

106

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

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Apoderado: Álvaro Correa Ordoñez.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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ASUNTO	Radicación	07-39669
	Trámite	002
	Evento	001
	Actuación	430
	Oficio	
	Folios	

12086

Patente de Invención



Vía PCT (fase nacional)



Cap.I



Cap. II



No. Sol. Internacional

PCT/US2005/039836

Fecha de Presentación Internal.

21/oct/2005

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

- 1.1. Descripción: Folios: 37 a 63 Como se radicó inicialmente.
- 1.2. Reivindicaciones: Folios 64 a 67 Como se radicó inicialmente.
- 1.3. Prioridad: Folio 1: 60/622.917, 28/oct/2004

2. Objeto de la invención

Título de la solicitud: USO DE UN INHIBIDOR mTOR EN EL TRATAMIENTO DE LEIOMA UTERINO.

El objeto de la invención es el uso de rapamicina, 42-0-(2-hidroxi)etil de rapamicina, CCI-779, para el tratamiento del leioma uterino.

- Reivindicaciones 1-7: Uso
- Reivindicaciones 8-14: Encabezadas como composición.
- Reivindicaciones 15-23: Encabezadas como paquete.

Se catalogó la invención, según la Clasificación Internacional de Patentes (IPC.):
A61K31/00, 31/46, 31/445, A61P15/00, 35/00.

3. De la solicitud de Patente

4.2 Reivindicaciones (artículo 30 de la Decisión 486 de la C.A.N. y/o art.22 Tratado PCT.

-Las reivindicaciones 8 y 9 no son claras, no definen técnicamente ninguna composición como tal, el texto de la reivindicación es una mezcla de uso, método terapéutico y materia deseada.

Concluir indicando que no cumplen con el requisito de claridad, concisión o sustento por la descripción de acuerdo al art. 30 de la Decisión 486.

4. Reivindicaciones relativas a un uso o a un uso distinto (artículos 14 y 21 D 486 de la C.A.N.)

-Las reivindicaciones 1-7 reclaman uso; de acuerdo con la legislación que nos rige, el uso no se considera para protección, como se lee en el artículo 14 de la Decisión 486 de la Comisión de la Comunidad Andina de Naciones "... Los Países Miembros otorgarán patentes para las invenciones sean de **productos o procedimientos** en todos los campos de la tecnología..."; aquí NO se hace alusión al uso.

Además los usos o segundos usos no son patentables, de conformidad con la sentencia del TJAC 89-AI-2000, los usos en general no son patentables.

5. Determinación del Estado de la Técnica

La fecha que se ha tenido en cuenta para determinar el estado de la técnica fecha de presentación de la solicitud de la cual se reclama prioridad, 28/oct/2004.

Teniendo en cuenta la fecha indicada se realizó la búsqueda del estado de la técnica, en bases de datos donde esta oficina tiene acceso libre, para búsqueda de documentos relacionados con la materia que se reclama como nueva e inventiva en presente solicitud, encontrándose los siguientes documentos más relevantes:

N°	Documento	Título	Fecha de Publicación*
D1	INDEX MERCK	Rapamycin	1974
D2	Rev. Med. Universidad de Navarra	Nuevos Medicamentos Sirolimus	2002
D3	J.Biol.Chem	AKT activity determines sensitivity to mammalian target of rapamycin (mTOR) inhibitors by regulating cyclin D1 and c-myc expression.	23/oct/2003
D4	Clinical Cancer Research	Pharmacodynamic evaluating of CCI-779, an inhibitor of mTOR, in Cancer patient	01/ago/2003
D5	Bulletin Du Cancer	Rapamycin and CCI-779	Oct/1999
D6	CERTICAN®	Everolimus	23/ago/2003

6. Análisis de patentabilidad (artículo 14 D486)

6.1. Novedad (artículo 16 D486 de la C.A.N.)

-La solicitud reclama “composiciones” que comprendan rapamicina o un derivado de rapamicina y los “paquetes” que contienen las “composiciones”.

-Las anterioridades enseñan composiciones específicas comerciales de rapamicina y de sus derivados y las formas de distribución comercial que en la solicitud las denomina paquetes.

-La rapamicina, el sirolimus, el CCI-779, EL tacrolimus, everolimus etc, son macrólidos ampliamente conocidos en el estado de la técnica señalado, por la actividad antiproliferativa in vitro frente a diversas líneas tumorales humanas de células trasplantadas y modelos tumorales. Por las anterioridades se conoce que inhibe la mTOR quinasa citoplasmática , que controla la iniciación de la traducción de ARNm en respuesta a factores de crecimiento tumoral, es el principal objetivo conocido de la rapamicina y sus derivados. Durante los ensayos clínicos, la rapamicina por vía oral como agentes inmunosupresores no mostraron toxicidad limitante de la dosis. Igualmente las anterioridades comprenden composiciones farmacéuticas específicas que los comprenden y hasta con nombre comercial.

Entonces las composiciones reclamadas en la solicitud no son nuevas, toda vez que las composiciones reclamadas en la solicitud, se reclama es el uso tácito de la rapamicina y sus derivados en la preparación de composiciones, entonces estas composiciones tienen sujeta su patentabilidad a la patentabilidad del principio activo. En otras palabras, dado que el objeto de esta reivindicación no es nuevo “per se” composición que comprende compuestos de rapamicina (del estado de la técnica), ya que se refiere a composiciones convencionales o de analogía a los conocidos en el estado de la técnica, su patentabilidad está conectada únicamente, a la del principio activo, lo mismo los “paquetes” que son la forma de distribución comercial de cualquier medicamento, que contenga el medicamento y las instrucciones de uso, por lo tanto la solicitud en estudio no cumple con lo exigido en artículo 16 D486 de la C.A.N.

-También es necesario recordar que Artículo 21.- Los productos o procedimientos ya patentados, comprendidos en el estado de la técnica, de conformidad con el artículo 16 de la presente Decisión, no serán objeto de nueva patente, por el simple hecho de atribuirse un uso distinto al originalmente comprendido por la patente inicial.

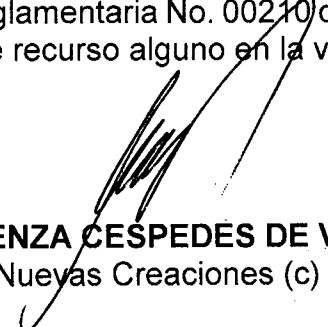
7. Conclusión:

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

Documento N°	Reivindicaciones Afectadas	Requisito que Afecta
D1	1-23	Novedad
D2	1-23	Novedad
D3	1-23	Novedad
D4	1-23	Novedad
D5	1-23	Novedad
D6	1-23	Novedad

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

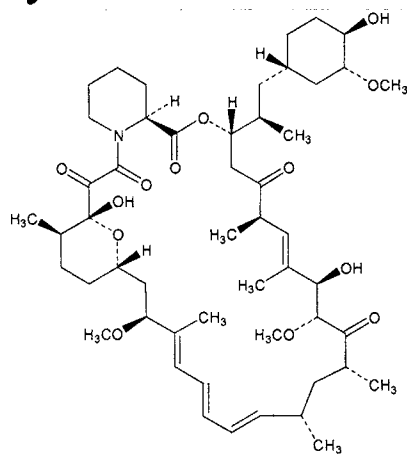
Notifíquese el presente oficio conforme a lo dispuesto en el artículo 8 de la resolución reglamentaria No. 00210 del 15 de enero de 2001, advirtiéndole que contra él no procede recurso alguno en la vía gubernativa.


ALIX CARMENZA CESPEDÉS DE VERGEL
Directora de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha _____ 17 SET 2010

CONCEPTO TECNICO No. 3257	EXAMINADOR TECNICO Código No. 202012
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Rapamycin



Search Monographs

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CambridgeSoft

Monograph Number: 8202

Title: Rapamycin

CAS Registry Number: 53123-88-9

Additional Names: Sirolimus; RAPA; RPM

Manufacturers' Codes: AY-22989; NSC-226080

Trademarks: Rapamune (Wyeth-Ayerst)

Molecular Formula: C₅₁H₇₉NO₁₃

Molecular Weight: 914.17.

Percent Composition: C 67.01%, H 8.71%, N 1.53%, O 22.75%

Literature References: Triene macrolide antibiotic isolated from *Streptomyces hygroscopicus*. Name derived from the native word for Easter Island, Rapa Nui. Isoln: S. N. Sehgal *et al.*, **DE 2347682**; *eidem*, **US 3929992** (1974, 1975 both to Ayerst McKenna Harrison); purification and characterization: C. Vézina *et al.*, *J. Antibiot.* **28**, 721 (1975); S. N. Sehgal *et al.*, *ibid.* 727. Inhibition of immune response: R. R. Martel *et al.*, *Can. J. Physiol. Pharmacol.* **55**, 48 (1977); of graft rejection in mice: C. P. Eng *et al.*, *Transplant. Proc.* **23**, 868 (1991). HPLC determ: K. L. Napoli, B. D. Kahan, *Clin. Chem.* **37**, 294 (1991). Pharmacokinetics in rabbit: N. Honcharik *et al.*, *Ther. Drug Monit.* **14**, 475 (1992). Potential utility as antiarthritic agent: R. P. Carlson *et al.*, *J. Pharmacol. Exp. Therap.* **266**, 1125 (1993). Total synthesis: K. C. Nicolaou *et al.*, *J. Am. Chem. Soc.* **115**, 4419 (1993); D. Romo *et al.*, *ibid.* 7906. Brief review of pharmacology: J. Y. Chang, S. N. Sehgal, *Brit. J. Rheumatol.* **30**, Suppl. 2, 62-65 (1991). Review of mechanism of action: N. H. Sigal, F. J. Dumont, *Ann. Rev. Immunol.* **10**, 519-560 (1992). Clinical trial in renal transplantation: B. D. Kahan *et al.*, *Lancet* **356**, 194 (2000).

Properties: Colorless crystalline solid from ether, mp 183-185°. uv max (95% ethanol): 267, 277, 288 nm (E_{1%^{1cm}} 417, 541, 416). [α]_{D²⁵} -58.2° (methanol). Sol in ether, chloroform, acetone, methanol and DMF; very sparingly sol in hexane and petr ether. Substantially insol in water. LD₅₀ in mice (mg/kg): ≈600 i.p.; >2,500 orally (Vézina).

Melting point: mp 183-185°

Optical Rotation: [α]_{D²⁵} -58.2° (methanol)

Absorption maximum: uv max (95% ethanol): 267, 277, 288 nm (E_{1%^{1cm}} 417, 541, 416)

Toxicity data: LD₅₀ in mice (mg/kg): ≈600 i.p.; >2,500 orally (Vézina)

Use: Tool for immunochemistry.

Therap-Cat: Immunosuppressant.

Sirolimus

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Introducción

El objetivo de la inmunosupresión en el trasplante de órganos es bloquear la respuesta inmune del paciente frente al injerto, evitando así el rechazo. Además debe lograrse que el umbral terapéutico sea el justo con el fin de evitar las infec-

obstante, y de manera frecuente pueden aparecer complicaciones asociadas como nefrotoxicidad, enfermedades

plante entre otras¹.

Sirolimus (Rapamicina), es un agente inmunosupresor nuevo y eficaz, aislado del *Streptomyces hygroscopicus*, una bacteria actinomiceta que inicialmente se estudió como antifúngico, y en la que posteriormente se descubrió actividad antitumoral e inmunosupresora en modelos animales². Es un producto natural obtenido por fermentación de esta bacteria y se descubrió en la Isla de Pascua (Rapa Nui, de ahí su nombre). Posee un mecanismo de acción un tanto distinto al de los inmunosupresores clásicos, como ciclosporina o tacrolimus, al no inhibir la calcineurina y producir una menor cantidad de efectos secundarios.

En 1999 la FDA aprobó su uso en la profilaxis del rechazo agudo post-trasplante renal basándose en una serie de ensayos clínicos dosis-dependiente.

Estructura química

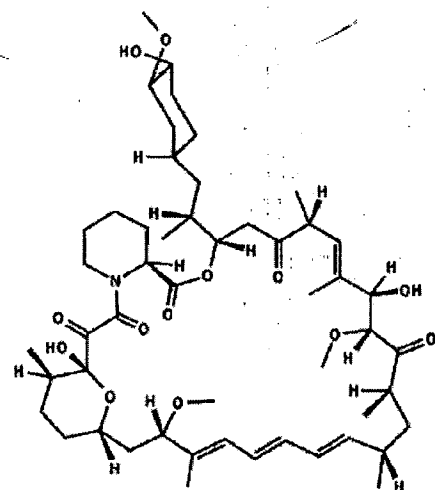
Sirolimus es una lactona macrocíclica, insoluble en agua y altamente lipofílica con un anillo macrolídico de 31 miembros. Su fórmula química es $C_{51}H_{90}NO_{13}$ y su peso molecular 914,2.

En la Figura 1 se representa su estructura molecular.

Mecanismo de acción

Sirolimus modula la respuesta inmune inhibiendo la actividad de una proteína con acción esencial en la coordinación de

Figura 1. Estructura molecular de Sirolimus



los procesos requeridos para que la célula pase de la fase G1 a la S del ciclo celular. Esta proteína no se encuentra influenciada por la acción de la ciclosporina u otro agente inmunosupresor, por este motivo el mecanismo de acción de sirolimus es único³.

Bloquea la proliferación celular, sin inhibir la actividad fosfatasa de la calcineurina. Interfiere la transducción de la señal provocada por la unión de la interleucina 2 (IL-2) a su receptor, deteniendo el ciclo de los linfocitos T en la transición de G1 a S e impidiendo por tanto su proliferación⁴.

Sirolimus se une a un receptor intracelular citosólico (inmunofilina), la proteína de unión al FK (FKBP), formando un complejo que inhibe la vía independiente del calcio mediante dos mecanismos: uno de acción directa y otro mediante la inhibición de dos enzimas diana específicas de rapamicina (TOR1 y TOR2). También inhibe la fosforilación de las quinasas p70 y p34 (inducidas por la IL-2), que son cruciales para la regulación del ciclo celular. Además sirolimus facilita la apoptosis de las células tumorales⁵⁻⁷.

El efecto inmunosupresor de sirolimus es sinérgico al de ciclosporina, tanto *in vivo* como *in vitro*, y el perfil de efectos adversos difiere del resto de agentes inmunosupresores. Por este motivo, sirolimus y ciclosporina pueden combinarse en un mismo régimen terapéutico⁸.

Farmacocinética

Absorción

Tras su administración oral sirolimus se absorbe de manera rápida. La concentración plasmática máxima (C_{max}) se alcanza a la hora de la administración de una dosis única y a las 2 horas (t_{max}) en pacientes trasplantados renales que han recibido dosis múltiples^{9,10}. La administración conjunta con alimentos grasos retrasa la velocidad de absorción; de esta forma la (C_{max}) disminuye en un 34%, la t_{max} se incrementa 3,5 veces, y el área bajo la curva de concentraciones plasmáticas vs. tiempo (ABC) también se incrementa en un 35% al compararlo con los valores obtenidos en ayunas¹¹.

Por este motivo y para disminuir la variabilidad, se recomienda administrar sirolimus de manera regular, bien sea con o sin alimentos¹¹. Puede administrarse con agua o zumos de fruta, a excepción de zumo de pomelo, ya que este puede alterar el metabolismo intestinal mediado por la isoenzima del citocromo P450 3A4 (CYP 3A4)⁹⁻¹¹.

La biodisponibilidad de sirolimus es aproximadamente del 14%. La baja magnitud de este valor es debida a un importante metabolismo por parte de la isoenzima CYP 3A4, tanto intestinal como hepática (efecto de primer paso) y por su eliminación contra gradiente por la glicoproteína P intestinal^{12,13}.

Distribución

Sirolimus se distribuye ampliamente en los elementos formes de la sangre, la fracción libre en plasma es de alrededor de 8%. En el ser humano se une principalmente con la albúmina sérica (97%), la α 1-glicoproteína ácida y lipoproteínas. Después de dosis múltiples en pacientes trasplantados renales, el volumen de distribución estimado (Vd) es de aproximadamente 1,7 l/kg^{9,10}.

Metabolismo

Sirolimus sufre un extenso metabolismo oxidativo a nivel hepático, mediante reacciones de desmetilación e hidroxilación. Origina un gran número de metabolitos, y en apariencia estos conservan parcialmente la actividad de la molécula original¹⁴.

Excreción

Su excreción es mayoritariamente fecal (91,1%), siendo la eliminación por vías urinarias baja (2,2%). Tal como se ha

podido comprobar tras la administración de una dosis única de sirolimus marcado radioactivamente a voluntarios sanos¹⁵.

Después de la administración de dosis múltiples a pacientes trasplantados renales, la semivida de eliminación ($t_{1/2}$) fue de 62 horas y el aclaramiento total (Cl/F) 210 ml/h/kg¹⁰.

Situaciones especiales

Insuficiencia hepática

Sirolimus se ha estudiado en pacientes con insuficiencia hepática clase A y B de la clasificación de Child-Pugh. Tras la administración de una sola dosis de 15mg vía oral ni la C_{max} ni la t_{max} se modificaron, pero el ABC se incrementó 61% y el aclaramiento disminuyó 33%. No hubo diferencias respecto al volumen de distribución en estado de equilibrio entre voluntarios sanos y pacientes con insuficiencia hepática. Por este motivo, la dosis de sirolimus en estos pacientes debe reducirse un tercio de la posología habitual realizándose una monitorización periódica de las concentraciones sanguíneas del fármaco¹⁶.

Insuficiencia renal

No se conoce el efecto de la insuficiencia renal sobre la farmacocinética del sirolimus, pero dado que su excreción es mayoritariamente fecal, no parece que se modifique en este grupo de pacientes¹⁶.

En un estudio de tolerancia en pacientes pediátricos en diálisis, tras la administración de sirolimus a dosis crecientes por vía oral, se observó un aumento estadísticamente significativo del aclaramiento total (Cl/F) en el grupo de pacientes de 5-11 años. Debido a que los niños más pequeños pueden requerir dosis mayores, la posología debe instaurarse en base a la superficie corporal total. Es también necesario realizar una monitorización periódica de las concentraciones de sirolimus¹⁶.

Otros

Los estudios clínicos realizados con sirolimus, no incluyeron un número suficiente de pacientes que permitan obtener conclusiones respecto a la influencia de la edad en su farmacocinética¹⁶.

No se han encontrado diferencias estadísticamente significativas entre los parámetros farmacocinéticos de individuos de raza negra y caucásica¹⁶.

Los varones tienen un aclaramiento total 12% menor que el de las mujeres, pero una $t_{1/2}$ significativamente más alta. No obstante, estas diferencias no requieren un ajuste especial de dosis¹⁶.

Eficacia clínica

Tras la realización de diferentes estudios, no se ha observado una clara diferencia de la dosis de 5mg frente a la de 2mg; incluso ciertos resultados sugieren un mejor perfil de seguridad a dosis bajas¹⁷. La información disponible señala que existe una correlación entre los niveles en sangre total y la eficacia de sirolimus. Sobre la base de un modelo de regresión logística, concentraciones medias superiores a 3,5 ng/ml (rango terapéutico de 3-18 ng/ml) se asocian a un riesgo menor de rechazo dentro de los primeros 75 días postrasplante, tal y como ha sido documentado mediante la realización de biopsias renales¹⁷.

Sirolimus se ha evaluado en administración conjunta con ciclosporina y corticosteroides en pacientes estables trasplantados renales. El Rapamune Global Study fue un ensayo multicéntrico, abierto, paralelo y aleatorizado realizado en Australia, Canadá, Europa y Estados Unidos en el que los pacientes recibieron sirolimus a dosis de 5 mg/día o placebo además de ciclosporina y prednisona. Sirolimus fue superior a placebo en la reducción de la incidencia de rechazo durante los primeros 6 meses después del trasplante, así como en relación al rechazo agudo¹⁸, pero no se observaron diferencias estadísticamente significativas al compararlo con ciclosporina a los doce meses¹⁹. Los pacientes tratados con sirolimus presentaban una función del injerto renal significativamente superior a lo largo del tiempo que los tratados con ciclosporina^{18,19}.

Por último en un ensayo de fase II denominado Régimen de Mantenimiento con Sirolimus (RMR), cuyo objetivo era evaluar la posibilidad de retirar ciclosporina del tratamiento inmunosupresor, se estudiaron 246 sujetos trasplantados renales durante doce meses que se dividieron en dos grupos de tratamiento. Al primero, se le administró una dosis fija de 2 mg/día de sirolimus (tras dosis de carga de 6mg), ciclosporina y corticosteroides; el segundo grupo recibió dosis decrecientes de sirolimus (hasta nivel valle-pico de 10-20 ng/ml), ciclosporina y corticosteroides. La dosis de ciclosporina fue progresivamente disminuyéndose hasta su retirada al tercer mes. No se observaron diferencias significativas entre el grupo tratado con sirolimus, ciclosporina y corticosteroides frente al que recibió sirolimus y corticosteroides en la supervivencia del injerto a los doce meses. La función renal mejoró significativamente con la retirada de ciclosporina¹⁹.

Resultados similares se hallaron al comparar el tratamiento entre sirolimus y azatioprina en trasplantados renales o en un estudio en trasplante simultáneo de páncreas y riñón^{20,21}.

Efectos adversos

Se ha descrito un aumento de incidencia de infecciones, linfoma y otros tipos de tumores (sobre todo cutáneos) secundarios al tratamiento crónico con sirolimus¹⁶. También se han comunicado casos de neumonitis intersticial que se resolvieron tras la suspensión del fármaco²².

En los tratamientos en asociación con ciclosporina existe una mayor incidencia de síndrome hemolítico urémico²³. Otro efecto adverso también descrito es la necrosis avascular de la cabeza del fémur²⁴.

Entre las reacciones adversas más frecuentemente descritas se encuentran dolor abdominal, diarrea, anemia, trombocitopenia, hipertensión arterial, hipercolesterolemia e hipotrigliceridemia (que a veces requiere tratamiento farmacológico para su control), hipopotasemia, artralgias, cefalea, faringitis y rinitis. Menos frecuentes son insomnio, tremor, taquicardia, estomatitis, exantema, acné y piodonitis¹⁶.

Interacciones medicamentosas

Sirolimus es metabolizado ampliamente por la isoenzima CYP 3A4 del citocromo P450 tanto en la pared intestinal como en el hígado y es también sustrato de la glicoproteína P intestinal.

Por este motivo la absorción y eliminación de sirolimus pueden verse alteradas.

Ciclosporina incrementa la C_{max} y el ABC de sirolimus. Probablemente el origen de esta interacción se encuentra en por la combinación de la inhibición competitiva del CYP 3A4 y de la glicoproteína P en el intestino delgado^{25,27}.

Los inhibidores de CYP 3A4 como los bloqueadores de los canales de calcio (diltiazem), antifúngicos (ketoconazol), macrólidos (claritromicina), agentes procinéticos (cisaprida), e inhibidores de la proteasa (ritonavir, indinavir) entre otros, incrementan la C_{max} y el ABC de sirolimus^{25,27}.

Por el contrario, los inductores de CYP 3A4 como rifampicina y anticonvulsivantes (carbamazepina) disminuyen la C_{max} y el ABC de sirolimus^{25,27}.

No se han demostrado interacciones significativas o importantes con aciclovir, digoxina, gliburida, prednisolona, cotrimoxazol o anticonceptivos orales^{25,27}.

Contraindicaciones y precauciones

Sirolimus está contraindicado en pacientes con hipersensibilidad al fármaco o a cualquiera de sus excipientes: propilenglicol, monoglicéridos, diglicéridos, etanol, ácidos grasos de soja y palmitato de ascorbilo¹⁶.

El uso de sirolimus se ha asociado frecuentemente a un incremento de los niveles séricos de colesterol y triglicéridos que pueden requerir tratamiento farmacológico^{16,28}.

Debido a la susceptibilidad de infecciones oportunistas, debe administrarse profilaxis antimicrobiana frente a citomegalovirus y *Pneumocystis carinii* durante los primeros 3 y 12 meses (respectivamente) siguientes al trasplante^{16,29}.

La administración de sirolimus a pacientes trasplantados hepáticos se ha asociado con trombosis hepática en el período inmediatamente posterior^{16,30}.

Indicaciones y posología

Sirolimus está indicado en la profilaxis del rechazo de órganos en pacientes de bajo o moderado riesgo inmunológico. Inicialmente fue utilizado en la profilaxis del rechazo renal, pero su uso se ha extendido al trasplante de otros órganos como páncreas, hígado y corazón.

La dosis de carga en adultos es 6 mg y está calculada en base a su semivida de eliminación prolongada (62 horas). En ausencia de esta, se requiere dos semanas para llegar al estado de equilibrio. La dosis de mantenimiento es de 2 mg¹⁶.

En niños a partir de 13 años, la dosis de mantenimiento es de 1 mg/m²/día, sin exceder un máximo de 2 mg/día. La dosis de carga es 6 mg/día¹⁶.

Presentación

Se presenta con el nombre comercial de Rapamune® (Laboratorios Wyeth-Ledrol), en frascos de 60ml de solución oral en la concentración de 1mg/ml a un precio de 271, 29 euros. La presentación de 30 sobres de 2mg/2ml de solución oral vale 271,29 euros y la de 30 sobres de 1mg/ml de solución oral 157,41 euros.

Bibliografía

- Ross S, Schroeder T, Hariharan S, et al. Prevention and management of the adverse effects associated with immunosuppressive therapy. *Drug Saf* 1993;9:104-31.
- Morris R. Rapamycins: Antifungal, antitumor, antiproliferative and immunosuppressive macrolides. *Transplant Rev* 1992;6:39-87.
- Abraham RT, Wiederrecht GJ. Immunopharmacology of rapamycin. *Annu Rev Immunol* 1996;14:483-510.
- Kahan BD. Sirolimus. A new agent for clinical renal transplantation. *Transplant Proc* 1997;29:48-50.
- Liu J, Albers MW, Wandless TJ, et al. Inhibition of T cell signalling by immunophytin-ligand complexes correlates with loss of calcineurin phosphatase activity. *Biochemistry* 1992;31:3896-901.
- Sabers CJ, Martin MM, Brunn GJ, et al. Isolation of protein target of FKBP12-rapamycin complexes in mammalian cells. *J Biol Chem* 1994;270:815-22.
- Terada N, Lucas JJ, Szepesi A, et al. Rapamycin blocks cell cycle progression of activated T cells prior to events characteristic of the middle to late G1 phase of the cell cycle. *J Cell Physiol* 1993;154:5-15.
- Sehgal SN. Therapeutic index and pharmacokinetic/pharmacodynamic study in orthotopic renal transplanted pigs after PO administration of rapamycin. Wyeth-Ayerst GTR-25118, 1994.
- MacDonald A, Scarola J, Burke JT, et al. Clinical pharmacokinetics and therapeutic drug monitoring of sirolimus. *Clin Ther* 2000;22 (Suppl. B): B101-B121.
- Zimmerman JJ, Kahan BD. Pharmacokinetics of sirolimus in stable renal transplant patients after multiple oral dose administration. *J Clin Pharmacol* 1997;37:405-15.
- Zimmerman JJ, Ferron GM, Heng-Keang L, et al. The effect of high fat meal on the oral bioavailability of the immunosuppressant sirolimus (Rapamycin). *J Clin Pharmacol* 1999;39:1155-61.
- Lampen A, Zhang Y, Hackbarth I, et al. Metabolism and transport of the macrolide immunosuppressant sirolimus in the small intestine. *J Pharmacol Exp Ther* 1998;285:1104-12.
- Sattler M, Guengerich FP, Yun C-H, et al. Cytochrome P-450 3A enzymes are responsible for biotransformation of FK 506 and rapamycin in man and rat. *Drug Metab Dispos* 1992;20:753-61.
- Leung LY, Lim H-K, Hicks D, et al. Sirolimus (rapamycin): Metabolite characterization in rat and human liver microsomal incubations and trough whole blood of renal transplant patients treated with sirolimus, cyclosporine and prednisone. *ISSX Proc* 1996;10:366.
- Leung LY, Zimmerman J, Lim HK. Rapamycin (sirolimus): Biotransformation in healthy male subjects after a single oral dose of the ¹⁴C radiolabeled drug (nominal 40 mg). Wyeth-Ayerst GTR-29528, 1997.
- Rapamune Prescribing Information. Wyeth-Ayerst Pharmaceutical, 2000.
- MacDonald A. A randomized trial of sirolimus, cyclosporine and prednisone vs cyclosporine and prednisone alone in recipients of mismatched first kidney grafts: Results at one year. *Transplantation* 1999; 67 (Suppl 9): S546. Abstract 15.
- Kahan BD, Julian BA, Pescovitz MD, et al. Sirolimus reduces the incidence of acute rejection episodes despite lower cyclosporine doses in caucasian recipients of mismatched primary renal allografts: A phase II trial. *Transplantation* 1999;68:1526-32.
- Vásquez E. Sirolimus: a new agent for prevention of renal allograft rejection. *Am J Health Syst Pharm* 2000;57(5):437-48.
- Kahan BD, et al. Efficacy of sirolimus compared with azathioprine for reduction of acute renal allograft rejection: a randomised multicentre study. *Lancet* 2000;356:194-202.
- Kaufman DB, Leventhal JR, Koffron AJ, et al. A prospective study of rapid corticosteroid elimination in simultaneous pancreas-kidney transplantation. *Transplantation* 2002;73:169-77.
- Singer SJ, Tiernan R, Sullivan E, et al. Interstitial pneumonitis associated with sirolimus therapy in renal-transplant recipients. *N Engl J Med* 2000;343:1815-6.
- Langer RM, Van Buren CT, Katz SM, et al. De novo hemolytic uremic syndrome after kidney transplantation in patients treated with cyclosporine and sirolimus combination. *Transplantation Proceedings* 2001;33:3236-7.
- Bhanani S, Eris J. Premature osteonecrosis and sirolimus treatment in renal transplantation. *BMJ* 2001;323:665.
- Kahan BD, Podbielski J, Napoli KL, et al. Immunosuppressive effects and safety of a sirolimus/cyclosporine combination regimen for renal transplantation. *Transplantation* 1998;66:1040-6.
- Murgia MG, Jordan S, Kahan BD. The side effect profile of sirolimus: A phase I study in quiescent cyclosporine-prednisone-treated renal transplant patients. *Kidney Int* 1996;49:209-16.
- Groth CG, Backman L, Morales JM, et al. Sirolimus (rapamycin)-based therapy in human renal transplantation: Similar efficacy and different toxicity compared with cyclosporine. *Transplantation* 1999;67:1036-42.
- Andany MA, Kasiskis BL. Dyslipidemia and its management after renal transplantation. *J Nephrol* 2001;14(Suppl 4): S81-S88.
- Dominguez J, Mahalik K, Kiberd B, et al. Conversion to rapamycin immunosuppression in renal transplant recipients: report of an initial experience. *Transplantation* 2000;70(8):1244-7.
- McAlister VC, Pettkian KM, Malatjalian DA, et al. Orthotopic liver transplantation using low-dose tacrolimus and sirolimus. *Liver Transpl* 2001;7(8):701-8.

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AKT activity determines sensitivity to mammalian target of rapamycin (mTOR) inhibitors by regulating cyclin D1 and c-myc expression.

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Abstract

Prior work demonstrates that AKT activity regulates sensitivity of cells to G(1) arrest induced by mammalian target of rapamycin (mTOR) inhibitors such as rapamycin and CCI-779. To investigate this, a novel high-throughput microarray polysome analysis was performed to identify genes whose mRNA translational efficiency was differentially affected following mTOR inhibition. The analysis also allowed the assessment of steady-state transcript levels. We identified two transcripts, cyclin D1 and c-myc, which exhibited differential expression in an AKT-dependent manner: High levels of activated AKT resulted in rapamycin-induced down-regulation of expression, whereas low levels resulted in up-regulation of expression. To ectopically express these proteins we exploited the finding that the p27(kip1) mRNA was efficiently translated in the face of mTOR inhibition irrespective of AKT activity. Thus, the p27(kip1) 5'-untranslated region was fused to the cyclin D1 and c-myc coding regions and these constructs were expressed in cells. In transfected cells, expression of cyclin D1 or c-myc was not decreased by rapamycin. Most importantly, this completely converted sensitive cells to a phenotype resistant to G(1) arrest. Furthermore, the AKT-dependent differential expression patterns of these two genes was also observed in a mouse xenograft model following in vivo treatment with CCI-779. These results identify two critical downstream molecular targets whose expression is regulated by AKT activity and whose down-regulation is required for rapamycin/CCI-779 sensitivity.

Advances in Brief

Pharmacodynamic Evaluation of CCI-779, an Inhibitor of mTOR, in Cancer Patients¹

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Abstract

CCI-779 is an ester of rapamycin and inhibitor of mammalian target of rapamycin (mTOR) currently in Phase II clinical development for the treatment of patients with cancer. CCI-779 interacts with mTOR and inhibits its kinase activity, resulting in inhibition of the mTOR-regulated translational controllers p70^{s6} kinase and 4E-BP1. Ultimately, CCI-779 decreases the translation of mRNAs involved in the control of the cell cycle, resulting in cell cycle arrest. The objective of this study was to develop a method to determine the pharmacodynamic effects of CCI-779 suitable for use in clinical trials. Exposure of Raji lymphoblastoid cells to increasing concentrations of rapamycin resulted in a linear concentration-dependent inhibition of p70^{s6} kinase activity, suggesting that p70^{s6} kinase activity could be an appropriate marker for mTOR-interacting agents. In subsequent experiments, treatment of nude mice bearing the CCI-779 susceptible breast cancer cell line MDA-468 with a single dose of 10 mg/kg CCI-779 resulted in a >80% inhibition of p70^{s6} kinase activity in peripheral blood mononuclear cells (PBMCs) 72 h after treatment. Importantly, the degree of p70^{s6} kinase inhibition was identical in PBMCs and simultaneously collected tumor tissue, suggesting that the PBMCs are an adequate surrogate tissue for p70^{s6} kinase activity *in vivo*. The intrasubject coefficient of variation of p70^{s6} kinase activity measured in PBMCs collected from five healthy volunteers on days 1, 4, and 8 was 14%, indicating that p70^{s6} kinase activity in PBMCs remains relatively stable over time. Finally, p70^{s6} kinase activity was measured in PBMCs from nine patients with renal cell

cancer treated with a single dose of 25, 75, or 250 mg of CCI-779 i.v. (three patients each). PBMCs were collected on days 2, 4, and 8 after CCI-779 treatment. In this small data set, eight of the nine patients had evidence of p70^{s6} kinase activity inhibition after treatment that was independent of the administered dose. There was a significant linear association between time to disease progression and inhibition of p70^{s6} kinase activity 24 h after treatment. In conclusion, these results indicate that the pharmacodynamic effects of CCI-779 can be determined using a p70^{s6} kinase assay in PBMCs. This assay is currently being incorporated in Phase I and II studies with CCI-779 to determine its relationship with dose and plasma concentration of the agent and its value as a predictor of treatment efficacy.

Introduction

CCI-779 is an ester of rapamycin, a natural macrolide antibiotic with antifungal, immunosuppressive, and antitumor properties (1), which is currently in Phase II clinical development for the treatment of cancer. CCI-779 forms a complex with FKBP-12, a member of the immunophilin family of FK506-binding proteins, and inhibits the activity of mTOR³ (2-5), a kinase that functions as a checkpoint for nutritional status (6) and a downstream mediator in the phosphatidylinositol 3'-kinase/Akt signaling pathway (7-9). Consequently, CCI-779 inhibits the transcriptional and translational functions regulated by mTOR. At the transcriptional level, mTOR positively controls RNA polymerase activity either by inactivation of the retinoblastoma protein, which inhibits the RNA polymerase I and III (10), or by stimulating the transcriptional activator STAT3, which is persistently activated in many human cancers and causes cellular transformation (11). Rapamycin treatment effectively blocked retinoblastoma protein phosphorylation and inactivation by mTOR (12). Likewise, the translational regulators p70^{s6} kinase and initiation factor 4E-BP1 are phosphorylated by mTOR (13-17). Ribosomal p70^{s6} kinase, when phosphorylated, attaches to the S6 subunit of the ribosome, and 4E-BP1, after being phosphorylated, is dissociated from the eukaryotic initiation factor eIF4E, allowing the formation of the 4F initiation complex. CCI-779, by inhibiting these translational regulators, leads to a decrease in overall protein synthesis of ~15% but strongly inhibits translation of specific mRNA that encodes ribosomal proteins and other components that promote cell growth, such as insulin-like growth factor-II, c-Myc, and cyclin D1 (18, 19).

CCI-779 has been evaluated in Phase I/II studies in patients with solid tumors (20-22). The preliminary results from Phase I clinical studies indicated that CCI-779 is generally well toler-

ated. Interestingly, the toxicity of the agent was not clearly dose related when administered on a weekly schedule of administration, complicating the selection of an appropriate dose for subsequent studies. In addition, encouraging evidence of antitumor activity was observed in Phase I clinical studies over a relatively broad range of doses and at different schedules of administration, further complicating the dose selection process. As a result, and because the maximum tolerated dose may not be the optimum dose for nontoxic agents, a range of dose levels was evaluated in Phase II trials.

The objectives of these translational studies were to develop a pharmacodynamic biomarker of CCI-779 for use in clinical research. The developmental efforts focused on measurement of p70^{s6} kinase activity because it is a direct downstream substrate of mTOR and can be quantitated using well-developed kinase assay methods. In addition, the PBMCs were selected to measure the pharmacodynamic effects of CCI-779 because p70^{s6} kinase is constitutively activated in these cells, PBMCs are easily collectable from patients enrolled in clinical trials, and previous studies had used PBMCs to evaluate the pharmacodynamics of rapamycin in studies evaluating the effects of rapamycin to prevent graft rejection in transplant patients (23). This article summarizes the development, validation, and feasibility application of this method.

Materials and Methods

Drugs. Rapamycin was obtained as a gift from the Drug Synthesis & Chemistry Branch, Developmental Therapeutics Program at the Division of Cancer Treatment and Diagnosis of the National Cancer Institute (Bethesda, MD). CCI-779 was provided by Wyeth Laboratories (Pearl River, NY).

Cell Lines. Human MDA-468 breast cancer and Raji (Burkitt's lymphoma) cells were obtained from the American Type Culture Collection. MDA-468 cells were cultured and grown until confluent in minimum essential medium containing 10% fetal bovine serum. Before injection into animals, cells were harvested in serum-free minimum essential medium. All cell culture media and reagents were purchased from Life Technologies, Inc. (Grand Island, NY). Raji cells were grown in RPMI 1640 with 2 mM glutamine modified by American Type Culture Collection to contain 10 mM HEPES, 1 mM sodium pyruvate, 4.5 grams/liter glucose, and 1.5 grams/liter sodium bicarbonate, supplemented with 10% fetal bovine serum.

In Vitro Studies. Raji cells were plated in a six-well plate at 3×10^5 cells/ml and treated for 30 min at the following rapamycin concentrations: 0, 0.1, 0.3, 0.5, 0.7, and 1 nM. All cell lines were incubated in a 37°C incubator containing 5% CO₂. After treatment, cells were harvested and rinsed with ice-cold PBS, and lysates were prepared for p70^{s6} kinase assay and immunoblotting as described below.

Animal Studies. Four- to 6-week-old nude female mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were housed at the University of Texas Health Science Center at San Antonio and treated under a protocol approved by the Institutional Animal Care Committee according to local and federal regulations. After a period of 5-6 days, mice were implanted s.c. with 2×10^6 growing MDA-468 human breast cancer cells. After 10 days, tumor diameters were deter-

mined, and the animals were randomized to the different treatment groups. Treatment was given by a single i.p. injection of 10 mg/kg CCI-779 or vehicle. Samples of PBMCs and tumor were collected from groups of five mice each at the following time points: (a) baseline; (b) 24 h; and (c) 72 h after treatment. Blood from each animal group of five mice was collected by cardiac puncture and pooled. PBMCs were extracted by a gradient centrifugation in lymphocyte separation media (Fisher Scientific, Suwanee, GA) at $1500 \times g$ for 20 min at room temperature. The isolated cells were snap frozen until analysis. Tumors were excised and snap frozen at -80°C until analysis. For the kinase activity analysis, lysis buffer was added to PBMCs and tumor tissues, and the subsequent protein extract was processed as described above.

Collection of PBMCs from Human Subjects. PBMCs from healthy human volunteers and cancer patients were extracted from whole blood in a CPT Vacutainer tube as recommended by the manufacturer. Briefly, 8 ml of whole blood were collected from a peripheral vein and centrifuged at $1500 \times g$ for 20 min at room temperature to isolate the PBMC fraction. The preparation was next transferred into a 15-ml conical tube, with PBS to fill the tube, and centrifuged at $600 \times g$ for 10 min at room temperature. Next, PBS was aspirated and disposed, and the PBMC pellet was snap frozen and stored at -80°C until use.

p70^{s6} Kinase Assay. The p70^{s6} kinase was immunoprecipitated from protein extracts and then assayed for kinase activity. Protein extracts from Raji cells, PBMCs, or tumor tissues were obtained after homogenization in lysis buffer [50 mM Tris (pH 7.5), 120 mM NaCl, 1 mM EDTA, 50 mM NaF, 40 mM 2-glycerophosphate, 0.1 mM sodium orthovanadate, 1 mM benzamidine, 0.5 mM phenylmethylsulfonyl fluoride, containing 1% NP40, and 10 μg/ml aprotinin, pepstatin, leupeptin, and antipain]. Protein concentration of cell lysates was determined using a Bradford assay. Equal amounts of lysate protein were incubated with antibody against p70^{s6} kinase (Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C, and then with 25 μl of protein G-agarose for 1 h. The immune complex beads were washed twice with lysis buffer and twice with kinase buffer [50 mM Tris (pH 7.5), 10 mM MgCl₂, 0.2 mM EGTA, 1 mM DTT, 1 mM benzamidine, and 0.5 mM phenylmethylsulfonyl fluoride]. The washed immunocomplexes were resuspended in 30 μl of kinase assay buffer containing 40 μM ATP, 2.5 μCi [³²P] ATP, 1 μM protein kinase A inhibitor peptide, and 250 μM p70^{s6} Rsk peptide substrate (Santa Cruz Biotechnology), and the reaction was incubated for 30 min at 30°C. Two different methods for the measurement of kinase activity were used to test the reliability of the assay; no significant changes were observed between results obtained by each method. In the first one, samples were subjected to SDS-PAGE, and the gel was stained with Coomassie Blue and vacuum dried. The dried gel was exposed with X-ray film at -80°C, and the kinase activity was measured by densitometry. In the second method, an aliquot of each sample was spotted onto P81 phosphocellulose paper. After extensive washing in 75 mM phosphoric acid, radioactivity on the papers was quantified by scintillation counting.

Immunoblotting. Equal amounts of Raji cell extract protein were subjected to SDS-PAGE, and proteins were transferred onto nitrocellulose membranes (Osmonics Inc., Minnetonka, MN). Membranes were blocked in 5% nonfat milk

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³ The abbreviations used are: mTOR, mammalian target of rapamycin; PBMC, peripheral blood mononuclear cell; CV, coefficient of variation.

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for 1 h and then incubated overnight at 4°C with either antibody against the phospho-p70^{s6} kinase (Thr389) or antibody against total p70^{s6} kinase as primary antibodies (Cell Signaling Technology, Beverly, MA). Then, the membranes were washed and incubated with antirabbit IgG conjugated to horseradish peroxidase for 1 h. After washing the membranes again, the immunoreactive proteins were detected using the enhanced chemiluminescence method (Amersham Pharmacia Biotech, Piscataway, NJ).

Healthy Volunteers Study. PBMCs from five healthy volunteers who were not taking CCI-779 were isolated from whole blood using the methodology described above. Blood samples were collected on days 0 (the day of first collection), 4 (3 days after day 0 collection), and 8 (7 days after day 0 collection).

Clinical Trial. Nine patients with advanced renal cell cancer and treated with different doses (25, 75, or 250 mg) of CCI-779 on a weekly schedule in a randomized Phase II study were studied (21). The details of this clinical trial will be reported separately. PBMCs were obtained from whole blood samples from each patient using CPT Vacutainer tubes, at the baseline visit, at 24- and 72-h post-treatment, and on day 8 before the second weekly dose. PBMCs were processed and analyzed as detailed above.

Statistical Analysis. In the measurement of p70^{s6} kinase activity, the values are expressed as the relative variation of the ³²P phosphorylation levels of the p70^{s6} Rsk peptide substrate between samples, according to the formula: relative p70^{s6} kinase activity = [(³²P substrate counts - background counts)/³²P Total counts]. The *in vitro* experiments were repeated three times, and the data presented represent the average of three experiments. The relationship between rapamycin concentration and p70^{s6} kinase inhibition in the Raji cells was determined by a linear regression analysis. The intersubject and intrasubject CV of p70^{s6} activity in PBMCs obtained from healthy volunteers was determined as the mean/SD. The decrement in p70^{s6} kinase activity in specimens collected from patients treated with CCI-779 was computed with the equation: proportional change = [(post-treatment - pretreatment)/pretreatment] × 100. The variation between the p70^{s6} kinase activity at the different time points was compared using a nonparametric test for multiple samples comparison. The relationship between the administered dose of CCI-779 and pharmacodynamic effects was explored using the non Kruskal-Wallis nonparametric test. The relationship between time to tumor progression and pharmacodynamic effects was explored using a linear regression analysis.

Results and Discussion

As mentioned above, inhibition of mTOR by CCI-779 inhibits the translational regulators 4E-BP-1 and p70^{s6} kinase. Previous studies have explored the analysis of changes in 4E-BP-1 phosphorylation to evaluate the biological effects of CCI-779. These studies have shown treatment with the agent results in a dose- and concentration-dependent inhibition in 4E-BP1 phosphorylation in *in vitro* and *in vivo* models (24-28). A recent study (28) reported a correlation between decreases in 4E-BP1 phosphorylation at site Thr70 and tumor growth inhibition.

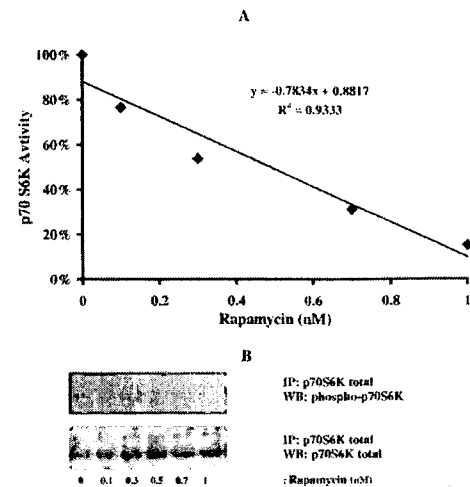


Fig. 1 Dose-response effect of rapamycin on p70^{s6} kinase activity in Raji cells. **A**, Raji cells were grown in full media and treated with increasing concentrations of rapamycin ranging from 0 to 1 nM. Cell lysates were analyzed for p70^{s6} kinase activity by scintillation counting, and the results are plotted as relative levels of kinase activity. **B**, immunoblotting analysis on phospho-p70^{s6} kinase expression in Raji cells and total p70^{s6} kinase as a loading control. Immunoprecipitates (IP) of total p70^{s6} kinase were analyzed by Western blot (WB) for either phospho-p70^{s6} or total p70^{s6} kinase. The panels show a representative result for at least three experiments with similar results.

These results suggest that an assay to measure decreases in phosphorylation for this particular site of 4E-BP1 may be a useful surrogate for determining inhibition of mTOR activity in clinical specimens. The use of 4E-BP1 to assess the pharmacodynamic effects of CCI-779, has, however, an important limitation from an analytical perspective. 4E-BP1 is not a kinase itself, and therefore, its activity cannot be measured using quantitative kinase assays as with p70^{s6} kinase. The availability of a quantitative assay for p70^{s6} kinase increases the rationale to use it rather than 4E-BP-1 for pharmacodynamic studies. For this reason, this study focuses on the development, validation, and feasibility testing of this approach.

Linear Concentration-Dependent Inhibition of p70^{s6} Kinase by Rapamycin in Raji Lymphoid Cells. The first experiment determined the effects of the mTOR inhibitor rapamycin on p70^{s6} kinase activity in the Raji cell line. Raji cells were selected for these experiments because they can be reliably cultured *in vitro* and have a lymphoid origin. As shown in Fig. 1A, p70^{s6} kinase activity decreased in a linear fashion (R² = 0.93) in response to increasing concentrations of rapamycin ranging from 0 to 1 nM. To confirm the results obtained with the kinase assay, we also performed Western blot analysis of protein extracts from Raji cells exposed to rapamycin with antibodies against total and active (phosphorylated) p70^{s6} kinase. As shown in Fig. 1B, exposure to rapamycin resulted in a concentration-dependent inhibition of p70^{s6} kinase activity in Raji cells

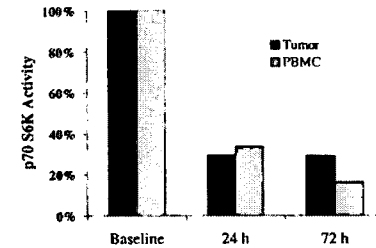


Fig. 2 p70^{s6} kinase activity in tumors and PBMCs in mice treated with CCI-779. Groups of five MDA-468 breast cancer cell-implanted mice were treated with a single dose of CCI-779 of 10 mg/kg and sacrificed before, and 24 and 72 h after, treatment. PBMCs were isolated from pooled blood collected from each mice group and cell lysates from extracted tumors and PBMCs processed for p70^{s6} kinase activity assay by scintillation counting. The average tumor size was 200 mg.

with no change in the expression of total protein, confirming the observations obtained with the quantitative kinase assay. These results, therefore, indicate that the activity of p70^{s6} kinase can be quantitated in lymphoid cells and varies in a predictable mode after exposure to an mTOR inhibitor, reinforcing the use of this method to evaluate CCI-779 pharmacodynamic effects.

CCI-779 Inhibits p70^{s6} Kinase Activity in PBMCs and Tumor Tissue *In Vivo*. The next question was to determine whether or not p70^{s6} kinase could be measured in PBMCs *in vivo*. To address this question, groups of five mice were treated with a single dose of CCI-779 of 10 mg/kg, a dose that has been shown to inhibit tumor growth in previous *in vivo* studies, and sacrificed 24 and 72 h after treatment. PBMCs were isolated from pooled blood collected from treated mice. As shown in Fig. 2, p70^{s6} kinase activity decreased by ~80% compared with the baseline activity. These results indicate that, indeed, p70^{s6} kinase activity in PBMCs is inhibited on treatment of mice with CCI-779 and can be quantitated. These results suggest that PBMCs, which are easily collected from patients in large scale clinical trials, could be an appropriate specimen to study the pharmacodynamics of CCI-779.

A major problem with the use of normal tissues to determine pharmacodynamic effects of anticancer agents is that the dose and/or concentration required to inhibit a biological target in tumor and normal tissue may not necessarily be similar. To address this problem, the p70^{s6} kinase activity was compared between PBMCs and simultaneously collected tumor tissues. For these experiments, we selected the PTEN-negative MDA-468 breast cancer cell line that has constitutive p70^{s6} kinase activity and has been shown to be susceptible to CCI-779 *in vitro* and *in vivo* experiments. As demonstrated in Fig. 2, the activity of p70^{s6} kinase decreased in a parallel fashion in tumor tissues and PBMCs, indicating that, indeed, the PBMCs could be an appropriate surrogate tissue to study the effects of CCI-779 on p70^{s6} kinase activity.

Although pharmacodynamic studies should ideally be conducted in tumor tissues rather than normal tissue, this is rarely possible in clinical trials in which only a minority of patients has accessible tumor tissues for sequential sampling. In addition, the

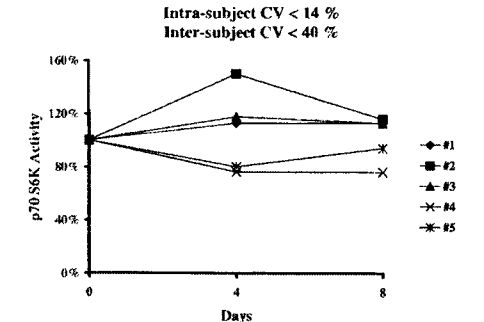


Fig. 3 p70^{s6} kinase activity in PBMCs in healthy volunteers. Five healthy volunteers not exposed to CCI-779 had PBMCs collected from whole blood at days 0, 4, and 8. p70^{s6} kinase activity assays by scintillation counting were performed in cell lysates, and the activity results were tested for the intra and intersubject CV.

collection of tumor tissues for biological studies in a sufficient number of patients to explore the relationship between pharmacodynamic effects and outcome is not possible. For this reason, the validation of a surrogate tissue that is easily collectable in a large number of patients in disease-oriented clinical trials is needed. The data reported here suggest that PBMCs may indeed be an appropriate surrogate tissue to examine the activity of p70^{s6} kinase on the basis of *in vivo* data. Additional clinical studies are currently in development to explore if this correlation also exists in patients in intensive studies in which tumor tissue and PBMCs will be simultaneously collected in a limited number of patients.

p70^{s6} Kinase Activity in PBMCs Collected from Human Subjects Remains Stable Over Time. Subsequently, we examined the degree of spontaneous variation of p70^{s6} kinase activity in PBMCs collected from normal individuals. Five healthy volunteers not exposed to CCI-779 had PBMCs collected at three consecutive time points, and p70^{s6} kinase activity was determined using the methods described previously. As illustrated in Fig. 3, although intersubject variability was marked with a CV of 40%, the intrasubject variability over a 1-week period was only 14%. These results indicate that p70^{s6} kinase activity in PBMCs does not change spontaneously over time, reinforcing its value as a potential relevant pharmacodynamic marker. In addition, the substantial variation observed among individuals at any given time point emphasizes the need to use each patient as his or her own control in the analysis of pharmacodynamic markers.

Variation in p70^{s6} Kinase Activity in PBMCs from Patients Treated with CCI-779. The feasibility of implementing this method in clinical trials was explored using a total of nine patients with renal cell carcinoma treated with CCI-779 at doses of 25, 75, and 250 mg in a double blind Phase II study. PBMCs were collected from these patients at baseline and on days 2, 4, and 8 after a single i.v. dose of CCI-779 (21). The activity of p70^{s6} kinase in PBMCs was determined as described above. Fig. 4 shows a representative example of changes in

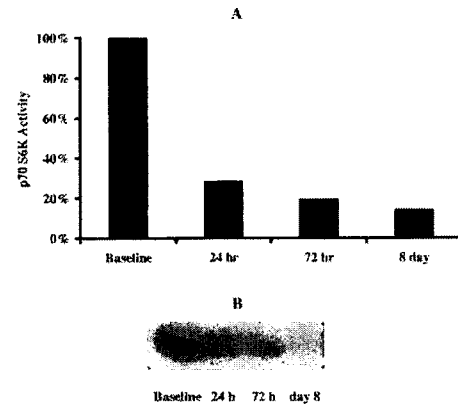


Fig 4. Representative example of p70^{S6} kinase activity methodology in PBMCs from cancer patients. A, graph of percentages of p70^{S6} kinase activities measured by gel densitometry in PBMC lysates collected from one cancer patient, treated with CCI-779 at a dose of 75 mg, at different time points. B, autoradiogram of an SDS polyacrylamide gel of samples of ³²P-p70 kinase activity assays used for the graph shown in A.

p70^{S6} kinase activity as a function of time. In the prototypical patient, p70^{S6} kinase activity decreased by 24 h after treatment and reached a maximum inhibition at day 8.

No relationship was observed between the administered dose of CCI-779 and inhibition of p70^{S6} kinase in PBMCs in this small cohort of patients, suggesting that CCI-779 exerts pharmacodynamic effects at doses as low as 25 mg. In this regard, preliminary data from randomized Phase II clinical trials of patients treated with CCI-779 do not suggest a clear association between dose and treatment outcome (21). Importantly, as illustrated in Fig 5, there was a linear association between inhibition of p70^{S6} kinase and time to tumor progression ($P < 0.04$). Although the limited sample size in this feasibility study does not permit one to draw firm conclusions regarding the value of this biomarker to predict the outcome of patients treated with the drug, the results support the incorporation of this test in additional larger studies.

On the basis of these results, this method has been implemented in additional Phase I and II clinical trials with CCI-779. The results of Phase I dose escalation and pharmacokinetic studies will permit one to explore the relationship between dose and pharmacokinetic parameters and pharmacodynamic effects and model the mathematical equations governing these relationships. Furthermore, the data from Phase II studies will be used to explore correlations between pharmacodynamic effects and outcome.

A key issue that needs to be considered in the development of pharmacodynamic end points, particularly when using normal tissues, is whether these end points are merely pharmacological end points whose target inhibition is not necessarily synonymous with antitumor effects. Indeed, for CCI-779, it has been demonstrated that the degree of inhibition of p70^{S6} kinase activity is not different in susceptible versus resistant cell lines,

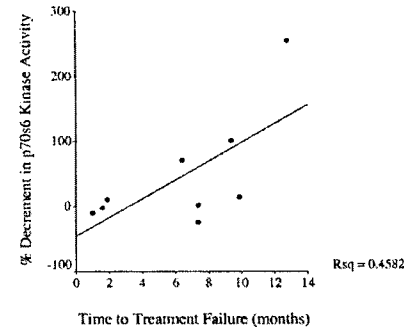


Fig 5. Relationship between proportional decrement in p70^{S6} kinase activity and time to tumor progression in nine patients with renal cell cancer treated with CCI-779. $P = 0.04$, $R^2 = 0.45$.

and other factors, such as the expression and modulation by CCI-779 of other oncogenes and cell cycle regulators, such as c-myc, cyclin D3, and p27, are also important. These studies, therefore, need to be followed by additional preclinical and clinical studies to determine the relationship between inhibition of the immediate intratumor target and other parameters of antitumor effects, such as cell cycle arrest, apoptosis, and tumor growth. Ultimately, as mentioned above, the final validation will be to prove the relationship between target inhibition and indices of patient outcome. These various aspects are currently the subjects of active investigation.

In summary, the results from these studies demonstrate that the pharmacodynamic effects of CCI-779 can be measured in PBMCs using a fully quantitative p70^{S6} kinase assay. The activity of p70^{S6} kinase decreases in a linear fashion after exposure to an mTOR inhibitor, and there is a good correlation in magnitude of p70^{S6} kinase inhibition between PBMCs and tumor tissues. In addition, this parameter appears to be relatively constant over time in healthy subjects. The combination of these findings reinforces the potential value of measuring p70^{S6} kinase activity as a pharmacodynamic marker of CCI-779. Studies in progress will help to elucidate the relationship between dose and pharmacokinetic parameters of CCI-779 and p70^{S6} kinase inhibition and the value of this measurement in predicting the clinical activity of this agent.

References

- Sehgal, S. N., Molnar-Kimber, K., Ocain, T. D., and Weichman, B. M. Rapamycin: a novel immunosuppressive macrolide. *Med. Res. Rev.*, 14: 1-22, 1994.
- Sabatini, D. M., Erdjument-Bromage, H., Lui, M., Tempst, P., and Snyder, S. H. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TOR1. *Cell*, 78: 35-43, 1994.
- Sabers, C. J., Martin, M. M., Brunn, G. J., Williams, J. M., Dumont, F. J., Wiederrecht, G., and Abraham, R. T. Isolation of a protein target of the FKBP12-rapamycin complex in mammalian cells. *J. Biol. Chem.*, 270: 815-822, 1995.
- Brown, E. J., Albers, M. W., Shin, T. B., Ichikawa, K., Keith, C. T., Lane, W. S., and Schreiber, S. L. A mammalian protein targeted by

- G1-arresting rapamycin-receptor complex. *Nature (Lond.)*, 369: 756-758, 1994.
- Chiu, M. L., Katz, H., and Berlin, V. RAPT1, a mammalian homolog of yeast Tor, interacts with the FKBP12/rapamycin complex. *Proc. Natl. Acad. Sci. USA*, 91: 12574-12578, 1994.
- Wiederrecht, G. J., Sabers, C. J., Brunn, G. J., Martin, M. M., Dumont, F. J., and Abraham, R. T. Mechanism of action of rapamycin: new insights into the regulation of G1-phase progression in eukaryotic cells. *Prog. Cell Cycle Res.*, 1: 53-71, 1995.
- Downward, J. Mechanisms and consequences of activation of protein kinase B/Akt. *Curr. Opin. Cell Biol.*, 10: 262-267, 1998.
- Scott, P. H., Brunn, G. J., Kohn, A. D., Roth, R. A., and Lawrence, J. C., Jr. Evidence of insulin-stimulated phosphorylation and activation of the mammalian target of rapamycin mediated by a protein kinase B signaling pathway. *Proc. Natl. Acad. Sci. USA*, 95: 7772-7777, 1998.
- Nave, B. T., Ouwens, M., Withers, D. J., Alessi, D. R., and Shepherd, P. R. Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. *Biochem. J.*, 344: 427-431, 1999.
- White, R. J. Regulation of RNA polymerases I and III by the retinoblastoma protein: a mechanism for growth control? *Trends Biochem. Sci.*, 22: 77-80, 1997.
- Bromberg, J. F., Wrzeszczynska, M. H., Devgan, G., Zhao, Y., Pestell, R. G., Albanese, C., and Darnell, J. E., Jr. Stat3 as an oncogene. *Cell*, 98: 295-303, 1999.
- Hashemolhosseini, S., Nagamine, Y., Morley, S. J., Desrivieres, S., Mereep, L., and Ferrari, S. Rapamycin inhibition of the G1 to S transition is mediated by effects on cyclin D1 mRNA and protein stability. *J. Biol. Chem.*, 273: 14424-14429, 1998.
- Sehgal, S. N. Rapamycin (Sirolimus, rapamycin): an overview and mechanism of action. *Ther. Drug Monit.*, 17: 660-665, 1995.
- Aoki, M., Blazek, E., and Vogt, P. K. A role of the kinase mTOR in cellular transformation induced by the oncoproteins p3k and Akt. *Proc. Natl. Acad. Sci. USA*, 98: 136-141, 2001.
- Brunn, G. J., Hudson, C. C., Sekulic, A., Williams, J. M., Hosoi, H., Houghton, P. J., Lawrence, J. C., Jr., and Abraham, R. T. Phosphorylation of the translational repressor PHAS-I by the mammalian target of rapamycin. *Science (Wash. DC)*, 277: 99-101, 1997.
- Gingras, A. C., Kennedy, S. G., O'Leary, M. A., Sonenberg, N., and Hay, N. 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt(PKB) signaling pathway. *Genes Dev.*, 12: 502-513, 1998.
- Hara, K., Yonezawa, K., Kozlowski, M. T., Sugimoto, T., Andrabi, K., Weng, Q. P., Kasuga, M., Nishimoto, I., and Avruch, J. Regulation of eIF-4E-BP1 phosphorylation by mTOR. *J. Biol. Chem.*, 272: 26457-26463, 1997.

- Nielsen, F. C., Ostergaard, L., Nielsen, J., and Christiansen, J. Growth-dependent translation of IGF-II mRNA by a rapamycin-sensitive pathway. *Nature (Lond.)*, 377: 358-362, 1995.
- Rosenwald, I. B., Kaspar, R., Rousseau, D., Gehrke, L., Lehoucq, P., Chen, J. J., Schmidt, E. V., Sonenberg, N., and London, I. M. Farkaryotic translation initiation factor 4E regulates expression of cyclin D1 at transcriptional and post-transcriptional levels. *J. Biol. Chem.*, 270: 21176-21180, 1995.
- Hidalgo, M., and Rowinsky, E. K. The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. *Oncogene*, 19: 6680-6686, 2000.
- Stadler, W., Logan, T., Dutcher, J. P., Hudes, G., Park, Y., Marshall, B., Bont, J., and Dukart, G. A randomized double-blind phase 2 study of intravenous CCI-779 administered weekly to patients with advanced renal cell carcinoma. *Proceedings of the 38th ASCO Annual Meeting*, 21: 10a, 2002.
- Chan, S., Johnston, S., Scheulen, M. E., Moss, K., Morant, R., Lahr, A., Feussner, A., Berger, M., and Kirsch, T. First report: a phase 2 study of the safety and activity of CCI-779 for patients with locally advanced or metastatic breast cancer failing prior chemotherapy. *Proceedings of the 38th ASCO Annual Meeting*, 21: 44a, 2002.
- Yatscoff, R. W., and Aspeslet, L. J. The monitoring of immunosuppressive drugs: a pharmacodynamic approach. *Ther. Drug Monit.*, 20: 459-463, 1998.
- Lin, T. A., Kong, X., Saltiel, A. R., Blackshear, P. J., and Lawrence, J. C., Jr. Control of PHAS-I by insulin in 3T3-L1 adipocytes. Synthesis, degradation, and phosphorylation by a rapamycin-sensitive and mitogen-activated protein kinase-independent pathway. *J. Biol. Chem.*, 270: 18531-18538, 1995.
- Beretta, L. A., Gingras, A. C., Svitnik, Y. V., Hall, M. N., and Sonenberg, N. Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation. *EMBO J.*, 15: 658-664, 1996.
- von Manteuffel, S. R., Gingras, A. C., Ming, X. F., Sonenberg, N., and Thomas, G. 4E-BP1 phosphorylation is mediated by the FRAP-p70S6 pathway and is independent of mitogen-activated protein kinase. *Proc. Natl. Acad. Sci. USA*, 93: 4076-4080, 1996.
- Yu, K., Toral-Barza, L., Discafani, C., Zhang, W. G., Skotnicki, J., Frost, P., and Gibbons, J. J. mTOR, a novel target in breast cancer: the effect of CCI-779, an mTOR inhibitor, in preclinical models of breast cancer. *Endocr. Relat. Cancer*, 8: 249-258, 2001.
- Dudkin, L., Dilling, M. B., Cheshire, P. J., Harwood, F. C., Hollingshead, M., Arbuck, S. G., Travis, R., Sausville, E. A., and Houghton, P. J. Biochemical correlates of mTOR inhibition by the rapamycin ester CCI-779 and tumor growth inhibition. *Clin. Cancer Res.*, 7: 1758-1764, 2001.

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Rapamycin and CCI-779

Bulletin du Cancer. Volume 86, Number 10, 808-11, Octobre 1999, Point sur...

Résumé Summary

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Summary : 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.

ARTICLE

La rapamycine (figure 1) ou sirolimus est un macrolide produit par *Streptomyces hygroscopicus*, proche de la ciclosporine et du FK506 (tacrolimus). Elle fut initialement identifiée comme un agent antifongique il y a maintenant trente ans [1]. Secondairement, elle a surtout été développée comme un agent immunosuppresseur (Rapamune[®], Wyeth-Léderlé). Plusieurs essais cliniques ont montré son intérêt dans le traitement du rejet de greffe d'organe [2]. Ses propriétés antitumorales sur différents modèles cellulaires sont également connues depuis plusieurs années, mais n'ont jamais fait l'objet de publications en clinique humaine [3].

L'originalité de la rapamycine tient à son mécanisme d'action. Elle inhibe spécifiquement une protéine kinase cytoplasmique, mTOR (pour mammalian target of rapamycin) qui intervient dans une voie de signalisation mitogénique réglant l'initiation de la traduction [4].

L'intérêt pour la rapamycine a récemment été relancé par le développement d'analogues tels que le CCI-779 (figure 1) témoignant, sur des modèles précliniques, d'une activité antiproliférative et d'un faible effet immunosuppresseur [5].

La rapamycine**Activité antitumorale**

In vitro, la rapamycine inhibe la croissance de plusieurs lignées tumorales humaines, en particulier d'ostéosarcome [6], de rhabdomyosarcomes Rh1 et Rh30 [7], de cancers bronchiques à petites cellules H69, H345 et H510 [8], d'hépatome H4 [9] et de cancer du sein hormonodépendant MCF7 [10]. La gamme des concentrations efficaces va de 0,3 à 50 nM. Dans plusieurs de ces lignées, il a été montré que la rapamycine bloque les cellules en phase G1 du cycle cellulaire. Cet effet cytostatique a été confirmé in vivo sur des modèles de xénogreffes de tumeurs humaines chez la souris nude [3]. Un effet proapoptotique a également été observé sur des lignées de rhabdomyosarcome cultivées sans facteur de croissance [11] et en association avec le cisplatine sur une lignée de carcinome ovarien [12].

Mécanisme d'action moléculaire

La rapamycine peut être considérée comme une « pro-drogue » dans la mesure où son action intracellulaire nécessite sa fixation à une immunophiline dénommée FKBP12 (FK506 binding protein, car elle fixe également le FK506) [13]. Le complexe ainsi formé est très stable (temps de demi-dissociation de 17,5 h) et autorise un effet biologique prolongé de la rapamycine (7 jours après une heure d'exposition in vitro) [11], suggérant que celle-ci puisse être administrée de façon discontinue.

La seule cible actuellement connue du complexe rapamycine/FKBP12 est la protéine mTOR. Celle-ci est une sérine-thréonine kinase de la famille des phosphatidyl inositol kinases [4]. Elle est activée par un grand nombre de facteurs de croissance, en particulier les interleukines 2, 4 et 6, l'insuline, et l'insulin-like growth factor 1. En réponse à ces stimuli mitogéniques, mTOR va activer l'initiation de la traduction d'un grand nombre d'ARNm par deux voies parallèles [4, 14] (figure 2) :

- le facteur d'initiation de la traduction 4E (eIF4E) est à l'état basal séquestré et inhibé par la protéine 4E-BP1. mTOR, en phosphorylant cette dernière, va permettre la libération de eIF4E et l'initiation de la traduction. Les protéines ainsi synthétisées sont supposées induire, directement ou indirectement, la transition G1/S ;

- mTOR active la p70/S6 kinase qui active alors à son tour la protéine ribosomique S6. Sous sa forme phosphorylée, cette dernière contrôle sélectivement la traduction d'ARNm possédant un domaine riche en pyrimidine à leur extrémité 5'. Ces ARNm codent pour des protéines ribosomiques et des facteurs d'élongation.

Les facteurs protéiques activés en amont de mTOR après fixation du facteur de croissance sur son récepteur sont moins bien connus. Une isoforme de la p85/PI3 kinase serait en particulier impliquée [15].

L'inhibition de la fonction biologique de mTOR par la rapamycine semble jouer un rôle essentiel dans son action cytostatique. Cependant, il reste encore beaucoup à apprendre sur, d'une part, les mécanismes exacts par lesquels mTOR contrôle la transition G1/S et, d'autre part, d'éventuelles autres cibles cellulaires de la rapamycine. Ainsi, il a été récemment montré sur des fibroblastes murins 3T3 stimulés par du sérum que la rapamycine diminuait le taux de cycline D1. Cependant, contrairement à ce que l'on pouvait croire, cela n'était pas dû à un défaut de synthèse mais à une dégradation accélérée de la protéine [16]. Sur d'autres modèles, la rapamycine induit l'accumulation de l'inhibiteur de cycline p27kip1 [17].

L'action proapoptotique de la rapamycine est également mal connue. Elle semble dépendante de l'inhibition de mTOR, mais pas de p53 [11], ni de bcl-2 [18].

Une meilleure connaissance des mécanismes d'action de la rapamycine au niveau moléculaire permettrait idéalement de prédire la sensibilité des tumeurs à cette molécule en fonction de l'expression ou de la non-expression de tel ou tel gène. Il a ainsi déjà été montré que des tumeurs surexprimant c-myc étaient résistantes à la rapamycine [19], de même que celles issues de patients atteints d'ataxie-télangiectasie [20]. De façon intéressante, le gène ATM, déficient au cours de l'ataxie-télangiectasie, code pour une phosphatidyl inositol kinase proche de mTOR.

Expérience clinique

Elle concerne essentiellement l'utilisation de la rapamycine comme agent immunosuppresseur, seule ou en association avec la ciclosporine. La rapamycine est administrée par voie orale et est métabolisée par le cytochrome P450 3A en plusieurs métabolites inactifs [21].

À la dose recommandée pour les phases II de 7 mg/m²/j, la toxicité porte essentiellement sur des paramètres biologiques [2]. Il existe une toxicité hématologique dose-dépendante prédominante sur les plaquettes, et généralement asymptomatique et ne nécessitant pas de transfusion. Cette hémato-toxicité est d'origine centrale et pourrait être liée à l'inhibition par la rapamycine des signaux mitogènes transmis par les cytokines. La rapamycine augmente le taux de triglycérides et de cholestérol parfois de façon considérable. Cette hyperlipémie pourrait surtout poser des problèmes lors d'une administration au long cours du fait de l'augmentation du risque cardiovasculaire. Il n'a jamais été observé de pancréatite aiguë liée à l'hypertriglycéridémie, ni d'autre manifestation clinique. Chez ces patients déjà lourdement immunodéprimés, la rapamycine ne semble pas augmenter le risque d'infections, sauf peut-être celles liées au virus herpès simplex. Cette constatation est à rapprocher du fait que la rapamycine augmente la traduction des ARNm de certains virus [22].

Un nouvel analogue de la rapamycine : le CCI-779

Dans plusieurs modèles murins, l'action antitumorale de la rapamycine est plus importante lorsqu'une administration parentérale est utilisée. Cependant, celle-ci est difficilement réalisable en pratique clinique courante du fait d'une faible solubilité de la molécule [5]. Des analogues de la rapamycine ont donc été développés dont les propriétés physicochimiques permettent une administration par voie intraveineuse aisée.

Le CCI-779 est l'un de ces analogues. Il présente une activité cytostatique sur plusieurs modèles de xénogreffes de tumeurs humaines à des souris nude, en particulier dans des glioblastomes, carcinomes de prostate, du pancréas et du sein [23] et des médulloblastomes [24].

Tout comme la rapamycine, la fixation du CCI-779 à la FKBP12 est une étape indispensable à son action [23]. Il reste cependant à déterminer si le mécanisme d'action du CCI-779 est entièrement superposable à celui de la rapamycine.

De façon intéressante, l'activité antitumorale décrite chez la souris nude est maintenue pendant 14 jours après une administration quotidienne de 5 jours alors que l'effet immunosuppresseur disparaît au bout de 24 h [23].

Des essais thérapeutiques de phase I sont en cours en France et aux États-Unis.

CONCLUSION

La rapamycine est le premier représentant d'une nouvelle classe d'anticancéreux au mécanisme d'action tout à fait original et au profil de toxicité favorable. Les études précliniques suggèrent pour ces molécules une action essentiellement cytostatique. Le CCI-779, premier analogue administrable par voie parentérale, fait actuellement l'objet d'essais cliniques de phase I. Parallèlement, une meilleure connaissance des cibles moléculaires de la rapamycine et de ses analogues pourrait guider la réalisation de futurs essais cliniques de phase II.

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 signalling 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 D1-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 I 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 S6 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, Liu 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. Médecine/sciences 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 p70S6K. J Biol Chem 1998 ; 273 : 4776-82.
16. Hashemolhosseini S, Nagamine Y, Morley SJ, Desrivieres S, Mercep L, Ferrari S. Rapamycin inhibition of the G1 to S transition is mediated by effects on cyclin D1 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 p27kip1 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. Trepanier 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).

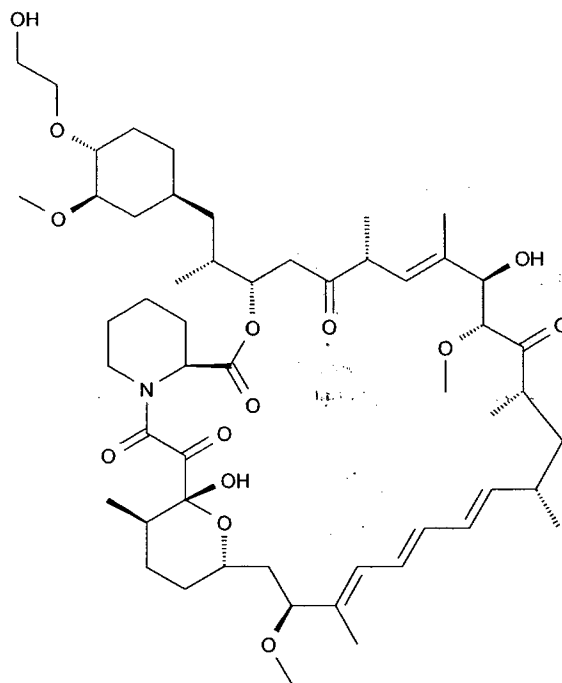
24. Geoerger B, Kerr K, Janss AJ, Sutton LN, Phillips PC. Rapamycin analog CCI-779 inhibits growth of human medulloblastoma xenografts. *Proc Am Ass Cancer Res* 1999 ; 40 : 603 (abstr. 3978).

CERTICAN[®]
(everolimus)

DESCRIPTION

The active ingredient of Certican is everolimus. The chemical name is 40-O-(2-hydroxyethyl)-rapamycin or 40-O-(2-hydroxyethyl)-sirolimus. Its molecular formula is $C_{53}H_{83}NO_{14}$ and its molecular weight is 958.2.
CAS number: 159351-69-6

The structural formula of everolimus is:



Everolimus is a white to faintly yellow powder practically insoluble in water but soluble in organic solvents such as ethanol and methanol.

Excipients:

Tablets: Butylated hydroxytoluene, magnesium stearate, lactose monohydrate, hypromellose, crospovidone, lactose anhydrous.

Dispersible tablets: Butylated hydroxytoluene, magnesium stearate, lactose monohydrate, hypromellose, crospovidone, lactose anhydrous, colloidal anhydrous silica.

PHARMACOLOGY

Pharmacodynamics

Everolimus, a proliferation signal inhibitor, prevents allograft rejection in rodent and non-human primate models of allotransplantation. It exerts its immunosuppressive effect by inhibiting the proliferation, and thus clonal expansion, of antigen-activated T cells which is driven by T cell-specific interleukins, e.g. interleukin-2 and interleukin-15. Everolimus inhibits an intracellular signaling pathway which is triggered upon binding of these T cell growth factors to their respective receptors, and which normally leads to cell proliferation. The blockage of this signal by everolimus leads to an arrest of the cells at the G₁ stage of the cell cycle.

At the molecular level, everolimus forms a complex with the cytoplasmic protein FKBP-12. In the presence of everolimus the growth factor-stimulated phosphorylation of the p70 S6 kinase is inhibited. Since p70 S6 kinase phosphorylation is under the control of FRAP (also called m-TOR), this finding suggests that the everolimus-FKBP-12 complex binds to and thus interferes with the function of FRAP. FRAP is a key regulatory protein which governs cell metabolism, growth and proliferation; disabling FRAP function thus explains the cell cycle arrest caused by everolimus.

Everolimus, has a different mode of action than cyclosporin. In preclinical models of allotransplantation, the combination of everolimus and cyclosporin was more effective than either drug alone.

The effect of everolimus is not restricted to T cells. It inhibits in general, growth factor-stimulated proliferation of haematopoietic as well as non-haematopoietic cells, like, for instance, that of vascular smooth muscle cells. Growth factor-stimulated vascular smooth muscle cell proliferation, triggered by injury to endothelial cells and leading to neointima formation, plays a key role in the pathogenesis of chronic rejection. Preclinical studies with everolimus have shown inhibition of neointima formation in a rat aorta allotransplantation model.

Pharmacokinetics

Absorption: After oral dosing, peak everolimus concentrations occur 1 to 2 h postdose. Everolimus blood concentrations are dose proportional over the dose range 0.25 to 15 mg in transplant patients. The relative bioavailability of the dispersible tablet compared with the tablet is 0.90 (90 % CI 0.76-1.07) based on the AUC-ratio.

Effects of Food: The C_{max} and AUC of everolimus are reduced by 60 % and 16 % when the tablet formulation is given with a high fat meal. To minimise variability, Certican should be taken consistently with or without food.

Distribution: The blood-to-plasma ratio of everolimus is concentration-dependent ranging from 17 % to 73 % over the range of 5 to 5000 ng/mL. Plasma protein binding is approximately 74 % in healthy subjects and patients with moderate hepatic impairment. The distribution volume associated with the terminal phase (V_z/F) in maintenance renal transplant patients is 342 ± 107 L.

Metabolism: Everolimus is a substrate of CYP3A4 and P-glycoprotein. The main metabolic pathways identified in man were mono-hydroxylations and O-dealkylations. Two main metabolites were formed by hydrolysis of the cyclic lactone. Everolimus

was the main circulating component in blood. None of the main metabolites are likely to contribute significantly to the immunosuppressive activity of everolimus.

Excretion: After a single dose of radiolabeled everolimus to transplant patients receiving cyclosporin the majority (80%) of radioactivity was recovered from the faeces, and only a minor amount (5%) was excreted in urine. Parent drug was not detected in urine nor faeces.

Steady-state pharmacokinetics: Pharmacokinetics were comparable for kidney and heart transplant patients receiving everolimus twice daily simultaneously with cyclosporin. Steady-state is reached by day 4 with an accumulation in blood levels of 2 to 3-fold compared with the exposure after the first dose. T_{max} occurs at 1 to 2 h postdose. C_{max} averages 11.1 ± 4.6 and 20.3 ± 8.0 ng/mL and AUC averages 75 ± 31 and 131 ± 59 ng.h/mL at 0.75 and 1.5 mg bid, respectively. Predose trough blood levels (C_{min}) average 4.1 ± 2.1 and 7.1 ± 4.6 ng/mL at 0.75 and 1.5 mg bid, respectively. Everolimus exposure remains stable over time in the first post-transplant year. C_{min} is significantly correlated with AUC yielding a correlation coefficient between 0.86 and 0.94. Based on a population pharmacokinetic analysis, oral clearance (CL/F) is 8.8 L/h (27% interpatient variation) and the central distribution volume (V_c/F) is 110 L (36% interpatient variation). Residual variability in blood concentrations is 31%. The elimination half-life is 28 ± 7h.

Hepatic impairment: Everolimus AUC was increased an average 2-fold in 8 patients with moderate hepatic impairment (Child-Pugh Class B) as compared with 8 healthy subjects. The AUC of everolimus tended to be greater than that of healthy subjects where bilirubin was >34 µmol/L, prothrombin time was >1.3 INR (> 4 sec prolongation), and/or albumin concentration was <35 g/L. The impact of severe hepatic impairment (Child-Pugh Class C) has not been assessed but the effect on everolimus AUC is likely to be as large or larger compared with moderate impairment.

Renal impairment: Post-transplant renal impairment (CL_{crea} range, 11-107 mL/min) did not affect the pharmacokinetics of everolimus.

Paediatrics: Everolimus CL/F increased in a linear manner with patient age (1 to 16 years), body surface area (0.49-1.92 m²), and weight (11-77 kg). Steady-state CL/F was 10.2 ± 3.0 L/h/m² and elimination half-life was 30 ± 11 h. Nineteen paediatric *de novo* renal transplant patients (1 to 16 years) received Certican dispersible tablets at a dose of 0.8 mg/m² (maximum 1.5 mg) twice-daily with cyclosporin microemulsion. They achieved an everolimus AUC of 87 ± 27 ng.h/mL which is similar to adults receiving 0.75 mg twice daily. Steady-state trough levels were 4.4 ± 1.7 ng/mL.

Elderly: A limited reduction in everolimus oral CL of 0.33 % per year was estimated in adults (age range studied was 16-70 years). No dose adjustment is considered necessary.

Exposure-response relationships: The average everolimus trough concentration over the first 6 months post-transplant was related to the incidence of biopsy-confirmed acute rejection and with thrombocytopenia in kidney and heart transplant patients (See Table 1 below).

Table 1. Drug Exposure-Response Relationships (Studies B251/B253)

Kidney transplantation (Study B251)					
Trough level (ng/mL)	< 3.4	3.5 - 4.5	4.6 - 5.7	5.8 - 7.7	7.8 - 15.0
Freedom from rejection	68 %	81 %	86 %	81 %	91 %
Thrombocytopenia (<100 x 10 ⁹ /L)	10 %	9 %	7 %	14 %	17 %
Heart transplantation (Study B253)					
Trough level (ng/mL)	< 3.5	3.6 - 5.3	5.4 - 7.3	7.4 - 10.2	10.3 - 21.8
Freedom from rejection	65 %	69 %	80 %	85 %	85 %
Thrombocytopenia (<75 x 10 ⁹ /L)	5 %	5 %	6 %	8 %	9 %

CLINICAL TRIALS

Kidney transplantation

Certican in fixed doses of 1.5 mg/day and 3 mg/day, in combination with standard doses of cyclosporin microemulsion and corticosteroids was investigated in two Phase III *de novo* renal transplant trials (Studies B201 and B251). Mycophenolate mofetil (MMF) 1 g twice a day was used as comparator. The co-primary composite endpoints were efficacy failure (biopsy-proven acute rejection, graft loss, death or loss to follow-up) at 6 months, and graft loss, death or loss to follow-up at 12 months. Certican was overall non-inferior to MMF in these trials. The incidence of biopsy-proven acute rejection at 6 months in the B201 study was 21.6 %, 18.2 %, and 23.5 % for the Certican 1.5 mg/day, Certican 3 mg/day and MMF groups, respectively. In the B251 study, the incidences were 17.1 %, 20.1 %, and 23.5 % for the Certican 1.5 mg/day, Certican 3 mg/day and MMF groups respectively.

Reduced allograft function with elevated serum creatinine was observed more frequently among subjects using Certican in combination with full dose cyclosporin microemulsion than in MMF patients. This effect is believed to be due to increased cyclosporin nephrotoxicity and may be reversible with cyclosporin dose reduction.

Two Phase IIIb studies (A2306 and A2307) evaluated efficacy and safety of Certican 1.5 and 3 mg per day (initial dosing, subsequent dosing based on target trough concentration ≥3ng/mL) in combination with reduced exposure to cyclosporin microemulsion monitored by C₂ in *de novo* transplant recipients. In study A2306, target C₂ concentrations were as follows: weeks 0-4, 1000-1400 ng/mL, weeks 5-8, 700-900 ng/mL, weeks 9-12, 550-650 ng/mL, weeks 13-52, 350-450 ng/mL. In study A2307 (combination with Simulect), target C₂ concentrations were as follows: weeks 0-8, 500-700 ng/mL, weeks 9-52, 350-450 ng/mL.

Actual trough concentrations (C₀) and C₂ for study A2306 are listed in Table2 below:

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Table 2: Summary statistics of CsA trough (C0) and C2 levels (Study A2306)

Visit	Mean CsA trough level (±SD)		Mean CsA C2 level (±SD)	
	Everolimus 1.5 mg	Everolimus 3 mg	Everolimus 1.5 mg	Everolimus 3 mg
Day 7	250 ± 114 N=110	262 ± 133 N=121	1259 ± 323 N=111	1224 ± 324 N=123
Day 28	239 ± 134 N=101	278 ± 207 N=110	1121 ± 359 N=103	1167 ± 410 N=119
Month 3	131 ± 85 N=91	140 ± 99 N=101	685 ± 284 N=95	754 ± 362 N=103
Month 6	82 ± 60 N=80	83 ± 67 N=96	534 ± 265 N=84	545 ± 318 N=99

In study A2306, 6 month results for the ITT population (N=237; 112 on Certican 1.5 mg/day and 125 on 3.0 mg/day) demonstrated incidences of biopsy-proven rejection of 25.0 % for the 1.5 mg/day group and 15.2 % for the 3 mg/day group. Across-study comparisons indicated that allograft function was better than seen with Certican in the full-dose cyclosporin microemulsion studies. On-treatment median serum creatinine values at Month 6 were 133 µmol/L in the 1.5 mg and 132 µmol/L in the 3 mg Certican group (mean: 147 and 140 µmol/L). On-treatment median creatinine clearance values according to Cockcroft-Gault were 66 and 63 mL/min in the 2 Certican groups (mean: 66 and 63 mL/min).

The A2307 study has a similar design to A2306 with the addition of 2 doses of basiliximab 20 mg at day 0 and day 4, and lower target C2 levels for cyclosporin through the first 8 weeks following transplantation. Analyses of the 6 month results (ITT, N=256; 117 on Certican 1.5 mg/day and 139 on 3.0 mg/day) demonstrated incidences of biopsy-proven rejection of 13.7 % for the 1.5 mg/day group and 15.1 % for the 3 mg/day group. On-treatment median serum creatinine values at Month 6 were 130 µmol/L in the 1.5 mg and 130 µmol/L in the 3 mg Certican group (mean: 137 and 136 µmol/L). On-treatment median creatinine clearance values according to Cockcroft-Gault were 67 and 64 mL/min in the 2 Certican groups (mean: 68 and 65 mL/min).

Heart transplantation

In the Phase III heart study (B253), both Certican 1.5 mg/day and 3 mg/day in combination with standard doses of cyclosporin microemulsion and corticosteroids, were investigated vs. azathioprine (AZA), 1-3 mg/kg/d. The primary endpoint was a composite of incidence of acute rejection ≥ISHLT grade 3A, acute rejection associated with haemodynamic compromise, graft loss, patient death or loss to follow-up at 6, 12 and 24 months. Both doses of Certican were superior to AZA at 6, 12 and 24 months. The incidence of biopsy proven acute rejection ≥ISHLT grade 3A at month 6 was 27.8 % for the 1.5 mg/d group, 19 % for the 3 mg/d group and 41.6% for the AZA group respectively (p = 0.003 for 1.5 mg vs control, < 0.001 for 3 mg vs control).

Based on coronary artery intravascular ultrasound data obtained from a subset of the study population, both Certican doses were statistically significantly more effective than AZA in preventing allograft vasculopathy (defined as an increase in maximum intimal thickness from baseline ≥0.5 mm in at least one matched slice of an automated pullback sequence), an important risk factor for long term graft loss.

Cyclosporin doses were based on target trough levels of: weeks 1-4: 250-400 ng/mL, months 1-6: 200-350 ng/mL, months 7-24: 100-300 ng/mL.

Actual trough concentrations (C0) for study B253 are listed in Table 3 below:

Table 3. Summary statistics of CsA trough (C0) levels (Study B253)

Visit	Mean CsA trough level ± SD	
	Everolimus 1.5 mg	Everolimus 3.0 mg
Week 1	220 ± 120 N = 167	226 ± 132 N = 167
Month 1	270 ± 119 N = 163	255 ± 111 N = 159
Month 6	201 ± 109 N = 112	185 ± 87 N = 115
Month 12	166 ± 84 N = 99	148 ± 70 N = 94
Month 24	150 ± 92 N = 65	137 ± 55 N = 58

Concentrations (ng/mL) are mean ± SD.

Elevated serum creatinine was observed more frequently among subjects using Certican in combination with full dose cyclosporin microemulsion than in AZA patients. This effect suggests that Certican increases the cyclosporin-induced nephrotoxicity. This may be reversible with cyclosporin dose reduction. However there are limited data in cardiac transplantation on dosing Certican with cyclosporin trough concentrations (C0) below 100ng/mL after 6 months.

INDICATIONS

Certican is indicated for the prophylaxis of organ rejection in adult patients at mild to moderate immunological risk receiving an allogeneic renal or cardiac transplant.

CONTRAINDICATIONS

Certican is contraindicated in patients with a known hypersensitivity to everolimus, sirolimus or to any of the excipients.

PRECAUTIONS

Certican has been administered in clinical trials concurrently with cyclosporin microemulsion, basiliximab and corticosteroids. Certican in combination with immunosuppressive agents other than these has not been adequately investigated.

The pharmacokinetics of everolimus have not been studied in patients with severe hepatic impairment. It is recommended that everolimus whole blood trough levels be closely monitored in hepatically impaired patients.

Co-administration with strong 3A4-inhibitors (e.g. ketoconazole, itraconazole, voriconazole, clarithromycin, telithromycin, ritonavir) and inducers (e.g. rifampicin, rifabutin) is not recommended unless the benefit outweighs the risk. It is recommended that everolimus whole blood trough levels be monitored whenever inducers or inhibitors of CYP3A4 are concurrently administered and following their discontinuation (see Interactions with other drugs).

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Patients receiving a regimen of immunosuppressive drugs, including Certican, are at increased risk of developing lymphomas or other malignancies, particularly of the skin. The absolute risk seems related to the duration and intensity of immunosuppression rather than to the use of a specific agent. Patients should be monitored regularly for skin neoplasms and advised to minimise exposure to UV light, sunlight and use appropriate sunscreen.

Patients on a regimen of immunosuppressive medicinal products, including Certican, are at increased risk of developing infections especially with opportunistic pathogens. Fatal infections and sepsis have been reported in patients treated with Certican.

In clinical trials with Certican, antimicrobial prophylaxis for *Pneumocystis jirovecii* (carinii) pneumonia was administered for the first 12 months following transplantation. Cytomegalovirus (CMV) prophylaxis was recommended for 3 months after transplantation, particularly for patients at increased risk for CMV disease.

The use of Certican with cyclosporin microemulsion in transplant patients has been associated with increased serum cholesterol and triglycerides that may require treatment. Patients receiving Certican should be monitored for hyperlipidemia and, if necessary, treated with lipid-lowering agents and appropriate dietary adjustments made. The risk/benefit should be considered in patients with established hyperlipidemia before initiating an immunosuppressive regimen including Certican. Similarly the risk/benefit of continued Certican therapy should be re-evaluated in patients with severe refractory hyperlipidemia.

During Certican therapy with cyclosporin microemulsion, patients administered Certican in conjunction with an HMG-CoA reductase inhibitor and/or fibrates should be monitored for the development of adverse effects associated with these agents.

Everolimus may potentiate the renal toxicity of cyclosporin. Regular monitoring of renal function is recommended in all patients. Appropriate adjustment of the immunosuppressive regimen, in particular reduction of cyclosporin dose, should be considered in patients with elevated serum creatinine levels. In patients receiving renal transplants, everolimus should not be used long-term together with full doses of cyclosporin (see Dosage and Administration). In patients receiving cardiac transplants, cyclosporin dose should be reduced as tolerated during the maintenance period, to prevent renal impairment. Caution should be exercised when co-administering other agents that are known to have a deleterious effect on renal function.

Administration of Certican to patients with rare hereditary problems of galactose intolerance, severe lactase deficiency or glucose-galactose malabsorption is not recommended.

A diagnosis of interstitial lung disease (ILD) should be considered in patients presenting with symptoms consistent with infectious pneumonia but not responding to antibiotic therapy and in whom infectious, neoplastic and other non-drug causes have been discounted through appropriate investigations. Cases of ILD have been reported with Certican which resolve on drug interruption with or without glucocorticoid therapy.

Mutagenesis, carcinogenesis and impairment of fertility:

Everolimus did not show genotoxicity in *in vitro* tests for gene mutation (bacteria and mammalian cells), and in an *in vitro* test and *in vivo* mouse micronucleus assay for clastogenic activity. Long-term carcinogenicity studies have been carried out in mice and rats

and no oncogenic responses were observed. Drug exposures (blood AUC) were up to 8-times the expected maximum human value in mice, but were less than the expected maximum human value in rats.

Everolimus completely impaired male rat fertility at an everolimus dose that resulted in a drug exposure (blood AUC) that was slightly above the expected maximum human value, and sperm number and motility were reduced. Testicular atrophy was observed in all animal species tested (mouse, rat, minipigs and monkey) at drug exposures similar to or slightly above the expected clinical exposure (blood AUC). There was evidence for partial recovery of fertility over a period approximately equivalent to the treatment period. Female rat fertility could not be assessed at dose resulting in an adequate drug exposure (blood AUC).

Use in Pregnancy (Category C)

There are no adequate data from the use of Certican in pregnant women and the potential risk to the fetus is unknown. In a rat study in which oral treatment started before mating and continued to the end of the period of organogenesis, treatment resulted in increased pre- and post-implementation losses. There was a low incidence of fetal cleft sternum, the significance of which is uncertain because it occurred at a dose giving a high fetal resorption rate. Systemic drug exposures (blood AUC) with the doses used in this study were below the expected maximum human value. Treatment of pregnant rabbits during the period of organogenesis slightly increased late fetal resorptions but did not otherwise affect fetal development. The highest dose used in this study gave a systemic drug exposure (blood AUC) that was slightly below the expected maximum human value. Women of childbearing potential should be advised to use effective contraception methods while they are receiving everolimus and up to 8 weeks after treatment has been stopped.

Use in Lactation

It is not known whether everolimus is excreted in human milk. In animal studies, everolimus and/or its metabolites were readily transferred into milk of lactating rats. Therefore, women who are taking Certican should not breast feed.

Interactions with Other Drugs

CYP3A4 is the main P450 enzyme involved in the microsomal metabolism of everolimus, and everolimus is a substrate for the multidrug efflux pump, p-glycoprotein (PgP). Therefore, absorption and subsequent elimination of systemically absorbed everolimus may be influenced by drugs that affect CYP3A4 and/or P-glycoprotein. Concurrent treatment with strong 3A4-inhibitors and inducers is not recommended unless the benefits outweigh the risk. Inhibitors of PgP may decrease the efflux of everolimus from intestinal cells and increase everolimus blood concentrations. *In vitro*, everolimus was a competitive inhibitor of CYP3A4 and of CYP2D6, potentially increasing the concentrations of drugs eliminated by these enzymes. Thus, caution should be exercised when co-administering everolimus with 3A4- and 2D6 substrates with a narrow therapeutic index. All *in vivo* interaction studies were conducted without concomitant cyclosporin.

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Cyclosporin (CYP3A4/PgP inhibitor): The bioavailability of everolimus was significantly increased by co-administration of cyclosporin microemulsion. In a single-dose study in healthy subjects, cyclosporin increased everolimus AUC by 168 % (range, 46 % to 365 %) and C_{max} by 82 % (range, 25 % to 158 %) compared with administration of everolimus alone. Dose adjustment of everolimus may be necessary if the cyclosporin dose is altered. Certican had a clinically minor influence on cyclosporin pharmacokinetics in renal and heart transplant patients receiving cyclosporin microemulsion.

Rifampicin (CYP3A4 inducer): Pre-treatment of healthy subjects with multiple-dose rifampicin followed by a single dose of Certican increased everolimus clearance nearly 3-fold, and decreased C_{max} by 58 % and AUC by 63 %. Combination with rifampicin is not recommended (see Precautions)².

Atorvastatin (CYP3A4-substrate) and **pravastatin** (PgP-substrate): Single-dose administration of Certican with either atorvastatin or pravastatin to healthy subjects did not influence the pharmacokinetics of atorvastatin, pravastatin and everolimus, nor, to a clinically relevant extent, the total HMG-CoA reductase bioreactivity in plasma. These results cannot be extrapolated to other HMG-CoA reductase inhibitors. Patients should be monitored for the development of rhabdomyolysis and other adverse events as described in the Product Information of HMG-CoA reductase inhibitors.

Other possible interactions: Inhibitors of CYP3A4 and PgP may increase everolimus blood levels (e.g. **antifungal agents:** fluconazole, ketoconazole, itraconazole; **macrolide antibiotics:** clarithromycin, erythromycin, **calcium channel blockers:** verapamil, nifedipine, diltiazem **protease inhibitors:** nelfinavir, indinavir, amprenavir² **other substances:** cisapride, metoclopramide, bromocriptine, cimetidine, danazol). Inducers of CYP3A4 may increase the metabolism of everolimus and decrease everolimus blood levels (e.g. St. John's wort (*Hypericum perforatum*), **anticonvulsants:** carbamazepine, phenobarbitone, phenytoin; **antibiotics:** rifabutin), **anti HIV drugs:** efavirenz, nevirapine².

Grapefruit and grapefruit juice affect cytochrome P450 and PgP activity and should therefore be avoided.

Vaccination: Immunosuppressants may affect response to vaccination and vaccination during treatment with Certican may be less effective. The use of live vaccines should be avoided.

ADVERSE REACTIONS

The frequency rates of the adverse drug reactions listed below were derived from three Phase III clinical trials and represent the pooled data from 1199 patients. These were 3 randomised, double blind, controlled, multi-centre trials: 2 *de novo* kidney transplant trials and 1 *de novo* heart transplant trial, in which Certican was administered at a dose of either 1.5 mg or 3.0 mg/day for at least 12 months in combination with cyclosporin microemulsion and corticosteroids.

Adverse reactions are listed according to the following categories:

Very common	≥10 %
Common	≥1 % and <10 %
Uncommon	≥0.1 % and <1 %
Rare	≥0.01 and <0.1 %

The adverse reactions reported as possibly or probably related to Certican seen in the Phase III clinical trials (renal and heart transplantation) occurring in more than 1% of patients are presented in Table 4.

Table 4. Adverse Reactions Possibly or Probably Related to Certican

Body system	Incidence	Adverse reaction
Blood and lymphatic system disorders	Very common	Leucopenia ¹
	Common	Thrombocytopenia ¹ , anaemia ¹ , coagulopathy, thrombotic thrombocytopenic purpura/hemolytic uraemic syndrome
	Uncommon	Hemolysis
Cardiac Disorder	Very Common	Pericardial effusion ²
Endocrine disorders	Uncommon	Hypogonadism male (testosterone decreased, LH increased)
Gastrointestinal disorders	Common	Abdominal pain, diarrhea, nausea, pancreatitis, vomiting
General disorders and administration site conditions	Common	Oedema, pain
Hepatobiliary disorders ¹	Uncommon	Hepatitis, hepatic dysfunction jaundice, elevated γ-GT, AST, ALT
Infections and infestations	Common	Viral, bacterial and fungal infections, sepsis
	Uncommon	Wound infection
Metabolic and nutrition disorders	Very common	Hypercholesterolemia, hyperlipidemia
	Common	Hypertriglyceridemia
Musculoskeletal system disorder	Uncommon	Myalgia
Vascular disorders	Common	Hypertension, lymphocele ³ , venous thromboembolism
Renal and urinary disorders	Common	Urinary tract infection
	Uncommon	Renal tubular necrosis ³ , pyelonephritis
Respiratory, thoracic and mediastinal disorders	Very Common	Pleural effusion ²
	Common	Pneumonia
	Uncommon	Interstitial lung disease
	Rare	Pulmonary alveolar proteinosis
Skin and subcutaneous tissue disorders	Common	Angioneurotic oedema ⁴ , acne, surgical wound complication
	Uncommon	Rash

¹ A dose dependent effect was established or a significantly higher incidence was seen in patients receiving 3 mg/day

² In cardiac transplantation

³ In renal transplantation

⁴ Predominantly in patients receiving concomitant ACE inhibitors

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Lymphoma or lymphoproliferative disease developed in 1.4 % of patients receiving Certican (1.5 mg or 3.0 mg/day) in combination with other immunosuppressants in controlled clinical trials followed for at least 1 year. Malignancies of the skin developed in 1.3 % of patients, other types of malignancies developed in 1.2 % of patients.

The occurrence of the adverse events may depend on the immunosuppressive regimen (i.e. degree and duration). Elevations in serum creatinine were observed in the pivotal studies more frequently in patients dosed with Certican in combination with full dose cyclosporin microemulsion than in control patients. The overall incidence of adverse events was lower with reduced dose cyclosporin.

Two open-label randomised studies in renal transplantation were performed using Certican doses of 1.5 or 3 mg/day and reduced exposure to cyclosporin. Certican dosing was adjusted based upon trough level monitoring to levels of ≥ 3 ng/mL, and cyclosporin was adjusted according to C2 monitoring as described in the "Clinical Trials" section. The safety profile of Certican in these trials was similar to that described above, except that elevation of serum creatinine was less frequent, and mean and median serum creatinine values were lower, than in the other phase III studies.

Cases of interstitial lung disease, implying lung intraparenchymal inflammation (pneumonitis) and/or fibrosis of non-infectious etiology, some fatal, have occurred in patients receiving rapamycins and their derivatives, including Certican. Mostly, the condition resolves after discontinuation of Certican and/or addition of glucocorticoids.

DOSAGE AND ADMINISTRATION

Treatment with Certican should only be initiated and maintained by physicians who are experienced in immunosuppressive therapy following organ transplantation. Everolimus should be used in combination with cyclosporin microemulsion and corticosteroids with cyclosporin exposure reduced over time post-transplantation (see *Therapeutic Drug Monitoring*).

An initial dose regimen of 0.75 mg twice a day is recommended for the general kidney and heart transplant population, administered as soon as possible after transplantation. The daily dose of Certican should always be given orally in two divided doses, consistently either with or without food.

Certican tablets should be taken whole and not crushed before use. For patients unable to swallow whole tablets, Certican dispersible tablets may be used as follows:

Administration in a 10mL oral syringe

The maximum amount of Certican that can be dispersed in a 10 mL syringe is 1.25 mg. Place the tablets into the syringe and add water to the 5 mL mark. Shake gently for 90 seconds. After dispersion administer orally directly from the syringe. Rinse the syringe with 5mL water and administer orally directly from the syringe. If required, a further 10 to 100 mL of water or flavoured drink can be administered.

Administration with a plastic cup

Place the Certican dispersible tablets in a plastic cup in approximately 25 mL of water. The maximum amount of Certican that can be dispersed in 25mL of water is 1.5mg. Allow the

tablets to dissolve for approximately 2 minutes. Swirl gently before drinking and immediately rinse the cup with 25 mL of water and drink completely.

Administration via nasogastric tube

Place the Certican dispersible tablets in a small plastic beaker in 10mL of water. The maximum amount of Certican that can be dispersed in 10mL of water is 1.25mg. Allow the tablets to dissolve for approximately 90 seconds and swirl gently. Place the dispersion into a syringe and inject slowly (within 40 seconds) into the nasogastric tube. Rinse the beaker (and the syringe) 3 times with 5 mL water and inject into the tube. Flush the tube with 10mL water. The nasogastric tube should be clamped for a minimum of 30 minutes after Certican administration. When cyclosporin microemulsion is administered via nasogastric tube, it should be administered before Certican. The two medicines should not be mixed.

The relative bioavailability of the dispersible tablet compared with the tablet is 0.90 based on the AUC-ratio of the two forms. Therefore in the case of a switch from one pharmaceutical form to another, it is recommended to monitor everolimus blood concentrations and to adjust dosages as necessary to achieve the desired target concentration (see *Therapeutic drug monitoring*).

There is insufficient experience to recommend the use of Certican in children and adolescents. Limited information is available in renal transplant paediatric patients.

Patients with renal impairment

No dosage adjustment is required.

Patients with mild or moderate hepatic impairment

Everolimus whole blood trough levels should be closely monitored in patients with impaired hepatic function. For patients with mild or moderate hepatic impairment (Child-Pugh Class A or B), the dose should be reduced to one half of the normal dose if two of the following apply: bilirubin >34 $\mu\text{mol/L}$ ($>2\text{mg/dl}$), albumin $<35\text{g/L}$ ($<3.5\text{g/dl}$), INR time >1.3 prothrombin ($>4\text{sec}$ prolongation). Further dose titration should be based on therapeutic drug monitoring. Everolimus pharmacokinetics have not been evaluated in patients with severe hepatic impairment (Child-Pugh Class C).

Therapeutic Drug Monitoring

Routine everolimus whole blood therapeutic drug level monitoring is recommended. Based on exposure-efficacy and exposure-safety analysis, patients achieving everolimus whole blood trough levels ≥ 3.0 ng/mL have been found to have a lower incidence of biopsy-proven acute rejection in both renal and cardiac transplantation compared with the patients whose trough levels are below 3.0 ng/mL. The upper limit to the therapeutic range is recommended at 8 ng/mL. Exposure above 12 ng/mL has not been studied. These recommended ranges for everolimus are based on chromatographic methods.

It is especially important to monitor everolimus blood concentrations, in patients with hepatic impairment, during concomitant administration of strong CYP3A4 inducers and inhibitors, when switching formulation and/or if cyclosporin microemulsion dosing is markedly reduced. Everolimus concentrations may be slightly lower following the dispersible tablet administration.

Optimally, dose adjustments of Certican should be based on trough levels obtained >4 -5 days after the previous dosing change. There is an interaction of cyclosporin on everolimus, and

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Suecia: Aprobado el medicamento de Novartis para trasplantes Certican®

EUROPA PRESS (España) 23-07-03

Los laboratorios Novartis anunciaron hoy que su nuevo medicamento indicado para los trasplantes Certican ha recibido la aprobación para su comercialización por parte de las autoridades sanitarias suecas, según informó la propia empresa en un comunicado. Este tratamiento ha sido autorizado para los trasplantes de corazón y riñón. Suecia será el miembro de referencia para el proceso de reconocimiento dentro de la Unión Europea.