

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO			
RAD: 11-111960- -11	FECHA: 2013-09-20 16:22:22		
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I		
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 6		
ACT: 519 PRESENTACION			

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
ALICIA LLOREDA RICAURTE

Asunto: Radicación: 11-111960- -11
 Trámite: 11
 Evento: 378
 Actuación: 519
 Oficio: 18208
 Folios: 6

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/EP2010/002005				
Fecha de Presentación Internacional	30/03/2010				

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

1. Documentos estudiados

Descripción:	Radicado No 11-111960-00000-0000	31/Agosto/2011
Reivindicaciones:	Radicado No 11-111960-00000-0000	30/marzo/2010
Dibujos:	Radicado No 11-111960-00000-0000	31/Agosto/2011
Secuencias	Radicado No 11-111960-00000-0000	31/Agosto/2011
Oposiciones:	Radicado No 11-111960-00008-0000	LafrancoI 16/Julio/2012
Respuesta del solicitante:	Radicado No 11-111960-00008-0000	18/Octubre/2012
Prioridad:	EP 090 051 103	07/Abril/2009

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Modificaciones no aceptadas (Art 34 D 486 y Art 19, 22 y 34 PCT)

Las modificaciones de las reivindicaciones 1 – 15,, folios 316 – 318,, no son aceptables porque no se pagó la tasa respectiva.

4. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada.

En el presente caso se estudiaran las reivindicaciones desde 1 a 15 (Radicado No 11-111960-00000-0000)

Título de la solicitud: “Anticuerpos biespecíficos anti-ERBB-3/anti C-MET”

El problema planteado en esta solicitud consiste en la necesidad de proporcionar, métodos de producción de anticuerpos biespecíficos anti ERBB3/ ANTI C-MET, composiciones farmacéuticas y usos, estas composiciones útiles para tratar enfermedades como el cáncer.

Para resolver este problema técnico la solicitud en estudio proporciona un anticuerpo biespecífico anti ErbB3 humana y anti c-met humana.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07K 16/28; C07K16/32; C07K16/46.

5. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de la reivindicación 15 no es patentable de acuerdo con el Art citado.

6. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El objeto de las reivindicaciones 12 – 14 no es patentable, de acuerdo con el Art 14.

7. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

1- La reivindicación 1 se refiere a un anticuerpo a partir de la internalización de la erb-3 no superior al 15 % cuando se mide después de 2 horas en un ensayo de citometría se flujo en células A431, si se compara con la internalización de la ErbB-3 en ausencia del anticuerpo, que es un resultado a alcanzar y no definen al anticuerpo, puesto que la reivindicación incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Se sugiere reemplazarlo por las características esenciales, estructurales

2- Las reivindicaciones 1-4 reclaman el anticuerpo solamente por el antígeno al cual se une.

Se sugiere definirlo a partir de su estructura, debe indicar la secuencia aminoácida de las regiones variables de la cadena ligera y pesada ó las secuencias de los 6 CDRs de las cadenas pesada y ligera

- 3- La reivindicación 10 reclama un ácido nucleico a partir del anticuerpo específico que codifica, lo cual no es aceptable. Se sugiere definirlo a partir de la secuencia nucleótida, indicando el número de la misma (SEQ ID N°)
- 4- La expresión: “no superior” en la reivindicación 1 es relativo por lo cual no permite distinguir la invención del estado de la técnica. Se sugiere sustituirlo por un término o rango concreto.
- 5- La reivindicación 5 define el anticuerpo sin tener en cuenta las 6 CDRs completas. Se sugiere definirlo a partir de su estructura, debe indicar la secuencia aminoácida de las regiones variables de la cadena ligera y pesada ó las secuencias de los 6 CDRs de las cadenas pesada y ligera.. También se refiere a dos anticuerpos diferentes se sugiere reclamarlos en reivindicaciones diferentes.

8. Análisis de las Oposiciones

Argumentaciones de Lafranco

Dice el opositor que esta solicitud pretende obtener patente de composiciones farmacéuticas que comprenden anticuerpos biespecíficos contra ErbB-3 humana y contra c-Met humana, sus métodos para su producción, sus composiciones farmacéuticas que comprenden dichos anticuerpos y sus respectivos usos son prácticas y eventos totalmente conocidos en la industria farmacéutica. El opositor argumenta que no existe novedad ni nivel inventivo y que sería inadecuado conceder un beneficio patentario.

El opositor manifiesta que las composiciones farmacéuticas de las cuales se hace referencia implícitamente, donde se involucran sutilmente agentes tales como Trastuzumab, Pertuzumab y Ertumaxomab no se puede pretender acceder a un beneficio patentario sobre estas composiciones que comprenden moléculas que se encuentran descubiertas y plenamente identificadas en el sector químico-farmacéutico, que en el documento “The Merck Index” en el que se encuentra el principio activo Trastuzumab se encuentra bajo el conocimiento al público desde un tiempo considerable y que además el mismo obtuvo patente WO 8906692 y US5677171 (año 1989 y 1997)

Dice el opositor que es imprescindible destacar que el documento del estado de la técnica WO2008/100624 describe un anticuerpo dirigido contra c-Met y ErbB3, en el que el ErbB3 específicamente es humano y presenta el isotipo IgG3 (página 15 II 21-22). Se presentan además los ácidos nucleicos que lo codifican (Página 18 II. 33-38), y su utilidad en el tratamiento de cáncer (página 38 II. 20-23) (ver además ejemplo 4),. El opositor manifiesta que aunque algunos detalles del anticuerpo como el porcentaje de internalización no han sido descritos previamente, dichas características son intrínsecas al constructo divulgado previamente en WO 2008/100624 y que por esta razón, la materia reclamada en la solicitud no cumple con el requisito de novedad.

Respuestas del Solicitante

El solicitante manifiesta que el opositor se limita exclusivamente a manifestar que las composiciones farmacéuticas que comprenden anticuerpos biespecíficos contra ErbB humana y contra c-Met humana son plenamente conocidos en la industria farmacéutica y que son analizadas a nivel nacional y en muchos otros países, simplemente destacando que el documento WO2008/100624 se describe un anticuerpo dirigido contra c-Met y ErbB3.

El solicitante manifiesta que no todos los anticuerpos biespecíficos que actúan en contra de las proteínas ErbB-3 humana y c-Met humana presentan las propiedades específicas mostradas por los anticuerpos revelados en la solicitud de la patente, para lo cual aporta anexo I. Dice el solicitante que en los datos experimentales que adjunta a la respuesta de la oposición, se destaca que los anticuerpos MH_BvAb32 presentan una fuerte internalización

de 28% y 24%, respectivamente, hecho que demuestra que las enseñanzas del documento WO2008/100624 no afectan la novedad y el nivel inventivo, ya que dicha anterioridad únicamente divulga anticuerpos HER3 que muestran una fuerte propiedad de internalización y no enseña anticuerpos que presenten una sorprendente propiedad de internalización reducida.

También manifiesta el solicitante que los documentos presentados por el opositor para demostrar que en el estado de la técnica ya se conocían sustancias como Trastuzumab, pertuzumab y Ertumaxomab, carecen de toda relevancia porque el solicitante de la invención en cuestión en ningún momento ha pretendido proteger tales sustancias per se.

Respecto a la oposición interpuesta respecto a las excepciones de patentabilidad, el solicitante dice que ha pagado la tasa establecida por reivindicaciones adicionales únicamente para la reivindicación 11 de la invención, y que es claro que las reivindicaciones 12 a 15 presentadas originalmente, dirigidas a proteger usos y métodos de tratamiento, han sido abandonadas.

Respuesta del Examinador Técnico

Respecto al documento aportado por el opositor como “The Merck Index”, no se evidencian las características técnicas esenciales del objeto de la invención, solamente se refiere la descripción de agentes como Trastuzumab, Pertuzumab y Ertumaxomab. El documento WO2008/100624 reportado por el opositor si revela las características técnicas esenciales del objeto de la invención como lo son anticuerpos biespecíficos de unión a ErbB-3 humana y a la c-Met humana (Pág. 15 II. 5-7), isotipo IgG3 (Pág.15 II 21-22), se presentan además los ácidos nucleicos que lo codifican (Página 18 II. 33-38). En cuanto a la utilidad de los anticuerpos como lo manifiesta el señor opositor, se aclara que este no es tenido en cuenta en el examen de patentabilidad por lo que corresponde a una excepción de patentabilidad de acuerdo a (Art 20 D 486 literal (d)).

Respecto a los argumentos del señor solicitante, se aclara que la internalización de los anticuerpos reclamados no corresponde a las características técnicas esenciales de la invención, sino que corresponde a un resultado a alcanzar y no definen al anticuerpo, puesto que incluiría no sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. También se le manifiesta que los documentos anexados a la respuesta de la posición no se tienen en cuenta puesto que no aportan información a las características esenciales del objeto de la invención de acuerdo a lo enseñado anteriormente; no se aceptan las modificaciones hechas al capítulo reivindicatorio, puesto que no se pagó la tasa respectiva y por consiguiente se estudió el capítulo reivindicatorio (Radicado No 11-111960-00000-0000).

El documento WO2008100624 presentado en la oposición desvirtúa el nivel inventivo del objeto de esta solicitud; por lo cual se considerará dentro del examen de patentabilidad.

9. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación internacional: abril 07 de 2009, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	WO2008/100624	“Antibodies against ERBB3 and uses thereof”	21/Agosto/2008
D2	Gold david, et al, Cancer research, american association for cancer research, Us Inkd., Vol. 68, no. 12, (Pág. 4819-4826).	“A Novel Bispecific, Trivalent Antibody Construct for Targeting Pancreatic Carcinoma”	15/Junio/2008
D3	US2005249722	“Monoclonal antibodies with enhanced ADCC function”	10/Noviembre/2005

El documento D1¹ divulga anticuerpos biespecífico (Pág. 14, líneas 19-32) que se unen a Erbb3 humana y a c-Met. (Pág. 14, líneas 19-32), isotipo IgG3 (página 15 II 21-22), se presentan además los ácidos nucleicos que lo codifican (Página 18 II. 33-38) y composiciones farmacéuticas (Pág. 1, renglones 35 – 36).

El documento D2² divulga anticuerpo biespecífico divalente (Pág. 4819, resumen), (Pág. 4820, Fig. 1).

El documento D3³ anticuerpos monoclonales caracterizados porque activan células efectoras : (Párr. 0025:(Párr. 0025), contienen Isotipo IgG1 (Párr. 0088), contienen sitio de glucosilación ASN297, en el que el contenido de fucosa es del 65% o menos (Párr. 0050).

10. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La reivindicación 1 consiste en *“un anticuerpo biespecífico que se une a ErbB3 humana y a la c-Met humana, que contiene un primer sitio de fijación sobre el antígeno que se une específicamente a ErbB3 humana y a un segundo sitio de fijación sobre el antígeno que se une específicamente a la c-Met humana, el anticuerpo presenta un porcentaje de internalización no superior al 15% en comparación con Erbb3 en ausencia del anticuerpo”*

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1
Anticuerpo biespecífico	Anticuerpo biespecífico(Pág. 14, líneas 19-32)
Se une a ErbB3 humana y a la c-Met Humana.	Anti c-Met y ErbB3 Humana (Pág. 14, líneas 19-32)

Las características esenciales de la composición de la reivindicación 1 habían sido divulgadas previamente en el documento D1, el cual, da a conocer anticuerpos biespecífico (Pág. 14, líneas 19-32) que se unen a Erbb3 humana y a c-Met. (Pág. 14, líneas 19-32), isotipo IgG3 (página 15 II 21-22), se presentan además los ácidos nucleicos que lo codifican (Página 18 II. 33-38) y composiciones farmacéuticas (Pág. 1, renglones 35 – 36).

En consecuencia, la reivindicación 1 carece de novedad, según lo divulgado en el documento D1.

Las reivindicaciones dependientes 3, 4, 8, 10, 11 no mencionan alguna característica que haga nueva a la composición de la reivindicación 1, por lo cual se considera que no son nuevas.

Nivel inventivo (Art18 D486)

La reivindicación 2 consiste en *“el anticuerpo biespecífico según la reivindicación 1, caracterizado por ser un anticuerpo biespecifico bivalente o trivalente, que se une a la ERBB-3 humana y a la c-Met humana y consta de uno o más sitios de unión a antígeno que se unen específicamente a la ErbB3 humana y un sitio de unión a antígeno que se une*

11http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=WO&NR=2008100624A2&KC=A2&FT=D&ND=4&date=20080821&DB=EPODOC&locale=en_EP

2<http://cancerres.aacrjournals.org/content/68/12/4819.full.pdf+html>

3http://worldwide.espacenet.com/publicationDetails/description?CC=US&NR=2005249722A1&KC=A1&FT=D&ND=3&date=20051110&DB=EPODOC&locale=en_EP

2

3

específicamente a la c-Met humana”

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1	D2
Anticuerpo biespecífico bivalente o trivalente	Anticuerpo biespecífico (Pág. 14, líneas 19-32)	Anticuerpo biespecífico divalente (Pág. 4819, resumen), (Pág. 4820, Fig. 1).
Se une a ErbB3 humana y a la c-Met humana.	Anti c-Met y ErbB3 Humana (Pág. 14, líneas 19-32)	No se menciona

El documento D1 se considera el estado de la técnica cercano a la invención definida en la reivindicación 2 porque divulga Anticuerpo biespecífico (Pág. 14, líneas 19-32) que se une a Anti c-Met y ErbB3 Humana (Pág. 14, líneas 19-32).

El documento D2 se considera el estado de la técnica cercano a la invención definida en la reivindicación 2 porque divulga Anticuerpo biespecífico divalente (Pág. 4819, resumen), (Pág. 4820, Fig. 1).

La diferencia entre la invención, definida en la reivindicación 2 y el anticuerpo de D1 consiste en que el anticuerpo biespecífico bivalente o trivalente

La diferencia entre la invención, definida en la reivindicación 2 y la invención de D2 es que el anticuerpo se une a ErbB3 humana y a la c-Met humana.

El efecto que logra el incluir un anticuerpo bivalente es la unión específica a c-Met y ErbB3 humana.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo modificar anticuerpos Anticuerpo biespecífico que se une a Anti c-Met y ErbB3 Humana conocidos en D1 para que tengan unión específica.

Sin embargo, la utilización de anticuerpos divalentes para uniones específicas a sus antígenos, ya está divulgada en el documento D2 que se refiere a anticuerpo monoclonal humanizado biespecífico divalente (Pág. 4819, resumen), (Pág. 4820, Fig. 1).

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría anticuerpos divalentes para uniones específicas a sus antígenos del documento D2 en la estructura de los anticuerpos divulgados en el documento D1 para llegar así al objeto de la reivindicación 2 de la solicitud. Por lo cual se considera obvia.

Conclusión:

La reivindicación 2, no cumple con el requisito de nivel inventivo (Art. 18).

La reivindicación 9 consiste en “*el anticuerpo biespecífico de las reivindicaciones 1-8, caracterizado porque dicho anticuerpo está glucosilado con una cadena azúcar en Asn297, en la que la cantidad de fucosa dentro de dicha cadena azúcar es del 55% o menos*”

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1	D3
Anticuerpo biespecífico bivalente o trivalente	Anticuerpo biespecífico (Pág. 14, líneas 19-32)	Anticuerpos monoclonales caracterizado por :(Párr. 0025)
De unión a ErbB3 humana y a la c-Met humana.	Anti c-Met y ErbB3 Humana (Pág. 14, líneas 19-32)	Que activan células efectoras (Párr. 0025)

Contiene región constante del subgrupo IgG1 o IgG3	isotipo IgG3	Isotipo IgG1 (Párr. 0088)
Glucosilado con una cadena de azúcar en ASN27, en el que la fucosa es de 65% o menos.	No menciona	Contiene sitio de glucosilación ASN297, en el que el contenido de fucosa es del 65% o menos (Párr. 0050)

El documento D1 se considera el estado de la técnica cercano a la invención definida en la reivindicación 9 porque divulga Anticuerpo biespecífico (Pág. 14, líneas 19-32) que se une a Anti c-Met y ErbB3 Humana (Pág. 14, líneas 19-32), con isotipo IgG3 (página 15 II 21-22)

El documento D3 se considera el estado de la técnica cercano a la invención definida en la reivindicación 9 porque divulga anticuerpos monoclonales caracterizados porque activan células efectoras (Párr. 0025:(Párr. 0025), contienen Isotipo IgG1 (Párr. 0088), contienen sitio de glucosilación ASN297, en el que el contenido de fucosa es del 65% o menos (Párr. 0050).

La diferencia entre la invención, definida en la reivindicación 9 y la invención de D1 consiste en que el anticuerpo biespecífico bivalente o trivalente esta glucosilado con una cadena de azúcar en ASN27, en el que la fucosa es de 65% o menos.

la diferencia entre la invención, definida en la reivindicación 9 y la invención de D2 es el anticuerpo biespecífico bivalente o trivalente que se une a ErbB3 humana y a la c-Met humana.

No se evidencia un efecto técnico mejorado en los anticuerpos biespecífico de unión a ErbB3 humana y a la c-met humana al glucosilarse con una cadena de azúcar en el sitio Asn297 puesto que dichos anticuerpos presentan la misma especificidad del anticuerpo del documento D1

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar anticuerpos biespecífico que se une a Anti c-Met y ErbB3 humana anticuerpos conocidos en D1 para que la unión a los antígenos sea específica”

Sin embargo, la obtención de anticuerpos monoclonales de unión a células efectoras donde su estructura presenta un sitio de glucosilación Asn297, ya está divulgada en el documento D3 que se refiere a anticuerpos monoclonales caracterizados porque activan células efectoras (Párr. 0025:(Párr. 0025), que contienen Isotipo IgG1 (Párr. 0088), contienen sitio de glucosilación ASN297, con contenido de fucosa es del 65% o menos (Párr. 0050).

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría la glucosilación con una cadena de azúcar en Asn297 del documento D3 en la estructura de los anticuerpos divulgados en el documento D1 para llegar así al objeto de la reivindicación 9 de la solicitud. Por lo cual se considera obvia.

Conclusión:

La reivindicación 9, no cumple con el requisito de nivel inventivo (Art. 18).

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO 2008/100624	1- 4, 8- 11	Novedad
D1	WO 2008/100624	2.	Nivel inventivo
D3	US2005249722	9	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2013-09-24

Elaboró: Edna Milena Bautista Rodriguez
Revisó: Jeny Paola Villate Becerra

Cancer Research

A Novel Bispecific, Trivalent Antibody Construct for Targeting Pancreatic Carcinoma

David V. Gold, David M. Goldenberg, Habibe Karacay, et al.

Cancer Res 2008;68:4819-4826.

Updated version	Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/68/12/4819
Supplementary Material	Access the most recent supplemental material at: http://cancerres.aacrjournals.org/content/suppl/2008/06/11/68.12.4819.DC1.html

Cited Articles	This article cites by 37 articles, 13 of which you can access for free at: http://cancerres.aacrjournals.org/content/68/12/4819.full.html#ref-list-1
Citing articles	This article has been cited by 6 HighWire-hosted articles. Access the articles at: http://cancerres.aacrjournals.org/content/68/12/4819.full.html#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org .
Permissions	To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org .

A Novel Bispecific, Trivalent Antibody Construct for Targeting Pancreatic Carcinoma

David V. Gold,¹ David M. Goldenberg,¹ Habibe Karacay,¹ Edmund A. Rossi,² Chien-Hsing Chang,^{2,3} Thomas M. Cardillo,³ William J. McBride,³ and Robert M. Sharkey¹

¹Garden State Cancer Center, Center for Molecular Medicine and Immunology, Belleville, New Jersey;

²TBC Pharmaceuticals, Inc.; and ³Immunomedics, Inc., Morris Plains, New Jersey

Abstract

Preclinical and clinical studies have demonstrated the application of radiolabeled mAb-PAM4 for nuclear imaging and radioimmunotherapy of pancreatic carcinoma. We have now examined the ability of a novel PAM4-based, bispecific monoclonal antibody (mAb) construct, TF10, to pretarget a radiolabeled peptide for improved imaging and therapy. TF10 is a humanized, bispecific mAb, divalent for mAb-PAM4 and monovalent for mAb-679, reactive against the histamine-succinyl-glycine hapten. Biodistribution studies and nuclear imaging of the radiolabeled TF10 and/or TF10-pretargeted hapten-peptide (IMP-288) were conducted in nude mice bearing CaPan1 human pancreatic cancer xenografts. ¹²⁵I-TF10 cleared rapidly from the blood, with levels decreasing to <1% injected dose per gram (ID/g) by 16 hours. Tumor uptake was $3.47 \pm 0.66\%$ ID/g at this time point with no accumulation in any normal tissue. To show the utility of the pretargeting approach, ¹¹¹In-IMP-288 was administered 16 hours after TF10. At 3 hours postadministration of radiolabeled peptide, imaging showed intense uptake within the tumors and no evidence of accretion in any normal tissue. No targeting was observed in animals given only the ¹¹¹In-peptide. Tumor uptake of the TF10-pretargeted ¹¹¹In-IMP-288 was $24.3 \pm 1.7\%$ ID/g, whereas for ¹¹¹In-IMP-288 alone it was only $0.12 \pm 0.002\%$ ID/g at 16 hours. Tumor/blood ratios were significantly greater for the pretargeting group ($\sim 1,000:1$ at 3 hours) compared with ¹¹¹In-PAM4-IgG ($\sim 5:1$ at 24 hours; $P < 0.0003$). Radiation dose estimates suggested that TF10/⁹⁰Y-peptide pretargeting would provide a greater antitumor effect than ⁹⁰Y-PAM4-IgG. Thus, the results suggest that TF10 pretargeting may provide improved imaging for early detection, diagnosis, and treatment of pancreatic cancer as compared with directly radiolabeled PAM4-IgG. [Cancer Res 2008;68(12):4819–26]

Introduction

Pancreatic cancer is an insidious disease with a particularly high mortality rate. In large measure, this is due to the location of the pancreas in the retroperitoneum, where the tumor can grow in a silent fashion. Symptoms that might suggest the patient seek medical assistance are usually not evident until an advanced stage

of tumor growth, and even at this time, the clinical presentation can be vague and representative of varying pathologies. Combined with the fact that at present, there are no effective means for treatment of this disease, patient outcomes are generally poor; 5-year survival is <5%. However, if detected early, when the cancer is still restricted to the pancreas, treatment by surgical resection, with or without chemotherapy and radiation therapy, can improve 5-year survival to roughly 25%; yet even this statistic is not very encouraging (1, 2). Consequently, considerable effort has been undertaken to find new methods to detect pancreatic cancer at an early stage of tumor growth (pancreatic intraepithelial neoplasia 3) before it becomes invasive, and to improve survival through earlier and more effective treatments.

We have identified a unique biomarker present on mucin expressed by >85% of invasive pancreatic adenocarcinomas, including early stage I disease and the precursor lesions, pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasia (3). The specific epitope, as detected by mAb-PAM4 (4), is absent from normal and inflamed pancreatic tissues, as well as most other malignant tissues. Thus, detection of the epitope provides a high diagnostic likelihood for the presence of pancreatic neoplasia. Early clinical studies using ¹³¹I- and ^{99m}Tc-labeled, murine PAM4 IgG or Fab', respectively, showed specific targeting in 8 of 10 patients with invasive pancreatic adenocarcinoma (5, 6). Of the two negative studies, one had a poorly differentiated pancreatic carcinoma that did not express the PAM4-epitope, whereas the other patient was later found to have pancreatitis rather than a malignant lesion. Accordingly, we believe that the high specificity of PAM4 for pancreatic cancer could be helpful in the detection and diagnosis of early disease. In addition to improved detection, ⁹⁰Y-PAM4 IgG was found to be effective in treating large human pancreatic cancer xenografts in nude mice (7), and when combined with gemcitabine, further improvements in therapeutic response were observed (8, 9). A Phase I therapy trial in patients who failed gemcitabine treatment was recently completed, finding the maximum tolerated dose of ⁹⁰Y-humanized PAM4 IgG to be 20 mCi/m² (10). Although all patients showed disease progression at or after week 8, initial shrinkage of tumor was observed in several cases. Clinical studies are now underway to evaluate a fractionated dosing regimen of ⁹⁰Y-hPAM4 IgG in combination with a radiosensitizing dose of gemcitabine.

Preclinical studies in xenograft models of colorectal cancer and lymphoma have suggested that delivery of radionuclides with pretargeting procedures can provide a significant therapeutic advantage over directly radiolabeled antibodies (11–16). In addition, we have shown that pretargeting procedures can be used in combination with ^{99m}Tc or ¹²⁴I for superior imaging capability over directly radiolabeled antibody fragments (17, 18). Thus, a pretargeting system based on the specificity of PAM4 for pancreatic

Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

Requests for reprints: David V. Gold, Garden State Cancer Center, 520 Belleville Avenue, Belleville, NJ 07109. Phone: 973-844-7025; Fax 1-973-844-7020; E-mail: dvgold@gscancer.org.

©2008 American Association for Cancer Research.

doi:10.1158/0008-5472.CAN-08-0232

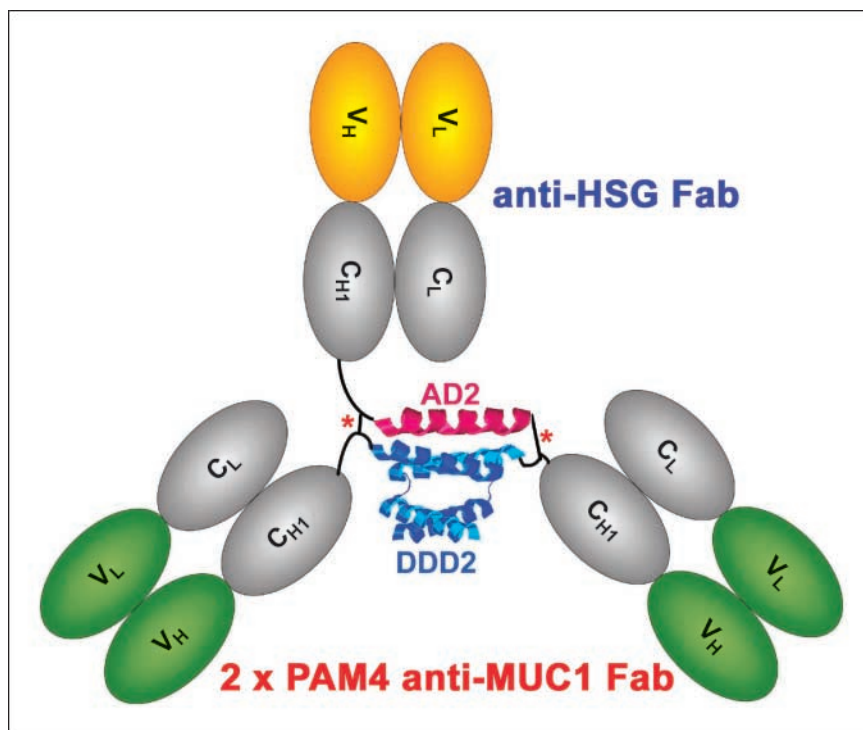


Figure 1. An illustration of TF10, created by the dock-and-lock method, showing two PAM4-Fab-DDD structures self-joined via the dimerization and docking domain (DDD) of human cAMP-dependent protein kinase A with mAb-679-Fab-AD [the anchoring domain (AD) from A-kinase anchor protein] binding to the DDD2 domain. Disulfide bridges (*) are formed between the DDD2 and AD domains to stabilize the final TF10 construct.

cancer could have an appreciable advantage over the directly radiolabeled conjugate. Previously, we reported initial pretargeting studies with a chemically conjugated bispecific antibody prepared by coupling the Fab' fragment of PAM4 to an anti-diethylene-triaminepentaacetic acid (DTPA) Fab', showing improved visualization of tumors with an ^{111}In -DTPA-peptide, as compared with directly radiolabeled PAM4 IgG (19). We now report the development of a novel recombinant, humanized bispecific monoclonal antibody (mAb), TF10, based on the targeting specificity of PAM4 to pancreatic cancer. This construct also binds to the unique synthetic hapten, histamine-succinyl-glycine (HSG), which has been incorporated in a number of small peptides that can be radiolabeled with a wide range of radionuclides suitable for single-photon emission computed tomography (SPECT) and positron emission tomography (PET) imaging, as well as for therapeutic purposes (11, 12, 17, 18). These studies illustrate the potential of this new construct to target pancreatic adenocarcinoma for imaging or therapeutic applications.

Materials and Methods

Construction of TF10, bispecific PAM4 antibody, and peptide hapten. TF10 and IMP-288, peptide hapten, were supplied by IBC Pharmaceuticals, Inc. TF10 was prepared as previously described for a similar bispecific mAb, TF2, an anti-carcinoembryonic antigen construct (17). Briefly, two engineered constructs were prepared based on the humanized forms of either mAb-PAM4 or mAb-679 (anti-HSG hapten). The first, hPAM4-Fab-DDD2, was created as a fusion product of the hPAM4-Fd fragment with the dimerization and docking domain (DDD) of human cyclic AMP (cAMP)-dependent protein kinase A. A *SacII/AgeI* restriction fragment containing the coding sequence for the heavy chain constant domains (C_{H1} - C_{H3}), which was excised from a pdHL2 vector for hPAM4 IgG expression, was replaced with a *SacII/AgeI* fragment that was excised from the plasmid vector CH1-DDD2-Fab-hMN-14-pdHL2, and contains the coding sequence for C_{H1} fused to a DDD2 peptide via a flexible linker.

The hPAM4-Fab-DDD2 vector (30 μg) was linearized by digestion with *SacI* restriction enzyme and stably transfected into Sp/ESF myeloma cells (2.8×10^6) by electroporation. After clonal selection in 0.2 $\mu\text{mol/L}$ methotrexate (Calbiochem), terminal batch cultures of a hPAM4-Fab-DDD2-expressing clone were grown in roller bottles in Hybridoma SFM (Invitrogen) supplemented with 0.8 $\mu\text{mol/L}$ methotrexate. The culture broth was clarified by centrifugation, filtered (0.2 μm), and concentrated 10-fold by diafiltration with exchange into 1 mmol/L EDTA, 0.04 mol/L PBS (pH 7.4).

TF10 was then prepared by combining 195 mg of hPAM4-Fab-DDD2 processed supernatant fluid and 85 mg of purified h679-Fab-AD2, a fusion construct prepared from the h679-Fd and the anchoring domain (AD) from A-kinase anchor protein. The mixture was reduced overnight at room temperature under mild conditions with 2 mmol/L reduced glutathione before the addition of 4 mmol/L oxidized glutathione and an additional overnight incubation. The conjugate was purified by HSG-peptide, affinity column chromatography, followed by Q Sepharose ion-exchange chromatography. The process resulted in a yield of 180 mg of highly purified TF10 at 3.2 mg/mL in 0.02% polysorbate-20, 0.04 mol/L PBS (pH 7.4).

In vitro and *in vivo* characteristics of the peptide IMP-288 [7,10-tetra-azacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA)-D-Tyr-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂], for use with ^{111}In , have been reported (15). The hapten-peptide is divalent with respect to reactivity with mAb-679 (anti-HSG). Production of DOTA-conjugated hPAM4, whole-IgG, has been described (7). *In vitro* characterization of the TF10, along with PAM4-IgG and other PAM4-based constructs, was conducted by enzyme immunoassay (4), SDS-PAGE (17), high-performance liquid chromatography (19), and low-pressure size-exclusion column chromatography (19).

Radiolabeling. Sodium iodide (^{125}I) and indium chloride (^{111}In) were obtained from Perkin-Elmer. TF10 was routinely labeled with ^{125}I by the iodogen method, with purification by use of size-exclusion spin columns. Radiolabeling of DOTA-peptide and DOTA-PAM4-IgG with $^{111}\text{InCl}$ was done as previously described (17, 18). Purity of the radiolabeled products was examined by size-exclusion high-performance liquid chromatography with the amount of free, unbound isotope determined by instant TLC.

Bispecific PAM4 biodistribution studies. Female athymic nude mice ~ 20 g (Taconic Farms), bearing s.c. CaPan1 human pancreatic cancer

xenografts, were injected with ^{125}I -TF10 (10 μCi ; 40 μg , 2.50×10^{-10} mol). At various time points, groups of mice ($n = 5$) were necropsied, with tumor and nontumor tissues removed and counted in a gamma counter to determine the percentage of injected dose per gram of tissue (%ID/g), with these values used to calculate blood clearance rates and tumor/nontumor ratios. All animal studies were carried out with approval from the Institutional Animal Care and Use Committee.

Pretargeting biodistribution studies. In a typical study, a bispecific mAb/radiolabeled peptide molar ratio of 10:1 was used. For example, a group of athymic nude mice bearing s.c. CaPan1 human pancreatic cancer xenografts was administered TF10 (80 μg , 5.07×10^{-10} mol), whereas a second group was left untreated. At 16 h postinjection of TF10, ^{111}In -IMP-288 hapten-peptide (30 μCi , 5.07×10^{-11} mol) was administered. Mice were necropsied at several time points, with tumor and nontumor tissues removed and counted in a gamma counter to determine the %ID/g. Tumor/nontumor ratios were calculated from these data. In a separate study, groups of mice were given ^{111}In -DOTA-PAM4-IgG (20 μCi , 50 μg , 3.13×10^{-10} mol) for the purpose of comparing biodistribution, nuclear imaging, and potential therapeutic activity. Radiation dose estimates were calculated from the time-activity curves with the assumption of no activity at zero time. Student's *t* test was used to assess significant differences.

Nuclear immunoscintigraphy. At 3 h postinjection of radiolabeled peptide or 24 h postinjection of radiolabeled hPAM4-IgG, tumor-bearing mice were imaged with a dual-head Solus gamma camera fitted with medium energy collimator for ^{111}In (ADAC Laboratories). Mice were imaged for a total of 100,000 cpm or 10 min, whichever came first.

Results

In vitro characterization of the bispecific mAb TF10. Figure 1 presents a model of the bispecific, trivalent TF10 construct created by the "dock-and-lock" method (17). The divalent hPAM4-Fab-DDD2 structure is dependent on the self-dimerization and docking domain (DDD) of human cAMP-dependent protein kinase A. The other arm of the bispecific construct, the monovalent h679-Fab-AD (anti-HSG) with anchoring domain (AD) from A-kinase anchor protein, naturally binds to the DDD2 domain, whereupon stable covalent disulfide bridges are formed. High-pressure size-exclusion chromatography showed a single peak eluting at approximately the same time as PAM4-IgG. The binding of TF10 to the target mucin antigen was analyzed by ELISA (Supplementary Fig. S1). The results showed nearly identical binding curves for the divalent TF10, PAM4-IgG, and PAM4-F(ab')₂ (half-maximal binding was calculated as 1.42 ± 0.10 , 1.31 ± 0.12 , and 1.83 ± 0.16 nmol/L, respectively;

$P > 0.05$ for all), whereas the monovalent bsPAM4 chemical conjugate (PAM4-Fab' $\times$ anti-DTPA-Fab') had a significantly lower avidity (half-maximal binding, 30.61 ± 2.05 nmol/L; $P = 0.0379$, compared with TF10), suggesting that TF10 binds in a divalent manner. The immunoreactive fraction of ^{125}I -TF10 bound to the mucin was 87%, with 9% found as unbound TF10 and 3% as free iodide (Supplementary Fig. S2A). Ninety percent of the ^{111}In -IMP-288 bound to TF10 (note that two peaks can be discerned that represent both monovalently and divalently bound products; Supplementary Fig. S2B). Of the total ^{111}In -IMP-288 bound to TF10, 92% eluted at higher molecular weight when excess mucin (200 μg) was added, with only 3% eluting with the non-mucin-reactive TF10 fraction. An additional 5% of the radiolabeled peptide eluted in the free peptide volume. None of the radiolabeled peptide bound to the mucin antigen in the absence of TF10 (not shown).

Biodistribution of ^{125}I -TF10 in CaPan1 tumor-bearing nude mice. TF10 showed a rapid clearance from the blood, starting with 21.03 ± 1.93 %ID/g at 1 hour and decreasing to just 0.13 ± 0.02 %ID/g at 16 hours (Table 1). The biological half-life was calculated to be 2.19 hours [95% confidence interval (95% CI), 2.11–2.27 hours]. Tissue uptake revealed enhanced activity in the liver, spleen, and kidneys at 1 hour, which cleared just as quickly by 16 hours [$T_{1/2} = 2.09$ hours (95% CI, 2.08–2.10), 2.84 hours (95% CI, 2.49–3.29), and 2.44 hours (95% CI, 2.28–2.63) for liver, spleen, and kidney, respectively]. Activity in the stomach most likely reflects the accretion and excretion of radioiodine, suggesting that the radioiodinated TF10 was actively catabolized, presumably in the liver and spleen, thereby explaining its rapid clearance from the blood. Nevertheless, by 16 hours, the concentration of radioiodine within the stomach was below 1% ID/g. A group of five non-tumor-bearing nude mice given ^{125}I -TF10 and necropsied at 16 hours showed similar tissue distribution, suggesting that the tumor had not affected the bispecific mAb distribution and clearance from normal tissues (data not shown). Of course, it is possible that differences occurred before the initial time point examined. Tumor uptake of TF10 peaked at 6 hours postinjection (7.16 ± 1.10 %ID/g) and had decreased to half maximum binding (3.47 ± 0.66 %ID/g) at 16 hours. Tumor uptake again decreased nearly 2-fold over the next 32 hours, but then was stable over the following 24 hours.

Table 1. Biodistribution of ^{125}I -TF10 in CaPan1 tumor-bearing nude mice

	%ID/g (mean $\pm$ SD)				
	1 h	6 h	16 h	48 h	72 h
Tumor	4.16 ± 0.42	7.16 ± 1.10	3.47 ± 0.66	1.65 ± 0.57	1.34 ± 0.24
Liver	10.8 ± 1.36	2.12 ± 0.22	0.15 ± 0.02	0.07 ± 0.01	0.07 ± 0.01
Spleen	11.8 ± 1.90	3.52 ± 0.93	0.17 ± 0.02	0.08 ± 0.02	0.04 ± 0.01
Kidneys	9.60 ± 1.45	2.35 ± 0.39	0.10 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Lung	6.17 ± 0.67	2.53 ± 0.13	0.17 ± 0.03	0.07 ± 0.01	0.05 ± 0.01
Blood	21.0 ± 1.93	4.33 ± 0.51	0.13 ± 0.02	0.03 ± 0.01	0.03 ± 0.01
Stomach	9.84 ± 2.22	28.9 ± 5.37	0.62 ± 0.19	0.12 ± 0.02	0.10 ± 0.03
Small intestine	2.59 ± 0.25	2.10 ± 0.21	0.18 ± 0.05	0.04 ± 0.00	0.03 ± 0.01
Large intestine	1.05 ± 0.17	2.15 ± 0.28	0.21 ± 0.06	0.06 ± 0.01	0.04 ± 0.01
Pancreas	1.77 ± 0.52	1.78 ± 0.28	0.06 ± 0.01	0.02 ± 0.00	0.01 ± 0.00
Tumor weight (g)	0.36 ± 0.14	0.35 ± 0.14	0.34 ± 0.06	0.47 ± 0.14	0.47 ± 0.13

Table 2. Biodistribution of ^{111}In -IMP-288 alone (no TF10) or pretargeted with varying amounts of TF10

	%ID/g at 3 h (mean $\pm$ SD)			
	5:1	10:1	20:1	No TF10
Tumor	19.0 $\pm$ 3.49	24.3 $\pm$ 1.71	28.6 $\pm$ 0.73	0.12 $\pm$ 0.00
Liver	0.09 $\pm$ 0.01	0.21 $\pm$ 0.12	0.17 $\pm$ 0.01	0.07 $\pm$ 0.00
Spleen	0.12 $\pm$ 0.04	0.16 $\pm$ 0.07	0.26 $\pm$ 0.10	0.04 $\pm$ 0.01
Kidneys	1.59 $\pm$ 0.11	1.72 $\pm$ 0.24	1.53 $\pm$ 0.14	1.71 $\pm$ 0.22
Lungs	0.19 $\pm$ 0.06	0.26 $\pm$ 0.00	0.29 $\pm$ 0.04	0.03 $\pm$ 0.00
Blood	0.01 $\pm$ 0.00	0.01 $\pm$ 0.01	0.01 $\pm$ 0.00	0.00 $\pm$ 0.00
Stomach	0.03 $\pm$ 0.02	0.02 $\pm$ 0.02	0.01 $\pm$ 0.00	0.02 $\pm$ 0.01
Small intestine	0.12 $\pm$ 0.08	0.08 $\pm$ 0.03	0.04 $\pm$ 0.01	0.06 $\pm$ 0.02
Large intestine	0.23 $\pm$ 0.10	0.39 $\pm$ 0.08	0.25 $\pm$ 0.08	0.33 $\pm$ 0.02
Pancreas	0.02 $\pm$ 0.00	0.02 $\pm$ 0.01	0.02 $\pm$ 0.00	0.02 $\pm$ 0.00
Tumor weight (g)	0.12 $\pm$ 0.03	0.32 $\pm$ 0.09	0.27 $\pm$ 0.01	0.35 $\pm$ 0.03

Biodistribution of TF10-pretargeted, ^{111}In -labeled peptide.

Although maximum tumor uptake of TF10 occurred at 6 hours, previous experience indicated that the radiolabeled peptide would need to be given at a time point when blood levels of TF10 had cleared to <1% ID/g (i.e., 16 hours; ref. 19). Higher levels of TF10 in the blood would lead to unacceptably high binding of the radiolabeled peptide within the blood (i.e., low tumor/blood ratios), whereas administering the peptide at a later time would mean the concentration of TF10 in the tumor would be decreased with consequently reduced concentration of radiolabeled peptide within the tumor. Thus, an initial pretargeting study was done using a 16-hour interval. With the amount of the ^{111}In -IMP-288 held constant (30 μCi , 5.07×10^{-11} mol), increasing amounts of TF10 were given so that the administered dose of TF10 and IMP-288 expressed as mole ratio varied from 5:1 to 20:1. As shown in Table 2, at 3 hours the amount of ^{111}In -IMP-288 in the blood was barely detectable (0.01%). Tumor uptake increased from $19.0 \pm 3.49\%$ ID/g to $28.55 \pm 0.73\%$ ID/g as the amount of bispecific mAb administered was increased 4-fold (statistically significant differences were observed for comparison of each TF10/peptide ratio, one group to another; $P < 0.03$ or better), but without any appreciable increase in normal tissue uptake. Tumor uptake in the animals given TF10 was >100-fold higher than when ^{111}In -IMP-288 was given alone. Comparison of ^{111}In activity in the normal tissues of the animals that either received or did not receive prior administration of TF10 indicated similar absolute values, which in most instances were not significantly different. This suggests that the bispecific mAb had cleared sufficiently from all tissues by 16 hours to avoid appreciable peptide uptake in these tissues. Tumor/blood ratios were >2,000:1, with other tissue ratios exceeding 100:1. Even tumor/kidney ratios exceeded 10:1. The highest tumor uptake of radioisotope with minimal targeting to nontumor tissues resulted from the 20:1 ratio; however, either of the TF10/peptide ratios could be used to achieve exceptional targeting to tumor, both in terms of signal intensity and contrast ratios. The 10:1 ratio was chosen for further study because the absolute difference in tumor uptake of radiolabeled peptide was not substantially different between the 10:1 ($24.3 \pm 1.71\%$ ID/g) and 20:1 ($28.6 \pm 0.73\%$ ID/g) ratios.

Images of the animals given TF10-pretargeted ^{111}In -IMP-288 at a bispecific mAb/peptide ratio of 10:1, or the ^{111}In -IMP-288 peptide alone, are shown in Fig. 2. The majority of these tumors were

≤ 0.5 cm in diameter, weighing ~ 0.25 g. The images show highly intense uptake in the tumor of the TF10-pretargeted animals (Fig. 2A, *top*). The intensity of the image background for the TF10-pretargeted animals was increased to match the intensity of the image taken of the animals given the ^{111}In -IMP-288 alone (Fig. 2B, *middle*). However, when the images were optimized for the TF10-pretargeted mice, the signal intensity and contrast were so high that no additional activity was observed in the body. No tumor localization was seen in the animals given the ^{111}In -IMP-288 alone, even when image intensity was enhanced.

An additional experiment was done to assess the kinetics of targeting ^{111}In -hPAM4 whole-IgG compared with that of the TF10-pretargeted ^{111}In -IMP-288 peptide (Table 3). Tumor uptake of the ^{111}In -peptide was highest at the initial time point examined, 3 hours ($15.99 \pm 4.11\%$ ID/g), whereas the blood concentration of radiolabeled peptide was only $0.02 \pm 0.01\%$ ID/g, providing a mean tumor/blood ratio of 946.3 ± 383.0 . Over time, radiolabeled peptide cleared from the tumor with a biological half-life of 76.04 hours. Among nontumor tissues, uptake was highest in the kidneys, averaging $1.89 \pm 0.42\%$ ID/g at 3 hours with a steady decrease over time (biological half-life, 33.6 hours). Liver uptake started at $0.15 \pm 0.06\%$ ID/g and remained essentially unchanged over time. In contrast to the TF10-pretargeted ^{111}In -IMP-288, the ^{111}In -hPAM4-IgG had a slower clearance from the blood, albeit there was a substantial clearance within the first 24 hours, decreasing from 30.1% ID/g at 3 hours to just $11.5 \pm 1.7\%$ ID/g at 24 hours. Variable elevated uptake in the spleen suggested that the antibody was likely being removed from the blood by a similar mechanism as previously described (i.e., targeting of secreted mucin that had become entrapped within the spleen; ref. 20). Tumor uptake peaked at 48 hours with $80.4 \pm 6.1\%$ ID/g, and remained at an elevated level over the duration of the monitoring period. The high tumor uptake, paired with a more rapid than expected blood clearance for an IgG, produced tumor/blood ratios of 5.2 ± 1.0 within 24 hours. Figure 2C (*bottom*) shows the images of the animals at 24 hours postadministration of ^{111}In -PAM4-IgG, illustrating that tumors could be visualized at this early time, but there was still considerable activity within the abdomen. Tumor/nontumor ratios were mostly higher for TF10-pretargeted ^{111}In -labeled hapten-peptide as compared with ^{111}In -hPAM4-IgG, except for the kidneys, where tumor/kidney ratios with the ^{111}In -IMP-288 and ^{111}In -hPAM4-IgG were similar at later times. However, tumor/kidney

ratios for the TF10-pretargeted ^{111}In -IMP-288 were high enough (e.g., $\sim 7:1$) at 3 hours to easily discern tumor from normal tissue.

Figure 3 illustrates the potential therapeutic capability of the direct and pretargeted methods to deliver radionuclide (^{90}Y). Although the concentration (%ID/g) of radioisotope within the tumor seems to be much greater when delivered by PAM4-IgG than by pretargeted TF10 at their respective maximum tolerated dose (0.15 mCi for ^{90}Y -hPAM4 and 0.9 mCi for TF10-pretargeted ^{90}Y -IMP-288), the radiation dose to tumor would be similar (10,080 and 9,229 cGy for ^{90}Y -PAM4-IgG and TF10-pretargeted ^{90}Y -IMP-288, respectively). The advantage for the pretargeting method would be the exceptionally low activity in blood (9 cGy), almost 200-fold less than with the ^{90}Y -hPAM4 IgG (1,623 cGy). It is also important to note that the radiation dose to liver, as well as other nontumor organs, would be much lower with the TF10-pretargeted ^{90}Y -IMP-288. The exception would be the kidneys, where the radiation dose would be similar for both protocols at their respective maximum dose (612 and 784 cGy for ^{90}Y -PAM4-IgG and TF10- ^{90}Y -IMP-288, respectively). The data suggest that for ^{90}Y -PAM4-IgG, as with most other radiolabeled whole-IgG mAbs, the dose-limiting toxicity would be hematologic; however, for the TF10 pretargeting protocol, the dose-limiting toxicity would be the kidneys.

Discussion

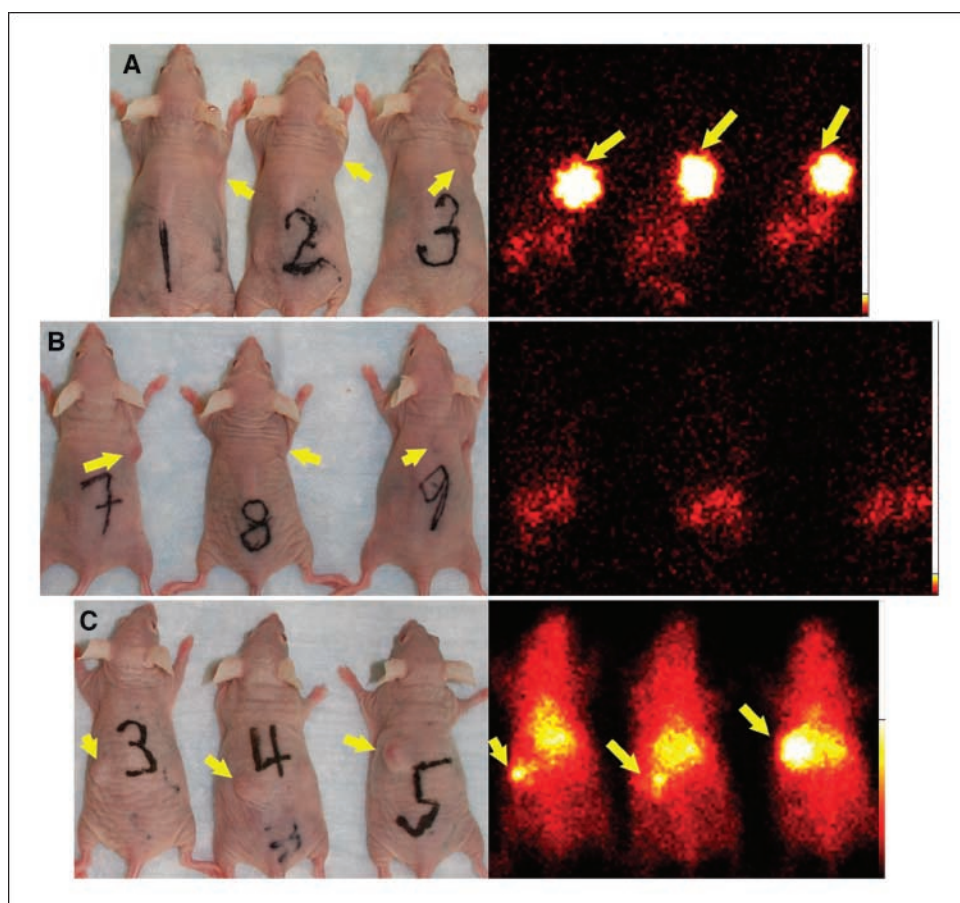
Current imaging procedures provide the critical information necessary for detection, diagnosis, and management of invasive pancreatic adenocarcinoma. Ultrasound, computerized tomogra-

phy (CT), and magnetic resonance imaging (MRI) technologies, which provide anatomic images, along with PET imaging of the metabolic environment, have routinely been found to provide high sensitivity in the detection of pancreatic masses; however, these data are, for the most part, based on detection of lesions >2 cm in a population that is already presenting clinical symptoms (21–24). At this time in the progression of the pancreatic carcinoma, the prognosis is rather dismal. To improve patient outcomes, detection of small, early pancreatic neoplasms in the asymptomatic patient will be necessary.

In the past few years, several institutions have undertaken a renewed effort at discovery of small pancreatic masses in asymptomatic, high-risk patient groups undergoing targeted screening protocols (25, 26). Canto and colleagues (27), using CT and endoscopic ultrasonography, screened a series of 78 patients considered at high-risk for pancreatic cancer. If endoscopic ultrasonography was abnormal, endoscopic ultrasonography-fine needle aspiration and endoscopic retrograde cholangiopancreatography were done. By use of this protocol, a significant number of early, potentially curable, neoplastic masses, as well as precursor lesions, were discovered in asymptomatic patients (10.2% diagnostic yield). However, the majority of patients examined presented with moderate to severe pancreatitis, a potentially confounding environment for accurate detection and diagnosis, especially of small neoplastic lesions.

Winter and colleagues (28), in a retrospective analysis of patients undergoing an abdominal imaging study for nonpancreatic disease ($n = 1,944$), reported discovery of "incidentalomas" (a periampullary

Figure 2. Immunoscintigraphy of CaPan1 human pancreatic cancer xenografts (~ 0.25 g). **A**, an image of mice that were injected with bispecific TF10 ($80\text{ }\mu\text{g}$, 5.07×10^{-10} mol) followed 16 h later by administration of ^{111}In -IMP-288 ($30\text{ }\mu\text{Ci}$, 5.07×10^{-11} mol). The image was taken 3 h later. The intensity of the image background was increased to match the intensity of the image obtained when ^{111}In -IMP-288 was administered alone ($30\text{ }\mu\text{Ci}$, 5.07×10^{-11} mol). **B**, no targeting was observed in mice given ^{111}In -IMP-288 alone. **C**, an image of mice that were given ^{111}In -DOTA-PAM4-IgG ($20\text{ }\mu\text{Ci}$, $50\text{ }\mu\text{g}$) with imaging done 24 h later. Although tumors are visible, considerable background activity is still present at this time point.



or pancreatic mass) in 86 patients (4.4% of patients examined). Others have recognized the challenge of managing the patient with incidentalomas or what seems to be cystic disease of the pancreas (i.e., to treat a potentially malignant disease by surgical resection, or wait and observe; ref. 29). Considering these incidentalomas can be identified retrospectively in almost 1% of all abdominal CT or MRI scans, this represents a large group of individuals from the general population for whom a significant diagnostic problem exists.

Imaging with a mAb-targeted approach, such as is described herein with mAb-PAM4, may provide the data necessary for diagnosis of these small, early cancers. Of prime importance is the specificity of the mAb. We have presented considerable data, including immunohistochemical studies of tissue specimens (3, 4) and immunoassay of patient sera (30), to show that mAb-PAM4 is highly reactive with a biomarker, the presence of which provides high diagnostic likelihood of pancreatic neoplasia. Furthermore, we determined that PAM4, although not reactive with normal adult pancreatic tissues nor active pancreatitis, is reactive with the earliest stages of neoplastic progression within the pancreas (pancreatic intraepithelial neoplasia 1 and intraductal papillary mucinous neoplasia) and that the biomarker remains at high levels of expression throughout the progression to invasive pancreatic adenocarcinoma (3). Preclinical studies with athymic nude mice bearing human pancreatic tumor xenografts have shown specific targeting of radiolabeled murine, chimeric, and humanized versions of PAM4 (7, 20).

Others have also examined the application of mAbs to target and provide *in vivo* imaging of pancreatic cancers, mostly as xenografts in athymic nude mice, although a few have been studied in clinical trials as well (31–35). While it is difficult to compare all of these previous studies, for the most part radiolabeled whole-IgG has been used, with the result that the highest tumor/blood ratios occurred between 72 and 96 hours postinjection and were in the range of 2:1 to 10:1; in other words, similar to the results we obtained with radiolabeled PAM4 whole-IgG. However, to the best of our knowledge, none of the other mAbs used *in vivo* in pancreatic cancer models or patients has shown the specificity for pancreatic cancer that we have reported for PAM4.

We appreciate that high-quality antibody-targeted tumor imaging in mice bearing human xenografts is quite different from what may be achieved clinically. In general, the difference in pharma-

cokinetics between the species provides for a substantially lower tumor uptake of radiolabeled antibody and lower tumor/nontumor ratios in the human than in the mouse. However, we have reported successful, specific imaging of invasive pancreatic adenocarcinoma with murine PAM4 whole-IgG, radiolabeled with ^{131}I or $^{99\text{m}}\text{Tc}$ (5, 6), and more recently as part of a phase I imaging and therapy trial using ^{111}In -DOTA humanized PAM4 (10).

Whereas imaging with radiolabeled antibodies (whole-IgG and/or their fragments and engineered constructs) has produced several Food and Drug Administration–approved imaging agents for several indications (e.g., arcitumomab, capromab, and others), the images usually suffer from relatively low signal strength (%ID/g) and insufficient contrast (tumor/nontumor ratio) for detection of small tumors. For detection of early, primary pancreatic cancer, which is tucked between several major organs and is more centrally located in the abdomen surrounded by major blood vessels, the sensitivity of the detection method will need to improve significantly.

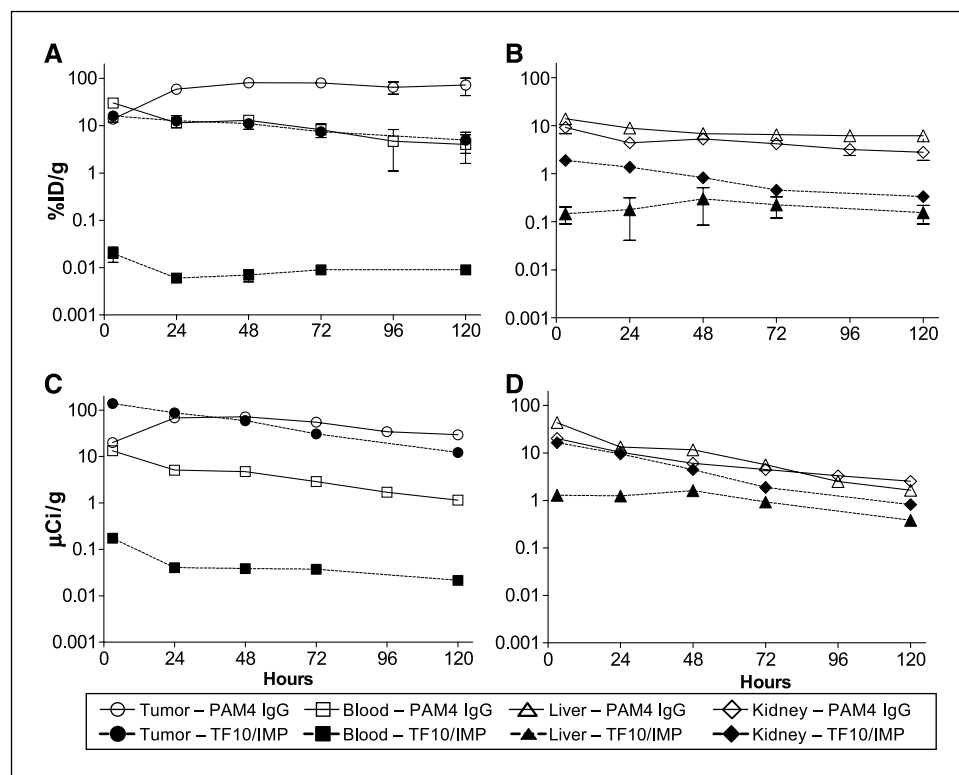
In the current studies, we have examined a next-generation, recombinant, bispecific PAM4-based construct, TF10, which is divalent for the PAM4 arm and monovalent for the anti-HSG hapten arm. There are several important characteristics of this pretargeting system's constructs, named dock-and-lock, including its general applicability and ease of synthesis (17, 36, 37). However, for the present consideration, the major differences from the previously reported chemical construct are the valency, which provides improved binding to tumor antigen, and, importantly, its pharmacokinetics. TF10 clearance from nontumor tissues is much more rapid than was observed for the chemical conjugate. Time for blood levels of the bispecific constructs to reach less than 1% ID/g was 40 hours postinjection for the chemical construct versus 16 hours for TF10. A more rapid clearance of the pretargeting agent has provided a vast improvement of the tumor/blood ratio, while maintaining high signal strength at the tumor site (%ID/g).

In addition to providing a means for early detection and diagnosis, the possibility to make use of the TF10 pretargeting system for therapy should not be overlooked. Consideration of the effective radiation dose to tumor and nontumor tissues favors the pretargeting method over directly radiolabeled PAM4-IgG. The dose estimates suggest that the two delivery systems have different dose-limiting toxicities: myelotoxicity for the directly radiolabeled

Table 3. Tumor/nontumor ratios for the biodistribution of TF10-pretargeted peptide or IgG

	^{111}In -hapten-peptide at 3 h	^{111}In -DOTA-PAM4 IgG at 24 h
	(mean $\pm$ SD)	
Tumor	1.0 $\pm$ 0.0	1.0 $\pm$ 0.0
Liver	128.8 $\pm$ 70.6	6.8 $\pm$ 1.4
Spleen	50.9 $\pm$ 31.1	5.6 $\pm$ 1.3
Kidneys	9.1 $\pm$ 3.4	13.7 $\pm$ 2.4
Lungs	54.9 $\pm$ 20.4	14.1 $\pm$ 2.0
Blood	915.2 $\pm$ 404.3	5.2 $\pm$ 1.0
Stomach	508.0 $\pm$ 271.5	61.0 $\pm$ 14.2
Small intestine	233.7 $\pm$ 100.0	18.9 $\pm$ 2.4
Large intestine	98.4 $\pm$ 47.1	35.1 $\pm$ 3.9
Pancreas	894.0 $\pm$ 855.0	35.4 $\pm$ 3.0
Tumor weight (g)	0.28 $\pm$ 0.21	0.10 $\pm$ 0.06

Figure 3. Extended biodistribution of ^{111}In -DOTA-PAM4-IgG (20 μCi , 50 μg) and TF10-pretargeted ^{111}In -IMP-288 (80 μg , 5.07×10^{-10} mol TF10 followed 16 h later with 30 μCi , 5.07×10^{-11} mol ^{111}In -IMP-288) in nude mice bearing CaPan1 human pancreatic cancer xenografts (mean tumor weight $\pm$ SD, 0.28 ± 0.21 and 0.10 ± 0.06 g for the pretargeting and IgG groups of animals, respectively). A and B, biological data; points, mean %ID/g ($n = 5$ mice per time point); bars, SD. C and D, effective activity, assuming mice were injected with their respective maximum tolerated dose, either 0.15 mCi of ^{90}Y -DOTA-PAM4-IgG or 0.9 mCi of TF10-pretargeted ^{90}Y -IMP-288.



PAM4 versus the kidney for the TF10 pretargeting system. This is of significance for the future clinical development of radiolabeled PAM4 as a therapeutic agent. Gemcitabine, the frontline drug of choice for pancreatic cancer, can provide significant radiosensitization of tumor cells. In previous studies, we showed that combinations of gemcitabine and directly radiolabeled PAM4-IgG provided synergistic antitumor effects compared with either arm alone (8, 9). The dose-limiting factor with this combination was overlapping hematologic toxicity. However, because the dose-limiting organ for TF10 pretargeting seems to be the kidney rather than hematologic tissues, combinations with gemcitabine should be less toxic, thus allowing increased administration of radioisotope with consequently greater antitumor efficacy.

The superior imaging achieved with TF10 pretargeting in preclinical models, as compared with directly radiolabeled DOTA-PAM4-IgG, provides a compelling rationale to proceed to clinical trials with this imaging system. The specificity of the tumor-targeting mAb for pancreatic neoplasms, coupled with the bispecific antibody platform technology providing the ability to conjugate various imaging compounds to the HSG-hapten-peptide for SPECT (^{111}In), PET (^{68}Ga), ultrasound (Au), or other contrast agents, or for that matter ^{90}Y or other radionuclides for therapy, provides high potential to improve overall patient outcomes (38).

In particular, we believe that a TF10-based ImmunoPET procedure will have major clinical value to screen individuals at high-risk for development of pancreatic cancer (e.g., genetic predisposition, chronic pancreatitis, smokers, etc.), as well as a means for follow-up of patients with suspicious abdominal images from conventional technologies and/or with indications due to the presence of specific biomarker(s) or abnormal biochemical findings. When used as part of an ongoing medical plan for following these patients, early detection of pancreatic cancer may be achieved. Finally, in combination with gemcitabine, TF10 pretargeting may provide a better opportunity for control of tumor growth than directly radiolabeled PAM4-IgG.

Disclosure of Potential Conflicts of Interest

D.M. Goldenberg, E.A. Rossi, C.H. Chang, T.M. Cardillo, and W.J. McBride declare financial interests (stock, options, and employment relationships) in Immunomedics, Inc., and/or IBC Pharmaceuticals, Inc.

Acknowledgments

Received 1/21/2008; revised 3/31/2008; accepted 4/21/2008.

Grant support: National Cancer Institute grants CA096924 and CA115755.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

1. Cleary SP, Gryfe R, Guindi M, et al. Prognostic factors in resected pancreatic adenocarcinoma: analysis of actual 5-year survivors. *J Am Coll Surg* 2004; 198:722-31.
2. Han SS, Jang JY, Kim SW, Kim WH, Lee KU, Park YH. Analysis of long-term survivors after surgical resection for pancreatic cancer. *Pancreas* 2006;32: 271-5.
3. Gold DV, Karanjawala Z, Modrak DE, Goldenberg DM, Hruban RH. PAM4-reactive MUC1 is a biomarker for early pancreatic adenocarcinoma. *Clin Cancer Res* 2007; 13:7380-7.
4. Gold DV, Lew K, Maliniak R, Hernandez M, Cardillo T. Characterization of monoclonal antibody PAM4 reactive with a pancreatic cancer mucin. *Int J Cancer* 1994;57:204-10.
5. Mariani G, Molea N, Bacciardi D, et al. Initial tumor targeting, biodistribution, and pharmacokinetic evaluation of the monoclonal antibody PAM4 in patients with pancreatic cancer. *Cancer Res* 1995;55:5911-5s.

6. Gold DV, Cardillo T, Goldenberg DM, Sharkey RM. Localization of pancreatic cancer with radiolabeled monoclonal antibody PAM4. *Crit Rev Oncol Hematol* 2001;39:147-54.
7. Cardillo TM, Ying Z, Gold DV. Therapeutic advantage of (90)yttrium- versus (131)iodine-labeled PAM4 antibody in experimental pancreatic cancer. *Clin Cancer Res* 2001;7:3186-92.
8. Gold DV, Schutsky K, Modrak D, Cardillo TM. Low-dose radioimmunotherapy ((90)Y-PAM4) combined with gemcitabine for the treatment of experimental pancreatic cancer. *Clin Cancer Res* 2004;10:3552-61.
9. Gold DV, Modrak DE, Schutsky K, Cardillo TM. Combined ⁹⁰Yttrium-DOTA-labeled PAM4 antibody radioimmunotherapy and gemcitabine radiosensitization for the treatment of a human pancreatic cancer xenograft. *Int J Cancer* 2004;109:618-26.
10. Gulec SA, Cohen SJ, Zuckier LS, et al. First clinical experience with ⁹⁰Y-radiolabeled humanized anti-MUC1 antibody (hPAM4) in patients with advanced pancreatic cancer: a phase I study. *Proc Amer Soc Clin Onc*, 43rd Annual Meeting, *J Clin Oncol* 2007;25(18S):636s (abstract #15034).
11. Karacay H, Brand PY, Sharkey RM, et al. Therapeutic advantage of pretargeted radioimmunotherapy using a recombinant bispecific antibody in a human colon cancer xenograft. *Clin Cancer Res* 2005;11:7879-85.
12. Sharkey RM, Karacay H, Chang CH, McBride WJ, Horak ID, Goldenberg DM. Improved therapy of non-Hodgkin's lymphoma xenografts using radionuclides pretargeted with a new anti-CD20 bispecific antibody. *Leukemia* 2005;19:1064-9.
13. Lin Y, Pagel JM, Axworthy D, Pantelias A, Hedin N, Press OW. A genetically engineered anti-CD45 single-chain antibody-streptavidin fusion protein for pretargeted radioimmunotherapy of hematologic malignancies. *Cancer Res* 2006;66:3884-92.
14. Goldenberg DM, Sharkey RM, Paganelli G, Barbet J, Chatal JF. Antibody pretargeting advances cancer radioimmunodetection and radioimmunotherapy. *J Clin Oncol* 2006;24:823-34.
15. Green DJ, Pagel JM, Pantelias A, et al. Pretargeted radioimmunotherapy for B-cell lymphomas. *Clin Cancer Res* 2007;13:5598-603s.
16. Pantelias A, Pagel JM, Hedin N, et al. Comparative biodistributions of pretargeted radioimmunoconjugates targeting CD20, CD22, and DR molecules on human B-cell lymphomas. *Blood* 2007;109:4980-7.
17. Rossi EA, Goldenberg DM, Cardillo TM, McBride WJ, Sharkey RM, Chang CH. Stably tethered multifunctional structures of defined composition made by the dock and lock method for use in cancer targeting. *Proc Natl Acad Sci U S A* 2006;103:6841-6.
18. McBride WJ, Zanzonico P, Sharkey RM, et al. Bispecific antibody pretargeting PET (ImmunoPET) with an ¹²⁴I-labeled hapten-peptide. *J Nucl Med* 2006;47:1678-88.
19. Cardillo TM, Karacay H, Goldenberg DM, et al. Improved targeting of pancreatic cancer: experimental studies of a new bispecific antibody, pretargeting enhancement system for immunoscintigraphy. *Clin Cancer Res* 2004;10:3552-61.
20. Gold DV, Alisauskas R, Sharkey RM. Targeting of xenografted pancreatic cancer with a new monoclonal antibody, PAM4. *Cancer Res* 1995;55:1105-10.
21. Scaglione M, Pinto A, Romano S, et al. Using multidetector row computed tomography to diagnose and stage pancreatic carcinoma: the problems and the possibilities. *JOP* 2005;6:1-5.
22. Dewitt J, Devereaux BM, Lehman GA, Sherman S, Imperiale TF. Comparison of endoscopic ultrasound and computed tomography for the preoperative evaluation of pancreatic cancer: a systematic review. *Clin Gastroenterol Hepatol* 2006;4:17-25.
23. Pakzad F, Groves AM, Ell PJ. The role of positron emission tomography in the management of pancreatic cancer. *Semin Nucl Med* 2006;36:248-56.
24. Schima W, Ba-Salamah A, Kolblinger C, Kulinna-Cosentini C, Puspoeck A, Gotzinger P. Pancreatic adenocarcinoma. *Eur Radiol* 2007;17:638-49.
25. Chari ST. Detecting early pancreatic cancer: problems and prospects. *Semin Oncol* 2007;34:284-94.
26. Canto MI. Strategies for screening for pancreatic adenocarcinoma in high-risk patients. *Semin Oncol* 2007;34:295-302.
27. Canto MI, Goggins M, Hruban RH, et al. Screening for early pancreatic neoplasia in high-risk individuals: a prospective controlled study. *Clin Gastroenterol Hepatol* 2006;4:766-81.
28. Winter JM, Cameron JL, Lillemoe KD, et al. Periampullary and pancreatic incidentaloma: a single institution's experience with an increasingly common diagnosis. *Ann Surg* 2006;243:673-83.
29. Spinelli KS, Fromwiller TE, Daniel RA, et al. Cystic pancreatic neoplasms: observe or operate. *Ann Surg* 2004;239:651-9.
30. Gold DV, Modrak DE, Ying Z, Cardillo TM, Sharkey RM, Goldenberg DM. New MUC1 serum immunoassay differentiates pancreatic cancer from pancreatitis. *J Clin Oncol* 2006;24:252-8.
31. Kaufmann LW, Vaillant JC, van Gulik TM, van Royen EA, Parc R, Obertop H. Efficacy of monoclonal antibody 131I-B72.3 immunoscintigraphy of pancreatic adenocarcinoma xenografts in nude mice. *Eur J Surg* 1999;165:659-64.
32. Hajjar G, Sharkey RM, Burton J, et al. Phase I radioimmunotherapy trial with iodine-131-labeled humanized MN-14 anti-carcinoembryonic antigen monoclonal antibody in patients with metastatic gastrointestinal and colorectal cancer. *Clin Colorectal Cancer* 2002;2:31-42.
33. Otsuji E, Matsumura H, Okamoto K, et al. Application of ^{99m}Tc labeled chimeric Fab fragments of monoclonal antibody A7 for immunoscintigraphy of pancreatic carcinoma. *J Surg Oncol* 2003;84:160-5.
34. Foss CA, Fox JJ, Feldmann G, et al. Radiolabeled anti-claudin 4 and anti-prostate stem cell antigen: initial imaging in experimental models of pancreatic cancer. *Mol Imaging* 2007;6:131-9.
35. Sabbah EN, Kadouche J, Ellison D, Finucane C, Decaudin D, Mather SJ. *In vitro* and *in vivo* comparison of DTPA- and DOTA-conjugated antiferritin monoclonal antibody for imaging and therapy of pancreatic cancer. *Nucl Med Biol* 2007;34:293-304.
36. Boswell CA, Brechbiel MW. Development of radioimmunotherapeutic and diagnostic antibodies: an inside-out view. *Nucl Med Biol* 2007;34:757-78.
37. Sharkey RM, Karacay H, McBride WJ, Rossi EA, Chang CH, Goldenberg DM. Bispecific antibody pretargeting of radionuclides for immuno single-photon emission computed tomography and immuno positron emission tomography molecular imaging: an update. *Clin Cancer Res* 2007;13:5577-85s.
38. Goldenberg DM, Rossi EA, Sharkey RM, McBride WJ, Chang CH. Multifunctional antibodies by the dock-and-lock method for improved cancer imaging and therapy by pretargeting. *J Nucl Med* 2008;49:158-63.