

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 13-015333-00000-0000

Fecha: 2013-01-28 16:37:35
Tra. 11 PATENTEIYII
Act. 411 PRESENTACION

Dep. 2020 DIR.NUEVASCR
Eve: 378 FASENACIONALI
Folios: 81



Industria y Comercio
SUPERINTENDENCIA

SOLICITUD

PATENTE DE INVENCION

21. EXPEDIENTE No.

13-015333

54. TÍTULO "DEPÓSITO FARMACÉUTICO PARA 5-FLUORO-2-[(1S)-1-(5-FLUORO-2-PIRIDIL)ETIL]AMINO]-6-[(5-ISOPROPOXI-1H-PIRAZOL-3-IL)AMINO]PIRIDIN-3-CARBONITRILO.

51. CLASIFICACIÓN INTERNACIONAL A61K 9/16

71. SOLICITANTE ASTRAZENECA AB

DOMICILIO SÖDERTALJE, SUECIA

74. REPRESENTANTE LEGAL ALICIA LLOREDA RICAURTE
T.P. 53.215

22. BOGOTÁ, D.C. 28 ENE 2013

(FORMA P 10)



Industria y Comercio
SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 13-015333- -00000-0000

Fecha: 2013-01-28 16:37:35
Tra. 11 PATENTEIYII
Act. 411 PRESENTACION

Dep. 2020 DIR.NUEVASOR
Eve: 378 FASENACIONALI
Folios: 81

ión

DIRECCIÓN DE NUEVAS CREACIONES
SOLICITUD FASE NACIONAL-PCT

1	(12) TIPO DE SOLICITUD	☒ Patente de invención ☒ Capítulo I	☐ Patente de Modelo de Utilidad ☐ Capítulo II
2	DATOS SOLICITUD INTERNACIONAL PCT (86)		
Solicitud Internacional No.		PCT/GB2011/051357	Fecha 19/07/11
Publicación Internacional No.		WO 2012/010883 A3	Fecha 26/01/12
3	(54) TÍTULO DE LA INVENCÓN:		
"DEPÓSITO FARMACÉUTICO PARA 5-FLUORO-2-[[[(1S)-1-(5-FLUORO-2-PIRIDIL)ETIL]AMINO]-6-[(5-ISOPROPOXI-1H-PIRAZOL-3-IL)AMINO]PIRIDIN-3-CARBONITRILÓ"			
4	CLASIFICACIÓN INTERNACIONAL (51) CIP		
A61K 9/16			
5	(71) SOLICITANTE (S)	☐ Esta persona también es inventor.	Para datos adicionales utilizar hoja de información complementaria
APELLIDOS O RAZÓN SOCIAL		NOMBRE	IDENTIFICACIÓN TIPO
4	ASTRAZENECA AB		
6	DATOS DEL SOLICITANTE		
DIRECCIÓN		S-151 85 Södertälje, Suecia.	No. TELÉFONO 3264270
CIUDAD		Södertälje	E-MAIL notificacionespatentes@lloredacamacho.com
DEPARTAMENTO/ESTADO			NACIONALIDAD O LUGAR DE CONSTITUCIÓN Suecia
PAÍS DE RESIDENCIA		Suecia	
7	(72) INVENTOR (ES) Para datos adicionales utilizar hoja de información complementaria		
APELLIDOS		NOMBRES	NACIONALIDAD
1. FULLER		Emily	Reino Unido
2. JANIN		Armelle	Francia
3. MACFAUL		Philip	Reino Unido
4. PUGH		Rachel	Reino Unido
5. NASH		Ian	Reino Unido
DIRECCIÓN DE CORREO ELECTRÓNICO: notificacionespatentes@lloredacamacho.com			
8	DATOS INVENTOR (ES)		
PAÍS RESIDENCIA		DEPARTAMENTO/ESTADO	CIUDAD DIRECCIÓN
Reino Unido			Cheshire Alderley Park, Macclesfield, Cheshire SK10 4TG, Reino Unido.
OTRO(S) SOLICITANTE(S) Y/O (OTRO(S)) INVENTOR(ES)			
☐ Los demás solicitantes y/o (demás) inventores se indican en una hoja a continuación.			
9	(74)	☒ REPRESENTANTE	☒ APODERADO
APELLIDOS		NOMBRES	IDENTIFICACIÓN
LLOREDA RICAURTE		ALICIA	C.C 39.690.713 T.P.53.215
DIRECCIÓN		Calle 72 No 5-83 Piso 5to	No. TELÉFONO 3264270
CIUDAD		Bogotá	E-MAIL notificacionespatentes@lloredacamacho.com
PAÍS		Colombia	No. DE PROTOCOLO EN LA OFICINA

10	(30) DECLARACIONES DE PRIORIDAD ☒ SI ☐ NO			
(33) PAÍS DE ORIGEN		CÓDIGO PAÍS	(31) NÚMERO	(32) FECHA (AAAA/MM/DD)
1. Estados Unidos de América		US	61/365,407	10/07/19
11	DECLARACIÓN SOBRE USO DE RECURSOS GENÉTICOS O BIOLÓGICOS			
<i>Declaro que el objeto de la presente solicitud de patente fue obtenido a partir de recursos genéticos o biológicos de los que cualquiera de los países miembros de la Comunidad Andina es país de origen.</i>				
☐ SI ☒ NO				
Nota: En caso afirmativo deberá anexar copia del contrato de acceso de recursos genéticos o productos derivados, o certificado o número de registro, expedido por la Autoridad competente.				
12	DECLARACIÓN SOBRE USO DE CONOCIMIENTOS TRADICIONALES			
<i>Declaro que el objeto de la presente solicitud de patente fue obtenido a partir de conocimientos tradicionales de comunidades indígenas, afroamericanas o locales de países miembros de la Comunidad Andina.</i>				
☐ SI ☒ NO				
Nota: En caso afirmativo deberá anexar la licencia o autorización de uso de conocimiento tradicional, o certificado, o número de registro expedido por la Autoridad competente.				
13	REDUCCIÓN DE TASAS			
<i>Declaro que carezco de medios económicos para presentar la solicitud de patente.</i>				
☐ SI ☒ NO				
Nota: En caso de ser persona natural y carecer de medios económicos, y por lo tanto, aplique la reducción de tasas a que se refiere la resolución vigente en tarifas, debe firmar la presente solicitud bajo la gravedad de juramento.				
Micro, pequeñas y medianas empresas		Universidades públicas o privadas	Entidades sin ánimo de lucro	
☐ Copia simple de la declaración de renta del año inmediatamente anterior, o en su defecto prueba documental idónea.		☐ Copia acto de reconocimiento institucional emitido por el Ministerio de Educación. Acreditación Colciencias.	☐ Copia de registro vigente en Cámara de comercio.	
☐ Documento de constancia de cumplimiento con lo establecido en la ley 905 de 2004.			☐ Hoja de información complementaria.	
			☐ Otros, especificar	
14	AUTORIZACIÓN DE NOTIFICACIÓN EN LÍNEA ☒ SI ☐ NO			
Manifiesto que he leído y entendido perfectamente los términos y condiciones de uso de medios electrónicos para las notificaciones en línea a través de Internet de los actos administrativos proferidos por la Superintendencia de Industria y Comercio que deben ser notificados personalmente y, en consecuencia, autorizo el servicio de notificación a través de internet.				
14	COMPROBANTE DE PAGO O PAGO ELECTRÓNICO	N° 12-136092	Fecha 10-12-2012	
15	FIRMA DEL SOLICITANTE, DEL APODERADO O DEL REPRESENTANTE LEGAL			
Junto a cada firma, indicar el nombre del firmante y su calidad (si tal calidad no es obvia al leer el petitorio)				
 ALICIA LLOREDA RICAURTE Representante C.C. 39.690.713 T.P. 53.215 Apoderado				
16	ORDEN Y RELACIÓN DE DOCUMENTOS QUE ACOMPAÑAN LA SOLICITUD			
Documentación Técnica Solicitud Internacional en castellano		Documentación Jurídica		
1. ☒ Descripción N° de folios: 26		9. ☒ Poderes, si fuera el caso.		
2. ☒ Reivindicaciones N° Reivindicaciones: 17		10. ☐ Documento que legalmente pruebe la cesión del inventor al solicitante o a su causante.		
3. ☒ Dibujos y/o figuras N° folios: 3		11. ☐ Copia del contrato de acceso de recursos genéticos o productos derivados, certificado o número de registro, si fuera el caso.		
4. ☒ Resumen.		12. ☐ Copia de la licencia o autorización de Conocimientos Tradicionales, certificado o número de registro, si fuera el caso.		
5. ☐ Certificado de depósito de material biológico si fuera el caso.		13. ☒ Documento que acredita la existencia y representación legal, cuando el solicitante es persona jurídica.		
6. ☐ Listado de secuencias de nucleótidos y/o aminoácidos en forma digital si fuera el caso.		14. ☒ Comprobante de pago de la tasa de presentación de la solicitud.		
7. ☐ Arte final 12 x 12.		15. ☐ Comprobante de pago por reivindicación de prioridad.		
8. ☐ Anexo formato digital.		16. ☐ Comprobante de pago de la tasa por concepto de excedente de palabras en la publicación.		
		17. ☐ Comprobante de pago por reivindicación adicional a 10.		

DEPÓSITO FARMACÉUTICO PARA 5-FLUORO-2-[[[(1S)-1-(5-
FLUORO-2-PIRIDIL)ETIL]AMINO]-6-[(5-ISOPROPOXI-1H-PIRAZOL-3-
IL)AMINO]PIRIDIN-3-CARBONITRILLO

5 La presente invención se refiere a un depósito farmacéutico
que comprende 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-
isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo o su sal
farmacéuticamente aceptable y a los usos del depósito farmacéutico.

WO2006/082392 describe derivados de pirazol, incluyendo 5-
10 fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-
pirazol-3-il)amino]piridin-3-carbonitrilo y sus sales farmacéuticamente
aceptables y describe que los derivados de pirazol tienen actividad
inhibitoria de la quinasa Trk.

Los receptores tirosina quinasas (RTK) son una subfamilia de
15 proteínas quinasas que juegan un papel crítico en la señalización
celular y están implicados en una diversidad de procesos relacionados
con el cáncer incluyendo proliferación, supervivencia, angiogénesis y
metástasis celular. Actualmente se han identificado hasta 100 RTK
diversos incluyendo quinasas relacionadas con tropomiosina (Trk).

20 Las Trk son los receptores de alta afinidad activados por un
grupo de factores de crecimiento solubles llamados neurotrofinas (NT).
La familia de receptores Trk tiene tres miembros: TrkA, TrkB y TrkC.
Entre las NT se encuentran (i) el factor de crecimiento nervioso (NGF)
que activa la TrkA, (ii) el factor neurotrófico derivado del cerebro
(BDNF) y NT-4/5 que activan la TrkB y (iii) la NT3 que activa la TrkC.
25 Cada receptor Trk contiene un dominio extracelular (unión del ligando),
una región transmembrana y un dominio intracelular (incluyendo el

dominio quinasa). Tras la unión del ligando, la quinasa cataliza la autofosforilación y provoca las sendas de transducción de señal corriente abajo.

5 Las Trk se expresan ampliamente en el tejido neuronal durante su desarrollo donde las Trk son críticas para el mantenimiento y la supervivencia de estas células. Sin embargo, aún se desconoce un papel postembrionario para el eje (o senda) de Trk/neurotrofina. Existen informes que muestran que las Trk desempeñan un papel importante tanto en el desarrollo como en la función del sistema nervioso (Patapoutian, A. et al *Current Opinion in Neurobiology*, 2001, 10 11, 272-280).

En la década pasada, se ha publicado una cantidad importante de documentos literarios que unen la señalización de Trk con el cáncer. Por ejemplo, mientras las Trk se expresan en niveles bajos fuera del sistema nervioso en el adulto, la expresión de Trk en los cánceres de 15 próstata avanzados aumenta. El tejido de próstata normal y los tumores de próstata dependientes de andrógeno expresan niveles bajos de Trk A y niveles indetectables de Trk B y C. Sin embargo, todas las isoformas de los receptores Trk así como sus ligandos cognados están sobrerregulados en el cáncer de próstata avanzado andrógeno- 20 independiente. Existe prueba adicional de que estas células de cáncer de próstata avanzado se vuelven dependientes del eje de Trk/neurotrofina para su supervivencia. Por lo tanto, los inhibidores de Trk pueden proporcionar una clase de agentes que inducen la apoptosis específicos para el cáncer de próstata andrógeno- 25 independiente (Weeraratna, A. T. et al *The Prostate*, 2000, 45, 140-148).

Asimismo, literatura muy reciente muestra también que la sobreexpresión, activación, amplificación y/o mutación de las Trk están asociadas con carcinoma secretor de mama (*Cancer Cell*, 2002, 2, 367-376), cáncer colorrectal (Bardelli et al *Science*, 2003, 300, 949-949) y cáncer de ovario (Davidson, B. et al *Clinical Cancer Research*, 2003, 9, 2248-2259).

Inesperadamente hemos descubierto que los derivados de pirazol descritos en WO2006/082392 son útiles en el tratamiento de otras enfermedades o afecciones médicas en las que se encuentra implicada la producción inadecuada de Trk. Dichas enfermedades y afecciones médicas pueden incluir enfermedades inflamatorias y alérgicas, tal como inflamación de las articulaciones (en especial artritis reumatoide, osteoartritis y gota). La osteoartritis es una afección particular.

Aunque la WO2006/082392 sugiere que los derivados de pirazol descritos en la misma pueden estar incluidos en una composición farmacéutica, por ejemplo en una forma adecuada para uso oral o tópico, para administración mediante inhalación o insuflación o para administración parenteral, no hay descripción en WO2006/082392 de un depósito farmacéutico que comprenda un derivado de pirazol como se describió en la misma y menos de dicho depósito farmacéutico que comprenda 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo o su sal farmacéuticamente aceptable.

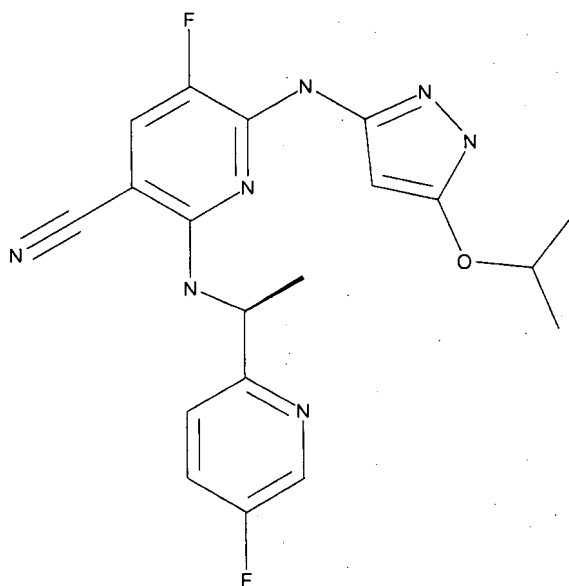
De acuerdo con la presente invención, se proporciona un depósito farmacéutico que comprende (i) 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-

piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo o su sal farmacéuticamente aceptable, como un agente farmacéutico (PA) y (ii) un polímero que se degrada para crear un microclima ácido, donde el PA se libera del polímero tras la degradación del polímero.

En el depósito farmacéutico de la presente invención, el agente farmacéutico (en lo sucesivo el PA) es 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo o su sal farmacéuticamente aceptable. De este modo, las referencias en la presente con respecto al PA incluyen el compuesto 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo por sí mismo, así como sus sales farmacéuticamente aceptables.

Como lo apreciará un experto en la técnica, un depósito farmacéutico es una composición que libera un PA, especialmente una cantidad farmacéuticamente efectiva de un PA (en la presente 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo o su sal farmacéuticamente aceptable) con el paso del tiempo, de modo de proporcionar la administración de liberación controlada y/o sostenida del PA comprendido en el mismo.

5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo tiene la estructura:



y se describe en el Ejemplo 119 de WO2006/082392. Un nombre alternativo es (S)-5-fluoro-2-(1-(5-fluoropiridin-2-il)etilamino)-6-(5-isopropoxi-1H-pirazol-3-ilamino) nicotinonitrilo.

Las sales farmacéuticamente aceptables de 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo para incluir en el depósito farmacéutico de la presente invención se basan en una opinión médica razonable como adecuadas para la administración a un sujeto, por ejemplo un animal de sangre caliente como el hombre, sin actividades farmacológicas no deseadas y sin toxicidad indebida. Las sales farmacéuticamente aceptables adecuadas incluyen sales de adición de ácido, por ejemplo, sales de adición de ácido con ácido orgánico o inorgánico, tales como ácido clorhídrico, bromhídrico, sulfúrico, fosfórico, trifluoroacético, cítrico, maleico, tartárico, fumárico, hemifumárico, succínico, hemisuccínico, mandélico, metanosulfónico, dimetanosulfónico, etan-1,2-sulfónico, bencenosulfónico, salicílico o 4-toluensulfónico.

El 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo y sus sales

farmacéuticamente aceptables pueden sintetizarse a partir de materiales de partida adecuados utilizando procesos estándar de química orgánica, por ejemplo como se describió en WO2006/082392.

El depósito farmacéutico de la presente invención permite la administración del PA utilizando una formulación de liberación controlada y/o sostenida de modo de mantener un nivel terapéutico del PA durante un período extendido de tiempo. Esto presenta ventajas ya que reduce la frecuencia de dosificación y proporciona un modo conveniente de administración del PA, lo que es, particularmente deseable para la administración del PA directamente en la articulación, es decir, mediante administración intra-articular. Las formulaciones de liberación controlada y/o sostenida pueden reducir también la gravedad y frecuencia de cualquier efecto colateral no deseado asociado con un PA particular. Las mejoras en la conveniencia de administración y aparición y gravedad reducidas de los efectos colaterales potencian a su vez el cumplimiento del paciente.

Varios compuestos que representan un PA son inadecuados para incluirse en los depósitos farmacéuticos, principalmente debido a factores tales como la inestabilidad de los compuestos en las formulaciones requeridas para la administración de liberación controlada y/o sostenida y/o intra-articular.

Asimismo, el PA incluido en el depósito farmacéutico de la presente invención puede proporcionarse en una alta concentración local sostenida del PA en el sitio de administración, por ejemplo en una articulación, para proporcionar la liberación controlada y/o sostenida del

PA. En otras palabras, el depósito farmacéutico es efectivo para liberar lentamente el PA de modo de alcanzar un efecto de larga duración.

De manera ventajosa, el PA puede incluirse en el depósito farmacéutico de la presente invención sin modificación química que sea requerida antes de su inclusión en el mismo.

Como lo apreciará un experto en la técnica, un "agente farmacéutico" (o PA) es un agente que causa un efecto farmacológico en un sujeto al que se le administra, por ejemplo en un animal de sangre caliente como un hombre, por ejemplo para tratar o evitar una enfermedad o afección médica. Tal como se describió anteriormente, el PA en el depósito farmacéutico de la presente invención es 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo o su sal farmacéuticamente aceptable, el cual se cree que causa un efecto farmacológico mediante la inhibición de los efectos de un Trk.

El PA que se incluye en el depósito farmacéutico de la presente invención es efectivo para el tratamiento de una enfermedad o afección inflamatoria, por ejemplo una afección causada por la inflamación de una articulación, tal como osteoartritis en la cual puede ocurrir la inflamación sinovial aguda y crónica. La osteoartritis (también conocida como enfermedad de artritis degenerativa o de articulaciones degenerativas) es la forma más común de artritis que presenta varios enfermos en el mundo. Se desean formulaciones mejoradas para la administración de los PA en el tratamiento de la osteoartritis.

Como lo apreciará un experto en la técnica, el PA está presente en el depósito farmacéutico de la presente invención en una

cantidad terapéuticamente eficaz. Una "cantidad terapéuticamente eficaz" es cualquier cantidad del PA (por ejemplo, como la contenida en el depósito farmacéutico) que, cuando se administra a un sujeto que sufre de una enfermedad o afección médica contra la cual es efectivo el PA, causa reducción, disminución o regresión de la enfermedad o afección médica.

La cantidad terapéuticamente eficaz del PA que se incluye en el depósito farmacéutico variará necesariamente dependiendo de la naturaleza y gravedad del trastorno a tratar y el paciente particular tratado, de acuerdo con principios médicos bien conocidos. De manera adicional, la cantidad terapéuticamente eficaz del PA que se incluye en el depósito farmacéutico variará necesariamente de acuerdo con el perfil de liberación controlada y/o sostenida, por ejemplo, dependiendo del período de tiempo durante el cual se requiere la liberación del PA y la concentración deseada del PA durante ese tiempo.

En adición al PA, el depósito farmacéutico de la presente invención comprende un polímero que se degrada para crear un microclima ácido, por ejemplo un polímero que se degrada en presencia de agua para crear un microclima ácido. En consecuencia, se denota un polímero que se degrada o descompone químicamente para proporcionar un pH ácido en un área pequeña y localizada (tal como una articulación) al cual se le administra el depósito farmacéutico.

De preferencia, el pH ácido es esencialmente uniforme en el área localizada y difiere del área circundante, que puede estar en un pH fisiológico (típicamente alrededor de pH 7.4). El pH ácido es

típicamente un pH de menos de aproximadamente 7.4, por ejemplo un pH en el rango de aproximadamente 1 a aproximadamente 7, tal como de aproximadamente 3 a aproximadamente 7; convenientemente de aproximadamente 1 a menos de 7 o de aproximadamente 3 a menos de 7.

Típicamente, el PA está disperso o encapsulado en el polímero, de modo que el PA esté continuamente liberado del polímero mientras el polímero se degrada en el tiempo para crear el microclima ácido. El PA que se incluye en el depósito farmacéutico de la presente invención es hidrolíticamente estable en el microclima ácido que se crea mediante la degradación del polímero. La liberación del PA del polímero proporciona la liberación controlada y/o sostenida del PA del depósito farmacéutico en un sujeto, por ejemplo un animal de sangre caliente como el hombre, al cual se le administra el depósito farmacéutico. De preferencia, una alta concentración local (es decir, en el área al cual se administra el depósito farmacéutico, tal como una articulación) para provocar el efecto terapéutico deseado y una concentración sistémica baja para mitigar contra cualquier toxicidad sistémica no deseada del PA se logran sobre la degradación del polímero y liberación del PA. De este modo, el depósito farmacéutico proporciona el PA al sujeto en concentraciones efectivas para el tratamiento de la enfermedad o afección médica particular durante un período de tiempo ininterrumpido.

Se puede utilizar cualquier polímero adecuado en el depósito farmacéutico de la presente invención siempre que el polímero se degrade para crear un microclima ácido, es decir, tras la administración

a un sujeto, por ejemplo a un animal de sangre caliente como el hombre y que sea biodegradable y biocompatible.

Como lo apreciará un experto en la técnica, el término "biocompatible" significa un material que es compatible con tejido vivo o un sistema vivo siendo no tóxico, dañino o fisiológicamente reactivo y que no causa rechazo inmunológico.

El término "biodegradable" significa un material que se degrada en un medio ambiente biológico.

Por ejemplo, un polímero puede ser "biodegradable" de modo que todo el polímero se biodegrade y no necesite ser eliminado luego de su uso, es decir, una vez que se liberó todo el PA. Dichos polímeros pueden comprender uniones de éster hidrolizables y enzimáticamente escindibles que se descomponen en condiciones biológicas (por ejemplo, en presencia de agua y enzimas biológicas encontradas en tejidos de animales de sangre caliente como humanos) para producir productos no tóxicos, biocompatibles y/o biodegradables. De manera alternativa, un polímero puede ser "biodegradable" por el hecho de tener una semivida limitada en un medio ambiente biológico. Por ejemplo, el polímero puede tener una semivida de 1 a 12 meses, tal como de 1 a 6 meses.

Típicamente, el polímero incluye al menos un grupo funcional ácido o al menos un grupo funcional que puede reaccionar para producir un grupo funcional ácido, es decir, dicho grupo funcional ácido es un grupo capaz de donar un protón a un grupo funcional básico, tal como una amina. Ejemplos de grupos funcionales ácidos adecuados incluyen grupos de ácidos carboxílicos (es decir, $-\text{CO}_2\text{H}$) y grupos de

ácidos sulfónicos (es decir, $-\text{S}(\text{O})_2\text{OH}$). Ejemplos de grupos funcionales adecuados que pueden reaccionar para producir un grupo funcional ácido incluyen ésteres (es decir, $\text{RC}(\text{O})\text{OR}$, donde R puede representar alquilo o arilo), dichos ésteres pueden reaccionar con agua para producir un grupo de ácido carboxílico correspondiente y un alcohol.

De preferencia, el polímero se selecciona de modo de degradar y liberar el PA durante un período de aproximadamente 30 a 90 días. Por ejemplo, el polímero puede degradar y liberar el PA durante un período de aproximadamente 30, aproximadamente 60 o aproximadamente 90 días. Por ejemplo, el polímero puede degradar y liberar el PA durante un período de aproximadamente 120, aproximadamente 150 o aproximadamente 180 días.

Los polímeros adecuados incluyen un poliéster de un ácido hidroxigraso y sus derivados (por ejemplo, ácido poliláctico, ácido poliglicólico, ácido policítrico, ácido polimálico, ácido poli- β -hidroxibutírico, polímero de apertura del anillo ϵ -capro-lactona, copolímero de ácido láctico-ácido glicólico, copolímero de ácido 2-hidroxibutírico-ácido glicólico, copolímero de ácido poliláctico-polietilenglicol o copolímero de ácido poliglicólico-polietilenglicol), un polímero de un α -cianoacrilato de alquilo (por ejemplo, poli(butil 2-cianoacrilato)), un oxalato de polialquileno (por ejemplo, oxalato de politrimetileno u oxalato de politetrametileno), un poli (orto) éster, un policarbonato (por ejemplo, carbonato de polietileno o carbonato de polietilenpropileno), un poliorto-carbonato, un ácido de poliamino (por ejemplo, poli- γ -L-alanina, ácido poli- γ -bencil-L-glutámico o ácido poli- γ -

metil-L-glutámico), un éster de ácido hialurónico y similares, y se pueden utilizar uno o más de estos polímeros.

Si los polímeros son copolímeros pueden ser copolímeros aleatorios, en bloque o de injerto. Cuando los ácidos α -hidroxicarboxílicos, ácidos hidroxidicarboxílicos y ácidos hidroxitricarboxílicos anteriores tienen actividad óptica en sus moléculas, se pueden utilizar cualquiera de los isómeros D, isómeros L e isómeros DL. Entre otros, se prefieren el polímero de ácido α -hidroxicarboxílico (de preferencia polímero de ácido láctico-ácido glicólico), su éster, ésteres de ácido poli- α -cianoacrílico, etc. y se prefiere aun más el copolímero de ácido láctico-ácido glicólico (también conocido como poli(láctido-co-glicólido) o (ácido)poli(láctico-co-glicólico) y en lo sucesivo referido como PLGA). De este modo, en un aspecto el polímero es PLGA. Tal como se utilizó en la presente, el término PLGA incluye los polímeros de ácido láctico (también conocido como poliláctido, (ácido)poli(láctico) o PLA).

Los polímeros de PLGA adecuados pueden tener una proporción molar de ácido láctico:ácido glicólico en el rango de 100:0 a 50:50, convenientemente en el rango de 95:5 a 50:50. Por ejemplo, el polímero de PLGA puede tener una proporción molar de ácido láctico:ácido glicólico de 95:5 o de 50:50.

Los polímeros de PLGA adecuados pueden tener una longitud de bloqueo en el rango de 1 a 5, preferentemente de 2 a 4.

Los polímeros de PLGA adecuados pueden tener un peso-peso molecular promedio de aproximadamente 3,000 a aproximadamente 50,000, preferentemente de aproximadamente 4,000 a

aproximadamente 40,000 y más preferentemente de aproximadamente 5,000 a aproximadamente 30,000 Daltones. El grado de dispersión (peso-peso molecular promedio/cantidad-peso molecular promedio, en lo sucesivo referido como polidispersidad) puede oscilar de aproximadamente 1.2 a aproximadamente 4.0, preferentemente de aproximadamente 1.3 a aproximadamente 3.5.

Como lo apreciará un experto en la técnica, el peso-peso molecular promedio, cantidad-peso molecular promedio y polidispersidad pueden determinarse mediante cualquier procedimiento o medio adecuado, por ejemplo mediante cromatografía de exclusión por tamaños (SEC) con sustancias de referencia de poliestireno con polidispersidad reducida con pesos moleculares pico de 1,000,000, 130,000, 50,000, 20,000, 10,000, 5,000, 2,000 y 580, respectivamente. La determinación puede llevarse a cabo utilizando una columna SEC Mixed Bed D de 5 μ m (fabricada por Polymer Laboratories Ltd., Reino Unido) y utilizando 5% de metanol en tetrahidrofurano como la fase móvil.

El PLGA puede prepararse mediante cualquier procedimiento convencional o puede estar comercialmente disponible. Por ejemplo, el PLGA puede producirse mediante polimerización de abertura del anillo con un catalizador adecuado a partir de láctido cíclico, glicólido, etc. (véase *Encyclopedic Handbook of Biomaterials and Bioengineering Part A: Materials*, Volumen 2, Marcel Dekker, Inc. (1995); EP-0058481B2; Effects of polymerization variables on PLGA properties: molecular weight, composition and chain structure y Dorta et al, *Int. J. Pharm.*, 100, pp 9-14 (1993)).

Se cree que el PLGA es biodegradable mediante la degradación de toda la composición del polímero sólido, debido a la descomposición de uniones de éster hidrolizables y enzimáticamente escindibles en condiciones biológicas (por ejemplo en presencia de agua y enzimas biológicas encontradas en tejidos de animales de sangre caliente como humanos) para formar ácido láctico y ácido glicólico. Tanto el ácido láctico como el ácido glicólico son productos no tóxicos solubles en agua de metabolismo normal, que pueden biodegradarse adicionalmente para formar dióxido de carbono y agua.

En otras palabras, se cree que el PLGA se degrada mediante hidrólisis de sus grupos de éster en presencia de agua, por ejemplo en el cuerpo de un animal de sangre caliente como un humano, para producir ácido láctico y ácido glicólico y crear el microclima ácido. Los ácidos láctico y glicólico son subproductos de varias sendas metabólicas en el cuerpo de un animal de sangre caliente como un humano en condiciones fisiológicas normales y por lo tanto son bien tolerados y producen toxicidad sistémica mínima.

El polímero se proporciona en cualquier forma adecuada en la que el PA puede dispersarse o encapsularse en el mismo antes de la degradación del polímero. Por ejemplo, el depósito farmacéutico puede comprender el polímero en la forma de micropartículas o nanopartículas o en una forma líquida, con el PA disperso o encapsulado en el mismo.

Las micropartículas adecuadas tienen típicamente un tamaño de partícula promedio en el rango de 0.1 a 1000 μ m, preferentemente de 1 a 750 μ m y más preferentemente de 10 a 500 μ m.

Las nanopartículas adecuadas tienen típicamente un tamaño de partícula promedio en el rango de 1 a 2000nm, preferentemente de 10 a 1000nm y más preferentemente de 50 a 500nm.

En particular, las micropartículas son de forma sustancialmente esférica (es decir, son microesferas).

Cuando el polímero tiene la forma de micropartículas, las micropartículas pueden prepararse utilizando cualquier procedimiento adecuado, tal como mediante un procedimiento de evaporación del solvente o extracción del solvente. Por ejemplo, en el procedimiento de evaporación del solvente, el PA y el polímero pueden disolverse en un solvente orgánico volátil adecuado (por ejemplo, una cetona como acetona, un hidrocarburo halogenado como cloroformo o cloruro de metileno, un hidrocarburo aromático halogenado, un éter cíclico como dioxano, un éster como acetato de etilo, un nitrilo como acetonitrilo o un alcohol como etanol) y pueden dispersarse en una fase acuosa que contenga un estabilizador de emulsión adecuado (por ejemplo, alcohol polivinílico, PVA). El solvente orgánico se evaporó luego para proporcionar micropartículas con el PA encapsulado en el mismo. En el procedimiento de extracción del solvente, el PA y el polímero pueden disolverse en un solvente polar (tal como acetonitrilo, diclorometano, metanol, acetato de etilo o formato de metilo) y luego dispersarse en una fase acuosa (tal como una solución de agua/PVA). Se produce una emulsión para proporcionar micropartículas con el PA encapsulado en el mismo. El secado por atomización es una técnica de fabricación alternativa para preparar las micropartículas.

En un aspecto, el depósito farmacéutico puede comprender el polímero (tal como, PLGA como se describió anteriormente) en la forma de micropartículas con el PA encapsulado en el mismo. Por ejemplo, el depósito farmacéutico puede comprender un polímero de PLGA que tenga una proporción molar láctido:glicólido de 50:50 en la forma de micropartículas con el PA encapsulado en el mismo. Dicho depósito farmacéutico puede ser adecuado para liberación controlada y/o sostenida del PA durante un período de aproximadamente 30 días. Adicionalmente, a modo de ejemplo, el depósito farmacéutico puede comprender un polímero de PLGA que tenga una proporción molar láctido:glicólido de 95:5 en la forma de micropartículas con el PA encapsulado en el mismo. Dicho depósito farmacéutico puede ser adecuado para la liberación controlada y/o sostenida del PA durante un período de aproximadamente 60 a 90 días. Dicho depósito farmacéutico puede ser adecuado para la liberación controlada y/o sostenida del PA durante un período de hasta 120, hasta 150 o hasta 180 días.

El depósito farmacéutico puede comprender el PA y el polímero en cualquier cantidad adecuada. Por ejemplo, el depósito farmacéutico puede comprender de 1 a 30% en peso del PA y de 70 a 99% en peso del polímero.

Por ejemplo, cuando el depósito farmacéutico de la presente invención comprende micropartículas de PLGA, el PLGA puede estar presente en una cantidad que oscila de aproximadamente 70% a aproximadamente 99% en peso de las micropartículas. Esta cantidad

de PLGA puede utilizarse cuando se carga de aproximadamente 1% a aproximadamente 30% en peso del PA en las micropartículas. Asimismo, se calcula esta cantidad del polímero para las micropartículas que comprenden el PA y el PLGA pero no otros excipientes farmacéuticos, por ejemplo utilizados para suspender las micropartículas antes de la liofilización. El PLGA puede utilizarse en una cantidad de aproximadamente 88% a aproximadamente 90% en peso de las micropartículas, cuando se carga de aproximadamente 10% a aproximadamente 12% en peso del PA en las micropartículas. La proporción del polímero depende típicamente de la fuerza de la actividad farmacológica del PA utilizado y de la velocidad y duración de liberación del PA.

El depósito farmacéutico puede comprender adicionalmente un diluyente o portador farmacéuticamente aceptable, que debería ser miscible en agua. Los diluyentes o portadores adecuados incluyen, por ejemplo, agentes modificadores de porosidad adecuados (tal como cloruro de sodio) que se disuelven rápidamente dejando poros y/o plastificadores adecuados para modificar la velocidad de difusión y/o reducir la porosidad (véase, por ejemplo, Burgess, D. J., Hickey, A. J., *Drugs and the Pharmaceutical Sciences* (149) pp 305-353).

El diluyente o portador puede incluirse en el depósito farmacéutico en cualquier cantidad adecuada. Por ejemplo, el diluyente o portador puede incluirse en una cantidad de 0 a 50% en peso de la composición total. De preferencia, el depósito farmacéutico no contiene un diluyente o portador adicional.

El depósito farmacéutico se proporciona típicamente para la administración local en un sitio de tratamiento deseado, tal como en una articulación.

El depósito farmacéutico puede formularse para su administración por inyección, tal como mediante inyección intra-articular. De este modo, en particular, el depósito farmacéutico puede proporcionarse en una forma inyectable (es decir, como un depósito farmacéutico inyectable). "Inyectable" quiere decir el depósito farmacéutico puede extraerse con una jeringa e inyectarse en un sujeto, por ejemplo en un animal de sangre caliente como un hombre, sin causar efectos adversos debido a la presencia de material sólido en el depósito. Por ejemplo, el depósito farmacéutico puede inyectarse en una articulación, tal como una articulación inflamada. En otras palabras, se proporciona un depósito farmacéutico para la inyección intra-articular. Las articulaciones adecuadas incluyen articulaciones de rodilla, cadera, hombro, tobillo, codo, muñeca, dedo del pie, dedo de la mano y facetas articulares. El depósito farmacéutico permanece en la articulación luego de la inyección en la misma y alcanza una administración local del PA de una manera controlada y sostenida, preferentemente durante un período de tiempo que oscila entre 30 y 90 días. Los depósitos farmacéuticos que alcanzan una administración local del PA de una forma controlada y sostenida durante un período de hasta 90 días son ventajosos porque esto minimiza la cantidad de inyecciones locales requeridas para realizar en una articulación, lo que permite que los depósitos cumplan las recomendaciones actuales para terapia intra-articular que aconseja no exceder de tres a cuatro

(aproximadamente 2 ml) inyecciones locales pequeñas en una articulación por año debido a posibles efectos adversos.

El depósito farmacéutico puede formularse para inyección en el espacio intra-articular de una articulación afectada, por ejemplo en una porción que contiene fluido sinovial de una articulación afectada, tal como en un sitio con osteoartritis. Como lo apreciaría un experto en la técnica, el fluido sinovial está contenido dentro de un espacio de articulación central definido por huesos opuestos de las articulaciones. Los inventores presentes han descubierto que tras la inyección del depósito farmacéutico en el fluido sinovial, se libera el PA e ingresa sustancialmente en el tejido circundante únicamente con cantidades menores que ingresen en la corriente sanguínea, es decir, para alcanzar una alta concentración local de PA en el área al cual se administra el depósito farmacéutico (tal como una articulación) y una concentración sistémica baja. De manera adicional, el depósito farmacéutico proporciona una "explosión" aceptable (es decir, liberación de PA) el primer día luego de la administración, que es ventajoso para su uso y es inesperada en vista de la descripción de la técnica previa, tal como en US-6,217,911, que describe que se prefiere poca liberación o que no haya explosión. El perfil de liberación eficiente proporcionado por el depósito farmacéutico de la presente invención no se habría predecido antes a partir de la técnica previa y asiste a la efectividad del depósito farmacéutico.

Preferentemente, el depósito farmacéutico proporciona una alta concentración local sostenida del PA en una articulación articular tras la

administración mediante inyección en la misma, tal como más de 100 nanomolar.

Los depósitos farmacéuticos inyectables pueden comprender una suspensión o dispersión de la combinación del PA y polímero en un diluyente o portador farmacéuticamente aceptable, que debería ser miscible en agua. Los diluyentes o portadores adecuados incluyen diluyentes o portadores acuosos tal como una solución acuosa isotónica de un potenciador de viscosidad (tal como carboximetilcelulosa de sodio), un tensoactivo (tal como polisorbato 80) y/o un ajustador de tonicidad (tal como cloruro de sodio). Los depósitos farmacéuticos inyectables pueden comprender agentes activos adicionales, tal como una anestesia local.

El depósito farmacéutico de la presente invención puede formularse para uso medicinal humano o veterinario. Por ejemplo, puede proporcionarse un depósito farmacéutico formulado para la inyección intra-articular para uso medicinal humano o veterinario.

La presente invención proporciona adicionalmente un depósito farmacéutico como se definió en la presente para su uso en la inhibición de los efectos de un Trk en un sujeto.

De acuerdo con otro aspecto de la presente invención, se proporciona el uso de un depósito farmacéutico como se definió en la presente para inhibir los efectos de un Trk en un sujeto.

De acuerdo con otro aspecto de la presente invención, se proporciona el uso de un depósito farmacéutico como se definió en la presente para la fabricación de un medicamento para su uso en la inhibición de los efectos de un Trk en un sujeto.

De acuerdo con otro aspecto de la presente invención, se proporciona un procedimiento para inhibir los efectos de un Trk en un sujeto que lo necesita, dicho procedimiento comprende administrar a dicho sujeto un depósito farmacéutico como se definió en la presente.

5 La presente invención proporciona adicionalmente un depósito farmacéutico como se definió en la presente para su uso en la prevención o tratamiento de una enfermedad inflamatoria, tal como osteoartritis, en un sujeto.

10 De acuerdo con otro aspecto de la presente invención, se proporciona el uso de un depósito farmacéutico como se definió en la presente para la prevención o el tratamiento de una enfermedad inflamatoria, tal como osteoartritis, en un sujeto.

15 De acuerdo con otro aspecto de la presente invención, se proporciona el uso de un depósito farmacéutico como se definió en la presente en la fabricación de un medicamento para su uso en la prevención o el tratamiento de una enfermedad inflamatoria, tal como osteoartritis, en un sujeto.

20 De acuerdo con otro aspecto de la presente invención, se proporciona un procedimiento para la prevención o el tratamiento de una enfermedad inflamatoria, tal como osteoartritis, en un sujeto que lo necesita, dicho procedimiento comprende administrar a dicho sujeto un depósito farmacéutico como se definió en la presente.

25 El "sujeto" al cual se le administrará el depósito farmacéutico de la invención es un animal, especialmente un animal de sangre caliente, tal como un animal doméstico o un hombre, particularmente un hombre.

La invención se ilustrará a continuación mediante los siguientes ejemplos no limitativos.

Ejemplo 1

5 Se preparó un depósito farmacéutico que comprendía micropartículas de PLGA que encapsulan 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo como el PA.

(i) Preparación de micropartículas

10 Se disolvieron 120 mg de 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo y 680 mg de PLGA (proporción molar láctido: glicólido de 50:50 y un peso molecular de 19.5 KD) en diclorometano (4 ml). Luego se dispersó esta solución en una fase acuosa de 0.5% de PVA p/v en
15 alto cizallamiento para formar una emulsión. El alto cizallamiento se creó utilizando una mezcla estática con una alta velocidad de flujo de fase acuosa, por ej., 1000mls/min. La emulsión resultante se agregó a agua (1250 ml) a 30°C y se agitó a 500rpm (utilizando un agitador Heidolph RZR1) durante 1 hora. La suspensión resultante se enfrió en
20 un baño de hielo y se permitió la sedimentación de las micropartículas durante 45 minutos. Se eliminó aproximadamente el 90% (en volumen) del sobrenadante, teniendo cuidado de no interrumpir las micropartículas sedimentadas. Se agregó agua (1 L) y se repitió el proceso. Se eliminó aproximadamente el 95% (en volumen) del
25 sobrenadante y se transfirieron las micropartículas al tubo de prueba de vidrio. El ciclo de lavado/sedimentación se repitió unas 2 veces

adicionales y las micropartículas se transfirieron a un recipiente liofilizado con un volumen mínimo de agua. El recipiente se congeló instantáneamente y se liofilizaron las micropartículas durante 48 horas.

(ii) Protocolo de liberación in vitro

Se suspendieron 0.9 mg de micropartículas que contenían 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo en 50:50 de PLGA en PBS que contenía 0.1% p.v de Tween 80 (20 ml). La suspensión resultante se mantuvo estática a 37°C y se tomaron las muestras en 24 horas mediante eliminación del medio (1 ml) seguida de la adición en el medio (1 ml) para asegurar que permaneció constante el volumen del medio dentro del experimento. Se tomaron muestras en intervalos regulares (véase Figura 1) hasta que el depósito no liberaba más 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo y se analizaron mediante HPLC. Los resultados se muestran en la Tabla 1 a continuación.

Tabla 1

Proporción molar de polímero láctido:glícólido/ MW (KD)	Carga de PA (% en peso)	Eficacia de encapsulación (%)	Explosión in vitro (%)	Liberación in vitro (%)
50:50/ 19.5	13.27	88.23	9.27	Día 16 - 98.8

Las micropartículas con 50:50 de PLGA proporcionaron buenas eficacias de encapsulación, produciendo cargas de 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo de aproximadamente 13%. Los datos de perfil de liberación in vitro se muestran en la Figura 1. Los estudios de liberación in vitro muestran que 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo en 50:50 de micropartículas de PLGA tuvo una buena explosión el día uno y se liberó durante 16 días in vitro.

Ejemplo 2

Eficacia in vivo en modelo MIA

Se evaluó el efecto de inyección intra-articular (IA) del inhibidor AZD6918 de Trk formulado con 50/50 de microesferas de PLGA en la artritis inducida por iodoacetato monosódico. Se inyectaron IA 50/50 de PLGA del compuesto 3 días luego de la inyección MIA (cuando la enfermedad se caracteriza por sinovitis activa) y se registró la carga de peso durante un período de 15 días.

La Figura 2 muestra un curso de tiempo de la asimetría de carga de peso luego de la administración del inhibidor de TRK 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo/50 50 de PLGA 3 días luego de la inyección MIA *p<0.05, **p<0.01, ***p<0.001.

ANOVA seguido de prueba Newman-Keuls post hoc con MIA indujo un déficit en la carga de peso, dando como resultado aproximadamente un 50% de reducción en la distribución del peso en

la pata inyectada. Se observó que este déficit se revirtió de manera importante en comparación con las microesferas de placebo con 200µg IA de 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo/PLGA de 2 a 8 días luego de la inyección del compuesto. El 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo (dosis de 200 µg) formulado con 50 50 de PLGA mostró buena eficacia cuando se revirtió la carga de peso espontáneo que lleva 3 días luego de MIA. La eficacia duró 8 días luego de la inyección IA del compuesto (11 días luego de MIA).

Procesos experimentales

Inyección intra-articular de 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo.

Se anestesiaron animales con isoflurano y 30µl de microesferas de PLGA que contenían 200µg inyectados en cada rodilla utilizando una jeringa Hamilton y una aguja 25G. El recipiente de microesferas se sometió a agitación vorticial antes de la inyección de cada animal para tratar de obtener una solución de inyección homogénea. Se recuperaron los animales y se devolvieron a sus jaulas hasta la muestra de plasma.

Muestra de plasma de vena caudal

Se calentaron los animales en una caja caliente durante 10 minutos a 42°C antes de colocarlos en una jaula y se tomaron muestras

de 200µl de sangre a partir de la vena caudal lateral en un tubo con heparina de litio en Sarsted Multivette 600 (cat no 15.1673) unido a una aguja 21G. Los tubos se colocaron en un rodillo hasta que se alcanzó la centrifugación a 13000rpm durante 5 minutos para la sedimentación de los glóbulos rojos. Se decantó el plasma y se almacenó a -20°C hasta el análisis por DMPK. Se tomaron las muestras 1h, 3h, 6h, 24h, 48h, 96h y 168h luego de la inyección.

La Figura 3 muestra los niveles de 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridli)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo en una formulación IA 50/50 de PLGA en ratas en plasma. Los niveles mostrados son para una dosis de 200ug de compuesto.

Conclusiones

Se observó una liberación de 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridli)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo durante 168 horas.

Los niveles plasmáticos concordaron bien con las predicciones teóricas basadas en perfiles de liberación in vitro.

REIVINDICACIONES

1. Un depósito farmacéutico que comprende (i) 5-fluoro-2-
[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-
5 il)amino]piridin-3-carbonitrilo o su sal farmacéuticamente aceptable,
como un agente farmacéutico (PA) y (ii) un polímero que se degrada
para crear un microclima ácido, donde el PA se libera del polímero tras
la degradación del polímero.

10 2. El depósito farmacéutico de acuerdo con la reivindicación
1, donde el polímero se selecciona de un poliéster de un ácido
hidroxigraso y sus derivados, un polímero de un α -cianoacrilato de
alquilo, un oxalato de polialquileno, un poli (orto) éster, un
policarbonato, un poliorto-carbonato, un ácido de poliamino, éster de
15 ácido hialurónico y sus mezclas.

3. El depósito farmacéutico de acuerdo con la reivindicación
2, donde el polímero es un copolímero de ácido láctico-ácido glicólico.

20 4. El depósito farmacéutico de acuerdo con la reivindicación
3, donde el copolímero de ácido láctico-ácido glicólico tiene una
proporción molar de ácido láctico:ácido glicólico en el rango de 100:0 a
50:50.

5. El depósito farmacéutico de acuerdo con la reivindicación 4, donde el copolímero de ácido láctico-ácido glicólico tiene una proporción molar de ácido láctico:ácido glicólico de 95:5.

5 6. El depósito farmacéutico de acuerdo con la reivindicación 4, donde el copolímero de ácido láctico-ácido glicólico tiene una proporción molar de ácido láctico:ácido glicólico de 50:50.

10 7. El depósito farmacéutico de acuerdo con cualquiera de las reivindicaciones 1 a 6, donde el depósito farmacéutico se formula para una liberación controlada y/o sostenida del PA durante un período de tiempo de aproximadamente 10 a 90 días.

15 8. El depósito farmacéutico de acuerdo con la reivindicación 7, donde el depósito farmacéutico se formula para una liberación controlada y/o sostenida del PA durante un período de tiempo de aproximadamente 14 días.

20 9. El depósito farmacéutico de acuerdo con la reivindicación 7, donde el depósito farmacéutico se formula para una liberación controlada y/o sostenida del PA durante un período de tiempo de aproximadamente 60 días.

25 10. El depósito farmacéutico de acuerdo con la reivindicación 7, donde el depósito farmacéutico se formula para una liberación

controlada y/o sostenida del PA durante un período de tiempo de aproximadamente 90 días.

5 11. El depósito farmacéutico de acuerdo con cualquiera de las reivindicaciones 1 a 10, que se formula para una administración mediante inyección.

12. El depósito farmacéutico de acuerdo con la reivindicación 11, que se formula para una administración mediante inyección intra-articular.

10 13. El depósito farmacéutico de acuerdo con cualquiera de las reivindicaciones 1 a 12, que se formula para uso medicinal humano.

14. El depósito farmacéutico de acuerdo con cualquiera de las reivindicaciones 1 a 12, que se formula para uso veterinario.

15 15. El depósito farmacéutico de acuerdo con cualquiera de las reivindicaciones 1 a 14, para la prevención o el tratamiento de osteoartritis.

20 16. El uso de un depósito farmacéutico de acuerdo con cualquiera de las reivindicaciones 1 a 14, para la prevención o el tratamiento de osteoartritis.

25 17. Un depósito farmacéutico como se describe generalmente en la presente.

Figura 1

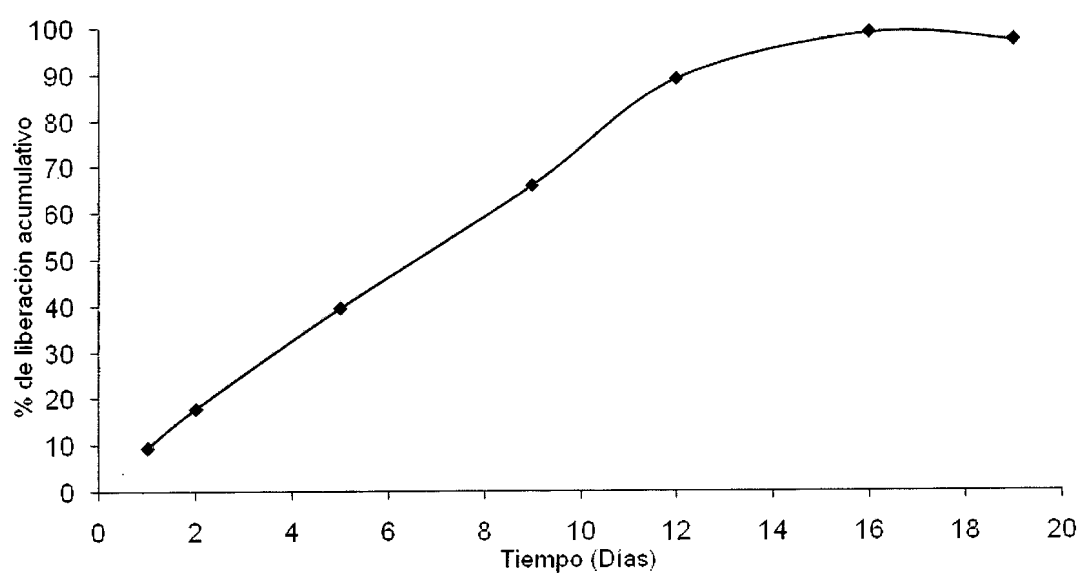


Figura 2

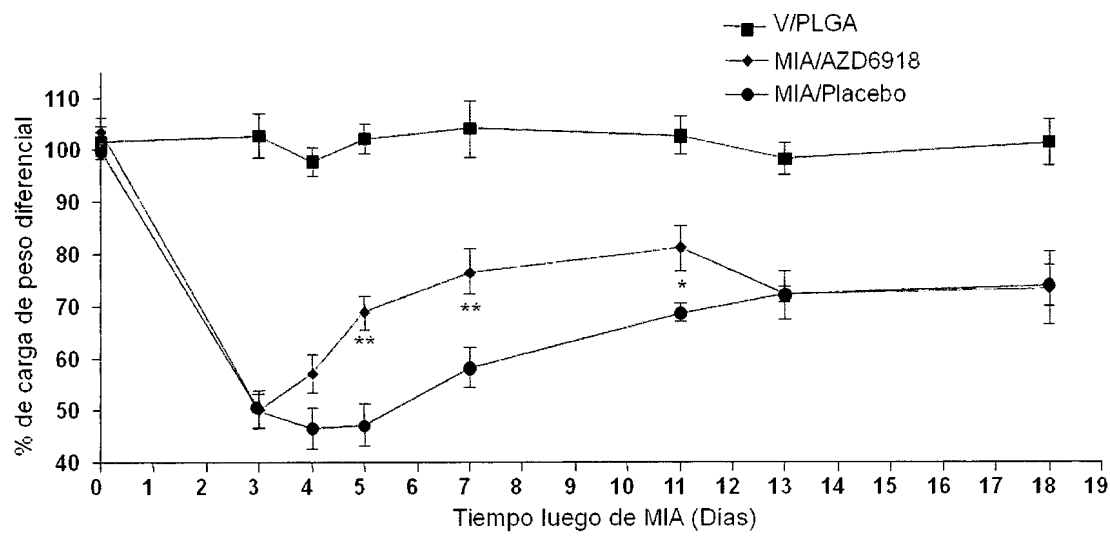
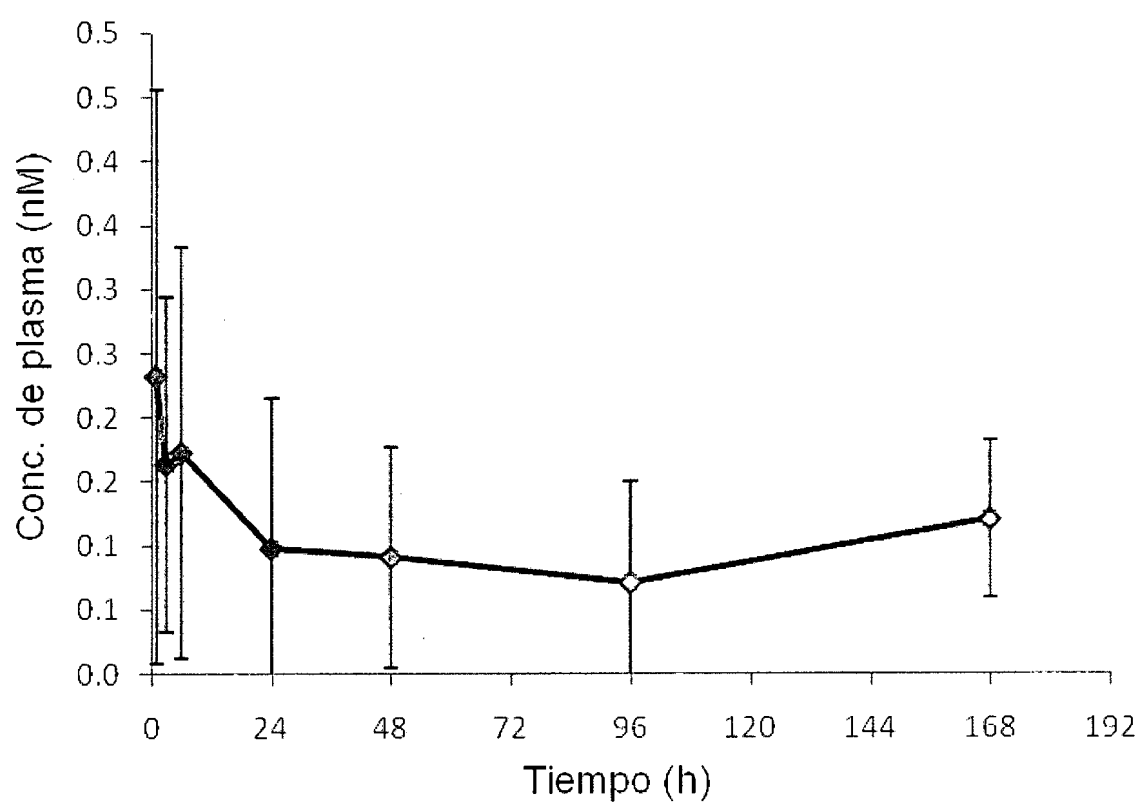


Figura 3

DEPÓSITO FARMACÉUTICO PARA 5-FLUORO-2-[[[(1S)-1-(5-FLUORO-2-
PIRIDIL)ETIL]AMINO]-6-[(5-ISOPROPOXI-1H-PIRAZOL-3-
IL)AMINO]PIRIDIN-3-CARBONITRIL

RESUMEN-OBJETO Y FINALIDAD DE LA INVENCION

Un depósito farmacéutico que comprende (i) 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo o su sal farmacéuticamente aceptable, como un agente farmacéutico (PA) y (ii) un polímero que se degrada para crear un microclima ácido, donde el PA se libera del polímero tras la degradación del polímero.

SECTOR: Industria farmacéutica; preparaciones médicas que contienen aglomerados de ingredientes orgánicos activos útiles en el tratamiento de desórdenes del sistema óseo.

 Industria y Comercio SUPERINTENDENCIA		SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO DIVISIÓN DE NUEVAS CREACIONES EXTRACTO PARA PUBLICACIÓN SOLICITUD DE PATENTE DE INVENCION PCT	
(21) No. de solicitud:		(51) Int Cl: A61K 9/16	
(22) Fecha de solicitud: 28-01-13		(71) Solicitante(s): ASTRAZENECA AB	
(30) Prioridad	(31) No. Prioridad 61/365,407	(32) Fecha 19-07-10	(33) País US
		(72) Inventor(es): FULLER, Emily; JANIN, Armelle; MACFAUL, Philip; PUGH, Rachel y NASH, Ian.	
		(74) Apoderado: ALICIA LLOREDA RICAURTE	
(85)	Fecha límite inicio fase nacional: 19-02-13		(87) Publicación internacional
(86)	Datos relativos a la presentación de la solicitud PCT		Fecha: 26-01-12
	Fecha de presentación de la solicitud: 19-07-11		No. publicación: WO 2012/010883 A3
	No. de solicitud: PCT/GB2011/051357		
(54) Título: "DEPÓSITO FARMACÉUTICO PARA 5-FLUORO-2-[[[(1S)-1-(5-FLUORO-2-PIRIDIL)ETIL]AMINO]-6-[[[5-ISOPROPOXI-1H-PIRAZOL-3-IL)AMINO]PIRIDIN-3-CARBONITRILO"			

Resumen - Reivindicación(es):

Resumen.-

Un depósito farmacéutico que comprende (i) 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[[[5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo o su sal farmacéuticamente aceptable, como un agente farmacéutico (PA) y (ii) un polímero que se degrada para crear un microclima ácido, donde el PA se libera del polímero tras la degradación del polímero.

Sector: Industria farmacéutica; preparaciones médicas que contienen aglomerados de ingredientes orgánicos activos útiles en el tratamiento de desórdenes del sistema óseo.



Industria y Comercio

SUPERINTENDENCIA

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

TARJETA ARCHIVO PROPIETARIOS

PATENTE DE INVENCION ☐ PCT ☒ MODELO DE UTILIDAD ☐ DISEÑO INDUSTRIAL ☐

(21) No. de solicitud:		(22) Fecha de solicitud: 28-01-13	
(51) Clasificación Internacional: A61K 9/16			
(71) Solicitante(s): ASTRAZENECA AB			
(72) Inventor(es): FULLER, Emily; JANIN, Armelle; MACFAUL, Philip; PUGH, Rachel y NASH, Ian.			
(74) Apoderado: ALICIA LLOREDA RICAURTE			
(30) Prioridad	(31) No. Prioridad 61/365,407	(32) Fecha 19-07-10	(33) País US
(85) Fecha límite inicio fase nacional: 19-02-13		(87) Publicación internacional	
(86) Datos relativos a la presentación de la solicitud PCT		Fecha: 26-01-12	
Fecha de presentación de la solicitud: 19-07-11		No. publicación: WO 2012/010883 A3	
No. de solicitud: PCT/GB2011/051357			
(54) Título: DEPÓSITO FARMACÉUTICO PARA 5-FLUORO-2-[[[(1S)-1-(5-FLUORO-2-PIRIDIL)ETIL]AMINO]-6-[(5-ISOPROPOXI-1H-PIRAZOL-3-IL)AMINO]PIRIDIN-3-CARBONITRILO.			

 Industria y Comercio SUPERINTENDENCIA		SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO DIVISIÓN DE NUEVAS CREACIONES TARJETA ARCHIVO TEMÁTICO PATENTE DE INVENCION _____ PCT <u>X</u> MODELO DE UTILIDAD _____ DISEÑO INDUSTRIAL _____	
(21) No. de solicitud: _____		(22) Fecha de solicitud: 28-01-13	
(51) Clasificación Internacional: A61K 9/16			
(71) Solicitante(s): ASTRAZENECA AB			
(72) Inventor(es): FULLER, Emily; JANIN, Armelle; MACFAUL, Philip; PUGH, Rachel y NASH, Ian.			
(74) Apoderado: ALICIA LLOREDA RICAURTE			
(30) Prioridad	(31) No. Prioridad 61/365,407	(32) Fecha 19-07-10	(33) País US
(85) Fecha limite inicio fase nacional: 19-02-13		(87) Publicación internacional:	
(86) Datos relativos a la presentación de la solíc. PCT Fecha de presentación de la solicitud: 19-07-11 No. de solicitud: PCT/GB2011/051357		Fecha: 26-01-12 No. Publicación: WO 2012/010883 A3	
(54) Título: DEPÓSITO FARMACÉUTICO PARA 5-FLUORO-2-[[[(1S)-1-(5-FLUORO-2-PIRIDIL)ETIL]AMINO]-6-[(5-ISOPROPOXI-1H-PIRAZOL-3-IL)AMINO]PIRIDIN-3-CARBONITRILO.			
(57) Resumen - Reivindicación(es): Resumen.- Un depósito farmacéutico que comprende (i) 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-piridil)etil]amino]-6-[(5-isopropoxi-1H-pirazol-3-il)amino]piridin-3-carbonitrilo o su sal farmacéuticamente aceptable, como un agente farmacéutico (PA) y (ii) un polímero que se degrada para crear un microclima ácido, donde el PA se libera del polímero tras la degradación del polímero. Sector: Industria farmacéutica; preparaciones médicas que contienen aglomerados de ingredientes orgánicos activos útiles en el tratamiento de desórdenes del sistema óseo.			

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(10) International Publication Number
WO 2012/010883 A3

(43) International Publication Date
26 January 2012 (26.01.2012)

- (51) International Patent Classification:
A61K 9/16 (2006.01) A61K 31/444 (2006.01)
A61K 47/34 (2006.01) A61P 19/02 (2006.01)
- (21) International Application Number:
PCT/GB2011/051357
- (22) International Filing Date:
19 July 2011 (19.07.2011)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
61/365,407 19 July 2010 (19.07.2010) US
- (71) Applicant (for all designated States except US): AS-TRAZENECA AB [—/SE]; S-151 85 Södertälje (SE).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): FULLER, Emily [GB/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). JANIN, Armelle [FR/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). MACFAUL, Philip [GB/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). PUGH, Rachel [GB/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB). NASH, Ian [GB/GB]; Alderley Park, Macclesfield, Cheshire SK10 4TG (GB).
- (74) Agent: YEADON, Mary Elizabeth; Greaves Brewster LLP, Indigo House, Cheddar Business Park, Wedmore Road, Cheddar, Somerset BS27 3EB (GB).

- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report (Art. 21(3))
(88) Date of publication of the international search report:
7 September 2012



WO 2012/010883 A3

(54) Title: PHARMACEUTICAL DEPOT FOR 5-FLUORO-2 [[(1S) -1- (5-FLUORO-2-PYRIDYL)ETHYL]AMINO]-6-[(5-ISO-PROPOXY-1H-PYRAZOL-3-YL)AMINO]PYRIDINE-3-CARBONITRILE

(57) Abstract: A pharmaceutical depot comprising (i) 5-fluoro-2-[[[(1S)-1-(5-fluoro-2- pyridyl)ethyl]amino]-6-[(5-isopropoxy-1H-pyrazol-3-yl)amino]pyridine-3-carbonitrile, or a pharmaceutically-acceptable salt thereof, as a pharmaceutical agent (PA) and (ii) a polymer which degrades to create an acidic microclimate, wherein the PA is released from the polymer upon polymer degradation.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference MEY/P2035PC0	FOR FURTHER ACTION see Form PCT/SA/220 as well as, where applicable, item 5 below.	
International application No. PCT/GB2011/051357	International filing date (day/month/year) 19/07/2011	(Earliest) Priority Date (day/month/year) 19/07/2010
Applicant ASTRAZENECA AB		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 08 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of:

- ☒ the international application in the language in which it was filed
☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))

b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).

c. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (See Box No. II)

3. ☐ **Unity of invention is lacking** (see Box No III)

4. With regard to the **title**,

- ☒ the text is approved as submitted by the applicant
☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

- ☒ the text is approved as submitted by the applicant
☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority

6. With regard to the **drawings**,

- a. the figure of the **drawings** to be published with the abstract is Figure No. _____
☐ as suggested by the applicant
☐ as selected by this Authority, because the applicant failed to suggest a figure
☐ as selected by this Authority, because this figure better characterizes the invention
b. ☒ none of the figures is to be published with the abstract

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2011/051357

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/16 A61K47/34 A61K31/444 A61P19/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2008/054292 A1 (ASTRAZENECA AB [SE]) 8 May 2008 (2008-05-08) page 1, lines 4-8 page 4, lines 29,30 page 13, lines 8-11 page 15, lines 7-11 page 18, lines 11-14 page 19, lines 3-8 page 20, line 12 - page 22, line 30	1-16
X	US 6 217 911 B1 (VAUGN WILLIAM M [US] ET AL) 17 April 2001 (2001-04-17) cited in the application	17
Y	the whole document	1-16
	----- -/--	

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family
---	---

Date of the actual completion of the international search 22 May 2012	Date of mailing of the international search report 08/06/2012
--	--

Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Gómez Gallardo, S
--	---

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2011/051357

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/125226 A1 (ASTRAZENECA AB [SE]; ASTRAZENECA UK LTD [GB]) 15 October 2009 (2009-10-15)	17
A	the whole document	1-16
X	US 2006/140988 A1 (CHEN GUOHUA [US] ET AL) 29 June 2006 (2006-06-29)	17
A	the whole document	1-16
X	US 2010/016257 A1 (BROWN LAURA J [US] ET AL) 21 January 2010 (2010-01-21)	17
A	the whole document	1-16
X	WO 99/01114 A1 (EURO CELTIQUE SA [LU]) 14 January 1999 (1999-01-14)	17
A	the whole document	1-16
X	BUTOESCU N ET AL: "Intra-articular drug delivery systems for the treatment of rheumatic diseases: A review of the factors influencing their performance", EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS, ELSEVIER SCIENCE PUBLISHERS B.V., AMSTERDAM, NL, vol. 73, no. 2, 1 October 2009 (2009-10-01), pages 205-218, XP026652681, ISSN: 0939-6411, DOI: 10.1016/J.EJPB.2009.06.009 [retrieved on 2009-06-21]	17
A	the whole document	1-16
X	US 2009/035250 A1 (DRAPEAU SUSAN J [US] ET AL) 5 February 2009 (2009-02-05)	17
A	page 1, paragraphs 8,13 page 2, paragraph 18-22 page 4, paragraphs 36,44 page 5, paragraph 46	1-16
A	WO 2006/082392 A1 (ASTRAZENECA AB [SE]; ASTRAZENECA UK LTD [GB]) 10 August 2006 (2006-08-10) cited in the application page 2, lines 21-28 page 29, lines 7-13 page 41, lines 20-23 page 88; example 119	1-17

-/--

INTERNATIONAL SEARCH REPORT

International application No PCT/GB2011/051357

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	AstraZeneca: "A Phase I, Open-Label, Dose-Escalation Study to Assess the Safety, Tolerability and Pharmacokinetics of AZD6918 Administered Daily as a Single Agent and in Combination Treatment in Adult Patients With Refractory Solid Malignancies", Clinical Study Report Synopsis; Drug Substance AZD6918: Study Code D2785C00002 16 June 2009 (2009-06-16), XP002676238, Retrieved from the Internet: URL:http://www.astrazenecaclinicaltrials.com/_mshost800325/content/clinical-trials/resources/pdf/D2785C00002 [retrieved on 2012-05-18] the whole document -----	1-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2011/051357

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008054292 A1	08-05-2008	CA 2685724 A1 EP 2164490 A1 TW 200826937 A US 2008108669 A1 WO 2008054292 A1	08-05-2008 24-03-2010 01-07-2008 08-05-2008 08-05-2008
US 6217911 B1	17-04-2001	NONE	
WO 2009125226 A1	15-10-2009	AU 2009235240 A1 CA 2725822 A1 CN 102056594 A CO 6311065 A2 EP 2271318 A1 JP 2011516535 A KR 20100135889 A US 2009281150 A1 WO 2009125226 A1	15-10-2009 15-10-2009 11-05-2011 22-08-2011 12-01-2011 26-05-2011 27-12-2010 12-11-2009 15-10-2009
US 2006140988 A1	29-06-2006	AR 052182 A1 AU 2005322231 A1 BR PI0519199 A2 CA 2587388 A1 EP 1827376 A1 JP 2008525471 A KR 20070091302 A US 2006140988 A1 WO 2006071694 A1	07-03-2007 06-07-2006 30-12-2008 06-07-2006 05-09-2007 17-07-2008 10-09-2007 29-06-2006 06-07-2006
US 2010016257 A1	21-01-2010	US 2007141160 A1 US 2010016257 A1	21-06-2007 21-01-2010
WO 9901114 A1	14-01-1999	AU 739384 B2 AU 8476598 A BR 9815499 A CA 2271750 A1 EP 1009390 A1 JP 2000511941 A NO 992373 A WO 9901114 A1	11-10-2001 25-01-1999 02-01-2001 14-01-1999 21-06-2000 12-09-2000 22-02-2000 14-01-1999
US 2009035250 A1	05-02-2009	NONE	
WO 2006082392 A1	10-08-2006	AR 056184 A1 AT 530545 T AU 2006210710 A1 BR PI0606793 A2 CA 2595834 A1 CN 101155800 A DK 1846394 T3 EP 1846394 A1 EP 2383268 A1 ES 2375735 T3 HR 20110962 T1 JP 2008528664 A KR 20070107107 A NZ 561145 A PT 1846394 E RS 52061 B	26-09-2007 15-11-2011 10-08-2006 01-12-2009 10-08-2006 02-04-2008 16-01-2012 24-10-2007 02-11-2011 05-03-2012 31-01-2012 31-07-2008 06-11-2007 25-02-2011 05-01-2012 30-04-2012

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2011/051357

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		US 2008139561 A1	12-06-2008
		UY 29360 A1	02-10-2006
		WO 2006082392 A1	10-08-2006

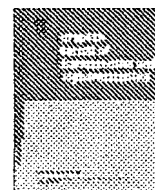
DOCUMENTOS ANEXOS AL REPORTE DE BÚSQUEDA INTERNACIONAL

- 1. "Intra-articular drug delivery systems for the treatment of rheumatic diseases: A review of the factors influencing their performance", EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS.**
- 2. XP002676238**



Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Review article

Intra-articular drug delivery systems for the treatment of rheumatic diseases: A review of the factors influencing their performance

N. Butoescu, O. Jordan*, E. Doelker

School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Geneva, Switzerland

ARTICLE INFO

Article history:

Received 10 December 2008

Accepted in revised form 16 June 2009

Available online 21 June 2009

Keywords:

Intra-articular

Drug delivery

Osteoarthritis

Rheumatoid arthritis

Liposomes

Nanoparticles

Microparticles

Depot formulation

Review

ABSTRACT

Osteoarthritis and rheumatoid arthritis are rheumatic diseases for which a curative treatment does not currently exist. Their management is directed towards pain relief achieved with different classes of drugs among which non-steroidal and steroidal anti-inflammatory substances are the most frequently used agents. Nevertheless, the oral or systemic administration of such drugs is hindered by numerous side effects, which could be overcome by their intra-articular (i-a.) administration as dosage forms capable of gradually releasing the active substance. The present review article summarises the research done in the field of drug delivery systems for i-a. injection vs. current management of osteoarthritis or rheumatoid arthritis. Aspects such as the influence of size, shape, polymer matrix or targeted drug on the i-a. retention time, phagocytosis and biological activity will be discussed. Finally, we will comment on the need for adapted delivery systems for the novel and very potent anti-inflammatory drugs, such as inhibitors of the p38 mitogen-activated protein kinase or the IL-1 β conversion enzyme, which to date cannot be properly used due to the severe side effects associated with their systemic administration.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Despite extended pharmaceutical and clinical research, there are still unmet needs in the treatment of rheumatic diseases such as osteoarthritis (OA) and rheumatoid arthritis (RA). For both conditions, no prevention methods are known, and optimal management is based on early diagnosis. Treatment of irreversible joint damage is achieved through oral, parenteral or intra-articular drugs. Despite some practical drawbacks, the direct delivery of a drug to an affected joint offers the possibility of reaching high drug concentrations at the site of action with limited systemic toxicity. The i-a. administration represents a valuable means to deliver to the joint drugs with low bioavailability, such as recombinant proteins, therapeutic genes or substances such as TNF α , matrix metalloproteinases or p38 mitogen-activated protein kinase inhibitors [1–6]. Nevertheless, depending on their chemical structure, some active compounds are rapidly cleared from the joint, thus requiring numerous injections, which could cause infection or joint disability [7]. An innovative way to maintain a therapeutic concentration over longer time periods is the administration of depot for-

mulations, which generally contain corticosteroids. Despite their clinically proven efficacy [8–11], these depot formulations suffer from a significant drawback, which limits their use. Actually, due to their crystalline nature, these drugs can generate inflammatory conditions upon i-a. injection, leading to crystal-induced arthritis, observed in 10% of the patients, but which disappears within a few days [12–14].

This reinforces the need to develop drug delivery systems for i-a. use that would function as depot gradually releasing the active substance and providing local sustained drug action. The potential of such drug delivery systems is high, because the release rate as well as the reservoir capacity can be tailored by specific technological parameters such as the polymer type and molecular weight or the formulation method. This review outlines current knowledge on the advantages and limitations of i-a. drug delivery systems, focusing on the factors that influence their biological performance, with particular emphasis on size, shape and chemical nature of the matrix. The authors have decided not to address issues related to the pharmacokinetics of drug solutions or suspensions in the synovial space, nor of their transport into cartilage, as these aspects have recently been presented in detail by Larsen et al. [15]. Other administration routes, involving joint-targeting strategies, have been reviewed elsewhere [16]. Moreover, aspects related to gene delivery [17] and cell-based therapies [18] are not presented in this article.

* Corresponding author. School of Pharmaceutical Sciences, University of Geneva, 30 Quai Ernest Ansermet, Sciences II, 1211 Geneva 4, Switzerland. Tel.: +41 223796586; fax: +41 223796567.

E-mail address: Oliver.Jordan@unige.ch (O. Jordan).

2. Normal vs. arthritic joint

Synovial joints (Fig. 1a) are highly specialised anatomical structures that are composed of two articulating bones stabilised by the surrounding muscles and ligaments [19]. Each joint has a unique configuration offering an appropriate contact area, which, combined with a suitable muscle action, allows for high-efficiency performance. To maintain its structural integrity, the joint is surrounded by a fibrous capsule, which is a firm structure consisting of dense connective tissue. Within the capsule, there are thick bands of parallel collagen fibres known as ligaments, and numerous nerve endings, which, along with those in the muscles, ligaments and tendons, are responsible for knee proprioceptive sense and deep pain perception [20]. The two bone connective surfaces are covered with cartilage, which is a resilient tissue that is composed of collagen and proteoglycan matrix. It provides the joint with almost frictionless mobility. Surprisingly, the cartilage surface is not smooth (Fig. 2) but shows irregular depressions and undulations, as demonstrated by a high number of scanning electron microscopy studies [21–23], optical microscopy [24] or atomic force microscopy [25,26]. In adults, cartilage is hypocellular, aneural, avascular and alymphatic. It receives nutrients by a two-step diffusion system [23], from the blood vessels into the synovial fluid and then from the synovium to the cartilage, in order to reach the cartilage cells, which are known as chondrocytes. Lining the inner joint space, the synovial membrane consists of two to three cell layers. It is responsible for providing nutrients to the cartilage and for secreting the synovial fluid. The composition of the synovial fluid is similar to that of plasma, but with reduced protein concentration and additional hyaluronic acid secreted by the synovial fibroblasts at concentrations between 2 and 4 mg/ml [27]. It provides the high viscosity characteristic to the synovial fluid and provides rheofluidifying properties, meaning that the more rapidly it flows, the more fluid it becomes [28]. Some joints have within their cavities a rudimentary fibrocartilaginous discoid structure called a meniscus. In other joints such as the knee or the temporomandibular joint, the meniscus is highly developed and well defined. The meniscus contributes to joint stability and shock absorption.

In people with OA, there is a progressive degeneration of the cartilage, first observed as fissures on the articular surface, which later infiltrate the damaged cartilage and eventually the subchondral bone (Fig. 1b). The early phase of proteoglycan loss is followed by a phase of increased matrix turnover, with net depletion of cartilage proteoglycans and collagens, finally leading to severe damage of the collagen network [29]. Cartilage breakdown is driven

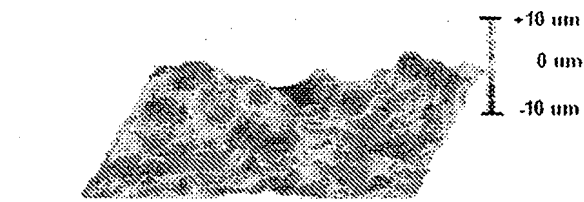


Fig. 2. Bovine knee cartilage surface topography visualized by atomic force microscopy. Modified from Shekhawat et al. [26].

by the production of numerous cytokines, growth factors and proteases. The matrix metalloproteinases (MMPs) play a major role in the cleavage of cartilage macromolecules, the by-products of which stimulate degradation through a positive feedback loop, implying cellular production of various cytokines such as IL-1. Cellular responses also lead to the production of growth factors, i.e., insulin-like growth factor-1 (IGF-1) and transforming growth factor β (TGF β). Bone remodelling and bone formation are observed in the form of osteophytes, bone protuberances into the joint space at the margins of the articular cartilage, and also in the form of subchondral bone, directly beneath the weight-bearing surface. Unlike RA, OA is a relatively non-inflammatory condition, although limited synovitis can be observed.

In RA, the synovial space undergoes a sustained inflammatory response (Fig. 1c), which is characterised by the presence of numerous inflammatory cell types including T cells, dendritic cells, macrophages, fibroblasts, mast cells, neutrophils and B cells. The activated T cells proliferate and stimulate the production of interferon- γ and other pro-inflammatory cytokines, which further stimulate macrophages, fibroblasts, chondrocytes and osteoclasts. Activated macrophages and fibroblasts secrete the tumour necrosis factor- α (TNF- α), as well as IL-1, IL-6, IL-15 and IL-18. In turn, these inflammatory mediators and various growth factors activate neutrophils and B cells. Finally, endothelial transformation enables enhanced cell recruitment to the joint. The synovial lining becomes hypertrophic and may thicken up to 10–12 cells in depth, with an increased proliferation of the fibroblast- and macrophage-like synoviocytes, as well as angiogenesis, and it finally develops into locally invasive pannus tissue. B cells and dendritic cells form aggregates with T cells and tissue macrophages [30,31]. The presence of all of these cell types creates an important change in the joint physiology, with increased synovial volume, pressure and temperature. In addition, acidosis, hypoxia, and the joint action

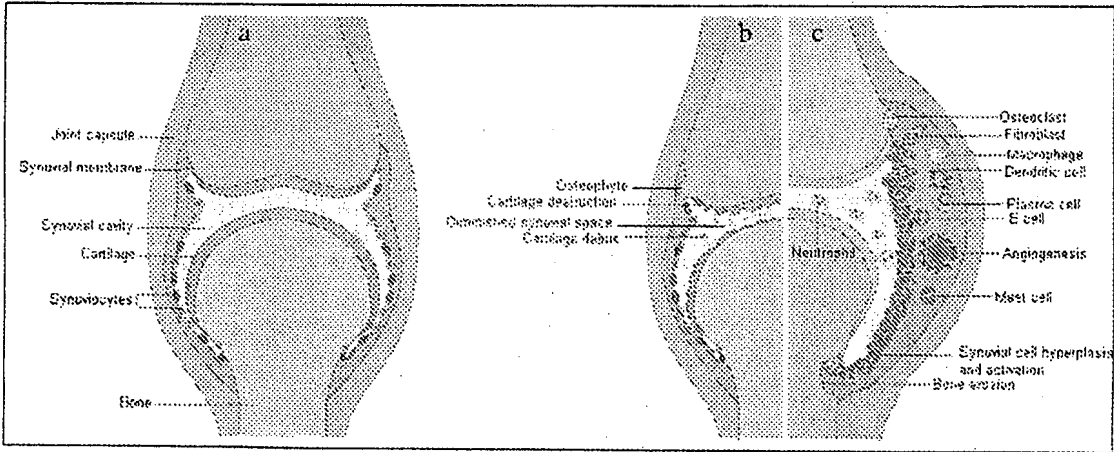


Fig. 1. (a) Normal joint; (b) OA joint with typical changes such as significant joint space narrowing, cartilage destruction and osteophyte formation; (c) RA joint presenting the pannus with numerous types of cells. Modified from Strand et al. [19].

of proteases and growth factors contribute to synovial inflammation, cartilage destruction and bone erosion [32–35].

3. Management of rheumatic diseases

3.1. Current management of osteoarthritis

OA usually has a slow and insidious onset, and it clinically manifests in the form of gradual pain development, joint stiffness and limitation in joint movements. Risk factors for OA progression include biomechanical factors such as joint injuries or deformities, obesity, occupational and non-occupational physical activity, but also systemic factors such as age, genetic factors, oestrogen deficiency, low bone mineral density, female sex, Caucasian ethnic origin and presence of crystals in joint fluid [36,37]. OA has a variable prevalence in developing countries, ranging from 3 to 20%, and it is the most common arthritic disease in developed countries. In the US, the prevalence, which was 15% in 1998, could reach 18.2% by the year 2020 [38]. Management of OA focuses on pain reduction and function restoration, which are achieved by a combination of prevention and non-pharmacological and pharmacological measures, as schematically presented in Fig. 3. Prevention measures consist mainly of weight reduction concomitant with strengthening of the periarticular muscles to provide improved joint support, as well as life-style changes, thermal cures or acupuncture. Medical management is usually conducted according to the guidelines developed by the American College of Rheumatology [39,40], the European League Against Rheumatism [41] or the Osteoarthritis Research International [42]. These guidelines indicate use of analgesics, NSAIDs, intra-articular agents and nutritional supplements, as is extensively discussed in previous review articles [36,43–48]. Unfortunately, a curative treatment has not been identified. Paracetamol is the first-line pharmacological agent recommended by all of the above-mentioned bodies and its effectiveness in the relief of pain associated with OA has recently been demonstrated by a meta-analysis of 10 randomised controlled trials [49]. NSAIDs are effective agents in the treatment of OA, but their use is generally accompanied by undesired gastrointestinal and renal effects. Selective cyclooxygenase-2 inhibitors, which are more effective than paracetamol, have the advantage of reduced gastrointestinal toxicity [50]. The Osteoarthritis Research International recommends topical agents and capsaicin as effective adjunctives or alternatives to oral analgesic/anti-inflammatory agents. For patients with moderate to severe pain and inflammation who do not respond satisfactorily to oral analgesic/anti-inflammatory agents, intra-articular corticosteroid injections should be considered [42]. The

beneficial effect of corticosteroids on cartilage can be attributed to several mechanisms; for example, suppression of the synthesis of metalloproteases or pro-inflammatory synovial factors such as IL-1 [46]. To reduce the risk of complications, corticosteroid i-a. injections should not be repeated within 3 to 6 months [41,42]. In an 84-patient clinical study reported by Gaffney et al. [10], a single i-a. injection of triamcinolone acetonide suspension resulted in short-term pain relief, the increased benefit being associated with synovial fluid aspiration. A few years later, the study conducted by Raynauld et al. [8] demonstrated long-term safety and efficacy of i-a. suspensions of triamcinolone. Generally, the corticosteroid is combined with lidocaine or bupivacaine and injected after joint aspiration. Potential side effects include post-injection flare or pain, crystal synovitis, haemarthrosis, joint sepsis and steroid cartilage atrophy, as well as systemic corticosteroid effects such as fluid retention or aggravation of hypertension. Viscosupplementation is another therapeutic approach in OA, aiming at replacing hyaluronic acid, whose level is reduced in the damaged knee, in order to restore the elasticity and viscosity of the synovial fluid to normal. One clinical study evaluating the efficacy of viscosupplementation compared to corticosteroid injections in a group of 100 patients demonstrated that both treatments were moderately effective [51]. Despite their delayed onset, the hyaluronic acid injections are characterised by prolonged duration and symptomatic benefit when compared to corticosteroid suspensions [42]. For patients who did not satisfactorily respond to any of the above-mentioned treatment methods, the guidelines suggest radioactive synovectomy. This method permits removal of the inflamed synovial lining, which leads to pain relief. Despite successful results, the fear of radioactivity leakage from the joint and the uncertain long-term biological hazards of radiation limited this form of therapy. Additional medications consisting of nutraceuticals, such as glucosamine and chondroitin, can be administered to OA patients to provide a symptomatic benefit [43,52,53]. Although progresses in OA therapy are numerous, there are currently no approved disease-modifying OA drugs (DMOADs) that stop and reverse cartilage destruction or restore the functional integrity of the joint. Given the complexity of this disease, it is necessary to first identify the major biochemical pathways that can be targeted by means of pharmacological or biological approaches in order to develop disease-modifying treatments.

3.2. Current management of rheumatoid arthritis

Similar to OA, RA does not benefit of the existence of a curative treatment. To date, the main objective of the RA treatments has

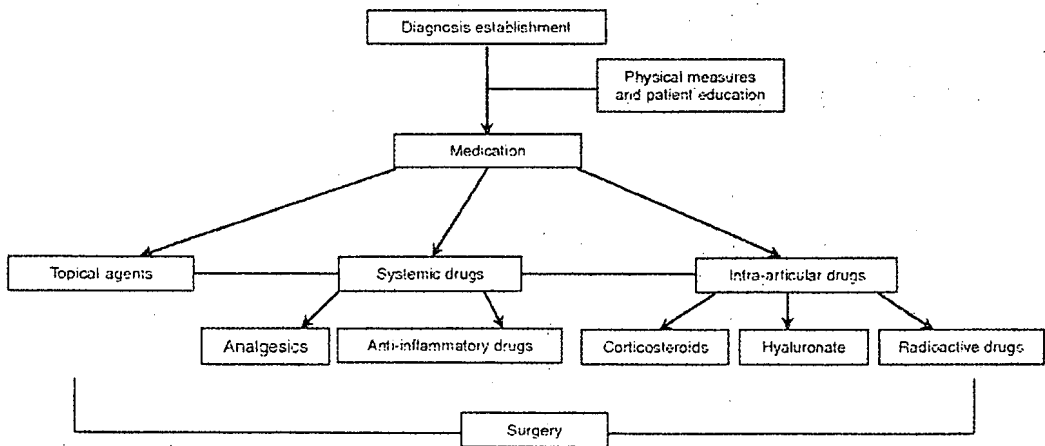


Fig. 3. Current management of OA. Modified from Lozada et al. [47].

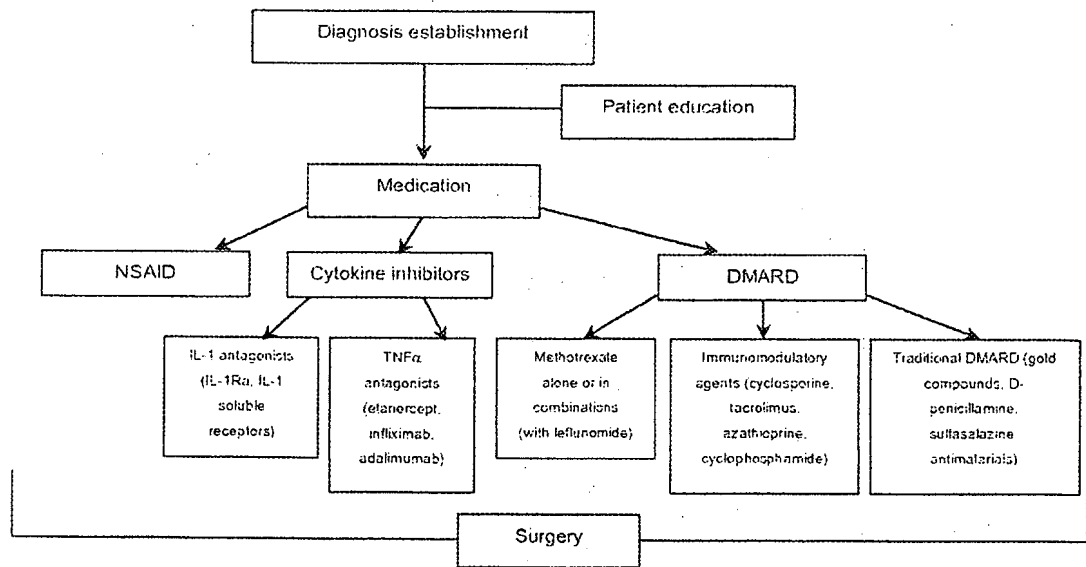


Fig. 4. Current management of RA.

been to reduce joint inflammation and pain, to maximise joint function, to prevent destruction and deformity and to reduce long-term patient disability. Early medical intervention has been shown to significantly improve the outcomes of the disease. Optimal treatment customised according to the gravity of the disease, type of joint involved, age, general health of the person, consists in a combination of medication, rest, joint-strengthening physiotherapy and patient education. The medical protocol [54] includes analgesics, NSAIDs, corticosteroids, conventional disease-modifying anti-rheumatic drugs (DMARDs) and biological agents such as TNF- α blockers or IL-1 receptor antagonist (IL-1Ra), as depicted in Fig. 4. Due to the progression of the disease, first-line medication, such as analgesics and NSAIDs, becomes rapidly ineffective, and i.a. administration of steroid depot formulations, which are able to relief pain and inflammation over a period of a few months, is required. Another stage in RA management is the introduction of DMARDs, the most current being salazopyrine and chloroquine, but gold compounds, penicillamine and hydroxychloroquine are also used. A number of immunosuppressive drugs are also employed to treat RA, for example, methotrexate, azathioprine, cyclophosphamide and cyclosporin. Due to their potential deleterious side effects, they are reserved for patients with very aggressive disease or with serious complications. Methotrexate is an exception, as its use is not associated with very notable side effects. For this reason, it has become the preferred second-line medication used alone or in combination therapy. Newer second-line drugs include leflunomide [55], which selectively inhibits de novo pyrimidine synthesis and thus, reduces the proliferation of activated CD4 T cells, which play a key role in RA pathogenesis. The immunosuppressive agent tacrolimus interferes with T cell proliferation via calcineurin inhibition. Furthermore, tacrolimus is currently in clinical trials [56–58]. Efforts to develop safer and more effective treatments for RA are based on an improved understanding of the role of inflammatory mediators such as TNF- α and IL-1 [59]. Treatments with etanercept – a soluble TNF- α type II receptor-IgG1 fusion protein – and with infliximab or adalimumab – monoclonal antibodies against TNF- α – have been approved by the Food and Drug Administration and the European Medicine Evaluation Agency for treatment of RA. Anakinra, a human IL-1 receptor antagonist, is another biological drug used to treat moderate to severe RA: it is generally used in combination with methotrexate. To-

gether with targeting TNF- α or IL-1, B cell targeting represents a novel approach. In this respect, rituximab, a chimeric anti-CD20 monoclonal antibody targeting B cells, has demonstrated efficacy in numerous clinical studies of arthritic patients [60]. Considering all of these aspects, i.a. administration of drug delivery systems that encapsulate these potent substances, which permits their controlled release over a long time period at doses that are significantly inferior to the systemic dose, might be a major breakthrough in limiting their side effects.

4. Intra-articular drug delivery systems

4.1. Intra-articular injection technique

Local administration is attractive for the delivery of high drug concentrations at the main site of inflammation and for minimising the side effects related to systemic administration. Aspirating and injecting the knee or other joints is a common technique for both diagnostic and therapeutic purposes, in spite of practical difficulties such as the lack of accessibility of the joint and thus, hindered needle placement [61–64]. The complications of i.a. injections like infection, post-injection flare, crystal-induced synovitis, cutaneous atrophy and steroid arthropathy [65], although rare, could result in dramatic side effects. The incidence of septic joints related to local steroid injection is about 1 in 10 000 injections, while for post-injection flare, a frequency of around 2% was recorded. Due to the crystalline nature of the steroids, crystal presence in the joint might produce transient synovitis in about 10% of the patients; this condition generally disappears after a few days [12–14]. Depending on the joint type and the injected substance, aspiration of the synovial fluid before injection might be necessary [14]. Moreover, post-injection rest is required in order to increase the residence time of the administered substance, and, hence, the clinical response [66]. Besides these rather serious side effects, the i.a. route of administration is also accompanied by some technical drawbacks related to the cost and time involved in the procedure, patient compliance and, most importantly, the rapid efflux of drugs from the joint cavity after instillation. This last shortcoming could be addressed by using drug delivery systems which ensure sustained release of the active substance in the joint, while minimising the systemic side effects.

4.2. Drug delivery system intra-articular residence time

4.2.1. Synovial membrane physiology

The synovium, which is the tissue between the fibrous joint capsule and the synovial cavity, is divided into three compartments with distinct functions, notably, the lining region; the sub-intimal stroma, which consists of connective tissue, lymphatic vessels and fat cells; and finally the vascularised region, with numerous capillaries. The synovial membrane, which is also referred to as the synovial lining, is an irregular membrane that is 3–4 cell-depth thin and is exempt of intercellular junctions. In normal conditions, it serves two functions, notably to provide nutrients to the cartilage cells and to secrete the synovial fluid, which lubricates the joint. The synovial membrane consists mainly of two types of cells, type A synoviocytes, which are similar to macrophages, and type B synoviocytes, which are similar to fibroblasts [67–71]. It has been demonstrated by Senda et al. that both types of synoviocytes possess the ability to phagocytose 240-nm latex particles [72]. Molecules entering or leaving the joint space must traverse both the capillary endothelium and the interstitial space between the synoviocytes [73]. The capillary endothelium presents fenestrations that face the joint cavity, thus favouring the influx of substances to the joint cavity. Regarding proteins, their synovial permeability is principally limited by the endothelium, while the permeability of small molecules appears to be limited by diffusion across the interstitial space [74]. Nevertheless, transport is a highly selective process, with small proteins such as albumin, entering more easily. In contrast, the clearance of all proteins is achieved by lymphatic drainage, while hyaluronan and proteoglycans have restricted access to the lymphatic system. When a joint becomes inflamed, the total protein concentration increases to values close to those of plasma, and the synovial fluid volume augments due to an increased permeability, which overwhelms the drainage capacity of the lymphatic system [73].

4.2.2. Retention times of drug delivery systems vs. solutions or suspensions

In terms of absorption and distribution into the systemic circulation, the i-a. route is equivalent to other non-i.v. parenteral routes of administration [75]. For this reason, drugs that are i-a. administered as solutions or suspensions have half-lives that can vary between 1 and 2 h for cortisone, naproxen or ketoprofen and up to 22–26 h for hyaluronan [76]. To increase the drug residence time in the synovial cavity, drug delivery systems may be used. Among them, thermally responsive elastin-like polypeptide gels that can spontaneously aggregate after i-a. injection represent a simple and innovative way to prolong the i-a. half-life of a drug. These aggregating elastin-like polypeptides form a drug-depot resulting in a 25-fold longer half-life than drugs administered with the non-aggregating protein. Research is ongoing to chemically couple the elastin-like polypeptide to proteins, such as the IL-1 receptor antagonist, in order to achieve higher residence times [77]. Besides the thermogelling approach, which is used to increase the retention time of a drug formulation in the joint, pH-sensitive gels, such as the holmium-166-chitosan complex, are interesting tools. I-a. injection of this type of composite material results in formation of a gel with minimal extra-articular leakage of the radioactive isotope. The safety of this complex as a radiation synovectomy agent was demonstrated in a Phase I/IIa study of 16 patients [78].

Liposomes, whose potential to increase the i-a. residence time of drugs was first suggested in 1976 by Shaw et al. [79], represent drug delivery systems with valuable clinical utility for i-a. drug delivery. Bonanomi et al. reported that the encapsulation of dexamethasone palmitate in liposomes resulted in an improved retention compared to microcrystalline triamcinolone acetate [80].

When encapsulating methotrexate in 1.2 μm liposomes, Williams et al. reported a 26.5% reduction in joint swelling of arthritic rats, compared to 3.5% for the free drug at 1 day after the injection. Moreover, the effect was still notable 20 days after the injection of liposomes (Fig. 5) [81]. The pharmacokinetics of liposomal lidocaine, a local anaesthetic that is frequently injected into joints for pain relief, was reported by Hou and co-workers after i-a. administration in healthy rabbit knees. They detected significantly different serum concentrations in rabbits treated with aqueous solution compared to liposomal lidocaine [82]. A highly versatile clinical option for the management of inflamed joints is photodynamic therapy with Verteporfin[®]. Generally used in oncology, this approach, whose *in vivo* efficacy depends mainly on photosensitizer uptake and accessibility in the target tissue, can also be used in RA. The kinetics and the drug clearance rate after i-v and i-a. administration of liposomal Verteporfin were studied, and interestingly, the drug was not detectable in the circulation after i-a. administration. This result supports the conclusion that photodynamic therapy offers a less invasive and safer alternative than surgical, chemical or radiation synovectomy [83]. The potential of liposomes was also explored in the field of radiographic markers. Edwards et al. studied the i-a. retention of liposomal iohexol compared to the free drug in sheep. In this case, the liposomes resulted in a significantly prolonged drug half-life, i.e., 134 h compared to only 3 h for the free iohexol [84].

Together with liposomes, microspheres injected i-a. represent one of the most studied means to decrease i-a. drug clearance. In this respect, Lu et al. reported that the mean residence time of flurbiprofen is doubled compared to injection of the drug suspension [85]. Similar observations were registered for a celecoxib solution compared to celecoxib-embedding chitosan microspheres, for which a 10-fold increase in the concentration of celecoxib in the joint was achieved after i-a. injection [86]. Methotrexate is a DMARD which largely benefits from local administration in the joint in the form of polymeric microparticles. Liang et al. formulated methotrexate-embedded poly(lactic acid) microparticles and tested their pharmacokinetics after i-a. injection in healthy rabbits [87,88]. As expected, the concentration of methotrexate in the synovial tissues following i-a. injection was significantly higher in the group treated with microparticles compared to the one treated with the drug solution [88].

Due to the limited number of reported clinical studies, most of them with liposomes, the i-a. drug delivery system approach remains experimental. In order to reach the clinical stage, more research is needed to demonstrate the *in vivo* benefits of such systems.

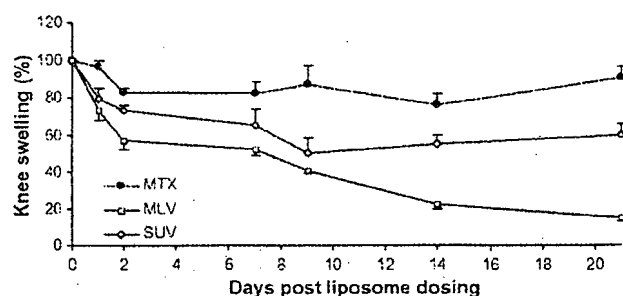


Fig. 5. The anti-inflammatory effect of liposome-conjugated methotrexate (methotrexate-small unilamellar vesicles, MTX-SUV, and methotrexate-multilamellar vesicles, MTX-MLV) as assessed by a reduction in knee swelling in rats with antigen-induced arthritis. One day after treatment, knee swelling (mean $\pm$ SEM) in MTX-MLV-treated rats was significantly less than in MTX-SUV-treated rats and than in rats treated with methotrexate solution (MTX) ($P < 0.04$). This difference remained significant at all time points up to day 21, demonstrating that MLV are well suited to treat the symptoms of arthritis. Adapted from Williams et al. [81].

4.2.3. Modulation of i-a. retention times

Obtaining high retention times in the joint, which is essential for the therapeutic success of i-a. drug delivery systems, can be achieved by both chemical and physical methods. The chemical methods deal with the functionalisation of the surface of liposomes, nano- or microparticles in order to achieve high binding to joint-specific targets, thus ensuring the persistence of these systems at the inflammatory site. When attempting to achieve selective cartilage binding using poly(propylene sulphide) nanoparticles functionalised with a special collagen II-binding peptide ligand, WYRGRL, Rothenfluh et al. [89] demonstrated that the peptide-decorated nanoparticles concentrated in the articular cartilage up to 72-fold more than nanoparticles displaying scrambled peptide sequence following i-a. injection in mice. Their innovative approach provides a means to target the articular cartilage, which is otherwise poorly accessible avascular tissue, in diseases such as OA. Another promising strategy that might be effective for the delivery of drugs to the inflamed synovium could be targeting of $\alpha v \beta 3$ integrin, which is up-regulated in neoangiogenic vessels. Currently, the efficacy of such an approach has been demonstrated in adjuvant-induced arthritis in rats after systemic administration of dexamethasone-containing liposomes with a surface-conjugated RGD peptide [90]. This approach, combined with i-a. administration, may achieve valuable improvement in the retention time. Alternative approaches that might also benefit from an i-a. administration in terms of limitation of non-specific systemic binding, are targeting of the folic acid receptor FR β , which is up-regulated on activated synovial macrophages, and targeting of E-selectin, which is an adhesion molecule that is up-regulated on the vascular endothelium of inflamed tissue [91].

Among the physical methods, one can consider the use of bioadhesive materials. An example of such material is collagomers, which are novel drug-carrier systems based on collagen-lipids conjugates [92]. However, studies are needed to demonstrate synovial tissue retention with this system. The use of a magnetic field also represents a recent approach to improve the accumulation of magnetic drug delivery systems at the targeted site. In this respect, Tanaka et al. used a 0.2 T permanent magnet implanted into the femur to increase the i-a. retention time of magnetic liposomes containing TGF β . To this end, a hole with a diameter of 4 mm and a depth of 8 mm was created into the bone. It was demonstrated that the presence of a magnet leads to a more efficient retention of the liposomes in the joint and to a significant diminution of cartilage defects at 12 weeks [93]. Butoescu et al. incorporated superparamagnetic iron oxide nanoparticles (SPIONs) in

PLGA microparticles loaded with dexamethasone and achieved a joint residence time of at least 3 months (Fig. 6) [94–96]. Regarding the possible toxicity of the SPIONs, Schulze et al. demonstrated that 30–40-nm PVA-coated SPIONs were biocompatible with articular and periarticular tissues in sheep [97]. Moreover, considering the magnetic properties of the SPIONs, they also investigated the effect of an extracorporeal magnet on nanoparticle persistence in the joint and have shown an increased local concentration in the presence of a magnet. Other studies on magnetic nanoparticles were conducted by Hellstern et al. [98], who employed light, fluorescence and confocal microscopy to assess the biocompatibility of the system with joint tissues, and the biodistribution of the drug delivery system in different organs. The authors established that the nanoparticles are taken up by the reticuloendothelial system, and that the main organs in which the nanoparticles are detected after i-a. administration are the liver and the spleen. Magnetic drug delivery systems need further investigation before the initiation of clinical trials to confirm the delivery of sufficient payloads to the joint, long-term retention and efficacy in arthritis or OA animal models. Magnetic field strengths used in these studies could be reproduced in patients wearing external magnets, but this issue should be further addressed.

4.3. Size

Unlike the systemic administration of drug delivery systems, i-a. injection in active RA or OA involves introducing the carrier into a pathological environment of excess protein and enzymes, many of which may interact with the drug carrier [99]. For this reason, the size, shape and type of carrier of the drug delivery system are essential and must be thoroughly studied to ensure that the carriers do not induce further inflammation or an immune response. A detailed review of these key aspects is presented hereafter. Concerning the size of the i-a. administered particles, the discussion of the work done in the field will focus on liposomes and on nano- and microparticles, emphasising aspects such as joint retention and phagocytosis as a function of size.

4.3.1. Liposomes

Regarding this type of vesicular carrier, research has focused mainly on increasing liposome retention as a function of particle size (surface area) and biocompatibility. Bonanomi et al. reported that increasing the size of dexamethasone palmitate encapsulating liposomes, ranging from 160 nm to 750 nm in diameter, resulted in a 2.6-fold increase in retention at 48 h post-injection [80,100]. A similar observation was described for liposomes containing methotrexate, for which a mean diameter of 1.2 μ m ensured a higher retention, and thus anti-inflammatory action, than 100 nm liposomes [81]. Together with traditional liposomes, niosomes, which are non-ionic surfactant-based liposomes, were also investigated for the local delivery of diclofenac sodium to treat arthritis [101]. Moreover, in order to further prolong the release of the active substance together with better joint retention, both liposome- and niosome-containing diclofenac were embedded in carbopol or sodium carboxymethyl cellulose gels, leading to novel drug delivery systems, lipogelosomes and niogelosomes, respectively. Radiolabelled diclofenac-containing lipogelosomes injected in arthritic rabbit joints presented the longest retention times compared with the other formulations. Moreover, the radioisotope was slowly released, 67% of the initially injected radioactivity being still present 24 h post-injection [101]. Liposomes have been shown to be very efficient in the local treatment of joint diseases in laboratory animals and also in humans. For example, when administered in human patients, hydrocortisone-entrapping liposomes remarkably improved subjective and objective indices of inflammation at 48 h, with a slow return to the pre-injected state

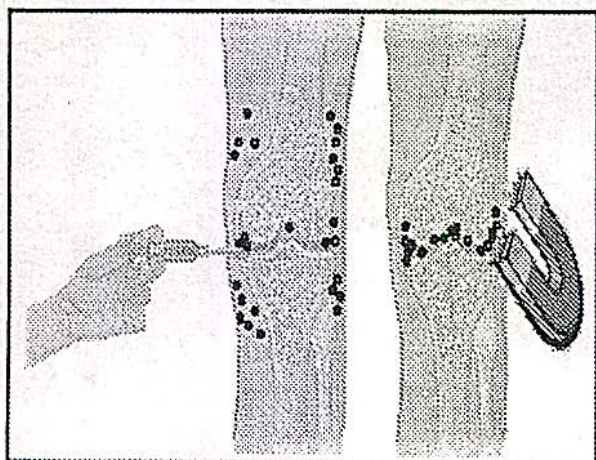


Fig. 6. An external magnet increases microparticle retention in the joint.

after 2 weeks [102]. In another clinical study, a single i-a. injection of clodronate-containing liposomes significantly reduced the expression of adhesion molecules, which is correlated with the extent of inflammation in the synovial lining (Fig. 7) [103]. These encouraging results in humans have stimulated further research of liposomes as a therapeutic method to locally treat inflammation and cartilage defects.

4.3.2. Nanoparticles

This type of carrier has mainly been studied in an attempt to target the articular cartilage in diseases such as OA. For this purpose, nanoparticles generally contain a specific cartilage-binding moiety. In addition, to achieve efficient carrier penetration into the cartilage, the carrier size may play an essential role. This aspect was demonstrated by Rothenfluh et al. [89] for nanoparticles coated with collagen II-binding peptide. The authors demonstrated by fluorescence measurement a 14.9-fold preferential accumulation of 38-nm mean diameter nanoparticles within the cartilage relative to 96-nm diameter nanoparticles. This significant difference was attributed to the 60-nm pore size of the dense collagen network.

4.3.3. Microparticles

Numerous reports focus on the influence of microparticle size on the phagocytosis of these systems once they are injected in the joint. It has been demonstrated that small particles are easily phagocytosed, with a reported limit ranging from the nanometre scale up to a few micrometers. Howie et al. [104] studied the response of the rat synovial tissue to the presence of polyethylene wear particles, similar to those commonly released from the articulating surfaces in joint prostheses. They reported that particles of about 5–15 μm , larger than the size of macrophages, tend to produce a multinuclear giant cell response, while particles smaller than 5 μm trigger a mononuclear macrophage response. Horisawa et al. [105] used fluorescently labelled PLGA nanoparticles (mean diameter 265 nm) and microparticles (mean diameter 26 μm) to evaluate synovial phagocytosis. As expected, nanoparticles were extensively phagocytosed and subsequently transported through the synovial membrane within 3–7 days. In contrast, microparticles of 26 μm in diameter were neither phagocytosed nor transported to the underlying synovial membrane, but they triggered a granulation reaction with multinuclear giant cells. Similar observations were registered by Nishide et al. who investigated the biodegradation and tissue response of PLLA microspheres with size fractions of 0–20, 20–100 and 100–200 μm i-a. injected into healthy rabbit joints [106]. The authors concluded that microparticles with diameters larger than 20 μm were not internalised into

macrophages, nor did they produce important inflammatory responses. In contrast, Liggins and co-workers demonstrated that PLGA microspheres in the size range of 1–20 μm produced significantly greater inflammatory responses than 35–105 μm particles, possibly explained by the large fraction of very small microparticles in the 1–20 μm batch. Interestingly, the phagocytosis of microspheres by synovial macrophages improved the retention of microparticles in the joint [107,108] and also ensured an increased drug concentration in inflammatory cells and delayed clearance from the joint, which minimised drug exposure to cartilage, thus reducing the side effects. Regarding drug concentration in the serum after i-a. administration of drug-encapsulating microparticles, Ramesh et al. pointed out that 400 min after the injection in the articular of dexamethasone encapsulated in PLA microparticles, no drug was detected in the serum of healthy rabbits, thus concluding that dexamethasone release is localised in the joint [109]. In contrast, a small quantity of methotrexate was detected in rabbit plasma 5 min post-injection of PLA microparticles, mostly due to a burst release of the drug from the particles [88]. Moreover, the microparticles, whose size is in the range 30–100 μm , did not induce acute inflammatory reactions.

Considering the diameters of all types of microparticles tested for i-a. delivery of drugs depicted in Table 1, it can be concluded that the most suitable size is between 5 and 10 μm . This particle size range ensures capture of the particles by the synovial macrophages, a process that results in a prolonged retention time in the joint. In addition, to limit the inflammatory reactions, the choice of adequate polymers is essential.

4.4. Quantity of i-a. injected drug delivery systems

Apart from size, the quantity of injected microparticles is also important to the appearance of an immune response, as shown in the studies conducted by Nishide et al. They demonstrated an enhanced number of white blood cells with increasing amounts of injected microparticles, from 5 to 40 mg per knee [106]. In contrast, no significant dose-dependent inflammatory effect was observed for paclitaxel-embedding PLGA microparticles after i-a. administration of 15 to 75 mg of particles per joint in healthy rabbit knees [110].

4.5. Shape

Not only the size, but also the shape of the particles injected into the joint is important for triggering an immune response. Irregularly shaped microparticles have been demonstrated to promote tissue inflammation in comparison with round-shaped drug

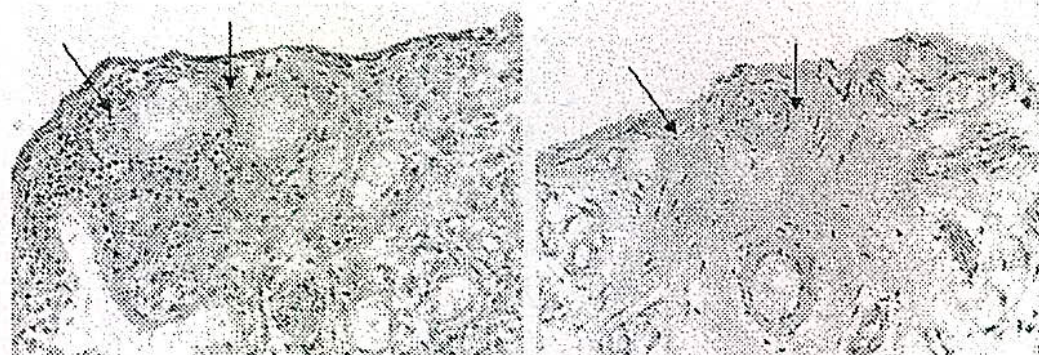


Fig. 7. Histological image of the synovial lining of a RA patient before and 7 days after clodronate liposome administration. Before the injection (left panel), numerous macrophage-like fibroblasts were present (indicated by arrows), while after the injection, normal features of the fibroblasts were recorded (right panel). (HE stain, original magnification 250 $\times$). Adapted from Barrera et al. [103].

Table 1
Drug delivery systems developed up to date for the i-a. administration.

Type	Targeted drug	Nature of the matrix	Diameter of particles	In vivo tests	Refs.
Gels	No drug	Elastin-like polypeptide, thermosensitive gel		I-a. injection in a rat knee	[77]
Liposomes	Clodronate	Egg phosphatidyl choline/cholesterol		Antigen-induced arthritis (AIA) model in sheep	[130]
		Phosphatidyl choline/cholesterol		Collagen-induced arthritis (CIA) in mice	[131]
		Egg phosphatidyl choline/cholesterol		I-a. injection in patients with RA	[103]
	Cortisol palmitate	Phosphatidic acid necessary for the full expression of anti-inflammatory activity		Bilateral inflammation induced by the i-a. injection of poly-D-lysine and hyaluronic acid	[79,132,133]
		Phosphatidic acid and dipalmitoyl phosphatidyl choline		AIA in rabbits	[114]
		Mixture of phospholipids		I-a. in human subjects with rheumatoid arthritis	[102]
	⁵¹ Cr	Lipid phase	100 nm	AIA in rabbits	[134]
	Daunorubicin	Not mentioned		I-a. injection in arthritic rabbits	[127]
	Dexamethasone palmitate	Egg phosphatidyl choline/phosphatidic acid	160–750 nm	I-a. injection in healthy and AIA rabbits	[80]
		Different phospholipid mixtures	100 nm to 30 µm	I-a. injection in healthy rabbits	[100]
	Diclofenac sodium	Different phospholipids	200–250 nm	AIA model in rabbits	[101]
	99mTc-labelled Eicosatetraenoic acid	Not mentioned		I-a. injection in acute synovitis joints	[127]
	Iohexol	Dipalmitoyl phosphatidyl choline/cholesterol/stearylamine		I-a. injection in healthy sheep	[84]
	Lactoferrin	Not mentioned		AIA in mouse	[135]
	Lidocaine	L-α-phosphatidyl choline/cholesterol		I-a. injection in healthy rabbits	[82]
Nanoparticles	Methotrexate	Different phospholipids	1 µm	AIA model in rabbits	[136,137]
		Different phospholipids	1.2 µm	AIA in rats	[81]
	Transforming growth factor-β1 (TGF-β1)	Egg yolk phosphatidyl choline/cholesterol/dipalmitoyl phosphatidic acid		Full-thickness cartilage defects in rabbits	[138]
		Egg yolk phosphatidyl choline/cholesterol	Magnetic liposomes	Full-thickness cartilage defects in rabbits	[93]
				Partial thickness defects in adult miniature pigs	[139]
	Triamcinolone acetonide 21-palmitate	Dipalmitoyl phosphatidyl choline/cholesterol		AIA model in rabbits	[140]
	Verteporfin [®]	Not known		AIA model in rabbits	[83]
	Betamethasone sodium phosphate	PLGA	370 nm	In rats: <i>in vivo</i> release study: dorsal air pouch model. In rabbits: efficacy on AIA model	[121]
	Flouresceinamine	PLGA	110–670 nm (mean 265 nm)	I-a. injection into rat joints	[105]
	No drug	Poly(propylene sulphide) functionalized with a collagen-binding ligand, WYRGRL	38 nm	I-a. administration in mice	[89]
Microparticles		SPIONs	30–40 nm	I-a. injection in sheep	[97,98,141]
	Celecoxib	Chitosan	8 µm	I-a. injection in rats	[86]
		Albumin	5.4 µm	AIA in rats	[142]
	Chondroitin sulphate	Gelatin	10 µm	I-a. injection in mice	[120]
	Basic Fibroblast Growth Factor (bFGF)	Gelatin hydrogel	70 µm	I-a. injection in joints of normal rabbits	[143]
	Dexamethasone	PDLLA	40–60 µm, 90–110 µm	I-a. injection in healthy rabbits	[109]
	Diclofenac sodium	PLGA	5–10 µm	AIA model in rabbits	[118]
	Doxycycline	PLGA	15–120 µm (mean 35 µm)	Not tested on animals	[144]
	Flurbiprofen	Gelatin	7.5 µm	I-a. injection in healthy rabbit joints	[85]
	Flouresceinamine	PLGA	3.1–60 µm (mean 26.5 µm)	I-a. injection into rat joints	[105]
	Insulin	PLGA		I-a. injection in healthy mice	[117]
	Methotrexate	PLLA	62–83 µm	I-a. injection in healthy rabbit joints	[87]
		PLLA	77 µm	I-a. injection in healthy rabbit joints	[88]
	Naproxen sodium	PLGA or BSA	5–10 µm	AIA model in rabbits	[116]
		PLGA	50 µm	Extracorporeal <i>in vivo</i> isolated horse metacarpophalangeal joint preparation	[145]
	Paclitaxel	PLGA, PLA, PCL, Chitosan	1–20 µm, 35–105 µm	AIA model in rabbits	[110]
		PLGA	50 µm	Extracorporeal <i>in vivo</i> isolated horse metacarpophalangeal joint preparation	[145]
	Prednisolone	Heat-stabilized albumin	23 µm	I-a. injection in rabbits	[146]
	Rose Bengal – model compound	Rabbit serum albumin	3.5 µm	AIA model in rabbits	[108]
	TGFβ	Calcium alginate		I-a. injection in rabbits with a full-thickness cartilage defect	[119]

Table 1 (continued)

Type	Targeted drug	Nature of the matrix	Diameter of particles	<i>In vivo</i> tests	Refs.
	Triamcinolone	Heat-stabilized albumin	23 µm	I-a. injection in rabbits	[146]
	¹⁶⁶ Ho	PLA	2–13 µm	I-a. injection in healthy rabbits	[129]
		Ferric hydroxide macroaggregates	1–5 µm		[147]
	¹⁸⁸ Re-labelled microparticles	Hydroxyapatite	2–20 µm	I-a. injection in rats	[148]
	¹⁶⁵ Dy	Ferric hydroxide macroaggregates	1–5 µm		[149]
	¹⁵³ Gd				[128]
	⁹⁰ Y				[150]
	⁵⁹ Fe				[129]
	¹⁴¹ Ce-labelled microparticles	Carbonized microparticles	10 and 14 µm	AIA model in rabbits	[128]
	^{99m} Tc-labelled microparticles	Denatured HSA	25, 10, 1–2 and 0.1 µm		
Miscellaneous	No drug	PLA, PBCA, Gelatin, Albumin	2–7.5 µm	I-a. injection in healthy rabbits	[107]
		PDLA	10, 100 or 200 µm	I-a injection in healthy rabbit joints	[106]
		Polyethylene Particles resulting from prostheses	1–200 µm	I-a. injection in healthy rats	[104]
	¹⁶⁶ Ho complex with chitosan	Chitosan		Phase I/IIa clinical study in humans	[78]
		Chitosan		I-a. injection in patients	[151]
	Transferosomes for epicutaneous ketoprofen	Lipids		Cutaneous application in humans with OA	[152,153]
	Diclofenac	Collagomers		Tribromethanol-induced OA model in rats	[92]

delivery systems. In this respect, Liggins et al. showed that irregular, milled chitosan particles induced joint inflammation despite the known articular biocompatibility of this biomaterial [110]. Similar histological observations showing marked inflammation in the synovial membrane and the subsynovial lining were made by Ratcliffe et al. for PLA and poly(butyl cyanoacrylate) microparticles obtained by simple polymer grinding [107]. Thus, to avoid inflammatory reactions subsequent to the administration of irregularly shaped particles, round-shaped particles are to be preferred for i-a. drug delivery.

4.6. Matrix materials

Regarding liposomes, research demonstrated that egg phospholipid liposomes were relatively fragile and prone to leakage. Dipalmitoyl phosphatidyl choline (DPPC) was chosen as the most important liposome phospholipid due to its gel–liquid crystalline phase transition temperature of 41 °C, which makes it more rigid, and thus stable, at the body temperature. From the point of view of biocompatibility, DPPC is very suitable for i-a. use as it is an endogenous component of the joints [111]. More precisely, it represents approximately 45% of the total synovial fluid lipid component. Cholesterol is often included in the composition of the liposomes for its capacity to improve *in vivo* stability and to prevent leakage of drug molecules through the liposomes bilayer [112]. Stearylamine represents another possible constituent of liposomes and is used to enhance repulsion between shell layers, and also to improve interaction with macrophages, due to its positive charge [113]. Moreover, changes in matrix composition can influence the therapeutic action of the formulation, as demonstrated for cortisol-containing liposomes whose anti-inflammatory activity decreases in the absence of phosphatidic acid [114]. In the formulation of nanoparticles, an innovative polymer matrix, poly(propylene sulphide), was used to obtain specially designed systems for targeting of the articular cartilage [89,115]. By varying the ratio between the polymer and the emulsifier, Pluronic 127, the authors achieved control of the size, ranging from 20 to 200 nm, and they obtained special nanoparticle core architecture, with a hydrophobic nucleus resulting from the disulphide cross-linking of poly(propylene sulphide), with hydrophilic domains consisting of polyethylene glycol moieties of the Pluronic and with the surface covered by peptide ligands to target the cartilage. Finally for

microparticle matrices, several materials have been studied, notably poly(lactide-co-glycolide) (PLGA) [116–118], poly(L-lactic acid) (PLA) [109], gelatin [85], albumin [108], poly(ε-caprolactone), chitosan [110], poly(butyl cyanoacrylate) [107], calcium alginate [119], hydroxyapatite and glucosamine–chondroitin [120], as displayed in Table 1. Among these materials, the release and degradation characteristics of albumin, gelatin, chitosan or alginate can be modulated by cross-linking methods [116]. Note that cross-linked albumin microspheres have been shown to be well tolerated by the synovial tissue [108,116]. Homopolymers and copolymers of lactic and glycolic acids are macromolecules degraded by simple hydrolysis of the ester bonds to produce non-toxic by-products, which can be easily cleared out from the joint. Due to its known biodegradability and to the fact that it is largely employed for numerous medical applications in animals and in humans, PLGA is the most frequently used polymer as particle matrix.

Regarding the encapsulation efficacy and drug loading, these two parameters varied in function of the active substance and the matrix material. Generally, literature reports very high encapsulation efficacy (70–80%) for i-a. drug delivery systems, corresponding to drug loadings of 1% [121], 5% [109,117,121], 10% [122] and up to 47% for hydrocortisone in PLGA microparticles [123]. As for the *in vitro* release, generally assessed in PBS, it varied from a few hours for BSA drug delivery systems [124] to around 100 h for chitosan microparticles [125]. In both cases, the release rate could be tailored by changing the cross-linker concentrations. For PLA- or PLGA-based systems, the release times were longer, up to 14 days in some reports [109,122]. Some authors also investigated the *in vivo* release in dorsal air pouch models in rodents [94,121] and demonstrated release times of 6–7 days.

4.7. Therapeutic agent

Due to an acute need for efficient therapeutic systems providing long-term pain relief, non-steroid and steroid anti-inflammatory drugs were, by far, the most tested drugs for encapsulation in either liposomes or nano- and microparticles, and their efficacy has been studied mostly on rabbit, sheep or rat arthritis models (Table 1). Another therapeutic class of active substances thoroughly studied as candidates for encapsulation is radioisotopes. In fact, the synovial lining of the arthritic joint becomes hyperplastic and may thicken to 10–12 cells in depth. To remove the

inflamed synovium lining, three methods are currently available, notably surgery (surgical synovectomy), chemical substances (chemical synovectomy), which are noxious agents used to kill the synoviocytes, and radionuclides (radiation synovectomy). Although very efficient in certain cases by providing pain relief up to 2–5 years [126], surgical synovectomy is limited by its technical complexity, and also by the fact that subsequent regrowth can occur. Chemical ablation uses noxious substances, usually chemotherapeutics, which kill the synovial lining. Encouraging results with chemical synovectomy were obtained with liposome-encapsulated daunorubicin [127], a potent anti-neoplastic agent, i.a. injected in arthritic rabbits. Compared to free daunorubicin, the liposomes elicited a reduction in temperature and diameter of the treated animal joint over a period of 24 days, demonstrating that a very small dose (5–10 µg) of substance can produce important beneficial effects. The third method is the ablation of inflamed synovium by intra-articular injection of beta-emitting radionuclide in colloidal or particulate form. The common drawback of the chemical and radiation synovectomy is the necessity to restrict the agent to the joint cavity, in order to limit the systemic side effects. This problem was solved by the encapsulation of the therapeutic agent into particulate systems, most generally liposomes or microparticles. Concerning radionuclides, a large panel of isotopes were embedded in either gels (¹⁶⁶Ho), liposomes (⁵¹Cr) or microparticles (¹⁸⁸Re, ^{99m}Tc, ¹⁴¹Ce, ¹⁶⁶Ho, etc.). Generally, an ideal radiopharmaceutical for radiation synovectomy should have appropriate beta-emission energy for effective tissular absorption and removal of inflamed synovium. In addition, the half-life needs to be short enough to reduce the risk of prolonged radiation *in vivo* but long enough to ensure adequate manufacturing and transport. Moreover, the radionuclide should bind irreversibly to the carrier particles, preferably having a diameter sufficiently small to allow effective phagocytosis by synoviocytes, but large enough to limit extra-articular leakage [78]. The carrier biodegradability is not compulsory, as long as the carriers do not induce inflammatory reactions into the joint. For example, Noble et al. used carbonised microparticles containing ¹⁴¹Ce and demonstrated that reducing the biodegradability of the particle or increasing the diameter results in the reduction of radioactivity loss from the knee joint [128], but a clinical use of such a system is hardly conceivable. Satisfactory rates of i.a. radioactivity retention were also achieved by using biodegradable materials such as hydroxyapatite [128] or PLA [129], with values superior to 98% after 48 h and 120 h, respectively. In contrast, larger radioactivity leakage was observed by Mumper et al. [129] for ferric hydroxide macroaggregates containing ⁵⁹Fe. The authors concluded that the 18.5% radioactivity loss after 5 days was due to instability of the ferric hydroxide macroaggregates in the presence of ⁵⁹Fe.

4.8. Drugs that might benefit from delivery system

Despite the significant progress done in the field of i.a. delivery, at present there is an acute need for specific drug delivery systems capable of reducing side effects and systemic toxicity either for classical anti-inflammatory drugs or for novel and very potent anti-inflammatory substances, which cannot be registered for the moment due to high toxicity (Table 2). For most RA or OA drugs, future development can only be achieved in the framework of adequate pharmaco-technological or biological development, to confine their action at the targeted site and hence to minimise the undesired effects. Such is the case of drugs targeting p38 mitogen-activated protein kinase (p38 MAPK), an enzyme that regulates cytokine levels and that plays an important role in the activation responses associated with inflammatory diseases [154–156]. An increasing number of potent and specific p38 MAPK inhibitors synthesised by numerous pharmaceutical companies are

Table 2
p38 MAPK inhibitors in clinical trials.

Compound	Company	Clinical status
AMG-548	Amgen	Phase I discontinued
AVE-9940	Sanofi-Aventis	Phase I discontinued
BIRB 796	Boehringer Ingelheim	Phase II
GSK-681323	GlaxoSmithKline	Phase I (2002)
PH-797804		Phase II (2007)
PS-540446	Bristol Meyers	Phase I (2005)
PS-516895	Squibb	Phase I (2004)
RO-4402257	Roche	Phase II (2006)
RO-3201195		Phase I
RWJ67657	Johnson & Johnson	Phase I discontinued
SB-203580	SmithKline	Discontinued
SB-281832	Beecham	Discontinued
SB-273005		Discontinued
SB-242235		Discontinued
SC80036	Pfizer	Discontinued
SCIO-323	Scios	Phase I
SCIO-469		Phase II (2005)
TAK-715	Takeda	Phase II (2005)
VX-702	Aventis/Vertex	Phase II (for acute coronary syndrome)
VX-745		Discontinued
VX-740 (Pralnacazan)		Phase II discontinued
VX-765 (prodrug of Pralnacazan)		Phase II discontinued

currently in clinical trials, generally for oral or parenteral administration in the case of RA. Early findings with compounds such as SB-203580 [157], SB-220025 or SB-242235 [158] prove that these substances are very potent *in vitro* inhibitors of p38 MAPK and that they produce significant anti-inflammatory response in different rodent models. Nevertheless, their development was stopped due to the lack of specificity or to the toxicity associated to the inhibition of liver p450 cytochrome [159,160]. A new p38 inhibitor, GSK-681323, is currently in early stages of clinical development [160], while SCIO-469, one of the most advanced compounds in clinical trials, is at the moment in Phase II [160]. VX-745 from Vertex is a promising compound, whose clinical evaluation was arrested in 2001 due to central nervous system toxicity associated with its high ability of crossing the brain–blood barrier [3,161]. A second-generation molecule developed by Vertex, VX-702, is currently under Phase II clinical trials [3,5,162,163]. Another class of anti-inflammatory compounds is represented by the IL-1β converting enzyme inhibitors, among which pralnacazan (VX-740) and an orally available prodrug (VX-765) are the most known and studied. Although in experimental models of OA and RA both pralnacazan and VX-765 reduced joint damage and RA progression [164–166], in clinical trials the use of pralnacazan was associated with liver abnormalities that led to the discontinuation of the trials.

Although very promising, an increasing number of novel and very active compounds fail to enter or are discontinued in the course of clinical trials due to adverse reactions. Nevertheless, these compounds may represent a major breakthrough if they were developed under an adapted drug delivery system, allowing on the one hand the administration of very small drug quantities and on the other hand the localisation of the compound at the targeted site, hence, avoiding side effects related to high drug concentrations or to systemic localisation.

5. Conclusions

In conclusion, i.a. drug delivery systems for OA or RA represent a valuable means to administer drugs directly in the joint cavity, while circumventing systemic toxicity and reducing leakage or exposure of other tissues and organs. Due to its encapsulation in a delivery system, the active substance is gradually released, and

it can act locally to diminish joint inflammation, in the case of non-steroidal or steroidal drugs, to remove the synovial lining, in the case of radioisotopes or anti-tumour drugs, or to target the articular cartilage, in the case of specific peptide drugs. The uptake by synovial macrophages is very important on the one hand to increase the retention of the drug delivery system in the joint and on the other hand to rapidly and effectively diminish inflammation by the direct targeting of the cells involved in inflammatory responses. Consequently, because of the environment of the arthritic joint, the drug delivery systems designed for i.a. administration must meet specific criteria concerning the lack of pro-inflammatory or pro-immunogenic activity, achieved through a careful selection of the carrier material and suitable size and shape.

Despite the extensive research done in the field, at present there is no polymeric drug delivery system registered for i.a. administration in humans, maybe due to stability issues, but also to a lack of definite evidence of pre-clinical and clinical efficacy. Nevertheless, among the different types of drug delivery systems, liposomes represent the most advanced carrier for i.a. administration, benefiting from preliminary evidence of efficacy obtained both in animal models and in small clinical studies. Further research is needed to improve the retention and to tailor the release rate of the encapsulated drug.

Research in this field should be directed towards the development of adapted delivery systems for potent anti-inflammatory drugs such as p38 MAPK or IL-1 β converting enzyme inhibitors. These types of agent block molecular functions that are involved in the immune response and signalling cascades. Systemic administration leads to an increased rate of infections and toxic side effects and thus, a local intra-articular administration may be of great benefit. Moreover, in order to increase the success of these intra-articular drug delivery systems, the research on encapsulation techniques should take into account specific targeting strategies for cellular receptors in the joint, which could represent a major breakthrough in the domain of rheumatic disease treatment.

Acknowledgements

The authors would like to thank Prof. Cem Gabay and Dr. Pierre-André Guerne for helpful discussion and interesting suggestions.

References

- [1] R.K. Studer, R. Bergman, T. Stubbs, K. Decker, Chondrocyte response to growth factors is modulated by p38 mitogen-activated protein kinase inhibition, *Arthritis Res. Ther.* 6 (2004) R56–R64.
- [2] S. Kang, M. Jung, C.W. Kim, D.Y. Shin, Inactivation of p38 kinase delays the onset of senescence in rabbit articular chondrocytes, *Mech. Ageing Dev.* 126 (2005) 591–597.
- [3] J.J. Haddad, VX-745, Vertex pharmaceuticals, *Curr. Opin. Invest. Drugs* 2 (2001) 1070–1076.
- [4] S. Kumar, B.J. Votta, D.J. Rieman, A.M. Badger, M. Gowen, J.C. Lee, IL-1- and TNF-induced bone resorption is mediated by p38 mitogen activated protein kinase, *J. Cell. Physiol.* 187 (2001) 294–303.
- [5] C. Pargellis, J. Regan, Inhibitors of p38 mitogen-activated protein kinase for the treatment of rheumatoid arthritis, *Curr. Opin. Invest. Drugs* 4 (2003) 566–571.
- [6] R.K. Studer, C.R. Chu, p38 MAPK and COX2 inhibition modulate human chondrocyte response to TGF- β , *J. Orthop. Res.* 23 (2005) 454–461.
- [7] C. Albert, O. Brocq, D. Gerard, C. Roux, L. Euler-Ziegler, Septic knee arthritis after intra-articular hyaluronate injection: two case reports, *Joint Bone Spine* 73 (2006) 205–207.
- [8] J.-P. Raynaud, C. Buckland-Wright, R. Ward, D. Choquette, B. Haraoui, J. Martel-Pelletier, I. Uthman, V. Khy, J. Tremblay, C. Bertrand, J. Martel-Pelletier, Safety and efficacy of long-term intraarticular steroid injections in osteoarthritis of the knee. A randomized, double-blind, placebo-controlled trial, *Arthritis Rheum.* 48 (2003) 370–377.
- [9] R.G. Gray, N.L. Gottlieb, Intra-articular corticosteroids, *Clin. Orthop. Rel. Res.* 177 (1983) 235–263.
- [10] K. Gaffney, J. Ledingham, J.D. Perry, Intra-articular triamcinolone hexacetonide in knee osteoarthritis: factors influencing the clinical response, *Ann. Rheum. Dis.* 54 (1995) 379–381.
- [11] G.V. Gordon, H.R. Schumacher, Electron microscopic study of depot corticosteroid crystals with clinical studies after intra-articular injection, *J. Rheumatol.* 61 (1978) 7–14.
- [12] M.H. Ellman, M.A. Becker, Crystal-induced arthropathies: recent investigative advances, *Curr. Opin. Rheumatol.* 18 (2006) 249–255.
- [13] T. Totsu, Steroid induced arthropathy, *J. Joint Surg.* 11 (1992) 87–95.
- [14] G.F. Moore, Arthrocentesis technique and intraarticular therapy, in: W.J. Koopman, L.W. Moreland (Eds.), *Arthritis and Allied Conditions – A Textbook of Rheumatology*, Lippincott Williams & Wilkins, Philadelphia, 2005, pp. 775–785.
- [15] C. Larsen, J. Øsergaard, S.W. Larsen, H. Jensen, S. Jacobsen, C. Lindegaard, A.H. Andersen, Intra-articular depot formulation principles: role in the management of postoperative pain and arthritic disorders, *J. Pharm. Sci.* 97 (2008) 4622–4654.
- [16] I.H. Tamer, U. Muller-Ladner, Drug delivery systems for the treatment of rheumatoid arthritis, *Exp. Opin. Drug Deliv.* 5 (2008) 1027–1037.
- [17] R.S. Traister, R. Hirsch, Gene therapy for arthritis, *Mod. Rheumatol.* 18 (2008) 2–14.
- [18] T. Hugle, J.M. van Laar, Stem cell transplantation for rheumatic autoimmune diseases, *Arthritis Res. Ther.* 10 (2008).
- [19] V. Strand, R. Kimberly, J.D. Isaacs, Biologic therapies in rheumatology: lessons, learned future directions, *Nat. Rev. Drug Discov.* 6 (2007) 75–92.
- [20] P.A. Revell, V. Mayston, P. Lalor, The synovial membrane in osteoarthritis: a histological study including the characterization of the cellular infiltrate present in inflammatory osteoarthritis using monoclonal antibodies, *Ann. Rheum. Dis.* 47 (1988) 300–307.
- [21] S.L.Y. Woo, K.N. An, S.P. Amoczek, Anatomy, biology, and biomechanics of tendon ligament and meniscus, in: S.R. Simon (Ed.), *Orthopaedic Basic Science*, American Academy of Orthopaedic Surgeons, Chicago, 1994, pp. 45–88.
- [22] F.N. Ghadially, Fine structure of joints, in: L. Sokoloff (Ed.), *The Joints and Synovial Fluid*, Academic, San Diego, 1978, pp. 105–168.
- [23] H.J. Mankin, V.C. Mow, Form and function of articular cartilage, in: S.R. Simon (Ed.), *Orthopaedic Basic Science*, American Academy of Orthopaedic Surgeons, Chicago, 1994, pp. 2–44.
- [24] D.L. Gardner, D.C. McGillivray, Living cartilage is not smooth, *Ann. Rheum. Dis.* 30 (1971) 3–9.
- [25] J.S. Jurvelin, D.J. Müller, M. Wong, D. Suder, Surface and subsurface morphology of bovine humeral articular cartilage by atomic force transmission electron microscopy, *J. Struct. Biol.* 117 (1996) 45–54.
- [26] V.K. Shekawat, M.P. Laurent, C. Muehlman, M.A. Wimmer, Surface topography of viable articular cartilage measured with scanning white light interferometry, *Osteoarthritis Cartil.*, 2009, in press.
- [27] F.M. Price, J.R. Levick, R.M. Mason, Glycosaminoglycan concentration in synovium and other tissues of rabbit knee in relation to synovial hydraulic resistance, *J. Physiol.* 495 (1996) 803–820.
- [28] D.V. Davies, Observations on the volume, viscosity and nitrogen content of synovial fluid with a note on the histological appearance of synovial membrane, *J. Anat.* 78 (2003) 68–78.
- [29] A.R. Poole, D.S. Howell, Etiopathogenesis of osteoarthritis, in: R.W. Moskowitz, D.S. Howell, R.D. Altman, J.A. Buckwalter, V.M. Goldberg (Eds.), *Osteoarthritis, Diagnosis and Medical/Surgical Management*, W.B. Saunders Company, Philadelphia, 2001, pp. 29–47.
- [30] S.E. Sweeney, G.S. Firestein, Rheumatoid arthritis: regulation of synovial inflammation, *Int. J. Biochem. Cell Biol.* 36 (2004) 372–378.
- [31] D.S. Springfield, M.E. Bolander, G.E. Friedlaender, N. Lane, Molecular and cellular biology of inflammation and neoplasia, in: S.R. Simon (Ed.), *Orthopaedic Basic Science*, American academy of orthopaedic surgeons, Rosemont, 1994, pp. 219–276.
- [32] M. Feldmann, F.M. Brennan, R.N. Maini, Role of cytokines in rheumatoid arthritis, *Ann. Rev. Immunol.* 14 (1996) 397–440.
- [33] D. Magne, G. Palmer, J. Barton, F. Mözin, D. Talabot-Ayer, S. Bas, T. Duffy, M. Noger, P.A. Guerne, M. Nicklin, C. Gabay, The new IL-1 family member IL-1F8 stimulates production of inflammatory mediators by synovial fibroblasts and articular chondrocytes, *Arthritis Res. Ther.* 8 (2006) R80.
- [34] E.H. Choy, G.S. Panayi, Cytokine pathways and joint inflammation in rheumatoid arthritis, *New Engl. J. Med.* 344 (2001) 907–916.
- [35] M. Chahaud, P. Garnero, J.M. Dayer, P.A. Guerne, F. Fossiez, P. Miossec, Contribution of interleukin 17 to synovium matrix destruction in rheumatoid arthritis, *Cytokine* 12 (2000) 1092–1099.
- [36] S.K. Das, A. Farooqi, Osteoarthritis, *Best Pract. Res. Clin. Rheumatol.* 22 (2008) 657–675.
- [37] L. Sharma, D. Kapoor, S. Issa, Epidemiology of osteoarthritis: an update, *Curr. Opin. Rheumatol.* 18 (2006) 147–156.
- [38] R.C. Lawrence, C.G. Helmick, F.C. Arnett, R.A. Deyo, D.T. Felson, E.H. Giannini, S.P. Heyse, R. Hirsch, M.C. Hochberg, G.G. Hunder, M.H. Liang, S.R. Pillemer, V.D. Steen, F. Wolfe, Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States, *Arthritis Rheum.* 41 (1998) 778–799.
- [39] M.C. Hochberg, R.D. Altman, K.D. Brandt, Guidelines for the medical management of osteoarthritis, *Arthritis Rheum.* 38 (1995) 1535–1540.
- [40] K.D. Brandt, A critique of the 2000 update of the American College of Rheumatology recommendations for management of hip and knee osteoarthritis, *Arthritis Rheum.* 44 (2001) 2451–2455.
- [41] K.M. Jordan, N.K. Arden, M. Doherty, B. Bannwarth, J.W.J. Bijlsma, P. Dieppe, K. Gunther, H. Hauselmann, G. Herrero-Beaumont, P. Kaklamanis, S. Lohmander,

- B. Leeb, M. Lequesne, B. Mazieres, E. Martin-Mola, K. Pavelka, A. Pendleton, L. Punzi, U. Serni, B. Swoboda, G. Verbruggen, I. Zimmerman-Gorska, M. Dougados, EULAR recommendations 2003: an evidence-based approach to the management of knee osteoarthritis: report of a task force of the standing committee for international clinical studies including therapeutic trials (ESCIIT), *Ann. Rheum. Dis.* 62 (2003) 1145–1155.
- [42] W. Zhang, R.W. Moskowitz, G. Nuki, S. Abramson, R.D. Altman, N. Arden, S. Bierma-Zeinstra, K.D. Brandt, P. Croft, M. Doherty, M. Dougados, M. Hochberg, D.J. Hunter, K. Kwok, L.S. Lohmander, P. Tugwell, OARS recommendations for the management of hip and knee osteoarthritis, part II: OARS evidence-based, expert consensus guidelines, *Osteoarthritis Cartil.* 16 (2008) 137–162.
- [43] D.T. Felson, Osteoarthritis of the knee, *New Engl. J. Med.* 354 (2006) 841–848.
- [44] L.T. Michael, T. Kelley, Nonsurgical management of osteoarthritis of the knee, *JAAAP* 19 (2006) 26–32.
- [45] M.S. Hogenmiller, C.J. Lozada, An update on osteoarthritis therapeutics, *Curr. Opin. Rheumatol.* 18 (2006) 256–260.
- [46] I. Uthman, J.-P. Raynauld, B. Haranui, Intra-articular therapy in osteoarthritis, *Postgrad. Med. J.* 79 (2003) 449–453.
- [47] C.J. Lozada, R.D. Altman, Management of osteoarthritis, in: W.J. Koopman, L.W. Moreland (Eds.), *Arthritis and Allied Conditions – A Textbook of Rheumatology*, Lippincott Williams & Wilkins, Philadelphia, 2005, pp. 2257–2276.
- [48] L.S. Simon, V. Stand, The pharmacological treatment of osteoarthritis, in: R.W. Moskowitz, D.S. Howell, R.D. Altman, J.A. Buckwalter, V.M. Goldberg (Eds.), *Osteoarthritis*, W.B. Saunders Company, Philadelphia, 2001, pp. 371–391.
- [49] W. Zhang, A. Jones, M. Doherty, Does paracetamol (acetaminophen) reduce the pain of osteoarthritis? – a meta-analysis of randomised controlled trials, *Ann. Rheum. Dis.* 63 (2004) 901–907.
- [50] T. Schnitzer, G.R. Burmester, E. Mysler, M.C. Hochberg, M. Doherty, E. Ehsrasm, X. Gitton, G. Krammer, B. Mellein, P. Matchaba, A. Glinona, C. Hawley, Comparison of luminacoxib with naproxen and ibuprofen in the Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET), reduction in ulcer complications: randomized controlled trial, *Lancet* 363 (2004) 665–674.
- [51] S.S. Leopold, B.B. Redd, W.J. Warme, P.A. Wehrle, P.D. Pettis, S. Shott, Corticosteroid compared with hyaluronic acid injections for the treatment of osteoarthritis of the knee. A prospective, randomized trial, *J. Bone Joint Surg. Am.* 85-A (2003) 1197–1203.
- [52] S.C. Vlad, M.P. LaValley, T.E. McAlindon, D.T. Felson, Glucosamine for pain in osteoarthritis: why do trial results differ?, *Arthritis Rheum* 56 (2007) 2267–2277.
- [53] T.E. McAlindon, B.A. Biggs, Nutritional factors and osteoarthritis: recent developments, *Curr. Opin. Rheumatol.* 17 (2005) 647–652.
- [54] E.S. El Desoky, Pharmacotherapy of rheumatoid arthritis: an overview, *Curr. Ther. Res.* 62 (2001) 92–112.
- [55] E.K. Li, L.-S. Tam, B. Tomlinson, Leflunomide in the treatment of rheumatoid arthritis, *Clin. Ther.* 26 (2004) 447–459.
- [56] K. Kitahara, S. Kawai, Cyclosporine and tacrolimus for the treatment of rheumatoid arthritis, *Curr. Opin. Rheumatol.* 19 (2007) 238–245.
- [57] S. Kawai, K. Yamamoto, Safety of tacrolimus, an immunosuppressive agent, in the treatment of rheumatoid arthritis in elderly patients, *Rheumatology (Oxford)* 45 (2006) 441–444.
- [58] D.E. Furst, K. Saag, M.R. Fleischmann, Y. Sherrer, J.A. Block, T. Schnitzer, J. Rutstein, A. Baldassare, J. Kaine, L. Calabrese, F. Dietz, M. Sack, R.G. Senter, C. Wiesenbutter, M. Schiff, C.M. Stein, Y. Sato, A. Matsumoto, J. Caldwell, R.E. Harris, L.W. Moreland, E. Hurd, D. Yocum, D.A. Stamler, Efficacy of tacrolimus in rheumatoid arthritis patients who have been treated unsuccessfully with methotrexate: a six-month, double-blind, randomized, dose-ranging study, *Arthritis Rheum.* 46 (2002) 2020–2028.
- [59] D.L. Scott, G.H. Kingsley, Tumor necrosis factor inhibitors for rheumatoid arthritis, *Reply, New Engl. J. Med.* 355 (2006) 2048.
- [60] J. Sibilia, J.E. Gottenberg, X. Mariette, Rituximab: a new therapeutic alternative in rheumatoid arthritis, *Joint Bone Spine* 75 (2008) 526–532.
- [61] C. Esenyel, M. Demirhan, M. Esenyel, M. Sonmez, S. Kahraman, B. Senel, T. Ozdes, Comparison of four different intra-articular injection sites in the knee: a cadaver study, *Knee Surg. Sports Traumatol. Arthrosc.* 15 (2007) 573–577.
- [62] H. Dabke V, Accuracy of needle placement into the intra-articular space of the knee, *J. Bone Joint Surg. Am.* 86-A (2004) 433–434.
- [63] J.H. Post III, Accuracy of needle placement into the intra-articular space of the knee, *J. Bone Joint Surg. Am.* 85-A (2003) 2481.
- [64] D.W. Jackson, N.A. Evans, B.M. Thomas, Accuracy of needle placement into the intra-articular space of the knee, *J. Bone Joint Surg. Am.* 84-A (2002) 1522–1527.
- [65] D.H. Neustadt, Intra-articular therapy, in: R.W. Moskowitz, D.S. Howell, R.D. Altman, J.A. Buckwalter, V.M. Goldberg (Eds.), *Osteoarthritis*, W.B. Saunders Company, Philadelphia, 2001, pp. 393–411.
- [66] X. Ayral, Injections in the treatment of osteoarthritis, *Best Pract. Res. Clin. Rheumatol.* 15 (2001) 609–626.
- [67] P.H. Wooley, M.J. Grimm, E.L. Radin, The structure and function of joints, in: W.J. Koopman, L.W. Moreland (Eds.), *Arthritis and Allied Conditions – A Textbook of Rheumatology*, Lippincott Williams & Wilkins, Philadelphia, 2005, pp. 149–175.
- [68] P.M. Graaback, Ultrastructural evidence for two distinct types of synoviocytes in rat synovial membrane, *J. Ultrastruct. Res.* 78 (1982) 321–339.
- [69] T.S. Momberger, J.R. Levick, R.M. Mason, Hyaluronan secretion by synoviocytes is mechanosensitive, *Matrix Biol.* 24 (2005) 510–519.
- [70] U. Müller-Ladner, R.E. Gay, S. Gay, Structure and functions of synoviocytes, in: W.J. Koopman, L.W. Moreland (Eds.), *Arthritis and Allied Conditions – A Textbook of Rheumatology*, Lippincott Williams & Wilkins, Philadelphia, 2005, pp. 271–287.
- [71] L.S. Wilkinson, A.A. Pitsillides, J.G. Worrall, J.C.W. Edwards, Light microscopic characterization of the fibroblast-like synovial intimal cell (synoviocyte), *Arthritis Rheum.* 35 (1992) 1179–1184.
- [72] H. Senda, E. Sakuma, I. Wada, H.J. Wang, H. Maruyama, N. Matsui, Ultrastructural study of cells at the synovium-cartilage junction: response of synovial cells of the rat knee joint to intra-articularly injected latex particles, *Acta Anat. Nippon.* 74 (1999) 525–535.
- [73] J.R. Levick, Permeability of the rheumatoid and normal synovium to specific plasma proteins, *Arthritis Rheum.* 24 (1981) 1550–1560.
- [74] P.A. Simkin, Synovial physiology, in: W.J. Koopman, L.W. Moreland (Eds.), *Arthritis and Allied Conditions – A Textbook of Rheumatology*, Lippincott, Williams & Wilkins, Philadelphia, 2005.
- [75] D.J. Schurman, G. Kajiyama, Antibiotic absorption from infected and normal joints using a rabbit knee joint model, *J. Orthop. Res.* 3 (1985) 185–188.
- [76] P.J. Coleman, D. Scott, J. Ray, R.M. Mason, J.R. Levick, Hyaluronan secretion into the synovial cavity of rabbit knees and comparison with albumin turnover, *J. Physiol.* 503 (1997) 645–656.
- [77] H. Betre, W. Liu, M.R. Zalutsky, A. Chilukoti, V.B. Kraus, L.A. Setton, A thermally responsive biopolymer for intra-articular drug delivery, *J. Control Rel.* 115 (2006) 175–182.
- [78] J. Song, C.H. Suh, Y.B. Park, S.H. Lee, N.C. Yoo, J.D. Lee, K.H. Kim, S.K. Lee, A phase I/IIa study on intra-articular injection of holmium-166-chitosan complex for the treatment of knee synovitis of rheumatoid arthritis, *Eur. J. Nucl. Med.* 28 (2001) 489–497.
- [79] I.H. Shaw, C.G. Knight, J.T. Dingle, Liposomal retention of a modified anti-inflammatory steroid, *Biochem. J.* 158 (1976) 473–476.
- [80] M.H. Bonanomi, M. Velvart, M. Stimpel, K.M. Roos, K. Fehr, H.G. Weder, Studies of pharmacokinetics and therapeutic effects of glucocorticoids entrapped in liposomes after intraarticular application in healthy rabbits and in rabbits with antigen-induced arthritis, *Rheumatol. Int.* 7 (1987) 203–212.
- [81] A.S. Williams, J.P. Camilleri, R.M. Goodfellow, B.D. Williams, A single intra-articular injection of liposomally conjugated methotrexate suppresses joint inflammation in rat antigen-induced arthritis, *Br. J. Rheumatol.* 35 (1996) 719–724.
- [82] S.-M. Hou, H.-Y. Yu, Comparison of systemic absorption of aqueous and liposomal lidocaine following intra-articular injection in rabbits, *J. Formos. Med. Assoc.* 96 (1997) 141–142.
- [83] R.K. Chowdhary, L.G. Ratkay, A.J. Canaan, J.D. Waterfield, A.M. Richter, J.G. Levy, Uptake of Verteporfin[®] by articular tissues following systemic and intra-articular administration, *Biopharm. Drug Dispos.* 19 (1998) 395–400.
- [84] S.H.R. Edwards, M.A. Cake, G. Spoelstra, R.A. Read, Biodistribution and clearance of intra-articular liposomes in a large animal model using a radiographic marker, *J. Liposome Res.* 17 (2007) 249–261.
- [85] Y. Lu, G. Zhang, D. Sun, Y. Zhong, Preparation and evaluation of biodegradable flurbiprofen gelatin micro-spheres for intra-articular administration, *J. Microencapsul.* 24 (2007) 515–524.
- [86] H. Thakkar, R.K. Sharma, A.K. Mishra, K. Chuttani, R.S.R. Murthy, Efficacy of chitosan microspheres for controlled intra-articular delivery of celecoxib in inflamed joints, *J. Pharm. Pharmacol.* 56 (2004) 1091–1099.
- [87] L. Liang, J. Jackson, W. Min, V. Risovic, K.M. Wasan, H.M. Burt, Methotrexate loaded poly(L-lactic acid) microspheres for intra-articular delivery of methotrexate to the joint, *J. Pharm. Sci.* 93 (2003) 943–956.
- [88] L.S. Liang, W. Wong, H.M. Burt, Pharmacokinetic study of methotrexate following intra-articular injection of methotrexate loaded poly(L-lactic acid) microspheres in rabbits, *J. Pharm. Sci.* 94 (2005) 1204–1215.
- [89] D.A. Rothenfluh, H. Bermudez, C.P. O'Neil, J.A. Hubbell, Biofunctional polymer nanoparticles for intra-articular targeting and retention in cartilage, *Nat. Mater.* 7 (2008) 248–254.
- [90] G.A. Koning, R.M. Schiffelers, M.H. Wauben, R.J. Kok, E. Mastrobattista, G. Molema, T.L.M. ten Hagen, G. Storm, Targeting of angiogenic endothelial cells at sites of inflammation by dexamethasone phosphate-containing RGD peptide liposomes inhibits experimental arthritis, *Arthritis Rheum.* 54 (2006) 1198–1208.
- [91] T. Garrood, C. Pitzalis, Targeting the inflamed synovium: the quest for selectivity, *Arthritis Rheum.* 54 (2006) 1055–1060.
- [92] R. Margalit, D. Peer, Lipidated glycoprotein particles and methods of use, *Patent No. WO/2006/017195*, 2006.
- [93] H. Tanaka, T. Sugita, Y. Yasunaga, S. Shimose, M. Deie, T. Kubo, T. Murakami, M. Ochi, Efficiency of magnetic liposomal transforming growth factor-beta 1 in the repair of articular cartilage defects in a rabbit model, *J. Biomed. Mater. Res. A* 73A (2005) 255–263.
- [94] N. Butoescu, O. Jordan, P. Burdet, C. Hebert, P. Stadelmann, A. Petri-Fink, H. Hofmann, E. Doelker, Dexamethasone-containing biodegradable superparamagnetic microparticles for intra-articular administration: physicochemical and magnetic properties, and in vitro and in vivo drug release, *Eur. J. Pharm. Biopharm.* 72 (2009) 529–538.
- [95] N. Butoescu, O. Jordan, A. Petri-Fink, H. Hofmann, E. Doelker, Co-encapsulation of dexamethasone 21-acetate and SPIONs into biodegradable polymeric microparticles designed for intra-articular delivery, *J. Microencapsul.* 25 (2008) 339–350.

- [96] N. Butoescu, C.A. Seemayer, G. Palmer, P.-A. Guerne, C. Gabay, E. Doelker, O. Jordan, Magnetically retainable microparticles for drug delivery to the joint: efficacy studies on an antigen-induced arthritis model in mice, *Arthritis Res. Ther.* 11 (2009) R72.
- [97] K. Schulze, A. Koch, B. Schopf, A. Petri, B. Steitz, M. Chastellain, M. Hofmann, H. Hofmann, B. von Rechenberg, Intraarticular application of superparamagnetic nanoparticles and their uptake by synovial membrane – an experimental study in sheep, *J. Magn. Magn. Mater.* 293 (2005) 419–432.
- [98] D. Hellstern, K. Schultze, B. Schopf, A. Petri-Fink, B. Steitz, S. Kamau, M. Hilbe, S. Koch-Schneidemann, L. Vaughan, M. Hottiger, H. Hofmann, M. Hofmann, B. von Rechenberg, Systemic distribution and elimination of plain and with Cy3.5 functionalized poly(vinyl alcohol) coated superparamagnetic maghemite nanoparticles after intraarticular injection in sheep in vivo, *J. Nanosci. Nanotechnol.* 6 (2006) 3261–3268.
- [99] I.H. Shaw, J.T. Dingle, Liposomes as steroid carriers in the intra-articular therapy of rheumatoid arthritis, in: G. Gregoriadis, A.C. Allison (Eds.), *Liposomes in Biological Systems*, John Wiley & Sons, Ltd., London, 1980, pp. 299–324.
- [100] M.H. Bonanomi, M. Velvart, H.G. Weder, Fate of different kinds of liposomes containing dexamethasone palmitate after intra-articular injection into rabbit joints, *J. Microencapsul.* 4 (1987) 189–200.
- [101] S. Türker, S. Erdogan, A.Y. Özer, E.L. Ergün, M. Tuncel, H. Bilgili, S. Deveci, Scintigraphic imaging of radiolabelled drug delivery systems in rabbits with arthritis, *Int. J. Pharm.* 296 (2005) 34–43.
- [102] M. De Silva, B.L. Hazleman, D.P. Page Thomas, P. Wraight, Liposomes in arthritis: a new approach, *Lancet* 313 (1979) 1320–1322.
- [103] P. Barrera, A. Blom, P.L. van Lent, L. van Bloois, J.H. Beijnen, N. Van Rooijen, M.C. Waal Malefijt, L.B. van de Putte, G. Storm, W.B. van den Berg, Synovial macrophage depletion with clodronate-containing liposomes in rheumatoid arthritis, *Arthritis Rheum.* 43 (2000) 1951–1959.
- [104] D.W. Howie, B. Manthey, S. Hay, B. Vernon-Roberts, The synovial response to intraarticular injection in rats of polyethylene wear particles, *Clin. Orthop. Relat. Res.* 292 (1993) 352–357.
- [105] E. Horisawa, K. Kubota, I. Tuhoi, K. Sato, H. Yamamoto, H. Takeuchi, Y. Kawashima, Size-dependency of DL-lactide/glycolide copolymer particulates for intra-articular delivery system on phagocytosis in rat synovium, *Pharm. Res.* 19 (2002) 132–139.
- [106] M. Nishide, S. Kamei, Y. Takakura, S. Tamai, Fate of biodegradable D,L-lactic acid oligomer microspheres in the articular, *J. Bioact. Compat. Polym.* 14 (1999) 385–398.
- [107] J.H. Ratcliffe, I.M. Hunneyball, A. Smith, C.G. Wilson, S.S. Davis, Preparation and evaluation of biodegradable polymeric systems for the intra-articular delivery of drugs, *J. Pharm. Pharmacol.* 36 (1984) 431–436.
- [108] J.H. Ratcliffe, I.M. Hunneyball, C.G. Wilson, A. Smith, S.S. Davis, Albumin microspheres for intra-articular drug-delivery: investigation of their retention in normal and arthritic knee joints of rabbits, *J. Pharm. Pharmacol.* 39 (1986) 290–295.
- [109] D.V. Ramesh, Y. Tabata, I. Ikada, Bioabsorbable microspheres for local drug release in the articular, *J. Bioact. Compat. Polym.* 14 (1999) 137–149.
- [110] R.T. Liggins, T. Cruz, W. Min, L. Liang, W.L. Hunter, H.M. Burt, Intra-articular treatment of arthritis with microsphere formulations of paclitaxel: biocompatibility and efficacy determinations in rabbits, *Inflamm. Res.* 53 (2004) 363–372.
- [111] A.V. Sarma, G.L. Powell, M. LaBerge, Phospholipid composition of articular cartilage boundary lubricant, *J. Orthop. Res.* 19 (2001) 671–676.
- [112] C. Kirby, J. Clarke, G. Gregoriadis, Effect of the cholesterol content of small unilamellar liposomes on their stability in vivo and in vitro, *Biochem. J.* 186 (1980) 591–598.
- [113] R.A. Schwendener, P.A. Lagocki, Y.E. Rahman, The effects of charge and size on the interaction of unilamellar liposomes with macrophages, *Biochim. Biophys. Acta, Biomembr.* 772 (1984) 93–101.
- [114] N.C. Phillips, D.P. Page Thomas, C.G. Knight, J.T. Dingle, Liposome-incorporated corticosteroids. II. Therapeutic activity in experimental arthritis, *Ann. Rheum. Dis.* 38 (1979) 553–557.
- [115] L. Setton, Polymer therapeutics: reservoir drugs, *Nat. Mater.* 7 (2008) 172–174.
- [116] S. Bozdog, S. Calis, H.S. Kas, M.T. Ercan, I. Peksoy, A.A. Hincal, *In vitro* evaluation and intra-articular administration of biodegradable microspheres containing naproxen sodium, *J. Microencapsul.* 18 (2000) 443–456.
- [117] L. Cai, F.W. Okumu, J.L. Cleland, M. Beresini, D. Hogue, Z. Lin, E.H. Filvaroff, A slow release formulation of insulin as a treatment for osteoarthritis, *Osteoarthritis. Cartil.* 10 (2002) 692–706.
- [118] M. Tuncay, S. Calis, H.S. Kas, M.T. Ercan, I. Peksoy, A.A. Hincal, Diclofenac sodium incorporated PLGA (50:50) microspheres: formulation considerations and in vitro/in vivo evaluation, *Int. J. Pharm.* 195 (2000) 179–188.
- [119] C.M. Mierisch, S.B. Cohen, L.C. Jordan, P.G. Robertson, G. Balian, D.R. Diduch, P.G. Robertson, Transforming growth factor- β in calcium alginate beads for the treatment of articular cartilage defects in the rabbit, *Arthroscopy* 18 (2002) 892–900.
- [120] K.E. Brown, K. Leong, C. Huang, R. Dalal, G.D. Green, H.B. Haimes, P.A. Jimenez, J. Bathon, Gelatin/chondroitin 6-sulphate microspheres for the delivery of therapeutic proteins to the joint, *Arthritis Rheum.* 41 (1998) 2185–2195.
- [121] E. Horisawa, T. Hirota, S. Kawazoe, J. Yamada, H. Yamamoto, H. Takeuchi, Y. Kawashima, Prolonged anti-inflammatory action of DL-lactide/glycolide copolymer nanospheres containing betamethasone sodium phosphate for intra-articular delivery system in antigen-induced arthritic rabbit, *Pharm. Res.* 19 (2002) 403–410.
- [122] L.S. Liang, J. Jackson, W. Min, V. Risovic, K.M. Wasan, H.M. Burt, Methotrexate loaded poly(L-lactic acid) microspheres for intra-articular delivery of methotrexate to the joint, *J. Pharm. Sci.* 93 (2004) 943–956.
- [123] M. Cavalier, J.P. Benoit, C. Thies, The formation and characterization of hydrocortisone-loaded poly(ϵ -lactide) microspheres, *J. Pharm. Pharmacol.* 38 (1986) 249–253.
- [124] S. Bozdog, S. Calis, H.S. Kas, M.T. Ercan, I. Peksoy, A.A. Hincal, *In vitro* evaluation and intra-articular administration of biodegradable microspheres containing naproxen sodium, *J. Microencapsul.* 18 (2001) 443–456.
- [125] H. Thakkar, R.K. Sharma, A.K. Mishra, K. Chuttani, R.S.R. Murthy, Efficacy of chitosan microspheres for controlled intra-articular delivery of celecoxib in inflamed joints, *JPP* 56 (2004) 1091–1099.
- [126] A.R. Taylor, J.S. Harbison, C. Pepler, Synovectomy of the knee in rheumatoid arthritis. II. Results of surgery, *Ann. Rheum. Dis.* 31 (1972) 159–161.
- [127] D.P. Page Thomas, N.C. Phillips, Intra-articular liposomal therapy, in: J.T. Dingle, P.J. Jacques, I.H. Shaw (Eds.), *Lysosomes in Applied Biology and Therapeutics*, North-Holland, Publishing Company, Amsterdam, 1979, pp. 601–623.
- [128] J. Noble, A.G. Jones, M.A. Davies, C.B. Sledge, R.I. Kramer, E. Livni, Leakage of radioactive particle systems from a synovial joint studied with a gamma camera. Its application to radioactive synovectomy, *J. Bone Joint Surg.* 65-A (1983) 381–389.
- [129] R.J. Mumper, B.J. Mills, U.Y. Ryo, M. Jay, Polymeric microspheres for radionuclide synovectomy containing neutron-activated holmium-166, *J. Nucl. Med.* 33 (1992) 398–402.
- [130] J. Highton, D. Guevremont, J. Thomson, B. Carlisle, I. Tucker, A trial of clodronate liposomes as anti-macrophage treatment in a sheep model of arthritis, *Clin. Exp. Rheumatol.* 17 (1999) 43–48.
- [131] P.L.E.M. van Lent, A.E.M. Holthuyzen, N. Van Rooijen, L.B.A. Van De Putte, W. van den Berg, Local removal of phagocytic synovial lining cells by clodronate liposomes decreases cartilage destruction during collagen type II arthritis, *Ann. Rheum. Dis.* 57 (1998) 408–413.
- [132] C.G. Knight, I.H. Shaw, Liposomes as carriers of anti-inflammatory steroids, in: J.T. Dingle, P.J. Jacques, I.H. Shaw (Eds.), *Lysosomes in Applied Biology and Therapeutics*, North-Holland Publishing Company, Amsterdam, 1979, pp. 575–601.
- [133] J.T. Dingle, J.L. Gordon, B.L. Hazleman, C.G. Knight, D.P. Page Thomas, N.C. Phillips, I.H. Shaw, F.J.T. Fildes, J.E. Oliver, G. Jones, E.H. Turner, J.S. Lowe, Novel treatment for joint inflammation, *Nature* 271 (1977) 372–373.
- [134] D.R. Bard, C.G. Knight, D.P. Page Thomas, The retention and distribution in the rabbit knee of a radionuclide complexed with a lipophilic chelator in liposomes, *Clin. Exp. Rheumatol.* 1 (1983) 113–117.
- [135] M. Trif, C. Guillen, D.M. Vaughan, J.M. Telfer, J.M. Brewer, A. Roseanu, J.H. Brock, Liposomes as possible carriers for lactoferrin in the local treatment of inflammatory diseases, *Exp. Biol. Med.* 226 (2001) 559–564.
- [136] W.C. Foong, K.L. Green, Retention and distribution of liposome-entrapped [^{3} H]methotrexate injected into normal or arthritic rabbit joints, *J. Pharm. Pharmacol.* 40 (1987) 464–468.
- [137] W.C. Foong, K.L. Green, Treatment of antigen-induced arthritis in rabbits with liposome-entrapped methotrexate injected intra-articularly, *J. Pharm. Pharmacol.* 45 (1993) 204–209.
- [138] T. Abe, H. Yamada, H. Nakajima, T. Kikuchi, H. Takaishi, T. Tadakuma, K. Fujikawa, Y. Toyama, Repair of full-thickness cartilage defects using liposomal transforming growth factor- β 1, *J. Orthop. Sci.* 8 (2003) 92–101.
- [139] E.B. Hunziker, Growth-factor-induced healing of partial-thickness defects in adult articular cartilage, *Osteoarthritis. Cartil.* 9 (2001) 22–32.
- [140] F. Lopez-Garcia, J.M. Vazquez-Auton, F. Gil, R. Latoore, F. Moreno, J. Villalain, J.C. Gomez-Fernandez, Intra-articular therapy of experimental arthritis with a derivative of triamcinolone acetonide incorporated in liposomes, *J. Pharm. Pharmacol.* 45 (1993) 576–578.
- [141] K. Schulze, A. Koch, A. Petri-Fink, S. Kamau, M. Hottiger, M. Hilbe, L. Vaughan, M. Hofmann, H. Hofmann, B. von Rechenberg, Uptake and biocompatibility of functionalized poly(vinylalcohol) coated superparamagnetic maghemite nanoparticles by synoviocytes in vitro, *J. Nanosci. Nanotechnol.* 6 (2006) 2829–2840.
- [142] H. Thakkar, R.K. Sharma, A.K. Mishra, K. Chuttani, R.S.R. Murthy, Albumin microspheres as carriers for the antiarthritic drug celecoxib, *AAPS Pharm. Sci. Tech.* 6 (2005) E65–E73.
- [143] A. Inoue, K.A. Takahashi, Y. Arai, H. Tonomura, K. Sakao, The therapeutic effects of basic fibroblast growth factor contained in gelatin hydrogel microspheres on experimental osteoarthritis in the rabbit knee, *Arthritis Rheum.* 54 (2006) 264–270.
- [144] M.C. Haerdi-Landerer, M.M. Suter, A. Steiner, M.M. Wittenbrink, A. Pickl, B.A. Gander, In vitro cell compatibility and antibacterial activity of microencapsulated doxycycline designed for improved localized therapy of septic arthritis, *J. Antimicrob. Chemother.* 61 (2008) 332–340.
- [145] B. Bragdon, A.L. Bertone, J. Hardy, E.J. Simmons, S.E. Weisbrode, Use of an isolated joint model to detect early changes induced by intra-articular injection of paclitaxel-impregnated polymeric microspheres, *J. Invest. Surg.* 14 (2001) 169–182.
- [146] D.J. Burgess, S.S. Davis, Potential use of albumin microspheres as a drug delivery system: II. In vivo deposition and release of steroids, *Int. J. Pharm.* 46 (1988) 69–76.

- [147] M. Chinol, S. Vallabhajosula, J.D. Zuckerman, S.J. Goldsmith, In-vivo stability of ferric hydroxide macroaggregates (FHMA). Is it a suitable carrier for radionuclides used in synovectomy? *Nucl. Med. Biol.* 17 (1990) 479–486.
- [148] K. Kothari, S. Suresh, H.D. Sanna, V. Meera, M.R.A. Pillai, 188Re-labeled hydroxyapatite particles for radiation synovectomy, *Appl. Radiat. Isot.* 58 (2003) 463–468.
- [149] D.J. Hnatowich, R.I. Kramer, C.B. Sledge, J. Noble, S. Shortkroff, Dysprosium-165-ferric hydroxide macroaggregates for radiation synovectomy, *J. Nucl. Med.* 19 (1978) 303–308.
- [150] M.A. Davies, M. Chinol, Radiopharmaceuticals for radiation synovectomy, *J. Nucl. Med.* 30 (1989) 1047–1055.
- [151] S.H. Lee, J.S. Suh, H.S. Kim, J.D. Lee, J. Song, S.K. Lee, MR evaluation of radiation synovectomy of the knee by means of intra-articular injection of holmium-166-chitosan complex in patients with rheumatoid arthritis: results at 4-month follow-up, *Korean J. Radiol.* 4 (2003) 170–178.
- [152] G. Cevc, A. Schatzlein, H. Richardsen, Ultradefinable lipid vesicles can penetrate the skin and other semi-permeable barriers unfragmented. Evidence from double label CLSM experiments and direct size measurements, *Biochim. Biophys. Acta, Biomembr.* 1564 (2002) 21–30.
- [153] M. Rother, B.J. Lavins, W. Kneer, K. Lehnhardt, E.J. Seidel, S. Mazgareanu, Efficacy and safety of epicutaneous ketoprofen in Transfersome (IDEA-033) versus oral celecoxib and placebo in osteoarthritis of the knee: multicentre randomized controlled trial, *Ann. Rheum. Dis.* 66 (2007) 1178–1183.
- [154] C. Gabay, Cytokines and cytokine receptors, in: W.J. Koopman, L.W. Moreland (Eds.), *Arthritis and Allied Conditions – A Textbook of Rheumatology*, Lippincott Williams & Wilkins, Philadelphia, 2005, pp. 423–476.
- [155] J.F. Schindler, J.B. Monahan, W.G. Smith, p38 pathway kinases as anti-inflammatory drug targets, *J. Dent. Res.* 86 (2007) 800–811.
- [156] R.J. Mayer, J.F. Callahan, p38 MAP kinase inhibitors: a future therapy for inflammatory diseases, *Drug Discov. Today* 3 (2006) 49–54.
- [157] A.M. Badger, M.N. Cook, M.W. Lark, T.M. Newman-Tarr, B.A. Swift, A.H. Nelson, F.C. Barone, S. Kumar, SB 203580 inhibits p38 mitogen-activated protein kinase, nitric oxide production, and inducible nitric oxide synthase in bovine cartilage-derived chondrocytes, *J. Immunol.* 161 (1998) 467–473.
- [158] A.M. Badger, D.E. Griswold, R. Kapadia, S. Blake, B.A. Swift, S.J. Hoffman, G.B. Stroup, E. Webb, D.J. Rieman, M. Gowen, J.C. Boehm, J.L. Adams, J.C. Lee, Disease-modifying activity of SB 242235, a selective inhibitor of p38 mitogen-activated protein kinase, in rat adjuvant-induced arthritis, *Arthritis Rheum.* 43 (2000) 175–183.
- [159] A.M. Badger, J.N. Bradbeer, B. Votta, J.C. Lee, J.L. Adams, D.E. Griswold, Pharmacological profile of SB 203580, a selective inhibitor of cytokine suppressive binding protein/p38 kinase, in animal models of arthritis, bone resorption, endotoxin shock and immune function, *J. Pharmacol. Exp. Ther.* 279 (1996) 1453–1461.
- [160] M.A. Palladino, F.R. Bahjat, E.A. Theodorakis, L.L. Moldawer, Anti-TNF- α therapies: the next generation, *Nat. Rev. Drug Discov.* 2 (2003) 736–746.
- [161] M.C. Bagley, T. Davis, M.C. Dix, M.J. Rokicki, D. Kipling, Rapid synthesis of VX-745: p38 MAP kinase inhibition in Werner syndrome cells, *Bioorg. Med. Chem. Lett.* 17 (2007) 5107–5110.
- [162] C. Ding, Drug evaluation: VX-702, a MAP kinase inhibitor for rheumatoid arthritis and acute coronary syndrome, *Curr. Opin. Invest. Drugs* 7 (2006) 1020–1025.
- [163] A.M. Badger, S. Blake, R. Kapadia, S. Sarkar, J. Levin, B.A. Swift, S.J. Hoffman, G.B. Stroup, W.H. Miller, M. Gowen, M.W. Lark, Disease-modifying activity of SB 273005, an orally active, nonpeptide $\alpha\beta 3$ (vitronectin receptor) antagonist, in rat adjuvant-induced arthritis, *Arthritis Rheum.* 44 (2001) 128–137.
- [164] M. Braddock, A. Quinn, Targeting IL-1 in inflammatory disease: new opportunities for therapeutic intervention, *Nat. Rev. Drug Discov.* 3 (2004) 1–10.
- [165] K. Rudolph, N. Gerwin, N. Verzijl, P. van der Kraan, W. van den Berg, Pralnacasan, an inhibitor of interleukin-1 β converting enzyme, reduces joint damage in two murine models of osteoarthritis, *Osteoarthr. Cartil.* 11 (2003) 738–746.
- [166] F. Lohrer, C. Bauer, N. Landauer, K. Schmall, B. Siegmund, H.A. Lehr, M. Dauer, M. Schoenharting, S. Endres, A. Eigler, The interleukin-1-converting enzyme inhibitor pralnacasan reduces dextran sulfate sodium-induced murine colitis and T helper 1T-cell activation, *J. Pharmacol. Exp. Ther.* 308 (2004) 583–590.

XP-002676238



Clinical Study Report Synopsis	
Drug Substance	AZD6918
Study Code	D2785C00002
Edition Number	1
Date	16 June 2009

A Phase I, Open-Label, Dose-Escalation Study to Assess the Safety, Tolerability and Pharmacokinetics of AZD6918 Administered Daily as a Single Agent and in Combination Treatment in Adult Patients with Refractory Solid Malignancies

Study dates:	First patient enrolled: 6 August 2008 Last patient completed: 12 March 2009
Phase of development:	Clinical pharmacology (I)

This study was performed in compliance with Good Clinical Practice, including the archiving of essential documents

This submission /document contains trade secrets and confidential commercial information, disclosure of which is prohibited without providing advance notice to AstraZeneca and opportunity to object.

Clinical Study Report Synopsis
Drug Substance AZD6918
Study Code D2785C00002
Edition Number 1
Date 16 June 2009

Study center(s)

This study was conducted at 2 study sites in the United States:

Publications

None at the time of writing this report.

Early termination of study

Study D2785C00002 was a Phase 1 study to evaluate the safety, tolerability and pharmacokinetics of an oral formulation of AZD6918 alone and in combination with either gemcitabine or pemetrexed in 2 separate arms. The first patient was enrolled on 6 August 2008, and the last patient completed the study on 12 March 2009. A decision to prematurely terminate the study was taken on 9 Feb 2009 and communicated to investigators. The reason for premature termination of the study was that the observed PK profile of AZD6918 in cancer patients showed that exposure was extremely low, as indicated by a lower C_{max} , C_{min} , and AUC, and that the apparent clearance was higher than predicted from animal studies. Based on this profile, as well as the variability observed among patients in the highest dose cohort (80 mg), the only cohort that included more than 1 patient, it was judged unlikely that an efficacious dose of AZD6918 could be reached. No safety issues were identified.

Objectives

The primary objective of this study was to identify a pharmacokinetically (PK)-guided estimated biologically effective dose (eBED) and maximum tolerated dose (MTD).

The secondary objectives were:

- To evaluate the safety and tolerability of AZD6918 as a single agent
- To evaluate the safety and tolerability of AZD6918 given in combination with chemotherapy (either pemetrexed or gemcitabine)
- To determine the pharmacokinetic profile of AZD6918 following single and multiple dose administration
- To evaluate the pharmacokinetics of AZD6918 and the selected chemotherapy agents when given in combination to determine if pharmacokinetics are altered by co-administration

Clinical Study Report Synopsis
 Drug Substance AZD6918
 Study Code D2785C00002
 Edition Number 1
 Date 16 June 2009

The exploratory objectives were:

- To seek preliminary evidence of the anti-tumor activity of AZD6918
- To correlate tumor biologic characteristics and clinical outcome using archival tumor tissue. All patients were required to consent although archival tissue did not have to be available at time of therapy initiation and was not a requirement for evaluability (samples were collected but have not been analyzed and are not presented in this Synopsis).

Optional: To obtain a blood sample for deoxyribonucleic acid (DNA) extraction for retrospective pharmacogenetic analysis (samples were collected but have not been analyzed and are not presented in this Synopsis).

Optional: Surplus blood or tissue samples may be analyzed for exploratory biomarkers to evaluate relationships between cancer related biomarkers and study treatments as part of a larger effort of biomarker evaluation (samples were collected but have not been analyzed and are not presented in this Synopsis).

Study design

Study D2785C00002 was a Phase 1, open-label, multi-center study to assess the safety, tolerability and pharmacokinetics of an oral formulation of AZD6918 (an inhibitor of tropomyosin-related kinase [Trk] receptors). The study was designed to include 2 parts, with each patient participating in only 1 part:

- **Part A (Dose Escalation Phase)** was designed to identify the safety, tolerability and PK of daily oral AZD6918 as a single agent up to eBED and MTD.
- In **Part B (Combination Expansion Phase)**, the eBED of AZD6918, if <MTD, (possibly MTD also, if significantly greater than eBED) was to be combined with gemcitabine or pemetrexed in 2 separate arms. Two dose levels of each chemotherapy were to be tested, and the highest dose at which <2/6 evaluable patients experienced dose-limiting toxicities (DLT) would be considered the MTD. Hence Part B was designed to assess safety and additional PK data of combination therapy.

No patients were enrolled in Part B of this study; therefore, the remainder of this Synopsis will address methods and results relating only to Part A.

During the Dose Escalation Phase, each dose cohort was to consist of 1 evaluable patient unless the patient experienced either of the following: 1) any CTCAE¹ Grade ≥ 2 non-disease

¹ Common Terminology Criteria Adverse Event, Version 3.0.

Clinical Study Report Synopsis
 Drug Substance AZD6918
 Study Code D2785C00002
 Edition Number 1
 Date 16 June 2009

related adverse event (AE) in the first cycle, or 2) a steady-state minimum AZD6918 plasma concentration of ≥ 80 nM at 24 hours on Day 9; in these cases, a dose cohort was expanded to include 2 additional evaluable patients (ie, a minimum of 3 evaluable patients). Criteria for expanding a cohort beyond 3 patients were outlined in the Clinical Study Protocol; however, there were no cohorts that included more than 3 patients in this study.

The starting dose of 20 mg was based on margins established through pre-clinical mouse and dog toxicology data and estimate of the Minimum Anticipated Biological Effect Level (MABEL). This dose represented $1/60^{\text{th}}$ of the $1/10^{\text{th}}$ the severely toxic dose (STD_{10}) in mice, $1/15^{\text{th}}$ of the no-observed-adverse-effect-level (NOAEL) in dogs and was approximately equivalent to the MABEL for tumor effect in an engineered mouse model. Dose Escalation was to be 100% in 1-patient cohorts until non-disease related CTCAE Grade ≥ 2 occurred during the first cycle. Subsequent dose escalations were to be 50% or 25%.

Target patient population and sample size

The target population included patients ≥ 18 years with histologically confirmed solid malignancy that was recurrent, metastatic, and/or unresectable, and for which standard curative or palliative measures did not exist or were no longer effective. A total enrollment of approximately 75 patients was planned for Parts A and B.

Investigational product and comparator(s): dosage, mode of administration and batch numbers

AZD6918 was supplied as a suspension (10 mg/mL) and administered orally once daily on an empty stomach. It was administered in doses of 20, 40, or 80 mg for the patients treated in this study. The formulations and batch numbers for this open-label investigational product were as follows: 1) Formulation Number F013630, Batch Numbers 2000116836 and 2000117034, and 2) Formulation Number F013650, Batch Numbers 2000117036 and 2000117387.

Duration of treatment

For Cycle 1 only, patients were to receive 21 days of AZD6918, followed by a 1-week observation period. If the patient remained on study, all subsequent cycles were to consist of 28 days of AZD6918 as continuous, once-daily dosing. At the Investigator's discretion, individual patients could receive consecutive treatment cycles provided: (1) they were continuing to benefit, and (2) they did not meet any other withdrawal criteria. Patients were to be followed for safety up to 30 days after last administration of AZD6918.

Criteria for evaluation - pharmacokinetics and efficacy (main variables)

PK variables: *AZD6918 (single dose)*: C_{max} , T_{max} , AUC, AUC (0-24), AUC (0-t), CL/F, V_z/F , $t_{1/2}$
AZD6918 (multiple dose): $C_{\text{max,ss}}$, $T_{\text{max,ss}}$, $C_{\text{min,ss}}$, $T_{\text{min,ss}}$, $C_{\text{avg,ss}}$, AUC $_{\tau,ss}$, AUC(0-t), CL_{ss}/F , V_{ss}/F , $t_{1/2\text{ss}}$, R_{ac} , MRT.

Efficacy variables: investigator assessment of overall best response per RECIST; number of patients with stable disease > 16 weeks.

Clinical Study Report Synopsis
 Drug Substance AZD6918
 Study Code D2785C00002
 Edition Number 1
 Date 16 June 2009

Criteria for evaluation - safety (main variables)

Safety variables: CTCAE grade and type of AEs and DLTs; changes in laboratory values, vital signs (including weight), physical exams, and ECGs (heart rate, PR, R-R and QT intervals and QTc[Fridericia]).

Because of the non-clinical/preclinical signs of reversible ataxia, which might reflect potential neurological activity, this study incorporated clinical safety assessment using the Mini-Mental State Examination (MMSE[®]), and International Co-operative Ataxia Rating Scale (ICARS) to assess for ataxia/cerebellar dysfunction. However, these assessments were not to be used for clinical decision-making in isolation, as the tools have not been validated in the target patient population. Pain assessment was measured on the clinical 0-10 scale, and pain medication use was recorded. The Karnofsky Performance Scale was used to assess a patient's ability to carry out activities of daily living.

As a result of preclinical findings in dogs of transient, dose-related decreases in systemic blood pressure with compensatory increases in heart rate, supine and standing blood pressures and pulse rates were measured at each visit.

Statistical methods

No formal statistical analyses were planned for this study. Because only 1 of the 3 dose levels investigated included more than 1 patient, summary output has been limited to demographic variables, disposition, PK variables, DLTs, and overview of AEs. Patient listings are provided for all variables collected in the study. Summary output, figures, and patient listings are included in the Clinical Study Report.

Subject population

A total of 7 patients were enrolled in this study (ie, signed informed consent); 5 of these received at least 1 dose of AZD6918 and comprised the safety analysis set. One patient was assigned to the 20 mg starting dose cohort and remained on treatment for a total of 112 days (ie, four 28-day cycles). One patient was assigned to the 40 mg escalation cohort and remained on treatment for 42 days. The first patient assigned to the 80 mg escalation cohort experienced two Grade 3 DLTs that were assessed by the investigator as possibly drug-related (see Summary of Safety Results, below). As a result (and per protocol), 2 additional patients were included in the 80 mg cohort. Treatment duration in the 80 mg cohort ranged from 13 to 57 days.

The dosed patients had a mean age of 58.0 years (range: 47 to 71 years). All 5 dosed patients were White and 4 (80%) were women. All patients had metastatic disease, with primary tumor types of colon (1), skin/soft tissue (2), breast (1), and melanoma²(1). Three of the

² In the study database, this patient's primary tumor was incorrectly coded as a CNS tumor; however, this patient had melanoma of unknown primary with metastatic deposits in the pituitary and liver.

Clinical Study Report Synopsis
 Drug Substance AZD6918
 Study Code D2785C00002
 Edition Number 1
 Date 16 June 2009

dosed patients discontinued because "condition under investigation worsened;" the other 2 dosed patients discontinued due to adverse event(s) (DAE).

Summary of pharmacokinetic results

All 5 patients dosed orally with AZD6918 were evaluable for pharmacokinetics. Plasma concentrations of AZD6918 were below the lower limit of quantification (LOQ: 1 ng/mL) at the lowest dose of 20 mg.

At the 40 mg dose level (n=1), exposure of AZD6918 on Day 1 and Day 8 (steady-state) of dosing were low, with values at steady-state of 1.39 ng/mL for C_{max} and 1.46 ng*h/mL for AUC_{0-last} . Following dosing at 80 mg (n=3), AZD6918 was rapidly absorbed with a median $T_{max,ss}$ of 0.75 h (range: 0.50 to 0.78 h). Plasma $C_{max,ss}$ (44.3 ng/mL, CV 270%) and $AUC_{0-last,ss}$ (67.2 ng*h/mL, CV 251%) were approximately 32- and 46-fold higher, respectively, compared to the 40 mg dose, although considerable inter-subject variability was observed. The geometric mean (gmean) $C_{min,ss}$ was 1.11 ng/mL (CV 12%) and was well below the estimated plasma concentration (32 ng/mL) required for biological activity.

The estimated AZD6918 steady-state apparent volume of distribution appeared to be large (V_{ss}/F 7320 L; CV 34%) with an apparent clearance of 1136 L/h (CV 216%). Since the absolute bioavailability (F) is unknown, it is difficult to interpret these findings. Plasma concentrations declined rapidly from C_{max} up to 4 h postdose and then more slowly, with a mean $t_{1/2,ss}$ of 8.64 h (range: 1.51 to 13.0 h).

The observed pharmacokinetic profile is characterized by a multi-exponential decline, lower C_{max} , C_{min} and AUC, and apparently higher clearance than predicted from pre-clinical studies. Based on this pharmacokinetic profile and the variability observed among patients at the 80 mg dose, a very high dose was likely to be necessary to achieve the plasma concentration required for biological activity (estimated to be 32 ng/mL). Since most of the predicted doses were above the maximal absorbable dose, it was judged unlikely that an appropriate biologically efficacious dose (BED) of AZD6918 could be reached.

Summary of efficacy results

All 5 dosed patients had measurable disease at baseline. Of these, 4 had postbaseline tumor assessments performed. There were no complete or partial responses according to RECIST.

Summary of safety results

The MTD for AZD6918 was not identified in this study. All dosed patients had at least 1 AE. There were no neurological AEs. Two patients (D2785C00002/E0001001 and D2785C00002/E0001002) died due to disease progression and also, in the case of Patient D2785C00002/E0001001, to liver dysfunction. Both of these patients also experienced DAEs. Additional details of each of these 2 cases are provided below:

- Patient D2785C00002/E0001001 (female/65 years old/melanoma/80 mg cohort) experienced 2 DLTs (Grade 3 bilateral lower extremity edema and abdominal

Clinical Study Report Synopsis
Drug Substance AZD6918
Study Code D2785C00002
Edition Number 1
Date 16 June 2009

edema); these were considered possibly related to study medication by the Investigator and by the AstraZeneca Study Team physician and Global Safety physician, and they resulted in discontinuation of study medication (DAEs). While these DLTs were not reported as SAEs by the Investigator and, therefore, do not appear as SAEs in the study database, the AstraZeneca Study Team physician and Global Safety physician considered the events to be medically important, and upgraded them to SAEs in the Global Safety database.

- Patient D2785C00002/E0001002 (female/47 years old/breast/80 mg cohort), who had liver metastases, experienced 4 DAEs, none considered related to study medication: Grade 3 elevated alkaline phosphatase, Grade 2 elevated AST and total bilirubin, and Grade 3 abdominal pain.

Although instances of acute, reversible decreases in systolic and/or diastolic blood pressure were recorded in some patients, interpretation of these results was confounded by concomitant anti-hypertensive medications in 3 of the 5 patients. ECG findings were unremarkable; there were no QT or QTc intervals >500 msec. Laboratory, vital signs, and physical exam data did not reveal any major safety concerns.

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No. PCT/GB2011/051357	International filing date (day/month/year) 19.07.2011	Priority date (day/month/year) 19.07.2010
--	--	--

International Patent Classification (IPC) or both national classification and IPC
INV. A61K9/16 A61K47/34 A61K31/444 A61P19/02

Applicant
ASTRAZENECA AB

1. This opinion contains indications relating to the following items:
- ☒ Box No. I Basis of the opinion
 - ☐ Box No. II Priority
 - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - ☐ Box No. IV Lack of unity of invention
 - ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
 - ☐ Box No. VI Certain documents cited
 - ☒ Box No. VII Certain defects in the international application
 - ☒ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:  European Patent Office P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Fax: +31 70 340 - 3016	Date of completion of this opinion see form PCT/ISA/210	Authorized Officer Gómez Gallardo, S Telephone No. +31 70 340-0
---	--	---



WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/GB2011/051357

Box No. I

Basis of the opinion

1.

With regard to the **language**, this opinion has been established on the basis of:

☒

the international application in the language in which it was filed

☐

a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2.

☐

This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3.

With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

a. (means)

☐

on paper

☐

in electronic form

b. (time)

☐

in the international application as filed

☐

together with the international application in electronic form

☐

subsequently to this Authority for the purposes of search

4.

☐

In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5.

Additional comments:

Box No. V

Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1.

Statement

Novelty (N)

Yes: Claims

1-16

No: Claims

17

Inventive step (IS)

Yes: Claims

No: Claims

1-17

Industrial applicability (IA)

Yes: Claims

1-17

No: Claims

2.

Citations and explanations

see separate sheet

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/GB2011/051357

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/GB2011/051357

Re Item V

Reasoned statement with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. CITED DOCUMENTS

Reference is made to the following documents:

- D1 WO 2008/054292 A1 (ASTRAZENECA AB [SE]) 8 May 2008 (2008-05-08)
- D2 US 6 217 911 B1 (VAUGN WILLIAM M [US] ET AL) 17 April 2001 (2001-04-17); cited in the application
- D3 WO 2009/125226 A1 (ASTRAZENECA AB [SE]; ASTRAZENECA UK LTD [GB]) 15 October 2009 (2009-10-15)
- D4 US 2006/140988 A1 (CHEN GUOHUA [US] ET AL) 29 June 2006 (2006-06-29)
- D5 US 2010/016257 A1 (BROWN LAURA J [US] ET AL) 21 January 2010 (2010-01-21)
- D6 WO 99/01114 A1 (EURO CELTIQUE SA [LU]) 14 January 1999 (1999-01-14)
- D7 BUTOESCU N ET AL: "Intra- articular drug delivery systems for the treatment of rheumatic diseases: A review of the factors influencing their performance", EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS, ELSEVIER SCIENCE PUBLISHERS B.V., AMSTERDAM, NL, vol. 73, no. 2, 1 October 2009 (2009-10-01), pages 205-218, XP026652681, ISSN: 0939-6411, DOI: 10.1016/ J.EJPB. 2009.06.009 [retrieved on 2009-06-21]
- D8 US 2009/035250 A1 (DRAPEAU SUSAN J [US] ET AL) 5 February 2009 (2009-02-05)
- D9 WO 2006/082392 A1 (ASTRAZENECA AB [SE]; ASTRAZENECA UK LTD [GB]) 10 August 2006 (2006-08-10); cited in the application

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/GB2011/051357

2. METHOD OF TREATMENT

The patentability of claim 16 is inter alia dependent upon its formulation as well as upon national and regional laws and no unifying criteria are provided in this field by the PCT. Its assessment will be carried out based on the alleged effects of the compounds/compositions searched in the International Search Report.

3. Rule 6.2(a) PCT

Claim 17 contravenes the requirements of Rule 6.2(a) PCT since it relies, in respect of the technical features of the invention, on references to the description ("*generally as herein described*"). Since claim 17 is drafted in indefinite terms, the pharmaceutical depot to which it refers is construed in its broadest sense when assessing the novelty of the present application.

4. CLARITY (Art. 6 PCT)

The use of the term "*about*", in claims 7-10, is indefinite and as such renders the scope of said claims unclear (Art. 6 PCT).

5. NOVELTY (Art. 33(2) PCT)

The present application does not meet the criteria of Article 33(1) PCT because the subject-matter of claim 17 is not new in the sense of Article 33(2) PCT.

All documents D2-D8 disclose pharmaceutical depots. Therefore, the subject-matter of claim 17 is not new in view of D2-D8 (Art. 33(2) PCT).

No document in the prior art discloses a pharmaceutical depot comprising (i) 5-fluoro-2-[[[(1S)-1-(5-fluoro-2-pyridyl)ethyl] amino] -6- [(5-isopropoxy-1H-pyrazol-3-yl) amino]pyridine-3-carbonitrile, or a salt thereof, and (ii) a polymer. Thus, claims 1-16 are novel (Art. 33(2) PCT).

6. INVENTIVE STEP (Art. 33(3) PCT)

6.1. Document D1 is regarded as being the prior art closest to the subject-matter of claim 1, and discloses (cf. passages cited in the search report) the use of (S)-5-fluoro-2-(1-(5-fluoropyridin-2-yl) ethylamino)-6-(5-isopropoxy-1H-pyrazol-3-ylamino)

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/GB2011/051357

nicotinonitrile for treating pain caused by osteoarthritis and rheumatoid arthritis. The above compound may be administered by injection into the joints. The document D9 discloses the same.

The subject-matter of claim 1 differs from D1 in that the pharmaceutical agent (S)-5-fluoro-2-(1-(5-fluoropyridin-2-yl)ethylamino)-6-(5-isopropoxy-1H-pyrazol-3-ylamino)nicotinonitrile is formulated in the form of a pharmaceutical depot comprising a polymer.

The effect of this difference is the controlled and/or sustained release of the pharmaceutical agent so as to maintain a therapeutic level of the drug over an extended period of time (cf. page 4, lines 13-15 of the present application).

The objective technical problem to be solved by the present application may therefore be regarded as the provision of a controlled and/or sustained release formulation of (S)-5-fluoro-2-(1-(5-fluoropyridin-2-yl)ethylamino)-6-(5-isopropoxy-1H-pyrazol-3-ylamino)nicotinonitrile.

All documents D2-D8 (cf. passages cited in the search report) disclose pharmaceutical depots for treating joint diseases such as osteoarthritis and rheumatoid arthritis. Said depots are effective to slowly release the pharmaceutical agent comprised therein so as to achieve a long acting effect. They comprise poly(DL-lactide-co-glycolide) and are injected directly into the affected joint (intra-articularly).

In view of the teaching of D2-D8, the person skilled in the art, faced with the above mentioned objective technical problem, would have been prompted to formulate the compound (S)-5-fluoro-2-(1-(5-fluoropyridin-2-yl)ethylamino)-6-(5-isopropoxy-1H-pyrazol-3-ylamino)nicotinonitrile in the form of a pharmaceutical depot comprising a polymer (such as poly(DL-lactide-co-glycolide)), thereby arriving at the subject-matter of claim 1.

It is also particularly worthy to note that document D1 discloses that (S)-5-fluoro-2-(1-(5-fluoropyridin-2-yl)ethylamino)-6-(5-isopropoxy-1H-pyrazol-3-ylamino)nicotinonitrile may be administered by injection into the joints. In view of the teaching of D2-D8, the skilled person seeking how to formulate (S)-5-fluoro-2-(1-(5-fluoropyridin-2-yl)

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/GB2011/051357

ethylamino)-6-(5-isopropoxy-1H-pyrazol-3-ylamino)nicotinonitrile so as to be injected directly into the joints would consider formulating said therapeutic agent in the form of a depot including a polymer.

For these reasons, the subject-matter of claim 1 appears to lack an inventive step (Art. 33(3) PCT).

6.2. The features of dependent claims 2-6 have already been employed for the same purpose in the documents D2-D8. It would, therefore, be obvious to the person skilled in the art to apply these features with their corresponding effect to the subject-matter of D1, thus arriving at the subject-matter of claims 2-6 (Art. 33(3) PCT).

6.3. The pharmaceutical depot of claims 7-10 is being defined in terms of results to be achieved, namely as being formulated for controlled and/or sustained release of the pharmaceutical agent over a certain period of time. These, however, merely amount to statements referring to the underlying problem with which the application is concerned and are not considered as technical features when assessing the inventive step of the present application. In view of this, claims 7-10 do not fulfill the requirements of Art. 33(3) PCT.

6.4. The features of claims 11-16 are known from D1. Thus, no inventive step can be acknowledged for these claims either (Art. 33(3) PCT).

6.5. Claim 17 is not new and, therefore, is not inventive either (Art. 33(3) PCT).

7. INDUSTRIAL APPLICABILITY (Art. 33(4) PCT)

Claims 1-17 satisfy the criterion of industrial applicability set forth in Art. 33(4) PCT.

Re Item VII

Certain defects in the international application

1. CLARITY (Art. 6 PCT)

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/GB2011/051357

The drawing pages are presently numbered as 1/3, 1/3 and 1/3 whereas they should be renumbered as 1/3, 2/3 and 3/3 (Art. 6 PCT).

Re Item VIII

Certain observations on the international application

1. CLARITY (Art. 6 PCT)

The term "*derivatives*", in claim 2, renders its scope unclear, since it is not known to the skilled reader which structures are intended to be encompassed by this term. Terms like "derivatives" include compounds obtained from another compound by a chemical reaction (including compounds which are structurally remote from the starting material), functional derivatives (such as compounds wherein hetero-atoms are exchanged by alternative atoms), compounds with numerous different types of side groups, etc. Consequently, the subject-matter of claim 2 does not fulfill the requirements of Art. 6 PCT.

ANEXO

El documento que acredita la calidad de REPRESENTANTES LEGALES en Bogotá de los doctores ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ para TODOS LOS ASUNTOS RELACIONADOS CON LA PROPIEDAD INDUSTRIAL de la sociedad peticionaria, que faculta a cada uno de dichos abogados para actuar individualmente, se está presentando con esta solicitud. En consecuencia, ruego a usted reconocer la personería de cada uno de dichos Representantes Legales para actuar dentro del presente expediente, a fin de que cualquiera de ellos pueda intervenir en cualquier tiempo respecto de la solicitud en él contenida.


ALICIA LLOREDA RICAURTE

T.P. No. 53.215

22

TRADUCCION OFICIAL No. 2004-08-09-397 de un documento escrito en Inglés, la cual para efectos del presente lleva el sello personal de **Martha Lucia Arciniegas M.**, Traductora e Intérprete Oficial en virtud de la Res 1523 /88 del Ministerio de Justicia.

Legalización de un Poder de Representación Legal conferido a: ALICIA LLOREDA, y/o GUSTAVO TAMAYO, y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ otorgado por **AstraZeneca AB**

Firmado por:

SIMON RICHARD REEVES

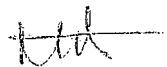
Certificación Notarial

Certificación de la firma precedente, debidamente legalizada por Notario Público,
John Haig Ekserdjian

[Sello Lacrado de Notario Público]

JOHN HAIG EKSERDJIAN

Notario Público de Wilmslow, Cheshire, Inglaterra


MARTHA LUCIA ARCINIEGAS M.
TRADUCTORA E INTERPRETE OFICIAL
RESOLUCION 1523/88 MINJUSTICIA

APOSTILLA

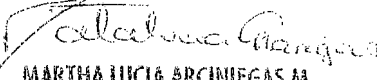
(Convención de La Haya del 5 de Octubre de 1961)

- 1. País: Reino Unido de Gran Bretaña e Irlanda del Norte
- Este documento público
- 2. ha sido firmado por **John Haig Erkserdjan**
- 3. actuando como **Notario Público**
- 4. y lleva el sello/estampilla **del Mencionado Notario Público**

Certificado

- 5. en Londres
- 6. el 2 de agosto del 2004
- 7. Por el Secretario Principal de Su Majestad para Asuntos Exteriores y del Commonwealth
- 8. No. **G479981**
- 9. Timbre/Sello
- 10. Firma
- Aparece el Sello de la Oficina
- E.M. Fitzgerald
- de Asuntos Exteriores y del Commonwealth

Según mi leal saber y entender, ésta en una traducción fiel y completa de su original en inglés, de la cual se deja copia para futuras referencias.


MARTHA LUCIA ARCINIEGAS M.
TRADUCTORA E INTERPRETE OFICIAL
RESOLUCION 1523/88 MINJUSTICIA

Los abajo firmados, AstraZeneca AB, una sociedad organizada y existente de conformidad con las leyes de Suecia,

con domicilio en

SE-15185 Sodertalje, Suecia,

29
The undersigned, AstraZeneca AB, a corporation organized and existing under the laws of Sweden,

domiciled at

SE-15185 Sodertalje
Sweden

nombramos por el presente a ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ, domiciliados en Bogotá, Colombia, con oficinas en dicha ciudad, Calle 72 No. 5-83, piso 6o., nuestros representantes legales y verdaderos en Bogotá, con facultades para recibir notificaciones y nombrar apoderados judiciales o extrajudiciales, con el fin de dar cumplimiento al parágrafo 1o. del artículo 543 del decreto 410 de 1971.

Los doctores ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ, o los apoderados que cualquiera de ellos designe, quedan especialmente facultados para representarnos ante las autoridades colombianas, administrativas, judiciales o de policía, en todos los trámites relativos a la propiedad industrial, a cuyo efecto les facultamos para dar ante dichas autoridades todos los pasos necesarios al objeto indicado; elevar solicitudes, declaraciones, reclamos, recursos y presentar desistimientos; formular descripciones, enmiendas, limitar el alcance de las solicitudes y los registros, presentar oposiciones y apelaciones; instaurar cancelaciones, renunciar total o parcialmente a los registros, abonar todos los impuestos y cuotas y efectuar todo otro pago determinado por la ley; recibir todos los documentos y valores, dando el descargo respectivo; llenar cualesquiera otros requisitos y tomar, en fin, todas las medidas que creyeran conducentes al resguardo de nuestros intereses; y adicionalmente quedan facultados para tomar intervención como demandantes o demandados ante los jueces que sean competentes, pudiendo transar, someter a árbitros, desistir, percibir, conciliar y apelar, con todas las demás facultades que resulten necesarias, y por el presente declaramos desde ahora válido y bueno cuanto dichos representantes y/o los apoderados que cualquiera de ellos nombre hicieren en nuestro beneficio.

Asimismo, facultamos a los doctores ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ, para solicitar en la Oficina de Propiedad Industrial la protocolización de este documento y de cualquier poder que cualquiera de ellos otorgue a nombre nuestro.

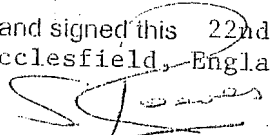
Dado y firmado el 22 de Julio de 2004
en Macclesfield, Inglaterra

hereby appoint, ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ, of Bogota, Colombia, with offices in said town, Calle 72 No. 5-83, Sixth Floor, our true and legal representatives in Bogotá, with power to receive notifications and to appoint judicial or extrajudicial agents, so as to comply with paragraph 1o. of article 543 of decree 410 of 1971.

Doctors ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ, or the agents by any one of them appointed, are especially empowered to represent us before the administrative, judicial or police authorities of Colombia, in all proceedings pertaining to industrial property, for which purpose we authorize them to take before the said authorities all the necessary steps for the said end; to file petitions, declarations, claims, recourses and withdrawals, to draw up specifications; to file amendments, to limit the scope of applications and registrations, file oppositions; file cancellations total or partially waive registrations, to pay all installments and effect all other payments prescribed by law; to receive proper receipts, to comply with all other requirements and, in short, to take all those measures which they may deem fit for the protection of our interests; and additionally we authorize said agents to act on our behalf, as complainants as well as defendants, before the competent judges, with full powers to make arrangements, to submit to arbitrators, to desist, to collect, to conciliate, to appeal and, in short, with all such further powers as may be required, and we hereby declare from now on as valid and good all that said representatives and/or the agents by any one of them appointed may do for our benefit.

We also authorize doctors ALICIA LLOREDA y/o GUSTAVO TAMAYO y/o IGNACIO SANTAMARIA y/o ENRIQUE ALVAREZ, to apply for protocolization of this document and of any power of attorney by any one of them conferred in our name, before the Office of Industrial Property.

Given and signed this 22nd day of July 2004
at Macclesfield, England


SIMON RICHARD REEVES
Duly Authorised Signatory

CERTIFICADO NOTARIAL (SOCIEDAD)

{ss

Hoy 26 de Julio de 2004, compareció
Simon Richard Reeves, a quien conozco y
de quien me consta que firmó el anterior documento,
y quien debidamente juramentado declaró y dijo que
él es firmante autorizado.

de, AstraZeneca AB,
la compañía nombrada en el documento que
antecede, una sociedad debidamente organizada y
existente conforme a las leyes de Suecia

, domiciliada en la
ciudad de Vastra Malarehamnen 9, SE-151
85 Sodertalje, Suecia,
legalmente constituida para un periodo de duración
que aún no ha terminado, que ejerce su objeto social
conforme a las leyes de Suecia

que el declarante está autorizado por dicha
Compañía para conferir este poder; que el sello
estampado por él al pie de tal poder, en nombre y en
representación de la expresada Compañía, es el
sello oficial de ella; y que el objeto para el cual se
confiere este instrumento está comprendido dentro
del de la sociedad.

El suscrito Notario ha visto los Estatutos de
incorporación firmados en Stockholm
, con fecha Oct. 31/1913
y certifica que de conformidad con la legislación de
dicho Estado la anterior declaración juramentada es
prueba suficiente de que los hechos sobre que ella
versa son ciertos o verdaderos, de lo cual da fe.

Notary Public (Notario Público).

NOTARIAL CERTIFICATE (CORPORATION)

{ss

On this 26th day of July, 2004,
personally Simon Richard Reeves
came

to me known, and known to me to be the person who
signed the foregoing instrument, who, being by me
duly sworn did depose and say that he is the a

duly authorised signatory of, AstraZeneca AB,
the company named within, a Corporation organized
and existing under the laws of Sweden

, domiciled at

Vastra-Malarehamnen 9, SE-151 85 Sodertalje,

Sweden

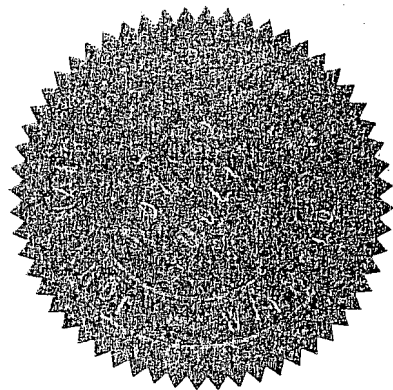
legally constituted for a period of tenure not yet
expired, which exercises its corporate purposes
according to the laws of Sweden

that the deponent is authorized by said Company to
confer this power of attorney; that the seal affixed
hereunto by the deponent in the name and on behalf
of said Corporation is the corporate seal thereof; and
that the purpose for which this instrument is granted
is included within the corporate purposes of the
Company.

The undersigned Notary Public has seen the articles
of Incorporation of said Corporation executed at
STOCKHOLM

under date of 31 October 1913
and hereby certifies that under the laws of said State
the foregoing acknowledgment is sufficient proof that
the facts therein contained are true, which he attests.

J. H. Hsing
Notary Public
Wilkesbarre
Pennsylvania
England



UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND

1. Country: United Kingdom of Great Britain and Northern Ireland
Pays: Royaume-Uni de Grande-Bretagne et d'Irlande du Nord

This public document / Le présent acte public

2. Has been signed by **John Haig Ekserdjian**
a été signé par

3. Acting in the capacity of **Notary Public**
agissant en qualité de

4. Bears the seal/stamp of **The Said Notary Public**
est revêtu du sceau/timbre de

5. at London/à Londres

Certified/Attesté

6. the/le **02 August 2004**

7. by Her Majesty's Principal Secretary of State for Foreign and Commonwealth Affairs /
par le Secrétaire d'Etat Principal de Sa Majesté aux Affaires Etrangères et du Commonwealth.

8. Number/sous No **G479981**

9. Stamp:
timbre:

10. Signature: **E. M. Fitzgerald**



For the Secretary of State / Pour le Secrétaire d'Etat

If this document is to be used in a country which is not party to the Hague Convention of 5 October 1961, it should be presented to the consular section of the mission representing that country. An apostille or legalisation certificate only confirms that the signature, seal or stamp on the document is genuine. It does not mean that the contents of the document are correct or that the Foreign & Commonwealth Office approves of the contents.

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
NIT : 800.176.089-2
-/-

 Industria y Comercio SUPERINTENDENCIA	RECIBO DE CAJA	No. 12 - 136092
		Bogotá D.C., Diciembre 10 de 2012 - 11:29:57

RECIBIDO DE : LLOREDA & CIA. S.A. NI 830.022.145

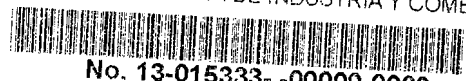
*** Soporte del Pago ***					
TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	VR. PAGO
CONSIGNACION	BANCO DE BOGOTA	062754387	495405666	10/12/2012	9.870.000.00

*** Conceptos Pagados ***			
CANT.	RENTISTICO	CONCEPTO	Vr. UNIDITARIO Vr. CONCEPTO
1	50005-01-09	OTROS SERVICIOS DE PROPIEDAD INDUSTRIAL	99 OTROS SERVCIOS ADMINISTRATIVOS
			500.000.00 500.000.00
			\$500.000.00

SON: **QUINIENTOS MIL PESOS MONEDA CORRIENTE**

Responsable: 
Recibo de Caja Aplicado al Expediente No. _____

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 13-015333- -00000-0000

Fecha: 2013-01-28 16:37:35 Dep. 2020 DIR. NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 81



No. 13-015333-00000-0000

Fecha: 2013-01-28 16:37:35 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 411 PRESENTACION Folios: 31

PATENTE DE INVENCION ☐

MODELO DE UTILI.

☐	Indicación que se solicita una patente.
☐	Datos de identificación del solicitante o de la persona que presenta la solicitud
☐	Descripción de la invención
☐	Dibujos de ser estos pertinentes
☐	Comprobante de pago de las tasas establecidas (De ser el caso formato de descuento)
Completa ☐	Incompleta ☐

PATENTE DE INVENCION PCT ☒

MODELO DE UTILIDAD PCT ☐

Art.33 Decisión 486/00, Circular Única

☒	Indicación que se solicita una PCT
☒	Copia de la solicitud en español, tal como fue presentada inicialmente (capítulo descriptivo, reivindicatorio, resumen)
☒	Dibujos de ser estos pertinentes
☒	Comprobante de pago de las tasas establecidas (de ser el caso formato de descuento)
Completa ☒	Incompleta ☐

DISEÑO INDUSTRIAL ☐

(Art. 119 Decisión 486/00)

☐	Indicación que se solicita Diseño industrial
☐	Datos de identificación del solicitante o de la persona que presenta la solicitud
☐	Representación gráfica y fotográfica del Diseño industrial o muestra del material que incorpora el diseño
☐	Comprobante de pago de las tasas establecidas
Completa ☐	Incompleta ☐

ESQUEMA DE TRAZADO ☐

(Art. 92 Decisión 486/00)

☐	Indicación que se solicita un esquema de trazado
☐	Datos de identificación del solicitante o de la persona que presenta la solicitud
☐	Representación gráfica de un esquema de trazado
☐	Comprobante de pago de las tasas establecidas
Completa ☐	Incompleta ☐