# **CL401**

# Dual TLR2 & TLR7 ligand

Catalog # tlrl-c401

For research use only

Version # 15B04-MM

## PRODUCT INFORMATION

#### **Content:**

- 500 µg CL401 provided as a lyophilized powder
- 1.5 ml endotoxin-free water

#### Storage:

- CL401 is shipped at room temperature. Store lyophilized product at -20 °C. Lyophilized product is stable for 1 year at -20 °C.
- Upon resuspension, store at 4 °C. Resuspended product is stable for 6 months at 4 °C. Do not store resuspended product in plastic tubes.

#### DESCRIPTION

CL401 is a small lipophilic molecule comprising an 8-hydroxyadenine compound conjugated with a Pam2C group. This bipartite structure confers to CL401 the ability to efficiently stimulate both TLR7 and TLR2, respectively. Intratumoral injection of CL401 (50 µg/mouse) leads to a significant antitumor activity.

#### **APPLICATIONS**

CL401 can be used *in vitro* to stimulate TLR2 and TLR7 or in animal models for its antitumoral or adjuvant activity.

## **BACKGROUND**

InvivoGen has developed a series of novel molecules designed to induce potent immune responses through the combined activation of several pattern recognition receptors (PRRs) that trigger different innate immune signaling pathways. These molecules are agonists for TLR2, TLR7 or both. Agonists that activate TLR2 are derived from the well-established TLR2 ligand, Pam2CSK4, and those recognized by TLR7 are derived from the 8-hydroxyadenine derivative CL264, a TLR7 agonist recently developed by InvivoGen (see Related Products overleaf).

TLR2 and TLR7 are two PRRs with distinct characteristics. TLR2 is a cell surface receptor expressed by many cell types, while TLR7 is an endosomal receptor expressed predominantly in plasmacytoid dendritic cells (pDC) and to a lesser extent in B cells. TLR2 signaling triggers the NF- $\kappa$ B pathway and the production of pro-inflammatory cytokines, such as TNF- $\alpha$ , whereas TLR7 signaling induces mainly the IRF pathway and the production of IFN- $\alpha$ . Combined activation of these different pathways results in robust immune responses with potential therapeutic effects. InvivoGen's multi-PRR agonists are promising candidates for antitumor and vaccine applications.

#### CHEMICAL PROPERTIES

**Synonym:** S-(2,3-bis(palmitoyloxy)-(2RS)propyl)-(R)-cysteinyl 4-((6-amino-2(butyl amino)-8-hydroxy-9H-purin-9-yl)methyl) aniline

Formula: C54H92N8O4S Molecular weight: 981 g/mol Solubility: DMSO (20 mg/ml)

Working concentration: 1 ng - 10 μg/ml (~1 nM - 10 μM)

Endotoxin level: <0.001 EU/µg

**Structure:** 

#### **METHODS**

#### Preparation of CL401 stock solution (1 mg/ml)

- 1. Add 50  $\mu l$  DMSO to 500  $\mu g$  CL401. Vortex until complete solubilization.
- 2. Once CL401 is solubilized, add 450  $\mu$ l endotoxin-free water (provided).

#### TLR stimulation with CL401 using HEK-Blue cells

CL401 can be used to stimulate TLR2 in HEK-Blue™ TLR2 cells and TLR7 in HEK-Blue™ TLR7 cells. These cells stably express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) and overexpress the appropriate TLR gene. For more information visit: www.invivogen.com/hek-blue-cells

- 1. Stimulate HEK-Blue<sup>TM</sup> TLR2 cells with 10 ng 1  $\mu$ g/ml CL401 and HEK-Blue<sup>TM</sup> TLR7 cells with 100 ng 10  $\mu$ g/ml CL401.
- 2. Incubate for 6 24 h at 37 °C, 5% CO2.
- 3. Determine TLR stimulation using a SEAP detection medium, such as QUANTI-Blue™ or HEK-Blue™ Detection (see Related Products, overleaf) or by assessing cytokine expression using an ELISA.



## RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR2 Cells	hkb-htlr2
HEK-Blue™ mTLR2 Cells	hkb-mtlr2
HEK-Blue™ hTLR7 Cells	hkb-htlr7
HEK-Blue™ mTLR7 Cells	hkb-mtlr7
RAW-Blue™ Cells (Mouse macrophage reporter cells)	raw-sp
HEK-Blue™ Detection (SEAP detection medium)	hb-det2
QUANTI-Blue™ (SEAP detection medium)	rep-qb1
Dual TLR2 & TLR7 ligands:	
Adilipoline™ (CL413; TLR2 & TLR7 ligand)	tlrl-c413
CL531 (TLR2 & TLR7 ligand)	tlrl-c531
CL572 (TLR2 (human) & TLR7 ligand)	tlrl-c572
TLR ligands & nucleic acid carriers:	
AdiFectin <sup>™</sup> (CL347; TLR7 ligand & nucleic acid carrier)	tlrl-c347
CL419 (TLR2 ligand & nucleic acid carrier)	tlrl-c419
PamadiFectin™ (CL553; TLR7 & TLR2 ligand & nucleic acid carrier)	tlrl-c553
TLR2 ligands:	
HKLM (Heat killed Listeria monocytogenes)	tlrl-hklm
FSL-1 (Synthetic diacylated lipoprotein)	tlrl-fsl
Pam2CSK4 (Synthetic diacylated lipoprotein)	tlrl-pm2s
TLR7 ligands:	
CL264 (8-hydroxyadenine derivative)	tlrl-c264e
CL307 (Spermine covalently linked to CL264)	tlrl-c307
Gardiquimod™ (Imidazoquinoline compound)	tlrl-gdqs

