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THE ALZET OSMOTIC PUMP NEWSLETTER–SPRING 2012

Special Delivery

Cancer Research

Before a cancer therapeutic is selected for clinical development, scientists must fully characterize its mechanism of action, antitumor activity, and safety profile in preclinical animal studies. Optimizing the schedule of drug administration is also addressed in order to achieve maximum therapeutic efficacy with the least burden of adverse effects. For some agents, continuous dosing is more efficacious compared to administration by immediate release methods, such as injections.

Since 1977, ALZET® Osmotic Pumps have been used in cancer studies for continuous delivery of experimental agents to lab animals. They maximize compound efficacy by maintaining constant levels in plasma or tissues within their therapeutic range during the entire treatment period. Also, significant therapeutic effects can be reached with lower drug doses, minimizing drug toxicity and unwanted adverse effects.

This Special Delivery issue highlights the effective use of ALZET pumps for evaluation of novel cancer treatments, such as peptoid therapies and IL-13-PE immunotherapy combined with chemotherapy. Contact ALZET Technical Services for additional information or references on any of these applications.

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Peptoids as Anti-cancer Therapeutics

–by Jose R Gadea

Peptoids have shown promise as anti-cancer agents and could potentially outperform therapeutic antibodies. While monoclonal antibodies have demonstrated clinical efficacy, they are notoriously difficult and expensive to manufacture in large quantities.[1,2] Researchers believe that peptoids may offer a more practical alternative. Peptoids are oligo-N-substituted glycines with antibody-like affinity and specificity, but far easier to synthesize.[2] Compared to antibodies and peptides, peptoids display enhanced serum stability and cell permeability properties.[3] In animal studies, peptoids were well tolerated.[2,4] With a proven track record delivering antibodies and other therapeutic peptides and proteins, ALZET Osmotic Pumps are well suited for studies designed to evaluate the *in vivo* efficacy of novel peptoids.

Researchers at the University of Texas Southwestern Medical Center are using ALZET pumps to investigate the potential use of peptoids as cancer therapeutics.[2,5] Their strategy is to develop highly specific peptoid ligands against vascular endothelial growth factor receptor-2 (VEGFR2), the dominant angiogenic signaling receptor, to stop vessel development and retard tumor growth. After screening thousands of peptoid libraries, the GU40C4 peptoid surfaced as their leading candidate for anti-angiogenesis therapy.[2]

Once GU40C4 was shown to have strong binding affinity to VEGFR2 *in vitro*, the investigators proceeded to evaluate *in vivo* efficacy in the A673 Ewing's sarcoma model. Athymic nude mice were injected with A673 cells to establish subcutaneous (SC) tumors and therapy started on the same day. ALZET pumps were used to administer GU40C4 (1.9 mg/kg/day), control peptoid, or saline continuously for 21 days. The study demonstrated that continuous GU40C4 infusion resulted in a potent therapeutic effect *in vivo*, effectively reducing tumor growth rate and volume. Compared to controls, tumors in GU40C4-treated mice were reduced up to 80% by day 25. Tumor growth remained suppressed in treated animals even 1 week after termination of therapy, and no adverse effects were observed with either GU40C4 or control peptoid. Histological analysis revealed that tumors from GU40C4-treated mice contained over 50% lower microvessel density (MVD) compared to controls, confirming the anti-angiogenic properties of GU40C4 peptoid.

Further studies were undertaken to optimize the GU40C4 peptoid and identify the minimum pharmacophore required for VEGFR2 molecular recognition.[6] These efforts led to the development of GU81, a GU40C4 derivative with reduced size and increased binding affinity.[5] Various *in vitro* studies validated the functional activity of GU81 where it was shown to share the same VEGFR2 binding site, but had superior potency over the parent compound, with a binding affinity of 12 nM (3-fold higher than GU40C4) and an IC_{50} value of ~430nM (2-fold lower than GU40C4).[5]

"continuous GU40C4 infusion resulted in a potent therapeutic effect in vivo, effectively reducing tumor growth rate and volume"

Having validated *in vitro* efficacy, the researchers evaluated the therapeutic effect of GU81, both alone and in combination with doxorubicin, in the MMTV-PyMT/Fvb transgenic breast cancer model.[5] GU81 peptoid was delivered intraperitoneally (IP) at a constant dose of 260 µg/day using ALZET pumps, while doxorubicin was given intravenously once weekly at 2 mg/kg. MMTV-PyMT/Fvb transgenic mice were treated for 19 days. Results showed that GU81 peptoid infusion alone did not have a significant therapeutic effect, but it did effectively augment the anti-tumor activity of doxorubicin. "Animals treated with the combination of GU81 and doxorubicin had decreased tumor burden, significantly reduced tumor invasion, increased tumor fat content, and a lower tumor growth index compared to animals from all other treatment groups."[5] (page 10) GU81 alone effectively reduced total vascular area by 50% and vessel size by nearly 30% in tumors, and significantly increased macrophage tumor infiltration compared to saline controls.

Although GU81 alone was only minimally effective in the MMTV-PyMT breast cancer model, a separate study demonstrated its effectiveness in the 4T1 breast cancer model.[7] BALB/c mice bearing 4T1 mammary tumors were treated with GU81 (120 mg/day; IP) administered continuously via ALZET pumps. After one and three weeks of therapy, GU81-treated mice had significantly smaller tumors and reduced MVD compared to controls. The investigators attribute these *in vivo* response variations to differences in the tumor model systems. They also speculate that a higher dose given for a longer period may be required to effectively treat MMTV-PyMT tumors. Studies are currently underway to further understand these variables and optimize GU81 peptoid therapy.

In general, these studies provide insights into the therapeutic potential of peptoid-based therapies. ALZET pumps may prove valuable as they are utilized in additional studies designed to evaluate and/or optimize the therapeutic efficacy of existing and novel peptoids, particularly where chronic delivery is required. Contact ALZET Technical Services to request a list of references on the use of ALZET pumps for administration of peptoids, antibodies, or other anti-cancer agents.

[1] Zhang et al. Cell Res 2007;17:89-99.
[2] Udugamasooriya et al. J Am Chem Soc 2008;130(17):5744-5752.
[3] Zuckermann et al. Current Opinion in Molecular Therapeutics 2009;11(3):299-307.
[4] Astle et al. Int J Ppt Res Ther 2008;14:223-227.
[5] Lynn et al. BMC Cancer 2010;10:397.
[6] Udugamasooriya et al. Bioorg Med Chem Lett 2008;18(22):5892-58894.
[7] Roland et al. PLoS ONE 2009;4(11):e7669.

IL-13-PE and Gemcitabine Combination Therapy for Pancreatic Cancer

–by Jose R Gadea

Pancreatic cancer is aggressive, usually carries a poor prognosis and the standard chemotherapy regimen (gemcitabine monotherapy) has not changed in over a decade. Although numerous phase III trials have evaluated new treatment options, none have significantly prolonged survival. New therapies are desperately needed, which led researchers at the US Food and Drug Administration and Yokohama City University to propose a novel approach by combining standard therapy with specific immunotherapy to tumor cell surface receptors. Fujisawa *et al.* identified IL-13Rα2, a high-affinity receptor for IL-13, as an ideal target for tumor immunotherapy since it was found to be overexpressed in many human cancers, including 71% of pancreatic ductal adenocarcinomas (PDA). To target the IL-13 receptor, the researchers developed a recombinant immunotoxin, named IL-13-PE, by linking IL-13 to a mutated form of the Pseudomonas exotoxin (PE).

In vitro studies demonstrated that IL-13-PE alone and combined with gemcitabine induced a potent and dose-dependent cytotoxic response against pancreatic tumor cell lines. With these encouraging results, Fujisawa *et al.* then evaluated the efficacy of IL-13-PE and gemcitabine in various mouse models of human PDA. Nude *nu/nu* mice were implanted with orthotopic pancreatic tumors derived from HS766T and MIA-PaCa2 cancer cells. Once tumors were visualized via real-time whole-body imaging, treatments were initiated either on day 5 for the early pancreatic cancer model or day 29 for the advanced cancer model. Tumor-bearing mice were treated with gemcitabine, IL-13-PE, or a combination of both agents. IL-13-PE was administered intraperitoneally at a dose of 100 µg/kg/day (or 25 µg/kg/day for the low-dose study) for 14 days, either by continuous infusion using ALZET Osmotic Pumps (IL-13-PE pump) or twice daily bolus injection (IL-13-PE bolus). In general, continuous ALZET pump infusion was superior to chronic injections for both, combined and monotherapy regimens. Infusion groups showed reduced tumor growth and enhanced survival, with combination therapy being most effective. In the early pancreatic cancer model, combination therapy with gemcitabine and continuous IL-13-PE infusion was the only treatment that resulted in complete eradication of established pancreatic tumors. Most mice (6/7) had no detectable tumors on day 21, compared with 4/7 mice from the gemcitabine + IL-13-PE bolus treatment group. Notably, 4/7 mice from the gemcitabine + IL-13-PE pump group remained tumor free throughout the course of the study (Figure 1A). These animals also survived much longer, with a mean survival time (MST) of >300 days, compared to 156 and 274 days in the no treatment and gemcitabine + IL-13-PE bolus groups, respectively (Figure 1B). Single therapy with IL-13-PE or gemcitabine also decreased tumor size and increased survival, but to a lesser extent (Figure 1).

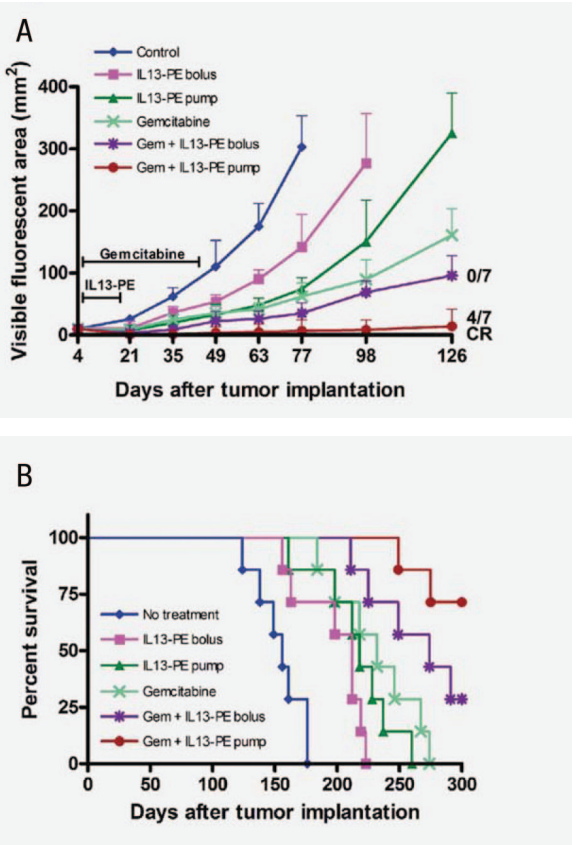


Figure 1. Quantification of tumor growth by real-time whole body imaging (A) and mice survival curves (B) in an early pancreatic cancer model using HS766T cells. Reprinted with permission from Fujisawa et al. Int. J. Cancer 2011;128:1221–1231

The antitumor effect of IL-13-PE was further evaluated in an advanced pancreatic tumor model with treatment initiated on day 29; an early pancreatic cancer model at a suboptimal dose of IL-13-PE (25 µg/kg/day for 14 days); and a cancer model using MIA-PaCa2 cells, which express lower levels of IL-13Rα2 compared to HS766T cells. In all cancer models, ALZET pump-infused IL-13-PE combined with gemcitabine was most effective at reducing tumors. Combination therapy also induced apoptosis and inhibited cell proliferation in pancreatic tumors. Gemcitabine was found to increase IL-13Rα2 expression in pancreatic cancer cells, explaining the enhanced therapeutic effect of IL-13-PE when combined with gemcitabine.

Fujisawa *et al.* demonstrated that IL-13-PE and gemcitabine work synergistically to achieve greater therapeutic effect in pancreatic cancer models. This treatment was also well tolerated, with no evidence of organ toxicity or other adverse effects. Furthermore, continuous administration of IL-13-PE produced a stronger response compared to bolus injections. Hence, the researchers propose that a similar therapeutic strategy may be beneficial for PDA treatment in humans. For more references on the use of ALZET pumps for administration of immunotoxins or chemotherapeutics, contact ALZET Technical Services.

Fujisawa et al. Int. J. Cancer 2011;128:1221–1231

Cancer Research Applications

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 maintain plasma levels in the therapeutic range, minimizing adverse effects and allowing the desirable effects to develop fully and reproducibly.

Intratumoral Delivery

ALZET pumps can easily be connected to a catheter to enable direct delivery of anti-cancer agents into tumors. This strategy has been shown to enhance the efficacy of some chemotherapeutics. Regional tumor therapy is more efficient at reaching significant therapeutic effects with lower drug doses. Furthermore, drug levels in systemic circulation are decreased, minimizing drug exposure in sensitive organs and reducing potential side effects.

In Vivo Imaging Applications

Bioluminescence imaging (BLI) is a useful experimental technique for *in vivo* imaging of small animals. It is a powerful tool for studying and monitoring ongoing biological processes (i.e., tumor growth) over time and in the same animal. ALZET pumps are increasingly being used in BLI studies as an effective means to facilitate steady-state delivery of bioluminescent substrates, such as luciferin. The pumps provide reliable and prolonged substrate delivery, thus eliminating repetitive injections and ensuring accurate detection of *in vivo* bioluminescence. ALZET pumps can be easily adapted for compatibility with BLI equipment.

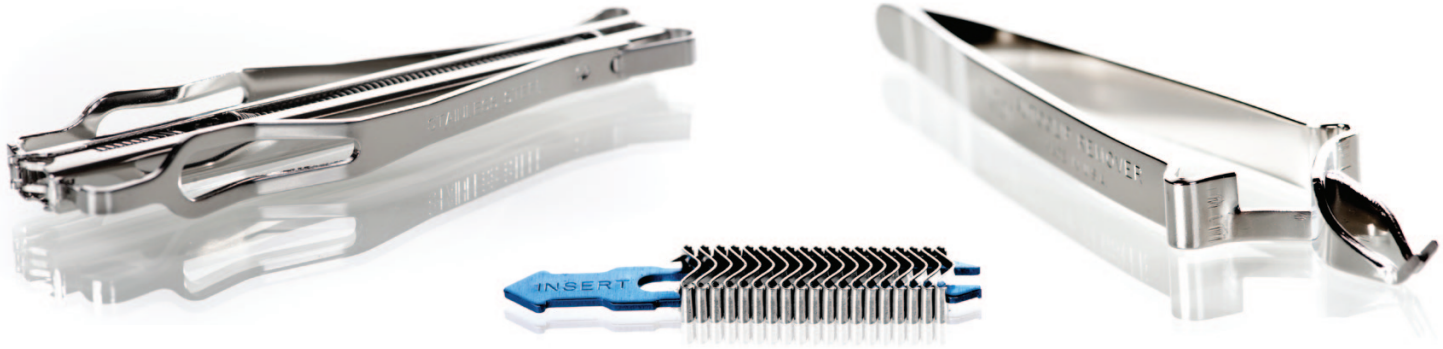
Measurement of Cell Proliferation

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.

Immunodeficient Mouse Models

The automatic operation and small size of ALZET pumps makes them an ideal infusion system for chronic dosing studies in nude and SCID mouse models, the two main strains of immunodeficient mice used in cancer research. No researcher intervention is required during infusion, and animal handling is kept to a minimum to reduce the risk of infection and stress. ALZET pumps have been used in immunodeficient mice since 1980, and over 320 publications are available as evidence of their research value in these species.

Streamline your pump implantation surgeries and save time!



The AutoClip & Reflex Wound Closure systems provide a fast and effective alternative to sutures for closing incisions made for ALZET pump implantation. The 9 mm AutoClips are ideal for use in rats, while the 7 mm Reflex clips are ideal for use in mice and young rats. Order today by contacting ALZET Customer Service at: alzetcs@durect.com or 877.922.5938

New Agents in the ALZET Bibliography

With more than 13,500 publications, ALZET pumps are commonly referenced in the scientific literature. We are constantly updating and adding to the ALZET bibliography. This table contains a list of new agents that have been recently administered via ALZET pumps.

Agent	Description / Therapeutic Category
Bevacizumab (Avastin)	Anti-VEGF monoclonal antibody
Cetuximab (Erbitux)	Anti-EGFR monoclonal antibody
Dobesilate	Vasoprotective; FGF inhibitor
EDL-155	Antineoplastic agent
Epirubicin (Farmorubicin)	Anthracycline drug
Gefitinib (Iressa)	EGFR inhibitor
GGTI-2418	Geranylgeranyltransferase I peptidomimetic inhibitor
GMX1777	Cyanoguanidinopyridine GMX1778 prodrug
GU81	VEGFR2 antagonist peptoid
Loop 6	Anti-angiogenic peptide; TIMP-2 C-terminal domain
Metacept 1	Hydroxamate-based histone deacetylase inhibitor derivative
MR1-1	Recombinant immunotoxin
PF-3758309	PAK4 pyrrolopyrazole inhibitor
PG-873637	CRF2R selective agonist
S 36578	αvβ3/αvβ5 inhibitor, non-peptide RGD-mimetic
Synstatins	Peptide inhibitors
Taurolidine	Antimicrobial with anti-LPS properties

References on these and other agents are available as a complimentary service. Contact ALZET Technical Services at 800.692.2990, or alzet@durect.com to request references specific to your research interest.

ALZET Pump Benefits in Cancer Research

- The only implantable pump small enough for use in mice
- Continuous and controlled delivery of anti-cancer agents
- Increased efficacy of therapeutic agents
- Improved bioavailability of drugs with short half-lives
- Reduced drug toxicity and side effects
- Reliable technology – over 35 years of research
- Well established research tool – over 13,500 publications
- Simple design with no electronics or batteries to fail
- Easy to use, with no programming or software to learn
- Targeted delivery into tumors, blood vessels, or other organs
- Convenient and cost-effective for chronic dosing of lab animals
- Reduced animal handling and stress
- Automatic nighttime and weekend dosing

Cancer Research Publications

Therapeutic antibodies, MMP inhibitors, angiogenesis modulators, cytokines, siRNAs, and oligonucleotides are all examples of agents that have been successfully delivered via ALZET pumps. New publications for these and hundreds of other experimental agents are constantly added to the ALZET bibliography. Contact us to request citations specific to your research interest.

ALZET Catheters

Specifically designed for use with ALZET pumps, ALZET specialized catheters are constructed with high-quality materials for increased patency and reduced vessel and tissue trauma. Each catheter incorporates design features to facilitate placement and stabilization, such as retention beads or suture patches. They are also customized for a specific target site (jugular and femoral vessel, peritoneum, spinal cord) and animal species (mouse, rat).

Surgical Training Video

Learn how to use and implant ALZET pumps, or train your staff on these procedures, with the ALZET Surgical Implantation Techniques video available on CD. Request a copy of this training tool today by visiting ALZET.com

For technical information about ALZET pumps, visit our website at www.alzet.com. You will find easy web page navigation, a detailed "Guide to use" for ALZET pumps, and many downloadable technical resources.

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Release Rates and Durations

ALZET pumps are available in 3 different sizes, durations ranging from 1 day to 42 days, and various release rates to meet your experimental research needs.

Pump Model	Reservoir Volume	Duration	Release Rate	Order #
1003D	100 µl	3 days	1.0 µl/hr	0000289
1007D	100 µl	1 week	0.5 µl/hr	0000290
1002	100 µl	2 weeks	0.25 µl/hr	0004317
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
2ML2	2 ml	2 weeks	5.0 µl/hr	0000325
2ML4	2 ml	4 weeks	2.5 µl/hr	0000327