is the underlying mechanism of synergism between EGFR/HER2 TKI and 5-FU in HER2-positive cancer cells.

mTOR

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Pharmacodynamics and anti-tumour activity of KU-0063794, a potent and specific inhibitor of mTOR kinase

B.R. Davies[1], C.M. Chresta[1], P. Dudley[1], N. Hewitt[1], M. Brady[1], M. Jacobsen[2], I. Hickson[3], N.M.B. Martin[3], G.C.M. Smith[3], M. Pass[1]. *[1]AstraZeneca, Cancer and Infection Research Area, Macclesfield, Cheshire, United Kingdom; [2]AstraZeneca, Global Safety Assessment, Macclesfield, Cheshire, United Kingdom; [3]KuDOS Pharmaceuticals Ltd, Biology, Cambridge, United Kingdom*

Background: The mTOR kinase is a critical regulator of cell growth, receiving stimulatory signals from growth factors via the phosphatidylinositol-3-OH kinase/AKT pathway, and integrating nutrient inputs in the form of amino acid, glucose and oxygen availability. In mammals, there are two distinct and mutually exclusive mTOR complexes, the substrate-defining subunits of which are raptor (the mTORC1 complex) and rictor (mTORC2). mTORC1 complexes are strongly inhibited by rapamycin and its analogues, whereas mTORC2 complexes are not. In some tumours, inhibition of mTORC1 by rapalogues removes a negative feedback mechanism resulting in activation of AKT, and this has been correlated with resistance to these agents. Here we describe the in vivo properties of KU-0063794, a potent and selective ATP-competitive inhibitor that directly targets mTOR kinase.
Materials and Methods: Anti-tumour and pharmacodynamic studies were carried out in immunodeficient rodents bearing either U87-MG or MCF-7 xenografts. Compound was dosed once daily by oral gavage. Pharmacokinetics were measured in plasma and pharmacodynamics in ex vivo tumour tissue by western blotting or immunohistochemistry.
Results: KU-0063794 inhibits phosphorylation of S6ser235/6 and AKTser473, which respectively are downstream targets of mTORC1 and mTORC2, in U87-MG xenografts growing in nude mice, and MCF-7 xenografts growing in severe combined immunodeficient (SCID) mice. Target inhibition correlated with plasma exposure to the drug, and significant inhibition of both targets was sustained for at least 24 h in U87-MG xenografts following an acute 75 mg/kg oral dose. Pharmacokinetic studies showed that exposure to the drug was similar in both mouse strains following an acute 75 mg/kg dose (AUCs = 573.6 and 614.4 mM.h in nude and SCID mice respectively), and exposure in nude mice scaled well with increasing dose (AUCs = 157.6 and 573.6 mM.h following doses of 18.75 and 75 mg/kg respectively). Chronic dosing of up to 75 mg/kg qd KU-0063794 was well tolerated, and resulted in a dose-dependent inhibition of U87-MG geometric mean delta tumour volume of up to 88%. In MCF-7 xenografts, an acute dose of KU-0063794 inhibited AKT, PRAS40 and S6 phosphorylation, whereas an acute dose of rapamycin resulted in activation of AKT, inhibition of S6 and no change in PRAS40 phosphorylation; these data are consistent with inhibition of mTORC1 and mTORC2 by KU-0063794 and inhibition of mTORC1 only by rapamycin.
Conclusions: The data show that inhibition of TOR kinase by KU-0063794 is well tolerated, results in dose-dependent anti-tumour activity, and offers a differentiated, potentially advantageous in vivo pharmacology to rapamycin and its analogues.

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Cellular characterization of OXA-01, a potent and selective dual mTORC1 and mTORC2 kinase inhibitor

S.V. Bhagwat[1], A.P. Crew[2], P.C. Gokhale[3], A. Cooke[3], J. Kahler[1], Y. Yao[1], A. Chan[1], L.D. Arnold[2], R. Wild[3], J.A. Pachter[1]. *[1]OSI Pharmaceuticals, Cancer Biology, Farmingdale, USA; [2]OSI Pharmaceuticals, Cancer Chemistry, Farmingdale, USA; [3]OSI Pharmaceuticals, In vivo Pharmacology, Boulder, USA*

Background: The PI3K/AKT/mTOR pathway is constitutively activated in many human cancers. mTOR is a clinically validated target in this pathway with rapamycin analogs such as CCI-779, RAD001 and AP23573 showing modest anti-tumor responses in patients. mTOR exists as a part of 2 complexes; mTORC1 (rapamycin-sensitive complex in vitro) and mTORC2 (rapamycin-insensitive complex in vitro). Rapamycin treatment of cells primarily inhibits mTORC1, resulting in inhibition of pS6K1 and p4E-BP1. However, in the majority (65%) of cell lines tested, rapamycin treatment also stimulates pAKT (mTORC2 activity), which may antagonize the anti-proliferative (mTORC1 mediated) effects of rapamycin in tumor cells.
Methods and Results: To identify compounds with greater antitumor efficacy than rapamycin analogs, we have generated a series of small molecule inhibitors that selectively inhibit the protein kinase activity of the mTOR catalytic subunit to directly inhibit both mTORC1 and mTORC2. One such selective compound, OXA-01 inhibits both mTORC1 and mTORC2 biochemical activities with IC50 values of 29 nM and 7 nM, respectively (100 μM ATP). In MDA-MB-231 breast cancer cells, this compound completely inhibited phosphorylation of 4E-BP1 and S6. AKT phosphorylation was also completely inhibited by OXA-01 in BT-474 breast cancer cells indicating inhibition of mTORC2 activity in cells. In addition, this compound inhibited proliferation of MDA-MB-231 and BT-474 breast cancer cell lines at concentrations that correlate with target inhibition. OXA-01 also induced apoptosis in a number of cell lines in the presence of 10% FCS as determined by DNA fragmentation ELISA and Caspase 3/7 assays. Oral dosing of OXA-01 at 50 mg/kg in mice resulted in sustained plasma levels significantly greater than the concentration required for cellular activity, and a good correlation was observed between inhibition of tumor phospho-4E-BP1 and tumor growth inhibition in the MDA-MB-231 xenograft model. Daily oral dosing at 75 mg/kg, twice a day for 14 consecutive days resulted in 100% mean tumor growth inhibition over the dosing period.
Conclusions: These data suggest that an mTOR kinase inhibitor such as OXA-01 may provide improved antitumor activity compared to rapamycin analogs.

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A phase I trial evaluating pharmacodynamics of deforolimus (AP23573, MK-8669) delivered orally on multiple dosing schedules

L. Berk[1], D. Miller[1], D. Clapham[1], J. Loewy[2], M. Mita[3], C. Britten[4], E. Poplin[5], C.L. Bedrosian[2], T. Clackson[1], V.M. Rivera[1]. *[1]ARIAD Pharmaceuticals, Research Department, Cambridge, MA, USA; [2]ARIAD Pharmaceuticals, Medical Department, Cambridge, MA, USA; [3]University of Texas Health Science Center, Cancer Therapy & Research Center's Institute for Drug Development, San Antonio, TX, USA; [4]UCLA Medical Center, Jonsson Comprehensive Cancer Center, Los Angeles, CA, USA; [5]Robert Wood Johnson Medical School, Cancer Institute of New Jersey, New Brunswick, NJ, USA*

Background: Deforolimus is a non-prodrug mTOR inhibitor that has shown promising anti-tumor activity when administered intravenously (IV) QDx5 every 2 wks. Rapid, potent and prolonged inhibition of mTOR activity in peripheral blood mononuclear cells (PBMCs) has been observed with this dosing regimen using a pharmacodynamic (PD) assay that measures phosphorylation of the mTOR target 4E-BP1. In a phase 1 trial (Trial 106), oral deforolimus delivered on various schedules demonstrated an activity and safety profile consistent with the IV form. Here, PD activity in this trial was assessed to determine the degree and duration of target inhibition and to compare to IV delivery.
Methods: Trial 106 was a dose escalation trial in adult patients (pts) with refractory or advanced solid tumors. Seven oral dosing regimens were investigated: QDx28, QDx21 Q28D, QDx6 QW, QDx5 QW and QDx4 QW. B.I.D. and loading dose schedules were also explored. Whole blood samples were collected prior to dosing and at up to 8 post-dose timepoints throughout the 28-day cycle, and protein extracts from PBMCs analyzed by Western blot. Levels of 4E-BP1 phosphorylated at Ser65/Thr70 (P-4E-BP1) were normalized to total levels and the median calculated for each dose group.
Results: Evaluable PD data were obtained from 141 of 147 treated pts. Rapid and potent mTOR inhibition was observed in PBMCs from all pts tested, with P-4E-BP1 levels decreasing by 83−93% within 24 hr at all dose levels (10–100 mg). Substantial mTOR inhibition was also maintained throughout the entire 28-day dosing cycle: in all schedules except QDx21 (7 day holiday), >70% inhibition was observed at most timepoints, even 4 days after dosing (QDx4). In the 24 patients dosed with the 40 mg QDx5 regimen, mTOR activity was inhibited by >90% within 24 hr after the first dose and inhibition was maintained at levels >75% throughout the 28-day cycle. These results are comparable to those obtained following IV dosing (3−28 mg).
Conclusions: Rapid, potent and prolonged inhibition of mTOR activity was observed in PBMCs following oral dosing with deforolimus. Based on these findings and previously reported activity and tolerability data, the 40 mg QDx5 regimen has been selected for further evaluation in SUCCEED, a global phase 3 trial of pts with metastatic soft-tissue and bone sarcoma in the maintenance setting.

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mTORC1/mTORC2 selective inhibitors: Identification and characterization of novel small molecules with anti-tumor activity

N. Miller[1]. *[1]Exelixis, Molecular Pharmacology, South San Francisco, CA, USA*

Background: mTOR is a key regulator of signaling pathways that control cell growth, proliferation, survival, autophagy and angiogenesis,