APPLICANT'S DETAILS

1	**Applicant's name**	s22 [redacted]
2	**Applicant's Business Address**	GPO Box 9848, Canberra ACT 2601, Australia
3	**Business name (if applicable)**	National Integrity of Sport Unit, Department of Health
4	**Date of submission**	04/09/2015
5	**Contact person**	s22 [redacted]
6	**E-mail Address of contact person**	s22 [redacted]
7	**Postal address of contact person**	GPO Box 9848, Canberra ACT 2601, Australia
8	**Phone Number of contact person**	s22 [redacted]

DECLARATION

I, s22 [redacted]

- declare that the information provided in this application is true and current;
- undertake to treat as confidential information, and not publicly disclose, the notice of interim decision in respect of this application, until (if relevant i.e. following referral to an expert advisory committee) the interim decision is published pursuant to subsection 42ZCZP of the Therapeutic Goods Regulations 1990, or the final decision is published pursuant to subsection 42ZCZS of the Therapeutic Goods Regulations 1990.

s22 [redacted]

s22 [redacted]

Sports Integrity Adviser
National Integrity of Sport Unit

Date: 4/09/2015

PART 1 – SUMMARY OF THE APPLICATION

PROPOSED SCHEDULING / RESCHEDULING OR OTHER CHANGE TO THE POISONS STANDARD

1. The National Integrity of Sport Unit (NISU) of the Department of Health requests the scheduling of the substances mentioned herein in Schedule 4 of the Standard for the Uniform Scheduling of Drugs and Poisons (Poison Standard or SUSMP) and requests consideration of an additional Appendix D listing. In the preparation of this application, expert analysis has been provided by Professor Andrew McLachlan, Professor of Pharmacy (Aged Care), University of Sydney, and Professor David Handelsman, Professor of Reproductive Endocrinology & Andrology, University of Sydney.

SUGGESTED SCHEDULING OR OTHER WORDING

Schedule 4 – New entries

Thymosin Beta 4 (Thymosin β4)

TB-500

Fibroblast Growth Factors

Appendix D – New entries

Thymosin Beta 4 (Thymosin β4)

TB-500

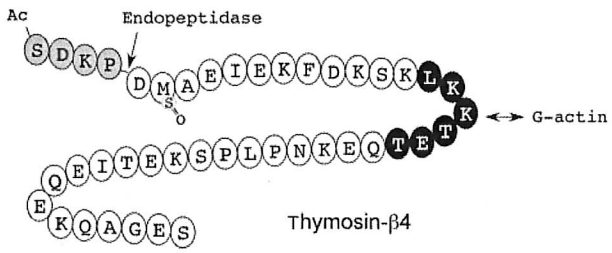
Fibroblast Growth Factors

SUBSTANCE SUMMARY

<u>Thymosin Beta 4 and TB-500</u>

2. This application is for Thymosin β4 and TB-500 (an analog of Thymosin β4) which is a growth factor affecting muscle, tendon or ligament, vascularisation and regenerative capacity. Thymosin β4 has never been approved by any major regulatory agency for human therapeutic use.

3. Thymosin β4 is a 43 amino acid peptide. The molecular structure of Thymosin β4 is shown in the diagram below where each letter indicates one of the 20 different amino acids.



4. TB-500, a short peptide analog of Thymosin β4, is presumed by design to have the same properties as Thymosin β4. TB-500 has not been marketed for human therapeutic use anywhere in the world.

5. These substances are currently used illicitly to enhance sporting performance and more broadly across the community often for body building and image enhancement purposes. The substances are banned by the World Anti-Doping Agency (WADA Prohibited List, 2015) for use by athletes, both in and out of competition.

6. Please refer to Attachment A for background on the pharmacology and structure of Thymosin β4 and TB500.

Fibroblast Growth Factors

7. Fibroblast Growth Factors (FGFs) are a family of growth factors involved in angiogenesis, wound healing, embryonic development and various endocrine signalling pathways. FGFs also have a role in the processes of proliferation and differentiation of wide variety of cells and tissues (Thisse and Thisse, 2005; Turner and Grose, 2010).

OVERVIEW

8. The substances included in this application are generally considered to be experimental and not approved for human use.

9. The substances have been identified as being used by athletes in Australia for the illegitimate enhancement of sporting performance in the February 2013 Australian Crime Commission Report *Organised Crime and Drugs in Sport* (ACC Report). The ACC Report outlined the emerging market in performance and image enhancing drugs and the involvement of criminal elements in their sale and distribution.

10. In August 2014, the NISU submitted an application to have a number of substances specifically identified in the ACC Report scheduled on the SUSMP. Following the November 2014 meeting of the Advisory Committee on Medicines Scheduling (ACMS #13), GHRP-6, CJC-1295, Ipamorelin, Pralmorelin (Growth Hormone Releasing Peptide-2), AOD 9604 and Hexarelin, and Growth Hormone Releasing Hormones (GHRHs), Growth Hormone Secretagogues (GHSs) and Growth Hormone Releasing Peptides (GHRPs) were included in Schedule 4 and Appendix D.

11. Despite the experimental status of these products they are readily available for purchase in Australia through online suppliers, based in Australia and overseas, and through compounding pharmacies and anti-ageing clinics.

12. The substances are advertised online and the lack of regulation allows suppliers to advertise them freely and make unproven assertions about the efficacy and safety of the substances.

13. Scheduling of these substances will assist to control the manner in which they are advertised, sold and accessed without legitimate purpose. It is incumbent on the regulator to schedule these substances to protect public health from the potential adverse impacts of these unapproved substances.

PART 2 – BODY OF THE APPLICATION

BACKGROUND

Importation into Australia

14. Organised networks of individuals and companies are involved in the acquisition and distribution of these substances. The ACC Report indicated that these substances are easily acquired from a multitude of online stores based in Australia and overseas.

15. The ACC *Australian Illicit Drug Report 2013-2014* identified that over the last decade, the number of performance and image enhancing drugs detected at the Australian border had increased, with 6885 detections in 2013-14, the third highest number in the past decade. In addition, the number of national arrests has continued to increase in 2013-14 providing the highest number on record.

16. International open source reports indicate countries including China, India, Pakistan, Thailand and others in Eastern Europe are primary source countries for these substances.

Public Health Concerns

17. The unregulated supply of these substances poses potentially serious health concerns as they are administered without reliable advice on appropriate dosage, frequency of administration, and exact content.

18. The ACC Report highlighted the supply of these substances is through unregulated means, including: bulk purchasing from overseas companies; use of compounding pharmacies to prepare products that include these substances; and distribution through diverse avenues including online suppliers, anti-ageing clinics and medical practitioners.

Evidence of Misuse

19. In recent years these substances have become increasingly popular among professional and amateur athletes, bodybuilders and the anti-ageing industry. A number of these groups are highly vulnerable. Sub-elite athletes are considered a high-risk group for doping due to the highly competitive nature of their events, the perceived advantage provided by these substances and the less stringent doping scrutiny to which they are subject.

20. The ACC Report identified widespread use of peptides and hormones by professional athletes in Australia in major sporting codes and other sports.

21. The ACC Report identified a number of vulnerabilities in Australia's regulatory and legislative framework that organised crime groups are exploiting. Scheduling of these substances on the SUSMP, and the consequential impacts in relation to penalties and enforcement, will serve to reduce these vulnerabilities and improve protection of the Australian community.

World Anti-Doping Agency

22. Thymosin β4 and TB-500 are classified as both Schedule 2 (S2) and Schedule 0 (S0) prohibited substances on the WADA Prohibited List for use by professional athletes both in and out of competition. FGFs are included under S2 on the list.

PART 2.1 CRITERIA WHICH MUST BE ADDRESSED – PROPOSALS TO CHANGE PART 4 OF THE POISONS STANDARD – SCHEDULING OR RESCHEDULING OF SUBSTANCES

(A) RISKS AND BENEFITS ASSOCIATED WITH THE USE OF A SUBSTANCE

23. There has been limited research regarding the harms and possible therapeutic benefits associated with the use of the substances proposed for scheduling.

24. The potential side effects of performance and image enhancing drugs, from a community health perspective, are wide ranging. Some of these substances, when used under medical supervision for legitimate therapeutic purposes, are considered safe and side effects minimal. There is, however, an inherent danger to individual health and wellbeing when substances are used unsupervised and for non-medical purposes.

(B) THE PURPOSES FOR WHICH A SUBSTANCE IS TO BE USED AND THE EXTENT OF USE OF THAT SUBSTANCE

25. The ACC Report highlighted these substances are being used by athletes and members of the broader community to promote muscle growth and maintain muscle gain.

26. Despite it being difficult to accurately estimate the extent of use of these substances, the ACC Report highlighted that available information including border seizures, national arrests and drug injecting data, suggest that the performance and image enhancing drugs market is expanding considerably in Australia.

27. Penalties exist in legislation for such improper practices, where sufficient evidence can be obtained to allow enforcement.

(C) TOXICITY AND SAFETY OF THE SUBSTANCE

<u>Thymosin β4 and TB-500</u>

28. While research on the effects of Thymosin β4 has been limited, findings to date include:

- Thymosin β4 administered in one phase I, one small therapeutic trial and an uncontrolled case series. The single phase I study investigated the effects of single and multiple doses of Thymosin β4 in healthy volunteers who underwent intravenous administration of a sterile pharmaceutical company manufactured product in doses ranging from 42 mg up to 1260 mg (Ruff, 2010). After a single dose to 40 participants, the multi-dose phase of the study was conducted involving 20 volunteers from the first single dose group plus

another 20 volunteers who all underwent daily injections for 14 days. A wide range of mostly mild and reversible adverse effects (as judged by a drug in development for therapy of patients with serious illness), more frequently in those receiving Thymosin β4 compared with placebo, were recorded but no serious adverse effects, dose-limiting toxicity or deaths were reported. Follow-up for risk of cancer promotion was limited to 6 months.

- A placebo-controlled therapeutic study involved 72 patients with venous stasis ulcers who were randomised to one of 3 doses (concentrations) of topical application of a dermal gel containing Thymosin β4 or placebo for 12 weeks. Despite a study design that was favourable to the trial product by excluding common underlying diseases that delay wound healing (eg arterial disease, diabetes), the study found no significant overall benefit of any dose of Thymosin β4 on wound healing. The failure of Thymosin β4 to effectively heal venous ulcers in a single study has many possible explanations which remains consistent with Thymosin β4 still being an effective drug. These reasons include suboptimal study design for some or all of the following reasons: wrong patient population, inadequate dosage regimen, too small a sample or too short treatment. For a first-in-human therapeutic trial, safety precautions always dictate the use of the minimum dosage regimen likely to be effective. This standard precaution may tend to underestimate the drug's optimal efficacy. Hence inadequate efficacy in the first human therapeutic trial is not surprising and does not mean the drug is necessarily ineffective. It is well understood that even if a drug does ultimately prove ineffective or unsafe for human therapeutic use, it may still be abused by elite athletes with doping intent.

29. No form of Thymosin ß4 is yet approved for human therapeutic use anywhere in the world. In concert, these findings would only support the safety of Thymosin β4 for therapeutic use using a pharmaceutical grade product under the ethical approval and supervision of a human research ethics committee (HREC) for a valid medical indication. No usage outside carefully monitored and ethically approved therapeutic trials is acceptable medical practice in 21st century Australia.

Fibroblast Growth Factors

30. There are no FGFs currently approved for human use in Australia. FGF-1 remains an experimental therapeutic substance that is not currently recommended for human use and has been mainly used experimentally in vitro or animal models. Trafermin is a human recombinant basic fibroblast growth factor (b-FGF) that promotes tissue granulation and the formation of new blood vessels. It is used as a topical liquid spray for the treatment of burns and intractable skin ulcers (Martindale, 2015).

(D) DOSAGE, FORMULATION, LABELLING, PACKAGING AND PRESENTATION OF A SUBSTANCE

Thymosin ß4 and TB-500

31. The use of Thymosin β4 in pre-registration human therapeutic trials is not the same as the drug having been approved or registered for marketing. Early,

pre-registration therapeutic trials for a new, unapproved drug are always conducted under the ethical jurisdiction of, and monitoring by, a HREC.

Fibroblast Growth Factors

32. The commercial product Fiblast (active ingredient: Trafermin) is marketed by Kaken Pharmaceticals in Japan for topical application (Martindale, 2015) and has been investigated in clinical trials for a possible therapeutic role in stroke (Bogousslavsky et al, 2002).

(E) POTENTIAL FOR MISUSE/ABUSE OF THE SUBSTANCE

33. A key finding of the ACC Report was that organised crime groups are taking advantage of the current legislative and regulatory situation whereby persons and entities who supply certain substances to athletes which are prohibited under the World Anti-Doping Code do not commit a crime in Australian jurisdictions. This is a significant legislative and regulatory vulnerability.

34. The unregulated supply of these substances poses serious health concerns as agents are being administered without appropriate trials in humans, without guidelines for the appropriate dosages and frequency of dosages and without knowledge of the exact contents of the product being administered.

35. The ACC Report also notes that '*the Australian PIEDs market has expanded rapidly in recent years*'. It observed that these substances are seen as a profitable and cost-effective product group and a large part of the reason for the increase in online and other suppliers is that the existing legislative and regulatory regime around the supply may not be consistently enforced.

36. The role that FGF, especially FGF-1 and b-FGF, has in angiogenesis, carcinogenesis and endocrine signalling pathways provides significant concern. Misuse or abuse has the potential to cause significant adverse health effects including cancer, cardiovascular problems and endocrinological health problems. Unrestricted access to FGFs could lead to substantial public health issues. Use of FGFs without appropriate medical supervision (or ethical clearances) poses a major risk to human health.

(F) ANY OTHER MATTER THAT MAY BE RELEVANT TO THE SCHEDULING OF A SUBSTANCE

37. Not applicable.

PART 2.2 CRITERIA WHICH MUST BE ADDRESSED – PROPOSALS TO CHANGE PARTS 1-3 OR PART 5 OF THE POISONS STANDARD

38. Not applicable.

CONCLUSION

39. The ACC has noted the performance and image enhancing drugs market in Australia has expanded rapidly in recent years, is large and diverse, with an extensive range of substances widely available to, and being used by, a broad cross section of the community.

40. The unregulated supply of these substances poses potentially serious health concerns beyond elite sports to the general community.

41. Scheduling on the SUSMP is an appropriate regulatory mechanism for these substances to ensure that those persons who wish to use them require a medical prescription to obtain, or be in possession of, these substances.

42. The scheduling of these substances on the SUSMP will provide an appropriate level of regulation that would align with the regulatory control of analogous substances including anabolic steroids, Selective Androgen Receptor Modulators and human Growth Hormones.

PART 3 – SUPPORTING DATA

SUPPORTING DATA SUMMARY

43. Supporting data is included at Attachment A.

SUPPORTING DATA DETAILS

44. Supporting data is included at Attachment A.

COPIES OF PAPERS REFERENCED

45. Copies of available references are included as part of the application.

PART 4 – BIBLIOGRAPHY

Australian Crime Commission (2013) *Organised Crime and Drugs in Sport – New Generation Performance and Image Enhancing Drugs and Organised Criminal Involvement in their use in Professional Sport*. Accessed 26th August 2014, https://www.crimecommission.gov.au/sites/default/files/organised-crime-and-drugs-in-sports-feb2013.pdf

Bogousslavsky J, Victor SJ, Salinas EO, Pallay A, Donnan GA, Fieschi C, Kaste M, Orgogozo JM, Chamorro A, Desmet A; European-Australian Fiblast (Trafermin) in Acute Stroke Group. Fiblast (trafermin) in acute stroke: results of the European-Australian phase II/III safety and efficacy trial. Cerebrovasc Dis. 2002;14(3-4):239-51.

Ruff D, Crockford D, Girardi G & Zhang Y. A randomized, placebo-controlled, single and multiple dose study of intravenous thymosin beta4 in healthy volunteers. Annals of the New York Academy of Sciences 1194:223-229.2010

Sweetman SC (ed), Martindale: The Complete Drug Reference [online] London: Pharmaceutical Press http://www.medicinescomplete.com/ (Accessed on 31 August 2015)

Thisse B, Thisse C. Functions and regulations of fibroblast growth factor signaling during embryonic development. Dev Biol. 2005 Nov 15;287(2):390-402

Turner N, Grose R. Fibroblast growth factor signalling: from development to cancer. Nature Reviews Cancer 10, 116-129 (February 2010)

Attachment A

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15 August 2015

Expert Report on WADA Doping Status of Thymosin Beta 4

This report is an expert statement on relevant physiology and pharmacology including the basis for prohibition of thymosin β4 (TB4) under the WADA Prohibited List. The comments herein include prefatory comments on the historical origin of the thymosin peptides including the relationship of TB4 (section 2) to the thymus extracted peptides (section 1) and to thymosin α1 (section 3).

These comments are up to date as of December 2014 and may not include more recent reports or developments.

My relevant professional expertise is summarised in an appendix.

1. *Historical Background of the Relationship of Thymus Extract Peptides, Thymomodulin and Thymosins*

1.1. Thymomodulin is a term that refers to a crude extract of calf thymus produced in Europe during the early to mid-20th century. It has sometimes been referred to as "thymic" or "thymus" hormones. At that time, prior to the modern detailed understanding of immunology, the thymus was known to be present at a young age and to virtually disappear by adulthood but its precise function was not yet known. By a process of little more than wishful thinking it was considered as a potential means of rejuvenation of youthful vigour and healing capacity. The calf thymus extract was described as a cell-free acid lysate so that, like any biological extract, it is a mixture of probably hundreds or thousands of active and inactive proteins – including ones that have opposing effects - making it subject to batch-to-batch variation in composition and effects. This makes it difficult, if not impossible, to standardise dosage or to evaluate therapeutic safety by modern standards.

1.2. Such crude extracts were used to important effect in the 19th and early 20th century laboratory research to identify and purify hormonal effects and ultimately to fully characterize the hormones we now know. Such crude extracts including thymomodulin were also popularly promoted by quack rejuvenation clinics, which proliferated in mid-20th century Europe. Till the middle of the 20th century crude biological extracts (eg dessicated thyroid extract, posterior pituitary snuff, equine estrogens) were still used therapeutically in medicine but have been supplanted by purified hormones as they became properly identified in the latter part of the 20th century. Crude extracts are an important first step along the discovery pathway of identifying important biological proteins, but they are definitely outmoded and unacceptable as therapeutic substances by the standards of medicine in the 21st century.

1.3. Thymomodulin was partially purified into subfractions called thymosins [1]. Some forms of thymomodulin continued to be marketed and used into the late 20th century in Europe [2, 3]. Thymosin fraction 5 (TF5) was used in some small therapeutic trials [4] but it appears never to have been formally marketed. TF5 was a family of at least 40 (and probably many more) mostly small acidic polypeptides with molecular weights 1,000 to 15,000 [5]. Subsequently, further purifications of TF5 by isoelectric focussing divided TF5 into 3 broad subsets, based on their pH, comprising highly acidic (α), acidic (β) and basic (γ) fractions. Each of these pH fractions comprised many distinct proteins which were then given numerical subscripts (α_1, α_2, α_3, β_1, β_2, β_3 etc) according to their appearance as bands on the purifying gels. However, even these gel fractions are not necessarily single proteins but can also be mixtures. Further work has clarified the precise molecular structure of many of these thymosins.

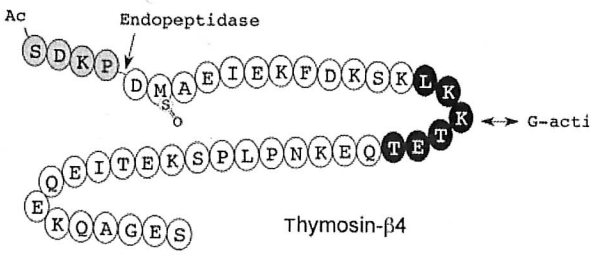
1.4. Thymosin α_1 and β_4 have been fully characterized structurally according to their precise amino acid sequence and developed for therapeutic trials.

2. Thymosin Beta-4

2.1. **WADA Status:** S0, S2

S2: Thymosin β4 is a growth factor affecting muscle, tendon or ligament, vascularisation and regenerative capacity.

S0: Thymosin β4 has never been approved by any major regulatory agency for human therapeutic use. If or when it is so approved for human therapeutic use, the substance would remain prohibited under S2.



Thymosin β-4 structure. From Hara Vitam Horm 201

2.2. **Chemical structure:**

Thymosin β4 is a 43 amino acid peptide. The molecular structure of Thymosin β4 is shown in the diagram opposite where each letter indicates one of the 20 different amino acids

2.3. **Physiology & Pharmacology:**
Thymosin β4 is a member of the family of thymosins, a highly conserved family of 40-60 small peptides originally purified from calf thymus. They now are divided into 3 groups (α, β, γ) according to their isoelectric points. The thymosin β family have a neutral pH (5.0-7.0) and includes ubiquitin (thymosin β1) but with thymosin β4 together with several others being highly homologous and having overlapping tissue regeneration and recovery functions [6].

2.4. **TB-500, an analog of thymosin β4**
TB-500, a short peptide analog of thymosin β4 has been identified in horse doping [7, 8] and the prospects of thymosin β4 as a doping agent has been outlined [9]. As TB-500 was invented as an analog of thymosin β4, it is presumed by design to have the same properties as thymosin β4. These include acting as a growth factor which affects muscle, tendon or ligament vascularisation and regenerative capacity hence banned under WADA category S2. TB-500 has not been marketed for human therapeutic use anywhere. Hence, TB-500 is banned under the WADA Prohibited List categories S0 and S2.

2.5. Thymosin β4 has both intracellular and extracellular functions [10]. The intracellular function is primarily as a G-actin monomers binding protein which acts to sequester the actin in the form of monofilaments in dynamic balance with F-actin polymers. These stabilise cellular shape and mobility including muscle contractility. Such intracellular functions are likely to be impervious to administration of exogenous thymosin β4.

2.6. The extracellular functions of thymosin β4 include angiogenesis [11-22], wound healing [23-32] and chemotaxis of cells involved in inflammation [33, 34] and tissue regeneration including skeletal and cardiac muscle [34-38]. The angiogenic effects vascularisation) involve interactions with hypoxia-inducing factor [14-16, 19] and Notch signalling [22, 39, 40], a pathway involving on hypoxia-inducing factor.

2.7. These functions of thymosin β4 may not be solely beneficial as noted in cautions from experimental studies suggesting that thymosin β4, via enhancing cell migration and angiogenesis, may promote the metastatic potential of certain cancers [41, 42].

2.8. Thus, as a growth factor affecting muscle, tendon or ligament, vascularisation and regenerative capacity as well as having interaction with hypoxia-inducing factor, thymosin beta 4 is considered a doping agent under section 2 of the Prohibited List.

2.9. **Safety:** Thymosin Beta-4 has been administered in one phase I, one small therapeutic trial and an uncontrolled case series. The single phase I study investigated the effects of single and multiple doses of thymosin β4 in healthy volunteers who underwent intravenous administration of a sterile pharmaceutical company manufactured product in doses ranging from 42 mg up to 1260 mg[43]. After a single dose to 40 participants, the multi-dose

phase of the study was conducted involving 20 volunteers from the first single dose group plus another 20 volunteers who all underwent daily injections for 14 days. A wide range of mostly mild and reversible adverse effects (as judged by a drug in development for therapy of patients with serious illness), more frequently in those receiving thymosin β4 compared with placebo, were recorded but no serious adverse effects, dose-limiting toxicity or deaths were reported. Follow-up for risk of cancer promotion was limited to 6 months.

A placebo-controlled therapeutic study involved 72 patients with venous stasis ulcers who were randomised to one of 3 doses (concentrations) of topical application of a dermal gel containing thymosin β4 or placebo for 12 weeks. Despite a study design that was favourable to the trial product by excluding common underlying diseases that delay wound healing (eg arterial disease, diabetes), the study found no significant overall benefit of any dose of thymosin β4 on wound healing. The failure of thymosin β4 to effectively heal venous ulcers in a single study has many possible explanations which remains consistent with thymosin β4 still being an effective drug. These reasons include suboptimal study design for some or all of the following reasons: wrong patient population, inadequate dosage regimen, too small a sample or too short treatment. For a first-in-human therapeutic trial, safety precautions always dictate the use of the minimum dosage regimen likely to be effective. This standard precaution may tend to underestimate the drug's optimal efficacy. Hence inadequate efficacy in the first human therapeutic trial is not surprising and does not mean the drug is necessarily ineffective. It is well understood that even if a drug does ultimately prove ineffective or unsafe for human therapeutic use, it may still be abused by elite athletes with doping intent.

A small and uncontrolled case series based on compassionate use approval claimed benefits of thymosin β4 ophthalmic solution for improving epithelial regrowth of chronic non-healing corneal ulcers [44].

2.9.1. The use of thymosin β4 in pre-registration human therapeutic trials is not the same as the drug having been approved or registered for marketing. Early, pre-registration therapeutic trials for a new, unapproved drug are always conducted under the ethical jurisdiction of, and monitoring by, a human research ethics committee (HREC). Among many other conditions, this requires the patient to provide written informed consent to the unproven treatment. Registration of a drug for therapeutic use requires a sequence of large and complex clinical therapeutic trials which must be completed satisfactorily before the drug dossier is submitted for registration. If it is successful, the drug is approved for general marketing as a proven treatment of a specific medical disease or condition. After registration the therapeutic use of the drug no longer requires ethical approval and informed consent for treatment and may be prescribed by a duly qualified and registered medical practitioner for that indication.

2.9.2. No form of thymosin $ß_4$ is yet approved for human therapeutic use anywhere in the world. In concert, these findings would only support the safety of thymosin β4 for therapeutic use using a pharmaceutical grade product under the ethical approval and supervision of a HREC for a valid medical indication. No usage outside carefully monitored and ethically

approved therapeutic trials is acceptable medical practice in 21st century Australia.

3. Thymosin α1

3.1. **WADA Status:** Not banned

Thymosin α1 is registered for human therapeutic use in several countries so is not S0. The countries that registered thymosin α1 for therapeutic use are less developed and developing countries with national drug regulatory affairs bureaus having limited within-agency expertise and uncertain transparency. Thymosin α1 is not registered by any major regulatory agency.

Although immune modifying effects can be considered as a growth factor for lymphocytes, thymosin α1 does not have any of the specific physiological or pharmacological growth factor properties outlined under S2.

3.2. **Chemical structure:**

Thymosin α1 is 28 amino acid peptide depicted in the adjacent figure using standard three letter codes for the different amino acids. The peptide is not glycosylated and the N terminus is acetyated.

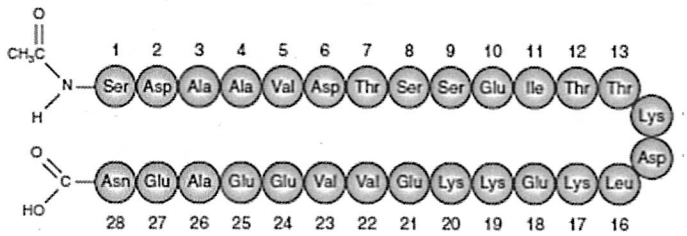


Figure 1. Structural formula of thymosin α_1.

3.3. **Physiological and pharmacological effects**

Thymosin α1 has a wide variety of physiological and pharmacological effects based on experimental studies in animals, cells and cell-free systems. The major physiological and pharmacological effects of thymosin α1 are immunomodulatory or immunostimulant effects that include induction of immune competence for maturing lymphocytes within the thymus, enhancing immune responses to infective agents or anti-cancer activity via stimulation of immune function of lymphocyte subpopulations.

Clinical therapeutic trials and registration

Thymosin α_1 has been marketed in a variety of countries for treatment of hepatitis B and C and "immune stimulant and adjuvant" effects involving co-ordinate activation of the innate and adaptive immune systems [45]. Other potential therapeutic benefits, none having sufficient proof to achieve marketing status, include adjuvant boosting of vaccine effectiveness, anti-cancer efficacy, enhancing recovery from infectious illness, immunodeficiency and cancer chemotherapy-induced myelosuppression [46-51]. It is notable that these approvals were solely in less developed and developing countries whose national regulatory agencies have limited in-agency drug regulatory expertise. They are often reliant on decisions of the major regulatory agencies in developed countries such as USA (FDA), Canada (Health Canada), UK (MHRA), Germany (BfARM), Sweden (MPA), Netherlands (MEB) and Australia (TGA). Notably thymosin α_1 is not approved by any of the major national regulatory agencies.

August 2015

References

1. Goldstein AL, Slater FD & White A. Preparation, assay, and partial purification of a thymic lymphocytopoietic factor (thymosin). *Proceedings of the National Academy of Sciences of the United States of America* 56:1010-1017.1966
2. Miric M, Vasiljevic J, Bojic M, Popovic Z, Keserovic N & Pesic M. Long-term follow up of patients with dilated heart muscle disease treated with human leucocytic interferon alpha or thymic hormones initial results. *Heart* 75:596-601.1996
3. Calsini P, Mocchegiani E & Fabris N. The pharmacodynamics of thymomodulin in elderly humans. *Drugs Under Experimental and Clinical Research* 11:671-674.1985
4. Wara DW, Goldstein AL, Doyle NE & Ammann AJ. Thymosin activity in patients with cellular immunodeficiency. *New England Journal of Medicine* 292:70-74.1975
5. Goldstein AL. History of the discovery of the thymosins. *Annals of the New York Academy of Sciences* 1112:1-13.2007
6. Hara T. Thymosins and muscle regeneration. *Vitamins and Hormones* 87:277-290.2011
7. Ho EN, Kwok WH, Lau MY, Wong AS, Wan TS, Lam KK, Schiff PJ & Stewart BD. Doping control analysis of TB-500, a synthetic version of an active region of thymosin beta(4), in equine urine and plasma by liquid chromatography-mass spectrometry. *Journal of Chromatography A* 1265:57-69.2012
8. Esposito S, Deventer K, Goeman J, Van der Eycken J & Van Eenoo P. Synthesis and characterization of the N-terminal acetylated 17-23 fragment of thymosin beta 4 identified in TB-500, a product suspected to possess doping potential. *Drug Test Anal* 4:733-738.2012
9. Davison G & Brown S. The potential use and abuse of thymosin beta-4 in sport and exercise science. *Journal of Sports Sciences* 31:917-918.2013
10. Crockford D, Turjman N, Allan C & Angel J. Thymosin beta4: structure, function, and biological properties supporting current and future clinical applications. *Annals of the New York Academy of Sciences* 1194:179-189.2010
11. Philp D, Huff T, Gho YS, Hannappel E & Kleinman HK. The actin binding site on thymosin beta4 promotes angiogenesis. *FASEB Journal* 17:2103-2105.2003
12. Philp D, Goldstein AL & Kleinman HK. Thymosin beta4 promotes angiogenesis, wound healing, and hair follicle development. *Mechanisms of Ageing and Development* 125:113-115.2004
13. Smart N, Rossdeutsch A & Riley PR. Thymosin beta4 and angiogenesis: modes of action and therapeutic potential. *Angiogenesis* 10:229-241.2007
14. Jo JO, Kim SR, Bae MK, Kang YJ, Ock MS, Kleinman HK & Cha HJ. Thymosin beta4 induces the expression of vascular endothelial growth factor (VEGF) in a hypoxia-inducible factor (HIF)-1alpha-dependent manner. *Biochimica et Biophysica Acta* 1803:1244-1251.2010
15. Moon EY, Im YS, Ryu YK & Kang JH. Actin-sequestering protein, thymosin beta-4, is a novel hypoxia responsive regulator. *Clinical and Experimental Metastasis* 27:601-609.2010
16. Yoon SY, Lee HR, Park Y, Kim JH, Kim SY, Yoon SR, Lee WJ, Cho BJ, Min H, Bang JW, Park H, Bang SI & Cho D. Thymosin beta4 expression correlates with lymph node metastasis through hypoxia inducible factor-alpha induction in breast cancer. *Oncology Reports* 25:23-31.2011
17. Dettin M, Ghezzo F, Conconi MT, Urbani L, D'Auria G, Falcigno L, Guidolin D, Nico B, Ribatti D, Di Bello C & Parnigotto PP. In vitro and in vivo pro-angiogenic effects of thymosin-beta4-derived peptides. *Cellular Immunology* 271:299-307.2011
18. Chiu LL & Radisic M. Controlled release of thymosin beta4 using collagen-chitosan composite hydrogels promotes epicardial cell migration and angiogenesis. *J Control Release* 155:376-385.2011
19. Ock MS, Song KS, Kleinman H & Cha HJ. Thymosin beta4 stabilizes hypoxia-inducible factor-1alpha protein in an oxygen-independent manner. *Annals of the New York Academy of Sciences* 1269:79-83.2012
20. Zachman AL, Crowder SW, Ortiz O, Zienkiewicz KJ, Bronikowski CM, Yu SS, Giorgio TD, Guelcher SA, Kohn J & Sung HJ. Pro-angiogenic and anti-inflammatory regulation by functional peptides loaded in polymeric implants for soft tissue regeneration. *Tissue Eng Part A* 19:437-447.2013

21. Kozaczuk A, Selmi A & Bednarek R. Bacterial expression, purification and angiogenesis-promoting activity of human thymosin beta4. *Protein Expression and Purification* 90:142-152.2013
22. Lv S, Cheng G, Zhou Y & Xu G. Thymosin beta4 induces angiogenesis through Notch signaling in endothelial cells. *Molecular and Cellular Biochemistry* 381:283-290.2013
23. Malinda KM, Sidhu GS, Mani H, Banaudha K, Maheshwari RK, Goldstein AL & Kleinman HK. Thymosin beta4 accelerates wound healing. *Journal of Investigative Dermatology* 113:364-368.1999
24. Goldstein AL, Hannappel E & Kleinman HK. Thymosin beta4: actin-sequestering protein moonlights to repair injured tissues. *Trends in Molecular Medicine* 11:421-429.2005
25. Philp D, Scheremeta B, Sibliss K, Zhou M, Fine EL, Nguyen M, Wahl L, Hoffman MP & Kleinman HK. Thymosin beta4 promotes matrix metalloproteinase expression during wound repair. *Journal of Cellular Physiology* 208:195-200.2006
26. Guarnera G, De. Rosa A & Camerini R. Thymosin beta-4 and venous ulcers: clinical remarks on a European prospective, randomized study on safety, tolerability, and enhancement on healing. *Annals of the New York Academy of Sciences* 1112:407-412.2007
27. Li X, Zheng L, Peng F, Qi C, Zhang X, Zhou A, Liu Z & Wu S. Recombinant thymosin beta 4 can promote full-thickness cutaneous wound healing. *Protein Expression and Purification* 56:229-236.2007
28. Treadwell T, Kleinman HK, Crockford D, Hardy MA, Guarnera GT & Goldstein AL. The regenerative peptide thymosin beta4 accelerates the rate of dermal healing in preclinical animal models and in patients. *Annals of the New York Academy of Sciences* 1270:37-44.2012
29. Xu TJ, Wang Q, Ma XW, Zhang Z, Zhang W, Xue XC, Zhang C, Hao Q, Li WN, Zhang YQ & Li M. A novel dimeric thymosin beta 4 with enhanced activities accelerates the rate of wound healing. *Drug Des Devel Ther* 7:1075-1088.2013
30. Wang X, Yang G, Li S, Gao M, Zhao P & Zhao L. The Escherichia coli-derived thymosin beta4 concatemer promotes cell proliferation and healing wound in mice. *Biomed Res Int* 2013:241721.2013
31. Xu B, Yang M, Li Z, Zhang Y, Jiang Z, Guan S & Jiang D. Thymosin beta4 enhances the healing of medial collateral ligament injury in rat. *Regulatory Peptides* 184:1-5.2013
32. Philp D, Badamchian M, Scheremeta B, Nguyen M, Goldstein AL & Kleinman HK. Thymosin beta 4 and a synthetic peptide containing its actin-binding domain promote dermal wound repair in db/db diabetic mice and in aged mice. *Wound Repair and Regeneration* 11:19-24.2003
33. Malinda KM, Goldstein AL & Kleinman HK. Thymosin beta 4 stimulates directional migration of human umbilical vein endothelial cells. *FASEB Journal* 11:474-481.1997
34. Tokura Y, Nakayama Y, Fukada S, Nara N, Yamamoto H, Matsuda R & Hara T. Muscle injury-induced thymosin beta4 acts as a chemoattractant for myoblasts. *J Biochem* 149:43-48.2011
35. Spurney CF, Cha HJ, Sali A, Pandey GS, Pistilli E, Guerron AD, Gordish-Dressman H, Hoffman EP & Nagaraju K. Evaluation of skeletal and cardiac muscle function after chronic administration of thymosin beta-4 in the dystrophin deficient mouse. *PLoS One* 5:e8976.2010
36. Smart N, Risebro CA, Clark JE, Ehler E, Miquerol L, Rossdeutsch A, Marber MS & Riley PR. Thymosin beta4 facilitates epicardial neovascularization of the injured adult heart. *Annals of the New York Academy of Sciences* 1194:97-104.2010
37. Evans MA, Smart N, Dube KN, Bollini S, Clark JE, Evans HG, Taams LS, Richardson R, Levesque M, Martin P, Mills K, Riegler J, Price AN, Lythgoe MF & Riley PR. Thymosin beta4-sulfoxide attenuates inflammatory cell infiltration and promotes cardiac wound healing. *Nat Commun* 4:2081.2013
38. Bao W, Ballard VL, Needle S, Hoang B, Lenhard SC, Tunstead JR, Jucker BM, Willette RN & Pipes GT. Cardioprotection by systemic dosing of thymosin beta four following ischemic myocardial injury. *Front Pharmacol* 4:149.2013
39. Gustafsson MV, Zheng X, Pereira T, Gradin K, Jin S, Lundkvist J, Ruas JL, Poellinger L, Lendahl U & Bondesson M. Hypoxia requires notch signaling to maintain the undifferentiated cell state. *Developmental Cell* 9:617-628.2005
40. Johnson EA. HIF takes it up a notch. *Sci Signal* 4:pe33.2011
41. Yamamoto T, Gotoh M, Kitajima M & Hirohashi S. Thymosin beta-4 expression is correlated with metastatic capacity of colorectal carcinomas. *Biochemical and*

Biophysical Research Communications 193:706-710.1993
42. Cha HJ, Jeong MJ & Kleinman HK. Role of thymosin beta4 in tumor metastasis and angiogenesis. *Journal of the National Cancer Institute* 95:1674-1680.2003
43. Ruff D, Crockford D, Girardi G & Zhang Y. A randomized, placebo-controlled, single and multiple dose study of intravenous thymosin beta4 in healthy volunteers. *Annals of the New York Academy of Sciences* 1194:223-229.2010
44. Dunn SP, Heidemann DG, Chow CY, Crockford D, Turjman N, Angel J, Allan CB & Sosne G. Treatment of chronic nonhealing neurotrophic corneal epithelial defects with thymosin beta 4. *Archives of Ophthalmology* 128:636-638.2010
45. Goldstein AL & Goldstein AL. From lab to bedside: emerging clinical applications of thymosin alpha 1. *Expert Opinion on Biological Therapy* 9:593-608.2009
46. Pierluigi B, D'Angelo C, Fallarino F, Moretti S, Zelante T, Bozza S, De Luca A, Bistoni F, Garaci E & Romani L. Thymosin alpha1: the regulator of regulators? *Annals of the New York Academy of Sciences* 1194:1-5.2010
47. Tuthill C, Rios I & McBeath R. Thymosin alpha 1: past clinical experience and future promise. *Annals of the New York Academy of Sciences* 1194:130-135.2010
48. Wolf E, Milazzo S, Boehm K, Zwahlen M & Horneber M. Thymic peptides for treatment of cancer patients. *Cochrane Database Syst Rev*:Cd003993.2011
49. Romani L, Moretti S, Fallarino F, Bozza S, Ruggeri L, Casagrande A, Aversa F, Bistoni F, Velardi A & Garaci E. Jack of all trades: thymosin alpha1 and its pleiotropy. *Annals of the New York Academy of Sciences* 1269:1-6.2012
50. Garaci E, Pica F, Serafino A, Balestrieri E, Matteucci C, Moroni G, Sorrentino R, Zonfrillo M, Pierimarchi P & Sinibaldi-Vallebona P. Thymosin alpha1 and cancer: action on immune effector and tumor target cells. *Annals of the New York Academy of Sciences* 1269:26-33.2012
51. Serafino A, Pierimarchi P, Pica F, Andreola F, Gaziano R, Moroni N, Zonfrillo M, Sinibaldi-Vallebona P & Garaci E. Thymosin alpha1 as a stimulatory agent of innate cell-mediated immune response. *Annals of the New York Academy of Sciences* 1270:13-20.2012

Appendix - Relevant expertise:

Current appointment:

- Inaugural Professor/Director, ANZAC Research Institute (1998-present)
- Inaugural Head, Andrology Department, Concord Hospital (1999-present)
- Professor of Reproductive Endocrinology & Andrology (1996, Personal Chair, Univ of Sydney)

Professional training:

- MB BS (1974, Univ of Melbourne)
- Medical specialist qualification in Endocrinology (1980, FRACP)

Research training:

- PhD (1984, Univ of Sydney)
- NHMRC Neil Hamilton Fairley Postdoctoral Fellow, Harbor-UCLA (1984-6)
- Wellcome Senior Research (Postdoctoral) Fellow, Univ of Sydney (1987-9)

Service to research, professional and health policy advisory bodies:

- WHO Human Reproduction Programme (1988-1994)
- Australian Drug Evaluation Committee (1994-1998)
- President, Endocrine Society of Australia (1992-4)
- Secretary, International Society of Andrology (1997-2001)
- Chair, Endocrine Society of Australia's writing group (2000) to create the 1st national testosterone prescribing guidelines; adopted and remain the PBS prescribing criteria
- NHMRC Grants (Reproduction, Endocrinology) & Fellowship Panels for >25 years
- Inaugural member, Board of Andrology Australia (1999-present)
- Inaugural Chair, Scientific Advisory Board, Freemasons Foundation Centre for Men's Health, University of Adelaide (2007-present)
- Crown expert witness, Full Bench, Federal Court of Australia, highest court hearing testimony from non-legal experts
- Invited submission, House of Representatives Standing Committee on Health and Ageing's review of impotence medications.

Anti-doping research and expertise:

- Expert advisory panel, Australian Sports Drug Medical Advisory Committee (1999-present)
- Anti-Doping Research Panel (2002-14)
- World Anti-Doping Agency's Health, Medicine and Research Committee (2011-6)
- ASADA Advisory Group (2011-present)
- WADA expert witness in successful CAS case (Dec 2014)

- ASADA expert witness in AFL Tribunal peptides case (2014-5)

Research track-record (since 1980):

- 360 peer-reviewed papers; 137 book chapters, reviews & reports; 446 scientific abstracts.
- Papers cited >12,500 times, average 23 citation/paper, h factor 59 (ISI Web of Science).
- Most actively cited author world-wide on "testosterone" (GOPUBMED database)
- Chapters in major textbooks of Endocrinology (De Groot's *Endocrinology*; Wass & Shalet's *Oxford Textbook of Endocrinology*) and Reproductive Biology (Knobil & Neill, *Physiology of Reproduction*).
- Served 14 editorial boards of peer-reviewed journals including Associate Editor, Male Reproduction, *JCEM* (2010-14) & Deputy Editor, *Asian Journal of Andrology* (2007-present).
- Invited ad hoc reviewer for 133 different peer-review journals
- Continuous peer-review research grant and contract funding since 1980 and pharma industry
- Awards: Royal Australasian College of Physician's Susman Prize (1994); inaugural AMA Men's Health Award (2003); Honorary Life Member, Endocrine Society of Australia (2008).
- Supervised or co-supervised 22 PhD and 11 other graduate students.