労災疾病臨床研究事業費補助金

石綿関連胸膜疾患における個別化治療とケアの確立

平成30年度～令和2年度 総合研究報告書

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目　次

Ⅰ．総合研究報告

Ⅱ．分担研究報告

Ⅰ．　総合研究報告

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総 合 研 究 報 告 書

【石綿関連胸膜疾患における個別化治療とケアの確立】

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研究要旨

切除不能悪性胸膜中皮腫に対する初回化学療法としてのシスプラチン、ペメトレキセド及びニボルマブの併用化学療法の臨床第 II 相試験を実施した。主要評価項目である Modified RECIST criteria による奏効率（中央判定）の評価を行ったところ、14 例において部分奏効が確認された（奏効率 77.8%）。化学療法あるいはニボルマブによると思われる消化器毒性、皮膚障害などの有害事象が認められたが既知の頻度、重症度と同等であり、コントロール可能であった。本試験は、シスプラチン、ペメトレキセドおよびニボルマブ併用化学療法の有用性と安全性を強く示唆するものと思われた。

また今後使用例の増加が予想されるニボルマブに関し、その効果を予測するためのバイオマーカーの確立が必須と考え、悪性胸膜中皮腫症例に対するニボルマブ投与における包括的免疫病態の変化を観察した。8 例の悪性中皮腫患者の免疫機能解析の結果、部分奏効の治療効果を示した 1 症例では、ナチュラルキラー細胞（NK）の IFN-γ 産生誘導能が高く、活性化細胞障害性リンパ球（CTL）が多く、制御性 T リンパ球（Treg）が多いことが確認された。抑制されていた強い NK 機能と CTL 機能が解放され、腫瘍抑制効果に至った可能性が示唆された。

メタボローム解析技術を用いたメタボローム解析は、オミクス解析の一つで、疾患における初期診断や薬剤に対するバイオマーカーに応用可能な生体内の代謝産物量を網羅的に測定することが可能である。葉酸代謝拮抗薬が良く効くあるいは効かない悪性胸膜中皮腫細胞株を同定した。また悪性胸膜中皮腫細胞株のペメトレキセド耐性化細胞株を樹立し、ペメトレキセドの標的酵素である thymidylate synthase（TYMS）の発現増加がペメトレキセド耐性化と関連があることを確認した。

石綿ばく露労働者に発症したびまん性胸膜肥厚における著しい呼吸機能障害の評価において、安静時には呼吸苦を訴えないが、歩行等の労作時に強い呼吸苦を訴える症例がある。6 分間歩行における歩行時の経皮的酸素飽和度（SpO_2）、脈拍、歩行距離を評価することで、より客観的な評価が可能であることを提唱した。

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A．研究目的

胸膜中皮腫に対する新たな治療法として、「切除不能悪性胸膜中皮腫に対する初回化学療法としてのシスプラチン、ペメトレキセドおよびニボルマブ併用化学療法の第 II 相試験」を医師主導治験（以下本治験）として行った。

また石綿関連胸膜疾患における個別化治療とケアの確立についての検討の中で、これまでの石綿曝露の免疫機能影響に関する基礎的情報および患者末梢血を用いた包括的免疫機能解析プラットフォームを活用し、悪性中皮腫症例へのニボルマブ投与前後の免疫動態解析を行い、治療効果の奏効に関わる免疫学的特徴の把握を試み、ニボルマブによる中皮腫治療効果を予測する免疫学的因子の探索を行った。さらに胸膜中皮腫における代謝産物プロファイルを解析することにより、抗がん剤の効果や耐性機序を予測し、患者を層別化できるバイオマーカーの発見を目的とした。また石綿ばく露労働者に発症するびまん性胸膜肥厚における著しい呼吸機能障害の基準は通常呼吸機能検査と動脈血ガス分析に基づき評価されるが、これらの検査値は良好であるにも関わらず日常生活動作が著しく低下する症例があり、そのような症例を適切に労災認定できるようにするため呼吸機能検査（1 次及び 2 次）に加え 6 分間歩行、アンケート調査（問診票 P-ADL）を行い、多角的に評価した。

B．研究方法

本治験では、外科的切除不能の進行又は転移性の悪性胸膜中皮腫を対象とし、中央判定による奏効率を主要評価項目として、ニボルマブを含む併用化学療法の有効性と安全性を検討する。主目的は、切除不能の進行又は転移性の悪性胸膜中皮腫に対し、初回化学療法としてシスプラチン（75 mg/m^2）、ペメトレキセド（500 mg/m^2）、ニボルマブ（360 mg/body）を 3 週間間隔で、最大 6 コース投与し、以後は中止基準に該当するまでニボルマブによる維持療法を 3 週間ごとに実施し、有効性及び安全性を多角的に検討する。実施医療機関は、岡山労災病院、岡山大学病院、四国がんセンター、山口宇部医療センターの 4 施設であり、実施可能性を考慮し、症例数は 18 例と設定した。

石綿による悪性胸膜中皮腫における免疫指標を包括的にスコアリングするため、実際にニボルマブによる治療を行う患者からニボルマブ投与前、投与 1 週後、また投与 3 か月後に末梢血を採取し、サイトカイン、単球・CD4 陽性細胞（Th）・CD8 陽性細胞（CTL）およびナチュラルキラー細胞（NK 細胞）の膜表面分子、遺伝子発現を観察した。

悪性胸膜中皮腫細胞株を用い、thymidylate synthase（TYMS）のノックダウン及び、レトロウイルスを用いたTYMSの過剰発現を行い、それらによって薬剤感受性が変化するかどうかを調べた。

胸部単純写真及びCT検査にてびまん性胸膜肥厚と診断された症例において、呼吸機能検査として、肺機能検査1次（%肺活量や1秒量、1秒率など）・2次（PaO_2や$AaDO_2$など）検査とともに6分間歩行試験を行った。6分間歩行試験では経皮的酸素飽和度（SpO_2）の最低値や歩行距離などをモニタリングした。

C．研究結果

本治験においては、2018年1月より症例登録を開始し、2019年5月までに予定した18例の登録を完了した。主要評価項目であるModified RECIST criteriaによる奏効率（中央判定）の評価を行ったところ、14例において部分奏功（PR）が確認された（奏効率 77.8%）。標的病変の腫瘍径和の変化率を図1、図2に示す。18例全例で少なくとも1ポイント以上において30%を超える腫瘍径和の減少が認められた。

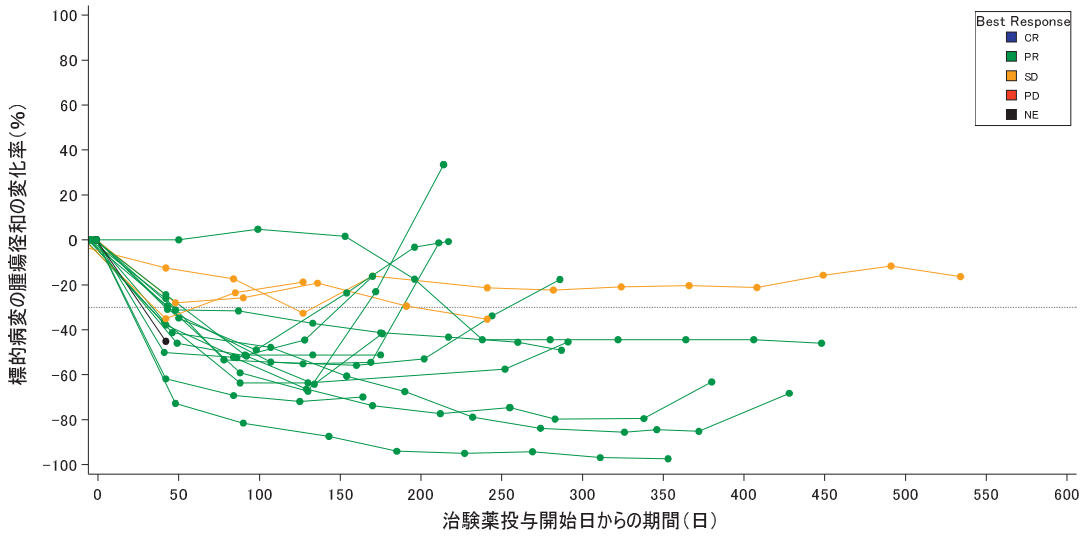

図1．標的病変の腫瘍径和の変化率のspider plot

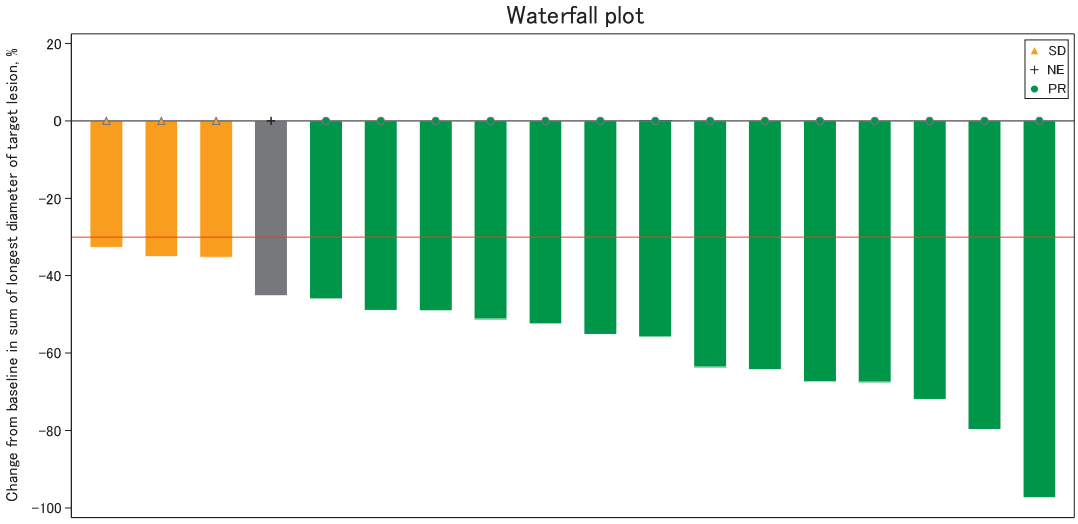

図２．最良の標的病変の腫瘍径和の変化率の waterfall plot

解析対象の 18 例全例に有害事象が発現し、主なものは悪心、食欲減退、しゃっくり、便秘、発疹、貧血、倦怠感、好中球数減少、上咽頭炎、不眠症、下痢、発熱、白血球数減少、末梢性ニューロパチーなどであった。有害事象により死亡した症例はなかった。

また実際にニボルマブを投与した悪性胸膜中皮腫患者から供与された末梢血中の免疫担当細胞における免疫指標を解析したところ、細胞表面分子では制御性 T 細胞の指標である GITR や CTLA-4 発現量の減少が顕著であった。細胞障害性 T 細胞（CTL）分化誘導の亢進を示す陽性細胞％の増加は見られなかった一方、CTL 機能を担うサイトカインである IFN-γ mRNA は投与３か月後に明瞭な増加を示し、PMA/ionomycin による刺激後の CTL では更に強い発現亢進を示した。血漿中サイトカインでは、IFN-γ や IL-17 濃度は治療に伴い徐々に増加した。他方、IL-1ß, TNF-α, IP-10 はじめ多くの炎症性サイトカイン濃度は治療に伴い著明に低下していた。そこで、症例数を蓄積し、包括的免疫機能解析によりニボルマブ投与前後の免疫動態を比較する共に、治療効果と関連する免疫学的特徴を探索した。(表１)。

表１．患者検体一覧

施設	患者ID	治療効果
岡山労災病院	MOP-1	SD
岡山労災病院	MOP-2	SD
四国がんセンター	MOP-3	PD
岡山労災病院	MOP-4	SD
岡山労災病院	MOP-5	SD
岡山労災病院	MOP-6	PD
岡山労災病院	MOP-7	PR
四国がんセンター	MOP-8	PD

ニボルマブ治療効果の内訳は、部分奏効（PR）１名、病状安定（SD）４名、増悪（PD）３名であった。そこで、８名を SD+PR 群５名と PD 群３名に分け群間で各指標の値およびその動態を比較すると共に、特に治療効果 PR を示す１名における免疫学的特徴の抽出を試みた。その結果、細胞表面分子、細胞内 mRNA レベル血中サイトカイン濃度の何れについても、安定（SD）+奏功（PR）群と病勢進行（PD）群の群間比較、および両群における治療前後の変化の特徴を捉えることは出来なかったものの、治療効果 PR を示した１例（MOP-7）については、他と比べて異なる

特徴を見いだすことができた。Th 上の CD25％と CTLA-4％および CTL 上の HLA-DR%、加えて刺激後 NK 中の IFN-γ mRNA レベルが継続して高い傾向であることが明らかとなった（図３）。

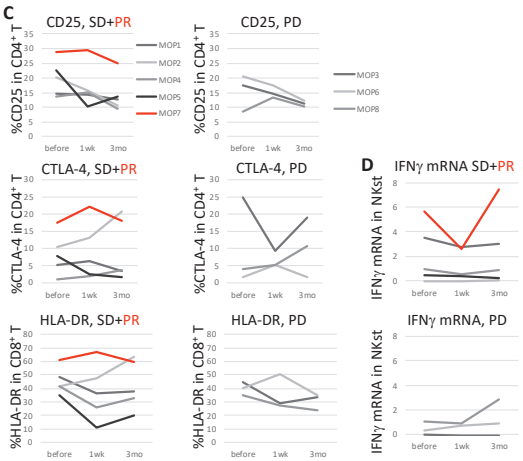

図３．PR を示す MOP-7 における特徴のまとめ

胸膜中皮腫細胞株を用いた実験では、ペメトレキセド感受性株である MSTO-211H 細胞、耐性株 NCI-H2452 細胞を用いてそれぞれの培養条件で代謝産物を抽出し、メタボローム解析を行ったところ、117 代謝産物の定量値が得られ、感受性株と耐性株で特徴的に違う代謝産物を示した。さらに、悪性胸膜中皮腫の治療に用いられるペメトレキセドの標的分子としてピリミジン生合成経路の TYMS に着目し、細胞株を用いて解析した。MSTO-211H 細胞株と TCC-MESO-2 細胞株いずれにおいてもコントロールと比較して、ペメトレキセド処理によって細胞増殖が抑制されたが、ペメトレキセドにチミジンを加えて処理した場合は、薬効が大きく消失していた 。この結果は、ペメトレキセドがピリミジン生合成経路を阻害することにより、悪性胸膜中皮腫の細胞増殖を抑制していることを示唆している。また MSTO-211H 細胞株と TCC-MESO-2 細胞株のそれぞれを親株とした２種の耐性化株を樹立したところ、いずれの耐性株においても TYMS の発現量が統計的に優位な増加を示していた。TYMS の発現量の増加がペメトレキセド耐性に繋がる一因ではないかと考えられた。

２種のペメトレキセド耐性化株のセルライセートを作成し、ウェスタンブロットを行ったところ、いずれの細胞株でも親株に比べてペメトレキセド耐性化株の方が TYMS の発現が増加していた（図４）。次に、TYMS の発現が増加しているペメトレキセド耐性化株を用いて、siRNA を用いて TYMS をノックダウンしたところ、薬剤耐性が有意に減少していた（図５）。これらのことから悪性胸膜中皮腫における薬剤耐性化は TYMS が関わっていることが示唆された。

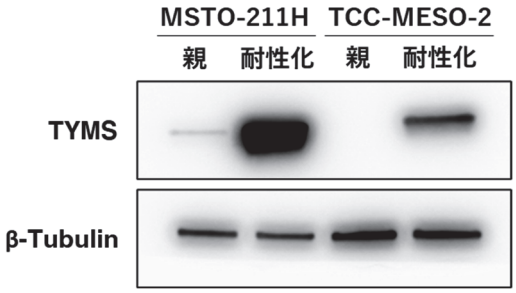

図４．薬剤耐性化によるタンパク質発現の変化

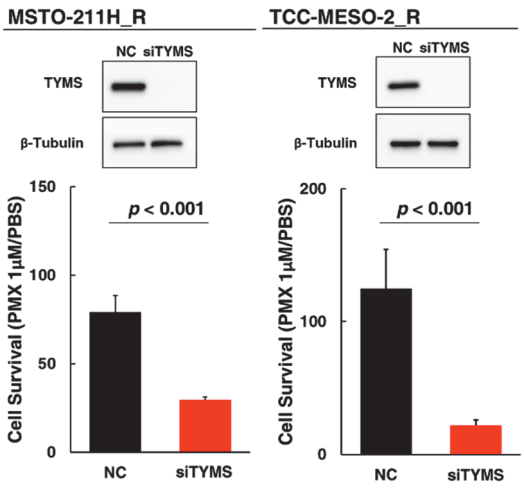

図５．TYMS の KD による薬剤耐性の変化

石綿ばく露によるびまん性胸膜肥厚に関する研究では、研究期間中に 12 名から研究参加同意を得た。12 名全員が男性であり、検査時年齢の中央値は 76.5 歳（67～87 歳）であった。12 名中 10 名が%VC < 60%で呼吸機能障害の基準を満たしていた。SpO_2 について、検査中の最低値は 86.2%（95%信頼区間 82-90%）であった。総歩行距離/予測値は 77.3%（95%信頼区間 63-91%）であった。また、P-ADL によるアンケート調査では、「階段」(24 点満点中，20.8 点）、「屋外歩行」(20 点満点中，17.5 点）の項目で、他の項目と比較して点数が低かった。

D．考察

本研究は、「石綿関連胸膜疾患における個別化治療とケアの確立」を課題とし、以下の研究項目を掲げた。1．切除不能悪性胸膜中皮腫に対するシスプラチン、ペメトレキセドおよびニボルマブ併用化学療法の医師主導治験、2．石綿ばく露による免疫動態の変化とニボルマブ投与における免疫修飾の観察、3．悪性胸膜中皮腫細胞における細胞内代謝産物プロファイルの解析(メタボローム解析)、4．びまん性胸膜肥厚における重症度を客観的に評価するための指標の作成、である。

悪性胸膜中皮腫に対する薬物治療としては、一次治療としてシスプラチン、ペメトレキセド療法が標準治療とされているがその治療成績は十分とは言えず、また同療法に抵抗性となった患者においては確立した治療法はなく、新たな治療法の開発が切望されている。

本研究は、現在の標準治療であるシスプラチン、ペメトレキセド療法とニボルマブの併用によりさらなる治療効果の上乗せを期待し、初回化学療法における有効性及び安全性を評価するものである。主要評価項目である中央判定における奏効率において、当初の想定を上回る高い奏効率が得られた。また安全性の評価においては、消化器毒性、皮膚障害などの有害事象が確認されたが、これらの頻度、重症度はこれまでの化学療法や免疫チェックポイント阻害薬をもちいた臨床試験の報告と同様であり、マネジメント可能なものであった。本試験は、シスプラチン、ペメトレキセドおよびニボルマブ併用化学療法の有用性と安全性を強く示唆するものであり、今後胸膜中皮腫におけるあらたな治療選択肢となる可能性がある。

また本試験は、悪性胸膜中皮腫に対する内科的治療において、医薬品の臨床試験に関する基準（Good Clinical Practice, GCP）に準拠する国内で初めての医師主導の臨床試験であり、今後さらに新規治療法を開発していくうえで極めて重要である。免疫チェックポイント阻害薬は今後、悪性胸膜中皮腫において中心的な役割を担うものと思われるが、他の疾患と同様に、悪性中皮腫に対しても一部で奏効する一方、一部では奏効しない状況である。そこで、これまでの石綿ばく露の免疫機能影響に関する基礎的情報および患者末梢血を用いた包括的免疫機能解析プラットフォームを活用し、悪性中皮腫症例へのニボルマブ投与前後の免疫動態解析を行い、治療効果の奏効に関わる免疫学的特徴の把握を試みた。その結果、細胞表面分子、細胞内 mRNA レベル血中サイトカイン濃度の何れについても、奏功例と不応例の比較、および両群における治療前後の変化の明確な特徴を捉えることは出来なかったものの、明確な治療効果を示した 1 例について他と比べて異なる特徴を見いだすことができた。現時点では治療効果の予測指標として確立されるには至っていないが、今後の一層の免疫機能解析によりニボルマブ治療の効果予測指標として確立されることが期待される。また本研究では、悪性胸膜中皮腫細胞株を用いた実験により、ペメトレキセド存在下においてチミジンの添加によって細胞生存率が大きく回復することを確認した。またペメトレキセド耐性化細胞株では、ペメトレキセドの標的酵素の一つである TYMS の mRNA の発現量が増大していることが観察された。TYMS の発現量が増大することで DNA 合成に関わる dTMP の細胞内濃度が必要十分となり、薬効が抑制、耐性となった可能性が考えられ

た。この結果は、悪性胸膜中皮腫組織や胸水のメタボローム解析が悪性胸膜中皮腫における化学療法の薬剤耐性症例の鑑別等に応用可能である可能性を示唆するものであったと考える。

胸膜中皮腫の治療成績は未だ不良であり、新たな治療戦略の確立が急務である。平成30 年８月にニボルマブが化学療法既治療の悪性胸膜中皮腫に対して適応を取得し、新たな治療選択肢となっている。研究代表者は、本研究期間中に胸膜中皮腫の薬物療法に関する複数の臨床試験に参画した。そのうちの一つである国際的な臨床試験において、免疫チェックポイント阻害薬であるニボルマブとイピリムマブの併用療法が、初回薬物療法において現在の標準治療であるシスプラチン、ペメトレキセド併用化学療法に全生存期間で上回ることが示され、今後新たな標準治療となる可能性がある。ただし、有害事象や初期無効症例など、ニボルマブとイピリムマブの併用療法には課題も残されており、本研究で取り組んだ化学療法と免疫チェックポイント阻害薬の併用療法はそれらの課題を克服しうる可能性があり、新たな治療法の選択肢として重要である。今後は、免疫チェックポイント阻害薬同士の併用療法、また化学療法と免疫チェックポイント阻害薬の併用療法などの有用性と安全性、また奏効例あるいは有害事象の可能性がある症例をあらかじめ選択するためのバイオマーカーの確立など、解決すべき課題は山積している。本研究は、臨床試験に加え、悪性胸膜中皮腫組織、胸水あるいは血液試料を用いた基礎研究により、悪性胸膜中皮腫の個別化治療の確立に向けて、治療に対する反応性の予測モデルや耐性メカニズムの解明を通し寄与したものと考えている。

また本研究では、石綿ばく露による良性胸膜疾患であるびまん性胸膜肥厚について、労災認定において重要な項目である著しい呼吸機能障害を評価する基準について検討した。胸部単純写真及び CT 検査にてびまん性胸膜肥厚と診断された症例において、肺機能検査とともに６分間歩行試験を行った。その結果、12 例中８例は歩行後の SpO_2 が 90%未満に低下していた。また P-ADL の結果から、「階段昇降」や「屋外歩行」など労作時に息切れなどを感じることが多いことが明らかとなった。以上の結果から、びまん性胸膜肥厚の労災認定基準の一つとして６分間歩行試験による「歩行時 SpO_2 の最低値として 90%以下あるいは総歩行距離/予測値の 90%以下とする基準にすること」を提唱した。本研究は、本事業の趣旨である石綿関連疾患の労災認定の適正化に大きく寄与するものと考える。

Ｅ．結論

「切除不能悪性胸膜中皮腫に対する初回化学療法としてのシスプラチン、ペメトレキセドおよびニボルマブ併用化学療法の第 II 相試験」を医師主導治験として実施した。本試験は、シスプラチン、ペメトレキセドおよびニボルマブ併用化学療法の有用性と安全性を強く示唆するものであり、今後胸膜中皮腫におけるあらたな治療選択肢となる可能性がある。

悪性中皮腫症例へのニボルマブ投与前後の包括的免疫動態解析を行った。ニボルマブに奏功した症例では、NK の IFN-γ 産生誘導能が高く、活性化 CTL が多く、Treg 細胞が多いことが確認された。中皮腫細胞株を用いた検討により、TYMS 遺伝子の発現誘導がペメトレキセドに対する耐性化の一因である可能性が考えられた。

石綿ばく露労働者に発症したびまん性胸膜肥厚における著しい呼吸機能障害の基準値において、６分間歩行試験における SpO_2 最低値 90%以下あるいは総歩行距離/予測値の 90%以下とすることを労災認定基準の一つとして提唱した。

Ｆ．健康危険情報

抗悪性腫瘍薬の使用に際しては製薬メーカーから提供される取り扱い情報に基づき適正に取り扱った。また実際の投与に際しては、厚生労働省労働基準局より発出された「発がん性等を有する化学物質を含有する抗がん剤等に対するばく露防止対策につ

いて」（基安化発 0529 第 1 号）に則り各施設で定められた抗がん剤ばく露対策マニュアルを遵守し、医師、薬剤師、看護師が薬剤に曝露しないようにした。また患者やその家族に対しても、薬剤の取扱いに関する情報を周知した。

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H. 知的財産権の出願・登録状況

1. 特許取得

該当するものなし。

2. 実用新案登録

該当するものなし。

3.その他

特記すべき事項なし。

Ⅱ．　分担研究報告

労災疾病臨床研究事業費補助金
分 担 研 究 報 告 書

【切除不能悪性胸膜中皮腫に対する初回化学療法としてのシスプラチン、ペメトレキセドおよびニボルマブ併用化学療法の第 II 相試験】

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研究要旨

悪性胸膜中皮腫は診断・治療ともに困難な疾患であり、診断から死亡に至るまでの生存期間中央値は 7.9 か月と予後不良である。化学療法治療歴のない悪性胸膜中皮腫の一次治療としてシスプラチンとペメトレキセドの併用療法が標準治療とされているが、同療法に抵抗性となった患者に対する確立した治療法はなく、新たな治療法の開発が切望されている。本研究では、抗 PD-1 抗体の有用性を検討するため「切除不能悪性胸膜中皮腫に対する初回化学療法としてのシスプラチン、ペメトレキセドおよびニボルマブ併用化学療法の第 II 相試験」を医師主導治験として企画、立案し、治験を実施した。平成 30 年１月より症例登録を開始し、令和元年５月までに予定した 18 例の登録を完了した。主要評価項目である Modified RECIST criteria による奏効率（中央判定）の評価を行ったところ、14 例において部分奏功（PR）が確認された（奏効率 77.8%）。化学療法あるいはニボルマブによると思われる消化器毒性、皮膚障害などの有害事象が認められたが既知の頻度、重症度と同等であり、コントロール可能であった。本試験は、シスプラチン、ペメトレキセドおよびニボルマブ併用化学療法の有用性と安全性を強く示唆するものと思われた。

Ａ．研究目的

1．治験の主目的

外科的切除不能の進行又は転移性の悪性胸膜中皮腫に対し、初回化学療法として、シスプラチン（75 mg/m^2）、ペメトレキセド(500 mg/m^2)、ニボルマブ（360 mg/body）を３週間間隔で、最大６コース投与し、以後は中止基準に該当するまでニボルマブによる維持療法を３週間ごとに実施したときの有効性（奏効率）を検討する。

2．治験の副目的

設定した有効性の副次評価項目及び安全性の評価項目を用いて、外科的切除不能の進行又は転移性の悪性胸膜中皮腫に対するシスプラチン、ペメトレキセドおよびニボルマブ併用化学療法の有効性及び安全性を多角的に検討する。

Ｂ．研究方法

１．対象

未治療の外科的切除不能の進行又は転移性の悪性胸膜中皮腫患者

２．選択基準

登録時に、下記のすべての基準を満たす被験者を選択する。なお、登録から３剤併用化学療法の初回投与前までに下記の基準を満たさないことが明らかとなった場合は、３剤併用化学療法の１コース目の投与を開始しない。

1) 年齢（同意取得時）：20 歳以上
2) 病理学的に悪性胸膜中皮腫と診断された患者
3) 未治療の外科的切除不能の進行又は転移性の悪性胸膜中皮腫患者
4) 登録前 28 日以内の画像診断において、CT 又は MRI により、Modified Response Evaluation Criteria in Solid Tumours（RECIST）criteria に定義される測定可能病変を一つ以上有する患者。ただし、測定可能病変が胸膜病変のみで胸膜癒着術の既往がある場合は、胸膜癒着術後の画像診断において測定可能病変を確認できた患者に限る。
5) PD-L1 発現解析に用いる腫瘍組織（保存組織又は直近で採取した生検組織）を提供できる患者
6) Eastern Cooperative Oncology Group（ECOG）performance status が 0 又は 1 の患者
7) 90 日以上の生存が期待される患者
8) 登録前 7 日以内に酸素補充を行わない状態で、安静時にパルスオキシメーターにて測定した経皮的酸素飽和度が 94%以上の患者。
9) 登録前 7 日以内に実施した最新の臨床検査値が下記の基準を満たす患者。なお、検査日前 14 日以内に顆粒球コロニー刺激因子（G-CSF 製剤）の投与又は輸血を受けていない臨床検査値とする。
   ① 好中球数が 1,500/mm^3 以上
   ② 血小板数が 100,000/mm^3 以上
   ③ ヘモグロビンが 9.0 g/dL 以上
   ④ AST(GOT)及び ALT（GPT）が施設基準値上限の 3.0 倍以下
   ⑤ 総ビリルビンが施設基準値上限の 2.0 倍以下
   ⑥ クレアチニンが施設基準値上限以下かつクレアチニンクリアランス（Cockcroft/Gault 式による推定値）が 60 mL/min を超える。
10) 妊娠する可能性のある女性（化学閉経などの医学的理由により月経がない患者も含む）
11) 男性の場合、ニボルマブ投与開始後からニボルマブ最終投与後少なくとも７ヵ月間（ニボルマブの５倍半減期と精子の代謝回転に要する期間の合計）の避妊に同意した患者、若しくは完全禁欲に同意した患者
12) 治験責任医師等より、本治験の内容について同意文書及び説明文書を用いて十分に説明を受け、自由意思により本治験参加に同意する患者

３．除外基準

登録時に、下記のいずれかの基準に該当すると考えられる被験者は除外する。なお、登録から３剤併用化学療法の初回投与前までに下記のいずれかの基準に抵触した場合は、３剤併用化学療法の１コース目の投与を開始しない。

1) 抗体製剤を含む他の薬剤に対する高度の過敏反応の合併又は既往を有する患者
2) 自己免疫疾患の合併又は慢性的あるいは再発性の自己免疫疾患の既往を有する患者。ただし、全身療法を必要としない皮膚疾患（白斑、乾癬、脱毛症など）又は外的誘因の非存在下では再発すると考えられない疾患、ホルモン補充療法により対処可能な甲状腺機能低下症を合併している患者は登録可能とする。
3) 重複がんを有する患者（完全切除された基底細胞がん、Stage I の有棘細胞がん、上皮内がん、粘膜内がん又は表在性膀胱がん、あるいは５年間以上再発が認められない他のがんの既往を有する患者は登録可能とする）
4) 脳又は髄膜に転移巣を有する患者。ただし、無症状かつ治療を必要としない患者は登録可能とする。また、本治験への登

録の 28 日以上前に同病巣に対する治療を終えて病状が安定しており、かつ本治験への登録の前 14 日間で全身性副腎皮質ホルモンの継続使用を要さない患者は登録可能とする。

5) 画像診断又は臨床所見により診断された間質性肺疾患若しくは肺線維症の合併又は既往を有する患者。ただし、放射線性肺臓炎については、線維化による安定化が確認され、再発の懸念がない患者は登録可能とする。
6) 憩室炎又は症候性消化管潰瘍疾患を合併している患者
7) ２週間に１回を超える頻度で排液を必要とする胸水の貯留を認める患者
8) 治療を必要とする心嚢液又は腹水の貯留を認める患者
9) 腫瘍に関連する疼痛が安定せず、管理不能な患者
10) 登録前 180 日以内に一過性脳虚血発作、脳血管発作、血栓症又は血栓塞栓症(肺動脈塞栓症又は深部静脈血栓症)の既往を有する患者
11) 下記の管理不能又は重大な心血管疾患を有する患者
    ① 登録前 180 日以内の心筋梗塞
    ② 登録前 180 日以内の管理不能な狭心症
    ③ New York Heart Association (NYHA) 心機能分類Ⅲ度又はⅣ度のうっ血性心不全
    ④ 適切な治療にもかかわらず管理不能な高血圧（収縮期血圧 150 mmHg 以上又は拡張期血圧 90 mmHg 以上が 24 時間以上持続するなど)
    ⑤ 管理不能な不整脈
12) 抗凝固療法（低用量アスピリンを含む抗血小板療法を除く）を受けている又はそれらを必要とする疾患を有する患者
13) 管理不能な糖尿病を合併している患者
14) 治療を必要とする全身性感染症を有する患者
15) HIV への感染が明らかな患者
16) HTLV-1 抗体検査、HBs 抗原検査又は HCV 抗体検査のいずれかが陽性の患者。また、HBs 抗原検査が陰性であるが、HBs 抗体検査又は HBc 抗体検査のいずれかが陽性かつ HBV-DNA 定量が検出感度以上の患者
17) 過去にニボルマブ（MDX-1106 又は BMS-936558)、抗 PD-1 抗体、抗 PD-L1 抗体、抗 PD-L2 抗体、抗 CD137 抗体、抗 CTLA-4 抗体又はその他の T 細胞制御を目的とした抗体療法若しくは薬物療法の前治療歴を有する患者
18) 登録前 14 日以内に局所又は表面麻酔を伴う手術療法を受けた患者
19) 登録前 28 日以内に全身麻酔を伴う手術療法を受けた患者
20) 登録前 14 日以内に胸膜癒着術を受けた患者（ピシバニールによるものを除く）
21) 登録前 28 日以内にピシバニールによる胸膜癒着術を受けた患者
22) 心膜癒着術あるいは腹膜癒着術の既往のある患者
23) 登録前 14 日以内に疼痛緩和を目的とした放射線療法を受けた患者
24) 登録前 56 日以内に放射性医薬品（検査及び診断を目的とした放射性医薬品の使用を除く）の投与を受けた患者
25) 登録前 28 日（抗体製剤の場合は 90 日）以内に他の未承認薬の投与(悪性胸膜中皮腫に対する効能・効果を有しない承認薬、臨床研究による投与や未承認の配合薬、新剤形薬も含む)を受けた患者
26) 登録前 28 日以内に全身性副腎皮質ホルモン（検査、アレルギー反応に対する予防投与又は放射線療法に伴う浮腫軽減などを目的とした一時的な使用を除く）又は免疫抑制剤の投与を受けた患者
27) 妊娠中、授乳中又は妊娠している可能性のある患者
28) 認知症の合併などにより同意能力を欠く状態であると判断される患者
29) その他、治験責任医師等が治験対象として不適当と判断した患者

４．投与量及び投与方法

本治験は外科的切除不能の進行又は転移性の悪性胸膜中皮腫を対象に、標準療法であるPC療法（シスプラチン、ペメトレキセド療法）にニボルマブを加えた３剤併用化学療法の有効性及び安全性を検討する多施設共同非盲検非対照試験である。本治験はスクリーニング期、治療期及び後観察期からなる。「選択基準」に示す基準を満たし、かつ「除外基準」に示す基準に該当せず、治験責任医師又は治験分担医師が本治験の対象として適格と判断した患者を組み入れる。試験の概要を図１に示した。治療期は、「３剤併用化学療法期」と「ニボルマブ単独維持療法期」から構成される。初回投与は登録から７日以内に行う。３剤併用化学療法期では、シスプラチン（75 mg/m^2）、ペメトレキセド（500 mg/m^2）、ニボルマブ（360 mg/body）の用量を３週間間隔で静脈内投与する。３週間を１コースとして、２コース間隔で画像診断（CT/MRI など）を実施し、「３剤併用化学療法期の投与継続基準」に示す基準をすべて満たす被験者は３剤併用化学療法を４～６コース行う。３剤併用化学療法期を完了するか、もしくは３剤併用化学療法の投与中止基準のいずれかに該当する場合は、ニボルマブ単独維持療法期への移行基準を確認のうえ、ニボルマブ単独維持療法期に移行する。移行期は「ニボルマブ単独維持療法期の投与中止基準」のいずれにも該当しない場合、３週間間隔で継続可能である。ニボルマブ単独維持療法期に移行できない場合や移行例が「ニボルマブ単独維持療法期の投与中止基準」のいずれかに該当する場合は、後観察期に移行する。

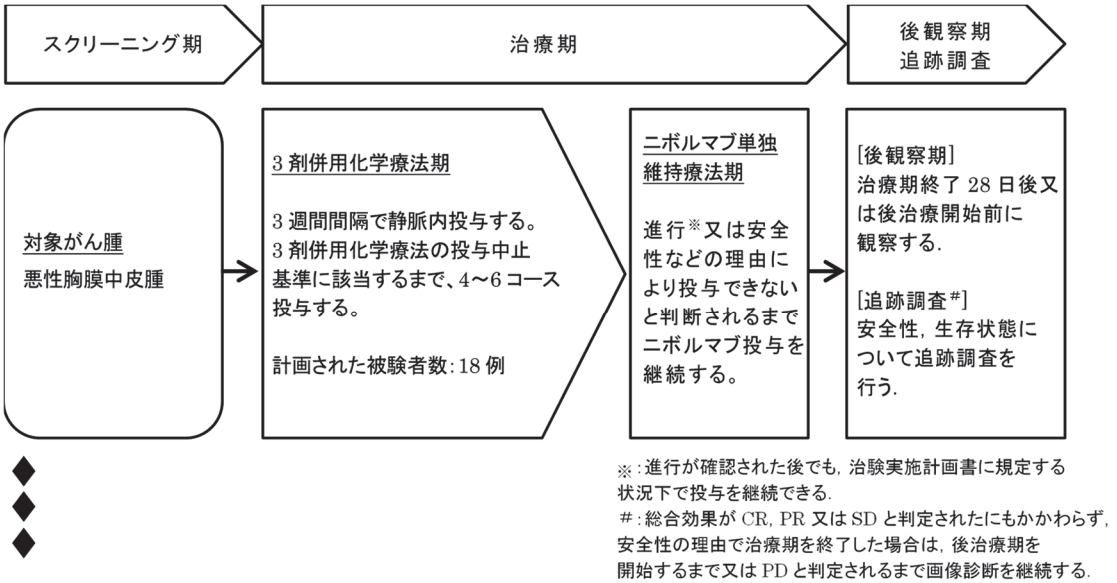

図１．試験の概要

５．実施医療機関

４施設（岡山労災病院、岡山大学病院、四国がんセンター、山口宇部医療センター）

６．計画された被験者数とその根拠

国内で施行されたシスプラチン、ペメトレキセド併用療法における奏効率は 36.8%と報告されている（Nakagawa et al. J J Clin Oncol 2008）。本治験を第 II 相試験として実施する場合、実施可能性を考慮のうえ、18 例と設定した。

本治験における奏効例数を５例～10 例と想定すると、奏効率は 35.7%～71.4%となるが、そのとき、奏効率の点推定値と Exact 法による下側信頼限界（信頼係数：両側 90%）の幅は 20%～25%となる。必要被験者数の算出で算出した必要被験者から数名の被験者が早期脱落などにより評価不能となる可能性を考慮し、目標被験者数を 18 名とした。

７．評価項目

１）有効性の評価項目

①主要評価項目

Modified RECIST criteria による奏効率（中央判定）

②副次評価項目

a. 奏効率（実施医療機関の医師による判定、Modified RECIST criteria）
b. 奏効率（中央判定、Modified RECIST criteria）
c. 病勢制御率（中央判定、Modified RECIST criteria）
d. 全生存期間
e. 無増悪生存期間（中央判定、Modified RECIST criteria）
f. 奏効期間（中央判定、Modified RECIST criteria）
g. 奏効に至るまでの期間（中央判定、Modified RECIST criteria）
h. 最良総合効果（中央判定、Modified RECIST criteria）
i. 標的病変の腫瘍径和の変化率（実施医療機関の医師による判定、Modified RECIST criteria）

２）安全性の評価項目

① 有害事象

a. 臨床検査（血液学的検査、生化学的検査、膵機能検査、血液凝固系検査、尿検査、免疫学的検査、ホルモン検査）
b. バイタルサイン（収縮期血圧／拡張期血圧、脈拍数、体温）、体重
c. 12 誘導心電図
d. 胸部 X 線
e. ECOG performance status

３）QOL 評価

QOL（EQ-5D、LCSS-Meso）

４）探索的評価項目

PD-L1 の免疫組織化学的解析

（倫理面への配慮）

本治験は治験実施計画書、ヘルシンキ宣言に基づく倫理的原則、医薬品、医療機器等の品質、有効性および安全性の確保等に関する法律第 14 条第３項、第 23 条の 25 第３項及び第 80 条の２に規定する基準並びに「医薬品の臨床試験の実施の基準に関する省令（GCP）」（平成９年厚生省令第 28 号）に則り実施するものとする。本治験は、実施に先立ち、各実施医療機関の治験審査委員会において、治験実施計画書、被験者の同意を得るのに使用される方法、治験薬概要書及びその他の必要な文書が審議され、本治験が倫理的及び科学的に妥当であるかどうか、その他、本治験が実施医療機関において行うのに適当であるかどうかの審査を受ける。被験者の登録および症例報告書における被験者の特定はデータ・試料管理担当者によって、被験者識別コード等で行うなど連結可能匿名化を行う。原資料の直接閲覧・取り扱い等においては被験者のプライバシー保護に十分配慮する。患者試料、中央判定のための画像なども、同様に被験者識別コード等で行うとともに、他施設への試料の移送などに際しては、この被験者識別コード等にて識別する。

C．研究結果

2018 年１月より症例登録を開始し、2019 年５月までに予定した 18 例の登録を完了した。登録症例の概要を表１に示す。

表 1. 登録症例の概要

項目	n (%)
性別	
男	15 (83.3)
女	3 (16.7)
年齢 (歳)	
平均値 ± 標準偏差	69.2 ± 4.1
中央値	69.0
最小値 ～ 最大値	64 ～ 78
組織型	
上皮型	14 (77.8)
肉腫型	2 (11.1)
二相型	2 (11.1)
病期分類	
Ⅰ期	8 (44.4)
Ⅱ期	0 (0.0)
Ⅲ期	9 (50.0)
Ⅳ期	1 (5.6)
ECOG Performance Status	
0	3 (16.7)
1	15 (83.3)
PD-L1 28-8 発現	
なし	1 (5.6)
あり	17 (94.4)

１．主要評価項目

Modified RECIST criteria による奏効率 (中央判定)： 18 例中 14 例が奏効と判定され、奏効率は 77.8%であった。なお、Clopper-Pearson 法に基づく 90%信頼区間は[56.1, 92.0]であった。

２．副次評価項目

1） 奏効率 (実施医療機関の医師による判定、Modified RECIST criteria) : 18 例中 14 例が奏効と判定され、奏効率は 77.8%であった。

2） 病勢制御率 (中央判定、Modified RECIST criteria) : 18 例中 17 例が病勢制御と判定され、病勢制御率は 94.4%であった。

3） 全生存期間：全生存期間は最短で 1.7 か月（打ち切り）、最長で 20.8 か月であった。

4） 無増悪生存期間（中央判定、Modified RECIST criteria）：無増悪生存期間は中央値 8.02 か月［90%信頼区間（5.75,14.06）］、最短で 1.7 か月（打ち切り）、最長で 14.7 か月（打ち切り）であった。

5） 奏効期間（中央判定、Modified RECIST criteria）：奏効期間の中央値は 6.70 か月［90%信頼区間（4.30,12.5）］、最短で 2.8 ヵ月、最長で 13.2 か月（打ち切り）であった。

6） 奏効に至るまでの期間（中央判定、Modified RECIST criteria）：奏効に至るまでの期間の中央値は 1.54 か月［90%信頼区間（1.38,1.64）］、最短で 1.4 か月、最長で 3.3 か月であった。

7） 最良総合効果（中央判定、Modified RECIST criteria）：最良総合効果は、CR 0 例、PR 14 例、SD 3 例、PD 0 例、NE 1 例であった。

8） 標的病変の腫瘍径和の変化率（実施医療機関の医師による判定、Modified RECIST criteria）（図２、図３）：最良総合効果が SD、NE であった症例も含め、18 例全例で少なくとも１ポイント以上の PR 評価が得られた標的病変の腫瘍径和の変化率は、PR 相当である 30%を超える減少が認められた。

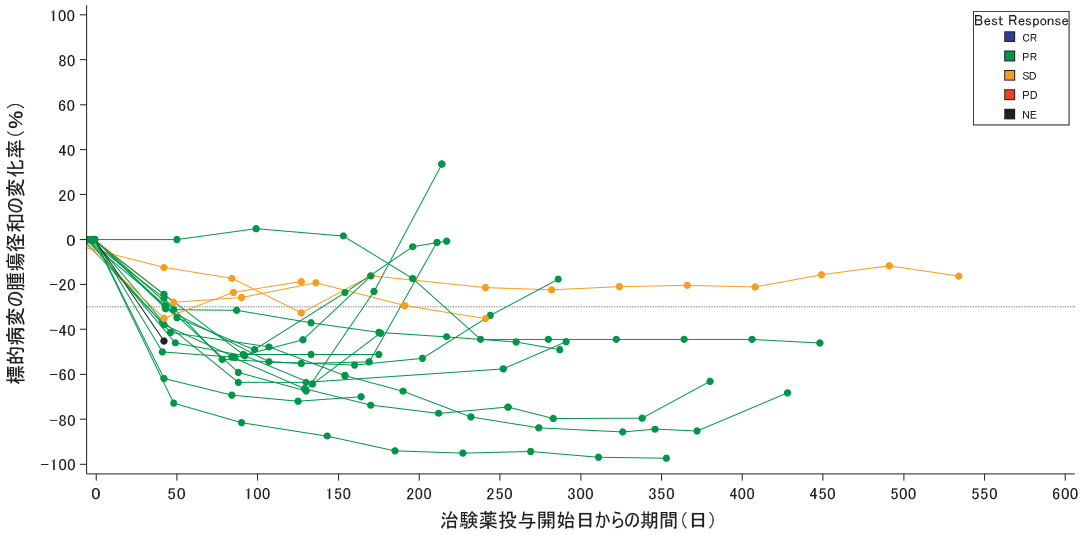

図２．標的病変の腫瘍径和の変化率の spider plot

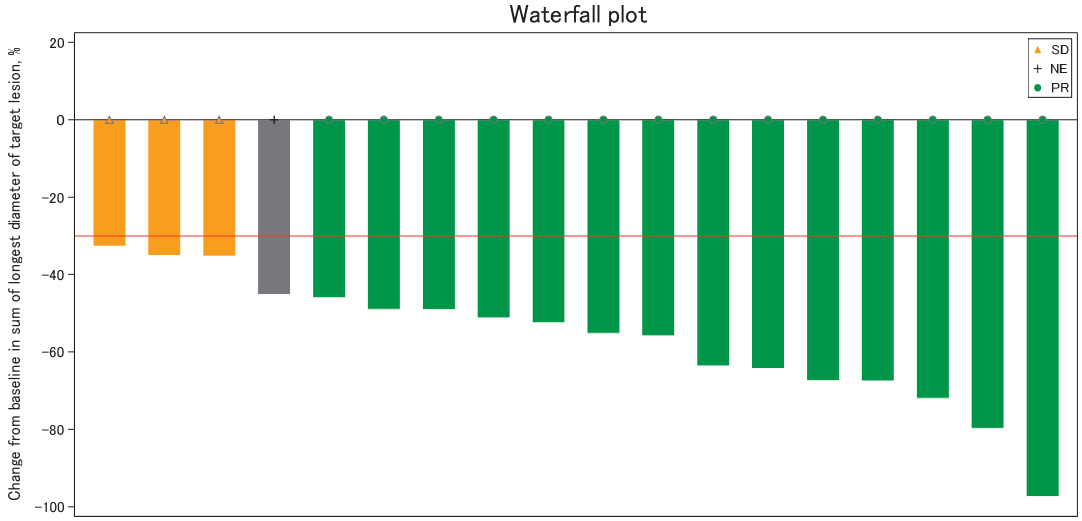

図３．最良の標的病変の腫瘍径和の変化率の waterfall plot

３．安全性の評価項目

１）　有害事象

解析対象の 18 例全例に有害事象が発現した。なお、有害事象により死亡した症例はなかった。ニボルマブとの関連が否定できない有害事象（副作用）は 16 例（88.9%）に発現した。

解析対象の 18 例の 10%以上で発現した有害事象は悪心 12 例（66.7%）、食欲減退 11 例（61.1%）、しゃっくり 11 例（61.1%）、便秘９例（50.0%）、発疹７例（38.9%）、貧血７例（38.9%）、倦怠感６例（33.3%）、好中球数減少５例（27.8%）、上咽頭炎５例（27.8%）、不眠症５例（27.8%）、下痢４例（22.2%）、発熱４例（22.2%）、白血球数減少４例（22.2%）、末梢性ニューロパチー４例（22.2%）、口内炎３例（16.7%）、肺炎３例（16.7%）、味覚障害３例(16.7%)、難聴３例（16.7%）、腹部不快感２例（11.1%）、口角口唇炎２例（11.1%）、低ナトリウム血症２例（11.1%）、筋肉痛２例（11.1%）、背部痛２例（11.1%）であった。

Grade3 以上の有害事象は、18 例中９例（50.0%）に発現した。内訳は、「代謝および栄養障害」６例(33.3%)［食欲減退５例（27.8%）、低ナトリウム血症２例（11.1%）］、「臨床検査」３例（16.7%）［白血球数減少１例（5.6%）、アラニンアミノトランスフェラーゼ増加１例（5.6%）、アスパラギン酸アミノトランスフェラーゼ増加１例（5.6%）、リンパ球数減少１例（5.6%）］、「感染症および寄生虫症」３例（16.7%）［肺炎１例(5.6%)、憩室炎１例（5.6%）、歯髄炎１例(5.6%)］、「血液およびリンパ系障害」３例(16.7%)［貧血３例（16.7%）］、「胃腸障害」２例（11.1%）［悪心１例（5.6%）、腸炎１例(5.6%)］、「呼吸器、胸郭および縦隔障害」１例（5.6%）［肺塞栓症１例（5.6%）］、「神経系障害」１例（5.6%）［末梢性ニューロパチー１例（5.6%）］、「筋骨格系および結合組織障害」１例（5.6%）［背部痛１例（5.6%）］であった。

４．QOL の評価

健康 VAS の平均値は、３剤併用化学療法期開始前に比べて、ニボルマブ単独維持療法期開始前には-5.6±24.2（-65～30）mm［平均値±標準偏差（最小値～最大値）、以下同様］、治療期終了時には 0.5±23.3（-40～30）mm であった。また、インデックススコアの平均値は、３剤併用化学療法期開始前に比べて、ニボルマブ単独維持療法期開始前には 0.0185±0.1389（-0.319～0.292）治療期終了時には、-0.0166±0.1912（-0.364～0.292）であった。

VAS（全平均）は、3剤併用化学療法期開始前に比べて、ニボルマブ単独維持療法期開始前には-0.01±13.57 (-21.1～28.1) mm、治療期終了時には -2.11±21.38 (-41.3～36.0) mm であった。これらの結果について、問題となるような QOL の変化はないと考えられた。

D．考察

悪性胸膜中皮腫に対する薬物治療としては、一次治療としてシスプラチン、ペメトレキセド療法が標準治療とされているがその治療成績は十分とは言えず、また同療法に抵抗性となった患者においては確立した治療法はなく、新たな治療法の開発が切望されている。

ニボルマブは、小野薬品工業株式会社とメダレックス社（現、ブリストル･マイヤーズ スクイブ社）が作製した、ヒト PD-1 (Programmed cell death-1) に対するヒト型モノクローナル抗体であり、小野薬品及び BMS 社が臨床開発を進めている。国内で行われた臨床試験「2nd/3rd ラインの悪性胸膜中皮腫（MPM）に対するニボルマブの第Ⅱ相試験（MERIT 試験)」において、シスプラチンあるいはカルボプラチンとペメトレキセドの併用療法に不応又は不耐となった悪性胸膜中皮腫 34 例が登録された。患者背景は男/女＝29/5, 年齢中央値 68 歳 (43-78 歳), PS0/1＝13/21, 上皮/肉腫/二相＝27/3/4, 前治療レジメン数 1/2＝24/10 であった。観察期間中央値16.8か月（1.8-20.2か月）の時点で奏効率は 29.4%（95%CI: 16.8-46.2), 無増悪生存期間及び全生存期間の中央値はそれぞれ 6.1 か月（95%CI: 2.9-9.9), 17.3か月（95%CI: 11.5-NR）であった。この結果に基づき、2次もしくは3次治療としてのニボルマブは有用な治療法であると結論づけられ、2018 年 8 月 21 日に「がん化学療法後に増悪した切除不能な進行・再発の悪性胸膜中皮腫」に対する適応を取得した。

本研究は、現在の標準治療であるシスプラチン、ペメトレキセド療法とニボルマブの併用によりさらなる治療効果の上乗せを期待し、初回化学療法における有効性及び安全性を評価するものである。主要評価項目である中央判定における奏効率において、当初の想定を上回る高い奏効率が得られた。また安全性の評価においては、消化器毒性、皮膚障害などの有害事象が確認されたが、これらの頻度、重症度はこれまでの化学療法や免疫チェックポイント阻害薬をもちいた臨床試験の報告と同様であり、マネジメント可能なものであった。本試験は、シスプラチン、ペメトレキセドおよびニボルマブ併用化学療法の有用性と安全性を強く示唆するものであり、今後胸膜中皮腫におけるあらたな治療選択肢となる可能性がある。

また本試験は、悪性胸膜中皮腫に対する内科的治療において、医薬品の臨床試験に関する基準（Good Clinical Practice, GCP）に準拠する国内で初めての医師主導の臨床試験であり、今後さらに新規治療法を開発していくうえで極めて重要である。

E．結論

「切除不能悪性胸膜中皮腫に対する初回化学療法としてのシスプラチン、ペメトレキセドおよびニボルマブ併用化学療法の第Ⅱ相試験」を医師主導治験として実施した。本試験は、シスプラチン、ペメトレキセドおよびニボルマブ併用化学療法の有用性と安全性を強く示唆するものであり、今後胸膜中皮腫におけるあらたな治療選択肢となる可能性がある。

F．健康危険情報

抗悪性腫瘍薬の使用に際しては製薬メーカーから提供される取り扱い情報に基づき適正に取り扱った。また実際の投与に際しては、厚生労働省労働基準局より発出された「発がん性等を有する化学物質を含有する抗がん剤等に対するばく露防止対策について」（基安化発 0529 第 1 号）に則り各施設で定められた抗がん剤ばく露対策マニュアルを遵守し、医師、薬剤師、看護師が薬剤に曝露しないようにした。また患者やその家族に対しても、薬剤の取扱いに関する情報を周知した。

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H．知的財産権の出願・登録状況

１．特許取得

該当するものなし。

２．実用新案登録

該当するものなし。

３．その他

特記すべき事項なし。

労災疾病臨床研究事業費補助金
分 担 研 究 報 告 書

【悪性胸膜中皮腫細胞株における葉酸代謝拮抗薬に応答する代謝変化解析】

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研究要旨

メタボローム解析技術を用いたメタボローム解析は、オミクス解析の一つで、疾患における初期診断や薬剤に対するバイオマーカーに応用可能な生体内の代謝産物量を網羅的に測定することが可能である。悪性胸膜中皮腫の早期診断や臨床で治療に使用されている抗がん剤に対するバイオマーカーの探索を目指して取り組んだ。

１年度目は、葉酸代謝拮抗薬が良く効くあるいは効かない悪性胸膜中皮腫細胞株を同定した。そして、葉酸代謝拮抗薬の標的酵素の代謝経路で下流に位置する代謝産物を加えることにより、薬効が完全に消失した。この実験条件下でメタボローム解析を行った結果、葉酸代謝拮抗薬処理により悪性胸膜中皮腫細胞内で、一炭素代謝経路に関連するアミノ酸や核酸の代謝産物量に変化が観察された。２年度目は TYMS に着目し、２つの悪性胸膜中皮腫細胞株においてペメトレキセド存在下でチミジンを培養液に添加した場合、いずれにおいても細胞生存率が約 80%まで回復することを確認した。さらに、悪性胸膜中皮腫が代謝産物を調整してペメトレキセド耐性となっているのではないかと考え、悪性胸膜中皮腫細胞株のペメトレキセド耐性化細胞株を樹立した。また、ペメトレキセドへの耐性化によってペメトレキセドの標的酵素である TYMS の発現が上昇していることを確認した。３年度目は耐性株が親株と比較して薬剤耐性化によってタンパク質レベルでも TYMS の発現が増加しているかどうかを確認した。さらに、TYMS と薬剤耐性との関連を調べるため TYMS の発現をノックダウンや過剰発現させたところ、ノックダウンによって薬剤耐性が低下し、過剰発現によって薬剤耐性が増加することがわかり、TYMS の発現増加がペメトレキセド耐性化と関連があることが示唆された。

Ａ．研究目的

質量分析計を用いる生体内の代謝産物を網羅的に解析するメタボローム解析技術は、400 以上におよぶ代謝産物を同定することができ、疾患の早期発見や患者の層別化を可能とするバイオマーカーを発見できる可能性を秘めている。

画像検査において悪性胸膜中皮腫が疑われる場合、胸水細胞診や胸膜生検の病理診断が必要である。中皮腫は、組織学的に上皮型、肉腫型とその両者が混ざり合って存在する二相型の３種類に分けられ、病理診断は難しい現状である。血液検査で悪性胸膜中皮腫を早期に発見する腫瘍マーカーの研究も進められているが、これまでのところ確実に診断する腫瘍マーカーは発見されていない。

悪性胸膜中皮腫は非常に治療が難しい病気の一つで、化学療法では葉酸代謝拮抗薬のペメトレキセド (Pemetrexed, PMX) とシスプラチンの併用療法である。しかし、PMX とシスプラチンの併用療法を受けた患者で奏効が認められる人は全体の 30～40%にとどまり、現在では寛解にいたる効

果は期待できない現状である。言い換えれば全体の 60～70%が薬剤耐性であると言える。PMX の標的分子は、ピリミジン生合成経路の thymidylate synthase (TYMS)、一炭素 (1C) 代謝経路の dihydrofolate reductase (DHFR)、プリン塩基新規生合成経路の glycinamide ribonucleotide formyltransferase (GART) の 3 種の酵素である。特に TYMS を第一標的としているが、この酵素は dUMP を基質として還元型メチル化反応により dTMP に変換する。シスプラチンの薬剤耐性のメカニズムはいくつか報告があるが、PMX についてはよく分かっていない。

そこで我々は本事業期間において、悪性胸膜中皮腫における代謝と薬剤の関連性を明らかににするため以下のことを目的に取り組んだ。

1）平成 30 年度

悪性胸膜中皮腫細胞株および患者由来細胞株を用いて、胸膜中皮腫細胞の代謝プロファイルと葉酸代謝拮抗薬処理時の細胞内代謝産物プロファイルを解析した。

2）令和 1 年度

平成 30 年度の研究では、TYMS の下流に存在する代謝産物であるチミジン (THY) と GART の下流に存在する代謝産物であるヒポキサンチン (HXN) の細胞内濃度が高い場合、PMX の薬効が失活する可能性があることを明らかにした。そこで、今年度は TYMS に着目し、2 つの悪性胸膜中皮腫において TYMS の下流の代謝産物 THY を培養液に添加し、それぞれの細胞生存率を測定した。昨年度の結果から、悪性胸膜中皮腫が代謝産物を調節して PMX 耐性を獲得すると仮定し、PMX 耐性となった悪性胸膜中皮腫細胞株の樹立や、親株と耐性化株における PMX 標的酵素の発現量の変化を調べた。

3）令和 2 年度

これまでの結果を基に TYMS の増加が PMX 耐性を誘導したのではないかと推察し、TYMS と PMX 耐性化との関連性を明らかにする。具体的には siRNA を用いた TYMS のノックダウン及び、レトロウイルスを用いた TYMS の過剰発現を行い、それらによって薬剤感受性が変化するかどうかを調べた。

B．研究方法

本事業において細胞株は市販されている悪性胸膜中皮腫細胞株と国立がん研究センターにおいて patient-derived xenograft (PDX) で樹立された細胞を用いた。また、それらを親株として令和 1 年度樹立した PMX 耐性化株 (MSTO-211H_R、TCC-MESO-2 _R) を用いた。葉酸代謝拮抗薬は PMX を用いた。

1）平成 30 年度

悪性胸膜中皮腫細胞株と PMX を用いて薬剤感受性試験を行った。PMX の濃度を変化させ、それぞれの細胞生存率を測定した。次に PMX の効果を標的分子の下流に存在する代謝産物、ピリミジン生合成経路 TYMS の下流に存在する THY とプリン塩基新規生合成経路 GART の下流に存在する HXN、これら 2 つが含まれる溶液 (HT) を培養液に添加し、細胞の生存率を測定した。最後に、① Phosphate Buffered Saline (PBS) をコントロールとし、②PMX(1 μM) 処理、③PMX + HT の 3 条件で、それぞれ悪性胸膜細胞から代謝産物を抽出し、Capillary electrophoresis-Mass Spectrometry (CE-MS)を用いて、メタボローム解析を行った。

2）令和 1 年度

MSTO-211H 細胞株と TCC-MESO-2 細胞株において、PBS 処理、PMX 処理 (1 μM)、PMX (1 μM) + THY (16 μM) 処理における細胞生存率を測定した。次に、昨年度算出した IC_{50} を基にして培養液中の PMX の濃度が 10 μM を超えるまで 2 日毎に培養液を変えながら漸増していき、薬剤耐性化株を樹立した。樹立した薬剤耐性化株は PMX の濃度を変化させた際のそれぞれの細胞生存

率を測定した。最後に、PMX の標的酵素である TYMS、DHFR、GART の mRNA の発現量が薬剤耐性化によって変化したのかどうかを RT-PCR 法を用いて調べた。

3）令和２年度

mRNA の発現と同様にタンパク質の発現も変化しているかどうかを確認するため、昨年度樹立した PMX 耐性化株２種とそれぞれの親株からセルライセートを作成し、ウェスタンブロットを行った。次に、PMX 耐性化株で発現が増加した TYMS が薬剤耐性化に関連するかどうかを確認するため遺伝子のノックダウン及び、過剰発現の試験を行った。TYMS のノックダウンでは、TYMS に特異的に作用する siRNA を用いて PMX 耐性化株２種の TYMS のノックダウンを行い、薬剤感受性が変化するかどうかを WST-8 によってその生細胞数を計測した。TYMS の過剰発現では、プラスミド(コントロール、TYMS)と Plat-A (Platinum Expression System Amphotropic cell) を用いて２種のレトロウイルスを作成し、PMX 耐性化株の親株である MSTO-211H と TCC-MESO-2 それぞれに感染させ、TYMS の過剰発現株とコントロール株を作成した。レトロウイルス感染後の培養には G418 (750 ug/ml) を含む培地で培養した。

(倫理面への配慮)

本事業における研究は、細胞株を用いた研究成果であり、ヒト由来検体を用いていないため、倫理面への配慮は不要である。

C．研究結果

1）平成 30 年度

PMX に対する悪性胸膜中皮腫細胞株の感受性は、MSTO-211H 細胞に対しては高く、NCI-H2452 細胞に対しては低かった(図 1) 。そこで、MSTO-211H 細胞を感受性株、NCI-H2452 細胞を耐性株として、感受性株と耐性株の違いを明らかにする目的で、種々の実験を行った。

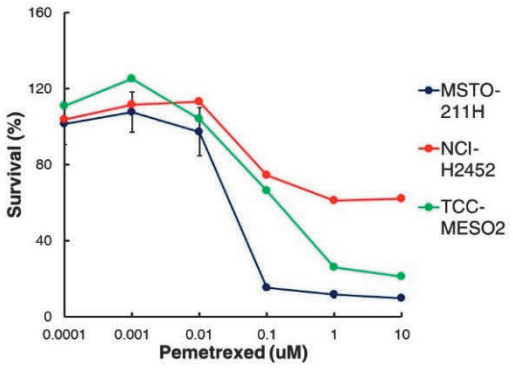

図１．PMX 濃度と生存曲線

PMX の標的分子は、一炭素 (1C) 代謝経路の DHFR、プリン塩基新規生合成経路の GART、ピリミジン生合成経路の TYMS の３種類の酵素である。MSTO-211H 細胞株では、コントロールの PBS 処理と比較して、葉酸代謝拮抗薬ペメトレキセド処理 (1 μM PMX) により細胞の増殖が抑制される。PMX 存在時に、

ピリミジン生合成経路 TYMS の下流に存在するチミジン (16 μM, THY) とプリン塩基新規生合成経路 GART の下流に存在するヒポキサンチン (100 μM, HXN)、これら２つが含まれる溶液 (HT) を添加すると、THY 添加により PMX の薬効が大きく消失した (図２)。

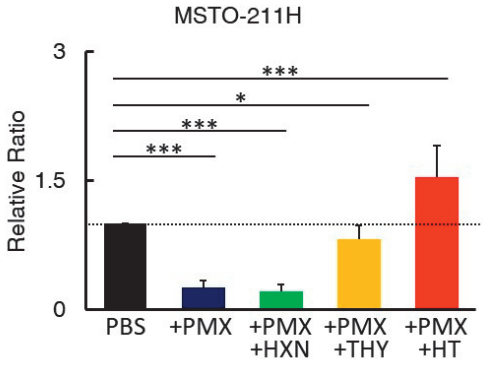

図２．薬剤感受性と下流代謝産物の影響

この結果は、PMX がピリミジン生合成経路を阻害することにより、悪性胸膜中皮腫の細胞増殖を抑制していることを示唆した。

PMX 感受性株である MSTO-211H 細胞、

耐性株 NCI-H2452 細胞を用いてそれぞれの培養条件で悪性胸膜中皮腫細胞から代謝産物を抽出し、CE-MS を用いてメタボローム解析を行った。117 代謝産物の定量値が得られ、感受性株と耐性株で特徴的に違う代謝産物を示した（図３）。

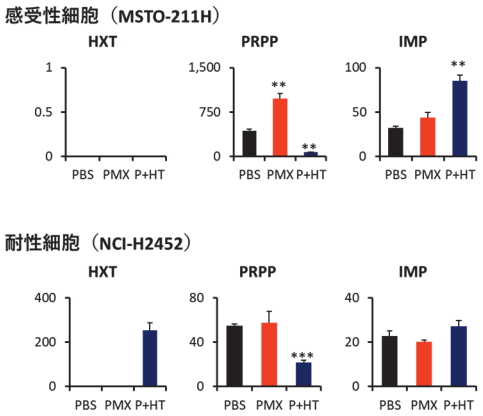

図３．特徴的な代謝産物量の変化
（pmol/10^6細胞）

HXT は感受性細胞株ではすべての条件で検出されず、耐性株では HT を添加することにより検出された。ピリミジン塩基やプリン塩基のリボースの元になる PRPP は、感受性株では PMX 処理により蓄積するが耐性株では変化が観察されない一方、HT 添加の実験条件下では、感受性株と耐性株共に低下した。HXT と PRPP から産生される IMP は、感受性株でのみ細胞内濃度の上昇が観察された。

２）令和１年度

MSTO-211H 細胞株と TCC-MESO-2 細胞株いずれにおいてもコントロールの PBS 処理と比較して、PMX 処理によって細胞増殖が抑制されたが、PMX + THY 処理の場合は、薬効が大きく消失することを確認した（図４）。

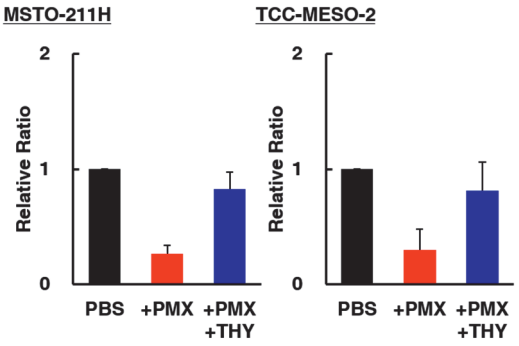

図４．薬剤感受性と下流代謝産物の影響

この結果は、PMX がピリミジン生合成経路を阻害することにより、悪性胸膜中皮腫の細胞増殖を抑制していることを示唆した。

MSTO-211H 細胞株と TCC-MESO-2 細胞株のそれぞれを親株とした２種の耐性化株を樹立した。これらの親株は、我々が以前報告したとおり、我々が保有する悪性胸膜中皮腫細胞株の中で PMX に対する感受性が高い細胞株である。いずれの耐性化株でも PMX 処理（1 μM）における細胞生存率が 50%を超え、76.5%（MSTO-211H 耐性化株）と 91.4%（TCC-MESO-2 耐性化株）であった（図５）。

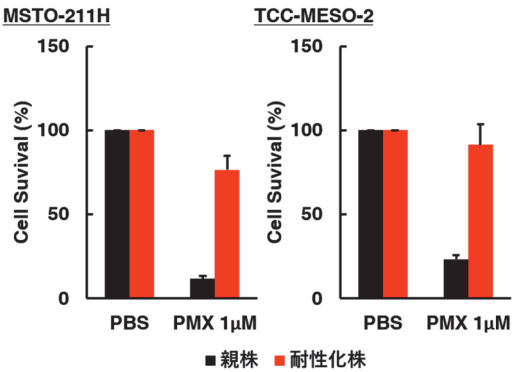

図５．PMX 処理による細胞生存率

樹立した PMX 耐性化株は、高い PMX 耐性を示したことから、PMX に対する耐性化によって標的酵素の発現量が変化したのではないかと考え、その発現量を調べた。

MSTO-211H 細胞と TCC-MESO-2 細胞のいずれの耐性株においても TYMS の発現量が統計的に優位な増加を示していることが分かった。一方で DHFR、GART については大きな違いが見られなかった（図６）。つまり、今回の２種の細胞株では、TYMS の発現量の増加が PMX 耐性に繋がる一因ではないかと考えられる。

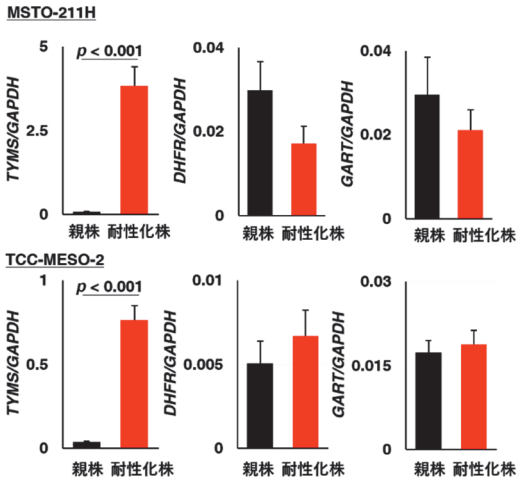

図６．mRNA の発現量

3）令和２年度

昨年度樹立した２種の PMX 耐性化株は PMX に対して強い耐性を有し、mRNA レベルで TYMS の発現が増加していることが分かっている。今年度はタンパク質レベルでも確認するため、MSTO-211H_R と TCC-MESO-2_R 及び、その親株のセルライセートを作成し、ウェスタンブロットを行った。その結果、いずれの細胞株でも親株に比べて PMX 耐性化株の方が TYMS の発現が増加していることが分かった（図７）。

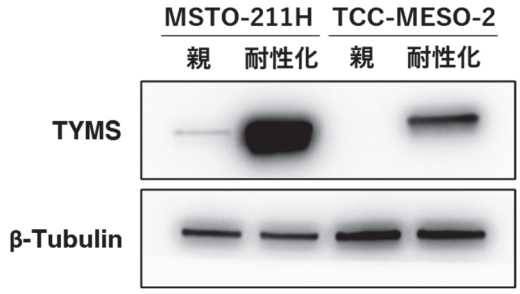

図７．薬剤耐性化によるタンパク質発現の変化

次に、TYMS の発現が増加していることが分かった PMX 耐性化株を用いて、siRNA による TYMS のノックダウンによって薬剤耐性が変化するかどうかを調べた。

２つの PMX 耐性化細胞株をそれぞれ Negative Control（NC）、TYMS 特異的 siRNA（siTYMS）でトランスフェクションし、48 hrs 後に回収した。回収した細胞の一部は、セルライセートを調製し、ウェスタンブロットを行った。残りの細胞は、PBS 処理と PMX 1 μM 処理を行い、WST-8 アッセイで生細胞数をカウントし、NC、siTYMS それぞれの PBS 処理群と PMX 処理群との比を算出した。

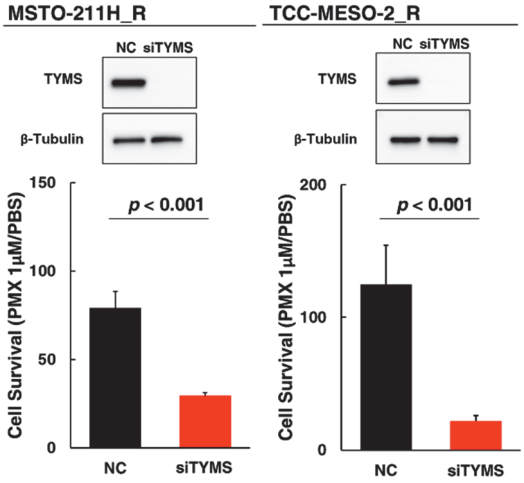

図８．TYMS の KD による薬剤耐性の変化

その結果、NC に比べて siTYMS において PMX によって生細胞数が有意に減少していることから、TYMS のノックダウンによって薬剤耐性が有意に減少したことがわかった（図８）。

次に TYMS を過剰発現させた場合、薬剤感受性が変わるかどうかを調べた。レトロウイルス発現システムである Plat-A cell に２種のプラスミド（コントロール、TYMS）をそれぞれトランスフェクションし、プラスミド由来の遺伝子を有する２種のレトロウイルスを作成した。得られたレトロウイルスを耐性化株の基となった２種の悪性胸膜中皮腫細胞株に感染させた。感染した細胞は G418 を含む培地で培養した後、TYMS

の発現をウェスタンブロットで調べた。その結果、レトロウイルスを用いた過剰発現系によりタンパク質レベルでTYMSの発現を増加させることができた。さらに、同じ細胞株を用いて、それぞれPBS処理、PMX 1 μM 処理を行い、その生細胞数をWST-8アッセイで調べ、PBS処理群とPMX処理群との比を算出した。その結果、TYMSを過剰発現させることにより、PMXに対する感受性が有意に低下することが分かった（図９）。

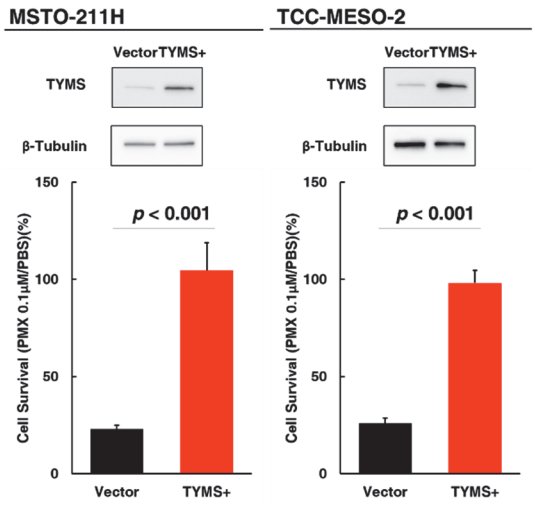

図９．TYMSの過剰発現による薬剤耐性の変化

これらのことから悪性胸膜中皮腫における薬剤耐性化はTYMSが関わっていることが示唆された。

D．研究発表

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E．知的財産権の出願・登録状況

1. 特許取得

記載事項なし。

2. 実用新案登録

記載事項なし。

3. その他

記載事項なし。

労災疾病臨床研究事業費補助金
分 担 研 究 報 告 書

【石綿曝露の免疫機能影響に関する基礎的知見に基づく悪性中皮腫症例の包括的免疫機能解析によるニボルマブ投与療法の効果予測因子の探索】

研究分担者　西村泰光　川崎医科大学　衛生学　准教授

研究要旨

悪性中皮腫症例へのニボルマブ投与前後の包括的免疫動態解析を行った。８例の悪性中皮腫患者の免疫機能解析の結果、部分奏効の治療効果を示す１症例では、NK の IFN-g 産生誘導能が高く、活性化 CTL が多く、Treg 細胞が多いことが確認された。抑制されていた強い NK 機能と CTL 機能が解放され、腫瘍抑制効果に至った可能性が示唆される。今後の一層の免疫機能解析によるニボルマブ治療効果予測指標の構築が期待される。

Ａ．研究目的

これまでに研究分担者らは石綿曝露の免疫機能影響に関する基礎的研究をヒト細胞株および末梢血細胞を用いて行い、石綿曝露下での培養により NK 細胞や T 細胞において特徴的な細胞表面分子の発現量変動を伴う機能低下および免疫抑制能の亢進を報告してきた。最近、それらの知見に基づき末梢血を検体とした包括的免疫機能解析を行い、石綿曝露者または悪性中皮腫患者の免疫学的特徴を明らかにしてきた。近年、免疫チェックポイント分子（IC）を標的とした IC 阻害薬（ICI）が開発され、種々の悪性疾患への適用が進んでおり、悪性中皮腫もその１つである。一方で、他の疾患と同様に、悪性中皮腫に対する ICI 治療効果は一部で奏効する一方、一部では奏効しない状況である。そこで、石綿関連胸膜疾患における個別化治療とケアの確立についての検討の中で、これまでの石綿曝露の免疫機能影響に関する基礎的情報および患者末梢血を用いた包括的免疫機能解析プラットフォームを活用し、悪性中皮腫症例へのニボルマブ投与前後の免疫動態解析を行い、治療効果の奏効に関わる免疫学的特徴の把握を試み、ニボルマブによる中皮腫治療効果を予測する免疫学的因子の探索を行った。

Ｂ．研究方法

研究体制の発足にあたり、これまでの石綿曝露の免疫機能影響に関する知見について研究班内で情報共有を進めた。さらに研究体制の整備にあたり、患者末梢血採血元となる施設との調整を進めた。具体的には、包括的免疫機能解析の実施には、0)採血管および採血管輸送器一式の採血元施設への適切な供給、１）採血元施設における同意を得た患者のエントリー、２）川崎医科大学中央研究センターにおける機器の確保(予約) および衛生学における人員の確保、３）採血管輸送委託先バイク便への正確な日時の情報伝達、４）採血管輸送器一式の施設への返送、といった多施設間による一連の作業が常に一定の条件で適切に円滑に進むことが必要不可欠である。これにあたり、作業フローの明示、担当者間の情報共有、適切なタイミングでの情報提供、を事前に進めた。それらの作業を完了・確認したのち、患者のエントリー、包括的免疫機能解析をスタートし、着実に結果を積み重ねていった。

準備した手順に従い、岡山労災病院または四国がんセンターにて同意された悪性中皮腫患者より末梢血を得た。採血はニボルマブ治療開始前、１週間後、３か月後の３点で行われた。採取された末梢血を川崎医科大学まで輸送し、翌日、血漿を遠心分離によ

り採取した後、lymphoprep を用いて末梢血単核細胞（PBMC）を調整した。PBMC の一部を用いて各種蛍光標識抗体にて染色し、CD4+T ヘルパー細胞（Th）・CD8+細胞傷害性 T リンパ球（CTL）・CD56+ナチュラルキラー細胞（NK）・単球の各細胞集団における細胞表面分子群の発現量をフローサイトメトリー（FCM）により陽性細胞比率（%）または平均蛍光強度（MFI）を測定した。PBMC の残りを FCM により 4 細胞集団にソートし、一部はそのまま凍結、残りは Th, CTL, NK については PMA/ionomycin 刺激下で、単球は無刺激下にて培養し翌日回収し凍結保存した。後日、凍結細胞を試料として total RNA を抽出し SYBR Green を用いて各種遺伝子の mRNA レベルを測定した。また、血漿については Luminex システムを用いて多項目のサイトカイン濃度を測定した（図 1）。最終的に、奏効例における特徴を示す免疫学的指標群について主成分分析を行い、治療効果と免疫学的動態の関わりを考察し、ニボルマブ治療効果予測指標確立の可能性を検討した。統計学的解析には GraphPad Prism9 および SPSS Statistics 27 を用いた。

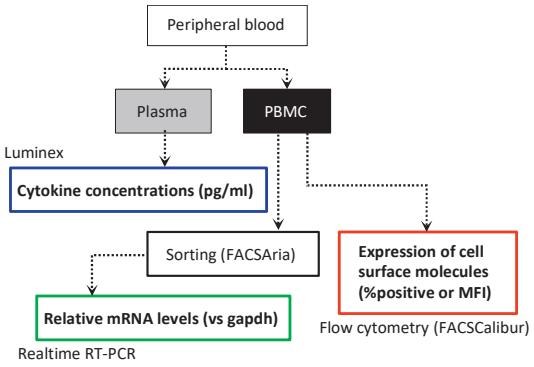

図 1．包括的免疫学的解析の作業フロー

(倫理面への配慮)

研究体制発足にあたり、川崎医科大学衛生学・大槻を研究代表者として、研究課題名「前治療不応性悪性胸膜中皮腫症例に対するニボルマブを含む化学療法における包括的免疫病態の変化の観察」として、倫理申請を行い承認された。令和 2 年度において、解析主体の交代に伴い、川崎医科大学衛生学・西村を研究代表者とする変更を申請し認められた。

C．研究結果

1)　研究体制の整備

本分担研究の遂行にあたり、基盤となる石綿曝露及び悪性中皮腫に関わる免疫機能動態の特徴に関する科学的基盤情報の共有、および研究遂行に必要な円滑で適切な作業フローの構築に必要な準備を行った。

①　石綿曝露及び悪性中皮腫に関わる免疫動態の特徴に関する科学的基盤情報の共有

研究の着実な遂行にあたり、研究分担者がこれまでに明らかにしてきた科学的基盤情報の共有を図った。研究分担者らは、ヒト細胞株およびヒト PBMC を石綿曝露下で培養する研究デザインに基づく一連の研究成果により、石綿曝露が NK 細胞や T 細胞の機能抑制を引き起こすことを明らかにしてきた。具体的には、NK においては石綿曝露により活性化受容体の細胞表面発現量の低下を伴う細胞傷害性低下が引き起こされ、中でも活性化受容体の 1 つ NKp46 の低下は石綿曝露下培養と悪性中皮腫患者末梢血で共通した観察された。また Th においては、石綿曝露と悪性中皮腫患者に共通してケモカイン受容体で Th1 機能指標である CXCR3 細胞表面発現量が低下していることが確認された。また CTL においては、石綿曝露が細胞傷害性低下と IFN-g 酸性低下が引き起こされ、悪性中皮腫患者では類似して細胞傷害性に寄与する perforin の産生誘導能が低下していた。また、石綿曝露は免疫抑制に働く Treg 細胞機能を亢進した。以上のように、研究分担者らは、石綿暴露による悪性中皮腫発症に関わる機序として石綿暴露による免疫抑制作用が有ることを実証してきた（図 2）。

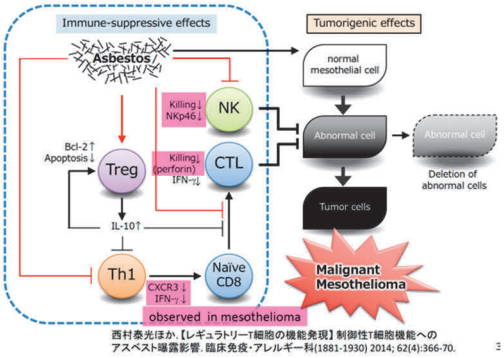

図２．石綿曝露の免疫抑制影響、およびその特徴の一部の悪性中皮腫患者における観察

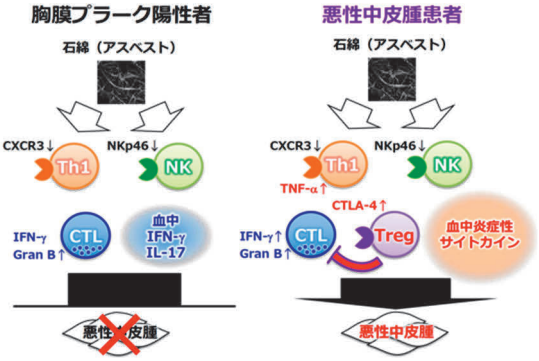

図３．胸膜プラーク陽性非胆癌者と悪性中皮腫患者の包括的免疫機能解析結果の差異のまとめ

それらの知見に基づき、これまでに胸膜プラーク陽性者（非胆癌）と悪性中皮腫患者の免疫学的特徴について健常人と比較した包括的免疫機能解析を行い、両者の特徴の差異を見いだした。胸膜プラークは石綿暴露の代表的な指標であるが、胸膜プラーク陽性で且つ非胆癌である場合には、石綿暴露による一部の免疫抑制作用が有っても、抗腫瘍免疫の健常性を保っている可能性があり、悪性中皮腫患者と胸膜プラーク陽性非胆癌者との間に免疫学的差異が確認できると予想された。包括的免疫機能解析の内容は本分担研究に類似の内容であった。解析の結果、胸膜プラーク陽性者と中皮腫患者は共通する特徴として、NK における NKp46 および Th における CXCR3 の細胞表面発現量低下が確認できた。両者の差異として、胸膜プラーク陽性者では CTL における Granzyme B mRNA レベルが高く維持され、同時に血中の IFN-g や IL-17 濃度が高く、抗腫瘍免疫機能が適切に維持されていると解釈できた。対照的に中皮腫患者では Treg 機能の亢進を示す CTLA-4 発現増加が見られ、また血中の炎症性サイトカイン濃度も高く、抗腫瘍免疫の抑制状態であり、中皮腫発症と免疫動態の差異を確認することができた（図３）。

更に、分担研究者らは同様の解析フローを用いることにより、悪性中皮腫患者とびまん性胸膜肥厚患者との免疫学的特徴の差異を明らかにすることができた。びまん性胸膜肥厚は石綿の吸入曝露が引き起こす疾患であり、両疾患の特徴を末梢血の免疫機能解析から捉えることができれば、診断に寄与する情報として有用性が期待された。包括的免疫機能解析から、悪性中皮腫患者と比較してびまん性胸膜肥厚患者では Treg 指標である GITR 発現量が低く、CTL の細胞傷害性に働く perforin mRNA 発現量が高いこと、両者の Th での転写因子 mRNA レベルの特徴は異なること、主成分分析の結果は両疾患は明瞭に異なる免疫学的特徴を持つことが示された。

以上の情報を中心とする、石綿曝露と悪性中皮腫に関わる免疫機能動態の情報を研究体制内で共有し、本研究課題の遂行にあたり、議論を繰り返し行った。これにより、以降において研究体制内で効果的な議論を可能とする環境を整備することができ、3）に示す具体的な研究成果に繋がる準備をすることが出来た。

② 包括的免疫機能解析の円滑で適切な作業フローの構築

多施設間において円滑に適切に患者採血検体が輸送され、計画どおりに包括的免疫機能解析が実行できるよう、情報の共有と備品の準備を行った。採血候補者のエントリーは患者の同意により確定するため、こ

れにあたり必要なスケジュールについて情報を整理した。具体的には、採血１週間前までには予定を確定さたいこと（機器確保、人員確保のため)、採血に都合の良い曜日があること（金曜は不可）、を確認した。採血管の輸送にはバイク便受託業者を利用すること、および遠方に当たっては鉄道などの公共交通機関とバイクを併用した輸送が行われることを確認した。また、採血管の輸送には特別な恒温ゲルおよび輸送器を用いることを説明した。それぞれの緊急の連絡先を確認した（図４)。また、輸送器の庫内温度を最適に維持するため、温度記録装置を用いること、および採血元施設担当者へ操作方法を指示した（図５)。加えて夏期など暑熱時期に行うべき工夫についても、文書をまとめ採血元施設担当者へ説明を行った。

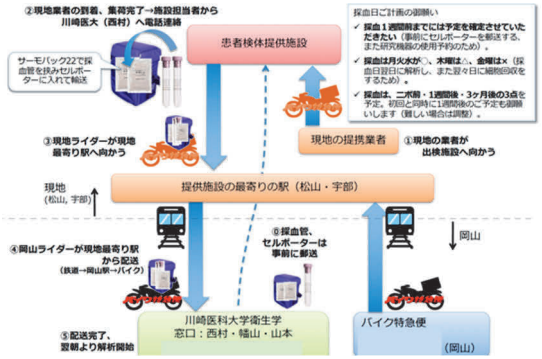

図４．患者検体輸送のながれ、および採血日計画のお願い

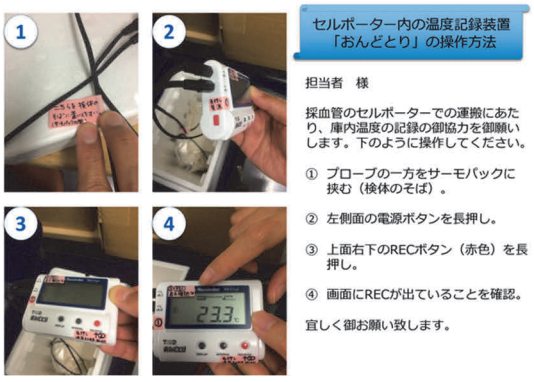

図５．温度記録装置の操作方法の説明

以上により、遠方より運搬される採血管を常に一定の条件で川崎医科大学まで輸送し、包括的免疫機能解析を一定の条件で実施する適切な環境を構築することができ、３)に示す研究成果を得ることが出来た。

2)　代表的１症例の包括的免疫機能解析の結果

包括的免疫機能解析の実施に当たり、運搬された血液検体を用いて適切に解析ができるか、治療前後の免疫機能動態を捉える事が出来るか、代表的１症例の解析を行うことにより確認することができた。岡山労災病院にて同意された悪性中皮腫患者１例の末梢血の解析を実施した。すると、Th や CTL, NK において、また血漿中サイトカイン濃度について、治療前後の変化を確認することができた。中でも、Treg 指標の低下、CTL の IFN-γ 産生能および細胞傷害性関連遺伝子発現亢進、NK 細胞の機能変化、血中サイトカインプロファイルの変化は明瞭であった（図６)。本１症例の解析結果から、引き続き症例数を蓄積し包括的免疫機能解析を実施することにより、ニボルマブ投与前後の免疫動態の変化、および治療効果予測する免疫学的指標の抽出が期待できることが確認された。

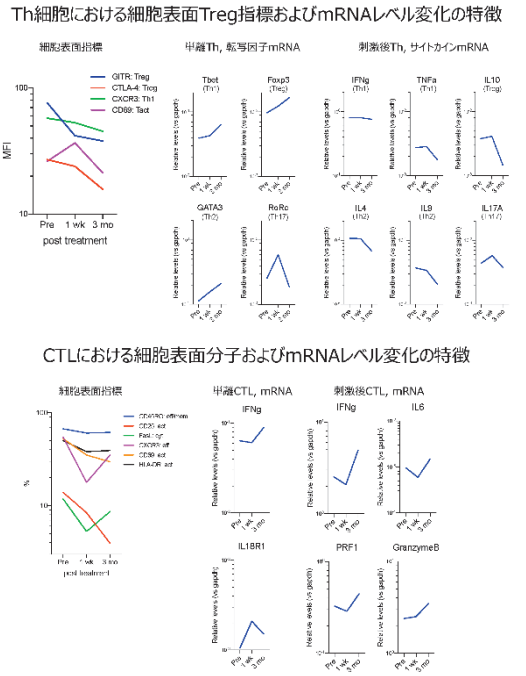

図６．代表的１症例の包括的免疫機能解析結果で確認できた治療前後の免疫動態の変化

3)　悪性中皮腫症例におけるニボルマブ投与療法の奏効に関わる免疫学的特徴の確認

これまでの準備、および代表的１例の解析結果より、本作業フローにより十分な解析結果が得られることが予想できた。そこで、症例数を蓄積し、包括的免疫機能解析によりニボルマブ投与前後の免疫動態を比較する共に、治療効果と関連する免疫学的特徴を探索した。合計８例についての解析を完了することが出来た（表１）。ニボルマブ治療効果の内訳は、部分奏効（PR）１名、病状安定（SD）４名、増悪（PD）３名であった。そこで、８名を SD+PR 群５名と PD 群３名に分け群間で各指標の値およびその動態を比較すると共に、特に治療効果 PR を示す１名における免疫学的特徴の抽出を試みた。また、PD 症例において３か月を待たずに治療を終了した場合には 1.5-2 か月時に採血が行われた（結果の表記では何れも３か月時と便宜表示)。

表１．患者検体一覧

施設	患者ID	治療効果
岡山労災病院	MOP-1	SD
岡山労災病院	MOP-2	SD
四国がんセンター	MOP-3	PD
岡山労災病院	MOP-4	SD
岡山労災病院	MOP-5	SD
岡山労災病院	MOP-6	PD
岡山労災病院	MOP-7	PR
四国がんセンター	MOP-8	PD

その結果、細胞表面分子、細胞内 mRNA レベル血中サイトカイン濃度の何れについても、SD+PR 群と PD 群の群間比較、および両群における治療前後の変化の特徴を捉えることは出来なかった。一方、治療効果 PR を示した１例 MOP-7 については、他と比べて異なる特徴を見いだすことができた。Th 上の CD25%と CTLA-4%および CTL 上の HLA-DR%、加えて刺激後 NK 中の IFN-g mRNA レベルが継続して高い傾向であることが明らかとなった（図７)。Th における CD25（IL-2Rα）の発現は活性化の指標であると同時に CD4+CD25+Treg 細胞との関わりも考えることができた。実際、CD25%は CTLA-4%有意な正の相関を示すことから、MOP-7 における CD25%の高値は活性化ではなく Treg 細胞の増加を意味していると解釈することができた。

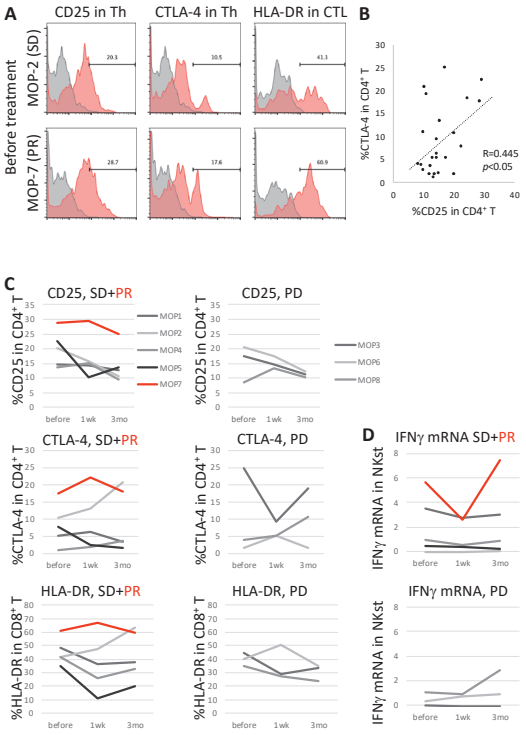

図７．PR を示す MOP-7 における特徴のまとめ

そこで、これら４因子について主成分分析を行ったところ、２つの主成分が抽出出来た。主成分１，２は PR を示す MOP-7 では常に高値を示し、SD,PD 群間に差は見られなかった。主成分１，２をプロットした図では、PR を示す MOP-7 は独立した座標に位置し、他とは異なる免疫学的特徴を持つことが明らかとなった（図８）。

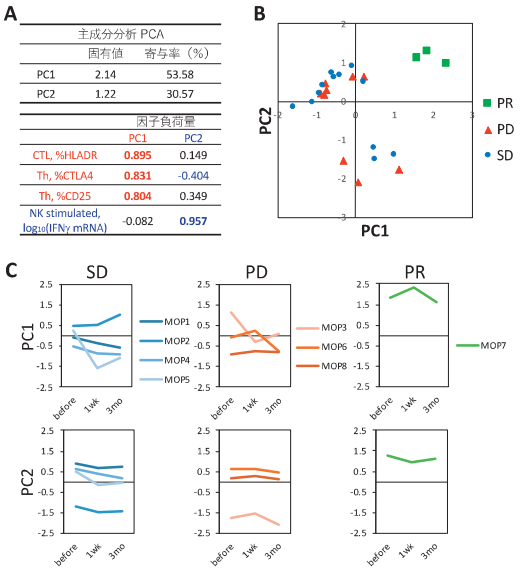

図８．主成分分析結果のまとめ

以上より、ニボルマブ投与による悪性中皮腫の治療奏効と関わる免疫学的特徴として、

1)NK の IFN-g 産生誘導能が高く
2)活性化 CTL が多く
3)Treg 細胞が多い

という３要素の重要性を見いだすことが出来た。

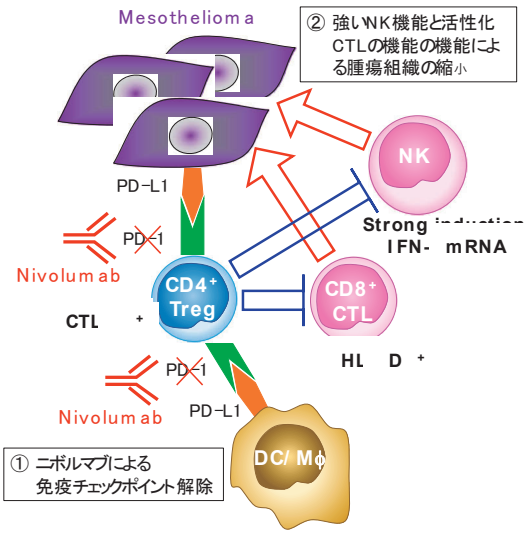

図９．ニボルマブ投与治療の奏効との関連が示唆される免疫学的機序

ニボルマブにより PD-1 分子を介した免疫抑制（Treg 機能）が解除されることで、備えるが抑制されていた強い NK 細胞機能と活性化 CTL の機能が解放され機能を発揮した結果、明瞭な腫瘍抑制効果に至った可能性が示唆される（図９）。解除すべき免疫抑制の標的が明瞭にあって、且つ発揮すべき潜在的な抗腫瘍免疫細胞の機能が保たれていることがニボルマブ治療の奏効に重要であると考えられる。今後の一層の免疫機能解析によるニボルマブ治療効果予測指標の構築が期待される。

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Ｅ．知的財産権の出願・登録状況

1. 特許取得

無し

2. 実用新案登録

無し

3.その他

特に無し

労災疾病臨床研究事業費補助金
分 担 研 究 報 告 書

【石綿ばく露によるびまん性胸膜肥厚の著しい呼吸機能障害に関する研究】

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研究要旨

石綿ばく露労働者に発症したびまん性胸膜肥厚における著しい呼吸機能障害の評価において、呼吸機能検査が出来ないあるいは安静時には呼吸苦を訴えないが、歩行等の労作時に強い呼吸苦を訴えるような場合には６分間歩行試験を施行することになっているが、労災認定のための明確な基準は存在しない。呼吸機能検査や動脈血ガス分析などの検査値は認定基準を満たさないが、労作時呼吸困難等の ADL 低下をアンケート形式で回答を得、どのように呼吸機能検査や６分間歩行結果と関連するかを調査した。その結果、対象となった労災補償を受けている症例のうちほぼ全員が階段昇降と屋外歩行の障害が多いと回答した。これら症例を労災認定できるようにするために、６分間歩行における歩行時の SpO_2、脈拍、歩行距離について呼吸機能検査結果と照合して基準値について検討したところ、歩行時の SpO_2 最低値の 95%信頼区間は 82～90%であり、総歩行距離/予測値の 95%信頼区間は 63～91%であった。びまん性胸膜肥厚症例の６分間歩行試験において、「歩行時 SpO_2 最低値 90%以下あるいは歩行距離が予測値の 90%以下であること」とすることが妥当であると判断した。

Ａ．研究目的

石綿ばく露労働者に発症したびまん性胸膜肥厚における著しい呼吸機能障害の基準は、(１) %肺活量 (%VC) が 60%未満、または(２)%VC が 60%以上 80%未満であって、次の(ア)または(イ)に該当する(ア)1 秒率 (FEV_1%) が 70%未満であり、かつ、%1 秒量 (%FEV_1) が 50%未満) 、(イ)動脈血酸素分圧 (PaO_2) が 60 Torr 以下である場合または肺胞気動脈血酸素分圧較差 ($AaDO_2$) が限界値を超える) 場合となっている。びまん性胸膜肥厚は主に臓側胸膜の病変であって肺疾患ではないが、肺活量が減少するため患者の大半は上り坂や階段を上がる際の労作時呼吸困難の訴えが最も多い。また、この自覚症状に対する治療方法についても気管支拡張剤の一部等に限られている。このように安静時の呼吸機能障害の指標のみならず、歩行時の呼吸機能障害を評価できる６分間歩行に注目して、その限界値について検討することを目的とした。この検査における著しい呼吸機能障害の基準の目安は歩行時あるいは歩行後の SpO_2 88% (PaO_2 55 mmHg) あるいは SpO_2 90% (PaO_2 60 mmHg) とされており、また歩行距離の測定による基準もあるが、明確な基準がないのが実情である。呼吸機能検査や動脈血ガス分析の検査値は現在の認定基準を満たさないが、ADL 低下が顕著な症例を労災認定できるようにするため、研究が必要と考えられる。じん肺法の呼吸機能検査１次及び２次検査、６分間歩行、また

アンケート調査（問診票 P-ADL）を行い、多角的に評価することで、６分間歩行試験における著しい呼吸機能障害の基準を設けることを目的として、本研究を立案した。

B．研究方法

1. 対象

以下の条件を全て満たすものを対象とする。

1）３年間以上の職業性石綿ばく露歴があり、労災認定基準を満たす胸部レントゲン及び CT 検査でびまん性胸膜肥厚と診断され、著しい呼吸機能障害を認めるため労災認定された症例とした。

2）胸部画像所見－特に CT 所見－

胸水貯留を認める症例は、胸水が器質化したことを確認するため、環境省石綿健康被害救済法の認定基準を参考とした。すなわち、

①胸水内部の不均一性(胸水の高吸収化)

②胸郭容積低下

③胸水貯留部位における“ Crow’s feet sign ”の存在

④胸水量の固定化（３か月以上の経過観察)

⑤胸水内のエアーの存在

※①③を必須とする３項目以上が合致した場合についてびまん性胸膜肥厚と判断した。ただし、②胸郭容積低下がある場合には３か月以上の胸水量の固定化があることを必要条件とした。

3）年齢 20 歳以上。

4）６分間歩行検査の実施、またアンケートへの回答が可能な症例とする。ただし、アンケートに関しては、家族・医療者の助けを借りることで回答可能なものは対象として構わない。

5）在宅酸素を導入している症例は除外とする。

2．方法

対象者には年齢、性別、職業性石綿ばく露歴と、その年数及び胸部レントゲン上のびまん性胸膜肥厚の両側性か片側性について調査した。CT 画像において、片側性の場合には左右のいずれか、器質化胸水の有無、上葉の Collapse があるかどうかについて検討した。

1）胸部レントゲン及び CT 検査にてびまん性胸膜肥厚と診断された症例において、呼吸機能検査として、肺機能検査１次（%VC や FEV_1、FEV_1%など）・２次（PaO_2 や $AaDO_2$ など）とともに６分間歩行試験を行う。６分間歩行試験では SpO_2 最低値や歩行距離などをモニタリングする。

なお、６分間歩行試験の禁忌と中止基準は以下の如くである。

(1) 絶対的禁忌

・前月の不安定狭心症と心筋梗塞

(2) 相対的禁忌

・安静時心拍数 > 120 bpm

・収縮期血圧 > 180 mmHg，および拡張期血圧 > 100 mmHg

(3) 中止基準

・胸痛、耐えられない呼吸困難、下肢の痙攣、ふらつき、多量の発汗、顔面蒼白またはチアノーゼの出現

・活動量計を装着し、評価を行う。

・日常生活の状況について P-ADL (別添１)を用いてアンケート調査を行う。（既往歴、内服、喫煙歴、職業歴などを併せて聴取する)

3. 実施期間・場所

調査は 2019 年４月から 2021 年３月まで行い、2021 年３月に調査を終了する。労働者健康安全機構アスベスト疾患研究・研修センターに研究事務局をおき、データマネジメントを行い、個人情報が外部へ流出しないような管理を行う。

(倫理的事項)

本試験の実施にあたって関係する法令や指針（ヘルシンキ宣言、ヒトゲノム・遺伝子

解析研究に関する倫理指針、疫学研究に関する倫理指針など）を遵守する。20 歳以上の患者が対象候補として参加を依頼され、その同意協力を考慮した上で、研究の意義、目的、方法、予測される結果、提供者が被るおそれのある不利益、試料保存及び使用方法、研究参加は自由意志によるもので、拒否・中止も自由でそれに伴う不利益もないこと等について十分な説明を行った上で同意を得る。また、一度同意を得ても患者の自由意志で撤回できる。よって、被検者の自由意志を尊重していると考える。実施などによって生ずる個人への利益、不利益の危険性、本研究で研究参加者が得られる利益はない。また、検査の施行やアンケートへの回答が可能であることが担当者（主治医）によって確認されたものを対象とすることで、対象者の保護を図る。

C．研究結果

対象とした 12 例は全例男性で、年齢は 67～87 歳（平均 76.5±5.5、中央値 76.5 歳）である。職業歴では 4 例が造船業の他、石綿吹付け、断熱保温、築炉、石綿スレート切断、配管、建設業、鋳物、船員が各 1 例であった。石綿ばく露期間は 0.5～50 年で平均 31.8±16.6 年、中央値 39.5 年であった。石綿吹付け作業を半年した症例では石綿肺 1 型を認めた。びまん性胸膜肥厚は両側性が 6 例、片側性が 6 例で、全例が右側のびまん性胸膜肥厚であった。このうち良性石綿胸水後の器質化胸水を認める症例が 4 例あった。また右肺尖部～上葉にかけて Collapse を認めた症例が 5 例あった。

呼吸機能検査と 6 分間歩行試験結果を表 1 に示す。呼吸機能検査では%VC は 32.6～63.7%（平均 48.7±10.1、中央値 49.6%）であり、%VC が 60%を超える症例が 2 例あったが、1 例の FEV_1%は 56.1%、%FEV_1 が 46.4%で、もう 1 例はこの度の検査では基準を満たしていないが、%VC、%FEV_1、FEV_1%のいずれも基準値下限であり、過去の%VC が 60%未満であったため既に認定も受けていた。

%FEV_1 は 56.1～100 %（平均 83.3±15.4%、中央値 89.5%）、%FEV_1 は 39.7～68.3%（平均 49.7±8.5、中央値 46.1%）であり、%FEV_1 は%VC 同様に低下していた。%VC と FEV_1%の相関性については図 1 に示す。

動脈血ガス分析結果では PaO_2 63.9～91.1 mmHg（平均 77.6±10.2 mmHg、中央値 76.5mmHg）、$AaDO_2$ は 10.7～34.9 mmHg（平均 23.5±7.6 mmHg、中央値 22.2 mmHg）と低 O_2 血症、著しい $AaDO_2$ の開大はなかった。

6 分間歩行開始時の SpO_2 は、91.3～97.5%（平均 95.4±1.8%、中央値 96%）であり、歩行中の最小値は 77～96%（平均 86.2±5.9%、中央値 85.5%）であった。歩行中 SpO_2 が 90%未満に低下したのは 12 例中 8 例であり、これら 8 例は 88%未満であった。歩行時の SpO_2 最低値の 95%信頼区間は 82～90%であった。

また、歩行距離は 106～469m（平均 354±89.6m、中央値 373m）であった。12 例中 1 例は 3 分間の休息が必要であったが、その他 11 例は 6 分間歩行を完遂できた。総歩行距離を予測値で割った値は表 1 に示す如く 29～110%（77.3±28.4）中央値 78%で、95%信頼区間は 63～91%であった。

また、脈拍数は歩行開始前が 75.8～97.2 /min（平均 82.7±6.1 /min、中央値 80.9 /min）であり、歩行中の最大値は 94～131 /min（平均 110.7±10.8 /min、中央値 110 /min）であった。

P-ADL に対する回答では表 2 に示すように食事や排泄、洗髪等では日常生活に困る症例はほとんどなく、更衣、会話のみならず、屋内歩行においても大半の症例で難なく可能であったが、しかし階段の昇降や屋外歩行においてほとんどの症例が制限されており、一部症例では大きな障害があることが判った。階段昇降と屋外歩行が一般人と同じ程度にできると回答した症例はわずか 1 例のみであった。

表１．肺機能検査、６分間歩行試験の各症例の結果

					肺機能検査			血ガス検査			6分間歩行試験													
											血圧		Sp02			PR			歩行/無歩行					
	測定時年齢（歳）	性別	身長（cm）	体重（kg）	%肺活量（%）	一秒率（%）	%一秒量（%）	PaO2（Torr）	PaCO2（Torr）	AaDO2（Torr）	開始前（mmHg）	終了後（mmHg）	開始前平均値（%）	歩行中最小値（%）	終了後最大値（%）	開始前平均値（bpm）	歩行中最大値（bpm）	終了後最小値（bpm）	実測行数（歩）	総歩行距離（m）	予測値（m）	割合（%）	中断・休息時間（分）	（秒）	合計（秒）
1	73	M	168.0	59.0	60.2	68.35	52.7	71.2	42.7	29.9	128 / 69	144 / 85	94.3	85	97	77.2	105	72	636	385	492	78	0	0	0
2	77	M	167.0	63.0	39.0	91.67	45.1	91.1	41.1	10.7	123 / 69	137 / 72	97.0	96	98	84.7	131	83	601	371	457	81	0	0	0
3	67	M	163.0	67.5	47.5	67.26	40.2	73.0	42.7	29.1	169 / 97	196 / 106	97.0	82	98	84.9	108	85	725	401	–	–	0	0	0
4	75	M	160.0	58.0	63.7	56.1	46.4	87.9	37.6	17.9	158 / 76	176 / 86	97.5	95	99	78.6	106	73	733	469	423	110	0	0	0
5	80	M	179.0	66.0	52.3	100	68.3	90.0	41.0	13.1	133 / 81	136 / 81	96.6	81	98	76.9	116	55	659	369	528	69	0	0	0
6	70	M	172.0	53.0	32.6	100	39.7	76.5	47.7	20.4	157 / 77	147 / 76	96.8	91	98	97.2	113	95	617	345	548	62	0	0	0
7	87	M	165.0	63.0	59.3	71.84	53.6	84.0	37.1	22.2	169 / 80	168 / 73	94.7	86	97	87.9	112	92	662	325	392	82	0	0	0
8	83	M	156.0	66.0	48.4	93.48	61.1	65.9	44.5	34.9	167 / 82	172 / 97	93.3	82	94	89.0	116	95	591	262	339	77	0	0	0
9	80	M	153.9	52.7	35.7	100	43.4	63.9	46.3	30.9	114 / 47	116 / 55	94.3	87	96	78.1	95	74	273	106	361	29	3	35	215
10	79	M	175.5	73.3	37.2	97.1	45.7	63.9	48.4	30.2	134 / 66	146 / 75	91.3	77	92	81.4	104	57	607	375	493	76	0	0	0
11	71	M	156.0	40.0	50.8	66.67	43.3				185 / 108	229 / 122	95.7	80	97	80.4	128	83	662	420	445	94	0	0	0
12	76	M	170.3	86.0	57.7	87.36	56.4	86.3	38.7	19.1	146 / 88	140 / 89	96.3	92	98	75.8	94	76	699	415	447	92	0	0	0

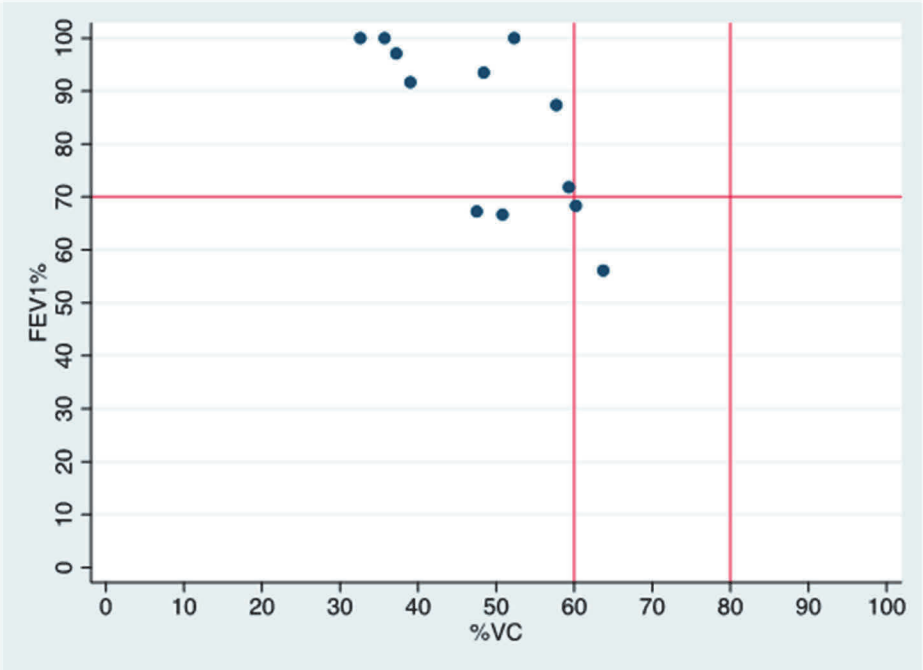

図１．呼吸機能分布図

%VC と FEV_1%の相関について検討したところ、混合性呼吸機能障害を示す症例は２例で、閉塞性呼吸機能障害が認められる症例が２例あった。この症例は%FEV_1が 50%未満であったため、労災認定を受けていた。

表２．P-ADL 調査表に対する各症例の回答結果

	測定時年齢（歳）	性別	食事	排出	入浴	洗髪	整容	更衣	屋内歩行	階段	屋外歩行	会話
1	73	M	22/24	21/24	21/24	22/24	20/24	16/20	19/24	15/24	14/20	13/16
2	77	M	23/24	22/24	24/24	24/24	24/24	20/20	22/24	18/24	17/20	15/16
3	67	M	24/24	24/24	24/24	24/24	24/24	20/20	24/24	23/24	20/20	16/16
4	75	M	24/24	24/24	22/24	24/24	24/24	20/20	24/24	22/24	14/20	16/16
5	80	M	24/24	24/24	24/24	24/24	24/24	20/20	24/24	20/24	18/20	16/16
6	70	M	22/24	24/24	23/24	24/24	24/24	19/20	22/24	20/24	17/20	15/16
7	87	M	24/24	24/24	24/24	24/24	24/24	20/20	24/24	24/24	20/20	16/16
8	83	M	24/24	24/24	24/24	24/24	24/24	20/20	24/24	19/24	17/20	15/16
9	80	M	24/24	24/24	18/24	24/24	24/24	20/20	24/24	23/24	18/20	15/16
10	79	M	24/24	24/24	24/24	24/24	24/24	20/20	24/24	23/24	19/20	16/16
11	71	M	23/24	24/24	23/24	21/24	23/24	19/20	23/24	22/24	19/20	16/16
12	76	M	回答なし									
減点スコア			**6**	**5**	**13**	**5**	**5**	**6**	**10**	**35**	**27**	**7**

D．考察

びまん性胸膜肥厚は石綿による非腫瘍性疾患であり、両側または片側の広範な胸膜肥厚を呈する疾患である。石綿ばく露労働者に発症したびまん性胸膜肥厚における著しい呼吸機能障害の基準において、呼吸機能検査が出来ない場合及び本疾患の性質上労作時呼吸困難が主症状であるため６分間歩行を施行するが、明確な基準が確立されていない。今回は現在の認定基準において労災認定された症例に肺機能検査１次・２次検査及び６分間歩行試験を施行した。

その結果、労災認定基準の著しい呼吸機能障害の%VC が 60%未満であった症例は 12 例中 10 例であったが、１例は閉塞性呼吸機能障害の%FEV_1は 50%以下を満たしていた。もう１例はこの度の検査では基準を満たしていないが、%VC、%FEV_1、FEV_1%のいずれも基準値下限であったが、過去の呼吸機能検査で%VC が 60%未満であったため既に労災認定されていた。

これら症例における６分間歩行時の特徴的所見は予想した通り歩行時の SpO_2 の低下であり、12 例中８例はSpO_2が 90%未満（88%未満）に低下していた。しかし 95%以上と低下しない症例も２例あった。

この結果を P-ADL 質問表から考察すると、階段昇降と屋外歩行では満点の 44 点から１点も引かれない症例はわずか１例なのに対して、最小で１点、最大で 15 点の減点がある症例を認めた。特に５点を超える減点となる６例中３例においてはSpO_2の最小値は 85%以下であった。しかし、その他の５例では 90%（88%）以下であった。

その他の指標である PaO_2、$AaDO_2$ では本疾患が肺障害ではないので著しく低値を示す症例が皆無であった。また、歩行距離に関しては予測値で割った値が 29～110%と差が大きかったが、95%信頼区間は 63～91%であった。

安静時の動脈血ガス分析あるいは呼吸機能検査の結果が良好であっても、本研究の P-ADL の結果に反映されているように、「階段昇降」や「屋外歩行」など労作時に息切れなどを感じることが多いことが回答から明らかとなった。

本疾患の特異性を考慮しても６分間歩行試験のような動的な試験を基準に組み込むことは妥当と思われる。特に歩行時の SpO_2 が一定以下に低下する例が多く、最小値が 90 %以下となる例が 12 例中８例で認められ、歩行時の SpO_2 最低値の 95%信頼区間は 82～90%であった。また、総歩行距離/予測値は 95%信頼区間が 63～91%であった。

以上の結果から「歩行時 SpO_2 の最低値として 90%以下あるいは総歩行距離/予測値の 90%以下とする基準にすること」が考慮されるべきであると思われた。

今後は著しい呼吸器障害を伴わないびまん性

胸膜肥厚症例を対照群とし、比較検討することにより今回の基準がより確立された基準となることが期待される。

E．結論

石綿ばく露労働者に発症したびまん性胸膜肥厚における著しい呼吸機能障害の基準値において、６分間歩行試験における労災認定基準を設けるため労災認定されている 12 例を対象として検討した結果、歩行時の SpO_2 最低値 90%以下あるいは総歩行距離/予測値の 90%以下とすることが妥当であると判断した。

F．研究発表

本研究の研究成果は、国内外の学会発表および英文雑誌で紙上発表することを目標とする。その際には完全な匿名化を行い、個人情報の特定ができないような措置を講ずる。また、発表媒体の倫理規定・投稿規定を遵守する。また、現在６分間歩行による著しい呼吸機能障害の明確な基準のない労災あるいは救済法の基準(案)として厚生労働省あるいは環境省に報告を送付して検討していただく。

G．知的財産権の出願・登録状況

１．特許取得

該当するものなし。

２．実用新案登録

該当するものなし。

３．その他

特記すべき事項なし。

別添１.

Ｐｕｌｍｏｎａｒｙ　ＡＤＬ：Ｐ－ＡＤＬ（Ｖｅｒ．２）　評価表

ご自宅での生活についてご記入下さい。（入院中の方は、入院直前の状況でお書き下さい）

氏名：　　**殿**

処方されている酸素量：①安静時（　　）Ｌ／分、②動作時（　　）Ｌ／分、
③睡眠時（　　）Ｌ／分
☆酸素量を変更する動作をお書きください：（　　）

※各項目のあてはまる番号（0～4）を一つずつ選んで○で囲んで下さい。

	達成方法	距　離	頻　度	速　度	息切れ	酸素量
食事	0　食べさせてもらう	0　自室(寝たままで)	0　毎回、食べさせてもらう	0　全く食べられない	0　耐えられない	0　自分で中止する
	1	1	1	1　かなり休みながら	1　かなりきつい	1　自分で変更する
	2　自分で食べる（刻み食など加工必要）	2　自室（寝床以外で）	2　状況により自分で食べる	2　途中でひと休み	2　きつい	2　ほぼ処方量を厳守
	3	3	3	3　休まずゆっくり	3　多少きつい	3　常に処方量を厳守
	4　自分で食べる（普通食）	4　自室以外（食堂など）	4　毎回、自分で食べる	4　スムーズにできる	4　何も感じない	4　処方されていない
排泄	0　差し込み便器を使用	0　ベット上	0　便所に行って排泄しない	0　全く便所に行かない	0　耐えられない	0　自分で中止する
	1　尿器・ポータブルトイレを使用	1	1　排便のみ便所	1　かなり休みながら	1　かなりきつい	1　自分で変更する
	2　夜間のみ尿器、ポータブルトイレ	2　ベットサイド	2　昼間便所に行くことがある	2　途中でひと休み	2　きつい	2　ほぼ処方量を厳守
	3　便所を使用し、介助を受ける	3	3　昼間は毎回便所に行く	3　休まずゆっくり	3　多少きつい	3　常に処方量を厳守
	4　便所を使用し、全く介助を受けない	4　便所	4　毎回（夜間も）便所に行く	4　スムーズにできる	4　何も感じない	4　処方されていない
入浴	0　清拭（体を拭く）してもらう	0　自室	0　全く入浴しない	0　全く自分でできない	0　耐えられない	0　自分で中止する
	1　自分で清拭（体を拭く）する	1	1	1　かなり休みながら	1　かなりきつい	1　自分で変更する
	2　ほとんど介助してもらう	2　浴室でシャワーのみ	2　たまに入浴を行う	2　途中でひと休み	2　きつい	2　ほぼ処方量を厳守
	3　一部介助してもらう	3	3	3　休まずゆっくり	3　多少きつい	3　常に処方量を厳守
	4　自分でできる	4　浴槽に入る	4　入浴日に毎回入浴する	4　スムーズにできる	4　何も感じない	4　処方されていない
洗髪	0　洗髪しない	0　ベット上	0　全く洗髪しない	0　全く自分でできない	0　耐えられない	0　自分で中止する
	1	1	1	1　かなり休みながら	1　かなりきつい	1　自分で変更する
	2　洗髪してもらう（理容院等を含む）	2　浴室以外（洗面所など）	2　入浴とは別に洗髪する	2　途中でひと休み	2　きつい	2　ほぼ処方量を厳守
	3	3	3	3　休まずゆっくり	3　多少きつい	3　常に処方量を厳守
	4　自分で洗髪する	4　浴室	4　入浴時に洗髪する	4　スムーズにできる	4　何も感じない	4　処方されていない
整容	0　寝たままで、介助を受ける	0　ベット上	0　洗面所で洗面歯磨きしない	0　全く自分でできない	0　耐えられない	0　自分で中止する
	1　座って、介助を受ける	1	1	1　かなり休みながら	1　かなりきつい	1　自分で変更する
	2　準備されれば座って自分で行える	2　洗面所以外（自室など）	2　たまに洗面所で洗面・歯磨き	2　途中でひと休み	2　きつい	2　ほぼ処方量を厳守
	3　座って自分でできる	3	3	3　休まずゆっくり	3　多少きつい	3　常に処方量を厳守
	4　立って、自分でできる	4　洗面所	4　毎回、洗面所で洗面・歯磨き	4　スムーズにできる	4　何も感じない	4　処方されていない

	達成方法	距　離	頻　度	速　度	息切れ	酸素量
更衣	0　更衣を手伝ってもらう		0　自分で更衣はできない	0　全く自分でできない	0　耐えられない	0　自分で中止する
	1		1	1　かなり休みながら	1　かなりきつい	1　自分で変更する
	2　準備されれば自分でできる		2　状況により自分で更衣を行う	2　途中でひと休み	2　きつい	2　ほぼ処方量を厳守
	3		3	3　休まずゆっくり	3　多少きつい	3　常に処方量を厳守
	4　自分でできる		4　毎回自分で更衣を行う	4　スムーズにできる	4　何も感じない	4　処方されていない
屋内歩行	0　全く歩けない	0　全く歩けない	0　全く歩けない	0　全く自分でできない	0　耐えられない	0　自分で中止する
	1　介助があれば歩ける	1　ベット周囲のみ	1	1　かなり休みながら	1　かなりきつい	1　自分で変更する
	2	2　自室内のみ	2　状況により歩くことができる	2　途中でひと休み	2　きつい	2　ほぼ処方量を厳守
	3　見守り（監視）があれば歩ける	3　便所・洗面所のみ	3	3　休まずゆっくり	3　多少きつい	3　常に処方量を厳守
	4　自分だけで歩ける	4　自宅内はすべて	4　いつでも歩くことができる	4　スムーズにできる	4　何も感じない	4　処方されていない
階段	0　自分では昇れない	0　全く昇れない	0　昇れない	0　全く自分でできない	0　耐えられない	0　自分で中止する
	1	1　2～3段	1	1　かなり休みながら	1　かなりきつい	1　自分で変更する
	2　介助があれば昇れる	2　５～６段	2　必要な時だけ昇る	2　途中でひと休み	2　きつい	2　ほぼ処方量を厳守
	3	3　2階まで	3	3　休まずゆっくり	3　多少きつい	3　常に処方量を厳守
	4　自分だけで昇れる	4　3階以上	4　いつでも昇ることができる	4　スムーズにできる	4　何も感じない	4　処方されていない

屋外歩行	0　全く歩けない	最長どのくらいの	0　全く歩けない	0　全く自分でできない	0　耐えられない	0　自分で中止する
	1　介助があれば歩ける	距離歩けますか？	1	1　かなり休みながら	1　かなりきつい	1　自分で変更する
	2		2　状況により歩くことができる	2　途中でひと休み	2　きつい	2　ほぼ処方量を厳守
	3　見守り（監視）があれば歩ける	（　　）m位	3	3　休まずゆっくり	3　多少きつい	3　常に処方量を厳守
	4　自分だけで歩ける		4　いつでも歩くことができる	4　スムーズにできる	4　何も感じない	4　処方されていない
会話	0　寝床（ベット上）で寝ながら	最長どのくらいの		0　全くできない	0　耐えられない	0　自分で中止する
	1	時間話せますか？		1　かなり休みながら	1　かなりきつい	1　自分で変更する
	2　車椅子や安楽椅子に座る			2　途中でひと休み	2　きつい	2　ほぼ処方量を厳守
	3	（　　）時間位		3　休まずゆっくり	3　多少きつい	3　常に処方量を厳守
	4　どこでも座っていればできる			4　スムーズにできる	4　何も感じない	4　処方されていない

特記事項

年　　月頃の状態　　記入日：　　年　　月　　日（記入者：　　）

Ⅲ.　研究成果の刊行に関する一覧表

＜研究成果の刊行に関する一覧表＞

【 書　籍 】

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
Fujimoto N.	Immunocheckpoint Blockade in Malignant Pleural Mesothelioma.	Prof. Tekemi Otsuki	Asbestos-related Diseases	Intech Open.	UK	2019	DOI: 10.5772/intechopen.89116

【 雑　誌 】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Fujimoto N, Aoe K, Kozuki T, Oze I, Kato K, Kishimoto T, Hotta K.	A phase II trial of first-line combination chemotherapy with cisplatin, pemetrexed, and nivolumab for unresectable malignant pleural mesothelioma: A study protocol.	Clin Lung Cancer.	19(5)	e705-e707	2018
Sato Y, Matsuda S, Maruyama A, Nakayama J, Miyashita T, Udagawa H, Umemura S, Yanagihara K, Ochiai A, Tomita M, Soga T, Tsuchihara K, Makinoshima H.	Metabolic Characterization of Antifolate Responsiveness and Non-responsiveness in Malignant Pleural Mesothelioma Cells.	Front Pharmacol.	9	1129 doi: 10.3389/fphar.	2018
Nagamatsu Y, Oze I, Aoe K, Hotta K, Kato K, Nakagawa J, Hara K, Kishimoto T, Fujimoto N.	Physician requests by patients with malignant pleural mesothelioma in Japan.	BMC Cancer.	19(1)	383	2019

＜研究成果の刊行に関する一覧表＞

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Okada M, Kijima T, Aoe K, Kato T, Fujimoto N, Nakagawa K, Takeda Y, Hida T, Kanai K, Imamura F, Oizumi S, Takahashi T, Takenoyama M, Tanaka H, Hirano J, Namba Y, Ohe Y.	Clinical Efficacy and Safety of Nivolumab: Results ofaMulticenter,Open-label,Single-arm,Japanese Phase II study in Malignant Pleural Mesothelioma (MERIT)	Clin Cancer Res.	25(18)	5485-5492	2019
Hotta K, Fujimoto N.	Current evidence and future perspectives of immune-checkpoint inhibitors in unresectable malignant pleural mesothelioma.	J Immunother Cancer.	8(1)	e000461 doi: 10.1136/jitc-2019-000461.	2020
Hotta K, Fujimoto N, Kozuki T, Aoe K, Kiura K.	Nivolumab for the treatment of unresectable pleural mesothelioma.	Expert Opin Biol Ther.	20(2)	109-114	2020
Fujimoto N.	An appropriate choice for immunotherapy in malignant pleural mesothelioma.	EBioMedicine.	62	doi: 10.1016/j.ebiom.2020.103057.	2020
Baas P, Scherpereel A, Nowak AK, Fujimoto N, Peters S, Tsao AS, Mansfield AS, Popat S, Jahan T, Antonia S, Oulkhouir Y, Bautista Y, Cornelissen R, Greillier L, Grossi F, Kowalski D, Rodríguez-Cid J, Aanur P, Oukessou A, Baudelet C, Zalcman G.	First-line nivolumab plus ipilimumab in unresectable malignant pleural mesothelioma (CheckMate 743): a multicentre, randomised, open-label, phase 3 trial.	Lancet.	397(10272)	375-386	2021

＜研究成果の刊行に関する一覧表＞

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Tanaka T, Miyamoto Y, Sakai A, Fujimoto N.	Nivolumab for malignant peritoneal mesothelioma.	BMJ Case Rep.	13(11)	e237721	2020
Fujimoto N, Okada M, Kijima T, Aoe K, Kato T, Nakagawa K, Takeda Y, Hida T, Kanai K, Hirano J, Ohe Y.	Clinical efficacy and safety of nivolumab in Japanese patients with malignant pleural mesothelioma: 3-year results of the MERIT study.	JTO Clin Res Rep	2（3）	https://doi.org/10.1016/j.jtocrr.2020.100135	2021

Ⅳ.　研究成果の別刷

Chapter

Immunocheckpoint Blockade in Malignant Pleural Mesothelioma

Nobukazu Fujimoto

Abstract

Targeting immunocheckpoint with immunomodulatory monoclonal antibodies has proven to be an effective antitumor strategy across a variety of cancers. The immunosuppressive tumor microenvironment in malignant pleural mesothelioma (MPM) has suggested that MPM might benefit from this kind of immunotherapy. In recent years, immunocheckpoint inhibitors (ICIs) have shown encouraging results for patients with MPM. Antibodies against programmed death 1 (PD-1) and PD-ligand 1 (PD-L1) have demonstrated favorable response, progression-free survival, and overall survival. The toxicity profiles were similar to those observed with ICIs in other malignancies, like melanoma and non-small cell lung cancer, and they appeared to be manageable. Nivolumab, an anti-PD-1 antibody, was approved in Japan for advanced or metastatic MPM patients resistant or intolerant to other chemotherapies. Important future issues include developing a combination therapy, where ICIs are combined with other agents (including other ICIs), and developing biomarkers for determining which patients might respond well and which might experience unacceptable toxicities.

Keywords: durvalumab, immunocheckpoint, nivolumab, pembrolizumab, PD-1

1. Introduction

Malignant pleural mesothelioma (MPM) is a rare pleural malignancy that is associated with asbestos exposure. Gemba et al. reported that more than 70% of malignant mesothelioma cases in Japan were associated with occupational or environmental asbestos exposure [1]. MPM is a highly aggressive neoplasm with a poor prognosis; the median overall survival (OS) is only about 12 months. Systemic chemotherapy with platinum plus pemetrexed is the recommended first-line systemic therapy for advanced MPM [2]. Some clinical trials have examined the efficacy of new agents to improve the results of the platinum/pemetrexed combination; however, no new agent has demonstrated significant clinical efficacy. Thus, the pemetrexed/platinum combination remains the standard treatment.

Currently, there is no recommended treatment option for MPM after first-line platinum/pemetrexed chemotherapy. Re-treatment with pemetrexed-based chemotherapy is a reasonable option for patients that achieved durable disease control with the first-line chemotherapy [3]. Other treatment options of salvage chemotherapy include vinorelbine and gemcitabine; however, the median OS with these agents only ranges from 5 to 10 months [4, 5]. Other experimental agents, such as angiogenesis inhibitors [6] or tyrosine kinase inhibitors [7], have not demonstrated efficacy.

Targeting immunocheckpoint with immunomodulatory monoclonal antibodies was shown to be an effective antitumor strategy across a variety of cancers [8]. The immunosuppressive tumor microenvironment in MPM has suggested that MPM might benefit from this kind of immunotherapy [9, 10]. In fact, in recent years, immunocheckpoint inhibitors (ICIs) have shown some encouraging results for patients with MPM.

In this chapter, we review recent clinical findings on several ICIs, including anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) antibody, anti-programmed death 1 (PD-1) antibody, and anti-PD-ligand 1 (PD-L1) antibody, for treating patients with MPM.

2. Anti-CTLA-4 antibody

Anti-CTLA-4 antibody was the first ICI described for treating MPM. Phase II studies demonstrated that tremelimumab, a selective human monoclonal antibody against CTLA-4, showed favorable activity as a second-line treatment for MPM [11, 12]. However, a double-blind study that compared tremelimumab to placebo in subjects with previously treated, unresectable malignant mesothelioma (DETERMINE study) failed to demonstrate differences in OS or progression-free survival (PFS) between the treatment and placebo groups [13]. After that, anti-CTLA-4 antibodies were studied in combination with an anti-PD-1 or anti-PD-L1 antibody.

3. Anti-PD-L1 antibody

Avelumab is a human IgG1 monoclonal antibody that targets PD-L1 [14]. A phase 1b open-label study (JAVELIN solid tumor) was conducted in patients with unresectable mesothelioma that progressed after platinum/pemetrexed treatment; patients were enrolled at 25 sites in three countries [15]. Of 53 patients treated, the objective response rate (RR) was 9% (95% confidence interval [95%CI]: 3.1–20.7%); one patient experienced a complete response, and four patients experienced a partial response. Responses were durable (median, 15.2 months; 95%CI: 11.1 to non-estimable) and occurred in patients with PD-L1-positive tumors (RR: 19%; 95%CI: 4.0–45.6) and PD-L1-negative tumors (RR: 7%; 95%CI: 0.9–24.3), based on a 5% or greater cutoff for PD-L1 expression. The median PFS was 4.1 months (95%CI: 1.4–6.2), and the 12-month PFS rate was 17.4% (95%CI: 7.7–30.4). The median OS was 10.7 months (95%CI: 6.4–20.2).

4. Anti-PD-1 antibody

4.1 Pembrolizumab

A nonrandomized, phase Ib trial was conducted to test pembrolizumab in patients with PD-1-positive MPM that had been treated previously. In the preliminary report, 20% of patients experienced an objective response, 72% experienced disease control, and the median OS was 18 months (95%CI: 9.4 to non-estimable) [16]. Then, a phase II trial assessed pembrolizumab activity in 65 unselected patients with MPM [17]. The objective RR was 19% and the disease control rate was 66%. The median PFS was 4.5 months (95%CI: 2.3–6.2), and the median OS was 11.5 months (95%CI: 7.6–14).

After those promising results, pembrolizumab was used off-label in Switzerland and Australia [18]. A total of 93 patients (48 from Switzerland and 45 from Australia) were treated. In those cohorts, the overall RR was 18%, the median PFS was 3.1 months, and the median OS was 7.2 months. Among patients with the non-epithelioid histological subtype, pembrolizumab treatment improved the objective RR (24% vs. 16%; $p = 0.54$) and the median PFS (5.6 vs. 2.8 months; $p = 0.02$).

4.2 Nivolumab

Another anti-PD-1 antibody, nivolumab, was first tested in recurrent MPM in the Netherlands [19]. In that single-center trial, patients with MPM received 3 mg/kg intravenous nivolumab every 2 weeks. Of the 34 patients included, eight patients (24%) displayed a partial response and another eight displayed stable disease, which resulted in a disease control rate of 47%. Japanese investigators also evaluated the efficacy and safety of nivolumab for advanced MPM in patients that were resistant or intolerant to prior chemotherapy [20]. Thirty-four patients were enrolled, and 10 patients (29.4%, 95%CI: 16.8–46.2) showed an objective response in a central assessment. Objective RRs were 25.9, 66.7, and 25.0% for epithelioid, sarcomatous, and biphasic histological subtypes, respectively (**Figure 1**). The median OS and PFS were 17.3 and 6.1 months, respectively (**Figure 2a** and **b**). Based on these findings,

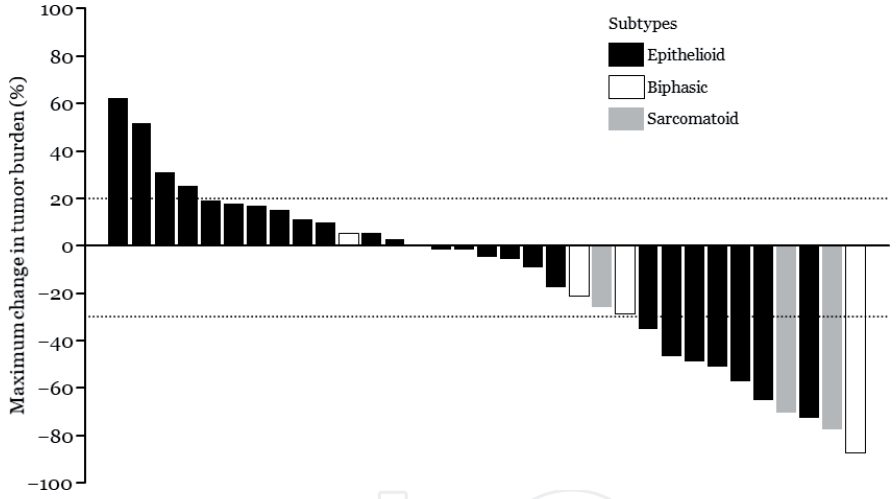

Figure 1.
A waterfall plot of the MERIT study results, which demonstrates the maximum percentage changes compared to baseline in target lesions of each patient, according to histological subtype (Ref. [20]).

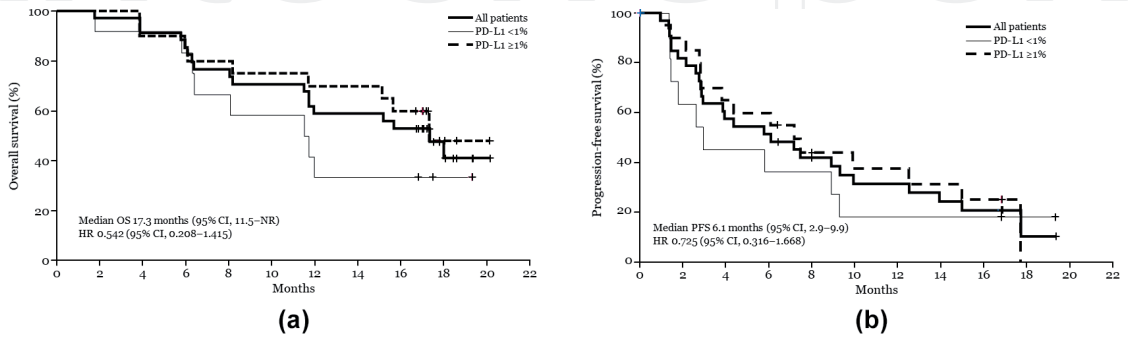

Figure 2.
Kaplan-Meier curves show survival for all patients and for patients grouped according to programmed death-ligand 1 (PD-L1) expression in the MERIT study (Ref. [20]). (a) Overall survival (OS); (b) progression-free survival (PFS). HRs compare the PD-L1 ≥ 1% group to the <1% group. CI, confidence interval; HR, hazard ratio; NR, not reached.

nivolumab was approved in Japan for patients with advanced or metastatic MPM that are resistant or intolerant to previous chemotherapy.

Although the effect requires confirmation in larger clinical trials, nivolumab and pembrolizumab might offer hope for patients with MPM.

5. Toxicity

The toxicity of these ICIs was acceptable in MPM. A study on pembrolizumab toxicity found grade 3 and 4 events, including adrenal insufficiency (3%), pneumonitis (3%), skin rash (3%), colitis (1.6%), confusion (1.6%), hepatitis (1.6%), and hyperglycemia (1.6%), and one grade 5 event of hepatitis (1.6%) [17]. In a study on nivolumab, adverse events of any grade occurred in 26 patients (76%), including fatigue (29%) and pruritus (15%) [19]. In that study, treatment-related grade 3 and 4 adverse events were reported in nine patients (26%); most events were pneumonitis, gastrointestinal disorders, and laboratory disorders. One treatment-related death was due to pneumonitis, but it was probably initiated by concurrent amiodarone therapy. These toxicity profiles were similar to those observed in other malignancies, including melanoma and non-small cell lung cancer (NSCLC), and they appeared to be manageable.

6. Future perspectives

Based on the promising results described above, ICIs could play a primary role in the treatment of MPM. An important issue for the future is whether ICIs can be combined with other agents, including other ICIs. For example, given the synergy between the PD-1/PD-L1 and CTLA-4 pathways in T-cell activation, a combination treatment with antibodies that target PD-1 or PD-L1 and CTLA-4 warrants investigation [22].

NIBIT-MESO-1 was an open-label, nonrandomized, phase II study that investigated the efficacy and safety of first- or second-line tremelimumab, a monoclonal antibody against CTLA-4, combined with durvalumab, a monoclonal antibody against PD-L1 [23]. In that study, patients with unresectable pleural or peritoneal mesothelioma received one dose of intravenous tremelimumab and durvalumab delivered every 4 weeks, for a total of four doses. This was followed by maintenance treatment with intravenous durvalumab. Of 40 patients, 11 (28%) displayed an objective response. The median PFS was 5.7 months (95%CI: 1.7–9.7), and the median OS was 16.6 months (95%CI: 13.1–20.1). Toxicity related to treatment was generally manageable and reversible.

Another multicenter, randomized, phase II study was conducted in France [24]. In that study, patients were randomly allocated to nivolumab or nivolumab plus ipilimumab. In the intention-to-treat population, the primary endpoint, 12-week disease control, was achieved by 25 (40%; 95%CI: 28–52) of 63 patients in the nivolumab group and by 32 (52%; 95%CI: 39–64) of 62 patients in the combination group. The most frequent grade 3 adverse events were asthenia (N = one [2%] with nivolumab vs. three [5%] with the combination), an asymptomatic increase in aspartate aminotransferase or alanine aminotransferase (N = none with nivolumab vs. four [7%] of each with the combination), and an asymptomatic increase in lipase (N = two [3%] with nivolumab vs. one [2%] with the combination). These findings indicated that the combination of anti-CTLA-4 and anti-PD1/PD-L1 antibodies appeared to be active and had a good safety profile in patients with MPM. Currently, there is an ongoing phase III, randomized, open-label trial for testing nivolumab in combination with ipilimumab vs. pemetrexed with cisplatin or carboplatin as a first-line therapy in unresectable MPM. The primary endpoint of the study, OS, will be reported in the near future.

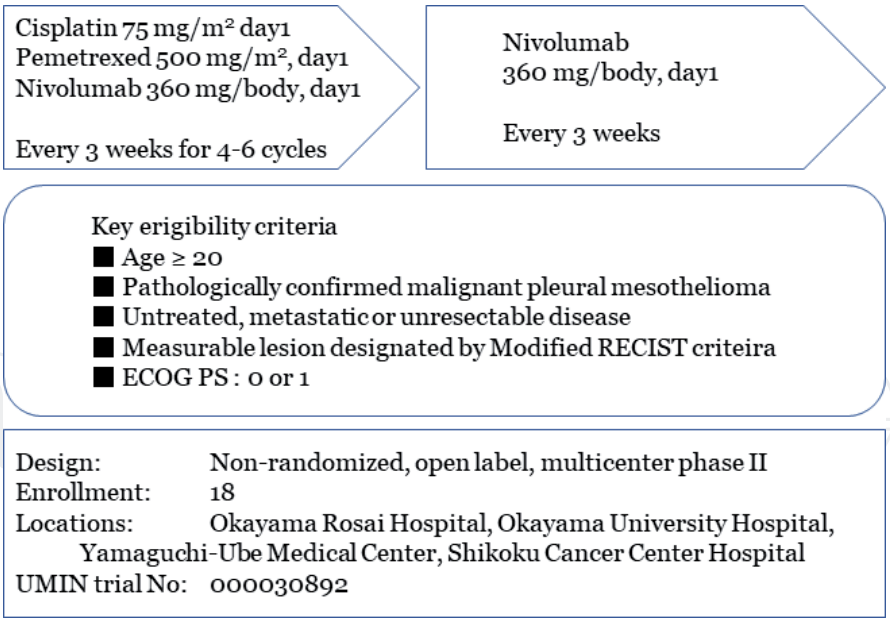

Figure 3.
Overview of a phase II trial for testing a first-line combination chemotherapy with cisplatin/pemetrexed and nivolumab for treating unresectable malignant pleural mesothelioma (Ref. [21]). RECIST, response evaluation criteria in solid tumors; ECOG, eastern cooperative oncology group; PS, performance status.

The combination of an anti-PD-1/PD-L1 antibody and conventional chemotherapy is also under investigation. Nowak et al. presented results from a phase II trial that tested durvalumab combined with cisplatin/pemetrexed in MPM [25]. The primary endpoint, PFS at 6 months, was 57% (N = 31/54; 95%CI: 45–68), the median PFS time was 6.9 months (95%CI: 5.5–9.0), and the objective RR was 48% (95%CI: 35–61). Grade 3–5 adverse events occurred in 36 patients, including neutropenia in 13%, nausea in 11%, anemia in 7%, fatigue in 6%, and any grade of peripheral neuropathy in 35%. The authors have conducted another phase II study to test the combination of nivolumab and cisplatin/pemetrexed, which is currently in progress (**Figure 3**) [21]. A large-scale randomized study for testing the combination of pembrolizumab and cisplatin/pemetrexed is also in progress. Based on whether these combination regimens, which include anti-PD1/PD-L1 antibodies, demonstrate sufficient activity, safety, and tolerability as first-line treatments, the standard regimen of cisplatin/pemetrexed might be replaced.

Another important issue is whether biomarkers can be developed to determine which patients might expect a response and which might expect unacceptable toxicity. Previous studies in patients with MPM have shown that tumors with positive PD-L1 expression were associated with worse survival outcomes compared to those with negative PD-L1 expression [26]. Although an optimal PD-L1 expression threshold could not be identified, a trend was observed, where a higher RR and more durable PFS were associated with increasing PD-L1 expression, in studies on pembrolizumab [17, 18] and nivolumab [20]. In some neoplasms, the tumor mutation burden or the tumor microenvironment was associated with the response to ICIs; however, those associations have not been established as biomarkers in MPM.

7. Conclusion

The prognosis of MPM remains poor. Recent encouraging results have suggested that a PD-1/PD-L1 blockade might be an effective treatment option

for MPM. Although the effect requires confirmation in larger clinical trials, nivolumab and pembrolizumab might offer hope for patients with MPM. Further study is warranted to develop more effective treatment strategies, such as combining ICI with other ICIs or with conventional chemotherapy, and to establish biomarkers for distinguishing patients that might respond to treatment from those likely to develop unacceptable toxicities.

Acknowledgements

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Conflict of interest

The author received consultancy fees from Boehringer Ingelheim, Ono, Bristol-Myers Squibb, Kyorin, and Kissei, and honoraria or research funding from Hisamitsu, Chugai, Ono, Taiho, Boehringer Ingelheim, Bristol-Myers Squibb, Novartis, GlaxoSmithKline, and MSD.

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A Phase II Trial of First-Line Combination Chemotherapy With Cisplatin, Pemetrexed, and Nivolumab for Unresectable Malignant Pleural Mesothelioma: A Study Protocol

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Abstract

Background: The purpose of this study is to assess the efficacy and safety of combination chemotherapy with cisplatin, pemetrexed, and nivolumab for unresectable malignant pleural mesothelioma (MPM). **Patients and Methods:** Patients with untreated, advanced, or metastatic MPM who meet the inclusion and exclusion criteria will be included. A total of 18 patients will be enrolled from 4 Japanese institutions within 1 year. Combination chemotherapy with cisplatin (75 mg/m^2), pemetrexed (500 mg/m^2), and nivolumab (360 mg/person) is administered every 3 weeks for a total of 4 to 6 cycles. Then, maintenance therapy with nivolumab will be administered until disease progression, unacceptable toxicities, or the patient's condition meets the withdrawal criteria. The primary end point is the centrally reviewed overall response rate. The secondary end points include the disease control rate, overall survival, progression-free survival, and adverse events. **Conclusion:** This phase II trial evaluating first-line combination chemotherapy for unresectable MPM commenced in January 2018. This is the first prospective trial to evaluate the effect of an anti-programmed death-1 antibody combined with cisplatin and pemetrexed for unresectable MPM.

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Keywords: Asbestos, Immune checkpoint inhibitor, Maintenance, Programmed death-1, Prospective study

Introduction

Malignant pleural mesothelioma (MPM) is a highly aggressive tumor that arises from mesothelial-lined surfaces and has a poor survival rate.[1] MPM occurs more frequently in men (80%) than in women, and the peak age of onset is between 60 and 80 years old.[2] The industrial use of asbestos has been banned in Japan since 2006, but the incidence of MPM is expected to continue to increase for the next few decades because of the past usage of asbestos.[3] Treatment of MPM is challenging. Most of the cases are diagnosed at an advanced stage and are treated with systemic chemotherapy. Combination chemotherapy with cisplatin and pemetrexed is the standard treatment regimen; however, the median overall survival (OS) is only approximately 12 .months.[4] Recently, the additional use of bevacizumab improved OS when used with cisplatin and pemetrexed in unresectable MPM.[5] However, the prolongation of the OS was <3 months. In addition, it can be administered only to bevacizumab-eligible patients. On the basis of these facts, cisplatin and pemetrexed is still considered the standard treatment regimen, thus, additional treatment options are urgently needed.

Nivolumab is a human monoclonal antibody that targets the programmed death (PD)-1 cluster of differentiation 279 cell surface membrane receptor. Binding of PD-1 to its ligands, PD ligands 1 and 2, results in the downregulation of lymphocyte activation.

This trial is registered in the UMIN Clinical Trials Registry: UMIN000030892.

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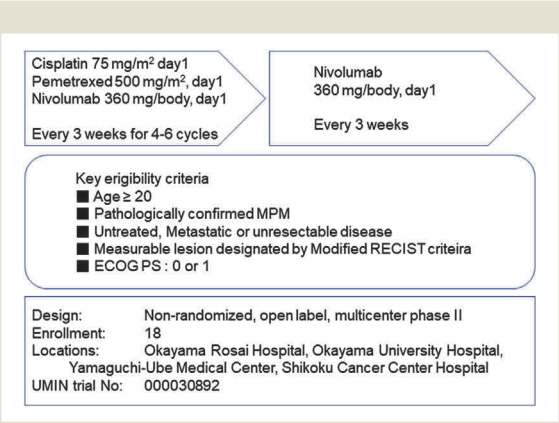
Figure 1 Overview of the Study Design



Abbreviations: ECOG = Eastern Cooperative Oncology Group; MPM = malignant pleural mesothelioma; PS = performance status; RECIST = Response Evaluation Criteria in Solid Tumors.

Nivolumab inhibits the interaction between PD-1 and its ligands, promotes immune responses, and triggers antitumor activity. It has already been approved by the Ministry of Health, Labor, and Welfare, Japan for multiple types of cancer including malignant melanoma, non–small-cell lung cancer, and gastric cancer in Japan. Additionally, a phase II trial showed there was a favorable response with nivolumab for previously treated MPM.[6]

A recent report indicated that platinum drugs enhance the effector immune response through modulation of PD-ligand 1.[7] These encouraging results might extend to the first-line treatment of MPM with the hope of enhancing the antitumor response, particularly in combination with the current standard chemotherapy. Unfortunately, no prospective clinical trial is being conducted to evaluate the combination of nivolumab and cisplatin/pemetrexed. Thus, we launched the current trial to assess combination chemotherapy with cisplatin, pemetrexed, and nivolumab for MPM.

Table 1 Key Inclusion Criteria

1. Age: older than 20 years at the date of informed consent
2. Pathologically-confirmed pleural malignant mesothelioma
3. Advanced or metastatic malignant pleural mesothelioma that is untreated and unresectable
4. Patients who have a measurable lesion designated according to modified RECIST criteria
5. Tumor sample available to test for programmed death-ligand 1 expression
6. Eastern Cooperative Oncology Group performance status is 0 or 1
7. Life expectancy is ≥90 days
8. Oxygen saturation measured using pulse oximeter is ≥94%
9. Meet the defined lab value criteria
10. Females of childbearing potential who agree to prevent pregnancy and lactation for at least 5 months after the last administration of nivolumab
11. Men who agree to contraception for at least 7 months after the last administration of nivolumab
12. Patients who understand the study contents and provide written consent by their own free will

Abbreviation: RECIST = Response Evaluation Criteria in Solid Tumors.

Table 2 Key Exclusion Criteria

1. History of anaphylaxis induced by any drug
2. Autoimmune disease
3. Double cancer
4. Metastasis to the brain or meninges
5. Interstitial lung disease or pulmonary fibrosis
6. Diverticulitis or peptic ulcer
7. Pleural effusion that requires drainage every 2 weeks or more
8. Pericardial effusion or ascites that requires drainage
9. Uncontrollable cancer pain
10. Transient ischemic attack, cerebrovascular accident, thrombosis, or thromboembolism within 180 days
11. Uncontrollable severe cardiovascular disease
12. Anticoagulant therapy
13. Uncontrollable diabetes
14. Receiving treatment for a systemic infection
15. Obviously positive for human immunodeficiency virus
16. HTLV-1 antibody-positive, HBs antigen-positive, or HCV antibody-positive. Either HBs antigen positive or HBc antibody-positive and HBV-DNA detection if HBs antigen is negative
17. History of treatment for T-cell regulation
18. Surgery with local or surface anesthesia within 14 days
19. Surgery with general anesthesia within 28 days
20. Pleurodesis within 14 days
21. Pleurodesis treated with picibanil within 28 days
22. Adhesion surgery of the pericardium or peritoneum
23. Radiation therapy for pain relief within 14 days
24. Radiopharmaceutical therapy within 56 days
25. Administration of unapproved drugs within 28 days or an unapproved antibody within 90 days
26. Administration of systemic adrenal cortical hormone or immunosuppressive agents
27. Women who are or might be pregnant or lactating
28. Patients who are incapable of giving consent (for example, because of dementia)
29. Any other inadequacy for this study

Abbreviations: HB = hepatitis B; HBV = hepatitis B virus; HCV = hepatitis C virus; HTLV = human T-cell leukemia virus.

Patients and Methods

Objectives/End Points

This study will assess the efficacy and safety of the first-line combination therapy of cisplatin, pemetrexed, and nivolumab for advanced or metastatic MPM. The primary end point is the centrally reviewed overall response rate. The secondary end points include efficacy evaluated according to the: (1) response rate assessed by investigators; (2) disease control rate; (3) OS; (4) progression-free survival; (5) duration of response; and (6) time to response. Safety and adverse events will also be evaluated.

Study Design/Study Setting

This is a single-arm, prospective, nonrandomized, noncomparative, open-label, multicenter, phase II trial. Figure 1 shows an overview of the study design.

Eligibility Criteria

All patients who meet the main inclusion and exclusion criteria (Tables 1 and 2) will be invited for screening. Written informed

consent must be obtained by an investigator from the patient before any screening or inclusion procedure. This study will be conducted in compliance with the principles of the Declaration of Helsinki, and the protocol was approved by the institutional review board of each of the participating hospitals.

Intervention

Treatment is composed of 2 sequential phases: the combination phase and the maintenance phase. In the former, cisplatin (75 mg/m^2), pemetrexed (500 mg/m^2), and nivolumab (360 mg/person) will be administered intravenously. Nivolumab was kindly provided by Ono Pharmaceutical Co, Ltd. This treatment will be repeated every 3 weeks with a total of 4 to 6 cycles. If patients have not progressed during the combination phase, maintenance therapy with nivolumab will be administered until disease progression, unacceptable toxicities, or the patient's condition meets the withdrawal criteria.

Nivolumab was administered at a dose of 240 mg/person biweekly in recent clinical trials[6,8] including the one for MPM that showed encouraging clinical utility and acceptable toxicity profile.[6] Both of cisplatin and pemetrexed are usually administered every 3 weeks. Under the consideration of practical utility and dose intensity, we planned to administer nivolumab every 3 weeks with the dose of 360 mg/person. The combination of nivolumab (10 mg/kg) and pemetrexed/cisplatin every 3 weeks showed an acceptable toxicity profile and encouraging antitumor activity in patients with advanced non–small-cell lung cancer.[9] On the basis of these findings, nivolumab will be administered at a dose of 360 mg/person, every 3 weeks, in the current study.

Outcome Measurement/Follow-up

Response is evaluated using the modified Response Evaluation Criteria in Solid Tumors.[10] The OS is defined as the duration from study registration until the date of death or the last patient visit.

Statistical Considerations

The target number of patients is 18 for the current phase II study. If we assume that there would be 6 to 12 patients with a response, the response rate would be 33.3% to 66.7%. In this case, the estimated accuracy indicates the range between the point estimate of the response rate and the lower confidence limit (2-sided 95% confidence coefficient on the basis of exact test) would be 18% to 22%. An OS curve will be constructed using the Kaplan–Meier product limit method.

Discussion

There is a medical need for improved treatments for MPM. This study is, to our knowledge, the first clinical trial to evaluate the effect of combining an immune checkpoint inhibitor and platinum-based chemotherapy for MPM. In addition, to our knowledge, this is the first investigator-initiated prospective clinical trial evaluating systemic chemotherapy for MPM that complies with Good Clinical Practice in Japan.

Conclusion

A phase II trial of first-line combination chemotherapy for unresectable MPM commenced in January 2018. This study is, to our knowledge, the first prospective trial to evaluate the effect of an anti–PD-1 antibody combined with cisplatin and pemetrexed for unresectable MPM.

Acknowledgments

This study is supported by a grant from Ono Pharmaceutical Co, Ltd, and also by grants-in-aid from the Ministry of Health, Labor, and Welfare, Japan. Nivolumab was kindly provided by Ono Pharmaceutical Co, Ltd.

Disclosure

Dr Fujimoto has received consultancy fees from Boehringer Ingelheim, Bristol-Myers Squibb, Kyorin, and Kissei, and received honoraria or research funding from Hisamitsu, Chugai, Ono, Taiho, Boehringer Ingelheim, Bristol-Myers Squibb, Novartis, GlaxoSmithKline, and MSD. Dr Aoe has received consultancy fees from Boehringer Ingelheim, Bristol-Myers Squibb, Ono, and received honoraria or research funding from Ono, Bristol-Myers Squibb, Novartis, MSD, AstraZeneca, and Eli Lilly. Dr Kozuki has received honoraria or research funding from Chugai, AstraZeneca, Eli Lilly, Pfizer, Ono, Boehringer Ingelheim, Bristol-Myers Squibb, Kyowa, Taiho, MSD, Merck, and Nippon Kayaku. Dr Hotta has received honoraria or research funding from AstraZeneca, Ono, Boehringer Ingelheim, Nippon Kayaku, Taiho, Chugai, Astellas, Novartis, Bristol-Myers Squibb, Eli Lilly, and MSD. The remaining authors have stated that they have no conflicts of interest.

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Metabolic Characterization of Antifolate Responsiveness and Non-responsiveness in Malignant Pleural Mesothelioma Cells

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Antifolates are a class of drugs effective for treating malignant pleural mesothelioma (MPM). The majority of antifolates inhibit enzymes involved in purine and pyrimidine synthesis such as dihydrofolate reductase (DHFR), thymidylate synthase (TYMS), and glycinamide ribonucleotide formyltransferase (GART). In order to select the most suitable patients for effective therapy with drugs targeting specific metabolic pathways, there is a need for better predictive metabolic biomarkers. Antifolates can alter global metabolic pathways in MPM cells, yet the metabolic profile of treated cells has not yet been clearly elucidated. Here we found that MPM cell lines could be categorized into two groups according to their sensitivity or resistance to pemetrexed treatment. We show that pemetrexed susceptibility could be reversed and DNA synthesis rescued in drug-treated cells by the exogenous addition of the nucleotide precursors hypoxanthine and thymidine (HT). We observed that the expression of pemetrexed-targeted enzymes in resistant MPM cells was quantitatively lower than that seen in pemetrexed-sensitive cells. Metabolomic analysis revealed that glycine and choline, which are involved in one-carbon metabolism, were altered after drug treatment in pemetrexed-sensitive but not resistant MPM cells. The addition of HT upregulated the concentration of inosine monophosphate (IMP) in pemetrexed-sensitive MPM cells, indicating that the nucleic acid biosynthesis pathway is important for predicting the efficacy of pemetrexed in MPM cells. Our data provide evidence that may link therapeutic response to the regulation of metabolism, and points to potential biomarkers for informing clinical decisions regarding the most effective therapies for patients with MPM.

Keywords: tumor metabolism, mesothelioma, antifolate therapy, purine, pyrimidine

Abbreviations: ECAR, extracellular acidification rate; IMP, inosine monophosphate; MPM, malignant pleural mesothelioma; MTA, 5-methylthioadenosine; MTAP, methylthioadenosine phosphorylase; PRPP, phosphoribosyl diphosphate.

INTRODUCTION

Malignant pleural mesothelioma is a locally invasive and rapidly fatal malignancy linked to asbestos exposure (Liu et al., 2017; Yap et al., 2017). MPM develops in the pleural cavity and is highly resistant to a number of therapeutics, with prognosis of patients remaining poor (Creaney and Robinson, 2017; Liu et al., 2017; Scherpereel et al., 2018). A combination of pemetrexed (PMX, also called Alimta or LY231514) and cisplatin has been the first line chemotherapy regimen for more than a decade (Vogelzang et al., 2003; Scagliotti et al., 2008; Liu et al., 2017). Pemetrexed is an antifolate that is able to simultaneously inhibit the synthesis of purines and pyrimidines (Shih et al., 1997). In clinical use, treatment with pemetrexed plus cisplatin and vitamin supplements resulted in superior survival, time to progression, and response rates compared to treatment with cisplatin alone in patients with MPM(Vogelzang et al., 2003; Scagliotti et al., 2008). Pemetrexed and its polyglutamated derivatives inhibit thymidylate synthase (*TYMS*), dihydrofolate reductase (*DHFR*), and glycinamide ribonucleotide transformylase (*GART*), all of which are involved in the *de novo* biosynthesis of thymidine and purine nucleotides (Shih et al., 1997;Yap et al., 2017). Antimetabolite agents, including pemetrexed, induce an imbalance in the cellular nucleotide pool and inhibit nucleic acid biosynthesis that results in arresting the proliferation of tumor cells and inducing cell death(Zhao and Goldman, 2003; Yap et al., 2017).

The discovery of oncogenic driver mutations has allowed the identification of druggable targets and development of new therapies using small molecule tyrosine kinase inhibitors (TKI) aimed at the relevant patient populations (Irmer et al., 2007; Levitzki, 2013; Hylebos et al., 2016). Comprehensive genomic analysis of MPM identified recurrent mutations, gene fusion and splicing alterations (Bueno et al., 2016). Through integrated analyses, alterations were identified in Hippo, mTOR, histone methylation RNA helicase and TP53 signaling pathways in MPM (Bueno et al., 2016). Other studies demonstrated that the most frequent genetic variations clustered into two main pathways (Hylebos et al., 2016). The first altered pathway was the TP53/DNA repair pathway with genetic variations in *TP53*, *BAP1* and *CDKN2A* genes, and the second pathway was the PI3K/AKT pathway, with genetic variations in *KIT*, *KDR*, *PIK3CA* and *NF2* genes, respectively (De Rienzo et al., 2016; Hylebos et al., 2016). However, there has been a paucity of new actionable mutations in MPM as drug targets.

Accumulating evidence shows that genetic mutations in cancer-driver genes, tumor suppressors, and amplified oncogenes are linked to specific alterations in metabolic pathways in cancer cells, involving proteins such as isocitrate dehydrogenase (IDH), fumarate hydratase (FH), MYC, K-RAS and BRAF (Levine and Puzio-Kuter, 2010; Cairns et al., 2011; Cheong et al., 2012; Dejure and Eilers, 2017; Palm and Thompson, 2017). The Warburg effect, the phenomenon in which cancer cells exhibit intense glucose consumption with production of lactate despite abundant oxygen availability, has been recognized since the 1930s (Vander Heiden et al., 2009; Lunt and Vander Heiden, 2011; Soga, 2013). Genetic mutations in tumor cells might cause several unique metabolic phenotypes that are critical for cancer cell proliferation in MPM. The frequent loss of CDKN2A (at 9p21) in MPM typically includes the homozygous co-deletion of MTAP (Illei et al., 2003). Specifically, MTAP catalyzes the reversible phosphorylation of MTA to the purine adenine and 5-methylthioribose-1-phosphate and PRMT5 inhibition induced metabolic vulnerability (Kryukov et al., 2016; Mavrakis et al., 2016; Yap et al., 2017). The MTAP protein plays a crucial role in polyamine metabolism involving salvage of adenosine and methionine from the substrate MTA (Bertino et al., 2011; Makinoshima et al., 2018).

One-carbon metabolism involving the folate and methionine cycle integrates carbon units from amino acids and generates diverse outputs, such as the biosynthesis of nucleotides, lipids and proteins in cancer cells (Yang and Vousden, 2016; Ducker and Rabinowitz, 2017; Newman and Maddocks, 2017). Glycine can be utilized for *de novo* purine biosynthesis by two mechanisms: direct incorporation into the purine backbone or further oxidation by the glycine cleavage system (GCS) to yield one-carbon units for nucleotide synthesis and cellular methylation reactions (Amelio et al., 2014; Newman and Maddocks, 2017). The GCS has also been implicated in cell transformation and tumorigenesis (Zhang et al., 2012). Given the high proliferation rate of cancer cells and the requirement of nucleotides for proliferation, cancer cells have a large demand for one-carbon units for nucleotide synthesis (Yang and Vousden, 2016; Ducker and Rabinowitz, 2017; Newman and Maddocks, 2017). To this day, chemical variants of these initial folate antagonists such as methotrexate and pemetrexed constitute a major class of cancer chemotherapy agents and are used as frontline chemotherapy for diverse cancers (Zhao and Goldman, 2003).

In this paper, we characterized the metabolic features of mesothelioma using a non-targeted metabolic profiling strategy based on capillary electrophoresis-mass spectrometry (CE/MS). MPM cell lines were categorized into two groups according to their susceptibility to pemetrexed treatment. Using end product rescue, we showed that treatment with pemetrexed mainly targets pyrimidine biosynthesis rather than purine biosynthesis in MPM cells. We also demonstrated a metabolic response including one-carbon cycle against pemetrexed treatment in sensitive or resistant MPM cells. Our results link the antifolate therapeutic response to the regulation of metabolism and imply that the levels of glycine and IMP are potential biomarkers that may inform the clinical utility of specific targeted therapies to treat patients with MPM.

EXPERIMENTAL PROCEDURES

Materials

MPM an aggressive malignancy affecting pleural surfaces, is divided into three main histological subtypes. The epithelioid and sarcomatoid subtypes are characterized by cuboid and fibroblastoid cells, respectively. The biphasic subtype contains a mixture of both. Commercially available cell lines were purchased from the American Type Culture Collection (ATCC), which included MSTO-211H (biphasic), NCI-H28 (epithelial),

NCI-H226 (epithelial), NCI-H2052 (epithelial) and NCI-H2452 (epithelial). 3 MPM cell lines (TCC-MESO1 (epithelial), TCC-MESO2 (epithelial), and TCC-MESO3 (biphasic)) were established from Japanese patients with MPM and some of the biological characteristics were analyzed in a previous report (Yanagihara et al., 2010). All cell lines were cultured in RPMI-1640 supplemented with 10% FBS. RPMI-1640 (R8758), glucose minus RPMI-1640 (R1383), phosphate buffered saline (PBS), hypoxanthine, thymidine, 2-deoxy-D-glucose (2DG) and MTA were purchased from Sigma–Aldrich (St. Louis, MO, United States). Fetal bovine serum (FBS) was purchased from Biowest (Nuaille, France). Dimethyl sulfoxide (DMSO) and glucose were purchased from Wako Pure Chemicals Industries (Osaka, Japan). Pemetrexed was purchased from Selleck (Houston, TX, United States). Cell Counting Kit-8 was purchased from Dojindo Laboratories (Kumamoto, Japan). FluxPak XF24 assay pack and XF glycolysis stress test kit was purchased from Seahorse Bioscience (North Billerica, MA, United States). Propidium iodide (PI) was purchased from Thermo Fisher Scientific (Waltham, MA, United States). Mini-PROTEAN TGX Precast Gel, Trans-Blot Turbo Transfer System and Trans-Blot Turbo Transfer Pack were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA, United States). Primary antibodies specific for DHFR, GART and TYMS were purchased from Abcam (Cambridge, United Kingdom) and primary antibody to phospho-RB1 (pRB1), RB1, p21 and GAPDH were purchased from Cell Signaling Technologies (Danvers, MA, United States). The peroxidase-linked secondary antibodies for WB, HRP-linked sheep anti-mouse IgG and donkey anti-rabbit IgG, were purchased from GE Healthcare Biosciences (Pittsburgh, PA, United States). Oligomycin was purchased from Merck Millipore (Darmstadt, Germany). SYBR Premix Ex Taq and primers were purchased from TaKaRa Bio (Shiga, Japan). Ribonuclease A (RNase A) was purchased from Roche Applied Science (Penzberg, Germany) and contaminating DNase was inactivated at 80°C for 30 min.

Cell Survival Assay and Proliferation Assay

MPM cells were seeded in RPMI-1640 containing various concentrations of pemetrexed in 96-well cell culture plates. After 72 h of incubation, cell viability was analyzed using a WST-8 assay using the Cell Counting Kit-8. The pemetrexed concentration against the percent of cell survival was plotted using all MPM cell lines. The IC_{50} values were calculated using Graph Pad Prism 7 software (GraphPad Software, Inc., La Jolla, CA, United States). For rescue experiments, MPM cells were cultured in complete medium supplemented with 100 μM hypoxanthine alone, 16 μM thymidine alone or mixed hypoxanthine plus thymidine in the presence of pemetrexed.

Cell Cycle Analysis

Cell cycle distribution was analyzed by flow cytometry. PBS, pemetrexed or pemetrexed + HT treated cells were harvested, washed twice with PBS, and fixed in 70% ethanol overnight at −20°C. Fixed cells were harvested and washed twice with PBS, incubated with 1 ml of PBS containing 40 μg/ml propidium iodide and 100 μg/ml RNase A for 30 min at 37°C. Stained cells were analyzed using a Becton Dickinson FACSMelody instrument (Franklin Lakes, NJ, United States). FlowJo software (FlowJo, LLC, Ashland, OR, United States) was used for data analysis and generation of graphs. Single cell populations were gated and the percentage of cells in the various stages of the cell cycle (G0/G1, S, and G2/M phases) was determined by the Watson Model.

Measurement of ECAR and OCR

ECAR and OCR were measured with a XF glycolysis stress test kit according to the manufacturer's instructions (Seahorse Bioscience, Agilent). In brief, 4.5×10^4 cells were plated onto XF24 plates in RPMI-1640 (10% FBS, 2 mM glutamine) and incubated at 37°C, 5% CO_2 overnight. Cells were washed with assay medium (minus glucose and unbuffered RPMI-1640 (SIGMA R1383)), replaced with assay medium, and then placed at 37°C in a CO_2-free incubator for 30 min. ECAR and OCR were monitored using a Seahorse Bioscience XF24 Extracellular Flux Analyzer over time and each cycle consisted of 3 min mixing, 3 min waiting, and 3 min measuring. Glucose, oligomycin and 2DG were diluted into XF24 media and loaded into the accompanying cartridge to achieve final concentrations of 10 mM, 5 μM, and 100 mM respectively. Injections of the drugs into the medium occurred at the time points specified.

Western Blotting

Cells were lysed in CST lysis buffer on ice for 2 min and centrifuged at 15,000 × *g* for 10 min. The protein content of supernatants was measured by BCA assay (Pierce). Equivalent amounts of protein samples were separated via 4–20% SDS/PAGE, transferred to PVDF membranes, and incubated overnight with primary antibodies (1:1000 dilution). The primary antibodies used in this study are listed in the materials. ECL anti-rabbit IgG HRP-linked whole antibody (1:10,000; GE Healthcare) was used as secondary antibody. Signals were detected using ECL Western Blotting Detection reagent (GE Healthcare) and FUSION FX (VILBER, France). The intensity of bands was quantified using Fusion Capt Advance FX7 software.

Quantitative RT-PCR

Cells were washed with PBS and total RNA from the MPM cell lines was isolated with TRIzol Reagent (Invitrogen). Complementary DNA (cDNA) was synthesized using the SuperScript VILO cDNA synthesis kit (Invitrogen). Primers for metabolic genes were purchased from TaKaRa Bio (Japan). Real-time RT–PCR was carried out with specific primers and a QuantStudio 3 Thermo Fisher Scientific (Waltham, MA, United States). GAPDH was used for normalization as control and the relative quantitation value compared to the calibrator for that target is expressed as $2^{-(Ct-Cc)}$.

Metabolite Measurements

Metabolic extracts were prepared from 1–5 × 10^6 MPM cells with methanol containing internal standard solution

(Human Metabolome Technologies; HMT, Inc., Tsuruoka, Japan) and analyzed using a capillary electrophoresis (CE)-connected electrospray ionization/time-of-flight mass spectrometry (ESI-TOFMS) and capillary electrophoresis tandem mass spectroscopy (CE-MS/MS) system (CARCINOSCOPE, Human Metabolic Technologies, Tsuruoka, Japan). Procedures for metabolite measurements were as previously described (Makinoshima et al., 2014, 2018). In brief, cells were washed twice in 5% mannitol solution and then treated with 800 μL of methanol and 550 μL of Milli-Q water containing internal standards (H3304-1002, HMT, Inc., Tsuruoka, Japan). The metabolite extract was transferred into a microfuge tube and centrifuged at 2,300 × *g* and 4°C for 5 min. Next, the upper aqueous layer was centrifugally filtered through a Millipore 5-kDa cutoff filter to remove proteins. The filtrate was centrifugally concentrated and resuspended in 50 μL of Milli-Q water for CE-MS analysis. Cationic compounds were analyzed in the positive mode of CE-TOFMS and anionic compounds were analyzed in the positive and negative modes of CE-MS/MS according to the methods developed by Soga and Heiger (2000) and Soga et al. (2002, 2003). To obtain peak information including *m/z*, migration time (MT), and peak area, detected peaks by CE-TOFMS and CE-MS/MS were extracted using automatic integration software (MasterHands, Keio University, Tsuruoka, Japan and MassHunter Quantitative Analysis B.04.00, Agilent Technologies, Santa Clara, CA, United States, respectively). Concentrations of metabolites were calculated by normalizing the peak area of each metabolite with respect to the area of the internal standard and by using standard curves obtained by three-point calibrations.

Statistical Analyses

Unless otherwise indicated, results were reported as the mean ± SD. Statistical analyses were done by two-tailed Student's *t*-test and *p*-values were indicated as * < 0.05, ** < 0.01 and *** < 0.005. For metabolomic data analysis we used Welch *t*-test and *p*-values were indicated as * < 0.05, ** < 0.01, and *** < 0.001.

RESULTS

IC_{50} Measurements in 8 Mesothelioma Cell Lines Treated With Pemetrexed

We analyzed the *in vitro* sensitivity of MPM cell lines to the antifolate inhibitor pemetrexed through proliferation assays. MPM cell growth inhibition dose-response curves of pemetrexed are shown in **Figure 1**. The cytotoxic effect of pemetrexed on human MPM cells was analyzed using a WST-8 cell counting kit after 72 h of exposure. The IC_{50} value was defined as the dose of pemetrexed required to produce a 50% reduction in the viability of MPM cells. IC_{50} values were measured in 8 MPM cell lines after 72 h treatment with pemetrexed and found to range from 47.4 nM to ≥10,000 nM (**Figure 1**) as follows: MSTO-211H (47.4 nM), NCI-H28 (84.1 nM), NCI-H226 (1950 nM), NCI-H2052 (135 nM), NCI-H2452 (>10,000 nM), TCC-MESO1 (435 nM), TCC-MESO2 (94.3 nM), TCC-MESO3 (883 nM).

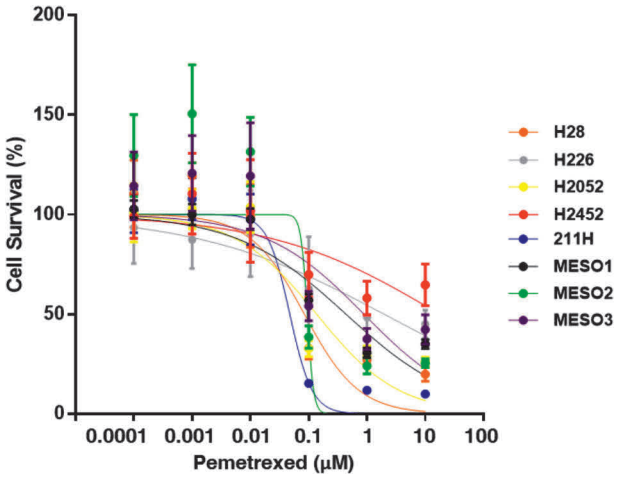

FIGURE 1 | Pemetrexed treatment represses cell growth of MPM cells. WST-8 assay with pemetrexed. Cells were treated with the indicated concentration of pemetrexed for 72 h, and their viability was assessed by the WST-8 assay. The % viability data are shown as the mean ± standard deviation (SD) ($n = 6$). Error bars indicates the range of SD. Blue line: MSTO-211H, Orange line: NCI-H28, Gray line: NCI-H226, Yellow line: NCI-H2052, Red line: NCI-H2452, Black line: TCC-MESO1, Green line: TCC-MESO2, and Purple line: TCC-MESO3. The *in vitro* half maximal inhibitory concentration (IC_{50}) of pemetrexed for the growth of MPM cell lines was calculated as the following values for each cell line: NCI-H28 = 84.1 nM, NCI-H226 = 1950 nM, NCI-H2052 = 135 nM, NCI-H2452 = >10,000 nM, MSTO-211H = 47.4 nM, TCC-MESO1 = 435 nM, TCC-MESO2 = 94.3 nM, and TCC-MESO3 = 883 nM.

We found that MPM cell lines could be categorized into two groups according to their susceptibility to pemetrexed treatment. In agreement with previous findings (Satoh et al., 2017), the NCI-H2452 cell line was resistant to pemetrexed treatment as compared to other MPM cell lines. MSTO-211H was highly sensitive to pemetrexed in the nanomolar range as compared to the pemetrexed-resistant cell line NCI-H2452 (**Figure 1**). For the rest of this paper, we utilized the MSTO-211H and TCC-MESO2 cell lines as pemetrexed-sensitive cells and the NCI-H2452 cell line as pemetrexed-resistant cells.

End Product Reversal Studies of Pemetrexed in MPM Cells

To confirm the target specificity of pemetrexed, we tested the ability of exogenous nucleotide precursors (hypoxanthine and thymidine) to rescue the antiproliferative effects of pemetrexed on either sensitive or resistant MPM cells (**Figure 2**). The cytotoxic effect of pemetrexed on human MPM cells was analyzed using the WST-8 cell counting kit after 72 h of exposure. The addition of either hypoxanthine (HXN) or thymidine (THY) did not change the cell proliferation rate as compared to PBS control (**Figures 2A–C**). Treatment with PMX significantly reduced cell growth in both sensitive and resistant MPM cells (**Figures 2A–C**). In agreement with previous findings (Shih et al., 1997), the addition of HXN partially rescued PMX cytotoxicity in TCC-MESO2 but had no effect for MSTO-211H and NCI-H2452 cell lines (**Figure 2**). Next, we found that the addition of THY

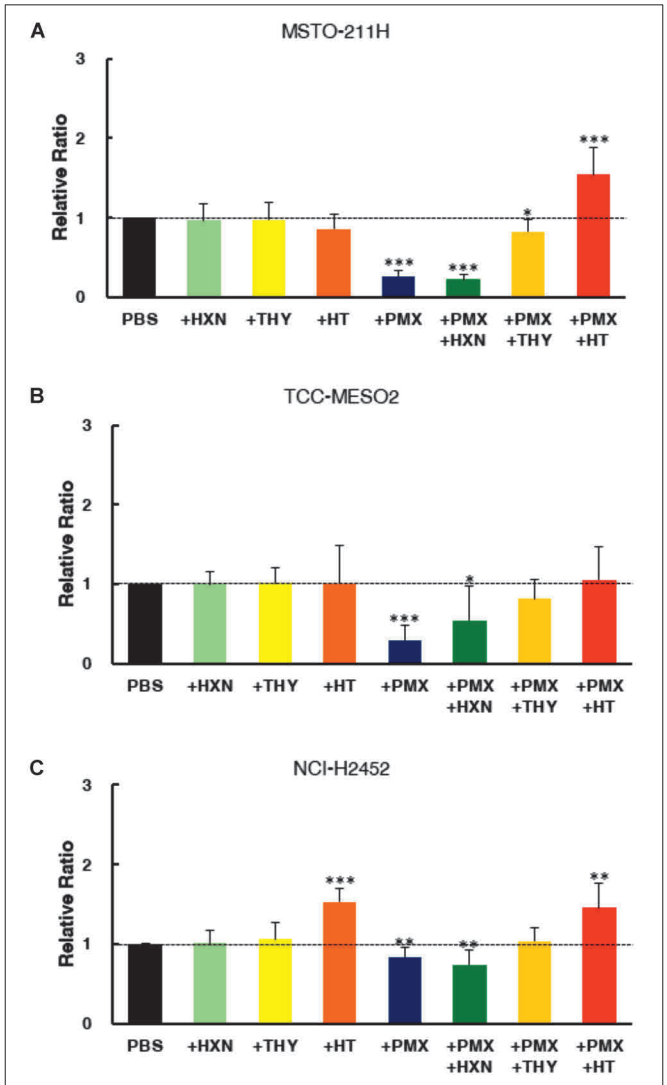

FIGURE 2 | End product reversal analysis of pemetrexed in MPM cells. Cell proliferation was measured with the WST-8 assay in the presence of either nucleotide precursor alone (thymidine: THY, hypoxanthine: HXN), pemetrexed (PMX) alone, or PMX + nucleotide precursor. Two pemetrexed-sensitive cell lines, MSTO-211H **(A)** and TCC-MESO2 **(B)**, and a pemetrexed-resistant cell line NCI-H2452 **(C)** were used- Black: PBS, Light Green: 100 μM hypoxanthine (HXN), Light Yellow: 16 μM thymidine (THY), Orange: 100 μM hypoxanthine +16 μM thymidine (HT), Blue: Pemetrexed (PMX) 1 μM, Green: PMX 1 μM + 100 μM HXN, Yellow: PMX 1 μM + 16 μM THY, Red: PMX 1 μM + HT. Dotted line denotes the level of proliferation by PBS control set at 1.0. Error bars indicate *s.d.* ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ vs. control by two-tailed Student's *t*-test.

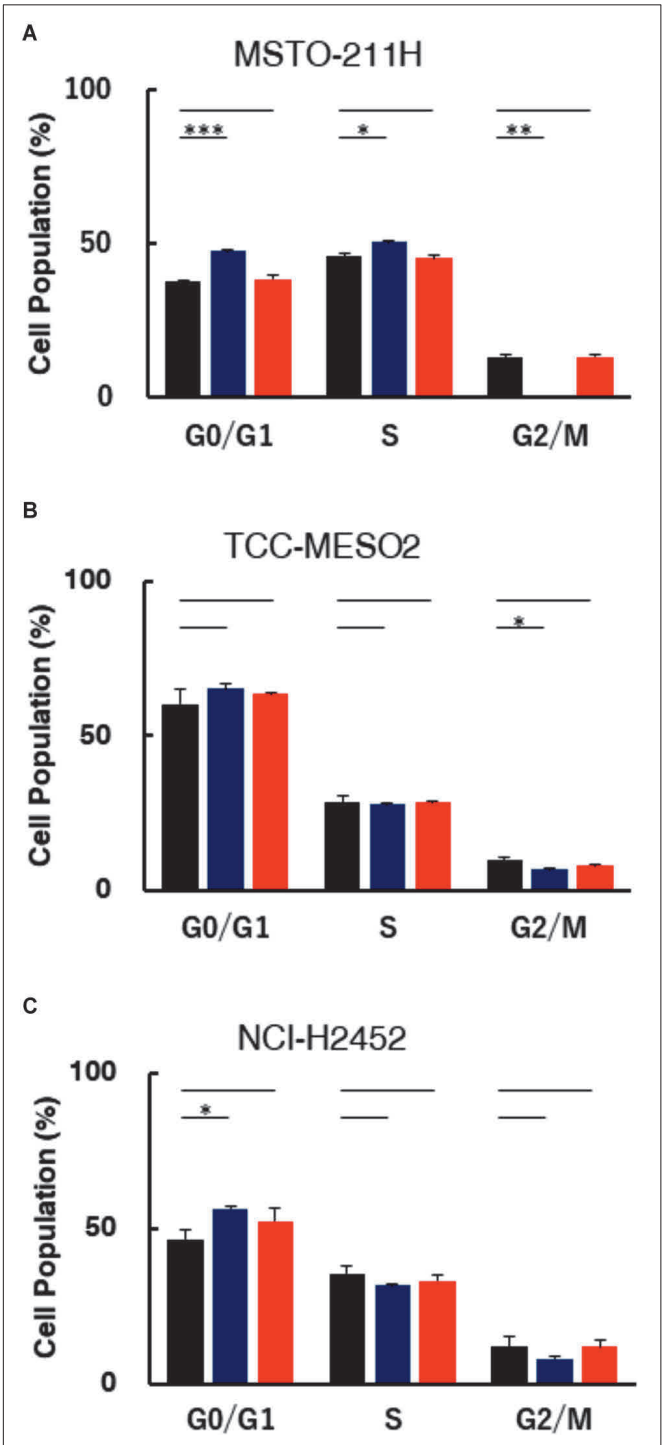

FIGURE 3 | Assessment of DNA content in MPM cells treated with pemetrexed. Treatment with pemetrexed arrested cell cycle in both the pemetrexed-sensitive MSTO-211H **(A)** and TCC-MESO2 **(B)**, and the PMX-resistant NCI-H2452 **(C)** MPM cells. After 6 h of 1 μM pemetrexed treatment, the cells were analyzed for DNA content via PI staining. The percentage of cell populations in the G0/G1, S, and G2/M phases. Black: PBS, Blue: Pemetrexed (PMX) 1 μM, Red: PMX 1 μM + 100 μM hypoxanthine + 16 μM thymidine (HT). The data were shown as means ± *s.d.* From at least three independent experiments ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control by two-tailed Student's *t*-test.

nullified PMX cytotoxicity in TCC-MESO2 (**Figure 2B**) and NCI-H2452 (**Figure 2C**) cells, and partially in MSTO-211H cells (**Figure 2A**). The combination of hypoxanthine plus thymidine (+HT) completely rescued the growth repression induced by pemetrexed and the cell numbers here were significantly higher than those seen with PBS control in MSTO-211H and NCI-H2452 cells. These results demonstrate the target specificity of pemetrexed in MPM cells.

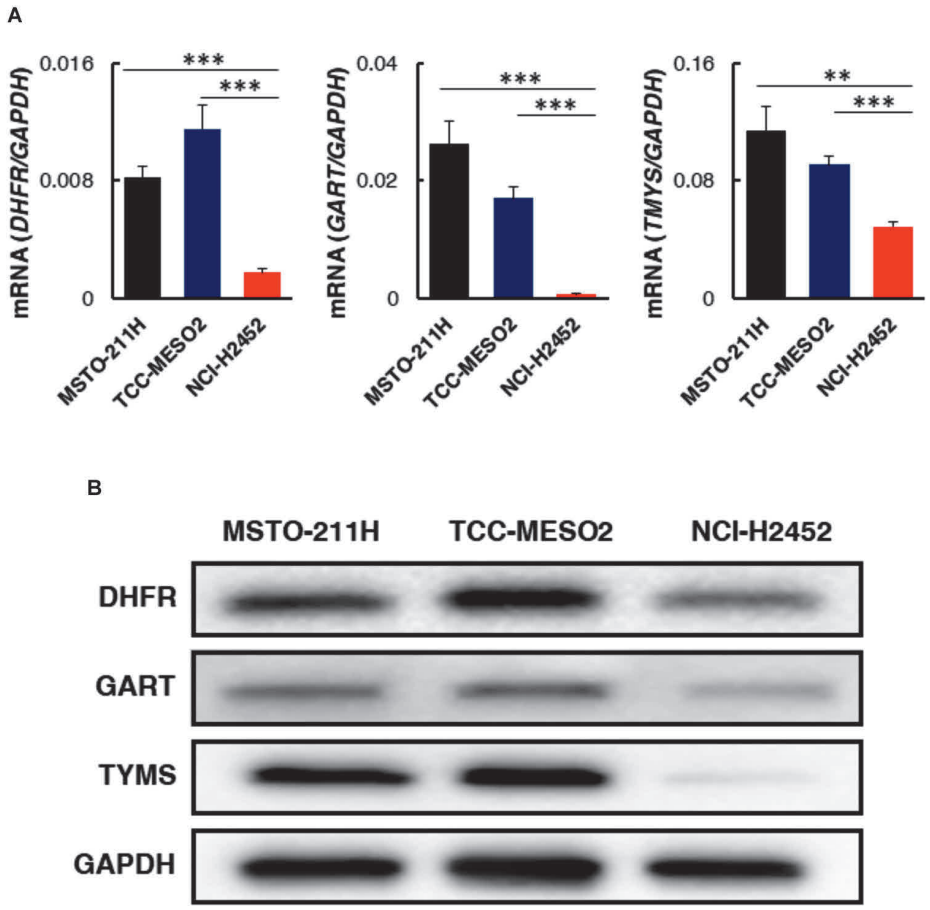

FIGURE 4 | Expression levels of target genes involved in one-carbon metabolism in pemetrexed-sensitive vs. pemetrexed-resistant MPM cells. **(A)** mRNA expression was evaluated by real-time RT-PCR using the $\Delta\Delta CT$ method normalized to the housekeeping gene GAPDH. Asterisks show significant difference from NCI-H2452 cells. Error bars indicate *s.d.* ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ vs. control by two-tailed Student's *t*-test. **(B)** Representative images of Western blotting (WB) characterizing proteins involved in one-carbon metabolism. Equivalent amounts of proteins from whole-cell lysates were subjected to WB analysis to detect the indicated proteins. GAPDH was used as a loading control.

Treatment of MPM Cells With Pemetrexed Alters Cell Cycle Populations

To characterize the impact of pemetrexed treatment on cell cycle progression, we performed cell cycle analysis in MPM cells fixed and stained with PI (propidium iodide). The DNA content of the cells treated with pemetrexed for 6 h is shown in **Figure 3**, while the data for 24 h is shown in **Supplementary Figure S1**. Cell cycle distributions were determined by flow cytometric analyses of MSTO-211H, TCC-MESO2 and NCI-H2452 cells treated with PBS or 1 μM pemetrexed, which is a relatively high concentration of pemetrexed for a short period of time (6 h). In pemetrexed-treated MSTO-211H cells, we observed a significant accumulation in the G0/G1 phase (47.7 ± 0.4%) of the cell cycle compared with 37.8 ± 0.1% in G1 when treated with PBS control (**Figure 3A**). The treatment of MSTO-211H cells with pemetrexed also induced S phase cell cycle arrest at 50.7 ± 0.1% of the cell population in the presence of pemetrexed as compared to 45.8 ± 1.2% with PBS control. Cell cycle arrest was released when HT solution was added into culture medium (**Figure 3A**). In both pemetrexed-sensitive cell lines, MSTO-211H and TCC-MESO2, the G2/M cell population was decreased after treatment with pemetrexed. MSTO-211H cells in G2/M were changed from 12.8% ± 0.1 to 0.0% and TCC-MESO2 cells in G2/M were altered from 9.5 ± 1.0% to 7.7 ± 0.4% (**Figures 3A,B**). Similarly, we observed statistically significant G1 arrest after treatment with pemetrexed in NCI-H2452 cells (**Figure 3C**). As the treatment with pemetrexed altered cell cycle in MPM cells, we analyzed the levels of proteins involved in cell cycle regulation using Western blot (WB). WB analyses of pRB1, RB1 and p21 did not evidence substantial changes in their levels upon pemetrexed treatment (**Supplementary Figure S2**).

Expression Level of Pemetrexed Target Molecules

Pemetrexed and its polyglutamated derivatives mainly inhibit thymidylate synthase (TYMS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide transformylase (GART), all of

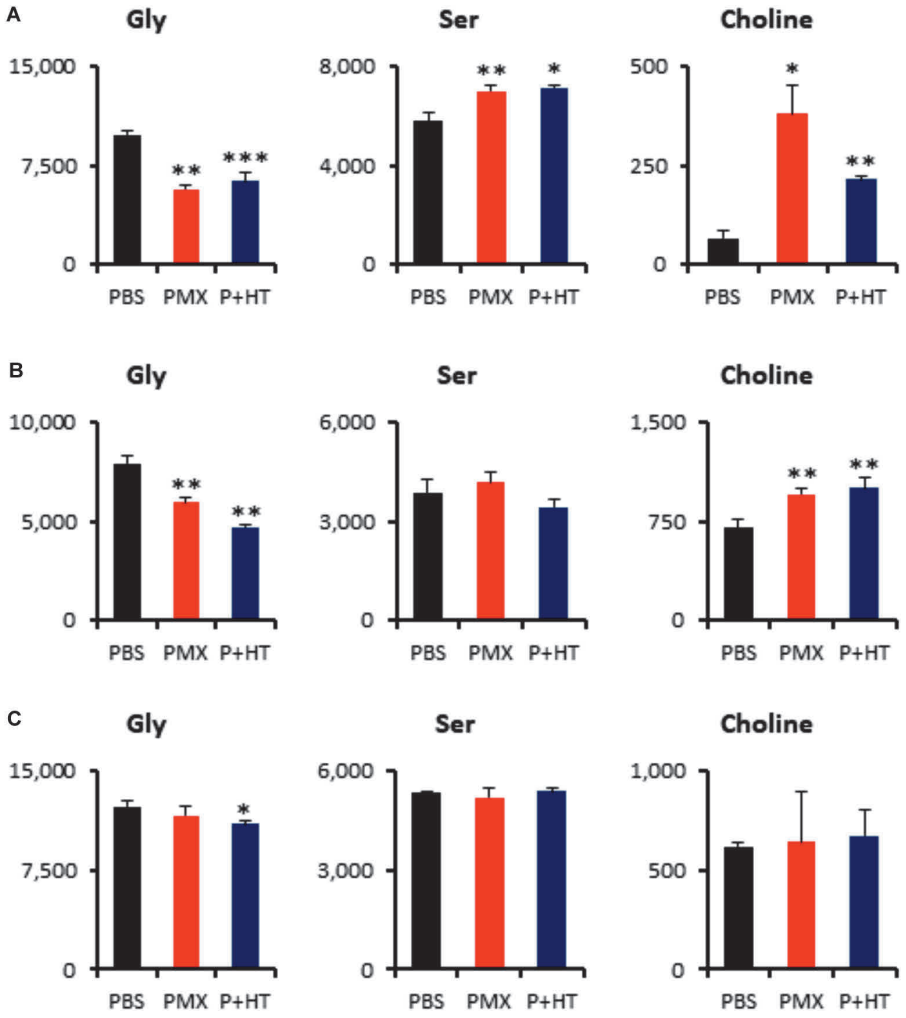

FIGURE 5 | Quantification of metabolites involved in the one-carbon pathway. Intracellular concentration (pmol/million cells) of key metabolites involved in the one-carbon metabolic pathway after treatment with pemetrexed is shown. Error bars indicate *s.d.* ($n = 3$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control by Welch *t*-test. Total metabolites were extracted with methanol from MSTO-211H **(A)**, TCC-MESO2 **(B),** or NCI-H2452 **(C)** cells treated with PBS (black), 1 μM PMX (red) or 1 μM PMX + HT (blue) for 6 h. Representative metabolites such as glycine (Gly), serine (Ser), and choline are shown.

which are involved in the *de novo* biosynthesis of thymidine and purine nucleotides (**Supplementary Figure S3**). To investigate the efficacy of one-carbon metabolic pathway inhibition against MPM cells, we investigated whether the expression levels of pemetrexed targets involved in this pathway were different in pemetrexed sensitive vs. resistant MPM cell lines. Several prior studies showed that *TYMS* overexpression is one of the major factors leading to resistance in pemetrexed-resistant cells and that regulation of *DHFR*, *RFC*, and *FPGS* expression is associated with acquired resistance to antifolate (Zhao and Goldman, 2003). Therefore, we measured the expression level of TYMS, DHFR and GART at the transcriptional and protein levels. Transcriptional expression of genes encoding pemetrexed-target enzymes was measured by RT-PCR (**Supplementary Table S1**). Interestingly, as shown in **Figure 4A**, we observed that DHFR, GART, and TYMS mRNA expression levels were all significantly higher in pemetrexed-sensitive cells as compared to the pemetrexed-resistant NCI-H2452 cells (DHFR: MSTO-211H = 4.7-fold, TCC-MESO2 = 6.5-fold; GART: MSTO-211H = 35.5-fold, TCC-MESO2 = 23.0-fold; TYMS: MSTO-211H = 2.3-fold, TCC-MESO2 = 1.9-fold). To confirm the mRNA results, we analyzed protein levels by WB. Representative images of WB results are shown in **Figure 4B** and quantitative analysis is shown in **Supplementary Figure S4**. Differences in DHFR protein levels in PMX-sensitive MSTO-211H and TCC-MESO2 cells as compared to PMX-resistant NCI-H2452 cells were not statistically significant (**Supplementary Figure S4**, $P = 0.055$). Consistent with the mRNA expression data, the expression of GART (MSTO-211H: 3.4-fold, TCC-MESO2: 3.3-fold), and TYMS proteins (MSTO-211H: 71.9-fold, TCC-MESO2: 81.4-fold) was found to be higher in pemetrexed-sensitive cells as compared to the pemetrexed-resistant NCI-H2452 cells (**Supplementary Figure S4**). Relative levels of GAPDH protein were not changed in MSTO-211H and

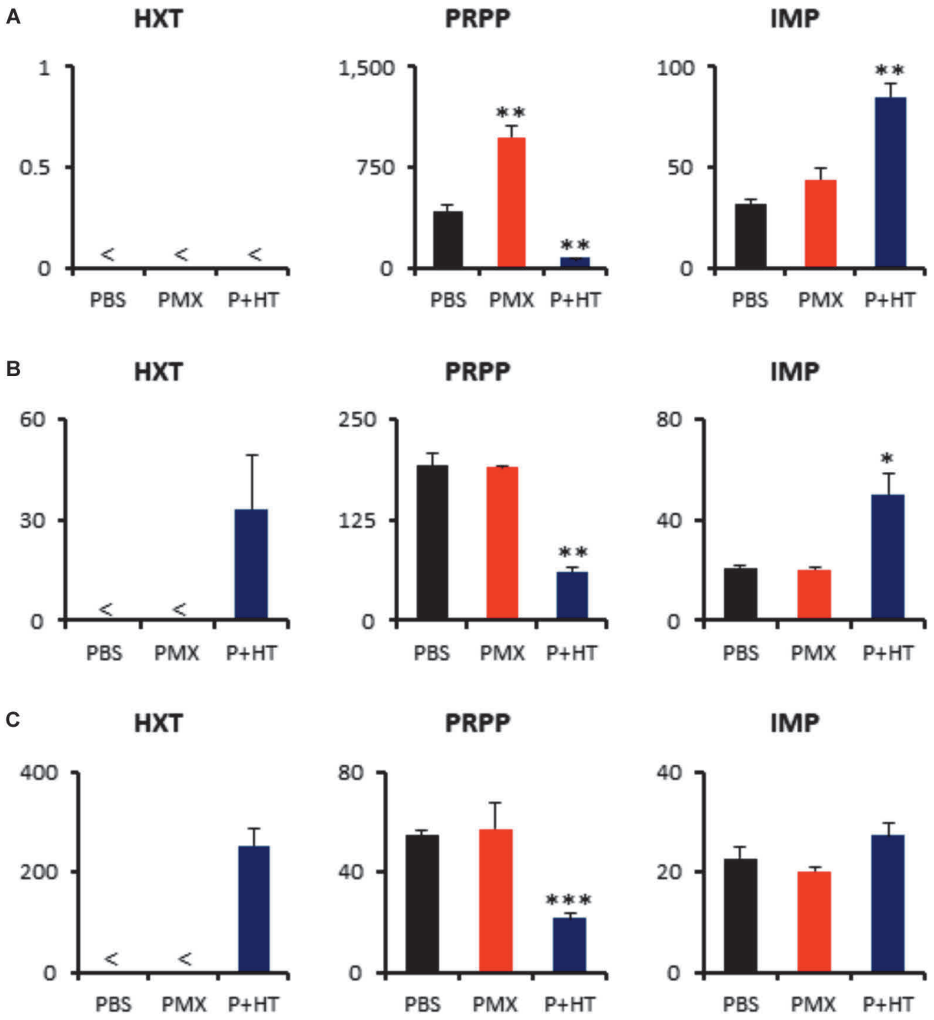

FIGURE 6 | Quantification of metabolites involved in purine salvage pathway. Intracellular concentration (pmol/million cells) of key metabolites involved in the purine salvage pathway after treatment of pemetrexed is shown. Error bars indicate *s.d.* ($n = 3$) and < indicates the metabolite was not detected (N.D.). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control by Welch *t*-test. Total metabolites were extracted with methanol from MSTO-211H **(A)**, TCC-MESO2 **(B)**, or NCI-H2452 **(C)** cells treated with PBS (black), 1 μM PMX (red) or 1 μM PMX + HT (blue) for 6 h. Representative metabolites including hypoxanthine (HXT), PRPP, and IMP are shown.

TCC-MESO2 as compared to NCI-H2452 cells (**Supplementary Figure S4**). The lower level of pemetrexed target molecules in the pemetrexed-resistant NCI-H2452 cells as compared to the sensitive cells suggests that the one-carbon metabolic pathway is altered and that the resistant cells may survive by bypassing folate related pathways.

Glycolytic Activities Are Not Altered in MPM Cells After Treatment With Pemetrexed

The expression level of enzymes in pemetrexed-sensitive cells was different from resistant cells, suggesting that metabolic profiles could be altered in cells sensitive or resistant to pemetrexed treatment. In cancer cells, the Warburg effect, whereby glucose is consumed with secretion of lactate despite abundant oxygen availability, has been recognized since the 1930s (Vander Heiden et al., 2009; Lunt and Vander Heiden, 2011; Soga, 2013). We hypothesized that glycolytic activity would be correlated with pemetrexed sensitivity. To test this idea, we measured the glucose-induced ECAR, an indicator of lactate production, and the oxygen consumption rate (OCR), an indicator of oxidative phosphorylation (OXPHOS), using a flux analyzer. Basal levels of ECAR at the beginning of measurements, which indicated non-glycolytic acidification, were low in all MPM cells (**Supplementary Figure S5**). Equivalent ECAR was observed in MPM cells both pre- and post-treatment with an ATPase inhibitor oligomycin to induce maximum cellular glycolytic capacity (**Supplementary Figure S5**). At the final step, the addition of 2-deoxy-D-glucose (2DG), an inhibitor of glycolysis, completely shut down extracellular acidification (**Supplementary Figure S5**). ECAR was not statistically different in sensitive or resistant MPM cells either with or without pemetrexed (**Supplementary Figure S5**).

Alterations in Metabolic Pathways in the Response to Pemetrexed Treatment

To investigate whether treatment of MPM cells with pemetrexed affects global cancer metabolism, we employed metabolomics analysis. Intracellular metabolites were extracted with methanol and analyzed using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) (Soga et al., 2003) and the profile of 117 quantified metabolites is shown in **Supplementary Table S2**. Principal component analysis (PCA) and heatmap analysis of metabolomics datasets within the indicated cell lines and culture conditions are shown in **Supplementary Figure S6**. The amount of adenosine triphosphate (ATP), which is the molecular unit of currency of intracellular energy transfer, was not changed in any of the three tested cell lines after pemetrexed treatment for 6 h (**Supplementary Table S2**). Thymidine was not detected in MPM cells under any experimental conditions (**Supplementary Table S2**). With respect to one-carbon metabolism, glycine was significantly decreased in pemetrexed-sensitive cells after treatment with pemetrexed, but not in the resistant NCI-H2452 cells (**Figure 5**). On the other hand, choline accumulated in pemetrexed-sensitive cells after pemetrexed treatment, but not in resistant NCI-H2452 cells (**Figure 5**). Although the folate metabolic pathway is linked with the methionine cycle and polyamine biosynthesis pathways (**Supplementary Figure S3**), intermediate metabolites were not changed by pemetrexed treatment in both pemetrexed-sensitive and resistant cells (**Supplementary Table S2**).

The addition of exogenous hypoxanthine and thymidine is sufficient to provide the end product of the pemetrexed block and to permit DNA synthesis for cell division (**Figure 2**). Although intracellular thymidine was not detected in MPM cells under any experimental condition (**Supplementary Table S2**), intracellular hypoxanthine was detected in TCC-MESO2 and NCI-H2452 cells (**Figure 6**). Metabolome analysis revealed that HT treatment with pemetrexed significantly decreased the levels of PRPP in both pemetrexed-sensitive and pemetrexed-resistant cell lines (**Figure 6**). On the other hand, IMP, which is a product of the purine salvage pathway, was increased in the pemetrexed-sensitive MPM cells treated with pemetrexed + HT solution (**Figures 6A,B**), but not in resistant NCI-H2452 cells (**Figure 6C**).

MTAP Expression and MTA Effect for MPM Cell Survival

A previous study has shown homozygous deletion of *CDKN2A* and co-deletion of the *MTAP* gene in the majority of clinical cases of MPM (Illei et al., 2003). To determine the status of MTAP expression in MPM cell lines used here, we quantified *MTAP* mRNA expression levels by RT-PCR. MTAP gene expression was detected in the pemetrexed-sensitive MSTO-211H and TCC-MESO2 cells, but not in pemetrexed-resistant NCI-H2452 cells (**Figure 7**). Given our observation that pemetrexed treatment with HT solution significantly decreased the levels of PRPP in all MPM cells whereas IMP was upregulated in pemetrexed-sensitive but not pemetrexed-resistant cells (**Figure 6**), we hypothesized that MTA changed the pemetrexed sensitivity of MPM cells. To test this, we added MTA to the culture medium and measured

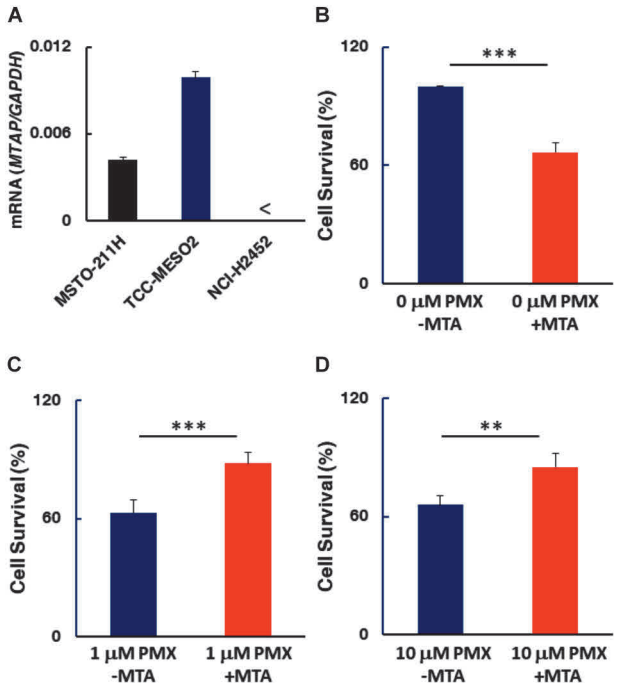

FIGURE 7 | MTAP expression in MPM cells and effect of MTA on MPM cell survival. **(A)** *MTAP* mRNA expression was evaluated by real-time RT-PCR using the ΔΔCT method normalized to the housekeeping gene GAPDH. < indicates not detected (N.D.). Error bars indicate *s.d.* (*n* = 6). **(B–D)** Addition of 5′-deoxy-5′-(methylthio)-adenosine (MTA) represses drug-resistant cells. NCI-H2452 cell survival (%) was measured in the presence of 0.1 mM MTA co-treated without **(B)** or with either 1 μM **(C)** or 10 μM pemetrexed **(D)**. The data are shown as the mean ± *s.d.* (*n* = 6). Error bars represent one *s.d.***P* < 0.05, ***P* < 0.01, ****P* < 0.005 vs. control by two-tailed Student's *t*-test.

survival of the NCI-H2452 pemetrexed-resistant cell line treated with 0, 1, or 10 μM pemetrexed (**Figure 7**). As predicted, the addition of MTA to the culture media of the drug-resistant cell line, NCI-H2452, resulted in a growth-repressive phenotype, as reflected by decreased cell survival (**Figure 7B**). In contrast, the addition of MTA to the culture media of NCI-H2452 cells treated with pemetrexed resulted in a more pemetrexed-resistant phenotype (**Figures 7C,D**). These results indicate that the vulnerability to targeting of the one-carbon metabolic pathway in MPM cells may be dependent on the purine biosynthesis pathway.

DISCUSSION

Antifolate is feasible chemotherapeutic option to treat MPM in the clinic. Most antifolate drugs including pemetrexed inhibit DHFR, TYMS and GART molecules involved in purine and pyrimidine biosynthesis. There still exists a need to develop predictive metabolic biomarkers that would allow stratification of patients for effective therapy with pemetrexed targeting the one-carbon metabolism pathway. In this paper, we found that MPM cell lines could be categorized into two groups based on their

sensitivity to pemetrexed treatment. Hypoxanthine and thymidine, which are end products of the pemetrexed-targeted metabolic pathway, are sufficient to cancel the cytotoxic effects of pemetrexed. Moreover, we found that the expression of pemetrexed-targeted enzymes was lower in pemetrexed-resistant MPM cells than in pemetrexed-sensitive cells on a molecular basis. Metabolomic analysis revealed that glycine and choline, which are involved in one carbon metabolism, were altered after treatment in pemetrexed-sensitive MPM cells, but not in resistant cells. In addition, the supplementation of HT induced higher concentrations of IMP in pemetrexed-sensitive MPM cells. Finally, we showed that the addition of MTA altered pemetrexed-sensitivity in MTAP-negative NCI-H2452 cells, indicating that the nucleic acid biosynthesis pathway is important for predicting the efficacy of pemetrexed in MPM cells.

The lower level of pemetrexed target molecules in the pemetrexed-resistant NCI-H2452 cells suggests that they may survive by bypassing folate related pathways (**Figure 4**). In addition, the level of intracellular glycine was decreased after treatment with pemetrexed in the sensitive MSTO-211H and TCC-MESO2 cells (**Figure 5**). In contrast to glycine, serine accumulated only in the MSTO-211H cells (**Figure 5**). Accumulating evidence indicates that serine and glycine metabolism is important for cancer growth and proliferation (Jain et al., 2012; Zhang et al., 2012; Kim et al., 2015; Ducker et al., 2017). The enzyme serine hydroxymethyltransferase (SHMT) catalyzes the conversion of serine and tetrahydrofolate (THF) into glycine and 5,10-methylene-THF (**Supplementary Figure S3**). Similarly, the enzyme glycine decarboxylase (GLDC) catalyzes the conversion of glycine and tetrahydrofolate (THF) into ammonia, carbon dioxide and 5,10-methylene-THF (**Supplementary Figure S3**). Although serine is the predominant source of one-carbon units in cancer cells (Labuschagne et al., 2014), the level of glycine was more dramatically down-regulated than serine (**Figure 5**). Glycine consumption and synthesis are correlated with rapid cancer cell proliferation and glycine metabolism supports *de novo* purine nucleotide biosynthesis. Moreover, the metabolic enzyme GLDC is critical for tumor-initiating cells in non-small cell lung cancer (NSCLC) (Zhang et al., 2012). Further investigation would be needed to characterize in greater detail the molecular mechanisms that control glycine metabolism in MPM cells.

Several studies have suggested that TYMS is a predictive biomarker for the use of pemetrexed in NSCLC (Bukhari and Goudar, 2013). However, these associations are controversial. Prior studies showed that TYMS overexpression was one of the major factors leading to resistance in pemetrexed-resistant cells (Zhao and Goldman, 2003). However, our study demonstrated the opposite results (**Figure 4**). This suggests that differences in degree of pemetrexed-resistance and patterns of resistance may be attributed to multiple factors that include drug transport and polyglutamination as well as changes in cellular folate pools that must be assessed and considered in pemetrexed-resistant MPM cells. A recent report showed that the balance between folic acid uptake, activation and utilization plays a crucial role in the response to pemetrexed-based chemotherapy and prognosis in MPM (Mairinger et al., 2017). We observed that the addition of HXN partially rescued PMX cytotoxicity in TCC-MESO2 cells but had no effect in MSTO-211H and NCI-H2452 cell lines (**Figure 2**). With regards to the difference between MSTO-211H and TCC-MESO2 cells in the treatment of PMX + HXN, we speculate that pemetrexed mainly inhibits the pyrimidine biosynthesis pathway in MSTO-211H cells and inhibits both pyrimidine and purine biosynthesis pathways in TCC-MESO2 cells. Our results showed that the combination of hypoxanthine plus thymidine completely rescued the growth repression induced by pemetrexed (**Figure 2**). We reasoned that the treatment with HT solution might induce the mRNA expression level of PMX-target genes involved in one-carbon metabolism. However, the mRNA levels in both PMX-sensitive vs. PMX-resistant MPM cells was almost equivalent even after treatment with HT solution (**Supplementary Figure S7**). Therefore, the measured concentrations of hypoxanthine and thymidine in the serum or within the tumor tissue of MPM patients may directly correlate with non-responsiveness for pemetrexed treatment. To test this idea, we will need to analyze nucleic acid metabolites in clinical samples for these and other potential biomarkers to predict therapeutic response in MPM.

AUTHOR CONTRIBUTIONS

HM, JN, SU, AO, MT, TS, and KT designed the study and contributed to analysis and interpretation of data. YS and HM wrote the initial draft of the manuscript. All other authors have contributed to data collection and interpretation, and critically reviewed the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2018.01129/full#supplementary-material

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RESEARCH ARTICLE Open Access

Physician requests by patients with malignant pleural mesothelioma in Japan

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Abstract

Background: Malignant pleural mesothelioma (MPM) is a fatal and rare disease that is caused by the inhalation of asbestos. Treatment and care requests made by MPM patients to their physicians were collected and analyzed.

Methods: This cross-sectional survey was part of a larger study ($N = 133$) regarding the quality of life of MPM patients. Specific responses to two open-ended questions related to patients' requests regarding treatment and care were quantified, analyzed and divided into categories based on content.

Results: Responses ($N = 217$) from MPM patients ($N = 73$) were categorized into 24 subcategories and then abstracted into 6 categories. The majority of requests were related to patient-physician communication. Patients wanted clear and understandable explanations about MPM and wanted their physician to deliver treatment based on the patient's perspective by accepting and empathizing with their anxiety and pain. Patients expected physicians to be dedicated to their care and establish an improved medical support system for MPM patients.

Conclusion: Patients with MPM had a variety of unmet needs from their physicians. Physicians who provide care to MPM patients should receive training in both communication skills and stress management. A multidisciplinary care system that includes respiratory and palliative care for MPM patients should be established.

Keywords: Asbestos, Communication, Mesothelioma, Patient-centered care, Support

Background

Globally, exposure to asbestos in the workplace is now considered one of the main causes of work-related deaths with one-half of these deaths attributable to cancers, including malignant pleural mesothelioma (MPM) [1]. The number of deaths from MPM in Japan was greater than 1400 in 2015 [2]. This number is expected to grow by 2040 [3]. MPM is fatal [4, 5] and causes debilitating physical symptoms, such as pain, dyspnea, fatigue, loss of appetite, and sweating [6]. Patients with MPM also experience emotional difficulties, including the shock of diagnosis [7], anxiety and depression [8], or guilt and shame [9]. In addition, patients have complained of a lack of information about the disease and a lack of compensation from their insurance providers [10]. Patients have also expressed anger toward their employers who did not alert them to the hazards of asbestos [8, 11], in response to their own ambivalence toward working in an unhealthy environment versus supporting their family [8], and as a result of the stress of dealing with asbestos-related lawsuits [8, 12, 13]. For patients with MPM, a multidisciplinary approach involving a psychologist specialized in taking care of cancer patients and their families is recommended [14]. In Japan, physicians are the major source of information and support for patients with MPM. Unfortunately, some patients with MPM have not been well informed, and physicians were unable to meet their needs. This lack of rapport and communication eventually led to dissatisfaction with their attending physician and had a negative impact on patients' quality of life (QOL) [10]. Given the importance of the physician-patient relationship, it is important to further investigate what MPM patients need from their physicians to address their current gap in knowledge of the disease. The current study is part of a larger study regarding the QOL of

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patients with MPM. The aim is to determine the needs of patients within the health services by quantifying the requests to their physicians and qualitatively analyzing their answers to two open-ended questions regarding these requests.

Methods

Study design

This study is a part of a major study about QOL and intention of care among MPM patients. This study is a cross-sectional descriptive study that used a mailed survey [15]. In brief, an invitation to participate in the study was sent to 422 cancer hospitals in Japan; 64 hospitals (15.2%) agreed to participate. In February 2016, the participating hospitals distributed 438 questionnaires to their patients with MPM. Additional questionnaires were mailed in March 2016 to 94 MPM patients who were identified through patient and family support groups, which have 15 branches in Japan. The completed questionnaires were mailed back to the researchers by the end of April 2016. Basic demographic and medical data of the participants were gathered using a separate researcher-constructed, patient self-administered questionnaire. The questionnaire contained 72 questions regarding the QOL of MPM patients and related factors. In total, 88 (20.1%) questionnaires were returned. Of the 94 questionnaires that were sent to the patients and family support groups, 45 (47.9%) were returned. In total, 133 questionnaires were collected, and 73 (54.9%) participants answered the two open-ended questions referred to as "requests to physicians." Table 1 describes the characteristics of the participants. In the current study, we evaluated the answers to open-ended questions: (1) "What do you request from your doctor about your diagnosis and treatment?" and (2) "Describe the attitude and words you want from your doctor (Additional file 1)."

Table 1 Demographic and Medical Characteristics of the Study Participants ($N = 73$)

Characteristic	Response	n	%
Gender	Male	61	83.6
	Female	12	16.4
Age in years (mean ± SD)		66.8 ± 11.3	
MPM Treatment Received			
Surgery	I did not have	43	58.9
	I had	30	41.1
Chemotherapy	I never had	13	17.8
	I had before	29	39.7
	I am having now	31	42.5
Radiotherapy	I never had	52	71.2
	I had before	19	26.0
	I am having now	2	2.7
Palliative care	I never had	39	53.4
	I had before	9	12.3
	I am having now	25	34.2
ECOG Performance Status	0	12	16.4
	1	40	54.8
	2	7	9.6
	3	13	17.8
	4	1	1.4
Relationship with Their Physician	Very good	30	41.1
	Good	31	42.5
	Moderate	9	12.3
	Not very good	2	2.7
	Poor	1	1.4

ECOG, Eastern Cooperative Oncology Group; SD, standard deviation

Data analysis

Basic medical and demographic information was tallied, and the percentages and mean values were calculated. The answers to the questions were analyzed using the qualitative content analysis procedures of Graneheim and Lundman [16]. Initial categories were created by grouping similar words and phrases. The authors discussed the definitions and examples that emerged through the content analysis to enhance the representation and add clarity to categories, definitions, and examples. Responses that were not easily ascribed to a specific category were discussed and assigned to an appropriate category when the research team achieved 100% consensus. This process was repeated until all the responses were coded [17]. Finally, two researchers verified all the answers and tallied the number of times each category and subcategory was mentioned. The prevalence was compared between patients who received palliative care and those who did not receive palliative care. Comparisons between independent groups were performed using the chi-square test.

Ethical considerations

Ethical approval for the study was obtained from the Okayama Rosai Hospital Ethics Review Board. Eligible MPM patients received written information about the study, including their right to confidentiality, to refuse participation, or to withdraw at any point in the study without penalty.

Results

Requests to the physician

The 217 requests by 73 respondents were categorized into 24 subcategories and were finally integrated into six

categories. Table 2 displays the categorized requests to physicians by MPM patients.

Understandable explanations to meet patient's needs

Among the 217 requests, 80 concerned explanations from their doctor. The most frequent requests were to tell the cause of the symptoms, explain the curability and prognosis of the disease, and provide a treatment plan ($n = 41$).

> *"A doctor told me 'You have 2 years to go.' However, I was so healthy and could not imagine how this could be happening. I was in a panic because I did not know what to do next. Later, another doctor said 'Live as you lived. When you have pain, I will introduce you to a doctor for pain.' This explanation gave me back my life."* (#18 Male)

The second most frequent request was to provide information about their disease in simple words ($n = 12$).

> *"'There is no change, the same as the last time.' [He] does not explain anything. How is it the same? Is it good or bad? Why does he think so? If he based his diagnosis upon data, show them to me."* (#47 Male)

Patients with MPM exhibited great concern regarding examinations. They wanted their physician to explain

Table 2 Requests to Physicians by MPM Patients (217 requests; $N = 73$)

Categories / Subcategories		Times mentioned	% of Sample
1. Understandable explanation to meet the patient's needs		80	
1.1	Explain the cause of the symptoms, curability and prognosis of the disease, and provide a treatment plan	41	56.2
1.2	Use simple words	12	16.4
1.3	Explain the purpose, benefits, risk and results of examinations	10	14.0
1.4	Inform about all treatment options	10	14.0
1.5	Give advice about daily activities	3	4.1
1.6	Spend enough time on explanations	2	2.7
1.7	Confirm patient's understanding and allow them to ask questions	2	2.7
2. Patient-centered treatment		39	
2.1	Minimize the physical impact of treatment	11	15.1
2.2	Do not give up on the treatment	10	14.0
2.3	Respect patient's intention	9	12.3
2.4	Careful clinical assessment to not miss clinical signs of progression	9	12.3
3. Improvement of treatment and support systems for MPM		35	
3.1	Develop country-wide specialized care system	16	21.9
3.2	Develop new drugs	10	14.0
3.3	Improve information systems	9	12.3
4. Emotional support		32	
4.1	Be kind and cheerful	11	15.1
4.2	Sympathize with patient's anxiety	10	14.0
4.3	Have a reliable attitude	6	8.2
4.4	Empathy for victims of asbestos	3	4.1
4.5	Visit patient as often as possible	2	2.7
5. Customize "breaking the bad news"		24	
5.1	Tell everything including bad news	17	23.3
5.2	Do not inform about bad news	5	6.8
5.3	Customize the contents and the way of informing	2	2.7
6. Dedication to the treatment of MPM		7	
6.1	Confront intractable disease	4	5.4
6.2	Learn about MPM	3	4.1

MPM, malignant pleural mesothelioma

the purpose, benefits and risks, and results of examinations (*n* = 10).*"Explain concretely why I need an examination and do not forget to tell me the results, including my data compared with normal levels. Being well-informed and knowing my results eases my anxiety and gives me a sense of control. I feel that I am not that bad yet."* (#72 Male)

"I want to know if the chemotherapy worked on my tumor." (#10 Male)

In addition, the respondents wanted to know all the treatment options (n = 10).*"I need to know the latest treatment."*(#81 Male)

"Does any treatment work for patients with MPM?" (#89 Male)

Furthermore, some respondents wanted advice about preparation. (*n* = 3)"*My doctor let me know the benefits of palliative care and advised me to introduce it at an early stage. It was helpful because I had time to prepare."* (#72 Male)

Patients with MPM wanted their physician to spend enough time on explanations (*n* = 2).*"I know doctors are very busy. However, please understand that each patient needs time to understand what you said. Please do give us information so that we can understand one thing and then go further with the explanation. If you only explain things one-by-one, we never understand and get confused."* (#2 Male)

Finally, patients with MPM wanted their physician to confirm their understanding of the explanation and allow them to ask questions (n = 2).*"My doctor always asks me 'Is there anything you want to ask me?' You will never know how greatly I appreciate him. It is the greatest gift for patients."* (#45 Male)

Patient-centered treatment

Eleven patients requested the minimization of the physical impact of the treatment.

"*I do not want to suffer from heavy treatment. Just relieve my pain and let me stay at home until the last day."* (#78 Male)

Other respondents wanted their physician to not give up on treatment (*n* = 10).*"My doctor said I cannot receive chemotherapy any more, but I really want to receive treatment. I hope my doctor never gives up on my treatment … .I feel safe as long as I receive treatment."* (#75 Male)

Nine respondents commented that their physician should respect patients' intentions because they were not treated in the way they wanted."*My doctor came to me and said, 'Move to another hospital. The members of the medical conference decided not to treat you here anymore.' How can they say that? Patients are completely reliant on their doctors; at the very least, treatment must include the patient's perspective."* (#120 Male)

"I hope my doctor not only treats my tumor but also takes care of me. I am not a box with cancer, but a living person." (#123 Male)

Another 9 patients with MPM wanted their physician to perform a careful clinical assessment to not miss clinical signs of progression (*n* = 9).*"I want my doctor to check very carefully to identify progress as soon as possible because MPM has no effective treatment. However, he repeats the same examination in a mechanical way. This makes me uneasy."* (#99 Male)

Need for improvement of treatment and a support system for MPM

Some patients described specific suggestions to improve support systems. The participants wanted the development of country-wide specialized care systems (*n* = 16), development of new drugs (*n* = 10), and improvement of information systems (*n* = 2).

"Because MPM is a difficult disease, I want to be treated by a specialist. I am disappointed that there is no specialist in my area." (#36 Male)

"Develop a test for early disease detection and develop a medical care service as soon as possible." (#12 Male)

"We need a liaison to consult with about MPM. It is so hard to collect information about the disease and hospitals for individual patients and their family." (#113 Male)

Emotional support

The participants wanted their physicians to be kind and cheerful (*n* = 11), to sympathize with patients' anxiety (n = 10), to have a reliable attitude (*n* = 9), and to visit the patient as often as possible (n = 2).

"No one can cheer me up but the doctor. I want my doctor to say, 'it is alright.' I was so happy when he said, 'Let's work together'."(#8 Male)

"When I am very anxious, I ask my doctor the same question many times. He says, 'I explained that before,

didn't I?' He is angry, and it makes me more anxious. I hope he allows me to ask questions as many times as I want." (#102 Male)

"My doctor pays attention to the computer and does not look at me. I hope he looks me in the eye." (#113 Male)

"My doctor came to me and smiled at me. It was only for a minute, but it worked and made me feel so relieved. I want him to come as often as possible." (#45 Male)

Furthermore, patients with MPM wanted to be considered as a victim of the use of asbestos and expected their physician to have empathy with victims of asbestos ($n = 3$).*"If I were to die from another cancer, I would not suffer like this. I am so resentful that I will die from asbestos; this feeling prevents me from facing my problems. How dare my doctor say 'patients with MPM are not the only ones who are suffering?'"* (#106 Male)

Customize "breaking the bad news"

Some of the participants wanted their physicians to inform them about everything including bad news ($n = 17$). In contrast, some did not want to be informed about bad news ($n = 5$) or requested that doctors customize the content and way of presenting bad news ($n = 2$).

"*I want my doctor to tell me everything, including bad news."* (#64 Male)

"I was already shocked to learn that I have MPM; it was cruel to tell me the time I had left." (#112 Male)

"Don't tell me the bad news. Just let me know something good." (#75 Female)

Dedication to the treatment of MPM

Patients wanted their physicians to confront the intractable disease ($n = 4$) and to learn more about MPM ($n = 3$).

"I hope my doctor has enough ambition and passion to battle the difficult disease of MPM." (#127 Male)

"My doctor's priority is to make money from us. They do not have the spirit to take care of us on our deathbed." (#120 Male)

"Doctors are the only hope for patients. I beg them to learn more about MPM." (#65 Male)

We compared these categorized requests according to MPM patients with or without palliative care. MPM patients who did not receive palliative care described more requests concerning understandable explanations, need for improvement of treatment and support systems, and dedication to the treatment of MPM than those who received palliative care. Among these requests, there was statistical significance concerning communication regarding the cause of the symptoms, curability and prognosis of the disease, and treatment plan ($p = 0.030$) (Additional file 2: Table S1).

Discussion

This study was part of a larger study about the QOL of MPM patients and sought to reveal their healthcare-related needs, particularly regarding interactions with their physician. Patients with MPM wanted their physicians to provide supportive communication, patient-centered care, and an attitude of dedication and commitment to their treatment. Most requests to their physicians concerned the content and method of communication. Patients wanted precise information about their condition, even if it was raw data from examinations. Patients also wanted the doctor to explain in laymen's terms how the condition would affect their daily lives. A previous study of patients with MPM also identified the difficulty of physicians in establishing rapport and engaging in a fruitful two-way communication [18]. The style of communication requested by patients with MPM was similar to studies of other cancers: a two-way exchange of information [19, 20]; and communication to provide the patient with data [21, 22]. Additionally, patients wanted to be allowed to ask questions [22], to be treated by physicians with insightful and empathetic attitudes [23, 24], and to be assured of on-going support [24].

The requests for emotional support were clearly evident in this study. The need for physicians to provide emotional support was documented in previous studies [23, 24], including one in which physicians were considered the most important source of psychological support [25]. In particular, our study indicated that MPM patients had an extra need for empathy due to their perception of being victims of asbestos. Additionally, the diagnosis of MPM engendered deep resentment given the circumstances surrounding their exposure to asbestos [10, 12, 26], feelings of injustice [12], and feelings of being traumatized [27].

This study also indicated that many patients with MPM wished for clear and complete information about their disease and its prognosis, while a smaller number of patients wanted the information to be delivered in a more indirect and vague manner. Yanagihara reported that Japanese patients wanted bad news to be minimized and to be conservative [28]. Patients with MPM were reported to have high levels of uncertainty and feelings of a lack of control leading to psychosocial distress since receiving their diagnosis [29]. Physicians should take

these differences into account when they present the diagnosis and prognosis of MPM to their patients.

It is fundamental that any treatment is the result of mutual decision-making between the patient and the physician. Our study demonstrated the frustration of some patients with MPM who could not receive chemotherapy due to a safety issue, leaving them feeling not cared for or abandoned. In addition, the current study indicated that patients who did not receive palliative care described more requests than those who received palliative care. One possible explanation would be a difficulty of physicians to tell the curability and prognosis of the disease to the patients. Miyashita et al. evaluated end-of-life cancer care in designated cancer centers and palliative care units and reported that care evaluation score was lower in designated cancer centers than in palliative care units concerning physical care by physician, help with decision making, and knowing what to expect about future condition [30]. Unfortunately, Japan has a limited care system for patients with MPM [31]. An integrated care and support system is urgently needed with a multidisciplinary approach that includes physicians, nurses, psychologists, support groups, and medical social workers.

Patients with MPM also expect their physicians to have updated knowledge about MPM and continued interest in searching for new methods of treatment. Patients certainly did not want their doctor to be stymied or to give up on them. Budych et al. previously indicated that patients with rare diseases prefer that their physician make most of the decisions regarding their care [32].

Limitations of this study include a low participation rate from hospitals (approximately 20%), although approximately half of the questionnaires were returned from the support groups. This study is also biased toward patients in the early stages of MPM and those with a good relationship with their physicians. However, given that other studies support the findings of this research, the risk of this bias is less of a concern. Further research should include a longitudinal, mixed-methods study that utilizes standardized instruments in addition to interviews with patients and physicians to shed more light on the specific needs of both groups.

Conclusion

This study indicated that patients with MPM had a variety of needs unmet by their physicians, even if they were in the early stages of the disease, and most had good relationships with their physicians. In addition, the current study indicated that patients who did not receive palliative care described more requests than those who received palliative care. Physicians should consider introducing shared decision-making and empathic verbal and nonverbal communication with dedication to the treatment of MPM. Physicians who provide care to MPM patients should receive training in both communication skills and stress management. A multidisciplinary care system that includes respiratory and palliative nurse specialists should be established for patients with MPM.

Additional file

Additional file 1: Questionnaire about quality of life of people with malignant pleural mesothelioma. (DOCX 17 kb)

Additional file 2: Table S1. (DOCX 22 kb)

Abbreviations

MPM: Malignant pleural mesothelioma; QOL: Quality of life

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Availability of data and materials

The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

Authors' contributions

YNIO and NF made substantial contributions to the conception and design. YN, KA, JN, and KHara made substantial contributions to data acquisition. YN, IO, KA, KHotta, KK, and TK made substantial contributions to data analysis and interpretation. YN and NF were involved in drafting the manuscript. NF provided the final approval of the version to be published.

Ethical approval and consent to participate

This study was approved by the institutional review board of Okayama Rosai Hospital (approval no. 2017–22). This study was also approved by the institutional review board of each hospital or institution that distributed the questionnaire to their patients, according to their policy. The study was conducted based on the ethical principles of avoiding harm, voluntary participation, anonymity, and protection of privacy and personal information. The purpose, procedures, and confidentiality of the study were explained in written format. The participants were informed that nonparticipation would not disadvantage them. Return of the answered questionnaire was considered to constitute the patient's consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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Clinical Trials: Immunotherapy

Clinical Cancer Research

Clinical Efficacy and Safety of Nivolumab: Results of a Multicenter, Open-label, Single-arm, Japanese Phase II study in Malignant Pleural Mesothelioma (MERIT)

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Abstract

Purpose: Malignant pleural mesothelioma (MPM) is a rare and aggressive malignancy with poor prognosis. Patients with MPM who do not respond to standard first-line chemotherapy have limited treatment options. We evaluated the efficacy and safety of nivolumab, an immune checkpoint inhibitor, for the treatment of advanced or metastatic MPM.

Patients and Methods: Japanese patients with unresectable, advanced, or metastatic MPM resistant or intolerant to ≤2 regimens of chemotherapy and ≥1 measurable lesion (s) were enrolled. Patients received nivolumab 240 mg intravenously every 2 weeks until progressive disease or unacceptable toxicity. The primary endpoint was objective response rate by central assessment according to the Modified Response Evaluation Criteria in Solid Tumors. Adverse events (AEs) and treatment-related AEs (TRAEs) were evaluated.

Results: Thirty-four patients were enrolled between July 2016 and October 2016. Median follow-up was 16.8 (range: 1.8–20.2) months. Ten (29%, 95% confidence interval, 16.8–46.2) patients showed a centrally assessed objective response. The objective response rates were 26% (7/27), 67% (2/3), and 25% (1/4) patients for epithelioid, sarcomatoid, and biphasic histologic subtypes, respectively. Median duration of response was 11.1 months with a 68% disease control rate. Median overall survival and progression-free survival were 17.3 and 6.1 months, respectively. The objective response rate was 40% with programmed death-ligand 1 expression ≥1% and 8% with <1%. Thirty-two patients (94%) experienced AEs and 26 (76%) experienced TRAEs.

Conclusions: Nivolumab met the primary endpoint as second- or third-line treatment for patients with MPM and showed promising efficacy with manageable toxicity.

See related commentary by Mansfield and Zauderer, p. 5438

Introduction

Malignant pleural mesothelioma (MPM) is a rare and aggressive malignancy, responsible for 1,550 malignancy-related deaths in Japan in 2016 (1). In Japan, MPM is more common in men than women given their increased likelihood of occupational exposure to asbestos, and MPM most commonly affects elderly people (median age, 68 years; ref. 2, 3), in part, because of the long latency of the effects of asbestos exposure, which typically occur 30–50 years postexposure (4).

The median survival for patients with MPM is 7.9 months based on studies of newly diagnosed patients in Japan (2, 5).

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Translational Relevance

Malignant pleural mesothelioma (MPM) is a rare malignancy with poor prognosis, and patients who do not respond to first-line chemotherapy have limited treatment options. In this (multicenter, open-label, single-arm, Japanese phase II study in malignant pleural mesothelioma) study, we evaluated the efficacy and safety of nivolumab, an immune checkpoint inhibitor, for the treatment of advanced or metastatic MPM in patients intolerant or resistant to ≤2 regimens of chemotherapy. Nivolumab yielded an objective response rate of 29%, median overall survival of 17.3 months, and progression-free survival of 6.1 months. Its efficacy appeared promising in all histologic subtypes (epithelioid, sarcomatoid, and biphasic) and in PD-L1 ≥1% and <1% patients, although our sample size was small. Nivolumab showed manageable toxicity. While our study lacked a comparator, our findings reflect those of similar trials and suggest that nivolumab provides a clinical benefit and is a potential second- or third-line treatment option for MPM.

Most patients are diagnosed with advanced-stage MPM and receive first-line chemotherapy with pemetrexed and cisplatin (PC). This regimen provides a survival benefit over cisplatin alone (12.1 months and 9.3 months, respectively; ref. 6). Carboplatin is less toxic and more convenient than cisplatin, and combination therapy for MPM with carboplatin and pemetrexed has been evaluated, yielding an overall survival (OS) and progression-free survival (PFS) comparable with that of PC (7–9). Furthermore, adding bevacizumab to PC significantly improved survival benefit by 2.7 months in comparison with PC (10). However, patients with MPM who do not respond to first-line treatment with PC have no standard treatment. National Comprehensive Cancer Network (NCCN) guidelines recommend treatment with nivolumab with or without ipilimumab (11) and pembrolizumab is also a treatment option, but no drug had yet been approved for second-line treatment of MPM before starting this study.

Programmed death ligand 1 (PD-L1) is the ligand to the human programmed death-1 (PD-1) receptor. It is expressed in the tumors of patients with MPM (12–15): in 40% of patients with MPM according to one clinical investigation (12) and in 70% according to data from archived patient tissue (13). PD-L1 expression is correlated with a poor prognosis in MPM (12–15). Nivolumab is a human mAb to the PD-1 receptor that inhibits the interaction between PD-1 and its ligands, PD-L1 or PD-L2. Furthermore, nivolumab is approved for the treatment of various subtypes of malignancies (16).

We hypothesized that nivolumab would be a potential second- or third-line treatment option for MPM. Thus, the multicenter, open-label, single-arm, Japanese phase II study in MPM (MERIT) study evaluated the clinical efficacy and safety of nivolumab in Japanese patients with advanced or metastatic MPM resistant/intolerant to ≤2 regimens of platinum-based chemotherapy in combination with pemetrexed. This study started before the NCCN guideline recommended nivolumab for second-line treatment of MPM (11).

Patients and Methods

Study design and patients

This was a multicenter, open-label, single-arm phase II study conducted from June 16, 2016 to March 14, 2018 (data cut-off date), at 15 centers in Japan (Supplementary Table S1). The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The study protocol was reviewed and approved by the institutional review board of each site before study initiation. This study is registered with clinicaltrials.jp (JapicCTI-163247). All patients provided written informed consent.

Selection and description of patients

Eligible patients were men and women ages ≥20 years with histologically confirmed MPM, unresectable advanced or metastatic MPM without surgery, or MPM resistant or intolerant to ≤2 regimens of chemotherapy including platinum-based combination therapy with pemetrexed; and had ≥1 measurable lesion(s) as defined in the Modified Response Evaluation Criteria in Solid Tumors (mRECIST) in MPM (17) and confirmed by imaging within 14 days before enrollment, available tumor tissue samples (fresh or archival) for analysis of PD-L1 expression, and an Eastern Cooperative Oncology Group performance status of 0 or 1. Main exclusion criteria were severe hypersensitivity reactions to any other drug, including antibody products; concurrent autoimmune disease or a history of chronic or recurrent autoimmune disease; multiple primary cancers; brain or meningeal metastases; current or history of interstitial lung disease or pulmonary fibrosis diagnosed on the basis of imaging or clinical findings; and previous treatment with nivolumab, anti-PD-1 antibody, anti-PD-L1, or PD-L2, or any other therapeutic antibodies or pharmacotherapies for T-cell regulation.

Procedures

Patients received 240-mg nivolumab via intravenous 30-min infusion every 2 weeks on day 1 of each cycle until any criterion for nivolumab discontinuation was met (Supplementary Table S2). Neither dose nor administration mode of nivolumab could be adjusted. Therapies prohibited during the study period included immunosuppressants, corticosteroids at doses exceeding 10 mg/day prednisone equivalent, antitumor therapies (e.g., chemotherapy, molecular-targeted therapy, and immunotherapy), concurrent radiotherapy, pleurodesis, and surgical therapies for malignant tumors.

Patients underwent tumor imaging by computed tomography or magnetic resonance imaging every three cycles. The target lesions in pleura were measured uni-dimensionally as the largest tumor thickness perpendicular to the chest wall or mediastinum according to modified RECIST (17); those in nonpleura were measured according to RECIST version 1.1.

PD-L1 expression analysis was performed in a central laboratory (Cancer Genetics, Inc.) using (fresh or archival) tumor tissue samples with 28-8 antibody (Dako). One or more formalin-fixed, paraffin-embedded (FFPE) blocks of tumor tissue samples collected by core needle biopsy, excisional biopsy, or incisional biopsy of ≥5 FFPE unstained slide samples (serial tissue sections) were analyzed for PD-L1 status. Each tumor tissue sample was required to contain ≥100 evaluable tumor cells. PD-L1–positive

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status was defined as membranous staining in ≥1% of tumor cells. Samples were classified as "not evaluable (NE)" if the biological conditions of the sample rendered the stained cell membranes difficult to assess, even if the samples otherwise met the evaluation criteria.

Outcomes

The primary endpoint was centrally assessed objective response according to mRECIST. The objective response rate was defined as the proportion of patients whose best overall response was complete response (CR) or partial response (PR). Secondary endpoints were investigator-assessed objective response rate and percent change in the sum of tumor sizes of target lesions; disease control rate, OS, PFS, duration of response, time to response, and best overall response assessed centrally. In addition, subgroup analyses of tumor response, PFS, OS by PD-L1 expression (<1% and ≥1%), and histologic subtype were performed.

OS was defined as the time from the first nivolumab dose to death from any cause. PFS was defined as the time from the first nivolumab dose to progressive disease (PD) or death from any cause. Disease control rate was the percentage of patients whose best overall response was CR, PR, or stable disease (SD).

Adverse events (AEs) and treatment-related AEs (TRAEs) were monitored throughout the study period and graded according to the Japanese translation (Japan Clinical Oncology Group edition) of the NCI Common Terminology Criteria for Adverse Events, version 4.0. AEs of special interest were prespecified as endocrine disorders, gastrointestinal toxicity, hepatotoxicity, pulmonary toxicity, nephrotoxicity, skin toxicity, and hypersensitivity/infusion reactions.

Statistical analysis

As there was no available standard treatment for the target population, the lower threshold for response was set at 5%, and an expected objective response rate of 19% was used for this study. We calculated that ≥29 patients would be required to detect a significant difference in the objective response rate with a power of 80% and a one-sided significance level of 0.025. To account for the estimated 10% dropout rate, we planned to recruit 32 patients. The full analysis set was used for the analysis of the efficacy endpoints, and the safety analysis set for the analysis of baseline demographic and clinical characteristics and safety endpoints. Frequency distribution and summary statistics were used for baseline characteristics. The objective response and disease control rates and their two-sided 95% confidence intervals (CI) were calculated. Medians and two-sided 95% CIs for OS, PFS, and duration of response were calculated using the Kaplan–Meier method. OS and PFS rates, and their two-sided 95% CIs, were calculated at 6 and 12 months depending on the duration of follow-up. The percentages of patients with best overall response of CR, PR, SD, PD, and NE were calculated. Statistical analyses were performed with SAS version 9.3 (SAS Institute Inc.).

Results

Most patients were male (29/34 patients, 85%), with a median age of 68.0 years; 27/34 patients (79%) had an epithelioid subtype (Table 1). Patients received a median of 12.5 (range, 1–42) doses; the median duration of treatment was 6.8 (range, 0.03–19.1) months. The median relative dose intensity was 96% (range, 62%–112%). Six patients (18%) were still on treatment, and 28 (82%) discontinued treatment at data cutoff. The reasons for discontinuation included PD (22 patients, 65%); unequivocal clinical progression attributable to PD (5 patients, 15%); development of grade ≥2 interstitial lung disease or pneumonitis (4 patients, 12%); lack of nivolumab administration for 6 weeks due to AE onset (2 patients, 6%); and continuation of treatment judged as inappropriate by the principal investigator (1 patient, 3%). Some patients had more than one reason for discontinuation. All 34 patients were included in both the full and safety analysis sets. Median follow-up was 16.8 (range, 1.8–20.2) months.

Table 1. Baseline demographic and clinical characteristics

	Nivolumab *N* = 34
Sex	
Male	29 (85)
Female	5 (15)
Age, years, median (range)	68.0 (43–78)
Body mass index, kg/m^2, median (range)	22.1 (15.8–29.0)
Number of prior treatment(s)	
1	24 (71)
2	10 (29)
Performance status	
0	13 (38)
1	21 (62)
Previous systemic therapy	
First line	
Pemetrexed + cisplatin/carboplatin	31 (91)
Pemetrexed + cisplatin + BBI608	2 (6)
Pemetrexed + cisplatin + bevacizumab	1 (3)
Second line	
Gemcitabine	3 (9)
Pemetrexed + cisplatin/carboplatin	3 (9)
Pemetrexed	2 (6)
Other	2 (6)
PD-L1 status	
≥1%	20 (59)
<1%	12 (35)
NE	2 (6)
Histological subtype	
Epithelioid	27 (79)
Biphasic	4 (12)
Sarcomatoid	3 (9)

NOTE: Data are *n* (%), unless otherwise stated.

Ten (29%; 95% CI, 16.8–46.2) of 34 patients had an objective response by central assessment (Table 2), and all were PR. The response rate by site according to mRECIST was identical. The disease control rate was 68% (95% CI, 50.8–80.9; Table 2). Regarding the best overall response, 10 (29%) patients had PR, 13 (38%) had SD, 9 (26%) had PD, and 2 (6%) were NE (Table 2). In addition, central review confirmed that 1 patient had no measurable lesions.

The Kaplan–Meier curves for OS and PFS are shown in Fig. 1A and B. Median OS was 17.3 months (95% CI, 11.5–not reached), with OS rates of 85% (95% CI, 68.2–93.6) and 59% (95% CI, 40.6–73.2) at 6 and 12 months, respectively. Median PFS was 6.1 months (95% CI, 2.9–9.9), with PFS rates of 52% (95% CI, 33.5–66.9) and 32% (95% CI, 16.4–47.9) at 6 and 12 months, respectively. At data cutoff, 3 of 10 patients (30%) had an ongoing response. The median duration of response was 11.1 months (95% CI, 3.5–16.2), with median time to response of 2.63 (range, 1.0–6.9) months. Among responders, the median reduction in target lesions from baseline (depth of response) was 61% (interquartile range, 48–72).

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Table 2. Efficacy of nivolumab

	N	Tumor response (95% CI)
Objective response rate ($n = 34$)	10	29% (16.8–46.2)
Epithelioid ($n = 27$)	7	26% (13.2–44.7)
Biphasic ($n = 4$)	1	25% (4.6–69.9)
Sarcomatoid ($n = 3$)	2	67% (20.8–93.9)
Disease control rate ($n = 34$)	23	68% (50.8–80.9)
Best overall response rate ($n = 34$)		
CR	0	0% (0.0–10.2)
PR	10	29% (16.8–46.2)
SD	13	38% (23.9–55.0)
PD	9	26%
NE	2	6%

NOTE: All results are from the central assessment according to mRECIST. 95% CIs were calculated using the Wilson method; 95% CIs were not calculated for the PD or NE categories.

Tumor shrinkage was observed in all histologic subtypes, especially in 6 of 7 patients with either sarcomatoid or biphasic histologic subtype, slight tumor growth was observed in 1 remaining patient. Therefore, the disease control rate in sarcomatoid/biphasic patients was 100% (Fig. 2A). Tumor shrinkage was observed, regardless of PD-L1 status. Among PD-L1 evaluable patients, tumor shrinkage occurred in 14 of 20 (70%) patients with PD-L1 expression ≥1% and 4 of 12 (33%) patients with PD-L1 expression <1% (Fig. 2A). A long duration of response was recorded with a median duration of 11.1 months (95% CI, 3.5–16.2; Fig. 2B). Patients with tumor shrinkage tended to maintain the tumor response (Fig. 3).

The objective response rate by histologic subtype is reported in Table 2. The objective response rates were 26%, 67%, and 25% for epithelioid, sarcomatoid, and biphasic histologic

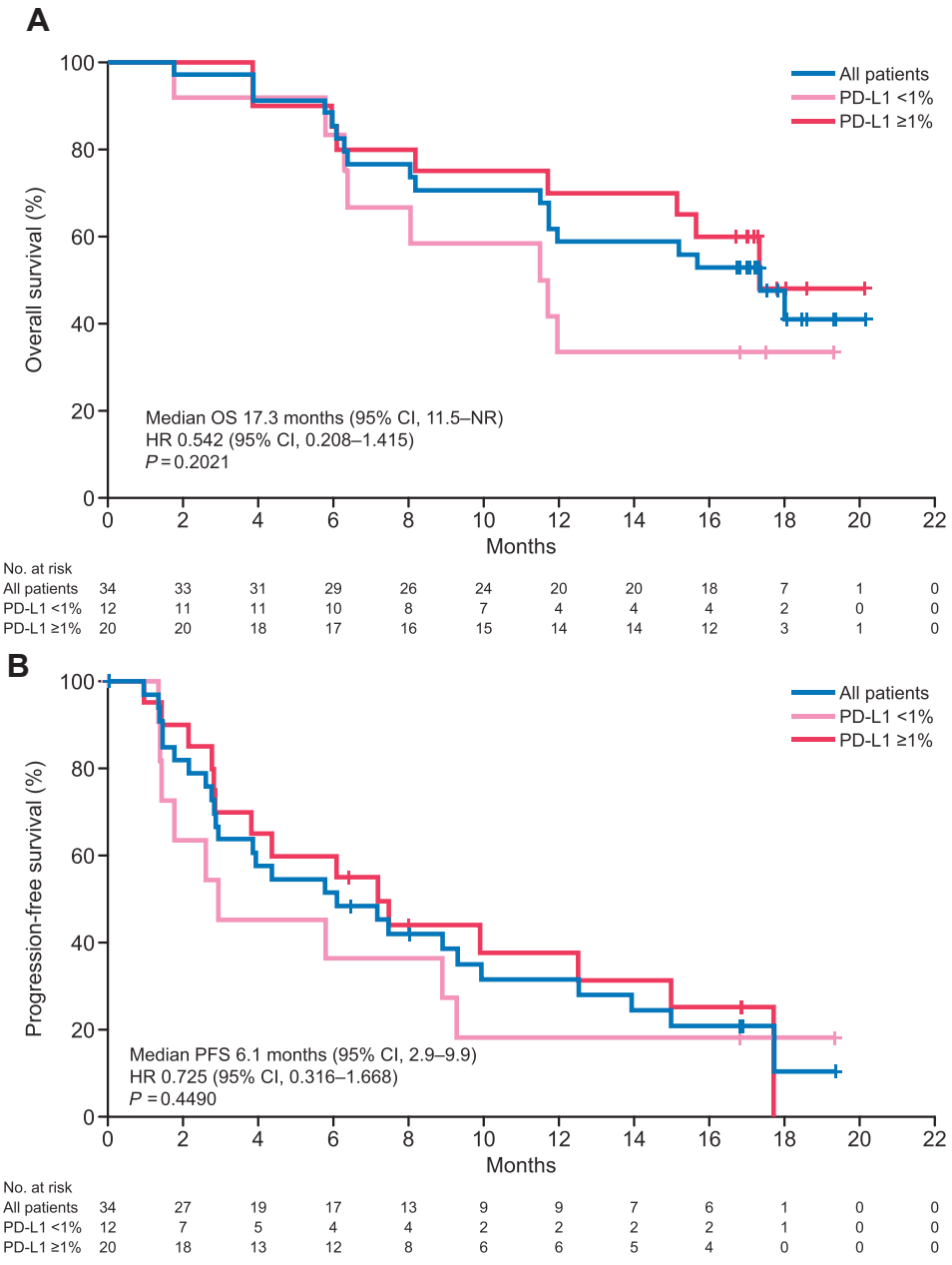

Figure 1.
Kaplan–Meier curves for OS (**A**) and PFS (**B**), for all patients and according to PD-L1 expression status. Median OS and PFS were calculated using values for all patients. HRs denote a comparison between the PD-L1 ≥1% and <1% groups. NR, not reached.

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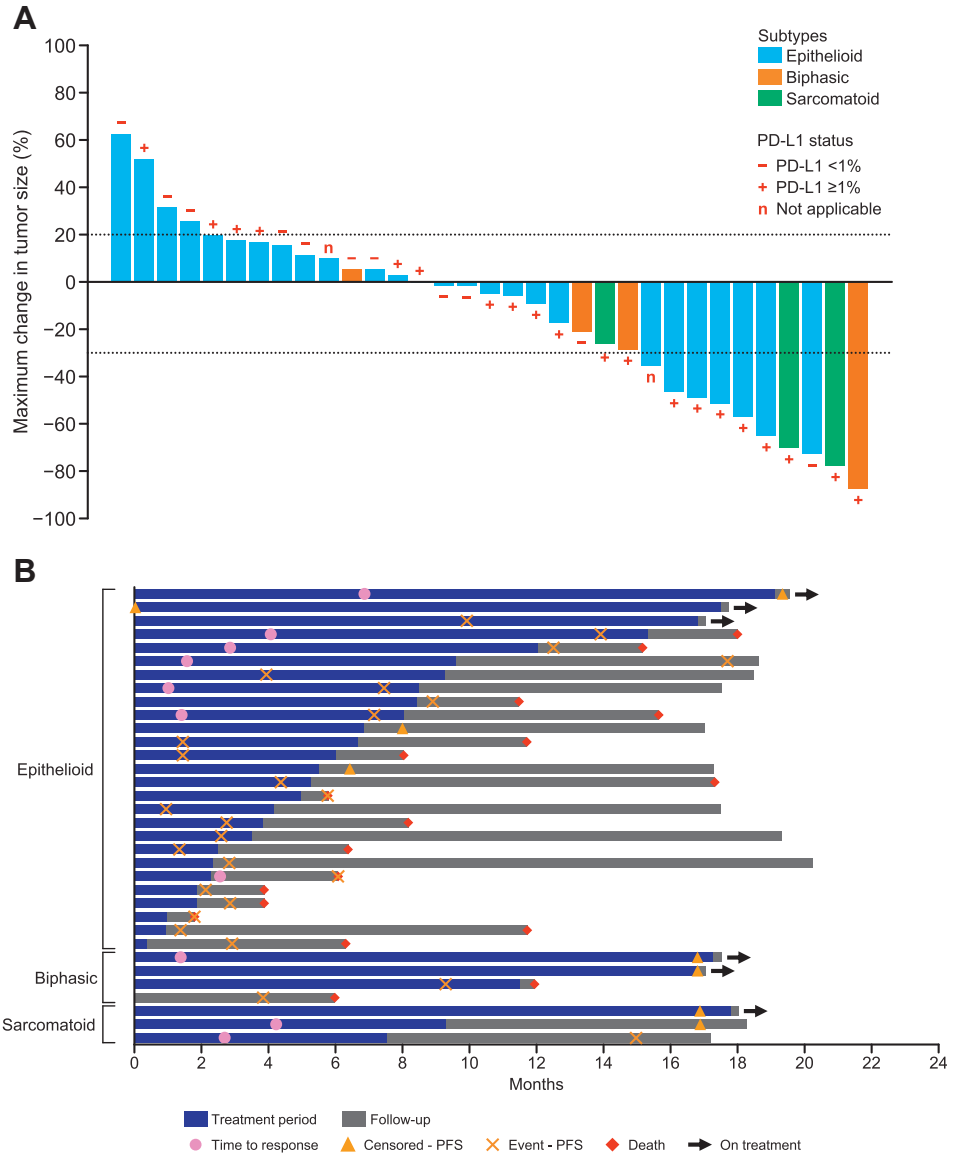

Figure 2.
Percent change in the sum of tumor size by histologic subtype and PD-L1 expression status. The waterfall plot shows the maximum percentage changes from baseline in target lesions by histologic subtype and PD-L1 expression status (**A**), with the length and direction of the bars indicating either an increase or decrease in the target lesion size of each patient. The swimmer's plot (**B**) shows treatment exposure and response duration, with bar length corresponding to the duration of treatment for each patient. Central assessment was performed with lesions defined as the largest tumor thickness perpendicular to the chest wall or mediastinum measured unidimensionally according to mRECIST.

subtypes, respectively. The subgroup analysis of OS and PFS by histologic subtype exhibited trends, with prolonged OS and PFS for patients with nonepithelioid subtype (Supplementary Fig. S1A and B). Results of tumor response analysis by PD-L1 expression are shown in Supplementary Table S3. The objective response rate differed by PD-L1 expression (40% for ≥1% vs. 8% for <1%, respectively). Similar trends were observed among patients with different PD-L1 expression levels (≥5% vs. <5% and ≥10% vs. <10%). The subgroup analysis of OS and PFS by PD-L1 status exhibited trends, with prolonged OS and PFS for patients with PD-L1 ≥1% versus <1% [hazard ratio (HR) for OS 0.542 (95% CI, 0.208–1.415; $P = 0.2021$); HR for PFS 0.725 (95% CI, 0.316–1.668; $P = 0.4490$); Fig. 1A and B].

All-cause AEs occurring in ≥5% of patients are shown in Table 3. Most patients (94%) experienced AEs and 16 (47%) patients experienced grade ≥3 AEs. A total of 26 patients (76%) experienced TRAEs, and 11 patients (32%) experienced Grade ≥3 TRAEs. Serious AEs occurred in 14 patients (41%), with 11 patients (32%) having serious TRAEs. Four patients (12%) experienced AEs leading to study treatment discontinuation [two events of interstitial pneumonia (1, grade 2; 1, grade 3) and two events of pneumonitis (both grade 3)]. No fatal AEs occurred between study start and either 28 days after the last nivolumab dose or the start of poststudy treatment. Regarding TRAEs with an incidence of ≥10%, rash occurred in 6 patients (18%); lipase increased, 5 (15%); and diarrhea and amylase increased, 4 each (12%).

The following AEs of special interest occurred: type 1 diabetes mellitus in 1 patient (3%), hypopituitarism in 1 patient (3%), hypothyroidism in 2 patients (6%); and blood thyroid stimulating hormone decreased, blood thyroid stimulating hormone increased, and thyroid function test abnormal in 1 patient (3%) each; diarrhea in 6 (18%) patients; gamma-glutamyltransferase increased in 2 patients (6%); alanine aminotransferase increased, aspartate aminotransferase increased, blood bilirubin increased, and blood alkaline phosphatase

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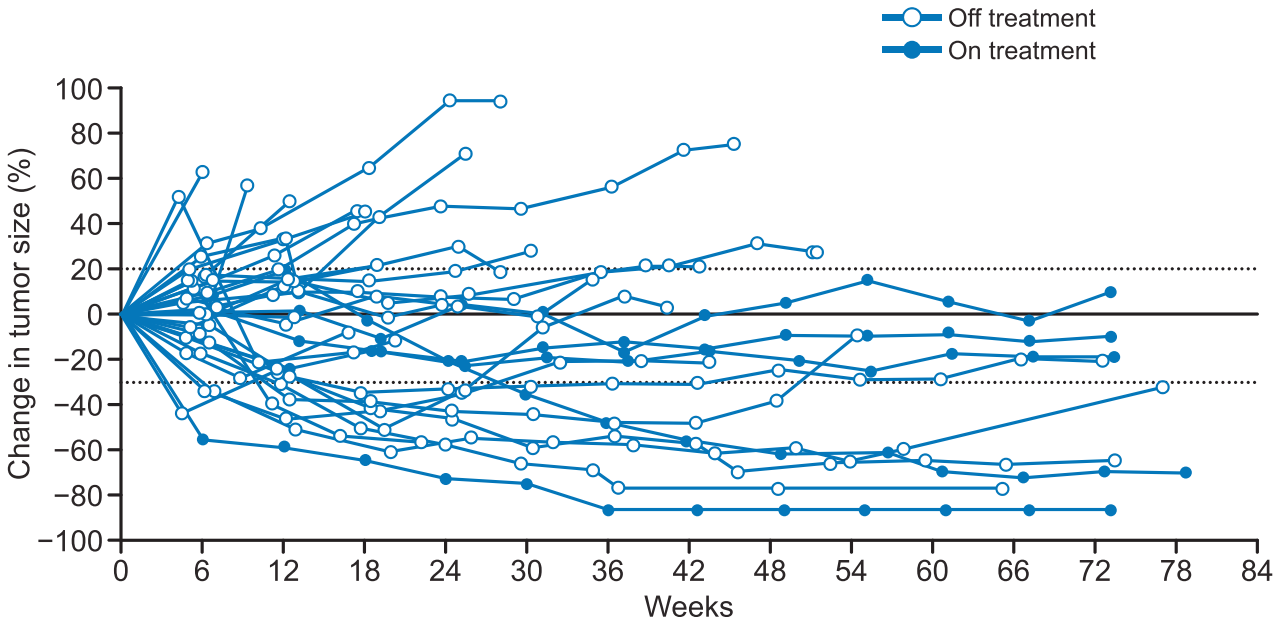

Figure 3.
Percent change in target tumor size over time. Central assessment was performed according to mRECIST.

increased in 1 patient (3%) each; interstitial lung disease and pneumonitis in 2 patients (6%) each; blood creatinine increased in 1 patient (3%); rash in 6 patients (18%), rash maculopapular in 2 patients (6%), and blister, eczema, rash pruritic, skin exfoliation, and urticaria in 1 patient (3%) each; and hypersensitivity in 1 patient (3%). Grade 3–4 AEs of special interest were diarrhea, gamma-glutamyltransferase increased, and pneumonitis in 2 patients (6%) each, and type 1 diabetes mellitus, hypopituitarism, alanine aminotransferase increased, aspartate aminotransferase increased, interstitial lung disease, and rash and hypersensitivity in 1 patient each (3%).

Discussion

MPM is a very aggressive malignancy with a poor prognosis. To develop better therapies for mesothelioma, recent research has focused on the role of immune cells within the tumor microenvironment. Treatment with immune checkpoint inhibitors, which reactivate immune responses that are silenced by immune checkpoints, has shown promising results (18).

The present results suggest that patients with advanced or metastatic MPM resistant or intolerant to the standard treatment may benefit from treatment with nivolumab. Previous studies of standard treatment in advanced or recurrent MPM reported response rates of 0%–2% with placebo or best supportive care and 0%–4.5% with investigational products (19–21). Efficacy of nivolumab for pretreated MPM was reported in previous studies (MAPS2 and NivoMes trials; ref. 22, 23). In addition, the KEYNOTE-028 study showed an objective response rate (investigator assessed according to RECIST guideline, version 1.1) of 20% (95% CI, 6.8–40.7) in previously treated patients with PD-L1–positive MPM receiving pembrolizumab 10 mg/kg every 2 weeks (24). In this study, an objective response rate of 29% was confirmed by central assessment according to mRECIST in patients with MPM and was concordant with the results of other similar studies (22–24). These results suggest that anti-PD-1 antibodies have a high potential for becoming a new treatment option for MPM.

Sarcomatoid or biphasic histologic subtypes are known predictors of poor prognosis (25), and PC therapy has little effect on these histologic subtypes (26). In this study, the objective response in patients with sarcomatoid and biphasic histologic subtypes was 2 of 3 and 1 of 4 patients, respectively. These results indicate that nivolumab had a beneficial effect in these histologic subtypes for which no previous treatment has been shown to be effective. This further supports the use of immune checkpoint inhibitors as potential treatment options to manage MPM. Interestingly, the PD-L1 expression rate was $\geq$50% in the three responders with sarcomatoid and biphasic histologic subtype (data not shown). However, these results should be interpreted with caution as there were only 7 patients with these subtypes. Further study in a larger number of patients with these histologic subtypes is warranted to confirm our findings.

Previous studies have shown that positive PD-L1 expression status has been associated with worse survival outcomes compared with negative PD-L1 expression status (14, 15). In this study, both PD-L1–positive and PD-L1–negative patients responded to nivolumab, and although not significant, differences in OS and PFS with PD-L1 expression status favored positive PD-L1 expression. While promising, these results must be considered in the context of the study design and size, and the fact that the PD-L1 analysis was exploratory. A greater number of patients showing PD-L1 expression responded to nivolumab, although some patients without PD-L1 expression also showed responses. This study was not powered to study differences in response or survival between categories of PD-L1 expression, but this is a critical area for future study in larger, comparative trials.

Patients who have PD after initial chemotherapy are generally expected to have a poor prognosis, advanced symptoms, and worsened condition compared with chemotherapy-naïve

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Table 3. AEs

	Nivolumab *N* = 34	
	Any grade	Grade 3–4
Any AEs	32 (94)	16 (47)
Most common AEs by preferred term (≥5% of patients)		
Viral upper respiratory tract infection	10 (29)	0 (0)
Weight decreased	7 (21)	0 (0)
Diarrhea	6 (18)	2 (6)
Rash	6 (18)	1 (3)
Pyrexia	6 (18)	0 (0)
Lipase increased	5 (15)	4 (12)
Stomatitis	5 (15)	1 (3)
Nausea	5 (15)	0 (0)
Amylase increased	4 (12)	2 (6)
Decreased appetite	4 (12)	2 (6)
Arthralgia	4 (12)	0 (0)
Vomiting	3 (9)	0 (0)
Fatigue	3 (9)	0 (0)
Malaise	3 (9)	0 (0)
Upper respiratory tract infection	3 (9)	0 (0)
Gamma-glutamyltransferase increased	2 (6)	2 (6)
Pneumonitis	2 (6)	2 (6)
Anemia	2 (6)	1 (3)
Hypophosphatemia	2 (6)	1 (3)
Interstitial lung disease	2 (6)	1 (3)
Hypothyroidism	2 (6)	0 (0)
Constipation	2 (6)	0 (0)
Dental caries	2 (6)	0 (0)
Mucosal inflammation	2 (6)	0 (0)
Edema peripheral	2 (6)	0 (0)
Lymphocyte count decreased	2 (6)	0 (0)
Hyperkalemia	2 (6)	0 (0)
Hypoalbuminemia	2 (6)	0 (0)
Myalgia	2 (6)	0 (0)
Dyspnea	2 (6)	0 (0)
Pneumothorax	2 (6)	0 (0)
Rash maculo-papular	2 (6)	0 (0)
AEs leading to discontinuation of study treatment	4 (12)	3 (9)
AEs leading to interruption of study treatment	15 (44)	10 (29)

NOTE: Data are presented as *n* (%).

patients. In fact, a PFS of 1.6–1.7 months and an OS of 5.4–4.9 months was reported in patients with MPM resistant/intolerant to standard treatment who received single-agent vinorelbine, single-agent gemcitabine, or both agents (27). Conversely, in this study, the median PFS and median OS were 6.1 months and 17.3 months, respectively, which were comparable with the results of previous studies in patients requiring second- and third-line treatment with nivolumab with or without ipilimumab (22, 23) and pembrolizumab (24). These findings suggest that nivolumab provides a clinical benefit and could be considered an option for second- or third-line treatment for MPM.

Regarding the safety profile, of the 34 patients receiving nivolumab, 32 (94%) and 26 (76%) patients experienced AEs and TRAEs, respectively. No deaths related to AEs were reported. Nivolumab is approved for the treatment of various cancer types and has been administered to many patients. In our opinion, the safety profile of nivolumab in this study did not differ greatly from that in other cancer types for which nivolumab has already been approved.

In conclusion, the primary endpoint was met in patients with advanced or metastatic MPM resistant or intolerant to maximally two regimens of chemotherapy including platinum-based combination therapy with pemetrexed who received nivolumab as second- or third-line treatment. Nivolumab showed a promising overall response rate of 29% and appeared to yield encouraging PFS and OS results across a range of histologic subtypes, and in patients with PD-L1 expression. Nivolumab had a manageable toxicity profile. Adequately powered, randomized, controlled trials are needed before definitive conclusions can be drawn regarding the survival benefits of nivolumab.

Disclosure of Potential Conflicts of Interest

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Clinical Cancer Research

Clinical Efficacy and Safety of Nivolumab: Results of a Multicenter, Open-label, Single-arm, Japanese Phase II study in Malignant Pleural Mesothelioma (MERIT)

Morihito Okada, Takashi Kijima, Keisuke Aoe, et al.

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Current evidence and future perspectives of immune-checkpoint inhibitors in unresectable malignant pleural mesothelioma

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ABSTRACT

Platinum-based chemotherapy is commonly used as the standard first-line treatment for unresectable malignant pleural mesothelioma (MPM). However, in recent times, immune-checkpoint inhibitors (ICIs) have led to a paradigm shift. Herein, we review relevant literature and ongoing trials of ICIs used as both first-line and salvage therapies. Specifically, in the Japanese single-arm, phase II trial, the MERIT trial, nivolumab, an antiprogrammed cell death 1 (PD-1) antibody showed favorable efficacy when used as a salvage therapy. Currently, multiple ICI monotherapy or combination therapy trials have been conducted, which could provide further evidence. Among available ICIs, the anti-PD-1 antibody is promising for unresectable MPM, despite the limited efficacy of anti-CTLA4 monotherapy. Ongoing studies will further confirm the potential efficacy of ICIs for MPM, as observed across other malignancies. It is also crucial to identify any clinically useful predictive biomarkers that could reveal ICIs with maximal effects in MPM.

INTRODUCTION

With increasing utilization of asbestos, the incidence of mesothelioma is considered to increase worldwide. Asbestos consumption in the USA has rapidly declined over the last 40 years, which has resulted in a considerable decline in mesothelioma incidence.[1] In Japan, the number of deaths had increased from 500 in 1995 to 1550 in 2016. Mesothelioma manifests mainly in the pleura, peritoneum and pericardium, although most commonly in the pleura.[2]

The major role of chronic inflammation and local tumor suppression in tumorigenesis observed in some experimental models led to the investigation of immunotherapy for malignant pleural mesothelioma (MPM).[3] There have been intensive investigations on the efficacy and safety of immune-checkpoint inhibitors (ICIs) in the treatment of unresectable advanced diseases.[4 5] Herein, we highlight relevant study results, as well as designs and concepts of ongoing studies in both first-line and salvage settings.

Known biology

Among approximately 400 different mineral fibers present in nature, six fibers (amphiboles fibers (crocidolite, actinolite, tremolite, anthophyllite and amosite) and serpentine fiber (chrysotile)) are called as 'asbestos'.[6] They are carcinogenic and have been associated with mesothelioma.[6 7] Furthermore, exposure of the chest to therapeutic ionizing radiation, usually performed to treat lymphomas, has been causally linked to mesothelioma, especially in young patients.[8–10]

The accumulation of genetic aberrations can induce malignancies. Recently, The Cancer Genome Atlas program investigated genetic alterations in mesotheliomas using next-generation sequencing (NGS).[11] The results revealed frequent mutations in BAP1, CDKN2A, NF2, TP53, LATS2 and SETD2.[11 12] Recently, a considerably higher number of genetic alterations in mesotheliomas has been detected than that detected by NGS, including point mutations, minute deletions and copy number changes.[13 14] Furthermore, the vast array of genetic alterations in mesothelioma may lead to producing neoantigens, which correlate with the clonal expansion of tumor-infiltrating T lymphocytes.[13 15] These findings suggest that, in contrast to the hypotheses based on NGS studies, mesothelioma may be immunogenic.[15]

Rationale for the development of immunotherapy

A hallmark of cancer is immune evasion, in which the immune system does not mount an effective antitumor response.[16] Programmed cell death 1 (PD-1) is a negative costimulatory receptor expressed primarily on the surface of activated T cells[17 18] and is involved in maintaining peripheral tolerance. The binding of PD-1 to one of its ligands, PD-L1 or PD-L2,

can inhibit a cytotoxic T-cell response.[19 20] Tumors can co-opt this pathway to escape T-cell-induced antitumor activity.[21–23]

The biology of MPM shows significant heterogeneity in both tumor and the microenvironment. Several studies, on T-cell-inhibitory receptors and chemokines, have indicated the prognostic role of lymphocytes and the occurrence of immunosuppression in MPM.[24 25] In a melanoma model, PD-1 blockade increased the proportion of antigen-specific CTLs that recognized melanoma targets by degranulation, suggesting increased recognition efficiency for cognate peptide.[26] The increased frequency and absolute number of antigen-specific CTLs by PD-1 blockade resulted from augmented proliferation, and not decreased apoptosis. These findings have led to the extensive development of agents blocking immunocheckpoints and their clinical investigation in various malignancies including MPM.

Biomarker in the ICI treatment of MPM

Some sensitive and specific immunohistochemistry markers including calretinin and WT1 are used for diagnosing mesothelioma.[4] However, markers for treatment efficiency have not been established. Generally, PD-L1 expression level is used as the representative maker for predicting the efficacy of ICIs. In the ICI monotherapy with the salvage setting in non-squamous cell non-small-cell lung cancer, the PD-L1 expression level affected the survival efficacy,[27] while its influence was weakened when combined with platinum-based chemotherapy in the first-line setting.[28]

In MPM, 20%–70% of the specimens tested are usually PD-L1 positive.[29] Such a wide range can be attributed to several factors. It could be because tumors are heterogeneous in nature.[4] It could be partially attributed to the antibodies used; SP-263 is the most commonly used antibody,[30–32] and the others include clones E1L3N and 28–8.[33] Furthermore, the histological subtype influences its frequency; PD-L1 expression is higher in non-epithelial mesotheliomas.[34] The cut-off levels of PD-L1 positivity vary among trials.[35] Considering that the positive rates were reported from different small studies with a small number of accrued patients, the data may be limited and actual rates of expression have hardly been studied. In addition to this, whether the ICI efficacy is truly dependent on the PD-L1 expression level is still controversial.

ICIs in the first-line settings

The standard treatment for unresectable, advanced malignant mesothelioma is chemotherapy, although with a very poor prognosis.[36] Similar to its use in non-small-cell lung cancer,[37–44] cisplatin (CDDP) and pemetrexed (PEM) combination therapy (CDDP/PEM) approved by the US Food and Drug Administration (FDA) in 2004, is strongly recommended as the first-line treatment for mesothelioma.[45] Moreover, molecularly targeted agents have been developed to augment cytotoxic chemotherapy. For instance, a randomized phase III MAPS study showed that adding bevacizumab to platinum doublets improved survival (HR of overall survival (OS) and progression-free survival (PFS): 0.77 (95% CI: 0.62 to 0.95); p=0.0167 and 0.61 (0.50 to 0.75); p<0.0001, respectively).[46] However, this regimen is yet to be approved by the FDA. A double-blind, randomized, placebo-controlled phase III study, the LUME-Meso trial of CDDP and PEM with or without nintedanib, a multikinase inhibitor for unresectable epithelioid MPM, showed that the primary endpoint, PFS, was not met.[47] Even with such an aggressive chemotherapy, OS for unresectable mesothelioma remains ≤12 months.[48]

Given the limitations in the efficacy of existing cytotoxic chemotherapy in MPM and recent advances in tumor immunology across various malignancies, ICIs have been investigated for the treatment of unresectable mesothelioma. A single-arm, Durvalumab with First-line Chemotherapy in Mesothelioma study examined treatment efficacy after adding durvalumab, a PD-L1 inhibitor, to CPPD/PEM, in 54 patients with untreated, unresectable MPM[49] (table 1). PFS (the primary endpoint) at 6 months was 57%, and the objective response rate (ORR) was 48%, with a median duration of response of 6.5 months. Immune-related adverse events of grade 3 and higher, occurred in eight patients (15%), including lipase elevation (n=1), pancreatitis (n=1) and renal impairment (n=1).

The Canadian Cancer Trials Group has launched a phase II/III study for unresectable MPM, to verify treatment efficacy following the addition of pembrolizumab, a PD-1 antibody, to the standard CPPD/PEM (NCT02784171) (table 2). The use of durvalumab as the first-line immunochemotherapy is also under evaluation, sponsored by PrECOG (NCT02899195). Japanese investigators are also conducting an exploratory phase II trial, using nivolumab combined with the standard CPPD/PEM, in patients with untreated, unresectable MPM.[50] Furthermore, a large-scale, randomized phase III study, the CheckMate 743 study is currently investigating the survival advantage of the nivolumab/ipilimumab combination immunotherapy, versus platinum/PEM, in 606 patients with untreated, unresectable MPM (NCT02899299).

Single-agent ICI therapy in the salvage setting

Although the salvage setting is discussed before advancements in the first-line setting, currently available agents in the salvage setting rarely work in MPM, with a median survival time (MST) of ≤6 months.[51] Vorinostat, a histone deacetylase inhibitor, was proven not to have any survival advantage in a placebo-controlled randomized phase III trial, the VANTAGE-014 trial,[52] without earlier trial result confirmation.

Thus far, four ICIs have been tested as an immunotherapy against relapsed tumors (table 1). A single-center, single-arm phase II study, the NivoMes trial, with single-agent nivolumab, an anti-PD-1 antibody showed that 16 (47%) of the 34 registered patients with recurrent MPM achieved disease control at 12 weeks (8 with

Table 1 Relevant trial results

Trial name	Year	Phase	RCT	Drug	Primary endpoint	No	PS 0–1	No of sarcomatoid histology	ORR	mPFS (mo)	MST (mo)	Pneumonitis*	Ref.
Frontline setting													
DREAM			No	Durvalumab	PFS, OR	54	100%	–	48%	6.9	–	NR	49
Salvage setting													
<Single agent>													
MERIT	2018	2	No	Nivolumab	OR	34	100%	3 (9%)	29%	6.1	17.3	6%	54
NivoMes	2018	2	No	Nivolumab	DCR	34	100%	2 (6%)	24%	2.6	11.8	12%	53
KN-028†,‡	2017	1b	No	Pembrolizumab	Safety	25	100%	2 (8%)	20%	5.4	18	NR	55
Chicago group	2018	2	No	Pembrolizumab	OR	65	100%	5 (8%)	19%	4.5	11.5	3%	56
JAVELIN	2019	1b	No	Avelumab	OR	53	100%	2 (4%)	9%	4.1	10.7	6%	57
Italian group	2013	2	No	Tremelimumab	OR	29	79%	3 (10%)	7%	6.2	10.7	NR	58
Italian group	2015	2	No	Tremelimumab	irOR	29	79%	1 (3%)	3%	–	–	NR	59
DETERMINE	2017	2b	Yes	Tremelimumab	OS	382	99%	22 (6%)	5%	2.8	7.7§	NR	60
				Placebo		189	100%	16 (8%)	1%	2.7	7.3	NR	
<Combination>													
NIBIT-MESO-1¶	2018	2	No	Tremelimumab/durvalumab	irOR	40	100%	2 (5%)	28%	5.7	16.6	NR	30
MAPS2	2019	2	Yes	Nivolumab/ipilimumab	DCR	62	98%	9 (15%)**	28%	5.6	15.9	2%	31
				Nivolumab		63	97%	11 (17%)**	19%	4.0	11.9	2%	
INITIATE	2019	2	No	Nivolumab/ipilimumab	DCR	34	100%	3 (9%)	29%	6.2	NR	NR	32

*Any grade.
†Those with the following conditions were eligible: (1) failed standard therapy and (2) unable to receive standard therapy.
‡Those with PD-L1-positive tumors were registered.
§OS-HR of 0.92 with a 95% CI 0.76 to 1.12.
¶Subjects who refused the first line platinum-based chemotherapy, or those with disease progression after a maximum of one line of platinum-based therapy, were eligible.
**Including biphasic histology.
DCR, disease control rate; DREAM, Durvalumab with First-Line Chemotherapy in Mesothelioma; irOR, immune-related objective response; MST, median survival time; NR, not reported; ORR, objective response rate; OS, overall survival; PD-L1, programmed cell death ligand 1; PFS, progression-free survival; RCT, randomized controlled trial.

Table 2 Ongoing relevant trials

Trial	Country	Phase	RCT	Regimen	Primary endpoint	No of planned pts	PS	Study start date	Registration no
Front-line setting									
Canadian group	Canada	2/3	Yes	Cis-pem±pembrolizumab	OS	126	0–1	07/10/16	NCT02784171
CM743	Global	3	Yes	Nivolumab/ipilimumab versus p-pem	OS	606	0–1	25/10/16	NCT02899299
PrE0505	USA	2	No	Cis-pem/durvalumab	OS	55	0–1	13/06/17	NCT02899195
JME-001	Japan	2	No	Cis-pem/nivolumab	OR	18	0–1	20/01/18	UMIN000030892
Salvage setting									
Confirm	UK	3	Yes	Nivolumab versus placebo	OS	336	0–1	28/03/17	NCT03063450

Cis-pem, cisplatin and pemetrexed; OS, overall survival; p-pem, platinum (cisplatin or carboplatin) and pemetrexed; PS, performance status; pts, patients; RCT, randomized controlled trial.

partial response (PR) and 8 with stable disease (SD)).[53] In this population, PD-L1 expression did not predict treatment responses. A Japanese single-arm phase II study, the MERIT study, also examined the efficacy and safety of nivolumab monotherapy in 34 patients with MPM with a history of prior chemotherapy.[54] The primary endpoint, ORR, was 29% (10/34), which was dependent on tumor PD-L1 expression, with an ORR of 40% and 8% when PD-L1 expression was ≥1% and <1%, respectively. The median PFS and MST were 6.1 and 17.3 months, respectively. Twenty-six patients (76%) experienced treatment-related adverse events (TRAEs). In essence, these results led to the approval of nivolumab in Japan for unresectable recurrent pleural mesothelioma.

A single-agent pembrolizumab, anti-PD-1 antibody trial (KEYNOTE-028) demonstrated that 5/25 (20%) of previously treated patients with MPM achieved PR, while 13 (52%) had SD, with no treatment-related deaths or discontinuations.[55] The Chicago group also conducted a pembrolizumab monotherapy phase II trial in 65 patients with pretreated mesothelioma.[56] Nineteen per cent of the patients achieved PR, without unexpected AEs. The ORR was associated with PD-L1 expression; 7%, 26%, and 31% in patients harboring tumors with PD-L1-expression level of <1%, 1%–49% and ≥50%, respectively. The study also showed a median PFS and OS of 4.5 and 11.5 months, respectively.

With avelumab, a human anti-PD-L1 IgG_1 antibody, a phase Ib monotherapy trial (JAVELIN) was conducted in 53 patients with pretreated malignant mesothelioma.[57] Despite the 9% response in the whole cohort, ORR seemed different, stratified by the PD-L1 expression level in patients with PD-L1-positive (19% (3 of 16)) vs PD-L1-negative tumors (7% (2 of 27)), considering a ≥5% PD-L1 cut-off. The median PFS was 4.1 months, whereas the MST extended to >10 months. Five patients (9%) had grades 3–4 TRAEs, without treatment-related deaths.

Tremelimumab, an anti-CTLA4 antibody, was also evaluated in a salvage setting. In Europe, two single-arm, phase II monotherapy trials showed preliminary efficacy, with an ORR of 3%–7%.[58 59] Following these trials, a randomized phase IIb study, the DETERMINE study, revealed that tremelimumab failed to significantly prolong OS compared with that of placebo, in 571 patients with previously treated malignant mesothelioma. The MST showed no difference between treatment groups, with 7.7 and 7.3 months in the tremelimumab and placebo arms, respectively (HR 0.92, 95% CI 0.76 to 1.12).[60]

ICI combination therapy in salvage settings

Given that enhanced immunogenicity can be achieved by combining PD1 or PDL1 and CTLA4 inhibitors,[3] several studies evaluating the combination of anti-CTLA-4 and anti-PD-[L]1 antibodies have been reported. A phase II study, the NIBIT-MESO-1 trial, investigated an ICI combination of tremelimumab and durvalumab for unresectable mesothelioma.[30] Subjects who had refused first-line platinum-based chemotherapy, or subjects with disease progression after a maximum of one line of platinum-based therapy, were enrolled. Eleven (28%) of 40 patients had an immune-related objective response. The median PFS and MST were 5.7 and 16.6 months, respectively. Baseline tumor PD-L1 expression did not correlate with the immune-related objective response, and seven patients (18%) had grades 3–4 TRAEs.

A combination therapy of nivolumab and ipilimumab, over nivolumab monotherapy, was examined in a randomized phase II trial (IFCT MAPS2).[31] A total of 125 patients with relapsed MPM were allocated to the combination therapy or monotherapy arm. Disease control rate (DCR), set as the primary endpoint, was 50% and 44%, whereas the ORR was 28% and 19%, respectively. As expected, the combination therapy had an increased risk of AE, with grades 3–4 of 26% and 14%, respectively. Three (5%) of 62 combination group patients had toxicities that led to death (hepatitis, encephalitis and acute kidney failure). When restricted to high PD-L1 tumors (>25%), either of

the regimens seemed effective, with ORRs of 63%–71% in the post hoc analyses.

Similar to this MAPS2 trial, a single-arm study, the INITIATE study,[32] evaluated the efficacy of nivolumab and ipilimumab in mesothelioma refractory to at least one line of platinum-based chemotherapy. Of the 34 patients included in efficacy assessment, 10 (29%) attained PR and 13 (38%) attained SD, resulting in a DCR (primary endpoint) of 68%. Despite the smaller-scale, non-randomized design, this study could reproduce the tolerance and efficacy results obtained from the MAPS2 trial. It also showed a relationship between tumor PD-L1 expression and the efficacy of this combination therapy.

Based on the aforementioned completed trials, several MPM trials are either ongoing or being initiated. The most pivotal is the one initiated by Cancer Research UK: a randomized, double blind placebo controlled CONFIRM trial of nivolumab versus placebo in patients with relapsed mesothelioma (NCT03063450). A total of 336 patients will be recruited from 25 institutes in the UK over a 4-year period. All patients will be treated for 12 months, except in situations of progress or withdrawal. It will be intriguing if this reproduces the Japanese MERIT study results.[54]

Overall, anti-PD-1 antibodies exhibited promising results when used alone as a salvage therapy after the first-line chemotherapy.[53–56]

Unresolved, unmet needs for MPM ICI therapy

Compared with clinical trials targeting other malignancies, the majority of prior MPM trials employed 'small-scale' and 'single-arm' designs, and their primary endpoints were set at only ORR or DCR. No clear survival advantage of ICI has been demonstrated through randomized trials. This is mainly because of the extremely small patient population, and mostly exploratory-type trials.[4] However, favorable responses and survival data could be observed across the studies, which are better than historical data. Considering the current limitations of treatment options in the salvage setting, ICI is now a potential rational and medically useful option for patients with unresectable, relapsed MPM, in the absence of any contraindications. Undoubtedly, well-designed randomized trials provide accurate and consistent data (ie, CONFIRM trial (NCT03063450); table 2). The accumulation of forthcoming relevant data through ongoing clinical trials is important for establishing better ICI use in daily practices.

Among toxicities induced by ICIs, pulmonary toxicity has to be properly managed, as it can be one of the most common causes of ICI-related death. The most common lung toxicity observed in patients receiving ICI treatment is pneumonitis.[61] In our review, as shown in table 1, it occurred in 2%–12% of the patients (median; 6%) in all the trials evaluating ICIs. This seemed almost consistent with that observed in other cancers. The patterns of onset and severity may also vary, and MPM often has characteristics of limited reserve in pulmonary function at the baseline. These findings suggest the importance of vigilance and rapid response. Thus, physicians still should recognize that the diagnosis of pneumonitis is particularly challenging and failure to detect and treat pneumonitis in a timely manner could lead to poor clinical outcomes.

Another unmet need is the identification of predictive biomarkers of ICI effects. Compared with other malignancies, progress in mesothelioma biomarker research is limited. Some of the single-arm ICI studies reveal the correlation between responses and higher PD-L1 expression. However, as insufficient survival data were generated, more established outcome data are needed to confirm the value of PD-L1 immunohistochemistry as a predictive biomarker for the OS effect. Recently, the tumor mutational burden (TMB) analysis using the whole exosome sequence has garnered attention in nivolumab therapy.[62] Moreover, in lung cancer, no association between TMB and PD-L1 expression was revealed.[62] Rather, a combination of them would be of value as a predictive biomarker. Nevertheless, only a few precise biomarkers for ICI efficacy assessments seem to exist in MPM clinical trials, besides PD-L1 expression. Further development of new biomarkers is also required for unresectable mesothelioma.

A majority of patients diagnosed with untreated, unresectable mesothelioma exhibit all expected symptoms at the initial presentation, and thus, do not meet the eligibility criteria to participate in clinical trials. Therefore, study results have to be interpreted cautiously, taking into consideration how each of them can be applied per in-care patient, during daily clinical practices.

In the future, more novel immunotherapy results will be made available, which could possibly lead to further drastic changes in unresectable MPM treatment. Our goal is to carefully evaluate any relevant information and deliver better patient treatment.

CONCLUSIONS

MPM prognosis has been poor with the standard platinum chemotherapy. Recently, in the salvage setting, anti-PD-1 antibodies yielded favorable ORR. Nivolumab is approved for use in Japan. Ongoing studies will further confirm the potential efficacy of ICIs for MPM, as observed across other malignancies. It is also crucial to identify any clinically useful predictive biomarkers that could reveal the ICIs with maximal effects in MPM.

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DRUG EVALUATION

Nivolumab for the treatment of unresectable pleural mesothelioma

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ABSTRACT

Introduction: Platinum-based chemotherapy is the current first-line standard therapy for unresectable malignant pleural mesothelioma (MPM). Recently, immune-checkpoint inhibitors (ICI) have been intensively investigated as treatment options for this disease. Nivolumab, an anti-programmed cell death (PD)-1 agent, was one of the first drugs used and is representative of available ICIs.

Areas covered: This review discusses previous relevant reports and current ongoing trials of nivolumab. The efficacy and safety of nivolumab have been investigated mostly in second-line or later treatment settings as both monotherapy and in combination with other ICIs. Particularly, nivolumab monotherapy yielded promising efficacy with an objective response rate of 29% and median overall survival of 17.3 months in salvage settings in the single-arm, Japanese phase 2 trial (MERIT). Notably, the study led to Japanese approval of nivolumab for unresectable recurrent MPM. Several trials with monotherapy or cotherapy with nivolumab have commenced, including randomized trials of nivolumab monotherapy vs. placebo in the salvage setting, and cotherapy with nivolumab and ipilimumab vs. the platinum doublet in the frontline setting.

Expert opinion: Nivolumab seems like a reasonable option for unresectable, relapsed MPM despite the lack of randomized trial data. Ongoing pivotal trials will confirm its efficacy.

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1. Introduction

Malignant pleural mesothelioma (MPM) is a rare and aggressive malignancy that occurs in the mesothelial surface of the pleural and peritoneal cavities, and the pericardium [1]. The disease is closely associated with asbestos exposure and approximately 80% of MPM cases are caused by occupational or environmental exposure [2–6]. Despite policies banning asbestos use in Western countries, MPM has continued to increase in many countries where asbestos is still extensively used. It is expected that 500,000 new cases of MPM will be diagnosed in men with occupational exposure in Europe alone [7]. The prognosis of MPM is poor, with a median survival time (MST) of 18 months and a 5-year overall survival (OS) rate of < 5% [8]. In particular, those with unresectable, advanced disease at the initial presentation characteristically have a worse prognosis than patients in earlier stages. This disappointing outcome is principally due to the lack of efficient screening methods and effective systemic therapy [9,10]. Therefore, innovative agents are urgently anticipated and required.

The role of peripheral immune tolerance with the co-inhibitory immune-checkpoint molecules cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) and its ligand (PD-L1) have been extensively investigated. PD-L1 is a transmembrane protein that binds to PD-1 and is expressed on cytotoxic T cells and other immune cells [11,12]. Various types of tumor cells have been shown to exhibit upregulated PD-L1 expression levels, which enables them to escape the immune response and keep proliferating [11]. Based on this background knowledge, anti-CTLA4, PD-1, and PD-L1 antibodies have been widely developed against various advanced malignancies. In this review, among the available immune-checkpoint inhibitors (ICI), we specifically discuss nivolumab, which blocks the PD-1 receptor, focusing on relevant previous trial reports and ongoing trials of unresectable MPM both in the first-line and salvage settings.

2. Basic information on nivolumab

Nivolumab is a human monoclonal antibody (HuMAb; immunoglobulin G4 [IgG4]-S228P) that targets the PD-1 cluster of the CD279 cell surface membrane receptor [13,14] (See Box 1). Nivolumab is expressed in Chinese hamster ovary cells and is produced using standard mammalian cell culture and chromatographic purification technologies. The agent was approved for the treatment of several types of tumors in various countries including the United States of America and Japan in 2014 and the European Union in 2015.

The interaction of PD-1 with its ligands, PD-L1 and PD-L2, can be blocked by nivolumab, leading to enhanced T-cell proliferation and interferon (IFN-γ) release *in vitro* [15].

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The supplementary data for this article can be accessed here.

Nivolumab binds with high affinity to activated human T-cells expressing cell surface PD-1 and cynomolgus monkey PD-1. Through a mixed lymphocyte reaction, nivolumab enhances reproducible IFN-γ release in a concentration-dependent manner [16].

In a population pharmacokinetic model, the overall distributions of nivolumab exposure are comparable after treatment with either 3 mg/kg or 240 mg nivolumab. The predicted range of nivolumab exposure following a 240 mg fixed dose across a 35 to 160 kg weight range is maintained well below corresponding exposure to the well-tolerated 10 mg/kg biweekly dosage of nivolumab. That is why a flat dose has been adopted in more recent nivolumab clinical trials.

The clinical activity and safety of nivolumab have been evaluated in patients with various malignancies including melanoma, non-small cell lung cancer (NSCLC), renal cell carcinoma, classical Hodgkin lymphoma, urothelial carcinoma, and head and neck carcinoma as a monotherapy or in combination with chemotherapy, targeted therapies, and other immunotherapies. In contrast, in mesothelioma, the clinical establishment of nivolumab has progressed slowly mainly because of the extremely small patient population and the difficulty associated with their accrual into relevant trials.

3. Nivolumab in the first-line setting

Patients with unresectable disease are often treated with systemic cytotoxic chemotherapy not as a cure but for disease management. Currently, the doublet chemotherapy of cisplatin and pemetrexed, antifolates, is the standard regimen for patients with frontline, unresectable MPM [17], followed by the regular approval in NSCLC [18–25]. However, the efficacy of this regimen is limited, with an objective response rate (ORR) of up to 30–40%, and some cancer-related symptoms can be relieved with the therapy, while the median OS is approximately 1 year in this disease setting [26].

Platinum agents can enhance the effector immune response through modulation of PD-L1 [27]. The observed encouraging results might extend ICI use to first-line treatment of MPM, particularly in combination with the standard platinum-based chemotherapy. Based on this background knowledge, ICIs have been tested in untreated, unresectable mesothelioma. Unfortunately, to date, no nivolumab trials have been reported (Table 1), while the potential benefit of adding durvalumab, a PD-L1 inhibitor, to the cisplatin and pemetrexed standard regimen was tested in 54 patients with untreated, unresectable MPM [28]. The study showed promising results and the primary endpoint of progression-free survival (PFS) at 6 months was 57%, with an ORR of 48% and median duration of response of 6.5 months.

In parallel with this promising trial, in January 2018 we commenced a phase 2 trial of nivolumab as a third agent in combination with the standard chemotherapy of cisplatin and pemetrexed for untreated, unresectable MPM [29] (Table 2). The primary endpoint is centrally reviewed ORR, while the secondary endpoints are disease control rate (DCR), OS, PFS, and adverse events (AEs). This is an exploratory trial with a target enrollment of 18 Japanese patients with good performance status.

As a different approach, the survival advantage of frontline combination immunotherapy with nivolumab and ipilimumab over platinum and pemetrexed is currently under investigation in 606 patients with unresectable MPM. This is the industry-sponsored, large-scaled, randomized phase 3, CheckMate 743 study (NCT02899299), initiated in October, 2016, with an estimated completion date of 15 April 2022.

4. Single-agent nivolumab in the salvage setting

No systemic treatment has been proven effective for mesothelioma refractory to first-line platinum doublet therapy in randomized clinical trials. Although multiple systemic therapeutic options have been investigated, there has been little progress [30]. Cotherapy with vinorelbine or gemcitabine or rechallenge with platinum therapy is often chosen in clinical practice, but is rarely effective [31,32]. Therefore, this challenging situation has created the most reasonable clinical setting for developing new treatment strategies using ICIs.

Currently, four ICIs have been tested in the second-line or later setting, including nivolumab as a monotherapy or in combination with other ICIs. Single-agent nivolumab was evaluated

Table 1. Relevant nivolumab trial results in the salvage setting.

Trial	Year	Phase	RCT	Drug	Primary endpoint	No.	ORR	mPFS (mo)	MST (mo)	Ref.
MERIT	2018	2	No	Nivolumab	OR	34	29%	6.1	17.3	[34]
NivoMes	2018	2	No	Nivolumab	DCR	34	24%	2.6	11.8	[33]
MAPS2	2019	2	Yes	Nivolumab/ipilimumab	DCR	62	28%	5.6	15.9	[36]
				Nivolumab		63	19%	4.0	11.9	
INITIATE	2019	2	No	Nivolumab/ipilimumab	DCR	34	29%	6.2	NR	[37]

Abbreviations: RCT; randomized controlled trial, ORR; objective response rate, mPFS; median progression-free survival, MST; median survival time, DCR; disease control rate, OS; overall survival, NR; not reached.

Table 2. Ongoing relevant nivolumab trials.

Trial	Country	Phase	RCT	Setting	Regimen	Primary endpoint	No. of planned pts	Study start date	Registration No.
CM743	Global	3	Yes	Frontline	Nivolumab/ipilimumab vs. p-pem	OS	606	25/10/16	NCT02899299
JME-001	Japan	2	No	Frontline	cis-pem/nivolumab	OR	18	20/01/18	UMIN000030892
CONFIRM	UK	3	Yes	Salvage	Nivolumab vs. placebo	OS	336	28/03/17	NCT03063450

Abbreviations: RCT, randomized controlled trial; pts, patients; cis-pem, cisplatin and pemetrexed; p-pem, platinum (cisplatin or carboplatin) and pemetrexed; OS, overall survival; OR, objective response.

in a single-center, single-arm phase 2 trial (NivoMes) for patients with recurrent MPM [33]. The study revealed a DCR at 12 weeks, set as the primary endpoint, of 47% (16 of 34), including eight partial responders [33], while PD-L1 expression failed to predict responses in this population. The median PFS and MST were 2.6 and 11.8 months, respectively, and nine (26%) patients developed grade ≥ 3 treatment-related AEs, including gastrointestinal disorders and pneumonitis. The investigators documented that single-agent nivolumab had meaningful clinical efficacy and a manageable safety profile in previously treated patients with MPM.

Japanese investigators conducted the single-arm phase 2 MERIT study, assessing the efficacy of nivolumab monotherapy in 34 previously treated patients with pleural MPM [34]. The primary endpoint was centrally defined ORR while AEs, PFS, and OS were also evaluated. The ORR was 29% (10/34, 95% confidence interval [CI]: 16.846.2), which was clearly affected by PD-L1 expression level, with an ORR of 40 and 8% in PD-L1 ≥ 1% and <1%, respectively. The ORR also seemed to be differently stratified by histologic subtypes: 26%, 67%, and 25% for epithelioid, sarcomatoid, and biphasic histologies, respectively. The survival data were also favorable with median PFS and MST of 6.1 and 17.3 months, respectively while 26 patients (76%) experienced treatment-related AEs. The results of this study led the Japanese government to approve nivolumab monotherapy for unresectable recurrent MPM.

5. Combination nivolumab and anti-CTLA-4 antibody in the salvage setting

Assuming that combining ICIs can enhance their upregulation of tumor immunogenicity [35], the combination of an anti-CTLA-4 antibody with nivolumab was investigated in several clinical trials. A randomized phase 2 trial (IFCT MAPS2) evaluated the benefits of a combination of nivolumab and ipilimumab over nivolumab monotherapy in MPM progression after first-line or second-line pemetrexed and platinum-based treatments (Supplemental Figure 1) [36]. A total of 125 relapsed MPM patients were allocated to the cotherapy or monotherapy arm. The primary endpoint of disease control at 12 weeks in the first 108 patients was met in both groups: 27 (50%, 95% CI: 37–63) of 54 in the combination arm and 24 (44%, 95% CI: 31–58) of 54 patients in the monotherapy arm reached centrally assessed disease control at 12 weeks. The efficacy of both regimens was enhanced especially in high PD-L1-expressing tumors (> 25%), with an ORR of 63% to 71%. Sixteen (26%) of 61 patients in the combination arm and nine (14%) of 63 in the monotherapy arm had grade ≥ 3 toxicities, and the most frequent were hepatic injury, asthenia, and lipase increase. The authors concluded that nivolumab monotherapy or nivolumab plus ipilimumab cotherapy both showed promising activity in relapsed patients with malignant pleural mesothelioma, without unexpected toxicity.

In addition to the MAPS2 trial, the efficacy of nivolumab plus ipilimumab was also investigated in the single-arm, phase 2 INITIATE trial in patients with mesothelioma refractory to platinum-based chemotherapy [37]. The primary endpoint was also set as disease control at 12 weeks. Thirty-four patients were evaluable for the response assessment at 12 weeks, and 10 (29%) and 13 (38%) achieved partial response (PR) and stable disease (SD), respectively, resulting in a DCR of 68% (23/34, 95% CI: 50–83). Notably, this study showed similar safety and efficacy results to those of MAPS2 trial [36,37]. This study also showed the association of tumor PD-L1 expression with the efficacy of the cotherapy. The most common AEs were skin disorders, infusion-related reactions, and fatigue. Grade 3 treatment-related AEs were reported in 12 (34%) of the 35 patients.

Along with these reported trials, UK investigators have commenced a randomized, placebo controlled, double blind trial (CONFIRM) comparing nivolumab monotherapy with a placebo in the salvage setting (NCT03063450). The study will recruit 336 patients with mesothelioma who have a history of at least one prior line of treatment at 25 institutes in the UK over a 4-year period. All patients are to be treated for 1 year. The primary endpoint is set as OS while the secondary endpoints are ORR, safety, and patient-oriented outcome. The actual study start date was 28 March 2017, and the estimated study completion date will be July 2021.

6. Conclusion

We have reviewed clinical trial results and ongoing trials related to nivolumab therapy in unresectable MPM. In the frontline setting, the addition of nivolumab to standard cytotoxic chemotherapy is being investigated to overcome the current poor prognosis. With the expectation of enhancing tumor immunogenicity, the combination of anti-CTLA-4 antibody and nivolumab is also under investigation. In the salvage setting, the single-arm, phase 2 MERIT trial showed a favorable ORR of 29% [34], leading to the approval of nivolumab monotherapy in Japan. Other trials have also successfully demonstrated similar efficacy of this agent. Although, to date, no randomized trials have demonstrated a robust survival advantage of nivolumab over other therapies, ongoing pivotal trial may confirm its efficacy.

7. Expert opinion

Nivolumab has been extensively evaluated for efficacy and safety in treating unresectable MPM (Table 2) [33,34,36,37], similar to investigations conducted in other malignancies [38]. However, in contrast to trials of other tumors, MPM trials were often designed as single-arm studies with small sample sizes and OS or PFS was not set as the primary endpoint [33,34,36,37]. Thus, in terms of activity, it is still unknown whether nivolumab monotherapy possesses true survival advantage over other therapies because of the insufficient efficacy data.

However, the following critical points should be considered a focus: 1) single-agent pembrolizumab, another PD-1 antibody, also showed an ORR of approximately 20% with MST of 12 to 18 months; 2) no clearly effective agents are currently available in the salvage setting; and 3) the ORR in the MERIT study was better than that in studies of other malignancies (i.e. ORR of 19%–20% in the study of nivolumab monotherapy for recurrent NSCLC [39,40]). Thus, some, but not all patients could benefit substantially from anti-PD-1 antibodies in the

Box 1. Drug summary box.

Drug name	Nivolumab (OPDIVO)
Phase	Approved
Indication	OPTIVO is indicated for the second – or later-line treatment of mesothelioma by the Ministry of Health, Labor and Welfare of Japan.
Pharmacology description	See the previously published review article [35].
Route of administration	Intravenous infusion
Chemical structure	See the previously published review article [35].
Pivotal trial	The MERIT study [34], a single-arm, Japanese, phase II clinical trial of nivolumab in the treatment of patients with malignant pleural mesothelioma in the second- or third-line setting. The study showed nivolumab monotherapy showed activity. This directly led to the approval of nivolumab for mesothelioma treatment in Japan.

salvage setting. Moreover, based on the low incidence of mesothelioma, we assume that the approval based only on the results of single-arm phase II clinical trials is reasonable, making the agent available to more patients.

However, it is important to note that after approval, the activity of nivolumab should be cautiously reevaluated through post-market surveillance and relevant research with larger study populations. In addition, verification of the approval in large-scale randomized trials is essential, and it is worth paying special attention to the expected results of the CONFIRM trial (NCT03063450). Whether the Japanese MERIT study results would be reproduced by this trial is of great interest [34].

In addition, Mansfield and colleagues stressed the importance of using contemporaneous synthetic control groups to develop surrogate/predictive markers for efficacy [41]. Such an approach would herald the next potential trend of strategies for designing clinical trials of ICIs in the treatment of rare malignancies including mesothelioma.

Similarly, in other malignancies including melanoma, renal cell carcinoma, and NSCLC [42], cotherapy with nivolumab and ipilimumab may also have a potent survival advantage even in untreated, unresectable MPM. Consequently, the Checkmate 743 trial (NCT02899299) may directly change the existing treatment strategy in the frontline setting. Further accumulation of forthcoming relevant data is strongly needed to improve the use of ICIs in daily clinical practice. Ongoing relevant studies are currently strongly expected to further confirm the role of immunotherapy in several disease settings, in addition to MERIT study results, hopefully leading to changes in the current historical prognosis of mesothelioma.

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• **This pivotal ranodimized study clearly showed the survival advantage of combination therapy of nivolumab plus ipilimumab over the standard sunitinib monotherapy.**

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Commentary

An appropriate choice for immunotherapy in malignant pleural mesothelioma

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In this article of *EBioMedicine*, Mankor and colleagues [1] report the results of immune monitoring of peripheral blood immune cell subsets in patients with malignant pleural mesothelioma (MPM) treated with so-called immune checkpoint inhibitors (ICIs). Combination treatment with anti-PD-1/anti-CTLA-4 antibodies induced an increase in the proliferation and activation of T cells. In addition, patients who responded to the combination treatment had low frequencies of naïve CD8 T cells and high frequencies of effector memory CD8 T cells expressing cytokines, such as granzyme-B and interferon-γ. These findings suggest that immune monitoring of peripheral blood immune cell subsets may provide information for predicting clinical benefit from ICI-ICI combination therapy.

MPM is strongly associated with asbestos exposure and has continued to increase in many developing countries. The combination of platinum and pemetrexed is considered a standard regimen, but median survival is approximately 1 year [2]. There is no established treatment option once cases are refractory or intolerable to the regimen. The immunosuppressive tumor microenvironment in MPM suggests that patients may benefit from this kind of immunotherapy. In recent years, some encouraging results of ICIs have been reported for MPM. In a Japanese single-arm phase II study examining the efficacy and safety of nivolumab monotherapy, the primary endpoint, objective response rate, was 29%, and the median progression-free and overall survival were 6.1 and 17.3 months, respectively [3]. These results led to the approval of nivolumab in Japan for unresectable recurrent MPM. However, the efficacy of anti-PD-1 antibody has not been established in randomised clinical studies.

Recently, the combination of nivolumab and ipilimumab was demonstrated to significantly improve overall survival compared to standard chemotherapy in the Checkmate-743 study [4]. An important clinical issue is to determine which patients can expect a response or unacceptable toxicity, as not all patients could benefit from the treatment, and some specific adverse events have been reported for the ICI-ICI combination. Some studies have revealed the correlation between responses and higher PD-L1 expression. In MPM, however, more established outcome data are needed to confirm the value of PD-L1 expression as a predictive biomarker. The tumor mutation burden and tumor microenvironment are associated with the response to ICIs in some neoplasms, but their roles as biomarkers have not been shown in MPM.

In this study, Mankor and colleagues show that patients who respond to combination treatment with nivolumab and ipilimumab have low frequencies of naïve CD8 T cells and high frequencies of cytokine-expressing effector memory CD8 T cells. A strength of this monitoring is that it can be performed before treatment induction. Notably, there are some limitations in this study, including a limited number of responding patients. However, the findings suggest that immune monitoring of peripheral blood immune cell subsets may act as a biomarker predicting a clinical benefit from ICI combination therapy. A prospective study with more subjects should be planned to validate these findings. In addition, basic or translational research to identify the mechanisms of action of T cells and cytokines against mesothelioma cells is warranted.

As a future perspective, the combination of an anti-PD-1 or anti-PD-L1 antibody and conventional chemotherapy is also under investigation. Nowak et al. recently presented favorable results from a phase II trial testing durvalumab, an anti-PD-L1 antibody, combined with cisplatin/pemetrexed in MPM [5]. A large-scale randomised study for testing the combination of pembrolizumab, another anti-PD-1 antibody, and cisplatin/pemetrexed is also ongoing. Platinum agents can enhance the effector immune response through modulation of PD-L1 [6]. Further development of new biomarkers to determine patients who would benefit from ICI-ICI combinations, ICI plus chemotherapy, or conventional chemotherapy is also needed.

A new era in systemic chemotherapy for MPM has just begun. Immune monitoring would be the key to choosing appropriate treatments.

Contributors

Dr Fujimoto wrote the commentary.

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First-line nivolumab plus ipilimumab in unresectable malignant pleural mesothelioma (CheckMate 743): a multicentre, randomised, open-label, phase 3 trial

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Summary

Background Approved systemic treatments for malignant pleural mesothelioma (MPM) have been limited to chemotherapy regimens that have moderate survival benefit with poor outcomes. Nivolumab plus ipilimumab has shown clinical benefit in other tumour types, including first-line non-small-cell lung cancer. We hypothesised that this regimen would improve overall survival in MPM.

Methods This open-label, randomised, phase 3 study (CheckMate 743) was run at 103 hospitals across 21 countries. Eligible individuals were aged 18 years and older, with previously untreated, histologically confirmed unresectable MPM, and an Eastern Cooperative Oncology Group performance status of 0 or 1. Eligible participants were randomly assigned (1:1) to nivolumab (3 mg/kg intravenously once every 2 weeks) plus ipilimumab (1 mg/kg intravenously once every 6 weeks) for up to 2 years, or platinum plus pemetrexed chemotherapy (pemetrexed [500 mg/m² intravenously] plus cisplatin [75 mg/m² intravenously] or carboplatin [area under the concentration-time curve 5 mg/mL per min intravenously]) once every 3 weeks for up to six cycles. The primary endpoint was overall survival among all participants randomly assigned to treatment, and safety was assessed in all participants who received at least one dose of study treatment. This study is registered with ClinicalTrials.gov, NCT02899299, and is closed to accrual.

Findings Between Nov 29, 2016, and April 28, 2018, 713 patients were enrolled, of whom 605 were randomly assigned to either nivolumab plus ipilimumab (n=303) or chemotherapy (n=302). 467 (77%) of 605 participants were male and median age was 69 years (IQR 64–75). At the prespecified interim analysis (database lock April 3, 2020; median follow-up of 29·7 months [IQR 26·7–32·9]), nivolumab plus ipilimumab significantly extended overall survival versus chemotherapy (median overall survival 18·1 months [95% CI 16·8–21·4] *vs* 14·1 months [12·4–16·2]; hazard ratio 0·74 [96·6% CI 0·60–0·91]; p=0·0020). 2-year overall survival rates were 41% (95% CI 35·1–46·5) in the nivolumab plus ipilimumab group and 27% (21·9–32·4) in the chemotherapy group. Grade 3–4 treatment-related adverse events were reported in 91 (30%) of 300 patients treated with nivolumab plus ipilimumab and 91 (32%) of 284 treated with chemotherapy. Three (1%) treatment-related deaths occurred in the nivolumab plus ipilimumab group (pneumonitis, encephalitis, and heart failure) and one (<1%) in the chemotherapy group (myelosuppression).

Interpretation Nivolumab plus ipilimumab provided significant and clinically meaningful improvements in overall survival versus standard-of-care chemotherapy, supporting the use of this first-in-class regimen that has been approved in the USA as of October, 2020, for previously untreated unresectable MPM.

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See Comment page 348

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Introduction

Malignant pleural mesothelioma (MPM) is a highly aggressive cancer and typically unresectable at diagnosis, with less than 10% of patients surviving 5 years or beyond.[1,2] Historically, age, sex, tumour grade and stage, and histology have been shown to be independent prognostic factors. Notably, worse prognosis has been reported for non-epithelioid histology versus the epithelioid subtype.[1–3] Until October, 2020, platinum agents plus folate antimetabolites, such as pemetrexed, have been the only approved first-line treatment regimens for MPM since 2004.[4,5] However, long-term survival outcomes remain poor with chemotherapy;[6–9] bevacizumab has been added to these regimens[10] but its use varies across regions. As such, there is an urgent need for new and effective therapeutic options.

Nivolumab, a fully human anti-programmed cell death 1 (PD-1) antibody, and ipilimumab, a fully human anti-cytotoxic T-lymphocyte 4 (CTLA-4) antibody are immune checkpoint inhibitors with distinct but

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Research in context

Evidence before this study

We searched PubMed and abstracts from major oncology congresses for studies published from database inception until Oct 2, 2020, relevant to unresectable malignant pleural mesothelioma (MPM) and cancer immunotherapy regimens, with a focus primarily on first-line phase 3 trials, using search terms that included, but were not limited to ("mesothelioma" AND "nivolumab") OR "chemotherapy" OR "pembrolizumab" OR "atezolizumab" OR "avelumab" OR "durvalumab" OR "ipilimumab" OR "tremelimumab" OR "PD-1" OR "PD-L1" OR "CTLA-4" (full names and abbreviations). Although we identified several studies assessing immunotherapy in MPM, we found no published randomised phase 3 studies investigating the efficacy or safety of immunotherapy regimens in the first-line setting. Various phase 1 and 2 studies in previously treated patients with MPM have suggested that immunotherapy regimens might provide clinical benefit. Notably, the multicentre, open-label, single-arm, phase 2 MERIT study led to the approval of nivolumab monotherapy for unresectable recurrent MPM in Japan. However, with recommended first-line systemic treatments limited to chemotherapy since 2004, with or without bevacizumab, there remains a need for new and effective therapeutic options. In the single-arm phase 2 DREAM study, first-line durvalumab plus chemotherapy exhibited promising activity in 54 patients with MPM, but the combination requires evaluation in a larger, randomised, phase 3 study. CheckMate 743 was designed to investigate the efficacy and safety of nivolumab plus ipilimumab versus chemotherapy. A previous non-comparative phase 2 trial (MAPS2) and single-arm phase 2 study (INITIATE) assessing nivolumab plus ipilimumab in MPM showed that this regimen was tolerable and exhibited encouraging clinical activity.

Added value of this study

Here we provide results from the randomised CheckMate 743 study, which is the first phase 3 study to show significant and clinically meaningful improvements in overall survival with immunotherapy versus standard-of-care platinum plus pemetrexed chemotherapy for first-line treatment of unresectable MPM. This regimen was found to show clinical benefit and tolerability, thus providing patients with a new first-line chemotherapy-free treatment option. Notably, survival with nivolumab plus ipilimumab was similar in patients with both non-epithelioid and epithelioid histologies, suggesting that the regimen could be considered for all patients with unresectable MPM. Responses were durable, with a 2-year duration of response rate of 32% of immunotherapy-treated patients. The safety profile of nivolumab plus ipilimumab was consistent with that observed in first-line non-small-cell lung cancer at this dose and schedule and no new safety signals were reported.

Implications of all the available evidence

Nivolumab plus ipilimumab can provide notable and clinically meaningful improvements in overall survival versus the current standard of care. Data from CheckMate 743 support a favourable clinical benefit–risk profile for nivolumab plus ipilimumab. Nivolumab plus ipilimumab is now indicated in the USA and Brazil as a first-line treatment for unresectable MPM.

complementary mechanisms of action. Ipilimumab induces T-cell proliferation and de-novo anti-tumour T-cell responses, including in memory T cells, whereas nivolumab restores the function of existing anti-tumour T cells.[11] Nivolumab plus ipilimumab is approved in various tumours[12] and has shown durable overall survival benefit in melanoma,[13] renal cell carcinoma,[14] and in non-small-cell lung cancer (NSCLC).[15] Furthermore, National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology (NCCN guidelines) recommend nivolumab with or without ipilimumab as a preferred treatment option (category 2A) in second-line or later MPM settings based on results from three phase 2 trials,[16–18] including the multicentre, open-label, randomised, non-comparative IFCT-1501 MAPS2 trial that showed encouraging clinical activity of the combination therapy.[16]

CheckMate 743 is a phase 3 study designed to assess efficacy and safety of first-line nivolumab plus ipilimumab versus platinum plus pemetrexed chemotherapy in unresectable MPM. Here we present results from the prespecified interim analysis, which has led to nivolumab plus ipilimumab gaining approval in the USA.[12] Additionally, NCCN guidelines recommend nivolumab plus ipilimumab as a preferred first-line option (category 2A) for patients with biphasic or sarcomatoid histology and is also an option for those with epithelioid histology.

For the **most recent and complete version of the NCCN guidelines** see NCCN.org

See Online for appendix

Methods

Study design and participants

CheckMate 743 is a global, open-label, randomised, controlled, phase 3 study run at 103 hospitals across 21 countries (appendix pp 2–4, 22). Eligible patients were aged 18 years or older with histologically confirmed unresectable MPM that was not amenable to curative therapy (surgery with or without chemotherapy), and an Eastern Cooperative Oncology Group performance status of 0 or 1.[19] Unresectability of the disease was determined by the investigator at individual sites using local standards. Patients must have completed any previous palliative radiotherapy 2 weeks or longer before initiating study treatment, with no residual signs of toxicity, and have measurable disease according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST)[20] for pleural mesothelioma. Patients without measurable pleural lesions but with metastatic non-pleural lesions

measurable per RECIST version 1.1 could be considered for inclusion after consultation with the study's medical monitor. Patients were required to have tumour samples available for programmed cell death ligand 1 (PD-L1) testing. Baseline laboratory tests required to assess eligibility included white blood cell counts, neutrophils, platelets, haemoglobin, serum creatinine, alanine aminotransferase, aspartate aminotransferase, and total bilirubin (appendix p 6).

Exclusion criteria included brain metastases (unless resected or treated with stereotactic radiotherapy and asymptomatic with no evolution within 3 months before study inclusion), autoimmune disease, and previous treatment with drugs targeting T-cell costimulation or checkpoint pathways. Patients were excluded if they presented with primitive peritoneal, pericardial, tunica vaginalis, or testis mesotheliomas. Other exclusion criteria included inadequate haematological, renal, or hepatic function; known HIV infection; or interstitial lung disease that was either symptomatic or might affect the detection or management of suspected drug-related pulmonary toxicity. Patients with current or previous malignancy with less than 3 years of complete remission (except for non-melanoma skin cancers and in-situ cancers) requiring or likely to require concurrent intervention during the study period were ineligible, as were patients requiring systemic corticosteroids (>10 mg daily prednisone or equivalent) or immunosuppressive medication within 14 days of the first dose of study drug. More detail on eligibility criteria are in the appendix (p 5) and study protocol (appendix pp 27–410).

An institutional review board or independent ethics committee at each study centre approved all versions of the protocol. An independent Data Monitoring Committee provided general oversight of efficacy and safety for the trial. The trial was done in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. All patients provided written informed consent.

Randomisation and masking

Patients were enrolled and randomly assigned (1:1) using an interactive web response system, stratified by sex and histology (epithelioid *vs* non-epithelioid [including sarcomatoid and mixed subtypes]) to nivolumab plus ipilimumab or platinum plus pemetrexed chemotherapy. The trial was open label and so patients and investigators were not masked to treatment assignment.

Procedures

Participants in both treatment groups were pretreated with folic acid (350–1000 µg orally daily) and vitamin B12 (1000 µg intramuscularly) 1 week before administration of the first dose of study drug (appendix p 5). Participants in the experimental group were given nivolumab (3 mg/kg intravenous infusion once every 2 weeks) plus ipilimumab (1 mg/kg intravenous infusion once every 6 weeks). Nivolumab was administered first, followed by ipilimumab. Participants in the chemotherapy group were given an intravenous infusion of cisplatin (75 mg/m²) or carboplatin (area under the concentration-time curve 5 mg/mL per min) plus pemetrexed (500 mg/m²) every 3 weeks for a maximum of six cycles. Treatment was continued until disease progression, unacceptable toxicity, or for 2 years for immunotherapy. Treatment with nivolumab plus ipilimumab was permitted beyond disease progression if prespecified requirements were met (appendix p 7). Dose reductions were permitted for chemotherapy, but not for nivolumab or ipilimumab; concomitant use of corticosteroids was permitted. Patients could receive subsequent therapy upon the discontinuation of study treatment in either group at the discretion of the investigator.

Tumour assessments were done 6 weeks after the date of the first dose of study drug and then every 6 weeks for the first 12 months. After 12 months, tumours were assessed every 12 weeks until blinded independent central review (BICR) confirmed disease progression per mRECIST or RECIST version 1.1 criteria, or both. At the time of investigator-assessed initial radiographic progression, the site had to request the blinded independent central review of progression from a third-party radiology vendor (E-research Technologies in St Louis, MO, USA); if progression was not confirmed, treatment could continue.

Adverse events were assessed at baseline and continuously throughout the study and during follow-up. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.0). Select adverse events consisted of a list of preferred terms with potential immune aetiology grouped by specific category (gastrointestinal adverse events, pulmonary adverse events, renal adverse events, hepatic adverse events, skin adverse events, infusion reactions, and endocrinopathies). The definition for serious adverse events is in the appendix (p 6). Treatment-related adverse events were defined as those reported between the first dose of study drug and 30 days after the last dose of study drug. According to study sponsor practice, only events that led to death within 24 h were documented as grade 5 events and reported as deaths here. Events leading to death more than 24 h after onset are reported with the worst grade before death.

Tumour histology was determined by individual sites using local protocols. Archival or fresh formalin-fixed paraffin-embedded tumour samples were collected before randomisation. Optional on-treatment fresh tumour samples were collected at weeks 6–8 and at disease progression, at the discretion of the investigator. Samples were sent to a central laboratory (Cancer Genetics, Rutherford, NJ, USA, and for patients in China, PD-L1 testing was done at Covance, Shanghai) to

determine the proportion of tumour cells showing plasma membrane PD-L1 staining of any intensity using the validated immunohistochemical 28-8 pharmDx assay (Dako, Carpinteria, CA, USA).

Laboratory tests were done within 14 days before randomisation and within 3 days before each dose. Full details of all assessments done are in the appendix (p 6). Hepatitis C RNA and HIV (where locally mandated) tests were done at screening only. All tests had to be done at follow-up visits 1 and 2.

Outcomes

The primary endpoint was overall survival in all patients randomly assigned to treatment after the US Food and Drug Administration provided guidance to change progression-free survival from a coprimary endpoint to a secondary endpoint (protocol amendment April 25, 2019; appendix p 7).[21] Overall survival was defined as the time from randomisation to the date of death due to any cause. Secondary endpoints were progression-free survival, objective response rate, time to response, duration of response, and disease control rate (radiographic tumour assessments per adapted mRECIST for pleural lesisons and RECIST [version 1.1] for the other lesions by BICR) in all patients randomly assigned to treatment, as well as overall survival, progression-free survival, and objective response rate by PD-L1 expression.

Progression-free survival was defined as the time from randomisation to the date of the first documented tumour progression or death due to any cause. Participants who died were considered to have progressed on the date of death. Participants who received subsequent therapy without previous reported progression were considered to have progressed on the date of death or were censored at the date of last evaluable tumour assessment before or on initiation of subsequent therapy. Objective response rate was defined as the proportion of patients with a best overall response of partial response or complete response and disease control rate was defined as the proportion of patients with a best overall response of complete response, partial response, or stable disease. Duration of response was defined as the time between the date of first response to the date of the first documented tumour progression, or death due to any cause, whichever occurred first.

Exploratory endpoints included safety and tolerability in all treated patients. Analysis of other exploratory endpoints that are ongoing but not reported here include pharmacokinetics, biomarkers, patient-reported outcomes, and immunogenicity; a full list is in the appendix (pp 119–122).

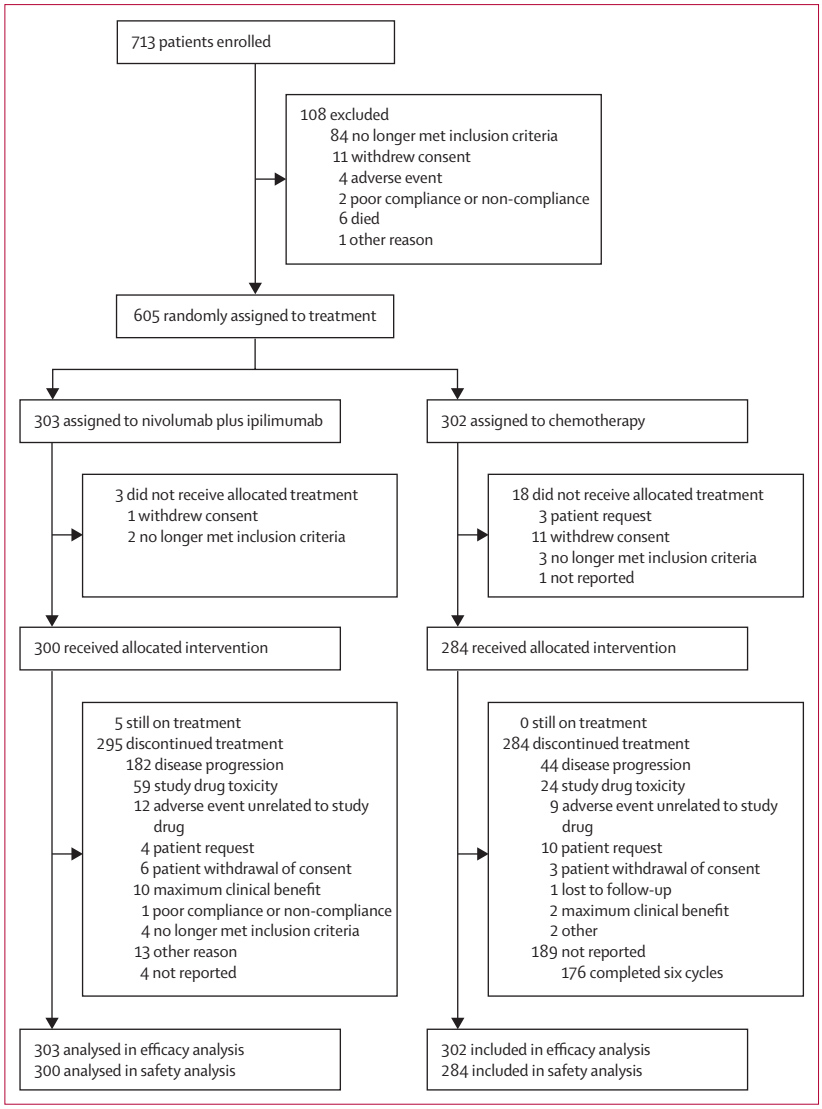


Figure 1: **Trial profile**

Statistical analysis

For the primary endpoint of overall survival, a sample of approximately 600 patients randomly assigned to treatment with 473 deaths would provide 90% power to detect a target hazard ratio (HR) of 0·72 with a two-sided type 1 error of 0·05, by means of a log-rank test. One prespecified interim analysis of overall survival was planned for superiority at approximately 403 deaths (85% of total anticipated events). At the time of database lock for the interim analysis, 419 patients had died (89% of total anticipated events); the boundary for declaring superiority for overall survival was a p value of less than 0·0345, based on the Lan-DeMets alpha spending function with O'Brien-Fleming boundaries. None of the secondary endpoints were included in the testing procedure; therefore, we did no formal statistical testing or allocation of alpha values for progression-free survival, objective response rate, and disease control rate.

We included all patients randomly assigned to treatment in demographic and efficacy analyses. We stratified analyses for overall survival and progression-free survival by sex and histology. We estimated HRs

and CIs (96·6% CIs for the overall survival primary analysis [alpha adjusted for interim analysis], and 95% CI elsewhere) using a stratified Cox proportional hazards model with treatment group as a single covariate. We checked the proportional hazards assumption only for the primary endpoint of overall survival by adding a time-dependent covariate, defined by treatment-by-time interaction, into the stratified Cox regression model of overall survival. We estimated survival curves and rates using the Kaplan-Meier method. We calculated exact two-sided 95% CIs for objective response and disease control rates using the Clopper-Pearson method. We did prespecified descriptive subgroup analyses for overall survival, summarised using HRs (with 95% CIs) calculated using an unstratified Cox proportional hazards model. Safety analyses included all patients who received at least one dose of study drug. We also did exposure adjusted safety analyses, taking into account all on-treatment events on the basis of the total exposure time. We calculated the person-year exposure as the sum over the participants' exposure expressed in years. More details on all analyses are in the appendix (pp 7–8).

We did all statistical analyses using SAS software (version 9.2). An independent Data Monitoring Committee reviewed efficacy and safety data on a periodic basis and at the time of the preplanned interim analysis. This trial is registered with ClinicalTrials.gov, NCT02899299.

Role of the funding source

The study was designed by the funder (Bristol Myers Squibb) and study steering committee. The funder had a role in data collection with the investigators, data analysis and interpretation in collaboration with the authors, and the writing of the report by funding professional medical writing assistance. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

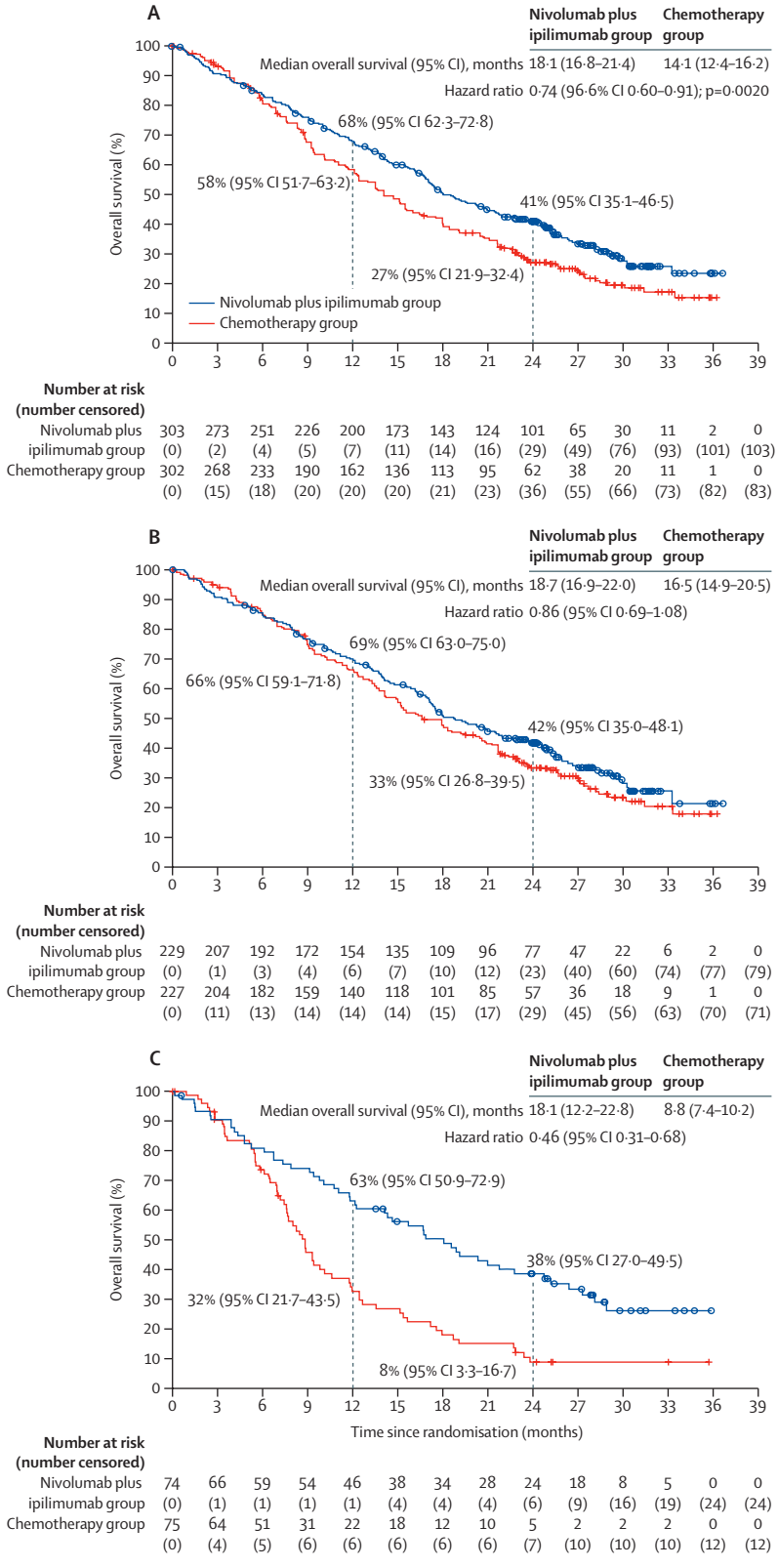
Between Nov, 29, 2016, and April 28, 2018, we enrolled 713 patients, of whom 605 were eligible and randomly assigned to nivolumab plus ipilimumab (n=303) or chemotherapy (n=302). 300 participants in the nivolumab plus ipilimumab group and 284 in the chemotherapy group received at least one dose of study drug (figure 1). At the prespecified interim analysis (database lock April 3, 2020), the median follow-up for overall survival was 29·7 months (IQR 26·7–32·9), with a minimum of 22·1 months. Baseline characteristics were well balanced between treatment groups (table 1). 467 (77%) of 605 participants were male and median age was 69 years (IQR 64–75). Overall, 456 (75%) of 605 patients had epithelioid tumour histology.

As of database lock, five (2%) of 300 patients in the nivolumab plus ipilimumab group who received treatment remained on treatment and no patients in the chemotherapy group remained on treatment (figure 1). The main reasons for treatment discontinuation in the nivolumab plus ipilimumab group were disease progression (182 [61%] of 300) and study drug toxicity

	Nivolumab plus ipilimumab group (n=303)	Chemotherapy group (n=302)
Age, years	69 (65–75)	69 (62–75)
<65	71 (23%)	96 (32%)
≥65 to <75	154 (51%)	127 (42%)
≥75	78 (26%)	79 (26%)
Sex		
Male	234 (77%)	233 (77%)
Female	69 (23%)	69 (23%)
Region		
North America	32 (11%)	27 (9%)
Europe	177 (58%)	175 (58%)
Asia	26 (9%)	39 (13%)
Rest of the world*	68 (22%)	61 (20%)
Eastern Cooperative Oncology Group performance status†		
0	114 (38%)	128 (42%)
1	189 (62%)	173 (57%)
Smoking status		
Current or former	173 (57%)	171 (57%)
Never	127 (42%)	122 (40%)
Unknown	3 (1%)	9 (3%)
Histology		
Epithelioid	229 (76%)	227 (75%)
Non-epithelioid	74 (24%)	75 (25%)
Sarcomatoid	35 (12%)	36 (12%)
Mixed or other	39 (13%)	39 (13%)
Stage		
1	12 (4%)	20 (7%)
2	23 (8%)	22 (7%)
3	103 (34%)	106 (35%)
4	160 (53%)	149 (49%)
Not reported	5 (2%)	5 (2%)
Previous cancer therapy		
Radiotherapy‡	29 (10%)	28 (9%)
Systemic therapy§	1 (<1%)	0
PD-L1 status		
Quantifiable	289 (95%)	297 (98%)
<1%¶	57/289 (20%)	78/297 (26%)
≥1%¶	232/289 (80%)	219/297 (74%)

Data are median (IQR) or n (%). PD-L1=programmed cell death ligand 1. *Includes Australia, Brazil, Chile, and South Africa. †On a score of 0 to 5, with higher scores indicating greater disability. One patient in the chemotherapy group had a baseline Eastern Cooperative Oncology Group performance status of 2 (protocol deviation). ‡Previous radiotherapy was provided for palliative support, pain management, or prophylactic track irradiation for tumour biopsy. §Due to incorrect data entry, one patient was reported as having previous systemic cancer therapy in the nivolumab plus ipilimumab group. ¶Calculated as a proportion of quantifiable patients.

Table 1: **Baseline characteristics**



(59 [20%]); 25 (8%) of 300 patients completed 2 years of immunotherapy. During the study, one patient in the nivolumab plus ipilimumab group discontinued study drug but received subsequent therapy from the investigator before BICR confirmation of disease progression. In the chemotherapy group, 176 (62%) of 284 patients completed all six cycles; 44 (16%) discontinued due to disease progression and 24 (8%) due to study drug toxicity. Median duration of treatment was 5·6 months (IQR 2·0–11·4) in the nivolumab plus ipilimumab group and 3·5 months (IQR 2·7–3·7) in the chemotherapy group (appendix p 9). The median number of nivolumab doses received was 12·0 (IQR 5·0–23·5) and of ipilimumab was 4·0 (2·0–7·0). After randomisation, 104 (34%) of 302 patients in the chemotherapy group were given cisplatin and 180 (60%) were given carboplatin; 29 (28%) of 104 patients given cisplatin switched to carboplatin after the first dose due to investigator decision. The median number of doses of cisplatin was 5·0 (IQR 3·0–6·0), of carboplatin was 6·0 (4·0–6·0), and of pemetrexed was 6·0 (4·0–6·0). Further information on treatment exposure is in the appendix (pp 9–10).

In the nivolumab plus ipilimumab group, 134 (44%) of 303 patients were given subsequent systemic therapy, ten (3%) were given subsequent immunotherapy, and 131 (43%) were given subsequent chemotherapy. In the chemotherapy group, 123 (41%) of 302 patients were given subsequent systemic therapy, 61 (20%) were given subsequent immunotherapy, and 95 (31%) were given subsequent chemotherapy (appendix p 11).

The study met its primary endpoint at the prespecified interim analysis according to the recommendation of the independent Data Monitoring Committee. Given that the study was able to reject the null hypothesis at the interim analysis, this analysis is considered final. Median overall survival was 18·1 months (95% CI 16·8–21·4) with nivolumab plus ipilimumab versus 14·1 months (12·4–16·2) with chemotherapy, with a stratified HR of 0·74 (96·6% CI 0·60–0·91; p=0·0020; figure 2). The p value for the time-dependent covariate was 0·9646, indicating that there was no evidence of a non-constant treatment effect over time. Overall survival rates at 1 year were 68% (95% CI 62·3–72·8) versus 58% (51·7–63·2) and at 2 years were 41% (35·1–46·5) versus 27% (21·9–32·4). Overall survival was similar between chemotherapy regimens: median overall survival was 13·7 months (95% CI 11·8–17·9) with pemetrexed plus cisplatin, and 15·0 months (12·2–17·9) with pemetrexed plus carboplatin (appendix p 25). Overall survival favoured nivolumab plus ipilimumab across most

Figure 2: **Overall survival in all randomised patients (A) and in patients with epithelioid tumour histology (B) and non-epithelioid tumour histology (C)**
The hazard ratio in part A is stratified by sex and histology. The hazard ratios in parts B and C are from unstratified Cox proportional hazard models.

subgroups, although survival in patients aged 75 years and older (n=157) was similar between treatment groups (figure 3). Notably, overall survival was improved with nivolumab plus ipilimumab versus chemotherapy regardless of histology (study stratification factor; figure 2). We found some evidence of higher treatment effect in patients with non-epithelioid histology (HR 0·46 [95% CI 0·31–0·68]) than in those with the epithelioid subtype (0·86 [0·69–1·08]). Median overall survival with nivolumab plus ipilimumab was similar between non-epithelioid and epithelioid subtypes (18·1 months [95% CI 12·2–22·8] *vs* 18·7 months [16·9–22·0]), as were 2-year overall survival rates (38% [95% CI 27·0–49·5] *vs* 42% [35·0–48·1]). By contrast, median overall survival with chemotherapy differed substantially between non-epithelioid and epithelioid subtypes (8·8 months [95% CI 7·4–10·2] *vs* 16·5 months [14·9–20·5]), as did 2-year overall survival rates (8% [95% CI 3·3–16·7] *vs* 33% [26·8–39·5]). Overall survival benefit by tumour PD-L1 expression level for nivolumab plus ipilimumab versus chemotherapy was greater in patients with tumour expression of PD-L1 of 1% or higher (HR 0·69 [95% CI 0·55–0·87]) than in patients with expression of less than 1% (0·94 [0·62–1·40]; figure 3; appendix pp 23–24). Nonetheless, median overall survival with nivolumab plus ipilimumab was similar in patients with tumours with PD-L1 expression of 1% or higher (18·0 months [95% CI 16·8–21·5]) and of less than 1% (17·3 months [95% CI 10·1–24·3]); 1-year survival rates were 70% (95% CI 63·4–75·3) and 59% (45·5–70·9); and 2-year survival rates were 41% (34·3–47·2) and 39% (25·9–51·3; appendix pp 23–24). Conversely, median overall survival with chemotherapy differed between patients with PD-L1 expression of 1% or higher (13·3 months [95% CI 11·6–15·4]) and less than 1% (16·5 months [13·4–20·5]); 1-year survival rates were 55% (95% CI 48·2–61·8) and 64% (52·3–73·9); and 2-year survival rates were 28% (22·1–34·7) and 25% (15·5–35·0; appendix pp 23–24).

The minimum follow-up for progression-free survival was 19·8 months. Median progression-free survival was similar between treatment groups: 6·8 months (95% CI 5·6–7·4) with nivolumab plus ipilimumab and 7·2 months (95% CI 6·9–8·0) with chemotherapy (HR 1·00 [95% CI 0·82–1·21]). However, progression-free survival rates at 2 years were numerically greater with nivolumab plus ipilimumab (16% [95% CI 11·7–21·5]) versus chemotherapy (7% [4·0–11·7]; figure 4).

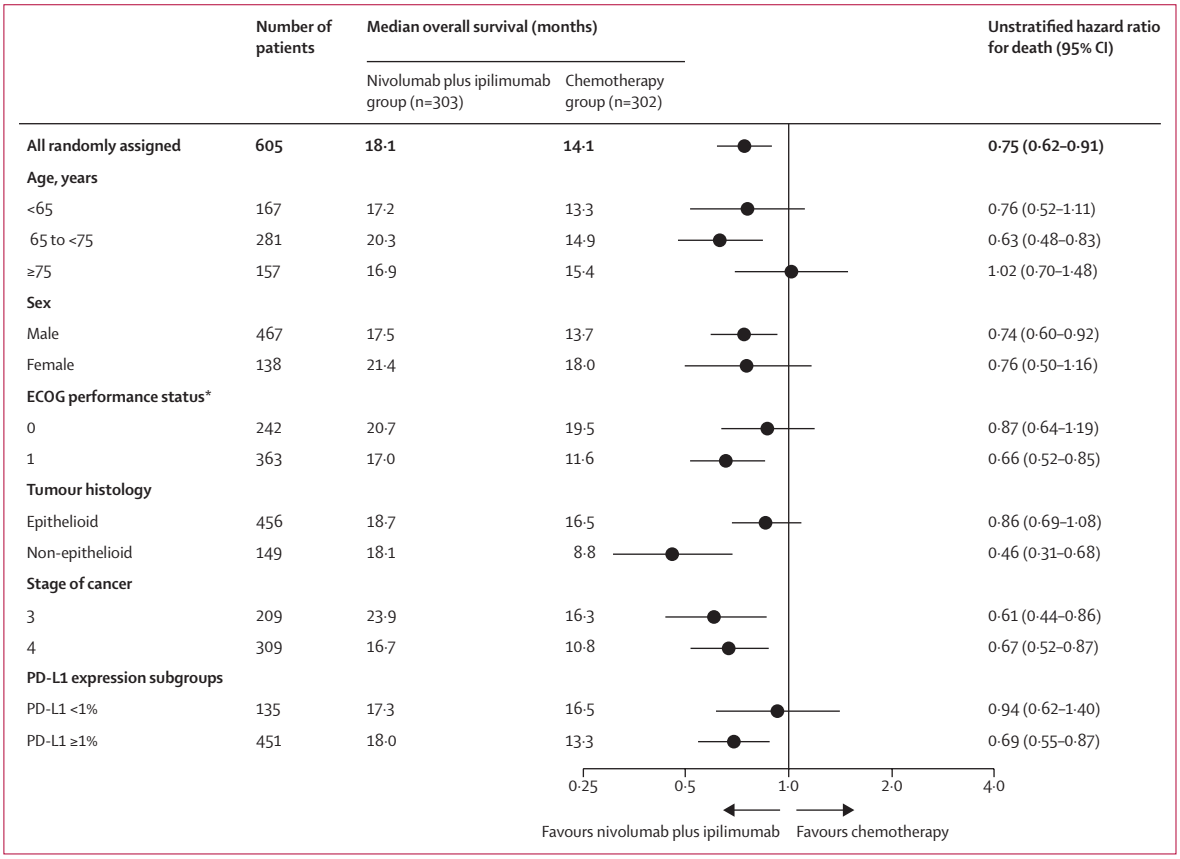


Figure 3: **Overall survival in predefined patient subgroups**
ECOG=Eastern Cooperative Oncology Group. PD-L1=programmed cell death ligand 1. *One patient in the chemotherapy group had a baseline performance status of 2 (protocol deviation).

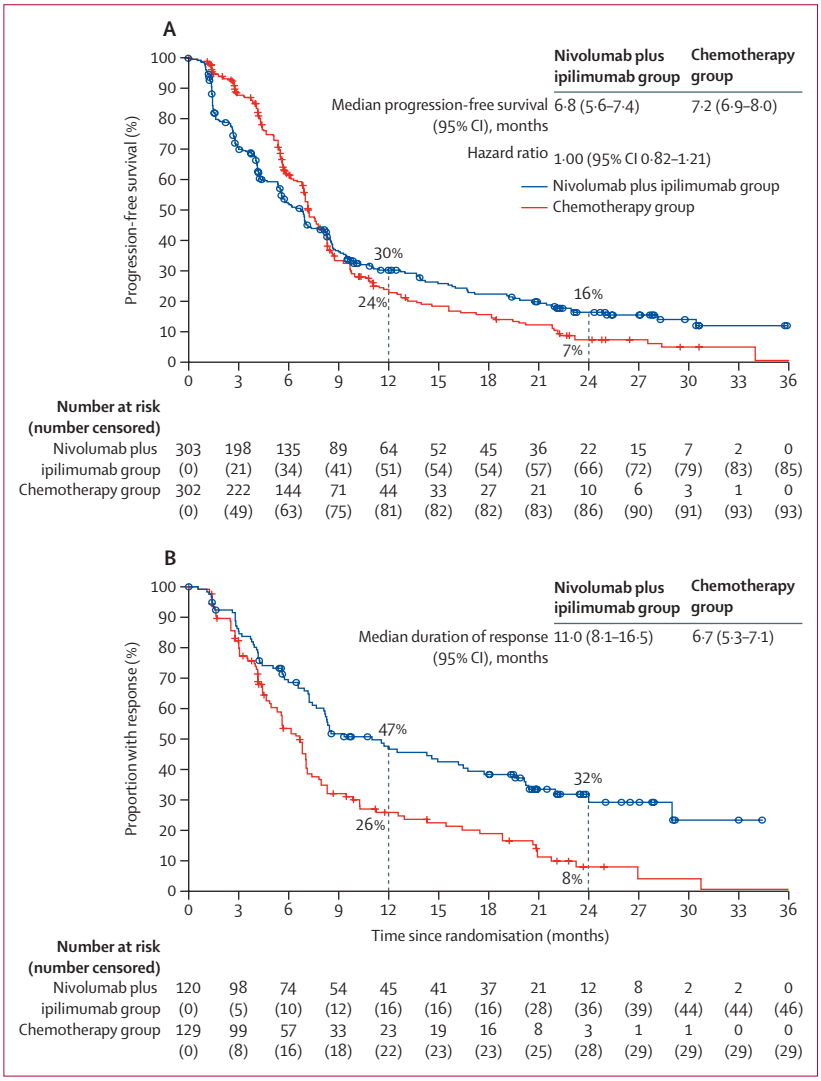


Figure 4: **Progression-free survival in all patients randomly assigned to treatment (A) and duration of response in confirmed responders (B)**

Progression-free survival and duration of response are both per blinded independent central review. The hazard ratio in part A is stratified by sex and histology.

	Nivolumab plus ipilimumab group (n=303)	Chemotherapy group (n=302)
Objective response rate		
n (%)	120 (40%)	129 (43%)
95% CI	34·1–45·4	37·1–48·5
Best overall response		
Complete response	5 (2%)	0
Partial response	115 (38%)	129 (43%)
Stable disease	112 (37%)	125 (41%)
Non-complete response and non-progressive disease	0	3 (1%)
Progressive disease	55 (18%)	14 (5%)
Unable to determine	4 (1%)	5 (2%)
Not reported	12 (4%)	26 (9%)
Disease control rate		
n (%)	232 (77%)	257 (85%)
95% CI	71·4–81·2	80·6–88·9
Time to response, months		
Median	2·7	2·5
IQR	1·45–3·27	1·41–3·02
Duration of response, months		
Median	11·0	6·7
95% CI	8·1–16·5	5·3–7·1
Proportion of patients with a response of at least 1 year or 2 years*		
At 1 year	47%	26%
95% CI	37–56	18–34
At 2 years	32%	8%
95% CI	23–41	3–15

Data are n (%), unless indicated otherwise. Minimum follow-up for objective response rate was 19·8 months. *Estimates are based on Kaplan-Meier estimates of duration of response.

Table 2: **Tumour response, as per blinded independent central review, in all patients randomly assigned to treatment**

An objective response was reported in 120 of 303 patients (40%; 95% CI 34·1–45·4) in the nivolumab plus ipilimumab group versus 129 of 302 patients (43%; 95% CI 37·1–48·5) in the chemotherapy group (table 2). Complete responses were only observed in the nivolumab plus ipilimumab group (five [2%] of 303 patients). Disease control was seen in 232 of 303 patients (77%; 95% CI 71·4–81·2) with a median time to response of 2·7 months (IQR 1·45–3·27) for the nivolumab plus ipilimumab group versus 257 of 302 (85%; 95% CI 80·6–88·9) with a median time to response of 2·5 months (IQR 1·41–3·02) for the chemotherapy group. Median duration of response in all confirmed responders was 11·0 months (95% CI 8·1–16·5) in the nivolumab plus ipilimumab group versus 6·7 months (95% CI 5·3–7·1) in the chemotherapy group (figure 4). The 2-year duration of response rate was 32% (95% CI 23–41) in the nivolumab plus ipilimumab group versus 8% (95% CI 3–15) in the chemotherapy group.

Safety is summarised in table 3, and all reported grade 3 and 4 treatment-related adverse events are listed in the appendix (pp 13–16). Of 300 patients treated with nivolumab plus ipilimumab, 28 (9%) discontinued ipilimumab early. In the chemotherapy group, dose reductions occurred in 89 (31%) of 284 participants who were given pemetrexed, 18 (17%) of 104 patients who were given cisplatin, and 85 (41%) of 209 participants who were given carboplatin, whereas dose reductions were not permitted for the nivolumab plus ipilimumab group. Grade 3–4 treatment-related adverse events were reported in 91 (30%) of 300 participants treated with nivolumab plus ipilimumab and 91 (32%) of 284 participants treated with chemotherapy. Any-grade serious treatment-related adverse events were reported in 64 (21%) patients treated with nivolumab plus ipilimumab versus 22 (8%) patients treated with chemotherapy; grade 3–4 treatment-related serious

	Nivolumab plus ipilimumab group (n=300)			Chemotherapy group (n=284)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
Any	148 (49%)	79 (26%)	12 (4%)	141 (50%)	73 (26%)	18 (6%)
Diarrhoea	52 (17%)	10 (3%)	0	19 (7%)	2 (1%)	0
Pruritus	46 (15%)	3 (1%)	0	1 (<1%)	0	0
Rash	40 (13%)	3 (1%)	0	15 (5%)	0	0
Fatigue	38 (13%)	3 (1%)	0	50 (18%)	5 (2%)	0
Hypothyroidism	32 (11%)	0	0	0	0	0
Nausea	29 (10%)	1 (<1%)	0	97 (34%)	7 (2%)	0
Anaemia	5 (2%)	1 (<1%)	0	70 (25%)	32 (11%)	0
Decreased appetite	27 (9%)	2 (1%)	0	48 (17%)	2 (1%)	0
Constipation	12 (4%)	0	0	41 (14%)	1 (<1%)	0
Vomiting	8 (3%)	0	0	35 (12%)	6 (2%)	0
Asthenia	25 (8%)	0	0	32 (11%)	12 (4%)	0
Increased lipase	7 (2%)	11 (4%)	2 (1%)	0	1 (<1%)	0
Colitis	3 (1%)	7 (2%)	0	1 (<1%)	1 (<1%)	0
Increased amylase	10 (3%)	6 (2%)	1 (<1%)	1 (<1%)	0	0
Thrombocytopenia	0	2 (1%)	0	16 (6%)	4 (1%)	6 (2%)
Neutropenia	0	1 (<1%)	1 (<1%)	28 (10%)	31 (11%)	12 (4%)

Data are n (%). Safety was assessed in all patients who received at least one dose of study drug. Treatment-related adverse events with an incidence of ≥10% in any group or grade 3 or 4 severity with an incidence of ≥2% in any group are shown. All grade 3 and 4 events are listed in the appendix (pp 13–16). Treatment-related adverse events included those reported between the first dose of study drug and 30 days after the last dose of study drug. *Only events that led to death within 24 h were documented as grade 5 and reported as deaths. Events leading to death >24 h after onset are reported with the worst grade before death.

Table 3: **Summary of treatment-related adverse events in all treated patients***

events were reported in 46 (15%) patients treated with nivolumab plus ipilimumab versus 17 (6%) treated with chemotherapy (appendix pp 17–19). Any-grade treatment-related adverse events that led to discontinuation (due to either component of the regimen) were reported in 69 (23%) of 300 patients treated with nivolumab plus ipilimumab and 45 (16%) of 284 patients treated with chemotherapy, and 45 (15%) patients treated with nivolumab plus ipilimumab and 21 (7%) patients treated with chemotherapy had grade 3–4 events that led to discontinuation (appendix p 20).

The most frequent any-grade treatment-related adverse events were diarrhoea in the nivolumab plus ipilimumab group (62 [21%] of 300 patients) and nausea in the chemotherapy group (104 [37%] of 284 patients). The most frequently reported any-grade serious treatment-related adverse events were colitis in the nivolumab plus ipilimumab group (nine [3%]) and anaemia in the chemotherapy group (six [2%]; appendix pp 17–19). The median exposure time was 6·5 months (IQR 2·99–12·22) for nivolumab plus ipilimumab and 4·5 months (3·65–4·68) for chemotherapy. Treatment exposure was 220·3 person-years with nivolumab plus ipilimumab and 94·5 person-years with chemotherapy. The overall exposure-adjusted incidence of treatment-related adverse events was 502·1 per 100 person-years with nivolumab plus ipilimumab versus 1355·3 per 100 person-years with chemotherapy.

A summary of treatment-related select adverse events (those with a potential immunological cause), time to onset and resolution of treatment-related select adverse events, the proportion of patients requiring immune-modulating concomitant medication (mostly corticosteroids), and the duration of use of immune-modulating concomitant medication are shown in the appendix (p 21). The most commonly reported any-grade treatment-related select adverse events with nivolumab plus ipilimumab were skin (108 [36%] of 300 patients) and gastrointestinal (66 [22%]) events. Overall, 198 (66%) of 300 patients who were given nivolumab plus ipilimumab died, with 183 (61%) deaths due to disease progression. 212 (75%) of 284 patients given chemotherapy died, with 199 (70%) deaths due to disease progression. Three (1%) treatment-related deaths occurred in the nivolumab plus ipilimumab group, due to pneumonitis, encephalitis, and heart failure. One (<1%) treatment-related death occurred in the chemotherapy group due to myelosuppression.

Discussion

To our knowledge, CheckMate 743 is the first large, randomised, phase 3 study to show significant and clinically meaningful improvement in overall survival with immunotherapy versus standard-of-care platinum plus pemetrexed chemotherapy for first-line treatment of unresectable MPM. Based on these results, in October, 2020, the US Food and Drug Administration approved nivolumab plus ipilimumab for this patient population.[12] With a median follow-up of 29·7 months, nivolumab plus ipilimumab provided durable survival benefit versus chemotherapy, with a 50% improvement

in the 2-year overall survival rate (41% *vs* 27%). Furthermore, estimated rates of patients who still had a response at 2 years was 8% with chemotherapy versus 32% with nivolumab plus ipilimumab. The safety profile of nivolumab plus ipilimumab in this study was consistent with that seen previously in NSCLC at this dose and schedule[15] and no new safety signals were reported.

The frequencies of grade 3 or 4 serious treatment-related adverse events and those leading to discontinuation were higher with nivolumab plus ipilimumab than with chemotherapy; however, most were manageable and resolved with steroids or supportive treatment. Moreover, when treatment-related adverse events were adjusted for exposure, the overall incidence of treatment-related adverse events was lower with nivolumab plus ipilimumab than with chemotherapy.

Benefit with nivolumab plus ipilimumab was observed in most subgroups assessed, with the exception of patients aged 75 years or older. However, these subgroups were small and did not have statistical power. As such, results from these subgroup analyses should be interpreted with caution. Importantly, benefits were observed across histological groups, albeit at different magnitudes. For example, median overall survival with nivolumab plus ipilimumab was consistent between patients with epithelioid histology (18·7 months; HR 0·86 [95% CI 0·69–1·08]) and non-epithelioid histology (18·1 months; HR 0·46 [0·31–0·68]), showing clinically meaningful survival improvements across both groups; 1-year and 2-year overall survival rates were also similar between the two histological subgroups. Notably, in the epithelioid subgroup, nivolumab plus ipilimumab showed an improvement of 2 months in median overall survival compared with chemotherapy, with an HR favouring nivolumab plus ipilimumab despite the 95% CI overlapping 1. Furthermore, the 2-year overall survival rate in the epithelioid subgroup showed a long-term benefit of nivolumab plus ipilimumab with a 9% absolute difference versus chemotherapy. The larger magnitude of benefit observed in the non-epithelioid subgroup was primarily driven by the inferior effect of chemotherapy in the non-epithelioid subtype, as previously reported.[3] This difference in outcomes between the subgroups treated with chemotherapy could not be attributed to the type of chemotherapy received because exploratory data from CheckMate 743 suggest that patients derive a similar overall survival benefit regardless of platinum backbone; median overall survival was similar between pemetrexed plus cisplatin and pemetrexed plus carboplatin.

Median progression-free survival and objective response rates were each numerically similar for nivolumab plus ipilimumab and chemotherapy. Median progression-free survival was similar to results from previously reported clinical trials in recurrent MPM.[16,18] The progression-free survival Kaplan-Meier curves crossed at approximately 8 months, reflecting more rapid, although not durable, disease control with chemotherapy. However, radiographic assessments in MPM can be challenging because of the absence of distinguishable tumour margins over time and successive CT evaluations.[22] Thus, overall survival is considered to be a more objective and reliable endpoint in this tumour type. Notably, nivolumab plus ipilimumab provided long-term overall survival benefit, although the slight early survival benefit observed with chemotherapy was not durable.

The duration of response and durable survival benefit observed with nivolumab plus ipilimumab in patients with MPM in CheckMate 743 builds on the existing body of evidence that shows extended survival benefit with this dual immunotherapy regimen across a number of other tumour types, including NSCLC.[13–15,23] Ipilimumab is hypothesised to drive memory T-cell production leading to durable responses when combined with nivolumab.[11] Results of the current study also corroborate the promising activity seen with anti-PD-1 or anti-PD-L1, and anti-CTLA-4 combination therapies in phase 2 studies in second-line or later settings of MPM,[16,18,24] and support the use of dual immunotherapy over single-agent anti-PD-1 or anti-CTLA-4 inhibitors, which have shown little benefit over chemotherapy.[25,26]

Some treatment guidelines (eg, NCCN guidelines) include the optional addition of the anti-angiogenic agent bevacizumab to platinum plus pemetrexed chemotherapy for first-line treatment of MPM in select patients, based on the survival benefit seen in a phase 3 trial;[5,10] however, this regimen is not approved by regulators. Nonetheless, given the durable survival benefit seen in CheckMate 743, combining nivolumab plus ipilimumab with other therapies, including anti-angiogenic agents or, as approved for NSCLC in May, 2020, a short course of chemotherapy,[12] merits investigation to determine whether survival outcomes can be further enhanced. Similarly, future trials assessing the benefit of second-line targeted therapies (eg, bevacizumab and ramucirumab) after nivolumab plus ipilimumab treatment are warranted.

Reliable biomarkers to predict the benefit of dual-agent immunotherapy in the treatment of MPM have not yet been identified. Although PD-L1 expression is an established biomarker for single-agent immunotherapy in NSCLC,[27] its role in predicting treatment outcomes with dual immunotherapy regimens has not been established. More specifically, in MPM trials investigating immunotherapies, the association between PD-L1 expression and efficacy is inconsistent.[17,18,24] In CheckMate 743, overall survival outcomes with nivolumab plus ipilimumab were similar in the subgroups with less than 1% and with 1% or higher PD-L1 expressions and better outcomes were seen with nivolumab plus ipilimumab than with chemotherapy at 2 years in both subgroups. However, survival with chemotherapy was better in patients with tumour PD-L1 expression of less than 1% than those with expression of 1% or higher. These observations suggest that absence of PD-L1 expression might be indicative of better prognosis

with chemotherapy. However, these descriptive and exploratory data should be interpreted with caution given their potential limitations—ie, PD-L1 expression was not a stratification factor in the study and the sample size of the PD-L1 expression less than 1% group was small. As such, the potential for imbalances in known or unknown prognostic factors does not allow us to draw definitive conclusions. Better characterisation of this heterogeneous disease using transcriptomic and epigenetic profiling should guide future patient selection and therapeutic strategies, and aid in the identification of novel biomarkers.[28,29]

In summary, first-line nivolumab plus ipilimumab provided a significant and clinically meaningful improvement in overall survival versus platinum plus pemetrexed chemotherapy. Nivolumab plus ipilimumab has a favourable clinical benefit–risk profile that has led to approval in the USA and should be considered as a new standard of care for previously untreated patients with unresectable MPM, regardless of histological subtype.

Contributors

PB, AS, AKN, NF, SPe, AST, ASM, SPo, TJ, PA, AO, CB, and GZ provided substantial contributions to the conception and design of the study. PB, AS, AKN, NF, SPe, AST, ASM, SA, YO, YB, RC, LG, FG, DK, JR-C, and GZ enrolled and treated patients. CB wrote the study statistical analysis plan, did all statistical analyses, and generated data. PB, AKN, NF, SPe, AST, ASM, SPo, TJ, PA, AO, CB, and GZ analysed and interpreted the data. PA and CB verified the underlying data from the study. All authors reviewed the data, contributed to the development of the manuscript, and approved the final version for publication.

Declaration of interests

PB has received institutional grant funding from Bristol Myers Squibb and MSD and has a consultancy or advisory role for Bristol Myers Squibb, MSD, Roche, Beigene, Epizyme, Takeda, Trizell, and Daichii-Sankyo (all honoraria are paid to his institute). AS has received grant funding and personal fees from Bristol Myers Squibb (for provided work on advisory boards, consultancy, service on the speaker's bureau, provision of expert testimony, and for travel or accommodation expenses) and their institution has also received support from Bristol Myers Squibb (payment for work as a principal investigator or coprincipal investigator in clinical trials); has received personal fees from AstraZeneca and MSD (for provided work on advisory boards, consultancy, service on the speaker's bureau, provision of expert testimony, and for travel or accommodation expenses) and their institution also received support from AstraZeneca and MSD (payment for work as a principal investigator or coprincipal investigator in clinical trials); and has received personal fees from Roche (for provided work on advisory boards, consultancy, service on the speaker's bureau, provision of expert testimony, and for travel or accommodation expenses) and their institution also received support from Roche (payment for work as a principal investigator or coprincipal investigator in clinical trials). AKN has received grant funding from Atara Biotherapeutics and Douglas Pharmaceuticals; received non-financial, travel support, and grant funding from AstraZeneca; received personal fees from Bayer Pharmaceuticals, Pharmabcine, and Trizell (honoraria and provided consulting); received personal fees, non-financial, and travel support from Boehringer Ingelheim (honoraria, served on the advisory board and travel funding); received personal fees from Douglas Pharmaceuticals, Merck Sharp Dohme, and Roche Pharmaceuticals (served on the advisory board and honoraria); and received personal fees from Atara Biotherapeutics (served on the advisory board). NF has received personal fees from Bristol Myers Squibb and Daiichi Sankyo (honoraria) and received grant funding and personal fees from ONO pharmaceutical (honoraria, and provided advice and consulting). 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For the **Bristol Myers Squibb policy on data sharing** see https://www.bms.com/researchers-and-partners/clinical-trials-and-research/disclosure-commitment.html

Data sharing

The Bristol Myers Squibb policy on data sharing is available online.

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Nivolumab for malignant peritoneal mesothelioma

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SUMMARY

Malignant peritoneal mesothelioma (MPeM) is a highly malignant neoplasm of the peritoneum, which carries a poor prognosis. A 70-year-old man, who was employed in the shipbuilding industry and exposed to asbestos for 50 years, was found to have a low-density lesion in the peritoneum around the liver and spleen, associated with multiple mediastinal and parasternal lymphadenopathy. Laparoscopic exploration was performed, and biopsy specimen analysis led to a diagnosis of MPeM. Initial systemic chemotherapy comprising cisplatin and pemetrexed yielded a modest cytoreductive effect. However, 4 months later, the patient presented with abdominal distension and anorexia. CT images revealed massive ascites, bowel obstruction and an enlarged intra-abdominal tumour, which was considered progression of the MPeM. The patient was treated with nivolumab. Bowel obstruction was improved after the first administration, and his sense of abdomen distension completely disappeared after the third administration. This case supports the utility of immunotherapy in MPeM.

BACKGROUND

Malignant peritoneal mesothelioma (MPeM) is a highly malignant neoplasm occurring in the peritoneum, which is associated with a poor prognosis. There is no established treatment strategy for this disease, and patients with MPeM are usually treated following the strategy for malignant pleural mesothelioma. In recent years, several encouraging reports have demonstrated that malignant pleural mesothelioma shows a positive clinical response to immunocheckpoint inhibitors. However, no clinical study has examined the utility and safety of immunocheckpoint inhibitors for MPeM treatment. Here, we report a case of MPeM which showed a significant clinical response to nivolumab treatment.

CASE PRESENTATION

A 70-year-old man, who had been employed in the shipbuilding industry and exposed to asbestos for 22 years between 16 and 38 years, was identified as hepatitis B virus-positive by a blood test. Further examination using abdominal CT scan revealed a low-density lesion in the peritoneum around the liver and spleen, associated with multiple mediastinal and parasternal lymphadenopathy. Positron emission tomography/CT scan revealed 18F-fluorodeoxyglucose (FDG) accumulation in the peritoneal lesion. Laparoscopic exploration was performed, and histopathological analyses of the biopsy specimen revealed a sheet-like proliferation of epithelial cells with round nuclei and conspicuous nucleoli. Immunohistochemical analyses demonstrated that these cells were positive for calretinin, D2-40 and cytokeratin 5/6, and negative for desmin, carcinoembryonic antigen and thyroid transcription factor-1. Based on these findings, the patient was diagnosed with MPeM, epithelioid subtype.

INVESTIGATIONS

This patient was referred to our hospital, where he received systemic chemotherapy comprising cisplatin and pemetrexed. Six cycles of this treatment yielded a modest cytoreductive effect. Four months later, the patient was admitted to another hospital due to bowel obstruction. He received conservative treatment, but continued to exhibit abdominal distension and anorexia. CT images showed massive ascites, bowel obstruction and an enlarged intra-abdominal tumour, which was considered to be the progression of the MPeM (figure 1A).

Nivolumab therapy was initiated as a salvage treatment. After the first nivolumab administration, the bowel obstruction was improved. The patient's sense of abdomen distension completely disappeared after the third nivolumab administration. After the fourth administration, CT images demonstrated remarkable reduction of the abdominal tumour (figure 1B). Nivolumab therapy did not result in any specific adverse event, except for grade 1 skin eruption (according to Common Toxicity Criteria of Adverse Event V.5).

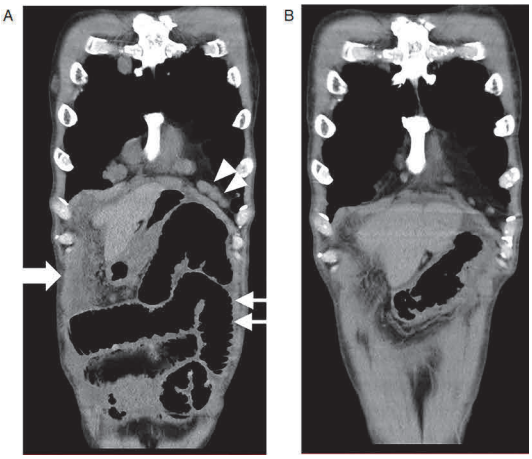

Figure 1 (A) CT images of the abdomen reveal a soft tissue lesion on the omentum (bold arrow); lymphadenopathy adjacent to pericardium fat (arrowheads) and dilatation and fluid accumulation of the small intestine, which indicate intestinal obstruction (narrow arrows). All of these findings suggest the progression of malignant peritoneal mesothelioma. (B) CT images after the fourth administration of nivolumab reveal significant improvement of all of the previous findings.

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OUTCOME AND FOLLOW-UP

The patient was administered 24 courses of nivolumab without disease regrowth. Further administration was suspended due to financial reasons. At this time, the patient had been progression free for 10 months after discontinuation without any cancer treatment.

DISCUSSION

Malignant mesothelioma (MM) is mainly found in the pleura and peritoneum, with some reports indicating that 80% occur in the pleura and 10%–20% in the peritoneum.[1] MPeM arises in peritoneal mesothelium cells, and is classified into epithelioid, sarcomatoid and biphasic subtypes. Yan *et al* found that 92% of cases were the epithelioid subtype and 8% the biphasic subtype.[2] Asbestos exposure is considered a main cause of MPeM, though the association is weaker than with malignant pleural mesothelioma.[3]

No standard MPeM treatment has yet been established. Selected patients receive cytoreductive surgery and hyperthermic intraperitoneal chemotherapy.[4] For patients with inoperable disease, systemic chemotherapy is the most common alternative treatment option, typically using a combination of cisplatin and pemetrexed, since pemetrexed has been approved for malignant pleural mesothelioma. One report of MPeM cases describes a chemotherapy response rate of 38%, and a median overall survival of 15.4 months.[5] In the salvage setting after chemotherapy failure, currently available agents rarely work against MM. In Japan in 2018, nivolumab was approved for malignant pleural mesothelioma that is refractory to primary chemotherapy, based on the favourable results of a phase II clinical study.[6] To date, no report has described the utility of immunocheckpoint inhibitors for MPeM treatment.

The drastic and durable clinical response to nivolumab in the current case of MPeM suggests the utility of immunotherapy in MPeM. A well-designed clinical study is warranted to examine whether nivolumab should be considered as a new treatment option for MPeM.

Patient's perspective

At diagnosis:

I had no symptoms and I was fully active, so I was very shocked when I was given the diagnosis of malignant peritoneal mesothelioma. I was exposed to asbestos when I worked at a shipyard, when I was 17–20 years old. I was afraid of what will happen to me in the future. I was getting in shape by going to the gym, so I was sure to overcome my disease. I want to set myself up as an example to other patients with mesothelioma who can survive long.

At the presentation of disease progression, small intestinal obstruction:

I was informed of the disease progression. My doctor said that there were few treatment options, and he suggested that I receive treatment with nivolumab. I thought it would be far better than doing nothing at all, so I decided to receive the nivolumab treatment.

After the third administration of nivolumab:

My abdomen has dented! I feel grateful that my doctor has treated me with nivolumab. I feel like people on the news, because I have watched the TV news that reported that the researchers who discovered programmed death-1 protein have been given the Nobel Prize. Now I can eat a lot, so I feel the benefit of nivolumab every day.

At the discontinuation of nivolumab:

I was very shocked to hear that I could not continue the nivolumab treatment because Worker's Compensation will not support the treatment anymore. I gradually feel more positive these days, so from now on, I will proactively do what I can.

Learning points

- There is no established treatment strategy for patients with malignant peritoneal mesothelioma (MPeM).
- Immunocheckpoint inhibitors have proven useful for malignant pleural mesothelioma in recent years.
- A well-designed clinical study is warranted to examine whether nivolumab should be considered as a new treatment option for MPeM.

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ORIGINAL ARTICLE

Clinical Efficacy and Safety of Nivolumab in Japanese Patients With Malignant Pleural Mesothelioma: 3-Year Results of the MERIT Study

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ABSTRACT

Introduction: We examined the long-term efficacy and safety of nivolumab, a human monoclonal antibody that inhibits interactions between the programmed cell death protein-1 receptor and its ligands (programmed death-ligand 1 and programmed death-ligand 2), in Japanese patients with malignant pleural mesothelioma (MPM).

Methods: Japanese patients with previously treated MPM (one or two regimens) were enrolled in a single-arm, phase 2 study and received nivolumab intravenously 240 mg every 2 weeks until progressive disease or unacceptable toxicity. The primary end point was the centrally assessed objective response rate. Other end points included overall survival (OS), progression-free survival (PFS), treatment-related adverse events, and patient-reported outcomes (Lung Cancer Symptom Scale for mesothelioma and EuroQOL visual analog scale). Patient enrollment started on June 16, 2016. Here, we report 3-year follow-up data (cutoff date: November 12, 2019).

Results: Thirty-four patients were enrolled. The centrally assessed objective response rate was previously reported (29.4%). The 2- and 3-year OS rates were 35.3% and 23.5%, respectively, and the corresponding PFS rates were 17.0% and 12.7%. Median OS and PFS were 17.3 and 5.9 months, respectively. Eight patients were alive at 3 years of follow-up. Nivolumab was well tolerated and no new safety signals were found. The patient-reported outcomes were maintained without marked deteriorations during the study.

Conclusions: Our results reveal clinically relevant long-term efficacy and safety of nivolumab for the treatment of MPM.

Keywords: Malignant pleural mesothelioma; Nivolumab; Programmed death-1; Japan

Introduction

Malignant pleural mesothelioma (MPM) is a rare, highly aggressive malignancy that is mostly due to occupational exposure to asbestos and is more common in older males.[1-3] In previous Japanese studies, the median survival of patients with newly diagnosed MPM was just 7.9 months, generally because most patients are diagnosed at an advanced stage.[1,2] The U.S. National Comprehensive Cancer Network guidelines for MPM recommend pemetrexed plus cisplatin (or carboplatin) with or without bevacizumab as first-line chemotherapy.[4] However, most patients fail to respond to first-line chemotherapy, necessitating subsequent systemic therapy, which may now involve pemetrexed (if not administered as first-line chemotherapy or as rechallenge), nivolumab with or without ipilimumab, or pembrolizumab.[4]

Nivolumab, a human monoclonal antibody that inhibits interactions between the programmed cell death protein-1 receptor and its ligands (programmed death-ligand 1 [PD-L1] and PD-L2), was approved in Japan (August 2018) for patients with pemetrexed–platinum doublet-treated MPM on the basis of the results of the Multicenter, Open-label, Single-arm, Japanese Phase II study in Malignant Pleural Mesothelioma (MERIT) study,[5] which enrolled 34 Japanese patients. After a median follow-up of 16.8 months, 10 patients had an objective response and the median overall survival (OS) was 17.3 months.[5]

To our knowledge, there are no published studies reporting the 3-year OS after second-line treatment. Here, we report the results obtained at the 3-year follow-up of patients enrolled in the MERIT study, including the efficacy outcomes for all patients and according to PD-L1 expression and MPM subtype (epithelioid or non-epithelioid), changes in quality of life (QOL) (determined using the EuroQOL visual analog scale [EQ-VAS] and Lung Cancer Symptom Scale for mesothelioma [LCSS-Meso] average symptom burden index), and treatment-related adverse events (TRAEs).

Materials and Methods

MERIT was an open-label, single-arm, phase 2 study performed at 15 centers in Japan. Its design is described in more detail in our previous report.[5] This study adhered to the Declaration of Helsinki and Good Clinical Practice and was registered on clinicaltrials.jp (JapicCTI-163247).

Patients

The full eligibility criteria are described in our previous report.[5] Briefly, males and females aged at least 20 years were eligible if they had histologically confirmed MPM, unresectable advanced or metastatic MPM without surgery, MPM resistant or intolerable to one or two previous chemotherapeutic regimens (platinum and pemetrexed), and at least one measurable lesion defined according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST) for MPM.[6] Key exclusion criteria included history of severe hypersensitivity reactions to other drugs (including antibody products), concurrent or history of autoimmune disease, multiple primary cancers, brain or meningeal metastases, current or history of interstitial lung disease or pulmonary

fibrosis, and previous treatment with immune checkpoint inhibitors (ICIs), therapeutic antibodies, or drugs targeting T-cell regulation. All patients provided written informed consent for participation in the study.

Study Design

All patients were treated with nivolumab at a dose of 240 mg by intravenous infusion every 2 weeks (one cycle) on day 1 of each cycle. Its dose or administration mode could not be adjusted. As previously explained,[5] nivolumab was to be continued until the patient met one of the discontinuation criteria: documentation of progressive disease (PD); unequivocal clinical progression; grade 2 or higher interstitial lung disease, grade 2 or higher eye disorder that did not improve to grade 1 or less with topical treatment, and a causal relationship with nivolumab could not be excluded; grade 3 or higher bronchospasm, neurotoxicity, hypersensitivity reaction, infusion reaction, or uveitis for which a causal relationship with nivolumab could not be excluded; no administration of nivolumab for 6 weeks after the previous dose (unless nivolumab is withheld for at least 6 weeks for steroid tapering); or the investigator or subinvestigator deemed it necessary to discontinue treatment in consideration of the efficacy or safety of nivolumab. Immunosuppressants, corticosteroids at doses of at least 10 mg/day prednisone equivalents, antitumor therapies, concurrent radiotherapy, pleurodesis, and surgical therapies for malignant tumors were prohibited. Tumor imaging (computed tomography or magnetic resonance imaging) was performed every three cycles. Target lesions in the pleura were measured unidimensionally as the largest tumor thickness perpendicular to the chest wall or mediastinum according to mRECIST.[6] Nonpleural lesions were measured according to RECIST version 1.1. PD-L1 expression was assessed as previously described.[5] PD-L1–positive status was defined as membranous staining in at least 1% of tumor cells.

End Points

The primary end point was the objective response rate (ORR), with central assessment according to mRECIST, and was defined as the proportion of patients with a complete response or partial response (PR). Secondary end points included the investigator-assessed ORR, changes in tumor size, disease control rate, OS, progression-free survival (PFS), duration of response, time to response, and best overall response (BOR) assessed centrally. Tumor responses were assessed in all patients combined and in patients divided into subgroups by PD-L1 expression ($<1\%$ or $\geq 1\%$) and histologic subtype (epithelioid, sarcomatoid, or biphasic) in prespecified analyses. QOL was assessed using the EQ-VAS and the LCSS-Meso symptom burden index[7] at enrollment and at each study visit. Safety was evaluated in terms of laboratory tests, AEs, and TRAEs. AEs and TRAEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

Statistical Analyses

As previously noted, a sample size of at least 29 patients was sufficient to detect a significant ORR with a power of 80% and a one-sided significance level of 0.025, on the basis of an expected ORR of 19%.[5] We also performed a landmark analysis of OS according to the BOR at 3 months for patients who survived for at least 3 months. All analyses were performed using standard methods at 95% confidence levels. Wilson's method was used to determine the 95% confidence intervals (CIs) for the ORR, disease control rate, and BOR. All analyses were conducted using SAS version 9.3 (SAS Institute Inc., Cary, NC).

Role of the Funding Source

This work was funded by Ono Pharmaceutical Co., Ltd., Japan, and Bristol-Myers Squibb, United States. The sponsors contributed to the study design, data collection, data analysis, data interpretation, and writing of the clinical study report.

Results

Patients

Patient enrollment started on June 16, 2016, and patients were followed up to the data cutoff date, November 12, 2019. Forty-three patients were screened (provided consent), and nine were excluded because they did not meet the inclusion criteria or withdrew their consent. A total of 34 patients were enrolled and treated with nivolumab, including 29 males (85.3%) and five (14.7%) females. Their characteristics are described in Supplementary Table 1 and in our previous report.[5] The minimum follow-up was 36 months. The median follow-up was 17.3 (range: 1.8–39.9) months for all 34 patients and 38.0 (range: 37.0–39.9) months for seven censored patients included in the end-of-study analysis.

Overall Response Rate

The centrally assessed ORR was unchanged from our previous report at 29.4% (95% CI: 16.8%–46.2%; 10 of 34 patients), with PR in 10 patients (Table 1). In most patients with PR or stable disease, their responses were maintained for a long period of time (Supplementary Fig. 1), up to approximately 2 years. Table 1 reveals the ORR in subgroups of patients, including the previously reported ORR by histologic subtype and PD-L1

Table 1. Responses to Nivolumab (N = 34)

Outcome	n/N (%)[a]	95% CI
BOR		
CR	0/34 (0.0)	0.0-10.2
PR	10/34 (29.4)	16.8-46.2
Stable disease	13/34 (38.2)	23.9-55.0
PD	9/34 (26.5)	n/c
NA	2/34 (5.9)	n/c
Response rate by subgroup		
Sex		
Male	7/29 (24.1)	12.2-42.1
Female	3/5 (60.0)	23.1-88.2
Age (y)		
<65	3/11 (27.3)	9.7-56.6
≥65	7/23 (30.4)	15.6-50.9
ECOG PS		
0	4/13 (30.8)	12.7-57.6
1	6/21 (28.6)	13.8-50.0
Histologic subtype		
Epithelioid	7/27 (25.9)	13.2-44.7
Sarcomatoid	2/3 (66.7)	20.8-93.9
Biphasic	1/4 (25.0)	4.6-69.9
Number of prior treatment(s)		
1	9/24 (37.5)	21.2-57.3
2	1/10 (10.0)	1.8-40.4
PD-L1 status		
≥1%	8/20 (40.0)	21.9-61.3
<1%	1/12 (8.3)	1.5-35.4
NA	1/2 (50.0)	9.5-90.5

BOR, best overall response; CR, complete response; ECOG PS, Eastern Cooperative Oncology Group performance status; NA, not assessable; n/c, not calculable; PD, progressive disease; PD-L1, programmed death ligand-1; PR, partial response.
[a]Percentages are calculated by the number (N) of patients within that subgroup.

status.[5] The present analyses newly revealed that the ORR was lower in patients with two previous treatments than in patients with one previous treatment.

OS and PFS

The 2- and 3-year OS rates were 35.3% and 23.5%, respectively, and the median OS was 17.3 months (95% CI: 11.5–26.6 months) (Fig. 1*A*). The 2- and 3-year PFS rates were 17.0% and 12.7%, respectively, and the median PFS was 5.9 months (Fig. 1*B*).

In PD-L1–positive patients, the 2- and 3-year OS rates were 35.0% and 15.0%, respectively, and the median OS was 19.1 months. The 2- and 3-year PFS rates were 18.9% and not calculable, respectively, and the median PFS was 7.2 months. In PD-L1–negative patients, the 2- and 3-year OS rates were both 33.3%, and the median OS was 11.6 months. The 2- and 3-year PFS rates were both 16.7%, and the median PFS in this subgroup was 2.9 months.

OS and PFS according to the histologic subtype of MPM are shown in Figure 2. Owing to the small numbers of patients with sarcomatoid or biphasic histologic subtypes, these patients were pooled together (as non-epithelioid subtype). In this subgroup, the median OS was 26.6 months, with 2- and 3-year OS rates of 57.1% and 42.9%, respectively (Fig. 2*A*). The median PFS was 18.2 months, whereas 2- and 3-year PFS rates were 42.9% and not calculable, respectively (Fig. 2*B*). In patients with the epithelioid histologic subtype, the median OS was 15.7 months and the 2- and 3-year OS rates were 29.6% and 18.5%, respectively (Fig. 2*A*). The median PFS, 2-year PFS, and 3-year PFS were 3.9 months, 9.6%, and 9.6%, respectively (Fig. 2*B*).

We also performed a landmark analysis of OS in patients with a best response of PR, stable disease, or PD (Supplementary Fig. 2). The median OS in these three subgroups was 20.9, 19.9, and 8.0 months, respectively.

Patient Status at 3 Years and Poststudy Treatments

Eight patients were alive at 3 years of follow-up, including seven at the database lock (Fig. 3). These seven patients were on a poststudy treatment at the cutoff date. They included four with epithelioid, two with biphasic, and one with sarcomatoid histologic subtypes. Four patients were treated with nivolumab for 2 years and one patient for 3 years. Eighteen patients received subsequent systemic treatments, as listed in Supplementary Table 2, including nivolumab in three patients. Nivolumab was not rechallenged as subsequent treatment in patients with PD, but one patient was switched to commercially available nivolumab after completing the clinical study, one patient started on commercially available nivolumab after the patient requested discontinuation of the clinical study upon approval of nivolumab in Japan, and one resumed nivolumab after discontinuation due to an AE.

Comparison of 3-Year Survivors and Nonsurvivors

In an exploratory analysis, we compared the characteristics and BOR between patients who survived for 3 years and nonsurvivors (Supplementary Table 3). Although there was an imbalance in the numbers of patients in these two groups, we observed no marked differences in their patient characteristics, except for the distribution of Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 and 1, with a significantly higher proportion of patients with ECOG PS of 0 among 3-year survivors ($p = 0.033$). The proportion of patients with a BOR of PR or stable disease was not significantly different between the two groups (75.0% in 3-year survivors and 65.4% in nonsurvivors, $p = 0.640$).

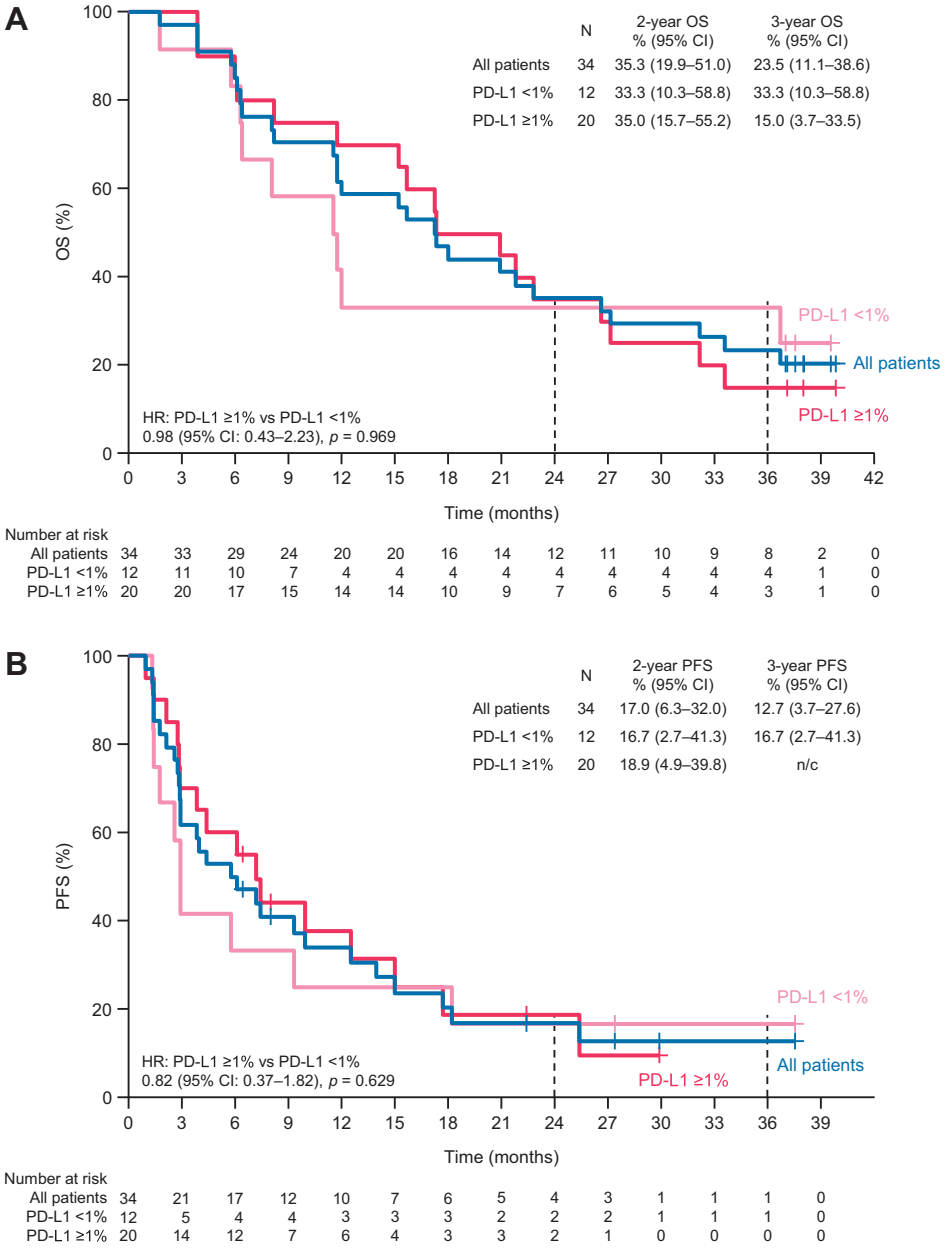

Figure 1. (*A*) OS and (*B*) PFS in all patients and in patients divided into subgroups by PD-L1 expression. CI, confidence interval; HR, hazard ratio; n/c, not calculable; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival.

Quality of Life

QOL was evaluated in terms of the EQ-VAS and LCSS-Meso symptom burden scale. Both outcomes were maintained over time among patients with available data (Fig. 4*A–D*).

Safety

We previously reported that TRAEs occurred in 26 patients (76.5%), including grade 3 to 4 TRAEs in 11 (32.4%) by the cutoff date of March 14, 2018,[5,8] and no additional TRAEs were observed thereafter until the cutoff date of November 12, 2019. There were no grade 5 TRAEs. The most common TRAEs were rash (six patients), lipase increased (five patients), and diarrhea and amylase increased (four patients each). Other TRAEs that occurred in at least two patients are listed in Table 2. Grade 3 or 4 TRAEs included lipase increased in four patients and diarrhea,

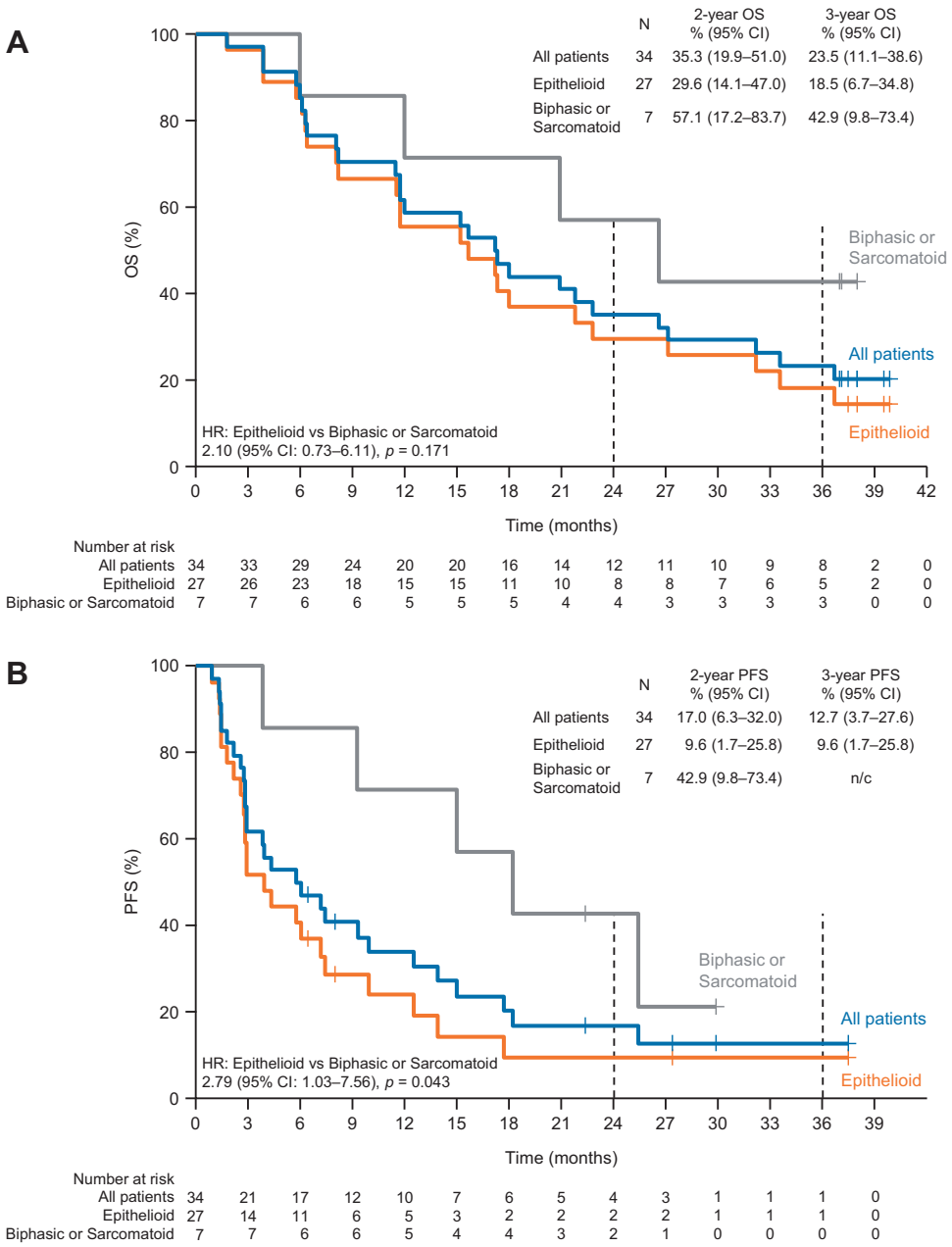

Figure 2. (*A*) OS and (*B*) PFS according to histologic subtype. Patients with biphasic or sarcomatoid histologic subtypes were pooled and compared with patients with the epithelioid histologic subtype. CI, confidence interval; HR, hazard ratio; n/c, not calculable; OS, overall survival; PFS, progression-free survival.

amylase increased, and pneumonitis in two patients each.

Discussion

The MERIT study evaluated the efficacy and safety of nivolumab in Japanese patients with MPM, and led to the approval of nivolumab for this indication in Japan. Until now, long-term survival rates of patients with MPM have remained poor, with limited benefit of chemotherapy. For example, second-line pemetrexed in combination with best supportive care (8.4 versus 9.7 months for best supportive care alone)[9] did not elicit marked improvements in OS. The introduction of ICIs has improved the prognosis of MPM. In the MAPS2 study, which enrolled patients with relapse after one or two lines of therapy, the median OS in nivolumab-treated patients was 11.9 months from the time of randomization (median follow-up of 20.1 months in the overall study population).[10] Therefore, we analyzed the OS and PFS at a 3-year follow-up in the MERIT study. We observed a

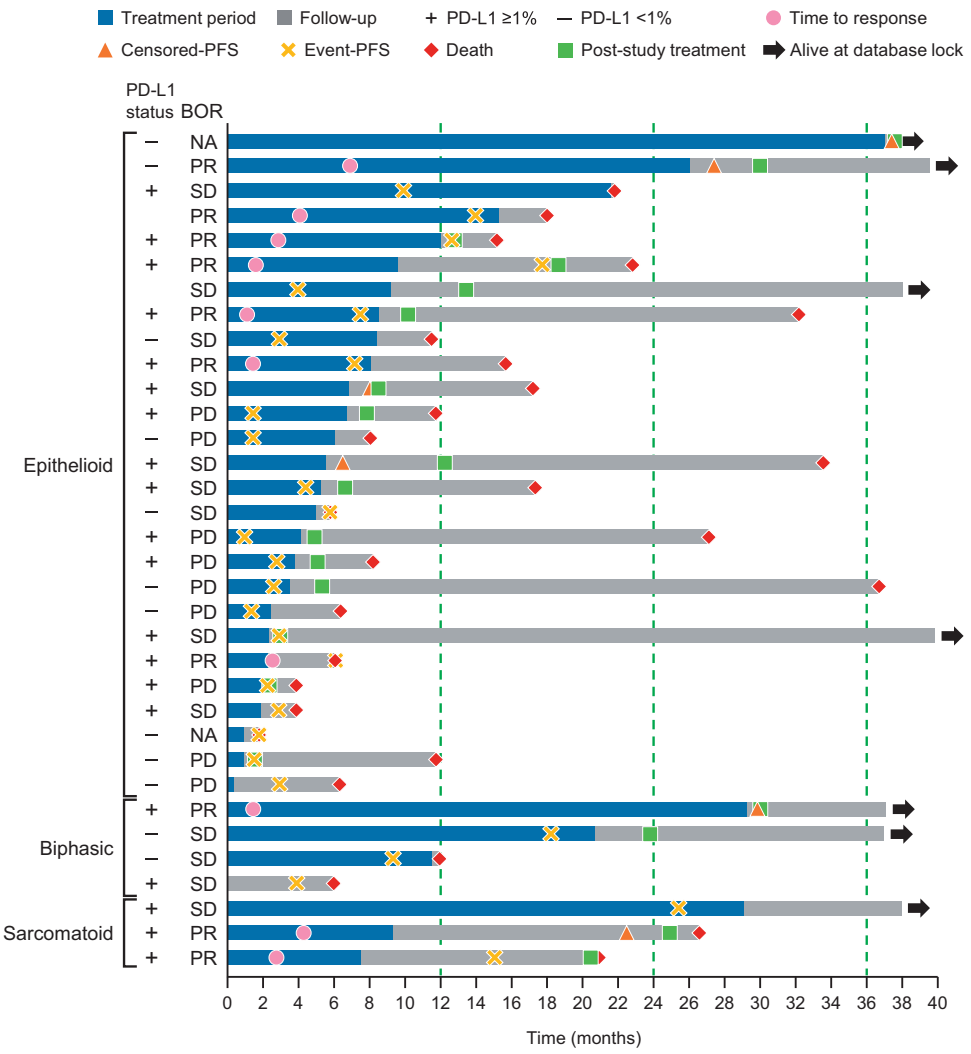

Figure 3. Swimmer plot of treatment duration, response to nivolumab, and follow-up period. BOR, best overall response; NA, not assessable; PD, progressive disease; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PR, partial response; SD, stable disease.

promising long-term survival of nivolumab-treated patients with a 3-year OS rate of 23.5%.

Although PD-L1 expression status was associated with the ORR, there were no significant differences in OS or PFS at 2 or 3 years between PD-L1–positive and PD-L1–negative patients. These results suggest that long-term survival in patients with nivolumab-treated MPM is not dependent on PD-L1 expression status. However, owing to the small number of patients, our findings may warrant confirmation in a future study with a larger number of patients or using a patient registry.

The histologic subtype of MPM is considered to be a prognostic factor for MPM, because patients with the biphasic or sarcomatoid histologic subtypes typically have worse prognosis after chemotherapy than patients with the epithelioid histologic subtype.[11,12] In the present analyses, the survival outcomes, especially PFS, were quite favorable in the patients with nonepithelioid subtypes. Furthermore, as in our previous report,[5] the ORR was also more favorable in patients with the nonepithelioid subtypes relative to that in patients with the epithelioid subtype. Thus, patients with nonepithelioid histologic subtypes tended to have better outcomes, although the reason for this is unknown. Further research is needed to investigate whether genomic alterations may explain the differences in survival with nivolumab between patients with nonepithelioid and epithelioid subtypes of MPM.

It is noteworthy that eight patients were alive at 3 years. There were no marked differences in patient characteristics between 3-year survivors and non-survivors except for ECOG PS at baseline.

Beyond assessing the efficacy of nivolumab in terms of tumor responses, we also examined its impact on QOL. We found that QOL, measured using the EQ-VAS and

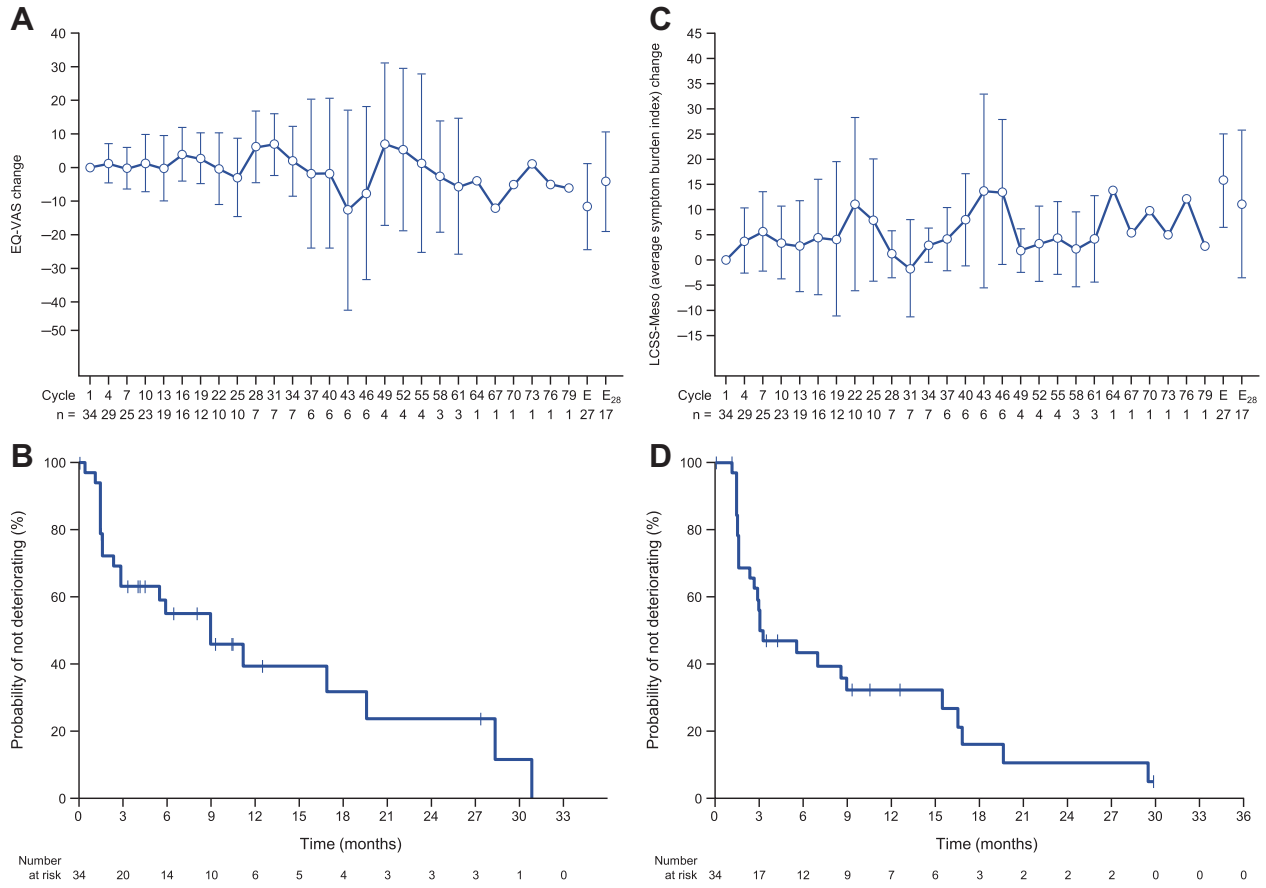

Figure 4. (*A*, *B*) Evolution of EQ-VAS and (*C*, *D*) LCSS-Meso average symptom burden index over time. Data are presented as means with 95% CIs. E, end of the treatment period (discontinuation); E_{28}, 28 days after the end of the treatment period; EQ-VAS, EuroQOL visual analog scale; LCSS-Meso, Lung Cancer Symptom Scale for mesothelioma.

LCSS-Meso symptom burden index, was maintained over time in this cohort of nivolumab-treated patients. The stability of QOL in nivolumab-treated patients observed here may reflect the potential clinical benefit of nivolumab in terms of long-term survival, especially in responders.

The MERIT study also monitored the safety of nivolumab in patients with MPM. Of note, despite the longer follow-up of patients in the present analyses, we detected no additional TRAEs (any grade or grades 3–4) since the previous cutoff date,[5,8] supporting the long-term safety of nivolumab in this patient population.

Another promising strategy for the treatment of MPM involves combining nivolumab with ipilimumab, a CTLA-4 antibody. This strategy was tested in the CheckMate 743 study, in which nivolumab plus ipilimumab significantly extended OS compared with chemotherapy (median: 18.1 versus 14.1 months, hazard ratio = 0.74, $p = 0.002$) with a median follow-up of 29.7 months.[13] Thus, this combination is expected to become a standard of care for MPM in the future. However, nivolumab monotherapy after second-line treatment may be useful for ICI-naive patients.

Our findings should be discussed in the context of the limitations of the study, notably the single-arm design and the sample size (34 patients). Furthermore, the subgroups included in the analyses of overall response and survival were small, which might introduce some bias because the study was not powered to detect differences among subgroups. Therefore, we must take care when generalizing the results to a broader population of patients treated with nivolumab in clinical practice, and our findings should be confirmed in future studies with more patients.

In conclusion, the 3-year follow-up of the MERIT study reveals the longer-term efficacy and safety of nivolumab with survival for more than 3 years in some patients and a clinical benefit as second- or third-line therapy for patients with MPM.

Data Availability

Qualified researchers may request Ono to disclose individual patient-level data from clinical studies through the following website: ClinicalStudyDataRequest.com. For more information on Ono's Policy for the Disclosure of

Table 2. TRAEs in Two or More Patients (N = 34)

AE	Any Grade	Grades 3-4
Any	26 (76.5)	11 (32.4)
Most common AEs by preferred term (in ≥2 patients)		
Rash	6 (17.6)	1 (2.9)
Lipase increased	5 (14.7)	4 (11.8)
Diarrhea	4 (11.8)	2 (5.9)
Amylase increased	4 (11.8)	2 (5.9)
Stomatitis	3 (8.8)	1 (2.9)
Weight decreased	3 (8.8)	1 (2.9)
Decreased appetite	3 (8.8)	1 (2.9)
Fatigue	3 (8.8)	0 (0.0)
Malaise	3 (8.8)	0 (0.0)
Arthralgia	3 (8.8)	0 (0.0)
Pneumonitis	2 (5.9)	2 (5.9)
Interstitial lung disease	2 (5.9)	1 (2.9)
Hypothyroidism	2 (5.9)	0 (0.0)
Nausea	2 (5.9)	0 (0.0)
Vomiting	2 (5.9)	0 (0.0)
Mucosal inflammation	2 (5.9)	0 (0.0)
Pyrexia	2 (5.9)	0 (0.0)
Lymphocyte count decreased	2 (5.9)	0 (0.0)
Rash maculopapular	2 (5.9)	0 (0.0)

Note: Data are presented as n (%).
AE, adverse event; TRAE, treatment-related AE.

Clinical Study Data, please see the following website: https://www.ono.co.jp/eng/rd/policy.html.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *JTO Clinical and Research Reports* at www.jtocrr.org and at https://doi.org/10.1016/j.jtocrr.2020.100135.

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