Bull Cancer 1999; 86 (10): 808-11

**POINT SUR...**



# Rapamycin and CCI-779

*Rapamycin and CCI-779*

**Jérôme ALEXANDRE**
**Eric RAYMOND**
**Jean-Pierre ARMAND**

Medicine Department, Institut Gustave-Roussy, Villejuif Cedex 94805.

**Abstract** - Rapamycin (sirolirnus) is a macrolide related to cyclosporine with immunosuppressive properties and antiproliferative activity in various human tumor cell lines and tumor xerograph models. The cytosolic kinase mTOR which controls the initiation of the translation of messenger RNA is the main known target of Rapamycin. During clinical studies, Rapamycin given by oral route as immunosuppressant did not show dose-limited toxicity and only asymptomatic thrombopenia and hyperlipemia were observed. In murine models, best antitumoral activity was observed using parental routes. CCI-779, an analog formulated for intravenous use has antitumor activity without significant immunosuppressive property in mice and is currently in Phase I trials in man.▲

**Key words**: Rapamycin, mTOR, CCI-779.

***Abstract*** *– Rapamycin (sirolimus) is a macrolide, related to cyclosporine with immunosuppressive properties and antiproliferative activity in various human tumor cells lines and tumor xenograft models. The cytosolic kinase mTOR which controls the initiation of the translation of messenger RNA is the main known target of rapamycin. During clinical studies, rapamycin given by oral route as immunosuppressant did not show dose-limited toxicity and only asymptomatic thrombopenia and hyperlipemia were observed. In murine models, best antitumoral activity was observed using parental routes. CCI-779, an analog formulated for intravenous use has antitumor activity without significant immunosuppressive property in mice and is currently in phase I trials in man.* ▲

***Key words:*** *rapamycin, mTOR, CCI-779.*

Article received on June 15, 1999, accepted after review on August 9, 1999.

Reprints: J. Alexander

Rapamycin (*Figure 1*) or Sirolimus is a macrolide produced by *Streptomyces hygroscopicus*, similar to cyclosporine and FK506 (tacrolimus). It was initially identified as an antifungal agent thirty years ago. [1] Secondarily, it has mainly been developed as an immunosuppressant agent (Rapamune®, Wyeth-Léderlé). Several clinical trials have shown its value in the treatment of organ graft rejection [2]. Its anti-tumor properties in different cell models have also been known for several years, but have never been the subject of publications on human clinical trials [3]. The originality of rapamycin is its mechanism of action. It specifically inhibits a cytoplasmic protein kinase, mTOR (for *mammalian Target Of Rapamycin*) that is involved in a path of mitogenic signaling which regulates translation initiation [4].

Interest in rapamycin was recently revived with the development of analogs, such as CCI-779 (*Figure 1*), showing, in preclinical models, an antiproliferative activity and a weak immunosuppressive effect [5].

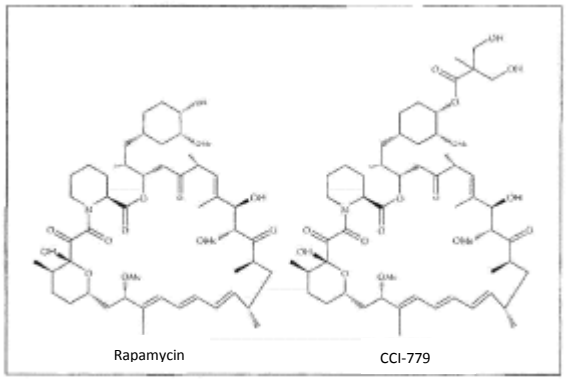



Figure 1. **Rapamycin and CCI-779**

# Rapamycin

## Antitumoral activity

*In vitro*, rapamycin inhibits the growth of several human tumor cell lines, especially osteosarcoma [6], Rh1 and Rh30 rhabdomyosarcoma [7], of H69, H345 and H510 small cell lung cancers [8], of H4 hepatoma [9] and of hormone-dependent MCF7 breast cancer [10]. The range of effective concentrations is from 0.3 to 50 nM. In many of these lines, it has been shown that rapamycin blocks the cells in the G1 phase of the cell cycle. This cytostatic effect was confirmed *in vivo* in human tumor xenograft models in nude mice. [3] A proapoptotic effect was also observed on rhabdomyosarcoma lines grown without growth factor [11] and in combination with cisplatin on an ovarian carcinoma cell line [12].

### Mechanisms of molecular action

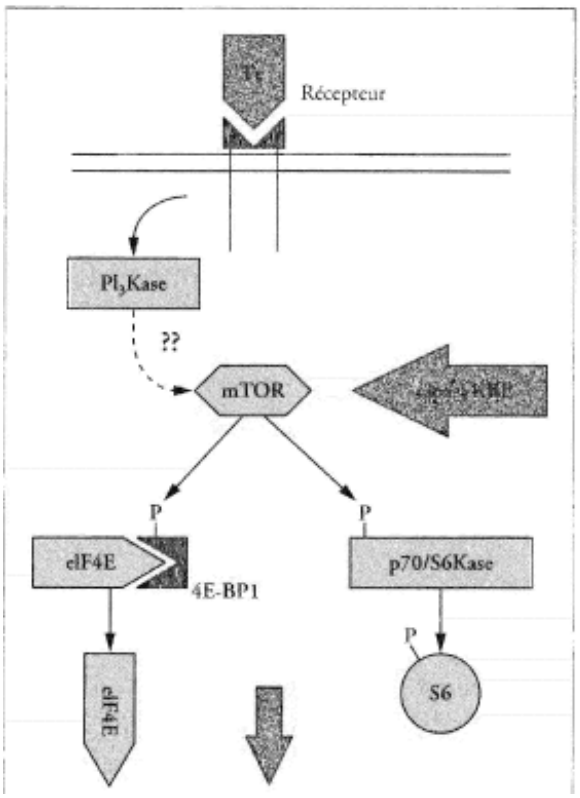
Rapamycin can be considered a "prodrug" insofar as its intracellular action requires attachment to an immunophilin called FKBP12 (FK506 binding protein because it also attaches FK506) [13]. The complex thus formed is very stable (duration of half-dissociation is 17.5 h) and allows a prolonged biological effect of rapamycin (7 days after one hour of exposure *in vitro*) [11], suggesting that the latter may be administered intermittently.

The only currently known target of the rapamycin/FKBP12 complex is mTOR. It is a serine/threonine kinase of the phosphatidylinositol kinases family [4]. It is activated by a large number of growth factors, in particular interleukins 2, 4 and 6, insulin, and insulin-like growth factor 1. In response to these mitogenic stimuli, mTOR activates the initiation of the translation of many mRNAs by two parallel pathways [4, 14] (*Figure 2*):

- Initiation factor 4E of the translation (eIF4E) is in the basal state sequestered and inhibited by the 4E-BP1 protein. mTOR, by phosphorylating the latter, will allow the release of eIF4E and the initiation of the translation. The thus synthesized proteins are assumed to induce, directly or indirectly, the G1/S transition;
- mTOR activates the p70/S6 kinase which itself then activates the ribosomal protein S6. In its phosphorylated form, the latter selectively controls the translation of mRNAs having a pyrimidine-rich domain at their 5' end. These mRNAs encode ribosomal proteins and elongation factors.

Protein activated factors upstream of mTOR after binding of the growth factor to its receptor are less known. An isoform of p85/PI kinase may, particularly, be involved [15].

The inhibition of the biological function of mTOR by rapamycin appears to play a key role in its cytostatic action. However, there is still much to learn on, firstly, the exact mechanisms by which mTOR controls the G1/S transition and, secondly, on any other cellular targets of rapamycin. Thus, it has recently been shown in 3T3 mouse fibroblasts stimulated with serum that rapamycin decreased the cyclin DI rate. However, unlike what one might believe, this was not due to a defective synthesis but to an accelerated degradation of the protein [16]. On other models, rapamycin induces




Initiation of the translation

Figure 2. Transduction pathway involving mTOR. Fc: growth factor; Pl, Kase: phosphatidylinositol 3 kinase; mTOR: mammalian target of rapamycin; rapa: rapamycin; FKBP: FK506 binding protein; p70/S6Kase: ribosomal protein S6 kinase; eIF4E: eukaryotic initiation factor 4E; 4E-BPI: eIF4E binding protein.

accumulation of the cyclin inhibitor $p27^{klp1}$ [17]. The proapoptotic action of rapamycin is also unclear. It seems dependent on mTOR inhibition, but not p53 [11], or bcl-2 [18]. A better understanding of the mechanisms of action of rapamycin at the molecular level would ideally predict tumor sensitivity to this molecule as a function of the expression or non-expression of a particular gene. It has already been shown that tumors overexpressing *c-myc* were resistant to rapamycin [19], as well as those from patients with ataxia telangiectasia [20]. Interestingly, the *ATM* gene, deficient in the ataxia telangiectasia, encodes a phosphatidylinositol kinase close to mTOR.

### Clinical experience

It concerns mainly the use of rapamycin as immunosuppressant agent, alone or in combination with ciclosporin. Rapamycin is

administered orally and is metabolized by cytochrome P450 3A in several inactive metabolites [21]

At the recommended dose for Phase II of 7 mg/m$^2$/day, toxicity mainly involves biological parameters [2]. There is a dose-dependent haematological toxicity predominant on platelets, and usually asymptomatic and not requiring transfusion. This haematotoxicity is of central origin and may be related to inhibition by rapamycin of mitogenic signals from the cytokines. Rapamycin increases the levels of triglycerides and cholesterol sometimes considerably. This hyperlipemia could be especially problematic when administered long-term due to the increased cardiovascular risk. No acute pancreatitis or other clinical manifestation associated with hypertriglyceridemia was ever observed. Among those already severely immunocompromised patients, rapamycin does not appear to increase the risk of infections, except perhaps those related to herpes simplex virus. This finding should be linked to the fact that rapamycin increases the translation of mRNAs of certain viruses [22].

## A new analog of rapamycin: CCI-779

In several mouse models, the antitumor action of rapamycin is most important when parenteral administration is used. However, it is difficult to achieve in routine clinical practice due to a low solubility of the molecule [5]. Thus analogues of rapamycin have been developed whose physicochemical properties allow for easy intravenous administration.

CCI-779 is one of these analogs. It has a cytostatic activity on several xenograft models of human tumors in nude mice, particularly in glioblastoma, prostate, pancreatic and breast carcinoma [23] and medulloblastoma [24].

Like rapamycin, attaching the CCI-779 to the FKBP12 is an essential step in its action [23]. It remains to determine whether it is possible to completely superimpose CCI-779's mechanism of action on that of rapamycin.

Interestingly, the antitumor activity described in nude mice is kept for 14 days after a daily administration over 5 days while the immunosuppressive effect disappears after 24 h [23].

Phase I clinical trials are underway in France and the United States.

## Conclusion

Rapamycin is the first representative of a new class of anticancer agents with a completely original mechanism of action and a favorable toxicity profile. Preclinical studies suggest that those molecules have an essentially cytostatic action. CCI-779, first analog administered parenterally, is currently the subject of Phase I clinical trials. In parallel, better knowledge of the molecular targets of rapamycin and its analogs could guide the achievement of future Phase II clinical trials.▼

## REFERENCES


**1**. Vezina C. Antimicrobial activity of streptomyces and fungi isolated from rapa Nui soil samples. *5th annual meeting of the Canadian Society of Chemotherapy*, Toronto, April 16, 1969.

**2**. Kahan BD. Rapamycin: personal algorithms for use based on 250 treated renal allograft recipients. *Transplant Proc* 1998; 30: 2185-8.

**3**. Morris RE. Rapamycins: antifungal, antitumor, antiproliferative, and immunosuppressive molecules. *Transplant Rev* 1992; 6: 39-87.

**4**. Thomas G, Hall MN. TOR signaling and. control of cell growth. *Curr Op Cell Biol* 1997; 9:782-7.

**5**. Sausville EA. New agents-noncytotoxics, antiangiogenesis. *ASCO Educational Book* 1998; 112-7.

**6**. Albers MW, Williams RT, Brown EJ, Tanaka A, Hall FL, Schreiber SL ,FKBP-rapamycin inhibits a cyclin-dependent kinase activity and a cyclin Dl-cdk association in early G1 of an osteosarcoma cell line. *J Biol Chem* 1993; 268; 22825-9.

**7**. Dilling MB, Dias P. Shapiro DN, Germain GS, Johnson RK, Houghton PJ. Rapamycin selectively inhibits the growth of childhood rhabdomyosarcoma cells through inhibition of signaling via the type 1 insulin-like growth factor receptor. *Cancer Res* 1994; 54: 903-7.

**8**. Seufferlein T, Rozengurt E. Rapamycin inhibits constitutive p70S6K phosphorylation, cell proliferation, and. colony formation in small cell lung cancer cells. *Cancer Res* 1996; 56; 3895-7.

**9**. Price DJ, Grove JR, Calvo V, Avruch J, Bierer BE. Rapamycin-induced inhibition of the 70-kilodalton 56 protein kinase. *Science* 1992; 257: 973-7.

**10**. Magge KP, Pryor AD, Marks JR, Iglehart JD, Miron A. Rapamycin (sirolimus) blocks estrogen mediated growth of breast cancer cell lines. *Proc Am Ass Cancer Res* 1999; 40: 636 (abstr. 4197).

**11**. Hosoi H, Dilling MB, Shikata T, Lia LN, Shu L, Ashmun RA. Rapamycin causes poorly reversible inhibition of mTOR and induces p53-dependent apoptosis in human rhabdomyosarcoma cells. *Cancer Res* 1999; 59: 886-94.

**12**. Shi Y, Frankel A, Radvanyi LG, Penn LZ, Miller RG, Mills GB. Rapamycin enhances apoptosis and increases sensitivity to cisplatin *in vitro*. *Cancer Res* 1995; 55: 1982-8.

**13**. Sabers CJ, Martin MM, Brunn GJ. Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. *J Biol Chem* 1995; 270: 815-22,

**14**. Beretta L, Grolleau A. La rapamycine; identification d'une nouvelle voie de signalisation des facteurs de croissance, réglant le début de la traduction. 1998; 14: 600-2.

**15**. Conus NM, Hemmings BA, Pearson RB. Differential regulation by calcium reveals distinct signaling requirements for the activation of Akt and p70$^{5GK}$. *J Biol Chem* 1998; 273: 4776-82.

**16**. Hashemolhosseini S, Nagamine Y, Morley SJ, Desrivieres S, Mercep L, Ferrari S. Rapamycin inhibition of the Gl to S transition is mediated by effects on cyclin Dl mRNA and protein stability. *J Biol Chem* 1998; 273; 14424-9.

**17**. Nourse J, Firpo E, Flanagan WM, Coats S, Polyak K, Lee MH, *et al* Interleukin-2-mediated elimination of the $p27^{kp1}$ cyclin-dependent kinase inhibitor prevented by rapamycin. Nature 1994; 372: 570-3.
**18**. Veverka KA, Germain GS, Dilling MB, Houghton PJ. Overexpression of bcl2 fails to protect rhabdomyosarcoma cells from rapamycin-induced apoptosis. *Proc Am Ass Cancer Res* 1998; 39: 571 (abstr. 3880).
**19**. Dilling MB, Hosoi H, Liu LN, Germain GS, Houghton PJ. Intrinsic resistance to rapamycin in human tumor cells may correlate with c-myc protein levels. *Proc Am Ass Cancer Res* 1999; 40: 428 (abstr. 2827).
**20**. Beamish H, Williams R, Chen P, Khanna KK, Hobson K, Watters D. Rapamycin resistance in ataxia-telangiectasia. *Oncogene* 1996; 13: 963-70.
**21**. Trépanier DJ, Gallant H, Legatt DF, Yatscoff RW. Rapamycin : distribution, pharmacokinetics and therapeutic range investigations: an update. *Clin Biochem* 1998; 31: 345-51.
**22**. Beretta L, Svitkin YV, Sonenberg N. Rapamycin stimulates viral protein synthesis and augments the shutoff of host protein synthesis upon picornavirus infection. *J Virol* 1996; 70: 8993-6.
**23**. Gibbons JJ, Discafani C, Peterson R, Hernandez R, Skotnicki J, Frost P. The effect of CCI-779, a novel macrolide anti-tumor agent, on the growth of human tumor cells *in vitro* and in nude mouse *xenografts in vivo*, *Proc Am Ass Cancer Res* 1999 ; 40: 301 (abstr. 2000).
**24**. Geoerger B, Kerr K, Janss AJ, Sutton LN, Phillips PC. Rapamycin analog CCI-779 inhibits growth of human medulloblastoma xenografts. *Proc Am Ass Cancer Res* 1999; 40; 603 (abstr. 3978),

November 2, 2016

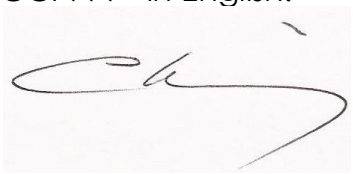
**Certification**

**Park IP Translations**

**TRANSLATOR'S DECLARATION:**

I, Claudine Joly-King, hereby declare:

That I possess advanced knowledge of the French and English languages. The attached French into English translation has been translated by me and to the best of my knowledge and belief, it is a true and accurate translation of the article entitled "La rapamycine et le CCI-779" in French and "Rapamycin and CCI-779" in English.



Claudine Joly-King

Project Number: FCHS_1610_027

15 W. 37th Street 8th Floor
New York, NY 10018
212.581.8870
ParkIP.com

DOCKET ALARM

# Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

## Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

## Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

## Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

## API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

**LAW FIRMS**
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

**FINANCIAL INSTITUTIONS**
Litigation and bankruptcy checks for companies and debtors.

**E-DISCOVERY AND LEGAL VENDORS**
Sync your system to PACER to automate legal marketing.