THE ALZET OSMOTIC PUMP NEWSLETTER – DECEMBER 2010

Special Delivery

▸ *Aging Research*

Aging is a significant risk factor for many diseases, including cancer, cardiovascular, and neurodegenerative diseases. Recent research shows that by manipulating particular signaling pathways and by balancing nutrition, the lifespan of many organisms can be extended.[1]

Resveratrol, a sirtuin activator, and rapamycin have emerged as potential anti-aging drug candidates for their positive roles in lifespan extension studies. Similarly, the regenerative potential of stem cells shows promise as novel therapy for many age-related degenerative disorders. These strategies can lead to significant health benefits, and even delay the onset of age-related disorders and improve longevity. This issue of the ALZET Special Delivery Newsletter highlights the key contributions of ALZET® pumps in exciting and promising anti-aging research. Please contact us if you would like further information on any of the research topics described below.

[1]*Kenyon, C. The genetics of ageing. Nature 2010;464:504-512*

In this issue:

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Sirtuin Research with ALZET Pumps

–by Jose R Gadea

The sirtuins, or silent information regulators (SIRT1-7), are a family of NAD^+-dependent enzymes which have been shown to control vital biological functions, such as apoptosis, cell cycle regulation, DNA damage repair, and muscle differentiation.[1] They have also been associated with lifespan extension and are the focus of intense investigation for their key roles as disease-modifiers in cardiovascular, metabolic, neurodegenerative and other aging disorders. As described below, ALZET Osmotic Pumps have played a key role in studies designed to further understand the involvement of sirtuins in human disease.

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SIRT1 Activation Improves Cardiovascular Disease (ALZET Use: Model of Hypertension)

Miyazaki *el al.* used ALZET pumps to establish an experimental model of hypertension to show that improvement of cardiovascular disease may be regulated through SIRT1 activation.[2] To test this hypothesis, the researchers studied the effects of resveratrol, a natural polyphenol found in grapes and known SIRT1 activator, on vascular smooth muscle cells (VSMCs) and aorta cells from hypertensive mice.

Experimental hypertension in C57/B6 mice was induced by chronic, subcutaneous administration of angiotensin II (Ang II) at a low dose of 490 ng/min/kg using ALZET pumps. Resveratrol was given via drinking water at an estimated dose of 10 mg/kg/day, while controls received water only. As most traditional effects of Ang II (i.e., vasoconstriction) are mediated by the Ang II type 1 receptor (AT1R), receptor levels in aorta extracts from hypertensive mice were measured to assess resveratrol activity. Western blot analysis revealed that AT1R levels were reduced by nearly 70% in the aortas from resveratrol-treated mice, compared to untreated controls (Figure 1A). Furthermore, Ang II-induced hypertension was returned to normal levels following resveratrol administration (Figure 1B). Resveratrol was also shown to significantly reduce AT1R protein levels in VSMCs in a time- and dose-dependent manner. AT1R expression was also suppressed in VSMCs overexpressing SIRT1. Incubation of these cells with nicotinamide, a noncompetitive SIRT1 inhibitor, increased AT1R expression, further confirming that SIRT1 is directly linked to AT1R downregulation.

Miyazaki *et al.* demonstrated that resveratrol suppressed AT1R expression in VSMCs and mice aortas through SIRT1 activation, and suggested that this mechanism may contribute to the anti-atherosclerosis and lifespan extension effects seen in other studies.

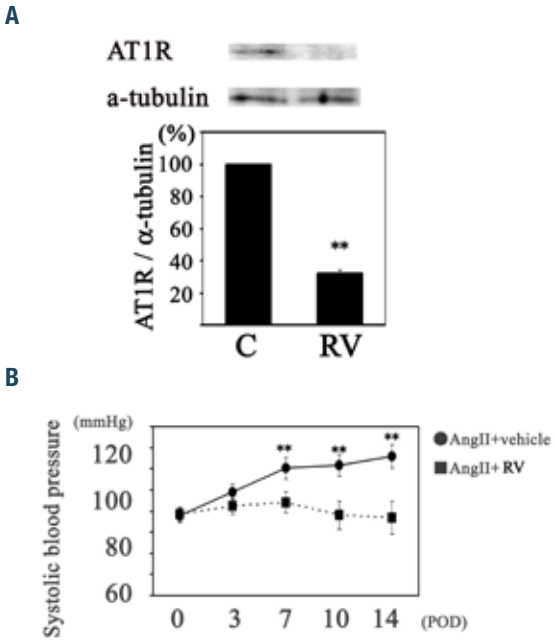

Figure 1. Effect of resveratrol on AT1R protein expression in the mouse aorta. A, AT1R expression in the membrane fractions of the aorta of mice treated with either resveratrol (RV) or water (C) was analyzed with Western blot analysis. The values are expressed as a percentage of water treated group (100%; n=4). B, Mice were treated with either resveratrol or vehicle (water). Ang II was administered in both groups and blood pressure was measured (n=4). POD indicates postoperative days after implatation of osmotic pump. Reproduced, with permission, from Miyazaki et al. Arterioscler Thromb Vasc Biol. 2008;28:1263-1269.

Antidiabetic Actions of Resveratrol (ALZET Use: Circumvent the Blood-Brain Barrier)

Resveratrol has also been shown to possess antidiabetic actions. Since SIRT1 is expressed in central nervous system (CNS) neurons that control glucose and insulin homeostasis, Ramadori *et al.* sought to demonstrate that the antidiabetic effects of resveratrol are mediated through SIRT1 activation in the brain.[3] ALZET pumps were used to infuse resveratrol directly to the CNS of mice since resveratrol penetrates the blood-brain-barrier poorly.

Model 1004 ALZET pumps were connected to brain cannulae and used to administer resveratrol (79.2 ng/day), or saline directly to the lateral ventricles (ICV) of diet-induced obese mice for 5 weeks. SIRT1 enzymatic activity was determined by measuring the amount of acetylated lysine in p53 (a SIRT1 target) by Western blot assay. Evaluation of forebrain lysate samples from resveratrol-infused mice showed lower levels of acetylated lysine, compared to those of saline-infused controls. Acetylated lysine levels in hepatic lysates were unchanged, suggesting that SIRT1 enzymatic activity was restricted to the brain. Furthermore, assessment of metabolic parameters showed that ICV infusion of resveratrol normalized glycemia and improved insulinemia in diet-induced obese and diabetic mice during the 5-week treatment period. Interestingly, resveratrol-treatment had no effect on body weight, food intake or circulating leptin levels. In addition, CNS resveratrol delivery was also shown to exert anti-inflammatory effects in the brain by repressing the hypothalamic nuclear factor-kB signaling pathway.

Ramadori *et al.* demonstrated that long-term, ICV administration of resveratrol enhanced SIRT1 enzymatic activity, and identified the brain as a key site for mediating the antidiabetic actions of resveratrol.

SIRT3 Blocks Cardiac Hypertrophy (ALZET Use: Model of Cardiac Hypertrophy)

Prolonged cardiac hypertrophy leads to congestive heart failure and sudden death due to arrhythmias. Sundaresan *et al.* demonstrated that SIRT3 protects the mouse heart by blocking the cardiac hypertrophic response.[4] The researchers used ALZET pumps to generate a stable model of cardiac hypertrophy in mice to study the effects of SIRT3 on cardiac disease. Cardiac hypertrophy was induced by chronic, intraperitoneal infusion of angiotensin II (Ang II) at 3.0 mg/kg/day for 14 days, phenylephrine (PE) at 75 mg/kg/day for 14 days, or isoproterenol (ISO) at 8.7 mg/kg/day for 7 days using ALZET pumps (Model 2002 or Model 2001).

Initial experiments using SIRT3-deficient mice (SIRT3-KO) showed that SIRT3 is required for blocking the cardiac hypertrophy response. Chronic administration of Ang II via ALZET pumps produced nearly 44% cardiac hypertrophy in SIRT3-KO mice, compared to only 22% hypertrophy in wild type (WT) mice. Similar results were obtained with ISO and PE.

To further investigate the antihypertrophic effects of SIRT3 *in vivo*, Sundaresan *et al.* generated Tg mice which, compared to non-Tg controls, contained a 3- to 4-fold higher level of SIRT3 expression in the heart. Non-Tg mice developed 25% cardiac hypertrophy after Ang II administration for 14 days, whereas SIRT3-Tg mice showed no noticeable increase in cardiac hypertrophy. SIRT3-Tg mice were also resistant to Ang II-induced cardiac fibrosis, whereas it was pronounced in non-Tg mice. Similar results were also obtained in the ISO-induced hypertrophy model. Left ventricular (LV) wall thickness was significantly higher in SIRT3-KO mice, compared to WT controls, and it was increased further after agonist infusion. Conversely, ISO infusion had no significant effect on the LV wall thickness of SIRT3-Tg.

In vitro experiments on cardiomyocytes showed that SIRT3 blocks the cardiac hypertrophic response through activation of Foxo-dependent antioxidants and reduction of reactive oxygen species (ROS) levels, leading to the suppression of transcription and translation factor signaling pathways responsible for the hypertrophic response. Collectively, these studies demonstrated that SIRT3 is a stress-responsive enzyme that protects the heart against hypertrophic stimuli.

Conclusion

The activation of sirtuin signaling pathways may provide a novel therapeutic strategy for preventing or delaying the onset of many human diseases. As these studies indicate, ALZET pumps proved useful in studies designed to investigate the effects of SIRT1 and SIRT3 activation in cardiovascular and metabolic diseases. In some cases, ALZET pumps enabled effective administration of sirtuin activators (i.e., resveratrol) directly into the CNS, thus circumventing the blood-brain-barrier. In other cases, ALZET pumps enabled the development of stable animal models in which to study the role of sirtuin activation on hypertension and cardiac hypertrophy. The availability of these models will facilitate the development of sirtuin-based therapeutics.

[1] *Finkel et al. Nature 2009;460:587-591*
[2] *Miyazaki et al. Arterioscler Thromb Vasc Biol. 2008;28:1263-1269*
[3] *Ramadori et al. Endocrinology 2009;150(12):5326-5333*
[4] *Sundaresan et al. J Clin Invest. 2009;119(9):2758-71*

ALZET Pumps Contribute to Stem Cell Research

–by Clarisa Peer

A decade after the first human stem cells were isolated by biologists at Johns Hopkins and the University of Wisconsin, therapies derived from human stem cells continue to advance toward what seems like inevitable human application. The first clinical trial is being planned by Geron Corporation, a biotech company in California. At the same time, ALZET pumps are enabling scientists at the preclinical stage. The following three studies demonstrate how researchers are using ALZET pumps to increase our understanding of potential stem cell therapies.

Model Creation

Emerging therapies depend upon the availability of a suitable animal model. Wu *et al.* used ALZET pumps to establish a rat model of Alzheimer's disease (AD) in order to evaluate treatment with transplanted neural stem cells (NSCs) genetically modified to express human nerve growth factor (hNGF).[1]

Model 2004 ALZET pumps connected to brain cannulae were used to infuse okadaic acid (OA) or artificial cerebrospinal fluid (aCSF) into the lateral ventricles (ICV) of male Wistar rats for 56 days. (Pumps were replaced on day 28 to extend delivery beyond the duration of a single pump.) The model was confirmed by observing neurobehavioral deficits relative to control animals, and also by the presence of neurofibrillary tangles (NFT) and plaque formation in the brains of treated animals. Following the infusion period, the experimental group received embryonic NSCs transduced with an adeno-associated virus containing genes for hNGF and enhanced green fluorescent protein (rAAV2-hNGF-eGFP). Controls received either unmodified NSC or culture media. Tests for spatial learning and exploratory locomotion were conducted both one week and one month later.

As expected with this model of AD, the OA group performed less well on the various tests, taking longer in the maze and spending less time in the target quadrant compared with aCSF-treated and sham-operated controls. The NSC-hNGF-eGFP group performed significantly better in both water maze and open-field testing compared with OA, NSC and media groups. The histopathology showed plaques and NFT in the cortex of OA-treated rats. Expression of hNGF was confirmed in various brain regions, along with the integration of transplanted NSC-hNGF-eGFP cell into surrounding host tissue. The authors deemed this AD model successful, in that OA infusion via ALZET pumps produced key features of the disease. In addition, the rAAV2 vector effectively inserted hNGF into the NSC. These transgenic cells integrated into the host tissue, where they secreted the neurotrophic factor, restoring certain functions of learning and memory.

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Neurogenesis Mechanisms Key to New Therapies

Abdipranoto *et al.* assert that the key to developing new therapies for neurodegenerative diseases lies in harnessing the neurogenesis which normally occurs in certain regions of the adult brain.[2] In fact, they argue that the clinical success of some current therapies may relate not only to their primary effects, such as alteration of synapse chemistry by SSRIs, but also to a stimulatory effect on neurogenesis. Abdipranoto-Cowley *et al.* sought to tease out the mechanisms of neurogenesis in a mouse model of neurodegenerative disease established by ICV injection of the excitotoxin, kainic acid (KA).[3] Neurogenesis is known to increase following acute excitotoxic injury, and the authors hypothesized a regulatory role in this process for the transforming growth factor- β (TGF- β) superfamily. An initial group received KA injections and ALZET pumps with vehicle, then was sacrificed shortly thereafter for evaluation of brain changes due excitotoxic insult. The authors observed an increase in mRNA for activin A. Activin A is a member of the TGF- β superfamily which is known to direct embryonic neurodevelopment, and is increased in models of acute and chronic brain damage.

The experimental group received KA injections, then ALZET pumps delivering either activin A or follistatin-288 (FS-288), a high-affinity activin A antagonist, for 3 days via ICV directed ALZET Brain Infusion Kits. During the infusion, bromodeoxyuridine (BrdU) was administered to quantify cell proliferation. Some animals also received indomethacin from the time of pump implantation through sacrifice. Brain tissues were harvested at 7 or 42 days for analysis.

The authors observed that not only did neurons express activin A after excitotoxic insult, but also that this expression was required for NSC proliferation and therefore neurogenesis. Activin A appeared to exert a powerful anti-inflammatory effect by suppressing microglia, gliosis and release of pro-inflammatory cytokines. FS-288 blocked neurogenesis after neurodegenerative injury, an effect reversed by NSAID administration.

Neurogenesis is a complex process that is not yet well understood. Using ALZET pumps, the authors elucidated a role for an anti-inflammatory agent, whether endogenous or exogenous, in restoring neuronal proliferation. Future study is required to learn whether this strategy may be effective in chronic neurodegenerative diseases as well.

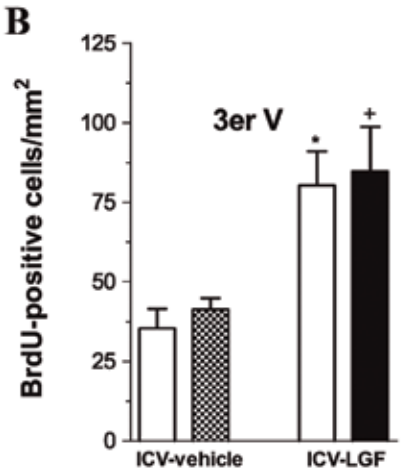

Figure 2. LGF increases BrdU incorporation in 6-OHDA-lesioned rats. ICV infusion of LGF (ICV-LGF) significantly increased the total number of BrdU-positive cells in the SVZ and denervated striatum ipsilateral to the LGF infusion (ICV-LGF, black bars) compared with the contralateral non-infused side (ICV-LGF, white bars) and with both sides of the vehicle group (ICV-vehicle). Note how ICV-vehicle, dark-dotted bars) compared with the contralateral non-infused side (ICV-LGF, white bars). Reproduced, with permission, from Gonzalo-Gobernado et al. J Histochem. Cytochem. 2009;57(5):491-502.

Promote Neurogenesis

Another approach to promoting neurogenesis is to test agents known to induce proliferation in other tissues. Gonzalo-Gobernado *et al.* used ALZET pumps to evaluate the neurogenic potential of liver growth factor (LGF) in a rat model of Parkinson's disease (PD), established by intracerebral injection of 6-OHDA.[4]

LGF is a known liver mitogen which has been shown to ameliorate certain behavioral and histopathological processes of PD when infused by ALZET pump.[5] Gonzalo-Gobernado *et al.* chose Model 2002 ALZET pumps for the 15-day infusion of LGF. ALZET Brain Infusion Kits directed the agent from the subcutaneously implanted pumps into the rat left ventricle. Pumps were implanted 6 weeks after 6-OHDA injection. BrdU was administered concurrently to label new cells.

Immunohistochemical analysis revealed that LGF increased the population of neuronal precursors in the subventricular zone and denervated striatum, as compared with the contralateral side, vehicle-infused animals and the non-infused side of the vehicle controls (Figure 2). In addition, LGF activated microglia and promoted astrogliosis, both of which may contribute to neurogenesis. The authors concluded that "LGF stimulates neurogenesis when infused into the lateral ventricle of 6-OHDA-lesioned rats. Because this factor also promotes the migration of newly generated neurons into the damaged striatum, we propose LGF as a novel factor that may be useful for neuronal replacement in neurodegenerative diseases such as PD."

For a list of references on the use of ALZET pumps in stem cell research, please contact ALZET Technical Support by email at alzet@durect.com.

[1] *Wu et al. Pathobiology 2008;75:186-194*
[2] *Abdipranoto et al. CNS & Neuro Disorders – Drug Targ 2008;7:187-210*
[3] *Abdipranoto-Cowley et al. Stem Cells 2009;27:1330-1346*
[4] *Gonzalo-Gobernado et al. J Histochem. Cytochem.2009;57(5):491-502*
[5] *Reimers et al. J Histochem. Cytochem. 2006;54(4):457-465*

Anti-Aging Effects of Rapamycin

By Laura Whitman and Jose R. Gadea

Rapamycin, a compound first isolated from bacteria from Easter Island, is most commonly known for its antifungal and potent immunosuppressive properties. It is already approved for use in humans for prevention of transplant rejection. New research indicates that rapamycin might also be used to prolong lifespan and prevent age-related diseases. A study published in the journal Nature reported the positive effects of rapamycin on the longevity of genetically heterogeneous mice.[1] Mean lifespan was increased 13% in female mice and 9% in male mice, while life expectancy at 600 days was increased 28% in males and 38% in females. Remarkably, rapamycin administration was initiated at 600 days of age, the equivalent of 60 years in humans. Rapamycin was given to mice in their food and had to be microencapsulated to improve its bioavailability and stability. The following studies demonstrate the use of ALZET pumps as an effective alternative means to deliver rapamacyn to mice to investigate its anti-aging properties.

Direct infusion was preferentially selected both because of its convenience as well as rapamycin's poor ability to cross into the CNS.

Recent research using ALZET pumps suggests that rapamycin's ability to activate the autophagy signaling cascade may account for some of its anti-aging effects. Crews *et al.* from the University of California, San Diego administered rapamycin or vehicle into the lateral ventricle of α-synuclein (α-syn) transgenic (Tg), 9 month old mice using ALZET pumps (Model 1007D).[2] Direct infusion was preferentially selected both because of its convenience as well as rapamycin's poor ability to cross into the CNS. The Tg mice express human wild type α-syn, have increased levels of mTor, develop α-syn-immunoreactive inclusion-like structures in the brain and display neurodegenerative deficits. Accumulation of α-syn has been linked to the pathogenesis of Parkinson's disease and dementia with Lewy bodies. Continuous ICV infusion of rapamycin resulted in reduced accumulation of α-syn in Tg mice and an amelioration of the associated neurodegenerative alterations seen in vehicle-treated or non-Tg controls. These results support the role of the mTor autophagy pathway in neuropathology as well as the potential neurotherapeutic effects of mTor inhibition by rapamycin.

Wang *et al.* used ALZET pumps to study the effects of rapamycin on vascular cell senescence, a cardiovascular disease associated with both aging and body mass index. Mice fed a high-fat diet show increased vascular senescence and dysfunction, and are more prone to peripheral and cerebral ischemia. All of these changes involve activation of the Akt-mTor pathway. Wang *et al.* from Harvard Medical School were able to show that blocking Akt phosphorylation with rapamycin leads to inhibition of vascular senescence in both Tg and obese mice.[3] Wild type, diet-induced obese mice and double Tg (myrAkt1) mice were treated with rapamycin or vehicle by continuous, subcutaneous administration using ALZET pumps. Preliminary rapamycin-infusion studies for 12 hours to 1 week showed both a dose- and time-dependent inhibition of Akt phosphorylation and mTorC2 complex formation. Treatment with rapamycin for 4 weeks reduced cerebral infarct size (post MCAO ischemia), attenuated neurological deficits in mice with Akt activation, and ameliorated obesity-induced vascular senescence. Rapamycin had no effect on vascular function and senescence in Akt1 null mice, further implicating the Akt-mTor pathway as the rapamycin target for reducing vascular senescence and improving vascular function. These studies provide a mechanistic link between diet-induced obesity and cellular senescence, and suggest that inhibition of Akt signaling with rapamycin therapy may have clinical benefits in obesity and age-related vascular disease. Contact ALZET Technical Services for a list of references on rapamycin administration using ALZET pumps.

[1] Harrison et al. Nature 2009;460:392-395
[2] Crews et al. PLoS ONE 2010;5(2):e9313
[3] Wang et al. Sci. Signal. 2009;2:ra11

Release Rates and Durations

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Pump Model	Reservoir Volume	Duration	Release Rate	Order #
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1004	100 µl	4 weeks	0.11 µl/hr	0009922
2001D	200 µl	1 day	8.0 µl/hr	0000294
2001	200 µl	1 week	1.0 µl/hr	0000292
2002	200 µl	2 weeks	0.5 µl/hr	0000296
2004	200 µl	4 weeks	0.25 µl/hr	0000298
2006	200 µl	6 weeks	0.15 µl/hr	0007223
2ML1	2 ml	1 week	10 µl/hr	0000323
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DECEMBER 2010
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New Agents in the ALZET Literature

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Agent	Descr./ Therapeutic Category
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AFL-4	*Murine VEGFR3-specific neutralizing antibody*
Artemin	*Member of the GDNF family*
Bevacizumab	*Anti-VEGF monoclonal antibody*
Bretazenil	*$GABA_A$ receptor partial agonist*
Carboxyfullerene, C3	*Carboxylic acid C60 derivative*
Cathepsin B	*Enzymatic protein*
Cetuximab	*Anti-EGFR monoclonal antibody*
Coenzyme Q10	*Superoxide dismutase mimetic*
CPT	*A1 receptor antagonist*
CXCL12	*Chemokine ligand 12*
EDL-155	*Tetrahydroisoquinoline derivative*
EphrinB3-Fc	*Anti-apoptotic ligand*
ETP-508	*Endothelin A receptor antagonist*
H158	*B1 receptor antagonist*
ISMN	*Isosorbide-5 mononitrate; a.k.a. Imdur*
JSH-23	*Nuclear factor-κ-B inhibitor*
LAP-RGD	*Prepropeptide latency-associated peptides*
Liquiritigenin	*Estrogen receptor β agonist*
M100907	*5-HT_{2A} receptor antagonists*
Metacept 1	*Histone deacetylase inhibitor*
MG132	*Proteasome inhibitor*
MLN-4760	*ACE2 inhibitor*
MR1-1	*Recombinant immunotoxin*
Ng219	*B2 receptor antagonist*
NIBR2130	*CXCR3 inhibitor*
Palmitic acid	*Saturated fatty acid*
PD 168393	*Irreversible erbB inhibitor*
PD149163	*NT1 selective analogue*
PETN	*Pentaerythritol tetranitrate*
PF-3758309	*PAK4 inhibitor*
SC-514	*IkB kinase inhibitor*
SR141716	*CB1 Antagonist*
Synstatins	*Angiogenesis inhibitors*
Taurolidine	*Antimicrobial derived from taurine*
TBOA	*Glutamate transporter blocker*
TTA-I1	*T-typed calcium channel antagonist*
U-75302	*BLT1 receptor antagonist*

References on these and other agents are available as a complementary service. Contact ALZET Technical Services at 800.692.2990, or via e-mail at alzet@durect.com to request a customized list of references in your area of interest.