

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List of abbreviations

AE	Adverse Event
AMG	Austrian Act on Pharmaceutical Products
CD10	Cluster of Differentiation 10
COX-2	Cyclooxygenase 2
CR	Complete Response
CRF	Case Report Form
CT	Computed Tomography
CTCL	Cutaneous T-cell lymphoma
CYP	Cytochrome P450 Enzymes
d	Days
ECG	Electrocardiogram
EGFR	Epidermal Growth Factor Receptor
EMA	European Medicines Agency
EORTC QLQ-30	European Organization for Research and Treatment of Cancer's core quality of life questionnaire
ER	Estrogen Receptor
ESS	Endometrial stromal sarcomas
ESS-1	Endometrial sarcoma cell line
FACS	Fluorescence activated cell sorting
FDA	US Food and Drug Administration
FL	Follicular lymphoma
FPFV	First patient, first visit
GCP	Good Clinical Practice
HAT	Histone acetylase
HDAC	Histone deacetylase Inhibitor
HG-ESS	High-grade endometrial stromal sarcoma
HIV	Human Immunodeficiency Virus
IC	Informed Consent
ICH	International Conference on Harmonization
IHC	Immunohistochemistry
JAZF1/JJAZ1	Genrearrangement bei LG-ESS
Ki67	Nuclear protein associated with cell proliferation
LG-ESS	Low-grade endometrial stromal sarcoma
LMS	Leiomyosarcoma

LPLV	Last patient, last visit
m	Months
MCL	Mantle cell lymphoma
MES-SA	Uterine sarcoma cell line
MF/HPF	mitotic figures per high power field
CS	Malignant mixed Muellerian tumors, carcinosarcomas
MRI	Magnetic Resonance Imaging
mTOR	Mechanistic Target of Rapamycin
MZL	Marginal zone lymphoma
NSCLC	Non-small cell lung cancer
ORR	Overall response rate
OSD	Overall stable disease
p53	Tumorprotein p53
PE	Physical examination
PFS	Progression-free Survival
PR	Progesterone Receptor
PR	Partial Response
PTCL	Peripheral T-cell lymphoma
PTEN	Phosphatase and Tensin homolog
RECIST	Response Evaluation Criteria in Solid Tumors
SAE	Serious Adverse Event
SAHA	Suberoylanilide hydroxamic acid
SD	Stable disease
SUSAR	Suspected Unexpected Serious Adverse Reaction
TKI	Tyrosine kinase inhibitors
TTP	Time to progression
UES	Undifferentiated endometrial sarcoma
UUS	Undifferentiated uterine sarcomas
WHO	World Health Organisation
YWHAE-FAM22	Genrearrangement bei HG-ESS

List of tables

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Confirmation of the final protocol

The signatories declare that they agree to fulfill their responsibilities within this study in accordance with local law, the Declaration of Helsinki, ICH-GCP and the study protocol as presented.

Principal Investigator /Sponsor representative

Prof. Dr Edgar Petru _____ _____

Date Signature

Responsible Pathologist

PD. Dr. Bernadette Liegl-Atzwanger _____ _____

Date Signature