28 February 2019
EMA/294785/2019
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Referral under Article 29(4) of Directive 2001/83/EC

Syner-Kinase and associated names

INN: urokinase

Procedure number: EMEA/H/A-29(4)/1472

Note:

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

Official address Domenico Scarlattilaan 6 ● 1083 HS Amsterdam ● The Netherlands
Address for visits and deliveries Refer to www.ema.europa.eu/how-to-find-us
Send us a question Go to www.ema.europa.eu/contact **Telephone** +31 (0)88 781 6000 An agency of the European Union

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1. Background Information

An application was submitted under the mutual recognition procedure for Syner-Kinase and associated names, 10,000 IU, 25,000 IU, 100,000 IU, 250,000 IU, 500,000 IU, powder for solution for injection or infusion on the basis of the marketing authorisation granted by UK on 29 September 2006.
The application was submitted to the concerned Member States (CMS): France, Germany, The Netherlands and Spain.

The names and MAHs of this medicinal product currently authorised are listed in Annex I of the CHMP opinion.

The mutual recognition procedure UK/H/6520/01-05/MR started on 04 January 2018.

On day 90, major issues on safety, efficacy and quality, raised by France, Germany, the Netherlands and Spain, remained unresolved; hence the procedure was referred to the Coordination Group for Mutual Recognition and Decentralised Procedures - Human (CMDh), under Article 29, paragraph 1 of Directive 2001/83/EC, by UK on 12 April 2018. The CMDh 60 day procedure was initiated on 7 May 2018.

Day 60 of the CMDh procedure was on 5 July 2018, and since there was no agreement between the involved MSs, the procedure was referred to the CHMP.

On 5 July 2018, the RMS UK therefore triggered a referral under Article 29(4) of Directive 2001/83/EC. France, Germany, the Netherlands and Spain raised objections on the grounds of potential serious risk to public health (PSRPH) in relation to the lack of bridging data between the product applied for and the product(s) described in literature that was used to demonstrate the benefit/risk balance of Syner-Kinase, the adventitious agent safety with respect to viral and prion clearance and the lack of adequate quality of the process validation of the semi-purified urokinase and lifetime of columns used for urokinase purification.

2. Scientific discussion

2.1. Introduction

Urokinase is a serine protease that catalyses conversion of plasminogen to plasmin with resultant fibrinolytic and thrombolytic properties. Urokinase is used for the lysis of blood clots in the following conditions:

- thrombosed intravascular catheters and cannulae
- extensive acute proximal deep vein thrombosis
- acute massive pulmonary embolism
- acute occlusive peripheral arterial disease with limb threatening ischemia

Urokinase is considered to have a well-established use in the above indications within the European Union and on 29 September 2006, Syner-Kinase was granted a marketing authorisation in the UK according to Article 10(a) of Directive 2001/83/EC.

Each vial of Syner-Kinase contains 10,000, 25,000, 100,000, 250,000 or 500,000 IU of urokinase produced from human male urine. The products are presented as powders for solution for injection or infusion. It is intended for intravascular administration after reconstitution with sterile physiological saline. The diluent is provided separately and was not part of these applications.

As mentioned above, the mutual recognition procedure was closed on day 90, with the four concerned Member States France, Germany, Spain and the Netherlands raising potential serious risk to public health in relation to a lack of bridging studies, adventitious agent safety with respect to viral and prion clearance, and quality. A referral was thus triggered at the CMD(h) but at D60 of the procedure, the PSRPH issues remained unresolved. The UK therefore triggered a referral under Article 29(4) of Directive 2001/83/EC.

As part of this procedure, the CHMP requested the applicant to justify that the available data on Syner-Kinase, including its comparison to the urokinase products mentioned in the literature, are adequate to support its positive benefit/risk balance in the proposed indications. The CHMP also requested the MAH/applicant to provide further information to support the viral and prion clearance capacity of the process and justify the adequacy of the procedures to support the suitability of viral and prion removal, including transmissible spongiform encephalopathies (TSE) infectivity reduction. Finally, the CHMP requested the MAH/applicant to provide further information to demonstrate that the manufacturing steps for semi-purified urokinase have been satisfactorily validated, and also that the control strategy for column lifetime during the manufacture of the active pharmaceutical ingredient is suitable. Furthermore, confirmation was required that the semi-purified urokinase is manufactured in accordance with GMP.

2.2. Assessment of the issues raised as a potential serious risk to public health

2.2.1. Lack of adequate bridging data to demonstrate safety and efficacy of Syner-Kinase

2.2.1.1. Comparability and consistency of Syner-Kinase since initial marking authorisation

The MAH/applicant provided a summary of the changes regarding the manufacture of the urokinase API contained in Syner-Kinase or the Syner-Kinase dosage forms that were introduced since the initial marketing authorisation:

- Replacement of the virus filter;
- Change of the manufacturer of the purified urokinase for Syner-Kinase from Gentium to BioAPI in Lugano, Switzerland and of the manufacturer of the semi-purified urokinase to a latest intermediate manufacturer.

  According to the MAH/applicant, the manufacturing processes followed by Gentium and by BioAPI for the urokinase API were identical; the equipment was the same, as were the operating personnel who came from Gentium and the scale of manufacture. The manufacturing processes followed by both manufacturer of the semi-purified urokinase consisted of a series of relatively straightforward well-understood chemical processes, as well as well-characterised analytical chromatographic methods in the early extraction process for urine-derived API's. The variation was granted by the MHRA in 2015.

  The MAH/applicant also highlighted that in introducing the new source for the semi-purified urokinase, due note had been taken of the *Guideline on the use of starting materials and intermediates collected from different sources in the manufacturing of non-recombinant biological medicinal products* (EMA/CHMP/BWP/429241/2013). The semi-purified urokinase is regarded as a process intermediate which could be produced by different suppliers using different processes provided that the quality of intermediates from variant sources and/or different process steps is sufficiently assured and that a comparable active substance is produced. To support such variation,

three batches of purified urokinase produced by BioAPI and derived from semi-purified urokinase from the latest intermediate manufacturer, had been compared with one batch of Gentium API.

The analysis has now been repeated using three batches of the BioAPI purified urokinase derived from semi-purified urokinase from the latest intermediate manufacturer and three batches of the API contained in Urokinase Vedim or Urokinase Crinos (see section 2.2.1.2 of this report).

- In May 2010 and 2015, GI Pharma was approved as an additional manufacturer by the UK MHRA. The manufacturing process is similar to the process followed by the original manufacturer, Sirton. All validation batches of Syner-Kinase produced by GI Pharma showed compliance with the specifications and stability results confirmed stability of the finished product regardless of the manufacturers of the semi-purified or purified urokinase.

2.2.1.2. Analytical data comparing the Syner-Kinase active ingredient (API) with the APIs in the urokinase products used in the literature studies cited in the submission

As discussed in the MAH/applicant's responses, the urokinase products used in the studies referred to in the literature provided (see references 1-13 in Annex) were either mentioned in the study reports or else could be inferred from the formulation available at the time in the appropriate dosage strengths in the Member States where the studies took place (between 1983 and 2017).

The availability of different urokinase-containing products in the EU is summarised in the table below:

Member state	Urokinase product	Licensed from	Licensed till	Licensed doses
Spain	Urokinase UCB (Vedim) https://cuidateplus.marca.com/medicamentos/urokinase-vedim-100000-ui-25-viales-25-ampollas-2-ml.html	01/09/1981	To date	100,000 IU, 250,000 IU
Italy	Urochinasi Crinos https://farmaci.torrinomedica.it/urochinasi_crinos/#P9	July 1987	To date	25,000 IU, 100,000 IU, 250,000 IU, 500,000 IU 1,000,000 IU
	Urokinasi Pfizer (formerly Hospira) https://farmaci.torrinomedica.it/urokinasi/#P9	25/11/2000	To date	100,000 IU, 1,000,000 IU
	Ukidan Serono https://farmaci.torrinomedica.it/ukidan/#P9 https://www.sec.gov/Archives/edgar/data/1117399/000115697303001773/u46842e6vk.htm	1975	2000	5,000 IU, 25,000 IU, 100,000 IU
Greece	Urochinasi Crinos https://www.galinos.gr/web/drugs/main/drugs/urochinasi	Only urokinase product in Greece	To date	25,000 IU, 100,000 IU, 1,000,000 IU

The urokinase products used in the list of references studies on which the MRP application was based would therefore have been Urochinasi Crinos from Italy and Greece or Urokinase Vedim from Spain. The urokinase in these products is and has always been manufactured by Gentium, Villa Guardia, Italy as was the case for the urokinase used to produce Syner-Kinase until 2015. The study which used Ukidan was withdrawn from the submission due to Ukidan no longer being available for comparison.

Authors	Urokinase product used
Petrakis *et al.*, 2000	Urochinasi Crinos (only urokinase product available at the time to have high enough doses)
Kalmanti M *et al*., 2002	Urochinasi Crinos (only urokinase rpoduct licenced and available in Greece to date)
Juve *et al*., 2003	Urokinase Vedim (mentioned by name)
De Gregorio *et al*., 2002	Urokinase Vedim (mentioned by name)
Fuentes *et al*., 1995	Urokinase Vedim (only urokinase product licensed in Spain since 1981 to date)
Bruzzese *et al*., 2016	Urochinasi Crinos (only urokinase product available at that time in Italy and at anywhere near the required dosage)
Giuntini *et al*., 1984	Ukidan (mentioned by name) *

*Giuntini study is no longer being considered as a reference because Ukidan is no longer marketed and hence no samples are available for analytical comparison.

In the context of this referral procedure, the applicant has identified further published clinical studies involving urokinase Vedim or Urochinasi Crinos and conducted in the same clinical settings and patients as indicated in the Syner-Kinase SmPC. The table below provides brief information about the design and results of these additional studies.

Authors	Design	Urokinase dosing regimen	Results	Urokinase product used
Martinez-Brotons., *et al*. 1987	20 acute arterial occlusion of limb patients treated with urokinase infusion through intra-arterial catheter.	Loading dose of 4,400 IU/Kg given over 15 minutes. Then infusion of 4,400 IU/Kg per hour administered for 12 hours.	Amputation was avoided in 2 out 3 patients with severe ischaemia. 17 patients suffered a less severe limb ischaemia. Complete lysis occurred in 60% and partial lysis in 20% of patients in less than 7 days. In occlusions lasting more than a week the figures were 14% and 28% respectively. Insertion site or local haematoma occurred in 5 cases.	Urokinase Vedim
Alonso *et al*. 2012	A 4 week study in 7 haemodialysis patients with permanent Catheters to restore patency and blood flow rate (BFR).	During the study period occluded catheters were locked with heparin Sodium 20 IU / ml (HS20) for 6 sessions and with 5% heparin (in 25% patients) or	Mean BFR was similar with both treatments; with HS20 279ml / min vs with PH 281ml / min, p = N.S. The mean venous pressure was the same (186 mmHg). The cost of PH was 2.78 time more than HS20.	Urokinase Vedim

		5,000 IU (PH) urokinase (in 75% patients) for 6 sessions as well.		
Lorenzo *et al*. 2005	Case reports of 16months follow up (8 months retrospective and 8 months prospective) of 3 HD patients with thrombosed catheters using two different urokinase dosing regimen to restore catheter patency.	In retrospective observation urokinase 5,000 IU dose was used whereas in prospective follow up urokinase 7,500 IU using push lock technique (0.3ml every 10 minutes) was used in same 3 HD.	The results were satisfactory to restore patency in totally obstructed catheters and improve all the blood flows.	Urokinase Vedim
Barberena 1983	11 patients treated for acute pulmonary thromboembolisms with urokinase.	Urokinase was administered at an initial dose of 2,700 lU/kg body weight over 5-10min, followed by 2,700 lU/kg body weight/hr for 12 hrs. In all patients after the urokinase infusion, 40 - 70mg of heparin was given intravenously every 4 hr.	Clear clinical improvement was demonstrated in all 11 patients, and electrocardiographically demonstrated improvement was seen in nine, with complete or nearly complete disappearance of the signs of acute pulmonary thromboembolism. 9 patients have urokinase or heparin related bleeding complications, majority at puncture sites. Other types of bleedings included subcutaneous hematoma, epistaxis and aspirated gastric bleeding.	Urokinase Vedim
González *et al*. 2017	A retrospective study of dysfunctional catheters in 30 haemodialysis patients with less than 6 months life expectancy.	Urokinase 100,000 IU lock was used to restore the patency of occluded catheters in 43.3% of cases whereas the rest did not need intervention.	16.7% patients required one urokinase lock. Frequent recurrence of dysfunction was observed in the remaining 26.6% of patients, contributing significantly towards overall cost.	Urokinase Vedim
Vignali *et al*. 1994	A retrospective study of efficacy and safety of	Patients were treated with t-PA doses of 10-20 mg	Recanalization was achieved in 55 of 59 (93%) patients treated with urokinase and	Urochinasi Crinos

	urokinase and t-PA in 83 patients suffering from acute occlusions of the peripheral arteries.	bolus followed from slow infusion of 5 mg/hr for up to 8 hours or urokinase 100,000-200,000 UI in bolus, followed by 50,000-75,000 IU/hr in infusion for 24 and 36 hours.	in 21 of 24 (88%) patients treated with recombinant t-PA (p >0.05). 10 patients with complications were in t-PA group including 8 cases of subcutaneous hematoma at the arterial access, one with subperitoneal extension and 2 cases of haemarthrosis. All complications resolved spontaneously without requiring any kind of intervention.	

In the original submission, a comparison of three batches of the urokinase used to produce Syner-Kinase and one batch of Gentium's urokinase from the intermediate manufacturing site in the initial marketing authorisation application was conducted using amino acid sequencing, relative molecular mass, molecular weight distribution by SDS-PAGE, SEC-HPLC, IEF and 2-D electrophoresis. The results demonstrated that urokinase from Gentium (hence the urokinase used to produce Urokinase Vedim and Urokinase Crinos) was comparable to the urokinase used to produce Syner-Kinase in respect of these physico-chemical parameters.

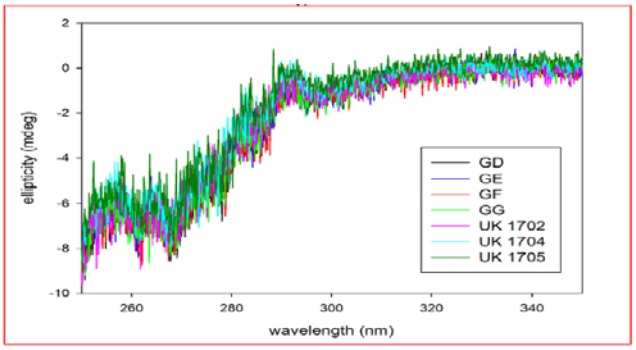
Information was provided as part of the referral procedure to establish analytical comparability with urokinase API manufactured by Bio API Switzerland from semi-purified urokinase material from the latest intermediate manufacturer (3 batches), with two batches of Urochinasi Crinos and one batch of Urokinase Vedim final products, both containing urokinase API manufactured by Gentium, Italy.

The analytical techniques used in the comparability study were the followings:

- Circular dichroism (secondary structure),
- N-terminal sequencing, amino acid composition, peptide mapping,
- SEC-HPLC (monitoring of molecular size, aggregation etc.)
- SDS-PAGE (molecular mass estimation and to confirm efficacy of separation and purification procedures).
- EIS-Q-TOF (electrospray-ionisation quadrupole time-of flight mass spectrometry for molecular mass determination).
- Iso-electric focusing (protein identification)

Circular Dichroism

CD spectra of the seven urokinase samples have been acquired at a protein concentration of 3.5 mg/mL, in a 2 mm path length cuvette, in the near UV region (250-350 nm, reporter of the tertiary structure of the protein) where possible differences in the structural organization of proteins should be more evident. CD spectra are characterized by the presence of a negative peak at 267 nm and a small positive peak at 291.5 nm.
All the spectra are comparable in term of shape and intensity of the CD signal. No differences are highlighted when the individual signals were overlaid.



Tryptic Digest Peptide Mapping

The table below show some of the results obtained for different replicates of the samples analyzed reporting: protein name, Coverage %, unique peptide.

Sample 2018/P38	Accession Number	Protein Name	Coverage%	Unique Peptide
S1-01	P00749	Urokinase-type plasminogen activator [OS=Homo sapiens]	53	21
S1-02	P00749	Urokinase-type plasminogen activator [OS=Homo sapiens]	53	25
S1-03	P00749	Urokinase-type plasminogen activator [OS=Homo sapiens]	61	23
S2-01	P00749	Urokinase-type plasminogen activator [OS=Homo sapiens]	40	17
S2-02	P00749	Urokinase-type plasminogen activator [OS=Homo sapiens]	48	20
S2-03	P00749	Urokinase-type plasminogen activator [OS=Homo sapiens]	48	21

This technique identifies N-terminal sequence, peptide map and amino acid sequences for comparison between manufacturers of urokinase. Tryptic digestion was followed by LC-MS to identify the peptide fragments. The method confirmed that all lots tested contained urokinase. All batches and lots showed similar coverage and identical sequences of the identified fragments. Although the method did not achieve 100% coverage, the sequences which were elucidated were identical between batches/manufacturers, confirming comparability of amino acid sequence.

Size Exclusion Chromatography.

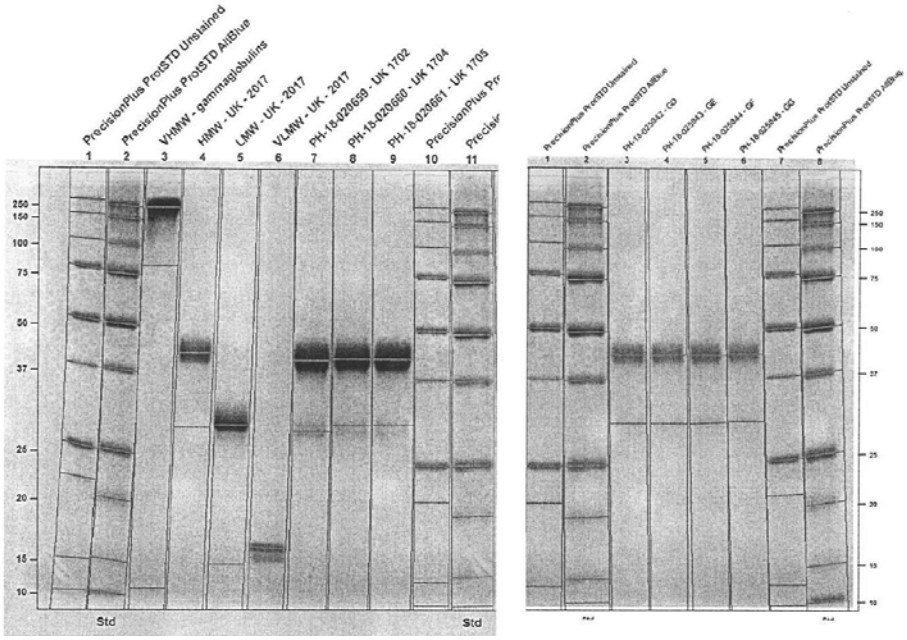
The comparison of chromatograms from the Finished Dosage Forms and from the Active Ingredient is shown below:

Sample description	% of High Molecular Weight	Retention time (min) HWM	% of Low Molecular Weight	Retention time (min) LWM
Urokinase, lot. UK.1702	96.4	30.44	3.6	32.68
Urokinase, lot. UK.1704	95.9	30.44	4.1	32.69
Urokinase, lot. UK.1705	96.3	30.43	3.7	32.69
Urokinase lyophilized GD	86.3	30.20	13.7	32.37
Urokinase lyophilized GE	86.3	30.22	13.7	32.40
Urokinase lyophilized GF	86.2	30.23	13.8	32.68
Urokinase lyophilized GG	86.3	30.23	13.7	32.68

All BioAPI samples have comparable quantities of HMW and LMW urokinase (LMW UK). After processing to DP, including freeze-drying, the HMW content increases from approx. 4% to approx. 14%. This is likely due to partial degradation of the product, but the Syner-Kinase FP is identical to the Crinos and Vedim products, also demonstrating comparability between Syner-Kinase and the literature products.

Reduced SDS-PAGE

The analysis of the three batches for the API and of the four batches for the finished products (see IEF below for a full explanation of the analysed batches) carried out using the technique of polyacrylamide gel electrophoresis in denaturing condition (SDS-PAGE) provided the following results:



All batches show identical migration patterns and essentially identical densities (visual and scanned), confirming identity and comparability of molecules.

ESI-Q-TOF

The high specificity of the mass spectrometry detector allows the molecular weight evaluation of each protein component (high molecular weight, low molecular weight, very low molecular weight urokinase).

Sample			Calculated Mw
	HMW-UK	LMW-UK	VLMW-UK
S3643 (UK 1702)	**46453**	32847	15392 + 15409
S3644 (UK 1704)	**46359**	32854	-
S3645 (UK 1705)	**46388**	32850	-
S3675 (GD)	**46585**	32661	15391 + 15407
S3677 (GF)	**46610**	32666	15392 + 15408
S3678 (GG)	**46471**	32668	15392 + 15409

The molecular weight of each fraction varies slightly from batch to batch, between manufacturers and between API and finished product, but only by a small margin. The variability is well within the range to be expected of heterogeneous molecules like urokinase.

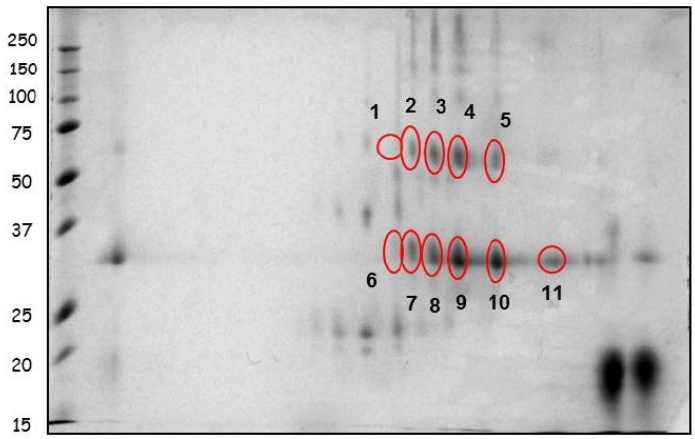
Iso-electric focusing

The iso-electric focusing report comparing BioAPI urokinase and the urokinase API contained in Urokinase Vedim or Urokinase Crinos, both produced by Gentium, the previous manufacturer of the urokinase API, was provided. Analysis was by reduced 2D gel electrophoresis.

Details of the samples tested and compared are provided below.

	Sample description	***Batch Number***	***Potency (IU/mL)***
	Urokinase manufactured by Bio API: Active Ingredient and Drug Product		
	Purified Urokinase	UK 1702	1'453'151
	Purified Urokinase	UK 1704	1'448'038
	Purified Urokinase	UK 1705	1'478'233
GE	Syner-KINASE	F02217004 / UK1701	50'000
	Drug Products acquired on the market:		
GD	Urokinasi Crinos/EG (Italy)	82305	50'000
GF	Urokinasi Crinos/EG (Italy)	82601	100'000
GG	Uroquinasa Vedim (Spain)	R-06/01	50'000

A representative 2D gel, along with the spot numbering system used in this comparison is shown below.



Three batches of purified urokinase API from BioAPI, one batch of Syner-Kinase finished product (with API from BioAPI), two batches of Urokinasi Crinos and one batch of Uroquinasa Vedim (all three batches with API from the previous Syner-Kinase API provider – Gentium) have been analysed by reduced 2D electrophoresis for pI. This experiment was performed to investigate comparability of pI between the current Syner-Kinase (BioAPI/Gipharma), pre-2015 Syner-Kinase (Gentium/Gipharma), and urokinase from literature references involved in the well-established use application for Syner-Kinase (Gentium/Crinos and Gentium/Vedim).

Visual examination of the 2D gels from all manufacturers show a very high degree of similarity in the number and position of spots.

Analysis of spot pI by PDQuest software supported the comparability of the post-2015 Syner-Kinase to pre-2015 Syner-Kinase and the urokinase reported in the literature (Gentium). Slight variability is due to the precision of the technique and it is noted that the reproducibility of pI is the same when comparing BioAPI vs. Gentium urokinase batches or BioAPI vs. BioAPI batches or Gentium vs. Gentium batches. This confirms the similarity of pI regardless of the source of the urokinase.

2.2.1.3. Clinical data

As part of this procedure, the MAH/applicant has provided a summary of the clinical data available with Syner-Kinase:

- Literature review

- A prospective audit was conducted by Kattenhorn (2009) using high dose Syner-Kinase infusion over 5 hours with doses 200,000 IU (44 occasions) or 250,000 IU (8 occasions). The mean pump speed prior to 200,000 IU intervention was 177.4 ml/min compared with 280 ml/min after intervention, and 140 ml/min compared with 295 ml/min post treatment in the 250,000 IU treatment group. The success rate of 52 infusions was 98%. There were no reports of any adverse events either during the infusion or post infusion.
- Dean and Foster (2009) designed a '4 step protocol' (Coventry and Warwickshire NHS protocol, 2009) using a range of doses. Data from 67 interventions with Syner-Kinase was collected. Steps 1 and 2 were timely and there was a 28% increase in BFR's when using Step 3a/b. No adverse effects were reported.
- In a UK retrospective audit, Spanos et al. (2015) studied low dose infusion of 12,500 IU per lumen (25,000 IU) over 3 hours. In total, 99 patients were given Syner-Kinase infusion and analysed. The infusion resulted in an improvement in BFR (262.6 ± 74 vs 322 ± 85.6ml/min, p=0.001). Ten patients needed a second infusion within 6 weeks. Sixty patients needed a central venous catheter-

exchange; median (IQR) 40.5 (16/115) days post urokinase infusion. Ten of those 60 patients had the additional indication of catheter removal (catheter related infection or cuff dislocation). Thirty-nine patients had fully restored catheter patency after the low dose urokinase infusion. The overall catheter survival rate was 46.9%. No adverse reactions to urokinase were seen, no bleeding nor allergic reactions in any patient.

- A prospective audit (PASSPORT) by Kumwenda et al. (2018) addressed the safety and efficacy of either low dose (12,000 – 25,000 IU) locking regimens or high dose (100,000 – 200,000+ IU) infusions in renal patients. Ten centres participated, in total 182 patients 84 females (45.4%) and 101 males (54.6%), mean age was 63.6 (25-93). Catheter clearance rate achieving BFR over 200 ml/min was 90.5% after the first intervention, 97% after the second intervention, and 99% in the third intervention.
- The same group in the PASSPORT study, Jackson et al. (2018) looked at catheter management in haematology/oncology patients. Syner-Kinase doses administered ranged between 5,000IU – 25,000IU. This was the first time the high dose of 25,000IU Syner-Kinase was administered for thrombolysis of haematology/oncology Central Venous Access Devices (CVAD). In total 138 patients 65 females (47.1%) and 73 males (52.9%), median age was 60 (46-68). Out of 76 patients who had persistent withdrawal occlusion (PWO), 81% restored patency with 5,000 IU, 78% had CVAD patency restored with 10,000 IU and 83% with 25,000IU. The overall success rate was 80% for patients with PWO. These results are comparable to patients with total occlusion (TO) who experience 89%, 100% and 100% success with 5,000 IU, 10,000IU and 25,000IU, respectively. The overall success rate was 89% for the first intervention to treat a total occlusion. No adverse effects were reported in the audit.

  - MAH generated post-authorisation data

- In addition, data from about 500 patients treated in the UK with Syner-Kinase were submitted to the UK MHRA in 2009 by Syner Medica to support the use of Syner-Kinase to maintain/restore the patency of central venous access devices for haemodialysis and haematology/oncology patients (type II variation procedure approved by the UK MHRA). These data were generated from a UK multicentre prospective audit conducted in 2008 over 4 months looking at the safety and efficacy of high-dose Syner-Kinase infusion. A total of 7 UK renal centres participated, data were collected from 148 patients with 233 episodes requiring Syner-Kinase intervention. Doses ranged between 100,000 IU – 400,000 IU. Out of 148 patients, 105 (70.9%) patients required single administration of Syner-Kinase, and 43 required more than one Syner-Kinase administration. In 233 patient episodes, 11 (4.7%) had a reduction in blood flow rate (BFR) after Syner-Kinase infusion, 18 (7.7%) had no change in BFR and 204 (87.6%) experienced an increase in BFR following Syner-Kinase infusion. Results were as follows: 226 patient episodes (97%) achieved 150 ml/min, 204 (88%) achieved 200 ml/min, 155 (67%) achieved 250 ml/min, 96 (41%) achieved 300 ml/min and 42 (18%) achieved more than 300 ml/min. In summary, 97% of cases had a successful dialysis session irrespective of their pre- and post- BFR. The improvement was clinically and statistically significant ($p<0.001$). Following Syner-Kinase infusion, only 7 cases (3%) had a BFR <150 ml/min requiring surgical intervention. No adverse events related to Syner-Kinase were reported either immediately after infusion or reported on the next dialysis visit. There were no reports during administration of pyrexia or hypersensitivity to Syner-Kinase or excipients, and no reports of embolic events post treatment. Syner-Kinase was not contraindicated in any patient and the treatment was well tolerated.

- Pharmacovigilance data

Since the grant of the marketing authorisation (MA) and by end of 2018, over 1,000,000 vials of Syner-Kinase had been sold in the EEA (UK, Ireland, Spain, Denmark, Austria, Germany, Portugal, the Netherlands, Malta, Cyprus and Iceland [Syner-Kinase has been approved for import by all local regulators in these Member States]). In addition, prior to the grant of the MA in 2006, over 155,000 vials had been sold in the UK on a named patient basis.

To date, no adverse events, serious adverse events or fatal reports involving Syner-Kinase have been reported.

2.2.1.4. Discussion on the safety and efficacy of Syner-Kinase

All the cited literature references upon which the 10a application is based used either Urochinasi Crinos from Italy & Greece or Urokinase Vedim from Spain. The API of both of these medicinal products is sourced from Gentium, Villa Guardia, Italy. Syner-Kinase also used API from Gentium, Villa Guardia, Italy, until 2015, when the manufacturer was changed by an approved variation to BioAPI, Switzerland.

Two aspects of comparability have been questioned. Firstly, the comparability of the urokinase in Syner-Kinase, versus the urokinase in the products in the literature used to support this well-established use application, which have now been identified as Urokinase Vedim and Urokinase Crinos. After the Marketing Authorisation, in 2015, comparability of 3 batches of Syner-Kinase versus one batch of Gentium urokinase was presented, comparing amino acid sequencing, relative molecular mass, molecular weight distribution by SDS-PAGE, SEC-HPLC, IEF and 2-D electrophoresis.

To increase confidence in the comparability of API between Vedim, Crinos and the API in Syner-Kinase, three batches of Gentium-derived urokinase (i.e. 1 batch from Urokinase Vedim and 2 batches from Urokinase Crinos) have also been analysed and compared to 3 batches of BioAPI derived urokinase. The new comparability study consists of, N-terminal sequencing, amino acid composition, peptide mapping, circular dichroism, SEC-HPLC, SDS-PAGE, iso-electric focusing and EIS-Q-TOF.

This additional study satisfies the request to show comparability of Syner-Kinase API with the urokinase API which was used in the supporting literature references. All techniques demonstrated comparability between the different sources of API.

The second aspect of comparability to be challenged was the comparability of the urokinase in Syner-Kinase when the product was initially authorised in the UK, versus the urokinase in the currently manufactured Syner-Kinase, due to several variations which have been approved during the product's life cycle. Gentium-derived urokinase is the API used in Urokinase Vedim and Urokinase Crinos as well as in the initially-authorised Syner-Kinase and so by analysing the product from these manufacturers, the urokinase in initially-authorised Syner-Kinase is also characterised. The comparability of this material to 3 batches BioAPI urokinase, which is the API in the current Syner-Kinase has been submitted within the procedure and demonstrated the comparability of Syner-Kinase API irrespective of the quality changes since the grant of the MA.

The Urokinase Vedim and Urokinase Crinos are only available as final products and the characterisation studies have therefore been performed on this material. An additional batch of Syner-Kinase dosage form (containing BioAPI urokinase) has also been characterised. Since the formulations are simple, the analysis of API and final product is considered acceptable; especially since a batch of BioAPI-Syner-Kinase final product is also included in the characterisation studies to facilitate a comparison of Gentium-urokinase final product with BioAPI-urokinase final product. Therefore, both requirements

have been addressed in one study, in which 3 batches of BioAPI-urokinase API have been compared to Gentium-Urokinase Vedim, Gentium-Urokinase Crinos and BioAPI-Syner-Kinase final product batches.

The purified urokinase batches tested were produced by the current suppliers of semi-purified and purified API. One of the tested batch concerns the final product manufactured at GI Pharma. The comparator batches from Crinos and Vedim are all final product batches manufactured by Gentium with the semi-purified intermediate manufactured by the intermediate manufacturing site in the initial marketing authorisation application. The comparability study is identical to the study discussed above to demonstrate comparability of Syner-Kinase with the literature batches of urokinase, and it was considered sufficient by the CHMP to conclude that comparability has been demonstrated.

Based on the above, it is considered that the studies provided demonstrate comparability between the urokinase in the current Syner-Kinase, the initially licensed Syner-Kinase, and the urokinase which is used in the literature references. The MAH has also provided real-world effectiveness data on the use of Syner-Kinase to maintain/restore the patency of central venous access devices (CVADs); improved clinical outcomes of patients with problematic catheters have been shown, which would allow in particular for a decrease in the number of lost dialysis sessions. In addition, no ADR report had been received from Syner-Kinase to date.

2.2.2. Viral and prion clearance

2.2.2.1. Validation and demonstration of viral and prion clearance capacity of the process

The protocols for the proposed virus removal, and TSE removal studies were submitted. The MAH/applicant also provided the protocol for the validation of downscaling of the nanofiltration step.

The protocols for both viral and TSE removal assessment are standard and were considered adequate by the CHMP.

The scale down protocol was provided for the Viresolve nanofiltration procedure to ensure it is correctly scaled down compared to the commercial process scale. All scalable parameters are appropriately scaled down.

The CHMP endorsed the strategy defined in the protocols. The MAH/applicant will provide the final reports of the virus and TSE removal studies and the updated risk assessment analysis by 31 May 2019 to the relevant national competent authorities.

2.2.2.2. Adequacy of the procedures to support the suitability of viral and prion removal

The manufacturing steps targeted to viral clearance are pasteurisation and nanofiltration.

In order to ensure the on-going effectiveness of the two virus clearance steps, each batch of purified urokinase is tested for hepatitis B, hepatitis C and HIV-1 and 2 using validated gene amplification methods. All the tests that have been performed over at least twelve years have shown negative results.

It is noted that no evidence of virus transmission by Syner-Kinase has been described since it was first marketed.

2.2.2.3. Discussion on viral and prion clearance

The MAH was requested to provide the study protocols for the scale down of the Viresolve nanofiltration procedure, for the removal of MVM and TSE by the scaled down nanofiltration procedure. Operating procedures were considered appropriate for both scales by the CHMP. No additional

information is required to demonstrate an appropriate scale down for the virus and TSE validation of the Viresolve step.

The viral/TSE removal protocols have been submitted and were considered acceptable by the CHMP. These are to be performed under GLP. The MAH/applicant will provide the final reports of the virus and TSE removal studies and the updated risk assessment analysis by 31 May 2019 to the relevant national competent authorities.

For the pasteurisation virus inactivation step, the MAH has submitted details of how this is performed, along with time/temperature charts for all process runs performed to date. This data demonstrates that the pasteurisation process is fully under control and the required temperature is attained for the required time interval. The described operating conditions ensure that the validation of the pasteurisation step was performed under worst case conditions.

The conditions under which the commercial nanofiltration process is performed have been described and compared to the down-scaled process for pathogen removal studies. It is demonstrated how the down-scaled process is operating under worst case conditions for protein/volume load, and under easily controlled conditions for pressure and temperature. Since a cut-off point for flux decay is given for the down-scaled process, it is not considered necessary to show comparable composition of the filtered material since the nature of the operation (filtration) gives very limited mechanisms to change the composition of the product.

Consequently, the CHMP considered that the virus removal/inactivation steps are appropriately controlled.

2.2.3. Processes validation and control strategy

Amongst the remaining concerns raised by the objecting Member States, were the validation and the good manufacturing practice (GMP) compliance of the manufacture of the semi-purified urokinase (intermediate API), as well as the control strategy for the chromatographic columns used to produce the purified urokinase.

2.2.3.1. Semi-purified urokinase

The latest intermediate manufacturing site has been producing the semi-purified urokinase from human urine by a process consisting of a series of chemical processes and chromatographic methods well defined and used in the early stages in the production of urine-derived products.

Certificates of analysis (CoA) for 3 consecutive batches of semi-purified urokinase prepared at the latest intermediate manufacturing site have been provided. All lots met the specification for fibrinolytic activity > 40,000 IU/mg, with results ranging from 66,702 to 81,193 IU/mg. Results for specific activity ranged from 162,386 to 166,159 IU/mg protein, which is in line with the specification.

All lots met the specification for HMW urokinase > 85% with results ranging from 91.3% to 94.3%. Results for moisture content ranged from 3.2% to 3.5% (specification is not more than 5%). Microbial counts were consistently less than 10 cfu/500,000 IU urokinase. All batches complied with the Q-PCR test specification for viral contaminants of “not detectable per 1,000,000 IU”.

Batch analysis results were provided for 3 lots of purified urokinase prepared from the above-mentioned batches of semi-purified urokinase, at the manufacturing site for Bio API in Switzerland.

All lots met the specification for fibrinolytic activity > 1,000,000 IU/g solution with results ranging from 1,205,087 to 1,346,334 IU/g solution. Results for specific activity ranged from 161,681 to 196,545 IU/mg protein (Specification = > 70,000 IU/mg protein which is the PhEur specification). All lots met

the specification for HMW urokinase > 85% with results ranging from 96.1% to 97.4%. Microbial counts were consistently 0 cfu/g.

The analyses showed that manufacturing process at the latest site, operated within established parameters, could perform effectively and reproducibly to produce an intermediate and API meeting their predetermined specifications and quality attributes and thus complying with ICH Q7.

During the course of the referral procedure, the MAH/Applicant informed the CHMP that the facilities of the above mentioned manufacturer of the semi-purified urokinase had been replaced. The MAH/applicant provided evidence that the new manufacturing site was approved by an EU regulatory authority as well as QP declarations that the new facility is operating in compliance with the principles of GMP.

2.2.3.2. Control strategy for the purified urokinase

The control strategy for the column lifetime was provided as requested as part of this referral procedure. According to the MAH/applicant, the continuous control of performance is better than setting a predetermined number of cycles after which the resins are replaced.

The SOPs for the control of the columns have also been provided.

2.2.3.3. Discussion on processes validation and control strategy

The semi-purified urokinase is produced at a very early stage in the full purification of human urine to produce the purified urokinase. The starting materials, human urine and crude urokinase, are heterogeneous materials. The specifications for specific activity and molecular size distribution for the resulting semi-purified material are not within an absolute range but are expressed as minimum criteria. Such flexibility in the specifications for biological substance intermediates is accepted by the *EU Guideline on the use of starting materials and intermediates collected from different sources in the manufacturing of non-recombinant biological medicinal products (EMA/CHMP/BWP/429241/2013)*, where semi-purified urokinase is given as an example.

The MAH/applicant has submitted data to demonstrate that 3 consecutive batches of semi-purified urokinase comply with pre-determined specifications. When the same batches of semi-purified urokinase were onward processed to API, this product also complied with all pre-determined specifications.

Taking into account the early stage in the manufacturing process and the requirements of the above cited guideline, the data provided are considered sufficient evidence that the manufacture of semi-purified urokinase is adequately validated and controlled.

Regarding the validation process of the column lifetime, Cleaning In Place conditions were provided and criteria which trigger repacking and cleaning or resin replacement were given. As such, no formal lifetime is defined for a batch of resin, but column performance is routinely monitored and appropriate action taken when a decline in performance is noted, whilst performance is still adequate to produce API of appropriate quality. The applicant has also provided SOPs describing the parameters controlled and acceptance criteria of the columns used at BioAPI for the manufacture of purified urokinase. Based on the information provided, BioAPI proposed performance of cycle by cycle controls on the Sephadex G-100 size exclusion column and regular monitoring of the anion exchange column is considered acceptable by the CHMP.

3. Benefit-risk balance

As part of this procedure, the MAH/applicant has provided relevant data to justify the extrapolation of the data available in the literature on the benefit and risks of urokinase in the indications applied for. The MAH/applicant has provided further comparative studies between Syner-Kinase active ingredient (API) and the API's in the urokinase products used in the literature studies cited in the submission, as well as data demonstrating the consistency of the product overtime despite the changes made to the product during its lifecycle.

Based on the data provided, it is considered that the comparability of Syner-Kinase and urokinase products used in the literature studies cited in the submission has been sufficiently demonstrated.

The MAH/applicant has successfully demonstrated that the virus removal/inactivation steps are appropriately controlled and will provide the final reports of the virus and TSE removal studies and the updated risk assessment analysis by 31 May 2019 to the relevant national competent authorities.

Finally, evidence has been provided that the manufacture of semi-purified urokinase is adequately validated and controlled and in compliance with good manufacturing practices and all concerns raised over the quality and manufacturing of Syner-Kinase are considered solved.

The CHMP considered, as a consequence, that the benefit-risk balance of Syner-Kinase is favourable.

4. Grounds for Opinion

Whereas

- The Committee considered the referral under Article 29(4) of Directive 2001/83/EC,
- The Committee considered the totality of the data submitted by the MAH/applicant in relation to the objections raised as potential serious risk to public health.
- The Committee concluded that Syner-Kinase is comparable to the urokinase products mentioned in the published literature, and that the data available are adequate to support its proposed use.
- The Committee concluded that the purification process of the active substance is suitable for the removal of possible viral and prion impurities.
- The Committee concluded that manufacture of the semi-purified urokinase is adequately validated and controlled, and that reassurance has been provided that this intermediate is manufactured at a site that complies with the principles and guidelines of Good Manufacturing Practice (GMP).

The Committee, as a consequence, considers that the benefit-risk balance of Syner-Kinase and associated names is favourable and therefore recommends the granting of the marketing authorisation(s) for the medicinal products referred to in Annex I of the CHMP opinion.

The product information remains as per the final version achieved during the Coordination group procedure as mentioned in Annex III of the CHMP opinion.