

## Product Data Sheet

Product Name: Everolimus (RAD001)

Cat. No.: GC13601

### Chemical Properties

Cas. No. 159351-69-6

SMILES OCCO[C@H]1[C@H](OC)C[C@H](C[C@H](C)[C@H](CC([C@H](C)/C=C(C)/[C@@H](O)[C@H]2OC=O)OC([C@@H]3CCCCN3C(C([C@@]4(O)[C@H](C)CC[C@@H](C[C@H](OC)/C(C)=C/C=C/C=C/[C@@H](C)C[C@@H](C)C2=O)O4)=O)=O)=O)CC1

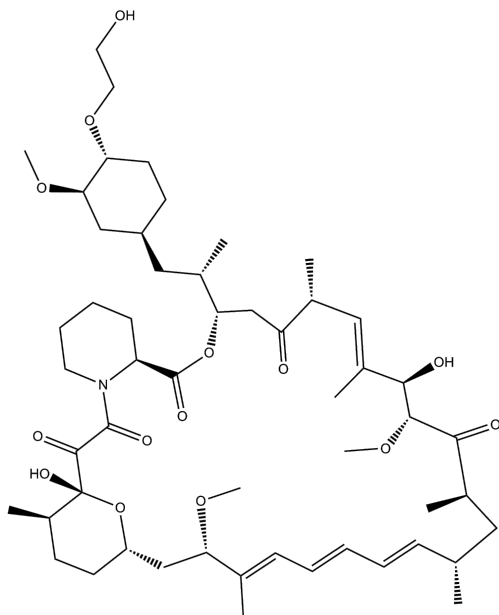
Formula  $C_{53}H_{83}NO_{14}$  M.Wt 958.22

Solubility  $\geq 47.911\text{mg/mL}$  in DMSO,  $\geq 122\text{mg/mL}$  in EtOH Storage Store at  $-20^{\circ}\text{C}$

General tips For obtaining a higher solubility, please warm the tube at  $37^{\circ}\text{C}$  and shake it in the ultrasonic bath for a while. Stock solution can be stored below  $-20^{\circ}\text{C}$  for several months.

Shipping Condition Evaluation sample solution: ship with blue ice All other available size: ship with RT, or blue ice upon request.

Structure



### Protocol

#### Cell experiment [1]:

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|-------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cell lines                    | NCI-H661 cells (human NSCLC cell line with wild-type PIK3CA and KRAS)                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Preparation Method            | NCI-H661 cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) at 37°C with 5% CO <sub>2</sub> . Cells were treated with 10nM or 0.1nM Everolimus for 24h, with partial groups receiving 4Gy X-ray irradiation and being detected at 0.5h and 24h post-irradiation; for clonogenic, migration and invasion assays, cells were pretreated with 0.1nM Everolimus for 24h before 4Gy X-ray irradiation, then cultured in drug-free medium for 14 days (clonogenic) or 24h (migration/invasion). |
| Reaction Conditions           | 0.1 or 10 nM; 24h                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Applications                  | Pretreatment with Everolimus enhanced the radiosensitivity of NCI-H661 cells, increased γ-H2AX focus formation at 0.5h and 24h post 4Gy irradiation in NCI-H661 cells, reduced the clonogenic survival, migration and invasion of irradiated NCI-H661 cells, and upregulated E-cadherin while downregulating vimentin expression in irradiated NCI-H661 cells.                                                                                                                                                                  |
| <b>Animal experiment [2]:</b> |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Animal models                 | Athymic nude mice (4-6 weeks old) bearing subcutaneous xenografts of UM-UC-3, UM-UC-6 or UM-UC-9 human bladder transitional cell carcinoma cells (1×10 <sup>6</sup> cells inoculated into the right flank, tumors reached ~5mm in diameter 1 week after inoculation)                                                                                                                                                                                                                                                            |
| Preparation Method            | Tumor-bearing mice received Everolimus at 5mg/kg via oral gavage twice weekly for 4 weeks. Mice were euthanized when tumor size reached ~1.5cm or at the end of 4-week treatment, and tumors were harvested for subsequent immunohistochemical analysis.                                                                                                                                                                                                                                                                        |
| Dosage form                   | 5mg/kg; oral gavage; twice weekly for 4 weeks                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |

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### Applications

Everolimus significantly inhibited the growth of subcutaneously implanted tumors derived from all three bladder cancer cell lines. Everolimus-treated UM-UC-3- and UM-UC-6-derived tumors showed reduced phosphorylated p70S6K expression, while phosphorylated p70S6K expression in Everolimus-treated UM-UC-9-derived tumors was unchanged. Everolimus treatment mildly decreased CD31-positive microvessel density in UM-UC-3-derived tumors, significantly decreased microvessel density in UM-UC-9-derived tumors.

### References:

[1] Chen Y, Li WW, Peng P, et al. mTORC1 inhibitor RAD001 (everolimus) enhances non-small cell lung cancer cell radiosensitivity in vitro via suppressing epithelial-mesenchymal transition. *Acta Pharmacol Sin.* 2019 Jul;40(7):1085-1094.

[2] Chiong E, Lee IL, Dadbin A, et al. Effects of mTOR inhibitor everolimus (RAD001) on bladder cancer cells. *Clin Cancer Res.* 2011 May 1;17(9):2863-73.

### Background

Everolimus (RAD001) is an orally effective second-generation mTOR inhibitor. Everolimus binds FKBP12 to allosterically inhibit mTORC1, thereby blocking protein translation, inducing G1 phase arrest, and downregulating HIF-1 $\alpha$ /VEGF to suppress angiogenesis. Everolimus reduces inflammation through autophagy regulation. Everolimus can be used in research related to malignant tumors and organ transplant anti-rejection<sup>[1-4]</sup>.

In vitro, treatment of nasopharyngeal carcinoma CNE-1 and HK1 cells with 30-60 $\mu$ M Everolimus for 72 hours inhibited cell proliferation<sup>[5]</sup>. Treatment of HONE-1 cells with 0.63nM Everolimus for 48 hours upregulated cleaved PARP and induced apoptosis<sup>[6]</sup>. Pretreatment of NCI-H661 cells with 0.1 or 10nM Everolimus for 24 hours followed by 4Gy irradiation enhanced radiosensitivity, increased  $\gamma$ -H2AX focus formation, and reduced migration and invasion capabilities<sup>[7]</sup>.

In vivo, continuous subcutaneous infusion of Everolimus (1.5mg/kg/day) for 12 weeks in ApoE<sup>-</sup>

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*-Fbn1*<sup>C1039G</sup>*-/-* mice with advanced atherosclerotic plaques reduced the proportion of circulating Ly6Chigh monocytes, depleted plaque macrophages, eliminated intraplaque neovascularization, alleviated cerebral cortical hypoxic injury, improved cardiac function, and increased survival rate<sup>[8]</sup>. Oral gavage of Everolimus (5mg/kg; twice per week; for 4 weeks) in nude mice subcutaneously inoculated with human bladder cancer UM-UC-3, UM-UC-6, and UM-UC-9 cells inhibited the growth of subcutaneous xenograft tumors<sup>[9]</sup>. Oral gavage of Everolimus (5mg/kg; twice per week) from 5 weeks of age to 20 weeks of age in TgMISIIR-TAg transgenic spontaneous ovarian cancer mice delayed ovarian tumor initiation and progression, reduced tumor burden, and decreased ascites formation and peritoneal dissemination<sup>[10]</sup>.

### References:

- [1] Hasskarl J. Everolimus. Recent Results Cancer Res. 2014;201:373-92.
- [2] Lee L, Ito T, Jensen RT. Everolimus in the treatment of neuroendocrine tumors: efficacy, side-effects, resistance, and factors affecting its place in the treatment sequence. Expert Opin Pharmacother. 2018 Jun;19(8):909-928.
- [3] Agarwala SS, Case S. Everolimus (RAD001) in the treatment of advanced renal cell carcinoma: a review. Oncologist. 2010;15(3):236-45.
- [4] Józwiak S, Stein K, Kotulska K. Everolimus (RAD001): first systemic treatment for subependymal giant cell astrocytoma associated with tuberous sclerosis complex. Future Oncol. 2012 Dec;8(12):1515-23.
- [5] Cai Y, Xia Q, Su Q, et al. mTOR inhibitor RAD001 (everolimus) induces apoptotic, not autophagic cell death, in human nasopharyngeal carcinoma cells. Int J Mol Med. 2013 Apr;31(4):904-912.
- [6] Ma BB, Lui VW, Hui EP, et al. The activity of mTOR inhibitor RAD001 (everolimus) in nasopharyngeal carcinoma and cisplatin-resistant cell lines. Invest New Drugs. 2010 Jun;28(3):413-420.
- [7] Chen Y, Li WW, Peng P, et al. mTORC1 inhibitor RAD001 (everolimus) enhances non-small cell lung cancer cell radiosensitivity in vitro via suppressing epithelial-mesenchymal transition. Acta Pharmacol Sin. 2019 Jul;40(7):1085-1094.

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[8] Kurdi A, Roth L, Van der Veken B, et al. Everolimus depletes plaque macrophages, abolishes intraplaque neovascularization and improves survival in mice with advanced atherosclerosis. *Vascul Pharmacol.* 2019 Feb;113:70-76.

[9] Chiong E, Lee IL, Dadbin A, et al. Effects of mTOR inhibitor everolimus(RAD001) on bladder cancer cells. *Clin Cancer Res.* 2011 May 1;17(9):2863-73.

[10] Mabuchi S, Altomare DA, Connolly DC, et al. RAD001(everolimus) delays tumor onset and progression in a transgenic mouse model of ovarian cancer. *Cancer Res.* 2007 Mar 15;67(6):2408-13.

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