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As. 122.923

Señor

**DIRECTOR DIVISIÓN DE NUEVAS CREACIONES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO**

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REF: Expediente No. **13 -001.846-** solicitud de patente de invención a favor de **ABRAXIS BIOSCIENCE, LLC**. Título: **MÉTODOS DE TERAPIA COMBINADA PARA TRATAR ENFERMEDADES PROFILERATIVAS.**

RECURSO DE REPOSICIÓN

JUAN PABLO CADENA SARMIENTO, portador de la tarjeta profesional No. 57.492 del C.S.J., obrando como apoderado de la sociedad solicitante, manifiesto a usted que debidamente notificado de la Resolución No. 72888 del 1º de Diciembre de 2014, interpongo contra ella en debido término, **RECURSO DE REPOSICIÓN**, para que sea revocada en su totalidad, y en su lugar se conceda el Privilegio de Patente, para el objeto de la solicitud en referencia.

Respaldo el RECURSO en los siguientes

HECHOS

1. La resolución No. 72888 arriba citada niega el Privilegio exclusivo de Patente de Invención para el objeto de la solicitud de la referencia, considerando que el capítulo reivindicatorio carece de nivel inventivo. Lo anterior, de acuerdo con las siguientes consideraciones.

i. Determinación del Estado de la Técnica

Consultadas las fuentes de información con que se cuenta se encontraron los siguientes documentos, que anticipan el objeto de esta solicitud.

No	Documento	Título	Fecha de publicación
D1	WO2006089290	<i>“Combinations and modes of administration of therapeutic agents and combination therapy”</i>	24/Agosto/2006
D8	ClinicalTrials.gov Identifier: NCT00842582	<i>“Azacitidine With Carboplatin and Paclitaxel for Newly Diagnosed Ovarian Cancer”</i>	11/febrero/2009
D7	Gene Ther Mol Biol Vol 12, 343-387, 2008	<i>“Microtubule-targeted antitumor drugs: chemistry, mechanisms and nanoparticle formulations”</i>	19/Diciembre/2008
D9	Mol Cell Biochem (2010) 342:101–109	<i>“Demethylating agent 5-aza-2-deoxycytidine enhances susceptibility of breast cancer cells to anticancer agents”</i>	9/Mayo/2010

Considera el examinador que la presente invención no cumple con el requisito de nivel inventivo en vista de los documentos D1, D7, D8 y D9.

RESUELVE

ARTÍCULO PRIMERO: Denegar la patente de invención a la creación titulada **“MÉTODOS DE TERAPIA COMBINADA PARA TRATAR ENFERMEDADES PROFILERATIVAS”**.

FUNDAMENTOS

Nos permitimos mostrar nuestro total desacuerdo frente a todas y cada una de las objeciones de la Resolución No. 72888 toda vez que en dicho análisis no se demostró de manera concluyente que la invención carecía de nivel inventivo. A continuación, me permito desvirtuar todas y cada una de las afirmaciones de su Despacho en contra del nivel inventivo de la invención y expuestas en la Resolución No. 72888.

Como primera medida, manifestamos nuestro desconcierto en torno a la decisión tomada por el señor Examinador pues en nuestro concepto, las enseñanzas de los citados documentos D1, D7, D8 y D9 sugieren o motivan el desarrollo de un kit de partes farmacéutico como el aquí reivindicado, ni mucho menos anticipan el efecto sinérgico derivado de la combinación específica que da lugar al mismo.

En este sentido, nos permitimos mencionar que ninguno de los documentos D1, D7, D8 y D9 divulga información relevante que permita a cualquier persona normalmente versada en la materia alcanzar el objeto de la presente solicitud de manera obvia, razón por la que consideramos que la decisión del señor Examinador carece completamente de fundamentos.

Inclusive, tras analizar los comentarios y objeciones manifestadas en la Resolución No. 72888, encontramos que el examen de patentabilidad ejecutado corresponde a un análisis en retrospectiva que, desde un enfoque altamente subjetivo, se limita a identificar de manera aislada e inconexa los diferentes elementos característicos de la presente invención, asumiendo que los mismos podrían ser combinados de manera obvia y sin ningún esfuerzo para desarrollar un kit de partes como el descrito en la reivindicación 1.

De este modo, consideramos que el señor Examinador no ha acatado los lineamientos del Instructivo de Examen de Solicitudes de Patentes de Invención y Modelo de Utilidad, el cual estipula la aplicación del método problema-solución para la determinación del nivel inventivo, aclarando además lo siguiente:

*“Si en el estado de la técnica en su conjunto hay un segundo documento que contiene una enseñanza que **indicaría** (no sólo que podría indicar, sino que indicaría) a la persona medianamente versada en la materia, enfrentada al problema técnico, **cómo modificar o adaptar el estado de la técnica más cercano para resolver el problema**, de la forma reivindicada, sin realizar una actividad inventiva, **la Invención se considera obvia**”*

Adaptado del Instructivo de Patentabilidad (Numeral 2.13.5.1)

En vista de lo anterior, solicitamos sea reconocido que ninguno de los documentos citados proporcionan indicaciones o instrucciones que especifiquen **cómo** llevar a cabo la combinación de (a) una composición de nanopartículas que comprenden taxano y una albúmina con (b) un agente adicional seleccionado entre decitabina y azacitidina, por lo cual insistimos en que las objeciones del señor Examinador carecen de total fundamento.

Más aún, cabe destacar que ninguno de los documentos D1, D7, D8 y D9 sugiere las posibles ventajas y efectos derivados del kit de partes farmacéutico reivindicado, por lo que de igual manera fallan en desvirtuar el nivel inventivo y la patentabilidad de la presente divulgación.

Así las cosas, procedemos a demostrar mediante argumentos y evidencias que la materia reivindicada en el actual pliego reivindicatorio no habría podido ser alcanzada de manera obvia, o sin implicar el uso de la actividad inventiva propia del versado en la materia, hecho que deseamos sea reconocido por su Despacho mediante el retiro de las respectivas objeciones.

Como primera medida, consideramos necesario llevar a cabo ahora una serie de aclaraciones relativas al sentido y alcance de la presente divulgación a fin de demostrar que pese a las divulgaciones de los documentos D1, D7, D8 y D9, no existe ningún motivo por el cual la presente invención pueda ser catalogada como un desarrollo obvio a la luz del estado de la técnica.

En primera instancia, es de notar que la invención reivindicada corresponde a un **kit de partes farmacéutico** que comprende:

- a) una composición de nanopartículas que comprenden taxano y una albúmina; y
- b) un agente adicional seleccionado entre decitabina y azacitidina, siendo dicha azacitidina adecuada para administración oral.

De la mano con lo anterior, es pertinente resaltar que dichas nanopartículas que comprenden taxano y una albúmina son ampliamente conocidas en el campo técnico

bajo la denominación **nab-paclitaxel**, y que estas se diferencian fundamental y estructuralmente del fármaco paclitaxel como tal.

A partir de lo anterior, reiteramos que la unión de paclitaxel a albúmina sérica para la formación de la estructura nanoparticulada específica del **nab-paclitaxel** constituye una **modificación mayor de la estructura del paclitaxel** simple o convencional, por lo cual ambas moléculas no son de ninguna manera comparables.

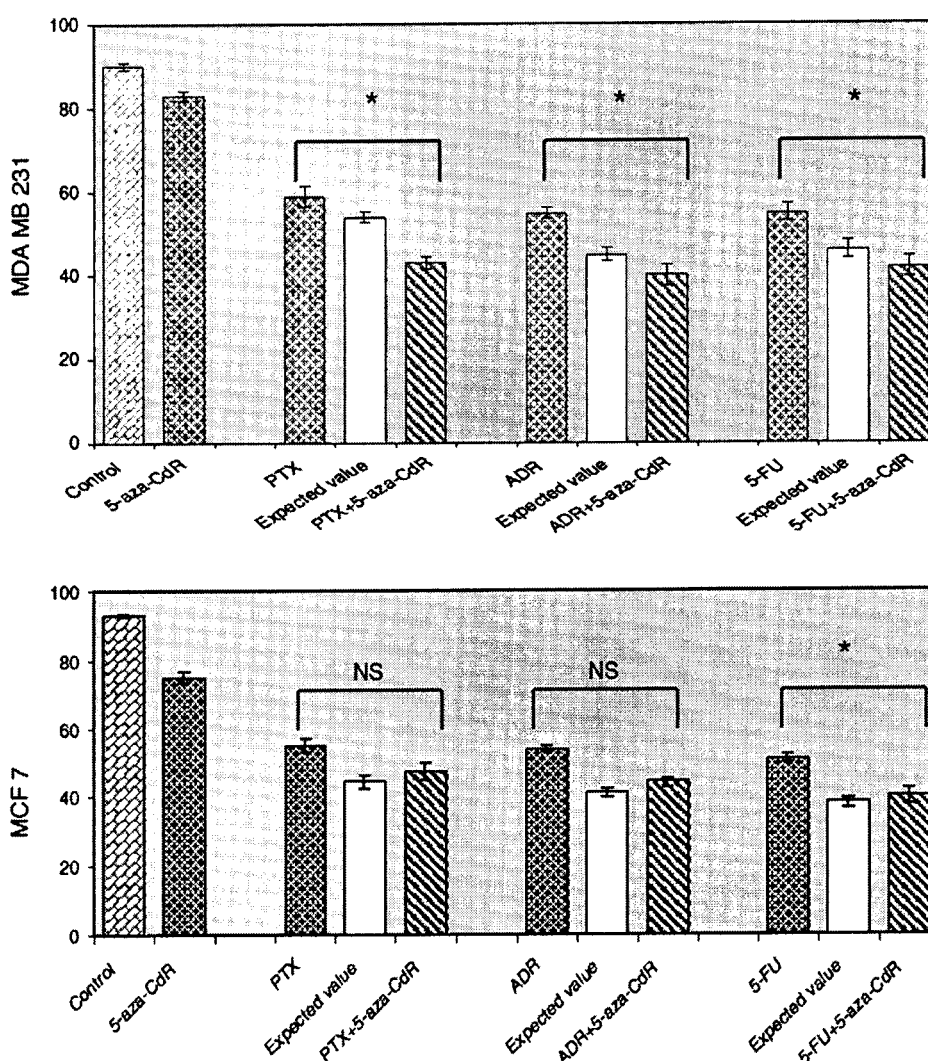
Dicha modificación estructural da lugar a una diferencia apreciable entre el paclitaxel simple o convencional y el **nab-paclitaxel** reivindicado, la cual impacta su vez los aspectos farmacológicos, biodistribución y farmacocinética puntuales de ambos fármacos, por lo que es claro que los mismos cuentan con **propiedades distintas**.

Debido a esta diferencia, consideramos que no existe ninguna razón por la cual las formulaciones de paclitaxel simple o convencional y **nab-paclitaxel** puedan ser consideradas equivalentes o intercambiables, razón por la que adicionalmente es imposible que el versado en la materia se vea motivado a sustituir de manera exitosa cualquier forma convencional de paclitaxel divulgada en el estado de la técnica por la composición de **nab-paclitaxel** que aquí se reivindica, alcanzando el mismo efecto técnico.

En concordancia con lo anterior, afirmamos que el versado en la materia no contaba con ninguna motivación para modificar las anterioridades descritas en los documentos D1 y D7 de acuerdo a lo divulgado en los documentos D8 y D9 respectivamente, pues estos últimos abordan el estudio de composiciones que contienen paclitaxel simple o convencional y por tanto, se **alejan** del alcance de la presente invención y del estado de la técnica más cercano a la misma.

Asimismo, solicitamos sea tenido en cuenta que, contrario a lo afirmado por el señor Examinador, los documentos D1, D7, D8 y D9 no proporcionan ninguna expectativa de éxito relacionada con el **efecto sinérgico** atribuido a la combinación de (a) una composición de nanopartículas que comprenden taxano y una albúmina con (b) un agente adicional seleccionado entre decitabina y azacitidina, a partir de lo cual es evidente que la presente invención cuenta con el debido nivel inventivo.

Con referencia a lo anterior, es nuestra intención aclarar que el documento D9 no proporciona ningún tipo de información relacionada con el efecto sinérgico que surge al combinar paclitaxel y azacitidina, sino que por el contrario, esta anterioridad comenta ampliamente que **el efecto derivado de las preparaciones farmacéuticas que comprenden paclitaxel resulta impredecible**, hecho que ilustra mejor la siguiente figura:



Adaptada de Figura 1; documento D9

Según revela la Figura 1 de dicho documento D9, el efecto sinérgico al cual se refiere el señor Examinador se observa únicamente en las células MDA MCB 231 (una línea celular de cáncer de mama); mientras no se observó ninguna ventaja considerable o significativa al comparar el efecto de la combinación de paclitaxel y decitabina frente

al efecto de paclitaxel como único activo en el tratamiento de células MCF-7 (otra línea celular de cáncer de mama).

A la luz de lo anterior, concluimos que ninguna de las referencias estudiadas por el señor Examinador, entre ellas el citado documento D9, anticipa de manera concreta el efecto derivado de la presente invención, razón por la cual consideramos que dicho estado de la técnica no representa sustento relevante para perjudicar la patentabilidad de la presente invención.

Igualmente, reiteramos que si una persona normalmente versada en la materia acudiera a las enseñanzas de los documentos citados por su Despacho como punto de partida, este no contaría con la motivación o guía suficiente para reemplazar el paclitaxel formulado de la manera convencional –dado a conocer por los documentos D8 y D9– por las composiciones de *nab*-paclitaxel de los documentos D1 y D7, por lo que es claro que este no lograría alcanzar la caracterización que da origen a la presente invención de manera obvia.

Dicho esto, consideramos que además de evaluar las anterioridades desde un punto de vista subjetivo, consideramos que el señor Examinador ha llevado a cabo interpretaciones erróneas de las mismas que, de cualquier manera, le restan validez a su decisión y respaldan nuestra defensa de la patentabilidad de la presente invención, hecho que solicitamos respetuosamente sea reconocido.

Adicional a lo ya expuesto, llamamos la atención sobre el hecho de que el fármaco paclitaxel se formula habitualmente con el solubilizante no iónico Cremophor –aceite de ricino polietoxilado– por los beneficios derivados de dicha combinación precisa, mientras que *nab*-paclitaxel representa una composición completamente libre de Cremophor, hecho que demuestra nuevamente que no existe ninguna razón por la cual dicho paclitaxel pueda ser reemplazado indistintamente por una composición de *nab*-paclitaxel como la que aquí se reivindica.

A este respecto, es preciso mencionar el excipiente Cremophor ha demostrado tener efectos sobre la actividad de paclitaxel e inclusive sobre otros fármacos que habitualmente se emplean en terapias de combinación, según expone el documento

titulado *“Differential modulation of cisplatin accumulation in leukocytes and tumor cell lines by the paclitaxel vehicle Cremophor EL”* anexo en formato digital al presente memorial (Anexo 1 – de Vos et al., *Annals of Oncology*, 1997, v.8, p.1145-1150).

Concretamente, de Vos et al. investigaron y analizaron el comportamiento del fármaco cisplatino en combinación con el excipiente Cremophor –el cual reiteramos se emplea habitualmente como vehículo de paclitaxel–, encontrando que dicho Cremophor juega un rol esencial en la disminución de los efectos adversos asociados a la acumulación de cisplatino al interior de los leucocitos y médula ósea, tal y como se aprecia en el siguiente fragmento:

“Nuestros resultados muestran que no son los taxanos en sí, sino los surfactantes polietoxilados Chremophor EL y Tween 80 los que inhiben selectivamente la absorción de cis-Pt en los leucocitos de la sangre periférica y en las células de médula ósea, y sugieren que estos vehículos son los principales factores determinantes de la mielotoxicidad reducida asociada a cis-Pt que se observa en los estudios clínicos de quimioterapia combinada con paclitaxel y docetaxel.”

Adaptado de Vos et al.; Anexo 1

A partir de estos hallazgos, de Vos et al. concluyen que la disminución de hasta 42% de la concentración de cisplatino al interior de los leucocitos analizados se debe precisamente a la presencia del vehículo Cremophor, que de este modo tiene un impacto directo en la reducción de la mielotoxicidad asociada a la acumulación intracelular de cisplatino sin perjuicio de la actividad quimioterapéutica de dicho fármaco.

En concordancia con lo anterior, es evidente que cualquier persona normalmente versada en la materia consciente de la favorabilidad asociada con la inclusión de Cremophor como parte de una composición antitumoral que contiene paclitaxel, optaría por mantener dicho componente en lugar de descartarlo, razón por la que consideramos que la presente invención **se opone** completamente a los conocimientos e instrucciones derivadas del arte previo y por tanto, no podría ser alcanzada de manera obvia.

Más allá de lo ya comentado, vale la pena agregar que en el momento en que fue desarrollada la presente invención, era imposible predecir la eficacia de las terapias de combinación que incluyen formulaciones de paclitaxel libres de Cremophor –como es el caso de la presente invención– dado que las ventajas del uso de composiciones conformadas por un único agente terapéutico **no pueden ser extrapoladas** con respecto a las composiciones que combinan varios agentes.

Como muestra de este hecho, procedemos a comparar los hallazgos y resultados expuestos en los siguientes dos documentos, anexos en formato digital:

Anexo 2: *“Polymer-Drug Conjugates: Recent Development in Clinical”*

Li et al., *Adv Drug Deliv*, 2008, v.60, p.886-898

Anexo 3: *“Phase III Trial Comparing Paclitaxel Poliglumex (CT-2103, PPX) in Combination with Carboplatin Versus Standard Paclitaxel and Carboplatin in the Treatment of PS 2 Patients with Chemotherapy-Naiˆve Advanced Non-small Cell Lung Cancer”*

Langer et al., *Journal of Thoracic Oncology*, 2008, v.3, p.623-630

En primer lugar, encontramos que el Anexo 2 (Li et al.) lleva a cabo un análisis comparativo del efecto antitumoral alcanzado por:

- i) Un conjugado polimérico que incluye paclitaxel en su forma conjugada con poli(ácido L-glutámico) y que carece de Cremophor, conocida en el campo técnico como CT-2103, PG-TXL o PPX: y
- ii) Taxol, una composición de paclitaxel que sí incluye Cremophor como vehículo.

Tras analizar pertinentemente los hallazgos de su estudio, Li et al. concluyen que el conjugado polimérico (i) exhibe una **eficacia antitumoral sorprendente y significativamente superior a la lograda por Taxol** al ser empleados de manera independiente como agentes quimioterapéuticos en el tratamiento de cáncer como el cáncer de células pulmonares no-pequeñas (NSCLC).

Teniendo en cuenta que las enseñanzas del Anexo 2 ilustran un caso en el que un conjugado polimérico de paclitaxel totalmente libre de Cremophor demuestra una **eficacia antitumoral superior** a la alcanzada por una composición que sí incluye dicho vehículo, es claro que cualquier versado en la materia esperaría que la inclusión de dicho conjugado como parte de composiciones terapéuticas alternativas resulte en la consecución de efectos terapéuticos destacados y favorables.

Sin embargo, el Anexo 3 (Langer et al.) reporta resultados derivados de la ejecución de un ensayo clínico de fase III que compara la efectividad de tratamientos combinados que comprenden carboplatino en conjunto con poli(ácido-L-glutámico)-paclitaxel o una formulación de paclitaxel en Cremophor, para el tratamiento de cáncer de células pulmonares no-pequeñas (NSCLC), que **se oponen** francamente a dicha expectativa.

Así pues, en contraste con las conclusiones y hallazgos de Li et al., Langer et al. observó que la combinación de carboplatino y poli(ácido-L-glutámico)-paclitaxel no presentó ninguna ventaja apreciable en comparación con la combinación que incluía una formulación convencional de paclitaxel.

Más en detalle, Langer et al. destaca que el tratamiento combinado que comprende poli(ácido-L-glutámico)-paclitaxel alcanzó un nivel de supervivencia media de apenas 7.8 meses, mientras que dicho parámetro fue superior para el caso de los pacientes tratados con la combinación que incluía una formulación de paclitaxel y Cremophor, resultado que ratifica lo ya mencionado.

A la luz de lo anterior, solicitamos sea reconocido que el hecho de que la formulación de poli(ácido-L-glutámico)-paclitaxel del Anexo 2 haya presentado resultados sorprendentes y ventajosos, no garantiza que todas y cada una de las composiciones subsecuentes que comprendan dicho conjugado sean igualmente exitosas y favorables, y que en este orden de ideas, **los resultados alcanzados por una combinación particular de paclitaxel no son extrapolables** a cualquier otra composición que incluya dicha forma de paclitaxel.

De este modo, concluimos que no existe una manera científica y concreta de predecir los efectos alcanzados por la combinación de (a) una composición de nanopartículas que comprenden taxano y una albúmina con (b) un agente adicional seleccionado entre decitabina y azacitidina pues el mismo arte previo revela estudios cuyos resultados se contradicen entre sí, lo cual respalda ampliamente la patentabilidad de la presente invención pues demuestra que al momento de su desarrollo, era **imposible predecir el efecto que la misma alcanzaría**.

Habiendo expuesto así las razones por las cuales la presente invención no podría haber sido alcanzada de manera obvia, o sin requerir de la aplicación de la habilidad inventiva propia del versado en la materia, consideramos necesario también demostrar ante su Despacho que la presente invención conlleva **ventajas valiosas** y por ende, simboliza un **aporte importante al campo técnico**, hecho que deseamos sea visto como una muestra adicional de su patentabilidad.

En este contexto, procedemos a presentar y comentar los resultados y conclusiones consignados en los siguientes posters científicos, los cuales también adjuntamos en formato digital:

Anexo 4: *“A Phase 1b Study of CC-486 (Oral Azacitidine) to Evaluate the Pharmacokinetics and Pharmacodynamics of Azacitidine Administered Alone and in Combination with Carboplatin or ABI-007 (nab®-Paclitaxel) in Patients with Solid Tumors”.*

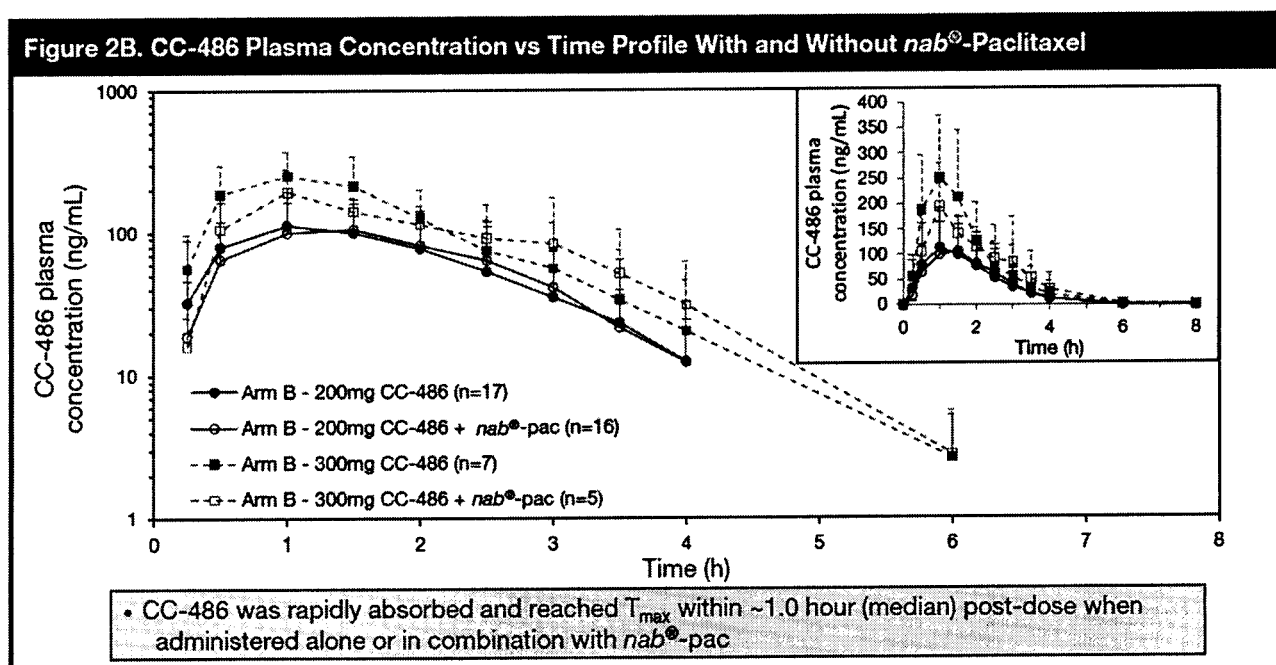
Laille et al. Presentado en la Conferencia Internacional de Blancos Moleculares y Terapéutica en Cáncer (AACR-NCI-EORTC), Octubre 19-23, 2013, Boston, MA, EUA.

Anexo 5: *“A Phase 1b Study of CC-486 (Oral Azacitidine) as a Priming Agent for Carboplatin or nab®-Paclitaxel in Patients with Relapsed or Refractory Solid Tumors”.*

LoRusso et al. Presentado en la Conferencia Internacional de Blancos Moleculares y Terapéutica en Cáncer (AACR-NCI-EORTC), Octubre 19-23, 2013, Boston, MA, EUA.

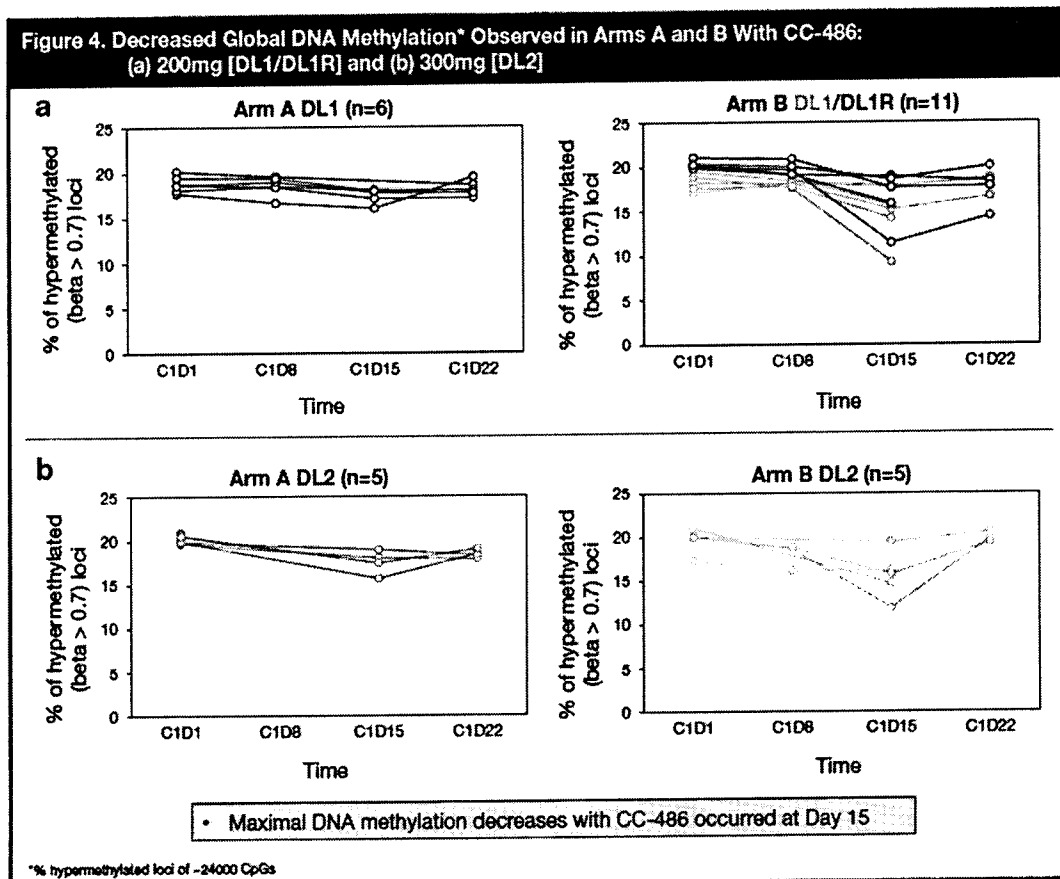
Debido a que los anteriores posters compilan y presentan datos relacionados con la farmacocinética, farmacodinamia, seguridad y eficacia de una formulación oral de azacitidina (CC-486) acorde con la presente invención, consideramos que su información es relevante y soporta nuestra posición en favor de la concesión del privilegio de patente.

La Figura 2B ilustrada a continuación evidencia la rápida absorción oral y biodisponibilidad que manifiesta la composición CC-486 con y sin la administración concomitante de *nab*-paclitaxel, lo cual corrobora la **favorabilidad** de la presente invención y su forma farmacéutica. Vale la pena destacar que la máxima concentración en plasma fue determinada trascurrida 1 hora desde la administración.



Adaptada de Laille et al.; Anexo 4

La Figura 4b enseña que tras la administración oral diaria de la composición CC-486 con y sin la administración concomitante de *nab*-paclitaxel, la máxima desmetilación del ADN fue lograda al día 15 de tratamiento; situación que demuestra la **eficacia** de la presente invención al ser incorporada como parte de un esquema quimioterapéutico.



Adaptada de Laille et al.; Anexo 4

Por otra parte, los resultados expuestos en el Anexo 5. (LoRusso et al) corresponden al estudio de dos tratamientos combinados mediante una metodología que evaluó la concentración de CC-486 bajo dos regímenes de dosificación para cada una de dichas composiciones, 300 mg y 200 mg diarios respectivamente. Las dos composiciones analizadas corresponden a las siguientes:

- (1) Composición CC-486 + carboplatino; y
- (2) Composición CC-486 + *nab*-paclitaxel,

Como resultado, LoRusso et al. refiere que ambos tratamientos combinados fueron tolerados por los pacientes, pero que el tratamiento (2) que combina 200 mg de CC-486 y 100 mg/m² de *nab*-paclitaxel demostró una **mejora en la velocidad de respuesta** comparada frente al comportamiento del tratamiento (1) que combina 200 mg de CC-486 y carboplatino.

De hecho, los resultados expuestos por LoRusso et al. señalan que aún al ser sometidos a la dosis más baja (200 mg de CC-486), tres pacientes que recibieron el

tratamiento combinado (2) manifestaron alcanzar una respuesta parcial al tratamiento, cuando ninguno de los pacientes tratados con 300 mg de CC-486 y carboplatino exhibieron respuesta de cualquier tipo.

Considerando entonces el conjunto de las enseñanzas de los Anexos 4 y 5, es indiscutible el hecho de que la presente invención conlleva efectos técnicos ventajosos y favorables que respaldan el cabal cumplimiento de los requisitos de patentabilidad de su parte, lo cual solicitamos respetuosamente sea sopesado por el señor Examinador a cargo del análisis del presente memorial.

Además, partiendo de que el efecto técnico alcanzado por la presente invención no habría podido ser previsto con base a lo divulgado en los documentos D1, D7, D8 y D9 citados por el señor Examinador y en el estado de la técnica general, insistimos en que el mismo no tiene ningún precedente en el campo técnico y, por tanto, respalda la patentabilidad de la presente invención en contraste con la decisión tomada por su Despacho.

Del mismo modo, dadas las falencias del examen de patentabilidad ya comentado, de la mano con nuestra argumentación al respecto, concluimos que dicho examen falla completamente en desvirtuar la patentabilidad de la presente invención ya que no proporciona sustento válido que sugiera o anticipe el objeto de la misma.

En este orden de ideas, afirmamos que la presente invención fue juzgada bajo un enfoque carente de fundamentos que demuestren la supuesta obviedad de la misma a la luz del estado de la técnica, el cual asumimos demostrado guarda total silencio respecto a cada uno de los elementos característicos del kit de partes reclamado en el actual pliego reivindicatorio, y mucho menos referencia las posibles ventajas derivadas de tal combinación en particular.

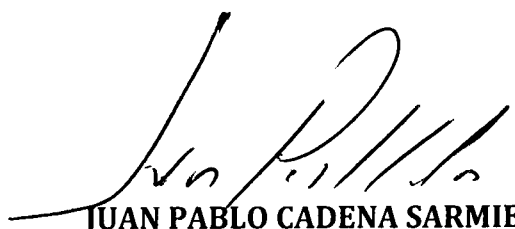
PETICIÓN

Por las razones anteriormente expuestas, solicito a ese despacho **REVOCAR** la decisión tomada mediante la Resolución No. 72888 del 1º de Diciembre de 2014 y tendiente a obtener la concesión del Privilegio de Patente de conformidad con los artículos 14 y 48 de la Decisión 486 de la comunidad Andina.

NOTIFICACIONES

Recibiré notificaciones en la Secretaría General de la Superintendencia de Industria y Comercio, o en su defecto, en mi oficina de Abogado, situada en la Calle 70ª No. 4-41 de esta ciudad de Bogotá.

Del Señor Director



JUAN PABLO CADENA SARMIENTO

T.P. No. 57.492 del C.S.J.

C.C. 80.410.337 de Usaquén

Calle 70 A No. 4 - 41

 Industria y Comercio SUPERINTENDENCIA		PLANILLA DE RADICACIÓN - DIGITALIZACIÓN DIARIA RELACIÓN DE ANEXOS	
Dependencia: 2020		Fecha: <u>15</u> / <u>1</u> / <u>15</u> AAAA MM DD	
Tipo de Radicación: Entrada: ☐ Salida: ☐ Traslado: ☐ Convenio: ☐		Recorrido:	
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Item	Unidad de Conservación
1	Bolsa
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3	Libro
4	Sobre
5	Tomo
6	Otro
Item	Soporte Documental
7	CD X
8	Disquete
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Original article

Differential modulation of cisplatin accumulation in leukocytes and tumor cell lines by the paclitaxel vehicle Cremophor EL

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Summary

Background: Several clinical studies have shown that polychemotherapy with the taxanes paclitaxel or docetaxel preceded or followed by cisplatin is associated with important schedule-dependent differences in toxicities, such as leukocytopenia. In general, the pharmacokinetics of both drugs during the combined treatment are unaltered, suggesting that a pharmacodynamic interaction might have occurred.

Materials and methods: In order to gain insight into this pharmacologic interaction, we performed *in vitro* drug accumulation studies using peripheral blood leukocytes and a panel of tumor and non-malignant cell lines with paclitaxel and docetaxel, as well as with their respective formulation vehicles Cremophor EL and Tween 80.

Results: Our results show a significant reduction in the intracellular cisplatin concentration in leukocytes of up to 42% in the presence of Cremophor EL and Tween 80 as compared to the control. This pharmacodynamic interaction of these surfactants with cisplatin seems to be specific for haematopoietic cells, and does not occur in solid tumor cells.

Conclusion: The present data suggest that the pharmaceutical vehicles Cremophor EL and Tween 80 might contribute to the reduced cisplatin-associated myelotoxicity observed in the clinical combination chemotherapy studies with paclitaxel and docetaxel.

Key words: cisplatin, Cremophor EL, drug accumulation, haematopoietic cells, leukocytes, paclitaxel

Introduction

Paclitaxel (Taxol), an antimicrotubule agent derived from the stem bark of the Western Yew tree (*Taxus brevifolia*), is one of the most promising drugs in the chemotherapeutic armamentory, with proven antitumor activity in wide variety of human neoplastic disorders [1]. Currently, optimization of the usage of paclitaxel is actively pursued, particularly by testing the efficacy of this drug in combination chemotherapy. One of the most obvious drug combinations to evaluate is paclitaxel followed by cisplatin (*cis*-Pt), since preclinical data indicated that this combination causes synergistic cytotoxicity in a variety of cell lines [2-4], and human tumor xenograft models [5, 6]. The only partially overlapping side effects of paclitaxel and *cis*-Pt, their different mechanisms of action, and paclitaxel's activity in *cis*-Pt-refractory ovarian cancer also made the combination of both drugs attractive for further clinical exploration [7].

Recently, exciting results have emerged from clinical combination chemotherapy studies with paclitaxel or its semisynthetic analogue docetaxel (Taxotere) and *cis*-Pt, testing two sequences of drug administration [8, 9]. These studies revealed important schedule-dependent differences in frequency and severity of toxicities, with

less severe myelotoxicity when the taxane was given before *cis*-Pt. Our own previous investigations demonstrated that in the sequence of docetaxel followed by *cis*-Pt, the reduced leukocytopenia was accompanied by a significant inhibition of *cis*-Pt uptake and DNA-adduct formation in peripheral blood leukocytes as compared to the alternate sequence [10, 11].

At present, convincing data on the fundamental mechanisms behind this clinically important pharmacologic interaction are lacking. This prompted us to further investigate the effects of paclitaxel and docetaxel, as well as of their respective formulation vehicles Cremophor EL and Tween 80, on the intracellular accumulation of *cis*-Pt in haematopoietic and tumor cells *in vitro*. Our results show that not the taxanes themselves, but the polyoxyethylated surfactants Cremophor EL and Tween 80 selectively inhibit *cis*-Pt uptake in peripheral blood leukocytes and bone marrow cells, and suggest that these vehicles are major determinants in the reduced *cis*-Pt-associated myelotoxicity observed in clinical combination chemotherapy studies with paclitaxel and docetaxel.

Materials and methods

Drugs and chemicals

The *cis*-Pt reference material was supplied by Pharmachemie (Haarlem, the Netherlands) as a solution containing 1 mg/ml of drug in D5NS (lot 95F29MC). Paclitaxel powder, the clinical paclitaxel formulation in Cremophor EL and dehydrated ethanol USP (1:1, v/v; Taxol) and carboplatin were obtained from Bristol-Myers Squibb (Princeton, NJ, USA). Docetaxel and the clinical docetaxel formulation in Tween 80 (Taxotere) originated from Rhône-Poulenc Rorer (Antony Cedex, France). Camptothecin lactone (Pharmacia Inc., New Mexico), doxorubicin (Farmitalia Carlo Erba, Milan, Italy), methotrexate (Pharmachemie) and vincristine (Eli Lilly, Nieuwegein, the Netherlands) were used as their pharmaceutical formulations for clinical use without further purification. Cremophor EL (polyoxyethyleneglycerol triricinoleate 35; lot 32H0925), Tween 80 (polyoxyethyleneglycerol sorbitan monooleate; lot 95H09251), castor oil and ricinoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Five reversed-phase HPLC fractions of Cremophor EL were kindly donated by Dr David Kessel (Wayne State University School of Medicine, Detroit, USA) [12]. Formic acid, tetrahydrofuran, ammonium hydroxide and Triton X-100 were from Baker (Deventer, the Netherlands). Coomassie Brilliant Blue G-250 was purchased from Bio-Rad Laboratories (Munich, Germany). All other chemicals and reagents were of analytical or HPLC grade and were obtained from Rathburn (Walkerburn, UK). Water was filtered and deionized with a Milli-Q-UF Plus system (Millipore, Bedford, MA, USA), and was used in all aqueous solutions.

Cell cultures

The human ovarian carcinoma cell lines A2780 and IGROV-1, the breast carcinoma cell line MCF-7, the human promyelocytic leukaemia cell line HL-60 and the murine lymphoid leukaemia cell line P388 were grown and maintained in RPMI1640 medium (Brunschwig, Amsterdam, the Netherlands). The non-malignant chinese hamster ovary (CHO) and the African green monkey kidney (VERO) cell lines were maintained in Dulbecco's modified Eagle's Medium (DMEM; Brunschwig). The cells were cultured in a humidified atmosphere in 5% CO₂ at 37 °C in media supplemented with 10% (w/v) of heat-inactivated bovine calf serum (Hyclone, Logan, UT, USA), 100 U/ml of penicillin, 100 µg/ml of streptomycin and 2 mM freshly added L-glutamine (all from Life Technologies, Gaithersburg, MD, USA). The cytotoxicity of Cremophor EL against the cells was estimated with the sulforhodamine B assay [13].

For peripheral blood leukocytes (PBL) isolation, 55 ml of a freshly prepared buffy coat suspension obtained from healthy volunteers was mixed with 50 ml of 0.1 M phosphate buffer and 300 ml of red cell lysing buffer (composed of 0.83% (w/v) of ammonium chloride and 0.1% (w/v) of potassium carbonate in 1 mM of EDTA). Prior to drug exposure, the mixture was incubated for 20 min at 4 °C in a water bath. After centrifugation, the PBL were washed with PBS (Unipath Ltd., Basingstoke, UK) and maintained in RPMI1640 medium for 45 min.

Isolation of granulocytes and mononuclear cells from heparinized human blood was performed by sequential ficoll-gradient centrifugation and methyl cellulose sedimentation at room temperature as described [14]. Contaminating erythrocytes were removed by hypotonic lysis in 0.22% (w/v) of sodium chloride for five minutes. Both post-ficoll cell fractions were handled further as described above for whole PBL.

Bone marrow samples were collected from female Wistar rats obtained from the Erasmus Animal Experimental Centre (Rotterdam, the Netherlands), that were housed in conventional plastic cages covered with filter bonnets, and provided with food and water *ad libitum*. The animals were sacrificed by cervical dislocation under diethylether anaesthesia, and femurs were removed surgically, scraped free of muscular tissues and other adhering debris, while maintaining the integrity of the knee and hip joints during dissection. The marrow

was harvested by snipping the tip of the femur and flushing with ice-cold PBS using a syringe fitted with a 27-gauge needle. Cell suspensions were stored on ice and used within one hour of collection.

Cellular drug accumulation

All cell lines were grown to 60%–70% confluence in 75 cm² cell culture flasks (Greiner, Alphen a/d Rijn, the Netherlands), and incubated simultaneously for two hours with Cremophor EL and *cis*-Pt (10 µg/ml). Immediately after drug incubation, the cells were rinsed twice with PBS, harvested by trypsinization, and washed three times with PBS.

For the accumulation studies in PBL and bone marrow cells, 6 ml of the cell suspension were incubated in 12.5 ml polypropylene screw-cap tubes (Greiner) with various concentrations of the drugs or surfactants and *cis*-Pt for two hours. During this incubation, the tubes were vortex-mixed at 30 min-intervals to prevent clogging of the cells. Next, samples were processed as described above for tumor cells.

Drug analyses

Cell pellets were digested with 500 µl of Triton X-100-water (1:500, v/v) by vortex-mixing (five minutes) and ultrasonication (15 minutes). A 100 µl-aliquot of this solution was removed for determination of the protein content by the method of Bradford [15]. The remaining cell lysate was then analyzed for total platinum by flameless atomic absorption spectrometry using a Perkin Elmer Model 4110 ZL atomic absorption spectrometer with Zeeman-background correction (Perkin Elmer Corp., Rockville, MD, USA) as described previously [16]. Each sample was analysed in duplicate by external standardization with a weighted linear regression analysis (1/x²), and the absorbance readings were averaged.

For the analysis of intracellular paclitaxel and docetaxel concentrations, PBL pellets were digested in 1000 µl of water, followed by liquid-liquid extraction and reversed-phase HPLC according to methods reported in detail previously [17, 18]. The HPLC equipment was composed of a constaMetric 3200 pump (LCD Analytical, Rivera Beach, CA, USA), a Waters Model 717 plus autosampler (Milford, MA, USA) and a UV-2000 detector (Spectra Physics, San Jose, CA, USA). Separations were achieved on an Inertsil ODS-80A column (GL Science, Tokyo, Japan) and a mobile phase of methanol-tetrahydrofuran-20% (w/v) ammonium hydroxide (60:2.5:37.5, v/v/v; pH 6.0), delivered at 1.00 ml/min. Detection was performed at 230 nm, with peak recording performed using the Chrom-Card data analysis system (Fisons, Milan, Italy). Calibration curves were fitted by weighted regression analysis (1/x²).

Cremophor EL concentrations in plasma collected from two patients receiving 100 and 225 mg/m² of paclitaxel (three-hour i.v. infusion), respectively, followed by 70 mg/m² of *cis*-Pt (three-hour i.v. infusion) were measured by a novel microassay that will be described in detail elsewhere [19]. In brief, the method is based on a selective binding of Coomassie Brilliant Blue G-250 to Cremophor EL following plasma protein precipitation with acetonitrile and liquid-liquid extraction with *n*-butylchloride. The increase in the absorption maximum of the dye from 465 to 595 nm after binding to Cremophor EL was monitored using a Titertec automated microplate reader (Flow Laboratories, Irvine, UK). Calibration curves constructed in blank human plasma over the concentration range of 0.05%–1.0% (v/v) of Cremophor EL were fitted by using unweighted second-order polynomial (quadratic) regression analysis.

Results

In order to gain insight into the pharmacologic mechanism of interaction involved in our clinical studies with paclitaxel or docetaxel and *cis*-Pt, *in vitro* drug accumulation studies were performed using both PBL, isolated

Table 1. Cellular accumulation of *cis*-Pt in PBL following a two-hour simultaneous *in vitro* incubation of various drugs and pharmaceutical formulation vehicles and *cis*-Pt (10 µg/ml). Data are presented as a mean percentage relative to a 100% control value ± SD.

Drug (formulation)	Concentration	<i>cis</i> -Pt accumulation (%)	n
Paclitaxel (Cremophor EL)	1.0 µg/ml	67 ± 12 ^a	3
Paclitaxel (DMSO)	1.0 µg/ml	99 ± 8	2
Docetaxel (Tween 80)	1.0 µg/ml	73 ± 13 ^a	3
Docetaxel (DMSO)	1.0 µg/ml	101 ± 13	3
Cremophor EL	1.0%	52 ± 9 ^a	2
	0.01%	58 ± 10 ^a	11
	0.001%	62 ± 11 ^a	5
	0.0001%	66 ± 11 ^a	2
	0.00001%	75 ± 2 ^a	2
Tween 80	0.01%	58 ± 8 ^a	3
Ethanol	0.01%	94 ± 7	3
Camptothecin	1.0 µg/ml	105 ± 9	3
Doxorubicin	1.0 µg/ml	101 ± 3	3
Methotrexate	1.0 µg/ml	104 ± 9	3
Vincristine	1.0 µg/ml	94 ± 16	3

^a $P < 0.05$ versus the control value.

from freshly prepared buffy coat suspensions, and various tumor and normal cell lines. The total cellular accumulation of the drugs used in these experiments was quantitated as a percentage relative to the 100% control value, which was assigned to the drug level achieved without co-incubation at the specified treatment interval.

Simultaneous incubation of PBL with Taxol (i.e., paclitaxel formulated in Cremophor EL) or Taxotere (i.e., docetaxel formulated in Tween 80) and *cis*-Pt resulted in a similar magnitude of *cis*-Pt uptake inhibition, with the intracellular concentrations of *cis*-Pt in PBL being significantly reduced by 33% and 27% for Taxol and Taxotere, respectively, as compared to the control values (Table 1).

Because the possibility existed that the reduced *cis*-Pt accumulation in PBL after co-incubation with Taxol or Taxotere was due to the large amounts of Cremophor EL and Tween 80 added to the cells, PBL were also treated with Cremophor EL or Tween 80 alone prior to *cis*-Pt (Table 1). In these experiments, a significant reduction in the intracellular *cis*-Pt concentration in PBL of 42% compared to the control was observed in the presence of 0.01% (v/v) of Cremophor EL or Tween 80 (i.e., the same concentrations present in 1.0 µg/ml of Taxol and Taxotere, respectively). Further studies revealed a clear concentration-dependent effect of Cremophor EL on the *cis*-Pt uptake in PBL, with maximal inhibition (approximately 50%) achieved at an extracellular concentration of 1.0% of Cremophor EL. It is of interest to note, however, that Cremophor EL concentrations as low as 0.00001% still resulted in a mere 25% decrease in *cis*-Pt uptake as compared to the control (Table 1). The increased *cis*-Pt levels observed in PBL incubated in higher concentrations of Cremophor EL ($\geq 10\%$) was not due primarily to interference with uptake, but rather to gross cellular damage (data not

Table 2. Cellular accumulation of paclitaxel, docetaxel and carboplatin in PBL following a two-hour simultaneous *in vitro* incubation with Cremophor EL or Tween 80 (0.01%). Data are presented as a mean percentage relative to a 100% control value ± SD.

Drug (formulation)	Concentration	Drug accumulation with Cremophor EL	n	Drug accumulation with Tween 80	n
Paclitaxel (DMSO)	1.0 µg/ml	94 ± 11	6	105 ± 14	4
Docetaxel (DMSO)	1.0 µg/ml	94 ± 6	3	96 ± 7	3
Carboplatin (water)	100 µg/ml	112 ± 13	4	95 ± 4	3

shown). The *cis*-Pt accumulation was unaffected in a similar set of experiments in which cells were exposed to paclitaxel or docetaxel formulated without Cremophor EL or Tween 80 (viz. DMSO) (Table 1). These findings indicate that the inhibition of *cis*-Pt accumulation in PBL observed with Taxol and Taxotere is exclusively caused by their formulation vehicles Cremophor EL and Tween 80.

Incubation of PBL with pure castor oil and ricinoleic acid, two major components present in the Cremophor EL vehicle prior to polyoxyethylation, showed no evidence of any decrease in *cis*-Pt accumulation (data not shown). Furthermore, experiments with five reversed-phase HPLC fractions of Cremophor EL (each with progressively increased hydrophobicity) indicated that the inhibitory effects of the unfractionated vehicle on *cis*-Pt uptake is not observed with the more hydrophilic components present in fraction I, mainly containing polyethylene glycol and oxyethylated glycerol (data not shown). The maximum concentration of ethanol, another component of the paclitaxel formulation, to which the cells were exposed was 0.01% (v/v). Although it was unlikely that such low concentrations would contribute to the observed effects, it was necessary to rule out this organic solvent as an important contributor to the decreased *cis*-Pt accumulation. To date, in the cases where cells were exposed to ethanol in the absence of drug, the cellular accumulation of *cis*-Pt was identical to the control (Table 1). In addition, a number of other drugs, formulated without a polyoxyethylated surfactant, including camptothecin, doxorubicin, methotrexate and vincristine had no effect on the accumulation of *cis*-Pt at the specified concentrations (Table 1).

PBL were also treated with paclitaxel, docetaxel (both formulated in DMSO) or the *cis*-Pt analogue carboplatin and the surfactants, to determine whether the observed mechanism of interaction was specific for *cis*-Pt. At drug concentrations chosen close to achievable plasma levels *in vivo*, both Cremophor EL and Tween 80 had negligible effects on drug accumulation (Table 2).

To test whether the pattern of *cis*-Pt uptake in the presence of Cremophor EL (at non-cytotoxic concen-

Table 3. Cellular accumulation of *cis*-Pt in various cell lines following a two-hour simultaneous *in vitro* incubation of Cremophor EL (0.01%) and *cis*-Pt (10 µg/ml). Data are presented as a mean relative to a 100% control value ± SD.

Cell line	Origin	<i>cis</i> -Pt accumulation	n	Cremophor EL IC ₅₀ (%) ^a	n
Malignant					
A2780	Human ovarian carcinoma	98 ± 8	3	0.047 ± 0.009	2
IGROV-1	Human ovarian carcinoma	96 ± 10	3	ND	—
MCF-7	Human breast carcinoma	102 ± 9	2	0.068 ± 0.008	2
HL-60	Human promyelocytic leukaemia	77 ± 12 ^b	5	ND	—
P388	Murine lymphoid leukaemia	84 ± 5 ^b	4	ND	—
Non-malignant					
CHO	Chinese hamster ovary	93 ± 4	3	0.092 ± 0.010	2
VERO	African green monkey kidney	111 ± 11	3	0.093 ± 0.015	2
Granulocytes	Human blood	82 ± 1 ^b	4	ND	—
Mononuclear cells	Human blood	76 ± 3 ^b	4	ND	—
Bone marrow	Wistar rat	84 ± 1 ^b	2	ND	—

^a Dose of Cremophor EL to produce a 50% inhibition of normal cell growth during a five-day continuous exposition.

^b *P* < 0.05 versus the control value.

trations; Table 3) was also altered in other mammalian cells, *cis*-Pt influx was examined in the tumor cell lines A2780, IGROV-1, MCF-7, HL-60 and P388, the non-malignant cell lines CHO and VERO, and rat bone marrow cells identically to incubations performed in PBL. As depicted in Table 3, a significant decrease in the intracellular *cis*-Pt concentration was only observed in the leukaemic cell lines and the rat bone marrow cells. Furthermore, the effect of Cremophor EL on the *cis*-Pt uptake in PBL was not lineage specific, as indicated by the results from incubation experiments using isolated granulocytes and mononuclear cells (Table 3). In all, these data indicate a selective protection of haematopoietic cells by Cremophor EL against *cis*-Pt uptake at concentrations that can be maintained in humans for several hours after i.v. paclitaxel administration (Figure 1).

Discussion

Cis-Pt is one of the most active anticancer agents. The drug is part of curative treatment in some diseases, however, most patients will eventually relapse after conventional three-weekly *cis*-Pt treatment as a result of cellular resistance to the drug. Our current hypothesis is that dose-intensification by shortening dose-intervals might be an approach to overcome clinical drug resistance. We have recently tested this concept in non-small cell lung cancer and ovarian cancer by giving *cis*-Pt weekly instead of three-weekly and demonstrated increased response rates of up to 52% and 90%, respec-

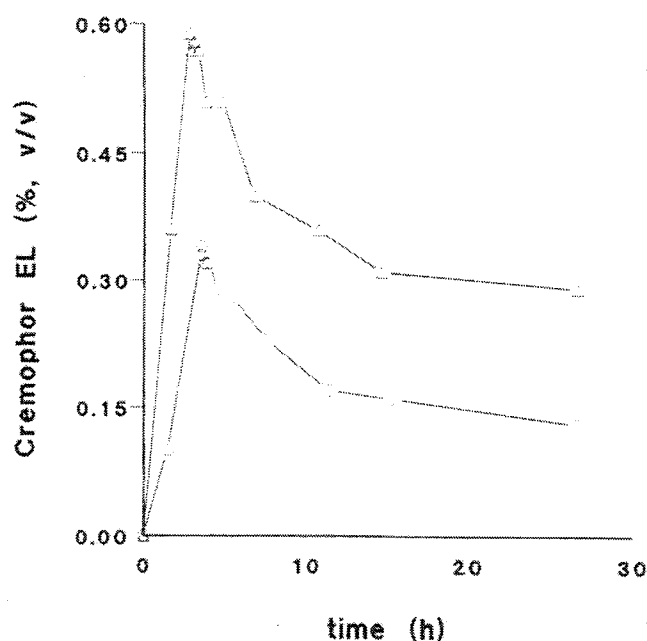


Figure 1. Plasma concentration versus time profile of Cremophor EL in two female patients with advanced ovarian cancer, treated with a three-hour intravenous infusion of paclitaxel at dose levels of 100 mg/m² and 225 mg/m². Paclitaxel was formulated at 6 mg/ml in a mixture of Cremophor EL-dehydrated ethanol USP (1:1, v/v), and diluted 20-fold in normal saline to a final concentration of 0.3 mg/ml within three hours before drug administration. Patients one (circles) and two (triangles) received absolute Cremophor EL doses of 13.8 and 27.9 ml, respectively.

tively [20, 21]. In these studies, however, *cis*-Pt-induced leukocytopenia appeared to be the dose-limiting toxicity. In contrast, weekly combination chemotherapy with *cis*-Pt and paclitaxel resulted in significantly less haematological toxicities than anticipated (unpublished data). The present study demonstrates that this effect may be related to a selective inhibition of *cis*-Pt accumulation in PBL and bone marrow cells by the paclitaxel vehicle Cremophor EL, which is not lineage specific. These data are in agreement with our previous findings of reduced *ex vivo* *cis*-Pt induced DNA-adduct levels in PBL obtained from patients three hours after treatment with paclitaxel compared to PBL from non-pretreated patients [16].

The different effects of Cremophor EL on the *in vitro* *cis*-Pt uptake in normal haematopoietic cells and (solid) tumor cells, respectively, suggest that administration of Cremophor EL could selectively provide protection against *cis*-Pt uptake in haematopoietic cells, without affecting antitumor activity. Support for this hypothesis can be derived from several clinical and pharmacokinetic phase I studies in patients with solid tumors, applying the sequence paclitaxel or docetaxel followed by *cis*-Pt in one group of patients and that of *cis*-Pt followed by paclitaxel or docetaxel in another group [8, 9]. These combination chemotherapy studies revealed clinically important schedule-dependent differences in frequency and severity of toxicities, whereas antitumor activity did not seem to be affected.

The extracellular levels of Cremophor EL used in our *in vitro* experiments were shown to be maintained in

patients for several hours after i.v. administration of paclitaxel, even at dose levels as low as 100 mg/m². These results are consistent with previously published measurements of Cremophor EL concentrations in plasma by pre-column derivatization and reversed-phase HPLC [22, 23]. Previous studies also demonstrated that the distribution volume of Cremophor EL in mice (0.14 l/kg) was less than the volume of the plasma and the extracellular compartment (approximately 0.2 l/kg), indicating that the tissue distribution of Cremophor EL is insignificant [24]. These data imply that even very low doses of Cremophor EL would still result in an extended exposure of bone marrow cells to levels of Cremophor EL providing protection against cellular *cis*-Pt uptake. Recent studies in mice showed that such low i.v. doses of Cremophor EL did not result in localized toxicity or marrow destruction [25], which is consistent with flow cytometric studies demonstrating that even very high concentrations of Cremophor EL (> 10%) did not lyse bone marrow cell membranes [26]. Moreover, the i.v. administration of Cremophor EL was associated with an increase in the incidence of both primitive and committed progenitors, and protected mice from irradiation-induced death [25]. These findings provide a rationale for attempts to circumvent *cis*-Pt-associated myelotoxicity by concomitant administration of Cremophor EL.

Cremophor EL is a complex heterogeneous liquid formed by the reaction of ethylene oxide with castor oil at a molar ratio of 35:1 [27]. Separation of Cremophor EL into a series of components based on reversed-phase HPLC provided additional information on the nature of the pharmacologically active constituents. These studies show that maximal inhibition of *cis*-Pt uptake in PBL was achieved with the more hydrophobic components of the mixture, mainly containing polyoxyethyleneglycerol triricinoleate along with fatty acids esters of polyethyleneglycol. As Tween 80 is a polyoxyethylated derivative of sorbitan monooleate, the active agents apparently contain hydrophilic polyoxyethylated side chains attached to hydrophobic moieties with no structural similarities.

The biochemical mechanism of the dose-dependent interaction of Cremophor EL with the cellular uptake of *cis*-Pt is not clear. It has been reported that Cremophor EL can have a major (reversible) effect on the microviscosity of cell membranes, thereby interfering with passive drug diffusion [26]. However, recent experiments demonstrated that *cis*-Pt accumulation in PBL was highly temperature dependent. Lowering the temperature from 37 to 4 °C led to 70% and 90% reduction of intracellular *cis*-Pt in the absence or presence of Cremophor EL, respectively (unpublished data). This would imply that besides passive diffusion, alternative transport systems for *cis*-Pt susceptible to modulation by Cremophor EL are present in PBL. Such mechanisms include facilitated diffusion through gated tunnels regulated by phosphorylation cascades initiated by activation of protein kinase C [28, 29]. These and several other possibilities are currently under investigation.

The lack of interaction between Cremophor EL and the *cis*-Pt analogue carboplatin in our *in vitro* model in PBL is in line with previous clinical observations demonstrating a sequence-independent effect on the myelotoxicity of the combination paclitaxel and carboplatin [30, 31]. In point of fact, in contrast to studies combining paclitaxel or docetaxel and *cis*-Pt, no diminution in expected leukocytopenia has been reported in any of the trials with paclitaxel/carboplatin combination chemotherapy [32]. Several recent studies, however, found that this combination was better tolerated than anticipated, particularly with respect to thrombocytopenia [33]. The mechanism underlying this observation remains to be elucidated.

In conclusion, this study provides evidence supportive of the conjecture that the pharmaceutical vehicles Cremophor EL and Tween 80 are major determinants in the reduced *cis*-Pt-associated myelotoxicity observed in clinical combination chemotherapy studies with paclitaxel and docetaxel. Furthermore, the pharmacokinetic selectivity for the central blood/bone marrow compartment, combined with a direct protection of normal haematopoietic cells by limitation of *cis*-Pt uptake make Cremophor EL a promising candidate for the selective modulation of *cis*-Pt-induced myelotoxicity in a clinical setting of short-interval *cis*-Pt administration. Presently, this concept is under investigation in a rodent tumor-bearing model.

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Polymer-Drug Conjugates: Recent Development in Clinical Oncology

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Abstract

Targeted drug delivery aims to increase the therapeutic index by making more drug molecules available at the diseased sites while reducing systemic drug exposure. In this update, we provide an overview of polymer-drug conjugates that have advanced into the clinical trials. These systems use synthetic water-soluble polymers as the drug carriers. The preclinical pharmacology and recent data in clinical trials with poly(L-glutamic acid)-paclitaxel (PG-TXL) are discussed first. This is followed by a summary of conjugates of a variety of polymeric conjugates with chemotherapeutic agents. Results from early clinical trials of these polymer-drug conjugates have demonstrated several advantages over the corresponding parent drugs, including fewer side effects, enhanced therapeutic efficacy, ease of drug administration, and improved patient compliance. Collectively, these data warrant further clinical development of polymer-drug conjugates as a new class of anticancer agents.

Keywords

Polymer-Drug Conjugates; Anticancer Drugs; Nanocarriers; Paclitaxel; PG-TXL

1. Introduction

Most chemotherapeutic drugs used clinically are limited by a relatively low therapeutic index, owing to toxic side effects. In the field of anticancer therapy, two strategies for improving the therapeutic efficacy of anticancer agents have emerged over the past several decades. The first approach is the design and development of agents that modulate the molecular processes and pathways specifically associated with tumor progression. The success of this approach is shown by the successful introduction of a new breed of molecularly targeted anticancer agents such as imatinib mesylate (Gleevec), gefitinib (Iressa), trastuzumab (Herceptin), and cetuximab (C225, Erbitux). Alternatively, existing anticancer agents can be made more effective by using nanocarriers that bring more drug molecules to the tumor site, compared with the conventional formulation, while reducing exposure of normal tissues to the drug.

The idea of covalently attaching chemotherapeutic agents to a water-soluble polymer was first proposed by Ringsdorf [1] in the mid-1970s. In this model, it was envisioned that not only could the pharmacokinetics of the drug attached to the polymeric carrier be modulated but also

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that active targeting could be achieved if a homing moiety was introduced to the same polymeric carrier (Fig. 1). Since then, polymer-drug conjugates have become a fast-growing field, with nearly a dozen polymeric conjugates advancing to the clinical trial stage (Table 1). Results from early clinical trials of these polymer-drug conjugates have demonstrated several advantages over the corresponding parent drugs, including fewer side effects, enhanced therapeutic efficacy, ease of drug administration, and improved patient compliance. Increased therapeutic efficacy is achieved primarily through an enhanced permeability and retention (EPR) effect of long-circulating polymers [2]. Among synthetic polymer-drug conjugates, poly (L-glutamic acid) (PG)-paclitaxel (PG-TXL) (CT-2103, Xyotax®) has advanced to Phase III clinical trials and is positioned to be the first of its class to reach the market. We previously reviewed the chemistry and applications of PG conjugates with various chemotherapeutic agents [3]. In this article, we will update the current clinical development of polymer-drug conjugates as novel anticancer agents, with particular emphasis on PG-TXL. Many excellent reviews with different focuses have been published, including those based on polymer-protein conjugates [4–7], most of which use polyethylene glycol (PEG) as the drug carrier [5–9]. These materials will not be covered here.

2. PG-TXL (CT-2103)

2.1. Antitumor Activity

Paclitaxel (Taxol®, TXL) is a mitotic spindle poison that promotes microtubular aggregation and interferes with essential cellular functions such as mitosis, cell transport, and cell motility [10]. Taxol® has shown a remarkable anti-neoplastic effect in a wide range of human cancers. Initially approved for the treatment of refractory ovarian cancer in 1992, it is now the first-line therapy for metastatic breast cancer and advanced ovarian cancer. In women with metastatic breast cancer, Taxol® produced an objective response rate of 32–62% [11]. Its effectiveness has also been demonstrated against non-small cell lung cancer (NSCLC), head and neck cancer, melanoma, and Kaposi's sarcoma. Because of its poor aqueous solubility, use of Taxol® requires a mixture of polyethoxylated castor oil (Cremophor) and ethanol as a vehicle, and it must be infused over 3 to 24 h.

PG-TXL was initially developed to overcome the poor aqueous solubility of Taxol® and hypersensitivity reaction associated with the use of Taxol® formulated with Cremophor/ alcohol. In PG-TXL, paclitaxel is conjugated to a synthetic polyamino acid, poly(L-glutamic acid) (PG) through its 2'-hydroxyl group via an ester linkage (Fig. 2). The resulting conjugate is highly water soluble (>20 mg/kg) and has demonstrated significantly enhanced antitumor efficacy and improved safety compared with paclitaxel in preclinical studies [12]. In a well-established syngeneic murine ovarian OCa-1 carcinoma model, a single dose of PG-TXL in saline (10 mg equiv. paclitaxel/ml) at a dose of 80 mg equiv. paclitaxel/kg caused significant tumor growth delay compared with the same dose of Taxol® in Cremophor/alcohol. Furthermore, a complete tumor cure was observed in all mice injected (n = 26) with PG-TXL at its maximal tolerated dose (MTD) of 160 mg equivalent paclitaxel/kg, which is two-fold higher than that of free paclitaxel. Similarly, treatment with PG-TXL caused complete tumor eradication at the MTD of PG-TXL (60 mg equiv. paclitaxel/kg) and at a lower dose of 40 mg/kg in Fischer rats with well-established rat breast 13762F adenocarcinoma. In contrast, paclitaxel at the dose of 40 mg/kg (its MTD) caused only a transient growth delay of approximately 15 days (Fig. 3).

The antitumor activity of PG-TXL was further evaluated in a panel of syngeneic murine tumors (MCA-4 and MCA-35 breast cancer, HCa-1 hepatocellular carcinoma, and F5a-II sarcoma; intramuscular inoculation), a human SKOV3ip1 ovarian tumor injected intraperitoneally, and an orthotopic human MDA-MB-435Lung2 breast tumor grown in the mammary fat pad of nude mice [13,14]. All treatments were given intravenously as a single bolus injection. All

murine tumors, whether sensitive or resistant to paclitaxel treatment, showed significant growth delay with PG-TXL at its maximum tolerated dose (160 mg equivalent paclitaxel/kg) or even at lower doses. In SKOV3ip1 tumor, PG-TXL significantly extended the survival of the mice. The median survival time for PG-TXL at 120 mg equivalent paclitaxel /kg was 75 days. In contrast, no difference in median survival time was found between paclitaxel -treated mice (56 days) and Cremophor vehicle-treated mice (59 days). In an orthotopic, metastatic MDA-MB-435Lung2 tumor model, PG-TXL at 120 mg equivalent paclitaxel/kg produced regression of the tumor in 50% of the cases. In the remaining mice with progressively growing tumors, micrometastases in the lung were found in only 25% of the animals. In comparison, treatment with paclitaxel at 60 mg/kg did not result in tumor regression, and metastases were found in 42% of the mice. In nude mice bearing i.p. implanted NMP-1 and HEY ovarian tumors, which were paclitaxel-resistant, significant improvement in survival and even some cures were observed after a single i.p. treatment with PG-TXL [14]. These studies demonstrated that PG-TXL has significant therapeutic activity against a broad range of solid tumors and metastatic disease.

2.2. Preclinical Pharmacology and Mechanism of Action

The observation that PG-TXL had antitumor activity superior to that of free paclitaxel and that PG-TXL was active against paclitaxel-resistant tumors in preclinical studies suggests that PG-TXL might possess favorable pharmacokinetic properties and/or have a mechanism of action different from that of paclitaxel. *In vitro*, both paclitaxel and PG-TXL induced extensive telomere erosion and telomeric associations, which are the early manifestations of programmed cell death [15]. Morphological analysis and biochemical characterizations in a panel of breast cancer cell lines confirmed that both PG-TXL and paclitaxel had similar abilities to induce apoptosis, independent of p53 status. Flow cytometric analysis further revealed that both PG-TXL and the free drug induced a characteristic G2/M arrest in the cell cycle [16]. However, compared with paclitaxel, PG-TXL appears to have reduced potency *in vitro*. For example, PG-TXL is >20 times less potent in supporting the growth of a paclitaxel-dependent CHO mutant cell line [12]. These data are consistent with the disturbance of microtubule polymerization as the major mechanism of action for PG-TXL and suggest that the release of paclitaxel or active species from PG-TXL is required for PG-TXL to exert its action.

In vivo, PG-TXL showed a biodistribution pattern different from that of free paclitaxel [17]. On the basis of area under the tissue concentration-time curve (AUC) values, tumor exposure to paclitaxel was five times greater when administered as PG-TXL than as paclitaxel formulated in Cremophor EL/alcohol vehicle. PG-TXL was retained much longer than free paclitaxel in the tumor due to a slower elimination of the drug conjugate. Furthermore, concentrations of free paclitaxel released from PG-TXL remained relatively constant in tumor tissue over a period of 144 h, whereas the concentration of free paclitaxel in the tumor of mice injected with paclitaxel in Cremophor EL/alcohol was reduced more than 6-fold by 144 h after injection. Singer et al. [18] showed that PG-TXL had a prolonged blood half-life and negligible release of the activity agent, paclitaxel (<1% of the total taxane). Thus, enhanced permeability and retention effect of PG-TXL are likely important contributing factors responsible for the superior therapeutic index of PG-TXL observed in preclinical studies.

Results comparing cellular uptake of tritium-labeled [³H]PG-TXL ([³H] labeled on PG polymer) and PG-[³H]TXL ([³H] labeled on paclitaxel) in tumor cells are intriguing. Whereas an insignificant amount of radioactivity was found taken up by MDA-MB-453 breast cancer cells when [³H]PG-TXL was used, there was a time-dependent increase of cell-associated radioactivity when PG-[³H]TXL was incubated with MDA-MB-453 cells. Moreover, cellular uptake of PG-[³H]TXL was more than 100 times higher than that of [³H]PG-TXL [16]. These results suggest that it was the free paclitaxel released into the culture media that had been taken

up by the cells. Intact PG-TXL was probably taken up by cancer cells through less efficient pinocytosis. PG-TXL, however, can be taken up efficiently by phagocytosis. Using HPLC/mass spectral analysis, Shaffer et al. [19] recently compared the profiles of PG-TXL metabolites in murine RAW 264.7 cells with monocyte/macrophage-like activity and in cancer cells (NCI-H460 lung cancer cells and HT-29 colon cancer cells). They showed that the major intracellular metabolites of PG-TXL resulting from the degradation of polymer backbone were monoglutamyl-2'-paclitaxel (Glu-2'-TXL) and diglutamyl-2'-paclitaxel (H₂N-Glu-Glu-2'-TXL). Significantly, the intracellular concentrations of Glu-2'-TXL and H₂N-Glu-Glu-2'-TXL were 100–1000 times higher in RAW 264.7 cells than in the cancer cells [19].

If PG-TXL could be efficiently taken up by macrophages, what is the role of macrophages residing in the tumors in intratumoral dispersion of PG-TXL? An *in vivo* imaging study using PG-DTPA-Gd, a magnetic resonance imaging (MRI)-visible PG surrogate, showed that PG-DTPA-Gd was initially localized to the perivascular space after its intravenous injection but was distributed to the necrotic zone of the tumors at later times (2–4 days). Interestingly, immunohistochemical analysis revealed that biotinylated PG-DTPA-Gd co-localized with macrophages in the necrotic areas (Fig. 4) [20]. These results suggest that PG-DTPA-Gd, and possibly other PG-drug conjugates, may be captured by resident macrophages in the necrotic areas or by migrating macrophages that eventually infiltrate into the necrotic tissues. Consistent with this hypothesis, recent studies using a monoclonal antibody that specifically recognizes PG-TXL showed that PG-TXL was taken up by macrophages *in vitro* through endocytosis [21] and that PG-TXL preferentially localized to perinecrotic areas in tumors *in vivo* [22]. Further study showed that enhancement of the necrotic tissue of the tumors was observed only with polymeric contrast agents and not with low-molecular-weight contrast agents, including oligo(L-glutamic acid)-DTPA-Gd [20], suggesting that prolonged blood circulation is required for the polymer to interact with macrophages.

Consistent with the MRI findings obtained with PG-DTPA-Gd, autoradiographic studies in mice injected with PG-[³H]TXL found that radioactivity was primarily located in the periphery of the tumor initially and was heterogeneously distributed throughout the tumor at a later time (i.e., by day 6) (Fig. 5) [17]. Three possible routes may account for the dispersion of radioactive species towards the center of the tumor: 1) diffusion of intact PG-[³H]TXL; 2) diffusion of small-molecular-weight metabolites of PG-[³H]TXL such as free [³H]paclitaxel, Glu-2'-TXL, and H₂N-Glu-Glu-2'-TXL; and 3) uptake of PG-[³H]TXL by macrophages and subsequent migration of macrophages within the tumor mass. Intact PG-TXL, as has been found with other macromolecules and nanoparticles, is more likely to localize in the perivascular space after extravasation from tumor microvessels with minimal penetration into the tumor matrix [23, 24]. Therefore, in addition to the possible role of macrophages in the dispersion of PG-TXL within the tumor interstitium, the role of PG-TXL degradation in perivascular fluid space on the dispersion of active species (i.e., radioactivity associated with PG-[³H]TXL and its metabolites) throughout the tumor mass should be further examined.

Recent studies suggest that selective proteolysis of the PG backbone through the action of cellular proteases in tumors, particularly cathepsin B, may be responsible for the increased site-specific delivery and enhanced antitumor activity of PG-TXL [19,25]. Cathepsin B is a lysosomal cysteine protease in normal cells and tissues. In premalignant and malignant lesions, the expression of cathepsin B is highly upregulated, and the enzyme is secreted and becomes associated with the cell surface [26]. Cathepsin B has been implicated in the degradation of the extracellular matrix, a crucial step in tumor dissemination and angiogenesis. Using a noninvasive near-infrared fluorescence optical imaging technique, we recently demonstrated selective degradation of L-PG polymer but not D-PG polymer in an orthotopic U87 glioma tumor model (Fig. 6) [25]. Such a technique can be very useful in investigating the spatial and

temporal distribution of the degradation products of PG-based polymeric drugs and the effect of polymer degradation on the antitumor activity of polymer-drug conjugates.

2.3. Clinical Findings

Results of clinical trials of CT-2103 (Xyotax®, PG-TXL) from data available in the literature are summarized in Table 1.

Phase I Studies—In the CT-2103 formulation used in clinical studies, the median molecular weight of PG-TXL is 48,000 Da, and the content of paclitaxel is about 37% by weight, equivalent to approximately one paclitaxel molecule for every 11 glutamic acid units in each PG polymer chain [18]. This formulation eliminated the use of Cremophor and alcohol and allows infusion of paclitaxel as PG-TXL over 30 minutes. In the initial clinical trials in the United Kingdom, the investigators gave PG-TXL (CT-2103) to cancer patients in a 30-minute infusion every 3 weeks at doses ranging from 30 to 720 mg/m² [27]. They detected CT-2103 in the plasma of all of the patients and observed a long plasma half-life of up to 185 h. CT-2103 could be detected in plasma 3 weeks after administration at a dose of 200 mg/m². Importantly, the AUC of free paclitaxel was about 1% to 2% of the AUC of CT-2103, which supported the *in vivo* stability of CT-2103 in the plasma and the slow, prolonged release of the active moiety [28]. The steady-state volume of distribution range was low ($V_{ss} = 2.7\text{--}13.2\text{ L}$), which suggests that the distribution of CT-2103 was restricted mainly to the plasma and other extracellular body fluids [29]. The MTD was 233 mg/m² with a 3-weekly schedule and 177 mg/m² with a more intense 2-weekly schedule. The dose-limiting toxicity (DLT) was primarily neuropathy. Notably, alopecia was almost absent in patients treated with CT-2103 compared with an incidence of over 50% following Taxol® as a 3- or 24-h infusion.

A second Phase I study evaluated the MTD of CT-2103 in combination with carboplatin in patients with a variety of solid tumors refractory to conventional treatment [30]. The MTD was determined to be 250 mg/m², and DLTs observed at 250 mg/m² were neutropenia and thrombocytopenia. Interestingly, the three patients who had partial responses in this series were all patients with ovarian cancer who had previously undergone unsuccessful Taxol® therapy.

Another Phase I study determined the MTD and DLTs of PG-TXL and concurrent radiation (50.4 Gy) in patients with esophageal and gastric cancer [31]. The MTD was found to be 70 mg/m²/week, with gastritis, esophagitis, neutropenia, and dehydration being the DLTs. Strong antitumor activity was observed in this Phase I study, with four (33%) of 12 patients with loco-regional disease undergoing a complete clinical response. An additional seven patients (58%) had partial response (>50% tumor reduction by post-treatment endoscopy). These promising preliminary results with combined PG-TXL and radiation indicate that PG-TXL warrants further evaluation as a radiosensitizer in other solid tumors.

Phase II Studies—A multicenter Phase II trial of PG-TXL to determine the efficacy and toxicity in heavily pretreated patients with advanced ovarian cancer has been completed [18, 32]. Ninety-nine patients registered in this trial received PG-TXL at a dose of 175 mg/m² every 3 weeks. The toxic effects were mild and consisted of grade 3 neuropathy (15%), grade 3 neutropenia (15%), and grade 4 neutropenia (9%). The observed response rates (28% in platinum-sensitive patients and 10% in platinum-resistant patients with one or two prior regimens) are in line with results seen in studies of other agents.

A recent report on a Phase II study in patients with metastatic breast cancer showed an unexpected incidence (22%) of hypersensitivity reactions [33]. This reaction is puzzling, considering that the approximate incidence of grade 3 or 4 hypersensitivity reaction in over 1,400 patients who have received CT-2103 in clinical trials was only 2%. Because the majority of women were postmenopausal at the study entry, one possible explanation is the differing

pharmacokinetics of CT-2103 in this population of patients. Further studies are needed to determine the possible roles of sex, hormonal status, and the duration of treatment in hypersensitivity reactions to CT-2103. Of the 18 patients treated with CT-2103, four (22%) had responses. In comparison, a study with Taxol® in a similar population of patients with largely HER2-negative metastatic breast cancer showed a 14% objective response rate in the first-line setting [33]. Unfortunately, because of the unexpected high incidence of hypersensitivity reaction, this Phase II study was terminated early, and the authors were unable to evaluate the clinical benefit rate or time to progression.

In an ongoing Phase II trial in patients with androgen independent prostate cancer, CT-2103 was found well tolerated and demonstrates activity measured by PSA response and PSA velocity changes [34]. Of the 21 patients enrolled to date (16 previously treated/5 untreated), 2 previously treated patients and 1 previously untreated responded based on a 50% PSA reduction that were maintained for at least 8 wks. In addition, 4 previously treated and 2 untreated patients demonstrated a delay in their PSA velocity. Adverse events included hypersensitivity reaction (1 patient), grade 2/3 neuropathy (7 patients), grade 3/4 neutropenia (8 patients) and grade 3 thrombocytopenia (1 patient). Currently, this trial continues to enroll patients.

Phase II studies in patients with resistant colorectal cancer and in previously untreated high-risk patients with NSCLC have been completed [18].

Phase III Studies—More than 1,700 patients with NSCLC have participated in Phase III trials of PG-TXL (CT-2103), which included two Phase III trials of CT-2103 as first-line treatment in performance status-2 (PS2) patients (STELLAR 3 and STELLAR 4) and one Phase III trial of CT-2103 as second-line treatment in PS0-2 patients (STELLAR 2) [18,35]. In the STELLAR 3 trial, CT-2103 plus carboplatin AUC 6 was compared with Taxol® plus carboplatin; in the STELLAR 4 trial, CT-2103 used as a single agent was compared with gemcitabine or vinorelbine; in STELLAR 2, CT-2103 used as a single agent was compared with docetaxel. In all studies, the primary efficacy end point was the duration of overall survival. The total numbers of patients in the STELLAR 2, STELLAR 3, and STELLAR 4 trials were 850, 400, and 477, respectively. As the results of the Phase III study completed in 2005, there was no significant overall improvement in survival of the patients evaluated. However, these studies did demonstrate similar efficacy, reduced side effects, and more convenient administration of CT-2103 compared with the control treatment arms [18,35].

Interestingly, in a subset evaluation of women with NSCLC, the premenopausal women fared significantly better as long as their estrogen levels were normal. Clinical data from a pooled analysis of CTI's STELLAR 3 and 4 trials showed that in the 198 women treated on those trials, superior survival was observed in those who received CT-2103 ($p = 0.03$). The most notable effect was among women less than 55 years old and presumably pre-menopausal who were treated with CT-2103 compared with standard chemotherapy (median survival 10.0 vs. 5.3 months, hazard ratio = 0.51, log rank $p = 0.038$), while a survival trend ($p = 0.134$) was observed in women 55 years of age and older (post-menopausal) [36]. The favorable antitumor activity among women less than 55 years stimulated the initiation of the PIONEER 1 clinical Phase III trial to evaluate prospectively CT-2103 given at a dose of 175 mg/m² once every 3 weeks in women with advanced NSCLC and PS2 status [37].

In general, CT-2103-related nonhematological toxicities were similar to those for other agents, with dose-related neurotoxicity as the most important clinical issue [35]. Neuropathy was a common toxicity associated with exposure to taxanes. In STELLAR 3, the frequency of neuropathy (all grades) was similar between treatment arms; grade 3 neuropathy occurred in 17% of patients receiving CT-2103 (210 mg/m²) versus 10% of patients receiving Taxol®.

CT-2103-induced neuropathy seemed to be dose dependent. Grade 3 neuropathy was limited to 4% of patients receiving single-agent CT-2103 175 mg/m² in STELLAR 4. Neurotoxicity occurred significantly later with CT-2103 (210 mg/m²) compared with Taxol® in STELLAR 3, suggesting that there might be slow accumulation of CT-2103 in peripheral nerves at doses greater than (175 mg/m²). Alternatively, the longer terminal half-life of CT-2103 might be related to increased neurotoxicity at higher CT-2103 doses. These observations suggest that significant neurotoxicity can be minimized by administering CT-2103 at 175 mg/m². Consequently, this dose will be used in subsequent trials.

A randomized Phase III trial of maintenance chemotherapy comparing 12 monthly cycles of single agent paclitaxel or Xyotax™ versus no treatment in women with stage III/IV ovarian epithelial or peritoneal cancer is currently recruiting participants (www.cancer.gov). This trial, sponsored by the National Cancer Institute and the Gynecologic Oncology Group (GOG), is projected to recruit 1550 eligible participants. The primary objective of this study is to determine whether Xyotax™ or paclitaxel, administered to women with advance ovarian cancer who have attained a clinically-defined complete response to primary platinum/taxane chemotherapy, will reduce the death rate, compared to retreatment at the time of documented disease progression. The secondary objective is to determine if, in this clinical setting, Xyotax™ produces a more favorable toxicity profile and superior quality of life compared to paclitaxel.

Summary—Compared with conventional paclitaxel-based treatment, PG-TXL showed three safety-related advantages. First, alopecia was rare, and complete hair loss was not observed. Second, nausea and vomiting were uncommon. Third, hypersensitivity reactions were rarely observed, and those that did occur were usually mild to moderate; thus, routinely used prophylactic premedications were not required. CT-2103 is active against a variety of cancers. Further studies to define toxicity and efficacy in patients with less prior therapy are needed to determine the role of CT-2103 in these cancers as first-line and second-line therapy. Of note is the observed improvement in overall survival for women receiving CT-2103 vs. control in combined log-rank analysis of STELLAR 3 and 4 Phase III trials. Consequently, a request for approval of CT-2103 for the treatment of NSCLC in women with normal estrogen levels (premenopausal) based on non-inferiority will be submitted to the EMEA, the European equivalent of the FDA, in 2008. In the United States, a confirmatory Phase III trial was initiated in 2007 on 450 women (normal premenopausal estrogen levels) for the treatment of NSCLC, comparing CT-2103 and carboplatin with paclitaxel and carboplatin. If results are favorable, CT-2103 will be submitted to the FDA for approval in 2009.

2.4. Modulation of Antitumor Therapy with External Radiation

Combining chemotherapy and radiotherapy has significantly improved response and survival rates in patients with many solid tumors. Many chemotherapeutic agents can increase the radiosensitivity of tumors, thus potentiating the tumor response to radiation-caused damage. We hypothesized that combining radiotherapy and chemotherapy using a polymer-drug conjugate could lead to a stronger radiosensitizing effect than using the parent drug owing to sustained release of the free drug from polymer-bound drug in the tumor. Irradiation can in turn potentiate the tumor response to polymer-drug conjugates by increasing tumor vascular permeability and thus the uptake of these conjugates into solid tumors. To test this hypothesis, PG-TXL was used as a model polymer-drug conjugate. Administration of PG-TXL delayed the growth of OCa-1 syngeneic murine ovarian tumors in C3Hf/Kam mice. However, when PG-TXL was given in combination with tumor irradiation, antitumor activity was significantly enhanced. Using tumor growth delay as an end point, enhancement factors ranging from 1.36 to 4.4 were observed; these values depended on the doses of PG-TXL and radiation delivered. Complete tumor regression was found with the use of increased radiation doses (>10 Gy) and

PG-TXL doses (>80 mg/kg equivalent paclitaxel) [38,39]. Similar results were observed in a mammary MCa-4 carcinoma model [40]. In contrast, combined radiotherapy and paclitaxel treatment yielded an enhancement factor of less than 1.0 in MCa-4 tumors, indicating that conjugation of paclitaxel with PG is necessary to improve the radiosensitization effect of paclitaxel. When the treatment end point was tumor cure, the enhancement factors were as high as 8.4 and 7.2 after fractionated and single-dose radiotherapy, respectively [41,42].

To determine whether prior irradiation affects tumor uptake of PG-TXL, [^3H]PG-TXL was injected into mice with OCa-1 ovarian tumors 24 h after local irradiation at 15 Gy [39]. The uptake of [^3H]PG-TXL in irradiated tumors was 28% to 38% higher than that in nonirradiated tumors at different times after injection of [^3H]PG-TXL, suggesting that irradiation increased the accumulation of PG-TXL in the tumors. Thus, the super-synergistic effect of combined radiotherapy and PG-TXL-based chemotherapy is partly ascribed to the enhanced permeability and retention effect of macromolecules caused by irradiation.

These promising preclinical studies have prompted the initiation of clinical trials combining PG-TXL with concurrent radiation in patients with esophageal and gastric cancer [31]. Data from this Phase I trial demonstrated major tumor responses in 91% of patients.

3. Other Polymer-Drug Conjugates in the Clinic

Over the last decade, nearly a dozen polymer-drug conjugates have entered clinical studies (Table 2). Early results have generally been promising, and antitumor activity has been seen in chemotherapy-refractory patients. On the basis of the current status in clinical studies, these conjugates can be categorized into three groups: 1) Conjugates that have progressed further to Phase II/III trials or conjugates for which further studies are planned. Polymer-drug conjugates belonging to this category include PK1, PK2, HPA copolymer-platinate, polymeric micelle NK911, carboxymethyl-dextran-bound CPT DE-310, and poly(L-glutamic acid)-paclitaxel XyotaxTM. 2) Conjugates that further clinical development has been either discontinued or suspended because of unacceptable toxicity, inadequate linker chemistry, or pharmacoeconomic considerations. HPA copolymer-camptothecin, HPA copolymer paclitaxel, PEG-camptothecin, PEG-paclitaxel, and PG-CPT (CT-2106) belong to this group. 3) Conjugates just entered into clinical trial studies, such as PEG-SN38 (EZN-2208) and a linear, cyclodextrin-bound CPT (IT101).

3.1. HPA Copolymer-Doxorubicin (PK1)

Doxorubicin is an anthracycline cytotoxic agent widely used in the treatment of solid tumors, lymphoma, and leukemia. The DLTs of doxorubicin include bone marrow suppression, mucositis, and cardiotoxicity. In an attempt to reduce its toxicity, doxorubicin was conjugated to a water-soluble synthetic polymer, *N*-(2-hydroxypropyl) methacrylamide (HPA)/methacrylic acid copolymers through a tetrapeptide linker (Gly-Phe-Leu-Gly) [43,44]. The tetrapeptide linker allowed selective release of the active drug in the tumors through the action of lysosomal enzymes. The conjugate, known as PK1, was found to be more potent than free doxorubicin in antitumor activity in preclinical animal studies [45,46]. PK1 was the first synthetic polymer-anticancer drug conjugate that entered clinical trials (in 1994) [47]. Data from Phase II trials for the treatment of breast, NSCLC, and colon cancers were presented in 2002 and showed positive responses in six of 63 patients, with manageable side effects [48]. The subject of development history, mechanism of action, and pharmacology for PK1 has been reviewed extensively [48–51].

3.2. HMPA Copolymer-Doxorubicin-Galactosamine (PK2)

PK2 is a 27-kDa HMPA copolymer derivatized with 6.5% mol/wt, <2% free doxorubicin, and 2 % mol/wt galactose, with efficient targeting of the asialoglycoprotein receptor selectively expressed on hepatocytes and in hepatomas. In preclinical murine models, 80% of an administered dose targeted the liver, associated with high anticancer activity. PK2 remained the only targeted polymeric conjugate to be tested clinically to date [52]. In Phase I/II trial, the maximum tolerated dose of PK2 was 160 mg/m², with neutropenia being the DLT. Hepatic targeting was confirmed by planar imaging and single photon emission computed tomography (SPECT) with ¹²³I-labeled PK2 co-injected with unlabeled PK2. The majority of conjugate was present in normal liver (16.9% after 24 h), with lower accumulations within the hepatic tumors (3.2%).

3.3. HMPA Copolymer-Camptothecin (MAG-CPT, PNU166148) and HMPA Copolymer-Paclitaxel (PNU166945)

The camptothecins (CPTs) are a family of synthetic and semisynthetic analogues of 20(S)-camptothecin that exhibit a broad range of anticancer activity by inhibiting topoisomerase-1 activity. Two properties of CPT compounds limit their therapeutic efficacy in humans: instability of the lactone form because of preferential binding of the carboxylate to serum albumin, and a lack of aqueous solubility. Conjugation of CPT to water-soluble polymeric carriers has in general resulted in improved stability of the lactone ring and increased aqueous solubility.

In HMPA-CPT (MAG-CPT, PNU166148), CPT is attached to the copolymer at the C-20 hydroxyl group of CPT through an ester linkage. A Phase I study in a total of 23 patients showed DLTs of myelosuppression, neutropenic sepsis, and diarrhoea. The maximum tolerated dose and dose recommended for further clinical study was 200 mg equivalent CPT/m². The plasma half-life of both MAG-CPT and released CPT was extended to > 6 days, indicating that the kinetics of free CPT was release rate dependent [53]. Results presented in an earlier clinical study showed serious bladder toxicity which became dose limiting. Patients with higher plasma AUC values had the more severe symptoms of renal toxicity [54].

In HMPA copolymer-paclitaxel (PNU166945), paclitaxel was linked to the same tetrapeptide linker used to create PK1 and PK2. In a Phase I clinical study in a small patient cohort (12 patients), one patient with advanced breast cancer had a partial response. PNU166945 showed toxicity consistent with commonly observed toxicities associated with paclitaxel [55]. One patient developed grade 3 neurotoxicity. Dose escalation was discontinued prematurely due to concerns about potential clinical neurotoxicity.

3.4. HMPA Copolymer-Platininate (AP5346)

The platinum-based compounds, cisplatin and carboplatin, are standard treatment regimens for a wide range of cancers. The DLTs of these drugs include nephrotoxicity, neurotoxicity, and myelosuppression. 1,2-Diaminocyclohexane (DACH) has received increasing attention in recent years because of its activity against cisplatin-resistant cancer cells. In AP5346, DACH-platinum was bound to HMPA via a pH-sensitive chelate. A methacrylamide monomer substituted with a triglycine aminomalonate group provided the primary binding site for the DACH-platinum moiety [56]. The conjugate contained approximately 10% platinum by weight and had a molecular weight of 25 kDa, which was designed to allow glomerular filtration while also being large enough to benefit from an EPR effect. While negligible in neutral solutions, release of the DACH-platinum moiety was increased at low pH so that platinum release was favored in environments such as the extracellular space of hypoxic tumors and the intracellular lysosomal compartment. In a clinical Phase I study, AP5346 was administered as a 1-hour intravenous infusion on days 1, 8, and 15 of a 28-day cycle. Twenty-six patients received 41

cycles. Antitumor activity included two partial responses in metastatic melanoma and ovarian cancer and an additional CA-125 normalization in a suspected ovarian cancer. AP5346 administered weekly for 3 weeks out of every 4 weeks was tolerated up to a dose of 640 mg Pt/m² on the first cycle. The pharmacokinetics of AP5346 indicated a prolonged half-life (mean terminal half-life $t_{1/2}$ = 72 h) and evidence of antitumor activity. Additional human clinical trials are planned for this agent.

3.5. PEG-Camptothecin (Pegamotecan) and PEG-SN38 (EZN-2208)

Pegamotecan was developed by Enzon Pharmaceuticals, Inc. The conjugate consists of two CPT molecules conjugated to a 40-kDa PEG using an alaninate ester linkage. Free CPT must be cleaved from the PEG to be pharmacologically active. The hydroxyl group (-OH) at the 20-position of CPT is the active portion of the molecule responsible for the conformational changes between the active lactone and relatively inactive carboxylate forms. The 20-OH of CPT in Pegamotecan, is blocked by the alaninate linker, which stabilizes the CPT molecule into its active lactone conformation [57]. The MTD of Pegamotecan when administered weekly for 3 of 4 weeks was 3,240 mg/m², with neutropenia being its DLT [58]. Other grade 3 and 4 toxic effects were anemia, thrombocytopenia, fatigue, prolonged partial thromboplastin time, hemorrhagic cystitis, dysuria, and urinary frequency. Of the 27 patients enrolled, two patients had unconfirmed partial responses. Further development of Pegamotecan appears to be discontinued; the drug is no longer listed in the company's drug portfolio/pipeline (www.enzon.com).

The main limitation of PEG as drug carrier is the presence of only two reactive groups per polymer chain, which leads to an intrinsically low drug payload. To overcome this limitation, the construction of a dendron structure at the PEG's end chain has been proposed. Enzon is currently developing a conjugate of SN38, an active metabolite of CPT, with a 40-kDa PEG containing four arms. EZN-2208 has shown activity in a panel of human tumor xenografts [59]. A Phase I study of EZN-2208 to evaluate the safety and tolerability of intravenous EZN-2208 in patients with advanced solid tumors or lymphoma is currently enrolling participants (<http://clinicaltrials.gov>).

3.6. PEG-poly(Aspartic Acid)-Doxorubicin Micelles (NK911)

The polymeric micelle consists of a PEG-poly(aspartic acid) block copolymer conjugated with doxorubicin (NK911) [60]. PEG is believed to form the outer shell of the micelle, producing a 'stealth' effect that prevents NK911 from being captured by the liver and spleen. The doxorubicin-conjugated poly(aspartic acid) chain is hydrophobic and is believed to form the hydrophobic inner core of the micelles in aqueous media. The hydrophobic inner core enables NK911 to entrap a sufficient amount of doxorubicin. NK911 has a diameter of about 40 nm. The plasma AUC and the tumor AUC of NK911 were lower than those of liposomal doxorubicin because NK911 was less stable in the bloodstream than doxorubicin [60]. Twenty-three patients participated in a Phase I study performed at the National Cancer Center Hospital, Tokyo, Japan. Neutropenia was the predominant hematological toxicity, and grade 3 or 4 neutropenia was observed at doses of 50 and 67 mg/m². The DLT occurred at a dose of 67 mg/m² (grade 4 neutropenia lasting more than 5 days). A partial response was obtained in one patient with metastatic pancreatic cancer among the 23 patients. A Phase II trial of NK911 for the treatment of metastatic pancreatic cancer is ongoing.

3.7. Cyclodextrin-Based Polymer-CPT (IT-101)

IT101 is a conjugate of CPT and a linear, cyclodextrin-based polymer (CDP) [61,62]. The components of CDP are beta-cyclodextrin and polyethylene-glycol (PEG). Camptothecin is covalently attached to CDP through a glycine linker, which preserves CPT in its active form and increases its water solubility. Preclinical efficacy of i.v. IT-101 (molecular weight ~90

KDa) demonstrated a significant antitumor effect with IT-101 in a range of mouse xenografts, including several tumors that were resistant to CPT [62]. Pharmacokinetic and biodistribution studies indicated that CDP–CPT conjugate gave prolonged plasma half-life, which led to enhanced distribution to tumor when compared to CPT alone [61]. These favorable results prompted the initiation of a Phase I trial designed to determine the safety, MTD, and the pharmacokinetics of IT-101 when administered intravenously to patients with relapsed or refractory cancer. A total enrollment of 36 patients is expected to complete by first quarter of 2008.

3.8. Carboxymethyldextran-Exatecan (DE-310)

In DE-310, DX-8951 (exatecan mesylate, a CPT analogue) is linked to carboxymethyldextran (Mw = 340 kDa) via a glycyl-glycyl-phenylalanyl-glycyl-peptidyl spacer. This peptide spacer is intended to provide sustained release of the active moiety DX-8951 within the tumor as a result of enzymatic cleavage of the peptide by cathepsin B and cathepsin L [63]. In Phase I trial, patients received DE-310 as a 3-hour infusion once every 2 weeks (dose, 1.0–2.0 mg/m²) or once every 6 weeks (dose, 6.0–9.0 mg/m²) [64]. Neutropenia and grade 3 thrombocytopenia, and grade 3 hepatotoxicity with veno-occlusive disease, were dose-limiting toxicities. Other hematologic and nonhematologic toxicities were mild to moderate and reversible. The apparent half-life of conjugated DX-8951, glycyl-DX-8951, and DX-8951 was 13 days. The observed long blood half-lives are no surprising considering that DE-310 had the highest molecular weight among all polymer-drug conjugates tested so far. Of the 27 patients enrolled into the study, 1 patient achieved a histologically proven complete remission, 1 patient confirmed partial remission, and 14 additional patients showed disease stabilization.

3.9. PG-Gly-CPT (CT-2106)

PG was an effective solubilizing carrier of CPT and stabilized the E-ring lactone structure in CPT. PG-CPT conjugate was obtained by directly coupling the hydroxy group at the C20(S)-position of CPT with the carboxylic acid of PG [65]. When given intravenously in four doses every 4 days at an equivalent CPT of 40 mg/kg, PG-CPT delayed the growth of established H322 human lung tumors grown subcutaneously in nude mice. In mice that received intratracheal inoculation of H322 cells, the same treatment prolonged the median survival duration in mice by four-fold when compared with that in untreated control mice. These results showed that PG was as efficient carrier of CPT [65].

Studies have systematically investigated the structural effects of the antitumor efficacy of PG-bound CPT, including linkers between PG and CPT, the point of attachment of PG on the CPT molecule, the polymer molecular weight, and drug loading [66–68]. Coupling through the 20 (S)-hydroxy group of CPT with or without a glycine linker yielded the most active conjugates, because this site is located in close proximity to the lactone ring; thus, the E-ring lactone is better protected by the linked PG chains. However, the linkers affected the maximum drug payload; for example, only 15% (by weight) of CPT loading could be achieved for direct conjugation of PG-CPT by the ester linkage because of steric hindrance, whereas up to 50% CPT loading could be achieved for the case with a glycine as linker [67]. Increasing the molecular weight of PG from 33 to 50 kDa improved the antitumor efficacy of PG-Gly-CPT, probably because of an increased plasma half-life and reduced renal clearance [66]. Considering its ease of synthesis, superior aqueous solubility and stability, and marked efficacy in various *in vivo* tumor models, PG-Gly-CPT with 30–35% CPT was selected for further study.

CT-2106 (PG-Gly-CPT) entered clinical Phase I trials in April 2004. Preliminary results on pharmacokinetics in 19 patients with CT-2106 administered as a 10 minute intravenous infusion every 3 weeks were recently presented [69,70]. The apparent termination $t_{1/2}$ of unconjugated CPT is quite long—approximately 40 h. Five days after the first administration

(cycle 1), the excretion in urine of CT-2106 and unconjugated CPT accounted for 27.9% and 5.1% of the administered dose, respectively. Thus, CT-2106 generates prolonged systemic exposures to unconjugated CPT in plasma, and the urine excretion levels of conjugated camptothecin are substantial. A major CPT conjugated species found in urine was glu-gly-CPT. DLs included grade 3/4 neutropenia with concomitant grade 3 thrombocytopenia (3 patients, 105 mg/m²) and grade 2 hematuria (1 patient, 25 mg/m²). Most adverse events have been mild or moderate. Four patients had stable disease (SD) for >9 weeks; 2 lung cancer patients (25 mg/m²) had SD for >27 weeks. In a second Phase I trial in 26 patients, the MTD, toxicity, pharmacokinetics, and response rate of CT-21206 when administered weekly for 3 consecutive weeks of every 4-week cycle were determined [71]. With this schedule, the MTD was 75 mg/m². DLTs observed were grade 3 or 4 neutropenia and concomitant grade 3 or 4 thrombocytopenia in the three patients treated at 105 mg/m² and grade 2 hematuria in a patient treated at 25 mg/m². Of the 25 patients assessable for response, no objective responses (complete or partial) were noted. Three patients had stable disease. The absence of objective responses (complete or partial) was not surprising in this heavily treated population.

Presently, Cell Therapeutics has no clinical trial material for additional trials. Further development of CT-2106 is delayed so that the company can focus resources on the development of Xyotax™ and other agents in the pipeline.

4. Conclusions

Results from early clinical trials of about a dozen polymer-drug conjugates have demonstrated several advantages over the corresponding parent drugs, including fewer side effects, enhanced therapeutic efficacy, ease of drug administration, and improved patient compliance. Owing to its favorable physicochemical properties, PG has been shown to be an excellent polymeric carrier of therapeutic agents. Xyotax™ (PG-TXL) has become the first polymer-drug conjugate for the delivery of cytotoxic chemotherapeutic agents to advance to clinical Phase III trials. Clinical data collected over the last decade warrant further development of polymer-drug conjugates as a new class of anticancer agents. Future generation of polymer-drug conjugates will have to meet a number of challenges, including the development of novel polymers with modulated rates of degradation, versatile conjugation chemistry allowing site-specific attachment of targeting moieties, and polymerization methods that allow accurate control of polymer molecular weights and molecular weight distributions. The incorporation of novel imaging techniques into the clinical trial design of new polymer-drug conjugates is also expected to facilitate noninvasive monitoring of drug delivery efficiency in the era of individualized therapy.

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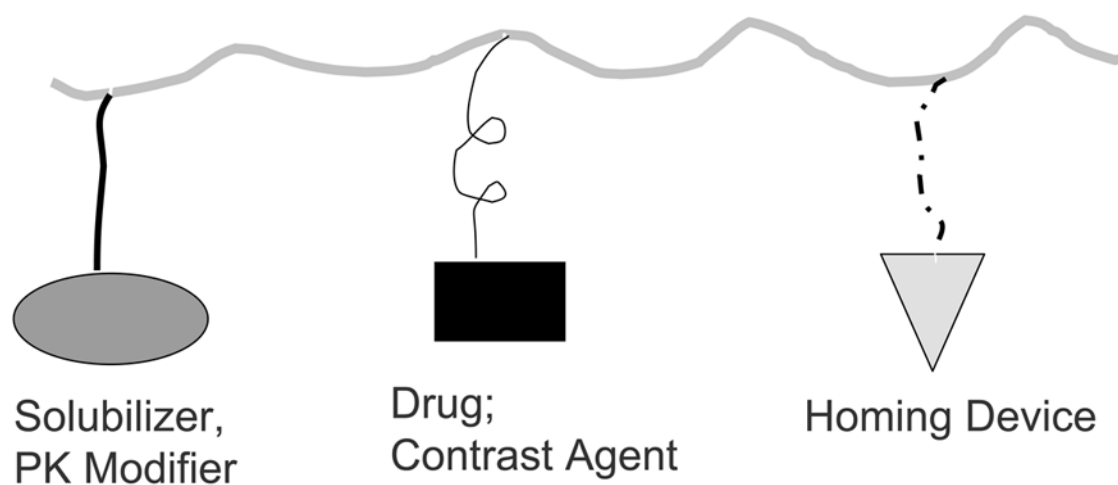


Fig. 1.

Model for targetable polymer-drug conjugates according to Ringsdorf [1], in which a solubility enhancer, a pharmacokinetic modifier, a homing device, and specific drugs or imaging probes could be attached to the same polymeric chain.

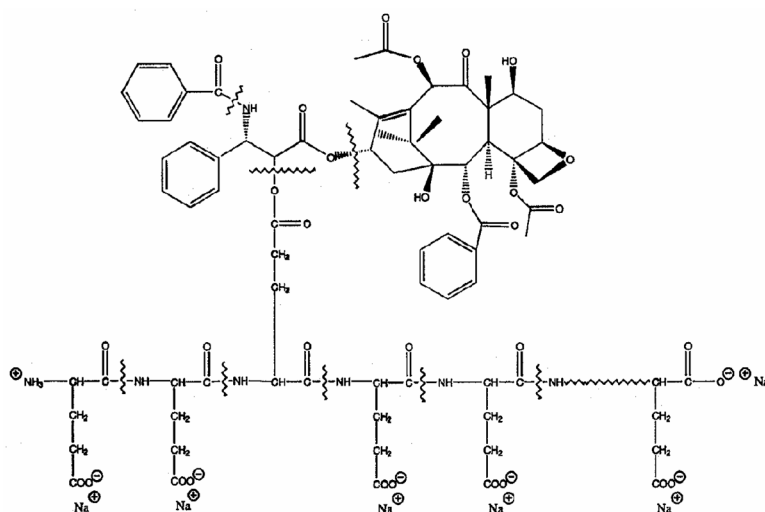


Fig. 2.
Structure of PG-TXL.

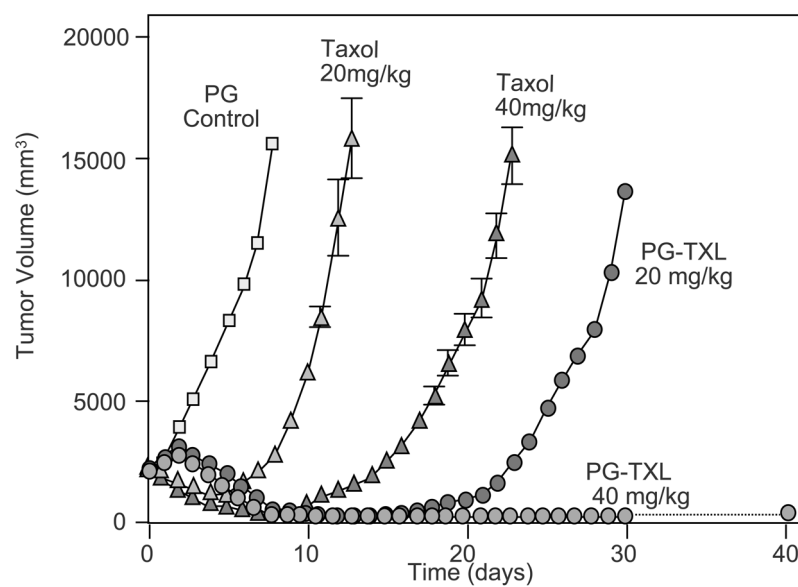
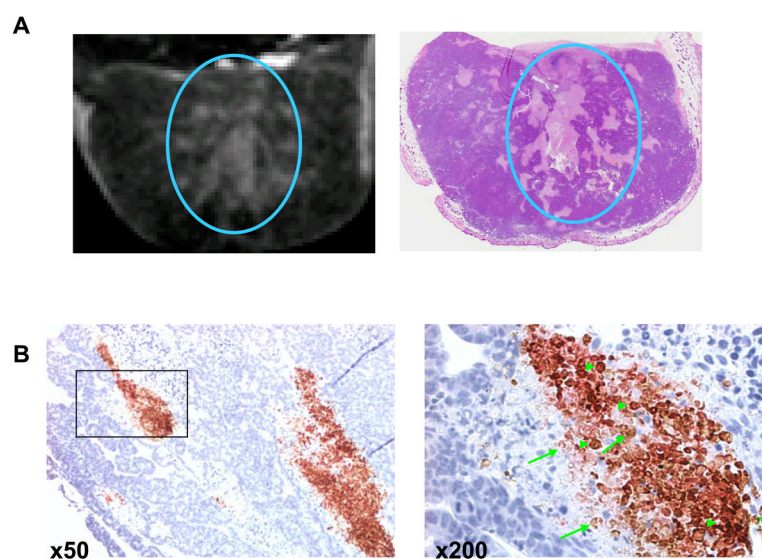


Fig. 3. Antitumor activity of PG-TXL in rats bearing rat breast tumor 13762F. Each drug was injected i.v. in a single dose at the indicated equivalent paclitaxel concentration. Data are presented as means $\pm$ SD of tumor volume (adapted from reference [12]).

**Fig. 4.**

A: Representative T_1 -weighted MRI image from a mouse bearing a subcutaneously inoculated Colo-205 tumor (left) and a photograph of the corresponding H&E-stained section of the tumor (right). The mouse received intravenous injections of L-PG-DTPA-Gd (0.04 mmol Gd/kg). L-PG-DTPA-Gd was distributed within the necrotic area (circle). B: Co-localization of macrophages and biotinylated L-PG-DTPA-Gd in the necrotic area in OCA-1 tumors. Staining with antibody directed against macrophage antigen F4/80 (reddish-pink) and with streptavidin-horseradish peroxidase and DAB for biotinylated L-PG-DTPA-Gd showed widespread macrophage infiltration in necrotic areas as well as distribution of the polymer and its degradation products to the necrotic area (brown). The sections were counterstained with hematoxylin (blue) to label the nuclei of viable tumor cells. Examples of biotin-L-PG-DTPA-Gd staining and dual F4/80 and biotin staining are indicated with arrows and arrowheads, respectively (from reference [20]).

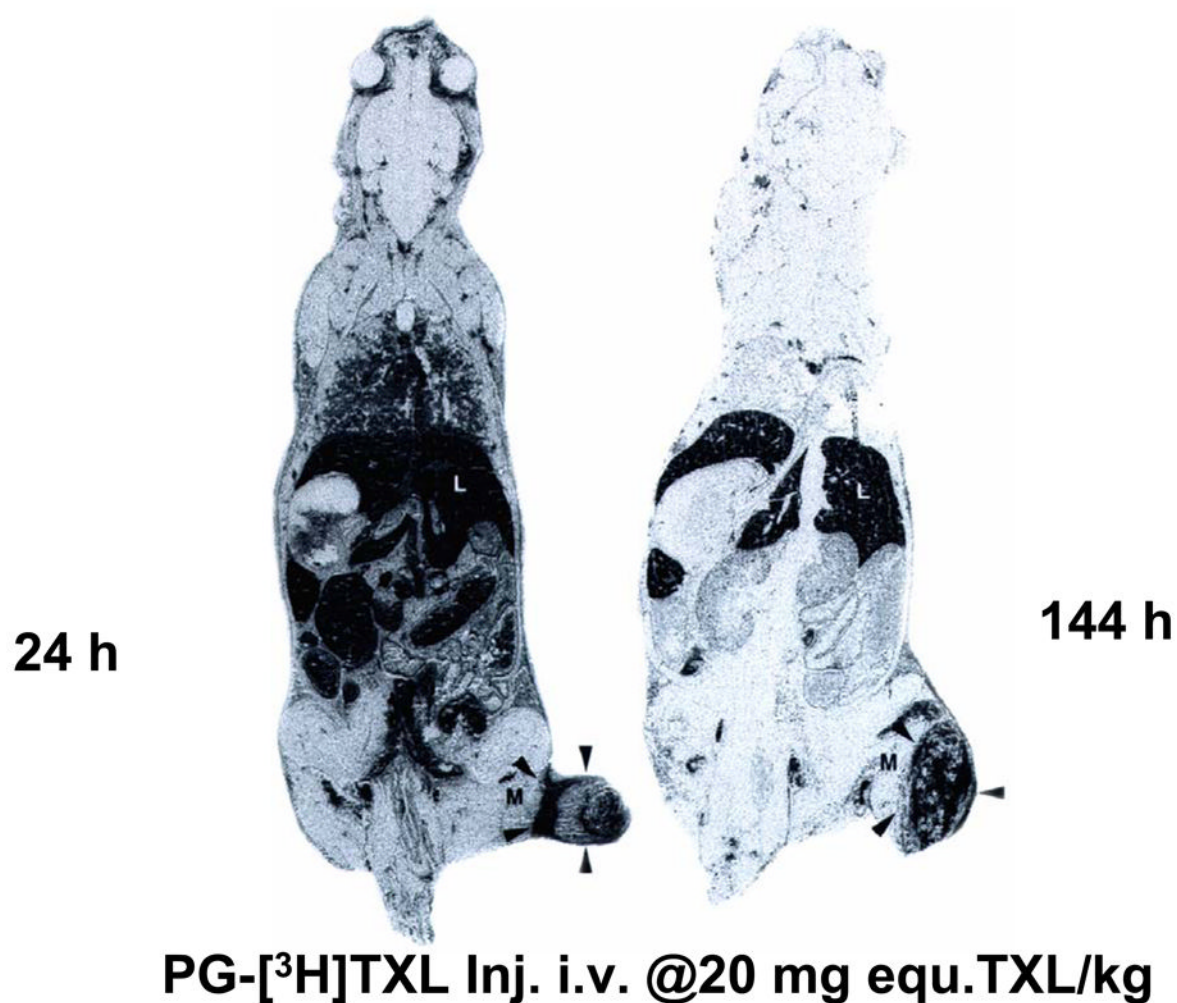


Fig. 5. Whole-body autoradiographs of mice killed 1 day (A) and 6 days (B) after tail vein injection of PG-[³H]TXL. Most radioactivity was localized to tumor periphery at 1 day after injection, but by day 6, radioactivity had diffused into the center of the tumor. L: liver; M: muscle; Arrow head: tumor (from reference [17]).

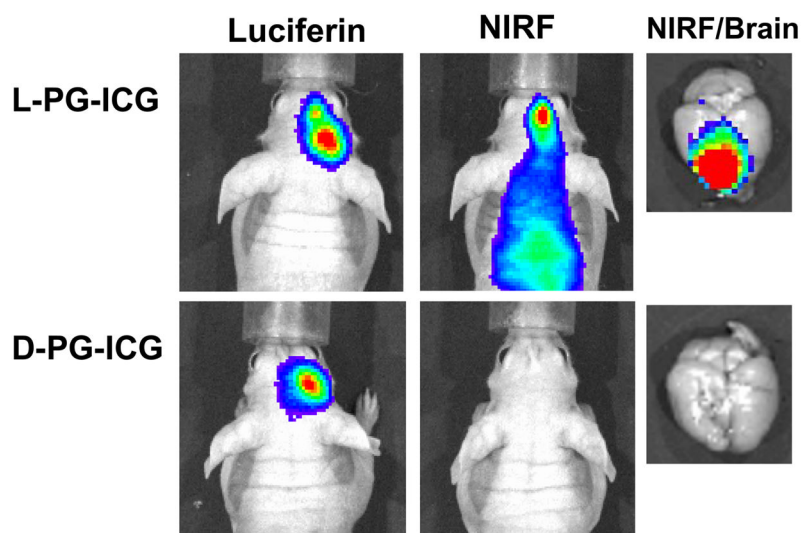


Fig. 6.

Representative *in vivo* near-infrared fluorescence (NIRF) images of cathepsin B activity in intracranially inoculated U87/TGL tumors. The mice used in the study had the same tumor burden, as indicated by the bioluminescence signal generated after intravenous injection of luciferin. NIRF Images were acquired 24 h after intravenous injection of L-PG-NIR813 (50 nmol/mouse) or D-PG-NIR813 (50 nmol/mouse) with the same NIR dye loading (10%) and the same molecular weight (17,500). The NIRF signal was visualized in the mouse injected with L-PG-NIR813 but not the mouse injected with D-PG-NIR813 (from reference [25]).

Table 1

Summary of clinical studies of PG-TXL (CT-2103)

Effect Studied	Status	Res
Safety and Pharmacokinetics	Phase Ia (n = 19 on 3- weekly schedule); Phase Ib (n = 11 on 2-weekly schedule)	Pha
Safety	Phase I (n = 22) PG-TXL used in combination with carboplatin; solid tumors	MT
Safety	Phase I (n = 21) PG-TXL plus concurrent radiation; esophageal and gastric cancer	MT
Safety and Efficacy	Phase II (n = 99) patients with heavily pretreated ovarian cancer	Eff
Safety and Efficacy	Phase II (n = 18) patients with metastatic breast cancer	Eff
Safety and Efficacy	Phase II (n = 21) patients with androgen independent prostate cancer	Eff
Efficacy	Phase III (n = 400) CT-2103 plus carboplatin compared to Taxol® plus carboplatin; STELLAR 3 trial in PS2 pts with NSCLC	Eff
Efficacy	Phase III (n = 477) CT-2103 as a single agent compared to gemcitabine or vinorelbine; STELLAR4 trial in PS2 patients with NSCLC	Eff
Efficacy	Phase III (n = 850) CT-2103 as a single agent compared to docetaxel; STELLAR 2 trial in PS0-2 patients with NSCLC	Eff

Table 2

Polymer-drug conjugates in clinical trials

Conjugates	Indication	Status	Company
HPMA-Doxorubicin(PK1; FCE28068)	Lung and breast cancers	Phase II as of 2002	Pfizer; Ca
HPMA-doxorubicin- galactosamine (PK2, FCE28069)	Hepatocellular carcinoma	Phase I/II	Pfizer; Ca
HPMA-camptothecin (PNU166148)	Solid tumors	Phase I; discontinued	Pfizer; Ca
HPMA-paclitaxel (PNU166945)	Solid tumors	Phase I; discontinued	Pfizer; Ca
HPMA-platinatate (AP5346, ProLindac)	Ovarian, melanoma, and colorectal cancers	Phase I	Access Pl
PEG-Camptothecin(Pegamotecan)	Solid tumors	Phase I; discontinued	Enzon
PEG-SN38 (EZN-2208)	Solid tumors	Phase I; initiated as of October 2007	Enzon
Polymeric Micelles(NK911)	Pancreatic cancer	Phase II	Nippon K
Cyclodextrin-Based Polymer-CPT (IT-101)	Solid tumors	Phase I	Insert The
Carboxymethyldextra n-Exatecan (DE-310)	Solid atumors	Phase I	Daiichi Pl
PG-TXL (CT-2103, Xyotax)	Lung, ovarian, colorectal, breast, and esophageal cancers	Phase III	Cell Ther
PG-camptothecin(CT2106)	Colorectal, lung, and ovarian cancers	Phase I	Cell Ther

Phase III Trial Comparing Paclitaxel Poliglumex (CT-2103, PPX) in Combination with Carboplatin Versus Standard Paclitaxel and Carboplatin in the Treatment of PS 2 Patients with Chemotherapy-Naïve Advanced Non-small Cell Lung Cancer

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Introduction: Performance status (PS) 2 patients with non-small cell lung cancer (NSCLC) experience more toxicity, lower response rates, and shorter survival times than healthier patients treated with standard chemotherapy. Paclitaxel poliglumex (PPX), a macromolecule drug conjugate of paclitaxel and polyglutamic acid, reduces systemic exposure to peak concentrations of free paclitaxel and may lead to increased concentrations in tumors due to enhanced vascular permeability.

Methods: Chemotherapy-naïve PS 2 patients with advanced NSCLC were randomized to receive carboplatin (area under the curve = 6) and either PPX (210 mg/m²/10 min without routine steroid premedication) or paclitaxel (225 mg/m²/3 h with standard premedication) every 3 weeks. The primary end point was overall survival.

Results: A total of 400 patients were enrolled. Alopecia, arthralgias/myalgias, and cardiac events were significantly less frequent with PPX/carboplatin, whereas grade ≥3 neutropenia and grade 3 neuropathy showed a trend of worsening. There was no significant difference in the incidence of hypersensitivity reactions despite the

absence of routine premedication in the PPX arm. Overall survival was similar between treatment arms (hazard ratio, 0.97; log rank $p = 0.769$). Median and 1-year survival rates were 7.9 months and 31%, for PPX versus 8 months and 31% for paclitaxel. Disease control rates were 64% and 69% for PPX and paclitaxel, respectively. Time to progression was similar: 3.9 months for PPX/carboplatin versus 4.6 months for paclitaxel/carboplatin ($p = 0.210$).

Conclusion: PPX/carboplatin failed to provide superior survival compared with paclitaxel/carboplatin in the first-line treatment of PS 2 patients with NSCLC, but the results with respect to progression-free survival and overall survival were comparable and the PPX regimen was more convenient.

Key Words: Non-small cell lung cancer, Paclitaxel poliglumex, PPX, CT-2103, PS 2, Toxicity.

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Patients with non-small cell lung cancer (NSCLC) usually present with inoperable, advanced disease. Untreated, these patients have a 1-year survival rate of approximately 10%.¹ Combination chemotherapy regimens provide a statistically significant survival benefit, with 1-year survival rates of 30% to 40% in performance status (PS) 0 to 1 individuals and 2-year survival rates exceeding 10%.^{2–5} Retrospective reviews and meta-analyses of phase III trials have shown that patients with compromised PS (e.g., PS 2) have significantly impaired survival compared with PS 0 to 1 patients.^{6–8} This may be due to highly aggressive disease or comorbid conditions and impaired organ function, which can exacerbate chemotherapy toxicity. Consequently, PS 2 patients are often excluded from chemotherapy trials. Toxicity may deter clinicians from using standard platinum-based combination regimens for PS 2 patients, who often receive palliative care or single-agent, non-platinum therapy instead.

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A recent, prospectively planned subanalysis of Cancer and Leukemia Group B (CALGB) 9730, however, demonstrated that PS 2 patients benefited from platinum-based combination therapy compared with a single agent.⁹ Of the nearly 600 patients enrolled in CALGB 9730, 99 were PS 2. Within this cohort, overall response rate, median survival, and 1- and 2-year survival rates were 24%, 4.7 months, 18%, and 9% for the paclitaxel/carboplatin regimen versus 10%, 2.4 months, 10%, and 0% for single-agent paclitaxel.

Paclitaxel polyglumex (PPX) is an anionic, polymeric macromolecule consisting of paclitaxel conjugated to glutamic acid residues.¹⁰ The large number of anionic charges renders this molecule water soluble. The ester bond between paclitaxel and the polyglutamate backbone is stable and resistant to spontaneous hydrolysis; therefore, PPX is not broken down by plasma esterases. Consequently, the circulating polymer is relatively less toxic to normal tissue and theoretically better at delivering paclitaxel to the target cells.¹¹ Aqueous solubility also permits rapid intravenous administration and obviates the need for toxic solubilizing agents such as Cremophor. Finally, this molecule capitalizes on enhanced tumor permeability and retention of macromolecules to maximize tumor paclitaxel exposure.

Preclinical models demonstrated activity in NSCLC lines and synergy with platinating agents, including carboplatin.^{12,13} In a phase II trial of 28 patients with treatment-naïve advanced NSCLC, PPX at a dose of 175 mg/m² every 3 weeks yielded a median survival of 8.1 months in a PS 0 to 1 population and 5.4 months in a PS 2 cohort. In phase I trials, PPX has been combined at doses as high as 225 mg/m² every 3 weeks with carboplatin (area under the curve [AUC] = 6) without untoward short-term toxicity.¹⁴ Based on cumulative neurotoxicity, the recommended phase II dose in this setting was 210 mg/m².

The observations from the phase II single-agent trial, the ability to combine PPX at full dose with standard doses of carboplatin, its convenient administration, and the potential of decreased toxicity compared with standard paclitaxel laid the groundwork for a phase III trial, PGT303. This trial, also known as Selected Targeted Efficacy in Lung Cancer to Lower Adverse Reactions 3 (STELLAR 3), compared standard paclitaxel/carboplatin with PPX/carboplatin in PS 2 patients.

MATERIALS AND METHODS

Study Design

This open-label, phase III study compared paclitaxel 225 mg/m² with PPX at 210 mg/m² each in combination with carboplatin (AUC = 6) given every 3 weeks for up to 6 cycles. Patients were excluded from the study for disease progression (PD), intolerable toxicity, withdrawal of consent, or investigator discretion.

Randomization was stratified based on disease stage (IV versus other), gender, history of brain metastases, and geographic location (US versus Western Europe/Canada versus Eastern Europe).

Antiemetic prophylaxis was permitted for carboplatin including dexamethasone to prevent delayed nausea and

vomiting. The paclitaxel arm required routine hypersensitivity prophylaxis; however, the use of routine antihistamines or hypersensitivity prophylaxis was prohibited on the PPX arm. Hematopoietic growth factor support was permitted according to American Society of Clinical Oncology guidelines.¹⁵

End Points

The primary study end point was survival. Secondary objectives included response rate, time to progression (TTP), safety, and quality of life. Radiographic response was assessed every 2 cycles. For patients who completed therapy without evidence of PD, indicator lesions were re-evaluated every 8 weeks until documentation of PD or start of second-line therapy.

Safety data were collected on all patients. Disease-related symptoms were measured by the Functional Assessment of Cancer Therapy Lung Cancer Subscale (FACT-LCS) at baseline and within 3 days of each treatment.

Eligibility Criteria

All enrollees had histologically or cytologically confirmed NSCLC, an Eastern Cooperative Oncology Group (ECOG) PS 2 and stage IV, stage IIIB (but not candidates for combined modality therapy with curative intent), or locally advanced or recurrent disease previously treated with radiation and/or surgery. Eligibility stipulated age ≥ 18 years; adequate organ function, including baseline absolute neutrophil count $\geq 1500/\mu\text{L}$; platelet count $\geq 100,000/\mu\text{L}$; creatinine ≤ 1.5 times the upper limit of normal (ULN); bilirubin $\leq$ ULN; transaminases ≤ 2.5 times ULN (≤ 5 times ULN in patients with hepatic metastases); and alkaline phosphatase ≤ 2.5 times ULN, unless bone metastases were present. Patients with stable, treated brain metastases were eligible. Adequate contraception was required for female patients of reproductive potential.

Exclusion criteria included any small cell or carcinoma histology; prior systemic therapy for NSCLC; active, untreated brain metastases; other active primary invasive malignancies requiring treatment; grade ≥ 2 neuropathy; clinically significant infection; exposure to other investigational agents within 4 weeks of study entry; and unstable concomitant medical conditions. The protocol was approved by the institutional review board at each participating institution. All patients gave informed consent.

Representative case report forms were carefully reviewed to make certain that patient characteristics supported PS 2 designations.

Efficacy Parameters and Statistical Considerations

Overall survival was defined as the interval between randomization and death from any cause. Patients remaining alive, including those lost to follow-up, were censored at the date of last contact. Nonstratified log-rank testing was used for the formal primary comparison of survival. This study targeted accrual of 370 evaluable patients with 80% power and 0.05 type I error to show a 1.5-month improvement in median survival from a projected baseline of 4 to 5.5 months. In addition, secondary analyses were conducted using Cox

regression models of prognostic factors associated with survival in patients with NSCLC.

Response was assessed by response evaluation criteria in solid tumors.¹⁶ Disease control was determined by the percentage of patients alive without PD for at least 12 weeks. A two-sided Fisher exact test with an α level of 0.05 for statistical significance was used. TTP was defined as the interval between randomization and PD and was analyzed using an unstratified log-rank test. Toxicities were compared between the treatment arms using the Fisher's exact test.

Quality of Life

Disease-related symptoms were measured by the FACT-LCS, a validated, five-point Likert-type scale ranging from 0 (not at all) to 4 (very much). The total LCS score ranged from 0 to 28, with higher scores indicative of fewer symptoms. The percentage of patients with at least two-point improvements in their LCS scores at the beginning of cycle 2 was compared by treatment arm.

RESULTS

Disposition of Patients

Four hundred patients were enrolled between December 2002 and December 2003; 199 patients were assigned to the PPX arm and 201 to the paclitaxel arm. Three patients, all in the paclitaxel treatment arm, did not receive study treatment: one developed PD before starting therapy, a second had an elevated baseline bilirubin and did not meet eligibility criteria, and a third was withdrawn from the study due to rapid PD and intercurrent pneumonitis.

The arms were well balanced with regard to baseline characteristics (Table 1). Seventy-seven percent of patients were male, 94% white, and 63% from Eastern Europe. Median age was 61 years on the PPX arm and 63 years on the paclitaxel arm. Seventy-three percent of patients had stage IV disease, and 7% had brain metastases at baseline. Russia contributed the largest proportion of patients (35%), followed by the United States (23%). The differences between Eastern European enrollees and their Western European, US, and Canadian counterparts are delineated in Table 2.

Treatment Completion Rates

The median number of cycles was four in each arm. In the PPX arm, 27% of patients completed six cycles of therapy compared with 36% in the paclitaxel arm ($p = 0.0411$; Fisher's exact test). The most frequent reasons for stopping treatment were PD (36% versus 34%, respectively) and adverse effects (27% versus 20%, respectively). Nine percent of patients withdrew consent in the PPX arm compared with 6% in the paclitaxel arm.

Efficacy

Median overall survival was 7.8 months in the PPX arm and 7.9 months in the paclitaxel arm (Fig. 1A). One-year survival was identical at 31%. The 18- and 24-month survival rates were numerically higher in the PPX arm (20 and 13%, respectively) compared with the paclitaxel arm (11 and 11%, respectively), but these differences were not statistically significant.

TABLE 1. Demographic and Baseline Characteristics

	PPX/Carboplatin (n = 199)	Paclitaxel/Carboplatin (n = 201)
Gender		
Male	151 (76%)	156 (78%)
Female	48 (24%)	45 (22%)
Race		
White	189 (95%)	188 (94%)
Black	8 (4%)	9 (5%)
Asian	2 (1%)	1 (<1%)
Hispanic	0 (0%)	2 (1%)
Other	0 (0%)	1 (<1%)
Age at randomization, (yr)		
Mean (SD)	61.1 (10.6)	61.5 (10.1)
Median (range)	61.0 (35–86)	63.0 (36–89)
Geographic site		
United States	46 (23%)	45 (22%)
Western Europe and Canada	30 (15%)	27 (13%)
Eastern Europe	123 (62%)	129 (64%)
Stage at randomization		
IIa	2 (1%)	0 (0%)
IIb	51 (26%)	55 (27%)
IV	146 (73%)	146 (73%)
History of brain metastases		
Yes	14 (7%)	15 (7%)
No	185 (93%)	186 (93%)
Smoking history		
Yes	170 (85%)	171 (85%)
No	29 (15%)	30 (15%)

PPX, paclitaxel poliglumex; SD, standard deviation.

TABLE 2. Demographic and Baseline Characteristics at Entry Based on Region

Region	US/Western Europe/Canada (n = 148)	Eastern Europe (n = 252)
≥5% weight loss within 6 mo of randomization	58 (39%)	104 (41%)
Histologic diagnosis		
Squamous cell carcinoma	43 (29%)	134 (53%)
Adenocarcinoma	78 (53%)	72 (29%)
Other	27 (18%)	46 (18%)
Stage at enrollment		
Stage IV/recurrent	122 (82%)	170 (67%)
Stage IIIb	26 (18%)	80 (32%)
History of brain metastases	21 (14%)	8 (3%)
Prior radiation	47 (32%)	40 (16%)
Median FACT-LCS score	18.7	17.0
Current use of tobacco	46 (31%)	113 (45%)
Baseline opioid use	19 (13%)	12 (5%)
Baseline hemoglobin ≥11 g/dL	110 (74%)	220 (87%)

FACT-LCS, functional assessment of cancer therapy lung cancer subscale.

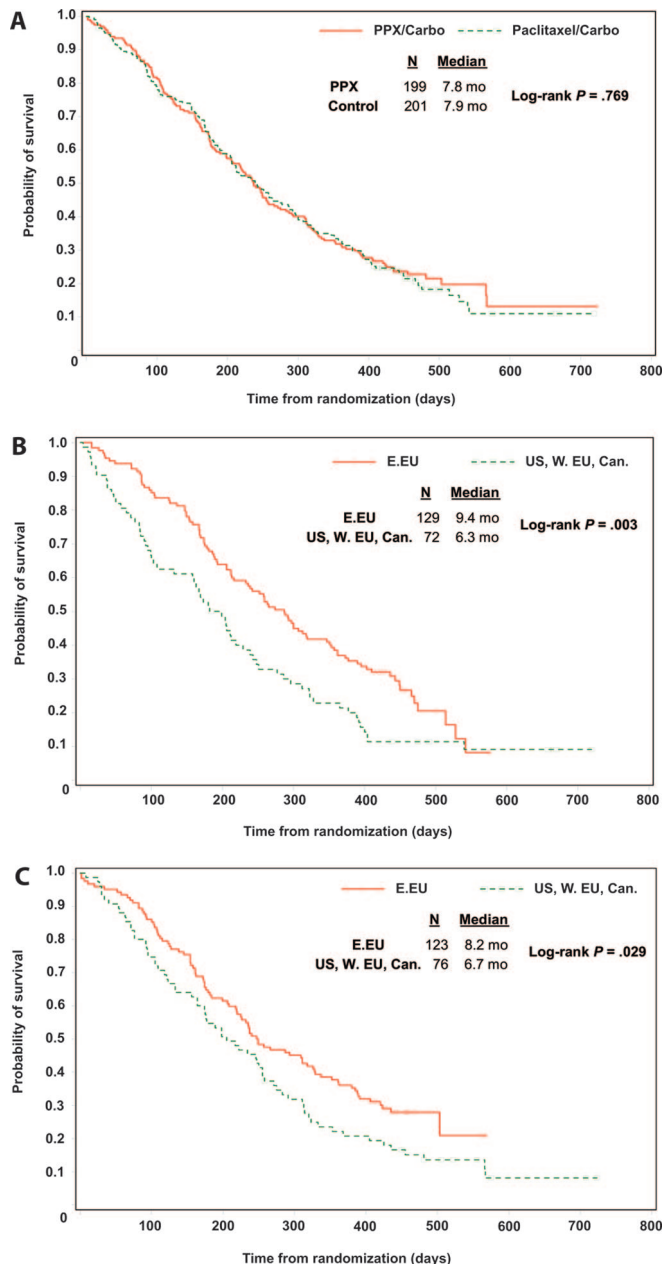


FIGURE 1. Overall survival using Kaplan-Meier estimation. (A) Paclitaxel poliglumex/carboplatin versus paclitaxel/carboplatin. (B) Paclitaxel/carboplatin—United States/Western Europe/Canada versus Eastern Europe. (C) PPX/carboplatin—United States/Western Europe/Canada versus Eastern Europe.

Prespecified analyses by stratification factors (Table 3) demonstrated that median survival in the paclitaxel arm was significantly better in Eastern Europe and Russia than in the United States and Western Europe (9.4 versus 6.3 months; $p = 0.003$; Fig. 1B). There was less difference in median survival on the PPX arm (8.2 versus 6.7 months; $p = 0.029$; Fig. 1C). Women on the paclitaxel arm had a median survival similar to those in the experimental arm (8.3 versus 7.9 months). However, at 12, 18, and 24 months, survival rates

were better on PPX compared with paclitaxel (37 versus 25%, 26 versus 5%, and 13 versus 5%, respectively). Survival for men was similar in both arms with virtually identical median survival (7.9 versus 7.9 months) and 1- (29 versus 33%) and 2-year (13 versus 13%) survival rates.

Cox multivariate stepwise modeling, with all other factors constant, demonstrated that weight loss before study entry, tobacco use, elevated lactate dehydrogenase levels, and elevated calcium levels significantly increased the probability of death.

Response and TTP

The overall response rate was 20% for the PPX arm with 1% complete responses and 37% for the paclitaxel arm with 2% complete responses; these results significantly favored the paclitaxel arm (Table 4). Blended response rates differed by geographic region: 35% for Eastern Europe, 23% for Western Europe and Canada, and 14% for the United States. Disease control rate was 64% in the PPX arm, compared with 69% in the paclitaxel arm.

Median TTP was 3.9 months in the PPX arm and 4.6 months on the paclitaxel arm ($p = 0.210$). The proportion of patients receiving subsequent therapy and the type of therapy administered were not different in the 2 arms. Eighteen percent of those enrolled in the PPX arm went on to radiation therapy, compared with 13% in the paclitaxel arm. Nearly 50% in each arm received additional chemotherapy; no specific agents predominated. Roughly 7% of patients received epidermal growth factor receptor tyrosine-kinase inhibitors as second-line or subsequent therapy.

Quality of Life

FACT-LCS evaluations were completed by 180 patients on the PPX/carboplatin arm and 172 patients on the paclitaxel/carboplatin arm. No significant difference in the FACT-LCS scores was seen.

Toxicity

Drug Delivery

Patients received nearly 90% of planned doses during the second and subsequent cycles. The mean delivered dose intensity per cycle was comparable in both arms. In Eastern Europe, 75% of patients received 4 to 6 cycles, compared with 44 and 49% in the United States and Western Europe/Canada, respectively. The mean normalized carboplatin dose per cycle per patient was the same for both arms (AUC = 5.6).

Relative Toxicities

Patients enrolled in the paclitaxel arm were significantly more likely to experience cardiac toxicity, alopecia, and musculoskeletal toxicity (Table 5). However, those in the PPX arm experienced significantly more nausea and vomiting. More grade 3/4 neuropathy was seen on the PPX arm (17 versus 10%), but this difference was not statistically significant. Time to first manifestation of neuropathy was generally later in the PPX arm—a median of 89 days compared with 54 days for the paclitaxel arm (log-rank $p < 0.001$). By day 100, the incidence was equal. Fatigue was higher in the PPX arm than the paclitaxel arm: 15 versus 8% ($p = 0.05$); however,

TABLE 3. Subgroup Analysis of Overall Survival

Subgroup	PPX/Carboplatin		Paclitaxel/Carboplatin		Hazard Ratio ^a	Log-Rank Test; <i>p</i>
	<i>n</i>	Median Survival, d (95% CI)	<i>n</i>	Median Survival, d (95% CI)		
Overall	199	237 (205–271)	201	239 (206–287)	0.97 (0.78–1.21)	0.769
Gender						
Male	151	237 (199–275)	156	239 (204–288)	1.04 (0.80–1.34)	0.780
Female	48	236 (174–380)	45	248 (167–323)	0.76 (0.48–1.20)	0.239
Geographic location						
United States	46	217 (164–291)	45	173 (100–248)	0.79 (0.50–1.23)	0.298
Western Europe/Canada	30	188 (118–275)	27	206 (103–322)	1.08 (0.63–1.86)	0.773
Eastern Europe	123	249 (219–326)	129	287 (233–347)	1.00 (0.75–1.33)	0.992
Disease stage at randomization						
IV	146	237 (189–283)	146	236 (205–277)	0.92 (0.71–1.19)	0.528
Other	53	246 (182–311)	55	274 (181–404)	1.09 (0.70–1.70)	0.715
History of brain metastases						
Yes	14	207 (145–320)	15	229 (87–328)	1.13 (0.51–2.50)	0.770
No	185	237 (206–275)	186	239 (206–292)	0.97 (0.77–1.22)	0.778

^a Hazard ratio (CT-2103 to paclitaxel) was estimated by Cox regression with treatment arm as a single covariate.
PPX, paclitaxel poliglumex; CI, confidence intervals.

TABLE 4. Efficacy Results in the Intent-to-Treat Population

	PPX/Carboplatin	Paclitaxel/Carboplatin
No. patients	199	201
Survival		
Median (d)	237	239
95% CI	205–271	206–287
Hazard ratio (95% CI); log-rank <i>p</i> value	0.97 (0.78–1.21); 0.769	
1-yr survival rate (%)	31	31
95% CI	24–37	25–38
2-yr survival rate (%)	13	11
95% CI	5–22	4–18
Time to progression		
Median (d)	118	139
95% CI	100–129	118–156
Hazard ratio (95% CI); log-rank <i>p</i> value	1.14 (0.93–1.40); 0.210	
Disease control, % (95% CI)	64 (57–70)	69 (62–75)
Response rate (patients with measurable disease only)		
No. patients	191	192
PR + CR, % (95% CI)	20 (15–27)	37 (30–44)
Confirmed PR + CR, % (95% CI)	13 (9–19)	28 (22–35)

PPX, paclitaxel poliglumex; CI, confidence intervals; PR, partial response; CR, complete response.

grade 3/4 fatigue was similar between arms: 2 versus 3% ($p = 0.66$).

The incidence of grade 3/4 thrombocytopenia was significantly higher in the PPX arm ($p < 0.001$) but was not associated with more thrombocytopenic bleeding or higher transfusion requirements. Five percent of patients on the PPX

arm required platelet transfusions, compared with 3% on the paclitaxel arm ($p = 0.42$). The incidence of febrile neutropenia was 6% in the PPX arm versus 3% in the paclitaxel arm ($p = 0.32$). The reported incidence of neutropenia in Eastern Europe was 24%, compared with 47% in the United States and 42% in Western Europe/Canada. The use of supportive care, including transfusions, erythropoietin, and granulocyte colony-stimulating factor, was similar between treatment arms but different based on geographic regions. The use of red blood cell growth factor support in the United States/Western Europe/Canada was 38% compared with less than 1% in Eastern Europe ($p < 0.001$); the use of white blood cell growth factor support in the United States/Western Europe/Canada was 31% compared with 7% in Eastern Europe ($p < 0.001$). Seven percent of those in Eastern Europe required red blood cell transfusions compared with 16% in the United States/Western Europe/Canada ($p = 0.006$).

Twelve percent of patients in each arm died within 30 days of treatment, but only 1% of all deaths were attributable to study drugs. There were 7.5% disease-related deaths, and 3.5% were due to comorbidities.

Total infusion time for the PPX combination was 48 minutes, compared with 224 minutes for the paclitaxel combination. The incidence of hypersensitivity reactions was 2% for the paclitaxel/carboplatin arm with standard premedications of steroids and H1 and H2 blockers, compared with 1% for the PPX/carboplatin arm without routine hypersensitivity reaction prophylaxis. However, 28% of PPX patients, mostly in the United States and Canada, received steroid prophylaxis and/or antihistamines beginning in cycle 1 as antiemetic prophylaxis for carboplatin.

DISCUSSION

STELLAR 3 is the first dedicated phase III trial ever conducted and reported in treatment-naïve PS 2 patients with

TABLE 5. No. (%) Patients with Treatment-Related Adverse Effects by Maximum NCI Common Toxicity Grade

Adverse Effects	PPX/Carboplatin (<i>n</i> = 199)			Paclitaxel/Carboplatin (<i>n</i> = 198)		
	All Grades	Grade 3	Grade 4	All Grades	Grade 3	Grade 4
Nonhematologic						
Infection and infestation	5 (3%)	1 (<1%)	1 (<1%)	4 (2%)	1 (<1%)	0
Neuropathy	99 (50%)	30 (15%)	3 (2%)	117 (59%)	18 (9%)	1 (<1%)
Cardiac	0	0	0	6 (3%)	1 (<1%)	0
Alopecia	27 (14%)	0	0	83 (42%)	0	0
Arthralgia	13 (7%)	2 (1%)	0	33 (17%)	2 (1%)	0
Myalgia	17 (9%)	2 (1%)	0	29 (15%)	5 (3%)	0
Fatigue	29 (15%)	4 (2%)	0	16 (8%)	5 (3%)	0
Nausea	97 (49%)	9 (5%)	0	70 (35%)	10 (5%)	0
Vomiting NOS	59 (30%)	8 (4%)	0	39 (20%)	3 (2%)	1 (<1%)
Hematologic						
Neutropenia	72 (36%)	18 (9%)	35 (18%)	53 (27%)	14 (7%)	17 (9%)
Febrile neutropenia	11 (6%)	4 (2%)	5 (3%)	5 (3%)	2 (1%)	2 (1%)
Thrombocytopenia	72 (36%)	38 (19%)	5 (3%)	25 (13%)	14 (7%)	0

NCI, National Cancer Institute; PPX, paclitaxel poliglumex; NOS, not otherwise specified.

NSCLC. Based on the preplanned statistical analysis, PPX failed to yield superior survival compared with paclitaxel in this setting, the primary end point, but disease control rates, TTP, and overall survival rates were comparable, as was dose delivery. Hence, potential utility for PPX in the PS 2 population still exists.

An unplanned subanalysis of women enrolled in this study by baseline estradiol levels, reported at the American Society of Clinical Oncology annual meeting in 2006, demonstrated a significant survival advantage for PPX in women with premenopausal estradiol levels (>30 pg/mL).¹⁷ This observation will be prospectively evaluated in a phase III study.

The overall survival for PS 2 patients enrolled in this trial was substantially longer than expected based on previously reported data. In the Lilenbaum et al. study (CALGB 9730),⁹ the median survival for PS 2 patients receiving paclitaxel and carboplatin was 4.7 months, compared with nearly 8 months for both arms in this trial. A previous phase II study of PPX alone at a lower dose of 175 mg/m² showed survival of 5.4 months in the PS 2 cohort. Whereas survival in North America and Western Europe mirrored recent experience with carboplatin and paclitaxel in PS 2 patients (Table 6), Eastern Europe differed substantially with far better sur-

vival observed in both arms. This may be due to a younger population with fewer comorbidities, less prior radiation, less advanced disease, less baseline anemia, and possibly lower incidence of readily recognizable central nervous system metastases. It may also be due to “contamination” by PS 0 to 1 patients, although auditing of case report forms does not support this theory, and there are no known geographic differences in the nature of PS evaluation. Still, PS designation remains highly subjective and open to debate.

PPX/carboplatin produced fewer myalgias and arthralgias, less alopecia, and delayed time to neuropathy, but more nausea, vomiting, and fatigue and overall worse neuropathy than paclitaxel/carboplatin. The increased incidence of nausea and vomiting in the PPX arm may have been due to lack of routine premedication for hypersensitivity reaction. Febrile neutropenia rates were comparable in each arm. Although the incidence of grade ≥3 thrombocytopenia was significantly higher for the PPX arm, it was readily manageable and did not result in more platelet transfusions. PPX administration was also more convenient than paclitaxel with a significantly shorter administration time (48 versus 224 minutes) and no significant increase in hypersensitivity reactions despite the absence of routine premedication.

TABLE 6. Comparative Analysis of Taxane-Based Regimens in Treatment-naïve PS 2 NSCLC in North America

Study	Regimen	No. Patients	Median Survival (mo)	1-yr Survival (%)
CALGB 9730 ⁸	Paclitaxel	50	2.4	10
CALGB 9730 ⁸	Paclitaxel/carboplatin	49	4.7	19
ECOG 1594 ⁴	Paclitaxel/carboplatin	15	4.6	13
ECOG 1599 ⁶	Paclitaxel/carboplatin	38	6.1	19
PGT303	Paclitaxel/carboplatin	201	8.0	31
PGT303	PPX/carboplatin	199	7.9	31

PS, performance status; NSCLC, non-small cell lung cancer; CALGB, Cancer and Leukemia Group B; ECOG, Eastern Cooperative Oncology Group; PPX, paclitaxel poliglumex.

The toxic death rates in this study were comparable with those observed in recent trials including ECOG 1594, ECOG 1599, and CALGB 9730.^{4,9,18} The reduction in toxic deaths compared with trials completed in the 1990s and earlier is likely due to better ancillary and supportive care management. The optimal approach to patients with NSCLC remains unknown, particularly in patients with compromised PS. STELLAR 3 showed that two platinum-based doublets are relatively well-tolerated with better than expected survival rates in PS 2 patients. The results of STELLAR 4, in which PPX at a dose of 175 mg/m² was compared with investigators' choice of single-agent gemcitabine or vinorelbine as front-line therapy for PS 2 patients and overall median survival exceeded 7 months, suggested that the addition of a platinating agent may not necessarily confer a survival benefit.¹⁹ Safety analysis of STELLAR 4 suggested a similar outcome and less neuropathy and neutropenia for the 175 mg/m² PPX dose compared with the 210 mg/m² dose used in STELLAR 3. No published PS 2-specific phase III trial to date has compared a platinum combination with a nonplatinum single agent. In the absence of a proven dose response for taxanes in NSCLC, and given the similar survival rates in STELLAR 3 and 4, future studies of PPX in NSCLC will be conducted at a dose of ≤ 175 mg/m².

In a Cox multivariate analysis of prognostic factors, the most significant negative prognostic factors included extrathoracic metastasis ($p = 0.006$), antecedent weight loss $\geq 5\%$ ($p = 0.006$), and LCS score < 18 ($p = 0.019$). Geographic site (Eastern Europe versus Western Europe/United States/Canada) had a clear-cut bearing on outcome ($p \leq 0.001$). These factors should be considered as stratifications in future trials.

Several other strategies are under investigation in the PS 2 population. The Southwest Oncology Group recently reported the results of a phase II trial investigating the role of erlotinib in PS 2 patients²⁰; and the CALGB has completed a PS 2-specific, randomized phase II trial evaluating docetaxel in combination with either bortezomib or cetuximab.²¹

Other chemotherapy "only" studies are also in progress. Gridelli et al.²² are completing a randomized phase II trial of pemetrexed either alone or followed after two cycles by gemcitabine in advanced NSCLC patients unsuitable for platinum-based treatment. To date, the sequential arm has not shown superiority to the single-agent arm.²² US Oncology, in a phase III trial, is comparing gemcitabine alone to combination gemcitabine/carboplatin. Single-agent versus doublet chemotherapy has been tested in several European trials in "elderly" NSCLC patients with somewhat conflicting results, but has never been tested exclusively in PS 2 patients.

CONCLUSIONS

PS 2 patients constitute a sizable percentage of new NSCLC patients; yet they have been largely excluded from or underrepresented in clinical trials in the last 10 to 15 years based on a general perception of overall poor outcome. Single-agent therapy is considered by many to be the standard approach despite data showing a benefit of combination therapy. The results of STELLAR 3 confirmed the tolerability

and efficacy of doublet treatment with carboplatin plus either PPX or paclitaxel. Future studies in PS 2 patients should focus on the nature of comorbidities, pretreatment disease characteristics, treatment-related quality of life, and toxicity management. Patients with poor PS require special attention to ensure clinically meaningful therapeutic benefits. In this vein, newer chemotherapeutics such as PPX have a potential role.

ACKNOWLEDGMENTS

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A Phase 1b Study of CC-486 (Oral Azacitidine) to Evaluate the Pharmacokinetics and Pharmacodynamics of Azacitidine Administered Alone and in Combination with Carboplatin or ABI-007 (*nab*[®]-Paclitaxel) in Patients with Solid Tumors

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BACKGROUND

- CC-486 (oral azacitidine) is an orally bioavailable formulation of azacitidine with demonstrated clinical activity in patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML)^{1,2}
- In these patient populations, CC-486 in extended dosing regimens (200mg/day and 300mg/day given 14 or 21 days of 28-day cycles), induces sustained global genomic hypomethylation^{2,3}
- Sustained exposure to CC-486 as a priming agent prior to introduction of cytotoxic agents may sensitize solid tumors to the effects of chemotherapy⁴

OBJECTIVES

- The effect of carboplatin or *nab*[®]-paclitaxel* (*nab*[®]-pac [ABI-007], Celgene, Summit, NJ) on the pharmacokinetics (PK) of CC-486
- The effect of CC-486 on the PK of carboplatin or *nab*[®]-pac
- The pharmacodynamic (PD; *i.e.*, DNA methylation) effects of CC-486 in combination with carboplatin or *nab*[®]-pac

**nab*[®] is a registered trademark of Celgene Corporation

METHODS

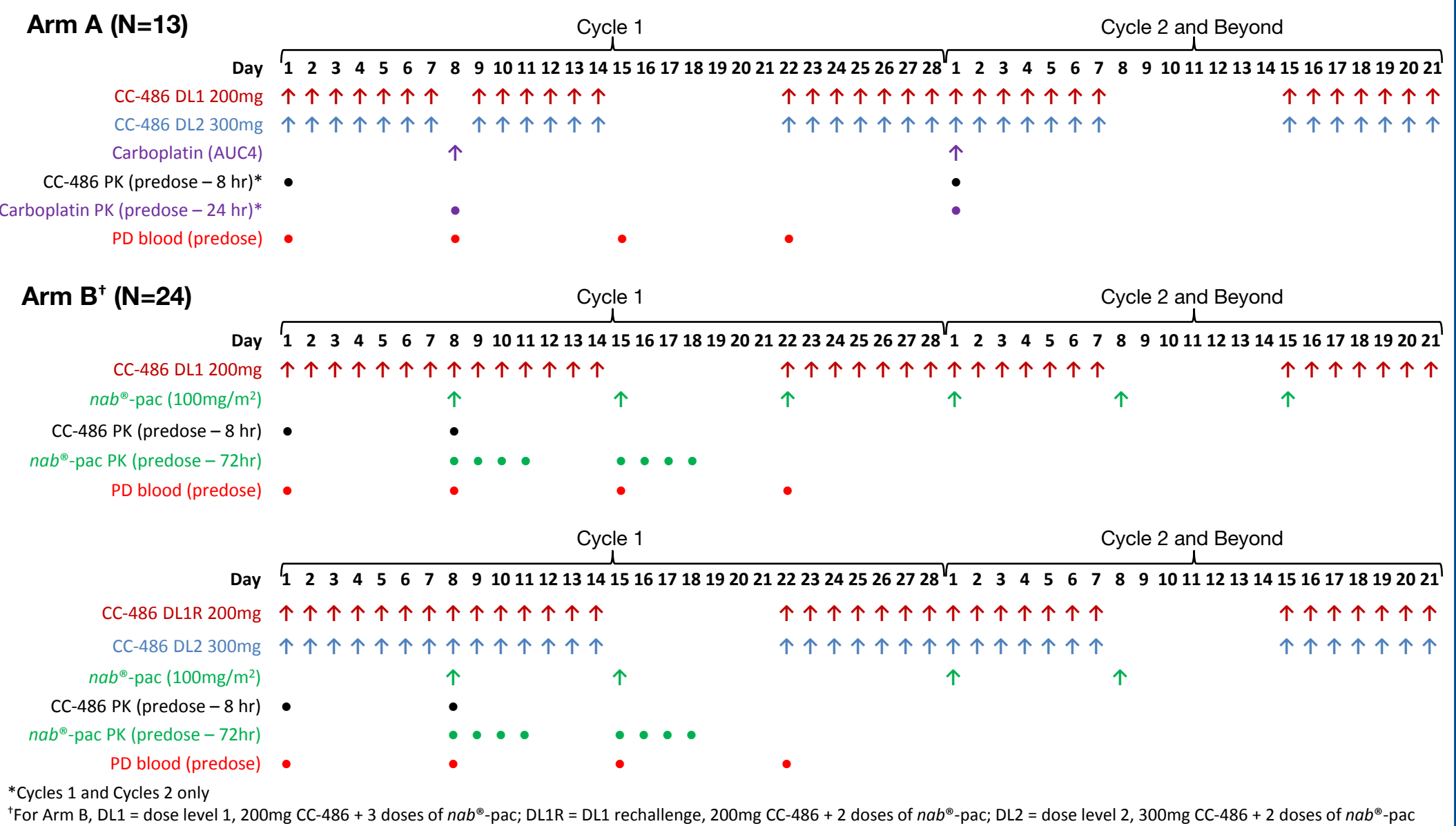
- Study design (Figure 1)**
- Phase 1b, multicenter, open-label study
 - Subjects ≥ 18 years of age with advanced relapsed or refractory solid tumors were included

- PK analysis**
- PK plasma samples were analyzed using validated HPLC-MS/MS methods

Sample collection and DNA methylation analysis

- Peripheral blood mononuclear cells (PBMCs) were purified from whole blood (12mL) by the Ficoll-Paque method and DNA was extracted from PBMCs (5x10⁶ cells) using the QIAamp DNA Mini Kit (Qiagen; Valencia, CA)
- 0.5µg DNA was used for HumanMethylation27 and HumanMethylation450 assays (Illumina; San Diego, CA). CpGs common to HumanMethylation27 and HumanMethylation450 platforms were included in analyses
- Percent of hypermethylated loci (beta value > 0.7) was calculated after removing low-quality probes (detection p-value ≤ 0.05 in < 50% of samples) and probes targeting loci on chromosomes X and Y
- The PD metric was change in the percent of hypermethylated loci from Cycle 1 Day 1 (C1D1) to Day 15 (C1D15) in each patient
- Statistical significance of a PK-PD correlation was evaluated using Pearson's correlation test

Figure 1. Study Design



RESULTS

Figure 2A. CC-486 Plasma Concentration vs Time Profile With and Without Carboplatin

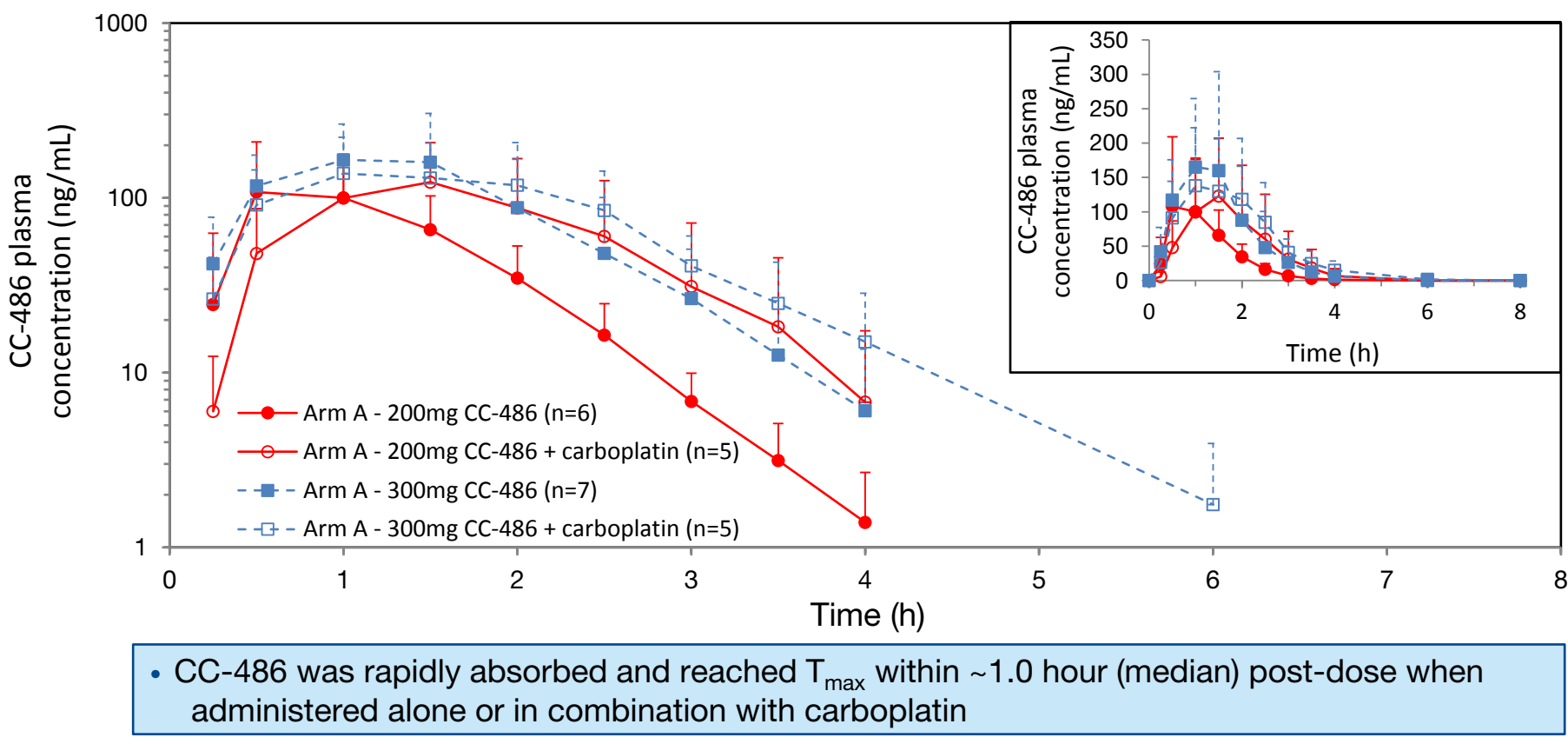


Figure 2B. CC-486 Plasma Concentration vs Time Profile With and Without *nab*[®]-Paclitaxel

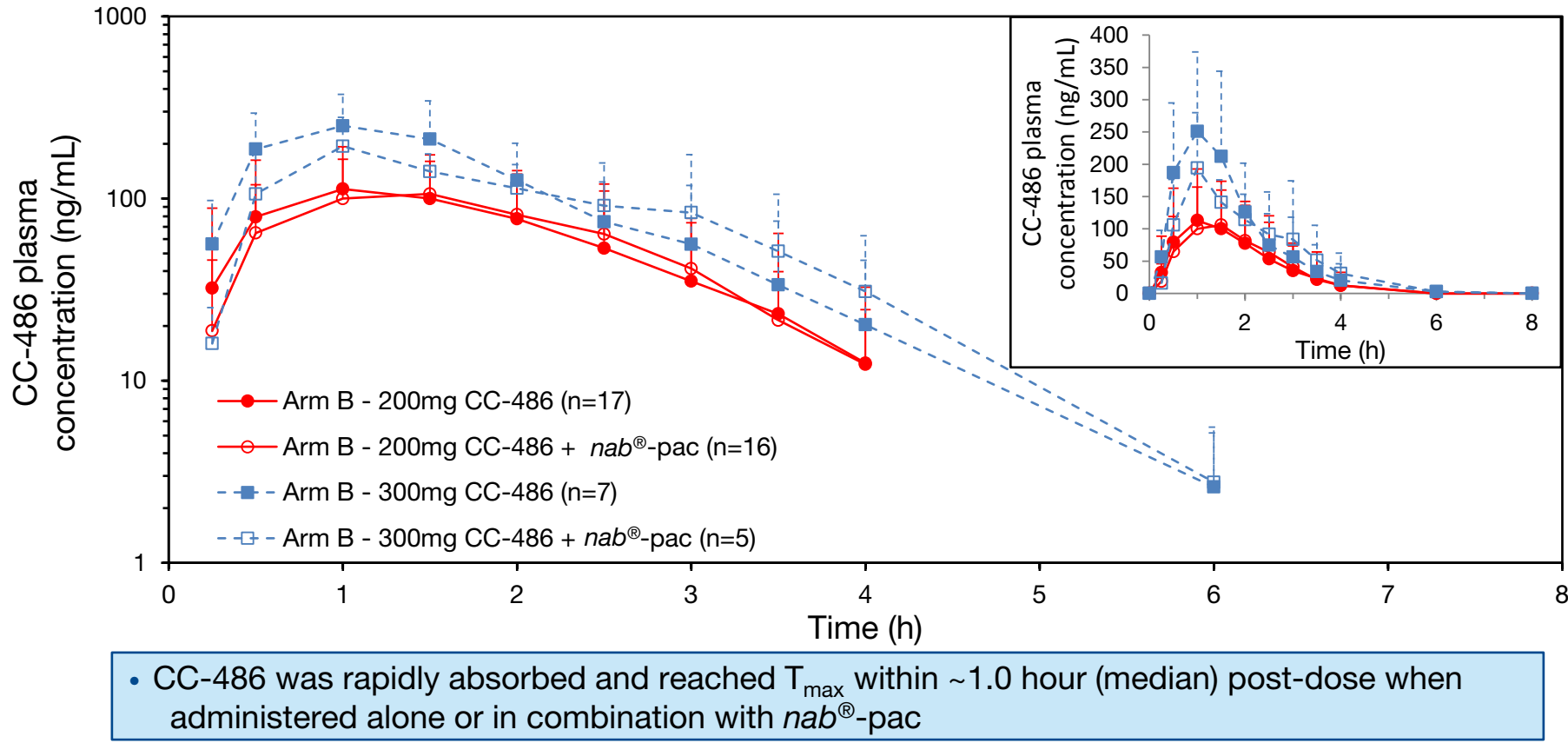


Figure 3. Individual and Mean AUC_∞ of CC-486 With and Without Carboplatin or *nab*[®]-Paclitaxel

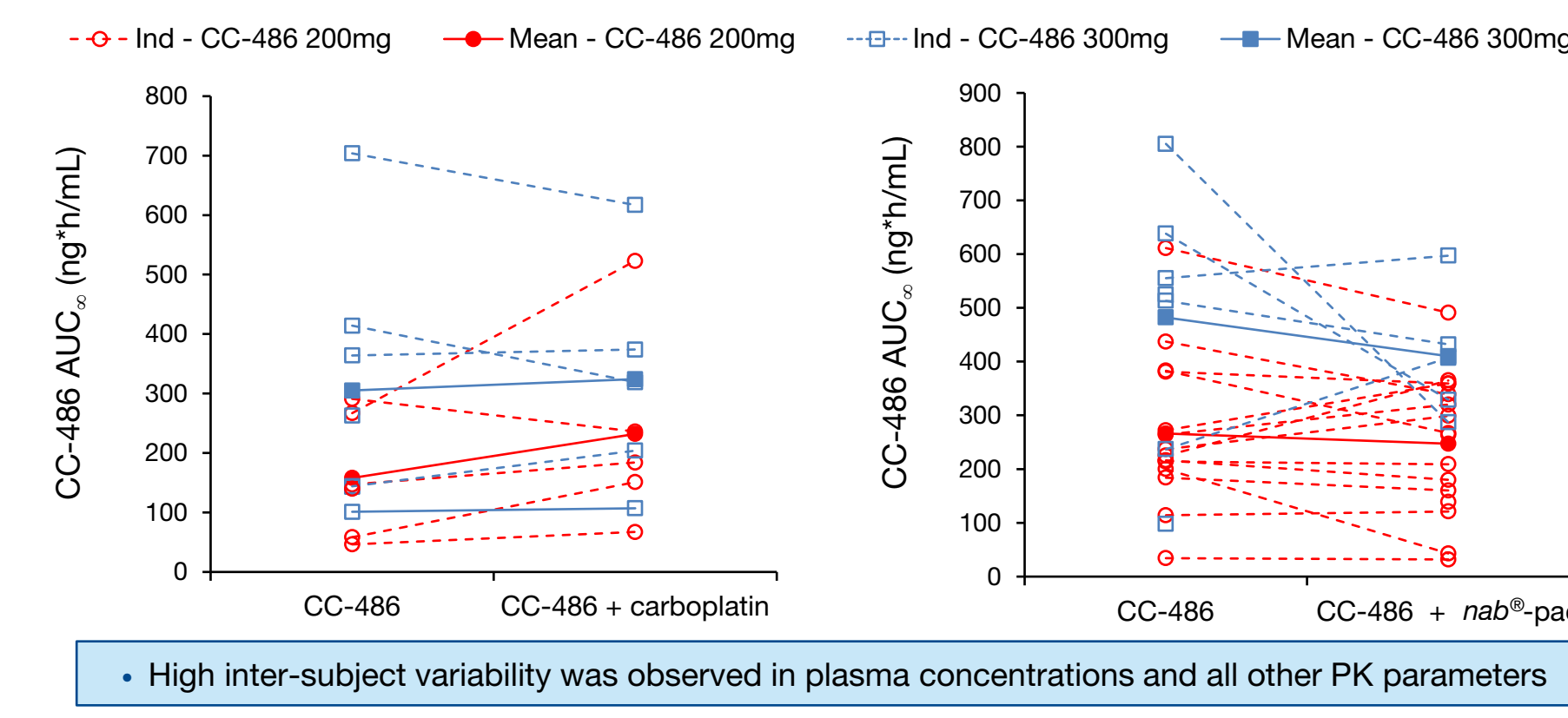


Table 1. Mean ± SD (%CV) CC-486 Plasma PK Parameters Following Administration of CC-486 Alone or in Combination With Carboplatin or *nab*[®]-Paclitaxel

Treatment Arm	CC-486 (dose/day)	n	AUC _∞ (ng·h/mL)	C _{max} (ng/mL)	CL/F (L/h)	t _{1/2} (h)	T _{max} * (h)	Vz/F (L)
Arm A: CC-486 alone	200mg	6	158 ± 102 (64.7)	118 ± 98 (82.7)	2000 ± 1510 (75.9)	0.45 ± 0.07 (15.3)	1.0 [0.5, 1.5]	1300 ± 992 (76.6)
		5	232 ± 174 (74.8)	140 ± 76.4 (54.7)	1320 ± 988 (74.7)	0.41 ± 0.05 (12.5)	1.0 [1.0, 1.5]	735 (63.6)
Arm A: CC-486 + carboplatin	300mg	7	305 ± 212 (69.6)	191 ± 120 (62.5)	1470 ± 932 (63.5)	0.45 ± 0.10 (21.7)	1.0 [0.5, 1.5]	897 ± 505 (56.3)
		5	324 ± 194 (59.7)	145 ± 82.2 (56.8)	1300 ± 915 (70.3)	0.70 ± 0.36 (52)	1.0 [0.5, 1.5]	1610 ± 2020 (125.9)
Arm B: CC-486 alone	200mg	17	266 ± 140 (52.5)	156 ± 81.8 (52.6)	1170 ± 1340 (114.3)	0.51 ± 0.20 (38.8)	1.0 [0.5, 4.0]	799 ± 819 (102.4)
		16	247 ± 128 (51.8)	133 ± 69.8 (52.4)	1430 ± 1640 (115.3)	0.57 ± 0.17 (30.3)	1.5 [0.5, 3.0]	1240 ± 1630 (130.8)
Arm B: CC-486 + <i>nab</i> [®] -pac	300mg	7	482 ± 240 (49.7)	268 ± 125 (46.8)	981 ± 963 (98.1)	0.75 ± 0.24 (32)	1.0 [0.5, 1.5]	1230 ± 1700 (138.2)
		5	410 ± 119 (29.1)	219 ± ±73 (33.3)	779 ± 209 (26.8)	0.58 ± 0.10 (17)	1.0 [1.0, 3.0]	656 ± 246 (37.5)

*Median [range]

- PK parameters were comparable for CC-486 alone or in combination with either carboplatin or *nab*[®]-pac. Similarly, carboplatin and *nab*[®]-pac PK parameters were similar when administered alone or in combination with CC-486 (data not shown)

Figure 4. Decreased Global DNA Methylation* Observed in Arms A and B With CC-486: (a) 200mg [DL1/DL1R] and (b) 300mg [DL2]

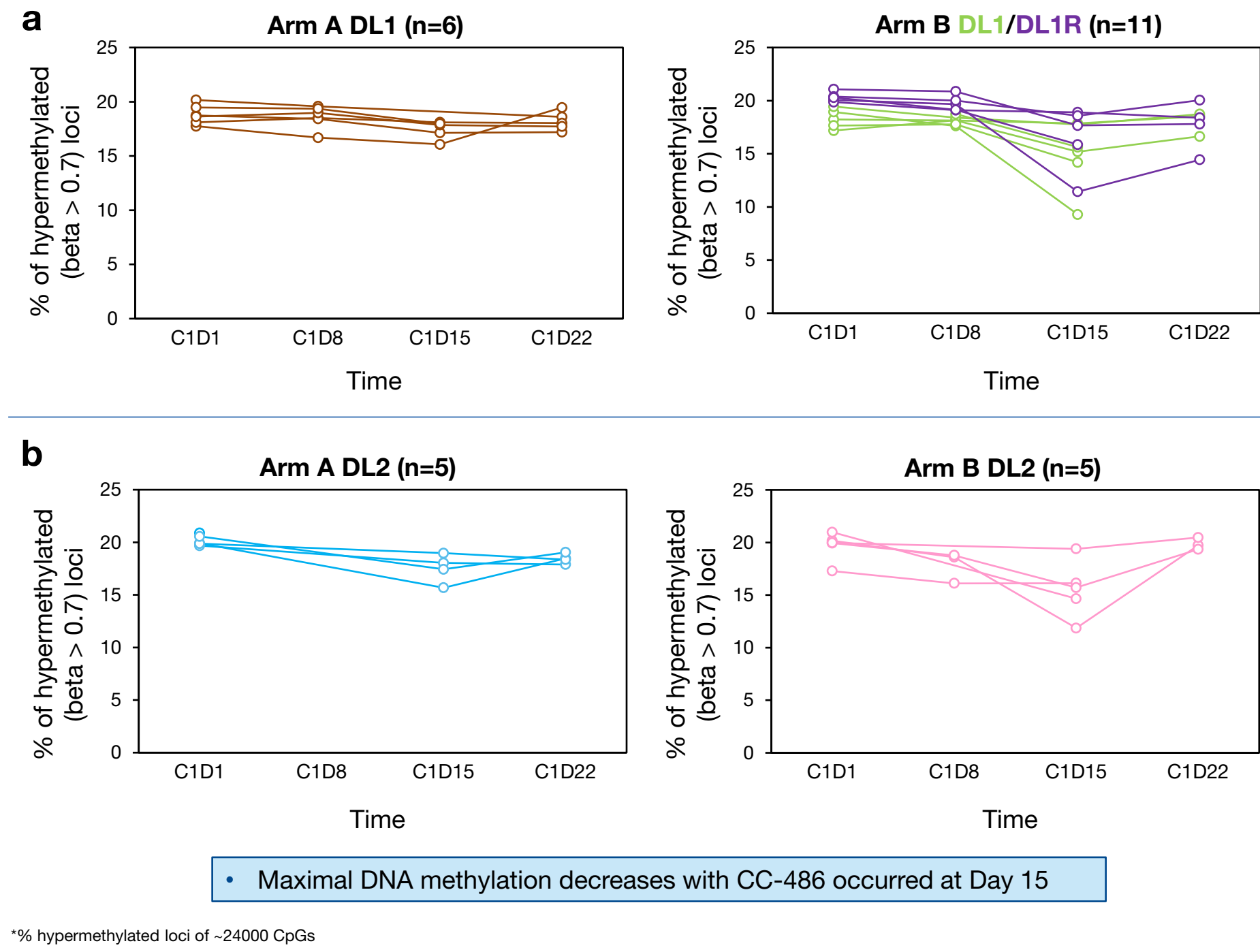
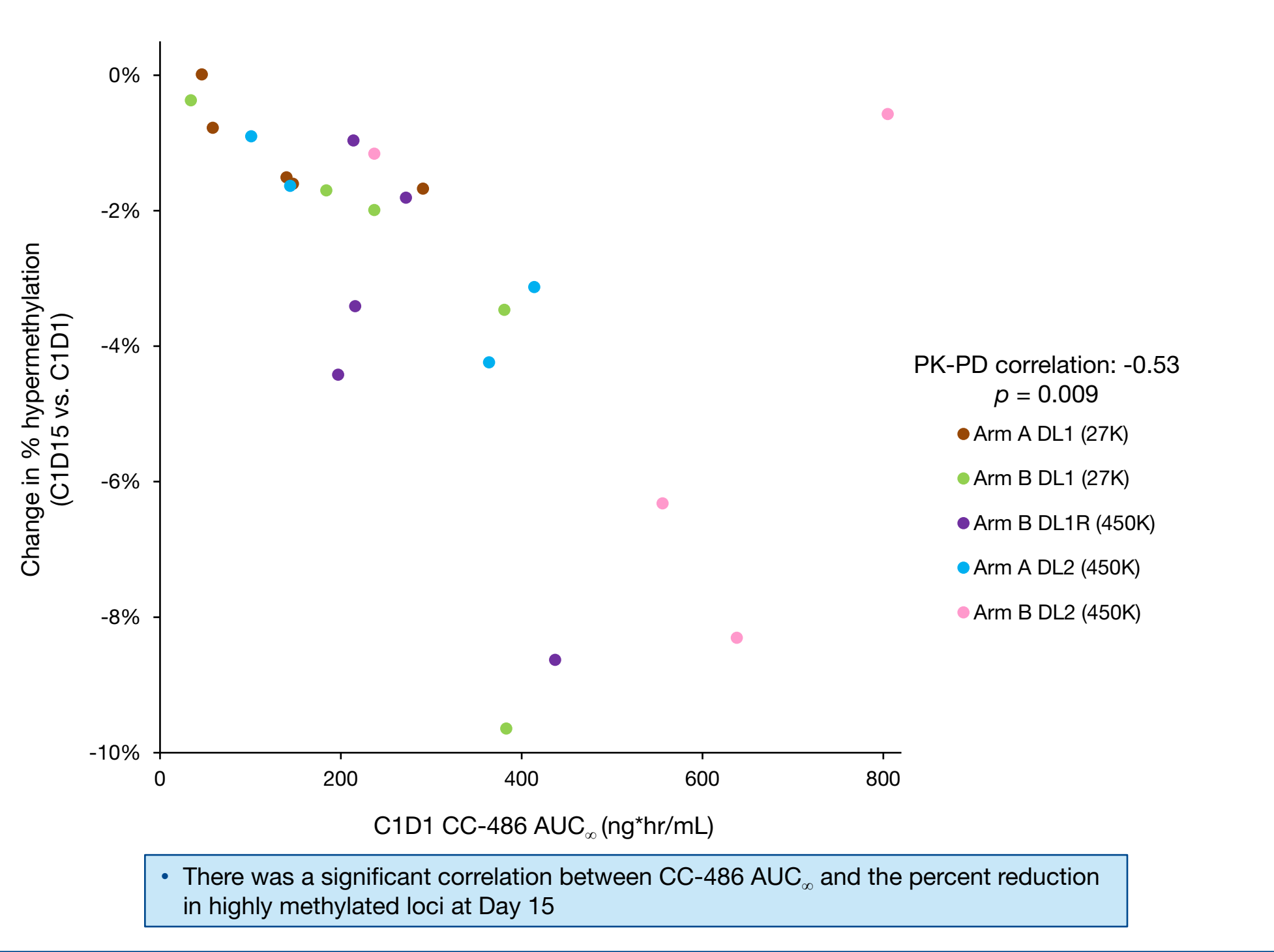


Figure 5. Correlation Between the PK and PD of CC-486



CONCLUSIONS

- Carboplatin and *nab*[®]-paclitaxel had minimal to no effect on CC-486 PK parameters, and vice versa
- Following administration of CC-486, global genomic hypomethylation was substantially correlated with azacitidine plasma AUC
 - Maximum demethylation occurred on Day 15 of the treatment Cycle
- The safety and anti-tumor effects of the treatment regimens in this study are shown in the AACR-NCI-EORTC 2013 LoRusso et al poster A120

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DISCLOSURES

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A Phase 1b Study of CC-486 (Oral Azacitidine) as a Priming Agent for Carboplatin or *nab*[®]-Paclitaxel in Patients with Relapsed or Refractory Solid Tumors

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BACKGROUND

- CC-486, an oral formulation of azacitidine, induces global genomic hypomethylation and clinical responses in myelodysplastic syndrome (MDS) patients at doses of 200mg/day and 300mg/day for 14 or 21 days of 28-day cycles with acceptable safety¹

OBJECTIVES

- This 2-part, open-label, multicenter, 3-arm Phase 1b study was designed to make a preliminary assessment of the safety, tolerability, and anti-tumor activity of CC-486, an epigenetic remodeling (priming) agent, in combination with carboplatin or *nab*[®]-paclitaxel* (*nab*[®]-pac [ABI-007]) in relapsed/refractory advanced unresectable solid tumors

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METHODS

Study design (Figure 1)

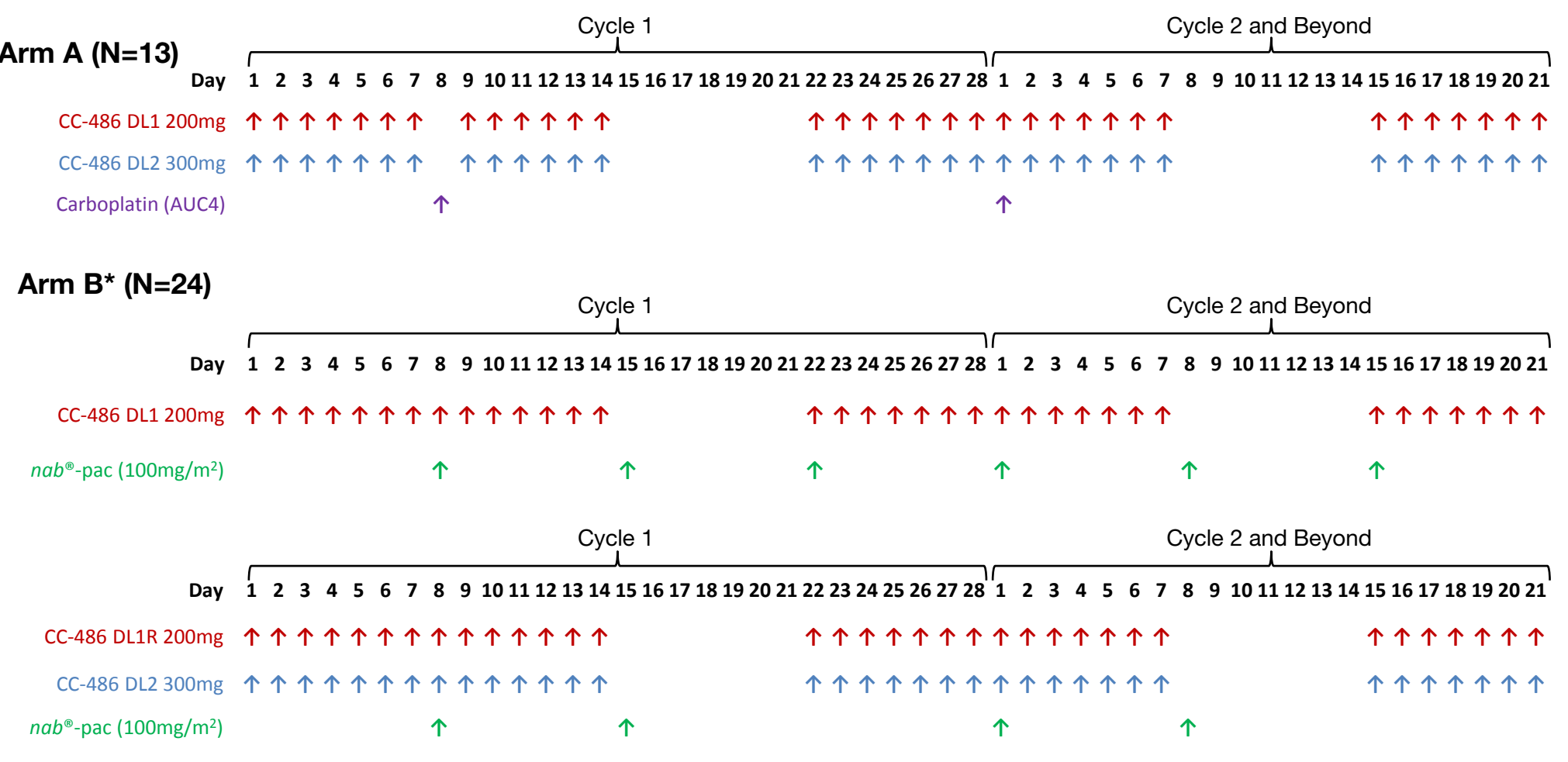
- CC-486 was given once-daily for 21 days of a 28-day cycle in Cycle 1 and for 14 days of 21-day cycles thereafter:
 - Arm A: CC-486 + carboplatin (AUC 4)
 - Arm B: CC-486 + *nab*[®]-pac
 - Arm C: CC-486 was given as single agent on a continuous and intermittent dosing schedule.

- Part 1** of the study assessed anti-tumor activity endpoints (response rate, duration of response, progression-free survival) according to the tumor-specific response criteria, Response Evaluation Criteria in Solid Tumors (RECIST)

- Part 2** of the study, which assesses the anti-tumor activity of CC-486 with carboplatin or *nab*[®]-pac in specific tumor types, is ongoing

- In this poster, we report findings from Part 1 Arms A and B only. Arm C will be reported separately and Part 2 is ongoing.**

Figure 1. Study Design



*In Arm B, DL1 = dose level 1, 200 mg CC-486 + 3 doses of *nab*[®]-pac; DL1R = DL1 re-challenge, 200 mg CC-486 + 2 doses of *nab*[®]-pac; DL2 = dose level 2, 300mg CC-486 + 2 doses of *nab*[®]-pac

RESULTS

Arm A (N=13)

- 2 Dose-limiting toxicities (DLTs) events occurred: Grade 3 thrombocytopenia with clinically significant bleeding (DL1, 200mg CC-486 QDx14/21 days), and Grade 4 neutropenia lasting > 7 days (DL2, 300mg CC-486 QDx14/21 days)
- Per protocol, CC-486 300mg QD was the maximum administered dose (MAD), and was tolerated in combination with carboplatin (AUC 4) every 3 weeks in this heavily pre-treated solid tumor patient population
- One patient on each DL discontinued treatment due to an adverse event (AE) (neutropenia and diarrhea in Cycle 2 on DL1; sepsis leading to death in Cycle 8 on DL2).

Arm B (N=24)

- 2 DLTs occurred on DL1: Grade 4 neutropenia for > 7 days, and febrile neutropenia. No DLTs occurred on DL1 re-challenge. 2 DLTs occurred on DL2 (Grade 4 neutropenia delayed the start of Cycle 2 > 7 days)
- Per protocol, CC-486 200mg QD was the MTD in combination with *nab*[®]-pac 100mg/m² on Days 8 and 15. This regimen was tolerated in these heavily pre-treated patients with solid tumors

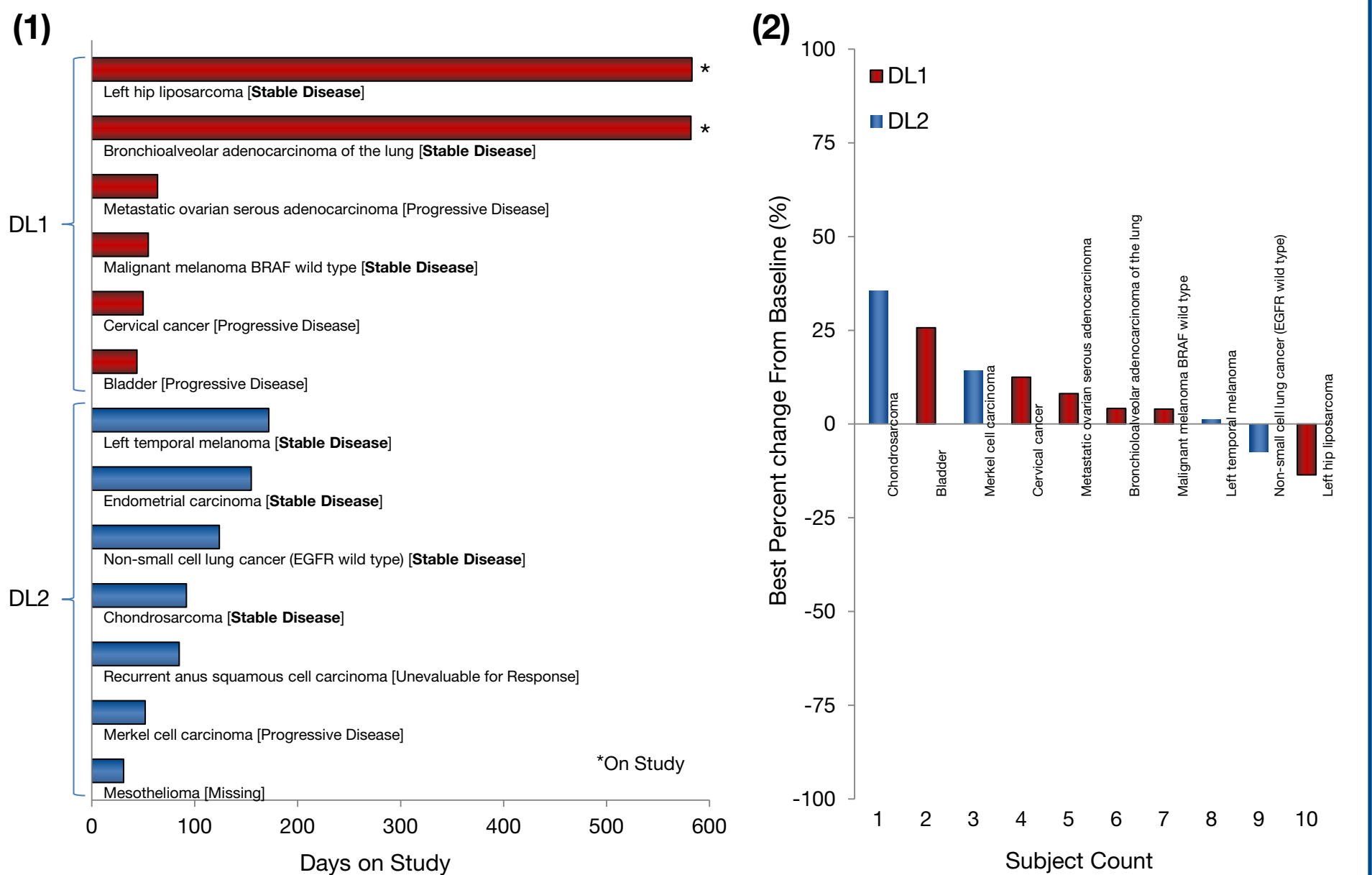
RESULTS

Table 1. Arm A - CC-486 + Carboplatin: Treatment-emergent Adverse Events (TEAEs)* and Drug-related TEAEs*

TEAEs Preferred Term	DL1 (n=6)		DL2 (n=7)		Total (N=13)
	Grade ≥ 3	All Grades	Grade ≥ 3	All Grades	All Grades
Anemia	1 (16.7)	4 (66.7)	1 (14.3)	3 (42.9)	7 (53.8)
Constipation	0	3 (50.0)	0	4 (57.1)	7 (53.8)
Decreased appetite	0	2 (33.3)	0	3 (42.9)	5 (38.5)
Diarrhoea	0	4 (66.7)	0	<20%	5 (38.5)
Fatigue	0	3 (50.0)	1 (14.3)	4 (57.1)	7 (53.8)
Leukopenia	0	<20%	1 (14.3)	<20%	<20%
Nausea	0	3 (50.0)	0	4 (57.1)	7 (53.8)
Neutropenia	2 (33.3)	3 (50.0)	3 (42.9)	3 (42.9)	6 (46.2)
Pyrexia	0	2 (33.3)	0	0	<20%
Thrombocytopenia	1 (16.7)	3 (50.0)	0	0	3 (23.1)
Vomiting	0	4 (66.7)	0	6 (85.7)	10 (76.9)
White blood cell count decreased	0	0	1 (14.3)	<20%	<20%
Drug-related TEAEs					
Preferred Term	DL1 (n=6)		DL2 (n=7)		Total (N=13)
	Grade ≥ 3	All Grades	Grade ≥ 3	All Grades	All Grades
Anemia	1 (16.7)	4 (66.7)	1 (14.3)	3 (42.9)	7 (53.8)
Decreased appetite	0	<20%	0	2 (28.6)	<20%
Diarrhoea	0	3 (50.0)	0	<20%	3 (23.1)
Fatigue	0	2 (33.3)	1 (14.3)	3 (42.9)	5 (38.5)
Leukopenia	0	<20%	1 (14.3)	<20%	<20%
Nausea	0	3 (50.0)	0	3 (42.9)	6 (46.2)
Neutropenia	2 (33.3)	3 (50.0)	3 (42.9)	3 (42.9)	6 (46.2)
Thrombocytopenia	1 (16.7)	3 (50%)	0	0	3 (23.1)
Vomiting	0	4 (66.7)	0	5 (71.4)	9 (69.2)
White blood cell count decreased	0	0	1 (14.3)	<20%	<20%

*Overall frequency ≥ 20% (any Grade) or Grade ≥ 3 (NCI-CTCAE v4.0)
DL1 = dose level 1, 200 mg CC-486 + 1 dose of carboplatin; DL2 = dose level 2, 300mg CC-486 + 1 dose of carboplatin

Figure 2. Arm A - CC-486 + Carboplatin: (1) Days on Study and Best Overall Response; (2) Percent Change from Baseline in Length of Target Lesions



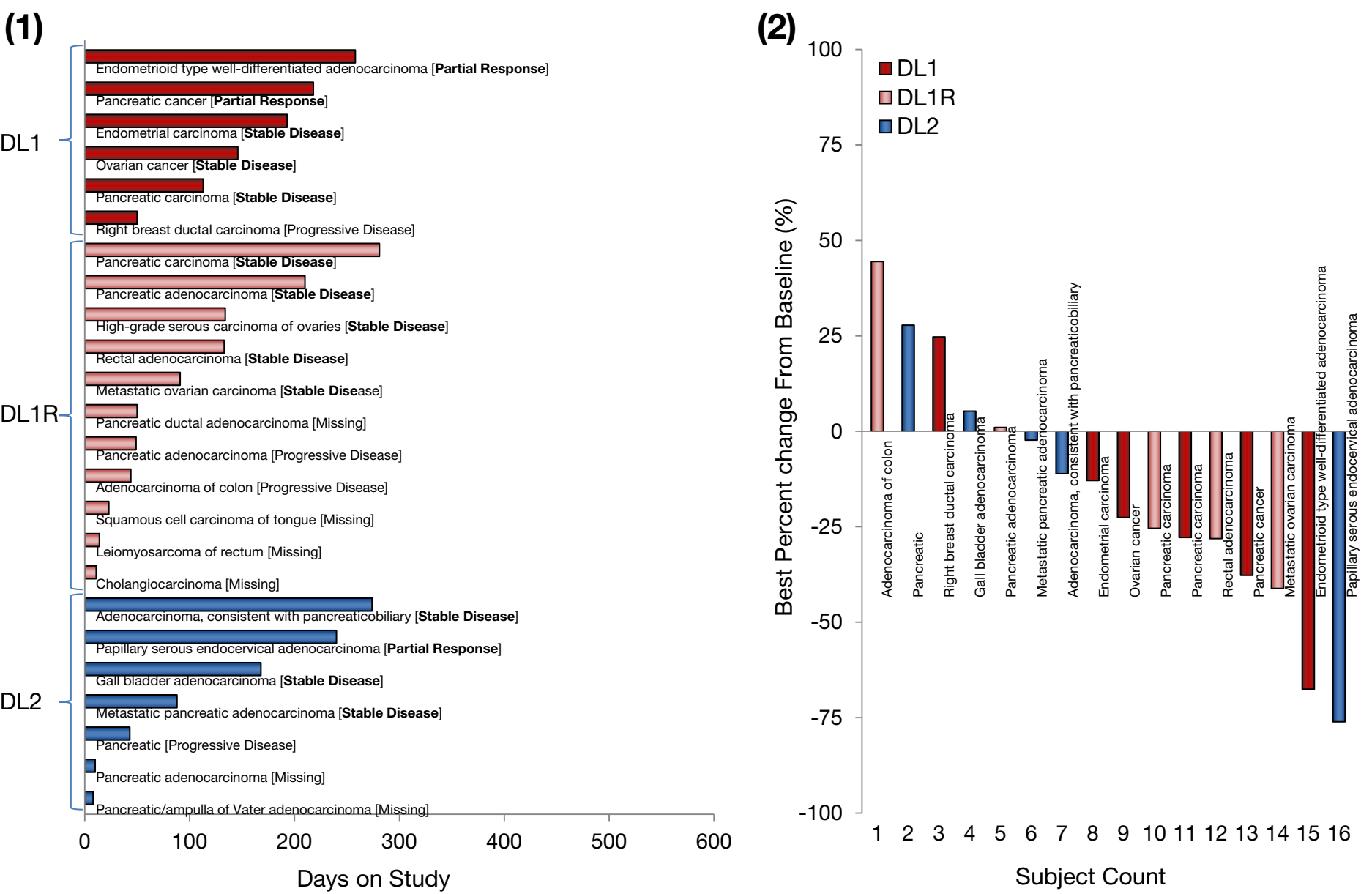
• 5/13 Patients in Arm A attained Stable Disease (>4 months)

Table 2. Arm B - CC-486 + *nab*[®]-Paclitaxel: TEAEs* and Drug-related TEAEs*

TEAEs Preferred Term	DL1 (n=6)		DL1R (n=11)		DL2 (n=7)		Total (N=24)
	Grade ≥ 3	All Grades	Grade ≥ 3	All Grades	Grade ≥ 3	All Grades	All Grades
Alopecia	0	5 (83.3)	0	4 (36.4)	0	5 (71.4)	14 (58.3)
Anemia	0	0	2 (18.2)	4 (36.4)	0	<20%	5 (20.8)
Constipation	0	<20%	0	3 (27.3)	0	3 (42.9)	7 (29.2)
Decreased appetite	0	<20%	0	5 (45.5)	0	0	6 (25.0)
Depression	0	2 (33.3)	0	0	0	0	<20%
Diarrhoea	0	5 (83.3)	0	4 (36.4)	0	2 (28.6)	11 (45.8)
Dysgeusia	0	2 (33.3)	0	<20%	0	2 (28.6)	5 (20.8)
Dyspnea	0	0	0	3 (27.3)	0	<20%	<20%
Fatigue	0	5 (83.3)	0	5 (45.5)	1 (14.3)	5 (71.4)	15 (62.5)
Febrile neutropenia	0	0	1 (9.1)	<20%	0	<20%	<20%
Insomnia	0	2 (33.3)	0	0	0	0	<20%
Nausea	0	4 (66.7)	0	7 (63.6)	0	<20%	12 (50.0)
Neutropenia	3 (50.0)	4 (66.7)	2 (18.2)	<20%	5 (71.4)	5 (71.4)	11 (45.8)
Peripheral sensory neuropathy	0	3 (50.0)	0	<20%	0	<20%	6 (25.0)
Pruritus	0	0	0	0	0	2 (28.6)	<20%
Vomiting	0	3 (50.0)	0	6 (54.5)	0	3 (42.9)	12 (50.0)
White blood cell count decreased	0	0	1 (9.1)	<20%	3 (42.9)	3 (42.9)	<20%
Drug-related TEAEs							
Preferred Term	DL1 (n=6)		DL1R (n=11)		DL2 (n=7)		Total (N=24)
	Grade ≥ 3	All Grades	Grade ≥ 3	All Grades	Grade ≥ 3	All Grades	All Grades
Alopecia	0	5 (83.3)	0	3 (27.3)	0	3 (42.9)	11 (45.8)
Anemia	0	0	1 (9.1)	3 (27.3)	0	<20%	<20%
Decreased appetite	0	<20%	0	4 (36.4)	0	0	<20%
Diarrhoea	0	5 (83.3)	0	3 (27.3)	0	2 (28.6)	10 (41.7)
Fatigue	0	3 (50.0)	0	5 (45.5)	0	2 (28.6)	10 (41.7)
Febrile neutropenia	0	0	1 (9.1)	<20%	0	<20%	<20%
Nausea	0	4 (66.7)	0	7 (63.6)	0	<20%	11 (45.8)
Neutropenia	3 (50.0)	4 (66.7)	2 (18.2)	<20%	5 (71.4)	5 (71.4)	11 (45.8)
Vomiting	0	3 (50.0)	0	6 (54.5)	0	3 (42.9)	12 (50.0)
White blood cell count decreased	0	0	1 (9.1)	<20%	3 (42.9)	3 (42.9)	<20%

*Overall frequency ≥20% (any Grade) or Grade ≥3 (NCI-CTCAE v4.0)
DL1 = dose level 1, 200 mg CC-486 + 3 doses of *nab*[®]-pac; DL1R = DL1 re-challenge, 200 mg CC-486 + 2 doses of *nab*[®]-pac; DL2 = dose level 2, 300mg CC-486 + 2 doses of *nab*[®]-pac

Figure 3. Arm A - CC-486 + *nab*[®]-Paclitaxel: (1) Days on Study and Best Overall Response; (2) Percent Change from Baseline in Length of Target Lesions



• 8/24 Patients attained Stable Disease (>4 months) and 3/24 patients attained Partial Response in Arm B

Figure 4. CC-486 Priming + *nab*[®]-Paclitaxel: Partial Response in Pancreatic Carcinoma

- 87-year-old male with pancreatic carcinoma
- One prior treatment regimen: gemcitabine × 8 cycles (Stable Disease)

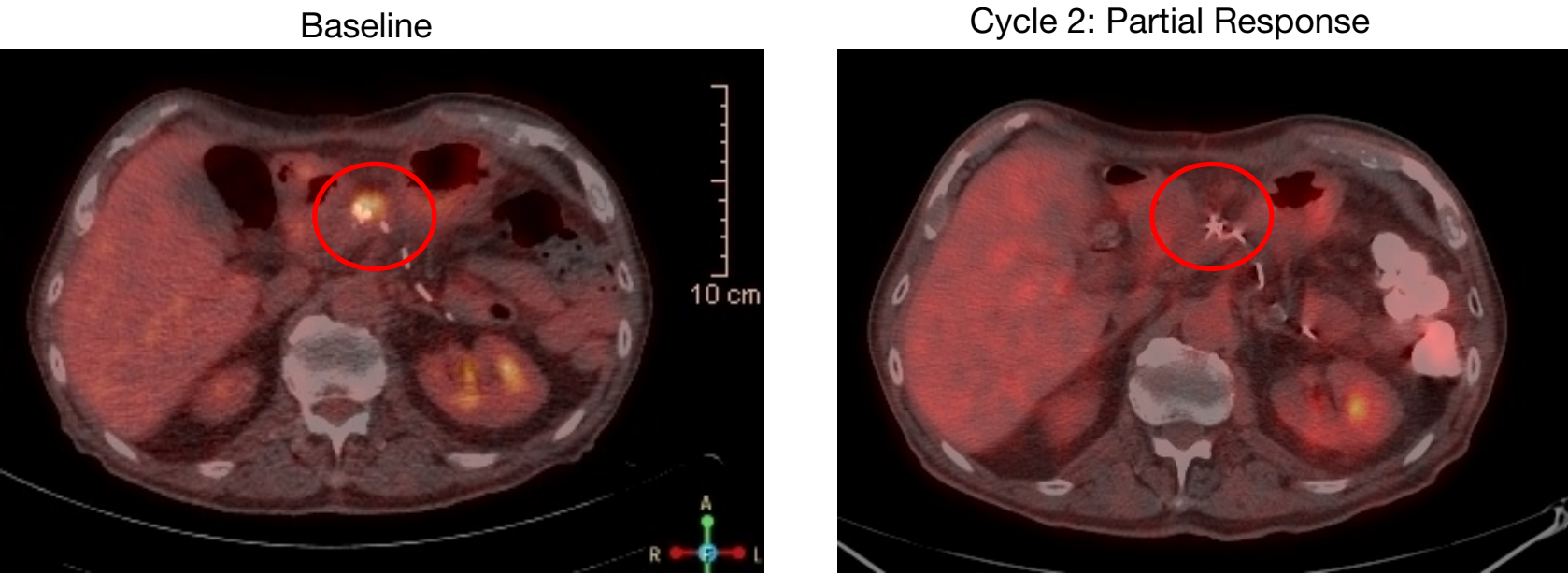
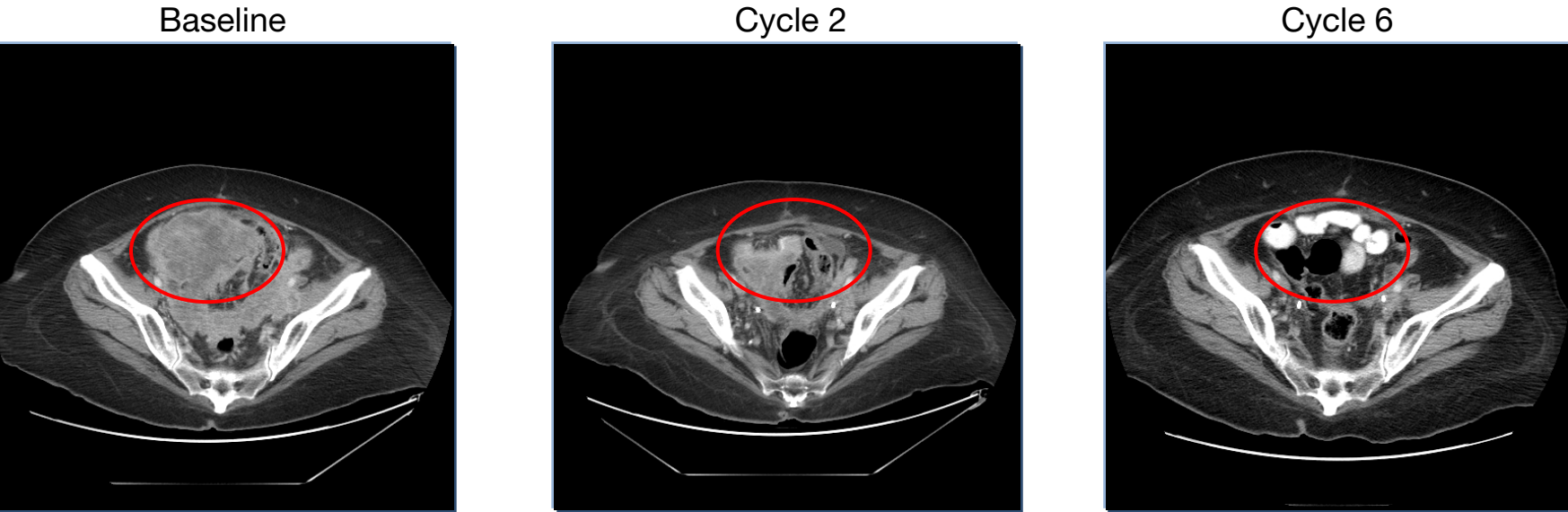


Figure 5. CC-486 Priming + *nab*[®]-Paclitaxel: Partial Response in Endometrial Carcinoma

- 57-year-old female with endometrial cancer who progressed after carbo/taxol, CC-122
- No evidence of disease at primary (some residual at other target lesions)



CONCLUSIONS

- CC-486 dosed 14 of 21 days is tolerated as a priming agent with carboplatin or with *nab*[®]-paclitaxel
- Incidence and severity of adverse events did not differ substantially between the two CC-486 dose levels; neutropenia was seen with both dose regimens and was dose-limiting in Arm B
- Both combination regimens showed evidence of anti-tumor activity
 - 5/13 Patients who received 300mg CC-486 + carboplatin achieved Stable Disease
 - 8/24 Patients who received 200mg CC-486 + *nab*[®]-paclitaxel achieved Stable Disease and 3/24 patients achieved Partial Response
- Expansion cohorts of Arm A (300mg CC-486 + carboplatin in relapsed/refractory bladder or ovarian carcinoma) and Arm B (200mg CC-486 + *nab*[®]-paclitaxel in relapsed/refractory NSCLC or pancreatic carcinoma) are ongoing
- Results of pharmacokinetic and pharmacodynamic assessments in Part 1 of this study are shown in the AACR-NCI-EORTC 2013 Laille et al poster B217

REFERENCES

- Laille et al. Presented at the 54th Annual Meeting of the American Society of Hematology (ASH), Atlanta, GA; December 8-11, 2012 . Poster 1337

DISCLOSURES

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