SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion and scientific discussion on procedures, which have been finalised before 1 February 2004. For scientific information on procedures after this date please refer to module 8B.

1. Introduction

Metalyse is a new thrombolytic agent containing the active substance tenecteplase (TNK tPA). It is intended for fibrinolytic treatment in the acute phase of myocardial infarction, and should be administered as an intravenous bolus over approximately 10 seconds in a weight-adjusted dose range from 30 to 50 mg. The clinical benefits of fibrinolytic therapy in patients with acute myocardial infarction (AMI) are well documented, and a close correlation between early treatment and survival is established. The rationale for the development of TNK-tPA was to find a fibrinolytic substance close to the native t-PA, which can be administered as a rapid, single bolus injection.

TNK-tPA is an enzyme (serine protease) that converts plasminogen to plasmin, primarily in the presence of fibrin. TNK-tPA also binds to fibrin in a thrombus and selectively converts thrombus-bound plasminogen to plasmin. This initiates local proteolysis of the fibrin matrix of the thrombus. Tenecteplase is a glycoprotein produced by recombinant DNA technique using Chinese Hamster Ovary (CHO) cells. It is a modified form of the naturally occurring human plasminogen activator. The molecule exhibits a well-known pronounced domain structure. Three sites of the molecule have been modified using site directed mutagenesis, thus tenecteplase has different pharmacokinetic properties from those of r-tPA.

Metalyse is presented as a lyophilised powder in a 20-ml glass vial with a rubber stopper and a vial cap with an integrated reconstitution device. It is available in three doses, 30 mg, 40 mg and 50 mg. The powder is reconstituted with water for injections from a pre-filled syringe.

Abbreviations used in this document:

ACT	Activated Clotting Time
AMI	Acute Myocardial Infarction
aPTT	Activated Partial Thromboplastin Time
ASA	Acetyl salicylic acid
AUC	Area Under the plasma concentration time Curve
CABG	Coronary Artery Bypass Graft
CI	Confidence Interval
DSMB	Data and Safety Monitoring Board
FDPs	Fibrin Degradation Products
IABP	Intra Aortic Balloon Pump
ICH	Intracranial Haemorrhage
IRA	Infarct Related Artery
i.v.	intravenous
MI	Myocardial Infarction
PAI-1	Plasminogen Activator Inhibitor-1 (physiological)
PD	Pharmacodynamics
PK	Pharmacokinetics
PTCA	Percutaneous Transluminal Coronary Angioplasty
TNK-tPA	Tenecteplase
rt-PA	Recombinant tissue-type Plasminogen Activator (alteplase)
SD	Standard Deviation
TIA	Transient Ischemic Attack
TIMI	Thrombolysis in Myocardial Infarction
U	Units

2. Chemical, pharmaceutical and biological aspects

Composition

Metalyse is available as powder and solvent for solution for injection. The active substance is available as a lyophilised powder in a 20-ml glass Type I vial with a bromobutyl rubber stopper and a vial cap with integrated reconstitution device. It is presented in three dosage forms, 30 mg, 40 mg and 50 mg. The powder is reconstituted with water for injection from a 10-ml polypropylene pre-filled syringe with a recommended volume of 6 ml, 8 ml or 10 ml, respectively.

Name of Ingredients	Function	Reference to Standards
Powder		
TNK-tPA	active substance	not applicable/Internal
Arginine Free Base	to increase solubility of TNK-tPA	Ph. Eur.
85% Phosphoric Acid	Buffering Agent	Ph. Eur.
Polysorbate 20	to prevent product loss due to non specific binding of TNK-tPA to equipment/containers	Ph. Eur.
Solvent		
Sterile water for Injection	solvent for reconstitution	Ph. Eur.

Development pharmaceutics: The choice of composition has been explained and is supported by results from the pharmaceutical development. Three vial strengths are manufactured from formulations that differ only in their amount of TNK-tPA.
An overfill for the three different dosage forms intended to ensure that the withdrawable contents of the vials are not less than the labelled contents has been sufficiently justified.

The manufacturing process is described in sufficient detail and adequately validated with overall consistent results for the three dosage forms.

Active substance

The test procedures and validation reports are included in the dossier and have been clearly presented.
As regards the choice of the routine tests it is considered that the list of quality characteristics to be tested is justified.

Potential impurities arising from the expression system include CHO-proteins. The removal of host cell derived DNA has been addressed by validation data. The "validation approach" as such appears justified. Thus, the absence of the quality characteristic "residual DNA" in the bulk specification for routine testing is considered to be acceptable.

Development Genetics
A mutant CHO cell line was used. This cell line had a long history of changes as it was already established from a subclone of the original cell line. A clone of this CHO cell line was selected and transfected with the TNK-tPA expression plasmid and co- transfected with a DHFR selection vector. Both plasmids are described in sufficient detail.
A detailed map of both plasmids and sequencing data are provided.

Cell Bank System
The master cell bank (MCB), working cell bank (WCB) and end-of-production cells (EOP) have been adequately characterised. The absence of adventitious agents, viral and non-viral contaminants has been confirmed by several methods.

The genetic stability of the MCB was examined. The specific productivity of EOP cells of 4 validation runs was compared to the specific productivity measured during the cell bank stability study. Data with regard to TNK-tPA cell banks, from various thawings over a period of storage exceeding 4 years

have shown that the length of storage has no negative effect on the viability of the cells. Additionally, information on the viability of individual cell banks (MCB and WCBs) used for manufacturing has been provided. In this context it should also be mentioned that whenever a new cell bank is prepared a variation should be filed to the EMEA.

Fermentation and Harvesting

The fermentation is generally started from the released WCB's, but exceptionally the applicant has confirmed that the fermentation may start using the MCB. The fermentation process is performed in three stages, a seed train, an inoculum train and a production culture.

The in-process controls for each step are provided and appear sufficient to control the process.

The only ingredient of animal origin is hydrolised peptone. This raw material is of bovine, porcine and equine origin. The starting material for the peptone comes from the USA, Canada, Australia and New Zealand. Certificates for all countries of origin have been submitted. Steam sterilisation of hydrolised peptone is performed at 121^{o}C for 20 minutes. A risk evaluation has been performed and is satisfactory.

Extensive data on the validation of the fermentation are provided.

Purification

A single batch of harvest cell culture fluid is produced from each 12,000 l fermenter and purified. The purification comprises chromatography steps, a viral inactivation step and a number of filtration steps. Sufficient details have been provided on reagents, columns and purification conditions.

Characterisation

Tenecteplase (TNK-tPA) is a mutated variant of recombinant tissue plasminogen activator alteplase (rt-PA). It is a multidomain protein involved in the regulation of the fibrinolytic pathway of haemostasis. Tenecteplase (TNK-tPA) was developed as a second generation molecule in which 6 amino acids (out of 527) were mutated. Tenecteplase results from the modification of alteplase at 3 specific sites: T (Thr 103 to Asn), N (Asn 117 to Gln) and K (Lys-His-Arg-Arg (296-299) to Ala-Ala-Ala-Ala). These amino acid substitutions have resulted in significant differences in the carbohydrate moiety of the molecule. The presence of 3 proteolytic cleavage products previously observed in alteplase was confirmed by N-terminal analysis. The amino acid sequence has been unequivocally determined. The observed amino acid sequence of tenecteplase has been shown to be consistent with the intended sequence as predicted by the DNA sequence by a combination of methods.

Analytical Development

Full details on the various tests used have been provided and are satisfactory.

Since there is no international reference material for TNK-tPA available, Boehringer Ingelheim developed and qualified a respective in-house reference material for in-process-, bulk and release testing of TNK-tPA.

Process validation

Testing EOP cultures for sterility, mycoplasma contamination, endotoxin level, retroviruses and adventititious viruses has shown there are no contaminating adventitious microorganisms. Measurement of RVLP in EOP cells results in RVLP concentrations typical for CHO cell lines and infectivity tests were always negative.

Process validation has been carried out for growth parameters.

Purification process

The process qualification requirements and acceptance criteria studies were conducted on 3 batches.
Data on the capacity to remove impurities are provided for each step of the purification process. The reduction of these impurities has been demonstrated. Re-use studies for the columns are provided and a cycle number has been established for each column.

Impurities

Detailed information on the impurity profile and the removal of impurities has been provided. These data demonstrate that the purification process produces active substance with acceptable residual impurities.

CHO proteins are the only impurity that is routinely tested in the active substance. For all other impurities, including host cell DNA, a validation approach is used. The validation approach as such appears justified on the basis of the results of the studies submitted on the removal of DNA. The omission of a specification limit for DNA in the bulk drug substance is acceptable, provided that an IPC for DNA is performed for a certain number of batches.

Whenever changes in the manufacturing process are made the removal of the impurities should be considered and a revalidation performed if relevant, especially for those impurities for which a validation approach was used.

Batch Analysis

Batch analyses of the bulk active substance have been performed on 4 batches produced at the 12,000-l scale at Boehringer Ingelheim. The bulks were derived from 4 different fermentation runs. These batches include the process validation batches. The assays for bulk analysis have been described.

The test results on 4 Batches of Bulk Drug Substance manufactured at Boehringer Ingelheim met the specifications. The company further provides a batch data assessment in which the results are summarised separately for each parameter. The results are also satisfactory. Product consistency for the batches from this process can therefore be established.

Control of the finished product

The finished product release specification for the 30, 40 and 50 mg dosage forms and test procedures have been provided. The results from batch analysis are satisfactory. Product consistency can be established.

Stability of the Product

Stability tests on the active substance

The stability testing of the Bulk active Substance of TNK-tPA was performed on 3 batches manufactured at the 12000 l scale and the data support the claimed shelf life.

Stability tests on the finished product

A total of 10 batches of the lyophilisate, comprising 30, 40 and 50 mg preparations, all of them manufactured at Boehringer Ingelheim, have been investigated in stability trials. Satisfactory 3-year stability data are now available. On this basis, a shelf-life of 3 years can be granted. The recommended storage temperature is up to 30°C.

Virological Documentation

The viral validation studies were performed in accordance with the appropriate ICH guidelines.
The MCB and EOP cells have been extensively investigated for contamination with viral agents. Particular attention was paid to the investigation of potential retrovirus contamination of the MCB, WCB and EOP cells. No infectious retrovirus was found. *In vitro* assays for adventitious viral contaminants have been performed on the MCB, WCB and EOP cells. To date no evidence of viral contamination has been observed.

The *in-vivo* assay for adventitious viral contaminants (test for the presence of inapparent, latent virus) was conducted with adult and suckling mice, guinea pigs and embryonated eggs.

Hamster and mouse antibody production test (HAP, MAP) were performed with the MCB, WCB and EOP cells.

In concentrated culture fluids from the WCB including EOP cells reverse transcriptase activity was detected, indicating the presence of particles containing reverse transcriptase, which is usual for CHO cells. Since the CHO cells used for the production contain endogenous retrovirus-like particles it is

important that the purification process should remove or inactivate these viruses as demonstrated with the X-MuLV model virus.

Purification process
BI to demonstrate the capacity of the purification process to eliminate viruses performed viral clearance studies. Full details of scale-down and the spiking experiments of the relevant purification steps have been provided. The choice of the model viruses in the BI study appears reasonable.

Solvent

The prefilled syringes with water for injections are manufactured at Abbott Laboratories, North Carolina 27801, USA. 10 ml propylene plastic syringes (Ansyr®) are used. Three different fill volumes are specified, 6 ml, 8 ml, and 10 ml. A short description of the product preparation is provided. The product is terminally sterilised. The specifications meet Ph Eur requirements.
The quality control testing and release of the prefilled water for injections syringes is performed at BI Pharma KG, Biberach, Germany.

Stability data are submitted for 3 batches representing the 10 ml, 8 ml and 6 ml volumes. The given shelf-life specifications meet the Ph Eur requirements in the monograph water for injection. Based on the provided data, the shelf life of 24 months as proposed by the company is acceptable.

Statement on GMP
The finished product is manufactured and released in the EU by Boehringer Ingelhein KG, Birkendorfer Strasse 65, D-88397 Biberach an der Riss, Germany. A copy of the current manufacturing licence has been provided.

Discussion on chemical, pharmaceutical and biological aspects

The substance is well characterised and the consistency of the product and the production process is deemed sufficient. The dossier takes into account current directives and guideline requirements as well as demonstrating that the medicinal product is made in compliance with the CPMP Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via medicinal products.

3. Toxico-pharmacological aspects

Pharmacodynamics

- *In vitro* studies

Results of *in vitro* studies demonstrated that clot lysis activity in human plasma induced by TNK-tPA was similar to that observed for tPA. At least a 10-fold higher fibrin **specificity** and an 80-fold higher resistance to inhibition by plasminogen activator inhibitor PAI-1 were found for TNK-tPA compared to tPA.

- *In vivo* studies

Thrombolytic efficacy, possible side effects as systemic fibrinolysis and bleeding tendencies were investigated in various animal models. It was shown that TNK-tPA was more potent in clot lysis than tPA. Furthermore, TNK-tPA decreased systemic fibrinolytic parameters to a lesser extent than tPA. The increased fibrin **specificity** of TNK-tPA reduces the propensity for causing peripheral bleeding and ameliorates the risk of cerebral haemorrhages in a rabbit model of embolic stroke.

Pharmacokinetics

Pharmacokinetics of TNK-tPA are highly consistent across animal species and humans. The initial volume of distribution of TNK-tPA (V_1) generally approximated plasma volume. These data suggest that TNK-tPA immediately distributes throughout the plasma compartment and, over time, exhibits limited extravascular distribution and/or tissue binding.

Dose-independent kinetics were observed in rats over the range of doses from 0.3 to 50 mg/kg. However, in beagle dogs, kinetics appeared to be dose-dependent from 0.3 to 30 mg/kg. At a dose level of 0.3 mg/kg, dogs have a TNK-tPA clearance similar to that in mice and rats (approximately 4 to 5 ml/min/kg) and approximately twice that observed in rabbits and monkeys at this dose level (2.0 to 2.4 ml/min/kg). Kinetics in humans were linear in the restricted dose range used in Phases II and III, but exhibited some nonlinearity over the wide dose range used in Phase I.

Possible effects of glycosylation on clearance mechanism of TNK-tPA were investigated in rats. TNK-tPA, like tPA is a glycoprotein, but with differing oligosaccharide structures. Indirect evidence suggests that the hepatic mannose receptor, which is important for tPA elimination, is not involved in TNK-tPA elimination, as TNK-tPA does not contain high mannose oligosaccharide. The hepatic low-density receptor related protein (LRP) could be involved in the clearance of both, TNK-tPA and tPA. Furthermore, the hepatic asialoglycoprotein receptor, which binds terminal galactose, may mediate TNK-tPA clearance.

In a whole body autoradiography study conducted in rats the tissue distribution following a single intravenous bolus injection of TNK-tPA, compared to t-PA. was assessed. The results clearly demonstrated that the liver was the major clearance organ for both [^{125}I]TNK-tPA and [^{125}I]tPA. It appeared that TNK-tPA was distributed to similar tissues as tPA, but with different kinetics.

Toxicology

Single dose toxicity

Single dose toxicity studies in rats rabbits and dogs using up to 36 times the intended clinical dose of Metalyse induced no effects other than the expected pharmacological changes of the coagulation system, primarily characterised by a transient decrease of α2-antiplasmin and fibrinogen.

Repeated dose toxicity studies were performed in rats and dogs with daily intravenous doses of Metalyse for two weeks. Toxicological signs were related to the exaggerated pharmacodynamic properties of Metalyse, i.e. the fibrinolytic action (haemorrhages at the injection site). No test article-related intercurrent deaths or unexpected toxicity occurred at doses up to 10 mg/kg, as long as antibody-formation did not confound the toxicological evaluation. The pharmacodynamic changes of the coagulation system observed in Metalyse-dosed dogs were similar in timing and magnitude to those produced by alteplase.

Reproductive toxicity was studied in the rabbit as a sensitive species during the period of organogenesis. Because antibody towards Metalyse are detectable at day 7, the period of treatment in the main embryofetal development study was divided into three equal periods. In a preliminary study single doses of 5 mg/kg, approximately 10 fold the clinical dose, did not show any bleeding complications when applied on either day 14, 15, 16 or 17 of pregnancy. The same holds true for repeated dosing during the period of early organogenesis in the main study. Pregnant Rabbits of all groups given Metalyse after gestation day 10, showed perivaginal bleeding, abortion and consecutive maternal death, with a high incidence. The results indicate that later stages of embryofetal development in rabbits appear to be more susceptible than earlier stages. Effects of Metalyse on fertility and on the perinatal and postnatal period were not studied. This is supported by the short duration of exposure, the mechanism of action and available data of such studies for alteplase, which were considered to be representative for this group of substances.

In conclusion, a comparison with other plasminogen activators shows that tenecteplase does not pose a higher risk to pregnant women than the other substances of this group. The results of reproductive toxicity testing are expressed adequately in the SPC.

Genotoxicity and carcinogenicity studies were not conducted. This is justified by the literature, not describing any genotoxic or carcinogenic potential of tissue plasminogen activator as well as the single dose administration to humans.

Local tolerance studies in rabbits and rats after intravenous, intraarterial and paravenous administration of Metalyse did not elicit irritation or bleeding at the injection site.

4. Clinical aspects

The clinical trials were performed according to GCP standards and agreed international ethical principles. The clinical trial programme consists of 4 studies.

Study Report	Design	N= Dose	Objective
TIMI 10 A	Open, uncontrolled Parallel-group Dose-finding	N=113 TNK-tPA 5/ 7,5/ 10/ 15/ 20/ 30/ 40/ 50 mg	Phase I single-dose study on pharmacokinetics, pharmacodynamics, safety and tolerance of TNK-tPA in AMI
TIMI 10 B	Open, controlled Parallel-group Dose-finding	N=555 TNK-tPA 30/ 40/ 50 mg N=325 rt-PA 100 mg	Phase II randomised angiographic trial on patency of infarct related artery (IRA), TIMI flow, effects on coagulation factors, fibrinogenolysis and pharmacokinetics, with AMI within 12 hours of symptom onset.
ASSENT I	Open, uncontrolled parallel-group	N=3235 TNK-tPA 30/ 40/ 50 mg	Phase II randomised trial on safety of TNK-tPA in AMI
ASSENT II	Double-blind Controlled Parallel-group	N=8461 TNK-tPA N=8488 rt-PA	Phase III trial on mortality of a single-bolus TNK-tPA vs. accelerated rt-PA in AMI

Clinical pharmacology

The clinical pharmacology of TNK-tPA was investigated in TIMI 10A and TIMI 10B studies involving 993 patients evaluating the pharmacodynamic effects and pharmacokinetic behaviour of the compound.

Pharmacodynamics

Data on coagulation and fibrinogenolysis were obtained during TIMI 10A study (n=79) and TIMI 10B study (n=159) in patients with acute myocardial infarction, treated concomitantly with ASA and heparin.

The pharmacodynamic effects of TNK-tPA in **TIMI 10A** study were assessed by measuring the responses of coagulation parameters to treatment and by evaluating patency of the infarct related artery using coronary angiography. TNK-tPA showed slight, not dose-dependent decrease of fibrinogen and plasminogen levels indicating a high degree of fibrin specificity. Coagulation parameters indicate an increase in the level of systemic plasmin generation at both 1 hour and 3 hours after administration which was consistent with the intended effect of plasminogen activation.

In **TIMI 10B**, patency, coagulation parameters as well as pharmacokinetics of TNK-tPA were investigated and compared with accelerated alteplase. The core coagulation laboratory data indicated that TNK-tPA had less impact on systemic clotting factors thanalteplase, i.e. was more fibrin specific than alteplase (e.g. less decrease of fibrinogen and plasminogen levels). Unlike accelerated rt-PA fibrinogen levels decreased by < 15% and plasminogen decreased by < 25% 3 hours after administration of TNK-tPA. Median levels of plasmin/α_2-antiplasmin were affected 3-5 times less by TNK-tPA than byalteplase.

TIMI 10B confirmed the results of TIMI 10A and showed comparable results of the coagulation parameters for the 30 mg, 40 mg and 50 mg dose groups, indicating reproducible and consistent effects.

Pharmacokinetics

Pharmacokinetics of TNK-tPA were studied in a total of 179 patients with acute myocardial infarction during the TIMI 10A (n=80) and TIMI 10B (n=99) trials. Comparative data with rt-PA were obtained in additional 53 patients. All formulations administered to patients consisted of a powder and solvent

(sterile water) for solution for injection (for intravenous use). The pharmacokinetic parameters indicated a dose-related increase in the TNK-tPA concentrations following i.v. bolus administration.

In **TIMI 10A** the concentration decreased in a biphasic manner following a bolus injection of TNK-tPA. The initial phase had a half-life of 11-20 minutes and the terminal phase had a half-life of 41-138 minutes for doses of 5-50 mg. The mean plasma clearance of TNK-tPA across the doses of 5-50 mg was 151 ml/min, ranging from 216 ml/min at a dose of 5 mg to 125 ml/min at a dose of 50 mg, indicating moderate non-linear pharmacokinetics in the lower dose range. The plasma clearance of TNK-tPA was 2- to 4-fold slower than that reported for rt-PA. The initial volume of distribution of TNK-tPA approximated the plasma volume.These PK results indicated that TNK-tPA administered as a bolus should result in a plasma concentration profile similar to that of the 90-minute accelerated rt-PA infusion.

In the **TIMI 10 B**, the determined PK parameters showed that the average maximum TNK-tPA plasma concentration (C_{max}) is increased in a dose-proportional manner (7.52-11.6 µg/ml) following i.v. bolus administration in the 30 - 50 mg dose range. The initial volume of distribution (V_1) ranged from 4.2 to 5.4 litres, approximating plasma volume, and was highly variable. The steady-state volume of distribution (V_{ss}) ranged from 6.1 - 8 L and was slightly larger than V_1, suggestive of some extravascular distribution or tissue binding. The mean residence time in the body was approximately 1 hour. The plasma clearance (CL) was approximately 100 ml/min and was similar for the 30-, 40- and 50 mg doses. TNK-tPA has a 4- to 5-fold slower plasma clearance compared to alteplase (453 ml/min). Following a bolus dose the plasma concentrations in most subjects decreased in a biphasic manner. The initial and terminal phase half-lives were approximately 22 and 90-129 minutes respectively.

The TIMI 10B study showed comparable results to TIMI 10A. In the group of patients for which complete PK and demographic data (e.g. age, weight, sex) were available (n=96), data showed that plasma clearance increased with increasing weight and decreasing age (weight accounted for 19% of the variability in plasma clearance of TNK-tPA).

The PK data of TNK-tPA differed from those of alteplase (longer half-life, approx. 4-fold slower plasma clearance, approx. 2-fold greater plasma exposure according to AUC) and support the intended single bolus administration.

No formal interaction studies have been performed to evaluate pharmacodynamic and pharmacokinetic interactions between TNK-tPA and other drugs commonly administered for the treatment of AMI (e.g. nitrates, heparin, platelet inhibitors, ß-blockers, ACE-inhibitors, etc.). No clinical trials were performed to investigate the pharmacokinetics (and Pharmacodynamics) of TNK-tPA in patients with hepatic or renal impairment.

Clinical efficacy

In total 12,364 patients were exposed to TNK-tPA in the submitted phase II and III clinical studies. The phase II programme included more than 4,000 patients and more than 3,700 of them received TNK-tPA (582 patients were treated between 6 to 12 hours). The phase III study was conducted in approx. 17,000 patients. The active comparator in the clinical trial programme wasalteplase, administered as the accelerated regimen.

Dose-response studies and main clinical studies

Dose response studies

The dose-response relationship was investigated in three trials including a total of 4228 patients with AMI up to 12 hours from symptoms onset.

In **TIMI 10 A**, 113 patients (< 70 years) with ST-segment elevation AMI presenting within 12 hours after symptom onset were treated with a single intravenous bolus of TNK-tPA over 5-10 seconds in a dose of 5 mg, 7.5 mg, 10 mg, 15 mg, 20 mg, 30 mg, 40 mg and 50 mg. The patients received concomitantly ASA (325 mg), and unfractionated heparin (i.v. bolus of 5000 U, followed by an infusion at 1000 U/hour for 48-72 hours, adjusted to maintain an aPTT of 55-85 seconds). Endpoints included the effect on coagulation parameters, evaluation of PK, angiographic assessment by TIMI grade flow, TIMI frame count, to quantify coronary flow objectively, and safety.

The percentage of patients achieving TIMI grade 3 flow at 90 minutes in the infarct related artery (IRA) was higher in patients treated with doses of 30, 40 and 50 mg as compared with lower doses. Statistical differences in the 30 mg, 40 mg and 50 mg doses were not apparent in this small trial.
Based on this pilot data and assuming a plateau-like effect of TIMI grade 3 flow from the 30 mg dose, the doses of 30 mg and 50 mg were selected for the subsequent phase II studies (TIMI 10B and ASSENT-I).

In the **TIMI 10B** study (March 1996-April 1997) the primary objective was to determine the percentage of subjects with TIMI grade 3 flow in the IRA 90 minutes after the start of treatment with bolus TNK-tPA compared with acceleratedalteplase. Secondary objectives included the evaluation of the effects of TNK-tPA on coagulation factors and fibrinogenolysis as well as the evaluation of the pharmacokinetics (as described in previous sections), safety and clinical efficacy of TNK-tPA. Moreover, the reocclusion rate at 18-36 hours after treatment initiation and of the formation of antibodies against TNK-tPA were to be evaluated.
Initially this trial was designed to evaluate coronary patency rates with 2 doses of TNK-tPA (30 mg and 50 mg), administered as i.v. bolus injections over 5-10 seconds as compared to a 90-minute accelerated infusion regimen of alteplase. In September 1996, the initial phase of the recruitment, the study protocol was amended (discontinuation of the 50 mg dose and replacement with 40 mg dose, adjustment of heparin dose, and age limit of 80 years as an inclusion criterion) following recommendation of the Data Safety Monitoring Board because 3 patients experienced intracranial haemorrhage (3/78).
Study population: 880 patients presenting with AMI were enrolled in this study and 877 were randomised. In general, patients were comparable across treatment groups with respect to demographic and baseline characteristics. The mean time for MI symptom onset to study drug initiation was 3.5 hours. Approximately 87% of patients were treated within 6 hours of symptom onset. The percentage of patients with >6 hours to 12 hours time from symptom onset to study drug initiation ranged from 11.6-13.2% in the different treatment groups.
The comparator treatment alteplase was administered as an accelerated, 90 minutes infusion regimen.
Concomitant medication:
ASA (150 mg -325 mg, p.o.) and i.v. heparin were given concomitantly, beginning at study entry.
A total of 362 patients was recruited before the (September 1996) protocol amendment mandating changes in heparin dosing and 518 patients were recruited thereafter. In addition, the company showed that the initial heparin use was comparable between the treatment groups. Other important concomitant medications were ß-blockers, calcium antagonists, nitrates and ACE-inhibitors with no relevant differences between treatment groups.

Results:
With regard to the primary endpoint TIMI grade 3 flow in the IRA 90 minutes the only statistically significant result was 30 mg TNK-tPA versus alteplase with a lower percentage of patients with TIMI grade 3 flow in the patients with 30 mg TNK-tPA (p=0.035).

Main Efficacy Results for TIMI 10B

	TNK-tPA 30 mg	**TNK-tPA 40 mg**	**TNK-tPA 50 mg**	**rt-PA**
Primary Endpoint: TIMI Grade 3 Flow at 90 Minutes				
	(n=302)	(n=148)	(n=76)	(n=311)
No. of patients (%)	164 (**54.3%**)[a]	93 (**62.8%**)	50 (**65.8%**)	195 (**62.7%**)
95 % CI	(48.5%, 60%)	(54.5%, 70.6%)	(54%, 76.3%)	(57.1%, 68.1%)
Secondary Endpoints: TIMI Grade 2 or 3 Flow at 90 Minutes				
	(n=302)	(n=148)	(n=76)	(n=311)
No. of patients (%)	232 (76.8%)	117 (79.1%)	67 (88.2%)	254 (81.7%)

[a] $p < 0.05$ compared with alteplase.

Subgroup analyses for 90-minute TIMI grade 3 flow and TIMI frame count by time to treatment and weight were performed.

In general, a shorter duration of time from symptom onset to treatment was associated with a higher percentage of subjects with TIMI grade 3 flow. It seems to be appropriate to use the conclusions drawn from the overall study as the basis for the justification of the dose regimen for the investigated patient population in the pivotal trial (AMI patients within 6 hours of symptom onset).

Furthermore, additional exploratory analyses - not pre-specified in the study protocol - were conducted to evaluate the relationship between weight-adjusted dose, TIMI grade flow, and TIMI frame counts.

A logistic regression model (Cox proportional hazard model) was used with TIMI grade as the dependent variables.

Significant predictors for TIMI grade 3 flow were log (dose/weight), age and infarct location. The percentage of patients with TIMI grade 3 flow seemed to increase with increasing dose per weight.

Furthermore, TIMI grade 3 flow after 90 minutes was assessed relative to systemic exposure of TNK-tPA over the first 90 minutes ($AUC_{2\text{-}90}$) showing a trend toward an increased percentage of patients who achieved TIMI grade 3 flow when AUC was greater.

In the safety study ASSENT I an exploratory analysis showed that mortality was directly related to dose/weight and, independently, inversely related to weight (i.e. an increase in dose/weight as well as a decrease in weight resulted in increased mortality).

It was determined on the basis of and integrated analysis of Phase I and Phase II data that the optimal regimen for TNK-tPA should be a weight-adjusted dose of approximately 0.53 mg/kg.

Main study

The ASSENT-II Study was conducted to demonstrate efficacy and safety in patients with acute myocardial infarction. This trial started on October 21, 1997, involving 29 countries (1,022 centres) and the enrolment of 17,005 patients was completed by December 29, 1998.

Study design:

The ASSENT-II Study is a multicentre, randomised, rt-PA-controlled, double-blind, double-dummy study in patients with AMI presenting within 6 hours of symptom onset. The patients were either treated with a bolus of TNK-tPA or the accelerated regimen of alteplase.

Patients 18 years and older who presented acute (≤ 6 hours) symptoms and prespecified ECG signs of myocardial infarction were included.

Dose regimens: The dose of TNK-tPA was a weight-adjusted rapid intravenous bolus over 5-10 seconds.

Subject weight	TNK-tPA dose
< 60 kg	30 mg
≥ 60 kg and <70 kg	35 mg
≥ 70 kg and <80 kg	40 mg
≥ 80 kg and < 90 kg	45 mg
≥ 90 kg	50 mg

The accelerated dose regimen of the comparator alteplase was also weight-adjusted (not to exceed 100 mg). For patients weighing:

> 67 kg: 15 mg as an IV bolus, followed by 50 mg infused over the first 30 minutes, then 35 mg infused over the next 60 minutes.

≤ 67 kg: 15 mg as an IV bolus, followed by 0.75 mg/kg infused over the first 30 minutes not exceeding 50 mg, then 0.50 mg/kg up to 35 mg infused over the next 60 minutes, not to exceed 35 mg.

Concomitant therapy:
ASA (150 – 325 mg) immediately upon study entry. Alternatively, if the patient was unable to ingest tablets, an initial dose of 100-250 mg ASA may have been given intravenously (in countries where this regimen is approved). Patients who had taken ASA within 12 hours before randomisation started ASA the next day.
Heparin was administered on a weight-adjusted basis as soon as possible after study entry for 48-72 hours.
For patients weighing ≤ 67 kg: 4000 U as bolus, 800 U/hour initial infusion rate
For patients weighing > 67 kg: 5000 U as bolus, 1000 U/hour initial infusion rate
The heparin infusion was adjusted to maintain an aPTT of 50-75 seconds.
The use of GP IIb/IIIa antagonists was discouraged during the first 24 hours after randomisation.

The primary efficacy objective was to demonstrate therapeutic equivalence in 30-day mortality between the TNK-tPA and alteplase dose regimens.

Secondary efficacy objectives:
- "net clinical" benefit, defined as the absence of mortality and non-fatal stroke at 30 days.
- the rate of the following non-fatal major cardiac in-hospital events:
  Recurrent MI, sustained hypotension, occurrence of pulmonary oedema and/or cardiogenic shock, major arrhythmias, invasive cardiac procedures, pericarditis, acute mitral regurgitation, pulmonary embolism, and tamponade
- 30-day mortality and net clinical benefit at 30 days according to age (≤ 75 y vs. > 75 y), time to treatment (0-2 hrs, >2-4 hrs, >4 hrs), infarct location (anterior vs. non- anterior) and history of previous MI (yes vs. no)

Statistical analysis
The primary endpoint (30-day mortality) was defined as death at or before 30 days after randomisation/enrolment. The upper bound of the two-sided 90 % confidence intervals for the absolute difference and the relative risk between the TNK-tPA and accelerated alteplase groups was used to demonstrate a clinical equivalence of 30-day mortality between the two treatment groups by showing that the 30-day mortality after TNK-tPA does not exceed the 30-day mortality after accelerated alteplase by more than 1 % or that the relative risk in 30-day mortality after TNK-tPA over accelerated alteplase does not exceed 14 %, whichever is smallest.

Three different analyses were performed for this trial: intent-to-treat, as treated, and randomised and treated. The protocol specified that the primary analysis of this study was to be performed on the intent-to-treat population. Therefore, efficacy and safety results are presented for this population only. The efficacy analysis of the intent-to-treat population included five prespecified covariates: age, Killip class, heart rate, systolic blood pressure, and infarct location.

(b) Study populations/accountability of patients
From 21 October 1997 to 29 November 1998, 17,005 patients were enrolled at 1,022 sites in 29 countries. Out of the 17,005 patients enrolled, 16,949 were randomised: 8,461 patients to TNK-TPA and 8,488 to alteplase. 445 patients were randomised but did not receive study drug (236 in the TNK-tPA and 209 in the alteplase group).

There were 16,557 patients in the as-treated population: 16,504 subjects were randomised according to baseline characteristics (e.g. sex, age, weights, medical history) and treated and 53 subjects were not randomised but treated. The analyses of this population were performed according to the treatment actually received. Of the 16,949 intent-to-treat patients, 16,943 (99.96 %) completed the 30-day follow-up. There was no significant difference between the two treatment groups with respect to baseline variables and medical history, being in addition very similar to the baseline characteristics recorded in previous thrombolytic studies such as GUSTO I and GUSTO III.

Efficacy Results

Thirty-Day Mortality:
A total of 1,045 deaths were observed in the intent-to-treat population: 521 patients were in the TNK-tPA group and 524 in the alteplase group. Data are missing for 6 patients.
The following table presents the relative risk and the absolute difference.

30-day mortality

	TNK-tPA	rt-PA	Relative Risk (90% Cl)	p-value	Absolute Difference (90% Cl)	p-value
Primary analysis Nonparametric adjusted rate (*)	6.179	6.151	1.004 (0.914,1.104)	0.0278	0.028 (-0.554,0.609)	0.0059
Secondary analysis Unadjusted rate	6.160	6.176	0.997 (0.904,1.101)	0.0264	-0.016 (0.624,0.592)	0.0060
Exploratory analysis Logistic regression (*)	6.089	6.140	0.992 (0.903,1.089)	0.0147	-0.051 (-0.623,0.522)	0.0025

The p-value is based on a test for equivalence
(*) Covariates used are age, infarct location, Killipp class, SBP and heart rate

Therapeutic equivalence could be demonstrated on the basis of the prespecified limit of 14 % for the relative risk and the prespecified boundary of 1 % for the absolute difference in all-cause mortality up to 30 days after initiation of study drug. The primary analysis as well as the additional analyses show similar results. According to the ICH 9 Guideline, the additionally calculated upper limit of the 95% CI for the nonparametric adjusted relative risk ratio (primary analysis) was 1.124 and thus, non-inferiority with respect to the non-inferiority margin of 14% can be concluded.
The mortality rates found in ASSENT II (approx. 6.2 % for both TNK-tPA and alteplase) are comparable to those of accelerated rt-PA in GUSTO I (6.3 %).

Thirty-Day Mortality-Subgroups:
A comparison of 30-day mortality rates for TNK-tPA and alteplase was considered for a range of subgroups as presented in the following table:

Thirty-Day Mortality Subgroups, Intent-to-Treat Population

	TNK-tPA (N=8461)	**rt-PA (N=8488)**	**Relative Risk (95% CI)**
Overall mortality (%)	521/8458 (6.16)	524/8485 (6.18)	0.997 (0.887,1.122)
Age (years)			
<= 75 (%)	338/7408 (4.56)	318/7410 (4.29)	1.063 (0.915,1.235)
> 75 (%)	182/1047 (17.38)	206/1070 (19.25)	0.903 (0.754,1.081)
Time to treatment (hours)			
0-2 (%)	125/2519 (4.96)	125/2563 (4.88)	1.017 (0.799,1.296)
>2-4 (%)	256/4036 (6.34)	214/3902 (5.48)	1.157 (0.970,1.379)
>4 (%)	129/1832 (7.04)	181/1970 (9.19)	0.766 (0.617,0.952)
Infarct location			
Anterior (%)	266/3332 (7.98)	279/3408 (8.19)	0.975 (0.830,1.146)
Other (%)	254/5112 (4.97)	245/5059 (4.84)	1.026 (0.865,1.218)
Previous MI			
Yes (%)	131/1335 (9.81)	118/1367 (8.63)	1.137 (0.897,1.441)
No (%)	389/7116 (5.47)	402/7098 (5.66)	0.965 (0.843,1.105)

Sex			
Male (%)	327/6518 (5.02)	314/6505 (4.83)	1.039 (0.894,1.209)
Female (%)	194/1940 (10.00)	210/1980 (10.61)	0.943 (0.784,1.134)
Heart rate(bpm)			
< 60 (%)	72/1454 (4.95)	56/1456 (3.85)	1.287 (0.915,1.812)
60-79 (%)	175/3871 (4.52)	185/3853 (4.80)	0.942 (0.769,1.152)
80-99 (%)	162/2346 (6.91)	165/2337 (7.06)	0.978 (0.794,1.205)
>= 100 (%)	110/774 (14.21)	115/820 (14.02)	1.013 (0.795,1.291)
Systolic blood pressure (mm Hg)			
< 100 (%)	89/462 (19.26)	83/469 (17.70)	1.089 (0.831,1.426)
100-139 (%)	256/4374 (5.85)	260/4321 (6.02)	0.973 (0.823,1.150)
140-174 (%)	162/3361 (4.82)	163/3390 (4.81)	1.002 (0.811,1.239)
>= 175 (%)	11/251 (4.38)	15/293 (5.12)	0.856 (0.401,1.830)
Killip class			
I (%)	351/7425 (4.73)	359/7462 (4.81)	0.983 (0.851,1.134)
II (%)	120/887 (13.53)	117/874 (13.39)	1.011 (0.797,1.281)
III (%)	27/93 (29.03)	24/98 (24.49)	1.185 (0.740,1.899)
IV (%)	18/35 (51.43)	22/36 (61.11)	0.842 (0.556,1.273)
Hypertension			
Yes (%)	255/3187 (8.00)	249/3267 (7.62)	1.050 (0.888,1.241)
No (%)	264/5263 (5.02)	271/5199 (5.21)	0.962 (0.816,1.135)
Diabetes			
Yes (%)	121/1383 (8.75)	116/1329 (8.73)	1.002 (0.786,1.278)
No (%)	398/7066 (5.63)	405/7140 (5.67)	0.993 (0.868,1.136)
Previous CABG			
Yes (%)	32/327 (9.79)	25/327 (7.65)	1.280 (0.776,2.111)
No (%)	488/8123 (6.01)	495/8136 (6.08)	0.987 (0.875,1.115)
Country (1)			
Europe (%)	259/4120 (6.29)	278/4112 (6.76)	0.930 (0.789,1.095)
Non-Europe (%)	262/4338 (6.04)	246/4373 (5.63)	1.074 (0.907,1.271)
Country (2)			
United States (%)	109/1811 (6.02)	100/1847 (5.41)	1.112 (0.854,1.447)
Non-US (%)	412/6647 (6.20)	424/6638 (6.39)	0.970 (0.851,1.106)
	TNK-tPA (N=8461)	**rt-PA (N=8488)**	**Relative Risk (95% CI)**
Race			
Caucasian (%)	468/7636 (6.13)	472/7637 (6.18)	0.992 (0.876,1.122)
African Descent (%)	5/116 (4.31)	3/114 (2.63)	1.638 (0.401,6.694)
Asian (Mongoloid) (%)	6/76 (7.89)	9/86 (10.47)	0.754 (0.281,2.022)
Other (%)	35/415 (8.43)	26/421 (6.18)	1.366 (0.837,2.227)
Weight (kg) (1)			
< 67 (%)	157/1556 (10.09)	149/1575 (9.46)	1.067 (0.862,1.320)
>= 67 (%)	357/6874 (5.19)	370/6879 (5.38)	0.966 (0.838,1.112)
Weight (kg) (2)			
< 60 (%)	75/629 (11.92)	67/653 (10.26)	1.162 (0.852,1.586)
60-69 (%)	117/1500 (7.80)	132/1479 (8.92)	0.874 (0.689,1.109)
70-79 (%)	136/2427 (5.60)	134/2437 (5.50)	1.019 (0.808,1.285)
80-89 (%)	97/2061 (4.71)	110/2026 (5.43)	0.867 (0.664,1.131)
>= 90 (%)	89/1813 (4.91)	76/1859 (4.09)	1.201 (0.890,1.620)

Results showed, as known from other thrombolytic agents, that factors such as age, time to treatment, infarct location, previous myocardial infarction, sex, heart rate, systolic blood pressure, Killip class, hypertension, diabetes and previous CABG can influence mortality.

Net Clinical Benefit:
Net clinical benefit was defined as the combination of death or non-fatal stroke at 30 days.
The next table presents the respective relative risks and absolute differences in the intent-to-treat population.

Death or nonfatal stroke

	TNK-tPA	rt-PA	Relative Risk (90% Cl)	p-value	Absolute Difference (90% Cl)	p-value
Primary analysis Nonparametric adjusted rate (*)	7.126	7.009	1.017 (0.931,1.110)	0.0328	0.118 (-0.503,0.739)	0.0194
Secondary analysis Unadjusted rate	7.106	7.036	1.010 (0.921,1.107)	0.0302	0.070 (-0.578,0.718)	0.0182
Exploratory analysis Logistic regression (*)	7.046	6.966	1.012 (0.927,1.104)	0.0251	0.080 (-0.533,0.693)	0.0136

The p-value is based on a test for equivalence
(*) Covariates used are age, infarct location, Killipp class, SPB and heart rate

The incidence of death or nonfatal stroke tended to be slightly higher in the TNK-tPA group compared to thealteplase group (7.11% vs 7.04%).

In-Hospital Events
During the in-hospital phase of the study, no significant difference between the two treatment groups was seen regarding reinfarction, sustained hypotension, pulmonary edema, cardiogenic shock, major arrthythmias, pericarditis, acute mitral regurgitation, pulmonary embolism, or tamponade. The incidence of invasive cardiac procedures during the in-hospital phase was similar in both treatment groups for PTCA, stent placement, and IABP.

Additional In-Hospital Events (Exploratory Variables)
No significant differences between the treatment groups were seen for “death or non-fatal ICH”, “death or nonfatal disabling stroke”, “death or non-fatal disabling ICH”, recurrent angina, acute ventricular septal defect, or TIA. Anaphylaxis occurred in 6 patients (0.07%) of the TNK-tPA group and in 16 patients (0.19%) of the alteplase group (p=0.0523). Anaphylaxis was considered possibly related to study drug in 1 out of 6 patients treated with TNK-tPA and in 8 out of 16 patients treated with alteplase.
In the TNK-tPA group more patients were in Killip Class I than in the alteplase group (93.91% vs. 93.03%, p=0.0255). In the TNK-tPA group fewer patients were in Killip Class IV than in the alteplase group (2.07% vs. 2.58%, p=0.032). There was no difference between the two groups with respect to patients presenting Killip Class II or Class III.

Conclusion on the efficacy results:
The intent-to-treat analysis (relative risk) demonstrated therapeutic equivalence for TNK-tPA and alteplase in 30-day mortality. The mortality rate of 6.2 % in both groups corresponds to that of alteplase in the GUSTO-I study (6.3 %, 30-day outcome). Risk groups have been identified and addressed in the SPC.

Clinical safety

Antibody formation:
Results of antibody formation were available for 543 TNK-tPA treated patients (at 30 days) in **TIMI 10B**. One patient had a positive titer antibody test, which was subsequently negative at 90 days. The observed titers were low and close to the detection limit of the assay.
This was in accordance to the respective data in **TIMI 10A.** Overall, the data on antibody formation that TNK-tPA does not seem to induce antibody formation.

Safety study ASSENT I

ASSENT I (Assessment of the safety of a new thrombolytic: TNK-tPA) was a randomised open-label uncontrolled safety study and included 3235 patients ≥ 18 years of age with AMI within 12 hours from symptom onset. This trial was performed at the same time as TIMI 10B and although there was no formal protocol amendment for this trial, the changes recommended by the DSMB (e.g. regarding the concomitant heparin dosage) were implemented at the same time that TIMI 10B was amended. In this study the dose regimen was not weight-adjusted and the patients were treated with fixed doses of 30, 40 or 50 mg TNK-tPA.
The primary objective was to evaluate the overall safety profile of TNK-tPA and the primary target variable was the rate of intracranial haemorrhages (ICH). Secondary target criteria were rates of death, total stroke (haemorrhagic and non-haemorrhagic), recurrent MI, cardiac revascularisation, cardiogenic shock, anaphylaxis, pulmonary oedema, and serious, life-threatening bleeding and the rate of death plus nonfatal strokes. Stroke events were reviewed by an Event Review Committee which classified events as ICHs or non-ICHs.

The study population consisted of patients ≥ 18 years with AMI who presented within 12 hours of symptom onset.

Number of TNK-tPA treated (safety evaluable) patients

	30 mg TNK-tPA	**40 mg TNK-tPA**	**50 mg TNK-tPA**	**Total**
No. of patients	1705	1457	73	3235
Discontinuation	139 (8.2%)	119 (8.2%)	4 (5.5%)	262 (8.1%)

The mean time from symptom onset to treatment was 3.8 hours. 16% of patients were treated >6 hours from symptom onset (30 mg: 14.8%, 40 mg: 17.1%, 50 mg: 21.9%).

Concomitant therapy: Patients were to be treated with 150-325 mg of oral ASA once daily (alternatively, an initial i.v. dose of 100-250 mg ASA was acceptable). Patients who had taken ASA within 24 hours prior to randomisation were to start ASA the next day.
Intravenous heparin was administered as soon as possible after study entry. Other medications (e.g. ß-blockers, calcium antagonists, ACE inhibitors, nitrates, etc.) were used at the discretion of the treating physician. There were no relevant differences seen between the study groups regarding important comedication.

Results:
A total of 25 patients experienced an **intracerebral haemorrhage (ICH**) within 30 days post treatment (0.9% in the 30 mg group, 0.6% in the 40 mg group and none in the 50 mg group). In both treatment groups patients with time to treatment of 0-6 hours had a lower rate of ICH (0.6%) as compared to patients with time to treatment > 6 hours (1.2%). Furthermore, the ICH rates tended to be lower after the protocol change time point: they were 1.6% before and 0.8% after the amendment in the 30 mg TNK-tPA group.
In addition, after the September 1996 protocol change the event rates were lower for the 30 mg group also for death (6.5% vs. 8.9%), death or non-fatal stroke (7.5% vs. 9.7%), serious bleeding (2.7% vs. 3.2%) and total stroke (1.4% vs. 2.4%).

The rates of selected clinical outcomes are summarised by treatment group in the following table:

Clinical outcomes at 30 day follow up

	30 mg TNK-tPA (n=1705)	**40 mg TNK-tPA** (n=1457)	**50 mg TNK-tPA** (n=73)	**Total** (n=3235)
Death	117 (6.9%)	88 (6.0%)	3 (4.1%)	208 (6.4%)
Recurrent MI	140 (8.2%)	86 (5.9%)	4 (5.5%)	230 (7.1%)
Total stroke[a]	26 (1.5%)	22 (1.5%)	0	48 (1.5%)
Pulmonary oedema[b]	73 (4.3%)	78 (5.4%)	5 (6.8%)	156 (4.8%)
Cardiogenic shock	62 (3.6%)	55 (3.8%)	3 (4.1%)	120 (3.7%)
Anaphylaxis	1 (0.1%)	3 (0.2%)	0	4 (0.1%)
Death or nonfatal stroke	133 (7.8%)	104 (7.1%)	3 (4.1%)	

Note: [a] Sum of haemorrhagic stroke, non-haemorrhagic stroke and cerebral infarction with haemorrhagic conversion
[b] Pulmonary oedema - Killip class III

There were no significant differences regarding the rates of death, total stroke, pulmonary oedema, cardiogenic shock, and anaphylaxis among the treatment groups. Fewer re-infarctions within 30 days occurred in the 40 mg TNK-tPA group compared with the 30 mg TNK-tPA group (5.9% vs. 8.2%, not significant).

The detailed results regarding the rate of death or non-fatal stroke are shown in the following table:

Death or non-fatal stroke at 30 days

	30 mg TNK-tPA (n=1705)	**40 mg TNK-tPA** (n=1457)	**50 mg TNK-tPA** (n=73)
Death or non-fatal stroke	133 (7.8%)	104 (7.1%)	3 (4.1%)
Time to treatment from symptom onset:	n=1440	N=247	n=57
≤ 6 hours	96 (6.7%)	81 (6.8%)	2 (3.5%)
> 6 hours	n=251	n=247	n=16
	31 (12.4%)	23 (9.3%)	1 (6.3%)

The rate of death or non-fatal stroke was not different across the treatment groups.

A total of 90 out of 3235 patients (2.8%) had at least one serious bleeding event (2.8%, 2.7% and 4.1% in the 30, 40 and 50 mg groups, respectively).

The majority of bleedings were non-instrumented site bleedings (86.2%). 43.6% of these events were probably related, and 27.7% were possibly related to the study drug.

At least one non-serious bleeding event (most frequently haemorrhage at the injection-site or at the catheter site, ecchymosis, epistaxis, haematuria) was reported in 32.9% of the patients (31.4%, 33.8% and 47.9% in the 30, 40 and 50 mg groups, respectively).

The percentage of patients experiencing at least one serious non-bleeding adverse event was 26.4%, 25.4% and 26.0% in the 30, 40 and 50 mg groups, respectively.

80-90% of all patients reported at least one non-serious, non-bleeding adverse event.
Platelet counts decreased in 13-15% of all patients at 24-48 hours after initiation of study drug but returned to baseline levels at hospital discharge.

ASSENT II study

a) Stroke

Total Stroke and Stroke Classification (Intent-to-Treat Population)

	TNK-tPA (n=8461)	rt-PA(n=8488)	Relative Risk (95% CI)	p-value
Total strokes (%)	151/8461 (1.78)	141/8488 (1.66)	1.074 (0.856,1.349)	0.5552
Primary ICH (%)	79/8461 (0.93)	80/8488 (0.94)	0.991 (0.727,1.350)	1.0000
Ischaemic stroke (*)(%)	61/8461 (0.72)	54/8488 (0.64)	1.133 (0.787,1.632)	0.5139
Haemorrhagic conversion (%)	6/8461 (0.07)	8/8488 (0.09)	0.752 (0.261,2.168)	0.7904
Unclassified (%)	11/8461 (0.13)	7/8488 (0.08)	1.576 (0.611,4.065)	0.3583

(*) Including haemorrhagic conversion

The incidence of total stroke tended to be higher in the TNK-tPA group (1.78 %) than in the alteplase group (1.66 %). The incidence of total stroke is consistent with the incidence observed in previous large-scale mortality studies, particularly for alteplase: 1.55 % in GUSTO I, and 1.79 % in GUSTO III. Primary ICHs occurred in 0.93 % of patients in the TNK-tPA group and 0.94 % of patients in the alteplase group, being virtually identical. When compared with the ICH rates in the accelerated rt-PA group in GUSTO I (0.72 %), GUSTO III (0.87 %), and COBALT (0.8 %), the rate for alteplase and TNK-tPA in ASSENT-II was slightly higher. This increase may be due to a better detection due to brain imaging. Another reason might be that the study did not have an upper age limit.
A breakdown of all strokes according to patients characteristics is provided in the following table:

ITT Population	TNK-tPA % of all patients	Alteplase % of all patients
Primary ICH	0.93	0.94
Ischaemic stroke	0.72	0.64
Any stroke	1.78	1.66
Age <= 75 years	1.59	1.27
Age > 75 years	3.15	4.39
Time to treatment 0-2 hr	1.39	1.05
Time to treatment >2-4 hr	2.01	1.79
Time to treatment >4 hr	1.80	2.18
Male	1.44	1.20
Female	2.94	3.18
Weight < 60 kg	3.33	4.29
Weight 60-69 kg	2.27	2.16
Weight 70-79 kg	2.10	2.52
Weight 80-89kg	1.55	1.33
Weight >= 90 kg	0.72	0.91

In subgroups results, the stroke rates, both total and ICHs, are higher in patients with a low body weight. These findings apply to both treatments and are in line with the results of the weight-subgroups for 30-day mortality. However, the total stroke and primary ICH rates tended to be higher in the alteplase group compared to the TNK-tPA group in patients with a low body weight.
A reason for the increased stroke rates in patients with low body weight might be the inclusion of risk groups in the low weight subgroup such as elderly or (light weight) females.

b) In-Hospital Disabling Non-Fatal Strokes

There was a trend towards a higher incidence in the TNK-tPA group compared to alteplase, particularly, for non-fatal disabling strokes.

	TNK-tPA	alteplase
Non-fatal disabling ICH	24/8456 (0.28%)	19/8485 (0.22 %)
Non-fatal disabling stroke	54/8453 (0.64%)	41/8483 (0.48%)

c) Bleeding Events (excluding ICH)

Bleeding occurred in fewer patients treated with TNK-tPA than in those treated with alteplase.

	TNK-tPA (N=8461)	alteplase (N=8488)	p-value
Major bleeds[a] (%)	394/8461 (4.66)	504/8484 (5.94)	0.0002
Total bleeds[a] (%)	2236/8461 (26.43)	2457/8488 (28.95)	0.0003
Units transfused blood			0.0013
None (%	7546/7881 (95.75)	7457/7890 (94.51)	
1-2 (%	204/7881 (2.59)	256/7890 (3.24)	
> 2 (%)	131/7881 (1.66)	177/7890 (2.24)	

[a] Excluding ICH

Significantly fewer major bleeding events occurred in the TNK-tPA group than in the alteplase group (p=0.0002). The difference in total bleeding events was also statistically significant (p=0.0003).

The site of major bleeding was most commonly identified by investigators as haematoma, and next common was gastrointestinal tract bleeding, regardles of which thrombolytic was used. Bruises, catheter site bleeding, and unspecified bleeding site made up the majority of the remaining major bleeds in both treatment groups.

Overall, about 95 % of the enrolled patients did not need blood transfusions during the in-hospital period. Significantly fewer patients treated with TNK-tPA needed blood transfusions than patients treated with alteplase (p=0.0113).

In all body weight classes, there was less bleeding in the TNK-tPA group compared to the alteplase group. These differences were statistically significant for the subgroups < 60 kg and between 60 and 70 kg. As already seen for the mortality and stroke results the number of patients with bleedings was higher in patients with low body weight. However, the difference was not as pronounced as seen for the mortality and stroke results.

d) Overall adverse events

Overall, 6305 patients (74.5 %) in the TNK-tPA group and 6406 patients (75.5 %) in the alteplase group experienced adverse events.

Adverse events classified by organ system class included: application-site disorders (1.4 % in both groups); cardiovascular disorders (general) (21 % in both groups); heart rate and rhythm disorders (42 % in both groups); liver and biliary system disorders (TNK-tPA 0.3 %, alteplase 0.4 %); metabolic and nutritional disorders (TNK-tPA 3.4 %, alteplase 3.9 %); platelet, bleeding, and clotting disorders (TNK-tPA 23.5 %, alteplase 25.4 %); respiratory symptom disorders (TNK-tPA 15.6 %, alteplase 16.2 %); urinary system disorders (TNK-tPA 7.7 %, alteplase 8.8 %).

The number of anaphylactic reactions was 6 under TNK-tPA and 16 under alteplase.

The non-serious adverse events show the standard profile of events seen during thrombolytic treatment of acute myocardial infarction. There was no qualitative difference between TNK-tPA and alteplase.

e) Serious adverse events

The cardiovascular serious adverse events are presented in the following table (patients with cardiogenic shock are classified under cardiac failure):

WHO System Organ Class WHO Preferred Term	ASSENT-II			
	TNK		rt-PA	
	N	%	N	%
Patients randomised	8461		8488	
CARDIOVASCULAR DISORDERS, GENERAL	427	5.0	463	5.5
ANEURYSM	12	0.1	11	0.1
CARDIAC FAILURE	281	3.3	316	3.7
CARDIAC FAILURE LEFT	3	0.0	9	0.1
CARDIAC FAILURE RIGHT	2	0.0	1	0.0
CARDIOMEGALY	2	0.0	0	0.0
CIRCULATORY FAILURE	1	0.0	5	0.1
CYANOSIS	0	0.0	1	0.0
ECG ABNORMAL	1	0.0	0	0.0
ECG ABNORMAL SPECIFIC	7	0.1	5	0.1
HEART DISORDER	0	0.0	1	0.0
HYPERTENSION	3	0.0	5	0.1
HYPERTENSION PULMONARY	2	0.0	0	0.0
HYPOTENSION	251	3.0	259	3.1

The events were mainly cardiac failure and hypotension. There was no difference between TNK-tPA and alteplase.

f) Causes of Deaths

During ASSENT-II, a total of 1045 patients died, 940 during the in-hospital phase and 105 after discharge until Day 30.

The cause of death is available for 938 out of 940 patients (99.8%) who died during the in-hospital period. The table below shows the causes of death, by classes, of patients who died in-hospital ("intent-to-treat" population in ASSENT-II).

In-hospital Causes of Death in Classes

ITT Population	Planned Treatment Code				ALL	
	TNK		alteplase			
	N	%	N	%	N	%
Cause of Death						
Reinfarction	30	6.38	25	5.34	55	5.86
Refractory Ischaemia	3	0.64	4	0.85	7	0.75
Cardiogenic Shock	186	39.57	185	39.53	371	39.55
Arrhythmias / Sudden Death	19	4.04	29	6.20	48	5.12
Asystoly / Cardiac Arrest	39	8.30	35	7.48	74	7.89
Cardiac Rupture / EMD	83	17.66	75	16.03	158	16.84
Other Cardiac Events	16	3.40	17	3.63	33	3.52
Stroke or Intracranial Haemorrhage	50	10.64	51	10.90	101	10.77
Major Bleeding (other than intracranial)	5	1.06	4	0.85	9	0.96
Other Non-cardiac Events	25	5.32	26	5.56	51	5.44
Other Significant Events	2	0.43	8	1.71	10	1.07
Unknown	12	2.55	9	1.92	21	2.24
ALL	470	100.00	468	100.00	938	100.00

Not unexpectedly, the in-hospital causes of death are mainly due the acute myocardial infarction and haemorrhagic stroke. These in-hospital causes of death are consistent both in nature and percentages with the 30 day phase II ASSENT-I safety trial.

The death rate and time to death were virtually identical, and causes of death were very similar for TNK-tPA treated patients as compared to those treated with alteplase.

g) ECG

In a relatively small number of cases the investigator determined an ECG finding to represent an adverse event, and reported the ECG interpretation along with the rest of the AE information: 35 cases on TNK-tPA, and 27 cases on alteplase. Of these events, for both treatment groups approximately two-thirds (24/35 on TNK-tPA and 18/27 on alteplase) were related to ST segment changes (elevation or depression). Eight were deemed SAEs in TNK-tPA treated patients, as were 5 events in alteplase treated patients.
TNK-tPA does not increase the incidence of ECG-related adverse events in comparison to alteplase.

Conclusion on the safety results:

The safety of TNK-tPA and alteplase in the ASSENT-II study was comparable with respect to the incidence of ICH (0.93 % vs. 0.94 %). TNK-tPA does not increase the risk of stroke (1.78% vs 1.65%). The stroke rates, both total and ICH, were higher in patients with a low body weight in both treatment groups which is in line with the results seen for 30-day mortality. The death rate and time to death were virtually identical, and causes of death were similar for TNK-tPA treated patients as compared to those treated with alteplase.
There were significantly fewer subjects with bleeding events, and blood transfusions in the TNK-tPA group compared with the alteplase group. Major bleeds occurred less frequently in patients treated with TNK-tPA than in those treated with alteplase (4.7% vs. 5.9%, p=0.0002).

5. Overall conclusions, benefit/risk assessment and recommendation

Quality

In general the substance is well characterised and the consistency of the product and the production process is deemed sufficient. The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.
Viral Safety and batch consistency have been documented and the relevant tests will be performed according to the agreed specifications.

Preclinical pharmacology and toxicology

Overall pharmacodynamic studies provided adequate evidence that tenecteplase induced clot lysis activity in human plasma as well as in various animal models. An increased fibrin specificity was demonstrated by *in vitro* and *in vivo* studies.
Safety pharmacology data in cynomolgus monkeys revealed reduction of blood pressure followed by changes of the ECG, but this occurred at multiple exposures that were considerably higher than the clinical exposure. Pharmacokinetic data showed that animals exhibit a reduction in clearance of tenecteplase relative to t-PA that is closely similar to that observed in humans, and a close similarity of other important pharmacokinetic parameters to humans.

Single and multiple dose toxicity studies in various animal species demonstrated dose dependant and reversible alterations of the coagulation parameters with local haemorrhages at the injection site, which was regarded as a consequence of the pharmacodynamic effect of tenecteplase. With regard to the indication and single dose administration in humans, reproductive toxicity testing was restricted to embryo foetal development in rabbits, as highly sensitive species. Most likely after repeated dosing at later gestation stages, pregnant rabbits showed bleeding and abortion with consecutive maternal death.

From these data it can be concluded that treatment with tenecteplase will subject pregnant women to an increased risk as compared to non pregnant women due to bleeding complications that are most likely of placental origin. In addition, the continuation of the pregnancy could be at risk during certain susceptible phases. However, a comparison with other plasminogen activators shows that tenecteplase does not pose a higher risk to pregnant women than other substances of this group.

Efficacy

The pharmacodynamic properties relevant to the indication sought for have been sufficiently evaluated. Study results concerning haemostasis and fibrinolysis parameters showed good consistency through the studies. The fibrin specificity of TNK-tPA is higher than that of alteplase.

Adequate pharmacokinetic studies have been conducted in patients with acute myocardial infarction. The results of pharmacokinetic studies were sufficiently consistent. The PK data of TNK-tPA differed from those of alteplase (longer half life, approx. 4-fold slower plasma clearance, 2-fold greater plasma exposure according to AUC) and support the intended single bolus administration. No pharmacokinetic studies have been performed in patients with renal or liver impairment. Even though laboratory data with respect to renal and hepatic function were not collected systematically, the ASSENT-II study revealed no differences between TNK-tPA and alteplase with respect to the adverse events linked to the system organ classification for the kidney and biliary system/liver. Nevertheless, due to the fact that TNK-tPA is metabolised by the liver, it is appropriate to consider severe hepatic impairment as a contra-indication to TNK-tPA treatment, as already mentioned in the SPC.

No clinical studies were aimed specifically at interactions between TNK-tPA and other medicinal drugs. However, in view of the relatively large data base of patients in the ASSENT-II study, grouped according to the concomitant medications, the conclusion of the Applicant that no relevant differences between TNK-tPA and alteplase can be detected regarding major clinical outcomes can be accepted.

Dose-finding was performed in three studies: two angiographic studies (TIMI 10A, TIMI 10B) and one safety study (ASSENT-I). In these trials a total of 4228 patients with AMI were included. The TIMI flow and TIMI frame count data indicate that there are dose-related effects of TNK-tPA. The percentage of patients with TIMI grade 3 flow and TIMI grade 2 or 3 flow at 90 minutes appeared to increase with increasing dose of TNK-tPA. The 30 mg dose appeared to be less effective than the other doses and was significantly less effective than alteplase in achieving higher grades of TIMI flow. The 40 and 50 mg doses were comparable to alteplase.
The risk of serious bleeding events is related to the dose of TNK-tPA.
The open uncontrolled safety study (ASSENT-I) shows no statistically significant differences between the dose regimens (30 mg, 40 mg and 50 mg) with respect to ICH rate, death, non-fatal stroke or death, and serious bleeding. Mortality tended to decrease with increasing dose. The dose selection for the phase III trial was mainly based on results and additional exploratory analyses of the TIMI 10B study, taking into account the safety data of ASSENT-I. It was shown, that the factors body weight and dose per weight are predictors of TIMI grade 3 flow. In a subset of patients TIMI grade 3 flow tended to increase with increased systemic exposure of TNK-tPA. Since pharmacokinetic analyses demonstrated that patient's body weight influenced the plasma clearance of TNK-tPA and consequently the systemic exposure, it was concluded to use a weight-adjusted dose of TNK-tPA in order to achieve a systemic exposure which should result in a greater likelihood of TIMI grade 3 flow.
Moreover, the fact that no specific safety problems arose in the large-scale phase III trial the recomendation of the proposed weight-adjusted dose regimen is acceptable, although not formally studied in phase II.

Efficacy was investigated in one pivotal confirmatory study (ASSENT-II). A total of 17,005 patients was exposed to TNK-tPA or to the comparator alteplase. Both dose regimens were weight-adjusted. Therapeutic equivalence could be demonstrated as predefined in the study protocol regarding 30-day mortality between a single bolus regimen of TNK-tPA and accelerated-infusion of alteplase. The mortality rates found in ASSENT-II (6.2 % in both groups) are comparable to those of accelerated rt-PA in GUSTO-I (6.3 %).

In summary, the results of the submitted clinical trials support the approved indication.

Safety

The safety profile of TNK-tPA in the weight-adjusted regimen compared to alteplase was investigated in particular in the ASSENT-II study. The incidence of ICH was comparable in the two treatment groups (0.93 % vs. 0.94 %). TNK-tPA does not increase the risk of stroke and ICH. The stroke rates, both total and ICH, were higher in patients with a low body weight in both treatment groups which is in line with the results seen for 30-day mortality.

The death rate and time to death were virtually identical, and causes of death were similar for TNK-tPA treated patients as compared to those treated with alteplase. There were significantly fewer subjects with bleeding events and blood transfusions in the TNK-tPA group compared with the alteplase group. Major bleeds occurred less frequently in patients treated with TNK-tPA than in those treated with alteplase (4.7% vs. 5.9%, p=0.0002).

Thus, the safety profile compared to alteplase seems to be in favour of TNK-tPA. No clinical circumstances have been identified where there is an excess risk attributable to TNK-tPA. Risk groups were identified and considered in the SPC.

There was no report of serious drug-related convulsions. Since convulsions are seen with other thrombolytic agents, a cumulative review of cases should be provided in the next PSUR to assess the risk of convulsions in patients receiving TNK-tPA.

Recommendation

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by consensus that the benefit/risk profile of Metalyse was favourable. Therefore, the CPMP recommended the granting of the marketing authorisation for Metalyse indicated for the thrombolytic treatment of suspected myocardial infarction with persistent ST elevation or recent left Bundle Branch Block within 6 hours after the onset of AMI symptoms.

6. Post authorisation

In November 2001 the MAH informed the EMEA of 4 cases involving Metalyse which suggested that the instructions for use of the product may not have been followed appropriately. It was decided to change the instructions for use and handling in the SPC and labelling by means of an Urgent Safety Restriction. The MAH subsequently sent a letter to healthcare professionals followed the publication of a statement on the EMEA website.

Adverse Drug reactions
Death and permanent disability have been reported in patients who have experienced stroke (including intracranial bleeding) and other serious bleeding episodes.
Isolated events related to the nervous system (e.g. somnolence, aphasia, convulsion) have been reported. Ischaemic or haemorrhagic cerebrovascular events may be contributing or underlying conditions.

In January 2004 the contraindication in diabetic patients (section 4.3 of the SPC) was deleted following the publication of a CPMP position statement concerning the use of iv fibrinolytics in diabetic patients. In addition, the term eye haemorrhage was aded to section 4.8 of the SPC.