

Enhanced immunotoxin therapy by continuous infusion of BB-3644.

Promising new antileukemia treatment: Continuous infusion of TT-232

Continuous infusion optimizes triple drug therapy against colon cancer.

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Special Delivery

► Cancer Focus

Cancer Research encompasses diverse aims, which extend beyond assessing the carcinogenic or anticarcinogenic potential of compounds. Researchers need reliable tools to study, for example, the mechanisms of tumor cell origination, the relationship between tumor growth and neovascularization, and to develop relevant *in vivo* models.

ALZET® Osmotic Pumps provide researchers with a dependable method for controlled, continuous dosing *in vivo*. For nearly 30 years, these pumps have been the recognized standard for infusion in unrestrained laboratory animals, including nude and SCID mice. When implanted subcutaneously or intraperitoneally, these miniature infusion pumps can sustain compound concentrations at predictable levels over prolonged periods of time. They can easily be connected to a catheter for delivery of agents directly into a specific target tissue, such as a tumor. ALZET pumps require no external connections or researcher intervention during the infusion period. Described below are select applications of ALZET pumps in cancer research, drawn from more than 9,500 published studies documenting the value of ALZET pumps in research. Please contact us if you would like references on any of the described applications, or a custom reference list relevant to your research area.



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Benefits of ALZET Pumps in Cancer Research Studies:

- The only implantable pump available for use in mice and young rats
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- Convenient and cost-effective for the chronic treatment of laboratory animals
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- Reproducible, consistent results
- No peak-and-trough fluctuations
- Automatic nighttime and weekend dosing
- Reduced handling and lower stress in laboratory animals



Use of ALZET pumps for successful implementation of *in vivo* imaging technologies.

Real-time *in vivo* imaging of small laboratory animals is now feasible through the novel use of luciferase reporters and bioluminescence imaging (BLI) technologies, such as the IVIS imaging system¹. This technology allows real-time monitoring of ongoing biological processes in living animals. BLI is based on the detection of visible light produced during luciferase-mediated oxidation of the molecular substrate, luciferin, when the enzyme is expressed *in vivo* as a molecular reporter. The luciferase reaction is highly dependent on substrate availability. In fact, studies show that if "exogenously administered luciferin is not abundantly present, light emission might not be a true representation of luciferase activity."² This constraint can be eliminated with the use of ALZET pumps, which facilitate steady-state delivery of bioluminescent substrates for extended periods of time.

A study by Gross *et al.*, published in Nature Methods, explored the benefits of continuous administration of D-luciferin via ALZET pumps to enable real-time imaging of I κ B kinase (IKK) inhibition in tumor xenografts.³ The study also looked at the pharmacodynamic properties of the drug candidate PS-1145, a selective IKK inhibitor. Nude mice

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bearing tumors expressing the $\text{I}\kappa\text{B}\alpha$ -firefly luciferase fusion reporter ($\text{I}\kappa\text{B}\alpha$ -Luc), or unfused luciferase, were subcutaneously implanted with 7-day ALZET pumps containing D-luciferin. Animals were also treated with increasing doses of PS-1145, and imaged with the IVIS imaging system at various time points before and after treatment. The authors found that PS-1145 induced a time-dependent increase in tumor bioluminescence that peaked 8-12 hours after drug administration, followed by a gradual

BLI was performed every 4 hours using the IVIS imaging system.

Animals in the injection protocol required D-luciferin injections every 4 hours for 44 hours, and were subjected to prolonged anesthesia during injection and imaging procedures. In contrast, rats implanted with pumps were anesthetized only briefly every 4 hours for imaging. Imaging results demonstrated rhythmic *Period1* activity in all olfactory bulbs of

“...the implanted pump allowed continuous real-time molecular imaging of reporter activity throughout the time course of a multi-day experiment...”

decrease over 32 hours to levels of vehicle treated mice.

The reporter provided continuous, noninvasive monitoring of target-specific IKK activation in real-time. This experimental approach allowed the authors to perform a complete time- and dose-dependent pharmacodynamic analysis of the IKK inhibitor using less than 30 animals. Gross *et al.* also concluded that “the utility of the reporter was further enhanced through innovative use of an implanted micro-osmotic pump for persistent and constant delivery of the bioluminescent substrate D-luciferin. . . . By eliminating constraints of intraperitoneal bolus reinjections of substrate, the implanted pump allowed continuous real-time molecular imaging of reporter activity throughout the time course of a multi-day experiment, while simultaneously allowing rapid analysis of drug action.”³

A separate study by Abraham *et al.* also describes the use of ALZET pumps for administration of bioluminescence substrates. The study published in the *Journal of Neuroscience* describes a unique, *in vivo* method to assess circadian rhythms in the olfactory bulb using BLI. This was possible with the use of the *Period1*-luciferase reporter, which was expressed in both intact and suprachiasmatic nuclei-lesioned (SCNX) rats used in the study. For substrate administration, one group of transgenic rats received D-luciferin via intracranial injection; another group was implanted with ALZET pumps (model 2ML1) delivering D-luciferin intraperitoneally.

pump-implanted rats, compared to only 70% in the luciferin-injected rats, indicating that “the pump-implantation procedure yielded a higher success rate.”³ The authors found that a circadian pattern of *Period1* driven activity was present in intact and SCNX rats, providing the first direct evidence of SCN-independent rhythms in the brain.

These studies show that BLI is a powerful tool for studying and monitoring *in vivo* biological processes, including tumor growth. The technique is non-invasive, simple to execute, and can help reduce the number of animals used for experimentation since data can be acquired from the same animal over time. Of equal importance is the appropriate method for substrate delivery. ALZET pumps proved to be an effective method for delivery of luciferin. They provide reliable and prolonged substrate delivery, thus eliminating complications of repetitive injections, and ensuring accurate detection of *in vivo* bioluminescence. **For additional information on the use of ALZET pumps for delivery of substrates, including luciferin, please contact ALZET Technical Services.**

1. Xenogen Corporation, a Caliper Life Sciences Inc. company, Alameda CA.
2. Sadikot, RT and Blackwell, TS. *Proc Am Thor Soc* 2005;2:537-540
3. Gross, S and Pivnicka-Worms, D. *Nat Methods* 2005;2(8):607-614
4. Abraham *et al.* *J. Neurosci.* 2005;25(38):8620-8626.

Enhanced immunotoxin therapy by continuous infusion of BB-3644

Hodgkin lymphoma cells (H-RS cells) are characterized by overexpression of the CD30 antigen, a lymphocyte activation marker. This antigen is a good target for immunotherapy as it is overexpressed in various malignant cell types, but not on normal cells. Immunotoxins constructed using anti-CD30 monoclonal antibodies (mAbs) linked to a recombinant toxin have shown antitumor activity against H-RS cells in mouse models and human clinical trials. Unfortunately, these mAbs have also been found to stimulate metalloproteinase-dependent shedding of soluble CD30 (sCD30) from H-RS cells *in vivo*, thereby hampering their anti-tumor activity.

Matthey *et al.* investigated the use of BB-3644, a metalloprotease inhibitor, to block the release of sCD30 and increase the efficacy of anti-CD30 immunotoxin. SCID mice were challenged with human Hodgkin's lymphoma cells; then, they were treated with a single injection of Ki-3(scFV)-ETA', a single chain immunotoxin against the CD30 antigen, with or without BB-3644. In follow-up experiments, BB-3644 was administered by subcutaneous continuous infusion via ALZET osmotic pumps (model 2001) for 8 days.

No therapeutic effect was observed in mice treated with vehicle alone, BB-3644 alone, immunotoxin alone, or even a 10-fold higher dose of the immunotoxin. The mean survival time (MST) in these animal groups was 35 days. In contrast, combined treatment with a single dose of immunotoxin and BB-3644 significantly increased the MST of animals from 35 to 92.5 days. More impressively, continuous administration of BB-3644 via ALZET pumps further enhanced the antitumor activity of the immunotoxin to more than 200 days of tumor-free survival. Matthey *et al.* reported that “the use of ALZET pumps allowed effective systemic levels of BB-3644 to be maintained for approximately 8 days”. A separate study confirmed that antigen-shedding inhibition by BB-3644 was responsible for survival in the animals. sCD30 antigen serum levels were significantly reduced from 79,000 to below 1,000 U/ml in animals implanted with ALZET pumps delivering BB-3644 for 7 days compared to vehicle controls. **Contact ALZET Technical Services to request a list of references on the use of ALZET pumps for administration of MMP inhibitors, or immunotoxins.**

Matthey *et al.* *Int. J. Cancer* 2004;111:568-574

Applications of ALZET Pumps in Cancer Research

Use in xenograft models:

ALZET pumps have been used in numerous *in vivo* cancer models to study the antiproliferative effects of agents and dosing schedules. Because the pumps are self-contained and require no handling during the infusion period, they are well suited for use in murine xenograft models in immunocompromised species such as SCID and nude rodents. They have been useful in the study of carcinomas of the breast, prostate, liver, skin, stomach and lung (small cell); sarcomas such as fibrosarcoma, Leydig cell, osteosarcoma; lymphomas including Burkitt's, EBV-related and EL4; and leukemias including myeloid and juvenile myelomonocytic, among others.

Lower toxicity by infusion:

Many chemotherapeutic agents have a relatively narrow therapeutic index, defined as a small gap between the toxic and therapeutic doses. Dosing by continuous infusion can serve to maintain plasma levels in the therapeutic range, minimizing adverse effects and allowing the desirable effects to develop fully and reproducibly.

Measurement of cell proliferation:

ALZET pumps have been used effectively to measure *in vivo* cell proliferation by continuous labeling with the base analog, 5-bromo-2'-deoxyuridine (BrdU). The pumps provide a continuous dose of the labeling agent over prolonged periods of time, which is essential for accurate measurement of slowly proliferating tissues, such as tumors. Because labeling occurs around-the-clock, results yield a true summation of the proliferative response for the entire labeling period. Continuous BrdU labeling using ALZET pumps has proven to be a more sensitive, reliable and convenient method for measuring chemically-induced cell proliferation.

Intratumoral delivery:

ALZET pumps can easily be connected to a catheter to enable delivery of anti-cancer agents directly into a tumor site. Regional tumor therapy is more efficient at reaching significant therapeutic effects with lower drug doses. Furthermore, drug levels in systemic circulation are decreased and drug exposure in sensitive organs is minimized, thus reducing potential side effects.



“The infusion of TT-232 by ALZET osmotic minipump resulted in 70-80% tumor growth inhibition and 20% tumor free survival.”



Promising new antileukemia treatment: ***Continuous infusion of TT-232***

TT-232, a novel somatostatin analog, activates somatostatin receptors (SSTR), which results in irreversible cell cycle arrest and apoptosis. In a recent study by Tejada *et al.*, TT-232 induced apoptosis in a dose- and administration mode-dependent manner. After implantation of either P-388 lymphoid or HL-60 human tumor cells, mice were treated with various TT-232 doses by either traditional injections for 14 days or continuous infusion via ALZET[®] Osmotic Pumps (model 2002) for 14-28 days.

The *in vivo* efficacy of TT-232 was significantly dependent upon the mode of administration.

While dose-dependent tumor inhibition was evident in each delivery method, constant infusion resulted in the most efficacious response in comparison to daily injections. In the P-388 tumor model, “the infusion of TT-232 by ALZET osmotic minipump resulted in 70-80% tumor growth inhibition and 20% tumor free survival”, whereas injections resulted in a modest 26-44% tumor growth inhibition with no impact on the survival of either tumor model.

The present study showed that high-dose infusion of TT-232 with ALZET pumps resulted in significant tumor growth inhibition. In addition, Tejada *et al.* reasoned that ALZET pumps would be preferable compared to injections since “serial injections represent significant stress to the animals.” Tejada *et al.* concluded, “TT-232 is a potent inhibitor of leukemia tumor *in vitro* and *in vivo* and [we] suggest infusion treatment as a beneficial application in clinical practice.”

Tejada et al. Anticancer Res 2005;25:325-330

Continuous infusion optimizes triple drug therapy against colon cancer.

Somatostatin analogs have been found to induce apoptosis and reduce proliferation of colon carcinoma cells in animal studies. Furthermore, triple therapy with octreotide (a somatostatin analog), galanin and serotonin has shown promise as a potential treatment for colon cancer, more so than single or double therapy.^{1,2} A study by El-Salhy demonstrated that the effectiveness of this triple drug therapy is also dependent on the mode and route of administration.¹ Nude mice were subcutaneously implanted with a human colon cancer xenograft.

Seven days later, mice were treated with octreotide, galanin, and serotonin, either via daily bolus injections or continuous infusion with ALZET pumps (model 1002). Drug treatments were maintained for 14 days and were given via the subcutaneous (SC) or intraperitoneal (IP) route.

Regardless of the route of administration, triple drug therapy generally induced apoptosis and reduced the tumor's volume, weight, proliferation index, vascularization, and number of viable cells. However, continuous IP infusion via ALZET pumps was the most effective treatment, decreasing tumor weight and volume by 70% compared to vehicle controls. The IP injection treatment only decreased tumor weight and volume by about 20% compared to vehicle controls. The IP infusion regimen also reduced the incidence of metastasis to the liver and lymph nodes.³ Triple drug therapy proved to be well

tolerated, as indicated by stable weights for both test and control animals throughout the study.

These agents, like most neuroendocrine gut peptides, have a short half-life. Therefore, El-Salhy attributed the success of this treatment to the low, but prolonged drug concentrations offered by the continuous administration method. On the contrary, intermittent injections provide high drug concentrations, but only during brief periods. Other studies have also optimized the therapeutic effects of anticancer compounds using ALZET pumps. For additional information, please contact ALZET Technical Services.

1. M. El-Salhy. *Histol Histopathol* 2005;20:19-25.
2. M. El-Salhy. *Oncol Rep* 2005;13(1):45-49
3. M. El-Salhy and V. Dennerqvist. *Oncol Rep* 2004; 11:1177-1182



ALZET Catheters

Medical grade polyethylene and vinyl catheters are available for multiple targeted delivery applications. Also available are a variety of specialized catheters, customized for a specific target and animal species. These catheters incorporate useful features, such as retention beads or suture patches to facilitate placement and stabilization in a vessel or tissue. For added convenience, they are available sterile and individually packaged. The following catheter types are available:

- Mouse and rat jugular catheters
- Rat intrathecal catheters
- Rat femoral catheters

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Recently Infused Agents

Continuous administration is a popular modality for drug administration in cancer studies. The following list represents new agents that have recently been infused via ALZET pumps:

Agent	Descr./ Therapeutic Category	Reference #
4F-benzoyl-TE14003	T140 analog	P6186
4F-benzoyl-TE14011	CXCR4 antagonist	P6771
ABT-510	Thrombospondin-1 mimetic peptide inhibitor	P7233
AM22-52	Adrenomedullin antagonist	P6799
Batimastat	Collagenase inhibitor	P6647
BB-3644	a.k.a. Solimastat; MMP inhibitor	P7054
Boronated porphyrin		P7283
DMXAA	a.k.a. 5,6-dimethylxanthenone-4-Acetic Acid	P6499
Epha2-Fc & Epha3-Fc soluble receptor	Eph A receptors	P6609
KT5823	Protein kinase G inhibitor	P6277
L-744832	Farnesyltransferase inhibitor	P7022
L-788123	Farnesyltransferase inhibitor	P7022
Luciferin	Bioluminescence substrate	P7435
ML-7	Myosin light chain kinase inhibitor	P7380
PNC-28	p-53 derived peptide	P7694
SJG-136	a.k.a. NSC-69450; DNA cross-linking agent	P7052
Suramin	Protein kinase C inhibitor	P6407
Thrombospondin-1	Glycoprotein G; Antiangiogenic	P6187
Troxacitabine	a.k.a. Troxatyl BCH-4556; DNA synthesis inhibitor	P6745