A L Z A

SPECIAL DELIVERY®

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Gene Therapy: Optimal Delivery with ALZET® Pumps

Gene therapy offers a panoply of potential treatments for conditions as diverse as cystic fibrosis, HIV infection, and certain tumors. The effectiveness of gene therapy is influenced by the selection of the appropriate vector, the immune response to the newly-synthesized protein, and the persistence of the transferred gene. Additionally, delivery options for introducing the gene must be weighed carefully. The optimal delivery system should increase transfection efficiency while minimizing side effects.

Recently, two research groups demonstrated effective gene transfer in animal models using ALZET pumps. The first demonstrated the efficacy of thymidine kinase gene transfer in a brain glioma model, which increased sensitivity to subsequent ganciclovir treatment. The second delivered adeno-associated virus which carried a marker gene to the cochlea of guinea-pigs.

Intracerebral Gene Delivery

Though sometimes localized, brain tumors are notoriously difficult to treat, and may be inoperable due to their proximity to critical brain structures. Gene therapy offers an interesting potential treatment for such tumors.

Zhu *et al*. investigated the antitumor effects of ganciclovir on brain F98 glioma cells.[1] This group introduced herpes simplex virus thymidine kinase (HSV*tk*) genes into brain F98 glioma cells to increase their sensitivity to the antitumor effects of ganciclovir. Initial *in vitro* studies were undertaken to assess the effects of ganciclovir on HSV*tk* transfected F98 cell lines. After HSV*tk* genes were transfected into glioma cells using liposomes, the dose-dependent cytotoxicity of ganciclovir to tumor cells was evaluated. The F98-tk cells exhibited a 1000-fold increase in sensitivity to the cytotoxic effects of ganciclovir when compared with wild-type F98 cells.

This group then investigated whether direct gene transfer using these cationic liposomes *in vivo* would cause glioma tumor regression. Liposomes were chosen over viral vectors as they are noninfectious, nonimmunogenic, and exhibit low toxicity *in vivo*.[2] Syngeneic F98 cells were injected into the caudate nucleus of Fischer rats. Seven days later, DNA-liposome complexes were continuously infused into the brain tumor via a Model 1003D pump attached to an ALZET brain infusion cannula. For comparison,

(Continued on Page 6, Column 1)

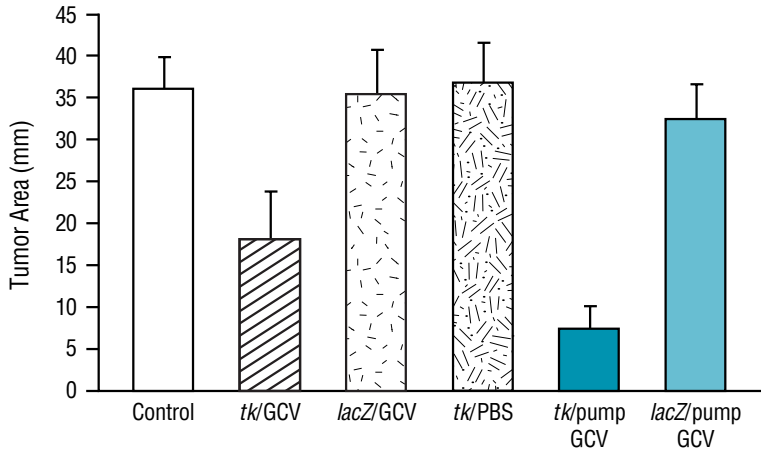


Figure 1. *Cross-sectional tumor size in different experimental groups.* ***Controls*** *received no treatment;* **tk/*GCV*** *animals were given a single injection of the HSV*tk *gene and ganciclovir;* **lacZ/*GCV*** *rats received a single injection of the* lacZ *gene plus ganciclovir; the* **tk/*PBS*** *group was given a single injection of the HSV*tk *gene and saline; the* **tk/*pump GCV*** *group received a continuous infusion of the HSV*tk *gene via ALZET pump along with ganciclovir; the* **lacZ/*pump GCV*** *animals received* lacZ *gene by infusion, plus ganciclovir.*

In This Issue

Very Special Delivery

Angiogenic Treatment with VEGF in an Ischemia Model

Coronary ischemia is the leading cause of human morbidity and mortality. One factor complicating the treatment of ischemia is reperfusion injury after the restoration of normal blood flow. Most treatments for ischemia reduce the oxygen demand on the heart, or increase the supply of oxygen by dilating the coronary arteries. An alternative therapeutic strategy is to restore myocardial blood supply through angiogenesis. One candidate for this approach, vascular endothelial growth factor (VEGF), is thought to be mitogenic for vascular endothelial cells, and has selective angiogenic effects *in vivo*.

A model of growth-factor stimulated angiogenesis was developed by Harada and his group from Beth Israel Hospital in Boston.[1] This group evaluated the effect of locally administered VEGF in stimulating angiogenesis after ischemia. A porcine model was chosen because the existing collateral development in pigs, like humans, is inadequate to prevent infarct after coronary artery occlusion.[2]

To create ischemia, a constrictor was placed around the left circumflex artery. The constrictor gradually occluded the artery, causing increasing ischemia over a three week period. Adjacent to the constrictor, a catheter attached to a Model 2ML4 ALZET pump directed flow to the myocardium near the circumflex artery (Figure 2). The pump and catheter were filled with human recombinant VEGF and heparin in saline. Controls received saline and heparin at a concentration insufficient to cause angiogenesis. The heparin increased the stability of the VEGF, and diminished its adsorption to both tubing and pump. *In vitro* studies showed that even with the addition of heparin, VEGF lost approximately 60% of its activity over 28 days at 37˚C.

After four weeks of VEGF delivery, ultrasonic crystals were sewn on the heart to measure myocardial wall motion. A micromanometer was introduced into the left ventricle to monitor pressure changes at rest and during rapid atrial pacing. Morphological assessment, as measured by left ventricular wall shortening during pacing, indicated that VEGF preserved wall motion. Moreover, treated animals had a smaller proportion of transmural infarcts when compared to controls (3 of 8 vs. 9 of 11). Myocardial function in VEGF-treated animals was improved relative to controls, as measured by fractional left ventricular shortening during pacing.

Animals treated with VEGF had significantly higher myocardial flow in the region of VEGF administration as determined by the relative concentrations of colored microspheres in blood and ventricular tissue ($p < 0.05$). Related analysis of left circumflex artery over total coronary flow indicated that there was significantly better preservation of left circumflex myocardial perfusion in animals treated with growth factor. Since the constrictor slowly decreased the flow through this artery, preservation of flow illustrates that angiogenesis was effective at preserving blood supply. Microscopic analysis showed significant

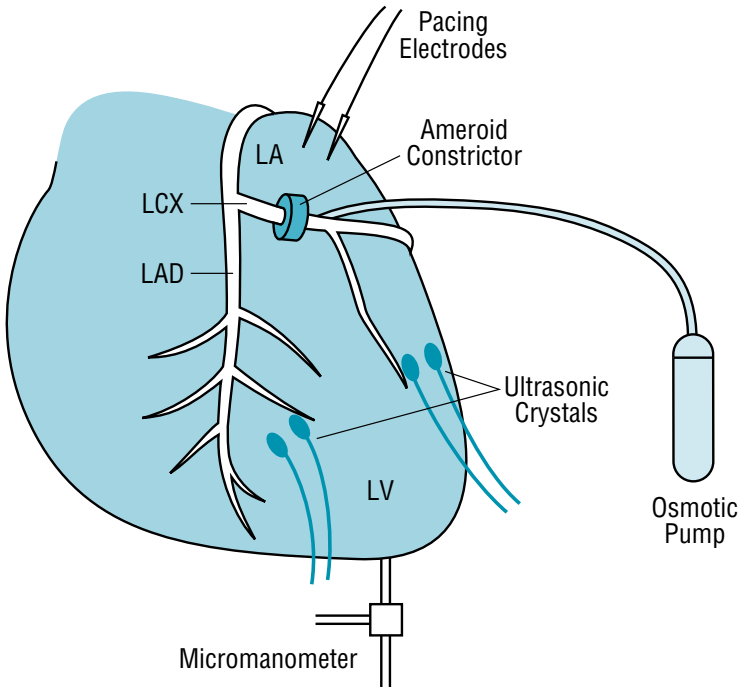


Figure 2.
Instrumentation placement on the porcine heart. An Ameroid constrictor was placed around the proximal left circumflex artery (LCX). A catheter connected to an ALZET pump delivered VEGF adventitially to the LCX myocardial area. After five to six weeks of recovery, a micromanometer inserted through the left ventricular apex was used to measure left ventricular pressure. Ultrasonic crystal pairs were implanted in the areas perfused by the LCX and left anterior descending aorta (LAD). LA refers to left atrium; LV is left ventricle.

neovascularization in the myocardial area infused with growth factor. Significantly lower coronary resistance in the left circumflex region of treated animals resulted in improved myocardial blood flow.

These results argue strongly for the potential value of VEGF after ischemia. By safely restoring blood flow to the ischemic myocardium, angiogenesis offers more than just symptomatic treatment. With new channels for blood flow, the need for surgical intervention and further pharmaceutical treatment may be mitigated.[3]

Harada *et al*. also stated that "...periadventitial delivery allows the use of a much smaller amount of VEGF than has been used with intravascular administration."[1] The dose of VEGF which proved effective at promoting angiogenesis was 450-fold lower than the systemic dosage used in a prior study.

Targeting delivery to a specific locale may beget a range of new research possibilities. Systemic delivery can cause widespread effects which may obscure clear cause and effect relationships. Indeed, Harada *et al*. were able to stimulate angiogenesis locally by directing catheter flow to the ischemic myocardium.

ALZET pumps have been used to target the delivery of a variety of growth factors. More information on these types of studies is available from ALZET Technical Services at (800) 692-2990.

1. Harada K, Friedman M, Lopez JJ, Wang SY, Li J, Prasad PV, Pearlman JD, Edelman ER, Sellke FW, Simons M. Vascular endothelial growth factor administration in chronic myocardial ischemia. *Am J Physiol 270 (Heart Circ Physiol 39)* 1996; H1791-H1802.
2. Sellke FW, Wang SY, Friedman M, Dai HB, Harada K, Lopez JJ, Simons M. β-Adrenergic modulation of the collateral-dependent coronary microcirculation. *J Surg Res* 1995; 59:185-190.
3. Lopez JJ and Simons M. Local extravascular growth factor delivery in myocardial ischemia. *Drug Delivery* 1996; 3:143-147.

Superovulation Techniques for Producing Transgenic Rats and Rabbits

Superovulation can allow researchers to learn more about oocyte maturation, fertilization, and embryo development. Optimally, it yields a large number of viable oocytes with normal developmental potential.

In the past, pregnant mare's serum gonadotropin was used to induce superovulation. While low doses may lead to viable oocytes, high doses can result in ovulatory variation, decreased fertilization rate, and abnormal embryo development and blastocysts. These problems compelled Armstrong and Opavsky to seek other methods for producing large numbers of viable oocytes and embryos in rats.[1]

These authors used continuous infusion to enhance the superovulatory activity of pituitary follicle stimulating hormone (FSH). When FSH was given by single or multiple injections, 1-2 out of 8 rats ovulated. When the same total dose was given via continuous infusion with an ALZET pump, superovulation occurred in all rats. A mean of 67 +/- 10 oocytes were recovered per rat.

Due to its short biological half-life, FSH is ineffective when administered as a single injection, and only marginally effective when injected twice daily. It is presumed that plasma levels must be maintained at an elevated level over a long enough period to enable recruitment of extra follicles.

Using these same techniques, Mullins *et al*. and Hammer *et al*. induced superovulation in rat and rabbit embryo donors.[2,3] ALZET osmotic pumps dispensed 1 IU/day of purified FSH for 60 hours. Two days after pump implantation, luteinizing hormone or human chorionic gonadotropin were injected intraperitoneally. Each donor yielded approximately 65 fertilized ova. Of these eggs, 80% were normal as judged by their ability to form blastocysts and morulae. The same research groups injected the animals with pregnant mare's serum gonadotropin and human chorionic gonadotropin, but found that the infused FSH yielded significantly better results. The authors state that "...it is likely that minipumps dispensing FSH will become the method of choice for supplying embryos for the generation of transgenic rats."[4]

1. Armstrong DT and Opavsky MA. Superovulation of immature rats by continuous infusion of follicle-stimulating hormone. *Biol Reprod* 1988; 39:511-518.
2. Mullins JJ, Peters J, Ganten D. Fulminant hypertension in transgenic rats harboring the mouse *Ren-2* gene. *Nature* 1990; 344:541-544.
3. Hammer RE, Malka SD, Richardson JA, Tang J-P, Taurog JD. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human β_2m: An animal model of HLA-B27-associated human disorders. *Cell* 1990; 63:1099-1112.
4. Robl JJ and Heideman JK, 1994. Production of transgenic rats and rabbits. In Pinkert CA (ed) *Transgenic Animal Technology: A Laboratory Handbook*. Academic Press, San Diego, CA, pp. 265-277.

In Situ Neuronal Cell Modulation

Neurodegenerative disorders are characterized by the irreversible loss of neurons in specific regions in the brain. Current treatments are little more than symptomatic, and do not retard or reverse neuronal loss. Growth factors hold therapeutic promise as they have the potential to stimulate the proliferation and differentiation of neurons and glia from neural precursor cells.

Recent *in vitro* studies suggest that the adult brain contains multipotential neural stem cells which proliferate indefinitely when exposed to epidermal growth factor (EGF). With EGF exposure, these cells demonstrate proliferation, the generation of differentiated progeny, and self-renewal. The source of these cells is the subependymal layer which lines the forebrain lateral ventricles.

To determine whether exogenous growth factors would affect the subependymal neural precursor cell population *in vivo*, Craig *et al*. from the University of Toronto infused growth factors into the mouse lateral ventricle.[1] Initially, a replication-deficient retrovirus containing a reporter gene was injected into the ventricles to label subependymal cells. One day later, Model 1007D ALZET pumps were connected to a 30 gauge cannulae implanted in the same location where the retrovirus was injected. EGF, basic fibroblast growth factor (FGF), nerve growth factor (NGF), and transforming growth factor-α (TGF-α) were infused for 6 days. Control animals received saline and mouse serum albumin.

The infusion of EGF or TGF-α into the mouse ventricle resulted in respective 18- and 14-fold increases in the total number of retrovirally labeled cells, when compared to vehicle controls. FGF resulted in a 2.4-fold increase in cell number, while NGF had little effect. The addition of FGF to EGF did not increase the number of labeled cells appreciably (Figure 3, page 5).

The retrovirally labeled cell populations migrated from the lateral ventricle into the normal brain parenchyma. One day after retrovirus injection, the labeled cells were within 50 µm of the lateral ventricle wall. After 6 days of EGF infusion, the labeled cells had migrated up to 400 µm away from the ventricle wall.

The results of these experiments indicate that EGF infusion into the adult mouse brain culminates in a dramatic increase in subependymal neural precursor cell populations. These cells migrated into the parenchyma, where they can differentiate into new CNS neurons and glia after growth factor withdrawal.

Whether the neurons which arise from adult neural precursor cells in the subependyma can be directed specifically to neuron-deficient CNS regions, and whether they will be able to make synaptic connections that significantly improve function, remains unclear. The work done by Craig *et al*. demonstrates that *in situ* growth factor modulation of endogenous CNS precursor cells may provide insight into how to replace neurons and glia lost to disease or trauma in the adult.

For more information on growth factor administration or work done in the mouse CNS, please contact ALZET Technical Services at (800) 692-2990. You may also return the attached response card.

1. Craig CG, Tropepe V, Morshead CM, Reynolds BA, Weiss S, van der Kooy D. *In vivo* growth factor expansion of endogenous subependymal neural precursor cell populations in the adult mouse brain. *J Neurosci* 1996; 16(8):2649-2658.

Intracerebroventricular Infusion in the Mouse

New strains of transgenic, selectively bred, and knockout mice provide genetically characterized disease models with well defined defects. For example, there is increased interest in using these animals as models for neurodegenerative diseases. In such research, direct infusion into the brain bypasses the blood-brain barrier, and can provide critically important information about neurological diseases. Unfortunately, there is a dearth of information about intracerebral infusion methodology in mice.

Four recent papers document the continuous infusion of growth factors or other peptides into the mouse lateral ventricles. Table 1 (page 5) summarizes the objective of each study, including the methodology and stereotaxic coordinates used.

Proper cranial coordinates for cannula implantation are essential. A new mouse brain atlas by Franklin and Paxinos has recently been published[1], while two older atlases have been cited with some frequency.[2,3]

Infusing agents into the mouse CNS can facilitate exciting new research opportunities. The low flow rate and small size of the new Model 1002 pump make it ideal for intracerebral delivery in mice. For more information about intraventricular infusion in mice or rats, or about the Model 1002 pump, please call the ALZET Technical Information line at (800) 692-2990.

1. Franklin BJK and Paxinos G, 1997. *The mouse brain in stereotaxic coordinates.* Academic Press, San Diego, CA.
2. Sidman RL, Angevine JB, Taber PE, 1971. *Atlas of the mouse brain and spinal cord.* Harvard University Press, Cambridge, MA.
3. Slotnick BM and Leonard CM, 1975. *A stereotaxic atlas of the albino mouse forebrain.* Rockville, Maryland: Alcohol, Drug Abuse, and Mental Health Administration.

Special thanks to David Kopf Instruments, Tujunga, California, for atlas information.

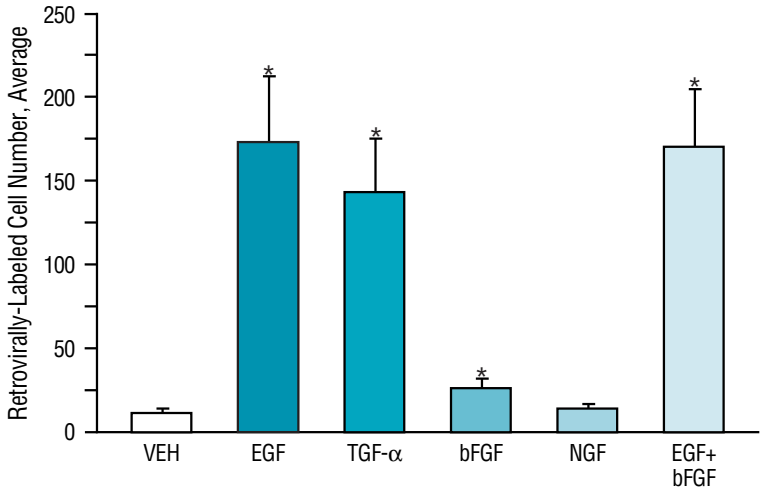


Figure 3.
Growth factor modulation of retrovirally labeled subependymal cells. The number of labeled cells after vehicle control (VEH) or growth factor treatments is shown. Bars marked with an asterisk differed from controls significantly ($p < 0.05$). EGF is epidermal growth factor, TGF-α is transforming growth factor-α, bFGF is basic fibroblast growth factor, and NGF is nerve growth factor.

Intraventricular Infusion of Peptides in Mice

Table 1

RESEARCH GROUP	NATURE OF STUDY	TARGET	COORDINATES	AGENTS INFUSED	PUMP MODEL
Craig CG, Tropepe V, Morshead CM, Reynolds BA, Weiss S, van der Kooy D. *J Neurosci* 1996; 16(8): 2649-2658.	Modulation of subependymal neural precursor cells	Lateral ventricle	Anteroposterior: +4.2 mm anterior to lambda Lateral: +0.7 mm Dorsoventral: -2.4 mm below dura	EGF FGF NGF TGF	1007D
Campbell AD and Erwin VG. *Peptides* 1995; 16(3):501-504.	Neurotensin receptor density changes following chronic neurotensin infusion	Lateral ventricle	1 mm lateral to bregma 3 mm below skull surface	Neurotensin	1003D 2001
Chadi G, Møller A, Rosèn L, Janson AM, Agnati LA, Goldstein M, Ögren S-O, Pettersson RF, Fuxe K. *Exp Brain Res* 1993; 97:145-158.	Protection of nigrostriatal dopamine neurons after MPTP treatment	Left lateral ventricle	Bregma: -0.4 mm Lateral: -0.4 mm Ventral: -1.7 mm	bFGF	1007D
Hadjiconstantinou M, Fitkin JG, Dalia A, Neff NH. *J Neurochem* 1991; 57:479-482.	Role of EGF as a neurotrophic factor for dopaminergic neurons	Right lateral ventricle	1 mm lateral to sagittal suture 1 mm caudal to bregma 2.5 mm below skull surface	EGF	2002

Gene Therapy *(Cont. from Page 1, Column 3)*

DNA-liposome complexes were also administered by intratumoral injection. Three days after DNA administration, animals received intraperitoneal injections of 100 mg/kg ganciclovir twice daily. Twenty-four days after tumor inoculation, rats were sacrificed, and tumor size was assessed.

Not only were tumors significantly smaller in the continuously-infused group, but tumor remission was complete in 4 out of 11 rats (36.4%). Figure 1 (page 1) shows differences in tumor size after HSV*tk* DNA infusion and injection followed by ganciclovir treatment. Ganciclovir was only effective when HSV*tk* DNA was administered, as control animals transfected with *lacZ* DNA showed little change in tumor size after ganciclovir treatment.

While a single injection of the DNA-liposome complex showed effect, significantly greater decreases in tumor size were seen in animals which received the HSV*tk* gene via ALZET pumps. There were no major histological abnormalities in animals which received continuous infusion, even though large doses were given.

The DNA-liposome mixture retained 90-98% of its transfection activity after 72 hours at 37°C. The authors concluded that "...this continuous delivery system could be a powerful method for the introduction of genetic materials for *in vivo* gene therapy."[1]

Intracochlear Infusion

To develop potential treatments for hearing impairment, Lalwani *et al*. delivered DNA directly into the peripheral auditory system of guinea-pigs.[3] Adeno-associated virus (AAV) was chosen as the transfection vector because it is nonpathogenic in animals and humans, and has a broad host range. It integrates into the genome of non-dividing cells with high frequency and stability. Animals were implanted with 1007D pumps filled with phosphate-buffered saline containing 10^6 viral particles per milliliter of AAV which included bacterial β-galactosidase (β-gal) gene as a marker. The pumps were connected to a catheter, which was introduced into the perilymphatic space of the cochlea 1 mm inferior to the round window. Controls received intracochlear saline infusion or no surgical intervention.

Cochlear sections were assayed via immunohistochemistry for β-gal expression. Animals which received AAV showed β-gal expression in nearly all cochlear tissue types, which indicated successful transfection. Much weaker staining was found in the contralateral ear, which may be indicative of migration via the cerebrospinal fluid. Both control groups were devoid of stain.

The tissues within and around the perfused cochlea were intact and free of inflammation following surgery and infusion. When comparing the advantages of injection and infusion, the authors stated that when compared to a single injection, "a slow infusion method for the delivery of virus . . . not only minimized trauma, but also increased the likelihood of the virus being available to sites distal to the infusion port within the cochlea."[3] They stated further that slow infusion did not disturb the cochlear fluid balance.

Intracochlear infusion of the AAV containing β-gal resulted in transfection and expression of the marker gene in a variety of cochlear tissues. The ability to transfect auditory hair and neuronal cells offers a potential avenue for the treatment of hearing loss with a variety of trophic factors. The work of Lalwani *et al*. suggests that gene delivery by constant infusion is superior to administration by injection.

Optimal Delivery

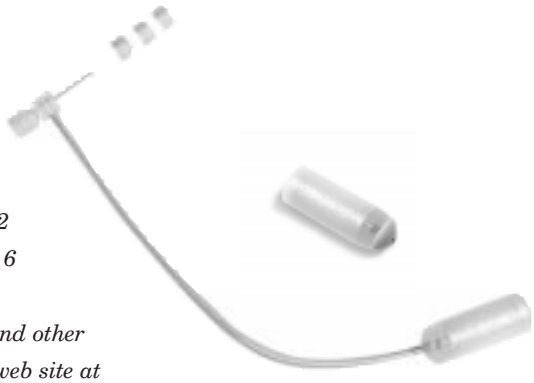
Gene therapy researchers face many hurdles, one of which is selecting the appropriate delivery mode to ensure optimal results. Both Lalwani *et al*. and Zhu *et al*. found that continuous infusion provided the slow delivery necessary for successful gene transfer in animal models. In addition, the ability to target gene delivery to a discrete area minimized side effects, and improved transfection efficiency.

ALZET pumps offer researchers both continuous infusion and targeted delivery capabilities. For other references on gene therapy or targeted delivery, please contact ALZET Technical Services at (800) 692-2990.

1. Zhu J, Zhang L, Hanisch UK, Felgner PL, Reszka R. A continuous intracerebral gene delivery system for *in vivo* liposome-mediated gene therapy. *Gene Therapy* 1996; 3:472-476.
2. The liposome mixture was a monovalent cationic formulation consisting of DMRIE, or 1,2-dimyristyloxypropyl-3-dimethylhydroxyethyl-ammonium bromide, and DOPE, which is dioleoylphosphatidyl ethanolamine.
3. Lalwani AK, Walsh BJ, Reilly PG, Muzyczka N, Mhatre AN. Development of *in vivo* gene therapy for hearing disorders: introduction of adeno-associated virus into the cochlea of the guinea pig. *Gene Therapy* 1996; 3:588-592.

New Model 1002 14-Day Pump Available

The Model 1002, which pumps at 0.25 microliters per hour for 14 days, was designed to provide long-term infusion in animals that weigh as little as 10 grams. The Model 1002 is a mere 15 millimeters long and 6 millimeters in diameter.



For more information on this and other ALZET osmotic pumps, visit our web site at www.alza.com/alzet. Technical inquiries may also be forwarded to (800) 692-2990, or e-mailed to alzet@alza.com.

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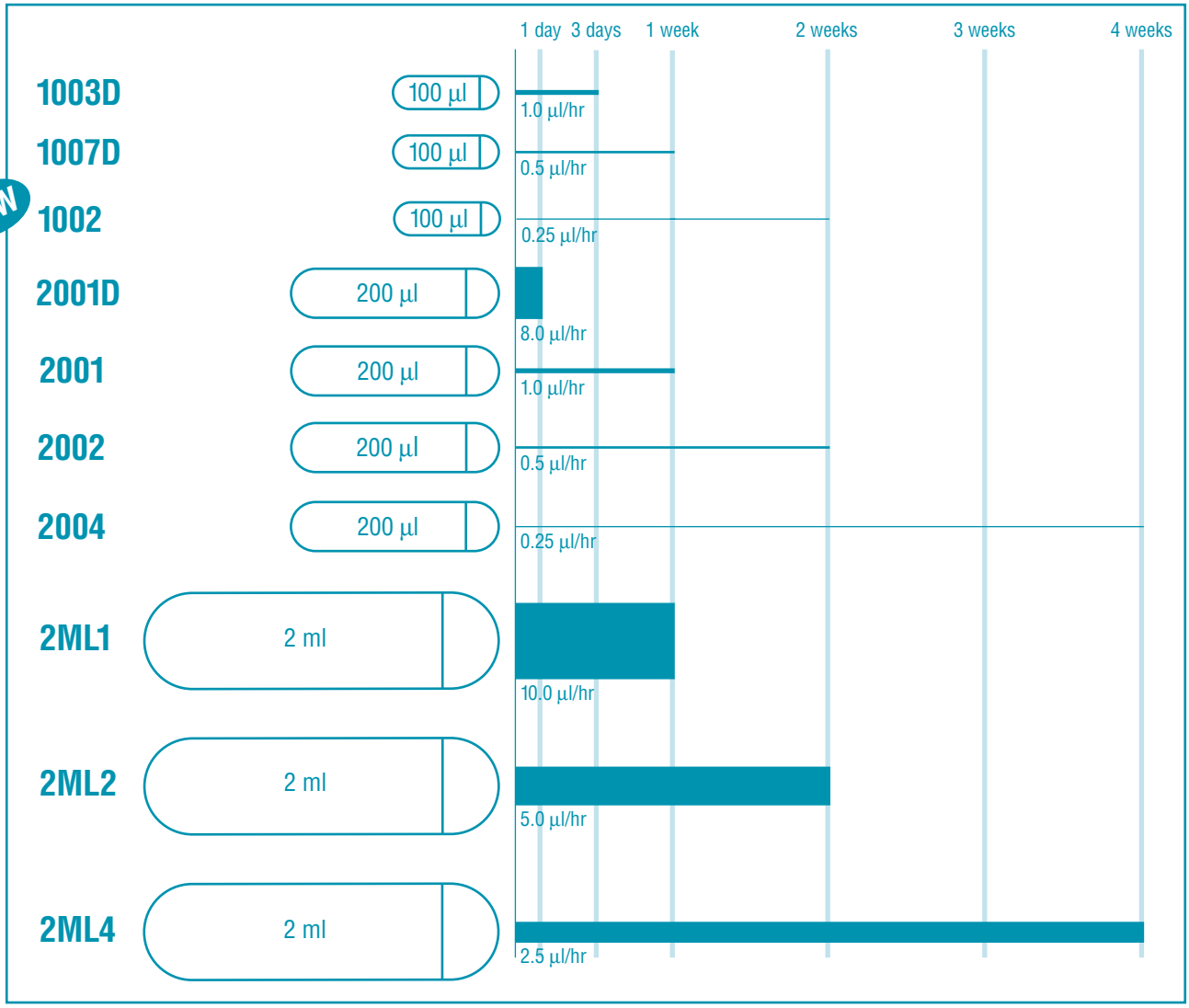

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New Agents in the ALZET Literature

With more than 5,000 references from the scientific literature, the ALZET pump bibliography is a valuable source of information on the controlled delivery of a wide variety of agents. Updates are frequent, and the most recent includes references on the delivery of:

Agent	Therapeutic Category
Adeno-Associated Virus	Gene Transfection Vector
Allylglycine	GABA Synthesis Inhibitor
Aminoguanidine	Nitric Oxide Synthase Inhibitor
Argatroban	Thrombin Inhibitor
BE-3333	Anti-Tumor Agent
CGS-26303	Endothelin-Converting Enzyme Inhibitor
Fluvastatin	HMG-CoA Reductase Inhibitor
Follistatin	Activin Binding Protein
HP5b	Hemoregulatory Peptide
Interleukin-13	Cytokine
Levonorgestrel	Progestin
Methoxyacetic Acid, 2-	Immunosuppressant
Mevalonate	Cholesterol Precursor
Mirtazapine	α_2-Adrenoceptor Antagonist
Nafadotride	Dopamine D_3 Antagonist
Naltriben	δ_2-Opioid Receptor Antagonist
Nigericin	Ionophore
Nilvadipine	Ca^{2+} Channel Blocker
Nitric Oxide Solution, Saturated	Vasodilator
Nitroindazole, 7-	Neuronal Nitric Oxide Synthase Inhibitor
Pancuronium	Nicotinic Receptor Antagonist
Plasminogen Activator Inhibitor-1	Plasminogen Activator System Antagonist
Renin	Proteinase
Tiagabine	Antiepileptic

References on these and other agents are available to you through ALZET Technical Services at (800) 692-2990. A bibliography search customized to your research interests can be mailed or faxed to you at no charge (please see the attached reply card).

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- ❑ A videotape of surgical implantation techniques (VIDEO)
- ❑ Model 1002 fact sheet
- ❑ Drug discovery brochure

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- ❑ Gene Therapy Research (GENE)
- ❑ Growth Factors (GF)
- ❑ Mouse ICV delivery (MCNS)
- ❑ Neurotrophic Factor Infusion (NEUR)
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