1. SYNOPSIS

Protocol Title	First in man study to evaluate the safety, tolerability and preliminary efficacy of the Fc-optimized FLT3 antibody FLYSYN for the treatment of acute myeloid leukemia patients with minimal residual disease
Investigational Product	FLYSYN (4G8-SDIEM), a chimeric Fc-optimized monoclonal antibody targeting the FLT3 receptor
Protocol Number	FLYSYN-101
EudraCT Number	2016-000236-17
ClinicalTrials.gov	NCT02789254
Indication	Diagnosis of acute myeloid leukemia (AML), ineligible for allogeneic hematopoietic stem cell transplantation in complete remission (CR) with molecular detection of minimal residual disease (MRD)
Sponsor and CRO	Sponsor: Synimmune GmbH Alte Landstrasse 42 15, 72072 Tuebingen Phone: +49-7071-7708381 FAX: +49-7071-7708383 Email: info@synimmune.de Clinical Research Organization: Center for Clinical Studies University Hospital Tuebingen ZKS Tuebingen Otfried-Mueller-Str. 45 D-72076 Tuebingen Phone: +49-7071-2985635 Fax: +49-7071-2925080 Email: zks@med.uni-tuebingen.de
Principal Investigator	Prof. Dr. H. R. Salih
Study Phase	First in man
Study Purpose/Rationale	The purpose of this study is to characterize the safety profile and preliminary efficacy of FLYSYN as monotherapy in adult subjects with MRD positive AML.
Study Objectives	Primary Objective • To determine the safety profile of the investigational product FLYSYN as monotherapy in MRD positive AML at various dose levels Secondary Objectives • To define the pharmacokinetics and pharmacodynamics profile of the investigational product FLYSYN • To evaluate the immunogenic potential of the investigational product FLYSYN • To evaluate the preliminary efficacy of FLYSYN in subjects with MRD positive AML

Study Endpoints	Primary Endpoint • Incidence and severity of adverse events (AE) (CTCAE V 4.03) until 28 days (i.e. Visit 7, day 29) after dosing for Cohorts 1 to 5 and until 35 days (i.e. Visit 9a, day 64) after last dosing for Cohort 6. Secondary Endpoints • Incidence and severity of adverse events (AE) (CTCAE V 4.03) until 180 days (i.e. Visit 11, day 180) after first dosing • Pharmacokinetics and pharmacodynamics • Immunogenicity of FLYSYN based on both absolute (number and percentage of subjects who develop HAMA/HAHA) and semi-quantitative (HAMA/HAHA titer determination of confirmed positive samples) assessments • Absolute and percent change from baseline in measurements of B, T, and NK cell populations and activation • Absolute changes from baseline in laboratory parameters • Change in cytokines from baseline • Overall molecular response rate, defined as MRD negativity (i.e. no detectable AML-MRD marker gene) or at least one log step reduction of at least one AML-MRD marker gene • Duration of molecular response, time to MRD progression (log step), time to relapse • Absolute change from baseline in overall quality of life scores (EORTC QLQ C-30)
Study Design	This is an open-label, single-arm, first in man multicenter trial to assess the safety and the preliminary efficacy of the antibody FLYSYN in MRD positive AML patients as monotherapy.
Length of study/ Time Lines	Total trial duration: 57 months Duration for individual patient: Study treatment: 18 months FSI (First patient In): 01.02.2017 LSI (Last patient In): 31.01.2020 LSO (Last patient last visit): 31.07.2021 Data base lock: 31.10.2021
Inclusion Criteria	• Age ≥18 years at the time of voluntarily signing an IEC-approved informed consent, there is no upper age limit • Diagnosis of AML according to WHO criteria • Confirmed FLT3 expression on leukemic cells • Known mutational status of FLT3 (FLT3-ITD, FLT3-TKD, FLT3 wild type) • Hematological CR (ANC count >1.000/µL, Thrombocytes > 100.000/µL), but MRD positivity (determined by NGS and NPM1 RT-PCR, where applicable) after any therapy except allogeneic stem cell transplantation • Life expectancy of > 3 months • ECOG performance status ≤ 2 • Subject must be willing to receive transfusion of blood products • Be willing and able to comply with the study protocol for the duration of the study

	• Females of childbearing potential (FCBP) must undergo repetitive pregnancy testing (serum or urine) and results must be negative
	• Reliable contraception should be maintained throughout the study and for 6 months after study treatment
	• Unless practicing complete abstinence from heterosexual intercourse, sexually active FCBP must agree to use adequate contraceptive methods
	• Males (including those who have had a vasectomy) must use an effective barrier method of contraception throughout the study and for 6 months after study treatment if sexually active with a female of childbearing potential
	• All subjects must:
	  ○ understand that the investigational product could have a potential teratogenic risk.
	  ○ be counseled about pregnancy precautions and risks of fetal exposure.
	  ○ be able to comply with all study-related procedures, medication use, and evaluations.

Exclusion Criteria	The presence of ANY of the following criteria will exclude a patient from study enrollment: • Patients proceeding to hematopoietic stem cell transplantation (suitable candidate and donor available, informed consent of patient) • Pregnant or breast feeding females • >5% blasts in bone marrow or extramedullary disease • Treatment with monoclonal antibody within 3 months before treatment with FLYSYN or known immunoglobulin intolerance • Known positivity for HIV, active HBV, HCV, or Hepatitis A infection • No consent for registration, storage and processing of the individual disease-characteristics and course as well as information of the family physician and/or other physicians involved in the treatment about study participation • No consent for biobanking • Presence of any medical/psychiatric condition or laboratory abnormalities which may limit full compliance with the study, increase the risk associated with study participation or study drug administration, or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for entry into this study • Prior history of malignancies, other than AML/myelodysplastic syndrome (MDS), unless the subject has been free of the disease for ≥ 2 years. Exceptions include the following: Basal cell carcinoma of the skin, carcinoma in situ of the cervix, carcinoma in situ of the breast, histological finding of prostate cancer of TNM stage T1 • Patients receiving any medication listed in the Appendix IV “Prohibited Medications” (within 14 days prior to the first dose of study drug) • Uncontrolled infection, e.g. infection progressing under adequate antimicrobial/antifungal/antiviral treatment • Patients under ongoing treatment with another investigational

	medication or having been treated with an investigational medication within 14 days of screening • Current treatment with immunosuppressive agents • Systemic diseases (cardiovascular, renal, hepatic, etc.) that would prevent study treatment (e.g., creatinine >1.5x upper normal serum level; bilirubin, AST or AP >2.5x upper normal serum level; heart failure NYHA III/IV; severe obstructive or restrictive ventilation disorder)
Total Number of Patients	The total number of patients will depend on the numbers of tested dose levels (DL`s) and patients per DL. Assuming that no dose limiting toxicity (DLT) occurs, the estimated maximum number of patients in the study will be 31.
STUDY DRUG **Study Drug and Formulation**	FLYSYN is an Fc-optimized, chimeric monoclonal antibody targeting the FLT3 membrane protein. FLYSYN is formulated in PBS with additives containing 1.7 mg/ml antibody Storage at 4 °C.
Route of Administration	IV infusion over a 3-hr duration
Prophylactic Treatment	Infusion-related reaction is an expected risk of FLYSYN. As a result, the sponsor is mandating prophylaxis with dimetindene, ranitidine and paracetamol prior to each FLYSYN infusion and inpatient application of FLYSYN with an 18 h observation period after the first application.
Recruitment and Dose Regimen	**Cohort 1:** Patient 1-3: FLYSYN 0.5 mg/m² body surface area (BSA) day 1 **Cohort 2:** Patient 4-6: FLYSYN 0.5 mg/m² body surface area (BSA) day 1 FLYSYN 1.0 mg/m² BSA day 2 **Cohort 3:** Patient 7-9: FLYSYN 0.5 mg/m² body surface area (BSA) day 1, FLYSYN 4.5 mg/m² BSA day 2 **Cohort 4:** Patient 10-12 and 13-18*: FLYSYN 0.5 mg/m² body surface area (BSA) day 1, FLYSYN 14.5** mg/m² BSA day 2 **Cohort 5:** Patient 19-21*: FLYSYN 0.5 mg/m² body surface area (BSA) day 1, FLYSYN 44.5** mg/m² BSA day 2 **Cohort 6:** Patient 22-24 and 25-31*: FLYSYN 0.5 mg/m² body surface area (BSA) day 1, FLYSYN 14.5** mg/m² BSA day 2, and 15 mg/m^2 BSA on day 15 and 15 mg/m² BSA on day 29 * If the maximum tolerated dose (MTD) is reached in Cohorts 1-5, the respective cohort will be expanded to a total of 16 patients for assessment of efficacy. ** The maximum upper limit for calculation of antibody dose is fixed at a body surface of 2.0 m^2, even if the calculated body surface exceeds this.

Dose Escalating Scheme

Cohort 1:

Patient No.	1st dose (Day 1)		2nd dose (Day 2)
1 + 2 + 3	→ 0,5 mg/m² *	1 day →	none

↓

21 days (DSMB review)

↓

Cohort 2:

Patient No.	1st dose (Day 1)		2nd dose (Day 2)
4 + 5 + 6	→ 0,5 mg/m²	1 day →	1,0 mg/m² *

↓

21 days (DSMB review)

↓

Cohort 3:

Patient No.	1st dose (Day 1)		2nd dose (Day 2)
7 + 8 + 9	→ 0,5 mg/m²	1 day →	4,5 mg/m² *

↓

21 days (DSMB review)

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Cohort 4:

Patient No.	1st dose (Day 1)		2nd dose (Day 2)
10 + 11 + 12	→ 0,5 mg/m²	1 day →	14,5 mg/m² *

↓

21 days (DSMB review)

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Patient No.	1st dose (Day 1)		2nd dose (Day 2)
13 - 18	→ 0,5 mg/m²	1 day →	14,5 mg/m² *

↓

21 days (DSMB review)

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Cohort 5:

Patient No.	1st dose (Day 1)		2nd dose (Day 2)
19 + 20 + 21	→ 0,5 mg/m²	1 day →	44,5 mg/m² *

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21 days (DSMB review)

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Cohort 6:

Patient No.	1.dose (d1)		2.dose (d2)		3.dose (d15)		4.dose (d29)
22 + 23 + 24	→ 0,5 mg/m²	1 day →	14,5 mg/m² *	→	15 mg/m²	→	15 mg/m²

↓

21 days (DSMB review)

↓

25 – 31	→ 0,5 mg/m²	1 day →	14,5 mg/m² *	→	15 mg/m²	→	15 mg/m²

* There will be a minimum of 48h between this treatment and the first treatment of the next patient within the same Cohort.

	In this study DLT are defined as the following treatment-related adverse events or laboratory abnormalities, graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03: 1. Grade 4 thrombocytopenia, anemia, neutropenia or respective decrease in any other sub-fraction of white lineage that does not resolve to grade 2 or less within 14 days unless attributable to AML progression. 2. Non-hematological toxicity grade 3 or 4 (excluding hypersensitivity reactions and fatigue) unless attributable to AML progression. The decision to proceed to the next dose Cohort will be taken by the principle investigator as well as the biostatistician based on safety data and the recommendations of the DSMB. The DSMB will monitor the study conduct and the safety aspects of the trial on a regular basis, and will give recommendations to the coordinating investigator/the sponsor whether to stop the trial or to amend the trial protocol. A report to the DSMB will be provided after the last patient of Cohorts 1 to 5 has completed visit 5 (day 15). If no patient experiences limiting toxicity and the DSMB has no concerns, the subsequent Cohort will be dosed. If one patient of a Cohort experiences limiting toxicity and the DSMB has no concerns, additional 3 patients will be included in the same Cohort and dosed accordingly. A report to the DSMB will be provided after the last patient of these additional three patients has completed visit 5 (day 15). If no patient of these additional three patients experiences limiting toxicity and the DSMB has no concerns, the subsequent Cohort will be treated. If two or more patients of the first three or six (as the case may be) patients of Cohort 2, 3 or 4 experience limiting toxicity and the DSMB has no concerns, the previous Cohort (i.e. the Cohort with maximum tolerated dose) will be expanded by additional 16 patients. Regular safety reports to the DSMB will be provided after 6 and 16 patients of these additional 16 patients have completed visit 5 (day 15). If two or more patients of the first three or six (as the case may be) patients of Cohort 1 experience limiting toxicity, the study will be terminated. If no patient of the first three patients or no more than one patient of the first six patients of Cohort 4 experience limiting toxicity and the DSMB has no concerns, Cohort 4 will be expanded by six or three (as the case may be) patients (to a total of 9 patients). A report to the DSMB will be provided after patient 9 of Cohort 4 has completed visit 5 (day 15). If the DSMB has concerns to proceed with Cohort 5 and has no concerns to expand Cohort 4, Cohort 4 will be expanded by ten additional patients (to a total of 19 patients). If the DSMB has no concerns, three patients of Cohort 5 will be treated. A report to the DSMB will be provided after the last of these three patients has completed visit 9 (day 43). If one patient of the first three patients of Cohort 5 experiences limiting toxicity and the DSMB has no concerns, additional 3 patients will be included in Cohort 5. A report to the DSMB will be provided after the last patient of these additional three patients has completed visit 5 (day 15). If the DSMB has concerns to proceed with Cohort 6 and has no concerns to expand Cohort 5, Cohort 5 will be expanded by seven or four (as the case may be) additional patients (to a total of 10 patients). If no

	patient of the first three patients or no more than one patient of the first six patients experiences limiting toxicity and the DSMB has no concerns, three patients of Cohort 6 will be treated. A report to the DSMB will be provided after the last of these three patients of Cohort 6 has completed visit 9 (day 43). If no patient experiences limiting toxicity and the DSMB has no concern, Cohort 6 will be expanded by seven patients (to a total of 10 patients). If one patient of the first three patients of Cohort 6 experiences limiting toxicity and the DSMB has no concerns, additional three patients will be treated in Cohort 6. A report to the DSMB will be provided after the last of these additional three patients has completed visit 9 (day 43). If no patient of these additional three patients experiences limiting toxicity and the DSMB has no concerns, Cohort 6 will be expanded by four patients (to a total of 10 patients). A regular safety report to the DSMB will be provided after ten patients of Cohort 6 have completed visit 9 (day 43). If two or more patients of the first three or six (as the case may be) patients of Cohort 6 experience limiting toxicity, Cohort 5 will be expanded by seven or four (as the case may be) additional patients (to a total of 10 patients). **Precautionary Measures** Precautionary measures adequate for a first-in-human study will be undertaken such as ECG and close clinical, in particular cardiovascular, monitoring during the 3-h infusion. Accordingly, the study site must have available emergency resuscitative equipment and personnel trained in the management of anaphylaxis in order to immediately treat systemic reactions under the direct supervision of an experienced physician.
Definition of LT	In this study LT is defined as the following treatment-related adverse events or laboratory abnormalities, graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03: 1. Grade 4 thrombocytopenia, anemia, neutropenia or respective decrease in any other sub-fraction of white lineage that does not resolve to grade 2 or less within 14 days unless attributable to AML progression. 2. Non-hematological toxicity grade 3 or 4 (excluding hypersensitivity reactions and fatigue) unless attributable to AML progression.
Study design and Biometrics	Cohort 1 will be treated as indicated and after the visit on day 15 the treated patients will be evaluated for limiting toxicity and cytopenia. If no limiting toxicity is identified, Cohort 2 will be treated. If limiting toxicity is identified in one patient of Cohort 1, additional 3 patients can be included in Cohort 1 and will be treated accordingly. If no limiting toxicity is identified in these additional 3 patients of cohort 1, cohort 2 will be treated. If limiting toxicity is identified in two or more of the first three or six (as the case may be) patients of Cohort 1, the study will be terminated. Cohort 2 will be treated as indicated and after the visit on day 15 the treated patients will be evaluated for limiting toxicity and cytopenia. If no limiting toxicity is identified, Cohort 3 will be treated. If limiting toxicity is identified in one patient of Cohort 2, additional 3 patients can be included in Cohort 2 and will be treated accordingly. If no limiting toxicity is identified in these additional 3 patients of cohort 2,

	cohort 3 will be treated. If limiting toxicity is identified in two or more of the first three or six (as the case may be) patients of Cohort 2, Cohort 1 will be expanded by 16 additional patients and treated accordingly. Cohort 3 will be treated as indicated and after the visit on day 15 the treated patients will be evaluated for limiting toxicity and cytopenia. If no limiting toxicity is identified, Cohort 4 will be treated. If limiting toxicity is identified in one patient of Cohort 3, additional 3 patients can be included in Cohort 3 and will be treated accordingly. If no limiting toxicity is identified in these additional 3 patients of Cohort 3, Cohort 4 will be treated. If limiting toxicity is identified in two or more of the first three or six (as the case may be) patients of Cohort 3, Cohort 2 will be expanded by 16 additional patients and treated accordingly. Cohort 4 will be treated as indicated and after the visit on day 15 the treated patients will be evaluated for limiting toxicity and cytopenia. If no limiting toxicity is identified, Cohort 4 will be extended by additional 6 patients (to a total of 9 patients) and treated accordingly. If limiting toxicity is identified in one patient of Cohort 4, additional 3 patients can be included in Cohort 4 and will be treated accordingly. If no limiting toxicity is identified in these additional 3 patients of Cohort 4, Cohort 4 will be extended by additional 3 patients (to a total of 9 patients). If limiting toxicity is identified in two or more of the first three or six (as the case may be) patients of Cohort 4, Cohort 3 will be expanded by 16 additional patients and treated accordingly. If no limiting toxicity is identified in 9 patients of Cohort 4, Cohort 5 will be treated, otherwise Cohort 4 will be expanded by additional 10 patients (to a total of 19 patients). Cohort 5 will be treated as indicated and after the visit on day 15 the treated patients will be evaluated for limiting toxicity and cytopenia. If no limiting toxicity is identified, Cohort 6 will be treated. If limiting toxicity is identified in one patient of Cohort 5, additional 3 patients can be included in Cohort 5 and will be treated accordingly. If no limiting toxicity is identified in these additional 3 patients of Cohort 5, Cohort 6 will be treated. If limiting toxicity is identified in two or more of the first three or six (as the case may be) patients of Cohort 5, Cohort 4 will be expanded by 10 additional patients (to a total of 19 patients) and treated accordingly. Cohort 6 will be treated as indicated and after the visit on day 43 the treated patients will be evaluated for limiting toxicity and cytopenia. If no limiting toxicity is identified, Cohort 6 will be extended by additional 7 patients (to a total of 10 patients) and treated accordingly. If limiting toxicity is identified in one patient of Cohort 6, additional 3 patients can be included in Cohort 6 and will be treated accordingly. If no limiting toxicity is identified in these additional 3 patients of cohort 6, cohort 6 will be extended by additional 4 patients (to a total of 10 patients). If limiting toxicity is identified in two or more of the first three or six (as the case may be) patients of Cohort 6, Cohort 5 will be expanded by seven or four (as the case may be) additional patients (to a total of 10 patients) and treated accordingly.

	There will be an interval of at least 48 hours between the completion of the first treatment (2nd dose) of a patient and the first treatment (1st dose) of the next individual patient within a Cohort. Regular safety assessment will be performed after visit 5 (day 15) of patient 9 and 19 (if applicable) of Cohort 4 and of patient 10 of Cohort 5 (if applicable) and after visit 9 (day 43) of patient 10 of Cohort 6 (if applicable). Efficacy parameters will be analyzed for each cohort. Statistical evaluation of efficacy parameter will be conducted for the cohort receiving the maximum tolerated dose consisting of 19 patients or for all patients of Cohort 4, Cohort 5 and Cohort 6 combined. The efficacy read out is defined as MRD negativity (i.e. no detectable AML-MRD marker gene) or one log reduction of at least one AML-MRD marker gene at day 43 which is defined as molecular response. An ineffective treatment is defined as a molecular response rate (P) equal or below 20%, whereas with an effective treatment a molecular response rate of 50% or higher is expected. If the number of molecular responses is 7 or more, the hypothesis that $P \leq 0.20$ is rejected with a target error rate of 0.10 and an actual error rate of 0.068. If the number of molecular responses is 6 or less, the hypothesis that $P \geq 0.50$ is rejected with a target error rate of 0.10 and an actual error rate of 0.084. Power is set at 90%.
Efficacy	If applicable, potential efficacy will be evaluated in terms of overall molecular response rate (ORR), SD, duration of molecular response, time to molecular progression (TTP), and molecular progression-free survival (PFS). The response evaluation will be based on blood and/or bone marrow levels of AML-MRD marker genes and blast cell count.
Safety	Adverse events occurring during the study, graded according to the NCI-CTC-AE version 4.03, will be recorded until study visit 11 (day 180 +/- 5 days). DATA SAFETY MONITORING BOARD The safety data are monitored on a continued basis by an Independent Drug Safety Monitoring Board (DSMB) comprising of 3 experienced physicians. In general, the DSMB will provide recommendations if the study can continue as planned in the protocol, if changes are needed from a safety point or if the MTD was reached.
Immunogenicity test	For measurement of the immunogenicity, anti-drug antibodies (ADA) will be analyzed in the blood samples. Samples will be taken for this examination as follows: Baseline and at Days 15, 22, 29, 43, 90, 180, 365 and 545. If ADA are not detected in Cohorts 1 – 5 at baseline and day 29, no further ADA measurements are required. If ADA are not detected in Cohort 6 at baseline and day 43, no further ADA measurements are required.
Pharmacokinetic sampling	Blood samples to assess pharmacokinetics of FLYSYN will be taken as follows: Cohort 1: Day 1: pre-dose 0, 1.5, 3, 6, 18 hours after start of infusion as well as at Days 2, 8, 15, 22, 29, 36, 43 and 90, Cohort 2, 3, 4 and 5: as Cohort 1 but additional samples are taken on

	Day 2 at pre-dose, 1.5, 3, 6 and 18 hours after start of infusion as well as one additional sample on Day 3. Cohort 6: as Cohorts 2, 3, 4 and 5 but additional samples are taken on days 15 and 29 at pre-dose, 1.5, 3 and 6 hours after start of infusion as well as at Day 64.
Analyses	Safety by CTC-AE (Version 4.03) until 180 (+/- 5) days after the last administration of test product. Cytokine panel including tumor necrosis factor (TNF), interleukin-2-receptor, interleukin-6 (IL-6), interleukin-10 (IL-10), interferon-γ (IFN-γ) at: Cohort 1: Day 1: pre-dose, 1.5, 3, 6 and 18 hours after start of infusion as well as at Days 2, 15 and 22. Cohort 2, 3, 4 and 5: as Cohort 1 but additional samples are taken on Day 2 at pre-dose, 1.5, 3, 6 and 18 hours after start of infusion as well as one additional sample on Day 3. Cohort 6: as Cohorts 2, 3, 4 and 5 but additional samples are taken on Day 15 and Day 29 at pre-dose, 1.5, 3 and 6 hours after start of infusion and at Day 43. No sample will be taken on Day 22. For Cohort 6 also at Day 64. NPM1 or MRD monitoring (in peripheral blood) at baseline, Days 1, 8, 15, 22, 29, 36, 43, 90, 180, 365 and 545. For Cohort 6 also at Day 64. Bone marrow aspirates (BMA) for NPM1 or MRD monitoring at baseline, Days 1, 15, 22, 43, 90, 180, 365 and 545 for Cohorts 1 to 5, and at baseline, Day 1, 15, 29, 43, 90, 180, 365 and 545 for Cohort 6. BMA for AML immunophenotyping at Days 1, 22, 43, 90, 180, 365 and 545 for Cohorts 1 to 5, and at Days 1, 15, 29, 43, 90, 180, 365, and 545 for Cohort 6. BMA for FLT-3 expression (if feasible) at baseline, Days 1, 15, 22 and 43 for Cohorts 1 to 5 and at baseline, Days 1, 15, 29 and 43 for Cohort 6.
Prior and/or Concomitant Medication	No other concurrent anti-cancer agents or therapies
Clinical Trial Centers	Department of Internal Medicine II, University Hospital of Tuebingen Department of Internal Medicine III, University Hospital of Ulm Department of Internal Medicine V, University Hospital of Heidelberg Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School