Kinase Assay:

Everolimus is an mTOR inhibitor of FKBP12 with IC50 of 1.6-2.4 nM.

Targets: mTOR (FKBP12)

IC50: 1.6-2.4 nM

In vitro: Everolimus exhibits the immunosuppressive activity which is comparable to that of rapamycin. Everolimus competes with immobilized FK 506 for binding to biotinylated FKBP12 and shows the inhibitory effect on a two-way MLR performed with spleen cells from BALB/c and CBA mice with IC50 of 0.12-1.8 nM. [1] Everolimus also shows antiangiogenic/cellular effects in VEGF-induced HUVEC proliferation with IC50 of 0.12 nM and bFGF-induced HUVEC proliferation with IC50 of 0.8 nM, respectively. [2] A recent study shows that Everolimus shows a dose-dependent inhibitory effects on both the total cells and the stem cells from the BT474 cell line and the primary breast cancer cells with IC50 of 156 nM in total cells of primary breast cancer cells and 71 nM in total cells of BT474 cells. In addition, combination treatment with Everolimus and trastuzumab produces the significantly increased inhibition on the growth of cancer stem cells with the inhibition rate increased by more than 50%. [3]

In vivo: Everolimus (0.1 to 10 mg/kg) dose-dependently inhibits growth of the primary (ear) and lymph node metastases of B16/B6 melanoma, with decreased total number of vessels and reduced mature vessels. [2] In a xenograft animal model of BT474 stem cells, Everolimus shows significant reductions in mean tumor sizes (590.6 mm3), compared to the control group with a tumor size of 698 mm3. Furthermore, treatment combination with Everolimus and trastuzumab significantly decreases the xenograft tumor size (410.8 mm3) more than Everolimus treatment alone. [3]

Clinical Trials: Everolimus is currently in Phase I clinical trials in patients with Unspecified Adult Solid Tumor.

Features:

Protocol (Only for Reference)

Kinase Assay: [1]

FKBP12 binding assay: Binding to the FK 506 binding protein (FKBP12) is indirectly assessed by means of an ELISA-type competition assay. FK 506 is included in each individual experiment as a standard, and the inhibitory activity is expressed as relative IC50 compared to FK 506 (nIC50 = IC50 Everolimus/IC50 FK 506). Mixed lymphocyte reaction (MLR): The immunosuppressive activities of RAP and its derivatives are assessed in a two-way MLR, using spleen cells of BALB/c and CBA mice. RAP is included in each individual experiment as a standard, and the inhibitory activity is expressed as relative IC50 compared to RAP (nIC50 = IC50 Everolimus/IC50 RAP).

Cell Assay: [2]

Cell Lines: BT474 cell line and the primary breast cancer cells

Concentrations: 0.001-10 μM

Incubation Time: 24 hours

Methods:

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye reduction assay (MTT assay) is used to compare the effects of Everolimus or trastuzumab on total breast cancer cells and breast CSCs. The total cells and the stem cells from the BT474 cell line and the primary breast cancer cells are respectively seeded into 96-well plates with different concentrations of the drugs, with five wells for each concentration, and the cells are cultured at 37 °C with 5 % CO2 in an incubator for 24 hours. The concentrations of Everolimus are 1 nM, 10 nM, 100 nM, 1 μM, 10 μM, 100 μM, and 1000 μM. The combinatorial inhibitory effect of Everolimus and Trastuzumab on the in vitro growth of breast CSCs is examined by MTT assay as well using 10 μg/mL trastuzumab in combination of increasing concentrations of everolimus (1 nM, 10 nM, 100 nM, and 1 μM). After drug treatment for 24 hours, 20 μL MTT (5 mg/mL) in phosphate buffered saline (PBS) is added to each well, and the cells are incubated at 37 °C with 5 % CO2 and saturated humidity for 4 hours. Following the subsequent removal of the supernatant, 150 μL dimethyl sulfoxide (DMSO) is added to each well, and the cells are vortexed for 10 minutes. The light absorbance (OD value) is measured for each well using an ELISA reader. Each experiment is repeated in triplicate, and dose–response curves are plotted. The probit software of the statistical software package SPSS 17.0 for Windows is used to calculate the inhibitory concentration (IC50) of Everolimus.
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<table>
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<th>Animal Models</th>
<th>Cultured BT474 stem cells are injected beneath the left breast pad of BALB/c nude mice.</th>
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**References**


**Animal Study**[3]

**Formulation**

Everolimus is dissolved in saline.

**Doses**

≤2 mg/kg

**Administration**

Administered via p.o.

**References**


**Customer Reviews**

Data from [Biochem Pharmacol, 2011 April, 82:216-226]

*Everolimus (RAD001)* purchased from Selleck

Inhibition of mTOR activity may be responsible for sorafenib-induced down-regulation of survivin. H1299 cells were treated with the indicated concentration of RAD001 or Rapamycin for 48 h. Then H1299 cells were incubated with or without 5 mM sorafenib, with or without 5 mM RAD001, and with or without 2 mM rapamycin for 48 h. The indicated protein levels were determined by Western blot analysis. β-Actin protein levels were measured as loading controls.

**PLEASE KEEP THE PRODUCT UNDER -20°C FOR LONG-TERM STORAGE.**

**NOT FOR HUMAN, VETERINARY DIAGNOSTIC OR THERAPEUTIC USE**

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