

# **Characterising the Role of Substance P in Human and Experimental Brain Tumours**

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December 2012

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

## **Dedication**

For Andrew Weldon and Jennifer Harford

## Declaration

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## Publications and Presentations

### Publications

Lewis, KM, **Harford-Wright E**, Vink R, Ghabriel MN (2012) Targeting classical but not neurogenic inflammation reduces peritumoral oedema in secondary brain tumours. *J Neuroimmunology*, 250(1-2) 59-65.

Lewis, KM, **Harford-Wright E**, Vink R, Ghabriel MN (2012) Walker 256 tumour cells increase substance P immunoreactivity locally and modify the properties of the blood-brain barrier during extravasation and brain invasion. *Clinical Experimental Metastasis*, [Epub ahead of print].

**Harford-Wright, E**, Lewis, K and Vink, R. (2011). Towards Drug Discovery for Brain Tumours: Interaction of Kinins and Tumours at the Blood Brain Barrier Interface. *Recent Patents on CNS Drug Discovery*, 6, pp 31-40.

**Harford-Wright E**, Thornton E and Vink R. (2010) Angiotensin-converting enzyme (ACE) inhibitors exacerbate histological damage and motor deficits after experimental traumatic brain injury. *Neuroscience Letters*, 481 p26-29

### Abstracts/Presentations

**Harford-Wright, E**, Lewis KM, Vink R, Ghabriel M. (2012) Substance P as a novel therapeutic target in the treatment of peritumoural oedema. *Proc. Aust Soc for Medical Research*.

**Lewis KM**, Harford-Wright E, Vink R, Ghabriel M. (2012) Effect of NK1 Receptor Antagonist Treatment on Tumor Growth In Vitro and in a Rat Model of Brain Metastases. *Proc. Aust Soc for Medical Research*.

**Harford-Wright E**, Lewis KM, Vink R, Ghabriel M. (2012) Characterising the role of substance P in the genesis of peritumoural oedema. *Proc. Aust Neurosci Soc*, 32<sup>nd</sup> annual meeting

Lewis KM, **Harford-Wright E**, Vink R, Ghabriel M. (2012) NK1 receptor antagonists do not prevent tumour cell extravasation and subsequent brain metastatic growth. *Proc. Aust Neurosci Soc*, 32<sup>nd</sup> annual meeting.

**Harford-Wright, E**, Lewis KM, Vink R, Ghabriel M. (2011) Characterising the expression of Substance P in human and experimental brain tumours. *Society for Neuroscience*

Lewis KM, **Harford-Wright E**, Vink R, Ghabriel M. (2011) Dexamethasone, but not NK1 receptor antagonist, treatment results in reduced brain tumor volume and edema. *Society for Neuroscience*

**Harford-Wright E**, Lewis KM, Vink R, Ghabriel M. (2011) Characterising the role of the neuropeptide substance P in experimental brain tumours. *Proc. Aust Soc for Medical Research*.

Lewis KM, **Harford-Wright E**, Vink R, Ghabriel M. (2011) Walker 256 metastatic brain tumour cells modify the properties of the blood-brain barrier locally during extravasation. *Proc. Aust Soc for Medical Research*.

## Acknowledgements

Undertaking this PhD has been both an extremely challenging and tremendously rewarding experience, and it is with great pleasure that I thank the following people who made it possible.

First and foremost, to my primary supervisor Professor Bob Vink who has been my mentor since my introduction to research over 5 years ago. Thank you for the opportunity to undertake a PhD in your laboratory, and a project that has been exceptionally engaging and fulfilling. I have thoroughly enjoyed my time in the lab, which manages to balance exciting science with a lot of fun. I've also really valued all your advice and perspective, which will no doubt hold me in good stead for the future.

To my co-supervisor Associate Professor Mounir Ghabriel, who has always been extremely generous in sharing his extensive research knowledge and experience. Thank you for being so approachable and patient, it has been a pleasure to be one of your students.

To the other senior members of the lab; Associate Professor Corinna Van Den Heuvel, Dr Stephen Helps, Dr Emma Thornton, Dr Renée Turner, Dr Jenna Ziebell and Dr Frances Corrigan, who have all been of great assistance in various aspects of this project.

In particular, to the amazing Emma and Renée, who have been such important parts of my PhD. I have been so lucky to work with such strong role models and even luckier to be able to count you as my friends. You are two incredible ladies and I couldn't have done this without all your encouragement and friendship.

Dr Frances Corrigan, not only are you a fabulous scientist, but also a lovely person. Thank you for going out of your way to help me, and for always providing me with some perspective when things were going wrong. A huge thank you for proof reading this thesis in record time, despite how busy you are!

I have been fortunate enough to share this experience with one of my best friends, Dr Anna Leonard. You have been my partner in crime for the past 5 years and I can honestly say I would not have arrived here without you. Thank you for your unconditional support, encouragement and

understanding as well as the countless wines, lunches, coffees and trips to the emergency room. You are one amazing lady and it has been such fun sharing this with you!

Kate Lewis, thank you for all your invaluable advice and input into this project. It was great being able to talk things through with someone in the same area, and to have someone to share in both the exciting times and the frustrations. Congratulations on finishing your PhD, and I wish you all the best in your next endeavours.

Kylie Ellis, who has helped to maintain my sanity, especially in these final few months. I can't wait to return the favour and celebrate the completion of your PhD very soon!

Joshua Burton for providing a number of the diagrams within this thesis, and who also patiently shared an office with some very excitable and at times crazy final year PhD students.

To the other members of the Vink Lab, past and present, who were there to celebrate the successes and commiserate in the not so successful times. It is you all who have made the lab such an enjoyable place to work.

The staff at the IMVS Centre for Neurological Diseases, for all their technical assistance as well as providing me with many entertaining moments whilst working in their lab. In particular, to Jim Manavis, who truly is an immunohistochemical guru. Thanks for sharing all your expertises with me and for always being so enthusiastic and helpful. Also to Kathryn Batra for sectioning all my human tissue without complaint!

The Neurosurgical Research Foundation, which provided the funding for this project, and whose support of our lab and our research is incredible.

To Kelly, Brigitt, Bec and the staff at the IMVS Animal Care Facility, thank you for taking such good care of my mice and being so easy and approachable to work with.

I am so blessed to have amazing family and friends who have been unbelievably supportive and encouraging over the last 3 and half years. Thank you for putting up with me! In particular, my parents for their unwavering support, as well as instilling in me the values that have been integral in the completion of this thesis. The successes of both your daughters are in large a reflection of you.

To my sister Hannah, my best friend and biggest cheerleader even from the other side of the world, thank you for “getting it.”

Finally, but most significantly, I would like to respectfully acknowledge the sacrifice of animal life involved in the generation of this thesis.

## Abbreviations

5-HT	Serotonin
ACE	Angiotensin Converting Enzyme
AQPs	Aquaporins
AQP-1	Aquaporin-1
AQP-4	Aquaporin-4
BBB	Blood-Brain Barrier
BSA	Blood Serum Albumin
Ca <sup>2+</sup>	Calcium
CCM	Complete Culture Medium
CGRP	Calcitonin Gene Related Peptide
Cl <sup>-</sup>	Chlorine
CNS	Central Nervous System
CO <sub>2</sub>	Carbon dioxide
CPP	Cerebral Perfusion Pressure
CSF	Cerebrospinal Fluid
CT	Computed Tomography
DAB	3'3'-diaminobenzidine
DMEM	Dulbeccos Modified Eagles Medium
DWI	Diffusion Weighted Imaging
EB	Evans Blue
ELISA	Enzyme-Linked Immunosorbent Assay
FBS	Fetal Bovine Serum
GBM	Glioblastoma Multiforme
GFAP	Glial Fibrillary Acidic Protein
H <sub>2</sub> O	Water
H <sub>2</sub> O <sub>2</sub>	Hydrogen Peroxide
H <sub>2</sub> SO <sub>4</sub>	Sulfuric Acid
H&E	Haematoxylin and Eosin
HRP	Horseradish Peroxidase
ICP	Intracranial Pressure
IL	Interleukin
K <sup>+</sup>	Potassium

LDH	Lactate Dehydrogenase
MRI	Magnetic Resonance Imaging
Na <sup>+</sup>	Sodium
NAT	N-acety-L-tryptophan
NBF	Neutral Buffered Formalin
NEP	Neutral Endopeptidase
NHS	Normal Horse Serum
O <sub>2</sub>	Oxygen
PBS	Phosphate Buffered Saline
PNS	Peripheral Nervous System
PPTA	Preprotachykinin A
PPTB	Preprotachykinin B
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SD	Standard Deviation
SEM	Standard Error of Mean
SP	Substance P
SPC	Streptavidin Peroxidase Conjugate
SRS	Stereotactic Radiosurgery
TAM	Tumour Associated Macrophages
TADC	Tumour Associated Dendritic Cells
TBI	Traumatic Brain Injury
TBS	Tris-Buffered Saline
TCA	Trichloroacetic Acid Solution
TNF	Tumour Necrosis Factor
WBRT	Whole Brain Radiotherapy

# Table of Contents

Dedication .....	i
Declaration .....	i
Publications and Presentations .....	ii
Publications .....	ii
Abstracts/Presentations .....	ii
Acknowledgements .....	iv
Abbreviations .....	vii
Table of Contents .....	ix
List of Tables.....	xvi
Abstract .....	xvii
<b>Chapter 1: Introduction .....</b>	<b>1</b>
<b>1.1 Epidemiology .....</b>	<b>2</b>
<b>1.2 Cancer .....</b>	<b>3</b>
<b>1.3 Primary Brain Tumours .....</b>	<b>5</b>
<b>1.4 Mechanisms of Metastasis .....</b>	<b>6</b>
1.4.1 Brain Metastasis .....	8
1.4.2 Features of Brain Metastasis .....	9
<b>1.5 Blood Brain Barrier .....</b>	<b>10</b>
1.5.1 Blood Brain Barrier and Tumours .....	11
<b>1.6 Cerebral Oedema.....</b>	<b>12</b>
1.6.1 Cytotoxic Brain Oedema .....	12
1.6.2 Vasogenic Brain Oedema .....	13
1.6.3 Peritumoural Oedema .....	13
<b>1.7 Inflammation and Cancer .....</b>	<b>15</b>
<b>1.8 Current Treatments .....</b>	<b>17</b>
1.8.1 Chemotherapy .....	17
1.8.2 Radiotherapy.....	18

1.8.3 Surgery .....	18
1.8.4 Stereotactic Radiosurgery.....	19
1.8.5 Corticosteroids .....	19
<b>1.9 Substance P .....</b>	<b>20</b>
1.9.1 Synthesis .....	21
1.9.2 Location .....	21
1.9.3 Receptors.....	21
1.9.4 NK1 Receptor .....	22
1.9.5 Metabolism.....	22
1.9.6 Behavioural Effects of SP .....	22
1.9.7 Neurogenic Inflammation .....	23
1.9.8 SP in Cancer.....	25
<b>1.10 Experimental Models of Brain Tumours .....</b>	<b>27</b>
1.10.1 Allograft Models .....	28
1.10.2 Xenograft Models.....	28
1.10.3 Stereotactic Implantation of Brain Tumours.....	29
1.10.4 Models of Metastasis .....	29
<b>1.11 Conclusion and Aims.....</b>	<b>31</b>
<b>Chapter 2: Materials and Methods .....</b>	<b>32</b>
<b>2.1 Animal Care .....</b>	<b>33</b>
2.1.1 Animal Ethics .....	33
2.1.2 General .....	33
<b>2.2 Experimental Procedures .....</b>	<b>33</b>
2.2.1 In Vitro Experiments: Cell Culture.....	33
Drug Treatments .....	35
<b>2.2.2 Anaesthesia .....</b>	<b>37</b>
2.2.3 Intracardiac Injection of A-375 Human Melanoma Cells.....	38
2.2.4 Stereotactic Implantation of A-375 Human Melanoma Cells .....	42
2.2.5 Post Surgical Recovery.....	44
<b>2.3 Drug Treatments.....</b>	<b>44</b>
<b>2.4 Oedema Measurements .....</b>	<b>44</b>
2.4.1 Wet Weight Dry Weight.....	44
2.4.2 Magnetic Resonance Imaging .....	45
<b>2.5 Blood Brain Barrier Permeability.....</b>	<b>45</b>

2.5.1 Evans Blue .....	45
<b>2.6 Histological Analysis .....</b>	<b>46</b>
2.6.1 Human Tissue .....	46
2.6.2 Tissue Processing .....	46
2.6.3 Haematoxylin & Eosin Staining .....	47
2.6.4 Immunohistochemistry .....	47
2.6.5 Immunohistochemical Detection .....	48
<b>2.7 Assessment of Histology .....</b>	<b>49</b>
2.7.1 Hamamatsu Nanozoomer .....	49
2.7.2 Qualitative Analysis .....	49
2.7.3 Tumour Volume .....	50
2.7.5 Colour Deconvolution .....	51
<b>2.8 SP ELISA .....</b>	<b>52</b>
2.8.1 Preparation of Brain Homogenate .....	52
2.8.2 Protein Extraction .....	53
2.8.3 Protein Estimation Assay .....	53
2.8.4 SP ELISA .....	54
<b>2.9 Statistical Analysis .....</b>	<b>54</b>
<b>Chapter 3: Investigation of Mediators of BBB Disruption and Oedema in Human Brain Tumour Tissue .....</b>	<b>55</b>
<b>3.1 Introduction .....</b>	<b>56</b>
<b>3.2 Experimental Design .....</b>	<b>58</b>
3.2.1 Histological Analysis .....	59
3.2.2 Statistical Analysis .....	59
<b>3.3 Results .....</b>	<b>60</b>
3.3.1 General Pathology-H&E .....	60
3.3.2 SP immunoreactivity is increased in primary and secondary brain tumours .....	63
3.3.2 NK1 Receptor staining is increased in primary and secondary brain tumours .....	67
3.3.3 Brain tumours have increased AQP-1 staining .....	71
3.3.5 Brain tumour vessels have decreased expression of Claudin-5 .....	79
<b>3.4 Discussion .....</b>	<b>82</b>
<b>Chapter 4: The Effects of NK1 Antagonist Treatment on Tumour Cells <i>in vitro</i> .....</b>	<b>87</b>

<b>4.1 Introduction .....</b>	<b>88</b>
<b>4.2 Methods.....</b>	<b>89</b>
4.2.1 Experimental Design.....	89
4.2.2 Immunohistochemistry.....	89
4.2.3 Trypan Blue Assay.....	89
4.2.4 SP ELISA.....	90
4.2.5 LDH ELISA.....	90
4.2.6 Statistical Analysis .....	90
<b>4.3 Results .....</b>	<b>91</b>
4.3.1 A-375 human melanoma cells express SP and the NK1 receptor <i>in vitro</i> .....	91
4.3.2 Treatment with the NK1 receptor antagonist Emend reduces A-375 cell viability <i>in vitro</i> .....	93
4.3.3 NK1 antagonist treatment increases SP levels in A-375 melanoma cells <i>in vitro</i> .....	95
4.3.4 NK1 antagonist treatment results in elevated LDH content in A-375 melanoma cells <i>in vitro</i> .....	97
<b>4.4 Discussion .....</b>	<b>99</b>
<b>Chapter 5: Characterisation of the Direct Inoculation Model of Brain Tumours .....</b>	<b>102</b>
<b>5.1 Introduction .....</b>	<b>103</b>
<b>5.2 Materials and Methods.....</b>	<b>104</b>
5.2.1 Implantation of Tumour Cells.....	104
5.2.2 Histological Analysis .....	104
5.2.3 Substance P ELISA .....	105
5.2.4 Wet Weight-Dry Weight .....	106
5.2.5 Evans Blue.....	106
5.2.6 Magnetic Resonance Imaging .....	107
5.2.7 Statistical Analysis .....	107
<b>5.3 Results .....</b>	<b>108</b>
5.3.1 Histological characterisation of tumour growth following a direct implantation model of brain tumours.....	108
5.3.2 Tumoural and peritumoural increase in SP and NK1 receptor levels following tumour cell inoculation.....	112
5.3.4 Direct implantation of tumour cells results in increased BBB disruption and oedema formation .....	117
<b>5.4 Discussion .....</b>	<b>121</b>

<b>Chapter 6: NK1 Antagonist Treatment Modulates Tumour Associated Oedema and Tumour Growth</b> .....	<b>124</b>
<b>6.1 Introduction</b> .....	<b>125</b>
<b>6.2 Materials and Methods</b> .....	<b>127</b>
6.2.1 Implantation of Tumour Cells .....	127
6.2.2 Treatment.....	127
6.2.3 Tissue Processing.....	128
6.2.4 Assessment of Lesion Volume.....	128
6.2.5 Immunohistochemistry .....	128
6.2.6 Wet Weight Dry Weight.....	129
6.2.7 Evans Blue .....	129
6.2.8 Statistical Analysis .....	129
<b>6.3 Results</b> .....	<b>130</b>
6.3.1 The effects of Emend and dexamethasone treatment on levels of SP and NK1 receptor following tumour cell inoculation .....	130
6.3.2 Effect of NK1 antagonist treatment on tumour associated BBB permeability and oedema formation .....	133
6.3.3 Histological assessment of NK1 antagonist treatment on tumour growth.....	136
6.3.4 The effect of NK1 antagonist treatment on tumour cell proliferation and apoptosis .....	138
<b>6.4 Discussion</b> .....	<b>140</b>
<b>Chapter 7: General Discussion</b> .....	<b>144</b>
7.1 Substance P and NK1 receptor are increased in brain tumours.....	145
7.2 Role of SP in an <i>in vitro</i> model of cancer.....	145
7.3 Effects of SP in an <i>in vivo</i> model of brain tumours .....	146
7.3.1 SP mediates tumour growth <i>in vivo</i> .....	147
7.3.2 SP mediates brain tumour associated oedema .....	150
7.2.3 Other potential roles of SP in carcinogenesis and future directions .....	151
7.4 Conclusion .....	152
<b>Reference List</b> .....	<b>153</b>

## List of Figures

Figure 1.1: Formation of Metastasis. ....	7
Figure 1.2: Blood Tumour Barrier. ....	14
Figure 2.1: A-375 Human Melanoma Cells <i>in vitro</i> . ....	34
Figure 2.2: Location of Intracardiac Injection. ....	39
Figure 2.3: Intracardiac injecting device. ....	40
Figure 2.4: Micrographs of Brains following Intracardiac Injection. ....	42
Figure 2.5: Stereotactic Implantation of Tumour Cells. ....	43
Figure 2.6: Determination of Tumour Volume. ....	50
Figure 2.7: Cell Counts of Proliferating and Apoptotic Cells. ....	51
Figure 2.8: Brain regions used for ELISA. ....	53
Figure 3.1: H&E stained sections of human normal brain and tumour tissue. ....	62
Figure 3.2: Median ranking of SP staining intensity in human primary and secondary brain tumours. ....	64
Figure 3.3: SP immunoreactivity in Tumour Tissue. ....	65
Figure 3.4: Representative perivascular SP staining in control and tumour tissue. ....	66
Figure 3.5: Median ranking of NK1 receptor immunoreactivity within the tumour mass of both primary and secondary brain tumours. ....	68
Figure 3.6: NK1 receptor immunolabelled sections. ....	69
Figure 3.7: Representative images of NK1 receptor immunoreactivity in the vasculature of controls and tumour tissue. ....	70
Figure 3.8: Median ranking of AQP-1 staining intensity within the tumour mass of both primary and secondary brain tumours. ....	72
Figure 3.9: AQP-1 staining within normal and tumour tissue. ....	73
Figure 3.10: AQP-1 immunoreactivity in the vasculature of control and tumour tissue. ....	74
Figure 3.11: Median ranking of AQP-4 staining within the tumour mass. ....	76
Figure 3.12: Representative images of AQP-4 staining within non-pathological and tumour tissue. ....	77
Figure 3.13: Representative images of AQP-4 staining around the microvessels of control and tumours. ....	78
Figure 3.14: Median ranking of claudin-5 expression within the vessels of normal and tumour tissue. ....	80
Figure 3.15: Claudin-5 immunolabelled sections within tumour vasculature. ....	81
Figure 4.1: Staining of A-375 human melanoma cells <i>in vitro</i> . ....	92
Figure 4.2: Percentage of viable cells following NK1 antagonist treatment. ....	94
Figure 4.3: SP content within A-375 human melanoma cells following treatment. ....	96

Figure 4.4: Levels of LDH within A-375 human melanoma cells following treatment. ....	98
Figure 5.1: Characterising the course of tumour growth over time. ....	109
Figure 5.2: Astrocytic response to tumour growth. ....	110
Figure 5.3: Immunohistochemistry for F 4/80 a marker of macrophages. ....	111
Figure 5.4: SP immunolabelled sections of peritumoural vessels and the tumour mass (40x). ....	113
Figure 5.5: Levels of SP at 4 weeks following tumour cell or culture medium injection. ....	114
Figure 5.6: NK1 receptor immunoreactivity. ....	115
Figure 5.7: Colour deconvolution of NK1 receptor staining at 3 weeks. ....	116
Figure 5.8: Albumin as a marker of BBB dysfunction. ....	118
Figure 5.9: Characterising oedema formation and BBB dysfunction. ....	119
Figure 5.10: MRI assessment of brain tumours. ....	120
Figure 6.1: SP immunolabelled sections following treatment. ....	131
Figure 6.2: NK1 receptor staining following Emend and dexamethasone treatment. ....	132
Figure 6.3: The effect of NK1 antagonist treatment on BBB permeability. ....	134
Figure 6.4: Effects of NK1 antagonist treatment on oedema formation. ....	135
Figure 6.5: The effect of NK1 antagonist treatment on tumour growth. ....	137
Figure 6.6: The effect of treatment on tumour cell proliferation and apoptosis. ....	139

## List of Tables

Table 1.1 Chronic Inflammatory Conditions Associated with Cancer .....	16
Table 2.1: Number and Location of Metastasis Following Intracardiac Injection .....	41
Table 3.1: Summary of Pathological Diagnosis, Age Range and Sex Distribution of Human Cases .....	58

## Abstract

Currently within Australia, brain tumours account for one death every eight hours. They are inherently difficult to treat, and even with the best current treatments, prognosis for these patients remains extremely poor. At present, treatment options for patients with either primary or secondary brain tumours are limited. Indeed most brain metastasis patients have a short survival time, despite the fact that initial treatment is often effective in reducing neurological deficits and tumour size.

One of the most serious complications of brain tumours is cerebral oedema, which is typically vasogenic in nature due to disruption of normal blood brain barrier function. Cerebral oedema is associated with many life-threatening complications such as raised intracranial pressure, reduction in cerebral perfusion pressure and in severe cases can result in brain herniation and death. Although treatments for cerebral oedema currently exist, they are associated with many deleterious side effects, prompting the search for alternative treatments.

The neuropeptide substance P and its NK1 receptor are reported to be upregulated in a number of different cancer types. This increase is thought to correspond with SP mediated increases in cellular proliferation, impairment of apoptosis and increased angiogenesis. Furthermore, SP has recently been implicated in the development of neurogenic inflammation in the brain, where it is associated with vasodilation, plasma extravasation and genesis of oedema. Accordingly, the current thesis sought to determine whether SP may provide a novel treatment target in both tumour associated oedema and tumour growth.

Immunohistochemical assessment of human primary and secondary brain tumour tissue demonstrated a marked increase in SP and the NK1 receptor in all tumour types investigated, confirming what has been previously observed in the literature. Of the metastatic tumours, melanoma demonstrated particularly elevated levels of SP and its receptor. Correspondingly, a human melanoma cell line, A-375, was examined *in vitro* and found to express both SP and the NK1 receptor. Furthermore, treatment with NK1 antagonists resulted in decreased cell viability of A-375 melanoma cells and an increase in cell death in this cell line *in vitro*, implicating SP in the facilitation of tumour growth.

An *in vivo* model of brain tumours using the same cell line, demonstrated increased SP and the NK1 receptor not only within the tumour, but also in peritumoural vessels. This increase corresponded

with a significant increase in brain water content and BBB permeability, implicating SP in the genesis of tumour associated oedema. Subsequent administration of the NK1 antagonist, Emend decreased BBB dysfunction and oedema formation and was more efficacious than the current clinical treatment choice, dexamethasone. Furthermore, treatment with Emend resulted in a decrease in tumour volume and in the number of proliferating cells, as well as an increase in apoptotic cells, an effect that was not seen with dexamethasone treatment. This indicates that SP may play multiple roles in tumour pathogenesis within the brain.

In conclusion, the present studies have identified a role for SP in tumour-associated oedema as well as in tumour growth and progression. Moreover, treatment with the NK1 antagonist Emend was more effective than the currently available clinical treatment, dexamethasone. Treatment with an NK1 antagonist may thus provide a novel therapeutic option, able to treat these two very different aspects of brain tumour pathogenesis.