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Everolimus, a further step in ‘targeted’ anti-neoplastic therapy?

Everolimus is a representative of a new class of antineoplastic agents offering new therapeutic options even for as-yet refractory carcinomas. A look at the biochemical pathways involved helps understand desired and adverse effects.

Introduction

On 30 March 2009, FDA approved everolimus (Afinitor) for the treatment of refractory renal cell carcinoma (RCC) [1]. For Europe, the approval by EMEA is expected imminently (positive opinion by the CHMP dated 29 May 2009). The mechanism of action is relatively new in cancer therapy, since everolimus is only the second compound of this group reaching marketability. It is anticipated that the area of application will not be limited to RCC.



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membered macrolide, containing both a lactone and a lactam, and 15 chiral centres. Everolimus differs from rapamycin, as does temsirolimus, only in the substituent on the cyclohexane ring side chain, but while in temsirolimus the hydroxyl group on this cyclohexane ring is esterified with dihydroxypivalic acid (2,2-bis(hydroxymethyl)propionic acid), in everolimus this hydroxyl group is etherified with ethylene glycol (see Figure 1). Therefore, everolimus can also be called a hydroxyethyl rapamycin. It is evident that an ether is much more stable than an ester.

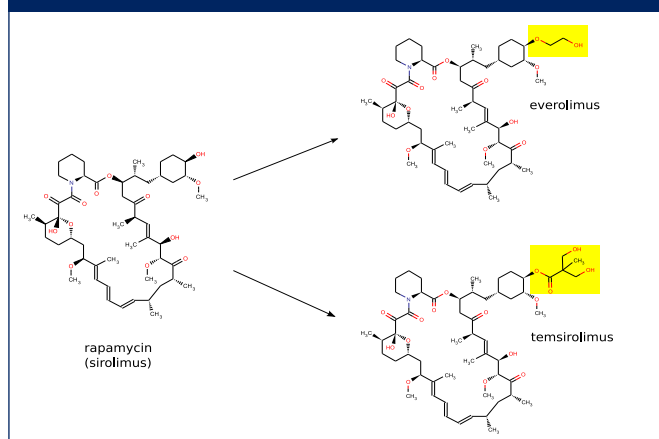
From Easter Island to mTOR inhibition

In the 1970s a new strain of the bacteria *Streptomyces hygroscopicus* was discovered in a soil sample taken from Easter Island. An antibiotic was isolated from this bacteria, and because of the Micronesian name of Easter Island, Rapa Nui, the new substance was called rapamycin. Rapamycin was identified as a potent antifungal agent. Later it was found to be also a potent suppressor of the immune system. Thus sirolimus (INN) was approved in 2000 by FDA and in 2001 by EMEA for the prophylaxis of organ rejection in patients receiving a renal transplant (Rapamune). Derivatives were also developed. One of them is everolimus, which was approved in 2004 in several European countries, but not in the US, for the prophylaxis of organ rejection in patients receiving a renal or heart transplant (Certican). At first, the mechanism of action of rapamycin and its analogues was not really clear. In 1993, during a screen for resistance to the antibiotic effect of rapamycin, it was found to bind to and inhibit two distinct enzymes in budding yeast, and so those enzymes were called ‘target of rapamycin’, TOR1 and TOR2 (Kunz et al., Helliwel et al., as cited in [2, 3]). Shortly afterwards, in 1994, a mammalian homologue was discovered, and as a consequence it got the name ‘mammalian target of rapamycin’ or mTOR (Brown et al., Chiu et al., Sabatini et al., as cited in [2, 3]). It was found that dysfunctions in the mTOR pathways were associated with several cancers. So the research on mTOR inhibitors such as rapamycin and its analogues got a new boost, and they were tested as potentially antineoplastic drugs. In 2007, the first rapamycin analogue got the approval for treatment of advanced RCC by FDA and EMEA: temsirolimus (Torisel). Everolimus is now the second mTOR inhibitor approved for cancer therapy.

Chemistry

As already mentioned, everolimus is a derivative of rapamycin, which is obtained via fermentation from a strain of the bacteria *Streptomyces hygroscopicus*. Rapamycin is a 31-

Figure 1: Chemical formulas of rapamycin, everolimus and temsirolimus



Pharmacokinetics

Everolimus is administered orally. For detailed pharmacokinetic data compared to temsirolimus (see Table 1). A high fat meal reduced C_{max} by 60% and AUC by 16% of the 1 mg dose. The blood-to-plasma ratio is concentration dependent. At blood concentrations observed in cancer patients following the recommended dose of 10 mg the blood-to-plasma ratio is about 20% [1].

Everolimus is a substrate of CYP3A4. Metabolites detected in human blood were scarcely active and included monohydroxylated and hydrolytic ring-opened products as well as a phosphatidylcholine conjugate. The main circulating compound is everolimus itself, accounting for about 40% of the AUC [1].

After hepatic metabolism of everolimus the metabolites are mainly excreted in the faeces. For patients with moderate hepatic impairment (Child–Pugh class B) the average AUC was found to be doubled which suggests a dose reduction to

Table 1: A comparison of everolimus and temsirolimus

INN	Everolimus	Temsirolimus
Other names	RAD001	CCI-779
Brand name	Afinitor	Torisel
Marketing authorisation holder	Novartis	Wyeth
FDA/EMEA approval	30 March 2009/ Expected 2009	19 November 2007
Indication	Advanced renal cell carcinoma after failure of treatment with sunitinib or sorafenib	First-line treatment of patients with advanced renal cell carcinoma who have at least three of six prognostic risk factors
Administration/dosage form	p.o./tablets	IV/concentrate and diluent for solution for infusion
Recommended dose	10 mg /day	25 mg/week
Regime	Continuous treatment	Continuous treatment
Available strengths	5 mg, 10 mg	30 mg/1.2 mL (25 mg/1 mL)
Storage	15–30°C Protect from light and moisture	2–8°C Protect from light
Shelf life	2 years	2 years 24 hours after reconstitution 6 hours (solution for injection)
Fraction absorbed	11%	n/a
T _{max} * (h)	1 (0.5 – 2.5)	n/a
C _{max} (ng/mL)*	64.4 ± 17.8	592.4 ± 101.9 (temsirolimus) 57.4 ± 14.3 (sirolimus)
AUC (ng·h/mL)*	510.1 ± 165.8	2276 ± 340 (temsirolimus) 5479 ± 1799 (sirolimus)
Clearance (L/h)*	20.6 ± 6.8	11.4 ± 2.4 (temsirolimus) 4.9 ± 1.2 (sirolimus)
T _{1/2} (h)*	36.9 ± 9.5	17.7 ± 4.5 (temsirolimus) 73.3 ± 23.2 (sirolimus)
Active metabolites	None	Sirolimus
Steady state	Within 2 weeks	n/a
Plasma protein binding	74%	87% (at 100 ng/mL)

* after single recommended dose in healthy subjects

5 mg. Everolimus should not be administered to patients with severe hepatic impairment (Child–Pugh class C). No dose reduction is required for patients with renal impairment [1].

Mechanism of action

Everolimus inhibits the so called mammalian target of rapamycin (mTOR). mTOR is a serine-threonine kinase, thus an enzyme catalysing the phosphorylation of proteins at amino acids serine and threonine, leading to activation or deactivation of this protein. mTOR exists in two distinct complexes, mTOR complex 1 and 2 (mTORC1, mTORC2). The two complexes are part of two contiguous, but distinct signalling pathways. It is remarkable that only mTORC1 is inhibited by rapamycin and its derivatives. However, recent investigations suggest that rapamycin can perturb mTORC2 assembly [2].

Everolimus does not bind straight to mTORC1. The real receptor for everolimus is the immunophilin FK506 binding protein (FKBP12), with which it forms a complex. This complex binds a region in the C terminus of mTORC1 called FRB (FKBP12 rapamycin binding), thereby inhibiting mTORC1 activity.

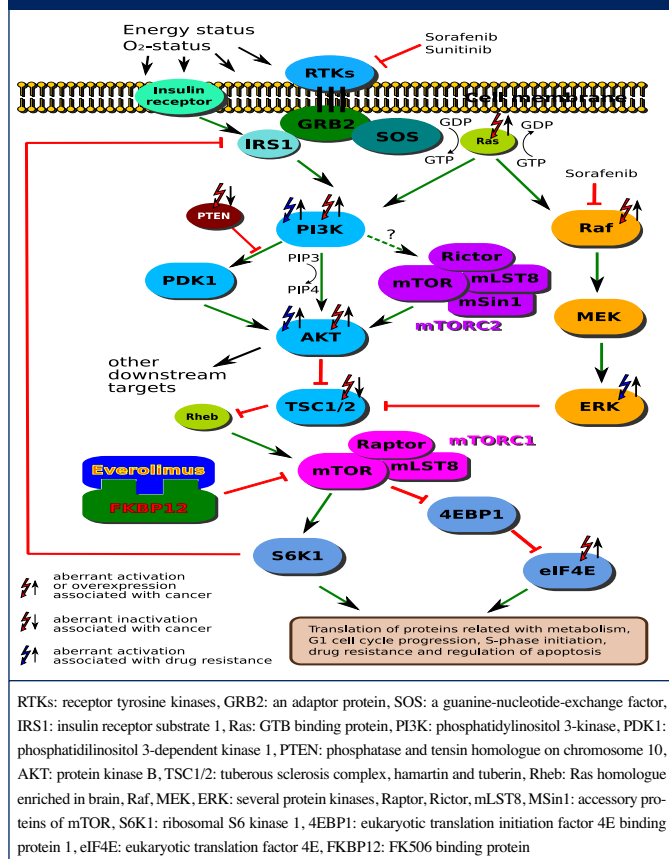
mTORC1 is a downstream target of the PI3K-AKT signalling pathway, a key regulator of cell cycle proliferation, cell growth and survival as well as glucose metabolism. It is frequently deregulated at various points in a wide range of tumour types (see Figure 2). The key enzymes are PI3K and AKT. Activation of several receptor tyrosine kinases (RTKs), for example IGF-1R (insulin-like growth factor 1 receptor) or EGFR (epidermal growth factor receptor), leads, amongst others via the GTP-binding protein Ras, to activation of PI3K. The major downstream target of PI3K is AKT. The activation of AKT via PI3K can be inhibited by PTEN protein. The encoding gene PTEN is known as tumour suppressor gene, and its loss or mutation is associated with several human cancers. AKT can also be activated by mTORC2. AKT has several downstream targets. Amongst others it inhibits the TSC1-TSC2 complex. By suppressing the GTP-binding protein Rheb, TSC1/2 is a negative regulator of mTORC1. Since AKT inhibits an inhibitor of mTORC1, the bottom line of AKT-activation is mTORC1-activation.

Beside the PI3K-AKT pathway there is another pathway leading to inhibition of TSC1/2 (and therefore to activation of mTORC1): the activation cascade RTKs-Ras-Raf-MEK-ERK.

Various points of the pathways outlined are deregulated in several human cancers [3]. Some of them are marked in Figure 2. All of them cause (over)activation of mTORC1. The main downstream targets of mTORC1 are 4EBP1 and p70S6K1 (S6K1).

Phosphorylation of S6K1 by mTOR leads to activation. S6K1 has several targets, including ribosomal proteins such as S6, elongation factors and insulin growth factor 2. 4EBP1 is inhibited by mTOR through phos-

Figure 2: Simplified view of mTOR pathways according to [2, 3]



phorylation and itself inhibits eIF4E. The mTORC1 pathway regulates the translation of mRNA encoding proteins required for G1 cell cycle progression and S-phase initiation via S6K1 and 4EBP1-eIF4E. Thus inhibition of mTOR activity by everolimus results in the arrest of G1 growth.

Efficacy

Preclinical *in vitro* studies demonstrated that everolimus inhibits most (but not all) of human tumour cell lines tested. The antiproliferative effect did not correlate with inhibition of S6K1 and 4EBP1 activation, but very well with the levels of AKT Serine 473 phosphorylation and S6 Serine 240 and 244 phosphorylation [2]. *In vitro* studies also showed antiangiogenic activity and inhibition of endothelial cell proliferation.

In a first phase I study in patients with advanced solid tumours everolimus was given weekly. It was well tolerated at a dose from 5 mg up to 30 mg. A second phase I study evaluated how the phosphorylation of the downstream targets 4EBP1, S6K and eIF4E was inhibited by weekly doses of 20, 50 or 70 mg and daily doses of 5 or 10 mg. Doses of 10 mg daily and 50–70 mg weekly resulted in almost complete inhibition of phosphorylated S6K and phosphorylated 4EBP1.

In several phase II studies the 10 mg daily regimen was further evaluated.

Based on these studies a double-blind, randomised, placebo-controlled phase III trial was initiated in 2006 for treatment of advanced RCC after progression on sunitinib, sorafenib or both (RECORD-1 Study) [4]. At this time standard treatment for advanced RCC was therapy with sunitinib (Sutent), sorafenib (Nexavar) or bevacizumab (Avastin) plus IFN- $\alpha 2\alpha$ (Roferon-A) [7], since temsirolimus (Torisel) was not approved at this time. After failure or progression there were no therapeutic alternatives. Four hundred and ten patients were randomly assigned in a two to one ratio either to oral everolimus 10 mg per day or to placebo. Primary end point was progression-free survival (PFS). Crossover to open label everolimus after disease progression was allowed. Final analysis was planned after 290 progression events, but after the second interim analysis the study was terminated, because the pre-specified efficacy stopping boundary was crossed. At the time of data cut off median PFS in the everolimus arm was four months compared to 1.9 months in the placebo arm (independent central review). This effect was almost exclusively due to stable disease, partial response was only seen in three patients (1%). Quality of life was sustained during therapy with everolimus. Due to the concession of crossover it was not possible to assess a benefit in overall survival.

Adverse effects

The term ‘targeted therapy’ suggests that a molecule graced by this term affects only aberrant cells and has only few and moderate adverse effects. This has only been a theoretical approach so far, as the targets are also present in normal cells playing a role in homeostasis.

Some of the adverse effects of everolimus can be deduced from its mechanism of action. Since mTOR downstream targets are involved in glucose and lipid metabolism, potential increases in cholesterol, triglycerides and glucose are not surprising. In fact elevation of these parameters was found in over 50% by blood tests, but fortunately clinically significant cases can mostly be managed and severe effects are rare. Everolimus also acts as an immunosuppressant, which increases the risk of potentially severe infections, especially with opportunistic pathogens.

Other frequent or severe adverse effects cannot easily be deduced from the mechanism of action.

The most common ($\geq 20\%$) adverse events observed in the RECORD-1 study were stomatitis (38%), anaemia (38%), asthenia (33%), diarrhoea (30%), cough (30%), rash (29%), nausea (26%), anorexia (25%), peripheral oedema (25%), pyrexia (20%), vomiting (20%), and hypercholesterolemia (20%). The most severe (grade 3/4) adverse reactions were anaemia (10%), dyspnoea (8%), hyperglycaemia (6%), fatigue (6%), and lymphopenia (4%). Pneumonitis (3%), dyspnoea (3%), lung disease (1%), fatigue (1%) and renal failure (1%) were the toxicities leading to treatment termination [1].

One specific side effect needs a closer look: non-infectious pneumonitis is a known class effect of rapamycin derivatives. It was reported in 14% of patients, also occurring at grade 3/4 (4%). As

this side effect can be fatal, appropriate monitoring is recommended. But it is notable that new or worsening CT changes were reported in almost 50%, although clinically reported pneumonitis occurred in only 14%. So CT results are only a hint of, but not evidence for, this adverse effect.

Drug interactions

Everolimus is a substrate of CYP3A4 and the multidrug efflux pump PgP, so strong or moderate inhibitors of CYP3A4 and PgP should not be used together with everolimus. With strong inducers of CYP3A4 or PgP a dose increase of everolimus should be considered. On the other hand everolimus is also an inhibitor of CYP3A4 and PgP, but there were no clinically significant pharmacokinetic interactions found due to this mechanism [1].

Future prospects

Other indications

Beside the approved treatment of refractory RCC, everolimus is also being investigated for the treatment of several other cancers. Recently the results of a phase II trial have been reported: 145 patients with relapsed lymphoma were treated with everolimus. The overall response rate was 33%, in the subgroup with Hodgkin's disease even 53%. Due to this outcome a phase III trial was initiated evaluating everolimus for adjuvant therapy in poor risk patients with diffuse large B-cell lymphoma [5].

Other phase III studies are investigating the efficacy of everolimus in the treatment of advanced gastric cancer, metastatic colorectal cancer, various neuroendocrine tumours and some benign tumours associated with tuberous sclerosis complex [6].

Combination therapy

Promising investigations deal with the combination of mTOR inhibitors with other antineoplastic agents. This is based on several rationales. The concept of vertical blockade means the use of agents inhibiting two or more different targets in the same signalling pathway with intent to break negative feedback loops. So the combination of everolimus with IGF-1R inhibitors showed an additive growth inhibitory effect [8]. The combination of everolimus with sorafenib (clinical trials phase I/II for RCC, hepatocellular carcinoma, lymphoma and multiple myeloma) is also in this category.

The most interesting concept deals with restoring sensitivity when drug resistance had occurred, for the PI3K-AKT and the Ras-Raf-MEK-ERK signalling pathways not only play a role in the onset of cancer but have also been implicated in multiple anticancer drug resistance. For example activation of AKT and/or PI3K is associated with resistance to trastuzumab, endocrine and paclitaxel therapy in breast cancer, to all-trans-retinoic acid in leukaemia, to imatinib in gastrointestinal stromal tumour, to cisplatin in ovarian, uterine, lung and breast cancer and to etoposide and doxorubicin in gastric cancer. ERK activation is involved in resistance to cisplatin in ovarian cancer, tamoxifen in breast cancer, doxorubicin in prostate cancer, 5FU in pancreatic cancer and to vincristine in leukaemia [3] (see Figure 2).

The results of a phase II trial concerning the neoadjuvant combination of everolimus and endocrine therapy (letrozole) in ER-positive breast cancer showed an increased clinical response rate of 68.1% vs. 59.1% for letrozole monotherapy [9]. Other ongoing clinical trials are investigating the combination of everolimus with exemestane (phase III), trastuzumab (phase I and II), trastuzumab plus paclitaxel (phase I/II and III), and paclitaxel plus cisplatin (phase I and II) in breast cancer. A big phase III trial is exploring the integration of everolimus, bevacizumab and lapatinib into current neoadjuvant chemotherapy regimes for primary breast cancer [6].

Conclusion

Rapamycin and its derivatives offer hope in the treatment of several carcinomas, as the target, mTOR, is part of an important signalling pathway associated with cancer genesis and drug resistance. Everolimus is the first mTOR-inhibitor approved for anti-neoplastic therapy, that can be administered orally. It has also filled the gap in the treatment of RCC resistant to VEGF inhibitors, as temsirolimus is only approved for advanced, but not refractory RCC. If expectations are fulfilled in ongoing trials, a broadening of indications can be expected. Future investigations also may elucidate, if there are further important clinical differences between the rapamycin analogues.

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