

P-2022/13418

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIRECCIÓN DE NUEVAS CREACIONES

E. S. D.

Re: Expediente No. **NC2022/0007361**Solicitud de patente a nombre de **INOVIO PHARMACEUTICALS, INC. Y REGENERON PHARMACEUTICALS, INC.**

Yo, CAROLINA DAZA MONTALVO identificada como aparece al pie de mi firma, domiciliada en la Carrera 11 No. 86-53, obrando como apoderada de las sociedades INOVIO PHARMACEUTICALS, INC, domiciliada en Pensilvania, Estados Unidos de América y REGENERON PHARMACEUTICALS, INC., domiciliada en Nueva York, Estados Unidos de América, solicitantes de la patente de referencia, en respuesta al oficio N° 10769 notificado electrónicamente el 10 de julio de 2025, relativo a los requisitos sustantivos de la solicitud, respetuosamente me permito presentar la siguiente información:

1. Título

El examinador afirma que el título de la invención "TERAPIA DE COMBINACIÓN PARA EL TRATAMIENTO DE CÁNCER CEREBRAL" no se relaciona con el objeto de la solicitud contenida en las reivindicaciones.

Para superar la objeción, el título se modifica de acuerdo con la sugerencia del examinador: "COMPOSICIÓN INMUNOGÉNICA QUE COMPRENDE TRANSCRIPTASA INVERSA DE LA TELOMERASA HUMANA (HTERT), TUMOR DE WILMS-1 (WT-1) Y ANTÍGENO DE MEMBRANA ESPECÍFICO DE LA PRÓSTATA (PSMA)".

2. Modificación al capítulo reivindicatorio:

Con fundamento en la facultad que confiere el artículo 34 de la Decisión 486 al solicitante para modificar la solicitud de patente en cualquier momento del trámite, presento junto a este memorial un nuevo capítulo reivindicatorio que consta de una

(1) reivindicación, el cual reemplaza completamente la versión presentada el 12 de diciembre de 2022. Específicamente, se realizaron las siguientes modificaciones:

- 2.1 Se eliminaron las antigua reivindicaciones 1 a 5 y 7 a 12. Además, se ajustó la numeración del documento.
- 2.2 Se modificó la antigua reivindicación 6 (nueva reivindicación 1), con el fin de incluir las características de las antiguas reivindicaciones 2, 4, 7 y 8.

Estas modificaciones no amplían de ninguna manera la materia objeto de la presente solicitud, ya que se encuentran sustentadas en la descripción originalmente presentada y se realizan con el fin de definir la materia reclamada de forma clara y superar las objeciones del examinador.

3. Pago de tasas

Considerando que el presente memorial se presenta junto con modificaciones a las reivindicaciones, se adjunta recibo por concepto de pago de tasa de modificación a la solicitud, según lo establecido en la Resolución 59669 de 2020.

Adicionalmente me permito resaltar que no se realiza el pago de la tasa por concepto de reivindicaciones adicionales, dado que el nuevo capítulo reivindicatorio cuenta con un menor número de cláusulas que el presentado y estudiado anteriormente por su despacho.

4. Reivindicaciones relativas a usos

El examinador indica que, de conformidad con lo señalado en el artículo 14 de la Decisión 486 de 2000 de la Comisión de la Comunidad Andina, solo son patentables las invenciones de producto o de procedimiento. En consecuencia, las reivindicaciones 9 a 12 no se consideran patentables.

Objeción del examinador:

Desde la perspectiva del examinador las reivindicaciones 9 a 12 reclaman una composición inmunológica para su uso en la elaboración de un medicamento para tratar un cáncer cerebral, en donde el cáncer cerebral es glioblastoma con MGMT metilado o sin metilar, lo cual se relaciona directamente con materia no patentable.

Argumentos del solicitante:

El solicitante en este sentido manifiesta respetuosamente su desacuerdo con el examinador, sin embargo, deja constancia de que las reivindicaciones 9 y 12 han sido eliminadas del pliego de reivindicaciones. De esta manera, no habría reivindicaciones relativas a uso, cumpliendo con lo establecido en el artículo 14 de la Decisión 486 de 2000 de la Comisión de la Comunidad Andina.

5. Claridad, concisión y soporte de las reivindicaciones

El solicitante manifiesta que mediante la presentación del nuevo capítulo reivindicatorio quedan subsanadas la totalidad de las objeciones presentadas por el examinador en su concepto técnico, de modo tal que las nuevas reivindicaciones reclaman de manera clara y concisa la invención descrita. Con respecto a las objeciones específicas del examinador, me permito manifestar lo siguiente:

- 5.1** El Examinador alega que la reivindicación 1 no es clara ni presenta sustento en la descripción, ya que no define una composición inmunogénica en términos de sus características técnicas esenciales completas. En particular, no se indican los componentes activos, excipientes y adyuvantes con sus respectivas cantidades y proporciones, ni se proporciona la estructura aminoacídica (con su respectivo SEQ ID NO.) de los elementos que conforman la composición, como hTERT, WT-1, PSMA, IL-12 y el anticuerpo anti-PD-1.

Se deja constancia de que la antigua reivindicación 1 ha sido eliminada del pliego de reivindicaciones.

Asimismo, el solicitante desea aclarar que la nueva reivindicación independiente 1 se dirige a una vacuna en la que se establece de manera precisa la estructura de aminoácidos de hTERT, WT-1, PSMA e IL-12, así como las secuencias de nucleótidos que los codifican. Adicionalmente, se incluye la caracterización del anticuerpo anti-PD-1 a partir de las SEQ ID NO correspondientes a sus CDRs, regiones variables y secuencias de cadena pesada y ligera, cumpliendo así con los requerimientos de caracterización estructural señalados por el examinador para la reivindicación independiente.

En vista de lo anterior, se solicita respetuosamente que se consideren superadas las objeciones planteadas en este aspecto.

- 5.2** El Examinador alega que la reivindicación 2 no es clara ni presenta sustento en la descripción, ya que no define una composición inmunogénica en términos de sus características técnicas esenciales completas. En particular, no se indican los componentes activos, excipientes y adyuvantes con sus respectivas cantidades y proporciones, ni se proporciona la estructura polinucleotídica (con su SEQ ID No.) de los elementos que conforman la composición, como hTERT, WT-1, PSMA, IL-12 y el anticuerpo anti-PD-1.

En este sentido, el solicitante desea resaltar que la antigua reivindicación 2 ha sido eliminada del pliego de reivindicaciones. En vista de lo anterior, se solicita respetuosamente que se tengan por superadas las objeciones planteadas en este aspecto.

- 5.3** El Examinador alega que las reivindicaciones 1 y 2 no son concisas, ya que ambas reclaman una composición inmunogénica en más de una reivindicación independiente.

En este sentido, el solicitante manifiesta su desacuerdo, sin embargo, como se mencionó en los apartados 5.1 y 5.2 las antiguas reivindicaciones 1 y 2 han sido eliminadas del pliego de reivindicaciones. En vista de lo anterior, se solicita respetuosamente que se tengan por superadas las objeciones planteadas en este aspecto.

- 5.4** El Examinador alega que la reivindicación 6 no es clara ni presenta sustento en la descripción, ya que no define una vacuna en términos de sus características técnicas esenciales estructurales completas. En particular, no se indican las secuencias de aminoácidos específicas que caracterizan la región variable de cadena pesada (VH) y la región variable de cadena ligera (VL) del anticuerpo anti-PD-1.

Con el fin de contribuir a dar continuidad al proceso, la antigua reivindicación 6 ha sido modificada con el fin de cumplir la sugerencia del Examinador, de modo que ahora reivindica las secuencias de aminoácidos específicas que caracterizan la región variable de cadena pesada (VH) y la región variable de cadena ligera (VL) del anticuerpo anti-

PD-1. De este modo, la vacuna reivindicada se define en términos de sus características técnicas esenciales estructurales completas.

En vista de lo anterior, se solicita respetuosamente que se tengan por superadas las objeciones planteadas en este aspecto.

5.5 El Examinador alega que la reivindicación 7 no es clara ni presenta sustento en la descripción, ya que se refiere a porcentajes de identidad de las secuencias de aminoácidos que caracterizan la región variable de cadena pesada (VH) y la región variable de cadena ligera (VL) del anticuerpo anti-PD-1. Sin embargo, no se justifica en la descripción el uso de variantes con al menos 90 % de identidad, ni se incluyen las secuencias completas de dichas variantes en el listado de secuencias con su respectivo SEQ ID No.

Se deja en constancia que la antigua reivindicación 7 ha sido eliminada del pliego de reivindicaciones. En vista de lo anterior, se solicita respetuosamente que se tengan por superadas las objeciones planteadas en este aspecto.

5.6 El Examinador alega que la reivindicación 7 no es clara, ya que incluye la expresión "*REGN2810 o un biosimilar o bioequivalente de este*", la cual remite a elementos de la descripción.

Como se mencionó anteriormente en el apartado 5.5 la antigua reivindicación 7 ha sido eliminada del pliego de reivindicaciones. En vista de lo anterior, se solicita respetuosamente que se tengan por superadas las objeciones planteadas en este aspecto.

6. Descripción

El examinador indica que la descripción no divulga la materia reclamada de manera suficiente para que una persona normalmente versada en la materia pueda ejecutarla o reproducirla, por lo que no cumpliría con las disposiciones del Art. 28 de la Decisión Andina 486.

Objeción del examinador:

En este sentido, el Examinador señala que la descripción no respalda el capítulo de reivindicación, ya que no hay evidencia concreta en la descripción de la obtención de

composiciones inmunogénicas que comprendan cualquier estructura para hTERT, WT-1 y PSMA, ni cualquier adyuvante. La solicitud únicamente presenta soporte técnico para una composición específica que incluye el plásmido INO-5401. Asimismo, se aclara al solicitante que, para reproducir la invención, es necesaria la estructura completa del anticuerpo, no solo las regiones CDRs, considerando que se trata de un anticuerpo humanizado y optimizado.

Argumento del solicitante:

Tal como se ha indicado en la sección 5 del presente memorial, el solicitante ha presentado un nuevo capítulo reivindicatorio en el que se elimina la antigua reivindicación 1. En consecuencia, ya no forma parte de la materia reclamada "cualquier" composición inmunogénica, ni las estructuras para la transcriptasa inversa de la telomerasa humana, el Tumor de Wilms-1, el antígeno de membrana específico de la próstata o un adyuvante en términos genéricos.

De esta manera, el alcance actual de la invención se deriva de una generalización razonable de lo efectivamente divulgado en la descripción, la cual proporciona información suficiente y clara para que una persona versada en la materia pueda reproducir la invención sin necesidad de realizar un esfuerzo inventivo indebido. A ello se suma que el alcance ha sido restringido a una caracterización estructural concreta de la vacuna, asegurando precisión técnica y sustento en la descripción. En particular, la nueva reivindicación 1 establece de forma precisa la estructura de aminoácidos de hTERT, WT-1, PSMA e IL-12, así como las secuencias de nucleótidos que los codifican. Asimismo, incorpora la caracterización del anticuerpo anti-PD-1 a partir de las SEQ ID NO correspondientes a sus CDRs, regiones variables y secuencias de cadena pesada y ligera.

En este marco, resulta pertinente recordar lo establecido en el artículo 51 de la Decisión 486, según el cual *"el alcance de la protección conferida por la patente estará determinado por el tenor de las reivindicaciones, y la descripción, los dibujos y, en su caso, las listas de secuencias o el depósito de material biológico servirán para interpretarlas"*. Esta disposición respalda una interpretación técnica coherente de la invención, que abarca todas las realizaciones divulgadas y ejecutables por una persona versada en la materia, las cuales se encuentran directamente vinculadas con el alcance de la vacuna definido en el nuevo capítulo reivindicatorio.

Por todo lo anterior, se concluye que la reivindicación independiente 1 reclama una razonable generalización de la materia divulgada, plenamente reproducible por una persona versada en la materia con sus conocimientos ordinarios en el campo, con base en la caracterización estructural incluida en la solicitud y en las limitaciones técnicas introducidas en el nuevo capítulo reivindicatorio. Ello se ajusta a lo previsto en la Guía de Examen de Patentabilidad de 2023 (Sección 4.4.2), la cual establece que:

"Una persona con conocimientos ordinarios en la materia debe ser capaz de llevar a cabo (o reproducir) la invención, y esto debe ser posible sin requerir un esfuerzo inventivo que exceda sus capacidades ordinarias."

De esta manera, encontramos que la materia divulgada en la descripción se relaciona de manera cercana con la materia definida en el capítulo reivindicatorio. Por lo tanto, la descripción proporciona toda la información necesaria para llevar a cabo la invención y cumple con los requisitos legales establecidos en la Decisión Andina 486.

7. Novedad

El examinador afirma en su concepto técnico que las antiguas reivindicaciones 1 a 6 no serían nuevas a la luz de las enseñanzas del documento D1 (WO2018064588).

En este sentido, quisiera destacar que el artículo 16 de la Decisión 486 de la Comisión de la Comunidad Andina establece que la invención se considera nueva cuando no está incluida en el estado de la técnica. Es decir, según el espíritu de la norma legal indicada, la invención no se considera nueva si está "*comprendida*" en el estado de la técnica, dicha norma se refiere, por supuesto, a la invención en su totalidad y no a sus componentes de forma aislada.

A este respecto, considero pertinente subrayar que, para considerar que el objeto de la solicitud no es nuevo, deben tenerse en cuenta al menos los siguientes aspectos:

- El objeto de la invención objeto de estudio debe estar contenido TOTAL y ABSOLUTAMENTE en el documento mencionado anteriormente; y
- La comparación del objeto de la invención en cuestión y el estado de la técnica no debe centrarse ni limitarse a los elementos de esta que se conocen previamente, sino de lo inédito y novedoso que la caracteriza. Esto significa que debe analizarse el valor añadido que aporta la invención con sus nuevas características al campo técnico al que pertenece la solicitud en estudio.

Esto significa que, para que un documento del estado de la técnica afecte a la novedad de una solicitud, debe revelar todas y cada una de las características técnicas que lo definen.

También es de señalar lo que se establece en el Manual Andino para el Examen de Patentes sobre el análisis de novedad.

"Para determinar la novedad de la invención, se debe comprobar si existen anticipaciones del estado de la técnica que contengan explícitamente todas las características técnicas esenciales de la invención. El examen de novedad se efectúa comparando elemento por elemento de la invención tal como está definido por las reivindicaciones con los del estado de la técnica." (El énfasis es nuestro)

"Si todas las características técnicas de la reivindicación independiente se encuentran descritas en un mismo antecedente y además se encuentran íntimamente relacionadas, el objeto de dicha reivindicación carece de novedad. Si una característica, aunque sea banal, no se encuentra contenida en el antecedente, la reivindicación es nueva" (El énfasis es nuestro)

"Modificaciones obvias o equivalentes del objeto descrito en el estado de la técnica no pueden ser citadas en contra de la novedad del objeto reivindicado sino cuando están descritas ellas mismas en el antecedente." (El énfasis es nuestro)

De lo anterior, puede interpretarse sin lugar a dudas que, si existe al menos una característica diferenciadora entre el objeto reivindicado y lo divulgado explícitamente en los antecedentes tomados en cuenta por sí solos, en este caso D1, incluso si dicha diferencia no es una característica esencial sino banal, debe reconocerse la novedad de la invención.

Objeción del examinador:

Cabe señalar que el examinador considera que el documento D1 anticipa el objeto de la presente solicitud porque divulga la transcriptasa inversa de la telomerasa humana (hTERT) con la secuencia de aminoácidos de SEQ ID NO: 20, el tumor de Wilms-1 (WT-1) con la secuencia de aminoácidos de SEQ ID NO: 26 y el antígeno de membrana específico de la próstata (PSMA) con la secuencia de aminoácidos de SEQ ID NO: 28 con un 100% de identidad y que adicionalmente la composición inmunogénica puede

ser una vacuna y combinarse adicionalmente con anticuerpos contra inhibidores de puntos de control.

Además, el examinador menciona que D1 menciona una composición inmunogénica para evaluar las respuestas inmunitarias inducidas en primates no humanos (NHP) que comprende pGX1434 (un plásmido para la expresión de transcriptasa inversa de la telomerasa humana (hTERT); pGX1404 (un plásmido para la expresión del antígeno de gen de tumor de Wilms-1 (WT1); pGX1108 (un plásmido para la expresión del antígeno PSMA); y pGX6006 (un plásmido para la expresión de rhIL-12).

Argumentos del solicitante:

En este sentido, el solicitante desea aclarar que la nueva reivindicación independiente 1 establece de manera expresa que el objeto a proteger es una vacuna. Si bien la referencia D1 divulga una formulación multivalente Wave 1 que comprende plásmidos que codifican los antígenos SynCon hTERT (SEQ ID NO: 45), Consensus WT1 (SEQ ID NO: 19) y Consensus PSMA (SEQ ID NO: 65), específicamente en el ejemplo 14 y en la figura 22B, dicha referencia no proporciona ninguna enseñanza ni divulgación concreta de una vacuna que combine simultáneamente una composición inmunogénica de plásmidos que codifican hTERT, PSMA y WT-1 con un anticuerpo anti-PD-1, tal como se recoge en la nueva reivindicación 1. Esta última incorpora además las características técnicas de las antiguas reivindicaciones 7 y 8, que no fueron objetadas por falta de novedad.

Adicionalmente, aun cuando el Examinador señala ciertas similitudes estructurales en los alineamientos 1 a 3, dichas similitudes no anticipan la totalidad de las características técnicas reivindicadas. La nueva reivindicación 1 no solo comprende las secuencias de nucleótidos que codifican los antígenos, sino también una caracterización estructural detallada del anticuerpo anti-PD-1 (incluyendo sus CDRs, regiones variables y cadenas pesada y ligera), lo que establece una clara diferenciación frente al documento D1.

Así pues, dado que el documento D1 no divulga de manera TOTAL Y ABSOLUTA las características técnicas esenciales de la materia definida en la reivindicación independiente de la presente solicitud, es posible concluir que la presente solicitud cumple el requisito de novedad estipulado en el artículo 16 de la Decisión 486.

8. Nivel inventivo

El Examinador considera que las reivindicaciones independientes 7 y 8, carecerían de nivel inventivo en vista de las enseñanzas del documento WO2018064588 (**D1**) y US2015/203579 (**D2**).

Con respecto a lo anterior, me permito resaltar las disposiciones del artículo 18 de la Decisión 486 de la Comisión de la Comunidad Andina, que establecen que “*se considerará que la invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica*”.

Lo anterior significa que el análisis de nivel inventivo **NO** es una comparación de los objetos de las anterioridades y la solicitud en estudio. Por el contrario, el análisis de nivel inventivo **debe demostrar** la obviedad de la invención y establecer cómo ésta **NO** contiene un salto cualitativo, que demuestre su altura inventiva, teniendo en cuenta que se hace **necesario demostrar** que la invención analizada carece de nivel inventivo y hubiese sido obvia para una persona versada en la materia.

El análisis del Examinador

El Examinador considera que el documento D1 representa el estado de la técnica más cercano a la invención definida en la reivindicación independiente 7 ya que divulga una vacuna multivalente que incluye una composición inmunogénica compuesta por los plásmidos pGX1434 (hTERT), pGX1404 (WT1), pGX1108 (PSMA) y pGX6006 (rhIL-12 como adyuvante). Se reporta que esta formulación indujo respuestas inmunitarias robustas en ratones, evidenciadas mediante ELISpot de IFN γ y activación de linfocitos T CD8+, además de una reducción de la carga tumoral y mayor supervivencia, según lo descrito en el Ejemplo 14 (págs. 205–206, figuras 22 a 28).

Ahora, es importante resaltar que el Examinador reconoció que la principal diferencia entre el anticuerpo divulgado en **D1** y el definido en la reivindicación 7 de la presente solicitud consiste en que la vacuna reclamada comprende un anticuerpo anti-PD-1 caracterizado porque comprende las regiones determinantes de complementariedad de cadena pesada (HCDR1, HCDR2 y HCDR3) de una región variable de cadena pesada (VH) que comprende la secuencia de aminoácidos de SEQ ID NO: 1 y tres regiones determinantes de complementariedad de cadena ligera (LCDR1, LCDR2 y LCDR3) de una región variable de cadena ligera (VL) que comprende la secuencia de aminoácidos de SEQ ID NO: 2.

Sin embargo, el Examinador considera que estas diferencias no están asociadas a un efecto técnico, ya que no se presentan pruebas que demuestren que incluir

específicamente un anticuerpo anti-PD-1 con una región VH correspondiente a la SEQ ID NO: 1 y una región VL correspondiente a la SEQ ID NO: 2 en una vacuna que comprende una composición inmunogénica con hTERT, WT-1 y PSMA, genere un efecto técnico distinto al ya divulgado en D1. En dicha referencia, ya se prevé que una vacuna que incluya un anticuerpo anti-PD-1 puede potenciar la respuesta inmunitaria celular y humoral al evitar la supresión de linfocitos T o B por PD-1 o PDL-1 (Página 11, líneas 11-18; Página 59, líneas 29-34; Página 60, líneas 1-29).

Sin embargo, el Examinador considera que estas metodologías no representan una contribución técnica adicional, ya que el documento **D2** ya sugería un anticuerpo anti-PD-1 que comprende una VH de SEQ ID NO: 162 y una VL de SEQ ID NO: 170 (Página 23, tabla 1), en donde la SEQ ID NO: 162 presenta 100% de identidad y cobertura con respecto a la SEQ ID NO: 1 que caracteriza la VH del anticuerpo anti-PD-1 reclamado (Ver alineamiento 4). Por otra parte, la SEQ ID NO: 170 presenta 100% de identidad y cobertura con respecto a la SEQ ID NO: 2 que caracteriza la VL del anticuerpo anti-PD-1 reclamado.

En consecuencia, el Examinador asegura que la persona normalmente versada en la materia estaría motivada a incluir el anticuerpo anti-PD-1 que comprende una VH de SEQ ID NO: 162 y una VL de SEQ ID NO: 170 del D2 en la vacuna de D1, para así llegar al objeto de la reivindicación 7. Por lo cual se considera obvia.

Por último, el Examinador afirma que la reivindicación 8 no menciona ninguna característica que, junto con las características de las reivindicaciones independiente, proporcione un nivel inventivo a la cerveza reivindicada, por lo que también carecen de nivel inventivo.

Ahora bien, dichas condiciones no se cumplen para el caso en cuestión, pues, tal como se mostrará más adelante, las características técnicas mencionadas en las nuevas reivindicaciones no pueden derivarse de manera obvia o evidente a partir de las enseñanzas del estado de la técnica representadas en el documento **D1 en combinación con D2**, y, por lo tanto, la materia reclamada es inventiva a la luz del arte previo.

La materia reclamada es inventiva a la luz del arte previo citado

Inicialmente, el solicitante manifiesta su desacuerdo con la opinión expresada por el examinador. No obstante, para efectos del presente debate, se acepta analizar el

documento **D1** y **D2** como base para evaluar la supuesta falta de nivel inventivo de las reivindicaciones.

Inicialmente se aclara que una persona experta en la materia, partiendo de D1 y D2, no habría llegado de manera evidente a la vacuna definida en la nueva reivindicación 1, ni habría tenido una expectativa razonable de éxito, considerando la naturaleza altamente impredecible del tratamiento del glioblastoma. D1 no enseña ni sugiere una vacuna que combine específicamente plásmidos codificantes de hTERT, PSMA y WT-1 con un anticuerpo anti-PD-1 como lo hace la nueva reivindicación 1.

Además, no existe una expectativa razonable de que las vacunas reivindicadas fueran efectivas contra el glioblastoma basándose únicamente en D1 y D2. En relación con el tratamiento de glioblastoma, D1 menciona que la combinación de un antígeno TERT con el antígeno tumoral CMV, opcionalmente junto con otros antígenos como BY-ESO-1, MAGE-A1 o WT1, podría aplicarse para el tratamiento o prevención del glioblastoma (página 185). Aunque el Ejemplo 14 y la Figura 28 de D1 sugieren una reducción de la carga tumoral tras el tratamiento con la formulación multivalente en ratones implantados con células TC-1, esta línea celular expresa E7 y proviene de células epiteliales pulmonares de ratón C57BL/6, inmortalizadas con HPV-16 E6/E7 y transformadas con un oncogén ras activado, lo cual limita su relevancia como modelo de glioblastoma (ver Anexo 2: Lin et al., Cancer Res. 1996; 56: 21–26).

Adicionalmente, el Examinador considera que no existe una expectativa razonable de que las vacunas reivindicadas fueran efectivas para el tratamiento del glioblastoma, basándose únicamente en las referencias D1 y D2, debido a la alta imprevisibilidad asociada a esta enfermedad en la fecha de prioridad. En ese momento, el tratamiento estándar para el glioblastoma multiforme (GBM) consistía en cirugía, seguida de radioterapia (RT) y quimioterapia con temozolomida (TMZ), administrada durante la RT y luego en ciclos de mantenimiento de 6 a 12 meses, según lo reportado por Stupp et al., 2005 (ver Anexo 3 a este memorial).

El glioblastoma es reconocido como el tipo más común y agresivo de tumor maligno primario del sistema nervioso central, con una tasa de supervivencia a cinco años inferior al 5%. A nivel mundial, se estima que 300,000 personas desarrollan cáncer cerebral o del sistema nervioso central cada año, y aproximadamente 241,000 fallecen por esta causa. Las opciones terapéuticas son limitadas, y desde 2005 no se ha aprobado ningún medicamento que mejore la supervivencia en pacientes con GBM recién diagnosticado (ver Anexos 4: *CheckMate 548 Trial* y Bristol-Myers Squibb, 2019).

En el estudio clínico CheckMate 548, se evaluó la adición del anticuerpo anti-PD-1 nivolumab al tratamiento estándar, comparado con el tratamiento estándar solo, en pacientes con GBM metilado en MGMT. El estudio no logró cumplir con el criterio principal de eficacia (supervivencia libre de progresión, PFS), lo que indica que la combinación no ofreció un beneficio clínico significativo. Según *Lim et al., Neuro-Oncology, 2022* (Anexo 5 de este memorial), la mediana de supervivencia global (OS) entre los pacientes tratados con nivolumab fue de 28.9 meses.

De manera similar al estudio CheckMate 548, el estudio clínico CheckMate 498 tampoco logró demostrar eficacia en el tratamiento del glioblastoma. Este estudio evaluó la combinación de radioterapia (RT) con el anticuerpo anti-PD-1 nivolumab frente al tratamiento estándar con quimiorradioterapia basada en temozolomida (TMZ) en pacientes con glioblastoma multiforme (GBM) no metilado en MGMT. Según el comunicado de *Bristol-Myers Squibb* (ver Anexo 6 de este memorial: CheckMate 498, 2019), el estudio no alcanzó su objetivo principal de mejorar la supervivencia global (OS).

Los resultados publicados por *Omuro et al., Neuro-Oncology, 2023* (ver Anexo 7 de este memorial), indican que la mediana de supervivencia fue de 13.4 meses en el grupo tratado con nivolumab, frente a 14.9 meses en el grupo control tratado con la terapia estándar. Estos datos refuerzan la conclusión de que, en la fecha de prioridad, no existía una expectativa razonable de que el uso de un anticuerpo anti-PD-1 en combinación con una composición inmunogénica fuera efectivo para el tratamiento del glioblastoma, dada la naturaleza altamente impredecible de esta enfermedad.

Adicionalmente, el análisis de supervivencia mediante el estimador de Kaplan-Meier mostró diferencias significativas entre los grupos evaluados en el estudio presentado por INOVIO en la reunión anual de ASCO 2022. En la Cohorte A (pacientes con promotor MGMT no metilado), la mediana de supervivencia global (OS) fue de 17.9 meses (IC 95%: 14.5–19.8), mientras que en la Cohorte B (pacientes con promotor MGMT metilado), la mediana de OS fue de 32.9 meses (IC 95%: 18.4–45.2). Estos resultados se obtuvieron en el contexto de una combinación terapéutica que incluye INO-5401, INO-9012 y el anticuerpo anti-PD-1 cemiplimab (Libtayo®), y fueron reportados en el comunicado oficial de INOVIO (ver Anexo 8 de este memorial)

Aunque el objetivo primario de supervivencia a 18 meses (OS18) no alcanzó el umbral preestablecido de mejora del 30 % sobre los valores históricos para pacientes con

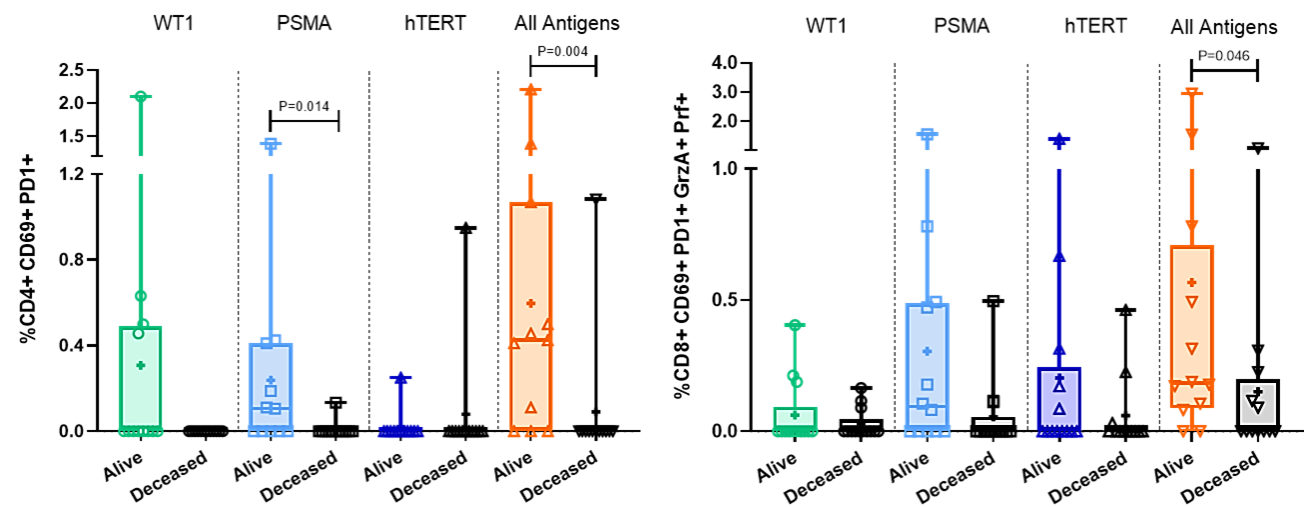
glioblastoma recién diagnosticado, ya sea con promotor MGMT metilado o no metilado, se observaron mejoras tanto en OS18 como en la mediana de supervivencia (OS) en comparación con dichos referentes históricos. En particular, un paciente de cada cohorte continúa en el estudio, con tiempos de tratamiento de aproximadamente 80 meses (~6.5 años) y 75 meses (~6.25 años), respectivamente.

A los 18 meses, el 50 % de los pacientes de la Cohorte A (MGMT no metilado) y el 70 % de la Cohorte B (MGMT metilado) seguían con vida. Estos resultados superan los valores históricos reportados por Gilbert et al. (ver Anexos 9 y 10 de este memorial: *J Clin Oncol*, 2013; *N Engl J Med*, 2014), donde se documentaron tasas de OS18 de 45 % y 60 % para pacientes con promotor MGMT no metilado y metilado, respectivamente. Asimismo, la mediana de OS en dichos estudios fue de 12.6 meses (no metilado) y 23.4 meses (metilado), mientras que en el estudio del solicitante se alcanzaron 17.9 y 32.9 meses, respectivamente.

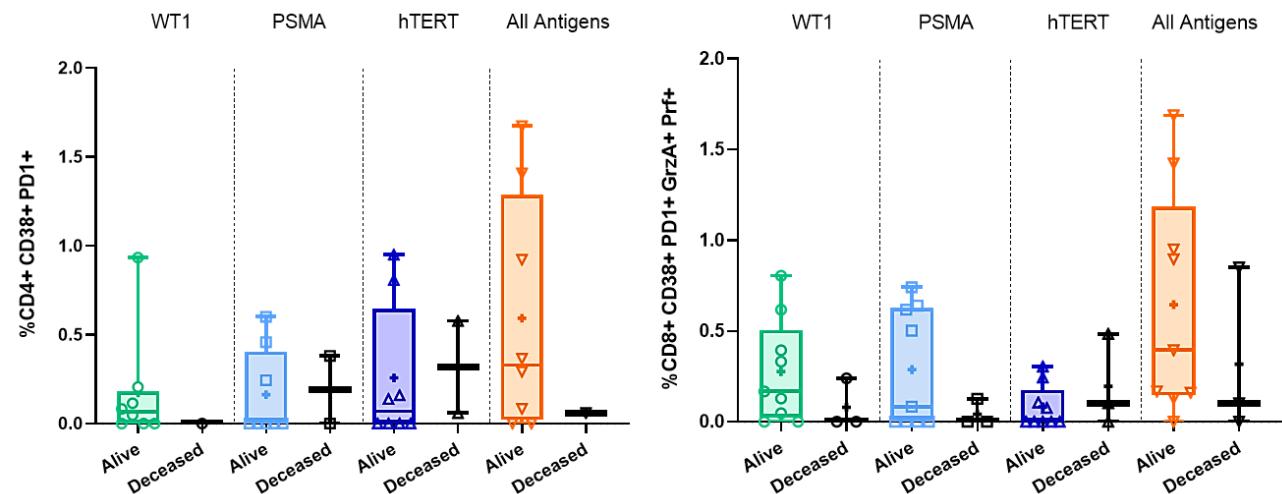
Además, los resultados de supervivencia del solicitante también son superiores a los reportados en los brazos control de los estudios fase 3 CheckMate-548 y CheckMate-498, donde la mediana de OS fue de 13.4 meses para pacientes MGMT no metilado y 28.9 meses para pacientes MGMT metilado tratados con nivolumab más terapia estándar.

Adicionalmente, los solicitantes realizaron análisis de citometría de flujo post-estudio para evaluar las respuestas de células T inducidas por INO-5401 + INO-9012 en combinación con cemiplimab en sangre periférica. Se obtuvieron muestras suficientes en el 81% de los pacientes de la Cohorte A (MGMT no metilado) y en el 67% de la Cohorte B (MGMT metilado). Se observaron respuestas inmunes específicas de INO-5401 en células T CD4+ y CD8+ en el 65% de los pacientes evaluables de la Cohorte A y en el 92% de los de la Cohorte B. Las respuestas incluyeron marcadores como CD69, PD-1, GrzA y perforina. La magnitud de la respuesta total a los antígenos de INO-5401 fue significativamente mayor en pacientes de la Cohorte A que sobrevivieron más de 18 meses, tanto en células T CD4+ como CD8+, en comparación con quienes sobrevivieron menos (Fig. 1A, C). En la Cohorte B, se observaron respuestas robustas en la mayoría de los pacientes (Fig. 1B).

Figura 1A.



B



C

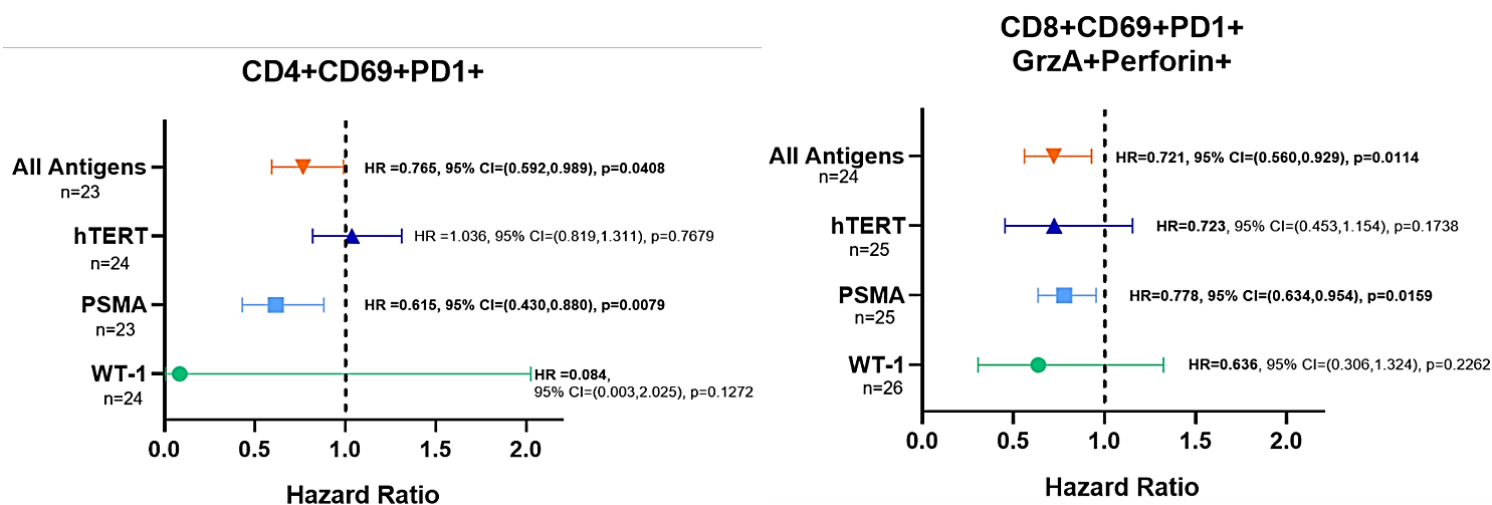


Fig. 1. INO-5401 + INO-9012 en combinación con cemiplimab induce respuestas de células T periféricas asociadas con un menor riesgo de muerte en pacientes de la cohorte A. A, B.

Se evaluó la frecuencia máxima postratamiento (%) de células T CD4+ activadas y con experiencia antigénica (Fig. A, B, paneles izquierdos) y células T CD8+ citotóxicas (Fig. A, B, paneles derechos) por encima del valor basal en respuesta a los antígenos de INO-5401 (WT1, PSMA, hTERT) y al total de antígenos (suma de las tres respuestas específicas por paciente), mediante citometría de flujo multiparamétrica, en pacientes vivos y fallecidos según la supervivencia global a 18 meses (OS18). Las comparaciones estadísticas entre grupos se realizaron con la prueba de rango suma de Wilcoxon bilateral, mostrando únicamente los valores de p significativos (Fig. A, B).

En las Figuras C y D, se analizó el riesgo de muerte a los 18 meses por cada aumento del 0.1% en la frecuencia de células T CD4+ y CD8+ en respuesta a los antígenos de INO-5401 y al total de antígenos, mediante razones de riesgo calculadas con el modelo de regresión de riesgos proporcionales de Cox.

- **Figuras A y C (Cohorte A, MGMT no metilado):** hasta $n = 12$ pacientes para CD4+CD69+PD-1+ y $n = 13$ para CD8+CD69+PD-1+GrzA+Prf+.

- **Figuras B y D (Cohorte B, MGMT metilado):** hasta $n = 9$ pacientes vivos y $n = 3$ fallecidos para CD4+CD38+PD-1+ y CD8+CD38+PD-1+GrzA+Prf+.

Los antígenos en las figuras se representan como: WT1 (círculo verde), PSMA (cuadro azul claro), hTERT (triángulo azul oscuro) y todos los antígenos (triángulo naranja). GrzA: granzima A; Prf: perforina.

En la Cohorte A, 12 de los 17 pacientes (71%) que presentaron respuestas inmunes a los antígenos de INO-5401 sobrevivieron al menos 18 meses, mientras que solo 1 de los 9 pacientes (11%) que no respondieron estaba vivo a los 18 meses. En la Cohorte B, 8 de los 11 pacientes (73%) con respuestas inmunes específicas también sobrevivieron al menos 18 meses.

Para profundizar en esta tendencia, se realizó un análisis de riesgos proporcionales de Cox para evaluar el impacto de las respuestas de células T periféricas sobre la supervivencia. Como se muestra en la **Fig. 1C**, en la Cohorte A, las respuestas de células T CD4+ a los antígenos combinados de INO-5401 se asociaron con una reducción del 23% en el riesgo de muerte a los 18 meses, mientras que las respuestas de células T CD8+ mostraron una reducción del 28%; ambas fueron estadísticamente significativas ($p < 0.05$ y $p < 0.025$, respectivamente).

En la **Cohorte B**, se observó un patrón similar de células T CD4+ y CD8+ activadas, con expresión de PD-1 y una tendencia hacia CD38, correlacionando con la supervivencia a 18 meses. Esta relación también fue evaluada mediante el modelo de Cox, y la **Fig. 1D** muestra que las respuestas de células T CD4+ se asociaron con una reducción del 19% en el riesgo de muerte, mientras que las respuestas de células T CD8+ se vincularon con una reducción del 11%.

En resumen, los pacientes que desarrollaron respuestas inmunes periféricas frente a los antígenos vacunales mostraron una mayor probabilidad de supervivencia a los 18 meses en comparación con aquellos que no presentaron dichas respuestas. Esta observación respalda una asociación entre las respuestas inmunes inducidas por inmunoterapia frente a antígenos tumorales y una mayor probabilidad de supervivencia a los 18 meses.

Por lo tanto, a la fecha de prioridad, una persona experta en la materia, incluso teniendo en cuenta las referencias D1 y D2, no habría esperado razonablemente los resultados clínicos obtenidos con las vacunas reivindicadas, dada la imprevisibilidad en el

tratamiento del glioblastoma. En consecuencia, la reivindicación solicitada debe considerarse inventiva respecto de las referencias citadas y el rechazo debe ser retirado.

Esta vacuna reivindicada no sería obvia ni predecible para los expertos en la materia, ni siquiera basándose en **D1**.

Teniendo en cuenta lo anteriormente expuesto, queda claro que las características técnicas de la materia definida en el capítulo reivindicatorio presentado junto al presente memorial no se derivan de manera obvia o evidente de las enseñanzas de **D1**. Por lo tanto, el objeto reclamado cumple con el requisito de Nivel inventivo establecido en el Art. 18 de la Decisión 486.

7. Conclusión

Con las modificaciones introducidas y los argumentos presentados puede concluirse que:

- i) El nuevo pliego reivindicatorio se ajusta a las disposiciones del Artículo 34 de la Decisión 486/00;
- ii) La invención de la presente solicitud es clara y cuenta con el debido sustento en el capítulo descriptivo, a la vez que se encuentra divulgada de manera suficientemente completa, cumpliendo así con los requerimientos de los Artículos 30 y 28 de la Decisión 486;
- iii) La presente invención se relaciona con materia susceptible de patentabilidad, cumpliendo así con los requerimientos del Artículo 14 de la Decisión 486;
- iv) La invención de la presente solicitud no resulta obvia ni se derivada de manera evidente del estado de la técnica, cumpliendo así con los requerimientos del Artículo 18 de la Decisión 486.

Por tanto, atentamente me permito solicitar a ese Despacho otorgar el privilegio de patente solicitado a la solicitud en estudio, toda vez que cumple a cabalidad con los requerimientos de la Decisión 486 de 2000.

Asimismo, respetuosamente solicito a ese Despacho pronunciarse en relación con todos los argumentos presentados en este memorial dando estricta aplicación al principio del debido proceso consagrado en el artículo 29 de la Constitución Nacional.

8. Anexos

1. Nuevo capítulo reivindicatorio conformado por una (1) reivindicación.
2. Ken-Yu Lin, Frank G. Guarnieri, Kevin F. Staveley-O'Carroll, Hyam I. Levitsky, J. Thomas August, Drew M. Pardoll, Tzyy-Chou Wu; Treatment of Established Tumors with a Novel Vaccine That Enhances Major Histocompatibility Class II Presentation of Tumor Antigen¹. *Cancer Res* 1 January 1996; 56 (1): 21–26.
3. Stupp et al., *N Engl J Med*. 2005 Mar 10;352(10):987–96
4. *CheckMate 548 Trial*, Bristol-Myers Squibb, 2019
5. *Lim et al., Phase III trial of chemoradiotherapy with temozolomide plus nivolumab or placebo for newly diagnosed glioblastoma with methylated MGMT promoter, Neuro-Oncology, Volume 24, Issue 11, November 2022, Pages 1935–1949, <https://doi.org/10.1093/neuonc/noac116>*
6. *CheckMate 498 Study Did Not Meet Primary Endpoint of Overall Survival*", 2019
7. *Omuro et al., Radiotherapy combined with nivolumab or temozolomide for newly diagnosed glioblastoma with unmethylated MGMT promoter: An international randomized phase III trial, Neuro-Oncology, Volume 25, Issue 1, January 2023, Pages 123–134, <https://doi.org/10.1093/neuonc/noac099>*
8. *INOVIO Announces Survival Results for INO-5401 + INO-9012 in Combination with Libtayo® (cemiplimab) in Patients with Newly Diagnosed GBM at ASCO Annual Meeting 2022*,
https://s23.q4cdn.com/479936946/files/doc_news/INOVIO-Announces-Survival-Results-for-INO-5401--INO-9012-in-Combination-with-Libtayo-cemiplimab-in-Patients-with-Newly-Diagnosed-GBM--8TI11.pdf.
9. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, Colman H, Chakravarti A, Pugh S, Won M, Jeraj R, Brown PD, Jaeckle KA, Schiff D, Stieber VW, Brachman DG, Werner-Wasik M, Tremont-Lukats IW, Sulman EP, Aldape KD, Curran WJ Jr, Mehta MP. A randomized trial of bevacizumab for newly diagnosed glioblastoma. *N Engl J Med*. 2014 Feb 20;370(8):699-708. doi: 10.1056/NEJMoa1308573. PMID: 24552317; PMCID: PMC4201043.
10. Gilbert MR, Wang M, Aldape KD, Stupp R, Hegi ME, Jaeckle KA, Armstrong TS, Wefel JS, Won M, Blumenthal DT, Mahajan A, Schultz CJ, Erridge S, Baumert B, Hopkins KI, Tzuk-Shina T, Brown PD, Chakravarti A, Curran WJ Jr, Mehta MP.

Dose-dense temozolomide for newly diagnosed glioblastoma: a randomized phase III clinical trial. J Clin Oncol. 2013 Nov 10;31(32):4085-91. doi: 10.1200/JCO.2013.49.6968. Epub 2013 Oct 7. PMID: 24101040; PMCID: PMC3816958.

11. Recibo de pago por concepto de modificaciones a las reivindicaciones.

Señora Directora,


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REIVINDICACIONES

1. Una vacuna que comprende:

- (a) Una composición inmunogénica que comprende un plásmido de ADN que codifica la transcriptasa inversa de la telomerasa humana (hTERT), en donde la hTERT comprende la secuencia de aminoácidos de la SEQ ID NO: 20,

en donde el plásmido de ADN que codifica el hTERT comprende la secuencia de ácido nucleico de la SEQ ID NO: 19;

un plásmido de ADN que codifica el Tumor de Wilms-1 (WT-1), en donde el WT-1 comprende la secuencia de aminoácidos de la SEQ ID NO: 26,

en donde el plásmido de ADN que codifica el WT-1 comprende la secuencia de ácido nucleico de la SEQ ID NO: 27; y

un plásmido de ADN que codifica el antígeno de membrana específico de la próstata (PSMA), en donde el PSMA comprende la secuencia de aminoácidos de la SEQ ID NO: 28,

en donde el plásmido de ADN que codifica el PSMA comprende la secuencia de ácido nucleico de la SEQ ID NO: 29;

- (b) un plásmido de ADN que comprende una secuencia de ácido nucleico que codifica la interleucina-12 (IL12), un plásmido de ADN que comprende una secuencia de ácido nucleico que codifica la subunidad p35 de IL12, un plásmido de ADN que comprende una secuencia de ácido nucleico que codifica la subunidad p40 de IL12, o un plásmido de ADN que comprende una secuencia de ácido nucleico que codifica la subunidad p35 de IL12 y una secuencia de ácido nucleico que codifica la subunidad p40 de IL12,

en donde la subunidad p35 de IL12 comprende la secuencia de aminoácidos de la SEQ ID NO: 23;

en donde el plásmido de ADN codifica la subunidad p35 de IL12 comprende la secuencia de ácido nucleico de la SEQ ID NO: 22; y

la subunidad p40 de IL12 comprende la secuencia de aminoácidos de la SEQ ID NO: 25,

en donde el plásmido de ADN que codifica la subunidad p40 de IL12 comprende la secuencia de ácido nucleico de la SEQ ID NO: 24; y

- (c) un anticuerpo anti receptor de muerte celular programada 1 (PD-1) en donde el anticuerpo anti-PD-1 comprende:

Una región determinante de complementariedad de cadena pesada 1 (HCDR1) que comprende la secuencia de aminoácidos de la SEQ ID NO:3, una región determinante de complementariedad de cadena pesada 2 (HCDR2) que comprende la secuencia de aminoácidos de la SEQ ID NO:4, y una región determinante de complementariedad de cadena pesada 3 (HCDR3) que comprende la secuencia de aminoácidos de la SEQ ID NO:5,

Una región determinante de complementariedad de cadena ligera 1 (LCDR1) que comprende la secuencia de aminoácidos de la SEQ ID NO:6, una región determinante de complementariedad de cadena ligera 2 (LCDR2) que comprende la secuencia de aminoácidos de la SEQ ID NO:7, y una región determinante de complementariedad de cadena ligera 3 (LCDR3) que comprende la secuencia de aminoácidos de la SEQ ID NO:8,

una región variable de cadena pesada (HCVR) que comprende la secuencia de aminoácidos de la SEQ ID NO: 1 y una región variable de cadena ligera (LCVR) que comprende la secuencia de aminoácidos de la SEQ ID NO: 2; o

una cadena pesada que comprende la secuencia de aminoácidos de la SEQ ID NO: 9 y una cadena ligera que comprende la secuencia de aminoácidos de la SEQ ID NO: 10.

Treatment of Established Tumors with a Novel Vaccine That Enhances Major Histocompatibility Class II Presentation of Tumor Antigen

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Treatment of Established Tumors with a Novel Vaccine That Enhances Major Histocompatibility Class II Presentation of Tumor Antigen¹

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Abstract

Presentation of antigenic peptides by MHC class II molecules to CD4⁺ T cells is critical to the generation of antitumor immunity. In an attempt to enhance MHC class II antigen processing, we linked the sorting signals of the lysosome-associated membrane protein (LAMP-1) to the cytoplasmic/nuclear human papilloma virus (HPV-16) E7 antigen, creating a chimera (Sig/E7/LAMP-1). Previously, we found that expression of this chimera *in vitro* and *in vivo* with a recombinant vaccinia vector targeted E7 to endosomal and lysosomal compartments and enhanced MHC class II presentation to CD4⁺ T cells compared to vaccinia expressing wild-type E7. In the current study, we tested these recombinant vaccinia for *in vivo* protection against an E7⁺ tumor, TC-1, which was derived from primary epithelial cells of C57BL/6 mice cotransformed with HPV-16 E6 and E7 and c-Ha-ras oncogenes. All mice vaccinated with 1 × 10⁷ plaque-forming units of wild-type E7-vaccinia showed progressive tumor growth when challenged with a tumorigenic dose of TC-1 tumor cells; in contrast, 80% of mice vaccinated with the chimeric Sig/E7/LAMP1 vaccinia remained tumor free 3 months after tumor injection. Furthermore, treatment with the Sig/E7/LAMP-1 vaccinia vaccine cured mice with small established TC-1 tumors, whereas the wild-type E7-vaccinia showed no effect on this established tumor burden. These findings point out the therapeutic limitations of recombinant vaccinia expressing unmodified tumor antigens. Further, they demonstrate that modifications that reroute a cytosolic tumor antigen to the endosomal/lysosomal compartment can profoundly improve the *in vivo* therapeutic potency of recombinant vaccines.

Introduction

It is becoming increasingly clear that CD4⁺ T cells are critical to the generation of potent antitumor immune responses. CD4⁺ T cells have been shown to be instrumental in generating immune responses against several solid malignancies in murine (1, 2) and human systems (3, 4). For example, several mouse tumors transfected with syngeneic MHC class II genes were effective vaccines against subsequent challenge with wild-type, MHC class II-negative tumors (5–7). In an adoptive transfer model, Greenberg *et al.* (8) demonstrated that CD4⁺ cells were critical in eliminating FBL tumors in mice. In addition, as crucial memory cells in the T-cell arm of the immune system, CD4⁺ cells may provide long-term immunity against specific tumor antigens (9, 10).

CD4⁺ T cells recognize antigen in the context of MHC class II molecules. In general, exogenous antigens are taken up by professional antigen-presenting cells through phagocytosis or endocytosis

and are degraded into antigenic peptides by acid proteases in low pH endosomal or lysosome-like compartments (11–13). The antigenic peptides then bind MHC class II molecules and are presented on the cell surface to CD4⁺, MHC class II-restricted T cells. On the other hand, cytoplasmic or nuclear proteins are generally processed and presented to CD8⁺ T cells through the MHC class I pathway. Cytoplasmic or nuclear proteins are degraded into peptides in the cytoplasm. The peptides are transported into the endoplasmic reticulum and complexed with MHC class I molecules, which present the antigenic peptides to CD8⁺ MHC class I-restricted T cells (reviewed in Ref. 14).

We previously described a novel molecular approach that directly routed a nuclear/cytoplasmic antigen, HPV-16 E7, into the endosomal and lysosomal compartments and enhanced the presentation of antigen to MHC class II-restricted CD4⁺ T cells (15). We then constructed a recombinant vaccinia virus containing the chimeric gene, *Sig/E7/LAMP-1*, in which E7 was linked to the endoplasmic reticulum translocation signal peptide, transmembrane domain, and lysosomal targeting domain of LAMP-1 (16–18). LAMP-1 is a type 1 transmembrane protein localized predominantly to lysosomes and late endosomes (19, 20). The cytoplasmic domain of LAMP-1 protein contains the amino acid sequence, Tyr-Gln-Thr-Ile, that mediates the targeting of LAMP-1 into the endosomal and lysosomal compartments (21, 22).

We chose the HPV-16 E7 as a model antigen for the following reasons: (a) HPVs, particularly HPV-16, are associated with most cervical cancers, and the HPV oncogenic proteins, E6 and E7, are important in the induction and maintenance of cellular transformation and are coexpressed in most HPV-containing cervical cancers. Therefore, vaccines or immunotherapies targeting E7 and/or E6 proteins may provide an opportunity to prevent and treat HPV-associated cervical malignancies; (b) HPV-16 E7 is a characterized cytoplasmic/nuclear protein and is more abundant than E6 in HPV-associated cancer cells; and (c) there were more immunologic assays performed on HPV-16 E7 compared to those on other HPV viral proteins. Therefore, more information is available on E7, and it potentially might be useful for designing immunological assays to study the E7-specific antitumor immune responses (reviewed in Ref. 23).

This specific targeting of HPV-16 E7 to the endosomal and lysosomal compartments allows antigenic peptides of E7 to complex with MHC class II molecules and enhances MHC class II presentation. Specifically, we showed that the *Sig/E7/LAMP-1* vaccinia *in vivo* generated greater E7-specific antibody production and CD4⁺ T cell-mediated lymphoproliferative responses than vaccinia expressing the wild-type HPV-16 E7 gene (15). In addition, E7-specific CTL responses were augmented as well, possibly as a consequence of enhanced CD4⁺ T-cell help (15).

To determine whether this MHC class II targeting strategy results

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³ The abbreviations used are: HPV, human papillomavirus; LAMP, lysosome-associated membrane protein; PFU, plaque-forming unit; MAb, monoclonal antibody.

directly in enhanced systemic antitumor responses, we generated an E7-expressing tumorigenic cell line, TC-1. Primary lung epithelial cells from C57BL/6 mice were immortalized by HPV-16 E6 and E7 and then transformed with an activated *ras* oncogene. The cotransformation produced a tumorigenic cell line expressing E6 and E7. This cell line mimics the natural sequence of tumor progression of cervical cancer in which HPV-16 E6 and E7 immortalizes cells and additional mutations transform the cells into advanced tumor cells with metastatic potential. This line thus provides an excellent model to compare the therapeutic potential of recombinant vaccinia expressing the wild-type *versus* the MHC class II-targeted forms of E7.

Materials and Methods

Construction of Mouse Tumor Cells by Cotransformation of HPV-16 E6 and E7 and Activated *ras* Oncogene. C57BL/6 mouse lungs were dispersed into a single-cell suspension by mechanical grinding, followed by digestion with collagenase at a concentration of 1 mg/ml in DMEM (GIBCO-BRL, Gaithersburg, MD). The primary lung cells were cultured *in vitro* in RPMI 1640, supplemented with 10% fetal calf serum, 50 units/ml penicillin/streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, and 2 mM nonessential amino acids, and grown at 37°C with 5% CO₂. Transduction of HPV-16 E6 and E7 genes into primary lung cells was performed with the LXSNI6E6E7 retroviral vector, a generous gift from Denise A. Galloway (Fred Hutchinson Cancer Research Center, Seattle, WA) (24). The HPV-16 E6 and E7 containing LXSNI6E6E7 were used to infect CRIP cells to generate recombinant virus with a wide host range. The primary lung cells were immortalized by transduction as described previously (24). Following transduction, the retroviral supernatant was removed, and the cells were grown in G418 (0.4 mg/ml) culture medium for an additional 3 days to allow for integration and expression of the recombinant retroviral genes. The immortalized lung (E6+E7) cells were then transduced with pVEJB expressing activated human c-Ha-*ras* gene, kindly provided by Chi V. Dang (The Johns Hopkins Hospital, Baltimore, MD), and selected with G418 (0.4 mg/ml) and hygromycin (0.2mg/ml). The presence of HPV-16 E7 was confirmed by PCR and immunofluorescent staining.

Tumor Growth Experiments. TC-1 tumor cells were injected in five C57BL/6 mice s.c. in the left leg at various doses, including 1×10^4 , 1×10^5 , and 1×10^6 cells/mouse. Mice were monitored regularly for tumor growth. The data were used to plot a graph of tumor growth kinetics. The animals were then sacrificed, and the tumor nodules were processed to check the presence of HPV-16 E7.

PCR of HPV-16 E7. PCRs were performed to determine the presence of E7 DNA in the tumor cells. The primers for HPV-16 E7 open reading frame were based on the published sequence of HPV-16 (25). The 5' primer containing 551–570 bp of HPV-16 sequence was 5'-CCCAGATCTAATCATG-CATG-3', and the 3' primer containing 840–859 bp of HPV-16 sequence was 5'-TATGGATCCTGAGAACAGAT-3'. The 100- μ l reactions containing 1 μ g of genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 6 mM MgCl₂, 0.5 μ M of the E7 primers, 0.2 mM each of dATP, dCTP, dTTP, and dGTP, and 2.5 units of ampli-Taq polymerase (Perkin-Elmer Cetus; Norwalk, CT) were subjected to 30 amplification cycles of 1 min of denaturation at 94°C, 1 min of primer annealing at 52°C, and 1 min of extension at 72°C with a thermal cycler (Perkin-Elmer Cetus). Aliquots of reaction products were size fractionated by 2% agarose gel electrophoresis.

Immunofluorescent Detection of HPV-16 E7 in TC-1 Tumor Cells. TC-1 cells in tissue culture were centrifuged onto a slide by cytospin, fixed with 4% paraformaldehyde, and permeabilized with 0.1% saponin. Explanted tumor cells were prepared from frozen sections. For the detection of the HPV-16 E7 protein, mouse anti-HPV-16 E7 MAb (Triton Corp., Alameda, CA) was used in conjunction with Texas Red-conjugated goat antimouse IgG (Calbiochem Corp., La Jolla, CA).

***In Vivo* Tumor Protection Experiments.** For vaccination, 1×10^7 PFUs of each vaccinia (including wild-type vaccinia, E7-vaccinia, and Sig/E7/LAMP-1 vaccinia) were injected i.p. into C57BL/6 mice. For challenge, 2×10^5 TC-1 cells/mouse were injected s.c. in the left leg 1 month after vaccination. TC-1 tumor cells growing *in vitro* culture were trypsinized, washed three times in serum-free $1 \times$ HBSS, and injected. Five mice were used

in each group. Mice were monitored twice a week for tumor growth and euthanized after the development of tumor.

***In Vivo* Tumor Regression Experiments.** Tumor cells for injection and the vaccinia for immunization were prepared as described above. Three $\times 10^4$ TC-1 cells were injected s.c. in the left leg. After 7 days, 1×10^7 PFU of recombinant vaccinia, either wild-type vaccinia, E7-vaccinia, or Sig/E7/LAMP-1-vaccinia, were injected i.p. into each C57BL/6 mouse. Five mice were used for each vaccinia. Mice were monitored twice weekly and euthanized after the development of tumor.

***In Vivo* Antibody Depletion Experiments.** *In vivo* antibody depletions have been described previously (26). Briefly, C57BL/6 mice were vaccinated with Sig/E7/LAMP-1 vaccinia at 1×10^7 PFU/mouse and challenged with TC-1 tumor cells at 2×10^5 cells/mouse 1 month later. Depletions were started 1 week prior to tumor inoculation. Five C57BL/6 mice were used in each group. MAb GK1.5 (27) was used for CD4 depletions, MAb 2.43 (28) was used for CD8 depletions, and MAb PK136 (29) was used for NK1.1 depletion. Depletion of lymphocyte subsets was assessed on the day of live tumor injection and weekly thereafter by flow cytometric analysis of spleen cells stained with 2.43 or GK1.5. For each time point of analysis, >99% depletion of the appropriate subset was achieved with normal levels of the other subsets. Depletion was terminated on day 45 after tumor inoculation.

Results

Creation of a Model Tumor Which Requires E₆ and E₇ For Malignant Phenotypes. Primary lung cells of C57BL/6 mice were immortalized with HPV-16 E6 and E7 genes and then transformed with pVEJB-expressing activated human c-Ha-*ras* gene. The cotransformation produced a tumorigenic cell line expressing E6 and E7. Morphologically, TC-1 tumor cells showed pleomorphic nuclear patterns, high mitotic index, abnormal mitosis, and high nuclear:cytoplasmic ratio, all commonly seen in malignant neoplasms (Fig. 1A). Immunocytochemical staining for cytokeratin, which is characteristic of epithelial-derived tumors, was positive for TC-1 (data not shown). In addition, the presence of E7 DNA was confirmed by PCR (Fig. 1B), and E7 proteins were detected by immunofluorescent staining (Fig. 1C). TC-1 is MHC class I⁺ and MHC class II⁻ (data not shown). Tumor growth kinetics were determined by injecting various doses of TC-1 cells into C57BL/6 mice. All the mice injected with TC-1 cells at 1×10^6 or 1×10^5 cells/mouse had tumor growth; at a dose of 1×10^4 cell/mouse, 80% (4 of 5) of mice injected with TC-1 tumor cells developed tumors (Fig. 2).

Vaccination with Sig/E7/LAMP-1 Vaccinia but not E7 Vaccinia Generates Systemic Responses against Challenge with the TC-1 Tumor. C57BL/6 mice were immunized i.p. with 1×10^7 PFU of either wild-type, E7, or Sig/E7/LAMP-1 vaccinia; 1 month later, the mice were challenged s.c. with 2×10^5 TC-1 cells. As shown in Fig. 3, 80% of mice receiving Sig/E7/LAMP-1 vaccinia remained tumor-free 3 months after tumor injection, while those injected with wild-type vaccinia or E7-vaccinia developed tumors within 3 weeks. Vaccination with E7-vaccinia slightly delayed tumor growth relative to wild-type vaccinia, but the effect was minimal. To assess treatment of established tumors, TC-1 cells were first injected into C57BL/6 mice s.c. at a dose of 3×10^4 /mouse in the left leg. Seven days later, these mice also received 1×10^7 PFU of either wild-type vaccinia, E7-vaccinia, or Sig/E7/LAMP-1-vaccinia i.p. As shown in Fig. 4, only those receiving Sig/E7/LAMP-1 vaccinia remained tumor free; all of the mice injected with wild-type vaccinia or E7-vaccinia showed progressive tumor growth within 2 weeks after tumor inoculation. Tumors that grew in mice vaccinated with E7-vaccinia still expressed E7 by PCR and immunofluorescence analysis (data not shown), indicating that the low therapeutic efficacy was not due to antigen loss.

CD4⁺, CD8⁺ T Cells, and NK1.1 Cells Were Essential for the Antitumor Immune Response Generated by Vaccination with Vaccinia Sig/E7/LAMP-1. To determine the types of lymphocytes that are important for the rejection of E7-positive tumor cells in our *in*

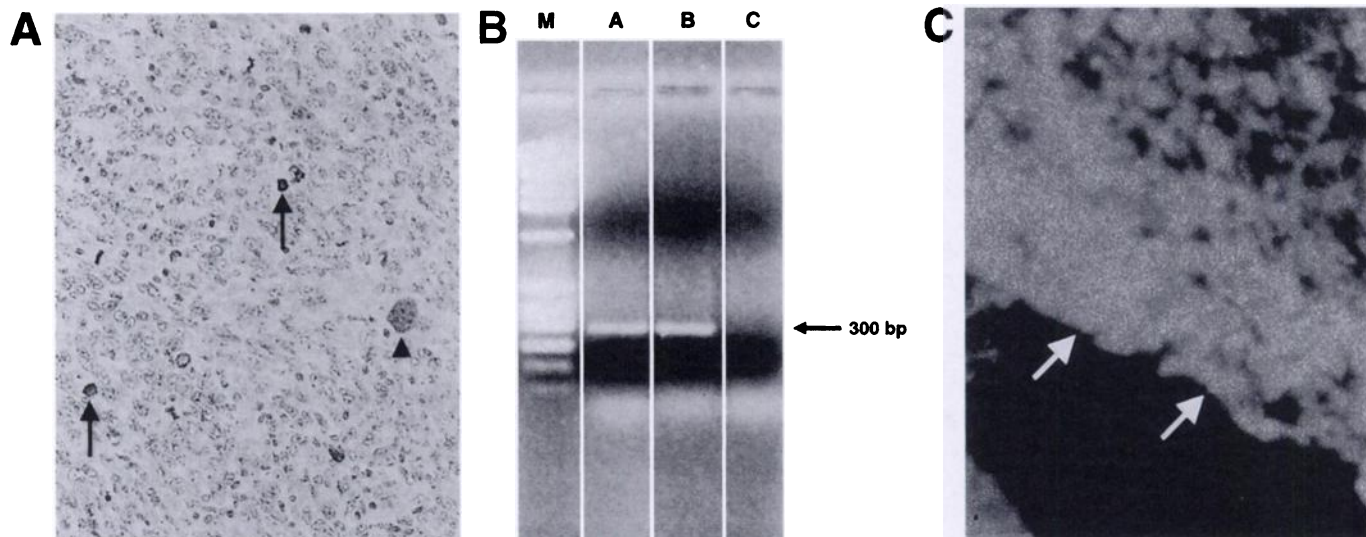


Fig. 1. Generation and characterization of E7-expressing TC-1 tumor. Tumors were explanted from C57BL/6 mice s.c. injected with TC-1 cells. **A**, hematoxylin and eosin staining of the explanted tumor. The tumor displays a high level of mitotic activity, pleomorphic nuclei (arrowhead), abnormal mitoses (arrows), and high nuclear:cytoplasmic ratio. $\times 250$. **B**, demonstration of the presence of E7 DNA within the genome of cells of explanted tumors by PCR and gel electrophoresis. Lane **M**, size marker. Lane **A**, positive control using DNA from CaSki cells, an HPV-16-containing cell line. Lane **B**, DNA from explanted TC-1 tumor. Lane **C**, negative control using HPV-negative human placenta DNA. A 300-bp DNA fragment corresponding to the size of the HPV-16 E7 open reading frame was observed in Lanes **A** and **B** as indicated by the arrow. **C**, immunofluorescent staining to demonstrate the expression of HPV-16 E7 protein in the explanted TC-1 tumor. Tumor sections were fixed, permeabilized, and stained with anti-HPV-16 E7 mouse MAb, followed by Texas red-conjugated goat antimouse IgG secondary antibodies. Arrows, the junction between infiltrating tumor and surrounding normal tissue. Diffuse cytoplasmic and nuclear staining were noted in the positive cells but not in the surrounding stromal tissues. $\times 250$.

vivo model, we performed *in vivo* antibody depletion experiments. Depletion of lymphocyte subsets was assessed on the day of tumor injection and weekly thereafter by flow cytometric analysis of spleen cells. More than 99% depletion of the appropriate subset was achieved with normal levels of the other subsets (data not shown). As shown in Fig. 5, all mice depleted of CD4⁺, CD8⁺ T cells or NK1.1 cells grew TC-1 tumors, in contrast to nondepleted mice which remained tumor free. Mice depleted of either CD4⁺ or CD8⁺ T cells grew tumors 2–3 weeks after injection; those depleted of NK1.1 cells grew tumors 4–5

weeks after tumor injections. These results suggest that CD4⁺, CD8⁺ T cells and NK1.1 cells are essential for the antitumor immunity generated by Sig/E7/LAMP-1 vaccinia.

Discussion

In this study, we have demonstrated that Sig/E7/LAMP-1 vaccinia induced potent E7-specific antitumor immunity. Vaccination with vaccinia Sig/E7/LAMP-1 protected mice from challenge and elimi-

Fig. 2. Tumor growth kinetics of TC-1. TC-1 tumor cells were injected into C57 BL/6 mice s.c. at various doses (1×10^6 , 1×10^5 , or 1×10^4 cells/mouse). The mice were monitored for evidence of tumor growth by palpation and inspection twice a week.

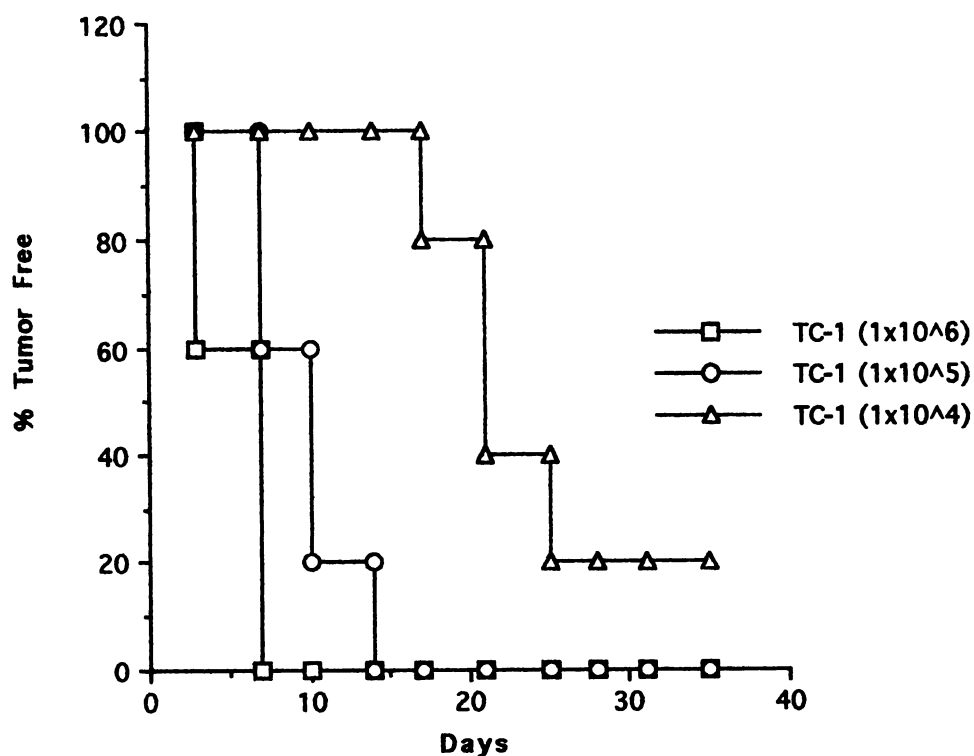
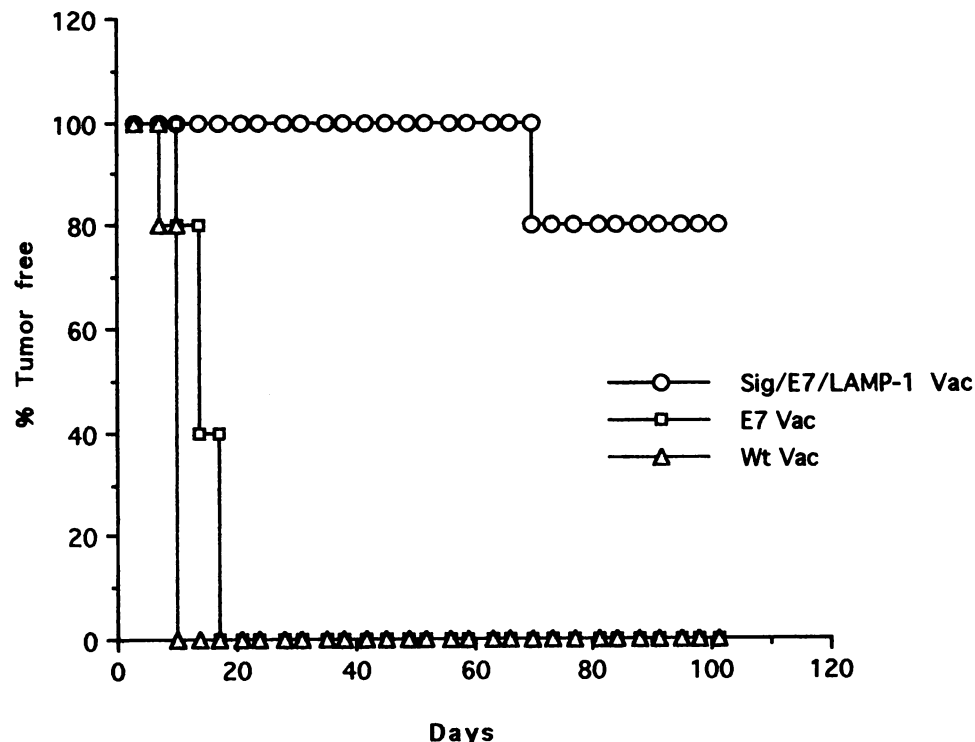


Fig. 3. Sig/E7/LAMP-1 vaccinia protects mice against challenge with TC-1 tumor. C57BL/6 mice were either not immunized or were immunized i.p. with wild-type (nonrecombinant) vaccinia, E7-vaccinia, or Sig/E7/LAMP-1-vaccinia at 1×10^7 PFUs/mouse. A month later, mice were challenged s.c. with TC-1 tumor at 2×10^5 cells/mouse. Tumor growth was assessed as in Fig. 2.

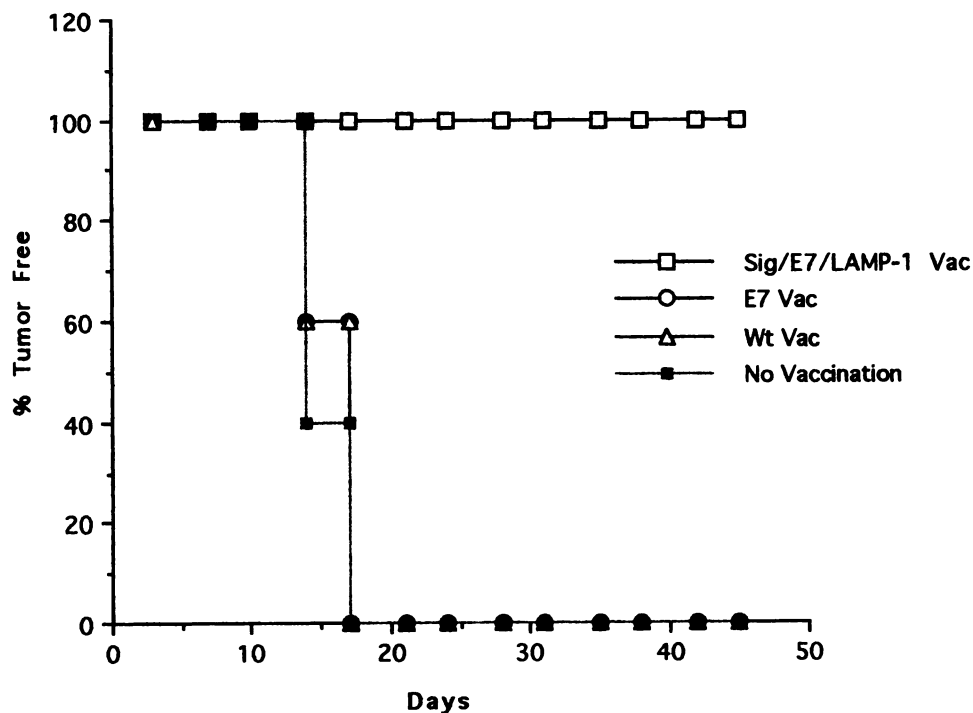


nated established E7-expressing tumors. We have demonstrated previously that Sig/E7/LAMP-1 vaccinia is capable of enhancing both CTL activity and helper T-cell proliferation (15). Thus, our results suggested that the potent antitumor immunity is correlated with the enhanced CD8⁺ and CD4⁺ T-cell responses generated by Sig/E7/LAMP-1 vaccinia and that both CD4⁺ and CD8⁺ T cells are important in the antigen-specific, antitumor immune responses. The *in vivo* antibody depletion studies also demonstrated the requirement of both CD4⁺ and CD8⁺ T cells for the generation of potent antitumor

immunity. Surprisingly, NK 1.1 cells were also found to be important in the antitumor immunity generated by Sig/E7/LAMP-1 vaccinia. We have demonstrated previously the corequirement for CD4⁺ T cells and NK cells in the immune response to tumors that are negative for MHC class I expression (26). In those studies, the depletion of NK cells resulted in the selective outgrowth of tumors that have down-regulated MHC class I expression.

Tumor growth in the vaccinated mice was not due to a consequence of antigen loss since E7 DNA and protein were present in the ex-

Fig. 4. Sig/E7/LAMP-1 vaccinia eliminates established TC-1 tumor. C57BL/6 mice were injected s.c. with TC-1 tumor at 3×10^4 cells/mouse and received either no treatment or immunization with wild-type vaccinia, E7-vaccinia, or Sig/E7/LAMP-1-vaccinia i.p. at 1×10^7 PFUs/mouse 7 days later. Tumor growth was assessed as in Fig. 2.



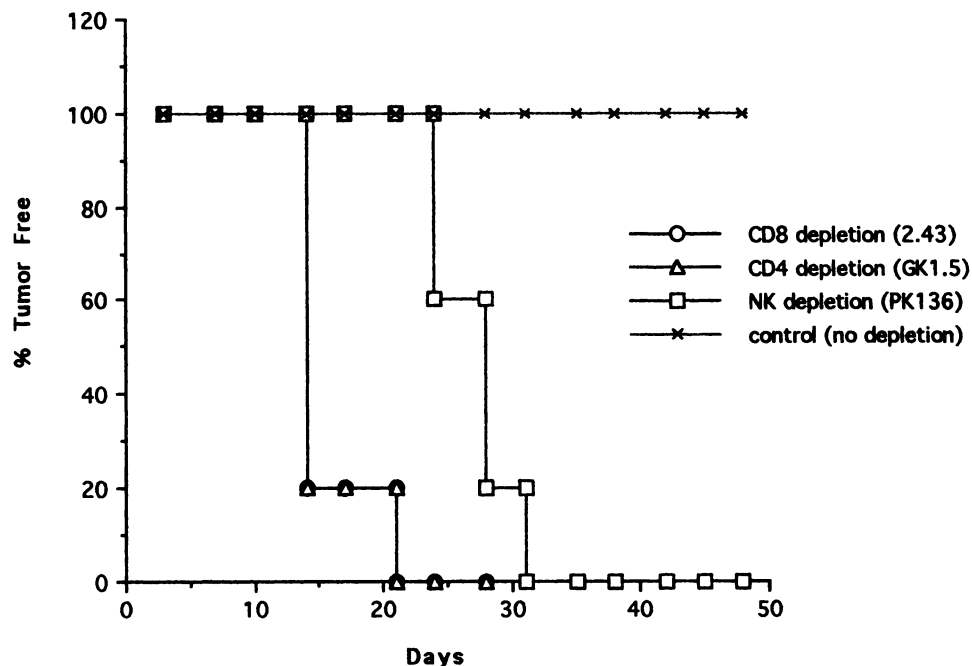


Fig. 5. Effect of lymphocyte subset depletions on the potency of Sig/E7/LAMP-1 vaccinia. C57BL/6 mice were immunized i.p. with Sig/E7/LAMP-1 vaccinia at 1×10^7 PFUs/mouse and challenged s.c. with TC-1 tumor at 2×10^5 cells/mouse 1 month later. Depletions were started 1 week prior to tumor inoculation. MAb GK1.5 (27) was used for CD4⁺ T-cell depletion (Δ), MAb 2.43 (28) was used for CD8⁺ T-cell depletion ($\circ$), and MAb PK136 (29) was used for NK1.1 cell depletion ($\square$). Mice without lymphocyte subset depletions were used as control ($\times$). Tumor growth was assessed as in Fig. 2.

planted TC-1 tumor cells. The ability of tumors to lose specific immunogenic antigens must be considered while evaluating an antigen-specific immunotherapy. An appropriate tumor model for HPV-associated cervical cancer should carry the E6 and E7 antigens in a form that is also required for the maintenance of its malignant phenotype; these tumor cells cannot avoid immune responses directed at the antigen by turning off or deleting the antigen since such cells would cease to be tumorigenic.

The new TC-1 tumor cell line was developed to circumvent the confounding issue of negatively selecting nonessential tumor markers in cancer vaccine experiments. Primary lung epithelial cells from C57BL/6 mice were immortalized with HPV-16 E6 and E7 and then transformed with the activated *ras* oncogene. This transformation process mimics the natural sequence in the pathogenesis of cervical cancer in which HPV-16 plays a critical role in carcinogenesis. Since E6 and E7 are required for the induction and maintenance of the malignant phenotype (30), the TC-1 tumor cells cannot avoid E7- and E6-directed immune recognition by losing E6 or E7 while remaining malignant.

As striking as the therapeutic effect of Sig/E7/LAMP-1 vaccinia is the lack of efficacy of E7-vaccinia. Our studies, therefore, suggest that modifications of recombinant poxvirus vaccines that enhance targeting of antigens into MHC processing pathways may greatly improve their clinical utility. Meneguzzi *et al.* reported protection by recombinant vaccinia expressing E6 and E7 in a different E6 and E7 expressing rat tumor but did not report any results on treatment of established tumors (31). It is possible that the combined immune responses targeted at both E6 and E7 resulted in more effective protection than we observed using only E7-vaccinia. Alternatively, the tumor used in those studies may have been more immunogenic or intrinsically less tumorigenic than TC-1.

In summary, we have demonstrated that the Sig/E7/LAMP-1 recombinant vaccinia can generate strong E7-specific antitumor immunity and that specific intracellular antigen targeting strategies might be successfully used to enhance the presentation of tumor-specific antigens, thereby increasing antigen-specific antitumor immune responses.

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ORIGINAL ARTICLE

Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma

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ABSTRACT

BACKGROUND

Glioblastoma, the most common primary brain tumor in adults, is usually rapidly fatal. The current standard of care for newly diagnosed glioblastoma is surgical resection to the extent feasible, followed by adjuvant radiotherapy. In this trial we compared radiotherapy alone with radiotherapy plus temozolomide, given concomitantly with and after radiotherapy, in terms of efficacy and safety.

METHODS

Patients with newly diagnosed, histologically confirmed glioblastoma were randomly assigned to receive radiotherapy alone (fractionated focal irradiation in daily fractions of 2 Gy given 5 days per week for 6 weeks, for a total of 60 Gy) or radiotherapy plus continuous daily temozolomide (75 mg per square meter of body-surface area per day, 7 days per week from the first to the last day of radiotherapy), followed by six cycles of adjuvant temozolomide (150 to 200 mg per square meter for 5 days during each 28-day cycle). The primary end point was overall survival.

RESULTS

A total of 573 patients from 85 centers underwent randomization. The median age was 56 years, and 84 percent of patients had undergone debulking surgery. At a median follow-up of 28 months, the median survival was 14.6 months with radiotherapy plus temozolomide and 12.1 months with radiotherapy alone. The unadjusted hazard ratio for death in the radiotherapy-plus-temozolomide group was 0.63 (95 percent confidence interval, 0.52 to 0.75; $P < 0.001$ by the log-rank test). The two-year survival rate was 26.5 percent with radiotherapy plus temozolomide and 10.4 percent with radiotherapy alone. Concomitant treatment with radiotherapy plus temozolomide resulted in grade 3 or 4 hematologic toxic effects in 7 percent of patients.

CONCLUSIONS

The addition of temozolomide to radiotherapy for newly diagnosed glioblastoma resulted in a clinically meaningful and statistically significant survival benefit with minimal additional toxicity.

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GLIOMASTOMA IS THE MOST FREQUENT primary malignant brain tumor in adults. Median survival is generally less than one year from the time of diagnosis, and even in the most favorable situations, most patients die within two years.¹⁻³ Standard therapy consists of surgical resection to the extent that is safely feasible, followed by radiotherapy; in the United States, adjuvant carmustine, a nitrosourea drug, is commonly prescribed.^{4,5} Cooperative-group trials have investigated the addition of various chemotherapeutic regimens to radiotherapy,⁶⁻⁹ but no randomized phase 3 trial of nitrosourea-based adjuvant chemotherapy has demonstrated a significant survival benefit as compared with radiotherapy alone, although there were more long-term survivors in the chemotherapy groups in some studies.¹⁰ A meta-analysis based on 12 randomized trials suggested a small survival benefit of chemotherapy, as compared with radiotherapy alone (a 5 percent increase in survival at two years, from 15 percent to 20 percent).¹¹ The meta-analysis included 37 percent of patients with prognostically more favorable, lower-grade gliomas.

Temozolomide, an oral alkylating agent, has demonstrated antitumor activity as a single agent in the treatment of recurrent glioma.¹²⁻¹⁴ The approved conventional schedule is a daily dose of 150 to 200 mg per square meter of body-surface area for 5 days of every 28-day cycle. Daily therapy at a dose of 75 mg per square meter for up to seven weeks is safe; this level of exposure to temozolomide¹⁵ depletes the DNA-repair enzyme O⁶-methylguanine-DNA methyltransferase (MGMT).¹⁶ This effect may be important because low levels of MGMT in tumor tissue are associated with longer survival among patients with glioblastoma who are receiving nitrosourea-based adjuvant chemotherapy.^{17,18}

A pilot phase 2 trial demonstrated the feasibility of the concomitant administration of temozolomide with fractionated radiotherapy, followed by up to six cycles of adjuvant temozolomide, and suggested that this treatment had promising clinical activity (two-year survival rate, 31 percent).¹⁹ The European Organisation for Research and Treatment of Cancer (EORTC) Brain Tumor and Radiotherapy Groups and the National Cancer Institute of Canada (NCIC) Clinical Trials Group therefore initiated a randomized, multicenter, phase 3 trial to compare this regimen with radiotherapy alone in patients with newly diagnosed glioblastoma.

METHODS

PATIENTS

Patients 18 to 70 years of age with newly diagnosed and histologically confirmed glioblastoma (World Health Organization [WHO] grade IV astrocytoma) were eligible for the study. Eligible patients had a WHO performance status of 2 or less and adequate hematologic, renal, and hepatic function (absolute neutrophil count, ≥ 1500 per cubic millimeter; platelet count, $\geq 100,000$ per cubic millimeter; serum creatinine level, ≤ 1.5 times the upper limit of normal in the laboratory where it was measured; total serum bilirubin level, ≤ 1.5 times the upper limit of normal; and liver-function values, < 3 times the upper limit of normal for the laboratory). Patients who were receiving corticosteroids had to receive a stable or decreasing dose for at least 14 days before randomization. All patients provided written informed consent, and the study was approved by the ethics committees of the participating centers.

STUDY DESIGN AND TREATMENT

Within six weeks after the histologic diagnosis of glioblastoma, we randomly assigned eligible patients to receive standard focal radiotherapy alone (the control group) or standard radiotherapy plus concomitant daily temozolomide, followed by adjuvant temozolomide. Randomization was performed at the EORTC Data Center, and patients were stratified according to WHO performance status, whether or not they had previously undergone debulking surgery, and the treatment center.²⁰ The assigned treatment had to begin within one week after randomization.

Radiotherapy consisted of fractionated focal irradiation at a dose of 2 Gy per fraction given once daily five days per week (Monday through Friday) over a period of six weeks, for a total dose of 60 Gy. Radiotherapy was delivered to the gross tumor volume with a 2-to-3-cm margin for the clinical target volume. Radiotherapy was planned with dedicated computed tomography (CT) and three-dimensional planning systems; conformal radiotherapy was delivered with linear accelerators with nominal energy of 6 MV or more, and quality assurance was performed by means of individual case reviews.²¹

Concomitant chemotherapy consisted of temozolomide (marketed as Temodal in Europe and Canada and Temodar in the United States; Schering-Plough) at a dose of 75 mg per square meter per

day, given 7 days per week from the first day of radiotherapy until the last day of radiotherapy, but for no longer than 49 days. After a 4-week break, patients were then to receive up to six cycles of adjuvant temozolomide according to the standard 5-day schedule every 28 days. The dose was 150 mg per square meter for the first cycle and was increased to 200 mg per square meter beginning with the second cycle, so long as there were no hematologic toxic effects. Because continuous daily temozolomide can cause lymphocytopenia, with a possible increased risk of opportunistic infections, patients in the radiotherapy-plus-temozolomide group were to receive prophylaxis against *Pneumocystis carinii* pneumonia, consisting of either inhaled pentamidine or oral trimethoprim-sulfamethoxazole,²² during concomitant treatment with radiotherapy plus temozolomide. Antiemetic prophylaxis with metoclopramide or a 5-hydroxytryptamine₃ antagonist was recommended before the initial doses of concomitant temozolomide and was required during the adjuvant five-day courses of temozolomide.

SURVEILLANCE AND FOLLOW-UP

The baseline examination included CT or magnetic resonance imaging (MRI), full blood counts and blood chemistry tests, and a physical examination that included the Mini-Mental State Examination (MMSE) and a quality-of-life questionnaire. During radiotherapy (with or without temozolomide), patients were to be seen every week. Twenty-one to 28 days after the completion of radiotherapy and every 3 months thereafter, patients underwent a comprehensive evaluation, including administration of the MMSE and the quality-of-life questionnaire and radiologic assessment of the tumor. During adjuvant temozolomide therapy, patients underwent a monthly clinical evaluation and a comprehensive evaluation at the end of cycles 3 and 6. Tumor progression was defined according to the modified WHO criteria as an increase in tumor size by 25 percent, the appearance of new lesions, or an increased need for corticosteroids.²³ When there was tumor progression or after two years of follow-up, patients were treated at the investigator's discretion, and the type of second-line therapy was recorded. Toxic effects were graded according to the National Cancer Institute Common Toxicity Criteria, version 2.0, with a score of 1 indicating mild adverse effects, a score of 2 moderate adverse effects, a score of 3 severe adverse effects, and a score of 4 life-threatening adverse effects.

STATISTICAL ANALYSIS

The primary end point was overall survival; secondary end points were progression-free survival, safety, and the quality of life. Overall survival and progression-free survival were analyzed by the Kaplan-Meier method, with use of two-sided log-rank statistics. This study had 80 percent power at a significance level of 0.05 to detect a 33 percent increase in median survival (hazard ratio for death, 0.75), assuming that 382 deaths occurred. All analyses were conducted on an intention-to-treat basis. The Cox proportional-hazards model was fitted to adjust for stratification factors and other confounding variables. Toxic effects are reported separately for the radiotherapy period, defined as extending from day 1 of radiotherapy until 28 days after the last day of radiotherapy, or until the first day of adjuvant temozolomide therapy. The adjuvant-therapy period was defined as extending from the first day of adjuvant temozolomide therapy until 35 days after day 1 of the last cycle of temozolomide. Findings with respect to the quality of life are not reported here.

ORGANIZATION OF THE TRIAL

The concept of the trial was developed by Dr. Stupp in collaboration with the EORTC Data Center, the EORTC Brain Tumor and Radiotherapy Groups, and the NCIC Clinical Trials Group, represented by Drs. Cairncross and Eisenhauer. The radiotherapy design and quality assurance were supervised by Dr. Mirimanoff. The trial was sponsored by the EORTC Brain Tumor and Radiotherapy Groups (trial 22981/26981) in Europe and the NCIC Clinical Trials Group (trial CE.3) in Canada. The trial was supported by an unrestricted educational grant from Schering-Plough, which also provided the study drug; however, Schering-Plough was not involved in trial design or analysis. All data were collected by the EORTC and NCIC data centers and reviewed by Drs. Stupp and Mirimanoff. The analysis was performed by the EORTC statistician, Mr. Gorrila. Histologic specimens were reviewed centrally (according to the revised WHO classification system²⁴) by a panel of three neuropathologists in Europe (Robert C. Janzer in Lausanne, Switzerland [chair]; Peter Wesseling in Nijmegen, the Netherlands; and Karima Mohktari in Paris) and a single neuropathologist in Canada (Samuel Ludwin, Kingston, Ont.). The article was written by Dr. Stupp with support from a medical writer and coauthors; all authors reviewed the manuscript.

Table 1. Demographic Characteristics of the Patients at Baseline.

Characteristic	Radiotherapy (N=286)	Radiotherapy plus Temozolomide (N=287)
Age — yr		
Median	57	56
Range	23–71	19–70
Age — no. (%) [*]		
<50 yr	81 (28)	90 (31)
≥50 yr	205 (72)	197 (69)
Sex — no. (%)		
Male	175 (61)	185 (64)
Female	111 (39)	102 (36)
WHO performance status — no. (%) ^{*†}		
0	110 (38)	113 (39)
1	141 (49)	136 (47)
2	35 (12)	38 (13)
Extent of surgery — no. (%) [*]		
Biopsy	45 (16)	48 (17)
Debulking	241 (84)	239 (83)
Complete resection	113 (40)	113 (39)
Partial resection	128 (45)	126 (44)
Time from diagnosis to radiotherapy — wk		
Median	5	5
Range	2.0–12.9	1.7–10.7
Baseline MMSE score — no. (%) [‡]		
30	91 (32)	100 (35)
27–29	97 (34)	96 (33)
≤26	86 (30)	81 (28)
Data missing	12 (4)	10 (3)
Corticosteroid therapy — no. (%)		
Yes	215 (75)	193 (67)
No	70 (24)	94 (33)
Data missing	1 (<1)	0
Slides available for pathological review — no. (%)	246 (86)	239 (83)
Findings on pathological review — no. (%)		
Glioblastoma	229 (93)	221 (92)
Anaplastic astrocytoma [§]	9 (4)	7 (3)
Inconclusive material	3 (1)	3 (1)
Other	5 (2)	8 (3)

^{*} This characteristic was used as a stratification factor at the time of randomization.

[†] A performance status of 0 denotes asymptomatic, 1 symptomatic and fully ambulatory, and 2 symptomatic and in bed less than 50 percent of the day.

[‡] The maximum score on the Mini-Mental State Examination (MMSE) is 30, and scores above 26 are considered to indicate normal mental status.

[§] Anaplastic astrocytoma included oligoastrocytoma.

RESULTS

PATIENTS

From August 2000 until March 2002, 573 patients from 85 institutions in 15 countries were randomly assigned to receive radiotherapy (286 patients) or radiotherapy plus temozolomide (287 patients). Nearly 50 percent of the patients were enrolled at 17 institutions. The characteristics of the patients in the two groups were well balanced at baseline (Table 1). The median age was 56 years, and 84 percent of patients had undergone debulking surgery. Slightly more patients in the radiotherapy group than in the radiotherapy-plus-temozolomide group were receiving corticosteroids at the time of randomization (75 percent vs. 67 percent). Histologic slides were submitted for 85 percent of patients, and central pathological review confirmed the diagnosis of glioblastoma in 93 percent of the reviewed cases; 3 percent had anaplastic astrocytoma or oligoastrocytoma (WHO grade III), and in 1 percent submitted material was insufficient for a definitive diagnosis.

DISPOSITION OF PATIENTS AND DELIVERY OF TREATMENT

The median time from diagnosis to the start of therapy was 5 weeks (range, 2.0 to 12.9) in the radiotherapy group and 5 weeks (range, 1.7 to 10.7) in the radiotherapy-plus-temozolomide group. Table 2 summarizes the details of treatment. Unplanned interruptions in radiotherapy were usually brief (median, four days) and interruptions due to the toxicity of therapy occurred in only 3 percent of the radiotherapy group and 4 percent of the radiotherapy-plus-temozolomide group. The other reasons were mainly administrative (e.g., holidays, radiotherapy equipment maintenance, or technical problems). One patient randomly assigned to radiotherapy alone received radiotherapy plus temozolomide. Among the 287 patients who were assigned to receive concomitant radiotherapy plus temozolomide, 85 percent completed both radiotherapy and temozolomide as planned. Thirty-seven patients (13 percent) prematurely discontinued temozolomide because of toxic effects (in 14 patients), disease progression (in 11), or other reasons (in 12).

After radiotherapy, 223 patients in the radiotherapy-plus-temozolomide group (78 percent) started adjuvant temozolomide and received a median of 3 cycles (range, 0 to 7); 47 percent of patients completed 6 cycles. The main reason for not

Table 2. Disposition of Patients and Intensity of Treatment.

Variable	Radiotherapy (N=286)	Radiotherapy plus Temozolomide (N=287)
Radiotherapy		
Never started radiotherapy — no. (%)	7 (2)	3 (1)
Dose — Gy		
Median	60	60
Range	12–62	12–62
No. of fractions		
Median	30	30
Range	6–33	5–33
Duration — wk		
Median	6.1	6.0
Range	1.3–7.6	0.6–10.3
Interruption or delay in radiotherapy — no. (%)	78 (27)	92 (32)
Delay due to toxicity — no. (%)	8 (3)	12 (4)
Received $\leq 90\%$ of planned dose — no. (%)	22 (8)	14 (5)
Early discontinuation of radiotherapy — no. (%)	19 (7)	14 (5)
Reason for discontinuation — no. (%)		
Disease progression	17 (6)	11 (4)
Other*	2 (1)	3 (1)
Concomitant temozolomide		
Never started concomitant temozolomide — no. (%)	—	6 (2)
Duration of therapy — days		
Median	—	42
Range	—	40–55
Received $\leq 90\%$ of planned dose — no. (%)	—	23 (8)
Early discontinuation of concomitant temozolomide — no. (%)	—	37 (13)
Reason for discontinuation of temozolomide — no. (%)		
Toxic effects	—	14 (5)
Disease progression	—	11 (4)
Other*	—	12 (4)
Adjuvant-therapy period		
Adjuvant temozolomide started — no. (%)		223 (78)
Cycles of temozolomide		
Median	—	3
Range	—	0–7
Patients completing 6 cycles — no. (%)	—	105 (47)
Dose escalated to 200 mg/m ² at cycle 2 — no. (%)	—	149 (67)
Adjuvant temozolomide discontinued — no. (%)		118 (53)
Reason for discontinuation — no. (%)		
Disease progression	—	86 (39)
Toxic effects	—	17 (8)
Decision by patient	—	8 (4)
Other	—	6 (3)
Missing data	—	1 (<1)

* Other reasons included any missed dose or patient or prescription error.

beginning or not completing adjuvant temozolomide therapy was disease progression. Only 8 percent of patients discontinued adjuvant temozolomide because of toxic effects. Beginning with cycle 2, the dose of temozolomide was increased to 200 mg per square meter in 67 percent of patients. Only 9 percent of patients did not receive the higher dose because of hematologic toxicity.

SURVIVAL AND PROGRESSION

At a median follow-up of 28 months, 480 patients (84 percent) had died. The unadjusted hazard ratio for death in the radiotherapy-plus-temozolomide group as compared with the radiotherapy group was 0.63 (95 percent confidence interval, 0.52 to 0.75; $P < 0.001$ by the log-rank test). These data indicate a 37 percent relative reduction in the risk of death for patients treated with radiotherapy plus temozolomide, as compared with those who received radiotherapy alone.

The median survival benefit was 2.5 months; the median survival was 14.6 months (95 percent confidence interval, 13.2 to 16.8) with radiotherapy plus temozolomide and 12.1 months (95 percent confidence interval, 11.2 to 13.0) with radiotherapy alone (Fig. 1 and Table 3). The two-year survival rate was

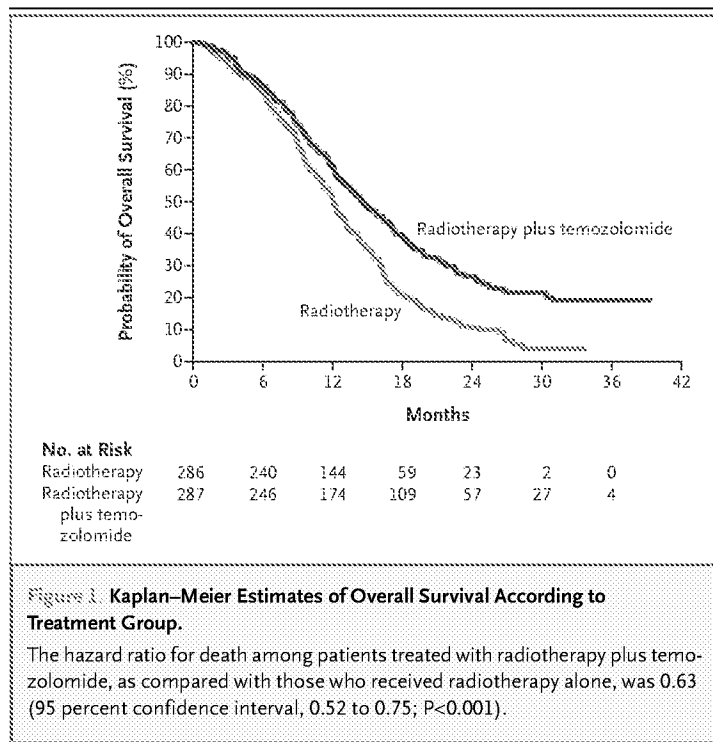
26.5 percent (95 percent confidence interval, 21.2 to 31.7 percent) in the group given radiotherapy plus temozolomide, as compared with 10.4 percent (95 percent confidence interval, 6.8 to 14.1 percent) with radiotherapy alone. The median progression-free survival was 6.9 months (95 percent confidence interval, 5.8 to 8.2) with radiotherapy plus temozolomide and 5.0 months (95 percent confidence interval, 4.2 to 5.5) with radiotherapy alone (hazard ratio for death or disease progression, 0.54 [95 percent confidence interval, 0.45 to 0.64]; $P < 0.001$ by the log-rank test) (Fig. 2).

The hazard ratio for death was adjusted by fitting the Cox proportional-hazard models. In addition to the stratification factors (the extent of surgery, WHO performance status, and treatment center), other possible confounding factors — age, use or nonuse of corticosteroids at randomization, sex, score on the MMSE, and tumor location — were included. The adjusted hazard ratio for death in the radiotherapy-plus-temozolomide group as compared with the radiotherapy group — 0.62 (95 percent confidence interval, 0.51 to 0.75) — was essentially the same as the unadjusted hazard ratio.

Survival according to prognostic factors, including age, sex, extent of surgery, WHO performance status, and use or nonuse of corticosteroids, was also analyzed (see Table 1 in the Supplementary Appendix, available with the full text of this article at www.nejm.org). Radiotherapy plus temozolomide was associated with a significant improvement in median overall survival in nearly all subgroups of patients (see Fig. 1 in the Supplementary Appendix); the exceptions were the small subgroup of 93 patients who underwent biopsy only and the 70 patients with a poor performance status.

SAFETY

We analyzed adverse events separately during radiotherapy (with or without concomitant temozolomide), the adjuvant-therapy period, and the entire study period (from study entry until disease progression or last follow-up). No grade 3 or 4 hematologic toxic effects were observed in the radiotherapy group. During concomitant temozolomide therapy, grade 3 or 4 neutropenia was documented in 12 patients (4 percent), and grade 3 or 4 thrombocytopenia occurred in 9 patients (3 percent) (Table 4). Overall, 19 patients (7 percent) had any type of grade 3 or 4 hematologic toxic effect. During adjuvant temozolomide therapy, 14 percent of patients



had any grade 3 or 4 hematologic toxic effect, 4 percent had grade 3 or 4 neutropenia, and 11 percent had grade 3 or 4 thrombocytopenia.

During the radiotherapy period, severe infections occurred in 6 patients in the radiotherapy group (2 percent) and in 9 patients in the radiotherapy-plus-temozolomide group (3 percent); during adjuvant temozolomide therapy, 12 patients (5 percent) had severe infections. The most common non-hematologic adverse event during radiotherapy was moderate-to-severe fatigue in 26 percent of patients in the radiotherapy group and 33 percent in the radiotherapy-plus-temozolomide group (Table 2 in the Supplementary Appendix). Thromboembolic events occurred in 28 patients (5 percent) — 16 in the radiotherapy group and 12 in the radiotherapy-plus-temozolomide group. Two patients in the radiotherapy-plus-temozolomide group died of cerebral hemorrhage in the absence of a coagulation disorder or thrombocytopenia. Pneumonia was reported in five patients in the radiotherapy group and three in the radiotherapy-plus-temozolomide group. Opportunistic infections occurred in two patients; one patient treated with radiotherapy alone had suspected *P. carinii* pneumonia, and one patient in the radiotherapy-plus-temozolomide group had proven bacterial and candida pneumonia.

TREATMENT AFTER DISEASE PROGRESSION

If disease progression occurred, further treatment was at the physician's discretion. At the cutoff date (May 10, 2004), 512 patients — 268 in the radiotherapy group (94 percent) and 244 in the radiotherapy-plus-temozolomide group (85 percent) — had disease progression. At the time of progression, 23 percent of patients in both treatment groups underwent a second surgery, and 72 percent of patients in the radiotherapy group and 58 percent in the radiotherapy-plus-temozolomide group received chemotherapy. Salvage chemotherapy consisted of temozolomide in 60 percent of patients in the radiotherapy group and 25 percent of patients in the radiotherapy-plus-temozolomide group. The response to salvage chemotherapy was not recorded as part of our study.

DISCUSSION

For more than 30 years, chemotherapy given as an adjunct to radiotherapy or before radiotherapy has been widely investigated in patients with malig-

nant glioma. Such treatment has had limited success.^{6-8,10,25-27} The present study demonstrates that the addition of chemotherapy to radiotherapy significantly prolongs survival among patients with newly diagnosed glioblastoma, with a median increase in survival of 2.5 months or a relative reduction in the risk of death of 37 percent. Unlike most previous studies, which included patients with both glioblastoma (WHO grade IV) and anaplastic astrocytoma (WHO grade III), who have a better prognosis, our study was designed to include only patients with glioblastoma. At two years, we found a clinically meaningful increase — by a factor of 2.5 — in the survival rate, from 10 percent with radiotherapy alone to 27 percent with radiotherapy plus temozolomide, consistent with the findings of the preceding phase 2 trial.¹⁹ An exploratory analysis of subgroups defined according to known prognostic factors demonstrated a survival benefit in nearly all subgroups.

The outcome for patients treated with radiotherapy alone in our trial compares favorably with the outcome in other trials.^{9,11,28} Patients being treated with corticosteroids received stable or decreasing doses before randomization and started radiotherapy within one week after randomization. These cri-

Table 3. Overall and Progression-free Survival According to Treatment Group.*

Variable	Radiotherapy (N=286)	Radiotherapy plus Temozolomide (N=287)
	value (95% CI)	
Median overall survival (mo)	12.1 (11.2–13.0)	14.6 (13.2–16.8)
Overall survival (%)		
At 6 months	84.2 (80.0–88.5)	86.3 (82.3–90.3)
At 12 months	50.6 (44.7–56.4)	61.1 (55.4–66.7)
At 18 months	20.9 (16.2–26.6)	39.4 (33.8–45.1)
At 24 months	10.4 (6.8–14.1)	26.5 (21.2–31.7)
Median progression-free survival (mo)	5.0 (4.2–5.5)	6.9 (5.8–8.2)
Progression-free survival (%)		
At 6 months	36.4 (30.8–41.9)	53.9 (48.1–59.6)
At 12 months	9.1 (5.8–12.4)	26.9 (21.8–32.1)
At 18 months	3.9 (1.6–6.1)	18.4 (13.9–22.9)
At 24 months	1.5 (0.1–3.0)	10.7 (7.0–14.3)

* A total of 160 patients in the radiotherapy group and 60 patients in the radiotherapy-plus-temozolomide group received temozolomide as salvage therapy. CI denotes confidence interval.

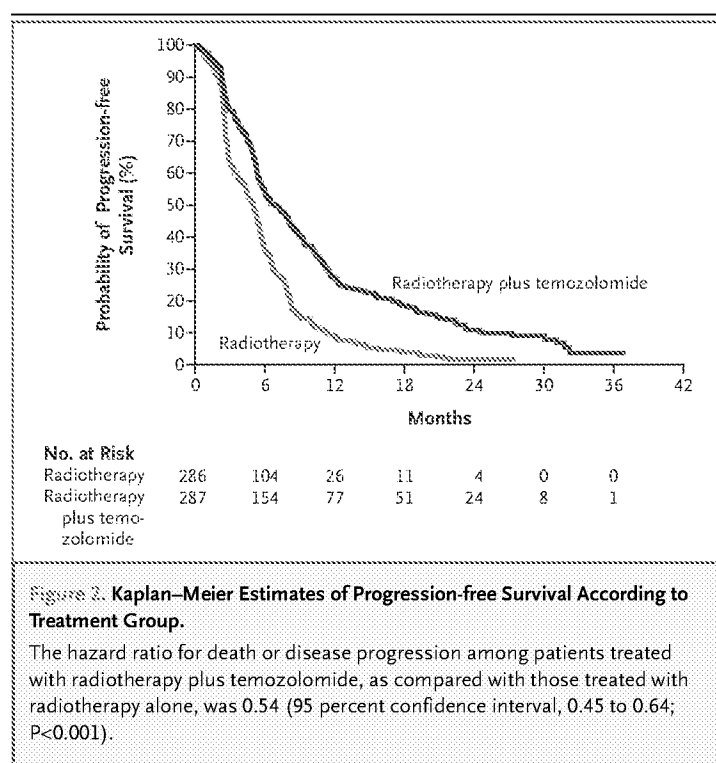


Table 4. Grade 3 or 4 Hematologic Toxic Effects in Patients Treated with Temozolomide.

Toxic Effect	Concomitant Temozolomide Therapy (N=284)	Adjuvant Temozolomide Therapy (N=223)	Entire Study Period* (N=284)
	number of patients (percent)		
Leukopenia	7 (2)	11 (5)	20 (7)
Neutropenia	12 (4)	9 (4)	21 (7)
Thrombocytopenia	9 (3)	24 (11)	33 (12)
Anemia	1 (<1)	2 (1)	4 (1)
Any	19 (7)	32 (14)	46 (16)

* The entire study period was defined as the period from study entry to seven days after disease progression.

teria may have served to exclude patients with the worst prognosis, who may not benefit from any therapy. Moreover, most patients had undergone debulking surgery. The relatively long survival after disease progression (approximately seven months in both groups) is also noteworthy. This extended survival may reflect either patient selection or the early detection of tumor progression by means of regular radiographic assessment. Furthermore, 72

percent of patients in the radiotherapy group and 58 percent of patients in the radiotherapy-plus-temozolomide group received salvage chemotherapy at the time of progression.

This trial was designed to determine whether the addition of temozolomide to radiotherapy early in the course of treatment prolongs survival among patients with glioblastoma, but it was not designed to distinguish between the effects of concomitant therapy with radiotherapy plus temozolomide and adjuvant treatment with temozolomide. At the time the trial was conceived, it was deemed most important to administer chemotherapy early in the course of the disease, for a sufficiently long time, and concurrently with radiotherapy. Temozolomide was given concomitantly with radiotherapy on a continuous schedule for several reasons. First, daily administration of low doses makes possible an increase by almost a factor of two in dose intensity, as compared with the standard regimen, without an increase in toxicity.¹⁵ Second, continuous administration of an alkylating agent depletes MGMT,¹⁶ an enzyme that may be induced by radiotherapy and that is necessary for repair of damage to DNA caused by alkylating agents.²⁹ In a companion translational study also reported in this issue of the *Journal*, we observed that methylation of the MGMT promoter, which results in gene silencing, is associated with a striking survival benefit in patients treated with radiotherapy plus temozolomide.³⁰ Third, synergy between temozolomide and radiotherapy has been observed in vitro.³¹⁻³³ The spontaneous conversion of temozolomide into the active metabolite and its ability to cross the blood-brain barrier also favors this regimen.³⁴ Finally, to ensure sufficient exposure to the drug, we added six cycles of adjuvant temozolomide after the completion of radiotherapy.

In the context of palliative care, chemotherapy-induced toxic effects should be manageable. Nausea was controlled with standard antiemetic agents. Severe myelosuppression was observed in 16 percent of patients, leading to the early discontinuation of chemotherapy in 5 percent. Whether the addition of chemotherapy increases the risk of radiotherapy-induced cognitive deficits cannot be assessed at this time. However, long-term monitoring and observational studies of late toxic effects will be important to guide treatment recommendations in the future. Furthermore, prolonged chemotherapy with alkylating agents has been associated with myelodysplastic syndromes and secondary leukemia occurring years after therapy.³⁵ In our trial, at a median

follow-up of approximately two years, there had been no evidence of any increase in treatment-induced late toxic effects. Such late toxicity may become a greater concern, however, if this regimen is used in patients with intermediate- or low-grade glioma, who have a more favorable prognosis in terms of survival.

In conclusion, the addition of temozolomide to radiotherapy early in the course of glioblastoma provides a statistically significant and clinically meaningful survival benefit. Nevertheless, the challenge remains to improve clinical outcomes further. For this reason, the regimen of radiotherapy plus temozolomide should serve as the new platform from which to explore innovative regimens for treating malignant gliomas. Many questions remain un-

answered regarding the applications of this regimen to lower grade gliomas and the optimal combination of radiotherapy and temozolomide.

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Bristol-Myers Squibb Provides Update on Phase 3 Opdivo (nivolumab)

CheckMate -548 Trial in Patients with Newly Diagnosed MGMT-

Methylated Glioblastoma Multiforme

09/05/2019

CATEGORY:

- [Corporate/Financial News](#)

PRINCETON, N.J.--([BUSINESS WIRE](#))--[Bristol-Myers Squibb Company](#) (NYSE: BMY) today announced that the Phase 3 CheckMate -548 trial evaluating the addition of *Opdivo* (nivolumab) to the current standard of care (temozolomide and radiation therapy) versus the standard of care alone did not meet one of its primary endpoints, progression-free survival (PFS), in patients with newly diagnosed glioblastoma multiforme (GBM) that is O6-methylguanine-DNA methyltransferase (MGMT)-methylated. The data monitoring committee recommended that the trial continue as planned to allow the other primary endpoint, overall survival (OS), to mature. The company remains blinded to all study data.

“Though CheckMate -548 did not show statistically significant improvement in progression-free survival, we are continuing to evaluate the benefit the addition of *Opdivo* to the standard of care treatment regimen may bring to patients suffering from GBM, an extremely aggressive and difficult disease to treat. We look forward to seeing the overall survival data when they are available,” said Fouad Namouni, M.D., head, Oncology Development, Bristol-Myers Squibb. “We are grateful to the patients, caregivers and investigators participating in this trial who make this research possible.”

About CheckMate -548

CheckMate -548 (NCT02667587) is a Phase 3 randomized, multi-center study evaluating *Opdivo* in addition to the current standard of care (temozolomide and radiation therapy), versus the standard of care alone, in patients with newly diagnosed GBM that is MGMT-methylated. The primary endpoints of the trial are PFS per blinded independent central review (BICR) and OS. The secondary endpoints are investigator assessed PFS, and OS rate up to two years.

About GBM

GBM is the most common and most aggressive type of primary malignant tumor of the central nervous system. The global incidence of GBM is less than 10 per 100,000 people. Standard treatment for patients with newly diagnosed GBM can include surgery followed by radiation and chemotherapy, but treatment options are limited. The last investigational medicine to improve survival for patients with newly diagnosed GBM was approved by the U.S. Food and Drug Administration in 2005. The five year survival rate of patients with GBM is less than five percent.

MGMT methylation status is among the most commonly used biomarkers in GBM for treatment guidance. Studies suggest MGMT methylation status may be predictive of the likelihood of patients with GBM to respond to therapy.

Bristol-Myers Squibb: Advancing Oncology Research

At Bristol-Myers Squibb, patients are at the center of everything we do. The focus of our research is to increase quality, long-term survival for patients and make cure a possibility. Through a unique multidisciplinary approach powered by translational science, we harness our deep scientific experience in oncology and Immuno-Oncology (I-O) research to identify novel treatments tailored to individual patient needs. Our researchers are developing a diverse, purposefully built pipeline designed to target different immune system pathways and address the complex and specific interactions between the tumor, its microenvironment and the immune system. We source innovation internally, and in collaboration with academia, government, advocacy groups and biotechnology companies, to help make the promise of transformational medicines, like I-O, a reality for patients.

About *Opdivo*

Opdivo is a programmed death-1 (PD-1) immune checkpoint inhibitor that is designed to uniquely harness the body's own immune system to help restore anti-tumor immune response. By harnessing the body's own immune system to fight cancer, *Opdivo* has become an important treatment option across multiple cancers.

Opdivo's leading global development program is based on Bristol-Myers Squibb's scientific expertise in the field of Immuno-Oncology, and includes a broad range of clinical trials across all phases, including Phase 3, in a variety of tumor types. To date, the *Opdivo* clinical development program has treated

<https://news.bms.com/news/corporate-financial/2019/Bristol-Myers-Squibb-Provides-Update-on-Phase-3-Opdivo-nivolumab-CheckMate--548-Trial-in-Patients-with-Newly-Diagnosed-MGMT-Methylated-Glioblastoma-Multiforme/default.aspx> <Accessed June 12, 2023>.

more than 35,000 patients. The *Opdivo* trials have contributed to gaining a deeper understanding of the potential role of biomarkers in patient care, particularly regarding how patients may benefit from *Opdivo* across the continuum of PD-L1 expression.

In July 2014, *Opdivo* was the first PD-1 immune checkpoint inhibitor to receive regulatory approval anywhere in the world. *Opdivo* is currently approved in more than 65 countries, including the United States, the European Union, Japan and China. In October 2015, the Company's *Opdivo* and *Yervoy* combination regimen was the first Immuno-Oncology combination to receive regulatory approval for the treatment of metastatic melanoma and is currently approved in more than 50 countries, including the United States and the European Union.

U.S. FDA-APPROVED INDICATIONS FOR OPDIVO®

OPDIVO® (nivolumab) as a single agent is indicated for the treatment of patients with unresectable or metastatic melanoma.

OPDIVO® (nivolumab), in combination with YERVOY® (ipilimumab), is indicated for the treatment of patients with unresectable or metastatic melanoma.

OPDIVO® (nivolumab) is indicated for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) with progression on or after platinum-based chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving OPDIVO.

OPDIVO® (nivolumab) is indicated for the treatment of patients with metastatic small cell lung cancer (SCLC) with progression after platinum-based chemotherapy and at least one other line of therapy. This indication is approved under accelerated approval based on overall response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

OPDIVO® (nivolumab) is indicated for the treatment of patients with advanced renal cell carcinoma (RCC) who have received prior anti-angiogenic therapy.

OPDIVO® (nivolumab), in combination with YERVOY® (ipilimumab), is indicated for the treatment of patients with intermediate or poor risk, previously untreated advanced renal cell carcinoma (RCC).

OPDIVO® (nivolumab) is indicated for the treatment of adult patients with classical Hodgkin lymphoma (cHL) that has relapsed or progressed after autologous hematopoietic stem cell transplantation (HSCT) and brentuximab vedotin or after 3 or more lines of systemic therapy that includes autologous HSCT. This indication is approved under accelerated approval based on overall response rate. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

OPDIVO® (nivolumab) is indicated for the treatment of patients with recurrent or metastatic squamous cell carcinoma of the head and neck (SCCHN) with disease progression on or after platinum-based therapy.

OPDIVO® (nivolumab) is indicated for the treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy. This indication is approved under accelerated approval based on tumor response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

OPDIVO® (nivolumab), as a single agent, is indicated for the treatment of adult and pediatric (12 years and older) patients with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer (CRC) that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan. This indication is approved under accelerated approval based on overall response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

OPDIVO® (nivolumab), in combination with YERVOY® (ipilimumab), is indicated for the treatment of adults and pediatric patients 12 years and older with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer (CRC) that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan. This indication is

<https://news.bms.com/news/corporate-financial/2019/Bristol-Myers-Squibb-Provides-Update-on-Phase-3-Opdivo-nivolumab-CheckMate--548-Trial-in-Patients-with-Newly-Diagnosed-MGMT-Methylated-Glioblastoma-Multiforme/default.aspx> <Accessed June 12, 2023>.

approved under accelerated approval based on overall response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

OPDIVO® (nivolumab) is indicated for the treatment of patients with hepatocellular carcinoma (HCC) who have been previously treated with sorafenib. This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

OPDIVO® (nivolumab) is indicated for the adjuvant treatment of patients with melanoma with involvement of lymph nodes or metastatic disease who have undergone complete resection.

IMPORTANT SAFETY INFORMATION

WARNING: IMMUNE-MEDIATED ADVERSE REACTIONS

YERVOY can result in severe and fatal immune-mediated adverse reactions. These immune-mediated reactions may involve any organ system; however, the most common severe immune-mediated adverse reactions are enterocolitis, hepatitis, dermatitis (including toxic epidermal necrolysis), neuropathy, and endocrinopathy. The majority of these immune-mediated reactions initially manifested during treatment; however, a minority occurred weeks to months after discontinuation of YERVOY.

Assess patients for signs and symptoms of enterocolitis, dermatitis, neuropathy, and endocrinopathy, and evaluate clinical chemistries including liver function tests (LFTs), adrenocorticotrophic hormone (ACTH) level, and thyroid function tests, at baseline and before each dose.

Permanently discontinue YERVOY and initiate systemic high-dose corticosteroid therapy for severe immune-mediated reactions.

Immune-Mediated Pneumonitis

OPDIVO can cause immune-mediated pneumonitis. Fatal cases have been reported. Monitor patients for signs with radiographic imaging and for symptoms of pneumonitis. Administer corticosteroids for Grade 2 or more severe pneumonitis. Permanently discontinue for Grade 3 or 4 and withhold until resolution for Grade 2. In patients receiving OPDIVO monotherapy, fatal cases of immune-mediated pneumonitis have occurred. Immune-mediated pneumonitis occurred in 3.1% (61/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, immune-mediated pneumonitis occurred in 6% (25/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated pneumonitis occurred in 4.4% (24/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated pneumonitis occurred in 1.7% (2/119) of patients.

In Checkmate 205 and 039, pneumonitis, including interstitial lung disease, occurred in 6.0% (16/266) of patients receiving OPDIVO. Immune-mediated pneumonitis occurred in 4.9% (13/266) of patients receiving OPDIVO: Grade 3 (n=1) and Grade 2 (n=12).

Immune-Mediated Colitis

OPDIVO can cause immune-mediated colitis. Monitor patients for signs and symptoms of colitis. Administer corticosteroids for Grade 2 (of more than 5 days duration), 3, or 4 colitis. Withhold OPDIVO monotherapy for Grade 2 or 3 and permanently discontinue for Grade 4 or recurrent colitis upon re-initiation of OPDIVO. When administered with YERVOY, withhold OPDIVO and YERVOY for Grade 2 and permanently discontinue for Grade 3 or 4 or recurrent colitis. In patients receiving OPDIVO monotherapy, immune-mediated colitis occurred in 2.9% (58/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, immune-mediated colitis occurred in 26% (107/407) of patients including three fatal cases. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated colitis occurred in 10% (52/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated colitis occurred in 7% (8/119) of patients.

In a separate Phase 3 study of YERVOY 3 mg/kg, severe, life-threatening, or fatal (diarrhea of ≥ 7 stools above baseline, fever, ileus, peritoneal signs;

Grade 3-5) immune-mediated enterocolitis occurred in 34 (7%) patients. Across all YERVOY-treated patients in that study (n=511), 5 (1%) developed intestinal perforation, 4 (0.8%) died as a result of complications, and 26 (5%) were hospitalized for severe enterocolitis.

Immune-Mediated Hepatitis

OPDIVO can cause immune-mediated hepatitis. Monitor patients for abnormal liver tests prior to and periodically during treatment. Administer corticosteroids for Grade 2 or greater transaminase elevations. For patients without HCC, withhold OPDIVO for Grade 2 and permanently discontinue OPDIVO for Grade 3 or 4. For patients with HCC, withhold OPDIVO and administer corticosteroids if AST/ALT is within normal limits at baseline and increases to >3 and up to 5 times the upper limit of normal (ULN), if AST/ALT is >1 and up to 3 times ULN at baseline and increases to >5 and up to 10 times the ULN, and if AST/ALT is >3 and up to 5 times ULN at baseline and increases to >8 and up to 10 times the ULN. Permanently discontinue OPDIVO and administer corticosteroids if AST or ALT increases to >10 times the ULN or total bilirubin increases >3 times the ULN. In patients receiving OPDIVO monotherapy, immune-mediated hepatitis occurred in 1.8% (35/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, immune-mediated hepatitis occurred in 13% (51/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated hepatitis occurred in 7% (38/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated hepatitis occurred in 8% (10/119) of patients.

In Checkmate 040, immune-mediated hepatitis requiring systemic corticosteroids occurred in 5% (8/154) of patients receiving OPDIVO.

In a separate Phase 3 study of YERVOY 3 mg/kg, severe, life-threatening, or fatal hepatotoxicity (AST or ALT elevations >5x the ULN or total bilirubin elevations >3x the ULN; Grade 3-5) occurred in 8 (2%) patients, with fatal hepatic failure in 0.2% and hospitalization in 0.4%.

Immune-Mediated Neuropathies

In a separate Phase 3 study of YERVOY 3 mg/kg, 1 case of fatal Guillain-Barré syndrome and 1 case of severe (Grade 3) peripheral motor neuropathy were reported.

Immune-Mediated Endocrinopathies

OPDIVO can cause immune-mediated hypophysitis, immune-mediated adrenal insufficiency, autoimmune thyroid disorders, and Type 1 diabetes mellitus. Monitor patients for signs and symptoms of hypophysitis, signs and symptoms of adrenal insufficiency, thyroid function prior to and periodically during treatment, and hyperglycemia. Administer hormone replacement as clinically indicated and corticosteroids for Grade 2 or greater hypophysitis. Withhold for Grade 2 or 3 and permanently discontinue for Grade 4 hypophysitis. Administer corticosteroids for Grade 3 or 4 adrenal insufficiency. Withhold for Grade 2 and permanently discontinue for Grade 3 or 4 adrenal insufficiency. Administer hormone-replacement therapy for hypothyroidism. Initiate medical management for control of hyperthyroidism. Withhold OPDIVO for Grade 3 and permanently discontinue for Grade 4 hyperglycemia.

In patients receiving OPDIVO monotherapy, hypophysitis occurred in 0.6% (12/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, hypophysitis occurred in 9% (36/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, hypophysitis occurred in 4.6% (25/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated hypophysitis occurred in 3.4% (4/119) of patients. In patients receiving OPDIVO monotherapy, adrenal insufficiency occurred in 1% (20/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, adrenal insufficiency occurred in 5% (21/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, adrenal insufficiency occurred in 7% (41/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, adrenal insufficiency occurred in 5.9% (7/119) of patients. In patients receiving OPDIVO monotherapy, hypothyroidism or thyroiditis resulting in hypothyroidism occurred in 9% (171/1994) of patients. Hyperthyroidism occurred in 2.7% (54/1994) of patients receiving OPDIVO monotherapy. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, hypothyroidism or thyroiditis resulting in hypothyroidism occurred in 22% (89/407) of patients. Hyperthyroidism occurred in 8% (34/407) of patients receiving this dose of OPDIVO with YERVOY. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, hypothyroidism or thyroiditis resulting in hypothyroidism occurred in 22% (119/547) of patients. Hyperthyroidism occurred in 12% (66/547) of patients receiving this dose of OPDIVO with YERVOY. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, hypothyroidism or thyroiditis resulting in hypothyroidism occurred in

15% (18/119) of patients. Hyperthyroidism occurred in 12% (14/119) of patients. In patients receiving OPDIVO monotherapy, diabetes occurred in 0.9% (17/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, diabetes occurred in 1.5% (6/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, diabetes occurred in 2.7% (15/547) of patients.

In a separate Phase 3 study of YERVOY 3 mg/kg, severe to life-threatening immune-mediated endocrinopathies (requiring hospitalization, urgent medical intervention, or interfering with activities of daily living; Grade 3-4) occurred in 9 (1.8%) patients. All 9 patients had hypopituitarism, and some had additional concomitant endocrinopathies such as adrenal insufficiency, hypogonadism, and hypothyroidism. Six of the 9 patients were hospitalized for severe endocrinopathies.

Immune-Mediated Nephritis and Renal Dysfunction

OPDIVO can cause immune-mediated nephritis. Monitor patients for elevated serum creatinine prior to and periodically during treatment. Administer corticosteroids for Grades 2-4 increased serum creatinine. Withhold OPDIVO for Grade 2 or 3 and permanently discontinue for Grade 4 increased serum creatinine. In patients receiving OPDIVO monotherapy, immune-mediated nephritis and renal dysfunction occurred in 1.2% (23/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, immune-mediated nephritis and renal dysfunction occurred in 2.2% (9/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated nephritis and renal dysfunction occurred in 4.6% (25/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated nephritis and renal dysfunction occurred in 1.7% (2/119) of patients.

Immune-Mediated Skin Adverse Reactions and Dermatitis

OPDIVO can cause immune-mediated rash, including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), some cases with fatal outcome. Administer corticosteroids for Grade 3 or 4 rash. Withhold for Grade 3 and permanently discontinue for Grade 4 rash. For symptoms or signs of SJS or TEN, withhold OPDIVO and refer the patient for specialized care for assessment and treatment; if confirmed, permanently discontinue. In patients receiving OPDIVO monotherapy, immune-mediated rash occurred in 9%

(171/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, immune-mediated rash occurred in 22.6% (92/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated rash occurred in 16.6% (91/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated rash occurred in 14% (17/119) of patients.

In a separate Phase 3 study of YERVOY 3 mg/kg, severe, life-threatening, or fatal immune-mediated dermatitis (eg, Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations; Grade 3-5) occurred in 13 (2.5%) patients. 1 (0.2%) patient died as a result of toxic epidermal necrolysis. 1 additional patient required hospitalization for severe dermatitis.

Immune-Mediated Encephalitis

OPDIVO can cause immune-mediated encephalitis. Evaluation of patients with neurologic symptoms may include, but not be limited to, consultation with a neurologist, brain MRI, and lumbar puncture. Withhold OPDIVO in patients with new-onset moderate to severe neurologic signs or symptoms and evaluate to rule out other causes. If other etiologies are ruled out, administer corticosteroids and permanently discontinue OPDIVO for immune-mediated encephalitis. In patients receiving OPDIVO monotherapy, encephalitis occurred in 0.2% (3/1994) of patients. Fatal limbic encephalitis occurred in one patient after 7.2 months of exposure despite discontinuation of OPDIVO and administration of corticosteroids. Encephalitis occurred in one patient receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg (0.2%) after 1.7 months of exposure. Encephalitis occurred in one RCC patient receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg (0.2%) after approximately 4 months of exposure. Encephalitis occurred in one MSI-H/dMMR mCRC patient (0.8%) receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg after 15 days of exposure.

Other Immune-Mediated Adverse Reactions

Based on the severity of the adverse reaction, permanently discontinue or withhold OPDIVO, administer high-dose corticosteroids, and, if appropriate, initiate hormone-replacement therapy. Across clinical trials of OPDIVO monotherapy or in combination with YERVOY, the following clinically significant immune-mediated adverse reactions, some with fatal outcome, occurred in <1.0% of patients receiving OPDIVO: myocarditis,

rhabdomyolysis, myositis, uveitis, iritis, pancreatitis, facial and abducens nerve paresis, demyelination, polymyalgia rheumatica, autoimmune neuropathy, Guillain-Barré syndrome, hypopituitarism, systemic inflammatory response syndrome, gastritis, duodenitis, sarcoidosis, histiocytic necrotizing lymphadenitis (Kikuchi lymphadenitis), motor dysfunction, vasculitis, aplastic anemia, pericarditis, and myasthenic syndrome.

If uveitis occurs in combination with other immune-mediated adverse reactions, consider a Vogt-Koyanagi-Harada-like syndrome, which has been observed in patients receiving OPDIVO and may require treatment with systemic steroids to reduce the risk of permanent vision loss.

Infusion Reactions

OPDIVO can cause severe infusion reactions, which have been reported in <1.0% of patients in clinical trials. Discontinue OPDIVO in patients with Grade 3 or 4 infusion reactions. Interrupt or slow the rate of infusion in patients with Grade 1 or 2. In patients receiving OPDIVO monotherapy as a 60-minute infusion, infusion-related reactions occurred in 6.4% (127/1994) of patients. In a separate study in which patients received OPDIVO monotherapy as a 60-minute infusion or a 30-minute infusion, infusion-related reactions occurred in 2.2% (8/368) and 2.7% (10/369) of patients, respectively. Additionally, 0.5% (2/368) and 1.4% (5/369) of patients, respectively, experienced adverse reactions within 48 hours of infusion that led to dose delay, permanent discontinuation or withholding of OPDIVO. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg every 3 weeks, infusion-related reactions occurred in 2.5% (10/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, infusion-related reactions occurred in 5.1% (28/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, infusion-related reactions occurred in 4.2% (5/119) of patients.

Complications of Allogeneic Hematopoietic Stem Cell Transplantation

Fatal and other serious complications can occur in patients who receive allogeneic hematopoietic stem cell transplantation (HSCT) before or after being treated with a PD-1 receptor blocking antibody. Transplant-related complications include hyperacute graft-versus-host-disease (GVHD), acute GVHD, chronic GVHD, hepatic veno-occlusive disease (VOD) after reduced

intensity conditioning, and steroid-requiring febrile syndrome (without an identified infectious cause). These complications may occur despite intervening therapy between PD-1 blockade and allogeneic HSCT.

Follow patients closely for evidence of transplant-related complications and intervene promptly. Consider the benefit versus risks of treatment with a PD-1 receptor blocking antibody prior to or after an allogeneic HSCT.

Embryo-Fetal Toxicity

Based on their mechanisms of action, OPDIVO and YERVOY can cause fetal harm when administered to a pregnant woman. Advise pregnant women of the potential risk to a fetus. Advise females of reproductive potential to use effective contraception during treatment with an OPDIVO- or YERVOY-containing regimen and for at least 5 months after the last dose of OPDIVO.

Increased Mortality in Patients with Multiple Myeloma when OPDIVO is Added to a Thalidomide Analogue and Dexamethasone

In clinical trials in patients with multiple myeloma, the addition of OPDIVO to a thalidomide analogue plus dexamethasone resulted in increased mortality. Treatment of patients with multiple myeloma with a PD-1 or PD-L1 blocking antibody in combination with a thalidomide analogue plus dexamethasone is not recommended outside of controlled clinical trials.

Lactation

It is not known whether OPDIVO or YERVOY is present in human milk. Because many drugs, including antibodies, are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from an OPDIVO-containing regimen, advise women to discontinue breastfeeding during treatment. Advise women to discontinue breastfeeding during treatment with YERVOY and for 3 months following the final dose.

Serious Adverse Reactions

In Checkmate 037, serious adverse reactions occurred in 41% of patients receiving OPDIVO (n=268). Grade 3 and 4 adverse reactions occurred in 42% of patients receiving OPDIVO. The most frequent Grade 3 and 4 adverse drug reactions reported in 2% to <5% of patients receiving OPDIVO were

abdominal pain, hyponatremia, increased aspartate aminotransferase, and increased lipase. In Checkmate 066, serious adverse reactions occurred in 36% of patients receiving OPDIVO (n=206). Grade 3 and 4 adverse reactions occurred in 41% of patients receiving OPDIVO. The most frequent Grade 3 and 4 adverse reactions reported in $\geq 2\%$ of patients receiving OPDIVO were gamma-glutamyltransferase increase (3.9%) and diarrhea (3.4%). In Checkmate 067, serious adverse reactions (74% and 44%), adverse reactions leading to permanent discontinuation (47% and 18%) or to dosing delays (58% and 36%), and Grade 3 or 4 adverse reactions (72% and 51%) all occurred more frequently in the OPDIVO plus YERVOY arm (n=313) relative to the OPDIVO arm (n=313). The most frequent ($\geq 10\%$) serious adverse reactions in the OPDIVO plus YERVOY arm and the OPDIVO arm, respectively, were diarrhea (13% and 2.2%), colitis (10% and 1.9%), and pyrexia (10% and 1.0%). In Checkmate 017 and 057, serious adverse reactions occurred in 46% of patients receiving OPDIVO (n=418). The most frequent serious adverse reactions reported in $\geq 2\%$ of patients receiving OPDIVO were pneumonia, pulmonary embolism, dyspnea, pyrexia, pleural effusion, pneumonitis, and respiratory failure. In Checkmate 032, serious adverse reactions occurred in 45% of patients receiving OPDIVO (n=245). The most frequent serious adverse reactions reported in at least 2% of patients receiving OPDIVO were pneumonia, dyspnea, pneumonitis, pleural effusions, and dehydration. In Checkmate 025, serious adverse reactions occurred in 47% of patients receiving OPDIVO (n=406). The most frequent serious adverse reactions reported in $\geq 2\%$ of patients were acute kidney injury, pleural effusion, pneumonia, diarrhea, and hypercalcemia. In Checkmate 214, serious adverse reactions occurred in 59% of patients receiving OPDIVO plus YERVOY and in 43% of patients receiving sunitinib. The most frequent serious adverse reactions reported in $\geq 2\%$ of patients were diarrhea, pyrexia, pneumonia, pneumonitis, hypophysitis, acute kidney injury, dyspnea, adrenal insufficiency, and colitis; in patients treated with sunitinib, they were pneumonia, pleural effusion, and dyspnea. In Checkmate 205 and 039, adverse reactions leading to discontinuation occurred in 7% and dose delays due to adverse reactions occurred in 34% of patients (n=266). Serious adverse reactions occurred in 26% of patients. The most frequent serious adverse reactions reported in $\geq 1\%$ of patients were pneumonia, infusion-related reaction, pyrexia, colitis or diarrhea, pleural effusion, pneumonitis, and rash. Eleven patients died from causes other than disease progression: 3 from adverse reactions within 30 days of the last OPDIVO dose, 2 from infection 8 to 9 months after completing OPDIVO, and 6 from complications of allogeneic HSCT. In Checkmate 141, serious adverse reactions occurred in 49% of

patients receiving OPDIVO (n=236). The most frequent serious adverse reactions reported in $\geq 2\%$ of patients receiving OPDIVO were pneumonia, dyspnea, respiratory failure, respiratory tract infection, and sepsis. In Checkmate 275, serious adverse reactions occurred in 54% of patients receiving OPDIVO (n=270). The most frequent serious adverse reactions reported in $\geq 2\%$ of patients receiving OPDIVO were urinary tract infection, sepsis, diarrhea, small intestine obstruction, and general physical health deterioration. In Checkmate 142 in MSI-H/dMMR mCRC patients receiving OPDIVO with YERVOY, serious adverse reactions occurred in 47% of patients. The most frequent serious adverse reactions reported in $\geq 2\%$ of patients were colitis/diarrhea, hepatic events, abdominal pain, acute kidney injury, pyrexia, and dehydration. In Checkmate 040, serious adverse reactions occurred in 49% of patients (n=154). The most frequent serious adverse reactions reported in $\geq 2\%$ of patients were pyrexia, ascites, back pain, general physical health deterioration, abdominal pain, and pneumonia. In Checkmate 238, Grade 3 or 4 adverse reactions occurred in 25% of OPDIVO-treated patients (n=452). The most frequent Grade 3 and 4 adverse reactions reported in $\geq 2\%$ of OPDIVO-treated patients were diarrhea and increased lipase and amylase. Serious adverse reactions occurred in 18% of OPDIVO-treated patients.

Common Adverse Reactions

In Checkmate 037, the most common adverse reaction ($\geq 20\%$) reported with OPDIVO (n=268) was rash (21%). In Checkmate 066, the most common adverse reactions ($\geq 20\%$) reported with OPDIVO (n=206) vs dacarbazine (n=205) were fatigue (49% vs 39%), musculoskeletal pain (32% vs 25%), rash (28% vs 12%), and pruritus (23% vs 12%). In Checkmate 067, the most common ($\geq 20\%$) adverse reactions in the OPDIVO plus YERVOY arm (n=313) were fatigue (62%), diarrhea (54%), rash (53%), nausea (44%), pyrexia (40%), pruritus (39%), musculoskeletal pain (32%), vomiting (31%), decreased appetite (29%), cough (27%), headache (26%), dyspnea (24%), upper respiratory tract infection (23%), arthralgia (21%), and increased transaminases (25%). In Checkmate 067, the most common ($\geq 20\%$) adverse reactions in the OPDIVO arm (n=313) were fatigue (59%), rash (40%), musculoskeletal pain (42%), diarrhea (36%), nausea (30%), cough (28%), pruritus (27%), upper respiratory tract infection (22%), decreased appetite (22%), headache (22%), constipation (21%), arthralgia (21%), and vomiting (20%). In Checkmate 017 and 057, the most common adverse reactions ($\geq 20\%$) in patients receiving OPDIVO (n=418) were fatigue, musculoskeletal

pain, cough, dyspnea, and decreased appetite. In Checkmate 032, the most common adverse reactions ($\geq 20\%$) in patients receiving OPDIVO (n=245) were fatigue (45%), decreased appetite (27%), musculoskeletal pain (25%), dyspnea (22%), nausea (22%), diarrhea (21%), constipation (20%), and cough (20%). In Checkmate 025, the most common adverse reactions ($\geq 20\%$) reported in patients receiving OPDIVO (n=406) vs everolimus (n=397) were fatigue (56% vs 57%), cough (34% vs 38%), nausea (28% vs 29%), rash (28% vs 36%), dyspnea (27% vs 31%), diarrhea (25% vs 32%), constipation (23% vs 18%), decreased appetite (23% vs 30%), back pain (21% vs 16%), and arthralgia (20% vs 14%). In Checkmate 214, the most common adverse reactions ($\geq 20\%$) reported in patients treated with OPDIVO plus YERVOY (n=547) vs sunitinib (n=535) were fatigue (58% vs 69%), rash (39% vs 25%), diarrhea (38% vs 58%), musculoskeletal pain (37% vs 40%), pruritus (33% vs 11%), nausea (30% vs 43%), cough (28% vs 25%), pyrexia (25% vs 17%), arthralgia (23% vs 16%), decreased appetite (21% vs 29%), dyspnea (20% vs 21%), and vomiting (20% vs 28%). In Checkmate 205 and 039, the most common adverse reactions ($\geq 20\%$) reported in patients receiving OPDIVO (n=266) were upper respiratory tract infection (44%), fatigue (39%), cough (36%), diarrhea (33%), pyrexia (29%), musculoskeletal pain (26%), rash (24%), nausea (20%) and pruritus (20%). In Checkmate 141, the most common adverse reactions ($\geq 10\%$) in patients receiving OPDIVO (n=236) were cough and dyspnea at a higher incidence than investigator's choice. In Checkmate 275, the most common adverse reactions ($\geq 20\%$) reported in patients receiving OPDIVO (n=270) were fatigue (46%), musculoskeletal pain (30%), nausea (22%), and decreased appetite (22%). In Checkmate 142 in MSI-H/dMMR mCRC patients receiving OPDIVO as a single agent, the most common adverse reactions ($\geq 20\%$) were fatigue (54%), diarrhea (43%), abdominal pain (34%), nausea (34%), vomiting (28%), musculoskeletal pain (28%), cough (26%), pyrexia (24%), rash (23%), constipation (20%), and upper respiratory tract infection (20%). In Checkmate 142 in MSI-H/dMMR mCRC patients receiving OPDIVO with YERVOY, the most common adverse reactions ($\geq 20\%$) were fatigue (49%), diarrhea (45%), pyrexia (36%), musculoskeletal pain (36%), abdominal pain (30%), pruritus (28%), nausea (26%), rash (25%), decreased appetite (20%), and vomiting (20%). In Checkmate 040, the most common adverse reactions ($\geq 20\%$) in patients receiving OPDIVO (n=154) were fatigue (38%), musculoskeletal pain (36%), abdominal pain (34%), pruritus (27%), diarrhea (27%), rash (26%), cough (23%), and decreased appetite (22%). In Checkmate 238, the most common adverse reactions ($\geq 20\%$) reported in OPDIVO-treated patients (n=452) vs ipilimumab-treated patients (n=453) were fatigue (57% vs 55%), diarrhea

<https://news.bms.com/news/corporate-financial/2019/Bristol-Myers-Squibb-Provides-Update-on-Phase-3-Opdivo-nivolumab-CheckMate--548-Trial-in-Patients-with-Newly-Diagnosed-MGMT-Methylated-Glioblastoma-Multiforme/default.aspx> <Accessed June 12, 2023>.

(37% vs 55%), rash (35% vs 47%), musculoskeletal pain (32% vs 27%), pruritus (28% vs 37%), headache (23% vs 31%), nausea (23% vs 28%), upper respiratory infection (22% vs 15%), and abdominal pain (21% vs 23%). The most common immune-mediated adverse reactions were rash (16%), diarrhea/colitis (6%), and hepatitis (3%).

In a separate Phase 3 study of YERVOY 3 mg/kg, the most common adverse reactions ($\geq 5\%$) in patients who received YERVOY at 3 mg/kg were fatigue (41%), diarrhea (32%), pruritus (31%), rash (29%), and colitis (8%).

Please see U.S. Full Prescribing Information for [OPDIVO](#) and [YERVOY](#), including **Boxed WARNING regarding immune-mediated adverse reactions** for YERVOY.

Checkmate Trials and Patient Populations

Checkmate 037—previously treated metastatic melanoma; **Checkmate 066**—previously untreated metastatic melanoma; **Checkmate 067**—previously untreated metastatic melanoma, as a single agent or in combination with YERVOY; **Checkmate 017**—second-line treatment of metastatic squamous non-small cell lung cancer; **Checkmate 057**—second-line treatment of metastatic non-squamous non-small cell lung cancer; **Checkmate 032**—small cell lung cancer; **Checkmate 025**—previously treated renal cell carcinoma; **Checkmate 214**—previously untreated renal cell carcinoma, in combination with YERVOY; **Checkmate 205/039**—classical Hodgkin lymphoma; **Checkmate 141**—recurrent or metastatic squamous cell carcinoma of the head and neck; **Checkmate 275**—urothelial carcinoma; **Checkmate 142**—MSI-H or dMMR metastatic colorectal cancer, as a single agent or in combination with YERVOY; **Checkmate 040**—hepatocellular carcinoma; **Checkmate 238**—adjuvant treatment of melanoma.

About the Bristol-Myers Squibb and Ono Pharmaceutical Collaboration

In 2011, through a collaboration agreement with Ono Pharmaceutical Co., Bristol-Myers Squibb expanded its territorial rights to develop and commercialize *Opdivo* globally, except in Japan, South Korea and Taiwan,

<https://news.bms.com/news/corporate-financial/2019/Bristol-Myers-Squibb-Provides-Update-on-Phase-3-Opdivo-nivolumab-CheckMate--548-Trial-in-Patients-with-Newly-Diagnosed-MGMT-Methylated-Glioblastoma-Multiforme/default.aspx> <Accessed June 12, 2023>.

where Ono had retained all rights to the compound at the time. On July 23, 2014, Ono and Bristol-Myers Squibb further expanded the companies' strategic collaboration agreement to jointly develop and commercialize multiple immunotherapies – as single agents and combination regimens – for patients with cancer in Japan, South Korea and Taiwan.

About Bristol-Myers Squibb

Bristol-Myers Squibb is a global biopharmaceutical company whose mission is to discover, develop and deliver innovative medicines that help patients prevail over serious diseases. For more information about Bristol-Myers Squibb, visit us at [BMS.com](https://www.bms.com) or follow us on [LinkedIn](#), [Twitter](#), [YouTube](#) and [Facebook](#).

Bristol-Myers Squibb Forward-Looking Statement

This press release contains “forward-looking statements” within the meaning of the Private Securities Litigation Reform Act of 1995 regarding, among other things, the research, development and commercialization of pharmaceutical products. All statements that are not statements of historical facts are, or may be deemed to be, forward-looking statements. Such forward-looking statements are based on historical performance and current expectations and projections about our future financial results, goals, plans and objectives and involve inherent risks, assumptions and uncertainties, including internal or external factors that could delay, divert or change any of them in the next several years, that are difficult to predict, may be beyond our control and could cause our future financial results, goals, plans and objectives to differ materially from those expressed in, or implied by, the statements. These risks, assumptions, uncertainties and other factors include, among others, the possibility of unfavorable results from further clinical trials involving the product candidate described in this release and whether such product candidate for the additional indication described in this release will be successfully developed and commercialized. No forward-looking statement can be guaranteed. Forward-looking statements in this press release should be evaluated together with the many risks and uncertainties that affect Bristol-Myers Squibb’s business and market, particularly those identified in

<https://news.bms.com/news/corporate-financial/2019/Bristol-Myers-Squibb-Provides-Update-on-Phase-3-Opdivo-nivolumab-CheckMate--548-Trial-in-Patients-with-Newly-Diagnosed-MGMT-Methylated-Glioblastoma-Multiforme/default.aspx> <Accessed June 12, 2023>.

the cautionary statement and risk factors discussion in Bristol-Myers Squibb's Annual Report on Form 10-K for the year ended December 31, 2018, as updated by our subsequent Quarterly Reports on Form 10-Q, Current Reports on Form 8-K and other filings with the Securities and Exchange Commission. The forward-looking statements included in this document are made only as of the date of this document and except as otherwise required by applicable law, Bristol-Myers Squibb undertakes no obligation to publicly update or revise any forward-looking statement, whether as a result of new information, future events, changed circumstances or otherwise.

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Phase III trial of chemoradiotherapy with temozolomide plus nivolumab or placebo for newly diagnosed glioblastoma with methylated *MGMT* promoter

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Abstract

Background. Nearly all patients with newly diagnosed glioblastoma experience recurrence following standard-of-care radiotherapy (RT) + temozolomide (TMZ). The purpose of the phase III randomized CheckMate 548 study was

to evaluate RT + TMZ combined with the immune checkpoint inhibitor nivolumab (NIVO) or placebo (PBO) in patients with newly diagnosed glioblastoma with methylated *MGMT* promoter (NCT02667587).

Methods. Patients (N = 716) were randomized 1:1 to NIVO [(240 mg every 2 weeks × 8, then 480 mg every 4 weeks) + RT (60 Gy over 6 weeks) + TMZ (75 mg/m² once daily during RT, then 150-200 mg/m² once daily on days 1-5 of every 28-day cycle × 6)] or PBO + RT + TMZ following the same regimen. The primary endpoints were progression-free survival (PFS) and overall survival (OS) in patients without baseline corticosteroids and in all randomized patients.

Results. As of December 22, 2020, median (m)PFS (blinded independent central review) was 10.6 months (95% CI, 8.9-11.8) with NIVO + RT + TMZ vs 10.3 months (95% CI, 9.7-12.5) with PBO + RT + TMZ (HR, 1.1; 95% CI, 0.9-1.3) and mOS was 28.9 months (95% CI, 24.4-31.6) vs 32.1 months (95% CI, 29.4-33.8), respectively (HR, 1.1; 95% CI, 0.9-1.3). In patients without baseline corticosteroids, mOS was 31.3 months (95% CI, 28.6-34.8) with NIVO + RT + TMZ vs 33.0 months (95% CI, 31.0-35.1) with PBO + RT + TMZ (HR, 1.1; 95% CI, 0.9-1.4). Grade 3/4 treatment-related adverse event rates were 52.4% vs 33.6%, respectively.

Conclusions. NIVO added to RT + TMZ did not improve survival in patients with newly diagnosed glioblastoma with methylated or indeterminate *MGMT* promoter. No new safety signals were observed.

Key Points

- NIVO did not improve survival in newly diagnosed glioblastoma with methylated *MGMT* promoter.
- No new safety signals were detected with NIVO + standard of care in this study.
- Nivolumab could be considered within future combination strategies.

Importance of the Study

The continued urgent need for novel treatment mechanisms to improve clinical outcomes in patients with glioblastoma and the demonstrated benefit of immune checkpoint inhibitors (ICIs) in various tumor types have led to the investigation of ICI efficacy in glioblastoma. Nearly all patients with newly diagnosed glioblastoma have recurrence after standard-of-care surgical resection followed by radiotherapy (RT) and temozolomide (TMZ). Methylation of the *MGMT* promoter is a positive prognostic factor and predictor of TMZ benefit in

this patient population. Here we report data from the largest phase III study in patients with glioblastoma and methylated *MGMT* promoter. Nivolumab vs placebo added to RT + TMZ did not improve survival. However, compared with previous trials, a higher median OS was observed in both treatment arms, potentially resulting from advances in patient care. Additionally, as no new safety signals were observed with nivolumab, this regimen can safely be considered for the basis of additional combination strategies.

Glioblastoma is the most common and aggressive primary malignant brain tumor in adults.^{1,2} Standard-of-care treatment for patients with newly diagnosed disease usually involves surgical resection followed by radiotherapy (RT) with concomitant and adjuvant temozolomide (TMZ).^{3,4} Approval of TMZ was based on a phase III study that showed overall survival (OS) improved from 12.1 months with RT alone to 14.6 months with TMZ-based chemoradiotherapy (hazard ratio [HR], 0.63; *P* < .001).^{3,5} More recently, the use of tumor-treating fields was also approved by the FDA for use in combination with adjuvant TMZ after standard-of-care surgery and RT + TMZ.⁶ However, no other therapies have been approved in patients with newly diagnosed glioblastoma, and nearly all treated patients experience recurrence, highlighting the need for novel therapies.²

O⁶-methylguanine DNA methyltransferase (*MGMT*) promoter status is a key prognostic factor in glioblastoma.⁷⁻¹¹

Epigenetic silencing of the *MGMT* gene via promoter methylation increases sensitivity to alkylating agents such as TMZ, and patients with tumors with a methylated *MGMT* promoter treated with TMZ achieve longer OS than those who have tumors with an unmethylated *MGMT* promoter.^{7,9,11-13} Observed rates of *MGMT* promoter methylation are variable and depend on the assay used; however, approximately 35% of patients with newly diagnosed glioblastoma are reported to have tumors with a methylated *MGMT* promoter.¹¹

Nivolumab (NIVO), a fully human immunoglobulin G4 monoclonal antibody that targets the programmed cell death-1 (PD-1) receptor, is approved for the treatment of multiple advanced cancers and has demonstrated anti-tumor activity in patients with melanoma with brain metastases.¹⁴ The immune checkpoint transmembrane protein programmed death-1 ligand 1 (PD-L1) is frequently expressed in primary glioblastomas, and high expression

levels have been associated with shorter survival.¹⁵ Preclinical data have suggested that RT induces cell death and the release of tumor antigens, which promote tumor-specific immune responses that could be amplified with immune-stimulating agents, such as immune checkpoint pathway inhibitors.¹⁶ Moreover, in murine glioma models, combination of a PD-1 inhibitor with RT improved OS compared with either treatment alone.¹⁷

Results from the randomized phase III CheckMate 143 study (NCT02017717) demonstrated that OS was comparable between NIVO and bevacizumab (9.77 vs 10.02 months) in patients with recurrent glioblastoma; however, a trend toward longer median OS was observed with NIVO vs bevacizumab (16.95 vs 10.12 months) in a subgroup of patients with a methylated *MGMT* promoter and no baseline corticosteroid use.¹⁸ A preliminary signal was also observed in the phase I cohorts 1c and 1d of the CheckMate 143 study in patients with newly diagnosed glioblastoma with a methylated or indeterminate *MGMT* promoter who were treated with NIVO in combination with RT + TMZ.¹⁹ Median progression-free survival (PFS) was 15.47 months (95% CI, 7.10 months to not estimable) in 15 patients with tumors with a methylated or indeterminate *MGMT* promoter compared with 6.47 months (95% CI, 4.14-10.18 months) in 16 patients with tumors with an unmethylated *MGMT* promoter; respective median OS was 33.38 months (95% CI, 16.20 months to not estimable) compared with 16.49 months (95% CI, 12.94-22.08 months).¹⁹ These findings supported further evaluation of NIVO in combination with standard-of-care RT + TMZ in patients with a methylated *MGMT* promoter.

Here we report the final analysis of the phase III CheckMate 548 trial (NCT02667587), which investigated the efficacy and safety of RT + TMZ in combination with NIVO or placebo (PBO) in patients with newly diagnosed glioblastoma with a methylated or indeterminate *MGMT* promoter.

Methods

Patients

Eligible patients had newly diagnosed, histologically confirmed supratentorial glioblastoma (WHO grade IV malignant glioma) and had not received treatment for glioblastoma other than surgery. Postoperative baseline MRI obtained either <72 hours or >14 days after surgery was required prior to randomization. A surgical resection of ≥20% of enhancing tumor was required, and patients must have fully recovered from surgery with no major ongoing safety issues.

Patients had to be ≥18 years of age, have a KPS (Karnofsky performance status) of ≥70, and be eligible to receive RT + concomitant TMZ. Screening for *MGMT* methylation status was performed in parallel with the phase III CheckMate 498 study (NCT02617589).^{20,21} Patients initially provided informed consent to participate and undergo screening in CheckMate 498 and then had *MGMT* status determined by an independent central laboratory. Patients with unmethylated *MGMT* promoter tumors proceeded with participation and randomization in CheckMate 498. Patients with methylated or indeterminate *MGMT*

promoter status were removed from CheckMate 498^{20,21} and became eligible to participate in this study. A total of 1002 prerandomized patients were therefore screened in CheckMate 498. Patients without a tumor sample were not eligible for participation in either study.

Patients receiving corticosteroids to manage glioblastoma symptoms at the time of screening were required to discontinue or taper use so that dose at randomization was ≤20 mg of prednisone or ≤3 mg of dexamethasone daily (or equivalent); inhaled or topical corticosteroids and adrenal replacement corticosteroid doses of >10 mg of prednisone daily (or equivalent) to manage other conditions were permitted in the absence of active autoimmune disease.

Other exclusion criteria included recurrent or secondary glioblastoma; metastatic extracranial or leptomeningeal disease; active, known, or suspected autoimmune disease; concomitant use of a carmustine wafer; use of any noninvasive anticancer medical device (eg, NovoTTF); and unresolved CNS hemorrhage.

Study Oversight

The study was conducted in accordance with Good Clinical Practice guidelines per the International Conference on Harmonisation and ethical principles of the European Union Directive and US Code of Federal Regulations and registered at ClinicalTrials.gov (NCT02667587). The protocol was approved by an institutional review board or independent ethics committee at each site before study activation. All patients provided written informed consent in accordance with the Declaration of Helsinki.

Study Design and Treatment

This study followed a single-blind, or “site-subject blinded,” design. Investigators, patients, and site staff were blinded to the therapy administered. Each investigative site had an unblinded pharmacist or designee, and designated BMS Research and Development staff were unblinded to facilitate drug supply and safety monitoring. Patients remained blinded to treatment conditions except in the event of a medical emergency or pregnancy in which knowledge of the treatment was critical for patient management and safety. Patients were randomly assigned, 1:1, to 2 treatment arms. The patients in the first arm received NIVO (240 mg every 2 weeks) for 8 doses and then 480 mg every 4 weeks) + RT (60 Gy over 6 weeks) + TMZ (75 mg/m² once daily during RT followed by a 4-week treatment break, and then 150-200 mg/m² once daily on days 1-5 of every 28-day cycle). The patients in the second arm received PBO (every 2 weeks for 8 doses and then every 4 weeks) + RT + TMZ following the same schedule and dosing regimen. Treatment continued until the occurrence of unacceptable toxicity or disease progression. However, NIVO treatment could be continued beyond suspected progression until confirmation of progression by follow-up MRI if evidence of investigator-assessed clinical benefit and tolerance of study drug were observed. Randomization was stratified according to the degree of surgical resection (complete vs partial) at baseline. Complete resection was defined as visible-total removal of an MRI-detectable tumor; however,

invasive glioblastoma still remained. Partial resection was defined as <90% of macroscopic removal of the tumor mass, or >10 mm residual.

The primary endpoints were PFS by blinded independent central review (BICR) in all randomized patients and OS in all randomized patients and in those without baseline corticosteroid use. Secondary endpoints included OS rate at 12 and 24 months and PFS per investigator assessment. Key exploratory endpoints included safety and tolerability and efficacy outcomes by tumor PD-L1 expression category.

Assessments

Tumor samples were assessed for *MGMT* promoter methylation status using a methylation-specific polymerase chain reaction assay from a central laboratory. A sample was determined to be *MGMT* methylated when the ratio of *MGMT* to β -actin control was ≥ 2 (calculated as [methylated *MGMT*/ β -actin] $\times$ 1000),¹³ and β -actin and *MGMT* were within the reportable range (β -actin ≥ 10 copies and *MGMT* ≥ 10 copies). A sample was determined to be *MGMT* unmethylated when the ratio of *MGMT* to β -actin control was <2 and as *MGMT* indeterminate when results were unable to be determined.

Disease status was assessed by investigators using contrast-enhanced MRI at baseline, approximately 4 weeks after completion of RT, every 8 weeks up to 24 months after randomization, and then every 12 weeks until progression according to Radiologic Assessment in Neuro-Oncology (RANO) criteria.²² RANO criteria recommend that within the first 12 weeks after completion of RT, when pseudoprogression is the most prevalent, progression can only be determined if the majority of the new enhancement was outside of the radiation field or if there was pathological confirmation of progressive disease (PD). Evidence suggests that patients treated with immunotherapy may derive clinical benefit despite initial evidence of disease progression; therefore, patients in the NIVO + RT + TMZ arm may have continued NIVO in the setting of suspected progression at investigator discretion until progression was confirmed.

PFS was defined as the time from randomization to documented progression or death from any cause, and OS was defined as the time from randomization to death.

An exploratory retrospective analysis of existing progression data from BICR per RANO criteria²² was used to estimate the rate of pseudoprogression as defined by immunotherapy RANO (iRANO) criteria in the NIVO + RT + TMZ arm.²³ Pseudoprogression was evaluated in patients treated with NIVO + RT + TMZ who had PFS of ≤ 6 months from the first NIVO dose. Patients with follow-up scans ≥ 3 -months post-PD and unconfirmed PD (no confirmation of PD worsening) while remaining on treatment were considered as having pseudoprogression.

Tumor PD-L1 expression was determined using a validated immunohistochemistry assay (PD-L1 IHC 28-8 pharmDx). PD-L1 positivity was defined as the percentage of tumor cells with membranous staining using 1% and 5% cutoff values. Patients were randomized to treatment regardless of tumor PD-L1 expression.

Adverse events were assessed continuously during the study per National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03.²⁴

Statistical Analysis

PFS and OS comparisons were based on a 2-sided log-rank test stratified by surgical resection at baseline (complete vs partial). OS analysis was conducted in the randomized population without baseline corticosteroids use and was planned approximately 20 months after completion of accrual or when 236 deaths were reported. OS and PFS curves, medians with 95% CIs, and OS rates at 12 and 24 months with 95% CIs were estimated using Kaplan-Meier methodology; HRs and corresponding 2-sided (95%) CIs were estimated using a Cox proportional hazards model, with treatment arm as a single covariate stratified by surgical resection (complete vs partial) at baseline. Baseline patient characteristics in all randomized patients and safety in all treated patients were characterized using descriptive statistics.

Results

Patients and Treatment

From May 11, 2016, through December 9, 2019, 716 patients with tumors with methylated or indeterminate *MGMT* promoter methylation status were randomized; 709 received study treatment with either NIVO + RT + TMZ (n = 355) or PBO + RT + TMZ (n = 354) (see [Supplementary Figure S1](#)). Patients were enrolled at 118 sites across 19 countries.

No marked imbalances in baseline characteristics or demographics were observed between the arms ([Table 1](#); [Supplementary Table S1](#)). Among all patients, 353 (98.6%) and 349 (97.5%) had a methylated *MGMT* promoter status, 4 (1.1%) and 7 (2.0%) had an indeterminate *MGMT* promoter status, and 1 (0.3%) and 2 (0.6%) had nonreported *MGMT* promoter status in the NIVO + RT + TMZ and PBO + RT + TMZ arms, respectively. Among patients with evaluable PD-L1 expression (n = 356 in each arm), baseline tumor PD-L1 expression was $\geq 1\%$ in 126 patients (35.4%) in the NIVO + RT + TMZ arm and in 118 patients (33.1%) in the PBO + RT + TMZ arm; baseline tumor PD-L1 expression was <1% in 230 patients (64.6%) and 238 patients (66.9%), respectively. Complete surgical resection had been performed in 199 patients (55.6%) in the NIVO + RT + TMZ arm and in 200 patients (55.9%) in the PBO + RT + TMZ arm. Most patients were not receiving corticosteroids at baseline (NIVO + RT + TMZ = 246/358 [68.7%]; PBO + RT + TMZ = 261/358 [72.9%]).

Median duration of NIVO treatment was 10.4 months (range, <0.1-52.5 months); the median duration of TMZ was 7.3 months (range, 0.2-43.5 months) in the NIVO + RT + TMZ arm and 7.4 months (range, <0.1-46.2 months) in the PBO + RT + TMZ arm. A median of 15.0 (range, 1-62) NIVO doses was received.

At data cutoff (December 22, 2020), most patients had discontinued treatment (318 patients [89.6%] in the NIVO + RT + TMZ arm and 310 patients [87.6%] in the PBO + RT

Table 1. Patient Demographics and Baseline Characteristics

Variable	Nivolumab + RT + TMZ n = 358 No. (%)	Placebo + RT + TMZ n = 358 No. (%)
Age		
Median (range), years	60.0 (24-79)	60.0 (18-81)
Age, years		
<65	245 (68.4)	237 (66.2)
≥65 to <75	90 (25.1)	104 (29.1)
≥75	23 (6.4)	17 (4.7)
Sex		
Male	205 (57.3)	197 (55.0)
Female	153 (42.7)	161 (45.0)
Histopathologic diagnosis		
Glioblastoma	353 (98.6)	353 (98.6)
Gliosarcoma	4 (1.1)	5 (1.4)
Not reported	1 (<1)	0
RPA class ^a		
III	32 (8.9)	23 (6.4)
IV	287 (80.2)	306 (85.5)
V	38 (10.6)	29 (8.1)
Not reported	1 (<1)	0
Extent of surgery ^b		
Complete resection	199 (55.6)	200 (55.9)
Partial resection	158 (44.1)	158 (44.1)
Not reported	1 (0.3)	0
Karnofsky performance status		
100	82 (22.9)	89 (24.9)
90	160 (44.7)	162 (45.3)
80	78 (21.8)	78 (21.8)
70	34 (9.5)	28 (7.8)
60	1 (<1)	0
Not reported	3 (<1)	1 (<1)
Time from initial diagnosis to randomization		
Median (range), weeks	5.29 (3.0-40.1 ^c)	5.36 (2.7-13.4)
MGMT promoter methylation status		
Methylated	353 (98.6)	349 (97.5)
Indeterminate	4 (1.1)	7 (2.0)
Not reported	1 (0.3)	2 (0.6)
Patients with evaluable PD-L1 expression		
PD-L1 expression level		
<1%	356 (99.4)	356 (99.4)
≥1%	230 (64.6)	238 (66.9)
≥1%	126 (35.4)	118 (33.1)
Corticosteroid use ^d		
Yes	112 (31.3)	97 (27.1)
≤3 mg/day	89 (24.9)	73 (20.4)
>3 mg/day	23 (6.4)	24 (6.7)
No	246 (68.7)	261 (72.9)

Abbreviations: MGMT, O⁶-methylguanine DNA methyltransferase; NIVO + RT + TMZ, nivolumab + radiotherapy + temozolomide; PD-L1, programmed cell death-1 ligand 1; PBO + RT + TMZ, placebo + radiotherapy + temozolomide; RPA, recursive partitioning analysis.

^aThe RPA classes were as follows: class III: age <50 years and Karnofsky performance status ≥90 (on a scale of 0-100, with higher scores indicating better function); class IV, <50 years and Karnofsky performance status <90 (or ≥50 years, Karnofsky performance status ≥70, complete or partial tumor resection, and ability to work); class V, ≥50 years, Karnofsky performance status ≥70, complete or partial tumor resection, and inability to work (or ≥50 years, Karnofsky performance status ≥70, and tumor-biopsy specimen only; or ≥50 years and Karnofsky performance status <70).⁸

^bThis characteristic was used as a stratification factor.

^cThe patient with 40.1 weeks from the initial diagnosis to the start of RT had two partial resections prior to randomization, with no RT or systemic cancer therapies in between.

^dBased on average corticosteroid use 5 days prior to the start of dosing or randomization date for patients not treated.

+ TMZ arm). The most common reasons for treatment discontinuation were disease progression (NIVO + RT + TMZ, $n = 177$ [49.9%]; PBO + RT + TMZ, $n = 222$ [62.7%]) and study drug toxicity (NIVO + RT + TMZ, $n = 74$ [20.8%]; PBO + RT + TMZ, $n = 18$ [5.1%]) ([Supplementary Figure S1](#)).

Efficacy

At data cutoff, the minimum potential follow-up was 12.5 months in the NIVO + RT + TMZ arm and 19.5 months in the PBO + RT + TMZ arm. Median PFS per BICR was 10.6 months (95% CI, 8.9-11.8 months) with NIVO + RT + TMZ vs 10.3 months (95% CI, 9.7-12.5) with PBO + RT + TMZ (HR, 1.1; 95% CI, 0.9-1.3) ([Figure 1A](#)). Median PFS per investigator assessment was 14.1 months (95% CI, 12.6-16.6 months) and 15.2 months (95% CI, 13.1-17.1 months), respectively (HR, 1.0; 95% CI, 0.9-1.2) ([Figure 1B](#)).

Among all patients, the median OS was 28.9 months (95% CI, 24.4-31.6 months) in the NIVO + RT + TMZ arm and 32.1 months (95% CI, 29.4-33.8 months) in the PBO + RT + TMZ arm (HR, 1.1; 95% CI, 0.9-1.3) ([Figure 2A](#)). The median OS was 31.3 months (95% CI, 28.6-34.8 months) and 33.0 months (95% CI, 31.0-35.1 months), respectively, in patients without baseline corticosteroid use (HR, 1.1; 95% CI, 0.9-1.4) ([Figure 2B](#)).

The 12-month OS rates in all patients were 82.7% (95% CI, 78.3%-86.3%) in the NIVO + RT + TMZ arm and 87.7% (95% CI, 83.8%-90.8%) in the PBO + RT + TMZ arm. The 24-month OS rates were 55.9% (95% CI, 50.5%-61.0%) in the NIVO + RT + TMZ arm and 63.3% (95% CI, 58.0%-68.2%) in the PBO + RT + TMZ arm. Among patients without baseline corticosteroid use, the 12-month OS rates were 85.5% (95% CI, 80.4%-89.4%) in the NIVO + RT + TMZ arm and 89.9% (95% CI, 85.5%-93.0%) in the PBO + RT + TMZ arm. The 24-month OS rates were 60.9% (95% CI, 54.4%-66.8%) in the NIVO + RT + TMZ arm and 67.1% (95% CI, 61.0%-72.6%) in the PBO + RT + TMZ arm.

Among patients with baseline PD-L1 expression $\geq 1\%$, median PFS was 10.6 months (95% CI, 8.1-12.1 months) in the NIVO + RT + TMZ arm and 9.7 months (95% CI, 6.5-11.8 months) in the PBO + RT + TMZ arm (HR, 1.1; 95% CI, 0.8-1.4) ([Figure 3A](#)). The median PFS in patients with PD-L1 $< 1\%$ was 11.2 months (95% CI, 8.5-12.3 months) in the NIVO + RT + TMZ arm and 11.5 months (95% CI, 9.9-13.2 months) in the PBO + RT + TMZ arm (HR, 1.0; 95% CI, 0.8-1.2) ([Figure 3B](#)). Median OS among patients with baseline PD-L1 expression $\geq 1\%$ was 29.8 months (95% CI, 23.3-34.6 months) in the NIVO + RT + TMZ arm and 31.0 months (95% CI, 26.5-34.5 months) in the PBO + RT + TMZ arm (HR, 1.0; 95% CI, 0.7-1.4) ([Figure 3C](#)). The median OS in patients with PD-L1 $< 1\%$ was 28.7 months (95% CI, 23.2-32.2 months) in the NIVO + RT + TMZ arm and 32.1 months (95% CI, 28.9-34.2 months) in the PBO + RT + TMZ arm (HR, 1.1; 95% CI, 0.9-1.4) ([Figure 3D](#)).

PFS and OS data by baseline PD-L1 expression $\geq 5\%$ are shown in [Supplementary Figure S2](#). Among patients with baseline PD-L1 expression $\geq 5\%$, median PFS was 8.4 months (95% CI, 6.2-12.3 months) in the NIVO + RT + TMZ arm and 9.9 months (95% CI, 6.5-13.1 months) in the PBO + RT + TMZ arm (HR, 1.1; 95% CI, 0.8-1.6). Median PFS in patients with PD-L1 $< 5\%$ was 11.5 months

(95% CI, 9.7-12.1 months) in the NIVO + RT + TMZ arm and 11.3 months (95% CI, 9.8-13.1 months) in the PBO + RT + TMZ arm (HR, 1.0; 95% CI, 0.8-1.2). Median OS was 29.2 months (95% CI, 21.8-42.9 months) and 28.9 (95% CI, 23.7-31.6 months) in the NIVO + RT + TMZ arm (HR, 1.0; 95% CI, 0.6-1.4) and 31.3 months (95% CI, 23.2-36.0 months) and 31.8 months (95% CI, 28.8-33.8 months) in the PBO + RT + TMZ arm (HR, 1.1; 95% CI, 0.9-1.4) in patients with $\geq 5\%$ and $< 5\%$ PD-L1 expression, respectively.

These results were consistent across several subgroup analyses ([Figure 4](#); [Supplementary Figure S3](#)).

Safety

Any-grade treatment-related adverse events (TRAEs) were reported in 92.4% of patients treated with NIVO + RT + TMZ and 83.6% of patients treated with PBO + RT + TMZ ([Table 2](#)). The most frequent TRAE was nausea (34.1%) in the NIVO + RT + TMZ arm and fatigue (32.8%) in the PBO + RT + TMZ arm. Rates of grade 3/4 TRAEs were 52.4% with NIVO + RT + TMZ and 33.6% with PBO + RT + TMZ. Neurological TRAEs occurred in 23.1% of patients treated with NIVO + RT + TMZ (grade 3/4, 5.1%) and 16.7% of patients treated with PBO + RT + TMZ (grade 3/4, 0.6%) ([Table 2](#)). The most frequent neurological TRAEs in both arms were headache (NIVO + RT + TMZ, 9.3%; PBO + RT + TMZ, 5.9%) and dysgeusia (NIVO + RT + TMZ, 5.6%; PBO + RT + TMZ, 4.2%). Any-grade serious TRAEs occurred in 105 patients (29.6%) treated with NIVO + RT + TMZ and 36 patients (10.2%) treated with PBO + RT + TMZ ([Table 2](#)). The most frequent serious TRAEs in both arms (NIVO + RT + TMZ/PBO + RT + TMZ) were tumor flare (2.5%/1.4%), pancytopenia (2.3%/0.6%), and thrombocytopenia (2.0%/1.7%).

Four treatment-related deaths were reported in the NIVO + RT + TMZ arm: respiratory failure, respiratory distress, pancytopenia, and pneumocystis pneumonia (1 each). No treatment-related deaths were reported in the PBO + RT + TMZ arm.

Any-grade TRAEs leading to discontinuation occurred in 81 patients (22.8%) in the NIVO + RT + TMZ arm and 32 patients (9.0%) in the PBO + RT + TMZ arm. Treatment-related immune-mediated AEs reported by category are shown in [Supplementary Table S2](#).

Pseudoprogression was evaluated in patients treated with NIVO + RT + TMZ who had PFS of ≤ 6 months from the first NIVO dose. Patients with follow-up scans ≥ 3 -months post-PD and unconfirmed PD (no confirmation of PD worsening) while remaining on treatment were considered as having pseudoprogression. Sixty-five patients in the NIVO + RT + TMZ arm were determined to be at risk for pseudoprogression among those with PD ($n = 237$). Forty of these patients had follow-up scans ≥ 3 -months post-PD, and of those, 20 were confirmed as having pseudoprogression and the other 20 had PD without pseudoprogression, per iRANO criteria.²³ Therefore, the rate of pseudoprogression among treated patients who had PD was 8.4%. Pseudoprogression could not be determined in the remaining 25 patients who had follow-up scans < 3 months (10.5% of patients with PD).

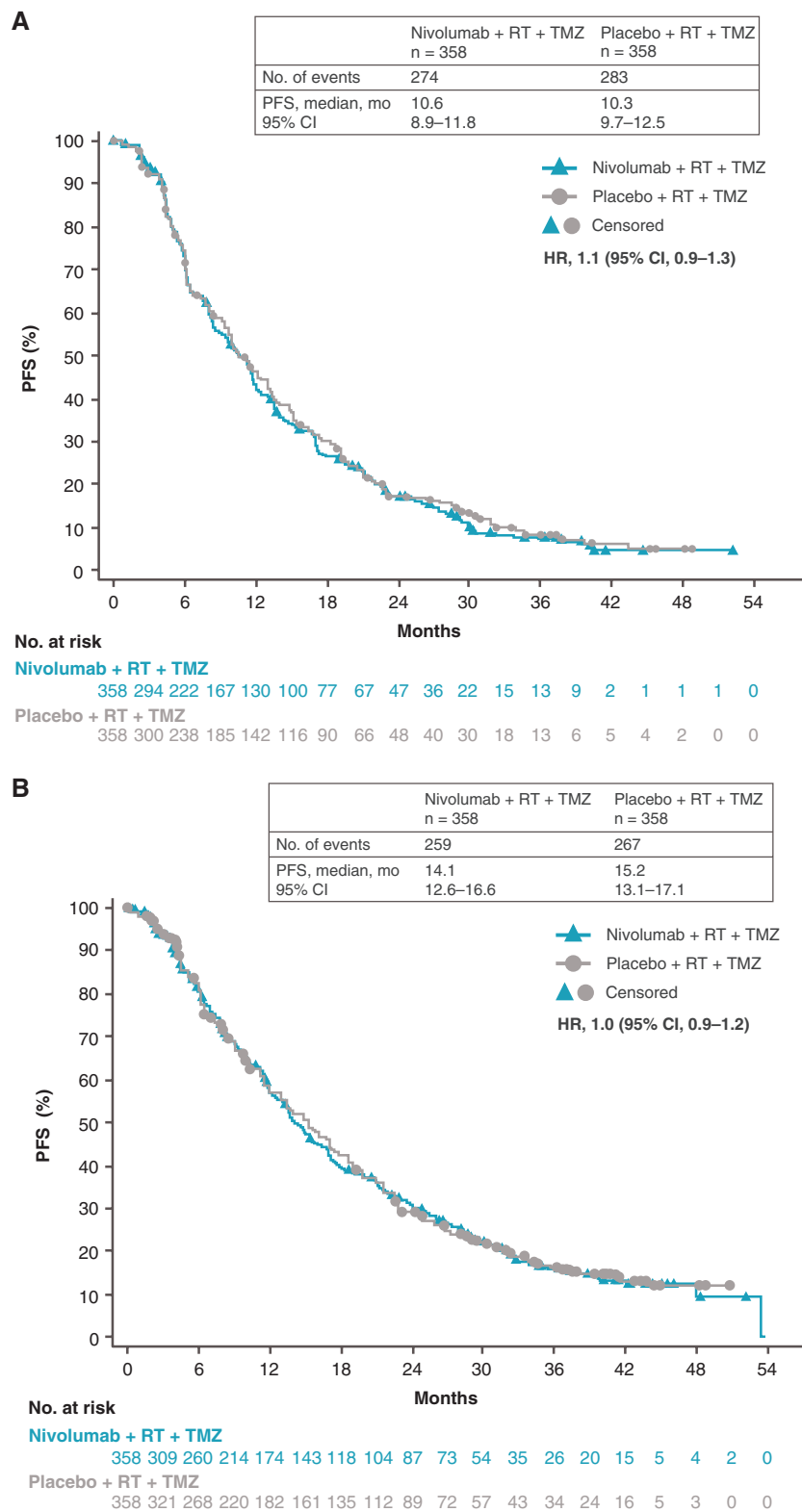


Fig. 1 Progression-free survival in all patients. Number of events, median PFS, and the Kaplan-Meier curve for PFS per blinded independent central review (A) and investigator (B) assessment. Symbols indicate censored observations. Abbreviations: HR, hazard ratio; PFS, progression-free survival; RT, radiotherapy; TMZ, temozolomide.

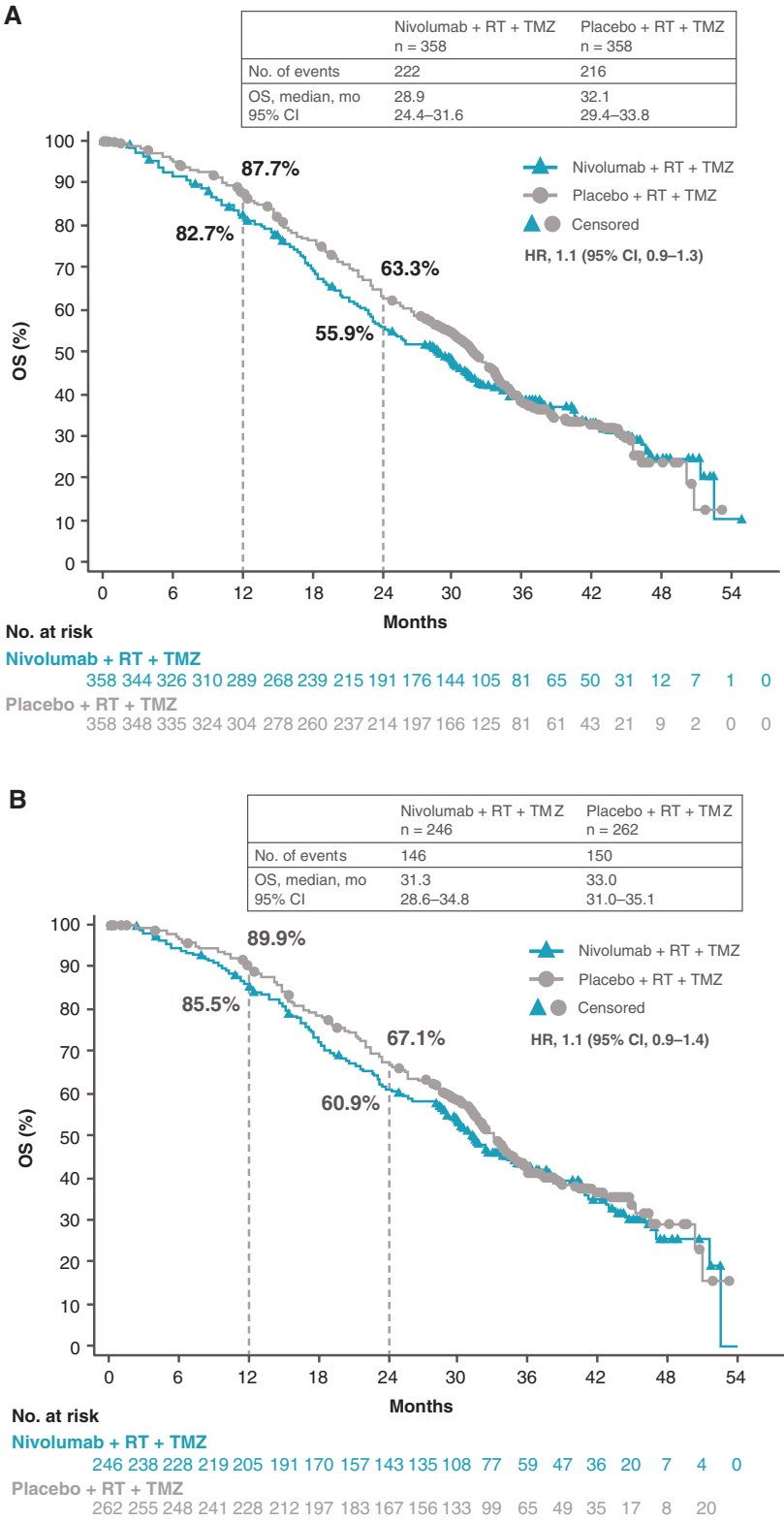


Fig. 2. Overall survival in all patients and patients without baseline corticosteroids. Number of events, median OS, and the Kaplan-Meier curve for OS in all patients (A) and in patients without baseline corticosteroid use (B). Symbols indicate censored observations. Abbreviations: OS, overall survival; RT, radiotherapy; TMZ, temozolomide.

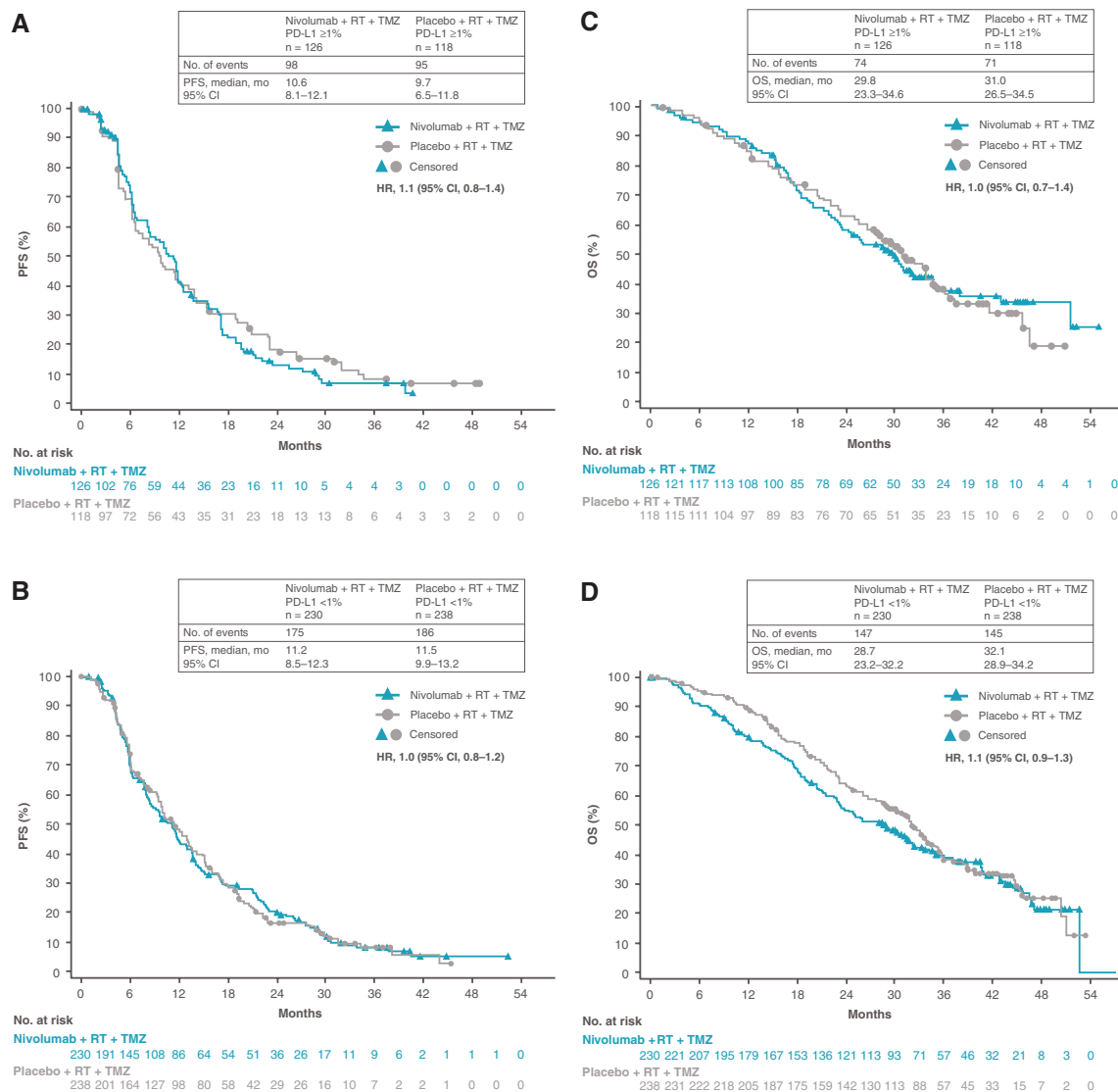


Fig. 3 Progression-free survival and overall survival by PD-L1 expression. Number of events, median PFS, and Kaplan-Meier curves for PFS in all patients with baseline PD-L1 expression $\geq 1\%$ (A) and $< 1\%$ (B). Number of events, median OS, and Kaplan-Meier curves for OS in all patients with baseline PD-L1 expression $\geq 1\%$ (C) and $< 1\%$ (D). Symbols indicate censored observations. Abbreviations: BICR, blinded independent central review; OS, overall survival; PD-L1, programmed death-1 ligand 1; PFS, progression-free survival; RT, radiotherapy; TMZ, temozolomide.

Discussion

CheckMate 548 was a randomized PBO-controlled phase III study investigating the efficacy and safety of NIVO added to RT + TMZ in patients with newly diagnosed glioblastoma with a methylated or indeterminate *MGMT* promoter. The study did not meet its primary endpoints of improved PFS by BICR and OS in the overall population and OS in the population without baseline corticosteroid use. There were no differences in PFS or OS according to PD-L1 expression $\geq 1\%$ or $\geq 5\%$ or within prespecified patient subgroups defined by baseline clinical characteristics.

Subgroup analyses for age and recursive partitioning analysis (RPA) class suggest potential trends for individuals aged > 75 years and in RPA class III for PFS, although the small number of patients in these categories precludes definitive conclusions.

Although NIVO has shown efficacy in several other cancer types, no survival benefit over that with standard of care was observed in patients with newly diagnosed glioblastoma with a methylated or indeterminate *MGMT* promoter. Median OS was 28.9 months (95% CI, 24.4–31.6 months) with NIVO + RT + TMZ vs 32.1 months (95% CI, 29.4–33.8 months) with PBO + RT + TMZ in this study. Prior studies of RT + TMZ in patients with newly diagnosed

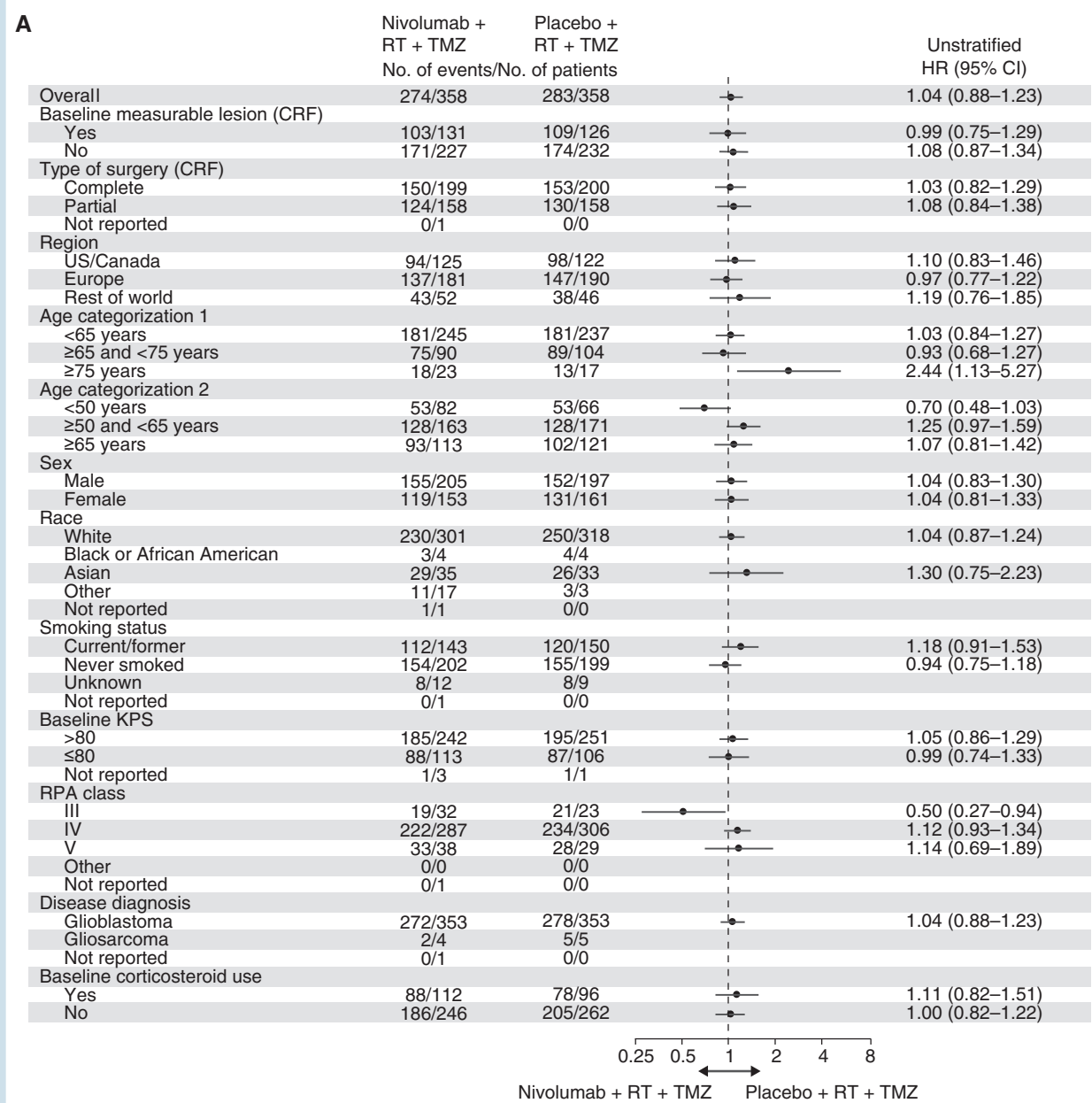


Fig. 4a Progression-free survival and overall survival in prespecified patient subgroups defined by baseline clinical characteristics. Forest plots of unstratified hazard ratios for progression per blinded independent central review (A) or death (B) in the analysis of treatment effect in prespecified patient subgroups according to baseline characteristics. Abbreviations: CRF, case report form; HR, hazard ratio; RPA, recursive partitioning analysis; RT, radiotherapy; TMZ, temozolomide.

glioblastoma with methylated *MGMT* promoter reported median OS of 21.7 months (95% CI, 17.4–30.4 months),⁹ 21.4 months (95% CI, 17.6–29.0 months),⁸ and 26.3 months (95% CI, 23.9–34.7 months).²⁵ However, eligibility criteria were slightly different, with this study excluding patients who had biopsy-only. Additional research is therefore needed to determine if the higher OS in this study compared with older studies could represent ongoing advances in surgery, monitoring, supportive care, or other clinical management aspects. Interestingly, some long-term

survivors in the NIVO arm had baseline PD-L1 of >5% (see [Supplementary Figure S2](#)); this observation may warrant further investigation.

No new safety signals were observed with the addition of NIVO to RT + TMZ. Increased rates of TRAEs, including serious TRAEs and TRAEs leading to discontinuation, were observed, but this finding most likely reflects toxicities due to the addition of NIVO to standard-of-care therapy. NIVO monotherapy has been associated with rare instances of life-threatening and/or fatal serious adverse reactions,

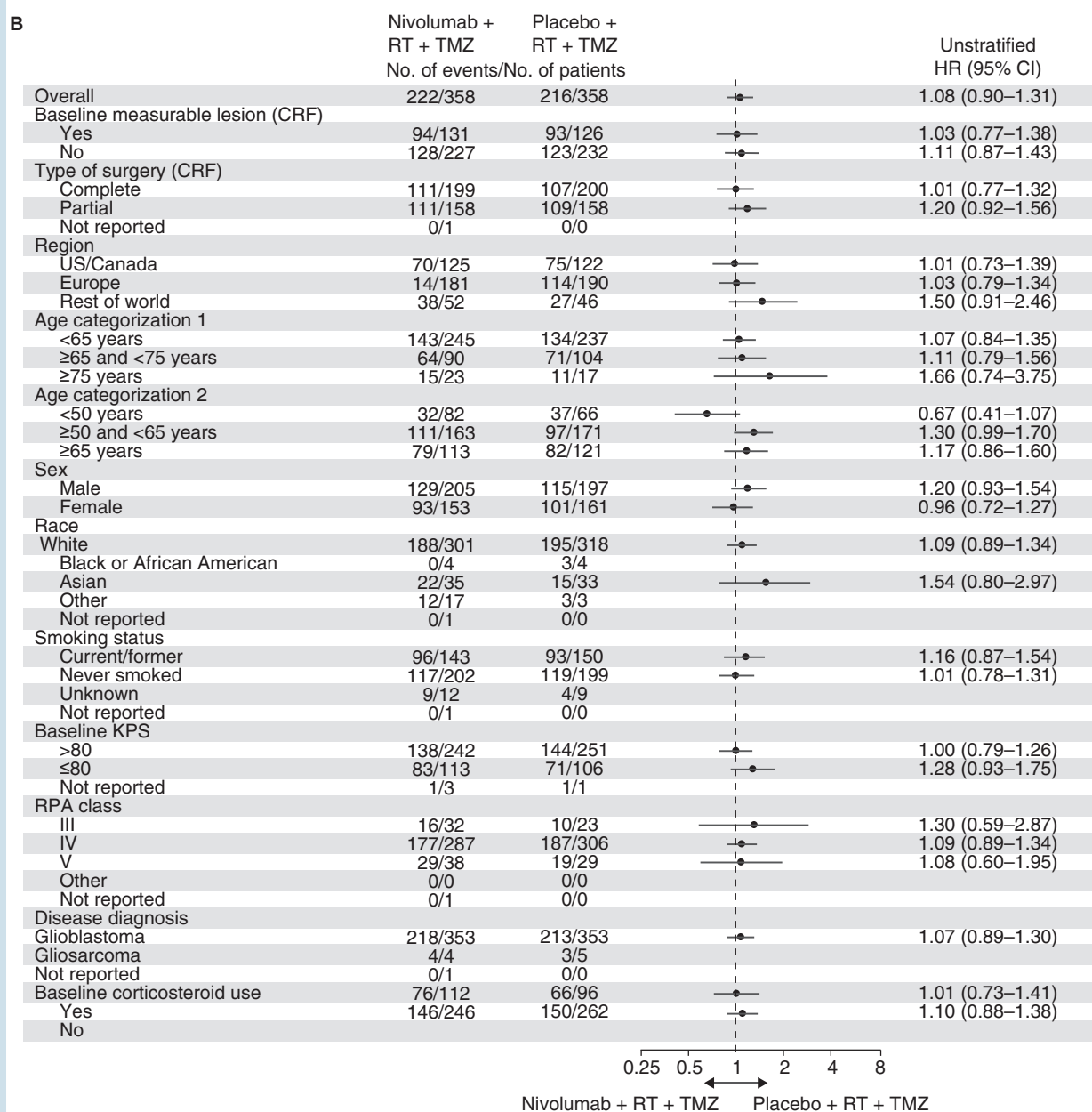


Fig. 4b Continued

including respiratory failure, respiratory distress, myocarditis, and pneumocystis pneumonia. Radiation and TMZ are independently associated with pancytopenia^{26,27} and secondary infections, including respiratory infections.^{13,28–33} Neurological toxicities as a whole seemed more prevalent in the NIVO + RT + TMZ arm, particularly headaches, although other events were relatively rare. Of note, lymphopenia rates were 10.7% and 8.5% in the NIVO + RT + TMZ and PBO + RT + TMZ arms, respectively, which may contribute to the immunosuppressive glioblastoma tumor microenvironment and impact outcomes of immunotherapy.^{34,35}

Pseudoprogression is a condition in which changes induced by immunotherapy, chemoradiation, or both

produce a transient increase in apparent tumor burden followed by tumor regression.^{36,37} This phenomenon is also known to happen after RT + TMZ therapy, occurring in 10% to 30% of patients with newly diagnosed glioblastoma, and introduces challenges with interpreting imaging changes.^{23,38–40} In an exploratory analysis, we evaluated radiographic findings with iRANO criteria²³ retrospectively, as these criteria were not available at the time of study design. Among the 237 patients with PD in the NIVO + RT + TMZ arm, 65 were considered at risk. Of those, 20 were confirmed as having pseudoprogression. Use of the iRANO guidelines²³ may allow for improvements in immunotherapy trial design and patient

Table 2. Treatment-Related Adverse Events

Event	Nivolumab + RT + TMZ, n = 355		Placebo + RT + TMZ, n = 354	
	No. (%)		No. (%)	
	Any Grade	Grade 3/4	Any Grade	Grade 3/4
Any TRAE	328 (92.4) ^a	186 (52.4)	296 (83.6)	119 (33.6)
TRAEs in ≥10% of patients in either arm				
Nausea	121 (34.1)	7 (2.0)	110 (31.1)	2 (0.6)
Fatigue	112 (31.5)	13 (3.7)	116 (32.8)	7 (2.0)
Constipation	72 (20.3)	3 (0.8)	62 (17.5)	0
Alopecia	67 (18.9)	0	52 (14.7)	1 (0.3)
Platelet count decreased	66 (18.6)	23 (6.5)	61 (17.2)	17 (4.8)
Lymphocyte count decreased	60 (16.9)	40 (11.3)	54 (15.3)	35 (9.9)
Thrombocytopenia	58 (16.3)	25 (7.0)	55 (15.5)	15 (4.2)
Vomiting	58 (16.3)	4 (1.1)	46 (13.0)	0
Decreased appetite	55 (15.5)	2 (0.6)	58 (16.4)	2 (0.6)
Pruritus	52 (14.6)	2 (0.6)	47 (13.3)	1 (0.3)
ALT increased	43 (12.1)	15 (4.2)	22 (6.2)	2 (0.6)
Rash	42 (11.8)	2 (0.6)	33 (9.3)	4 (1.1)
Lymphopenia	38 (10.7)	19 (5.4)	30 (8.5)	19 (5.4)
WBC count decreased	31 (8.7)	1 (0.3)	36 (10.2)	13 (3.7)
Neurological TRAEs	82 (23.1)	18 (5.1)	59 (16.7)	2 (0.6)
Neurological TRAEs in ≥2% of patients in either arm				
Headache	33 (9.3)	2 (0.6)	21 (5.9)	0
Dysgeusia	20 (5.6)	0	15 (4.2)	0
Dizziness	10 (2.8)	0	11 (3.1)	0
Cognitive disorder	8 (2.3)	0 (0)	2 (0.6)	0
Hemiparesis	7 (2.0)	3 (0.8)	3 (0.8)	0
Memory impairment	7 (2.0)	0 (0)	6 (1.7)	0
Serious TRAEs	105 (29.6) ^a	81 (22.8)	36 (10.2)	26 (7.3)
Serious TRAEs in ≥2% of patients in either arm				
Pancytopenia	8 (2.3)	8 (2.3)	2 (0.6)	2 (0.6)
Thrombocytopenia	7 (2.0)	7 (2.0)	6 (1.7)	6 (1.7)
Tumor flare	9 (2.5)	5 (1.4)	5 (1.4)	3 (0.8)
TRAEs leading to discontinuation	81 (22.8) ^a	60 (16.9)	32 (9.0)	20 (5.6)

Abbreviations: ALT, alanine aminotransferase; RT, radiotherapy; TMZ, temozolomide; TRAE, treatment-related adverse events; WBC, white blood cells.

^aOne grade 5 event (respiratory distress) occurred with NIVO + RT + TMZ.

management in the future. However, given the overlap in the PFS curves across both arms and similar duration of TMZ treatment, it is unlikely that an excess in pseudoprogression and potential early discontinuation of treatment would account for the lack of efficacy in the NIVO arm compared with the PBO arm.

Recent clinical trials have suggested that immune checkpoint inhibitors may affect the tumor microenvironment of glioblastomas, including enhanced expression of cytokine and chemokine transcripts, higher immune-cell infiltration, and augmented T-cell receptor clonal diversity among tumor-infiltrating T lymphocytes.^{41–43} Results of a small study suggest that the

addition of neoadjuvant pembrolizumab prior to salvage surgery followed by continued adjuvant therapy may extend survival.⁴³ In contrast, and in line with the results of our trial, recent results from the phase III CheckMate 498 study (NCT02617589) in patients with newly diagnosed glioblastoma with an unmethylated *MGMT* promoter demonstrated that immunotherapy with NIVO did not improve survival.^{20,21}

As the addition of a single immunotherapy agent was not detrimental for outcomes in this study, additional considerations for treatment failure that could be further investigated include differences in the glioblastoma tumor microenvironment, T-cell exhaustion, and the potential for

rapid tumor growth to leave insufficient time for immune system function. Additionally, novel combination checkpoint strategies, or combinations with other agents, such as a myeloid modulator, may be next considerations.

Limitations of the study include the lack of immune-predictive biomarkers and comprehensive genomic characterization due to the limited availability of tumor samples; therefore, novel biomarkers associated with this tumor type remain to be further explored.

In conclusion, CheckMate 548 was the largest phase III study conducted to date in patients with glioblastoma with a methylated or indeterminate *MGMT* promoter. Although results indicated that immunotherapy with NIVO did not add clinical benefit to standard-of-care RT + TMZ, it could be considered within new combination treatment strategies. Glioblastoma remains a difficult disease to treat, with few effective treatment options, and the role of immunotherapy in this treatment landscape remains an area for further investigation.

Supplementary Material

Supplementary material is available at *Neuro-Oncology* online.

Keywords

glioblastoma | *MGMT* promoter | nivolumab | PD-L1 | temozolomide

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Conflict of interest statement. M. Lim received research support from Accuray, Arbor Pharmaceuticals, Biohaven, Bristol Myers Squibb, Kirin-Kyowa, Tocagen, and UroGen; received personal and consultant fees from Biohaven, Black

Diamond Therapeutics, Century Therapeutics, Hemispherian, InCando, InCephalo Therapeutics, Insightec, Novocure, Noxxon, Pyramid Bio, Sanianoia, Stryker, VBI; and has shares in Egret Therapeutics. M.W. received other funding from Bristol Myers Squibb; grants from Merck (EMD), MSD, Novocure, and Quercis; and personal fees from AbbVie, Bristol Myers Squibb, Merck (EMD), MSD, Orbus, and Y-mAbs. A.I. received research grants from Carthera, Transgene, Sanofi, Air Liquide, Servier Pharmaceuticals, Nutritheragene; travel funding from Novocure, Carthera, and Leo Pharma; served on advisory boards for Leo Pharma and Novocure. J.S. received other funding from AbbVie; received personal fees from Medac, Med-Update, Novocure, Roche, and Seagen; and served on advisory boards for Novocure and Seagen. G.F. received other funding from Genenta. R.R.R. has nothing to disclose. G.A. has nothing to disclose. J.B. has served as a consultant for Bristol Myers Squibb. J.W.T. received grants from AbbVie, Agios, and Navio and personal fees from Medlink. J.H. has nothing to disclose. K.P. has nothing to disclose. F.D.V. received funding from Agios, Bristol Myers Squibb, Novartis, and Roche. A.W. has nothing to disclose. A.S. received grant funding from Kura Oncology and Exelixis; other funding from Bristol Myers Squibb, Novocure, Merck, Bayer, AbbVie, Oncocutics, and Caris Life Sciences. S.S. received grant funding from Bristol Myers Squibb, Brooklyn Immunotherapeutics, and Merck; other funding from Boehringer Ingelheim, Eli Lilly, Merck; and personal fees from AbbVie. I.K.M. received research funding from Amgen, General Electric, Lilly, Kazia Therapeutics, and Servier Pharmaceuticals; other funding from Agios, Black Diamond Therapeutics, Debiopharm Group, Puma Biotechnology, Servier Pharmaceuticals, Voyager Therapeutics, DC Europa Ltd, Kazia Therapeutics, Novartis, Cardinal Health, Roche, Vigeo Therapeutics, Samus Therapeutics, Prelude Therapeutics, and AstraZeneca. M.K. has nothing to disclose. M.R. is an employee and received stocks from Bristol Myers Squibb. R.S. is an employee of Bristol Myers Squibb. D.W. is an employee of Bristol Myers Squibb. D.L. was an employee and received stocks from Bristol Myers Squibb. M. Lee is an employee of Bristol Myers Squibb. D.A.R. received other funding from Acerta Pharma, Agenus, Celldex, EMD Serono, Incyte, Inovio, Midatech, Omniox, and Tragara and personal fees from AbbVie, Advantagene, Agenus, Amgen, Bayer, Bristol Myers Squibb, Celldex, DelMar, EMD Serono, Genentech/Roche, Inovio, Merck, Merck KGaA, Monteris, Novocure, Oncorus, Oxigene, Regeneron, Stemline, and Taiho Oncology, Inc. A.O. has served as a consultant on ad hoc advisory boards for KIYATEC, Merck, Pyramid, and Ono Pharmaceutical Company Ltd, and has received grant funding from Agios, Arcus Biosciences, and Denovo.

Authorship statement. Study conception and design: M. Lim, M.W., G.F., R.R.R., A.S., S.S., D.A.R., and A.O. Data acquisition: M. Lim, M.W., A.I., J.S., G.F., G.A., J.B., J.T., J.H., K.P., F.D.V., A.W., A.S., S.S., I.M., M.K., D.A.R., and A.O. Data analyses: M.R., R.S., D.W., and M. Lee. Data interpretation: all authors. Contribution to and approval of manuscript: all authors.

Data Availability

The Bristol Myers Squibb policy on data sharing may be found at <https://www.bms.com/researchers-and-partners/independent-research/data-sharing-request-process.html>.

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Bristol-Myers Squibb Announces Phase 3 CheckMate -498 Study Did Not Meet Primary Endpoint of Overall Survival with Opdivo (nivolumab) Plus Radiation in Patients with Newly Diagnosed MGMT-Unmethylated

Glioblastoma Multiforme

05/09/2019

CATEGORY:

- [Corporate/Financial News](#)

PRINCETON, N.J.--([BUSINESS WIRE](#))--[Bristol-Myers Squibb Company](#) (NYSE: BMY) today announced the Phase 3 CheckMate -498 trial evaluating *Opdivo* (nivolumab) plus radiation versus temozolomide plus radiation in patients with newly diagnosed O6-methylguanine-DNA methyltransferase (MGMT)-unmethylated glioblastoma multiforme (GBM) did not meet its primary endpoint of overall survival (OS) at final analysis. The safety profile of *Opdivo* was consistent with previously reported studies in solid tumors. The Company will complete a full evaluation of the data from CheckMate -498 and work with investigators on the future presentation and publication of the results.

“While we are disappointed the CheckMate -498 trial did not meet its primary endpoint, GBM is a notoriously aggressive cancer,” said Fouad Namouni, M.D., head, oncology development, Bristol-Myers Squibb. “We are grateful to all those who participated in this trial and remain committed to researching the potential of immunotherapy to address the important unmet medical need of patients who suffer from this devastating disease.”

Opdivo is also being studied in patients with newly diagnosed MGMT-methylated GBM in the Phase 3 CheckMate -548 (NCT02667587) study, in which *Opdivo* is added to the current standard of care, radiation plus temozolomide.

About CheckMate -498

CheckMate -498 (NCT02617589) is a Phase 3 randomized, multi-center study evaluating *Opdivo* and radiation versus temozolomide and radiation in patients with newly diagnosed MGMT-unmethylated GBM. After surgery, patients in the experimental arm received *Opdivo* every two weeks concurrent

<https://news.bms.com/news/corporate-financial/2019/Bristol-Myers-Squibb-Announces-Phase-3-CheckMate--498-Study-Did-Not-Meet-Primary-Endpoint-of-Overall-Survival-with-Opdivo-nivolumab-Plus-Radiation-in-Patients-with-Newly-Diagnosed-MGMT-Unmethylated-Glioblastoma-Multiforme/default.aspx> <Accessed June 12, 2023>.

with radiation, followed by maintenance with *Opdivo* every four weeks until disease progression or unacceptable toxicity. The primary endpoint of the trial was OS. Secondary endpoints included progression-free survival, and OS rate at two years.

About Glioblastoma Multiforme

Glioblastoma multiforme (GBM) is the most common and most aggressive type of primary malignant tumor of the central nervous system. Globally, an estimated 300,000 patients develop, and 241,000 patients die from, brain or central nervous system cancer each year. Standard treatment for patients with newly diagnosed GBM can include surgery followed by radiation and chemotherapy, but treatment options are limited. The last investigational medicine to improve survival for patients with newly diagnosed GBM was approved by the U.S. Food and Drug Administration in 2005. The five year survival rate of patients with GBM is less than five percent.

MGMT methylation status is the most commonly used biomarker in GBM and, studies suggest, may be predictive of the likelihood of patients with GBM to respond to alkylating chemotherapy such as temozolomide. Patients with MGMT-unmethylated GBM are generally known to have a worse prognosis than patients with MGMT-methylated GBM.

Bristol-Myers Squibb: Advancing Oncology Research

At Bristol-Myers Squibb, patients are at the center of everything we do. The focus of our research is to increase quality, long-term survival for patients and make cure a possibility. Through a unique multidisciplinary approach powered by translational science, we harness our deep scientific experience in oncology and Immuno-Oncology (I-O) research to identify novel treatments tailored to individual patient needs. Our researchers are developing a diverse, purposefully built pipeline designed to target different immune system pathways and address the complex and specific interactions between the tumor, its microenvironment and the immune system. We source innovation internally, and in collaboration with academia, government, advocacy groups and biotechnology companies, to help make the promise of transformational medicines, like I-O, a reality for patients.

About *Opdivo*

<https://news.bms.com/news/corporate-financial/2019/Bristol-Myers-Squibb-Announces-Phase-3-CheckMate--498-Study-Did-Not-Meet-Primary-Endpoint-of-Overall-Survival-with-Opdivo-nivolumab-Plus-Radiation-in-Patients-with-Newly-Diagnosed-MGMT-Unmethylated-Glioblastoma-Multiforme/default.aspx> <Accessed June 12, 2023>.

Opdivo is a programmed death-1 (PD-1) immune checkpoint inhibitor that is designed to uniquely harness the body's own immune system to help restore anti-tumor immune response. By harnessing the body's own immune system to fight cancer, *Opdivo* has become an important treatment option across multiple cancers.

Opdivo's leading global development program is based on Bristol-Myers Squibb's scientific expertise in the field of Immuno-Oncology, and includes a broad range of clinical trials across all phases, including Phase 3, in a variety of tumor types. To date, the *Opdivo* clinical development program has enrolled more than 25,000 patients. The *Opdivo* trials have contributed to gaining a deeper understanding of the potential role of biomarkers in patient care, particularly regarding how patients may benefit from *Opdivo* across the continuum of PD-L1 expression.

In July 2014, *Opdivo* was the first PD-1 immune checkpoint inhibitor to receive regulatory approval anywhere in the world. *Opdivo* is currently approved in more than 65 countries, including the United States, the European Union, Japan and China. In October 2015, the Company's *Opdivo* and *Yervoy* combination regimen was the first Immuno-Oncology combination to receive regulatory approval for the treatment of metastatic melanoma and is currently approved in more than 50 countries, including the United States and the European Union.

U.S. FDA-APPROVED INDICATIONS FOR OPDIVO®

OPDIVO® (nivolumab) as a single agent is indicated for the treatment of patients with unresectable or metastatic melanoma.

OPDIVO® (nivolumab), in combination with YERVOY® (ipilimumab), is indicated for the treatment of patients with unresectable or metastatic melanoma.

OPDIVO® (nivolumab) is indicated for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) with progression on or after platinum-based chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving OPDIVO.

<https://news.bms.com/news/corporate-financial/2019/Bristol-Myers-Squibb-Announces-Phase-3-CheckMate--498-Study-Did-Not-Meet-Primary-Endpoint-of-Overall-Survival-with-Opdivo-nivolumab-Plus-Radiation-in-Patients-with-Newly-Diagnosed-MGMT-Unmethylated-Glioblastoma-Multiforme/default.aspx> <Accessed June 12, 2023>.

OPDIVO® (nivolumab) is indicated for the treatment of patients with metastatic small cell lung cancer (SCLC) with progression after platinum-based chemotherapy and at least one other line of therapy. This indication is approved under accelerated approval based on overall response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

OPDIVO® (nivolumab) is indicated for the treatment of patients with advanced renal cell carcinoma (RCC) who have received prior anti-angiogenic therapy.

OPDIVO® (nivolumab), in combination with YERVOY® (ipilimumab), is indicated for the treatment of patients with intermediate or poor risk, previously untreated advanced renal cell carcinoma (RCC).

OPDIVO® (nivolumab) is indicated for the treatment of adult patients with classical Hodgkin lymphoma (cHL) that has relapsed or progressed after autologous hematopoietic stem cell transplantation (HSCT) and brentuximab vedotin or after 3 or more lines of systemic therapy that includes autologous HSCT. This indication is approved under accelerated approval based on overall response rate. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

OPDIVO® (nivolumab) is indicated for the treatment of patients with recurrent or metastatic squamous cell carcinoma of the head and neck (SCCHN) with disease progression on or after platinum-based therapy.

OPDIVO® (nivolumab) is indicated for the treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy. This indication is approved under accelerated approval based on tumor response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

OPDIVO® (nivolumab), as a single agent, is indicated for the treatment of adult and pediatric (12 years and older) patients with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer

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(CRC) that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan. This indication is approved under accelerated approval based on overall response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

OPDIVO® (nivolumab), in combination with YERVOY® (ipilimumab), is indicated for the treatment of adults and pediatric patients 12 years and older with microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer (CRC) that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan. This indication is approved under accelerated approval based on overall response rate and duration of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in confirmatory trials.

OPDIVO® (nivolumab) is indicated for the treatment of patients with hepatocellular carcinoma (HCC) who have been previously treated with sorafenib. This indication is approved under accelerated approval based on tumor response rate and durability of response. Continued approval for this indication may be contingent upon verification and description of clinical benefit in the confirmatory trials.

OPDIVO® (nivolumab) is indicated for the adjuvant treatment of patients with melanoma with involvement of lymph nodes or metastatic disease who have undergone complete resection.

IMPORTANT SAFETY INFORMATION

WARNING: IMMUNE-MEDIATED ADVERSE REACTIONS

YERVOY can result in severe and fatal immune-mediated adverse reactions. These immune-mediated reactions may involve any organ system; however, the most common severe immune-mediated adverse reactions are enterocolitis, hepatitis, dermatitis (including toxic epidermal necrolysis), neuropathy, and endocrinopathy. The majority of these immune-mediated reactions initially manifested during treatment; however, a minority occurred weeks to months after discontinuation of YERVOY.

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Assess patients for signs and symptoms of enterocolitis, dermatitis, neuropathy, and endocrinopathy, and evaluate clinical chemistries including liver function tests (LFTs), adrenocorticotrophic hormone (ACTH) level, and thyroid function tests, at baseline and before each dose.

Permanently discontinue YERVOY and initiate systemic high-dose corticosteroid therapy for severe immune-mediated reactions.

Immune-Mediated Pneumonitis

OPDIVO can cause immune-mediated pneumonitis. Fatal cases have been reported. Monitor patients for signs with radiographic imaging and for symptoms of pneumonitis. Administer corticosteroids for Grade 2 or more severe pneumonitis. Permanently discontinue for Grade 3 or 4 and withhold until resolution for Grade 2. In patients receiving OPDIVO monotherapy, fatal cases of immune-mediated pneumonitis have occurred. Immune-mediated pneumonitis occurred in 3.1% (61/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, immune-mediated pneumonitis occurred in 6% (25/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated pneumonitis occurred in 4.4% (24/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated pneumonitis occurred in 1.7% (2/119) of patients.

In Checkmate 205 and 039, pneumonitis, including interstitial lung disease, occurred in 6.0% (16/266) of patients receiving OPDIVO. Immune-mediated pneumonitis occurred in 4.9% (13/266) of patients receiving OPDIVO: Grade 3 (n=1) and Grade 2 (n=12).

Immune-Mediated Colitis

OPDIVO can cause immune-mediated colitis. Monitor patients for signs and symptoms of colitis. Administer corticosteroids for Grade 2 (of more than 5 days duration), 3, or 4 colitis. Withhold OPDIVO monotherapy for Grade 2 or 3 and permanently discontinue for Grade 4 or recurrent colitis upon re-initiation of OPDIVO. When administered with YERVOY, withhold OPDIVO and YERVOY for Grade 2 and permanently discontinue for Grade 3 or 4 or

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recurrent colitis. In patients receiving OPDIVO monotherapy, immune-mediated colitis occurred in 2.9% (58/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, immune-mediated colitis occurred in 26% (107/407) of patients including three fatal cases. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated colitis occurred in 10% (52/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated colitis occurred in 7% (8/119) of patients.

In a separate Phase 3 study of YERVOY 3 mg/kg, severe, life-threatening, or fatal (diarrhea of ≥ 7 stools above baseline, fever, ileus, peritoneal signs; Grade 3-5) immune-mediated enterocolitis occurred in 34 (7%) patients. Across all YERVOY-treated patients in that study (n=511), 5 (1%) developed intestinal perforation, 4 (0.8%) died as a result of complications, and 26 (5%) were hospitalized for severe enterocolitis.

Immune-Mediated Hepatitis

OPDIVO can cause immune-mediated hepatitis. Monitor patients for abnormal liver tests prior to and periodically during treatment. Administer corticosteroids for Grade 2 or greater transaminase elevations. For patients without HCC, withhold OPDIVO for Grade 2 and permanently discontinue OPDIVO for Grade 3 or 4. For patients with HCC, withhold OPDIVO and administer corticosteroids if AST/ALT is within normal limits at baseline and increases to >3 and up to 5 times the upper limit of normal (ULN), if AST/ALT is >1 and up to 3 times ULN at baseline and increases to >5 and up to 10 times the ULN, and if AST/ALT is >3 and up to 5 times ULN at baseline and increases to >8 and up to 10 times the ULN. Permanently discontinue OPDIVO and administer corticosteroids if AST or ALT increases to >10 times the ULN or total bilirubin increases >3 times the ULN. In patients receiving OPDIVO monotherapy, immune-mediated hepatitis occurred in 1.8% (35/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, immune-mediated hepatitis occurred in 13% (51/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated hepatitis occurred in 7% (38/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated hepatitis occurred in 8% (10/119) of patients.

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In Checkmate 040, immune-mediated hepatitis requiring systemic corticosteroids occurred in 5% (8/154) of patients receiving OPDIVO.

In a separate Phase 3 study of YERVOY 3 mg/kg, severe, life-threatening, or fatal hepatotoxicity (AST or ALT elevations >5x the ULN or total bilirubin elevations >3x the ULN; Grade 3-5) occurred in 8 (2%) patients, with fatal hepatic failure in 0.2% and hospitalization in 0.4%.

Immune-Mediated Neuropathies

In a separate Phase 3 study of YERVOY 3 mg/kg, 1 case of fatal Guillain-Barré syndrome and 1 case of severe (Grade 3) peripheral motor neuropathy were reported.

Immune-Mediated Endocrinopathies

OPDIVO can cause immune-mediated hypophysitis, immune-mediated adrenal insufficiency, autoimmune thyroid disorders, and Type 1 diabetes mellitus. Monitor patients for signs and symptoms of hypophysitis, signs and symptoms of adrenal insufficiency, thyroid function prior to and periodically during treatment, and hyperglycemia. Administer hormone replacement as clinically indicated and corticosteroids for Grade 2 or greater hypophysitis. Withhold for Grade 2 or 3 and permanently discontinue for Grade 4 hypophysitis. Administer corticosteroids for Grade 3 or 4 adrenal insufficiency. Withhold for Grade 2 and permanently discontinue for Grade 3 or 4 adrenal insufficiency. Administer hormone-replacement therapy for hypothyroidism. Initiate medical management for control of hyperthyroidism. Withhold OPDIVO for Grade 3 and permanently discontinue for Grade 4 hyperglycemia.

In patients receiving OPDIVO monotherapy, hypophysitis occurred in 0.6% (12/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, hypophysitis occurred in 9% (36/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, hypophysitis occurred in 4.6% (25/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated hypophysitis occurred in 3.4% (4/119) of patients. In patients receiving OPDIVO monotherapy, adrenal insufficiency occurred in 1% (20/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, adrenal insufficiency occurred in 5% (21/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with

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YERVOY 1 mg/kg, adrenal insufficiency occurred in 7% (41/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, adrenal insufficiency occurred in 5.9% (7/119) of patients. In patients receiving OPDIVO monotherapy, hypothyroidism or thyroiditis resulting in hypothyroidism occurred in 9% (171/1994) of patients. Hyperthyroidism occurred in 2.7% (54/1994) of patients receiving OPDIVO monotherapy. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, hypothyroidism or thyroiditis resulting in hypothyroidism occurred in 22% (89/407) of patients. Hyperthyroidism occurred in 8% (34/407) of patients receiving this dose of OPDIVO with YERVOY. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, hypothyroidism or thyroiditis resulting in hypothyroidism occurred in 22% (119/547) of patients. Hyperthyroidism occurred in 12% (66/547) of patients receiving this dose of OPDIVO with YERVOY. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, hypothyroidism or thyroiditis resulting in hypothyroidism occurred in 15% (18/119) of patients. Hyperthyroidism occurred in 12% (14/119) of patients. In patients receiving OPDIVO monotherapy, diabetes occurred in 0.9% (17/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, diabetes occurred in 1.5% (6/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, diabetes occurred in 2.7% (15/547) of patients.

In a separate Phase 3 study of YERVOY 3 mg/kg, severe to life-threatening immune-mediated endocrinopathies (requiring hospitalization, urgent medical intervention, or interfering with activities of daily living; Grade 3-4) occurred in 9 (1.8%) patients. All 9 patients had hypopituitarism, and some had additional concomitant endocrinopathies such as adrenal insufficiency, hypogonadism, and hypothyroidism. Six of the 9 patients were hospitalized for severe endocrinopathies.

Immune-Mediated Nephritis and Renal Dysfunction

OPDIVO can cause immune-mediated nephritis. Monitor patients for elevated serum creatinine prior to and periodically during treatment. Administer corticosteroids for Grades 2-4 increased serum creatinine. Withhold OPDIVO for Grade 2 or 3 and permanently discontinue for Grade 4 increased serum creatinine. In patients receiving OPDIVO monotherapy, immune-mediated nephritis and renal dysfunction occurred in 1.2% (23/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, immune-

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mediated nephritis and renal dysfunction occurred in 2.2% (9/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated nephritis and renal dysfunction occurred in 4.6% (25/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated nephritis and renal dysfunction occurred in 1.7% (2/119) of patients.

Immune-Mediated Skin Adverse Reactions and Dermatitis

OPDIVO can cause immune-mediated rash, including Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), some cases with fatal outcome. Administer corticosteroids for Grade 3 or 4 rash. Withhold for Grade 3 and permanently discontinue for Grade 4 rash. For symptoms or signs of SJS or TEN, withhold OPDIVO and refer the patient for specialized care for assessment and treatment; if confirmed, permanently discontinue. In patients receiving OPDIVO monotherapy, immune-mediated rash occurred in 9% (171/1994) of patients. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg, immune-mediated rash occurred in 22.6% (92/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated rash occurred in 16.6% (91/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, immune-mediated rash occurred in 14% (17/119) of patients.

In a separate Phase 3 study of YERVOY 3 mg/kg, severe, life-threatening, or fatal immune-mediated dermatitis (eg, Stevens-Johnson syndrome, toxic epidermal necrolysis, or rash complicated by full thickness dermal ulceration, or necrotic, bullous, or hemorrhagic manifestations; Grade 3-5) occurred in 13 (2.5%) patients. 1 (0.2%) patient died as a result of toxic epidermal necrolysis. 1 additional patient required hospitalization for severe dermatitis.

Immune-Mediated Encephalitis

OPDIVO can cause immune-mediated encephalitis. Evaluation of patients with neurologic symptoms may include, but not be limited to, consultation with a neurologist, brain MRI, and lumbar puncture. Withhold OPDIVO in patients with new-onset moderate to severe neurologic signs or symptoms and evaluate to rule out other causes. If other etiologies are ruled out, administer corticosteroids and permanently discontinue OPDIVO for immune-mediated encephalitis. In patients receiving OPDIVO monotherapy, encephalitis

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occurred in 0.2% (3/1994) of patients. Fatal limbic encephalitis occurred in one patient after 7.2 months of exposure despite discontinuation of OPDIVO and administration of corticosteroids. Encephalitis occurred in one patient receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg (0.2%) after 1.7 months of exposure. Encephalitis occurred in one RCC patient receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg (0.2%) after approximately 4 months of exposure. Encephalitis occurred in one MSI-H/dMMR mCRC patient (0.8%) receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg after 15 days of exposure.

Other Immune-Mediated Adverse Reactions

Based on the severity of the adverse reaction, permanently discontinue or withhold OPDIVO, administer high-dose corticosteroids, and, if appropriate, initiate hormone-replacement therapy. Across clinical trials of OPDIVO monotherapy or in combination with YERVOY, the following clinically significant immune-mediated adverse reactions, some with fatal outcome, occurred in <1.0% of patients receiving OPDIVO: myocarditis, rhabdomyolysis, myositis, uveitis, iritis, pancreatitis, facial and abducens nerve paresis, demyelination, polymyalgia rheumatica, autoimmune neuropathy, Guillain-Barré syndrome, hypopituitarism, systemic inflammatory response syndrome, gastritis, duodenitis, sarcoidosis, histiocytic necrotizing lymphadenitis (Kikuchi lymphadenitis), motor dysfunction, vasculitis, aplastic anemia, pericarditis, and myasthenic syndrome.

If uveitis occurs in combination with other immune-mediated adverse reactions, consider a Vogt-Koyanagi-Harada-like syndrome, which has been observed in patients receiving OPDIVO and may require treatment with systemic steroids to reduce the risk of permanent vision loss.

Infusion Reactions

OPDIVO can cause severe infusion reactions, which have been reported in <1.0% of patients in clinical trials. Discontinue OPDIVO in patients with Grade 3 or 4 infusion reactions. Interrupt or slow the rate of infusion in patients with Grade 1 or 2. In patients receiving OPDIVO monotherapy as a 60-minute infusion, infusion-related reactions occurred in 6.4% (127/1994) of patients. In a separate study in which patients received OPDIVO monotherapy as a 60-minute infusion or a 30-minute infusion, infusion-related reactions occurred in 2.2% (8/368) and 2.7% (10/369) of patients, respectively.

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Additionally, 0.5% (2/368) and 1.4% (5/369) of patients, respectively, experienced adverse reactions within 48 hours of infusion that led to dose delay, permanent discontinuation or withholding of OPDIVO. In patients receiving OPDIVO 1 mg/kg with YERVOY 3 mg/kg every 3 weeks, infusion-related reactions occurred in 2.5% (10/407) of patients. In RCC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, infusion-related reactions occurred in 5.1% (28/547) of patients. In MSI-H/dMMR mCRC patients receiving OPDIVO 3 mg/kg with YERVOY 1 mg/kg, infusion-related reactions occurred in 4.2% (5/119) of patients.

Complications of Allogeneic Hematopoietic Stem Cell Transplantation

Fatal and other serious complications can occur in patients who receive allogeneic hematopoietic stem cell transplantation (HSCT) before or after being treated with a PD-1 receptor blocking antibody. Transplant-related complications include hyperacute graft-versus-host-disease (GVHD), acute GVHD, chronic GVHD, hepatic veno-occlusive disease (VOD) after reduced intensity conditioning, and steroid-requiring febrile syndrome (without an identified infectious cause). These complications may occur despite intervening therapy between PD-1 blockade and allogeneic HSCT.

Follow patients closely for evidence of transplant-related complications and intervene promptly. Consider the benefit versus risks of treatment with a PD-1 receptor blocking antibody prior to or after an allogeneic HSCT.

Embryo-Fetal Toxicity

Based on their mechanisms of action, OPDIVO and YERVOY can cause fetal harm when administered to a pregnant woman. Advise pregnant women of the potential risk to a fetus. Advise females of reproductive potential to use effective contraception during treatment with an OPDIVO- or YERVOY-containing regimen and for at least 5 months after the last dose of OPDIVO.

Increased Mortality in Patients with Multiple Myeloma when OPDIVO is Added to a Thalidomide Analogue and Dexamethasone

In clinical trials in patients with multiple myeloma, the addition of OPDIVO to a thalidomide analogue plus dexamethasone resulted in increased mortality.

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Treatment of patients with multiple myeloma with a PD-1 or PD-L1 blocking antibody in combination with a thalidomide analogue plus dexamethasone is not recommended outside of controlled clinical trials.

Lactation

It is not known whether OPDIVO or YERVOY is present in human milk. Because many drugs, including antibodies, are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from an OPDIVO-containing regimen, advise women to discontinue breastfeeding during treatment. Advise women to discontinue breastfeeding during treatment with YERVOY and for 3 months following the final dose.

Serious Adverse Reactions

In Checkmate 037, serious adverse reactions occurred in 41% of patients receiving OPDIVO (n=268). Grade 3 and 4 adverse reactions occurred in 42% of patients receiving OPDIVO. The most frequent Grade 3 and 4 adverse drug reactions reported in 2% to <5% of patients receiving OPDIVO were abdominal pain, hyponatremia, increased aspartate aminotransferase, and increased lipase. In Checkmate 066, serious adverse reactions occurred in 36% of patients receiving OPDIVO (n=206). Grade 3 and 4 adverse reactions occurred in 41% of patients receiving OPDIVO. The most frequent Grade 3 and 4 adverse reactions reported in $\geq 2\%$ of patients receiving OPDIVO were gamma-glutamyltransferase increase (3.9%) and diarrhea (3.4%). In Checkmate 067, serious adverse reactions (74% and 44%), adverse reactions leading to permanent discontinuation (47% and 18%) or to dosing delays (58% and 36%), and Grade 3 or 4 adverse reactions (72% and 51%) all occurred more frequently in the OPDIVO plus YERVOY arm (n=313) relative to the OPDIVO arm (n=313). The most frequent ($\geq 10\%$) serious adverse reactions in the OPDIVO plus YERVOY arm and the OPDIVO arm, respectively, were diarrhea (13% and 2.2%), colitis (10% and 1.9%), and pyrexia (10% and 1.0%). In Checkmate 017 and 057, serious adverse reactions occurred in 46% of patients receiving OPDIVO (n=418). The most frequent serious adverse reactions reported in $\geq 2\%$ of patients receiving OPDIVO were pneumonia, pulmonary embolism, dyspnea, pyrexia, pleural effusion, pneumonitis, and respiratory failure. In Checkmate 032, serious adverse reactions occurred in 45% of patients receiving OPDIVO (n=245). The most frequent serious adverse reactions reported in at least 2% of

patients receiving OPDIVO were pneumonia, dyspnea, pneumonitis, pleural effusions, and dehydration. In Checkmate 025, serious adverse reactions occurred in 47% of patients receiving OPDIVO (n=406). The most frequent serious adverse reactions reported in $\geq 2\%$ of patients were acute kidney injury, pleural effusion, pneumonia, diarrhea, and hypercalcemia. In Checkmate 214, serious adverse reactions occurred in 59% of patients receiving OPDIVO plus YERVOY and in 43% of patients receiving sunitinib. The most frequent serious adverse reactions reported in $\geq 2\%$ of patients were diarrhea, pyrexia, pneumonia, pneumonitis, hypophysitis, acute kidney injury, dyspnea, adrenal insufficiency, and colitis; in patients treated with sunitinib, they were pneumonia, pleural effusion, and dyspnea. In Checkmate 205 and 039, adverse reactions leading to discontinuation occurred in 7% and dose delays due to adverse reactions occurred in 34% of patients (n=266). Serious adverse reactions occurred in 26% of patients. The most frequent serious adverse reactions reported in $\geq 1\%$ of patients were pneumonia, infusion-related reaction, pyrexia, colitis or diarrhea, pleural effusion, pneumonitis, and rash. Eleven patients died from causes other than disease progression: 3 from adverse reactions within 30 days of the last OPDIVO dose, 2 from infection 8 to 9 months after completing OPDIVO, and 6 from complications of allogeneic HSCT. In Checkmate 141, serious adverse reactions occurred in 49% of patients receiving OPDIVO (n=236). The most frequent serious adverse reactions reported in $\geq 2\%$ of patients receiving OPDIVO were pneumonia, dyspnea, respiratory failure, respiratory tract infection, and sepsis. In Checkmate 275, serious adverse reactions occurred in 54% of patients receiving OPDIVO (n=270). The most frequent serious adverse reactions reported in $\geq 2\%$ of patients receiving OPDIVO were urinary tract infection, sepsis, diarrhea, small intestine obstruction, and general physical health deterioration. In Checkmate 142 in MSI-H/dMMR mCRC patients receiving OPDIVO with YERVOY, serious adverse reactions occurred in 47% of patients. The most frequent serious adverse reactions reported in $\geq 2\%$ of patients were colitis/diarrhea, hepatic events, abdominal pain, acute kidney injury, pyrexia, and dehydration. In Checkmate 040, serious adverse reactions occurred in 49% of patients (n=154). The most frequent serious adverse reactions reported in $\geq 2\%$ of patients were pyrexia, ascites, back pain, general physical health deterioration, abdominal pain, and pneumonia. In Checkmate 238, Grade 3 or 4 adverse reactions occurred in 25% of OPDIVO-treated patients (n=452). The most frequent Grade 3 and 4 adverse reactions reported in $\geq 2\%$ of OPDIVO-treated patients were diarrhea and increased

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lipase and amylase. Serious adverse reactions occurred in 18% of OPDIVO-treated patients.

Common Adverse Reactions

In Checkmate 037, the most common adverse reaction ($\geq 20\%$) reported with OPDIVO (n=268) was rash (21%). In Checkmate 066, the most common adverse reactions ($\geq 20\%$) reported with OPDIVO (n=206) vs dacarbazine (n=205) were fatigue (49% vs 39%), musculoskeletal pain (32% vs 25%), rash (28% vs 12%), and pruritus (23% vs 12%). In Checkmate 067, the most common ($\geq 20\%$) adverse reactions in the OPDIVO plus YERVOY arm (n=313) were fatigue (62%), diarrhea (54%), rash (53%), nausea (44%), pyrexia (40%), pruritus (39%), musculoskeletal pain (32%), vomiting (31%), decreased appetite (29%), cough (27%), headache (26%), dyspnea (24%), upper respiratory tract infection (23%), arthralgia (21%), and increased transaminases (25%). In Checkmate 067, the most common ($\geq 20\%$) adverse reactions in the OPDIVO arm (n=313) were fatigue (59%), rash (40%), musculoskeletal pain (42%), diarrhea (36%), nausea (30%), cough (28%), pruritus (27%), upper respiratory tract infection (22%), decreased appetite (22%), headache (22%), constipation (21%), arthralgia (21%), and vomiting (20%). In Checkmate 017 and 057, the most common adverse reactions ($\geq 20\%$) in patients receiving OPDIVO (n=418) were fatigue, musculoskeletal pain, cough, dyspnea, and decreased appetite. In Checkmate 032, the most common adverse reactions ($\geq 20\%$) in patients receiving OPDIVO (n=245) were fatigue (45%), decreased appetite (27%), musculoskeletal pain (25%), dyspnea (22%), nausea (22%), diarrhea (21%), constipation (20%), and cough (20%). In Checkmate 025, the most common adverse reactions ($\geq 20\%$) reported in patients receiving OPDIVO (n=406) vs everolimus (n=397) were fatigue (56% vs 57%), cough (34% vs 38%), nausea (28% vs 29%), rash (28% vs 36%), dyspnea (27% vs 31%), diarrhea (25% vs 32%), constipation (23% vs 18%), decreased appetite (23% vs 30%), back pain (21% vs 16%), and arthralgia (20% vs 14%). In Checkmate 214, the most common adverse reactions ($\geq 20\%$) reported in patients treated with OPDIVO plus YERVOY (n=547) vs sunitinib (n=535) were fatigue (58% vs 69%), rash (39% vs 25%), diarrhea (38% vs 58%), musculoskeletal pain (37% vs 40%), pruritus (33% vs 11%), nausea (30% vs 43%), cough (28% vs 25%), pyrexia (25% vs 17%), arthralgia (23% vs 16%), decreased appetite (21% vs 29%), dyspnea (20% vs 21%), and vomiting (20% vs 28%). In Checkmate 205 and 039, the most common adverse reactions ($\geq 20\%$) reported in patients receiving OPDIVO

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(n=266) were upper respiratory tract infection (44%), fatigue (39%), cough (36%), diarrhea (33%), pyrexia (29%), musculoskeletal pain (26%), rash (24%), nausea (20%) and pruritus (20%). In Checkmate 141, the most common adverse reactions ($\geq 10\%$) in patients receiving OPDIVO (n=236) were cough and dyspnea at a higher incidence than investigator's choice. In Checkmate 275, the most common adverse reactions ($\geq 20\%$) reported in patients receiving OPDIVO (n=270) were fatigue (46%), musculoskeletal pain (30%), nausea (22%), and decreased appetite (22%). In Checkmate 142 in MSI-H/dMMR mCRC patients receiving OPDIVO as a single agent, the most common adverse reactions ($\geq 20\%$) were fatigue (54%), diarrhea (43%), abdominal pain (34%), nausea (34%), vomiting (28%), musculoskeletal pain (28%), cough (26%), pyrexia (24%), rash (23%), constipation (20%), and upper respiratory tract infection (20%). In Checkmate 142 in MSI-H/dMMR mCRC patients receiving OPDIVO with YERVOY, the most common adverse reactions ($\geq 20\%$) were fatigue (49%), diarrhea (45%), pyrexia (36%), musculoskeletal pain (36%), abdominal pain (30%), pruritus (28%), nausea (26%), rash (25%), decreased appetite (20%), and vomiting (20%). In Checkmate 040, the most common adverse reactions ($\geq 20\%$) in patients receiving OPDIVO (n=154) were fatigue (38%), musculoskeletal pain (36%), abdominal pain (34%), pruritus (27%), diarrhea (27%), rash (26%), cough (23%), and decreased appetite (22%). In Checkmate 238, the most common adverse reactions ($\geq 20\%$) reported in OPDIVO-treated patients (n=452) vs ipilimumab-treated patients (n=453) were fatigue (57% vs 55%), diarrhea (37% vs 55%), rash (35% vs 47%), musculoskeletal pain (32% vs 27%), pruritus (28% vs 37%), headache (23% vs 31%), nausea (23% vs 28%), upper respiratory infection (22% vs 15%), and abdominal pain (21% vs 23%). The most common immune-mediated adverse reactions were rash (16%), diarrhea/colitis (6%), and hepatitis (3%).

In a separate Phase 3 study of YERVOY 3 mg/kg, the most common adverse reactions ($\geq 5\%$) in patients who received YERVOY at 3 mg/kg were fatigue (41%), diarrhea (32%), pruritus (31%), rash (29%), and colitis (8%).

Please see U.S. Full Prescribing Information for [OPDIVO](#) and [YERVOY](#), including **Boxed WARNING regarding immune-mediated adverse reactions** for YERVOY.

Checkmate Trials and Patient Populations

<https://news.bms.com/news/corporate-financial/2019/Bristol-Myers-Squibb-Announces-Phase-3-CheckMate--498-Study-Did-Not-Meet-Primary-Endpoint-of-Overall-Survival-with-Opdivo-nivolumab-Plus-Radiation-in-Patients-with-Newly-Diagnosed-MGMT-Unmethylated-Glioblastoma-Multiforme/default.aspx> <Accessed June 12, 2023>.

Checkmate 037—previously treated metastatic melanoma; **Checkmate 066**—previously untreated metastatic melanoma; **Checkmate 067**—previously untreated metastatic melanoma, as a single agent or in combination with YERVOY; **Checkmate 017**—second-line treatment of metastatic squamous non-small cell lung cancer; **Checkmate 057**—second-line treatment of metastatic non-squamous non-small cell lung cancer; **Checkmate 032**—small cell lung cancer; **Checkmate 025**—previously treated renal cell carcinoma; **Checkmate 214**—previously untreated renal cell carcinoma, in combination with YERVOY; **Checkmate 205/039**—classical Hodgkin lymphoma; **Checkmate 141**—recurrent or metastatic squamous cell carcinoma of the head and neck; **Checkmate 275**—urothelial carcinoma; **Checkmate 142**—MSI-H or dMMR metastatic colorectal cancer, as a single agent or in combination with YERVOY; **Checkmate 040**—hepatocellular carcinoma; **Checkmate 238**—adjuvant treatment of melanoma.

About the Bristol-Myers Squibb and Ono Pharmaceutical Collaboration

In 2011, through a collaboration agreement with Ono Pharmaceutical Co., Bristol-Myers Squibb expanded its territorial rights to develop and commercialize *Opdivo* globally, except in Japan, South Korea and Taiwan, where Ono had retained all rights to the compound at the time. On July 23, 2014, Ono and Bristol-Myers Squibb further expanded the companies' strategic collaboration agreement to jointly develop and commercialize multiple immunotherapies – as single agents and combination regimens – for patients with cancer in Japan, South Korea and Taiwan.

About Bristol-Myers Squibb

Bristol-Myers Squibb is a global biopharmaceutical company whose mission is to discover, develop and deliver innovative medicines that help patients prevail over serious diseases. For more information about Bristol-Myers Squibb, visit us at [BMS.com](https://www.bms.com) or follow us on [LinkedIn](#), [Twitter](#), [YouTube](#) and [Facebook](#).

Bristol-Myers Squibb Forward-Looking Statement

<https://news.bms.com/news/corporate-financial/2019/Bristol-Myers-Squibb-Announces-Phase-3-CheckMate--498-Study-Did-Not-Meet-Primary-Endpoint-of-Overall-Survival-with-Opdivo-nivolumab-Plus-Radiation-in-Patients-with-Newly-Diagnosed-MGMT-Unmethylated-Glioblastoma-Multiforme/default.aspx> <Accessed June 12, 2023>.

This press release contains "forward-looking statements" within the meaning of the Private Securities Litigation Reform Act of 1995 regarding the research, development and commercialization of pharmaceutical products. All statements that are not statements of historical facts are, or may be deemed to be, forward-looking statements. Such forward-looking statements are based on historical performance and current expectations and projections about our future financial results, goals, plans and objectives and involve inherent risks, assumptions and uncertainties, including internal or external factors that could delay, divert or change any of them in the next several years, and could cause our future financial results, goals, plans and objectives to differ materially from those expressed in, or implied by, the statements. No forward-looking statement can be guaranteed. Forward-looking statements in this press release should be evaluated together with the many uncertainties that affect Bristol-Myers Squibb's business, particularly those identified in the cautionary factors discussion in Bristol-Myers Squibb's Annual Report on Form 10-K for the year ended December 31, 2018, as updated by our subsequent Quarterly Reports on Form 10-Q, Current Reports on Form 8-K and other filings with the Securities and Exchange Commission. The forward-looking statements included in this document are made only as of the date of this document and except as otherwise required by federal securities law, Bristol-Myers Squibb undertakes no obligation to publicly update or revise any forward-looking statement, whether as a result of new information, future events, changed circumstances or otherwise.

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<https://news.bms.com/news/corporate-financial/2019/Bristol-Myers-Squibb-Announces-Phase-3-CheckMate--498-Study-Did-Not-Meet-Primary-Endpoint-of-Overall-Survival-with-Opdivo-nivolumab-Plus-Radiation-in-Patients-with-Newly-Diagnosed-MGMT-Unmethylated-Glioblastoma-Multiforme/default.aspx> <Accessed June 12, 2023>.

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Radiotherapy combined with nivolumab or temozolomide for newly diagnosed glioblastoma with unmethylated *MGMT* promoter: An international randomized phase III trial

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Abstract

Background. Addition of temozolomide (TMZ) to radiotherapy (RT) improves overall survival (OS) in patients with glioblastoma (GBM), but previous studies suggest that patients with tumors harboring an unmethylated *MGMT* promoter derive minimal benefit. The aim of this open-label, phase III CheckMate 498 study was to evaluate the efficacy of nivolumab (NIVO) + RT compared with TMZ + RT in newly diagnosed GBM with unmethylated *MGMT* promoter.

Methods. Patients were randomized 1:1 to standard RT (60 Gy) + NIVO (240 mg every 2 weeks for eight cycles, then 480 mg every 4 weeks) or RT + TMZ (75 mg/m² daily during RT and 150–200 mg/m²/day 5/28 days during maintenance). The primary endpoint was OS.

Results. A total of 560 patients were randomized, 280 to each arm. Median OS (mOS) was 13.4 months (95% CI, 12.6 to 14.3) with NIVO + RT and 14.9 months (95% CI, 13.3 to 16.1) with TMZ + RT (hazard ratio [HR], 1.31; 95% CI, 1.09 to 1.58; $P = .0037$). Median progression-free survival was 6.0 months (95% CI, 5.7 to 6.2) with NIVO + RT and 6.2 months (95% CI, 5.9 to 6.7) with TMZ + RT (HR, 1.38; 95% CI, 1.15 to 1.65). Response rates were 7.8% (9/116) with NIVO + RT and 7.2% (8/111) with TMZ + RT; grade 3/4 treatment-related adverse event (TRAE) rates were 21.9% and 25.1%, and any-grade serious TRAE rates were 17.3% and 7.6%, respectively.

Conclusions. The study did not meet the primary endpoint of improved OS; TMZ + RT demonstrated a longer mOS than NIVO + RT. No new safety signals were detected with NIVO in this study. The difference between the study treatment arms is consistent with the use of TMZ + RT as the standard of care for GBM. ClinicalTrials.gov NCT02617589

Key Points

- NIVO did not improve survival in newly diagnosed GBM with unmethylated *MGMT* promoter.
- No new safety signals were detected with NIVO + standard of care in this study.
- Immunotherapy with NIVO is not a suitable replacement for chemotherapy with TMZ.

Importance of the Study

Given the survival benefits of immunotherapy in cancer, it was hypothesized that it may also offer promise in difficult-to-treat cancers, such as glioblastoma (GBM). Worse outcomes are observed in patients with GBM with unmethylated versus methylated *MGMT* promoter. Temozolomide (TMZ), the standard chemotherapy, is associated with limited efficacy in unmethylated *MGMT* tumors. Here we report data

from the largest phase III study in patients with GBMs and unmethylated *MGMT* promoter and the first prospective phase III study examining TMZ omission in this chemoresistant phenotype. Nivolumab + radiotherapy (NIVO + RT) showed a shorter survival benefit vs TMZ + RT, suggesting that NIVO is not a suitable replacement for TMZ. Results also suggest that in the absence of other treatment options, TMZ should continue to be the standard of care for all patients with GBM regardless of *MGMT* promoter status.

Glioblastoma (GBM), the most common primary malignant brain tumor, is associated with a dismal prognosis and poor quality of life.^{1–4} The mainstay of treatment for newly diagnosed disease is surgical resection followed by radiotherapy (RT) with concomitant and adjuvant temozolomide (TMZ).^{4–6} The benefit of this treatment was demonstrated in a phase III study, which showed improved overall survival (OS) from 12.1 months with RT alone to 14.6 months with TMZ chemoradiotherapy (hazard ratio [HR], 0.63; $P < .001$).⁵

Chemosensitivity to alkylating agents has been strongly linked to epigenetic silencing of the *MGMT* gene in various cancers.^{7,8} Methylation of the *MGMT* promoter results in decreased *MGMT* expression, which reduces DNA repair capacity and confers chemosensitivity.^{8–10} Analyses from the pivotal phase III study validating TMZ in GBM suggested that patients with tumors harboring a methylated *MGMT* promoter derived a survival benefit from TMZ + RT (median, 21.7 vs. 15.3 months), whereas patients with unmethylated *MGMT* promoter derived

minimal and statistically insignificant benefit (median, 12.7 vs 11.8 months).⁸ *MGMT* promoter methylation is also an independent prognostic factor in GBM; patients with a methylated *MGMT* promoter achieve significantly better outcomes.^{8,10,11} Given the lack of treatment alternatives, TMZ is offered to all patients with GBM, regardless of tumor *MGMT* promoter methylation status—with or without tumor-treating fields.^{4,6,12} Because of TMZ's minimal benefit and known toxicities, reassessment of its role in GBM with unmethylated *MGMT* promoter remains of interest, and novel treatment alternatives are clearly needed for this patient population.¹³

Nivolumab (NIVO) is a fully human immunoglobulin G4 monoclonal antibody targeting the programmed cell death 1 protein (PD-1) immune checkpoint. NIVO has been shown to improve survival in multiple cancers, including melanoma, lung cancer, and renal cell carcinoma, and has demonstrated activity in brain metastasis from melanoma.^{14–16} Gliomas have been shown to express PD-1 ligand (PD-L1),

and expression levels have been associated with tumor grade.^{17,18} Preclinical studies in GBM models suggest that efficacy of PD-1 inhibitors could be enhanced through combination with RT.¹⁹ RT may expose antigenic mutations, induce the expression of peptides that can activate T cells, and recruit antigen-presenting and immune effector cells to the tumor microenvironment (TME).^{20–22} Given the chemoresistance observed in tumors with unmethylated *MGMT* promoter, we conducted a phase III study to evaluate whether immunotherapy with NIVO could improve survival when combined with RT (NIVO + RT) compared with conventional chemoradiotherapy with TMZ + RT in this patient population.

Materials and Methods

Study Design and Participants

In this open-label, phase III study, patients were stratified by degree of tumor resection (complete vs. partial) at baseline and randomized 1:1 to receive NIVO + RT or TMZ + RT. In both arms, focal RT consisted of 60 Gy in 2-Gy fractions. In the NIVO + RT arm, RT was combined with NIVO 240 mg every 2 weeks for eight doses followed by NIVO 480 mg every 4 weeks until unacceptable toxicity or disease progression. In the TMZ + RT arm, RT was combined with the standard TMZ regimen, 75 mg/m² once daily during RT (concomitant),²³ followed by a 4-week treatment break and then adjuvant treatment with TMZ 150 to 200 mg/m² once daily on days 1 to 5 of a 28-day cycle for ≤ 6 cycles (maintenance). The median dose and duration of RT was 60.0 Gy and 6.1 weeks in both arms, respectively. Per investigator's discretion, patients receiving NIVO were permitted to continue treatment beyond suspected progression until confirmation of progression on follow-up MRI.

Eligible patients were aged ≥ 18 years and had newly diagnosed, histologically confirmed, supratentorial GBM with unmethylated *MGMT* promoter determined centrally by a methylation-specific polymerase chain reaction assay.²⁴ Other key eligibility criteria included no prior treatment for GBM beyond surgery and Karnofsky Performance Scale (KPS) ≥ 70. At randomization, patients must have been receiving ≤ 20 mg prednisone or ≤ 3 mg dexamethasone (or equivalent). Patients were excluded if they had recurrent or secondary GBM; undergone biopsy only for GBM at surgery; tumors harboring *IDH-1* or *-2* mutation; unresolved CNS hemorrhage; metastatic extracranial or leptomeningeal disease; active, known, or suspected autoimmune disease; tumor-treating fields therapy (not a recommended treatment at time of study start); or used a biodegradable carmustine wafer.

Procedures

Tumor samples were assessed for *MGMT* promoter methylation status; testing was performed by Covance laboratory services. A sample was determined to be *MGMT* unmethylated when the ratio of the gene copy numbers of methylated *MGMT* to control (β -actin) $\times$ 1000 was < 2 and the gene copy numbers of *MGMT* and control were within the reportable range (β -actin ≥ 10 copies and

MGMT ≥ 10 copies). Disease status was assessed using contrast-enhanced MRI at baseline and beginning 4 weeks ($\pm$ 7 days) after RT completion. Then, disease status was evaluated every 8 weeks ($\pm$ 7 days) until progression per Response Assessment in Neuro-Oncology (RANO) criteria.²⁵ As detailed in the RANO criteria, classification of tumor progression during the first 12 weeks after completion of RT requires either that the new enhancement be located outside of the radiation field (beyond the high-dose region or the 80% isodense line) or unequivocal pathological confirmation of progressive disease. Confirmation was determined at a subsequent MRI performed within 8 weeks after the initial radiological assessment of progression. Theoretically, patients treated with immunotherapy may derive clinical benefit despite initial evidence of disease progression; therefore, patients in the NIVO + RT arm were allowed to continue NIVO in the setting of suspected progression at investigator discretion until progression was confirmed. Progression-free survival (PFS) was defined as time from randomization to documented progression or death from any cause. OS was defined as time from randomization to death from any cause. Tumor-sample sections for PD-L1 expression were retrospectively assessed centrally (LabCorp Clinical Trials, Research Triangle Park, NC, USA); PD-L1 positivity was defined as percentage of membranous staining of tumor cells with 1% and 5% cutoff values. Adverse events (AEs) were assessed continuously during the study per National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03. Patient-reported outcomes (PROs) related to patients' health-related quality of life (HRQoL) were assessed using the European Organisation for Research and Treatment of Cancer QLQ-C30 and EQ-5D-3L questionnaires, collected at baseline, week 11, and then every 8 weeks during treatment until disease progression. The time to deterioration in PRO score was assessed by time from randomization to first worsening of PRO score from baseline during treatment—meeting or exceeding the minimal change in responder definition threshold—without subsequent improvement based on the responder definition.²⁶

Outcomes

The primary endpoint was OS. Secondary endpoints included investigator-assessed PFS based on RANO criteria and OS at 24 months using Kaplan-Meier methodology. Key exploratory endpoints included safety and tolerability, HRQoL, and efficacy based on tumor PD-L1 expression.

Treatment Beyond Suspected Progression

Patients in the NIVO + RT arm with evidence of progression in imaging findings were allowed to continue study therapy until disease progression was confirmed.

Statistical Analyses

OS, defined as the time between the date of randomization and the date of death due to any cause, was compared between treatment arms using a two-sided log-rank test

stratified by extent of surgical resection (complete or partial). The final OS analysis was planned after follow-up of ≥ 23 months or when ≥ 390 deaths were reported, providing $\approx 90\%$ power with an overall type I error of 0.05. At the time of the database lock, some patients had < 24 months of follow-up. However, given the number of events at the time of the database lock, it was considered that the number of patients with follow-up of < 24 months at the time of the current analysis would not have affected the data maturity or interpretability of the results. Kaplan-Meier methodology was used to estimate OS and PFS curves, medians with 95% CIs, and OS and PFS rates at fixed time points with 95% CIs. HRs and corresponding two-sided 95% CIs were estimated using a stratified Cox proportional hazards model. A stratified Cox proportional hazards regression model was used to estimate the HR between treatment groups. Baseline characteristics in all randomized patients and safety in all treated patients were assessed using descriptive statistics.

Study Oversight

The study was conducted in accordance with Good Clinical Practice guidelines per the International Conference on Harmonisation and with ethical principles of the European Union Directive and US Code of Federal Regulations. The study is registered at ClinicalTrials.gov (NCT02617589). The protocol was approved by an institutional review board or independent ethics committee at each site before study activation. All patients provided written informed consent in accordance with the Declaration of Helsinki.

Role of the Funding Source

The study was designed by the authors in collaboration with the funder (Bristol Myers Squibb). The authors and funder were responsible for data collection, and the funder was responsible for data analysis. The authors and funder were involved in data interpretation, development of the report, and the decision to submit. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

Results

Patients and Treatment

From March 1, 2016, through October 25, 2018, 560 patients with newly diagnosed GBM with unmethylated *MGMT* promoter were randomized to receive NIVO + RT ($n = 280$) or TMZ + RT ($n = 280$) (Figure 1). Patients were enrolled at 124 sites across 19 countries. Of 560 randomized patients, 278 of 280 (99.3%) in the NIVO + RT arm and 275 of 280 (98.2%) in the TMZ + RT arm eventually received treatment. No marked imbalances were observed in baseline characteristics or demographics between arms (Table 1 and Supplementary Table S1).

Complete surgical resection had been performed in 151 patients (53.9%) in the NIVO + RT arm and 144 patients (51.4%) in the TMZ + RT arm. Baseline PD-L1 expression was $\geq 1\%$ in 104 patients (37.8%) in the NIVO + RT arm and 125 patients (44.6%)

in the TMZ + RT arm; PD-L1 expression was $< 1\%$ in 171 patients (62.2%) and 155 patients (55.4%), respectively (PD-L1 was not evaluable in one patient and tumor tissue samples were not collected for four patients in the NIVO + RT arm). Seventy-eight patients (27.9%; $n = 280$) in the NIVO + RT arm and 95 patients (33.9%; $n = 280$) in the TMZ + RT arm were receiving corticosteroids at baseline, with 5.7% and 7.9% of patients receiving > 3 mg/day of dexamethasone equivalents, respectively.

The median duration of study treatment was 22.1 weeks (range, 0.1–140.9) in the NIVO + RT arm and 6.1 weeks (range, 0.6–8.3; concomitant) and 15.4 weeks (range, 0.1–121.1; maintenance) in the TMZ + RT arm. A median of 10.0 doses of NIVO was received (range, 1–40); the median number of TMZ cycles for all patients who entered the maintenance phase was 4.0 (range, 1–31).

At data cutoff, four patients (1.4%) in the NIVO + RT arm and one patient (0.4%) in the TMZ + RT arm were still receiving treatment. Among treated patients, discontinuations occurred in 274 patients (98.6%) in the NIVO + RT arm and 274 patients (99.6%) in the TMZ + RT arm. The most common reasons for treatment discontinuation were disease progression (NIVO + RT, $n = 214$ [77.0%]; TMZ + RT, $n = 137$ [49.8%]) and study drug toxicity (NIVO + RT, $n = 26$ [9.4%]; TMZ + RT, $n = 20$ [7.3%]) (Figure 1). In the TMZ + RT arm, 76 patients (27.6%) completed treatment.

Efficacy

At data cutoff (March 21, 2019), the median follow-up time for OS was 13.0 months (range, 0.6–32.4) in the NIVO + RT arm and 14.2 months (range, 0–32.6) in the TMZ + RT arm. The final analysis was performed after 462 OS events had occurred. The median OS (mOS) was 13.4 months (95% CI, 12.6 to 14.3) in the NIVO + RT arm and 14.9 months (95% CI, 13.3 to 16.1) in the TMZ + RT arm (HR, 1.31; 95% CI, 1.09 to 1.58; $P = .0037$) (Table 2 and Figure 2A). The 24-month OS rates were 10.3% (95% CI, 6.8 to 14.6) in the NIVO + RT arm and 21.2% (95% CI, 16.4 to 26.5) in the TMZ + RT arm.

Among patients with baseline PD-L1 expression $\geq 1\%$, mOS was 12.6 months ($n = 104$; 95% CI, 11.3 to 14.2) in the NIVO + RT arm and 15.5 months ($n = 125$; 95% CI, 13.2 to 17.2) in the TMZ + RT arm (HR, 1.4; 95% CI, 1.1 to 1.9) (Supplemental Figure S1A). The mOS in patients with PD-L1 $< 1\%$ was 13.8 months ($n = 171$; 95% CI, 13.0 to 14.6) in the NIVO + RT arm and 14.7 months ($n = 155$; 95% CI, 12.6 to 16.0) in the TMZ + RT arm (HR, 1.2; 95% CI, 0.9 to 1.5) (Supplemental Figure S1B). OS data by baseline PD-L1 expression $\geq 5\%$ are shown in Supplemental Figures S1C and S1D. The results were consistent across several subgroup analyses, including complete tumor resection (Figure 3).

Exploratory analyses (not protocol defined) showed balanced distributions of *MGMT* scores across both arms. Median PFS was 6.0 months (95% CI, 5.7 to 6.2) with NIVO + RT versus 6.2 months (95% CI, 5.9 to 6.7) with TMZ + RT (HR, 1.38; 95% CI, 1.15 to 1.65) (Table 2; Figure 2B). The 12-month PFS rate was 5.7% (95% CI, 3.2 to 9.1) with NIVO + RT and 17.7% (95% CI, 13.3 to 22.7) with TMZ + RT.

The investigator-assessed objective response rate per RANO criteria was 7.8% (9/116; 95% CI, 3.6 to 14.2) in the NIVO + RT arm and 7.2% (8/111; 95% CI, 3.2 to 13.7) in the TMZ + RT arm (Supplemental Table S2). Duration of

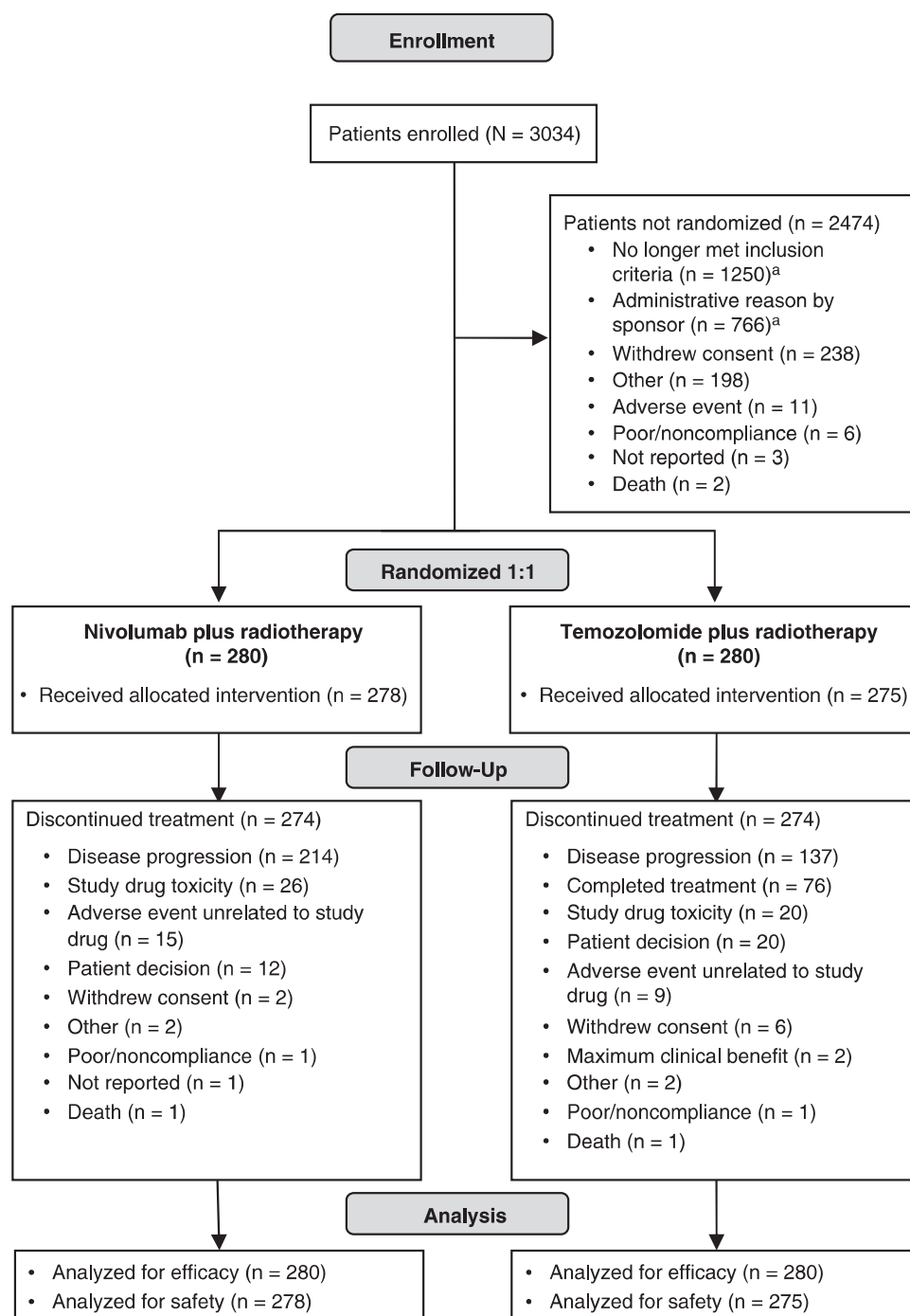


Fig. 1 Trial profile. ^aThe majority of the nonrandomized population was excluded due to methylation status (cutoff, ratio of the gene copy numbers of methylated *MGMT* to control (β -actin) $\times 1000 < 2$).

response data are presented in [Supplemental Table S2](#). Demographic and disease characteristics of responders are presented in [Supplemental Table S3](#).

Subsequent cancer therapy (any therapy) was received by 63.6% and 53.6% of patients in the NIVO + RT and TMZ + RT groups, respectively, of which 52.9% and 46.1%, respectively, received subsequent systemic cancer therapy ([Supplemental Table S4](#)). In the NIVO + RT group, 41.1% of

patients received subsequent treatment with alkylating agent (including 38.9% receiving TMZ therapy), and 27.5% received vascular endothelial growth factor antibody. In the TMZ + RT group, 30.7% of patients received alkylating agent, and 28.9% received vascular endothelial growth factor antibody. Three patients (1.1%) in the NIVO + RT arm and 7 (2.5%) in the TMZ + RT arm received subsequent immunotherapy ([Supplemental Table S4](#)).

Table 1. Patient Demographics and Baseline Characteristics

	Nivolumab plus radiotherapy (n = 280)	Temozolomide plus radiotherapy (n = 280)
Age, years		
Median	59.5	56.0
Range	18–83	23–81
Age, no. (%)		
< 65 years	190 (67.9)	207 (73.9)
≥ 65 to < 75 years	76 (27.1)	61 (21.8)
≥ 75 years	14 (5.0)	12 (4.3)
Sex, no. (%)		
Male	190 (67.9)	175 (62.5)
Female	90 (32.1)	105 (37.5)
Histopathologic diagnosis, no. (%)		
Glioblastoma	272 (97.1)	270 (96.4)
Gliosarcoma	8 (2.9)	10 (3.6)
RPA class, no. (%) ^a		
III	20 (7.1)	42 (15.0)
IV	219 (78.2)	202 (72.1)
V	41 (14.6)	36 (12.9)
Other	0	0
Extent of surgery, no. (%) ^b		
Complete resection	151 (53.9)	144 (51.4)
Partial resection	129 (46.1)	136 (48.6)
KPS, no. (%)		
100	76 (27.1)	91 (32.5)
90	122 (43.6)	118 (42.1)
80	54 (19.3)	47 (16.8)
70	28 (10.0)	20 (7.1)
Not reported	0	4 (1.4)
Time from diagnosis to randomization, weeks		
Median	4.93	5.14
Range	(4.1–5.6)	(4.3–5.9)
Patients with evaluable PD-L1 expression, no. (%)		
PD-L1 expression level, no. (%) ^c		
< 1%	275 (99.6)	280 (100.0)
≥ 1%	171 (62.2)	155 (55.4)
≥ 1%	104 (37.8)	125 (44.6)
Not quantifiable	1 (0.4)	0
Corticosteroid use, no. (%) ^d		
Yes	78 (27.9)	95 (33.9)
≤ 3 mg/day	62 (22.1)	73 (26.1)
> 3 mg/day	16 (5.7)	22 (7.9)
No	202 (72.1)	185 (66.1)

KPS, Karnofsky Performance Scale; PD-L1, programmed cell death ligand 1; RPA, recursive-partitioning analysis.

^aThe RPA classes were as follows: class III, age < 50 years and KPS ≥ 90 (on a scale of 0–100, with higher scores indicating better function); class IV, < 50 years and KPS < 90 (or ≥ 50 years, KPS ≥ 70, complete or partial tumor resection, and ability to work); class V, ≥ 50 years, KPS ≥ 70, complete or partial tumor resection, and inability to work (or ≥ 50 years, KPS ≥ 70, and tumor-biopsy specimen only; or ≥ 50 years and KPS < 70).³⁵

^bThis characteristic was used as a stratification factor as recorded in the interactive voice response system at time of randomization. Information presented as collected in the case report form.

^cPercentages were based on the number of patients with evaluable PD-L1 expression.

^dBased on average corticosteroid use 5 days before start of dosing or randomization date for patients not treated (in dexamethasone equivalent). Patients enrolled at doses > 3 mg/day were tapered off; treatment did not commence until the dose was ≤ 3 mg/day.

Table 2. Overall Survival and Progression-Free Survival Rates Per Investigator Assessment

	Nivolumab plus radiotherapy (n = 280)	Temozolomide plus radiotherapy (n = 280)
Overall survival, months		
Median (95% CI)	13.4 (12.6 to 14.3)	14.9 (13.3 to 16.1)
Overall survival rate, (95% CI) %		
6 months	88.5 (84.1 to 91.7)	88.7 (84.4 to 91.9)
12 months	58.3 (52.2 to 63.9)	62.3 (56.3 to 67.8)
18 months	28.5 (23.3 to 34.0)	36.4 (30.7 to 42.2)
24 months	10.3 (6.8 to 14.6)	21.2 (16.4 to 26.5)
Progression-free survival, months		
Median (95% CI)	6.0 (5.7 to 6.2)	6.2 (5.9 to 6.7)
Progression-free survival rate, (95% CI) %		
6 months	50.5 (44.3 to 56.3)	54.6 (48.4 to 60.4)
9 months	14.8 (10.7 to 19.4)	30.9 (25.3 to 36.6)
12 months	5.7 (3.2 to 9.1)	17.7 (13.3 to 22.7)
18 months	3.0 (1.3 to 5.8)	8.1 (5.1 to 11.9)

Safety

Any-grade treatment-related AEs (TRAEs) were reported in 72.7% of patients treated with NIVO + RT and 75.6% of patients treated with TMZ + RT. The most frequent TRAE was fatigue (any grade, 19.1%) in the NIVO + RT arm and nausea (any grade, 29.1%) in the TMZ + RT arm (Table 3; Supplemental Table S5). Rates of grade 3/4 TRAEs were 22.0% with NIVO + RT and 25.1% with TMZ + RT. Three treatment-related deaths were reported in the NIVO + RT arm: vasogenic cerebral edema, sudden death, and respiratory failure (1 each), after receiving 10, 7, and 10 infusions of NIVO, respectively. The patient who died from sudden death had previously experienced hyperglycemia and grade 3 rash and had been treated with insulin and corticosteroids. No deaths attributed to treatment were reported in the TMZ + RT arm. Neurological TRAEs occurred in 16.5% (grade 3/4, 1.8%) of patients treated with NIVO + RT and 9.5% (grade 3/4, 0%) of patients treated with TMZ + RT. Any-grade serious TRAEs occurred in 17.3% (NIVO + RT) and 7.6% (TMZ + RT) of patients. Any-grade TRAEs leading to discontinuation occurred in 24 patients (8.6%) in the NIVO + RT arm and 16 patients (5.8%) in the TMZ + RT arm. Treatment-related, immune-mediated AEs reported by category are shown in Supplemental Table S6. Most patients were not receiving corticosteroids at baseline (NIVO + RT = 200/278 [71.9%]; TMZ + RT = 180/275 [65.5%]). The median dose of corticosteroid was 0 mg/day (dexamethasone equivalents) in the NIVO + RT arm throughout the study treatment, except at weeks 11–18 and 91–98 when median corticosteroid use was 0.21 and 0.44 mg/day, respectively. Similarly, in the TMZ + RT arm, median dose of corticosteroid was 0 mg/day except at weeks 1–6 and 11–18 when median corticosteroid use was 0.24 and 0.66 mg/day, respectively.

PROs

In all randomized patients, median time to deterioration of HRQoL scores was 4.6 months with NIVO + RT and

3.1 months with TMZ + RT (HR, 0.76; 95% CI, 0.59 to 0.99; $P = .039$). A trend of delayed time to deterioration was observed in the NIVO + RT arm compared with the TMZ + RT arm for most domains of HRQoL and similarly for general health utilities (EQ-5D-3L index and visual analog scale; Supplemental Figure S2). However, these results for time to deterioration were affected by heavy censoring and should be interpreted with caution.

Discussion

CheckMate 498 is a randomized phase III study investigating the efficacy of NIVO + RT compared with conventional TMZ + RT chemoradiation in patients with newly diagnosed GBM with unmethylated *MGMT* promoter. Although patients in both arms fared better than historical controls, the primary endpoint was not met. TMZ + RT was associated with superior OS compared with NIVO + RT (mOS, 14.9 vs. 13.4 months), suggesting that NIVO is not a substitute for TMZ in this patient population.

Although NIVO has shown notable efficacy in several other cancer types, it did not demonstrate a survival benefit in patients with newly diagnosed GBM with unmethylated *MGMT* promoter compared with TMZ. Likewise, in a subgroup analysis of the CheckMate 143 phase III study, NIVO did not demonstrate a survival benefit versus bevacizumab in patients with recurrent GBM with unmethylated *MGMT* promoter.^{27,28} PD-L1 expression in this study ($\approx 41\%$ of all patients expressed PD-L1 $\geq 1\%$) was similar to that observed in other GBM studies.¹⁸ However, it did not predict survival benefit with NIVO, suggesting that other factors may hinder successful immune responses in this tumor type. Notably, recent results from the CheckMate 548 study (NCT02667587) in newly diagnosed GBM with methylated *MGMT* have also shown the addition of NIVO to TMZ + RT does not prolong PFS or OS compared with TMZ + RT alone.²⁹ Taken together, these results clearly highlight

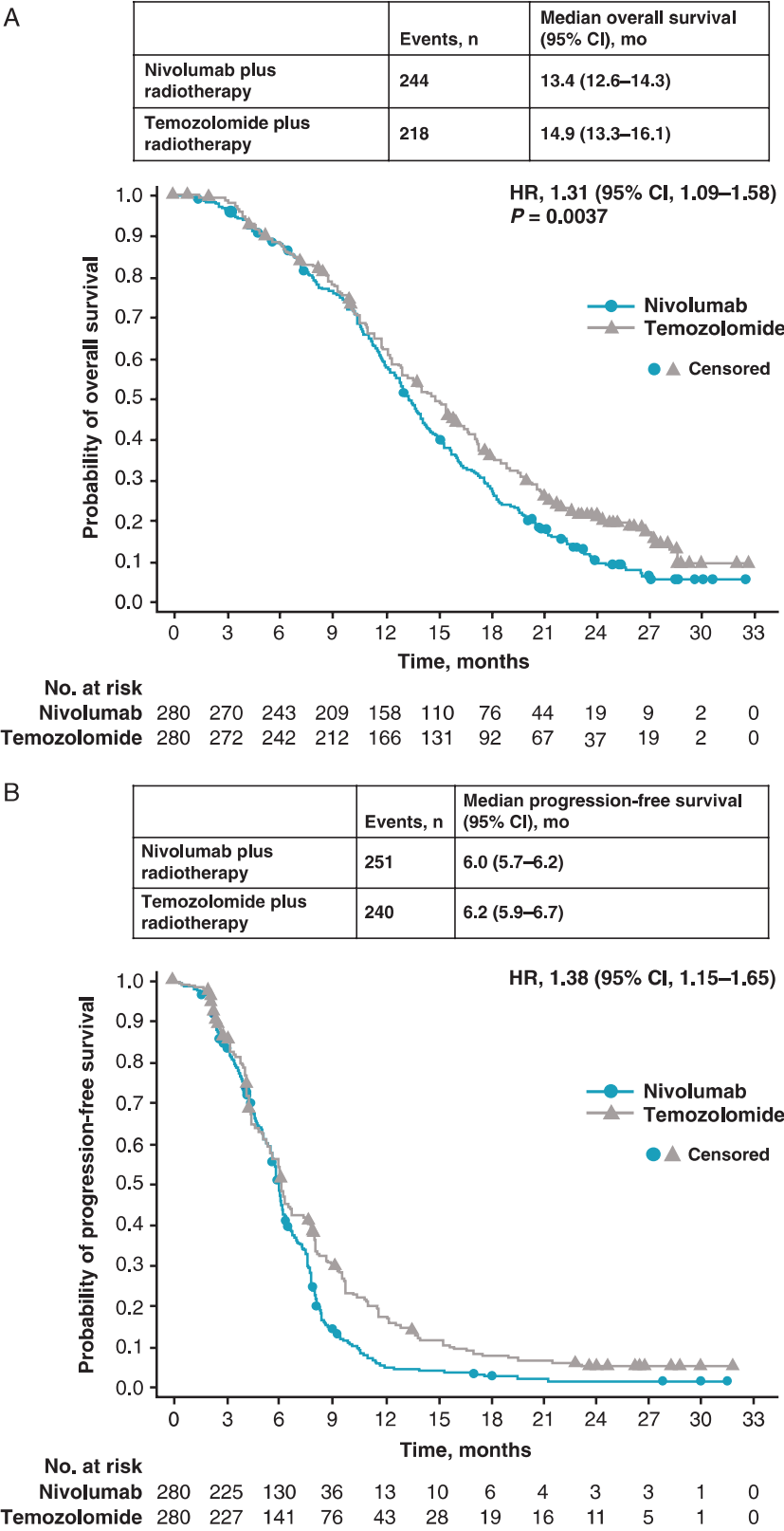


Fig. 2 OS and PFS in all patients. (A) Shows the number of events, median OS, and the Kaplan-Meier curve for OS in all patients treated with nivolumab plus radiotherapy or temozolomide plus radiotherapy. (B) Shows the number of events, median PFS, and the Kaplan-Meier curve for PFS per investigator assessment in patients treated with nivolumab plus radiotherapy or temozolomide plus radiotherapy. Symbols indicate censored observations. Hazard ratios and 95% CIs were estimated using a Cox proportional hazards model. OS, overall survival; PFS, progression-free survival.

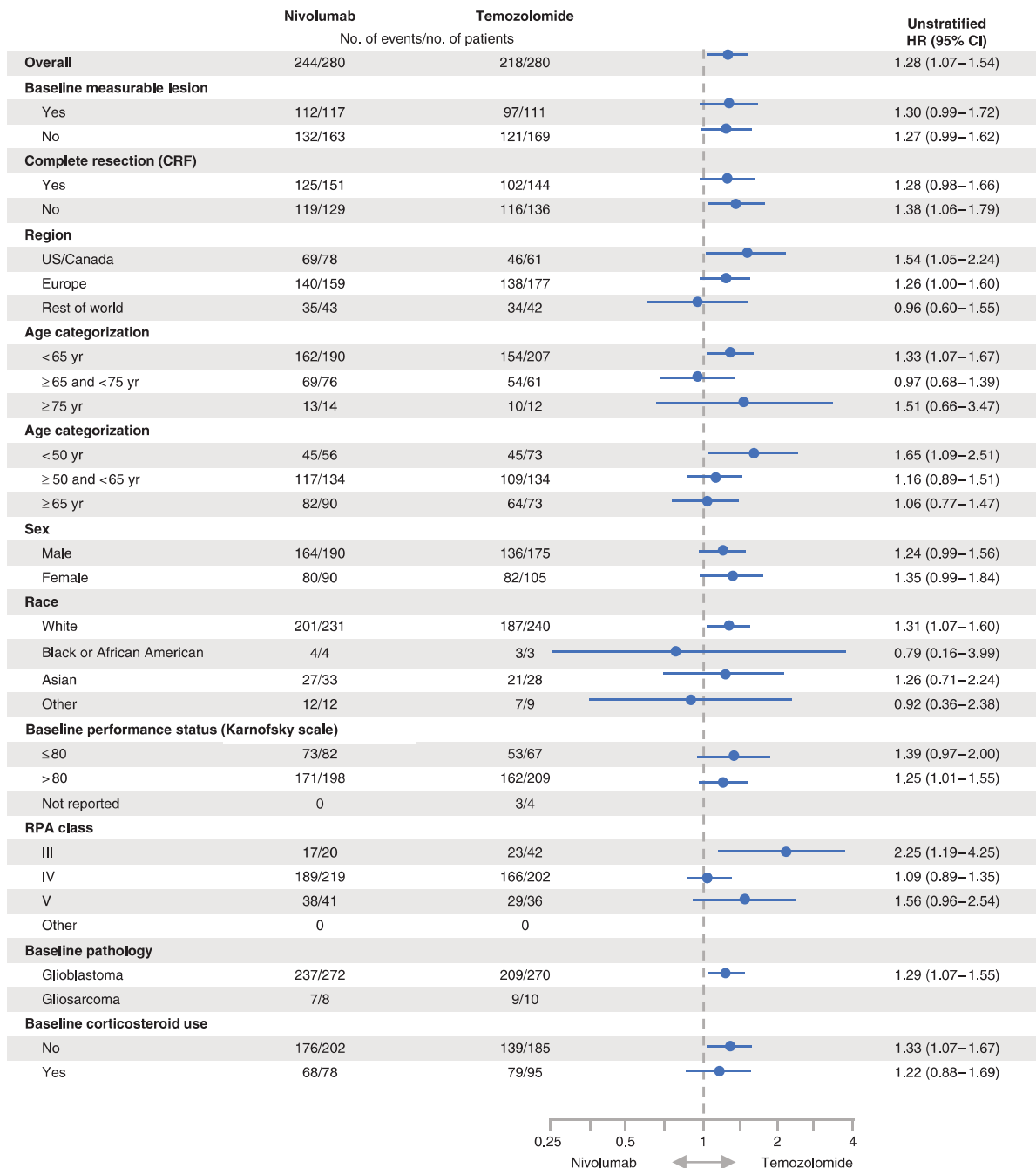


Fig. 3 Overall survival in prespecified patient subgroups defined by baseline clinical characteristics. This figure shows a forest plot of unstratified hazard ratios for death in the analysis of treatment effect in prespecified patient subgroups according to baseline characteristics.

a need for better understanding the mechanisms of immune evasion in GBM to improve the efficacy of immunotherapies. In addition to PD-L1 expression, multiple factors have been implicated in the maintenance of an immunosuppressive microenvironment in gliomas. These include both tumor- and brain-specific mechanisms (eg, low tumor mutational burden, recruitment of other immune checkpoints, decreased T-cell responsiveness, inhibitory cytokine production, interactions between CNS microenvironment

and microglia, predominance of myeloid cells, and paucity of lymphocytes in TME), in addition to frequent corticosteroid use.^{30–33}

A key limitation of our study was the lack of immune-predictive biomarkers and comprehensive genomic characterization due to limited availability of tumor samples; therefore, novel biomarkers remain to be further explored. In addition, this study did not consider potential effects of the timing of PD-1 blockade relative to RT administration,

Table 3. Treatment-Related Adverse Events^a

	Nivolumab plus radiotherapy (n = 278)		Temozolomide plus radiotherapy (n = 275)	
	Any grade	Grade 3/4	Any grade	Grade 3/4
Any treatment-related adverse event, no. (%) ^b	202 (72.7)	61 (21.9)	208 (75.6)	69 (25.1)
Fatigue	53 (19.1)	1 (0.4)	77 (28.0)	3 (1.1)
Pyrexia	15 (5.4)	4 (1.4)	2 (0.7)	0
Alopecia	31 (11.2)	0	48 (17.5)	1 (0.4)
Rash	28 (10.1)	5 (1.8)	6 (2.2)	1 (0.4)
Pruritus	20 (7.2)	1 (0.4)	11 (4.0)	0
Diarrhea	22 (7.9)	2 (0.7)	8 (2.9)	0
Nausea	18 (6.5)	0	80 (29.1)	2 (0.7)
Headache	16 (5.8)	0	11 (4.0)	0
Radiation skin injury	21 (7.6)	0	17 (6.2)	0
Hypothyroidism/autoimmune hypothyroidism	16 (5.8)	2 (0.8)	0	0
Decreased appetite	15 (5.4)	0	34 (12.4)	0
Lymphocyte count decreased/lymphopenia	12 (4.3)	4 (1.5)	51 (18.6)	28 (10.2)
Asthenia	10 (3.6)	0	17 (6.2)	1 (0.4)
Vomiting	8 (2.9)	0	39 (14.2)	0
Constipation	7 (2.5)	0	40 (14.5)	0
Neutrophil count decreased/neutropenia	4 (1.4)	3 (1.1)	28 (10.2)	12 (4.4)
Platelet count decreased/thrombocytopenia	3 (1.1)	1 (0.4)	66 (24.0)	30 (10.9)
Treatment-related adverse event leading to discontinuation, no. (%)	24 (8.6)	20 (7.2)	16 (5.8)	12 (4.4)

^aData are based on a March 21, 2019, database lock. The safety analysis included all patients who received ≥ 1 dose of study drug. Some patients had > 1 adverse event. Includes events reported between first dose and 30 days after last dose of study therapy. Three treatment-related deaths were reported in the nivolumab arm due to vasogenic cerebral edema, sudden death, and respiratory failure (1 each); no treatment-related deaths were reported in the temozolomide arm.

^bThese treatment-related adverse events were reported in $\geq 5\%$ of the patients in either study arm. The full-length treatment-related adverse events table is included in [Supplement Table S5](#).

which has subsequently been demonstrated to be of possible consequence in some preclinical, non-GBM settings.³⁴

The overall safety profile with NIVO + RT in this study was similar to that reported in CheckMate 143 for NIVO alone, with no new safety signals observed.²⁸ However, in some AE categories, as expected, more AEs were reported in the NIVO + RT arm than in the TMZ + RT arm. One of the reasons for the omission of TMZ in the NIVO + RT arm was to manage lymphopenia and immunosuppression; indeed, lymphopenia was more frequent in the TMZ + RT arm than in the NIVO + RT arm. Interestingly, HRQoL deteriorated numerically more rapidly in the TMZ + RT arm than in the NIVO + RT arm, consistent with expected effects of chemotherapy. However, time to deterioration results were affected by heavy censoring and should be interpreted with caution.

Our study found a statistically significant survival benefit with TMZ + RT over NIVO + RT despite tumor *MGMT* unmethylated status. mOS was 14.9 months (95% CI, 13.3 to 16.1) with TMZ + RT and 13.4 months (95% CI, 12.6 to 14.3) with NIVO + RT. These results were similar to those of previous studies, including the study conducted by Gilbert et al.,³⁵ which reported an mOS of 14.6 months (95% CI, 13.2 to 16.5) with TMZ + RT. The study conducted by Hegi et al.⁸ also produced similar results: an mOS of 12.7 months (95% CI, 11.6 to 14.4) with TMZ + RT and 11.8 months (95% CI, 9.7 to 14.1)

with RT alone. Several potential differences in studies exist, including patient selection, study design, and patient management. However, one caveat is that NIVO was compared with TMZ, and any potential benefit of combining NIVO with TMZ was not evaluated to fully assess the effects of NIVO.

In summary, we report on the largest phase III study conducted to date in patients with GBM molecularly selected for unmethylated *MGMT* promoter and the first to prospectively examine the omission of TMZ in this population. Overall, our results indicate that immunotherapy with NIVO is not a suitable replacement for chemotherapy with TMZ despite the chemoresistance of this difficult-to-treat patient population. Further immunotherapy efforts in GBM include alternative immune checkpoint inhibitors, vaccines, oncolytic viruses, and cell therapies. Additionally, immune checkpoint inhibitors combined with each other or with vaccines may also be explored.

Supplementary Material

Supplementary material is available at *Neuro-Oncology* online.

Keywords

newly diagnosed glioblastoma | nivolumab | radiotherapy | temozolomide | unmethylated *MGMT*

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Data Availability

The data sets presented in this article are not readily available because requestors must complete a data request on the BMS investigator portal. Requests to access the data sets should be directed to <https://fastrack-bms.force.com/Login>. Bristol Myers Squibb's policy on data sharing may be found at <https://www.bms.com/researchers-and-partners/independent-research/data-sharing-request-process.html>.

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INOVIO Announces Survival Results for INO-5401 + INO-9012 in Combination with Libtayo® (cemiplimab) in Patients with Newly Diagnosed GBM at ASCO Annual Meeting 2022

5/27/2022

INOVIO's DNA medicines immunotherapy in combination with Libtayo® elicits vaccine-associated immune responses when administered with RT/TMZ to newly diagnosed GBM patients

INO-5401 + INO-9012 + Libtayo® elicits cancer antigen-specific T cells

55% of MGMT methylated subjects remain alive at a median of 32.5 months

Dr. David Reardon, Principal Investigator, to present on June 6, 2022 at ASCO

PLYMOUTH MEETING, Pa., May 27, 2022 /PRNewswire/ -- INOVIO (NASDAQ: INO) announced results from the company's novel Phase 1/2 trial of INO-5401 and INO-9012 in combination with PD-1 inhibitor Libtayo® (cemiplimab) in the treatment of newly diagnosed glioblastoma (GBM), including encouraging median overall survival (OS) data from fifty-two subjects. Median OS duration in unmethylated MGMT (Cohort A) was 17.9 months. Median OS data in MGMT Methylated patients (Cohort B) are being presented for the first time, at a median of 32.5 months, which compares favorably to historical comparisons (23.2-25 months).

Overall, INO-5401 + INO-9012 is demonstrated to be tolerable and immunogenic when administered with Libtayo and RT/TMZ (radiation and temozolomide) to newly diagnosed GBM patients. Notably, INO-5401 elicited antigen-specific T cells that may infiltrate GBM tumors. The data from this study was selected to be presented in an oral presentation by Dr. David Reardon on Monday, June 6, 2022, at the 2022 American Society of Clinical Oncology (ASCO) at the McCormick Place Convention Center in Chicago, Illinois.

Presentation Details: June 6, 2022, 12:42 – 12:54 p.m. CDT

Presenting Author: David A. Reardon
Central Nervous System Tumors Session

Abstract #2004: Intramuscular (IM) INO-5401 + INO-9012 with electroporation (EP) in combination with cemiplimab (REGN2810) in newly diagnosed glioblastoma

Fifty-two subjects were enrolled: 32 in Cohort A; 20 in Cohort B (35% women; median age 60 years [range 19-78 years]). The adverse event profile was consistent with known single-agent (INO-5401, INO-9012, EP or Libtayo) events; most events were $\leq$ Grade 2 and no related events were Grade $\geq$ 4. Median OS durations in Cohorts A and B were 17.9 months (95% CI 14.5-19.8) and 32.5 months (95% CI 18.4-not reached), respectively. Flow cytometry revealed activated, antigen specific CD4+CD69+PD1+ and CD8+CD69+PD1+ T cells, the latter with lytic potential as defined by presence of perforin and granzyme A. Both subsets exhibited HR < 1.0 and p < 0.05 when accounting for a 0.1% T cell frequency change, translating to a 23% and 28% reduced risk of death at 18 months, respectively.

A post-hoc exploratory analysis showed that gene expression levels of INO-5401 antigens and immune cell markers from pre-treatment tumor tissues were similar between alive and deceased groups; however, the alive group displayed significant differential expression of genes regulating apoptosis, proliferation, and immune responses. Post-treatment tumor tissue displayed altered gene expression for immune-related markers versus pre-treatment tissue, including markers of T cell infiltration, activation, and lytic potential.

Dr. David Reardon, Clinical Director, Center for Neuro-Oncology of Dana-Farber Cancer Institute and coordinating principal investigator of the study said, "GBM remains one of the most aggressive and hard-to-treat cancers. The fact that we have seen this novel combination trial of a T cell generating DNA medicine combined with a PD-1 checkpoint benefit a large percent of trial participants past 32 months is very encouraging. These latest results and continued development are welcoming as it continues to improve upon a standard of care which was defined 17 years ago and remains sub-optimal for our patients with GBM."

Dr. Jeffrey Skolnik, INOVIO's Senior Vice President, Clinical Development, said, "We, along with our collaborative partner Regeneron, remain encouraged with the progress to date from this novel combination therapy study. As concluded in the abstract, INO-5401 + INO-9012 has an acceptable risk/benefit profile and elicits robust immune responses that may correlate with a potentially enhanced survival when administered with Libtayo and RT/TMZ to newly diagnosed GBM patients. Our goal is to build upon INO-5401's ability to elicit antigen-specific T cells that can infiltrate GBM tumors and complement the clinically-active profile of Libtayo to a potentially larger study in the future."

INO-5401, INO-9012, Libtayo, and the combination of these products have not been approved or evaluated by any Regulatory Authority worldwide for the treatment of newly diagnosed GBM.

Study Design

The trial was designed to evaluate safety, immunogenicity and efficacy of INO-5401 and INO-9012 in combination with Libtayo, with radiation and chemotherapy, in subjects with newly diagnosed glioblastoma (GBM). This is a Phase 1/2, open-label, multi-center trial conducted in 52 evaluable patients with GBM. There are two cohorts in this trial. Cohort A includes 32 participants with a tumor with an unmethylated O6-methylguanine-deoxyribonucleic acid (DNA) methyltransferase (MGMT) promoter. Cohort B includes 20 participants with a tumor with a MGMT methylated promoter. Both cohorts received INO-5401 and INO-9012 and Libtayo at the same doses and on the same dosing schedule, and both cohorts received radiation and TMZ. For more information of the clinical study, see www.clinicaltrials.gov, identifier NCT03491683.

About INO-5401 and INO-9012

INO-5401 encodes for INOVIO's SynCon® antigens for hTERT, WT1, and PSMA, and has the potential to be a powerful cancer immunotherapy in combination with checkpoint inhibitors. The National Cancer Institute previously highlighted hTERT, WT1, and PSMA among a list of important cancer antigens, designating them as high priorities for cancer immunotherapy development. These three antigens were reported to be over-expressed, and often mutated, in a variety of human cancers including glioblastoma, and targeting these antigens may prove efficacious in the treatment of patients with cancer. INO-9012 encodes for IL-12, which is a T cell immune activator.

About Glioblastoma (GBM)

GBM is the most common and aggressive type of brain cancer and remains a devastating disease for both patients and caregivers. Its prognosis is extremely poor, with very few new therapies approved over the last 10 years. The median overall survival for patients receiving standard of care therapy is approximately 15 to 22 months and the median progression-free survival is approximately 7-10 months. In the U.S., the estimated annual incidence of GBM is 11,362 cases or 3.21 cases per 100,000 persons and the median age at diagnosis is 65 years.

About INOVIO

INOVIO is a biotechnology company focused on developing and commercializing DNA medicines to help protect people from infectious diseases and help treat people with cancer and HPV-associated diseases. Our DNA medicines are delivered using our proprietary smart device to produce a robust and tolerable immune response against targeted pathogens and cancers.

Partners and collaborators include Advaccine, ApolloBio Corporation, AstraZeneca, The Bill & Melinda Gates Foundation, Coalition for Epidemic Preparedness Innovations, Defense Advanced Research Projects Agency/Joint

Program Executive Office for Chemical, Biological, Radiological and Nuclear Defense/Department of Defense, HIV Vaccines Trial Network, International Vaccine Institute, Kaneka Eurogentec, Medical CBRN Defense Consortium, National Cancer Institute, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Ology Bioservices, the Parker Institute for Cancer Immunotherapy, Plumblin Life Sciences, Regeneron, Richter-Helm BioLogics, Thermo Fisher Scientific, University of Pennsylvania, Walter Reed Army Institute of Research, and The Wistar Institute. For more information, visit www.inovio.com.

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This press release contains certain forward-looking statements relating to our business, including our plans to develop DNA medicines, our expectations regarding our research and development programs, including the planned initiation and conduct of preclinical studies and clinical trials and the availability and timing of data from those studies and trials, and our ability to successfully manufacture and produce large quantities of our product candidates if they receive regulatory approval. Actual events or results may differ from the expectations set forth herein as a result of a number of factors, including uncertainties inherent in pre-clinical studies, clinical trials, product development programs and commercialization activities and outcomes, our ability to secure sufficient manufacturing capacity to mass produce our product candidates, the availability of funding to support continuing research and studies in an effort to prove safety and efficacy of electroporation technology as a delivery mechanism or develop viable DNA medicines, our ability to support our pipeline of DNA medicine products, the ability of our collaborators to attain development and commercial milestones for products we license and product sales that will enable us to receive future payments and royalties, the adequacy of our capital resources, the availability or potential availability of alternative therapies or treatments for the conditions targeted by us or our collaborators, including alternatives that may be more efficacious or cost effective than any therapy or treatment that we and our collaborators hope to develop, issues involving product liability, issues involving patents and whether they or licenses to them will provide us with meaningful protection from others using the covered technologies, whether such proprietary rights are enforceable or defensible or infringe or allegedly infringe on rights of others or can withstand claims of invalidity and whether we can finance or devote other significant resources that may be necessary to prosecute, protect or defend them, the level of corporate expenditures, assessments of our technology by potential corporate or other partners or collaborators, capital market conditions, the impact of government healthcare proposals and other factors set forth in our Annual Report on Form 10-K for the year ended December 31, 2021, our Quarterly Report on Form 10-Q for the quarter ended March 31, 2022 and other filings we make from time to time with the Securities and Exchange Commission. There can be no assurance that any product candidate in our pipeline will be successfully developed, manufactured or commercialized, that

final results of clinical trials will be supportive of regulatory approvals required to market products, or that any of the forward-looking information provided herein will be proven accurate. Forward-looking statements speak only as of the date of this release, and we undertake no obligation to update or revise these statements, except as may be required by law.

View original content:<https://www.prnewswire.com/news-releases/inovio-announces-survival-results-for-ino-5401--ino-9012-in-combination-with-libtayo-cemiplimab-in-patients-with-newly-diagnosed-gbm-at-asco-annual-meeting-2022-301556446.html>

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A Randomized Trial of Bevacizumab for Newly Diagnosed Glioblastoma

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ABSTRACT

BACKGROUND

Concurrent treatment with temozolomide and radiotherapy followed by maintenance temozolomide is the standard of care for patients with newly diagnosed glioblastoma. Bevacizumab, a humanized monoclonal antibody against vascular endothelial growth factor A, is currently approved for recurrent glioblastoma. Whether the addition of bevacizumab would improve survival among patients with newly diagnosed glioblastoma is not known.

METHODS

In this randomized, double-blind, placebo-controlled trial, we treated adults who had centrally confirmed glioblastoma with radiotherapy (60 Gy) and daily temozolomide. Treatment with bevacizumab or placebo began during week 4 of radiotherapy and was continued for up to 12 cycles of maintenance chemotherapy. At disease progression, the assigned treatment was revealed, and bevacizumab therapy could be initiated or continued. The trial was designed to detect a 25% reduction in the risk of death and a 30% reduction in the risk of progression or death, the two coprimary end points, with the addition of bevacizumab.

RESULTS

A total of 978 patients were registered, and 637 underwent randomization. There was no significant difference in the duration of overall survival between the bevacizumab group and the placebo group (median, 15.7 and 16.1 months, respectively; hazard ratio for death in the bevacizumab group, 1.13). Progression-free survival was longer in the bevacizumab group (10.7 months vs. 7.3 months; hazard ratio for progression or death, 0.79). There were modest increases in rates of hypertension, thromboembolic events, intestinal perforation, and neutropenia in the bevacizumab group. Over time, an increased symptom burden, a worse quality of life, and a decline in neurocognitive function were more frequent in the bevacizumab group.

CONCLUSIONS

First-line use of bevacizumab did not improve overall survival in patients with newly diagnosed glioblastoma. Progression-free survival was prolonged but did not reach the prespecified improvement target. (Funded by the National Cancer Institute; ClinicalTrials.gov number, NCT00884741.)

From the University of Texas M.D. Anderson Cancer Center (M.R.G., T.S.A., J.S.W., P.D.B., I.W.T.-L., E.P.S., K.D.A.) and the University of Texas Health Science Center School of Nursing (T.S.A.), Houston; American College of Radiology (J.J.D., S.P., M.W.) and Thomas Jefferson University (M.W.-W.) — both in Philadelphia; the University of Chicago, Chicago (J.J.D.); Tel-Aviv Medical Center, Tel Aviv, Israel (D.T.B.); Cleveland Clinic, Cleveland (M.A.V.); the University of Utah, Salt Lake City (H.C.); Ohio State University, Columbus (A.C.); University of Wisconsin, Madison (R.J.); Mayo Clinic, Jacksonville, FL (K.A.J.); University of Virginia, Charlottesville (D.S.); Southeast Cancer Control Consortium, Winston-Salem, NC (V.W.S.); Barrow Neurologic Institute, Phoenix, AZ (D.G.B.); Emory University, Atlanta (W.J.C.); and the University of Maryland, Baltimore (M.P.M.). Address reprint requests to Dr. Gilbert at the Department of Neuro-Oncology, University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Unit 431, Houston, TX 77030.

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GLIOMASTOMA IS THE MOST COMMON primary malignant brain tumor in adults. After maximal surgical tumor resection, the current standard of care is based on a phase 3, randomized clinical trial conducted by the European Organization for Research and Treatment of Cancer and the National Cancer Institute of Canada, which showed that concurrent treatment with daily temozolomide and radiotherapy followed by maintenance temozolomide was superior to radiotherapy alone.^{1,2}

Despite the improvement in outcomes with this combined chemoradiotherapy approach, few patients survive beyond 5 years; therefore, new therapeutic strategies are needed.³ Angiogenesis is a prominent feature of glioblastoma, most commonly attributed to the autocrine and paracrine production of vascular endothelial growth factor A (VEGF-A), which up-regulates the VEGF signal-transduction pathway.^{4,5} Several approaches have been used to target this prominent component of the tumor biology. Small-molecule tyrosine kinase inhibitors of this pathway, such as cediranib and sorafenib, have shown minimal efficacy.^{6,7} Bevacizumab is a humanized monoclonal antibody against the VEGF-A ligand that binds to its circulating target, altering the kinetics of ligand binding to endothelial cells and down-regulating angiogenesis.⁸ Initial studies that explored the efficacy of bevacizumab in adults with recurrent glioblastoma showed clinical activity, including a reduction in tumor size, a prolongation of progression-free survival, and an overall lowering of glucocorticoid requirements to control tumor-related edema.^{9,10} These results led to the accelerated Food and Drug Administration approval of bevacizumab for patients with recurrent glioblastoma.

Preclinical models suggest that antiangiogenic therapies cause temporary vascular normalization, leading to improved blood flow, which in turn should improve the delivery of oxygen and chemotherapeutic agents, potentially enhancing the efficacy of both radiotherapy and chemotherapy.¹¹ As a collaborative effort of the Radiation Therapy Oncology Group (RTOG), the North Central Cancer Treatment Group (NCCTG), and the Eastern Cooperative Oncology Group (ECOG), we conducted a randomized, placebo-controlled, double-blind, phase 3 trial, called RTOG 0825, to test the hypothesis that antiangiogenic therapy improves the efficacy of the standard chemoradiotherapy for glioblastoma.

METHODS

STUDY PATIENTS

Patients were eligible for the study if they were at least 18 years of age and had newly diagnosed glioblastoma (World Health Organization [WHO] grade IV astrocytoma), as confirmed on central review. Additional eligibility criteria included a Karnofsky performance status of at least 70 (on a scale from 0 to 100, with higher numbers indicating a higher activity level) and adequate hematologic, renal, and hepatic function (Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). Patients with active cardiac disease or recent cerebrovascular events were excluded. In addition, patients were required to undergo an imaging study (computed tomography [CT] or magnetic resonance imaging [MRI]) performed within 1 week before study registration to rule out recent intracranial hemorrhage. Patients who were receiving glucocorticoids had to have received a stable or decreasing dose for the 5 days before study registration. Also required was the submission of a paraffin-embedded tumor-tissue block with a minimum of 1 cm² of tumor surface area before the initiation of radiotherapy.

All patients provided written informed consent. The study was approved by the institutional review board or the equivalent panel at each study center before patient enrollment.

STUDY TREATMENT

Patients had to be registered before the start of the concomitant radiotherapy and chemotherapy (for details, see Fig. S1 in the Supplementary Appendix). Fractionated, conformal radiotherapy or intensity-modulated radiotherapy (IMRT) was given at a daily dose of 2 Gy. Treatment was delivered 5 days a week for 6 weeks, for a total dose of 60 Gy. Conformal therapy was delivered to an initial volume consisting of the area of enhancement, the postoperative cavity plus surrounding edema (or other abnormality as seen on fluid-attenuated inversion recovery [FLAIR] images on MRI), and a 2-cm margin, for a total dose of 46 Gy in 23 fractions, followed by a boost of 14 Gy in 7 fractions to the area of enhancement plus the cavity and a 2.5-cm margin. IMRT was permitted within protocol-defined guidelines at institutions that fulfilled IMRT-specific quality requirements, and all patients underwent radiotherapy quality assurance with the use of pre-

defined guidelines. Treatment with temozolomide, at a dose of 75 mg per square meter of body-surface area, was started at the initiation of radiotherapy and was continued daily until the completion of radiotherapy, with a maximum of 49 doses.

Patients were randomly assigned to receive either bevacizumab or placebo in a permuted-block design.¹² Stratification factors were status with respect to O-6-methylguanine–DNA methyltransferase (MGMT) and a tumor-based molecular profile based on expression of nine genes.¹³ MGMT status was determined with the use of a quantitative methylation-specific polymerase-chain-reaction (PCR) assay performed centrally by OncoMethylome Sciences.¹⁴ The nine-gene assay was performed with the use of a PCR technique optimized for paraffin-embedded tumor samples, and results were dichotomized as either favorable or unfavorable.¹³

Bevacizumab (or placebo) was administered intravenously at a dose of 10 mg per kilogram of body weight every 2 weeks, starting at week 4 of radiotherapy, until disease progression, severe treatment-related toxicity, or completion of adjuvant therapy (maximum number of doses, 24 over 12 cycles).

Maintenance treatment with temozolomide began 4 weeks after the completion of radiotherapy at a starting dose of 150 mg per square meter for 5 consecutive days of a 28-day cycle, with an increase to 200 mg per square meter for subsequent cycles if no treatment-related adverse events of grade 2 or higher were noted. Treatment was planned for 6 cycles with the option of extension to a total of 12 cycles if there were no or only low-grade adverse events and there was evidence of continued benefit. Antiemetic therapy with the use of a 5-hydroxytryptamine receptor antagonist was strongly recommended. Pneumocystis prophylaxis was recommended for patients with CD4 counts of less than 200 per cubic millimeter. At the time of tumor progression, patients could be informed about their study-group assignment and either begin or continue a bevacizumab-containing regimen provided as part of the study.

PATIENT EVALUATION AND FOLLOW-UP

At baseline, all the patients underwent a physical examination that included a neurologic assessment, complete blood counts, blood chemical analyses (including tests of renal and hepatic function), and tumor imaging with either MRI (preferred) or CT, as well as a serum pregnancy test in women

of child-bearing age. Patients were invited to participate in a longitudinal evaluation of the net clinical benefits of the treatment (NCB substudy) with the use of the M.D. Anderson Symptom Inventory–Brain Tumor Module (MDASI-BT), a neurocognitive-function test battery (Hopkins Verbal Learning Test-Revised [HVLTR], Trail Making Test [TMT], and Controlled Oral Word Association [COWA]), and the European Organization for Research and Treatment of Cancer quality-of-life questionnaire with a brain-cancer module (EORTC QLQ-C30/BN20).^{15–18} Patients were administered the NCB substudy measures at the time of imaging studies. During radiotherapy, patients were assessed for adverse events weekly and underwent weekly complete blood counts and monthly blood chemical analyses. During the maintenance phase of treatment, patients underwent blood counts and blood chemical analyses on days 21 and 28 of each cycle.

A repeat tumor-imaging study was performed approximately 4 weeks after completion of radiotherapy and then before the initiation of cycle 4 of maintenance treatment (as well as before the initiation of cycles 7 and 10, if administered). Patients who completed adjuvant treatment underwent tumor imaging every 3 months until tumor progression. Response was assessed with the use of serial measures of the product of the two largest cross-sectional diameters, and progression was defined as an increase in tumor size by at least 25% or the development of a new lesion.¹⁹ Since early reactions to radiotherapy may emulate tumor progression, investigators were encouraged not to declare tumor progression within the first 12 weeks after completion of radiotherapy unless there was a new lesion or neurologic worsening.²⁰ Toxic effects were recorded and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 3.0.

PRIMARY END POINTS

The coprimary end points were the duration of overall survival from randomization, which was defined as the time until death from any cause, and the duration of progression-free survival, which was defined as the time until either disease progression or death.

STUDY OVERSIGHT

The trial, which was sponsored by the National Cancer Institute (which also provided the study

drug), was developed by the first and last authors in collaboration with the RTOG Brain Committee, the RTOG Statistical Group, the Cancer Therapy Evaluation Program at the National Cancer Institute, the NCCTG, and the ECOG. An unrestricted educational grant for support of the study was provided by Genentech, which had no role in the collection of data, analysis of findings, or preparation of this report. All treatment data were collected by the RTOG data center and reviewed by the first author. The analyses were performed by RTOG statisticians. Central review was performed on all pathological specimens. The first draft of the manuscript was written by the first author with support from all coauthors; all authors reviewed and approved the manuscript. No one who is not an author contributed to the preparation of the manuscript. All the authors vouch for the completeness and accuracy of the data and confirm that the study was conducted according to the protocol, which is available at NEJM.org.

STATISTICAL ANALYSIS

The trial was designed to concurrently provide a power of 80% for the detection of a 25% relative reduction in the risk of death (hazard ratio, 0.75) and a 30% relative reduction in the risk of either disease progression or death (hazard ratio, 0.70) in the bevacizumab group as compared with the placebo group. To control for type I errors in testing for the coprimary end points by means of the log-rank test,²¹ the threshold for statistical significance was set at a two-sided P value of 0.046 for overall survival and 0.004 for progression-free survival. The enrollment goal was 612 eligible patients, and a definitive analysis would be performed after 390 deaths had occurred. Interim monitoring with early stopping criteria for efficacy and futility was performed, as described in the study protocol, and was overseen by the RTOG data and safety monitoring committee.

We used the Kaplan–Meier method to estimate survival distributions and a Cox proportional-hazards model to calculate hazard ratios.^{22,23} To determine whether a molecularly defined subgroup had a selective survival benefit from the addition of bevacizumab to standard treatment, we performed protocol-specified subset analyses for each tumor molecular factor and for combinations of molecular profile and MGMT status. We used the Cox model to perform additional

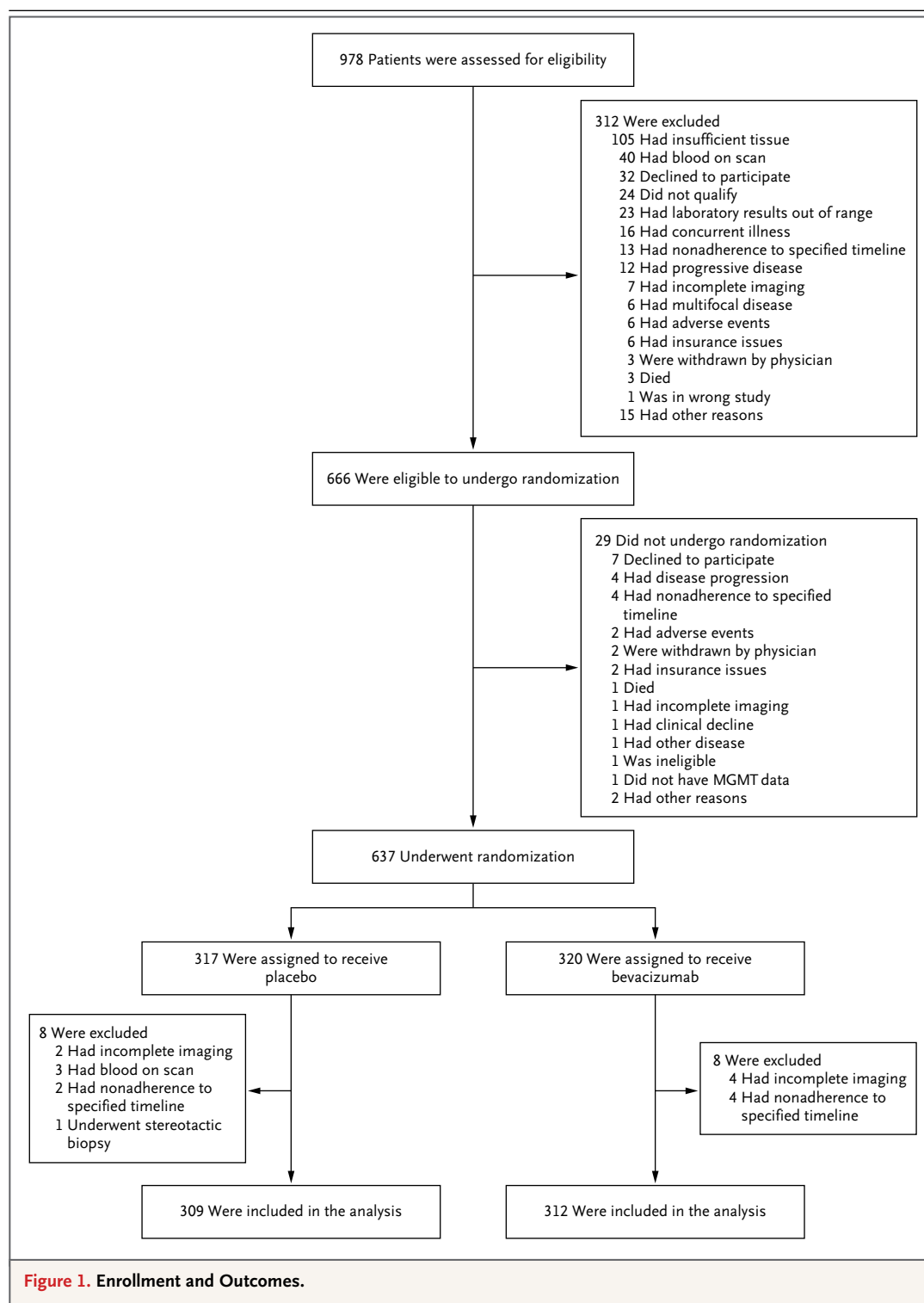
analyses that examined the effects of these factors and recursive partitioning analysis (RPA) class,¹³ a compilation of clinical factors that define a patient's prognosis, with classes ranging from I to VI and higher classes indicating a worse prognosis. This study enrolled patients in RPA classes III, IV, and V. For all these analyses, we used a likelihood-ratio test to evaluate differential treatment effects (interactions). We evaluated the proportionality of hazards using a test based on model residuals and smoothed hazard plots.^{24,25}

In the NCB substudy,¹⁸ we assessed net clinical benefits to determine whether there were differences in changes between the two study groups from baseline to week 46 in patient-reported outcomes (on the basis of the MDASI-BT and EORTC QLQ-C30/BN20) or neurocognitive function (HVL-R, TMT, and COWA). As specified in the trial protocol, these analyses were restricted to patients who were deemed to be progression-free at the time of the assessment. General linear models were used for longitudinal assessments, with fixed effects for study group and time factors and inclusion of MGMT status and RPA class to adjust for prognostic status. A treatment-by-time interaction effect was added to the model to determine whether there were between-group differences in patterns of response over time, with a P value of 0.05 considered to indicate statistical significance.

RESULTS

STUDY PATIENTS

From April 2009 through May 2011, we enrolled 978 patients, of whom 341 were deemed to be ineligible or otherwise unable to undergo randomization (Fig. 1). Patients who underwent randomization had clinical characteristics that were similar to those who did not undergo randomization (Table S2 in the Supplementary Appendix). A total of 637 patients (65% of those who were enrolled and 96% of those who were deemed to be eligible) were randomly assigned to receive either placebo (317 patients) or bevacizumab (320 patients); 621 patients were included in the final analysis. One patient who was found to have disease progression before randomization was not included in the analysis of progression-free survival. Baseline characteristics were well balanced between the two study groups (Table S3 in the Supplementary Appendix). The characteristics of patients who con-



sented to participate in the NCB substudy did not differ from the characteristics of those who were unwilling or unable to participate (Table S4 in the Supplementary Appendix). More than 80% of

the patients who underwent randomization consented to participate in the assessment of net clinical benefits. Only 20 patients could not participate because of native language constraints

(Table S5 in the Supplementary Appendix). The rate of completion of the test instruments remained high from baseline to week 46 (Table S7 in the Supplementary Appendix).

STUDY TREATMENT

In the bevacizumab group, 163 of 312 patients (52.2%) received at least 6 cycles of treatment (median number, 6). In the placebo group, 110 of 309 patients (35.6%) received at least 6 cycles of maintenance treatment (median number, 3). The full 12 cycles were administered in 57 patients (18.3%) in the bevacizumab group and in 59 patients (19.1%) in the placebo group. Tumor progression or death prompted treatment cessation in 73 of 268 patients (27.2%) in the bevacizumab group and in 135 of 274 patients (49.3%) in the placebo group. Toxicity or intercurrent illness resulted in treatment cessation in 88 patients (32.8%) in the bevacizumab group and in 45 patients (16.4%) in the placebo group.

At the time of this analysis, the treatment assignment had been revealed for 155 of 320 patients (48.4%) in the bevacizumab group and 178 of 317 patients (56.2%) in the placebo group. Among patients with disease progression, salvage treatment was planned for 87 of 155 patients (56.1%) in the bevacizumab group and 128 of 178 patients (71.9%) in the placebo group. Among these patients, the protocol-related bevacizumab regimen was continued in 39 patients (25.2%) in the bevacizumab group and was started in 86 patients (48.3%) in the placebo group.

TREATMENT OUTCOMES

Primary Analysis

At the time of analysis (December 2012), 208 of the 621 patients who were included in the primary analysis (33.5%) were still alive, with a median follow-up time of 20.5 months. The median overall survival was 15.7 months (95% confidence interval [CI], 14.2 to 16.8) in the bevacizumab group and 16.1 months (95% CI, 14.8 to 18.7) in the placebo group (hazard ratio for death in the bevacizumab group, 1.13; 95% CI, 0.93 to 1.37; $P=0.21$ by the log-rank test) (Fig. 2A).

Tumor progression or death occurred in 512 patients (82.4%). The median duration of progression-free survival was 10.7 months (95% CI, 10.0 to 12.2) in the bevacizumab group as compared with 7.3 months (95% CI, 5.9 to 7.9) in the placebo group (hazard ratio for progression or death, 0.79; 95% CI, 0.66

to 0.94; $P=0.007$ by the log-rank test) (Fig. 2B). The treatment effect for progression-free survival varied over time ($P<0.001$ for proportionality of hazards).

MGMT Status, Molecular Profile, and RPA Class

MGMT status was prognostic regardless of the study treatment. The median overall survival was 14.3 months (95% CI, 13.6 to 15.3) for patients with MGMT unmethylated tumors as compared with 23.2 months (95% CI, 20.1 to 28.3) for those with methylated tumors (hazard ratio for death in patients with unmethylated tumors, 2.10; 95% CI, 1.65 to 2.68; $P<0.001$). The median progression-free survival was 8.2 months (95% CI, 7.5 to 9.2) for patients with MGMT unmethylated tumors and 14.1 months (95% CI, 10.5 to 16.1) for those with methylated tumors (hazard ratio, 1.67; 95% CI, 1.36 to 2.05; $P<0.001$).

The nine-gene assay, which was developed in a separate trial involving more than 500 patients with glioblastoma who did not receive bevacizumab, was not prognostic in either study group on the basis of a specific favorable or unfavorable categorization of continuous data and a uniform setting of analytic specifications.

The RPA class was prognostic regardless of the study treatment. The median duration of overall survival was 20.1 months (95% CI, 16.7 to 35.7) for patients in class III, 15.6 months (95% CI, 14.6 to 17.2) for those in class IV, and 13.2 months (95% CI, 10.2 to 14.6) for those in class V ($P<0.001$). The median progression-free survival was 12.5 months (95% CI, 9.1 to 16.6) for patients in class III, 9.6 months (95% CI, 8.5 to 10.4) for those in class IV, and 7.1 months (95% CI, 5.3 to 8.7) for those in class V ($P=0.001$). Subset analyses that were based on the individual molecular components as well as various combinations of MGMT status and molecular profile were performed. No subset had improved survival with the addition of bevacizumab. These treatment effects were similar across RPA classes (Table 1).

Similarly, treatment effects after adjustment for MGMT status, molecular profile, and RPA class were unchanged: hazard ratio for death, 1.12 (95% CI, 0.91 to 1.37; $P=0.30$); and hazard ratio for disease progression or death, 0.80 (95% CI, 0.66 to 0.97; $P=0.02$). Testing for statistical interaction did not identify a significant differential effect of treatment according to combinations of MGMT status and molecular profiles or those factors individually.

NET CLINICAL BENEFITS

In the NCB substudy, we found greater deterioration over time in the bevacizumab group than in the placebo group on the basis of the between-group difference in the composite scores on the neurocognitive-function test battery ($P=0.05$), as well as the scores for the Controlled Oral Word Association Test ($P=0.003$) and the Trail Making Test, Part A ($P=0.04$). Longitudinal evaluation also revealed greater deterioration in the bevacizumab group on the basis of the MDASI-BT composite symptom score ($P=0.02$), composite symptom-interference score ($P<0.001$), and the scores for activity-related symptom interference ($P=0.004$), mood-related symptom interference ($P<0.001$), affective factors ($P=0.04$), cognitive factors ($P=0.01$), treatment factors ($P=0.03$), and generalized or disease factors ($P=0.01$), as well as EORTC QLQ-C30/BN20 scores for cognitive functioning ($P=0.008$), motor dysfunction ($P=0.02$), and communication deficit ($P=0.003$) (Table S8 in the Supplementary Appendix).

SAFETY AND TOXICITY

Safety and toxicity data were available for 300 patients in the placebo group and 303 patients in the bevacizumab group. During chemoradiotherapy with bevacizumab or placebo, serious lymphopenia was the most common toxic effect, occurring in approximately 10% of patients in the two study groups but without clinically significant opportunistic infection. Serious neutropenia was more common in the bevacizumab group than in the placebo group (7.3% vs. 3.7%), as was serious thrombocytopenia (10.2% vs. 7.7%) (Table 2).

During the maintenance phase, serious adverse events were more prevalent in the bevacizumab group than in the placebo group, including hypertension (4.2% vs. 0.9%), thromboembolic disease (7.7% vs. 4.7%), wound dehiscence (1.5% vs. 0.9%), fatigue (13.1% vs. 9.0%), visceral perforation (1.2% vs. 0.4%), and serious hemorrhage (1.5% vs. 0.9%). Serious neutropenia was more common in the bevacizumab group (10.0% vs. 5.1%), but thrombocytopenia was slightly less common (11.1% vs. 11.7%) (Table 2).

DISCUSSION

Two phase 2 studies have evaluated the use of bevacizumab in patients with newly diagnosed glioblastoma. Vredenburgh and colleagues treat-

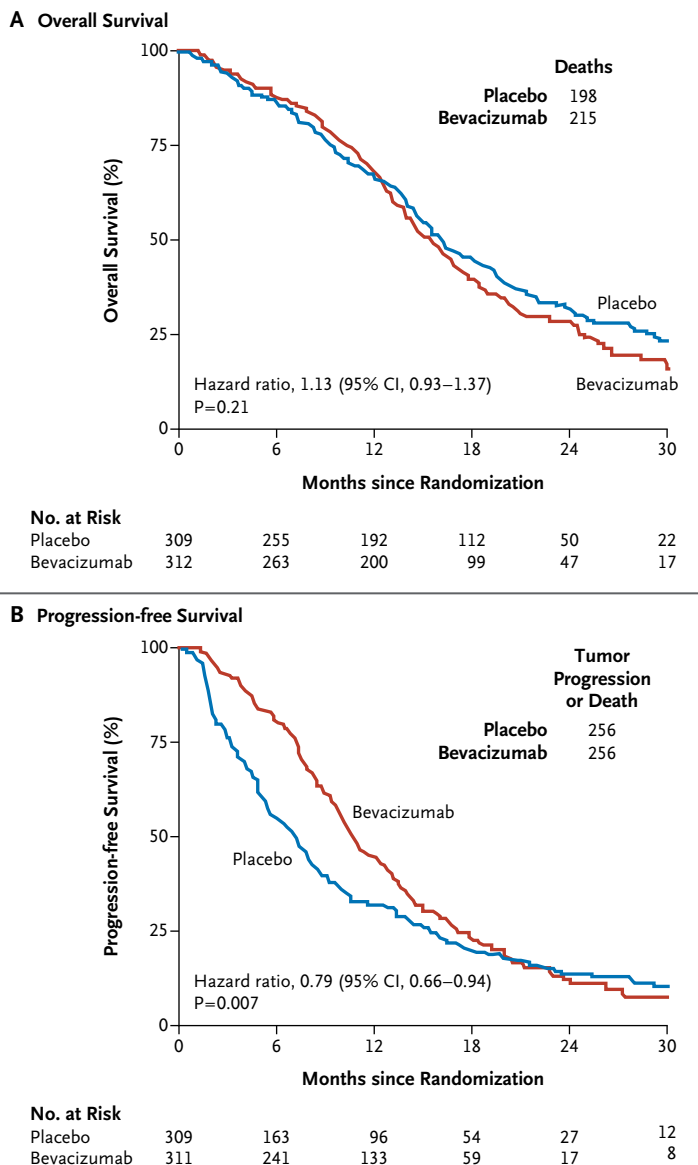


Figure 2. Primary End Points, According to Study Group.

The median rates of overall survival were similar in the bevacizumab and placebo groups (Panel A). The median rate of progression-free survival was higher in the bevacizumab group than in the placebo group but did not reach the prespecified threshold for significance ($P<0.004$) (Panel B).

ed 75 patients with radiotherapy, temozolomide, and bevacizumab, followed by adjuvant temozolomide, irinotecan, and bevacizumab.²⁶ The median overall survival was 21.2 months, which compared favorably with survival in historical controls. These results were very similar to those reported by Lai and collaborators, but they compared results in the study patients with those in a contemporary group of patients who received

Table 1. Study End Points, According to Genetic, Molecular, and Clinical Prognostic Subgroups.*

End Point and Subgroup	Bevacizumab <i>mo</i>	Placebo	Hazard Ratio (95% CI) [†]	P Value
All patients				
Median overall survival	15.7	16.1	1.13 (0.93–1.30)	0.21
Median progression-free survival	10.7	7.3	0.79 (0.66–0.94)	0.007
MGMT status and molecular profile				
Methylated MGMT				
Favorable molecular profile				
Median overall survival	16.7	25.0	2.27 (0.91–5.68)	0.07
Median progression-free survival	13.0	13.5	1.39 (0.67–2.89)	0.38
Unfavorable molecular profile				
Median overall survival	21.1	25.3	1.24 (0.73–2.12)	0.43
Median progression-free survival	16.9	8.4	0.63 (0.40–0.98)	0.04
Unmethylated MGMT				
Favorable molecular profile				
Median overall survival	13.9	14.6	1.02 (0.66–1.57)	0.94
Median progression-free survival	10.1	7.3	0.72 (0.48–1.07)	0.10
Unfavorable molecular profile				
Median overall survival	14.0	14.6	1.13 (0.86–1.49)	0.36
Median progression-free survival	9.8	5.4	0.86 (0.67–1.11)	0.25
RPA class				
III				
Median overall survival	20.6	19.8	0.98 (0.54–1.81)	0.96
Median progression-free survival	14.9	9.5	0.74 (0.43–1.25)	0.25
IV				
Median overall survival	15.7	15.6	1.14 (0.90–1.44)	0.29
Median progression-free survival	10.8	7.3	0.78 (0.63–0.96)	0.02
V				
Median overall survival	12.6	13.3	1.01 (0.66–1.56)	0.96
Median progression-free survival	9.8	4.4	0.70 (0.46–1.06)	0.10

* The recursive-partitioning analysis (RPA) classes are as follows: class III: an age of less than 50 years and a Karnofsky performance score of 90 or more (on a scale of 0 to 100, with higher scores indicating better function); class IV, an age of less than 50 years and a Karnofsky performance score of less than 90 (or an age of 50 years or more, a Karnofsky performance score of 70 or more, a gross total or partial tumor resection, and an ability to work); class V, an age of 50 years or more, a Karnofsky performance score of 70 or more, a gross total or partial tumor resection, and an inability to work (or an age of 50 years or more, a Karnofsky performance score of 70 or more, and tumor-biopsy specimen only; or an age of 50 years or more and a Karnofsky performance score of less than 70). CI denotes confidence interval.

[†] Hazard ratios are for death (for overall survival) and tumor progression or death (for progression-free survival) in the bevacizumab group, as compared with the placebo group.

standard temozolomide and radiotherapy, which showed an improvement in progression-free survival with bevacizumab (13.6 vs. 7.6 months) but not in overall survival (19.6 vs. 21.1 months).²⁷

Our study was designed to determine whether adding bevacizumab to the first-line treatment for glioblastoma would improve patient outcomes. We chose the coprimary end points, overall survival

Table 2. Serious Adverse Events.

Event	During Chemoradiotherapy				During Adjuvant Treatment			
	Bevacizumab (N=303)		Placebo (N=300)		Bevacizumab (N=260)		Placebo (N=233)	
	Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4	Grade 3	Grade 4
	<i>number of patients (percent)</i>							
Anemia	1 (0.3)	1 (0.3)	1 (0.3)	0	4 (1.5)	2 (0.8)	3 (1.3)	0
Leukopenia	10 (3.3)	6 (2.0)	4 (1.3)	3 (1.0)	20 (7.7)	2 (0.8)	14 (6.0)	0
Neutropenia	7 (2.3)	15 (5.0)	5 (1.7)	6 (2.0)	20 (7.7)	6 (2.3)	7 (3.0)	5 (2.1)
Lymphopenia	24 (7.9)	8 (2.6)	22 (7.3)	5 (1.7)	27 (10.4)	7 (2.7)	23 (10)	8 (3.4)
Thrombocytopenia	10 (3.3)	21 (6.9)	17 (5.7)	6 (2.0)	18 (6.9)	11 (4.2)	23 (10)	4 (1.7)
Fatigue	7 (2.3)	0	8 (2.7)	0	32 (12.3)	2 (0.8)	21 (9.0)	0
Nausea and vomiting	2 (0.7)	0	1 (0.3)	0	11 (4.2)	0	4 (1.7)	0
Wound dehiscence	3 (1.0)	0	1 (0.3)	0	3 (1.2)	1 (0.4)	2 (0.9)	0
Hypertension	4 (1.3)	0	1 (0.3)	0	11 (4.2)	0	2 (0.9)	0
Thromboembolic disease	6 (2.0)	8 (2.6)	3 (1.0)	8 (2.7) and 1 grade 5 (0.3)	11 (4.2)	8 (3.1) and 1 grade 5 (0.4)	7 (3.0)	4 (1.7)
Hemorrhage	0	0	1 (0.3)	0	3 (1.2)	1 grade 5 (0.4)	2 (0.9)	0
Visceral perforation	1 (0.3)	0	0	1 (0.3)	2 (0.8)	1 (0.4)	1 (0.4)	0

al and progression-free survival, because of the crossover design of the study. At the time of disease progression, the treatment assignment in the bevacizumab group could be revealed and patients could begin or continue a regimen containing bevacizumab. Therefore, the end point of overall survival was used to determine whether first-line use of bevacizumab was superior to use as a salvage regimen, and the end point of progression-free survival was used to evaluate the potential early benefits of combined treatment.

We found no benefit in overall survival with early administration of bevacizumab. Progression-free survival was prolonged for the patients receiving first-line bevacizumab, but the hazard ratio (0.79) and P value (0.007) did not reach the predefined criteria. However, we did not assess the efficacy of first-line treatment with bevacizumab in patients with unresected tumors, since the requirement for a tumor specimen excluded patients who had undergone only a diagnostic biopsy. In additional analyses of subgroups of patients defined prospectively on the basis of molecular markers, we did not identify a subgroup of patients who had a selected survival benefit from the early

administration of bevacizumab. Further investigations of molecularly defined subgroups may uncover a predictive marker panel for bevacizumab, which would require additional prospective testing.

A similar study, Avastin [bevacizumab] in Glioblastoma (AVAGlio), which also evaluated the role of bevacizumab as first-line treatment in patients with newly diagnosed glioblastoma, was recently completed.²⁸ That study showed a result for overall survival that was similar to our finding but showed a different result for progression-free survival on the basis of a statistical design that included a different prespecified alpha level for progression ($P < 0.01$). The AVAGlio study also evaluated patient-reported outcomes but restricted those data primarily to health-related quality-of-life measures, whereas we also collected measures of symptom burden and interference and the results of objective tests of neurocognitive function.

In our study, we evaluated longitudinal changes in both objective and patient-reported neurocognitive function and in self-reported quality of life and other symptoms in patients who were deemed to be progression-free. These assessments consistently showed that patients receiving bevacizumab, as compared with placebo, had greater deteriora-

tion in scores on objective tests of neurocognitive function as well as in perceived cognitive function, suggesting either unrecognized tumor progression or bevacizumab-related neurotoxicity.^{29,30} In addition, among patients without tumor progression on imaging studies, those who were initially treated with bevacizumab reported greater deterioration in the perceived severity of their symptoms, as assessed on the basis of both patient-reported outcomes and symptom-associated interference with daily activities.

In conclusion, we did not observe an overall survival advantage with first-line use of bevacizumab in patients with newly diagnosed glioblastoma. Furthermore, higher rates of neurocog-

nitive decline, increased symptom severity, and decline in health-related quality of life were found over time among patients who were treated with bevacizumab.

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Dose-Dense Temozolomide for Newly Diagnosed Glioblastoma: A Randomized Phase III Clinical Trial

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See accompanying article on page 4076

ABSTRACT

Purpose

Radiotherapy with concomitant and adjuvant temozolomide is the standard of care for newly diagnosed glioblastoma (GBM). O⁶-methylguanine-DNA methyltransferase (*MGMT*) methylation status may be an important determinant of treatment response. Dose-dense (DD) temozolomide results in prolonged depletion of *MGMT* in blood mononuclear cells and possibly in tumor. This trial tested whether DD temozolomide improves overall survival (OS) or progression-free survival (PFS) in patients with newly diagnosed GBM.

Patients and Methods

This phase III trial enrolled patients older than age 18 years with a Karnofsky performance score of ≥ 60 with adequate tissue. Stratification included clinical factors and tumor *MGMT* methylation status. Patients were randomly assigned to standard temozolomide (arm 1) or DD temozolomide (arm 2) for 6 to 12 cycles. The primary end point was OS. Secondary analyses evaluated the impact of *MGMT* status.

Results

A total of 833 patients were randomly assigned to either arm 1 or arm 2 (1,173 registered). No statistically significant difference was observed between arms for median OS (16.6 v 14.9 months, respectively; hazard ratio [HR], 1.03; $P = .63$) or median PFS (5.5 v 6.7 months; HR, 0.87; $P = .06$). Efficacy did not differ by methylation status. *MGMT* methylation was associated with improved OS (21.2 v 14 months; HR, 1.74; $P < .001$), PFS (8.7 v 5.7 months; HR, 1.63; $P < .001$), and response ($P = .012$). There was increased grade ≥ 3 toxicity in arm 2 (34% v 53%; $P < .001$), mostly lymphopenia and fatigue.

Conclusion

This study did not demonstrate improved efficacy for DD temozolomide for newly diagnosed GBM, regardless of methylation status. However, it did confirm the prognostic significance of *MGMT* methylation. Feasibility of large-scale accrual, prospective tumor collection, and molecular stratification was demonstrated.

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INTRODUCTION

Glioblastoma (GBM) is the most common primary malignant brain tumor in adults. The recent phase III randomized clinical trial performed by the European Organisation for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) established the current standard of care for newly diagnosed GBM, which involves surgery or regional radiotherapy with concomitant temozolomide daily during the radiation therapy.¹ Following completion of radiotherapy, patients undergo six cycles of single-agent temozolomide (5

days of a 28-day cycle). However, even with this treatment, the outcome for most patients with GBM remains grim, with a 2-year survival rate of only 27%.

Tumor specimens from a subset of patients enrolled onto the EORTC/NCIC clinical trial were evaluated for epigenetic changes in the promoter region of the O⁶-methylguanine-DNA methyltransferase (*MGMT*) DNA repair gene.² The *MGMT* enzyme has been established as a major mechanism of resistance to alkylating agents, such as temozolomide.³ In this study of 203 tumor samples, epigenetic silencing by methylation of the promoter

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region of the *MGMT* gene was associated with statistically significant improvement in overall survival (OS) that was most marked in patients receiving the combined radiotherapy and temozolomide regimen. These findings suggest that modulation of *MGMT* enzymatic activity may increase sensitivity and response to temozolomide regimens.

Exposure to radiation has been shown to upregulate *MGMT*, whereas prolonged exposure to alkylating agents has been shown to deplete intracellular *MGMT* in peripheral blood mononuclear cells.^{4,5} Dose-dense (DD) schedules of temozolomide, designed to reduce *MGMT* levels and exhaust activity, have been tested in patients with recurrent or progressive GBM (reviewed in Wick et al⁶). Nonrandomized studies that tested a variety of treatment schedules reported treatment efficacy that compares favorably with the established temozolomide single-agent schedule without an apparent increase in treatment-related toxicity.^{7,8} The impact of two temozolomide schedules on *MGMT* activity were compared by using peripheral blood mononuclear cells as a surrogate for tumor.⁵ Both the alternating 7-days-on, 7-days-off and the 21-of-28-days schedules were shown to deplete *MGMT* activity. However, the latter schedule resulted in more prolonged and lower *MGMT* activity.

To test the hypothesis that prolonged exposure to temozolomide improves survival in patients with newly diagnosed GBM, a randomized phase III clinical trial comparing the standard adjuvant temozolomide treatment (days 1 through 5 of a 28-day cycle) with the DD schedule (days 1 through 21 of a 28-day cycle) was initiated as a collaborative effort involving the Radiation Therapy Oncology Group (RTOG), the EORTC, and the North Central Cancer Therapy Group (NCCTG).

serum AST $\leq 3 \times$ the upper limit for the laboratory). Patients taking corticosteroids had to be taking a stable or decreasing dose for the 5 days before study registration. Submission of a tumor tissue block with a minimum of 1 cm² of tumor by day 14 of radiotherapy was a requirement. Before patient accrual, all patients were required to provide informed consent after study approval by an institutional review board or ethics committee.

Study Design and Treatment

The study schema is provided in Figure 2. Patients were required to be registered before the initiation of the concomitant radiotherapy and chemotherapy. Radiotherapy consisted of fractionated, conformal radiation given at a daily dose of 2 Gy. Treatment was delivered 5 days a week for a total of 6 weeks to a total dose of 60 Gy. Two radiotherapy protocols were allowed. In North America (RTOG, NCCTG), an initial volume consisting of enhancement, postoperative cavity, plus surrounding edema (or fluid-attenuated inversion-recovery [FLAIR] abnormality defined by magnetic resonance imaging [MRI]) and a 2-cm margin received 46 Gy in 23 fractions followed by a boost of 14 Gy in seven fractions to the area of enhancement plus the cavity and a 2.5-cm margin. In European (EORTC) centers, a single planning volume was used to deliver 60 Gy in 30 fractions to the area of enhancement and the cavity with a 2- to 3-cm margin. Temozolomide at a dose of 75 mg/m² was started along with the radiotherapy and was continued on a daily basis until completion of radiation treatment, with a maximum of 49 doses. During the concomitant radiotherapy and temozolomide treatment, prophylaxis against *Pneumocystis jiroveci* pneumonia was required. Antiemetic prophylaxis was recommended at initiation of the concomitant radiotherapy and chemotherapy regimen.

Patients were randomly assigned after completion of the concomitant radiotherapy and chemotherapy treatment to either standard or DD temozolomide in a permuted block design by using the method described by Zelen.⁹ Stratification factors included the RTOG recursive partitioning analysis (RPA) class (a compilation of clinical prognostic factors including age, performance status, extent of tumor resection, and neurologic function¹⁰), *MGMT* gene promoter methylation status, and radiotherapy plan (RTOG or EORTC). *MGMT* gene promoter methylation status was determined by using a quantitative methylation-specific polymerase chain reaction assay as described¹¹ and performed centrally by Oncomethylome Science.

Adjuvant temozolomide treatment was initiated 4 weeks after completion of radiotherapy. Patients on the standard treatment arm received temozolomide at a starting dose of 150 mg/m² for 5 consecutive days of a 28-day cycle, and temozolomide was increased for subsequent cycles to 200 mg/m² if no treatment-related adverse events greater than grade 2 were noted. Treatment was planned for six cycles with the potential to extend treatment to a total of 12 cycles if treatment was well tolerated and there was evidence of continued benefit defined as either continued tumor response based on serial MRI, progressive improvement in the patient's performance status or neurologic

PATIENTS AND METHODS

Patients

Patients were eligible for the study if they were older than age 18 years with a newly diagnosed, histologically confirmed GBM (WHO grade 4 astrocytoma; Fig 1). Additional eligibility criteria included a Karnofsky performance score of at least 60 and adequate hematologic, renal, and hepatic function (defined as an absolute neutrophil count $\geq 1,500$ /mL, platelet count $\geq 100,000$ /mL, serum creatinine ≤ 1.7 mg/dL, serum blood urea nitrogen ≤ 25 mg/dL, serum total bilirubin ≤ 2.0 mg/dL, and serum ALT and

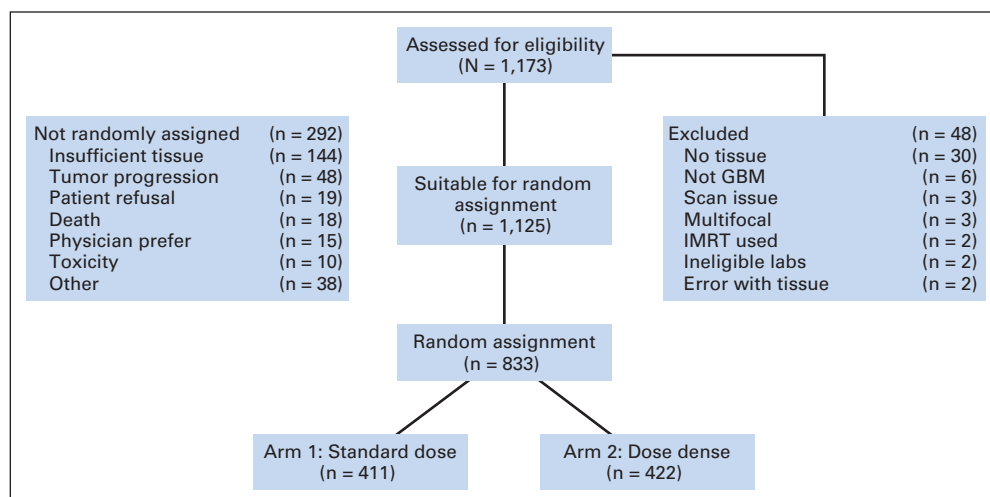


Fig 1. CONSORT diagram outlining accrual and eligibility. GBM, glioblastoma; IMRT, intensity-modulated radiotherapy.

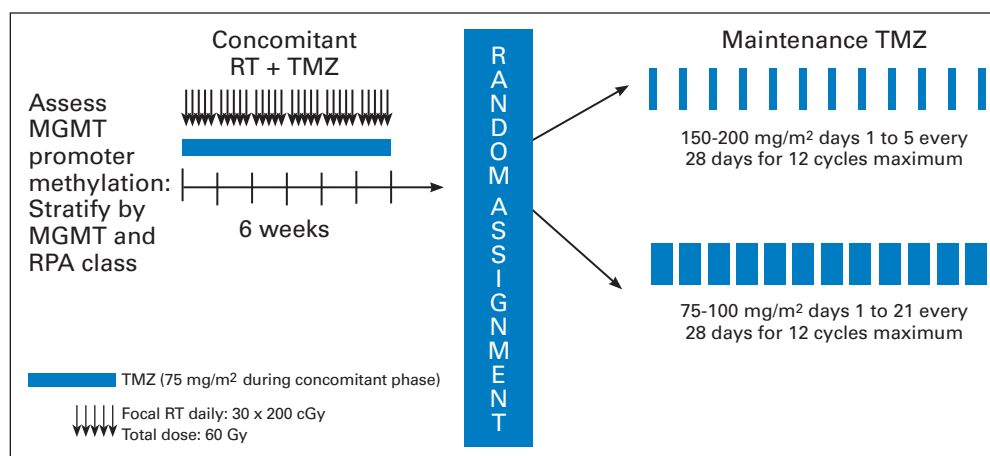


Fig 2. Protocol schema. MGMT, O⁶-methylguanine-DNA methyltransferase; RPA, recursive partitioning analysis; RT, radiotherapy; TMZ, temozolomide.

function, or a decreasing requirement for corticosteroids. Patients randomly assigned to the DD treatment arm received an initial dose of 75 mg/m² for 21 consecutive days of a 28-day cycle, which was increased for subsequent cycles to 100 mg/m² if no treatment-related adverse events greater than grade 2 were noted. As with the standard dose arm, six cycles were planned with the potential to extend to a total of 12 cycles if the previously described criteria for benefit were met. Antiemetic therapy using a 5-hydroxytryptamine antagonist was strongly recommended for all patients. *P. jiroveci* pneumonia prophylaxis was recommended for patients with CD4 counts less than 200/mL.

Patient Evaluation and Follow-Up

Baseline evaluation included a neurologic evaluation, complete blood counts, blood chemistries including renal and hepatic function, a serum pregnancy test (as appropriate), and tumor imaging with either MRI scans (preferred) or computed tomography. A subset of patients were enrolled onto the Net Clinical Benefits study to evaluate the impact of tumor and treatment. They underwent baseline as well as longitudinal testing with the MD Anderson Symptom Inventory for Brain Tumors (MDASI-BT); a neurocognitive function battery; and the EORTC QLQ30/BN20 (the EORTC general quality-of-life and brain cancer-specific quality-of-life questionnaire).¹²⁻¹⁴ During radiotherapy, patients were assessed weekly for adverse events and underwent weekly complete blood counts and monthly blood chemistries. During the adjuvant phase of treatment, patients in the standard adjuvant treatment arm had blood counts and blood chemistries measured on days 21 and 28 of each cycle; patients in the DD treatment arm had blood testing on days 14, 21, and 28 of each cycle. A repeat tumor imaging study was performed approximately 4 weeks after completion of radiotherapy and before initiation of cycle 4 (and 7 and 10, if given). Patients who completed adjuvant treatment underwent tumor imaging every 3 months until tumor progression. Assessment of response was made by using serial measures of the product of the two largest cross-sectional diameters, and progression was defined as an increase in tumor size by at least 25% or development of a new lesion.¹⁵ In light of the recognition of early radiation reactions emulating tumor progression, investigators were encouraged not to declare tumor progression within the first 12 weeks after completion of radiation unless there was either a new lesion or the patient had neurologic worsening.¹⁶ Toxicities were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events version 3.0.

Statistical Considerations

The primary end point was OS. Secondary end points included progression-free survival (PFS) and toxicities associated with each treatment. The hypothesis was that there would be at least 20% reduction in hazard rate for the DD temozolomide arm, corresponding to a median survival time of 17.5 months. A one-sided log-rank test at a significance level of 0.025 would have 80% power to detect this survival difference with a sample size of 750 randomly assigned patients (647 deaths were required for the final analysis).

With 750 analyzable patients, the study had at least 90% power to detect a 2-month increase in PFS for patients treated with DD temozolomide.

The statistical analysis is based on the modified intent-to-treat principle (including all of the eligible and randomly assigned patients, regardless of treatment receipt). OS and PFS were estimated by using the Kaplan-Meier method, and differences between treatment groups were tested by using the log-rank test.^{17,18} The Cox proportional hazards model was used to estimate the treatment hazard ratios (HRs) associated with each end point while adjusting for stratification factors.¹⁹ OS was defined as the interval from random assignment to death as a result of any cause or the last follow-up date on which the patient was reported alive. PFS was defined as the interval from random assignment to progression or death or being censored at the last clinical or radiology assessment date on which the patient was reported alive without progression. For all end points except the OS comparison between two treatments for the study, a *P* value of less than .05 (two-sided) was defined as the significance threshold. All of the analyses were performed by using SAS version 9 (SAS Institute, Cary, NC).

RESULTS

Patient Demographics

The study opened for accrual in January 2006 and closed to accrual in June 2008 with a total of 1,173 patients entered (Fig 1). Among registered patients, 1,125 were eligible for concurrent radiotherapy and temozolomide and 48 (4%) were ineligible. A total of 833 patients (74% of those enrolled) were randomly assigned for the adjuvant stage of treatment. Inadequate tissue (*n* = 144) and early disease progression (*n* = 48) were the most frequent reasons for not being randomly assigned to one of the two study arms. The characteristics of the two treatment groups were well balanced at baseline (Table 1). Tumor tissue blocks were submitted from 1,141 (97%) of the enrolled patients. All randomly assigned patients had centrally confirmed GBM. MGMT gene promoter methylation status determination was available for 91% of samples.

Treatment Administration

At the completion of radiotherapy, patients were randomly assigned to receive either standard dose (*n* = 411) or DD temozolomide (*n* = 422). For the standard dose arm, the median number of cycles was three, and 155 patients (37%) received at least six cycles of temozolomide, whereas for the DD arm, the median number of cycles was

Table 1. Patient Characteristics

Characteristic	Standard Dose Temozolomide (arm 1)		Dose-Dense Temozolomide (arm 2)	
	No.	%	No.	%
Age, years				
< 50	112	27	111	26
≥ 50	299	73	311	74
Sex				
Male	239	58	237	56
Female	172	42	185	44
Race/ethnicity				
White	319	78	328	78
Nonwhite	13	3	15	3
Unknown	79	19	79	19
KPS				
60-80	138	34	146	35
90-100	273	66	276	65
Surgery				
Biopsy	14	3	13	3
Partial resection	167	41	188	45
Total resection	230	56	221	52
Radiation				
RTOG/NCCTG	337	82	349	83
EORTC	74	18	73	17
Neurologic function				
No symptoms	140	34	147	35
Minor symptoms	185	45	196	45
Moderate symptoms	84	20	75	18
Severe symptoms	2	1	4	1
MGMT status				
Methylated	122	30	123	29
Unmethylated	254	62	263	62
Unknown (invalid, indeterminate)	35	8	36	9
RPA class				
III	85	21	86	20
IV	251	61	259	61
V	75	18	77	19

Abbreviations: EORTC, European Organisation for Research and Treatment of Cancer; KPS, Karnofsky performance status; MGMT, O⁶-methylguanine-DNA methyltransferase; NCCTG, North Central Cancer Therapy Group; RPA, recursive partitioning analysis; RTOG, Radiation Therapy Oncology Group.

four, and 181 patients (43%) received at least six cycles of treatment. More than six cycles of adjuvant treatment were given to 117 and 122 patients on the standard and DD arms, respectively. The full 12 cycles of adjuvant treatment were administered to 57 and 59 patients on the standard and DD arms, respectively. Tumor progression or death prompted treatment cessation in 255 patients (62%) on the standard dose arm and 211 patients (50%) on the DD arm. Toxicity or intercurrent illness resulted in treatment cessation in 49 patients (12%) on the standard dose arm and 94 patients (22%) on the DD arm. The median total dose of temozolomide administered was 7,500 mg in the standard dose arm and 17,010 mg in the DD arm.

Treatment Outcomes

At the time of final study analysis (using data through January 6, 2011), 219 patients (20%) were still alive, with a median follow-up time of 31.9 months. The median OS and PFS (from the time of

Table 2. Outcomes Data From Study Registration

Patient Group	No. of Patients	OS (months)	PFS (months)	2-Year Survival Rate (%)
All eligible	1,120	16.0	7.5	
All randomly assigned	833	17.7	8.2	
Arm 1	411	18.9	7.5	34.2
Arm 2	422	16.8	8.8	33.9
MGMT				
Methylated	245	23.2	10.5	47.3
Unmethylated	517	16.0	7.8	25.4
Methylated				
Arm 1	122	23.5	8.8	
Arm 2	123	21.9	11.7	
Unmethylated				
Arm 1	254	16.6	7.1	
Arm 2	263	15.4	8.2	

Abbreviations: MGMT, O⁶-methylguanine-DNA methyltransferase; OS, overall survival; PFS, progression-free survival.

registration) were 16 months (95% CI, 15.2 to 16.7 months) and 7.5 months (95% CI, 7.1 to 8.0 months), respectively.

For the primary analyses of this study, OS and PFS were measured from the time of random assignment. For reference, summary outcome data are provided from study registration in Table 2. There were 652 deaths among the 833 randomly assigned patients, with 22% of patients still alive. The median follow-up from random assignment was 31.5 months. The median OS was 16.6 months (95% CI, 14.9 to 18.0 months) for patients on the standard dose arm and 14.9 months (95% CI, 13.7 to 16.5 months) for patients on the DD arm (Fig 3A). The *P* value from the one-sided log-rank test was .63 with an associated HR of 1.03 (95% CI, 0.88 to 1.20) for DD versus standard dose. The protocol-specified six cycles of maintenance therapy were completed in 336 patients, but 75 (22%) stopped because of progression or toxicity. Only 22 (7%) stopped at treatment completion, and 239 (71%) received additional cycles. The median OSs for those 22 and 239 patients are 24.9 months (95% CI, 19.2 to 36.2 months) and 30.2 months (95% CI, 25.5 to 35.4 months), respectively.

At the time of analysis, 753 patients (91%) had experienced tumor progression or had died. The median PFS was 5.5 months (95% CI, 4.7 to 6.1 months) for the standard dose arm and 6.7 months (95% CI, 6.2 to 7.7 months) for the DD arm, yielding a two-sided *P* value of .06 with an associated HR of 0.87 (95% CI, 0.75 to 1.00; Fig 3B).

MGMT methylation status was available for 762 of the randomly assigned patients. In this cohort, there were 595 deaths and disease progression occurred in an additional 93 patients. The median OS was 21.2 months (95% CI, 17.9 to 24.8 months) and 14.0 months (95% CI, 12.9 to 14.7 months) for tumors with methylated and unmethylated MGMT gene promoter, respectively. The HR (methylated *v* unmethylated) was 0.58 (95% CI, 0.48 to 0.69; *P* < .001; Fig 4A). Similarly, the median PFS was 8.7 months (95% CI, 6.6 to 11.2 months) and 5.7 months (95% CI, 5.1 to 6.1 months), respectively, for tumors with methylated and unmethylated MGMT gene promoter with an HR (methylated *v* unmethylated) of 0.61 (95% CI, 0.52 to 0.73; *P* < .001; Fig 4B).

For patients with tumors that were designated as MGMT unmethylated (*n* = 517), there were 433 deaths and 53 additional patients with tumor progression. The median OS was 14.6 months (95%

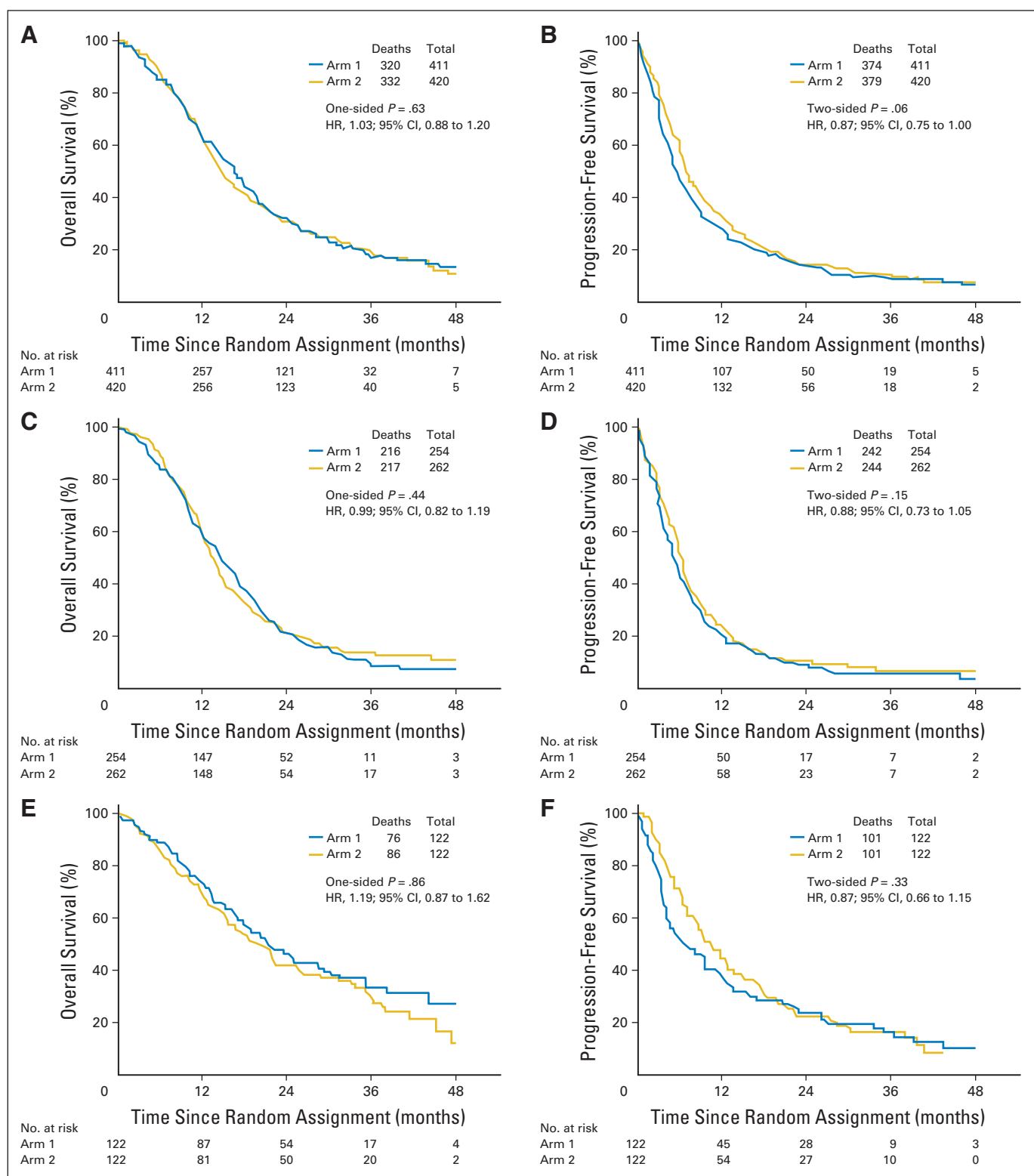


Fig 3. Outcomes comparing standard and dose-dense temozolomide therapy. (A, B) Overall and progression-free survival for all randomly assigned patients. (C, D) Overall and progression-free survival for patients with O^6 -methylguanine-DNA methyltransferase (*MGMT*) unmethylated tumors. (E, F) Overall and progression-free survival for patients with *MGMT* methylated tumors. HR, hazard ratio.

CI, 13.2 to 16.5 months) for standard dose and 13.3 months (95% CI, 12.3 to 14.3 months) for the DD arm (HR, 0.99; 95% CI, 0.82 to 1.19; $P = .44$; Fig 3C). Median PFS was 5.1 months (95% CI, 4.3 to 5.7 months) for the standard arm and 6.0 months (95% CI, 5.5 to 6.5

months) for the DD arm (HR, 0.88; 95% CI, 0.73 to 1.05; $P = .15$; Fig 3D). For patients with *MGMT* methylated tumors ($n = 245$), there were 162 deaths and an additional 40 patients with tumor progression. The median OS was 21.4 months (95% CI, 17.6 to 29.0 months) for

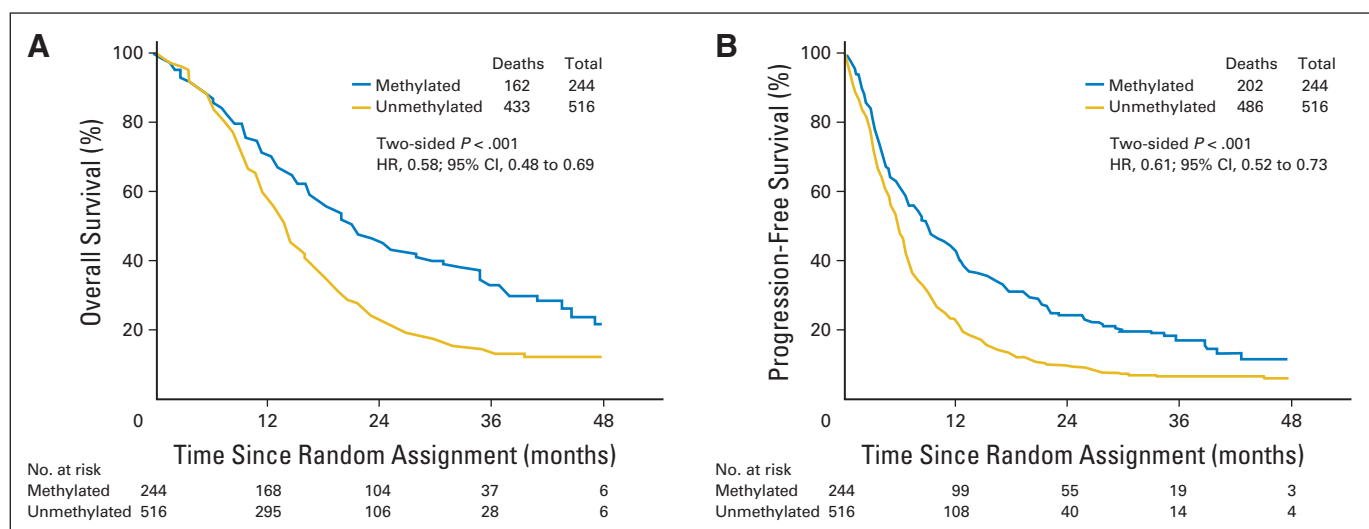


Fig 4. Outcomes based on tumor O⁶-methylguanine-DNA methyltransferase (MGMT) methylation status. (A) Overall survival. (B) Progression-free survival. HR, hazard ratio.

the standard arm and 20.2 months (95% CI, 15.4 to 25.1 months) for the DD arm (HR, 1.19; 95% CI, 0.87 to 1.62; $P = .86$; Fig 3E). Median PFS was 6.5 months (95% CI, 4.1 to 9.6 months) for the standard arm and 10.1 months (95% CI, 7.9 to 12.4 months) for the DD arm (HR, 0.87; 95% CI, 0.66 to 1.15; $P = .33$; Fig 3F).

Cox proportional hazard modeling for OS and PFS included recursive partitioning analysis (RPA) class (III, IV, V), tumor MGMT status (methylated, unmethylated), and radiation type (European ν US). For both the OS and PFS end points, RPA class and MGMT status remained in the model. After adjusting for these factors, the OS HR for DD versus standard treatment was 1.06 (95% CI, 0.90 to 1.24; $P = .51$) and the PFS HR for DD versus standard treatment was 0.89 (95% CI, 0.77 to 1.03; $P = .12$). Neither a global test for interaction of treatment with covariates of MGMT methylation status, RPA class, or radiation type nor any pairwise tests of interaction were significant.

Safety and Toxicity

Adverse events were evaluated separately during the concurrent chemotherapy and radiotherapy and the adjuvant treatment phase. Grade 3 or 4 lymphopenia was the most common toxicity occurring in 12% of patients but without significant opportunistic infections. Serious neutropenia and thrombocytopenia occurred in 3.6% and 6.8%, respectively, of all patients undergoing radiation. There was one treatment-related death as a result of neutropenia.

During the adjuvant phase of treatment, grade 3 to 5 adverse events were more prevalent with the DD ($n = 194$ of 369) compared with standard dose treatment ($n = 120$ of 351). However, most of the difference was because of a higher number of patients with lymphopenia (107 ν 51) and fatigue (33 ν 12). Overall, neutropenia and thrombocytopenia were infrequent and were similar between the two arms (Table 3).

DISCUSSION

The landmark study performed by the EORTC and NCIC provided the first Level 1 evidence for the clinical benefit of chemotherapy for patients with newly diagnosed GBM.¹ MGMT has been proposed as a

major mechanism of resistance to alkylating agents and, therefore, tumor cells with low levels of MGMT expression were expected to have a better response to these drugs.⁴ A companion correlative subset study to the EORTC trial demonstrated a relationship between the tumor promoter region methylation status of the MGMT gene and outcome.² Tumor cells with high levels of MGMT protein are thought to be resistant to alkylating agents, and it would follow that successful depletion of intracellular MGMT would be expected to enhance treatment response.

The study reported here sought to determine whether prolonged exposure to an alkylating agent with known activity in GBM would improve patient outcomes as measured by OS or PFS. The DD schedule was designed to deplete cellular MGMT and restore sensitivity to temozolomide.⁵ Alternatively, in tumor cells with no or low MGMT expression, the DD schedule was projected to improve response by an enhancement of tumor cell exposure, as has been demonstrated with other cancer regimens, particularly in breast cancer.²⁰ Unfortunately, no therapeutic benefits were detected for the DD regimen. A recent study that evaluated temozolomide dosing regimens in chemotherapy-naïve patients with recurrent high-grade gliomas did not demonstrate improved survival for the DD temozolomide regimen using the same

Table 3. Serious Toxicities Associated with Standard Dose and Dose-Dense Temozolomide Treatment

Category	Grade 3				Grade 4			
	Standard Dose		Dose Dense		Standard Dose		Dose Dense	
	No.	%	No.	%	No.	%	No.	%
Anemia	4	1	4	1	0		0	
Leukopenia	13	4	33	9	7	2	3	1
Neutropenia	16	5	29	8	8	2	7	2
Lymphopenia	41	12	76	20	10	3	31	9
Thrombocytopenia	20	6	18	5	13	4	8	2
Fatigue	12	3	33	9	0		0	
Nausea/vomiting	5	1	8	2	0		0	

21-of-28-days schedule.²¹ The investigators postulated that peak temozolomide concentrations, rather than prolonged exposure, may be most important for treatment efficacy. Furthermore, comparison of the OS results with those in prior studies is limited by differences in eligibility (age, need for tumor resection) and loss of patients before random assignment.

Although a therapeutic advantage for DD temozolomide was not established, the study provides important information and precedence regarding the design and feasibility of large-scale, international, collaborative cancer clinical trials. The high rate of tumor tissue acquisition (97%) from the eligible patients provided the opportunity to confirm the prognostic significance of *MGMT* gene promoter methylation and this tissue repository has provided samples for advanced multiplatform molecular analyses. Patient outcomes instruments, including tests of neurocognitive function, symptom burden, and quality of life were completed by a subset of patients. These analyses, reported separately, provide an unprecedented opportunity to examine the comparative impact of the study treatments on patient outcomes. This study design was successfully implemented for a recently completed international phase III study in patients with newly diagnosed GBM.

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TERAPIA DE COMBINACIÓN PARA EL TRATAMIENTO DE CÁNCER CEREBRAL

Detalle del pago

Id.	Rentístico	Concepto y Servicio	Cant.	Val. Unit.	Subtotal
1	50005-01-03	Modificaciones / Correcciones al registro Patente PCT (DESCUENTO INTERNET)	1	\$ 225,000	\$ 225,000
TOTAL					\$ 225,000