Everolimus

Jens Hasskarl

Abstract

Everolimus (RAD001, Afinitor®) is an oral protein kinase inhibitor of the mammalian target of rapamycin (mTOR) serine/threonine kinase signal transduction pathway. The mTOR pathway regulates cell growth, proliferation, and survival and is frequently deregulated in cancer. Everolimus has been approved by the FDA and the EMA for the treatment of advanced renal cell carcinoma (RCC), subependymal giant cell astrocytoma (SEGA) associated with tuberous sclerosis (TSC), pancreatic neuroendocrine tumors (PNET), in combination with exemestane in advanced hormone-receptor (HR)-positive, HER2-negative breast cancer. Everolimus shows promising clinical activity in additional indications. Multiple phase 2 and phase 3 trials of everolimus alone or in combination are ongoing and will help to further elucidate the role of mTOR in oncology. For a review on everolimus as immunosuppressant, please consult other sources.

Contents

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1 Introduction

Everolimus is an analog of the naturally occurring macrolide rapamycin. Rapamycin (sirolimus) was isolated from a Streptomyces species from soil of the Easter Island (Rapa Nui) (Sehgal et al. 1975). Rapamycin is a macrolide with antifungal and immunosuppressive properties (Eng et al. 1991). The identification of the mammalian target of rapamycin (mTOR) signaling pathway spurred the development of rapamycin analogs (so-called rapalogs) in the following years (Brown et al. 1994; Sabatini et al. 1994). Several rapalogs are under clinical use, and further investigations to harness their immunosuppressive and antiproliferative potential are ongoing. These are sirolimus (rapamycin) (Sehgal 1995), temsirolimus (CCI-779) (Geoerger et al. 2001), everolimus (RAD001) (Schuler et al. 1997), and deforolimus (AP23573) (Mita et al. 2008).

Rapalogs bind to the FK506-binding protein-12 (FKBP12). This complex inhibits the mTOR, a protein kinase that regulates cell growth, proliferation, and survival (Fig. 1). mTOR can form two functionally distinct complexes that differ in their sensitivity to rapamycin (Jacinto et al. 2004). mTOR complex 1 (mTORC1) regulates translation and cell growth via phosphorylation of S6 kinase (S6K) and eukaryotic initiation factor eIF4E binding protein (4E-BP) and is very sensitive to inhibition by rapamycin. The second mTOR complex (mTORC2) is resistant to rapamycin and is involved in (re)organization of the actin cytoskeleton. mTORCs integrate signals from multiple upstream pathways and relay the information through the regulation of multiple downstream pathways (Laplante and Sabatini 2012; Houghton 2010; O'Reilly and McSheehy 2010). In essence, the mTOR pathway is activated via the phosphatidylinositol 3-kinase (PI3K) pathway and the tuberous sclerosis complex (TSC1/2) (Mak and Yeung 2004; Manning and Cantley 2003; Levine et al. 2006). Mutations in these components or in the tumor suppressor protein PTEN, a negative regulator of PI3K, may result in their dysregulation. Various preclinical models have confirmed the role of this pathway in tumor development (Manning and Cantley 2003; Podsypanina et al. 2001; Chan 2004).

There is evidence that the mTOR pathway holds several feedback loops and that it is interconnected with various other signaling pathways. Inhibition of mTORC1 by everolimus releases the inhibitory action of S6K on IRS1, allowing further activation of PI3K and compensatory activation of AKT and its downstream targets (Majumder et al. 2004). Inhibition of mTORC1 by everolimus also results in a feedback activation of the mitogen-activated protein kinase (MAPK) pathway (Carracedo et al. 2008). mTORC1 is mainly regulated by TSC1 and TSC2. Loss of function mutations of the TSC1 or TSC2 genes lead to uncontrolled signaling of mTORC1 and formation of harmatomas throughout the entire body.

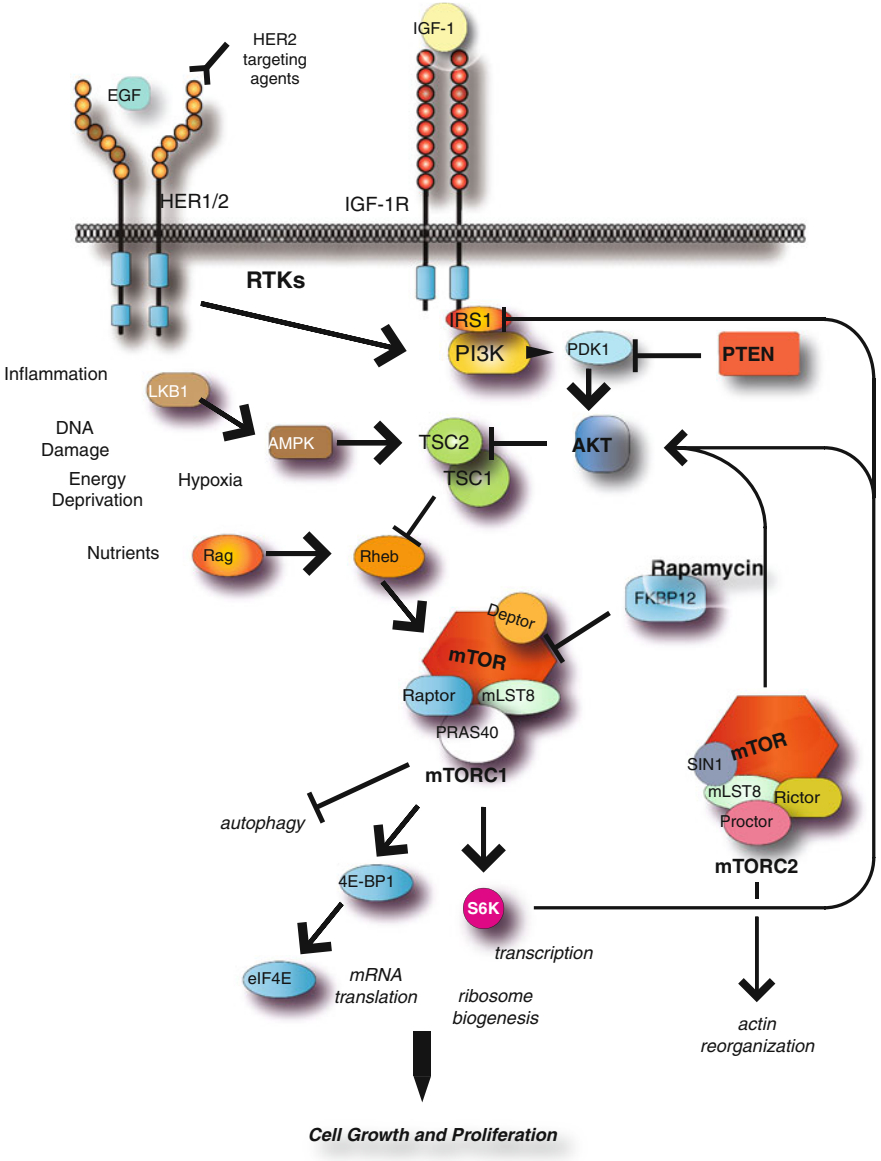


Fig. 1 The mTOR pathway [modified from Laplante and Sabatini (2012), Houghton (2010), O'Reilly and McSheehy (2010), Levine et al. (2006)]. *Deptor* DEP domain-containing mTOR-interacting protein; *EGF* epidermal growth factor; *eIF4E* Eukaryotic translation initiation factor 4E; *4E-BP* eukaryotic initiation factor 4E (eIF-4E) binding protein; *FKBP* FK506 binding protein; *HER* human epidermal growth factor receptor; *IGF(R)* insulin-like growth factor (receptor); *IRS1* insulin receptor substrate 1; *LKB1* liver kinase B1; *AMPK* adenine monophosphate-activated protein kinase, *mLST8* mammalian lethal with SEC13 protein 8; *mTOR* mammalian target of rapamycin; complex; *PDK1* 3-phosphoinositide-dependent protein kinase-1; *PI3K* Phosphatidylinositide 3-kinase; *PRAS* Proline-rich AKT1 substrate 1; *Proctor* protein observed with Rictor; *PTEN* Phosphatase and tensin homolog; *Rag & Rheb* small GTPases; *Raptor* regulatory-associated protein of mTOR; *Rictor* rapamycin-insensitive companion of mTOR; *S6K* Ribosomal protein S6 kinase; *SIN* stress-activated protein kinase interacting protein 1

From an oncologist's perspective, the PI3K/mTOR pathway is an interesting therapeutic target as it is involved in many cellular processes (Bjornsti and Houghton 2004):

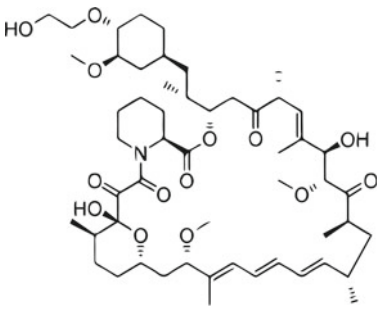
- mTOR functions as a sensor of mitogens, growth factors, and energy and nutrient levels.
- mTOR facilitates G1-S cell cycle progression.
- The PI3K/mTOR/PTEN pathway is frequently dysregulated in human cancers.
- mTOR is involved in the production of pro-angiogenic factors (i.e., VEGF) and inhibition of endothelial cell growth and proliferation.
- mTOR can inactivate eukaryotic initiation factor 4E binding proteins and activate the 40S ribosomal S6 kinases, regulating protein translation, including the HIF-1 proteins.
- Oncogenic transformation may sensitize tumor cells to mTOR inhibition.

2 Structure and Mechanism of Action

Everolimus [RAD001, Afinitor® (40-O-(2-hydroxyethyl)-rapamycin)] is a derivative of rapamycin (sirolimus) (Fig. 2). It is an orally available selective inhibitor of mTOR. Like Rapamycin, it binds FKBP12 and inhibits the mTORC1 complex (Fig. 1), abrogating downstream signaling of this pathway. mTORC1 is a downstream signal transducer of the PI3K pathway, which is frequently activated in human malignancies. Everolimus, like rapamycin, does not affect the activity of mTORC2 complex. Based on its mechanism of action, everolimus is not expected induce rapid cell death but rather to slow tumor growth.

3 Preclinical Data

Everolimus and other rapalogs inhibit the proliferation of various human tumor cell lines and human umbilical vein endothelial cells in vitro. The IC_{50} (dose at which growth is inhibited by 50 %) ranges from sub-nanomolar to micromolar, depending on the cell type. In vitro everolimus reduces expression of HIF1 and VEGF, suggesting that everolimus may also act as an anti-angiogenic agent. This anti-angiogenic activity of everolimus was confirmed in vivo. Mice with primary and metastatic tumors treated with everolimus showed a significant reduction in blood vessel density when compared to controls (Lane et al. 2009). The pharmacokinetic profile of everolimus in rats and mice showed sufficient tumor penetration, above what was needed to inhibit the proliferation of endothelial cells and tumor cell lines in vitro, and below concentrations reached in humans (O'Reilly et al. 2010). Everolimus administered daily p.o. potently inhibited tumor growth in multiple different mouse and rat xenograft models.



International non-proprietary name:	Everolimus
Synonyms:	RAD001
Molecular Weight:	958.2 Daltons
Molecular Formula:	$C_{53}H_{83}NO_{14}$

Chemical Name:	(1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R)-1,18-dihydroxy-12-(1R)-2-[(1S,3R,4R)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]-1-methylethyl}-19,30-dimethoxy-15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-aza-tricyclo[30.3.1.04,9] hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentaone
Formulations:	Four strengths of tablets (2.5 mg, 5 mg, 7.5 mg, 10 mg). Store dry at room temperature, away from heat, moisture, and light.

Source:
CSID:24747358,http://www.chemspider.com/Chemical-Structure.24747358.html (accessed 20:52, May 6, 2013)

Fig. 2 Chemical structure of everolimus

4 Clinical Data

In addition to being a potent immunosuppressive agent, everolimus is currently being investigated as an anticancer agent based on its potential to act directly on the tumor by inhibiting tumor cell growth and proliferation and indirectly by inhibiting angiogenesis (via potent inhibition of tumor cell VEGF production and VEGF-induced proliferation of endothelial cells). At time of writing 212 active interventional, investigator-initiated or industry-sponsored phase I–IV trials were registered at www.cinicaltrials.gov (Table 1). Of those, 147 trials are actively recruiting patients (35 phase I, 43 phase I/II, 50 phase II, 2 phase II/III, 9 phase III, and 8 phase IV).

4.1 Pharmacokinetics and Pharmacodynamics

In a dose-escalation study of everolimus in 92 patients with advanced cancer patients, everolimus was rapidly absorbed after oral administration, with a median time to peak blood levels (tmax) of 1–2 hours after administration. Maximum tolerated dose (MTD) was not reached. The blood concentration was dose proportional over the dose range tested while maximum blood concentration C_{max} appeared to plateau at dose levels higher than 20 mg/week (O'Donnell et al. 2008). The terminal half-life was 30 h (range, 26–38 h) similar to that in healthy volunteers. Inter-patient variability was moderate. High-fat meals alter the absorption of everolimus. Everolimus is metabolized and excreted into the feces >80 %.

Table 1 Active clinical trials with everolimus

Indication	I	I/II	II	II/III	III	IV
Advanced cancer	13	1	4			1
Brain tumors	2	5	2			
Breast cancer	1	3	15		8	2
Gastroesophageal cancer	1	3	2		2	
Hepatocellular cancer (HCC)	1	1	6		1	
GI (CRC, pancreatic cancer, BTC)		5	1			
GYN (Ovarian, endometria, cervical cancer)	2		5			
Head and neck cancer		5	2			
Acute lymphoblastic leukemia	1	1				
Acute myelogenous leukemia	1					
Lung cancer (NSCLC)	3	1				
Non-Hodgkin lymphoma (NHL)	3	10	5		1	
Hodgkin lymphoma (HL)		1	1			
Neuroendocrine tumors (NET)	4	4	7		3	2
Other (CML (uveal) melanoma, EBV-driven tumors, germ cell tumors, PKD, CUP, mesothelioma)	1	1	6	1		
Prostate cancer	2	1	5			
Kidney cancer (RCC)	1	4	19		4	3
Sarcoma		3				
Osteosarcoma		2	1			
Thyroid cancer		3	1			
Tuberous sclerosis complex (TSC)		3	1	1	3	
Urothelial cancer	1	1	2			

GI gastrointestinal cancer; *HCC* hepatocellular cancer; *CRC* colorectal cancer; *BTC* biliary tract cancer; *GYN* gynecological cancer; *NSCLC* non-small-cell lung cancer; *CML* chronic myelogenous leukemia; *EBV* Epstein–Barr virus; *PKD* polycystic kidney disease; *CUP* carcinoma of unknown primary; *RCC* renal cell carcinoma

Pharmacodynamic modeling based on S6 kinase inhibition in peripheral blood mononuclear cells suggested 5–10 mg daily to be an adequate dose to produce a high degree of sustained target inhibition (O'Donnell et al. 2008).

4.2 Clinical Development of Everolimus

Based on the mode of action, preclinical results and early clinical activity of everolimus across different tumor types, Novartis launched the WIDE (Worldwide Initiative to Develop Everolimus) program to develop everolimus in a broad range

of malignancies as well as TSC. Main indications in which everolimus is developed are as follows:

- Breast cancer (BOLERO: breast cancer trials of oral everolimus).
- Gastric cancer (GRANITE: gastric antitumor trial with everolimus).
- Hepatocellular cancer (EVOLVE: everolimus for liver cancer evaluation).
- Liver cancer (EVOLVE: everolimus for liver cancer evaluation).
- Lymphoma (PILLAR: pivotal lymphoma trials of RAD001).
- Neuroendocrine tumors (RADIANT: RAD001 in advanced neuroendocrine tumors).
- Renal cell carcinoma (RECORD: renal cell cancer treatment with oral RAD001 given daily).
- TSC (EXIST: examining everolimus in a study of TSC).

The majority of these trials are in late stage, have either results or results are shortly awaited. In the following, the major indications in which everolimus has been or is being investigated, either as single agent or in combination with other agents, will be discussed.

4.2.1 Clinical Studies in Breast Cancer

Hormone-Receptor-Positive, HER2-Negative Breast Cancer

The development of everolimus in breast cancer followed a very strong lead from preclinical results, which translated nicely into early clinical activity. Proliferation of breast cancer cells is driven by the estrogen receptor (ER) and the human epidermal growth factor receptor (HER) family. The PI3K/AKT/mTOR pathway modulates these signals and can support resistance to endocrine therapy. mTORC1 activates S6K, which then can phosphorylate and activate the ER. Combination of everolimus with aromatase inhibitors inhibited proliferation and induced apoptosis in MCF7 cells (Boulay et al. 2005).

A phase I trial of everolimus in combination with letrozole reported promising clinical responses, with a manageable safety profile of the combination (Awada et al. 2008). Based on these results, a neoadjuvant, randomized phase II trial (NCT00107016) was launched. A total of 270 postmenopausal women were randomized to receive either 4 months of letrozole (2.5 mg/day) plus everolimus (10 mg/day) or letrozole plus placebo. Response rate and biomarker inhibition were higher in the everolimus arm (Baselga et al. 2009).

The BOLERO-2 trial was the logical continuation of these trials of everolimus in combination with hormonal therapy. This randomized phase III trial compared the efficacy of exemestane (25 mg/day) in combination with everolimus (10 mg/day) versus exemestane in combination with placebo. A total of 724 patients with HR-positive, advanced progressive or recurrent breast cancer who were refractory to letrozole or anastrozole were randomized 2:1 to everolimus or placebo. The primary end point was progression-free survival. Both arms were well balanced. At time of a preplanned interim analysis after 359 PFS events had been reported, median PFS was 6.9 months with exemestane plus placebo versus A total of

2.8 months with exemestane plus placebo (HR 0.43; 95 % CI 0.35–0.54; $p < 0.001$) based on local assessment, and 10.6 versus 4.1 months according to central assessment (HR 0.36; 95 % CI 0.27–0.47; $p < 0.001$) (Baselga 2012). This led to the approval of everolimus in combination with exemestane for treatment of postmenopausal women with advanced hormone-receptor (HR)-positive, HER2-negative breast cancer with recurrence or progression after treatment with letrozole or anastrozole in July 2012 by the FDA and the EMA.

Multiple other trials of everolimus in various combinations are currently active, e.g., BOLERO-4 (Open-label, Phase II, Study of Everolimus Plus Letrozole in Postmenopausal Women With ER+ Metastatic Breast Cancer), BOLERO-6 (A Phase II Study of Everolimus in Combination With Exemestane Versus Everolimus Alone Versus Capecitabine in Advance Breast Cancer), and VICTORIA (Study to Compare Vinorelbine In Combination With the mTOR Inhibitor Everolimus versus Vinorelbine monotherapy for Second-line Treatment in Advanced Breast Cancer).

HER2-Positive Breast Cancer

Preclinical studies suggested that PI3K inhibitors could overcome PTEN loss-induced resistance to trastuzumab in HER2-positive breast cancer cells in vitro and in vivo (Lu et al. 2007; Nagata et al. 2004). Clinical evidence of activity of everolimus in combination with a trastuzumab-containing regimen came from two phase I/II studies.

Study NCT00426556 was a single-arm, open-label dose-escalation trial designed to evaluate the feasibility, dose, and schedule for combining everolimus with weekly paclitaxel and trastuzumab (Andre et al. 2010). A total of 33 patients with HER2-positive advanced breast cancer previously treated with trastuzumab were treated with everolimus 5 mg/day, 10 mg/day, or 30 mg/week in combination with paclitaxel (80 mg/m^2 days 1, 8, and 15 every 4 week) and trastuzumab (2 mg/kg/week). Neutropenia (Grade 3 to 4) was the most common toxicity observed ($n = 17$ patients). On the basis of observed dose-limiting toxicities and overall safety considerations, everolimus 10 mg/day was chosen for further development. Among patients with measurable disease ($n = 27$), ORR was 44 %. Median PFS was promising (34 weeks; 95 % CI 29.1–40.7 weeks).

The second phase I/II study (NCT00426530) investigated trastuzumab and vinorelbine plus everolimus. Fifty patients with HER2-positive metastatic breast cancer pretreated with trastuzumab were enrolled in this Bayesian dose-escalation study to receive everolimus 5 mg/day, 20 mg/week, or 30 mg/week plus vinorelbine (25 mg/m^2 on day 1 and 8 every 3 week) and trastuzumab (2 mg/kg/week). Again, neutropenia (grade 3/4) was the most frequently observed toxicity (DLT), and everolimus 5 mg/day was selected for further development. Disease control was achieved in 83 % of patients; the median duration of response was 32.7 weeks for CR/PR and 38.6 weeks for SD (Jerusalem et al. 2011). Based on these results, 2 phase III trials, BOLERO-1&3 were launched.

The BOLERO-1 phase III trial (NCT00876395) is comparing the efficacy of placebo or everolimus in combination with trastuzumab and paclitaxel, as first-line

therapy advanced HER2-positive breast cancer. Recruitment has been completed, and results are awaited in the near future.

The BOLERO-3 phase III trial (NCT01007942) compared the combination of trastuzumab and vinorelbine with everolimus versus trastuzumab and vinorelbine with placebo in patients with HER2-positive advanced breast cancer previously treated with a taxane and who were resistant to trastuzumab. A total of 569 patients were randomized in a 1:1 ratio and stratified by prior lapatinib use. Primary endpoint of the study was PFS. Study treatment was continued until tumor progression or intolerable toxicity. Study results were recently presented at ASCO 2013 (O'Regan R, et al. ASCO 2013, abstract #505). Arms were well balanced. The primary endpoint was PFS by local assessment. The primary efficacy analysis showed a statistically significant prolongation of median PFS from 5.8 months in the placebo arm to 7.0 months in the everolimus arm corresponding to an estimated 22 % risk reduction for PFS (HR = 0.78; 95 % CI 0.65–0.95; $p < 0.0067$). Subgroup analyses favored the everolimus arm, and no difference in global quality of life was noted. At time of the cut-off date (March 15, 2013), OS data were immature. In the light of newly available HER2-targeting treatment options like pertuzumab (Swain et al. 2013, 2012) and T-DM1 (Verma et al. 2012), the clinical implications of the BOLERO-3 results need to be carefully evaluated.

Triple Negative Breast Cancer

Data on everolimus in triple negative breast cancer might be of interest but await confirmation in larger patient cohorts (Singh et al. 2012 San Francisco Breast Cancer Symposium, abstract #108).

4.2.2 Clinical Studies in Gastric Cancer

Based on results from few smaller phase II trials, which had shown limited activity of everolimus (Doi et al. 2010; Taguchi et al. 2011; Yoon et al. 2012); GRANITE-1 (NCT00879333) was designed. Results of this phase III trial in previously treated patients with advanced gastric cancer were presented at the ASCO Gastrointestinal Cancers Symposium 2012 (J Clin Oncol 30, 2012 suppl 4; abstr LBA3). In this trial, 656 patients were randomized 2:1 to receive everolimus (10 mg/day) plus BSC or placebo plus BSC. Baseline characteristics were well balanced. The primary endpoint, prolongation of OS, was not reached. Median OS was 5.4 months with everolimus versus 4.3 months with placebo (HR 0.90; 95 % CI 0.75–1.08; $p = 0.124$). Secondary endpoints included PFS and ORR. Median PFS per local assessment was 1.7 versus 1.4 months with PBO (HR 0.66; 95 % CI 0.56–0.78; $p < 0.0001$).

4.2.3 Clinical Studies in Liver Cancer

Preclinical evidence for a possible role of mTOR in HCC came from xenograft models, in which everolimus suppressed xenograft growth, provided the rationale for investigation of everolimus in HCC (Huynh et al. 2009; Villanueva et al. 2008). One phase I/II trial in 28 patients with HCC determined 10 mg/day as recommended

dose for phase II. Although possible clinical activity was noted, the trial did not reach its phase II stage (Zhu et al. 2011). One phase III study in HCC compared the efficacy of everolimus (10 mg/day) versus placebo (EVOLVE-1). In this trial, 546 patients with HCC after failure of sorafenib were randomized (2:1) to receive everolimus 7.5 mg/day or placebo. The primary endpoint of prolongation of overall survival was not met.[1]

4.2.4 Clinical Studies in Lymphoma

Preclinical results showed increased sensitivity of everolimus-treated diffuse large B-cell lymphoma (DLBCL) cells to rituximab in vitro (Wanner et al. 2006), and an increased cytotoxic effect when combined with other agents in mantle cell lymphoma (MCL) (Haritunians et al. 2007; Nishioka et al. 2008), and in other models (Crazzolara et al. 2009; Saunders et al. 2011; Xu et al. 2013).

Everolimus showed promising clinical activity as single agent in heavily pretreated Hodgkin lymphoma (HL). Of nineteen patients treated with everolimus (10 mg/day), eight patients achieved a PR and one patient achieved a CR. Median time to progression was 7.2 months (Johnston et al. 2010).

Study NCT00516412 evaluated the activity of everolimus in MCL (Renner et al. 2012). In thirty-five evaluable patients (median age 69), ORR was 20 % (95 % CI 8–37(, median PFS was 5.5 months (95 % CI 2.8–8.2). Another phase II trial investigated everolimus in 77 patients with relapsed/refractory aggressive NHL (47 DLBCL, 19 MCL, 8 FL, 3 other). Median age was 70 years, median number of prior therapies 3 (range 1–15). ORR was 30 % (95 % CI 20–41 %). ORR for patients with DLBCL was 30, 32 % for MCL and 38 % for FL. Median time to progression was 3.4 months (95 % CI 2.1–4.2), median progression-free survival was 3.0 months (95 % CI 2.1–3.9), and median overall survival was 8.1 months (95 % CI 5.3–12.5) (Witzig et al. 2011). Combination of everolimus with rituximab in 26 patients with relapsed DLBCL led to a response rate of 38 % (90 % CI 21–56). Median duration of response was 8.1 months (Barnes et al. 2013).

The PILLAR-1 trial (NCT00702052) was an open-label, single-arm, phase II study evaluating everolimus (10 mg/day) in patients with bortezomib-refractory MCL. The primary endpoint was ORR, secondary endpoints included PFS, OS, and duration of response. Preliminary results were presented at ASH 2010 (O'Connor et al. 2010) and updated at ASH 2012 (Wang et al. 2012). Full results are published in the Novartis Clinical Trial Results Database.[2] In this trial in 58 patients with heavily pretreated MCL, everolimus only showed very modest activity with an ORR of 8.6 % (90 % CI 3.5–17.3), thus failing the primary endpoint.

PILLAR-2 (NCT00790036) is an ongoing randomized, placebo-controlled phase III trial evaluating everolimus as maintenance therapy in patients with poor risk DLBCL who have achieved CR after rituximab-containing first-line therapy.

[1] Novartis press release http://www.novartis.com/newsroom/media-releases/en/2013/1721562.shtml.

[2] http://www.novctrd.com/ctrdWebApp/clinicaltrialrepository/displayFile.do?trialResult=8443

The primary endpoint is disease-free survival (DFS). Secondary endpoints are OS, lymphoma-specific survival, and safety.

4.2.5 Clinical Studies in Neuroendocrine tumors

Phase II Studies in NET

Two initial phase II studies were conducted in NET. The first trial conducted by J. Yao at the MD Anderson Cancer Center evaluated treatment with everolimus 5 or 10 mg/day plus depot octreotide 30 mg (LAR) every 28 days in patients with metastatic or unresectable, well-differentiated, neuroendocrine tumors (Yao et al. 2008). The overall median PFS of patients treated with octreotide LAR and everolimus was 60 weeks (95 % CI 54–66 weeks). Stratified by tumor group, median PFS of patients with carcinoid and islet cell tumors was 63 weeks (95 % CI 55–71 weeks) and 50 weeks (95 % CI 31–70 weeks), respectively (HR 1.2; 95 % CI 0.7–2.2).

An additional open-label, non-randomized phase II study in 160 patients with pancreatic neuroendocrine tumors (PNET) stratified by ongoing octreotide therapy at study entry (Yao et al. 2010). Patients who were not being treated with octreotide at study entry were assigned to Stratum 1 ($n = 115$, everolimus 10 mg/day), and patients treated with octreotide LAR for at least 3 consecutive months at study entry were assigned to Stratum 2 ($n = 45$, everolimus 10 mg/day and octreotide LAR every 28 days). Median PFS was 9.7 months (95 % CI 8.3–13.3 months) in Stratum 1, and 16.7 months (95 % CI 11.1 months-NA) in Stratum 2. Median OS in Stratum 1 was 24.9 months (95 % CI 20.2–27.1 months). Median OS had not been reached for Stratum 2 at the time of data cutoff.

Phase III Studies in NET

Two Phase III clinical trials have investigated the efficacy and safety of everolimus in NETs, the RADIANT 2&3 trials.

RADIANT-3 was an international, multicenter, double-blind, phase III study to compare the efficacy of everolimus against placebo in patents with advanced progressive PNET (Yao et al. 2011). A total of 410 patients from 18 countries were randomly assigned to receive everolimus (207 patients) or placebo (203 patients) until disease progression or intolerable toxicity. Patients assigned to placebo were allowed to crossover to everolimus upon progression. The median PFS (the primary end point) by local investigator was 11.0 months (95 % CI 8.4–13.9) in the everolimus group, as compared with 4.6 months (95 % CI 3.1–5.4) in the placebo group (HR 0.35; 95 % CI 0.27–0.45; $p < 0.001$). Median overall survival was not reached, and no significant difference between the groups was observed (HR 1.05; 95 % CI 0.71–1.55; $p = 0.59$).

Based on this trial, everolimus was approved in 2011 by the FDA for the treatment of progressive neuroendocrine tumors of pancreatic origin (PNET) in patients with unresectable, locally advanced or metastatic disease and by the EMA for the treatment of unresectable or metastatic, well- or moderately differentiated neuroendocrine tumors (NET) of pancreatic origin in adults with progressive disease.

RADIANT-2 was a prospective, randomized, double-blind, multicenter, placebo-controlled phase III study to evaluate the safety and efficacy of everolimus 10 mg/day plus octreotide LAR or matching placebo plus octreotide LAR in patients with advanced carcinoid tumor (Pavel et al. 2011). Patients enrolled had to have a progressive, advanced, well-differentiated carcinoid tumors and had to have symptoms related to carcinoid syndrome at enrollment or prior to enrollment ("functional NET"). Four hundred and twenty-nine patients with advanced functional NET were enrolled to this study worldwide, 216 were randomized to treatment with octreotide +everolimus and 213 to treatment with octreotide plus placebo. Primary endpoint was again PFS. This trial was complicated by several factors: Imbalances at baseline and opposing/conflicting results in local and central response assessment interpretations. Results as per the amended primary endpoint [PFS assessed by an independent adjudication radiology committee (IAC)] showed a 5.1-month prolongation in median PFS from 11.3 months for octreotide plus placebo to 16.4 months for octreotide plus everolimus (HR 0.77). Nevertheless, statistical significance was not reached, as the prespecified statistical boundary was missed. No statistically significant difference was evident in terms of overall survival, although numerically more deaths were reported from the everolimus treatment group (HR 1.22; 95 % CI: 0.91, 1.62; $p = 0.908$).

The RADIANT-4 trial (NCT01524783) is currently recruiting patients with advanced non-functioning NET of gastrointestinal or lung origin to compare the efficacy of everolimus + best supportive care (BSC) versus placebo + BSC. As this trial excludes patients with functional NET, somatostatin analogs are not allowed as concomitant medication. Other recruiting trials evaluating everolimus in NET are, for example, the LUNA trial (lung and thymic NET, NCT01563354) and the COOPERATE trials (gastroenteropancreatic NET, NCT01374451, NCT01263353).

4.2.6 Clinical Studies in Kidney Cancer

Based on strong preclinical rationale and early clinical results, several phase II and III trials in RCC were launched.

RECORD-1 (NCT00410124) was a randomized, double-blind, placebo-controlled phase III trial of everolimus in patients with metastatic RCC after progression on VEGF-targeted therapy. Four hundred and sixteen patients were randomized 2:1 to receive everolimus (10 mg/day) ($n = 272$) or placebo ($n = 138$). The primary endpoint was PFS, assessed by central review. Results at the second prespecified interim analysis suggested a significant difference in efficacy between arms, and the trial was stopped early after 191 PFS events had been observed. Median PFS was 4.0 months (95 % CI 3.7–5.5) versus 1.9 months (95 % CI 1.8–1.9) (Motzer et al. 2008). Final results confirmed the early results with a median PFS of 4.9 months (95 % CI 4.0–5.5) with everolimus versus 1.9 months (95 % CI 1.8–1.9) with placebo (HR 0.33; 95 % CI 0.25–0.43; $p < 0.001$). OS was similar in both arms (Median OS 14.8 vs. 14.4 months; HR 0.87; 95 % CI 0.65–1.15; $p = 0.162$) but was likely confounded by a high percentage (80 %) crossover to everolimus (Motzer et al. 2010). Based on

RECORD-1, the FDA and EMA approved everolimus for the treatment of patient with advanced RCC after failure of sunitinib or sorafenib.

The first data on combination of everolimus and bevacizumab in RCC came from trial NCT00323739 (Hainsworth et al. 2010). Eighty patients with advanced RCC (50 treatment naïve, 30 previously treated) received bevacizumab (10 mg/kg on days 1 and 15) and everolimus (10 mg/day). Median PFS in treatment naïve and previously treated patients were 9.1 and 7.1 months. Based on promising preliminary data from this trial, two larger randomized studies investigating the combination of everolimus and bevacizumab were launched.

RECORD-2 (NCT00719264) was a randomized, open-label, multicenter phase II study comparing the efficacy and safety of everolimus in combination with bevacizumab (EB) versus interferon-α in combination with bevacizumab (IB) as first-line treatment for patients with metastatic RCC. Patients were stratified according to their MSKCC risk status (favorable vs. intermediate vs. poor). Primary endpoint was PFS; secondary endpoints included OS, ORR, and duration of response, safety, and QoL. Final results for OS and safety were presented at ASCO 2013 demonstrating that EB was not superior to IB. Median OS was 27.1 months (95 % CI 19.9–35.3) in the EB arm, and 27.1 months (95 % CI 20.4–30.8) in the IB arm (HR 1.01; 95 % CI 0.75–1.34; $p = 0.96$) (Ravaud et al., ASCO 2013, abstract # 4576). Both arms showed similar PFS, response rates, and time to definitive deterioration of QoL.

The CALGB-90802 study (NCT01198158), a large randomized phase III trial, is comparing everolimus plus bevacizumab versus everolimus plus placebo after failure of $\geq$ 1 prior VEGFR TKI.

RECORD-3 (NCT00903175) was recently presented at ASCO 2013 (Motzer et al., ASCO 2013 abstract #4504). RECORD-3 was a randomized phase II trial comparing sequential first-line everolimus and second-line sunitinib versus first-line sunitinib and second-line everolimus in patients with metastatic RCC. Primary objective was to show PFS non-inferiority of first-line everolimus compared with first-line sunitinib. Secondary objectives included the comparison of combined PFS for the two sequences of treatment, ORR, and OS. A total of 471 treatment-naïve patients with metastatic RCC were included. The trial failed to show non-inferiority. Median PFS in first-line with everolimus was 7.85 months compared to 10.71 months with sunitinib (HR = 1.43; 95 % CI 1.15–1.77). ORR clearly favored sunitinib (26.6 %; 95 % CI 21.1–32.8) over everolimus (8 %; 95 % CI 4.9–12.2). The analysis of the combined PFS also clearly favored sunitinib as first-line. PFS in the everolimus-sunitinib arm was 21.13 months compared to 25.79 months in the sunitinib-everolimus arm (HR = 1.28; 95 % CI 0.94–1.73). Results presented clearly showed that a VEGF-TKI should be standard first-line treatment for advanced RCC, and that everolimus is a good option for second-line therapy.

The currently recruiting RECORD-4 (NCT01491672) trial will assess efficacy (PFS) of everolimus in second-line treatment of advanced RCC in three different cohorts. Patients are enrolled in one of three cohorts based upon their first-line therapy: (1) prior cytokines, (2) prior sunitinib, or (3) prior anti-VEGF therapy other than sunitinib.

4.2.7 Clinical Studies in TSC

TSC is an autosomal dominant genetic disorder that results from mutations in the TSC1 or TSC2 genes. TSC is characterized by development of benign tumors (harmatomas) throughout the body. Manifestations of TSC vary from individual to individual, ranging from mild symptoms to physical and intellectual disabilities (Orlova and Crino 2010). Approximately 1/3 of cases are inherited, whereas 2/3 are de novo mutations. TSC1 mutations appear to be more common in familial (inherited) cases of TSC, while mutations in the TSC2 gene occur more frequently in sporadic cases. Inactivating mutations in TSC1 and TSC2 release their inhibitory effect on mTORC1 and subsequent hyperproliferation. Accordingly, mTOR inhibitors were very attractive molecules to find novel treatment options for TSC. Meikle and colleagues demonstrated very good activity of rapalogs in a mouse model for TSC1 (Meikle et al. 2008), where median survival was prolonged from 33 to >100 days. Rapamycin also improved cognitive defects in a TSC2-deficient mouse model (Ehninger et al. 2008). Building on this strong preclinical rationale, an investigator-initiated phase I/II trial (NCT00411619) in children and adults with TSC suffering from subependymal giant cell astrocytomas (SEGA) was conducted. A total of 28 patients were enrolled to receive everolimus 3 mg/day. There was a clinically meaningful reduction in volume of the primary SEGA ($p < 0.001$ for baseline versus 6 months (Krueger et al. 2010). Based on these results, a full clinical development program (EXIST) was launched.

EXIST-1 was a randomized, double-blind phase III trial to assess the efficacy and safety of everolimus in patients with SEGA associated with TSC. A total of 117 patients were randomized 2:1–4.5 mg/m^2/day (titrated to achieve blood trough concentrations of 5–15 ng/ml) everolimus ($n = 78$) or placebo ($n = 39$). A total of 27 (35 %) patients in the everolimus arm had a ≥50 % reduction in SEGA volume versus none in the placebo group ($p < 0.0001$) (Franz et al. 2013).

EXIST-2 (NCT00790400) was a randomized phase III trial in adult patients with angiomyolipoma associated with TSC. A total of 118 patients were randomized 2:1 to receive everolimus 10 mg/day ($n = 79$) or matching placebo ($n = 39$). The primary endpoint was the proportion of patients with confirmed ≥50 % reduction in total volume of target angiomyolipomas relative to baseline. The angiomyolipoma response rate was 42 % (95 % CI 31–53) for everolimus versus 0 % (95 % CI 0–9) in the placebo group (Bissler et al. 2013).

Based on EXIST-1&2, everolimus was approved for treatment of adults with renal angiomyolipoma and TSC, not requiring immediate surgery, and pediatric and adult patients with TSC who have SEGA that requires therapeutic intervention but cannot be curatively resected.

There is accumulating evidence that mTOR activation might be involved not only in TSC development but also drive seizures in TSC patients (Wong 2012). EXIST-3 is a three-arm, randomized, double-blind, placebo-controlled study of the efficacy and safety of 2 trough ranges of everolimus as adjunctive therapy in patients TSC who have refractory partial-onset seizures. Patients are randomized 1:1:1 to receive either everolimus titrated to 3–7 ng/ml or to 9–15 ng/ml, or

matching placebo. This multicenter study will enroll 345 patients globally, at approximately 125 sites. Participants must have a definite diagnosis of TSC based on the modified Gomez criteria and a diagnosis of partial-onset epilepsy according to the classification of the International League Against Epilepsy prior to enrollment. Primary objective is to compare the reduction in frequency of partial-onset seizures on each of 2 trough ranges of everolimus versus placebo in patients with TSC who are taking 1–3 anti-seizure drugs.

5 Toxicity

Everolimus has been investigated in over 30,000 patients in clinical studies and in post-marketing experience. In cancer patients, the main adverse events reported with everolimus were: stomatitis, non-infectious pneumonitis, infections, and renal failure. In addition, laboratory abnormalities, mainly hyperglycemia, hyperlipidemia, anemia, neutropenia, and thrombocytopenia were reported. For a recent and complete list of adverse drug reactions, please refer to your local drug label or package insert.

6 Drug Interactions

Everolimus is mainly metabolized by CYP3A4 in the liver and to some extent in the intestinal wall. Everolimus is also a substrate of P-glycoprotein (PGP). Therefore, absorption and subsequent elimination of systematically absorbed everolimus may be influenced by medications that interact with CYP3A4 and/or PGP. In vitro studies showed that everolimus is a competitive inhibitor of CYP3A4 and of CYP2D6 substrates, potentially increasing the concentrations of medicinal products eliminated by these enzymes. Strong inhibitors of CYP3A4 (azoles, antifungals, cyclosporine, erythromycin) have been shown to reduce the clearance of everolimus therapy, thereby increasing everolimus blood levels. Similarly, Rifampin, a strong inducer of CYP3A4, increases the clearance of everolimus thereby reducing everolimus blood levels. Caution should be exercised when co-administering everolimus with CYP3A4 inhibitors or inducers.

7 Biomarkers

To date, no valid predictive or prognostic biomarker for everolimus across all indications tested has been identified. S6K1 activity in peripheral blood mononuclear cells seems one of the most reliable biomarkers for target inhibition by everolimus (O'Reilly and McSheehy 2010). Hortobagyi presented a tissue-based biomarker analysis at ASCO 2013 (Abstract #505). In this retrospective exploratory analysis, 309 archival tissue samples from BOLERO-2 3230 exons of 182 oncogenes and tumor suppressor genes were analyzed using next generation

sequencing. No predictive marker for response to treatment with everolimus could be identified, as treatment effect was similar in all molecular subgroups analyzed. Only a small subset of patients whose tumors showed amplification of the FGF receptors (FGFR) 1 or 2 seemed to derive smaller benefit of everolimus than patients with FGFR wild type tumors (HR 0.59, 95 % CI 0.31–1,14 vs. HR 0.36, 95 % CI 0.24–0.53, $n = 48$ vs. 114).

8 Summary and Perspectives

Everolimus is an inhibitor of the mTOR pathway, specifically mTORC1. Based on its ubiquitous expression and central role multiple cellular signaling pathways, mTOR is an interesting target for cancer therapy. So far, clinical investigations based on sound preclinical rationale have led to the approval of everolimus for the treatment of the following:

- postmenopausal women with advanced HR-positive, HER2-negative breast cancer (advanced HR+ BC) in combination with exemestane after failure of treatment with letrozole or anastrozole
- adults with progressive neuroendocrine tumors of pancreatic origin (PNET) that are unresectable, locally advanced or metastatic. The safety and effectiveness of AFINITOR in the treatment of patients with carcinoid tumors have not been established
- adults with advanced renal cell carcinoma (RCC) after failure of treatment with sunitinib or sorafenib
- adults with renal angiomyolipoma and TSC, not requiring immediate surgery
- pediatric and adult patients with TSC who have SEGA that requires therapeutic intervention but cannot be curatively resected.

Several registration trials have failed, as the data at the time of decision to move into a phase III trial were not too convincing. Nevertheless, compared to other development programs in the industry, the story if everolimus is clearly a success story. More data from phase III trials are awaited in the not too far future. Hopefully, these trials will open new treatment options for our patients.

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