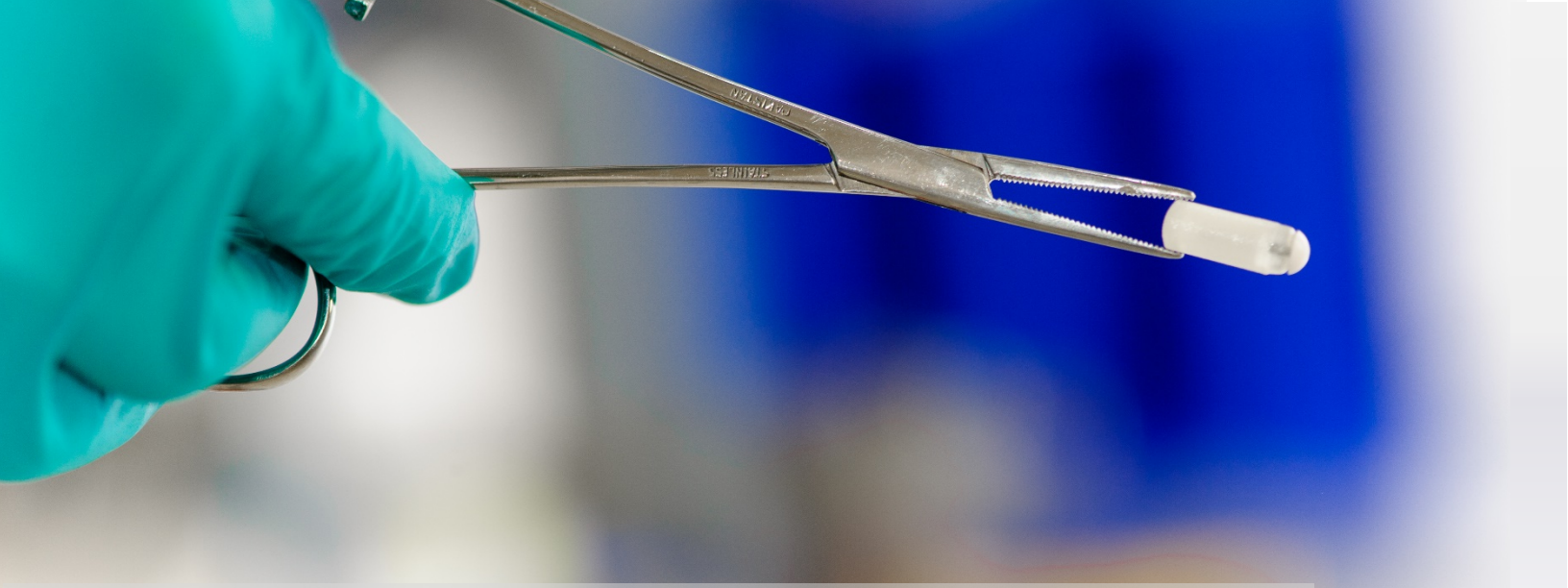

ALZET Research Application

Humanized Mouse Models

The nude mouse and severe combined immunodeficient (SCID) mouse have traditionally been used as recipients for human cells or tissues because they lack host immunity and easily accept heterologous cells. The introduction of the non-obese diabetic (NOD)/SCID mouse led to the development of highly immunodeficient strains, able to engraft human cells and tissues more efficiently, which are more appropriate for generating humanized mouse models.

The humanized mouse – a mouse carrying functional human genes, cells, tissues, and/or organs – is now a powerful research tool for the *in vivo* study of human biology and disease. Humanized mouse models enable a better understanding of disease pathways and ultimately improve the translational value of preclinical studies. Various humanized mouse models have been developed for the study of infectious diseases, autoimmunity, transplantation, vaccine development, cancer immunotherapy, regenerative medicine, cell development, and more.

ALZET® Osmotic Pumps are used extensively with immunodeficient mice, and hundreds of publications attest to their research value in these species. These implantable infusion pumps offer a convenient alternative to repetitive injections for continuous dosing of unrestrained lab animals. Their automatic operation, small size and simple design make them suitable for chronic dosing studies in humanized mouse models. No researcher intervention is required during infusion, and animal handling is kept to a minimum to reduce the risk of infection and stress. Read on for research summaries describing the use of ALZET pumps in humanized mouse models.

ALZET Pump Highlights

- Small size for implantation
- 9 pump models for mice
- Continuous and controlled delivery of agents
- Minimize side effects and experimental variables
- Convenient and cost-effective dosing method
- Reduced animal handling and stress
- Delivery rates ranging from 0.11 µl/hr to 8.0 µl/hr
- Delivery durations ranging from 1 day to 6 weeks

Immunodeficient Strains*

Nude Mouse

SCID Mouse

NOD/SCID Mouse

NSG Mouse

NOG Mouse

NRG Mouse

* See page 4 for complete descriptions

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alzet® OSMOTIC PUMPS

Use of ALZET Pumps in Humanized Mouse Models

Dixit *et al.* Skeletal Response to Insulin in the Naturally Occurring Type 1 Diabetes Mellitus Mouse Model. *JBMR Plus* 2021;5(5):e10483

Purpose	Determine the molecular and cellular mechanisms that contribute to impaired bone morphology and composition in type 1 diabetes mellitus (T1DM). Once determined, evaluate the impact of insulin treatment.
Mouse Model	D-NOD, ND-NOD, NOR
Role of ALZET	Continuous, subcutaneous infusion of Humulin R using ALZET Model 1002
Key Findings	• Decreased bone volume in diabetic mice was associated with two changes: increased sclerostin expression in osteocytes, and attenuation of bone formation indices. • Decreased bone volume in diabetic mice occurred without changes in bone resorption, suggesting that T1DM did not affect the bone mineralization process, but instead resulted in microenvironmental alterations that favored mineral loss from the bone matrix. • Dysregulation of genes involved in fatty acid oxidation, transport, and synthesis was found in the bones of diabetic NOD mice. • Exposure to insulin resulted in an increase of pyruvate dehydrogenase kinase isoenzyme 4 and glucose transporter 1 levels, and a decrease of phosphorylated-AKT levels in diabetic NOD mice. • Osteoblasts and osteocytes undergo metabolic shifts in response to T1DM that may promote demineralization of the bone matrix.

Turner *et al.* Identification of synergistic drug combinations using breast cancer patient-derived xenografts. *Scientific Reports* 2020;10(1):1493

Purpose	Identify promising targeted therapeutic candidate combinations for triple-negative breast cancer (TNBC) through *in vitro* screening of 1,363 drugs in patient-derived xenograft (PDX) models and evaluate the most promising drug pair *in vivo*.
Mouse Model	Patient-derived xenograft model of basal-like TNBC HCI01 cells in female NOD/SCID gamma (NSG) mice
Role of ALZET	Continuous, 7-day subcutaneous infusion of YM-155 via ALZET Model 1007D
Key Findings	• Identified that the combination of afatinib (epidermal growth factor receptor (EGFR) inhibitor) and YM155 (inhibitor of baculoviral inhibitor of apoptosis repeat-containing 5 (BIRC5; survivin) expression) is cytotoxic across multiple models of basal-like TNBC and works to reduce PDX mammary tumor growth *in vivo* • YM155 reduces EGFR expression in TNBC cells • High expression of EGFR and BIRC5 reduces metastasis-free survival • Both EGFR and BIRC5 are highly expressed in basal-like PDXs, cell lines, and patients, suggesting that co-targeting of these proteins could be promising for clinical success in TNBC.

Oh *et al.* Elevated GCN5 expression confers tamoxifen resistance by upregulating AIB1 expression in ER-positive breast cancer. *Cancer Letters* 2020;495:145-155

Purpose	Investigate the role general control no-derepressible 5 (GCN5) and its target genes play in tamoxifen-resistant (TamR) breast cancer
Mouse Model	NOD-SCID mice subcutaneously injected with MCF7-GCN5, MCF7-vec, and MCF7-GCN5-shAIB1
Role of ALZET	Continuous, subcutaneous infusion of tamoxifen into mice with tumors at least 40mm^3 in size
Key Findings	• Increased GCN5 in TamR breast cancer cells was linked to proteasomal sparing • GCN5 overexpression upregulated the expression of amplified in breast cancer 1 (AIB1), resulting in decreased p53 stability and tamoxifen resistance. • Tamoxifen sensitivity of GCN5-AIB1overexpressing MCF7 cells was restored by forced p53 expression. • GCN5 promotes AIB1 expression and tamoxifen resistance in breast cancer by reducing p53 levels. • GCN5 may be a therapeutic target for tamoxifen resistance in breast cancer and could serve as a novel prognostic marker.

Use of ALZET Pumps in Humanized Mouse Models

Gartung, *et al.* Suppression of chemotherapy-induced cytokine/lipid mediator surge and ovarian cancer by a dual COX-2/sEH inhibitor. *Proceedings of the National Academy of Sciences* 2019;116(5):1698-1703

Purpose	To demonstrate that ovarian tumor cell debris generated by first-line platinum- and taxane-based chemotherapy accelerates tumor progression by stimulating a macrophage-derived "surge" of proinflammatory cytokines and bioactive lipids.
Mouse Model	SCID and C57BL/6 mice injected with ovarian tumor cells
Role of ALZET	Continuous, intraperitoneal infusion of PTUPB an inhibitor of both COX-2 and sEH
Key Findings	• Ovarian tumor cell debris generated by front-line chemotherapy promotes tumor growth by stimulating the release of proinflammatory cytokines and lipid mediators in the tumor microenvironment. • PTUPB inhibited debris-stimulated tumor growth in an ovarian cancer model, which resulted in sustained survival for over 120 days. • PTUPB prevented the chemotherapy-induced cytokine and lipid surge. • Dual COX-2/sEH inhibition may have clinical implications for use in combination with cytotoxic cancer therapies to alleviate debris-mediated inflammation and resultant tumorigenesis.

Zhang, *et al.* Targeting histone methyltransferase G9a inhibits growth and Wnt signaling pathway by epigenetically regulating HP1a and APC2 gene expression in non-small cell lung cancer. *Molecular Cancer* 2018;17(1):153

Purpose	Investigate the impact of dysregulated histone methyltransferase G9a on tumor growth and the signaling pathways in non-small cell lung cancer (NSCLC).
Mouse Model	Xenograft of H1299 cells in NOD/SCID/IL2Rgamma null (NSG) mice
Role of ALZET	Continuous, intraperitoneal infusion of UNC0638, a selective G9a inhibitor, for 14 days via ALZET Model 1002
Key Findings	• Overexpression of G9a was found in 43.2% of 213 NSCLC tissues. • Targeting G9a by the specific inhibitor UNC0638 down-regulated HP1a, and epigenetically restored expression of APC2 and other tumor suppressors through promoter demethylation • Restoring HP1a and silencing APC2 respectively reduced the inhibitory effects on cell proliferation and Wnt signaling pathway in cancer cells in which G9a was silenced or suppressed. • Targeting G9a might result in suppressed tumor growth and Wnt signaling pathway partially through down-regulating HP1a and epigenetically restoring tumor suppressors like APC2, which are silenced in NSCLC. • Overexpressed G9a is a promising therapeutic target

Examples of Humanized Mouse Models

BLT humanized (hBLT) mouse	PBMC humanized (hPBMC) mouse
• Modification of the SCID-Hu model • NOD/SCID mice engrafted with human liver and thymus tissues, along with autologous CD34⁺ HSCs • Robust human hemato-lymphoid system observed between 12-16 weeks post engraftment • Most functional immune system of any current humanized mouse model • Average lifespan of ~8.5 months	• NSG mice engrafted with human peripheral blood mononuclear cells (hPBMC) • Perfect for short-term experiments where T cell engraftment is of primary concern • Enable short-term studies requiring human T cells without the 12 week T-cell maturation waiting period
CD34+ humanized (hCD34+) mouse	**GTL, or NSG-BLT, mouse**
• Robust multi-lineage engraftment of human immune cells within the NSG background, including very good T- cell maturation and function • hCD34+ mice have longer life spans than BLT mice, enabling execution of longer duration studies	• Modification of the BLT mouse: engraftment on the NSG instead of the NOD/SCID background • Allows longer term experiments compared with BLT mice since NSG mice do not develop thymic lymphomas as in the NOD/SCID

Immunodeficient mouse strains used to generate humanized mouse models

Nude Mice

- Spontaneous mutation (*Foxn1*) results in lack of body hair and deteriorated or absent thymus
- Incapable of cell-mediated immunity due to lack of T cells; partially defective B cell development
- Intact innate immunity
- Recipient of allogenic and xenogenic grafts

SCID Mice

- Recessive mutation results in deficient activity of an enzyme involved in DNA repair, causing impaired cellular and humoral immune response
- Intact innate immunity; NK cell activity limits engraftment
- Some functional T and B cells develop with age (referred as "leakiness")
- Model organisms for immune system, cell transplantation, infectious disease, and vaccine research
- Humanized models: SCID mice engrafted with human fetal tissues (SCID-Hu), or peripheral blood mononuclear cells (Hu-PBL-SCID)

NOD SCID (NOD.CB17-Prkdc$^{scid/J}$)

- SCID mutation transferred to NOD background
- Lacks functional T and B cells
- Reduced NK cell activity, impaired complement pathway, and reduced "leakiness" of residual T and B cells than SCID strain
- Accepts allogeneic and xenogeneic grafts very efficiently; used for transplantation of normal and malignant human cells and tissues, including isolates from hematopoietic cancers
- Develops thymic lymphomas by 8-9 months; best used in short term experiments
- Adoptive transfer recipient for study of autoimmune type 1 diabetes

NSG mouse (NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ)

- a.k.a NOD/SCID gamma, or NOD/SCID IL-2 receptor gamma chain knockout
- NOD/SCID mice bearing a targeted mutation in the IL-2 receptor-γ chain resulting in deficient signaling of multiple cytokines (IL2, IL4, IL7, IL9, IL15 and IL21)
- Severe defects in innate and adaptive immunity: lacks T and B cells, natural killer (NK) cells, and complement activity; reduced macrophage and dendritic cell function
- No T and B cell "leakiness" associated with aging
- Resistant to lymphoma, allowing for long-term experiments
- Improved engraftment of primary human cells, including PBMCs and HSCs
- Enhanced model for human hematopoiesis, cancer (leukemia, multiple myeloma), visceral diseases, autoimmune type 1 diabetes, infectious disease (AIDS), regenerative medicine, and hematology

NRG mouse (NOD.Cg-Rag1^{tm1Mom} Il2rg^{tm1Wjl}/SzJ)

- Also called B6 Rag1; B6 inbred background simplifies creation of compound immunodeficient mutants
- Similar to the NSG strain, but the SCID mutation is substituted for the Rag1 knockout mutation, which provides greater tolerance for irradiation and chemotherapy
- Support higher levels of human cord blood stem cell engraftment following irradiation-conditioning
- Model for human lymphohematopoietic cell engraftment studies that require a radiation-resistant host

NOG mouse (NOD/Shi-scid/IL-2Rγ^{null})

- Combination of the NOD/SCID and the IL-2 receptor-γ chain knockout (IL2rγKO) mouse
- Similar to the NSG mouse (The Jackson Laboratory), but developed by the Central Institute for Experimental Animals (Japan)
- Triggering through the IL-2 γ chain receptor is disabled due to a truncated intracytoplasmic tail (receptor completely knocked down in NSG mice)
- Vulnerable to developing lymphomas after irradiation, but yield similar engraftment results (compared to NSG) even when not irradiated

Resources:

- Ito *et al.* Cellular & Molecular Immunology 2012;9:208-214
- Shultz *et al.* Nature Reviews 2007;7:118-130
- https://www.jax.org/
- www.ciea.or.jp/en

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