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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. S. D.

Re: Expediente N° 07-010.436

Solicitud de Patente de Invención Titulada: "ANTICUERPOS ANTAGONISTAS DE IL-17"

Solicitante: NOVARTIS A.G.

Nuestra Ref.: P2007/000001

RESPUESTA AL OFICIO No.01049, NOTIFICADO POR FIJACIÓN EN LISTA No. 23 DE FECHA 26 DE ENERO DE 2012

(PRIMER EXAMEN DE FONDO)

CARLOS R. OLARTE, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal previsto en el artículo 45 de la Decisión 486, en los siguientes términos:

1. En primer lugar, con el ánimo de brindar mayor claridad a la materia reivindicada, me permito presentar un **Capítulo Reivindicatorio Modificado**, que reemplaza en su totalidad al Capítulo Reivindicatorio presentado al momento de radicar la presente solicitud.

Así entonces, el Capítulo Reivindicatorio Modificado contiene los siguientes cambios:

- se eliminaron las Reivindicaciones 1, 7, 8, y 11 a 14;
- se limita la expresión "una molécula de enlace de IL-17" por "un anticuerpo de enlace de IL-17 y fragmentos del mismo", lo cual encuentra soporte en el Capítulo Descriptivo, página 6, párrafo 2, líneas 6 a 11;
- se incluye la caracterización de la antigua Reivindicación 1 en las nuevas Reivindicaciones 1 y 2 (Reivindicaciones originales 2 y 3), particularmente, las características funcionales que definen claramente las equivalentes CDR o CDR' directas (al menos un 95% de homología total), lo anterior encuentra soporte en el Capítulo Descriptivo, página 24, líneas 10 a 18;

- se introduce la nueva Reivindicación 7, la cual reclama un set de dos vectores de expresión compatibles capaces de replicarse en una línea celular procariótica o eucariótica, en la cual, un vector codifica por lo menos el dominio V_H del anticuerpo de enlace de IL-17 o un fragmento del mismo, y el otro vector y un vector que codifica al menos el dominio V_L del anticuerpo de enlace de IL-17 o un fragmento del mismo, de acuerdo con las nuevas Reivindicaciones 1 a 3;
- se introducen las nuevas Reivindicaciones 10 a 12, las cuales reclaman modalidades específicas que se encuentran dentro del alcance de las nuevas Reivindicaciones 1 y 2.

Valga anotar que las modificaciones efectuadas al Capítulo Reivindicatorio cumplen a cabalidad con lo estipulado en el Artículo 34 de la Decisión 486, ya que éstas de ninguna manera implican una ampliación de la protección correspondiente a la divulgación contenida en la solicitud inicial, por el contrario, éstas corresponden a una franca limitación de la materia reivindicada.

2. a- La Examinadora señala que las Reivindicaciones 11 a 14 reclaman materia no patentable.

b- Considero superada la anterior objeción, en tanto las Reivindicaciones 11 a 14 se eliminaron del Capítulo Reivindicatorio Modificado adjunto.

3. a- La Examinadora señala que el Capítulo Reivindicatorio presenta los siguientes problemas de claridad y concisión:

- la molécula de enlace de la Reivindicación 1 se define a partir de los parámetros de concentración inicial del antígeno;
- las Reivindicaciones 2 y 3 deben modificarse de manera que se refieran a un anticuerpo caracterizado a partir de todas las regiones CDRs (3 CDR de la cadena pesada, 3 CDR de la cadena ligera);
- las Reivindicaciones 2 y 3 incluyen términos ambiguos como "cuando menos un sitio de enlace" o "sus equivalentes CDR directos";
- el anticuerpo de la Reivindicación 5 debe caracterizarse a partir de las secuencias de las regiones variables de cadena pesada y ligera;
- la construcción de ADN debe caracterizarse a partir de la secuencia de nucleótidos que la conforma;
- la construcción de ADN de las Reivindicaciones 7 y 8 debe referirse a una secuencia particular y no a una sustancialmente homóloga o equivalente, puesto que no es posible establecer la estructura de la secuencia reclamada;

- el vector de expresión de la Reivindicación 9 debe referirse a las secuencias nucleótidas completas que codifican el anticuerpo, por lo tanto, debe retirarse la expresión "comprende cuando menos una construcción";
- el proceso para la producción de la molécula de la Reivindicación 10 debe referirse al anticuerpo de la solicitud, definiendo en su literal a) la célula huésped (o microorganismo) que se transforma con el vector. Así, la referencia a un organismo general carece de sustento pues, de acuerdo con el Capítulo Descriptivo, la transformación se realizó con *E. coli*;
- la composición farmacéutica de la Reivindicación 15 debe referirse a la reivindicación en la cual se define la estructura completa del anticuerpo, identificando todos los elementos que la componen y proporciones de los mismos.

b- i) En primer lugar, llamo la atención de la Examinadora sobre las limitaciones introducidas en el Capítulo Reivindicatorio Modificado adjunto, atendiendo a las objeciones de claridad elevadas. En particular, las Reivindicaciones 1, 7, 8 y 11 a 14 se eliminaron, y se especifica que la "molécula de enlace de IL-17" es particularmente "un anticuerpo de enlace de IL-17 y fragmentos del mismo", lo cual encuentra soporte en el Capítulo Descriptivo, página 6, párrafo 2, líneas 6 a 11.

Adicionalmente, dicho anticuerpo de las nuevas Reivindicaciones 1 y 2 (Reivindicaciones originales 2 y 3), comprende un dominio variable de cadena pesada y un dominio variable de la cadena ligera, cada uno de los cuales se encuentra definido por tres CDR, en las cuales se identifica para cada una la secuencia a la cual corresponde. Por ejemplo, el dominio variable de cadena pesada de la Reivindicación 1 presenta tres CDR (CDR1, CDR2 y CDR3), que tienen las secuencias SEQ ID NO:1, SEQ ID NO:2 y SEQ ID NO:3.

De la misma forma, se incluyó en las nuevas Reivindicaciones 1 y 2 la característica funcional que define las equivalentes CDR o CDR' directas, las cuales presentan al menos un 95% de homología total. Lo anterior encuentra soporte en el Capítulo Descriptivo, página 24, líneas 10 a 18.

En conclusión, las nuevas Reivindicaciones 1 y 2 reclaman de manera apropiada un anticuerpo que contiene seis CDR, tres CDRs de la cadena ligera y tres CDRs de la cadena pesada, definidas específicamente por las secuencias correspondientes. Dichas reivindicaciones contienen anticuerpos que están definidos en dos categorías: 1) seis CDRs, o 2) seis CDRs y la propiedad inhibitoria previamente reclamada. Los anticuerpos que hacen parte de la categoría 2 están

definidos mediante la inclusión de las equivalentes CDR o CDR' directas, lo cual simplemente significa que dichas equivalentes presentan las mismas CDRs pero pueden diferir en cualquier parte de la región variable, mientras presenten la propiedad inhibitoria reclamada. Teniendo en cuenta lo anterior, se introdujeron las nuevas Reivindicaciones 10 a 12, las cuales hacen parte de la categoría 1 y solamente definen los anticuerpos de la invención mediante las secuencias correspondientes.

ii) Habiendo caracterizado de forma clara los anticuerpos de la presente invención en las Reivindicaciones 1 y 2 (y las nuevas Reivindicaciones 10 a 12), considero respetuosamente que limitaciones a las reivindicaciones dependientes constituirían una restricción al concepto inventivo desarrollado por los inventores.

Como es sabido, un inventor está en su derecho de reclamar protección para la totalidad de un concepto inventivo desarrollado **sin importar la amplitud de éste**, siempre y cuando se conserve el principio de unidad de invención. Adicionalmente, vale la pena anotar que el uso de diferentes términos que implican la posibilidad de obtener la invención en diversas modalidades, es una práctica aceptada a nivel mundial en el ámbito de las patentes, ya que una patente de invención no se limita a una única modalidad, si no que cubre un concepto inventivo global que abarca diferentes modalidades (diversas materializaciones de la invención) ligadas por un efecto técnico común que finalmente es el que delimita el alcance de la invención.

Teniendo en cuenta lo anterior, considero que limitar el significado de las reivindicaciones dependientes, tales como las nuevas Reivindicaciones 4 a 6, 8 y 9 (Reivindicaciones originales 5, 6, 9, 10 y 15), las cuales se han limitado de acuerdo con las nuevas Reivindicaciones 1 y 2, sólo limitaría indebidamente la protección de la patente y restringiría el alcance al que el inventor tiene derecho.

Por ejemplo, las nuevas Reivindicaciones 5 a 7 (Reivindicaciones originales 6 y 9, y la nueva Reivindicación 7, respectivamente), que reclaman un constructo de ADN que codifica el anticuerpo debidamente caracterizado en las nuevas Reivindicaciones 1 y 2, define claramente, para una persona medianamente versada en la materia, los límites y alcance de la materia reclamada. De la nueva Reivindicación 6 (Reivindicaciones original 9) se ha eliminado la frase "por lo menos una", con lo cual es claro que el vector de expresión codifica los dominios V_H y V_L del anticuerpo. De la misma forma, la nueva Reivindicación 7 establece que cada vector en el set de vectores codifica el dominio V_H o el dominio V_L del anticuerpo, pero no los dos. La

producción del anticuerpo reclamado se puede alcanzar mediante el uso de un vector que codifica para las dos cadenas V_H y V_L (Reivindicación 6), así como mediante el uso de vectores de expresión, uno que codifica para la cadena V_H y otro para la cadena V_L del anticuerpo (Reivindicación 7).

De la misma forma, la nueva Reivindicación 8 (antigua Reivindicación 10) es dependiente de las Reivindicaciones 6 y 7, lo cual implica que el proceso de manufactura expresa constructos de ADN que codifican particularmente los anticuerpos aquí reclamados. De nuevo, limitar dicho proceso, sólo restringiría el derecho del inventor sobre el proceso desarrollado.

Por último, la nueva Reivindicación 9 (antigua Reivindicación 15) reclama una composición farmacéutica que contiene un anticuerpo de cualquiera de las Reivindicaciones 1 a 4, las cuales se encuentran caracterizadas apropiadamente mediante seis CDR, tres CDRs para el dominio variable de la cadena pesada, y tres CDRs para el dominio variable de la cadena ligera.

En conclusión, y a la luz de las modificaciones realizadas, resulta claro el alcance de la materia reclamada, pudiéndose determinar qué está protegido y qué no. En consecuencia, considero que el Capítulo Reivindicatorio Modificado adjunto cumple con los requisitos de claridad y concisión, permitiéndole a la persona medianamente versada en la materia determinar el alcance de la presente invención.

4. a- La Examinadora señala que la patente WO02/058717 (D1) afecta la novedad de la Reivindicación 1, y el documento "Human IL-17 Antibody Monoclonal Mouse IgG2B Clone #41809[1]" (D2), afecta el nivel inventivo de la Reivindicación 1.

La Examinadora argumenta que D1 divulga moléculas antagonistas a la IL-17 humana, entre ellas, las moléculas de constructo Fc del receptor IL-17 (Ejemplo 1), así como su uso en el tratamiento de artritis. La Examinadora concluye que D1 divulga todas las características esenciales de la materia reclamada en la Reivindicación 1, por lo que carece de novedad.

En cuanto al nivel inventivo, la Examinadora argumenta que D2 divulga el anticuerpo comercial MAB317 que se une a IL-17 humana. Dicho anticuerpo presenta actividad sobre la inhibición de citoquina IL-17 (Tabla 9) en menor proporción al anticuerpo de la presente invención. La principal diferencia entre la materia reclamada en la presente solicitud y D2 es la disminución en

[1] R&D Systems, CAtalog N°: MAB317. http://www.rndsystems.com/pdf/mab317.pdf

la actividad de IL-17 reportada para la molécula aquí reclamada. El efecto que produce dicha diferencia es que la molécula de la presente invención podría tener mayor afinidad por el IL-17 que el anticuerpo de D2.

Sin embargo, la Examinadora señala que no es evidente cómo el anticuerpo de la presente invención presenta mayor afinidad, ya que la diferencia estructural no es clara.

b- i) En primer lugar, llamo nuevamente la atención de la Examinadora sobre las limitaciones realizadas en el Capítulo Reivindicatorio Modificado adjunto, en donde se establece claramente que la presente invención reclama un anticuerpo que contiene seis CDRs, tres CDRs de la cadena ligera y tres CDRs de la cadena pesada que presentan las secuencias SEQ ID NO:1 a 3 y SEQ ID NO:11 a 13 para el dominio variable de la cadena pesada (V_H), y SEQ ID NO:4 a 6 para el dominio variable de la cadena ligera (V_L).

En este punto vale la pena recordar que para fundamentar una afectación válida contra la novedad de una invención, el arte previo debe divulgar explícitamente todas y cada una de las características de la invención.[2] Es más, el Tribunal Andino de Justicia (TAJ) ha determinado que para establecer una objeción válida contra la novedad, la divulgación del arte previo debe ser lo suficientemente detallada para que una persona versada en la materia pueda usar dicha información para reproducir y explotar la invención.[3] En el presente caso, habiendo limitado y caracterizado las secuencias de los anticuerpos de las Reivindicaciones 1 y 2 de la presente invención, es claro que ni D1 ni D2 enseñan o sugieren un anticuerpo que presente los CDRs de las cadenas pesadas y ligeras aquí reclamados, por lo que dichos documentos **no** afectan la novedad de la presente solicitud.

ii) De otra parte, los documentos citados por la Examinadora se refieren o a un anticuerpo murino anti IL-17, o a proteínas de fusión del receptor anti IL-17. D1 divulga una proteína de fusión del receptor IL-17, particularmente una proteína de fusión del receptor IL-17 Fc IgG1, usada en un modelo de artritis en ratón, no un anticuerpo para IL-17, por lo que divulga materia no relacionada con la presente invención. De otra parte, el anticuerpo divulgado en D2 es un anticuerpo murino anti IL-17, particularmente el anticuerpo MAB317.

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[2] Manual Andino de Patentes. (2004). Sección 9.4. (disponible en http://www.comunidadandina.org/public/patentes.pdf).
[3] Proceso 06-IP-89 (http://intranet.comunidadandina.org/Documentos/Procesos/209-IP-2005.doc);
y Proceso 36-IP-2004 (http://www.notinet.com.co/serverfiles/servicios/archivos/30jul04/ca36-04.htm)

En primer lugar es importante reconocer que los anticuerpos murinos presentan una vida media corta *in vivo* en humanos (debido a formación de complejos inmunes), así como penetración limitada en los tejidos objetivo, y una recopilación inadecuada de funciones efectoras del huésped. Entre otros problemas mayores asociados con los anticuerpos murinos, se incluyen: estimulación reducida de citotoxicidad y formación de complejos luego de una administración repetida, los cuales pueden resultar en reacciones alérgicas, y en algunos casos, shock anafiláctico. Adicionalmente, el epítope de unión a un anticuerpo particular (determinado por los CDRs del anticuerpo) y la fuerza y especificidad mediante la cual dicho anticuerpo se une al epítope, determina si el anticuerpo funcionará en un régimen de tratamiento en humanos.

Como se evidencia en los tres resúmenes que acompañan el presente memorial, los anticuerpos de la presente solicitud se unen claramente a un epítope de IL-17 humana, que media el tratamiento en humanos. No hay evidencia alguna sobre la unión del anticuerpo divulgado en D2, que permita el tratamiento en humanos. Es más, incluso si el anticuerpo de D2 se uniera al epítope de IL-17 humana que está involucrado en la mediación de la terapia humana, no existe evidencia alguna de que dicha unión se realice con suficiente fuerza y especificidad para que afecte de alguna manera la terapia humana. Por último, muchos factores están involucrados en la farmacocinética de los anticuerpos monoclonales, tales como, la estructura del anticuerpo, el tamaño, modificaciones, y su inmunogenidicad[4], como se establece por ejemplo en el Anexo 1. Como resultado, mientras los anticuerpos de la presente solicitud tienen claramente valor terapéutico, no existe evidencia de que el anticuerpo murino de D2 presente una actividad o función similar.

iii) Ahora bien, como se evidencia de la presente invención, tal y como se muestra en el Capítulo Descriptivo, página 69, Ejemplo 2, el anticuerpo AIN457 tiene una altísima afinidad por IL-17 humana (K_D = 122± 22 pM) y una alta actividad neutralizante para IL-17 (IC_{50}= 2.1 ± 0.1nM a una concentración de IL-17 humano de 1.87nM en fibroblastos humanos), dicha actividad neutralizante se mide por la capacidad del AIN457 de bloquear la producción de la IL-6 a partir de fibroblastos dérmicos humanos estimulados con IL-17. Así, el anticuerpo AIN457 puede bloquear la señal de IL-17 en una relación molar de, aproximadamente, 1:1, entre los anticuerpos y el blanco. Adicionalmente, y como puede observarse en la Tabla 4, página 74 del Capítulo Descriptivo, el AIN457 es bastante específico para la IL17, incluso en comparación con otros primates. Como se muestra en la Tabla 9 del Capítulo Descriptivo, el

[4] Samaranayake et al "Challenges in monoclonal antibody-based therapies". Annals of Medicine. Vol. 41, pp. 322-331. 2009 (Anexo 1).

AIN457 suprime la secreción de la IL-6 humana dependiente de IL-17 a partir de fibroblastos dérmicos humanos en un grado comparable al receptor señuelo humano IL-17R/Fc, siendo el AIN457 muy superior al anticuerpo monoclonal murino anti IL-17 humano comercialmente disponible de D2 (MAB317).

La Examinador cita el anticuerpo MAB317 de D2 como referencia, sin embargo, la Tabla 9 demuestra claramente que el anticuerpo AIN457 de la presente invención es, aproximadamente, **seis veces más potente** que el anticuerpo MAB317.

Así, el anticuerpo AIN457 de la presente invención, exhibe efectos sorprendentemente superiores e inesperados, los cuales no están descritos, o siquiera sugeridos, en ninguno de los documentos citados (D1 y /o D2). Dichas propiedades sorprendentes y superiores, se podrían esperar de cualquier otro anticuerpo dentro del alcance del Capítulo Reivindicatorio Modificado adjunto, ya que todos los anticuerpos reclamados comparten los seis CDRs que se encuentran en el anticuerpo AIN457.

Por último, me permito adjuntar al presente memorial diferentes resúmenes de varios congresos científicos en los que se muestra la actividad altamente valiosa e importante del anticuerpo AIN457, el cual se encuentra dentro del alcance del Capítulo Reivindicatorio Modificado adjunto:

- Resumen presentado en el ACR/ARHP Annual Scientific Meeting, realizado en Filadelfia, Estados Unidos, el 16-21 de octubre de 2009 (Anexo 2). Este resumen se produce como resultado de un estudio clínico usando AIN457 para el tratamiento de artritis reumatoidea (RA), del cual se concluye que un mayor porcentaje de los pacientes tratados con AIN457 alcanzan una respuesta ACR20[5] comparada con placebo. En adición, las proporciones de ACR50 y ACR70 mostraron un aumento numérico significativo en el tratamiento con AIN457 frente al placebo. Los resultados demostraron que el anticuerpo AIN457, el cual presenta los CDRs aquí reclamados es útil en el tratamiento de artritis.[6]
- Resumen presentado en el decimoctavo congreso de la European Academy of Dermatology and Venereology (EADV), realizado en Berlín el 07-11 de octubre de 2009 (Anexo 3), el cual detalla los resultados de ensayos clínicos realizados usando AIN457 para el tratamiento de psoriasis. Los resultados demostraron que el grupo

[5] Cirterio de respuesta para estudios clínicos sobre artritis reumatoidea.
[6] Se anexa un resumen adicional relacionado con el tratamiento de artritis reumatoide con el anticuerpo AIN457 (Anexo 6).

en tratamiento con AIN457 redujo en un 63% en calificación PASI (Psoriasis Area and Severity Index) mientras que el placebo obtuvo una reducción del 9%. Adicionalmente, los sujetos tratados con AIN457 exhibieron una rápida respuesta en la reducción de PASI tempranamente, a la dos 2 semanas después de la dosificación (P =0.0001 vs. placebo). Este estudio concluyó que el anticuerpo AIN457 es útil en el tratamiento de psoriasis.

- Resumen presentado en la reunión de la ARVO (The Association of Research in Vision and Ophtalmology), llevada a cabo en Fort Lauderdale, Estados Unidos, el 2-6 de mayo de 2010 (Anexo 4), el cual detalla los resultados más recientes de estudios clínicos para determinar el efecto del anticuerpo AIN457 para el tratamiento de uveítis. Este estudio concluyó que el anticuerpo AIN457 es útil en el tratamiento de dicha enfermedad.

Adicionalmente, se adjunta a este memorial, como Anexo 5, un resumen que presenta los resultados del anticuerpo AIN457 en el tratamiento de artritis reumatoidea, psoriasis y uveítis.

Ninguno de los anteriores resultados obtenidos por el anticuerpo AIN457, que se encuentra dentro del alcance de la materia aquí reclamada, podrían haber sido previstos a partir de las enseñanzas de D1 y/o D2, así como tampoco se podía prever sus características particulares.

Lo anterior cobra importancia particularmente porque la evaluación del nivel inventivo debe centrarse únicamente en la cuestión de si, para la persona medianamente versada en la materia, la materia reivindicada habría podido derivarse de manera obvia a partir del arte previo y sin necesidad de investigación adicional alguna. En el presente caso, la persona medianamente versada en la materia, siguiendo las enseñanzas de D2 no habría podido prever la estructura particular de los anticuerpos reclamados, es decir, los CDRs específicos que caracterizan los dominios de las cadenas pesadas y ligeras, y menos aún, habría podido prever la actividad sorprendente e inesperada de los anticuerpos reclamados para el tratamiento de enfermedades como artritis reumatoidea, psosiaris y uveítis.

Finalmente, y teniendo en cuenta que según los lineamientos del Manual Andino de Patentes (MAP), las invenciones en el campo de la química tienen nivel inventivo cuando tienen una estructura inesperada (no derivable por la persona medianamente versada en la materia), o

presentan un efecto técnico inesperado o mejorado impredecible del arte previo[7], la invención aquí reivindicada reviste nivel inventivo.

5. Finalmente, deseo poner bajo su conocimiento que para el concepto inventivo comprendido en la presente solicitud, el privilegio de patente ha sido otorgado hasta el momento por parte de las Oficinas de Patentes de Argentina (AR050200), Australia (AU2005268857 y AU2010201689), Chile (CL46543), Estonia (EE005930), Europa (EP1776142), Grecia (GR3076050), Hong Kong (HK1101277), Japón (4682200), República de Corea (KR852523), Marruecos (MA28982), Nueva Zelanda (NZ552658), **Perú** (PE5590), Pakistán PK140934), Rusia (RU2426741), Singapur (SG155186), Turquía (TR201110270), Taiwán (TWI359153), Estados Unidos (US8119131 y US7807155) y Suráfrica (ZA2007/00242).

Si bien es cierto que las concesiones de patentes en oficinas de otros países no son vinculantes en el sentido que obliguen a la SIC a tomar una decisión en el mismo sentido, considero que dichas concesiones sugieren el cumplimiento de los requisitos de patentabilidad de la materia reivindicada, ya que los criterios de evaluación y estudio aplicados para concluir la aceptación de dichas patentes resultan equivalentes a los que la SIC debe emplear.

6. Por todo lo anterior, considero que las objeciones presentadas por la Examinadors en su examen de fondo han sido resueltas y por consiguiente solicito respetuosamente que el Despacho tenga por cumplidos los requisitos de patentabilidad y proceda con la concesión de la patente.

Atentamente,

CARLOS R. OLARTE
C.C. No. 79.782.747 de Bogotá
T.P. 74.295
Anexo:

- Capítulo Reivindicatorio Modificado.
- Anexo 1: Samaranayake et al "Challenges in monoclonal antibody-based therapies". Annals of Medicine. Vol. 41, pp. 322-331. 2009.

[7] Manual Andino de Patentes. (2004). Sección 10.8.1 (disponible en http://www.comunidadandina.org/public/patentes.pdf).

- Anexo 2: Tak et al., 73rd Annu Sci Meet Am Coll Rheumatol (October 16-21, Philadelphia) 2009, Abst 1922.
- Anexo 3: Stoll, D.; Draelos, Z.; Gold, M.H.; Kircik, L.; et al. 18th Congr Eur Acad Dermatol Venereol (EADV) (October 7-11, Berlin) 2009, Abst P1138.
- Anexo 4: Buggage et al., Annu Meet Assoc Res Vision Ophthalmol (ARVO) (May 2-6, Fort Lauderdale) 2010, Abst 3779/D1066.
- Anexo 5: Hueber (2010) Sci. Transl. Med. 2:52rs72.
- Anexo 6: Durez et al. Annu Eur Congr Rheumatol (EULAR) (June 10-13, Copenhagen) 2009, Abst LB0002)

CAPÍTULO REIVINDICATORIO MODIFICADO
(Primer Examen de Fondo)

1. Un anticuerpo de enlace de IL-17 o fragmento del mismo, que comprende ambos dominios variables de cadena pesada (V_H) y de cadena ligera (V_L); en donde dicho anticuerpo de enlace de IL-17 o fragmento del mismo comprende cuando menos un sitio de enlace de antígeno que comprende:

a) un dominio variable de cadena pesada de inmunoglobulina (V_H), el cual comprende, en secuencia, las regiones hipervariables CDR1, CDR2, y CDR3, dicha CDR1 tiene la secuencia de aminoácidos de la SEQ ID NO:1, dicha CDR2 tiene la secuencia de aminoácidos de la SEQ ID NO:2, y dicha CDR3 tiene la secuencia de aminoácidos de la SEQ ID NO:3, o sus equivalentes de CDR directas; y

b) un dominio variable de cadena ligera de inmunoglobulina (V_L), el cual comprende, en secuencia, las regiones hipervariables CDR1', CDR2', y CDR3', dicha CDR1' tiene la secuencia de aminoácidos de la SEQ ID NO:4, dicha CDR2' tiene la secuencia de aminoácidos de la SEQ ID NO:5, y dicha CDR3' tiene la secuencia de aminoácidos de la SEQ ID NO:6, o sus equivalentes de CDR' directas.

en donde el dominio V_H que comprende una o más CDR directas equivalentes, tiene al menos un 95% de homología total con la secuencia del dominio V_H especificado en a) y el dominio VL que comprende una o más CDR' directas equivalentes tiene al menos un 95% de homología total con la secuencia del dominio V_L especificado en b), comprendiendo una CDR1', CDR2' y CDR3'; y en donde el anticuerpo de enlace de IL-17 o fragmento del mismo comprende uno o más equivalentes directos es capaz de inhibir la actividad la IL-17 humana 1 nM en una concentración menor a 5 nM por el 50%, en donde dicha actividad inhibidora se mide sobre la producción de IL-6 inducida por huIL-17 en fibroblastos dérmicos humanos, y donde las secuencias CDR están de acuerdo con la definición Kabat.

2. Un anticuerpo de enlace de IL-17 o un fragmento del mismo, que comprende ambos dominios variables de cadena pesada (V_H) y de cadena ligera (V_L); en donde dicho anticuerpo de enlace de IL-17 o fragmento del mismo comprende cuando menos un sitio de enlace de antígeno que comprende:

a) un dominio variable de cadena pesada de inmunoglobulina (V_H), el cual comprende, en secuencia, las regiones hipervariables CDR1-x, CDR2-x, y CDR3-x, dicha CDR1-x tiene la secuencia de aminoácidos de la SEQ ID NO:11, dicha CDR2-x tiene la secuencia de aminoácidos de la SEQ ID NO:12, y dicha CDR3-x tiene la secuencia de aminoácidos de la SEQ ID NO:13, o sus equivalentes de CDR-x directos; y

b) un dominio variable de cadena ligera de inmunoglobulina (V_L), el cual comprende, en secuencia, las regiones hipervariables CDR1', CDR2', y CDR3', dicha CDR1' tiene la secuencia de aminoácidos de la SEQ ID NO:4, dicha CDR2' tiene la secuencia de aminoácidos de la SEQ ID NO:5, y dicha CDR3' tiene la secuencia de aminoácidos de la SEQ ID NO:6, o sus equivalentes de CDR' directos;

en donde el dominio V_H que comprende una o más CDR directas equivalentes, tiene al menos un 95% de homología total con la secuencia del dominio V_H especificado en a) y el dominio VL que comprende una o más CDR' directas equivalentes tiene al menos un 95% de homología total con la secuencia del dominio V_L especificado en b), comprendiendo una CDR1', CDR2' y CDR3'; en donde el anticuerpo de enlace de IL-17 o fragmento del mismo comprende uno o más equivalentes directos es capaz de inhibir la actividad la IL-17 humana 1 nM en una concentración menor a 5 nM por el 50%, en donde dicha actividad inhibidora se mide sobre la producción de IL-6 inducida por huIL-17 en fibroblastos dérmicos humanos, y donde las secuencias CDR están de acuerdo con la definición Chotia.

3. El anticuerpo de enlace de IL-17 o un fragmento del mismo, de acuerdo con cualquiera de las Reivindicaciones 1 o 2, la cual es un anticuerpo humano recombinante.

4. Un anticuerpo de enlace de IL-17 o fragmento del mismo, el cual comprende cuando menos un sitio de enlace de antígeno que comprende:

a) un primer dominio que tiene una secuencia de aminoácidos 95% homóloga a la mostrada a la SEQ ID NO:8, empezando con el aminoácido en la posición 1, y finalizando con el aminoácido en la posición 127; o

b) un primer dominio que tiene una secuencia de aminoácidos 95% homóloga a la mostrada a la SEQ ID NO:8, empezando con el aminoácido en la posición 1, y finalizando con el aminoácido en la posición 127, y un segundo dominio que tiene una secuencia de aminoácidos sustancialmente homóloga a la mostrada en la SEQ ID NO:10, empezando con el aminoácido en la posición 1, y finalizando con el aminoácido en la posición 109, en donde el anticuerpo de enlace de IL-17 o fragmento del mismo comprende uno o más equivalentes directos es capaz de inhibir la actividad la IL-17 humana 1 nM en una concentración menor a 5 nM por el 50%, en donde dicha actividad inhibidora se mide sobre la producción de IL-6 inducida por huIL-17 en fibroblastos dérmicos humanos.

5. Un constructo de ADN que codifica un anticuerpo de enlace de IL-17 o un fragmento del mismo, de acuerdo con cualquiera de las Reivindicaciones 1 a 4.

6. Un vector de expresión capaz de replicarse en una línea celular procariótica o

eucariótica, el cual comprende el constructo de ADN de acuerdo con la Reivindicación 5.

7. Un set de dos vectores de expresión capaces de replicarse en una línea celular procariótica o eucariótica, un vector codifica por lo menos el dominio V_H del anticuerpo de enlace de IL-17 o un fragmento del mismo de acuerdo con las Reivindicaciones 1 a 3, y un vector que codifica al menos el dominio V_L del anticuerpo de enlace de IL-17 o un fragmento del mismo de acuerdo con las Reivindicaciones 1 a 3.

8. Un proceso para la producción de un anticuerpo de enlace de IL-17 o un fragmento del mismo, el cual comprende: (i) cultivar un organismo que se transforma con el vector de expresión de acuerdo con la Reivindicación 6 o el set de vectores de expresión de acuerdo con la Reivindicación 7; y (ii) recuperar el anticuerpo de enlace de IL-17 del cultivo.

9. Una composición farmacéutica, la cual comprende un anticuerpo para IL-17 de acuerdo con las Reivindicaciones 1 a 4, en combinación con un excipiente, diluyente, o vehículo farmacéuticamente aceptable.

10. El anticuerpo de enlace de IL-17 de acuerdo con la Reivindicación 1, que comprende:

a) un dominio variable de cadena pesada de inmunoglobulina (V_H), el cual comprende, en secuencia, las regiones hipervariables CDR1, CDR2, y CDR3, dicha CDR1 tiene la secuencia de aminoácidos de la SEQ ID NO:1, dicha CDR2 tiene la secuencia de aminoácidos de la SEQ ID NO:2, y dicha CDR3 tiene la secuencia de aminoácidos de la SEQ ID NO:3;y

b) un dominio variable de cadena ligera de inmunoglobulina (V_L), el cual comprende, en secuencia, las regiones hipervariables CDR1', CDR2', y CDR3', dicha CDR1' tiene la secuencia de aminoácidos de la SEQ ID NO:4, dicha CDR2' tiene la secuencia de aminoácidos de la SEQ ID NO:5, y dicha CDR3' tiene la secuencia de aminoácidos de la SEQ ID NO:6.

11. El anticuerpo de enlace de IL-17 de acuerdo con la Reivindicación 1, que comprende:

a) un dominio variable de cadena pesada de inmunoglobulina (V_H), el cual comprende, en secuencia, las regiones hipervariables CDR1-x, CDR2-x, y CDR3-x, dicha CDR1-x tiene la secuencia de aminoácidos de la SEQ ID NO:11, dicha CDR2-x tiene la secuencia de aminoácidos de la SEQ ID NO:12, y dicha CDR3-x tiene la secuencia de aminoácidos de la SEQ ID NO:13; y

b) un dominio variable de cadena ligera de inmunoglobulina (V_L), el cual

comprende, en secuencia, las regiones hipervariables CDR1', CDR2', y CDR3', dicha CDR1' tiene la secuencia de aminoácidos de la SEQ ID NO:4, dicha CDR2' tiene la secuencia de aminoácidos de la SEQ ID NO:5, y dicha CDR3' tiene la secuencia de aminoácidos de la SEQ ID NO:6.

12. El anticuerpo de enlace de IL-17 o un fragmento del mismo, de acuerdo con cualquiera de las Reivindicaciones 10 o 11, la cual es un anticuerpo humano.

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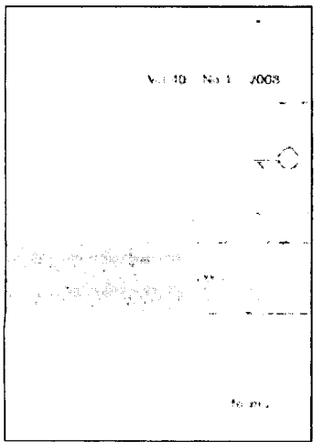

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Challenges in monoclonal antibody-based therapies

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REVIEW ARTICLE

Challenges in monoclonal antibody-based therapies

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Abstract

Therapeutic monoclonal antibodies (mAbs) are the fastest growing class of new therapeutic molecules. They hold great promises for the treatment of a variety of diseases, including chronic inflammatory diseases and cancer. However, the current manufacturing and purification processes cause limitations in the production capacity of therapeutic antibodies, leading to an increase in cost. Genetic delivery of therapeutic monoclonal antibodies by *in vivo* production offers a new potential solution to these problems. Firstly, therapeutic efficacy can be improved by maintaining stable therapeutic, non-toxic levels within the blood circulation over a long period of time. Repeated high-dose bolus injections could be avoided, thereby reducing the possibility of side-effects. Secondly, the high cost of manufacturing and purification of the therapeutic antibodies could be reduced, making an *in vivo*/*ex vivo* mAb gene transfer an economically viable and attractive option.

In general, three approaches can be used for the stable long-term expression and secretion of therapeutic antibodies *in vivo*: 1) direct *in vivo* administration of integrating vectors carrying a mAb gene, 2) grafting of *ex vivo* genetically modified autologous cells, and 3) implantation of an encapsulated antibody producing heterologous or autologous cells.

This paper describes the key factors and problems associated with the current antibody-based immunotherapies and reviews prospects for genetic *in vivo* delivery of therapeutic antibodies.

Key words: *Antibody therapy, cell encapsulation, genetic delivery, in vivo production, safety*

Introduction

Behring and his colleagues probably conducted the first antibody-based therapy in 1890. They immunized rats, guinea-pigs, and rabbits with attenuated forms of infectious agents causing diphtheria and tetanus. Serum produced in these animals was injected into non-immunized animals that were previously infected with the fully virulent bacteria. By doing so, the ill animals could be cured through the administration of the serum, and it was already in the following year the first successful therapeutic serum treatment of a child suffering from diphtheria occurred. Since then γ-globulin and related therapies have become clinical practice.

But it was not until 1975 that it became possible to produce identical antibodies specific to a given antigen using the mouse hybridoma technology described by Köhler and Milstein (1). It paved the way for the emergence of therapeutic monoclonal antibodies (mAbs). However, the utility of these murine antibodies as human therapeutics turned out to be limited. Two major problems plagued the use of murine mAbs: First, although they had great specificity for their targets, they did not always trigger human effector functions (e.g. complement system or professional phagocytes). Secondly, murine antibodies were recognized by the immune system, and they stimulated the production of human anti-mouse antibodies (HAMA response). Also, the therapeutic use of murine antibodies was limited by their short serum half-life (2). An exception to these is the mouse monoclonal antibody orthoclone OKT3 used in the prevention of organ graft rejection. It also became obvious that the incidence of immunogenicity is variable and depends

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Key messages

- Therapeutic monoclonal antibodies (mAbs) are the fastest growing class of new therapeutic molecules.
- Current manufacturing and purification processes cause limitations in the production capacity of therapeutic antibodies.
- Genetic delivery of therapeutic monoclonal antibodies by *in vivo* production offers a new potential solution in antibody therapy.

on the nature of the target antigen, disease process being treated, and the schedule of administration. It was shown that about 50%–70% of patients with solid tumours develop human anti-mouse antibodies after exposure to mouse antibodies, whereas only about 30% of patients with relapsed B-cell malignancies treated with mouse antibodies developed HAMA.

Current therapeutic antibodies

Monoclonal antibodies now comprise the majority of recombinant proteins in clinical testing, with more than 150 trials worldwide (2). About a quarter of new biotech drugs in development are monoclonal antibodies (3). These entities represent also the fastest growing class of new therapeutic molecules (4). Seven of the approved monoclonal antibody products each had global sales of over US$1 billion in 2006. Three of the products, infliximab and adalimumab, both indicated for the treatment of autoimmune disorders, and rituximab indicated for non-Hodgkin's lymphoma, B-cell leukaemia, and autoimmune disorders, had sales over US$2 billion each. Global sales expectation for monoclonal antibodies is around US$16 billion by 2010 (5) (Table I).

The first mAb approved for human therapy was the murine monoclonal antibody, muromonab-CD3 (Orthoclone OKT3) in June 1986, for the treatment of transplant rejection. The platelet aggregation inhibitor, abciximab (ReoPro) was the first chimeric and the first mAb fragment to be approved by the Food and Drug Administration (FDA), in December 1994. The first humanized mAb approved was daclizumab (Zenapax) in December 1997 for the prevention of transplant rejection. The first human mAb approved by FDA was adalimumab (Humira) in December 2002, for the treatment of rheumatoid arthritis and other inflammatory conditions. Of the 21 FDA-approved mAbs, 3 are murine, 5 are chimeric, 11 are humanized, and 2 are human mAbs (Table I). Gemtuzumab ozogamicin conjugated with immunotoxin calicheamicin was approved in 2000, and ibritumomab tiuxetan (Zevalin) was the first approved mAb conjugated with the radionuclide yttrium-90 in 2002, for the treatment of acute myelogenous leukaemia and non-Hodgkin's lymphoma, respectively. In 1997, rituximab (Rituxan), the first mAb for the treatment of cancer, was approved by the FDA (Table I).

By March 2008, 21 therapeutic mAbs had been approved by the FDA for human therapy in both haematological and solid tumours, and autoimmune, inflammatory, infectious, and cardiovascular diseases (6–8) (Table I). Non-Hodgkin's lymphoma, metastatic breast cancer, acute myeloid leukaemia, chronic lymphocytic leukaemia, and metastatic colorectal cancer are some of the cancers where mAbs are indicated for therapy (2,8,9). Acute transplant rejection is the major autoimmune indication, with Crohn's disease and rheumatoid arthritis being inflammatory indications for therapy. In addition to these, mAbs are used for the treatment of respiratory syncytial viral infection, prevention of blood clotting, in refractory unstable angina, and as a snake venom antidote (8,9).

Genetic engineering of mAbs

In an attempt to reduce immunogenicity of mouse antibodies, genetic engineering was used to generate chimeric antibodies, that is, antibodies with human constant regions and mouse variable regions. This technique has been central to the clinical use of antibodies. Chimeric antibodies were less immunogenic, with the ability to trigger human effector functions and increased circulation half-life. Even though chimeric antibodies were perceived as less foreign, and therefore less immunogenic, human anti-chimeric antibody responses (HACAs) have been observed (10). Further minimization of the mouse component of antibodies was achieved through complementarity-determining region (CDR) grafting. In such 'humanized' antibodies, only the CDR loops that are responsible for antigen binding are inserted into the human variable-domain framework. The ability to manipulate antibodies into more human-like variants finally made antibodies in the main stream of clinical use. With the isolation of genes encoding for human variable regions, their successful expression in *Escherichia coli*, and the introduction of phage-display technology along with the developments in hybridoma technology, the task of selecting fully human variable domains has been greatly simplified (11). Recent advancements in transgenic

Table I. Monoclonal antibodies approved by the US Food and Drug Administration (FDA).

	Generic name	Trade name	Antibody category	Therapeutic category	First approval date (US FDA)	Company	Sales 2006 (US$ millions)
1	Muromonab-CD3	Orthoclone OKT3	Murine, IgG2a, anti-CD3	Immunological	19 Jun 86	Johnson & Johnson (J&J)/Ortho Biotech	n/a
2	Abciximab	ReoPro	Chimeric, IgG1, anti-GPIIb/IIIa; Fab	Haematological	22 Dec 94	Centocor/J&J	n/a
3	Rituximab	Rituxan	Chimeric, IgG1κ, anti-CD20	Oncological	26 Nov 97; 02 Jun 98 (EU)	Genentech/Roche/Biogen Idec	2,037 (US only)
4	Daclizumab	Zenapax	Humanized IgG1κ, anti-CD25	Immunological	10 Dec 97; 26 Feb 99 (EU)	Hoffman-La Roche	n/a
5	Basiliximab	Simulect	Chimeric, IgG1κ, anti-CD25	Immunological	12 May 98; 09 Oct 98 (EU)	Novartis	n/a
6	Palivizumab	Synagis	Humanized, IgG1κ, antirespiratory syncytial virus	Anti-infective	19 Jun 98; 13 Aug 99 (EU)	MedImmune	1,100 (global)
7	Infliximab	Remicade	Chimeric, IgG1κ, anti-TNFα	Immunological	24 Aug 98; 13 Aug 99 (EU)	Centocor/J&J	3,013 (J&J-global);1,239 (Sc-P-global)
8	Trastuzumab	Herceptin	Humanized, IgG1κ, anti-HER2	Oncological	25 Sep 98; 28 Aug 00 (EU)	Genentech/Roche	1,230 (US only)
9	Gemtuzumab ozogamicin	Mylotarg	Humanized IgG4κ, anti-CD33; immunotoxin	Oncological	17 May 00;	Wyeth	n/a
10	Alemtuzumab	Campath-1H	Humanized, IgG1κ, anti-CD52	Oncological	07 May 01; 06 Jul 01 (EU)	Genzyme	n/a
11	Ibritumomab tiuxetan	Zevalin	Murine, IgG1κ, anti-CD20; yttrium-90 conjugated	Oncological	19 Feb 02; 16 Jan 04 (EU)	Biogen Idec	n/a
12	Adalimumab	Humira	Human, IgG1κ, anti-TNFα	Immunological	31 Dec 02; 01 Sep 03 (EU)	Abbott	2,040 (global)
13	Omalizumab	Xolair	Humanized, IgG1κ, anti-IgE	Immunological	20 Jun 03; 27 Oct 05 (EU)	Genentech/Novartis/Roche	425 (US only)
14	Tositumomab-I131	Bexxar	Murine, IgG2aλ, Iodine131	Oncological	27 Jun 03	Corixa	n/a
15	Efalizumab	Raptiva	Humanized, IgG1κ, anti-CD11a	Immunological	27 Oct 03; 20 Sep 04 (EU)	Genentech/Roche	90 (US only)
16	Cetuximab	Erbitux	Chimeric, IgG1κ, anti-EGFR	Oncological	12 Feb 04; 29 Jun 04 (EU)	Imclone Systems/ Bristol-Myers Squibb/Merck	652 (US only); 1,100 (global)
17	Bevacizumab	Avastin	Humanized, IgG1, anti-VEGF	Oncological	26 Feb 04; 12 Jan 05 (EU)	Genentech/Roche	1,750 (US only); 1,250 (Q1-Q3-global)
18	Natalizumab[a]	Tysabri	Humanized, IgG4κ, anti-α 4-integrin	Immunological	23 Nov 04; 29 Jun 06 (EU)	Biogen	8 (Q1-Q3; US only)
19	Ranibizumab	Lucentis	Humanized, IgG1κ fragment, anti-VEGF-A	AMD	30 Jun 06; 24 Jan 07 (EU)	Genentech	380 (US only)
20	Panitumumab	Vectibix	Human, IgG2κ, anti-EGFR	Oncological	27 Sep 06	Amgen	39 (Q4-US only)
21	Eculizumab	Soliris	Humanized IgG 2/4 against complement protein C5	Haematological, immunological	16 Mar 07; 20 Jun 07 (EU)	Alexion Pharmaceutical Inc.	

[a]Voluntary suspension from the market from 28 February 2005 and FDA approval for remarketing under restricted distribution programme from 5 June 2006. Approved for the maintenance treatment of moderate to severe Crohn's disease in January 2008.

AMD = age related macular degeneration; EGFR = epidermal growth factor; EU = European Union approval status and dates; J&J = Johnson & Johnson; n/a = sales data not available for year 2006; Q = quarter; Sc-P = Schering- Plough; TNFα = tumour necrosis factor α; VEGF = vascular endothelial growth factor.

animal techniques too enabled the production of fully human mAbs in animals such as humanized mouse (12).

Technical advances in antibody development further enabled the generation of modified antibodies with enhanced properties and less immunogenicity (4). Different types of variable domain architectures, such as diabodies, triabodies, and bispecific diabodies, are engineered to better serve specific therapeutic applications. Bivalent antibody-binding fragments (F(ab)2), single chain variable fragments (scFv), minibodies with two scFv regions fused to heavy-chain constant region (C_H3), and bispecific diabodies with two different scFv regions are some examples of modified mAbs.

Limitations of current antibody therapies

Production

Different methods using hybridoma technology in bacterial, yeast, and mammalian cell lines and transgenic animals are used in the production of different types of mAbs. In all these methods the biological materials used in the production process have to be free of microbial contamination by mycoplasma, fungi, bacteria, viruses, and prions. Animals have to be specific pathogen-free (SPF) quality. Currently, recombinant expression technology in mammalian cell culture is the principal means for the commercial production of antibodies. Human mAbs produced in cell lines immortalized by introduction of Epstein-Barr virus (EBV) could carry a potential risk of cancer. Risk of contamination of different cell lines, production of endogenous immunoglobulin chains, genetic instability of the cell line, and level and consistency of the expression of both heavy and light chains are other aspects that need further attention. Another problem encountered during mAb production is to find ways of generating stoichiometric amounts of both heavy and light chains in the production cell lines, because such imbalances in the chain production can be toxic to cells or can result in non-functional immunoglobulin chains in the supernatant that can complicate the purification process (13).

Tedious purifications, characterizations, and validations have to be done during the production process for the establishment of consistency with regard to identity, purity, and potency between different batches. Changes or adaptations in the production process such as transition of *in vivo* production to *in vitro* production, changes in culture procedure or culture conditions, changes in purification procedure, different types of fermentation technology in different plants, hollow fibre versus suspension culture, or additional modifications of the antibody molecule can lead to an altered form of the antibody with compromised specificity (5,14).

Apart from the high material cost and long cycle times, the mammalian cell culture-based production systems provide a limited control over the glycosylation of the mAbs (15). The glycosylation profile is a major determinant of the stability, biological activity including receptor binding and effector function, pharmacokinetics, biodistribution, and solubility of mAbs. The glycosylation pattern is dependent on the protein's three-dimensional structure, host cell enzymatic system in the endoplasmic reticulum and Golgi apparatus, efficiency of protein expression, physiological status of the host cells, availability of the sugar-nucleotide donors, and the fermentation conditions (16–18).

Due to the need for high doses over a long period of time, production capacity of mAbs in mammalian cell cultures may become a limiting factor (5,19–22) with forecasts of a shortage of the capacity for the production in near future (5,8). Transgenic animals provide an alternative and potentially a more economical source for the production as compared to traditional cell culture and fermentation systems especially when needed in high doses for a longer period of time (23).

Cost

The start-up cost for six 15,000-litre bioreactors will be in the range of 100 million euros. In addition, investments in the range of 300–500 million euros along with the need to hire and train 400–500 employees will be required every 5–10 years (5). Large-scale production of monoclonal antibodies needs economical purification processes with high throughput that can deliver a high yield and process reliability while producing an extremely pure product (24). The success of production systems depend on intrinsic characteristics of hybridomas such as cell growth and antibody production ability in large-scale production (3).

The cost of therapy is a major limiting factor associated with mAbs, with monthly costs of certain drugs reaching nearly US$10,000 and annual costs of about US$100,000 per patient leading to serious concerns (25). Both the high doses and the longer period of time have aggravated the issue within the industry justifying the high cost as the inherent value of life-sustaining therapies (6,8,25). Monoclonal antibodies are also differentially priced according to the therapeutic category. Monoclonal antibodies for oncological conditions with a smaller market are

priced much more highly than those for immunological diseases with a larger market. High treatment costs have raised the necessity for biosimilars, although these have not been successful yet due to complexity of production, lack of regulatory pathways for approval, and patent protection covering all the recently approved products (2).

Pharmacokinetics

Many factors, such as antigen distribution and antigen concentration, antibody structure, size and engineering, host factors, concurrent medications and immunogenicity, are involved in the pharmacokinetics of monoclonal antibodies (7,26,27). The half-lives of murine antibodies are much shorter than chimeric or humanized antibodies in man: murine (2–3 days) <chimeric (8–10 days) <humanized (20–23 days) (7). Smaller modified antibodies are rapidly cleared from plasma (half-life 0.5–30 hours) due to the lack of Fc domain, though they have a better penetration ability especially in the treatment of cancer (9,28,29). In fact, only 0.001%–0.01% of the injected antibody dose accumulates per gram of solid tumour in patients (30).

Elimination of mAbs might be altered by interactions with the target antigen which manifests as a dose-dependent clearance rate and half-life, with a shorter half-life at low antibody concentrations that do not saturate the antigens, whereas an increased half-life and decreased clearance rate is observed with a higher concentration that can saturate the antigens (7). Monoclonal antibodies show nonlinear pharmacokinetic and pharmacodynamic properties which are not properly understood with individual variability (31). Effects of demographic variables, such as age, gender, and renal and hepatic function, on the pharmacokinetics of monoclonal antibodies are somewhat controversial (7). Large proteins in general have slower kinetics than small molecules with limited tissue penetration properties. Some concomitant medications, such as methotrexate, azothioprine, and mycophenolate mofetil, have been reported to reduce the clearance of some antibodies, and paclitaxel has been shown to increase serum concentrations of some antibodies (3).

Safety

Most of the current mAbs have to be administered in large doses, ranging from 5 to 20 milligrams per kilogram body weight at a time, to achieve a high and sustained therapeutic plasma concentration of around several hundred μg per millilitre. This is not only leading to problems such as high cost, but also to problems related to administration, bioavailability, and increased immunogenicity (3,5,8,22,25, 32–38). Most mAbs have to be given as hour-long intra-venous (i.v.) infusions needing hospital environments with skilled staff and equipment, and repeated over a long period of time (8). Due to infusion-based treatment the concentration of the molecule varies between subtoxic to subtherapeutic levels resulting in variations in bioavailability with a higher probability of neutralizing anti-idiotypic immune response (8).

HAMA response results in rapid elimination of mouse antibodies from the host with a reduced efficiency, possible immune complex damage to the kidneys, anaphylaxis, and allergic reactions (2,3,7,9,13,39,40). These were reduced with the development of chimeric antibodies, humanized antibodies (41), and the development of fully human antibodies through transgenic mice (42,43) or through generation of human hybridomas (9,39, 41). Two types of HAMA responses have been reported: anti-isotypic and anti-idiotypic (3). All mAbs in clinical use have exhibited some level of immunogenicity (7). Chimeric, humanized, and human mAbs have demonstrated immunogenicity rates of 5%–10% (2,43). The protein structure, immune modulatory effect of protein, formulation, contaminants and impurities, route, dose, frequency and duration of administration, major histocompatibility complex (MHC) genotype of the patient, associated diseases, and concomitant therapy are factors that directly affect immunogenicity, whereas timing and frequency of sampling, assay method, and expression of titres are factors that affect the measured immune response (38). Identification of anti-monoclonal antibodies is highly dependent on the sensitivity and specificity of the assay, making comparisons difficult (2). Lack of precise methods to determine immunogenicity and lack of specific guide-lines for immunogenicity assay validation are major limitations in the antibody therapy (44).

Like all other classes of therapeutic agents, monoclonal antibodies can cause side-effects. These effects are related to one of three major mechanisms: xenogenic nature of the antibodies, suppression of physiological functions in line with the specificity of the antibody, and activation of inflammatory cells and mediators after binding to the target (3). Monoclonal antibodies are generally considered less toxic than cytotoxic chemotherapeutic agents used in cancer treatment. Mechanism-independent toxicities are due to hypersensitivity reactions and can rarely be severe enough to cause anaphylactic reactions.

Mechanism-dependent toxicities include cardiac toxicity with trastuzumab (40,45), first-dose toxicity related to rapid lysis of malignant cells by rituximab (46), skin eruptions with cetuximab (47,48), and hypertension, bleeding, thrombosis, and proteinuria with bevacizumab (49). Hypersensitivity reactions leading to serum sickness are generally rare, but fatal infusion reactions have been reported with some mAbs (3).

Potential improvements of future antibody therapies

Because of the limiting factors described above, as well as the inconvenience of weekly infusions over a long period of time, new technological solutions have been explored. One solution to the problems mentioned above could be the use of gene transfer technology for the production of therapeutic antibodies *in vivo*.

The aim of genetic delivery of therapeutic mAb genes is to avoid the costly and laborious procedure of repeated intravenous mAb infusions. The genetic delivery of mAb genes can be achieved with viral and non-viral gene transfer methods. In an optimal situation, a single vector injection would result in a continuous and stable release of low but therapeutically relevant quantities of mAbs within the patient. Monoclonal antibodies produced by the patient have the advantage of being potentially less immunogenic than recombinantly produced mAbs with an improved bioavailability of the therapeutic proteins. Possible side-effects and immune responses against therapeutic proteins could be minimized by avoiding repeated administration of high doses of these therapeutic antibodies.

Stable long-term expression and secretion of therapeutic antibodies *in vivo* can be achieved *in vivo* by three approaches: 1) direct *in vivo* administration of mAb gene carrying vectors, 2) grafting of *ex vivo* genetically modified autologous cells, and 3) implantation of *ex vivo* transduced cells, encapsulated within artificial semi-permeable membranes (Figure 1).

The ability to regulate the antibody production very tightly and consistently is essential if the therapy is no longer required or in the case of development of undesired side-effects. Tetracycline-dependent transcriptional switches, *lac* operator, rapamycin and progesterone analogue-inducible systems have been successfully employed in several preclinical gene therapy studies using different vector systems (50), though the authors could not find specific examples of regulation of *in vivo* antibody production. However, the leakiness of the regulation systems, lack of penetration of the inducer drug to the target tissue/organ, immune responses against the activator proteins, and modulation of endogenous gene expression are some of the drawbacks of these systems (50). Another possibility is to incorporate a suicide gene such as *herpes simplex* virus thymidine kinase in the transgene cassette along with the antibody gene. Administration of the pro-drug such as ganciclovir will specifically kill the cells expressing the transgene.

Ex vivo gene transfer

Ex vivo gene transfer involves the collection of cells which will be genetically modified, their maintenance in cell culture, and *in vitro* gene transfer. Successfully transduced cells will be selected, expanded and introduced into the recipient (Figure 1a). Once transplanted, the gene-modified cells produce therapeutic proteins. Benefits of this approach include the ability to target gene transfer to a specific cell type and the possibility to select for correctly modified cells before transplantation. Possible vector inactivation by the host immune system is also avoided. *Ex vivo* gene transfer to the patient-derived cells is, however, only applicable to those cells and tissues that can be safely removed, are susceptible to gene transfer, and can be reimplanted.

In addition to the patient's own cells, transplantation of *ex vivo* modified non-self cells is possible if cells are microencapsulated prior to transplantation. In microencapsulation, the modified cells are enclosed within artificial semi-permeable membranes. The membrane is designed to allow bidirectional diffusion of nutrients, oxygen, and waste products, while preventing the immune cells from destroying the enclosed cells (Figure 1b). Many types of polymers can be used for microencapsulation to allow long-term function of the graft. (51,52).

In vivo gene transfer

Several studies have shown that production and secretion of antibodies can also be achieved through direct *in vivo gene transfer* using different viral vectors and plasmid DNA (pDNA) (8,22,53,54) (Figure 1c; Table II). In animal studies, vectors have mostly been injected into skeletal muscles (i.m.) and blood circulation. After i.v. vector injection, the main site of transduction is the liver, thus making hepatocytes key players in antibody expression. Transgene proteins expressed by hepatocytes may actually be less immunogenic

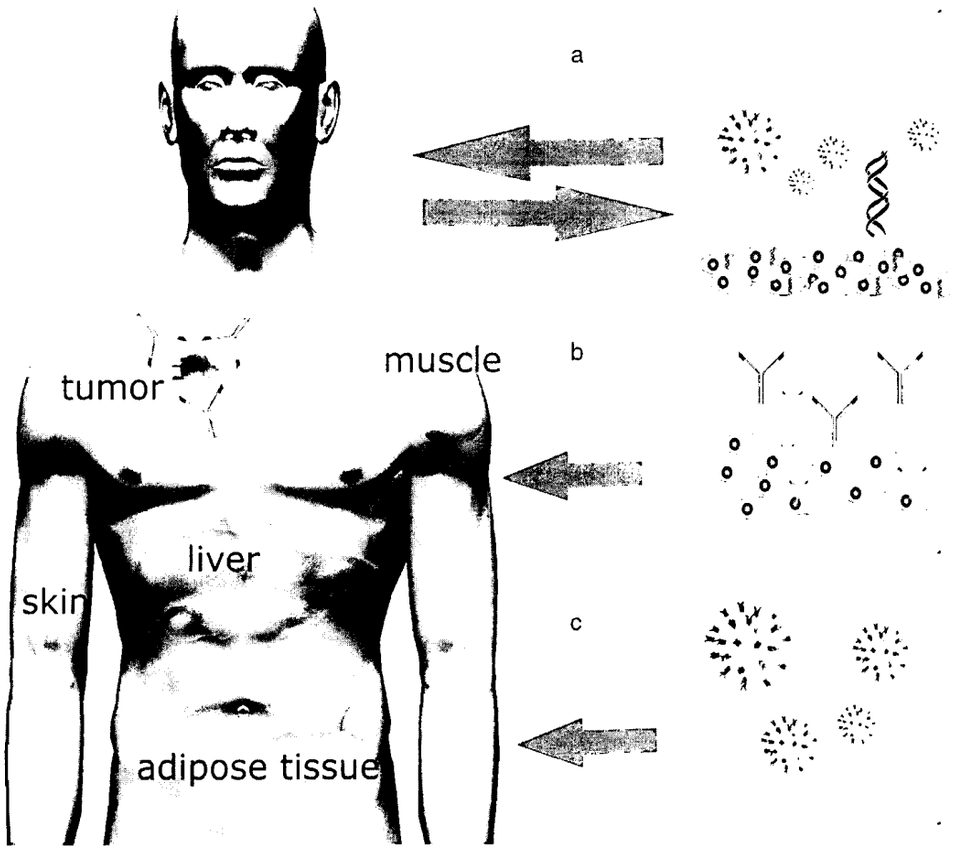

Figure 1. There are three approaches through which stable long-term expression and secretion of therapeutic antibodies *in vivo* could be achieved: A) grafting of *ex vivo* genetically modified *autologous* cells, B) implantation of *ex vivo* transduced cells encapsulated within artificial semi-permeable membranes, and C) direct *in vivo* administration of mAb gene-carrying vectors.

than proteins expressed by other cells, which is an advantage of i.v. injections (55). Muscle cells are also known to be capable of expressing correctly folded and functional antibody molecules (56–58) (Table II). The data available from animal studies imply that after i.m. vector injection antibody blood concentrations have generally been lower and more variable than after i.v. administration (54,57,59). Adenovirus (Ad) vectors do not integrate, which leads to declining antibody expression after the initial production peaks at around one week (59–61). The majority of adeno-associated virus (AAV) vector genomes persist in infected cells as extra-chromosomal episomes (62–64) which promote more sustained antibody expression than Ads (22,58). Lentivirus vectors efficiently catalyse transduction and transgene integration in various cell types, and intravenously injected they have promoted high and rather stable blood mAb concentrations, the major site of transduction being the liver (54). Intravenously administered lentiviral vectors or rAAVs thus seem to be the most suitable vectors for long-term *in vivo* mAb production. For short-term production, non-integrating Ads or plasmid DNA would be more suitable. The use of integrating vectors for *in vivo* mAb gene transfer raises safety issues and ethical concerns, because the unpredictable integration pattern of the currently available vectors can adversely result in insertional mutagenesis and disruption of cellular genes (65,66). This risk can be overcome if the transduced cells are selected *in vitro* before implantation into the recipient. The development of site-specifically integrating vectors would also enable safer *in vivo* mAb gene transfer. Also, by using non-integrating vectors the risks of insertional mutagenesis can be minimized, and rather long-term mAb secretion can be achieved if non-dividing cells and tissues, such as the muscle, are targeted for gene transfer (67).

Table II. *In vivo* antibody production by different vectors in animal models.

Vector	Vector load/dose	Animal species	Delivery route—i.v./i.m.	Long-term average serum concentration	Peak serum concentration (time point)	Duration of expression	Immune responses towards vector (v) or product (p)	Reference
Ad	4×10^9–1×10^{11} pfu	mice	i.v.	0.001–0.02 μg/mL	10 μg/mL (d2)	20 days	yes (v)	(68)
Ad-Tg10	1×10^6–10^9 IP	mice	i.v.	0.1–0.3 μg/mL	250 μg/mL (d6–30)	196 days (7 months)	yes (p and v)	(57)
	1×10^6–10^9 IP		i.m.	0.1 μg/ mL	0.8–3 μg/mL	196 days (7 months)	yes (p and v)	(57)
Ad	5×10^9 pfu	nude mice	i.v.	n/a	n/a	10 days	n/a	(59)
Ad	2×10^9 pfu	mice	i.v.	>40 μg/mL	158 ± 38.5 μg/mL	35 days	n/a	(61)
rAAV	5×10^{10} DRP	mice	i.m.	0.5–1.0 μg/mL	1 μg/mL	168 days (6 months)	n/a	(58)
	5×10^{11}		i.m.	4–5 μg/mL	8 μg/mL	168 days (6 months)	n/a	
rAAV8	$1/2/4 \times 10^{11}$ vg	mice	i.v.	>1000 μg/mL	8000 μg/mL	112 days (4 months)	n/a	(22)
EIAV	1×10^7 TU, 2 injections	mice	i.v.	1–4 μg/mL	3–8.4 μg/mL	130 days	yes (v) VSV-G; yes (p) Myc-His-TagscFvB7	(54)
	$2 \times 5 \times 10^6$ TU		i.m.	2–14 μg/mL	0.8–20 μg/mL	130 days	-/-	
pDNA+ep	40–200 μg	mice	i.m.	0.01–0.4 μg/mL	1.5 μg/mL (d14)	140 days	n/a	(69)
pDNA+ep	10–50 μg	sheep	i.m.	0.05–0.2 μg/mL (mouse)	0.06–0.1 μg/mL	196 days (7 months)	yes (p)	(67)
	2×100μg		i.m.	0.025–0.01 μg/mL (sheep)	0.055 μg/mL	28 days	yes (p)	

Ad = adenovirus; d = days; DRP = DNase-resistant particles; EIAV = equine infectious anaemia virus; ep = electroporation; i.m. = intramuscular; IP = infectious particles; i.v. = intravenous; n/a = data not available; pDNA = plasmid DNA; pfu = plaque-forming units; rAAV = recombinant adeno-associated virus; Tg = Transgene; TU = transdusing units; vg = vector genomes; VSV-G = vesicular stomatitis virus G-protein.

Conclusion

Therapeutic mAbs are the fastest growing class of new therapeutic molecules. They hold great promise for the treatment of a variety of diseases. However, with the rising demand for these therapeutics, there is a need for new technological innovations regarding mAb manufacturing and the therapeutical approach itself. The idea of the genetic delivery of therapeutic mAbs either by *ex vivo* gene transfer or *in vivo* gene transfer represents an exciting technique to further improve monoclonal antibody-based therapies. Genetic delivery of therapeutic proteins have clear advantages: First the therapeutic efficacy of the therapeutic antibody could most likely be improved by maintaining stable therapeutic and non-toxic levels of the therapeutic antibody within the blood circulation over a long period of time. Repeated high-dose bolus injections are avoided, thereby reducing the possibility of side-effects. Secondly, highly expensive production and manufacturing costs of the therapeutic proteins can be reduced, making a one-time gene transfer procedure available to the patients.

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ANEXO 2[22]

Presentation: AIN457 Shows a Good Safety Profile and Clinical Benefit in Patients with Active Rheumatoid Arthritis (RA) Despite Methotrexate Therapy: 16-Weeks Results From a Randomized Proof-of-Concept Trial (ACR/ARHP Annual Scientific Meeting)

1922 - AIN457 Shows a Good Safety Profile and Clinical Benefit in Patients with Active Rheumatoid Arthritis (RA) Despite Methotrexate Therapy: 16-Weeks Results From a Randomized Proof-of-Concept Trial

Tuesday, October 20, 2009: 2:30 PM
Auditorium (Pennsylvania Convention Center)

Paul P. Tak[1], *P. Durez*[2], *JJ. Gomez-Reino*[3], *B. Wittmer*[4], *V. Chindalore*[5], *F. Di Padova*[6], *AM. Wright*[6], *G. Bruin*[6] *and V Hueber*[6], [1]*Academic Medical Center/ University of Amsterdam, Amsterdam, Netherlands,* [2]*University of Louvain, Brussels, Belgium,* [3]*University Clinical Hospital, Santiago de Compostela, Spain,* [4]*Commonwealth Biomedical Researcl Madisonville, KY,* [5]*Pinnacle Research Group, Anniston, AL,* [6]*Novartis Pharma AG, Basel, Switzerland*

Presentation Number: 1922

Purpose: : AIN457 is a fully human IgG1κ monoclonal anti-IL17 antibody that selectively neutralizes IL-17A. IL-17 is overexpressed in synovial tissues in RA and preclinical studies support that IL-17A is a key driver of synovial inflammation and degradation of cartilage and bone. This study explores IL-17A blockade with AIN457 as a novel therapeutic approach to RA. The objective is to determine the safety, tolerability and initial evidence of clinical efficac of AIN457 in-patients with active RA despite methotrexate (MTX) therapy up to 16 weeks.

Method: : This was a multicenter, randomized, double blind, parallel-group study. A total of 52 patients with active R received either, 2 doses of 10 mg/kg AIN457 (*N*=26) three weeks apart or placebo (*N*=26). All patients were followed up to 16 weeks. This abstract expands on the previously reported 6-week results to include secondary endpoints and response rates up to 16 weeks.

Results: : Patients characteristics at baseline were comparable between AIN457 and placebo groups (mean age 49.9 *vs* 49.8 yrs, median disease duration 3.9 *vs.* 2.9 yrs; median weekly MTX dose 15 mg *vs.* 12.5 mg, mean DAS28 scor 5.88 *vs.* 5.84, mean tender joint count 17.6 *vs.* 17.1, mean swollen joint count 11.6 *vs.* 11.1, median C-reactive protein (CRP) 7.6 *vs.* 9.8 mg/L). AIN457 was well tolerated and has a favorable consistent PK profile with a long elimination half life of 23 days. Adverse event types and incidence rates were comparable between the groups, with a slightly higher frequency in the AIN457 group (81% *vs.* 65%). The overall rate of infections was identical in the AIN4! and placebo group (9 events [35%] in each). No cases of neutropenia or immunogenicity were reported. Exploratory analysis showed that a greater percentage of patients treated with AIN457 2x10mg/kg achieved an ACR20 response over 16 weeks compared to placebo [*P*=0.08] (Table 1). Both ACR50 and ACR70 rates showed numerical improvemer with AIN457 compared to placebo. DAS28 assessment showed greater reductions with AIN457 2x10mg/kg compared placebo over the16-weeks period (EULAR response rates are shown in Table 1). AIN457 induced fast onset of respons and sustained reductions in CRP levels through week 16 (Figure 1). This was paralleled by improvements in the patier global assessment (PGA) (Figure 2) and HAQ scores.

Conclusion: : The good safety, rapid onset and encouraging efficacy of AIN457 suggest a novel therapeutic concept with the potential for improved patient outcomes. These positive results support development of AIN457 in RA and potentially other immune-mediated disorders.

Table 1: Rates of ACR and DAS28-based EULAR response in the treatment groups

Weeks since dosing	AIN457 10mg/kg (*N*=26)			Placebo (*N*=26)		
	ACR20	ACR50	ACR70	ACR20	ACR50	ACR70
16	14(54%)	7(27%)	2(8%)	8(31%)	4(15%)	0(0%)

Weeks since dosing	AIN457 10mg/kg (*N*=26)			Placebo (*N*=26)		
	None	Moderate	Good	None	Moderate	Good
16	10(38%)	8(31%)	8(31%)	16(62%)	5(19%)	5(19%)

Figure 2. Geometric mean (+/- SE) CRP concentrations over time in the treatment groups.

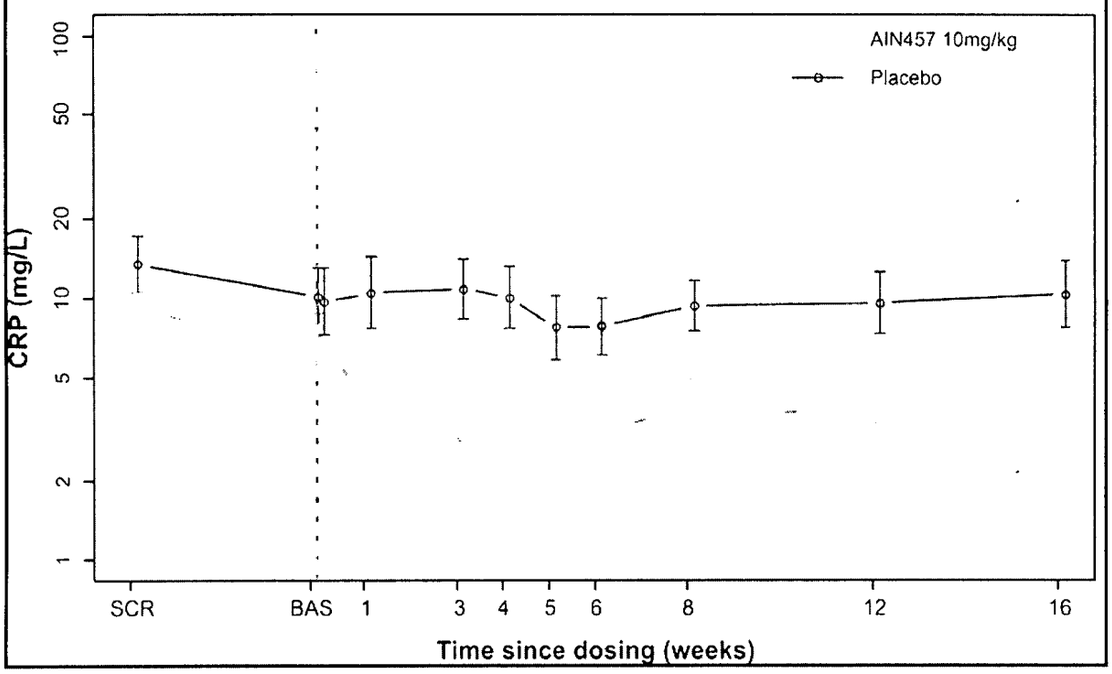
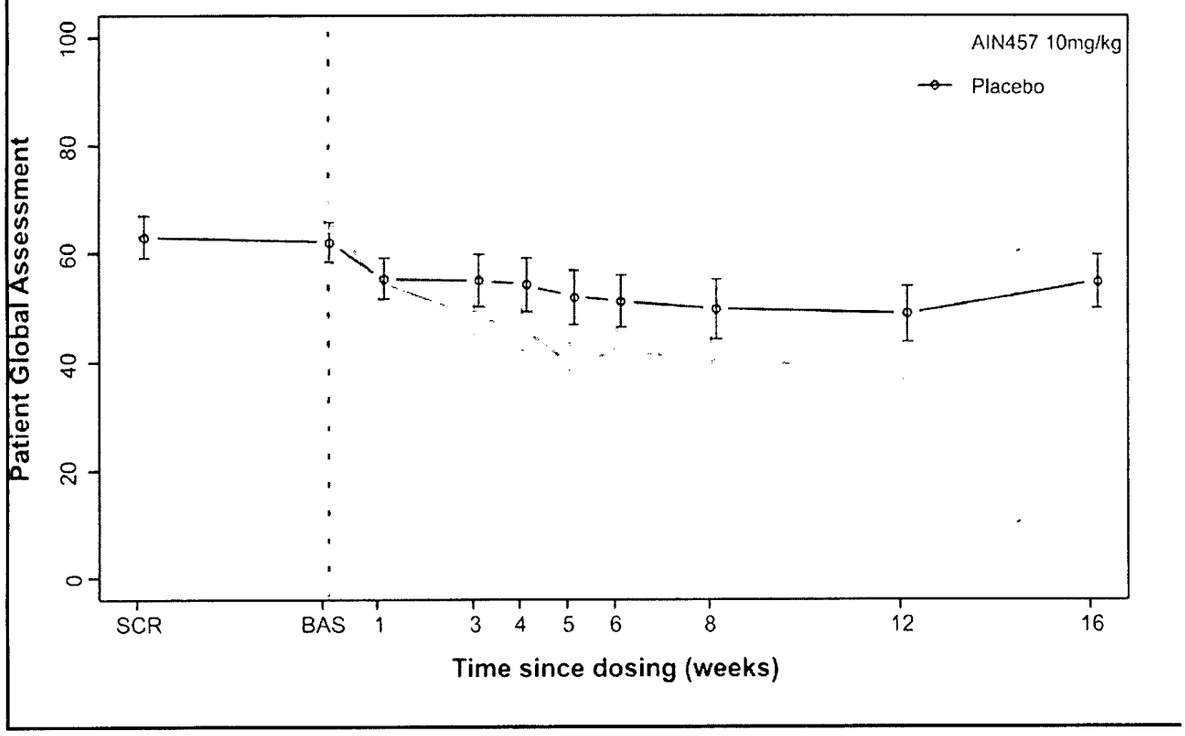


Figure 2. The mean (+/- SE) patient global assessment score over time in the treatment group



Keywords: cytokines, methotrexate (MTX) and rheumatoid arthritis, treatment

Disclosure: P. P. Tak, Novartis Pharmaceutical Corporation, 2, Novartis Pharmaceutical Corpora 5 ; **P. Durez**, None; **J. Gomez-Reino**, Roche Pharmaceuticals, Schering Plough, 5, Roche Pharmaceuticals, Schering Plough, Bristel-Myers Squibb, Abbott, 8 ; **B. Wittmer**, Novartis Pharmaceutical Corporation, 5 ; **V. Chindalore**, Novartis Pharmaceutical Corporation, 5 ; **F. I**

Padova, Novartis Institutes for Biomedical Research, 3 ; **A. Wright**, Novartis Pharmaceutica Corporation, 3, Novartis Pharmaceutical Corporation, 1 ; **G. Bruin**, Novartis Pharmaceutical Corporation, 3 ; **W. Hueber**, Novartis Pharmaceutical Corporation, 3 .

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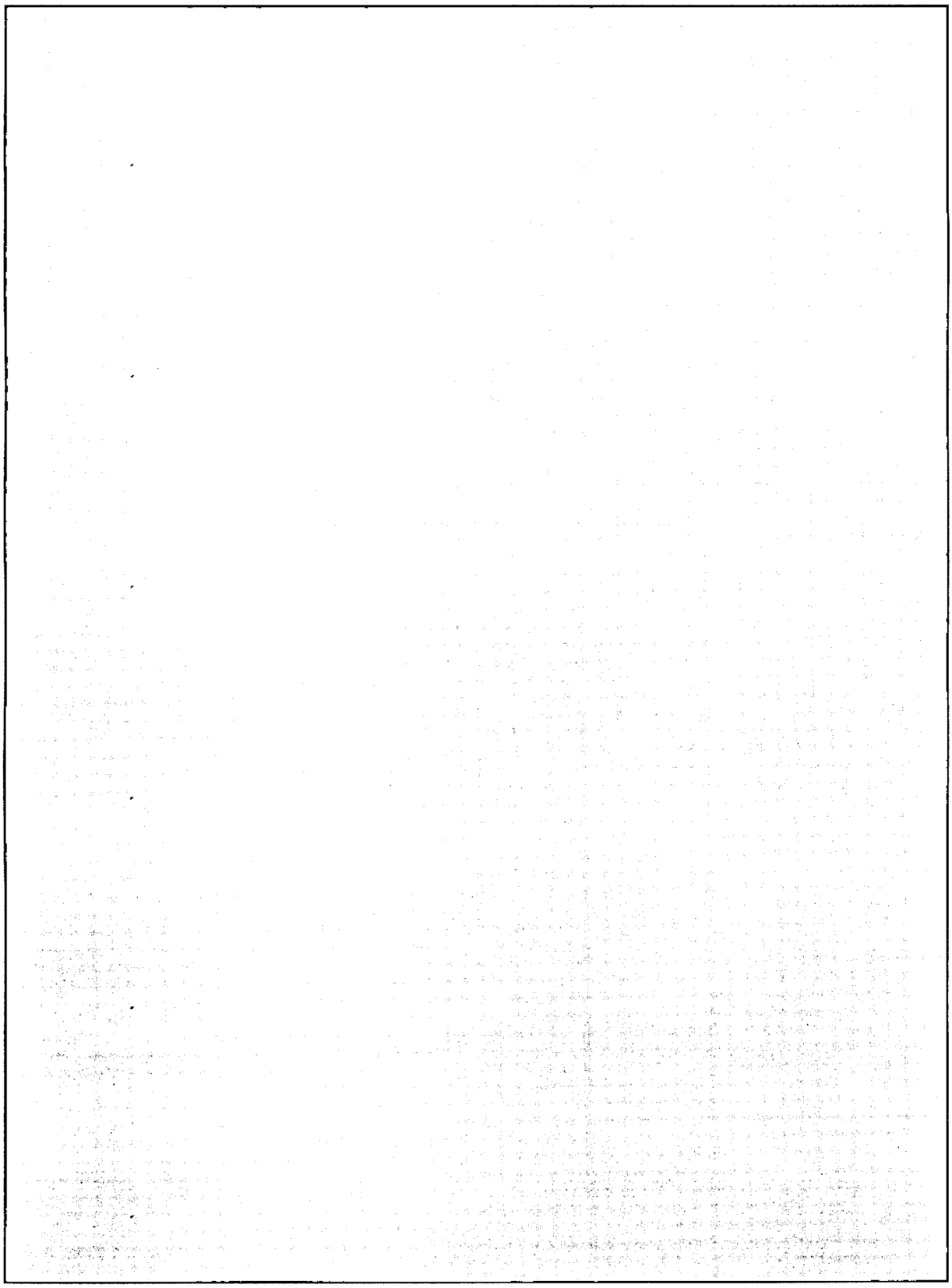

Reference
18th Congr Eur Acad Dermatol Venereol (EADV) (October 7-11, Berlin) 2009, Abst P1138

Title
AIN457 demonstrated to be an effective, fast acting drug for the treatment of chronic plaque type psoriasis in a proof of concept trial

Anti-IL-17 mAb (418942)
AIN-457 is a fully human monoclonal antibody which targets human IL-17A, a proinflammatory cytokine found to be upregulated in lesional skin. The antibody was recently evaluated for the treatment of plaque psoriasis in a proof-of-concept, phase II trial. Patients with a diagnosis of moderate to severe chronic plaque psoriasis (N = 36) for at least 6 months were randomized to receive a single-dose intravenous (i.v.) infusion of AIN-457 (3 mg/kg) or placebo (n = 18/group). The primary endpoints were determined as psoriasis area and severity index (PASI) and investigator's global assessment (IGA) scores at 12 weeks. The study revealed reductions from baseline in mean PASI scores of 63% and 9%, respectively, for the AIN-457 and placebo groups. AIN-457-treated subjects exhibited a rapid response in the reduction of PASI as early as 2 weeks postdose (P = 0.0001 vs. placebo). Reductions in IGA scores were also seen in the AIN-457-treated participants. The incidence of adverse events, which were described as mild and transient, was found to be similar in both treatment groups. A single i.v. infusion of AIN-457 did not elicit the production of detectable anti-drug antibodies and displayed pharmacokinetic characteristics that are typical for a human IgG1 antiserum with a long elimination half-life of approximately 29.2 days. The study supports the development of AIN-457 for the treatment of psoriasis and other immune-mediated conditions.

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Presentation Abstract

Program#/Poster#:	3779/D1066
Abstract Title:	**The Study of IL-17A Expression as a Biomarker for Patients With Active Noninfectious Uveitis Treated With AIN457**
Presentation Start/End Time:	Tuesday, May 04, 2010, 3:45 PM - 5:30 PM
Session Number:	383
Session Title:	Clinical Ocular Inflammatory Disease
Location:	Hall B/C
Reviewing Code:	301 non-infectious uveitis: clinical and translational studies – IM
Author Block:	*R.R. Buggage[1], H.B. Richards[2], M.-A. Valentin[2], I. Koroleva[3], T.B. Dryja[3].* [1]Novartis Pharmaceuticals Corp, New York, NY; [2]Novartis Institutes for Biomedical Research, Basel, Switzerland; [3]Novartis Institutes for Biomedical Research, Cambridge, MA.
Keywords:	742 uveitis-clinical/animal model, 466 clinical (human) or epidemiologic studies: treatment/prevention assessment/controlled clinical trials, 556 inflammation

Abstract Body:

Purpose: Higher IL-17 expression has been detected in patients with active uveitis and Behçet's disease compared to patients with inactive disease and healthy controls. AIN457 offers a novel therapeutic strategy for noninfectious uveitis including Behçet's uveitis. The effect of AIN457 on IL-17A expression and the correlation between baseline IL-17A and treatment response were evaluated in an open-label study.

Methods: 16 patients with active noninfectious uveitis of various etiologies were treated with 2 intravenous doses of AIN457 at day 0 and 21 and followed through week 8. Serum IL-17A was measured on day 0 using a highly sensitive immunoassay coupled to single photon detection and mRNA levels were measured on day 0, weeks 1,2,3,4 and 8 by RT-PCR.

Results: Treatment with AIN457 reduced intraocular inflammation, improved visual acuity or allowed for reduction of concomitant corticosteroids in 11/16 patients. While the mean baseline IL-17A level in patients with active uveitis was higher than in healthy subjects (0.47 vs 0.26 pg/ml; p=0.05), the difference was mainly due to a single outlier. After excluding this patient, there was no significant difference in baseline IL-17A levels comparing study patients and controls. Poor correlation existed between baseline serum IL-17A protein levels and IL-17A mRNA expression in whole blood. No obvious relationship existed between IL-17A mRNA levels in whole blood and response to treatment.

Conclusions: Although baseline serum levels and expression of IL-17A were not elevated in this active uveitis study population, the clinical findings support the role of IL-17A in the pathogenesis of human uveitis and suggest that IL-17A inhibition with AIN457 may be efficacious for the treatment of noninfectious uveitis. Moreover these results indicate that ocular elevation of IL-17A may be locally confined and not measurable in the circulation. Additional studies are needed to understand the value of measuring circulating IL-17A in patients with noninfectious uveitis as a potential biomarker predictive of response to therapy with AIN457.

CommercialRelationships: **R.R. Buggage**, Novartis, E; **H.B. Richards**, Novartis, E; **M.-A. Valentin**, Novartis, E; **I. Koroleva**, Novartis, E; **T.B. Dryja**, Novartis, E.

Support: None

Clinical Trial: www.clinicaltrials.gov NCT00685399

ANEXO 5. 27

Science Translational Medicine
AAAS

Effects of AIN457, a Fully Human Antibody to Interleukin-17A, on Psoriasis, Rheumatoid Arthritis, and Uveitis

Wolfgang Hueber, *et al.*

Sci Transl Med **2**, 52ra72 (2010);
DOI: 10.1126/scitranslmed.3001107

RESEARCH ARTICLE

AUTOIMMUNE DISEASE

Effects of AIN457, a Fully Human Antibody to Interleukin-17A, on Psoriasis, Rheumatoid Arthritis, and Uveitis

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Interleukin-17A (IL-17A) is elaborated by the T helper 17 (T_H17) subset of T_H cells and exhibits potent proinflammatory properties in animal models of autoimmunity, including collagen-induced arthritis, experimental autoimmune encephalomyelitis, and experimental autoimmune uveitis. To determine whether IL-17A mediates human inflammatory diseases, we investigated the efficacy and safety of AIN457, a human antibody to IL-17A, in patients with psoriasis, rheumatoid arthritis, and chronic noninfectious uveitis. Patients with chronic plaque-type psoriasis (n = 36), rheumatoid arthritis (n = 52), or chronic noninfectious uveitis (n = 16) were enrolled in clinical trials to evaluate the effects of neutralizing IL-17A by AIN457 at doses of 3 to 10 mg/kg, given intravenously. We evaluated efficacy by measuring the psoriasis area and severity index (PASI), the American College of Rheumatology 20% response (ACR20) for rheumatoid arthritis, or the number of responders for uveitis, as defined by either vision improvement or reduction in ocular inflammation or corticosteroid dose. AIN457 treatment induced clinically relevant responses of variable magnitude in patients suffering from each of these diverse immune-mediated diseases. Variable response rates may be due to heterogeneity in small patient populations, differential pathogenic roles of IL-17A in these diseases, and the different involvement or activation of IL-17A–producing cells. The rates of adverse events, including infections, were similar in the AIN457 and placebo groups. These results support a role for IL-17A in the pathophysiology of diverse inflammatory diseases including psoriasis, rheumatoid arthritis, and noninfectious uveitis.

INTRODUCTION

The homodimeric molecule interleukin-17A (IL-17A) is a pleiotropic cytokine that is key to the definition of the $CD4^+$ T helper 17 (T_H17) cell lineage (*1*, *2*). IL-17A and IL-17F are two IL-17 family members that are secreted by T_H17 cells and share the same receptors, IL-17 receptor A (IL-17RA) and IL-17 receptor C (IL-17RC) (*3*). IL-17RA and IL-17RC are ubiquitously expressed, including in keratinocytes, macrophages, fibroblasts, osteoblasts, and epithelial, endothelial, and dendritic cells (*4*), revealing the pleiotropic nature of IL-17A. Cell activation by IL-17A can cause release of proinflammatory cytokines and mediators of tissue destruction (*5*–*7*). IL-17A is mainly produced by T_H17 cells, which derive from naïve $CD4^+$ T cells after stimulation by transforming growth factor–β (TGF-β) and IL-6 (*1*, *2*). Macrophages, astrocytes, Langerhans cells, and mast cells also contribute to IL-17A production (*8*, *9*). Moreover, T_H17 cells express the IL-23 receptor (IL-23R), and IL-23 is an important survival factor for T_H17 cells and production of IL-17A (*10*, *11*). A subset of T_H17 cells also secretes IL-21, IL-22, IL-26, and interferon-γ (IFN-γ) (*12*).

IL-17A contributes to the pathogenesis of animal models of T_H1-driven autoimmunity (*13*–*15*). Recent insights into the T_H17/IL-17 biology from these models have led to the hypothesis that the effects of T_H17 cells and their signature cytokine IL-17A can more profoundly explain T cell–dependent pathobiology in these models, replacing the T_H1 paradigm, in which T_H1 cytokines are the paramount mediators of tissue damage, with an expanded T_H1/T_H17 paradigm (*16*).

Although the etiology of psoriasis, noninfections uveitis, and rheumatoid arthritis (RA) is unknown and clinical presentation of these diseases is diverse, activated (auto)reactive T_H cells seem to initiate and drive organ-specific inflammatory processes in all of them (*17*–*19*). Moreover, the recent discovery that polymorphisms in IL-23R can control susceptibility to psoriasis, psoriatic arthritis, inflammatory bowel disease, RA, and ankylosing spondylitis (*20*) emphasizes the importance of the T_H17 pathway and IL-17A in these and related human immune-mediated diseases.

Psoriasis is a common skin disease characterized by excessive turnover of skin cells. Activated T_H cells are recognized among the primary pathogenic modulators (*21*). IL-23 and its downstream T_H17 cytokines

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are likely pathogenic in human psoriatic skin, because messenger RNA (mRNA) for the T_H17 cytokines IL-17A, IL-17F, and IL-22 accumulates in cutaneous lesions (*17*). Indeed, inhibition of p40, the shared subunit of IL-23 and IL-12, is effective in the treatment of psoriasis (*22*).

RA is an autoimmune disease of synovial joints, characterized by recruitment and activation of immune cells such as autoreactive T and B cells, production of proinflammatory cytokines, and cartilage and bone destruction (*23*). Unlike psoriasis and uveitis, which are associated with human leukocyte antigen (HLA) class I and lack specific autoantibody responses, strong HLA class II association and specific autoantibodies are hallmarks of RA. Hence, it was postulated, contrary to the earlier T_H1 paradigm of RA (*18*), that autoreactive B cells contribute to RA pathogenesis (*24*), as confirmed later by the effectiveness of B cell–targeting therapies in RA (*25*). Nevertheless, in support of T cell involvement, in vitro–expanded $CD4^+$ T cells from RA patients contain more T_H17 cells than do controls and produce more IL-17A, as measured in synovial fluid (SF) (*23*). To complicate matters, non-T cells such as mast cells are also involved in synovial production of IL-17A (*9*). Together, these recent data suggest that IL-17A is involved in the pathogenesis of RA.

Noninfectious uveitis is a form of intraocular inflammation that remains a major cause of visual loss. Uveitis is viewed as a prototypical organ-specific T cell–driven autoimmune disease of an immune-privileged site, with T_H1 cells forming the pathogenic subset (*19*). Recent data from the experimental autoimmune uveitis (EAU) model show a distinct role for IL-23 in eliciting disease (*26*), fuelling the hypothesis that both IL-17A–producing T_H17 cells and T_H1 cells are culprits in tissue damage. Moreover, up-regulation of IL-23 and IL-17A occurs in patients with active uveitis suffering from Behçet's or Vogt-Koyanagi-Harada (VKH) disease, as measured by mRNA in peripheral blood mononuclear cells (PBMCs), serum enzyme-linked immunosorbent assay (ELISA), or production of IL-17A in supernatants of PBMCs (*27*, *28*).

Together, these data suggest that IL-17A may be a therapeutic target in T cell–driven inflammatory disorders. Here, we explored IL-17A blockade with AIN457, a highly specific, human immunoglobulin G1κ (IgG1κ) monoclonal antibody, to treat psoriasis, RA, and chronic noninfectious uveitis. The objective was to assess the safety and possible efficacy of AIN457 in these disorders.

RESULTS

Baseline characteristics and follow-up

We enrolled a total of 104 patients (60 AIN457- and 44 placebo-treated patients) in three clinical trials. All patients participated until the specified endpoints, except for three patients in the RA trial and one in the uveitis trial. Baseline characteristics were comparable between the active treatment and placebo arms in the psoriasis and RA trials; in the open-label uveitis trial, all patients received active treatment (Table 1).

Table 1. Baseline demographics and disease characteristics for the three proof-of-concept trials: BMI, body mass index; CV, coefficient of variation; ND, not determined; VAS, visual analog scale; PGA, patient global assessment; CRP, C-reactive protein; DAS28, 28-joint Disease Activity Score; DMARD, disease-modifying antirheumatic drug; IGA, investigator global assessment; PASI, psoriasis area-and-severity index; SD, standard deviation.

	Psoriasis		Rheumatoid arthritis		Uveitis
	AIN457 (3 mg/kg)	Placebo	AIN457 (10 mg/kg)	Placebo	AIN457 (10 mg/kg)
	n = 18	*n* = 18	*n* = 26	*n* = 26	*n* = 16
Age (years) (mean ± SD)	50.7 ± 8.73	50.9 ± 12.04	49.9 ± 8.53	49.8 ± 15.19	42.6 ± 14.88
Male, *n* (%)	11 (61)	13 (72)	7 (27)	6 (23)	4 (25)
Caucasians, *n* (%)	17 (94)	17 (94)	23 (88)	22 (85)	7 (44)
BMI (kg/m^2) (mean ± SD)	32.97 ± 7.44	33.79 ± 10.54	28.48 ± 6.66	28.13 ± 5.58	ND
PASI (mean ± SD)	18.5 ± 8.7	18.3 ± 8.1			
IGA score, *n* (%)					
2 (mild)	0 (0)	1			
3 (moderate)	13 (72)	11 (61)			
4 (severe)	5 (28)	5 (28)			
5 (very severe)	0 (0)	1 (6)			
Rheumatoid factor positive (≥10), *n* (%)			14	16 (62)	
Disease duration (years), median (range)			3.9 (1–20)	2.9 (1–37)	
DAS28 (mean ± SD)			6.48 ± 5.73	7.44 ± 8.85	
CRP (mg/L), geometric mean (CV%)			6.25 (113.2)	10.11 (150.0)	
Previous DMARD, *n* (%)					
1			23 (88%)	24 (92%)	
2			3 (12%)	2	
PGA on 100-mm VAS (mean ± SD)			67.3 ± 15.84	62.1 ± 19.09	
IGA on 100-mm VAS (mean ± SD)			62.3 ± 17.85	65.0 ± 19.20	

In the psoriasis trial, after a single administration of AIN457 (3 mg/kg), disease severity (the mean PASI) was reduced relative to baseline by 58% and 4% in AIN457- and placebo-treated patients, respectively (P = 0.0001). Fifteen (83%) AIN457-treated patients showed significant reductions in the investigator global assessment (IGA) score categories at week 4 relative to baseline and to placebo-treated patients (11%) (P = 0.0004). The differences in PASI and IGA were maintained at week 12, with mean reductions in PASI relative to baseline of 63% for AIN457 and 9% for placebo, respectively (P = 0.0005) (Fig. 1, panel 1). All PASI response rates (PASI50, PASI75, and PASI90, defined as proportion of patients who achieved 50%, 75%, and 90% reductions in their PASI, respectively) were higher with AIN457 treatment than placebo at week 12 (Fig. 1, panels 2 to 4). Photographs of a representative patient before and after a single infusion of AIN457 (3 mg/kg) are shown in Fig. 1, which shows a reduction in typical disease features (redness, induration, and scaling).

Immunostained micrographs at baseline (week 0) and week 4 of 14 placebo- and 16 AIN457-treated patients with psoriasis showed colocalization of IL-17A and CD3 (Fig. 2, A to E). AIN457 treatment resulted in a significant decrease in the area occupied by dermal IL-17A$^+$ CD3$^+$ T cells (P = 0.042) (in mean pixel counts) by week 4 (Fig. 2F). Skin samples from typical psoriasis plaques, assessed with reverse transcription polymerase chain reaction (RT-PCR) and microarray analysis of gene expression for a wide array of cytokines and chemokines implicated in autoimmunity, showed substantial changes, primarily down-regulation, in response to AIN457 treatment (Fig. 3). Notably, a relatively minor change in tumor necrosis factor–α (TNF-α) expression was observed, in line with reports that TNF-α expression in psoriatic skin lesions is primarily regulated posttranscriptionally (*29*).

In the RA trial, the American College of Rheumatology 20% response (ACR20) rates were higher with AIN457 than with placebo at week 6 (46% versus 27%, P = 0.12; treatment difference was a priori considered statistically significant if $P < 0.2$) (Fig. 4A). The response to treatment was rapid: ACR20 responder rates reached 50% for AIN457 and 31% for placebo as early as week 4 (P = 0.13) and was maintained at week 16 (54% versus 31%; P = 0.08). The 28-joint disease activity scores (DAS28) and C-reactive protein (CRP) values decreased over time, with greater reductions for AIN457-treated patients than for placebo at week 6 (P = 0.16 and P = 0.0013, respectively) (Fig. 4, B and C). The treatment response to AIN457 measured by the area under the response-time curve (AUC) for ACR20, DAS28, and CRP was significantly higher for AIN457 than placebo (P = 0.011, P = 0.027, and P = 0.002, respectively).

All patients in the uveitis trial had active uveitis at baseline, with an anterior chamber cell score of at least 1+ for anterior uveitis cases or a vitreous haze score of at least 1+ for posterior segment uveitis (intermediate, posterior, or panuveitis). Thirteen patients showed at least a one-step decrease in ocular inflammation at week 8 compared to baseline at week 0. Two patients had no change in ocular inflammation, and one patient (with anterior uveitis and unknown HLA-B27 status) had worsening of uveitis during the 2 weeks after the first dose and dropped out of the trial (Fig. 5). The response to treatment was rapid, with 50% of patients initially achieving an anterior chamber cell score of 0 to trace and a vitreous haze score of 0 to trace by week 2. Overall, 11 of the 16 patients had responded at week 8 (Fig. 5). Of five patients with anterior uveitis, three achieved quiescence while taking no topical or systemic corticosteroids. Of 11 patients with posterior segment uveitis, 8 were categorized as responders to treatment by meeting the following criteria: 3 patients had a 15-letter improvement in visual acuity, 4 patients had a two-step reduction or complete resolution of vitreous haze,

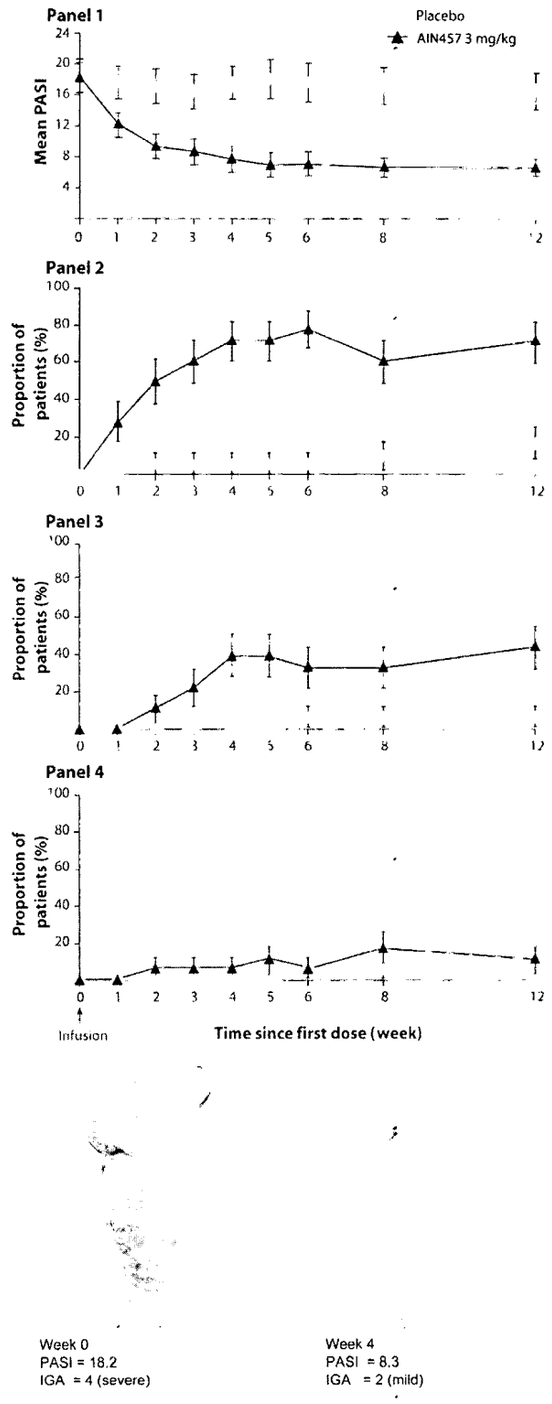

Fig. 1. Clinical response to AIN457 in patients with chronic plaque psoriasis. Patients were treated with a single intravenous infusion of AIN457 (3 mg/kg) between week 0 and week 12. Panel 1: mean reduction in PASI; panels 2 to 4: proportion of patients with PASI50, PASI75, and PASI90, respectively. Error bars, SE. Arrow: visit at which drug or placebo was administered. (Lower panels) Clinical improvement in psoriasis in a patient treated with a single intravenous infusion of AIN457 (3 mg/kg).

and 4 patients were able to stop topical and systemic corticosteroids without worsening of uveitis.

Safety

No deaths occurred and no safety events of concern were observed in the cohort of 104 patients participating in the three studies. No antibodies to AIN457 were detected. All observed infections were not serious, and their rates were similar in the AIN457 and placebo groups.

In the psoriasis trial, one serious adverse event (SAE) (worsening of preexisting congestive cardiac disease) occurred in the AIN457 group. Adverse events (AEs) were reported in nine AIN457- and eight placebo-treated patients. Two AEs were classified as severe, whereby one occurred in an AIN457-treated patient who experienced severe pruritus. All other reported AEs were mild or moderate. None of the patients discontinued treatment because of AEs or SAEs. Infections, reported in five AIN457- and three placebo-treated patients, were all rated mild and were mostly in the upper respiratory tract.

In the RA trial, one SAE (laryngeal abscess due to RA of the cricoarytenoid joint) occurred in an AIN457-treated patient after study termination. Two other SAEs (interstitial lung disease and brachial plexopathy), both requiring hospitalization, were reported in the placebo-treated group. The overall AE incidence rate was slightly higher in the AIN457 group compared to placebo (Table 2). A total of 18 patients, 9 in each group, had not-serious infections. Most frequently observed infections were nasopharyngitis and upper respiratory tract infections. Two patients treated with AIN457 and one with placebo discontinued the study because of worsening of arthritis.

In the uveitis trial, no SAEs were reported. Ten (63%) of the 16 participating patients experienced AEs, with the most frequent AEs being headache (56%), upper abdominal pain (19%), and conjunctival hyperemia (19%).

DISCUSSION

Here, we report our exploration of the therapeutic use of a monoclonal antibody to IL-17A for the treatment of three immune-mediated human diseases: psoriasis, RA, and chronic noninfectious uveitis. One or two doses of AIN457 significantly reduced the disease activity compared to the placebo-treated group (for psoriasis and RA) or had effects comparable to those of historical control subjects for chronic noninfectious uveitis treated with a biologic therapy (*30*).

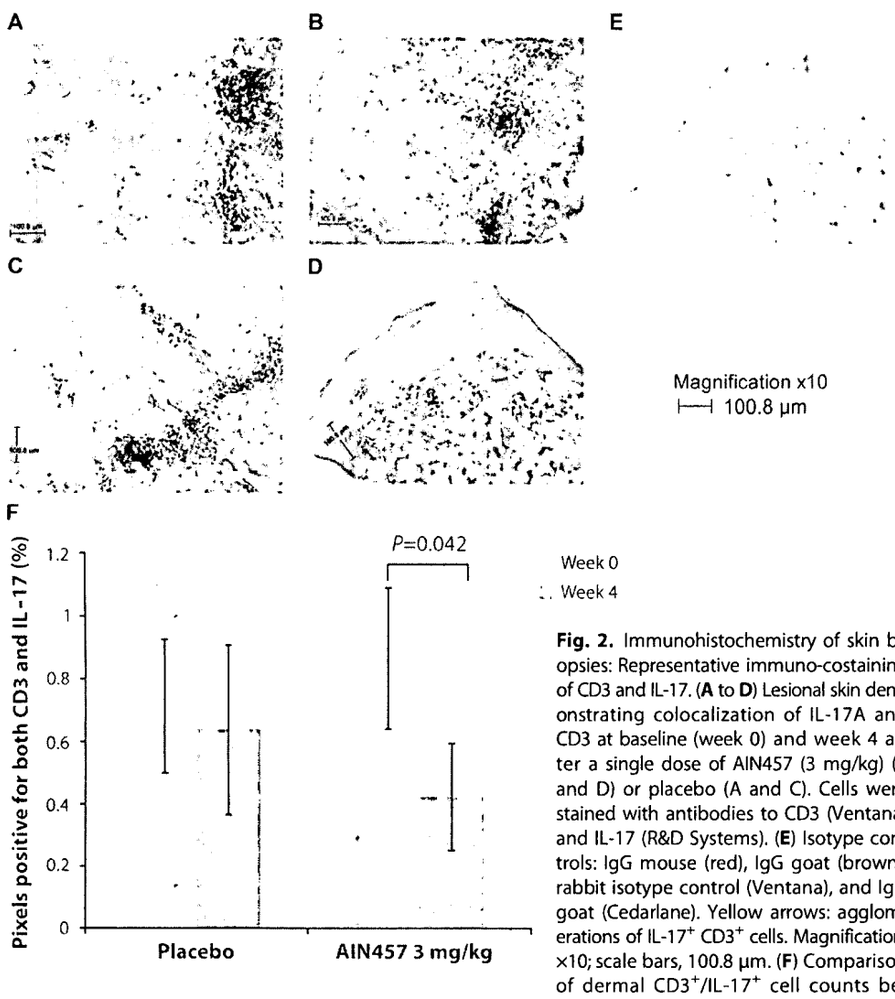

Fig. 2. Immunohistochemistry of skin biopsies: Representative immuno-costaining of CD3 and IL-17. (**A** to **D**) Lesional skin demonstrating colocalization of IL-17A and CD3 at baseline (week 0) and week 4 after a single dose of AIN457 (3 mg/kg) (B and D) or placebo (A and C). Cells were stained with antibodies to CD3 (Ventana) and IL-17 (R&D Systems). (**E**) Isotype controls: IgG mouse (red), IgG goat (brown), rabbit isotype control (Ventana), and IgG goat (Cedarlane). Yellow arrows: agglomerations of IL-17$^+$ CD3$^+$ cells. Magnification, ×10; scale bars, 100.8 µm. (**F**) Comparison of dermal CD3$^+$/IL-17$^+$ cell counts between week 0 and week 4. Error bars, SE. T cell counts were done manually by a pathologist. Percent of dermal CD3$^+$/IL-17$^+$ pixels was calculated with Aperio ImageScope software.

In psoriasis patients, we noted faster (as early as week 2) and greater benefits with AIN457 treatment than with placebo at all time points up to week 12. Because PASI50 or greater response rates are generally considered a relevant difference, almost 75% of patients on AIN457 achieved a clinically meaningful reduction of disease activity. These clinical responses in patients treated with AIN457 were associated with reductions of histomorphological signs of acanthosis and epidermal hyperplasia, and changes in gene expression of markers of the IL-17A pathway. As shown by RT-PCR and array analysis, expression of IL-17A and IL-22 was markedly reduced after therapy with AIN457. Blockade of IL-17A had broad anti-inflammatory effects as shown by down-regulation of many mediators, including upstream cytokines such as IL-12B (the p40 subunit of IL-23 and IL-12) or downstream cytokines such as CCL20 [chemokine (C-C motif) ligand 20]. Moreover, our immunohistochemical data demonstrated large numbers of CD3$^+$ T cells in the affected skin that colocalized with IL-17A, suggesting that T cells are the probable source of IL-17A. Additionally, T cell numbers were reduced after AIN457 therapy (Fig. 2F). Our obser-

vations suggest that IL-17A is pathogenic in psoriasis, and we hypothesize that IL-17A augments a feedback loop involving IL-23, possibly TNF, dendritic cells, and T_H17 cells as main producers of IL-17A. In line with this hypothesis, therapies that block the activation or the differentiation of T cells, such as antibodies to p40, which target both IL-23 and IL-12 (*17*, *22*), or to cyclosporine A (CsA) (*31*), are very effective against psoriasis.

In the RA trial, AIN457 had effects rapidly after the first infusion, and the improvements were maintained up to 13 weeks after the second infusion, indicating that AIN457 has the potential to rapidly suppress synovial inflammation and provide sustained benefit. One measure of disease severity (AUC of ACR20, DAS28, and CRP)

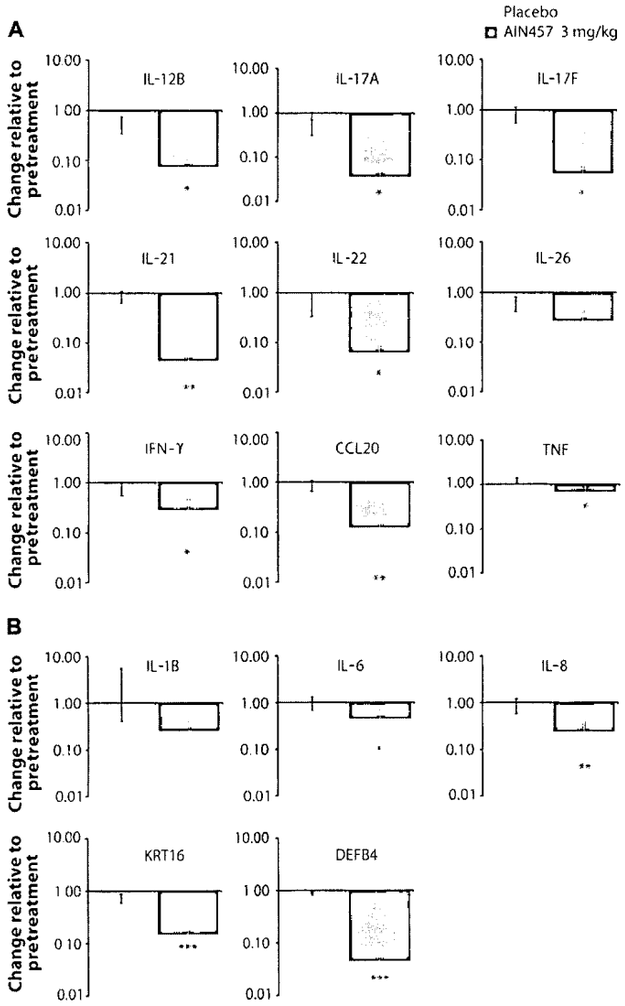

Fig. 3. Gene expression changes in skin of patients with psoriasis treated with AIN457. Subjects were given a single dose of AIN457 (3 mg/kg) ($n = 3$) or placebo ($n = 5$) at week 4; data are expressed as compared to baseline (week 0). (**A**) Real-time RT-PCR analysis showing mean fold change (±SE) at week 4 relative to baseline mRNA expression normalized to human control gene [glucuronidase β (GUSB)]. *$P < 0.05$; **$P < 0.01$. (**B**) Affymetrix HG-U133 Plus 2.0 chip analysis showing mean fold change (±SE) at week 4 relative to baseline mRNA expression and ANOVA. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

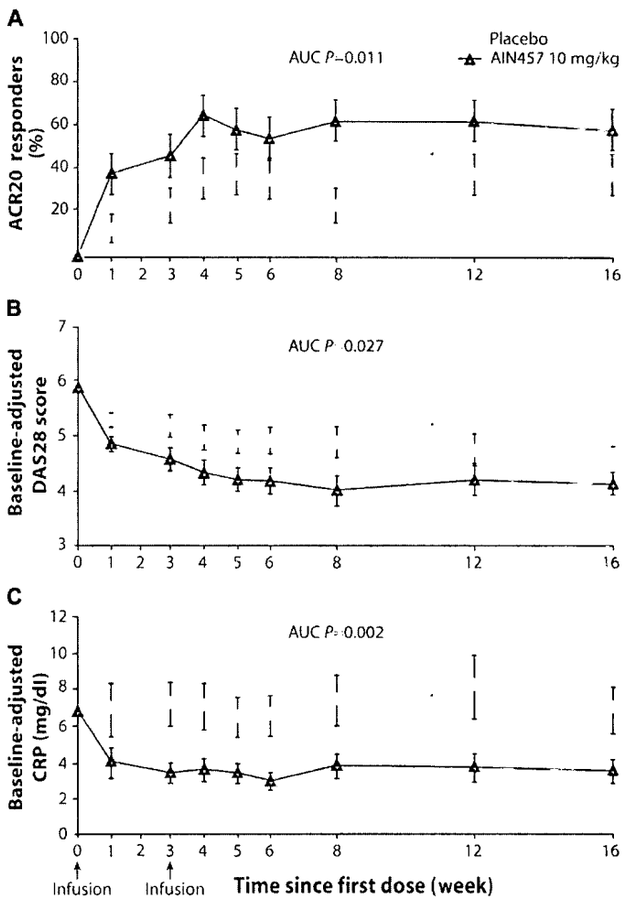

Fig. 4. ACR20, DAS28, and CRP changes for a two-dose infusion regimen of AIN457 (10 mg/kg) versus placebo. (**A**) The proportion of ACR20 responders (±SE) is presented for each treatment group at each visit. (**B** and **C**) Appropriate baseline-adjusted means (±SE) from the statistical models of DAS28 (B) and CRP (C) are presented for each treatment group at each visit.

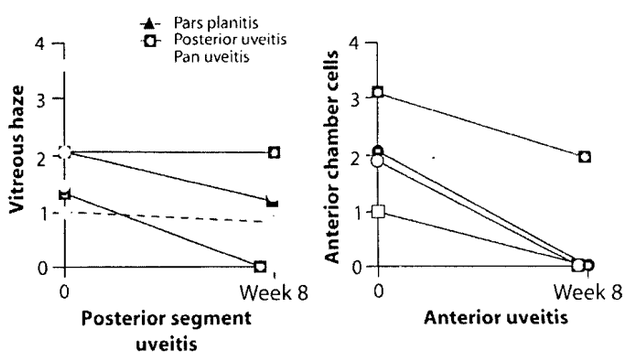

Fig. 5. Vitreous haze (posterior segment uveitis) or anterior chamber cells (anterior uveitis) at week 8 versus week 0. Posterior segment uveitis includes patients with intermediate, posterior, and pan uveitis. One patient with anterior uveitis discontinued at week 2 because of worsening uveitis, and thus, week 8 data were not available for this. Each line represents one individual patient.

showed significant differences between the AIN457- and placebo-treated groups, strengthening the notion that AIN457 may be efficacious in RA.

One concept of RA describes T cells as principle drivers of the autoimmune responses (*18*), and recently, their involvement in the production of IL-17A in RA has been proposed (*23*, *32*, *33*). Our findings

Table 2. Adverse events. (A) Number and percent of patients in each treatment group who (i) had any AE, (ii) had an SAE, (iii) died, or (iv) discontinued the study because of an AE. AEs were assigned to a primary body system using the Medical Dictionary for Regulatory Activities (MedDRA). In each treatment group, the number (%) of patients who experienced an AE in each primary body system was calculated. (B) Body systems in which >10% of patients in at least one of the active AIN457 groups experienced an AE. Individual AEs with an event rate of >10% in at least one of the active AIN457 groups are also presented underneath the related body system. Body systems and AEs (within relevant body system) are displayed in alphabetical order. A subject experiencing multiple AEs within a body system is counted only once in the respective body system category row.

Events	Psoriasis		Rheumatoid arthritis		Uveitis
	AIN457 (3 mg/kg)	Placebo	AIN457 (10 mg/kg)	Placebo	AIN457 (10 mg/kg)
	n = 18	*n* = 18	*n* = 26	*n* = 26	*n* = 16
(A) General, *n* (%)					
Any AE	9 (50)	8 (44)	21	17 (65)	10 (63)
Any SAE	1 (6)	0	1 (4)	2	0
Death	0	0	0	0	0
Discontinuation of follow-up owing to AE	0	0	2	1 (4)	0
(B) Body system, *n* (%)					
Eye disorders	0	1 (6)	1 (4)	0	4 (25)
Eye pain	0	0	0	0	2 (13)
Ocular hyperemia	0	0	0	0	3 (19)
Vision blurred	0	0	0	0	2 (13)
Gastrointestinal disorders	1 (6)	1 (6)	3 (12)	5 (19)	6 (38)
Abdominal pain, upper	0	0	0	0	3 (19)
Vomiting	0	0	1 (4)	3 (12)	2 (13)
General disorders and administration site conditions	3 (17)	1 (6)	6 (23)	1 (4)	2 (13)
Fatigue	2 (11)	1 (6)	2	1 (4)	2 (13)
Infections and infestations	5 (28)	3 (17)	9 (35)	9 (35)	2 (13)
Injury, poisoning, and procedural complications	0	0	3 (12)	2	0
Investigations	3 (17)	3 (17)	0	0	1 (6)
Blood cholesterol increased	2 (11)	0	0	0	0
Blood glucose increased	2 (11)	1 (6)	0	0	0
Blood triglycerides increased	2 (11)	2 (11)	0	0	1 (6)
Metabolism and nutrition disorders	2 (11)	0	2	2	0
Musculoskeletal and connective tissue disorders	0	1 (6)	8 (31)	4 (15)	4 (25)
Back pain	0	0	3 (12)	1 (4)	2 (13)
Muscle spasm	0	0	2	0	2 (13)
Rheumatoid arthritis	0	0	3 (12)	2	0
Nervous system disorders	1 (6)	1 (6)	5 (19)	6 (23)	9 (56)
Dizziness	0	0	3 (12)	3 (12)	1 (6)
Headache	1 (6)	1 (6)	1 (4)	2	9 (56)
Sinus headache	0	0	0	0	2 (13)
Respiratory, thoracic, and mediastinal disorders	1 (6)	0	4 (15)	3 (12)	1 (6)
Skin and subcutaneous tissue disorders	2 (11)	0	4 (15)	2	0
Vascular disorders	2 (11)	1 (6)	3 (12)	2	1 (6)
Hypertension	2 (11)	1 (6)	2	0	0

suggest that IL-17A participates in RA pathogenesis because patients show relevant responses to inhibition of IL-17A. On the basis of earlier observations of elevated concentrations of IL-17A in SF (*34*) and recent reports of a correlation between SF T_H17 cells and SF IL-17A (*23*), it is reasonable to infer that T_H17 cells are among the main producers of IL-17A in RA. Mast cells were recently described to also produce IL-17A in synovial tissue (*9*); however, relative contributions of synovial cell types to IL-17A production need further investigation.

In the uveitis trial, patients with posterior segment uveitis had disease severity comparable to that of patients in an open-label trial of infliximab, an antibody to TNF-α (*35*). Most patients with uveitis involving the posterior segment responded to AIN457 with an improvement in visual acuity, a reduction in ocular inflammation, or the ability to stop corticosteroid therapy, with similar proportions of patients responding in these ways as did patients treated with infliximab. In these patients, there was a mean increase in visual acuity of more than two lines using Early Treatment of Diabetic Retinopathy Study (ETDRS) charts (see Materials and Methods) and a rapid reduction in vitreous haze that was sustained during the 8-week follow-up. Five patients with anterior uveitis had experienced one or more years of continuous or recurrent severe disease that was resistant to topical corticosteroids and that required repeated or systemic immunosuppression or periocular injections of corticosteroids. Although our study was short (2 months), without a placebo or other control group, and the primary objective of the study was to evaluate the safety of AIN457 in the treatment of uveitis, the reductions in ocular inflammation suggest that IL-17A inhibition may be beneficial in severe, chronic noninfectious uveitis requiring systemic immunosuppressive therapy.

The evolution of T_H1 into a T_H1/T_H17 paradigm, first proposed for animal models of autoimmunity and later expanded to human diseases (*16*), finds further support in clinical results reported here. Our data suggest that IL-17A contributes to the pathogenesis of psoriasis, RA, and noninfectious uveitis, conditions in which T_H1 cells are implicated in disease initiation and perpetuation (*17–19*). We hypothesize that in psoriasis and uveitis, IL-17A may be produced mainly by T_H17 cells, corroborated for psoriasis by our colocalization studies, and thus may be the pathogenic driver in these two diseases. This notion is supported by the responsiveness of psoriasis and uveitis to the T cell inhibitor CsA, which is able to effectively block production of IL-17A by T_H17 cells (*36*). Unlike psoriasis and uveitis, RA is not unequivocally responsive to CsA (*37*). We observed comparatively lower response rates in RA, potentially attributable to a more complex interplay of pathogenic cells including T cell subsets (*38*), mast cells (*9*), and B cells (*25*).

A total of 60 patients in three proof-of-concept trials were exposed to AIN457 at doses ranging from 1 × 3 mg/kg to 2 × 10 mg/kg. The most frequent AEs observed in these studies were as expected for the respective diseases and populations and were consistent with previously reported effects of a fully human monoclonal antibody of the IgG1κ class (*22*). There was no clear-cut increase in AEs or SAEs after AIN457 compared with placebo treatment. Safety observations reported herein need to be confirmed in future trials with more diverse populations and ethnicities over longer observation periods for evaluation of long-term host defense risks in relation to therapeutic benefits.

Our proof-of-concept studies had limitations, including small patient numbers, use of preliminary dosing regimens that may require adjustment to provide optimal benefit (dose-ranging studies are ongoing), and the short follow-up periods that limit detection of infrequent or late-occurring adverse effects and demonstration of long-term benefits. In the RA trial, we cannot exclude that observed differences at the primary endpoint occurred at a 12% chance level. Therefore, our results need to be confirmed in larger trials. However, the observed difference between AIN457 and placebo effects at all time points and the significant result ($P < 0.05$) of the AUC analysis support the notion that AIN457 may have therapeutic effects in RA. Moreover, results reported recently from a small proof-of-concept study with a humanized monoclonal antibody to IL-17A (LY2439821) in patients with RA on background immunosuppressants demonstrated efficacy comparable to that observed in our trial (*39*).

In conclusion, our findings indicate that targeted inhibition of the proinflammatory cytokine IL-17A with AIN457 is a valid therapeutic approach and may be useful in the treatment of psoriasis, RA, and noninfectious uveitis. These positive results support development of AIN457 therapy for these and potentially other immune-mediated disorders where medical need continues to exist.

MATERIALS AND METHODS

Three proof-of-concept studies were conducted in patients with psoriasis, RA, and chronic noninfectious uveitis (see fig. S1). All patients provided informed consent. Studies were approved by the institutional review boards at the participating centers and conducted in accordance with the principles of the Declaration of Helsinki.

Study design

The psoriasis study was a single-dose, double-blind, placebo-controlled, parallel-group study in which 36 patients were randomly assigned to receive a single infusion of either AIN457 (3 mg/kg) or saline (placebo) and monitored for 12 weeks.

The RA first-in-human study was designed in three parts to assess safety, tolerability, and pharmacokinetics of single or multiple doses of AIN457. In this report, we provide data from the cohort of patients randomly assigned to receive either two infusions of AIN457 (10 mg/kg) (the highest dose of the trial) or saline placebo. The interval of 3 weeks between the first and the second administration reflected the expected half-life of AIN457 ($t_{1/2}$, 3 to 4 weeks). A total of 52 patients, 26 per group, were enrolled in this 16-week study.

The uveitis trial was an open-label study in which patients with active chronic noninfectious uveitis who required systemic immunosuppression were given two infusions of AIN457 (10 mg/kg): the first at baseline and the second 3 weeks later. Thus, responses were compared with those described in an open-label trial of infliximab, an antibody to TNF-α that is considered an effective therapy for refractory uveitis (*35*).

In all three trials, AIN457 was administered as a 2-hour intravenous infusion.

Patients

In the psoriasis trial, patients aged 18 to 69 years with active disease of at least 6 months of duration were eligible for inclusion if they had a coverage of at least 10% of body surface area with plaques, a score of 3 or more on the IGA scale, and a PASI of 12 or higher (*40–42*). We excluded patients who received biological treatment for psoriasis within 2 months, systemic drugs for psoriasis and phototherapy within 1 month, and topical treatment for psoriasis within 2 weeks of randomization.

In the RA trial, patients aged 18 to 75 years with active disease were eligible for inclusion if they were taking a stable dose of methotrexate (≤25 mg per week) for at least 3 months and the condition had failed to respond to this therapy. We excluded patients who received biological

treatment for RA within 2 or 3 months (respective to drug half-life), disease-modifying antirheumatic drugs (DMARDs) except for stable methotrexate within 1 month, intra-articular and systemic steroid injections for RA within 1 month, and investigational agents for RA within 3 months of randomization.

In the uveitis trial, patients with uveitis aged 18 to 75 years who had visual acuity of 20/40 or worse in at least one eye were eligible for inclusion. Sixteen patients were enrolled: four with HLA-B27–associated anterior uveitis, one with anterior uveitis with HLA-B27 status unknown, four with idiopathic panuveitis, three with sarcoidosis-associated uveitis (panuveitis, posterior uveitis, and intermediate uveitis), two with VKH disease, one with idiopathic posterior uveitis, and one with Behçet's-associated panuveitis. The anterior uveitis patients had disease sufficiently severe and recurrent that a systemic immunosuppressive drug was indicated. Patients with posterior segment uveitis were either intolerant or refractory to systemic corticosteroids or at a stage in their disease course where a corticosteroid-sparing immunosuppressant therapy was required. Exclusion criteria included infections, uveitis of uncertain etiology, and treatment with any immunosuppressant therapy within 3 months before study start.

Endpoints and assessments

In the psoriasis study, the co-primary endpoints were PASI and IGA at week 4. Both parameters were assessed at screening, baseline, weekly until week 6, and then at weeks 8 and 12. Secondary endpoints included pharmacokinetic and pharmacodynamic assessments. Analyses of samples collected at baseline (week 0) and after treatment (week 4) included enumeration of dermal T cells (Fig. 2F) and immunohistology measures of psoriasis plaques (acanthosis, parakeratosis, epidermal thickening, and Ki-67 staining; detailed data for 18 placebo- and 18 AIN457-treated patients available upon request). Gene expression analysis was performed by quantitative RT-PCR for IL-12B (the shared p40 subunit of IL-23 and IL-12), IL-17A, IL-17F, IL-21, IL-22, IL-26, IFN-γ, CCL20, and TNFα, and by array analysis with HG-U133 Plus 2.0 chips for IL-1β, IL-6, IL-8, defensin β4 (DEFB4), and keratin 16 (KRT16). Statistical comparisons between baseline and week 4 measurements were done by Student's *t* tests for quantitative RT-PCR and CD3/IL-17$^+$ T cell enumerations, and analysis of variance (ANOVA) for array data.

In the RA study, the primary endpoint was the ACR20 response rate at week 6. The assessment of components of ACR20, ACR50, and ACR70 measures of disease improvement was performed at weeks 1, 3, 4, 5, 6, 8, 12, and 16. Patients were considered to be ACR20 responders per criteria defined previously (*43*) using CRP. The DAS28 score using the CRP was also calculated at each visit (*43*).

In the chronic noninfectious uveitis study, the primary objective was to assess the safety and tolerability of AIN457. The secondary efficacy objective was to determine the number of patients who responded to therapy at week 8 relative to baseline, with a responder defined with criteria similar to but somewhat more stringent than those reported in an open-label trial of infliximab. In particular, to be a responder, a patient had to have a study eye either with at least a two-step improvement in anterior chamber cells or vitreous haze score to be able to stop topical prednisolone or oral prednisone therapy being taken at baseline, or with three-line improvement in visual acuity (*44*, *45*). Visual acuity was measured with the ETDRS method (*46*).

Safety assessments

Because the RA study was the first-in-human study, a data safety monitoring board was in place to assess potential early safety signals and guide transition to higher-dose levels. Safety was assessed by recording AEs and SAEs, physical examination, and regular monitoring of vital signs, body weight, hematology, blood chemistry, and urine tests. Clinical signs and symptoms of infection were assessed throughout the studies, and immunogenicity to AIN457 was measured.

Statistical analysis

Sample size calculations are described in table S1. Demographic and baseline characteristics were summarized by treatment group. Continuous variables were summarized by mean and SD. SEM was calculated where applicable. Categorical variables were summarized by absolute frequencies and percentages.

In the psoriasis study, the Wilcoxon's rank-sum test was used to compare the mean change from baseline in PASI between active treatment and placebo at each visit. The proportion of patients who achieved 50%, 75%, and 90% reductions in their PASI (PASI50, PASI75, and PASI90, respectively) was summarized by treatment group. The change in IGA score (that is, the number of levels reduced from baseline) was treated as a categorical variable and, at each visit, compared globally across treatment groups with Pearson χ^2 test.

In the RA trial, Fisher's exact test was used at each time point to compare the ACR20 response rate in the two treatment groups. Similar analyses were performed to compare the ACR50 and ACR70 response rates. For analysis of DAS28, a linear mixed-effect model was fitted with treatment group, time point, treatment-by-time interaction, and baseline DAS28 as fixed effects. An unstructured correlation matrix was fitted to adjust for the correlation within patients; a similar analysis was performed on CRP concentrations. In addition, for the ACR20, DAS28, and CRP variables, the AUC for each subject was calculated. The mean AUC in each treatment group was compared with a *t* test (for ACR20) and analysis of covariance with baseline as a covariate (for DAS28 and CRP). Due to the skewed nature of the data, the CRP concentrations were log-transformed before the analysis.

In the uveitis trial, for continuous and ordered categorical variables, differences between baseline and week 8 were compared with analysis of covariance models.

SUPPLEMENTARY MATERIAL

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Materials and Methods

Fig. S1. Patient disposition for the psoriasis study, the rheumatoid arthritis study, and the noninfectious uveitis study.

Investigators in the study listed by indication and site.

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Reference
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Title
AIN457, an anti-IL17 antibody, shows good safety and induces clinical responses in patients with active rheumatoid arthritis (ra) despite methotrexate therapy in a randomized, double-blind proof-of-concept trial

Anti-IL-17 mAb (418942)
AIN-457, a fully human monoclonal antibody against the key regulator of synovial, cartilage and bone pathology in rheumatoid arthritis (RA) --IL-17A-- was recently evaluated as a novel therapeutic modality in patients with active RA despite previous treatment with methotrexate (MTX) in a randomized, double-blind, proof-of-concept trial. A total of 52 patients were randomized to receive 2 intravenous doses of 10 mg/kg AIN-457 (n = 26) or placebo (n = 26) at 3 weeks apart. A statistically significant (based on a predefined level) larger number of patients treated with AIN-457 (46%) achieved an American College of Rheumatology (ACR)20 response rate compared to placebo-treated patients (27%; P = 0.12) at 6 weeks. A clinically important improvement was also seen in the ACR50 (27 vs. 15%) but not the ACR70 (8 vs. 8%) rates in patients treated with AIN-457 compared to placebo. The safety profile was one expected for this type of drug. Early efficacy data indicate rapid reduction of RA disease activity and improvement of RA symptoms following AIN-457 treatment. Analysis of later time points is ongoing.