



Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E. S. D.

Superintendencia de Industria y Comercio



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TC

TÍTULO SOLICITADO: "CONJUGADOS DE ANTICUERPO Y FÁRMACO
BASADOS EN ERIBULINA Y MÉTODOS PARA SU
USO"

PUBLICACIÓN: Gaceta 837 de 31 de agosto de 2018.

PRIORIDAD: US 62/302,562 02/03/2016

SOLICITANTE: EISAI R [&] D MANAGEMENT CO., LYD.

APODERADO: CARLOS REINALDO OLARTE GARCIA

TRÁMITE: REMISIÓN DE INFORMACIÓN

JUAN PABLO REYES VILLAMIZAR, identificado como aparece al pie de mi firma, obrando en calidad de apoderado de la sociedad **LABORATORIOS LEGRAND S.A.**, en el trámite de la referencia, y con el propósito de coadyuvar en la decisión sobre la solicitud **NC2018/0008667**, de manera respetuosa me permito presentar argumentos adicionales para demostrar que la misma incumple los requisitos de patentabilidad aplicables, y no merece por ello, recibir el beneficio solicitado.

I. PETICIÓN

Sírvase negar el beneficio patentario a la solicitud contenida en el expediente NC2018/0008667, por no cumplir con lo dispuesto en la Decisión 486 de 2000, con base en los fundamentos que se expondrán a continuación.

II. LEGÍTIMO INTERÉS

Asiste a **LABORATORIOS LEGRAND S.A.**, legítimo interés para presentar esta oposición, pues la eventual concesión de la solicitud restringiría injustificadamente el empleo de ciertas sustancias, derivados y procesos que a nuestro juicio carecen de nivel inventivo, en detrimento de sus legítimos intereses comerciales. De igual manera mis clientes están interesados, por su misma condición de industrias farmacéuticas, en evitar la concesión de privilegios de patente que incumplan los requisitos materiales de patentabilidad establecidos en las normas vigentes, y que supongan un riesgo para la libre competencia.

III. FUNDAMENTOS DE LA OPOSICIÓN

La solicitud colombiana en trámite NC2018/0008667, que se refiere a conjugados de anticuerpo y fármaco (ADC) que se unen a antígenos diana de oncología tales como el receptor de folato alfa y/o proporcionan actividad del fármaco anti-tubulina, no cumple los requisitos materiales para otorgársele el privilegio de patente de invención, tal como puede determinarse al valorar los siguientes argumentos:

3.1. El capítulo reivindicatorio carece de claridad y concisión

En primer lugar, la solicitud carece de concisión ya que presenta 182 reivindicaciones y en éstas no define específicamente las características técnicas del conjugado anticuerpo-fármaco ya que incluye una fórmula general (I) en donde el anticuerpo o fragmento de unión al antígeno no se divulga mediante su estructura, así mismo, el enlazador tampoco es divulgado y el fármaco Eribulina es ampliamente conocido, por lo tanto no se evidencia una real invención a partir de las reivindicaciones.

Adicional a lo anterior, no se puede considerar que la reivindicación 1 sea clara ya que no hay definición técnica mediante su estructura completa, dejando lugar a múltiples sustituciones que pueden hacer que el conjugado tenga estructuras y funciones completamente diferentes y contrarias, tal como se puede observar a continuación:

«Un conjugado de anticuerpo-fármaco de fórmula (I): Ab-(L-D) p (I) en donde Ab es un anticuerpo de internalización o fragmento de unión al antígeno de internalización de 5 este que se dirige a una célula tumoral; D es eribulina; L es un enlazador escindible que une covalentemente Ab con D; y p es un entero entre 1 y 20.»

En cuanto a los componentes del conjugado, el “anticuerpo de internalización” no define estructuralmente la molécula, así como el “enlazador escindible” no limita técnicamente la invención, sino que se refieren a características funcionales que no definen los límites de lo reclamado, haciendo la solicitud ambigua y especulativa.

De igual forma, las restricciones a lo largo del capítulo no son claras ya que como se puede apreciar en la reivindicación 95 y en relación con los anticuerpos, se definen dichas moléculas por su antígeno y no por su estructura; de otro lado, las definiciones estructurales de las reivindicaciones 96 y 97 para tales anticuerpos que se unen al receptor folato alfa, presentan únicamente estructuras de CDRs sin tener en cuenta la complejidad total del anticuerpo.

En este mismo sentido, se reivindican otros anticuerpos, tal es el caso del que se une al receptor 2 del factor de crecimiento epidérmico humano (HER2), dentro de los que se puede encontrar el anticuerpo comercial conocido como Trastuzumab, definido únicamente por parte de sus secuencias CDRs y no por su estructura completa. Otros anticuerpos que involucra la invención son aquellos definidos como anticuerpo-antimesotelina, también definidos de forma deficiente y entendiendo que tanto la estructura como la función de los conjugados que involucran los anticuerpos es diferente y por lo tanto no implican un único concepto inventivo.

Así las cosas, la solicitud en cuestión falla en cumplir con lo dispuesto por el Artículo 30 de la D. 486 que precisa lo siguiente: *“Las reivindicaciones definirán la materia que se desea proteger mediante la patente. Deben ser claras y concisas y estar enteramente sustentadas por la descripción.”*

La solicitud además falla en sustentar todas y cada una de las modalidades enseñadas por las reivindicaciones en la descripción debido a que en la misma no se divulgan todos los anticuerpos y enlazadores reclamados ya que se define una cantidad de posibles combinaciones de agentes que no son especificados.

Por otro lado, las reivindicaciones establecen anticuerpos diferentes (como ya fue mencionado antes) que incluyen regiones funcionalmente diferentes que no se relacionan ni en su estructura ni en su función, entre ellos podríamos mencionar a los anticuerpos anti receptor de folato alfa, anti-HER2 y anti-mesotelina, por lo que la solicitud además incumple con el requisito de unidad de invención tal como lo determina el Art. 25 de la D. 486: *“La solicitud de patente sólo podrá comprender una invención o un grupo de invenciones relacionadas entre sí, de manera que conformen un único concepto inventivo”*.

En este sentido, la solicitud no solo comprende diferentes categorías desligadas entre si de las características esenciales, sino que además incluye una cantidad no determinada de posibles invenciones que pueden comprender macromoléculas con funciones y estructuras completamente diferentes a la aportada por la descripción de la presente solicitud.

En cuanto a las reivindicaciones 124 a 150, involucran métodos de tratamiento los cuales no pueden ser considerados objeto de patente, por su parte, las reivindicaciones 157 – 168 comprenden el uso de los conjugados lo cual no está contemplado dentro de la Decisión 486 en su Art. 14: *“Los Países Miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial.”*

Así las cosas, la materia de las reivindicaciones y en general la solicitud en estudio, no puede ser objeto de patente pues incumple las normas mínimas de presentación, como es la claridad, concisión, soporte, unidad de invención, métodos de tratamiento y usos.

3.2. En el estado de la técnica, los documentos WO 2014/089335 A2, el documento de Zheng W et al., “Macrocyclic ketone analogues of halichondrin B”, y el documento Nikolaos Diamantis et al., “Antibody-drug conjugates—an emerging class of cancer treatment” anticipan la materia reivindicada por la presente solicitud:

La publicación internacional WO 2014/089335 A2 **“Bcma antigen binding proteins”**, de Armitage Richard J. [US], Blake Michelle [US], Fanslow III, William [US], O’Neill Jason Charles [US], Kannan Gusasekaran [US] Xu Jiangchun [US] Tometsko Mark Edward [US], Wang Zhulun [US], Sudom Athena [US]., publicada

el 12.06.2014, se refiere a proteínas de unión a antígeno BCMA (antígeno de maduración de células B), tales como anticuerpos, secuencias de polinucleótidos que codifican dichas proteínas de unión a antígeno y composiciones y métodos para diagnosticar y tratar enfermedades de la misma forma que la materia de la solicitud en estudio:

«The present invention relates to BCMA (B-Cell Maturation Antigen) antigen binding proteins, such as antibodies, polynucleotide sequences encoding said antigen binding proteins, and compositions and methods for diagnosing and treating diseases. The present invention also relates to BCMA antibody drug conjugates and uses thereof.»

(Abstract, WO 2014/089335 A2)

En este caso, el documento WO 2014/089335 A2 divulga anticuerpos que se unen al antígeno BCMA y conjugados de los mismos, en donde se sugiere adicionalmente al análogo de Halicondrina el E7389 más conocido como Eribulina, como un medicamento aceptable:

There are a number of compounds that affect cellular microtubules that were obtained from natural sources-extracts, and semisynthetic and synthetic analogs that appear to possess potential as toxins for the generation of immunoconjugates. Such molecules and examples of drug products utilizing them, include the following: Colchicine-site Binders (Curacin), Combretastatins (AVE806, Combretastatin A-4 prodrug (CA4P), Oxi-4503), Cryptophycins (LY355703), Discodermolide, Dolastatin and Analogs (Auristatin PHE, Dolastatin 10, ILX-65 1, Symplostatin 1, TZT-1027), Epothilones (BMS-247550, BMS-3 10705, EP0906, KOS-862, ZK-EPO), Eleutherobin, FR1 82877, Halichondrin B (E7389), Halimide (NPI-2352 and NPI-2358), Hemiasterlins (HTI-286), Laulimalide, Maytansinoids ("DM1," "DM3" or "DM4") (Bivatuzumab mertansine, Cantuzumab mertansine, huN90 1-DM1/BB-1090 ITAP, MLN59 1DM1, My9-6-DM1, Trastuzumab-DM1), PC-SPES, Peloruside A, Resveratrol, S-allylmercaptocysteine (SAMC), Spongistatins, Vitilevuamide, Molecular Motor-Kinesins (SB-7 15992), Designed Colchicine-Site Binders (A-289099, A-293620/A-3 183 15, ABT-75 1/E70 10, D-2485 1/D-641 31, ZD6 126), Other Novel Spindle Poisons (2-Methoxyestradiol (2-ME2), Bezimidazole Carbamates (ANG 600 series, Mebendazole), CP248/CP46 1, HMN-2 14, R440, SDX-103, T67/T607). Further, additional marine derived toxins are reviewed in Mayer, A. M. 5. Marine Pharmacology in 1998: Antitumor and Cytotoxic Compounds. The Pharmacologist. 41(4): 159-164 (1999).

(Pág. 109; WO 2014/089335 A2)

En cuanto a las repeticiones de los agentes terapéuticos dentro de los que se encuentra eribulina se pueden encontrar de 1 a 20 motivos de agentes por anticuerpo lo que equivale al factor p de la solicitud la solicitud en trámite:

ratio, or DAR). A BCMA ADC may have 1 to 20 chemotherapeutic agents per antibody.

Compositions of BCMA ADCs may be characterized by the average number of drug moieties per antibody molecule in the composition. The average number of drug moieties may be determined by conventional means such as mass spectrometry, immunoassay, and HPLC (*e.g.*, see EXAMPLE 7).

- 5 In some instances, a homogeneous BCMA ADC population may be separated and purified by means of reverse phase HPLC or electrophoresis. Thus, pharmaceutical BCMA ADC compositions may contain a heterogeneous or homogeneous population of antibodies linked to 1, 2, 3, 4, 5, 6, 7 or more drug moieties. Embodiments of the invention include compositions comprising an average number of maytansinoids (*e.g.*, DM1) molecules per BCMA ADC is between 1 and 10, between 2 and 7, or
- 10 preferably between 3 and 5. In preferred embodiments, the composition of a BCMA ADC has an average number of maytansinoids (*e.g.*, DM1) molecules per BCMA ADC of about 3.0, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9, about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, or about 5.0. Such a composition may contain a therapeutically effective amount of the BCMA ADC and may be
- 15 lyophilized. In particularly preferred embodiments, the BCMA ADC comprises antibody Ab-1, Ab-2, or Ab-3, as variously described herein, conjugated to DM1 by a MCC linker at the ratios described above.

(Pág. 111; WO 2014/089335 A2)

El uso de anticuerpos monoclonales dentro del conjugado, puede incluir gran diversidad de anticuerpos entre ellos el anti-HER2 trastuzumab:

Streptozocin, SU1 1248, Sunitinib, Sutent®, Tamoxifen, Tarceva®, Targretin®, Taxol®, Taxotere®, Temodar®, Temozolomide, Temsirolimus, Teniposide, TESPA, Thalidomide, Thalomid®, TheraCys®, Thioguanine, Thioguanine Tabloid®, Thiophosphoamide, Thioplex®, Thiotepa, TICE®, Toposar®, Topotecan, Toremifene, Torisel®, Tositumomab, Trastuzumab, Treanda®, Tretinoin, Trexall™, Trisenox®, TSPA, TYKERB®, VCR, Vectibix™, Velban®, Velcade®, VePesid®, Vesanoid®, Viadur™, Vidaza®, Vinblastine, Vinblastine Sulfate, Vincasar, Pfs®, Vincristine, Vinorelbine, Vinorelbine tartrate, VLB, VM-26, Vorinostat, VP- 16, Vumon®, Xeloda®, Zanosar®, Zevalin™, Zinecard®, Zoladex®, Zoledronic acid, Zolinza, Zometa®, and the like.

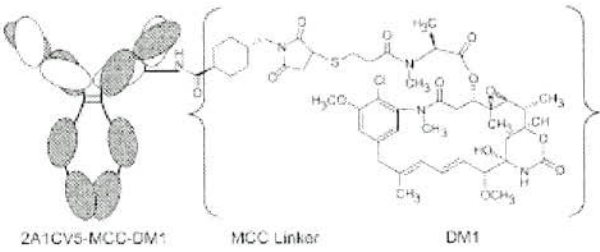
(Pág. 135; WO 2014/089335 A2)

Adicionalmente, este documento incluye el uso de composiciones farmacéuticas para el uso en el tratamiento de cáncer, por ejemplo, la reivindicación 200 de dicho documento incluye el mismo uso y función:

200. A method of treating a disease, comprising administering an effective amount of the compositions of any of claims 197 to 199, wherein the disease is selected from the group consisting of: B-cell cancers, multiple myeloma, malignant plasma cell neoplasms, Kahler's disease and Myelomatosis; Plasma cell leukemia; Plasmacytoma; B-cell prolymphocytic leukemia; Hairy cell leukemia; B-cell non-Hodgkin's lymphoma (NHL); Acute myeloid leukemia (AML); Chronic myeloid leukemia (CML); Acute lymphocytic leukemia (ALL); Chronic lymphocytic leukemia (CLL); Follicular lymphoma (including follicular non-Hodgkin's lymphoma types); Burkitt's lymphoma (Endemic Burkitt's lymphoma; Sporadic Burkitt's lymphoma); Marginal zone lymphoma (Mucosa-Associated Lymphoid Tissue; MALT / MALToma; Monocytoid B cell lymphoma; Splenic lymphoma with villous lymphocytes); Mantle cell lymphoma; Large cell lymphoma (Diffuse large cell; Diffuse Mixed Cell; Immunoblastic Lymphoma; Primary Mediastinal B Cell Lymphoma; Angiocentric Lymphoma - pulmonary B cell); Small lymphocytic lymphoma (SLL); Precursor B-lymphoblastic lymphoma; Myeloid leukemia (granulocytic; myelogenous; Acute myeloid leukemia; Chronic myeloid leukemia; Subacute myeloid leukemia; Myeloid sarcoma; Chloroma; Granulocytic sarcoma; Acute promyelocyte leukemia; Acute myelomonocytic leukemia); Waldenstrom's macroglobulinemia, or other B-cell lymphoma.

(Pág. 236-237; WO 2014/089335 A2)

Ahora bien, con respecto a las diferentes partes del conjugado, en este caso el enalizador y el anticuerpo en vista de que pueden ser criterios intercambiables y por lo tanto opcionales, en la técnica no puede esperarse que su inclusión presente un efecto sorprendente demostrado, por lo tanto, la solicitud en trámite en la estructura general presentada no cumple con el criterio de novedad con respecto al documento WO 2014/089335 A2 tal como se observa en la siguiente tabla:

Reiv	Exp. NC 2018/0008667	WO 2014/089335A2
1	Un conjugado de anticuerpo-fármaco de fórmula (I): Ab-(L-D) p (I) en donde	
	Ab es un anticuerpo de internalización o fragmento de unión al antígeno de internalización de este que se dirige a una célula tumoral;	Abstract, (Trastuzumab pg. 135- entre otros-)
	D es eribulina;	Pág 22, 28, 42, 109, 210, 217, 235,
	L es un enlazador escindible que une covalentemente Ab con D;	Pag. 26
	y p es un entero entre 1 y 20.»	Pag. 111

Por lo tanto, se concluye que la solicitud en estudio no cumple con el criterio de novedad.

3.3. Las características técnicas esenciales de la presente solicitud habían sido anticipadas previamente, por lo que no se encuentra que los compuestos reclamados por la solicitante sean inventivos.

En cuanto a modalidades particulares como la presentada en la reivindicación 6:

6. El conjugado de anticuerpo-fármaco de cualquiera de las reivindicaciones de 1 a 5 en donde el enlazador escindible comprende un resto escindible que está posicionado de manera tal que ninguna parte del enlazador o el anticuerpo o fragmento de unión al antígeno permanece unida a eribulina tras la escisión.

Tenemos que la solicitud en trámite no cumple con el requisito de nivel inventivo en vista del documento de titulado **“Macrocyclic ketone analogues of halichondrin B”**, por Wanjun Zheng, Boris M. Seletsky, Monica H. Palme, Paul J. Lydon, Lori A. Singer Charles E., Chase Charles A. Lemelin, Yongchun Shen, Heather Davis, Lynda Tremblay, Murray J. Towle, Kathleen A. Salvato, Bruce F. Wels, Kimberley K. Aalfs, Yoshito Kishi, Bruce A. Littlefield, Melvin J. Yu., publicado el 15.11.2004, el cual también anticipa la materia objeto de las reivindicaciones de la solicitud en estudio, al referirse a los análogos de la cetona macrocíclica de halicondrina B estructuralmente simplificados (como se ha visto un sinónimo del compuesto eribulina), los cuales retienen la actividad inhibitoria del crecimiento celular in vitro, la estabilidad en suero de ratón y la eficacia in vivo del producto natural. Veamos:

«Structurally simplified macrocyclic ketone analogues of halichondrin B were prepared by total synthesis and found to retain the potent cell growth inhibitory activity in vitro, stability in mouse serum, and in vivo efficacy of the natural product.»

(Abstract, Zheng et al)

Este documento además de incluir eribulina (E7389), divulga compuestos con un enlace escindible (clivable) amida o ester y un motivo (sitio específico de un anticuerpo) adicional sustituido en fenil, benzoil, etil o propil en la amina C35:

Modifications to the C.32 side chain had little impact on in vitro biological activity (Table I). Interestingly, amine **4** (E7389, previously ER-086526, also NSC-707389) exhibited a reversibility ratio of one in U937 cells. Several other amine (34–37) and amide (38–40) derivatives were subsequently prepared and also found to exhibit a low reversibility ratio (e.g., **34**). E7389 demonstrated remarkable efficacy (0.05–1 mg/kg) in a variety of human tumor xenograft models.²³ This compound was selected for development as a new potential anticancer chemotherapeutic agent, and is currently in collaborative Phase I clinical trials with the United States NCI.

(p. 5554, Zheng et al)

Por último, el documento **“Antibody-drug conjugates—an emerging class of cancer treatment”** por Nikolaos Diamantis, Udai Banerji., publicado el 16.02.2016, se refiere a que los conjugados anticuerpo-fármaco (ADC) son una nueva clase emergente de agentes de tratamiento contra el cáncer que combina la selectividad del tratamiento dirigido con la potencia citotóxica de los fármacos de quimioterapia:

«Antibody-drug conjugates (ADCs) are an emerging novel class of anticancer treatment agents that combines the selectivity of targeted treatment with the cytotoxic potency of chemotherapy drugs. New linker technology associated with novel highly potent cytotoxic payloads has permitted the development of more effective and safe ADCs. In recent years, two ADCs have been licensed, T-DM1 and brentuximab vedotin, and are already establishing their place in cancer treatment. A plethora of ADCs are being investigated in phases I and II trials, emerging data of which appears promising. As we deepen our understanding of what makes a successful ADC, an increasing number of ADCs will likely become viable treatment options as single agents or in combination with chemotherapy. This review will present the philosophy underlying ADCs, their main characteristics and current research developments with a focus on ADCs in solid tumours.»

(Abstract, Diamantis et al)

Adicionalmente menciona que, la nueva tecnología de enlace asociada con las novedosas cargas útiles citotóxicas altamente potentes ha permitido el desarrollo de ADCs más efectivos y seguros. En los últimos años, dos ADC han sido autorizados, T-DM1 y brentuximab vedotin, y ya están estableciendo su lugar en el tratamiento del cáncer. Se está investigando una gran cantidad de ADC en los ensayos de las fases I y II, cuyos datos emergentes parecen prometedores. A medida que profundizamos nuestra comprensión de lo que hace que un ADC sea

exitoso, un número creciente de ADC probablemente se volverán opciones de tratamiento viables como agentes individuales o en combinación con quimioterapia. Esta revisión presentará la filosofía subyacente de los ADC, sus características principales y los desarrollos de investigación actuales con un enfoque en los ADC en tumores sólidos.

De acuerdo con este documento, antes de la fecha de presentación de la solicitud, existía razonamiento normal en la técnica por el cual una persona versada en la materia habría empleado los conjugados del documento WO 2014/089335 A2 en el uso, el tratamiento y como composiciones farmacéuticas para el tratamiento de cáncer empleando -entre otros- anticuerpos conocidos como Trastuzumab, el agente Eribulina y un enlazador.

En consecuencia, rogamos se analicen los argumentos expuestos con el rigor y detenimiento habituales y que en consecuencia se decrete, además de la negación de la solicitud en comento, el archivo del expediente que la contiene.

IV. FUNDAMENTOS DE DERECHO

Son fundamentos de derecho el Capítulo I, (Requisitos de Patentabilidad) y el artículo 42 (Oposiciones) de la Decisión 486 de 2000.

V. PRUEBAS

Ruego que se tenga como tal, sin perjuicio de aquellas que el Despacho considere oficiosamente, las siguientes:

- Publicación Internacional WO 2014/089335 A2, ***"Bcma antigen binding proteins"***, por Armitage Richard et al., publicada el 12.06.2014, Portada, Pág 22, 26, 28, 42, 109, 111, 135, 210, 217, 235,
- La publicación científica ***"Macrocyclic ketone analogues of halichondrin B"***, por Wanjun Zheng, Boris M. Seletsky, Monica H. Palme, Paul J. Lydon, Lori A. Singer Charles E., Chase Charles A. Lemelin, Yongchun Shen, Heather Davis, Lynda Tremblay, Murray J. Towle, Kathleen A. Salvato, Bruce F. Wels, Kimberley K. Aalfs, Yoshito Kishi, Bruce A. Littlefield, Melvin J. Yu., publicado el 15.11.2004. Todo el documento.

- La publicación científica ***“Antibody-drug conjugates—an emerging class of cancer treatment”*** por Nikolaos Diamantis et al., publicado el 16.02.2016. Todo el documento.

VI. ANEXOS

Para efectos del trámite correspondiente, me permito anexar al presente escrito los siguientes anexos:

1. Certificado de existencia y representación legal de **LABORATORIOS LEGRAND S.A.**
2. Poder para actuar.
3. Constancia de pago de las tasas de tramitación de oposición.
4. El mencionado en el acápite de pruebas.

VII. NOTIFICACIONES

Las recibiré en la secretaría de su Despacho o en mi oficina localizada en la Carrera 17 No. 88-23, Oficina 207, E-mail: info@reyes-abogados.com

Señor Superintendente,



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61/775,125 8 March 2013 (08.03.2013) US
61/890,064 11 October 2013 (11.10.2013) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: BCMA ANTIGEN BINDING PROTEINS

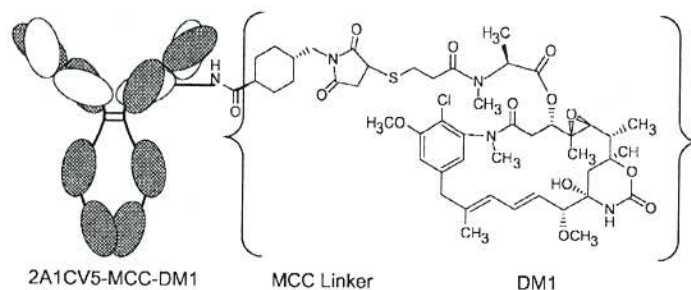


Fig. 2

(57) Abstract: The present invention relates to BCMA (B-Cell Maturation Antigen) antigen binding proteins, such as antibodies, polynucleotide sequences encoding said antigen binding proteins, and compositions and methods for diagnosing and treating diseases. The present invention also relates to BCMA antibody drug conjugates and uses thereof.

BCMA ANTIGEN BINDING PROTEINS

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CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119 of U.S. Provisional Application Serial Number 61/734,767, filed December 7, 2012, U.S. Provisional Application Serial Number 61/759,702, filed February 1, 2013, U.S. Provisional Application Serial Number 61/775,125, filed
10 March 8, 2013, and U.S. Provisional Application Serial Number 61/890,064, filed October 11, 2013 which are hereby incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to B-Cell Maturation Antigen (BCMA) antigen binding
15 proteins, particularly antibodies, polynucleotide sequences encoding said antigen binding proteins, and compositions and methods for treating diseases. Aspects of the invention also include antibody drug conjugates and uses thereof.

BACKGROUND

20 B-cell maturation antigen (BCMA), B-cell activating factor receptor (BAFF-R), and transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) promote B-cell survival at distinct stages of development by engaging a proliferation-inducing ligand (APRIL) and/or BAFF (B-cell activating factor). Dysregulated signaling can promote B-cell survival and proliferation, causing autoimmunity and neoplasia. For a review, see Rickert, *et al.*, *Immunological*
25 *Reviews* 2011, Vol. 244: 115-133.

BAFF and APRIL exhibit structural similarity and overlapping yet distinct receptor binding specificity. BAFF and APRIL bind BCMA, but the APRIL:BCMA interaction is of higher affinity, whereas BAFF-R only binds BAFF with high affinity. The negative regulator TACI binds to both BAFF and APRIL with similar affinity. BCMA expression in the B cell lineage is restricted to
30 germinal center B cells, memory B cells (in humans), and plasma cells. BCMA is a receptor for BAFF and APRIL engagement on bone marrow plasma cells, although TACI is also expressed on these cells. The survival of long-lived plasma cells does not require BAFF but is dependent upon APRIL.

BCMA expression is highly restricted to plasma cells. Multiple myeloma is caused by a
35 clonal expansion of malignant plasma cells. By 2020, the total number of incident cases of multiple myeloma (MM) in the major markets will increase from 46,400 to 55,300. Newly diagnosed cases of myeloma are similarly distributed between three disease stages with stage 1, 2 and 3 accounting for about 33% of the diagnosed cases each. In the US, approximately 20,500 people are diagnosed with

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multiple myeloma each year and almost 11,000 MM patients die each year. Despite advances in therapy, multiple myeloma remains an incurable disease, see Jemal A, *et al. CA Cancer J Clin.* 2008;58:7 1-96; Kyle RA, *et al. Mayo Clin Proc.* 2003;778:2 1-33; Bergsagel DE, *et al. Blood.* 1999, 94: 1174- 1182.

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BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the amino acid sequence of human BCMA (SEQ ID NO:285) and cynomolgus (*Macaca fascicularis*) BCMA (SEQ ID NO:286).

FIGURE 2 is a schematic of one embodiment of an antibody drug conjugate showing the structures of an exemplary linker and toxin.

FIGURE 3 shows that BCMA mRNA is expressed on >99% of multiple myeloma patient samples.

FIGURES 4A and B are schematics of the monoclonal antibody generation, screening, and characterization for the two campaigns.

FIGURES 5A-C are data tables for the monoclonal antibody generation, screening, and characterization for the two campaigns.

FIGURE 6 shows binding of the 2A1 and 1E1 mAbs to the myeloma cell line H929.

FIGURE 7 shows the 2A1 mAb bound BCMA on various myeloma and B-cell lines.

FIGURE 8 is a graph of the ADCC activity for the 2A1 and 1E1 antibodies (EC_{50} of 11 ng/ml for 2A1 and 76 ng/ml for 1E1).

FIGURE 9 shows that the 2A1 antibody was readily internalized following binding to BCMA on H929 cells.

FIGURE 10 is various 2A1-MCC-DM1 ADCs analyzed by mass spectrometry.

FIGURE 11 depicts two different antibodies (2A1 and 1E) that were conjugated with different linkers and maytansine molecules and analyzed for their capacity to kill myeloma cells.

FIGURES 12A-F shows 2A1-MCC-DM1 ADC was tested against various multiple myeloma cell lines, including H929, U266, MMIR, MMIS, the EW36 B-cell lymphoma cell line, and the RAMOS Burkitt's lymphoma cell line.

FIGURE 13 graphically illustrates that various cell lines exposed to free DM1 are in fact killed by free DM1.

FIGURES 14A and B show the T_m for various 2A1 mAb covariance muteins.

FIGURE 15 is a graph showing the binding of various 2A1 mAb covariance muteins to H919 cells.

FIGURES 16A and B show that some 2A1 mutein antibodies retained comparable binding to BCMA relative to parental 2A1 (2A1 WT), whereas the 2A1 covariance 3, 8, 9 and 11-19 antibodies had reduced binding to BCMA.

FIGURE 17 shows the comparative ADCC activity of various 2A1 mAb covariance muteins.

TMI); eleutherobin; pancratiastatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlormaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, 5 nimustine, ranimustine; antibiotics, such as the enediyne antibiotics (e.g. calicheamicin, especially calicheamicin .gamma and calicheamicin theta I, see, e.g., Angew Chem. Intl. Ed. Engl. 33:183-186 (1994); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomorphores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabacin, caminomycin, carzinophilin; 10 chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, nitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites, such as 15 methotrexate and 5-fluorouracil (5-FU); folic acid analogues, such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs, such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as, ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens, such as calusterone, dromostanolone propionate, epitioastanol, mepitioastane, testolactone; anti-adrenals, such as 20 aminoglutethimide, mitotane, trilostane; folic acid replenisher, such as frolinic acid; aceglutone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids, such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; 25 podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK.RTM.; razoxane; rhizoxin; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL.TM., Bristol-Myers Squibb Oncology, 30 Princeton, N.J.) and doxetaxel (TAXOTERE.RTM., Rhone-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP- 16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-1 1; topoisomerase inhibitor RFS 2000; difluoromethylomithine (DMFO); 35 retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors, such as anti-estrogens including for example tamoxifen, raloxifene, aromatase



inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY1 170 18, onapristone, and toremifene (Fareston); and anti-androgens, such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; siRNA and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Other chemotherapeutic agents that can be used with the present invention are disclosed in US

- 5 Publication No. 20080 171040 or US Publication No. 20080305044 and are incorporated in their entirety by reference.

There are a number of compounds that affect cellular microtubules that were obtained from natural sources-extracts, and semisynthetic and synthetic analogs that appear to possess potential as toxins for the generation of immunoconjugates. Such molecules and examples of drug products

- 10 utilizing them, include the following: Colchicine-site Binders (Curacin), Combretastatins (AVE806, Combretastatin A-4 prodrug (CA4P), Oxi-4503), Cryptophycins (LY355703), Discodermolide, Dolastatin and Analogs (Auristatin PHE, Dolastatin 10, ILX-65 1, Symplostatin 1, TZT- 1027), Epothilones (BMS-247550, BMS-3 10705, EP0906, KOS-862, ZK-EPO), Eleutherobin, FRI 82877, Halichondrin B (E7389), Halimide (NPI-2352 and NPI-2358), Hemiasterlins (HTI-286), Laulimalide, 15 Maytansinoids ("DM1," "DM3" or "DM4") (Bivatuzumab mertansine, Cantuzumab mertansine, huN90 1-DM 1/BB- 1090 ITAP, MLN59 IDM1, My9-6-DM1, Trastuzumab-DM1), PC-SPES, Peloruside A, Resveratrol, S-allylmercaptocysteine (SAMC), Spongistatins, Vitilevuamide, Molecular Motor-Kinesins (SB-7 15992), Designed Colchicine-Site Binders (A-289099, A-293620/A-3 183 15, ABT-75 1/E70 10, D-2485 1/D-641 3 1, ZD6 126), Other Novel Spindle Poisons (2-Methoxyestradiol (2- 20 ME2), Bezimidazole Carbamates (ANG 600 series, Mebendazole), CP248/CP46 1, HMN-2 14, R440, SDX- 103, T67/T607). Further, additional marine derived toxins are reviewed in Mayer, A. M. S. Marine Pharmacology in 1998: Antitumor and Cytotoxic Compounds. The Pharmacologist. 4 1(4): 159- 164 (1999).

- In preferred embodiments, the BCMA ADC comprises an antibody as described herein 25 conjugated to one or more maytansinoid molecules, which are mitotic inhibitors that act by inhibiting tubulin polymerization. Maytansinoids, including various modifications, are described in US Pat. Nos. 3896 111; 4 15 1042; 4 137230; 4248870; 4256746; 4260608; 42658 14; 4294757; 43070 16; 4308268; 4309428; 43 13946; 43 15929; 43 1782 1; 4322348; 433 1598; 436 1650; 4364866; 44242 19; 4450254; 4362663; 437 1533; and WO 2009/099728. Maytansinoid drug moieties may be isolated 30 from natural sources, produced using recombinant technology, or prepared synthetically. Exemplary maytansinoids include C- 19-dechloro (US Pat No. 4256746), C-20-hydroxy (or C-20-demethyl) +/- C- 19-dechloro (US Pat. Nos. 43070 16 and 4361 650), C-20-demethoxy (or C-20-acyloxy (-OCOR), +/- dechloro (US Pat. No. 4294757), C-9-SH (US Pat. No. 4,424,2 19), C- 14-alkoxymethyl (demethoxy/CH20R) (U.S. Pat. No. 4,33 1,598), C- 14-hydroxymethyl or acyloxymethyl (CH20H or 35 CH20Ac) (U.S. Pat. No. 4,450,254), C- 15-hydroxy/acyloxy (U.S. Pat. No. 4,364,866), C- 15-methoxy (U.S. Pat. Nos. 4,3 13,946 and 4,3 15,929), C- 18-N-demethyl (U.S. Pat. Nos. 4,362,663 and 4,322,348), and 4,5-deoxy (U.S. Pat. No. 4,37 1,533). Various positions on maytansinoid compounds

may be used as the linkage position, depending upon the type of link desired. For example, for forming an ester linkage, the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group are all suitable (US Pat. Nos. 5208020, RE39151, and 6913748; US Patent Appl.

5 Pub. Nos. 20060167245 and 20070037972, and WO 2009099728).

Preferred maytansinoids include those known in the art as DM1, DM3, and DM4 (US Pat. Appl. Pub. Nos. 2009030924 and 20050276812, incorporated herein by reference). Ansamitocin P-3 is synthetically altered to produce the warhead DM1. DM1 is reported to bind to tubulin in the vinca alkaloid binding site and to inhibit tubulin polymerization.

10 ADCs containing maytansinoids, methods of making such ADCs, and their therapeutic use are disclosed in US Patent Nos. 5208020 and 5416064, US Pat. Appl. Pub. No. 20050276812, and WO 2009099728 (all incorporated by reference herein). Linkers that are useful for making maytansinoid ADCs are known in the art (US Pat. No. 5208020 and US Pat. Appl. Pub. Nos. 2005016993 and 20090274713; all incorporated herein by reference). Maytansinoid ADCs
15 comprising an SMCC linker may be prepared as disclosed in US Pat. Publ. No. 2005/0276812.

Embodiments of the present invention include Ab-1-MCC-DM1, Ab-2-MCC-DM1, Ab-3-MCC-DM1, Ab-4-MCC-DM1, Ab-5-MCC-DM1, Ab-6-MCC-DM1, Ab-7-MCC-DM1, Ab-8-MCC-DM1, Ab-9-MCC-DM1, Ab-10-MCC-DM1, Ab-11-MCC-DM1, Ab-12-MCC-DM1, Ab-13-MCC-DM1, Ab-14-MCC-DM1, Ab-15-MCC-DM1, Ab-16-MCC-DM1, and Ab-17-MCC-DM1.

20

Drug Loading

A major drawback with existing antibody-drug conjugates is their inability to deliver a sufficient concentration of drug to the target site because of the limited number of targeted antigens and the relatively moderate cytotoxicity of cancerostatic drugs like methotrexate, daunorubicin and
25 vincristine. In order to achieve significant cytotoxicity, linkage of a large number of drug molecules either directly to the antibody or through a polymeric carrier molecule becomes necessary. However such heavily modified antibodies often display impaired binding to the target antigen and fast *in vivo* clearance from the blood stream.

Current methods of conjugation of maytansinoids with cell binding agents (such as
30 antibodies) are well-known in the art and may be used to create the BCMA ADCs. Conjugation methods may involve two reaction steps, as described in U.S. Pat. No. 5,208,020. A one-step process for conjugation of maytansinoids may be used to create the BCMA ADCs and is described in U.S. Pat. No. 6,441,163. Maytansinoid-based immunotoxin technology is available from ImmunoGen Corporation (Cambridge, Mass.).

35 The process of conjugating maytansinoids (*e.g.*, DM1) to a BCMA antibody as described herein will produce a composition comprising a population of maytansinoids (*e.g.*, DM1)-conjugated antibodies having a range of maytansinoids (*e.g.*, DM1) molecules per antibody (a drug-antibody

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ratio, or DAR). A BCMA ADC may have 1 to 20 chemotherapeutic agents per antibody.

Compositions of BCMA ADCs may be characterized by the average number of drug moieties per antibody molecule in the composition. The average number of drug moieties may be determined by conventional means such as mass spectrometry, immunoassay, and HPLC (*e.g.*, see EXAMPLE 7).

- 5 In some instances, a homogeneous BCMA ADC population may be separated and purified by means of reverse phase HPLC or electrophoresis. Thus, pharmaceutical BCMA ADC compositions may contain a heterogeneous or homogeneous population of antibodies linked to 1, 2, 3, 4, 5, 6, 7 or more drug moieties. Embodiments of the invention include compositions comprising an average number of maytansinoids (*e.g.*, DM1) molecules per BCMA ADC is between 1 and 10, between 2 and 7, or
- 10 preferably between 3 and 5. In preferred embodiments, the composition of a BCMA ADC has an average number of maytansinoids (*e.g.*, DM1) molecules per BCMA ADC of about 3.0, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9, about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, or about 5.0. Such a composition may contain a therapeutically effective amount of the BCMA ADC and may be
- 15 lyophilized. In particularly preferred embodiments, the BCMA ADC comprises antibody Ab-1, Ab-2, or Ab-3, as variously described herein, conjugated to DM1 by a MCC linker at the ratios described above.

- Aspects of the invention include isolated monoclonal antibodies comprising the six CDRs of Ab-1, wherein said antibody further comprises a linker and a drug. Aspects of the invention include
- 20 isolated monoclonal antibodies comprising the six CDRs of Ab-2, wherein said antibody further comprises a linker and a drug. Aspects of the invention include isolated monoclonal antibodies comprising the six CDRs of Ab-3, wherein said antibody further comprises a linker and a drug. Aspects of the invention include isolated monoclonal antibodies comprising the six CDRs of Ab-4, wherein said antibody further comprises a linker and a drug. Aspects of the invention include isolated
- 25 monoclonal antibodies comprising the six CDRs of Ab-5, wherein said antibody further comprises a linker and a drug. Aspects of the invention include isolated monoclonal antibodies comprising the six CDRs of Ab-6, wherein said antibody further comprises a linker and a drug. Aspects of the invention include isolated monoclonal antibodies comprising the six CDRs of Ab-7, wherein said antibody further comprises a linker and a drug. Aspects of the invention include isolated monoclonal
- 30 antibodies comprising the six CDRs of Ab-8, wherein said antibody further comprises a linker and a drug. Aspects of the invention include isolated monoclonal antibodies comprising the six CDRs of Ab-9, wherein said antibody further comprises a linker and a drug. Aspects of the invention include isolated monoclonal antibodies comprising the six CDRs of Ab-10, wherein said antibody further comprises a linker and a drug. Aspects of the invention include isolated monoclonal antibodies
- 35 comprising the six CDRs of Ab-11, wherein said antibody further comprises a linker and a drug. Aspects of the invention include isolated monoclonal antibodies comprising the six CDRs of Ab-12, wherein said antibody further comprises a linker and a drug. Aspects of the invention include isolated

cell; Diffuse Mixed Cell; Immunoblastic Lymphoma; Primary Mediastinal B Cell Lymphoma; Angiocentric Lymphoma - pulmonary B cell); Small lymphocytic lymphoma (SLL); Precursor B-lymphoblastic lymphoma; Myeloid leukemia (granulocytic; myelogenous; Acute myeloid leukemia; Chronic myeloid leukemia; Subacute myeloid leukemia; Myeloid sarcoma; Chloroma; Granulocytic
 5 sarcoma; Acute promyelocyte leukemia; Acute myelomonocytic leukemia); Waldenstrom's macroglobulinemia, or other B-cell lymphoma.

The BCMA ADCs, particularly Ab-1, Ab-2, or Ab-3 ADC, and more particularly Ab-1 ADC, as variously described herein, may be used in combination with standard cancer therapies, such as, but not limited to surgery, radiation therapy, and chemotherapy, as well as proteasome inhibitors,
 10 corticosteroids, and stem cell transplants. The BCMA ADCs, particularly Ab-1, Ab-2, or Ab-3 ADC, and more particularly Ab-1 ADC, as variously described herein, may be used in combination with administration of a chemotherapeutic agent. The chemotherapeutic agent may be administered prior to, concurrent with, or subsequent to administration of one or more BCMA ADCs, particularly Ab-1, Ab-2, or Ab-3 ADC, and more particularly Ab-1 ADC, as variously described herein. A
 15 chemotherapeutic agent is a compound used in the treatment of cancer. Examples of chemotherapeutic agents include, but is not limited to: 13-cis-Retinoic Acid, 2-CdA 2-Chlorodeoxyadenosine, 5-Azacitidine 5-Fluorouracil 5-FU, 6-Mercaptopurine, 6-MP 6-TG 6-Thioguanine, Abraxane, Accutane®, Actinomycin-D, Adriamycin®, Aducanumab®, Agrylin®, Ala-Cort®, Aldesleukin, Alemtuzumab, ALIMTA, Alitretinoin, Alkaban-AQ®, Alkeran®, All-transretinoic Acid,
 20 Alpha Interferon, Altretamine, Amethopterin, Amifostine, Aminoglutethimide Anagrelide, Anadron®, Anastrozole, Arabinosylcytosine, Ara-C, Aranesp®, Aredia®, Arimidex®, Aromasin®, Arranon®, Arsenic Trioxide, Asparaginase, ATRA, Avastin®, Azacitidine, BCG, BCNU, Bendamustine, Bevacizumab, Bexarotene, BEXXAR®, Bicalutamide, BiCNU, Blenoxane®, Bleomycin, Bortezomib, Busulfan, Busulfex®, C225, Calcium Leucovorin, Campath®, Camptosar®,
 25 Camptothecin-11, Capecitabine Carac™, Carboplatin, Carmustine, Carmustine Wafer, Casodex®, CC-5013, CCI-779, CCNU, CDDP, CeeNU, Cerubidine®, Cetuximab, Chlorambucil, Cisplatin, Citrovorum Factor, Cladribine, Cortisone, Cosmegen®, CPT-11, Cyclophosphamide, Cytadren®, Cytarabine, Cytarabine Liposomal, Cytosar-U®, Cytoxan®, Dacarbazine, Dacogen, Dactinomycin, Darbepoetin Alfa, Dasatinib, Daunomycin, Daunorubicin, Daunorubicin Hydrochloride,
 30 Daunorubicin Liposomal, DaunoXome®, Decadron, Decitabine, Delta-Cortef®, Deltasone®, Denileukin, Diftitox, DepoCyt™, Dexamethasone, Dexamethasone Acetate, Dexamethasone Sodium Phosphate, Dexasone, Dexrazoxane, DHAD, DIC, Diodex, Docetaxel, Doxil®, Doxorubicin, Doxorubicin Liposomal, Droxia™, DTIC, DTIC-Dome®, Duralone®, Efudex®, Eligard™, Ellence™, Eloxatin™, Elspar®, Emcynt®, Epirubicin, Epoetin Alfa, Erbitux, Erlotinib, Erwinia L-asparaginase, Estramustine, Ethyol, Etopophos®, Etoposide, Etoposide Phosphate, Eulexin®,
 35 Evista®, Exemestane, Fareston®, Faslodex®, Femara®, Filgrastim, Floxuridine, Fludara®, Fludarabine, Fluoroplex®, Fluorouracil, Fluoxymesterone, Flutamide, Folinic Acid, FUDR®,



- Fulvestrant, G-CSF, Gefitinib, Gemcitabine, Gemtuzumab, ozogamicin, Gemzar, Gleevec™, Gliadel® Wafer, GM-CSF, Goserelin, Halotestin®, Herceptin®, Hexadrol, Hexalen®, Hexamethylmelamine, HMM, Hycamtin®, Hydrea®, Hydrocort Acetate®, Hydrocortisone, Hydrocortisone Sodium Phosphate, Hydrocortisone Sodium Succinate, Hydrocortone Phosphate,
- 5 Hydroxyurea, Ibritumomab, Ibritumomab Tiuxetan, Idamycin®, Idarubicin, Ifex®, IFN-alpha, Ifosfamide, Imatinib, mesylate, Imidazole, Carboxamide, Interferon alfa, Interferon Alfa-2b (PEG Conjugate), Interleukin-2, Interleukin-11, Intron A® (interferon alfa-2b), Iressa®, Irinotecan, Isotretinoin, Ixabepilone, Ixempra™, Kidrolase, Lanacort®, Lapatinib, L-asparaginase, LCR, Lenalidomide, Letrozole, Leucovorin, Leukeran, Leukine™, Leuprolide, Leurocristine, Leustatin™,
- 10 Liposomal Ara-C, Liquid Pred®, Lomustine, L-PAM, L-Sarcosyl, Lupron®, Lupron Depot®, Matulane®, Maxidex, Mechlorethamine, Mechlorethamine Hydrochloride, Medralone®, Medrol®, Megace®, Megestrol, Megestrol Acetate, Melphalan, Mercaptopurine, Mesna, Mesnex™, Methotrexate, Methotrexate Sodium, Methylprednisolone, Meticorten®, Mitomycin, Mitomycin-C, Mitoxantrone, M-Prednisol®, MTC, MTX, Mustargen®, Mustine, Mutamycin®, Myleran®,
- 15 Mylocel™, Mylotarg®, Navelbine®, Nelarabine, Neosar®, Neulasta™, Neumega®, Neupogen®, Nexavar®, Nilandron®, Nilutamide, Nipent®, Nitrogen Mustard, Novaldex®, Novantrone®, Octreotide, Octreotide acetate, Oncospar®, Oncovin®, Ontak®, Onxal™, Oprevelkin, Orapred®, Orasone®, Oxaliplatin, Paclitaxel, Paclitaxel Protein-bound, Pamidronate, Panitumumab, Panretin®, Paraplatin®, Pediapred®, PEG Interferon, Pegaspargase, Pegfilgrastim, PEG-INTRON™, PEG-L-asparaginase, PEMETREXED, Pentostatin, Phenylalanine Mustard, Platinol®, Platinol-AQ®,
- 20 Prednisolone, Prednisone, Prelone®, Procarbazine, PROCRIT®, Proleukin®, Prolifeprospan 20 with Carmustine Implant, Purinethol®, Raloxifene, Revlimid®, Rheumatrex®, Rituxan®, Rituximab, Roferon-A® (Interferon Alfa-2a), Rubex®, Rubidomycin hydrochloride, Sandostatin®, Sandostatin LAR®, Sargramostim, Solu-Cortef®, Solu-Medrol®, Sorafenib, SPRYCEL™, STI-571,
- 25 Streptozocin, SU1 1248, Sunitinib, Sutent®, Tamoxifen, Tarceva®, Targretin®, Taxol®, Taxotere®, Temodar®, Temozolomide, Temsirolimus, Teniposide, TESP, Thalidomide, Thalomid®, TheraCys®, Thioguanine, Thioguanine Tabloid®, Thiophosphoamide, Thioplex®, Thiotepa, TICE®, Toposar®, Topotecan, Toremfene, Torisel®, Tositumomab, Trastuzumab, Treanda®, Tretinoin, Trexall™, Trisenox®, TSPA, TYKERB®, VCR, Vectibix™, Velban®, Velcade®, VePesid®,
- 30 Vesanoid®, Viadur™, Vidaza®, Vinblastine, Vinblastine Sulfate, Vincasar, Pfs®, Vincristine, Vinorelbine, Vinorelbine tartrate, VLB, VM-26, Vorinostat, VP-16, Vumon®, Xeloda®, Zanosar®, Zevalin™, Zinecard®, Zoladex®, Zoledronic acid, Zolinza, Zometa®, and the like.

As mentioned above, the BCMA ADCs, particularly Ab-1, Ab-2, or Ab-3 ADC, and more particularly Ab-1 ADC, as variously described herein, may be used in combination with standard

35 cancer therapies, such as, but not limited to proteasome inhibitors, and in particular carfilzomib (such as Kyprolis®).

2352 and NPI-2358), Hemiasterlins (HTI-286), Laulimalide, Maytansinoids ("DM1," "DM3" or "DM4") (Bivatuzumab mertansine, Cantuzumab mertansine, huN90 1-DM 1/BB- 10901 TAP, MLN591DM1, My9-6-DM1, Trastuzumab-DM1), PC-SPES, Peloruside A, Resveratrol, S-allylmercaptocysteine (SAMC), Spongistatins, Vitilevuamide, Molecular Motor-Kinesins (SB-715992), Designed Colchicine-Site Binders (A-289099, A-293620/A-318315, ABT-751/E7010, D-24851/D-64131, ZD6126), Other Novel Spindle Poisons (2-Methoxyestradiol (2-ME2), Bezimidazole Carbamates (ANG 600 series, Mebendazole), CP248/CP461, HMN-214, R440, SDX-103, T67/T607).

193. The antibody or fragment of claim 192, wherein drug or chemotherapeutic agent is selected from the group consisting of: Maytansinoids ("DM1," "DM3" or "DM4").

194. The antibody or fragment of claim 193, wherein said drug or chemotherapeutic agent is DM1.

195. The antibody or fragment of claims 193 or 194, wherein said antibody or fragment has a drug to antibody/antibody fragment ratio of between 1 and 10, inclusive.

196. The antibody or fragment of claims 195, wherein said antibody or fragment has a drug to antibody/antibody fragment ratio of between 2 and 5, inclusive.

197. A composition, comprising the antibody or fragments thereof of any of claims 108 to 196.

198. A pharmaceutical composition, comprising the antibody or fragments thereof of any of claims 108 to 196.

199. The pharmaceutical composition of claim 198, comprising a pharmaceutically acceptable diluent, carrier, solubilizer, emulsifier, and/or a preservative.

200. A method of treating a disease, comprising administering an effective amount of the compositions of any of claims 197 to 199, wherein the disease is selected from the group consisting of: B-cell cancers, multiple myeloma, malignant plasma cell neoplasms, Kahler's disease and Myelomatosis; Plasma cell leukemia; Plasmacytoma; B-cell prolymphocytic leukemia; Hairy cell leukemia; B-cell non-Hodgkin's lymphoma (NHL); Acute myeloid leukemia (AML); Chronic myeloid leukemia (CML); Acute lymphocytic leukemia (ALL); Chronic lymphocytic leukemia (CLL); Follicular lymphoma (including follicular non-Hodgkin's lymphoma types); Burkitt's lymphoma (Endemic Burkitt's lymphoma; Sporadic Burkitt's lymphoma); Marginal zone lymphoma (Mucosa-Associated Lymphoid Tissue; MALT / MALToma; Monocytoid B cell lymphoma; Splenic lymphoma with villous lymphocytes); Mantle cell lymphoma; Large cell lymphoma (Diffuse large

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cell; Diffuse Mixed Cell; Immunoblastic Lymphoma; Primary Mediastinal B Cell Lymphoma; Angiocentric Lymphoma - pulmonary B cell); Small lymphocytic lymphoma (SLL); Precursor B-lymphoblastic lymphoma; Myeloid leukemia (granulocytic; myelogenous; Acute myeloid leukemia; Chronic myeloid leukemia; Subacute myeloid leukemia; Myeloid sarcoma; Chloroma; Granulocytic sarcoma; Acute promyelocyte leukemia; Acute myelomonocytic leukemia); Waldenstrom's macroglobulinemia, or other B-cell lymphoma.

201. The method of claim 200, wherein the disease is selected from the group consisting of: B-cell cancers, multiple myeloma, malignant plasma cell neoplasms, or other B-cell lymphoma.

202. The method of claim 201, wherein the disease is multiple myeloma.

203. The method of any of claims 200 to 202, further comprising administering one of more additional treatments selected from the group consisting of: chemotherapy, radiation therapy, surgery, proteasome inhibitors, corticosteroids, and stem cell transplants.

204. The method of claim 203, wherein the administering of said additional treatment is prior, concurrent, or subsequent, or a combination of each, to the administration of the compositions of any of claims 197 to 199.

Macrocyclic ketone analogues of halichondrin B

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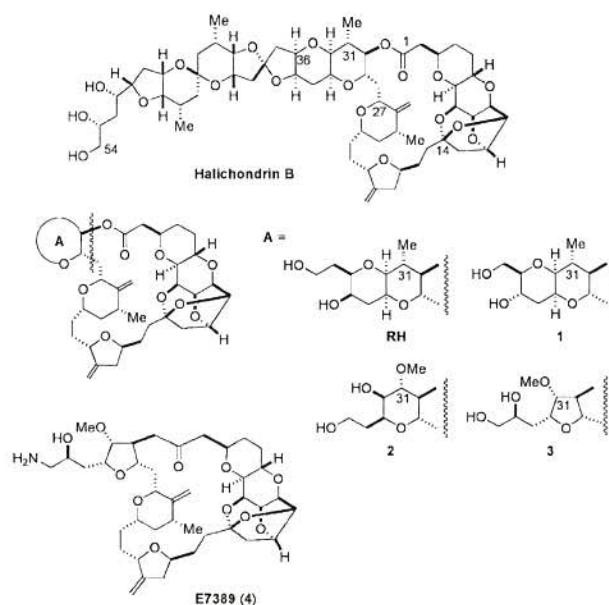
This paper is dedicated to memory of Bruce F. Wels, our friend and colleague

Abstract—Structurally simplified macrocyclic ketone analogues of halichondrin B were prepared by total synthesis and found to retain the potent cell growth inhibitory activity in vitro, stability in mouse serum, and in vivo efficacy of the natural product.
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Halichondrin B (HB) is a structurally complex marine natural product.^{1,2} Despite extensive synthetic studies, the only successful total synthesis of HB and norhalichondrin B was reported by Kishi and co-workers in 1992.³ The discovery that the right half (RH) of HB and analogue **1** exhibited potent cell growth inhibitory activity in vitro^{4,5} and antitumor efficacy in vivo,⁶ respectively, provided a compelling starting point for a drug discovery program. In the preceding paper⁷ we reported our SAR investigation and identification of a series of macrolactone analogues (e.g., **2** and **3**) that retained the remarkable in vitro biological profile of HB. However, these compounds failed to demonstrate salutary effects in vivo. Herein, we report our efforts to optimize the in vivo properties of these structurally simplified analogues, culminating in the discovery of E7389 (**4**), a fully synthetic analogue of HB that is currently undergoing Phase I clinical evaluation.

Instability of the macrolactone acyl moiety was regarded as the most likely explanation for the observed lack of in vivo efficacy associated with the structurally simplified macrolactone series. Consistent with this hypothesis, in vitro activity of octahydropyrano[3,2-*b*]pyran analogue **1** was not affected by inclusion of up to 1%

mouse serum in the 3–4 day growth inhibition assay, whereas activity of the tetrahydropyran or tetrahydrofuran series of analogues exemplified by **2** and **3**, respectively, were completely abrogated by mouse serum at concentrations as low as 0.01%. Since the seco acid of



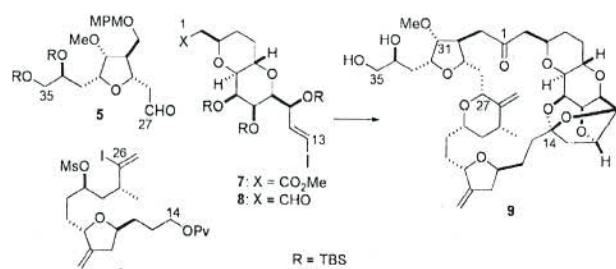
* Corresponding author. Tel.: +1 978 661 7202; fax: +1 978 657 7715; e-mail: melvin_yu@eri.eisai.com

RH was not active, the lack of in vivo efficacy for structurally simplified macrolactone analogues in the mouse xenograft model was most likely attributed to sensitivity of the macrolactone ring to nonspecific esterases present in serum.

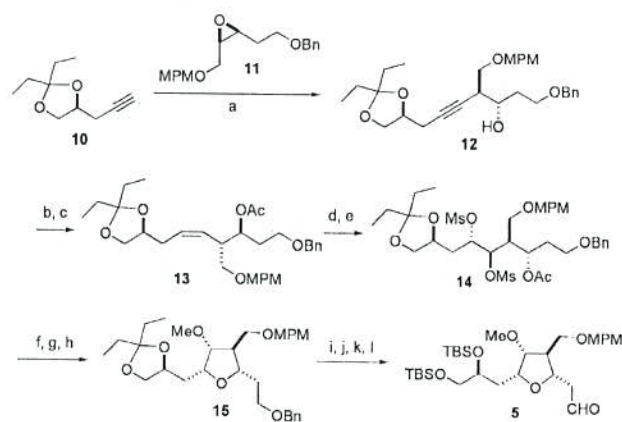
To further test this hypothesis, nonhydrolyzable ester bioisosteres were prepared to identify derivatives that would retain activity in the presence of mouse serum. Of the surrogate groups examined, only the ketone bioisostere provided analogues with sufficient in vitro potency to warrant further investigation.

The macrocyclic ketone analogues were prepared in a convergent manner from three major fragments **5**, **6**,^{3a,8} and **8**⁹ in a way similar to that developed by Kishi and co-workers for the total synthesis of HB (Scheme 1).

Aldehyde **5** was prepared as outlined in Scheme 2. Regioselective opening of epoxide **11**¹⁰ with the lithium anion of **10**¹¹ in the presence of $\text{BF}_3 \cdot \text{OEt}_2$ furnished a 3:1 mixture of structural isomers favoring desired product **12**.¹² Hydrogenation using Lindlar's catalyst followed by acetylation furnished *cis*-olefin **13**. Alkene dihydroxylation with osmium tetroxide afforded an 8:1 mixture of diastereomers, which was directly converted



Scheme 1. Final assembly from key fragments.

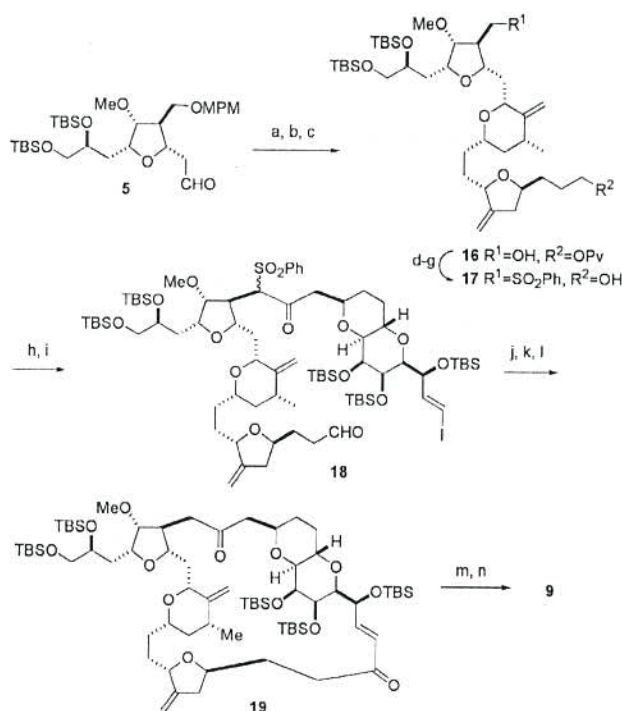


Scheme 2. Synthesis of aldehyde **5**. Reagents and conditions: (a) *n*-BuLi, **11**, $\text{BF}_3 \cdot \text{OEt}_2$, toluene, -50°C , 69%; (b) H_2 , 5% Pd/CaCO₃, quinoline, hexanes/ CH_2Cl_2 , rt, 99%; (c) Ac_2O , pyridine, DMAP, CH_2Cl_2 , rt, 99%; (d) OsO_4 , NMO, *t*-BuOH/ H_2O , rt; (e) MsCl , pyridine, rt, 58%; (f) Triton B, MeOH, rt; (g) MeMgBr , THF, 45°C ; (h) *t*-BuOK, MeI, THF, 0°C , 84%; (i) HCl, THF/MeOH/ H_2O , rt; (j) TBSCl, imidazole, DMF, rt; (k) H_2 , Raney Ni, EtOH, 84%; (l) $(\text{COCl})_2$, DMSO, Et₃N, CH_2Cl_2 , -60 to 0°C , 73%.

to the corresponding dimesylates. The desired isomer **14** was separated by column chromatography and subjected to cyclization in the presence of Triton B. Methylmagnesium bromide mediated desulfonylation¹³ followed by methylation of the corresponding alcohol furnished **15**. Adjusting protecting groups on the C.32 side chain, selective cleavage of the benzyl ether under Raney-Nickel conditions¹⁴ and Swern oxidation¹⁵ afforded aldehyde **5**.

Nozaki-Hiyama-Kishi coupling¹⁸ of aldehyde **5** with vinyl iodide **6**^{3a} and subsequent KHMDS-promoted cyclization afforded a 3:1 mixture of two C.27 diastereomers favoring the desired product (Scheme 3). The MPM-ethers were cleaved using DDQ¹⁹ and two C.27 isomers were separated by flash chromatography. The major product **16** was converted to sulfone **17** in four steps. Coupling of sulfone **17** with aldehyde **8** followed by oxidation resulted in **18**. SmI_2 mediated desulfonylation²⁰ followed by Nozaki-Hiyama-Kishi macrocyclization¹⁸ and subsequent allylic alcohol oxidation gave enone **19**. Treatment with TBAF and PPTS afforded the desired macrocyclic ketone **9**.

The vicinal diol on the C.32 side chain provided a convenient handle for subsequent derivatization. Analogues

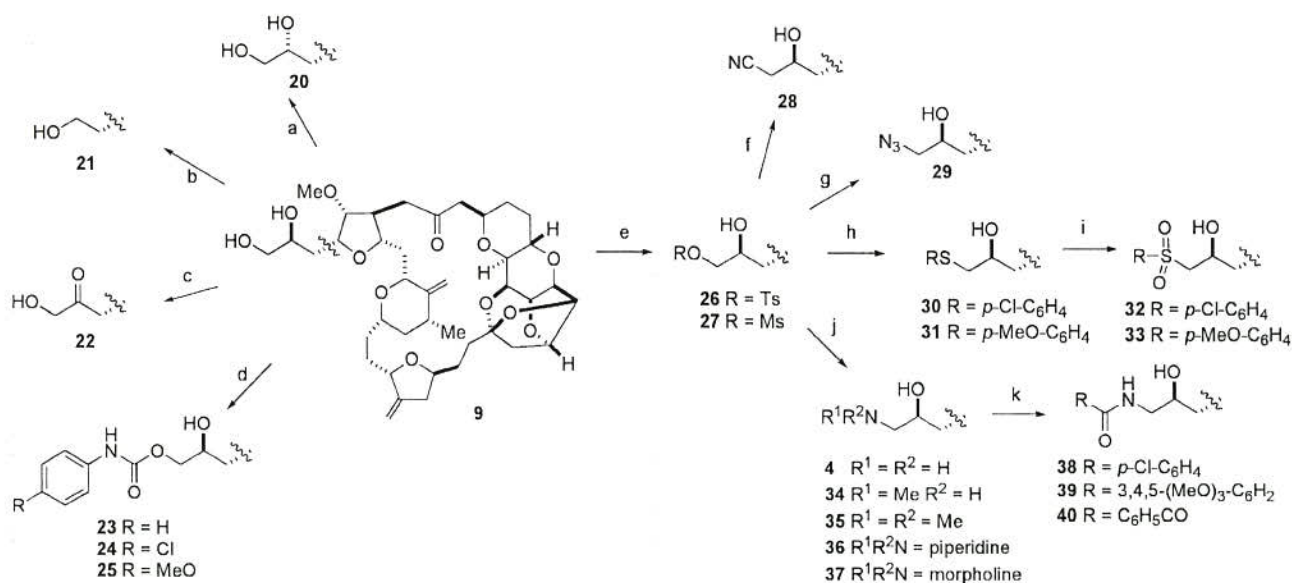


Scheme 3. Synthesis of macrocyclic ketone **9**. Reagents and conditions: (a) 0.5% $\text{NiCl}_2/\text{CrCl}_2$, **6**, THF/DMF (4:1), rt; (b) KHMDS, THF, 0°C , 75%; (c) DDQ, $\text{CH}_2\text{Cl}_2/\text{pH}$ 7 buffer, rt, 59%; (d) MsCl , Et₃N, CH_2Cl_2 , 0°C , 98%; (e) PhSH, *i*-Pr₂NEt, DMF, rt, 96%; (f) TPAP, NMO, CH_3CN , rt, 81%; (g) DIBAL, CH_2Cl_2 , -78°C , 97%; (h) *n*-BuLi, **8**, DME, -40 to -26°C ; (i) Dess-Martin,¹⁶ CH_2Cl_2 , rt, 82%; (j) SmI_2 , THF/MeOH, -78°C , 82%; (k) 1% $\text{NiCl}_2/\text{CrCl}_2$, THF/DMF (4:1), rt; (l) Dess-Martin,¹⁶ CH_2Cl_2 , rt, 73%; (m) TBAF/imidazole-HCl, THF, rt; (n) PPTS, CH_2Cl_2 , rt, 90%.¹⁷

20–40 were prepared from diol 9 using standard transformations summarized in Scheme 4.

The compounds were evaluated for cell growth inhibitory activity against DLD-1 human colon cancer cells

under continuous exposure conditions, and the ability to maintain a complete mitotic block (CMB) 10h after drug washout using flow cytometric analysis of U937 human histiocytic lymphoma cells (reversibility assay).²¹ In addition, susceptibility to P-glycoprotein (PgP)



Scheme 4. C.32 Side chain modifications. Reagents and conditions: (a) i. DEAD, Ph₃P, *p*-NO₂C₆H₄CO₂H, Et₂O, rt; ii. K₂CO₃, MeOH, rt, 60%; (b) i. NaIO₄, MeOH–H₂O (4:1), rt; ii. NaBH₄, MeOH–CH₂Cl₂ (4:1), –78 °C to rt, 89%; (c) i. TBDPSCl, imidazole, DMF, rt; ii. Dess–Martin reagent, CH₂Cl₂, rt; iii. TBAF, imidazole–HCl, THF, rt, 77%; (d) ArNCO, Et₃N, CH₂Cl₂, rt, 83–92%; (e) TsCl, pyridine, CH₂Cl₂, rt, 72%; or MsCl, collidine, CH₂Cl₂, 0 °C, 90%; (f) KCN, DMSO, 60 °C, 67% from 26; (g) *n*-Bu₄NN₃, DMF, 80 °C, 85% from 26; (h) i. NaI, acetone, 60 °C; ii. ArSH, DMF, 59–85% from 26, rt; (i) *m*CPBA, CH₂Cl₂, 0 °C, 61–74%; (j) R¹R²NH, MeOH, rt, 90–100% from 27; (k) RCO₂H, EDCl, CH₂Cl₂, rt, 31–70%.

Table 1. In vitro activity profile for macrocyclic ketone analogues of HB

Compound	DLD-1 ^a IC ₅₀ , nM ^a (<i>n</i>)	U937 ^b IC ₉₉ , 0h, nM	U937 ^b IC ₉₉ , 10h, nM (<i>n</i>)	Reversibility ratio	FR ^c
HB	0.74 ± 0.03 (6)	8	25 (4)	3	2.8
1	3.4 ± 0.40 (4)	9	220 (6)	24	12
2	1.8 ± 0.00 (3)	10	300 (3)	30	10
3	0.67 ± 0.09 (3)	2	15 (4)	8	6.2
4 (E7389)	13.0 ± 0.20 (10)	10	10 (4)	1	15
9 (ER-076349)	1.0 ± 0.10 (18)	3	38 (2)	13	9.7
20	1.9 ± 0.10 (4)	3	100 (1)	33	13
21	0.66 ± 0.11 (4)	3	100 (1)	33	8.5
22	1.1 ± 0.20 (5)	3	30 (2)	10	3.7
23	1.0 ± 0.20 (4)	3	65 (2)	22	5.7
24	1.3 ± 0.06 (3)	3	65 (2)	22	8.6
25	0.86 ± 0.07 (3)	3	30 (3)	10	5.8
26	2.0 ± 0.20 (3)	7	30 (2)	4	2.4
27	0.80 ± 0.01 (3)	3	30 (1)	10	3.0
28	0.49 ± 0.07 (4)	2	30 (2)	15	7.0
29	0.53 ± 0.01 (4)	3	30 (2)	10	1.9
30	1.2 ± 0.10 (3)	10	200 (2)	20	2.0
31	0.71 ± 0.12 (3)	3	30 (2)	10	1.7
32	0.66 ± 0.06 (4)	3	30 (2)	10	3.2
33	0.74 ± 0.03 (3)	3	30 (2)	10	3.4
34	3.3 ± 0.10 (3)	10	10 (2)	1	7.0
35	1.2 ± 0.20 (3)	1	10 (2)	10	22
36	0.47 ± 0.08 (3)	1	10 (2)	10	17
37	0.43 ± 0.03 (3)	3	20 (2)	7	4.7
38	0.69 ± 0.03 (4)	3	20 (2)	7	5.9
39	0.72 ± 0.06 (4)	2	10 (2)	5	20
40	0.87 ± 0.03 (4)	3	10 (2)	3	3.6

^a Cell growth inhibition under continuous exposure conditions for 3–4 days, IC₅₀ ± SEM (*n* = number of experiments).

^b Mitotic block reversibility assay.

^c Fold resistance calculated as the IC₅₀ ratio between retrovirally transformed P388/VMDRC.04 and parental P388 cells.

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mediated drug efflux was determined using murine P388/VMDRC.04 cells²² (Table 1).

Macrocyclic ketone **9** (ER-076349, NSC 707390) exhibited an in vitro profile very similar to the corresponding macrolactone analogue **3**, thus confirming bioisosteric equivalence of the ketone and ester functional groups. Compound **9** remained active in vitro even after inclusion of 100% mouse serum, and exhibited antitumor efficacy in a variety of human tumor xenograft models.²³

Modifications to the C.32 side chain had little impact on in vitro biological activity (Table 1). Interestingly, amine **4** (E7389, previously ER-086526, also NSC-707389) exhibited a reversibility ratio of one in U937 cells. Several other amine (**34–37**) and amide (**38–40**) derivatives were subsequently prepared and also found to exhibit a low reversibility ratio (e.g., **34**). E7389 demonstrated remarkable efficacy (0.05–1 mg/kg) in a variety of human tumor xenograft models.²³ This compound was selected for development as a new potential anticancer chemotherapeutic agent, and is currently in collaborative Phase I clinical trials with the United States NCI.

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Keywords: antibody-drug conjugate; target antigen; cytotoxic payload; linker; T-DM1; brentuximab vedotin; MMAE; resistance

Antibody-drug conjugates—an emerging class of cancer treatment

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Antibody-drug conjugates (ADCs) are an emerging novel class of anticancer treatment agents that combines the selectivity of targeted treatment with the cytotoxic potency of chemotherapy drugs. New linker technology associated with novel highly potent cytotoxic payloads has permitted the development of more effective and safe ADCs. In recent years, two ADCs have been licensed, T-DM1 and brentuximab vedotin, and are already establishing their place in cancer treatment. A plethora of ADCs are being investigated in phases I and II trials, emerging data of which appears promising. As we deepen our understanding of what makes a successful ADC, an increasing number of ADCs will likely become viable treatment options as single agents or in combination with chemotherapy. This review will present the philosophy underlying ADCs, their main characteristics and current research developments with a focus on ADCs in solid tumours.

Paul Ehrlich's vision of a rational targeted strategy against invading microbes or malignant cells has been a driving research throughout the past century, bringing a scientific and therapeutic revolution in cancer treatment. Although antibody-drug conjugates (ADCs) have been under investigation for decades, it is only recently with the advent of significant advances in engineering new linker and conjugation technologies together with highly potent cytotoxic drugs that we have realised the ADC's true potential (Panowski *et al*, 2014). Monoclonal antibodies have proved to have an important role in cancer treatment with drugs such as trastuzumab, pertuzumab, cetuximab and rituximab becoming the standard of care in selected solid tumours and lymphomas. Classic chemotherapy, the mainstay of anticancer treatment, demonstrates limited selectivity against cancer cells leading to a small therapeutic window, thus limiting its efficacy.

Antibody-drug conjugates could bring these two classes of drugs with their complementing properties together, in creating a highly selective and highly cytotoxic cancer treatment with an increased therapeutic window, as envisaged by Paul Ehrlich (Hughes, 2010). Looking back into the reasons for previous failures gives us an opportunity to identify the crucial characteristics that will make ADC an effective anticancer treatment. The low chemotherapy drug potency, unstable linkers and low antigen selectivity are the most commonly identified weaknesses limiting the efficacy of an ADC (Perez *et al*, 2014). In addition, in some of the first clinical trials murine antibodies were used leading to high rates of immunogenicity and, therefore, to low efficacy (Hughes, 2010).

Antibody-drug conjugates have a complex structure with many moving parts, each of which has different properties and desirable characteristics. An ADC can be divided into three main structural units: the antibody, the cytotoxic agent and the linker, which will be discussed below.

ANTIBODY-DRUG CONJUGATES

Target antigen. The ideal target antigen should be: (a) highly expressed with limited heterogeneity across the tumour and with low normal tissue expression, for example, T-DM1 targets HER-2, a receptor that reaches levels of expression of 2×10^6 on HER-2-positive cancer cells compared with 2×10^4 on other cells (Shefet-Carasso and Benhar, 2015); (b) there should be minimal antigen shedding to prevent the antibody binding to its target within the circulation; and (c) the antibody should be well internalised by receptor-mediated endocytosis and should not be modulated during endocytosis. The target antigen should not be down-regulated after treatment with the ADC (Mack *et al*, 2014). The minimum threshold of the different variables that are required to make a tumour antigen an effective target is still undetermined and interdependent. Studies in lymphoma and prostate cancer have shown that a minimum value of tumour-antigen density is a prerequisite for ADC efficacy. The biomarker analysis study for TDM1 showed that although it was active across different HER-2 expression subgroups, patients with tumours who

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expressed HER-2 more highly derived the greatest benefit (Baselga *et al*, 2013). The desirable cutoff value of antigen expression varies greatly and depends on other target antigen properties such as the internalisation rate and binding affinity, as well as other ADC characteristics, such as cytotoxic payload and linker stability. There is evidence that ADCs can be effective even when they target antigens with low expression given minimal normal tissue expression (Perez *et al*, 2014). The problem of non-homogeneous expression of the target antigen in solid tumours could potentially be addressed by the bystander effect, that is, the process by which membrane-permeable free cytotoxic payload is able to induce cell death to the neighbouring cells after being internalised and cleaved from the linker. Conversely, the bystander effect can increase the ADCs off-target systemic toxicity. Various factors can affect the rate of internalisation of the ADC in the cancer cell, which is a poorly understood process. One such factor is the epitope on the target antigen. For example, different epitopes of the HER-2 receptor have resulted in significantly different rates of internalisation and degradation of the mAb-Ag molecule. In addition, there are other difficulties while targeting cancer cell surface antigens, such as the high interstitial tumour pressure, downregulation of the antigen and the presence of other physical and kinetic barriers that diminish the cytotoxic payload uptake (Mack *et al*, 2014; Perez *et al*, 2014). Although most licensed or at an advanced research stage ADCs target tumour antigens, alternative approaches are actively being investigated to overcome some of the above mentioned limitations (Casi and Neri, 2012).

Another approach is to target antigens in stroma and vasculature. There is evidence in the preclinical and clinical setting that components of the neovasculature subendothelial extracellular matrix and of the tumour stroma could be valuable target antigens. A characteristic example is the extra-domain B (ED-B) of fibronectin which is a marker of angiogenesis, specifically highly expressed in vasculature of aggressive solid tumours (Palumbo *et al*, 2011) (Figure 1). The human mAb L19 which targets the ED-B has been combined with different effector molecules and has been studied extensively as a potential ADC. This target was successfully exploited by conjugating it with tubulin inhibitors showing that non-internalising vascular targeting ADCs could offer other treatment approaches (Perrino *et al*, 2014), (Table 1).

A further interesting approach to targeting antigens is to target the tumour-initiating cells or cancer stem cells (TICs). The hypothesis is that since TICs comprise an aggressive sub-population of tumour cells that are responsible for tumour growth, metastasis

and recurrence, targeting these cells will have a big impact on the disease control (Visvader and Lindeman, 2008). Sapra *et al* (2013) have developed an ADC combined with a tubulin inhibitor that targets 5T4 oncofetal antigen, which is an antigen expressed in TICs in NSCLC and has been associated with an invasive phenotype. A phase I trial investigating this ADC is currently recruiting patients with NSCLC and other solid tumours (Figure 1).

Antibody selection. Antibody engineering has made breakthroughs over recent years making it feasible to produce humanised and fully human antibodies as the basic components of ADCs. The early generation ADCs used murine antibodies causing significant immunogenicity, with many patients producing human anti-mouse antibodies thus reducing the efficacy of treatment.

The most commonly used antibody format currently is human IgG isotypes and in particular IgG1 (Hughes, 2010; Perez *et al*, 2014). The antibodies once part of the ADC can retain their original properties and activate immune functions such as antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (Perez *et al*, 2014). They could still act as receptor inhibitors or signal modulators. One example of complementary action is T-DM1 whose Ab's retain effector functions have been shown to activate ADCC (Junttila *et al*, 2011). The independent function of the antibody is not always beneficial and complementary to ADC efficacy, especially when Ab binding is sufficient to produce a cytotoxic effect. The Ab's independent effector functions could lead to increased toxicity, reduced tumour localisation and internalisation of the ADC (Sapra and Shor, 2013).

Linkers and conjugation. The linker plays a crucial role since its properties greatly influence the ADC's pharmacokinetics, therapeutic index and efficacy (Hughes, 2010; Flygare *et al*, 2013; Shefet-Carasso and Benhar, 2015). The ideal linker should be stable so that the ADC does not release the cytotoxic drug before reaching its target and causing off-target toxicity. At the same time it should be able to release the drug efficiently once internalised (Teicher and Chari, 2011).

Another important factor is how many of the drug molecules will be loaded onto the antibody: the drug-antibody ratio (DAR). Attaching too few of the drug molecules will lead to decreased efficacy. Attach too many and the ADC will become unstable with altered pharmacokinetic properties, increased plasma clearance, reduced half-life and increased systemic toxicity (Perez *et al*, 2014). The currently licensed ADCs with proven activity are produced by nonspecific conjugation to lysine residues and to some degree

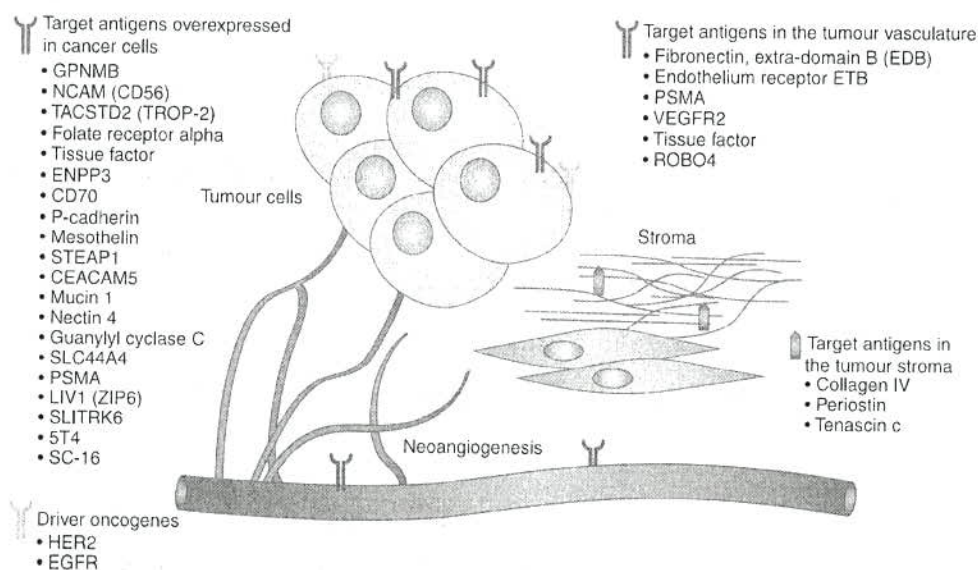


Figure 1. Target antigens for ADCs in solid tumours.

Table 1. Target antigens in solid tumours under research		
Name	ADC	Lead indication
Target antigens overexpressed in cancer cells		
GPNMB	Glembatumumab vedotin [1]	Breast cancer and melanoma
CD56	Lorvotuzumab mertansine (IMGN-901) [2]	SCLC
TACSTD2 (TROP2)	sacituzumab govitecan (IMMU-132) [3]	TNBC and pancreatic cancer
CEACAM5	Labetuzumab SN-38 [4]	Colorectal cancer
Folate receptor- α	- Mirvetuximab soravtansine (IMGN-853) [5] - Vintafolide [6]	Ovarian and endometrial cancer
Mucin 1 (Sialoglycope CA6)	SAR-566658 [7]	Breast, ovarian, cervical, lung and pancreatic cancer
STEAP1	Vandortuzumab vedotin RG-7450 [8]	Prostate cancer
Mesothelin	- DMOT4039A [9] - Anetumab ravtansine (BAY-94-9343) [10]	Ovarian, pancreatic cancer and mesothelioma
Nectin 4	- Enfortumab vedotin (ASG-22M6E) [11] - ASC-22CE [12]	Bladder, breast, lung and pancreatic cancer
ENPP3	AGS-16M8F [13]	Renal cell carcinoma, liver carcinoma and prostate cancer
Guanylyl cyclase C (GCC)	Indusatumab vedotin (MLN-0264) [14]	Pancreatic and colorectal cancer
SLC44A4	ASG-5ME [15]	Pancreatic, gastric and prostate cancer
NaPi2b	- anti-NaPi2b ADC, Lifestuzumab vedotin [16] - DNIB0600A [17] - AMG-172 [18] - MDX-1203 [19] - Vorsetuzumab mafodotin SGN-75 [20]	Non-small cell lung cancer and platinum-resistant ovarian cancer
CD70 (TNFSF7)		Renal cell carcinoma
CA9, Carbonic anhydrase	BAY79-4620 [21]	Solid tumours
ST4 (TPBG)	PF 06263507 [22]	Solid tumours
SLTRK6	ASG-15ME [23]	Bladder cancer
SC-16 (anti-Fyn3)	SC16LD6.5 [24]	SCLC
Tissue factor	HuMax-TF-ADC (TF-011-MMAE) [25]	NSCLC and ovarian cancer
LIV-1 (ZIP6)	SGN-LIV1A [26]	Solid tumours
P-Cadherin	PCA062 [27]	Breast cancer
PSMA	- MLN2704 [28] - PSMA-ADC [29]	Solid tumours
		Prostate cancer
Target antigens in the tumour vasculature and stroma		
Fibronectin Extra-domain B (ED-B)	Human mAb L19 and F8 [30]	Solid tumours
Endothelin receptor ETB	RG-7636 [31]	Melanoma
VEGFR2 (CD309)	Anti-VEGFR-2ScFv-As2O3-stealth Nanoparticles [32]	Solid tumours
Tenascin c	Anti-TnC-A1 antibody SIP(F16) [33]	Solid tumours
Collagen IV	Cytotoxic immunoconjugates [34]	Solid tumours
Periostin	Anti-periostin antibody [35]	Solid tumours
Target antigens regulated from driver oncogenes		
HER 2	- T-DM1[36] - ARX788 [37] - SYD985 [38]	Breast cancer
EGFR	- ABT-414 [39] - IMGN289 [40] - AMG-595 [41]	Glioblastoma, NSCLC, head and neck, breast, oesophageal
Target antigens in haematological malignancies		
CD30	- Brentuximab vedotin [42] - Iratumumab MDX-060 [43]	HL and ALCL
CD22	- Inotuzumab ozogamicin (CMC-544) [44] - Pinatuzumab vedotin [45] - Epratuzumab SN38 [46]	NHL and ALL
CD79b	Polatuzumab vedotin [47]	DLBCL and follicular NHL
CD19	- Coltuximab ravtansine [48] - SAR-3419 [49]	DLBCL and ALL
CD138	Indatuximab ravtansine [50]	Multiple myeloma
CD74	Milatuzumab doxorubicin [51]	CLL, NHL and multiple myeloma
CD37	IMGN-529 [52]	NHL and CLL
CD33	- IMGN779 [53] - SGN CD33 A [54]	AML
CD19	SGN-CD19A [55]	ALL and NHL,
CD98	IGN523 [56]	AML
Abbreviations: ADC = antibody-drug conjugate; ALL = acute lymphocytic leukemia; AML = acute myelogenous leukemia; CLL = chronic lymphocytic leukemia; DLBCL = diffuse large B-cell lymphoma; HL = Hodgkin lymphoma; NHL = non-Hodgkin lymphoma; NSCLC = non-small cell lung cancer; SCLC = small cell lung cancer; TNBC = triple-negative breast cancer. Please refer to the Supplementary Data File for all references cited in this table		

consist of an undesirable heterogeneous mixture of ADCs containing drug molecules with high DAR. There are ongoing efforts for more homogeneous ADCs with increased number of drug molecules stably linked to the Ab. The optimal DAR is undetermined and highly dependent on other ADC variables; however, more commonly the ADCs aim to attain a DAR close to 4. (Hamblett *et al*, 2004; Teicher and Chari, 2011). Site-specific conjugation, has been an important step in ADC development, enabled the production of homogeneous ADCs with the desired and pre-specified DAR by using techniques such as engineered cysteine

residue, non-canonical amino acid incorporation or modification of peptide tags (Panowski *et al*, 2014; Agarwal and Bertozzi, 2015).

Linkers can be divided in non-cleavable and cleavable. Non-cleavable linkers have the characteristic that following ADC lysosomal degradation the cytotoxic payload remains active while still being attached to the linker and an amino acid residue. T-DM1 is an ADC that uses a non-cleavable linker (Junttila *et al*, 2011; Shefet-Carasso and Benhar, 2015).

Cleavable linkers use different methods to release cytotoxic drug, increasing the possibility of the bystander effect. There are acid-sensitive linkers that will release the free drug in the low pH conditions in lysosomes or endosomes. Such a mechanism was used by gemtuzumab ozogamicin and its linker was characterised by certain plasma instability (Hamann *et al*, 2002; Shefet-Carasso and Benhar, 2015). Inotuzumab ozogamicin, now in phase II trials, uses the same linker and it is more stable (Boghaert *et al*, 2008). Another type of cleavable linker are lysosomal protease-sensitive linkers with the licensed example of brentuximab vedotin (Senter and Sievers, 2012). A third type are glutathione-sensitive linkers that benefit from the higher concentration of glutathione in tumour cells. (Sapra *et al*, 2011). Each approach has different advantages and disadvantages, so the choice of the linker can only be determined by taking into account all the other components of the ADC.

Cytotoxic payload. To create an effective ADC, it is imperative to have a potent cytotoxic payload. The first generation of ADCs used classical chemotherapy drugs such as doxorubicin and methotrexate with the benefit of a well-known toxicity profile (Shefet-Carasso and Benhar, 2015). Repeated studies, however, have shown that the actual concentration of the cytotoxic payload in tumour cells is minimal with only 1–2% of the administered dose reaching the tumour (Teicher and Chari, 2011). It is evident that the optimal chemotherapy drug used should be extremely potent, being effective at picomolar or nanomolar concentrations.

There are two main categories of cytotoxic drugs used in ADC development: microtubule inhibitors and DNA-damaging drugs. Auristatins block tubulin assembly and cause G2/M phase cell cycle arrest; they are the most commonly used payloads, accounting for a majority of cytotoxic payloads used in ADCs currently investigated (Sapra and Shor, 2013; Bouchard *et al*, 2014). Monomethyl auristatin E, an auristatin derivative (MMAE) is the cytotoxic payload of brentuximab vedotin and has a free drug IC_{50} : 10^{-11} – 10^{-9} allowing it to be effective in the low nanomolar range (Gerber *et al*, 2009). Maytansinoids, another class of tubulin inhibitors, have also been used successfully in the ADC development. The cytotoxic payload of trastuzumab emtansine (T-DM1) DM1 is a highly potent maytansinoid, developed by Immunogen, with a free drug IC_{50} : 10^{-11} – 10^{-9} . Another maytansinoid drug, DM4 incorporated on SAR3419, is being investigated in phase II trials exhibiting significant 'bystander effect' as the ADC's metabolites have lipophilic properties allowing crossing of the cellular membrane (Blanc *et al*, 2011). Tubulysins are a promising new class of tubulin inhibitors. Tubulysin analogues were successfully conjugated to trastuzumab forming a stable and potent ADC (Cohen *et al*, 2014). The DNA-damaging agents have the ability to be active throughout the different cell cycle phases. Duocarmycin is a powerful cytotoxic alkylating compound that binds to the minor groove of DNA and has shown activity against various multidrug-resistant models. Duocarmycin-based ADCs are currently under investigation in a phase I trial setting conjugated to the anti-HER-2 antibody, trastuzumab (Van Herpen *et al*, 2015).

Calicheamicin is a potent antitumour antibiotic that causes double-strand DNA breaks and rapid cell death by binding to the DNA's minor groove. It is less dependent on cell cycle progression making it potentially useful against TICs who have lower rates of proliferation (Gupta *et al*, 2009; Sapra *et al*, 2011). Gemtuzumab ozogamicin and other ADCs such as inotuzumab ozogamicin in

non-Hodgkin lymphoma and MDX-1203 in renal cancer are using these agents (Owonikoko *et al*, 2014).

A potential new drug under investigation is α -amanitin, an RNA polymerase II inhibitor in the picomolar range, derived from the mushroom, *Amanita phalloides* (Moldenhauer *et al*, 2012). Other topoisomerase inhibitors under investigation is the SN38, the active metabolite of irinotecan (Palakurthi, 2015). Another new category is the pyrrolobenzodiazepines (PBDs) that bind to discrete DNA sequences causing lethal lesions and have interestingly not been found to have cross-resistance with common chemotherapeutic agents (Bouchard *et al*, 2014).

A different approach, but in line with ADC principles, are the radioimmunoconjugates where radionuclides are the cytotoxic payloads linked to monoclonal antibodies. Two radioimmunoconjugates targeting CD20 have been approved for treatment of refractory Hodgkin's lymphoma (Yttrium-90-ibritumomab tiuxetan and iodine-131-tositumomab). Despite its efficacy due to under-utilisation tositumomab manufacturer has discontinued its production. In solid tumours, radioimmunoconjugates are being investigated, particularly in treating minimal residual disease in prostate and colorectal cancer among others (Kraeber-Bodere *et al*, 2014).

CLINICAL EXPERIENCE

T-DM1, Kadcyla. The only ADC licensed in non-haematological malignancies is ado-trastuzumab emtansine (T-DM1, Kadcyla, Roche, Genentech), the antibody used is the well-known trastuzumab, a humanised IgG1 anti-HER-2 Ab linked with a stable non-cleavable linker to the maytansinoid DM1 (LoRusso *et al*, 2011; Verma *et al*, 2012). T-DM1 has been approved in the second line setting by the FDA in 2013 for HER-2-positive patients who had previously received treatment with trastuzumab and taxane chemotherapy. T-DM1 has an overall tolerable toxicity profile with most common adverse events being fatigue, transaminitis, nausea, thrombocytopenia and rash. In USA, T-DM1 carries black box warnings for hepatotoxicity, embryo-fetal and cardiac toxicity ($P < 0.001$) (Verma *et al*, 2012).

T-DM1 is also being investigated as a single agent compared to docetaxel in previously treated gastric cancer with results expected in 2015 (GATSBY trial, NCT01641939) (Shah, 2015).

Brentuximab vedotin, Adcetris. Brentuximab vedotin (BV, Adcetris, Seattle Genetics) is composed of an anti-CD30 mAb connected with a cleavable peptide to the highly potent tubulin inhibitor MMAE discussed previously. CD30 is a member of the tumour necrosis factor (TNF) family identified on Reed–Sternberg cells of classical Hodgkin lymphoma (HL). Binding of BV to the cell surface will lead to internalisation and lysosomal proteolytic cleavage of the linker releasing the MMAE (Senter and Sievers, 2012; Sievers and Senter, 2013).

BV has gained approval for the treatment of patients with relapsed or refractory CD30+ HL following autologous stem cell transplant (ASCT) or patients not eligible for ASCT who have failed at least two other chemotherapy treatments. BV has also been approved for patients with anaplastic large cell lymphoma (ALCL) as a second line. The accelerated approval for Hodgkin's lymphoma was based on a single-arm phase II clinical trial, where there was a 73% response rate, 32% complete remission and a median duration 20.5 months (Younes *et al*, 2012). The indication for ALCL was established based on the impressive results of the phase II study. Patients at this study had an 86% overall response rate and 54% complete responses (Pro *et al*, 2012). The most common adverse reactions were peripheral sensory neuropathy, neutropenia, fatigue, nausea and thrombocytopenia. In USA, BV carries a black box warning for progressive multifocal leukoencephalopathy (Younes *et al*, 2012).

Gemtuzumab ozogamicin, Mylotarg. Gemtuzumab ozoogamicin (GO, Mylotarg, Pfizer) was the first ADC to be approved by the FDA in 2000. It was licensed as a monotherapy in patients over the age of 60 with acute myelogenous leukemia (AML) who were not candidates for cytotoxic chemotherapy. GO consisted of a humanised IgG4 mAb directed against CD33 a surface antigen present in 85–90% of AML linked to a calicheamicin cytotoxin with positive results coming from the first single-agent phase II studies achieving 30% remissions (Tsimberidou *et al*, 2006). In 2010, the results of a post-approval phase III study showed no clinical benefit. In fact, the patients in the GO arm had a higher risk of fatal AEs and this trial led to the retraction of its FDA licence approval (Petersdorf *et al*, 2013). The failures were attributed to an unstable linker allowing premature release of the cytotoxic payload and to the not-so-selective antibody target (ten Cate *et al*, 2009).

More recent trials such as the ALFA-0701 using intermittent dosing regimens have showed good results, improving event-free survival and overall survival in patients with AML, reigniting discussions about the future of GO (Castaigne *et al*, 2012).

Clinical trial setting. Research in ADCs has been very active with almost 120 total clinical trials with the majority focusing on haematological malignancies. Interest in solid tumours is evident with 52 open phase I/II studies. There are approximately 50 unique ADCs under investigation, with 35 ADCs investigated in solid tumours (Mullard, 2013).

Putative mechanisms of resistance. Cancer cells under the pressure of any treatment develop resistance mechanisms that will allow them to survive. These mechanisms are complex and variable confronting the ADC in each of its different components, the cytotoxic drug, the monoclonal antibody or by activating survival signalling pathways (Shefet-Carasso and Benhar, 2015).

The cytotoxic payload of the ADC is subject to the same multidrug resistance mechanisms as conventional chemotherapy drugs. Some of the most commonly used drugs in the new generation ADCs such as auristatins (MMAE) and calicheamicin are substrates of P-glycoprotein. Resistance can also emerge from mechanisms that will limit the intracellular concentration of the ADC. Barok *et al* (2014) have described such mechanisms in their work with T-DM1, that is, downregulation of the target antigen, reduced internalisation of the ADC, diminished lysosomal degradation or increased ADC recycling to the cell surface and masking of the antigen epitope. There is also evidence that activation of the PI3K/AKT, MEK/ERK and JAK/STAT pathways leads to increased ADC resistance indicating that a potential way ahead could be combinations with small molecule inhibitors (Shefet-Carasso and Benhar, 2015). Clonal expansion of resistant cells and intratumoural heterogeneity representing populations of cells that are resistant *de novo* within the same tumour, are likely to influence resistance to ADCs (Gerlinger *et al*, 2012).

CONCLUSION: FUTURE PROSPECTS

Predictive biomarkers are essential to ensure we are offering the most effective treatments to the group of patients most likely to benefit from them. Development of specific tests that will determine the chances of responding to treatment to an ADC by investigating the level of expression of the target antigen similar to the immunohistochemical (IHC) confirmation of HER-2 expression are already being explored (Mack *et al*, 2014). Efforts are ongoing in order to move away from IHC-based techniques that require tissue biopsy and use circulating tumour cells or imaging techniques to identify the patient population more likely to respond (Mack *et al*, 2014).

Another approach to ADCs is to use high-affinity molecules like folic acid or growth hormones as cytotoxic payload carriers or small antibody fragments such as diabodies or minibodies. The rationale

behind this approach is that it might result in more effective drug delivery and increased antitumour activity. However, this approach will also limit the pharmacokinetic benefits of an ADC resulting in more rapid clearance and larger volume of distribution.

Combination strategies are actively explored in many ongoing clinical trials. T-DM1 is being investigated in combination with pertuzumab (Phillips *et al*, 2014). Other combinations of ADCs investigated are with conventional chemotherapies, for example, brentuximab with modified AVD, PI3K inhibitors like BYL719 with T-DM1 or with TKI inhibitors like neratinib.

There remain major hurdles that ADCs need to overcome: low delivery efficiency, target antigens expressed in normal tissues, the heterogeneity of target antigen expression in the tumour and more (Teicher and Chari, 2011). The future of ADCs seems promising as the combination of new linker technologies and more powerful cytotoxic payloads leads to the emergence of more stable and effective ADCs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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