Title of Study: A multi-centre, multi-national open study in patients with hepatic cirrhosis to characterise the association between the pharmacokinetics of NRL972 and disease severity	
Co-ordinating Investigator: Prof. Dr. med. Guido Gerken	
Study Centres: Multi-national, multi-centre study in 97 (planned: up to 130) qualified centres with suitable provisions for the care and management of patients with hepatic cirrhosis	
Publication (reference): Not applicable	
Studied period: 25Mar2008 30Jun2009	Phase of development: III
Objectives: To provide proof of influence of disease severity (primary: Child-Turcotte-Pugh [CTP] class; secondary: composite reference criteria [extended CTP, multifactorial matrix]) on the pharmacokinetics (PK) of NRL972 (2-point sample analysis using the C(30):C(10) concentration ratio versus a 60 min pharmacokinetic profile in patients with histologically confirmed hepatic cirrhosis).	
Methodology: Multi-centre, multi-national, open study assessing the PK of NRL972 in patients with hepatic cirrhosis CTP-classes A, B, and C (histologically confirmed by liver biopsy). In the case of a CTP score ≥ 10 points the histological confirmation of hepatic cirrhosis and available histological material for review could be replaced by an objective imaging study (CT or NMR scan) within 3 months of the screening visit confirming hepatic cirrhosis (scans were collected and reviewed).	
Number of patients (planned and analysed): <u>Planned</u>: Eligible: up to 1400 patients. Screened: minimum 1200 with: CTP class A: at least ≥ 600 patients; CTP class B: ≥ 200 patients; CTP class C: ≥ 400 patients. <u>Analysed:</u> Screened: 1400 patients, Eligible for V1: 1332 patients. Safety population / intention-to-treat (ITT): 1305 patients. Analysed (keypoint available for primary criterion): 1240 patients (ITT-KPA) CTP class A: 605 patients; CTP class B: 273 patients; CTP class C: 362 patients.	

Per protocol population 1 (PP1): 1234 patients
PP2 (with confirmation of hepatic cirrhosis by reference pathologist or radiologist): 1067 patients

Diagnosis and main criteria for inclusion:

- Written informed consent;
- Male and female (non-child-bearing potential = post-menopausal or medically adequate contraception) of any ethnicity;
- 18 to 80 years of age;
- Patients with histologically established diagnosis of hepatic cirrhosis and available histological material for review by the central histopathologist. In the case of a CTP score ≥ 10 points the histological confirmation of hepatic cirrhosis and available histological material for review could be replaced by an objective imaging study (CT or NMR scan) within 3 months of the screening visit confirming hepatic cirrhosis (scans were collected and reviewed). Patients with the diagnosis of primary biliary cirrhosis, primary sclerosing cholangitis, and cystic fibrosis-associated liver disease were to be excluded;
- Present CTP-class A, B, or C;
- Medically fit to undergo the protocol-defined procedures without undue risk and discomfort;
- Predicted life-expectancy ≥ 6 months based on clinical judgement.

Test product, dose and mode of administration, batch number:
Single dose of 2 mg of NRL972 (batch no. NORs003) in 5 mL solution for injection (0.4 mg/mL) administered intravenously (i.v.) as a 15-sec bolus on one occasion.

Duration of treatment:
Evaluation of the investigational parameter and reference criteria over the duration of 2-5 days with test procedures (medical history, physical examination, clinical laboratory tests, ultrasound [US]-investigations and imaging studies, gastroscopy, NRL972- and monoethylglycinexylidide [MEGX']-test) scheduled such that undue stress and discomfort was avoided.

Reference therapy, dose and mode of administration, batch number:
NA

Criteria for evaluation:
Efficacy:
"Investigational parameter" (PK of NRL972 after a 15-sec i.v. injection):
Primary: 2-point fractional recovery C(30):C(10) concentration ratio.
Secondary: Secondary: C(30):C(15), C(45):C(10) and C(45):C(15) concentration ratios; clearance (CL) and apparent terminal disposition half-life ($t_{½}$) based on C(30):C(10) or other 2-point analyses and 60 min pharmacokinetic profile, as well as the concentration at 60 min after injection. Determination of severity stages based on NRL972 C(30):C(10) concentration ratio. Establishment of a multifactorial matrix (factor analysis) for staging of cirrhosis patients into severity classes. Investigation on the influence of concomitant medications and concomitant diseases. Accuracy of different hepatic scores to detect cirrhosis. Test-retest reliability of the CTP score. Biological profile of NRL972 C(30):C(10) concentration ratio class in relation to disease severity.

Safety:

- Well-being and adverse events (throughout);
- Clinical laboratory tests (using a central laboratory for the assessment) at the screening visit and within 12-24 h after NRL972-test;
- Vital functions (systolic blood pressure [SBP]/diastolic blood pressure [DBP] and pulse rate [PR]): at the screening visit, before and 1 h after NRL972-injection.

Reference criteria:
Based on clinical relevance for staging of hepatic cirrhosis:

- CTP-class and sum of scores (based on seconds for prothrombin time);
- Extended CTP-class and multifactorial reference matrix derived within the study by multifactorial analysis from criteria based on baseline features, medical history, physical examination, mental state, clinical laboratory tests, abdominal ultrasound (qualitative, semi-quantitative and selected quantitative criteria) and objective imaging study (optional), doppler-ultrasound (qualitative criteria; optionally: quantitative criteria), gastroscopy and MEGX'-test;
- Hepatic disease scores relevant for cirrhosis (calculated on the basis of the above determined reference criteria): Model for Endstage Liver Disease (MELD) score, D'Amico stage, Erasme Score, Ekindjian Score, Hepatic Dysfunction Score, Liver Damage Score, Maddrey's Discriminant Function score.

Statistical methods:

- The primary variable of the study was the 2-point fractional recovery C(30):C(10) concentration ratio of NRL972 (where $C(t_i)$ = concentration at t_i minutes after i.v. injection of NRL972). An ANOVA model for the investigation of the influence of CTP staging on the 2-point fractional recovery C(30):C(10) concentration ratio of NRL972 was composed (the effect considered in the ANOVA model was the CTP-class). The following hypotheses were tested on an alpha-level of 5%: H_0: $\pi_1 = \pi_2 = \pi_3$ (no difference between the effects π_i, where π_i is the effect if CTP class i) vs. H_1: $\pi_r \neq \pi_s$ (for at least one couple (r,s) of values). Descriptive statistics were also performed for the 2-point fractional recovery C(30):C(10) concentration ratio of NRL972 by CTP-class and overall.
- The secondary efficacy variables were analysed using either descriptive methods or an adapted analysis similar to the primary analysis. A score for staging of cirrhosis severity was obtained by means of factor analysis based on a multifactorial matrix, which contained diagnostic parameters for cirrhosis (selected by an expert panel). It was investigated if the CTP staging could be extended by means of this tool. Standardised scores for cirrhosis staging derived from this matrix were mapped to NRL972 C(30):C(10) concentration ratios using linear regression. Odds ratios for the probability of patients to belong to certain severity stages were also calculated by means of a logistic regression model. Cut-off values of the NRL972 C(30):C(10) ratio for allocating patients to NRL972-based severity stages derived from CTP and D'Amico stages were determined using discriminant analysis or ROC analysis.

SUMMARY – CONCLUSIONS

EFFICACY RESULTS:

The primary endpoint of the present study was to provide confirmation of the influence of CTP staging on the PK of NRL972 in patients with confirmed hepatic cirrhosis. The investigation of the influence of CTP staging on the 2-point fractional recovery C(30):C(10) concentration ratio of NRL972 confirmed that the CTP class had a significant effect on the NRL972 concentration ratio in all analysed populations ($p < 0.0001$). Respectively, the lowest mean value of NRL972 C(30):C(10) ratio was documented for patients with CTP class A (0.507 (SD = 0.183), ITT-KPA population), thereafter CTP class B (0.704 (SD = 0.157), ITT-KPA population) and the highest for patients with CTP class C (0.791 (SD = 0.131), ITT-KPA population). By applying an ANOVA model with the C(30):C(10) concentration ratio of NRL972 as a dependent variable and the CTP class as effect, all p-values for pairwise contrasts between CTP classes were significant ($p < 0.0001$). All results for the primary criterion are shown for the ITT-KPA population.

Similarly to the primary criterion, values of the 2-point fractional recovery concentration ratios, i.e. C(45):C(15), C(30):C(15) and C(45):C(10) of NRL972 were influenced by CTP class (analyses performed for ITT-KPA population). In patients with CTP class A, the respective mean concentration ratios were 0.416 (SD = 0.172), 0.616 (SD = 0.155), and 0.349 (SD = 0.180). In CTP class B, the mean concentration ratios were 0.630 (0.149), 0.778 (0.117), and 0.574 (0.173). In CTP class C, the mean ratios were 0.737 (0.123), 0.846 (0.096), and 0.692 (0.147). By applying an ANOVA model with the concentration ratios of NRL972 as a dependent variable and the CTP class as an effect, all the p-values for pairwise contrast were significant ($p < 0.0001$) for all concentration ratios. Clearance (all numbers given as [mL/min]) was fastest in patients with CTP class A (mean = 183.785 (SD = 140.969)) and decreased in the subsequent CTP classes (CTP B: 87.490 (SD = 116.883), CTP C: 47.635 (SD = 36.974)) according to 60 min non-compartmental pharmacokinetic (PK) assessment. Pairwise comparison of CTP classes revealed that the clearance was significantly different ($p < 0.0001$) between all CTP classes. The mean clearance values obtained by fractional 2-point analysis were 123.523 (SD = 68.805), 127.527 (SD = 78.586), 127.470 (SD = 76.521), and 120.156 (SD = 67.830) for CTP class A, 71.795 (SD = 55.823), 71.395 (SD = 69.351), 72.581 (SD = 71.017), and 69.637 (SD = 54.869) for CTP class B, and 47.878 (SD = 30.751), 45.094 (SD = 27.914), 46.765 (SD = 30.288), and 45.393 (SD = 27.959) for CTP class C; all numbers are given for T(30):T(10), T(45):T(15), T(30):T(15) and T(45):T(10) in this order. The CTP class could be shown to have a significant ($p < 0.0001$) influence on clearance as derived from 2-point analysis, no matter which pair of measurements was chosen. The shortest mean terminal half-life ($t_{½}$, all numbers given as [min]) was found in CTP A patients (32.244 (SD = 38:566)), the longest in CTP C patients (101.213 (SD = 84.128)) as obtained by 60 min non-compartmental analysis. ANOVA showed that the CTP class significantly influenced the $t_{½}$. Moreover, estimates of the $t_{½}$ were derived by two-point fractional analysis, i.e. T(30):T(10), T(45):T(15), T(30):T(15) and T(45):T(10). The respective mean values were 28.098 (SD = 50.607), 28.971 (SD = 34.693), 33.573 (SD = 76.032), and 28.082 (SD = 39.644) for CTP class A, 64.715 (SD = 171.314), 74.642 (SD = 324.969), 60.721 (SD = 67.918), and 56.740 (SD = 46.377) for CTP class B, and 86.392 (SD = 80.021), 88.002 (SD = 55.272), 103.336 (SD = 1127.748), 84.785 (SD = 54.291) for CTP class C. For all these pairs of measurements it was true that the $t_{½}$ was lowest in CTP A patients and highest in CTP C patients. $t_{½}$ values obtained by 2-point analysis significantly influenced by the CTP group, except for the contrast between CTP B and C for the time points T(45):T(15) . The mean NRL972 C(60) concentrations (all numbers given as [ng/mL]) were 116.1 (SD = 89.60) in CTP class A, 216.3 (SD = 103.09) in CTP class B, and 279.5 (SD = 98.60) in CTP class C.

(Note: all following numbers are shown for the PP2 population, if not indicated otherwise)

Most patients were allocated to CTP A (approx. 41%, all numbers based on prothrombin prolongation), whereas CTP group B contained the lowest number of patients (approx. 20%). There were no obvious differences between the scores derived from either prothrombin time or INR. The highest mean CTP total scores were 11.50 in CTP class C, the lowest in class A were close to the minimum of 5 points (5.23). A shift analysis taking into account the time points V0, V1 prior to NRL972 injection, and 12-24 h after the NRL972 test showed that 5.9% to 17.7% of class A patients had a change to class B, 7.6% to 14.2% of

class B patients to class C, 15.5% to 31.0% from class B to class A, and 8.3% to 11.8% from class C to class B (CTP calculated using prothrombin prolongation, results for INR-based CTP were similar).

Based on the results of the measurements of the C(30):C(10) concentration ratio, clearance, $t_{1/2}$, and concentration after 60 min(C(60)) of NRL972 it was attempted to define cut-off values for each of these parameters which allowed the staging of cirrhosis patients into the classes A, B, and C derived from CTP classes (this and all following analyses were performed for the PP2 population). This was done by both discriminant analysis and ROC analysis. The correlation coefficients between PK parameter and CTP class (Spearman's correlation coefficient) were 0.63675 for the NRL972 C(30):C(10) concentration ratio, -0.65039 for clearance, 0.69693 for $t_{1/2}$, and 0.61235 for C(60).

The cut-off values for allocation of NRL972 PK to groups derived from CTP classes as well as the corresponding sensitivities and specificities were calculated for one half of the PP2 population (determination step). The cut-offs were then applied to the other half of the same population (validation step) and sensitivity and specificity were determined for this subpopulation. The sensitivity and specificity values of both subpopulations were then compared using the 95%-CI; an overlap of CIs indicated that there were no statistically significant differences between the sensitivities and specificities of the two subpopulations. This was the case for all PK parameters and for the distinction of all three CTP-derived groups. The results for ROC and discriminant analysis were approximately in the same range. Therefore only the ROC values determined for part 1 of the PP2 population are shown in the following table:

Cut-offs for classification of NRL972 PK values to CTP-derived groups A, B, or C as obtained by ROC analysis (PP2 population, N = 1067)

	NRL972 pharmacokinetic parameter			
	C(30):C(10) ratio	**Clearance**	**Terminal half-life**	**Concentration at end of test: C(60)**
Group A vs. B				
Cut-off	0.62121	79.823 mL/min	31.260 min	117.0 ng/mL
Sensitivity	79.83%	66.39%	84.87%	83.19%
Specificity	70.20%	74.90%	63.92%	57.65%
Group B vs. C				
Cut-off	0.76871	52.305 mL/min	66.120 min	226.0 ng/mL
Sensitivity	72.05%	71.43%	72.67%	70.81%
Specificity	65.55%	68.91%	72.27%	64.71%

It was investigated whether original PK parameters should be corrected for age, gender and BMI prior to determination of cut-off values. Analysis of covariance showed a significant influence of the factor 'gender' on the NRL972 C(30):C(10) concentration ratio ($p = 0.0447$). However, the difference of the least squares means (lsmean) of the concentration ratio values between both genders was considered not clinically relevant (difference: 0.008; male patients: 0.6448, female patients: 0.6370). The covariate 'age' had a significant influence on the NRL972 C(30):C(10) concentration ratio, ($p < 0.0001$), so the correlation between the NRL972 C(30):C(10) concentration ratio and age was calculated (Spearman correlation coefficient: 0.11545, Pearson correlation coefficient: 0.13581). As these coefficients were smaller than 0.7, no further steps were performed for a correction of original NRL972 C(30):C(10) concentration ratios by means of linear regression prior to determination of cut-off values. The covariates 'age' and 'BMI' were shown to have significant influence on the clearance of NRL972 (age: $p < 0.0001$, BMI: $p = 0.0022$). Therefore, correlation coefficients according to Pearson and Spearman were calculated for correlations between clearance and age but also clearance and BMI. The coefficients for the correlation between clearance and age were -0.17534 (Pearson) and -0.13196 (Spearman). The coefficients for the correlation between clearance and BMI were 0.09465 (Pearson) and 0.11809 (Spearman). As these coefficients were smaller than 0.7, no further steps were performed. There was no significant influence of the factor

'gender' or any covariate on the variance of the $t_{1/2}$ of NRL972. Therefore, for all three parameters no correction of original parameter values by means of respective linear regression formulas was performed prior to determination of cut-off values.

In order to create a more comprehensive staging system, a multifactorial matrix was constructed comprising parameters which were considered as clinically relevant for cirrhosis staging by an expert panel. A factor analysis performed on these parameters resulted in a set of 38 factors with an eigenvalue >1 (Kaiser criterion) and including items with a factor loading >0.45 (selection by varimax rotation). These factors included liver specific and non-specific combinations of parameters. Based on these factors, specific and total scores for the severity assessment of cirrhosis were constructed. For the total score (based on all factors), the Cronbach's α was 0.7898, and for the specific score (based on specific factors only) it was 0.7802. Repetition of the factor analysis with the CTP class as an additional item resulted in the CTP class being grouped in factor 1, i.e. not forming a factor of its own, which led to the conclusion that the classification could not be improved by addition of further parameters.

Standardised total and specific scores were calculated on an individual patient basis using the factors identified in the factor analysis. The ranges of the scores were 56.37 to 122.04 for the total score and 14.48 to 49.37 for the specific score. The scores were split into six classes C1 to C6 at the 10th, 25th, 50th, 75th, and 90th percentiles of the empirical distribution of the respective score. These percentiles were then mapped to NRL972 C(30):C(10) concentration ratio values by linear regression, with the concentration ratio as a dependent variable and the respective score as an independent variable. This resulted in five NRL972 C(30):C(10) concentration ratio values which served as cut-off values to define six classes of NRL972 concentration ratios (termed N1 to N6).

Mapping of the Clinical Cirrhosis Staging Matrix Total Score to the NRL972 classes showed that approximately two thirds of all patients were found to belong to the classes N1 or N6 (N1: 31.1%; N6: 37.3%). This was due to the fact that many patients of the classes C1 to C3 (72.0%, 52.2%, and 45.7%) belonged to class N1 and many patients of the classes C4 to C6 (45.7%, 65.6%, and 76.4%) belonged to class N6. For the specific score, in principle the same was true, but here the allocation of patients with NRL972-classes N1 to N6 to classes C1 to C6 was not as marked as for the total score, especially for the NRL972 classes N3 and N4. Here, the classes N1 and N6 shared approximately 50% of all patients (N1: 24.5%; N6: 27.6%), which is also markedly more than the 20% allocated to C1 and C6. The same method of cross-tabulation was performed for classes N1 to N6 and the CTP classes A, B, and C. Most patients in class N1 (total score) were classified CTP A (56.2%), and most patients in class N6 were classified as CTP B (44.7%) or C (74.1%). For the specific score, the results were similar, but less marked than for the total score (44.6% of N1 patients were classified as CTP A, 29.5% of N6 patients were classified as CTP B, and 62.0% of patients in class N6 were classified as CTP C). For both the total and the specific score, the classes N1 to N6 could not be clearly separated based on the Clinical Cirrhosis Staging Matrix Score, and vice versa, due to the high variation of values in each class (applicable to both NRL972 classes N1 to N6 and matrix-derived classes C1 to C6). The obtained cut-offs for the concentration ratios as obtained by ROC analysis were as follows:

Cut-offs of the NRL972 C(30):C(10) concentration ratio based on Clinical Cirrhosis Staging Matrix classes C1 to C6

Clinical cirrhosis staging matrix standardized total score class	Cut-off (NRL972 C(30):C(10) concentration ratio)	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Youden-Index
C1 – C2	0.47083	57.50%	60.75	68.66%	48.87%	0.18248
C2 – C3	0.54725	58.43%	55.00%	6842%	44.22%	0.13427

C3 – C4	0.62295	77.15%	52.81%	62.05%	69.80%	0.29963
C4 – C5	0.74160	60.63%	50.56%	42.36%	68.18%	0.11187
C5 – C6	0.80856	55.66%	57.50%	46.46%	66.19%	0.13160

By means of a logistic regression analysis odds ratios for the risk of patients to belong to either NRL972 concentration ratio class N1 or N6 were calculated. The odds ratios which were most different from 1 (1 = no influence on the risk to belong to class N6) for the risk to belong to class N6 ratios were found for 'splenic venous tract visualised' (yes vs. no, odds ratio = 2.866), 'location of varices' (based on a score from 1 to 3 for the location, with 1 meaning "inferior", 2 "median", and 3 "superior"; odds ratio = 2.005), 'HbA1c' (odds ratio = 1.666), 'oesophagitis' (present vs. absent, odds ratio = 0.379), 'gender' (male vs. female, odds ratio = 0.405), 'diabetes mellitus' (yes vs. no, odds ratio = 0.229) and 'ligation of varices' (yes vs. no, odds ratio = 0.137). An increase in total bilirubin by 1 unit slightly elevated the chance to belong to N6 (odds ratio = 1.160), whereas an increase in direct bilirubin decreased this chance (odds ratio = 0.853). The Goodness-of-Fit test by Hosmer and Lemeshow resulted in a p-value of 0.1774, which does not question the goodness of fit. Observed and predicted classification of patients to either class N1 or N6 according to the regression model were cross-tabulated, showing that 9.6% of patients with an observed N1 class were allocated to predicted class N6, and 7.3% with an observed N6 class were allocated to predicted class N1. The majority of cases mapped correctly with frequencies above 90%.

Analysis of correlation between the NRL972 C(30):C(10) concentration ratio and established hepatic disease scores showed Pearson correlation coefficients of >0.5 to the CTP class, the integrated Model of End-stage LiverDisease (iMELD) score, the Erasme score, the hepatic dysfunction score, the liver damage scores, the modified Cirrhosis Discrimination Score (CDS), the Hepascore, the Health Utility Índex (HUI), the Shasta index, hyaluronic acid, and the Fibrotest. The correlation coefficient (Pearson) between the NRL972 C(30):C(10) concentration ratio and the standardised total score of the Clinical Cirrhosis Staging Matrix was 0.53829.

The test-retest-reliability of the CTP total score was assessed and overall revealed a high correlation between all pairs of assessments (V0 vs. V1 prior to injection, V0 vs. V1 12-24 h after test, V1 prior to injection vs. V1 12-24 h after test) with a Pearson's correlation coefficient over 0.9, indicating a high test-retest reliability of the CTP total score.

By means of ANOVA the effects of concomitant medications and concomitant diseases on NRL972 C(30):C(10) concentration ratio, clearance, and $t_{1/2}$ were analysed (results shown for PP2 population). Besides CTP class, the NRL972 C(30):C(10) concentration ratio was also significantly influenced by 'drugs for gastrointestinal disorders' ($p = 0.0169$) and the interaction between CTP class and 'drugs for acid-related disorders' ($p = 0.0035$). The $t_{1/2}$ was, besides CTP class alone, significantly influenced by 'diuretics' ($p = 0.0091$) and the interaction between CTP class and 'diuretics' ($p = 0.0154$). The direction of drug effects on the PK parameters of NRL972 was investigated using lsmeans. For the concentration ratio, drugs for acid-related disorders did not have a significant influence themselves, but in interaction with CTP class there was a minor influence (up to 0.5%). Regarding the changes in lsmeans observed for the interaction between drugs for acid-related disorders and CTP class, it was likely that the CTP class was the main factor for the observed change. With drugs for GI disorders, the influence was significant for the drugs themselves, but not for the interaction of drug and CTP class; however, the difference in lsmeans was small and probably not clinically relevant (approximately 6%). For $t_{1/2}$, administration of diuretics led to an improvement, expressed by a decrease in lsmeans of around 18 minutes. The interaction between diuretics and CTP class was also significantly improved for CTP B and CTP C plus use of diuretics, without loss of the overall CTP-dependent impairment of $t_{1/2}$ values with increasing severity.

Oesophageal varices ($p = 0.0005$), hepatic encephalopathy ($p = 0.0355$), and cytolytic hepatitis ($p = 0.0033$) had a significant influence on the NRL972 concentration ratio. The clearance was significantly influenced by hepatomegaly ($p = 0.0105$), anaemia ($p = 0.0466$), cell death ($p = 0.0278$), cytolytic hepatitis ($p = 0.0016$), and the interactions between CTP class and thrombocytopenia ($p = 0.0162$), menopause ($p = 0.0036$), and cytolytic hepatitis ($p = 0.0314$). Significant influences in $t_{1/2}$ were anaemia

(p = 0.0115), prolonged aPTT (p = 0.0076), cell death (p = 0.0103), as well as the interactions between CTP class and hepatomegaly (p = 0.0305), splenomegaly (p = 0.0110), increased gamma-GT (p = 0.0088), thrombocytopenia (p = 0.0273), prolonged aPTT (p = 0.0006), and cell death (p = 0.0016). The CTP class significantly influenced all three parameters in the medication analysis, but only the concentration ratio in the diseases analysis. Differences in lsmeans for diseases with a statistically significant influence on NRL972 PK parameters were investigated. All diseases with significant influence (worsening) were considered to be complications of progressing cirrhosis, and therefore deteriorations in NRL972 PK values are most likely linked with the progression of cirrhosis itself. An exception was 'hepatomegaly', which had a positive influence on the clearance of NRL972 (approximately 24 mL/min).

Medications and their indications were analysed to determine if there was a significant influence on NRL972 PK parameters. Highly significant influences (p <0.0001) on the NRL972 C(30):C(10) concentration ratio were the interaction between antihaemorrhagics and oesophageal varices, between drugs for GI disorders and nephrolithiasis, between drugs for GI disorders and hyperbilirubinaemia, and between drugs for GI disorders and increased blood bilirubin. The $t_{1/2}$ was influenced by the interaction between diuretics and ascites (p <0.0001).

In analogy to the definition of cut-off values of the NRL972 C(30):C(10) concentration ratio for CTP-derived classes, the same type of analyses were applied using the D'Amico scale. This included the comparison of 95%-CIs between subpopulations of the entire population for validation of sensitivity and specificity values. In the following table the results for NRL972 PK values-derived groups I, II, and III+IV (combined, as the groups could not be distinguished by the 95%-CIs of the mean NRL972 concentration ratios) as well as the combined groups C (compensated, groups I and II) and D (decompensated, groups III and IV) according to ROC analysis are shown. Comparison of the CIs between determination and validation step showed no statistically significant differences between any sensitivity or specificity values for any parameter.

Cut-offs for classification of NRL972 PK values to CTP-derived groups C and D, as well as I, II, II+IV as obtained by ROC analysis (PP2 population, N = 1067)

	NRL972 pharmacokinetic parameter		
	C(30):C(10) ratio	**Clearance**	**Terminal half-life**
Group C vs. D			
Cut-off	0.69509	76.529 mL/min	57.539 min
Sensitivity	71.22%	74.54%	63.10%
Specificity	73.00%	70.72%	83.65%
Group I vs. II			
Cut-off	0.63636	90.433 mL/min	32.892 min
Sensitivity	48.68%	48.68%	54.61%
Specificity	81.08%	74.77%	72.07%
Group II vs. III+IV			
Cut-off	0.76871	67.840 mL/min	50.737 min
Sensitivity	51.66%	69.00%	65.68%
Specificity	82.89%	69.74%	75.00%

The capability of NRL972 C(30):C(10) concentration ratio to distinguish between stages of the MELD score, the D'Amico score, and the LDS bases on either IgG or gamma globulins was investigated using ANOVA. Significant influences on the concentration ratio were found for pairwise comparison of all MELD levels except levels 1 vs. 2, all D'Amico stages except stages III vs. IV, and all LDS stages regardless whether LDS was based on gamma globulins or IgG.

The accuracy of different established non-invasive markers to detect cirrhosis was determined by application of these scales/scores to the patients of the PP2 population. As all these patients had confirmed cirrhosis, all patients categorised as 'normal' by any scale were considered as false negative findings. For the NRL972 concentration ratio a cut-off value for differentiation between normal and abnormal liver function of 0.313 was used. Of all cirrhosis markers tested based on the PP2 population, the NRL972 C(30):C(10) concentration ratio delivers the lowest percentage of false negative results with 7.2%. All other scores delivered at least 10% false negative results, with the Fibrotest in combination with APRI being the score with the highest number of false negative diagnoses (72.3%). The inclusion of patients with missing values did not alter the distribution of correct classification in absence or presence of cirrhosis.

The most prominent causes of cirrhosis were chronic viral hepatitis (only cause, 25.9% to 59.5%) and alcohol abuse (only cause, 26.3% to 62.0%). Alcoholism was the most frequent origin of cirrhosis in patients with CTP class B and C, whereas post-viral hepatitic cirrhosis was most frequent in CTP A patients. A relatively small number of patients had an aetiology of both chronic alcoholism and chronic viral hepatitis (A: 4.7%; B: 13.1%; C: 9.3%). The NRL972 C(30):C(10) concentration ratios in each CTP class did not obviously differ between patients with alcoholic and post-viral hepatitis cirrhosis. ANOVA resulted in significant influences on the concentration ratios for the CTP class ($p < 0.0001$), but not for aetiology ($p = 0.3907$) or the interaction of both ($p = 0.3219$). Pairwise comparison of the parameters showed that only the CTP classes had significant effects on the concentration ratio (A vs. B: $p < 0.0001$; B vs. C: $p = 0.0013$; A vs. C: $p < 0.0001$).

Investigation of the effect of liver volume on the NRL972 C(30):C(10) concentration ratio showed that most patients with a reduced volume were CTP class C (37.0%) (this analysis was based on the ITT-KPA population). The majority of patients with an enlarged liver belonged to CTP class B (40.7%). Most normal livers were found in patients with CTP class A (40.2%). For the groups A, B, and C based on cut-offs for NRL972 PK parameters this outcome was similar, but enlarged livers were most frequent in NRL972 C(30):C(10) ratio-derived group C (38.8%), clearance-based group A (36.0%), and $t_{1/2}$-based group C (38.9%). Reduced liver volumes were most frequent in NRL972 C(30):C(10) ratio-based group C (27.4%), clearance-based group C (33.0%), and $t_{1/2}$-based group C (29.5%). In order to investigate whether the liver volume had a significant influence on the PK of NRL972, linear regression analyses and ANCOVA modelling were applied. The linear regression analyses showed that the liver volume was significantly related to the NRL972 C(30):C(10) ratio (p 0.0006), but not to clearance ($p = 0.4475$) or $t_{1/2}$ ($p = 0.0659$). ANCOVA with the NRL972 C(30):C(10) ratio as a dependent variable revealed a significant influence of CTP class ($p < 0.0001$), liver volume ($p < 0.0001$), and the interaction of both variables ($p = 0.0079$). For the clearance of NRL972, only the CTP class influenced the dependent variable significantly ($p < 0.0001$) in the ANCOVA model, but not the liver volume ($p = 0.4178$) or the interaction of both ($p = 0.4334$). The $t_{1/2}$ was significantly influenced by the CTP class ($p < 0.0001$) and the liver volume ($p = 0.0251$), but not the interaction of both ($p = 0.1252$).

The patients' Quality of Life was assessed using the CLDQ score, which comprises 29 questions grouped to six domains (abdominal symptoms, fatigue, systemic symptoms, activity, emotional function, worry). For all domains and the total score there was a decrease in mean scores as the severity of the cirrhosis increased. None of the mean scores exceeded 5.51 or was lower than 3.20 (PP2 population; note that a decrease in the CLDQ score indicates a worsening of well-being).

SAFETY RESULTS:

In the safety population (N = 1305), 5 patients (0.4%) died during the course of the study or shortly after its termination. All cases were attributed to the disease progression and considered by the investigator to be unrelated to the administration of NRL972.

Altogether, 15 treatment-emergent Serious Adverse Event (SAE) symptoms occurred in 10 patients (3 patients of CTP class B and 7 patients of CTP class C). All SAEs were assessed as unrelated to the administration of the IMP. Three patients (0.2%, all CTP class C patients) prematurely withdrew from the study due to 4 Treatment Emergent Adverse Event (TEAE) symptoms which all were 'serious' and

assessed as unrelated by the investigator. The difference in the number of treatment-emergent SAEs per patient between the patients of the different CTP classes was statistically significant ($p = 8.495*10^{-4}$).

There were 3 patients (0.2%) classified as CTP class C who prematurely terminated the study due to TEAEs. All three patients died. The difference in the number of TEAEs leading to premature discontinuation per patient between the patients of the different CTP classes is statistically significant ($p = 0.0358$) and related to the decompensated CTP C cirrhosis patients with rapid disease progression.

TEAEs were reported for patients in all hepatic cirrhosis CTP classes. In total, the number of patients affected by any TEAE was 105 (8.0%) (CTP class A: 66 (10.4%), CTP class B: 16 (5.6%), CTP class C: 23 (6.0%)). The number of episodes reported was 148 (CTP class A: 91, CTP class B: 24, CTP class C: 33), and the number of symptoms was 161 (CTP class A: 99, CTP class B: 24, CTP class C: 38). The difference in the number of TEAEs per patient between the patients of the different CTP classes was statistically significant ($p = 0.0109$).

In the causality assessment to the administration of the IMP by the investigator, the majority of cases were assessed as unrelated (141 TEAEs (95.3%)), 5 TEAEs (3.4%) were assessed as 'possible' and 2 TEAEs (1.4%) as 'probable' (CTP class A: 4 (4.4%) as 'possible' and 2 (2.2%) as 'probable', CTP class B: 1 (4.2%) as 'possible' and 0 (0.0%) as 'probable', CTP class C: 0 TEAEs (0.0%) as 'possible' and as 'probable' each).

In the investigator's causality assessment to lidocaine, the majority of AEs were unrelated to lidocaine application (90 TEAEs (60.8%)). 15 TEAEs (10.1%) were assessed as 'possible', and 42 TEAEs (28.4%) as 'probable' (CTP class A: 13 (14.3%) as 'possible' and 31 (34.1%) as 'probable'; CTP class B: 1 TEAE (4.2%) as 'possible' and 3 (12.5%) as 'probable'; CTP class C: 1 TEAE (3.0%) as 'possible' and 8 (24.2%) as 'probable'.

The intensity of the AE symptoms was reported as 'mild' in 121 cases (81.8%), 'moderate' in 16 cases (10.8%) and as 'severe' in 11 cases (7.4%) (CTP class A: 'mild' in 82 cases (90.1%), 'moderate' in 9 cases (9.9%) and 'severe' in 0 cases (0.0%); CTP class B: 'mild' in 16 cases (66.7%), 'moderate' in 5 cases (20.8%) and 'severe' in 3 cases (12.5%); CTP class C: 'mild' in 23 cases (69.7%), 'moderate' in 2 cases (6.1%) and 'severe' in 8 cases (24.2%).

The following primary System Organ Classes (SOC) were the most prominent with regard to the patients with the respective symptoms: 26 patients (2.0%) were affected by 31 TEAE symptoms of 'nervous system disorders' (CTP class A: 19 patients (3.0%) were affected by 22 symptoms, CTP class B: 1 patient (0.3%) was affected by 1 symptom, CTP class C: 6 patients (1.6%) were affected by 8 symptoms), 22 patients (1.7%) were affected by 29 TEAE symptoms of 'gastrointestinal disorders' (CTP class A: 16 patients (2.5%) were affected by 20 symptoms, CTP class B: 1 patient (0.3%) was affected by 2 symptoms, CTP class C: 5 patients (1.3%) were affected by 7 symptoms) and 22 patients (1.7%) were affected by 25 symptoms of 'ear and labyrinth disorders' (CTP class A: 15 patients (2.4%) were affected by 17 symptoms, CTP class B: 3 patients (1.0%) were affected by 3 symptoms, CTP class C: 4 patients (1.0%) were affected by 5 symptoms).

The most frequent TEAE symptoms by preferred term (PT) were 'dizziness' (19 patients (1.5%) were affected by 19 symptoms, CTP class A: 14 patients (2.2%) were affected by 14 symptoms, CTP class B: 1 patient (0.3%) was affected by 1 symptom, CTP class C: 4 patients (1.0%) were affected by 4 symptoms), 'tinnitus' (12 patients (0.9%) were affected by 12 symptoms, CTP class A: 10 patients (1.6%) were affected by 10 symptoms, CTP class B: 1 patient (0.3%) was affected by 1 symptom, CTP class C: 1 patient (0.3%) was affected by 1 symptom) and 'vertigo' (10 patients (0.8%) were affected by 10 symptoms, CTP class A: 7 patients (1.1%) were affected by 7 symptoms, CTP class B: 2 patients (0.7%) were affected by 2 symptoms, CTP class C: 1 patient (0.3%) was affected by 1 symptom).

In summary, the reported AE profile within this study was in accordance with the safety profile of NRL972 as shown in the previously conducted studies.

All 1305 patients (100.0%) of the safety population had at least one laboratory value outside the normal range limits at the eligibility visit V0; 1 patient (0.1%, CTP class A) had no laboratory value outside the normal range limits at the test visit V1 while the data for 26 patients (2.0%) at V1 were missing. With

regard to the pre-post differences of the median values of the safety laboratory parameters between the test visit V1 and the eligibility visit V0, only minor changes were recorded. In the vital signs, none of the parameters assessed showed systematically or relevant changes during the course of the study.

In conclusion, the administration of NRL972 proved to be generally well tolerated, and the safety data were in-line with the so far known safety profile of the substance and support the use in all cirrhosis population irrespectively of the degree of medical impairment.

CONCLUSION:

The purpose of this study was to provide confirmation of the association between the severity of hepatic cirrhosis and the pharmacokinetics of NRL972. With the CTP staging being an established reference for assessment of cirrhosis severity, it could be shown by means of ANOVA that NRL972 pharmacokinetics (C(30):C(10) concentration ratio, clearance, $t_{1/2}$, and concentration at the end of the NRL972 test C(60)) were significantly different between the CTP classes. In consequence, cut-off values for NRL972 C(30):C(10) concentration ratio, clearance, $t_{1/2}$, and concentration at the end of the NRL972 test C(60) were determined to allocate patients into class A, B, or C derived from CTP class A, B, and C using NRL972 PK values.

Sensitivities and specificities of cut-off values were determined using discriminant and ROC analyses, a direct comparison of both statistical approaches is not possible, but for all four PK parameters investigated (NRL972 C(30):C(10) concentration ratio, clearance, $t_{1/2}$, and concentration at 60 min after the injection of NRL972 C(60)), the respective cut-offs were similar, which indicates the robustness of the results. As the ROC analysis can be seen as more universal for the CTP-derived groups in the range of statistical applications, the focus of the discussion was placed on this approach. The cut-off values and the associated sensitivities and specificities were first determined for one half of the PP2 population, and afterwards sensitivity and specificity were validated by the second half of the population (validation group) using the cut-off determined for the first subpopulation. Comparison of the 95%-CIs of each sensitivity and specificity value within each PK parameter showed that the respective results between the PP2 subpopulations did not differ with statistical significance. This was true for both sensitivity and specificity within all four parameters investigated. The sensitivities obtained by ROC analysis reached 70% in most cases, with the cut-off between clearance-derived groups A and B and concentration ratio-derived groups B and C being exceptions. For the NRL972 C(30):C(10) concentration ratio almost 80% were reached for the cut-off between groups A and B and almost 70% for the cut-off between groups B and C; for the C(60) concentration 83% for the cut-off between groups A and B was reached, and 70% for the cut-off between groups B and C, which indicates the good suitability of NRL972 pharmacokinetics for severity assessment of cirrhosis.

Analysis of covariance for the factor, gender and the covariates age and BMI showed significant influences on the NRL972 C(30):C(10) concentration ratio (gender, age) and clearance (age and BMI), but these influences were assessed not to be clinically relevant according to a priori definitions.

The CTP score comprises only three laboratory parameters and two subjective clinical criteria. A shift analysis of patients showed that a considerable number of patients had a shift in CTP classification from A to B and B to C and vice versa (up to 17% per CTP group) within a 12 to 24 hour period during this study, demonstrating the need for a more biologically accurate approach to assess the severity and/or stage of hepatic cirrhosis. This was done using a factor analysis which included an extensive set of parameters selected by an expert panel. These items could be grouped to 38 factors with an eigenvalue >1; not all of these factors were considered specific for liver cirrhosis or liver functionality. Based on this Clinical Cirrhosis Staging Matrix, an evaluation score could be developed on a standardised individual basis (total score); with only the specific factors taken into account, a specific score was the result. This Clinical Cirrhosis Staging Matrix and the resulting scores can be considered one of the most comprehensive approaches to staging of the disease and representing a validated outer criterion for the severity of hepatic cirrhosis for further comparison. The standardised scores were then subdivided into six classes C1 to C6 based on the 10^{th}, 25^{th}, 50^{th}, 75^{th}, and 90^{th} percentile of the empirical distribution of standardised scores.

The cut-off values could then be mapped to NRL972 concentration ratios via linear regression, resulting in six concentration ratio classes N1 to N6. Cross-tabular analysis showed that for most concentration ratio-classes a majority of patients was allocated into the respective Clinical Cirrhosis Staging Matrix Class. This result is a considerable indication that changes in the NRL972 C(30):C(10) concentration ratio reflects the true progression of hepatic cirrhosis, instead of being correlated to single factors which by themselves may not reliably indicate the progression of the disease. This was also true for a cross-tabulation of concentration ratio classes with CTP classes A, B, and C. However, it showed that a considerable percentage of patients (up to 10%) with CTP class A belonged to concentration ratio classes N5 and N6. It is therefore possible that the CTP A classified patients have more severe hepatic impairment and are therefore incorrectly classified as having mild disease. Furthermore, using the standardised total score, approximately two thirds of all patients were allocated to NRL972 concentration ratio classes N1 or N6. Using the standardised specific score, it still was 50% of all patients. Therefore, a logistic regression analysis was performed resulting in odds ratios for the patients' probability to belong to class N6.

The logistic regression model showed that more than 90% of patients were correctly predicted to belong to either class N1 or N6 based on the parameters processed by the model. The odds ratios show that patients with a visualised splenic venous tract, as well as varices in the upper part of the oesophagus have an increased chance to belong to class N6; both symptoms are known to occur more frequently in patients with severe cirrhosis due to advanced portal hypertension. Increase in HbA1c also elevated the chance to belong to class N6. The presence of oesophagitis indicated an approximately threefold higher chance for patients to belong to class N1 than N6, which may be due to the continuous treatment of this condition in patients with more severe cirrhosis (labelled as N6). The same may be true for the ligation of varices, which increased the chance of belonging to class N1 approximately 7-fold. The presence of diabetes mellitus diminished the chance of patients to belong to class N6 by the factor 4, and being male diminished this chance by the factor 2.5.

The analysis on the influence of concomitant medications showed that the NRL972 C(30):C(10) concentration ratio was significantly influenced by drugs for GI disorders and the interaction between CTP class and drugs for acid-related disorders, whereas the clearance was not influenced by any drug class. The $t_{1/2}$ was influenced by diuretics and the interaction between CTP class and diuretics. Most of the differences between the lsmeans indicated that these influences were not of clinical relevance, except for the influence of diuretics on the $t_{1/2}$; intake of diuretics led to an inprovement in this parameter ($t_{1/2}$). In both the ANOVA regarding concomitant medications and the ANOVA regarding concomitant diseases, the CTP class was included. In the ANOVA for concomitant medications, the CTP class significantly influenced all three PK parameters; however, in the ANOVA on concomitant diseases only for the NRL972 C(30):C(10) concentration ratio a significant influence was found.

The analysis on the influence of concomitant diseases showed that a number of conditions which are related to hepatic cirrhosis and other clinical signs of relevant liver impairment (e.g. varices, anaemia, cell death, cytolytic hepatitis), or conditions in interaction with the CTP class (e.g. hepatomegaly, splenomegaly, increase of gamma-GT, thrombocytopenia; these factors alone had no significant influence in this case) significantly influenced the PK results for NRL972. All these conditions can be seen as complications to the progression of cirrhosis itself, therefore their worsening on the different PK parameters reflects mainly severity of the cirrhosis. Presence of hepatomegaly improved the clearance, which may be explained by an increase in functional liver mass in mild to moderate stages of the disease. In combination with CTP class, there was no significant influence.

In combination with the treated indications, only drugs for GI disorders used for treatment of nephrolithiasis or increased blood bilirubin, as well as antihaemorrhagics used for treatment of oesophageal varices influenced the NRL972 concentration ratio; this may be due to the fact that these indications occur more often with increasing progression of the disease, which also causes elevation of the NRL92 C(30):C(10) concentration ratio. There was also a significant influence of diuretics and ascites on the half-life of NRL972.

Analogously to the determination of CTP-derived groups, cut-off values and their corresponding sensitivity and specificity for correct staging of cirrhosis patients were determined for the D'Amico scale. This scale is divided into four severity stages (I, II, II and IV), which can be combined to indicate compensated (stages I and II) or decompensated cirrhosis (II and IV). As with CTP-derived groups; sensitivity and specificity were first calculated for one half of the population, and then validated using the other half by comparison of 95%-CIs. The result of these comparisons was that sensitivities and specificities did not differ with statistical significance for any PK parameter (C(30):C(10) concentration ratio, clearance, $t_{1/2}$) nor for any cut-off value separating different severity stages. The highest sensitivities (75%) for distinction between compensated and decompensated cirrhosis were reached for the clearance of NRL972, the highest specificities (>80%) for $t_{1/2}$. That is, NRL972 pharmacokinetic measurements are also suitable for distinction of NRL972-based severity stages derived from D'Amico stages.

Furthermore, the capability of the NRL972 C(30):C(10) concentration ratio to distinguish between stages of the MELD score, the D'Amico score and the LDS was examined. Pairwise comparison of stages with ANOVA indicated that a distinction between all MELD levels except 1 and 2 is possible, as well as a differentiation between all D'Amico stages except stages III and IV (this finding is in concordance with the results of the cut-off analysis for D'Amico stages). This might indicate a reduced capability of D'Amico staging to differentiate between patients with progressed cirrhosis (particularly for patients at risk of bleeding), and of MELD to differentiate between progressed stages of cirrhosis. LDS stages, regardless of whether calculated with IgG or gamma globulins, could be distinguished significantly.

As the PP2 population by definition only comprised patients with confirmed cirrhosis, any patient classified as 'normal' by any marker that indicates or excludes cirrhosis could be considered a false negative result. Thus, a rating of cirrhosis markers based on the frequency of false negative and true positive findings was possible. Based on a cut-off value of 0.313 as found in a previous study, the NRL972 C(30):C(10) concentration ratio as cirrhosis marker delivered the lowest percentage of false negative results (7.2%). Other markers failed to accomplish this accuracy; some markers even delivered more than 50% of false negative findings. This was true even if the high number of missing values (up to 20% for some markers) was taken into account. This finding supports the potential utility of the NRL972 C(30):C(10) concentration ratio to detect the presence of hepatic cirrhosis; it may also be an indicator that most hepatic scores are based on too few parameters, especially if the good correlation of the NRL972 C(30):C(10) concentration ratio to the standardised scores obtained by comprehensive factor analysis is taken into account.

The liver volume was found to significantly influence the C(30):C(10) concentration ratio of NRL972, but not the clearance nor the termination half-life (PP2 population, for the ITT population a significant influence was found), which was probably due to the fact that most patients with enlarged liver volume had CTP class B, whereas most patients with reduced liver volume were found in CTP class C.

Analyses with respect to the aetiology of hepatic cirrhosis showed that the cause of the disease, be it chronic alcohol consumption, chronic viral hepatitis, or both, does not have a significant influence on the C(30):C(10) concentration ratio of NRL972. This finding supports the suitability of NRL972 as a comprehensive tool for staging and severity assessment of hepatic cirrhosis, where biological elimination is independent from and unaltered by the aetiology of cirrhosis.

The safety profile of NRL972 as assessed in this study did not differ from previous studies. No SAEs were assessed as to be related to the administration of NRL972. Five TEAEs out of 148 were assessed as probably related, and two as possibly related to IMP administration. The causality assessment to lidocaine, which was administered for performance of the MEGX test, showed that TEAEs were approximately eight times more frequently assessed as possibly or probably related to administration of lidocaine than to NRL972, which indicates a more acceptable risk profile for the NRL972 test compared to the MEGX test. The overall tolerability with respect to vital signs and changes in laboratory parameters was good, as no major changes after IMP administration were documented.

Overall, NRL972 PK parameters provide a promising tool to detect hepatic cirrhosis and/or assess the

severity and the stages of cirrhosis without major undue safety risks in a medically compromised patient population.

Date of the report: 21 Apr 2011

Norgine discontinued the development of NRL972 in July 2013.