

## PATENT ASSIGNMENT

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SUBMISSION TYPE:	NEW ASSIGNMENT
NATURE OF CONVEYANCE:	ASSIGNMENT
CONVEYING PARTY DATA	
Name	Execution Date
COVITA LIMITED	10/22/2010
RECEIVING PARTY DATA	
Name:	MDX BIOTECH PTY LIMITED
Street Address:	BRIGHTON EAST
City:	VICTORIA
State/Country:	AUSTRALIA
Postal Code:	3187
PROPERTY NUMBERS Total: 1	
Property Type	Number
Application Number:	12376289
CORRESPONDENCE DATA	
Fax Number:	(949)760-9502
Phone:	949-760-0404
Email:	EFILING@KNOBBE.COM
<i>Correspondence will be sent to the e-mail address first; if that is unsuccessful, it will be sent via US Mail.</i>	
Correspondent Name:	KNOBBE MARTENS OLSON & BEAR LLP
Address Line 1:	2040 MAIN STREET
Address Line 2:	14TH FLOOR
Address Line 4:	IRVINE, CALIFORNIA 92614
ATTORNEY DOCKET NUMBER:	AJPARK40.004APC
NAME OF SUBMITTER:	Raymond D. Smith (Reg. No. 55,634)
Total Attachments: 21 source=ASSIGNMENT_5#page1.tif source=ASSIGNMENT_5#page2.tif source=ASSIGNMENT_5#page3.tif	

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## DEED OF ASSIGNMENT OF INTELLECTUAL PROPERTY

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### PARTIES

COVITA LIMITED at Hamilton, New Zealand ('Covita')

AGRESEARCH LIMITED at Hamilton, New Zealand ('AgResearch')

MDX BIOTECH PTY LIMITED (ACN 133 150 884) at Brighton East, Victoria 3187, Australia ('the Assignee')

### BACKGROUND

- A. The Assignors own the Intellectual Property.
- B. The Assignors have agreed to assign the Intellectual Property to the Assignee on the terms of this Deed and upon the further terms of a Sale and Purchase of Business Assets to be executed by the Parties on or about the date of execution of this Deed.

### THIS DEED PROVIDES

#### 1. Definitions

##### 1.1 In this Deed:

'Assignors' means Covita and AgResearch;

'Disclosures' means:

- (a) John Hopkins patents as identified in AJ Parks freedom to operate opinion dated 9 June 2006;
- (b) the patents and patents applications set out in Schedule 1 attached hereto, together with continuations, divisionals, reissues, re-examinations thereof
- (c) all disclosures made on behalf of AgResearch and/or Covita by AJ Park relating to any of the patents and patent applications set out in Schedule 1

'Intellectual Property' means

- (a) the Patents;
- (b) the Patent Applications;
- (c) the MSV invention described in Schedule 4;

- (d) all patent applications (including foreign applications) that are filed or may later be filed based on or corresponding to the Patent Applications;
- (e) all divisional and continuation, in whole or in part, applications and reissue applications based on any of the Patent Applications;
- (f) all issued and unexpired patents resulting from the Patent Applications;
- (g) all issued and unexpired reissue, re-examination, renewal, or extension patents that may be based on the foregoing; and
- (h) IP claimed in any of the foregoing referred to in paragraphs (a) to (g);
- (i) Improvements to any of the foregoing, up to the date of this Deed, and the IP in any such Improvements; and
- (j) the right to apply for a patent or equivalent protection in all countries and to claim priority under any international convention from any such application(s), in relation to the foregoing.

'Improvements' means all developments of, improvements to, additions to or alterations to the Intellectual Property, made by the Assignors.

'IP' means:

- (a) an invention or discovery; manner, method or process of manufacture; method or principle of construction; chemical composition or formulation; biological material; computer program; integrated circuit, circuit layout or semiconductor chip layout or design; plan, drawing or design; or scientific, technical or engineering information or document;
- (b) patent, application for a patent, right to apply for a patent or similar rights for or in respect of any of the foregoing;
- (c) trade secret, know-how, or right of secrecy or confidentiality in respect of any information or document referred to in the foregoing; and
- (d) copyright or other rights in the nature of copyright subsisting in any works or other subject matter referred to in the foregoing.

'Patents' means the granted patents referred to in Schedule 2.

'Patent Applications' means the patent applications referred to in Schedule 2.

'Records' means the documents and thing referred to in Schedule 3.

2: Joint & Several

2.1 The covenants and obligations on the part of the Assignors contained in this Deed are binding upon each of them jointly and severally.

3. The Assignment

3.1 The Assignors hereby assign all their right, title and interest in and to the Intellectual Property to the Assignee.

3.2 The Assignors assign to the Assignee the right to sue for and recover damages and other relief in relation to any infringement of the Intellectual Property that may have occurred before the date of this Deed.

3.3 Upon the date of this Deed, the Assignors must give written instructions to AJ Parks patent attorneys that all its files relating to the maintenance and prosecution of the Intellectual Property, from the date of this Deed, are held by it on behalf of the Assignee.

4. Records

4.1 The title to the Records passes from the Assignors to the Assignee upon the date of this Deed.

4.2 Within 30 days of the date of this Deed, the Assignors must deliver the Records to the Assignee.

4.3 The Assignors may retain one copy of the Records for archival purposes.

4.4 If at any time the Assignee reasonably requires any document that relates to the Intellectual Property which the Assignors still has possession of, the Assignors will:

(a) use the Assignors' reasonable efforts to locate that document; and

(b) provide a copy of that document, or the original, to the Assignee.

5 Further Assurance

5.1. The Assignors must on demand by the Assignee, perform all such acts and execute all such agreements, assurances and other documents and instruments as the Assignee reasonably requires either to perfect the rights and powers afforded, created or intended to be afforded or created by this Deed or to give full force and effect to, or facilitate the performance of, the transactions provided for in this Deed.

5.2. Without limiting the generality of clause 5.1, the Assignors must sign all such documents as shall be required to assign to the Assignee all Patent

Applications pending, and all Patents granted, that are included in the Intellectual Property.

- 5.3. All reasonable costs incurred by the Assignors in complying with the Assignee's requests, including the costs of any notary public, shall be paid by the Assignee.

## 6 General Warranties

- 6.1 The Assignors warrant to the Assignee that except as disclosed in the Disclosures to the best of the Assignors' knowledge as at the date of this Deed:

- (a) either jointly or separately the Assignors are the sole owners of all the Intellectual Property both legally and beneficially;
- (b) the Intellectual Property has not been licensed, made the subject of an unexpired option, or other contract, deed, arrangement or understanding (granting a third party rights to the Intellectual Property), or encumbered, mortgaged, or charged in any way by the Assignors, nor made subject to any lien by the Assignors;
- (c) without limiting the generality of the foregoing neither Assignor is aware of any patent granted anywhere in the world that the Intellectual Property infringes as at the date of this Deed;
- (d) the Assignors are not aware of any litigation pending in respect of the Intellectual Property; and
- (e) no claim or demand has been received by either Assignor from any person in relation to the Intellectual Property.

- 6.2 For the purpose of clause 6.1 the expression 'to the best of the Assignors' knowledge':

- (a) means and is limited to the recollection of Dr Ian Boddy an employee of AgResearch Limited who was also a director of Orico between 30 June 2006 and 31 December 2008 (when the company was struck off);
- (b) does not include documents, agreements and other business records of or relating to Orico Limited (in particular from the period prior to the date on which AgResearch Limited acquired a controlling interest in Orico Limited), or any other predecessor in title of either Assignor, with which Dr Ian Boddy is unfamiliar or has no recollection of, notwithstanding that the documents, agreements and other business records are or may be in the possession of AgResearch or Covita;

- (c) in respect of the warranty in clause 6.1(c), does not include AgResearch or Covita having undertaken any freedom to operate or patent searching beyond those disclosed in the Disclosures.

The Assignee acknowledges and agrees that information within a document in the possession of the AgResearch or Covita is not deemed to be within the Assignors' knowledge for the purpose of the above warranties.

## 7. Warranties In Relation To Patents

### 7.1 The Assignors warrant to the Assignee that:

- (a) all Patent Applications have been made in the prescribed form and in the prescribed manner;
- (b) the inventors named in each Patent Application are the only inventors in relation to the subject matter of each Patent Application;
- (c) no person who is an inventor has been omitted from being named as such in a Patent Application;
- (d) no person has been included as an inventor in a Patent Application, who is not in fact an inventor;
- (e) in relation to each Patent that is granted, all the Patents subsist and the particulars of each patent set out in the Schedule are correct;
- (f) in relation to each Patent that is granted, all maintenance, continuation, renewal and other fees payable in relation to each Patent before the date of this Deed have been paid;
- (g) any previous assignment of a Patent is valid;
- (h) the Assignors and have not done or failed to do and will not do or fail to do anything that may cause any of the Patents or any patent claim to be revoked or to be declared or held to be wholly or partially invalid or unenforceable;
- (i) the Assignors have no notice of any challenge to the validity of any of the Patents and are not aware of any actual, suspected or threatened claim in relation to any of the Patents;
- (j) those of the inventors who at the time of making the invention were employees of AgResearch were subject to an employment agreement with AgResearch containing an intellectual property clause the same or substantially similar to those contained in Schedule 5.

### 7.2 AgResearch and Covita make no warranty as to the existence or validity of any assignment of the Intellectual Property from any other inventors who

were not employees of AgResearch or Covita and mdx confirms it has conducted its own due diligence concerning these matters.

8. Other Warranties

8.1 The Assignors warrant that:

- (a) the Assignors have the legal right and power to enter into this Deed;
- (b) the Assignors have full legal capacity and power to enter into this Deed and to carry out the transactions that this Deed contemplates; and
- (c) the execution of this Deed has been duly and validly authorised by all necessary corporate action on behalf of the Assignors.

9. Limitation Of Liability

9.1 All warranties given by the Assignors are limited to those expressly set out in this Deed with the intent and effect that, to the extent permitted by law, all conditions and warranties implied by law are excluded.

9.2 All warranties in relation to documents, reports or assessments provided or prepared by any third party (including the freedom to operate opinions in respect of the Wyeth and John Hopkins patents, given by AJ Parks patent attorneys) are hereby expressly excluded by the Assignors.

9.3 To the extent permitted by law, the total joint liability of the Assignors in relation to or arising from this Deed and the Agreement for Sale and Purchase of Business Assets dated on or about the date of this Deed (pursuant to which this Deed was entered including without limitation in relation to or arising from a breach of any of the warranties made by the Assignors, as qualified by this Deed, shall be limited to NZ\$2,000,000 provided that:

- (a) the Assignors shall have no liability to the Assignee for any indirect or consequential loss or damage; and
- (b) the Assignors shall have no liability for any claim not notified to them by the Assignee within 3 years of the date of this Deed.

10. Disclosure And Use Of Intellectual Property

10.1 Subject to the terms of a Patent License between the Assignee and AgResearch Limited made on or about the date of this Deed, the Assignors must:

- (a) maintain the Intellectual Property in strictest confidence;



- (b) not disclose the Intellectual Property to any person without the prior written consent of the Assignee; and
  - (c) not use the Intellectual Property in any way without the prior written consent of the Assignee.
- 10.2 Clause 10.1(a) and (b) cease to apply to such parts of the Intellectual Property as enter the public domain after the date of this Deed other than by a breach of this Deed by the Assignors.
- 11. Governing Law
  - 11.1 This Deed and any disputes relating to it shall be governed by and construed in all respects in accordance with the laws of New Zealand.
  - 11.2 Each party to this Deed submits to the non-exclusive jurisdiction of the courts of New Zealand.

Executed as a Deed this 22<sup>nd</sup> day of October 2010

SIGNED for and on behalf of Covita Limited \*

Scott Balme      SLBalm      Director  
Name                      Signature                      Position in Company

In the presence of

JAN BODDY      Jubaddy      770 B Gordon Rd      General Manager  
Full Name                      Signature                      Address                      Occupation

SIGNED for and on behalf of AgResearch Limited \*

Tom Richardson      TERich      CEO  
Name                      Signature                      Position in Company

In the presence of

JAN BODDY      Jan Boddy      770 B Gordon Rd      General Mgr  
Full Name                      Signature                      Address                      Occupation

SIGNED for and on behalf of mdx Biotech Pty Ltd \*

KEVIN HEALEY      Kevin Healey      DIRECTOR  
Name                      Signature                      Position in Company

In the presence of

CHRIS BOSTON      C. Boston      44 MACKAY ST      DIRECTOR  
Full Name                      Signature                      Address                      Occupation  
YARRAVILLE VIC 3013

\* This Deed must be signed by:

- (a) Two or more directors of the company; or
- (b) A single director of the company and a witness; or
- (c) (If the company's constitution allows it), any other person and a witness; or
- (d) One or more persons with a power of attorney to act on the company's behalf.

## SCHEDULE 1: DISCLOSURES

WIPO Patent Number	Title
WO 2002 010214	Growth differentiation factor receptors, agonists and antagonists thereof, and methods of using same
WO 1994 021681	Growth differentiation factor-8 (GDF-8)
US 7737116	Modified and stabilized GDF propeptides and uses thereof
WO 2004 037861	Neutralizing antibodies against GDF-8 and uses therefor
WO 2003 027248	Antibody inhibitors of GDF-8 and uses therefor
WO 2000 043781	Growth differentiation factor inhibitors and uses therefor
WO 2007 067616	Uses of myostatin antagonists

SCHEDULE 2: PATENTS

	Status	Application Number	Application Date	Patent Number	Grant Date
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**Myostatin Antagonists (Myostatin Antagonists)**

USA	Filed	60/835,525	03/08/2007		
Australia	Filed	2007279456	02/08/2007		
Canada	Filed	2693378	02/08/2007		
China PR	Filed	2.0078E+11	02/08/2007		
European Patent Convention	Filed	7834817.4	02/08/2007		
Hong Kong	Filed	09109360.4	02/08/2007		
Japan	Filed	2009-522733	02/08/2007		
New Zealand	Filed	575138	02/08/2007		
USA	Filed	12/376289	02/08/2007		

**Myostatin Isoform (Myostatin Isoform)**

Canada	Filed	2,582,940	30/09/2005		
European Patent Convention	Filed	5790714.9	30/09/2005		
Japan	Filed	507103400	30/09/2005		
New Zealand	Granted	538396	30/09/2005	538396	07/06/2007
USA	Filed	11/576,449	30/09/2005		
	<b>Status</b>	<b>Application Number</b>	<b>Application Date</b>	<b>Patent Number</b>	<b>Grant Date</b>

**Myostatin Muscle Regeneration (Methods and Compositions for Improving Muscle Regeneration)**

Australia	Filed	2006211813	07/02/2006		
Canada	Filed	2,597,152	07/02/2006		
China PR	Filed	200680009234.6	07/02/2006		
European Patent Convention	Filed	06716787.4	07/02/2006		
Hong Kong	Filed	08105540.6	07/02/2006		
India	Filed	3460/CHENP/2007	07/02/2006		
Japan	Filed	2007-554036	07/02/2006		
New Zealand	Granted	545079	14/02/2006	545079	11/09/2008
USA	Filed	11/883,854	07/02/2006		

**Myostatin Wound Healing (Methods and Compositions for Improving Wound Healing)**

Australia	Filed	2006211812	07/02/2006		
Canada	Filed	2,597,146	07/02/2006		
China PR	Filed	200680009255.8	07/02/2006		
European Patent Convention	Filed	6716786.6	07/02/2006		
Hong Kong	Filed	08105538.0	07/02/2006		
India	Filed	3880/CHENP/2007	07/02/2006		
Japan	Filed	2007-554035	07/02/2006		
New Zealand	Granted	538097	14/02/2006	538097	09/11/2006
USA	Filed	11/883,871	07/02/2006		

**Synthetic Myostatin Antagonists**

USA Provisional	Filed	61/247,821	01/10/2009		
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### SCHEDULE 3

#### RECORDS:

1. Originals of such laboratory notebooks owned and in the possession of the Assignors at the date of this Deed.
2. If requested by the Assignee, the Assignors will make available to the Assignees copies of any science reports, business contracts or agreements in its possession at the Settlement Date relating to the Intellectual Property or its prior work in respect of the Intellectual Property, that are or might reasonably be expected to be of importance to the Assignee in terms of its ongoing management and protection of the Intellectual Property, subject to any obligations of confidentiality owed by each Assignor to any third party, and subject to the right of the Assignors to such redaction as is considered necessary by the Assignors to protect any commercially sensitive information of the Assignors.
3. The Assignors may retain a copy of all original records transferred to the Assignee as required to meet its obligations under the Public Records Act 2006.
4. In the case of the provision of any lab books, science reports or business contracts to the Assignors, such lab books, science reports will be deemed provided without warranty of any kind including as to content, accuracy, fitness for purpose or otherwise, acknowledging that the Assignee has had the opportunity to peruse all such documents prior to entry into this Deed and associated Sale and Purchase Agreement. The Assignee shall be deemed to use the information contained in any such records at its own risk. The Assignee shall not copy, distribute, publish or otherwise disclose any science report, business contract or agreement provided to it by the Assignors, or any extract therefrom without the prior written consent of the Assignors.

## SCHEDULE 4: DESCRIPTION OF MSV INVENTION

### Summary of Invention

1. That a truncated MSV can be made synthetically and that does not contain a reactive cysteine residue in the  $\alpha$ -helix 1 region.
2. That a truncated MSV peptide that contains the sequence QFX1SX2LX3E is essential for bioactivity (where X1 can be C, A, S or Y and X2 can be I or V and X3 can be G or E).
3. That inclusion of aspartate and serine residues at the N- and C-termini improves solubility without affecting bioactivity.
4. That any combination of residues D, S, E, V, Y, I, W, G or F must precede the QFXSILGE peptide sequence and any combination of residues A, T, V, D, F, L or S must follow the QFXSILGE peptide sequence.
5. That the truncated MSV peptide can differ by more the 70% similarity to any of the prior sequences listed in patent WO 2006/036074 A1 (Myostatin Isoform).

### Background

MSV arises from alternative splicing in exon 3 of the myostatin gene. Briefly, a novel 195 bp open reading frame is appended to the first 21 bp of exon3 to generate a novel 65 amino acid C-terminal sequence (Figure 1). Therefore, the canonical mature myostatin peptide (the moiety that binds activin2b receptors) is removed.

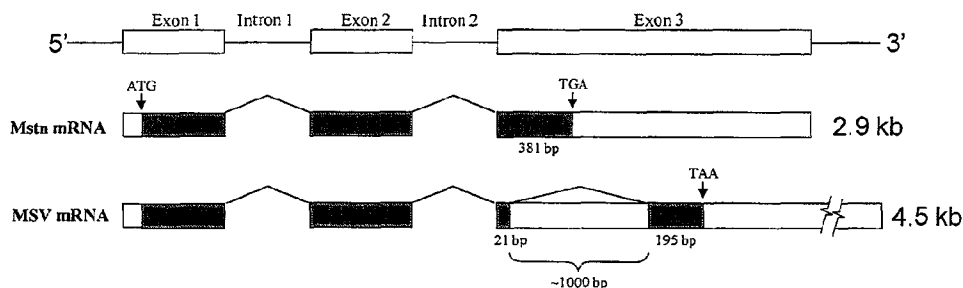


Figure 1: Splicing for myostatin and MSV.

There is a putative precursor convertase cleavage site ((R/K)-(X)<sub>n</sub>-(R/K)↑I), suggesting that the C-terminal 47 amino acid sequence of the MSV precursor is cleaved. Western immunoblotting provided evidence that the cleavage does occur in skeletal muscle (data not shown). Livestock species have two alpha helices, while other species have a single alpha helix (Figure 2). We have previously shown that either a single (truncated ovine or full human) or a double-alpha helix form of MSV (ovine) stimulates proliferation and/or differentiation of myoblasts (primary ovine, murine and primary human). We have also demonstrated that MSV (ovine) binds with high affinity (10<sup>-9</sup> Kd) to mature myostatin and to activin2b receptors.



Therefore, MSV acts in part by binding directly to myostatin to reduce its bioavailability. Secondly, MSV binds directly to activin2b receptors with similar affinity to that of myostatin. Both peptides stimulate canonical Smad signalling cascade. However, non-Smad pathways are also used by TGF- $\beta$  family members. We speculate that in addition to activation of Smad2/3, MSV activates a non-Smad pathway to oppose the actions of myostatin (Moustakas & Heldin 2005).

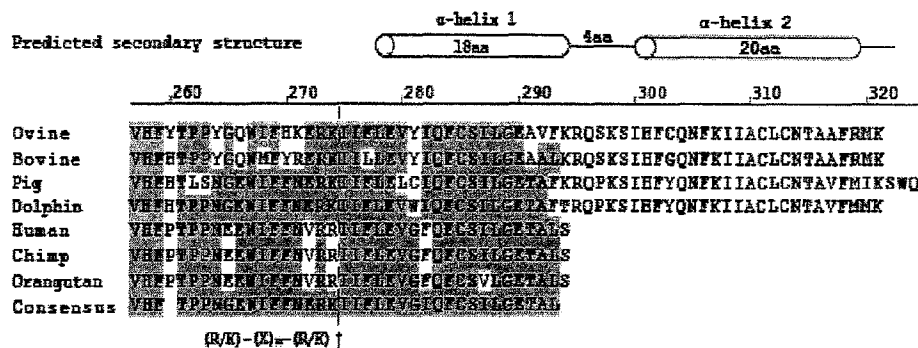


Figure 2. Alignment of cetartiodactyl and primate MSV peptides.

### Bioactive region

All peptides made, either recombinantly or synthesised, that contain the alpha1 region ( $\alpha$ -helix 1) stimulate proliferation of human, mouse and sheep myoblasts (see patent). Therefore, the major bioactive region must reside in this sequence. However, it is noteworthy to add that recent myoblast proliferation assays showed bioactivity for an alpha2 synthetic peptide. This was not claimed in the first patent, but the sequence will be covered by that patent. We have made peptides of variable length within the alpha1 domain and have found that all that contain the central consensus QFCSILGE are active (Table 1). However, the synthesised QFCSILGE alone has no activity when tested in a proliferation assay. Therefore, we postulate that a further two to three amino acids either side are essential to confer activity. Recently, two human MSV peptides were synthesised (VGFQFCSILGETALS and VGFQFASILGETALS) (Auspep Ltd) and tested on myoblasts to determine if cysteine is essential for activity. Both peptides were bioactive in our proliferation assay suggesting that the problematic cysteine residue in the alpha1 core sequence may not be required for activity (Figure 3). Another ovine alpha1 peptide LEVYIQFASILGEA made with the substitution of cysteine to alanine tested bioactive in the myoblast proliferation assay confirming that the cysteine is dispensable in the alpha1 core sequence (Figure 4). A synthetic scrambled alpha 1 (shMSVscramble) or a random peptide (shMSCsrc) had no bioactivity highlighting a specific order of residues for MSV and that the presence of a "reactive cysteine" residue is insufficient to provide bioactivity.

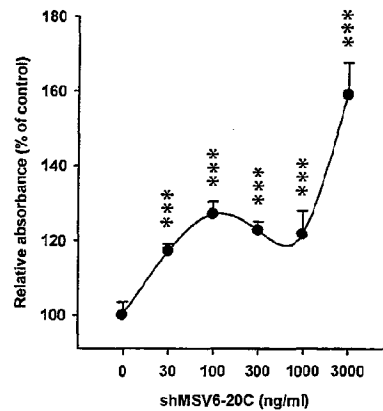
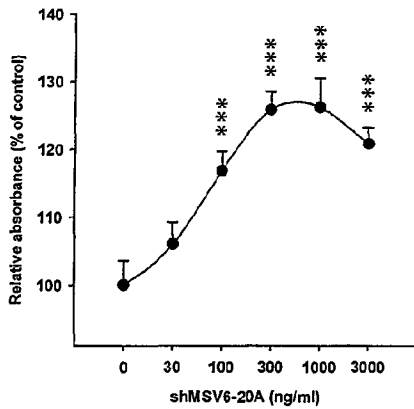


Figure 3. Synthetic human MSV peptides with an internal cysteine (shMSV6-20C) or the cysteine substituted with alanine (shMSV6-20A) stimulates the proliferation of C2C12 murine myoblasts. Proliferating C2C12 myoblasts were treated with 0, 30, 100, 300, 1000 and 3000 ng/ml of synthetic peptide for 72h and cell replication was determined using the methylene blue assay (Oliver et al. 1989). Shown are mean relative absorbance  $\pm$  SEM expressed as a percentage of no protein control. Asterisks indicate significance relative to no protein control using unpaired T-test (\*\* $P < 0.01$ , \*\*\*  $P < 0.001$ ).

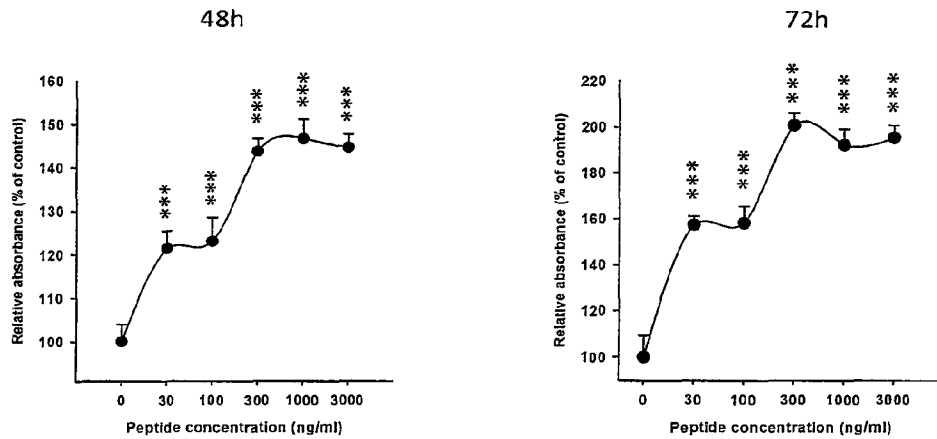


Figure 4. A synthetic ovine MSV peptide with an internal cysteine to alanine substitution (oMSV4-17A) stimulates the proliferation of C2C12 murine myoblasts. Proliferating C2C12 myoblasts were treated with 0, 30, 100, 300, 1000 and 3000 ng/ml of synthetic peptide for 48 or 72h and cell replication was determined using the methylene blue assay (Oliver et al. 1989). Shown are mean relative absorbance  $\pm$  SEM expressed as a percentage of no protein control. Asterisks indicate significance relative to no protein control using unpaired T-test (\*\* $P < 0.01$ , \*\*\*  $P < 0.001$ ).

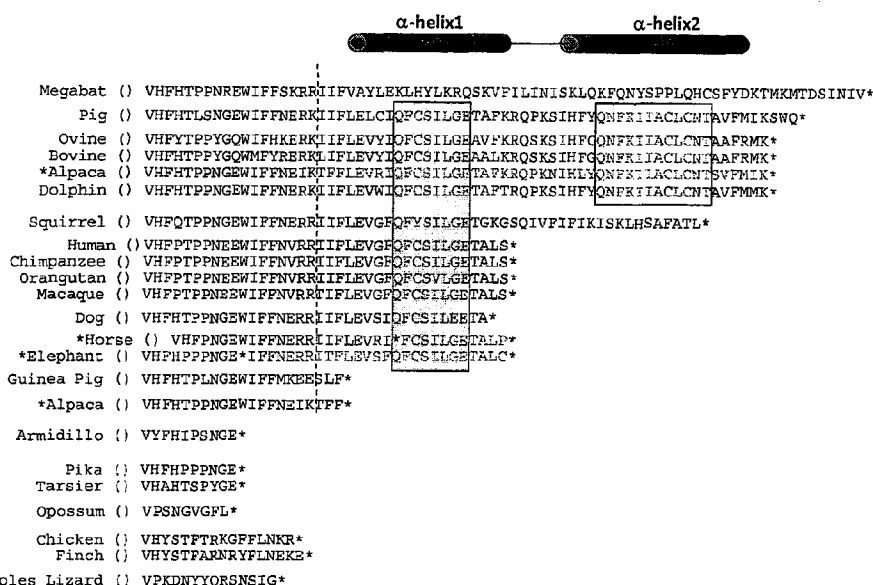
	(1)	Position					Proliferation assays		
		1	10	20	30	40	50	65	Murine
roMSV47	(1)	-----IIFLEVYIQFCSILGETDLS-----RAVFKRQSKSIHFQNFKIIACLNTAAFRMK					+	+	+
roMSV65	(1)	VHFYTPPYGQWIFHKRRIIFLEVYIQFCSILGETDLS-----RAVFKRQSKSIHFQNFKIIACLNTAAFRMK					+	+	NT
rhMSV38	(1)	VHFYTPPNBEWIFFNVRRIIFLEVYIQFCSILGETDLS-----RAVFKRQSKSIHFQNFKIIACLNTAAFRMK					+	NT	+
rhMSV20	(1)	-----IIFLEVYIQFCSILGETDLS-----RAVFKRQSKSIHFQNFKIIACLNTAAFRMK					+	NT	+
roMSValpha1	(1)	-----IIFLEVYIQFCSILGETDLS-----RAVFKRQSKSIHFQNFKIIACLNTAAFRMK					+	+	NT
roMSValpha2	(1)	-----IIFLEVYIQFCSILGETDLS-----IHFQNFKIIACLNTAAFRMK					+	+	NT
sbMSValpha2	(1)	-----IIFLEVYIQFCSILGETDLS-----IHFQNFKIIACLNTAAFRMK					+	NT	NT
shMSV scramble	(1)	-----CLVTFISIRGIFVGSGLAIQF-----					-	-	NT
shMSV1-10	(1)	-----IIFLEVYIQFCSILGETDLS-----					-	-	NT
shMSV1-15	(1)	-----IIFLEVYIQFCSILGETDLS-----					-	-	NT
shMSV1-20	(1)	-----IIFLEVYIQFCSILGETDLS-----					+	-	NT
shMSV11-20	(1)	-----IIFLEVYIQFCSILGETDLS-----					+	+	NT
shMSV6-15	(1)	-----VGFQKSIHFQNFKIIACLNTAAFRMK-----					+	+	NT
shMSV6-20	(1)	-----VGFQKSIHFQNFKIIACLNTAAFRMK-----					+	+	NT
shMSV6-20A	(1)	-----VGFQKSIHFQNFKIIACLNTAAFRMK-----					+	NT	NT
shMSV6-20C	(1)	-----VGFQKSIHFQNFKIIACLNTAAFRMK-----					+	NT	NT
shMSVsrc	(1)	-----TSRIVKTANCFHGNFQASV-----					-	NT	NT
MSV alpha1 ab	(1)	-----KLEVYIQFCSILGETDLS-----					-	-	NT
soMSV18	(1)	-----FYTFPPYQWIFHKRRIIFLEVYIQFCSILGETDLS-----					-	-	NT
soMSValpha1 core	(1)	-----IIFLEVYIQFCSILGETDLS-----					-	-	NT
soMSValpha1 new	(1)	-----IIFLEVYIQFCSILGETDLS-----					+	NT	NT
soMSV linker	(1)	-----RAVFKRQSKSIHFQNFKIIACLNTAAFRMK-----					-	-	NT
so/hMSVcore C	(1)	-----SDEVYIQFCSILGETDLS-----					NT	NT	NT
so/hMSVcore S	(1)	-----SDEVYIQFSSILGETDLS-----					NT	NT	NT
Consensus	(1)	EV QFCSILGE							

Table 1. Screening of recombinant and synthetic peptides made for MSV and tested in proliferation assays of murine and/or ovine and/or human myoblasts (s = synthesised, r = recombinantly expressed in *E. coli*, h = human, m = murine, o = ovine, + bioactive, -not bioactive, NT = not tested, highlighting with blue shows homology with a consensus peptide sequence).

### Next steps

A key requirement is to develop a peptide that can be consistently made to a high purity (>95%), that is soluble and has a reasonable half-life in vivo (>6 h after a bolus injection administered sc). An outstanding problem at present is the solubility of these synthetic peptides. To overcome this problem, we have had to solubilise them in dimethylsulfoxide (DMSO). To protect the reactive cysteine from oxidation and the subsequent peptide dimer formation, mercaptoethanol was added to the peptide solutions. However, these agents are toxic to cells and inhibit proliferation. Intriguingly, these truncated MSV peptides still stimulate proliferation, or, potentially, also promote cell survival in this assay. To overcome the problem of solubility and the use of DMSO and mercaptoethanol, we have ordered and received two new synthetic peptides with more soluble residues added either side of the active core region, which is also a combination of ovine and human sequence (SDEVYIQFCSILGETDLS and SDEVYIQFSSILGETDLS). These two peptides are soluble and both stimulated proliferation of murine myoblasts (C2C12) in our myoblast proliferation assay. The assay will be repeated to confirm the result.

We anticipate changing the residues in these two peptides further by substituting the most conserved residues identified in this region from the alignment in Figure 5 to obtain a peptide that is soluble and bioactive. A further step is to stabilise the peptide in a manner that we speculate will prolong the half-life in vivo by adding a cysteine residue at the N- and C-termini (this step may not be necessary). This will allow the formation of a cyclic peptide in oxidative conditions and it may prevent exposure of terminal amino acids to enzymatic degradation.



*Figure 5. Alignment of MSV peptides from species available on Ensembl ([www.ensembl.com](http://www.ensembl.com)) for which the MSV coding region was available. In general, MSV is present placental mammals and primarily in primates and cetartiodactyls (see Figure 6). Note that Orangutans, Dogs and Squirrels have differing residues in the core sequence in  $\alpha$ -helix1. The red lettering is a theoretical sequence and is added because a stop codon prevents translation (Horse and Elephant) or an Indel changes the reading frame (Alpaca).*

In summary, recombinant and synthetic peptides are equally bioactive. Key features are:

1. Myoblasts, irrespective to species, respond similarly to MSV treatment indicating a common molecular target and we speculate that this is via binding to myostatin and/or activating a non-Smad pathway to block myostatin signalling. In support, MSV binds with high affinity to myostatin and activin2b receptors using Surface Plasmon Resonance.
2. The core sequence for activity is QFX1SX2LX3E is essential for bioactivity (where X1 can be C, A, S or Y and X2 can be I or V and X3 can be G or E). See Figure 5 for substitution rationale.
3. That a truncated MSV can be made synthetically and that does not contain a reactive cysteine residue in the  $\alpha$ -helix 1 region.
4. That inclusion of aspartate and serine residues at the N- and C-termini improves solubility without affecting bioactivity.
5. That a combination of two to three residues of D, S, E, V, Y, I, W, G or F must precede the QFX1SX2LX3E peptide sequence and a combination of two to three residues of A, T, V, D, F, L or S must follow the QFX1SX2LX3E peptide sequence.

6. That the truncated MSV peptide can differ by more the 70% similarity to any of the prior sequences listed in patent WO 2006/036074 A1 (Myostatin Isoform).
7. The alpha2 sequence also increases proliferation on its own but when it is combined with alpha1 it may have a synergistic effect on myoblast proliferation (not yet tested).

#### References

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Springer MS, Murphy WJ, Eizirik E, O'Brien SJ. Placental mammal diversification and the Cretaceous-Tertiary boundary. Proc Natl Acad Sci U S A. 2003 Feb 4;100(3):1056-61.

## SCHEDULE 5: CLAUSES IN AGRESEARCH CONFIDENTIALITY

Extract From AgResearch Collective Employment Agreement July 2004

### **Confidentiality, intellectual property and privacy**

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Extract From Ag Research Collective Employment Agreement July 2001

### **Confidentiality, IP and Privacy**

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Extract From AgResearch Collective Employment Agreement 1996

### **23. CONFIDENTIALITY AND INTELLECTUAL PROPERTY**

23.1 Employees must keep confidential all information relating to the employer's research and commercial activities, including all transactions, records, genetic material and any documentation which is not in the public domain, and must not use any such information for any unauthorised purpose. The employer shall from time to time issue policy statements setting out

guidelines for the authorised release of information by employees, and these guidelines shall be strictly observed.

- 23.2 All inventions, discoveries, patents, designs, copyright or other intellectual property or proprietary rights arising from an employee's employment shall be the property of the employer unless otherwise agreed in writing.
- 23.3 The obligations contained in this clause shall continue after the termination of employment.
- 23.4 This clause shall not apply to employees who have entered into an individual confidentiality and/or intellectual property agreement with the employer.

Extract From AgResearch Collective Employment Agreement 1993

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