Synopsis of results: TMD Prevention 2007

COORDINATING INVESTIGATOR	Prof. Dr. Dirk Reinhardt Pediatric Hematology and Oncology Hannover Medical School Carl-Neuberg-Str.1, 30625 Hannover Current address Kinderklinik III, Universitätsklinikum Essen Hufelandstr. 55 45147 Essen
SPONSOR	Medizinische Hochschule Hannover Carl-Neuberg-Str. 1 30625 Hannover, Germany Tel.: +49 (0)511-532 6000 Fax: +49 (0)511-532 6003
TITLE OF STUDY	Elimination of the preleukemic clone in children with Down syndrome (DS) and transient myeloproliferative disorder (TMD) to prevent AML - Model of leukemia prevention TMD Prevention 2007 Version 1 1.2.2007 TMD Prevention 2007 Version 2 11.6.2007 final
CONDITION	Transient myeloproliferative disorder
OBJECTIVE(S)	To demonstrate that monitoring of the GATA1s positive preleukemic clone in TMD and elimination of the clone with cytarabine can reduce the risk of DS-AMKL
INTERVENTION (S)	Experimental intervention: Monitoring of GATA1s positive preleukemic clones and low-dose cytarabine treatment in children with persisting GATA1s clone according to the treatment algorithm outlined in Figure 1 (below). Control intervention: none (historical control) Duration of intervention per patient: 3 weeks
INVESTIGATIONAL MEDICINAL PRODUCT (IMP)	Cytarabine (all approved medicinal products), provided by hospital pharmacy. Dose: Cytarabine 1.5 mg/kg body weight i.v. or s.c. on day 1,2,3,4,5,6,7 (= one course)
INCLUSION AND EXCLUSION CRITERIA	Inclusion criteria: • Diagnosis in one of the study centers (see below) • Age: Newborns/Infants below 3 months of age • Evidence of Down Syndrome / trisomy 21 mosaic (constitutional trisomy 21) • Morphological and/or immunological detection of myeloid blasts (≥5%) in peripheral blood or bone marrow and/or proven GATA1-mutation • Informed consent of parents/custodians Exclusion criteria • Severe concomitant disease which prevents diagnostic measures or therapy • Severe anemia (Hb< 9g/dl), thrombocytopenia (<100.000/µl) or neutropenia (<500/µl), not caused by the TMD (e.g. immunthrombocytopenia, congenital neutropenia, hemolytic anemia)

Synopsis of results: TMD Prevention 2007

OUTCOME(S)	Primary efficacy endpoint: Incidence of acute megakaryoblastic leukemia Key secondary endpoint(s): GATA1s negativity (sensitivity 10^{-4}) at week 12
STUDY TYPE	Non-randomised, against historical control
STATISTICAL ANALYSIS	Efficacy: Main target criterion is the proportion of patients who survive the TMD and develop AMKL within 3 years after diagnosis. The null hypothesis (no difference to the historical control group, AMKL-rate 22%) has been tested with a one-sided 95% confidence interval for the difference of the 3-years cumulative incidence of AMKL. Descriptive comparisons to the historical control group of 146 patients (also including the calculation of cumulative incidences – competing risk: death of any cause - and descriptive Gray's tests) are provided. Adverse effects of treatment are monitored and analyzed with descriptive methods.
SAMPLE SIZE	Planned: To be assessed for eligibility (n = 130) To be allocated to trial (n = 120) To be analysed (n = 100) Study conduct: 104 patients have been included. 3 patients had to be excluded from survival analysis because of insufficient follow-up. Number of courses: 43 patients were treated with Cytarabine, 27 patients received one course, 10 two courses and six three courses of chemotherapy
TRIAL DURATION	Planned Duration: First patient in to last patient out: 5 years, duration of the entire trial: 8 years First patient in: 1.8.2007 Last patient out: 8.2.2015
PARTICIPATING CENTERS	Planned: All 69 children's hospitals with a department of pediatric hematology/oncology, who are participating in the AML-BFM Studies Participating centers: ASKLEPIOS Clinic St. Augustin AMC, Amsterdam Charité Campus Virchow, Berlin Cnopf'sche Kinderklinik, Nürnberg Dr. Horst-Schmidt-Kinderklinik, Wiesbaden Dutch Childhood Oncology Erasmus MC, Rotterdam Ev. Krankenhaus Bielefeld Georg-August-Universität Göttingen HELIOS Klinikum Erfurt GmbH I. Kinderklinik des KZVA, Augsburg Johannes Wesling Klinikum Minden Kinder- u. Poliklinik des Klinikums rechts der Isar der Technischen Universität München Klinik f. Kinder- u. Jugendmedizin, Göttingen

	Klinikum Chemnitz Klinikum Dortmund Klinikum Oldenburg Klinikum d. J. W. Goethe-Universität, Frankfurt Martin-Luther-Univ. Halle-Wittenberg Med. Hochschule Hannover UKL / Klinik f. Kinder- u. Jugendmedizin, Lübeck UMCN, Nijmegen UMCG, Groningen UMCU, Utrecht Univ.-Klinikum Carl-Gustav-Carus, Dresden Universitäts-Kinder- und Jugendklinik Rostock Universitätskinderklinik Mannheim Universitätsklinik f. Kinder u. Jugendliche, Erlangen Universitätsklinik f. Kinder- u. Jugendmedizin, Ulm Universitätsklinikum Bonn Universitätsklinikum Freiburg Universitätsklinikum Hamburg-Eppendorf Universitätsklinikum Heidelberg Universitätsklinikum Münster Vestische Kinderklinik Datteln VUMC, Amsterdam
Summary – Conclusions: Efficacy Results, Safety Results, Conclusion	**Patients:** Here we report a cohort of 104 patients (male: 60, female:44) diagnosed with TMD. The median age at diagnosis was 4 days. As common in children with Down syndrome, many of the patients presented with comorbidities (cardiac defects: 68%, other malformations: 15%); 37% were delivered preterm (Table 1). Results are compared to a historical control group (N=146, *Klusmann et al: Treatment and prognostic impact of transient leukemia in neonates with Down syndrome. Blood. 2008. 111: 2991-2998*). **Efficacy:** 43 patients received low-dose cytarabin treatment, 61 patients did not receive this treatment. There was no difference in the three years cumulative incidence (CI) of AMKL (17 events in 101 patients vs. 29 in 146, p(Fisher)=0.62, CI 19.9±3.5% vs. 19.7±4.3%, difference -0.2%, lower limit of the 95% confidence interval -8.9%, p(Gray)=0.91, Figure 2). Overall, patients in this trial do not show a significantly better event-free survival (EFS; 72±4% vs. 63±4%, p=0.17) and overall survival (OS; 90±3% vs. 85±3%, p=0.16, Figure 3) than the historic control group (n=146). Especially the rate of early death (within 6 months) was low (Figure 4). Patients which presented with TMD-related clinical symptoms (n=42; symptoms: hyperleucocytosis [WBC>100,000], hepatopathy, ascites, hydrops fetalis) had a tendency for a better EFS (58±8% vs. 44±8%, p=0.12) and OS (80±7% vs. 67±7% p=0.11). For the progression to myeloid leukemia (ML)-DS there was no significant difference between the two groups (21±7% vs. 23%±7%, p=0.96). For patients which do not show any of the TMD-related symptoms (n=59), no significant differences were observed regarding EFS (81±5% vs. 71±5%, p=0.27), OS (98% vs. 93±3%, p=0.16) and CI of ML-DS (19±6% vs. 22±4%, p=0.95) compared to patients without TMD-related symptoms in the historic control (n=101). **Safety:** There were no SAEs according to the principal investigator. The rate of adverse events (graded according to the *NCI* Common Terminology Criteria for Adverse Events, modified by GPOH; CTC

	version 2.0) were tolerable (Table 2, for CRF see page 14). Elevation of liver enzymes is a symptom of the underlying disease. **Conclusion:** The investigated treatment strategy to eliminate persistent GATA-Clones didn't present with unexpected safety issues, but was unable to improve the rate of AMKL in patients with TMD.
Date of report	03.12.2017

Figure 1: Study design

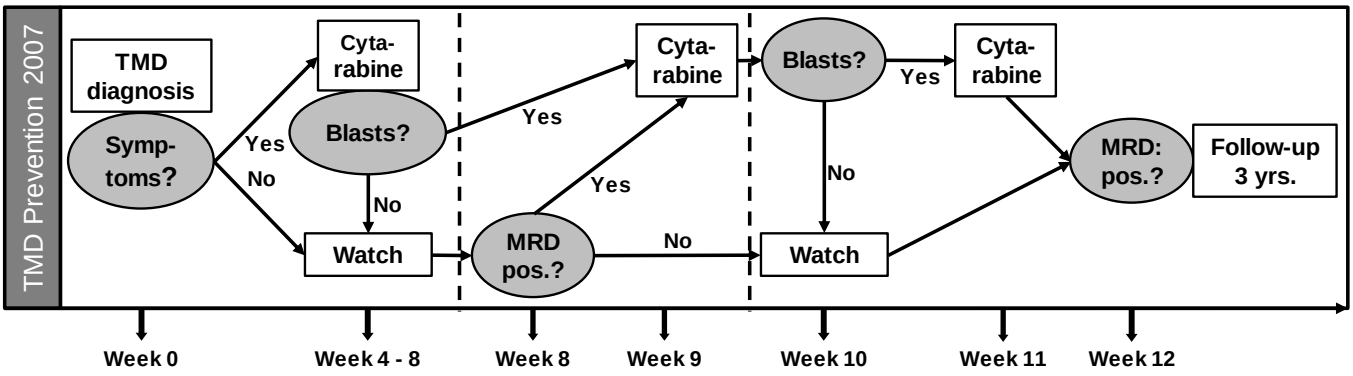

Figure 2: Control vs TMD 2007, cumulative incidence of AMKL
Three-years cumulative incidence 19.9±3.5% vs. 19.7±4.3%

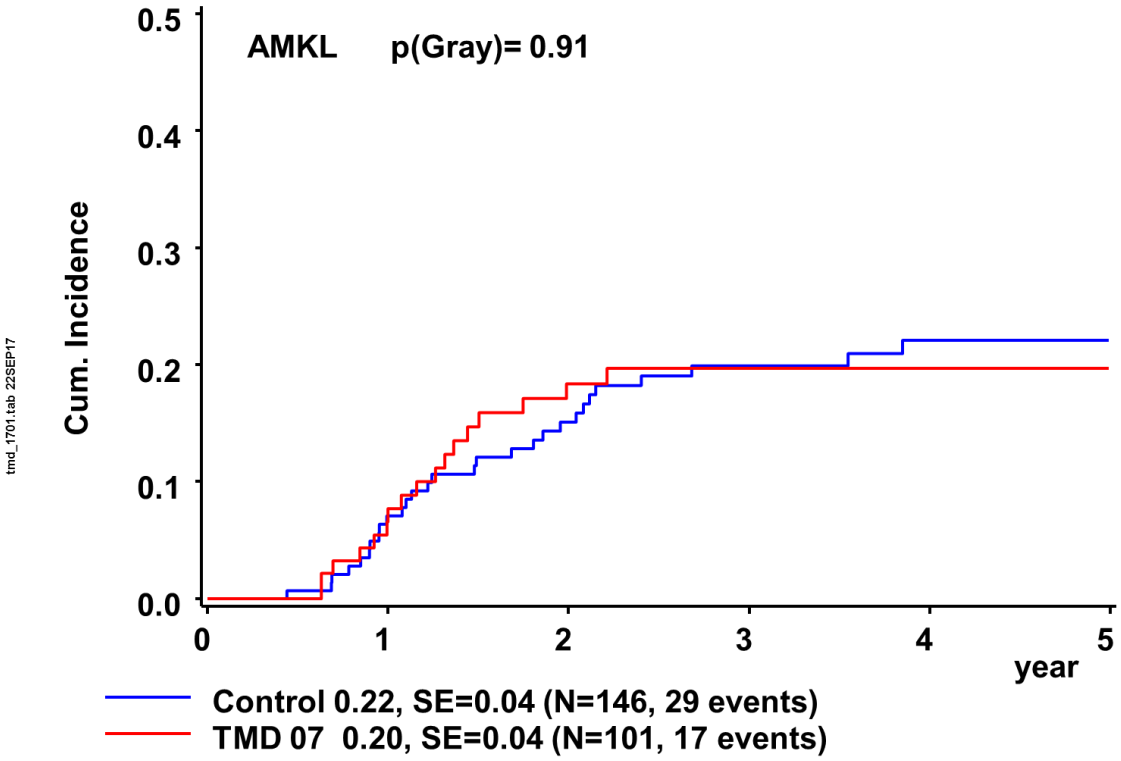

Figure 3: Control vs TMD 2007, Survival

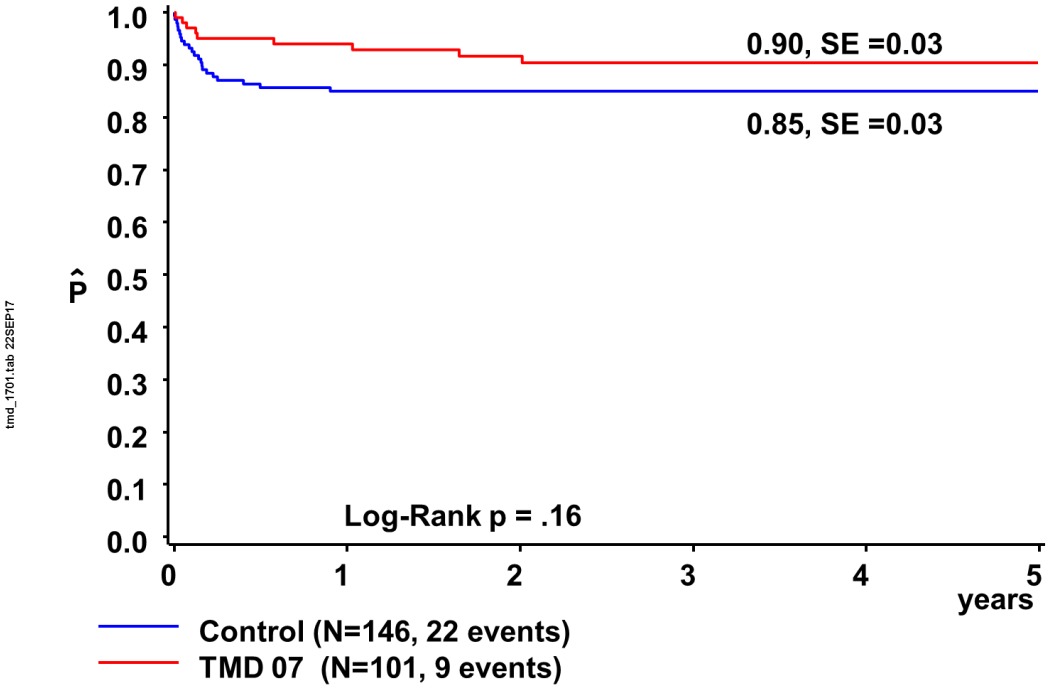

Figure 4: Control vs TMD 2007, incidence of early death (up to 6 months)

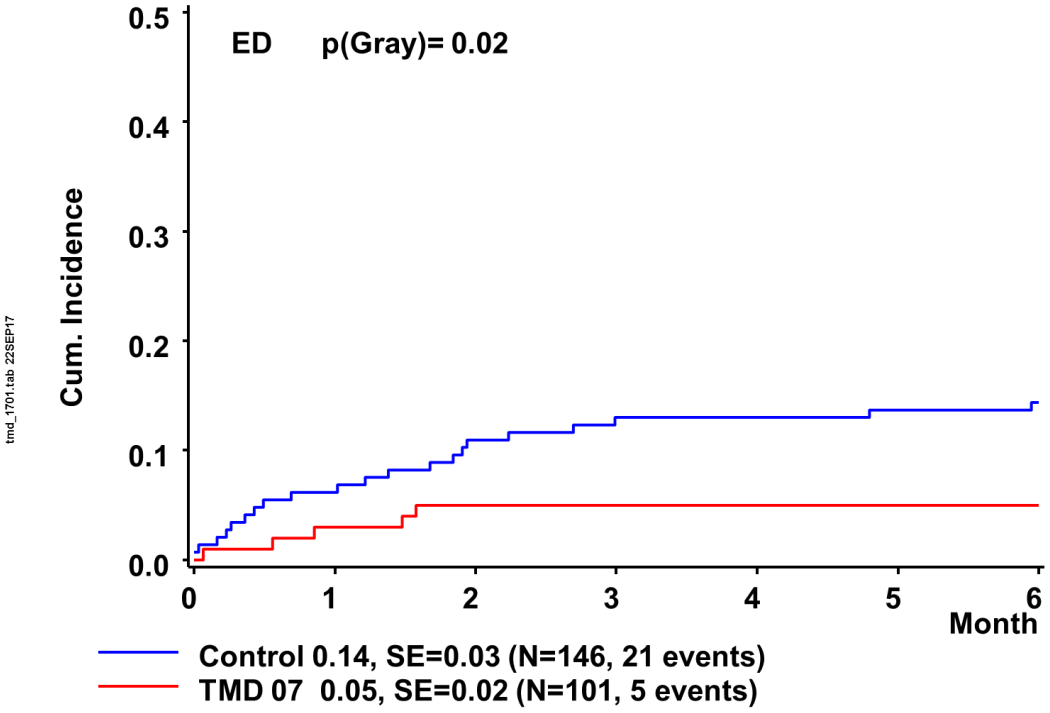

Table 1: Patient characteristics

p-values**:** Historical Control Group vs All Patients, Pat with Early Death vs Pat without Early Death, Pat with ML-DS vs Pat without ML-DS, Pat. with Therapy vs Pat. without Therapy. Categorical variables: p(Fisher), continuous p(Mann-Whitney-U)

	All (n=104)				Historical Control Group (n=146)					Early Death (n=5)					ML-DS (n=17)					Therapy (n=43)				
	Median	Min	Max	N	Median	Min	Max	N	P	Median	Min	Max	N	P	Median	Min	Max	N	P	Median	Min	Max	N	P
Birth weight (kg)	2,9	1,3	5,5	101	2,9	1,3	5,6	104	0,339	2,6	1,3	4,0	4	0,430	3,2	1,5	5,5	17	0,027 *	2,8	1,3	4,4	43	0,063
Birth lenght (cm)	48,0	38,5	58,5	87	48,0	34,0	66,0	46	0,610	50,0	38,5	51,0	3	0,856	50,0	39,0	58,5	14	0,046 *	48,0	38,5	53,0	37	0,099
Gestational age (weeks)	37,0	28,0	43,0	101	37,0	30,0	41,0	78	0,888	38,0	31,0	38,0	5	0,973	37,0	30,0	39,0	17	0,936	37,0	28,0	40,0	43	0,129
WBC count, (x 10^9/L)	24	3	306	104	40	4	556	128	0,163	43	20	171	5	0,112	23	3	149	17	0,473	50	3,2	306,0	43	<0,001 *
Platelets (x 10^9/L)	105	13	901	103	119	4	1047	128	0,738	80	32	242	5	0,316	94	15	505	17	0,726	124	30,0	606,0	43	0,352
Hemoglobin (g/L)	15,0	5,6	24,2	103	14,5	4,8	25,7	128	0,291	15,3	9,6	16,8	5	0,657	13,1	7,0	20,2	17	0,444	13,6	7,3	20,6	43	0,077
Blasts PB (%)	26,5	0,0	91,0	102	39,0	2,0	95,0	128	0,441	29,0	2,0	59,0	5	0,744	41,0	0,0	89,0	17	0,314	49,0	3,0	91,0	43	<0,001 *
Blasts KM (%)	14,3	6,0	60,0	6	32,0	5,0	93,0	104	0,550						8,0	8,0	8,0	1		41,0	8,0	60,0	3	0,400
		%	(no.)	N		%	(no.)	N	P		%	(no.)	N			%	(no.)	N				%	(no.)	N	
Elevated AST		19%	16	84		28%	27	96	0,166		80%	4	5	0,004 *		12%	2	17	0,506		26%	11	43	0,166
Elevated ALT		31%	27	88		24%	23	96	0,324		80%	4	5	0,029 *		24%	4	17	0,569		42%	18	43	0,037 *
Patholgic coagulation		36%	19	53		22%	23	104	0,086		33%	1	3	1,000		33%	3	9	1,000		52%	15	29	0,010 *
Hydrops fetalis		7%	7	98		5%	7	146	0,576		40%	2	5	0,040 *		6%	1	17	1,000		9%	4	43	0,696
Pleural effusion		6%	6	96		16%	24	146	0,027 *		25%	1	4	0,231		0%	0	17	0,587		10%	4	41	0,397
Pericardial effusion		21%	20	97		12%	17	146	0,069		40%	2	5	0,273		12%	2	17	0,511		30%	13	43	0,045 *
Ascites		17%	17	99		8%	12	146	0,043 *		60%	3	5	0,034 *		18%	3	17	1,000		26%	11	43	0,063
Splenomegaly		28%	27	98		42%	44	104	0,039 *		60%	3	5	0,127		29%	5	17	1,000		42%	18	43	0,007 *
Hepatomegaly		44%	43	98		60%	62	104	0,034 *		80%	4	5	0,165		53%	9	17	0,433		65%	28	43	<0,001 *
Cholestasis		23%	21	93		15%	16	104	0,207		50%	2	4	0,219		13%	2	16	0,511		41%	15	37	0,002 *
Hepatopathy		31%	30	98		44%	32	72	0,077		80%	4	5	0,030 *		29%	5	17	1,000		53%	23	43	<0,001 *
Intensive care		54%	41	76		25%	37	146	<0,001 *		100%	5	5	0,058		64%	7	11	0,533		71%	27	38	0,005 *
Ventilation required		40%	31	78		29%	30	104	0,153		100%	5	5	0,008 *		36%	4	11	1,000		50%	19	38	0,105
Cardiac defects		68%	69	101		47%	68	146	<0,001 *		40%	2	5	0,323		76%	13	17	0,571		74%	31	42	0,388
Therapy applied		43%	43	101		19%	28	146	<0,001 *		60%	3	5	0,648		53%	9	17	0,423		100%	43	43	<0,001 *

Table 2: Toxicity grading

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
General condition							
0	7	24.1	2	18.2	1	20.0	10
1	8	27.6	7	63.6	4	80.0	19
2	6	20.7	1	9.1	.	.	7
3	2	6.9	.	.	.	.	2
4	6	20.7	1	9.1	.	.	7

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Nausea							
0	10	40.0	7	63.6	4	80.0	21
1	3	12.0	2	18.2	.	.	5
2	7	28.0	1	9.1	1	20.0	9
3	2	8.0	.	.	.	.	2
4	3	12.0	1	9.1	.	.	4

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Vomiting							
0	22	73.3	8	72.7	3	60.0	33
1	3	10.0	1	9.1	1	20.0	5
2	5	16.7	2	18.2	.	.	7
3	.	.	.	.	1	20.0	1

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Stomatitis							
0	30	100.0	11	100.0	5	100.0	46

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Diarrhea							
0	23	76.7	9	81.8	4	80.0	36
1	2	6.7	.	.	.	.	2
2	3	10.0	1	9.1	1	20.0	5
3	2	6.7	1	9.1	.	.	3

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Skin changes							
0	27	93.1	10	90.9	5	100.0	42
1	1	3.4	.	.	.	.	1
3	.	.	1	9.1	.	.	1
4	1	3.4	.	.	.	.	1

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Creatinine							
0	26	83.9	10	90.9	5	100.0	41
1	3	9.7	.	.	.	.	3
2	2	6.5	1	9.1	.	.	3

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Bilirubine							
0	13	44.8	6	54.5	3	60.0	22
1	2	6.9	.	.	.	.	2
2	3	10.3	.	.	1	20.0	4
3	6	20.7	2	18.2	.	.	8
4	5	17.2	3	27.3	1	20.0	9

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
SGOT/SGPT							
0	15	50.0	4	36.4	1	20.0	20
1	8	26.7	2	18.2	2	40.0	12
2	4	13.3	1	9.1	1	20.0	6
3	3	10.0	4	36.4	1	20.0	8

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Arrhytmia							
0	28	100.0	11	100.0	5	100.0	44

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Cardiac function							
0	19	100.0	6	100.0	2	100.0	27

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Echokardio: LV-SF							
0	16	94.1	6	100.0	2	100.0	24
1	1	5.9	.	.	.	.	1

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Lung problems							
0	9	34.6	9	81.8	4	80.0	22
1	4	15.4	.	.	1	20.0	5
2	1	3.8	.	.	.	.	1
3	6	23.1	1	9.1	.	.	7
4	6	23.1	1	9.1	.	.	7

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Thrombosis							
0	28	100.0	10	90.9	5	100.0	43
2	.	.	1	9.1	.	.	1

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Central neurotoxicity							
0	24	96.0	10	100.0	5	100.0	39
2	1	4.0	.	.	.	.	1

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Peripheral neurotoxicity							
0	24	100.0	10	100.0	5	100.0	39

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Infection							
0	19	65.5	7	63.6	3	60.0	29
1	2	6.9	.	.	.	.	2
2	7	24.1	2	18.2	1	20.0	10
3	1	3.4	2	18.2	1	20.0	4

Synopsis of results: TMD Prevention 2007

Grade	Course no.						All
	1		2		3		
	N	%	N	%	N	%	N
Fever							
0	20	69.0	7	63.6	4	80.0	31
1	8	27.6	4	36.4	1	20.0	13
2	1	3.4	.	.	.	.	1

Synopsis of results: TMD Prevention 2007

TMD 2007 2/2007

Akut-Toxizität während der Cytarabintherapie **Kurs-Nr. .....**

Patient-ID oder Initialen:..... Klinik-Nr::.....

Bitte kreuzen Sie für jeden Parameter das entsprechende Feld an!

Grad	0	1	2	3	4	5	nicht durchgeführt
Allgemein-zustand	Normale Aktivität	Geringe Beeinträchtigung	Altersentspr. Aktivität stark eingeschränkt	Bettlägerig, pflegebedürftig	Intensive Behandlung, schwerstkrank	Tod	❑
Übelkeit	keine	ausreichende Nahrungsaufnahme	deutl. verminderte Aufnahme	praktisch keine Nahrungsaufnahme	TPN erforderlich	Tod	❑
Erbrechen (Anzahl Episoden in 24h)	0	1	2 - 5	6 - 10	>10 / TPN erforderlich	Tod	❑
Stomatitis	keine	schmerzlose Ulzera, Erythem	schmerzende Ulzera, kann essen	schmerzende Ulzera, kann nicht essen	TPN wegen Stomatitis erforderlich	Tod	❑
Diarrhoe (Anstieg Stuhlfrequenz/-Tag)	keine	2 - 3	4-6 o. nächtl. Stuhl o. leichte Bauchkrämpfe	7 - 9 oder Inkontinenz oder starke Bauchkrämpfe	> 10 o. blutiger Durchfall o. TPN erforderlich	Tod	❑
Hautverän-derungen	keine	Erythem	trockene Desquamation, Vaskulitis, Pruritus	feuchte Desquamationen, Ulzerationen	Exfoliative Dermatitis, Nekrosen	Tod	❑
Creatinin	Altersnorm	≤ 1.5 x N	> 1.5. - 3.0 x N	> 3.0 - 6.0 x N	> 6.0 x N	Tod	❑
Bilirubin	Altersnorm	≤ 1.5 x N	> 1.5 – 3.0 x N	> 3.0 - 10.0 x N	> 10.0 x N	Tod	❑
SGOT / SGPT	Altersnorm	≤ 2.5 x N	> 2.5 - 5.0 x N	> 5.0 - 20.0 x N	> 20.0 x N	Tod	❑
Arrhythmie	keine	asympt., keine Therapie	rekurr./persist., keine Therapie	Therapie erforderlich	Hypotension, ventr. Arrhyt.,Defibrillation	Tod	❑
Herzfunktion	Altersnorm	EF ↓ <20% vom Ausgangswert	EF ↓ >20% vom Ausgangswert	Milde Herzinsuffiz., therapeut. kompens.	Schwere / refraktäre Herzinsuffizienz	Tod	❑
Echokardio: LV-SF	> 30 %	≥ 24 % - < 30 %	≥ 20 % - < 24 %	>15 % - < 20 %	≤15 %	Tod	❑
Klinische Kardiomyopathie			Nein [ ] Ja [ ]				
Medikamentöse Therapie			Nein [ ] Ja [ ] welche:				
Lungen-probleme	keine	Tachypnoe	Dyspnoe	O_2 notwendig	Beatmung	Tod	❑
Thrombose	keine	Katheterthrombose	tiefe Venenthrombose ohne Antikoagulation	tiefe Venenthrombose mit Notwendigkeit der Antikoagulation	Embolie, Sinusvenenthrombose	Tod	❑
Zentrale Neurotoxizität	keine	vorübergehende Lethargie	Somnolenz < 50%/Tag, mäßige Desorientierung	Somnolenz ≥ 50%/Tag, erhebl. Desorientierung, Halluzination	Koma, Krämpfe	Tod	❑
Periphere Neurotoxizität	keine	Parästhesien u./o. herabgesetzte Sehnenreflexe	schwere Parästhesien u./o. milde Schwäche	unerträgliche Parästhesien, ausgeprägte motorische Verluste	Paralyse	Tod	❑
Infektion	keine	leicht	kein Erreger; i.v. Antibiotika	Erreger; i.v. Antibiotika	septischer Schock	Tod	❑
Fieber (°C)	keines	38 -39	> 39 - 40	> 40 für < 24 Std.	> 40 für ≥ 24 Std.	Tod	❑

Fieber[1] >38° .....(Tage), Erregernachweis Nein [ ], wenn Ja bitte ankreuzen

([1]wenn antipyrestische Therapie erforderlich = Fieber, kein Fieber= Temp < 38°C ohne Antipyrese)

		Erreger			Nachweis durch		
		Bakterien	Pilze	Viren	Kultur	Rö / CT-Thorax	Histologie
Sepsis							
Pneumonie							
lokal							

Blutung: Nein [ ] Ja [ ]	Petechien	Hämaturie	Hirn
wenn Ja, bitte entsprechendes Feld ankreuzen	Hämatome	Magen-Darm	Sonstige

Aplasiedauer Neutrophile<500/µl nein ❑ ja ❑ von |__|__|.|__|__|.|__|__| bis |__|__|.|__|__|.|__|__|

Thromboz. <20 000/µl nein ❑ ja ❑ von |__|__|.|__|__|.|__|__| bis |__|__|.|__|__|.|__|__|

Transfusionen / Anzahl:Thrombozyten: .....Erythrozyten:.....

system. Antimykose: Nein [ ] / Ja [ ] prophylaktisch [ ] empirisch [ ] therapeutisch [ ]

Andere Komplikationen: .....

.....