

Bogotá D.C., 11 de agosto de 2016

Doctor
JOSÉ LUIS SALAZAR LÓPEZ
Dirección de Nuevas Creaciones
Superintendencia de Industria y Comercio
Ciudad

Superintendencia de Industria y Comercio

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Tipo aplicación: Respuesta a requerimiento

Expediente	14 - 155963
Patente	NUEVOS DERIVADOS FOSFATOS, SU PROCEDIMIENTO DE PREPARACIÓN Y LAS COMPOSICIONES FARMACEUTICASQUE LOS CONTIENEN.
Solicitante	LES LABORATORIES SERVIER y VERNALIS (R&D) LIMITED
Asunto	RESPUESTA AL OFICIO 5549 del 12 de mayo de 2016

CLAUDETTE VERNOT HERNÁNDEZ, mayor de edad, identificada con la cédula de ciudadanía No. 51.722.517 expedida en Bogotá, portadora de la tarjeta profesional No. 57.111 del Consejo Superior de la Judicatura, actuando en calidad de apoderada de LES LABORATORIES SERVIER Y VERNALIS (R&D) LIMITED, conforme a los poderes que obran en el expediente, por medio del presente escrito y encontrándome dentro del término concedido, me dirijo a usted para dar respuesta al requerimiento efectuado mediante Oficio 5549 del 12 de mayo de 2016.

I. RESPUESTA A LOS REQUERIMIENTOS

• Excepción a la patentabilidad

El Examinador considera que el objeto de las **reivindicaciones 16, 18, 19, 20, 21 y 26** que se refieren a métodos de tratamiento, no es patentable de acuerdo con lo dispuesto en el literal d) del artículo 20 de la Decisión 486 de la Comisión de la Comunidad Andina.

Por lo anterior, las reivindicaciones 16, 18, 19, 20, 21 y 26 han sido eliminadas.

• Reivindicaciones relativas a un segundo uso

El Examinador considera que el uso de las **reivindicaciones 14, 15, 17, 24 y 25** no es patentable, de acuerdo con lo dispuesto en el artículo 14 de la Decisión 486 de la Comisión de la Comunidad Andina.

Por lo anterior, las reivindicaciones 14, 15, 17, 24 y 25 han sido eliminadas.

2

- De la solicitud de patente

1. Título

Manifiesta el examinador que el título de la Patente no es claro porque no menciona la estructura base o farmacofórica de los compuestos, por lo cual sugiere realizar las aclaraciones necesarias.

En relación con este requerimiento proponemos modificar el título de la siguiente manera:

"NUEVOS DERIVADOS DE FOSFATO DE 1,2,3,4-TETRAHIDROISOQUINOLINA AMIDA FENILO, MÉTODO DE PREPARACIÓN DE LOS MISMO Y COMPOSICIONES FARMACÉUTICAS QUE LOS CONTIENEN".

2. Reivindicaciones

- Manifiesta el examinador que las **reivindicaciones 9 y 10** no son claras cuando mencionan la expresión "representan ventajosamente", lo cual es considerado subjetivo y ambiguo ya que no permite caracterizar adecuadamente el objeto de la invención, por lo cual sugiere eliminar dicha expresión del pliego reivindicatorio.

En relación con este requerimiento y de acuerdo a lo propuesto por el Examinador, aceptamos eliminar el término "ventajosamente" de las reivindicaciones 9-10.

- Considera el examinador que en la **reivindicación 12** la expresión "...determinados grupos (hidroxi, amino...)...", se considera ambigua, ya que no permite reconocer cuales son exactamente los grupos a los que se pretende proteger en el proceso de dicha reivindicación, por lo cual sugiere adicionar todas las características necesarias para dar la mayor claridad a la invención.

En relación con este requerimiento, modificamos la reivindicación 12 como sigue:

"[...] entendiéndose que, en cualquier momento que se considere apropiado en el curso del proceso descrito anteriormente, los grupos hidroxi y amino de los reactivos o intermediarios de síntesis pueden ser protegidos y luego desprotegidos de acuerdo a los requisitos de la síntesis."

- Considera el examinador que la **reivindicación 22** no es concisa ya que se pretende reclamar una "asociación" con "un agente anticanceroso elegido entre los agentes genotóxicos, los venenos mitóticos, los anti-metabolitos, los inhibidores del proteasoma, los inhibidores de quinasas o los anticuerpos", con los cuales se podrían presentar múltiples combinaciones que no tienen sustento en el capítulo descriptivo, por lo que sugiere eliminar cualquier referencia a asociaciones en el pliego reivindicatorio. Lo anterior aplica para la **reivindicación 23**.

En relación con lo dicho frente a las reivindicaciones 22 y 23, no estamos de acuerdo con la opinión del examinador, por cuanto las terapias de combinación en oncología se prueban actualmente con el fin de mejorar la eficacia de los tratamientos, así como la

2

calidad de vida de los pacientes (ver Komarova et al Nature 2013 291; Ann N.Y. Acad Sci 2005 1059: 61 a 69). Tales estrategias terapéuticas son operaciones de rutina para los médicos en oncología.

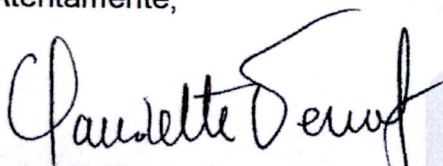
Por lo tanto, la combinación de las reivindicaciones 22 y 23 son modalidades específicas de la invención. Como estas combinaciones genéricas y la correspondiente Markush de fórmula (I) se reivindican en la misma solicitud de patente, no hay necesidad de probar ningún efecto sinérgico en el presente caso. La patentabilidad de las combinaciones se evalúa a partir de la patentabilidad de los compuestos de fórmula (I); estos deben ser considerados como novedosos e inventivos con base en el hecho que los compuestos de fórmula (I) son novedosos e inventivos.

No obstante, lo anterior, y por razones de claridad, sugerimos sustituir el término "asociación" con el término "combinación farmacéutica" en las reivindicaciones 22-23.

II. ANEXOS

1. Nuevo capítulo de Reivindicaciones.
2. Artículo titulado "Preclinical Modeling of Combination Treatments: Fantasy or Requirement?" – Shannon Decker and Edward Sausville.
3. Artículo titulado: "Calculated treatment" – Natalia L. Komarova.

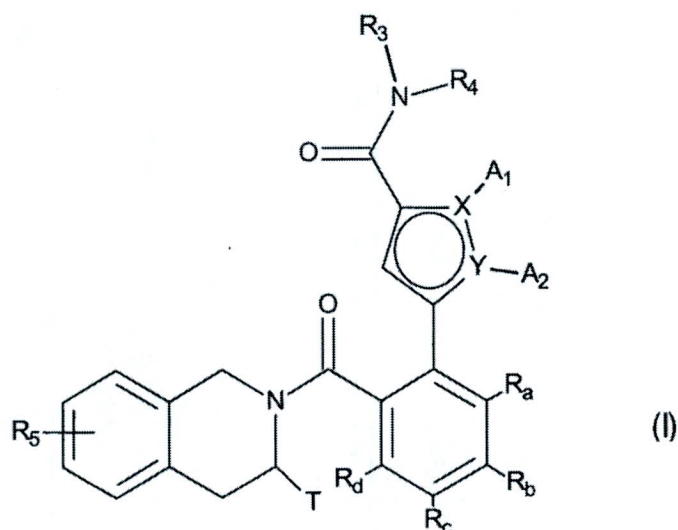
Atentamente,



Claudette Vernot Hernández
C.C. No. 51.722.517 de Bogotá.
T.P. No. 57.111 del C.S de la J.

REIVINDICACIONES

1. Compuesto fosfato de fórmula (I):



en la que :

- ♦ X e Y representan un átomo de carbono o un átomo de nitrógeno, entendiéndose que no pueden representar simultáneamente dos átomos de carbono o dos átomos de nitrógeno,
- ♦ A₁ y A₂ forman junto con los átomos a los que están unidos un heterociclo Het sustituido opcionalmente, aromático o no, constituido por 5, 6 ó 7 eslabones, y que puede contener, además del nitrógeno representado por X o por Y, uno a 3 heteroátomos elegidos independientemente entre oxígeno, azufre y nitrógeno, entendiéndose que el nitrógeno en cuestión puede estar sustituido con un grupo que representa un átomo de hidrógeno, un grupo alquilo (C₁-C₆) lineal o ramificado o un grupo -C(O)-O-Alk en el que Alk es un grupo alquilo (C₁-C₆) lineal o ramificado,
- o bien, A₁ y A₂ representan independientemente el uno del otro un átomo de hidrógeno, un polihalógenoalquilo (C₁-C₆) lineal o ramificado, un grupo alquilo (C₁-C₆) lineal o ramificado o un cicloalquilo,

- ◆ T representa un átomo de hidrógeno, un alquilo (C_1-C_6) lineal o ramificado sustituido opcionalmente con uno a tres átomos de halógeno, un grupo alquil(C_1-C_4)- NR_1R_2 , o un grupo alquil(C_1-C_4)- OR_6 ,
- ◆ R_1 y R_2 representan independientemente el uno del otro un átomo de hidrógeno o un grupo alquilo (C_1-C_6) lineal o ramificado,
o bien R_1 y R_2 forman con el átomo de nitrógeno al que están unidos un heterocicloalquilo,
- ◆ R_3 representa un grupo alquilo (C_1-C_6) lineal o ramificado, alquénilo (C_2-C_6) lineal o ramificado, alquinilo (C_2-C_6) lineal o ramificado, cicloalquilo, cicloalquil(C_3-C_{10})alquilo (C_1-C_6) lineal o ramificado, heterocicloalquilo, arilo o heteroarilo, entendiéndose que uno o varios átomos de carbono de los grupos anteriores, o de sus sustituyentes opcionales, puede(n) estar deuterado(s),
- ◆ R_4 representa un grupo arilo, heteroarilo, cicloalquilo o alquilo (C_1-C_6) lineal o ramificado, entendiéndose que uno o varios átomos de carbono de los grupos anteriores, o de sus sustituyentes opcionales, puede(n) estar deuterado(s),
- ◆ R_5 representa un átomo de hidrógeno o de halógeno, un grupo alquilo (C_1-C_6) lineal o ramificado, o un grupo alcoxi (C_1-C_6) lineal o ramificado,
- ◆ R_6 representa un átomo de hidrógeno o un grupo alquilo (C_1-C_6) lineal o ramificado,
- ◆ R_a , R_b , R_c y R_d representan independientemente los unos de los otros R_7 , un átomo de halógeno, un grupo alcoxi (C_1-C_6) lineal o ramificado, un grupo hidroxí, un grupo polihalógenoalquilo (C_1-C_6) lineal o ramificado, un grupo trifluorometoxi, $-NR_7R_7'$, nitro, $R_7-CO-alquil(C_0-C_6)-$, $R_7-CO-NH-alquil(C_0-C_6)-$, $NR_7R_7'-CO-alquil(C_0-C_6)-$, $NR_7R_7'-CO-alquil(C_0-C_6)-O-$, $R_7-SO_2-NH-alquil(C_0-C_6)-$, $R_7-NH-CO-NH-alquil(C_0-C_6)-$, $R_7-O-CO-NH-alquil(C_0-C_6)-$, un grupo heterocicloalquilo, o bien los sustituyentes de una de las parejas (R_a, R_b), (R_b, R_c) o (R_c, R_d) forman junto con los átomos de carbono a los que están unidos un ciclo constituido por 5 a 7 eslabones, que puede contener de uno a 2 heteroátomos elegidos entre oxígeno y azufre, entendiéndose también que uno o varios átomos de carbono del ciclo definido anteriormente puede(n) estar deuterado(s) o sustituidos con uno a 3 grupos elegidos entre halógeno o alquilo (C_1-C_6) lineal o ramificado,
- ◆ R_7 y R_7' representan independientemente el uno del otro un hidrógeno, un alquilo

(C₁-C₆) lineal o ramificado, un alquenilo (C₂-C₆) lineal o ramificado, un alquinilo (C₂-C₆) lineal o ramificado, un arilo o un heteroarilo, o bien R₇ y R₇' forman junto con el átomo de nitrógeno al que están unidos un heterociclo constituido por 5 a 7 eslabones,

siendo el compuesto de fórmula (I) tal que al menos uno de los átomos de carbono que lo constituyen está sustituido con uno de los grupos fosfatos siguientes: -OPO(OM)(OM'), -OPO(OM)(O⁻M₁⁺), -OPO(O⁻M₁⁺)(O⁻M₂⁺), -OPO(O⁻)(O⁻)M₃²⁺, -OPO(OM)(O[CH₂CH₂O]_nCH₃), o -OPO(O⁻M₁⁺)(O[CH₂CH₂O]_nCH₃), en los que M y M' representan independientemente el uno del otro un átomo de hidrógeno, un grupo alquilo (C₁-C₆) lineal o ramificado, un grupo alquenilo (C₂-C₆) lineal o ramificado, un grupo alquinilo (C₂-C₆) lineal o ramificado, un cicloalquilo o un heterocicloalquilo, los dos constituidos por 5 a 6 eslabones, mientras que M₁⁺ y M₂⁺ representan independientemente el uno del otro un catión monovalente farmacéuticamente aceptable, M₃²⁺ representa un catión divalente farmacéuticamente aceptable y n es un número entero comprendido entre 1 y 5,

entendiéndose que:

- por "arilo", se entiende un grupo fenilo, naftilo, bifenilo o indenilo,
- por "heteroarilo", se entiende cualquier grupo mono o bicíclico constituido por 5 a 10 eslabones, que posee al menos una parte aromática, y que contiene de 1 a 4 heteroátomos elegidos entre oxígeno, azufre o nitrógeno (incluyendo los nitrógenos cuaternarios),
- por "cicloalquilo", se entiende cualquier grupo carbocíclico no aromático, mono o bicíclico, que contiene 3 a 10 eslabones,
- por "heterocicloalquilo", se entiende cualquier grupo no aromático mono o bicíclico, fusionado o espiro, constituido por 3 a 10 eslabones, y que contiene de 1 a 3 heteroátomos elegidos entre oxígeno, azufre, SO, SO₂ o nitrógeno,

pudiendo estar sustituidos los grupos arilo, heteroarilo, cicloalquilo y heterocicloalquilo así definidos y los grupos alquilo, alquenilo, alquinilo, alcoxi, con 1 a 3 grupos elegidos entre alquilo (C₁-C₆) lineal o ramificado sustituido opcionalmente, espiro (C₃-C₆), alcoxi (C₁-C₆)

lineal o ramificado sustituido opcionalmente, alquil(C₁-C₆)-S-, hidroxilo, oxo (o N-óxido llegado el caso), nitro, ciano, -COOR', -OCOR', NR'R'', polihalógenoalquilo (C₁-C₆) lineal o ramificado, trifluorometoxi, alquil(C₁-C₆)sulfonilo, halógeno, arilo sustituido opcionalmente, heteroarilo, ariloxi, ariltio, cicloalquilo, heterocicloalquilo sustituido opcionalmente con uno o varios átomos de halógeno o grupos alquilo, entendiéndose que R' y R'' representan, independientemente el uno del otro, un átomo de hidrógeno o un grupo alquilo (C₁-C₆) lineal o ramificado sustituido opcionalmente,

pudiendo estar sustituido el grupo Het definido en la fórmula (I) con uno a tres grupos elegidos entre alquilo (C₁-C₆) lineal o ramificado, hidroxilo, alcoxi (C₁-C₆) lineal o ramificado, NR₁'R₁'', o halógeno, entendiéndose que R₁' y R₁'' tienen las mismas definiciones que los grupos R' y R'' mencionados anteriormente,

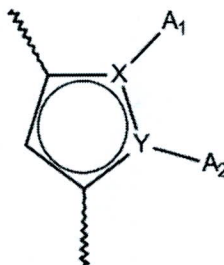
sus enantiómeros y diastereoisómeros, así como sus sales de adición a un ácido o a una base farmacéuticamente aceptable.

2. Compuesto de fórmula (I) según la reivindicación 1 en el que R₄ representa un fenilo sustituido en posición *para* con un grupo de fórmula -OPO(OM)(OM'), -OPO(OM)(O⁻M₁⁺), -OPO(O⁻M₁⁺)(O⁻M₂⁺), -OPO(O⁻)(O⁻)M₃²⁺, -OPO(OM)(O[CH₂CH₂O]_nCH₃), o -OPO(O⁻M₁⁺)(O[CH₂CH₂O]_nCH₃), en los que M y M' representan independientemente el uno del otro un átomo de hidrógeno, un grupo alquilo (C₁-C₆) lineal o ramificado, un grupo alqueno (C₂-C₆) lineal o ramificado, un grupo alquino (C₂-C₆) lineal o ramificado, un cicloalquilo o un heterocicloalquilo, los dos constituidos por 5 a 6 eslabones, mientras que M₁⁺ y M₂⁺ representan independientemente el uno del otro un catión monovalente farmacéuticamente aceptable, M₃²⁺ representa un catión divalente farmacéuticamente aceptable y n es un número entero comprendido entre 1 y 5, entendiéndose que el grupo fenilo puede estar sustituido opcionalmente con uno o varios átomos de halógeno.

3. Compuesto de fórmula (I) según la reivindicación 1 en el que R₄ representa un fenilo sustituido en posición *para* con un grupo de fórmula -OPO(O⁻Na⁺)(O⁻Na⁺).

4. Compuesto de fórmula (I) según una de las reivindicaciones 1 a 3 en el que X representa un átomo de carbono e Y representa un átomo de nitrógeno.

5. Compuesto de fórmula (I) según una de las reivindicaciones 1 a 3 en el que el grupo:



representa una 5,6,7,8-tetrahidroindolizina, una indolizina o un pirrol dimetilado.

6. Compuesto de fórmula (I) según una de las reivindicaciones 1 a 5 en el que T representa un grupo metilo, (morfolin-4-il)metilo ó 3-(morfolin-4-il)propilo.

7. Compuesto de fórmula (I) según una de las reivindicaciones 1 a 6 en el que R_a y R_d representan cada uno un átomo de hidrógeno y (R_b, R_c) forman junto con los átomos de carbono a los que están unidos un grupo 1,3-dioxolano, un grupo 1,4-dioxano, o bien R_a , R_c y R_d representan cada uno un átomo de hidrógeno y R_b representa un hidrógeno o un halógeno.

8. Compuesto de fórmula (I) según una de las reivindicaciones 1 a 6 en el que R_a y R_d representan cada uno un átomo de hidrógeno, R_b representa un átomo de halógeno y R_c un grupo metoxi.

9. Compuesto de fórmula (I) según una de las reivindicaciones 1 a 6 en el que R_a , R_b y R_d representan cada uno un átomo de hidrógeno y R_c representa un grupo $NR_7R_7'-CO-$ alquil(C_0-C_6)-O-.

10. Compuesto de fórmula (I) según una de las reivindicaciones 1 a 9 en el que R_3 representa un grupo elegido entre fenilo, 1H-indol, 1H-pirrolo[2,3-

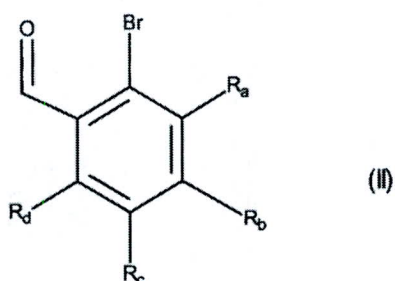
b]piridina, piridina, 1*H*-pirazol, 1*H*-pirrol, y 2,3-dihidro-1*H*-pirrolo[2,3-*b*]piridina, conteniendo estos grupos opcionalmente uno o varios sustituyentes elegidos entre alquilo (C₁-C₆) lineal o ramificado, ciano, o trideuteriometil.

11. Compuesto de fórmula (I) según la reivindicación 1 elegido en la lista siguiente:

- 4-[[[3-(6-[[[(3*S*)-3-(morfolin-4-ilmetil)-3,4-dihidroisoquinolin-2(1*H*)-il]carbonil]-1,3-benzodioxol-5-il)-5,6,7,8-tetrahidroindolizin-1-il]carbonil}(fenil)amino]fenil fosfato de disodio,
- 4-[[[5-(5-cloro-2-[[[(3*S*)-3-(morfolin-4-ilmetil)-3,4-dihidroisoquinolin-2(1*H*)-il]carbonil}fenil)-1,2-dimetil-1*H*-pirrol-3-il]carbonil}(piridin-4-il)amino]fenil fosfato de disodio,
- 4-([5-(5-cloro-2-[[[(3*S*)-3-(morfolin-4-ilmetil)-3,4-dihidroisoquinolin-2(1*H*)-il]carbonil}fenil)-1,2-dimetil-1*H*-pirrol-3-il]carbonil}[1-(trideuteriometil)-1*H*-pirazol-4-il]amino]fenil fosfato de disodio,
- 4-[[[5-(5-cloro-2-[[[(3*S*)-3-(morfolin-4-ilmetil)-3,4-dihidroisoquinolin-2(1*H*)-il]carbonil}fenil)-1,2-dimetil-1*H*-pirrol-3-il]carbonil}(5-ciano-1,2-dimetil-1*H*-pirrol-3-il)amino]fenil fosfato de disodio,
- 4-[[[5-(5-cloro-2-[[[(3*S*)-3-(morfolin-4-ilmetil)-3,4-dihidroisoquinolin-2(1*H*)-il]carbonil}fenil)-1,2-dimetil-1*H*-pirrol-3-il]carbonil}(5-ciano-1-metil-1*H*-pirrol-3-il)amino]fenil fosfato de disodio,
- 4-[[[5-(5-cloro-2-[[[(3*S*)-3-(morfolin-4-ilmetil)-3,4-dihidroisoquinolin-2(1*H*)-il]carbonil}fenil)-1,2-dimetil-1*H*-pirrol-3-il]carbonil}(1-metil-1*H*-pirazol-4-il)amino]fenil fosfato de disodio,
- 4-[(5-ciano-1,2-dimetil-1*H*-pirrol-3-il){[5-(5-fluoro-2-[[[(3*S*)-3-(morfolin-4-ilmetil)-3,4-dihidroisoquinolin-2(1*H*)-il]carbonil}fenil)-1,2-dimetil-1*H*-pirrol-3-il]carbonil}amino]fenil fosfato de disodio,
- 4-[[[5-(5-fluoro-2-[[[(3*S*)-3-(morfolin-4-ilmetil)-3,4-dihidroisoquinolin-2(1*H*)-il]carbonil}fenil)-1,2-dimetil-1*H*-pirrol-3-il]carbonil}(1-metil-1*H*-pirazol-4-il)amino]fenil fosfato de disodio,

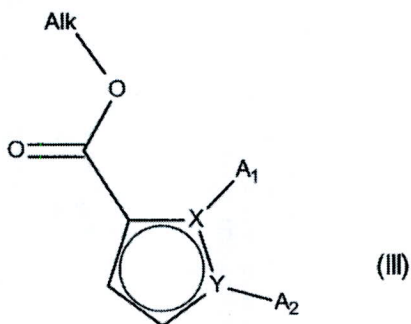
sus enantiómeros y diastereoisómeros, así como sus sales de adición a un ácido o a una base farmacéuticamente aceptable.

12. Procedimiento de preparación de los compuestos de fórmula (I) según la reivindicación 1 caracterizado porque se utiliza como producto de partida el compuesto de fórmula (II):



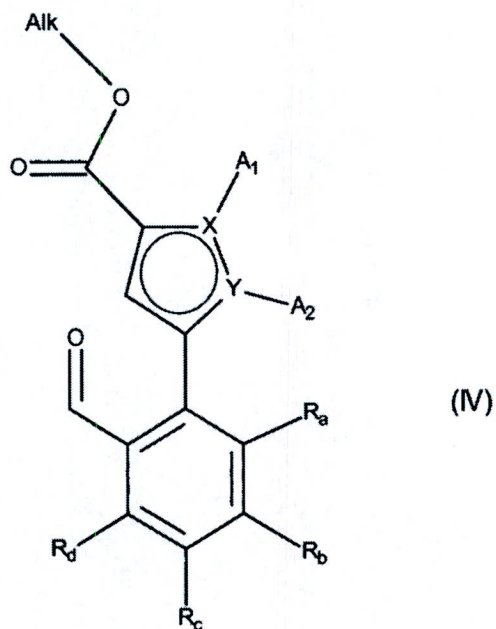
en la que R_a , R_b , R_c y R_d son tales como se han definido en la reivindicación 1,

compuesto de fórmula (II) que se somete a una reacción de Heck, en medio acuoso u orgánico, en presencia de un catalizador de paladio, de una base, de una fosfina y del compuesto de fórmula (III):



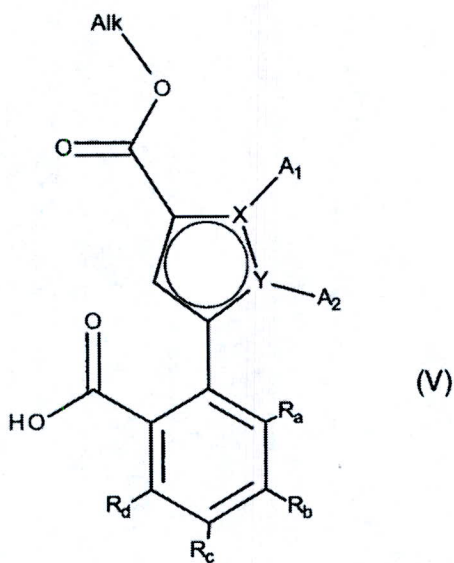
en la que los grupos A_1 , A_2 , X e Y son tales como se han definido en la reivindicación 1 y Alk representa un alquilo (C_1 - C_6) lineal o ramificado,

para obtener el compuesto de fórmula (IV):



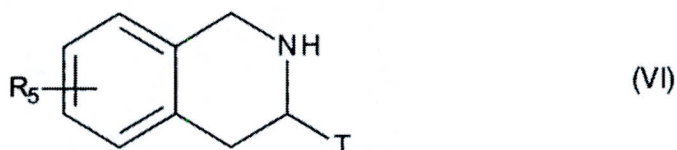
en la que A_1 , A_2 , X , Y , R_a , R_b , R_c y R_d son tales como se han definido en la reivindicación 1 y Alk es tal como se ha definido anteriormente,

compuesto de fórmula (IV) cuya función aldehído se oxida en ácido carboxílico para formar el compuesto de fórmula (V):



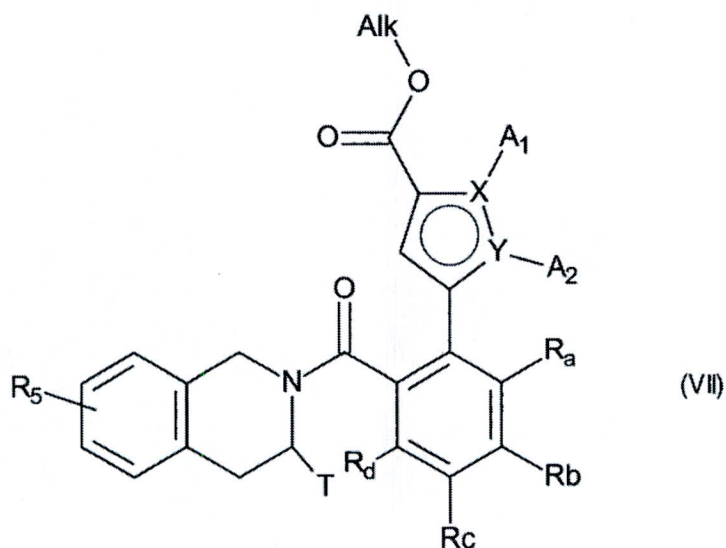
en la que A_1 , A_2 , X , Y , R_a , R_b , R_c y R_d son tales como se han definido en la reivindicación 1 y Alk es tal como se ha definido anteriormente,

compuesto de fórmula (V) que experimenta un acoplamiento peptídico con un compuesto de fórmula (VI):



en la que T y R₅ son tales como se han definido en la reivindicación 1,

para dar lugar al compuesto de fórmula (VII):



en la que A₁, A₂, X, Y, R_a, R_b, R_c, R_d, T y R₅ son tales como se han definido en la reivindicación 1 y Alk es tal como se ha definido anteriormente,

compuesto de fórmula (VII) cuya función éster se hidroliza para dar lugar al ácido carboxílico o al carboxilato correspondiente, el cual puede convertirse en un derivado de ácido tal como el cloruro de acilo o el anhídrido correspondiente, antes de acoplarse con una amina NHR₃R₄, en la que R₃ y R₄ tienen el mismo significado que en la reivindicación 1, antes de someterse a la acción de un derivado pirofosfato, fosfonato o fosforilo en condiciones básicas, pudiendo hidrolizarse o hidrogenolizarse opcionalmente el compuesto así obtenido, para dar lugar al compuesto de fórmula (I),

compuesto de fórmula (I) que puede purificarse según una técnica clásica de separación, que se transforma, si se desea en sus sales de adición a un ácido o a una base farmacéuticamente aceptable y del que se separan opcionalmente los isómeros según una técnica clásica de separación,

entendiéndose que en cualquier momento que se juzgue oportuno en el curso del procedimiento descrito anteriormente, los grupos hidroxilo y amino de los reactivos o intermediarios de síntesis pueden ser protegidos y luego desprotegidos de acuerdo a los requisitos de la síntesis.

13. Composición farmacéutica que contiene un compuesto de fórmula (I) según una cualquiera de las reivindicaciones 1 a 11 o una de sus sales de adición con un ácido o una base farmacéuticamente aceptable en combinación con uno o varios excipientes farmacéuticamente aceptables.

14. Combinación farmacéutica de un compuesto de fórmula (I) según cualquiera de las reivindicaciones 1 a 11 con un agente anticanceroso elegido entre los agentes genotóxicos, los venenos mitóticos, los anti-metabolitos, los inhibidores del proteasoma, los inhibidores de quinasas o los anticuerpos.

15. Composición farmacéutica que contiene una combinación farmacéutica según la reivindicación 14 en combinación con uno o varios excipientes farmacéuticamente aceptables.

Preclinical Modeling of Combination Treatments: Fantasy or Requirement?

SHANNON DECKER AND EDWARD A. SAUSVILLE

*University of Maryland Marlene and Stewart Greenebaum Cancer Center,
Baltimore, Maryland 21201, USA*

ABSTRACT: Combination chemotherapy is considered the “norm” in cancer chemotherapy. While the development of classical combination regimens took into account factors such as single-agent activity, different toxicity profiles, and advantageous pharmacology of the component drugs, in the era of targeted therapies there are additional considerations such as new mechanisms of resistance due to modulation of pathway dependence. Since it is not feasible to test all of the possible combinations clinically, some method for preclinically identifying and prioritizing promising combinations is necessary. While *in vivo* animal models can be used for safety testing and some pharmacokinetics, even they can quickly become prohibitively resource intensive for the purpose of efficacy determinations. Therefore, factors such as biologic rationale, reliable *in vitro* results in more than one tumor type, achievable *in vivo* pharmacology, and selectivity of primary tumor cell activity become important in the evaluation of potential combination regimens.

KEYWORDS: combination chemotherapy; cancer; synergy; preclinical models

COMBINATIONS ARE THE “NORM” IN CANCER CHEMOTHERAPY

In cancer treatment, combination chemotherapy has been found more effective than single-drug regimens and, in fact, is accepted as the “norm.” Combination therapies have been the basis of the major success stories in the treatment of various cancers. Initial treatment for acute lymphoblastic leukemia (ALL) usually consists of a regimen such as vincristine, prednisone, and anthracycline with or without asparaginase, resulting in complete remissions in a large majority of cases. More than 75% of all newly diagnosed patients with adult Hodgkin’s lymphoma can be cured with combination chemotherapy and/or radiation therapy. One of the most effective and least toxic chemotherapy regimens for this disease is known as ABVD and consists of a combination of adriamycin, bleomycin, vinblastine, and dacarbazine. In lung cancer, the combination of carboplatin and paclitaxel is clinically useful. Even in epithelial cancers, such as testis or adjuvant therapies for breast or colon cancers, combination therapies are standard.

Address for correspondence: Edward A. Sausville, University of Maryland Marlene and Stewart Greenebaum Cancer Center, 22 S. Greene Street, Baltimore, MD 21201. Voice: 410-328-7394; fax: 410-328-6896.
esausville@umm.edu

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One empirical basis for understanding the success of certain combination therapies is that they represent pharmacologically tolerable combinations of drugs that affect the same target but that have often been selected due to different "large" organ toxicities.¹ Three of the four drugs in the ABVD regimen target DNA. While three of the four in the regimen cause decreases in white blood cells and platelets, the non-hematologic toxicities vary. Adriamycin produces cardiac toxicity, vinblastine causes neurotoxicity, and bleomycin has pulmonary side effects.

A different view of how to produce useful combination regimens suggests that in addition to having different spectra of toxicity, preferably with minimal cross resistance, other important criteria are that each of the drugs have proven efficacy as single agents, and they actually have different mechanisms of action in eliciting an anti-proliferative effect.² Ideally, the drugs should be administered at the maximum tolerated dose for the shortest time compatible with normal cell compartment recovery. In the lung cancer regimen mentioned above, both carboplatin and paclitaxel have some single-agent activity, with carboplatin targeting DNA and paclitaxel acting on tubulin. Both drugs lower white blood cell counts and platelets, with paclitaxel having neurotoxicity as an additional side effect.

TARGETED THERAPY AND COMBINATION DESIGN

In addition to empirical approaches to combination design, the development of targeted therapies has created the possibility for a "rational" basis for constructing combinations where the drugs are selected to affect different targets and/or circumvent resistance. For example, the use of combinations of reverse transcriptase inhibitors and protease inhibitors is the standard of care in the treatment of AIDS and seems to prevent the facile development of mutant resistant strains of the HIV virus. In this case, two distinct targets—each crucial to creating a successful viral replication cycle—convey a profound antiviral effect when coincidentally inhibited. An analogous strategy is used in the treatment of tuberculosis, which rapidly becomes resistant to monotherapy.

This rational approach to combination design requires consideration of the targets and pathways involved. For instance, one can take into account whether a linear or concentric model of target action applies (FIG. 1). In the classic linear model with a single pathway affected, a series of drugs each acting on its own component of that linear pathway could be imagined as conveying benefit when used in combination. Alternatively and somewhat more likely, the tumor could exist in a "super state" where there are multiple pathways that all contribute to tumor survival and growth. One could then design a combination drug strategy that affects each of the targets contributing to the super state.

Another method for approaching targets and pathways in the context of combination design is to consider what has been described as the six essential alterations in cell physiology in malignancy: evasion of apoptosis; self-sufficiency in growth signals; insensitivity to anti-growth signals; sustained angiogenesis; limitless replicative potential; and capacity for tissue invasion and metastasis.³ The first three of these alterations can be thought of as the targets of more classical cancer chemotherapy agents, while the latter three are the focus of intense interest for the development of many new targeted therapies. Acting on the more "classical" targets, such as DNA

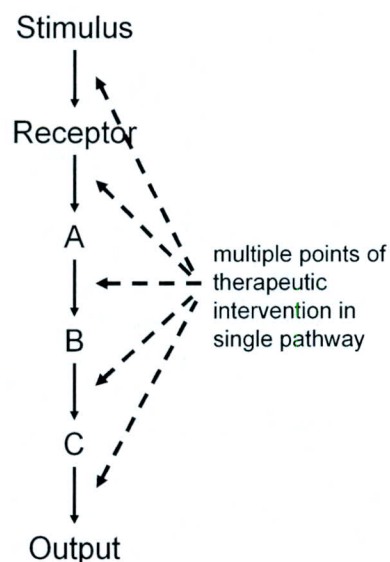
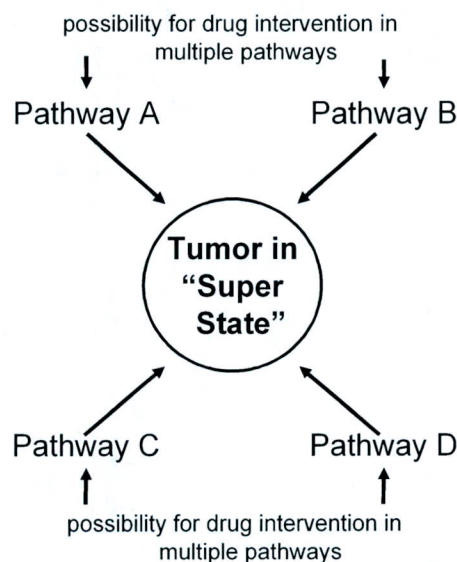
A. Linear**B. Concentric**

FIGURE 1. Models of target action. (A) In a classic linear model, a single pathway needs to be affected by candidate agents. (B) In a concentric model, multiple pathways contribute to tumor survival and growth.

or tubulin, is in essence a means of evoking a "stress response" leading to the induction of apoptosis. For DNA-interactive agents, this could mean affecting the p53 checkpoint response or p53-induced genes, while for a tubulin-targeted drug the stress evokes a mitotic checkpoint response. Useful targeted agents will also evoke a stress response depending on how important the drug's target is in sustaining an anti-apoptotic influence.

While minimal cross resistance was a desired characteristic of a classical combination regimen, inclusion of targeted therapies in combination regimens offers potential additional avenues of resistance. In addition to the classical mechanisms of drug resistance related to the drug itself, such as efflux, metabolism, and target expression, another means of resistance that arises with targeted agents is through the modulation of pathway dependence. In the super state example in FIGURE 1, if a drug affecting only pathway A were used, the tumor could alter its growth and survival so as to exist without pathway A, rendering that drug alone ineffective. This was actually recently illustrated by the development of resistance to A431 squamous cell carcinoma xenografts to anti-epidermal growth factor receptor (EGFR) monoclonal antibodies for two cycles.⁴ A431 cell variants were developed from recurring resistant tumors which grew faster and were clearly less responsive to the anti-EGFR antibodies than the parental line. The investigators looked at the relative expression

17

levels of a number of different targets in these variants, including relative VEGF expression. In five of the six resistant cell variants, VEGF expression levels were elevated relative to the parent line. These results suggest that, at least in this example, acquired resistance can develop to a targeted therapy and can proceed, in part, by selecting advantageous tumor subpopulations which upregulate a different, parallel growth-modulating pathway, in this case modifying the tumor stroma. Resistance does not appear therefore to have arisen in relation to the drug-affected pathway, but with another pathway having been called into play.

Taking into account target, pathway, and resistance issues, one could imagine designing a combination of targeted agents for the treatment of bulk tumor that consisted of an anti-proliferative, a pro-apoptotic, and an anti-vascular therapy. A recent clinical trial of an anti-vascular agent with an anti-proliferative regimen demonstrated that this sort of combination could be beneficial in a patient population.⁵ The trial placed patients with previously untreated metastatic colorectal carcinoma into one of three groups: bolus IFL (5-fluorouracil, leucovorin, and irinotecan) + placebo; bolus IFL + the anti-vascular antibody bevacizumab; or 5-fluorouracil, leucovorin, and bevacizumab. The overall response rate for IFL + placebo was 34.7% with a duration of 7.1 months, while IFL + bevacizumab was 44.9% with a duration of 10.4 months ($P = 0.029$). Improvement was also seen in progression-free survival, 6.2 months for IFL + placebo versus 10.6 months for IFL + bevacizumab ($P < 0.00001$).

Other clinical results from the combination of classical regimens with targeted agents include those with the RAF-kinase and VEGFR inhibitor BAY43-9006. In a phase II randomized discontinuation trial in a variety of advanced solid tumors, there were no complete responses to the single agent, but partial responses in renal (35%), sarcoma (10%), thyroid (40%), and melanoma (2%).⁶ A phase II trial in melanoma of BAY-43-9006 with carboplatin and paclitaxel, however, achieved 40% PR and 43% stable disease.⁷ Taken together, these data raise the possibility of modulation of sensitivity *in vivo* to platinum by BAY43-9006. One potential mechanism is better delivery of the drug because of the effects of the anti-vascular compound on interstitial fluid pressure.⁸

In contrast to a classical combination regimen where each component of the regimen had some level of activity as a single agent, the possibility exists that the targeted agents may be relatively to completely inactive as single agents. Another design strategy therefore is to combine two or more anti-signaling molecules that alone may not be toxic, but in combination they affect two or more critical pathways or have an augmented effect on one pathway. For preventive therapy, a strategy that combines an anti-mutator, an anti-telomerase, and an anti-invasive could be envisioned.

PRECLINICAL TESTING OF COMBINATIONS

Clinical trials of combination regimens are extremely expensive and time-consuming. Furthermore, the number of potential combinations is virtually unlimited. So the question is whether it is possible to determine preclinically which combinations will be effective. In April 2003, the U.S. National Cancer Institute Developmental Therapeutics Program (NCI DTP) held a workshop on defining the

17

efficacy of combinations.⁹ Some of the goals of the workshop were to attempt to determine what preclinical data would represent convincing evidence that a combination should be tested clinically, and how best to prioritize the possibilities. The outcome of the workshop suggested that one should utilize the *in vitro* data to prioritize for those regimens clearly showing synergy as opposed to mere lack of antagonism. *In vitro* data should represent more than one tumor type. There should be a biologic rationale for the combination. Ideally, evidence should exist for greater activity against tumor cells as opposed to normal cells. *In vivo* tumor efficacy of the combination was not considered necessary, although obviously a plus if present. If possible, clear evidence of likely safety in the *in vivo* models should be demonstrated. Pharmacokinetics, however, should be utilized from early human studies, where available, to model regimens that approximate non-antagonistic interactions *in vitro*. The concentrations studied *in vitro* should clearly be those that are relevant clinically.

SYNERGY

Given the enormous number of possible combinations of drugs, the idea of prioritizing synergistic combinations and not selecting antagonistic ones is obviously desirable. Synergism in fact is almost critical to define in those situations where the single agents are relatively ineffective on their own. Defining synergy, however, is no simple matter, and a variety of techniques for doing so has been covered in great detail elsewhere.¹⁰

One of the more straightforward methods for determining synergy is known as fractional effect analysis, where the effect of the drugs alone is multiplied and compared with the effect of the combination at the same drug concentrations. Although simple, it has the significant limitation of assuming a linear concentration–effect relationship for each agent as well as the combination.

The isobologram representation is one of the most commonly used methods for calculating synergy. Concentration–effect curves are calculated for each drug alone and for the effect of a variable concentration of one drug in the presence of a fixed concentration of the other. The “apparent” IC_{50} (50% inhibitory concentration) of the combination is then plotted, representing expected additivity. If the observed effect of the combination falls to the left of the expected curve, there is synergy and to the right, antagonism. In addition to being a very labor-intensive method that does not allow quantitation of synergism, this method also occasionally produces sigmoidal observed curves that lie on both sides of the expected curve. Although there are complicated three-dimensional response surface approaches that overcome some of the limitations of isobolograms, their complexity often dictates against them.

The well-known Chou and Talalay median-drug effect analysis is a somewhat more accessible method to overcome the sigmoidicity problem found with isobolograms.¹¹ The drug mixture is at constant concentration ratios so that the relative effects of each drug in the mixture are identical. The growth inhibitory effect of each drug, the sigmoidicity of the curves, and an estimated IC_{50} are used to calculate a combination index (CI) that serves as a quantitative measure of synergism. This method has the advantage of allowing evaluation of drug combinations where the drugs interact in the same pathway or in completely separate pathways.

PROBLEMS IN DESIGNING SIGNALING COMBINATIONS

Definition of synergy is by no means the only issue that remains unresolved in the design of signaling combinations in cancer treatment. We do not know yet how to diagnose the "mixture" of pathways acting in a tumor, and what would be a basis for selecting certain combination regimen drug members. Also unknown is the best time in the natural history of the tumor for intervention with agents affecting combined pathways, whether "up front" or after a certain sequence of standard and investigational therapies. It would also be useful to know whether there are any qualitative differences in the results that are achievable between certain drug types, such as antibodies versus small molecules or between different types of small molecules. If so, why and how can they be exploited?

COMBINATIONS OF KINASE-DIRECTED AGENTS

A look at any of a number of schematic illustrations of the principal pathways involved for some of the new kinase-directed agents in development reveals the complexity of attempting to select the best possible combinations. Among the combinations of new agents that have been tried preclinically are the cyclin-dependent kinase (CDK) inhibitor flavopiridol with the erbB receptor-directed monoclonal antibody trastuzumab. Flavopiridol has been shown to inhibit CDK4 activity after cyclin D1 expression in MCF7 breast cancer cells.¹² On Western blots, cyclin D1 starts to diminish after 6 h of exposure to flavopiridol and is almost completely gone by 10 h. CDK4 activity follows slightly behind, beginning to diminish at 8 h. Effects on steady-state mRNA levels after treatment with 300 nM flavopiridol show a similar time course. Cyclin D1 is downstream of erbB2, and Nahta *et al.* demonstrated that cyclin D1 functions are essential and rate limiting for erbB2 transformation.¹³ These results suggested the combination of drugs active against erbB2 and cyclin D1 (trastuzumab and flavopiridol). They found that these two drugs were synergistically cytotoxic against erbB2-positive breast cancer cell lines and that adding flavopiridol to trastuzumab synergistically lowered erbB2 levels in these cells.

The PDK1-Akt survival pathway (FIG. 2) has also been the focus of numerous drug discovery efforts. The alkylphospholipid perifosine, which is currently in clinical trials, causes almost complete loss of Akt phosphorylation on at least two key sites.¹⁴ Perifosine was further shown to decrease plasma membrane localization of Akt at approximately 5 μ M. In patients, a loading dose followed by maintenance dosing produces approximately these levels.^{16,17} UCN-01 produced this effect by inhibiting the upstream kinase, PDK1. The combination of perifosine and UCN-01 was tested *in vitro* because these two drugs both produce loss of phosphorylation of Akt, but through different mechanisms.¹⁸ Using the Chou and Talalay method, this combination was shown to be moderately synergistic. Further, cell cycle analysis of the effect of the combination showed an increase in the percentage of cells arresting in G0/G1, 87% for the combination versus 51% for controls, 52% for perifosine-treated, and 69% for UCN-01-treated.

UCN-01 was also tested *in vitro* in combination with the geldanamycin analogue 17-AAG, an antagonist of the molecular chaperone hsp90. Synergistic induction of apoptosis and loss of mitochondrial membrane potential was observed in leukemia

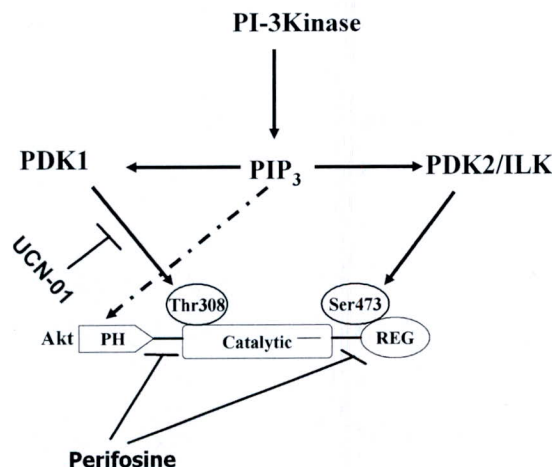


FIGURE 2. The PDK1-Akt survival pathway. Points of action of perifosine and UCN-01 are shown. Numerous drug discovery efforts not reviewed here have focused on various other targets in the pathway.

cells.¹⁹ Explication of the basis of this synergy indicated that 17-AAG potentiates UCN-01 cytotoxicity and that interference with both the Akt and Raf-1/MEK/MAP kinase cytoprotective signaling pathways contributes to this effect.

ANIMAL MODELS AND COMBINATIONS

While the above examples all demonstrate synergy *in vitro*, the question often arises whether *in vitro* combination data are sufficient to justify moving forward to human clinical trials. There are, however, a number of problems with using animal models for the assessment of combinations. An appropriately powered animal experiment with untreated controls, vehicle controls, a Drug A alone group, a Drug B alone group, and a combination group easily exceeds over 100 animals. Logistical and cost issues aside, the pharmacology in mice, or whatever species is desired, must approximate the pharmacology in humans, and even then it will be difficult to predict the pharmacokinetic interactions. One must either control for or actively design pharmacology into the study. Finally, the results can easily show not only additive or synergistic efficacy, but also additive or synergistic toxicity that may or may not be relevant to the human experience.

Animal models have both logistical and technical limitations; therefore, in attempting to define a "roadmap" to the clinic for new combination therapies, the guidelines emerging from the NCI DTP workshop on prioritizing on synergy, biologic rationale, selectivity, likely safety, and favorable pharmacokinetics can serve as an initial series of filters to weed out combinations that do not merit animal or clinical testing. Given that combination therapies have always been a major basis of success in the treatment of cancer and that the nature of the new generations of drugs

that is emerging makes it even less likely that a monotherapy will suffice, it is hoped that what will materialize after applying a series of appropriate *in vitro* filters will be a select and manageable number of combination designs for more advanced testing in a clinical setting.

[COMPETING INTERESTS: Dr. Edward A. Sausville is on the Scientific Advisory Board for Kosan Biosciences and is a consultant for Kosan Biosciences and Keryx Pharmaceuticals. The author declares a competing financial interest.]

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explored by comparing the rate of a process — such as the rate at which a particle will decay into other, lighter particles — with the rate of its CP-related process, in which all the initial and final particles are replaced with the corresponding antiparticles. If these rates are measured to be different, then a ‘CP asymmetry’ has been observed. Measurements of CP asymmetries are interesting for two reasons. First, they provide stringent tests of the standard model of particle physics. Second, they may provide hints that could point scientists in the direction of a solution to the mystery of the Universe’s matter–antimatter imbalance.

Among the four known forces of nature — the standard model’s weak, strong and electromagnetic forces, and the gravitational force — the weak force is the only one that distinguishes between matter and antimatter. But even the weak force would not do so if it were not that three quark particles exist for each of two charge types: the down, strange and bottom quarks, which have a charge that is minus one-third of the proton’s charge; and the up, charm and top quarks, which have a charge that is two-thirds of the proton’s charge. With three quarks of either charge, the weak force has a single coupling constant (a parameter that quantifies the force’s strength), which is different for particles and antiparticles^{2,3}. If, as the standard model predicts, all CP asymmetries are proportional to this single coupling constant, then the sizes of these asymmetries are correlated. By contrast, most theories that go beyond the standard model have many independent coupling constants that distinguish particles from antiparticles, and thus predict deviations from the standard-model correlations.

Mesons are bound states of one quark and one antiquark. Until the LHCb measurement was made, CP asymmetries had been observed in the decays of three types of meson: K^0 mesons, B^0 mesons, and $B^\pm$ mesons. The LHCb experiment is the first to measure CP asymmetry in the decay of a fourth type of meson, the B_s^0 meson. The fact that, within the standard model, the difference in the laws of physics followed by matter and antimatter is encoded in a single parameter provides a particularly strong correlation between this asymmetry and an asymmetry in B^0 decay that has been measured by several experiments with high accuracy. Although neither of these asymmetries, nor the corresponding decay rates, can be theoretically predicted with any accuracy, the product of the CP asymmetry with the decay rate should, according to the standard model, be equal (to a good approximation) between the B_s^0 decay and the B^0 decay^{4,5}. The LHCb measurement is consistent with the predicted equality at the level of a few per cent, implying yet another triumph of the standard model.

In many extensions of the standard model, new particles are predicted to interact more strongly with the heavier quarks (strange, bottom, charm and top) than with the lighter ones

(up and down). The K^0 mesons, the B^0 mesons and the $B^\pm$ mesons all have either a down (anti)quark or an up (anti)quark. But the B_s^0 mesons are different: they are made up of a strange quark and a bottom antiquark. Their antiparticles are composed of a bottom quark and a strange antiquark. Thus, scientists had hoped that although the effects of new physics were negligibly small in all previously measured CP asymmetries, they would be large enough to be observed in B_s^0 decays. Frustratingly, this seems not to be the case.

“The LHCb measurement reinforces the standard model’s explanation of how the weak force distinguishes matter from antimatter.”

However, the standard model fails to explain the Universe’s matter–antimatter imbalance. All structures in the Universe, from clusters of galaxies to human cells, are made of matter: protons, neutrons and electrons. Their antiparticles — the antiprotons, antineutrons and positrons — are not found in the Universe at large. If the laws of nature were identical for matter and antimatter, then particles and antiparticles would have been created in equal amounts and would then have annihilated each other,

leaving only pure radiation and no matter structures in the Universe. Our very existence is possible only because of CP asymmetries. The standard model allows all antimatter to disappear from the Universe, but it predicts that the amount of surviving matter is many orders of magnitude smaller than observed.

Therefore, there must exist a force, as yet unknown to us, that distinguishes matter from antimatter in a way that is much stronger than that of the weak force. Theorists have made various suggestions as to what this new force might be. To disclose the nature of this force, further hints from experiments are needed. Searching for CP asymmetries in neutrino ‘oscillations’ of one type into another and for the electric-dipole moments of the neutron and the electron, in addition to measuring CP asymmetries in B_s^0 -meson decays, seem the most promising avenues through which to obtain such hints. The consistency of the LHCb measurement with the standard model provides further motivation to pursue the other searches even more vigorously. ■

Yosef Nir is in the Department of Particle Physics, Weizmann Institute of Science, Rehovot 76100, Israel.
e-mail: yosef.nir@weizmann.ac.il

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CANCER

Calculated treatment

Mathematical modelling linked with patient data suggests that combination therapy is more effective than sequential treatment at preventing drug resistance in cancer. This predictive approach may pave the way for personalized therapies.

NATALIA L. KOMAROVA
& C. RICHARD BOLAND

In a study published in *eLIFE*, Bozic *et al.*¹ use a mathematical approach to examine tumour evolution and response to chemotherapy. In one example, they describe a patient who had the skin cancer melanoma characterized by an estimated tumour burden of 9.8×10^{10} cells and 8 metastatic lesions. Their modelling predicts a 0% chance of disease control using a single drug, but that the likelihood of successful treatment could rise to 88% during combined therapy with two drugs. The approach offers a brave, quantitative look at designing targeted therapy for cancer.

The search for cancer treatments has traversed a long and thorny path, with more

failures and disappointments than glimpses of success. A major breakthrough was achieved with the development of a drug called imatinib in the 1990s. This inhibitor of tyrosine kinase enzymes showed breathtaking success for treating chronic myelogenous leukaemia (CML). Imatinib and other small-molecule inhibitors ‘recognize’ and attack cancer cells, but spare normal cells, thereby reducing side effects compared with conventional chemotherapy. Since the discovery of imatinib, dozens of other such inhibitors have been developed for treating different cancers. However, the initial excitement surrounding these drugs was tempered by the appearance of drug resistance — the phenomenon in which disease returns a few months after initial treatment success².

Cancer is a process of Darwinian evolution played out in a particular organ. Normal cells divide and die, and each division brings a small chance of genetic change. Most such mutations are deleterious, and the cells die without leaving offspring, but some confer new properties that promote growth or survival and can lead to cancer. The greatest challenge for drugs targeted at such cells is the further evolution of mutations that confer drug resistance. Combining multiple drugs that have distinct mechanisms of action might provide a solution to this problem. The concept of a 'drug cocktail' was introduced in 1996 in the context of treating AIDS. There, the emergence of viral strains resistant to single drug treatments rendered all previous attempts to control the disease unsuccessful. Viral evolution in a patient is conceptually similar to the evolution of cancer cells, so similar treatment strategies might work well for both diseases.

Current common practice for treating cancers with small-molecule inhibitors is to administer the agents sequentially, starting with a 'first-line' drug and switching to 'second-line' therapies if the tumour relapses. Bozic *et al.* assessed the effectiveness of this approach using sophisticated mathematical techniques and data from patients with melanoma or with pancreatic or colorectal cancers. They convincingly demonstrate that a sequential strategy "precludes any chance for cure", even in the best-case scenario in which no single mutants confer resistance to both drugs. However, they show that simultaneously combining two or more drugs can provide much-needed hope for patients.

As demonstrated last year³, drug-resistant mutants typically exist at low levels in tumours before the beginning of treatment. Treatment with a single drug gives a competitive advantage to mutants resistant to that drug, such that by the time of the switch to a second-line therapy, there is a high chance that a mutant that is also resistant to the second drug (a doubly resistant mutant) has already emerged (Fig. 1). But combination therapy eliminates cells that are singly resistant to either drug and therefore — because the likelihood of a doubly mutated cell emerging in such a population is low — greatly increases the chance of success.

The greatest obstacle for combination treatments is the phenomenon of cross-resistance, in which a single mutation confers resistance to more than one drug. But even if such mutants are generated, the authors estimate that combination treatment can be beneficial in some cases, whereas single-drug and sequential strategies offer no hope.

Previous mathematical analyses have also shown⁴ that cyclic treatments are ineffective compared with combination treatments, and have led to the proposal⁵ that a combination of three anticancer drugs would be needed to treat CML. It has also already been argued⁶ that, even in the presence of cross-resistant

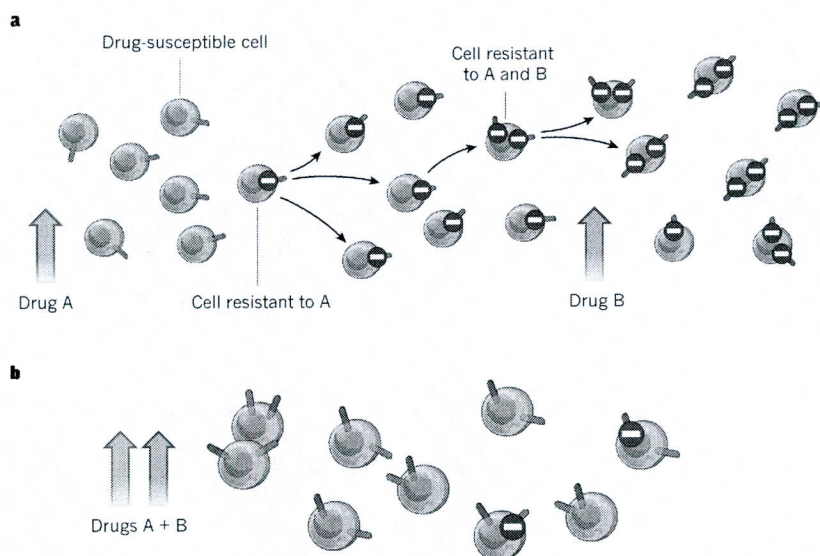


Figure 1 | Single-drug versus combination therapy. **a**, During therapy with one drug alone, a cell that acquires a mutation that confers resistance to the drug will be at a proliferative advantage. By the time this is recognized and treatment with a second drug is started, it is likely that a cell resistant to both drugs will already have emerged. **b**, Starting therapy with both drugs simultaneously means that cells acquiring single resistance will be immediately eliminated by the other drug. Bozic *et al.*¹ use mathematical modelling to show that this approach increases the chance of effective treatment.

mutations in CML, combination treatments give patients a better chance of cure than do single-drug treatments. And *in vitro* studies⁷ that compared CML cells treated with one small-molecule inhibitor with those treated with a combination of two or three demonstrated that the combined therapy suppressed cell proliferation more effectively. But with Bozic and colleagues' success in synthesizing theoretical and experimental methods and applying the analysis to solid tumours, these modelling studies have taken a leap forward.

Even more significantly, the authors' paper outlines a roadmap for future personalized therapies, by showing that specific parameters for a patient can be measured and used in a mathematical model to calculate the probability of treatment success and to design the best possible treatment strategy. The authors extracted tumour parameters — including its size at presentation, cell division and cell death rates, and changes in the associated kinetic parameters following treatment — from 20 patients with melanoma who were treated with the small-molecule inhibitor vemurafenib. With this information, they were able to predict the most likely outcome of single, dual and triple therapies for each patient.

There is a bright future for this approach. As new drugs and more information on the exact mechanisms of drug action become available, the model can be iteratively improved. For example, there is currently a strong research focus on cellular plasticity, the heterogeneity of cells within a tumour and the role of

cancer stem cells. But it is not known how the presence of cancer cells with differing properties affects a tumour's susceptibility to targeted treatments. Moreover, the evolutionary costs of resistance for a cell have not been quantified in most cases, nor have mutation rates for molecular changes of various kinds, although estimates have been made for the number of mutations conferring resistance to certain drugs in CML^{7,8}. The potential complications of drug cocktails — including toxicity and undesirable drug interactions — must also be taken into account. But the overall message is loud and clear: mathematics can help to calculate treatment strategies, and the best hope so far lies in combination therapies. ■

Natalia L. Komarova is in the Departments of Mathematics and Ecology and Evolutionary Biology, University of California Irvine, Irvine, California 92697, USA. **C. Richard Boland** is in the Department of Gastroenterology, Baylor University Medical Center, Dallas 75246, Texas, USA.
e-mail: komarova@uci.edu

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